Isoxazolone Based Inhibitors of p38 MAP Kinases

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SAR of N-alkylated isoxazolones as p38 MAP kinase inhibitors was realized. The data herein show the possibility of transferring the SAR study and evaluation from N-1-substituted imidazole to isoxazolones. Optimization of substituent was realized.

Introduction

In a continuous effort to develop improved p38 MAP[a] kinase inhibitors, we focused our attention on the suitability of the isoxazolone ring as bioisosteric replacement for the imidazole ring of SB203580 (Figure 1).

A large body of evidence indicates that p38 activity is critical for the normal immune and inflammatory responses.[1] The requirement for p38 activation in cellular responses has been defined largely through the use of experimental pyridinylimidazole anti-inflammatory drugs, the cytokine-suppressive anti-inflammatory drugs (CSAIDs). The most extensively characterized is the compound SB203580.

p38 inhibitors are potent inhibitors of LPS-mediated TNF-α production in macrophages.[2] The ability of p38 inhibitors to block TNF-α synthesis can be exploited in the treatment of inflammatory diseases.

Previous work from our laboratories described the synthesis and SAR of N-1-substituted imidazole p38 inhibitors, exemplified by **1** (Figure 1),[3] which contain the prototypical vicinal bis-aryl pharmacophore. In the cited publication, we reported optimization of a novel structural class of tetrasubstituted imidazoles inhibitors of p38 MAP kinase[4,5] by imidazole N-1 substitutions. The work has demonstrated that by utilization of an in vitro enzymatic assay as a primary screening tool, N-1 substitution can successfully lead to potent and efficacious p38 MAP kinase inhibitors.

The development of the imidazole based p38 MAP kinase inhibitors into anti-inflammatory drugs was obstructed by their severe liver toxicity, as the pyridinylimidazoles were found to interact with the hepatic cytochrome P450 enzymes involved in drug metabolism.[6] In this prospective we investigated the potential of the isoxazole as bioisosteric replacement of the imidazole heterocycle.[7] This led to the development of isoxazoles and then consequentially to isoxazolones as an emerging leading series in our project. Herein, we report the synthesis and SAR of 5-alkoxyisoxazoles (**2**, Figure 1) and of N-substituted 5-isoxazolones (**3**, Figure 1). Some of the reported structures are claimed as anticytokine agents by Procter & Gamble Pharmaceuticals;[8] nevertheless, to date, no p38 MAP kinase inhibition data have been published.[9] Our investigation shows that the structure–activity study realized on the N-1-substituted imidazoles[3] could be transferred to the isoxazolone series but not to the isoxazoles.

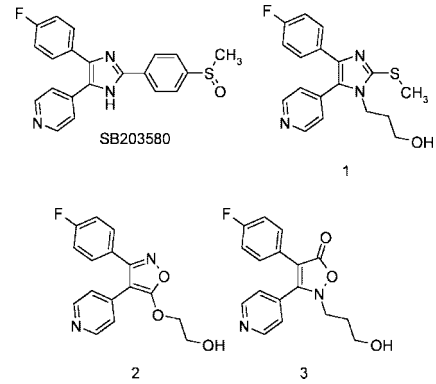
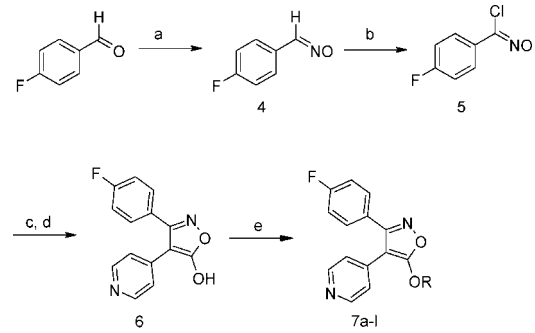


Figure 1. Prototypical pyridinylimidazole p38 inhibitor SB203580 and related vicinal bis-aryl p38 MAP kinase inhibitors. $IC_{50} \pm$ SEM (μM) p38α: **1**, 0.813 ± 0.04; **2**, 14.7% inhibition at 10 μM; **3**, 1.15 ± 0.03. IC_{50} SB203580, 0.045 ± 0.01.

