Journal of Medicinal Chemistry Article

J. Med. Chem. **2009,** *52,* 5531–5545 **5531**
DOI: 10.1021/jm9008604

Design, Synthesis, and Structure−Activity Relationships of Aminopyridine *N*-Oxides, a Novel Scaffold for the Potent and Selective Inhibition of p38 Mitogen Activated Protein Kinase†

Wenceslao Lumeras,*,‡ Francisco Caturla,‡ Laura Vidal,‡ Cristina Esteve,‡ Cristina Balagué,§ Adelina Orellana,§ María Domínguez,∥ Ramón Roca,⊥ Josep M. Huerta,⊥ Núria Godessart,§ and Bernat Vidal‡

‡*Department of Medicinal Chemistry and* §*Department of Biology and* ∥*Department of ADME and* ⊥*Department of Computational and Structural Drug Discovery, Almirall Research Center, Almirall S.A., Ctra. Laureà Miró 408, E-08980 Sant Feliu de Llobregat, Barcelona, Spain*

Received June 12, 2009

A novel series of aminopyridine *N*-oxides were designed, synthesized, and tested for their ability to inhibit p38α MAP kinase. Some of these compounds showed a significant reduction in the LPS-induced TNFα production in human whole blood. Structure−activity relationship studies revealed that *N*-oxide oxygen was essential for activity and was probably a determinant factor for a marked selectivity against other related kinases. Compound **45** was identified as a potent and selective p38α inhibitor with an appropriate balance between potency and pharmacokinetics. In vivo efficacy of **45** was demonstrated in reducing TNFα levels in an acute murine model of inflammation (ED_{50} = 1 mg/kg in LPS-induced TNFα production when dosed orally 1.5 h prior to LPS administration). The oral efficacy of **45** was further demonstrated in a chronic model of adjuvant arthritis in rats with established disease when administered orally (ED_{50} = 4.5 mg/kg).

Introduction

The p38α mitogen activated protein (MAP[a]) kinase is an intracellular serine/threonine (Ser/Thr) kinase that is activated by a range of environmental stimuli such as TNFα, IL-1β, and stress.[1,2] Activation of p38α occurs through bisphosphorylation by the dual-specificity Ser/Thr MAP kinases MKK3 and MKK6 on the Thr180-Gly181-Tyr182 motif located on the activation loop.[2,3] In its activated state, p38α phosphorylates a range of intracellular protein substrates that post-transcriptionally regulate the biosynthesis of TNFα and IL-1β. The pathophysiological consequence of excessive production of TNFα and IL-1β is thought to be significant mediation of the progression of many inflammatory diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease.[4−7] The proven ability of p38α MAP kinase to efficiently regulate both the release and the activity of those pro-inflamatory cytokines has attracted numerous pharmaceutical companies and independent researchers into pursuing p38α inhibitors primarily as novel anti-inflammatory drugs.[8]

Since the first report in 1994 of the pyridylimidazole-based p38α inhibitor SB203580 (Figure 1),[9] several compounds have advanced into clinical trials and some of them have dropped out due to various reasons.[10,11] Candidates from Vertex (VX-745, Figure 1),[12,13] Boehringer Ingelheim (BIRB-796),[14,15] Pfizer (SD-006),[16] Amgen (AMG-548),[17,18] and Scios (SCIO-469)[19,20] have maintained, or in some cases improved, potency and/or minimized liabilities that were identified in the original pyridylimidazole series.

VX-745 was one of the first orally bioavailable discovered compounds that progressed to phase IIb clinical trials. This inhibitor was shown to have anti-inflammatory activity in rodent models and established proof-of-principle in RA patients[21] before being discontinued due to issues associated with adverse neurological effects in animal models.[22]

The prototypical p38α inhibitor SB203580 contained a 4-aryl 5-(4-pyridinyl) motif and was found to interact competitively with the p38α ATP binding site. An example of the binding orientation is shown in Figure 2, where key interactions of this inhibitor with the active site of the enzyme are depicted. Two key interactions with the hinge region connecting the N- and C- terminal lobes of the kinase domain are observed with this compound, as well as with most known[23] p38α inhibitors: a hydrogen bond through the pyridyl nitrogen to the backbone amide NH of Met109 and a significant lipophilic interaction of the inhibitor with the deep hydrophobic pocket I. This pocket I (also called the "selectivity pocket") displayed in our proposed binding model (Figure 2) is a region not occupied by the ATP when bound to the kinase. It is known[24] that the binding of an aryl group into this pocket imparts selectivity for p38 against other related kinases.

†Coordinates and structure factors have been deposited in the RCSB Protein Data Bank (access code 3HRB for complex of p38α with **45**).

*To whom correspondence should be addressed. Phone: 34 93 312 86 14. Fax: 34 93 312 86 35. E-mail: wenceslao.lumeras@almirall.com.

[a] Abbreviations: MAP, mitogen activated protein; TNFα, tumor necrosis factor α; IL-1β, interleukin 1 β; MKK3, MAP kinase kinase-3; MKK6, MAP kinase kinase-6; RA, rheumatoid arthritis; ERK1, mitogen activated protein kinase 1; JNK1−3, c-Jun N-terminal kinase 1−3; MK2, MAP kinase activated protein kinase-2; COPD, chronic obstructive pulmonary disease; TMEDA, tetramethylethylenediamine; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; S-Phos, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl; LPS, lipopolysaccharide; THP-1, human acute monocytic leukemia cell line; SAR, structure−activity relationships; HWB, human whole blood; CHO, Chinese hamster ovarian; hERG, human ether-a-go-go-related gene; HEK, human embryonic kidney; CI, confidence interval; AIA, adjuvant-induced arthritis.

Published on Web 08/14/2009

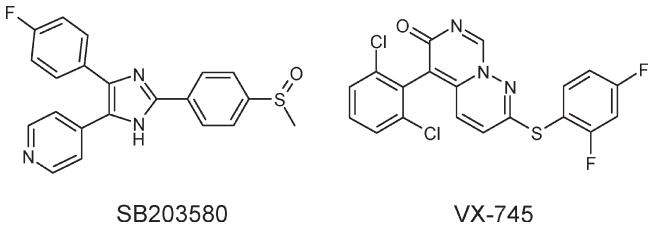


Figure 1. Structure of some selected p38α MAP kinase inhibitors.

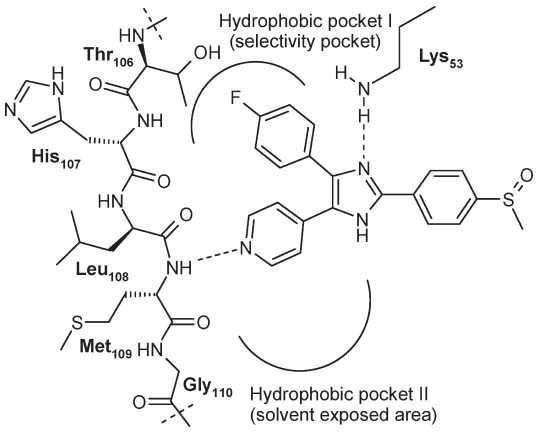


Figure 2. Schematic representation of the interactions of prototypical p38α inhibitor SB203580 in the active site of the enzyme, as shown in X-ray complex 1A9U.[23]

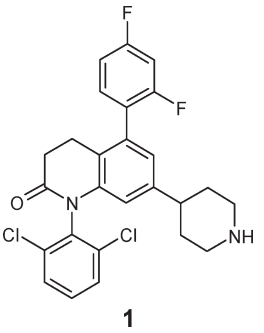


Figure 3. Merck's p38α inhibitor **1**.

Many of the early compounds presented a diaryl heterocyclic scaffold and have been shown to bind to the p38α active site in an orientation similar to SB203580.[25−28] Of the nonvicinal diaryl scaffolds, Merck inhibitor **1** (Figure 3) has been shown to be a potent and very selective inhibitor. This p38α MAP kinase inhibitor displayed potent activity (p38α IC_{50} = 0.74 nM) and selectivity for p38α over a variety of other closely related kinases, including ERK1 and JNK1−3 (> 10000 fold).[29] The exquisite selectivity of Merck's compound could be based on an optimum interaction with the hydrophobic pocket I as well as on the ability of the carbonyl oxygen of this ligand to establish two key hydrogen bond contacts with both the backbone amide NH of Met109 and the NH of Gly110, inducing a conformational change in the hinge region of the enzyme, as depicted in Figure 4a. This disposition can be clearly confirmed in the X-ray crystallographic studies carried out with **1** in the active site of p38α.[29] Although many kinases show a high degree of analogy at their catalytic sites, this conformational flexibility is an almost exclusive feature of p38α and p38*β* due to the presence of the small Gly110. Other related kinases contain bulkier residues on this position, which makes the conformational change virtually impossible.[29]

As part of our strategy to identify novel, potent, and selective p38α MAP kinase inhibitors, we focused our interest in the unprecedented level of selectivity exhibited in **1**. This compound is derived from a dihydroquinolinone core[30,31] and is characterized by the presence of two pendant aromatic rings that are critical for the enzymatic potency. The combined fine-tuned occupation of the hydrophobic pocket I with the already mentioned conformational change (peptide flip of the Gly110) approach provides a great opportunity to design novel and selective p38α inhibitors, which should interact within the catalytic site in a unique way.

With the aim of identifying a potent and selective p38α inhibitor based on a simple core which displays a binding mode similar to **1**, we focused on published Bayer pyridinones **A** (Figure 5). This original structure had been claimed by Bayer scientists as p38 MAP kinase inhibitors for the treatment of different inflammatory conditions (in particular asthma and COPD), showing enzymatic inhibition values in the submicromolar range.[32,33] On the basis of this core, we proposed an isosteric change, using a novel aminopyridine *N*-oxide scaffold **B** as a suitable alternative for the development

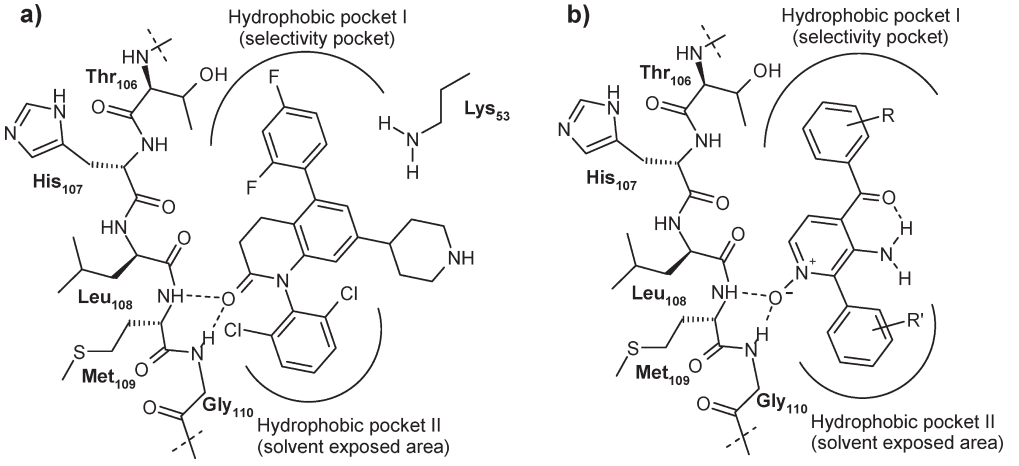


Figure 4. (a) Schematic representation of key interactions of the dihydroquinolinone-based Merck's inhibitor **1** in the active site of p38α, derived from X-ray complex 1OVE.[29] (b) Schematic drawing of predicted key interactions of the novel aminopyridine *N*-oxide inhibitor **B** in the active site of p38α.

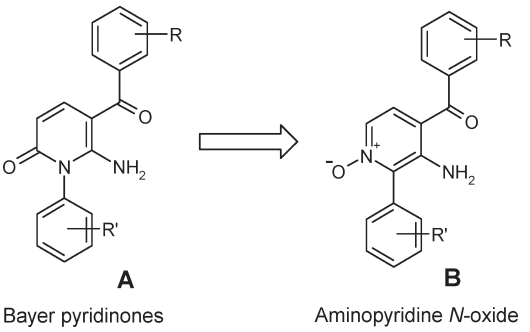


Figure 5. Derivation of the novel aminopyridine *N*-oxide core **B** from Bayer pyridinones **A**.[32,33]

of potential new p38α inhibitors (Figure 5). A plausible intramolecular hydrogen bond between the carbonyl's oxygen and the amino group would arrange a pseudobicyclic scaffold and place the rest of substituents in a suitable conformation within the active site of the enzyme. As depicted in Figure 4b, the *N*-oxide oxygen would be located in an appropriate disposition to simultaneously interact with the backbone amide NH of Met109 and the NH of Gly110 within the hinge region, resulting in the described conformational change (peptide flip of the Gly110) to reach good potency and an improved selectivity profile, while the pendant aromatic rings are assumed to make essential interactions with the enzyme by occupying the two key hydrophobic regions.

