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Discovery of 1,4-Disubstituted 3-Cyano-2-pyridones: A New Class of Positive Allosteric Modulators of the Metabotropic Glutamate 2 Receptor

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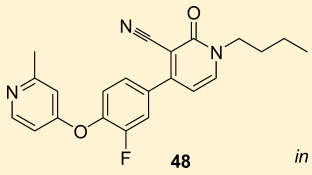
Supporting Information

ABSTRACT: The discovery and characterization of compound **48**, a selective and in vivo active mGlu2 receptor positive allosteric modulator (PAM), are described. A key to the discovery was the rational exploration of the initial HTS hit **13** guided by an overlay model built with reported mGlu2 receptor PAM chemotypes. The initial weak in vitro activity of the hit **13** was quickly improved, although compounds still had suboptimal druglike properties. Subsequent modulation of the physicochemical properties resulted in compounds having a more balanced profile, combining good potency and in vivo pharmacokinetic properties. Final refinement by addressing cardiovascular safety liabilities led to the discovery of compound **48**. Besides good potency, selectivity, and ADME properties, compound **48** displayed robust in vivo activity in a sleep−wake electroencephalogram (sw-EEG) assay consistent with mGlu2 receptor activation, in accordance with previous work from our laboratories.



48

mGlu2 EC_{50} = 361 nM
mGlu2 E_{MAX} (%) = 233
RLM (% metabolized) = 50
HLM (% metabolized) = 33

in vivo active sw-EEG at 10 mg/kg s.c.

INTRODUCTION

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) of vertebrates and modulates the synaptic response via activation of either ionotropic (iGlu) or metabotropic glutamate (mGlu) receptors.[1−3] Eight G-protein-coupled mGlu receptor subtypes are known and have been classified into three groups based on homology, pharmacology, and signaling pathways: group I, mGlu1 and mGlu5 receptors; group II, mGlu2 and mGlu3 receptors; group III, mGlu4, -6, -7, and -8 receptors.[4,5] Group I receptors are primarily expressed postsynaptically, whereas both groups II and III are mainly presynaptic where they reduce glutamate release.[6,7] The group II mGlu2 receptor is widely distributed in the brain, and high levels are found in forebrain and limbic areas such as prefrontal cortex, hippocampus, and amygdala where excessive glutamate neurotransmission may be implicated in the pathophysiology of anxiety and schizophrenia.[8−11] It is therefore expected that activation of mGlu2 receptors may provide anxiolytic and/or antipsychotic effects.[12−15] This was confirmed in a phase II trial with LY2140023, a prodrug of the mixed mGlu2/3 receptor agonist LY404039, which showed improvements in positive and negative symptoms in schizophrenic patients.[16] In addition, anxiolytic efficacy of the agonist LY354740 was demonstrated in a CO_2 inhalation study by reduction in number and severity of panic symptoms in patients with DMS-IV panic disorder.[17] Multiple preclinical studies have shown the efficacy of mGlu2 receptor activation in animal models of disorders such as anxiety/stress and depression.[18,19]

Constrained glutamate agonists such as LY404039 and LY354740 bind at the orthosteric site, and behavioral studies in knockout mice suggest that their activity is mGlu2 receptor mediated.[20,21] Receptor activation with positive allosteric modulators (PAMs) which act via alternative allosteric site(s) offers several advantages. First, allosteric molecules are likely to be less polar and have improved CNS penetration, as they do not require amino acid functional groups necessary at the orthosteric site. The allosteric site(s) can be less conserved and therefore allow improved mGlu receptor subtype selectivity. They only act in the presence of endogenous glutamate and so may be less prone to cause receptor desensitization,[22,23] and they respond to physiological glutamate fluctuations. Given these potential advantages, the number of reported mGlu2 receptor PAM chemical series has increased over the past years

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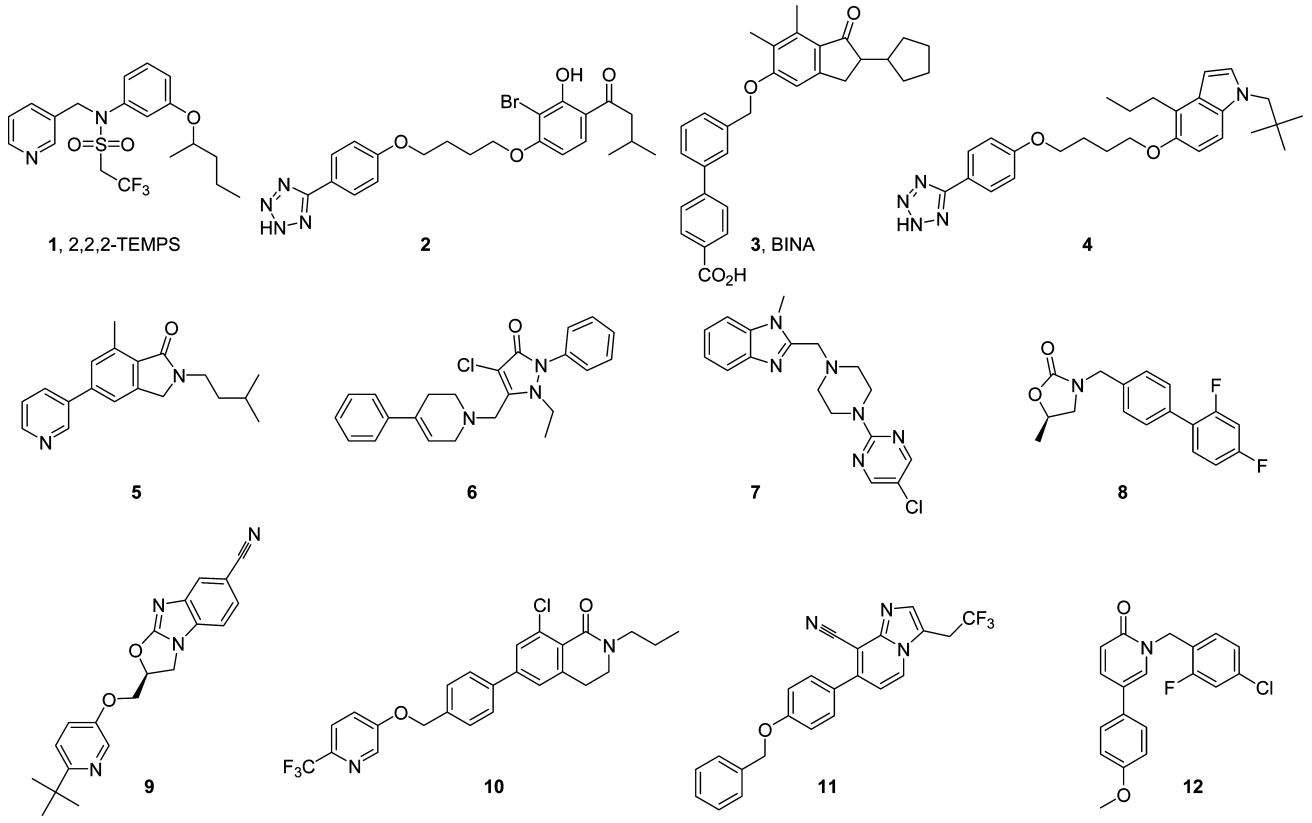

Figure 1. Examples of reported mGlu2 receptor PAMs.

(Figure 1).[24] Pyridylmethylsulfonamides (2,2,2-TEMPS, **1**),[25−27] acetophenones (**2**),[28−31] bicyclic ketones (BINA, **3**),[32,33] and indoles (**4**)[34] were the earliest reported mGlu2 receptor PAMs. Subsequently AstraZeneca and NPS Pharmaceuticals filed patent applications for isoindolones (**5**)[35] and pyrazolones (**6**);[36] the former compounds were included in a recent article presenting BINA analogues with improved pharmacokinetic (PK) properties.[37] Newer series of benzimidazoles (**7**),[38−40] oxazolidinones (**8**),[41,42] and oxazolobenzimidazoles (**9**)[43] have been reported, as well as isoquinolones (**10**),[44,45] imidazopyridines (**11**),[46] and 1,5-disubstituted-2-pyridones (**12**)[47] from our laboratories (Figure 1).

We have recently reported the discovery and exploration of a series of isoquinolones[44,45] and 1,5-disubstituted-2-pyridones[47] for which we have shown that combining appropriate potency and metabolic stability was a challenge. As a result, lead compounds from these chemical classes suffered from poor pharmacokinetics (PK) properties that precluded their further development.[48] Here we report the identification and in vivo evaluation of advanced leads from a novel 1,4-disubstituted-2-pyridone series of mGlu2 receptor PAMs.[49−51]

2-Pyridone **13** was identified as a hit from a high throughput screening (HTS) of the Addex Pharmaceuticals compound collection. The molecule displayed low micromolar activity in a mGlu2 receptor PAM fluorometric imaging plate reader (FLIPR) assay which was subsequently confirmed in a follow-up [^{35}S]GTPγS functional assay with an EC_{50} of 8 μM and E_{MAX} [52] of 117%. The HTS hit **13** was considered in terms of its 3D similarity to mGlu2 receptor PAMs **2**, **3**, **5**, and **6** known at the time. There was good overlap between the pyridone scaffold, matching the carbonyl acceptor (feature F1) and the ring centers present in other mGlu2 receptor PAMs. In addition, there was excellent match of the isopentyl group in hydrophobic region (F2), while the cyano compared well to the methyl, bromo, and chloro species present within a small volume (V1) (Figure 2). However, the HTS hit was clearly lacking the large

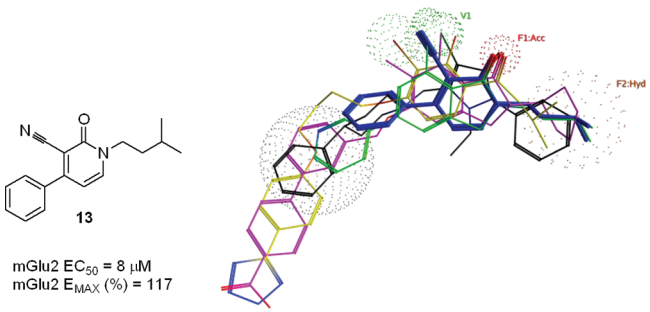

Figure 2. Origin of the 1,4-disubstituted 2-pyridone mGlu2 receptor PAM series. Shown are HTS hit **13** and overlay comparison of **13** (blue) with other known mGlu2 receptor PAMs: **2** (yellow), **3** (magenta), **5** (green), and **6** (black). Dotted spheres show similar overlapping features. F1 is a scaffold acceptor. F2 is a hydrophobic group. V1 is a volume containing a small substituent such as Br, Me, or Cl.

substituent in the 4 position on the pyridone ring present in the other reference molecules. A lead optimization strategy is described here to identify in vivo active mGlu2 receptor PAMs via modification at the 4 position of the HTS hit **13**. In this report we describe our optimization efforts that led to the discovery of the advanced lead **48**. This molecule showed good mGlu2 receptor PAM in vitro activity and selectivity, acceptable preliminary safety profile, and good brain exposure. We have shown previously that mGlu2 receptor activation with the agonist LY354740 and/or PAM BINA (**3**) shows common

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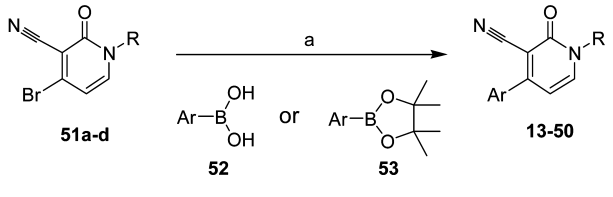
changes of rapid eye movement (REM) sleep variables in a sleep-wake electroencephalogram (sw-EEG) assay, and that combining both compounds produces synergistic effects.[53] Compound **48** displayed robust in vivo activity in the same model, consistent with mGlu2 receptor activation.

CHEMISTRY

The general synthesis of target compounds **13**–**50** are shown in Scheme 1. They were prepared via Suzuki coupling of the corresponding 4-bromopyridones **51a**–**d** with an array of either boronic acids (**52**) or esters (**53**). The chemical structures of compounds **13**–**50** are shown in Tables 1, 2, and 4.

Scheme 1. Synthesis of Final Compounds 13–50[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, $NaHCO_3$, H_2O/1,4-dioxane, 150 °C, 10 min, microwave.

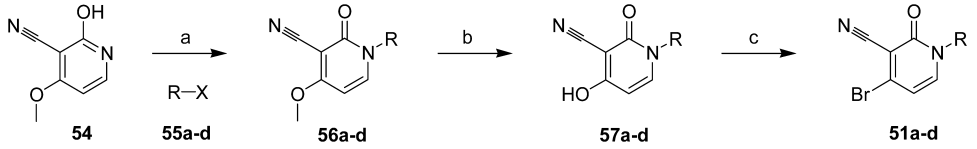
The key bromopyridone intermediates **51a**–**d** and non-commercially available boronic acids **52** and esters **53** were prepared following the procedures shown in Schemes 2–5. Intermediate pyridones **51a**–**d** were obtained via the three-step sequence outlined in Scheme 2. Thus, treatment of 2-hydroxypyridine derivative **54** with the required alkyl halides **55a**–**d** afforded the N-alkylated 2-pyridones **56a**–**d**. Subsequent cleavage of the methyl ether followed by treatment of the resulting hydroxypyridones **57a**–**d** with $POBr_3$ yielded the target intermediates **51a**–**d**.

The synthesis of the tetrahydropyranyl substituted boronates **53a,b** was accomplished in two steps (Scheme 3). The oxygen-linked tetrahydropyranyl derivative **53a** was prepared via Mitsunobu reaction of 4-hydroxytetrathydropyran **58** with 4-bromo-2-chlorophenol **59** followed by palladium catalyzed reaction of the intermediate bromophenyl ether **60** with bis-(pinacolato)diboron. In the case of the nitrogen-linked analogue **53b**, the required intermediate bromoaniline **63** was obtained by reductive amination of the pyranone **61** with 4-bromo-2-chloroaniline **62**.

The 3-pyridyloxy substituted boronates **53c**–**g** were prepared following the synthesis sequence shown in Scheme 4. Nucleophilic aromatic substitution of 4-fluoronitrobenzenes **65a,b** with 3-hydroxypyridines **64a**–**c** led to the 3-pyridyl 4-nitrophenyl ethers **66a**–**e**. Subsequent reduction of the nitro group followed by Sandmeyer reaction on the intermediate anilines **67a**–**e** yielded the bromophenylethers **68a**–**e** that were finally transformed into the boronates **53c**–**g** by palladium catalyzed reaction with bis(pinacolato)diboron.

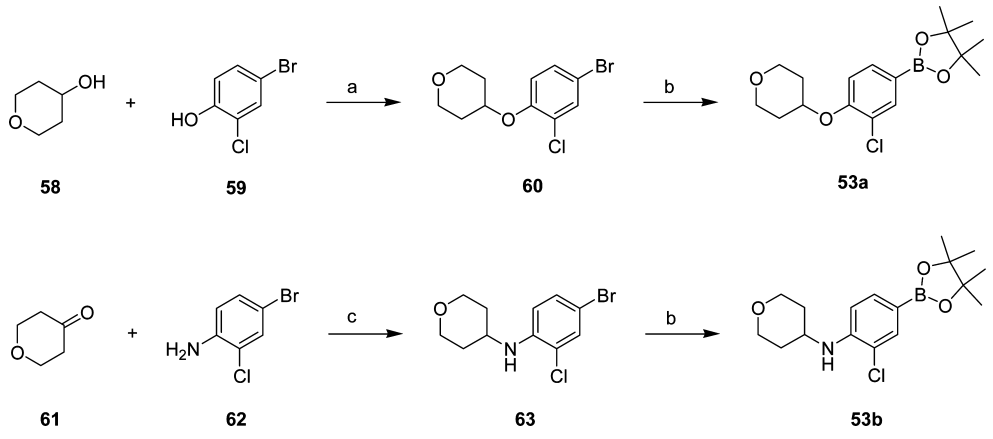
The 4-pyridyloxy substituted boronates **53h**–**k** were prepared by reaction of 4-bromophenol **70a**–**c** with the corresponding 4-chloropyridines **69a**–**c** followed by standard "bromo-to-boron" transformation of the intermediate bromophenyl ethers **71a**–**d** (Scheme 5). In the case of the more electron deficient halogen-substituted 4-bromophenols **73a**–**c**, the nucleophilic substitution with 4-chloropyridines **69a**–**c** failed and starting materials were recovered. Nevertheless, the use of the more electrophilic 4-nitropyridine *N*-oxide **72** solved the problem and the required bromophenyl ethers **74a**–**c** could be obtained. Interestingly, the standard "bromo-to-boron" transformation led

Scheme 2. Preparation of 4-Bromopyridone Building Blocks 51a–d[a]



[a]Reagents and conditions: (a) K_2CO_3, MeCN, 100 °C, 18 h; (b) NaOH, H_2O, reflux, 18 h; (c) $POBr_3$, DMF, 110 °C, 3 h.

