Design, Synthesis, and Biological Evaluation of Novel Tri- and Tetrasubstituted Imidazoles as Highly Potent and Specific ATP-Mimetic Inhibitors of p38 MAP Kinase: Focus on Optimized Interactions with the Enzyme's Surface-Exposed Front Region

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The synthesis, biological testing, and SAR of novel 2,4,5- and 1,2,4,5-substituted 2-thioimidazoles are described. Amino, oxy, or thioxy substituents at the 2-position of the pyridinyl moiety were evaluated for their contributions to inhibitor potency and selectivity against p38 mitogen activated protein kinase (p38 MAPK) as well as for the ability to minimize cytochrome P450 (CYP450) inhibition. Incorporation of polar substituted (cyclo)aliphatic amino substituents (e.g., tetrahydropyranylamino), which positively interacted with the surface-exposed front region (hydrophobic region II) of the enzyme led to the identification of extremely potent p38 MAPK inhibitors with p38 IC_{50} values in the low nanomolar range. Approximately 90 pyridinylimidazole-based compounds with a range of potencies against p38α MAP kinase were further investigated for their ability to inhibit the release of tumor necrosis factor-α (TNFα) and/or interleukin-1β (IL-1β) from human whole blood. Some of the most promising drug candidates underwent selectivity profiling against a panel of 17 different kinases besides p38α and/or were tested for their interaction potential toward a number of metabolically relevant CYP450 isozymes.

Introduction

Protein kinases are critical enzymes in cellular signal transduction cascades. Numerous diseases including inflammatory and autoimmune diseases such as rheumatoid arthritis (RA) or inflammatory bowel disease IBD), neurodegenerative conditions, cardiovascular events, and even cancer could be associated with atypical regulation of protein kinase-mediated cell signaling.[1] The progress of chronic inflammation is driven by an amplified systemic occurrence of several proinflammatory cytokines like tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), whose biosynthesis and release is elevated by the activity of serine/threonine/tyrosine kinases, e.g., the signal molecule p38 mitogen-activated protein kinase (p38, p38 MAPK[a]).[2,3] While p38 is mainly activated by ambient cell stress (e.g., UV radiation, osmotic shock, mechanic stress, lipopolysaccharide (LPS) exposure), this kinase can also be phosphorylated upon TNFα or IL-1β receptor binding and functions in the cell signaling network responsible for the up-regulation of these inflammatory mediators both at the transcriptional and at the translational level.[4] Several small molecule p38 MAPK inhibitors have been shown to effectively block the production of IL-1, TNF, and other cytokines in vitro and in animal tests.[5–8] Many of these "ATP mimetics" were derived from the prototypical pyridin-4-ylimidazole SB203580 (Figures 1 and 2), which decisively contributed to the identification and characterization of the p38 MAPK signaling pathway as a valid therapeutic target in inflammation, and the structural requirements for p38 inhibition have been reported.[8–10]

The essential interactions of pyridinylimidazole inhibitors with the ATP-binding cleft are briefly summarized as follows (see Figure 4):[11–13] (1) hydrogen donor/acceptor functions of the 2-aminopyridyl residues within the hinge region (mainly gaining activity); (2) space-filling lipophilic aryl residues binding to the hydrophobic back pocket (also hydrophobic region I, mainly gaining selectivity); (3) interactions with the hydrophobic front region (also hydrophobic region II, Figure 3, gaining both activity and selectivity); (4) further interactions with both the sugar pocket and the phosphate binding region (importance less clear, preferred positions to modify physicochemical properties).

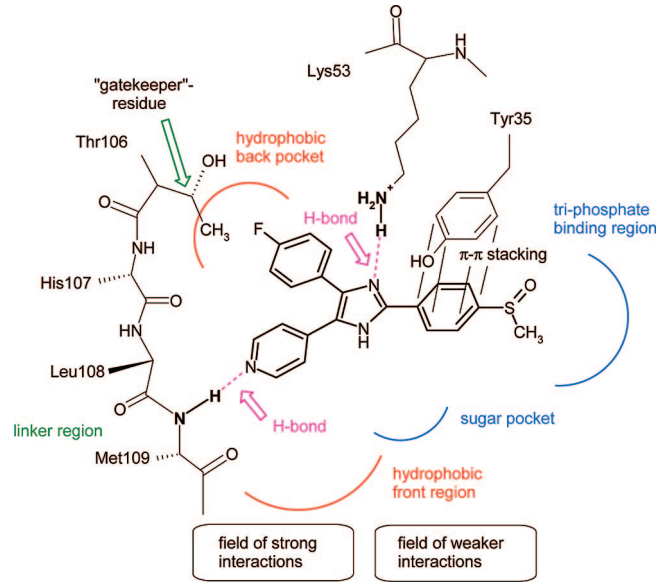

Figure 1. Essential interactions of SB203580 with p38α MAP kinase. The base of the hydrophobic back pocket defined by the residue of Thr106 (gatekeeper) is more effectively occupied by selective 4-fluorophenyl ring containing inhibitors. Sufficient contacts between the side chain of the respective gatekeeper residue and this 4-fluorophenyl substituent are not possible within other MAPK (family members), e.g., p38δ, p38γ, or ERK2, thus gaining selectivity.

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[a] Abbreviations: p38 MAP kinase, p38 mitogen activated protein kinase; ATP, adenosine triphosphate; CYP450, cytochrome P450; LPS, lipopolysaccaride; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; SAR, structure−activity relationship.

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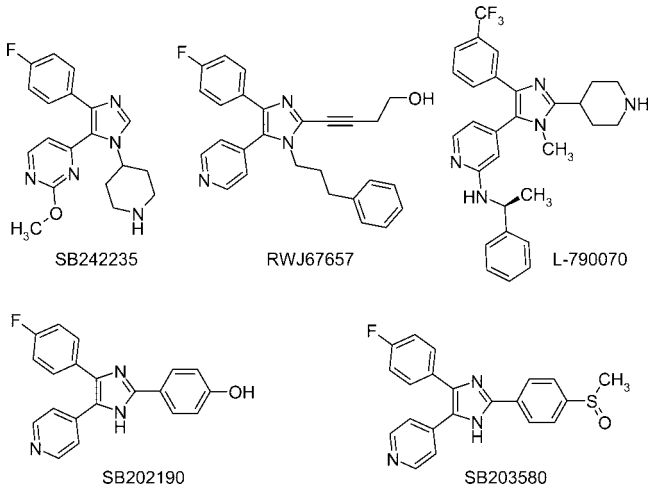


Figure 2. Pyridinyl- and pyrimidinylimidazole-based p38 MAP kinase inhibitors, which have been advanced to preclinical or clinical studies for therapeutic intervention of acute and chronic inflammatory diseases.

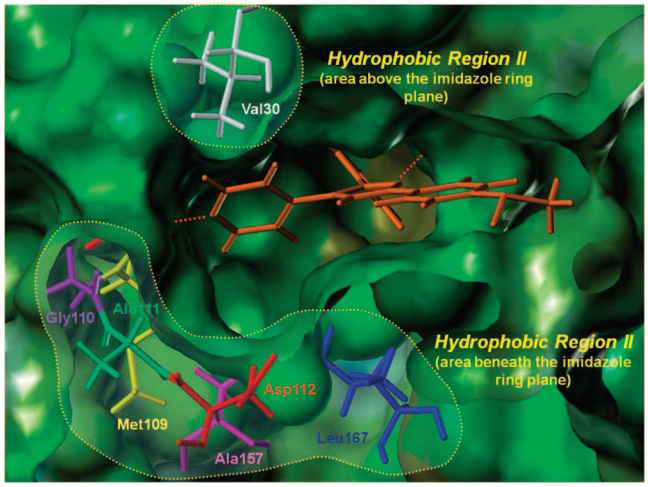


Figure 3. View into the ATP-binding site of p38 with the bound pyridinylimidazole prototype inhibitor SB203580 (brownish formula). Corresponding amino acids (differently colored) of both areas of the hydrophobic region II, stretching above as well as beneath the ring plane of the imidazole scaffold, are highlighted. The hydrophobic region II is widely opened to the solvent area (in the front). In the case of p38 this hydrophobic region seems to be capable of accommodating large and electronically diverse substituents linked to the 2-position of the pyridine of the respective pyridinylimidazole inhibitor (figure generated with Sybyl on the basis of PDB structure 2ewa).

Although almost 20 compounds under investigation as anticytokine agents entered clinical studies, only six p38 MAPK inhibitor candidates (BIRB796, SCIO469, VX702, pamapimod (Roche), SB-681323, and GW-856553)[9,14] for the treatment of rheumatoid arthritis reached phase II clinical trials.[15] Poor kinetic properties and unacceptable safety profiles including side effects in the liver and in the CNS were mainly responsible for this failure. Infections, dermatological diseases, or renal problems have also been observed.[16,17] Although the toxicity of the p38 inhibitor class is believed to be primarily mechanism-derived, there are also strong indications for nonmechanistic, CYP450-negotiated, harmful effects.

First generation pyridinylimidazoles, e.g., SB203580, are liver-selective and have been associated with CYP450 inhibition. Both imidazoles and pyridines coordinate well to the heme iron of the human cytochrome P450 (CYP450) system, a common characteristic associated with the electron density of these heterocycles, and related compounds possessing these substructures are often potent inhibitors of P450 enzymes.[18–20] However, the exposed pyridin-4-yl ring of the diaryl heterocyclic inhibitors, which is obligatory to promote strong binding to the backbone Met109 within the linker region of p38 than the better shielded core heterocycle (imidazole), is regarded as the ligand more likely to coordinate to the CYP450 system.[18] Inhibitors of human hepatic cytochrome P450 can potentially cause drug−drug interactions or lead to other hepatic changes such as P450 enzyme induction. Furthermore SB-like small molecule p38 inhibitors occasionally possess genotoxic potential.

The selectivity profile, i.e., the extent of p38 inhibition versus inhibition of other protein kinases, represents a critical feature of inhibitor design as well. Until now 518 different kinases have been identified in the human genome (=kinome).[21] Nearly one-fourth of the druggable kinome consists of kinases involved in signal transduction.[22–24] All these kinases possess a catalytic domain, which is highly conserved in sequence and structure and wherein the native cosubstrate ATP binds in a very similar manner.[21] The general architecture of the ATP binding site in protein kinases has been sufficiently reviewed.[25–28] The design of a small, highly specific synthetic inhibitor directed against the ATP-binding site of a single kinase is hampered by the remarkable consistencies in the catalytic mechanisms of the kinases, the high degree of sequence homology, identical protein folding topologies, and the common cosubstrate ATP.[17,23,29]

Nonetheless, many selective p38 MAPK inhibitor candidates are based upon the imidazole scaffold and bind at the ATP site located in the cleft between the N- and the C-terminal domains of p38 (Figure 2).[30] This class of compounds is exemplified by the p38 inhibitors SB203580, SB202190, SB242235, L-790070, RWJ67657, and SB202190 (Figure 2) and targets all major interaction sites.[31]

Because first generation SB-like p38 inhibitors suffered from nonmechanistic side effects, more structural modification will be necessary. Thus, attempts to optimize newer pyridinylimidazole inhibitors have included incorporating different substituents on the imidazole core itself and/or by introducing additional (amino) substituents at the ortho position of the pyridin-4-yl moiety.[7,32] Particularly, the substituents at the imidazole N1 and C2 positions have been varied extensively to improve physicochemical properties and to reduce toxicity.[33,34] The different design strategies have led to several inhibitors with high potency and enhanced selectivity among closely related kinases.[8,35] Here, 2-sulfanylimidazoles and their active in vivo metabolic transformation products (sulfoxides, sulfones) demonstrated decisive advantages over prototype SB203580-like 2-arylimidazoles, e.g., fewer interactions with metabolic enzymes like CYP450 and better kinetic and metabolic properties.[36–39]

The 2-alkylsulfanyl-5-(pyridin-4-yl)imidazoles **III** and **IV** (Figure 5) have been recently recognized as strong inhibitors of both the isolated p38 MAP kinase and cytokine release in PBMCs as well as in human whole blood, with IC_{50} values in the low micromolar range, comparable to those of the potent model compound SB203580.[36] Furthermore, CYP450-dependent toxic side effects were decisively reduced, thus showing a general advantage of 2-thioimidazoles compared to prototypical SB-like 2-aryl-subtituted derivatives. Compounds **III** and **IV** can be viewed as open chain analogues of the early lead compound SK&F86002 (Figure 5) and are derived from their parent by opening of the imidazothiazole ring system via the biologically active derivatives **I** (starting point for trisubstituted

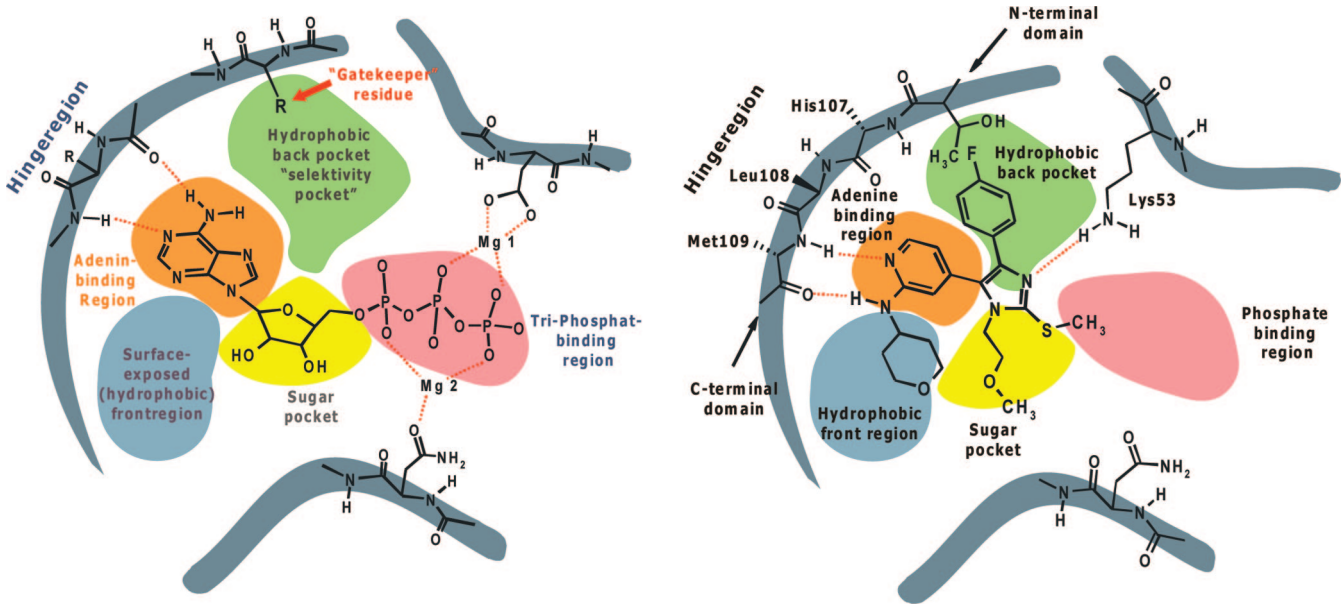


Figure 4. Binding mode of ATP (left) and proposed binding mode of an exemplary inhibitor molecule (compound **35i**, right) to the ATP-binding site of p38α MAP kinase. The hinge part of the kinase polypeptide backbone is shown on the left. The hydrophobic back pocket and the surface-exposed front region are not utilized for interactions with the enzyme's native cosubstrate ATP.

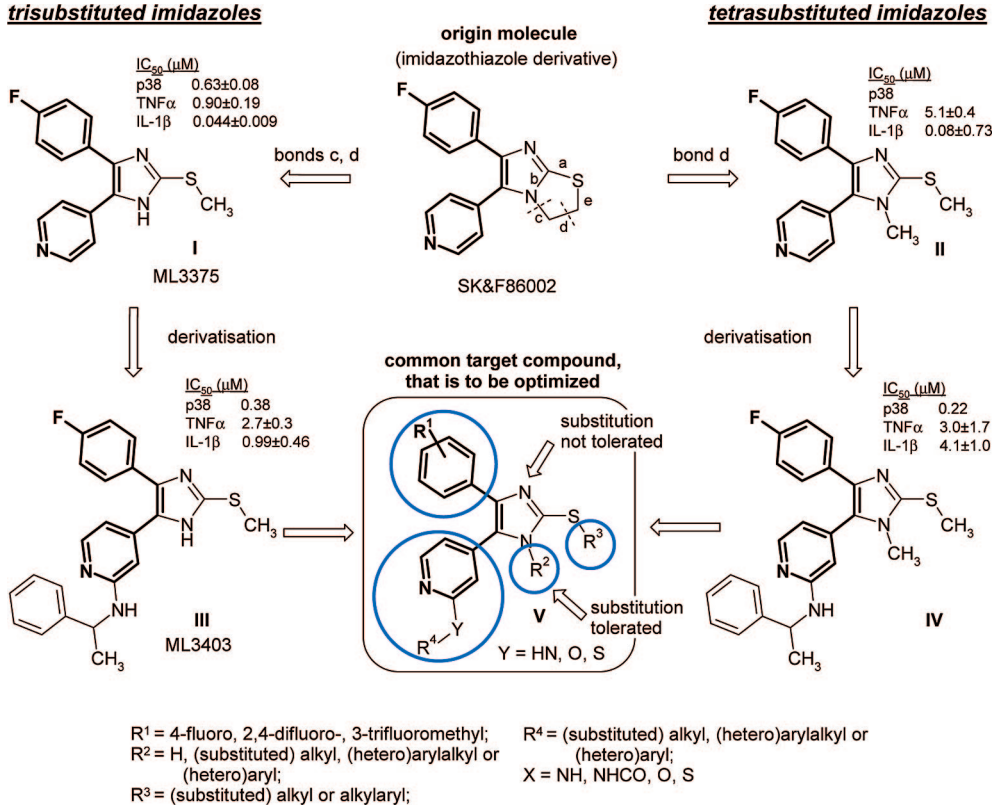
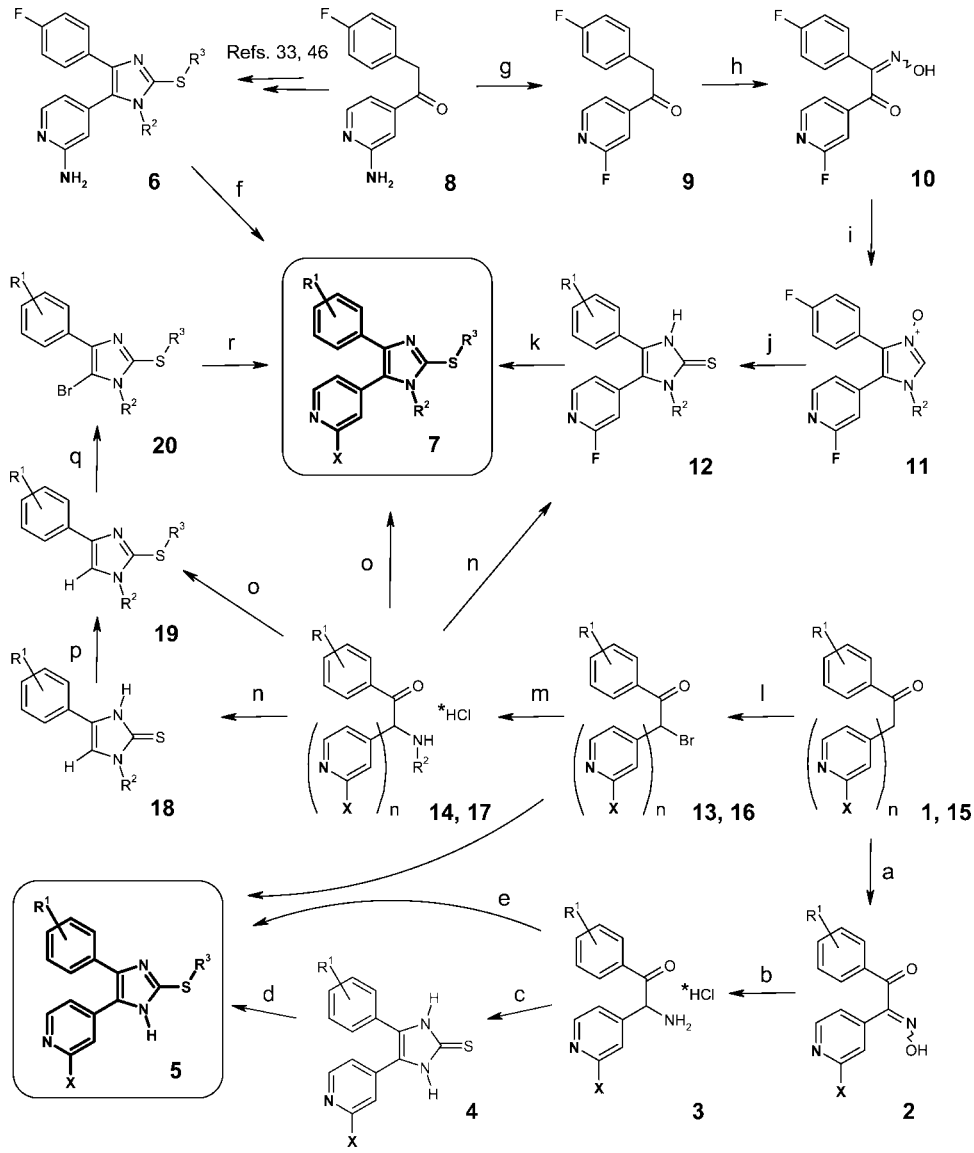


Figure 5. Structural deduction of the general target molecule **V** from the early lead compound SK&F86002. The partial structures that are to be varied and optimized in order to improve relevant parameters like activity, selectivity, toxicity, or physicochemical properties are encircled in blue. The fundamental vicinal diaryl system of this compound class is highlighted as well (bold). Reported p38 IC_{50} data for compounds **I**−**IV** descend from an old in-house test model, where the average test values were about 1 order of magnitude (10-fold) higher than those of the newly synthesized and here presented pyridnylimidazoles, which were tested using a new, partly automated ELISA assay.