Chemistry

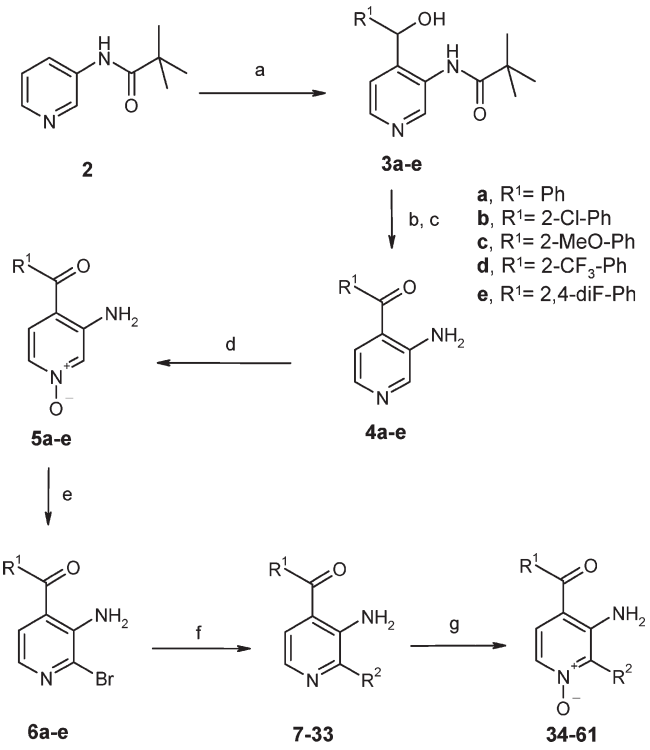
Aminopyridine *N*-oxides **34**−**61** were prepared following the synthetic route depicted in Scheme 1. Starting from *N*-(pyridin-3-yl)pivalamide **2**,[34] an ortho-directed lithiation at the 4-position of the pyridine followed by subsequent addition of the corresponding aldehydes gave carbinols **3a**−**e**. Benzylic oxidation with MnO_2 followed by the removal of pivaloyl group in acidic media afforded ketones **4a**−**e**, which were converted into their corresponding *N*-oxides **5a**−**e** via a smooth oxidation with *m*CPBA. Treatment of the latter with $POBr_3$ furnished, in a regioselective way, key bromopyridines **6a**−**e**, which were reacted with the appropriate boronic acids or esters (method A) under standard palladium-mediated coupling conditions[35,36] to afford compounds **7**−**33**. For the preparation of **20** and **22**, the arylation of bromopyridine **6e** with sterically hindered phenylboronic acids was achieved in good to acceptable yields by using a mixture of *S*-Phos and $Pd_2(dba)_3$ in toluene[37] (method B). In the case of **21** and **28**, the introduction of the *o*,*o*-difluorophenyl rings was successfully carried out by taking advantage of a Negishi[38,39] coupling reaction between **6e** and the corresponding in situ formed *o*,*o*-difluorophenyl zinc derivatives (method C). Subsequent *m*CPBA oxidation at the pyridine nitrogen cleanly provided *N*-oxides **34**−**61**.

Alternatively, compounds **58** and **59** were synthesized in an additional step by standard alkylation or amide formation reactions, respectively, following the *N*-oxidation reaction (Scheme 2) in order to avoid interferences with other basic nitrogen atoms present in the molecule.

Results and Discussion

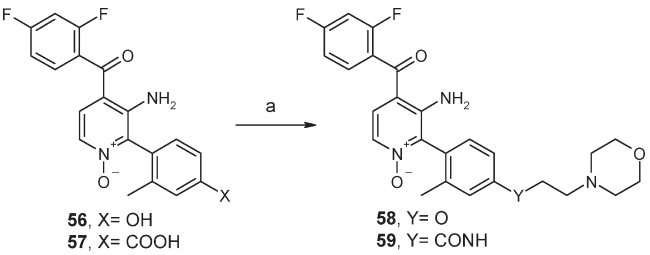
On the basis of our initial design of a simple aminopyridine template **B** (Figure 5), non-*N*-oxide compound **7** (precursor of **34**) was tested for its ability to inhibit in vitro the p38α MAP kinase. Some enzymatic inhibition was seen (p38α IC_{50} = 2.2 μM) and revealed **7** as an initial hit for further optimization. To our delight, we could observe that the presence of an oxygen attached to the nitrogen of the pyridine core (aminopyridine *N*-oxide analogue **34**, Table 1), which would be responsible for the postulated interactions with the hinge region, afforded a reasonable inhibitory potency in the enzymatic (p38α IC_{50} = 0.967 μM) and in the cellular (LPS-induced TNFα release in THP-1 cells, IC_{50} = 1.63 μM) assays. To our knowledge, this was the first example of a highly

Scheme 1[a]



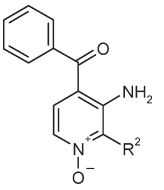
[a] Reagents and conditions: (a) *n*-BuLi, TMEDA, Et_2O, R^1CHO, −78 to 0 °C, 5 h, 35−65%. (b) MnO_2, $CHCl_3$, rt, 24−48 h, 85−100%. (c) HCl, EtOH, 100 °C, 6−24 h, 75−95%. (d) *m*CPBA, CH_2Cl_2, rt, 24−48 h, 70−90%. (e) $POBr_3$, CH_2Cl_2, 60 °C, 3 h, 40−60%. (f) Method A: R^2-$B(OH)_2$ or 4,4,5,5-tetramethyl-2-R^2-1,3,2-dioxaborolane, Pd_2Cl_2-dppf·DCM, 2M Cs_2CO_3, dioxane, 80−100 °C, 18 h, 15−97%; method B: R^2-$B(OH)_2$, $Pd_2(dba)_3$, *S*-Phos, K_3PO_4, toluene, 100 °C, 18−72 h, 25−50%; method C: 1,3-difluorobenzene or 1,3-difluoro-5-methoxybenzene, *n*-BuLi, THF, −78 °C, 30 min, then $ZnCl_2$, THF, −50 °C, 20 min, and then $Pd(PPh_3)_4$, THF, 40 °C, 48−72 h, 21−93%; (g) *m*CPBA, CH_2Cl_2, rt, 18 h, 40−100%.

Scheme 2[a]



[a] Reagents and conditions: (a) For **58**: 4-(2-chloroethyl)morpholine hydrochloride, **56**, K_2CO_3, CH_3CN, 80 °C, 18 h, 54%. For **59**: (2-morpholin-4-ylethyl)amine, **57**, HBTU, DIEA, DMF, rt, 18 h, < 10%.

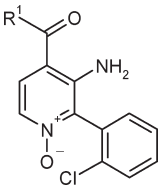
Table 1. 3-Amino-2-aryl-4-benzoylpyridine 1-Oxides: R^2 Group Exploration



compd	R^2	IC_{50} (nM)[a] obtained in the enzymatic and cellular assays: p38α	TNF-α cell (THP-1)	TNF-α HWB
34	phenyl	967 (1.13)	1633 (1.62)	> 10000
35	2-Cl-Ph	174 (1.47)	168 (1.68)	705 (1.75)
36	2-Me-Ph	411 (1.28)	nd	1487 (2.44)
37	4-Cl-Ph	2530 (1.60)	nd	nd

[a] Values are reported as the geometric mean of at least two independent determinations along with the geometric SD (in brackets). nd = not determined.

Table 2. 3-Amino-4-aroyl-2-(2-chlorophenyl)pyridine 1-Oxides: R^1 Group Optimization



compd	R^1	IC_{50} (nM)[a] obtained in the enzymatic and cellular assays: p38α	TNF-α cell (THP-1)	TNF-α HWB
38	2-Cl-Ph	24 (1.26)	n.d.	152 (1.09)
39	2-MeO-Ph	149 (1.00)	281 (1.51)	> 1000
40	2-CF_3-Ph	1655 (1.77)	nd	nd
41	2,4-diF-Ph	39 (1.33)	75 (1.24)	79 (1.09)

[a] Values are reported as the geometric mean of at least two independent determinations along with the geometric SD (in brackets). nd = not determined.

decorated 3-aminopyridine *N*-oxide scaffold to be identified as an inhibitor of p38α MAP kinase.

Preliminary SAR studies were developed in this series in order to further explore its full potential. Our binding model of **B** proposes a fit of the phenyl ring located into the hydrophobic pocket II (Figure 4b), a relatively open solvent exposed area. As demonstrated in Table 1, compounds **35** and **36**, possessing ortho substituted phenyl rings in R^2, were markedly more potent than their unsubstituted analogue **34**. The role of this substituent seems to be the maintenance of the ring in an appropriate (orthogonal) orientation with respect to the aminopyridine core, increasing the lipophilic interactions with the hydrophobic pocket II. The postulated binding model is consistent with the observed SAR. Substitution at the para position of the aryl ring by lipophilic groups with no substitution at the ortho positions, as in **37**, did not improve the enzymatic inhibition (p38α IC_{50} = 2.53 μM).

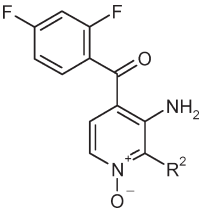
The postulated binding model (Figure 4b) suggests that not very large substituents would be tolerated in the para position of the phenyl group in R^1 due to its proximity toward the hinge region.[40] In contrast, relatively bulky substituents would be allowed in the ortho position. To investigate the effect of substitution on this group, a small number of compounds (**38**−**41**) were synthesized, keeping the 2-Cl-phenyl substitution pattern in R^2, and their potency in the enzymatic assay measured (Table 2). As postulated above, a chlorine atom in the ortho position (**38**) was well tolerated and significantly increased the p38 inhibitory potency (7-fold) with respect to the R^1 unsubstituted lead **35**. This substituent also afforded a substantial improvement (5-fold) with respect to **35** in the LPS-induced TNFα production assay in human whole blood (HWB). We put particular emphasis on this assay as a key driver for compound selection[41] because it provided surrogate efficacy in a physiologically relevant environment in the presence of serum albumin and other proteins.

As the size of the ortho substituents on R^1 notably increased, there was a remarkable loss in potency (enzymatic and cellular), as can be seen in compounds **39** and **40**, suggesting that this deep hydrophobic pocket is very sensitive to accommodate larger substitutions on this ring.[40] More interestingly, compound **41**, which combines two fluorine substituents in the ortho and para positions, as in Merck inhibitor **1**, exhibited good potency in both the enzymatic (p38α IC_{50} = 39 nM) and cellular assays (TNFα in HWB IC_{50} = 79 nM).

After this preliminary exploration, the 2,4-difluorophenyl ring was considered as an optimum R^1 substitution pattern. Taking this into consideration, a thorough analysis of the SAR around the R^2 moiety was thus carried out with the aim of finding suitable substituents that would optimally fit the hydrophobic pocket II, providing more potent analogues.

As we have previously mentioned, ortho substitution on the R^2 aryl group is preferred over para and meta, as can be seen in compounds **41**−**43** (Tables 2 and 3). The introduction of bulkier groups in the ortho position resulted in a loss in potency (analogue **46**). Interestingly, the ortho substitution by an electron-donating group, as in derivative **44**, gave a very potent compound in the enzymatic assay (IC_{50} = 23 nM) but there was a clear shift in the cellular assay (TNFα in HWB IC_{50} = 291 nM). However, 2-methyl analogue **45** displayed good potency in both assays (p38α IC_{50} = 21 nM and TNFα in HWB IC_{50} = 170 nM). We have already assessed that the increased activity of these analogues is in agreement with the fact that a substituent in ortho forces the orthogonality of the rings, optimizing the lipophilic interactions with the hydrophobic pocket II.[42−44] Following this premise, a series of 2,6-disubstituted phenyl analogues in R^2 were synthesized in order to maximize the lipophilic interactions in this region. Gratifyingly, we could observe that 2,6-dichlorophenyl analogue **47** exhibited and excellent in vitro enzymatic potency (p38α IC_{50} = 9 nM), 4-fold more potent than its monosubstituted analogue **41**. Surprisingly, this compound suffered from a considerable drop in potency in the cellular HWB assay (77-fold loss). The presence of the smaller fluorine atoms in **48** afforded the most potent compound of this series tested in the cell-based assays (TNFα in HWB IC_{50} = 37 nM) showing also a very good enzymatic activity (p38α IC_{50} = 17 nM). 2,6-Dimethylphenyl analogue **49** revealed a similar trend to **48**, with excellent values in all assays. In a comparable manner to **44**, electron-donating groups on the ring in **50** give nice enzymatic values but fail again in the HWB assay (41-fold drop). In general, the 2,6-disubstituted derivatives showed an increase in the enzymatic potency with respect to their monosubstituted analogues.

Table 3. 3-Amino-2-aryl-4-(2,4-difluorobenzoyl)pyridine-1-oxides: R^2 Group Optimization



compd	R^2	IC_{50} (nM)[a] obtained in the enzymatic and cellular assays		
		p38α	TNF-α cell (THP-1)	TNF-α HWB
42	3-Cl-Ph	228 (1.27)	1118 (1.08)	> 1000
43	4-Cl-Ph	802 (1.15)	nd	4460 (1.35)
44	2-MeO-Ph	23 (1.29)	nd	291 (1.97)
45	2-Me-Ph	21 (1.81)	46 (1.43)	170 (2.10)
46	2-^{i}Pr-Ph	208 (1.17)	658 (1.52)	> 1000
47	2,6-diCl-Ph	9 (1.87)	14 (2.37)	692 (1.24)
48	2,6-diF-Ph	17 (1.18)	36 (1.16)	37 (1.24)
49	2,6-diMe-Ph	17 (1.93)	31 (1.83)	50 (1.59)
50	2,6-diMeO-Ph	15 (1.57)	291 (1.58)	616 (1.52)
51	2,3-diMeO-Ph	37 (1.13)	1483 (1.75)	> 10000
52	1,3-benzodioxol-4-yl	24 (1.28)	215 (1.60)	11076 (1.42)
53	2,4-diF-Ph	126 (1.41)	nd	283 (1.21)
54	2-Me-4-Cl-Ph	38 (2.13)	211 (1.08)	470 (1.29)
55	2,6-diF-4-MeO-Ph	5 (1.00)	145 (1.05)	1500 (1.07)

[a] Values are reported as the geometric mean of at least two independent determinations along with the geometric SD (in brackets). nd = not determined.

Table 4. Solubility Values (μg/mL) of the Selected Compounds Measured at different pH

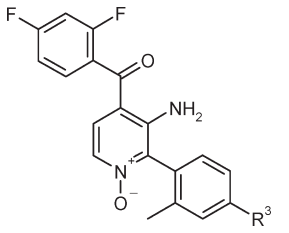
entry	compd[a]	soln pH = 0.1	soln pH = 7.4	soln pH = 9.0
1	**41**	66 ± 6	59 ± 17	39 ± 4
2	**45**	110 ± 5	63 ± 1	74 ± 4
3	**48**	0 ± 0	4 ± 0	2 ± 0
4	**49**	40 ± 1	69 ± 6	70 ± 2
5	**56**	44 ± 6	37 ± 2	nd
6	**57**	41 ± 2	> 1000 ± 10	> 1000 ± 3
7	**58**	> 1000 ± 17	51 ± 4	nd
8	**60**	24 ± 3	67 ± 1	89 ± 3
9	**61**	54 ± 2	46 ± 1	49 ± 1

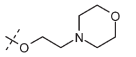
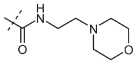
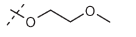
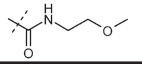
[a] Results expressed as the mean of six independent determinations ± SD. nd = not determined.

