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# Discovery, Structure–Activity Relationship, and Antiparkinsonian Effect of a Potent and Brain-Penetrant Chemical Series of Positive Allosteric Modulators of Metabotropic Glutamate Receptor 4

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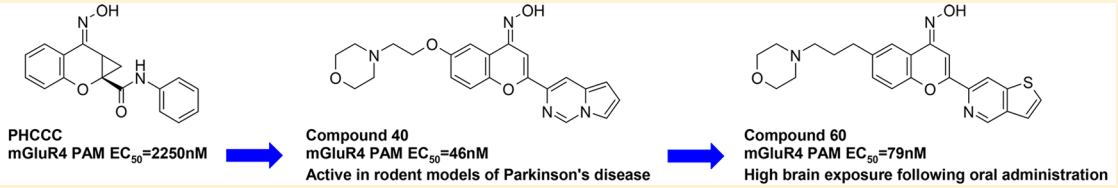
Supporting Information




**ABSTRACT:** The metabotropic glutamate receptor 4 (mGluR4) is an emerging target for the treatment of Parkinson's disease (PD). However, since the discovery of its therapeutic potential, no ligand has been successfully developed enough to be tested in the clinic. In the present paper, we report for the first time the medicinal chemistry efforts conducted around the pharmacological tool (−)-PHCCC. This work led to the identification of compound **40**, a potent and selective mGluR4 positive allosteric modulator (PAM) with good water solubility and demonstrating consistent activity across validated preclinical rodent models of PD motor symptoms after intraperitoneal administration: haloperidol-induced catalepsy in mouse and the rat 6-hydroxydopamine (6-OHDA) lesion model. Moreover, we also describe the identification of compound **60** a close analogue of compound **40** with improved pharmacokinetic profile after oral administration. On the basis of its favorable and unique preclinical profile, compound **60** (PXT002331, now foliglurax) was nominated as a candidate for clinical development.

## INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disorder affecting more than six million people worldwide.[1] It results from the loss of dopamine neurons in the basal ganglia (BG), a brain region responsible for the control of motor functions. Available treatments, mainly based on dopamine replenishment, are only effective at managing early PD symptoms.[2] As the disease progresses, these treatments become less effective and produce debilitating side effects.[3,4] Among them, dyskinesia (or L-dopa-induced dyskinesia, LID), characterized by involuntary movements appearing after several years of L-dopa therapy, represents a serious challenge for late-stage PD treatment.

Over the past decade, novel therapeutic strategies targeting nondopaminergic transmissions have emerged.[5] Among them, modulation of presynaptic glutamate receptors such as metabotropic glutamate receptor 4 (mGluR4) has proven to be a promising approach to normalize the BG circuitry.[6,7] To date, eight mGluRs have been cloned and classified in three groups according to their sequence homologies, pharmacological properties, and signal transduction mechanisms: group I includes mGluR1 and mGluR5, group II mGluR2 and mGluR3, and group III mGluR4, mGluR6, mGluR7, and mGluR8.[8] Given its expression in desired regions of the BG motor circuit, its presynaptic localization, and its physiological function to decrease neurotransmitter release, the mGluR4 receptor has received much interest as a therapeutic target for a L-dopa-sparing strategy in PD. Indeed, mGluR4 localizes presynaptically at striatopallidal fibers where its activation circumvents dopamine action via the indirect pathway.[9]

Initial modulation of mGluR4 was made with nonselective group III mGluR agonists (activating mGluR4, mGluR6, mGluR7, and mGluR8) such as L-AP4 or ACPT-I (Figure 1). These highly polar pharmacological tools were used to demonstrate the potential of group III activation in several in vitro and in vivo paradigms of PD.[10,11] More selective tools came later with positive allosteric modulators (PAMs) such as

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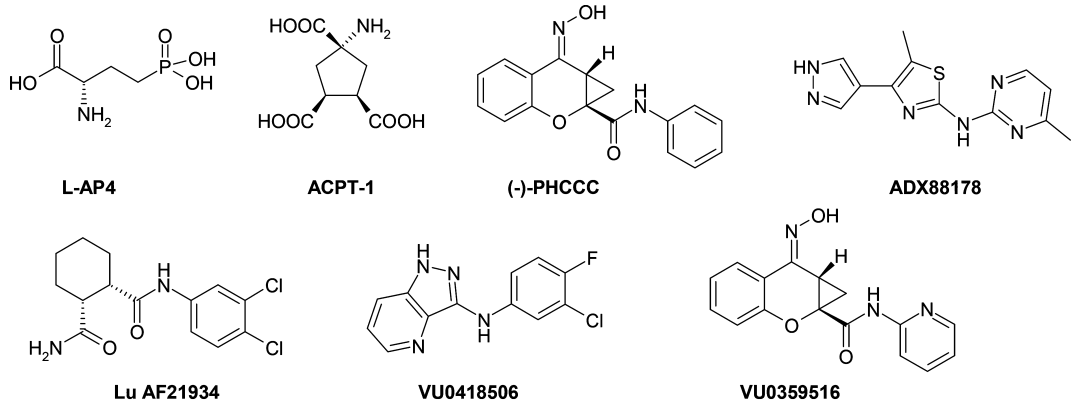
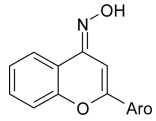
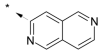



**Figure 1.** Structure of reference mGluR4 ligands.

**Table 1. Exploration of the Aromatic Substituents of Chromenone Oxime Scaffold**



| Compounds | Aromatic group | mGluR4 PAM $EC_{50}$ (μM)[a] | Compounds | Aromatic group | mGluR4 PAM $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| PHCCC | / | 2.25 ± 1.07 | **11** |  | 0.31 ± 0.18 |
| **1** |  | 14.2 ± 3.75 | **12** |  | 1.27 ± 0.31 |
| **2** |  | NA | **13** |  | 0.24 ± 0.07 |
| **3** |  | >30 | **14** |  | 0.11 ± 0.03 |
| **4** |  | NA | **15** |  | 0.13 ± 0.09 |
| **5** |  | >30 | **16** |  | 0.20 ± 0.14 |
| **6** |  | NA | **17** |  | 0.28 ± 0.08 |
| **7** |  | NA | **18** |  | 0.86 ± 0.32 |
| **8** |  | NA | **19** |  | 0.050 ± 0.08 |
| **9** |  | 3.96 ± 1.03 | **20** |  | NA |
| **10** |  | 6.99 ± 0.66 | **21** |  | NA |

[a]Values are the mean (±SD) of a minimum of three independent experiments. NA: not active.

(−)-PHCCC,[12] ADX88178,[13,14] Lu AF21934,[15] or VU0418506[16,17] (Figure 1). Allosteric modulation offers several advantages over orthosteric approaches such as the possibility to obtain subtype selectivity and the access to druggable compounds more amenable to medicinal chemistry strategies. However, despite more than a decade of chemical optimization, none of these mGluR4 PAMs has progressed in the clinic.

In this paper, we report for the first time the medicinal chemistry work based on (−)-PHCCC, which led to a novel chromenone-oxime series with improved properties. We demonstrate that this effort led to the identification of compounds with an improved profile, up to the discovery of compound **60** (PXT002331),[18] which is currently in clinical development. We also describe more in depth compound **40**



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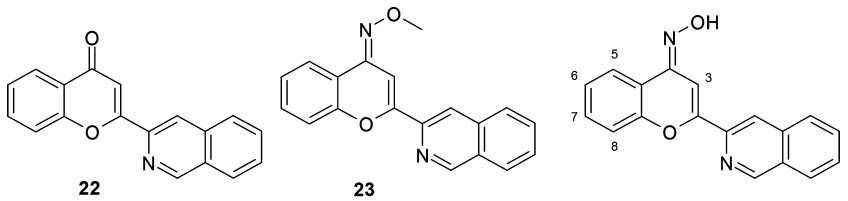
Table 2. Exploration of the Core Chomenone Oxime of Compound 11




| compd | substituents | mGluR4 PAM $EC_{50}$ ($\mu$M)[a] | compd | substituents | mGluR4 PAM $EC_{50}$ ($\mu$M)[a] |
|---|---|---|---|---|---|
| **11** | H | 0.31 ± 0.18 | **30** | 7-Br | 1.72 ± 0.11 |
| **22** | | NA | **31** | 7-OMe | 1.16 ± 0.31 |
| **23** | | NA | **32** | 8-Cl | NA |
| **24** | 3-Me | NA | **33** | 6-cyclopropyl | 1.01 ± 0.07 |
| **25** | 6-Me | 0.98 ± 1.08 | **34** | 6-O-$(CH_2)_2$-OMe | 0.75 ± 0.40 |
| **26** | 6-$CF_3$ | 2.47 ± 1.72 | **35** | 6- O-$(CH_2)_2$-methylpiperazine | 9.84 ± 4.21 |
| **27** | 6-$OCF_3$ | 1.63 ± 1.59 | **36** | 6- NH-$(CH_2)_3$-methylpiperazine | 5.26 ± 2.85 |
| **28** | 6-Br | 0.69 ± 0.04 | **37** | 7-O-$(CH_2)_2$-OMe | 3.01 ± 2.84 |
| **29** | 6-OMe | 1.39 ± 0.56 | **38** | 7-$(CH_2)_2$-phenyl | 1.74 ± 0.76 |

[a]Values are the mean (±SD) of a minimum of three independent experiments. NA: non active.

(6-(2-morpholin-4-yl-ethoxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one oxime, PXT001687)[19] as a representative candidate of the series with significant antiparkinsonian activity in preclinical rodent models of Parkinson's disease.

## SAR FROM (−)-PHCCC TO COMPOUND 40

(−)-*N*-Phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxamide ((−)-PHCCC, Figure 1) is the first partially selective mGluR4 PAM described in the literature.[12] It constitutes an unprecedented pharmacological tool that, in its racemic form, was used by multiple teams to demonstrate the therapeutic potential of mGluR4 potentiation in several disease paradigms including Parkinson's disease,[20,21] anxiety,[22] medulloblastoma,[23] pain,[24,25] and multiple sclerosis.[26] However, (−)-PHCCC does not constitute a good drug candidate as it suffers from weak micromolar potency ($EC_{50}$ = 2.25 $\mu$M on mGluR4 with the racemic form), residual mGluR1 NAM activity, and poor brain penetration, forcing pharmacologists to use central administration or toxic DMSO vehicles. Several medicinal chemistry explorations were conducted around (−)-PHCCC in order to improve its properties, but limited successes were reported and this scaffold was long considered as "flat" with any chemical modifications resulting in a loss of mGluR4 PAM activity.[27−29] The only optimized derivative found was VU0359516[30] (Figure 1), a 2-pyridyl amide analogue described with a 10-fold improvement in potency and no longer side activity on mGluR1.[30]

Despite these disappointing reports, we decided to re-examine the potential of (−)-PHCCC scaffold by checking importance of the cyclopropane and amide moieties. Indeed, we considered that removal of the chiral centers of (−)-PHCCC and replacement of the amide right-hand side were key in order to simplify the scaffold for an optimization effort and to increase chances of brain penetration. Analogue **1** (Table 1) was synthesized and was found to retain some level of PAM activity on mGluR4 ($EC_{50}$ = 14.2 $\mu$M). Interestingly, this molecule **1** was previously reported by a team from Vanderbilt University as being inactive at 30 $\mu$M in a Gqi5 functional test (compound **1i** in Supporting Information of ref 28), whereas it behaves as a full PAM in our chimeric Gi/Gq (GqTOP) assay. This illustrates the differences that can be measured depending on the in vitro model systems used[31] and underlines the importance of validating the compounds activity in animal models in the early steps of an optimization program.

Synthetic routes of the different compounds described in this article are detailed in the Supporting Information (SI).

Encouraged by the result obtained with **1**, we decided to further explore the styryl moiety and prepared a library of rigidified analogues (see Table 1). First, rigidification with a naphthyl or several 5−6 and 6−6 bicyclic heterocyclic groups led to very weak or inactive analogues **2**−**8**. However, quinolinyl **9**−**10** and isoquinolinyl **11** were active, and position of the nitrogen $\alpha$ to the link with the chromenone oxime central core seems to be important to reach submicromolar potency, compound **11** showing a 7-fold improved potency compared with PHCCC. This was further confirmed with good levels of potency obtained with other 6−6 and 6−5 bicyclic heterocycles, **12**−**19** all having a nitrogen atom positioned similarly as in isoquinolinyl **11**, and with the absence of activity of compound **20**, isomer of the active compounds **14** or **17**. Interestingly, although a bicyclic heterocyle with a "rightly" positioned nitrogen is crucial for activity, it seems to be sensitive to decoration as the methyl substitution of **21** prevents mGluR4 PAM activity.

In parallel to the study of aromatic substituents of the chromenone oxime described in Table 1, we decided to expand the exploration of the core scaffold with analogues of the representative compound **11** (Table 2). First, we observed that modifications of the oxime function, changed for a keto function in **22** or substituted by a methyl group in **23**, resulted in inactive molecules. As a matter of fact, this observation correlates with the results obtained by Williams et al., showing that modifications of the oxime moiety of VU0359516 always result in loss of activity.[30] Second, we explored the influence of small substituents on the core group. We observed that substitution of position 3, close to the oxime moiety, with a methyl group (compound **24**) was not tolerated. This was not the case for position 6 (compounds **25**−**29**) and position 7 (compounds **30** and **31**) that do tolerate small substituents ranging from methyl to bromine atom. However, position 8 appeared to be more sensitive as the chloro substituted **32** was inactive on mGluR4. We then explored the possibility to add larger groups at positions 6 and 7 that are chemically more accessible. All the tested bulkier groups resulted in compounds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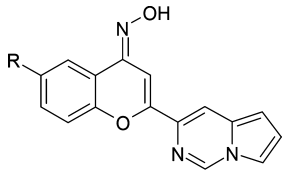
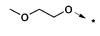
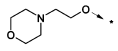
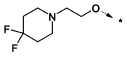
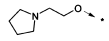
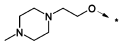
still potent on mGluR4 with $EC_{50}$ ranging between 0.75 to 9.8 $\mu$M (compounds **33**−**38**). Both polar and nonpolar groups were introduced, illustrating the fact that positions 6 and 7 can constitute a handle to improve different properties of the molecules.

On the basis of the observations made with compound **11** analogues, we next investigated the effects of introductions of polar groups on position 6 of compound **14** that showed mGluR4 PAM activity with a potency of 110 nM. The objective of this investigation was to increase the solubility of **14** that was rather poor (0.2 $\mu$M in Milli-Q water), making the in vivo characterization of this molecule challenging. It was found that, to a greater extent than with compound **11**, introduction of polar groups was well tolerated and even slightly improved the mGluR4 PAM activity with compound **40** that seemed to bear an ideal substituent in terms of polarity and/or basicity (Table 3). Water solubility was also clearly improved, with a 53000-fold increase for the HCl salt of compound **40** compared with **14**. However, all polar groups were not similarly tolerated, as illustrated with compounds **42** and **43** that exert more than 10-fold decrease of activity compared with compound **14** (Table 3).

**Table 3. Introduction of Polar Groups on Molecule 14**




| Compounds | R | mGluR4 PAM $EC_{50}$ ($\mu$M)[a] | Water solubility ($\mu$M)[b] |
|---|---|---|---|
| **14** | H | 0.11 ± 0.03 | 0.2 |
| **39** |  | 0.16 ± 0.08 | 16.3 |
| **40** |  | 0.046 ± 0.018 | 10 600 |
| **41** |  | 0.15 ± 0.09 | ND |
| **42** |  | 2.34 ± 0.67 | ND |
| **43** |  | 1.71 ± 0.98 | ND |

[a]Values are the mean (±SD) of a minimum of three independent experiments. [b]Thermodynamic solubility measured in milli-Q water. ND: not determined.

## IN VITRO PROPERTIES OF COMPOUND 40

Having identified compound **40**, a potent mGluR4 PAM with clearly improved water solubility, we further extended its characterization in vitro. Its mode of action on mGluR4 was studied using the human recombinant receptor expressed in a transfected human cell line (HEK 293 cells). These cells were also transfected with a plasmid encoding a chimeric G protein that allowed redirection of the activation signal to intracellular calcium pathway. Receptor activity was then detected by changes in intracellular calcium, measured using a fluorescent calcium-sensitive dye (Fluo4AM, Molecular Probes). Agonist and PAM activities of compound **40** were consecutively evaluated on the same cell plate. Agonist activity was first tested for 10 min with the addition of the compound alone in the cell media. Cells were then stimulated by addition of glutamate at a concentration that resulted in 20% of the maximal effect ($EC_{20}$), and fluorescence was recorded for an additional 3 min. Agonist or PAM activities were evaluated in comparison to, respectively, basal signals evoked either by the buffer or an $EC_{20}$ glutamate concentration. For potency and efficacy determination, a concentration−response test of compound **40** was performed. In addition, the mode of action of compound **40** was further characterized in experiments assessing the shift of glutamate concentration−response curves. In this alternative setting, 10 concentrations of glutamate (ranging over 4.5 logs) were tested alone or in the presence of 4 concentrations of compound **40** (ranging from 0.1 to 3 $\mu$M). Potency of glutamate, alone or in the presence of compound **40**, was determined and used to calculate the fold-increase in the apparent affinity of glutamate. Results showed that in these cell lines, compound **40** had a low agonist activity, with an average stimulation of the receptor of 19 ± 12% at 3 $\mu$M. However, as a PAM, compound **40** potentiated the response of human mGluR4 to glutamate with a potency of 46 ± 18 nM and amplified the effects of the $EC_{20}$ glutamate concentration to 77 ± 2% of the maximal response to glutamate (Figure 2A). For comparison, in the same cellular assay, PHCCC exerts mGluR4 PAM effects with a potency of 2.25 ± 1.07 $\mu$M, which represents an approximately 50-fold improvement in potency for compound **40**. It should be noted here that no clear structure−activity relationship was observed with regard to efficacy modulation as most of the active PAMs described in this study resulted in an efficacy between 75% and 100% of the maximal response to glutamate. Moreover, increasing concentrations of compound **40** progressively produced a leftward shift in the glutamate concentration−response relationship and increased the potency of glutamate for mGluR4 by approximately 10-fold at 3 $\mu$M (Figure 2B). Compound **40** had a similar profile in a distinct cell assay that measured the cAMP response following mGluR4 stimulation. Indeed, in this second cellular assay, compound **40** potentiated the response of human mGluR4 to a glutamate $EC_{20}$ with a potency of 70 nM (compared to PHCCC, which had a potency of 2.5 $\mu$M; data not shown) and induced a leftward shift of the glutamate concentration−response curve. In cell lines expressing the rat mGluR4, compound **40** was slightly more potent with an $EC_{50}$ of 27 ± 2 nM, and the responses were enhanced to 75 ± 2% of the maximal response to glutamate (SI, Figure S1).

Then the selectivity of compound **40** for mGluR4 was investigated using human cell lines (HEK 293) expressing each of the other human mGluRs as well as by measuring inhibition of orthosteric ligand binding on the other glutamate receptors, namely NMDA, AMPA, and kainate, on membranes from rat cerebral cortex. Compound **40** had no effect on iGluRs, neither on group I (mGluR1 and 5) nor on group II (mGluR2 and 3) mGluRs up to 10 $\mu$M, the highest concentration tested. Among group III mGluRs, compound **40** showed 13-times higher selectivity for mGluR4 than for mGluR6, was not active on mGluR7 and had a very partial agonist activity on mGluR8 with an average stimulation of the receptor of 23 ± 7% at 10 $\mu$M (Figure 2C). It has to be noted that expression of mGluR6 is strictly restricted to the retina.[32] Thus, it is not expected that an activity of compound **40** on mGluR6 may be confounding with a potential antiparkinsonian effect in animal models. Finally,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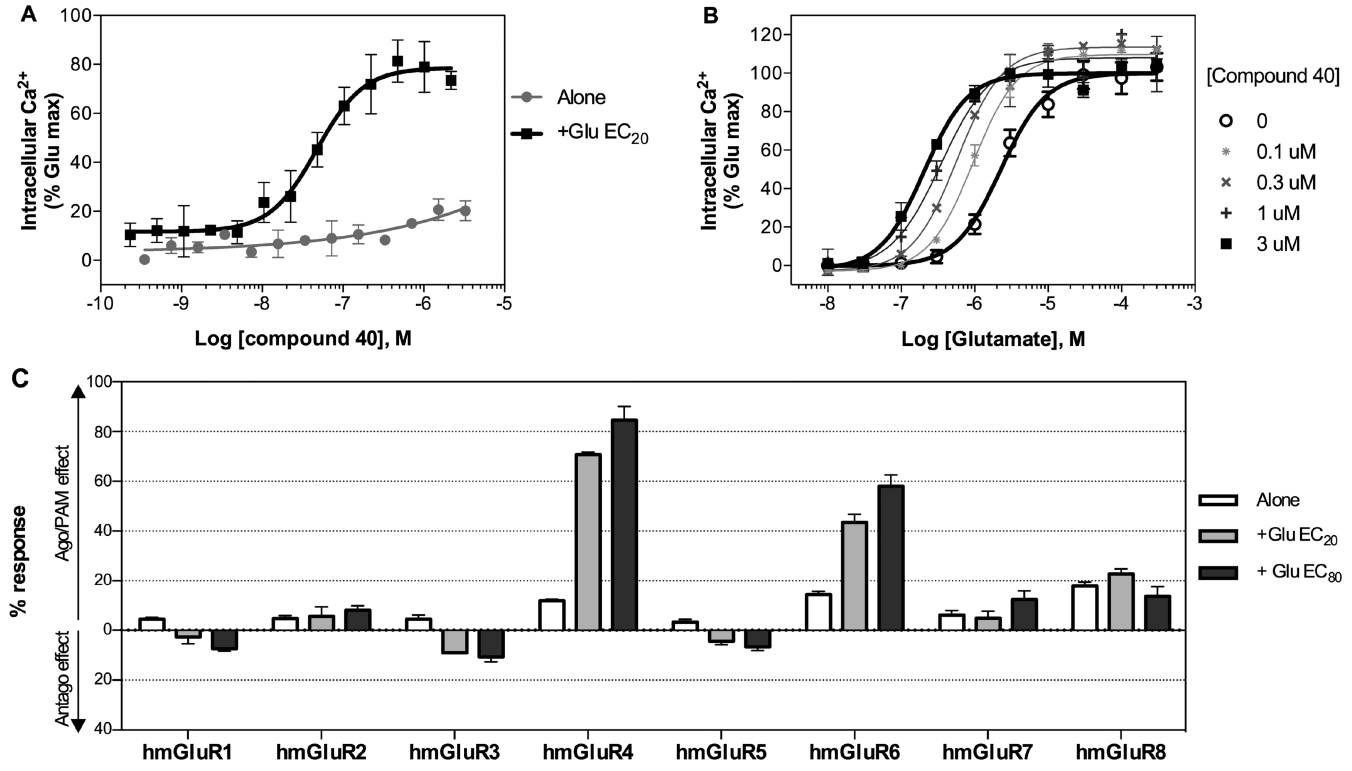


**Figure 2.** In vitro properties of compound **40**. (A) Agonist and PAM activities of compound **40** were consecutively evaluated in HEK293 cells expressing human mGluR4. In these cell lines, compound **40** potentiated the increases in intracellular calcium concentrations induced by an $EC_{20}$ glutamate (PAM effect) but had a minor agonist activity in absence of glutamate. (B) Increasing concentrations of compound **40** induced a 10-fold leftward shift of the glutamate concentration−response curves in these cell lines. (A,B) Each point represents the mean (±SD) of duplicate determination from a representative experiment. (C) Selectivity profile of compound **40** among mGluRs. Compound **40** was tested at 1 $\mu$M on HEK293 cells expressing each of the human mGluR, alone or in the presence of either an $EC_{20}$ or an $EC_{80}$ glutamate. The corresponding glutamate concentrations were determined for each receptor subtype. L-AP4 has been used instead of glutamate on hmGluR7. Each bar represents the mean (+SEM) of activities measured in at least two (when not active) or three experiments.

