Targeting the Ribose and Phosphate Binding Site of p38 Mitogen-Activated Protein (MAP) Kinase: Synthesis and Biological Testing of 2-Alkylsulfanyl-, 4(5)-Aryl-, 5(4)-Heteroaryl-Substituted Imidazoles

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Three series of substituted 2-alkylsulfanyl-4-(4-fluorophenyl)imidazoles, 5-pyridinyl-, 1-methyl-5-pyridinyl-, and 5-(2-aminopyridin-4-yl)-imidazoles, were prepared and tested for their ability to inhibit p38 MAP kinase and TNF-α release. These compounds were prepared by using different synthetic routes. They were tested by applying a nonradioactive p38 MAP kinase assay and by measurement of TNF-α release in human whole blood. Potent inhibitors (IC_{50}values in the low nanomolar range, as low as 2 nM in the enzyme assay and 37 nM in the human whole blood test) were identified by variation of substituents at the imidazole-C2-thio position as well as at the 2-aminopyridinyl functionality. In contrast to other known kinase inhibitors, these novel imidazole derivatives with the substituents at the imidazole-C2-thio position may interact with the ribose as well as with the phosphate binding site of the p38 MAP kinase.

Introduction

The p38 mitogen-activated protein (MAP[a]) kinase, a serine/threonine kinase, is one of the best characterized kinases in the inflammatory process.[1,2] Among the four identified p38 isoforms (p38α, p38β, p38γ, and p38δ), the α-form is the most fully studied. Cellular stress (osmotic shock, UV radiation, mechanic stress) activates predominantly the p38 MAP kinase. This kinase plays a central role in the biosynthesis of the proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) at the translational and transcriptional levels.[3–5] Antagonism of these proinflammatory cytokines has been recognized as an effective possibility for the development of new drug candidates for the treatment of, for example, rheumatoid arthritis, psoriasis, or inflammatory bowel disease.[1,6] One of the first lead compounds as a p38 MAP kinase inhibitor was the pyridinylimidazole SB203580.[7,8] Crystallographic data[9–11] showed that SB203580 binds in the adenosine triphosphate (ATP) binding site of p38 MAP kinase with a crucial hydrogen bond between the pyridin-4-yl moiety and the backbone NH of Met109 in the hinge region (Figure 1).[10] The 4-fluorophenyl ring binds to the hydrophobic pocket I, mainly gaining selectivity. Another possible ligand−protein interaction is a hydrogen bond between Lys53 and N-3 of the imdazole core as well as a $\pi-\pi$ stacking between Tyr35 and the phenyl system. The hydrophobic region II remains unoccupied. According to Schwalbe,[12] SB203580 seems to bind to both p38α DFG-in and DFG-out conformation. Further clinical development of those first generation p38 inhibitors was obstructed by their severe liver toxicity associated with the interference with hepatic cytochrome P450 enzymes.[13] It still remains unclear if this unwanted effect was attributed to the imidazole or the pyridine ring.[14]

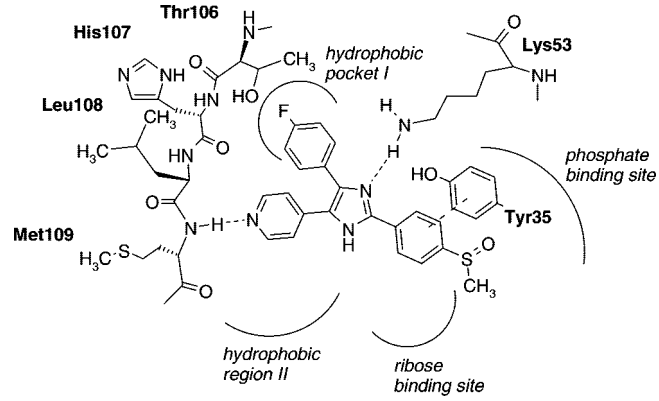

Figure 1. Schematic drawing of important interactions between the competitive inhibitor SB203580 and the ATP binding site of p38α.

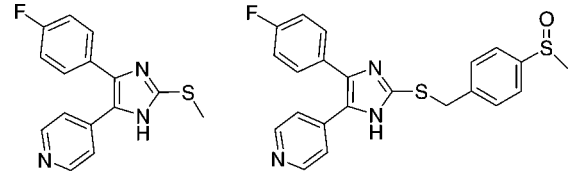

Figure 2. Pyridinyl imidazole inhibitors ML3163 and ML3375 of MERCKLE.[15]

We previously reported that trisubstituted imidazoles structurally related to SB203580, namely, ML3375[15](Figure 2), which bears a small *S*-methyl substituent at the imidazole-C-2 position, was 6−10 times more potent than the aromatic analogue ML3163[15] (Figure 2). This greater inhibitory potential of ML3375 was attributed to a better entry of the smaller inhibitor molecule (ML3375) into the binding cleft of p38 MAP kinase, leading to stronger enzyme−drug interactions.[15] With respect to these earlier results, we first focused on the variation at the imidazole-C2-thio position with aliphatic moieties. We prepared two series of analogues of ML3375, imidazole N-1-methylated analogues **2** and as non-N-methylated analogues **1** (Figure 3).

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[a] Abbreviations: ATP, adenosine triphosphate; IL-1β, interleukin-1β; MAP, mitogen-activated protein; NaHMDS, sodium hexamethylendisilazan; TNF-α, tumor necrosis factor-α.

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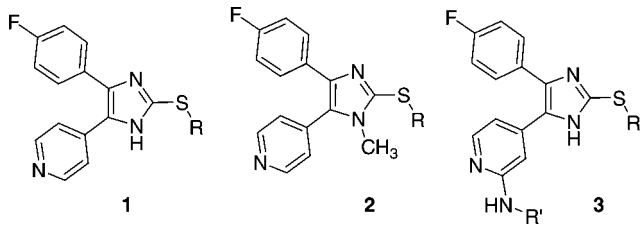


Figure 3. 2-Alkylsulfanyl-4-(4-fluorophenyl)-5-pyridinyl-1*H*-imidazoles **1**, 2-alkylsulfanyl-4-(4-fluorophenyl)-1-methyl-5-pyridinyl-1*H*-imidazoles **2**, and 2-alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-1*H*-imidazoles **3**.

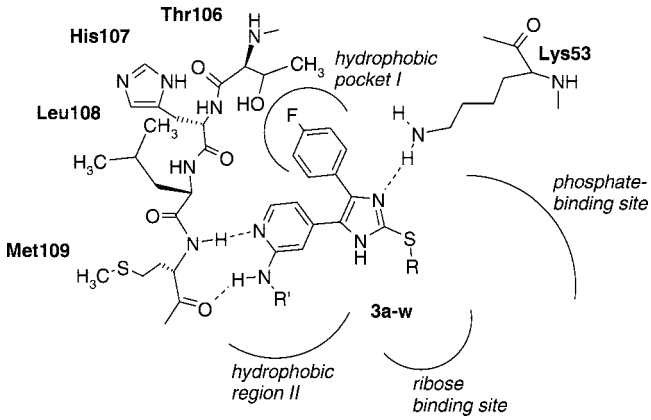


Figure 4. Putative inhibitors **3a**−**w** and the important interactions at the ATP binding site of p38 MAP kinase.

We especially chose polar aliphatic substituents, since substitution at the imidazole-C2-thio position may allow an interaction with the ribose binding site as well as with the phosphate binding site. Moreover, S-substitution with polar groups may be associated with an improvement in cell penetration and in physicochemical properties. The imidazole-C2-thio derivatives show less interactions with the metabolic enzyme cytochrome P450 compared to 2-arylimidazoles, like the prototypical inhibitor SB203580.[16] Furthermore, we also synthesized a broad range of 2-alkylsulfanyl-5-(2-aminopyridin-4-yl)-substituted imidazoles **3** (Figure 3). In contrast to substrates **1** and **2**, compound **3** may interact with the hydrophobic region II and may form an additional hydrogen bond to the carbonyl function of Met109 of the backbone (Figure 4). The residues (R) at the 2-thioether moiety can also interact with the ribose and/or the phosphate binding site of the enzyme. A combination of variations at the C2-thio position of the imidazole and the pyridine substituents led to compounds exhibiting extremely high potency in inhibition of p38 MAP kinase and TNF-α release. The four different synthetic approaches toward the target compounds **1**−**3** allowing the introduction of this broad range of substituents are presented. Data for inhibition of p38 MAP kinase and cytokine TNF-α release (from human whole blood) are provided.

Chemistry

The key intermediate for the synthesis of the 4-(4-(4-fluorophenyl)-2-(alkylthio)-1*H*-imidazol-5-yl)pyridines **1**, thione **10**, was synthesized according to the procedure of Lantos[17] as outlined in Scheme 1. The starting materials (4-fluorophenyl)-acetonitrile and methyl isonicotinate were converted to cyanoketone **4** in a condensation reaction. The cyano group was hydrolyzed and decarboxylated to yield ethanone **5**, which was treated with sodium acetate and hydroxylamine hydrochloride to afford the corresponding oxime **6**. After tosylation of the oxime **6**, tosylate **7** was reacted in a Neber rearrangement[18] to the aziridine **8**. The α-amino ketone **9** derived from the aziridine **8** as an intermediate by treatment with aqueous hydrochloride was reacted in situ with KSCN to yield the thione **10**. The conversion to the title compounds **1a**−**f** was finally accomplished by reaction of thione **10** with suitable alkyl bromides and *t*-BuOK (Scheme 1).

Preparation of the 2-alkylsulfanyl-4-(4-fluorophenyl)-1-methyl-5-pyridinyl-1*H*-imidazole **2** (Scheme 2), exemplified by 3-(4-(4-fluorophenyl)-1-methyl-5-(pyridin-4yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (**2a**), was accomplished via 2-(4-fluorophenyl)-1-(pyridin-4-yl)ethan-1,2-dion-2-oxime (**11**).[19] Reaction of the oxime **11** with 1,3,5-trimethyl-1,3,5-triazinane resulted in the formation of the *N*-oxide **12**, which was finally treated with 2,2,4,4-tetramethylcyclobutane-1,3-dithione to yield 4-(4-fluorophenyl)-1-methyl-5-(pyridin-4-yl)-1*H*-imidazole-2-thione (**13**). Subsequent reaction of thione **13** with NaOMe and 3-bromopropane-1,2-diol led to the title compound **2a**.

