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Bioavailability Studies and in vitro Profiling of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitor UCPH-102

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Although the selective excitatory amino acid transporter subtype 1 (EAAT1) inhibitor UCPH-101 has become a standard pharmacological tool compound for in vitro and ex vivo studies in the EAAT research field, its inability to penetrate the blood–brain barrier makes it unsuitable for in vivo studies. In the present study, per os (p.o.) administration (40 mg kg^{-1}) of the closely related analogue UCPH-102 in rats yielded respective plasma and brain concentrations of 10.5 and 6.67 μM after 1 h. Three analogue series were designed and synthesized to improve the bioavailability profile of UCPH-102, but none displayed substantially improved properties in this respect. In vitro profiling of UCPH-102 (10 μM) at 51 central nervous system targets in radioligand binding assays strongly suggests that the compound is completely selective for EAAT1. Finally, in a rodent locomotor model, p.o. administration of UCPH-102 (20 mg kg^{-1}) did not induce acute effects or any visible changes in behavior.

Introduction

In the mammalian central nervous system (CNS), uptake of glutamate (Glu) is mediated by a family of high-affinity Na^+-dependent excitatory amino acid transporters (EAAT) comprising five subtypes: EAAT1–5.[1–4] Although EAAT2 is the most abundant subtype in the adult brain and is believed to be responsible for more than 90% of total Glu uptake, the EAAT1 and EAAT3 subtypes are also expressed throughout the CNS, albeit at lower densities. Finally, EAAT4 is expressed almost exclusively on GABAergic Purkinje cells of the cerebellum and is considered to be a low-capacity Glu transporter, and EAAT5 is only expressed in the retina. Changes in the expression and/or functions of the EAATs have been linked to several disease states such as amyotrophic lateral sclerosis, Alzheimer's disease, chronic pain, and obsessive compulsive disorder.

In the course of our previous work on the EAATs we discovered the first class of selective EAAT1 inhibitors[5,6] and subsequently conducted elaborate structure–activity relationship (SAR) studies to optimize the inhibitory potencies as well as the pharmacokinetic properties of this inhibitor class.[7–10] The analogue UCPH-101 has become a standard tool compound in the EAAT field (Figure 1); however, as it does not penetrate the blood–brain barrier (BBB), it is not suitable for in vivo studies.[11–14] Herein we report that the close analogue UCPH-102, in contrast to UCPH-101, is able to cross the BBB, and we present

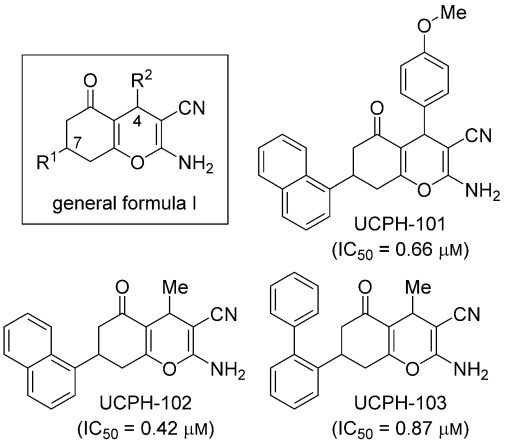

Figure 1. General formula I of the first class of selective EAAT1 inhibitors and structures of three of the most potent analogues UCPH-101, UCPH-102, and UCPH-103.

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Table 1. Bioavailability in rat (p.o. administration, $n=3$) of UCPH-101, UCPH-102, and UCPH-103.

Compd	Dose [mg kg^{-1}]	Plasma concentration [μM] (ng mL^{-1}) 0.5 h	1 h	2 h	Brain concentration [μM] (ng mL^{-1}) 0.5 h	1 h	2 h
UCPH-101[7]	10	1.66	0.66	0.16	0	0	0
UCPH-103[6]	10	0.67	–[a]	1.24	–[b]	–[a]	0.75
UCPH-102	40	9.30 (3070 ± 125)	10.5 (3457 ± 324)	–[a]	4.02 (1327 ± 48)	6.67 (2203 ± 269)	–[a]

[a] Not determined. [b] Below detection limit of 250 ng mL^{-1}.

SAR studies aimed at improving the potency, bioavailability, and pharmacokinetic properties of this EAAT1 inhibitor (compound series **1a–k**, **2a–e**, and **3a–d**). Finally, we describe tritium labeling of UCPH-102 ([^{3}H]UCPH-102) for potential use in in vitro studies.

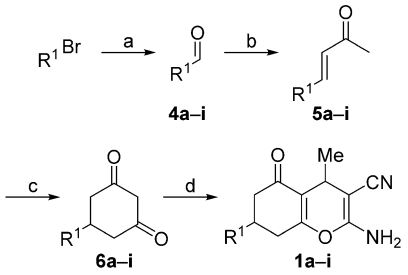
Results and Discussion

We previously published data on the oral bioavailability and BBB penetration for the two analogues UCPH-101[6] and UCPH-103[8] in rats (Figure 1, Table 1). No BBB penetration could be detected for UCPH-101 (2 h, 10 mg kg^{-1}), and the brain concentration of UCPH-103 after 2 h (10 mg kg^{-1}) was determined to be only 0.75 μM. We speculated that the different abilities to penetrate the BBB could be due to the size of the R^2 substituent (4-methoxyphenyl versus methyl). We therefore investigated the oral bioavailability and BBB penetration of UCPH-102 at a dose of 40 mg kg^{-1}. As can be seen from Table 1, this resulted in a brain concentration of 6.67 μM after 1 h (Table 1). We next determined brain protein binding (fraction unbound, FU [%]) of UCPH-102 by in vitro equilibrium dialysis, and found the unbound fraction of the compound to be 3.8% (Table 2).

Design and synthesis of UCPH-102 analogues

Given the inverse correlation between lipophilicity and %FU,[15] we next sought to optimize the physicochemical properties of UCPH-102 by decreasing its lipophilic character, while maintaining (or improving) its inhibitory potency at EAAT1. This was attempted by the systematic introduction of a nitrogen atom into the highly lipophilic naphthyl group of UCPH-102. The nine quinoline/isoquinoline analogues **1a–i** were designed and their synthesis pursued in accordance with the previously described one-pot three-component reaction (Scheme 1). For analogues **1a,f** commercially available bromoisoquinolines were firstly converted into their corresponding carbaldehydes **4a,f**. All nine carbaldehydes **4a–i** were condensed with diethyl (2-oxopropyl)phosphonate to afford enones **5a–i** in good yields. Subsequent reaction with diethyl malonate followed by basic ester hydrolysis and acidic decarboxylation afforded the corresponding 1,3-diketones **6a–i** in moderate to good yields. The target compounds **1a–i** were finally obtained by a one-pot three-component reaction by addition of malononitrile and acetaldehyde.

The synthesis of target analogues **1j,k** commenced with conversion of the corresponding acetophenones **7j,k** into hydroxylamines **8j,k** by reaction with hydroxylamine hydrochloride salt.[16] These were then cyclized to their corresponding benzisoxazoles by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and triphenylphosphine in high yields.[17] The free phenol of series **j** was then converted into its triflate form by use of triflic anhydride in good yield.[18] Heck coupling of analogues **9j**[19] and **9k** afforded the ketones **5j,k**, which were next converted into the 1,3-diketones **6j,k** by reaction with diethylmalonate followed by aqueous ester hydrolysis and acidic decarboxylation. Finally, the one-pot, three-component reaction using malononitrile and acetaldehyde gave the final compounds **1j,k** (Scheme 2).

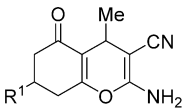


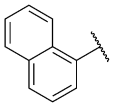
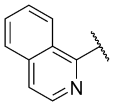
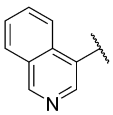
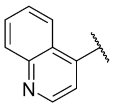
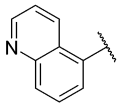
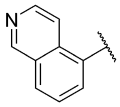
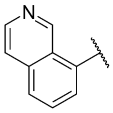
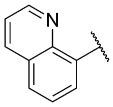
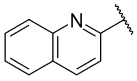
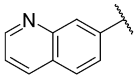
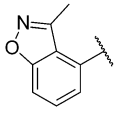
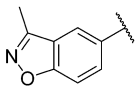
Scheme 1. Synthesis of quinoline and isoquinoline analogues **1a–i** by a one-pot three-component reaction. *Reagents and conditions*: a) sBuLi, DMF, THF, −78 °C→RT (58 and 76%); b) diethyl (2-oxopropyl)phosphonate, NaH, THF, RT (60–83%); c) diethyl malonate, NaOEt/EtOH, then NaOH (reflux), then H_2SO_4 (reflux) (18–84%); d) malononitrile, acetaldehyde *N*-methylmorpholine, EtOH, 0 °C→RT (43–68%).

The pharmacological properties of the 11 analogues **1a–k** were characterized at stable EAAT1-, EAAT2-, and EAAT3-HEK293 cells in a conventional [^{3}H]D-Asp uptake assay,[20] and the results are presented in Table 2. All of the analogues were inactive at EAAT2 and EAAT3 in the concentration ranges tested (Table 2). Analogues **1a–g** displayed between 5- and 100-fold decreases in inhibitory potency at EAAT1 relative to UCPH-102, depending on the position of the introduced nitrogen atom. Analogously, analogues **1h,i** also exhibited ~10-fold lower inhibitory potencies in comparison with a similar 2-naphthyl analogue previously reported.[5,6] Finally, the two benzisoxazoles **1j,k** displayed distinct inhibitory potencies (respective IC_{50} values of 14 and 100 μM) depending on the substitution point. This difference was not unexpected, as we have previously observed a similar pattern for 1-/2-naphthyl groups as substituents at the 7-position of this scaffold.[5,6]

The unbound fraction of the most potent analogues **1a,e,g** in the series were determined to be 4.7, 6.9, and 3.8%, respectively. Thus, for **1a** and **1e** this property was improved relative

Table 2. Inhibitory potencies of analogues **1a–k** at EAAT1, calculated log*P* values, and percent fraction unbound (FU) of selected analogues.[a]



Compd	R^1	IC_{50} [μM] ($pIC_{50} \pm SEM$)[b]	cLog*P*	FU [%][c]
UCPH-102		0.42[7]	2.71	3.8
1a		2.5 (5.61 ± 0.06)	1.44	4.7
1b		4.6 (5.34 ± 0.08)	1.47	–
1c		11 (4.95 ± 0.09)	1.69	–
1d		~50 (~4.3)	1.69	–
1e		2.2 (5.65 ± 0.07)	1.48	6.9
1f		~30 (~4.5)	1.48	–
1g		2.1 (5.67 ± 0.06)	1.69	3.8
1h		~100 (~4.0)	1.66	–
1i		~100 (~4.0)	1.73	–
1j		14 (4.88 ± 0.06)	1.51	–
1k		~100 (~4.0)	1.55	–

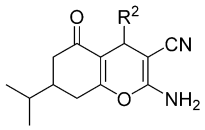
[a] All analogues displayed no inhibitory potency at EAAT2 or EAAT3 at concentrations of 100 μM ($IC_{50} > 100$ μM). [b] Values are the mean ± SEM of $n = 3$ experiments. [c] Residual brain protein binding.

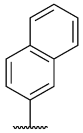
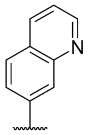
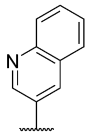
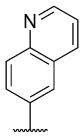
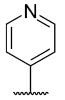
to UCPH-102 (Table 2). However, in light of their ~5-fold lower inhibitory potencies at EAAT1, the modestly increased %FU values were not sufficiently prominent for these analogues to be considered superior to UCPH-102 as in vivo candidates. In summary, the 1-series did not lead to a better in vivo candidate than UCPH-102.

Design and synthesis of analogues of 2a

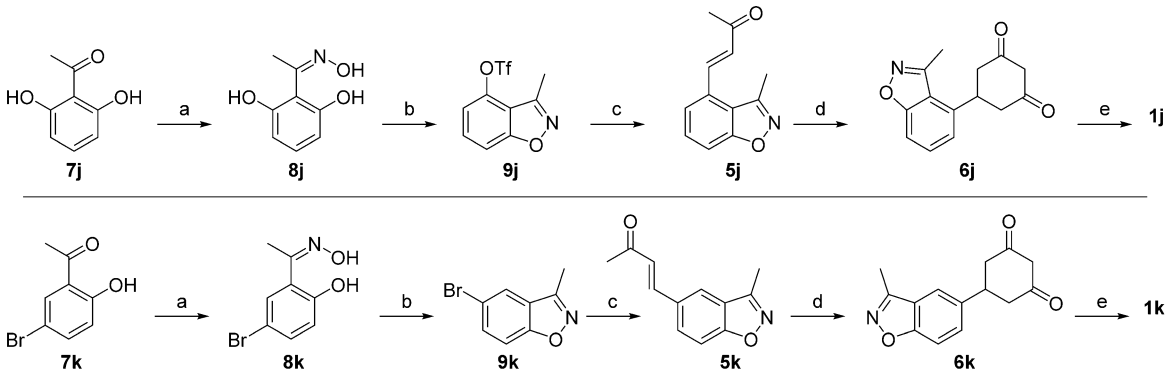
Recently, we have shown that analogue **2a** (Table 3) displays low-micromolar inhibitory potency at EAAT1. However, percentage unbound to brain protein (%FU) of this analogue was determined to be 10-fold lower than UCPH-102 (0.3%, Table 3). Nevertheless, we decided to follow the same strategy as that outlined for **1a–i** and incorporated a nitrogen atom into the synthetically feasible positions of the naphthyl group in **2a** (analogues **2b–d**). Further to this, we also synthesized two an-

Table 3. Inhibitory potencies of analogues **2a–f** at EAAT1, calculated log*P* values, and percent fraction unbound (FU) of selected analogues.[a]

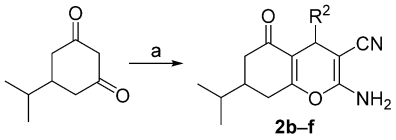


Compd	R^2	IC_{50} [μM] ($pIC_{50} \pm SEM$)[b]	cLog*P*	FU [%][c]
2a		1.6[21]	3.89	0.3
2b		35 (4.63 ± 0.21)	2.87	–
2c		8.8 (5.09 ± 0.09)	2.87	–
2d		15 (4.90 ± 0.13)	1.40	–
2e		~100 (< 4.0)	1.40	–
2f		~100 (< 4.0)	–	–

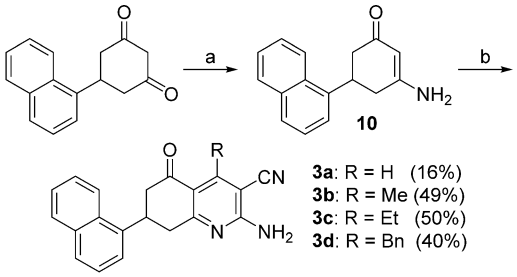
[a] All analogues displayed no inhibitory potency at EAAT2 or EAAT3 at concentrations of 100 μM ($IC_{50} > 100$ μM). [b] Values are the mean ± SEM of $n = 3$ experiments. [c] Residual brain protein binding.



Scheme 2. Synthesis of target analogues **1j,k** by a one-pot three-component reaction. *Reagents and conditions*: a) hydroxylamine hydrochloride, EtOH/H_2O, 70 °C (80 and 99%); b) PPh_3, DDQ, CH_2Cl_2, RT, 10 min, (63 and 84%), for **8j** also triflic anhydride, pyridine, CH_2Cl_2, 0 °C→RT, 18 h, 87%; c) **5j**: $PdCl_2(PPh_3)_2$, NEt_3, DMF, 110 °C, 18 h, quant., **5k**: $Pd(OAc)_2$, P(*o*-tol)$_3$, K_2CO_3, DMF, MW, 100 °C, 1 h, 53%; d) diethyl malonate, NaOEt/EtOH, then NaOH (reflux), then H_2SO_4 (reflux) (47 and 49%); e) malononitrile, acetaldehyde, *N*-methylmorpholine, EtOH, 0 °C→RT (both 21%).