**Table 4. Mean PK Parameters of Compound 40 Following Intravenous Administration at 1 mg/kg in Rat, and at 2 mg/kg in Mouse**

| 40 | $C_{max}$ (ng/mL) | $t_{1/2}$ (min) | $AUC_{0-\infty}$ (h·ng/mL) | $CL_p$ (mL/min/kg) | $V_d$ (L/kg) | brain/plasma |
|---|---|---|---|---|---|---|
| 1 mg/kg iv (rat) | 444 ± 93[a] | 44 ± 9[a] | 166 ± 36[a] | 113 ± 29[a] | 6.5 ± 0.75[a] | 3.3 ± 0.04[a,c] |
| 2 mg/kg iv (mouse) | 753 ± 80[b] | 12 ± 1.8[a] | 269 ± 39.8[a] | 123 ± 18.2[a] | 6 ± 0.8[a] | 3.6 ± 0.3[b,d] |

[a]Values are the mean (±SEM) of 3 animals [b]Values are the mean (±SEM) of 2 animals. [c]Ratio calculated at 1 h postdose. [d]Ratio calculated at 30 min postdose.

**Table 5. Mean PK Parameters of Compound 40 in Rat Following Intraperitoneal or Oral Administration and in Mouse Following Oral Administration[a]**

| 40 | plasma $C_{max}$ (ng/mL) | plasma $T_{max}$ (min) | plasma $T_{1/2}$ (h) | plasma $AUC_{0-inf}$ (h·ng/mL) | $[brain]_{30\ min}$ (ng/g) | brain/plasma[b] | brain/$rEC_{50}$[b] |
|---|---|---|---|---|---|---|---|
| 10 mg/kg ip (rat) | 1498 ± 62 | 15 ± 0 | 4.0 ± 0.16 | 2245 ± 269 | 5647 ± 765 | 3.1 ± 0.3 | 3.6 |
| 30 mg/kg ip (rat) | 9324 ± 745 | 15 ± 0 | 4.5 ± 0.7 | 11349 ± 1 433 | 22792 ± 1 111 | 3.1 ± 0.05 | 14.5 |
| 10 mg/kg po (rat) | 135 ± 39 | 20 ± 5 | 7.3 ± 1.7 | 903 ± 223 | 454 ± 28 | 2.3 ± 0.4 | 0.3 |
| 10 mg/kg po (mouse) | 229 ± 50 | 30 ± 0 | ND | ND | 390 ± 78 | 1.7 ± 0.34 | 0.2[c] |

[a]ND: not determined. $rEC_{50}$: $EC_{50}$ measured in cell lines overexpressing the rat mGluR4 receptor. Values are the mean (±SEM) of 3 rats or 2 mice. [b]Ratio calculated at 30 min postdose. [c]The mouse $EC_{50}$ was assumed to be the same as the $rEC_{50}$.

not only the potency but also the selectivity for mGluR4 has been improved compared with (−)-PHCCC because compound **40** has no longer any activity on mGluR1.

Additionally, compound **40** was also evaluated on other targets of importance for Parkinson's disease. Compound **40** at 10 $\mu$M showed no functional activity (neither agonist nor PAM nor antagonist activity) on D1, $D2_L$, and $A_{2A}$ receptors. No effect was observed up to 10 $\mu$M on COMT, MAO-A, and MAO-B, reducing the risk for interaction with L-dopa metabolism in animal models and in Parkinson's disease patients. Finally, activity of compound **40** was also assessed on a panel of diverse kinases (e.g., Flt3, GSK3$\beta$, IRAK4, JAK3, TAK1). Except for a 64% inhibition of Flt3, no kinase inhibition was detected at 10 $\mu$M. Altogether, these results demonstrate that compound **40** is both potent and selective for mGluR4.



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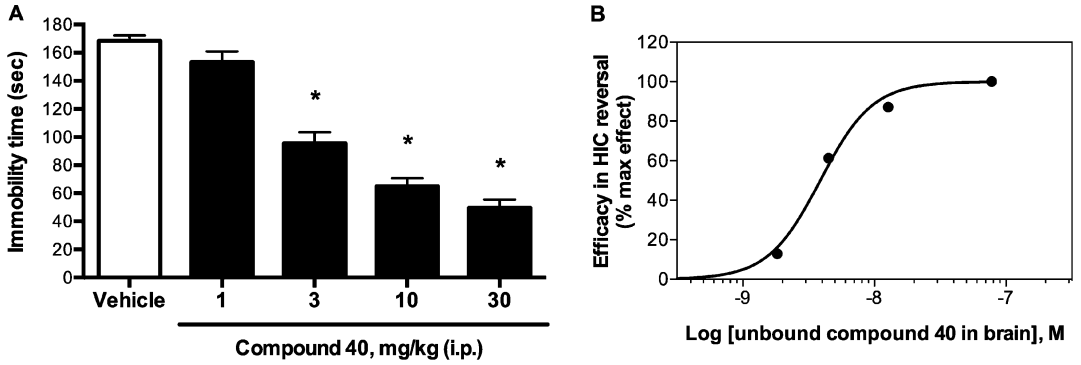
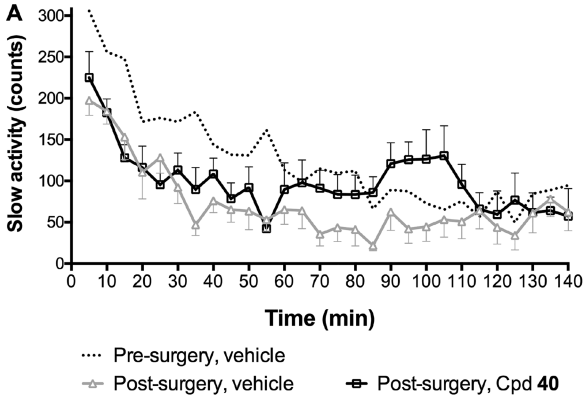



**Figure 3.** Reversal of haloperidol-induced catalepsy in mice by compound **40**. Mice were administered haloperidol (0.5 mg/kg, ip), and 1 h later, when catalepsy was present, they received an ip dose of compound **40** or vehicle. Catalepsy was assessed 40 min after compound **40** dosing. (A) Each bar represents the mean (+SD) latency to move on a vertical grid (immobility time). *$p < 0.05$ when compared to Vehicle (one-way ANOVA followed by Dunn's multiple comparison) ($n$ = 8/group). (B) Relationship between efficacy in reversal of haloperidol-induced catalepsy and compound **40** unbound fraction exposed in the brain.




B

| Time (min) | Plasma (ng/mL) | Brain (ng/g) | Brain unbound [a] (ng/g) | Brain unbound /rEC$_{50}$ [b] |
|---|---|---|---|---|
| 30 | 7396 | 22792 | 173 | 14.5 |
| 60 | 3557 | 12027 | 91 | 7.6 |
| 120 | 535 | 1739 [c] | 13 | 1.1 |
| 240 | 99 | 322 [c] | 2 | 0.2 |

a: calculated using the unbound fraction in rat brain (0.76%)
b: EC$_{50}$ measured in cell lines overexpressing the rat mGluR4 (12 ng/mL)
c: calculated using the mean Brain/Plasma ratio measured at 30 and 60 min (3.25)

**Figure 4.** Antiparkinsonian effect of stand-alone compound **40** treatment on locomotor activity of 6-OHDA-lesioned rats. (A) Time-course of spontaneous locomotor activity in adult male Sprague–Dawley rats before ("Presurgery, vehicle", dotted line) or after 6-OHDA-induced striatal lesions. Lesioned rats were treated either with vehicle ("Postsurgery, vehicle", gray curve) or with compound **40** at 30 mg/kg ip ("Postsurgery, **40**", black curve). Each time-point represents the mean counts (±SEM) for every 5 min. Compound **40** was administered ip at T0. ($n$ = 10). (B) Compound **40** plasma and brain exposures in rats dosed with 30 mg/kg ip. Brain exposure was measured 30 and 60 min after administration. Brain levels at 120 and 240 min (c) were extrapolated from measured plasma exposure by using an average brain/plasma ratio of 3.25 as determined at 30 and 60 min. Unbound fraction of compound **40** in brain (a) was calculated by using the brain protein binding previously measured (99.24%, i.e. 0.76% unbound fraction). Brain unbound over rEC$_{50}$ ratio (b) was then calculated by using the in vitro rat mGluR4 EC$_{50}$ obtained in HEK293 cells ($n$ = 3).

## PHARMACOKINETIC PROPERTIES OF COMPOUND 40

We next evaluated the pharmacokinetic properties of compound **40** in rat and mouse. Following intravenous administration, compound **40** had a very similar pharmacokinetic profile in both species, with a high clearance (113 and 117 mL/min/kg in rat and in mouse, respectively), a high volume of distribution (6.5 and 6 L/kg in rat and mouse, respectively), and a short half-life (44 and 12 min in rat and mouse, respectively) (Table 4). In both mouse and rat, compound concentrations were 3-fold higher in the brain than in plasma, indicating that the compound is CNS penetrant and has a preferential exposure in brain (Table 4).

Compound **40** was administered intraperitoneally at 10 and 30 mg/kg or orally at 10 mg/kg in rats (Table 5). Higher than dose-proportional increases were observed in plasma $C_{max}$ and $AUC_{0-\infty}$ following intraperitoneal administration from 10 to 30 mg/kg, and plasma concentrations were at maximum 15 min after administration. Measurements made after oral administration of 10 mg/kg revealed that the compound has a medium oral bioavailability in rats ($F$ = 54%). All animals were exposed to compound **40** at the first sampling time, 15 min postoral dose, confirming drug absorption, with $C_{max}$ reached 20 min after administration (Table 5). Following a single dose of compound **40**, complete elimination was observed, with concentrations between 2% and 10% of the maximum concentrations observed at 24 h postdose. The mean half-life was 7.3 h with an oral dose of 10 mg/kg. The pharmacokinetics of compound **40** in mouse is qualitatively similar to those in rat.

This PK profile, together with the high brain protein binding measured in rat brain homogenate (99.24%), revealed that brain exposures are greater than the in vitro EC$_{50}$ values after intraperitoneal administration of 10 and 30 mg/kg but not after oral administration of 10 mg/kg in rat (Table 5). Assuming a linear PK profile after oral administration, a dose of 100 mg/kg would have to be administered in rats in order to reach brain exposures greater than EC$_{50}$ and expect to see a pharmacodynamic effect in vivo.

On the basis of these in vitro and in vivo parameters, compound **40** was chosen for further characterization in rodent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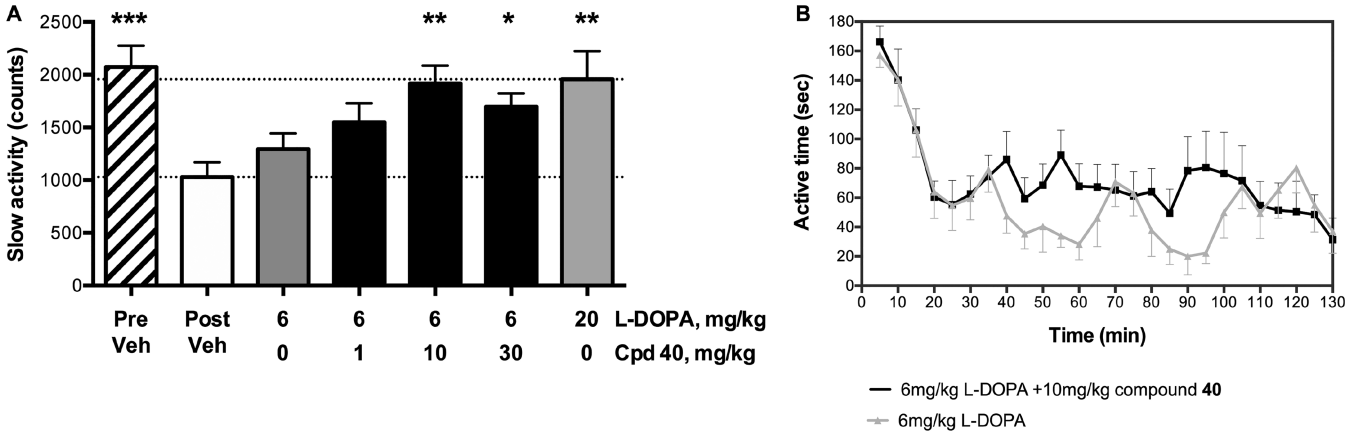


**Figure 5.** Antiparkinsonian effect of the combined treatment with compound **40** and a low suboptimal dose of L-dopa on locomotor activity of 6-OHDA-lesioned rats. (A) Total spontaneous locomotor activity values are presented. Data are expressed as mean of group (+SEM) and analyzed using one-way analysis of variance (ANOVA) repeated measures followed by Dunnett's multiple comparisons. ***$p < 0.001$, **$p < 0.01$, * $p < 0.05$ vs Post Veh. The dotted horizontal lines represent the mean locomotor activity of the control conditions in 6-OHDA-lesioned rats: optimal L-dopa (upper line) and vehicle (lower line). Pre Veh: rats activity before the surgery, after vehicle ip administration. Post Veh: activity of the same animals after the surgery and stabilization of the 6-OHDA-induced striatal lesion, after vehicle ip administration; compound **40** ip administration. ($n$ = 10). (B) Time-course of spontaneous locomotor activity of 6-OHDA-lesioned rats treated either with a low suboptimal dose of L-dopa alone ("6 mg/kg L-dopa", gray curve) or with addition of compound **40** at 10 mg/kg ip ("6 mg/kg L-dopa + 10 mg/kg compound **40**", black curve). Each time-point represents the mean counts (±SEM) for every 5 min ($n$ = 10).

models of Parkinson's disease, and SAR efforts were pursued in order to improve the oral PK profile of the series.

## ■ PARKINSON'S DISEASE MODELS

As a preliminary model of Parkinson's disease motor symptoms, the ability of compound **40** to reverse haloperidol-induced catalepsy was assessed in mouse. Mice received an injection of haloperidol (0.5 mg/kg, ip) to induce catalepsy, and 1 h later, while catalepsy was present, animals were administered an ip dose of compound **40**. Catalepsy was then assessed 40 min after compound **40** dosing. As shown in Figure 3A, compound **40** produced a dose-dependent reversal of catalepsy, with significant effects obtained between 3 and 30 mg/kg ip, which is in accordance with the prediction based on the PK profile of compound **40**. In this study, we measured the compound exposure in mouse brain at the end of the experiment to determine the in vivo $EC_{50}$. Using the free fraction previously determined in rat brain (0.76%), we extrapolated the concentrations of free compound **40** in brain from the measured total brain concentrations (SI, Table S1). The corresponding PK/PD relationship is shown in Figure 3B and indicates that the in vivo $EC_{50}$ in brain is around 5 nM, which corresponds well with the in vitro $EC_{50}$ on rat mGluR4 (27 nM), inferring that the effect seen in this model is mGluR4-related.

We next evaluated the effects of compound **40** in a more elaborated rodent model of Parkinson's disease motor symptoms. Compound **40** was administered to rats that had previously undergone a bilateral lesion of the striatum induced by 6-hydroxydopamine (6-OHDA). In most models utilizing the neurotoxin 6-OHDA, animals are dopamine depleted only unilaterally.[33,34] While dopamine loss in human PD can be asymmetrical at the earliest stages of the disease, it ultimately results in dopamine loss in both hemispheres.[35,36] To select a context that is closer to the human condition, we chose a model where dopamine is being depleted bilaterally. Male Sprague–Dawley rats received bilateral injections of 6-OHDA into the striatum, which induced parkinsonian motor deficits indicated by reduced spontaneous activity and rearing in the open field arena (Figure 4A, SI, Figure S2a). Thus, active time, slow activity (i.e., nonstereotyped activity), and rearing time were significantly reduced to 64%, 66%, and 68% respectively, by the 6-OHDA-induced bilateral lesion compared with presurgery (SI, Figure S2a, "Post" versus "Pre"). When administered alone, compound **40** (30 mg/kg, ip) induced an increase in active time, slow activity, and rearing time compared with vehicle administration (SI, Figures S2a and 4a). The time course analysis reveals that effects of compound **40** on slow activity were observable during the 30–120 min postadministration period (Figure 4A). Comparison of pharmacokinetics and pharmacodynamic effects demonstrates an excellent correlation between the increase of compound **40** exposure in brain (Figure 4B) and the efficacy in improving the slow activity (Figure 4A). Indeed, compound **40** effects become observable from 30 min postadministration, and at 30 and 60 min after ip dosing at 30 mg/kg, brain exposures were respectively 14.5- and 7.6-fold greater than the in vitro $EC_{50}$ at rat mGluR4 (Figure 4B), supporting that the effect seen in this model is mGluR4-related. Assuming a linear brain/plasma ratio over time, brain exposure would barely reach the mGluR4 $EC_{50}$ at 120 min after administration, which is in accordance with the loss of effect of compound **40** at that time point in this rat model (Figure 4A,B).

In a recent study, Jenkins et al.[37] have shown that treatment of rodents with 6-OHDA may slightly decrease the expression of mGluR4 in brain. Although we have used a different model, where 6-OHDA induced a milder bilateral lesion, it was important to do this PK/PD correlation in order to support target engagement in the antiparkinsonian effects of compound **40**.

Then, parkinsonian rats received L-dopa either alone or in combination with compound **40**. As expected, L-dopa was effective in improving motor performance, inducing a dose-dependent increase in locomotor activity compared with vehicle administration (Figure 5A). Dose–response curves performed with L-dopa (3, 6, and 20 mg/kg) showed that 20


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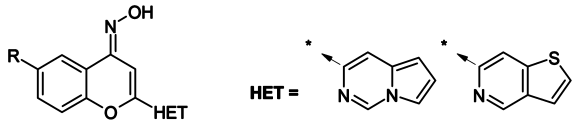
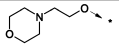
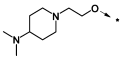
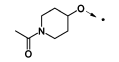
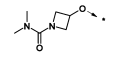
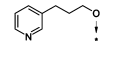
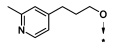
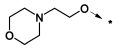
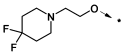
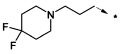
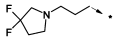
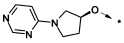
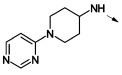
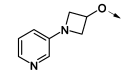
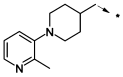
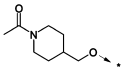
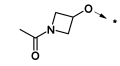
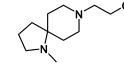
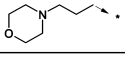
Table 6. Further Optimization of Brain Penetration




| Compounds | HET | R | mGluR4 PAM $EC_{50}$ (nM) [a] | $[Brain]_{30min}$ (ng/g) [b] | $[Brain]_{60min}$ (ng/g) [b] | $[Brain]_{90min}$ (ng/g) [b] |
|---|---|---|---|---|---|---|
| **40** | A |  | 46 ± 18 | 454 | 262 | ND |
| **44** | A |  | 165 ± 7 | 2.8 | 2.9 | ND |
| **45** | A |  | 10 ± 1 | 24.1 | 12.1 | ND |
| **46** | A |  | 35 ± 8 | 25.5 | 8.8 | ND |
| **47** | A |  | 50 ± 16 | 3.4 | 2.3 | ND |
| **48** | A |  | 19 ± 7 | 77.3 | 82.5 | ND |
| **49** | B |  | 35 ± 8 | 408 | 521 | 281 |
| **50** | B |  | 90 ± 14 | 239 | 207 | 194 |
| **51** | B |  | 106 ± 20 | 157 | 201 | 100 |
| **52** | B |  | 25 ± 5 | 275 | 197 | 137 |
| **53** | B |  | 17 ± 4 | 20 | 9.5 | ND |
| **54** | B |  | 53 ± 20 | ND | BLQ | ND |
| **55** | B |  | 4 ± 0 | 33.4 | 26.7 | ND |
| **56** | B |  | 4 ± 1 | 7.4 | BLQ | ND |
| **57** | B |  | 6 ± 2 | 10.7 | 6.5 | ND |
| **58** | B |  | 53 ± 6 | BLQ | BLQ | ND |
| **59** | B |  | 60 ± 5 | BLQ | BLQ | ND |
| **60** | B |  | 79 ± 19 | 458 | 785 | 818 |

[a]Values are the mean (±SD) of a minimum of three independent experiments. [b]Values of brain concentrations are mean of three rats following oral administration at 10 mg/kg. BLQ: concentration below lower limit of quantification (1 ng/mL). ND: not determined.

mg/kg L-dopa represented an optimal antiparkinsonian dose, restoring the motor activity of the rats to normal levels (Figure 5A), while the dose of 6 mg/kg L-dopa was selected as an ineffective subthreshold dose for use in subsequent experiments. When compound **40** was coadministered with the subthreshold low dose of L-dopa, we observed a significant improvement in motor performance that showed dose dependence for compound **40** (Figure 5A). Thus, the combination of 6 mg/kg L-Dopa and 10 mg/kg compound **40** was able to fully reverse the motor deficit of parkinsonian rats and reached the same level of efficacy as the high dose of 20 mg/kg L-dopa (Figure 5A). Consequently, in this rat model of Parkinson's disease, administration of compound **40** (10 mg/kg, ip) allowed a decrease by 70% of the dose of L-dopa while maintaining the same antiparkinsonian efficacy. As shown in Figure 5A,B, compound **40** induced a dose- and time-dependent antiparkinsonian effect, with the highest improvement obtained at the dose of 10 mg/kg (Figure 5A), from 30 to 120 min postadministration of compound **40** (Figure 5B). At the highest dose of 30 mg/kg, the antiparkinsonian effect of



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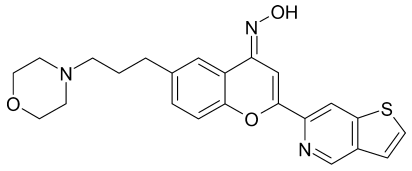
Table 7. Summary of Key Parameters for Compound 60



Compound 60 (PXT002331)

| | in vitro pharmacology[a] | | | | | |
|---|---|---|---|---|---|---|
| | hum ago $EC_{50}$ | hum PAM $EC_{50}$ | (hum fold shift) | | | rat PAM $EC_{50}$ |
| mGluR4 | >15 $\mu$M | 79 nM | 10 (at 3 $\mu$M) | 10 (at 3 $\mu$M) | 10 (at 3 $\mu$M) | 47 nM |
| selectivity | group I<br>not active | group II<br>not active | mGlu6<br>15× | mGlu7<br>110× | mGlu8<br>50× | NMDA, AMPA, kainate<br>not active |
| specificity | COMT<br>not active | MAO-A<br>not active | MAO-B<br>not active | MAO-B<br>not active | MAO-B<br>not active | |
| | in vivo pharmacokinetics (rat)[b] | | | | | |
| 1 mg/kg (iv) | plasma $C_{max}$ (ng/mL) | $t_{1/2}$ (h) | plasma $AUC_{0-inf}$ (h·ng/mL) | $CL_p$ (mL/min/kg) | $V_d$ (L/kg) | brain/plasma[c] |
| | 325 + 30 | 0.90 + 0.15 | 163 + 15 | 104 + 10 | 8.02 + 1.28 | 8.4 + 0.7 |
| 10 mg/kg po | plasma $C_{max}$ (ng/mL) | plasma $T_{max}$ (h) | plasma $AUC_{0-inf}$ (h·ng/mL) | brain $AUC_{0-inf}$ (h·ng/g) | | brain/plasma[d] |
| | 94 + 21 | 1.17 + 0.44 | 432 + 126 | 2713 + 544 | | 6.6 + 0.6 |

[a]Activities were measured at least in duplicates. Values are the mean of at least three experiments. [b]Values are the mean (±SEM) of three rats. [c]Ratio calculated at 1 h postdose. [d]Ratio of $AUC_{0-inf}$.

compound **40** in combination with 6 mg/kg of L-dopa has reached a plateau with a maximal efficacy equivalent to the normal activity of prelesion rats (Figure 5A).

