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New Insight into the Structure–Activity Relationships of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors UCPH-101 and UCPH-102

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In the present study, we made further investigations on the structure–activity requirements of the selective excitatory amino acid transporter 1 (EAAT1) inhibitor, 2-amino-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (UCPH-101), by exploring 15 different substituents (R^1) at the 7-position in combination with eight different substituents (R^2) at the 4-position. Among the 63 new analogues synthesized, we identified a number of compounds that unexpectedly displayed inhibitory activities at EAAT1 in light of understanding the structure–activity relationship (SAR) of this inhibitor class extracted from previous studies. Moreover, the nature of the R^1 and R^2 substituents were observed to contribute to the functional properties of the various analogues in additive and non-additive ways. Finally, separation of the four stereoisomers of analogue **14g** (2-amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene) was carried out, and in agreement with a study of a related scaffold, the *R* configuration at C4 was found to be mandatory for inhibitory activity, while both the C7 diastereomers were found to be active as EAAT1 inhibitors. A study of the stereochemical stability of the four pure stereoisomers **14g-A–D** showed that epimerization takes places at C7 via a ring-opening, C–C bond rotation, ring-closing mechanism.

Introduction

The five excitatory amino acid transporter (EAAT) subtypes EAAT1–5 mediate the synaptic reuptake of the major excitatory neurotransmitter glutamate in the central nervous system.[1–4] In the course of our search for subtype-selective inhibitors, a series of highly selective EAAT1 inhibitors with the general formula I (Figure 1) was discovered, with the most potent analogues being UCPH-101 and UCPH-102.[5–9] UCPH-101 has subsequently been applied as a pharmacological tool in several studies exploring the physiological functions and therapeutic potential in EAAT1 and other EAATs.[10–13]

Six key conclusions from our previously published structure–activity relationship (SAR) studies on this class of inhibitors are: 1) An aryl or heteroaryl group is required as the R^1 substituent. 2) A wide range of substituents is allowed at the 4-position (R^2), but not hydrogen. 3) Any chemical change to the 5-oxo group, the 3-CN group, the 2-NH_2 group, or the 1-oxy functionality leads to complete loss of EAAT1 inhibitory activity.[5,9,14] 4) The stereochemical requirement at C4 was shown to be the *R* configuration.[9] 5) An in silico study concluded that a C7 substituent occupies roughly the same region in space regardless of stereochemistry, which, together with the fact that inhibitory activity is present in both pairs of diastereomers (Figure 1), led to the conclusion that the absolute stereochemistry at C7 is less important.[5] 6) This class of inhibitors bind to the EAAT1 protein in a reversible manner.[15]

The present study arose from an unexpected observation made for the four analogues **2–5b** (see Table 3 below), which were synthesized in an attempt to optimize the pharmacokinetic properties of this class of inhibitors. Interestingly, the observed inhibitory properties of these four analogues contrasted with SAR conclusions previously drawn for this inhibitor class. This finding prompted us to explore the SAR of this compound series by a systematic approach. This was realized through an elaborate matrix SAR study, in which 15 and 8 substituents (R^1 and R^2, respectively) were varied systematically. Amongst the 74 compounds within this matrix of a theoretical total of 120 compounds that were characterized in the present study, several analogues displayed unexpected pharmacological properties and a pattern of cooperativity between the bulkiness and identities of the R^1 and R^2 substituents with respect to EAAT1 activity that would not necessarily have emerged from a conventional stepwise SAR exploration.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201500525: Overview of post-purification diastereomeric ratios for all analogues; HPLC chromatograms of **14g** and the four stereoisomers **14g-A–D**; ^{1}H NMR spectra of **14g-A** in [D_6]ethanol/D_2O (1:1) at $t = 0$, 24, 48, and 120 h.

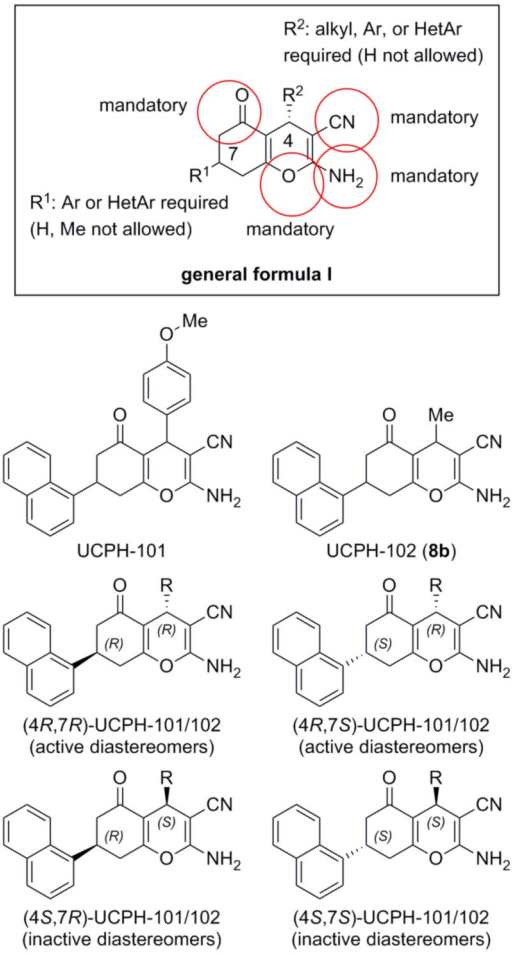
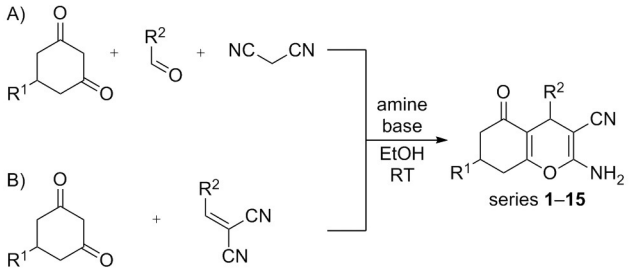


Figure 1. Parental skeleton of the EAAT1 inhibitor series (general formula I) and SAR conclusions based on previous studies, chemical structures of analogues UCPH-101 and UCPH-102 (compound **8b**) and their respective two pharmacologically active diastereomers.

Results and Discussion

Synthesis

All new analogues reported herein were synthesized by a two-step protocol (series **a**–**d**) or a one-pot three-component reaction (series **e**–**h**) as previously described for this compound class (Scheme 1).[5,14] The 1,3-diketone components were obtained from commercial suppliers (compound series **1**–**4**, **6**, **7**, and **12**–**15**), synthesized in due course (**16** for use in compound series **5**), or synthesized in accordance with published procedures (compound series **8** and **9**–**11**). In general, the crude product was a ~1:1 ratio of diastereomers as determined by ^{1}H NMR spectroscopy. However, this ratio would then vary according to the method of purification: while being retained by column chromatography, precipitation and/or crystallization gave diastereomeric ratios up to 1:9 (Table S1, Supporting Information). Analogues **12a** and **13a** proved unstable in aqueous media and were therefore not included in the study.



Scheme 1. General pathways for the synthesis of target compounds (series **1**–**15**) by either a A) one-pot or B) two-step reaction. See Table 3 for definitions of R^1 and R^2 groups.

Separation and pharmacological characterization of the four stereoisomers of 14g

Exhaustive attempts to separate the four stereoisomers of UCPH-101 and UCPH-102 were carried out, all of which failed due to insufficient chromatographic separation of stereoisomers or problems of solubility in the respective solvent systems. Several analogues were investigated, and finally analogue **14g** proved suitable both in terms of separation and solubility. The resolution was performed on a Daicel Chiralpac IF column to afford the four stereoisomers **14g-A**, **14g-B**, **14g-C**, and **14g-D** in high stereochemical purity (>98%). The four stereoisomers were subsequently characterized by NMR (Table 1). From these data we concluded that **14g-A**,**D** and **14g-B**,**C** are pairs of enantiomers. The four stereoisomers were characterized pharmacologically as inhibitors of EAAT1, and the results are summarized in Table 1. Whereas **14g-A**,**B** are inactive, stereoisomers **14g-C**,**D** were found to inhibit EAAT1-mediated transport with respective IC_{50} values of 1.6 and 1.9 μM.

Table 1. HPLC peak identity, ^{1}H NMR chemical shift of H4, and inhibitory potencies at EAAT1 of the four stereoisomers of **14g** (**14g-A**–**D**).[a]

Compd	HPLC ID	δ_{H4} [ppm][b]	EAAT1 IC_{50} [μM] (pIC_{50} ± SEM)
14g-A	Peak 1	4.36	>100 (<4.0)
14g-B	Peak 2	4.38	>100 (<4.0)
14g-C	Peak 3	4.38	1.6 (5.88 ± 0.15)
14g-D	Peak 4	4.36	1.9 (5.76 ± 0.09)

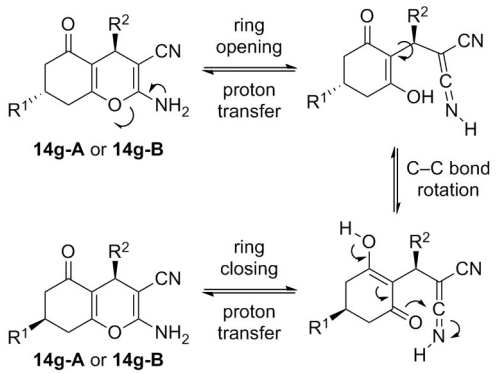
[a] All four analogues were inactive at EAAT2 and EAAT3 (IC_{50} > 100 μM). [b] Determined in [D_6]ethanol/D_2O (1:1). [c] Values are the mean ± SEM of n = 3 replicates.

The stereochemical stability was investigated for diastereomers **14g-A** and **14g-C** by dissolving the pure stereoisomer in a 1:1 mixture of ethanol/water with a few drops of dimethyl sulfoxide to aid solubility at room temperature. The solution was monitored by HPLC at 3, 24, and 48 h (see Figure S3, Supporting Information). For both **14g-A** and **14g-C** a time-dependent epimerization was observed to form **14g-B** and **14g-D**, respectively. While negligible epimerization was observed up to 3 h, as much as 18/21% was observed after 48 h. With respect to the method of pharmacological characterization,

stereochemical stability up to 3 h at room temperature is considered sufficient (see Experimental Section below).

To determine the mechanism of epimerization, an ^{1}H NMR experiment was performed: stereoisomer **14g-A** was dissolved in [D_6]ethanol/D_2O (1:1), and ^{1}H NMR spectra were collected at regular time intervals. The formation of **14g-B** was clearly evident with no incorporation of deuterium [at time *T* with percentage of **14g-D** in parentheses: $t = 0$ min (0%), 24 h (5%), 48 h (9%), and 120 h (18%)]. These findings, together with the fact that the stereochemistry at C4 is essential for inhibitory activity,[15] led us to conclude that epimerization takes place at C7 by a stepwise ring-opening, C4–C5 bond rotation, ring-closing mechanism, as outlined in Table 2. Unfortunately, we were unable to determine the respective *cis–trans* relationships of the four **14g** stereoisomers by X-ray crystallography. However, importantly, the two 7-epimers **14g-C** and **14g-D**, which are comprised in each of their respective diastereomer pairs, are equipotent EAAT1 inhibitors. Therefore, we conclude that the obtained variation in percent diastereomeric excess due to method of purification (Table S1, Supporting Information) is unlikely to influence the pharmacological properties of the different compounds.

Table 2. Stereochemical stability of **14g-A**,**C** in EtOH/H_2O/DMSO at RT (HPLC) and mechanism for epimerization at C7 shown for the **14g-A**↔**14g-B** pair based on ^{1}H NMR spectroscopic investigations in [D_6]ethanol/D_2O (1:1).



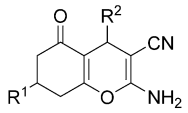
Compd	HPLC	3 h	24 h	48 h
14g-A	Peak 1	<2% **14g-B**	8% **14g-B**	18% **14g-B**
14g-B	Peak 2	–	–	–
14g-C	Peak 3	<1% **14g-D**	10% **14g-D**	21% **14g-D**
14g-D	Peak 4	–	–	–

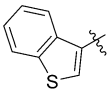
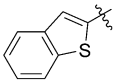
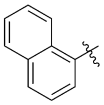
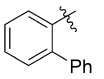
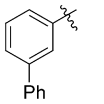
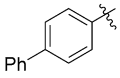
2D SAR matrix study

In our ongoing work to optimize the pharmacokinetic properties of this series of EAAT1 inhibitors to facilitate their use as pharmacological tools for in vivo studies, we attempted to decrease the lipophilicity of UCPH-102 by substituting the 1-naphthyl group with a heterocycle. Thus, we designed and synthesized furyl analogues **2b** and **3b** and thiophenyl analogues **4b** and **5b**. Their functional properties were characterized at EAAT1 in a conventional [^{3}H]D-Asp uptake assay, none of the four analogues displayed significant inhibitory activity at concentrations up to 300 μM (Table 3). This finding was quite surprising, as the corresponding 4-phenyl analogue **2e** was previously found to be a fairly potent EAAT1 inhibitor (IC_{50} = 7.3 μM).[14] To investigate this observation further, we synthesized the respective 4-phenyl analogues **3e**, **4e**, and **5e**, which, analogously to **2e**, were found to inhibit EAAT1 uptake, exhibiting IC_{50} values ranging from 3.8 to 24 μM at the transporter (Table 3). The trend was further supported by the fact that increasing the size of the R^2 substituent by the introduction of a *para*-biphenyl group in these furyl and thiophenyl analogues resulted in even more potent EAAT1 inhibitors (**2g**, **3g**, **4g**, and **5g**; Table 3, and light-grey boxes, Table 4). To investigate whether this correlation between the relative sizes of R^1 and R^2 groups and EAAT1 activity of the analogue is also present in the inverted situation—that is, smaller R^1 groups versus larger R^2 groups—we synthesized **13g**, which has a methyl group at the R^1 position and a *para*-biphenyl moiety at the R^2 position. According to our previous SAR observations, this analogue would be predicted to be inactive, but interestingly, **13g** potently inhibited EAAT1-mediated uptake (IC_{50} = 1.9 μM; Table 3, and white box in Table 4). Notably, the introduction of groups smaller than the *para*-biphenyl at the R^2 position led to completely inactive analogues (**13c–f**).