2-alkylsulfanylimidazoles) and **II** (starting point for trisubstituted 2-alkylsulfanylimidazoles) and placement of additional phenylethylamino substituents at the pyridine component.

In response to the need for promising development candidates and to overcome the lack of specificity as well as the CYP450-dependent side effects (hepatotoxicity) of the diaryl-substituted heterocyclic class of p38 inhibitors, we aimed to synthesize highly substituted 2-thioimidazoles targeting all relevant regions within the binding site of the kinase. By exploitation of less-conserved surrounding kinase areas, those not or only marginally involved in ATP binding, it was proposed to improve the pharmacological profile of the small molecule inhibitors (Figure

Scheme 1. Summary of the Synthetic Approaches toward the 2-Halogenopyridinylimidazole Scaffolds **5** and **7**[31,41,a]



[a] Reagents and conditions: (a) $NaNO_2$, glacial acetic acid, room temp; (b) H_2, Pd−C 10%, 1 atm, 2-propanolic hydrogen chloride, room temp; (c) DMF, KSCN, reflux; (d) iodomethane, ethanol/THF 8 + 2, reflux;[36] (e) DMF, methyl-/ethyl-/benzylthiocyanate, reflux;[41] (f) 50% HBF_4 (aq), $NaNO_2$, 0 °C then 45 °C;[74] (g) $NaNO_2$, 70% HF in pyridine (Olah's reagent), −15 to −10 °C → room temp; (h) $NaNO_2$, glacial acetic acid, room temp; (i) $(H_2CNR^2)_3$, ethanol, reflux; (j) 2,2,4,4-tetramethylcyclobutane-1,3-dithione, dichloromethane, room temp; (k) Hal-R^3, K_2CO_3, methanol, room temp; (l) Br_2, glacial acetic acid, room temp;[45] (m) excess H_2N-R^2, DCM (+MeOH), −5 to 0 °C, then 1.25% HCl/EtOH, diethyl ether/acetone 1 + 1; (n) KSCN, DMF, reflux; (o) R^3-SCN, DMF, reflux; (p) Hal-R^3, K_2CO_3, methanol, room temp; (q) NBS, CCl_4, 0 °C then room temp; (r) $Pd(PPh_3)_4$ resp. $PdCl_2(PPh_3)_2/PPh_3$, argon, 2 M Na_2CO_3 or K-acetate, toluol or DMF, 120 or 80 °C.[75]

4).[17,26] These sites of incomplete interaction with the bound cosubstrate ATP vary widely among the 518 identified human kinases and thus offer great capacity for selective inhibitor binding (Figure 4).[23,40] Here, the often neglected surface-exposed front region (Figures 3 and 4) seemed to be best suited for further improvements in potency and selectivity of known inhibitor molecules.[23] In the present work, the 2-alkylsulfanylimidazole derivatives (**III** and **IV**) served as starting compounds for additional structural optimizations. Moreover, introduction of substituents at the pyridine ring that are sterically demanding or electronically shielding, but at the same time metabolically stable, should reduce interferences with the CYP450 system and reduce in vivo toxicity.

Chemistry

Synthesis of the 2,4,5-trisubstituted imidazole scaffolds **5** bearing a substitutable 2-halogenopyridin-4-yl at the 4- or 5-position (depending on the present tautomeric form) was performed starting from ethanones **1** via the intermediates **2** and **3** (and **4**) and pursuing either a previously published four-step nitrosation/reduction/cyclization strategy[36] or a newly optimized and fully regioselective three-step variant with generally high overall yields (lower part of Scheme 1, Table 1).[41]

Alternatively, **5** could be obtained in only two steps but in lower yields by direct cyclization of an α-bromoketone with *S*-methylisothiourea (Scheme 2).[42–44] To achieve the 2-chloropyridin-4-ylimidazole target compound **5d**, the appropriate 1-aryl-2-heteroaryl-substituted ethanone **1b** was subjected to acid catalyzed α-monofunctionalization reaction with Br_2 at ambient temperature.[45] The resulting unstable α-bromoethanone intermediate **13c** then was immediately reacted with *S*-methylisothiuronium sulfate in DMF under reflux conditions to obtain a mixture of cyclization products from which the imidazole-2-

Table 1. Substitution of Compounds **1**−**20** According to Scheme 1

compd	R^1	R^2	R^3	*n*	X
1a	4-F			1	F
1b	4-F			1	Cl
1c	4-F			1	Br
1d	3-CF_3			1	F
2a, **3a**, **4a**	4-F				F
2b, **3b**, **4b**	4-F				Cl
2c, **3c**[a]	4-F				Br
2d, **3d**	3-CF_3				F
3e	4-F				H
5a	4-F		Me		F
5b	4-F		Et		F
5c	4-F		Bn		F
5d	4-F		Me		Cl
5e	4-F		Et		Cl
5f	4-F		Bn		Cl
5g	4-F		Me		H
5i	3-CF_3		Me		F
5j	3-CF_3		Et		F
5k	3-CF_3		Bn		F
6a		Me	Me		
6b		$(CH_2)_2OMe$	Me		
7a	4-F	Me	Me		F
7b	4-F	$(CH_2)_2OMe$	Me		F
7c	4-F	$(CH_2)_2OMe$	Bn		F
7d	4-F	$(CH_2)_2OMe$	$CH_2CH(OH)CH_2OH$		F
7e	4-F	Me	Me		H
11a		Me			
11b		$(CH_2)_2OMe$			
12a	4-F	Me			
12b	4-F	$(CH_2)_2OMe$			
12c	4-F	$(CH_2)_2OMe$			
13a	4-F			1	F
13b	3-CF_3			1	F
14a	4-F	Me		1	F
14b	4-F	$(CH_2)_2OMe$		1	F
14c	3-CF_3	$(CH_2)_2OMe$		1	F
15a	H			0	
15b	4-F			0	
16a	H			0	
16b	4-F			0	
17a	H	$(CH_2)_2OMe$		0	
17b	H	Bn		0	
17c	4-F	Me		0	
17d	4-F	$(CH_2)_2OMe$		0	
18a	H	$(CH_2)_2OMe$			
18b	H	Bn			
18c	4-F	Me			
18d	4-F	$(CH_2)_2OMe$			
19a	H	$(CH_2)_2OMe$	Me		
19b	H	Bn	Me		
19c	4-F	Me	Me		
19d	4-F	$(CH_2)_2OMe$	Me		
20a	4-F	Me	Me		
20b	4-F	$(CH_2)_2OMe$	Me		

[a] As hydrogen sulfate.

thioether **5d** could be isolated in 12% yield by column chromatography.[42] As side products (SP), the related 2-aminothiazole derivative (SP1, $m/z = 305$), an obvious S-oxidation artifact of **5d** (SP2, $m/z = 335$), and higher amounts of debrominated starting material (ethanone **1b**) were identified (GC−MS) and separated.

We also applied the same reaction conditions to the reaction of **13c** with *N*,*S*-dimethylisothiourea in an attempted regioselective preparation of 1,2,4,5-tetrasubstituted imidazoles. However, contrary to the results of Dodson et al. for a structurally similar compound, no product accumulation was observed.[44] Instead, decomposition of the starting material(s) took place.

The required 1,2,4,5-tetrasubstituted fluoropyridinylimidazole precursors **7**, which allow the subsequent S_NAr-like introduction of chiral *N*-, *O*- or S-(aryl)alkyl or aryl substituents at the 2-position of the pyridine, could be obtained via four different regioselective syntheses,[31] which are outlined in Scheme 1 (upper part) starting from 5-(2-aminopyridin-4-yl)imidazoles **6**,[33,46] 1-(2-aminopyridin-4-yl)ethanone **8**,[33] or 2-(2-fluoropyridin-4-yl)ethanones **1**[36,41] and acetophenones **15**,[47] respectively. Table 1 summarizes the substitution pattern of all intermediates as well as of the target compounds **7**.

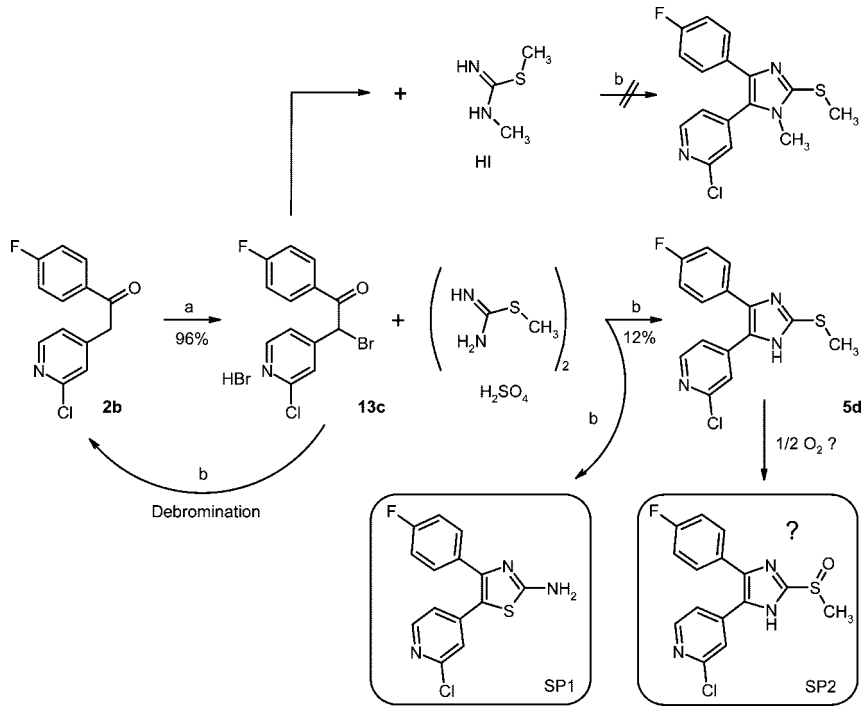
The most elegant access to compounds **7** with moderate overall yields was accomplished from 2-(2-fluoropyridin-4-yl)ethanones **1** via the obligatory intermediates **13** and **14** and the optional imidazole-2-thiones **12** by a novel and extremely flexible synthetic methodology that allowed the regioselective introduction and variation of all relevant substituents in only three (or four) steps.[31]

2-Fluoropyridin-4-yl compounds **7** were well suited for the synthesis of target compounds **35** (Table 8) upon substitution with all kinds of nucleophiles (Scheme 3).

In a final step, a variety of different (hetero)aryl, (hetero)arylalkyl, and (cyclo)alkyl residues could be introduced at the pyridin-4-yl moiety of the obtained halogenopyridinylimidazoles via known S_NAr methods using suitable amines, alcohols, or thioalcohols as the corresponding nucleophiles, giving p38 inhibitors with maximum structural diversity.[32,48] Starting points for further derivatization primarily were the methylsulfanylimidazoles **5a**, **5d**, **5i**, **7a**, and **7b** (Scheme 1, Table 1) with a 2-chloro- or a 2-fluoropyridinyl moiety. The functional requirement for the reactivity of the pyridine component was achieved by the above-mentioned syntheses. C2 of the pyridine is activated for S_NAr reactions. Substituents are directed ortho (or para) by the −M effect of the heterocyclic sp^2-N. The S_NAr mechanism allows the introduction of chiral building blocks (N-, O-, or S-nucleophiles) and even tolerates aniline-like substituents [e.g., **33c**, **34a** (Table 7), **35e** (Table 8)], which generally cannot be coupled when using aminopyridines **6** and substituted alkyl halides as reactants and executing a S_N reaction.[31] Commercial availability of several primary or secondary amines allows rapid variation leading to a broad scope of substitution patterns.

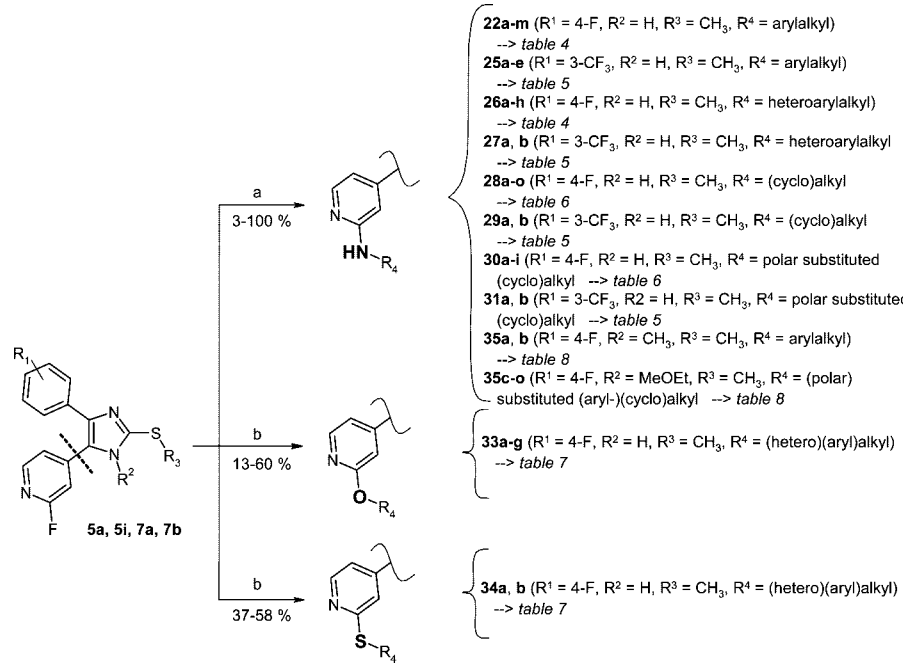
The chloropyridinylimidazoles **5d**−**f** proved to be rather inactive toward conventional nucleophilic aromatic substitution reactions, with several model primary amines (e.g., benzylamine) requiring harsh conditions (excess of amine, high temperatures, long reaction times, solvent-free operation) that led to multiple side products. Our initial intention was to reinforce the coupling tendency of such 2-chloropyridine intermediates (**5d**) toward amines (here, 1,2-diphenylethylamine) with the aid of Pd catalysts conducting a cross-coupling-like C−N formation method. However, an attempted literature procedure using a chelate complex of either (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl [(±)BINAP] or 1,3-bis(diphenyl-phosphino)propane (dppp) with $Pd_2(DBA)_3$ as the catalytic system, NaO-*t*-Bu as base, and toluene (or DMF) as solvent at 70−80 °C under argon was not successful.[49] None of the desired substitution product was detected. Furthermore, attempts to deprotonate some amines with NaH in DMF giving the putatively more reactive ionic nitrogen nucleophiles also failed, regularly leaving unstirrable gels that prevented completion of this reaction. Dimethylformamide (DMF) reacts as an equivalent of dimethylamine in nucleophilic substitution of active halogen atoms implicating dimethylaminopyridines (exemplified by **32a**) as side products.[50] Therefore, the points of origin for further derivatization at the pyridyl moieties of the starting imidazole-2-thioethers were the presumably more reactive 2-fluoropyridines **5a**, **5i** as well as **7a** and **7b**. Here, the reactivity is additionally enhanced by the high electronegativity of the fluorine atom strongly polarizing the pyridine-C_2−F bond, making it the preferred leaving group.

Scheme 2. Condensation with *S*-Methylisothiuronium Sulfate or *N*,*S*-Dimethylisothiuronium Iodide[a]



[a] (a) Br_2, glacial acetic acid, room temp; (b) DMF, reflux.

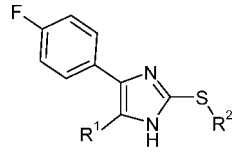
Scheme 3. Nucleophilic Aromatic Displacement of the Fluorine at the Pyridine Moieties[a]



[a] Reagents and conditions: (a) 5- to 20-fold excess of neat primary or secondary amine (aliphatic or aromatic), reflux or 150−170 °C, argon; (b) NaH, DMF, excess of aliphatic or aromatic alcohol or thioalkohol, 160 °C, argon.

