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Article

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Discovery, structure-activity relationship and anti-parkinsonian effect of a potent and brain-penetrant chemical series of positive allosteric modulators of metabotropic glutamate receptor 4

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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 analog of compound **40** with improved pharmacokinetic profile after oral administration. Based on its favorable and unique preclinical profile, compound **60** (PXT002331, now foliglurax) was nominated as 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]. Amongst 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 non-dopaminergic transmissions have emerged [5]. Amongst 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 non-selective 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 (-)-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** (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 anti-parkinsonian activity in preclinical rodent models of Parkinson's disease.

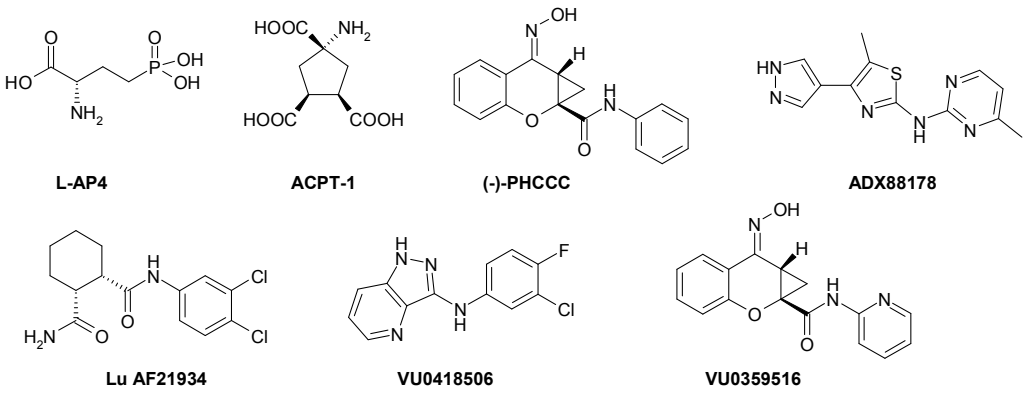


Figure 1. Structure of reference mGluR4 ligands.

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 μM 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 analog described with a 10-fold improvement in potency and no longer side activity on mGluR1 [30].

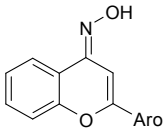
Despite these disappointing reports, we decided to re-examine the potential of (-)-PHCCC scaffold by checking importance of the cyclopropane and amide moieties. Indeed, we considered that removal of the chiral centers of (-)-PHCCC and replacement of the amide right-hand side were key in order to simplify the scaffold for an optimization effort and to increase chances of brain penetration. Analog **1** (Table 1) was synthesized and was found to retain some level of PAM activity on mGluR4 (EC_{50}=14.2 μM). Interestingly, this molecule **1** was previously reported by a team from Vanderbilt University as being inactive at 30 μM in a Gqi5 functional test (compound 1i in supplementary information of reference [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.

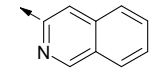
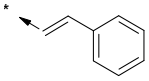
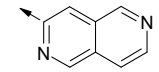
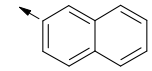
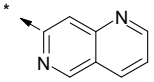
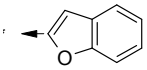
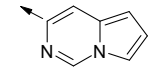
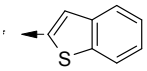
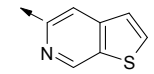
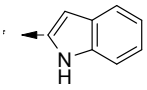
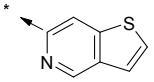
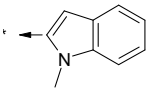
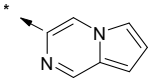
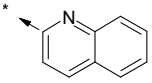
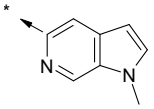
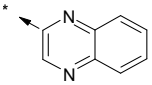
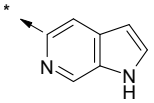
Syntheses and all analytical characterizations of the different compounds described in this article are detailed in the Supporting information (SI).

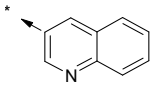
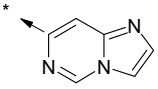
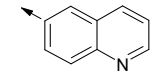
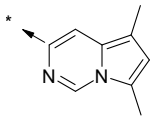
Encouraged by the result obtained with **1**, we decided to further explore the styryl moiety and prepared a library of rigidified analogs (see Table 1). First, rigidification with a naphtyl or several 5-6 and 6-6 bicyclic heterocyclic groups lead to very weak or inactive analogs **2-8**. However, quinolinyl **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 sub-micromolar 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** prevent mGluR4 PAM activity.