Scheme 1[a]



[a] Reagents: (a) hydroxylamine chloride, NaOH 50%, H_2O/ice/ethanol, <10 °C then room temp, 1 h; (b) *N*-chlorosuccinimide, DMF, room temp; (c) NaHDMS, ethyl 4-pyridylacetate, THF, room temp, 6 h; (d) conc HCl; (e) alkyl halide, Et_3N, CH_2Cl_2.

Chemistry

The synthesis of the 5-alkoxyisoxazoles was based on O-alkylation of the pyridinylisoxazolone **6** synthesized from commercially available starting materials (Scheme 1).

According to Scheme 1, **6** was synthesized via 1,3-dipolar cycloaddition,[10] where the 1,3-dipole is 4-fluorophenylnitrile oxide generated from the corresponding hydroxamic acid chloride **5**, which was obtained by a high yield two-step procedure starting from 4-fluorobenzaldehyde. Ethyl 4-pyridylacetate is deprotonated

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[a] Abbreviations: p38 MAP, p38 mitogen activated protein; CSAIDs, cytokine-suppressive anti-inflammatory drugs; LPS, lipopolysaccaride; TNF-α, tumor necrosis factor-α; SAR, structure–activity relationship.

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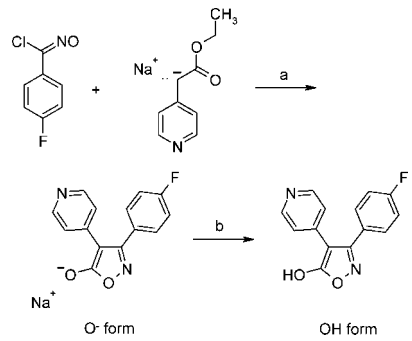
Scheme 2[a]



[a] Reagents: (a) THF, room temp, 6 h; (b) conc HCl.

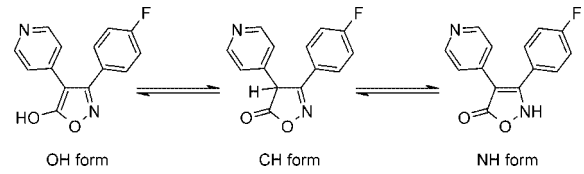
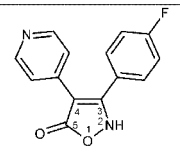


Figure 2. Tautomerism of 5-hydroxyisoxazole, **6**.

Table 1. ^{13}C NMR Chemical Shifts for **6** in DMSO and Aqueous Systems



Chemical shifts δ (ppm)			
Solvent System	C-3	C-4	C-5
DMSO-d_6	155.19	90.00	166.19
D_2O[a]	147.94	84.60	163.05
99. 8% D_2O /0. 2% NaOH[a]	149.6	87.1	165.3

[a] In aqueous solutions, shifts calculated from DMSO=39.52 ppm relative to Me4Si.

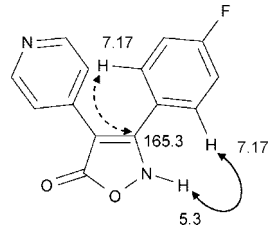


Figure 3. $^1H-^1H$ NMR NOESY (bold line) and $^1H-^{13}C$ NMR HMBC (dashed line) correlations in **6** (chemical shift in ppm).

by sodium hexamethyldisilazide (NaHDMS) and added to an equimolar amount of 4-fluorophenyl chloride oxime. The in situ generated nitrile oxide acts on the deprotonated ester to give the conjugated base (O^- form) of **6**. Column chromatography over SiO_2 allows removal of starting material and side products by the elution with ethyl acetate. Elution with methanol allows isolation of the conjugated base. The sodium salt of the base is then converted to the protonated OH and crystallized. The ring closure yielding the O^- form of isoxazolone is mapped in Scheme 2.