Carrying out a variation at the phenyl substitution pattern, it was seen that both the 2,3-disubstituted analogues **51** and **52** were potent against p38α activity but again showed a significant shift in the cellular HWB assay. The 2,4-disubstitution pattern on the phenyl R^2 ring offered a new approach to interact with the hydrophobic pocket II. 2,4-Difluorophenyl compound **53** displayed modest potency compared to its 2,6-difluorophenyl analogue **48** (7-fold drop in both assays). A combination of methyl and chlorine in **54** gave a better p38 inhibitory potency (IC_{50} = 38 nM), but it was still far from the 2,6-disubstituted derivatives. Interestingly, the 2,4,6-trisubstituted compound **55** significantly increased the enzymatic inhibitory activity, leading to the most potent molecule found in the series (p38α IC_{50} = 5 nM); unfortunately, its lack of potency in the HWB cellular assay (40-fold loss with respect to **48**) meant that this compound was not characterized further.

Among all of the compounds synthesized up to this point, analogues **41**, **45**, **48**, and **49** displayed the best p38α inhibitory and cellular (THP-1 and HWB) potency values. However, these molecules exhibited rather limited solubility (Table 4, entries 1−4), increasing the risk of a poor oral bioavailability.[45] For this reason, our next strategy focused on the improvement of this physicochemical property, installing either polar or ionizable functional groups directly or indirectly attached (via an appropriate linker) to the para position of the R^2 aryl ring, pointing out toward the solvent exposed area (Table 5). The presence of a hydroxyl group in **56** slightly improved the enzymatic potency (2.6-fold compared to **45**), but there was a big drop in the cellular assays, particularly in

Table 5. 3-Amino-2-(2-methylphenyl)-4-(2,4-difluorobenzoyl)pyridine-1-oxide: R^3 Group Optimization



Compd.[c]	R^3	IC_{50} (nM)[a] obtained in the enzymatic and cellular assays		
		p38α	TNF-α cell (THP-1)	TNF-α HWB
56	-OH	8 (2.35)	83 (1.27)	7047 (1.01)
57	-COOH	491 (1.20)	n.d.	> 1000
58		14 (1.04)	1269 (1.64)	> 10000
59		42 (1.77)	n.d.	> 1000
60		20 (1.64)	938 (1.25)	> 10000
61		70 (1.41)	1723 (2.29)	> 10000

[a] Values are reported as the geometric mean of at least two independent determinations along with the geometric SD (in brackets). nd = not determined.

Table 6. Pharmacokinetic Parameters of **41** and **45** in the Rat after Intravenous (iv) and Oral (po) Administration[a]

compd	dose po (mg/kg)	dose iv (mg/kg)	C_{max} po (μM)	t_{max} po (h)	AUC_{0-6}[b] po (μM h)	$t_{1/2}$ iv (h)	Cl[c] iv (mL/min/kg)	V_{ss}[d] iv (L/kg)	F_{0-6}[e] (%)
41	10	1	11.9 ± 3.2	3.5 ± 0.7	54.4 ± 13	14.8 ± 8	1.7 ± 0	1.8 ± 0.4	23 ± 6
45	10	0.5	4.7 ± 0.3	0.75 ± 0.3	22.2 ± 4.7	1.6 ± 0.02	16.7 ± 3	2.3 ± 0.1	75 ± 1.3

[a] Results expressed as the mean ± SD of $n = 2$. [b] AUC = area under the curve. [c] Cl = clearance. [d] V_{ss} = volume of distribution steady state. [e] F = bioavailability.

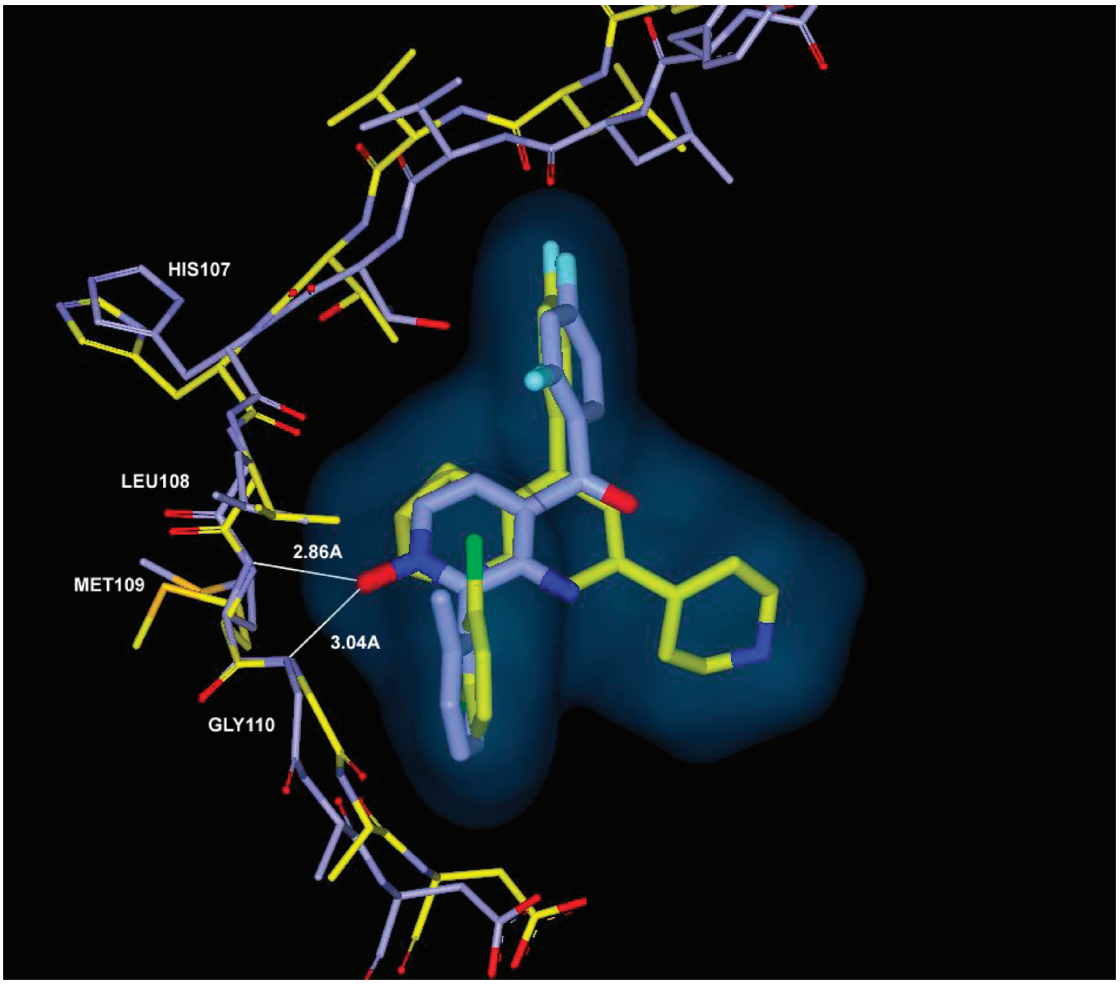


Figure 6. X-ray overlay of compounds **45** (shown in light violet)[46] and **1** (shown in yellow, taken from the published X-ray structure)[29] complexed to p38α active site. The conformational change of Gly110 can be seen in both proteins, revealing significant hydrogen bond interactions between Met109/Gly110 NHs and *N*-oxide oxygen (in **45**) or amide carbonyl (in **1**). Both compounds occupy in a similar manner the deep hydrophobic pocket I (selectivity pocket) that is not accessed by ATP.

HWB ($IC_{50} = 7$ μM) and, to our surprise, displayed poorer solubility values than its precursor **45** (Table 4, entry 5). When a carboxylic acid was installed in this position (**57**), the potency clearly decreased (24-fold loss in p38α with respect to **45**), although its solubility at neutral and basic pH dramatically increased (Table 4, entry 6). The insertion of a residue containing a basic group (compounds **58** and **59**), as expected, led to a dramatic improvement in solubility at acidic pH (Table 4, entry 7). Unfortunately, although **58** and **59** retained or slightly improved the enzymatic activity, a tremendous drop in the cellular assay potency was observed. On the basis of these results, the introduction of nonbasic solubilizing polar substituents was considered. Unfortunately, solubility was not increased (Table 4, entries 8 and 9) and the synthesized derivatives **60** and **61** did not demonstrate noticeable advantages with respect to their basic counterparts **58** and **59**. At this point, we could speculate that the tremendous drop between the enzymatic and HWB assays in **56**−**61** could be related to the increased polarity of the compounds, although we were not able to find a common trend to all the compounds of the series.

Despite the efforts to increase the solubility, compounds **41** and **45** still displayed a useful compromise between desirable physical properties (solubility) and potency in both enzymatic and cellular (HWB) assays and were selected as suitable leads for further characterization.

The pharmacokinetic parameters of compounds **41** and **45** in rat are summarized in Table 6. Compound **41** was slowly absorbed ($t_{max} = 3.5$ h), showing very good oral exposure (AUC_{0-6}). The low values of clearance (Cl) and volume of distribution in the steady state (V_{ss}) translated into a very long terminal half-life ($t_{1/2} = 14.8$ h). Moreover, this compound

exhibited a modest bioavailability (F = 23%). On the other hand, compound **45**, with a more rapid absorption (t_{max} = 0.75 h) and still good oral exposure, displayed moderate clearance and low volume of distribution in the steady state, affording a shorter half-life ($t_{1/2}$ = 1.6 h). Additionally, analogue **45** exhibited a 3-fold increase in rat oral bioavailability (F = 75%), which led to its selection as an advanced lead for additional profiling.

To confirm the proposed binding mode for our aminopyridine *N*-oxide scaffold **B** (Figure 5), compound **45** was cocrystallized with unphosphorylated p38α.[46] Pleasantly, the X-ray structure (Figure 6) revealed a novel binding mode according to that previously proposed in Figure 4b and was consistent with the structure activity relationships (SAR) generated. Of particular interest were the two hydrogen bond interactions generated between the *N*-oxide oxygen, the backbone amide NH of Met109, and the NH of Gly110, resulting in the described conformational change ("Gly flip"). Overlying X-ray crystal structures of **45**[46] with that of Merck inhibitor **1** (Figure 6)[28] clearly confirmed the similarities of the key hydrogen bond interactions of both compounds with the hinge region of the enzyme. More interestingly, the suggested pseudobicyclic scaffold of **B** was nicely established in the crystal structure of **45**, with the formation of an intramolecular hydrogen bond between the carbonyl oxygen and the amino group, placing the pending aryl rings in a similar conformation to those of **1** within the p38α active site.

The selectivity of **45** was assessed by the screening of this compound against a panel of 21 tyrosine and serine/threonine kinases.[47] Impressively, **45** was found to be highly selective for p38α/β over all tested kinases (e.g., c-Raf, JNK1−3, MK2, IC_{50} > 10 μM).

Its ability to inhibit different cytochrome P450 isoforms was also evaluated. Compound **45** did not inhibit any of the most relevant P450 isoforms (1A2, 3A4, 2C9, 2C19, and 2D6) at a concentration of 10 μM.

Compound **45** was tested for in vitro cytotoxicity against a Chinese hamster ovarian (CHO) cell line, showing a clean cytotoxic profile (IC_{50} > 200 μM).

In relation to the cardiovascular safety package, analogue **45** did not have a significant effect on the inhibition of the human ether-a-go-go-related gene (hERG) channel (4.7% at 3 μM) expressed in human embryonic kidney (HEK-293) cells.

In Vivo Pharmacological Evaluation. Key compounds were tested in vivo in an acute model based on rat-LPS induced TNFα (Table 7). In this model, compounds were dosed orally one hour prior to LPS administration and the amount of TNFα in plasma was measured 1.5 h later (coinciding with the peak of TNFα production). Compound **34** dose-dependently inhibited TNFα production with and ED_{50} similar to that obtained for BIRB-796 reference compound. In contrast, compound **45** was much more potent at inhibiting TNFα production in this model with an ED_{50} of 1.03 mg/kg (95% CI, 0.5−2). These results are in accordance with those obtained in enzymatic and cellular assays for both compounds.

The adjuvant-induced arthritis (AIA) model was selected as a chronic disease model to evaluate compound efficacy (Table 7). Upon arthritis induction, compounds were administered orally once daily for 7 days. Compound **34** dosed at 10 mg/kg produced a slight inhibition (17%) of the contralateral paw volumes (versus vehicle-treated animals). In contrast, **45** dose-dependently inhibited arthritis progression with an ED_{50} of 4.5 mg/kg (95% CI, 2−11). These results demonstrate a superior efficacy of **45** versus other clinically tested reference compounds like BIRB-796 and VX-745 when dosed in the same manner (Figure 7 and Table 7).

Table 7. Summary of in Vivo Efficacy Assays

compd	rat LPS-induced TNF ED_{50} (mg/kg)[a]	AIA ED_{50} (mg/kg) or % inhibition
34	8.4 (6−12)	> 10 (17% at 10 mg/kg)
45	1.03 (0.5−2)	4.5 (2−11)
BIRB-796	7.9 (3−22)	8.6 (4−19)
VX-745	nd	> 100

[a] Results represent the calculated ED_{50} along with the lower and upper 95% confidence limits (in brackets). nd = not determined.

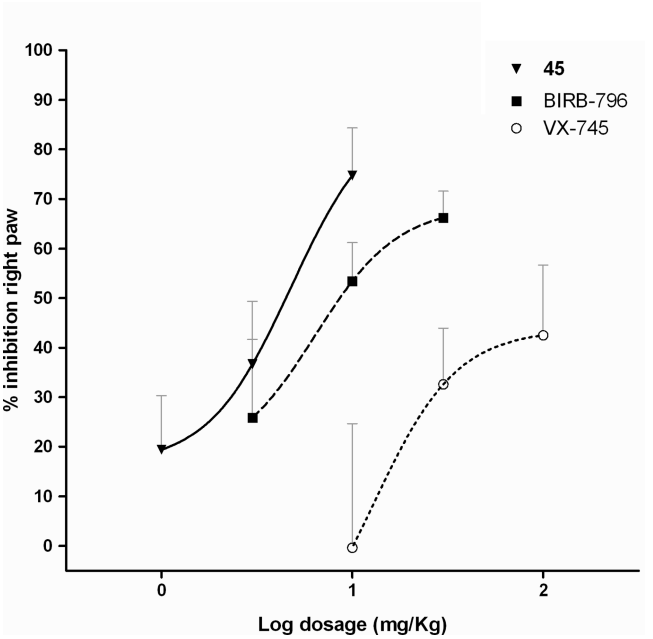


Figure 7. Compound efficacy in the adjuvant-induced arthritis model. Each point represents the mean ± SEM of individual values.