Scheme 3. Synthesis of **2b–f** starting from commercially available 5-isopropylcyclohexa-1,3-dione and the appropriate commercially available aldehyde R^2CHO, by use of a one-pot three-component reaction. *Reagents and conditions*: a) malononitrile, appropriate commercial aldehyde (R^2CHO), amine base, EtOH, RT (22–62%).



Scheme 4. Synthesis of pyridine analogues **3a–d**. *Reagents and conditions*: a) NH_4OAc, 3 Å MS, EtOH, reflux (84%); b) RCHO, $CH_2(CN)_2$, *n*PrOH, 110 °C.

alogues with a lower relative carbon content (pyridine analogues **2e,f**). The five analogues **2b–f** were synthesized following the standard route (Scheme 3) and subsequently characterized as inhibitors at EAAT1, EAAT2, and EAAT3 in the [^{3}H]D-Asp uptake assay (Table 3).[20] Analogues **2b–d** displayed 5- to 20-fold decreases in inhibitory potencies at EAAT1 relative to **2a**, and a complete loss of inhibitory activity was observed for pyridine analogues **2e,f**. Given these results, the %FU values were not determined for this 2-series.

Design and synthesis of pyridine analogues

As a final attempt to optimize the in vivo properties of UCPH-102, the 4*H*-pyran ring was substituted for a pyridine ring. The four analogues **3a–d** were synthesized, varying the 3-substituent from hydrogen to alkyl groups of increasing length (Scheme 4). None of the four analogues displayed inhibitory activity at EAAT1–3 at concentrations up to 300 μM (IC_{50} > 300 μM) in the [^{3}H]D-Asp uptake assay.[20]

In summary, we pursued the optimization of the brain protein binding properties of UCPH-102 with the aim of nominating an in vivo candidate for animal studies. Three compound series **1**–**3** were designed and synthesized, but none of the analogues displayed improved properties in terms of potency versus %FU.

In vitro profiling of UCPH-102

UCPH-101 and UCPH-102 were shown in previous studies to be completely selective inhibitors of EAAT1 over the four other EAAT subtypes.[5,6,21] To elucidate the overall selectivity profile of UCPH-102, the compound was subjected to an elaborate in vitro screening on a selection of neurotransmitter receptors and transporters in a total of 51 radioligand binding assays (performed by the US National Institute of Mental Health's Psychoactive Drug Screening Program) and at other CNS targets in in-house functional assays. When tested at an assay concentration of 10 μM in the competition binding assays, UCPH-102 did not display significant binding affinity to the majority of receptors and transporters for serotonin, dopamine, norepinephrine, histamine, acetylcholine, GABA, glutamate, and opioids included in the screenings (Table 4). However, UCPH-102 inhibited radioligand binding to the α_{2C} norepinephrine receptor, the norepinephrine transporter (NET), and the histamine H_2 receptor, as well as to the benzodiazepine binding sites of native rat $GABA_A$ receptors, by approximately 50% (i.e., IC_{50} ~ 10 μM at these targets). In the functional assays, UCPH-102 was found to be completely inactive at GABA transporters, $GABA_B$ receptors, and the $\alpha_4\beta_2$ nicotinic acetylcholine receptor when tested at an assay concentration of 100 μM (Table 4).

A caveat associated with the use of the competition binding assays in this screening is that ligands acting through allosteric

Table 4. Pharmacological properties of UCPH-102 at a selection of neurotransmitter receptors and transporters.[a]

Target	Assay	IC_{50} [nM] (inhibition [%])
Serotonin (5-HT)		
5-HT_{1A} (h)	[^{3}H]8-OH-DPAT binding	>10000 (15)
5-HT_{1B} (h)	[^{3}H]GR125743 binding	>10000 (0.2)
5-HT_{1D} (h)	[^{3}H]GR125743 binding	>10000 (−13)
5-HT_{1E} (h)	[^{3}H]5-HT binding	>10000 (−1.7)
5-HT_{2A} (h)	[^{3}H]ketanserin binding	>10000 (−9.6)
5-HT_{2B} (h)	[^{3}H]LSD binding	>10000 (34)
5-HT_{2C} (h)	[^{3}H]mesulergine binding	>10000 (23)
5-HT_3 (h)	[^{3}H]LY278584 binding	>10000 (3.2)
5-HT_{5A} (h)	[^{3}H]LSD binding	>10000 (3.0)
5-HT_6 (h)	[^{3}H]LSD binding	>10000 (29)
5-HT_7 (h)	[^{3}H]LSD binding	>10000 (−13)
SERT (h)	[^{3}H]citalopram binding	>10000 (−6.6)
Dopamine (DA)		
D_1 (h)	[^{3}H]SCH23390 binding	>10000 (18)
D_2 (h)	[^{3}H]*N*-methylspiperone binding	>10000 (20)
D_3 (h)	[^{3}H]*N*-methylspiperone binding	>10000 (22)
D_4 (h)	[^{3}H]*N*-methylspiperone binding	>10000 (−0.9)
D_5 (h)	[^{3}H]SCH23390 binding	>10000 (16)
DAT (h)	[^{3}H]WIN35428 binding	>10000 (−12)
Norepinephrine (NE)		
α_{1A} (h)	[^{3}H]prazosin binding	>10000 (8.9)
α_{1B} (h)	[^{3}H]prazosin binding	>10000 (12)
α_{1D} (h)	[^{3}H]prazosin binding	>10000 (−0.7)
α_{2A} (h)	[^{3}H]rauwolscine binding	>10000 (−3.0)
α_{2B} (h)	[^{3}H]rauwolscine binding	>10000 (32)
α_{2C} (h)	[^{3}H]rauwolscine binding	~10000 (46)
β_1 (h)	[^{125}I]pindolol binding	>10000 (−0.9)
β_2 (h)	[^{3}H]CGP 12177 binding	>10000 (−2.8)
β_3 (h)	[^{3}H]CGP 12177 binding	>10000 (9.2)
NET (h)	[^{3}H]nisoxetine binding	~10000 (44)
Acetylcholine		
m_1 (h)	[^{3}H]QNB binding	>10000 (0.2)
m_2 (h)	[^{3}H]QNB binding	>10000 (−20)
m_3 (h)	[^{3}H]QNB binding	>10000 (−5.7)
m_4 (h)	[^{3}H]QNB binding	>10000 (−7.5)
m_5 (h)	[^{3}H]QNB binding	>10000 (8.7)
$\alpha4\beta2$[b] (m)	FLIPR membrane potential	>100000 (EC_{50} >100000)
Histamine		
H_1 (h)	[^{3}H]pyrilamine binding	>10000 (9.0)
H_2 (h)	[^{125}I]tiotidine binding	~10000 (54)
H_3 (gp)	[^{3}H]α-methylhistamine binding	>10000 (4.2)
H_4 (h)	[^{3}H]histamine binding	>10000 (12)
GABA		
Rat forebrain	[^{3}H]muscimol binding (GABA-A)	>10000 (22)
Rat brain	[^{3}H]flunitrazepam binding (GABA-A)	~10000 (52)
GAT1[b] (h)	[^{3}H]GABA uptake	>100000
BGT1[b] (h)	[^{3}H]GABA uptake	>100000
GAT2[b] (h)	[^{3}H]GABA uptake	>100000
GAT3[b] (h)	[^{3}H]GABA uptake	>100000
$GABA_{B1a,2}$[b] (r)	Ca^{2+}/Fluo4 assay (with Gqi5)	>100000 (EC_{50} >100000)
$GABA_{B1b,2}$[b] (r)	Ca^{2+}/Fluo4 assay (with Gqi5)	>100000 (EC_{50} >100000)
Opioid		
δ (h)	[^{3}H]DADLE binding	>10000 (10)
κ (h)	[^{3}H]U69593 binding	>10000 (13)
μ (h)	[^{3}H]DAMGO binding	>10000 (−0.7)
Sigma		
σ1 (rat brain)	[^{3}H]pentazocine(+) binding	>10000 (−14)
σ2 (rat, PC12)	[^{3}H]DTG binding	>10000 (−0.9)

[a] The binding properties of UCPH-102 (10 μM) at various receptors and transporters were determined in competition binding assays (using radioligand concentrations at or near the K_D value for the specific target) performed by the NIMH Psychoactive Drug Screening Program (PDSP). Percent inhibition or percent potentiation of control radioligand binding at 10000 nM UCPH-102 is given in parentheses (positive and negative values represent % inhibition and % potentiation, respectively). Inhibition of control binding by >50% is considered significant by the PDSP. Data are based on four independent determinations. [b] The functional properties of UCPH-102 at selected transporters and receptors were determined in in-house functional assays (IC_{50} and EC_{50} values given in nM); h: human, gp: guinea pig, m: mouse, r: rat.

sites will not necessarily compete with, or modulate, orthosteric radioligand binding to a target. However, the majority of targets assayed by radioligand binding in this study are class A 7-transmembrane receptors, and to our knowledge few (if any) allosteric modulators of these receptors have been reported to not affect orthosteric radioligand binding. Hence, although we cannot exclude the possibility that UCPH-102 could target an allosteric site in some of the receptors, the inactivity of the drug in the binding assays is likely to be a true reflection of its pharmacology at them. In conclusion, considering the considerably higher functional potency displayed by UCPH-102 as an EAAT1 inhibitor (IC_{50} = 0.42 μM) relative to the targets included in this screening, it is reasonable to conclude that the compound is quite selective for EAAT1.

Following the in vitro profiling, we continued with an in vivo study of possible acute behavioral effects 30–90 min after p.o. administration of UCPH-102 at doses of 1, 5, and 20 mg kg^{-1} on mouse locomotor activity (Figure 2). None of the three drug doses induced significant changes in activity counts in comparison with vehicle (Figure 2). Furthermore, visual inspection of the mice did not disclose any changes in basal behavior.

Based on these results it seems reasonable to conclude that UCPH-102 has no high-affinity off-targets that result in acute visible effects. The free brain concentration of the two active diastereomers of UCPH-102 after 40 mg kg^{-1} p.o. administration can be calculated to 0.13 μM (6.67/2×0.038). In view of this, EAAT1 target occupancy must be expected to be lower than the in vitro IC_{50} value determined for UCPH-102 in the [^{3}H]D-Asp uptake assay. This fact should be considered when evaluating the effects of EAAT1 inhibition in the aforementioned in vivo study as well as in the planning of further studies.[22]

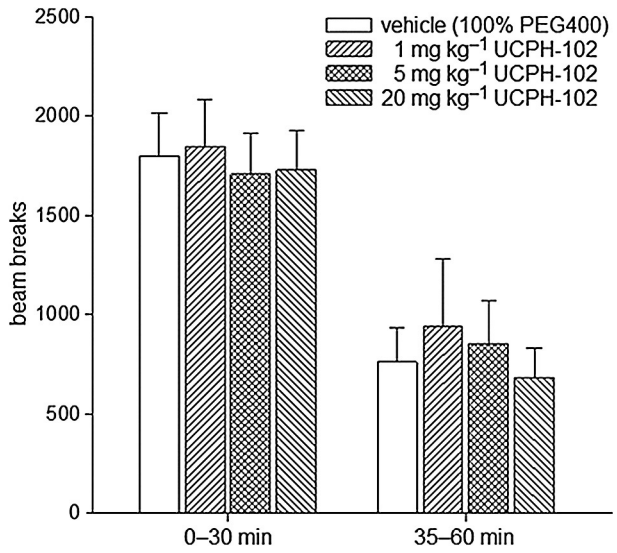
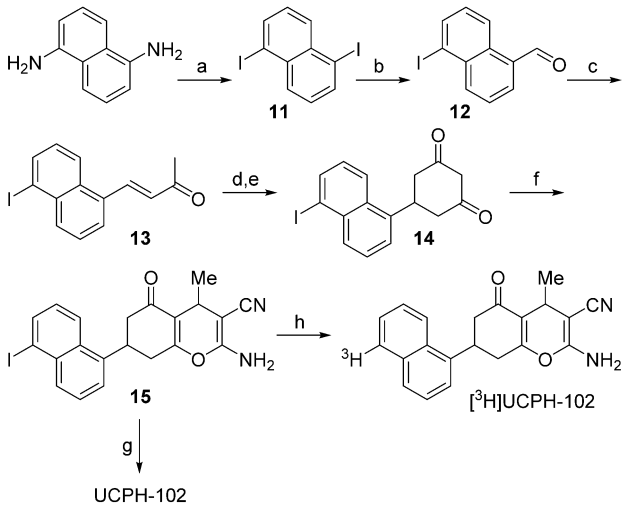


Figure 2. Locomotor activity 0–30 min and 35–60 min after p.o. dosing of 1, 5, or 20 mg of UCPH-102. Data are the mean ± SD of $n = 8$ mice. Activity was assessed using standard assay conditions and custom-made equipment, as described in the Experimental Section.

Synthesis of [^{3}H]UCPH-102

We were interested in obtaining a tritium-labeled analogue of UCPH-102 for potential use in numerous in vitro studies. A retrosynthetic analysis suggested the iodinated compound **15** as a suitable precursor for the synthesis of [^{3}H]UCPH-102 by palladium-catalyzed reduction with tritium gas (Scheme 5). Starting from commercially available naphthalene-1,5-diamine (**10**), diazotization by use of sodium nitrite, concentrated sulfuric acid, and potassium iodide in water gave diiodonaphthalene **11**.[23,24] Under optimized conditions, treatment of **11** with two equivalents of *tert*-butyllithium in tetrahydrofuran (THF) at −78 °C, followed by quenching with one equivalent of *N*,*N*-dimethylformamide, afforded the desired carbaldehyde **12** in 58% yield. This intermediate was then converted into enone **13** by reaction with diethyl (2-oxopropyl)phosphonate and sodium hydride in dry THF.[6] Subsequent reaction with diethyl malonate, 21% *w*/*w* sodium ethoxide in absolute ethanol followed by aqueous ester hydrolysis and acidic decarboxylation provided 1,3-diketone **14**. Finally, a three-component reaction of diketone **14**, malononitrile, and acetaldehyde gave the desired precursor **15** in 72% yield.[6] To explore reaction conditions for the tritiation step, hydrodehalogenation of iodine **15** under palladium on carbon and hydrogen gas afforded UCPH-102 in quantitative yield. Next, tritium labeling of iodine **15** was performed on a tritium manifold system using carrier-free tritium gas and Pd/C as the catalyst to afford [^{3}H]UCPH-102. The radioligand was stable when dissolved and stored in 10% ethanol in dimethyl sulfoxide.



Scheme 5. Synthesis of iodine precursor **15** and its application to the synthesis of [^{3}H]UCPH-102. *Reagents and conditions*: a) $NaNO_2$, KI, conc. H_2SO_4, H_2O, 80 °C, 21 h, (38%); b) *t*BuLi, DMF, THF, −78 °C, 1.5 h, (58%); c) diethyl (2-oxopropyl)phosphonate, NaH, THF, 20.5 h, (73%); d) diethyl malonate, 21% *w*/*w* NaOEt, absolute EtOH, reflux, 15 h; e) 2 M NaOH, H_2O, 85 °C, 3 h, 2 M H_2SO_4 (pH ≈ 1), 100 °C, 1 h, (71%); f) malononitrile, acetaldehyde, *N*-methylmorpholine, absolute EtOH, RT, 23 h, (72%); g) 10% Pd/C, Et_3N, $H_{2(g)}$, ~101 kPa, DMSO, RT, 90 min, quant.; h) 10% Pd/C, $^3H_{2(g)}$, 40 kPa, DMSO, 60 min.

Conclusions

In the present study we identified the selective EAAT1 inhibitor UCPH-102 as a better-suited candidate for in vivo studies than UCPH-101, due to its BBB-penetration ability. We pursued the structural optimization of UCPH-102 with respect to its lipophilicity and brain protein binding properties. Despite the synthesis and evaluation of series **1**–**3**, UCPH-102 remained the best-suited in vivo candidate in evaluating the parameters of oral bioavailability, BBB penetration, and brain protein binding against potency. An elaborate in vitro screening of UCPH-102 (10 μM) at 51 relevant CNS targets advocated that UCPH-102 is EAAT1-selective. Furthermore, p.o. administration of UCPH-102 at 1, 5, and 20 mg kg^{-1} in a rodent locomotor model produced no significant effects. Also, visual inspection of the mice disclosed no changes in basal behavior.