Table 1: Exploration of the aromatic substituents of chromenone oxime scaffold



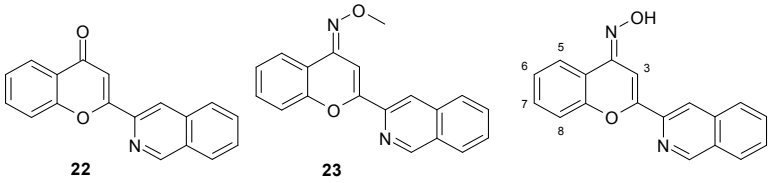
Compounds	Aromatic group	mGluR4 PAM EC_{50} (μM)[a]	Compounds	Aromatic group	mGluR4 PAM EC_{50} (μM)[a]
PHCCC	/	2.25 ± 1.07	**11**		0.31 ± 0.18
1		14.2 ± 3.75	**12**		1.27 ± 0.31
2		NA	**13**		0.24 ± 0.07
3		>30	**14**		0.11 ± 0.03
4		NA	**15**		0.13 ± 0.09
5		>30	**16**		0.20 ± 0.14
6		NA	**17**		0.28 ± 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 3 independent experiments. NA: not active.

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 analogs 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]. Secondly, 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** to **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 still potent on mGluR4 with EC_{50} ranging between 0,75 µM to 9,8 µM (compounds **33** to **38**). Both polar and non-polar groups were introduced illustrating the fact that positions 6 and 7 can constitute a handle to improve different properties of the molecules.

Table 2: Exploration of the core chomenone oxime of compound 11



22 23

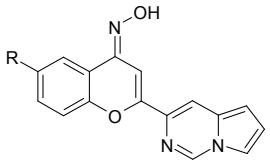
Compounds	Substituents	mGluR4 PAM EC_{50} (μM)[a]	Compounds	Substituents	mGluR4 PAM EC_{50} (μM)[a]
11	H	0.31 ± 0.18	**30**	7-Br	1.72 ± 0.11
22	/	NA	**31**	7-OMe	1.16 ± 0.31
23	/	NA	**32**	8-Cl	NA
24	3-Me	NA	**33**	6-cyclopropyl	1.01 ± 0.07
25	6-Me	0.98 ± 1.08	**34**	6-O-$(CH_2)_2$-OMe	0.75 ± 0.40
26	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

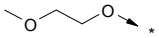
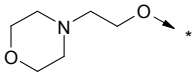
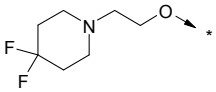
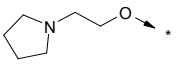
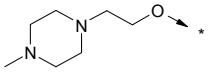
[a] Values are the mean (± SD) of a minimum of 3 independent experiments. NA: non active.

Based on the observations made with compound **11** analogs, 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 μM in MilliQ 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 53 000 -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} (µM)[a]	Water solubility (µM)[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 3 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 minutes 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 minutes. 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 presence of 4 concentrations of compound **40** (ranging from 0.1 to 3 μM). Potency, of glutamate alone or in 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 μM. 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 μM, 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 regards 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 μM (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 μM – 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).

