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Substituted Isoxazoles as Potent Inhibitors of p38 MAP Kinase

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*In a continuous effort to develop improved p38 MAP (mitogen-activated protein) kinase inhibitors, we focused our attention on the suitability of the isoxazole ring as a bioisosteric replacement for the imidazole ring of SB-203580. 3,4- and 4,5-disubstituted as well as 3,4,5-trisubstituted isoxazole derivatives were synthesized. These compounds were tested in an in vitro enzyme-linked immunosorbent assay of isolated p38 MAP kinase and for inhibitory potency against cytochrome P450. Compound **4a** displays a highly promising profile for development as an anti-inflammatory agent owing to its enhanced suppression of cytokine release, decreased affinity for cytochrome P450 and a twofold decrease in IC_{50} toward isolated p38 MAP kinase.*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory and destructive disease of the joints. RA is characterized by synovial inflammation and ensuing cartilage destruction leading to acute pain and serious disability in the long term. Its prevalence (≈1% of the population) and impact on patient quality of life mean that improved therapies are an important objective in pharmaceutical research.

Cytokines such as tumor necrosis factor (TNF-α) and interleukin-1 (IL-1) are detected at both protein and mRNA levels[1] in arthritic joints. High levels of these pro-inflammatory cytokines underlie a number of serious inflammatory diseases.[2] The destruction of the cartilage observed in rheumatic disease is mediated mainly by proteinases, which can degrade cartilage matrix components including type II collagen and aggrecan. Of these proteinases, matrix metalloproteinases (MMPs), enzymes produced by activated macrophages and fibroblasts in response to pro-inflammatory cytokines such as IL-1 and TNF-α, have been considered the main enzymes responsible for degradation of aggrecan and collagens in cartilage[3] and were thought to play a significant role in RA pathology.[4] Although matrix metalloproteinases are chiefly involved,[5] these well-known enzymes do not appear likely to mediate specific proteolyses known in disease; recent studies have shown that disintegrin and metalloproteinase (ADAM) families represent alternative candidates for this.[6–9] Inhibitors of their activity are being actively sought to protect cartilage from degradation, especially in the more prevalent disease osteoarthritis (OA).[10] Several lines of evidence in RA and in animal arthritis models support a role for osteoclasts in the pathogenesis of bone erosion. Osteoclastogenesis is promoted by the cells found in inflammatory bone lesions and can be enhanced by pro-inflammatory cytokines such as TNF-α and IL-1.[11]

Cytokine regulation, therefore, represents a potentially important therapeutic approach in the treatment of rheumatoid and inflammatory diseases. An approach to control cytokine levels involves disruption of the signal-transduction pathway that leads to their release from stimulated inflammatory cells. A key element in this pathway is p38 mitogen activated protein (MAP) kinase.[12] Activation of this kinase under a variety of stress stimuli[13,14] results in diphosphorylation at a Thr-Gly-Tyr motif located in the activation loop. Once activated, p38 phosphorylates and activates other kinases and transcription factors on serine or threonine residues, some of which are responsible for the transcriptional regulation of expression of genes that encode inflammatory cytokines.[15–17]

Several p38 MAPK inhibitors have been shown to block the production of IL-1, TNF, and other cytokines.[13] The inhibition of cytokine production seems to result from the combined effect at the transcriptional and translational levels.[17]

An initial series of pyridinylimidazole anti-inflammatory agents served as tools to elucidate the role of p38 MAP kinase in inflammation.[18] In 1993, the structure–activity relationships (SARs) for the inhibition of cytokine synthesis by bicyclic imidazoles were described. These studies explored dual 5-lipoxygenase/cyclooxygenase (LO/COX) and cytokine inhibition as a potential mechanism for the activity of these compounds.[19] Subsequently, SB-203580 and other 2,4,5-triaryl imidazoles were prepared as pharmacological tools to be used in the search for the molecular target involved in cytokine regulation.[20] Pyridinylimidazoles containing pyridin-4-yl and 4-fluorophenyl groups were the first representative inhibitors shown to bind to the ATP-binding pocket of p38 MAP kinase.[12] The binding mode of SB-203580 as an ATP-competitive compound is well-described[21–23] and is shown in Figure 1.

The general architecture of the ATP-binding site in protein kinases has been reviewed recently.[24] The linker region between the N- and C-terminal domains, surrounded by two hy-

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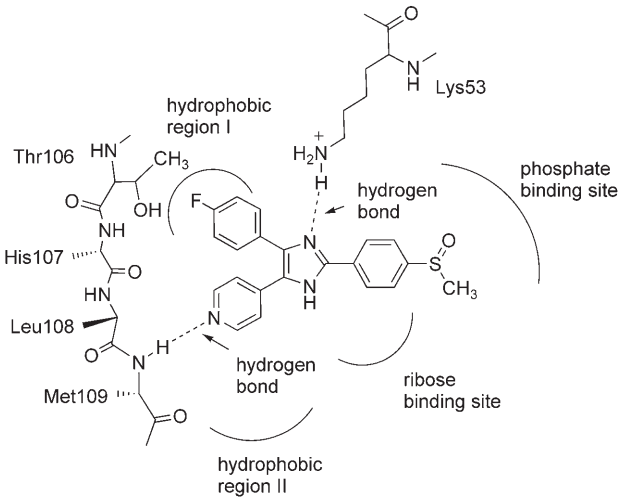


Figure 1. Important interactions between the prototypical pyridin-4-yl imidazole inhibitor SB-203580 and the ATP-binding site of p38; the hydrophobic area below the linker region is not occupied by SB-203580.

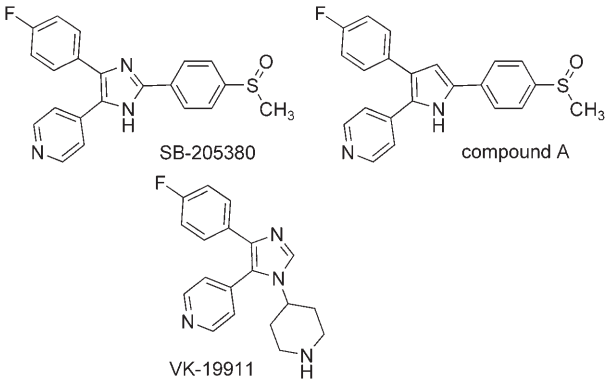


Figure 2. Inhibitors of p38 MAP kinase: SB-203580 (IC_{50} = 39 nM),[31] compound A (IC_{50} = 1220 nM),[31] and VK-19911.[25]

drophobic regions, serves as a highly significant anchor for the binding of ATP-site-directed kinase inhibitors (Figure 1).

The crystal structures of p38 kinase complexed with various pyridin-4-yl imidazole derivatives described so far contain a common set of features that can be exemplified by the binding mode of SB-203580: notably, the formation of a hydrogen bond between the backbone NH group of Met 109 in the linker region and the pyridine nitrogen atom of the inhibitor (Figure 1).[21,22,25] As observed with various inhibitors of p38 MAP kinase,[26,27] replacement of the pyridin-4-yl moiety with a pyridin-3-yl ring results in a decrease in the inhibition of cytokine release. This loss of potency could be explained by a disturbance of the pivotal hydrogen bond with Met 109, thus underscoring the crucial importance of the pyridine ring for biological activity.

The 4-fluorophenyl ring binds to a hydrophobic pocket with walls formed by the N-terminal domain core at the back of the active site. The selectivity of these compounds for p38α has been attributed to this interaction, which is mediated by the presence of Thr 106 in the ATP-binding site. Other MAP kinases, except p38β, have either a methionine or a glutamine residue in this position, and the larger side chains of these residues prevent the binding of the fluorophenyl ring of the inhibitors. This hypothesis has been confirmed by site-directed mutagenesis experiments.[25,28]

Still open to debate is the relevance of the hydrogen bond between N3 of the imidazole ring and Lys 53 of p38 MAP kinase. Although several studies indicate the imidazole ring as a critical determinant for the binding of pyridinylimidazoles to p38 MAP kinase,[17,21–23,29,30] some authors[23,28,31] suggest a role for the imidazole as scaffold for positioning the fluorophenyl and pyridine rings. The modest inhibitory activity of compounds that are unable to form a hydrogen bond to Lys 53, such as compound A[31,32] (Figure 2), underscore the need for further investigations.

In 1997, Wilson et al., in their discussion of the complex between compound VK-19911 (Vertex Pharmaceuticals) (Figure 2) and p38 MAP kinase,[25] suggested that a nitrogen atom bearing a lone pair in position 3 of the imidazole ring could be necessary to avoid a repulsive interaction with the positively charged side chain of Lys 53, rather than to form an attractive interaction with p38 MAP kinase. This hypothesis seems to suggest that the nitrogen in position 3 could be easily substituted with another heteroatom bearing a suitable lone pair; lack of inhibition would, alternatively, support the idea of the requirement for hydrogen bonding with p38. As studies of the hydrogen-bonding properties of oxygen and nitrogen acceptors in aromatic heterocycles[33] have shown, nitrogen is a significantly better H-bond acceptor than oxygen. As a consequence, a ligand in which a nitrogen has been replaced by an oxygen acceptor is not expected to retain the hydrogen bond with the protein. This is supported by the much greater number of known structures[33] that have hydrogen bonds to nitrogen atoms than the number of structures showing hydrogen bonds to oxygen, especially for cases in which the two different heteroatoms are in a competitive situation (for example, in the same ring).