Experimental Section

Chemistry

All reactions involving dry solvents or sensitive agents were performed under a nitrogen or argon atmosphere, and glassware was dried prior to use. Commercially available chemicals were used without further purification. THF, DMF, and CH_2Cl_2 were dried using an SG water solvent purification system. Reactions were monitored by analytical thin-layer chromatography (TLC, Merck silica gel 60 F_{254} aluminum sheets). Flash chromatography and dry vacuum chromatography was carried out using Merck silica gel 60A (35–70 and 15–30 μm, respectively). ^{1}H NMR spectra were recorded on a 300, 400, or 600 MHz Avance Bruker instrument, and ^{13}C NMR spectra on a 75, 100, or 150 MHz Avance Bruker instrument. HPLC was performed with a Dionex UltiMate 3000 pump and photodiode array detector (λ 200 and 210 nm, respectively) installed with an XTerra MS C_{18} column (3.5 μm, 4.6 mm × 150 mm), using a 5–95% MeCN gradient in H_2O containing 0.1% TFA. LC–MS spectra were recorded using an Agilent 1200 series solvent delivery system

equipped with an auto-injector coupled to an Agilent 6400 series triple quadrupole mass spectrometer equipped with an electrospray ionization source. Gradients of 5% aqueous MeCN + 0.1% HCO_2H (solvent A), and 95% aqueous MeCN + 0.05% HCO_2H (solvent B) were used. Melting points were measured using an MPA 100 Optimelt automatic melting point system and are uncorrected. The purity of compounds submitted for pharmacological characterization was determined by HPLC to be >95%.

Apparatuses and general setup for the tritiation experiment: The tritiation reaction was performed on a custom-designed tritium manifold system manufactured by RC Tritec AG, Switzerland. Tritium gas stored as uranium tritide on a uranium bed and was released by heating to ~500 °C. HPLC analysis and purification of tritium samples were performed using a Waters 1524 binary pump, a Waters 2487 dual absorbance detector (set at λ 220 and 254 nm), equipped with Bioscan Hidex liquid scintillation counter. HPLC-grade water obtained by purification using an Aurium 611 UV system (Sartorius, Germany) was used. Safety handling with tritium gas was monitored by a Canberra T100DSi Tritium air monitor. Liquid scintillation counting was performed by RackBeta 1214 and Hidex 300SL scintillation counters.

2-Amino-7-(5-^{3}H-naphth-1-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile ([^{3}H]UCPH-102). Iodine **15** (3.4 mg) was dissolved in DMSO (1.0 mL) then added to a round-bottomed reaction vessel (1 mL) already loaded with the palladium catalyst (3.8 mg, 10% Pd/C). The reaction vessel was mounted onto the tritium manifold system and frozen with liquid nitrogen, then evacuated to $<5\times10^{-3}$ mbar. The vessel was then filled with an atmosphere of argon before it was allowed to warm to RT. It was stirred vigorously for a couple of minutes before the freeze; the evacuation and stir procedure was repeated three times to effectively ensure deoxygenation of the reaction mixture. The flask was yet again frozen with liquid nitrogen, evacuated to $<5\times10^{-3}$ mbar before carrier-free tritium gas (8.9 Ci, $^3H_2$, 400 mbar) was added. The reaction mixture was stirred at RT for 1 h, then carefully pulled out of the vessel and diluted with H_2O (2.0 mL). The dilution was filtered through a disposable Whatman glass microfiber filter (grade GF/C, 1.2 μm pore size, 13 mm Ø) for removal of the solid catalyst. The filter was flushed with additional H_2O (1.0 mL). The crude mixture was subjected to three consecutive freeze pump thaw repetitions for the removal of labile tritium. After freeze-drying of the crude mixture the product was purified by RP-HPLC using a C_{18} column and a gradient of MeCN/H_2O as the eluent. The fraction containing the product was lyophilized and then re-solubilized in 25 mL 10% EtOH in DMSO. The radiochemical yield was analyzed to be 75 mCi, with a specific activity of 18.1 Ci mmol^{-1}. The radioligand was chemically stable in this solvent mixture, while water should be avoided due to hydrolysis over time. Preparative HPLC: column: Princeton Spher C_{30} (250 mm × 10 mm, 5 μm); flow rate: 4 mL min^{-1}; eluents: A = 99.9% purified H_2O, 0.1% TFA; B = 90% MeCN, 9.9% purified H_2O, 0.1% TFA; gradient: 0–1 min 30% B, 1–20 min 30–100% B, 20–21 min 100% B, 21–22 min 100–30% B, 22–25 min 30% B. The product [^{3}H]UCPH-102 was collected at 8.8 min. Analytical HPLC: column: Phenomenex Luna C_{18} (2) (250 mm × 4.6 mm); flow rate: 1.5 mL min^{-1}; eluents: A = 94.9% purified H_2O, 5% MeCN, 0.1% TFA; B = 99.9% MeCN, 0.1% TFA; gradient: 0–0.50 min 10% B, 0.5–18 min 10–75% B, 18–19 min 75–100% B, 19–20 min 100% B, 20–21 min 100–10% B, 21–23 min 0% B. The retention time of [^{3}H]UCPH-102 was detected at 11.3 min.

2-Amino-7-(isoquinolin-1-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 a). 5-(Isoquinoline-1-yl)cyclohexan-1,3-dione (40.0 mg, 0.17 mmol) was dissolved in absolute EtOH (8 mL) and malononitrile (15 mg, 0.24 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (27 μL, 0.50 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The mixture was warmed to RT and stirred for 18 h. The solvent was evaporated and the residue purified by flash column chromatography (CH_2Cl_2/EtOAc 9:1 to 5:1) to give the title compound as a mixture of diastereomers as a white solid (45 mg, 0.13 mmol, 81%); R_f = 0.24 and 0.34 (CH_2Cl_2/EtOAc, 5:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.46 (d, J = 5.6 Hz, 1H), 8.45 (d, J = 8.5 Hz, 1H), 8.38 (t, J = 8.1 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.81–7.77 (m, 1H), 7.75 (d, J = 5.6 Hz, 1H), 7.74–7.69 (m, 2H), 6.87 (s, 2H), 6.80 (s, 0.8H), 4.64 (d, J = 6.3 Hz, 0.4H), 4.60–4.54 (m, 1H), 3.19–3.14 (m, 1H), 3.11 (dd, J = 17.5, 10.2 Hz, 1H), 2.94 (qd, J = 17.3, 7.0 Hz, 1H), 2.85 (t, J = 4.0 Hz, 1H), 2.84–2.79 (m, 1H), 2.76 (dd, J = 17.8, 4.5 Hz, 1H), 2.69 (dd, J = 16.3, 4.6 Hz, 1H), 2.64 (d, J = 3.8 Hz, 2H), 1.18 (d, J = 4.5 Hz, 3H), 1.05 ppm (d, J = 7.0 Hz, 1.2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.4, 163.0, 160.4, 158.8, 141.4, 135.9, 130.3, 127.7, 127.5, 125.5, 124.7, 119.9, 114.7, 57.8, 56.0, 42.2, 35.1, 31.9, 24.8, 23.0, 22.3, 18.5 ppm; mp: 117.3–120.8 °C (decomp.); LC–MS: (*m*/*z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-7-(isoquinolin-4-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 b). 5-(Isoquinolin-4-yl)cyclohexan-1,3-dione (49.0 mg, 0.21 mmol) was dissolved in absolute EtOH (10 mL) and malononitrile (17 mg, 0.26 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (35 μL, 0.63 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The mixture was warmed to RT and stirred for three days. The formed precipitate was then filtered off and washed with cold EtOH (3 mL) and dried to give the title compound as a white solid (30 mg, 0.09 mmol, 43%); R_f = 0.45 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.24 (d, J = 4.8 Hz, 1H), 8.52 (d, J = 42.1 Hz, 1H), 8.30 (dd, J = 27.4, 8.5 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.86–7.82 (m, 1H), 7.72 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 3.4 Hz, 2H), 4.37–4.30 (m, 0.35H), 4.25–4.18 (m, 0.6H), 3.19 (dt, J = 13.1, 6.5 Hz, 1H), 3.10–3.02 (m, 1H), 2.94 (ddd, J = 24.2, 16.1, 12.3 Hz, 1H), 2.81–2.73 (m, 1H), 2.71–2.61 (m, 1H), 1.19 (d, J = 6.5 Hz, 3H), 1.12 ppm (d, J = 6.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.5, 163.2, 158.8, 148.4, 142.7, 140.0, 135.8, 130.3, 125.8, 125.7, 124.8, 120.9, 120.0, 114.8, 57.8, 43.3, 33.7, 31.7, 24.8, 22.9 ppm; LC–MS: (*m*/*z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-3-cyano-4-methyl-5-oxo-7-(4-quinolinyl)-5,6,7,8-tetrahydro-4*H*-chromene (1 c). 5-(Quinolin-4-yl)cyclohexane-1,3-dione (50 mg, 0.21 mmol) was suspended in absolute EtOH (10 mL) and malononitrile (14 mg, 0.21 mmol) was added. The suspension was evacuated and backfilled with N_2 several times, cooled to 0 °C and acetaldehyde (34 μL, 0.63 mmol) was added. The reaction mixture was stirred at 0 °C for 20 min before *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The reaction mixture was left in the ice-bath to gradually warm up to RT during 16 h during which further precipitation occurred. The suspension was filtered and the resulting white solid was washed with cold EtOH (3 mL) and dried to give the title compound as a white solid (43 mg, 0.13 mmol, 62%) containing 6% EtOH *w*/*w*. R_f = 0.5 (two spots, EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.90–8.86 (m, 1H), 8.36 (d, J = 8.3 Hz, 0.3H), 8.31 (d, J = 8.3 Hz, 0.7H), 8.06 (d, J = 8.4 Hz, 1H), 7.81–7.75 (m, 1H), 7.68–7.62 (m, 1H), 7.55 (d, J = 4.6 Hz, 0.3H), 7.48 (d, J = 4.6 Hz, 0.7H), 6.90 (s, 2H), 4.49–4.39 (m, 1H), 3.24–3.15 (m, 1H), 3.07–2.95 (m, 1H), 2.95–2.52 (m, 3H), 1.19 (d, J = 6.5 Hz, 0.9H), 1.12 ppm (d, J = 6.5 Hz, 2.1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.4, 162.6, 158.8, 150.3, 148.0, 147.9,

129.9, 129.3, 126.8, 126.0, 123.4, 119.9, 118.4, 114.9, 57.6, 42.4, 32.6, 32.6, 24.9, 22.3 ppm; mp: 174.6–178.8 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-3-cyano-4-methyl-5-oxo-7-(quinolin-5-yl)-5,6,7,8-tetrahydro-4*H*-chromene (1 d). 5-(Quinolin-5-yl)cyclohexane-1,3-dione (50 mg, 0.21 mmol) was suspended in absolute EtOH (20 mL) and malononitrile (14 mg, 0.21 mmol) was added. The suspension was evacuated and backfilled with N_2 three times, cooled to 0 °C and acetaldehyde (34 μL, 0.63 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min before *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The reaction mixture was left in the ice-bath to gradually warm up to RT during 16 h during which further precipitation occurred. The volume was decreased in vacuo to ~5 mL and the resulting suspension was filtered and the white solid was washed with cold EtOH (3 mL) and dried to give the title compound as a white solid (47 mg, 0.142 mmol, 68%) containing 12% EtOH *w/w*. R_f = 0.5 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.95–8.88 (m, 1H), 8.74 (dd, *J* = 18.5, 8.6 Hz, 1H), 7.94 (dd, *J* = 8.4, 2.7 Hz, 1H), 7.80–7.70 (m, 1H), 7.68–7.52 (m, 2H), 6.89 (s, 2H), 4.44–4.35 (m, 0.6H), 4.31–4.23 (m, 0.4H), 3.25–3.15 (m, 1H), 3.05–2.79 (m, 2H), 2.78–2.54 (m, 2H), 1.20 (d, *J* = 6.5 Hz, 1.8H), 1.12 ppm (d, *J* = 6.5 Hz, 1.8H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.8, 195.5, 163.3, 162.8, 158.8, 158.8, 150.2, 148.1, 148.1, 139.5, 139.4, 131.8, 131.7, 129.1, 129.0, 128.3, 128.2, 125.8, 125.8, 123.7, 123.7, 121.4, 121.4, 119.9, 114.8, 114.8, 57.8, 57.6, 43.2, 33.6, 33.5, 32.7, 32.2, 24.9, 24.9, 23.0, 22.4 ppm; mp: 201.8–204.6 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-7-(isoquinolin-5-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 e). 5-(Isoquinoline-5-yl)-cyclohexan-1,3-dione (52.0 mg, 0.22 mmol) was dissolved in absolute EtOH (10 mL) and malononitrile (16 mg, 0.24 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (40 μL, 0.64 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The mixture was warmed to RT and stirred for 18 h. The formed precipitate was then filtered off and washed with cold EtOH (3 mL) and dried to give the title compound as one diastereomer as a white solid (42 mg, 0.13 mmol, 58%); R_f = 0.48 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.33 (s, 1H), 8.54 (d, *J* = 6.0 Hz, 1H), 8.11 (d, *J* = 6.1 Hz, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 7.75 (d, *J* = 7.1 Hz, 1H), 7.69–7.65 (m, 1H), 6.90 (s, 2H), 4.38–4.30 (m, 1H), 3.20 (dd, *J* = 12.9, 6.5 Hz, 1H), 2.97 (dd, *J* = 17.1, 4.9 Hz, 1H), 2.90 (dd, *J* = 15.9, 11.7 Hz, 1H), 2.75 (dd, *J* = 17.0, 4.9 Hz, 1H), 2.65 (dd, *J* = 15.7, 3.9 HZ, 1H), 1.12 ppm (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.8, 162.7, 158.8, 153.2, 143.3, 137.9, 133.2, 128.6, 127.5, 127.1, 126.9, 119.9, 116.1, 114.8, 57.6, 42.9, 33.2, 32.7, 24.9, 22.4 ppm; mp: 208.6–223.1 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-7-(isoquinolin-8-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 f). 5-(Isoquinoline-8-yl)cyclohexan-1,3-dione (30.0 mg, 0.12 mmol) was dissolved in absolute EtOH (8 mL) and malononitrile (9 mg, 0.12 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (25 μL, 0.49 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The mixture was warmed to RT and stirred for two days. The formed precipitate was then filtered off and washed with cold EtOH (3 mL) and dried to give the title compound as a mixture of two diastereomers as a white solid (20 mg, 0.06 mmol, 51%); R_f = 0.72 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.75 (s, 1H), 8.73 (s, 1H), 8.54 (d, *J* = 5.6 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.85 (d, *J* = 5.6 Hz, 1H), 7.79–7.75 (m, 1H), 7.70 (d, *J* = 7.2 Hz, 1H), 6.90 (s, 2H), 4.53–4.47 (m, 1H), 3.19 (dd, *J* = 12.4, 5.9 Hz, 1H), 3.01 (dd, *J* = 17.1, 10.3 Hz, 1H), 2.88 (dd, *J* = 16.2, 13.1 Hz, 1H), 2.75 (dd, *J* = 17.6, 4.2 Hz, 1H), 2.64–2.59 (m, 1H), 1.20 ppm (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.5, 163.2, 158.8, 148.4, 142.7, 140.0, 135.8, 130.3, 125.8, 125.7, 124.8, 120.9, 120.0, 114.8, 57.8, 43.3, 33.7, 31.7, 24.8, 22.9 ppm; mp: 208.2–220.3 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-4-methyl-5-oxo-7-(quinolin-8-yl)- 5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 g). 5-(Quinoline-8-yl)cyclohexan-1,3-dione (50.0 mg, 0.21 mmol) was dissolved in absolute EtOH (10 mL) and malononitrile (16 mg, 0.24 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (35 μL, 0.63 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The mixture was warmed to RT and stirred for 18 h. The formed precipitate was filtered off and washed with cold EtOH (3 mL) and dried to give the title compound as a mixture of two diastereomers as a white solid (38 mg, 0.11 mmol, 55%); ratio: 1:1.27; R_f = 0.64 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.96 (d, 1H), 8.54 (d, *J* = 6.0 Hz, 1H), 8.11 (d, *J* = 6.1 Hz, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 7.75 (d, *J* = 7.1 Hz, 1H), 7.69–7.65 (m, 1H), 6.90 (s, 2H), 4.38–4.30 (m, 1H), 3.20 (dd, *J* = 12.9, 6.5 Hz, 1H), 2.97 (dd, *J* = 17.1, 4.9 Hz, 1H), 2.90 (dd, *J* = 15.9, 11.7 Hz, 1H), 2.75 (dd, *J* = 17.0, 4.9 Hz, 1H), 2.65 (dd, *J* = 15.7, 3.9 Hz, 1H), 1.12 ppm (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.8, 162.7, 158.8, 153.2, 143.3, 137.9, 133.2, 128.6, 127.5, 127.1, 126.9, 119.9, 116.1, 114.8, 57.6, 42.9, 33.2, 32.7, 24.9, 22.4 ppm; mp: 208.7–222.8 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-4-methyl-5-oxo-7-(quinolone-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 h). 5-(Quinolin-2-yl)cyclohexan-1,3-dione (36 mg, 0.15 mmol) was dissolved in absolute EtOH (8 mL) and malononitrile (12 mg, 0.18 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (50 μL, 0.90 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (10 μL, 0.08 mmol) was added. The mixture was warmed to RT and stirred for 20 h. The solvent was evaporated and the residue purified by flash column chromatography (CH_2Cl_2/EtOAc 5:1) to give the title compound as a white foam (10.27 mg, 0.03 mmol, 21%); R_f = 0.14 and 0.11 (CH_2Cl_2/EtOAc 5:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.73 (s, 1H), 8.54 (d, *J* = 5.6 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.85 (d, *J* = 5.6 Hz, 1H), 7.79–7.75 (m, 1H), 7.70 (d, *J* = 7.2 Hz, 1H), 6.90 (s, 2H), 4.53–4.47 (m, 1H), 3.19 (dd, *J* = 12.4, 5.9 Hz, 1H), 3.01 (dd, *J* = 17.1, 10.3 Hz, 1H), 2.88 (dd, *J* = 16.2, 13.1 Hz, 1H), 2.75 (dd, *J* = 17.6, 4.2 Hz, 1H), 2.64–2.59 (m, 1H), 1.20 ppm (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.4, 195.3, 163.2, 161.6, 161.5, 158.8, 146.9, 146.6, 136.8, 129.6, 128.6, 127.8, 126.8, 126.7, 126.2, 120.6, 119.9, 115.2, 115.0, 57.8, 57.6, 41.9, 41.1, 31.7, 31.6, 24.8, 24.7, 23.0, 22.4 ppm; mp: 79.0–98.8 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-4-methyl-5-oxo-7-(quinolin-7-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1 i). 5-(Quinolin-7-yl)cyclohexan-1,3-dione (40 mg, 0.15 mmol) was dissolved in absolute EtOH (8 mL) and malononitrile (15 mg, 0.19 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (50 μL, 0.90 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (5 μL, 0.04 mmol) was added. The mixture was warmed to RT and stirred for 20 h. The solvent was evaporated and the residue purified by prep. TLC (CH_2Cl_2/EtOAc 2:1) to give the title compound as an off-white solid (10.31 mg, 0.03 mmol, 19%); R_f = 0.16 (CH_2Cl_2/