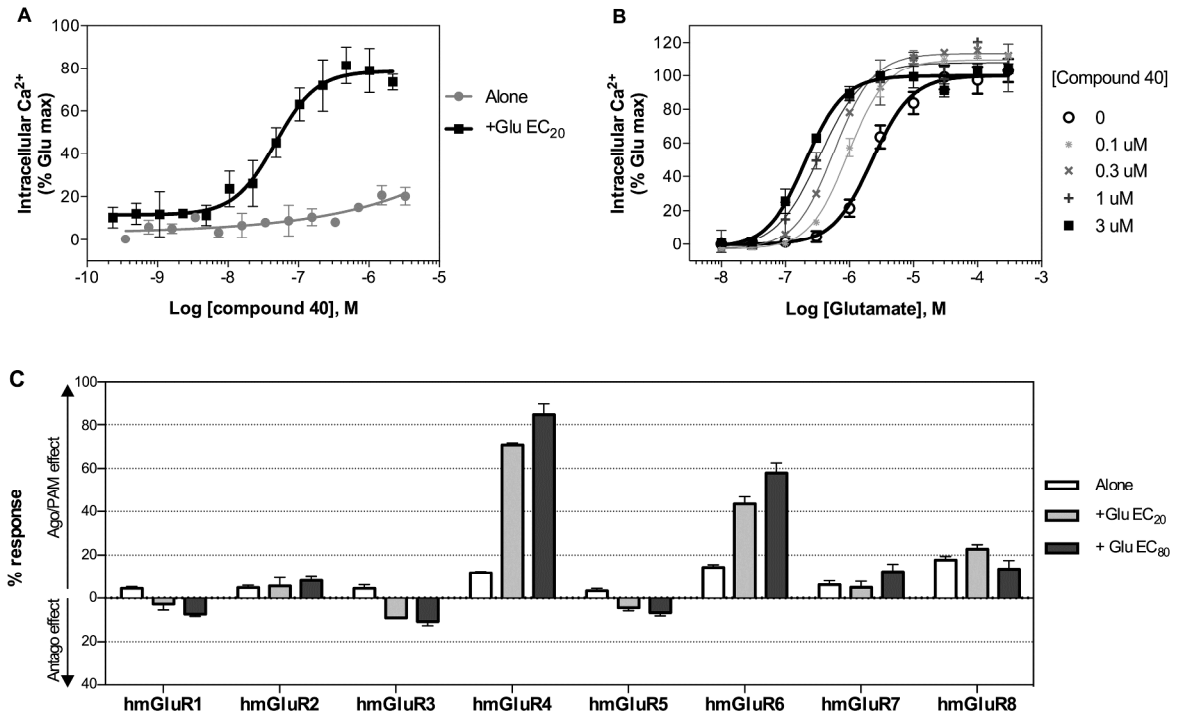


Figure 2: ***In vitro*** **properties of compound 40. A**, Agonist and PAM activities of compound **40** were consecutively evaluated in HEK293 cells expressing human mGluR4. In these cell lines, compound **40** potentiated the increases in intracellular calcium concentrations induced by an EC_{20} glutamate (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 μM on HEK293 cells expressing each of the human mGluR, alone or in 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.

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 μM, 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 μM (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 anti-parkinsonian effect in animal models. Finally, not only the potency but also the selectivity for mGluR4 has been improved compared with (-)-PHCCC since 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 μM showed no functional activity (neither agonist, nor PAM and antagonist activity) on D1, $D2_L$ and A_{2A} receptors. No effect was observed up to 10 μM 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, GSK3beta, IRAK4, JAK3, TAK1). Except a 64%-inhibition of Flt3, no kinase inhibition was detected at 10 μM. Altogether, these results demonstrate that compound **40** is both potent and selective for mGluR4.

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).

Table 4: Mean PK parameters of compound 40 following intravenous administration at 1 mg/kg in rat, and at 2 mg/kg in mouse.

Compound 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 i.v. (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 i.v. (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]

ND: not determined. Values are the mean (± SEM) of [a] 3 or [b] 2 animals. Ratio calculated at [c] one hour or [d] 30 min post-dose.

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 post-oral 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 hours post-dose. The mean half-life was 7.3 hours with an oral dose of 10 mg/kg. The pharmacokinetics of compound **40** in mouse is qualitatively similar to those in rat.

Table 5: Mean PK parameters of compound 40 in rat following intraperitoneal or oral administration, and in mouse following oral administration

Compound 40	Plasma C_{max} (ng/mL)	Plasma T_{max} (min)	Plasma $T_{1/2}$ (h)	Plasma $AUC_{0\text{-inf}}$ (h.ng/mL)	$[Brain]_{30\ min}$ (ng/g)	Brain /Plasma [a]	Brain /rEC_{50}
10 mg/kg i.p. (rat)	1 498 ± 62	15 ± 0	4.0 ± 0.16	2 245 ± 269	5 647 ± 765	3.1 ± 0.3	3.6
30 mg/kg i.p. (rat)	9 324 ± 745	15 ± 0	4.5 ± 0.7	11 349 ± 1 433	22 792 ± 1 111	3.1 ± 0.05	14.5
10 mg/kg p.o. (rat)	135 ± 39	20 ± 5	7.3 ± 1.7	903 ± 223	454 ± 28	2.3 ± 0.4	0.3
10 mg/kg p.o. (mouse)	229 ± 50	30 ± 0	ND	ND	390 ± 78	1.7 ± 0.34	0.2 [b]

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. [a] Ratio calculated at 30 min post-dose. [b] The mouse EC_{50} was assumed to be the same as the rEC_{50}.

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*.

Based on these *in vitro* and *in vivo* parameters, compound **40** was chosen for further characterization in rodent models of Parkinson's disease, and SAR efforts were pursued in order to improve the oral PK profile of the series.

Parkinson's Disease (PD) 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 one hour later, while catalepsy was present, animals were administered an *i.p.* dose of compound **40**. Catalepsy was then assessed 40 minutes 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 *i.p.*, 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.