In this model consisting of a partial bilateral lesion of the striatum induced by 6-OHDA, the dose of 20 mg/kg L-dopa did not induce dyskinesia. However, this dose produced an increase of rearing activity that is above normal activity measured before the lesion (SI, Figure S3a). This abnormally high rearing activity is considered as an early sign of potential dyskinesia development in this model. Thus, it is noteworthy that none of the conditions tested with compound **40** induced an overactive rearing behavior, neither the high dose of compound **40** alone nor any dose of compound **40** in combination with L-dopa (SI, Figure S3b). Consequently, in this model of Parkinson's disease, compound **40** completely reversed the motor deficits without increasing the risk of developing dyskinesia.

As a first conclusion, in the 6-OHDA rat model of Parkinson's disease motor symptoms, administration of compound **40** allowed a decrease by 70% of the dose of L-dopa while maintaining the same antiparkinsonian efficacy without inducing any adverse event such as overactive rearing, stereotyped behavior or dyskinesia. These results show supportive evidence that compound **40** could be developed as a potential L-dopa-sparing treatment in PD, preserving maximal antiparkinsonian benefits without the need to use high L-dopa dosage. Importantly, compound **40** demonstrated consistent antiparkinsonian activities in two rodent models and exhibited robust PK/PD relationship.

## FURTHER IMPROVEMENTS OF PK PROPERTIES, IDENTIFICATION OF CANDIDATE 60

Encouraged by the promising results in PD models obtained with ip administration of compound **40**, we next decided to pursue the optimization of the PK profile with the objective of obtaining an optimized compound with increased brain exposure over time and thus improved potential target engagement after oral administration. Table 6 and SI, Figure S4 show the brain exposures of diverse molecules with good mGluR4 PAM activities ($EC_{50}$ between 4 and 165 nM). As a comparator, concentrations of 454 and 262 ng/g of compound **40** were measured in rat brains 30 and 60 min postadministration of an oral dose of 10 mg/kg (Tables 5 and 6). Within the same pyrrolopyrimidine series (HET = A in Table 6), we did not identify substituent that give rise to a better brain exposure. Indeed, replacements of the morpholine moiety of compound **40** by a 4-(dimethylamino)-piperidine (compound **44**), a close amide (compound **45**) or urea (compound **46**), or pyridine moieties (compounds **47** and **48**) were detrimental to brain exposures (Table 6). As a consequence, none of these first analogues of compound **40** had brain exposure at concentrations higher than 100 ng/g at 30 or 60 min when given orally at 10 mg/kg. By contrast, changing the right-hand side heterocycle moiety for a thienopyridine (HET = B in Table 6) seemed rather beneficial for brain exposure. Indeed, compound **49** showed much higher brain concentrations 60 min postdosing compared with the close compound **40** and was even detected with concentrations above 200 ng/g 90 min after oral administration (Table 6). This encouraged us to explore further thienopyridine with similar basic left-hand side chains (compounds **50**–**52**), but these three compounds did not show improvement compared with **49** despite a similar or more potent activity. Similarly to what was observed in pyrrolopyrimidine series, introduction of an aromatic group (compounds **53**–**56**) or modifications of the morpholine moiety for close piperidine or azetidine analogues (compounds **56**–**59**) also resulted in very low brain exposures (Table 6). Interestingly, however, some of these compounds showed a clear increase in their mGluR4 PAM potency as illustrated with molecules **55**–**57**. Finally, the only chemical change in **49** resulting in an increased brain exposure was the replacement of the oxygen atom linking the chromenone oxime scaffold with the left-hand side chain by a $-CH_2-$ group. Indeed, compound **60** was found at concentrations well-above 400 ng/g (~1 $\mu$M) up to



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90 min after dosing (Table 6). This isosteric modification did not alter the mGluR4 PAM activity because compound **60** exerts a PAM activity on mGluR4 with an $EC_{50}$ that is similar to the ones of close analogues **49** or **40** (Table 6).

Having identified compound **60** as a potent mGluR4 PAM with clearly improved brain exposure following oral administration, we further extended its characterization. Key characteristics of compound **60** are summarized in Table 7. The in vitro properties of compound **60** are very close to those of compound **40**. Indeed, compound **60** had minor agonist activity, with an average stimulation of the receptor of 32 ± 8% at 15 $\mu$M, and acted as a PAM on mGluR4, with a potency of 79 ± 19 nM, amplifying the effects of the $EC_{20}$ glutamate concentration to 70 ± 5% of the maximal response to glutamate (SI, Figure S5). Compound **60** increased the apparent affinity of glutamate for mGluR4 by 10-fold (Table 7, SI, Figure S5) and had no effect on NMDA, AMPA, kainate, group I (mGluR1 and 5), or group II (mGluR2 and 3) mGluRs up to 10 $\mu$M, the highest concentration tested. Among group III mGluRs, compound **60** showed more than 15-, 110- and 50-times higher selectivity for mGluR4 than for mGluR6, -7, and -8, respectively (Table 7, SI, Figure S5). Compound **60** had no effect on COMT, MAO-A, or MAO-B at 10 $\mu$M in enzymatic assays (Table 7), reducing the risk for interaction with L-dopa metabolism.

PK characteristics of compound **60** in rats are given in Figure 6 and Table 7. Compound **60** displayed excellent CNS

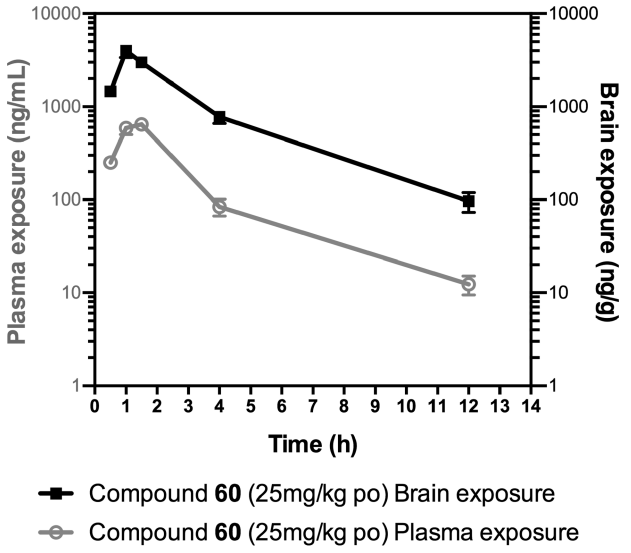


**Figure 6.** PK profile of compound **60** in plasma and brain following oral administration at 25 mg/kg (in water) to Sprague–Dawley rats.

penetration after oral dosing, with more than 6-fold (and up to 13.5-fold depending on the dose) higher exposure in brain than in plasma (Table 7). Moreover, the close correlation between plasma and brain concentration curves over time indicates that plasma concentrations are good predictors of compound **60** concentration levels and kinetics in the brain (Figure 6).

Altogether, these characteristics demonstrate that compound **60** is a potent and selective mGluR4 PAM, with high brain exposure measured after oral administration. The compound **60** will be extensively characterized in another article, to be published in due course.

## CONCLUSION

After a thorough development from (−)-PHCCC and extensive knowledge gained on the medicinal chemistry and pharmacology of mGluR4 PAMs, it has been possible to generate a novel series of compounds with an improved profile and with significant antiparkinsonian activity demonstrated in validated rodent models of PD motor symptoms. On the basis of its complete preclinical profile, compound **60** has been identified as the most promising candidate, which could be further evaluated in the clinic.

## EXPERIMENTAL SECTION

**Chemistry.** All reagents were commercial grade and used without further purification. Commercially available anhydrous solvents were used for reactions conducted under inert atmosphere. Silica gel generally used for column chromatography was SDS silica gel (60AAC 40–63 $\mu$M). Thin layer chromatography was carried out using precoated silica gel F-254 plate. $^1$H NMR spectra were recorded on a Bruker AMX-400 operating at 400.33 MHz. $^{13}$C NMR spectra were recorded on a Bruker AVANCE I 400 Fourier transform spectrometer, operating at 100.67 MHz, using a 5 mm QNP probe operating at 300 K. Proton chemical shifts are listed relative to residual $CDCl_3$ (7.27 ppm), DMSO-$d_6$ (2.51 ppm) or $D_2O$ (4.60 ppm). Splitting patterns are designated as s (singlet), d (doublet), dd (double–doublet), t (triplet), tt (triplet–triplet), td (triplet-doublet), q (quartet), quint (quintuplet), sex (sextuplet), sept (septuplet), m (multiplet), b (broad). For $^{13}$C NMR spectrum: The chemical shifts ($\delta$) were measured in ppm and referenced from the central peak of DMSO-$d_6$ (39.5 ppm). Electrospray MS spectra were obtained on a Waters micromass platform LCMS spectrometer. All mass spectra were full-scan experiments (mass range 100–800 amu). Mass spectra were obtained using electrospray ionization. The HPLC system was a Waters platform with a 2767 sample manager, a 2525 pump, and a photodiode array detector (190–400 nM). The column used was an XBridge C18 3.5 $\mu$M (4.6 mm × 50 mm) in analytical mode and an XBridge C18 OBD 5 $\mu$M (30 mm × 100 mm) in preparative mode. The mobile phase in both cases consisted in an appropriate gradient of A and B. A was water with 0.05% of TFA and B was MeOH with 0.05% of TFA. Flow rate was 1 mL per min in analytical mode and 25 mL min in preparative mode. All LCMS were performed at room temperature. At the end of each preparative HPLC, the tubes were collected and TFA was neutralized with potassium carbonate before extraction or filtration of the product. Microwave experiments were performed on a Biotage Initiator. The microwave modulates the power in order to reach the selected temperature as fast as possible. The time of each experiment is the time at the selected temperature. High resolution mass spectroscopy was measured for compounds **40** and **60** using a LC-UV-MS/MS Agilent 1200 with a QToF 6520 detector in + ESI. Melting points are measure on a Barnstead Electrothermal 9100 and are not corrected. Oxime final compounds were isolated as >95% of isomer *E* according to NMR analysis. The purity of final compounds was measured by HPLC and was found to be above 95%.

General synthetic routes of all compounds described in this article are detailed in the Supporting Information. A few key intermediates were prepared for all these syntheses, they were numbered with three-digit numbers, and their preparations are described after the description of compound **1**–**60** syntheses.

*2-Styryl-chromen-4-one Oxime (**1**).* 2-Styryl-chromen-4-one was prepared in a manner analogous to **2** (73%), starting from cinnamoyl chloride. LCMS, $m/z$ = 249.0 $[M + H]^+$. Then, oxime formation was obtained as follows: a mixture of 2-styryl-chromen-4-one (150 mg, 0.60 mmol) and hydroxylamine hydrochloride (84 mg, 1.2 mmol) in anhydrous methanol (12 mL) was subjected to microwave irradiation at 130 °C for 20 min. Methanol was removed under vacuum, and the crude solid was purified by column chromatography on silica gel (using 0–30% ethyl acetate in cyclohexane as eluent) to give 2-styryl-chromen-4-one oxime (**1**, 65 mg, 41%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.98 (s, 1H), 7.86 (dd, $J$ = 7.9, 1.6 Hz, 1H),


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7.68–7.65 (m, 2H), 7.52–7.46 (m, 1H), 7.45–7.33 (m, 5H), 7.27–7.22 (m, 1H), 7.11 (d, $J$ = 16.1 Hz, 1H), 6.70 (s, 1H). MS (ESI+): 264.1 $[C_{17}H_{13}NO_2 + H]^+$ ($m/z$); mp 196–199 °C.

*2-Naphthalen-2-yl-chromen-4-one oxime (**2**).* To a cold suspension of 2-naphthoic acid (2.0 g, 11.6 mmol) in dichloromethane (60 mL) were added oxalyl chloride (1.1 mL, 12.6 mmol) and dimethylformamide (50 μL). The reaction mixture was stirred at room temperature for 2 h and concentrated to dryness to give crude 2-naphthoic acid chloride (2.5 g). The crude acid chloride was dissolved in dry pyridine (50 mL), cooled to 0 °C, and 2-hydroxy-acetophenone (1.43 g, 10.5 mmol) added. The reaction mixture was heated at 60 °C for 2 h before being poured onto ice-cold water (150 mL). The solution was acidified to pH 1 with concentrated hydrochloric acid, and the precipitate was collected by filtration, washed with water, and dried under vacuum to give naphthalene-2-carboxylic acid 2′-acetyl-phenyl ester (2.7 g, 80%). The crude ester was dissolved in DMSO (30 mL), and crushed potassium hydroxide (1.4 g, 25.8 mmol) was added. The reaction mixture was stirred at room temperature for 14 h before being poured onto ice-cold water and acidified to pH 3–4 with a 6N aqueous hydrochloric acid solution. The resulting precipitate was collected by filtration, washed with water, and dried under vacuum to give 1-(2-hydroxy-phenyl)-3-naphthalen-2-yl-propane-1,3-dione (1.44 g, 86%). The crude diketone was dissolved in DMSO (25 mL), and *para*-toluenesulfonic acid monohydrate (660 mg, 3.47 mmol) was added. The reaction mixture was heated at 90 °C for 4 h before being poured onto ice-cold water. The resulting precipitate was collected by filtration, then dissolved in dichloromethane, dried over sodium sulfate, and concentrated under vacuum. Purification by column chromatography on silica gel (using cyclohexane/ethyl acetate: 80/20 as eluent) gave 2-naphthalen-2-yl-chromen-4-one (956 mg, 70%) as a brown solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.50 (s, 1H), 8.26 (dd, $J$ = 8.1, 1.3 Hz, 1H), 8.02–7.87 (m, 4H), 7.74 (td, $J$ = 7.8, 1.7 Hz, 1H), 7.65 (dd, $J$ = 8.2, 1.0 Hz, 1H), 7.62–7.55 (m, 2H), 7.45 (td, $J$ = 8.1, 1.1 Hz, 1H), 6.99 (s, 1H).

**2** was prepared in a manner analogous to **11** (69%, 2 steps), starting from 2-naphthalen-2-yl-chromen-4-one. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.06 (s, 1H), 8.56 (s, 1H), 8.14–8.06 (m, 1H), 8.04–7.95 (m, 3H), 7.92 (dd, $J$ = 8.1, 1.3 Hz, 1H), 7.64–7.56 (m, 2H), 7.55–7.47 (m, 2H), 7.34–7.26 (m, 1H), 7.28 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 153.3, 151.1, 142.0, 134.3, 133.6, 132.6, 130.5, 129.4, 128.7, 127.6, 127.1, 126.9, 125.2, 122.7, 122.3, 118.6, 117.7, 107.3, 93.9 ppm. MS (ESI+): 288.0 $[C_{19}H_{13}NO_2 + H]^+$ ($m/z$); mp 224–226 °C.

*2-Benzofuran-2-yl-chromen-4-one Oxime (**3**).* The title compound was prepared in a manner analogous to **2** (13%), starting from 2-benzofuran-2-yl-chromen-4-one. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.23 (s, 1H), 7.90 (dd, $J$ = 7.8, 1.6 Hz, 1H), 7.77 (d, $J$ = 7.5 Hz, 1H), 7.72 (d, $J$ = 8.2 Hz, 1H), 7.57 (s, 1H), 7.54–7.28 (m, 5H), 7.14 (s, 1H). MS (ESI+): 277.98 $[C_{17}H_{11}NO_3 + H]^+$ ($m/z$).

*2-Benzo[b]thiophen-2-yl-chromen-4-one Oxime (**4**).* The title compound was prepared in a manner analogous to **2** (26%, overall yield), starting from benzo[b]thiophene-2-carbonyl chloride. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.19 (s, 1H), 8.14 (s, 1H), 8.07–8.04 (m, 1H), 7.97–7.94 (m, 1H), 7.90 (dd, $J$ = 8.0, 1.6 Hz, 1H), 7.56–7.26 (m, 5H), 7.04 (s, 1H). MS (ESI+): 294.02 $[C_{17}H_{11}NO_2S + H]^+$ ($m/z$).

*2-(1H-Indol-2-yl)-chromen-4-one Oxime (**5**).* The title compound was prepared in a manner analogous to **2** (1%, overall yield), starting from 1*H*-indole-2-carboxylic acid. It was purified by preparative HPLC. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.89 (s, 1H), 11.00 (s, 1H), 7.90 (dd, $J$ = 7.9, 1.6 Hz, 1H), 7.63 (d, $J$ = 7.9 Hz, 1H), 7.55–7.50 (m, 1H), 7.44 (dd, $J$ = 8.0, 0.8 Hz, 1H), 7.40 (dd, $J$ = 8.1, 0.8 Hz, 1H), 7.30–7.26 (m, 1H), 7.23–7.19 (m, 2H), 7.11 (d, $J$ = 1.6 Hz, 1H), 7.10–7.04 (m, 1H). MS (ESI+): 277.0 $[C_{17}H_{12}N_2O_2 + H]^+$ ($m/z$).

*2-(1-Methyl-1H-indol-2-yl)-chromen-4-one Oxime (**6**).* The title compound was prepared in a manner analogous to **2** (1%, overall yield), starting from 1-methyl-1*H*-indole-2-carboxylic acid. It was purified by preparative HPLC. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.02 (s, 1H), 7.91 (dd, $J$ = 7.9, 1.6 Hz, 1H), 7.63 (d, $J$ = 7.9 Hz, 1H), 7.57 (d, $J$ = 8.4 Hz, 1H), 7.55–7.49 (m, 1H), 7.42 (dd, $J$ = 8.4, 1.2 Hz, 1H), 7.33–7.26 (m, 2H), 7.12–7.07 (m, 1H), 7.06 (s, 1H), 6.93 (s, 1H), 3.93 (s, 3H). MS (ESI+): 291.06 $[C_{18}H_{14}N_2O_2 + H]^+$ ($m/z$); mp 194–196 °C.

*2-Quinolin-2-yl-chromen-4-one Oxime (**7**).* The title compound was prepared in a manner analogous to **2** (24%, overall yield), starting from quinaldoyl chloride. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.20 (s, 1H), 8.59 (d, $J$ = 8.5 Hz, 1H), 8.19 (d, $J$ = 8.5 Hz, 1H), 8.14 (d, $J$ = 8.5 Hz, 1H), 8.07 (d, $J$ = 8.5 Hz, 1H), 7.93 (d, $J$ = 8.5 Hz, 1H), 7.85 (td, $J$ = 7.5, 1.3 Hz, 1H), 7.80 (s, 1H), 7.68 (td, $J$ = 7.6, 1.3 Hz, 1H), 7.60–7.49 (m, 2H), 7.31 (td, $J$ = 7.5, 1.3 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 152.3, 151.0, 149.8, 147.2, 142.0, 137.7, 130.7, 130.5, 129.2, 128.0, 127.9, 127.5, 125.2, 122.3, 118.9, 117.9, 117.6, 95.6 ppm. MS (ESI+): 289.0 $[C_{18}H_{12}N_2O_2 + H]^+$ ($m/z$); mp 232–235 °C.