We found these observations highly interesting and therefore decided to expand this two-dimensional SAR study to a matrix study of the R^1 and R^2 substituents covering lipophilic groups of various size, which would neither engage in hydrogen bonding nor salt-bridge formation with the transporter protein. The substituents chosen were: R^1 = H (series **12**), Me (series **13**), *i*Pr (series **14**), *i*Bu (series **15**), and 11 different aryl or heteroaryl groups (series **1–11**); and R^2 = H (series **a**), Me (series **b**), *i*Pr (series **c**), *i*Bu (series **d**), and five different aryl groups (series **e–h**). The pharmacological activities of 74 analogues (63 new and 11 previously published) at EAAT1 in the [^{3}H]D-Asp uptake assay are summarized in Table 3 (with a color-coded representation of the data in Table 4). A number of interesting observations were made from this matrix study. Comparison of the pharmacological profile of the four analogues **8b**, **8g**, **14b**, and **14g** (Table 3, and dark-grey boxes in Table 4) shows that if both R^1 and R^2 are either small or large, inhibitory activity at EAAT1 is completely lost (compounds **14b** and **8g**), whereas both small–large size combinations lead to the most potent analogues of the series (**8b** and **14g**). The three analogues **12b**, **12e**, and **12g**, which hold no substituent at the R^1 position (R^1 = H), but have methyl, phenyl, or *para*-biphenyl as their respective R^2 substituent, are completely inactive. On the other hand, analogues with small (methyl) or bulky (isopropyl) aliphatic R^1 substituents (series **13** and **14**) displayed everything from complete inactivity to potent inhibition of EAAT1-mediated transport. In fact, there is a highly pronounced trend between R^2 substituent size and transporter activity (**13b–h**, **14b–h**). A general comparison of the two latter series revealed that analogues with a methyl group as the R^1 substituent require considerably larger substituents at the R^2 position in order to inhibit EAAT1 activity (**13b–h**) than analogues with isopropyl as the R^1 substituent (**14b–h**). Analogues with large aromatic R^1 substituents generally display potent in-

Table 3. Inhibitory potencies at EAAT1 in the [^{3}H]D-Asp uptake assay.[a]

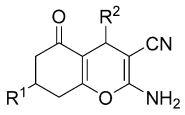


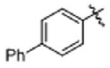
Series	R^1	R^2 = H (a)	Me (b)	*i*Pr (c)	*i*Bu (d)	Ph (e)	2-Naph (f)	Ph-*p*Ph (g)	Ph-*p*Naph (h)
12	H	–[b]	>300 [<3.5]	–	–	>300 [<3.5]	–	>100 [<4.0]	–
13	Me	–[b]	>300 [<3.5]	>300 [<3.5]	~300 [~3.5]	>100 [<4.0]	>100 [<4.0]	1.9 [5.73±0.07]	1.3 [5.88±0.03]
14	*i*Pr	–	>300 [<3.5]	~30 [~4.5]	~100 [~4.0]	11 [4.95±0.03]	1.6 [5.80±0.14]	0.64 [6.20±0.08]	4.2 [5.37±0.07]
15	*i*Bu	–	–	–	–	11 [4.96±0.14]	–	3.2 [5.48±0.07]	–
1		>300[16]	9.2[16]	6.3 [5.19±0.04]	~100 [~4.0]	3.9[16]	3.6[14]	2.1[14]	~30 [~4.5]
2		>300 [<3.5]	>300 [<3.5]	15 [4.83±0.09]	~100 [~4.0]	7.3[14]	>100 [<4.0]	1.2 [5.96±0.11]	5.3 [5.28±0.12]
3		>300 [<3.5]	>300 [<3.5]	–	–	24 [4.34±0.05]	–	1.9 [5.73±0.07]	–
4		>300 [<3.5]	>300 [<3.5]	–	–	10 [5.04±0.09]	–	2.0 [5.69±0.11]	–
5		>300 [<3.5]	~300 [~3.5]	–	–	3.8 [5.42±0.06]	–	2.1 [5.75±0.18]	–
6		21 [4.73±0.15]	2.6 [5.68±0.22]	–	–	5.6 [6.33±0.19]	–	>100 [<4.0]	–
7		>300 [<3.5]	>300 [<3.5]	–	–	2.3 [5.71±0.17]	–	>30 [<4.5]	–
8		13 [4.91±0.1]	0.42[16]	0.6 [6.82±0.08]	1.0 [6.00±0.13]	0.93 [6.11±0.20]	0.62 [6.21±0.12]	>300 [<3.5]	–
9		12 [5.00±0.23]	0.87[13]	–	–	1.2[13]	–	>300 [<3.5]	–
10		>300 [<3.5]	~10[13]	–	–	>300 [<3.5]	–	>300 [<3.5]	–
11		>300 [<3.5]	>300[13]	–	–	>300 [<3.5]	–	>100 [<4.0]	–

[a] All analogues were characterized as mixtures of *cis*/*trans* diastereomers. IC_{50} values are given in μM, with pIC_{50} ± SEM values in square brackets (n = 3–5). None of the analogues displayed significant inhibitory activity at EAAT2 and EAAT3 when tested at concentrations up to 30 μM (IC_{50} > 30 μM), 100 μM (IC_{50} > 100 μM), or 300 μM (IC_{50} > 300 μM) (depending on the solubility of the compounds in the assay buffer). [b] Analogues **12a** and **13a** proved unstable in aqueous buffer solution and are therefore not suited for pharmacological characterization.

hibition, except those bearing either H or the largest substituent (*para*-biphenyl) at the R^2 position, for which EAAT1 inhibitory activity is detrimentally decreased (**6a**,**b**,**e**,**g**, **7a**,**b**,**e**,**g**, **8a**–**g**, and **9a**,**b**,**e**,**g**), thus defining the upper and lower limits for R^2 substituent size in analogues with bulky aromatic R^1 substituents. This overall trend was also true for the R^1 phenyl analogues **1a**–**h**, but the presence of a smaller aromatic substituent at the R^1 position made it possible for the molecule to accommodate the large *para*-biphenyl group as R^2 substituent (**1g**) and retain EAAT1 inhibitory activity, whereas introduction of an even larger aromatic group (*para*-naphthylphenyl, **1h**) dramatically decreased transporter activity (Table 3). The inactivity or weak EAAT1 inhibitory activity displayed by analogues bearing *meta*- or *para*-biphenyl as R^1 substituents and H,

Table 4. Sorting of R^1 groups according to bulk, and color representation of the inhibitory potencies of the analogues at EAAT1.[a]



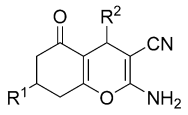
Series	R¹	R² H (a)	Me (b)	*i*Pr (c)	*i*Bu (d)	Ph (e)	2-Naph (f)	Ph-*p*Ph (g)	Ph-*o*Naph (h)
12	H	—	●	—	—	●	—	●	—
13	Me	—	●	●	●	●	●	●	●
14	*i*Pr	—	●	●	●	●	●	●	●
15	*i*Bu	—	—	—	—	●	—	●	—
1		●	●	●	●	●	●	●	●
2		●	●	●	●	●	●	●	●
3		●	●	—	—	●	—	●	—
4		●	●	—	—	●	—	●	—
5		●	●	—	—	●	—	●	—
6		●	●	—	—	●	—	●	—
7		●	●	—	—	●	—	●	—
8		●	●	●	●	●	●	●	--
9		●	●	—	—	●	—	●	—
10		●	●	—	—	●	—	●	—
11		●	●	—	—	●	—	●	—

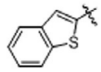
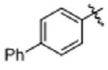
[a] ●: IC_{50} <1 μM, ●: IC_{50} 1–5 μM, ●: IC_{50} 5–25 μM, ●: IC_{50} >30, >100, or >300 μM; the color scheme is based on the specific values listed in Table 1.

methyl, phenyl, or *para*-biphenyl as R^2 substituents (**10a**,**b**,**e**,**g** and **11a**,**b**,**e**,**g**) deviate from this general pattern, which is also shared by the R^1 *ortho*-biphenyl analogues. This can most likely be ascribed to a steric clash between these biphenyl groups with the transporter protein, and it may suggest that *meta*- or *para*-biphenyl groups in these analogues project into regions of the EAAT1 binding pocket that an *ortho*-biphenyl R^1 substituent does not.

To address the influence of lipophilicity of the analogues on the inhibitory potency at EAAT1, cLog $P_{(o/w)}$ values were calcu-

Table 5. Lipophilicity (cLog $P_{(o/w)}$) of series **1**–**15** in numbers and grouped by background color.[a]

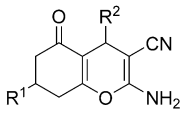


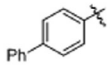
Series	R^1	R^2 H (a)	Me (b)	*i*Pr (c)	*i*Bu (d)	Ph (e)	2-Naph (f)	Ph-*p*Ph (g)	Ph-*p*Naph (h)
12	H	–0.2	0.2	1.0	1.4	1.5	2.7	3.4	4.7
13	Me	0.2	0.6	1.4	1.8	1.8	3.1	3.8	5.0
14	*i*Pr	2.0	1.4	2.2	2.6	2.6	3.9	4.6	5.8
15	*i*Bu	2.9	1.8	2.6	3.0	3.1	4.3	5.0	6.3
1		1.1	1.5	2.3	2.7	2.8	4.0	4.7	5.9
2		–0.2	0.2	1.0	1.5	1.5	2.7	3.4	4.7
3		–0.2	0.2	1.0	1.5	1.5	2.7	3.4	4.7
4		0.7	1.0	1.8	2.3	2.3	3.6	4.3	5.5
5		0.7	1.0	1.8	2.3	2.3	3.6	4.3	5.5
6		2.1	2.5	3.3	3.7	3.8	5.0	5.7	6.9
7		2.1	2.5	3.3	3.7	3.8	5.0	5.7	6.9
8		2.3	2.7	3.5	4.0	4.0	5.2	5.9	7.2
9		3.1	3.5	4.3	4.7	4.7	6.0	6.7	7.9
10		3.1	3.5	4.3	4.7	4.7	6.0	6.7	7.9
11		3.1	3.5	4.3	4.7	4.7	6.0	6.7	7.9

[a] ■: ≤1, ■: 1≤3, ■: 3<5, ■: ≥5.

lated (Tables 5 and 6). Comparison of the lipophilicities of the analogues with their respective EAAT1 activities did not reveal a uniform and conventionally assumed correlation. For example, analogues of series **14** (R^1 = *i*Pr, R^2 = Me to 2-Naph, **14b**–**g**) displayed a substantial correlation between lipophilicity and inhibitory potency. However, the analogues of series **8** (R^2 = Me to 2-Naph, **8b**–**g**) all exhibited high inhibitory potencies at EAAT1 regardless of their lipophilicities, and analogues from series **13** (R^1 = Me, R^2 = Me to 2-Naph, **13b**–**g**), characterized by the same lipophilicity range, were all either inactive or low-po-

Table 6. Lipophilicity (cLog $P_{(o/w)}$) as background color,[a] and inhibition at EAAT1 as dot color.[b]



Series	R^1	R^2 H (a)	Me (b)	*i*Pr (c)	*i*Bu (d)	Ph (e)	2-Naph (f)	Ph-*p*Ph (g)	Ph-*o*Naph (h)
12	H	–	●	–	–	●	–	●	–
13	Me	–	●	●	●	●	●	●	●
14	*i*Pr	–	●	●	●	●	●	●	●
15	*i*Bu	–	–	–	–	●	–	●	–
1		●	●	●	●	●	●	●	●
2		●	●	●	●	●	●	●	●
3		●	●	–	–	●	–	●	–
4		●	●	–	–	●	–	●	–
5		●	●	–	–	●	–	●	–
6		●	●	–	–	●	–	●	–
7		●	●	–	–	●	–	●	–
8		●	●	●	●	●	●	●	–
9		●	●	–	–	●	–	●	–
10		●	●	–	–	●	–	●	–
11		●	●	–	–	●	–	●	–

[a] ■: ≤1, ■: 1≤3, ■: 3<5, ■: ≥5. [b] ●: IC_{50} <1 μM, ●: IC_{50} 1–5 μM, ●: IC_{50} 5–25 μM, ●: IC_{50} >30, >100, or >300 μM. The color schemes are based on the specific values listed in Table 1.

tency inhibitors. Moreover, whereas the similar log*P* and IC_{50} values of analogues **9b** and **14g** could suggest that lipophilicity plays a major role in EAAT1 inhibitory activity, analogues **8b** and **13f** are examples of inversely correlated lipophilicity and potency. Overall, this analysis leads to the conclusion that lipophilicity alone does not determine the EAAT1 inhibitory activity of this inhibitor class.