Despite their good in vitro profiles and proven efficacy in animal models, first-generation pyridinylimidazoles as p38 MAP kinase inhibitors cause severe liver toxicity[34] mainly through interference with hepatic cytochrome P450 (CYP) enzymes, as pyridine and imidazole are heterocyclic ligands for the heme iron of cytochrome P450.[35] It remains unclear if the toxicity results from the action of one or both the heterocycles. Different investigators had attempted to elucidate and overcome the toxicity problems that hindered the clinical development of pyridinylimidazoles. The pyridine[34,36] ring has been replaced with other suitable heterocycles, and sterically demanding substituents have been introduced at the imidazole N1[26] or at position 2 of the pyridine ring[36] to decrease CYP inhibition. Although the majority of the work has focused on imidazoles, several groups have undertaken systematic core modifications, replacing the imidazole ring with other heterocycles[27,31,37,38] such as oxazoles.[39–41] Some of those compounds are shown in Figure 3. The IC_{50} data from Merck for compounds C and D (Figure 3) underscore nitrogen as a better H-bond acceptor

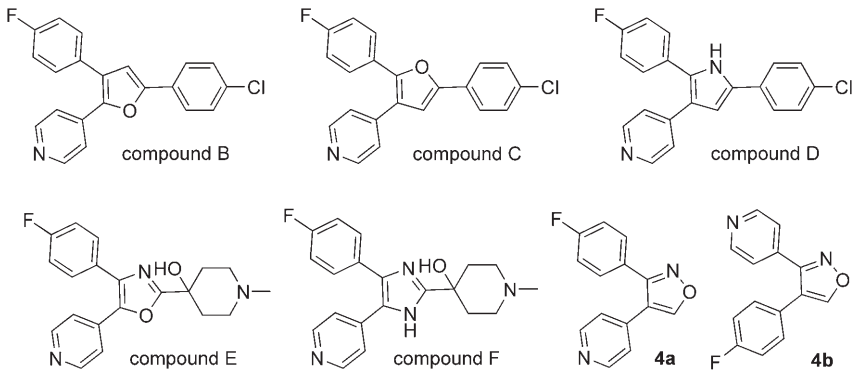


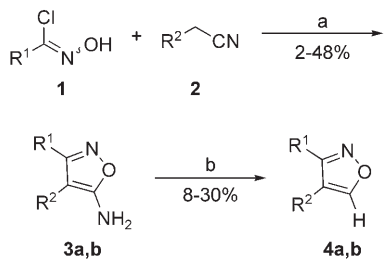
Figure 3. Inhibitors of p38 MAP kinase: compound B (IC_{50} = 530 nM),[31] compound C (IC_{50} = 630 nM),[31] compound D (IC_{50} = 200 nM),[31] compound E (IC_{50} = 350 nM),[41] compound F (IC_{50} = 90 nM),[41] **4a**, and **4b**.

than oxygen, reflected in the threefold greater potency of the pyrrole regioisomer than that of the furan.

Researchers at Novartis[41] reported an improved profile for the oxazole compound E (Figure 3) toward cytochrome P450, but the decrease in CYP inhibition was not accompanied by adequate inhibitory potency toward p38 MAP kinase. In fact, compound E shows greater than threefold decreased potency relative to that of the related imidazole compound F. Theoretical calculations[33] show that the energy of a hydrogen bond to an sp^2 nitrogen or oxygen atom is significantly affected by the neighboring atoms in the ring. This effect could explain the greater inhibitory activity of the tautomeric imidazole (compound F) in comparison with the stable oxazole (compound E).

Herein, we report the effect of replacing the core imidazole with an isoxazole ring. The described SAR studies for isoxazoles as p38 MAP kinase inhibitors show that the isoxazole nucleus provides a bioisosteric replacement of imidazole. Furthermore, it is associated with a diminished interaction with CYP. The 3,4-diaryl-substituted 5-aminoisoxazole compounds **3a** and **3b** reported as p38 MAP kinase inhibitors by Teikoku Hormone Manufacturing Co.[42] were modified to obtain **4a** and **4b** (Scheme 1). Although some of the derivatives have already been described in patents,[43] no biological data are available yet.

To investigate the role of Lys53 in interaction with these compounds, we prepared two sets of 4,5-diaryl-substituted isoxazoles. Data gathered from this series suggests that the interaction between Lys53 and the isoxazole nitrogen atom cannot be established with the isoxazole oxygen atom. Moreover, we introduced substituents at position 2 of the pyridine ring to fill the hydrophobic pocket II, which is normally unoccupied by unsubstituted pyridin-4-yl inhibitors. In this way, we were able to establish a further interaction between the inhibitor and Met109 of p38 MAP kinase.



Scheme 1. Reagents and conditions: a) NaOEt, EtOH, THF, room temperature, then 0 °C; b) acetic acid, H_2O, $NaNO_2$, room temperature.

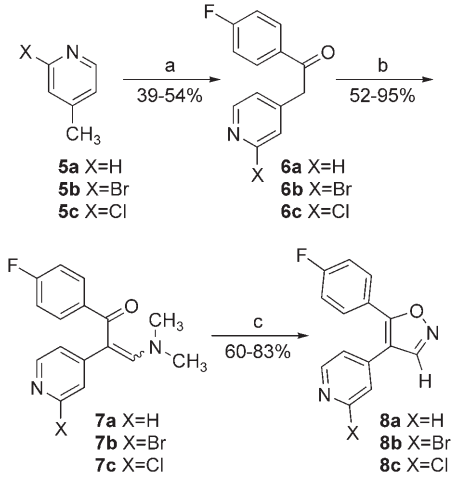
Results and Discussion

Chemistry

The synthesis of 3,4- and 4,5-disubstituted isoxazoles proceeded as follows: compounds **4a** and **4b** were prepared from the corresponding 5-aminoisoxazoles **3a** and **3b**[42] according to the method of Becalli et al.[44] (Scheme 1).

The ring closure[42] of compounds **3a**,**b** was obtained in 1,3-dipolar cycloaddition reactions by using nitrile oxides derived from the corresponding hydroxyiminoyl chlorides as 1,3-dipoles, and substituted nitriles as dipolarophiles in the presence of sodium ethoxide as base. Subsequent diazotization with sodium nitrite yielded C3-unsubstituted isoxazoles **4a** and **4b**.

4,5-disubstituted isoxazoles were prepared according to well-known procedures[36,43,45] with 1-(fluorophenylpyridine)-2-(pyridin-4-yl)ethanone (Schemes 2 and 3). Compounds **6a**–**d** were obtained according to the low-temperature protocol described by Liverton et al.[36] 4-Methylpyridine **5a** and 2-halogen-4-methylpyridine **5b**–**d** were deprotonated with LDA, and the resulting picolyllithium derivatives reacted with Weinreb amide[30,46] and 4-fluoro-*N*-methoxy-*N*-methylbenzamide to yield the ketones **6a**–**d**. Compounds **8a**–**d** were synthesized according to the procedure described by Oku et al.[43] The enaminoketones[47] **7a**–**d** were produced by reflux with *N*,*N*-dimethylformamide–*N*,*N*-dimethylacetal in toluene.

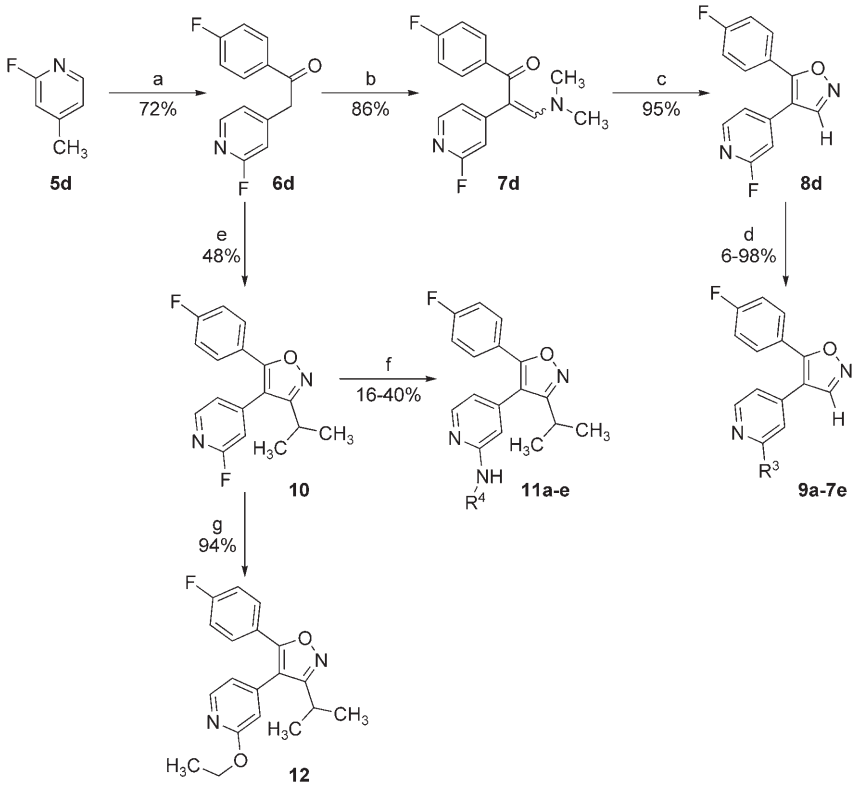


Scheme 2. Reagents and conditions: a) LDA, THF, −78 °C, then 4-fluoro-*N*-methoxy-*N*-methylbenzamide; b) DMF–DMA, toluene, reflux; c) hydroxylamine hydrochloride, Na_2CO_3, aqueous methanol (60%), acetic acid, reflux. LDA = lithium diisopropylamide.

The 4,5-diarylisoxazoles **8a**–**b** were obtained directly from treatment with hydroxyamine hydrochloride in aqueous methanol (60%, pH 5, AcOH). The series of 4,5-diarylisoxazoles substituted on position 2 of pyridin-4-yl by amines was realized starting from compound **8d** (Scheme 3). The displacement of the fluorine atom was carried out at pH 1 with different alcohols to provide compounds **9a**–**e**.

Similar syntheses cannot be applied toward a series of analogues in which the pyridine fluorine atom is replaced by an NHR^4 group; in fact, a displacement with amines would introduce a basic environment that cannot be tolerated by 3-unsubstituted isoxazoles. It is known that the basic treatment of C3-unsubstituted isoxazoles induces a ring-opening and elimination reaction to give β-ketonitriles, owing to facile deprotonation at C3.[48,49] To solve this problem, we synthesized compound **10**, which bears an isopropyl group in position 3. The choice of an isopropyl substituent was guided by the combination of favorable features such as small size, lipophilicity, and ease of synthesis. 1-(4-Fluorophenyl)-2-(2-fluoropyridin-4-yl)-ethanone **6d** was used as the key intermediate for subsequent transformations.