For the S_NAr with N-nucleophiles these fluoropyridine reactants were either directly dissolved or suspended in a 5- to 10-fold excess of the respective liquid amino reactant and the mixture was stirred (refluxed) under argon at 150−170 °C until the starting material completely disappeared, an augmented appearance of side products was observed, or the product accumulation ceased. In the case of solid but low melting amines, the substitution reaction was carried out in the molten material under otherwise identical conditions. Amines with low boiling points (≪100 °C), on the other hand, only could be effectively coupled under elevated pressure in a glass or metal bomb. When the 2-fluoropyridines were used, the nucleophilicity of the introducible and mostly primary amines or amino alcohols was sufficient for S_NAr and the latter did not have to be activated by basic deprotonation in advance. When amino alcohols were reacted, possible concomitant ether formation at the specified position of the pyridnylimidazole was never detected and only the respective aminopyridine derivatives were isolated.[51] In

Table 2. Biological Activity of 2-Halogen-Substituted Pyridin-4-yl- and Quinolin-4-ylimidazole Derivatives with a 4-Fluorophenyl Moiety at Imidazole C4 or C5[a]



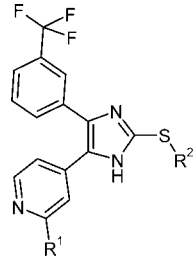
#	R^1	R^2	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
5a		-CH_3	0,559±0.002 (n=2)	29,9±0,529 (n=4)	n. t.
5d		-CH_3	1,04 (n=1)	n. t.	n. t.
ML 3375 (5g)		-CH_3	0,069±0,024 (n=4)	8,65±2,63 (n=8)	n. t.
5h[41]		-CH_3	0,411±0,056 (n=4)	16,8±1,39 (n=4)	35,0±10,9 (n=4)
5b		-C_2H_5	0,213 (n=1)	n. t.	n. t.
5c			0,258 (n=1)	n. t.	n. t.
5e		-C_2H_5	0,372±0,108 (n=4)	36,0±9,83 (n=4)	n. t.
5f			2,90±1,94 (n=4)	50,9±2,23 (n=4)	n. t.
Reference SB203580			0.062±0,006 (n = 164)	2,30±0,105 (n=81)	1,98±0,32 (n=18)

[a] *n*: number of experiments. n.t.: not tested.

contrast, less nucleophilic alcohols and thioalcohols merely reacted as the corresponding anionic salts (alcoholates or thioalcoholates, respectively). Consequently, quite a few N-, O- and S-nucleophiles could be readily introduced at the 2-position of the respective pyridine moiety using known S_NAr methods (Tables 1–10). In the case of 2,4,5-trisubstituted imidazoles, nucleophilic aromatic substitutions frequently could not be driven to completion leaving unreacted starting material and traces of undefined side products. In contrast, when the corresponding 1,2,4,5-tetrasubstituted imidazoles were produced, reaction times were even shorter and yields were significantly higher (up to 100%). Usually no side products could be observed, as both the starting materials (**7a**, **7b**) and the resulting products **35a**−**o** (Table 8) proved to be surprisingly thermostable. Because of steric hindrance, primary amines having an additional α-(aryl)alkyl branch on their (hetero)(aryl)alkyl side chain on average gave lower product yields than those with a linear aliphatic residue. Attempts to couple, for example, α,α-dimethylpropylamine with the 2-fluoropyridinyl of **5a** using comparable reaction conditions completely failed and could not be accomplished, even with attempts to accelerate the reaction time drastically by microwave radiation.

We also proposed to undertake pyridine amination of analogous 2-halogenopyridin-4-ylimidazole-2-thioethers with an exocyclic *S*-ethyl or *S*-benzyl substituent instead of the small methyl group. Because of the obligatory long reaction times, initial attempts to specifically react the chloropyridinyl analogue **5f** by means of S_NAr with 1-phenylethylamine resulted in the concomitant nucleophilic attack at the benzylic position of the benzylsulfanyl substituents by the excessive amine. The unwanted cleavage of the benzyl residue at the exocyclic sulfur combined with the actually expected amination at the fluoropyridine ring inevitably resulted in the production of the imidazol-2-thione **24** (Scheme 4), as the single isolated and detected product.[36] To avoid this problem, we chose to prepare the 2-aminopyridin-4-yl imidazole-2-thione **24** first as a main product that should be consequently S-alkylated in a final step (Scheme 4). This inverse process then would probably tolerate the subsequent introduction of functionalized S-residues as well. However, reacting the starting 2-fluoropyridin-4-ylimidazol-2-thione **4a** with a 10-fold excess of 1-phenylethylamine yielded a mixture of several products, from which **24** could not be isolated as a pure solid by any accepted method (LC/MS purity: ∼50−60%).

Table 3. Biological Activity of 2-Halogen-Substituted Pyridin-4-ylimidazole Derivatives with a 3-Trifluoromethylphenyl Moiety at Imidazole C4 or C5



#	R^1	R^2	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
5i	-F	$-CH_3$	1,09±0,396 (n=4)	44,2±8,06 (n=4)	n. t.
5j	-F	$-C_2H_5$	0,657±0,139 (n=4)	71,5±13,9 (n=4)	n. t.
5k	-F		1,95±0,345 (n=4)	83,6±2,09 (n=2)	n. t.
Reference SB203580			0.062±0,006 (n = 164)	2,30±0,105 (n=81)	1,98±0,32 (n=18)

^{1}H (and ^{13}C NMR) spectra of the prepared (and in general relatively hydrophilic) amino or (thi)oxypyridinylimidazole compounds **22** and **25**−**35** (see Scheme 3) were preferably collected in deuteromethanol. Whenever the ^{1}H NMR spectra were collected in the more viscous DMSO at ambient temperature, we observed two sets of signals (in nearly 1:1 ratio), indicating a slow (on the NMR time scale) equilibrium between two isomers (atropisomers).[52] This effect could be attributed to the sterically restricted free rotation of the substituted pyridinyl ring around its σ bond with the imidazole core, particularly at compounds with bulky (aryl)alkyl residues [for example, at compounds **5h** (spectrum is in the Supporting Information), **22e**, **26a**, **c**, **28a**, **30c**, and **33d**]. The appearance of different atropisomers, however, repeatedly complicated the interpretation of the spectra. In deuteromethanol the interconversion of the two isomers is fast on the NMR time scale and the resonances are averaging to a single signal.

Biological Testing

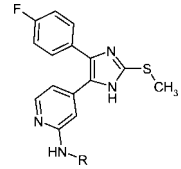
All compounds were primarily screened in an isolated p38α MAP kinase assay.[53,54] Inhibition of cytokine release (TNFα, IL-1β) from immune cells was accomplished using diluted fresh human whole blood of at least two anonymized donors.[55–57] CYP interactions were investigated by BDGentest Corp.[58] The selectivity profiling of selected compounds against a panel of 18 different kinases (including p38α) was performed by Upstate.[59]

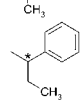
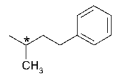
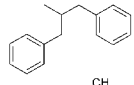
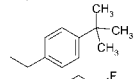
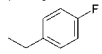
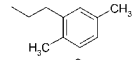
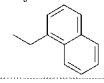
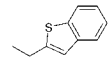
Biological Discussion

2,4,5-Trisubstituted Imidazoles. Since the objective 2-thioimidazole derivatives are structurally similar to SB203580, they may bind similarly to the p38 MAP kinase through comparable interactions with active site residues, with the exception of $\pi-\pi$ stacking with Tyr35.[34] Structure−activity-relationships (SAR) comparable to those of SB203580 or analogous literature examples have been described for different series of variable tri- or tetrasubstituted 2-(aryl)alkylsulfanylimidazoles whose structures are based on the pyridinylimidazole p38 inhibitors ML3163 and ML3375 (Figure 6).[8,33,35,36] These earlier studies established that small (methyl) substituents located at the exocyclic sulfur atom (ML3375) significantly improved inhibition of activity in both the isolated kinase assay and of cytokine release from human whole blood. This greater inhibitory potential was attributed to better entry of the inhibitor molecule into the binding cleft of p38, leading to stronger enzyme−drug interactions.[34] Furthermore, diverse C2-S-substituents were investigated as ligands possibly targeting the kinase phosphate binding region, in close analogy to the triphosphate end of ATP.[60] Attempts to develop compounds that bind at positions within the p38 kinase that are unoccupied by first generation inhibitors like ML3375 or SB203580 were conducted mainly by regioselective introduction and structural variation of additional functionalities, e.g., at the imidazole nitrogen that is located next to the pyridyl part and/or at the 2-position of the pyridine ring.[33,36] These novel interactions included the formation of a second hydrogen bond between the backbone Met109 inside the linker region and a primary or secondary amino function ortho to the pyridine ring nitrogen, hydrophilic interactions with less-conserved areas of the ribose pocket via appropriate imidazole N1 substituents, and finally, lipophilic vdW contacts and H-bonds between kinase residues or backbone atoms around the hydrophobic front region or its surrounding solvent accessible area by pyridine-C2 adducts. As the structural modifications at the imidazole N1 had been already extensively reported, this article will now focus on the introduction and wide variation of substituents at the pyridine part of these 2-thioimidazoles bridged by different heteroatoms (NH, O, S).

The strength of the H-bonding between the pyridine-N of the inhibitor and the amide-NH of Met109 is influenced by the electron donating or withdrawing capacity of the substituents. The p38 inhibitory potency has been correlated with the electron density at the respective heterocyclic ring nitrogen.[36] With reference to the parent compound ML3375 (Table 2), an electronegative halogen atom located at the 2-position of the pyridin-4-yl moiety significantly decreased the biological activity. Fluoropyridine **5a**, for example, exhibited a 2.8-fold[76] worse inhibitory potency toward the isolated enzyme, and a similar 3.5-fold decrease in the suppression of cytokine release (TNFα)

Table 4. Biological Activity of Different (Hetero)arylalkylaminopyridines (2-Thioimidazole Derivatives with a 4-Fluorophenyl ring)[a]

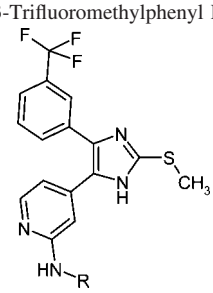


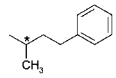
#	R	Yield[$]	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
22a[36]		41%[36]	0,139±0,001 (n=2)	n. t.	n. t.
ML3403[36] **(22b)**		17%	0,04±0,005 (n=4)	4,33±1,83 (n=4)	n. t.
22c		13%	0,068±0,014 (n=6)	0,672±0,154 (n=4)	7,64±3,08 (n=4)
22d		15%	0,028±0,003 (n=6)	2,67±0,573 (n=4)	n. t.
22e		20%	0,047±0,018 (n=4)	2,81±0,843 (n=4)	16,2±5,93 (n=12)
22f		65%	0,017±0,007 (n=4)	3,93±0,189 (n=4)	10,1±4,37 (n=8)
22g		20%	0,013±0,002 (n=6)	0,35±0,066 (n=4)	2,37±0,109 (n=4)
22h		10%	0,348±0,054 (n=4)	43,5±3,43 (n=4)	n. t.
22i[†]		14%	2,15±0,46 (n=8)	43,6±4,18 (n=4)	n. t.
22j		64%	1,34±0,311 (n=14)	52,6±7,73 (n=4)	n. t.
22k		59%	0,173±0,028 (n=4)	22,2±8,25 (n=4)	n. t.
22l		52%	0,082±0,031 (n=6)	51,5±9,02 (n=4)	59,3±0,377 (n=4)
22m		33%	0,171±0,083 (n=4)	28,0±5,7 (n=4)	n. t.
26a		34%	0,046±0,011 (n=6)	4,88±0,982 (n=4)	n. t.
26b		43%	0,023±0,002 (n=6)	14,5±4,82 (n=4)	23,5±11,3 (n=4)
26c[‡]		43%	0,314±0,085 (n=4)	55,5±1,16 (n=4)	n. t.
26d		32%	0,06±0,02 (n=4)	5,52±0,613 (n=4)	n. t.
26e		56%	0,113±0,072 (n=6)	7,43±1,84 (n=8)	n. t.
26f		31%	0,07±0,012 (n=4)	4,32±1,13 (n=4)	n. t.
26g		28%	0,098±0,024 (n=4)	9,85±1,96 (n=4)	n. t.
26h		19%	0,119±0,056 (n=4)	6,22±3,53 (n=4)	n. t.
Reference SB203580			0.062±0,006 (n = 164)	2,30±0,105 (n=81)	1,98±0,32 (n=18)

[a] Key for the symbols. $: last step (nucleophilic aromatic substitution). †: The 1-benzyl-2-phenyl-ethylamine used for the S_NAr reaction was produced by (catalytic) reductive amination (Leuckart−Wallach reaction) of 1,3-diphenylpropan-2-one according to Allegretti.[70] Yield: 53% (free base) over two steps. ‡: The C-benzo[*b*]thiophen-2-yl-methylamine used for the S_NAr reaction was produced in advance by reduction of benzo[*b*]thiophen-2-carboxamide with $LiAlH_4$ in 41% yield.

was also observed. For compound **5f**, the large benzylsulfanyl moiety at the 2-position of the imidazole core diminished the p38 inhibitory potency in contrast to its sterically less demanding ethylsulfanyl analogue **5e** (3.8-fold) and particularly with respect

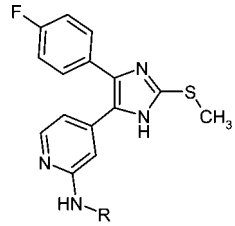
Table 5. Biological Activity of Different (Hetero)arylalkylaminopyridines, (Cyclo)alkylaminopyridines and (Cyclo)alkylaminopyridines with an Additional Polar Residue (2-Thioimidazole Derivatives with a 3-Trifluoromethylphenyl Ring)

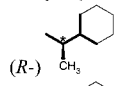
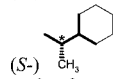
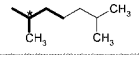


#	R	Yield	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
25a		35%	0,282±0,108 (n=4)	38,1±10,4 (n=4)	n. t.
25b		22%	0,199±0,089 (n=4)	12,2±5,54 (n=4)	n. t.
25c		11%	0,248±0,110 (n=4)	21,2±5,93 (n=4)	10,3±3,33 (n=4)
25d		42%	0,093±0,02 (n=4)	23,0±9,18 (n=4)	n. t.
25e		24%	0,049±0,014 (n=4)	4,64±2,43 (n=8)	1,29±0,069 (n=4)
27a		45%	0,239±0,028 (n=4)	26,8±12,2 (n=4)	n. t.
27b		45%	0,191±0,028 (n=4)	14,8±3,48 (n=8)	n. t.
29a		20%	0,036±0,004 (n=4)	9,89±2,83 (n=4)	n. t.
29b		3%	0,034±0,003 (n=8)	2,93±0,529 (n=4)	1,36±0,463 (n=4)
31a		33%	0,061±0,015 (n=4)	5,19±0,64 (n=4)	n. t.
31b		57%	0,011±0,0009 (n=10)	1,68±0,021 (n=4)	n. t.
Reference SB203580			0.062±0,006 (n = 164)	2,30±0,105 (n=81)	1,98±0,32 (n=18)

to the "small" methylsulfanyl derivatives ML3375, **5a**, and **5d** (17.8-, 6.4- and 2.8-fold, respectively). Moreover, comparison of TNFα-inhibition data of **5f** and ML3375 shows that the lesser p38α inhibitory activity of **5f** is largely translated into a diminished activity in the whole blood model (9.5-fold decrease compared to ML3375). The presence of the less electronegative chlorine atom (instead of a fluorine atom) in compounds **5d**, **5e**, and **5f** was not found to have significant influence on the electron-withdrawing properties at the pyridine, as the inhibition toward the isolated enzyme was comparable to the fluoropyridine derivatives (**5a**−**c**). These findings suggest that for the imidazole-2-thioethers the size of the substituent at the proximal sulfur and the resulting (in)ability of the inhibitor molecule to enter into the ATP cleft deeply have greater influence than the nature of the halogen atom at the pyridine-C2 (Figure 7). In the kinase activity assay, the ethylsulfanyl substituent of **5e** proved to be nearly bioisosteric to the well-defined methylsulfanyl substituent (e.g., at compound **5a**, divergence factor of 1.7), additionally confirming the idea of stronger binding of the 4-fluorophenyl/pyridin-4-yl pharmacophore inside the ATP site, when substituents at the exocyclic sulfur are not bulky. The slight difference (1.6-fold) in TNFα-release between alkylsulfanyls **5a** and **5e** implied similar cell penetration behavior and plasma protein binding for both compounds. The ethyl vs benzyl functionalities of **5e** and **5f**, respectively, caused significant differences in p38 inhibition, which were completely canceled out in the cell-based assay system. Perhaps the benzylsulfanyl derivative **5f** penetrated the cellular membrane lipid bilayers more efficiently and/or other meaningful influencing factors adherent to whole blood systems (i.e., enzymatic metabolism

Table 6. Biological Activity of Different (Cyclo)alkylaminopyridines and (Cyclo)alkylaminopyridines with an Additional Polar Group (2-Thioimidazole Derivatives with a 4-Fluorophenyl Ring)[a]

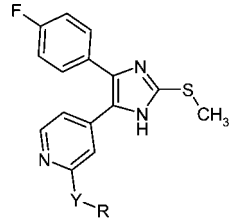


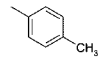
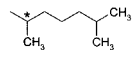
#	R	Yield	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
28a		38%	0,08±0,018 (n=4)	5,94±0,582 (n=4)	n. t.
28b		27%	0,009±0,001 (n=8)	3,30±1,59 (n=4)	n. t.
28c		27%	0,153±0,017 (n=6)	27,6±12,1 (n=4)	n. t.
28d		40%	0,02±0,004 (n=6)	0,362±0,04 (n=2)	n. t.
28e		14%	0,013±0,005 (n=4)	0,458±0,08 (n=4)	0,136±0,007 (n=2)
28f *cis*		12%*	0,031±0,006 (n=8)	3,68±0,673 (n=4)	n. t.
28g *trans*		18%*	0,015±0,002 (n=6)	15,8±1,10 (n=4)	n. t.
28h *cis/trans*		25%*	0,02±0,005 (n=4)	5,40±1,72 (n=4)	n. t.
28i		20%	0,023±0,005 (n=4)	0,622±0,034 (n=4)	2,76±0,522 (n=4)
28j		49%	0,09±0,036 (n=10)	2,43±0,439 (n=4)	n. t.
28k		57%	0,126±0,041 (n=4)	4,54±0,546 (n=4)	n. t.
28l		8%	0,011±0,002 (n=10)	0,167±0,051 (n=4)	0,63±0,088 (n=4)
28m		12%	0,023±0,004 (n=6)	0,435±0,194 (n=8)	n. t.
28n		9%	0,026±0,003 (n=10)	0,7±0,102 (n=4)	n. t.
28o		26%	0,02±0,004 (n=6)	3,99±2,38 (n=8)	7,56±2,81 (n=4)
30a		20%	0,045±0,004 (n=4)	2,04±0,303 (n=4)	n. t.
30b		40%	0,021±0,005 (n=8)	0,416±0,162 (n=4)	1,89±0,73 (n=4)

#	R	Yield	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
30c		24%	0,035±0,005 (n=4)	0,515±0,019 (n=4)	0,25±0,053 (n=4)
30d		80%	0,006±0,002 (n=4)	0,439±0,098 (n=8)	n. t.
30e		34%	0,01±0,001 (n=4)	0,239±0,056 (n=4)	n. t.
30f		20%	0,034±0,006 (n=6)	1,06±0,393 (n=4)	n. t.
30g		29%	0,015±0,003 (n=6)	0,68±0,176 (n=4)	n. t.
30h†		22%	0,005±0,0003 (n=6)	0,201±0,004 (n=4)	n. t.
30i		40%	0,003±0,0005 (n=4)	0,386±0,061 (n=8)	n. t.
Reference SB203580			0.062±0,006 (n = 164)	2,30±0,105 (n=81)	1,98±0,32 (n=18)

[a] Key for the symbols. ∗: The *cis*-**28f** and the *trans*-**28g** isomers were not especially produced but separated from the cis/trans mixture (**28h**) by column chromatography. †: The tetrahydropyran-4-ylamine used for the S_NAr reaction was produced by (catalytic) reductive amination (Leuckart−Wallach reaction) of tetrahydropyran-4-one according to the protocol of Allegretti.[70] Yield: 46% (free base) over two steps.

Table 7. Biological Activity of Different 2-(Thi)oxypyridinylimidazoles[a]



#	Y	R	Yield	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM [μM]	IL-1β IC_{50}±SEM [μM]
33a	O		39%	0,436±0,086 (n=4)	44,7±5,48 (n=4)	n. t.
33b	O		60%	0,639±0,083 (n=4)	44,3±5,41 (n=4)	n. t.
33c	O		32%	0,033±0,01 (n=6)	18,3±5,04 (n=8)	27,4±8,45 (n=4)
33d	O		38%	0,424±0,095 (n=4)	38,4±0,415 (n=4)	n. t.
33e	O		13%	0,542±0,145 (n=8)	11,2±4,01 (n=4)	n. t.
33f	O		n. d.*	n. t. (impure)	n. t.	n. t.
33g	O		56%	0,641±0,044 (n=4)	42,7±0,859 (n=4)	n. t.
34a	S		58%	n. t. (impure)*	n. t.	n. t.
34b	S		37%	0,087±0,026 (n=4)	24,1±8,40 (n=4)	61,3±32,3 (n=4)
Reference SB203580				0.062±0,006 (n = 164)	2,30±0,105 (n=81)	1,98±0,32 (n=18)

[a] Asterisk (∗) indicates not determined.

reactions, plasma protein binding, cell−cell interaction) are responsible for these effects.