It is well-known[11,12] that 5-hydroxyisoxazoles are in equilibrium with the other two tautomeric forms: CH and NH forms (Figure 2). According to literature[11,13–16] for 3-substituted isoxazolin-5-ones in a solvent of low dielectric constant, the predominant form is the CH. The NH form becomes more favored when substituents on C-4 are introduced and in more polar solvents. For 5-hydroxyisoxazoles, contrary to what was reported for 3-hydroxyisoxazolones,[17] the OH form is rarely observed; it was detected only when chelation took place between the hydroxyl group and the 4-substituent.[11]

Analytic on **6** seems to indicate that in solution it assumes an oxo form,[11,13,14] as most of the compounds with a hydroxyl

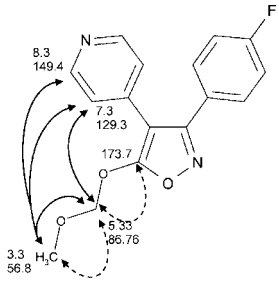
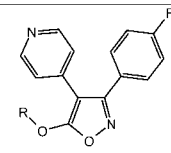
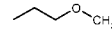
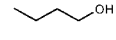


Figure 4. $^1H-^1H$ NMR NOESY (bold line) and $^1H-^{13}C$ NMR HMBC (dashed line) correlations in **7e** (chemical shift in ppm).

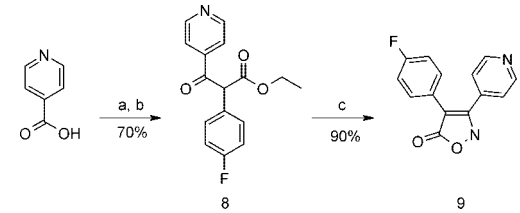
Table 2. Inhibition of p38 MAP Kinase[a]



Cmpd	R	IC_{50}±SEM (μM) p38α[b]
7a		26,5@10 μM
7b		13,6@10 μM
7c		23,7@10 μM
7d		26,4@10 μM
7e		27,8@10 μM
7f		14,4@10 μM
7g		9,8@10 μM
7h		14,7@10 μM
7i		11,9@10 μM

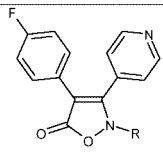
[a] IC_{50} SB203580 = 0.045 ± 0.011 μM. [b] Percentage of inhibition at 10 μM (concentration of test compound). Inhibition data from p38 kinase assay, ref 20.

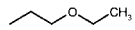
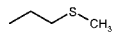
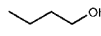
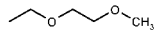
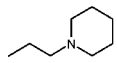
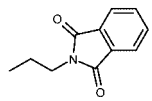
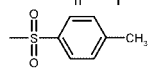
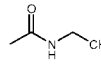
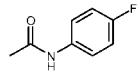
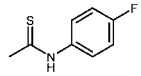
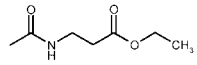
Scheme 3[a]



[a] Reagents: (a) CDI, DMF, room temp; (b) (4-fluorophenyl)acetic acid ethyl ester, NaH, room temp; (c) $NH_2OH \cdot HCl$, H_2O, MeOH, 80 °C.

Table 3. Inhibition of p38 MAP Kinase[a]



Cmpd	R	IC_{50}±SEM (μM) p38α[a]
10a		1.63±0.04 (n=3)
10b		42.2@10μM[b]
10c		2.13±0.08 (n=3)
10d		3.83±0.05 (n=3)
10e		2.13±0.08 (n=3)
10f		1.32±0.02 (n=3)
10g		3.9±0.09 (n=3)
10h		2.5±0.04 (n=3)
10i		1.15±0.03 (n=3)
10l		3.83±0.05 (n=3)
10m		38.3@10μM[b]
10n		41.86@10μM[b]
10o		27.5@10μM[b]
10p		0.48±0.02 (n=3)
10q		0.12±0.02 (n=3)
10r		0.23±0.05 (n=3)
10s		0.32±0.03 (n=3)
10t		0.35±0.03 (n=3)
10u		0.97±0.05 (n=3)

[a] IC_{50} SB203580 = 0.045 ± 0.011 μM. [b] Percentage of inhibition at 10 μM (concentration of test compound). Inhibition data from p38 kinase assay, ref 20.

function in the position α or γ to a nitrogen atom. Chemical shifts from ^{13}C NMR experiments in DMSO-d_6, 90% D_2O/10% DMSO-d_6, and 89.98% D_2O/10% DMSO-d_6/0.02% NaOH for **6** are listed in Table 1.