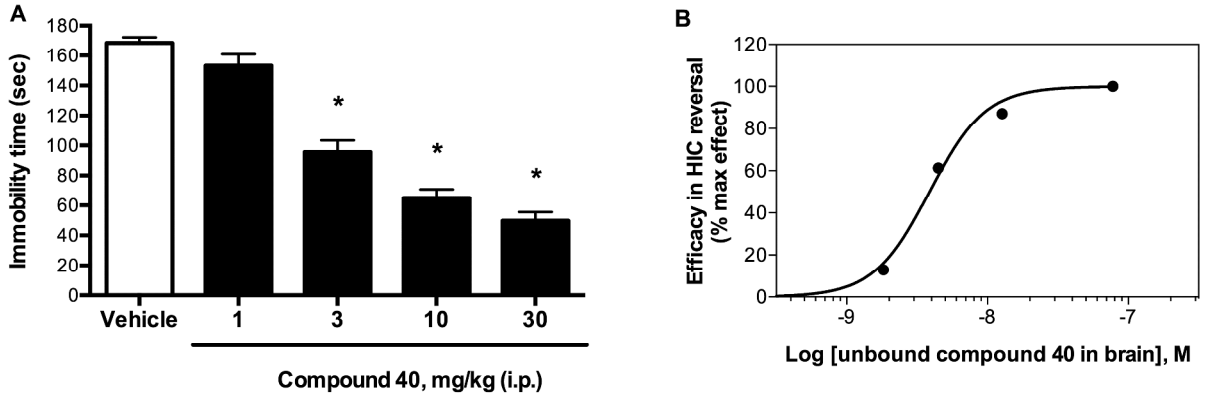


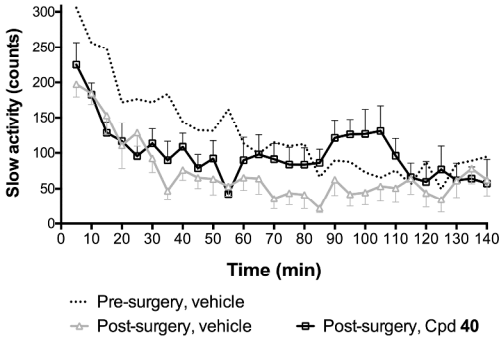
Figure 3: Reversal of haloperidol-induced catalepsy in mice by compound 40. Mice were administered haloperidol (0.5 mg/kg, *ip*) and one hour later, when catalepsy was present, they received an *ip* dose of compound **40** or vehicle. Catalepsy was assessed 40 minutes after compound **40** dosing. **A**, 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.

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]. In order 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.* non-stereotyped activity) and rearing time were significantly reduced to 64%, 66% and 68% respectively, by the

6-OHDA-induced bilateral lesion compared with pre-surgery (Figure S2a, "Post" versus "Pre"). When administered alone, compound **40** (30 mg/kg, *i.p.)* induced an increase in active time, slow activity and rearing time compared with vehicle administration (Figure S2a and 4a). The time course analysis reveals that effects of compound **40** on slow activity were observable during the 30-120 min post-administration 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 post-administration and at 30 and 60 min after *i.p.* 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 and 4b).

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 anti-parkinsonian effects of compound **40**.

A



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 ("Pre-surgery, vehicle", dotted line) or after 6-OHDA-induced striatal lesions. Lesioned rats were treated either with vehicle ("Post-surgery, vehicle", grey curve) or with compound **40** at 30 mg/kg *ip* ("Post-surgery, Cpd **40**", black curve). Each time-point represents the mean counts (+/- SEM) for every 5 minutes. 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).

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 mg/kg L-Dopa represented an optimal anti-parkinsonian 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 sub-threshold dose for use in subsequent experiments. When compound **40** was co-administered with the sub-threshold 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, *i.p.*) allowed a decrease by 70% of the dose of L-Dopa while maintaining the same anti-parkinsonian efficacy. As shown in Figures 5a and 5b, compound **40** induced a dose- and time-dependent anti-parkinsonian effect, with the highest improvement obtained at the dose of 10 mg/kg (Figure 5a), from 30 to 120 min post-administration of compound **40** (Figure 5b). At the highest dose of 30 mg/kg, the anti-parkinsonian effect of 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 pre-lesion rats (Figure 5a).