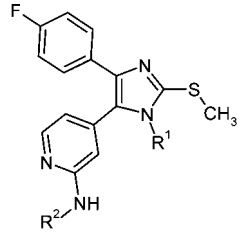
Comparable SARs were found within the series of 2-(aryl)-alkylsulfanyls having a congruent 5-(2-halogeno)pyridine-4-yl moiety but bearing a 3-trifluoromethylphenyl system instead of the classical 4-fluorophenyl ring at the 4-position of the imidazole core (Table 3). For these 3-trifluoromethylphenylimidazole derivatives, p38 inhibition is generally less efficient (see later, 2-sulfanylimidazole derivatives with additional amino substituents at the pyridine ring). In the p38 assay, compounds **5i** and **5j** (Table 3) with a methylsulfanyl and an ethylsulfanyl substituent, respectively, at the imidazole-C2, were ∼2.5-fold better than the benzylsulfanyl analogue **5k**, with both **5i** and **5j** exhibiting similar IC_{50} values. However, in the whole blood model, differences in inhibition of cytokine release were less drastic among all three representatives.

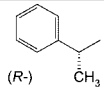
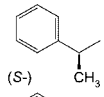
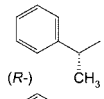
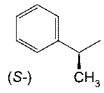
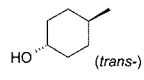
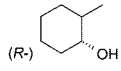
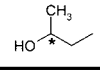
Replacement of the essential 4-pyridine ring in ML3375 by a 4-quinoline ring (**5h**) did not improve the inhibitory efficacy in the kinase assay or the inhibition of cytokine release from immune cells. Compound **5h** exhibited ∼25% of the p38 kinase inhibitory activity of ML3375. Differences in the inhibition of TNFα release were even less significant, with ML3375 being 1.6-fold more effective than **5h.** The restricted rotation of the bulkier quinolin-4-yl moiety around its σ bond to the imidazole C5 combined with a planar alignment of this condensed aromatic system probably led to a suboptimal H-bond formation between the quinoline-N and the amide-NH of Met109.

Introduction of Arylalkylamino Substituents at the Pyridine C2. Hydrogen bonding between the pyridine ring N of the inhibitor and the backbone amide-NH of Met109 in the otherwise lipophilic linker region (Ala51, Thr106, His107, Leu108, and Met109) is essential for the biological activity of the vicinal diaryl-substituted heterocyclic compound class.[11−13] Appropriate amino substituents at the 2-position of the pyridine can enhance activity toward unsubstituted (e.g., ML3375) and toward halogenated pyridine derivatives (Tables 1 and 2), as they are able to form a second H-bond with the linker region next to the existing one (here, between the exocyclic 2-amino group as H-donor and the main chain carbonyl oxygen of Met109 as H-acceptor).[13,36] On the other hand, an electron donating amino group should increase the electron density at the pyridine, thereby intensifying the affinity of the inhibitor for the enzyme.[32,61,62] Moreover, by adequate lipophilic side chains, further interactions with the surface-exposed front region (hydrophobic region II) can be achieved.[36] Therefore, we prepared a series of analogues by introducing a number of primary arylalkylamines at the pyridinyl ring of ML3375.

The biological results of these novel arylalkylamino-substituted pyridinylimidazole analogues **22a**−**m** are summarized (Table 4). Structural analyses of the more potent

Table 8. Biological Activity of Different Tetrasubstituted Aminopyridinylimidazoles



#	R^1	R^2	Yield	p38α IC_{50}±SEM [μM]	TNFα IC_{50}±SEM[μM]
35a	-CH_3		80%	0,96±0,42 (n=4)	> 20 (n=4)
35b	-CH_3		81%	0,07±0,03 (n=4)	1,71±0,855 (n=4)
35c			84%	0,61±0,02 (n=4)	9,86±1,5 (n=4)
35d			60%	0,04±0,01 (n=4)	0,58±0,05 (n=4)
35e			65%	0,051±0,004 (n=4)	n. t.
35f			69%	0,359±0,097 (n=8)	28,0±1,91 (n=4)
35g			62%	0,042±0,0005 (n=8)	3,57±1,03 (n=8)
35h			100%	0,235±0,051 (n=4)	11,5±0,901 (n=4)
35i			94%	0,038±0,005 (n=6)	5,88±1,49 (n=4)
35j			97%	0,003±0,0002 (n=4)	1,65±0,75 (n=4)
35k			38%	0,211±0,017 (n=2)	n. t.
35l			100%	0,292±0,023 (n=6)	17,4±0,02 (n=4)
35m			99%	0,485±0,025 (n=4)	n. t.
35n			94%	n. t. (impure)	n. t.
35o			95%	0,146±0,020 (n=4)	6,38±1,19 (n=4)
Reference SB203580				0.062±0,006 (n = 164)	2,30±0,105 (n=81)

inhibitor candidates demonstrated a certain tolerance for differently constituted and configurated arylalkyl moieties; however, no clear tendency for optimum SAR could be found. In general, sufficiently lipophilic arylalkylamino residues favorably affected biological activity (compounds **22b**−**g**,**l**). For condensed aromatic ring systems (**22d**,**e**,**m**), the spatial geometry seemed to play a crucial role; however, multiple separate aromatic cycles in the aliphatic side chain resulted in increased IC_{50} values (**22h**,**i**). The most potent representatives of this series (**22b**−**g**) commonly possess an α- or β-methyl or ethyl group at the introduced amino substituent (**22b**,**c**,**f**,**g**) and/or a more flexible spacer of up to three carbon atoms between the exocyclic amino-N and the terminal phenyl ring (**22e**,**f**,**k**, Figure 8). Alternatively, the α-methyl branch can be ring-closed to either a penta- or a hexaycyclic indane or a tetrahydronaphthalene structure element with the adjacent phenyl ring (compounds

Table 9. Kinase Selectivity Profile of Selected Compounds[a]

kinases[71] 78	BIRB796	SB203580	SB202190	**22c**	**22d**	**22g**	**26b**	**28o**	**30c**	**35g**
CaMK-II	no data	0	no data	0	0	0	0	0	61	12
c-Raf	(91)[59] 79	85	no data	31	36	19	53	7	0	0
ERK1	(14)	no data	no data	23	28	32	46	28	18	0
MAPK2/ERK2	28	15	11	21	0	17	31	6	8	5
JNK1α1/SAPK1c	1	0	7	13	62	88	72	86	46	85
MEK1	(14)	0	no data	4	1	3	1	2	20	0
JNK2α2	50	32	no data	4	79	94	90	91	86	99
JNK3	9	86	no data	63	100	99	99	99	96	89
SAPK2a/p38α	97	98	100	99	98	97	99	94	95	100
SAPK2b/p38β2	97	90	97	98	90	96	99	96	96	100
SAPK3/p38γ	97	4	20	17	9	0	0	6	8	0
SAPK4/p38δ	89	7	13	0	0	11	9	2	8	n.t.
PKA	21	4	34	0	0	0	4	0	1	0
PKCα	30	11	8	0	0	0	0	0	0	0
PKBα	0	38	47	14	12	5	1	6	12	6
GSK3β	0	34	39	0	0	0	1	0	10	7
ROCK-II	22	23	39	0	0	0	0	0	0	7
LCK	82	68	63	25	26	30	28	47	26	25

[a] The inhibitory potency was evaluated by the Upstate Kinase Profiler carried out at 100 μM ATP concentration. Values are reported as %-inhibition at a inhibitor concentration of 10 μM.

Table 10. Inhibition of the Activity of Relevant CYP450 Isozymes at 10 μM Inhibitor Concentration[a]

compd	1A2	2C9	2C19	2D6	3A4
22c	55	80	88	73	81
22d	68	84	79	68	76
22g	38	88	89	78	95
26a	83	82	78	51	83
26b	83	93	94	88	89
26d	76	71	63	66	81
26f	**53**	**52**	**47**	**9**	**53**
26g	67	77	81	54	93
28a	62	73	80	63	80
28l	45	75	76	53	59
28o	23	76	84	66	71
30b	**29**	**39**	**25**	**5**	**33**
30c	**70**	**47**	**51**	**22**	**52**
33c	94	81	86	0	95
35a	52	78	87	53	85
35b	**60**	**66**	**76**	**27**	**87**
35c	25	71	83	64	87
35d	5	85	95	25	81
35g	0	73	80	54	81
35j	**23**	**40**	**58**	**24**	**80**
SB203580	**38**	**87**	**92**	**73**	**77**

[a] Results in % inhibition. Each case originated from a single-point detection using a standardized method.[59]

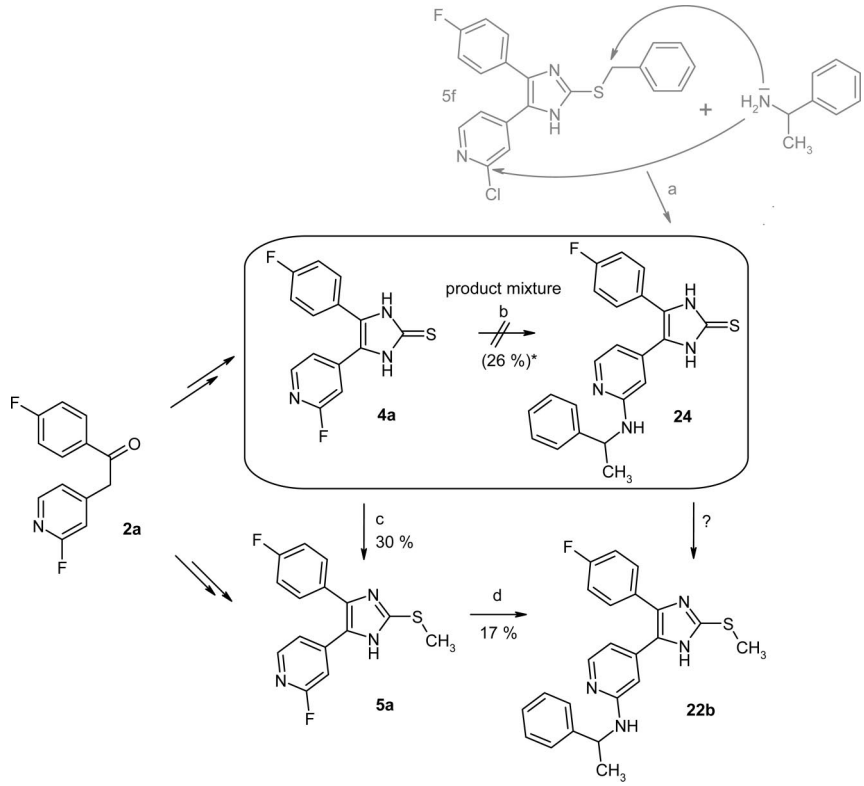
22d and **22e**, Figure 9). A possible binding model using compound **22f** was generated, illustrating the possible orientation of the branched (β-methyl) arylalkyl side chain within the hydrophobic region II (Figure 10). Here, the 2-phenylpropyl residue is more directed toward the lipophilic subarea that is located above the imidazole ring plane (area A). The best compound in this series, the 1-methyl-3-phenylpropylaminopyridine **22g**, proved to be 5 times as effective as the standard SB203580 with respect to the inhibition of isolated p38 MAPK and was also significantly superior to the prototype in suppression of TNFα release. Contrary to our expectations, introduction of sterically demanding (and rather hydrophilic) arylalkyl residues at the pyridin-4-yl ring could not reduce interactions of the inhibitor with the heme iron of the CYP450 system compared to SB203580. This modification was inadequate to satisfactorily prevent the inhibition of metabolically essential CYP isozymes, with the exception of CYP450 1A2 (**22c**,**g**; compare Table 10) and consequently was not qualified for decreasing in vivo liver toxicity.

Besides the positive interactions of the substituted 5-pyridin-4-yl moiety with both the linker region and the surface-exposed front area of the enzyme, resulting in increased activity as well as selectivity, the (halogenated) aryl ring in the 4-position of the imidazole is mostly responsible for gaining selectivity to p38α over other (related) kinases. This important structural element occupies the deep hydrophobic back pocket of p38, comprising the residues Val38, Ala51, Val52, Lys53, Leu75, Ile85, Leu86, Leu104, Val105, and Thr106 that correspond to the hydrophobic region I (Figures 3 and 4).[7] Small (halogen or alkyl) substituents are tolerated in the 2-, 3-, and 4-position of the aryl system, and other researchers have varied these aryl substituents in an effort to improve the selectivity profile of these diaryl heterocyclic p38 inhibitors.[7,10] The formal introduction of a 3-trifluoromethylphenyl group instead of the para halogen atom at the phenyl ring of 2,4,5-trisubstituted imidazoles only slightly affected the p38 inhibitory activity but led to a 30-fold selectivity toward the c-raf kinase (vs equipotent inhibition of p38 and c-raf with the analogous 4-chlorophenyl derivative (Figure 11, left-hand side).[7] Combination of individually optimized substituents at the N1- and C2-position of the imidazole and the introduction of adequately substituted 2-amino functions at the pyridine or the pyrimidine ring resulted in additive increases in p38 potency. Simultaneously, a more than 100-fold selectivity toward both the c-raf and JNKa1 resulted (Figure 11, right-hand side).[7]

Against this background, we replaced the classical 4-fluorophenyl component of our objective alkylsulfanylimidazoles by a 3-trifluoromethylphenyl ring. To attain synergistic effects caused by the concurrent location of key substituents at the relevant positions of the inhibitor molecules, we varied the already tested arylalkylamino residues at the 2-position of the pyridine ring while retaining the small methylsulfanyl substituent at the 2-position of the imidazole.

The 4-(3-trifluoromethylphenyl) groups of the explored compounds **27a**,**b** (Table 5) are supposed to target the hydrophobic region I composed of the β-strands 3, 4, and 5 as well as the α-helix C, in close analogy to the 4-fluorophenyl residue of SB203580 (Figure 12).[25] The selectivity of those pyridin-4-yl-/3-trifluoromethylphenyl-substituted imidazole analogues is assumed to depend on the presence of a Thr in position 106 of the p38α MAPK ("gatekeeper residue").[12,13,63] In other kinases, this explicit position is occupied by sterically more demanding amino acid side chains (Met, Glu) that do not tolerate such bulky 3-CF_3-aryl substituents of putatively specific p38 inhibitors. The arylalkyl residues introduced at the pyridine part lie in the

Scheme 4. Amination of Imidazol-2-thione **23**[a]



[a] (a) 10-fold excess of 1-phenylethylamine, 150-160 °C, 22 h; (b) 10-fold excess of 1-phenylethylamine, 160 °C, 4 h; (c) iodomethane (3 equiv), EtOH/THF 1 + 1, reflux, 40 h; (d) 10-fold excess of 1-phenylethylamine, 160 °C, 15 h; (∗) LC/MS purity: ∼50−60%.

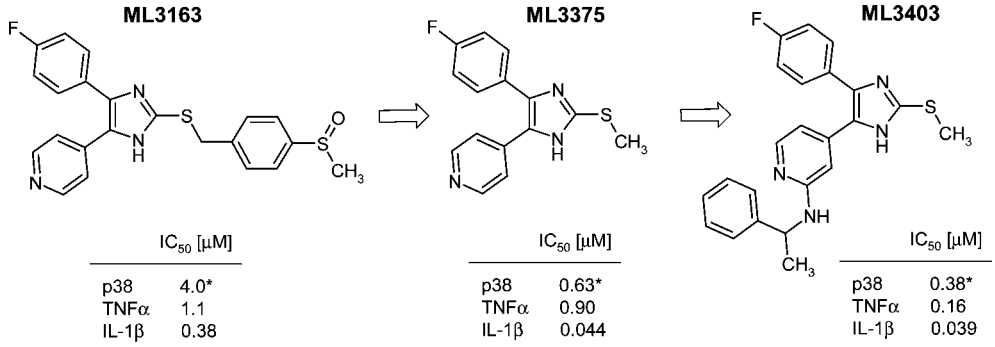


Figure 6. (Aryl)Alkylsulfanylimidazole lead structures from Merckle. The asterisk (∗) indicates that the given p38 IC_{50} data come from a precursor ELISA assay, where test values generally proved to be about 10-fold higher than with the new procedure mentioned at the beginning of this section, which was used for all newly tested compounds in the present work. The corresponding biological activities of the reference SB203580 are p38 0.29, TNFα 0.59, IL-1β 0.037.

hydrophobic region II within the vdW distances of the side chain atoms of Val30, Ile108, Asp112 and the main chain atoms of Ala111 (Figure 12).[25] This pocket, which is quite spacious in the inactive p38 MAPK, could function as a major selectivity determinant for such compounds bearing bulky NH-alkylphenyl-like residues at the pyridine for p38 compared to other kinases.

The 3-trifluoromethylphenyl analogues **25a**−**e** (Table 5) can form up to two hydrogen bonds with residues that also contribute to the ATP binding in the kinase linker (hinge) region (His107-Ala110). Here, the 2-aminopyridine ring interacts with the main chain nitrogen and the main chain oxygen atoms of Met109 (H donor−acceptor interactions, Figure 12). In a structurally related inhibitor molecule with a 3-trifluoromethylphenyl system, the conformation of this peptide region in the bound p38α was identical to that in the apo enzyme.[10] In contrast to SB203580 and similar compounds,[13] the side chain nitrogen of the conserved Lys53 does not interact with the N3 of the imidazole ring of this representative compound (Figure 13, right).[10]The crystal structure shows the differing spatial orientation of the present imidazole compound within the ATP site in comparison to the exemplified 4-fluorophenyl derivative (Figure 13, left). The imidazole-N3 of this inhibitor is separated from the side chain amino group of Lys53 by 4 Å, too far to allow the formation of an H-bond. Thus, this excessive distance may explain the poorer p38 inhibition potency of the 4-(3-trifluoromethylphenyl)-2-thioimidazole compounds in Table 5 compared to the analogous 4-fluorophenyl derivatives (Table 4) having similar substitution patterns. Calculated binding distances of the respective aminopyridine moieties to the backbone residues of the kinase linker region (field of strong interactions) in the cocrystallized structures 1BL7 and 1OUK (Figure 13) should have promised stronger enzyme−drug interactions for the 3-trifluoromethylphenyl-substituted imidazoles.

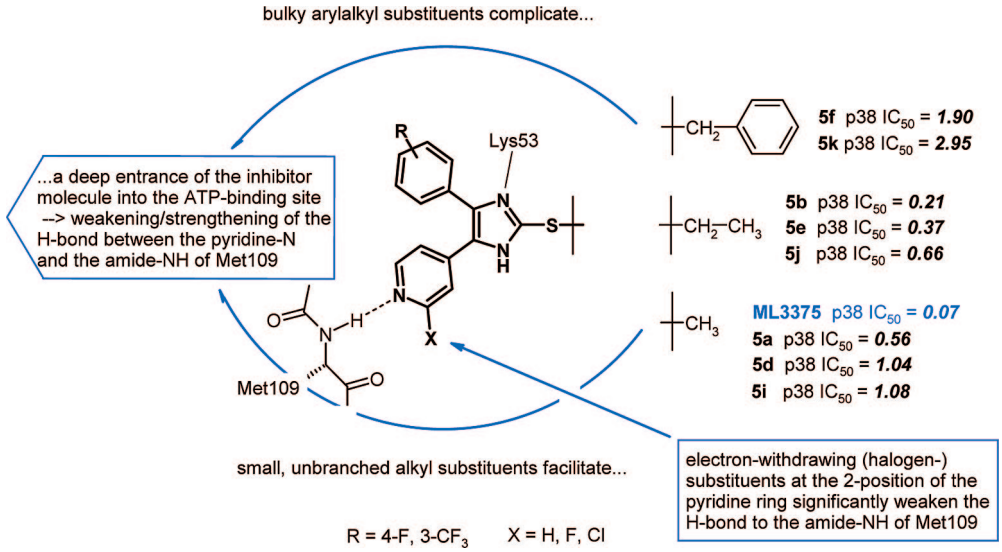


Figure 7. Electron-withdrawing halogen atoms at the 2-position of the pyridine ring of the investigated 2-thioimidazoles as well as short arylalkyl residues at the proximal sulfur lead to a reduced p38 inhibition potency, weakening the H-bond of the pyridine ring N and the backbone amide NH of Met109.