The isoxazole ring closure was carried out according to a modified version of the procedures described by Bravo et al.[50] and Renzi et al.[51] (Scheme 3). The 2-fluoro-4-[5-(4-fluorophenyl)-isoxazol-4-yl]-pyridine **10** was formed by the subsequent deprotonation of ketone **6d** with LDA at −78 °C followed by ring closure through a 1,3-cycloaddition with the isobutyronitrile *N*-oxide precursor generated in situ from the corresponding hydroxyiminoyl chloride. Starting from isoxazole **10**, nucleophilic displacement was feasible not only with appropriate amines[36,52] to give compounds **11a**–**d**, but also with ethanolic HCl to produce compound **12**, showing that the outlined synthetic pathway allows the introduction of a variety of substituents on the pyridine ring.



Scheme 3. Reagents and conditions: a) LDA, THF, −78 °C, then 4-fluoro-*N*-methoxy-*N*-methylbenzamide; b) DMF–DMA, toluene, reflux; c) hydroxyamine hydrochloride, Na_2CO_3, aqueous methanol (60%), acetic acid, reflux; d) alcoholic HCl, 70 °C; e) *n*BuLi, DIPEA, THF, −78 °C, hydroxyiminoyl chloride; f) R^4NH_2, 70 °C; g) ethanol saturated with HCl, 70 °C. DIPEA = *N*,*N*-diisopropylethylamine.

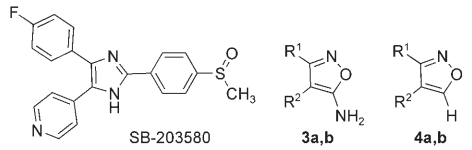
Inhibition of p38 MAP kinase

The pyridinylimidazole SB-203580 and pyridinylpyrrole L-167307 are known to be ATP-competitive, potent inhibitors of p38 MAP kinase, able to inhibit cytokine release. Replacement of the imidazole ring with an isoxazole ring to keep the pyridin-4-yl and 4-fluorophenyl pharmacophore intact lead us to active pyridinylisoxazoles. These were tested in an in vitro enzyme-linked immunosorbent assay (ELISA) of isolated p38 MAP kinase.[53]

Starting with compounds **3a**–**b** from Teikoku Hormone Manufacturing Co., removal of the amino function in position 5 of the isoxazole ring led to compounds **4a**–**b**. A comparison of the activity of these compounds (Table 1) shows that the basicity in position 5 of the isoxazole ring is not necessary for p38 inhibition; compounds **4a** and **3a** are twofold more potent than SB-203580. These inhibitors also demonstrate that the sulfinylphenyl moiety of SB-203580 may not be essential.

In Table 2, the experimental IC_{50} values of **4a** and SB-203580 are reported along with the published data for the imidazole analogue compound G reported by Ortho–McNeil Pharmaceutical in 1999.[36,54] The IC_{50} value we determined for SB-203580 differs substantially from the previously reported value, as there is a significant difference between the test systems used: the activity of p38 inhibition was assayed at Merck according a radioactive protocol at an ATP concentration of 10 μM,[36] whereas the activities of compound **4a** and SB-203580 were determined in our laboratories with an immunosorbent non-radioactive assay and a 10-fold higher ATP concentration.[53] Therefore, the potencies of **4a** and compound G are compared with that of SB-203580 in Table 2. Compound G is 0.23-fold as active as SB-203580; in contrast, replacement of the imidazole ring with isoxazole (as in **4a**) resulted in a 2.5-

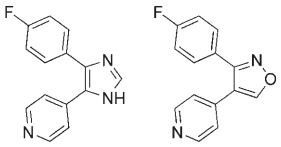
Table 1. Inhibition of p38 MAP kinase.



Compd	R^1	R^2	$IC_{50} \pm SEM$ [μM][a]
3a	4-F-C_6H_4	4-Pyr	0.20 ± 0.03 ($n=3$)
3b	4-Pyr	4-F-C_6H_4	1.61 ± 0.02 ($n=3$)
4a	4-F-C_6H_4	4-Pyr	0.20 ± 0.02 ($n=3$)
4b	4-Pyr	4-F-C_6H_4	1.22 ± 0.02 ($n=3$)
SB-203580	–	–	0.5 ± 0.3 ($n=12$)

[a] n = number of experiments.

Table 2. Inhibition of p38 MAP kinase.



Compd	$IC_{50} \pm SEM$ [μM][a]	$IC_{50} \pm SEM$ [μM][a,b]	Relative Potency[c]
compound G	–	0.17	0.23
4a	0.20 ± 0.02 ($n=3$)	–	2.5
SB-203580	0.5 ± 0.3 ($n=12$)	0.039 ± 0.11 ($n=70$)	–

[a] n = number of experiments. [b] Data from reference [36, 54]. [c] (IC_{50} SB-203580)/(IC_{50} test compound).

fold greater activity than that of SB-203580 which suggests that isoxazole has better scaffold properties. Furthermore, compound G can undergo annular tautomerism (Figure 4),

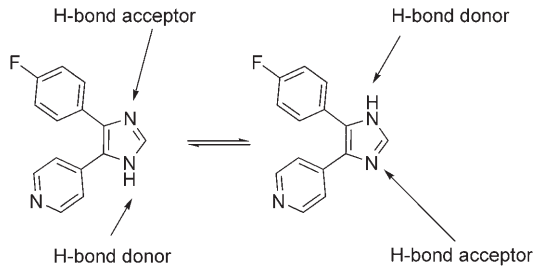


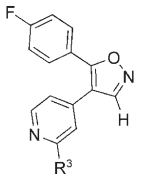
Figure 4. The annular tautomerism of compound G[36,54].

which inverts the positions of hydrogen-bond acceptor and donor in the molecule. This could lead to unfavorable positioning of the aromatic moieties, and is a potentially negative feature that is not observed for compound **4a**. The results suggest that heterocycles that are stable as regioisomers, such as isoxazole (in contrast to the tautomeric imidazoles), are worthy of further investigation.

The difference in biological activity between **4a** and **4b** (Table 1), in accordance with the above consideration, suggests the importance of an atom bearing lone-pair electrons which are able to give a dynamic or static interaction[25,29,30,55] with Lys53 for proper positioning of the aromatic moieties. This underscores the importance of the core heterocycle in providing an optimum scaffold for both the pyridin-4-yl and 4-fluorophenyl moieties. In this regard, **4a** would have the proper alignment as exhibited by its good biological activity (IC_{50} = 0.2 μM, Table 1).

As shown in Table 3, the 4,5-diarylisoxazole **8a** displays no significant inhibitory activity. Data for **8a** and **4a** seem to indicate a dynamic interaction of the core heterocycle with Lys53,

Table 3. Inhibition of p38 MAP kinase.



Compd	R^3	$IC_{50} \pm SEM$ [μM][a]
8a	H	41 ± 10 ($n=3$)
8b	Br	89
8c	Cl	42 ± 10 ($n=3$)
8d	F	24% (100 μM)
9a	H_3CO	50
9b	$H_3C(CH_2)O$	82
9c	$(CH_3)_2(CH)O$	31 ± 6 ($n=3$)
9d	$H_3C(CH_2)_3O$	21 ± 2 ($n=3$)
9e	$(C_6H_5)(CH_2)_2O$	34 ± 2 ($n=3$)

[a] n = number of experiments.

contrary to the suggestion given in the report of the crystal structure of p38 MAP kinase complexed with VK-19911.[25] Furthermore, the isoxazole ring oxygen atom behaves as weaker H-bond acceptor than the nitrogen atom in position 2 (Figure 5). This result is in good agreement with results of the

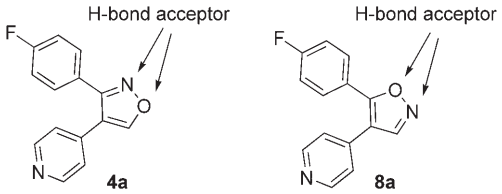


Figure 5. The hydrogen-bonding properties of isoxazoles **4a** and **8a**.

study by Nobeli et al.[33] and also with unpublished results reported by researchers at Novartis[41] with regard to the compounds shown in Figure 6. Compound H shows up to fivefold lower p38 inhibitory activity than the isomer E, presumably due to the better hydrogen bonding for the latter compound.

In the second part of this study, we focused on the interdependency of biological effects exerted by substitution at the pyridine ring for a series of 3-substituted and unsubstituted 4,5-diarylisoxazoles. If position 2 of the pyridine in the 3-unsub-

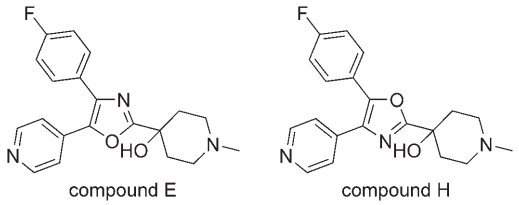


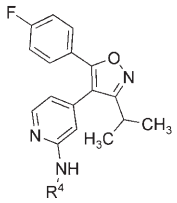
Figure 6. Oxazole compounds (Novartis):[41] compound E ($IC_{50}=0.350$ μM),[41] compound H ($IC_{50}=2$ μM).[41]

stituted 4,5-diarylisoxazole series is replaced by alkoxy groups (compounds **9a**–**e**), biological activity is very poor (Table 3). Increased inhibitory potency was not observed over a range of small (in **9a**) to large substituents (in **9e**); this finding does not agree with biological data observed for the pyridinylimidazole series.[52] For the halogenated pyridines **8b**–**d**, the loss of potency can be attributed to the decreased electron density at the pyridine ring nitrogen atom and the subsequent weakening of the hydrogen bond with the amide NH group of Met 109.

Examination of SAR at position 2 for the 3-substituted 4,5-diarylisoxazole series (Table 4) shows that small substituents such as propylamine (in **11a**) or cyclopropylmethylamine (in **11c**) are generally well-tolerated in contrast to the pentylamine-modified **11b.** Attachment of 1-phenylethylamine to position 2 of the pyridine moiety (for **11e**) leads to a more potent kinase inhibitor with good inhibitory activity. This could be explained through an additional hydrogen bond between the amine and carbonyl groups of the enzyme backbone (Figure 7). Furthermore, the side chain could exhibit an improved interaction with the hydrophobic pocket II.