Physical form of inhibitors when bound to EAAT1

As demonstrated for analogue **14g** (Table 2), this class of inhibitors seems to equilibrate between closed-ring and open-ring forms in aqueous solution. Combining the cooperative nature of the R^1 and R^2 substituents as a key determinant for EAAT1 activity with the previously reported observation that chemical changes to the 1-oxy, 2-NH_2, 3-CN, and 5-oxo functionalities results in loss of inhibitory activity, we speculate that the ring-open form of the inhibitor could play a key role in its inhibition of EAAT1-mediated glutamate uptake. Because the open-ring form is far more flexible than the closed-ring form, the corporative nature of the R^1 and R^2 groups in determining the EAAT1 inhibitory activity might be rooted in this mechanism. Furthermore, the stereochemistry at C7 is abolished in the open form due to the symmetry plane of the diketone ring (Figure 2), which is in agreement with the observation that the

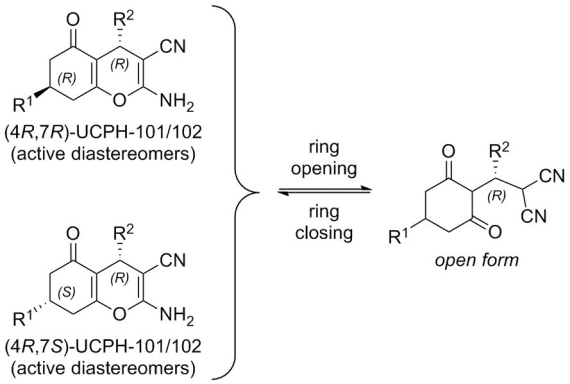


Figure 2. Suggested open form of EAAT1 inhibitors (see Table 2 for mechanism of epimerization at C7).

absolute stereochemistry at C7 has little influence on EAAT1 inhibitory activity (Table 1). On the other hand, it is also a distinct possibility that EAAT1 binds the closed form of the inhibitor and that the highly diverse EAAT1 activities exhibited by the 74 analogues with different R^1/R^2 combinations thus arise from their respective capacities to fit into the binding pocket in the transporter protein. In the absence of solid experimental evidence for either of the two scenarios, we will refrain from drawing conclusions as to which physical form of the inhibitor (Figure 2) actually binds to the EAAT1 protein.

Conclusions

From the present SAR study we conclude that the inhibitory activity of this class of chromene-based EAAT1 inhibitors is related to the relative sizes and identities of R^1 and R^2 substituents in a cooperative manner. We find it truly intriguing that this finely tuned interplay between the two substituents is a key determinant in EAAT1 inhibitory activity of this compound series. The four stereoisomers of the potent analogue **14g** (designated **14g-A**, **14g-B**, **14g-C**, and **14g-D**) were separated, and it was unequivocally confirmed that the stereochemistry at C7 is not vital for EAAT1 inhibitory activity. Moreover, an NMR spectroscopic study revealed time-dependent epimerization at C7 via a ring-opening, C–C bond rotation, ring-closing mechanism. As discussed above, this raises the question as to whether these inhibitors bind EAAT1 in their ring-closed or ring-open forms.

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 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 was carried out using Merck silica gel 60A (35–70 μm). ^{1}H NMR spectra were recorded on a 300, 400, or 600 MHz Bruker Avance instrument, and ^{13}C NMR spectra on a 75, 100, or 150 MHz Bruker Avance. HPLC was performed using a Dionex UltiMate 3000 pump and photodiode array detector (λ 200 and 210 nm) 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.

Preparative HPLC was carried out on an Agilent Prep HPLC system with an Agilent 1100 series pump, an Agilent 1200 series diode array, a multiple wavelength detector (G1365B), and an Agilent Prep HT High-Performance Preparative Cartridge Column (Zorbax, 300SB-C_{18} Prep HT, 21.2×250 mm, 7 μm). A Daicel Chiralpac IF column (10 mm Ø, 25 cm) was used for preparative separation of the four stereoisomers of **14g** (**14g-A**–**D**). 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 with an MPA 100 Optimelt automatic melting point system and are uncorrected. Compounds were dried under high vacuum using a Holm & Halby Heto LyoPro 6000 freeze drier. The purity of compounds submitted for pharmacological characterization was determined by elemental analysis and/or HPLC to be >95%.

2-Amino-7-(furan-2-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2a). 5-(Furan-2-yl)cyclohexane-1,3-dione (0.135 g, 0.757 mmol) was suspended in CH_2Cl_2 (10 mL). Malononitrile (2.50 g, 37.8 mmol) was added followed by *N*-methylmorpholine (84 μL, 0.757 mmol). Then formaldehyde (37 wt% in H_2O; 57 μL, 0.757 mmol) was added and the mixture was stirred at RT for 4.5 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched by addition of 1 M NaOH (40 mL). The two phases were separated, and the organic phase was washed further with 1 M NaOH (3× 40 mL), dried using anhydrous $MgSO_4$, filtered, and evaporated to give a yellow solid (0.066 g). The crude product was triturated with Et_2O to give the title compound (0.043 g, 22%) as an orange solid. R_f (EtOAc/MeOH/AcOH, 100:10:1) 0.92; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 7.59 (dd, 1H, J = 0.7 Hz, J = 1.7 Hz), 6.93 (bs, 2H), 6.40 (dd, 1H, J = 1.9 Hz, J = 3.2 Hz), 6.20 (ddd, 1H, J = 0.9 Hz, J = 0.9 Hz, J = 3.2 Hz), 3.62–3.53 (m, 1H), 2.84–2.72 (m, 4H), 2.70 (dd, 1H, J = 4.8 Hz, J = 16.3 Hz), 2.59 (dd, 1H, J = 10.3 Hz, J = 16.3 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 195.1, 162.6, 159.0, 155.6, 141.9, 120.3, 110.4, 109.8, 105.1, 50.3, 40.3, 31.1, 30.8, 18.7; LC–MS: two

peaks in UV ($\lambda = 254$ nm): Peak 1: $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_3$ 257.09, found 257.1, Peak 2: $[M+H_2O+H]^+$ calcd for $C_{14}H_{15}N_2O_4$ 275.10, found 275.2; Anal. calcd for $C_{14}H_{12}N_2O_3$: C 65.62, H 4.72, N 10.93, found: C 65.53, H 4.75, N 11.02; mp: 172.0–173.6 °C (1 °C min^{-1}, decomp.).

2-Amino-7-(furan-3-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (3 a). 5-(Furan-3-yl)cyclohexane-1,3-dione (0.136 g, 0.757 mmol) was suspended in CH_2Cl_2 (10 mL). Malononitrile (2.50 g, 37.8 mmol) was added followed by *N*-methylmorpholine (84 μL, 0.757 mmol). Then formaldehyde (37 wt% in H_2O; 57 μL, 0.757 mmol) was added and the mixture was stirred at RT for 5 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched by addition of 1 M NaOH (40 mL). The phases were separated and the organic phase was washed further with 1 M NaOH (3×40 mL), dried using anhydrous $MgSO_4$, filtered and evaporated to give a yellow solid (0.065 g). The crude product was triturated with Et_2O to give the title compound (0.043 g, 13%) as an orange solid. R_f (EtOAc/MeOH/AcOH, 100:10:1) 0.92; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.60$ (dd, 1H, $J = 1.7$ Hz, $J = 1.7$ Hz), 7.53 (ddd, 1H, $J = 0.9$ Hz, $J = 0.9$ Hz, $J = 1.8$ Hz), 6.91 (bs, 2H), 6.54 (dd, 1H, $J = 0.9$ Hz, $J = 1.7$ Hz), 3.37–3.27 (m, 1H), 2.77 (s, 2H), 2.72–2.65 (m, 2H), 2.62 (dd, 1H, $J = 4.3$ Hz, $J = 16.3$ Hz), 2.51 ppm (dd, 1H, $J = 11.5$ Hz, $J = 16.3$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 195.8$, 163.3, 159.0, 143.4, 138.7, 127.1, 120.3, 109.8, 109.7, 50.3, 42.3, 32.7, 28.8, 18.7 ppm; LC–MS: two peaks in UV ($\lambda = 254$ nm): Peak 1: $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_3$ 257.09, found 257.1, Peak 2: $[M+H_2O+H]^+$ calcd for $C_{14}H_{15}N_2O_4$ 275.10, found 275.1; Anal. calcd for $C_{14}H_{12}N_2O_3$: C 65.62, H 4.72, N 10.93, found: C 65.58, H 4.75, N 10.90; mp: 171.3–172.1 °C (1 °C min^{-1}, decomp.).

2-Amino-7-(thiophen-3-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (4 a). 5-(Thiophen-3-yl)cyclohexane-1,3-dione (0.056 g, 0.288 mmol) was suspended in CH_2Cl_2 (3.8 mL). Malononitrile (0.952 g, 14.4 mmol) was added followed by *N*-methylmorpholine (32 μL, 0.288 mmol). Then formaldehyde (37 wt% in H_2O; 22 μL, 0.288 mmol) was added and the mixture was stirred at RT for 4 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched by addition of 1 M NaOH (40 mL). The phases were separated and the organic phase was washed further with 1 M NaOH (3×40 mL), dried using anhydrous $MgSO_4$, filtered and evaporated to give the title compound (0.019 g, 24%) as light-yellow solid. R_f (EtOAc/MeOH/AcOH, 100:10:1) 0.91; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.51$ (dd, 1H, $J = 3.0$ Hz, $J = 5.0$ Hz), 7.30 (ddd, 1H, $J = 1.2$ Hz, $J = 1.2$ Hz, $J = 3.0$ Hz), 7.16 (dd, 1H, $J = 1.3$ Hz, $J = 5.0$ Hz), 6.91 (bs, 2H), 3.55–3.45 (m, 1H), 2.83–2.57 ppm (m, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 195.8$, 163.4, 159.0, 143.8, 127.0, 126.4, 120.5, 120.3, 109.8, 50.3, 42.9, 33.2, 33.1, 18.7 ppm; LC–MS: two peaks in UV ($\lambda = 254$ nm): Peak 1: $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_2S$ 273.07, found 273.1, Peak 2: $[M+H_2O+H]^+$ calcd for $C_{14}H_{15}N_2O_3S$ 291.08, found 291.1; Anal. calcd for $C_{14}H_{12}N_2O_2S$: C 61.75, H 4.44, N 10.29, found: C 61.65, H 4.45, N 10.26; mp: 180.5–181.9 °C (decomp.).

2-Amino-5-oxo-7-(thiophen-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (5 a). 5-(Thiophen-2-yl)cyclohexane-1,3-dione (**16**; 0.147 g, 0.757 mmol) was suspended in CH_2Cl_2 (10 mL). Malononitrile (2.50 g, 37.8 mmol) was added followed by *N*-methylmorpholine (84 μL, 0.757 mmol). Then formaldehyde (37 wt% in H_2O; 57 μL, 0.757 mmol) was added and the mixture was stirred at RT for 4 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched by addition of 1 M NaOH (40 mL). The phases were separated and the organic phase was washed further with 1 M NaOH (3×40 mL), dried using anhydrous $MgSO_4$, filtered and evaporated to give a yellow solid (0.066 g). The crude product was triturated with Et_2O to give the title compound (0.044 g, 21%) as yellow solid. R_f (EtOAc/MeOH/AcOH, 100:10:1) 0.92; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.41$–7.38 (m, 1H), 7.00–6.97 (m, 2H), 6.93 (bs, 2H), 3.81–3.71 (m, 1H), 2.86–2.61 ppm (m, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 195.1$, 162.8, 159.0, 146.2, 127.0, 124.1, 124.0, 120.3, 110.0, 50.3, 43.7, 34.3, 33.0, 18.7 ppm; LC–MS conditions: two peaks in UV ($\lambda = 254$ nm): Peak 1: $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_2S$ 273.07, found 273.1, Peak 2: $[M+H_2O+H]^+$ calcd for $C_{14}H_{15}N_2O_3S$ 291.08, found 291.1; Anal. calcd for $C_{14}H_{12}N_2O_2S$: C 61.75, H 4.44, N 10.29, found: C 61.65, H 4.42, N 10.31; mp: 175.6–176.5 °C (decomp.).