EtOAc 2:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.73 (s, 1H), 8.54 (d, J = 5.6 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 5.6 Hz, 1H), 7.79–7.75 (m, 1H), 7.70 (d, J = 7.2 Hz, 1H), 6.90 (s, 2H), 4.53–4.47 (m, 1H), 3.19 (dd, J = 12.4, 5.9 Hz, 1H), 3.01 (dd, J = 17.1, 10.3 Hz, 1H), 2.88 (dd, J = 16.2, 13.1 Hz, 1H), 2.75 (dd, J = 17.6, 4.2 Hz, 1H), 2.64–2.59 (m, 1H), 1.20 ppm (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 195.4, 195.3, 163.2, 161.6, 161.5, 158.8, 146.9, 146.6, 136.8, 129.6, 128.6, 127.8, 126.8, 126.7, 126.2, 120.6, 119.9, 115.2, 115.0, 57.8, 57.6, 41.9, 41.1, 31.7, 31.6, 24.8, 24.7, 23.0, 22.4 ppm; mp: 180.1–204.8 °C (decomp.); LC–MS: (m/z) calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.1, found: 332.1.

2-Amino-4-methyl-7-(3-methylbenzo[*d*]isoxazol-4-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1j). 5-(3-Methylbenzo[*d*]isoxazol-4-yl)cyclohexan-1,3-dione (70 mg, 0.29 mmol) was dissolved in absolute EtOH (15 mL) and malononitrile (26 mg, 0.39 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (50 μL, 0.90 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (10 μL, 0.08 mmol) was added. The mixture was warmed to RT and stirred for 20 h. The solvent was evaporated and the residue purified by flash chromatography (CH_2Cl_2/EtOAc 2:1) to give the title compound as a white solid (10.3 mg, 0.03 mmol, 21%); R_f = 0.14 and 0.11 (CH_2Cl_2/EtOAc 5:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 7.56 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 6.90 (s, 2H), 3.89–3.81 (m, 1H), 3.59 (d, J = 5.7 Hz, 1H), 3.47 (s, 1H), 3.16 (q, J = 6.4 Hz, 1H), 3.11 (dd, J = 17.2, 11.6 Hz, 1H), 2.88 (dd, J = 16.3, 13.4 Hz, 1H), 2.77 (dd, J = 17.3, 4.1 Hz, 1H), 2.63 (s, 3H), 1.15 ppm (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 195.4, 195.3, 163.2, 161.6, 161.5, 158.8, 146.9, 146.6, 136.8, 129.6, 128.6, 127.8, 126.8, 126.7, 126.2, 120.6, 119.9, 115.2, 115.0, 57.8, 57.6, 41.9, 41.1, 31.7, 31.6, 24.8, 24.7, 23.0, 22.4 ppm; LC–MS: (m/z) calcd for $C_{19}H_{18}N_3O_3$ $[M+H]^+$: 336.1, found: 336.1.

2-Amino-4-methyl-7-(3-methylbenzo[*d*]isoxazol-5-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1k). 5-(3-Methylbenzo[*d*]isoxazol-5-yl)cyclohexan-1,3-dione hydrate (29.8 mg, 0.11 mmol) was dissolved in absolute EtOH (6 mL) and malononitrile (8 mg, 0.11 mmol) was added at RT. The mixture was cooled to 0 °C and acetaldehyde (20 μL, 0.34 mmol) was added. The mixture was stirred for 10 min and then *N*-methylmorpholine (3 μL, 0.08 mmol) was added. The mixture was warmed to RT and stirred for 20 h. The solvent was evaporated and the residue purified by flash chromatography (CH_2Cl_2/EtOAc 5:1 to 2:1) to give the title compound as a yellow solid (8.0 mg, 0.024 mmol, 21%); R_f = 0.57 (CH_2Cl_2/EtOAc 2:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.64 (s, 1H), 9.32 (s, 1H), 7.62 (s, 1H), 6.94 (dd, J = 8.3, 2.0 Hz, 1H), 6.88 (s, 1H), 6.81 (d, J = 8.3 Hz, 2H), 3.44 (dd, J = 7.0, 5.1 Hz, 1H), 3.29–3.22 (m, 2H), 3.12 (dt, J = 6.6, 5.2 Hz, 1H), 2.73 (dd, J = 16.4, 11.0 Hz, 2H), 2.62–2.54 (m, 3H), 2.45 (dd, J = 16.3, 3.1 Hz, 1H), 2.08 (s, 3H), 1.11 ppm (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 195.7, 169.0, 163.3, 158.8, 146.7, 133.1, 126.2, 122.9, 120.9, 119.9, 115.9, 114.9, 57.7, 43.7, 36.7, 34.0, 24.8, 23.6, 23.0 ppm; mp: 189.1–221.6 °C (decomp.); LC–MS: (m/z) calcd for $C_{19}H_{18}N_3O_3$ $[M+H]^+$: 336.1, found: 336.1.

2-Amino-7-isopropyl-5-oxo-4-(pyridin-3-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2b). Nicotinaldehyde (10.7 mg, 0.1 mmol) and malononitrile (7.3 mg, 0.11 mmol) were dissolved in absolute EtOH (2 mL) at RT under N_2. 5-Isopropyl-1,3-hexanedione (16.9 mg, 0.11 mmol) and *N*-methylmorpholine (1.4 μL, 0.015 mmol) were added, and the reaction mixture was stirred for 24 h at RT. The reaction mixture was then evaporated to dryness and purified by flash column chromatography (CH_2Cl_2/EtOAc 10:1 to 10:3). Recrystallization from CH_2Cl_2/Petroleum ether afforded the title compound as yellowish crystals (10 mg, 0.03 mmol, 33%); R_f = 0.19 (CH_2Cl_2/EtOAc 10:1); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.45–8.35 (m, 2H), 7.54 (tdd, J = 10.3, 2.4, 1.6 Hz, 1H), 7.15 (dddd, J = 7.8, 5.0, 4.1, 0.9 Hz, 1H), 4.61 (d, J = 3.9 Hz, 2H), 4.37 (d, J = 1.6 Hz, 1H), 2.52 (ddd, J = 17.8, 4.7, 1.6 Hz, 1H), 2.49–2.24 (m, 3H), 2.08–1.87 (m, 3H), 1.80 (dddd, J = 17.8, 8.5, 4.6, 2.5 Hz, 1H), 1.61–1.47 (m, 1H), 1.19 (s, 1H), 0.91–0.74 ppm (m, 7H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 196.1, 163.9, 157.9, 149.2, 149.2, 148.4, 138.7, 135.5, 123.4, 123.4, 114.1, 77.3, 77.2, 77.0, 76.7, 62.3, 40.7, 39.8, 38.7, 33.5, 31.8, 31.7, 30.9, 19.5, 19.4, 19.4 ppm; mp: 184.1–184.4 °C; LC–MS: (m/z) calcd for $C_{18}H_{20}N_3O_2$ $[M+H]^+$: 310.1, found: 310.1; HPLC: >96% purity.

2-Amino-7-isopropyl-5-oxo-4-(pyridin-4-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2c). Carried out as described for **2b** starting with pyridine-4-aldehyde. Recrystallization from CH_2Cl_2/Petroleum ether afforded the title compound as white crystals (10 mg, 0.03 mmol, 33%); R_f = 0.29 (CH_2Cl_2/EtOAc 10:1); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.45 (t, J = 4.8 Hz, 2H), 7.10 (dd, J = 14.7, 5.1 Hz, 2H), 4.73 (d, J = 4.4 Hz, 2H), 4.34 (s, 1H), 2.66–2.22 (m, 3H), 2.14–1.87 (m, 1H), 1.54 ppm (dq, J = 12.8, 6.5 Hz, 1H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 195.1, 195.0, 163.3, 162.6, 157.1, 157.0, 150.7, 150.5, 149.0, 149.0, 121.8, 121.7, 117.2, 112.6, 112.5, 76.3, 76.2, 76.0, 75.7, 60.4, 60.3, 40.1, 39.7, 38.8, 37.7, 34.2, 34.0, 30.8, 30.7, 29.9, 29.7, 18.5, 18.4, 18.4, 18.3 ppm; mp: 177.5–178.3 °C; LC–MS: (m/z) calcd for $C_{18}H_{20}N_3O_2$ $[M+H]^+$: 310.1, found: 310.1; HPLC: >98% purity.

2-Amino-7-isopropyl-5-oxo-4-(quinolin-7-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2d). Carried out as described for **2b** starting with quinoline-7-carboxaldehyde. Recrystallization from CH_2Cl_2/Petroleum ether afforded the title compound as white crystals (8 mg, 0.02 mmol, 22%); R_f = 0.07 (CH_2Cl_2/EtOAc 10:1); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.79 (ddd, J = 4.6, 2.9, 1.7 Hz, 2H), 8.06 (dq, J = 8.5, 1.5 Hz, 2H), 7.81–7.67 (m, 4H), 7.55 (ddd, J = 13.7, 8.3, 1.9 Hz, 2H), 7.28 (ddd, J = 8.3, 4.2, 1.4 Hz, 2H), 4.52 (dd, J = 19.6, 3.5 Hz, 7H), 2.55–2.25 (m, 6H), 2.09–1.82 (m, 5H), 1.50 (s, 16H), 1.19 (s, 1H), 0.87 ppm (td, J = 8.9, 7.3 Hz, 16H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 196.3, 163.9, 157.7, 150.5, 145.4, 136.0, 128.0, 127.6, 127.2, 120.9, 114.6, 77.3, 77.2, 77.0, 76.7, 40.8, 38.6, 35.5, 31.7, 30.9, 19.5, 19.4, 19.4 ppm; mp: 179.1–179.4 °C; LC–MS: (m/z) calcd for $C_{22}H_{22}N_3O_2$ $[M+H]^+$: 360.2, found: 360.1; HPLC: >99% purity.

2-Amino-7-isopropyl-5-oxo-4-(quinolin-3-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2e). Carried out as described for **2b** starting with quinoline-3-carboxaldehyde. Filtration of the reaction mixture afforded the title compound as white crystals (18 mg, 0.05 mmol, 51%); R_f = 0.14 (CH_2Cl_2/EtOAc 10:1); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.77 (dd, J = 14.8, 2.3 Hz, 1H), 8.06 (dd, J = 8.8, 2.0 Hz, 2H), 7.82 (dt, J = 8.1, 2.0 Hz, 1H), 7.68 (ddd, J = 8.4, 6.5, 1.3 Hz, 1H), 7.58–7.35 (m, 1H), 4.72 (d, J = 4.2 Hz, 1H), 4.63 (q, J = 1.4 Hz, 1H), 2.67–2.25 (m, 3H), 2.16–1.96 (m, 1H), 1.05–0.77 ppm (m, 5H); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 199.1, 196.2, 196.1, 163.9, 163.2, 157.8, 150.5, 147.6, 135.5, 134.6, 128.6, 128.0, 126.7, 118.3, 113.7, 77.2, 77.0, 76.8, 62.4, 39.8, 38.7, 31.7, 30.7, 19.5 ppm; mp: 210.5–212.4 °C; LC–MS: (m/z) calcd for $C_{22}H_{22}N_3O_2$ $[M+H]^+$: 360.2, found: 360.1; HPLC: >98% purity.

2-Amino-7-isopropyl-5-oxo-4-(quinolin-6-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2f). Carried out as described for **2b** starting with quinoline-6-carboxaldehyde. Filtration of the reaction mixture afforded the title compound as white crystals (22 mg, 0.06 mmol, 62%); R_f = 0.10 (CH_2Cl_2/EtOAc 10:1); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.79 (dt, J = 4.2, 1.5 Hz, 1H), 8.07 (dddd, J = 8.3, 3.5, 1.7, 0.8 Hz, 1H), 7.97 (dd, J = 8.8, 5.0 Hz, 1H), 7.67 (dd, J = 11.5, 2.0 Hz, 1H), 7.47 (ddd, J = 17.1, 8.7, 2.1 Hz, 1H), 7.31 (ddd, J = 8.3, 4.2,

1.1 Hz, 1H), 4.61–4.53 (m, 3H), 2.56 (ddd, J=17.8, 4.7, 1.6 Hz, 1H), 2.52–2.26 (m, 3H), 2.09–1.87 (m, 1H), 1.81 (dddd, J=17.9, 8.4, 6.5, 4.3 Hz, 1H), 1.17 (t, J=7.0 Hz, 1H), 0.91–0.81 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=196.2, 163.8, 163.1, 157.8, 150.2, 141.0, 136.3, 130.1, 130.0, 128.9, 128.8, 128.1, 126.7, 126.6, 121.3, 114.5, 77.3, 77.2, 77.0, 76.7, 63.0, 41.3, 40.8, 39.9, 38.7, 35.8, 35.6, 31.8, 31.7, 30.9, 30.8, 19.5, 19.4, 19.4 ppm; mp: 214.0–214.3 °C; LC–MS: (m/z) calcd for $C_{22}H_{22}N_3O_2$ $[M+H]^+$: 360.2, found: 360.1; HPLC: >98% purity.