These explanations are further supported by a comparison of compounds **12** and **9b**, in which the activity is observed to improve through substitution at position 3 (Table 5).

Table 4. Inhibition of p38 MAP kinase.



Compd	R^4	$IC_{50} \pm$ SEM [μM][a]
11a	$H_3C(CH_2)_2$	4.0
11b	$H_3C(CH_2)_4$	82.0
11c	$(C_3H_5)CH_2$	1.3 ± 0.28 ($n=3$)
11d	(C_3H_5)	2.0
11e	$(C_6H_5)CH(CH_3)$	0.55 ± 0.33 ($n=3$)

[a] n = number of experiments.

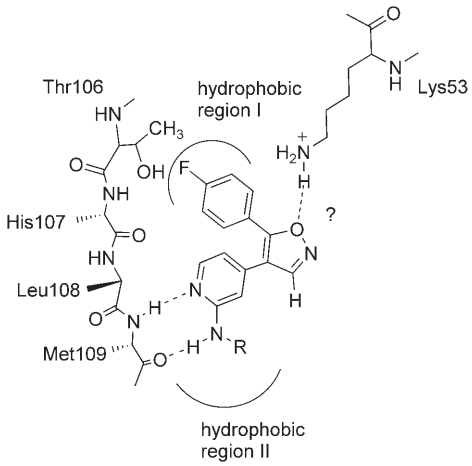
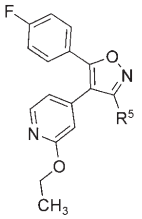


Figure 7. Binding interactions for compound **11d** with p38 MAP kinase.

Table 5. Inhibition of p38 MAP kinase.



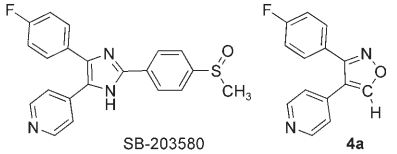
Compd	R^5	$IC_{50} \pm$ SEM [μM][a]
9b	H	82
12	$(H_3C)_2CH$	56 ± 2.0 ($n=3$)

[a] n = number of experiments.

Inhibitory potency toward cytochrome P450

Isoxazole **4a** and SB-203580 were compared in an assay by BD GenTest.[56] SB-203580, known to be a ligand for the heme iron of isoforms of cytochrome P450, displayed a high affinity to CYP2D6 and CYP3A4. In contrast to SB-203580, the isoxazole **4a** did not interfere with the two isoforms of cytochrome P450 (Table 6). This shows that the isoxazole nucleus is a suitable bioisosteric replacement for the imidazole ring, and minimizes interaction with CYP450. This finding might be important for future drug design.

Table 6. Inhibition data toward two CYP450 isoforms.[a]



Compd	CYP2D6	CYP3A4
4a	34	13
SB-203580	73	76

[a] Given as % inhibition at 10 μM.

Conclusions

We have shown that bioisosteric replacement of the core heterocycle with isoxazole is possible, and that it may provide a better scaffold than imidazoles. A decreased interaction with cytochrome isoenzymes is a further advantage of the scaffold. Isoxazole **4a** showed excellent inhibitory potency in the isolated enzyme assay. The 4-[3-(4-fluorophenyl)isoxazol-4-yl]-pyridine **4a** is the more effective regioisomer owing to proper alignment for hydrogen bonding with the enzyme backbone. These data support the importance of hydrogen-bond formation with Lys 53 for inhibitors of p38 MAP kinase and are consistent with the different roles of nitrogen and oxygen atoms as H-bond acceptors. The inhibitory potency of 4-[5-(4-fluorophenyl)isoxazol-4-yl]-pyridine **8a** can be markedly increased by the introduction of appropriate substituents at position 2 of the pyridine ring. Specifically, 1-phenylethylamine **11e** showed good biological results owing to the fact that: 1) it does not decrease the electron density of the pyridine nitrogen atom, 2) it is able to form another hydrogen bond from the amine group to the carbonyl group of the enzyme backbone, and 3) it is able to interact with hydrophobic pocket II. From the aforementioned points, amine substituents at position 2 are generally more favorable than alkoxy substituents, which were even observed to be detrimental to biological activity. Pyridinylisoxazoles are potent inhibitors of p38 MAP kinase, as shown by an in vitro screening assay of isolated kinase. Moreover, they are unlikely to act as nonspecific inhibitors of redox systems such as cytochrome P450 enzymes.

Experimental Section

General methods: All commercially available solvents and reagents were used as received. Melting points were determined on a Büchi Melting Point 545 apparatus and were thermodynamically corrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Spectrospin AC 200 at 200 MHz. GC–MS analyses were carried out on a Hewlett–Packard HP 6890 Series GC system with a 5% phenylmethylsiloxane column (HP-5MS, 30 m) with an inlet temperature of 250 °C and a temperature program of: 160 °C, 1.0 min; 10 °C min^{-1} → 240 °C, 5 min; 10 °C min^{-1} → 270 °C, 15.0 min; elution with helium (64.1 mL min^{-1}), and a Hewlett–Packard HP 5973 mass-selective detector.

General procedure[42,44] for the preparation of 3,4-diarylisoxazoles (Scheme 1); 3-(4-fluorophenyl)-4-pyridin-4-yl-isoxazol-5-yl-amine (3a): A solution of sodium ethoxide (1.7 g, 25 mmol) in dry ethanol (40 mL) was added to a stirred solution of substituted pyridinyl-4-acetonitrile **2** (2.97 g, 25 mmol) in dry THF (30 mL) at room temperature. The reaction mixture was cooled to 0 °C, and the substituted hydroxamic acid chloride **1** (4.35 g, 25 mmol) in dry ethanol (8 mL) was added over 10 min. The reaction mixture was stirred for 1 h at 0 °C and then for a further 1 h at 45 °C. The solvent was evaporated in vacuo, and the residue taken up in 200 mL water. The compound was precipitated by the addition of CH_2Cl_2 and subsequently purified by recrystallization (CH_2Cl_2) to afford **3a** (48%): mp: 192 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 4.91 (s, 2H, NH_2, exchangeable), 7.03–7.12 (m, 4H, 4-F-C_6H_4 and 4-Pyr), 7.38–7.45 (m, 2H, 4-F-C_6H_4), 8.54 ppm (d, 2H, $^3J_{H,H} = 5.1$ Hz, 4-Pyr). Anal. ($C_{14}H_{10}FN_3O$): C, H, N.

4-(4-Fluorophenyl)-3-pyridin-4-yl-isoxazol-5-yl-amine (3b): The title compound was prepared as described for **3a** from 4-fluorophenylacetonitrile **2** (0.68 g, 5 mmol) and substituted hydroxamic acid chloride **1** (0.78 g, 5 mmol) to give **3b** (1.6%): mp: 157 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 4.66$ (s, NH_2, exchangeable), 7.06–7.22 (m, 4H, 4-F-C_6H_4), 7.36 (dd, 2H, $^3J_{H,H} = 1.7$, 2.8 Hz, 4-Pyr), 8.60 ppm (dd, 2H, $^3J_{H,H} = 1.7$, 2.8 Hz, 4-Pyr). Anal. ($C_{14}H_9FN_3O$): C, H, N.

4-[3-(4-Fluorophenyl)isoxazol-4-yl]pyridine (4a): 5-Aminoisoxazole **3a** (0.7 g, 2.7 mmol) was dissolved in a mixture of acetic acid, water, and THF (8 mL) and $NaNO_2$ was added over a period of 15 min at room temperature. The reaction mixture was taken up in water, stirred for 30 min, and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were successively washed with a solution of $NaHCO_3$, brine, and water. After evaporation of the organic solvent, the residue was purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 8:2) to yield **4a** (8%): mp: 55 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 7.08–7.20 (m, 4H, 4-F-C_6H_4 and 4-Pyr), 7.44–7.51 (m, 2H, 4-F-C_6H_4), 8.60–8.62 (dd, 2H, $^3J_{H,H} = 1.7$, 2.8 Hz, 4-Pyr), 8.67 ppm (s, 1H, CH); GC–MS 9.15 min, *m/z*(%): 240(100) [M^+], 212(75) [2H-azirine], 184(35), 158(25), 122(8), 95(25) [$C_6H_4F^+$]. Anal. ($C_{14}H_9FN_2O$): C, H, N.

4-[4-(4-Fluorophenyl)isoxazol-3-yl]pyridine (4b): The title compound was prepared from **3b** (0.4 g, 1.5 mmol) as described for the synthesis of **4a**; (29%): mp: 99 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 7.05–7.14 (m, 2H, 4-F-C_6H_4), 7.19–7.24 (m, 2H, 4-F-C_6H_4), 7.40 (dd, 2H, $^3J_{H,H} = 1.6$, 2.8 Hz, 4-Pyr), 8.56 (s, 1H, CH), 8.65 ppm (dd, 2H, $^3J_{H,H} = 1.6$, 2.8 Hz, 4-Pyr); GC–MS 9.21 min, *m/z*(%): 240(100) [M^+], 211(10) [2H-azirine], 157(25), 134(45), 120(10), 107(90), 95(10), [$C_6H_4F^+$]. Anal. ($C_{14}H_9FN_2O$): C, H, N.

General procedure[42,44] for the preparation of 4,5-diarylisoxazoles (Schemes 2 and 3); 1-(4-fluorophenyl)-2-pyridin-4-yl-ethanone (6a): *n*-Butyllithium (210 mmol, 15% in *n*-hexane) was added dropwise to a stirred solution of diisopropylamine (200 mmol) in dry THF (150 mL) at −78 °C. The reaction mixture was allowed to stir for 1 h. A solution of picoline (5.58 g, 60 mmol) in dry THF (15 mL) was then added, and the reaction mixture was stirred for 1 h. 4-Fluoro-*N*-methoxy-*N*-methylbenzamide (11 g, 60 mmol) in dry THF (15 mL) was rapidly added; after 1 h, the reaction mixture was allowed to warm to room temperature, was poured into saturated aqueous sodium bicarbonate solution, and was extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4. Removal of the solvent produced the crude compound **6a** (39%), which was brought into the next step without further purification: mp: 91 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS); $\delta =$ 4.27 (s, 2H, CH_2), 7.12–7.21 (m, 4H, 4-F-C_6H_4 and 4-Pyr), 7.99–8.07 (m, 2H, 4-F-C_6H_4), 8.58 ppm (d, 2H, $^3J_{H,H} = 4.8$ Hz, 4-Pyr).