2-Amino-7-(benzo[*b*]thiophen-3-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (6 a). 5-(Benzo[*b*]thiophen-3-yl)cyclohexane-1,3-dione (50 mg, 0.21 mmol) was suspended in CH_2Cl_2 (3.5 mL) at RT. Malononitrile (676 mg, 10.23 mmol) was added followed by *N*-methylmorpholine (23 μL, 0.21 mmol). Then formaldehyde (37 wt% in H_2O (15 μL, 0.21 mmol) was added and the reaction mixture was stirred at RT for 18 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched with 1 M NaOH (40 mL). The two phases were separated and the organic phase was washed further with 1 M NaOH (3×40 mL) and dried over anhydrous $MgSO_4$. After concentration in vacuo the crude product was purified by column chromatography on silica gel to afford the title compound as a white solid (11 mg, 34 μmol, 17%): R_f (EtOAc/heptane 3:2) 0.40; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.90$ (dd, $J = 7.2$, 1.6 Hz, 1H), 7.75 (dd, $J = 7.2$, 1.6 Hz, 1H), 7.45–7.37 (m, 2H), 7.18 (s, 1H), 4.51 (s, 2H), 3.90–3.82 (m, 1H), 3.03 (s, 2H), 2.98–2.71 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 195.9$, 162.7, 158.0, 140.8, 137.5, 136.7, 124.8, 124.3, 123.3, 121.4, 121.2, 119.1, 111.2, 56.5, 42.6, 33.3, 32.4, 18.6 ppm; LC–MS: $[M+H]^+$, calcd for $C_{18}H_{14}N_2O_2S$ 323.38, found 323.2; HPLC: purity ($\lambda = 254$ nm) >95%; mp: 208–210 °C (decomp.).

2-Amino-7-(benzo[*b*]thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (7 a). 5-(Benzo[*b*]thiophen-2-yl)cyclohexane-1,3-dione (50 mg, 0.21 mmol) was suspended in CH_2Cl_2 (3.5 mL) at RT. Malononitrile (672 mg, 10.3 mmol) was added followed by *N*-methylmorpholine (23 μL, 0.21 mmol). Then formaldehyde (37 wt% in H_2O (15 μL, 0.21 mmol) was added and the reaction mixture was stirred at RT for 22 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched with 1 M NaOH (40 mL). The two phases were separated and the organic phase was washed further with 1 M NaOH (3×40 mL), dried over anhydrous $MgSO_4$, filtered and evaporated to give the title compound as a yellow solid (20 mg, 62 μmol, 29%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.80$ (dd, $J = 7.2$, 1.6 Hz, 1H), 7.72 (dd, $J = 7.2$, 1.6 Hz, 1H), 7.39–7.31 (m, 2H), 7.11 (s, 1H), 4.49 (s, 2H), 3.83–3.75 (m, 1H), 3.00 (s, 2H), 2.93–2.72 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 194.9$, 162.0, 157.9, 146.1, 139.5, 138.8, 124.6, 124.5, 123.4, 122.3, 120.5, 119.0, 111.5, 56.6, 43.8, 34.8, 34.7, 18.6 ppm; LC–MS: $[M+H]^+$ calcd for $C_{18}H_{14}N_2O_2S$, 323.38 found 323.21; HPLC: purity ($\lambda = 254$ nm) >95%; mp: 190–192 °C (decomp.).

2-Amino-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (8 a). 5-(naphthalen-1-yl)cyclohexane-1,3-dione (0.110 g, 0.462 mmol) was suspended in CH_2Cl_2 (6 mL). Malononitrile (1.525 g, 23.0 mmol) was added followed by *N*-methylmorpholine (51 μL, 0.462 mmol). Then formaldehyde (37 wt% in H_2O; 35 μL, 0.462 mmol) was added and the mixture was stirred at RT for 5 h. The mixture was diluted with CH_2Cl_2 (10 mL) and then quenched by addition of 1 M NaOH (10 mL). The two phases were separated and the organic phase was washed further with 1 M NaOH (2×10 mL), dried using anhydrous $MgSO_4$, filtered and evaporated to give a yellow solid (0.087 g). The crude product was recrystallized from absolute EtOH to give the title compound

(0.033 g, 23%) as a light-yellow solid. R_f (EtOAc/heptane 1:1) 0.32; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.24 (d, 1H, J = 7.6 Hz), 7.96 (dd, 1H, J = 2.1 Hz, J = 7.5 Hz), 7.85 (dd, 1H, J = 2.5 Hz, J = 6.6 Hz), 7.60–7.48 (m, 4H), 6.93 (bs, 2H), 4.39–4.30 (m, 1H), 2.98 (dd, 1H, J = 10.6 Hz, J = 17.0 Hz), 2.88 (dd, 1H, J = 12.3 Hz, J = 16.2 Hz), 2.86 (s, 2H), 2.72 (dd, 1H, J = 4.2 Hz, J = 17.0 Hz), 2.63 ppm (dd, 1H, J = 3.7 Hz, 16.2 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 196.6, 164.2, 159.6, 139.0, 134.0, 131.1, 129.3, 127.8, 126.9, 126.2, 126.1, 123.7, 123.5, 120.9, 110.1, 50.9, 43.4, 33.9, 33.4, 19.4 ppm; LC–MS: two peaks in UV (λ = 254 nm): Peak 1: $[M+H]^+$ calcd for $C_{20}H_{17}N_2O_2$ 317.13, found 317.1, Peak 2: $[M+H_3O]^+$ calcd for $C_{20}H_{19}N_2O_3$ 335.14, found 335.1; Anal. calcd for $C_{20}H_{16}N_2O_2 \cdot 0.1\,C_2H_6O$: C 75.59, H 5.21, N 8.73, found: C 75.52, H 5.13, N 8.67; mp: 194.5–196.9 °C (decomp.).

7-([1,1′-Biphenyl]-2-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (9a). 5-(1,1′-Biphenyl)cyclohexane-1,3-dione (0.200 g, 0.757 mmol) was suspended in CH_2Cl_2 (10 mL). Malononitrile (2.498 g, 37.8 mmol) was added followed by *N*-methylmorpholine (84 μL, 0.757 mmol). Then formaldehyde (37 wt% in H_2O; 57 μL, 0.757 mmol) was added and the mixture was stirred at RT for 4 h. The mixture was diluted with CH_2Cl_2 (40 mL) and then quenched by addition of 1 M NaOH (40 mL). The two phases were separated and the organic phase was washed further with 1 M NaOH (2×40 mL), dried using anhydrous $MgSO_4$, filtered and evaporated to give a yellow solid (0.157 g). The crude product was recrystallized from absolute EtOH to give the title compound (0.061 g, 24%) as a white solid. R_f (EtOAc/heptane, 1:1) 0.42; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.61 (d, 1H, J = 7.8 Hz), 7.47–7.28 (m, 7H), 7.17 (dd, 1H, J = 1.5 Hz, J = 7.8 Hz), 6.87 (bs, 2H), 3.48–3.37 (m, 1H), 2.93 (dd, 1H, J = 11.5 Hz, J = 17.3 Hz), 2.76 (dd, 1H, J = 13.6 Hz, J = 16.3 Hz), 2.71 (s, 2H), 2.39–2.29 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.6, 163.4, 158.9, 141.3, 140.7, 139.7, 130.1, 128.9, 128.4, 127.9, 127.2, 126.6, 126.4, 120.2, 109.5, 50.4, 43.2, 34.2, 33.4, 18.6 ppm; LC–MS: two peaks in UV spectrum (λ = 254 nm): Peak 1: $[M+H]^+$ calcd for $C_{22}H_{19}N_2O_2$ 343.14, found 343.2, Peak 2: $[M+H_2O+H]^+$ calcd for $C_{22}H_{21}N_2O_3$ 361.16, found 361.2; Anal. calcd for $C_{22}H_{18}N_2O_2$: C 77.17, H 5.30, N 8.18, found: C 77.22, H 5.30, N 8.31; mp: 194.3–197.8 °C (decomp.).

7-([1,1′-Biphenyl]-3-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (10a). To a suspension of dione (53 mg, 0.20 mmol) in CH_2Cl_2 (2.6 mL) was added first malononitrile (569 mg, 10.0 mmol) followed by *N*-methylmorpholine (22 μL, 0.20 mmol). To the resulting solution was finally added formaldehyde (37% aqueous solution, 17 μL, 0.2 mmol) and the reaction mixture was allowed to stir at RT for 16 h. The resulting mixture was diluted with CH_2Cl_2 (30 mL) and the reaction was then quenched with 1 M aqueous NaOH (10 mL). The two phases were separated and the organic phase was further washed with 1 M aqueous NaOH (3×10 mL), dried over $MgSO_4$, filtered and evaporated to give a yellow crude. Purification by column chromatography (5% EtOAc in CH_2Cl_2) gave the title compound as a pale-yellow solid (24 mg, 35%). R_f (EtOAc/CH_2Cl_2, 5:95) 0.27; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57 (d, J = 7.24 Hz, 2H), 7.52 (d, J = 7.82 Hz, 1H), 7.45 (q, J = 7.40 Hz, 4H), 7.37 (t, J = 7.31 Hz, 1H), 7.22 (d, J = 7.68 Hz, 1H), 4.51 (s, 2H), 3.54–3.43 (m, 1H), 3.04–2.96 (m, 2H), 2.86–2.65 ppm (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 196.2, 163.1, 158.2, 142.5, 142.2, 140.9, 129.5, 129.0, 127.7, 127.3, 126.4, 125.7, 125.5, 119.4, 111.3, 56.4, 43.7, 38.8, 34.6, 18.8 ppm; HPLC: >98%, t_R = 1.20 min, reversed phase, H_2O/MeCN/TFA, gradient run, 5 mL min^{-1}; LC–MS: $[M+H]^+$ (decomp.); mp: 163.3–165.2 °C (decomp.).

7-([1,1′-Biphenyl]-3-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (11a). To a suspension of dione (100 mg, 0.378 mmol) in CH_2Cl_2 (4.9 mL) was added first malononitrile (1.24 g, 18.9 mmol) followed by *N*-methylmorpholine (42 μL, 0.38 mmol). To the resulting suspension was finally added formaldehyde (37% aqueous solution, 31 μL, 0.38 mmol) and the reaction mixture was allowed to stir at RT for 16 h. The solvent was then evaporated and the resulting residue was dissolved in the minimum amount of CH_2Cl_2 and purified directly by gradient column chromatography (0–5% EtOAc in CH_2Cl_2). Further purification was performed by recrystallization in CH_2Cl_2/MeOH (1:1) giving the title compound as yellow crystals (41 mg, 32%). R_f (EtOAc/CH_2Cl_2 8:92) 0.34; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.70–7.60 (m, 4H), 7.45 (ddq, J = 2.23, 6.50, 8.71 Hz, 4H), 7.39–7.32 (m, 1H), 6.94 (s, 2H), 3.49 (ddt, J = 4.41, 10.65, 12.43 Hz, 1H), 2.90 (ddt, J = 2.15, 10.84, 17.06 Hz, 1H), 2.84–2.70 (m, 3H), 2.58 ppm (ddd, J = 3.32, 16.53, 29.08 Hz, 2H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 195.9, 163.6, 159.1, 142.0, 139.8, 138.7, 128.9, 128.8, 127.6, 127.5, 127.3, 127.0, 126.8, 126.6, 126.6, 120.3, 109.8, 50.4, 43.0, 37.3, 33.5, 18.8 ppm; HPLC: >96%, t_R = 1.22 min, reversed phase, H_2O/MeCN/TFA, gradient run, 5 mL min^{-1}; LC–MS: $[M+H]^+$ (decomp.); mp: 200.5–204.3 °C (decomp.).

2-Amino-7-(furan-2-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2b). Synthesized in accordance with procedure published for **9b**. Stirring at RT for four days gave the title compound (0.091 g, 60%) as a yellow solid in a 2:1 mixture of diastereomers. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.58 (dd, 0.7H, J = 0.8 Hz, J = 1.8 Hz), 7.56 (d, 0.3H, J = 0.8 Hz, J = 1.7 Hz), 6.88 (bs, 1.3H), 6.87 (bs, 0.7H), 6.39 (dd, 0.7H, J = 1.9 Hz, J = 3.2 Hz), 6.38 (dd, 0.3H, J = 1.9 Hz, J = 3.2 Hz), 6.22–6.19 (m, 0.7H), 6.15–6.13 (m, 0.3H), 3.62–3.43 (m, 1H), 3.14–3.07 (m, 1H), 2.89–2.51 (m, 4H), 1.10 (d, 2H, J = 6.5 Hz), 0.99 ppm (d, 1H, J = 6.5 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 194.8, 162.4, 161.6, 158.7, 155.6, 141.93, 141.86, 119.8, 115.0, 110.4, 105.2, 105.1, 57.7, 57.6, 40.8, 40.5, 31.1, 31.0, 30.8, 24.7, 22.8, 22.4 ppm; LC–MS: $[M+H]^+$ calcd for $C_{15}H_{15}N_2O_3$, 271.10; found 271.2; HPLC: >97%, t_R = 1.50 min, reversed phase, H_2O/MeCN/TFA, gradient run, 5 mL min^{-1}; mp: 179.9–180.3 °C (1 °C min^{-1}).