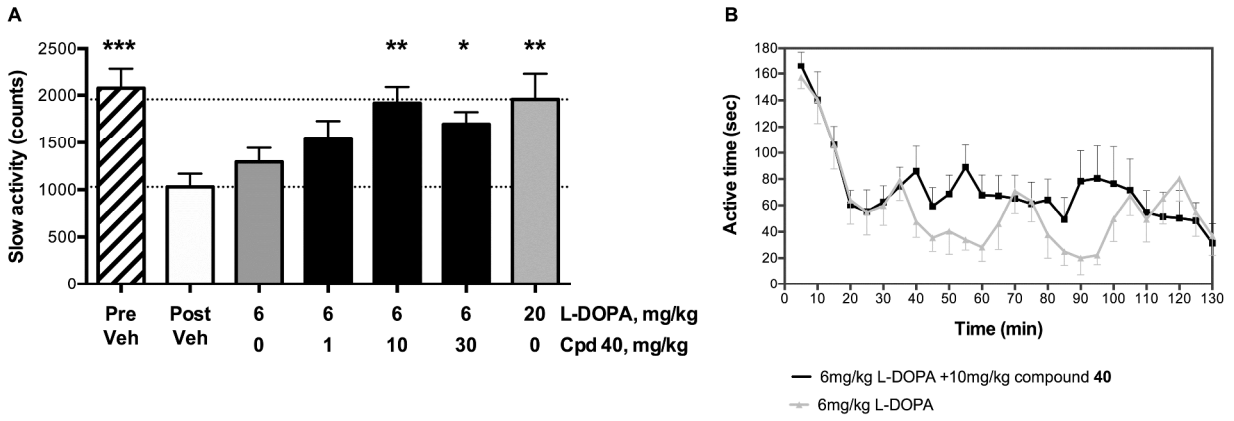


Figure 5: Antiparkinsonian effect of the combined treatment with compound 40 and a low sub-optimal dose of L-Dopa on locomotor activity of 6-OHDA-lesioned rats. A, Total spontaneous locomotor activity values are presented. Data are expressed as mean of group (+SEM) and analyzed using one-way analysis 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 sub-optimal dose of L-Dopa alone ("6mg/kg L-DOPA", grey curve) or with addition of compound **40** at 10 mg/kg ip ("6mg/kg L-DOPA +10mg/kg compound **40**", black curve). Each time-point represents the mean counts (+/- SEM) for every 5 minutes. (n = 10).

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.

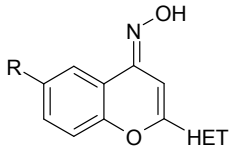
As a first conclusion, in the 6-OHDA rat model of Parkinson's disease motor symptoms, administration of compound **40** allowed a decrease by 70% of the dose of L-Dopa while maintaining the same anti-parkinsonian efficacy, without inducing any adverse event such as overactive rearing, stereotyped behavior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

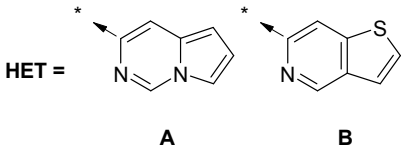
Further improvements of PK properties, identification of candidate **60**

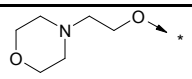
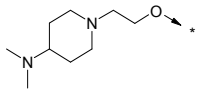
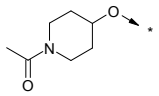
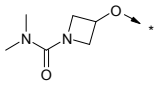
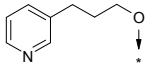
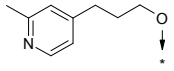
Encouraged by the promising results in PD models obtained with *ip* administration of compound **40**, we next decided to pursue the optimization of the PK profile with the objective of obtaining an optimized compound with increased brain exposure over time and thus improved potential target engagement 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 nM 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 post-administration 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** & **48**) were detrimental to brain exposures (Table 6). As a consequence, none of these first analogs 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 post-dosing 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** to **52**), but these two 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** to **56**) or modifications of the morpholine moiety for close piperidine or azetidine analogs (compounds **56** to **59**) also resulted in very low brain exposures (Table 6). Interestingly however, some of these compounds showed a clear increase in their

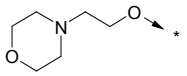
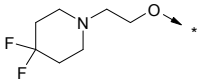
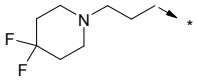
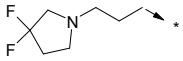
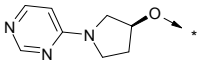
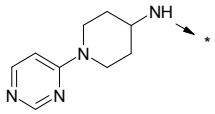
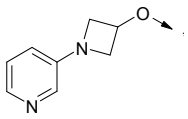
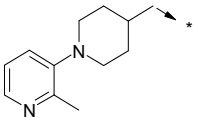
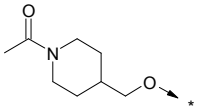
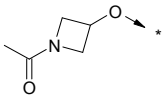
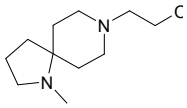
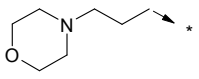
mGluR4 PAM potency as illustrated with molecules **55** to **57**. Finally, the only chemical change in **49** resulting in an increased brain exposure was the replacement of the oxygen atom linking the chromenone oxime scaffold with the left-hand side chain by a -CH_2- group. Indeed, compound **60** was found at concentrations well-above 400 ng/g (~1 μM) up to 90 min after dosing (Table 6). This isosteric modification did not alter the mGluR4 PAM activity since compound **60** exerts a PAM activity on mGluR4 with an EC_{50} that is similar to the ones of close analogs **49** or **40** (Table 6).

