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Chiral Sulfoxides as Metabolites of 2-Thioimidazole-Based p38α Mitogen-Activated Protein Kinase Inhibitors: Enantioselective Synthesis and Biological Evaluation

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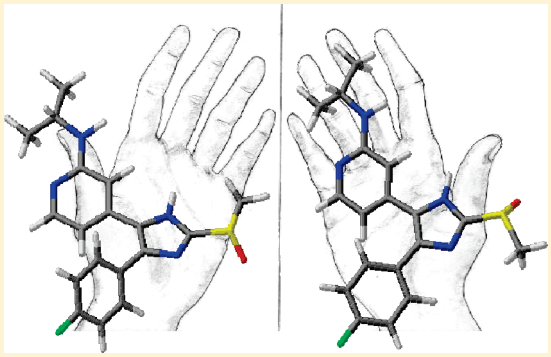
S Supporting Information

ABSTRACT: A number of pharmaceutically important drugs contain asymmetric sulfinyl moieties, so the biological evaluation of chiral sulfoxides as human drug metabolites is important for the development of safe and effective pharmaceuticals. Asymmetric oxidation is one of the most attractive ways to prepare chiral sulfoxides. In combination with different chiral ligands, the iron- and titanium-catalyzed asymmetric oxidations of tri- and tetrasubstituted 2-thioimidazoles afford the corresponding sulfoxides with enantiomeric excesses up to 99% as novel p38α mitogen-activated protein kinase (p38α MAPK) inhibitors. The enantiomerically pure sulfoxides were evaluated on their inhibitory potency against p38α MAPK compared to the respective sulfides and sulfoxide racemates and showed differences in their affinities for the enzyme with IC_{50} in the low nanomolar range. In addition, the ability to inhibit the release of tumor necrosis factor-α (TNF-α) from human whole blood (HWB) was examined. Some pyridinylimidazole derivatives showed excellent HWB activity with IC_{50} as low as 52 nM.



INTRODUCTION

Protein kinases are crucial components in signal transduction for the propagation and regulation of immunologic responses.[1] They transmit extracellular signals to the nucleus, where phosphorylation and activation of transcription factors induce the transcription of specific genes.[2] Dysregulation of protein kinase activity[3] is directly involved in many human diseases characterized by acute and chronic inflammation, such as eczema and psoriasis, rheumatoid arthritis, and inflammatory bowel disease.[1] A causal role in the pathogenesis of chronic inflammation is an increased gene expression of several proinflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), whose biosynthesis and release are regulated by the activation of the p38α mitogen-activated protein kinase (p38α MAPK) as a serine/threonine kinase.[4,5] Hence, the p38α MAPK is a valid target for the development and improvement of highly potent and selective inhibitors of this enzyme to treat diseases where tissue inflammation and proinflammatory cytokines have been implicated.[6]

Favored synthetic scaffolds for compounds targeting the p38α MAPK are pyridinylimidazoles.[7,8] Derived from the adenosine triphosphate (ATP) competitive p38α MAPK inhibitor **1** (SB203580)[1,9] (Figure 1), the synthesis of recently published tri- and tetrasubstituted 2-thioimidazole derivatives **2** (Figure 1) provided efficient inhibitors with reduced cytochrome P450 (CYP450) interactions and improved pharmacokinetic and metabolic properties related to the prototype inhibitor **1**.[10]

Furthermore, the second generation pyridinylimidazole inhibitor **3** (ML3403)[11−13] (Figure 1), which is another representative of this class of inhibitors with high potency and selectivity for p38α MAPK, was investigated in CYP-mediated metabolism studies.[11,12,14] In liver microsomal incubations, a rapid oxidation of the sulfanyl group of **3** to the corresponding sulfoxide **4** (ML3603)[11,12] (Figure 1) as the active phase I metabolite was observed and represents the key step for the metabolic biotransformation of 2-thioimidazole derivatives. In an extension of the in vitro study, the in vivo pharmacokinetics of **3** in Wistar rats were examined after oral gavage[12] and supported the in vitro liver microsome studies by demonstrating that **3** was also oxidized to the pharmacologically active metabolite **4** comparable to the in vitro findings. The systemic exposure of the metabolite was approximately 3-fold higher compared to the parent drug, suggesting that metabolic sulfoxidation may contribute to the

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activity of this class of compounds in vivo.[15] The new stereocenter at the sulfur atom of the heterocyclic sulfoxide derivatives formed by oxidation, such as in compound **4**, generates two possible sulfoxide enantiomers, which can differ in pharmacodynamic and/or pharmacokinetic properties as a consequence of the stereoselective interaction with the enzyme.[16] Since one sulfoxide enantiomer can have a higher affinity for the p38α MAPK, combined with an increase of potency, we must undertake a detailed investigation of the biological activities of both single enantiomers against the p38α MAPK, compared to the sulfoxide racemate.

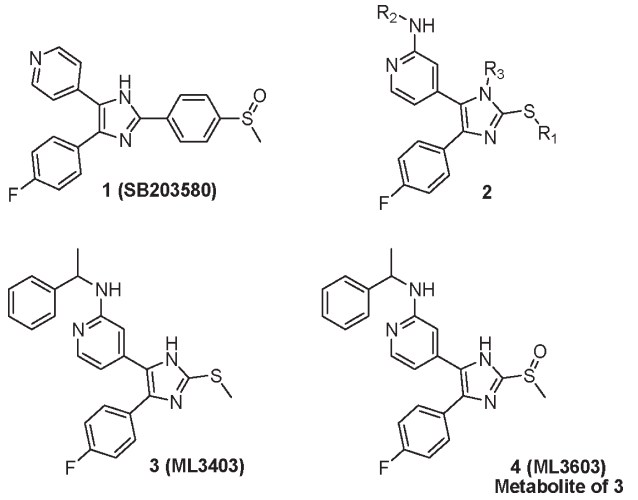
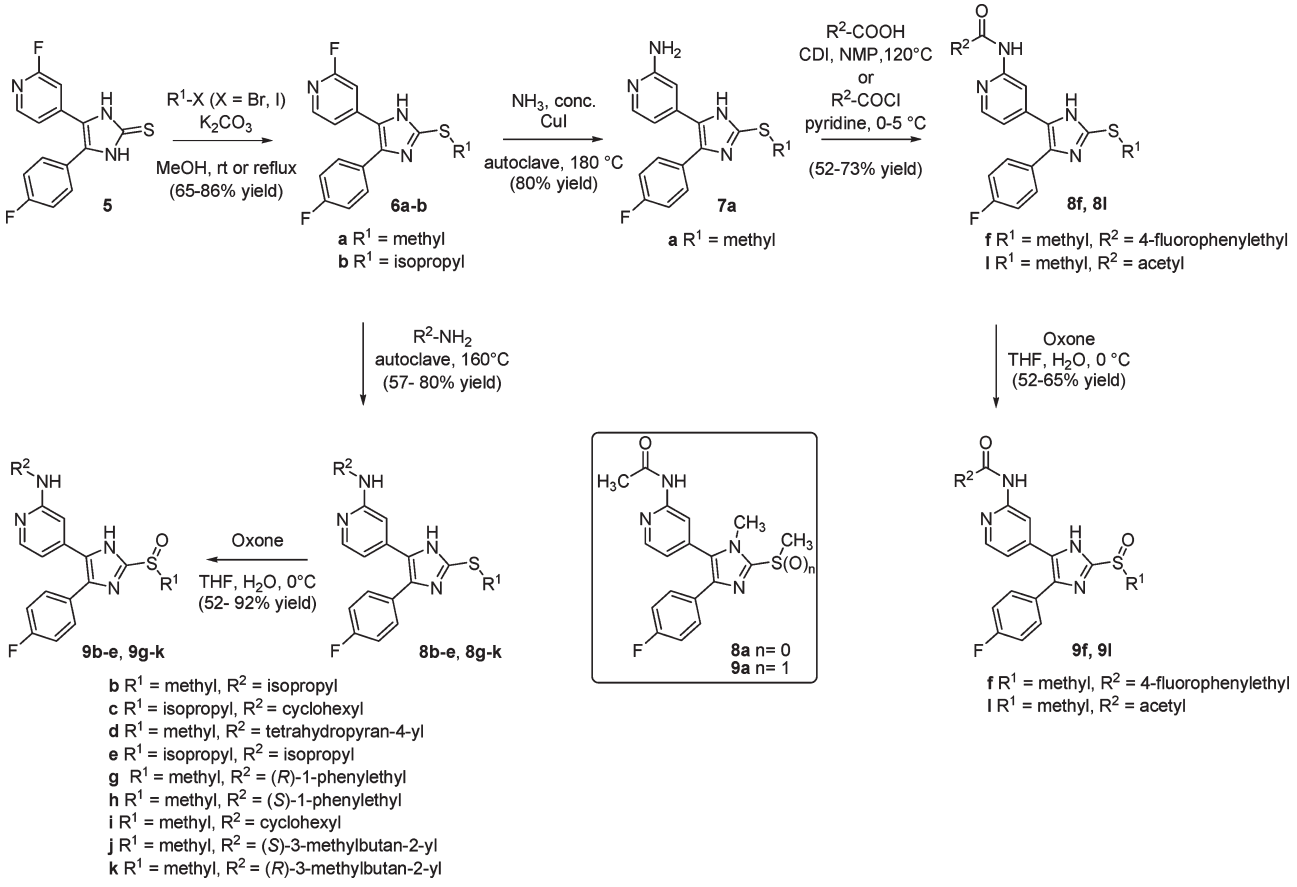


Figure 1. Pyridinylimidazole inhibitors of p38α MAP kinase.

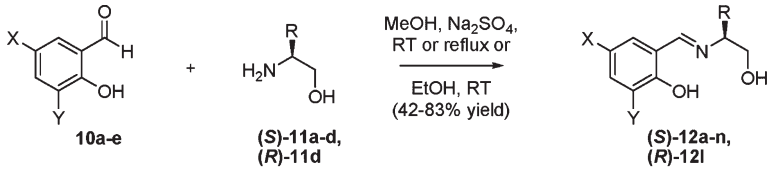
The number of marketed enantiopure compounds has increased tremendously, and many chiral sulfoxides exhibit interesting biological activities and show promise as therapeutic agents,[17] e.g., the proton-pump inhibitor omeprazole and some of its derivatives[18−20] as well as the platelet adhesion inhibitor (*S*)-(+)-3,4-dihydro-6-[3-(1-*o*-tolyl-2-imidazolyl)sulfinylpropoxy]-2(1*H*)-quinolinone (OPC-29030).[21,22] Therefore, we considered the development of practical synthetic routes to prepare new enantiopure heterocyclic sulfoxides, including pyridinylimidazole derivatives such as p38α MAPK inhibitors. Our intention was to examine the possible advantages of using a single enantiomer compared to the sulfoxide racemate.