*2-Quinoxalin-2-yl-chromen-4-one Oxime (**8**).* The title compound was prepared in a manner analogous to **2** (7%, overall yield), starting from 2-quinoxaloyl chloride. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.29 (s, 1H), 9.57 (s, 1H), 8.22–8.15 (m, 2H), 7.95–7.90 (m, 3H), 7.76 (s, 1H), 7.56–7.54 (m, 2H), 7.34–7.28 (m, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 151.0, 150.9, 144.9, 142.3, 142.1, 141.7, 141.0, 131.3, 131.2, 130.8, 129.4, 129.0, 125.4, 122.2, 118.7, 118.0, 96.9 ppm. MS (ESI+): 290.09 $[C_{17}H_{11}N_3O_2 + H]^+$ ($m/z$); mp 263–265 °C.

*2-Quinolin-3-yl-chromen-4-one Oxime (**9**).* The title compound was prepared in a manner analogous to **2** (10%, overall yield), starting from quinoline-3-carboxylic acid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.18 (s, 1H), 9.43 (d, $J$ = 2.4 Hz, 1H), 9.97 (d, $J$ = 2.4 Hz, 1H), 8.16 (dd, $J$ = 8.3, 1.2 Hz, 1H), 8.09 (d, $J$ = 8.3 Hz, 1H), 7.92 (dd, $J$ = 8.0, 1.4 Hz, 1H), 7.86 (ddd, $J$ = 8.4, 6.9, 1.4 Hz, 1H), 7.71 (ddd, $J$ = 8.0, 6.9, 1.1 Hz, 1H), 7.58–7.50 (m, 2H), 7.40 (s, 1H), 7.31 (m, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 151.4, 151.0, 147.8, 147.1, 141.8, 132.9, 130.8, 130.6, 128.9, 128.7, 127.5, 126.9, 125.3, 125.1, 122.2, 118.6, 117.7, 94.8 ppm. MS (ESI+): 289.06 $[C_{18}H_{12}N_2O_2 + H]^+$ ($m/z$); mp 225–228 °C.

*2-Quinolin-6-yl-chromen-4-one Oxime (**10**).* The title compound was prepared in a manner analogous to **2** (4%, overall yield), starting from quinoline-6-carboxylic acid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.13 (s, 1H), 8.97 (dd, $J$ = 4.3, 1.7 Hz, 1H), 8.65 (d, $J$ = 1.9 Hz, 1H), 8.54 (dd, $J$ = 8.3, 1.0 Hz, 1H), 8.28 (dd, $J$ = 8.9, 1.9 Hz, 1H), 8.12 (d, $J$ = 8.9 Hz, 1H), 7.92 (dd, $J$ = 7.9, 1.0 Hz, 1H), 7.63 (dd, $J$ = 8.3, 4.3 Hz, 1H), 7.55–7.48 (m, 2H), 7.34–7.28 (m, 2H). MS (ESI+): 289.02 $[C_{18}H_{12}N_2O_2 + H]^+$ ($m/z$); mp 222–224 °C.

*2-Isoquinolin-3-yl-chromen-4-one Oxime (**11**).* To a suspension of 2-isoquinolin-3-yl-chromen-4-one (**22**, 459 mg, 1.67 mmol) in methanol (11 mL) was added *O-tert*-butyl hydroxylamine hydrochloride (421 mg, 3.35 mmol). The mixture was subjected to microwave irradiation at 130 °C for 30 min. Methanol was removed under vacuum, and the residue was purified by column chromatography on silica gel (using a gradient of 0–5% ethyl acetate in cyclohexane as eluent) to give 2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (387 mg, 67%) as a yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.29 (s, 1H), 8.30 (s, 1H), 8.10 (dd, $J$ = 7.9, 1.5 Hz, 1H), 8.01 (d, $J$ = 7.7 Hz, 1H), 7.95 (d, $J$ = 7.9 Hz, 1H), 7.80 (s, 1H), 7.75 (td, $J$ = 7.0, 1.1 Hz, 1H), 7.65 (td, $J$ = 7.5, 1.1 Hz, 1H), 7.42 (td, $J$ = 7.7, 1.7 Hz, 1H), 7.34 (dd, $J$ = 8.3, 1.3 Hz, 1H), 7.21 (td, $J$ = 7.4, 1.3 Hz, 1H), 1.43 (s, 9H).

To an ice-cooled solution of 2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (136 mg, 0.39 mmol) in dichloromethane (10 mL) was cautiously added a 1 M solution of titanium tetrachloride in dichloromethane (1.2 mL, 1.2 mmol). The reaction mixture was stirred at 0 °C for 2 h then at room temperature for 3 h. The reaction mixture was poured onto ice-cold water (100 mL), basified using a 6N aqueous solution of sodium hydroxide until pH 10, and the resulting yellow precipitate collected by filtration. The solid was washed with water, dried under vacuum, and purified by column chromatography on silica gel (using a gradient of cychlohexane/ethyl acetate/dichloromethane: 80/10/10 to 0/50/50 as eluent) to give 2-isoquinolin-3-yl-chromen-4-one oxime (**11**, 71 mg, 62%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11,08 (s, 1H), 9.42 (s, 1H), 8.50 (s, 1H), 8.22 (d, $J$ = 7.9 Hz, 1H), 8.15 (d, $J$ = 7.9 Hz, 1H), 7.93 (dd, $J$ = 8.1, 1.2 Hz, 1H), 7.88 (td, $J$ = 7.5, 1.1 Hz, 1H), 7.78 (td, $J$ = 7.4, 1.1 Hz, 1H), 7.77 (s, 1H), 7.60–7.48 (m, 2H), 7.31 (td, $J$ = 7.4,


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1.3 Hz, 1H). MS (ESI+): 289.3 $[C_{18}H_{12}N_2O_2 + H]^+$ ($m/z$); mp 247−249 °C.

*2-[2,6]Naphthyridin-3-yl-chromen-4-one Oxime (**12**).* The title compound was prepared in a manner analogous to **11**, starting from [2,6]naphthyridine-3-carboxylic acid methyl ester. It was purified by preparative HPLC. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.17 (s, 1H), 9.61 (s, 1H), 9.57 (s, 1H), 8.81 (d, $J$ = 5.6 Hz, 1H), 8.67 (s, 1H), 8.13 (d, $J$ = 5.6 Hz, 1H), 7.93 (dd, $J$ = 8.0, 1.2 Hz, 1H), 7.79 (s, 1H), 7.57 (td, $J$ = 8.4, 1.2 Hz, 1H), 7.52 (d, $J$ = 7.6 Hz, 1H), 7.32 (td, $J$ = 8.0, 1.2 Hz, 1H). MS (ESI+): 290.1 $[C_{17}H_{11}N_3O_2 + H]^+$ ($m/z$).

*[2,6]Naphthyridine-3-carboxylic Acid Methyl Ester.* Compound was prepared as follows: To a cold solution of 4-dimethoxymethyl-pyridine-3-carbaldehyde[38] (400 mg, 1.91 mmol) in dichloromethane (10 mL) was slowly added a solution of acetylamino-(dimethoxy-phosphoryl)-acetic acid methyl ester[39] (503 mg, 2.1 mmol) and 1.8-diazabicyclo[5.4.0]undec-7-ene (0.31 mL, 2.10 mmol). The reaction mixture was stirred at 0 °C for 1 h, then at room temperature for 18 h, before being poured onto a cold saturated solution of sodium bicarbonate and extracted twice with dichloromethane. The combined organic extracts were dried over sodium sulfate and concentrated to dryness. The residue was dissolved in toluene (49 mL), and *para*-toluenesulfonic acid (315 mg, 1.66 mmol) was added. The reaction mixture was refluxed for 18 h before being concentrated under vacuum. The brown residue was dissolved in ethyl acetate, washed with a saturated solution of sodium bicarbonate and brine, dried over sodium sulfate, and concentrated under vacuum to give [2,6]-naphthyridine-3-carboxylic acid methyl ester (241 mg, 62%) as a brown solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.49 (s, 1H), 9.43 (s, 1H), 8.87 (d, $J$ = 5.6 Hz, 1H), 8.72 (s, 1H), 7.88 (d, J = 5.6 Hz, 1H), 4.09 (s, 3H).

*2-[1,6]Naphthyridin-3-yl-chromen-4-one Oxime (**13**).* The title compound was prepared in a manner analogous to **11**, starting from [1,6]naphthyridine-3-carboxylic acid methyl ester. It was purified by preparative HPLC. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.16 (s, 1H), 9.52 (s, 1H), 9.25−9.15 (m, 1H), 8.70−8.60 (m, 1H), 8.50 (s, 1H), 7.93 (dd, $J$ = 7.6, 1.2 Hz, 1H), 7.83 (s, 1H), 7.82−7.72 (m, 1H), 7.65−7.50 (m, 2H), 7.35−7.25 (m, 1H). MS (ESI+): 290.1 $[C_{18}H_{12}N_2O_2 + H]^+$ ($m/z$).

*[1,6]Naphthyridine-3-carboxylic Acid Methyl Ester.* Compound was prepared in a manner analogous to [2,6]naphthyridine-3-carboxylic acid methyl ester (43%), starting from 3-diethoxymethyl-pyridine-2-carbaldehyde[38] instead of 4-dimethoxymethyl-pyridine-3-carbaldehyde. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.39 (d, $J$ = 2.4 Hz, 1H), 9.21 (dd, $J$ = 4.3, 1.9 Hz, 1H), 8.81 (s, 1H), 8.41−8.37 (m, 1H), 7.67 (ddd, $J$ = 8.3, 4.1, 2.0 Hz, 1H), 4.09 (s, 3H).

*2-Pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one Oxime (**14**).* The title compound was prepared in a manner analogous to **11** (43%, overall yield), starting from pyrrolo[1,2-$c$]pyrimidine-3-carboxylic acid methyl ester.[40] $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.97 (s, 1H), 9.22 (s, 1H), 8.05 (s, 1H), 7.88 (dd, $J$ = 7.9, 2.4 Hz, 1H), 7.82 (d, $J$ = 2.4 Hz, 1H), 7.54−7.42 (m, 2H), 7.46 (s, 1H), 7.27 (t, $J$ = 6.8 Hz, 1H), 6.97 (dd, $J$ = 3.7, 2.9 Hz, 1H), 6.74 (d, $J$ = 3.7 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 152.0, 151.0, 142.1, 139.5, 132.1, 130.5, 130.1, 124.8, 122.2, 118.8, 117.6, 117.0, 114.2, 109.7, 103.4, 93.6 ppm. MS (ESI+): 278.1 $[C_{16}H_{11}N_3O_2 + H]^+$ ($m/z$); mp 260−263 °C.

*2-Thieno[2,3-c]pyridin-5-yl-chromen-4-one Oxime (**15**).* The title compound was prepared in a manner analogous to **11** (21%, overall yield), starting from thieno[2,3-$c$]pyridin-5-carboxylic acid methyl ester.[41] $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.04 (s, 1H), 9.38 (s, 1H), 8.52 (s, 1H), 8.24 (d, $J$ = 5.3 Hz, 1H), 7.92 (dd, $J$ = 7.9, 1.4 Hz, 1H), 7.72 (d, $J$ = 5.3 Hz, 1H), 7.71 (s, 1H), 7.58−7.45 (m, 2H), 7.30 (t, $J$ = 7.4 Hz, 1H). MS (ESI+): 295.0 $[C_{16}H_{10}N_2O_2S + H]^+$ ($m/z$); mp 243−245 °C.

*2-Thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime (**16**).* The title compound was prepared in a manner analogous to **11** (43%, overall yield), starting from thieno[3,2-$c$]pyridin-6-carboxylic acid methyl ester.[41] $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.05 (s, 1H), 9.25 (s, 1H), 8.76 (s, 1H), 8.03 (d, $J$ = 5.3 Hz, 1H), 7.91 (dd, $J$ = 7.9, 1.3 Hz, 1H), 7.72 (d, $J$ = 5.3 Hz, 1H), 7.71 (s, 1H), 7.58−7.46 (m, 2H), 7.30 (t, $J$ = 7.5 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 150.4, 148.8, 145.2, 143.5, 140.5, 139.9, 134.2, 129.0, 128.3, 122.7, 120.6, 120.0, 116.6, 115.5, 111.9, 92.6 ppm. MS (ESI+): 295.0 $[C_{16}H_{10}N_2O_2S + H]^+$ ($m/z$); mp 272−275 °C.

*2-Pyrrolo[1,2-a]pyrazin-3-yl-chromen-4-one Oxime (**17**).* The title compound was prepared in a manner analogous to **11** (41% overall yield), starting from pyrrolo[1,2-$a$]pyrazine-3-carboxylic acid methyl ester. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.96 (s, 1H), 8.97 (s, 1H), 8.93 (s, 1H), 7.92 (s, 1H), 7.90 (dd, $J$ = 7.9, 1.5 Hz, 1H), 7.53 (td, $J$ = 7.8, 1.3 Hz, 1H), 7.48 (s, 1H), 7.40 (d, $J$ = 8.3 Hz, 1H), 7.28 (t, $J$ = 7.5 Hz, 1H), 7.00 (dd, $J$ = 3.9, 2.4 Hz, 1H), 6.96 (d, $J$ = 3.9 Hz, 1H). MS (ESI+): 278.0 $[C_{16}H_{11}N_3O_2 + H]^+$ ($m/z$); mp 276−277 °C.

*Pyrrolo[1,2-a]pyrazine-3-carboxylic Acid Methyl Ester.* Compound was prepared as follows: a suspension of methyl 2-{bis[(*tert*-butoxy)carbonyl]amino}prop-2-enoate[42] (1.65 g, 4.5 mmol), potassium carbonate (3.7 g, 27.0 mmol), and pyrrole-2-formyl (428 mg, 4.5 mmol) in dry acetonitrile (45 mL) was stirred at room temperature for 16 h. The reaction mixture was filtered off, and the filtrate was concentrated under vacuum to give 1.8 g of a pale-yellow oil. The crude oil (500 mg, 1.26 mmol) was dissolved in TFA (4 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was poured onto ice−water, neutralized with sodium bicarbonate, and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness. The residue was purified by column chromatography on silica gel (using 0−100% ethyl acetate in cyclohexane as eluent) to give 3,4-dihydro-pyrrolo[1,2-$a$]pyrazine-3-carboxylic acid methyl ester (169 mg, 75%) as an orange oil. The crude oil was dissolved in dichloromethane (5 mL), and manganese dioxide (800 mg, 9.2 mmol) was added in one portion. The reaction mixture was stirred at 40 °C for 1 h, filtered off, and the filtrate concentrated to dryness to give pyrrolo[1,2-$a$]pyrazine-3-carboxylic acid methyl ester (130 mg, 80%) as yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.83 (s, 1H), 8.76 (s, 1H), 7.55 (d, $J$ = 2.4 Hz, 1H), 7.00 (dd, $J$ = 4.0, 2.4 Hz, 1H), 6.89 (d, $J$ = 4.0 Hz, 1H), 3.97 (s, 3H).

*2-(1-Methyl-1H-pyrrolo[2,3-c]pyridin-5-yl)-chromen-4-one Oxime (**18**).* At 0 °C, sodium hydride (60% in mineral oil, 10 mg, 0.25 mmol) was slowly added to a solution of 2-(1*H*-pyrrolo[2,3-$c$]pyridin-5-yl)-chromen-4-one *O*-*tert*-butyl-oxime (**19a**, 77 mg, 0.23 mmol) in dimethylformamide (5 mL), and the reaction mixture was stirred at room temperature for 1 h. At 0 °C, iodomethane (16 $\mu$L, 0.25 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 1 h before being poured into brine and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness to give crude 2-(1-methyl-1*H*-pyrrolo[2,3-$c$]pyridin-5-yl)-chromen-4-one *O*-*tert*-butyl-oxime **18a** as a brown solid. *tert*-Butyl removal was performed in a manner analogous to **11**, and purification by preparative HPLC afforded 2-(1-methyl-1*H*-pyrrolo[2,3-$c$]pyridin-5-yl)-chromen-4-one oxime (**18**, 17 mg, 23%, 2 steps) as a yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.00 (bs, 1H), 9.05 (s, 1H), 8.39 (s, 1H), 7.91 (dd, $J$ = 7.8, 1.5 Hz, 1H), 7.83 (s, 1H), 7.61 (s, 1H), 7.54 (ddd, $J$ = 8.3, 6.9, 1.5 Hz, 1H), 7.49 (dd, $J$ = 8.3, 1.3 Hz, 1H), 7.30 (ddd, $J$ = 7.8, 6.9, 1.3 Hz, 1H), 6.76 (d, $J$ = 2.6 Hz, 1H), 4.02 (s, 3H). MS (ESI+): 292.1 $[C_{17}H_{13}N_3O_2 + H]^+$ ($m/z$); mp 270−275 °C.

*2-(1H-Pyrrolo[2,3-c]pyridin-5-yl)-chromen-4-one Oxime (**19**).* Under argon, a solution of 2-hydroxyacetophenone (1.68 g, 12.4 mmol) in tetrahydrofuran (120 mL) was cooled to −78 °C and a 1 M solution of lithium hexamethyldisilazane in THF (2.25 mL, 2.25 mmol) was added dropwise. The reaction mixture was stirred at −78 °C for 1 h and at −10 °C for 2 h, then cooled again at −78 °C, before addition of a solution of 4-methyl-5-nitro-pyridine-2-carboxylic acid methyl ester[43] (2.42 g, 12.4 mmol) in THF (60 mL). The resulting dark-red solution was stirred at −78 °C for 1 h then allowed to reach room temperature for 18 h. The reaction mixture was poured into an ice-cold 1 N solution of hydrochloric acid (200 mL) and extracted twice with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated to dryness. The residue was dissolved in acetic acid (60 mL), treated with sulfuric acid (0.33 mL), and heated at 100 °C for 30 min. After cooling to room temperature, the mixture was concentrated under vacuum and the residue was


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neutralized with an aqueous solution of sodium bicarbonate. The resulting precipitate was collected by filtration, washed with water, and dried under vacuum to give 2-(4-methyl-5-nitro-pyridin-2-yl)-chromen-4-one (2.33 g, 66%) as a brown solid. A mixture of the previous chromen-4-one (770 mg, 2.72 mmol) and *tert*-butyl-hydroxylamine hydrochloride (685 mg, 5.45 mmol) in methanol (20 mL) was subjected to microwave iradiation at 130 °C for 30 min. Methanol was removed under vacuum, and the crude solid was purified by column chromatography on silica gel (using a gradient of 0−20% dichloromethane in cyclohexane as eluent) to give 2-(4-methyl-5-nitro-pyridin-2-yl)-chromen-4-one *O*-*tert*-butyl-oxime (394 mg, 41%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.23 (s, 1H), 8.08 (dd, $J$ = 7.9, 1.7 Hz, 1H), 7.89 (s, 1H), 7.78 (s, 1H), 7.42 (td, $J$ = 7.6, 1.7 Hz, 1H), 7.32−7.19 (m, 2H), 2.76 (s, 3H), 1.42 (s, 9H). LCMS, $m/z$ = 354.0 $[M + H]^+$.

To a suspension of 2-(4-methyl-5-nitro-pyridin-2-yl)-chromen-4-one *O*-*tert*-butyl-oxime (250 mg, 0.70 mmol) in dimethylformamide (6 mL) was added dimethylformamide-dimethylacetal (127 $\mu$L, 0.95 mmol), and the reaction mixture was stirred at 90 °C for 2.5 h. DMF was removed under vacuum, and the residue was dissolved in absolute ethanol. Then 10% palladium on charcoal (50 mg) was added, and the suspension was stirred under 1 atm of hydrogen at room temperature for 16 h. The catalyst was removed by filtration, and the filtrate was purified by column chromatography on silica gel (using a gradient of 0−20% ethyl acetate in cyclohexane as eluent) to give 2-(1*H*-pyrrolo[2,3-*c*]pyridin-5-yl)-chromen-4-one *O*-*tert*-butyl-oxime (**19a**, 165 mg, 70%) as a yellow solid. *tert*-Butyl removal was performed in a manner analogous to **11**, and purification by preparative HPLC afforded 2-(1*H*-pyrrolo[2,3-*c*]pyridin-5-yl)-chromen-4-one oxime (**19**, 39 mg, 70%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.90 (bs, 1H), 10.80 (s, 1H), 8.84 (s, 1H), 8.25 (s, 1H), 7.89 (dd, $J$ = 6.9, 1.5 Hz, 1H), 7.68 (dd, $J$ = 2.5, 1.5 Hz, 1H), 7.57 (bs, 1H), 7.53−7.47 (m, 2H), 7.26 (ddd, $J$ = 7.8, 6.9, 1.3 Hz, 1H), 6.67 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 154.0, 151.2, 142.4, 138.4, 134.7, 133.3, 132.2, 130.4, 130.3, 124.7, 122.2, 119.0, 117.7, 111.9, 101.9, 93.0 ppm. MS (ESI+): 278.1 $[C_{16}H_{11}N_3O_2 + H]^+$ ($m/z$); mp 270−275 °C.

*2-Imidazo[1,2-a]pyridin-7-yl-chromen-4-one Oxime (**20**).* The title compound was prepared in a manner analogous to **11** (10%, overall yield), starting from imidazo[1,2-*a*]pyridine-7-carboxylic acid ethyl ester.[44] $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.09 (s, 1H), 8.61 (d, $J$ = 7.3 Hz, 1H), 8.16 (s, 1H), 8.05 (s, 1H), 7.87 (d, $J$ = 7.8 Hz, 1H), 7.69 (s, 1H), 7.51−7.49 (m, 2H), 7.41 (dd, $J$ = 7.3, 1.8 Hz, 1H), 7.29−7.24 (m, 1H), 7.21 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 149.6, 149.1, 142.3, 140.0, 133.0, 128.7, 126.0, 125.3, 123.1, 120.3, 116.7, 116.0, 112.4, 111.7, 106.6, 92.5 ppm. MS (ESI+): 278.1 $[C_{16}H_{11}N_3O_2 + H]^+$ ($m/z$); mp 265−267 °C.