Scheme 3. Synthesis of Tetrahydropyranyl Substituted Boronates 53a,b[a]



[a]Reagents and conditions: (a) polymer supported PPh_3, DBAD, CH_2Cl_2, rt, 2 h; (b) $PdCl_2$dppf, AcOK, DMF/1,4-dioxane, 150 °C, 10 min, microwave; (c) $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 16 h.

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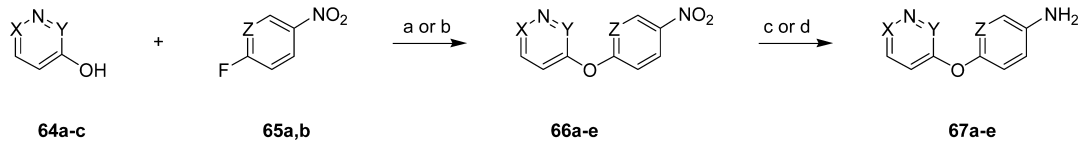
Scheme 4. Synthesis of 3-Pyridyloxy Substituted Boronates 53c–g[a]



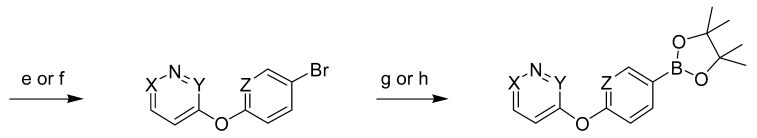
64a-c | **65a,b** | **66a-e** | **67a-e**

64a: X,Y = CH
64b: X = CH; Y = CMe
64c: X,Y = CMe

65a: Z = CH
65b: Z = CCl

66a: X,Y,Z = CH
66b: X,Z = CH; Y = CMe
66c: X,Y = CMe; Z = CH
66d: X = CH; Y = CMe; Z = CCl
66e: X,Y = CMe; Z = CCl

67a: X,Y,Z = CH
67b: X,Z = CH; Y = CMe
67c: X,Y = CMe; Z = CH
67d: X = CH; Y = CMe; Z = CCl
67e: X,Y = CMe; Z = CCl



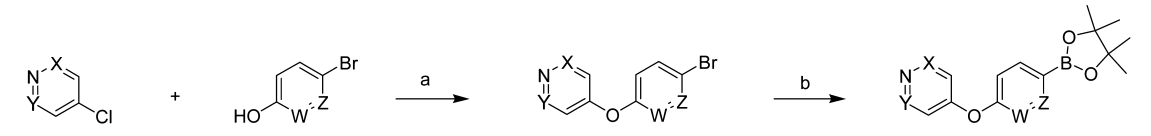
68a-e | **53c-g**

68a: X,Y,Z = CH
68b: X,Z = CH; Y = CMe
68c: X,Y = CMe; Z = CH
68d: X = CH; Y = CMe; Z = CCl
68e: X,Y = CMe; Z = CCl

53c: X,Y,Z = CH
53d: X,Z = CH; Y = CMe
53e: X,Y = CMe; Z = CH
53f: X = CH; Y = CMe; Z = CCl
53g: X,Y = CMe; Z = CCl

[a]Reagents and conditions: (a) Cs_2CO_3, DMF, 170 °C, 30 min, microwave; (b) Cs_2CO_3, DMF, 130 °C, 16–24 h; (c) H_2 (1 atm), Pd/C, EtOAc, rt, 16 h; (d) $SnCl_2$, EtOH, 90 °C, 1 h; (e) $NaNO_2$, HBr, H_2O, rt, 2 h; (f) CuBr, rt, 2 h, then 140 °C, 2 h; (g) bis(pinacolato)diboron, $PdCl_2$dppf, AcOK, DMSO, 120 °C, 5 h; (h) bis(pinacolato)diboron, $PdCl_2$dppf, AcOK, 1,4-dioxane, DMF, 150 °C, 10 min, microwave.

Scheme 5. Synthesis of 4-Pyridyloxy Substituted Boronates 53h–n[a]



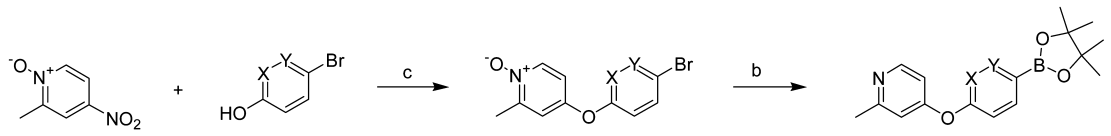
69a-c | **70a-c** | **71a-d** | **53h-k**

69a: X,Y = CH
69b: X = CH; Y = CMe
69c: X,Y = CMe

70a: W,Z = CH
70b: W = CH; Z = Cl
70c: W = CF; Z = CH

71a: X,Y,W,Z = CH
71b: X,W,Z = CH; Y = CMe
71c: X,W = CH; Y = CMe; Z = CCl
71d: X,Y = CMe; Z = CH; W = CF

53h: X,Y,W,Z = CH
53i: X,W,Z = CH; Y = CMe
53j: X,W = CH; Y = CMe; Z = CCl
53k: X,Y = CMe; Z = CH; W = CF



72 | **73a-c** | **74a, c** | **53l-n**

73a: X = CCl; Y = CH
73b: X = CF; Y = CH
73c: X = CH; Y = CF

74a: X = CCl; Y = CH
74b: X = CF; Y = CH
74c: X = CH; Y = CF

53l: X = CCl; Y = CH
53m: X = CF; Y = CH
53n: X = CH; Y = CF

[a]Reagents and conditions: (a) NaH or K_2CO_3, DMF, 170 °C, 30 min, microwave; (b) bis(pinacolato)diboron, $PdCl_2$dppf, AcOK, DMSO, 120 °C, 5 h; (c) NaH or K_2CO_3, solvent, 150–180 °C, 3 h to 2 days.

to simultaneous reduction of the pyridine *N*-oxide, yielding the final boronates **53l–n**.

The required boronic acids or esters required for the preparation of compounds **13–19**, **24**, **25**, **28**, **30**, and **31** are commercially available.

RESULTS AND DISCUSSION

For our initial exploration we kept constant the isopentyl group at position 1 of the cyanopyridone core and explored different substituents and decoration patterns of the C-4 phenyl ring (**13–31**). In general, most compounds showed excellent potentiation with E_{MAX} values ranging from 135% to 249% (Table 1). Initial SAR showed that substitution at the para-position was preferred for activity, as moving the 4-methoxy group of compound **14** to meta-position (**15**) and ortho-position (**16**) resulted in a decrease in activity. Introduction of a fluorine atom adjacent to the methoxy group **17** resulted in activity comparable to that of **15**. Better results were obtained with groups such as chlorine or methoxy, and compounds **18** and **19** had EC_{50} values of 363 and 260 nM, respectively. In vitro

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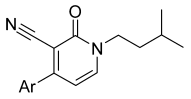
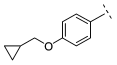
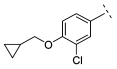
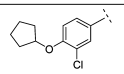
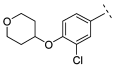
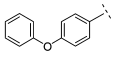
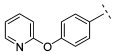
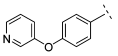
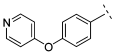
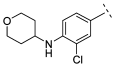
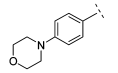
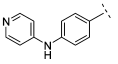
Table 1. Functional Activity and Metabolic Stability Data of Representative mGlu2 Receptor PAMs 13-31[a]



13-31

Compd	Ar	mGlu2 EC_{50}[a] (nM)	mGlu2 E_{MAX} (%)[a]	HLM (%)[b]	RLM (%)[b]
13		8,510	135	36	100
14		1,150	160		
15		3,169	176	74	--
16		>10,000	120	38	--
17		1,170	160	35	--
18		363	200	43	99
19		260	242	26	--
20		290	192	17	16
21		110	206	17	72
22		100	199	--	--
23		186	197	36	54
24		42	188	36	32
25		646	249	20	36
26		479	231	21	29
27		550	245	27	41
28		460	186	--	--
29		174	247	42	47
30		2,040	236	38	48
31		1,479	186	25	33

[a]Values are the mean of three experiments. Only differences in pEC_{50} up to 0.6 log units (SD < 0.5) were considered as reproducible and were maintained. [b]HLM and RLM data refer to percent of compound metabolized after incubation with microsomes for 15 min at 5 μM.

metabolic stability for **18** was comparable to **13**. In general, replacement of the methoxy group by larger alkyloxy substituents **20**–**27** provided compounds with improved potency, indicating that increased lipophilicity in that area of the molecule is advantageous for potency. Compound **20**, having a bulkier and more lipophilic ether, was significantly more potent (EC_{50} = 290 nM) than **13**. In addition, **20** was found to be metabolically more stable in both human liver microsomes (HLM, 17%) and rat liver microsomes (RLM, 16%). As in the case of **18**, introduction of an adjacent chlorine atom to the cyclopropylmethyloxy group of compound **20** gave compound **21**, which had increased potency (EC_{50} = 110 nM) but higher metabolic turnover in RLM (72%). The same trend in activity was observed with compound **22** (EC_{50} = 100 nM). The less liphophilic tetrahydropyranyl derivative **23** was slightly less potent than **21** and **22** and showed a reduced metabolism in RLM (54%). Following the trend shown by previous analogues the more liphophilic biaryl ether **24** showed higher potency than previous ethers (EC_{50} = 42 nM) and was metabolically more stable in both HLM and RLM (36% and 32%, respectively). Unfortunately, the aqueous solubility of **24** was rather poor (<0.001 mg/mL at pH 4–7.4), which could present a challenge in terms of oral bioavailability and would also complicate its formulation for in vivo studies.

We therefore focused on the design of compounds bearing hydrophilic/polar groups as substituents of the C-4 phenyl ring, **25**–**31**. In an attempt to improve aqueous solubility, the set of pyridyl ethers **25**–**27** was prepared. These compounds were found to be at least 10-fold less active than the biaryl ether analogue **24** with EC_{50} values ranging from 479 to 646 nM. Metabolic stability for these pyridine analogues was promising. As anticipated, the basic pyridine nitrogen had a beneficial effect for the aqueous solubility of these analogues, particularly pyridine derivatives **26** and **27** (≥4 mg/mL at 20% HP-β-CD buffer, pH 4).

To complete the SAR analysis, we examined the effect of replacing the ether linker by *N*-alkyl substitutients. Relative to the ether **14**, compound **28** bearing a methylamino substituent was ~4-fold more potent (EC_{50} = 460 nM). This trend was not confirmed by other N-linked analogues such as **29**, which showed potency comparable to that of its ether analogue **23**. Tertiary amines **30** were overall less potent. Finally, compound **31** turned out to be 3-fold less potent than its oxygen analogue **27**. In this case, the increased basicity of the pyridine nitrogen could account for the loss of activity.

Analysis of the structure–activity relationship (SAR) generated from the compounds in Table 1 showed that both potency and fair metabolic stability could be achieved in this novel 1,4-disubstituted 2-pyridone series. However, the most potent compounds (**21**, **22**, and **24**) suffered from poor solubility. Taking this into account, compounds **26** and **27** combining moderate activity, metabolic stability, and good solubility were selected for further evaluation.

Further profiling of **27** and related analogues revealed some potential safety issues associated with these pyridyl derivatives. For instance, compound **27** showed significant CYP450 inhibition[54,55] (>50% at1 μM) of the major isozymes, 3A4

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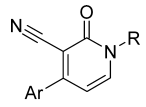
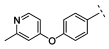
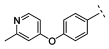
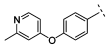
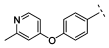
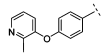
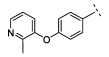
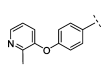
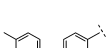
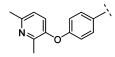
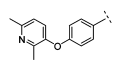
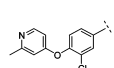
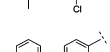
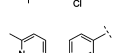
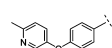
Table 2. Functional Activity and Metabolic Stability Data of Representative Pyridyloxyaryl-Substituted mGlu2 Receptor PAMs 32−47[a]



32-47

Compd	Ar	R	mGlu2 EC_{50} (nM)[a]	mGlu2 E_{MAX}(%)[a]	HLM[b] (%)	RLM[b] (%)	hERG[c]
32			447	266	61	47	--
33			126	233	36	44	80
34			398	243	31	44	53
35			832	199	9	19	--
36			347	228	30	50	31
37			275	256	57	45	36
38			1,072	155	36	39	--
39			138	239	51	60	--
40			100	246	59	49	--
41			263	183	35	42	68
42			166	245	35	51	--
43			933	187	34	47	--
44			100	236	29	60	56
45			230	180	37	22	75
46			30	231	34	50	80
47			64	196	25	27	89

[a]Values are the mean of three experiments. Only differences in pEC_{50} up to 0.6 log units (SD < 0.5) were considered as reproducible and were maintained. [b]HLM and RLM data refer to percent of compound metabolized after 15 min at 5 μM. [c]Experiments were performed using HEK293 cells stably transfected with the hERG potassium channel. Whole-cell currents were recorded with an automated patch-clamp system (PatchXpress 7000A). Three concentrations of compound were tested (0.1, 0.3, and 3 μM; n = 3) and compared to vehicle (0.1% DMSO; n = 3). Data are presented as percent inhibition at the highest concentration tested (3 μM).

(70%), 2C9 (82%), 2D6 (84%), 1A2 (80%), and 2C19 (97%), which could translate into potential drug−drug interactions in the case of coadministration with other drugs. Moreover, **27** showed a moderate hERG channel interaction (52% inhibition at 3 μM) in a patch clamp assay, which may result in QTc prolongation and lead to serious cardiac arrhythmias. The optimization of the pyridyl derivatives, besides improving potency, also focused on ways to attenuate the possible safety concerns derived from CYP450 and hERG channel interactions. Table 2 shows representatives that best illustrate our SAR understanding and progress.

Introduction of a methyl group in the adjacent position of the pyridine nitrogen led to a remarkable improvement of the CYP450 profile, and compound **32** showed less than 50% inhibition toward the 5 isoforms at 5 μM. **32** was slightly more potent than **27**; however, it showed a worse metabolic stability profile in HLM (61% vs 21% for **27**). Metabolite identification studies after in vitro incubation with RLM and HLM revealed that oxidation of the *N*-isopentyl chain was the preferred metabolic pathway for **32**. Looking for compounds with better metabolic stability led us to prepare **33**−**35**, where the metabolically unstable isopentyl chain was replaced with a variety of aliphatic groups. A 3-fold increase in activity was found with the *N*-cyclobutylmethyl analogue **33** (EC_{50} = 126 nM); unfortunately, this increase in potency was accompanied by a strong interaction with the hERG channel (80% inhibition at 3 μM). A better overall profile was identified in **34** having an *N*-butyl substituent, which showed good potency and CYP450 profile comparable to those of **27**. Furthermore its metabolic stability in HLM was enhanced (31% vs 61% for **32**). Compound **35**, contained a less lipophilic *N*-cyclopropylmethyl substituent and showed 2-fold reduction in potency relative to **32**. The data generated for **32**−**35** proved that while increasing lipophilicity around the pyridone nitrogen was beneficial for primary activity, it also increased the liability of oxidative metabolic pathways. A similar trend was found in the 3-pyridyl derivatives **36**−**38**. Interestingly, compound **36** proved to be cleaner in the hERG patch clamp assay compared to its corresponding regioisomer compound **34**. Although not reported here, all attempts to increase potency and metabolic stability by polar/hydrophilic substituents on the pyridinyl ring were fruitless.