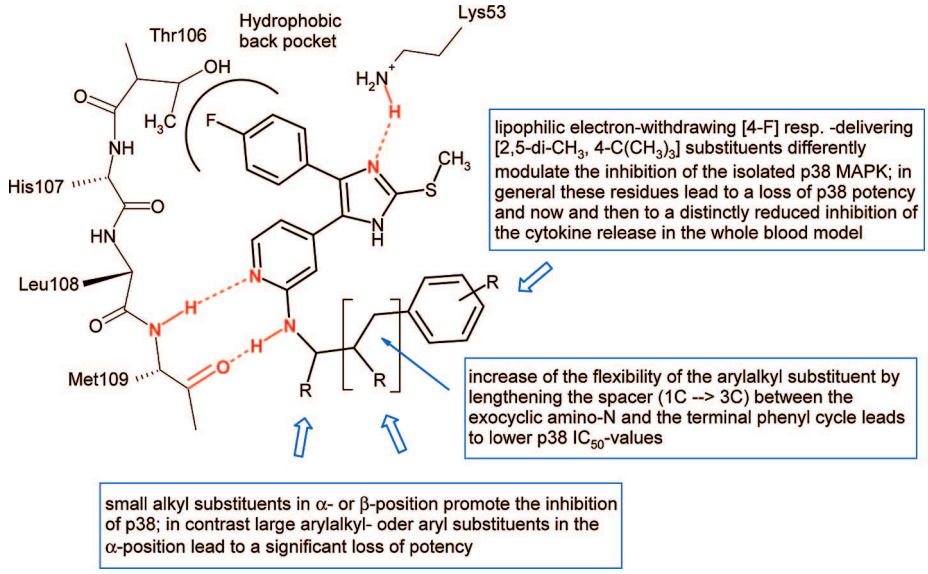


Figure 8. Binding model for 2-alkylsulfanyl-4(5)-pyridin-4-yl-imidazole p38 inhibitors having additional arylalkyl substituents at pyridine C2.

Each of the compounds **25a**−**e** (Table 5) on average exhibited only a fifth of the p38 inhibitory potency of the corresponding pyridinylimidazole derivatives with a 4-fluorophenyl residue (see Table 4). SAR of the introduced arylalkylamino substituents at the pyridinyl moiety of these 3-trifluoromethylphenyl-substituted imidazoles generally correlated with the SAR of the 4-fluorophenyl analogues. For both series of inhibitors, a qualitative as well as a reasonably quantitative effect was caused by the introduction of the same arylalkylamino substituent. Thus, the abolition of the H-bond between the imidazole-N and the amino group of Lys53 was more important for inhibitor potency than the nature of the pyridine substituents. Consequently, the least displaced orientation of those modified "bulkier" drug molecules within the active site was responsible for the consistently reduced activity of the 3-trifluoromethylphenyl-substituted imidazoles. In contrast, the most favorable arylalkylaminopyridine residues among the 4-fluorophenylimidazole derivatives (Table 4) led to a relatively significant enhanced kinase inhibition compared to the fluorinated pyridinylimidazole precursor **5i** (Table 3) and were also able to (over)compensate for this loss in potency. As a result, compound **25e**, bearing the already proven and tested 1-methyl-3-phenylpropylamino substituent at the pyridine, is at least as potent as the reference SB203580. The elevated lipophilicity of the 3-trifluoromethylphenylimidazoles **25a**−**e** promotes good plasma protein binding with probable accumulation in the lipophilic cell membranes, qualifying these compounds as relatively poor inhibitors of cytokine release (TNFα, IL-1β) in an almost double-digit micromolar concentration array (**25a**,**c**,**d**).

In a related consideration, the increased lipophilicity resulting in presumed low water solubility makes these 3-CF_3-phenyl derivatives less feasible for peroral administration.[64]

Introduction of Heteroarylalkylamino Substituents at the Pyridine C2. Beside the wide-ranging modifications at the aliphatic spacer bearing the (terminal) phenyl ring(s) (Tables 3 and 4) another promising route was to replace the six-membered aromatic system by diverse heteroaromatic cyclic structures. Moreover, suitably located O- or N-containing ring systems (H-acceptors) probably would make additional electrostatic interactions with polar backbone residues or with the solvent area

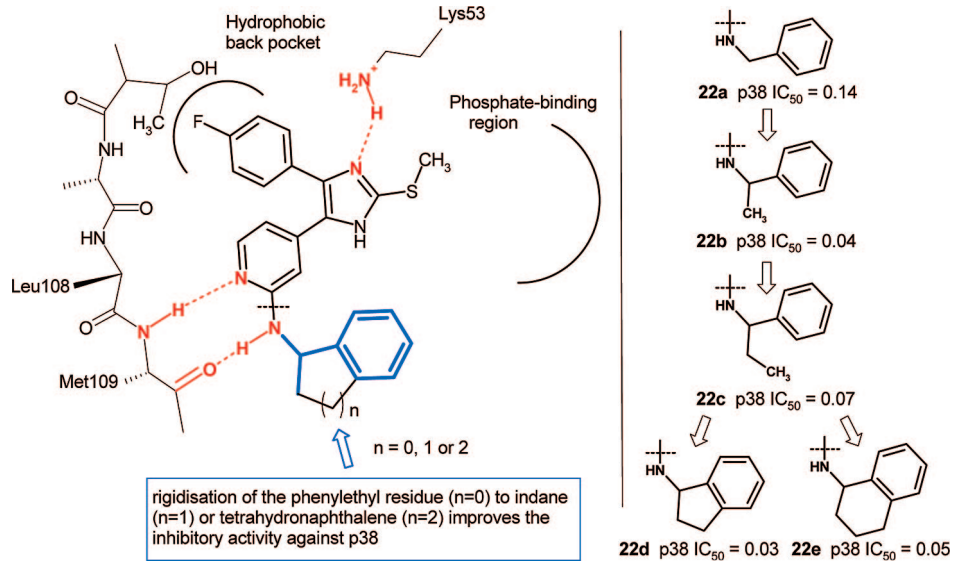


Figure 9. Binding model for 2-alkylsulfanyl-4(5)-pyridin-4-ylimidazole p38 inhibitors with a (rigid) phenylethylamino substituent in the 2-position of the pyridine.

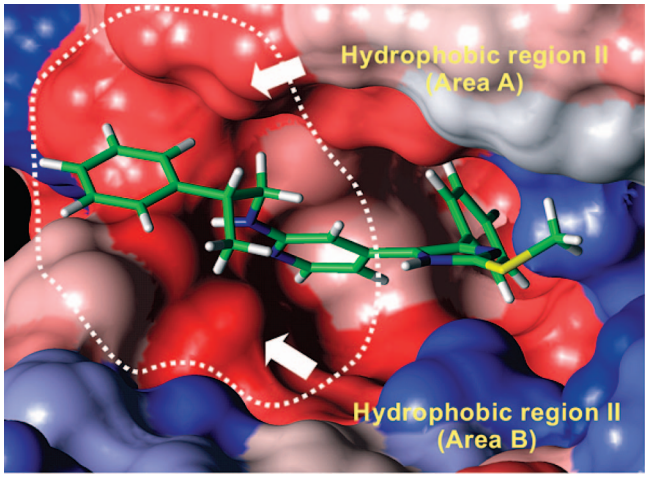


Figure 10. Binding model of **22f** (*S*-enantiomer) in the ATP-site of p38 based on the PDB structure 1a9u. Colors correspond with the grade of lipophilicity of the side chains of the amino acids (blue = hydrophilic, red = lipophilic). The spatial position of **22f** in the kinase was received by docking with FlexX (version 1.13).[72] Arrows indicate the subareas of the hydrophobic region II (framed in white color) above (area A, Val30, Ala40, Leu108) and beneath (area B, Ile84, Met109, Ala157, Leu167) the imidazole plane of the inhibitor in proximity to the linker region. Between these two hydrophobic surfaces the 2-phenylpropyl residue, bound in the 2-position of the pyridine via a nitrogen atom, is optimally buried. The figure was provided with Insight II.[73]

surrounding the hydrophobic region II. Hypothetical SARs for the heteroarylalkylamino substituents of compounds **26a**−**h** (Table 4), partly corresponding to those of the arylalkylaminopyridines, are schematized in Figure 14.

Placement of flexible amino substituents bearing a side chain heteroaromatic ring led to a clearly enhanced inhibitory potency (p38 IC_{50} values) in the isolated kinase assay comparable in magnitude of that of the unsubstituted pyridinylimidazole ML3375 (e.g., **26e**,**g**,**h**) or even to the potent reference SB203580 (**26a**,**d**,**f**) except for compound **26c** with a condensed heterocycle and the 3-trifluoromethylphenyl compounds **27a** and **27b** (Table 5). A 2-thienyl ring one or two carbon atoms distant from the exocyclic NH group in the shape of a flexible linear aliphatic chain gave the best results (Tables 5 and 6). There was no need for an α-methyl branch in this linear spacer to increase potency up to 2.5-fold of the activity of SB203580 (compound **27b**) as was required with the arylalkylamino residues (compare Tables 3 and 4).

Substitution of the sulfur in the terminal thiophene ring by a more electronegative oxygen atom led to a marked loss in activity (**26d**, **27b**). The position of the heteroatom within the heterocycle was also important. In a series of pyridinyl position isomers [5-(pyridin-2-, 3-, 4-ylmethylaminopyridin-4-yl)imidazoles **26f**−**h**] a clear preference was identified for the derivative in which the terminal pyridyl ring is bound in ortho position to the methylene spacer (**26f**) whereas the 4-pyridyl analogue **26h** was notably less active (Table 5).

For the specified examples **27a** and **27b** (Table 5) of the series of 3-trifluoromethylphenyl derivatives, identical binding modes and thus equal binding energies of the matching heteroarylalkylamino substituents in the hydrophobic region II can be predicted, as they also existed for the 4-fluorophenyl analogues. Once more, this prediction is reinforced because the furfurylaminopyridine **27b** is 1.4-fold discriminated by its sulfur pendant **27a** and this difference in activity is almost identical to that between **26a** and **26d** (Table 4). Furthermore, as already found for the related arylalkylaminopyridines (Tables 3 and 4), the p38 inhibitory potency of each of these two 3-trifluoromethylphenyl-substituted imidazoles proved to be ~5-fold lower than its identical pyridine substituted analogue from the 4-fluorophenyl series. In turn, the comparatively reduced activity can be attributed to the lack of the H-bond between the sp^2-hybridized imidazole-N and Lys53, the quantitatively different lipophilic interactions of the fluorinated aryl ring at C4 of the imidazole, and the deviating orientation of the inhibitor molecule in the adenine region of the kinase.

Despite the good inhibition in the p38 assay, prevention of TNFα (and IL-1β) release by these manufactured heteroarylalkylaminopyridines lagged behind that of the SB prototype. The still relatively high lipophilicity of the introduced heteroarylalkyl residues may be mainly responsible for this failure. As expected, the comparatively more hydrophilic heteroarylalkylamino derivatives (**26a**) **26d** and **26f**−**h** exhibited the lowest TNFα IC_{50} values in this series.

Introduction of (Cyclo)alkylamino Substituents at the Pyridine C2. With regard to a future oral bioavailability of these drug candidates, we also aimed to improve the expected in vivo

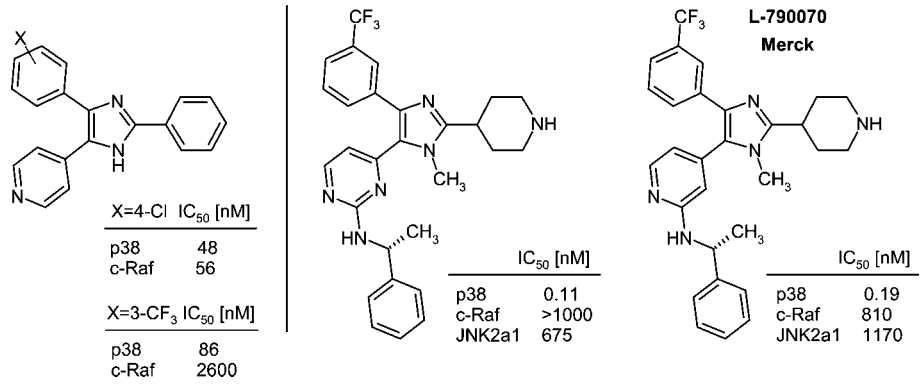


Figure 11. Several published Merck compounds with a 3-trifluoromethylphenyl ring at the imidazole-C4.[7,10]

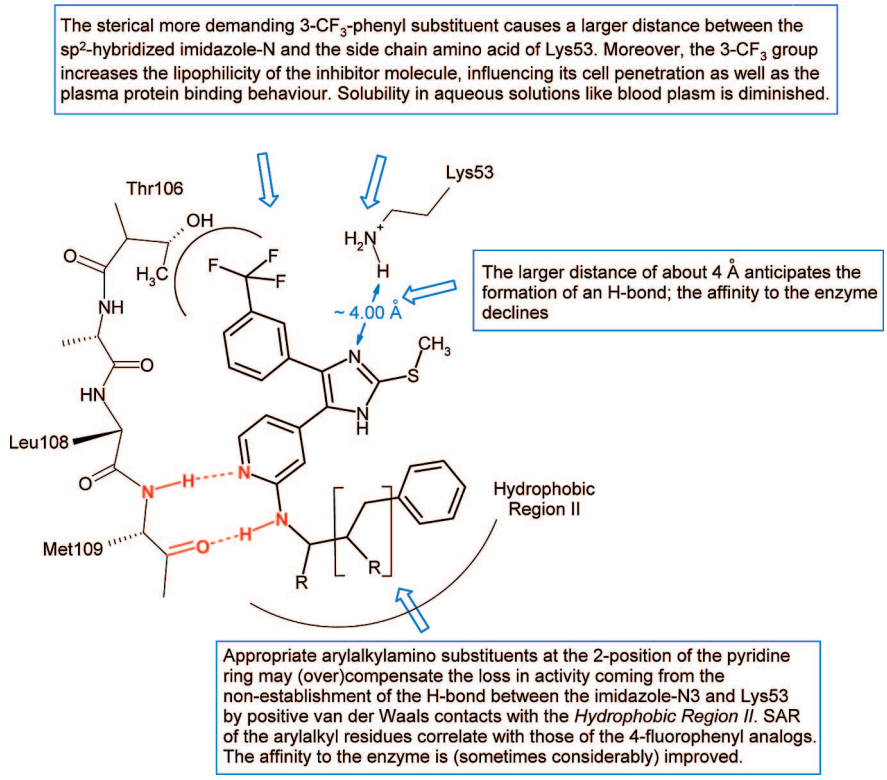


Figure 12. Binding model of substituted 5-pyridin-4-yl-2-thioimidazoles in which the 4-fluorophenyl component at the 4-position of the imidazole is replaced by an analogous 3-trifluoromethylphenyl residue.

absorption or permeation behavior of the more potent aryl(alkyl)aminopyridinylimidazoles by enhancing their hydrophilicity (solubility) to meet the Lipinski rule criteria.[64] In particular, the putatively more selective but quite lipophilic derivatives with a 3-trifluoromethylphenyl moiety are anticipated to be poorly soluble in aqueous media and become worse resorbed in the blood circuit and furthermore are expected to distribute better to more lipophilic compartiments and thus will possibly exhibit lower plasma levels after oral gavage [e.g., calculated log *P* values: **25e** (Table 5), 6.03 ± 0.64; **22g** (Table 4) 5.08 ± 0.65; SB203580, 4.19 ± 0.67].[7] Structurally similar inhibitors of p38 MAPK have been previously prepared in which the arylalkylamino residues at the pyridine (or at the bioisosteric pyrimidine) were replaced by "smaller" (cyclo)aliphatic amino residues.[32] These modified compounds showed suitable hydrophilicity (solubility) and occupied better log *P* values[32] but also claimed a higher metabolical stability upon further substitution compared to analogous diaryl heterocyclic inhibitors bearing methylbenzylamine-like residues at the pyridine/pyrimidine C2.[37,38] Some of these derivatives exhibited highly potent inhibition of cytokine release, particularly the suppression of the TNFα release.[32]

2-Thioimidazoles in Table 6 were modified in an analogous way with either an aliphatic or a cycloaliphatic amino substituent at the 2-position of their pyridine-4-yl moiety. With the exception of compounds **28a**,**c**,**j**,**k**, substances out of this series exhibited a good to excellent p38 inhibitory activity, as clearly reflected by IC_{50} concentrations ranging from the submicromolar to the single-digit nanomolar range (e.g., **28b**,**l**). The most potent representatives **28b** and **28l** were 4- to 4.5-fold superior to SB203580. The disintegration of the planarity of the aromatic system introduced with the alkylamino side chain at the pyridine moiety of the substituted imidazoles (Figure 15) may be considered as a cause of the general increase in activity of these arylalkylaminopyridines (Tables 3 and 4). Starting from compound **28a** with a cyclohexylmethylamino substituent, it was again very effective to position an additional α-methyl branch in the aliphatic spacer next to the exocyclic NH-nitrogen. The resulting asymmetry of the residue was of great importance, as

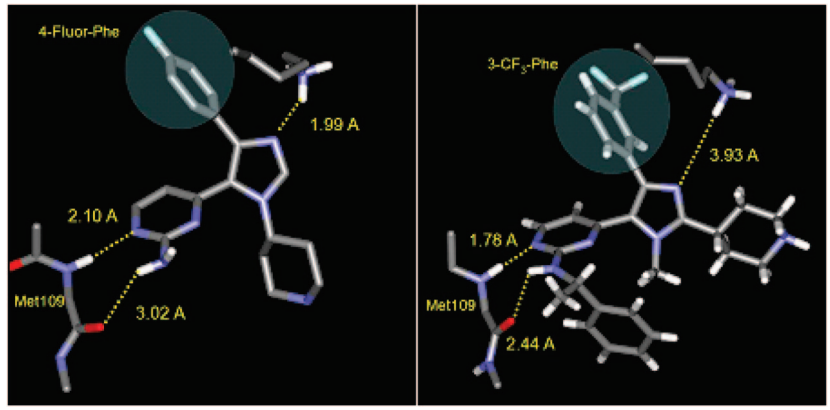


Figure 13. Cocrystal structures of the p38α MAPK with different ATP-competitive diaryl-substituted imidazole inhibitors: (left) compound from SmithKlineBeecham with a 4-fluorophenyl ring (PDB entry 1BL7);[13] (right) compound from Merck with a 3-trifluoromethylphenyl ring (PDB entry 1OUK).[10] Pictured are the spatial distances of the possible H-bonds between the respective drug molecule and the backbone amino acids Met109 as well as Lys53. Because of the differing orientation of the MERCK compound (with a bulkier 3-CF_3 residue) in the adenine-binding region of the kinase and the resulting larger distance of the imidazole-N3 to the side chain of Lys53, the corresponding ligand−enzyme H-bond is missing.