Conclusions

The initial hypothesis of inducing a conformational change in the hinge region of p38α to develop inhibitors with an improved selectivity profile against other kinases has generated a variety of selective inhibitors based on a novel aminopyridine *N*-oxide scaffold. After an extensive SAR exercise, compounds **41** and **45** were identified as potent p38α inhibitors and were able to effectively inhibit the production of TNFα in LPS-stimulated THP-1 cells as well as in human whole blood. The proposed binding mode for this novel series of inhibitors is consistent with the crystallography results obtained from inhibitor **45** within the p38α active site. This compound displayed an excellent selectivity for p38α/β versus a panel of 21 related kinases as well as good pharmacokinetic properties in Wistar rats.

In vivo pharmacological data for **45** confirms that this compound is orally bioavailable and is active both in acute and chronic inflammation models, clearly reflecting the contribution of its potent cellular activity and the plasma levels attained.

Experimental Section

Chemistry. Nonaqueous reactions were performed under argon or nitrogen atmosphere at room temperature, unless otherwise noted. All commercial reagents and anhydrous

solvents were purchased from Aldrich and were used without further purification or distillation unless otherwise stated.

Routine ^{1}H nuclear magnetic resonance spectra were recorded on the following instruments: Varian Gemini 300 MHz, in a Varian Mercury plus NMR spectrometer operating at a frequency of 200 MHz or in a Varian Mercury plus NMR spectrometer at 400 MHz. Samples were dissolved in deuterated chloroform ($CDCl_3$) or deuterated methylsulfoxide (DMSO-d_6) and tetramethylsilane (TMS) was used as reference.

Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}. Compounds were visualized by UV light and/or stained with either potassium permanganate or cerium molybdate solutions followed by heating. Flash column chromatography was performed on SDS silica gel 60 (particle size of 40−63 μm).

HPLC analysis was performed on a Waters Alliance 2795 chromatographer equipped with a Waters 2996 diode-array detector and a Waters ZQ mass spectrometer detector. HPLC analysis was conducted following the next method: chromatography performed on a Symmetry C18 column (100 mm × 2.16 mm, 3.5 μm). The mobile phase, at a flow of 0.4 mL/min, was a 20 min binary gradient of water (containing 0.01 M ammonium formate at pH 3.0) and a mixture acetonitrile−methanol 50:50 (containing 0.01 M ammonic formiate 0.01M) (0−95%). The total run time was 26 min. The retention time (rt) is expressed in min and UV chromatograms were processed at 210 nm with blank subtraction. All key compounds were proven by this method to show ≥95% purity. Additionally, elemental analyses were performed for key compounds. Elemental analyses were performed in FISONS EA-1108 CHNS analyzer using acetanilide as standard. A table summarizing key target compounds can be found at the Supporting Information.

General Method for the Synthesis of *N*-{4-[(Aryl) hydroxymethyl]pyridin-3-yl}-2,2-dimethylpropanamides 3a, 3b, and 3e. *N*-{4-[Hydroxy(phenyl)methyl]pyridin-3-yl}-2,2-dimethylpropanamide (3a). *n*-BuLi (2.5 M in hexanes, 11.2 mL, 28 mmol) was dropwise added to a solution of 2,2-dimethyl-*N*-pyridin-3-ylpropanamide (**2**) (2 g, 11.2 mmol) in dry tetrahydrofuran (28 mL) at −78 °C under argon and the resulting mixture was stirred at that temperature for 15 min and at 0 °C for 3 h. Then, the reaction mixture was cooled down to −78 °C and benzaldehyde (1.72 mL, 16.8 mmol) in 2.8 mL of tetrahydrofuran was carefully added. After 15 min, the cooling bath was removed and the mixture stirred overnight at room temperature. Subsequently, water was added to the flask and it was extracted with ethyl acetate (3 × 50 mL), the organic solution was washed with brine, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica flash, using hexane/ethyl acetate (1:2 to ethyl acetate) as eluents, to yield the title compound (2.16 g, 54%) as a solid. LCMS (*m*/*z*): 285 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.35 (brs, 1H), 8.82 (s, 1H), 8.30 (d, J = 5 Hz, 1H), 7.40 (d, J = 5 Hz, 1H), 7.18−7.38 (m, 5H), 6.78 (brs, 1H), 5.85 (s, 1H), 1.05 (s, 9H).

***N*-{4-[(2-Chlorophenyl) (hydroxy) methyl]pyridin-3-yl}-2,2-dimethylpropanamide (3b).** This compound was prepared from 2,2-dimethyl-*N*-pyridin-3-ylpropanamide (**2**) and 2-chlorobenzaldehyde as described in the synthesis of **3a**. Yield: 33%. LCMS (*m*/*z*): 319, 321 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.10 (s, 1H), 8.21 (d, J = 6 Hz, 1H), 7.49−7.53 (m, 1H), 7.30−7.43 (m, 4H), 6.91 (d, J = 6 Hz, 1H), 6.15 (s, 1H), 1.31 (s, 9H).

***N*-(4-((2,4-Difluorophenyl)(hydroxy)methyl)pyridin-3-yl)-2,2-dimethyl-propanamide (3e).** This compound was prepared from 2,2-dimethyl-*N*-pyridin-3-ylpropanamide (**2**) and 2,4-difluorobenzaldehyde as described in the synthesis of **3a**. Yield: 57%. LCMS (*m*/*z*): 321 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.11 (s, 1H), 8.95 (brs, 1H), 8.18 (d, J = 5 Hz, 1H), 7.30−7.40 (m, 1H), 6.95 (d, J = 5 Hz, 1H), 6.80−7.00 (m, 2H), 6.08 (s, 1H), 1.27 (s, 9H).

General Method for the Synthesis of *N*-{4-[(Aryl) hydroxymethyl]pyridin-3-yl}-2,2-dimethylpropanamides 6c and 6d. *N*-{4-[Hydroxy(2-methoxyphenyl)methyl]pyridin-3-yl}-2,2-dimethylpropanamide (3c). *n*-BuLi (2.5 M in hexanes, 56 mL, 140.5 mmol) was dropwise added to a solution of 2,2-dimethyl-*N*-pyridin-3-ylpropanamide (**2**) (10 g, 56.2 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) (20.93 mL, 140.5 mmol) in diethyl ether (338 mL) at −78 °C under argon, and the resulting mixture was stirred at that temperature for 15 min and at −10 °C for 2 h. Then, the reaction mixture was cooled down to −78 °C, and 2-methoxybenzaldehyde (16.97 mL, 140.5 mmol) in 34 mL of dry tetrahydrofuran was carefully added. After 15 min, the cooling bath was removed and the mixture stirred at room temperature for 2 h. Subsequently, water was added to the flask (400 mL) and it was extracted with ethyl acetate (4 × 200 mL), the organic solution was washed with brine, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was treated with EtOAc (50 mL) and stored at 4 °C for 18 h. The final compound crystallized out to yield the title compound (11.1 g, 63%) as a solid. LCMS (*m*/*z*): 315 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.98 (s, 1H), 8.45 (brs, 1H), 8.30 (d, J = 5 Hz, 1H), 7.42−7.55 (m, 1H), 7.25−7.30 (m, 1H), 6.90−7.10 (m, 2H), 7.00 (d, J = 5 Hz, 1H), 6.05 (s, 1H), 3.75 (s, 3H), 1.12 (s, 9H).

***N*-{4-[Hydroxy(2-trifluoromethylphenyl)methyl]pyridin-3-yl}-2,2-dimethyl-propanamide (3d).** This compound was prepared from 2,2-dimethyl-*N*-pyridin-3-ylpropanamide (**2**) and 2-trifluoromethylbenzaldehyde as described in the synthesis of **3c**. Yield: 57%. LCMS (*m*/*z*): 353 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.00 (s, 1H), 8.40 (brs, 1H), 8.35 (d, J = 5 Hz, 1H), 7.75−7.80 (m, 1H), 7.60−7.70 (m, 2H), 7.30−7.40 (m, 1H), 6.95 (d, J = 5 Hz, 1H), 6.05 (s, 1H), 1.10 (s, 9H).

General Method for the Synthesis of (3-Aminopyridin-4-yl)-(aryl)methanones 4a−e. (3-Aminopyridin-4-yl)(phenyl)methanone (4a). Compound **3a** (2.16 g, 7.6 mmol) was dissolved in chloroform (65 mL) and activated manganese(IV) oxide (6.61 g, 76 mmol) was portionwise added during 1 h. The suspension was stirred at room temperature for 16 h. The mixture was filtered through celite, washed with more chloroform, and the solvent evaporated to afford the corresponding oxidized derivative, which was directly dissolved in 23 mL of ethanol, treated with HCl 5N (70 mL), and heated to 98 °C for 6 h. The reaction mixture was cooled down, poured into ice water, and the pH adjusted to 9−10 with concentrated aqueous ammonia. The solution was extracted with ethyl acetate (2 × 250 mL), the organic layer was washed with brine, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was triturated with hexane/diethyl ether (5:1) to yield the corresponding compounds **4a** (0.9 g, 60%) as a yellowish solid. LCMS (*m*/*z*): 199 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (s, 1H), 7.95 (d, J = 5 Hz, 1H), 7.40−7.75 (m, 5H), 7.15−7.25 (m, 1H), 5.90 (brs, 2H).

(3-Aminopyridin-4-yl)(2-chlorophenyl)methanone (4b). This compound was prepared from **3b** as described in the synthesis of **4a**. Yield: 92%. LCMS (*m*/*z*): 233, 235 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (s, 1H), 7.86 (d, J = 6 Hz, 1H), 7.31−7.50 (m, 4H), 6.95 (d, J = 6 Hz, 1H), 6.31 (brs, 2H).

(3-Aminopyridin-4-yl)(2-methoxyphenyl)methanone (4c). This compound was prepared from **3c** as described in the synthesis of **4a**. Yield: 84%. LCMS (*m*/*z*): 229 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (s, 1H), 7.85 (d, J = 5 Hz, 1H), 7.40−7.55 (m, 1H), 7.25−7.35 (m, 1H), 7.00−718 (m, 3H), 6.20 (brs, 2H), 3.78 (s, 3H).

(3-Aminopyridin-4-yl)[2-(trifluoromethyl)phenyl]methanone (4d). This compound was prepared from **3d** as described in the synthesis of **4a**. Yield: 78%. LCMS (*m*/*z*): 267 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.77−7.83 (m, 2H), 7.59−7.69 (m, 2H), 7.34−7.38 (m, 1H), 6.83 (d, J = 5 Hz, 1H), 6.38 (brs, 2H).

(3-Aminopyridin-4-yl)(2,4-difluorophenyl)methanone (4e). This compound was prepared from **3e** as described in the synthesis of **4a**. Yield: 74%. LCMS (*m*/*z*): 235 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$,

300 MHz) δ 8.35 (s, 1H), 7.90 (d, J = 6 Hz, 1H), 7.40−7.58 (m, 1H), 6.90−7.10 (m, 3H), 6.20 (brs, 2H).

General Method for the Synthesis of (3-Amino-1-oxidopyridin-4-yl)(aryl)methanones 5a−e. (3-Amino-1-oxidopyridin-4-yl)-(phenyl)methanone (5a). To a solution of **4a** (800 mg, 4 mmol) in dichloromethane (20 mL) at 0 °C was added portionwise *meta*-chloroperbenzoic acid (6 mmol) and the reaction mixture was stirred overnight at room temperature. Then, more dichloromethane was added (50 mL) and the solution was washed with aqueous sodium bicarbonate 4% (3 × 30 mL) and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to give a residue that was triturated in a mixture of hexane and ethyl acetate (9:1) and filtered to afford **5a** (778 mg, 90%) as a yellow solid. LCMS (*m*/*z*): 215 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.85 (s, 1H), 7.42−7.65 (m, 6H), 7.35 (d, J = 6 Hz, 1H), 6.35 (brs, 2H).

(3-Amino-1-oxidopyridin-4-yl)(2-chlorophenyl)methanone (5b). This compound was prepared from **4b** as described in the synthesis of **5a**. Yield: 88%. LCMS (*m*/*z*): 249, 251 (M + 1)$^+$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.90 (d, J = 4 Hz, 1H), 7.64 (brs, 2H), 7.45−7.57 (m, 4H), 7.31 (dd, J = 2 and 6 Hz, 1H), 6.86, (d, J = 6 Hz, 1H).

(3-Amino-1-oxidopyridin-4-yl)(2-methoxyphenyl)methanone (5c). This compound was prepared from **4c** as described in the synthesis of **5a**. Yield: 80%. LCMS (*m*/*z*): 245 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (s, 1H), 7.40−7.55 (m, 2H), 7.15−7.20 (m, 1H), 6.95−7.08 (m, 3H), 6.50 (brs, 2H), 3.78 (s, 3H).

(3-Amino-1-oxidopyridin-4-yl)[2-(trifluoromethyl)phenyl]-methanone (5d). This compound was prepared from **4d** as described in the synthesis of **5a**. Yield: 90%. LCMS (*m*/*z*): 283 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.77−7.83 (m, 2H), 7.59−7.69 (m, 2H), 7.34−7.38 (m, 1H), 6.90 (d, J = 5 Hz, 1H), 6.58 (brs, 2H).

(3-Amino-1-oxidopyridin-4-yl)(2,4-difluorophenyl)methanone (5e). This compound was prepared from **4e** as described in the synthesis of **5a**. Yield: 80%. LCMS (*m*/*z*): 283 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.77−7.83 (m, 2H), 7.59−7.69 (m, 2H), 7.34−7.38 (m, 1H), 6.90 (d, J = 5 Hz, 1H), 6.58 (brs, 2H).

General Method for the Synthesis of (3-Amino-2-bromopyridin-4-yl)(aryl)methanones 6a−e. (3-Amino-2-bromopyridin-4-yl)(phenyl)methanone (6a). (3-Amino-1-oxidopyridin-4-yl)-(phenyl)methanone (**5a**) (520 mg, 2.43 mmol) was dissolved in 15 mL of dry dichloromethane and phosphorus oxybromide (2.08 g, 7.28 mmol) added portionwise. The mixture was stirred at 60 °C for 3 h. The reaction was cooled down, poured into ice water, and the pH adjusted to 10−11 with concentrated aqueous ammonia. The solution was extracted with ethyl acetate (2 × 200 mL), the organic layer was washed with brine, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica flash, using hexane/ethyl acetate (3:1) as eluents, to yield **6a** (390 mg, 58%) as a bright-yellow solid. LCMS (*m*/*z*): 277, 279 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.78 (m, 6H), 7.22 (d, J = 6 Hz, 1H), 6.40 (brs, 2H).