The metal-catalyzed asymmetric oxidation of prochiral sulfides is an attractive way to generate optically active sulfoxides. In this article, to prepare novel p38α MAPK inhibitors, we describe three catalyst systems to oxidize more complex structural units like tri- and tetrasubstituted 2-thioimidazoles to the corresponding sulfoxides with good to high enantioselectivities by using transition metals as catalyst precursors in the presence of a chiral ligand and a hydroperoxide as oxidant. Depending on the application of the chiral ligand, the asymmetric oxidation can be controlled with respect to a single enantiomer so that both enantiomers E_1 and E_2 of the pyridinylimidazole sulfoxides are synthetically accessible. The enantiomerically pure sulfoxides thus obtained were evaluated for their inhibitory potency against p38α MAPK as well as for their ability to inhibit TNF-α release in human whole blood in comparison to the respective sulfides

Scheme 1. Synthesis of the Trisubstituted 2-Thioimidazoles and Their Respective Sulfoxide Racemates



Scheme 2. Synthesis of the Chiral Schiff Base Ligands (*S*)-12a−n and (*R*)-12l from 3,5-Disubstituted Salicylaldehyde Analogues and Chiral Amino Alcohols



10a: X= Y= H
10b: X= Y= Br
10c: X= Y= I
10d: X= Y= *t*- Bu
10e: X= NO_2,Y= *t*- Bu

(*S*)-11a: R= *i*- Pr
(*S*)-11b: R= Bn
(*S*)-11c: R= *i*- Bu
(*S*)-11d: R= *t*- Bu
(*R*)-11d: R= *t*- Bu

(*S*)-12a: X= Y= H, R= *i*- Pr
(*S*)-12b: X= Y= H, R= Bn
(*S*)-12c: X= Y= H, R= *i*- Bu
(*S*)-12d: X= Y= H, R= *t*- Bu
(*S*)-12e: X= Y= Br, R= *i*- Pr
(*S*)-12f: X= Y= Br, R= Bn
(*S*)-12g: X= Y= Br, R= *i*- Bu
(*S*)-12h: X= Y= Br, R= *t*- Bu
(*S*)-12i: X= Y= I, R= *i*- Pr
(*S*)-12j: X= Y= I, R= Bn
(*S*)-12k: X= Y= I, R= *i*- Bu
(*S*)-12l: X= Y= I, R= *t*- Bu
(*S*)-12m: X= Y= *t*- Bu, R= *t*- Bu
(*S*)-12n: X= NO_2, Y= *t*- Bu, R= *t*- Bu
(*R*)-12l: X= Y= I, R= *t*- Bu

and sulfoxide racemates. Our goal was to identify possible differences between the enantiomers of the inhibitors with respect to their interaction behaviors with the enzyme.

CHEMISTRY

We first examined three different methodologies for the enantioselective sulfoxidation of tri- and tetrasubstituted 2-alkylsulfanylimidazoles with titanium- or iron-based asymmetric catalysts as applied to six model compounds **8a**−**f**, which differed in the substituents on the imidazole core itself and also at the ortho-position of the pyridine ring[23] (Scheme 1). We compared the performance of the three oxidation methods with regard to the enantioselectivity of the relevant reaction with the aim of transferring these results to further pyridinylimidazole derivatives **8g**−**l**. The synthetic method of the trisubstituted 2-thioimidazole derivatives **8b**−**f** and their corresponding sulfoxide racemates **9b**−**f** is depicted in Scheme 1.

Starting from compound **5**, which we obtained using a previously described four-step synthesis,[13,23] the trisubstituted 2-thioimidazoles **8b**−**f** were synthesized via an alkylation of the sulfur atom (compounds **6a**[13,24] and **6b**) followed by a nucleophilic aromatic substitution on the pyridine moiety with ammonia or alkylamines. A subsequent acylation of compound **7a**[11,25] with 4-fluorophenylacetic acid yielded the desired compound **8f**. To validate the enantiomeric excesses, we oxidized the 2-alkylsulfanylimidazoles **8b**−**f** to the corresponding sulfoxide racemates **9b**−**f** under standard oxidation conditions using potassium peroxomonosulfate. The synthesis of the tetrasubstituted imidazole derivatives **8a** and **9a** (Scheme 1) is known from the research literature.[26−28]

The initial asymmetric oxidations of the tri- and tetrasubstituted 2-thioimidazoles **8a**−**d** and **8f** were achieved by titanium-mediated oxidation with cumene hydroperoxide (CHP) in the presence of diethyl D-tartrate (D-DET or (*S*,*S*)-DET). The most favorable solvent in which to run the reaction was dichloromethane, and the optimum temperature was $T \approx -18$ °C (Table S1 in Supporting Information).

The enantiomeric excesses were strongly dependent on the amount of the catalyst and on the substitution pattern of the 2-thioimidazoles **8a**−**d** and **8f** (Table S1). A change in the catalyst system from the combination of Ti(O-*i*-Pr)$_4$/D-DET/H_2O/CHP = 1:2:1:2, as described by Kagan et al.,[29,30] to a ratio of Ti(O-*i*-Pr)$_4$/D-DET/H_2O/CHP = 2:4:1:4 increased the enantiomeric excess of the sulfoxide **9a** of the tetrasubstituted 2-thioimidazole **8a** from 46% (Table S1, entry 1) to 76% (Table S1, entry 2). The application of Hünig's base (*N*,*N*-diisopropylethylamine) in the Ti(O-*i*-Pr)$_4$/tartrate system for improvement of the enantioselectivity, which has also been published in connection with the asymmetric synthesis of esomeprazole,[18] led to the formation of the sulfoxide racemate **9a**. This outcome is in accordance with the publication of Seenivasaperumal et al.,[31] where the oxidation of an N-methylated 2-methylsulfanylimidazole yielded the racemic product. The addition of water to the catalyst system is essential to ensure high enantiomeric excesses.[29] The above-mentioned catalyst system Ti(O-*i*-Pr)$_4$/D-DET/H_2O/CHP = 2:4:1:4 produced an overoxidation of the trisubstituted 2-thioimidazoles **8b**, **8d**, and **8f** to the corresponding sulfones. Hence, the amount of the chiral titanium catalyst must be decreased for the trisubstituted 2-thioimidazoles **8b**, **8d**, and **8f**. By use of the combinations Ti(O-*i*-Pr)$_4$/D-DET/H_2O/CHP = 1:2:1:2 (**8b** and **8d**) and Ti(O-*i*-Pr)$_4$/D-DET/H_2O/CHP = 1.5:3:1:3 (**8f**), the sulfoxides **9b**, **9d**, and **9f** could be isolated with low to moderate enantiomeric excesses (7−31% ee) (Table S1, entries 3, 5, 6). Presumably, the steric effects of the bulky groups on the pyridine ring or the substituents on the sulfur atom account for the different outcomes of the enantioselective sulfoxidations involving the chiral titanium catalyst. Another explanation may be the stabilizing electronic interactions between the substrate and the ligand.[31] Similar results, but with inverse configurations of the respective sulfoxides **9a**−**d** and **9f**, were obtained by using diethyl L-tartrate (L-DET or (*R*,*R*)-DET) instead of D-DET (Table S1, entries 7−12).

The influence of the application of Hünig's base in the Ti(O-*i*-Pr)$_4$/tartrate system on the enantiomeric excess was also examined on the non-N-methylated imidazole compound **8b**. In the presence of an imidazole ring with unsubstituted nitrogen

atom,[31] the ee value of the sulfoxide **9b** was increased from 20% (Table S1, entry 3) to 56% ee (D-DET) (Figure S1 in Supporting Information) and from 31% (Table S1, entry 9) to 53% ee (L-DET) (Figure S2 in Supporting Information), but the conversion to the sulfoxide **9b** was only moderate. A further disadvantage is the sulfone formation in relatively large amounts, as shown in Figures S1 and S2.

The tri- and tetrasubstituted 2-thioimidazoles **8a**−**d** can also be oxidized enantioselectively under mild reaction conditions by chiral iron complexes, generated in situ from $Fe(acac)_3$ and chiral Schiff base ligands using 32% aqueous hydrogen peroxide as the terminal oxidant[32] in dichloromethane at room temperature. To improve the enantioselectivity of the metal-catalyzed reactions, 4-methoxybenzoic acid was used as an additive to stabilize the catalytic system.[32] Therefore, a series of already published[32−39] and newly synthesized chiral Schiff bases with *S*-configuration (*S*)-**12a**−**n** were prepared from 3,5-disubstituted salicylaldehyde analogues **10a**−**e** and chiral amino alcohols (*S*)-**11a**−**d** with different side chains (Scheme 2). The selection of these chiral Schiff bases (*S*)-**12a**−**n** was based on previous studies by Legros,[32] Zeng,[39] and Gama,[33] in which iron− and vanadium−Schiff base complexes were successfully used to oxidize different sulfide substrates to chiral sulfoxides. During vanadium-catalyzed sulfoxidation, Gama found that the presence of electron-withdrawing bromine along with bulky substituents on the ligand enhances the enantioselectivity in the sulfide to sulfoxide oxidation,[33] while Legros reported that iron−Schiff base complexes derived from chiral *tert*-leucinol are effective catalysts for the enantioselective sulfoxidation.

To examine the influence of the substitution pattern of the chiral Schiff base ligands (*S*)-**12a**−**n** on the enantioselectivity of the sulfoxidation process, 14 chiral Schiff bases (*S*)-**12a**−**n** were tested on the tetrasubstituted 2-thioimidazole **8a**, which was chosen as the model substrate for this study. This compound is interesting from a pharmaceutical point of view, since the corresponding sulfoxide racemate **9a** displayed an immunomodulating and cytokine release-inhibiting effect with a better metabolic stability, an increased oral bioavailability, and an increased systemic exposure compared to its sulfanyl analogue.[26,40] Numerous trials revealed that only the chiral ligands (*S*)-**12d**,[38] (*S*)-**12h**,[33] and (*S*)-**12l**[32] with the hydrogen or the halogen atoms on the aromatic ring and the *tert*-butyl group at the imino alcohol side chain in combination with $Fe(acac)_3$ afforded the sulfoxide **9a** with moderate to good enantioselectivity of the second eluted enantiomer E_2 (Table S2, entries 1−5; see Supporting Information).

When we extended these studies to the trisubstituted 2-thioimidazoles **8b**−**d**, the corresponding sulfoxides **9b**−**d** were isolated with remarkable enantiomeric excesses (up to 96%) of the second eluted enantiomer E_2 (Table S2, entries 6−14). Notably, the chiral Schiff base (*S*)-**12d** was much less efficient in this latter system than the other two ligands (*S*)-**12h** and (*S*)-**12l**. This "halogen effect" cannot be explained at this time.[32] The exchange of $Fe(acac)_3$ for $VO(acac)_2$ led to the isolation of the sulfoxide racemates in all cases. The corresponding results are not listed here in detail. Hence, the iron atom of the metal catalyst may promote a preferred coordination to the chiral ligands and to the substrate. Furthermore, the enantiomeric excess values of the sulfoxides **8a**−**d** are influenced by the amount of the catalyst. For the tetrasubstituted 2-thioimidazole **8a**, the system worked best when the ratio of sulfide/$Fe(acac)_3$/ligand/H_2O_2 ranged from 1:0.02:0.04:1.2 to 1:0.04:0.08:1.6, whereas the best combination for the trisubstituted 2-thioimidazoles **8b**−**d** was $Fe(acac)_3$/ligand/H_2O_2 = 0.2:0.4:1.2. Possibly the NH group of the imidazole core interacts directly with the iron atom of $Fe(acac)_3$ so that the construction of the metal−ligand catalyst is prevented when the concentration of the metal compound is low.

With the chiral Schiff base (*R*)-**12l** with the *R*-configuration (Scheme 2), the first eluted enantiomer E_1 predominated (Table S2, entry 15), indicating that the inverse enantiomer was obtained in excess (60% ee).

The best results with respect to the enantiomeric excess of the sulfoxides **9a**,**b**, **9d**−**l** were achieved by using either of the metal catalyst system Ti(O-*i*-Pr)$_4$ (0.1 equiv)/*S*-BINOL (0.2 equiv)/H_2O (0.2 equiv) or Ti(O-*i*-Pr)$_4$ (0.1 equiv)/*R*-BINOL (0.2 equiv)/H_2O (0.2 equiv) with *tert*-butyl hydroperoxide (TBHP) (70% solution of TBHP in water, 2.0 equiv) as oxidant,[21,41] with dichloromethane as solvent. This titanium-catalyzed procedure is a tandem sulfoxidation−kinetic resolution process, which consists of a combination of asymmetric oxidation (at lower temperature) and kinetic resolution (at room temperature).[21] The optimal temperature to carry out the reaction was T = 5 °C for the 2-thioimidazole derivatives **8a**, **8b**, **8d**, and **8f**, followed by stirring at room temperature, whereas the asymmetric oxidation of the 2-sulfanylimidazole derivative **8e** was successful at T = 25 °C.