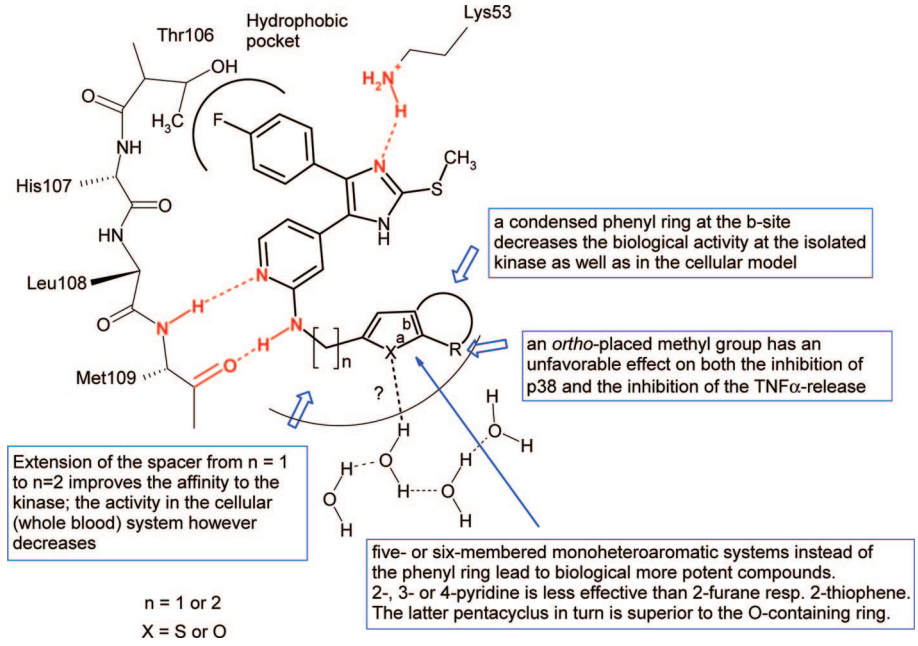


Figure 14. Binding model for heteroarylalkylaminopyridines.

both enantiomers differently inhibited the p38, whereas in the case of the objective cyclohexylethyl residue, the preference clearly was in favor of the *R*-enantiomer (**28b** vs **28c**, 16-fold difference in activity). This chiral residue seems to be best orientated between the two hydrophobic subareas of the hydrophobic region II (Figure 3), while the more flexible cyclohexylmethyl substituent of **28a** probably cannot optimally orientate at either the upper (area A) or the lower (area B) lipophilic surface of this solvent-exposed front region (compare ML3403 vs **22a**, Table 4). Decisive benefits could be also achieved by either shortening the spacer (**28d**−**h**) or lengthening this side chain for one to three CH_2 units (**28i**,**j**,**n**,**o**) and also by a formal ring opening and narrowing of the aliphatic terminus to a dimethyl group (at compounds **28i**,**j**,**l**,**n**,**o**). Placement of an additional methyl group in the ortho position of the directly NH-linked cyclohexyl ring in **28e** further improved the p38 inhibitory activity compared to the corresponding nor compound **28d**. In contrast, additional para-placed methyl groups offered only small advantages for the activity (**28f**−**h**). The trans-configured 4-methylcyclohexyl residues gave better results than their cis-configured counterparts (**28f** vs **28g**). Inhibition data for the suppression of TNFα release were more diverse than the values for inhibition of the kinase, varying over 2 decimal powers from the 2-digit micromolar to the 3-digit nanomolar range. The smaller and less flexible the alkyl residues at the exocyclic NH groups were, the lower the cytokine IC_{50} values tended to be. The best results were obtained with the compounds whose alkylamino substituents at the pyridine formally resemble the biologically interesting 1,2-dimethylpropyl structure of compound **28l**, as proved by the analogues **28d**,**e**,**l**,**m** and partially by **28i** and **28n**. These derivatives dominate with their cytokine inhibitory potency elevated up to 1 order of magnitude (factor 10) over the model compound SB203580. The corresponding SARs were also found for the less active analogues from Table 5 bearing a 3-trifluoromethylphenyl ring at their imidazole-C4 (**29a**,**b**), which exhibited decreased inhibition of TNFα release owing to their higher lipophilicity. In addition, calculated log *P* values of the most potent inhibitors **28** (Table

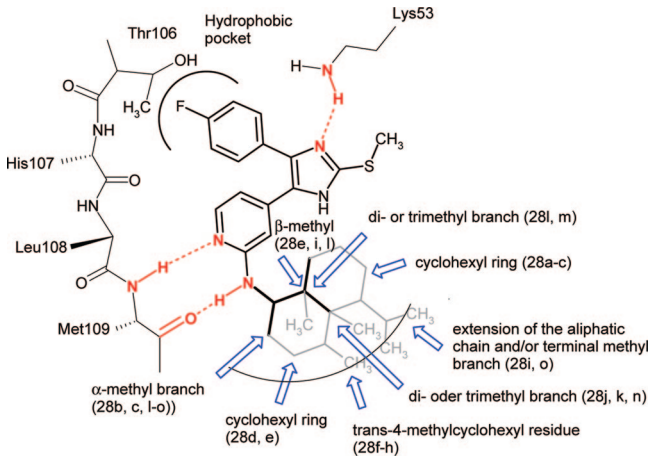


Figure 15. Possible binding model for the alkylaminopyridinylimidazole derivatives with superimposed alkylamino substituents of all compounds from Table 6. Highlighted is the 1,2-dimethylpropyl residue of the most potent compound **28l**, which formally represents a central structure part in the other potent derivatives.

6, e.g., **28l**, log $P = 3.93 \pm 0.65$) and **29** (Table 5, e.g., **29b**, log $P = 4.87 \pm 0.64$) fit much better with the Lipinski criteria than those of the analogous arylalkylaminopyridine compounds (Tables 4 and 5), which exhibited similar p38 activities, qualifying them (**28** and **29**) as the preferred candidates for potential oral application.

Introduction of (Cyclo)alkylamino Substituents with an Additional Polar Group at the Pyridine C2. We considered that placement of an additional polar group in the (cyclo)alkylamino residue may permit the formation of a further H-bond with polar atoms of the protein backbone proximal to the hydrophobic region II or its surrounding solvent-exposed area. This change may improve the affinity of the inhibitor for p38 and increase the inhibitory potency compared to analogues with simple alkyl substituents. Moreover, unwanted interactions with the CYP450 system may be minimized by changing the electronic properties of the substituted (cyclo)alkyl residues.

Indeed, even more biologically active 5-pyridin-4-yl-2-thioimidazoles were achieved by the introduction of different (cyclo)aliphatic primary amines with additional polar groups (Tables 5 and 6). On the basis of the hydroxyethylaminopyridine **30a** (Table 6), which had already been proved to be equipotent to SB203580, the efficacy in the p38 assay was further improved by extending the spacer (**30g**) or by the successful placement of a small α- or β-alkyl group (next to the nitrogen atom) in the aliphatic chain. However, the most potent representatives generally possessed an α-methyl or ethyl branch in the side chain of the amino substituent, as illustrated by the comparison of **30d** and **30e** relative to **30b** and **30f** (Figure 16). The p38 IC_{50} value of **30d** averaged 6 nM. The functionalities giving the greatest advantage for the p38 activity are residues that can be formally derived from cyclohexylamine, e.g., tetrahydropyran-4-ylamine (at **30h**) or 4-hydroxycyclohexylamine (at **30i**). For the latter substituent, as the trans form, the inhibitory potency of **30i** could be increased more than 20-fold as opposed to SB203580 (Figure 17). Compound **30h** exhibited a p38 IC_{50} of 5 nM, while compound **30i** averaged an IC_{50} of only 3 nM. Accordingly, the identical substituent, introduced at the fluorinated pyridinyl moiety in the 3-trifluoromethylphenyl-substituted imidazoles series, led to a 5-fold boost of the activity over the reference compound (**31b**, Table 5, $IC_{50} = 11$ nM) and overcame the lost binding energy arising from the absence of the H-bond between the imidazole-N and Lys53. The p38 IC_{50} values of the remainder of the compounds in Tables 5 and 6 were in the submicromolar sector as well (between 61 and 10 nM). For a better understanding of the binding mode, a binding model for compound **31b** in the p38α MAPK was calculated by molecular computer modeling based on the PDB structure 2ewa (Figure 18). The substituted *trans*-cyclohexyl ring fits optimally between the two lipophilic surfaces of the hydrophobic region II. Beside the positive vdW contacts within this region an additional hydrogen bond could be identified linking the exposed 4-hydroxy group at the cyclohexyl ring and the carbonyl side chain of Asn115.

This series of relatively hydrophilic compounds (**30a**−**i**, Table 6), including the two derivatives with a 3-trifluoromethylphenyl residue (**31a**,**b**, Table 5), consistently proved to be excellent inhibitors of cytokine release from human whole blood, as documented by the corresponding low micromolar IC_{50} concentrations for the inhibition of TNFα release. Here, the inhibitory constants vary from 5.2 μM for compound **31a** to 0.4 μM for **30h** and **30i**, which denotes, in the worst case, an equipotent inhibitor and, at best (**30i**), a more than 8-fold increased activity with respect to the reference SB203580. Better water solubility, a strongly reduced plasma protein binding behavior, and a possibly accelerated cell penetration with only minor accumulation in the lipid membrane may have contributed to this improvement. Furthermore, some of these more "hydrophilic" pyridinylimidazoles were characterized by a significantly diminished CYP450 interaction.

Besides the general positive effect on the physicochemical properties, functionalized (polar) (cyclo)alkylresidues (e.g., with additional hydroxy groups) in the 2-position of the pyridine may further improve the activity profile by providing sites for an even more flexible derivatization, e.g., via the subsequent introduction of additional substituents in the already installed side chain of the amino residues, etherification, oxidation, etc.

Introduction of (Hetero)(aryl)alkyloxy or Thioxy Substituents at the Pyridine C2. In the (hetero)(aryl)alkylaminopyridines presented here, the contribution of the exocyclic NH function to the interaction with the p38 MAP kinase could be quantitatively assessed by replacing the nitrogen atom in the side chain of the pyridine with an oxygen or a sulfur or by substituting the exocyclic amino group with an additional methyl group (dimethylaminopyridine). Because of a disproportionate steric demand within the targeted enzyme region, the latter modification led in the case of **32** (Figure 19) only to a moderate p38 inhibition, emphasizing the importance of the "free" NH function in making an additional H-bond in the linker region.[36] Because the benzyloxypyridine **33a** (Table 7) and the 1-phenylethyloxypyridine **33b**, each with concurrent arylalkyl residues complementary to their analogous aminopyridines ML3403, exhibited a strongly decreased biological activity (5.1- and 16-fold, respectively), it became evident that the nitrogen atom in the side chain could not be replaced by an oxygen without accepting losses of affinity and, consequently, of the activity (Figure 19).

The (hetero)arylalkyl- and (cyclo)alkyloxy- or thioxypyridines (Table 7), except compound **33c** which most likely had an alternative binding mode, generally exhibited worse biological activity profiles in the isolated p38 MAPK assay as well as in the whole blood assay, compared to the standard SB203580 (here about 1 magnitude, factor 10) or to the "unsubstituted" pyridinylimidazole ML3375. The reduced activity can be attributed to the abolition of the second hydrogen bond to Met109 within the linker region (Figure 20). On the other hand, factors like the atom size and the electronegativity as it affects

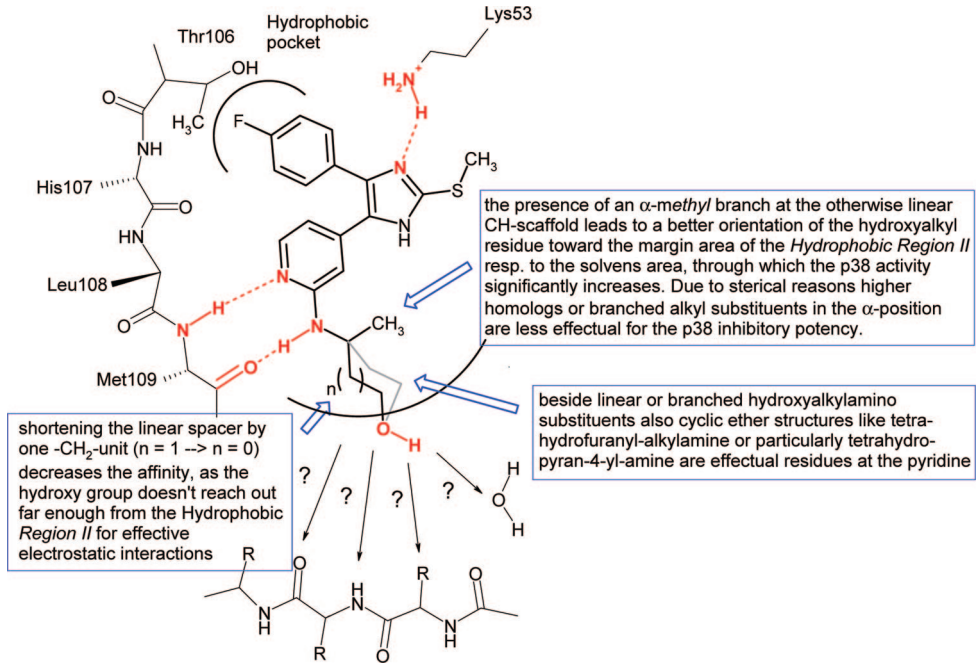


Figure 16. Common binding model for substituted pyridinylimidazoles with an additional polar group at the aliphatic or cyclicoaliphatic amino substituent.

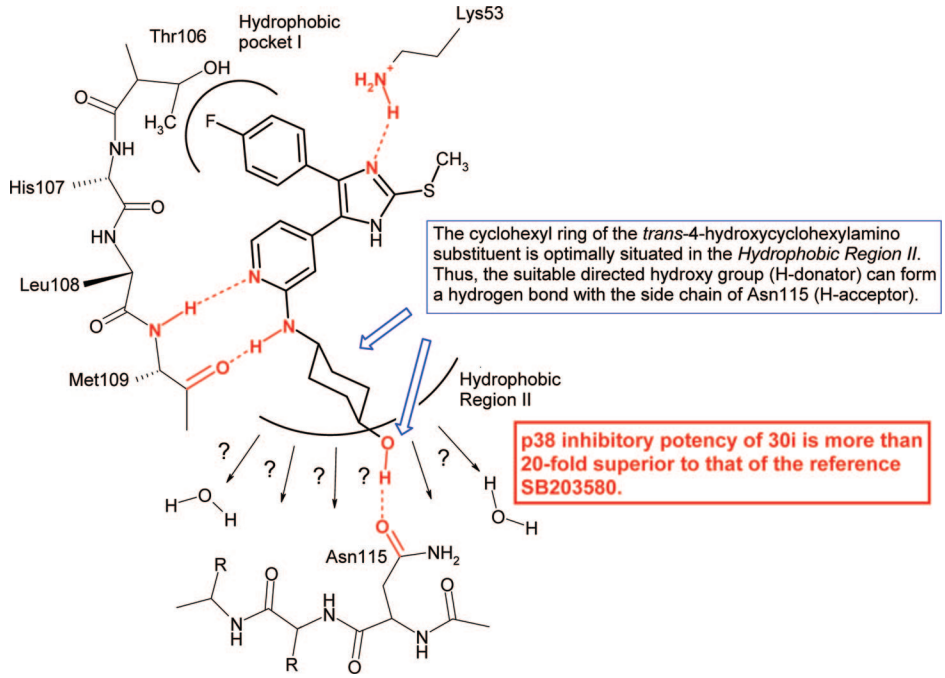


Figure 17. Possible interactions of compound **33i** with the p38 MAPK.

polarizability of the exocyclic heteroatom at the 2-position of the pyridine as well as differing binding angles of the linked residues and subsequent changes in the spatial geometry play a role. Small, short chain, and less voluminous but flexible (hetero)arylalkyl (**33a**,**b**) or, even better, simple alkyl substituents (**34b**, Table 7) still achieved the best results, whereas long chain or branched (chiral) residues (e.g., with α-methyl groups) at compounds **33b** and **33g** were counterproductive. In the series of (hetero)(aryl)alkylaminopyridines, the development of an asymmetric center by introduction of an α-methyl group in the alkyl chain of the substituent at the pyridine always led to a significant increase of the inhibitory activity of the respective derivative in comparison to the underlying nor derivative. Presumably, the resulting unfavorable conformation of compounds **33b** and **33g** averted a deep entry of the drug molecule into the active site of the kinase. Finally, interpretation of the biological data led to the conclusion that there is no correlation in the binding behavior of identical residues within the hydrophobic region II between these (thi)oxypyridines and the corresponding aminopyridine analogues. Compound **34b** is almost as active as the reference SB203580, indicating that promising results could be also reached without the implied H-bond by positive interactions (or no negative interactions) with suitable but sterically less demanding substituents. For the more potent *p*-tolyloxypyridine derivative **33c** [and optionally, for its untested phenylthioxy analogue **34a** (Table 7)] a different

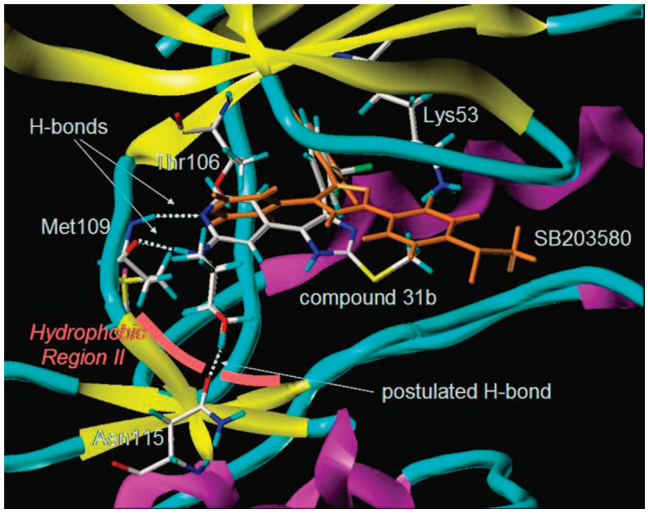


Figure 18. Hypothetical binding mode of compound **31b** (differently colored) within the active site of p38 superposed by the reference compound SB203580 (brownish). The docking is based on the PDB structure 2ewa and was generated by FlexX implemented in Sybyl 7.2. The 4-hydroxy functionality at the cyclohexyl moiety can form a hydrogen bond with the carbonyl group of the side chain of Asn115, which is located marginal the linker region.

binding mode inducing a peptide flip between Met109 and Gly110 in the linker region was speculated.[10,65] In turn, an additional H-bond (here, between the oxygen atom in position 2 of the pyridine and the amide-NH of Gly110) led to a much stronger binding of the investigated molecule in the ATP site (Figure 21). Gly110 is an amino acid that is specific for the α-, β-, and γ-isoforms of p38.[10] Bulkier residues, which predominate in other MAP kinases, would make this peptide flip energetically unfavorably, possibly determining the high selectivity of this compound class (**33c**). In the isolated kinase, **33c** is 2-fold as active as the prototype SB203580. However, all of the compounds from Table 7 were identified only as very poor inhibitors of TNFα release compared with their aminopyridine analogues, presumably because of their higher lipophilicity.