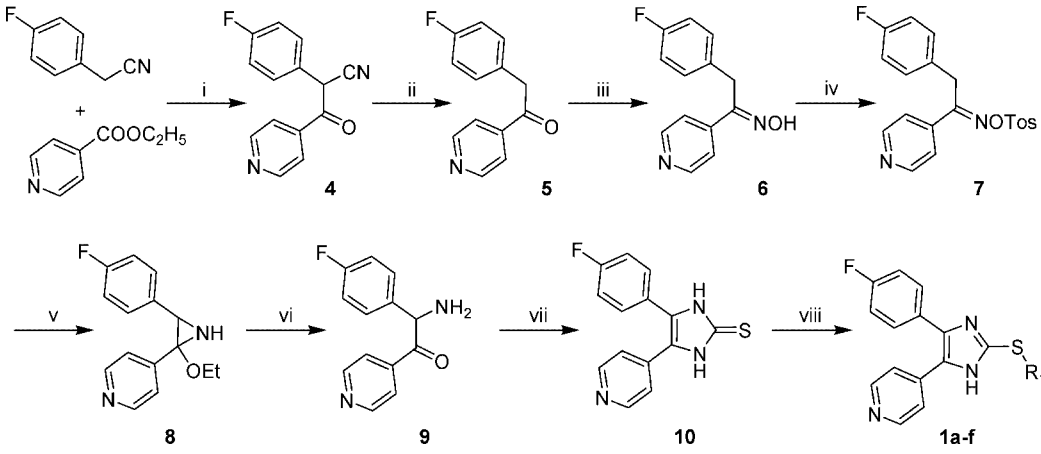
The analogues **3a**−**w** were prepared by two separate ways. The synthetic route to 2-alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazoles **3a**−**v** was published recently.[20] The key intermediates of this synthetic strategy toward compounds **3a**−**v** are *N*-boc-protected *N*-alkyl-4-methylpyridin-2-ylamines **15**, which were synthesized according to two different routes (Scheme 3). The pathway A starts from 2-amino-4-methylpyridine that was initially boc-protected. The following step, a nucleophilic substitution reaction employing primary and benzylic halides, yielded in picolines **15a**−**e**. Operating according to route B allowed the introduction of different moieties at the amino function via Buchwald−Hartwig reactions,[21] using 2-bromo-4-methylpyridine and secondary amines as the staring materials. The *N*-alkyl-4-methylpyridin-2-ylamines prepared via the aromatic C−N cross-coupling reaction were subsequently boc-protected to compounds **15f**−**i**. Routes A and B enabled the variations at the amino function of the title compounds **3a**−**v**. The 5-(2-aminopyridin-4-yl)-substituted imidazoles **3a**−**v** were prepared via a straightforward five-step synthesis (Schemes 4 and 5). Picolines **15a**−**i** were converted into the ethanones **16a**−**i** by use of ethyl 4-fluorobenzoate and NaHMDS. Upon treatment with an excess of sodium nitrite in acetic acid at room temperature, ethanones **16a**−**i** were converted into the α-hydroxyiminoketones **17a**−**i**. The oximes **17a**−**i** were reduced to the corresponding hydrochlorides **18a**−**i** accompanied by deprotection of the boc group using methanolic hydrogen chloride and Pd/C under hydrogen atmosphere at atmospheric pressure and room temperature. Cyclization was achieved by treatment with potassium thiocyanate (Scheme 4). Finally the thiones **19a**−**i** were converted into the title compounds **3a**−**v** by nucleophilic substitution reactions with appropriate alkyl halides and *t*-BuOK or NaOMe (Scheme 5).

Compound **3w** was synthesized starting from 4-(4-fluorophenyl)-5-(2-fluoropyridin-4-yl)-1,3-dihydroimidazole-2-thione[16] (**20**) (Scheme 6). Thione **20** and 2-bromoethanol were reacted in a substitution reaction to 2-(4-(4-fluorophenyl)-5-(2-fluoropyridin-4-yl)-1*H*-imidazol-2-ylthio)ethanol (**21**). In the final step the fluorine atom of **21** was displaced by a solvent-free reaction in a microwave reactor with an excess of the amine.

Biological Testing

The inhibitory potency of the title compounds was evaluated using an isolated p38α kinase assay wherein SB203580 is used

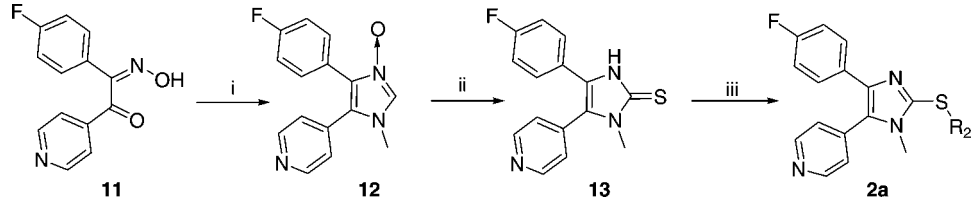
Scheme 1. Preparation of 2-Alkylsulfanyl-4-(4-fluorophenyl)-1-methyl-5-pyridinyl-1*H*-imidazoles **1a**−**f**[a]



a R^1 = 2,3-dihydroxypropyl **b** R^1 = 1,3-diethoxy-1,3-dioxopropan-2-yl **c** R^1 = 3-hydroxypropyl **d** R^1 = 2-hydroxyethyl **e** R^1 = 4-methoxy-4-oxobutyl **f** R^1 = 2-methoxy-2-oxoethyl

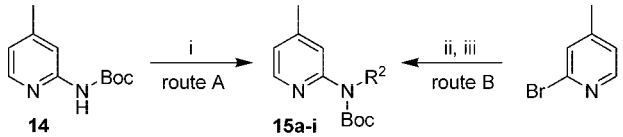
[a] Reagents and conditions: (i) NaOMe, MeOH, reflux temperature; (ii) HBr (48%), reflux temperature, then addition of ice and NH_3; (iii) NH_2OH, $MeOH/H_2O$, reflux temperature; (iv) TsCl, Py; (v) EtOK, EtOH; (vi) 10% HCl; (vii) KSCN, reflux temperature, (viii) R^1-Br, *t*-BuOK, MeOH.

Scheme 2. Preparation of 2-Alkylsulfanyl-4-(4-fluorophenyl)-5-pyridinyl-1*H*-imidazole **2a**[a]



[a] Reagents and conditions: (i) 1,3,5-trimethyl-1,3,5-triazinane, EtOH, reflux temperature, then 4 °C; (ii) 2,2,4,4-tetramethylcyclobutane-1,3-dithione, $CHCl_3$, 0 °C, then room temp; (iii) 3-bromopropane-1,2-diol, MeONa, MeOH, room temp.

Scheme 3. Preparation of *N*-boc-Protected *N*-Alkyl-4-methyl-pyridine-2-amines **15a**−**i** by Two Different Synthetic Routes[a]



a R^2 = phenylethyl **b** R^2 = benzyl **c** R^2 = isobutyl **d** R^2 = ethyl **e** R^2 = methyl **f** R^2 = 3-methylbut-2-yl **g** R^2 = *sec*-butyl **h** R^2 = isopropyl **i** R^2 = tetrahydropyran-4-yl

[a] Reagents and conditions: (i) R^2-Br, NaH, DMF, 0 °C; (ii) R^2-NH_2 or R^2-NH_2HCl, $Pd_2(dba)_3$, *t*-BuONa, BINAP, toluene, reflux temperature; (iii) Boc_2O, DMAP, CH_2Cl_2, room temp.

as a reference.[22] The TNF-α release was tested using a human whole blood assay (see Experimental Section).

Biological Results and Disscusion

The effects of the S-substituent on inhibition of p38 and TNF-α release are shown in Table 1. The compounds **1a**−**f** of the first series are structurally related to SB203580, the reference compound. Thus, the binding mode at the ATP binding site of p38 MAP kinase is the same except for the $\pi-\pi$-stacking with Tyr35 (Figure 1). All the compounds with alcohol groups showed comparable potency in both assays (**1a**, **1c**, **1d**, Table 1), but whereas the esters **1e** and **1f** (Table 1) showed similar IC_{50} values in the kinase assay, they showed clearly diminished potency in the human whole blood assay compared to the alcohol derivatives. This result may be attributed to a reduced solubility of the ester derivatives in the TNF-α release assay. The 2-hydroxyethyl residue **1d** showed better inhibition in the isolated p38 MAP kinase assay than the 2,3-dihydroxypropyl residue **1a**, while in the whole blood test, this result is inverted. By far, the least active compound in both assays was the malonate **1b** (Table 1).

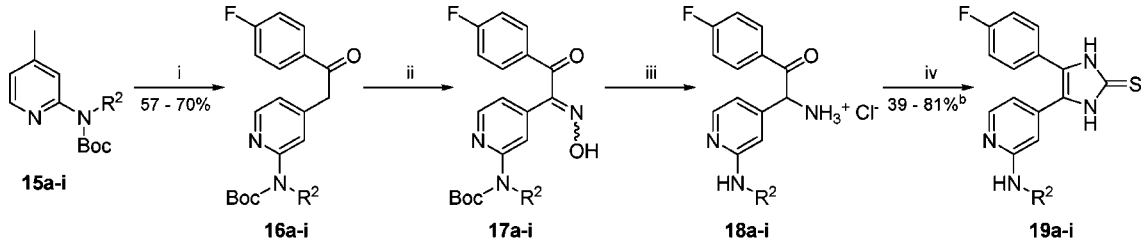
The influence on imidazole N-1 methylation is shown in Table 2. Because of the very distinctive decrease of compound **2a** in activity (compared to the nonmethylated compound **1a**) in the p38 MAP kinase assay, we focused in the following on compounds **3a**−**w**.

In contrast to SB203580, ML3375, ML3163, **1a**-**f**, and **2a**, the analogues of the third series **3a**−**w** have additional possibilities of interaction with the ATP binding site. The introduction of an amino function at the 2-position of the pyridine ring resulted in a second hydrogen bond to Met109 of the backbone.