2-Amino-7-(furan-3-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (3b). 5-(Furan-3-yl)cyclohexane-1,3-dione (0.178 g, 1.00 mmol) was dissolved in absolute EtOH (25 mL). The mixture was cooled to 0 °C and malononitrile (0.066 g, 1.00 mmol) was added followed by acetaldehyde (176 μL, 3.00 mmol). After 15 min, *N*-methylmorpholine (10 μL, 0.09 mmol) was added and the mixture was stirred at RT for 2.5 h. The mixture was evaporated to dryness and the residue was recrystallized from absolute EtOH to give the title compound (0.149 g, 54%) as an off-white crystalline solid in a 3:1 mixture of diastereomers. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.60–7.56 (m, 1H), 7.53–7.51 (m, 0.75H), 7.49–7.47 (m, 0.25H), 6.89 (bs, 2H), 6.55–6.52 (m, 0.75H), 6.51–6.49 (m, 0.25H), 3.30–3.18 (m, 1H), 3.10 (q, 1H, J = 6.6 Hz), 2.73–2.45 (m, 4H), 1.09 (d, 2.25H, J = 6.6 Hz), 1.03 ppm (d, 0.75H, J = 6.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 199.8, 196.1, 163.7, 162.9, 159.3, 144.0, 139.3, 139.2, 127.6, 120.5, 115.6, 115.5, 110.3, 58.4, 58.2, 43.5, 33.6, 33.5, 29.6, 29.4, 25.5, 23.7, 23.2 ppm; LC–MS: $[M+H]^+$ calcd for $C_{15}H_{15}N_2O_3$ 271.11, found 271.2; Anal. calcd for $C_{15}H_{14}N_2O_3 \cdot 0.2\,H_2O$: C 66.78, H 5.30, N 10.23, found: C 66.02, H 5.07, N 9.92; mp: 179.0–181.4 °C (decomp.).

2-Amino-7-(furan-3-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (4b). 5-(Furan-3-yl)cyclohexane-1,3-dione (0.178 g, 1.00 mmol) was dissolved in absolute EtOH (25 mL). The mixture was cooled to 0 °C and malononitrile (0.066 g, 1.00 mmol)

was added followed by acetaldehyde (176 μL, 3.00 mmol). After 15 min, *N*-methylmorpholine (10 μL, 0.09 mmol) was added and the mixture was stirred at RT for 2.5 h. The mixture was evaporated to dryness and the residue was recrystallized from absolute EtOH to give the title compound (0.149 g, 54%) as an off-white crystalline solid in a 3:1 mixture of diastereomers. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.60–7.56 (m, 1H), 7.53–7.51 (m, 0.75H), 7.49–7.47 (m, 0.25H), 6.89 (bs, 2H), 6.55–6.52 (m, 0.75H), 6.51–6.49 (m, 0.25H), 3.30–3.18 (m, 1H), 3.10 (q, 1H, J = 6.6 Hz), 2.73–2.45 (m, 4H), 1.09 (d, 2.25H, J = 6.6 Hz), 1.03 ppm (d, 0.75H, J = 6.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 199.8, 196.1, 163.7, 162.9, 159.3, 144.0, 139.3, 139.2, 127.6, 120.5, 115.6, 115.5, 110.3, 58.4, 58.2, 43.5, 33.6, 33.5, 29.6, 29.4, 25.5, 23.7, 23.2 ppm; LC–MS: $[M+H]^+$ calcd for $C_{15}H_{15}N_2O_3$ 271.11, found 271.2; Anal. calcd for $C_{15}H_{14}N_2O_3 \cdot 0.2H_2O$: C 66.78, H 5.30, N 10.23, found: C 66.02, H 5.07, N 9.92; mp: 179.0–181.4 °C (decomp.).

2-Amino-4-methyl-5-oxo-7-(thiophen-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (5b). 5-(Thiophen-2-yl)cyclohexane-1,3-dione (**16**; 0.147 g, 0.757 mmol) was dissolved in absolute EtOH (20 mL). Malononitrile (0.051 g, 0.757 mmol) was added followed by *N*-methylmorpholine (10 μL, 0.091 mmol) and acetaldehyde (0.2 mL, 3.56 mmol). The mixture was stirred at RT for 3 h, and then the solvent was decreased in vacuo to 1/3 to induce crystallization. The precipitate was filtered off and washed with absolute EtOH to give the title compound (0.097 g, 45%) as off-white crystals in a 2:3 mixture of diastereomers. R_f (EtOAc/heptane 1:1) 0.52 and 0.57; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.39 (dd, 0.6H, J = 1.9 Hz, J = 4.4 Hz), 7.37 (dd, 0.4H, J = 1.6 Hz, J = 4.8 Hz), 7.01–6.94 (m, 2H), 6.89 (bs, 1.2H), 6.88 (bs, 0.8H), 3.84–3.76 (m, 0.4H), 3.75–3.64 (m, 0.6H), 3.13 (q, 1H, J = 6.5 Hz), 2.93–2.58 (m, 4H), 1.11 (d, 1.8H, J = 6.5 Hz), 1.04 ppm (d, 1.2H, J = 6.5 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 194.89, 194.87, 162.7, 161.8, 158.73, 158.71, 146.3, 146.2, 127.0, 126.9, 124.2, 124.0, 123.9, 119.8, 115.2, 57.7, 57.6, 44.2, 43.9, 34.4, 34.2, 32.93, 32.87, 24.8, 24.7, 22.9, 22.3 ppm; LC–MS: $[M+H]^+$ calcd for $C_{15}H_{15}N_2O_2S$ 287.09, found 287.1; HPLC: >95%, t_R = 1.53 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 176.5–180.7 °C (1 °C min^{-1}).

2-Amino-7-(benzo[*b*]thiophen-3-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (6b). 5-(Benzo[*b*]thiophen-3-yl)cyclohexane-1,3-dione (0.448 g, 2.00 mmol) was dissolved in absolute EtOH (50 mL). The mixture was cooled to 0 °C and then malononitrile (0.132 g, 2.00 mmol) was added followed by acetaldehyde (350 μL, 6.00 mmol) After 15 min, *N*-methylmorpholine (20 μL, 0.18 mmol) was added, and the mixture was stirred at RT for 3 h and left to stand for 16 h. The resulting precipitate was filtered off and recrystallized from absolute EtOH to give the title compound (0.220 g, 33%) as an off-white crystalline solid in a 15:2 mixture of diastereomers. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.02–7.95 (m, 2H), 7.56 (s, 0.88H), 7.48 (s, 0.12H), 7.43–7.32 (m, 2H), 6.91 (bs, 2H), 3.92–3.78 (m, 1H), 3.16 (q, 1H, J = 6.3 Hz), 2.96–2.61 (m, 4H), 1.16 (d, 2.65H, J = 6.3 Hz), 1.09 ppm (d, 0.35H, J = 6.5 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 196.0, 163.8, 159.3, 140.4, 138.22, 138.16, 125.1, 124.8, 123.6, 122.7, 122.5, 120.6, 115.5, 58.4, 43.4, 33.5, 32.1, 25.6, 23.8 ppm; LC–MS: $[M+H]^+$ calcd for $C_{19}H_{17}N_2O_2S$ 337.10, found 337.2; Anal. calcd for $C_{19}H_{16}N_2O_2S \cdot 0.05H_2O$: C 67.66, H 4.81, N 8.30, found: C 67.37, H 4.50, N 8.12; mp: 194.4–200.1 °C (decomp.).

2-Amino-7-(benzo[*b*]thiophen-2-yl)-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (7b). 5-(Benzo[*b*]thiophen-2-yl)cyclohexane-1,3-dione (0.140 g, 0.57 mmol) was suspended in absolute EtOH (15 mL). Malononitrile (0.038 g, 0.57 mmol) was added followed by acetaldehyde (95 μL, 1.71 mmol) After 10 min, *N*-methylmorpholine (7.5 μL, 0.074 mmol) was added and the mixture was stirred at RT for 16 h. The resulting precipitate was filtered off and washed with absolute EtOH to give the title compound (0.125 g, 62%) as an off-white crystalline solid in a 2:1 mixture of diastereomers. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.92–7.85 (m, 2H), 7.78–7.71 (m, 2H), 7.37–7.22 (m, 3H), 6.93 (bs, 2H), 3.95–3.74 (m, 1H), 3.13 (q, 1H, J = 6.6 Hz), 2.98–2.67 (m, 4H), 1.12 (d, 2H, J = 6.6 Hz), 1.05 ppm (d, 1H, J = 6.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 195.2, 163.1, 162.3, 159.2, 147.7, 147.6, 139.9, 138.7, 125.0, 124.7, 123.8, 123.0, 121.4, 121.1, 121.1, 120.5, 115.8, 58.4, 58.3, 44.3, 44.1, 34.7, 34.4, 34.3, 25.5, 23.7, 23.1 ppm; LC–MS: $[M+H]^+$ calcd for $C_{19}H_{17}N_2O_2S$ 337.10, found 337.1; Anal. calcd for $C_{19}H_{16}N_2O_2S$: C 67.84, H 4.79, N 8.33, found: C 67.55, H 4.56, N 7.94; mp: 190.1–195.7 °C (decomp.).

2-Amino-3-cyano-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (12b). Cyclohexane-1,3-dione (55 mg, 0.490 mmol) was dissolved in absolute EtOH (6 mL) and malononitrile (35 mg, 0.530 mmol) was added. The solution was cooled to 0 °C before acetaldehyde (80 μL) was added. The reaction mixture was stirred for 40 min before *N*-methylmorpholine (10 μL, 0.097 mmol) was added. The reaction mixture was left in the ice bath to gradually warm up to RT over 16 h during which a precipitation has occurred. The suspension was filtered and the white solid was washed with cold EtOH (3×1 mL) and dried giving the title compound as a white solid (71 mg, 0.348 mmol, 71%). R_f (2% MeOH in CH_2Cl_2) 0.5; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.83 (s, 2H), 3.09 (q, J = 6.5 Hz, 1H), 2.50–2.43 (m, 2H), 2.39–2.24 (m, 2H), 2.00–1.80 (m, 2H), 1.06 ppm (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 196.46, 163.91, 158.71, 119.92, 115.16, 57.67, 36.38, 26.35, 24.69, 22.83, 19.85 ppm; LC–MS: $[M+H]^+$ calcd 205.10, found 205.1 HPLC: >95%, t_R = 1.12 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 202.3–204.2 °C (decomp.).

2-Amino-3-cyano-4,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (13b). 5-Methylcyclohexane-1,3-dione (82 mg, 0.620 mmol) and malononitrile (43 mg, 0.65 mmol) were dissolved in absolute EtOH (10 mL) and the flask was flushed with N$_2$ and cooled to 0 °C before acetaldehyde (100 μL, 1.82 mmol) was added. The reaction was stirred at 0 °C for 30 min before *N*-methylmorpholine (10 μL, 0.09 mmol) was added. The reaction mixture was stirred for further 1 h at 0 °C then left to warm up to RT. After a total of 16 h the solvent was reduced and the resulting white precipitate was filtered off and washed with cold absolute EtOH (2×1 mL) and dried giving the title compound as a white solid (82 mg, 0.373 mmol, 57%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (400 MHz, CD_3Cl): δ = 4.43 (s, 2H), 3.39–3.28 (m, 1H), 2.54–2.41 (m, 2H), 2.34–2.01 (m, 3H), 1.21 (dd, J = 6.5, 2.5 Hz, 3H), 1.10 ppm (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.9, 196.8, 163.0, 162.3, 157.8, 119.1, 119.1, 116.3, 116.1, 77.4, 77.2, 77.0, 63.8, 63.6, 45.4, 45.3, 35.1, 34.9, 28.5, 28.0, 25.00, 24.9, 23.0, 22.4, 20.9, 20.9 ppm; LC–MS: $[M+2H]^+$ calcd 219.11 $[M+2H]^+$ found 219.1; HPLC: >95%, t_R = 1.25 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 193.7–197.3 °C (decomp.).

2-Amino-3-cyano-7-isopropyl-4-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (14b). 5-Isopropylcyclohexane-1,3-dione (100 mg, 0.648 mmol) and malononitrile (43 mg, 0.651 mmol) was dissolved in absolute EtOH (20 mL) and the flask was evacuated and backfilled with N$_2$ three times. The resulting solution was cooled to 0 °C before acetaldehyde (100 μL, 1.82 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min before *N*-methylmorpholine (10 μL, 0.090 mmol) was added. The reaction mixture was then stirred for 16 h during which it was allowed to warm to RT. The solvent was removed in vacuo and the resulting yellow solid was pu-

rified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent 1% MeOH in CH_2Cl_2). The colorless though visible band (R_f 0.2) was collected and the solvent was removed giving the title compound as an off-white solid (148 mg, 0.601 mmol, 93%). R_f (1% MeOH in CH_2Cl_2) 0.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.45 (s, 2H), 3.39–3.26 (m, 1H), 2.62–2.00 (m, 4H), 2.00–1.82 (m, 1H), 1.69–1.52 (m, 1H), 1.22 (d, J = 5.9 Hz, 1.5H), 1.20 (d, J = 5.9 Hz, 1.5H), 0.99–0.87 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.4, 197.2, 163.7, 163.0, 157.9, 157.9, 119.1, 119.1, 116.3, 116.1, 63.7, 63.5, 41.6, 41.1, 40.0, 39.0, 32.0, 31.9, 31.0, 30.9, 25.0, 25.0, 23.0, 22.3, 19.7, 19.6, 19.6 ppm; LC–MS: $[M+H]^+$ calcd 147.1, found 147.1 HPLC: >92% (unstable), t_R = 1.50 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 170.6–175.2 °C (decomp.).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-isobutyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (1c). 5-Phenylcyclohexane-1,3-dione (62 mg, 0.329 mmol) and malononitrile (16 mg, 0.394 mmol) was dissolved in absolute EtOH (3 mL) before isobutyraldehyde (88 μL, 0.973 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 16 h during which the reaction mixture became yellow. The solvent was removed giving a yellow semisolid which was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent 1% MeOH in CH_2Cl_2). The colorless band (R_f 0.3, 2% MeOH in CH_2Cl_2) was isolated and the solvent evaporated giving the title compound as an off-white solid (87 mg, 0.282 mmol, 86%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (600 MHz, CD_3Cl): δ = 7.39–7.33 (m, 2H), 7.31–7.27 (m, 1H), 7.24 (dd, J = 11.2, 7.4 Hz, 2H), 4.54 (s, 2H), 3.52–3.45 (m, 0.4H), 3.41 (d, J = 2.2 Hz, 1H), 3.39–3.31 (m, 0.6H), 2.86 (dd, J = 17.4, 9.3 Hz, 0.4H), 2.78–2.68 (m, 3H), 2.59 (dd, J = 16.8, 13.7 Hz, 0.6H), 1.95–1.85 (m, 1H), 1.06 (d, J = 7.0 Hz, 1.8H), 1.02 (d, J = 7.0 Hz, 1.2H), 0.79 (d, J = 6.9 Hz, 1.8H), 0.65 ppm (d, J = 6.9 Hz, 1.2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.9, 195.7, 164.2, 163.2, 160.1, 160.0, 141.9, 141.9, 129.1, 129.0, 127.6, 127.5, 126.8, 126.8, 120.3, 120.2, 115.9, 115.4, 58.5, 57.9, 44.2, 43.8, 38.6, 38.5, 35.5, 35.4, 34.8, 34.5, 33.7, 33.1, 20.4, 20.2, 17.1, 16.8 ppm; LC–MS: $[M+H]^+$ calcd 309.16 $[M+H]^+$ found 309.1; HPLC: >98%, t_R = 2.17 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 159.7–162.3 °C (decomp.).