1,2,4,5-Tetrasubstituted Imidazoles. Introduction of Different Amino Substituents at the Pyridine C2. For the investigated pyridinylimidazoles, by the final combination of optimized amino substituents at the 2-position of the pyridine (deduced from the trisubstituted imidazole series discussed above) with recently approved[33,36,46,66] N1 (particularly methyl and methoxyethyl) and C2 (preferably methylsulfanyl) substituents, we sought to gain synergistic effects that would result in improvements in affinity and activity, the physicochemical properties, the metabolical stability, and the reduction of harmful interactions with different CYP450 isozymes. Introduction of large substituents at the 1 and 2 positions of the core imidazole resulted in a general loss of activity. This effect could be overcome by introducing appropriate substituents at the pyridinyl moiety. Besides the well-known H-donor/acceptor functions of the 2-aminopyridin-4-yl pharmacophore part with the p38 hinge region, the NH-linked aryl, arylalkyl or (substituted) (cyclo)alkyl side chains can make further hydrophobic and/or electrostatic interactions with the surface-exposed front region and its bordering areas leading to an even stronger binding of the small molecule. For the N1 substituent, particularly of the 2-methoxyethyl residue, which was found to have the most favorable effects on the biological activity, the exact mode of binding remains unclear.[33] Because of steric interferences especially with (bulkier) pyridine substituents, differing binding modes of the flexible 2-methoxyethyl (perhaps within the ribose pocket) are likely to occur. Thus, the potency of the inhibitors is largely codetermined by the nature of the N1 residue.[33]

Inhibition concentrations of compounds **35a**−**o** (Table 8) in the kinase test assay vary from 960 to 3 nM. A modestly reduced p38 activity, a general weakness for the N1-substituted methylsulfanylimidazole derivatives compared to the corresponding N1 unsubstituted imidazoles (with identical or similar pyridine moieties), was acceptable, as additional substituents at this position of the imidazole core can further modify physicochemical (hydrophilicity, solubility, electron density of the core heterocycle, stability) and toxic properties[33,36] (see Table 10). Nevertheless, in some cases (**35b**,**d**,**g**,**i**,**j**), the introduction of structurally diverse but sterically capable amino substituents at the pyridine part in addition to the 2-methoxyethyl substituent on the imidazole N1-position implied very effective interactions with p38, leading to biological activities that clearly discriminated the reference SB203580. The chirality of introduced amines is also relevant, as evidenced by the IC_{50} values of enantiomers **35a** and **35b** as well as **35c** and **35d**. Here, the *S*-enantiomers (**35a** and **35d**) are identified as the predominant stereoisomers in clear contrast to amino residues at the pyridine moieties in the 2,4,5-trisubstituted imidazole series, where the *R*-enantiomer was the more active form (see Table 6, **28b** vs **28c**).[36] This finding is also consistent with the disappointing results of the tetrasubstituted imidazole **35f** (Table 8) bearing an *R*-configured amino substituent at its pyridine. The inverted activities can be most likely attributed to steric discrepancies of the introduced (chiral) 2-aminopyridine substituents with the adjacent imidazole N1 residues (2-methoxyethyl) causing a "switch" in the conformation of the more extended inhibitor molecules, leading to a change in the binding mode of the (aryl)alkyl side chain at the exocyclic NH group in the hydrophobic region II. In the presence of the 2-methoxyethyl group at position N1, bulkier pyridine substituents are less well accepted. At this point, the α-methyl group in the side chain of the aliphatic spacer at the 2-aminopyridine sometimes seemed to be rather harmful, as can be inferred from the biological results of **35l** and **35o**. If the N1-methoxyethyl residue makes a constant contibution to the affinity with p38, a different binding mode for each of these pyridine substituents inside the hydrophobic region II (compared to the N-unsubstituted imidazole analogues) can be postulated. This reasoning may explain the lack of correlation in the IC_{50} values between analogous tri- and tetrasubstituted imidazoles having identical (chiral) substituents at the pyridine.

To clarify the binding mode of the more potent compounds in this series, an energetically minimized binding mode was generated for the tetrahydropyran-4-ylaminopyridine **35i** by molecular modeling (Figure 22). Analogue **35i** is nearly equipotent to the cyclohexylaminopyridine **35g** both in the p38 test assay and in the whole blood test model. Among the listed 1,2,4,5-tetrasubstituted imidazoles (Table 8) compound **35j** exhibited the best results, with a relative p38 IC_{50} value of only 0.048 (IC_{50} = 3 nM!). Here, the substituents at all relevant positions of the pyridinylimidazole scaffold seem to be best possibly combined. In analogy to **35i**, the introduced *trans*-4-hydroxycyclohexyl residue of **35j** optimally fits into the surface-exposed front region making hydrophobic as well as additional electrostatic interactions and significantly increasing the activity and probably the selectivity. Furthermore, **35j** also maintained its high potency in the cytokine test, whereas TNFα results of the other compounds were more widespread. Substituents placed at the 2 position of the imidazole (here, 2-methoxyethyl) contribute to bioactivity only to a lesser extent. By steric

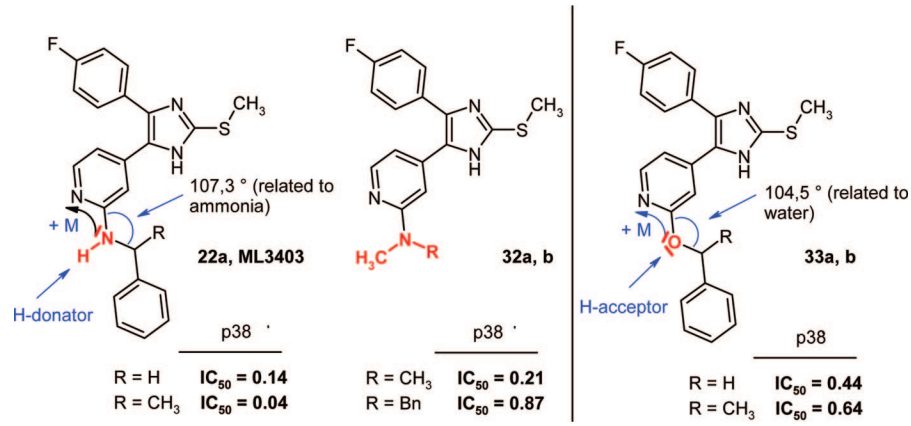


Figure 19. Influence of the exocyclic NH functionality to the inhibition of p38.

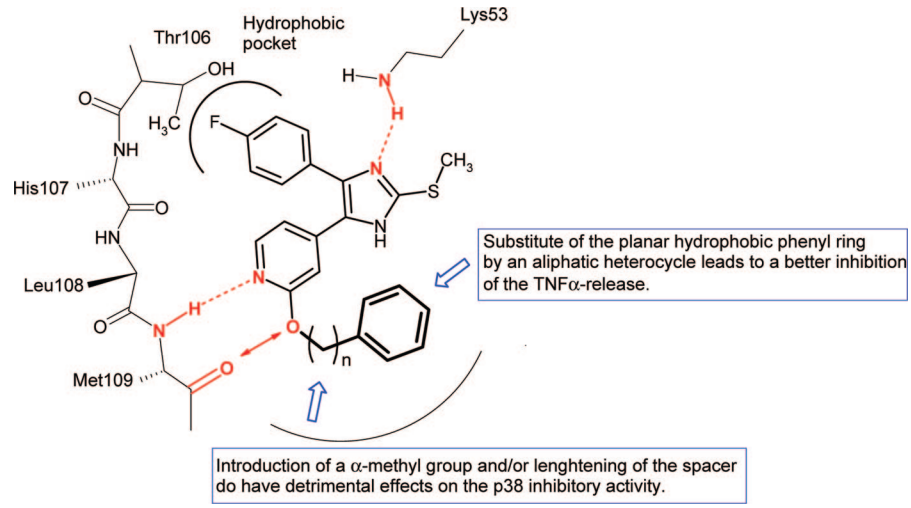


Figure 20. SAR of the (hetero)(aryl)alkyloxypyridines.

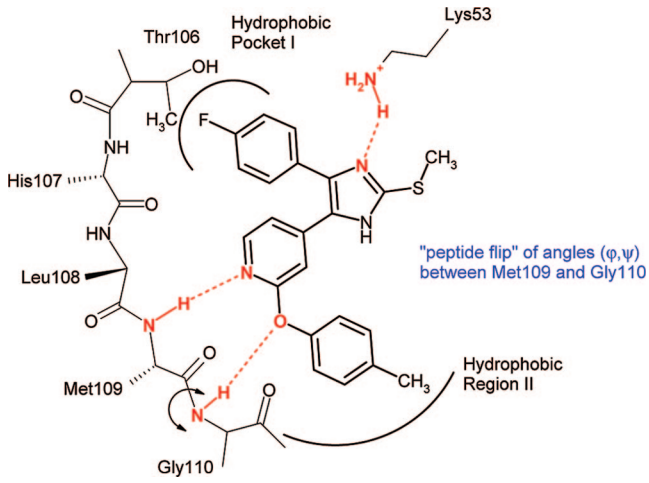


Figure 21. Possible binding model of the aryloxypyridine **33c**, which gives consideration to the experimentally detected high p38 inhibitory activity compared to the less potent (hetero)(aryl)alkyloxypyridine derivatives.

shielding and/or changing of the electronical properties of the critical imidazole core, these N1 substituents reduced negative CYP450 interactions.

Many kinase inhibitors that are currently in clinical or preclinical development states are directed to the ATP binding site of the enzyme. Many of these ATP-competitive inhibitors lack of selectivity. Binding specificity and affinity of the potential inhibitors, however, cannot be simply predicted on the basis of present sequence or structure information, requiring extensive testing of the compounds against multiple kinases. The specificity among many of the known kinase inhibitor molecules that were either advanced to clinical trials or admitted for therapeutic use widely varies, and the specificity could not be strongly correlated with the chemical structure or the identity of the intended target structure.[30] Of 20 inhibitor molecules that had been profiled against a panel of 113 different kinases, the pyridinylimidazoles SB203580 and SB202190 were among the most selective compounds, binding only to a few other kinases apart from their primary target.

Selectivity profilings of a selection of our substituted pyridinyl-2-thioimidazole inhibitors, summarized in Table 9, also indicate a clear preference for p38 compared to other relevant human kinases selected from different categories of the phylogenetic kinase tree. In this test (of 17 kinases beside p38α) compounds **22c**,**d**,**g**, **26b**, **28o**, **30c**, and **35g** exhibited excellent p38α IC_{50} values between 13 and 68 nM. The p38β form was on average 96% inhibited at an inhibitor concentration of 10 μM, whereas the γ- and the δ-forms were not inhibited by these compounds. The documented tri- and tetrasubstituted imidazole derivatives only exhibited an inhibitory activity toward the very closely related JNKs. However, while compounds **22d**,**g**, **26b**, **28o**, and **35g** inhibited all three JNK subtypes (JNK 1α1, JNK 2α2, and JNK3) nearly equipotently, the most selective 1-phenylethylaminopyridine derivative **22c** of this series shows only a dominant inhibition toward JNK3, whereas both other isoforms

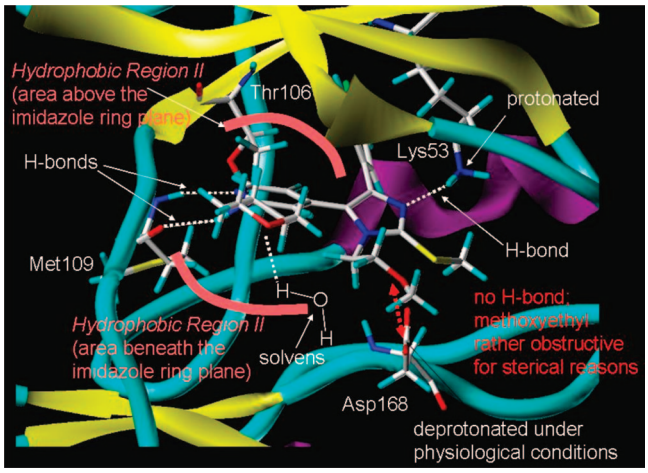


Figure 22. Proposed binding mode of compound **35i** in the active site of the 38α MAPK. The docking was performed with FlexX implemented in Sybyl 7.2 based on the PDB structure 1BL7. One possible H-bond is detected between the basic imidazole N (sp^2) and Lys53. Moreover, the 2-aminopyridine ring can execute the essential H-donor/acceptor functions with backbone Met109 in the linker region. The tetrahydropyran-4-yl side chain in the 2-position of the pyridine ring is optimally located in the hydrophobic region II. The ring oxygen atom of the pyrane can possibly make further electrostatic interactions (e.g., with or via a water molecule of the solvent area surrounding the surface-exposed front region) leading to a significant increase in the activity. Because of the inability to form an H-bond with the (deprotonated) Asp168 under physiological conditions, the importance of the flexible 2-methoxyethyl substituent at the N1 position for the inhibitor potency remains less clear. A sterical antagonism with the respective substituent at the pyridine ring (here, tetrahydropyran-4-ylamino) is most likely leading to a "conformational switch" of the compound compared to analogous 2,4,5-trisubstituted imidazoles, which lack an additional substituent at the imidazole N1. However, adequate aliphatic substituents at the N1 position of the imidazole can possibly further improve the physicochemical properties (e.g., log *P* values) of the inhibitor molecule.

(JNK2 and JNK3) were not addressed. Similarly, the tetrahydrofuranylmethylaminopyridine **30c** exhibited a clearly reduced inhibition of the JNK1 subtype.

Despite the fact that the shown inhibitors (Table 9) quasi-mimic the enzymatic cosubstrate and thus compete with ATP for binding to the active site of the kinase(s), they are much more selective to p38α and β than to the p38γ and the δ isoforms (e.g., in comparison to the "allosteric" DFGout binder BIRB796) as well as toward most of the other members of the MAPK family. In addition, these compounds have virtually no activity against other kinases including the tyrosine kinases.[7]

Most of the interactions of these exemplified compounds (**22c**,**d**,**g**, **26b**, **28o**, **30c**, and **35g**) with p38α occur with areas of the protein that are not directly involved in the binding of ATP and thus are less highly conserved among the kinases but that decisively contribute to the recognized selectivity. By introduction of suitable substituents at the 2-position of the pyridine of the diaryl heterocyclic imidazoles, which can effectively interact with the kinase's surface-exposed front region (hydrophobic region II), not only the inhibitory potency can be significantly enhanced but also additional selectivity to p38 with regard to other kinases can be gained.

In order to minimize the toxic effects arising from unwanted CYP interactions, future generations of pyridinylimidazoles will have to be modified by introducing sterically demanding and electronically modifying substituents at the imidazole and/or the pyridine ring. These variations could be successfully accomplished at both the tri- and the tetrasubstituted 2-thioimidazole series of the present work. To determine if the introduced residues actually can reduce the affinity of these compounds with the CYP450 system, some of the more potent representatives were additionally subjected to a screening assay with relevant CYP 450 isozymes. Among the 20 inhomogeneous pyridinyl-2-thioimidazoles (Table 10), there are differences in the inhibition of the tested CYP isozymes. These differences are particularly apparent when examining the metabolically relevant isozymes 1A2 and 2D6. Compounds **22c**,**g**, **26f**, **28l**,**o**, **30b**, **35a**, and **35c**−**j** inhibited CYP450 1A2 less than 55%. Moreover, the isoform 2D6 is also less inhibited by compounds **26a**,**f**,**g**, **28l**, **30b**,**c**, **33c**, and **35a**,**b**,**d**,**g**,**j** compared with the model inhibitor SB203580, whereas the other candidates were stronger inhibitors of these two isoforms. Of the remaining tested CYP450 isozymes, only slight improvements could be achieved over SB203580 by the introduction of the respective substituents at the pyridine ring (inhibition of 2C9, 2C19, and 3A4 less than 55% by **26f**, **30b**,**c** as well as by **35j**). It is remarkable that the investigated CYP450 enzymes are commonly better inhibited by compounds with nonpolar substituents (e.g., **22d**, **26b**,**d**,**g**, **28a**, or **33c**). Accordingly, the relatively more hydrophilic compounds **26f**, **30b**,**c** as well as **35b** and **35j** exhibited the lowest affinity (inhibition less than 55%) compared to almost all tested CYP isozymes (except CYP450 1A2 at **30c** and 3A4 at **35b**,**j**). These compounds would be expected to have the lowest pharmacological toxicity in vivo too and therefore represent the most promising developmental candidates. This result is gratifying, as the most potent inhibitors presented here were gained by the introduction of polar amino alcohols at the pyridine. Furthermore, as indicated by the examples in Table 10, a reduction of the CYP450-mediated toxicity can be achieved only by a sterically shielded and electronically modified pyridine ring without additional N1 substituents, thus making the pyridine the more critical structural determinant, with respect to toxicology, of this inhibitor class.

Conclusions

The optimized and, on the whole, extremely flexible synthetic concepts illustrated herein allowed us to regioselectively produce numerous highly (2,4,5-tri- and 1,2,4,5-tetra-) substituted and structurally diverse 2-thioimidazole derivatives starting from comparatively few ethanone structures. In the p38-MAPK test assay, many of the represented (amino)pyridinylimidazole analogues were superior to the current standard SB203580 with respect to the inhibitory activity. Because of their suitable cell penetration properties, the most potent compounds also inhibited the release of relevant cytokines (TNFα, IL-1β) from human whole blood with high efficacy.

In this study the vast importance of the kinase's surface-exposed front region (hydrophobic region II) as an important binding area of protein kinases (here, the p38α MAPK) for gaining additional activity *and* selectivity was impressively demonstrated.

By introduction of additional substituents at the pyridine ring of the 2-thioimidazole derivatives, several inhibitors could be generated whose specificity for p38 and whose toxic pharmacological profile may suggest further developments to anti-inflammatory drugs.[4,67,68] On the basis of these biological primary screenings, a number of compounds were selected for follow-up investigation (in particular, **22c**,**g**, **26b**,**f**, **28l**, **29a**, **30c**,**d**,**h**,**i**, **31b**, **33c**, **35b**,**d**,**g**,**i**, **j**).

With regard to the potent prototype SB203580, the most promising inhibitory properties useful for a further preclinical development are mainly offered by those compounds that

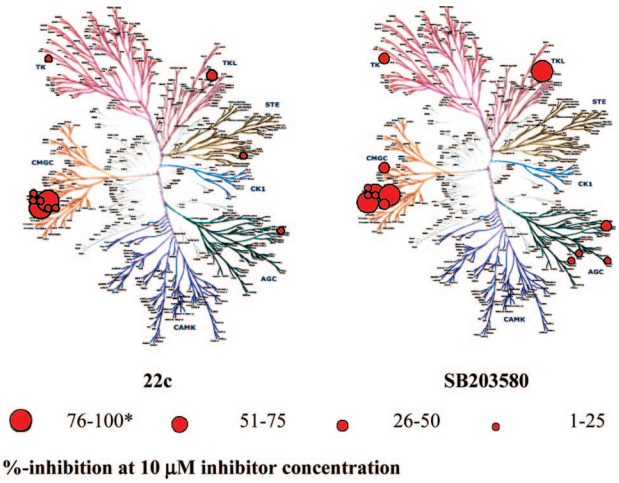


Figure 23. Selectivity of **22c** for p38 toward other kinases (left) in comparison with SB203580 (right), modified according to Fabian et al.[30] (both compounds were tested against a panel of 17 different kinases of the human genome).

optimally combine excellent enzyme-inhibitory concentrations (in the low nanomolar range) and cell penetration promoting characteristics. This is in particular realized for compounds **22c** and **22g**, **26f**, **28l**, **30b**,**c**, **30h**, **35b**,**d**, and **35g**. However, for compounds **26b**, **29b**, **30d**, **33c**, **35b**, and **35h**−**k** the good inhibitory potencies in the p38 kinase test assay are offset by unfavorable cell permeation properties, and so effective inhibition is not expressed in the cellular (whole blood) model. Furthermore, with regard to the molecular weights, the calculated log *P* values, and the number of available H-bond donors and acceptors, the Lipinski rule of five criteria have been fulfilled by all selected imidazole compounds, additionally qualifying them as promising drug candidates for an oral administration.[64]

Moreover, cytochrome P450 interaction was very efficiently reduced through introduction of sterically and also electronically shielding (amino)substituents at the 2-position of the pyridine moiety particularly in compounds **26f**, **30b**,**c**, **35b**, and **35j**.[18] Other derivatives also exhibited a clearly decreased CYP450 interaction potential, especially with the metabolically relevant isozymes 1A2 and 2D6.