*2-(5,7-Dimethyl-pyrrolo[1,2-c]pyrimidin-3-yl)-chromen-4-one Oxime (**21**).* The title compound was prepared in a manner analogous to **11** (19% overall yield), starting from 5,7-dimethyl-pyrrolo[1,2-*c*]pyrimidine-3-carboxylic acid methyl ester. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.89 (s, 1H), 8.89 (s, 1H), 7.94 (d, $J$ = 1.3 Hz, 1H), 7.88 (d, $J$ = 7.9 Hz, 1H), 7.54−7.45 (m, 2H), 7.41 (s, 1H), 7.26−7.24 (m, 1H), 6.61 (s, 1H), 2.54 (s, 3H), 2.35 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 152.2, 151.0, 142.3, 136.9, 130.3, 129.3, 126.9, 124.6, 122.2, 122.0, 118.9, 117.7, 117.6, 112.2, 108.2, 92.8, 10.7, 10.0 ppm. MS (ESI+): 306.1 $[C_{18}H_{15}N_3O_2 + H]^+$ ($m/z$); mp >250 °C.

*5,7-Dimethyl-pyrrolo[1,2-c]pyrimidine-3-carboxylic Acid Methyl Ester.* Compound was prepared as follows: a solution of 3,5-dimethylpyrrole-2-carbaldehyde (1.0 g, 8.1 mmol), 1.8-diazabicyclo[5.4.0]undec-7-ene (1.3 mL, 8.9 mmol), and ethyl isocyanoacetate (1.0 mL, 8.9 mmoL) in dioxane (18 mL) was stirred at room temperature for 24 h before being hydrolyzed with cold water, neutralized with acetic acid (5%), and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness. The residue was purified by column chromatography on silica gel (using a gradient of 0−100% ethyl acetate in cyclohexane as eluent) to give 5,7-dimethyl-pyrrolo[1,2-*c*]pyrimidine-3-carboxylic acid methyl ester (522 mg, 29%) as a yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.51 (s, 1H), 8.12 (s, 1H), 6.55 (s, 1H), 4.42 (q, $J$ = 7.1 Hz, 1H), 2.51 (s, 3H), 2.34 (s, 3H), 1.41 (t, $J$ = 7.1 Hz, 1H).

*2-Isoquinolin-3-yl-chromen-4-one (**22**).* To a suspension of sodium hydride (60% in mineral oil, 227 mg, 5.70 mmol) in dry pyridine (4 mL) was added dropwise a solution of methyl isoquinoline-3-carboxylate (390 mg, 2.08 mmol) and 2-hydroxyacetophenone (257 mg, 1.89 mmol) in dry pyridine (4 mL). The reaction mixture was heated at 90 °C for 15 min before being cooled to room temperature and poured into an ice-cooled 1 N aqueous solution of hydrochloric acid. The product was extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness. The residue was dissolved in acetic acid (10 mL), and sulfuric acid (40 $\mu$L) was added. The resulting solution was heated at 100 °C for 30 min. The solvents were removed under vacuum, and the crude solid was triturated in water. The solid was collected by filtration, washed with an aqueous solution of sodium bicarbonate, and dried under vacuum to give 2-isoquinolin-3-yl-chromen-4-one (**22**, 459 mg, 89%) as a beige solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.32 (s, 1H), 8.49 (s, 1H), 8.27 (d, $J$ = 7.2 Hz, 1H), 8.06 (d, $J$ = 7.9 Hz, 1H), 8.01 (d, $J$ = 7.9 Hz, 1H), 7.85−7.63 (m, 4H), 7.59 (s, 1H), 7.44 (t, $J$ = 7.2 Hz, 1H); mp 170−175 °C.

*2-Isoquinolin-3-yl-chromen-4-one O-Methyl-oxime, Mixture of Isomers E and Z (**23**).* To a suspension of 2-isoquinolin-3-yl-chromen-4-one (**22**, 40 mg, 0.14 mmol) in methanol (2 mL) was added methoxylamine hydrochloride (24 mg, 0.29 mmol). The reaction mixture was subjected to microwave irradiation at 130 °C for 30 min. Methanol was removed under vacuum, and the residue was purified by column chromatography on silica gel (using a gradient of 0−20% ethyl acetate in cyclohexane as eluent) to give a 1:3 mixture of *Z*/*E* 2-isoquinolin-3-yl-chromen-4-one *O*-methyl-oxime (28 mg, 66%) as a yellow solid. $^1$H NMR of the main isomer (300 MHz, $CDCl_3$) $\delta$ 9.26 (s, 1H), 8.30 (s, 1H), 8.04 (dd, $J$ = 7.9, 1.6 Hz, 1H), 8.00 (d, $J$ = 7.9 Hz, 1H), 7.93 (d, $J$ = 8.0 Hz, 1H), 7.82 (s, 1H), 7.76−7.71 (m, 1H), 7.67−7.62 (m, 1H), 7.46−7.39 (m, 1H), 7.35 (dd, $J$ = 8.3, 1.2 Hz, 1H), 7.24−7.18 (m, 1H), 4.03 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 153.7, 152.8, 151.2, 142.7, 142.6, 135.3, 131.5, 131.1, 128.9, 128.6, 127.9, 127.6, 125.2, 122.5, 117.8, 117.7, 117.2, 94.7, 61.7 ppm. MS (ESI+): 303.0 $[C_{19}H_{14}N_2O_2 + H]^+$ ($m/z$); mp 170−176 °C.

*2-Isoquinolin-3-yl-3-methyl-chromen-4-one Oxime (**24**).* The title compound was prepared in a manner analogous to **11** (4% overall yield), starting from 2-hydroxypropiophenone instead of 2-hydroxyacetophenone. It was purified by preparative HPLC. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.13 (s, 1H), 9.49 (s, 1H), 8.44 (s, 1H), 8.22 (d, $J$ = 8.4 Hz, 1H), 8.16 (d, $J$ = 8.4 Hz, 1H), 7.88 (t, $J$ = 7.2 Hz, 1H), 7.79 (t, $J$ = 7.2 Hz, 1H), 7.42 (d, $J$ = 7.6 Hz, 1H), 7.38 (d, $J$ = 7.6 Hz, 1H), 7.05 (d, $J$ = 8.0 Hz, 1H), 6.98 (t, $J$ = 7.6 Hz, 1H), 2.27 (s, 3H). MS (ESI+): 303.1 $[C_{19}H_{14}N_2O_2 + H]^+$ ($m/z$).

*2-Isoquinolin-3-yl-6-methyl-chromen-4-one Oxime (**25**).* The title compound was prepared in a manner analogous to **11** (21% overall yield), starting from 2-hydroxy-5-methyl-acetophenone instead of 2-hydroxyacetophenone. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.04 (s, 1H), 9.42 (s, 1H), 8.48 (s, 1H), 8.22 (d, $J$ = 8.1 Hz, 1H), 8.15 (d, $J$ = 8.1 Hz, 1H), 7.88 (td, $J$ = 7.9, 1.3 Hz, 1H), 7.78 (td, $J$ = 7.9, 1.3 Hz, 1H), 7.75 (s, 1H), 7.71 (s, 1H), 7.42 (d, $J$ = 8.3 Hz, 1H), 7.36 (dd, $J$ = 8.8, 1.7 Hz, 1H), 2.37 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 151.6, 151.5, 148.1, 142.0, 141.0, 134.2, 133.0, 130.3, 130.2, 127.5, 127.4, 126.7, 126.4, 120.9, 117.4, 116.3, 115.6, 93.7, 19.4 ppm. MS (ESI+): 303.4 $[C_{19}H_{14}N_2O_2 + H]^+$ ($m/z$); mp 260−264 °C.

*2-Isoquinolin-3-yl-6-trifluoromethyl-chromen-4-one Oxime (**26**).* The title compound was prepared in a manner analogous to **11** (10% overall yield), starting from 2-hydroxy-5-trifluoromethyl-acetophenone instead of 2-hydroxyacetophenone. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.39 (s, 1H), 9.43 (s, 1H), 8.54 (s, 1H), 8.23 (d, $J$ = 7.9 Hz, 1H), 8.15 (m, 2H), 7.94−7.85 (m, 2H), 7.79 (td, $J$ = 7.5, 1.3 Hz, 1H), 7.78 (s, 1H), 7.73 (d, $J$ = 8.7 Hz, 1H). MS (ESI+): 357.1 $[C_{19}H_{11}F_3N_2O_2 + H]^+$ ($m/z$); mp 245−247 °C.

*2-Hydroxy-5-trifluoromethyl-acetophenone.* Compound was prepared as follows: at −78 °C, to solution of 2-methoxy-5-trifluoromethyl-acetophenone (650 mg, 3.0 mmol) in dry dichloro-



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methane (40 mL) was slowly added a 1 M solution of boron trichloride in dichloromethane (7.5 mL, 7.5 mmol), keeping the internal temperature below −70 °C. The brown−orange solution was slowly warmed up to room temperature within 2 h. At 0 °C, the reaction mixture was hydrolyzed with a 1 N aqueous hydrochloride solution (40 mL) and extracted with dichloromethane. The organic layer was washed with water, dried over sodium sulfate, and concentrated to dryness. The residue was purified by column chromatography on silica gel (using a gradient of 0−10% ethyl acetate in cyclohexane) to give 2-hydroxy-5-trifluoromethyl-acetophenone (467 mg, 77%) as a pale-yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 12.55 (s, 1H), 7.99 (d, $J$ = 2.0 Hz, 1H), 7.70 (dd, $J$ = 8.8, 2.0 Hz, 1H), 7.08 (d, $J$ = 8.8 Hz, 1H), 2.69 (s, 3H).

*2-Isoquinolin-3-yl-6-trifluoromethoxy-chromen-4-one Oxime (**27**).* The title compound was prepared in a manner analogous to **11** (15% overall yield), starting from 2-hydroxy-5-trifluoromethoxy-acetophenone instead of 2-hydroxyacetophenone. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.34 (s, 1H), 9.43 (s, 1H), 8.51 (s, 1H), 8.22 (d, $J$ = 7.9 Hz, 1H),), 8.15 (d, $J$ = 7.9 Hz, 1H), 7.89 (td, $J$ = 7.6, 1.3 Hz, 1H), 7.79 (td, $J$ = 7.5, 1.3 Hz, 1H), 7.78−7.75 (m, 1H), 7.75 (s, 1H), 7.66 (d, $J$ = 9.0 Hz, 1H), 7.57 (dd, $J$ = 9.0, 2.5 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 152.9, 152.8, 149.5, 144.8, 142.6, 141.4, 135.3, 131.5, 128.8, 128.5, 127.9, 127.5, 123.5, 120.3, 120.0, 120.0 (q, $J$ = 260 Hz, $OCF_3$), 117.1, 113.9, 94.7 ppm. MS (ESI+): 373.1 $[C_{19}H_{11}F_3N_2O_3 + H]^+$ ($m/z$); mp 250−254 °C.

*6-Bromo-2-isoquinolin-3-yl-chromen-4-one Oxime (**28**).* To an ice-cooled solution of 6-bromo-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl oxime (**103**, 50 mg, 0.12 mmol) in dichloromethane (3 mL) was cautiously added a 1 M solution of titanium tetrachloride in dichloromethane (0.35 mL, 0.35 mmol). The reaction mixture was stirred at 0 °C for 2 h, then at room temperature for 2 more hours, before being poured onto ice-cold water (50 mL). The mixture was basified using a 6 N aqueous sodium hydroxide solution until pH 10, and the yellow precipitate was collected by filtration. Recrystallization in hot chloroform gave 6-bromo-2-isoquinolin-3-yl-chromen-4-one oxime (**28**, 37 mg, 85%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.31 (s, 1H), 9.41 (s, 1H), 8.49 (s, 1H), 8.22 (d, $J$ = 7.9 Hz, 1H), 8.13 (d, $J$ = 7.9 Hz, 1H), 7.97 (d, $J$ = 2.4 Hz, 1H), 7.88 (td, $J$ = 7.5, 1.3 Hz, 1H), 7.78 (td, $J$ = 7.9, 1.3 Hz, 1H), 7.75 (s, 1H), 7.72 (dd, $J$ = 8.8, 2.4 Hz, 1H), 7.50 (d, $J$ = 8.8 Hz, 1H). MS (ESI+): 369.3 $[C_{18}H_{11}BrN_2O_2 + H]^+$ ($m/z$); mp 266−269 °C.

*2-Isoquinolin-3-yl-6-methoxy-chromen-4-one Oxime (**29**).* At 0 °C, to a solution of 6-hydroxy-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**203**, 80 mg, 0.22 mmol) in DMF (2.5 mL) was added sodium hydride (60% in mineral oil, 13 mg, 0.33 mmol), and the reaction mixture was stirred at room temperature for 1 h. At 0 °C, iodomethane (15 μL, 0.24 mmol) was added dropwise. The resulting solution was stirred at room temperature for 20 h before being hydrolyzed and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum. Purification by column chromatography on silica gel (using a gradient of 0−20% ethyl acetate in cyclohexane as eluent) afforded 2-isoquinolin-3-yl-6-methoxy-chromen-4-one oxime *O-tert*-butyl oxime (59 mg, 67%) as a yellow solid. *tert*-Butyl removal was performed in a manner analogous to **11**. Purification by column chromatography on silica gel (using a gradient of 0−10% methanol in dichloromethane as eluent) afforded 2-isoquinolin-3-yl-6-methoxy-chromen-4-one oxime (**29**, 31 mg, 62%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.04 (s, 1H), 9.41 (s, 1H), 8.47 (s, 1H), 8.21 (d, $J$ = 8.1 Hz, 1H), 8.14 (d, $J$ = 8.1 Hz, 1H), 7.87 (t, $J$ = 7.7 Hz, 1H), 7.77 (t, $J$ = 7.7 Hz, 1H), 7.75 (s, 1H), 7.47 (d, $J$ = 9.0 Hz, 1H), 7.34 (d, $J$ = 3.0 Hz, 1H), 7.15 (dd, $J$ = 9.0, 3.0 Hz, 1H), 3.81 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 156.1, 152.7, 152.6, 145.4, 143.7, 142.2, 135.3, 131.4, 128.6, 128.5, 127.9, 127.5, 119.4, 119.0, 118.1, 116.8, 104.2, 94.2, 55.4 ppm. MS (ESI+): 319.0 $[C_{19}H_{14}N_2O_3 + H]^+$ ($m/z$); mp 250−255 °C.

*7-Bromo-2-isoquinolin-3-yl-chromen-4-one Oxime (**30**).* The title compound was prepared in a manner analogous to **28** (37%) starting from 7-bromo-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**106**). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.26 (s, 1H), 9.42 (s, 1H), 8.52 (s, 1H), 8.22 (d, $J$ = 8.1 Hz, 1H), 8.10 (d, $J$ = 7.9 Hz, 1H), 7.89 (td, $J$ = 7.6, 1.3 Hz, 1H), 7.86−7.75 (m, 3H), 7.74 (s, 1H), 7.49 (dd, $J$ = 8.5, 1.9 Hz, 1H). MS (ESI+): 369.3 $[C_{18}H_{11}BrN_2O_2 + H]^+$ ($m/z$); mp 279−283 °C.

*2-Isoquinolin-3-yl-7-methoxy-chromen-4-one Oxime (**31**).* The title compound was prepared in a manner analogous to **29** (18%, two steps), starting from 7-hydroxy-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**206**). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.85 (s, 1H), 9.42 (s, 1H), 8.48 (s, 1H), 8.22 (d, $J$ = 8.0 Hz, 1H), 8.12 (d, $J$ = 8.0 Hz, 1H), 7.88 (t, $J$ = 6.9 Hz, 1H), 7.85−7.75 (m, 2H), 7.75 (s, 1H), 7.09 (d, $J$ = 2.9 Hz, 1H), 6.91 (dd, $J$ = 8.8, 2.9 Hz, 1H), 3.87 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 161.0, 152.8, 152.7, 152.3, 143.1, 142.0, 135.4, 131.4, 128.6, 128.5, 127.9, 127.4, 123.4, 116.7, 113.2, 111.6, 101.3, 95.0, 55.6 ppm. MS (ESI+): 319.0 $[C_{19}H_{14}N_2O_3 + H]^+$ ($m/z$); mp 246−248 °C.

*8-Chloro-2-isoquinolin-3-yl-chromen-4-one Oxime (**32**).* The title compound was prepared in a manner analogous to **11** (12% overall yield), starting from 3-chloro-2-hydroxyacetophenone instead of 2-hydroxyacetophenone. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.34 (s, 1H), 9.44 (s, 1H), 8.38 (s, 1H), 8.23 (d, $J$ = 8.1 Hz, 1H), 8.17 (d, $J$ = 8.1 Hz, 1H), 7.89 (t, $J$ = 7.4 Hz, 1H), 7.87 (d, $J$ = 7.0 Hz, 1H), 7.79 (t, $J$ = 7.4 Hz, 1H), 7.79 (s, 1H), 7.71 (d, $J$ = 7.9 Hz, 1H), 7.31 (t, $J$ = 7.9 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 153.0, 152.2, 146.6, 142.7, 141.6, 135.2, 131.5, 130.6, 128.8, 128.5, 127.9, 127.6, 125.4, 121.7, 121.1, 120.7, 116.8, 95.3 ppm. MS (ESI+): 323.1 $[C_{18}H_{11}ClN_2O_2 + H]^+$ ($m/z$); mp 270−272 °C.

*6-Cyclopropyl-2-isoquinolin-3-yl-chromen-4-one Oxime (**33**).* A solution of 6-bromo-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl oxime (**103**, 100 mg, 0.24 mmol), palladium acetate (3 mg, 0.014 mmol), potassium phosphate (175 mg, 0.83 mmol), dicyclohexylbiphenylphosphine (8 mg, 0.024 mmol), and cyclopropylboronic acid pinacol ester (99 mg, 0.59 mmol) in toluene (3 mL) was degassed with argon for 10 min. The reactor was sealed and the reaction mixture was heated at 120 °C for 18 h before being poured onto a saturated aqueous solution of ammonium chloride and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude solid was purified by column chromatography on silica gel (using a gradient of 0−80% dichloromethane in cyclohexane as eluent) to give 6-cyclopropyl-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl oxime (18 mg, 20%) as a yellow solid. LCMS, $m/z$ = 385.1 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **28** (60%), and the title product was purified by preparative HPLC. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.48 (s, 1H), 9.38 (s, 1H), 8.36 (s, 1H), 8.19 (d, $J$ = 8.1 Hz, 1H), 8.12 (d, $J$ = 8.1 Hz, 1H), 7.90−7.65 (m, 2H), 7.41 (d, $J$ = 8.4 Hz, 2H), 7.29 (d, $J$ = 7.7 Hz, 1H), 7.08 (s, 1H), 2.02−1.98 (m, 1H), 1.00−0.96 (m, 2H), 0.69−0.65 (m, 2H). MS (ESI+): 329.2 $[C_{21}H_{26}N_2O_2 + H]^+$ ($m/z$).

*2-Isoquinolin-3-yl-6-(2-methoxy-ethoxy)-chromen-4-one Oxime (**34**).* The title compound was prepared in a manner analogous to **29** (67%, two steps), starting from 6-hydroxy-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**203**) and 2-bromoethylmethyl ether instead of iodomethane. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.04 (s, 1H), 9.41 (s, 1H), 8.47 (s, 1H), 8.21 (d, $J$ = 8.1 Hz, 1H), 8.14 (d, $J$ = 8.1 Hz, 1H), 7.87 (t, $J$ = 7.7 Hz, 1H), 7.77 (t, $J$ = 7.9 Hz, 1H), 7.73 (s, 1H), 7.46 (d, $J$ = 8.9 Hz, 1H), 7.34 (d, $J$ = 3.0 Hz, 1H), 7.16 (dd, $J$ = 8.9, 3.0 Hz, 1H), 4.16−4.12 (m, 2H), 3.41−3.37 (m, 2H) 3.32 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 155.3, 152.8, 152.7, 145.5, 143.2, 142.2, 135.3, 131.4, 128.6, 128.5, 127.9, 127.5, 119.4, 119.0, 118.6, 116.8, 104.9, 94.2, 70.3, 67.4, 58.2 ppm. MS (ESI+): 363.2 $[C_{21}H_{18}N_2O_4 + H]^+$ ($m/z$); mp 198−204 °C.

*2-Isoquinolin-3-yl-6-[2-(4-methyl-piperazin-1-yl)-ethoxy]-chromen-4-one Oxime (**35**).* At 0 °C, to a mixture of 6-hydroxy-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**203**, 110 mg, 0.33 mmol), 1-(2-hydroxyethyl)-4-methylpiperazine (50 mg, 0.34 mmol), and triphenylphosphine (130 mg, 0.49 mmol) in THF (3 mL) was added dropwise a 40% solution of diethyl azodicarboxylate in toluene (225 μL, 0.49 mmol). The reaction mixture was stirred at room temperature for 3 days before being poured onto a 1 N aqueous solution of HCl. The aqueous phase was washed twice with


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dichloromethane, neutralized by addition of a 6 N solution of NaOH, and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum to give 2-isoquinolin-3-yl-6-[2-(4-methyl-piperazin-1-yl)-ethoxy]-chromen-4-one *O*-*tert*-butyl oxime (51 mg, 31%) as a yellow solid. *tert*-Butyl removal was performed in a manner analogous to **11**. Purification by column chromatography on silica gel (using a gradient of 0−10% methanol in dichloromethane as eluent) afforded 2-isoquinolin-3-yl-6-[2-(4-methyl-piperazin-1-yl)-ethoxy]-chromen-4-one oxime (**35**, 21 mg, 53%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.03 (s, 1H), 9.41 (s, 1H), 8.47 (s, 1H), 8.21 (d, $J$ = 8.1 Hz, 1H), 8.14 (d, $J$ = 8.1 Hz, 1H), 7.87 (t, $J$ = 7.5 Hz, 1H), 7.77 (t, $J$ = 7.5 Hz, 1H), 7.73 (s, 1H), 7.46 (d, $J$ = 9.2 Hz, 1H), 7.33 (d, $J$ = 2.5 Hz, 1H), 7.15 (dd, $J$ = 9.2, 2.5 Hz, 1H), 4.11 (t, $J$ = 5.6 Hz, 2H), 2.70 (t, $J$ = 5.6 Hz, 2H), 2.60−2.40 (m, 4H), 2.40−2.20 (m, 4H), 2.14 (s, 3H). MS (ESI+): 431.3 $[C_{25}H_{26}N_4O_3 + H]^+$ ($m/z$); mp 233−236 °C.