2-(2-bromopyridin-4-yl)-1-(4-fluorophenyl)ethanone (6b): The title compound was prepared from 2-bromopicoline **5b** as described for the synthesis of **6a**; (56%): mp: 114 °C; ^{1}H NMR ($CDCl_3$): $\delta =$ 4.25 (s, 2H, CH_2), 7.11–7.26 (m, 3H, 2-Br-Pyr and 4-F-C_6H_4), 7.42 (s, 1H, 3-H 2-Br-Pyr), 7.99–8.06 (m, 2H, 4-F-C_6H_4), 8.36 ppm (d, 1H, $^3J_{H,H} = 5.1$ Hz, 6-H 2-Br-Pyr); GC–MS 11.05 min, *m/z*(%): 293(2) [M^+], 123(100) [$C_7H_4FO^+$], 95(43) [$C_6H_4F^+$], 75(12).

2-(2-chloropyridin-4-yl)-1-(4-fluorophenyl)ethanone (6c): The title compound was prepared from 2-chloropicoline **5c** as described for the synthesis of **6a**; (54%): mp: 113 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 4.27 (s, 2H, CH_2), 7.11–7.26 (m, 4H, 3-H/5-H, 2-Cl-Pyr, and 4-F-C_6H_4), 7.99–8.06 (m, 2H, 4-F-C_6H_4), 8.34 ppm (dd, 1H, $^3J_{H,H} = 0.6$, 5.1 Hz, 6-H 2-Cl-Pyr); GC–MS

10.29 min, *m/z*(%): 249(3) [M^+], 123(100) [$C_7H_4FO^+$], 95(44) [$C_6H_4F^+$].

1-(4-Fluorophenyl)-2-(2-fluoropyridin-4-yl)ethanone (6d): The title compound was prepared from 2-chloropicoline **5c** as described for the synthesis of **6a**; (72%): mp: 116 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 4.31$ (s, 2H, CH_2), 6.85 (m, 1H, 3-H 2-F-Pyr), 7.13–7.26 (m, 3H, 5-H, 2-F-Pyr, and 4-F-C_6H_4), 8.00–8.07 (m, 2H, 4-F-C_6H_4), 8.19 ppm (d, 1H, $^3J_{H,H} = 5.1$ Hz, 6-H 2-F-Pyr); GC–MS 8.77 min, *m/z*(%): 233(6) [M^+], 123(100) [$C_7H_4FO^+$], 95(94) [$C_6H_4F^+$], 75(56).

3-Dimethylamino-1-(4-fluorophenyl)-2-(pyridin-4-yl)prop-2-en-1-one (7a): An excess of *N*,*N*-dimethylformamide–*N*,*N*-dimethylacetate (30 mmol) was added to a solution of **6a** (2.58 g, 12 mmol) in toluene (60 mL) and stirred under reflux for 2.5 h. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 6:4) to yield **7a** (52%) as an oil: ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 2.81$ (s, 6H, 2× CH_3), 6.94–7.08 (m, 4H, 4-F-C_6H_4 and 4-Pyr), 7.40–7.47 (m, 3H, CH and 4-F-C_6H_4), 8.48–8.50 ppm (m, 2H, 4-Pyr).

2-(2-Bromopyridin-4-yl)-3-dimethylamino-1-(4-fluorophenyl)-prop-2-en-1-one (7b): The title compound was synthesized from **6b** as described for the synthesis of **7a**; (64%): ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 2.82$ (s, 6H, 2×CH_3), 6.99–7.08 (m, 3H, 4-F-C_6H_4 and 5-H 2-Br-Pyr), 7.29 (s, 1H, CH), 7.43 (s, 1H, 3-H 2-Br-Pyr) 7.44–7.50 (m, 2H, 4-F-C_6H_4), 8.23 ppm (d, 1H, $^3J_{H,H} = 5.1$ Hz, 6-H 2-Br-Pyr).

2-(2-Chloropyridin-4-yl)-3-dimethylamino-1-(4-fluorphenyl)prop-2-en-1-one (7c): The title compound was synthesized from **6c** as described for the synthesis of **7a**; (73%): ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 2.80$ (s, 6H, 2×CH_3), 6.99–7.05 (m, 3H, 4-F-C_6H_4 and 5-H 2-Cl-Pyr), 7.17 (s, 1H, CH), 7.37 (s, 1H, 3-H 2-Cl-Pyr), 7.47–7.53 (m, 2H, 4-F-C_6H_4), 8.24 ppm (d, 1H, $^3J_{H,H} = 5.1$ Hz, 6-H 2-Cl-Pyr).

3-Dimethylamino-1-(4-fluorphenyl)-2-(2-fluorpyridin-4-yl)prop-2-en-1-one (7d): The title compound was synthesized from **6d** as described for the synthesis of **7a**; (86%): mp: 97 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 2.83$ (s, 6H, 2×CH_3), 6.95–7.01 (m, 3H, 4-F-C_6H_4 and 5-H 2-F-Pyr), 7.07 (s, 1H, CH), 7.17 (s, 1H, 3-H 2-F-Pyr), 7.37–7.49 (m, 2H, 4-F-C_6H_4), 8.11 ppm (d, 1H, $^3J_{H,H} = 5.1$ Hz, 6-H 2-F-Pyr).

4-[5-(4-Fluorophenyl)isoxazol-4-yl]pyridine (8a): The enaminoketone **7a** (1.9 g, 6.7 mmol) was dissolved in methanol/water (30:20 *v/v*). A mixture of Na_2CO_3 (3.8 mmol) and hydroxyamine hydrochloride (7.1 mmol) was then added, and the solution was adjusted to pH 5 with acetic acid. The reaction mixture was heated at reflux for 2.5 h and was neutralized with concentrated ammonia. Compound **8a** was precipitated from the reaction mixture by adding ice; (95%): mp: 99 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.09$–7.18 (m, 2H, 4-F-C_6H_4), 7.30 (dd, 2H, $^3J_{H,H} = 1.6$, 3.0 Hz, 4-Pyr), 7.59–7.66 (m, 2H, 4-F-C_6H_4), 8.43 (s, 1H, CH), 8.65 ppm (dd, 2H, $J = 1.6$, 3.0 Hz, 4-Pyr); GC–MS 6.79 min, *m/z*(%): 240(100) [M^+], 212(15) [2H-azirine], 184(18), 157(38), 145(12), 123(95) [$C_7H_4FO^+$], 108(14), 95(64) [$C_6H_4F^+$], 75(22), 63(16), 51(8). Anal. ($C_{14}H_9FN_2O$): C, H, N.

2-Bromo-4-[5-(4-fluorophenyl)isoxazol-4-yl]pyridine (8b): The title compound was synthesized from **7b** as described for the synthesis of **8a**; (90%): mp: 139 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.13$–7.23 (m, 3H, 5-H 2-Br-Pyr and 4-F-C_6H_4), 7.52 (s, 1H, 3-H 2-Br-Pyr), 7.58–7.65 (m, 2H, 4-F-C_6H_4), 8.42 ppm (d, 1H, $^3J_{H,H} =$ 5.1 Hz, 6-H 2-Br-Pyr), 8.44 (s, 1H, CH).

2-Chloro-4-[5-(4-fluorophenyl)isoxazol-4-yl]pyridine (8c): The title compound was synthesized from **7c** as described for the synthesis of **8a**; (90%): mp: 102 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.13$–7.26 (m, 3H, 5-H 2-Cl-Pyr and 4-F-C_6H_4), 7.52 (s, 1H, 3-H 2-Cl-Pyr), 7.58–7.66 (m, 2H, 4-F-C_6H_4), 8.35 ppm (dd, 2H, $^3J_{H,H} =$ 1.6, 3.0 Hz, 6-H 2-Cl-Pyr), 8.43 (s, 1H, CH).

2-Fluoro-4-[5-(4-fluorophenyl)isoxazol-4-yl]pyridine (8d): The title compound was synthesized from **7d** as described for the synthesis of **8a**; (95%): mp: 122 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS) $\delta = 6.95$ (s, 1H, 3-H 2-F-Pyr), 7.12–7.21 (m, 3H, 4-F-C_6H_4 and 5-H 2-F-Pyr), 7.58–7.65 (m, 2H, 4-F-C_6H_4), 8.11 (dd, $^3J_{H,H} = 1.6$, 3.0 Hz, 1H, 6-H 2-F-Pyr), 8.45 ppm (s, 1H, CH); GC–MS 6.44 min, *m/z*(%): 258(100) [M^+], 230(35), 202(45), 175(17), 163(8), 123(90) [$C_7H_4FO^+$], 108(26), 95(86) [$C_6H_4F^+$], 75(22). Anal. ($C_{14}H_8F_2N_2O$): C, H, N.

4-[5-(4-Fluorophenyl)isoxazol-4-yl]-2-methoxypyridine·HCl (9a): Compound **8d** (0.15 g, 0.6 mmol) was dissolved in a solution of methanol (2 g) saturated with HCl and was stirred for 2.5 h at 70 °C. The solvent was evaporated in vacuo to produce **9a** (76%) as a white solid: mp: 134 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 3.99$ (s, 3H, CH_3), 6.79 (s, 1H, 3-H 2-MeO-Pyr), 6.88 (d, 1H, $^3J_{H,H} = 5.2$ Hz, 5-H 2-MeO-Pyr), 7.09–7.18 (m, 2H, 4-F-C_6H_4), 7.60–7.67 (m, 2H, 4-F-C_6H_4), 8.20 (d, 1H, $^3J_{H,H} = 5.2$ Hz, 6-H 2-MeO-Pyr), 8.45 ppm (s, 1H, CH); GC–MS 7.74 min, *m/z*(%): 270(100) [M^+], 240(54), 211(27), 184(32), 145(43), 123(94) [$C_7H_4FO^+$], 109(3), 95(78) [$C_6H_4F^+$], 75(32), 51(15). Anal. ($C_{15}H_{11}FN_2O_2$·HCl): C, H, N.