Table 6: Further optimization of brain penetration





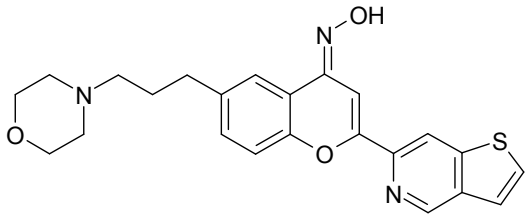
Compounds	HET	R	mGluR4 PAM EC_{50} (nM) [a]	$[Brain]_{30min}$ (ng/g) [b]	$[Brain]_{60min}$ (ng/g) [b]	$[Brain]_{90min}$ (ng/g) [b]
40	A		46 ± 18	454	262	ND
44	A		165 ± 7	2.8	2.9	ND
45	A		10 ± 1	24.1	12.1	ND
46	A		35 ± 8	25.5	8.8	ND
47	A		50 ± 16	3.4	2.3	ND
48	A		19 ± 7	77.3	82.5	ND

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 3 independent experiments. [b] Values of brain concentrations are mean of 3 rats following oral administration at 10 mg/kg. BLQ: concentration below lower limit of quantification (1 ng/mL); ND: not determined.

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 µM, 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, 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 µM, 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, Figure S5). Compound **60** had no effect on COMT, MAO-A or MAO-B at 10 µM in enzymatic assays (Table7), reducing the risk for interaction with L-Dopa metabolism.

Table 7: Summary of key parameters for compound 60



Compound 60 (PXT002331)

In vitro Pharmacology [a]

mGluR4	hum ago EC_{50}	hum PAM EC_{50}	hum fold shift	rat PAM EC_{50}

	> 15 μM	79 nM	10 (at 3 μM)			47 nM
Selectivity	Group I	Group II	mGlu6	mGlu7	mGlu8	NMDA, AMPA, kainate
	Not active	Not active	15x	110x	50x	Not active
Specificity	COMT	MAO-A	MAO-B			
	Not active	Not active	Not active			

In vivo Pharmacokinetics (rat) [b]

1 mg/kg *i.v.*	Plasma C_{max} (ng/mL)	$t_{1/2}$ (h)	Plasma $AUC_{0\text{-inf}}$ (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 *p.o.*	Plasma C_{max} (ng/mL)	Plasma T_{max} (h)	Plasma $AUC_{0\text{-inf}}$ (h.ng/mL)	Brain $AUC_{0\text{-inf}}$ (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 3 rats. [c] Ratio calculated at 1 hour post-dose. [d] Ratio of $AUC_{0\text{-inf}}$.

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

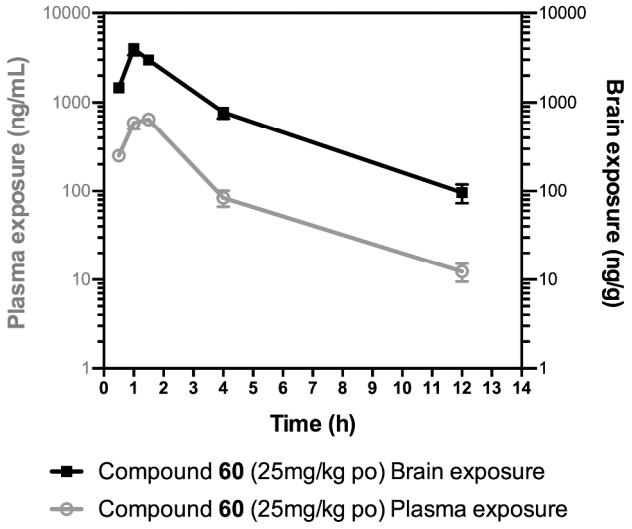


Figure 6: PK profile of compound 60 in plasma and brain following oral administration at 25 mg/kg (in water) to Sprague-Dawley rats.