Table 3 summarizes the amino substitution SAR at the outset of our effort in this series. The benzylic compounds **3a** and **3b** showed moderate inhibition of p38 MAP kinase and TNF-α release. It was evident that an α-methyl substituent at the 2-amino moiety benefits p38 inhibition as well as TNF-α release in the human whole blood test. Introduction of aliphatic (**3c**−**h**) or cycloaliphatic (**3i**) amino substituents at the 2-position of the pyridine moiety resulted in an increase in inhibition of p38 MAP kinase and TNF-α release relative to **3a** and **3b**, except **3e**.

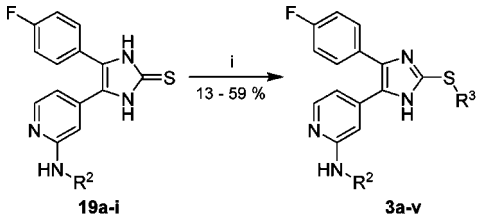
The aliphatic analogues **3c**−**h** were optimized toward their p38 MAP kinase inhibitory activity starting from **3f** by the successive removal of a methyl group (Figure 5). The order of the inhibitory activity of the amino substituents improved from the IC_{50} value of 15 nM for the 3-methylbut-2-yl derivative **3f**

Scheme 4. Preparation of the 2-(*N*-Alkylamino)pyridinyl-Substituted 1,3-Dihydroimidazole-2-thiones Scaffolds **19a**−**i**[a]



[a] Reagents and conditions: (i) NaHMDS, ethyl 4-fluorobenzoate, THF, 0 °C to room temp; (ii) $NaNO_2$, acetic acid, 10 °C to room temp; (iii) Pd/C 10%, MeOH/ HCl, H_2, atmospheric pressure, room temp; (iv) KSCN, DMF, reflux temperature, 3h. [b] Yield over three steps.

Scheme 5. Preparation of Compounds **3a**−**v** via Nucleophilic Substitution of Imidazole-2-thiones **19a**−**i**[a]



a R^2 = 1-phenylethyl
b R^2 = benzyl
c R^2 = isobutyl
d R^2 = ethyl
e R^2 = methyl
f R^2 = 3-methylbut-2-yl
g R^2 = *sec*-butyl
h R^2 = isopropyl
i R^2 = tetrahydropyran-4-yl

a R^2 = 1-phenylethyl, R^3 = 2,3-dihydroxypropyl
b R^2 = benzyl, R^3 = 2,3-dihydroxypropyl
c R^2 = isobutyl, R^3 = 2,3-dihydroxypropyl
d R^2 = ethyl, R^3 = 2,3-dihydroxypropyl
e R^2 = methyl, R^3 = 2,3-dihydroxypropyl
f R^2 = 3-methylbut-2-yl, R^3 = 2,3-dihydroxypropyl
g R^2 = *sec*-butyl, R^3 = 2,3-dihydroxypropyl
h R^2 = isopropyl, R^3 = 2,3-dihydroproxypyl
i R^2 = tetrahydropyran-4-yl, R^3 = 2,3-dihydroxypropyl
j R^2 = 3-methylbut-2-yl, R^3 = 3-hydroxypropyl
k R^2 = 3-methylbut-2-yl, R^3 = 2-hydroxyethyl
l R^2 = 3-methylbut-2-yl, R^3 = (*R*)-2,3-dihydroxypropyl
m R^2 = 3-methylbut-2-yl, R^3 = (*S*)-2,3-dihydroxypropyl
n R^2 = isopropyl, R^3 = (*R*)-2,3-dihydroxypropyl
o R^2 = isopropyl, R^3 = (*S*)-2,3-dihydroxypropyl
p R^2 = 1-phenylethyl, R^3 = methylacet-2-yl
q R^2 = 1-phenylethyl, R^3 = carboxymethyl
r R^2 = 1-phenylethyl, R^3 = (diethoxyphosphoryl)methyl
s R^2 = 3-methylbut-2-yl, R^3 = methylacet-2-yl
t R^2 = isopropyl, R^3 = methylacet-2-yl
u R^2 = isopropyl, R^3 = 2-hydroxyethyl
v R^2 = tetrahydropyran-4-yl, R^3 = 2-hydroxyethyl

[a] Reagents: (i) R^3-X (X = Cl, Br, I), *t*-BuOK or EtONa, MeOH.

to an IC_{50} value of 5 nM for the *sec*-butyl **3g**, which was then exceeded by the isopropyl derivative **3h** (IC_{50}= 3 nM). An additional methyl-branching in the β-position obviously decreased the inhibitory activity (see values for **3f**, **3g**, and **3h**; Table 3). The IC_{50} value decreased slightly for the ethyl derivative **3d** and dramatically for the methyl derivative **3e**. The methyl group was apparently too small for interaction with the hydrophobic region II. Interestingly, the tetrahydropyran-4-yl residue **3i** was equipotent with respect to **3h** in the enzyme inhibition assay as well as in the TNF-α release assay in whole blood (Table 3).

Differences in activity between the racemic 2,3-dihydroxypropyl moiety and the corresponding compounds with *S*- or *R*-configuration were negligible for the enzyme assay and for the human whole blood test (compare compounds **3f**,**l**,**m** and compounds **3h**,**n**,**o**; Tables 3 and 4). The products with *R*-configuration tended to result in a little higher activity.

Table 5 shows the influence of the imidazole-C2-S substituents in the presence of the 3-methylbut-2-yl substituent at the 2-aminopyridine moiety. The analogues of Table 5 showed similar potency in inhibition of the p38 MAP kinase. In the TNF-α release assay, however, the alcohol residues were dramatically superior to the ester residue. The 2-hydroxyethyl seems to be the best of the tested hydroxy residues in both inhibition the p38 MAP kinase and TNF-α release (as low as 37 nM).

Table 6 shows compounds that retained the alkylsulfanyl moiety of the compounds (2-hydroxyethyl) and then varied the amino functionality.

Variation of the best amino residues (R^2) and the best imidazole-C2-S residue (2-hydroxyethyl, R^3) led to compounds with IC_{50}values in the low single-digit nanomolar range in the p38 assay (down to 2 nM) and in the double-digit nanomolar range in TNF-α release in human whole blood (down to 37 nM). Compared to the S-methylated compound **22**[23] (Table 6) our compounds **3i** and **3v** with the same 2-amino substitution exhibited higher potency in inhibition, especially in the human whole blood test.

In Table 7, the effects of esters and carboxylic acid as imidazole-C2-S substituents are compared. All the esters and the free carboxylic acid showed a dramatic collapse of activity in TNF-α release in comparison with all the diols as well as with the compound bearing a single OH group. These results are in accordance with the observations already made with compounds **1a**, **1c**, **1d**, **1e**, and **1f**. Interestingly, the phosphonate **3r** showed a decrease in p38 inhibition relative to **3p** and **3q** but a more potent inhibition of TNF-α release in human whole blood relative to **3p** and **3q**.

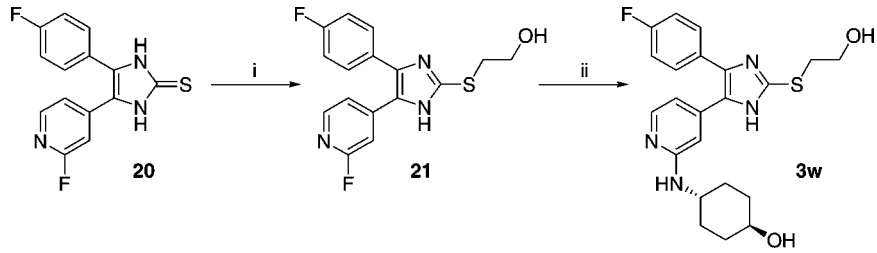
In Figure 6 a suggested binding mode for compound **3h** is presented. Of note is a possible interaction of the 2,3-dihydroxypropyl residue with the p38 conserved residue Asp168-Phe169-Gly170 (DFG), in particular hydrogen bonding between a hydroxy group and Asp168.

Conclusion

We have presented syntheses and biological data of several 2-thio-substituted 4-(4-fluorophenyl)-5-pyridinyl-1*H*-imidazoles, allowing us to prepare the very potent p38 MAP kinase inhibitors **3h**, **3i, 3u**, **3v**, and **3w**. Substituents at the 2-thio position contribute to bioactivity only to a small extent. For example, the IC_{50} values vary only a small range in the enzyme assay for compounds **3f**, **3j**-**m**, and **3s** and compounds **3h**, **3n**, **3o**, **3t**, and **3u** compared to the change of IC_{50} values achieved by variation of the pyridine-C2-amino moiety.

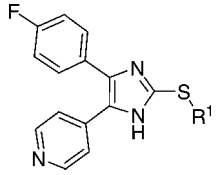
Comparison of compounds **1a** (Table 1), **2a** (Table 2), and **3h** and **3i** (Table 3), all bearing the 2,3-dihydroxypropyl moiety at the 2-position of the imidazole core, showed a clear decline in inhibitory activity of p38 MAP kinase arising by introduction

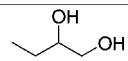
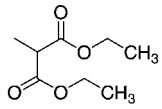
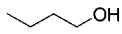
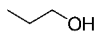
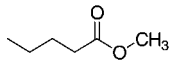
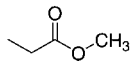
Scheme 6. Preparation of Compound **3w**: Nucleophilic Substitution of the Sulfur Atom of Imidazole-2-thione **20** Followed by Nucleophilic Aromatic Displacement of the Fluorine Atom at the Pyridine Moiety via a Solvent-Free Microwave Reaction[a]



[a] Reagents and conditions: (i) 2-bromoethanol, EtONa, MeOH, room temp; (ii) *trans*-4-aminocyclohexanol, microwave, 130 °C.

Table 1. Biological Data of 2-Alkylsulfanyl-4-(4-fluorophenyl)-5-pyridin-4-yl-1*H*-imidazoles **1a**−**f** (Variation of the Alkylsulfanyl Moiety R^1)



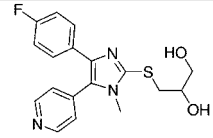
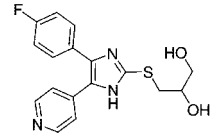
compd	R^1	IC_{50}±SEM [μM] p38α[a]	IC_{50}±SEM [μM] TNF-α[a]
1a		0.392±0.052	3.38±0.65
1b		2.24±0.268	25.38±3.79
1c		0.215±0.056	6.69±3.99
1d		0.184±0.050	4.21±0.87
1e		0.487±0.101	31.92±7.04
1f		0.158±0.029	31.93±2.07

[a] Results are from three experiments. [b] Tests were carried out in duplicate.

of a methyl group at the imidazole N1-position and an enormous increase in activity by introduction of optimal 2-amino substituents.