Under these conditions, the sulfoxidations for the trisubstituted 2-thioimidazoles **8b**, **8d**, and **8f** proceeded with excellent enantioselectivities (95−99% ee) (Table S3, entries 2, 3, and 5 and Table S4, entries 2, 3 and 5; see Supporting Information) and with high conversions up to 99%. When *S*-BINOL was used as the chiral auxiliary, the first eluted enantiomers E_1 of the sulfoxides were obtained (Table S3, entries 1−5), whereas *R*-BINOL afforded the corresponding second eluted enantiomers E_2 (Table S4, entries 1−5).

The high ee values of the sulfoxides may result from two independent stereoselective processes, i.e., the asymmetric oxidation producing sulfoxide and its subsequent kinetic resolution via further oxidation to the sulfone.[42] By the use of both aforementioned catalytic systems, the sulfone formations were very low (<5%) after stirring at room temperature for 45−90 min. Thus, the high ee values of the sulfoxides do not arise from a selectivity enhancement caused by kinetic resolution of the chiral sulfoxide but rather are the direct result of the enantioselective oxidation.[42] This argument was confirmed by the time profiles of the asymmetric oxidation of the 2-thioimidazole derivative **8b** (Figures S3 and S4; see Supporting Information). The ee values of the sulfoxide **9b** did not change significantly during the reaction process, but the yields of the sulfoxide **9b** were dramatically enhanced from 57% to 92% (*S*-BINOL) (Figure S3) and from 53% to 84% (*R*-BINOL) (Figure S4) when the reaction temperature was increased from T = 5 °C to room temperature after 5 h. Even 3 h after the mixture was stirred at room temperature, sulfones were formed only in very low quantities (∼3%) and were associated with a small decrease of the ee values of the sulfoxide **9b** using the system Ti(O-*i*-Pr)$_4$/*R*-BINOL/H_2O/TBHP (Figure S4).

Furthermore, the enantiomeric excesses are dependent on the substitution pattern of the 2-thioimidazoles **8a**,**b** and **8d**−**f**. If the methyl group of the sulfur atom at the C2 position of the imidazole core in compound **8b** was replaced by a bulky isopropyl group (compound **8e**), the ee value of the sulfoxide **9e** decreased significantly from 99% to 62% ee (*S*-BINOL)

(Table S3, entries 2 and 4) and from 98% to 58% ee (*R*-BINOL) (Table S4, entries 2 and 4). Presumably, the optimal coordination between the substrate and the ligand is prevented by steric hindrance of the bulky isopropyl group. Lower ee values were also observed for the tetrasubstituted 2-thioimidazole derivative **8a** (Table S3, entry 1 and Table S4, entry 1), which can also be attributed to unfavorable interactions between the substrate and the ligand. To confirm this hypothesis and to confirm the influence of the methyl group on the imidazole ring nitrogen on the enantiomeric excess, we oxidized enantioselectively the analogue trisubstituted 2-thioimidazole derivative **8l** under the same reaction conditions, which resulted in significant increases of the ee of the sulfoxide **9l** from 51% to 89% ee in the reaction with *S*-BINOL (Table S3, entries 1 and 11) and from 26% to 87% ee with *R*-BINOL (Table S4, entries 1 and 11). Clearly, 2-thioimidazole derivatives with an unsubstituted NH group at the imidazole-N1 position and with an *S*-methyl group on the C2 position of the imidazole core are most suitable for the asymmetric oxidations using either metal catalyst system $Ti(O\text{-}i\text{-}Pr)_4$/*S*-BINOL or *R*-BINOL/$H_2O$/TBHP.

We continued our investigation with a variety of trisubstituted 2-thioimidazoles **8g**−**k** with additional functionalities in the ortho-position of the pyridine ring (Scheme 1). The trisubstituted 2-thioimidazoles **8g**−**k** were successfully and enantioselectively oxidized to the corresponding sulfoxides **9g**−**k** with ee values up to 99% (Table S3, entries 6−10 and Table S4, entries 6−10). Obviously, the different substituents in the ortho-position of the pyridine ring of the 2-thioimidazoles **8g**−**k** had little or no influence on the reaction outcomes with respect to the enantiomeric excesses of the sulfoxides **9g**−**k**.

The identification of the absolute configurations of the sulfur atom of the 2-thioimidzole derivatives was accomplished by the X-ray crystal structures of both enantiomers E_1 and E_2 of the sulfoxide **9b** (Figure S5 and Figure S6; see Supporting Information).[43] According to the Cahn−Ingold−Prelog nomenclature, the first eluted enantiomer E_1 is in the *R*-configuration and the second eluted enantiomer E_2 is in the *S*-configuration. This assignment of the absolute configuration for the sulfur atom has been confirmed by the crystal structure of the first eluted enantiomer E_1 of the analogous compound **9a**, which has already been published[44] and showed the *R*-configuration. The X-ray crystal structure of the second eluted enantiomer E_2 of compound **9a** with *S*-configuration has been registered in The Cambridge Crystallographic Data Centre.[45] These classifications of the absolute configurations of both enantiomers can be transferred to the other 2-thioimidazole derivatives **8c**−**l** listed in Tables S3 and S4.

BIOLOGICAL TESTING

To analyze, the influence of stereochemistry on the biological activity, the inhibitory potencies of the enantiomerically pure sulfoxides were evaluated in comparison to the sulfides and sulfoxide racemates in a nonradioactive immunosorbent p38α MAPK enzyme assay, in which ATP competes with the inhibitor for the same binding pocket in the catalytic site of p38α MAPK.[46] According to the effectiveness of the pyridinylimidazole inhibitor and the resulting p38α kinase activity, the phosphorylation reaction of the activating transcription factor 2 (ATF-2), the endogenous protein substrate of p38 MAPK, is suppressed to a greater or lesser extent so that the amount of phosphorylated ATF-2 inversely correlates with the inhibitory activity of the p38α MAPK inhibitor.[46]

In addition, the inhibition of the release of tumor necrosis factor-α (TNF-α) from human whole blood after LPS stimulation was also investigated for several test compounds that displayed good inhibition in the p38α MAPK enzyme assay. In contrast to the isolated cell assay, the human whole blood assay takes into consideration pathophysiological conditions and other important parameters such as solubility, cell−cell interactions, plasma−protein binding, diffusion processes, metabolism, and different enzyme expression.[47] In this assay, the enzyme activity is measured by determining the levels of TNF-α secretion with a nonradioactive enzyme-linked immunosorbent assay (ELISA) procedure.[47] The known inhibitor **1** was used as a reference compound in both the whole blood TNF-α release assay and in the isolated p38α MAPK enzyme assay.

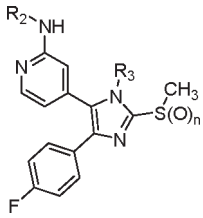
BIOLOGICAL DISCUSSION

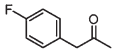
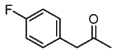
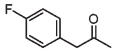
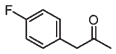
As we have already shown in previous publications,[48,49] 2-acylaminopyridinylimidazole derivatives are potent inhibitors for p38α MAPK and therefore are interesting compounds for the investigation of the inhibitory potency of the enantiomerically pure sulfoxides. The biological activities of the tri- and tetrasubstituted pyridinylimidazole derivatives with an additional 2-acylamino moiety in the ortho-position of the pyridine ring, which were synthesized in the present study, are listed in Table 1. By comparison of the tetrasubstituted 2-thioimidazole derivative **8a**, functionalized with an acetylamino substituent in the C2 position of the pyridine ring, with the analogous trisubstituted compound **8l**, it is obvious that the affinity of the enzyme for the compound **8a** is strongly reduced by the presence of the alkyl substituent on the imidazole core nitrogen atom, expressed as a 7.5-fold decrease of activity with respect to the trisubstituted 2-thioimidazole derivative **8l** with an IC_{50} of 37 nM (Table 1).

Docking studies provided no conclusive explanation for the influence of the additional *N*1-alkyl substitution on the imidazole core on the interactions with the ATP binding site. On the other hand, earlier investigations showed that the 2-acetylamino residue on the pyridine ring is well tolerated and caused at least a 2-fold increase in inhibitory activity compared with the reference substance **1** by fostering optimal interactions with the kinase's hydrophobic region II and by the formation of an additional hydrogen bond from Met109 in the hinge region to the hydrogen donor function of the amide NH.[10,49]

The corresponding sulfoxides derived from sulfide compounds **8a** and **8l** with enhanced hydrophilic character showed a significant loss of activity toward p38 MAPK relative to the sulfides (Table 1). In the case of the tetrasubstituted sulfoxides, the sulfoxide racemate **9a** and the individual enantiomers (*R*)-**9a** and (*S*)-**9a** had similar biological activities against p38α MAPK with IC_{50} in the range 1.03−1.27 μM so that the interactions with the enzyme are independent of the binding geometry of the enantiomers. Therefore, no stereopreference was observed. The decrease of inhibitory activity of the sulfoxide derivatives might be explained by an effect on entropy, which influences the strength of the protein−ligand interactions. For the binding with the protein, water molecules, situated in the binding pocket, must be displaced, thereby increasing the degree of disorder and the associated entropy. The contribution to the binding affinity is greater when more water molecules are displaced from the hydrophobic surroundings. Because of the hydrogen bond acceptor function of the polar sulfoxide group in the respective derivatives, their contribution to the binding affinity to the

Table 1. Biological Activity of Tri- and Tetrasubstituted 2-Acylaminopyridinylimidazoles



Compound	*ee* [%]	R_2	R_3	p38α IC_{50}±SEM [μM][a]	RP[b]	TNF-α IC_{50}±SEM [μM][c]	RP[b]
8a (n= 0)			-CH_3	0.188±0.024 (**1**: 0.052±0.005)	0.28	n. t.	
9a (n= 1)			-CH_3	1.072±0.217 (**1**: 0.045±0.003)	0.042	n. t.	
(*R*)-**9a** (n= 1)	85		-CH_3	1.030±0.258 (**1**: 0.036±0.001)	0.035	n. t.	
(*S*)-**9a** (n= 1)	76		-CH_3	1.265±0.126 (**1**: 0.036±0.001)	0.028	n. t.	
8l (n= 0)			-H	0.037±0.002 (**1**: 0.078±0.006)	2.11	n. t.	
9l (n= 1)			-H	0.403±0.067 (**1**: 0.041±0.003)	0.10	n. t.	
(*R*)-**9l** (n= 1)	89		-H	0.161±0.002 (**1**: 0.089±0.005)	0.55	n. t.	
(*S*)-**9l** (n= 1)	87		-H	0.197±0.009 (**1**: 0.089±0.005)	0.45	n. t..	
8f (n= 0)			-H	0.009±4.7e^{-4} (**1**: 0.031±0.001)	3.44	2.98±0.13 (**1**: 1.95±0.50)	0.66
9f (n= 1)			-H	0.069±0.003 (**1**: 0.077±0.005)	1.16	5.55±0.27 (**1**: 1.70±0.07)	0.31
(*R*)-**9f** (n= 1)	95		-H	0.077±0.006 (**1**: 0.077±0.005)	1.0	6.41±1.20 (**1**: 1.70±0.49)	0.27
(*S*)-**9f** (n= 1)	97		-H	0.141±0.014 (**1**: 0.077±0.005)	0.55	7.53±2.81 (**1**: 1.70±0.49)	0.23

[a] Mean ± SEM of three experiments. [b] Relative potency IC_{50}(**1**)/IC_{50}(test compound) to allow better comparison of test data from different assays; e.g., differences in HWB samples underlie significant interindividual variations. [c] Mean ± SEM of two experiments. n.t.: not tested.