2-Ethoxy-4-[5-(4-fluorophenyl)isoxazol-4-yl]pyridine·HCl (9b): The title compound was prepared according to the procedure described for **9a**; compound **8d** (0.25 g, 1 mmol) in a solution of ethanol (2 g) saturated with HCl was stirred for 5 h at 70 °C to produce **9b** (98%) as a white solid: mp: 150 °C; ^{1}H NMR (200 MHz, [D_6]DMSO, 25 °C, TMS): $\delta = 1.31$ (t, 3H, $^3J_{H,H} = 7.0$ Hz, CH_3), 4.32 (q, 2H, $^3J_{H,H} = 7.0$ Hz, CH_2), 6.93 (s, 1H, 3-H 2-EtO-Pyr), 6.97 (d, 1H, $^3J_{H,H} = 4.8$ Hz, 5-H 2-EtO-Pyr), 7.36–7.45 (m, 2H, 4-F-C_6H_4), 7.65–7.72 (m, 2H, 4-F-C_6H_4), 8.17 (d, 1H, $^3J_{H,H} = 4.8$ Hz, 6-H 2-EtO-Pyr), 9.09 ppm (s, 1H, CH); GC–MS 8.15 min, *m/z*(%): 284(36) [M^+], 269(80), 255(45), 240(42), 213(23), 184(14), 172(45), 157(30), 145(43), 123(100) [$C_7H_4FO^+$], 95(85) [$C_6H_4F^+$], 75(67), 63(15), 51(12). Anal. ($C_{16}H_{13}FN_2O_2$·HCl): C, H, N.

4-[5-(4-Fluorophenyl)isoxazol-4-yl]-2-isopropoxypyridine·HCl (9c): The title compound was prepared according to the procedure described for **9a**; compound **8d** (0.15 g, 0.6 mmol) in a solution of isopropanol (2 g) saturated with HCl was stirred for 5 h at 70 °C to produce **6c** (5.5%) as a white solid: mp: 150 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 1.39$ (s, 6H, 2×CH_3), 5.27–5.40 (m, 1H, CH), 6.72 (s, 1H, 3-H 2-*i*Pr-*O*-Pyr), 6.80 (d, 1H, $^3J_{H,H} = 4.7$ Hz, 5-H 2-*i*Pr-*O*-Pyr), 7.13–7.22 (m, 2H, 4-F-C_6H_4), 7.61–7.69 (m, 2H, 4-F-C_6H_4), 8.16 (d, 1H, $^3J_{H,H} = 4.7$ Hz, 6-H 2-*i*Pr-*O*-Pyr), 8.39 ppm (s, 1H, CH); GC–MS 6.49 min, *m/z*(%): 298(55) [M^+], 283(100), 255(95), 240(90), 213(58) [$C_7H_4FO^+$], 198(12), 172(36), 145(38), 123(97), 95(80) [$C_6H_4F^+$], 75(38). Anal. ($C_{17}H_{15}FN_2O_2$·HCl): C, H, N.

2-Butoxy-4-[5-(4-fluorophenyl)isoxazol-4-yl]-pyridine·HCl (9d): The title compound was prepared according to the procedure described for **9a**; compound **8d** (0.15 g, 0.6 mmol) in a solution of *n*-butanol (2 g) saturated with HCl was stirred for 5 h at 70 °C to produce **9d** (41%) as a white solid: mp: 141 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.98$ (t, 3H, $^3J_{H,H} = 7.7$ Hz, CH_3), 1.52 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 4.69 (t, 2H, $^3J_{H,H} = 6.3$ Hz, OCH_2), 7.13 (s, 1H, 3-H 2-Bu-*O*-Pyr), 7.20–7.28 (m, 3H, 4-F-C_6H_4 and 5-H 2-Bu-*O*-Pyr), 7.58–7.65 (m, 2H, 4-F-C_6H_4), 8.33–8.36 (d, 1H, 6-H 2-Bu-*O*-Pyr), 8.57 ppm (s, 1H, CH); GC–MS 9.90 min, *m/z*(%): 312(5) [$M+$], 292(15), 269(18), 255(38), 239(4), 228(13), 213(21), 184(9), 172(12),

157(6), 145(11), 133(5), 123(100) [$C_7H_4FO^+$], 95(45) [$C_6H_4F^+$], 75(10), 57(4). Anal. ($C_{18}H_{17}FN_2O_2$·HCl): C, H, N.

4-[5-(4-Fluorophenyl)isoxazol-4-yl]-2-phenethoxypyridine·HCl (9e): The title compound was prepared according to the procedure described for **9a**; compound **8d** (0.2 g, 0.8 mmol) in a solution of 2-phenylethanol (2 g) saturated with HCl was stirred for 5 h at 70 °C to produce **9e** (20%) as a white solid: mp: 99 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.09 (t, 2H, J = 7.0 Hz, CH_2), 4.55 (t, 2H, J = 7.0 Hz, CH_2), 6.73 (s, 1H, 3-H 2-Oxy-Pyr), 6.77 (d, 1H, $^3J_{H,H}$ = 5.5 Hz, 5-H 2-Oxy-Pyr), 7.10–7.30 (m, 7H, Ph and 4-F-C_6H_4), 7.57–7.64 (m, 2H, 4-F-C_6H_4), 8.12–8.15 (d, 1H, $^3J_{H,H}$ = 5.5 Hz, 6-H 2-Oxy-Pyr), 9.08 ppm (s, 1H, CH); GC–MS 17.40 min, *m/z*(%): 360(100) [M^+], 339(49), 323(8), 304(8), 294(3), 275(11), 249(11), 175(6), 165(10), 154(11), 139(43), 127(23), 114(27), 102(18), 88(12), 75(12), 63(18). Anal. ($C_{22}H_{17}FN_2O_2$·HCl): C, H, N.

2-Fluoro-4-[5-(4-fluorophenyl)-3-isopropylisoxazol-4-yl]-2-pyridine (10): A solution of *n*-buthyllithium (13 mL, 30 mmol, 15% in *n*-hexane) was added to a solution of diisopropylamine (30 mmol) in 50 mL absolute THF at −78 °C under an argon atmosphere and was stirred for 45 min. After a solution of **6d** (1.16 g, 5 mmol) in 10 mL absolute THF was added, the reaction mixture was stirred for a further 45 min. The in situ prepared substituted hydroxamic acid chloride was then added. The reaction was stopped by the addition of 50 mL water after 5 h at −78 °C. The aqueous phase was extracted with ether (3×50 mL), and the organic extracts were dried over Na_2SO_4. The reaction mixture was purified by column chromatography (SiO_2 60, CH_2Cl_2/EtOAc 4:6) to produce **10** (48%): mp: 148.3 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.29 (s, 6H, 3×CH_3), 2.97 (m, 1H, CH), 6.89 (s, 1H, 3-H 2-F-Pyr), 7.02–7.14 (m, 3H, 4-F-C_6H_4 and 5-H 2-F-Pyr), 7.26–7.48 (m, 2H, 4-F-C_6H_4), 8.31–8.33 ppm (d, 1H, $^3J_{H,H}$ = 5.7 Hz, 6-H 2-F-Pyr); GC–MS 7.30 min, *m/z*(%): 300(100) [M^+], 285(20), 270(10), 257(40), 239(10), 230(20), 123(95), 95(55), 75(20): Anal. ($C_{17}H_{14}F_2N_2O$): C, H, N.

4-[5-(4-Fluorophenyl)-3-isopropylisoxazol-4-yl]-pyridin-2-yl-propylamine (11a): Compound **10** (100 mg, 0.3 mmol) was dissolved in an excess of propylamine (2 g, 33 mmol) and heated at reflux for 3 h. The reaction mixture was allowed to cool to room temperature and was quenched with a solution of 10% aqueous citric acid (10 mL). This was adjusted to pH 5 with the addition of a solution of 20% aqueous NaOH. The resulting emulsion was extracted with ethyl acetate (3×50 mL). The combined organic phases were washed successively with citric acid (10%, pH 5), aqueous Na_2CO_3, and brine, and were dried over Na_2SO_4. The solvent was removed in vacuo. The residue was purified by column chromatography (SiO_2 60, CH_2Cl_2/EtOAc 4:6) to afford **11a** (60%): mp: 127 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.94 (m, 3H, CH_3), 1.29 (s, 6H, 2×CH_3), 1.63 (m, 2H, CH_2), 2.97 (m, 1H, CH), 3.23 (m, 2H, CH_2), 4.75 (s, 1H, NH), 6.28 (s, 1H, 3-H 2-Amino-Pyr), 6.50 (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 5-H 2-Amino-Pyr), 6.98–7.08 (m, 2H, 4-F-C_6H_4), 7.50–7.57 (m, 2H, 4-F-C_6H_4), 8.14 ppm (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 6-H 2-Amino-Pyr); GC–MS 11.11 min, *m/z*(%): 339(45) [M^+], 324(40), 310(100), 297(40), 282(10), 268(10), 254(35), 239(8), 123(60) [$C_7H_4FO^+$], 95(25) [$C_6H_4F^+$]. Anal. ($C_{20}H_{22}FN_3O$): C, H, N.

4-[5-(4-Fluorophenyl)-3-isopropylisoxazol-4-yl]-pyridin-2-yl-pentylamine (11b): Starting from compound **7** (0.2 g, 0.6 mmol) and an excess of pentylamine (2 g, 23 mmol), **11b** was produced by using the procedure described for **11a** (5 h at 90 °C, 90%): mp: 107 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.90 (t, 3H, CH_3), 1.29 (s, 6H, 2×CH_3), 1.34–1.39 (m, 6H, 3×CH_2), 2.96 (m, 1H, CH), 3.23 (q, 2H, NH–CH_2), 4.62 (s, 1H, NH), 6.27 (s, 1H, 3-H 2-Amino-Pyr), 6.50 (d, 1H, $^3J_{H,H}$ = 5.2 Hz, 5-H 2-Amino-Pyr), 6.98–7.07 (m, 2H, 4-F-C_6H_4), 7.50–7.57 (m, 2H, 4-F-C_6H_4), 8.14 ppm (d, 1H, $^3J_{H,H}$ = 5.2 Hz, 6-H 2-Amino-Pyr); GC–MS 14.10 min, *m/z*(%): 367(40) [M^+], 351(16), 324(85), 310(100), 297(40), 282(15), 268(10), 254(35), 239(8), 212(8), 123(70) [$C_7H_4FO^+$], 95(55) [$C_6H_4F^+$]. Anal. ($C_{22}H_{26}FN_3O$): C, H, N.