Substituents located at the imidazole-C2 position exhibited a major impact toward inhibition of TNF-α release (compare, for example, compounds **3a** and **3p**, **3s** and **3f**, and **3h** and **3t**). Within both series **1** and **3**, the 2-hydroxyethyl substituent at the 2-thio position showed the highest activity in the kinase assay, followed by the 3-hydroxypropyl residue. The 2,3-dihydroxypropanol moiety exhibited a little less activity in the kinase assay, and the same result was observed in the whole blood test. Substitution of polar aliphatic moieties at the imidazole-C2-thio position was well tolerated. These polar aliphatic substituents were superior to the S-methylated analogue **22** in inhibition of p38 MAP kinase and especially in TNF-α release. Moreover, these imidazole derivatives may interact with the ribose as well as with the phosphate binding site because of the moieties at the 2-thio position. These observations suggest that for the imidazole-2-thioethers **1** and **3**, the additional interaction with the ATP binding cleft has a greater influence than the ability of the inhibitor molecule to enter more deeply into the binding cleft. On the other hand, considering the significant improvement in inhibition in TNF-α release, it seems very likely that the 2-thio substituents have a major influence on physicochemical properties (like cell penetration, solvation, and lower lipophilicity). The most promising candidates are presently under investigation in animal models of inflammation.

Table 2. Biological Data of 3-(4-(4-Fluorophenyl)-1-methyl-5-(pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol **2a** (Influence on Imidazole N-1 Methylation)

compd	IC_{50}±SEM [μM] p38α[a]
2a 	1.22
1a 	0.392±0.052 (n=3)

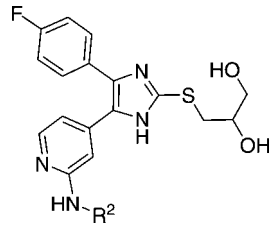
[a] Results are from one experiment except where otherwise stated.

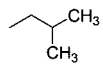
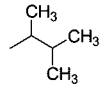
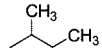
Experimental Section

General. All commercially available reagents and solvents were used without further purification. The microwave reaction was performed on a CEM Discover system. Flash chromatography was performed using a LaFlash system (VWR) with Merck silica gel (PharmPrep 60 CC 25−40 μm). NMR data were recorded on a Bruker Spectrospin AC 200 at ambient temperature. Chemical shifts are reported in ppm from the solvent resonance. IR data were determined on a Perkin-Elmer Spectrum One spectrometer (ATR Technique). High-resolution spectra (FT-ICR) were obtained on a Bruker APEX II with electron spray ionization. Elemental analysis results for carbon, hydrogen, and nitrogen were obtained from a Carlo Erba Strumentazione model 1106 instrument and were within ±0.4%. The purity of the final compounds was determined by HPLC on a Hewlett-Packard HP 1090 series II liquid chromatograph using a Betasil C8 column (150 mm × 4.6 mm i.d., dp = 5 μm, Thermo Fisher Scientific, Waltham, MA) at 230 and 254 nm employing a gradient of 0.01 M KH_2PO_4 (pH 2.3) and methanol as the solvent system with a flow rate of 1.5 mL/min. All final compounds were >97% pure (see Supporting Information for details).

General Procedure for the Synthesis of the Title Compounds 1a−f (General Procedure A). To a solution of 4-(4-fluorophenyl)-5-(pyridin-4-yl)-1,3-dihydroimidazole-2-thione[17] (**10**) (200 mg, 0.73 mmol) and *t*-BuOK (86 mg, 0.77 mmol) in dry MeOH (15 mL) was added under argon atmosphere after 15 min the appropriate

Table 3. Biological Activity of 2-(2,3-Dihydroxypropyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-1*H*-imidazoles **3a**−**i** (Variation of the Amino Function R^2)



compd	R^2	IC_{50}±SEM [μM] p38α[a]	IC_{50}±SEM [μM] TNF-α[b]
3a		0.041±0.007	0.936±0.15
3b		0.087±0.034	2.67±0.54
3c		0.013±0.002	0.168±0.023
3d		0.027±0.005	0.777±0.003
3e		0.102±0.036	5.724±0.081
3f		0.015±0.002 (n=8)	0.183±0.016 (n=3)
3g		0.005±6.3e^{-4}	0.156±0.008 (n=8)
3h		0.003±3.7e^{-4} (n=6)	0.126±0.005 (n=3)
3i		0.003±2.8e^{-4}	0.131±0.007 (n=5)

[a] Results are from three experiments except where otherwise stated. [b] Tests were carried out in duplicate except where otherwise stated.

alkyl halide (0.77 mmol). The solution was heated to reflux temperature (**1a**, **1c**−**f**) or stirred at room temperature overnight (**1b**). After extraction with water and EtOAc, the organic phase was washed twice with water, dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash chromatography.

3-(4-(4-Fluorophenyl)-5-(pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (1a). Compound **1a** was prepared according to general procedure A using 3-bromopropane-1,2-diol (119 mg) as alkyl halide. The reaction mixture was heated to reflux temperature for 2.5 h and purified by flash chromatography (SiO_2, from MeOH/DCM 5:95 to MeOH/DCM 15:85). Yield: 82 mg (32%). ^{1}H NMR (CD_3OD) δ 3.17−3.41 (m, 2H, CH_2, solvent peak), 3.64 (d, J = 5.4 Hz, 2H, CH_2), 3.86−3.94 (m, 1H, CH), 7.14−7.23 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 4H, C^3-/C^5-H Pyr, C^2-/C^6-H 4-F-Phe), 8.42 (d, J = 6.2 Hz, 2H, C^2-/C^6-H Pyr). HRMS-ESI, m/z ($C_{17}H_{16}FN_3O_2S$): calcd, 346.1020 $[M + H]^+$; found, 346.1022.

Diethyl 2-4(4-(4-Fluorophenyl)-5-(pyridin-4-yl)-1*H*-imidazol-2-ylthio)malonate (1b). Compound **1b** was prepared according to general procedure A using diethyl 2-bromomalonate (184 mg) as alkyl halide. The reaction mixture was stirred at room temperature overnight and purified by flash chromatography (SiO_2, from SiO_2, from EtOAc/DCM 7:3 to EtOAc/DCM 8:2). Yield: 39 mg (10%). ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 7.0 Hz, 6H, 2 × CH_3), 4.18 (q, J = 7.1 Hz, 4H, 2 × CH_2), 5.28 (s, 1H, CH), 7.22−7.52 (m, 6H, 4 × 4-F-Phe, C^3-/C^5-H Pyr), 8.46−8.58 (m, 2H, C^2-/C^6-H Pyr), 13.10 (s, 1H, NH). HRMS-ESI, m/z ($C_{21}H_{20}FN_3O_4S$): calcd, 430.1231 $[M + H]^+$; found, 430.1232.

3-(4-(4-Fluorophenyl)-5-(pyridin-4-yl)-1*H*-imidazol-2-ylthio)propan-1-ol (1c). Compound **1c** was prepared according to general procedure A using 3-bromopropan-1-ol (107 mg) as alkyl halide. The reaction mixture was heated to reflux temperature for 2 h and purified by flash chromatography (SiO_2, from MeOH/DCM 3:97 to MeOH/DCM 1:9). Yield: 53 mg (22%). ^{1}H NMR (CD_3OD) δ 1.84−1.97 (m, 2H, CH_2), 3.21 (t, J = 7.1 Hz, 2H, CH_2), 3.71 (t, J = 6.1 Hz, 2H, CH_2), 7.14−7.22 (m, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 4H, C^3-/C^5-H Pyr, C^2-/C^6-H 4-F-Phe), 8.40−8.46 (m, 2H, C^2-/C^6-H Pyr). Anal. ($C_{17}H_{16}FN_3OS$) C, H, N.

2-(4-(4-Fluorophenyl)-5-(pyridin-4-yl)-1*H*-imidazol-2-ylthio)-ethanol (1d). Compound **1d** was prepared according to general procedure A using 2-bromoethanol (96 mg) as alkyl halide. The reaction mixture was heated to reflux temperature for 2 h and purified by flash chromatography (SiO_2, from MeOH/DCM 5:95 to MeOH/DCM 1:9). Yield: 94 mg (40%). ^{1}H NMR (CD_3OD) δ 3.23 (t, J = 6.3 Hz, 2H, CH_2), 3.83 (t, J = 6.2 Hz, 2H, CH_2), 7.14−7.23 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 4H, C^3-/C^5-H Pyr, C^2-/C^6-H 4-F-Phe), 8.41 (d, J = 6.1 Hz, 2H, C^2-/C^6-H Pyr). HRMS-ESI, m/z ($C_{16}H_{14}FN_3OS$): calcd, 316.0914 $[M + H]^+$; found, 316.0915.

Methyl 4-(4-(4-Fluorophenyl)-5-(pyridin-4-yl)-1*H*-imidazol-2-ylthio)butanoate (1e). Compound **1e** was prepared according to general procedure A using methyl 4-bromobutyrate (139 mg) as alkyl halide. The reaction mixture was heated to reflux temperature for 1 h and purified by flash chromatography (SiO_2, from DCM/EtOAc 1:1 to DCM/EtOAc 2:3). Yield: 118 mg (43%). ^{1}H NMR (DMSO-d_6) δ 1.88−1.95 (m, 2H, CH_2), 2.44−2.50 (m, CH_2, solvent peak), 3.14 (t, J = 7.0 Hz, 2H, CH_2), 3.57 (s, 3H, CH_3), 7.27−7.38 (m, 4H, C^3-/C^5-H 4-F-Phe, C^3-/C^5-H Pyr), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 8.43−8.46 (m, 2H, C^2-/C^6-H Pyr), 12.79 (bs, 1H, NH). ^{1}H NMR (CD_3OD) δ 1.90−2.04 (m, 2H, CH_2), 2.52 (t, J = 7.1 Hz, 2H, CH_2), 3.14 (t, J = 7.1 Hz, 2H, CH_2), 3.64 (s, 3H, CH_3), 7.14−7.22 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.61 (m, 4H, C^2-/C^6-H 4-F-Phe, C^3-/C^5-H Pyr), 8.40−8.43 (m, 2H, C^2-/C^6-H Pyr). Anal. ($C_{19}H_{18}FN_3O_2S$) C, H, N.