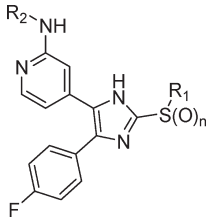
enzyme is significantly less than in the case of the sulfides and is associated with a decrease of activity.

However, both trisubstituted sulfoxide enantiomers (*R*)-**9l** and (*S*)-**9l** showed distinct differences in their binding affinities to the enzyme in comparison with the sulfoxide racemate **9l**. While (*R*)-**9l** (IC_{50} = 0.161 μM) binds to the p38α MAPK with an affinity comparable to its optical antipode (IC_{50} = 0.197 μM), the two single enantiomers (*R*)-**9l** and (*S*)-**9l** displayed a 5.5-fold or 4.5-fold improved inhibition of p38α MAPK relative to the sulfoxide racemate **9l** having an IC_{50} of 0.403 μM (Table 1).

Furthermore, the trisubstituted 2-thioimidazole derivative **8f** could be identified as a potent p38α MAPK inhibitor with an IC_{50} in the single digit nanomolar range by introduction of a 2-(4-fluorophenyl)acetamide moiety at the pyridine C2 position. Apart from the specific interactions with the hydrophobic region II of the kinase by optimal arrangement on the lipophilic surface of the solvent-exposed front region, additional polar interactions of the 4-fluorosubstituent are possible with the solvent area surrounding the hydrophobic region II to increase the biological activity of compound **8f**.[10,49] A comparison between the two enantiomers (*R*)-**9f** and (*S*)-**9f** and the corresponding sulfoxide racemate **9f** showed that the two single enantiomers (*R*)-**9f** and (*S*)-**9f** differed again in their affinities for the enzyme by a factor of 1.8, whereas the enantiomer with *R*-configuration was the more active form ((*R*)-**9f**, IC_{50} = 77 nM; (*S*)-**9f**, IC_{50} = 141 nM) and had an inhibitory potency comparable to that of the sulfoxide racemate **9f** (IC_{50} = 69 nM, Table 1).

Despite the good inhibition in the p38α assay, the 2-thioimidazole derivative **8f** exhibited a weaker effect on the suppression of TNF-α release (**8f**, IC_{50} = 2.98 μM) (Table 1) compared to the prototype inhibitor **1** (IC_{50} = 1.95 μM). The lower cytokine inhibitory potency may be due to increased plasma protein binding or to the accumulation in the lipophilic cell membrane[10] caused by the lipophilic character of the 4-fluorophenylacetamide

Table 2. Biological Activity of Trisubstituted 2-Alkylaminopyridinyl Imidazoles: Effect of Isopropylamino and Alkylsulfanyl Substituents



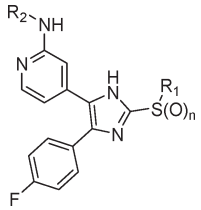
Compound	*ee* [%]	R_1	R_2	p38α IC_{50}±SEM [μM][a]	RP[b]	TNF-α IC_{50}±SEM [μM][c]	RP[b]
8b (n= 0)		$-CH_3$		0.002±2.6e^{-4} (**1**: 0.036±0.001)	18	0.13±0.02 (**1**: 1.56±0.61)	12.0
9b (n= 1)		$-CH_3$		0.002±2.3e^{-4} (**1**: 0.015±0.003)	7.5	0.47±0.09 (**1**: 1.23±0.49)	2.62
(*R*)-9b (n= 1)	99	$-CH_3$		0.012±0.001 (**1**: 0.061±0.004)	5.08	0.53±0.04 (**1**: 1.92±0.27)	3.62
(*S*)-9b (n= 1)	98	$-CH_3$		0.024±0.001 (**1**: 0.061±0.004)	2.54	0.84±0.15 (**1**: 1.92±0.27)	2.29
8e (n= 0)				0.004±0.9e^{-4} (**1**: 0.015±0.003)	3.75	0.12[d] (**1**: 2.33[d])	19.42
9e (n= 1)				0.003±3.5e^{-4} (**1**: 0.015±0.003)	5	0.25±0.02 (**1**: 1.23±0.49)	4.92
(*R*)-9e (n= 1)	62			0.004±4.2e^{-4} (**1**: 0.037±0.007)	9.25	0.45±0.10 (**1**: 1.91±0.16)	4.24
(*S*)-9e (n= 1)	58			0.006±2.2e^{-4} (**1**: 0.037±0.007)	6.17	0.61±0.18 (**1**: 1.91±0.16)	3.13

[a] Mean ± SEM of three experiments. [b] Relative potency IC_{50}(**1**)/IC_{50}(test compound). [c] Mean ± SEM of two experiments. [d] One experiment.

residue in compound **8f**. The ability to inhibit the TNF-α release was further reduced by the oxidation of the sulfur atom on the imidazole core C2 position, as indicated by the elevated IC_{50} in Table 1. In contrast to the p38α assay, the sulfoxide derivatives **9f**, (*R*)-**9f**, and (*S*)-**9f** were poor inhibitors of TNF-α release, with no significant differences among their IC_{50} values: stereochemistry had no apparent effect on inhibiting the release of this cytokine. These results can be explained by means of the plasma protein binding, involving multiple binding proteins like albumin, lipoproteins and glycoproteins, whereas the binding protein concentrations may vary in the plasma of the two anonymous donors. With regard to the fact that the binding proteins have a large number of drug binding sites, e.g., five to seven binding sites in albumin, it is possible that the inhibitors may simultaneously attach to more than one binding site by nonspecific interactions with the protein, independent of a preferred binding geometry of the enantiomers and without consideration of the stereospecificity. As a result of the nonspecific bindings, both enantiomers have the same inhibitory effect. Additional post-translational modifications such as glycosylation and other meaningful factors like cell−cell interactions, diffusion processes, and different enzyme expression levels[47] can have effects on the drug binding in immune cells.

In earlier studies, 2-alkylaminopyridinylimidazole derivatives could be identified as potent inhibitors of the p38α MAP kinase and for cytokine release in the human whole blood.[10] Therefore, we continued our investigation of the inhibitory potency of the enantiomerically pure sulfoxides with a variety of trisubstituted 2-thioimidazoles with additional (cyclo)alkylamino substituents in the C2 position of the pyridine ring to enhance the hydrophilic character and to satisfy the Lipinski rules.[10,50] The introduction of an isopropylamino substituent at the C2 position of the pyridine-4-yl moiety led to p38α MAPK inhibitors with good to excellent potency, as expressed by IC_{50} concentrations in the double-digit to the single-digit nanomolar range (Table 2). In the isolated p38α MAP kinase assay, the 2-thioimidazole derivative **8b** with an IC_{50} of 2 nM exhibited an 18-fold improved inhibitory activity related to the reference compound **1**. Even though the oxidation of the sulfur atom is associated with a loss of activity, the sulfoxide derivatives **9b**, (*R*)-**9b**, and (*S*)-**9b** were nevertheless very efficient inhibitors for the p38 MAP kinase, and the p38α enzyme discriminated stereoselectively between the two enantiomers. In contrast to the

Table 3. Biological Activity of Trisubstituted 2-Alkylaminopyridinyl Imidazoles: Effect of Cyclohexyl and Tetrahydropyranyl Substituents



Compound	*ee* [%]	R_1	R_2	p38α IC_{50}±SEM [μM][a]	RP[b]	TNF-α IC_{50}±SEM [μM][c]	RP[b]
8i (n= 0)		-CH_3		0.027±0.002 (**1**: 0.078±0.006)	2.89	0.17±0.04 (**1**: 1.23±0.49)	7.24
9i (n= 1)		-CH_3		0.034±0.001 (**1**: 0.057±0.001)	1.68	0.62±0.20 (**1**: 1.23±0.49)	1.98
(*R*)-**9i** (n= 1)	97	-CH_3		0.029±0.001 (**1**: 0.074±0.01)	2.55	0.55±0.07 (**1**: 1.70±0.07)	3.09
(*S*)-**9i** (n= 1)	98	-CH_3		0.050±0.001 (**1**: 0.074±0.01)	1.48	0.59±0.22 (**1**: 1.23±0.49)	2.08
8c (n= 0)		H_3C CH_3		0.012 (n=1) (**1**: 0.050±0.002)	4.17	0.65±0.26 (**1**: 1.86±0.08)	2.86
9c (n= 1)		H_3C CH_3		0.018±0.001 (**1**: 0.039±0.004)	2.17	1.08±0.44 (**1**: 1.86±0.08)	1.72
(*R*)-**9c** (n= 1)	20	H_3C CH_3		0.011±0.3e^{-4} (**1**: 0.034±0.003)	3.09	0.25±0.10 (**1**: 1.91±0.16)	7.64
(*S*)-**9c** (n= 1)	95	H_3C CH_3		0.021±3.4e^{-4} (**1**: 0.034±0.003)	1.62	0.34±0.07 (**1**: 1.91±0.16)	5.62
8d (n= 0)		-CH_3		0.005±2.0e^{-4} (**1**: 0.049±0.014)	9.8	0.18±0.03 (**1**: 1.92±0.27)	10.67
9d (n= 1)		-CH_3		0.012±2.8e^{-4} (**1**: 0.056±0.003)	4.67	0.34±0.11 (**1**: 1.23±0.49)	3.62
(*R*)-**9d** (n= 1)	98	-CH_3		0.011±2.9e^{-4} (**1**: 0.049±0.014)	4.45	0.79±0.25 (**1**: 1.70±0.49)	2.15
(*S*)-**9d** (n= 1)	95	-CH_3		0.034±0.002 (**1**: 0.047±0.004)	1.38	0.83±0.34 (**1**: 1.70±0.49)	2.05

[a] Mean ± SEM of three experiments. [b] Relative potency IC_{50}(**1**)/IC_{50}(test compound). [c] Mean ± SEM of two experiments.

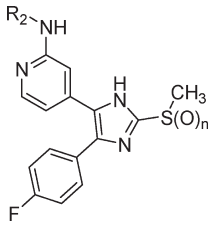
sulfoxide enantiomer (*R*)-**9b**, a 2-fold reduced binding affinity to the enzyme was observed for the enantiomer (*S*)-**9b**. Consequently, the enantiomer (*R*)-**9b** must account for the majority of the inhibitory potency of the sulfoxide racemate **9b** (Table 2).

Results of similar quality were achieved in the human whole blood assay, in which these derivatives proved to be efficient inhibitors of cytokine release with low micromolar IC_{50} values. Here again, the enantiomer (*R*)-**9b** is the more active form and determines the inhibitory activity of the sulfoxide racemate **9b**. The 2- to 12-fold improvement in the inhibition of TNF-α release of the derivatives **8b**, **9b**, (*R*)-**9b**, and (*S*)-**9b** can be attributed to a multitude of factors like improved water solubility or reduced plasma protein binding, associated with a low accumulation in the lipophilic cell membrane.