2-Amino-3-cyano-7-(2-furyl)-4-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (2c). 5-(2-Furyl)cyclohexane-1,3-dione (58 mg, 0.326 mmol) and malononitrile (26 mg, 0.394 mmol) was dissolved in absolute EtOH (3 mL) before isobutyraldehyde (88 μL, 0.973 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 16 h during which the reaction mixture became yellow. The solvent was removed giving a yellow semisolid which was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent 1% MeOH in CH_2Cl_2). The colorless band (R_f 0.3, 2% MeOH in CH_2Cl_2) was isolated and the solvent evaporated giving the title compound as an off-white solid (89 mg, 0.298 mmol, 92%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (600 MHz, CD_3Cl): δ = 7.38–7.30 (m, 1H), 6.31 (ddd, J = 24.4, 3.2, 1.9 Hz, 1H), 6.07 (dd, J = 23.2, 3.2 Hz, 1H), 4.55 (d, J = 15.0 Hz, 2H), 3.60–3.54 (m, 1H), 3.50–3.42 (m, 1H), 3.40–3.32 (m, 1H), 2.91 (ddd, J = 11.3, 6.1, 3.4 Hz, 1H), 2.86–2.77 (m, 2H), 2.75–2.67 (m, 1H), 2.55 (dd, J = 16.8, 12.9 Hz, 1H), 1.92–1.84 (m, 1H), 1.82–1.75 (m, 1H), 1.04 (d, J = 7.0 Hz, 2H), 0.95 (d, J = 7.0 Hz, 1H), 0.76 (d, J = 6.8 Hz, 2H), 0.53 ppm (d, J = 6.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.0, 195.0, 163.6, 162.2, 160.0, 159.9, 155.0, 154.8, 142.1, 141.9, 120.2, 120.1, 115.7, 115.5, 110.5, 110.4, 106.0, 105.2, 58.4, 58.1, 41.6, 40.8, 35.3, 33.7, 33.3, 32.1, 32.1, 31.6, 31.4, 20.2, 20.1, 17.0, 16.6 ppm; LC–MS: $[M+H]^+$ calcd 299.14 $[M+H]^+$ found 299.1; HPLC: >98%, t_R = 2.17 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 138.9–140.8 °C (decomp.).

2-Amino-3-cyano-4-isopropyl-7-(1-naphthyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (8c). 5-(1-Naphthyl)cyclohexane-1,3-dione (200 mg, 0.839 mmol) was dissolved in absolute EtOH (15 mL). Isobutyraldehyde (191 mg, 1.052 mmol), malononitrile (66 mg, 0.999 mmol) and *N*-methylmorpholine (12 μL, 0.109 mmol) were added. The reaction mixture was stirred at RT for 16 h. The solvent was removed in vacuo and the resulting yellow oil was purified by gradient column chromatography (3 cm Ø, 100 mL SiO_2, eluent 0–2% MeOH in CH_2Cl_2) and the colorless band (R_f 0.2, 2% MeOH in CH_2Cl_2) was isolated and the solvent was removed in vacuo giving the title compound as a white solid (287 mg, 0.801 mmol, 94%). R_f (2% MeOH in CH_2Cl_2) 0.2; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.22 (t, J = 8.8 Hz, 1H), 7.99–7.91 (m, 1H), 7.84 (t, J = 7.1 Hz, 1H), 7.63–7.37 (m, 4H), 6.97 (s, 1H), 6.95 (s, 1H), 4.51–4.33 (m, 0.5H), 4.32–4.16 (m, 0.5H), 3.17 (t, J = 2.8 Hz, 1H), 3.08–2.56 (m, 4H), 1.92–1.69 (m, 1H), 0.98 (d, J = 7.0 Hz, 1.6H), 0.92 (d, J = 7.0 Hz, 1.4H), 0.80 (d, J = 6.8 Hz, 1.6H), 0.60 ppm (d, J = 6.8 Hz, 1.4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.9, 195.5, 164.9, 164.0, 161.2, 161.1, 138.4, 138.3, 133.6, 133.5, 130.6, 130.5, 128.9, 128.8, 127.3, 127.2, 126.4, 125.7, 125.6, 125.3, 123.3, 123.3, 123.0, 123.0, 121.2, 121.2, 114.2, 113.9, 52.3, 51.6, 43.4, 42.6, 35.5, 35.3, 33.5, 33.4, 33.3, 33.00, 32.9, 32.6, 20.2, 20.1, 16.9, 16.5 ppm; LC–MS: $[M+H]^+$ calcd 359.18 $[M+2H]^+$ found 359.1, $[M+2H]^+$ calcd 360.18, $[M+2H]^+$ found 360.1; HPLC: >98%, t_R = 2.00 min (split peak, ~1:1 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 203.1–204.6 °C (decomp.).

2-Amino-3-cyano-4-isopropyl-7-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (13c). 5-Methylcyclohexane-1,3-dione (41 mg, 0.325 mmol) and malononitrile (26 mg, 0.394 mmol) were dissolved in absolute EtOH (3 mL) before isobutyraldehyde (88 μL, 0.973 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 40 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with cold EtOH (2×2 mL) and dried giving the title compound as an off-white solid (43 mg, 0.175 mmol, 54%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.53 (s, 2H), 3.38–3.32 (m, 1H), 2.59–2.47 (m, 2H), 2.40–2.15 (m, 2H), 2.10–2.01 (m, 1H), 1.93–1.79 (m, 1H), 1.11 (d, J = 6.2 Hz, 3H), 1.05–0.98 (m, 3H), 0.74–0.69 ppm (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.7, 196.6, 164.6, 163.6, 160.1, 160.0, 120.4, 120.3, 115.4, 115.2, 58.4, 57.8, 45.4, 45.1, 35.4, 35.3, 35.3, 34.7, 33.6, 33.2, 28.3, 28.1, 20.9, 20.8, 20.4, 20.2, 16.9, 16.9 ppm; LC–MS: $[M+H]^+$ calcd 247.14 $[M+H]^+$ found 247.1; HPLC: >98%, t_R = 1.43 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 198.3–204.6 °C (decomp.).

2-Amino-3-cyano-4-isopropyl-7-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (14c). 5-Isopropylcyclohexane-1,3-dione (50 mg, 0.324 mmol) and malononitrile (26 mg, 0.394 mmol) were dissolved in absolute EtOH (3 mL) before isobutyraldehyde (88 μL, 0.973 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 40 h during which it became yellow. The solvent was removed and the resulting yellow semisolid was subjected to column chromatography (2 cm Ø, 50 mL SiO_2, eluent 2% MeOH in CH_2Cl_2). The light-yellow band (R_f 0.3) was isolated and the solvent evaporated giving the title compound as a pale-yellow foam (88 mg, 0.321 mmol, 99%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (600 MHz, CD_3Cl): δ = 4.52 (s, 2H), 3.38–3.35 (m, 0.4H), 3.35–3.32 (m, 0.6H), 2.60–2.48 (m, 1H), 2.47–2.36 (m, 1H), 2.31–2.15 (m, 1H), 2.11–2.02 (m, 1H), 1.99–1.91 (m, 1H), 1.91–1.80 (m, 1H), 1.65–1.59 (m, 1H), 1.04–0.99 (m, 3H), 0.98–0.90 (m, 9H), 0.73 (d, J = 6.8 Hz, 1.8H), 0.70 ppm (d, J = 6.8 Hz, 1.2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 197.2, 197.0, 165.1, 164.3,

160.2, 160.0, 120.4, 120.3, 115.5, 115.1, 58.4, 57.7, 41.5, 41.2, 40.3, 39.0, 35.5, 35.3, 33.6, 33.1, 31.9, 31.8, 31.1, 30.8, 20.4, 20.2, 19.8, 19.7, 19.7, 19.6, 17.0, 16.9 ppm; LC–MS: $[M+H]^+$ calcd 275.18 $[M+H]^+$ found 275.1; HPLC: >95%, t_R = 1.68 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 140.8–143.8 °C (decomp.).

2-Amino-3-cyano-4-isobutyl-7-phenyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (1 d). 5-Phenylcyclohexane-1,3-dione (40 mg, 0.213 mmol) and malononitrile (17 mg, 0.257 mmol) were mixed in a vial and this was evacuated and backfilled with argon four times. Another vial with absolute EtOH (3 mL) was also evacuated and backfilled with argon four times before isovaleraldehyde (67 μL, 0.638 mmol) and *N*-methylmorpholine (3 μL, 0.027 mmol) were added and the resulting solution was added to the first vial under argon. The reaction mixture was stirred in the dark at RT for 16 h. The solvent was removed and the resulting pale-yellow semisolid was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent CH_2Cl_2/EtOH 20:1) and the colorless band (R_f 0.23) was isolated and evaporated to give the title compound as a pale-yellow solid (64 mg, 0.199 mmol, 93%). R_f (CH_2Cl_2/EtOAc 20:1) 0.23; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.39–7.33 (m, 2H), 7.32–7.27 (m, 1H), 7.26–7.21 (m, 2H), 4.49 (bs, 2H), 3.49–3.41 (m, 1.4H), 3.39–3.31 (m, 0.6H), 2.85–2.55 (m, 4H), 1.92–1.85 (m, 0.6H), 1.85–1.77 (m, 0.4H), 1.43–1.38 (m, 1.2H), 1.38–1.34 (m, 0.8H), 1.02 (d, J = 6.5 Hz, 1.8H), 0.99 (d, J = 6.6 Hz, 1.2H), 0.91 (d, J = 6.6 Hz, 1.8H), 0.86 ppm (d, J = 6.6 Hz, 1.2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.8, 195.6, 163.4, 162.6, 158.9, 141.9, 129.2, 129.1, 129.1, 129.0, 127.6, 127.5, 126.8, 119.8, 116.9, 116.8, 62.3, 62.0, 47.2, 46.1, 44.1, 44.1, 38.9, 38.4, 34.8, 34.6, 27.6, 27.5, 24.9, 24.9, 24.1, 24.0, 22.0 ppm; LC–MS: $[M+H]^+$ calcd 323.18 $[M+H]^+$ found 324.1; HPLC: >95%, t_R = 1.84 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 171.2–180.2 °C (decomp.).