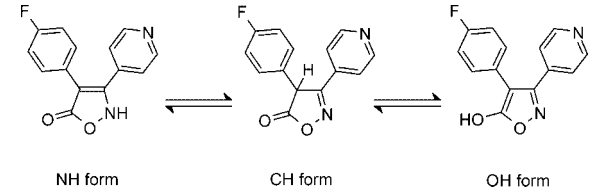


Figure 5. Tautomeric equilibrium for **9**.

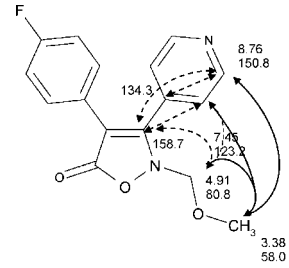
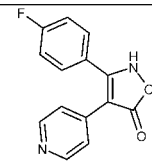
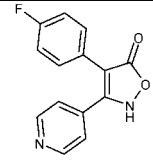


Figure 6. $^1H-^1H$ NMR NOESY (bold line) and $^1H-^{13}C$ NMR HMBC (dashed line) correlations in **10e** (chemical shift in ppm).

Scheme 4. Synthesis of Ureas and Thioureas Derivatives

Table 4. p38 Inhibition

cmpd	Structure	IC_{50}±SEM (μM) p38α[a]
6		1.40±0.01 (n=3)
9		0.39±0.01

The data are in close agreement with literature,[13] and they indicate that **6**, also in basic conditions, assumes the NH form. A ring sp^2 carbon bonded to one heteroatom (C-3) lies upfield from a second sp^2 carbon bonded to two heteroatom (C-5). Furthermore, 2D NMR experiments confirmed those data (Figure 3).

Derivatives of **6** were prepared by alkylation. As pointed out, **6** exists, in polar solvents such as DMSO, in the NH form. This experimental evidence, nevertheless, does not imply absence of tautomeric equilibrium undetectable by NMR. Different tautomers could potentially lead to different alkylation products: N-alkylation for the NH form and O-alkylation for the OH form. In the literature it is possible to find examples of alkylation of 3-hydroxyisoxazoles[18] as well as 3-hydroxyisothiazoles[19] that show that alkylation depends on the relative rates of reaction of the two tautomers rather than on their relative proportions.

In the case of **6**, alkylation reactions had been realized in different solvent systems (DMF and $CHCl_3$) to investigate

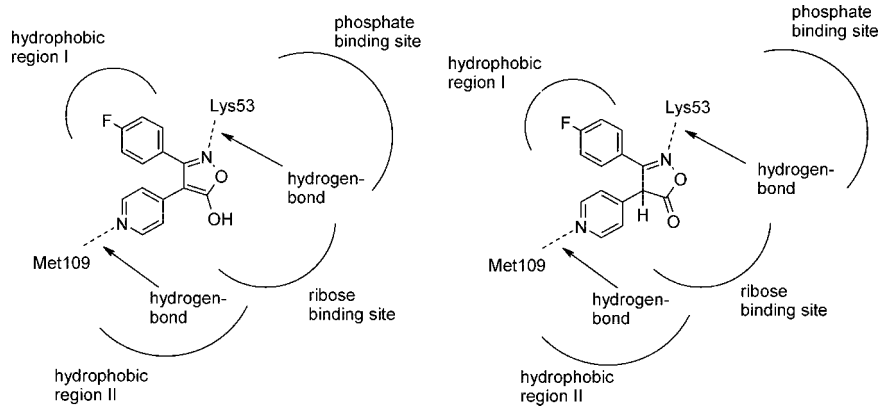


Figure 7. Suggested binding modus of **6** in the ATP pocket of p38 MAPK.

possible dependence of reaction product from solvent polarity, but in all examined cases no dependency was observed and in all cases the O-alkylation product was obtained versus the N-alkylation one (Scheme 1).

The alkylation products were identified by $^1H-^1H$ NMR NOESY and by $^1H-^{13}C$ NMR HMBC (Figure 4).