To investigate the possible interactions of these compounds with the phosphate binding region of the kinase,[10] we replaced the C2-*S*-methyl substituent in the 2-thioimidazole derivative **8b** with a bulky isopropyl group to create the 2-thioimidazole derivative **8e** (Table 2). In contrast to the sterically less hindered analogue compound **8b**, new compound **8e** exhibited a significant 4.8-fold decrease in the p38α relative inhibitory potency. Possibly, access to the entrance in the binding pocket of the p38α MAPK is rendered more difficult by space-filling substituents on the sulfur atom of the imidazole core, and weaker enzyme–ligand interactions occur.[10] While the introduction of the isopropyl group on the sulfur atom in compound **8e** had a negative effect on the p38α inhibition, there was no significant difference in TNF-α release between the 2-alkylsulfanylimidazole derivatives **8b** (IC_{50} = 0.13 μM) and **8e** (IC_{50} = 0.12 μM), perhaps because of similar degrees of cell penetration and plasma protein binding.

In contrast to the aforementioned sulfoxide derivatives, the sulfoxide racemate **9e** as well as both single enantiomers (*R*)-**9e** and (*S*)-**9e** displayed increased inhibitory activity in the isolated p38 enzyme assay compared with the corresponding

Table 4. Biological Activity of Trisubstituted 2-Alkylaminopyridinylimidazoles: Effect of Chiral Substituents



Compound	*ee*	R_2	p38α IC_{50}±SEM [μM][a]	RP[b]	TNF-α IC_{50}±SEM [μM][c]	RP[b]
8j (n= 0)		(S)-	0.026±2.7e^{-4} (**1**: 0.047±0.004)	1.81	0.05±0.01 (**1**: 1.56±0.61)	31.20
9j (n= 1)		(S)-	0.017±0.002 (**1**: 0.058±0.004)	3.41	0.15±0.04 (**1**: 1.56±0.61)	10.40
(*R*)-9j (n= 1)	96	(S)-	0.019±0.004 (**1**: 0.058±0.004)	3.05	0.20±0.06 (**1**: 1.70±0.49)	8.50
(*S*)-9j (n= 1)	93	(S)-	0.027±0.002 (**1**: 0.058±0.004)	2.15	0.25±0.11 (**1**: 1.70±0.49)	6.80
8k (n= 0)		(R)-	0.040±0.002 (**1**: 0.072±0.005)	1.80	0.18±0.03 (**1**: 1.56±0.61)	8.67
9k (n= 1)		(R)-	0.061±0.004 (**1**: 0.072±0.005)	1.18	1.34±0.39 (**1**: 1.23±0.49)	0.92
(*R*)-9k (n= 1)	93	(R)-	0.031±0.001 (**1**: 0.072±0.005)	2.32	0.90±0.22 (**1**: 1.55±0.16)	1.72
(*S*)-9k (n= 1)	94	(R)-	0.036±0.006 (**1**: 0.052±0.005)	1.44	0.75±0.03 (**1**: 1.55±0.16)	2.07

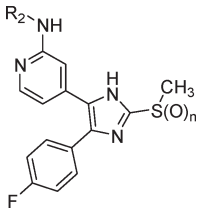
[a] Mean ± SEM of three experiments. [b] Relative potency IC_{50}(**1**)/IC_{50}(test compound). [c] Mean ± SEM of two experiments.

2-alkysulfanylimidazole derivative **8e**. Although the ee values of 62% (*R*-enantiomer) and 58% (*S*-enantiomer) are only moderate, the p38α enzyme showed a clear preference for the enantiomer with *R*-configuration (IC_{50} = 4 nM), as indicated by the 9-fold enhanced relative inhibitory activity with respect to the standard **1**. The outcome also suggests that the *R*-enantiomer contributes to the potency of the sulfoxide racemate **9e** to a great extent. Possibly, the polar sulfoxide group can form additional interactions with the phosphate binding or ribose regions to amplify the biological activity of these derivatives. Unfortunately, the increased activity of the sulfoxide derivatives in comparison to the 2-alkylsulfanylimidazole derivative **8e** was not maintained in the whole blood system, but their cytokine inhibitory potency still exceeded that of the reference substance **1**, whereas the sulfoxide racemate **9e** and the *R*-enantiomer had an equivalent effect on the TNF-α release.

We then prepared a series of compounds modified with a cyclohexylamino or a tetrahydropyran-4-ylamino residue at the C2 position of the pyridine-4-yl moiety to investigate possible interactions with the hydrophobic region II and its surrounding solvent-exposed area. These analogues were very potent inhibitors in the isolated p38α enzyme assay with IC_{50} in the low double-digit to single-digit nanomolar range, and they were also effective suppressors of TNF-α release in the human whole blood model (Table 3). The enantiomers with *R*-configuration had generally higher affinities for the p38α enzyme, combined with an increase of potency, than the corresponding optical antipodes. For example, the enantiomer (*R*)-**9d** (IC_{50} = 11 nM) was equipotent with the sulfoxide racemate **9d** (IC_{50} = 12 nM), but was 3 times better than the enantiomer (*S*)-**9d** (IC_{50} = 34 nM). Consequently, the biological activity of the sulfoxide racemates is predominately determined by the binding affinity of the *R*-enantiomer, whereas the *S*-enantiomer plays a subordinate role. The two sulfoxide enantiomers (*R*)-**9c** and (*S*)-**9c**, bearing an isopropyl group on the sulfur atom of the imidazole core, were more effective for the prevention of TNF-α release compared to the sulfide **8c** and the sulfoxide racemate **9c** (Table 3). Although the enantiomeric excess of the enantiomer (*R*)-**9c** was very low (20% ee), it shows a 2.7- or 4.4-fold increase of inhibitory potency relative to compounds **8c** and **9c**, respectively. The results obtained with these more hydrophobic compounds may be attributed to a combination of different factors as mentioned above.

Apart from the stereogenic center generated by oxidation of the sulfur atom in the heterocyclic sulfoxide derivatives, additional chirality from different amino substituents at the C2 position of the pyridine ring may encourage interactions with the p38α enzyme, leading to improved biological activities related to the reference

Table 5. Biological Activity of Trisubstituted 2-Arylalkylaminopyridinyl Imidazoles: Effect of Chiral Substituents



Compound	*ee*	R_2	p38α IC_{50}±SEM [μM][a]	RP[b]	TNF-α IC_{50}±SEM [μM][c]	RP[b]
8g (n= 0)		(R)-	0.065±0.003 (**1**: 0.078±0.006)	1.20	n. t.	
9g (n= 1)		(R)-	0.207±0.010 (**1**: 0.057±0.001)	0.28	n. t.	
(*R*)-**9g** (n= 1)	93	(R)-	0.168±0.004 (**1**: 0.083±0.003)	0.49	n. t.	
(*S*)-**9g** (n= 1)	93	(R)-	0.20±0.008 (**1**: 0.083±0.003)	0.42	n. t.	
8h (n= 0)		(S)-	0.050±0.003 (**1**: 0.050±0.002)	1.0	1.93±0.34 (**1**: 1.70±0.07)	0.88
9h (n= 1)		(S)-	0.095±0.004 (**1**: 0.050±0.002)	0.53	6.28±3.16 (**1**: 1.56±0.61)	0.25
(*R*)-**9h** (n= 1)	97	(S)-	0.025±0.001 (**1**: 0.028±0.002)	1.12	10.02±6.08 (**1**: 1.56±0.61)	0.16
(*S*)-**9h** (n= 1)	99	(S)-	0.045±0.002 (**1**: 0.028±0.002)	0.62	5.52±2.47 (**1**: 1.56±0.61)	0.28

[a] Mean ± SEM of three experiments. [b] Relative potency IC_{50}(**1**)/IC_{50}(test compound). [c] Mean ± SEM of two experiments. n.t.: not tested.

molecule **1** (Table 4). While the differences in the biological activities in the isolated p38α enzyme assay are only marginal for the both 2-thioimidazole derivatives **8j** and **8k** and their corresponding sulfoxide derivatives, a significant increase in the cytokine relative inhibitory potency was observed for the pyridinylimidazole derivatives **8j**, **9j**, (*R*)-**9j**, and (*S*)-**9j** modified with the *S*-configurated 3-methylbutan-2-amino side chain residue at the pyridine moiety. The 2-thioimidazole derivative **8j** with an IC_{50} of 52 nM exhibited a 30-fold improvement in the suppression of TNF-α release with respect to the reference **1**, but the oxidation of the sulfur atom (**9j**) is associated with a loss of activity (Table 4). However, the sulfoxide derivatives **9j**, (*R*)-**9j**, and (*S*)-**9j** were very efficient inhibitors for the suppression of TNF-α release with IC_{50} concentrations in the low micromolar range. The highly potent inhibition of cytokine release by these pyridinylimidazole derivatives can also be explained by the aforementioned parameters in the context of preferred stereoselective interactions with the enzyme, evoked by the chirality of the alkylamino side chain residue and by the discrimination of the optical antipode.

Furthermore, the introduction of the chiral phenylethylamino residue at the C2 position of the pyridine ring, analogous to the two known compounds **3** and **4**, also indicates that the *S*-enantiomer of the residue is the predominant stereoisomer in combination with the *R*-configured sulfur atom such as in the sulfoxide derivative (*R*)-**9h** (Table 5), which has similar relative biological activity against the p38 MAPK as the corresponding sulfide derivative **8h**. A 2-fold increased relative potency of (*R*)-**9h** was observed in comparison to the sulfoxide racemate **9h** and the *S*-enantiomer (*S*)-**9h**. The resulting fluctuations in the data derived from the human whole blood model using samples from two anonymous donors can also be ascribed to the above-mentioned parameters and are intensified by the high lipophilic character of the introduced arylalkyl residue.

CONCLUSION

We present three practical and convenient methods to achieve high enantiomeric excesses up to 99% in the asymmetric

oxidation of a wide range of tri- and tetrasubstituted 2-thioimidazoles to the corresponding sulfoxides by the use of suitable metal−ligand catalysts. Depending on the application of the chiral ligands, the asymmetric oxidation can be controlled with respect to a single enantiomer so that both enantiomers E_1 and E_2 are synthetically accessible by the methods described here. We have also shown the influence of the substitution pattern of the heterocyclic 2-thioimidazoles on the enantioselectivity of the oxidation reaction. The identification of the absolute configuration of the sulfur atom was performed by X-ray analysis, and the first eluted enantiomer E_1 was shown to be in the *R*-configuration and the second eluted enantiomer E_2 was in the *S*-configuration. The pyridinylimidazole derivatives were evaluated in the isolated p38α enzyme assay and in the human whole blood model and were shown to be very potent inhibitors of the p38 MAPK. The enantiomerically pure sulfoxides with the *R*-configuration displayed a generally higher affinity for the enzyme, which was associated with an increased inhibitory potency in comparison with the optical antipode.

EXPERIMENTAL SECTION

General. All reagents and solvents were of commercial quality and used without further purification. NMR data were recorded at ambient temperature on a Bruker Avance 200 at 200 and 50 MHz or on a Bruker Avance 400 at 400 and 100 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the solvent resonance. High-resolution mass spectral analyses were performed on a Bruker APEX II spectrometer using electron spray ionization (ESI) or on a Finnigan Sektorfield mass spectrometer using electron impact (EI). HPLC separation of the sulfoxide enantiomers was achieved on Daicel Chiralpak IA (250 mm × 4.6 mm, dp = 5 μm, CH_2Cl_2/MeOH/TEA 98:2:0.1) on an HP 1090 instrument using 0.8 mL/min flow rate and UV detection at 254 nm. The purities of the final compounds were determined by HPLC on a HPLC Hewlett-Packard HP 1090 series II liquid chromatograph equipped with a UV diode array detector (DAD) (detection at 230 and 254 nm) using a Thermo Betasil C_8 column (150 mm × 4.6 mm, dp = 5 μm) or a ZORBAX Eclipse XDB-C_8 column (150 mm × 4.6 mm, dp = 5 μm), 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 have a purity of >95% (see Supporting Information for details).