2-Amino-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3a). To a solution of 3-amino-5-(naphthalen-1-yl)cyclohex-2-en-1-one (50 mg, 0.21 mmol) in *n*-propanol (0.9 mL) was added malononitrile (620 mg, 9.39 mmol). Additional *n*-propanol (0.5 mL) was added to bring all reagents into solution. Formaldehyde (37% aqueous solution, 51 μL, 0.63 mmol) was added and the reaction mixture was heated at 110 °C in a sealed flask. After 6 h, the solvent was evaporated and the resulting residue was purified by flash column chromatography (0→10% EtOAc in CH_2Cl_2). Further purification by recrystallization from MeOH afforded the title compound as a beige solid (11 mg, 0.03 mmol, 16%); R_f=0.37 (CH_2Cl_2/EtOAc 9:1); ^{1}H NMR (400 MHz, $CDCl_3$/[D_4]MeOH (1:1)): δ=8.36 (s, 1H), 8.05 (d, J=8.4 Hz, 1H), 7.92–7.85 (m, 1H), 7.77 (d, J=7.8 Hz, 1H), 7.56–7.38 (m, 4H), 4.37–4.27 (m, 1H), 3.37–3.26 (m, 2H), 3.03–2.95 ppm (m, 2H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=193.7, 168.1, 160.8, 160.8, 141.6, 139.1, 133.5, 130.6, 128.8, 127.1, 126.3, 125.7, 125.6, 123.0, 117.7, 116.1, 89.0, 44.2, 33.3 ppm; LC–MS: (m/z) calcd for $C_{20}H_{16}N_3O$ $[M+H]^+$: 314.1, found: 314.1.

2-Amino-4-methyl-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3b). To a solution of 3-amino-5-(naphthalen-1-yl)cyclohex-2-en-1-one (42 mg, 0.18 mmol) and malononitrile (39 mg, 0.59 mmol) in *n*-propanol (0.8 mL) was added acetaldehyde (33 μL, 0.60 mmol). The reaction mixture was then heated at 110 °C in a sealed flask for 6 h. Precipitation occurred upon cooling the reaction to RT. The solid was filtered off and washed with cold EtOH. Further purification by trituration in MeOH afforded the title compound as an off-white solid (29 mg, 0.09 mmol, 49%); R_f=0.19 (CH_2Cl_2/EtOAc 8:2); ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.19 (d, J=7.8 Hz, 1H), 7.99–7.90 (m, 1H), 7.83 (dd, J=2.1, 7.0 Hz, 1H), 7.64 (br s, 2H), 7.60–7.43 (m, 4H), 4.35–4.22 (m, 1H), 3.31 (dd, J=16.6, 11.3 Hz, 1H), 3.19–3.08 (m, 1H), 3.00 (dd, J=16.3, 12.0 Hz, 1H), 2.86–2.76 (m, 1H), 2.74 ppm (s, 3H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=195.8, 168.6, 160.2, 156.2, 139.0, 133.5, 130.6, 128.8, 127.1, 126.3, 125.7, 125.6, 123.0, 122.8, 116.7, 115.7, 91.1, 46.2, 40.7, 32.9, 20.4 ppm; mp: 257.8–259.3 °C; LC–MS: (m/z) calcd for $C_{21}H_{18}N_3O$ $[M+H]^+$: 328.1, found: 328.1.

2-Amino-4-ethyl-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3c). To a solution of 3-amino-5-(naphthalen-1-yl)cyclohex-2-en-1-one (50 mg, 0.21 mmol, 1 equiv) and malononitrile (42 mg, 0.63 mmol) in *n*-propanol (0.8 mL) was added propionaldehyde (55 μL, 0.63 mmol). The resulting clear solution was then heated at 110 °C in a sealed flask overnight. The reaction mixture was cooled to RT and evaporated. The resulting residue was suspended in *n*-propanol (0.3 mL) and cooled in the freezer for further precipitation. The solid was filtered off and triturated in MeOH (1 mL) to afford the title compound as an off-white solid (36 mg, 0.10 mmol, 50%); R_f=0.47 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.21 (d, J=7.9 Hz, 1H), 8.02–7.91 (m, 1H), 7.83 (dd, J=7.1, 1.9 Hz, 1H), 7.65 (br s, 2H), 7.59–7.42 (m, 4H), 4.30 (tt, J=11.4, 3.6 Hz, 1H), 3.39–3.26 (m, 1H), 3.26–3.09 (m, 3H), 3.02 (dd, J=16.3, 12.0 Hz, 1H), 2.90–2.74 (m, 1H), 1.20 ppm (t, J=7.4 Hz, 3H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=195.4, 169.1, 161.9, 160.3, 139.1, 133.5, 130.7, 128.8, 127.1, 126.3, 125.7, 125.6, 123.1, 122.8, 115.9, 115.4, 90.2, 46.3, 40.9, 32.9, 25.9, 14.1 ppm; mp: 206.3–207.7 °C; LC–MS: (m/z) calcd for $C_{22}H_{20}N_3O$ $[M+H]^+$: 342.2, found: 342.1.

2-Amino-4-benzyl-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3d). To a solution of 3-amino-5-(naphthalen-1-yl)cyclohex-2-en-1-one (50 mg, 0.21 mmol) and malononitrile (42 mg, 0.63 mmol) in *n*-propanol (0.8 mL) was added phenylacetaldehyde (74 μL, 0.63 mmol). The resulting mixture was then heated at 110 °C in a sealed flask overnight. The reaction mixture was cooled to RT and evaporated. The resulting brown oil was purified by flash column chromatography (0→10% EtOAc in CH_2Cl_2) followed by recrystallization in CH_2Cl_2/MeOH to afford the title compound as off-white crystals (34 mg, 0.08 mmol, 40%); R_f=0.45 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.21 (d, J=8.1 Hz, 1H), 7.98–7.91 (m, 1H), 7.83 (d, J=8.0 Hz, 1H), 7.72 (s, 2H), 7.60–7.50 (m, 2H), 7.50–7.39 (m, 2H), 7.32–7.25 (m, 2H), 7.23–7.12 (m, 3H), 4.71 (d, J=13.9 Hz, 1H), 4.58 (d, J=13.9 Hz, 1H), 4.38–4.27 (m, 1H), 3.39–3.29 (m, 2H), 3.27–3.18 (m, 1H), 3.03 (dd, J=16.3, 11.4 Hz, 1H), 2.87–2.77 ppm (m, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=195.5, 169.3, 160.5, 157.3, 138.9, 138.0, 133.5, 130.6, 128.8, 128.3, 128.3, 127.1, 126.3, 126.1, 125.7, 125.5, 123.1, 122.9, 116.4, 115.7, 91.7, 46.1, 40.9, 36.9, 32.7 ppm; mp: 217.8–219.3 °C; LC–MS: (m/z) calcd for $C_{27}H_{22}N_3O$ $[M+H]^+$: 404.2, found: 404.1.

Isoquinoline-1-carbaldehyde (4a). A solution of 1-bromoisoquinoline (769 mg, 3.70 mmol) in dry THF (300 mL) was cooled to −78 °C under a N_2 atmosphere. *s*BuLi (4.5 mL, 5.80 mmol, 1.4 M in cyclohexane) was added dropwise followed by addition of dry DMF (6.0 mL, 75.9 mmol). The reaction mixture was allowed to warm to RT and stirred for 30 min. Saturated NH_4Cl (300 mL) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (2×300 mL), dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (heptane/EtOAc 5:1) afforded the title compound as an orange solid (326 mg, 2.08 mmol, 58%); R_f=0.20 (heptane/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.40 (s, 1H), 9.34–9.32 (m, 1H), 8.76 (d, J=5.5 Hz, 1H), 7.90–7.89 (m, 2H), 7.78–7.76 ppm (m, 2H). The ^{1}H NMR data are in agreement with published values.[18]

Isoquinoline-8-carbaldehyde (4f). A solution of 8-bromoisoquinoline (1.50 g, 7.21 mmol) in dry THF (400 mL) was cooled to −78 °C under a N_2 atmosphere. *s*BuLi (9.5 mL, 6.79 mmol, 1.4 M in cyclohexane) was added dropwise followed by addition of dry DMF (10 mL, 129.98 mmol). The reaction mixture was allowed to warm to RT and stirred for 30 min. Saturated NH_4Cl (400 mL) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (3×400 mL), dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (heptane/EtOAc 5:1) afforded the title compound as a yellow solid (0.86 g, 5.48 mmol, 76% yield); R_f=0.21 (heptane/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.63 (s, 1H), 10.43 (s, 1H), 8.72 (d, J=5.8 Hz, 1H), 8.19 (dd, J=7.0, 1.2 Hz, 1H), 8.16 (d, J=8.3 Hz, 1H), 7.98 (dd, J=8.3, 7.0 Hz, 1H), 7.89 ppm (d, J=5.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.2, 149.2, 143.0, 137.2, 136.5, 133.5, 132.2, 130.1, 125.2, 121.2 ppm; mp: 81.7–84.3 °C; LC–MS: (m/z) calcd for $C_{10}H_8NO$ $[M+H]^+$; 158.1, found; 158.1.

4-(Isoquinolin-1-yl)but-3-en-2-one (5a). A suspension of NaH (60% *w/w* in mineral oil, 59 mg, 2.27 mmol) in dry THF (3 mL) was stirred under a N_2 atmosphere for 15 min. A solution of diethyl 2-oxopropylphosphonate (0.45 mL, 2.27 mmol) in dry THF (1 mL) was slowly added at 0 °C over 15 min. The clear yellow solution was warmed to RT and stirred for 2 h after which 1-isoquinoline-carbaldehyde (325 mg, 6.58 mmol) in dry THF (2 mL) was added dropwise. The reaction was left to stir for 19 h and then quenched with

sat. NH_4Cl (10 mL). The phases were separated and the aqueous layer was extracted with Et_2O (3×10 mL). The combined organic phases were washed with H_2O (20 mL), dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (heptane/EtOAc 3:1) afforded the title compound as green solid (250 mg, 1.27 mmol, 61%); R_f=0.15 (heptane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.26 (s, 1H), 8.77 (s, 1H), 8.21 (d, J=16.1 Hz, 1H), 8.14 (d, J=8.5 Hz, 1H), 8.03 (d, J=8.2 Hz, 1H), 7.81 (ddd, J=8.4, 7.0, 1.3 Hz, 1H), 7.71–7.65 (m, 1H), 7.49 (dd, J=8.3, 4.2 Hz, 1H), 6.89 (d, J=16.1 Hz, 1H), 2.47 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.5, 154.1, 141.6, 136.9, 133.8, 131.3, 130.4, 128.4, 128.1, 127.7, 125.7, 122.4, 28.4 ppm; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.1.

4-(Isoquinolin-4-yl)but-3-en-2-one (5b). Carried out as described for **5a** starting with isoquinoline-4-carbaldehyde. Purification by flash column chromatography (CH_2Cl_2/EtOAc 4:1) afforded the title compound as yellow–orange solid (1.06 g, 5.35 mmol, 81%); R_f= 0.15 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.26 (s, 1H), 8.77 (s, 1H), 8.21 (d, J=16.1 Hz, 1H), 8.14 (d, J=8.5 Hz, 1H), 8.03 (d, J=8.2 Hz, 1H), 7.81 (ddd, J=8.4, 7.0, 1.3 Hz, 1H), 7.71–7.65 (m, 1H), 7.49 (dd, J=8.3, 4.2 Hz, 1H), 6.89 (d, J=16.1 Hz, 1H), 2.47 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.5, 154.1, 141.6, 136.9, 133.8, 131.3, 130.4, 128.4, 128.1, 127.7, 125.7, 122.4, 28.4 ppm; mp: 107.9–113.7 °C; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.1.

4-(Quinolin-4-yl)but-3-en-2-one (5c). Carried out as described for **5a** starting with quinoline-4-carbaldehyde. Purification by flash column chromatography (heptane/EtOAc 4:1) afforded the title compound as beige powder (2.45 g, 12.4 mmol, 86%); R_f=0.45 (heptane/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.95 (d, J= 7.0 Hz, 1H), 8.22 (d, J=21.1 Hz, 1H), 8.16 (t, J=12.7 Hz, 2H), 7.68 (t, J=11.3 Hz, 1H), 7.61 (t, J=11.3 Hz, 1H), 7.51 (d, J=7.0 Hz, 1H), 6.83 (d, J=22.1 Hz, 1H), 2.49 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=198.1, 151.0, 149.0, 140.5, 137.9, 133.1, 131.0, 130.8, 127.9, 126.2, 123.8, 118.5, 29.1 ppm; mp: 91.7–92.5 °C; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.1.

4-(Quinolin-5-yl)but-3-en-2-one (5d). Carried out as described for **5a** starting with quinoline-5-carbaldehyde. Purification by flash column chromatography (heptane/EtOAc 1:1) afforded the title compound as slightly yellow oil (0.36 g, 1.83 mmol, 60%); R_f=0.17 (heptane/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.99 (dd, J= 4.3, 1.8 Hz, 1H), 8.54 (ddd, J=8.6, 1.5, 0.9 Hz, 1H), 8.29 (d, J= 16.1 Hz, 1H), 8.19 (d, J=8.3 Hz, 1H), 7.86 (d, J=7.0 Hz, 1H), 7.75 (dd, J=8.2, 7.5 Hz, 1H), 7.52 (dd, J=8.8, 4.3 Hz, 1H), 6.87 (d, J= 16.1 Hz, 1H), 2.48 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 197.7, 150.8, 148.5, 138.2, 132.1, 132.0, 131.6, 130.1, 129.1, 126.8, 125.4, 121.7, 28.4 ppm; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.1.

4-(Isoquinolin-5-yl)but-3-en-2-one (5e). Carried out as described for **5a** starting with isoquinoline-5-carbaldehyde. Purification by flash column chromatography (CH_2Cl_2/EtOAc 4:1) afforded the title compound as pale-yellow solid (1.05 g, 5.33 mmol, 83%); R_f=0.11 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.30 (d, J= 0.7 Hz, 1H), 8.63 (d, J=6.0 Hz, 1H), 8.25 (d, J=16.0 Hz, 1H), 8.05 (d, J=8.2 Hz, 1H), 8.00 (d, J=7.3 Hz, 1H), 7.96 (d, J=6.1 Hz, 1H), 7.65 (t, J=7.8 Hz, 1H), 6.86 (d, J=16.0 Hz, 1H), 2.47 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.7, 153.3, 143.9, 137.8, 133.2, 130.9, 130.2, 128.9, 128.8, 127.0, 116.1, 28.2 ppm; mp: 56.1–65.2 °C; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.1.

4-(Isoquinolin-8-yl)but-3-en-2-one (5f). Carried out as described for **5a** starting with isoquinoline-8-carbaldehyde. Purification by flash column chromatography (CH_2Cl_2/EtOAc 4:1) afforded the title compound as yellow solid (338 mg, 1.71 mmol, 81%); R_f=0.15 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.67 (s, 1H), 8.77 (s, 1H), 8.62 (d, J=5.8 Hz, 1H), 8.36 (d, J=16.0 Hz, 1H), 7.93 (d, J= 8.2 Hz, 1H), 7.89 (d, J=7.2 Hz, 1H), 7.79 (dd, J=10.2, 5.2 Hz, 1H), 6.86 (d, J=16.0 Hz, 1H), 2.50 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.6, 147.4, 141.6, 137.5, 136.7, 133.4, 131.8, 131.0, 128.9, 126.8, 126.1, 121.7, 28.0 ppm; mp: 80.8–83.5 °C; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.1.