An SAR trend on liphophilicity vs potency was observed with analogues having a second methyl on the pyridine ring, **39**−**41**. These compounds showed on average a 3-fold increase in potency when compared to their corresponding monomethylated versions **36** and **38**. The introduction of an additional methyl group had little effect on metabolism of these compounds. Moreover, we examined the influence of the introduction of a chlorine atom on the phenyl ring in the pyridine series. As previously observed for compounds **20** and **21**, chloro-substituted pyridyloxy derivatives **44**−**47** showed a 3- to 4-fold increase in potency when compared to their corresponding nonchlorinated

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analogues. Interestingly, the chloro substituent was preferred in the position adjacent to the ether linker, as moving it to an alternative location, as in **43**, led to a substantial loss of activity. This might be explained as a result of possible conformational changes in the molecule due to an unfavorable interaction between the cyano and chloro groups. The additional chloro substituent had little or no effect on the microsomal stability of these compounds, which ranged from moderate to good in all cases. The introduction of the liphophilic chlorine atom had an effect not only on primary activity but also on the hERG interaction. Thus, compounds **44**−**47** showed inhibition values greater than 55% at 3 μM.

To better understand the origin of this hERG liability, additional compounds were further profiled in the patch-clamp assay and relevant physicochemical properties (cLogP and pK_a) were calculated. The results are shown in Table 3. A careful analysis of data showed that there was a relationship between the basicity of the pyridine nitrogen and the percent of inhibition in the patch-clamp test. Thus, the less basic 2- and 3-pyridyloxy derivatives **25** and **26** showed a less pronounced hERG interaction when compared to their regioisomeric 4-pyridyloxy analogue **27**. This effect was more pronounced for **31** where the strong hERG affinity (91%) is consistent with a remarkable increase in basicity when compared to **27**. The same effect was found with the methyl substituted pyridyloxy analogues **34**, **44**, and **46**, where additional methyl groups enhanced the basicity. Thus, the dimethyl derivative **46** showed higher affinity for the hERG channel than its monomethyl analogue **44**. These data also suggest that the overall lipophilicity of the compounds contributes to their interaction with the hERG channel, with the more liphophilic analogues having greater inhibition values.

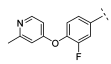
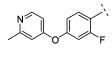
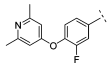
Table 3. Patch-Clamp Data and Calculated Physicochemical Properties of Analogues 25−27, 31, 34, 44, and 46

compd	hERG[a]	pK_a[b]	cLogP
25	33	3.2	4.1
26	30	4.4	4.1
27	52	6	4.1
31	91	7.6	4.3
34	53	6.7	4.2
44	56	5.5	4.7
46	80	5.8	5.2

[a]Experiments were performed using HEK293 cells stably transfected with the hERG potassium channel. Whole-cell currents were recorded with an automated patch-clamp system (PatchXpress 7000A). Three concentrations of compound were tested (0.1, 0.3, and 3 μM; n = 3) and compared to vehicle (0.1% DMSO; n = 3). Data are presented as percent inhibition at the highest concentration tested (3 μM). [b]Calculated with ACD/pKa, version 12.0.

On the basis of these SAR trends, we focused on analogues having an electron withdrawing and less lipophilic fluorine substituent on the phenyl ring in an attempt to modulate the basicity of the distal pyridine ring (pK_a < 6) while keeping the overall lipophilicity in a more optimal range (cLogP ≈ 4). Three selected examples (**48**−**50**) from this exploration are shown in Table 4. In relation to primary activity, compound **48** was 2-fold less potent than its chloro analogue **42**. On the contrary, a 3-fold improvement in activity was observed with **49** over its chloro-substituted equivalent **43**. In this case the smaller fluorine atom may have less interaction with the cyano group and may therefore not affect the conformation of the molecule. The introduction of a second methyl group as in **50** did not result in a potency increase. Compounds **48**−**50** showed good to moderate metabolic stability across human and rat liver microsomes. More interestingly, our hypothesis for hERG interaction was confirmed with analogue **48**, which when compared to compound **34** showed a significant reduction in its hERG inhibition (21% for **48** vs 53% for **34** at 3 μM). This reduction was consistent with the lower basicity of the pyridine (**48** pK_a = 5.9 vs **34** pK_a = 6.7) and equal cLogP for both molecules. The reduction in basicity of the pyridine nitrogen was less pronounced when the fluorine atom was in a position meta to the ether linker, and consequently **49** showed hERG inhibition similar to that of **34** (52% for **49** vs 53% for **34** at 3 μM). Despite the fluorine substituent, the introduction of a second methyl group on the pyridine ring was detrimental for hERG interaction, and compound **50** showed 71% inhibition. In this latter case, the additional methyl increased basicity and lipophilicity, which translated into the stronger hERG inhibition.

Table 4. Functional Activity, Metabolic Stability, Patch-Clamp Data, and Calculated Physicochemical Properties of Analogues 48−50

Compd	Ar	R	mGlu2 EC_{50}[a]	mGlu2 E_{MAX}(%)[a]	HLM[b] (%)	RLM[b] (%)	hERG[c]	pKa[d]	cLogP
48			316	233	33	50	21	5.9	4.2
49			390	232	31	50	52	6.1	4.4
50			372	217	16	41	71	6.6	4.7

[a]Values are the mean of three experiments. Only differences in pIC_{50} up to 0.6 (SD < 0.5) were considered as reproducible and were maintained. [b]HLM and RLM data refer to percent of compound metabolized after 15 min at 5 μM. [c]Experiments were performed using HEK293 cells stably transfected with the hERG potassium channel. Whole-cell currents were recorded with an automated patch-clamp system (PatchXpress 7000A). Three concentrations of compound were tested (0.1, 0.3, and 3 μM; n = 3) and compared to vehicle (0.1% DMSO; n = 3). Data are presented as percent inhibition at the highest concentration tested (3 μM). [d]Calculated with ACD/pKa, version 12.0.

A selected group of representative compounds from the pyridyloxy class, **34**, **36**, and **46**−**48**, were evaluated in brain penetration studies in mice. The results obtained are shown in Table 5. Using compound **34** as a baseline, we observed that

Table 5. Brain Kinetics after a Single Subcutaneous Dose at 10 mg/kg of Compounds 34, 36, and 46−48 in the Swiss Mouse[a]

	brain level ± SEM[b] (ng/g)			
compd	0.5 h	1 h	1.5 h	B/P (1 h)
34	1156 ± 259	994 ± 122	592 ± 115	0.5
36	822[c]	294[c]	100[c]	0.3
46	1480 ± 173	830 ± 482	281 ± 196	0.4
47	750 ± 220	357 ± 15	240 ± 47	0.1
48	1280[c]	1117[c]	1018[c]	0.6

[a]Study in male Swiss mice dosed at 10 mg/kg formulated in 20% HP-β-CD at pH 4. [b]Data are expressed as geometric mean values of at least two runs ± the standard error of the mean (SEM). [c]Only one animal was dosed.

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after subcutaneous administration at 10 mg/kg dose in mice the compound exhibited moderate exposures even 1.5 h postdose. A subtle modification of compound **34** to its 3-pyridine version **36** led to a poorer brain penetration with lower brain concentrations, which rapidly declined. Despite their strong hERG liability, we evaluated the effect of the left-hand side in compounds **46** and **47**, which rendered these compounds most potent, on plasma and brain exposures. Thus, compound **46** showed brain exposures similar to those of compound **36** but only at 1 h after administration, being much lower after 1.5 h. Compound **47**, having a different N-substitution pattern, showed a reduction in *B*/*P* ratios combined with poor exposures. The best results were obtained with compound **48**, suggesting that the introduction of a fluorine atom is beneficial in terms of in vivo PK properties. Exposure of this compound in brain was comparable to that of analogue **34** at the 1 h time point and was 2-fold higher at 1.5 h, likely related to reduced clearance. In summary, despite their excellent in vitro potency, compounds **46** and **47** were precluded from further in vivo evaluation because of low brain exposures. Compound **48** appeared to be the most suitable for further in vivo profiling.

The ability of compound **48** to shift the in vitro concentration response curve (CRC) of glutamate was investigated further. As shown in Figure 3, the CRC of glutamate shifted

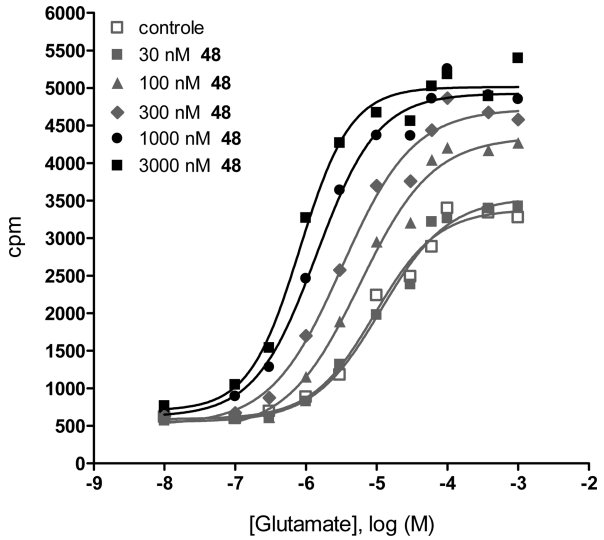

Figure 3. Glutamate CRC in presence of varying concentration of compound **48**, demonstrating an approximate 11-fold decrease in glutamate EC_{50} at 3 μM **48** (experiment performed with CHO cells expressing cloned human mGlu2 receptor).

to the left and upward with increasing concentrations of compound **48**. An ~11-fold shift in the glutamate EC_{50} was observed in the presence of 3 μM **48**. This result is in line with a positive allosteric interaction between glutamate and compound **48** and indicates that **48** as PAM increases both efficacy and potency of glutamate.

Targeting the allosteric site of mGlu receptors is expected to improve the chance of identifying selective ligands. Thus, compound **48** was tested for agonist or antagonist activity on mGlu receptors in fluorescent Ca^{2+} assays using HEK293 cells expressing human mGlu1, mGlu3, and mGlu5 receptors or in [^{35}S]GTPγS assays using L929 cells expressing human mGlu4 or using CHO cells expressing rat mGlu6 receptors. Compound **48** was found to be selective and did not show activity against the other mGlu receptors. In addition, compound **48** was profiled against ~50 targets in a selectivity screen performed at CEREP.[56] Selectivity overall was good, and the only interaction greater than 50% at 10 μM was with the norepinephrine transporter.

Given the high expression of mGlu2 receptor in forebrain regions and the relationship between glutamate neurotransmission and sleep process, we decided to evaluate the acute pharmacological effect of **48** in sw-EEG in rats. The results that we obtained were in accordance with the findings from earlier animal studies in our laboratories, in which modulation of the mGlu2 receptor elicited common changes in a rodent's sleep−wake architecture.[53] Relative to control, acute subcutaneous administration of a dose of 10 mg/kg, **48** exerted significant effect in suppressing REM sleep during the first 2 h without clear effects on the other sleep−wake stages (Figure 4). Remarkably, at this dose of 10 mg/kg REM sleep reduction was accompanied by a significant lengthening of REM sleep onset latency (bottom right panel). Analysis of sw-EEG parameters indicated that reduction in REM sleep time resulted from a reduction in both number of periods and their mean duration. No clear disturbances in total time spent in different vigilance states or sleep variables were found either during or after these effects of the compound, apart from the reduction in intermediate sleep transients, which are directly linked to (the reduction in) REM sleep occurrence. Last, no indications of sleep fragmentation were revealed by examination of total number of transitions from sleep states toward waking.

The effects on sw-EEG architecture in rats demonstrate that compound **48** (10 mg/kg) was centrally active in rats. Remarkably, **48** produced a robust inhibition of REM sleep during the first 2 h associated with an increase in REM sleep onset latency while no subsequent homeostatic changes were observed over the recording session.

CONCLUSION

In summary, the discovery and optimization of a novel series of 1,4-disubstituted 2-pyridones in search of mGlu2 receptor PAMs with in vivo activity are reported. The lead optimization strategy started from the hit compound **13** and was guided by an overlay model built with different mGlu2 receptor PAMs known to date. That exploration led quickly to compounds with remarkably increased potency but poor druglike properties. Further optimization of physicochemical properties of the compounds and subsequent improvement of CV liabilities led to the discovery of compound **48**. This molecule showed good mGlu2 receptor PAM in vitro activity and selectivity, acceptable preliminary safety profile, and good brain exposure. Furthermore, compound **48** displayed robust in vivo activity in a sw-EEG assay consistent with mGlu2 receptor activation, in accordance with previous work from our laboratories.

EXPERIMENTAL SECTION

Chemistry. Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was carried out on silica gel 60 F254 plates (Merck). Flash column chromatography was performed on silica gel, particle size 60 Å, mesh = 230−400 (Merck), under standard techniques. Microwave assisted reactions were performed in a single-mode reactor, Biotage Initiator Sixty microwave reactor (Biotage), or in a multimode reactor, MicroSYNTH Labstation (Milestone, Inc.). Nuclear magnetic resonance (NMR) spectra were recorded with either a Bruker DPX-400 or a Bruker AV-500 spectrometer (broaduker AG) with standard pulse sequences

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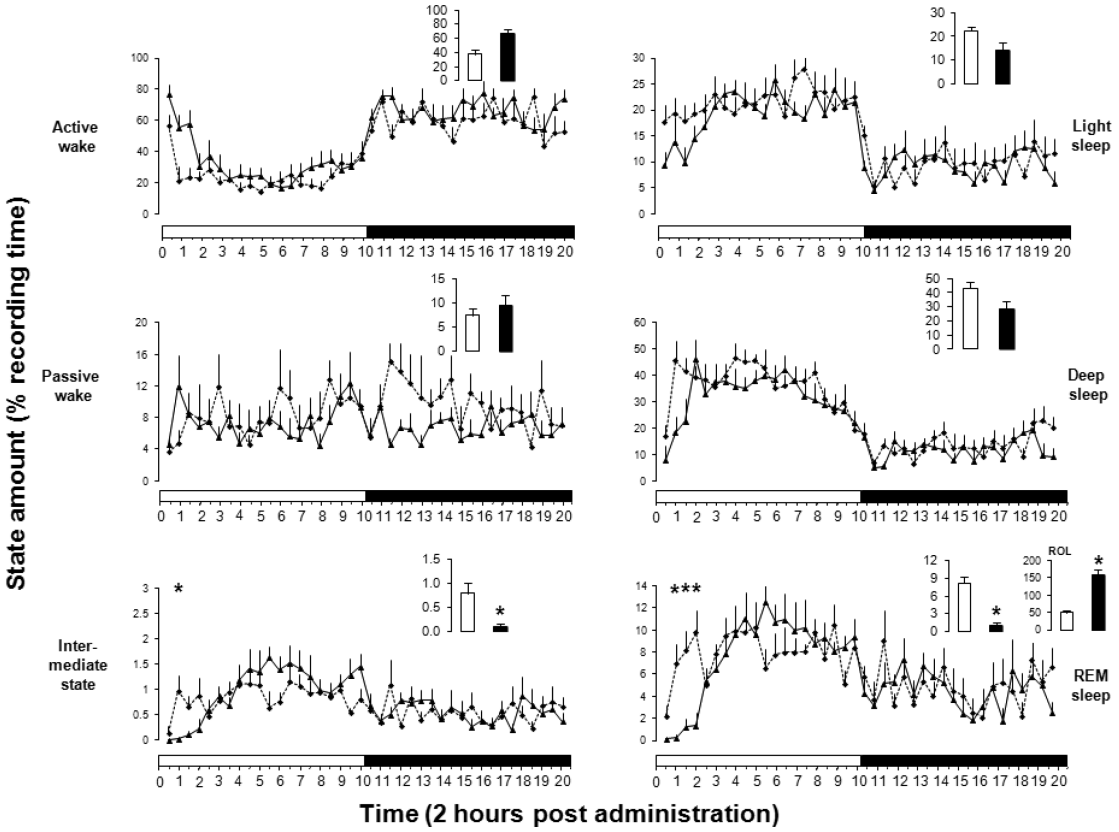