General Procedure A for the Synthesis of Tri- and Tetrasubstituted 2-Thioimidazoles. The reaction mixture, composed of the specific amine (3.0−23.0 equiv) and the 2-thioimidazole derivative (1.0 equiv), was heated at T = 160 °C in a high pressure reactor (Berghof). The progress was monitored until HPLC analysis indicated complete conversion. After the mixture was cooled to room temperature, an aqueous saturated solution of $NaHCO_3$ was added (pH 8−9). The mixture was extracted with EtOAc, dried over Na_2SO_4, and evaporated. The crude product was purified by recrystallization from an appropriate solvent.

4-(4-(4-Fluorophenyl)-2-(methylthio)-1*H*-imidazol-5-yl)-*N*-isopropylpyridin-2-amine 8b. According to general procedure A, compound **6a** (20.0 g, 66.0 mmol) and isopropylamine (15.13 g, 251.0 mmol) were heated for 20 h at T = 160 °C in a high pressure reactor. After extraction with a solvent mixture of EtOAc/THF, the crude product was purified by recrystallization from EtOAc to yield 16.10 g (71.2%) of the pale yellow product **8b**. ^{1}H NMR (200 MHz, methanol-d_4): δ [ppm] = 1.09 (d, J = 6.3 Hz, 6H, CH(CH_3)$_2$), 2.54 (s, 3H, SCH_3), 3.71−3.90 (m, 1H, CH(CH_3)$_2$), 6.39−6.50 (m, 2H, C^3-$H_{Pyridine}$, C^5-$H_{Pyridine}$), 6.99−7.13 (m, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.31−7.45 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.74 (d, J = 5.9 Hz, 1H, C^6-$H_{Pyridine}$). HR-MS (ESI) calculated for $C_{18}H_{19}FN_4S$ [M + H$^+$] m/z = 343.1387, measured m/z = 343.1386.

***N*-Cyclohexyl-4-(4-(4-fluorophenyl)-2-(isopropylthio)-1*H*-imidazol-5-yl)pyridin-2-amine 8c.** According to general procedure A, compound **6b** (5.0 g, 15.1 mmol) and cyclohexylamine (19.97 g, 197 mmol) were heated for 18 h at T = 160 °C in a high pressure reactor. After extraction with a solvent mixture of EtOAc/THF and evaporation, the title compound **8c** precipitated as a colorless solid of high analytical quality, which was filtered off and washed with isopropylether (4.10 g, 68.3%). ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 0.96−1.24 (m, 5H, CH_2, cyclohexyl), 1.31 (d, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 1.46−1.74 (m, 3H, CH_2, cyclohexyl), 1.75−1.92 (m, 2H, CH_2, cyclohexyl), 3.38−3.54 (m, 1H, CH, cyclohexyl), 3.60 (sept, J = 6.7 Hz, 1H, CH(CH$_3$)$_2$), 6.28 (d, J = 7.8 Hz, $NH_{Cyclohexyl}$), 6.40 (d, J = 5.6 Hz, 1H, C^5-$H_{Pyridine}$), 6.51 (s, 1 H, C^3-$H_{Pyridine}$), 7.21 (t, J = 8.8 Hz, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.40−7.56 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.83 (d, J = 5.3 Hz, 1H, C^6-$H_{Pyridine}$), 11.85 (brs, 1H, $NH_{Imidazole}$). HR-MS (ESI) calculated for $C_{23}H_{27}FN_4S$ [M + H$^+$] m/z = 411.2013, measured m/z = 411.2013.

4-(4-(4-Fluorophenyl)-2-(isopropylthio)-1*H*-imidazol-5-yl)-*N*-isopropylpyridin-2-amine 8e. According to general procedure A, the title compound **8e** was obtained from compound **6b** (1.50 g, 4.6 mmol) and isopropylamine (6.38 g, 106.0 mmol) after heating for 27 h at T = 160 °C in a high pressure reactor and treatment with isopropyl ether as a colorless solid (1.30 g, 79.7%). ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 1.07 (d, J = 5.3 Hz, 6H, SCH(CH_3)$_2$), 1.31 (d, J = 6.2 Hz, 6H, NHCH(CH_3)$_2$), 3.48−3.74 (m, 1H, SCH(CH_3)$_2$), 3.77−4.10 (m, 1H, NHCH(CH_3)$_2$), 6.19−6.64 (m, 2H, C^3-$H_{Pyridine}$, C^5-$H_{Pyridine}$), 7.02−7.37 (m, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.38−7.63 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.74−7.94 (m, 1H, C^6-$H_{Pyridine}$), 12.66 (brs, 1H, $NH_{Imidazole}$). HR-MS (ESI) calculated for $C_{20}H_{23}FN_4S$ [M + H$^+$] m/z = 371.1700, measured m/z = 371.1700.

2-(4-Fluorophenyl)-*N*-(4-(4-(4-fluorophenyl)-2-(methylthio)-1*H*-imidazol-5-yl)pyridin-2-yl)acetamide 8f. Under an argon atmosphere, *N*,*N*′-carbonyldiimidazole (2.59 g, 15.7 mmol) was added to a stirred solution of 4-fluorophenylacetic acid (2.31 g, 14.7 mmol) in 12.0 mL of dry NMP at room temperature. When gas evolution ceased, compound **7a** (1.50 g, 5.0 mmol) was added and the resulting reaction mixture was stirred at T = 120 °C for 2 h. After the mixture was cooled to room temperature, an aqueous saturated solution of $NaHCO_3$ was added (pH 8−9) and the mixture was extracted with EtOAc, dried over Na_2SO_4, and concentrated in vacuo. The title compound **8f** was achieved as a yellow solid (1.56 g, 72.8%) of high analytical quality without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 2.59 (s, 3H, SCH_3), 3.67 (s, 2H, CH_2), 6.99 (d, J = 5.1 Hz, 1H, C^5-$H_{Pyridine}$), 7.06−7.53 (m, 8H, C^2-$H_{4\text{-Fluorophenyl}}$, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$, C^2-$H_{4\text{-Fluorophenylacetamide}}$, C^3-$H_{4\text{-Fluorophenylacetamide}}$, C^5-$H_{4\text{-Fluorophenylacetamide}}$, C^6-$H_{4\text{-Fluorophenylacetamide}}$), 8.11 (d, J = 5.3 Hz, 1H, C^6-$H_{Pyridine}$), 8.29 (s, 1H, C^3-$H_{Pyridine}$), 10.58 (s, 1H, NHCO), 12.69 (s, 1H, $NH_{Imidazole}$). HR-MS (ESI) calculated for $C_{23}H_{18}F_2N_4OS$ [M + H$^+$] m/z = 437.1242, measured m/z = 437.1246.

4-(4-(4-Fluorophenyl)-2-(methylthio)-1*H*-imidazol-5-yl)-*N*-((*S*)-3-methylbutan-2-yl)pyridin-2-amine 8j. According to general procedure A, compound **6a** (2.0 g, 6.60 mmol) and (*S*)-(+)-3-methyl-2-butylamine (2.47 g, 27.7 mmol) were heated for 20 h at T = 160 °C in a high pressure reactor. After extraction with EtOAc, the crude product was purified by flash chromatography on silica gel (EtOAc/hexane 2:3) to yield 1.40 g (57.3%) of the pale yellow product **8j**. ^{1}H NMR (methanol-d_4, 400 MHz) δ = 0.82−0.97 (m, 6H, CH(CH_3)$_2$), 1.08 (d, J = 6.6 Hz, 3H, NHCHCH_3), 1.65−1.81 (m, 1H, CH(CH_3)$_2$), 2.63 (s, 3H, S-CH_3), 3.44−3.57 (m, 1H, NHCHCH$_3$) 6.46−6.60 (m, 2H, C^5-$H_{Pyridine}$ and C^3-$H_{Pyridine}$), 7.14 (t, J = 8.5 Hz, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$) 7.40−7.51 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.80 (d, J = 5.1 Hz, 1H, C^6-$H_{Pyridine}$). HR-MS calculated for $C_{20}H_{23}FN_4S$ m/z = 370.1622, measured m/z = 370.1629.

4-(4-(4-Fluorophenyl)-2-(methylthio)-1*H*-imidazol-5-yl)-*N*-((*R*)-3-methylbutan-2-yl)pyridin-2-amine 8k. According to general procedure A, compound **6a** (0.76 g, 2.5 mmol) and (*R*)-(−)-3-methyl-2-butylamine (0.67 g, 7.5 mmol) were heated for 24 h at T = 160 °C in a high pressure reactor. After extraction with EtOAc, the crude product was purified by flash chromatography on silica gel (EtOAc/hexane 2:3) to yield 0.56 g (60.5%) of the pale yellow product **8k**. ^{1}H NMR (methanol-d_4, 400 MHz) δ = 0.85−0.97 (m, 6H, CH(CH_3)$_2$), 1.09 (d, J = 6.6 Hz, 3H, NHCHCH_3), 1.67−1.80 (m, 1H, CH(CH_3)$_2$), 2.63 (s, 3H, S-CH_3), 3.46−3.56 (m, 1H, NHC$H$$CH_3$), 6.48−6.63 (m, 2H, C^5-$H_{Pyridine}$ and C^3-$H_{Pyridine}$), 7.16 (t, J = 8.3 Hz, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.42−7.52 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.80 (d, J = 4.6 Hz, 1H, C^6-$H_{Pyridine}$). HR-MS calculated for $C_{20}H_{23}FN_4S$ m/z = 370.1622, measured m/z = 370.1620.

***N*-(4-(4-(4-Fluorophenyl)-2-(methylthio)-1*H*-imidazol-5-yl) pyridin-2-yl)acetamide 8l.** Compound **7a** (0.51 g, 1.70 mmol) was dissolved in 5.0 mL of pyridine. The solution was cooled in an ice bath, and acetylchloride (0.47 g, 6.0 mmol) was added dropwise. The resulting mixture was stirred at T = 0−5 °C. After 1 h, the solution was allowed to warm to room temperature and an aqueous solution of $NaHCO_3$ was added. The aqueous/organic mixture was extracted with EtOAc, dried over Na_2SO_4, and the solvents of the combined organic phases were removed under reduced pressure. The crude product was purified by flash chromatography (silica gel, EtOAc/EtOH 9/1) to obtain 0.3 g (52.4%) of the yellow solid **8l**. ^{1}H NMR (methanol-d_4, 400 MHz) δ = 2.14 (s, 3H, CO-CH_3), 2.65 (s, 3H, S-CH_3), 7.11−7.19 (m, 3H, C^5-$H_{Pyridine}$ and C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorphenyl}}$), 7.40−7.52 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 8.05−8.22 (m, 2H, C^6-$H_{Pyridine}$ and C^3-$H_{Pyridine}$). HR-MS calculated for $C_{17}H_{15}FN_4OS$ m/z = 342.0945, measured m/z = 342.0978.

General Procedure B for the Synthesis of the Racemic Sulfoxides. The respective 2-thioimidazole derivative was dissolved in THF, and water was added. The mixture was cooled in an ice bath, and an aqueous solution of potassium peroxomonosulfate (Oxone) was added dropwise. The resulting mixture was stirred at T = 0 °C. The progress was monitored until HPLC analysis indicated complete conversion. After reaction completion, the solution was allowed to warm to room temperature and an aqueous solution of $NaHCO_3$ (pH 8) was added. The aqueous/organic mixture was extracted with EtOAc, dried over Na_2SO_4, and the solvents of the combined organic phases were removed under reduced pressure. The crude product was purified by recrystallization from an appropriate solvent or by flash chromatography.