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. Based on 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 μM). Thin layer chromatography was carried out using pre-coated silica gel F-254plate. ^{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-*D6* (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 (δ) 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 electro spray ionization. The HPLC system was a Waters platform with a 2767 sample manager, a 2525 pump, a photodiode array detector (190-400 nM). The column used was an XBridge C18 3.5 μM (4.6 x 50 mm) in analytical mode and an XBridge C18 OBD 5 μM (30 x 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 document. A few key intermediates were prepared for all these syntheses, they are numbered with 3-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 minutes. Methanol was removed under vacuum and the crude solid was purified by column chromatography on silica gel (using 0% to 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 (300MHz, DMSO-*d6*) δ 10.98 (s, 1H), 7.86 (dd, J = 7.9, 1.6 Hz, 1H), 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-naphtoic 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-naphtoic 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) was 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 (300MHz, DMSO-*d6*) δ 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 (300MHz, DMSO-*d6*) δ 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-*d6*) δ 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 (300MHz, DMSO-*d6*) δ 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 (300MHz, DMSO-*d6*) δ 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 1H-indole-2-carboxylic acid. It was purified by preparative HPLC. ^{1}H-NMR (300MHz, DMSO-*d6*) δ 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-1H-indole-2-carboxylic acid. It was purified by preparative HPLC. ^{1}H-NMR (300MHz, DMSO-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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 (300MHz, DMSO-*d6*) δ 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-*d6*) δ 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

(300MHz, DMSO-*d6*) δ 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-*d6*) δ 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 (300MHz, DMSO-*d6*) δ 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% to 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 (300MHz, $CDCl_3$) δ 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 1M 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 was 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 (300MHz, DMSO-*d6*) δ 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, 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-*d6*) δ 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 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, 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, $CDCl_3$) δ 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-*d6*) δ 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 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, $CDCl_3$) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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 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), pyrrole-2-formyl (428 mg, 4.5 mmol) in dry acetonitrile (45 mL) was stirred at room temperature for 16h. 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 1h. 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% to 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 1h, 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 (300MHz, $CDCl_3$) δ 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-(1H-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 µl, 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-1H-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-1H-pyrrolo[2,3-c]pyridin-5-yl)-chromen-4-one oxime (**18**, 17 mg, 23%, 2 steps) as a yellow solid. ^{1}H-NMR (300MHz, DMSO-*d6*) δ 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 1M 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 1N 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 minutes. After cooling to room temperature, the mixture was concentrated under vacuum and the residue was 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 minutes. Methanol was removed under vacuum and the crude solid was purified by column chromatography on silica gel (using a gradient of 0% to 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-*d6*) δ 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 µl, 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. 10% palladium on charcoal (50 mg) was added and the suspension was stirred under 1 atmosphere 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% to 20% ethyl acetate in cyclohexane as eluent) to give 2-(1H-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-(1H-pyrrolo[2,3-c]pyridin-5-yl)-chromen-4-one oxime (**19**, 39 mg, 70%) as a yellow solid. ^{1}H-NMR (300MHz, DMSO-*d6*) δ 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-*d6*) δ 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 (300MHz, DMSO-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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 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 24h, 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% to 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 (300MHz, $CDCl_3$) δ 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-hydroxy-acetophenone (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 1N 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 µl) 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 (300MHz, $CDCl_3$) δ 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% to 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 (300MHz, $CDCl_3$) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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). (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-*d6*) δ 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 was prepared as follows: at -78 °C, to solution of 2-methoxy-5-trifluoromethyl-acetophenone (650 mg, 3.0 mmol) in dry dichloromethane (40 ml) was slowly added a 1M 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 hours. At 0 °C, the reaction mixture was hydrolyzed with a 1N 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% to 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, $CDCl_3$) δ 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-*d6*) δ 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-*d6*) δ 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 1M 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 6N 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 (400MHz, DMSO-*d6*) δ 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). (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 1h. At 0°C, iodomethane (15 µl, 0.24 mmol) was added dropwise. The resulting solution was stirred at room temperature for 20h, 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% to 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% to 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 (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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). (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 (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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-*d6*) δ 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 minutes. 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% to 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 (400MHz, DMSO-*d6*) δ 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-bromoethylmethylether instead of iodomethane. ^{1}H NMR (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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 1N aqueous solution of HCl. The aqueous phase was washed twice with dichloromethane, neutralized by addition of a 6N 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% to 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 (400MHz, DMSO-*d6*) δ 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% to 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% to 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 (400MHz, DMSO-*d6*) δ