2-Amino-3-cyano-7-(2-furyl)-4-isobutyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (2 d). 5-(2-Furyl)cyclohexane-1,3-dione (38 mg, 0.213 mmol) and malononitrile (17 mg, 0.257 mmol) were mixed in a vial which was then evacuated and backfilled with argon four times. Another vial with absolute EtOH (3 mL) was also evacuated and backfilled with argon four times before isovaleraldehyde (67 μL, 0.638 mmol) and *N*-methylmorpholine (3 μL, 0.027 mmol) were added and the resulting solution was added to the first vial under argon. The reaction mixture was stirred in the dark at RT for 16 h. The solvent was removed and the resulting pale-yellow semisolid was purified by column chromatography (2 columns of 2 cm Ø, 50 mL SiO_2, eluent CH_2Cl_2/EtOH, 20:1). The two pairs of diastereomers were partly separated by the column chromatography giving one fraction (R_f 0.35, CH_2Cl_2/EtOH 20:1) of two enantiomers (20 mg, 0.064, 30%) separated from a (4:6) mixture of the four isomers (R_f 0.35 + 0.26, CH_2Cl_2/EtOH, 20:1). In total, this gave the title compound as a pale-yellow solid (32 mg, 0.166 mmol, 78%). R_f (CH_2Cl_2/EtOH, 20:1) 0.35 + 0.26. Data for the mixed fraction. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.34 (dd, J = 18.0, 1.2 Hz, 1H), 6.31 (ddd, J = 17.0, 3.2, 1.9 Hz, 1H), 6.07 (dd, J = 18.0, 3.2 Hz, 1H), 4.47 (d, J = 9.2 Hz, 2H), 3.58–3.50 (m, 0.6H), 3.49–3.38 (m, 1.4H), 2.91–2.49 (m, 4H), 1.89–1.81 (m, J = 13.5, 6.5 Hz, 0.4H), 1.76–1.68 (m, 0.6H), 1.41–1.23 (m, 2H), 1.00 (d, J = 6.5 Hz, 1.2H), 0.95 (d, J = 6.5 Hz, 1.8H), 0.89 (d, J = 6.6 Hz, 1.2H), 0.82 ppm (d, J = 6.6 Hz, 1.8H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.0, 194.9, 161.8, 158.8, 142.1, 141.9, 119.6, 116.9, 110.4, 105.6, 105.2, 47.0, 46.1, 41.5, 41.1, 32.1, 32.1, 32.0, 31.6, 27.6, 27.5, 24.9, 24.8, 24.0, 23.9, 22.0 ppm; LC–MS: $[M+H]^+$ calcd 313.16 $[M+H]^+$ found 313.1; HPLC: >98%, t_R = 1.70 + 1.76 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 154.5–155.7 °C (decomp.). Data for the fraction with one enantiomeric pair ^{1}H NMR (600 MHz, CD_3Cl): δ = 7.36 (d, J = 1.2 Hz, 1H), 6.32 (dd, J = 3.2, 1.9 Hz, 1H), 6.08 (d, J = 3.2 Hz, 1H), 4.48 (s, 2H), 3.49–3.39 (m, 2H), 2.87–2.76 (m, 2H), 2.74–2.65 (m, 1H), 2.56 (dd, J = 16.8, 12.5 Hz, 1H), 1.85 (dq, J = 13.0, 6.5 Hz, 1H), 1.40–1.32 (m, 2H), 1.00 (d, J = 6.5 Hz, 3H), 0.89 ppm (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 194.9, 162.8, 158.8, 155.1, 142.1, 119.6, 116.9, 110.4, 105.2, 62.3, 47.0, 41.5, 32.1, 32.1, 27.5, 24.9, 24.0, 22.0 ppm; HPLC: >98%, t_R = 1.77 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; LC–MS: $[M+H]^+$ calcd 313.16 $[M+H]^+$ found 313.1.

2-Amino-3-cyano-4-isobutyl-7-(1-naphthyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (8 d). 5-(1-Naphthyl)cyclohexane-1,3-dione (50 mg, 0.210 mmol) was dissolved in absolute EtOH (7 mL). Isovaleraldehyde (66 μL, 0.630 mmol), malononitrile (17 mg, 0.281 mmol) and *N*-methylmorpholine (3 μL, 0.027 mmol) were added. The reaction mixture was stirred at RT for 16 h. The solvent was removed in vacuo and the resulting pale-yellow oil was purified by gradient column chromatography (2 cm Ø, 50 mL SiO_2, eluent 0–2% MeOH in CH_2Cl_2) and the colorless band (R_f 0.2, 2% MeOH in CH_2Cl_2) was isolated and the solvent was removed in vacuo giving the title compound as a light-yellow solid (59 mg, 0.157 mmol, 75%). R_f (1% MeOH in CH_2Cl_2) 0.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (t, J = 8.3 Hz, 1H), 7.94–7.87 (m, 1H), 7.80 (dd, J = 8.1, 5.2 Hz, 1H), 7.61–7.42 (m, 3H), 7.37 (dd, J = 16.8, 7.1 Hz, 1H), 4.48 (bs, 2H), 4.36–4.25 (m, 0.4H), 4.25–4.13 (m, 0.6H), 3.54–3.46 (m, 1H), 3.00–2.68 (m, 4H), 2.01–1.91 (m, 0.6H), 1.90–1.78 (m, 0.4H), 1.51–1.44 (m, 1.2H), 1.43–1.36 (m, 0.8H), 1.06 (d, J = 6.5 Hz, 1.7H), 1.01 (d, J = 6.5 Hz, 1.3H), 0.96 (d, J = 6.6 Hz, 1.7H), 0.89 ppm (d, J = 6.6 Hz, 1.3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.1, 195.9, 163.7, 162.9, 158.9, 158.9, 137.7, 137.6, 134.2, 134.2, 131.0, 131.0, 129.5, 129.5, 128.2, 128.1, 126.8, 126.7, 126.1, 126.0, 125.7, 125.5, 123.1, 122.9, 122.5, 122.5, 119.7, 116.8, 116.8, 62.4, 62.1, 47.4, 46.2, 43.9, 43.6, 34.5, 34.2, 34.1, 33.6, 27.7, 27.5, 25.0, 24.9, 24.1, 24.0, 22.0, 22.0 ppm; LC–MS: $[M+H]^+$ calcd 373.19 $[M+2H]^+$ found 373.1, $[M+2H]^+$ calcd 374.2, $[M+2H]^+$ found 374.1; HPLC: >95%, t_R = 2.09 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 94.8–114.9 °C (decomp.).

2-Amino-3-cyano-4-isobutyl-7-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (13 d). 5-Methylcyclohexane-1,3-dione (27 mg, 0.213 mmol) and malononitrile (17 mg, 0.257 mmol) were mixed in a vial and this was evacuated and backfilled with argon four times. Another vial with absolute EtOH (2 mL) was also evacuated and backfilled with argon four times before isovaleraldehyde (67 μL, 0.638 mmol) and *N*-methylmorpholine (3 μL, 0.027 mmol) were added and the resulting solution was added to the first vial under argon. The reaction mixture was stirred in the dark at RT for 16 h. The solvent was removed and the resulting pale-yellow semisolid was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent CH_2Cl_2/EtOH 20:1) and the colorless band (R_f 0.16) was isolated and evaporated to give the title compound as a pale-yellow solid (54 mg, 0.207 mmol, 97%). R_f (CH_2Cl_2/EtOH 20:1) 0.16; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.46 (s, 2H), 3.43–3.36 (m, 1H), 2.54–2.43 (m, 2H), 2.36–2.01 (m, 3H), 1.88–1.77 (m, 1H), 1.37–1.31 (m, 2H), 1.13–1.08 (m, 3H), 0.99 (d, J = 6.5 Hz, 3H), 0.86 ppm (t, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.7, 196.6, 163.8, 162.9, 158.9, 158.9, 119.8, 119.8, 116.5, 116.4, 62.3, 62.0, 46.9, 46.2, 45.4, 45.3, 35.3, 34.9, 28.5, 28.0, 27.6, 27.5, 24.9, 24.0, 24.0, 22.1, 22.0, 20.9, 20.8 ppm; LC–MS: $[M+H]^+$ calcd 261.16 $[M+H]^+$ found 161.1; HPLC: >98%, t_R = 1.51 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 161.0–162.5 °C (decomp.).

2-Amino-3-cyano-4-isobutyl-7-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (14 d). 5-Isopropylcyclohexane-1,3-dione (33 mg, 0.214 mmol) and malononitrile (17 mg, 0.257 mmol) were mixed in

a vial and this was evacuated and backfilled with argon four times. Another vial with absolute EtOH (2 mL) was also evacuated and backfilled with argon four times before isovaleraldehyde (67 μL, 0.638 mmol) and *N*-methylmorpholine (3 μL, 0.027 mmol) were added and the resulting solution was added to the first vial under argon. The reaction mixture was stirred in the dark at RT for 16 h. The solvent was removed and the resulting pale-yellow semisolid was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent CH_2Cl_2/EtOH 20:1) and the colorless bands (R_f 0.45, CH_2Cl_2/EtOH 10:1) was isolated and evaporated to give the title compound as a pale-yellow solid (60 mg, 0.263 mmol, 97%). R_f (CH_2Cl_2/EtOH, 10:1) 0.45; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.45 (s, 2H), 3.43–3.35 (m, 1H), 2.58–2.06 (m, 4H), 1.97–1.86 (m, 1H), 1.85–1.77 (m, 1H), 1.65–1.58 (m, 1H), 1.34 (t, J = 6.6 Hz, 2H), 0.99 (dd, J = 6.5, 4.8 Hz, 3H), 0.96–0.93 (m, 6H), 0.88 (d, J = 6.6 Hz, 2H), 0.85 ppm (d, J = 6.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.9, 164.3, 158.9, 119.8, 116.5, 47.0, 46.0, 41.5, 41.2, 40.1, 39.0, 32.0, 31.9, 31.1, 30.9, 27.6, 27.5, 24.9, 24.8, 24.0, 22.0, 22.0, 19.7, 19.7, 19.6, 19.6 ppm; LC–MS: $[M+H]^+$ calcd 289.19 $[M+H]^+$ found 289.1; HPLC: >98%, t_R = 1.51 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 128.1–135.3 °C (decomp.).

2-Amino-7-(furan-3-yl)-5-oxo −4-phenyl −5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (3e). 5-(Furan-3-yl)cyclohexane-1,3-dione (0.178 g, 1.00 mmol) was dissolved in absolute EtOH (25 mL). Malononitrile (0.065 g, 1.00 mmol) was added followed by benzaldehyde (101 μL, 1.00 mmol) After 30 min, *N*-methylmorpholine (10 μL, 0.09 mmol) was added and the mixture was stirred at RT for 16 h. The precipitate was filtered off and recrystallized from absolute EtOH to give the title compound (0.174 g, 52%) as off-white crystalline solid in a 7:1 mixture of diastereomers. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.60–7.55 (m, 1H), 7.52 (bs, 0.87H), 7.39 (bs, 0.13H), 7.31–6.96 (m, 7H), 6.54 (bs, 0.87H), 6.49 (bs, 0.13H), 4.19 (s, 0.87H), 4.16 (s, 0.13H), 3.31–3.18 (m, 1H), 2.91–2.69 (m, 2H), 2.62–2.41 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 195.5, 164.3, 159.0, 145.2, 144.0, 139.2, 128.9, 127.7, 127.6, 127.2, 120.3, 114.2, 110.3, 58.9, 43.4, 36.1, 33.8, 29.4 ppm; LC–MS: $[M+H]^+$ calcd for $C_{20}H_{17}N_2O_3$ 333.12, found 333.2; Anal. calcd for $C_{20}H_{16}N_2O_3$·0.15H_2O: C 71.69, H 4.90, N 8.36, found: C 71.44, H 4.61, N 8.22; mp: 175.4–182.1 °C (decomp.).

2-Amino-5-oxo-4-phenyl-7-(thiophen-3-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (4e). 5-(Thiophen-3-yl)cyclohexane-1,3-dione (0.145 g, 0.75 mmol) was dissolved in absolute EtOH (20 mL). The mixture was cooled to 0 °C, and then malononitrile (0.050 g, 0.75 mmol) was added followed by benzaldehyde (76 μL, 0.75 mmol). After 15 min, *N*-methylmorpholine (10 μL, 0.09 mmol) was added and the mixture was stirred at RT overnight. The precipitate was filtered off and recrystallized from absolute EtOH to give the title compound (0.153 g, 59%) as an off-white crystalline solid in a 3:4 mixture of diastereomers. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.50–7.45 (m, 1H), 7.32–6.94 (m, 9H), 4.20 (s, 0.43H), 4.17 (s, 0.57H), 3.62–3.50 (m, 0.57H), 3.50–3.37 (m, 0.43H), 3.06–2.82 (m, 2H), 2.67–2.51 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 195.4, 164.3, 163.4, 159.0, 158.8, 145.2, 144.9, 144.2, 144.1, 128.9, 128.7, 127.7, 127.6, 127.5, 127.1, 126.9, 121.3, 121.1, 120.3, 114.2, 113.9, 58.8, 43.9, 43.7, 36.14, 36.09, 34.1, 33.9, 33.8, 33.6 ppm; LC–MS: $[M+H]^+$ calcd for $C_{20}H_{17}N_2O_2S$ 349.10, found 349.2; Anal. calcd for $C_{20}H_{16}N_2O_2S$·0.15H_2O: C 68.42, H 4.68, N 7.98, found: C 68.19, H 4.45, N 7.84; mp: 200.4–204.2 °C (decomp.).

2-Amino-5-oxo-4-phenyl-7-(thiophen-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (5e). 5-(thiophen-2-yl)cyclohexane-1,3-dione (**16**; 0.100 g, 0.515 mmol) was dissolved in absolute EtOH (9 mL). Malononitrile (0.039 g, 0.515 mmol) was added followed by benzaldehyde (53 μL, 0.515 mmol). Then *N*-methylmorpholine (9.1 μL, 0.082 mmol) was added and the mixture was stirred at RT for 19 h. The solvent was decanted off and the residue was triturated with Et_2O to give the title compound (0.117 g, 65%) as a white powder in a 3:7 mixture of diastereomers. R_f (EtOAc/MeOH/AcOH 100:10:1) 0.93; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.41–7.37 (m, 1H), 7.33–7.27 (m, 0.6H), 7.23–7.11 (m, 3H), 7.06–6.92 (m, 4.7H), 6.90–6.87 (m, 0.7H), 4.22 (s, 0.3H), 4.18 (s, 0.7H), 3.87–3.78 (m, 0.7H), 3.76–3.66 (m, 0.3H), 3.04–2.85 (m, 2H), 2.76–2.57 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 194.3, 194.1, 163.3, 162.5, 158.4, 158.3, 146.1, 144.6, 144.2, 128.3, 128.1, 127.2, 126.98, 126.96, 126.6, 126.4, 124.2, 124.1, 124.02, 123.98, 119.6, 113.7, 113.5, 58.3, 44.1, 43.6, 35.4, 34.6, 34.2, 32.8 ppm; LC–MS: $[M+H]^+$ calcd for $C_{20}H_{17}N_2O_2S$ 349.10, found 349.2; HPLC: >95%, t_R = 1.70 and 1.77 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 194.8–195.9 °C (1 °C min^{-1}).