4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)-*N*-isopropylpyridin-2-amine 9b. According to general procedure B, the title compound **9b** was obtained by the sulfoxidation of compound **8b** (9.31 g, 27.2 mmol), dissolved in 177.0 mL of THF, with an aqueous solution of potassium peroxomonosulfate (Oxone) (8.76 g, 14.3 mmol in 183 mL water) after stirring for 2 h at T = 0 °C and recrystallization from EtOAc as a beige solid (7.97 g, 81.9%). ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 1.10 (d, J = 6.3 Hz, 6H, NHCH(CH_3)$_2$), 3.07 (s, 3H, SOCH_3), 3.74−4.07 (m, 1H, CH(CH_3)$_2$), 6.34 (s, 1H, NHCH(CH_3)$_2$, exchangeable with D_2O), 6.42 (d, J = 5.2 Hz, 1H, C^5-$H_{Pyridine}$), 6.58 (s, 1H, C^3-$H_{Pyridine}$), 7.26 (t, J = 8.6 Hz, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.42−7.63 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.89 (d, J = 5.1 Hz, 1H, C^6-$H_{Pyridine}$), 13.73 (s, 1H, $NH_{Imidazole}$, exchangeable with D_2O). HR-MS (ESI) calculated for $C_{18}H_{19}FN_4OS$ [M + H$^+$] m/z = 359.1336, measured m/z = 359.1336.

***N*-Cyclohexyl-4-(4-(4-fluorophenyl)-2-(isopropylsulfinyl)-1*H*-imidazol-5-yl)pyridin-2-amine 9c.** According to general procedure B, the title compound **9c** was obtained by the sulfoxidation of compound **8c** (0.31 g, 0.76 mmol), dissolved in 15.0 mL of THF and 3.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.25 g, 0.4 mmol in 4.5 mL of H_2O) after stirring for 3.5 h at T = 0 °C as a colorless solid without further purification (0.21 g, 65.8%). ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 0.96−1.24 (m, 7H, CH_2, cyclohexyl and SOCH(CH_3)$_2$), 1.19−1.34 (4H, CH_2, cyclohexyl and SOCH(CH_3)$_2$), 1.44−1.90 (m, 5H, CH_2, cyclohexyl), 3.33−3.62 (m, 2H, CH, cyclohexyl and SOCH(CH_3)$_2$), 6.29 (brs, 1H, $NH_{Cyclohexyl}$, exchangeable with D_2O), 6.39 (d, J = 5.6 Hz, 1H, C^5-$H_{Pyridine}$), 6.53 (s, 1H, C^3-$H_{Pyridine}$), 7.12−7.34 (m, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.40−7.56 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.83 (s, 1H, C^6-$H_{Pyridine}$), 13.66 (s, 1H, $NH_{Imidazole}$, exchangeable with D_2O). HR-MS (ESI) calculated for $C_{23}H_{27}FN_4OS$ [M + H$^+$] m/z = 427.1962, measured m/z = 427.1964.

4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)-*N*-(tetrahydro-2*H*-pyran-4-yl)pyridin-2-amine 9d. According to general procedure B, the title compound **9d** was obtained by the sulfoxidation of compound **8d** (8.0 g, 20.8 mmol), dissolved in 154.0 mL of THF and 52.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (7.0 g, 11.4 mmol in 77.0 mL of H_2O) after stirring for 2 h at T = 0 °C and recrystallization from MeOH as a colorless solid (6.86 g, 85.7%). ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 1.24−1.53 (m, 2H, C^3-$H_{Tetrahydropyranyl}$, C^5-$H_{Tetrahydropyranyl}$), 1.67−1.90 (m, 2H, C^3-$H_{Tetrahydropyranyl}$, C^5-$H_{Tetrahydropyranyl}$), 3.06 (s, 3H, SOCH_3), 3.22−3.44 (m, 2H, C^2-$H_{Tetrahydropyranyl}$, C^6-$H_{Tetrahydropyranyl}$), 3.64−3.94 (m, 3H, C^2-$H_{Tetrahydropyranyl}$, C^4-$H_{Tetrahydropyranyl}$, C^6-$H_{Tetrahydropyranyl}$), 6.47 (d, J = 5.1 Hz, 1H, C^5-$H_{Pyridine}$), 6.50−6.71 (m, 2H, C^3-$H_{Pyridine}$ and $NH_{Tetrahydropyranyl}$, exchangeable with D_2O), 7.27 (t, J = 8.7 Hz, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.43−7.62 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.89 (brs, 1H, C^6-$H_{Pyridine}$), 13.75 (s, 1H, $NH_{Imidazole}$, exchangeable with D_2O). HR-MS (ESI) calculated for $C_{20}H_{21}FN_4O_2S$ [M + H$^+$] m/z = 401.1442, measured m/z = 401.1442.

4-(4-(4-Fluorophenyl)-2-(isopropylsulfinyl)-1*H*-imidazol-5-yl)-*N*-isopropylpyridin-2-amine 9e. According to general procedure B, the title compound **9e** was obtained by the sulfoxidation of compound **8e** (0.25 g, 0.68 mmol), dissolved in 5.0 mL of THF and 1.7 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.24 g, 0.4 mmol in 1.3 mL of H_2O) after stirring for 3 h at T = 0 °C and treatment with isopropyl ether as a beige solid (0.21 g, 83.8%). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.08 (d, J = 6.2 Hz, 6H, NHCH(CH_3)$_2$), 1.15 (d, J = 7.0 Hz, 3H, SOCH(CH_3)$_2$), 1.25 (d, J = 6.8 Hz, 3H, SOCH(CH_3)$_2$), 3.37−3.55 (m, 1H, SOCH(CH_3)$_2$), 3.77−4.02 (m, 1H, NHCH(CH_3)$_2$), 6.27−6.47 (m, 2H, C^5-$H_{Pyridine}$ and NHCH(CH_3)$_2$, exchangeable with D_2O), 6.59 (s, 1H, C^3-$H_{Pyridine}$), 7.09−7.38 (m, 2H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$), 7.42−7.60 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 7.85 (s, 1H, C^6-$H_{Pyridine}$), 13.69 (s, 1H, $NH_{Imidazole}$, exchangeable with D_2O). HR-MS (ESI) calculated for $C_{20}H_{23}FN_4OS$ [M + H$^+$] m/z = 387.1649, measured m/z = 387.1649.

2-(4-Fluorophenyl)-*N*-(4-(4-(4-fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)pyridin-2-yl)acetamide 9f. According to general procedure B, the title compound **9f** was obtained by the sulfoxidation of compound **8f** (0.30 g, 0.69 mmol), dissolved in 5.0 mL of THF and 0.9 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.23 g, 0.4 mmol in 1.3 mL of water) after stirring for 1.5 h at T = 0 °C and flash chromatography on silica gel (THF/hexane 1:1) as a yellow solid (0.16 g, 51.5%). ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 3.06 (s, 3H, SOCH_3), 3.68 (s, 2H, CH_2), 7.01 (d, 1H, J = 5.1 Hz, 1H, C^5-$H_{Pyridine}$), 7.13 (t, J = 8.7 Hz, 2H, C^3-$H_{4\text{-Fluorophenylacetamide}}$, C^5-$H_{4\text{-Fluorophenylacetamide}}$), 7.20−7.41 (m, 4H, C^3-$H_{4\text{-Fluorophenyl}}$, C^5-$H_{4\text{-Fluorophenyl}}$, C^2-$H_{4\text{-Fluorophenylacetamide}}$, C^6-$H_{4\text{-Fluorophenylacetamide}}$), 7.43−7.60 (m, 2H, C^2-$H_{4\text{-Fluorophenyl}}$, C^6-$H_{4\text{-Fluorophenyl}}$), 8.20 (d, 1H, J = 5.1 Hz, 1H, C^6-$H_{Pyridine}$), 8.30 (s, 1H, C^3-$H_{Pyridine}$), 10.70 (s, 1H, NHCO, exchangeable with D_2O), 13.89 (s, 1H, $NH_{Imidazole}$, exchangeable with D_2O). HR-MS (ESI) calculated for $C_{23}H_{18}F_2N_4O_2S$ [M + Na$^+$] m/z = 475.1011, measured m/z = 475.1013.

4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)-*N*-((*R*)-1-phenylethyl)pyridin-2-amine 9g. According to general procedure B, the title compound **9g** was obtained by the sulfoxidation of compound **8g** (0.12 g, 0.30 mmol), dissolved in 3.0 mL of THF and 0.5 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.10 g, 0.17 mmol in 1.0 mL of water) after stirring for 2.5 h at T = 0 °C and flash chromatography (silica gel, EtOAc/MeOH 9:1) as a pale yellow solid (0.08 g, 63.4%). ^{1}H NMR (methanol-d_4, 400 MHz) δ = 1.42−1.50 (m, 3H, CHCH_3), 3.11 (s, 3H, SO-CH_3), 4.63−4.73 (m, 1H, CHCH$_3$), 6.56 (s, 1H, C^3-H$_{Pyridine}$), 6.65 (d, J = 5.3 Hz, 1H, C^5-H$_{Pyridine}$), 7.06−7.20 (m, 3H, C^3-H$_{4\text{-Fluorophenyl}}$, C^5-H$_{4\text{-Fluorophenyl}}$, C^4-H$_{Phenylethylamine}$), 7.21−7.31 (m, 4H, C^2-H$_{Phenylethylamine}$, C^3-H$_{Phenylethylamine}$, C^5-H$_{Phenylethylamine}$, C^6-H$_{Phenylethylamine}$), 7.39−7.49 (m, 2H, C^2-H$_{4\text{-Fluorophenyl}}$, C^6-H$_{4\text{-Fluorophenyl}}$), 7.85 (d, J = 5.3 Hz, 1H, C^6-H$_{Pyridine}$). HR-MS calculated for $C_{23}H_{21}FN_4OS$ m/z = 420.1415, measured m/z = 420.1418.

4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)-*N*-((*S*)-1-phenylethyl)pyridin-2-amine 9h. According to general procedure B, the title compound **9h** was obtained by the sulfoxidation of compound **8h** (12.14 g, 30.0 mmol), dissolved in 273.0 mL of THF and 90.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (9.77 g, 15.9 mmol in 176.0 mL water) after stirring for 2.5 h at T = 0 °C and treatment with a solvent mixture of cyclohexane and isopropyl ether as a colorless solid (11.26 g, 91.8%). ^{1}H NMR (methanol-d_4, 400 MHz) δ = 1.45−1.49 (m, 3H, CHCH_3), 3.11 (s, 3H, SO-CH_3), 4.68 (q, J = 6.6 Hz, 1H, CHCH$_3$), 6.57 (s, 1H, C^3-H$_{Pyridine}$), 6.60−6.69 (m, 1H, C^5-H$_{Pyridine}$), 7.07−7.20 (m, 3H, C^3-H$_{4\text{-Fluorophenyl}}$, C^5-H$_{4\text{-Fluorophenyl}}$, C^4-H$_{Phenylethylamine}$), 7.20−7.30 (m, 4H, C^2-H$_{Phenylethylamine}$, C^3-H$_{Phenylethylamine}$, C^5-H$_{Phenylethylamine}$, C^6-H$_{Phenylethylamine}$), 7.38−7.51 (m, 2H, C^2-H$_{4\text{-Fluorophenyl}}$, C^6-H$_{4\text{-Fluorophenyl}}$), 7.85 (d, J = 5.6 Hz, 1H, C^6-H$_{Pyridine}$). HR-MS calculated for $C_{23}H_{21}FN_4OS$ m/z = 420.1415, measured m/z = 420.1419.