In Table 2 the 5-alkoxyisoxazole series is listed. The nature of the introduced substituents in the 5 position at the isoxazole core was chosen on the basis of an N-1-substituted imidazole study undertaken in our research group.[3] According to the cited study, systematic optimization of the imidazole N-1 substituent resulted in the identification of rests able to confer high inhibitory activity to the molecule. For those compounds the range of the experimental IC_{50} was (6–9) $\times$ 10^{-2} μM. The undertaken study showed that alkoxy substitution in the 5 position of the isoxazole core is not tolerated, and it was not possible to calculate any IC_{50} for any of the series members.

Compound **9**, regioisomer of **6**, was synthesized according to the procedure outlined in Scheme 3. The first step of the synthesis is a Claisen condensation of 2-aryl acetate ester and pyridylimidazolide to give the β-ketoester **8** by using NaH as the base system. The reaction had been also realized by using harpoon bases such as LDA, NaHDMS, and sodium *tert*-butoxide and by using, according to a classical Claisen condensation scheme, sodium ethanolate. In these cases, no reaction (LDA, sodium ethanolate) or just traces of product (NaHDMS, *tert*-butoxide) were observed. The low conversions result primarily from the fact that the product 1,3-dicarbonyl compound is a carboxylic acid that quenches unreacted bases at a rate that exceeds acylation.

Similar to what is described for **6**, derivatives of **9** were prepared (Table 3). The same considerations about tautomery and reactivity toward alkylation done previously can be applied to compound **9**, in solution the different tautomeric forms could theoretically interconvert according to the equilibrium in Figure 5. Alkylation reactions were realized by treating **9** with the appropriate alkyl halide using triethylamine as base. Also in this case, as it was observed for **6**, experiments realized in $CHCl_3$ and DMF showed absence of dependency of reaction products by solvent nature. A 2D NMR study allowed identification of the products as *N*-alkyl-5-isoxazolones, underlying a difference in reactivity for the two tautomers (Figure 6). The presence of the 4-fluorophenyl moiety in the 4 position on the isoxazolone ring may affect the reactivity of the molecule toward nucleophilic substitution, leading to *N*-alkyl-5-isoxazolones. The series of analogues was then completed by reacting **9** with isocyanates and isothiocyanates, introducing carboxamides functions in the 2 position of the ring. Substituted ureas and thioureas were obtained by *N*-hydro-*C*-alkylamino addition reactions according to Scheme 4. Compound **9** was added to the isocyanate (or isothiocyanate) in a polar solvent such as DMF in the presence of sodium hydride as a base.

Biological Results and Discussion

The biological activity for the analogues in Table 2 shows that alkoxy substitution in 5 position of the isoxazole core is not tolerated; the unsatisfactory inhibition activity may be related to sterical effects. The biological activity of **6** and **9** are reported in Table 4. Of note is that the hydrogen bond acceptor for **9**, as shown by cocrystallization in mutated p38 active side,[9] is the carbonyl oxygen, and accepting the binding mode suggested in Figure 7 is the ring nitrogen for **6**. Different studies on the hydrogen bonding properties of oxygen and nitrogen acceptors in aromatic heterocycles have been published[21,22] showing that nitrogen is a significantly better H-bond acceptor than oxygen. In particular, studies of Boehm et al.[22] of the hydrogen bond capabilities of oxygen atoms covalently bonded to an sp^2 hybridized atom showed that these were often considerably weaker acceptors than equivalent nitrogen atoms. This is in contrast to the common expectations, based solely on electronegativity, that both are good acceptors. Nevertheless, for the two regioisomers **6** and **9**, it must be taken into account that the nitrogen is endocyclic while the carbonyl oxygen exocyclic, this fact, influencing the length of the H-bond, and the part of the molecules exposed to the ribose binding site, is different in the two cases.

As the data in Table 4 show, the alkylated derivatives exert relatively good activity, but they did not represent a real optimization of the inhibitory potency of **9**. The most potent alkyl substituent was the propanol **10i** and the ethoxyethyl **10f**. These data are in accordance with the already cited optimization study of the imidazole N-1 substituents undertaken in our research group and recently published (Figure 1).[3] This evidence is relevant because it allows application of the SAR of isoxazolones to imidazole and vice versa. The most promising compounds were the urea and the amide derivatives **10p**–**u**. The good activity of the urea derivatives is in agreement with the findings of researchers at Procter & Gamble.[9] Laughlin nevertheless reported significant metabolism in an in vitro rat hepatocyte metabolic stability assay. For this reason, the good inhibitory activity of amide-type **10p** and **10q** can be considered a satisfying result.