Cyclopropylmethyl-4-[5-(4-fluorophenyl)-3-isopropylisoxazol-4-yl]-pyridin-2-yl-amine (11c): Starting from compound **7** (0.2 g, 0.6 mmol) and an excess of pentylamine (2 g, 33 mmol), **11c** was produced by using the procedure described for **11a** (5 h at 50 °C, 22%): mp: 137 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.26 (m, 2H, CH_2), 0.55 (m, 2H, CH_2), 0.52–0.59 (m, 1H, CH, *c*Pr), 1.30 (s, 6H, 2×CH_3), 2.95–3.09 (m, 1H, CH, *i*Pr), 3.11–3.15 (m, 2H, CH_2), 5.33 (s, 1H, NH), 6.33–6.34 (s, 1H, 3-H 2-Amino-Pyr), 6.52 (d, 1H, $^3J_{H,H}$ = 6.0 Hz, 5-H 2-Amino-Pyr), 7.00–7.09 (m, 2H, 4-F-C_6H_4), 7.50–7.57 (m, 2H, 4-F-C_6H_4), 8.12 ppm (d, 1H, $^3J_{H,H}$ = 6.0 Hz, 6-H 2-Amino-Pyr); GC–MS 14.12 min, *m/z*(%): 351(25) [M^+], 336(20), 322(100), 309(10), 296(18), 280(10), 254(10), 211(5), 198(10), 184(15), 157(20), 144(7), 123(30) [$C_7H_4FO^+$], 95(25) [$C_6H_4F^+$]. Anal. ($C_{21}H_{22}FN_3O$): C, H, N.

Cyclopropyl-4-[5-(4-fluorophenyl)-3-isopropylisoxazol-4-yl]pyridin-2-yl-amine (11d): Starting from compound **10** (0.2 g, 0.6 mmol) and an excess of cyclopropylamine (2 g, 33 mmol), **11d** was produced by using the procedure described for **11a** (4 h at 90 °C, 22%): mp: 128 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.37 (m, 2H, CH_2), 0.61 (m, 2H, CH_2), 1.58 (s, 6H, 2×CH_3), 2.49–2.63 (m, 1H, CH), 4.59–5.65 (m, 1H, CH), 5.17 (s, 1H, NH), 6.01 (s, 1H, 3-H 2-Amino-Pyr), 6.44 (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 5-H 2-Amino-Pyr), 6.91–6.99 (m, 2H, 4-F-C_6H_4), 7.37–7.44 (m, 2H, 4-F-C_6H_4), 8.12 ppm (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 6-H 2-Amino-Pyr); GC–MS 11.59 min, *m/z*(%): 337-(100) [M^+], 322(80), 309(10), 294(10), 282(15), 267(10), 253(10), 157(35), 144(15), 123(60) [$C_7H_4FO^+$], 95(25) [$C_6H_4F^+$]. Anal. ($C_{20}H_{20}FN_3O$): C, H, N.

4-[5-(4-Fluorophenyl)-3-isopropylisoxazol-4-yl]-pyridin-2-yl-(1-phenylethyl)amine (11e): Starting from compound **10** (0.2 g, 0.6 mmol) and an excess of phenylethylamine (2.4 g, 20 mmol), **11e** was produced by using the procedure described for **11a** (4 h at 90 °C, 19%): mp: 139 °C; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.12 (d, 3H, J = 6.9 Hz, CH_3), 1.58 (s, 6H, 2×CH_3), 2.49–2.63 (m, 1H, CH), 4.59–5.65 (m, 1H, CH), 5.17 (s, 1H, NH), 6.01 (s, 1H, 3-H 2-Amino-Pyr), 6.44 (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 5-H 2-Amino-Pyr), 6.91–6.99 (m, 2H, 4-F-C_6H_4), 7.23–7.30 (m, 5H, Ph), 7.37–7.44 (m, 2H, 4-F-C_6H_4), 8.12 ppm (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 6-H 2-Amino-Pyr); GC–MS 17.60 min, *m/z*(%): 401(100) [M^+], 386(90), 358(8), 254(10), 239(8), 120(65), 105(45), 95(55) [$C_6H_4F^+$]. Anal. ($C_{25}H_{24}FN_3O$): C, H, N.

2-Ethoxy-4-[5-(4-fluorphenyl)-3-isopropylisoxazol-4-yl]pyridine·HCl (12): Compound **10** (0.15 g, 0.5 mmol) was dissolved in a solution of ethanol (2 g) saturated with HCl and stirred for 5 h at 70 °C. The solvent was evaporated in vacuo to produce **12** (94%) as a white solid: mp: 133 °C; ^{1}H NMR (200 MHz, [D_6]DMSO, 25 °C, TMS): δ = 1.04–1.26 (m, 6H, 2×3H, CH_3, *i*Pr), 1.35 (t, 3H, $^3J_{H,H}$ = 7.0 Hz, CH_3), 2.96–3.08 (m, 1H, CH), 4.36 (q, 2H, $^3J_{H,H}$ = 7.0 Hz, CH_2), 6.47 (s, 1H, 3-H 2-Amino-Pyr), 7.00 (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 5-H 2-Amino-Pyr), 7.28–7.40 (m, 2H, 4-F-C_6H_4), 7.51–7.67 (m, 2H, 4-F-C_6H_4), 8.28 ppm (d, 1H, $^3J_{H,H}$ = 4.0 Hz, 6-H 2-Amino-Pyr); GC–MS 8.50 min, *m/z*(%): 326(30) [M^+], 311(100), 297(45), 282(20), 269(18), 255(24), 240(5), 123(40) [$C_7H_4FO^+$], 95(30) [$C_6H_4F^+$], 75(3). Anal. ($C_{19}H_{19}FN_2O_2$·HCl): C, H, N.

p38 MAP kinase assay: Test compounds were assayed in concentrations ranging from 10^{-4} to 10^{-8} M according to protocols described previously.[53] Results are given as IC_{50} values (μM).

Cytochrome P450 assay: The inhibition of cytochrome P450 isoenzymes by selected compounds at a concentration of 10 μM in phosphate buffer (pH 7.4, 0.1% DMSO) was determined as reported.[56]