Figure 4. Effects of subcutaneous administration of **48** (10 mg/kg) or vehicle (20% CD + 2H2T) on sleep−wake organization in rats during 20 consecutive hours. Mean percentage of occurrence per 30 min period is indicated for each sleep−wake state. Black bar under the graphs indicates dark period. Small bar charts indicate amounts of vigilance states in min (plus SEM) during the first 2 h postadministration. For REM sleep, the REM sleep onset latency (ROL) is indicated additionally in the small bottom right panel. $N = 8$ for each group. ∗ indicates $p < 0.05$: Wilcoxon−Mann−Whitney rank sum tests compared to vehicle values.

operating at 400 and 500 MHz, respectively, using $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane ($\delta = 0$). Coupling constants are reported in hertz. Splitting patterns are defined by s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), quin (quintet), sex (sextet), sep (septet), or m (multiplet). Liquid chromatography combined with mass spectrometry (LC−MS) was performed either on a HP 1100 HPLC system (Agilent Technologies) or Advanced Chromatography Technologies system composed of a quaternary or binary pump with degasser, an autosampler, a column oven, a diode array detector (DAD), and a column as specified in the respective methods. Flow from the column was split to a MS spectrometer. The MS detector was configured with either an electrospray ionization source or an ES-CI dual ionization source (electrospray combined with atmospheric pressure chemical ionization). Nitrogen was used as the nebulizer gas. Data acquisition was performed with MassLynx-Openlynx software or with Chemsation-Agilent Data Browser software. Detailed information about the different LCMS methods employed can be found in the Supporting Information. Retention time (t_R) is expressed in min. Gas chromatography combined with mass spectrometry (GC−MS) was performed using a 6890 series gas chromatograph (Agilent Technologies) system comprising a 7683 series injector and autosampler, a column oven, and a J&W HP-5MS coupled to a 5973N MSD mass selective detector (single quadrupole, Agilent Technologies). The MS detector was configured with an electronic impact ionization source/chemical ionization source (EI/CI). EI low-resolution mass spectra were acquired by scanning from 50 to 550 at a rate of 14.29 scans/s. The source temperature was maintained at 230 °C. Helium was used as the nebulizer gas. Data acquisition was performed with Chemstation-Open Action software. Melting point (mp) values are peak values and were obtained with experimental uncertainties that are commonly associated with this analytical method and were determined in open capillary tubes on a Mettler FP62 apparatus with a temperature gradient of 10 °C/min. Maximum temperature was 300 °C.

Purities of all new compounds were determined by analytical reverse phase (RP) HPLC using the area percentage method on the UV trace recorded at a wavelength of 254 nm and were found to have ≥95% purity unless otherwise specified.

1-Isopentyl-2-oxo-4-phenylpyridine-3-carbonitrile (13). To a stirred suspension of **51a** (0.12 g, 0.40 mmol) and phenylboronic acid (0.06 g, 0.50 mmol) in a saturated aqueous solution of $NaHCO_3$ (3 mL) and 1,4-dioxane (3 mL) was added $Pd(PPh_3)_4$ (0.046 g, 0.04 mmol). The mixture was heated at 150 °C for 10 min under microwave irradiation. The mixture was cooled to room temperature and filtered through a Celite pad. The filtrate was diluted with water (20 mL) and extracted with EtOAc (2 × 15 mL). The organic layer was washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude was purified by flash column chromatography (silica gel, EtOAc in DCM, 0/100 to 20/80) to give the desired product **13** as a white solid (0.06 g, 51%), mp 140.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.64−1.75 (m, 3H), 3.98−4.07 (m, 2H), 6.34 (d, J = 7.0 Hz, 1H), 7.48−7.53 (m, 3H), 7.53 (d, J = 7.0 Hz, 1H), 7.58−7.65 (m, 2H). LC−MS m/z 267 $[M + H]^+$, t_R = 4.25 min.

1-Isopentyl-4-(4-methoxyphenyl)-2-oxopyridine-3-carbonitrile (14). Starting from **51a** (0.06 g, 0.20 mmol) and 4-methoxyphenylboronic acid (0.03 g, 0.22 mmol) and following the procedure described for **13**, compound **14** was obtained as a light yellow solid (0.031 g, 43%), mp 99 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (d, J = 6.3 Hz, 6H), 1.50−1.60 (m, 3H), 3.81 (s, 3H), 3.90−4.00 (m, 2H), 6.26 (d, J = 7.1 Hz, 1H), 6.95 (d, J = 8.6 Hz, 2H), 7.40 (d, J = 7.1 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H). LC−MS m/z 297 $[M + H]^+$, t_R = 4.07 min.

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1-Isopentyl-4-(3-methoxyphenyl)-2-oxopyridine-3-carbonitrile (15). Starting from **51a** (0.06 g, 0.20 mmol) and 3-methoxyphenylboronic acid (0.03 g, 0.22 mmol) and following the procedure described for **13**, compound **15** was obtained as a white solid (0.025 g, 35%), mp 96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (d, J = 6.0 Hz, 6H), 1.50−1.60 (m, 3H), 3.80 (s, 3H), 3.90−4.00 (m, 2H), 6.29 (d, J = 7.1 Hz, 1H), 7.00 (dd, J = 8.3, 2.6 Hz, 1H), 7.05−7.15 (m, 2H), 7.35 (dd, J = 8.0, 8.0 Hz, 1H), 7.40 (d, J = 7.1 Hz, 1H). LC−MS m/z 297 $[M + H]^+$, t_R = 4.31 min.

1-Isopentyl-4-(2-methoxyphenyl)-2-oxopyridine-3-carbonitrile (16). Starting from **51a** (0.06 g, 0.20 mmol) and 2-methoxyphenylphenylboronic acid (0.03 g, 0.22 mmol) and following the procedure described for **13**, compound **16** was obtained as a yellow oil (0.025 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (d, J = 6.2 Hz, 6H), 1.64−1.74 (m, 3H), 3.87 (s, 3H), 3.98−4.04 (m, 2H), 6.31 (d, J = 6.8 Hz, 1H), 7.02 (broad d, J = 8.5 Hz, 1H), 7.06 (broad t, J = 7.5 Hz, 1H), 7.31 (dd, J = 7.6, 1.8 Hz, 1H), 7.42−7.47 (m, 1H), 7.46 (d, J = 7.0 Hz, 1H). LC−MS m/z 297 $[M + H]^+$, t_R = 4.11 min.

1-Isopentyl-4-(3-fluoro-4-methoxyphenyl)-2-oxopyridine-3-carbonitrile (17). Starting from **51a** (0.1 g, 0.33 mmol) and 3-fluoro-4-methoxyphenylboronic acid (0.06 g, 0.36 mmol) and following the procedure described for **13**, compound **17** was obtained as a yellow solid (0.05 g, 70%), mp 133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.63−1.72 (m, 3H), 3.96 (s, 3H), 3.98−4.04 (m, 2H), 6.29 (d, J = 7.0 Hz, 1H), 7.08 (t, J = 8.5 Hz, 1H), 7.34 (dd, J = 11.8, 2.3 Hz, 1H), 7.46−7.50 (m, 1H), 7.50 (d, J = 7.0 Hz, 1H). LC−MS m/z 315 $[M + H]^+$, t_R = 4.30 min.

1-Isopentyl-4-(3-chloro-4-methoxyphenyl)-2-oxopyridine-3-carbonitrile (18). Starting from **51a** (0.4 g, 1.48 mmol) and 3-chloro-4-methoxyphenylboronic acid (0.28 g, 1.48 mmol) and following the procedure described for **13**, compound **18** was obtained as a white solid (0.46 g, 95%), mp 149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (d, J = 6.2 Hz, 6H), 1.49−1.64 (m, 3H), 3.95 (s, 3H), 3.96−4.02 (m, 2H), 6.56 (d, J = 7.0 Hz, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.67 (dd, J = 8.7, 2.3 Hz, 1H), 7.77 (d, J = 2.3 Hz, 1H), 8.15 (d, J = 7.0 Hz, 1H). LC−MS m/z 331 $[M + H]^+$, t_R = 4.33 min.

1-Isopentyl-4-(3,4-dimethoxyphenyl)-2-oxopyridine-3-carbonitrile (19). Starting from **51a** (0.3 g, 1.11 mmol) and 3,4-dimethoxyphenylboronic acid (0.22 g, 1.22 mmol) and following the procedure described for **13**, compound **19** was obtained as a yellow solid (0.32 g, 65%), mp 52 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.62−1.74 (m, 3H), 3.95 (s, 3H), 3.95 (s, 3H), 3.98−4.04 (m, 2H), 6.35 (d, J = 7.0 Hz, 1H), 7.19−7.26 (m, 3H), 7.48 (d, J = 7.3 Hz, 1H). LC−MS m/z 327 $[M + H]^+$, t_R = 4.03 min.

1-Isopentyl-4-[4-(cyclopropylmethoxy)phenyl]-2-oxopyridine-3-carbonitrile (20). Starting from **51a** (0.11 g, 0.4 mmol) and 2-(4-cyclopropylmethoxyphenyl)-4,4,5,5-tetramethyl[1,3,2]-dioxaborolane (0.31 g,1.62 mmol) and following the procedure described for **13**, compound **20** was obtained as a white solid (0.22 g, 64%), mp 114.1 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.32−0.43 (m, 2H), 0.61−0.73 (m, 2H), 0.99 (d, J = 6.4 Hz, 6H), 1.24−1.34 (m, 1H), 1.63−1.71 (m, 3H), 3.86 (d, J = 6.9 Hz, 2H), 3.97−4.03 (m, 2H), 6.32 (d, J = 6.9 Hz, 1H), 6.98−7.03 (m, 2H), 7.47 (d, J = 7.2 Hz, 1H), 7.57−7.63 (m, 2H). LC−MS m/z 337 $[M + H]^+$, t_R = 4.72 min.

1-Isopentyl-4-[3-chloro-4-(cyclopropylmethoxy)phenyl]-2-oxopyridine-3-carbonitrile (21). Starting from **51a** (0.11 g, 0.4 mmol) and 3-chloro-4-cyclopropyloxyphenyl-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane (0.11 g, 0.44 mmol) and following the procedure described for **13**, compound **21** was obtained as a white solid (0.88 g, 64%), mp 294 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.36−0.48 (m, 2H), 0.63−0.75 (m, 2H), 0.99 (d, J = 6.4 Hz, 6H), 1.29−1.40 (m, 1H), 1.62−1.72 (m, 3H), 3.96 (d, J = 6.6 Hz, 2H), 3.98−4.04 (m, 2H), 6.30 (d, J = 7.0 Hz, 1H), 7.01 (d, J = 9.3 Hz, 1H), 7.49 (d, J = 7.0 Hz, 1H), 7.57−7.61 (m, 2H). LC−MS m/z 371 $[M + H]^+$, t_R = 5.40 min.

1-Isopentyl-4-[3-chloro-4-(cyclopentoxy)phenyl]-2-oxopyridine-3-carbonitrile (22). Starting from **51a** (0.11 g, 0.4 mmol) and 3-chloro-4-cyclopenyloxy)phenyl)-4,4,5,5-tetramethyl[1,3,2]-dioxaborolane (0.24 g, 0.7 mmol) and following the procedure described for **13**, compound **22** was obtained as a pale yellow solid (0.53 g, 37%), mp 155.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (d, J = 6.1 Hz, 6H), 1.61−1.72 (m, 5H), 1.81−1.90 (m, 2H), 1.90−1.99 (m, 4H), 3.96−4.04 (m, 2H), 4.88 (quin, J = 4.1 Hz, 1H), 6.30 (d, J = 7.2 Hz, 1H), 7.02 (d, J = 8.1 Hz, 1H), 7.49 (d, J = 6.9 Hz, 1H), 7.55−7.63 (m, 2H). LC−MS m/z 285 $[M + H]^+$, t_R = 5.88 min.

1-Isopentyl-4-(3-chloro-4-tetrahydropyran-4-yloxyphenyl)-2-oxopyridine-3-carbonitrile (23). Starting from **51a** (0.65 g, 2.42 mmol) and 2-(3-chloro-4-tetrahydropyran-4-yloxyphenyl)-4,4,5,5-tetramethyl[1,3,2]dioxaborolane **53a** (0.9 g, 2.66 mmol) and following the procedure described for **13**, compound **23** was obtained as a white solid (0.175 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (d, J = 6.4 Hz, 6H), 1.63−1.75 (m, 3H), 1.86−1.97 (m, 2H), 2.03−2.13 (m, 2H), 3.66 (ddd, J = 11.4, 7.4, 3.6 Hz, 2H), 3.97−4.10 (m, 4H), 4.64−4.72 (m, 1H), 6.32 (d, J = 7.0 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 7.0 Hz, 1H), 7.59−7.64 (m, 2H). LC−MS m/z 401 $[M + H]^+$, t_R = 4.58 min.

1-Isopentyl 4-(4-phenyloxyphenyl)-2-oxopyridine-3-carbonitrile (24). Starting from **51a** (0.15 g, 0.57 mmol) and 4-phenoxyphenylboronic acid (0.12 g, 0.57 mmol) and following the procedure described for **13**, compound **24** was obtained as a white solid (0.1 g, 50%), mp 151.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (d, J = 6.2 Hz, 6H), 1.50−1.65 (m, 3H), 3.91−4.06 (m, 2H), 6.53 (d, J = 7.0 Hz, 1H), 7.09−7.17 (m, 4H), 7.22−7.27 (m, 1H), 7.43−7.51 (m, 2H), 7.66−7.73 (m, 2H), 8.15 (d, J = 7.0 Hz, 1H). LC−MS m/z 359 $[M + H]^+$, t_R = 5.13 min.

1-Isopentyl-4-[4-(pyridin-2-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (25). Starting from **51a** (0.11 g, 0.4 mmol) and commercially available 2-[-4-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenoxy]pyridine (0.25 g, 0.8 mmol) and following the procedure described for **13**, compound **25** was obtained as a white solid (0.084 g, 56%), mp 155.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (d, J = 6.2 Hz, 6H), 1.53−1.65 (m, 3H), 3.97−4.04 (m, 2H), 6.58 (d, J = 7.0 Hz, 1H), 7.15 (broad d, J = 8.1 Hz, 1H), 7.20 (ddd, J = 7.3, 5.0, 0.8 Hz, 1H), 7.28−7.33 (m, 2H), 7.70−7.75 (m, 2H), 7.92 (ddd, J = 8.3, 7.0, 2.1 Hz, 1H), 8.17 (d, J = 7.0 Hz, 1H), 8.20 (ddd, J = 4.8, 2.1, 0.7 Hz, 1H). LC−MS m/z 360 $[M + H]^+$, t_R = 4.23 min.

1-Isopentyl-4-[4-(pyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (26). Starting from **51a** (0.48 g, 1.79 mmol) and **53c** (0.8 g, 2.69 mmol) and following the procedure described for **13**, compound **26** was obtained as a cream solid (0.42 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, J = 6.2 Hz, 6H), 1.55−1.68 (m, 3H), 3.92−3.98 (m, 2H), 6.26 (d, J = 7.0 Hz, 1H), 7.05 (broad d, J = 8.7 Hz, 2H), 7.28−7.35 (m, 1H), 7.35−7.41 (m, 1H), 7.46 (d, J = 7.0 Hz, 1H), 7.58 (broad d, J = 8.7 Hz, 2H), 8.32−8.48 (m, 2H). LC−MS m/z 360 $[M + H]^+$, t_R = 4.07 min.