Experimental Section. Typical procedures

Synthesis of 5-Alkoxyisoxazoles. General Procedure I. To a suspension of **6** in DMF (2 mL/1mmol of **6**) was added 3 equiv of Et_3N, and the mixture was refluxed for 2 h. The limpid solution was then combined with halogen halide (1.8 equiv) and

the mixture stirred under reflux for another 2 h and subsequently at room temperature overnight. The mixture was poured into water, and the two phases were partitioned. The water phase was extracted twice with DCM. The collected organic phases were washed with H_2O, brine, and H_2O, dried over Na_2SO_4, and concentrated under vacuum. The final product was purified by column chromatography on SiO_2.

4-[3-(4-Fluorophenyl)-5-methoxyisoxazol-4-yl]pyridine (7a). 7a was obtained according to general procedure I by reacting 111 μL (1.8 mmol) of methyl iodide and 256 mg (1 mmol) of **6** and subsequent purification by column chromatography on SiO_2 (eluent THF). Yield 160 mg (22%); $C_{15}H_{11}FN_2O_2$ (MW 270.26); mp 229 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 3.84 (s, 3H, -CH_3), 7.29–7.51 (m, 6H), 8.03–8.06 (d, 2H); ^{13}C NMR (DMSO-d_6) δ (ppm) 45.0, 85.4 (C^4 Isox), 115.7, 116.3 (J=22.0, C^4 4FPh), 128.9 (J = 2.9, C^2 4FPh), 129.3 (J = 8.5, C^3/C^5 4FPh), 142.1, 149.1, 161.4 (C^3 Isox), 163.0 (J = 229.0 Hz, C^1 4FPh), 174.0 (C^5 Isox); FTIR 3049, 2965, 1676, 1619, 1517, 1475, 1443, 1415, 1366, 1219, 1202, 956, 883, 844 cm^{-1}; MS 271 (M + 1), 254, 243, 227, 212, 134, 94. Anal. ($C_{15}H_{11}FN_2O_2$) C, H, N, O.

N-Alkylation of 9. General Procedure II. To a suspension of **9** (1 equiv) in DMF (2 mL/1mmol **9**) was added 1.8 equiv of Et_3N, and the mixture was refluxed for 2 h. The cooled mixture (room temperature) was combined with alkyl halide (1.5 equiv) (if not specified otherwise, the chloride was used) and stirred for 3 h at room temperature. The reaction was worked up following method a, b, or c depending on the nature of the residue.

4-(4-Fluorophenyl)-3-(pyridin-4-yl)-2-tosylisoxazol-5(2*H*)-one (10p). 10p was synthesized according to general procedure II by reacting 1.36 g (7.2mmol) of 4-methylbenzenesulfonyl chloride with 1 g (3.9 mmol) of **9**. Purification following method c (crystallization from acetone) afforded the title compound. Yield 50 mg (3%); $C_{21}H_{15}FN_2O_4S$ (MW 410.07); mp 198 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.51 (s, 3H, $-CH_3$), 6.96–7.26 (m, 4H), 7.37–7.43 (m, 4H), 7.64 (dd, J = 0.5/1.4 Hz; 2H, Py), 8.78 (dd, J = 0.5/1.4 Hz; 2H, Py); ^{13}C NMR (DMSO-d_6) δ (ppm) 21.8, 114.9 (C^4 isoxazolone), 116.1 (J = 19.1 Hz, C^2/C^6 4FPh), 121.4 (J = 3.5, C^4 4FPh), 121.9, 125.8, 126.9, 127.6, 130.3 (J = 8.4 Hz, C^3/C^5 4FPh), 140.5, 147.6, 149.9, 155.6 (C^3 isoxazolone), 163.0 (J = 251.0 Hz, C^1 4FPh), 167.0 (C^5 isoxazolone); FTIR 3053, 1770, 1638, 1590, 1509, 1384, 1228, 1173, 1158, 954, 848, 812, 690 cm^{-1}. Anal. ($C_{21}H_{15}FN_2O_4S$) C, H, N, O.