(3-Amino-2-bromopyridin-4-yl)(2-chlorophenyl)methanone (6b). This compound was prepared from **5b** following the experimental procedure described for the synthesis of **6a**. Yield: 57%. LCMS (*m*/*z*): 311, 313, 315 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.65 (d, J = 4 Hz, 1H), 7.29−7.49 (m, 4H), 6.98 (d, J = 4 Hz, 1H), 6.91 (brs, 2H).

(3-Amino-2-bromopyridin-4-yl)(2-methoxyphenyl)methanone (6c). This compound was prepared from **5c** following the experimental procedure described for the synthesis of **6a**. Yield: 61%. LCMS (*m*/*z*): 307, 309 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62 (d, J = 6 Hz, 1H), 7.42−7.58 (m, 1H), 7.27−7.35 (m, 1H), 6.99−7.12 (m, 3H), 6.80 (bs, 2H).

(3-Amino-2-bromopyridin-4-yl)(2-trifluoromethylphenyl)-methanone (6d). This compound was prepared from **5d** following the experimental procedure described for the synthesis of **6a**. Yield: 49%. LCMS (*m*/*z*): 345, 347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78−7.83 (m, 1H), 7.61−7.68 (m, 3H), 7.33−7.37 (m, 1H), 6.91 (brs, 2H). 6.87 (d, J = 6 Hz, 1H).

(3-Amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (6e). This compound was prepared from **5e** following the experimental procedure described for the synthesis of **6a**. Yield: 52%. LCMS (*m*/*z*): 313, 315 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (d, J = 6 Hz, 1H), 7.46−7.54 (m, 1H), 7.12 (dd, J = 2, 4 Hz, 1H), 6.88−7.09 (m, 2H), 6.75 (brs, 2H).

General Procedure for the Synthesis of (3-Amino-1-oxido-2-arylpyridin-4-yl)(aryl)methanones 7−33. Method A. In a Schlenk tube were charged the compounds **6a−e** (0.50 mmol), the corresponding boronic acids, or 4,4,5,5-tetramethyl-2-aryl-1,3,2-dioxaborolanes (0.75 mmol), cesium carbonate (2 M aqueous solution, 1.50 mmol), and dioxane (1.4 mL). The mixture was submitted to three vacuum−argon cycles, and then [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (1:1) (0.035 mmol) was added and the mixture purged in the same way. The reaction was stirred at 80 °C under argon for 17 h. Subsequently, water was added to the cold reaction mixture and it was extracted with ethyl acetate (3 × 50 mL), the organic solution was washed with brine, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica flash, using a mixture of hexanes and ethyl acetate as eluent, to yield the compounds **7−19**, **24−27**, **29**, and **30**.

Method B. In a Schlenk tube were charged the compounds **6a−e** (0.50 mmol), the corresponding boronic acids (1.00 mmol), potassium carbonate (1.50 mmol), and toluene (4 mL). The mixture was submitted to three vacuum−argon cycles, and then S-PHOS (0.030 mmol) and tris(dibenzylideneacetone)dipalladium(0) (0.015 mmol) were added and the mixture purged in the same way. The reaction was stirred at 100 °C under argon for 2 days. Subsequently, water was added to the cold reaction mixture and it was extracted with ethyl acetate (3 × 50 mL), the organic solution was washed with brine, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica flash, using a mixture of hexanes and ethyl acetate as eluent to afford compounds **20**, **22**, and **23**.

Method C. *n*-BuLi (2.5 M in hexanes, 1.20 mmol) was dropwise added to a solution of the corresponding benzene derivative (1.30 mmol) in dry tetrahydrofuran (2 mL) at −78 °C under argon, and the resulting mixture was stirred at that temperature for 30 min. Then, the reaction mixture was warmed up to −50 °C and $ZnCl_2$ (0.5 M in THF, 1.30 mmol) carefully added. After 20 min, the corresponding compound **6a−e** (0.65 mmol, in 1.5 mL of THF) and tetrakis(triphenylphosphine)palladium(0) (0.061 mmol) were sequentially added. The mixture was then submitted to three vacuum−argon cycles and warmed, first to room temperature for 15 min and then to 40 °C for 48 h. After this time, the reaction was cooled down and the solvent evaporated under reduced pressure. The resulting crude was purified by column chromatography on silica flash using a mixture of hexanes and ethyl acetate as eluent to yield the compounds **21** and **28**.

(3-Amino-2-phenylpyridin-4-yl)(phenyl)methanone (7). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(phenyl)methanone (**6a**) and phenylboronic acid following the general method A. Yield: 72%. LCMS (*m*/*z*): 275 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (d, J = 5.3 Hz, 1H), 7.4−7.81 (m, 9H), 7.21 (d, J = 6.7 Hz, 2H), 6.1 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)pyridin-4-yl](phenyl)methanone (8). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(phenyl)methanone (**6a**) and (2-chlorophenyl)boronic acid following the general method A. Yield: 67%. LCMS (*m*/*z*): 309−311 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (d, J = 5.0 Hz, 1H), 7.53−7.59 (m, 1H), 7.38−7.49 (m, 7H), 7.26 (m, 1H), 7.05 (d, J = 5.0 Hz, 1H), 6.1 (brs, 2H).

[3-Amino-2-(2-methylphenyl)pyridin-4-yl](phenyl)methanone (9). This compound was prepared starting from (3-amino-2-bromo-

pyridin-4-yl)(phenyl)methanone (**6a**) and (2-methylphenyl)boronic acid following the general method A. Yield: 78%. LCMS (*m*/*z*): 289 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (d, J = 5.3 Hz, 1H), 7.47−7.80 (m, 5H), 7.33 (m, 4H), 5.83 (brs, 2H), 2.23 (s, 3H).

[3-Amino-2-(4-chlorophenyl)pyridin-4-yl](phenyl)methanone (10). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(phenyl)methanone (**6a**) and (4-chlorophenyl)boronic acid following the general method A. Yield: 71%. LCMS (*m*/*z*): 309−311 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (d, J = 5.0 Hz, 1H), 7.46−7.80 (m, 9H), 7.26 (m, 1H), 6.07 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)pyridin-4-yl](2-chlorophenyl)-methanone (11). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2-chlorophenyl)methanone (**6b**) and (2-chlorophenyl)boronic acid following the general method A. Yield: 43%. LCMS (*m*/*z*): 343−345−347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, J = 6.0 Hz, 1H), 7.53−7.59 (m, 1H), 7.38−7.49 (m, 7H), 7.05 (d, J = 6.0 Hz, 1H), 6.30 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)pyridin-4-yl](2-methoxyphenyl)-methanone (12). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2-methoxyphenyl)methanone (**6c**) and (2-chlorophenyl)boronic acid following the general method A. Yield: 60%. LCMS (*m*/*z*): 339−341 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, J = 6.0 Hz, 1H), 7.55−7.60 (m, 1H), 7.10−7.50 (m, 7H), 7.05 (d, J = 6.0 Hz, 1H), 6.30 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)pyridine-4-yl](2-trifluoromethylphenyl)methanone (13). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2-trifluoromethylphenyl)-methanone (**6d**) and (2-chlorophenyl)boronic acid following the general method A. Yield: 72%. LCMS (*m*/*z*): 377−379 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, J = 6.0 Hz, 1H), 7.53−7.59 (m, 1H), 7.38−7.49 (m, 7H), 7.05 (d, J = 6.0 Hz, 1H), 6.30 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (14). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2-chlorophenyl)boronic acid following the general method A. Yield: 49%. LCMS (*m*/*z*): 345−347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 6.0 Hz, 1H), 7.88 (m, 1H), 7.47−7.75 (m, 4H), 7.18 (d, J = 5.5 and 2.8 Hz, 1H), 6.92−7.10 (m, 2H), 6.02 (brs, 2H).

[3-Amino-2-(3-chlorophenyl)pyridine-4-yl](2,4-difluorophenyl)-methanone (15). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (3-chlorophenyl)boronic acid following the general method A. Yield: 71%. LCMS (*m*/*z*): 345−347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, J = 5.5 Hz, 1H), 7.67 (m, 1H), 7.43−7.60 (m, 4H), 7.13 (dd, J = 5.1 and 2.8 Hz, 1H), 6.90−7.08 (m, 2H), 6.44 (brs, 2H).

[3-Amino-2-(4-chlorophenyl)pyridine-4-yl](2,4-difluorophenyl)-methanone (16). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (4-chlorophenyl)boronic acid following the general method A. Yield: 84%. LCMS (*m*/*z*): 345−347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 6.7 Hz, 1H), 7.50−7.70 (m, 5H), 6.93−7.13 (m, 3H), 6.40 (brs, 2H).

[3-Amino-2-(2-methoxyphenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (17). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2-methoxyphenyl)boronic acid following the general method A. Yield: 81%. LCMS (*m*/*z*): 341 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 5.5 Hz, 1H), 7.37−7.57 (m, 3H), 6.91−7.14 (m, 5H), 6.30 (brs, 2H), 3.85 (s, 3H).

[3-Amino-2-(2-methylphenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (18). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2-methylphenyl)boronic acid following the general method A. Yield: 85%. LCMS (*m*/*z*): 325 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 5.1 Hz), 7.48−7.59 (m, 1H), 7.31−7.37 (m, 4H), 7.13 (dd, J = 5.1 and 2.7 Hz, 1H), 6.91−7.09 (m, 2H), 6.17 (brs, 2H), 2.21 (s, 3H).

[3-Amino-2-(2-isopropylphenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (19). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2-isopropylphenyl)boronic acid following the general method A. Yield: 80%. LCMS (*m*/*z*): 353 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 5.5 Hz, 1H), 7.24−7.59 (m, 5H), 7.13 (dd, J = 5.4 and 2.7 Hz, 1H), 6.91−7.09 (m, 2H), 6.16 (brs, 2H), 2.77 (hept, J = 7.1 Hz, 1H), 1.21 (d, J = 7.0 Hz, 3H), 1.16 (d, J = 7.1 Hz, 3H).

[3-Amino-2-(2,6-dichlorophenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (20). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2,6-dichlorophenyl)boronic acid following the general method B. Yield: 50%. LCMS (*m*/*z*): 379, 381, 383 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09 (d, J = 5.4 Hz, 1H), 7.33−7.61 (m, 4H), 7.23 (dd, J = 5.5 and 3.1 Hz, 1H), 6.91−7.10 (m, 2H), 6.06 (brs, 2H).

[3-Amino-2-(2,6-difluorophenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (21). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and 2,6-difluorobenzene following the general method C. Yield: 88%. LCMS (*m*/*z*): 347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, J = 5.5 Hz, 1H), 7.39−7.59 (m, 2H), 7.22 (dd, J = 5.4 and 3.1 Hz, 1H) 6.93−7.14 (m, 4H), 6.20 (brs, 2H).

[3-Amino-2-(2,6-dimethylphenyl)pyridin-4-yl](2,4-difluorophenyl)-methanone (22). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2,6-dimethylphenyl)boronic acid following the general method B. Yield: 44%. LCMS (*m*/*z*): 339 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.04 (d, J = 5.5 Hz, 1H), 7.55 (m, 1H), 6.91−7.30 (m, 6H) 6.08 (brs, 2H), 2.08 (s, 6H).

[3-Amino-2-(2,6-dimethoxyphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (23). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2,6-dimethoxyphenyl)boronic acid following the general method B. Yield: 44%. LCMS (*m*/*z*): 371 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (d, J = 4 Hz, 1H), 7.43−7.57 (m, 1H), 7.40 (t, J = 8 Hz, 1H), 7.10 (dd, J = 4 and 2 Hz, 1H), 6.90−7.05 (m, 2H), 6.70 (d, J = 8 Hz, 2H), 6.20 (brs, 2H), 3.76 (s, 6H).

[3-Amino-2-(2,3-dimethoxyphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (24). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2,3-dimethoxyphenyl)boronic acid following the general method A. Yield: 84%. LCMS (*m*/*z*): 371 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 5.1 Hz, 1H), 7.53 (m, 1H), 7.20 (d, J = 8.2 Hz, 1H), 7.14 (dd, J = 5.5 and 3.2 Hz, 1H), 6.90−7.09 (m, 4H), 6.36 (brs, 2H), 3.94 (s, 3H), 3.71 (s, 3H).

[3-Amino-2-(1,3-benzodioxol-4-yl)pyridin-4-yl](2,4-difluorophenyl)methanone (25). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and 1,3-benzodioxol-4-ylboronic acid following the general method A. Yield: 56%. LCMS (*m*/*z*): 355 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (d, J = 6 Hz, 1H) 7.46−7.57 (m, 1H), 7.16 (dd, J = 2 and 6 Hz, 1H), 6.90−7.11 (m, 5H), 6.8 (brs, 2H), 6.06 (s, 2H).

[3-Amino-2-(2,4-difluorophenyl-4-yl)pyridin-4-yl](2,4-difluorophenyl)methanone (26). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2,4-difluorophenyl)boronic acid following the general method A. Yield: 32%. LCMS (*m*/*z*): 347 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (d, J = 6 Hz, 1H) 7.46−7.59 (m, 2H), 7.18 (dd, J = 2 and 4 Hz, 1H), 6.90−7.11 (m, 4H), 6.25 (brs, 2H).

[3-Amino-2-(2-methyl-4-chlorophenyl-4-yl)pyridin-4-yl](2,4-difluorophenyl)methanone (27). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)-methanone (**6e**) and (2-methyl-4-chlorophenyl)boronic acid following the general method A. Yield: 83%. LCMS (*m*/*z*): 359 (M + 1)$^+$.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 6 Hz, 1H) 7.49−7.60 (m, 1H), 7.25−7.36 (m, 3H), 7.15 (dd, J = 2 and 4 Hz, 1H), 6.90−7.25 (m, 2H), 6.12 (brs, 2H), 2.19 (s, 3H).

[3-Amino-2-(2,6-difluoro-4-methoxyphenyl-4-yl)pyridin-4-yl]-(2,4-difluorophenyl)methanone (28). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and 2-bromo-1,3-difluoro-5-methoxybenzene following the general method C. Yield: 21%. LCMS (m/z): 377 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (d, J = 6 Hz, 1H) 7.47−7.58 (m, 1H), 7.19 (dd, J = 2 and 4 Hz, 1H), 6.90−7.00 (m, 2H), 6.62 (m, 2H), 6.23 (brs, 2H), 3.86 (s, 3H).