4-(Quinolin-8-yl)but-3-en-2-one (5g). Carried out as described for **5a** starting with quinoline-8-carbaldehyde. Purification by flash column chromatography (heptane/EtOAc 3:1) afforded the title compound as green solid (0.96 g, 4.86 mmol, 76%); R_f=0.15 (heptane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.00 (dd, J=4.2, 1.8 Hz, 1H), 8.91 (d, J=16.7 Hz, 1H), 8.20 (dd, J=8.3, 1.6 Hz, 1H), 8.06 (dd, J=7.3, 0.8 Hz, 1H), 7.90 (dd, J=8.2, 1.2 Hz, 1H), 7.60 (t, J=7.7 Hz, 1H), 7.49 (dd, J=8.3, 4.2 Hz, 1H), 6.97 (d, J=16.7 Hz, 1H), 2.55 ppm (s, 3H). The ^{1}H NMR data are in agreement with published values.[19]

4-(Quinolin-2-yl)but-3-en-2-one (5h). Carried out as described for **5a** starting with quinoline-2-carbaldehyde. Purification by flash column chromatography (heptane/EtOAc 3:1) afforded the title compound as light-brown solid (601 mg, 3.05 mmol, 48%); R_f= 0.28 (heptane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.20 (d, J= 8.5 Hz, 1H), 8.11 (d, J=8.5 Hz, 1H), 7.83 (d, J=8.2 Hz, 1H), 7.78–7.70 (m, 2H), 7.67 (dd, J=8.5, 2.7 Hz, 1H), 7.61–7.55 (m, 1H), 7.16 (dd, J=16.4, 1.6 Hz, 1H), 2.47 ppm (d, J=1.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=198.7, 153.5, 143.0, 136.9, 131.9, 130.2, 129.7, 128.1, 127.6, 127.5, 120.0, 27.5 ppm; mp: 66.3–71.0 °C; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.0.

4-(Quinolin-7-yl)but-3-en-2-one (5h). Carried out as described for **5a** starting with quinoline-7-carbaldehyde. Purification by flash column chromatography (CH_2Cl_2/EtOAc 4:1) afforded the title compound as light-brown solid (442 mg, 2.24 mmol, 47%); R_f=0.17 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.97 (dd, J=4.2, 1.5 Hz, 1H), 8.52 (d, J=8.6 Hz, 1H), 8.27 (d, J=16.0 Hz, 1H), 8.17 (d, J=8.5 Hz, 1H), 7.84 (d, J=7.3 Hz, 1H), 7.73 (dd, J=8.4, 7.3 Hz, 1H), 7.49 (dd, J=8.6, 4.2 Hz, 1H), 6.84 (d, J=16.0 Hz, 1H), 2.45 ppm (d, J=0.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=198.1, 150.9, 142.4, 136.2, 130.5, 129.3, 128.8, 128.6, 124.7, 122.0, 27.7 ppm; mp: 61.2–64.5 °C; LC–MS: (*m*/*z*) calcd for $C_{13}H_{12}NO$ $[M+H]^+$: 198.1, found: 198.0.

4-(3-Methylbenzo[*d*]isoxazol-4-yl)but-3-en-2-one (5j). 3-Methylbenzo[*d*]isoxazol-4-yl trifluoromethanesulfonate (0.38 mg, 1.35 mmol) was dissolved in degassed DMF (15 mL) and $PdCl_2(PPh_3)_2$ (0.10 g, 0.13 mmol, 10 mol%), and NEt_3 (0.27 mL, 2.03 mmol) were added followed by methyl vinyl ketone (0.55 mL, 6.75 mmol). The reaction was stirred at 110 °C under N_2 atm. for 18 h, then cooled to RT and diluted with H_2O (30 mL). The mixture was extracted with EtOAc (3×30 mL) and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography (heptane/EtOAc 2:1) afforded the title compound as an orange solid (271 mg, 1.35 mmol, quant.); R_f=0.18 (heptane/EtOAc 3:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ=7.85 (d, J=16.3 Hz, 1H), 7.49 (d, J= 8.1 Hz, 1H), 7.46 (d, J=7.7 Hz, 1H), 7.39 (d, J=16.3 Hz, 1H), 7.31 (t, J=7.9 Hz, 1H), 2.69 (s, 3H), 2.45 ppm (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=198.9, 164.6, 151.3, 140.6, 138.6, 130.4, 126.3, 124.6, 124.5, 111.7, 27.7, 14.6 ppm; mp: 165.5–169.9 °C; LC–MS: (*m*/*z*) calcd for $C_{12}H_{12}NO_2$ $[M+H]^+$: 202.2, found: 202.1.

4-(3-Methylbenzo[*d*]isoxazol-5-yl)but-3-en-2-one (5k). 5-Bromo-3-methylbenzo[*d*]isoxazole (0.98 g, 4.62 mmol) was dissolved in degassed DMF (18 mL) and $Pd(OAc)_2$ (0.11 g, 0.48 mmol, 10 mol%), tri-*o*-toluoylphosphine (0.28 g, 0.94 mmol, 20 mol%) and K_2CO_3 (0.97 g, 6.99 mmol) were added followed by methyl vinyl ketone (0.45 mL, 5.55 mmol). The microwave vial was sealed and purged with N_2. The reaction was stirred in the microwave at 100 °C for 1 h, then cooled to RT and diluted with EtOAc (40 mL) and H_2O (40 mL). The organic phase was washed with H_2O (2×20 mL), dried over $MgSO_4$, filtered and evaporated. Flash column chromatography (heptane to heptane/EtOAc 3:1) afforded the title compound as a yellow solid (522 mg, 2.59 mmol, 56%); R_f = 0.17 (heptane/EtOAc 3:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.83 (d, J = 1.6 Hz, 1H), 7.61 (d, J = 16.2 Hz, 1H), 7.53–7.47 (m, 2H), 6.73 (d, J = 16.2 Hz, 1H), 2.66 (s, 3H), 2.40 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.2, 165.1, 152.4, 143.3, 142.3, 131.0, 126.8, 125.0, 119.3, 110.7, 27.6, 14.6 ppm; mp: 116.3–118.8 °C; LC–MS: (*m*/*z*) calcd for $C_{12}H_{12}NO_2$ $[M+H]^+$: 202.2, found: 202.1.

5-(Isoquinolin-1-yl)cyclohexan-1,3-dione (6a). A solution of NaOEt in EtOH (2.68 M, 0.5 mL) was added dropwise to a solution of diethylmalonate (0.2 mL, 1.32 mmol) in absolute EtOH (13 mL) over 10 min at RT and stirred for 30 min. 4-(Isoquinolin-1-yl)but-3-en-2-one (236 mg, 1.20 mmol) in absolute EtOH (13 mL) was next added dropwise over 10 min and the mixture was now heated at reflux and stirred for 4 h. The mixture was cooled to RT and the solvent evaporated in vacuo. The residue was dissolved in H_2O (13 mL) and evaporated. This was repeated three times and then the residue was dried under vacuum overnight. The solid was suspended in 2 M NaOH (3.4 mL), heated at 90 °C and stirred at that temperature for 3 h. The mixture was cooled to RT and acidified with 2 M H_2SO_4 to pH 5–6. The mixture was heated at reflux and stirred for 2 h before cooling to RT. The reaction was made alkaline (pH > 10) using 5 M NaOH. The aqueous phase was washed with EtOAc (13 mL) and CH_2Cl_2 (13 mL) and then filtered. The mixture was acidified with 2 M H_2SO_4 to pH 5–6 upon which a precipitate formed. Precipitation was allowed to occur in the fridge overnight. The precipitate was filtered off and dried to afford the title compound as a beige solid (152 mg, 0.63 mmol, 53%); R_f = 0.69 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.15 (br s, 1H), 8.46 (d, J = 5.6 Hz, 1H), 8.37 (d, J = 8.5 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.81–7.75 (m, 1H), 7.73 (d, J = 5.6 Hz, 1H), 7.70 (dd, J = 11.3, 4.1 Hz, 1H), 5.32 (br s, 1H), 4.56–4.39 (m, 1H), 2.94–2.68 (m, 2H), 2.58–2.49 ppm (m, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 161.1, 141.4, 135.9, 130.2, 127.7, 127.5, 125.2, 124.5, 119.7, 103.4, 40.1, 36.2 ppm; mp: 115.2–129.1 °C (decomp.); LC–MS: (*m*/*z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Isoquinolin-4-yl)cyclohexan-1,3-dione (6b). A solution of NaOEt in EtOH (2.68 M, 0.8 mL) was added dropwise to a solution of diethylmalonate (0.35 mL, 2.15 mmol) in absolute EtOH (20 mL) over 10 min at RT and stirred for 30 min. 4-(Isoquinolin-4-yl)but-3-en-2-one (355 mg, 1.77 mmol) in absolute EtOH (20 mL) was next added dropwise over 10 min and the mixture was now heated at reflux and stirred for 2.5 h. The mixture was cooled to RT and the solvent evaporated in vacuo. The residue was dissolved in H_2O (13 mL) and evaporated. This was repeated three times and then the residue was dried under vacuum overnight. The solid was suspended in 2 M NaOH (4 mL), heated at 90 °C and stirred at that temperature for 3 h. The mixture was cooled to RT and acidified with 2 M H_2SO_4 to pH 5–6. The mixture was heated at reflux and stirred at that temperature for 2 h before cooling to RT. The reaction was made alkaline (pH > 10) using 5 M NaOH. The aqueous phase was washed with EtOAc (20 mL) and CH_2Cl_2 (20 mL) and then filtered. The mixture was acidified with 2 M H_2SO_4 to pH 5–6 upon which a precipitate formed. Precipitation was allowed to occur in the fridge overnight. The precipitate was filtered off and dried to afford the title compound as an off-white solid (360 mg, 1.50 mmol, 85%); R_f = 0.15 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.22 (br s, 1H), 9.24 (s, 1H), 8.52 (s, 1H), 8.22 (d, J = 8.6 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 7.88–7.81 (m, 1H), 7.74–7.68 (m, 1H), 5.36 (s, 1H), 3.09–2.69 ppm (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 151.5, 140.3, 133.2, 132.1, 130.9, 128.5, 127.9, 127.2, 122.3, 103.5, 40.1, 32.2 ppm; mp: 127.8–138.2 °C (decomp.); LC–MS: (*m*/*z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Quinolin-4-yl)cyclohexan-1,3-dione (6c). A solution of NaOEt in EtOH (2.68 M, 2.1 mL, 5.6 mmol) was added dropwise over 40 min at RT to diethylmalonate (0.85 mL, 5.6 mmol) in absolute EtOH (50 mL). The solution was stirred at RT for 30 min, after which 4-(quinolin-4-yl)but-3-en-2-one (1.01 g, 5.0 mmol) in absolute EtOH (50 mL) was added dropwise over 70 min at RT. The solution was stirred at reflux for 2 h and then cooled to RT. The solvent was evaporated and the residue was dried in vacuo. The residue was dissolved in 2 M NaOH (2.8 mL, 5.6 mmol) and the mixture was heated at 90 °C and stirred at this temperature for 2.5 h. The mixture was cooled to RT and acidified with 2 M H_2SO_4 to pH 5–6. After addition of toluene (1.5 mL), the reaction mixture was stirred at 70 °C for 30 min and then stirred at reflux for 1 h. The brown solution with the white precipitate was put on the ice-bath to induce further precipitation. The mixture was then filtered and washed thoroughly with ice-cold H_2O. The mother liquor was evaporated to dryness and the residue was re-dissolved in EtOH. The brown mother liquor was evaporated to dryness and the crude residue was purified by dry column vacuum chromatography (EtOAc/MeOH/AcOH 100:10:1). The beige precipitate filtered off and yellowish-white powder obtained from dry column chromatography were both identified as the title compound (865 mg, 3.61 mmol, 61%); R_f = 0.18 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.85 (d, J = 4.6 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.76 (t, J = 7.0 Hz, 1H), 7.63 (t, J = 7.0 Hz, 1H), 7.50 (d, J = 4.5 Hz, 1H), 5.34 (s, 1H), 4.31–4.19 (m, 1H), 2.80–2.66 (m, 3H), 2.58 ppm (s, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 150.3, 148.8, 147.9, 129.9, 129.2, 126.7, 126.1, 123.3, 118.3, 103.5, 33.2 ppm; mp: 219.0–222.7 °C (decomp.); LC–MS: (*m*/*z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Quinolin-5-yl)cyclohexan-1,3-dione (6d). Under a nitrogen atmosphere diethyl malonate (0.30 mL, 1.97 mmol) was dissolved in absolute EtOH (20 mL). NaOEt in EtOH (0.75 mL, 1.97 mmol) was added dropwise and the mixture was stirred at RT for 30 min, after which 4-(quinolin-5-yl)but-3-en-2-one (354 mg, 1.80 mmol) in absolute EtOH (20 mL) was added slowly over 10 min. The mixture was heated at reflux and stirred for 3 h after which it was cooled to RT. The mixture was evaporated to dryness and then suspended in 2 M NaOH (4 mL). The suspension was heated at 90 °C and stirred at that temperature for 3 h. The mixture was allowed to cool to RT, after which it was acidified by addition of 2 M H_2SO_4 to pH 5–6. The mixture was heated at reflux and stirred for 105 min. The mixture was cooled to RT and then made alkaline (pH > 14) by addition of 5 M NaOH. The mixture was washed with EtOAc (20 mL) and CH_2Cl_2 (20 mL). The aqueous phase was filtered and the pH was adjusted to pH 5–6 by addition of 2 M H_2SO_4. Precipitation was allowed to occur at 5 °C, and was then filtered off and dried to give the title compound as beige powder (263 mg, 1.10 mmol, 61%); R_f = 0.38 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.22 (br s, 1H), 8.91 (dd, J = 4.1, 1.5 Hz, 1H), 8.66 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.74 (dd, J = 8.0, 7.3 Hz,