1-Isopentyl-4-(4-pyrid-4-yloxyphenyl)-2-oxopyridine-3-carbonitrile (27). Starting from **51a** (0.72 g, 2.7 mmol) and **53h** (0.95 g, 3.2 mmol) and following the procedure described for **13**, compound **27** was obtained as a white solid (0.40 g, 41%), mp 139 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.95 (d, J = 6.4 Hz, 6H), 1.53−1.65 (m, 3H), 3.97−4.05 (m, 2H), 6.58 (d, J = 6.9 Hz, 1H), 7.01−7.07 (m, 2H), 7.34−7.39 (m, 2H), 7.76−7.82 (m, 2H), 8.19 (d, J = 6.9 Hz, 1H), 8.49−8.58 (m, 2H). LC−MS m/z 360 $[M + H]^+$, t_R = 4.09 min.

1-Isopentyl-4-(4-methylaminophenyl)-2-oxopyridine-3-carbonitrile (28). Starting from **51a** (0.116 g, 0.4 mmol) and 4-methylaminophenyl-4,4,5,5-tetramethyl[1,3,2]dioxaborolane (0.16 g, 0.5 mmol) and following the procedure described for **13**, compound **27** was obtained as a bright yellow solid (0.04 g, 31%), mp 189 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (d, J = 6.2 Hz, 6H), 1.49−1.63 (m, 3H), 2.75 (d, J = 5.0 Hz, 3H), 3.89−3.98 (m, 2H), 6.43−6.50 (m, 1H), 6.48 (d, J = 7.0 Hz, 1H), 6.65 (broad d, J = 8.9 Hz, 2H), 7.52 (broad d, J = 8.7 Hz, 2H), 7.99 (d, J = 7.3 Hz, 1H). LC−MS m/z 296 $[M + H]^+$, t_R = 3.97 min.

1-Isopentyl-4-[3-chloro-4-(tetrahydropyran-4-ylamino)-phenyl]-2-oxopyridine-3-carbonitrile (29). Starting from **51a** (1.3 g, 4.94 mmol) and **53b** (2 g, 5.93 mmol) and following the procedure described for **13**, compound **29** was obtained as a pale yellow solid (0.196 g, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.51−1.72 (m, 5H), 2.02−2.11 (m, 2H), 3.55 (td, J = 11.5, 2.3 Hz, 2H), 3.58−3.68 (m, 1H), 3.95−4.07 (m, 4H), 4.63 (broad d, J = 7.7 Hz, 1H), 6.29 (d, J = 7.0 Hz, 1H), 6.76 (broad d, J = 9.1 Hz, 1H), 7.44 (d, J = 7.0 Hz, 1H), 7.53−7.61 (m, 2H). LC−MS m/z 400 $[M + H]^+$, t_R = 4.48 min.

1-Isopentyl-4-(4-morpholinophenyl)-2-oxopyridine-3-carbonitrile (30). Starting from **51a** (0.56 g, 2.70 mmol) and 4-(morpholin-1-yl)phenylboronic acid (0.72 g, 2.7 mmol) and following the procedure described for **13**, compound **30** was obtained as a yellow solid (0.62 g, 65%), mp 171 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.62−1.73 (m, 3H), 3.24−3.31 (m, 4H), 3.84−3.90 (m, 4H), 3.95−4.03 (m, 2H), 6.33 (d, J = 6.9 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 7.2 Hz, 1H), 7.62 (d, J = 9.0 Hz, 2H). LC−MS m/z 352 $[M + H]^+$, t_R = 4.09 min.

1-Isopentyl-4-[4-(2-methylpyridin-4-ylamino)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (31). Starting from **51a** (0.54 g, 1.99 mmol) and 4-(4-pyridylamino)phenylboronic acid (0.51 g, 2.36 mmol) and following the procedure described for **13**, compound **29** was obtained as a pale yellow solid (0.25 g, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (d, J = 6.2 Hz, 6H), 1.51−1.64 (m, 3H), 3.93−4.03 (m, 2H), 6.54 (d, J = 7.0 Hz, 1H), 7.03−7.07 (m, 2H), 7.32−7.37 (m, 2H), 7.65−7.70 (m, 2H), 8.12 (d, J = 7.0 Hz, 1H), 8.26−8.31 (m, 2H), 9.21 (s, 1H). LC−MS m/z 359 $[M + H]^+$, t_R = 3.35 min.

1-Isopentyl-4-[4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (32). Starting from **51a** (0.74 g, 2.84 mmol) and **53i** (0.88 g, 2.84 mmol) and following the procedure described for **13**, compound **32** was obtained as a white solid (0.35 g, 36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (d, J = 6.1 Hz, 6H), 1.57−1.68 (m, 3H), 2.51 (s, 3H), 3.93−4.00 (m, 2H), 6.27 (d, J = 6.9 Hz, 1H), 6.70−6.76 (m, 2H), 7.14 (broad d, J = 8.7 Hz, 2H), 7.47 (d, J = 6.9 Hz, 1H), 7.63 (broad d, J = 8.7 Hz, 2H), 8.35 (d, J = 5.8 Hz, 1H). LC−MS m/z 374 $[M + H]^+$, t_R = 4.27 min.

1-Cyclobutylmethyl-4-[4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (33). Starting from **51d** (0.56 g, 2.1 mmol) and **53i** (0.65 g, 2.1 mmol) and following the procedure described for **13**, compound **33** was obtained as a white solid (0.48 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.71−1.81 (m, 2H), 1.81−1.95 (m, 2H), 2.00−2.09 (m, 2H), 2.55 (s, 3H), 2.78 (sep, J = 7.8 Hz, 1H), 3.98 (d, J = 7.5 Hz, 2H), 6.25 (d, J = 6.9 Hz, 1H), 6.75−6.79 (m, 2H), 7.15 (broad d, J = 8.7 Hz, 2H), 7.45 (d, J = 6.9 Hz, 1H), 7.64 (broad d, J = 8.7 Hz, 2H), 8.34−8.38 (m, 1H). LC−MS m/z 372 $[M + H]^+$, t_R = 4.07 min.

1-Butyl-4-[4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (34). Starting from **51b** (1.44 g, 5.68 mmol) and **53i** (1.76 g, 5.68 mmol) and following the procedure described for **13**, compound **34** was obtained as a white solid (0.7 g, 36%), mp 121 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 3H), 1.31−1.39 (m, 2H), 1.69−1.77 (m, 2H), 2.52 (s, 3H), 3.95 (t, J = 7.5 Hz, 2H), 6.27 (d, J = 6.9 Hz, 1H), 6.71−6.77 (m, 2H), 7.15 (broad d, J = 8.7 Hz, 2H), 7.47 (d, J = 6.9 Hz, 1H), 7.63 (broad d, J = 8.7 Hz, 2H), 8.35 (d, J = 5.8 Hz, 1H). LC−MS m/z 360 $[M + H]^+$, t_R = 3.97 min.

1-Cyclopropylmethyl-4-[4-(2-methylpyridin-4-yloxy)-phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (35). Starting from **51c** (0.48 g, 1.91 mmol) and **53i** (1.05 g, 3.39 mmol) and following the procedure described for **13**, compound **35** was obtained as a white solid (0.33 g, 48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.40−0.47 (m, 2H), 0.49−0.58 (m, 2H), 1.21−1.34 (m, 1H), 2.45 (s, 3H), 3.86 (d, J = 7.2 Hz, 2H), 6.59 (d, J = 6.9 Hz, 1H), 6.84 (dd, J = 5.8, 2.6 Hz, 1H), 6.93 (d, J = 1.7 Hz, 1H), 7.34 (broad d, J = 8.7 Hz, 2H), 7.79 (broad d, J = 8.7 Hz, 2H), 8.22 (d, J = 7.2 Hz, 1H), 8.39 (d, J = 5.5 Hz, 1H). LC−MS m/z 358 $[M + H]^+$, t_R = 3.7 min.

1-Butyl-4-[4-(2-methylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (36). Starting from **51b** (0.61 g, 2.41 mmol) and **53d** (0.9 g, 2.89 mmol) and following the procedure described for **13**, compound **36** was obtained as a white solid (0.51 g, 60%), mp 136.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 3H), 1.36−1.46 (m, 2H), 1.75−1.83 (m, 2H), 2.50 (s, 3H), 4.00 (t, J = 7.5 Hz, 2H), 6.31 (d, J = 7.2 Hz, 1H), 6.98−7.03 (m, 2H), 7.18 (dd, J = 8.1, 4.9 Hz, 1H), 7.30 (dd, J = 8.1, 0.9 Hz, 1H), 7.49 (d, J = 7.2 Hz, 1H), 7.59−7.64 (m, 2H), 8.39 (dd, J = 4.6, 1.2 Hz, 1H). LC−MS m/z 360 $[M + H]^+$, t_R = 3.96 min.

1-Isopentyl-4-[4-(2-methylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (37). Starting from **51a** (0.64 g, 2.41 mmol) and **53d** (0.9 g, 2.89 mmol) and following the procedure described for **13**, compound **37** was obtained as a white solid (0.69 g, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.63−1.73 (m, 3H), 2.50 (s, 3H), 3.98−4.05 (m, 2H), 6.31 (d, J = 7.2 Hz, 1H), 6.98−7.03 (m, 2H), 7.19 (dd, J = 8.1, 4.6 Hz, 1H), 7.31 (dd, J = 8.1, 1.2 Hz, 1H), 7.50 (d, J = 6.9 Hz, 1H), 7.59−7.64 (m, 2H), 8.39 (dd, J = 4.8, 1.3 Hz, 1H). LC−MS m/z 374 $[M + H]^+$, t_R = 4.12 min.

1-Cyclopropylmehtyl-4-[4-(2-methylpyridin-3-yloxy)-phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (38). Starting from **51c** (0.39 g, 1.53 mmol) and **57d** (0.57 g, 1.83 mmol) and following the procedure described for **13**, compound **38** was obtained as a yellow solid (0.39 g, 72%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.38−0.46 (m, 2H), 0.48−0.56 (m, 2H), 1.20−1.32 (m, 1H), 2.39 (s, 3H), 3.83 (d, J = 7.2 Hz, 2H), 6.54 (d, J = 6.9 Hz, 1H), 7.08 (broad d, J = 9.0 Hz, 2H), 7.34 (dd, J = 8.1, 4.9 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.71 (broad d, J = 9.0 Hz, 2H), 8.18 (d, J = 7.2 Hz, 1H), 8.37 (dd, J = 4.6, 1.1 Hz, 1H). LC−MS m/z 358 $[M + H]^+$, t_R = 3.67 min.

1-Butyl-4-[4-(2,6-dimethylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (39). Starting from **51b** (0.59 g, 2.33 mmol) and **53e** (0.91 g, 2.80 mmol) and following the procedure described for **13**, compound **39** was obtained as a pale yellow solid (0.57 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 3H), 1.35−1.46 (m, 2H), 1.74−1.83 (m, 2H), 2.46 (s, 3H), 2.57 (s, 3H), 4.00 (t, J = 7.4 Hz, 2H), 6.32 (d, J = 6.9 Hz, 1H), 6.95−7.01 (m, 2H), 7.05 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 6.9 Hz, 1H), 7.57−7.63 (m, 2H). LC−MS m/z 374 $[M + H]^+$, t_R = 4.24 min.

1-Isopentyl-4-[4-(2,6-dimethylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (40). Starting from **51a** (0.63 g, 2.33 mmol) and **53e** (0.91 g, 2.80 mmol) and following the procedure described for **13**, compound **40** was obtained as a pale yellow solid(0.57 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.4 Hz, 6H), 1.61−1.74 (m, 3H), 2.45 (s, 3H), 2.56 (s, 3H), 3.96−4.06 (m, 2H), 6.32 (d, J = 7.0 Hz, 1H), 6.94−7.01 (m, 2H), 7.05 (d, J = 8.3 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.51 (d, J = 7.0 Hz, 1H), 7.57−7.63 (m, 2H). LC−MS m/z 388 $[M + H]^+$, t_R = 4.54 min.

1-Cyclopropylmethyl-4-[4-(2,6-dimethylpyridin-3-yloxy)-phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (41). Starting from **51c** (0.39 g, 1.53 mmol) and **53e** (0.6 g, 1.84 mmol) and following the procedure described for **13**, compound **41** was obtained as a white solid (0.18 g, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.38−0.50 (m, 2H), 0.61−0.76 (m, 2H), 1.28 (m, 1H), 2.45 (s, 3H), 2.56 (s, 3H), 3.87 (d, J = 7.3 Hz, 2H), 6.29−6.37 (m, 1H), 6.95−7.01 (m, 2H), 7.04 (d, J = 8.3 Hz, 1H), 7.22 (d, J = 8.3 Hz, 1H), 7.58−7.66 (m, 3H). LC−MS m/z 372 $[M + H]^+$, t_R = 4.0 min.

1-Butyl-4-[3-chloro-4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (42). Starting from **51b** (0.39 g, 1.53 mmol) and **53l** (0.57 g, 1.83 mmol) and following the procedure described for **13**, compound **42** was obtained as a white solid (0.38 g, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.37−1.46 (m, 2H), 1.76−1.84 (m, 2H), 2.54 (s, 3H), 4.03 (t, J = 7.4 Hz, 2H), 6.33 (d, J = 6.9 Hz, 1H), 6.69 (dd, J = 5.8, 2.6 Hz, 1H), 6.73 (d, J = 2.6 Hz, 1H), 7.25 (d, J = 8.7 Hz, 1H), 7.57 (d, J = 6.9 Hz, 1H), 7.62 (dd, J = 8.4, 2.3 Hz, 1H), 7.73 (d, J = 2.3 Hz, 1H), 8.41 (d, J = 5.8 Hz, 1H). LC−MS m/z 394 $[M + H]^+$, t_R = 4.25 min.

1-Butyl-4-[2-chloro-4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (43). Starting from **51b** (0.3 g, 1.17 mmol) and **53j** (0.41 g, 1.2 mmol) and following the procedure described for **13**, compound **43** was obtained as a white solid (0.15 g, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.00 (t, J = 7.4 Hz, 3H), 1.43 (broad sex, J = 7.5 Hz, 2H), 1.82 (broad quin, J = 7.6 Hz, 2H), 2.56 (s, 3H), 4.04 (t, J = 7.5 Hz, 2H), 6.30 (d, J = 6.9 Hz, 1H), 6.76 (dd, J = 5.8, 2.6 Hz, 1H), 6.81 (d, J = 2.6 Hz, 1H), 7.10 (dd, J = 8.4, 2.6 Hz, 1H), 7.24 (d, J = 2.6 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 6.9 Hz, 1H), 8.44 (d, J = 5.5 Hz, 1H). LC−MS m/z 394 $[M + H]^+$, t_R = 4.25 min.

1-Butyl-4-[3-chloro-4-(2-methylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (44). Starting from **51b** (0.51 g, 2 mmol) and **53f** (0.90 g, 2.6 mmol) and following the procedure described for **13**, compound **44** was obtained as a pale yellow solid (0.54 g, 69%), mp 137.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (t, J = 7.4 Hz, 3H), 1.39−1.47 (m, 2H), 1.77−1.85 (m, 2H),

2.57 (s, 3H), 4.04 (t, J = 7.4 Hz, 2H), 6.33 (d, J = 6.9 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 7.19−7.26 (m, 2H), 7.52−7.58 (m, 2H), 7.74 (d, J = 2.3 Hz, 1H), 8.41 (dd, J = 4.5, 1.3 Hz, 1H). LC−MS m/z 394 $[M + H]^+$, t_R = 4.27 min.

1-Cyclopropylmethyl-4-[3-chloro-4-(2-methylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (45). Starting from **51c** (0.6 g, 2.37 mmol) and **53f** (0.69 g, 2 mol) and following the procedure described for **13**, compound **45** (0.4 g, 52%) was obtained as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.38−0.47 (m, 2H), 0.48−0.56 (m, 2H), 1.21−1.32 (m, 1H), 2.43 (s, 3H), 3.84 (d, J = 7.2 Hz, 2H), 6.59 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 8.7 Hz, 1H), 7.33 (dd, J = 8.1, 4.9 Hz, 1H), 7.41 (broad d, J = 8.4 Hz, 1H), 7.63 (dd, J = 8.5, 2.2 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 8.21 (d, J = 6.9 Hz, 1H), 8.35−8.38 (m, 1H). LC−MS m/z 392 $[M + H]^+$, t_R = 4.06 min.