The crystal structure of **1e** has been proven by X-ray analysis: Enraf-Nonius CAD-4, Cu Kα, SIR92, SHELXL97. Further details of the crystal structure analysis are available in ref 24.

Methyl 2-(4-(4-Fluorophenyl)-5-(pyridin-4-yl)-1*H*-imidazole-2-ylthio)acetate (1f). Compound **1f** was prepared according to general procedure A using methyl bromoacetate (118 mg) as alkyl halide. The reaction mixture was heated to reflux temperature for 2 h and purified by flash chromatography (SiO_2, from EtOAc/DCM 1:1 to EtOAc/DCM 2:1). Yield: 134 mg (53%). ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 3H, CH_3), 4.07 (s, 2H, CH_2), 7.12−7.36 (m, 4H, C^3-/C^5-H 4-F-Phe, C^3-/C^5-H Pyr), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 8.43−8.51 (m, 2H, C^2-/C^6-H Pyr), 12.87 (s, 1H, NH). HRMS-ESI, m/z ($C_{17}H_{14}FN_3O_2S$): calcd, 344.0864 $[M + H]^+$; found, 344.0864.

3-(4-(4-Fluorophenyl)-1-methyl-5-(pyridin-4yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (2a). Thione **13**[19] (1.10 g, 3.86 mmol) was suspended in dry MeOH (20 mL), and NaOMe (648 mg, 12.0 mmol) was added. The mixture was stirred at room temperature until a clear solution was obtained before 3-bromopropane-1,2-diol (794 mg, 5.12 mmol) was added dropwise. The mixture was stirred at room temperature for 2 h, neutralized (HCl 10%), and concentrated to dryness. The residue was purified by column chromatography (SiO_2, DCM/EtOH 95:5) to yield 600 mg (43%) of **2a**. ^{1}H NMR ($CDCl_3$) δ 3.42 (d, J = 5.4 Hz, 2H, CH_2), 3.59 (s, 3H, *N*-CH_3), 3.69−3.85 (m, 2H, CH_2), 3.85−4.10 (m, 1H, CH), 6.93 (t, J = 8.8 Hz, 2H, C^3-/C^5-H 4-F-Phe), 7.22 (d, J = 6.0 Hz, 2H, C^3-/C^5-H Pyr), 7.26−7.33 (m, 2H, C^2-/C^6-H 4-F-Phe), 8.70 (d, J = 6.0 Hz, 2H, C^2-/C^6-H Pyr). HRMS-ESI, m/z ($C_{18}H_{18}FN_3O_2S$): calcd, 359.1177 $[M + H]^+$; found, 359.1176.

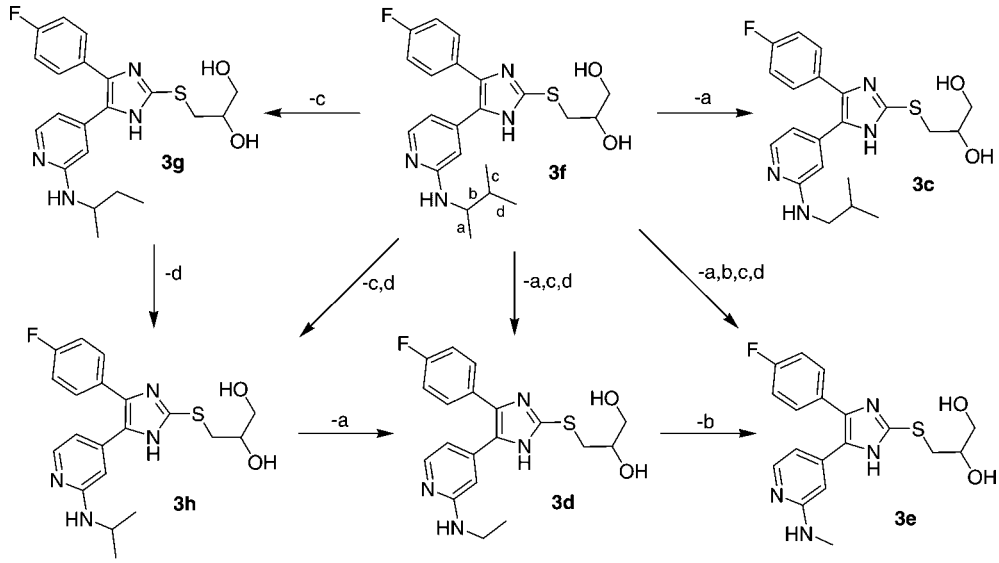
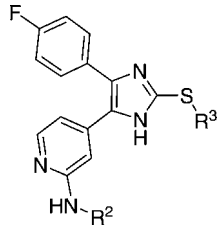
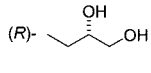
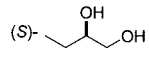
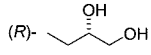
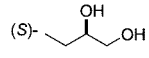


Figure 5. Compound **3f** as a starting point of optimization in the aliphatic series.

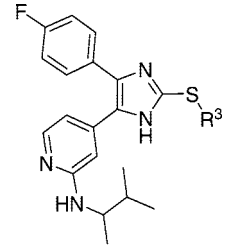
Table 4. Biological Activity of the 2-(2,3-Dihydroxypropyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-1*H*-imidazole Analogues (Influence of Enantiomers at Imidazole-C2-S Substituents)

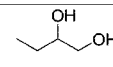
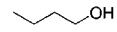
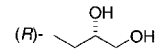
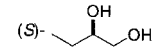
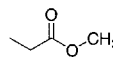


compd	R^2	R^3	IC_{50}±SEM [μM] p38α[a]	IC_{50}±SEM [μM] TNF-α[b]
3l			0.018±0.003	0.167±0.023
3m			0.010±0.004	0.139±0.009
3n			0.006±3.8e^{-4}	0.180±0.061 (n=3)
3o			0.003±1.0e^{-4}	0.123±0.019 (n=4)

[a] Results are from three experiments. [b] Tests were carried out in duplicate except where otherwise stated.

Table 5. Biological Data of 2-Alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-1*H*-imidazoles (Effect on S-Substitution R^3)



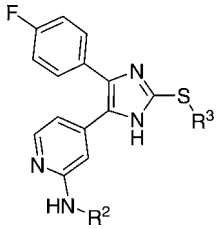
compd	R^3	IC_{50}±SEM [μM] p38α[a]	IC_{50}±SEM [μM] TNF-α[b]
3f		0.015±0.002 (n=8)	0.183±0.016
3j		0.012±0.002	0.145±0.001
3k		0.011±8.8e^{-4}	0.037±0.004
3l		0.018±0.003	0.167±0.023
3m		0.010±0.004	0.139±0.009
3s		0.019±0.008	8.94±1.89

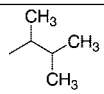
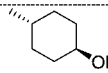
[a] Results are from three experiments except where otherwise stated. [b] Tests were carried out in duplicate.

General Procedure for the Synthesis of the Title Compounds 3b−p and 3r−v (General Procedure H). To a solution of 5-(2-(alkyl/phenylalkylamino)pyridin-4-yl)-4-(4-fluorphenyl)-1,3-dihydroimidazol-2-thiones **19a−i** (1.0 equiv) and *t*-BuOK (1.1 or 1.2 equiv) in dry MeOH was added under argon atmosphere the appropriate alkyl halide (1.1 or 1.2 equiv). The solution was heated to reflux temperature until complete disappearance of the starting material (thiones) and cooled to room temperature. After extraction with water and EtOAc the organic phase was washed twice with water, dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash chromatography or by crystallization.

3-(4-(4-Fluorophenyl)-5-(2-(1-phenylethylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3a). To a solution of **19a** (130 mg, 0.33 mmol) and NaOEt (27 mg, 0.40 mmol) in dry MeOH (15 mL) was added under argon atmosphere 3-bromopropane-1,2-diol (62 mg, 0.40 mol). The solution was stirred for 5 h at room temperature and 3 h at 50 °C. Afterward the reaction mixture was cooled to room temperature and the crude product was purified by flash chromatography (SiO_2, from DCM/EtOH 9:1 to DCM/EtOH 1:1) to yield 73 mg (48%) of compound **3a**. ^{1}H NMR (CD_3OD) δ 1.45 (d, J = 6.9 Hz, 6H, 2 × CH_3), 3.12−3.33 (m, CH_2, solvent peak), 3.63 (d, J = 5.5 Hz, 2H, CH_2), 3.82−3.94 (m, 1H, CH), 4.65−4.76 (m, 1H, CH), 6.48 (s, 1H, C^3-H Pyr), 6.57 (dd, J_1 = 5.5 Hz, J_2 = 1.5 Hz, 1H, C^5-H Pyr), 7.06−7.26 (m, 7H, C^3-/C^5-H 4-F-Ph, 5 Phe), 7.36−7.44 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (dd, J_1 = 5.5 Hz, J_2 = 0.6 Hz 1H, C^6-H Pyr). Anal. ($C_{25}H_{25}FN_4O_2S$) C, H, N.

Table 6. Biological Activity of 2-(2-Hydroxyethyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-1*H*-imidazole Derivatives



compd	R^2	R^3	IC_{50}±SEM [μM] p38α[a]	IC_{50}±SEM [μM] TNF-α[b]
3k			0.011±8.8e^{-4}	0.037±0.004
3u			0.003±2.6e^{-4}	0.129±0.013
3v			0.002±3.2e^{-4}	0.080±0.017
22[23]			0.005±2.7e^{-4}	0.201±0.004
3w			0.002±4.6e^{-4}	0.157±0.031

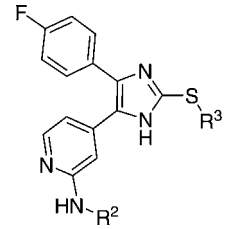
[a] Results are from three experiments. [b] Tests were carried out in duplicate.