[3-Amino-2-(2-methyl-4-hydroxyphenyl-4-yl)pyridin-4-yl](2,4-difluorophenyl)methanone (29). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and (2-methyl-4-hydroxyphenyl)boronic acid following the general method A. Yield: 58%. LCMS (m/z): 341 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.97 (d, J = 6 Hz, 1H) 7.48−7.59 (m, 1H), 7.18 (dd, J = 2 and 4 Hz, 1H), 6.90−7.10 (m, 3H), 6.58−6.62 (m, 2H), 6.25 (brs, 2H), 2.08 (s, 3H).

4-[3-Amino-4-(2,4-difluorobenzoyl)pyridin-2-yl]-3-methylbenzoic Acid (30). This compound was prepared starting from (3-amino-2-bromopyridin-4-yl)(2,4-difluorophenyl)methanone (**6e**) and 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid following the general method A. Yield: 60%. LCMS (m/z): 369 $(M + 1)^+$. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.00−8.09 (m, 2H) 7.88 (d, J = 6 Hz, 1H), 7.58−7.70 (m, 1H), 7.42 (d, J = 8 Hz, 1H), 7.26 (dd, J = 2 and 4 Hz, 1H), 7.10−7.20 (m, 2H), 2.23 (s, 3H).

(3-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid was prepared as follows: In a Biotage microwave vial (10−20 mL) was placed a mixture of 4-bromo-3-methylbenzoic acid (1 g, 4.65 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (1.77 g, 6.97 mmol), potassium acetate (2.3 g, 23.2 mmol), and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (1:1) (379 mg, 0.46 mmol) in dry DMF (20 mL). The mixture was submmited to microwave irradiation at 120 °C for 15 min on an Initiator Sixty system from Biotage. The solvent was evaporated and the residue suspended on a 1:1 mixture 2N HCl/EtOAc. The aqueous phase was extracted with EtOAc. The combined organic layers were dried and the solvents removed to afford a brown oil, which was submitted to column chromatography on silica flash using a mixture of hexanes and ethyl acetate (8:2) as eluent to yield the title compound (1.1 g, 90%) as a white solid).

{3-Amino-2-[2-methyl-4-(2-morpholin-4-ylethoxy)phenyl]-pyridin-4-yl}(2,4-difluorophenyl)methanone (31). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)-(2,4-difluorophenyl)methanone (**6e**) and 4-{2-[3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy]ethyl}morpholine following the general method A. Yield: 61%. LCMS (m/z): 454 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, J = 4 Hz, 1H) 7.47−7.58 (m, 1H), 7.24 (d, J = 4 Hz, 1H), 7.10 (dd, J = 2 and 4 Hz, 1H), 6.84−7.04 (m, 4H), 6.19 (brs, 2H), 4.18 (t, J = 6 Hz, 2H), 3.77 (t, J = 6 Hz, 4H), 2.85 (t, J = 6 Hz, 2H), 2.62 (t, J = 6 Hz, 4H), 2.18 (s, 3H).

(4-{2-[3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenoxy]ethyl}-morpholine was prepared starting from 4-[2-(4-bromo-3-methylphenoxy)ethyl]-morpholine following the general method used for (3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (see **30**). Yield: 71%).

{3-Amino-2-[4-(2-methoxyethoxy)-2-methylphenyl]pyridin-4-yl}(2,4-difluoro-phenyl)methanone (32). This compound was prepared starting from from (3-amino-2-bromopyridin-4-yl)-(2,4-difluorophenyl)methanone (**6e**) and 2-[4-(2-methoxyethoxy)-2-methylphenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane following the general method A. Yield: 42%. LCMS (m/z): 399 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, J = 6 Hz, 1H) 7.47−7.58 (m, 1H), 7.25 (d, J = 6 Hz, 1H), 7.10 (dd, J = 2 and 4 Hz, 1H), 6.87−7.04 (m, 4H), 6.20 (brs, 2H), 4.18 (t, J = 6 Hz, 2H), 3.79 (t, J = 6 Hz, 2H), 3.48 (s, 3H), 2.18 (s, 3H).

(2-[4-(2-Methoxyethoxy)-2-methylphenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane was prepared starting from 1-bromo-4-(2-methoxyethoxy)-2-methylbenzene following the general method used for (3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (see **30**). Yield: 64%).

4-[3-Amino-4-(2,4-difluorobenzoyl)pyridin-2-yl]-*N*-(2-methoxyethyl)-3-methyl-benzamide (33). To a mixture of 4-[3-amino-4-(2,4-difluorobenzoyl)pyridin-2-yl]-3-methylbenzoic acid (**30**) (100 mg, 0.27 mmol), 2-methoxyethanamine (24 μL, 0.27 mmol), and HATU (104 mg, 0.27 mmol) in dimethylformamide (2 mL) was added DIEA (107 μL, 0.61 mmol), and the resulting suspension was stirred at room temperature for 2 h. Ethyl acetate (40 mL) and water (20 mL) were added, the aqueous phase was separated, and the organic phase was washed with water (20 mL) and brine (20 mL), dried over anhydrous sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica flash, using hexane/ethyl acetate (7:3), to yield the title compound as a yellow solid (85 mg, 73%). LCMS (m/z): 426 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.02 (d, J = 6 Hz, 1H) 7.70−7.80 (m, 2H), 7.50−7.60 (m, 1H), 7.42 (d, J = 6 Hz, 1H), 7.16 (dd, J = 4 and 6 Hz, 1H), 6.90−7.00 (m, 2H), 6.58 (brt, J = 6 Hz, 1H), 6.10 (brs, 2H), 3.68 (m, 2H), 3.62 (dd, J = 4 and 8 Hz, 2H), 3.42 (s, 3H), 2.26 (s, 3H).

(3-Amino-1-oxido-2-phenylpyridin-4-yl)(phenyl)methanone (34). To a solution of (3-amino-2-phenylpyridin-4-yl)(phenyl)methanone (**7**) (137 mg, 0.5 mmol) in dichloromethane (3 mL) at 0 °C was portionwise added *meta*-chloroperbenzoic acid (130 mg, 0.75 mmol), and the reaction mixture was stirred overnight at room temperature. Then, more dichloromethane was added (30 mL) and the solution was washed with aqueous sodium bicarbonate 4% (3 × 30 mL) and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to give a residue that was purified by crystallization from a mixture of hexane, diethyl ether, and ethyl acetate to yield the title compound (113 mg, 75%) as a yellow solid. LCMS (m/z): 291 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45−7.68 (m, 12H), 7.36 (d, J = 6.0 Hz, 1H), 6.32 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)-1-oxidopyridin-4-yl](phenyl)-methanone (35). This compound was prepared starting from [3-amino-2-(2-chlorophenyl)pyridin-4-yl](phenyl)methanone (**8**) following the experimental procedure described for the synthesis of **34**. Yield: 98% LCMS (m/z): 325, 327 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.69 (m, 11H), 6.3 (brs, 2H).

[3-Amino-2-(2-methylphenyl)-1-oxidopyridin-4-yl](phenyl)-methanone (36). This compound was prepared starting from [3-amino-2-(2-methylphenyl)pyridin-4-yl](phenyl)methanone (**9**) following the experimental procedure described for the synthesis of **34**. Yield: 96%. LCMS (m/z): 305 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23−7.73 (m, 11H), 6.27 (brs, 2H), 2.23 (s, 3H). Anal. ($C_{19}H_{16}N_2O_2$) C, H, N.

[3-Amino-2-(4-chlorophenyl)-1-oxidopyridin-4-yl](phenyl)-methanone (37). This compound was prepared starting from [3-amino-2-(4-chlorophenyl)pyridin-4-yl](phenyl)methanone (**10**) following the experimental procedure described for the synthesis of **34**. Yield: 100%. LCMS (m/z): 325, 327 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33−7.73 (m, 11H), 6.3 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)-1-oxidopyridin-4-yl](2-chlorophenyl)-methanone (38). This compound was prepared starting from [3-amino-2-(2-chlorophenyl)pyridin-4-yl](2-chlorophenyl)methanone (**11**) following the experimental procedure described for the synthesis of **34**. Yield: 48%. LCMS (m/z): 359−361−363 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, J = 8 Hz, 1H), 7.37−7.67 (m, 8H), 7.08 (d, J = 6 Hz, 1H), 6.47 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)-1-oxidopyridin-4-yl](2-methoxyphenyl)methanone (39). This compound was prepared starting from [3-amino-2-(2-chlorophenyl)pyridin-4-yl](2-methoxyphenyl)methanone (**12**) following the experimental procedure described for the synthesis of **34**. Yield: 83%. LCMS (m/z):

355−357 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.68−7.61 (m, 1H), 7.60 (d, J = 6 Hz, 1H), 7.54−7.31 (m, 5H), 7.19 (d, J = 6 Hz, 1H), 7.12−7.01 (m, 2H), 3.82 (s, 3H), 6.39 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)-1-oxidopyridin-4-yl](2-trifluoromethylphenyl)methanone (40). This compound was prepared starting from [3-amino-2-(2-chlorophenyl)pyridin-4-yl](2-trifluoromethylphenyl)methanone (**13**) following the experimental procedure described for the synthesis of **34**. Yield: 54%. LCMS (m/z): 393−395 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80−7.84 (m, 1H), 7.61−7.71 (m, 3H), 7.58 (d, J = 6 Hz, 1H), 7.53−7.40 (m, 4H), 6.98 (d, J = 6 Hz, 1H), 6.42 (brs, 2H).

[3-Amino-2-(2-chlorophenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (41). This compound was prepared starting from [3-amino-2-(2-chlorophenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**14**) following the experimental procedure described for the synthesis of **34**. Yield: 72%. LCMS (m/z): 361−363 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62−7.67 (m, 2H), 7.38−7.58 (m, 4H), 7.24 (dd, J = 7.0 and 2.8 Hz, 1H), 6.92−7.10 (m, 2H), 6.38 (brs, 2H). Anal. ($C_{18}H_{11}ClF_2N_2O_2$) C, H, N.

[3-Amino-2-(3-chlorophenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (42). This compound was prepared starting from [3-amino-2-(3-chlorophenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**15**) following the experimental procedure described for the synthesis of **34**. Yield: 75%. LCMS (m/z): 361−363 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, J = 7.0 Hz, 1H), 7.45−7.56 (m, 4H), 7.37 (m, 1H), 7.20 (dd, J = 7.0 and 3.1 Hz, 7H), 6.91−7.10 (m, 2H), 6.47 (brs, 2H).

[3-Amino-2-(4-chlorophenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (43). This compound was prepared starting from [3-amino-2-(4-chlorophenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**16**) following the experimental procedure described for the synthesis of **34**. Yield: 79%. LCMS (m/z): 361−363 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43−7.70 (m, 6H), 7.20 (dd, J = 7.0 and 3.1 Hz, 1H), 6.90−7.10 (m, 2H), 6.5 (brs, 2H).

[3-Amino-2-(2-methoxyphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (44). This compound was prepared starting from [3-amino-2-(2-methoxyphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**17**) following the experimental procedure described for the synthesis of **34**. Yield: 79%. LCMS (m/z): 357 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62 (d, J = 7.0 Hz, 1H), 7.44−7.57 (m, 2H), 7.31 (m, 1H), 7.31 (m, 1H), 6.45 (brs, 2H), 3.85 (s, 3H).

[3-Amino-2-(2-methylphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (45). This compound was prepared starting from [3-amino-2-(2-methylphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**18**) following the experimental procedure described for the synthesis of **34**. Yield: 95%. LCMS (m/z): 341 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, J = 7.0 Hz, 1H), 7.35−7.57 (m, 4H), 7.29 (m, 1H), 7.20 (dd, J = 7.0 and 3.1 Hz, 1H), 6.91−7.10 (m, 2H), 6.40 (brs, 2H), 2.21 (s, 3H). Anal. ($C_{19}H_{14}F_2N_2O_2$) C, H, N.

[3-Amino-2-(2-isopropylphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (46). This compound was prepared starting from [3-amino-2-(2-isopropylphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**19**) following the experimental procedure described for the synthesis of **34**. Yield: 93%. LCMS (m/z): 369 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (d, J = 7.4 Hz, 1H), 7.36−7.58 (m, 4H), 7.18−7.23 (m, 2H), 6.92−7.10 (m, 2H), 6.38 (brs, 2H), 2.59 (hep, J = 7.0 Hz, 1H), 1.27 (d, J = 7.0 Hz, 3H), 1.19 (d, J = 6.6 Hz, 3H).

[3-Amino-2-(2,6-dichlorophenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (47). This compound was prepared starting from [3-amino-2-(2,6-dichlorophenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**20**) following the experimental procedure described for the synthesis of **34**. Yield: 87%. LCMS (m/z): 395, 397, 399 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, J = 7.0 Hz, 1H), 7.40−7.59 (m, 4H), 7.28 (dd, J = 7.0 and 2.7 Hz, 1H), 6.92−7.11 (m, 2H), 6.35 (brs, 2H).

[3-Amino-2-(2,6-difluorophenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (48). This compound was prepared starting from [3-amino-2-(2,6-dichlorophenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**21**) following the experimental procedure described for the synthesis of 34. Yield: 73%. LCMS (m/z): 363 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, J = 7.1 Hz, 1H), 7.46−7.60 (m, 2H), 7.27 (m, 1H), 6.92−7.17 (m, 4H), 6.49 (brs, 2H). Anal. ($C_{18}H_{10}F_4N_2O_2$) C, H, N.

[3-Amino-2-(2,6-dimethylphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (49). This compound was prepared starting from [3-amino-2-(2,6-dimethylphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**22**) following the experimental procedure described for the synthesis of **34**. Yield: 73%. LCMS (m/z): 355 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.67 (d, J = 7.1 Hz, 1H), 7.53 (m, 1H), 7.19−7.38 (m, 4H), 6.92−7.10 (m, 2H), 6.35 (brs, 2H), 2.14 (s, 6H).