1-Butyl-4-[3-chloro-4-(2,6-dimethylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (46). Starting from **51b** (0.51 g, 2 mmol) and **53g** (0.79 g, 2.2 mmol) and following the procedure described for **13**, compound **46** was obtained as a white solid (0.36 g, 44%), mp 268.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (t, J = 7.4 Hz, 3H), 1.37−1.48 (m, 2H), 1.76−1.85 (m, 2H), 2.50 (s, 3H), 2.59 (s, 3H), 4.03 (t, J = 7.4 Hz, 2H), 6.32 (d, J = 7.0 Hz, 1H), 6.80 (d, J = 8.5 Hz, 1H), 7.06 (d, J = 8.3 Hz, 1H), 7.19 (d, J = 8.3 Hz, 1H), 7.51 (dd, J = 8.5, 2.3 Hz, 1H), 7.55 (d, J = 7.0 Hz, 1H), 7.72 (d, J = 2.3 Hz, 1H). LC−MS m/z 408 $[M + H]^+$, t_R = 4.58 min.

1-Cyclopropylmehtyl-4-[3-chloro-4-(2,6-dimethylpyridin-3-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (47). Starting from **51c** (0.51 g, 2 mmol) and **53g** (0.79 g, 2.2 mmol) and following the procedure described for **13**, compound **47** was obtained as a white solid (0.49 g, 60%), mp 201 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.41−0.52 (m, 2H), 0.66−0.77 (m, 2H), 1.25−1.36 (m, 1H), 2.50 (s, 3H), 2.59 (s, 3H), 3.90 (d, J = 7.2 Hz, 2H), 6.34 (d, J = 6.9 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 8.5, 2.5 Hz, 1H), 7.70 (d, J = 7.2 Hz, 1H), 7.73 (d, J = 2.3 Hz, 1H). LC−MS m/z 406 $[M + H]^+$, t_R = 4.34 min.

1-Butyl-4-[3-fluoro-4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (48). Starting from **51b** (0.45 g, 1.77 mmol) and **53m** (0.58 g, 1.77 mmol) and following the procedure described for **13**, compound **48** was obtained as a white solid (0.45 g, 68%), mp 125.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.42 (broad sex, J = 7.5 Hz, 2H), 1.77−1.85 (m, 2H), 2.54 (s, 3H), 4.03 (t, J = 7.4 Hz, 2H), 6.32 (d, J = 6.9 Hz, 1H), 6.72 (dd, J = 5.8, 2.3 Hz, 1H), 6.76 (d, J = 2.6 Hz, 1H), 7.29 (t, J = 8.2 Hz, 1H), 7.46−7.52 (m, 2H), 7.56 (d, J = 6.9 Hz, 1H), 8.41 (d, J = 5.8 Hz, 1H). LC−MS m/z 378 $[M + H]^+$, t_R = 3.94 min.

1-Butyl-4-[2-fluoro-4-(2-methylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (49). Starting from **51b** (0.45 g, 1.77 mmol) and **53n** (0.58 g, 1.77 mmol) and following the procedure described for **13**, compound **49** was obtained as a white solid (0.45 g, 68%), mp 177.1 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.42 (sex, J = 7.5 Hz, 2H), 1.77−1.84 (m, 2H), 2.56 (s, 3H), 4.03 (t, J = 7.4 Hz, 2H), 6.34 (dd, J = 6.9, 1.7 Hz, 1H), 6.79 (dd, J = 5.6, 2.2 Hz, 1H), 6.83 (d, J = 2.0 Hz, 1H), 6.94 (dd, J = 10.8, 2.2 Hz, 1H), 6.99 (dd, J = 8.5, 1.9 Hz, 1H), 7.51−7.58 (m, 2H), 8.45 (d, J = 5.5 Hz, 1H). LC−MS m/z 378 $[M + H]^+$, t_R = 4.02 min.

1-Butyl-4-[3-fluoro-4-(2,6-dimethylpyridin-4-yloxy)phenyl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (50). Starting from **51b** (0.25 g, 0.98 mmol) and **53k** (0.37 g, 1.08 mmol) and following the procedure described for **13**, compound **50** was obtained as a white solid (0.22 g, 59%), mp 204 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 3H), 1.42 (dq, J = 15.2, 7.4 Hz, 2H), 1.76−1.84 (m, 2H), 2.50 (s, 6H), 4.03 (t, J = 7.4 Hz, 2H), 6.32 (d, J = 6.9 Hz, 1H), 6.58 (s, 2H), 7.27 (t, J = 8.4 Hz, 1H), 7.45−7.51 (m, 2H), 7.56 (d, J = 6.9 Hz, 1H). LC−MS m/z 392 $[M + H]^+$, t_R = 4.23 min.

4-Bromo-1-butyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (51b). $POBr_3$ (13.69 g, 47.7 mmol) was added portionwise to a solution of **57b** (4.59 g, 23.87 mmol) in DMF (96 mL). The mixture was stirred at 110 °C for 3 h. The mixture was cooled to 0 °C, carefully treated with water, and extracted with EtOAc. The organic layer was separated, dried (Na_2SO_4), and filtered, and the solvents were evaporated in vacuo. The crude product was purified by flash column chromatography (silica gel, DCM). The desired fractions were collected and concentrated in vacuo. The solid residue was washed with diethyl ether to yield **51b** as a white solid (3.96 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (t, J = 7.37 Hz, 3H), 1.32−1.42 (m, 2H), 1.69−1.78 (m, 2H), 3.95 (t, J = 7.37 Hz, 2H), 6.50 (d, J = 7.22 Hz, 1H), 7.37 (d, J = 7.22 Hz, 1H). LC−MS m/z 257 $[M + H]^+$.

Compounds **51a,c,d** were prepared following the same synthesis procedure as **51b**.

4-Bromo-1-isopentyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (51a). Starting from **57a** (3 g, 14.5 mmol) and following the procedure described for **51b**, compound **51a** was obtained as a white solid (3.55 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (d, J = 6.2 Hz, 6H), 1.56−1.68 (m, 3H), 3.92−4.00 (m, 2H), 6.51 (d, J = 7.2 Hz, 1H), 7.39 (d, J = 7.4 Hz, 1H). LC−MS m/z 269 $[M + H]^+$.

4-Bromo-1-cyclopropylmethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (51c). Starting from **57c** (4.68 g, 24.6 mmol) and following the procedure described for **51b**, compound **51c** was obtained as a white solid (4.09 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.36−0.42 (m, 2H), 0.45−0.55 (m, 2H), 1.15−1.28 (m, 1H), 3.78 (d, J = 7.26 Hz, 2H), 6.82 (d, J = 7.26 Hz, 1H), 8.12 (d, J = 7.26 Hz, 1H). LC−MS m/z 252 $[M + H]^+$.

4-Bromo-1-cyclobutylmethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (51d). Starting from **57d** (6.17 g, 30.2 mmol) and following the procedure described for **51b**, compound **51d** was obtained as a white solid (5.41 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.72−1.84 (m, 2H), 1.85−2.01 (m, 2H), 2.02−2.13 (m, 2H), 2.77 (sep, J = 7.7 Hz, 1H), 3.97 (d, J = 7.4 Hz, 2H), 6.49 (d, J = 7.4 Hz, 1H), 7.36 (d, J = 7.2 Hz, 1H). LC−MS m/z 267 $[M + H]^+$.

2-(3-Chloro-4-tetrahydropyran-4-yloxyphenyl)-4,4,5,5-tetramethyl[1,3,2]dioxaborolane (53a). To a suspension of **60** (1.8 g, 6.17 mmol), bis(pinacolato)diboron (1.88 g, 7.4 mmol), and AcOK (1.81 g, 18.5 mmol) in a mixture of previously degassed 1,4-dioxane (8 mL) and DMF (1 mL) was added $PdCl_2$dppf (0.15 g, 0.18 mmol). The mixture was heated at 150 °C for 10 min under microwave irradiation. The mixture was cooled to room temperature and then filtered through a Celite pad. The filtrate was diluted with water (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, DCM in heptane, 0/100 to 100/0) to yield **53a** as a pale yellow oil (2 g, 66%). ^{1}H NMR (500 MHz, $CDCl_3$-d) δ 1.33 (s, 12H), 1.81−1.90 (m, 2H), 1.97−2.06 (m, 2H), 3.61 (ddd, J = 11.34, 7.44, 3.47 Hz, 2H), 4.00 (td, J = 7.59, 3.61 Hz, 2H), 4.58−4.65 (m, 1H), 6.92 (d, J = 8.38 Hz, 1H), 7.63 (dd, J = 8.09, 1.44 Hz, 1H), 7.82 (d, J = 1.45 Hz, 1H). GC−MS (EI): m/z 339 $[M]^+$.

[2-Chloro-4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-phenyl](tetrahydropyran-4-yl)amine (53b). To a suspension of **62** (3.66 g, 12.6 mmol) in previously degassed 1,4-dioxane (10 mL) were added bis(pinacolato)diboron (3.84 g, 15.1 mmol), AcOK (3.71 g, 37.8 mmol), and $PdCl_2$dppf (0.55 g, 0.75 mmol). The mixture was heated at 95 °C for 12 h. The mixture was cooled to room temperature and then filtered through a Celite pad. The filtrate was diluted with water (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc/heptane, 1/9) to yield **53a** as colorless oil (2.87 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 12H), 1.49−1.62 (m, 2H), 1.99−2.09 (m, 2H), 3.49−3.65 (m, 3H), 4.01 (dt, J = 11.8, 3.7 Hz, 2H), 4.48 (broad d, J = 7.9 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 7.55 (dd, J = 8.2, 0.8 Hz, 1H), 7.70 (d, J = 0.7 Hz, 1H). LC−MS m/z 338 $[M + H]^+$.

3-[4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)phenoxy]-2-methylpyridine (53d). Starting from **68b** (0.66 g, 2.5 mmol) and following the procedure described for **53a**, compound **53d** was obtained as a dark brown oil (0.65 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 12H), 2.48 (s, 3H), 6.89 (broad d, J = 8.7 Hz, 2H), 7.13 (dd, J = 7.9, 4.8 Hz, 1H), 7.21 (d, J = 8.1 Hz, 1H), 7.78 (broad d, J = 8.4 Hz, 2H), 8.33 (broad d, J = 4.6 Hz, 1H). LC−MS m/z 312 $[M + H]^+$.

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3-[4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)phenoxy]-2,6-dimethylpyridine (53e). To a degassed suspension of **68c** (0.90 g, 3.23 mmol), bis(pinacolato)diboron (0.81 g, 3.23 mmol), and AcOK (0.95 g, 9.69 mmol) in DMSO (20 mL) was added $PdCl_2$dppf (0.08 g, 0.1 mmol). The mixture was heated at 117 °C for 5 h. The mixture was cooled to room temperature and filtered through a Celite pad. The filtrate was diluted with water (10 mL) and extracted with EtOAc (2 × 10 mL). The organic layer was concentrated in vacuo to yield **53e** as dark brown oil (0.85 g, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 12H), 2.42 (s, 3H), 2.54 (s, 3H), 6.85 (broad d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.1 Hz, 1H), 7.15 (d, J = 8.1 Hz, 1H), 7.76 (broad d, J = 8.4 Hz, 2H). LC−MS m/z 326 $[M + H]^+$.

3-[2-Chloro-4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-phenoxy]-2-methylpyridine (53f). Starting from **68d** (2.4 g, 8.03 mmol) and following the procedure described for **53e**, compound **53f** was obtained as a dark brown oil (2.32 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 12H), 2.53 (s, 3H), 6.77 (d, J = 8.1 Hz, 1H), 7.06−7.14 (m, 2H), 7.62 (d, J = 8.1 Hz, 1H), 7.92 (s, 1H), 8.32 (broad d, J = 3.5 Hz, 1H). LC−MS m/z 346 $[M + H]^+$.

3-[2-Chloro-4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-phenoxy]-2,6-dimethylpyridine (53g). Starting from **68e** (2.4 g, 8.03 mmol) and following the procedure described for **53e**, compound **53g** was obtained as a dark brown oil (1.98 g, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32−1.36 (m, 12H), 2.45 (s, 3H), 2.54 (s, 3H), 6.67 (d, J = 8.09 Hz, 1H), 6.98 (d, J = 8.38 Hz, 1H), 7.06 (d, J = 8.67 Hz, 1H), 7.58 (dd, J = 8.09, 1.44 Hz, 1H), 7.90 (d, J = 1.45 Hz, 1H). LC−MS m/z 360 $[M + H]^+$.

4-[-4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-phenoxy]-2-methylpyridine (53i). To a degassed solution of **71b** (2.22 g, 8.33 mmol) in 1,4-dioxane (8 mL) and DMF (3 mL) were added bis(pinacolato)diboron (6.35 g, 24.98 mmol), AcOK (2.45 g, 24.98 mmol), and $PdCl_2$dppf (0.27 g, 0.33 mmol). The resulting mixture was heated at 150 °C for 10 min under microwave irradiation. The reaction mixture was then cooled to room temperature, diluted with water, and extracted with EtOAc. The organic layer was washed with brine and then dried over Na_2SO_4 and concentrated in vacuo to yield a crude residue that was purified using column chromatography, eluting with 0−10 7 N NH_3·MeOH in DCM to yield of **53i** (2.31 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 12H), 2.49 (s, 3H), 6.65−6.72 (m, 2H), 7.07 (broad d, J = 8.3 Hz, 2H), 7.87 (broad d, J = 8.3 Hz, 2H), 8.32−8.38 (m, 1H). LC−MS m/z 312 $[M + H]^+$.

4-[-4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-3-chloro-phenoxy]-2-methylpyridine (53j). To a degassed solution of **71c** (2.4 g, 2.0 mmol) in DMSO (10 mL) were added bis(pinacolato)-diboron (0.58 g, 2.30 mmol), AcOK (0.59 g, 6.00 mmol), and $PdCl_2$dppf (0.049 g, 0.06 mmol). The resulting mixture was heated at 150 °C for 10 min under microwave irradiation. The reaction mixture was then cooled to room temperature, diluted with water, and extracted with EtOAc. The organic fraction was separated, washed with brine, and then dried over Na_2SO_4 and concentrated in vacuo to yield **53j** (3.00 g, 100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 12H), 2.50 (s, 3H), 6.66−6.72 (m, 2H), 6.96 (dd, J = 8.2, 1.9 Hz, 1H), 7.09 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 8.37 (d, J = 6.1 Hz, 1H). LC−MS m/z 346 $[M + H]^+$.

4-[-4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-2-fluoro-phenoxy]-2,6-dimethylpyridine (53k). Starting from **71d** (0.50 g, 1.67 mmol) and following the procedure described for **53i**, compound **53k** was obtained as an oil (0.30 g, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 12H), 2.46 (s, 6H), 6.50 (s, 2H), 7.14 (t, J = 7.7 Hz, 1H), 7.58−7.67 (m, 2H). LC−MS m/z 344 $[M + H]^+$.

4-[-4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-2-chloro-phenoxy]-2-methylpyridine (53l). To a degassed solution of **74a** (2.43 g, 7.73 mmol) and following the procedure described for **53i**, compound **53l** was obtained as an oil (2.97 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 12H), 2.49 (s, 3H), 6.61−6.65 (m, 2H), 7.13 (d, J = 8.1 Hz, 1H), 7.74 (dd, J = 8.1, 1.4 Hz, 1H), 7.94 (d, J = 1.4 Hz, 1H), 8.33 (d, J = 6.2 Hz, 1H). LC−MS m/z 346 $[M + H]^+$.