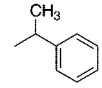
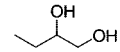
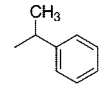
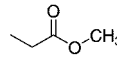
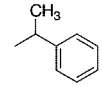
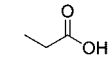
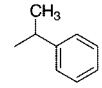
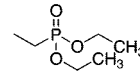
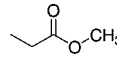
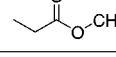
3-(5-(2-(1-Benzylamino)pyridin-4-yl)-4-(4-fluorophenyl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3b). Compound **3b** was prepared according to general procedure H from **19b** (150 mg, 0.40 mmol), *t*-BuOK (54 mg, 0.48 mmol), 3-bromopropane-1,2-diol (74 mg, 0.44 mol), and MeOH (8 mL). The reaction mixture was heated to reflux temperature for 4.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 49 mg (27%). ^{1}H NMR (CD_3OD) δ 3.12−3.36 (m, CH_2, solvent peak), 3.63 (d, J = 5.4 Hz, 2H, CH_2), 3.83−3.94 (m, 1H, CH), 4.39 (s, 2H, CH_2), 6.58−6.61 (m, 2H, C^3-/C^5-H Pyr), 7.05−7.14 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.17−7.27 (m, 5H, Phe), 7.37−7.46 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.83 (d, J = 5.9 Hz, C^6-H Pyr). HRMS-ESI, *m*/*z* ($C_{24}H_{23}FN_4O_2S$): calcd, 451.1596 $[M + H]^+$; found, 451.1599.

3-(4-(4-Fluorophenyl)-5-(2-(isobutylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3c). Compound **3c** was prepared according to general procedure H from **19c** (150 mg, 0.44 mmol), *t*-BuOK (59 mg, 0.53 mmol), 3-bromopropane-1,2-diol (82 mg, 0.53 mol), and MeOH (8 mL). The reaction mixture was heated to reflux temperature for 2.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 7:3). Yield: 53 mg (29%). ^{1}H NMR (CD_3OD) δ 0.92 (d, J = 6.6 Hz, 6H, 2 × CH_3), 1.81 (sept, J = 6.7 Hz, 1H, CH), 2.97 (d, J = 7.0 Hz, 2H, CH_2), 3.13−3.38 (m, CH_2, solvent peak), 3.64 (d, J = 5.4 Hz, 2H, CH_2), 3.84−3.95 (m, 1H, CH), 6.54−6.57 (m, 2H, C^3-/C^5-H Pyr), 7.10−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.42−7.52 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (d, J = 6.0 Hz, 1H, C^6-H Pyr). HRMS-ESI, *m*/*z* ($C_{21}H_{25}FN_4O_2S$): calcd, 417.1755 $[M + H]^+$; found, 417.1756.

3-(5-(2-(Ethylamino)pyridin-4-yl)-4-(4-fluorophenyl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3d). Compound **3d** was prepared according to general procedure H from **19d** (200 mg, 0.64 mmol), *t*-BuOK (86 mg, 0.76 mmol), 3-bromopropane-1,2-diol (118 mg, 0.76 mol), and MeOH (10 mL). The reaction mixture was heated to reflux temperature for 3 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 41 mg (17%). ^{1}H NMR (CD_3OD) δ 1.18 (t, J = 7.2 Hz, 3H, CH_3), 3.13−3.38 (m, 2 × CH_2, solvent peak), 3.62−3.65 (m, 2H, CH_2), 3.83−3.94 (m, 1H, CH), 6.54−6.57 (m, 2H, C^3-/C^5-H Pyr), 7.11−7.20 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.81 (d, J = 5.2 Hz, 1H, C^6-H Pyr). ^{1}H NMR (DMSO-d_6) δ 1.08 (t, J = 7.1 Hz, 3H, CH_3), 3.09−3.33 (m, 4H, 2 × CH_2), 3.39−3.42 (m, 2H, CH_2), 3.69−3.78 (m, 1H, CH), 4.87 (bs, 1H, OH, exchangeable), 5.31 (bs, 1H, OH exchangeable), 6.41−6.57 (m, 3H, C^3-/C^5-H Pyr, NH exchangeable), 7.14−7.32 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.79−7.91 (m, 1H, C^6-H Pyr), 12.68 (bs, 1H, NH exchangeable). HRMS-ESI, *m*/*z* ($C_{19}H_{21}FN_4O_2S$): calcd, 398.1442 $[M + H]^+$; found, 398.1440.

Table 7. Biological Activity of 2-Alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-1*H*-imidazoles (Ester and Carboxylic Acid)



compd	R^2	R^3	IC_{50}±SEM [μM] p38α[a]	IC_{50}±SEM [μM] TNF-α[b]
3a			0.041±0.007	0.936±0.15
3p			0.036±0.008	45.48±2.46
3q			0.037±0.007	65.70±10.72
3r			0.400±0.047	19.40±4.48
3s			0.019±0.008	8.94±1.89
3t			0.004±0002	12.77±0.72

[a] Results are from three experiments. [b] Tests were carried out in duplicate.

3-(4-(4-Fluorophenyl)-5-(2-(methylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3e). Compound **3e** was prepared according to general procedure H from **19e** (150 mg, 0.5 mmol), *t*-BuOK (67 mg, 0.6 mmol), 3-bromopropane-1,2-diol (92 mg, 0.6 mmol), and MeOH (8 mL). The reaction mixture was heated to reflux temperature for 3 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 47 mg (25%). ^{1}H NMR (CD_3OD) δ 2.80 (s, 3H, CH_3), 3.14−3.38 (m, CH_2, solvent peak), 3.64 (d, J = 5.4 Hz, CH_2), 3.84−3.95 (m, 1H, CH), 6.54−6.56 (m, 2H, C^3-/C^5-H Pyr), 7.09−7.18 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.42−7.49 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, J = 5.4 Hz, 1H, C^6-H Pyr). HRMS-ESI, *m*/*z* ($C_{18}H_{19}FN_4O_2S$): calcd, 375.1286 $[M + H]^+$; found, 375.1284.

3-(4-(4-Fluorophenyl)-5-(2-(3-methylbutan-2-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3f). Compound **3f** was prepared according to general procedure H from **19f** (100 mg, 0.28 mmol), *t*-BuOK (35 mg, 0.31 mmol), 3-bromopropane-1,2-diol (48 mg, 0.31 mol), and MeOH (6.5 mL). The reaction mixture was heated to reflux temperature for 3 h and purified by flash chromatography (SiO_2, from DCM/MeOH 95:5 to DCM/EtOH 8:2). Yield: 50 mg (41%). ^{1}H NMR (CD_3OD) δ 0.83−0.93 (m, 6H, 2

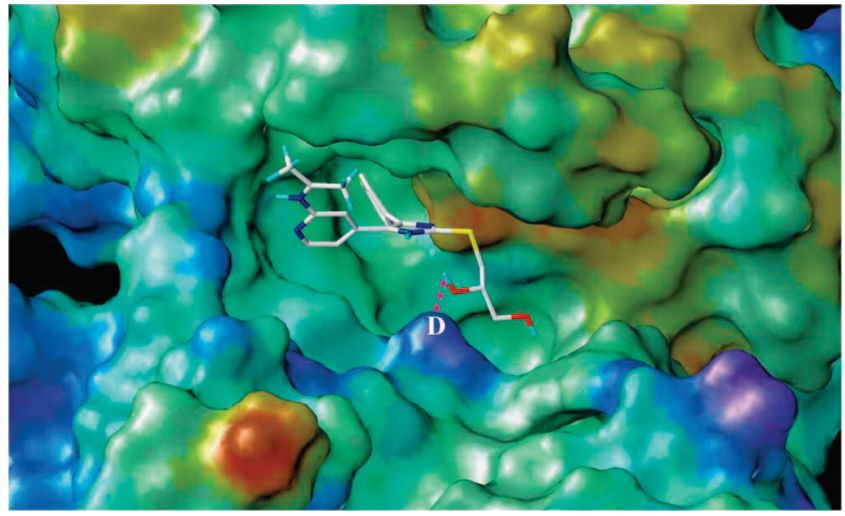


Figure 6. A suggested binding mode for compound **3h**. After geometric optimization in the MMFF94 force field, the molecule was docked into the p38 active center by using the docking program FlexX. As protein model, the X-ray structure 1bl7.pdb[10] was used. The Connolly surface, e.g., the water accessible surface of the protein, is colored by lipophilicity (green = lipophilic, blue = hydrophilic). Asp168 is labeled with a white D, and the possible hydrogen bond is shown as a dashed magenta line. Some hydrogens are omitted for clarity.

× CH_3), 1.05−1.08 (m, 3H, CH_3), 1.68−1.78 (m, 1H, CH), 3.13−3.37 (m, CH_2, solvent peak), 3.49−3.55 (m, 1H, CH), 3.64 (d, J = 5.4 Hz, CH_2), 3.86−3.91 (m, 1H, CH), 6.50−6.52 (m, 2H, C^3-/C^5-H Pyr), 7.10−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.37−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (d, J = 4.8 Hz, 1H, C^6-H Pyr). Anal. ($C_{22}H_{27}FN_4O_2S$) C, H, N.

3-(4-(4-Fluorophenyl)-5-(2-(*sec*-butylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3g). Compound **3g** was prepared according to general procedure H from **19g** (100 mg, 0.29 mmol), *t*-BuOK (50 mg, 0.32 mmol), 3-bromopropane-1,2-diol (33 mg, 0.29 mol), and MeOH (8 mL). The reaction mixture was heated to reflux temperature for 3 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 8:2). Yield: 31 mg (26%). ^{1}H NMR (CD_3OD) δ 0.87−0.95 (m, 3H, CH_3), 1.14 (d, J = 6.4 Hz, 3H, CH_3), 1.43−1.56 (m, 2H, CH_2), 3.17−3.38 (m, CH_2, solvent peak), 3.55−3.65 (m, 3H, CH, CH_2), 3.84−3.93 (m, H, CH), 6.54−6.59 (m, 2H, C^3-/C^5-H Pyr), 7.11−7.20 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.52 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.78 (d, J = 5.4 Hz, 1H, C^6-H Pyr). Anal. ($C_{21}H_{25}FN_4O_2S$) C, H, N.