Many first generation p38 inhibitors, which imitate the cosubstrate ATP, are limited by insufficient specificity because of the sequential and structural similarities of the ATP-binding sites of a multiplicity of the 518 known protein kinases. Thus, the design of specific ATP site directed kinase inhibitors (ATP mimetics) is still considered a major challenge to be met by medicinal chemistry.[23] Here, the prototypical pyridinylimidazoles SB203580 and SB202190 still rank among the most selective inhibitor representatives[30] in their class, whose selectivity profile is even better than, for example, that of the "allosteric" urea-based inhibitor BIRB796 binding in the so-called DFG out region hindering ATP from entering into the ATP site.[69]

By considering and exploiting the less conserved areas at the active site of p38 that are not occupied by ATP, we have attempted to design inhibitors that target hydrophobic region II with the aim to improve p38 MAPK selectivity. The novel 2-alkylsulfanyl-5-pyridin-4-ylimidazole compounds of the present work having additional (amino-) substituents in the 2-position of the pyridine ring, which make efficient interactions with residues of the hydrophobic region II and beyond, show a clearly enhanced specificity for p38 over other kinases that exceeds the reference SB203580 (Figure 23, exemplary for **22c**).

Experimental Section

Chemistry. All reagents and solvents were of commercial quality and used without further purification. General procedures A and B were followed for the preparation of target compounds via nucleophilic aromatic substitution of 2-halogenopyridines **5** and **7**.

General Procedure A for the Preparation of N-Substituted 2-Aminopyridines. Variant 1. Under an inert atmosphere of argon, the respective 5-(2-halogenopyridin-4-yl)imidazole (1 equiv) was suspended in an excess volume of the respective (primary) amine (5−10 equiv). The mixture was stirred at the appropriate temperature until the imidazole starting material had been consumed completely and no further product formation took place or increasing amounts of any side products were detected (TLC, SiO_2 60, DCM/ethyl acetate, 1/2). The reaction was cooled to room temperature and suspended in an aqueous solution of citric acid (10%), which had been brought to pH 5 with an aqueous solution of NaOH (20%). The emulsion was extracted with ethyl acetate (3×). The combined organic extract was washed with citric acid (10%, pH 5), an aqueous solution of Na_2CO_3 (10%), and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. The crude oily residue was mostly purified by column chromatography or preparative TLC and seldom by recrystallization from ethanol. Crystallization/solidification of the pure compound occurred upon evaporation with little diethyl ether.

Variant 2. After the 5-(2-halogenopyridin-4-yl)imidazole was reacted with an excess of the respective amine according to variant 1, the cold reaction mixture was taken up/dissolved in the required volume of ethyl acetate (6−90 mL/mmol starting material) and the remaining amine was removed by vacuum distillation (amines with relatively low boiling points) or by multiple washing of the organic solution with water (4−10 mL/mmol starting material) or brine. The pH of the final aqueous extraction phase should be almost neutral (pH 6−7). The pooled aqueous extract then was once again extracted with ethyl acetate. The entire organic extract was dried over Na_2SO_4 and the solvent rotary evaporated in vacuo. In some cases only (e.g., with less hydrophilic amino nucleophiles), the resulting oily residue had to be additionally purified by a chromatographic method. Otherwise, the concentrated and commonly analytically pure compound was either crystallized from *n*-hexane or solidified upon evaporation with diethyl ether as an amorphous or a glasslike substance.

General Procedure B for the Preparation of O- and S-Substituted 2-Oxy- or Thioxypyridines. To a suspension of NaH (moistened with oil, 55−65%, 1.05 equiv) in dry DMF under argon was dropwise added the respective liquid alcohol or thioalcohol (1 equiv). The mixture was stirred at room temperature until gas release (H_2) ceased and deprotonation of the nucleophilic reagent was complete (1−2 h, bubble counter). Subsequently the respective 5-(2-halogenopyridin-4-yl)imidazole (0.1 equiv) was added in one portion to this suspension and the mixture was heated to reflux temperature and left stirring at this temperature for the indicated time. Then the mixture was cooled to room temperature and was hydrolyzed with the respective volume of water or 10% citric acid (pH 4−5). The aqueous/organic mixture was extracted with ethyl acetate (4 × 50 mL), and after it was optionally dried over Na_2SO_4, the solvent of the combined organic phases was evaporated as far as possible (removal of residual DMF!). To obtain the pure substitution product, the resulting dark oily residue was purified by using a chromatographic method. Further purification techniques could follow (e.g., recrystallization procedures or washing steps). The initially sticky product was finally dried and solidified by treatment with diethyl ether.

Typical Procedures. {4-[4-(4-Fluorophenyl)-2-methylsulfanyl-1*H*-imidazol-5-yl]pyridin-2-yl}-(2-phenylpropyl)amine (22f). According to general procedure A (variant 1) the title compound was obtained from 2-fluoro-4-[5-(4-fluorophenyl)-2-methylsulfanyl-3*H*-imidazol-4-yl]pyridine **5a** (0.5 g, 1.6 mmol) and (*RS*)-2-phenylpropylamine (2.23 g, 16.5 mmol) after 8 h at 160 °C. The crude product was purified by column chromatography (SiO_2 60, CH_2Cl_2/EA 1 + 2) to yield 0.45 g (65.2%) of **22f**: $C_{24}H_{23}FN_4S$ (M_r 418.54);

mp 107 °C; ^{1}H NMR (CD_3OD) δ (ppm) 1.18−1.27 (m, 3H, $-CH_3$), 2.60 (s, 3H, $-SCH_3$), 2.89−3.00 (m, 1H, methine-H), 3.25−3.34 (m, 2H, N$-CH_2-$, superposed by the CD_3OD signal), 6.50−6.56 (m, 2H, C^3-/C^5-H 2-amino-Pyr), 7.06−7.26 (m, 7H, C^3-/C^5-H 4-F-Phe and Phe), 7.40−7.47 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (dd, 1H, J = 0.71/5.48 Hz, C^6-H 2-amino-Pyr); IR (ATR) 1220 (C−F) cm^{-1}; GC (method 2) 23.3 min; MS *m/z* (%) 418 (6, M^+), 313 (100), 298 (8), 105 (5, $C_8H_9^+$), 91 (1, tropylium$^+$), 77 (2), 65, 51; HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{24}H_{24}FN_4S$, 419.1700; found, 419.1690.

Furan-2-ylmethyl-{4-[2-methylsulfanyl-4-(3-trifluoromethylphenyl)-1*H*-imidazol-5-yl]pyridin-2-yl}amine (27b). According to general procedure A (variant 1), the title compound was obtained from 2-fluoro-4-[2-methylsulfanyl-5-(3-trifluoromethyl-phenyl)-3*H*-imidazol-4-yl]pyridine **5i** (0.3 g, 0.8 mmol) and furfurylamine (1.0 g, 10.3 mmol) after 8.5 h under reflux. The crude product was purified by column chromatography (SiO_2 60, CH_2Cl_2/EA 1 + 2) to yield 164 mg (44.9%) of **27b**: $C_{21}H_{17}F_3N_4OS$ (M_r 430.46); mp 105 °C; ^{1}H NMR (CD_3OD) δ (ppm) 2.64 (s, 3H, $-SCH_3$), 4.41 (s, 2H, $-CH_2-$), 6.14−6.16 (m, C^3-H furyl−), 6.29−6.31 (m, 1H, C^4-H furyl−), 6.56−6.62 (m, 2H, C^3-/C^5-H 2-amino-Pyr), 7.37−7.39 (m, 1H, C^5-H furyl−), 7.51−7.71 (m, 3H, $C^4-/C^5-/C^6-$H 3-trifluoromethyl-Phe), 7.78 (s, 1H, C^2-H 3-trifluoromethyl-Phe), 7.90 (d, 1H, J = 5.43 Hz, C^6-H 2-amino-Pyr); IR (ATR) 1122 (CF_3) cm^{-1}; GC (method 2) 12.2 min; MS *m/z* (%) 430 (100, M^+), 415 (3, $M^+ - CH_3$), 401 (71), 375 (12), 335 (6), 262 (2), 205 (13), 171 (3), 145 (2, 3-trifluoromethylphenyl$^+$), 96 (7, furfurylamino$^+$), 81 (25, furfuryl$^+$), 53 (11); HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{21}H_{18}F_3N_4OS$, 431.1148; found, 431.1150.

((*R*)-1-Cyclohexylethyl)-{4-[4-(4-fluorophenyl)-2-methylsulfanyl-1*H*-imidazol-5-yl]pyridin-2-yl}amine (28b). According to general procedure A (variant 1), the title compound was obtained from 2-fluoro-4-[5-(4-fluorophenyl)-2-methylsulfanyl-3*H*-imidazol-4-yl]pyridine **5a** (0.5 g, 1.6 mmol) and (*R*)-(−)-1-cyclohexylethylamine (2.1 g, 16.5 mmol) after 8.5 h at 155−160 °C. The crude product was purified by column chromatography (SiO_2 60, CH_2Cl_2/EA 1 + 2) to yield 180 mg (26.6%) of **28b**: $C_{23}H_{27}FN_4S$ (M_r 410.56); mp 117 °C; ^{1}H NMR (CD_3OD) δ (ppm) 0.93−1.40 (m, 9H, $C^3-/C^4-/C^5-H_2$ cyclohexyl and $-CH_3$), 1.72−1.82 (m, 5H, C^2-/C^6-H_2 and C^1-H cyclohexyl), 2.62 (s, 3H, $-SCH_3$), 3.50 (quint, 1H, J = 6.51 Hz, NCH<), 6.48−6.52 (m, 2H, C^3-/C^5-H 2-amino-Pyr), 7.09−7.18 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.41−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7,78 (d, 1H, J = 5.80 Hz, C^6-H 2-amino-Pyr); IR (ATR) 1221 (C−F) cm^{-1}; GC (method 2) 18.7 min; MS *m/z* (%) 410 (13, M^+), 395 (3, $M^+ - CH_3$), 327 (100, M^+-cyclohexyl), 311 (12), 300 (28), 285 (3), 267 (3), 163 (5), 147 (15), 95 (1, 4-fluorophenyl$^+$), 55 (4); HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{23}H_{28}FN_4S$, 411.2013; found, 411.2009.

2-{4-[4-(4-Fluorophenyl)-2-methylsulfanyl-1*H*-imidazol-5-yl]pyridin-2-ylamino}propan-1-ol (30d). According to general procedure A (variant 1), the title compound was obtained from 2-fluoro-4-[5-(4-fluorophenyl)-2-methylsulfanyl-3*H*-imidazol-4-yl]pyridine **5a** (0.5 g, 1.6 mmol) and (*RS*)-2-amino-1-propanol (2.48 g, 33.0 mmol) after 8 h at 155 °C. The reaction mixture was combined with 10% citric acid (pH 4−5, 15 mL) and subsequently extracted with ethyl acetate (5 × 30 mL). Washing the organic extract conformable to the specifications with 10% $NaCO_3$(aq), 10% citric acid (pH 4−5) and saturated NaCl(aq) left the analytically pure product that could be solidified as amorphous foam upon multiple evaporating with diethyl ether. A chromatographic purification was not necessary. Yield: 0.47 g (79.6%) of **30d**; $C_{18}H_{19}FN_4OS$ (M_r 358.44); mp 92 °C; ^{1}H NMR (CD_3OD) δ (ppm) 1.17 (d, 3H, J = 6.57 Hz, -CH_3), 2.63 (s, 3H, $-SCH_3$), 3.51−3.55 (m, 2H, $-CH_2-$), 3.82 (sext, 1H, J = 6.58 Hz, methine-H), 6.53 (dd, 1H, J = 1.52/5.51 Hz, C^5-H 2-amino-Pyr), 6.60 (s, 1H, C^3-H 2-amino-Pyr), 7.09−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.42−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, 1H, J = 5.53 Hz, C^6-H 2-amino-Pyr); IR (ATR) 1221 (C−F) cm^{-1}; LC 11.0 min, 99.9%; HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{18}H_{20}FN_4OS$, 359.1336; found, 359.1324.

4-[4-(4-Fluorophenyl)-2-methylsulfanyl-1*H*-imidazol-5-yl]-2-(1-phenylethoxy)pyridine (33b). According to general procedure B, the title compound was obtained from NaH (0.79 g, 18.1 mmol, moistened with oil, 55−65%), (*RS*)-1-phenylethanol (2.1 g, 17.2 mmol), and 2-fluoro-4-[5-(4-fluorophenyl)-2-methylsulfanyl-3*H*-imidazol-4-yl]pyridine **5a** (0.5 g, 1.6 mmol) in absolute DMF (7 mL) after 4.5 h at 155 °C. The crude product was prepurified by column chromatography (SiO_2 60, CH_2Cl_2/EA 4 + 1), and after complete evaporation of the organic solvent(s) the residue dissolved in methanol. Upon dropwise addition of water, pure **33b** precipitated from the alcoholic solution as fine white crystals. The suspension was ultracentrifugated and the surmounting solvent mixture (including residual phenylethanol) decanted. The sediment was dried by multiple evaporation with little diethyl ether. Yield, 0.4 g (59.8%) of **33b**; $C_{23}H_{20}FN_3OS$ (M_r 405.50); mp 62−64 (82) °C; ^{1}H NMR (CD_3OD) δ (ppm) 1.56 (d, 3H, J = 6.50 Hz, -CH_3), 2.61 (s, 3H, $-SCH_3$), 5.96 (q, 1H, J = 6.48 Hz, methine-H), 6.82−6.93 (m, 2H, C^3-/C^5-H 2-oxy-Pyr), 7.08−7.44 (m, 9H, 4-F-Phe and Phe), 7.92 (d, 1H, J = 5.30 Hz, C^6-H 2-oxy-Pyr); IR (ATR) 1222 (C−F) cm^{-1}; GC (method 2) 17.8 min; MS *m/z* (%) 405 (85, M^+), 390 (22, $M^+ - CH_3$), 328 (6), 300 (100, M^+ − phenylethyl), 285 (39), 268 (28), 228 (9), 172 (8), 121 (4), 105 (76, $C_8H_9^+$), 79 (12), 77 (11), 51 (2); HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{23}H_{21}FN_3OS$, 406.1384; found, 406.1371.

2-Ethylsulfanyl-4-[4-(4-fluorophenyl)-2-methylsulfanyl-1*H*-imidazol-5-yl]pyridine (34b). According to general procedure B, the title compound was obtained from 2-fluoro-4-[5-(4-fluorophenyl)-2-methylsulfanyl-3*H*-imidazol-4-yl]pyridine **5a** (0.4 g, 1.3 mmol) and sodium ethanthiolate (1.11 g, 13.2 mmol) in absolute DMF (5 mL) after 7 h at 155−160 °C. The cold reaction mixture was combined with ice−water (∼150 mL) and acidified with 20% HCl to pH 3−4. From the cloudy solution a fluffy yellow-beige precipitate dropped down, which was filtered off and dried in vacuo. The crude product was two times purified by column chromatography (SiO_2, CH_2Cl_2/EA 1 + 2 and SiO_2, CH_2Cl_2/EA 1 + 1) to finally yield 0.17 g (37.3%) of **34b**. Crystallization of **34b** occurred upon multiple evaporation with little diethyl ether: $C_{17}H_{16}FN_3S_2$ (M_r 345.46); mp 60 °C; ^{1}H NMR (CD_3OD) δ (ppm) 1.23−1.37 (m, 3H, -$CH_2-\underline{CH_3}$), 2.63 (s, 3H, $-SCH_3$), 2.94−3.05 (m, 2H, $-S-CH_2-$), 7.09−7.27 (m, 3H, C^3-/C^5-H 4-F-Phe and C^5-H 2-thioxy-Pyr), 7.37 (s, 1H, C^3-H 2-thioxy-Pyr), 7.43−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 8.22 (d, 1H, J = 5.46 Hz, C^6-H 2-thioxy-Pyr); IR (ATR) 1223 (C−F) cm^{-1}; GC (method 2) 10.5 min; MS *m/z* (%) 345 (100, M^+), 330 (48, $M^+ - CH_3$), 312 (71), 285 (24), 269 (9), 212 (7), 121 (6), 95 (4, 4-fluorophenyl$^+$); HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{17}H_{17}FN_3S_2$, 346.0842; found, 346.0850.

{4-[4-(4-Fluorophenyl)-3-(2-methoxyethyl)-2-methylsulfanyl-1*H*-imidazol-5-yl]pyridin-2-yl}(tetrahydro-pyran-4-yl)amine (35i). Starting from 0.3 g (0.8 mmol) of 2-fluoro-4-[5-(4-fluorophenyl)-3-(2-methoxyethyl)-2-methylsulfanyl-3*H*-imidazol-4-yl]pyridine **7b** and 0.84 g (8.3 mmol) tetrahydropyran-4-ylamine, an amount of 345 mg (93.9%) of compound **35i** was obtained according to the general procedure A (variant 2). Reaction time, 16 h; $C_{23}H_{27}FN_4O_2S$ (M_r 442.56); mp 59−62 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.40−1.60 (m, 2H, C^3-/C^5-H tetrahydropyranyl−), 1.93−2.00 (m, 2H, C^3-/C^5-H tetrahydropyranyl−), 2.72 (s, 3H, $-SCH_3$), 3.27 (s, 3H, $-OCH_3$), 3.42−3.56 (m, 4H, C^2-/C^6-H tetrahydropyranyl− and $-CH_2-O-$), 3.65−3.81 (m, 1H, methine-H), 3.93−4.07 (m, 4H, C^2-/C^6-H tetrahydropyranyl− and >N$-CH_2-$), 4.64 (d, 1H, exchangeable, J = 7.65 Hz, >NH), 6.36 (s, 1H, C^3-H 2-amino-Pyr), 6.57 (dd, 1H, J = 1.36/5.21 Hz, C^5-H 2-amino-Pyr), 6.88−6.96 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 8.14 (dd, 1H, J = 0.55/5.22 Hz, C^6-H 2-amino-Pyr); IR (ATR) 1219 (C−F) cm^{-1}; GC (method 2) 16.1 min; MS *m/z* (%) 442 (100, M^+), 427 (4, $M^+ - CH_3$), 413 (8), 397 (9), 385 (50), 370 (8), 357 (64, M^+ − tetrahydropyranyl), 342 (3, M^+ − tetrahydropyranylamino$^+$), 325 (11), 299 (8), 267 (16), 146 (4), 121 (4), 100 (2, tetrahydropyran-4-ylamino$^+$), 59 (4, methoxyethyl$^+$), 55 (5); HRMS (ESI, pos mode) *m/z* $[M + H]^+$ calcd for $C_{23}H_{28}FN_4O_2S$, 443.1912; found, 443.1909.

The syntheses and analytical properties of compounds **1a**−**d**, **2a**−**d**, **3a**−**e**, **4a**,**b**, **5a**−**k**, **6a**,**b**, **7a**−**e**, **8**−**10**, **11a**,**b**, **12a**−**c**, **13a**−**c**, **14a**−**c**, **16b**, **17a**−**d**, **18a**−**d**, **19a**−**d**, **20a**,**b**,and **32b** have been published elsewhere.[31,36,41] The following commercial starting materials were used: **15a**,**b** and **16a**.

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Supporting Information Available: Synthetic procedures, routine spectroscopic and HRMS data of all target compounds, and (HP)LC and ^{13}C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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