4-[-4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-2-fluoro-phenoxy]-2-methylpyridine (53m). Starting from **74b** (0.50 g, 1.67 mmol) and following the procedure described for **53i**, compound **53m** was obtained as an oil (0.30 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 12H), 2.49 (s, 3H), 6.63−6.68 (m, 2H), 7.15 (t, J = 7.9 Hz, 1H), 7.59−7.68 (m, 2H), 8.35 (d, J = 6.7 Hz, 1H). LC−MS m/z 330 $[M + H]^+$.

4-[-4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-3-fluoro-phenoxy]-2-methylpyridine (53n). Starting from **74c** (0.50 g, 1.77 mmol) and following the procedure described for **53i**, compound **53n** was obtained as an oil (0.58 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (s, 12H), 2.51 (s, 3H), 6.69−6.74 (m, 2H), 6.76 (dd, J = 9.8, 2.2 Hz, 1H), 6.86 (dd, J = 8.2, 2.2 Hz, 1H), 7.78 (dd, J = 8.3, 6.9 Hz, 1H), 8.38 (d, J = 5.5 Hz, 1H). LC−MS m/z 330 $[M + H]^+$.

1-Isopentyl-4-methoxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (56a). Isopentyl bromide **55a** (24.1 mL, 160 mmol) was added to a stirred suspension of 2-hydroxy-4-methoxynicotinonitrile **54** (20 g, 133 mmol) and K_2CO_3 (55.2 g, 400 mmol) in CH_3CN (600 mL). The mixture was stirred at reflux for 20 h. The cooled mixture was filtered through a Celite pad and concentrated in vacuo. The residue thus obtained was precipitated by treatment with diisopropyl ether. The resulting solid was filtered off and dried in the vacuum oven (50 °C) to yield **56a** (24 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (d, J = 6.22 Hz, 6H), 1.42−1.58 (m, 3H), 3.86−3.92 (m, 2H), 3.98 (s, 3H), 6.44 (d, J = 7.88 Hz, 1H), 8.12 (d, J = 7.88 Hz, 1H). LC−MS m/z 221 $[M + H]^+$.

1-Butyl-4-methoxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (56b). Starting from butyl bromide **55b** (11.21 mL, 103 mmol) and **54** (8 g, 53.3 mmol) and following the procedure described for **56a**, compound **56b** was obtained as a white solid (11.15 g, quantitative), mp 78.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 3H), 1.36 (sex, J = 7.5 Hz, 2H), 1.71 (quin, J = 7.5 Hz, 2H), 3.93 (t, J = 7.4 Hz, 2H), 3.99 (s, 3H), 6.07 (d, = 7.8 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H). LC−MS m/z 207 $[M + H]^+$.

1-Cyclopropylmethyl-4-methoxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (56c). Starting from bromomethylcyclopropane **55c** (11.95 g, 103 mmol) and **54** (8 g, 53.3 mmol) and following the procedure described for **56a**, compound **56c** was obtained as a white solid (10.9 g, quantitative), mp 139.1 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.34−0.45 (m, 2H), 0.58−0.70 (m, 2H), 1.17−1.27 (m, 1H), 3.80 (d, J = 7.2 Hz, 2H), 4.01 (s, 3H), 6.13 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.5 Hz, 1H). LC−MS m/z 205 $[M + H]^+$.

1-Cyclobutylmethyl-4-methoxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (56d). Starting from bromomethylcyclobutane **55d** (11.36 g, 76.2 mmol) and **54** (10 g, 66.6 mmol) and following the procedure described for **56a**, compound **56d** was obtained as a white solid (14.4 g, quantitative), mp 117.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.70−1.83 (m, 2H), 1.83−1.99 (m, 2H), 2.00−2.10 (m, 2H), 2.76 (sep, J = 7.8 Hz, 1H), 3.95 (d, J = 7.5 Hz, 2H), 3.99 (s, 3H), 6.05 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H). LC−MS m/z 219 $[M + H]^+$.

1-Isopentyl-4-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (57a). A mixture of **56a** (24 g, 110 mmol) in 1 N aqueous NaOH (40 mL) was stirred at 100 °C for 20 h. The mixture was cooled to 0 °C and acidified with 2 N aqueous HCl to pH 2−3. The product precipitated in the mixture, and it was filtered, washed with cold diisopropyl ether, and dried at 50 °C in a vacuum oven to yield **57a** as a white solid (21.5 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (d, J = 6.22 Hz, 6H), 1.34−1.60 (m, 3H), 3.73−3.91 (m, 2H), 6.04 (d, J = 7.67 Hz, 1H), 7.84 (d, J = 7.67 Hz, 1H). LC−MS m/z 207 $[M + H]^+$.

1-Butyl-4-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (57b). Starting from **56b** (11 g, 53.3 mmol) and following the procedure described for **57a**, compound **57b** was obtained as a white solid (7.58 g, 74%), mp 192.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, J = 7.4 Hz, 3H), 1.25 (dq, J = 15.1, 7.4 Hz, 2H), 1.50−1.62 (m, 2H), 3.82 (t, J = 7.2 Hz, 2H), 6.04 (d, J = 7.4 Hz, 1H), 7.83 (d, J = 7.6 Hz, 1H), 12.64 (broad s, 1H). LC−MS m/z 193 $[M + H]^+$.

1-Cyclopropylmethyl-4-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (57c). Starting from **56c** (10.88 g, 53.3 mmol) and following the procedure described for **57a**, compound **57c** was obtained as a white solid (8.2 g, 81%), mp 231.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.30−0.37 (m, 2H), 0.39−0.55 (m, 2H), 1.06−1.22 (m, 1H), 3.67 (d, J = 7.26 Hz, 2H), 6.04 (d, J = 7.46 Hz, 1H), 7.87 (d, J = 7.46 Hz, 1H). LC−MS m/z 191 $[M + H]^+$.

1-Cyclobutylmethyl-4-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (57d). Starting from **56d** (14.53 g, 66.6 mmol) and following the procedure described for **57a**, compound **57d** was obtained as a white solid (6.17 g, 45%), mp 296.7. °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.64−2.00 (m, 6H), 2.62 (sep, J = 7.7 Hz, 1H), 3.85 (d, J = 7.4 Hz, 2H), 6.04 (d, J = 7.6 Hz, 1H), 7.83 (d, J = 7.6 Hz, 1H), 12.66 (broad s, 1H). LC−MS m/z 205 $[M + H]^+$.

4-(4-Bromo-2-chlorophenoxy)tetrahydropyran (60). To a cooled suspension (0 °C) of 4-hydroxytetrahydropyrane **58** (2.2 mL, 23.1 mmol), 3-chloro-4-hydroxybromobenzene **59** (4 g, 19.3 mmol), and polymer supported PPh_3 (17.29 g, 39.6 mmol) in dry DCM (250 mL) was added di-*tert*-butyl azodicarboxylate (6.65 g, 28.9 mmol) portionwise. The mixture was stirred at 0 °C for 5 min and at room temperature for 2 h. The resin was filtered off and washed with DCM (2 × 100 mL). The filtrates were concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (silica gel, 7 N solution of ammonia in MeOH in DCM, 0/100 to 2/98) to give **60** as a colorless oil (5.38 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79−1.89 (m, 2H), 1.96−2.05 (m, 2H), 3.59 (ddd, J = 11.4, 7.5, 3.7 Hz, 2H), 4.00 (ddd, J = 11.4, 7.2, 3.6 Hz, 2H), 4.51 (tt, J = 7.3, 3.7 Hz, 1H), 6.83 (d, J = 8.8 Hz, 1H), 7.30 (dd, J = 8.8, 2.3 Hz, 1H), 7.52 (d, J = 2.5 Hz, 1H). GC−MS (EI): m/z 290 $[M]^+$.

(4-Bromo-2-chorophenyl)tetrahydropyranylamine (63). 4-Bromo-2-choroaniline **62** (4.0 g, 19.37 mmol) was added to stirred suspension of tetrahydropyranone **61** (2.69 mL, 29.05 mmol), sodium triacetoxyborohydride (6.12 g, 29.05 mmol), and 4 Å molecular sieves in dry DCM (100 mL). The mixture was stirred at room temperature for 72 h and then filtered over a Celite pad. The filtrate was concentrated in vacuo. The resulting crude product was purified by column chromatography (silica gel, 7 M solution of ammonia in MeOH/DCM, 0/100 to 5/95) to yield **16** as a white solid (4.83 g, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.49−1.59 (m, 2H), 1.98−2.06 (m, 2H), 3.45−3.56 (m, 3H), 4.01 (dt, J = 11.9, 3.9 Hz, 2H), 4.21 (broad d, J = 8.1 Hz, 1H), 6.55 (d, J = 8.7 Hz, 1H), 7.21 (dd, J = 8.7, 2.3 Hz, 1H), 7.39 (d, J = 2.3 Hz, 1H). LC−MS m/z 291 $[M + H]^+$.

3-(4-Nitrophenoxy)-2-methylpyridine (66b). 4-Fluoronitrobenzene **65a** (2.58 g, 18.3 mmol) and Cs_2CO_3 (8.9 g, 27.5 mmol) were added to a mixture of 3-hydroxy-2-methylpyridine **64b** (2 g, 18.3 mmol) in dry DMF (5 mL). The mixture was heated at 170 °C for 30 min under microwave irradiation. After the mixture was cooled to room temperature, the solids were filtered off and the filtrate was concentrated in vacuo. The resulting residue was treated with water (10 mL) and extracted with DCM (2 × 10 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The resulting crude product was purified by column chromatography (silica gel, MeOH in DCM, 0/100 to 5/95) to yield **66b** as a bright yellow solid (4.01 g, 95%), mp 76.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.45 (s, 3H), 6.95 (broad d, J = 9.0 Hz, 2H), 7.23 (dd, J = 7.9, 4.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 8.23 (broad d, J = 9.0 Hz, 2H), 8.45 (d, J = 4.6 Hz, 1H). LC−MS m/z 231 $[M + H]^+$.

3-(4-Nitrophenoxy)-2,6-dimethylpyridine (66c). Starting from 4-fluoronitrobenzene **65a** (3.41 g, 24.3 mmol) and 2,6-dimethyl-3-hydroxypyridine **64c** (3 g, 24.3 mmol) and following the procedure described for **66b**, compound **66c** was obtained as a yellow solid (5.2 g, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 (s, 3H), 2.58 (s, 3H), 6.90−6.95 (m, 2H), 7.08 (d, J = 8.1 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 8.19−8.24 (m, 2H). LC−MS m/z 244 $[M + H]^+$.

3-(2-Chloro-4-nitrophenoxy)-2-methylpyridine (66d). A mixture of 3-hydroxy-2-methylpyridine **64b** (1.4 g, 17.2 mmol), 3-chloro-4-fluoronitrobenzene **65b** (3 g, 17.2 mmol), and Cs_2CO_3 (8.43 g, 26.1 mmol) in DMF (25 mL) was stirred at 130 °C for 16 h in a sealed tube. After cooling to room temperature the solvent was evaporated in vacuo. The residue thus obtained was purified by column chromatography (silica gel, MeOH/DCM, 2/98) to yield **66d** as a bright yellow solid (3.69 g, 81%), mp 96.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 6.73 (d, J = 9.2 Hz, 1H), 7.24 (dd, J = 8.1, 4.6 Hz, 1H), 7.30 (dd, J = 8.1, 1.4 Hz, 1H), 8.07 (dd, J = 9.0, 2.8 Hz, 1H), 8.42 (d, J = 2.8 Hz, 1H), 8.47 (dd, J = 4.7, 1.5 Hz, 1H). LC−MS m/z 264 $[M + H]^+$.

3-(2-Chloro-4-nitrophenoxy)-2,6-dimethylpyridine (66e). Starting from 2,6-dimethyl-3-hydroxypyridine **64c** (4.24 g, 34.5 mmol) and 3-chloro-4-fluoronitrobenzene **65b** (6 g, 34.2 mmol) and following the procedure described for **66d**, compound **66e** was obtained as a yellow solid (3.30 g, 34%), mp 77.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H), 2.58 (s, 3H), 6.68 (d, J = 9.0 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 8.05 (dd, J = 9.1, 2.7 Hz, 1H), 8.40 (d, J = 2.5 Hz, 1H). LC−MS m/z 279 $[M + H]^+$.

4-(2-Methylpyridin-3-yloxy)phenylamine (67b). Palladium (10% on carbon, 0.2 g) was added to a mixture of **66b** (4 g, 17.4 mmol) in EtOAc (150 mL). The mixture was hydrogenated at room temperature for 24 h. The mixture was filtered through a Celite pad and the filtrate evaporated in vacuo to yield **67b** as a pale pink solid (3.2 g, 92%), mp 85.3 °C. (3.2 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 3H), 3.61 (broad s, 2H), 6.65−6.72 (m, 2H), 6.78−6.84 (m, 2H), 6.98−7.06 (m, 2H), 8.19 (dd, J = 3.9, 2.1 Hz, 1H). LC−MS m/z 201 $[M + H]^+$

3-(4-Aminophenoxy)-2,6-dimethylpyridine (67c). Starting from **66d** (4 g, 16.38 mmol) and following the procedure described for **67b**, compound **67c** was obtained as a white solid (3.2 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 2.50 (s, 3H), 6.62−6.69 (m, 2H), 6.73−6.79 (m, 2H), 6.86−6.92 (m, 1H), 6.94−7.00 (m, 1H). LC−MS m/z 215 $[M + H]^+$.

4-(2-Methylpyridin-3-yloxy)-3-chlorophenylamine (67d). $SnCl_2$ (11.54 g, 51.2 mmol) was added at once to a stirred solution of **66d** (2.73 g, 10.23 mmol) in EtOH (100 mL). The mixture was stirred at 90 °C for 1 h in a sealed tube, and then it was cooled to room temperature and the volatiles were evaporated in vacuo. The residue thus obtained was taken up in EtOAc and washed twice with an aqueous K_2CO_3 saturated solution. The organic layer was separated, dried (Na_2SO_4) and the solvent evaporated in vacuo to yield **67d** as a pale cream solid (2.36 g, 98%), mp 75.9 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.61 (s, 3H), 3.70 (broad s, 2H), 6.57 (dd, J = 8.4, 2.6 Hz, 1H), 6.80 (d, J = 2.3 Hz, 1H), 6.81−6.87 (m, 2H), 7.01 (dd, J = 8.1, 4.9 Hz, 1H), 8.18 (d, J = 4.3 Hz, 1H). LC−MS m/z 235 $[M + H]^+$.

4-(2,6-Dimethylpyridin-3-yloxy)-3-chlorophenylamine (67e). Starting from **66e** (3.3 g, 11.7 mmol) and following the procedure described for **67d**, compound **67e** was obtained as a pale cream solid (2.78 g, 95%), mp 126.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.48 (s, 3H), 2.56 (s, 3H), 3.66 (broad s, 2H), 6.55 (dd, J = 8.5, 2.5 Hz, 1H), 6.75−6.82 (m, 2H), 6.84−6.89 (m, 1H), 7.26 (s, 1H). LC−MS m/z 249 $[M + H]^+$.

3-(4-Bromophenoxy)-2-methylpyridine (68b). A solution of $NaNO_2$ (2.85 g, 40.9 mmol) in water (20 mL) was added dropwise over 45 min to a mixture of **67b** (4 g, 19.9 mmol) in 48% aqueous HBr (25 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, and then CuBr (4.28 g, 29.8 mmol) was added slowly at 0 °C. The reaction mixture was stirred at room temperature for 2 h and then at 140 °C for 20 h. After the mixture was cooled to room temperature, the crude product was carefully treated with an aqueous $NaHCO_3$ saturated solution and extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue thus obtained was purified by column chromatography (silica gel, EtOAc in heptane, 1/4) to yield **68b** as a brown oil (3.5 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 6.78−6.83 (m, 2H), 7.13 (dd, J = 8.3, 4.6 Hz, 1H), 7.18 (dd, J = 8.1, 1.6 Hz, 1H), 7.41−7.47 (m, 2H), 8.32 (dd, J = 4.6, 1.6 Hz, 1H). LC−MS m/z 265 $[M + H]^+$.