3-(4-(4-Fluorophenyl)-5-(2-(isopropylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3h). Compound **3h** was prepared according to general procedure H from **19h** (100 mg, 0.30 mmol), *t*-BuOK (34 mg, 0.33 mmol), 3-bromopropane-1,2-diol (52 mg, 0.33 mol), and MeOH (8 mL). The reaction mixture was stirred at 55 °C for 3.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 70 mg (59%). ^{1}H NMR (CD_3OD) δ 1.15 (d, J = 6.3 Hz, 6H, 2 × CH_3), 3.13−3.37 (m, CH_2, solvent peak), 3.62−3.65 (m, 2H, CH_2), 3.75−3.91 (m, 2H, 2 × CH), 6.51−6.53 (m, 2H, C^3-/C^5-H Pyr), 7.10−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80−7.82 (m, 1H, C^6-H Pyr). HRMS-ESI, m/z ($C_{20}H_{23}FN_4O_2S$): calcd, 403.1599 $[M + H]^+$; found, 403.1598.

3-(4-(4-Fluorophenyl)-5-(2-(tetrahydro-2*H*-pyran-4-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3i). Compound **3i** was prepared according to general procedure H from **19i** (150 mg, 0.41 mmol), *t*-BuOK (54 mg, 0.48 mmol), 3-bromopropane-1,2-diol (75 mg, 0.48 mol), and MeOH (8 mL). The reaction mixture was heated to reflux temperature for 3.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 58 mg (31%). ^{1}H NMR (DMSO-d_6) δ 1.24−1.46 (m, 2H, C^3-/C^5-H thp), 1.77−1.83 (m, 2H, C^3-/C^5-H thp), 3.08−3.39 (m, 6H, CH_2, CH, CH thp, C^2-/C^6-H thp), 3.74−3.86 (m, 4H, CH_2, C^2-/C^6-H thp), 4.86 (s, 1H, OH, exchangeable), 5.30 (s, 1H, OH, exchangeable), 6.42−6.63 (m, 3H, C^3-/C^5-H Pyr, NH, exchangeable), 7.18−7.32 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.81−7.91 (m, 1H, C^6-H Pyr), 12.68 (bs, 1H, NH, exchangeable). HRMS-ESI, m/z ($C_{22}H_{25}FN_4O_3S$): calcd, 445.1703 $[M + H]^+$; found, 445.1703.

3-(4-(4-Fluorophenyl)-5-(2-(3-methylbutan-2-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propan-1-ol (3j). Compound **3j** was prepared according to general procedure H from **19f** (300 mg, 0.84 mmol), *t*-BuOK (104 mg, 0.93 mmol), 3-bromo-1-propanol (129 mg, 0.93 mol), and MeOH (20 mL). The reaction mixture was heated to reflux temperature for 1 h and purified by flash chromatography (SiO_2, from DCM/MeOH 95:5 to DCM/MeOH 85:15). Yield: 140 mg (40%). ^{1}H NMR (CD_3OD) δ 0.88 and 0.90 (2d, J = 6.8 Hz and J = 6.8 Hz, 6H, 2 × CH_3), 1.06 (d, J = 6.6 Hz, 3H, CH_3), 1.67−1.81 (m, 1H, CH), 1.85−1.95 (m, 2H, CH_2), 3.17 (t, J = 7.1 Hz, 2H, CH_2), 3.44−3.56 (m, 1H, CH), 3.70 (t, J = 6.0 Hz, 2H, CH_2), 6.50−6.54 (m, 2H, C^3-/C^5-H Pyr), 7.07−7.16 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.41−7.48 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (dd, J_1 = 5.1 Hz, J_2 = 0.7 Hz, 1H, C^6-H Pyr). Anal. ($C_{22}H_{27}FN_4OS$) C, H, N.

2-(4-(4-Fluorophenyl)-5-(2-(3-methylbutan-2-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)ethanol (3k). Compound **3k** was prepared according to general procedure H from **19f** (300 mg, 0.84 mmol), *t*-BuOK (113 mg, 1.01 mmol), 3-bromoethanol (126 mg, 1.01 mol), and MeOH (20 mL). The reaction mixture was heated to reflux temperature for 1.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 85:15). Yield: 128 mg (38%). ^{1}H NMR (CD_3OD) δ 0.90 and 0.92 (2d, J = 6.8 Hz and J = 6.8 Hz, 6H, 2 × CH_3), 1.07 (d, J = 6.6 Hz, 3H, CH_3), 1.65−1.81 (m, 1H, CH), 3.19 (t, J = 6.3 Hz, 2H, CH_2), 3.47−3.55 (m, 1H, CH), 3.81 (t, J = 6.3 Hz, 2H, CH_2), 6.50−6.52 (m, 2H, C^3-/C^5-H Pyr), 7.10−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.42−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.81 (d, J = 6.2 Hz, 1H, C^6-H Pyr). HRMS-ESI, m/z ($C_{21}H_{25}FN_4OS$): calcd, 401.1806 $[M + H]^+$; found, 401.1805.

(2*R*)-3-(4-(4-Fluorophenyl)-5-(2-(3-methylbutan-2-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3l). Compound **3l** was prepared according to general procedure H from **19f** (300 mg, 0.84 mmol), *t*-BuOK (104 mg, 0.93 mmol), (*R*)-(−)-3-chloropropane-1,2-diol (103 mg, 0.93 mol), and MeOH (20 mL). The reaction mixture was heated to reflux temperature for 1.25 h and purified by flash chromatography (SiO_2, from DCM/MeOH 95:5 to DCM/MeOH 85:15). Yield: 170 mg (47%). ^{1}H NMR (CD_3OD) δ 0.89 and 0.91 (2d, J = 6.7 Hz and J = 6.8 Hz, 6H, 2 × CH_3), 1.06 (d, J = 6.6 Hz, 3H, CH_3), 1.64−1.81 (m, 1H, CH), 3.17−3.35 (m, CH_2, solvent peak), 3.46−3.54 (m, 1H, CH), 3.64 (d, J = 5.4 Hz, CH_2), 3.84−3.95 (m, 1H, CH), 6.49−6.52 (m, 2H, C^3-/C^5-H Pyr), 7.08−7.17 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.42−7.49 (m, 2H,

C^2-/C^6-H 4-F-Phe), 7.79 (d, $J = 6.1$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{22}H_{27}FN_4O_2S$): calcd, 431.1912 [M + H]$^+$; found, 431.1909.

(2*S*)-3-(4-(4-Fluorophenyl)-5-(2-(3-methylbutan-2-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3m). Compound **3m** was prepared according to general procedure H from **19f** (300 mg, 0.84 mmol), *t*-BuOK (104 mg, 0.93 mmol), (*S*)-(+)-3-chloropropane-1,2-diol (103 mg, 0.93 mol), and MeOH (20 mL). The reaction mixture was heated to reflux temperature for 1.25 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/MeOH 8:2). Yield: 210 mg (58%). ^{1}H NMR (CD_3OD) δ 0.90 and 0.91 (2d, $J = 6.8$ Hz and $J = 6.8$ Hz, 6H, 2 × CH_3), 1.07 (d, $J = 6.6$ Hz, 3H, CH_3), 1.65−1.82 (m, 1H, CH), 3.14−3.35 (m, CH_2, solvent peak), 3.47−3.55 (m, 1H, CH), 3.64 (d, $J = 5.4$ Hz, CH_2), 3.83−3.94 (m, 1H, CH), 6.50−6.53 (m, 2H, C^3-/C^5-H Pyr), 7.10−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.81 (d, $J = 6.4$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{22}H_{27}FN_4O_2S$): calcd, 431.1912 [M + H]$^+$; found, 431.1910.

(2*R*)-3-(4-(4-Fluorophenyl)-5-(2-(isopropylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3n). Compound **3n** was prepared according to general procedure H from **19h** (200 mg, 0.61 mmol), *t*-BuOK (75 mg, 0.67 mmol), (*R*)-(−)-3-chloropropane-1,2-diol (74 mg, 0.67 mol), and MeOH (15 mL). The reaction mixture was heated to reflux temperature for 1 h and crystallized from MeOH/Et_2O/*n*-hexane. Yield: 113 mg (46%). ^{1}H NMR (DMSO-d_6) 1.08 (d, $J = 6.4$ Hz, 6H, 2 × CH_3), 3.13−3.42 (m, 2 × CH_2, H_2O in DMSO-d_6), 3.67−3.92 (m, 2H, 2 × CH), 6.29 (d, $J = 7.3$ Hz, 1H), 6.37 (dd, $J_1 = 5.4$ Hz, $J_2 = 1.3$ Hz, 1H, C^5-H Pyr), 6.47 (s, 1H, C^3-H Pyr), 7.17−7.26 (m, 2H, C^3/C^5-H 4-F-Phe), 7.43−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.84 (d, $J = 4.7$ Hz, 1H, C^6-H Pyr). Anal. ($C_{20}H_{23}FN_4O_2S$) C, H, N.

(2*S*)-3-(4-(4-Fluorophenyl)-5-(2-(isopropylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)propane-1,2-diol (3o). Compound **3o** was prepared according to general procedure H from **19h** (200 mg, 0.61 mmol), *t*-BuOK (75 mg, 0.67 mmol), (*S*)-(+)-3-chloropropane-1,2-diol (74 mg, 0.67 mol), and MeOH (15 mL). The reaction mixture was heated to reflux temperature for 1 h and crystallized from MeOH/Et_2O/*n*-hexane. Yield: 119 mg (49%). ^{1}H NMR (DMSO-d_6) δ 1.08 (d, $J = 6.4$ Hz, 6H, 2 × CH_3), 3.08−3.43 (m, 2 × CH_2, H_2O in DMSO-d_6), 3.69−3.93 (m, 2H, 2 × CH), 6.30 (d, $J = 7.6$ Hz, 1H), 6.39 (d, $J = 5.3$ Hz, 1H, C^5-H Pyr), 6.49 (s, 1H, C^3-H Pyr), 7.17−7.26 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.85 (d, $J = 5.2$ Hz, 1H, C^6-H Pyr). Anal. ($C_{20}H_{23}FN_4O_2S$) C, H, N.