1H), 7.62 (d, J=7.0 Hz, 1H), 7.56 (dd, J=8.8, 4.0 Hz, 1H), 5.35 (s, 1H), 4.27–4.17 (m, 1H), 2.80–2.65 (m, 2H), 2.59–2.45 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=150.1, 148.2, 140.2, 131.7, 129.1, 128.0, 125.8, 123.6, 121.4, 103.5, 33.2 ppm; mp: 196.5–209.1 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Isoquinolin-5-yl)cyclohexan-1,3-dione (6e). A solution of NaOEt in EtOH (2.68 M, 2.0 mL) was added dropwise to a solution of diethylmalonate (0.8 mL, 5.30 mmol) in absolute EtOH (55 mL) over 10 min at RT and stirred for 30 min. 5-(Isoquinolin-5-yl)but-3-en-2-one (950 mg, 4.82 mmol) in absolute EtOH (36 mL) was next added dropwise over 10 min and the mixture was then heated at reflux and stirred for 2 h. The mixture was cooled to RT and the solvent evaporated in vacuo. The residue was dissolved in H_2O (30 mL) and evaporated. This was repeated three times and then the residue was dried under vacuum overnight. The solid was suspended in 2 M NaOH (15 mL), heated at 90 °C and stirred at that temperature for 3 h. The mixture was cooled to RT and acidified with 2 M H_2SO_4 to pH 5–6. The mixture was heated at reflux and stirred at that temperature for 2 h before cooling to RT. The reaction was made alkaline (pH>10) using 5 M NaOH. The aqueous phase was washed with EtOAc (15 mL) and CH_2Cl_2 (15 mL) and then filtered. The mixture was acidified with 2 M H_2SO_4 to pH 5–6 upon which a precipitate formed. Precipitation was allowed to occur in the fridge overnight. The precipitate was filtered off, triturated with EtOH and dried to afford the title compound as an off-white solid (511 mg, 2.13 mmol, 44%); R_f=0.17 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=9.31 (s, 1H), 8.52 (d, J=6.1 Hz, 1H), 8.02 (d, J=2.5 Hz, 1H), 8.01 (d, J=4.8 Hz, 1H), 7.77 (d, J=7.1 Hz, 1H), 7.66 (t, J=7.7 Hz, 1H), 5.18 (br s, 1H), 4.16–4.05 (m, 1H), 2.63 (dd, J=16.5, 11.1 Hz, 2H), 2.55–2.49 ppm (m, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ=172.0, 153.2, 143.2, 139.3, 133.3, 128.6, 127.4, 127.1, 126.4, 116.1, 102.6, 33.5, 21.1 ppm; mp: 203.4–206.5 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Isoquinolin-8-yl)cyclohexan-1,3-dione (6f). A solution of NaOEt in EtOH (2.68 M, 0.70 mL) was added dropwise to a solution of diethylmalonate (0.3 mL, 1.88 mmol) in absolute EtOH (19 mL) over 10 min at RT and stirred for 30 min. 5-(isoquinolin-8-yl)but-3-en-2-one (336 mg, 1.70 mmol) in absolute EtOH (36 mL) was next added dropwise over 10 min and the mixture was heated at reflux and stirred for 4 h. The mixture was cooled to RT and the solvent evaporated in vacuo. The residue was dissolved in H_2O (19 mL) and evaporated. This was repeated three times and then the residue was dried under vacuum overnight. The solid was suspended in 2 M NaOH (3.8 mL), heated at 90 °C and stirred at that temperature for 3 h. The mixture was cooled to RT and acidified with 2 M H_2SO_4 to pH 5–6. The mixture was heated at reflux and stirred at that temperature for 2 h before cooling to RT. The reaction was made alkaline (pH>10) using 5 M NaOH. The aqueous phase was washed with EtOAc (15 mL) and CH_2Cl_2 (15 mL) and then filtered. The mixture was acidified with 2 M H_2SO_4 to pH 5–6 upon which a precipitate formed. Precipitation was allowed to occur in the fridge overnight. The precipitate was filtered off and dried to afford the title compound as an off-white solid (189 mg, 0.78 mmol, 46%); R_f= 0.63 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=11.21 (br s, 1H), 9.62 (s, 1H), 8.53 (d, J=5.6 Hz, 1H), 7.89–7.83 (m, 2H), 7.77–7.72 (m, 1H), 7.67 (d, J=7.0 Hz, 1H), 5.36 (s, 1H), 4.47–4.38 (m, 1H), 2.87–2.73 (m, 2H), 2.52–2.49 ppm (m, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ=148.2, 142.6, 140.6, 135.9, 130.3, 125.7, 125.6, 124.7, 121.0, 103.6, 40.1, 32.8 ppm; mp: 198.5–216.1 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Quinolin-8-yl)cyclohexan-1,3-dione (6g). A solution of NaOEt in EtOH (2.68 M, 2.0 mL) was added dropwise over 10 min to a solution of diethyl malonate (0.8 mL, 5.26 mmol) in absolute EtOH (54 mL) at RT under N_2. The solution was stirred for 30 min before 4-(quinolin-8-yl)but-3-en-2-one (0.94 g, 4.79 mmol) in absolute EtOH (36 mL) was added dropwise over 10 min. The mixture was heated at reflux and stirred at that temperature for 2.5 h. The reaction was cooled to RT and the solvent was removed in vacuo. The residue was dissolved in H_2O (30 mL) and concentrated. This was repeated three times and the residue was dried under vacuum overnight. The residue was then dissolved in 2 M NaOH (15 mL) and stirred at 90 °C for 3 h. After cooling to RT the mixture was acidified with 2 M H_2SO_4 until pH 5–6 and stirred at reflux for 2 h. The mixture was cooled to RT and treated with aqueous 5 M NaOH until pH>10. The aqueous phase was next washed with EtOAc (13 mL) and CH_2Cl_2 (13 mL). The pH of the mixture was adjusted to pH 5–6 upon which a fine precipitate formed. The suspension was cooled in an ice bath to induce further precipitation. The precipitate was filtered off and dried. The mother liquor was concentrated in vacuo, dissolved in 2 M NaOH (15 mL) and the pH was adjusted to pH 5–6. The precipitate formed was filtered and dried. This was repeated one more time to afford the title compound as a beige solid (404 mg, 1.69 mmol, 35%); R_f=0.43 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=11.16 (br s, 1H), 8.97 (dd, J=4.1, 1.8 Hz, 1H), 8.40 (dd, J=8.3, 1.7 Hz, 1H), 7.90 (dd, J= 8.2, 1.0 Hz, 1H), 7.75 (d, J=6.7 Hz, 1H), 7.61 (t, J=6.1 Hz, 1H), 7.59 (dd, J=8.2, 4.1 Hz, 1H), 5.34 (s, 1H), 4.60 (m, 1H), 2.80 (dd, J=16.5, 11.8 Hz, 2H), 2.60–2.53 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=149.6, 145.4, 141.1, 136.7, 128.1, 126.8, 126.5, 126.4, 121.4, 103.3, 40.1, 33.2 ppm; mp: 163.9–172.8 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Quinolin-2-yl)cyclohexan-1,3-dione (6h). A solution of NaOEt in EtOH (2.68 M, 0.3 mL) was added dropwise to a solution of diethylmalonate (0.15 mL, 0.80 mmol) in absolute EtOH (8 mL) at RT under N_2. The solution was stirred for 30 min before 4-(quinolin-2-yl)but-3-en-2-one (140 mg, 0.71 mmol) in absolute EtOH (8 mL) was added dropwise over 10 min. The mixture was heated at reflux and stirred at that temperature for 16 h. The reaction was cooled to RT and the solvent was removed in vacuo and dried under vacuum. The residue was then dissolved in 2 M NaOH (1.6 mL) and stirred at 90 °C for 3 h. After cooling to RT the mixture was acidified with 2 M H_2SO_4 until pH 5–6 and stirred at reflux for 4 h. The mixture was cooled to RT and treated with aqueous 5 M NaOH until pH>10. The aqueous phase was next washed with EtOAc (5 mL) and CH_2Cl_2 (5 mL). The pH of the mixture was adjusted to pH 5–6 with 2 M H_2SO_4 upon which a fine precipitate formed. The suspension was cooled in an ice bath to induce further precipitation. The precipitate was filtered off and dried. The mother liquor was concentrated in vacuo, dissolved in 2 M NaOH (15 mL) and the pH was adjusted to pH 5–6. The precipitate formed was filtered and dried. This was repeated one more time to afford the title compound as an off-white solid (58 mg, 0.24 mmol, 34%); R_f=0.67 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ= 11.18 (s, 1H), 8.33 (d, J=8.4 Hz, 1H), 7.99–7.92 (m, 2H), 7.74 (ddd, J=8.3, 6.8, 1.6 Hz, 1H), 7.58 (ddd, J=10.8, 7.1, 4.5 Hz, 2H), 5.30 (s, 1H), 3.69 (ddd, J=11.2, 6.6, 4.6 Hz, 1H), 2.97 (ddd, J=25.6, 12.1, 5.4 Hz, 1H), 2.83 (ddd, J=17.7, 16.0, 9.3 Hz, 1H), 2.73–2.58 ppm (m, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ=162.3, 147.0, 136.7, 129.5, 128.6, 127.7, 126.8, 126.1, 120.5, 103.3, 41.0, 40.1 ppm; mp: 135.5–

184.5 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

5-(Quinolin-7-yl)cyclohexan-1,3-dione (6i). A solution of NaOEt in EtOH (2.68 M, 0.75 mL) was added dropwise to a solution of diethylmalonate (0.30 mL, 1.97 mmol) in absolute EtOH (20 mL) at RT under N_2. The solution was stirred for 30 min before 4-(quinolin-7-yl)but-3-en-2-one (354 mg, 1.79 mmol) in absolute EtOH (20 mL) was added dropwise over 5 min. The mixture was heated at reflux and stirred at that temperature for 3 h. The reaction was cooled to RT and the solvent was removed in vacuo and dried under vacuum. The residue was then dissolved in 2 M NaOH (1.6 mL) and stirred at 90 °C for 3 h. After cooling to RT the mixture was acidified with 2 M H_2SO_4 until pH 5–6 and stirred at reflux for 4 h. The mixture was cooled to RT and treated with 5 M NaOH until pH > 10. The aqueous phase was next washed with EtOAc (5 mL) and CH_2Cl_2 (5 mL). The pH of the mixture was adjusted to pH 5–6 with 2 M H_2SO_4 upon which a fine precipitate formed. The suspension was cooled in an ice bath to induce further precipitation. The precipitate was filtered off and dried. The mother liquor was concentrated in vacuo, dissolved in 2 M NaOH (15 mL) and the pH was adjusted to pH 5–6 with 2 M H_2SO_4. The precipitate formed was filtered and dried. This was repeated one more time to afford the title compound as an off-white solid (74 mg, 0.31 mmol, 18%); R_f = 0.37 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 11.28 (br s, 1H), 8.88 (d, J = 4.1 Hz, 1H), 8.33 (d, J = 8.3 Hz, 1H), 7.98–7.83 (m, 2H), 7.66 (d, J = 8.5 Hz, 1H), 7.52–7.46 (m, 1H), 5.34 (s, 1H), 3.62–3.52 (m, 1H), 2.83–2.58 (m, 2H), 2.59 ppm (m, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 172.7, 150.6, 147.8, 145.0, 135.6, 128.2, 126.7, 126.4, 126.1, 121.1, 103.6, 48.5, 30.1 ppm; mp: 197.2–216.9 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{15}H_{14}NO_2$ $[M+H]^+$: 240.1, found: 240.1.

4-(3-Methylbenzo[*d*]isoxazol-4-yl)cyclohexane-1,3-dione (6j). A solution of NaOEt in EtOH (2.68 M, 0.41 mL) was added dropwise over 10 min to a solution of diethylmalonate (0.16 mL, 1.09 mmol) in absolute EtOH (11 mL) at RT under N_2. The solution was stirred for 40 min before 4-(3-methylbenzo[*d*]isoxazol-4-yl)but-3-en-2-one (199 mg, 0.99 mmol) in absolute EtOH (11 mL) was added dropwise over 7 min. The mixture was heated at reflux and stirred at that temperature for 5 h. The reaction was cooled to RT and the solvent was removed in vacuo. The residue was dried under vacuum overnight. The residue was then dissolved in 2 M NaOH (2.2 mL) and stirred at 90 °C for 4 h. The mixture was cooled to RT and acidified with 2 M H_2SO_4 until pH 1–2 and continued to stir at RT for 4 h. The formed precipitate was filtered off, washed with H_2O and dried to afford the title compound as a brown foam (110 mg, 0.45 mmol, 46%); R_f = 0.54 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.11 (br s, 1H), 8.98 (s, 1H), 7.11–7.04 (m, 1H), 6.86 (d, J = 8.2, 7.7 Hz, 1H), 6.75 (d, J = 7.8 Hz, 1H), 3.80–3.73 (m, 1H), 3.38–3.31 (m, 1H), 2.92–2.81 (m, 2H), 2.33–2.24 (m, 2H), 1.99 ppm (s, 3H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 169.4, 168.6, 156.2, 153.3, 142.1, 124.5, 122.3, 121.7, 108.8, 103.5, 100.4, 41.6, 35.2, 14.1 ppm; mp: > 250 °C; LC–MS: (*m/z*) calcd for $C_{14}H_{16}NO_4$ $[M+H^+ + H_2O]$: 262.1, found: 262.1.

5-(3-Methylbenzo[*d*]isoxazol-5-yl)cyclohexane-1,3-dione (6k). A solution of NaOEt in EtOH (2.68 M, 0.51 mL) was added dropwise over 10 min to a solution of diethylmalonate (0.21 mL, 1.37 mmol) in absolute EtOH (14 mL) at RT under N_2. The solution was stirred for 30 min before 4-(3-methylbenzo[*d*]isoxazol-5-yl)but-3-en-2-one (0.25 g, 1.24 mmol) in absolute EtOH (14 mL) was added dropwise over 10 min. The mixture was heated at reflux and stirred at that temperature for 4 h. The reaction was cooled to RT and the solvent was removed in vacuo. The residue was dissolved in H_2O (30 mL) and concentrated. This was repeated three times and the final residue was dried under vacuum overnight. The residue was then dissolved in 2 M NaOH (12 mL) and stirred at RT for 17 h. The mixture was then acidified with 2 M H_2SO_4 until pH 1–2 and continued to stir at RT for 5 h. The formed precipitate was filtered off and dried to afford the title compound as a brown solid (149 mg, 0.61 mmol, 49%); R_f = 0.23 (EtOAc/MeOH/AcOH 100:10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 11.11 (br s, 1H), 7.57 (s, 1H), 6.91 (d, J = 9.9 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 5.26 (s, 1H), 3.22–3.15 (m, 1H), 2.67–2.63 (m, 2H), 2.36–2.25 (m, 2H), 2.08 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 169.5, 150.8, 147.1, 134.5, 126.6, 125.8, 123.4, 121.5, 116.4, 104.0, 38.5, 24.0 ppm; mp: 195.4–197.0 °C (decomp.); LC–MS: (*m/z*) calcd for $C_{14}H_{16}NO_4$ $[M+H^+ + H_2O]$: 262.1, found: 262.1.

1-(2,6-Dihydroxyphenyl)ethan-1-one oxime (8j). A solution of 2,6-dihydroxyacetophenone (2.01 g, 13.15 mmol) in absolute EtOH (100 mL) was warmed to 70 °C. A solution of hydroxylamine hydrochloride (25.53 g, 0.37 mol) in H_2O (65 mL) was added and the reaction mixture was stirred at that temperature for 20 h. The solution was cooled to RT and diluted with H_2O (200 mL). The mixture was extracted with EtOAc (3 × 100 mL), the combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography (heptane/EtOAc 3:1) afforded the title compound as a yellow solid (1.77 g, 10.61 mmol, 81%); R_f = 0.33 (heptane/EtOAc 3:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 10.94 (s, 1H), 9.72 (s, 2H), 6.93 (t, J = 8.1 Hz, 1H), 6.32 (d, J = 8.1 Hz, 2H), 2.10 ppm (s, 3H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 156.6, 153.8, 129.3, 111.3, 106.7, 15.4 ppm; mp: 134.8–138.5 °C; LC–MS: (*m/z*) calcd for $C_8H_{10}NO_3$ $[M+H]^+$: 168.1, found: 168.1.

1-(5-Bromo-2-hydroxyphenyl)ethan-1-one oxime (8k). A solution of 5-bromo-2-hydroxyacetophenone (2.02 g, 9.32 mmol) in absolute EtOH (49 mL) was warmed to 70 °C. A solution of hydroxylamine hydrochloride (19.21 g, 0.27 mol) in H_2O (73 mL) was added and the reaction mixture was stirred at that temperature for 2 h. The solution was cooled to RT and diluted with H_2O (240 mL). The mixture was extracted with EtOAc (3 × 150 mL), the combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to afford the title compound as an off-white solid (2.14 g, 9.30 mmol, quant.); R_f = 0.18 (heptane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.15 (s, 1H), 7.52 (d, J = 2.4 Hz, 1H), 7.34 (dd, J = 8.7, 2.4 Hz, 1H), 7.30 (s, 1H), 6.86 (d, J = 8.7 Hz, 1H), 2.34 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.8, 156.8, 133.5, 130.1, 120.1, 119.2, 110.9, 10.8 ppm; mp: 155.6–158.5 °C; LC–MS: (*m/z*) calcd for $C_8H_9BrNO_2$ $[M+H]^+$: 230.0, 232.0, found: 230.0, 232.0.