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-bromoethylmethylether instead of iodomethane. ^{1}H NMR (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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 18h. 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% to 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 atmosphere of hydrogen. The reaction mixture was stirred at room temperature for 5h, 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 $[M + H]^+$. Tertbutyl 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 (400MHz, DMSO-*d6*) δ 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-bromoethylmethylether instead of iodomethane. ^{1}H NMR (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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% to 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 1M 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 1.2N 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 (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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), 2.60-2.30 (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 minutes. 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% to 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% to 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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 minutes. 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 (400MHz, DMSO-*d6*) δ 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% to 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 (400MHz, DMSO-*d6*) δ 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, 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 under vacuum. The crude yellow solid was purified by column chromatography on silica gel (using a gradient of 20% to 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 (400MHz, DMSO-*d6*) δ 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 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 1N 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 16h. 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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 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 20h. 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 was 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 2h 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 (400MHz, DMSO-*d6*) δ 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-4H-chromen-6-yl)-propionaldehyde (**301**) and 4,4-difluoropiperidine instead of morpholine. ^{1}H-NMR (400MHz, DMSO-*d6*) δ 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-4H-chromen-6-yl)-propionaldehyde (**301**) and 3,3-difluoropyrrolidine instead of morpholine. ^{1}H-NMR (400MHz, DMSO-*d6*) δ 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 16h. The reaction mixture was concentrated under vacuum and the crude residue was purified by column chromatography on silica gel (using a gradient of 0 to 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-*d6*) δ 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 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% to 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 (400MHz, DMSO-*d6*) δ 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 2h. 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% to 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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% to 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 minutes. 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 (400MHz, DMSO-*d6*) δ 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% to 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 1M 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 isopropanol (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 isopropanol 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 (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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% to 40% ethyl 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 3h. The resulting solution was diluted with dichloromethane, washed with a saturated aqueous solution of potassium carbonate and brine, 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 16h. 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 (400MHz, DMSO-*d6*) δ 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-4H-chromen-6-yl)-propionaldehyde (**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% to 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 1.2M 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 (400MHz, DMSO-*d6*) δ 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-*d6*) δ 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 1h, 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-(tertbutyl)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 (400MHz, DMSO-*d6*,) δ 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-*d6*) δ 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 (300MHz, $CDCl_3$) δ 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 (400MHz, DMSO-*d6*) δ 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 (400MHz, DMSO-*d6*) δ 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 (300MHz, $CDCl_3$) δ 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).

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 1.5N 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% to 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, $CDCl_3$) δ 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 (300MHz, $CDCl_3$) δ 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 (400MHz, $CDCl_3$) δ 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 (300MHz, $CDCl_3$) δ 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 0.5M 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 1h. THF was removed under vacuum and the resulting crude yellow oil was purified by column chromatography on silica gel (using 10% to 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 3N 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-4H-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-iodomethyl-piperidine (738 mg, 2.27 mmol) in anhydrous DMA (2.0 mL) was added dropwise and the reaction mixture was stirred at room temperature for 1h. 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 24h, 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 to 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 over-expressing one of the 8 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% non-essential 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 Gα15, 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 24h after electro-transfection. The day of the screening, cells were first deprived from FCS for 3 hours,

then washed with freshly prepared assay buffer (1x HBSS supplemented with 20 mM HEPES, 1 mM $MgSO_4$, 3.3 mM Na_2CO_3, 1.3 mM $CaCl_2$, 2.5mM Probenecid, and 0.1% BSA) and loaded for 1h30 with assay buffer containing 1 µM Fluo4-AM and 0.1 mg/mL pluronic acid. After washing, cells were incubated in assay buffer (50 µL 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 3x compound solution (prepared in buffer). Then, cells were stimulated by 50 µL (or 20 µL for 384 WP format) of 3x 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 sec 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 sigmoidal dose-response (variable slope) analysis in XLfit Scientific Curve Fitting pour 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 presence of various concentrations (typically 0.1, 0.3, 1, 3, 10 µM) of each compound. Concentration-response curves were fitted

using the sigmoidal dose-response (variable slope) analysis in XLfit Scientific Curve Fitting pour Excel (IDBS). Potency of glutamate alone or in 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.

ANCILLARY INFORMATION

Supporting Information: General methods for the synthesis and characterization of all compounds, and methods for the *in vitro* and *in vivo* DMPK protocols and supplemental figures. Molecular formula strings and the associated biological data were also provided as a CSV file.

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***Abbreviations Used*:** AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; BG, basal ganglia; COMT, Catechol-O-methyltransferase; L-DOPA, 3,4-dihydroxy-Lphenylalanine; 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.

Keywords**:** Metabotropic glutamate receptors; mGluR4 ; mGlu4 ; allosteric modulation ; PAM ; Parkinson's disease ; haloperidol-induced catalepsy ; 6-OHDA ; structure-activity relationship ; brain penetration ; chromenone ; oxime ; L-Dopa-sparing ; Basal Ganglia.

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