*2-Isoquinolin-3-yl-6-[3-(4-methyl-piperazin-1-yl)-propylamino]-chromen-4-one Oxime (**36**).* Under inert atmosphere, a mixture of 6-bromo-2-isoquinolin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**103**, 150 mg, 0.35 mmol), 3-(4-methylpiperazin-1-yl)propylamine (83 mg, 0.53 mmol), potassium *tert*-butoxide (59 mg, 0.53 mmol), and [1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene](3-chloropyridyl)palladium(II) dichloride (5 mg, 0.01 mmol) in 1,2-dimethoxyethane (2 mL) was heated at 110 °C for 3 days. The solvent was removed under vacuum, and the crude mixture was purified by column chromatography on silica gel (using a gradient of 5−20% methanol in dichloromethane as eluent) to give 2-isoquinolin-3-yl-6-[3-(4-methyl-piperazin-1-yl)-propylamino]-chromen-4-one *O*-*tert*-butyl oxime (159 mg, 90%) as a yellow solid. *tert*-Butyl removal was performed in a manner analogous to **11**. Purification by column chromatography on silica gel (using a gradient of 20−30% methanol in dichloromethane and 5% ammonium hydroxide as eluent) afforded 2-isoquinolin-3-yl-6-[3-(4-methyl-piperazin-1-yl)-propylamino]-chromen-4-one oxime (**36**, 100 mg, 75%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.83 (s, 1H), 9.40 (s, 1H), 8.43 (s, 1H), 8.20 (d, $J$ = 8.1 Hz, 1H), 8.13 (d, $J$ = 8.1 Hz, 1H), 7.85 (t, $J$ = 7.3 Hz, 1H), 7.76 (t, $J$ = 7.3 Hz, 1H), 7.69 (s, 1H), 7.28 (d, $J$ = 8.9 Hz, 1H), 6.94 (d, $J$ = 3.0 Hz, 1H), 6.82 (dd, $J$ = 8.9, 3.0 Hz, 1H), 5.88 (m, 1H), 3.04 (m, 2H) 2.45−2.20 (m, 10H), 2.14 (s, 3H), 1.70 (m, 2H). MS (ESI+): 444.5 $[C_{26}H_{29}N_5O_2 + H]^+$ ($m/z$); mp 208−211 °C.

*2-Isoquinolin-3-yl-7-(2-methoxy-ethoxy)-chromen-4-one Oxime (**37**).* The title compound was prepared in a manner analogous to **29** (22%, two steps), starting from 7-hydroxy-2-isoquinolin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**206**) and 2-bromoethylmethyl ether instead of iodomethane. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.84 (s, 1H), 9.42 (s, 1H), 8.50 (s, 1H), 8.22 (d, $J$ = 8.0 Hz, 1H), 8.13 (d, $J$ = 8.0 Hz, 1H), 7.89 (t, $J$ = 7.0 Hz, 1H), 7.85−7.75 (m, 2H), 7.75 (s, 1H), 7.11 (d, $J$ = 2.9 Hz, 1H), 6.91 (dd, $J$ = 8.8, 2.9 Hz, 1H), 4.24−4.20 (m, 2H), 3.74−3.70 (m, 2H), 3.34 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 160.3, 152.8, 152.6, 152.2, 143.1, 142.0, 135.3, 131.5, 128.6, 128.5, 127.9, 127.4, 123.4, 116.7, 113.6, 111.7, 101.8, 95.0, 70.2, 67.4, 58.2 ppm. MS (ESI+): 363.2 $[C_{21}H_{18}N_2O_4 + H]^+$ ($m/z$); mp 223−225 °C.

*2-Isoquinolin-3-yl-7-phenethyl-chromen-4-one Oxime (**38**).* Under inert atmosphere, a mixture of 7-bromo-2-isoquinolin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**106**, 100 mg, 0.24 mmol), phenylacetylene (31 μL, 0.28 mmol), triethylamine (49 μL, 0.35 mmol), copper iodide (9 mg, 0.05 mmol), and bis-(triphenylphosphine)palladium(II) dichloride (17 mg, 0.02 mmol) in DMF (5 mL) was heated at 90 °C for 18 h. The reaction mixture was cooled, neutralized with a 0.5N aqueous solution of HCl, and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness. Purification by column chromatography on silica gel (using a gradient of 20−60% dichloromethane in cyclohexane) afforded the phenylethynyl intermediate (62 mg, 59%) as a beige solid. LCMS, $m/z$ = 445.0 $[M + H]^+$. The solid was dissolved in methanol (2 mL) and THF (9 mL), and the solution was degassed with argon. Lindlar's catalyst (22 mg) was added, and the suspension was placed under 1 atm of hydrogen. The reaction mixture was stirred at room temperature for 5 h before being filtered off. The filtrate was concentrated under vacuum to give the crude phenethyl product (61% purity) as a greenish oil. LCMS, $m/z$ = 449.0 $[M + H]^+$. *tert*-Butyl removal was perfomed in a manner analogous to **11**, and purification by preparative HPLC afforded 2-isoquinolin-3-yl-7-phenethyl-chromen-4-one oxime (**38**, 13 mg, 14% overall yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.04 (s, 1H), 9.50 (s, 1H), 8.56 (s, 1H), 8.30 (d, $J$ = 8.0 Hz, 1H), 8.21 (d, $J$ = 8.0 Hz, 1H), 7.97 (t, $J$ = 7.2 Hz, 1H), 7.90−7.80 (m, 3H), 7.49 (s, 1H), 7.40−7.35 (m, 4H), 7.35−7.20 (m, 2H), 3.06 (s, 4H). MS (ESI+): 393.1 $[C_{26}H_{20}N_2O_2 + H]^+$ ($m/z$).

*6-(2-Methoxy-ethoxy)-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one oxime (**39**).* The title compound was prepared in a manner analogous to **29** (45%, two steps), starting from 6-hydroxy-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**204**) and 2-bromoethylmethyl ether instead of iodomethane. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.93 (s, 1H), 9.21 (s, 1H), 8.03 (s, 1H), 7.81 (d, $J$ = 2.8 Hz, 1H), 7.43 (s, 1H), 7.40 (d, $J$ = 9.0 Hz, 1H), 7.30 (d, $J$ = 3.0 Hz, 1H), 7.13 (dd, $J$ = 9.0, 3.0 Hz, 1H), 6.98 (dd, $J$ = 3.8, 2.8 Hz, 1H), 6.73 (d, $J$ = 3.8 Hz, 1H), 4.16−4.11 (m, 2H), 3.69−3.64 (m, 2H), 3.39 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 155.2, 152.1, 145.3, 142.2, 139.5, 132.2, 130.2, 119.3, 118.9, 118.5, 116.9, 114.2, 109.6, 104.9, 103.3, 92.9, 70.3, 67.3, 58.2 ppm. MS (ESI+): 352.1 $[C_{19}H_{17}N_3O_4 + H]^+$ ($m/z$); mp 212−215 °C.

*6-(2-Morpholin-4-yl-ethoxy)-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one oxime Hydrochloride (**40**).* A mixture of 6-hydroxy-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**204**, 100 mg, 0.29 mmol), 4-(2-chloroethyl)morpholine hydrochloride (80 mg, 0.43 mmol), and potassium carbonate (119 mg, 0.86 mmol) in dry acetone (2.5 mL) was heated at 60 °C for 20 h. Acetone was removed under vacuum, and the crude residue was treated with water and extracted twice with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated under vacuum. Purification by column chromatography on silica gel (using a gradient of 20−100% ethyl acetate in cyclohexane as eluent) afforded 6-(2-morpholin-4-yl-ethoxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (89 mg, 67%) as a yellow solid. LCMS, $m/z$ = 463.3 $[M + H]^+$.

At 0 °C, to a stirred solution of 6-(2-morpholin-4-yl-ethoxy)-2-pyrrolo[1,2,-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (80 mg, 0.17 mmol) in dichloromethane (1.0 mL) was added a 1 M solution of titanium tetrachloride in dichloromethane (0.5 mL, 0.52 mmol). The reaction mixture was stirred at 0 °C for 2 h then at room temperature for 24 h before being neutralized to pH = 10 by addition of a 6N aqueous solution of NaOH. The resulting yellow precipitate was collected by filtration, washed with water, and dried under vacuum before being treated with a 1.2 N solution of HCl in methanol. Concentration to dryness and trituration in diethyl ether gave 6-(2-morpholin-4-yl-ethoxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one oxime hydrochloride (**40**, 38 mg, 51%) as an orange solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.99 (bs, 1H), 10.67 (bs, 1H), 9.22 (s, 1H), 8.05 (s, 1H), 7.82 (d, $J$ = 2.8 Hz, 1H), 7.46 (d, $J$ = 9.0 Hz, 1H), 7.44 (s, 1H), 7.39 (d, $J$ = 3.0 Hz, 1H), 7.20 (dd, $J$ = 9.0, 3.0 Hz, 1H), 6.98 (dd, $J$ = 3.6, 2.8 Hz, 1H), 6.74 (d, $J$ = 3.6 Hz, 1H), 4.46−4.43 (m, 2H), 3.99−3.96 (m, 2H), 3.90−3.70 (m, 2H), 3.65−3.45 (m, 4H), 3.30−3.12 (m, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 155.2, 152.1, 145.3, 142.2, 139.5, 132.7, 130.2, 119.3, 118.8, 118.5, 117.0, 114.2, 109.6, 105.1, 103.3, 92.9, 66.2 (2C), 65.8, 56.9, 53.6 (2C) ppm. HRMS, $m/z$ calcd for $C_{22}H_{22}N_4O_4$ $[(M + H)^+]$ 406.1641, found 406.1639; mp >270 °C.

*6-[2-(4,4-Difluoro-piperidin-1-yl)-ethoxy]-2-pyrrolo[1,2-c]-pyrimidin-3-yl-chromen-4-one Oxime Hydrochloride (**41**).* The title compound was prepared in a manner analogous to **42** (48%, two steps), using 4,4-difluoropiperidine instead of pyrrolidine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.27 (bs, 1H), 10.99 (bs, 1H), 9.23 (s, 1H), 8.06 (s, 1H), 7.82 (d, $J$ = 2.8 Hz, 1H), 7.47 (d, $J$ = 9.0 Hz, 1H), 7.45 (s, 1H), 7.41 (d, $J$ = 3.0 Hz, 1H), 7.22 (dd, $J$ = 9.0, 3.0 Hz, 1H), 6.99 (dd, $J$ = 3.7, 2.8 Hz, 1H), 6.74 (d, $J$ = 3.7 Hz, 1H), 4.52−4.47 (m, 2H), 3.90−3.70 (m, 2H), 3.66−3.62 (m, 2H), 3.29−3.33 (m, 2H), 2.60−



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2.30 (m, 4H). MS (ESI+): 441.1 $[C_{23}H_{22}F_2N_4O_3 + H]^+$ ($m/z$); mp >250 °C.

*6-(2-Pyrrolydin-1-yl-ethoxy)-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one Oxime Hydrochloride (**42**).* A suspension of 6-hydroxy-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**204**, 140 mg, 0.40 mmol), potassium carbonate (277 mg, 2.0 mmol), and 1,2-dichloroethane (1.0 mL, 12.6 mmol) in dry DMF (6.5 mL) was subjected to microwave irradiation at 130 °C for 90 min. The reaction mixture was poured onto a saturated aqueous solution of ammonium chloride and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness. Purification by column chromatography on silica gel (using a gradient of 0−20% ethyl acetate in cyclohexane as eluent) gave 6-(2-chloro-ethoxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl oxime (48 mg, 29%) as a yellow solid. This reaction was realized twice and the products were combined. LCMS, $m/z$ = 412.0 $[M + H]^+$.

A mixture of 6-(2-chloro-ethoxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl oxime (64 mg, 0.16 mmol), potassium carbonate (64 mg, 0.46 mmol), and pyrrolidine (19 μL, 0.24 mmol) in acetonitrile (1.5 mL) was heated in a sealed reactor at 100 °C for 18 h. The reaction mixture was cooled to room temperature, and the precipitate was filtered off and washed with ethyl acetate. The filtrate was concentrated to dryness and purified by column chromatography on silica gel (using a gradient of 0−2% methanol in ethyl acetate as eluent) to give 6-(2-pyrrolydin-1-yl-ethoxy)-2-pyrrolo[1,2-*c*]-pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl oxime (49 mg, 71%) as a yellow solid. LCMS, $m/z$ = 447.1 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **40**. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.94 (bs, 1H), 10.16 (bs, 1H), 9.22 (s, 1H), 8.04 (s, 1H), 7.82 (d, $J$ = 2.8 Hz, 1H), 7.46 (d, $J$ = 9.0 Hz, 1H), 7.44 (s, 1H), 7.38 (d, $J$ = 3.0 Hz, 1H), 7.20 (dd, $J$ = 9.0, 3.0 Hz, 1H), 6.99 (dd, $J$ = 3.6, 2.8 Hz, 1H), 6.74 (d, $J$ = 3.6 Hz, 1H), 4.40−4.25 (m, 2H), 3.45−3.69 (m, 4H), 3.11−3.22 (m, 2H), 2.00−2.08 (m, 2H), 1.88−1.95 (m, 2H). MS (ESI+): 391.2 $[C_{22}H_{22}N_4O_3 + H]^+$ ($m/z$); mp >260 °C.

*6-[2-(4-Methyl-piperazin-1-yl)-ethoxy]-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one Oxime Dihydrochloride (**43**).* The title compound was prepared in a manner analogous to **42** (26%, two steps), using 4-methylpiperazine instead of pyrrolidine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.56 (bs, 1H), 10.98 (bs, 1H), 9.22 (s, 1H), 8.05 (s, 1H), 7.82 (d, $J$ = 2.8 Hz, 1H), 7.46 (d, $J$ = 9.0 Hz, 1H), 7.45 (s, 1H), 7.39 (d, $J$ = 3.0 Hz, 1H), 7.21 (dd, $J$ = 9.0, 3.0 Hz, 1H), 6.99 (dd, $J$ = 3.7, 2.8 Hz, 1H), 6.74 (d, $J$ = 3.7 Hz, 1H), 4.46−4.42 (m, 2H), 4.00−3.20 (m, 10H), 2.84 (s, 3H). MS (ESI+): 420.2 $[C_{23}H_{25}N_5O_3 + H]^+$ ($m/z$); mp >230 °C.

*6-[2-(4-Dimethylaminopiperidin-1-yl)-ethoxy]-2-pyrrolo[1,2-c]-pyrimidin-3-yl-chromen-4-one Oxime Dihydrochloride (**44**).* The title compound was prepared in a manner analogous to **42** (14%, two steps), using 4-dimethylaminopiperidine instead of pyrrolidine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.08 (s, 1H), 7.99 (s, 1H), 7.73 (m, 1H), 7.42 (m, 3H), 7.41 (s, 1H), 7.17 (m, 1H), 6.95 (s, 1H), 6.71 (m, 1H), 4.36 (m, 2H), 3.70 (m, 2H), 3.53 (m, 2H), 3.16 (m, 2H), 2.78 (s, 6H), 2.50 (m, 1H), 2.28 (m, 2H), 2.03 (m, 2H). Two exchangeable protons were not observed. MS (ESI+): 448.3 $[C_{25}H_{29}N_5O_3 + H]^+$ ($m/z$); mp >255 °C.

*6-(1-Acetyl-piperidin-4-yloxy)-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one Oxime (**45**).* The title compound was prepared in a manner analogous to **58** (22%, four steps), starting from 6-iodo-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**105**) and using 1-boc-4-hydroxypiperidine instead of 1-boc-3-hydroxyazetidine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.01 (bs, 1H), 9.22 (s, 1H), 8.05 (s, 1H), 7.82 (s, 1H), 7.43−7.37 (m, 3H), 7.17 (dd, $J$ = 9.0, 2.9 Hz, 1H), 6.99−6.98 (m, 1H), 6.75−6.73 (m, 1H), 4.66−4.57 (m, 1H), 3.89−3.81 (m, 1H), 3.72−3.64 (m, 1H), 3.39−3.30 (m, 1H), 3.28−3.19 (m, 1H), 2.02 (s, 3H), 1.99−1.86 (m, 2H), 1.71−1.59 (m, 1H), 1.57−1.48 (m, 1H). MS (ESI+): 419.1 $[C_{23}H_{22}N_4O_4 + H]^+$ ($m/z$); mp >250 °C.

*3-(4-Hydroxyimino-2-pyrrolo[1,2-c]pyrimidin-3-yl-4H-chromen-6-yloxy)-azetidine-1-carboxylic Acid Dimethylamide (**46**).* 6-(1-Boc-azetidin-3-yloxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime was prepared in a manner analogous to **58** (first step, 40%), starting from 6-iodo-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime **105**. LCMS, $m/z$ = 505.3 $[M + H]^+$.

To a solution of 6-(1-boc-azetidin-3-yloxy)-2-pyrrolo[1,2-*c*]-pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (235 mg, 0.47 mmol) in dichloromethane (23 mL) was added trifluoroacetic acid (0.5 mL), and the reaction mixture was stirred at room temperature for 30 min. The reddish solution was carefully neutralized by addition of a saturated aqueous solution of potassium carbonate at 0 °C and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness to give 6-(azetidin-3-yloxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (195 mg, quant) as a yellow solid. LCMS, $m/z$ = 405.3 $[M + H]^+$.

The title compound was prepared in a manner analogous to **58** (84%, 2 steps), starting from 6-(azetidin-3-yloxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime and using dimethylcarbamyl chloride instead of acetyl chloride. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.93 (s, 1H), 9.09 (s, 1H), 7.92 (s, 1H), 7.69 (d, $J$ = 2.5 Hz, 1H), 7.32−7.30 (m, 2H), 7.04 (d, $J$ = 3.0 Hz, 1H), 6.98 (dd, $J$ = 9.0, 3.0 Hz, 1H), 6.85 (dd, $J$ = 3.7, 2.5 Hz, 1H), 6.61 (d, $J$ = 3.7 Hz, 1H), 4.90 (m, 1H), 4.18 (dd, $J$ = 9.1, 6.5 Hz, 2H), 3.76 (dd, $J$ = 9.1, 3.8 Hz, 2H), 2.60 (s, 6H). MS (ESI+): 420.3 $[C_{22}H_{21}N_5O_4 + H]^+$ ($m/z$); mp >250 °C.

*6-(3-Pyridin-3-yl-propoxy)-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one Oxime (**47**).* A mixture of 6-hydroxy-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**204**, 300 mg, 0.86 mmol), 3-(3-chloropropyl)-pyridine hydrochloride[45] (422 mg, 1.89 mmol), and potassium carbonate (475 mg, 3.43 mmol) in dry acetonitrile (8.5 mL) was heated at 85 °C for 3 days. Acetonitrile was removed under vacuum, and the crude residue was treated with water and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum. Purification by column chromatography on silica gel (using a gradient of 0−50% ethyl acetate in cyclohexane as eluent) afforded 6-(3-pyridin-3-yl-propoxy)-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (320 mg, 80%) as a yellow solid. LCMS, $m/z$ = 469.4 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **60**. The title compound was purified by preparative HPLC and isolated as a free base after neutralization of the collected tubes with potassium carbonate and collection by filtration (9%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.95 (bs, 1H), 9.22 (s, 1H), 8.87 (s, 1H), 8.78 (d, $J$ = 5.6 Hz, 1H), 8.50 (d, $J$ = 7.8 Hz, 1H), 8.04 (s, 1H), 7.99 (dd, $J$ = 7.8, 5.6 Hz, 1H), 7.82 (d, $J$ = 2.5 Hz, 1H), 7.44 (s, 1H), 7.41 (d, $J$ = 9.1 Hz, 1H), 7.30 (d, $J$ = 3.0 Hz, 1H), 7.08 (dd, $J$ = 9.1, 3.0 Hz, 1H), 6.98 (dd, $J$ = 3.5, 2.5 Hz, 1H), 6.74 (d, $J$ = 3.5 Hz, 1H), 4.06 (t, $J$ = 6.1 Hz, 2H), 3.00 (t, $J$ = 7.5 Hz, 2H), 2.19−2.12 (m, 2H). MS (ESI+): 413.3 $[C_{24}H_{20}N_4O_3 + H]^+$ ($m/z$); mp >250 °C.

*6-[3-(2-Methyl-pyridin-4-yl)-propoxy]-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one Oxime, Hydrochloride (**48**).* At 0 °C, to a solution of 3-(2-methyl-pyridin-4-yl)-propan-1-ol (230 mg, 1.52 mmol) in dichloromethane (7.6 mL) and triethylamine (0.25 mL, 1.82 mmol), methanesulfonyl chloride (118 μL, 1.52 mmol) was slowly added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane, washed with a saturated aqueous solution of potassium carbonate and brine, and then dried over sodium sulfate and concentrated under vacuum. The crude methanesulfonic acid 3-(2-methyl-pyridin-4-yl)-propyl ester (350 mg) was directly engaged in the next step. LCMS, $m/z$ = not detected.

To a solution of 6-hydroxy-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl-oxime (**204**, 150 mg, 0.43 mmol) in acetonitrile (9.0 mL) were added potassium carbonate (180 mg, 1.28 mmol) and methanesulfonic acid 3-(2-methyl-pyridin-4-yl)-propyl ester (147 mg, 0.64 mmol). The reaction mixture was stirred at 100 °C overnight before being cooled to room temperature, neutralized with water, and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated


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under vacuum. The crude yellow solid was purified by column chromatography on silica gel (using a gradient of 20−50% ethyl acetate in cyclohexane as eluent) to afford 6-[3-(2-methyl-pyridin-4-yl)-propoxy]-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O*-*tert*-butyl oxime (100 mg, 32%) as a yellow solid. LCMS, $m/z$ = 483.3 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **60**. The title compound was purified by preparative HPLC and isolated as a free base after neutralization of the collected tubes with potassium carbonate and collection by filtration (61%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.93 (s, 1H), 9.24 (s, 1H), 8.68 (d, $J$ = 6.2 Hz, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 7.84−7.81 (m, 2H), 7.46 (s, 1H), 7.44 (d, $J$ = 9.1 Hz, 1H), 7.36 (d, $J$ = 3.0 Hz, 1H), 7.12 (dd, $J$ = 9.1, 3.0 Hz, 1H), 7.00 (dd, $J$ = 3.8, 2.8 Hz, 1H), 6.76 (d, $J$ = 3.8 Hz, 1H), 4.08 (t, $J$ = 6.2 Hz, 2H), 3.04 (t, $J$ = 7.2 Hz, 2H), 2.72 (s, 3H), 2.21−2.14 (m, 2H). One exchangeable proton was not observed. MS (ESI+): 427.3 $[C_{25}H_{22}N_4O_3 + H]^+$ ($m/z$); mp >250 °C.