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[1] M. Feldmann, F. M. Brennan, R. N. Maini, *Cell* **1996**, *85* 307 – 310.
[2] J. C. Lee, S. Kassis, S. Kumar, A. Badger, J. L. Adams, *Pharmacol. Ther.* **1999**, *82* 389 – 397.
[3] Y. Okada in *Kelly's Textbook of Rheumatology* (Eds.: S. Ruddy, E. J. Harris, C. Sledge), WB Saunders, Philadelphia, **2001**, pp. 55 – 72.
[4] M. Feldmann, F. M. Brennan, R. N. Maini, *Annu. Rev. Immunol.* **1996**, *14* 397 – 440.
[5] J. Martel-Pelletier, D. J. Welsch, J. P. Pelletier, *Best Pract. Res., Clin. Rheumatol.* **2001**, *15* 805 – 829.
[6] H. Yamada, Y. Yoshihara, *Clin. Calcium* **2003**, *13* 702 – 708.
[7] M. D. Tortorella, T. C. Burn, M. A. Pratta, I. Abbaszade, J. M. Hollis, R. Liu, S. A. Rosenfeld, R. A. Copeland, C. P. Decicco, R. Wynn, A. Rockwell, F. Yang, J. L. Duke, K. Solomon, H. George, R. Bruckner, H. Nagase, Y. Itoh, D. M. Ellis, H. Ross, B. H. Wiswall, K. Murphy, M. C. Hillman, Jr., G. F. Hollis, R. C. Newton, R. L. Magolda, J. M. Trzaskos, E. C. Arner, *Science* **1999**, *284*, 1664 – 1666.
[8] I. Abbaszade, R. Q. Liu, F. Yang, S. A. Rosenfeld, O. H. Ross, J. R. Link, D. M. Ellis, M. D. Tortorella, M. A. Pratta, J. M. Hollis, R. Wynn, J. L. Duke, H. J. George, M. C. Hillman, Jr., K. Murphy, B. H. Wiswall, R. A. Copeland, C. P. Decicco, R. Bruckner, H. Nagase, Y. Itoh, R. C. Newton, R. L. Magolda, J. M. Trzaskos, T. C. Burn, *J. Biol. Chem.* **1999**, *274*, 23443 – 23450.
[9] H. Nagase, M. Kashiwagi, *Arthritis Res. Ther.* **2003**, *5*, 94 – 103.
[10] M. Feldmann, *Nat. Immunol.* **2001**, *2*, 771 – 773.
[11] K. Redlich, S. Hayer, R. Ricci, J. P. David, M. Tohidast-Akrad, G. Kollias, G. Steiner, J. S. Smolen, E. F. Wagner, G. Schett, *J. Clin. Invest.* **2002**, *110*, 1419 – 1427.
[12] J. C. Lee, J. T. Laydon, P. C. McDonnell, T. F. Gallagher, S. Kumar, D. Green, D. McNulty, M. J. Blumenthal, J. R. Heyes, *Nature* **1994**, *372*, 739 – 746.
[13] J. L. Adams, A. M. Badger, S. Kumar, J. C. Lee, *Prog. Med. Chem.* **2001**, *41*, 1 – 60.
[14] J. M. Kyriakis, J. Avruch, *Physiol. Rev.* **2001**, *81*, 807 – 869.
[15] L. A. Tibbles, J. R. Woodgett, *Cell. Mol. Life Sci.* **1999**, *55*, 1230 – 1254.
[16] G. Lominadze, M. J. Rane, M. Merchant, J. Cai, R. A. Ward, K. R. McLeish, *J. Immunol.* **2005**, *174*, 7257 – 7267.
[17] S. Kumar, J. Boehm, J. C. Lee, *Nat. Rev. Drug Discovery.* **2003**, *2*, 717 – 726.
[18] I. Lantos, P. E. Bender, K. A. Razgaitis, B. M. Sutton, M. J. DiMartino, D. E. Griswold, D. T. Walz, *J. Med. Chem.* **1984**, *27*, 72 – 75.
[19] J. C. Lee, A. M. Badger, D. E. Griswold, D. Dunnington, A. Truneh, B. Votta, J. R. White, P. R. Young, P. E. Bender, *Ann. N. Y. Acad. Sci.* **1993**, *696*, 149 – 170.
[20] T. F. Gallagher, S. M. Fier-Thompson, R. S. Garigipati, M. E. Sorenson, J. M. Smietana, D. Lee, P. E. Bender, J. C. Lee, J. T. Laydon, D. E. Griswold, M. C. Chabot-Fletcher, J. J. Breton, J. L. Adams, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1171 – 1176.
[21] L. Tong, S. Pav, D. M. White, S. Rogers, K. M. Crane, C. L. Cywin, M. L. Brown, C. A. Pargellis, *Nat. Struct. Biol.* **1997**, *4*, 311 – 316.
[22] Z. Wang, B. J. Canagarajah, J. C. Boehm, S. Kassisa, M. H. Cobb, P. R. Young, S. Abdel-Meguid, J. L. Adams, E. J. Goldsmith, *Structure* **1998**, *6*, 1117 – 1128.
[23] R. J. Gum, M. M. Mclaughlin, S. Kumar, Z. Wang, M. J. Bower, J. C. Lee, J. L. Adams, G. P. Livi, E. J. Goldsmith, P. R. Young, *J. Biol. Chem.* **1998**, *273*, 15605 – 15610.
[24] L. M. Toledo, N. B. Lydon, D. Elbaum, *Curr. Med. Chem.* **1999**, *6*, 775 – 805.
[25] K. P. Wilson, P. G. McCaffrey, K. Hsiao, S. Pazhinisamy, V. Galullo, G. W. Bemis, M. J. Fitzgibbon, P. R. Caron, M. A. Murcko, M. S. S. Su, *Chem. Biol.* **1997**, *4*, 423 – 431.
[26] J. C. Boehm, J. M. Smietana, M. E. Sorenson, R. S. Garigipati, T. F. Gallagher, P. L. Sheldrake, J. Bradbeer, A. M. Badger, J. T. Laydon, J. C. Lee, L. M. Hillegass, D. E. Griswold, J. J. Breton, M. C. Chabot-Fletcher, J. L. Adams, *J. Med. Chem.* **1996**, *39*, 3929 – 3937.
[27] S. A. Laufer, G. K. Wagner, *J. Med. Chem.* **2002**, *45*, 2733 – 2740.
[28] C. E. Fitzgerald, S. B. Patel, J. W. Becker, P. M. Cameron, D. Zaller, V. B. Pikounis, S. J. O'Keefe, G. Scapin, *Nat. Struct. Biol.* **2003**, *10*, 764 – 769.
[29] P. R. Young, M. M. McLaughlin, S. Kumar, S. Kassis, M. L. Doyle, D. McNulty, T. F. Gallagher, S. Fisher, P. C. McDonnell, S. A. Carr, M. J. Huddleston, G. Seibel, T. G. Porter, G. P. Livi, J. L. Adams, J. C. Lee, *J. Biol. Chem.* **1997**, *272*, 12116 – 12121.
[30] T. F. Gallagher, G. L. Seibel, S. Kassis, J. T. Laydon, M. J. Blumenthal, J. C. Lee, D. Lee, J. C. Boehm, S. M. Fier-Thompson, J. W. Abt, M. E. Soreson, J. M. Smietana, R. F. Hall, R. S. Garigipati, P. E. Bender, K. F. Erhard, A. J. Krog, G. A. Hofmann, P. L. Sheldrake, P. C. McDonnell, S. Kumar, P. R. Young, J. L. Adams, *Bioorg. Med. Chem.* **1997**, *5*, 49 – 64.
[31] S. E. de Laszlo, D. Visco, L. Agarwal, L. Chang, J. Chin, G. Croft, A. Forsyth, D. Fletcher, B. Frantz, C. Hacker, W. Hanlon, C. Harper, M. Kostura, B. Li, S. Luell, M. MacCoss, N. Mantlo, E. A. O'Neill, C. Orevillo, M. Pang, J. Parsons, A. Rolando, Y. Sahly, K. Sidler, W. R. Widmer, S. J. O'Keefe, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2689 – 2694.
[32] S. E. de Laszlo, *Preparation of pyridylpyrroles and analogues as cytokine inhibitors and glucagon antagonists*. [WO97/16442] 96-US18539, 178, **1996**.
[33] I. Nobeli, S. L. Price, J. P. M. Lommerse, R. Taylor, *J. Comput. Chem.* **1997**, *18*, 2060 – 2074.
[34] J. L. Adams, J. C. Boehm, S. Kassis, P. D. Gorycki, E. F. Webb, R. Hall, M. Sorenson, J. C. Lee, A. Ayrton, D. E. Griswold, T. F. Gallagher, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3111 – 3116.
[35] B. Testa, P. Jenner, *Drug Metab. Rev.* **1981**, *12*, 1 – 117.
[36] N. J. Liverton, J. W. Butcher, C. F. Claiborne, D. A. Claremon, B. E. Libby, K. T. Nguyen, S. M. Pitzenberger, H. G. Selnick, G. R. Smith, A. Tebben, J. P. Vacca, S. L. Varga, L. Agarwal, K. Dancheck, A. J. Forsyth, D. S. Fletcher, B. Frantz, W. A. Hanlon, C. F. Harper, S. J. Hofsess, M. Kostura, J. Lin, S. Luell, E. A. O'Neill, C. J. Orevillo, M. Pang, J. Parsons, A. Rolando, Y. Sahly, D. M. Visco, S. J. O'Keefe, *J. Med. Chem.* **1999**, *42*, 2180 – 2190.
[37] S. Ohkawa, K. Naruo, S. Miwatashi, H. Kimura, *Preparation of 5-(4-pyridyl)thiazoles as p38 MAP kinase inhibitors and inhibitors of TNF-α production*. Takeda Chemical Industries, Ltd. Japan. [2001074811] 2001-JP2629, 288, **2001**, WO.29-3-2001.
[38] S. Ohkawa, K. Naruo, S. Miwatashi, H. Kimura, S. Miwatashi, *Preparation of thiazole derivatives as p38 MAP kinase inhibitors and inhibitors of TNF-α production*. [WO2001010865] 2000-JP5198, 166, **2000**.
[39] L. Revesz, F. E. Di Padova, T. Buhl, R. Feifel, H. Gram, P. Hiestand, U. Manning, R. Wolf, A. G. Zimmerlin, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2109 – 2112.
[40] L. Revesz, *Preparation of azolylazines as p38 MAP kinase inhibitors*. [WO2000063204] 2000-EP3290, 87, **2000**.
[41] L. Revesz, F. E. Di Padova, T. Buhl, R. Feifel, H. Gram, P. Hiestand, U. Manning, A. G. Zimmerlin, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1261 – 1264.
[42] N. Minami, M. Sato, K. Hasumi, N. Yamamoto, Keino, M. Katsuyuki, K. Teruaki, O. Arihiro, S. Shuji, S. Norihisa, A. Shuichiro, D. Akira, K. Satoshi, S. Motohiro, S. Asano, *Preparation of 5-aminoisoxazole derivatives as inhibitors of p38 MAP kinase*. [JP2000086657] 98-272467, 28, **1998**.
[43] T. Oku, Y. Kawai, H. Marusawa, H. Yamazaki, Y. Abe, H. Tanaka, *Preparation of condensed pyrazole derivatives with interleukin-1 and tumor necrosis factor inhibitory activity*. [EP531901] 92-115154, 84, **1992**.
[44] E. M. Beccalli, A. Manfredi, A. Marchesini, *J. Org. Chem.* **1985**, *50*, 2372 – 2375.
[45] Y. Kawai, H. Yamazaki, H. Tanaka, T. Oku, *Preparation of pyrazolo[5,1-c]-[1,2,4]triazines with interleukin-1 and tumor necrosis factor inhibitory activity*. [WO94/19350] 94-JP213, 108, **1994**.
[46] S. Nahm, S. M. Weinreb, *Tetrahedron Lett.* **1981**, *22*, 3815 – 3818.

[47] R. SanMartin, E. Martinez de Marigorta, E. Dominguez, *Tetrahedron* **1994**, *50*, 2255 – 2264.
[48] E. Dominguez, E. Ibeas, E. Martinez de Marigorta, J. K. Palacios, R. SanMartin, *J. Org. Chem.* **1996**, *61*, 5435 – 5439.
[49] B. J. Wakefield, D. J. Wright, *Adv. Heterocycl. Chem.* **1979**, *17*, 147 – 204.
[50] P. Bravo, D. Diliddo, G. Resnati, *Tetrahedron* **1994**, *50*, 8827 – 8836.
[51] G. Renzi, V. Dal Piaz, S. Pinzauti, *Gazz. Chim. Ital.* **1969**, *99*, 753 – 761.
[52] S. A. Laufer, G. K. Wagner, D. A. Kotschenreuther, W. Albrecht, *J. Med. Chem.* **2003**, *46*, 3230 – 3244.
[53] S. Laufer, S. Thuma, C. Peifer, C. Greim, Y. Herweh; A. Albrecht, F. Dehner, *Anal. Biochem.* **2005**, *344*, 135 – 137.
[54] M. Wachter, *Preparation of 2-substituted imidazoles useful in the treatment of inflammatory diseases*. [WO99/3837] 98-US13419, 55, **1999**.
[55] T. F. Gallagher, S. M. Fier-Thompson, R. S. Garigipati, M. E. Sorenson, J. M. Smietana, D. Lee, P. E. Bender, J. C. Lee, J. T. Laydon, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1171 – 1176.
[56] Gentest Corporation (Woburn, MA, USA), Technical Bulletin, Version 3, **1998**.

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