[3-Amino-2-(2,6-dimethoxyphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (50). This compound was prepared starting from [3-amino-2-(2,6-dimethoxyphenyl)pyridin-4-yl]-(2,4-difluorophenyl)methanone (**23**) following the experimental procedure described for the synthesis of **34**. Yield: 83%. LCMS (m/z): 387 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, J = 8 Hz, 1H), 7.55−7.43 (m, 2H), 7.14 (dd, J = 2,4 Hz, 1H), 6.90−7.08 (m, 2H), 6.72 (d, J = 8 Hz, 2H), 6.46 (brs, 2H), 3.80 (s, 6H). Anal. ($C_{20}H_{16}F_2N_2O_4$) C, H, N: calcd, 62.18; found, 61.68.

[3-Amino-2-(2,3-dimethoxyphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (51). This compound was prepared from [3-amino-2-(2,3-dimethoxyphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**24**) following the experimental procedure described for the synthesis of **34**. Yield: 67%. LCMS (m/z): 387 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, J = 7.0 Hz, 1H), 7.51 (m, 1H), 7.24−7.32 (m, 1H), 7.19 (dd, J = 7.0 and 2.7 Hz, 1H), 6.85−7.13 (m, 4H), 6.44 (brs, 2H), 3.94 (s, 3H), 3.84 (s, 3H).

[3-Amino-2-(1,3-benzodioxol-4-yl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (52). This compound was prepared from [3-amino-2-(1,3-benzodioxol-4-yl)pyridin-4-yl](2,4-difluorophenyl)methanone (**25**) following the experimental procedure described for the synthesis of **34**. Yield: 43%. LCMS (m/z): 371 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, J = 8.0 Hz, 1H), 7.43−7.55 (m, 1H), 7.19 (dd, J = 4.0 and 8.0 Hz, 1H), 6.88−7.09 (m, 5H), 6.62 (brs, 2H), 6.06 (dd, J = 2 and 12 Hz, 2H).

[3-Amino-2-(2,4-difluorophenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (53). This compound was prepared from [3-amino-2-(2,4-difluorophenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**26**) following the experimental procedure described for the synthesis of **34**. Yield: 90%. LCMS (m/z): 363 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.65 (d, J = 8.0 Hz, 1H), 7.38−7.57 (m, 2H), 7.24 (dd, J = 2.0 and 4.0 Hz, 1H), 6.91−7.15 (m, 4H), 6.47 (brs, 2H).

[3-Amino-2-(4-chloro-2-methylphenyl)-1-oxidopyridin-4-yl]-(2,4-difluorophenyl)methanone (54). This compound was prepared from [3-amino-2-(4-chloro-2-methylphenyl)pyridin-4-yl]-(2,4-difluorophenyl)methanone (**27**) following the experimental procedure described for the synthesis of **34**. Yield: 84%. LCMS (m/z): 375 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, J = 8.0 Hz, 1H), 7.36−7.57 (m, 3H), 7.19−7.25 (m, 2H), 6.90−7.10 (m, 2H), 6.37 (brs, 2H), 2.20 (s, 3H).

[3-Amino-2-(2,6-difluoro-4-methoxyphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (55). This compound was prepared from [3-amino-2-(2,6-difluoro-4-methoxyphenyl)-pyridin-4-yl](2,4-difluorophenyl)methanone (**28**) following the experimental procedure described for the synthesis of **34**. Yield: 72%. LCMS (m/z): 393 (M + 1)$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.65 (d, J = 8.0 Hz, 1H), 7.45−7.56 (m, 1H), 7.23 (dd, J = 8.0 and 4.0 Hz, 1H), 6.92−7.10 (m, 2H), 6.63−6.72 (m, 2H), 6.54 (brs, 2H), 3.88 (s, 3H).

[3-Amino-2-(4-hydroxy-2-methylphenyl)-1-oxidopyridin-4-yl]-(2,4-difluorophenyl)methanone (56). This compound was prepared from [3-amino-2-(4-hydroxy-2-methylphenyl)pyridin-4-yl](2,4-difluorophenyl)methanone (**29**) following the experimental procedure described for the synthesis of **34**. Yield: 99%. LCMS (*m*/*z*): 357 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60−7.70 (m, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.40−7.50 (m, 1H), 7.20−7.30 (m, 1H), 7.14 (dd, J = 8.0 and 2.0 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.88 (brs, 2H), 6.70−6.80 (m, 2H), 1.94 (s, 3H).

4-[3-Amino-4-(2,4-difluorobenzoyl)-1-oxidopyridin-2-yl]-3-methylbenzoic Acid (57). This compound was prepared from 4-[3-amino-4-(2,4-difluorobenzoyl)pyridin-2-yl]-3-methylbenzoic acid (**30**) following the experimental procedure described for the synthesis of **34**. Yield: 57%. LCMS (*m*/*z*): 385 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (brs, 2H), 7.24−7.70 (m, 6H), 7.89−7.98 (m, 2H).

{3-Amino-2-[2-methyl-4-(2-morpholin-4-ylethoxy)phenyl]-1-oxidopyridin-4-yl}(2,4-difluorophenyl)methanone (58). To a solution of [3-amino-2-(4-hydroxy-2-methylphenyl)-1-oxidopyridin-4-yl](2,4-difluorophenyl)methanone (**56**) (200 mg, 0.56 mmol) in 6 mL of acetonitrile were added 4-(2-chloroethyl)morpholine hydrochloride (156 mg, 0.84 mmol) and potassium carbonate (301 mg, 2.18 mmol), and the mixture was heated to 80 °C for 18 h. The reaction was cooled down and filtered through a pad of celite, washing with acetonitrile (10 mL). The solvent was removed under reduced pressure to give a crude oil, which was purified by column chromatography on silica flash using dichloromethane/ methanol (95:5) as eluents. The resulting solid was further purified by crystallization from a mixture of diisopropylether and ethyl acetate (2/1) to yield the title compound (142 mg, 54%) as a bright-yellow solid. LCMS (*m*/*z*): 470 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62 (d, J = 6.0 Hz, 1H), 7.45−7.56 (m, 1H), 7.14−7.21 (m, 2H), 6.90−7.10 (m, 4H), 6.43 (brs, 2H), 4.17 (t, J = 4.0 Hz, 2H), 3.76 (t, J = 4.0 Hz, 2H), 2.84 (t, J = 4.0 Hz, 2H), 2.61 (t, J = 6.0 Hz, 2H), 2.17 (s, 3H).

4-[3-Amino-4-(2,4-difluorobenzoyl)-1-oxidopyridin-2-yl]-3-methyl-*N*-(2-morpholin-4-ylethyl)benzamide (59). To a solution of 4-[3-amino-4-(2,4-difluorobenzoyl)-1-oxidopyridin-2-yl]-3-methylbenzoic acid (**57**) (50 mg, 0.14 mmol) in 2 mL of *N*,*N*-dimethylformamide were added (2-morpholin-4-ylethyl)amine (25 mg, 0.19 mmol), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) (65 mg, 0.17 mmol), and diisopropyl ethyl amine (301 mg, 2.18 mmol) and the mixture was stirred overnight under argon. The reaction was diluted with ethyl acetate, washed with 5% citric acid, water, brine, and dried over sodium sulfate. Removal of the solvent under reduced pressure afforded a residue which was purified by column chromatography on silica flash, using dichloromethane/ethanol (95:5) as eluent, to yield 4-[3-amino-4-(2,4-difluorobenzoyl)-1-oxidopyridin-2-yl]-3-methyl-*N*-(2-morpholin-4-ylethyl)benzamide (59) (1%) as a bright-yellow solid. LCMS (*m*/*z*): 497 $(M + 1)^+$. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.83−7.90 (m, 2H), 7.56−7.68 (m, 2H), 7.36−7.43 (m, 2H), 7.12−7.21 (m, 2H), 3.72−3.77 (m, 4H), 3.61 (t, J = 8.0 Hz, 2H), 2.64−2.75 (m, 6H), 2.23 (s, 3H).

{3-Amino-2-[4-(2-methoxyethoxy)-2-methylphenyl]-1-oxidopyridin-4-yl}(2,4-difluorophenyl)methanone (60). This compound was prepared from {3-amino-2-[4-(2-methoxyethoxy)-2-methylphenyl]pyridin-4-yl}(2,4-difluorophenyl)methanone (**32**) following the experimental procedure described for the synthesis of **34**. Yield: 72%. LCMS (*m*/*z*): 415 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, J = 8.0 Hz, 1H), 7.45−7.56 (m, 1H), 7.14−7.21 (m, 2H), 6.91−7.01 (m, 4H), 6.43 (brs, 2H), 4.18 (t, J = 4.0 Hz, 2H), 3.78 (t, J = 4.0 Hz, 2H), 3.47 (s, 3H), 2.17 (s, 3H).

4-[3-Amino-4-(2,4-difluorobenzoyl)-1-oxidopyridin-2-yl]-*N*-(2-methoxyethyl)-3-methylbenzamide (61). This compound was prepared from 4-[3-amino-4-(2,4-difluorobenzoyl)pyridin-2-yl]-*N*-(2-methoxyethyl)-3-methylbenzamide (**33**) following the experimental procedure described for the synthesis of **34**. Yield: 60%. LCMS (*m*/*z*): 442 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75−7.84 (m, 2H), 7.65 (t, J = 4.0 Hz, 2H), 7.49−7.60 (m, 1H), 7.37 (t, J = 8.0 Hz, 2H), 7.23 (t, J = 4.0 and 8.0 Hz, 2H), 6.92−7.10 (m, 2H), 6.57 (brt, J = 6.0 Hz, 1H), 6.34 (brs, 2H), 3.67 (m, 2H), 3.60 (dd, J = 8.0 and 4.0 Hz), 3.41 (s, 3H), 2.26 (s, 3H).

Biological Methods. p38α Kinase Inhibition Assay. Enzymatic activity assay was performed in 96-well microtiter plates (Corning, catalogue no. 3686) using a total volume of 50 μL of an assay buffer composed of 50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 1.75 mM Na_3VO_4.

Various concentrations of the test compound or vehicle controls were preincubated for one hour with 0.055 μg/mL of the human p38α (SAPKa) enzyme (obtained from University of Dundee). The reaction started by addition of biotinylated ATF2 substrate and ATP in concentrations around their K_m values (final concentration 0.62 and 60 μM, respectively) and took place for one hour at 25 °C. Addition of the detection reagents, streptavidin−XL665 and antiphosphoresidue antibody coupled to Europium cryptate, caused the juxtaposition of the cryptate and the XL665 fluorophore, resulting in fluorescence energy transfer (FRET). The FRET intensity depends on the amount of bounded cryptate antibody, which is proportional to the extent of substrate phosphorylation. FRET intensity was measured using Victor 2 V spectrofluorometer.

Data were analyzed by nonlinear regression (Hill equation) to generate a dose−response curve. The calculated IC_{50} value is the concentration of the test compound, which caused a 50% decrease in the maximal FRET intensity.

Inhibition of TNFα Production Induced by LPS in the Human Monocytic Cell Line THP-1 Assay. For this purpose, 2×10^5 cells/well were plated in tissue-culture treated round-bottom 96-well plates in RPMI (containing 10% FCS, L-Gln 2 mM, Hepes buffer 10 mM, sodium pyruvate 1 mM, glucose 4.5 g/L, $NaHCO_3$ 1.5 g/L and β-mercaptoethanol 50 μM), together with compounds at the desired test concentration and LPS (Sigma, L2630) at a final 10 μg/mL concentration. Compounds were resuspended in 100% DMSO at a concentration of 1 mM and titrated thereof in 10× dilutions in medium. Controls included unstimulated and stimulated cells treated with the highest concentration of compound vehicle (1% DMSO). Cells were incubated for 5 h at 37 °C in a 5% CO_2 atmosphere. Cell supernatant was recovered by centrifugation and diluted 5-fold prior to testing in a standard human TNFα ELISA (RnD systems).

Inhibition of TNFα Production Induced by LPS in Human Whole Blood Assay. Healthy volunteer donor blood was collected by venipuncture in heparinized tubes. Two μL of 10-fold compound concentrations in 100% DMSO were mixed with 200 μL of LPS-stimulated blood in microtiter plates. Controls included unstimulated and stimulated blood treated with the highest concentration of compound vehicle (1% DMSO). Plates were incubated for 24 h at 37 °C with shaking. Supernatant was recovered by centrifugation and diluted one-sixth prior to testing in a standard TNFα ELISA (RnD systems).

Data were analyzed by nonlinear regression (Hill equation) to generate a dose−response curve. The calculated IC_{50} value corresponds to the concentration of the test compound, causing a 50% decrease in the maximal TNFα production (absolute IC_{50}).

In Vivo Assays. LPS-Induced TNFα in the Rat. One hour prior to LPS administration, rats were dosed orally with the compounds suspended in 0.5% methylcellulose/0.1% Tween-80. LPS (5 mg/kg) was administered intraperitoneally, and 1.5 h later, rats were anesthesized and retroorbital blood collected in heparin tubes. Plasma was separated by centrifugation and diluted one-fifth prior to assaying in a standard rat TNF-alpha ELISA (RnD Systems).

Adjuvant-Induced Arthritis (AIA) Model. Arthritis was induced by intraplantar administration of 100 μL *Mycobacter-*

ium tuberculosis suspension (5 mg/mL in paraffin oil) in the left paw of male Wistar rats (Day 0). On day 11 postinoculation, animals were weighed and paw volume measured by plethismometry. Animals with left paw volumes ranging between 3.5 and 5 mL and right paw volumes between 2 and 3 mL were randomized in the required treatment groups (7 animals/group). Treatment was started and continued for 7 consecutive days. On each day, animals were weighed and appropriately dosed orally with the indicated doses of compounds suspended in 0.5% methylcellulose/0.1% Tween 80. Paw volumes were monitored every other day. A healthy control group was inoculated with paraffin oil on day 0 and monitored thereafter for paw volumes and weight. At the end of the study, animals were euthanized with CO_2. Inhibition percentage was calculated using last day contralateral (right) paw volumes.

Acknowledgment. We thank Victor Segarra from the Department of Computational and Structural Drug Discovery for his kind and valuable support. We gratefully acknowledge Phil Leonard, Marieke Lamers and Martin Fisher from BioFocus DPI (formerly at Sareum) for their kind assistance in the crystallographic studies of compound **45**.