General Procedure III. To a solution of **9** (1 equiv) in DMF (1 mL/1mmol of **9**), sodium hydride (1 equiv) and the appropriate isocyanate (1.5 eq) were added. The mixture was stirred for 12 h at room temperature and then combined with water. Filtration of the formed precipitate afforded the pure title compound. A further purification step was not necessary.

***N*-Ethyl-4-(4-fluorophenyl)-5-oxo-3-(pyridin-4-yl)isoxazole-2(5*H*)-carboxamide (10r). 10r**was synthesized according to the general procedure III starting from 1 g (3.9mmol) of **9** and 0.4 g (5.8 mmol) of ethyl isocyanate. Yield 293 mg (23%); $C_{17}H_{14}FN_3O_3$ (MW 327.1); mp 200 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 1.03 (t, 3H), 3.08 (m, 2H), 7.08–7.25(m, 4H, 4FPh), 7.45 (d, 2H, J = 1.6 Hz, Py), 8.45 (t, 1H, exch.), 8.65 (d, 2H, J = 1.6 Hz, Py); ^{13}C NMR (DMSO-d_6) δ (ppm) 14.9, 22.5, 105.5 (C^4 isoxazolone), 115.9 (J = 21.3 Hz, C^2/C^6 4FPh), 123.0 (J =3.1, C^4 4FPh), 124.2, 131.0 (J = 7.9 Hz, C^3/C^5 4FPh), 136.9, 148.2, 149.9, 154.4 (C^3 isoxazolone), 161.9 (J = 240.0 Hz, C^1 4FPh), 166.2 (C^5 isoxazolone); FTIR 3072, 2923, 2854, 1750, 1723, 1579, 1508, 1219, 954, 841, 826 cm^{-1}. Anal. ($C_{17}H_{14}FN_3O_3$) C, H, N, O.

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Supporting Information Available: General synthetic procedures, spectral and analytical and biochemical experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ono, K.; Han, J. The p38 signal transduction pathway. Activation and function. *Cell. Signalling* **2000**, *12*, 1–13.

(2) Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heyes, J. R. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **1994**, *372*, 739–746.

(3) Laufer, S. A.; Zimmermann, W.; Ruff, K. J. Tetrasubstituted imidazole inhibitors of cytokine release: probing substituents in the N-1 position. *J. Med. Chem.* **2004**, *47*, 6311–6325.

(4) Laufer, S. A.; Striegel, H. G.; Wagner, G. K. Imidazole inhibitors of cytokine release: probing substituents in the 2 position. *J. Med. Chem.* **2002**, *45*, 4695–4705.

(5) Laufer, S.; Striegel, H.-G.; Tollmann, K.; Albrecht, W. Preparation of 2-Methylsulfanyl-3*H*-imidazoles and Related Compounds as Immunomodulators. 2002-10238045[10238045], 31; DE. 20-8-2002, 2004; Merckle G.m.b.H.Chem.-Pharm.Fabrik,Germany.

(6) Adams, J. L.; Boehm, J. C.; Kassis, S.; Gorycki, P. D.; Webb, E. F.; Hall, R.; Sorenson, M.; Lee, J. C.; Ayrton, A.; Griswold, D. E.; Gallagher, T. F. Pyrimidinylimidazole inhibitors of CSBP/p38 kinase demonstrating decreased inhibition of hepatic cytochrome P450 enzymes. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3111–3116.

(7) Laufer, S. A.; Margutti, S.; Fritz, M. D. Substituted isoxazoles as potent inhibitors of p38 MAP kinase. *ChemMedChem* **2006**, *1*, 197–207.

(8) Clark, M. P.; Djung, J. F.-J.; Laughlin, S. K.; Tullis, J. S.; Natchus, M. G.; De, B. Isoxazolones Useful in Treating Diseases Associated with Unwanted Cytokine Activity. 2002-US15431[2002094266], 69; WO 15-5-2002, 2002; The Procter & Gamble Company.