***N*-Cyclohexyl-4-(4-(4-fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)pyridin-2-amine 9i.** According to general procedure B, the title compound **9i** was obtained by the sulfoxidation of compound **8i** (0.15 g, 0.4 mmol), dissolved in 4.0 mL of THF and 1.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.14 g, 0.22 mmol in 1.0 mL water) after stirring for 2 h at T = 0 °C and flash chromatography (silica gel, EtOAc/MeOH 9:1) as a colorless solid (0.09 g, 56.5%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 1.04−1.34 (m, 5H, CH_2, cyclohexyl), 1.49−1.91 (m, 5H, CH_2, cyclohexyl), 3.07 (s, 3H, SO-CH_3), 3.47−3.58 (m, 1H, CH, cyclohexyl), 6.38 (d, J = 7.1 Hz, 1H, $NH_{Cyclohexyl}$), 6.44 (d, J = 5.1 Hz, 1H, C^5-H$_{Pyridine}$), 6.54 (s, 1H, C^3-H$_{Pyridine}$), 7.27 (t, J = 8.5 Hz, 2H, C^3-H$_{4\text{-Fluorphenyl}}$, C^5-H$_{4\text{-Fluorphenyl}}$), 7.53 (dd, J_1 = 8.5 Hz, J_2 = 5.7 Hz, 2H, C^2-H$_{4\text{-Fluorphenyl}}$, C^6-H$_{4\text{-Fluorphenyl}}$), 7.89 (d, J = 4.8 Hz, 1H, C^6-H$_{Pyridine}$), 13.83 (brs, 1H, $NH_{Imidazole}$). HR-MS calculated for $C_{21}H_{23}FN_4OS$ m/z = 398.1571, measured m/z = 398.1580.

4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)-*N*-((*S*)-3-methylbutan-2-yl)pyridin-2-amine 9j. According to general procedure B, the title compound **9j** was obtained by the sulfoxidation of compound **8j** (0.93 g, 2.51 mmol), dissolved in 20.0 mL of THF and 5.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.79 g, 1.30 mmol in 22.0 mL water) after stirring for 2.0 h at T = 0 °C and treatment with a solvent mixture of EtOAc and isopropyl ether as a colorless solid (0.74 g, 78.5%). ^{1}H NMR (methanol-d_4, 400 MHz) δ = 0.92 (t, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 1.09 (d, J = 6.6 Hz, 3H, NHCHCH_3), 1.67−1.81 (m, 1H, CH(CH$_3$)$_2$), 3.15 (s, 3H, SO-CH$_3$), 3.46−3.60 (m, 1H, CH(CH$_3$)$_2$), 6.60 (dd, J_1 = 5.6 Hz, J_2 = 1.3 Hz, 1H, C^5-H$_{Pyridine}$), 6.63 (s, 1H, C^3-H$_{Pyridine}$), 7.13−7.24 (m, 2H, C^3-H$_{4\text{-Fluorophenyl}}$, C^5-H$_{4\text{-Fluorophenyl}}$), 7.56−7.58 (m, 2H, C^2-H$_{4\text{-Fluorophenyl}}$, C^6-H$_{4\text{-Fluorphenyl}}$), 7.84 (d, J = 5.6 Hz, 1H, C^6-H$_{Pyridine}$). HR-MS calculated for $C_{20}H_{23}FN_4OS$ m/z = 386.1571, measured m/z = 386.1559.

4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)-*N*-((*R*)-3-methylbutan-2-yl)pyridin-2-amine 9k. According to general procedure B, the title compound **9k** was obtained by the sulfoxidation of compound **8k** (0.38 g, 1.03 mmol), dissolved in 8.0 mL of THF and 3.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.32 g, 0.5 mmol in 9.0 mL water) after stirring for 4.0 h at T = 0 °C and flash chromatography on silica gel (EtOAc/EtOH 95:5) as a beige solid (0.24 g, 61.1%). ^{1}H NMR (methanol-d_4, 400 MHz) δ = 0.93 (t, J = 6.2 Hz, 6H, CH(CH_3)$_2$), 1.11 (d, J = 6.6 Hz, 3H, NHCHCH_3), 1.69−1.82 (m, 1H, CH(CH$_3$)$_2$), 3.15 (s, 3H, SO−CH$_3$), 3.48−3.58 (m, 1H, CH(CH$_3$)$_2$), 6.66 (d, J = 5.8 Hz, 1H, C^5-H$_{Pyridine}$), 6.72 (s, 1H, C^3-H$_{Pyridine}$), 7.21 (t, J = 8.6 Hz, 2H, C^3-H$_{4\text{-Fluorophenyl}}$, C^5-H$_{4\text{-Fluorphenyl}}$), 7.54 (dd, J_1 = 8.1 Hz, J_2 = 5.3 Hz, 2H, C^2-H$_{4\text{-Fluorophenyl}}$, C^6-H$_{4\text{-Fluorophenyl}}$), 7.82 (d, J = 5.6 Hz, 1H, C^6-H$_{Pyridine}$). HR-MS calculated for $C_{20}H_{23}FN_4OS$ m/z = 386.1571, measured m/z = 386.1587.

***N*-(4-(4-(4-Fluorophenyl)-2-(methylsulfinyl)-1*H*-imidazol-5-yl)pyridin-2-yl)acetamide 9l.** According to general procedure B, the title compound **9l** was obtained by the sulfoxidation of compound **8l** (0.28 g, 0.82 mmol), dissolved in 6.0 mL of THF and 1.0 mL of H_2O, with an aqueous solution of potassium peroxomonosulfate (Oxone) (0.28 g, 0.45 mmol in 1.5 mL water) after stirring for 4.0 h at T = 0 °C and flash chromatography on silica gel (EtOAc/EtOH 95:5) as a colorless solid (0.19 g, 64.7%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 2.02 (s, 3H, CO-CH_3), 3.05 (s, 3H, SO-CH_3), 7.09 (d, J = 4.0 Hz, 1H, C^5-H$_{Pyridine}$), 7.21 (t, J = 8.1 Hz, 2H, C^3-H$_{4\text{-Fluorophenyl}}$, C^5-H$_{4\text{-Fluorophenyl}}$), 7.36−7.49 (m, 2H, C^2-H$_{4\text{-Fluorophenyl}}$, C^6-H$_{4\text{-Fluorophenyl}}$), 8.06−8.21 (m, 2H, C^6-H$_{Pyridine}$, C^3-H$_{Pyridine}$). HR-MS calculated for $C_{17}H_{15}FN_4O_2S$ m/z = 358.0894, measured m/z = 358.0901.

Typical Experimental Procedure for the Asymmetric Oxidation of Tri- and Tetrasubstituted 2-Thioimidazoles with Ti(O-*i*-Pr)$_4$, D-DET or L-DET, and CHP. Ti(O-*i*-Pr)$_4$ (1.0−2.0 equiv) was added rapidly to a solution of D-DET or L-DET (2.0−4.0 equiv) in 10.0 mL of dichloromethane at room temperature. After 2.5 min, water (1.0 equiv) was added slowly. The resulting mixture was stirred initially for 20 min at room temperature, followed by cooling to T = −18 °C for further 20 min. After addition of the respective 2-thioimidazole **8a**−**d**, **8f** (1.0 equiv), and CHP (2.0−4.0 equiv), the resulting mixture was stirred at T = −18 °C until HPLC analysis indicated complete conversion. The mixture was combined with water and extracted with dichloromethane. The organic layer was washed with brine and H_2O, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel to yield the pure sulfoxide. The enantiomeric ratio of the sulfoxides **9a**−**d**, **9f** was determined by enantioselective, analytical HPLC (Daicel Chiralpak IA, 4.6 mm × 250 mm, 5 μm, CH_2Cl_2/MeOH/TEA 98:2:0.1, λ = 254 nm, 0.8 mL/min flow rate).

Typical Experimental Procedure for the Asymmetric Oxidation of Tri- and Tetrasubstituted 2-Thioimidazoles with Fe(acac)$_3$, H_2O_2, and Chiral Schiff Bases (*S*)-12d,[38] (*S*)-12h,[33] (*S*)-12l,[32] and (*R*)-12l. Fe(acac)$_3$ (0.02−0.2 equiv) and the specific chiral Schiff base (*S*)-**12d**, (*S*)-**12h**, (*S*)-**12l**, or (*R*)-**12l** (0.04- 0.4 equiv) were dissolved in 4.0 mL of dichloromethane. After the mixture was stirred at room temperature for 1 h, the resulting solution was added to a suspension of 4-methoxybenzoic acid (0.01−0.2 equiv) in 1.0 mL of dichloromethane. The mixture was stirred for 10 min, followed by the addition of the respective 2-thioimidazole **8a**−**d** (1.0 equiv). The solution was then treated with 32% aqueous H_2O_2 (1.2−2.0 equiv) and was stirred at room temperature until HPLC analysis indicate complete conversion. The mixture was combined with water and extracted with dichloromethane. The organic extract was washed with brine and water and dried over Na_2SO_4. After rotary evaporation, the crude product was purified by flash chromatography on silica gel to obtain the pure sulfoxide. The enantiomeric ratio of the sulfoxides **9a**−**d**

was determined by enantioselective, analytical HPLC (Daicel Chiralpak IA, 4.6 mm × 250 mm, 5 μm, 0.8 mL/min, CH_2Cl_2/MeOH/TEA 98:2:0.1, λ = 254 nm, 0.8 mL/min flow rate).

Typical Experimental Procedure for the Asymmetric Oxidation of Tri- and Tetrasubstituted 2-Thioimidazoles with Ti(O-*i*-Pr)$_4$, *S*-BINOL or *R*-BINOL, and TBHP. Ti(O-*i*-Pr)$_4$ (0.1 equiv) was added dropwise to a solution of *S*-BINOL or *R*-BINOL (0.2 equiv) in 6.0 mL of dichloromethane. After the mixture was stirred for 20 min at room temperature, water (0.2 equiv) and the specific 2-thioimidazole **8a**,**b**, **8d**−**l** (1.0 equiv) were added. The resulting mixture was stirred initially at room temperature for 30 min and was then cooled to T = 5 °C (or room temperature for compound **8e**) for 20 min, followed by the dropwise addition of TBHP (70% in water, 2.0 equiv). The progress of the reaction was monitored until HPLC analysis indicated complete conversion. After reaction completion, the reaction mixture was allowed to warm to room temperature and was stirred for another 45−90 min before being diluted with CH_2Cl_2 and dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography on silica gel to yield the pure sulfoxide. The enantiomeric ratio of the sulfoxides **9a**,**b**, **9d**−**l** was determined by enantioselective analytical HPLC (Daicel Chiralpak IA, 4.6 mm × 250 mm, 5 μm, 0.8 mL/min, CH_2Cl_2/MeOH/TEA 98:2:0.1, λ = 254 nm, 0.8 mL/min flow rate).

■ ASSOCIATED CONTENT

Supporting Information. Tables S1, S2, S3, and S4; Figures S1, S2, S3, S4, S5, and S6; experimental procedures; characterization data for new compounds; X-ray crystallographic data for E_1 and E_2 of **9b**; and chiral HPLC traces of the compounds shown in Tables S1, S2, S3, and S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

p38α MAPK, p38α mitogen-activated protein kinase; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide; HWB, human whole blood; IL-1β, interleukin-1β; ATP, adenosine triphosphate; CYP450, cytochrome P450; CHP, cumene hydroperoxide; D-DET, diethyl D-tartrate; L-DET, diethyl L-tartrate; *S*-BINOL, (*S*)-(−)-1,1′-bi(2-naphthol); *R*-BINOL, (*R*)-(+)-1,1′-bi(2-naphthol) TBHP, *tert*-butyl hydroperoxide; ATF-2, activating transcription factor 2; ELISA, enzyme-linked immunosorbent assay

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