Supporting Information Available: Detailed elemental analysis and HPLC purity data for final compounds and kinase selectivity panel for **45**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Han, J.; Lee, J. D.; Bibbs, L.; Ulevitch, R. J. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* **1994**, *265*, 808–811.
(2) Raingeaud, J.; Gupta, S.; Rogers, J. S.; Dickens, M.; Han, J.; Ulevitch, R. J.; Davis, R. J. Characterization of the structure and function of a novel MAP kinase kinase (MKK6). *J. Biol. Chem.* **1996**, *271*, 2886–2891.
(3) Raingeaud, J.; Whitmarsh, A. J.; Barrett, T.; Derijard, B.; Davis, R. J. MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol. Cell. Biol.* **1996**, *16*, 1247.
(4) Badger, A. M.; Bradbeer, J. N.; Votta, B.; Lee, J. C.; Adams, J. L.; Griswold, D. E. Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1453–1461.
(5) Feldmann, M.; Brennan, F. M.; Maini, R. N. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.* **1996**, *14*, 397–440.
(6) Rutgeerts, P.; D'Haens, G.; Targan, S.; Vasiliauskas, E.; Hanauer, S. B.; Present, D. H.; Mayer, L.; van Hogezand, R. A.; Braakman, T.; DeWoody, K. L.; et al. Efficacy and safety of retreatment with anti-tumour necrosis factor antibody (Infliximab) to maintain remission in Chron's disease. *Gastroenterology* **1999**, *117*, 761–769.
(7) Foster, M. L.; Halley, F.; Souness, J. E. Potential of p38 inhibitors in the treatment of rheumatoid arthritis. *Drug News Perspect.* **2000**, *13*, 488–497.
(8) Kumar, S.; Boehm, J.; Lee, J. C. p38 MAP kinases: key signaling molecules as therapeutic targets for inflammatory diseases. *Nature Rev. Drug Discovery* **2003**, *2*, 717–726.
(9) Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heyes, J. R.; Landvatter, S. W.; Strickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Young, P. R. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **1994**, *372*, 739–746.
(10) Vertex USA: Vertex has suspended development of VX-745, a p38 MAP kinase inhibitor, which was in phase II trials in the USA and Europe as potential treatment for rheumatoid arthritis. Vertex Inc., press release (September, 2001).
(11) SmithKline Beecham (now GlaxoSmithKline) has discontinued development of SB 242235, a cytokine suppressor (SmithKline Beecham, April 1999). This product was in phase I clinical studies in the UK (SmithKline Beecham, March 1999) and was being investigated as a potential treatment for rheumatoid arthritis (SmithKline Beecham, April 1999). R&D Focus Report, March 27, 2001.
(12) Ferraciola, G. F. VX-745 Vertex Pharmaceutical. *Curr. Opin. Anti-Inflammatory Immunomodul. Invest. Drugs* **2000**, *2*, 74–77.
(13) Haddad, J. J. VX-745 (Vertex Pharmaceuticals). *Curr. Opin. Investig. Drugs* **2001**, *2*, 1070–1076.
(14) Zimmiti, C. S.; Schwartz, R.; Torcellini, C. A.; Pargellis, C. A.; Madwed, J. B.; Weldon, S. M. Suppression of p38 activity in vitro and THF alpha production in vivo with BIRB-796 BS, a novel p38 kinase inhibitor. *Arthritis Rheum.* **2001**, *44*, S114.
(15) Regan, J.; Capolino, A.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Hickey, E.; Kroe, R. R.; Madwed, J.; Moriak, M.; Nelson, R.; Pargellis, C. A.; Swinamer, A.; Torcellini, C.; Tsang, M.; Moss, N. Structure−Activity Relationships of the p38α MAP Kinase Inhibitor 1-(5-*tert*-Butyl-2-*p*-tolyl-2*H*-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)napthalen-1-yl]urea (BIRB-796). *J. Med. Chem.* **2003**, *46*, 4676–4686.
(16) Devraj, R. V. Discovery and Development of Orally Active p38 Kinase Inhibitors as Anti-TNF Agents. *Abstract of Papers,* 229th National Meeting of the American Chemical Society, San Diego, CA, March 13−17, 2005; American Chemical Society: Washington, DC, 2005; MEDI-296.
(17) Zack, D. J.; Campagnuolo, G.; Middleton, S.; Koch, A.; Zhu, L.; Bolon, B.; Feige, U. Efficacy of AMG 548, a novel p38 kinase inhibitor, in rats with adjuvant-induced or collagen-induced arthritis. 67th Annual Scientific Meeting of the American College of Rheumatology, Orlando, FL, October 23−28, 2003, Abstract 834.
(18) Domínguez, C; Liu, L.; Zhang, D.; Tamayo, N.; Powers, D.; Min, W.; Feige, F.; Harris, R.; Wild, S.; Neervannan, S.; Rashid, S.; Harvey, T. p38 MAP kinase: an exciting target for the treatment of inflammatory diseases. *Abstract of Papers*, 228th National Meeting of the American Chemical Society, Philadelphia, PA; American Chemical Society: Washington, DC, *2004*; MEDI-189.
(19) Nikas, S. N.; Drossos, A. A. SCIOS-469 (Scios Inc). *Curr. Opin. Investig. Drugs* **2004**, *5*, 1205–1212.
(20) Perumattan, J. 2-[6-Chloro-5-[4-(4-fluoro-benzyl)-2*R*,5*S*-dimethyl-piperazine-1-carbonyl]-1*H*-indol-3-yl]-2-oxo-acetamides as potent inhibitors of p38 alpha MAP kinase. *Drugs Future* **2006**, *31* (Suppl. A), Abstract O19.
(21) Weisman, M.; Furst, D.; Schiff, M.; Kauffman, R.; Merica, E.; Martin-Munley, S. FRI0018 A double-blind, placebo controlled trial of VX-745, an oral p38 MAP kinase inhibitor, in patients with rheumatoid arthritis (RA). *Ann. Rheum. Dis.* **2002**, *61*, Suppl. 1.
(22) Vertex Pharmaceuticals, Inc. Press Release (September 24, 2001).
(23) Wang, Z.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; Abdel-Meguid, S.; Adams, J. L.; Goldsmith, E. J. Structural basis of inhibitor selectivity in MAP kinases. *Structure* **1998**, *6*, 1117–1128.
(24) Gallagher, T. F.; Seibel, G. L.; Kassis, S.; Laydon, J. T.; Blumenthal, M. J.; Lee, J. C.; Lee, D.; Boehm, J. C.; Fier-Thompson, S. M.; Abt, J. W.; Sorenson, M. E.; Smietana, J. M.; Hall, R. F.; Garigipati, R. S.; Bender, P. E.; Erhard, K. F.; Krog, A. J.; Hoffmann, G. A.; Sheldrake, P. L.; McDonnell, P. C.; Kumar, S.; Young, P. R.; Adams, J. L. Regulation of stress-induced cytokine production by pyridinylimidazoles; inhibition of CSBP kinase. *Bioorg. Med. Chem.* **1997**, *5*, 49–64.
(25) Liverton, N. J.; Butcher, J. W.; Claiborne, C. F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. Design and Synthesis of Potent, Selective, and Orally Bioavailable Tetrasubstituted Imidazole Inhibitors of p38 Mitogen-Activated Protein Kinase. *J. Med. Chem.* **1999**, *42*, 2180–2190.
(26) Jackson, P. F.; Bullington, J. L. Pyridinylimidazole based p38 MAP kinase inhibitors. *Curr. Top. Med. Chem.* **2002**, *2*, 1011–1020.
(27) Collis, A. J.; Foster, M. L.; Halley, F.; Maslen, C.; McLay, I. M.; Page, K. M.; Redford, E. J.; Souness, J. E.; Wilsher, N. E. RPR203494 a Pyrimidine Analogue of the p38 Inhibitor RPR200765A with an Improved in Vitro Potency. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 693–696.
(28) Revesz, L.; Dipadova, F. E.; Buhl, T.; et al. SAR of 4-Hydroxypiperidine and Hydroxyalkyl Substituted Heterocycles as Novel p38 MAP Kinase Inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1261–1264.
(29) Fitzgerald, C. E.; Patel, S. B.; Becker, J. W.; Cameron, P. M.; Zaller, D.; Pikounis, V. B.; O'Keefe, S. J.; Scapin, G. Structural Basis for p38α MAP Kinase Quinazolinone and Pyrido-Pyrimidine Inhibitor Specificity. *Nat. Struct. Biol.* **2003**, *10*, 764–769.
(30) Hunt, J. A.; Kallashi, F.; Ruzek, R. D.; Sinclair, P. J.; Ita, I.; McCormick, S. X.; Pivnichny, J. V.; Hop, C. E. C.; Kumar, S.; m

Wang, Z.; O'Keefe, S. J.; O'Neill, E. A.; Porter, G.; Thompson, J. E.; Woods, A.; Zaller, D. M.; Doherty, J. B. p38 Inhibitors: piperidine- and 4-aminopiperidine-substituted naphthyridinones, quinolinones, and dihydroquinazolinones. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 467–470.

(31) Bao, J.; Hunt, J. A.; Miao, S.; Rupprecht, K. M.; Stelmach, J. E.; Liu, L.; Ruzek, R. D.; Sinclair, P. J.; Pivnichny, J. V.; Hop, C. E. C. A.; Kumar, S.; Zaller, D. M.; Shoop, W. L.; O'Neill, E. A.; O'Keefe, S. J.; Thompson, C. M.; Cubbon, R. M.; Wang, R.; Zhang, W. X.; Thompson, J. E.; Doherty, J. B. p38 MAP kinase inhibitors: Metabolically stabilized piperidine-substituted quinolinones and naphthyridinones. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 64–68.

(32) Alonso-Alija, C.; Michels, M.; Schirok, H.; Schlemmer, K.-H.; Bell, J.; Fitzgerald, M. F.; Dodd, S.; Gill, A. Monocyclic aroylpyridinones as antiinflammatory agents. Patent WO03076405, September, **2003**.

(33) Schirok, H.; Alonso-Alija, C.; Benet-Buchholz, J.; Goller, A. H.; Grosser, R.; Michels, M.; Paulsen, H. Efficient Regioselective Synthesis of 6-Amino-5-benzoyl-1-Substituted 2(1*H*)-Pyridinones. *J. Org. Chem*. **2005**, *70*, 9463–9469.

(34) Guengoer, T.; Marsais, F.; Queguiner, G. *ortho*-Functionalization of Aminopyridines. Regioselective Lithiation of 3-Pivaloylaminopyridines. *Synthesis* **1982**, *6*, 499–500.

(35) (a) Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev*. **1995**, *95*, 2457−2483 (Review). (b) Stanforth, S. P. Catalytic cross-coupling reactions in biaryl synthesis. *Tetrahedron* **1998**, *54*, 263−303 (review).

(36) Jin, R.; Bian, Z.; Kang, C.; Guo, H.; Gao, L. Synthesis of 3,3′-Di(2-pyridyl)-1,1′-bi-2-naphthol Derivatives. *Synth. Commun*. **2005**, *35*, 1897–1902.

(37) Walker, S. D.; Barder, T. E.; Martinelli, J. R.; Buchwald, S. L. A Rationally Designed Universal Catalyst for Suzuki−Miyaura Coupling Processes. *Angew. Chem., Int. Ed*. **2004**, *43*, 1871–1876.

(38) Negishi, E.-I.; King, A. O.; Okukado, N. Selective carbon−carbon bond formation via transition metal catalysis. 3. A highly selective synthesis of unsymmetrical biaryls and diarylmethanes by the nickel- or palladium-catalyzed reaction of aryl- and benzylzinc derivatives with aryl halides. *J. Org. Chem*. **1977**, *42*, 1821–1823.

(39) Negishi, E.-I.; Luo, F.-T.; Frisbee, R.; Matsushita, H. A Regiospecific Synthesis of Carbosubstituted Heteroaromatic Derivatives via Pd-Catalyzed Cross Coupling. *Heterocycles* **1982**, *18*, 117–122.

(40) Wang, Z.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; Abdel-Meguid, S.; Adams, J. L.; Goldsmith, E. J. Structural Basis of Inhibitor Selectivity in MAP Kinases. *Structure* (*London*) **1998**, *6*, 1117–1128.

(41) McClure, K. F.; Letavic, M. A.; Kalgutkara, A. S.; Gabel, C. A.; Audoly, L.; Barberiaa, J. T.; Braganzaa, J. F.; Cartera, D.; Cartya, T. J.; Cortinaa, S. R.; Dombroskia, M. A.; Donahuea, K. M.; Elliotta, N. C.; Gibbonsa, C. P.; Jordana, C. K.; Kupermana, A. V.; Labasia, J. F.; LaLibertea, R. E.; McCoya, J. M.; Naimana, B. M.; Nelsona, K. L.; Nguyena, H. T.; Peesea, K. M.; Sweeneya, F. J.; Taylora, T. J.; Trebinoa, C. E.; Abramova, Y. A.; Lairda, E. R.; Volberga, W. A.; Zhoua, J.; Bacha, J.; Lombardo, F. Structure−activity relationships of triazolopyridine oxazole p38 inhibitors: Identification of candidates for clinical development. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 4339–4344.

(42) Hauser, D. R.; Scior, T.; Domeyer, D. M.; Kammerer, B.; Laufer, S. A. Synthesis, Biological Testing, and Binding Mode Prediction of 6,9-Diarylpurin-8-ones as p38 MAP Kinase Inhibitors. *J. Med. Chem*. **2007**, *9*, 2060–2066.

(43) Wan, Z.; Boehm, J. C.; Bower, M. J.; Kassis, S.; Lee, J. C.; Zhao, B.; Adams, J. L. *N*-Phenyl-*N*-purin-6-yl ureas: The design and synthesis of p38α MAP kinase inhibitors. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 1191–1194.

(44) Natarajan, S. W.; Wisnoski, D. D.; Singh, S. B.; Stelmach, J. E.; O'Neill, E. A.; Schwartz, C. D.; Thompson, C. M.; Fitzgerald, C. E.; O'Keefe, S. J.; Cornelis, S. K.; Hop, E. C.; Zaller, D. M.; Schmatz, D. M.; Doherty, J. B. p38 MAP kinase inhibitors. Part 1: Design and development of a new class of potent and highly selective inhibitors based on 3,4-dihydropyrido[3,2-*d*]pyrimidone scaffold. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 273–276.

(45) Clark, R. D.; Wolohan, P. R. Molecular Design and Bioavailability. *Curr. Top. Med. Chem*. **2003**, *3*, 1269–1288.

(46) Crystallographic coordinates for the structure of unphosphorylated p38α complexed with **45** have been deposited at the Protein Data Bank (www.rscb.org) with entry accession code 3HRB.

(47) See Supporting Information (p S32).