(9) Laughlin, S. K.; Clark, M. P.; Djung, J. F.; Golebiowski, A.; Brugel, T. A.; Sabat, M.; Bookland, R. G.; Laufersweiler, M. J.; VanRens, J. C.; Townes, J. A.; De, B.; Hsieh, L. C.; Xu, S. C.; Walter, R. L.; Mekel, M. J.; Janusz, M. J. The development of new isoxazolone based inhibitors of tumor necrosis factor-alpha (TNF-α) production. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2399–2403.

(10) Dannhardt, G.; Laufer, S.; Obergrusberger, I. A new synthesis of 3,4-diaryl-5-oxo-4,5-dihydroisoxazoles and their transformation to 5-[*N*-(w-aminoalkyl)amino]isoxazoles and 5-(2-aminoethylthio)isoxazoles. *Synthesis* **1989**, *27*, 5–280.

(11) Elguero, J., Marzin, C., Katritzky, A. R., Linda, P., Eds. *Advances in Heterocyclic Chemistry, Supplement 1: The Tautomerism of Heterocycles*; Academic Press: New York, 1976; p 656.

(12) Kohler, E. P.; Blatt, A. H. Diphenylisoxazolone. Study of the tautomerism of isoxazolones. *J. Am. Chem. Soc.* **1928**, *50*, 504–515.

(13) Katritzky, A. R.; Barczynski, P.; Ostercamp, D. L.; Yousaf, T. I. Mechanisms of heterocyclic ring formations. 4. A carbon-13 NMR study of the reaction of b-keto esters with hydroxylamine. *J. Org. Chem.* **1986**, *51*, 4037–4042.

(14) Boulton, A. J.; Katritzky, A. R. Tautomerism of heteroaromatic compounds with five-membered rings. I. 5-Hydroxyisoxazoles-5-isoxazolones. *Tetrahedron* **1961**, *12*, 41–50.

(15) Franchini, P. F. Dipole moments and tautomerism of isoxazolin-5-ones. *Corsie Semin. Chim.* **1968**, *14*, 23–25.

(16) Cencioni, R.; Franchini, P. F.; Orienti, M. Dipole moments of 5-substituted isoxazoles. I. Dipole moments and tautomerism of isoxazolin-5-ones. *Tetrahedron* **1968**, *24*, 151–166.

(17) Jacquier, R.; Petrus, C.; Petrus, F.; Verducci, J. Azoles. LXXII. Unequivocal synthesis and tautomerism of 3-isoxazolones. *Bull. Soc. Chim. Fr.* **1970**, *197*, 8–1985.

(18) Frolund, B.; Jensen, L. S.; Guandalini, L.; Canillo, C.; Vestergaard, H. T.; Kristiansen, U.; Nielsen, B.; Stensbol, T. B.; Madsen, C.; Krogsgaard-Larsen, P.; Liljefors, T. Potent 4-Aryl- or 4-arylalkyl-substituted 3-isoxazolol GABAA antagonists: synthesis, pharmacology, and molecular modeling. *J. Med. Chem.* **2005**, *48*, 427–439.

(19) Chan, A. W. K.; Crow, W. D.; Gosney, I. Isothiazole chemistry. X. Acylation, alkylation, and tautomerism in 3-hydroxyisothiazole. *Tetrahedron* **1970**, *26*, 2497–2506.

(20) Laufer, S.; Thuma, S.; Peifer, C.; Greim, C.; Herweh, Y.; Albrecht, A.; Dehner, F. An immunosorbent, nonradioactive p38 MAP kinase assay comparable to standard radioactive liquid-phase assays. *Anal. Biochem.* **2005**, *344*, 135–137.

(21) Nobeli, I.; Price, S. L.; Lommerse, J. P. M.; Taylor, R. Hydrogen bonding properties of oxygen and nitrogen acceptors in aromatic heterocycles. *J. Comput. Chem.* **1997**, *18*, 2060–2074.

(22) Boehm, H. J.; Brode, S.; Hesse, U.; Klebe, G. Oxygen and nitrogen in competitive situations: which is the hydrogen-bond acceptor? *Chem.—Eur. J.* **1996**, *2*, 1509–1513.

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