*3-(2-Methyl-pyridin-4-yl)-propan-1-ol.* Compound was prepared as follows: To a solution of 2-methyl-pyridine-4-carbaldehyde (100 mg, 1.03 mmol) in toluene (4.0 mL) was added (carbethoxymethylene)triphenylphosphorane (431 mg, 1.24 mmol), and the reaction mixture was stirred at room temperature overnight. Toluene was removed under vacuum, and petroleum ether (20 mL) was added. The solid was triturated and filtered off. The filtrate was concentrated under vacuum to give 3-(2-methyl-pyridin-4-yl)-acrylic acid ethyl ester as a colorless oil. LCMS, $m/z$ = 192.1 $[M + H]^+$. To a solution of crude 3-(2-methyl-pyridin-4-yl)-acrylic acid ethyl ester in absolute ethanol (9.0 mL), sodium borohydride (1.56 g, 41.2 mmol) was slowly added and the suspension was stirred at room temperature for 3 days. The reaction mixture was neutralized by addition of a 1 N aqueous solution of HCl at 0 °C, diluted with water, and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum to give 3-(2-methyl-pyridin-4-yl)-propan-1-ol (230 mg, 74%) as a pale-yellow oil. LCMS, $m/z$ = 152.1 $[M + H]^+$.

*6-(2-Morpholin-4-yl-ethoxy)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**49**).* A mixture of 6-hydroxy-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**201**, 100 mg, 0.27 mmol), 4-(2-chloroethyl)morpholine hydrochloride (62 mg, 0.41 mmol), and potassium carbonate (113 mg, 0.82 mmol) in dry acetonitrile (5.5 mL) was heated under reflux for 16 h. The reaction mixture was poured into ice−water, and the resulting precipitate was collected by filtration and dried under vacuum to give 6-(2-morpholin-4-yl-ethoxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (108 mg, 82%) as a yellow solid. LCMS, $m/z$ = 480.3 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **40** (77%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.14 (bs, 2H), 9.27 (s, 1H), 8.78 (s, 1H), 8.06 (d, $J$ = 5.4 Hz, 1H), 7.74 (d, $J$ = 5.4 Hz, 1H), 7.70 (s, 1H), 7.52 (d, $J$ = 9.0 Hz, 1H), 7.43 (d, $J$ = 2.9 Hz, 1H), 7.25 (dd, $J$ = 9.0, 2.9 Hz, 1H), 4.52−4.49 (m, 2H), 4.00−3.97 (m, 2H), 3.87−3.81 (m, 2H), 3.60−3.51 (m, 4H), 3.26−3.18 (m, 2H). MS (ESI +): 424.3 $[C_{22}H_{21}N_3O_4S + H]^+$ ($m/z$); mp >230 °C.

*6-[2-(4,4-Difluoro-piperidin-1-yl)-ethoxy]-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**50**).* The title compound was prepared in a manner analogous to **49** (42%, two steps), starting from 6-hydroxy-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**201**) and 1-(2-chloroethyl)-4,4-difluoropiperidine hydrochloride instead of 4-(2-chloroethyl)-morpholine hydrochloride. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.36 (bs, 1H), 11.06 (bs, 1H), 9.26 (s, 1H), 8.77 (s, 1H), 8.05 (d, $J$ = 5.4 Hz, 1H), 7.73 (d, $J$ = 5.4 Hz, 1H), 7.69 (s, 1H), 7.49 (d, $J$ = 9.0 Hz, 1H), 7.42 (d, $J$ = 2.9 Hz, 1H), 7.24 (dd, $J$ = 9.0, 2.9 Hz, 1H), 4.51−4.48 (m, 2H), 3.92−3.88 (m, 2H), 3.74−3.69 (m, 2H), 3.65−3.63 (m, 2H), 3.45−3.26 (m, 2H), 2.43−2.33 (m, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 154.4, 152.6, 147.4, 145.8, 145.7, 142.8, 142.1, 137.4, 136.4, 131.2, 122.9 (t, $J$ = 247 Hz, $CF_2$), 119.5, 119.1, 118.6, 114.1, 105.7, 94.0, 62.8, 55.9, 53.9 (2C), 49.2 (2C) ppm. MS (ESI+): 458.3 $[C_{23}H_{21}F_2N_3O_3S + H]^+$ ($m/z$); mp 225−230 °C.

*1-(2-Chloroethyl)-4,4-difluoropiperidine Hydrochloride.* Compound was prepared as follows: a suspension of 4,4-difluoropiperidine hydrochloride (1.1 g, 6.98 mmol), 2-bromo-1-ethanol (470 μL, 6.63 mmol), and potassium carbonate (780 mg, 5.65 mmol) in dry acetonitrile (20 mL) was heated at 90 °C for 20 h. After cooling, the suspension was filtered off and the filtrate was concentrated under vacuum. The residue was taken in chloroform, filtered off, and the filtrate concentrated to dryness to give 2-(4,4-difluoro-piperidin-1-yl)-ethanol (1.1 g, 95%) as a yellow oil. The alcohol was dissolved in dry toluene (10 mL) and thionyl chloride (580 μL, 7.25 mmol) was added. The solution was heated at 120 °C for 2 h before being cooled with an ice bath. The resulting precipitate was collected by filtration and washed with diethyl ether. The residue was recrystallized in hot 1-butanol and triturated in diethyl ether to give 1-(2-chloroethyl)-4,4-difluoropiperidine hydrochloride (685 mg, 64%) as colorless crystals. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.25 (bs, 1H), 4.05−4.03 (m, 2H), 3.26−3.16 (m, 2H), 3.73−3.60 (m, 4H), 2.45−2.32 (m, 4H).

*6-[3-(4,4-Difluoro-piperidin-1-yl)-propyl]-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**51**).* The title compound was prepared in a manner analogous to **60** (39%, two steps), starting from 3-(4-*tert*-butoxyimino-2-thieno[3,2-*c*]pyridin-6-yl-4*H*-chromen-6-yl)-propionaldehyde (**301**) and 4,4-difluoropiperidine instead of morpholine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.30 (bs, 1H), 11.02 (bs, 1H), 9.25 (s, 1H), 8.75 (s, 1H), 8.04 (d, $J$ = 5.4 Hz, 1H), 7.78−7.68 (m, 3H), 7.44 (s, 2H), 3.66−3.55 (m, 2H), 3.20−3.05 (m, 6H), 2.76−2.66 (m, 2H), 2.38−2.24 (m, 2H), 2.16−2.02 (m, 2H). MS (ESI +): 456.0 $[C_{24}H_{23}N_3O_2F_2S + H]^+$ ($m/z$); mp 201−206 °C.

*6-[3-(3,3-Difluoro-pyrrolidin-1-yl)-propyl]-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**52**).* The title compound was prepared in a manner analogous to **60** (25%, two steps), starting from 3-(4-*tert*-butoxyimino-2-thieno[3,2-*c*]pyridin-6-yl-4*H*-chromen-6-yl)-propionaldehyde (**301**) and 3,3-difluoropyrrolidine instead of morpholine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.50 (bs, 1H), 11.01 (bs, 1H), 9.26 (s, 1H), 8.76 (s, 1H), 8.05 (d, $J$ = 5.4 Hz, 1H), 7.78−7.70 (m, 3H), 7.48−7.40 (m, 2H), 4.20−4.00 (m, 2H), 3.45−3.30 (m, 2H), 3.30−3.20 (m, 2H), 2.75−2.65 (m, 2H), 2.08−1.90 (m, 2H), 1.55−1.30 (m, 2H). MS (ESI+): 444.2 $[C_{22}H_{19}F_2N_3O_3S + H]^+$ ($m/z$); mp 226−229 °C.

*(R)-6-(1-Pyrimidin-4-yl-pyrrolidin-3-yloxy)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**53**).* (*R*)-6-(Pyrrolidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl oxime hydrochloride was prepared in a manner analogous to **58** (99%, two steps), starting from 6-iodo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**102**) and using (*R*)-(−)-*N*-boc-3-pyrrolidinol instead of 1-boc-3-hydroxyazetidine. LCMS, $m/z$ = 436.1 $[M + H]^+$.

A mixture of (*R*)-6-(pyrrolidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl oxime hydrochloride (100 mg, 0.21 mmol), 4-bromopyrimidine hydrochloride (83 mg, 0.42 mmol), and diisopropylethylamine (0.11 mL, 0.64 mmol) in ethanol (1.0 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated under vacuum, and the crude residue was purified by column chromatography on silica gel (using a gradient of 0−2% of methanol in dichloromethane as eluent) to give (*R*)-6-(1-pyrimidin-4-yl-pyrrolidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl oxime (95 mg, 87%) as a yellow solid. LCMS, $m/z$ = 514.1 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **58** (39%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.13 (bs, 1H), 9.32 (s, 1H), 8.92 (s, 1H), 8.83 (s, 1H), 8.42 (d, $J$ = 6.9 Hz, 1H), 8.11 (d, $J$ = 5.4 Hz, 1H), 7.79 (d, $J$ = 5.4 Hz, 1H), 7.75 (s, 1 H), 7.56 (d, $J$ = 9.0 Hz, 1H), 7.45 (d, $J$ = 3.0 Hz, 1H), 7.28 (dd, $J$ = 9.0, 3.0 Hz, 1H), 7.05 (d, $J$ = 6.9 Hz, 1H), 5.39−5.36 (m, 1H), 4.07−4.03 (m, 1H), 3.98−3.93 (m, 2H), 3.76−3.71 (m, 1H), 2.44−2.40 (m, 2H). One exchangeable proton was not observed. MS (ESI+): 458.0 $[C_{24}H_{19}N_5O_3S + H]^+$ ($m/z$); mp >250 °C.

*6-(1-Pyrimidin-4-yl-piperidin-4-ylamino)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**54**).* Under inert atmosphere, a mixture of 6-iodo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**102**, 500 mg, 1.05 mmol), 1-boc-4-aminopiperidine (420 mg, 2.10 mmol), potassium phosphate (668 mg, 3.15 mmol), copper iodide (40 mg, 0.21 mmol), and ethylene glycol (0.12 mL, 2.10 mmol) in *n*-butanol (2.5 mL) was heated at 100 °C for



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2 days. The reaction mixture was cooled to room temperature, filtered off, and the residue washed with ethyl acetate. The filtrate was washed with water and brine. The organic phase was dried over sodium sulfate, filtered, and concentrated under vacuum. The crude solid was purified by flash column chromatography on silica gel (using 10−30% ethyl acetate in cyclohexane as eluent) to afford 6-(1-boc-piperidin-4-ylamino)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (390 mg, 68%) as a yellow solid. LCMS, $m/z$ = 549.2 $[M + H]^+$.

The title compound was prepared in a manner analogous to **53** (76%, three steps) starting from 6-(1-boc-piperidin-4-ylamino)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.12 (bs, 1H), 9.27 (s, 1H), 8.83 (s, 1H), 8.79 (s, 1H), 8.35 (d, $J$ = 7.5 Hz, 1H), 8.07 (d, $J$ = 5.4 Hz, 1H), 7.75 (d, $J$ = 5.4 Hz, 1H), 7.71 (s, 1H), 7.50−7.44 (m, 2H); 7.30−7.18 (m, 2H), 4.96−4.68 (m, 2H), 3.79 (bs, 1H), 3.54−3.35 (m, 2H), 2.19−2.11 (m, 2H), 1.52−1.68 (m, 2H). Two exchangeable protons were not observed. MS (ESI+): 471.0 $[C_{25}H_{22}N_6O_2S + H]^+$ ($m/z$); mp >250 °C.

*6-(1-Pyridin-3-yl-azetidin-3-yloxy)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime (**55**).* Under inert atmosphere, a mixture of 6-(azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime hydrochloride obtained in example **58** (100 mg, 0.22 mmol), 3-bromopyridine (31 μL, 0.33 mmol), palladium(II) acetate (5 mg, 0.02 mmol), (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene (27 mg, 0.04 mmol), and sodium *tert*-butoxide (63 mg, 0.65 mmol) in toluene (3.5 mL) was heated at 120 °C for 2 h. The reaction mixture was poured onto ice−water and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness. The resulting yellow oil was purified by column chromatography on silica gel (using 0−10% methanol in dichloromethane as eluent) to afford 6-[1-(pyridin-4-yl)-azetidin-3-yloxy]-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one-*O*-*tert*-butyl oxime (70 mg, 60%) as a yellow solid. LCMS, $m/z$ = 499.0 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **58**. The title compound was purified by preparative HPLC and isolated as a free base after neutralization of the collected tubes with potassium carbonate and extraction with dichloromethane (21%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.08 (bs, 1H), 9.23 (s, 1H), 8.74 (s, 1H), 8.02 (d, $J$ = 5.4 Hz, 1H), 7.92−7.88 (m, 2H), 7.72 (d, $J$ = 5.4 Hz, 1H), 7.66 (s, 1H), 7.50 (d, $J$ = 8.9 Hz, 1H), 7.21 (d, $J$ = 2.9 Hz, 1H), 7.19−7.11 (m, 2H), 6.94−6.87 (m, 1H), 5.31−5.29 (m, 1H), 4.34 (dd, $J$ = 6.5, 8.6 Hz, 2H), 3.89 (dd, $J$ = 3.8, 8.6 Hz, 2H). MS (ESI+): 443.0 $[C_{24}H_{18}N_4O_3S + H]^+$ ($m/z$); mp >250 °C.

*6-(2′-Methyl-3,4,5,6-tetrahydro-2H-[1,3′]bipyridinyl-4-ylmethyl)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime, Hydrochloride (**56**).* The title compound was prepared in a manner analogous to **55** (63%), starting from 6-piperidin-4-ylmethyl-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime hydrochloride (**401**) and using 3-bromo-2-methyl-pyridine instead of 3-bromopyridine. *tert*-Butyl removal was performed in a manner analogous to **60** (40%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.98 (bs, 1H), 9.26 (s, 1H), 8.76 (s, 1H), 8.36 (d, $J$ = 5.4 Hz, 1H), 8.08 (d, $J$ = 8.0 Hz, 1H), 8.04 (d, $J$ = 5.4 Hz, 1H), 7.79 (dd, $J$ = 8.0, 5.6 Hz, 1H), 7.74−7.71 (m, 3H), 7.44 (d, $J$ = 8.3 Hz, 1H), 7.40 (d, $J$ = 8.3, 2.0 Hz, 1H), 3.24−3.21 (m, 2H), 2.77−2.71 (m, 2H), 2.68−2.65 (m, 2H), 2.65 (s, 3H), 1.79−1.69 (m, 3H), 1.49−1.36 (m, 2H). One exchangeable proton was not observed. MS (ESI+): 485.3 $[C_{27}H_{24}N_4O_3S + H]^+$ ($m/z$); mp >210 °C.

*6-(1-Acetyl-piperidin-4-ylmethoxy)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime (**57**).* The title compound was prepared in a manner analogous to **58** (30%, 4 steps), using 1-boc-4-piperidinemethanol instead of 1-boc-3-hydroxyazetidine. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.01 (bs, 1H), 9.25 (s, 1H), 8.76 (s, 1H), 8.03 (d, $J$ = 5.4 Hz, 1H), 7.72 (d, $J$ = 5.4 Hz, 1H), 7.68 (s, 1H), 7.45 (d, $J$ = 9.1 Hz, 1H), 7.33 (d, $J$ = 3.1 Hz, 1H), 7.15 (dd, $J$ = 9.1, 3.1 Hz, 1H), 4.43−4.38 (m, 1H), 4.00−3.85 (m, 3H), 3.15−3.00 (m, 1H), 2.57−2.51 (m, 1H), 2.05−2.00 (m, 1H), 1.99 (s, 3H), 1.86−1.74 (m, 2H), 1.34−1.06 (m, 2H). MS (ESI+): 450.1 $[C_{24}H_{23}N_3O_4S + H]^+$ ($m/z$); mp >235 °C.

*6-(1-Acetyl-azetidin-3-yloxy)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime (**58**).* Under inert atmosphere, a mixture of 6-iodo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**102**, 255 mg, 0.52 mmol), 1-boc-3-hydroxyazetidine (456 mg, 2.62 mmol), copper iodide (20 mg, 0.10 mmol), 1,10-phenanthroline (38 mg, 0.21 mmol), and cesium carbonate (513 mg, 1.57 mmol) in toluene (1.0 mL) was heated at 120 °C for 24 h. The reaction mixture was cooled to room temperature, and the precipitate was filtered off and washed with ethyl acetate. The filtrate was concentrated to dryness and purified by column chromatography on silica gel (using a gradient of 0−40% ethyl acetate in cyclohexane as eluent) to give 6-(1-boc-azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (185 mg, 68%) as a yellow solid. LCMS, $m/z$ = 522.3 $[M + H]^+$.

To a solution of 6-(1-boc-azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (182 mg, 0.35 mmol) in dichloromethane (1.8 mL), was added dropwise a 2N solution of HCl in $Et_2O$ (1.8 mL, 3.5 mmol), and the reaction mixture was stirred at room temperature for 30 min. The yellow precipitate was collected by filtration, washed with a little amount of diethyl ether, and dried under vacuum to give 6-(azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime hydrochloride (159 mg, 99%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.46 (bs, 1H), 9.29 (bs, 1H), 9.27 (s, 1H), 8.79 (s, 1H), 8.06 (d, $J$ = 5.4 Hz, 1H), 7.74 (d, $J$ = 5.4 Hz, 1H), 7.59 (s, 1H), 7.52 (d, $J$ = 9.0 Hz, 1H), 7.29 (d, $J$ = 3.0 Hz, 1H), 7.15 (dd, $J$ = 9.0, 3.0 Hz, 1H), 5.22−5.18 (m, 1H), 4.44−4.04 (m, 2H), 4.08−4.02 (m, 2H), 1.40 (s, 9H). LCMS, $m/z$ = 422.3 $[M + H]^+$.

At 0 °C, to a solution of 6-(azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime hydrochloride (159 mg, 0.35 mmol) in triethylamine (0.2 mL) and dichloromethane (3.2 mL) was added acetyl chloride (60 μL, 0.84 mmol). The reaction mixture was stirred at 0 °C for 6 h before being poured onto a saturated aqueous solution of ammonium chloride and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to dryness. Purification by column chromatography on silica gel (using a gradient of 0−10% methanol in dichloromethane as eluent) gave 6-(1-acetyl-azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl oxime (100 mg, 62%) as a yellow solid. LCMS, $m/z$ = 464.3 $[M + H]^+$.

At 0 °C, to a solution 6-(1-acetyl-azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl oxime (97 mg, 0.21 mmol) and 2,2,2-trifluoroethanol (11 μL, 0.15 mmol) in dichloromethane (10 mL) was added dropwise a 1 M solution of titanium chloride in dichloromethane (0.4 mL, 0.42 mmol). The reaction mixture was stirred at room temperature for 16 h before being treated with an anhydrous solution of 2-propanol (25 mL) to give an orange solution. The mixture was concentrated under vacuum until an orange precipitate formed in 2−3 mL of remaining solvent. The precipitate was collected by filtration, washed with a little amount of 2-propanol and $Et_2O$, and dried under vacuum to give 6-(1-acetyl-azetidin-3-yloxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one oxime (**58**, 80 mg, 94%) as an orange solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.10 (bs, 1H), 9.26 (s, 1H), 8.78 (s, 1H), 8.05 (d, $J$ = 5.4 Hz, 1H), 7.73 (d, $J$ = 5.4 Hz, 1H), 7.69 (s, 1H), 7.50 (d, $J$ = 9.0 Hz, 1H), 7.20 (d, $J$ = 3.0 Hz, 1H), 7.12 (dd, $J$ = 9.0, 3.0 Hz, 1H), 5.12−5.07 (m, 1H), 4.54 (dd, $J$ = 9.5, 6.5 Hz, 1H), 4.27 (dd, $J$ = 10.6, 6.5 Hz, 1H), 4.13 (dd, $J$ = 9.5, 3.6 Hz, 1H), 3.82 (dd, $J$ = 10.6, 3.6 Hz, 1H), 1.81 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 169.9, 153.2, 152.7, 147.7, 145.9, 145.4, 142.4, 142.2, 136.5, 131.6, 122.9, 119.5, 118.9, 114.5, 105.4, 94.2, 67.5, 65.6, 56.9, 54.3, 52.0 ppm. MS (ESI+): 408.2 $[C_{21}H_{17}N_3O_4S + H]^+$ ($m/z$); mp 230−246 °C.

*6-[2-(1-Methyl-1,8-diaza-spiro[4.5]dec-8-yl)-ethoxy]-2-thieno-[3,2-c]pyridin-6-yl-chromen-4-one Oxime Dihydrochloride (**59**).* A suspension of 6-hydroxy-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**201**, 200 mg, 0.55 mmol), potassium carbonate (453 mg, 3.28 mmol), and 2-bromoethanol (174 μL, 2.45 mmol) in dry acetonitrile (5.5 mL) was heated at 100 °C for 3 days. The reaction mixture was concentrated under vacuum and purified by column chromatography on silica gel (using a gradient of 0−40% ethyl



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acetate in cyclohexane as eluent) to give 6-(2-hydroxy-ethoxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (187 mg, 83%) as a yellow solid. LCMS, $m/z$ = 411.3 $[M + H]^+$.

At 0 °C, to a solution of 6-(2-hydroxy-ethoxy)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (185 mg, 0.45 mmol) in dichloromethane (4.5 mL) and triethylamine (75 μL, 0.54 mmol), methanesulfonyl chloride (42 μL, 0.54 mmol) was slowly added and the reaction mixture was stirred at room temperature for 3 h. The resulting solution was diluted with dichloromethane, washed with a saturated aqueous solution of potassium carbonate and brine, and then dried over sodium sulfate and concentrated o dryness. The crude methane sulfonic ester was obtained as a yellow solid and engaged in the next step without purification. LCMS, $m/z$ = 489.3 $[M + H]^+$.