3-(4-Bromophenoxy)-2,6-dimethylpyridine (68c). Starting from **67c** (4 g, 18.58 mmol) and following the procedure described for **68b**, compound **68c** was obtained as a brown oil (4.1 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 2.53 (s, 3H), 6.71−6.80 (m, 2H), 6.97 (d, J = 8.1 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.34−7.43 (m, 2H). LC−MS m/z 278 $[M + H]^+$.

3-(4-Bromo-2-chlorophenoxy)-2-methylpyridine (68d). Starting from **67d** (2.36 g, 10.1 mmol) and following the procedure described for **68b**, compound **68d** was obtained as a brown oil (2.4 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (s, 3H), 6.72 (d, J = 8.8 Hz, 1H), 7.03 (dd, J = 8.3, 1.4 Hz, 1H), 7.11 (ddd, J = 8.1, 4.4, 0.5 Hz, 1H), 7.34 (dd, J = 8.7, 2.4 Hz, 1H), 7.63 (d, J = 2.3 Hz, 1H), 8.31 (dd, J = 4.7, 1.5 Hz, 1H). LC−MS m/z 299 $[M + H]^+$.

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3-(2-Chloro-4-bromophenoxy)-2,6-dimethylpyridine (68e). Starting from **67e** (2.78 g, 11.2 mmol) and following the procedure described for **68b**, compound **68e** was obtained as a brown oil (3.13 g, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.47 (s, 3H), 2.53 (s, 3H), 6.63 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 8.1 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 7.29 (dd, J = 8.7, 2.3 Hz, 1H), 7.61 (d, J = 2.3 Hz, 1H). LC−MS m/z 314 $[M + H]^+$.

4-(4-Bromophenoxy)-2-methylpyridine (71b). To a suspension of NaH (0.055 g, 13.8 mmol) in DMF (14 mL) at 0 °C was added portionwise 4-bromophenol **70a** (2.00 g, 11.6 mmol). The reaction mixture was stirred at 0 °C for 10 min, and then 4-chloro-2-picoline **69b** (1.47 g, 11.6 mmol) was added. The resulting mixture was heated at 180 °C for 45 min under microwave irradiation. After cooling to room temperature the reaction mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo. The residue thus obtained was purified by column chromatography (silica gel, 0−3% MeOH in DCM) to yield **71b** as a yellow oil (2.22 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 6.65 (dd, J = 5.5, 2.3 Hz, 1H), 6.68 (d, J = 2.3 Hz, 1H), 6.94−6.99 (m, 2H), 7.49−7.54 (m, 2H), 8.35 (d, J = 5.8 Hz, 1H). GC−MS m/z 264 $[M]^+$.

4-(4-Bromo-3-chlorophenoxy)-2-methylpyridine (71c). To a solution of 4-bromo-3-chlorophenol **70b** (4.80 g, 23.5 mmol) in xylenes (50 mL) were added 4-chloropicoline **69b** (3.00 g, 23.5 mmol) and K_2CO_3 (3.90 g, 28.5 mmol). The resulting solution was then heated at 150 °C for 5 days. The reaction mixture was then filtered through a Celite pad. The filtrate was concentrated in vacuo to yield a residue that was purified by column chromatography (silica gel, 0−3% MeOH in DCM) to yield **71c** as a pale brown solid (4.95 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.52 (s, 3H), 6.66−6.73 (m, 2H), 6.88 (dd, J = 8.8, 2.7 Hz, 1H), 7.21 (d, J = 2.9 Hz, 1H), 7.64 (d, J = 8.7 Hz, 1H), 8.39 (d, J = 5.5 Hz, 1H). LC−MS m/z 299 $[M + H]^+$.

4-(4-Bromo-2-fluorophenoxy)-2,6-dimethylpyridine (71d). Starting from 4-bromo-2-fluorophenol **70c** (3.10 g, 16.2 mmol) and 4-chloro-2,6-dimethylpyridine **69c** (3.00 g, 16.2 mmol) and following the procedure described for **71c**, compound **71d** was obtained as a pale brown solid (3.20 g, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.47 (s, 6H), 6.49 (s, 2H), 7.05 (t, J = 8.4 Hz, 1H), 7.33 (broad d, J = 8.7 Hz, 1H), 7.40 (dd, J = 9.5, 2.0 Hz, 1H). LC−MS m/z 297 $[M + H]^+$.

4-(4-Bromo-2-chlorophenoxy)-2-methylpyridine 1-Oxide (74a). To a solution of 4-bromo-2-chlorophenol **73a** (5.37 g, 26.0 mmol) in DMF (10 mL) at 0 °C was added NaH (0.93 g, 38.9 mmol) portionwise. The mixture was stirred for 10 min at 0 °C, and then 4-nitro-2-picoline *N*-oxide **72** (4 g, 25.9 mmol) was added. Stirring was continued for 1 h at room temperature, and then the mixture was heated at 180 °C for 40 min under microwave irradiation. The reaction mixture was cooled to room temperature, filtered through a Celite pad, and the filtrate was diluted with water and extracted with EtOAc (4 × 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue thus obtained was purified by column chromatography (silica gel, EtOAc/heptane, 50/50) to yield **74a** (4.03 g, 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.69 (dd, J = 7.2, 3.5 Hz, 1H), 6.76 (d, J = 3.5 Hz, 1H), 7.04 (d, J = 8.7 Hz, 1H), 7.47 (dd, J = 8.7, 2.3 Hz, 1H), 7.67 (d, J = 2.3 Hz, 1H), 8.19 (d, J = 6.9 Hz, 1H). LC−MS m/z 315 $[M + H]^+$.

4-(4-Bromo-2-fluorophenoxy)-2-methylpyridine 1-Oxide (74b). To a solution of 4-bromo-3-fluorophenol **73b** (2.00 g, 10.4 mmol) in *N*-methylpyrrolidone (14 mL) at 0 °C was added NaH (0.054 g, 13.6 mmol) portionwise. The mixture was stirred for 10 min at 0 °C, and then **72** (1.77 g, 11.5 mmol) was added. The reaction mixture was heated at 240 °C for 60 min under microwave irradiation. The reaction mixture was cooled to room temperature, filtered through a Celite pad, and the filtrate was diluted with Et_2O and washed with water (4 × 20 mL). The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo to give a residue that was purified by column chromatography (silica gel, 0−3% MeOH·NH_3 (7 N) in DCM) to yield **74b** (2.35 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.73 (dd, J = 6.9, 3.0 Hz, 1H), 6.80 (broad s, 1H), 7.08 (t, J = 8.4 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.42 (dd, J = 9.7, 1.6 Hz, 1H), 8.19 (d, J = 7.2 Hz, 1H). LC−MS m/z 297 $[M + H]^+$.

4-(4-Bromo-3-fluorophenoxy)-2-methylpyridine 1-Oxide (74c). Starting from 4-bromo-3-fluorophenol **73c** (6 g, 31.4 mmol) and following the procedure described for **74a**, compound **74c** was obtained as an oil (3.61 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (s, 3H), 6.78−6.84 (m, 2H), 6.87−6.92 (m, 2H), 7.61 (dd, J = 8.8, 7.6 Hz, 1H), 8.25 (d, J = 7.2 Hz, 1H). LC−MS m/z 297 $[M + H]^+$.

Biology. Membrane Preparation. CHO cells were cultured to preconfluence and stimulated with 5 mM butyrate for 24 h, prior to washing in PBS, and then collection was by scraping in homogenization buffer (50 mM Tris-HCl buffer, pH 7.4, 4 °C). Cell lysates were homogenized briefly (15 s) using an ULTRA-TURRAX homogenizer. The homogenate was centrifuged at 23500*g* for 10 min and the supernatant discarded. The pellet was resuspended in 5 mM Tris-HCl, pH 7.4, and centrifuged again (30000*g*, 20 min, 4 °C). The final pellet was resuspended in 50 mM HEPES, pH 7.4, and stored at −80 °C in appropriate aliquots before use. Protein concentration was determined by the Bradford method (Bio- Rad, U.S.) with bovine serum albumin as standard.

[^{35}S]GTPγS Binding Assay. Measurement of mGluR2 positive allosteric modulatory activity of test compounds in membranes containing human mGluR2 was performed using frozen membranes that were thawed and briefly homogenized prior to preincubation in 96-well microplates (15 mg/assay well, 30 min, 30 °C) in assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 3 mM $MgCl_2$, 50 μM GDP, 10 mg mL^{-1} saponin) with increasing concentrations of positive allosteric modulator (from 0.3 nM to 50 μM) and either a minimal predetermined concentration of glutamate (PAM assay) or no added glutamate. For the PAM assay, membranes were preincubated with glutamate at EC_{25} concentration, i.e., a concentration that gives 25% of the maximal response glutamate. After addition of [^{35}S]GTPγS (0.1 nM, fc) to achieve a total reaction volume of 200 μL, microplates were shaken briefly and further incubated to allow [^{35}S]GTPγS incorporation on activation (30 min, 30 °C). The reaction was stopped by rapid vacuum filtration over glass-fiber filter plates (Unifilter 96-well GF/B filter plates, Perkin-Elmer, Downers Grove, IL, U.S.), using a 96-well plate cell harvester (Filtermate, Perkin-Elmer, U.S.), and then by washing three times with 300 μL of ice-cold wash buffer ($Na_2PO_4·2H_2O$, 10 mM, $NaH_2PO_4·H_2O$, 10 mM, pH 7.4). Filters were then air-dried, and 40 mL of liquid scintillation cocktail (Microscint-O) was added to each well. Membrane-bound [^{35}S]GTPγS was measured in a 96-well scintillation plate reader (Top-Count, Perkin-Elmer, U.S.). Nonspecific [^{35}S]GTPγS binding is determined in the presence of cold 10 mM GTP. Each curve was performed at least three times using duplicate sample per data point and at 11 concentrations.

Data Analysis. The concentration−response curves in the presence of added EC_{25} of mGluR2 agonist glutamate to determine positive allosteric modulation (PAM), were generated using the Prism GraphPad software (Graph Pad Inc., San Diego, USA). The curves were fitted to a four-parameter logistic equation (Y = bottom + (top − bottom)/(1 + $10^{((\log EC_{50}-X)(\text{Hill slope}))}$) allowing determination of EC_{50} values. The EC_{50} is the concentration of a compound that causes a half-maximal potentiation of the glutamate response. This is calculated by subtracting the maximal responses of glutamate in the presence of a fully saturating concentration of a positive allosteric modulator from the response of glutamate in the absence of a positive allosteric modulator. The concentration producing the half-maximal effect is then calculated as EC_{50}. The pEC_{50} values are calculated as the −log EC_{50} (wherein EC_{50} is expressed in mol L^{-1}).

Patch Clamp Assay. Experiments were performed using HEK293 cells stably expressing the HERG potassium channel. Cells were grown at 37 °C and 5% CO_2 in culture flasks in MEM medium supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine−penicillin−streptomycin solution, 1% nonessential amino acids (100×), 1% sodium pyruvate (100 mM), and 0.8% Geneticin (50 mg/mL). Before use, the cells were subcultured in MEM medium in the absence of 5 mL of L-glutamine−penicillin−streptomycin. For use in the automated patch-clamp system PatchXpress 7000A (Axon Instruments) cells were harvested to obtain cell suspension of single cells. Extracellular solution contained the following (mM): 150 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 5 glucose (pH 7.4 with

NaOH). Pipette solution contained the following (mM): 120 KCl, 10 HEPES, 5 EGTA, 4 ATP-Mg_2, 2 $MgCl_2$, 0.5 $CaCl_2$ (pH 7.2 with KOH). Patch-clamp experiments were performed in the voltage-clamp mode, and whole-cell currents were recorded with an automated patch-clamp assay utilizing the PatchXpress 7000A system (Axon Instruments). Current signals were amplified and digitized by a Multiclamp amplifier and stored and analyzed by using the PatchXpress, DataXpress software, and Igor 5.0 (Wavemetrics). The holding potential was −80 mV. The HERG current (K^+-selective outward current) was determined as the maximal tail current at −40 mV after a 2 s depolarization to +60 mV. Pulse cycling rate was 15 s. Before each test pulse a short pulse (0.5 s) from the holding potential to −60 mV was given to determine (linear) leak current. After whole-cell configuration and a stability period were established, the vehicle was applied for 5 min followed by the test substance with increasing concentrations of 10^{-7}, 3×10^{-7}, and 3×10^{-6} M. Each concentration of the test substance was applied twice. The effect of each concentration was determined after 5 min as an average current of three sequential voltage pulses. To determine the extent of block, the residual current was compared with vehicle pretreatment.

Method for Identification of Main Metabolites. The test compounds were incubated with human and rat liver microsomes, at 0.4% final solvent concentration (0.16% DMSO and 0.24% MeCN). The study was conducted to identify the main metabolites formed after 0 min (control) and 60 min of incubation time in the presence of NADPH generating system at 37 °C. Test concentration of the compound was 5 μM. Samples were compared to the control incubations in which compound was added at termination. Data were acquired on a Waters UPLC/QToF Premier mass spectrometer using a 10 min generic UPLC method and a generic MSe method. Complementary MS/MS experiments were performed when necessary. An Acquity UPLC C18 (2.1 mm × 100 mm, 1.8 μm) column was used. Interpretation of data was performed with Waters Metabolynx software.

Sleep−Wake EEG. Animals, Drug Treatment, and Experimental Procedure. All in vivo experimental procedures were performed according the applicable European Communities Council Directive of November 24, 1986 (86/609/EEC), and approved by the Animal Care and Use Committee of Janssen Pharmaceutical Companies of Johnson & Johnson and by the local ethical committee.

Male Sprague−Dawley rats (Charles River, France) weighing 250−300 g were used. Animals were chronically implanted with electrodes for recording the cortical electroencephalogram (EEG), electrical neck muscle activity (EMG), and ocular movements (EOG). All animals were housed in individually ventilated cages under environmentally controlled conditions (ambient temperature, 22 °C; humidity, 60%) on a 12 h light/dark cycle (lights on from 12:00 a.m. to 12:00 p.m., illumination intensity of ∼100 lx). The animals had free access to food and tap water.

The effects of the tested molecule and vehicle on sleep−wake distribution during the lights-on period were investigated in 16 rats (n = 8 each group). Two EEG recording sessions were performed: the first recording session started at 13:30 h and lasted 20 h following oral administration of saline. The second recording session was performed during the same consecutive circadian time and for the same duration following administration of either vehicle (20% CD + 2H2T) or tested compound.

Sleep polysomnographic variables were determined offline using a sleep stages analyzer, scoring each 2 s epoch before averaging stages over 30 min periods. Sleep−wake state classifications were assigned based on a combination of dynamics of five EEG frequency domains, integrated EMG, EOG, and body activity level: active wake (AW); passive wake (PW); intermediate stage (pre-REM transients); rapid eye movement sleep (REM); light non-REM sleep (lSWS); deep non-REM sleep (dSWS). Different sleep−wake parameters were investigated over 20 h postadministration: amount of time spent in each vigilance state, sleep parameters, latencies for first REM sleep period, and the number of transitions between states.

Statistical Analysis. Time spent in each vigilance state (AW, PW, lSWS, dSWS, IS, and REMS) was expressed as a percentage of the recording period. A statistical analysis of the obtained data was carried out by a nonparametric analysis of variance of each 30 min period, followed by a Wilcoxon−Mann−Whitney rank sum test of comparisons with the control group.

■ ASSOCIATED CONTENT

Supporting Information

Details of LC−MS methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADMET, absorption, distribution, metabolism, excretion, and toxicity; BBB, blood−brain barrier; CNS, central nervous system; DMS-IV, diagnostic statistical manual of mental disorders volume IV; GPCR, G-protein-coupled receptor; HLM, human liver microsome; iv, intravenous; mGlu2, metabotropic glutamate 2; ND, not determined; nm, not measurable; PAM, positive allosteric modulator; PK, pharmacokinetics; SAR, structure−activity relationship; SEM, standard error of the mean; po, oral; REM, rapid eye movement; RLM, rat liver microsome; ROL, rapid eye movement sleep onset latency; sc, subcutaneous; SD, standard deviation; sw-EEG, sleep−wake electroencephalogram

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