3-Methylbenzo[*d*]isoxazol-4-ol. PPh_3 (2.84 g, 10.83 mmol) was dissolved in dry CH_2Cl_2 (36 mL) and DDQ (2.47 g, 10.88 mmol) was added at RT. The brown reaction mixture was stirred at that temperature for 3 min before 1-(2,6-dihydroxyphenyl)ethan-1-one oxime (**8j**) (1.20 g, 7.18 mmol) was added. The suspension was stirred for 16 h and the solvent was evaporated in vacuo. Purification by flash column chromatography (heptane/EtOAc 3:1) afforded the title compound as a white solid (900 mg, 6.03 mmol, 84%); R_f = 0.23 (heptane/EtOAc 3:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.91 (s, 1H), 7.21 (t, J = 8.1 Hz, 1H), 7.03 (dd, J = 8.1, 0.9 Hz, 1H), 6.88 (dd, J = 8.1, 0.8 Hz, 1H), 2.70 ppm (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 163.4, 152.1, 148.1, 129.0, 125.8, 111.1, 101.8, 14.1 ppm; mp: 129.8–131.5 °C; LC–MS: (*m/z*) calcd for $C_8H_8NO_2$ $[M+H]^+$: 150.0, found: 150.1.

3-Methylbenzo[*d*]isoxazol-4-yl trifluoromethanesulfonate (9j). 3-Methylbenzo[*d*]isoxazol-4-ol (0.80 g, 5.36 mmol) which was prepared as described above was dissolved in dry CH_2Cl_2 (5 mL) and pyridine (0.86 mL, 10.72 mmol) was added at RT. The reaction was

cooled to 0 °C and Tf_2O (1.1 mL, 6.44 mmol) in CH_2Cl_2 (2.5 mL) was added dropwise. The reaction was warmed to RT and stirred for 18 h. The reaction was diluted with Et_2O (8 mL) and 2 M HCl (3 mL). The reaction mixture was washed with sat. $NaHCO_3$ (5 mL) and brine (5 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by flash column chromatography (heptane/EtOAc 6:1) afforded the title compound as a yellow solid (1.32 g, 4.69 mmol, 87%); R_f = 0.33 (heptane/EtOAc 6:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51 (dd, J = 8.2, 0.9 Hz, 1H), 7.34 (t, J = 8.2 Hz, 1H), 7.24 (dd, J = 8.3, 0.8 Hz, 1H), 2.69 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.6, 152.7, 139.0, 134.8, 124.8, 116.9, 110.6, 14.6 ppm; ^{19}F NMR (400 MHz, $CDCl_3$): δ = 73.0 ppm; mp: 51.4–55.5 °C; LC–MS: (*m/z*) calcd for $C_9H_7F_3NO_4S$ $[M+H]^+$: 282.0, found: 282.1.

5-Bromo-3-methylbenzo[*d*]isoxazole (9k). PPh_3 (2.08 g, 7.96 mmol) was dissolved in dry CH_2Cl_2 (26 mL) and DDQ (1.79 g, 7.88 mmol) was added at RT. The brown reaction mixture was stirred at that temperature for 5 min before 1-(5-bromo-2-hydroxyphenyl)ethan-1-one oxime (1.21 g, 5.23 mmol) was added. The suspension was stirred at RT for 10 min and the solvent was evaporated in vacuo. Purification by flash column chromatography (heptane/EtOAc 3:1) afforded the title compound as an off-white solid (991 mg, 4.67 mmol, 89%); R_f = 0.32 (heptane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, J = 1.9 Hz, 1H), 7.41 (dd, J = 8.6, 1.9 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 2.64 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.1, 150.0, 143.2, 127.5, 122.5, 116.8, 111.4, 14.5 ppm; LC–MS: (*m/z*) calcd for C_8H_7BrNO $[M+H]^+$: 212.0, 214.0, found: 212.1, 214.1.

3-Amino-5-(naphthalen-1-yl)cyclohex-2-enone (10). To a solution of 5-(naphthalen-1-yl)-cyclohexan-1,3-dione (600 mg, 2.52 mmol) in absolute EtOH (11.5 mL) containing some 3 Å molecular sieves was added NH_4OAc (584 mg, 7.55 mmol) and the reaction mixture was stirred at reflux overnight. The resulting brown cloudy suspension was evaporated to dryness. Saturated $NaHCO_3$ (50 mL) was added and the resulting mixture was extracted with EtOAc (3×75 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated to afford the title compound as a yellow solid (505 mg, 84%). The product was used in the following steps without any further purification. R_f = 0.16 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (400 MHz, $CDCl_3$/[D$_4$]MeOH (1:1)): δ = 8.13 (d, J = 8.3 Hz, 1H), 7.89 (dd, J = 8.1, 1.4 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.60–7.42 (m, 4H), 5.37 (s, 1H), 4.21 (tt, J = 10.4, 5.1 Hz, 1H), 2.91–2.75 (m, 2H), 2.74–2.58 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$/[D$_4$]MeOH (1:1)): δ = 199.0, 172.5, 140.2, 135.6, 132.5, 130.1, 128.5, 127.3, 126.7, 126.5, 124.0, 123.7, 98.0, 43.3, 36.4, 36.1 ppm; mp: 203.8–205.4 °C; LC–MS: (*m/z*) calcd for $C_{16}H_{16}NO$ $[M+H]^+$: 238.1, found: 238.1.

1,5-Diiodonaphthalene (11). $NaNO_2$ (5.23 g, 75.9 mmol) in H_2O (20 mL) was added dropwise to a solution of naphthalen-1,5-diamine (4.00 g, 25.3 mmol), conc. H_2SO_4 (25 mL) and H_2O (20 mL) at −15 °C over 40 min. The reaction mixture was stirred for an additional 10 min, then KI (12.6 g, 75.9 mmol) in H_2O (20 mL) was added dropwise over 30 min. The reaction mixture was stirred at 80 °C for 21 h and quenched with KOH (pH ≈ 10) at 0 °C. The black solid residue was filtered off and the aqueous phase was extracted with diethyl ether (3×50 mL). The combined organic phases were washed with 1 M HCl (30 mL), sat. $Na_2S_2O_3$ (30 mL), 1 M NaOH (30 mL) and brine (30 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by flash column chromatography afforded the title compound as a brown solid (3.68 g, 9.69 mmol, 38%); R_f = 0.75 (heptane/CH_2Cl_2 5:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.12 (d, J = 2.1 Hz, 2H), 8.09 (s, 1H), 7.24 ppm (t, J = 8.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.4, 134.5, 133.5, 128.3, 99.7 ppm; mp: 146–148 °C.

5-Iodonaphthalene-1-carbaldehyde (12). *t*BuLi (1.49 M, 3.5 mL, 5.26 mmol) was added dropwise to a solution of 1,5-diiodonaphthalene (**11**) (1.00 g, 2.6 mmol) in dry THF (50 mL) at −78 °C under a N_2 atmosphere. DMF (0.20 mL, 2.6 mmol) was added slowly and the reaction was stirred at that temperature for 1.5 h. The reaction mixture was warmed to RT, diluted with H_2O (50 mL) and extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine (30 mL), dried over MgSO4, filtered and evaporated. Purification by flash column chromatography afforded the title compound as a yellow solid (371 mg, 1.51 mmol, 58%); R_f = 0.42 (heptane/EtOAc 5:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.39 (s, 1H), 9.30 (d, J = 8.4 Hz, 1H), 8.43 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 7.2 Hz, 1H), 8.02 (d, J = 7.2 Hz, 1H), 7.70 (t, J = 8.4 Hz, 1H), 7.36 ppm (t, J = 8.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 192.9, 139.5, 138.9, 137.4, 135.5, 134.6, 131.8, 130.0, 126.7, 125.8, 100.2 ppm; mp: 114–116 °C.

(*E*)-4-(1-Iodonaphthalen-5-yl)but-3-en-2-one (13). A solution of diethyl-2-oxopropylphosphonate (0.24 mL, 1.23 mmol) in dry THF (5 mL) was added dropwise over 20 min to a solution of NaH (60% dispersion in mineral oil, 49 mg, 1.23 mmol) in dry THF (5 mL) at RT under a N_2 atmosphere. The lightly yellow solution was stirred for an additional 1.5 h at RT after which a solution of carbaldehyde **12** (330 mg, 1.17 mmol) in dry THF (5 mL) was added at 0 °C. The reaction mixture was stirred at RT for 21 h. The reaction mixture was quenched with H_2O (10 mL) and extracted with EtOAc (3× 30 mL). The combined organic phases were washed with brine (30 mL), dried over Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography afforded the title compound as a yellow solid (276 mg, 0.85 mmol, 73%); R_f = 0.20 (heptane/EtOAc 5:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.27 (d, J = 15.6 Hz, 1H), 8.17–8.09 (m, 3H), 7.74 (d, J = 6.9 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.21 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 15.6 Hz, 1H), 2.46 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 197.8, 139.4, 138.0, 134.8, 134.3, 132.4, 132.0, 130.2, 127.6, 127.1, 125.9, 124.1, 100.5, 28.1 ppm; mp: 118–120 °C; LC–MS: (*m/z*) calcd for $C_{14}H_{11}IO$ $[M+H]^+$: 323.1, found: 323.1; HPLC: purity (λ 254 nm) > 98%.

5-(1-Iodonaphthalen-5-yl)cyclohexane-1,3-dione (14). A solution of NaOEt (0.52 mL, 1.39 mmol) in absolute EtOH (5 mL) was added dropwise over 10 min to a stirred solution of diethylmalonate (0.22 mL, 1.39 mmol) in absolute EtOH (15 mL) at RT under a N_2 atmosphere. This yellow solution was stirred at RT for an additional 40 min after which a solution of enone **13** (300 mg, 0.93 mmol) in a mixture of abs EtOH/THF (12 mL, 3:1) was added dropwise over 10 min. The reaction mixture was stirred at RT for 30 min and then at reflux for 15 h. The reaction mixture was concentrated and the brown sticky residue was dried under vacuum. To this residue H_2O (10 mL) and 2 M NaOH (2.3 mL, 4.6 mmol) were added and the reaction mixture was stirred at 85 °C for 3 h and then cooled to RT. The solution was adjusted to pH 2 by adding 2 M H_2SO_4 and stirred at 100 °C for 1 h. After concentration in vacuo, the crude product was dissolved in EtOAc (30 mL) and extracted with 1 M NaOH (3× 20 mL). The combined aqueous phases were acidified with conc. H_2SO_4 (pH ≈ 1) and extracted with EtOAc (3×20 mL). The combined organic phases were washed with H_2O (20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo to afford the title compound as a yellow solid (241 mg, 0.66 mmol, 71%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.23 (s, 0.5H), 8.27–8.12 (m, 2H), 7.97–7.88 (m, 1H), 7.64–7.55 (m, 2H), 7.35–7.27 (m, 1H), 5.33 (s, 0.5H), 4.24–4.17 (m, 1H), 2.77–2.40 ppm (m, 4H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 197.3, 140.8, 138.0,

134.5, 132.2, 131.4, 128.3, 124.8, 104.1, 101.6, 61.2, 34.3 ppm; mp: 162–164 °C; LC–MS: (*m/z*) calcd for $C_{16}H_{13}IO_2$ $[M+H]^+$: 365.2, found: 365.2; HPLC: purity (λ 254 nm) >95%.

2-Amino-5,6,7,8-tetrahydro-7-(1-iodonaphthalen-5-yl)-4-methyl-5-oxo-4*H*-chromene-3-carbonitrile (15). *N*-Methylmorpholine (27 μL, 0.24 mmol) was added to a solution of 1,3-dione **14** (180 mg, 0.49 mmol), acetaldehyde (56 μL, 0.98 mmol) and malononitrile (65 mg, 0.98 mmol) in abs EtOH (20 mL) at 0 °C and the mixture was stirred at RT for 17 h. After concentration in vacuo, the crude product was purified by column chromatography to afford the title compound as a yellow solid (161 mg, 0.35 mmol, 72%); R_f=0.37 (heptane/EtOAc 1:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ= 8.38 (d, J=8.0 Hz, 1H), 8.20 (d, J=8.0 Hz, 1H), 8.00 (t, J=4.0 Hz, 1H), 7.65 (d, J=4.0 Hz, 2H), 7.34 (t, J=8.0 Hz, 1H), 6.90 (br s, 2H), 4.45–4.26 (m, 1H), 3.60–3.48 (m, 1H), 2.97–2.59 (m, 4H), 1.20 (d, J=8.0 Hz, 2H), 1.12 ppm (d, J=8.0 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=195.6, 163.3, 158.8, 139.5, 137.5, 133.9, 131.5, 130.9, 127.6, 124.3, 119.9, 114.8, 100.8, 57.8, 43.7, 32.6, 24.8, 23.0, 17.4 ppm; mp: 202–204 °C; LC–MS: (*m/z*) calcd for $C_{21}H_{17}IN_2O_2$ $[M+H]^+$: 457.3, found: 457.3; HPLC: purity (λ 254 nm) >95%.

Nonspecific brain protein binding

Nonspecific brain protein binding of UCPH-102 and its analogues was determined in rat brain homogenate using in vitro equilibrium dialysis. Rat brains for nonspecific protein binding studies were harvested from animals in house according to the European Community Guidelines and Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised in 1985). The procedures were reviewed and approved by the Finnish National Animal Experiment Board (license ESAVI/6317/04.10.03/2011). Brain tissues were homogenized in three volumes of phosphate-buffered saline at pH 7.4 on ice by using an ultrasonic homogenizer after which the investigated compounds were added into the homogenate. Equilibrium dialysis was performed for 400 μL of tissue homogenate and 600 μL of buffer for 4 h at 37 °C in a single-use RED Plate with an 8 kDa cutoff dialysis membrane (Thermo Scientific, Rockford, IL, USA). After the dialysis concentrations were determined by HPLC, the buffer-to-homogenate concentration ratio of the investigated compounds was calculated. The concentration ratio was used to calculate the unbound fraction in brain (%FU). A previously described approach to account for the effect of tissue dilution in the homogenate was used to calculate the %FU in brain.[25]

HPLC analyses

The purity of the investigated compounds in equilibrium dialysis samples were determined by an HPLC system, which consisted of an Agilent 1100 binary pump (Agilent Technologies Inc., Wilmington, DE, USA), a 1100 micro vacuum degasser, an HP 1050 Autosampler, an HP 1050 variable-wavelength detector (operated at λ 254 nm). Chromatographic separations were carried out on an Agilent Zorbax SB-C$_{18}$ analytical column (4.6 mm×250 mm, 5 μm; Agilent Technologies Inc.) by isocratic elution of MeCN and 0.1% HCO_2H in H_2O at a ratio of 50:50–70:30 (*v/v*), depending on the compound, at a flow rate of 0.8 mL min^{-1} at room temperature. Retention times for the compounds were between 3.0 and 6.0 min.

Bioavailability studies

Bioavailability studies were performed in rats as described earlier.[6] All animal experiments were carried out in accordance with Danish legislation according to the European Union regulation (directive 2010/63 of September 22, 2010), granted by the animal welfare committee, appointed by the Danish Ministry of Food, Agriculture and Fisheries—Danish Veterinary and Food Administration.

Locomotor activity studies

Locomotor activity was assessed using standard assay conditions and custom-made equipment. Briefly, 30 min after dosing with UCPH-102, mice were placed in a novel test cage, situated in a U-frame equipped with 2×8 infrared light sources and photocells. The light beams crossed the cage 1.8 cm above the bottom surface, which was covered by a thin layer of standard bedding material. Recording of a motility count required the interruption of adjacent light beams, thus avoiding counts induced by stationary movements of the mouse. All animal experiments were carried out in accordance with Danish legislation according to the European Union regulation (directive 2010/63 of September 22, 2010), granted by the animal welfare committee, appointed by the Danish Ministry of Food, Agriculture and Fisheries—Danish Veterinary and Food Administration.

In silico studies

All in silico studies were performed using the MOE 2013.08 software package (Molecular Operating Environment, Chemical Computing Group) using the built-in mmff94x force field and the GB/SA continuum solvation model. Calculation of the octanol/water partition coefficient (cLog*P*) was performed using the built-in function by the same name.

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Keywords: blood–brain barrier · EAAT1 · inhibitors · in vitro profiling · in vivo administration · structure–activity relationships

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