A suspension of the freshly obtained methane sulfonic ester (100 mg, 0.20 mmol), potassium carbonate (85 mg, 0.61 mmol), and 1-methyl-1.8-diazaspiro(4.5)decane dihydrochloride (70 mg, 0.31 mmol) in dry acetonitrile (2.0 mL) was heated at 100 °C for 16 h. The reaction mixture was partitioned between ethyl acetate and water and extracted twice with ethyl acetate. The combined organic extracts were washed brine, dried over sodium sulfate, and concentrated to dryness to give 6-[2-(1-methyl-1,8-diaza-spiro[4.5]dec-8-yl)-ethoxy]-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (66 mg, 59%) as a yellow solid. LCMS, $m/z$ = 447.5 $[M + H]^+$.

*tert*-Butyl removal was performed in a manner analogous to **40**. The title compound was purified by preparative HPLC, and salt formation was realized in a manner analogous to **40** (26%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.32 (bs, 1H), 11.21 (bs, 1H), 11.13 (bs, 1H), 9.23 (s, 1H), 8.85−8.82 (m, 1H), 8.11 (d, $J$ = 5.4 Hz, 1H), 7.79 (d, $J$ = 5.4 Hz, 1H), 7.75 (s, 1H), 7.57 (d, $J$ = 9.0 Hz, 1H), 7.48 (d, $J$ = 3.0 Hz, 1H), 7.31 (dd, $J$ = 9.0, 3.0 Hz, 1H), 4.60−4.51 (m, 2H), 3.82−3.71 (m, 2H), 3.66−3.57 (m, 3H), 3.39−3.12 (m, 3H), 2.85−2.67 (m, 3H), 2.49−2.31 (m, 3H), 2.22−1.90 (m, 5H). MS (ESI+): 491.4 $[C_{27}H_{30}N_4O_3S + H]^+$ ($m/z$); mp >200 °C.

*6-(3-Morpholin-4-yl-propyl)-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one Oxime Hydrochloride (**60**).* A mixture of 3-(4-*tert*-butoxyimino-2-thieno[3,2-*c*]pyridin-6-yl-4*H*-chromen-6-yl)-propional-dehyde (**301**, 360 mg, 0.89 mmol), morpholine (116 mg, 1.33 mmol), sodium triacetoxyborohydride (283 mg, 1.33 mmol), and 1 drop of acetic acid in THF (4.0 mL) was stirred at room temperature for 16 h. The reaction mixture was poured onto ice−water and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under vacuum. The resulting crude yellow oil was purified by column chromatography on silica gel (using 10−100% ethyl acetate in cyclohexane as eluent) to give 6-(3-morpholin-4-yl-propyl)-2-thieno-[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (300 mg, 70%) as a yellow solid. LCMS, $m/z$ = 478.3 $[M + H]^+$.

To a solution of 6-(3-morpholin-4-yl-propyl)-2-thieno[3,2-*c*]-pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (80 mg, 0.17 mmol) in dichloromethane (8 mL) was slowly added TFA (8 mL). The reaction mixture was heated at 45 °C for 24 h before being carefully neutralized by an aqueous solution of sodium bicarbonate at 0 °C and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under vacuum. The crude yellow solid was purified by preparative HPLC and treated with a 1.2 M solution of HCl in methanol. Concentration to dryness and trituration in diethyl ether afforded 6-(3-morpholin-4-yl-propyl)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one oxime hydrochloride (**60**, 42 mg, 55%) as a yellow powder. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.04 (bs, 1H), 10.72 (bs, 1H), 9.26 (s, 1H), 8.77 (s, 1H), 8.05 (d, $J$ = 5.4 Hz, 1H), 7.76−7.70 (m, 3H), 7.47−7.41 (m, 2H), 3.95 (m, 2H), 3.76 (m, 2H), 2.42, (m, 2H), 3.06 (m, 4H), 2.71 (m, 2H), 2.10 (m, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 152.5, 149.6, 147.9, 145.3, 142.4, 142.1, 137.5, 136.4, 131.7, 130.9, 123.0, 121.6, 118.4, 117.8, 114.5, 94.9, 63.1(2C), 55.5, 50.9(2C), 31.5, 24.4 ppm. HRMS, $m/z$ calcd for $C_{23}H_{23}N_3O_3S$ $[(M + H)^+]$ 421.1460, found 421.1454; mp 193−200 °C.

*6-Bromo-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one O-tert-Butyl-oxime (**101**).* To a suspension of sodium hydride (5.5 g, 60% in mineral oil, 139.4 mmol) in anhydrous pyridine (50 mL) was added a solution of 5-bromo-2-hydroxyacetophenone (10.0 g, 46.50 mmol) in pyridine (50 mL) followed by a solution of thieno[3,2-*c*]pyridine-6-carboxylic acid methyl ester (10.7 g, 55.80 mmol) in pyridine (50 mL). After complete addition, the reaction mixture was heated at 90 °C for 1 h before it was cooled to room temperature. The reaction mixture was poured into a cold aqueous HCl solution (3N, 625 mL). The resulting solid was collected by filtration, washed with water, and dried under suction. The solid was suspended in glacial acetic acid (100 mL), and 0.5 mL of concentrated $H_2SO_4$ was added. The resulting suspension was heated at 110 °C for 1 h, before it was cooled to room temperature and concentrated to dryness under reduced pressure. The resulting residue was suspended in ice−water (200 mL) and neutralized with an aqueous NaOH solution (10%). The resulting precipitate was collected by filtration, washed with water, hexane, and dried under suction to give 6-bromo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one (13 g, 78%) as a green solid. LCMS, $m/z$ = 358.0 $[M + H]^+$.

In a sealed tube, a suspension of 6-bromo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one (9.0 g, 25.28 mmol) and *O*-(*tert*-butyl)-hydroxylamine hydrochloride (6.3 g, 50.56 mmol) in anhydrous EtOH (90 mL) was heated at 120 °C for 12 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was collected by filtration, washed with cold EtOH (50 mL), and dried under vacuum to give 6-bromo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O*-*tert*-butyl-oxime (**101**, 6.8 g, 57%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$,) $\delta$ 9.27 (s, 1H), 8.78 (s, 1H), 8.07−8.03 (m, 2H), 7.75−7.71 (m, 2H), 7.61 (s, 1H), 7.53−7.49 (m, 1H), 1.39 (s, 9H). LCMS, $m/z$ = 430.9 $[M + H]^+$.

*6-Iodo-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one O-tert-Butyl-oxime (**102**).* The title compound was prepared in a manner analogous to **101** (50%), starting from 5-iodo-2-hydroxyacetophenone[46] instead of 5-bromo-2-hydroxyacetophenone. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.26 (s, 1H), 8.77 (s, 1H), 8.22 (s, 1 H), 8.06 (d, $J$ = 5.2 Hz, 1H), 7.87 (d, $J$ = 8.3 Hz, 1H), 7.73 (d, $J$ = 5.2 Hz, 1H), 7.61 (s, 1 H), 7.34 (d, $J$ = 8.3 Hz, 1H), 1.39 (s, 9H). LCMS, $m/z$ = 476.9 $[M + H]^+$.

*6-Bromo-2-isoquinolin-3-yl-chromen-4-one O-tert-Butyl-oxime (**103**).* The title compound was prepared in a manner analogous to **101** (76%), starting from isoquinoline-3-carboxylic acid methyl ester. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.28 (s, 1H), 8.27 (s, 1H), 8.19 (d, $J$ = 2.4 Hz, 1H), 8.02 (d, $J$ = 8.3 Hz, 1H), 7.94 (d, $J$ = 8.3 Hz, 1H), 7.78 (s, 1H), 7.76 (td, $J$ = 8.1, 1.3 Hz, 1H), 7.67 (td, $J$ = 7.5, 1.3 Hz, 1H), 7.50 (dd, $J$ = 8.6, 2.4 Hz, 1H), 7.22 (d, $J$ = 8.6 Hz, 1H), 1.43 (s, 9H).

*6-Bromo-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one O-tert-Butyl-oxime (**104**).* The title compound was prepared in a manner analogous to **101** (42%) starting from pyrrolo[1,2-*c*]pyrimidine-3-carboxylic acid ethyl ester instead of thieno[3,2-*c*]pyridine-6-carboxylic acid methyl ester. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.21 (s, 1H), 8.07 (s, 1H), 8.00 (s, 1H), 7.83 (d, $J$ = 2.4 Hz, 1H), 7.70−7.67 (m, 1H), 7.44 (d, $J$ = 8.8 Hz, 1H), 7.34 (s, 1H), 6.99 (d, $J$ = 3.7 Hz, 1H), 6.75 (d, $J$ = 3.7 Hz, 1H), 1.36 (s, 9H). LCMS, $m/z$ = 413.1 $[M + H]^+$.

*6-Iodo-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one O-tert-Butyl-oxime (**105**).* The title compound was prepared in a manner analogous to **101** (50%), starting from pyrrolo[1,2-*c*]pyrimidine-3-carboxylic acid ethyl ester and 5-iodo-2-hydroxyacetophenone instead of 5-bromo-2-hydroxyacetophenone. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.22 (s, 1H), 8.20 (d, $J$ = 2.3 Hz, 1H), 8.07 (s, 1H), 7.86−7.83 (m, 2H), 7.37 (s, 1H), 7.30 (d, $J$ = 8.6 Hz, 1H), 7.00 (dd, $J$ = 3.8, 2.9 Hz, 1H), 6.76 (d, $J$ = 3.8 Hz, 1H), 1.38 (s, 9H). LCMS, $m/z$ = 460.1 $[M + H]^+$.

*7-Bromo-2-isoquinolin-3-yl-chromen-4-one O-tert-Butyl-oxime (**106**).* The title compound was prepared in a manner analogous to **101** (77%), starting from isoquinoline-3-carboxylic acid methyl ester and 4-bromo-2-hydroxyacetophenone instead of 5-bromo-2-hydroxyacetophenone. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.28 (s, 1H), 8.26 (s, 1H), 8.02 (d, $J$ = 8.1 Hz, 1H), 7.94 (d, $J$ = 8.5 Hz, 1H), 7.93 (d, $J$ = 8.1 Hz, 1H), 7.78 (s, 1H), 7.76 (td, $J$ = 7.6, 1.3 Hz, 1H), 7.66 (td, $J$ = 7.5, 1.3 Hz, 1H), 7.53 (d, $J$ = 1.9 Hz, 1H), 7.31 (dd, $J$ = 8.5, 1.9 Hz, 1H), 1.42 (s, 9H).



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*6-Hydroxy-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one O-tert-Butyl-oxime (**201**).* A suspension of 6-bromo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O-tert*-butyl-oxime (**101**, 400 mg, 1.16 mmol), tris(dibenzylideneacetone)dipalladium (60 mg, 0.06 mmol), 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (51 mg, 0.12 mmol), and potassium hydroxide (390 mg, 6.96 mmol) in dioxane (2.5 mL) was degassed with argon for 10 min. Water (2.5 mL) was added, and the reaction mixture was further degassed for 10 min. The tube was sealed, and the reaction mixture was heated at 100 °C for 12 h under vigorous stirring. The reaction mixture was cooled to room temperature, dioxane was removed under vacuum, and the aqueous layer was neutralized with a 1.5 N aqueous solution of HCl. The mixture was extracted twice with ethyl acetate, and the combined organic extracts were dried with brine, sodium sulfate, and concentrated under vacuum. Purification by column chromatography on silica gel (using a gradient of 10−40% ethyl acetate in cyclohexane as eluent) afforded 6-hydroxy-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O-tert*-butyl-oxime (**201**, 376 mg, 88%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.15 (s, 1H), 8.45 (s, 1H), 7.69 (s, 1 H), 7.58 (d, $J$ = 5.4 Hz, 1H), 7.50−7.48 (m, 2H), 7.20 (d, $J$ = 9.0 Hz, 1H), 6.92 (dd, $J$ = 9.0, 3.6 Hz, 1 H), 5.42 (s, 1H), 1.40 (s, 9H). LCMS, $m/z$ = 367.2 $[M + H]^+$.

*6-Hydroxy-2-isoquinolin-3-yl-chromen-4-one O-tert-Butyl-oxime (**203**).* The title compound was prepared in a manner analogous to **201** (89%) starting from 6-bromo-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**103**). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.28 (s, 1H), 8.29 (s, 1H), 8.01 (d, $J$ = 7.7 Hz, 1H), 7.94 (d, $J$ = 8.5 Hz, 1H), 7.75 (t, $J$ = 7.4 Hz, 1H), 7.75 (s, 1H), 7.65 (t, $J$ = 7.5 Hz, 1H), 7.50 (d, $J$ = 3.0 Hz, 1H), 7.28−7.22 (m, 1H), 6.90 (dd, $J$ = 8.5, 3.0 Hz, 1H), 5.08 (s, 1H), 1.42 (s, 9H).

*6-Hydroxy-2-pyrrolo[1,2-c]pyrimidin-3-yl-chromen-4-one O-tert-Butyl-oxime (**204**).* The title compound was prepared in a manner analogous to **201** (57%) starting from 6-bromo-2-pyrrolo[1,2-*c*]pyrimidin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**104**). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.79 (s, 1H), 7.90 (s, 1H), 7.52 (d, $J$ = 2.9 Hz, 1H), 7.49 (s, 1H), 7.44 (d, $J$ = 3.0 Hz, 1H), 7.17 (d, $J$ = 8.8 Hz, 1H), 6.95−6.88 (m, 2H), 6.63 (d, $J$ = 3.8 Hz, 1H), 5.56 (bs, 1H), 1.41 (s, 9H). LCMS, $m/z$ = 350.3 $[M + H]^+$.

*7-Hydroxy-2-isoquinolin-3-yl-chromen-4-one O-tert-Butyl-oxime (**206**).* The title compound was prepared in a manner analogous to **201** (75%) starting from 7-bromo-2-isoquinolin-3-yl-chromen-4-one *O-tert*-butyl-oxime (**106**). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.26 (s, 1H), 8.25 (s, 1H), 8.01−7.91 (m, 3H), 7.75 (s, 1H), 7.72 (dd, $J$ = 7.9, 1.0 Hz, 1H), 7.66−7.61 (m, 1H), 6.79 (d, $J$ = 2.4 Hz, 1H), 6.69 (dd, $J$ = 8.5, 2.4 Hz, 1H), 5.45 (s, 1H), 1.39 (s, 9H).

*3-(4-tert-Butoxyimino-2-thieno[3,2-c]pyridin-6-yl-4H-chromen-6-yl)-propionaldehyde (**301**).* Under inert atmosphere, to a mixture of 6-bromo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O-tert*-butyl-oxime (**101**, 1.0 g, 2.3 mmol), palladium(II) acetate (51 mg, 0.23 mmol), and 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (195 mg, 0.46 mmol) in anhydrous THF (4 mL) was added a 0.5 M solution of 2-(1,3-dioxolan-2-yl)ethylzinc bromide in THF (9.2 mL, 4.6 mmol). The reaction mixture was subjected to microwave irradiation at 100 °C for 1 h. THF was removed under vacuum and the resulting crude yellow oil was purified by column chromatography on silica gel (using 10−30% ethyl acetate in cyclohexane as eluent) to give 6-(2-[1,3]dioxolan-2-yl-ethyl)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one *O-tert*-butyl-oxime (0.87 g, 84%) as a yellow solid. LCMS, $m/z$ = 451.2 $[M + H]^+$.

To a solution of 6-(2-[1,3]dioxolan-2-yl-ethyl)-2-thieno[3,2-*c*]-pyridin-6-yl-chromen-4-one *O-tert*-butyl-oxime (400 mg, 0.89 mmol) in THF (8.0 mL) was added a 3 N aqueous solution of HCl (2.5 mL). The resulting yellow mixture was stirred at room temperature for 24 h to give a thick-yellow emulsion. The reaction mixture was heated at 60 °C for 4 h before being cooled to room temperature. The resulting emulsion was neutralized by addition of an aqueous saturated solution of sodium bicarbonate and extracted twice with dichloromethane. The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under vacuum to give 3-(4-*tert*-butoxyimino-2-thieno[3,2-*c*]pyridin-6-yl-4*H*-chromen-6-yl)-propionaldehyde (**301**, 360 mg, quant) as a yellow solid. The product was used in the next step without purification. LCMS, $m/z$ = 407.3 $[M + H]^+$.

*6-Piperidin-4-ylmethyl-2-thieno[3,2-c]pyridin-6-yl-chromen-4-one O-tert-Butyl-oxime Hydrochloride (**401**).* Under inert atmosphere, to a suspension of zinc dust (223 mg, 3.41 mmol) in anhydrous DMA (0.5 mL) was added 1,2-dibromoethane (32 μL) and trimethylsilyl chloride (49 μL). The resulting slurry was stirred at room temperature for 15 min. A solution of 1-boc-4-iodomethylpiperidine (738 mg, 2.27 mmol) in anhydrous DMA (2.0 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. After decantation, the supernatant was collected by syringe and added to a mixture of 6-bromo-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one-*O-tert*-butyl-oxime (**101**, 325 mg, 0.76 mmol), copper iodide (9 mg, 0.045 mmol), and [1,1′-bis(diphenylphosphino)ferrocene]-dichloropalladium(II), complex with dichloromethane (19 mg, 0.023 mmol) in anhydrous DMA (2.0 mL). The reaction mixture was sealed under inert atmosphere and heated at 80 °C for 24 h before being filtered off on Celite and washed with ethyl acetate. The filtrate was concentrated to dryness, and the crude residue was purified by column chromatography on silica gel (using a gradient of 0−10% of ethyl acetate in cyclohexane as eluent) to afford 6-(1-boc-piperidin-4-ylmethyl)-2-thieno[3,2-*c*]pyridin-6-yl-chromen-4-one-*O-tert*-butyl-oxime (330 mg, 79%) as a yellow solid. LCMS, $m/z$ = 548.4 $[M + H]^+$. Boc removal was performed in a manner analogous to **58** (quant). LCMS, $m/z$ = 448.3 $[M + H]^+$.

**Calcium Functional Assay on Human mGluRs.** Compounds were tested successively for their agonist and allosteric modulator activities on HEK-293 cells transiently overexpressing one of the eight subtypes of human or rat mGlu receptors. Compounds exerted agonist activity if, by themselves in absence of glutamate, they were able to activate the tested mGluR subtype and they exerted positive (PAM) or negative (NAM) allosteric modulator activity if they increased or decreased the effect of glutamate (or L-AP4 for mGluR7), respectively.

HEK-293 cells were cultured in Modified Eagle's Medium (MEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin and 1% nonessential amino acids at 37 °C/5% $CO_2$ and transfected by electroporation as previously described.[47,48] Plasmids encoding human mGluRs were constructed from a pRK backbone and human mGluR cDNAs (either cloned from SK-NSH or human cerebellum mRNA extract or purchased from BioXTal or Genecopoeia). Plasmids encoding the promiscuous G protein G$\alpha$15 or the chimeric Gqi9 or Gi/Gq (GqTOP) G proteins (used to deviate the natural coupling of the group II and group III mGluRs from inhibition of cAMP production to $Ca^{2+}$ release pathway) were described previously.[47,49,50] Receptor activity was detected by changes in intracellular calcium, as measured using the fluorescent $Ca^{2+}$ sensitive dye, Fluo4-AM (Molecular Probes). Cells were cultured for 24 h after electrotransfection. The day of the screening, cells were first deprived from FCS for 3 h, then washed with freshly prepared assay buffer (1× HBSS supplemented with 20 mM HEPES, 1 mM $MgSO_4$, 3.3 mM $Na_2CO_3$, 1.3 mM $CaCl_2$, 2.5 mM Probenecid, and 0.1% BSA) and loaded for 1 h 30 min with assay buffer containing 1 μM Fluo4AM and 0.1 mg/mL pluronic acid. After washing, cells were incubated in assay buffer (50 or 20 μL for assay performed in 96 well-plate (WP) or 384 WP format, respectively), then agonist and allosteric modulator activities of compound were consecutively evaluated on the same cell plate. Agonist activity was first tested during 10 min with the addition of 50 μL (or 20 μL for 384 WP format) of 3× compound solution (prepared in buffer). Then, cells were stimulated by 50 μL (or 20 μL for 384 WP format) of 3× glutamate solution (prepared in buffer) at $EC_{20}$ or $EC_{80}$ for PAM and NAM tests, respectively, and fluorescence was recorded for additional 3 min; $EC_{20}$ and $EC_{80}$ glutamate concentrations are the concentrations resulting in 20% or 80% of the maximal glutamate response, respectively. Successive compound additions and measurement of fluorescence signals (excitation, 485 nm; emission, 525 nm) at sampling intervals of 1 s were performed using microplate reader FLIPRTetra (Molecular Devices).

For potency determination, a concentration−response test was performed using 20 concentrations (ranging over 6 logs) of each compound. Concentration−response curves were fitted using the


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sigmoidal dose−response (variable slope) analysis in XLfit Scientific Curve Fitting for Excel (IDBS). Potency and efficacy (expressed in percentage of maximal glutamate response) of agonist/positive allosteric modulator effects were calculated. Experiments were performed in duplicate, three times independently.

For experiments of shift in glutamate concentration−response curve, 10 or 20 concentrations of glutamate (ranging over 4.5 logs) were tested alone or in the presence of various concentrations (typically 0.1, 0.3, 1, 3, 10 $\mu$M) of each compound. Concentration−response curves were fitted using the sigmoidal dose−response (variable slope) analysis in XLfit Scientific Curve Fitting for Excel (IDBS). Potency of glutamate alone or in the presence of compound was determined and used to calculate fold increase in the apparent affinity for glutamate. Experiments were performed in duplicate, three times independently.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00991.

General methods for the synthesis of all compounds, and methods for the in vitro and in vivo DMPK protocols and supplemental figures (PDF)

Molecular formula strings and the associated biological data (XLSX)

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The manuscript was written by D.C and S.S. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare the following competing financial interest(s): All authors of this manuscript are or were employees of the different institutions involved in this program (Prexton Therapeutics, Domain Therapeutics, Prestwick Chemical, EMD Serono). Prexton Therapeutics is actively developing mGluR4 PAMs as therapeutic agents.

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## ■ ABBREVIATIONS USED

AMPA, $\alpha$-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; BG, basal ganglia; COMT, catechol-*O*-methyltransferase; L-DOPA, 3,4-dihydroxy-L-phenylalanine; MAO, monoamine oxidase; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; NAM, negative allosteric modulator; PAM, positive allosteric modulator; PD, Parkinson's disease; PK, pharmacokinetic(s); 6-OHDA, 6-hydroxydopamine

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