Methyl 2-(4-(4-Fluorophenyl)-5-(2-(1-phenylethylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)acetate (3p). Compound **3p** was prepared according to general procedure H from **19a** (200 mg, 0.51 mmol), *t*-BuOK (63 mg, 0.56 mmol), methyl bromoacetate (86 mg, 0.56 mol), and MeOH (5 mL). The reaction mixture was stirred at 55 °C for 1 h and purified by flash chromatography (SiO_2, from DCM/EtOAc 1:1 to DCM/EtOAc 1:2). Yield: 109 mg (46%). ^{1}H NMR (CD_3OD) δ 1.45 (d, $J = 6.8$ Hz, 3H, CH_3), 3.69 (s, 3H, CH_3), 3.86 (s, 2H, CH_2), 6.48 (s, 1H, C^3-H Pyr), 6.58 (d, $J = 5.3$ Hz, 1H, C^5-H Pyr), 7.11−7.26 (m, 7H, 5 × Phe, C^3-/C^5-H 4-F-Phe), 7.36−7.43 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, $J = 5.4$ Hz, 1H, C^6-H Pyr). Anal. ($C_{25}H_{23}FN_4O_2S$) C, H, N.

2-(4-(4-Fluorophenyl)-5-(2-(1-phenylethylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)acetic Acid (3q). To a solution of **19a** (100 mg, 0.25 mmol) and NaOEt (20 mg, 0.30 mmol) in dry MeOH (15 mL) was added under argon atmosphere 2-bromoacetic acid (41 mg, 0.30 mmol). The solution was stirred at 50 °C for 5 h and cooled to room temperature. The solvent evaporated under reduced pressure and the crude product was purified by flash chromatography (SiO_2, from EtOAc/MeOH 85:15 to 100% MeOH) to yield 23 mg (21%) of compound **3q**. ^{1}H NMR (CD_3OD) δ 1.45 (d, $J = 6.0$ Hz, 3H, CH_3), 3.67 (s, 2H, CH_2), 6.50 (s, 1H, C^3-H Pyr), 6.57 (d, $J = 5.3$ Hz, 1H, C^5-H Pyr), 7.05−7.25 (m, 7H, 5 × Ph, C^3-/C^5-H 4-F-Phe), 7.38−7.44 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (d, $J = 5.5$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{24}H_{21}FN_4O_2S$): calcd, 449.1442 [M + H]$^+$; found, 449.1443.

Diethyl (4-(4-Fluorophenyl)-5-(2-(1-phenylethylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)methylphosphonate (3r). Compound **3r** was prepared according to general procedure K from **19a** (200 mg, 0.51 mmol), *t*-BuOK (63 mg, 0.56 mmol), diethyl iodomethylphosphonate (157 mg, 0.56 mol) and MeOH (15 mL). The reaction mixture was stirred at 55 °C for 3.5 h and purified by flash chromatography (SiO_2, from *n*-hexane/EtOAc 3:7 to *n*-hexane/EtOAc 0:1). Yield: 129 mg (47%). ^{1}H NMR (CD_3OD) δ 1.22 (t, $J = 7.0$ Hz, 6H, 2 × CH_3), 1.45 (d, $J = 6.8$ Hz, 3H, CH_3), 3.50 (d, $J = 12.4$ Hz, 2H, CH_2), 4.02−4.16 (m, 4H, 2 × CH_2), 4.60−4.70 (m, 1H, CH), 6.47 (s, 1H, C^3-H Pyr), 6.58 (d, $J = 5.3$ Hz, 1H, C^5-H Pyr), 7.06−7.24 (m, 7H, 5 × Ph, C^3-/C^5-H 4-F-Phe), 7.36−7.43 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, $J = 5.5$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{27}H_{30}FN_4O_3PS$): calcd, 541.1833 [M + H]$^+$; found, 541.1833.

Methyl 2-(4-(4-Fluorophenyl)-5-(2-(3-methylbutan-2-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)acetate (3s). Compound **3s** was prepared according to general procedure H from **19f** (300 mg, 0.84 mmol), *t*-BuOK (104 mg, 0.93 mmol), methyl 2-bromoacetate (142 mg, 0.93 mmol), and MeOH (20 mL). The reaction mixture was heated to reflux temperature for 1 h and purified by flash chromatography (SiO_2, from petroleum ether/EtOAc 3:7 to EtOAc 100%). Yield: 140 mg (42%). ^{1}H NMR (CD_3OD) δ 0.85−0.94 (m, 6H, 2 × CH_3), 1.08 (d, $J = 6.6$ Hz, 3H, CH_3), 1.65−1.82 (m, 1H, CH), 3.45−3.58 (m, 1H, CH), 3.72 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 6.52−6.56 (m, 2H, C^3-/C^5-H Pyr), 7.11−7.20 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.80 (d, $J = 5.5$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{22}H_{25}FN_4O_2S$): calcd, 429.1755 [M + H]$^+$; found, 429.1753.

Methyl 2-(4-(4-Fluorophenyl)-5-(2-(isopropylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)acetate (3t). Compound **3t** was prepared according to general procedure H from **19h** (200 mg, 0.61 mmol), *t*-BuOK (75 mg, 0.67 mmol), methyl 2-bromoacetate (102 mg, 0.67 mmol), and MeOH (15 mL). The reaction mixture was heated to reflux temperature for 1 h and purified by flash chromatography (SiO_2, from petroleum ether/EtOAc 3:7 to EtOAc 100%). Yield: 108 mg (44%). ^{1}H NMR (CD_3OD) δ 1.17 (d, $J = 6.4$ Hz, 6H, 2 × CH_3), 3.72 (s, 3H, CH_3), 3.74−3.84 (m, 1H, CH), 3.89 (s, 2H, CH_2), 6.53−6.55 (m, 2H, C^3-/C^5-H Pyr), 7.11−7.20 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.44−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, $J = 5.0$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{20}H_{21}FN_4O_2S$): calcd, 401.1442 [M + H]$^+$; found, 401.1443.

2-(4-(4-Fluorophenyl)-5-(2-(isopropylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)ethanol (3u). Compound **3u** was prepared according to general procedure H from **19h** (150 mg, 0.46 mmol), *t*-BuOK (56 mg, 0.50 mmol), 2-bromoethanol (63 mg, 0.50 mol), and MeOH (10 mL). The reaction mixture was stirred at 55 °C for 3.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 67 mg (39%). ^{1}H NMR (CD_3OD) δ 1.16 (d, $J = 6.4$ Hz, 6H, 2 × CH_3), 3.19 (t, $J = 6.3$ Hz, 2H, CH_2), 3.74−3.87 (m, 3H, CH, CH_2), 6.52−6.54 (m, 2H, C^3-/C^5-H Pyr), 7.07−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.51 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, $J = 5.8$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{19}H_{21}FN_4OS$): calcd, 373.1493 [M + H]$^+$; found, 373.1494.

2-(4-(4-Fluorophenyl)-5-(2-(tetrahydro-2*H*-pyran-4-ylamino)pyridin-4-yl)-1*H*-imidazol-2-ylthio)ethanol (3v). Compound **3v** was prepared according to general procedure H from **19i** (150 mg, 0.40 mmol), *t*-BuOK (50 mg, 0.45 mmol), 2-bromoethanol (56 mg, 0.45 mol), and MeOH (10 mL). The reaction mixture was stirred at 55 °C for 3.5 h and purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 75:25). Yield: 82 mg (50%). ^{1}H NMR (CD_3OD) δ 1.39−1.56 (m, 2H, C^3-/C^5-H thp), 1.86−1.92 (m, 2H, C^3-/C^5-H thp), 3.20 (t, $J = 6.2$ Hz, 2H, CH_2), 3.42−3.52 (m, 2H, C^2-/C^6-H thp), 3.66−3.84 (m, 3H, CH, CH_2), 3.91−3.97 (m, 2H, C^2-/C^6-H thp), 6.52−6.57 (m, 2H, C^3-/C^5-H Pyr), 7.11−7.20 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.83 (d, $J = 4.8$ Hz, 1H, C^6-H Pyr). HRMS-ESI, *m/z* ($C_{19}H_{21}FN_4OS$): calcd, 415.1599 [M + H]$^+$; found, 415.1600.

(1*R*,4*R*)-4-(4-(4-(4-Fluorophenyl)-2-(2-hydroxyethylthio)-1*H*-imidazol-5-yl)pyridin-2-ylamino)cyclohexanol (3w). Compound **3w** was prepared by irradiating **21** (160 mg, 0.48 mmol) and *trans*-4-

aminocyclohexanol (440 mg, 3.84 mmol) in a sealed tube at 135 °C for 3.5 h by moderating the initial microwave power (250 W). After the mixture was cooled to room temperature in a stream of compressed air, the yellow solid was dissolved in MeOH and transferred into a round-bottomed flask and the solvent was evaporated. The crude product was purified by flash chromatography (SiO_2, from DCM/EtOH 95:5 to DCM/EtOH 8:2). Yield: 82 mg (40%). ^{1}H NMR (CD_3OD) δ 1.18−1.38 (m, 4H, cyclohexyl), 1.93−2.07 (m, 4H, cyclohexyl), 3.19 (t, J = 6.2 Hz, 2H, CH_2), 3.39−3.55 (m, 2H, 2 × CH cyclohexyl), 3.81 (t, J = 6.2 Hz, CH_2), 6.50−6.56 (m, 2H, C^3-/C^5-H Pyr), 7.10−7.19 (m, 2H, C^3-/C^5-H 4-F-Phe), 7.43−7.50 (m, 2H, C^2-/C^6-H 4-F-Phe), 7.82 (d, J = 5.2 Hz, 1H, C^6-H Pyr). HRMS-ESI, m/z ($C_{22}H_{25}FN_4O_2S$): calcd, 429.1755 $[M + H]^+$; found, 429.1752.

Whole Blood Assay. The fresh human whole blood was diluted with FBS (fetal bovine serum). An amount of 400 μL of the diluted blood was pipetted into each well. After addition of the different dilutions of test compounds to the corresponding wells, a 15 min incubation time (at 5% CO_2) was allowed. The next step was pipetting LPS into the wells for stimulation and compound data. DPBS-gentamicin was put into the wells for the basal level. After another incubation time (incubation at 37 °C, 5% CO_2, 2.5 h), the cellular blood compounds were centrifuged, plasma (supernatant) was taken out, and the amount of TNF-α was measured using an ELISA assay.

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Supporting Information Available: Full experimental procedures and spectroscopic data for compounds **15a**−**i**, **16a**−**i**, **17a**−**i**, **18a**−**i**, **19a**−**i**, and **21**; elemental analysis results and HRMS and HPLC data for the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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