2-Amino-7-(benzo[*b*]thiophen-3-yl)-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (6e). *N*-Methylmorpholine (9 μL, 82 μmol, 1 equiv) was added to a stirred suspension of 5-(benzo[*b*]thiophen-3-yl)cyclohexane-1,3-dione (20 mg, 82 μmol) and 2-benzylidenemalononitrile (13 mg, 82 μmol) in absolute EtOH (3 mL) at RT and stirred for 18 h. The resulting white solid was filtered and washed with cold absolute EtOH. This afforded the title compound as a white solid (23 mg, 57 μmol, 70% yield). ^{1}H NMR (400 MHz, [D$_6$]DMSO): (~3:4 ratio of diastereomers) δ = 8.07–7.96 (m, 2H), 7.44–7.14 (m, 8H), 7.02 (d, J = 4.0 Hz, 2H), 4.27 (s, 0.60H), 4.26 (s, 0.40H), 4.05–3.99 (m, 0.39H), 3.89–3.84 (m, 0.61H), 3.10–2.94 (m, 2H), 2.84–2.60 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 194.9, 163.9, 163.0, 158.4, 144.7, 144.4, 139.8, 137.6, 137.5, 137.3, 128.3, 128.2, 127.3, 127.2, 126.6, 126.5, 124.5, 124.2, 124.1, 123.1, 123.0, 122.2, 122.1, 121.9, 119.7, 119.6, 58.4, 58.1, 42.3, 35.5, 32.9, 32.5, 31.3, 31.2 ppm; LC–MS: $[M+H]^+$ calcd for $C_{24}H_{18}N_2O_2S$ $[M+H]^+$, 399.1, found 399.1; HPLC: >98%; mp: 213–215 °C (decomp.).

2-Amino-7-(benzo[*b*]thiophen-2-yl)-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (7e). *N*-Methylmorpholine (9 μL, 82 μmol) was added to a stirred suspension of 5-(benzo[*b*]-thiophen-2-yl)cyclohexane-1,3-dione (20 mg, 82 μmol) and 2-benzylidenemalononitrile (13 mg, 82 μmol) in absolute EtOH (3 mL) at RT and stirred for 18 h. The white solid was filtered off and washed with cold abs EtOH. This afforded the title compound as a white solid (19 mg, 48 μmol, 58% yield). ^{1}H NMR (400 MHz, [D$_6$]DMSO): (~3:4 ratio of diastereomers) δ = 7.92 (dd, J = 10.4, 7.6 Hz, 1H), 7.78 (d, J = 7.2 Hz, 0.40H), 7.70 (d, J = 7.2 Hz, 0.60H), 7.39–7.20 (m, 5H), 7.08–6.88 (m, 5H), 4.25 (s, 0.43H), 4.20 (s, 0.57H), 3.97–3.91 (m, 0.61H), 3.88–3.80 (m, 0.39H), 3.19–2.98 (m, 2H), 2.86–2.67 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 194.1, 193.9, 163.2, 162.3, 158.4, 158.3, 147.0, 144.5, 144.1, 139.5, 139.4, 138.2, 138.1, 128.3, 128.0, 127.2, 126.9, 126.6, 126.3, 124.4, 124.1, 123.3, 123.2, 122.4, 122.3, 121.0, 120.5, 119.7, 119.6, 113.8, 113.5, 58.3, 43.5, 43.0, 35.5, 35.4, 34.0, 33.7, 33.5, 33.3 ppm; LC–MS: $[M+H]^+$ calcd for $C_{24}H_{18}N_2O_2S$ $[M+H]^+$, 399.1, found 399.1; HPLC: >98%; mp: 244–246 °C (decomp.).

2-Amino-7-(naphthalen-1-yl)-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (8e). 5-(naphthalen-1-yl)cyclohexane-1,3-dione (0.200 g, 0.839 mmol) was dissolved in absolute EtOH (15 mL). Malononitrile (0.057 g, 0.839 mmol) was added followed by benzaldehyde (85 μL, 0.839 mmol). Then *N*-methylmorpholine (15 μL, 0.134 mmol) was added and the mixture was stirred at RT for 7.5 h, and then allowed to induce crystallization. The solvent was filtered off and the precipitate was washed with absolute EtOH to give the title compound (0.215 g, 65%) as white powder

in a 1:1 mixture of diastereomers. R_f (EtOAc/heptane 1:1) 0.40; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.26 (d, 0.5 H, J = 9.0 Hz), 8.23 (d, 0.5 H, J = 9.5 Hz), 7.98–7.93 (m, 1 H), 7.85 (d, 0.5 H, J = 8.3 Hz), 7.83 (d, 0.5 H, J = 8.8 Hz), 7.61–7.49 (m, 3 H), 7.43–7.13 (m, 6 H), 7.01 (bs, 1 H), 6.99 (bs, 1 H), 4.46–4.38 (m, 0.5 H), 4.30–4.20 (m, 0.5 H), 4.27 (bs, 1 H), 3.15–3.02 (m, 1 H), 2.98–2.75 (m, 2 H), 2.66 (dd, 0.5 H, J = 4.8 Hz, J = 16.3 Hz), 2.54 ppm (dd, 0.5 H, J = 4.0 Hz, J = 16.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 195.21, 195.18, 164.1, 163.1, 158.5, 144.7, 144.4, 138.4, 138.2, 133.5, 130.6, 130.5, 128.9, 128.8, 128.4, 128.2, 127.4, 127.3, 126.6, 126.5, 126.4, 126.3, 125.7, 125.6, 125.4, 123.4, 123.3, 123.1, 123.0, 119.7, 113.4, 113.3, 58.5, 58.2, 43.0, 42.9, 35.7, 35.6, 33.7, 33.3, 32.9, 32.5 ppm; LC–MS: $[M+H]^+$ calcd for $C_{26}H_{21}N_2O_2$ 393.16, found 393.2; HPLC: > 95 %, t_R = 2.12 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 204.9–210.9 °C (decomp.).

7-([1,1′-Biphenyl]-3-yl)-2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (10 e). To a suspension of the dione (53 mg, 0.20 mmol) and benzylidenemalononitrile (33 mg, 0.22 mmol) in absolute EtOH (1 mL) was added piperidine (4 μL, 0.038 mmol). Additional piperidine (2 μL, 0.02 mmol) was added and the resulting clear solution was allowed to stir at RT for 4 h. The solvent was reduced to half the volume and the resulting precipitate was filtered off and washed with cold absolute EtOH to give the title compound as a white solid (64 mg, 76 %). R_f (EtOAc/CH_2Cl_2 1:9) 0.47; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.75–7.59 (m, 3 H), 7.58–7.28 (m, 7 H), 7.27–7.08 (m, 4 H), 7.03 (d, J = 16.08 Hz, 2 H), 4.25 (d, J = 8.27 Hz, 1 H), 3.68–3.40 (m, 1 H), 3.24–3.00 (m, 1 H), 2.88–2.72 (m, 2 H), 2.61–2.43 ppm (m, 1 H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): (two diastereomers) δ = 195.1, 195.0, 164.0, 163.1, 158.6, 158.4, 144.7, 144.4, 143.4, 140.41, 140.39, 140.1, 129.12, 129.07, 128.9, 128.8, 128.4, 128.2, 127.4, 127.2, 127.1, 126.8, 126.6, 126.5, 126.2, 126.0, 125.6, 125.4, 125.2, 119.7, 113.63, 113.55, 58.3, 58.2, 43.3, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 37.7, 37.5, 35.6, 35.4, 33.8, 33.5 ppm; LC–MS: $[M+H]^+$ calcd for $C_{28}H_{23}N_2O_2$ 419.18, found 419.1; mp: 197.3–198.5 °C (decomp.).

7-([1,1′-Biphenyl]-4-yl)-2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (11 e). To a suspension of the dione (35 mg, 0.13 mmol) and benzylidenemalononitrile (22 mg, 0.15 mmol) in absolute EtOH (0.7 mL) was added piperidine (1.3 μL, 0.013 mmol). Additional piperidine (1.3 μL, 0.013 mmol) was added and the resulting clear solution was allowed to stir at RT overnight. The reaction mixture was then cooled on ice bath to promote further precipitation and the resulting precipitate was filtered off. Purification by trituration in Et$_2$O gave the title compound as an off-white solid (37 mg, 67 %). R_f (EtOAc/CH_2Cl_2 1:9) 0.50; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.71–7.56 (m, 4 H), 7.46 (ddd, J = 2.07, 7.01, 7.92 Hz, 3 H), 7.43–7.29 (m, 3 H), 7.29–7.14 (m, 3 H), 7.14–7.08 (m, 1 H), 7.03 (d, J = 15.56 Hz, 2 H), 4.29–4.22 (m, 1 H), 3.64–3.39 (m, 1 H), 3.15–2.95 (m, 1 H), 2.87–2.65 (m, 2 H), 2.61–2.40 ppm (m, 1 H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): (two diastereomers) δ = 195.0, 163.9, 163.1, 158.4, 144.7, 144.4, 141.9, 141.8, 138.7, 128.91, 128.89, 128.4, 128.2, 127.6, 127.3, 127.23, 127.18, 126.8, 126.7, 126.60, 126.56, 126.54, 126.49, 113.64, 113.56, 58.3, 58.2, 40.1, 40.0, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 37.3, 37.0, 35.6, 35.5 ppm; LC–MS: $[M+H]^+$ calcd for $C_{28}H_{23}N_2O_2$ 419.18, found 419.1; mp: 249.0–251.2 °C (decomp.).

2-Amino-3-cyano-4-phenyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (12 e). 2-benzylidenemalononitrile (58 mg, 0.376 mmol) was dissolved in absolute EtOH (6 mL) before cyclohexane-1,3-dione (46 mg, 0.413 mmol) and *N*-methylmorpholine (6.2 μL, 0.056 mmol) was added. The reaction mixture was stirred at RT for 16 h during which a precipitation occurred. The suspension was filtered and the white solid was washed with cold absolute EtOH (3 × 1 mL) and dried giving the title compound as a white solid (81 mg, 0.304 mmol, 81 %). R_f (2 % MeOH in CH_2Cl_2) 0.5; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.33–7.23 (m, 2 H), 7.23–7.12 (m, 3 H), 6.97 (s, 2 H), 4.18 (s, 1 H), 2.71–2.52 (m, 2 H), 2.38–2.17 (m, 2 H), 2.06–1.79 ppm (m, 2 H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 196.3, 164.9, 158.9, 145.2, 128.8, 127.6, 127.0, 120.2, 114.3, 58.7, 36.8, 35.9, 26.9, 20.3 ppm; LC–MS: $[M+H]^+$ calcd 267.11, $[M+2H]^+$ found 267.0; HPLC: > 95 %, t_R = 1.30 min (mixture of two diastereomers), reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 199.1–202.2 °C (decomp.).

2-Amino-3-cyano-7-methyl-4-phenyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (13 e). 5-Methylcyclohexane-1,3-dione (45 mg, 0.357 mmol) and 2-benzylidenemalononitrile (51 mg, 0.33 mmol) were dissolved in absolute EtOH (10 mL). *N*-methylmorpholine (5 μL, 0.045 mmol) was added. The reaction mixture was stirred for 4 h during which a white precipitate was formed. The white solid was filtered off and washed with cold absolute EtOH (3 × 1 mL) and dried giving the title compound as a white solid (82 mg, 0.293 mmol, 88 %). R_f (2 % MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 7.28 (td, J = 7.8, 1.4 Hz, 2 H), 7.21–7.10 (m, 3 H), 6.98 (d, J = 8.4 Hz, 2 H), 4.19–4.14 (m, 1 H), 2.63–2.55 (m, 1 H), 2.53–2.46 (m, 1 H), 2.43–2.23 (m, 2 H), 2.19–2.02 (m, 1 H), 1.02 (d, J = 6.1 Hz, 1.5 H), 0.99 ppm (d, J = 6.5 Hz, 1.5 H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 195.8, 195.7, 164.1, 163.2, 158.5, 158.4, 144.8, 144.7, 128.3, 128.3, 127.1, 127.1, 126.5, 126.5, 119.8, 119.7, 113.4, 113.2, 58.2, 58.2, 44.3, 44.3, 35.6, 35.4, 34.3, 33.9, 27.6, 27.4, 20.3, 20.2 ppm; LC–MS: $[M+2H]^+$ calcd 281.13 $[M+2H]^+$ found 281.1 HPLC: > 96 %, t_R = 1.41 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 252.1–260.3 °C (decomp.).

2-Amino-3-cyano-7-isopropyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene (14 e). 2-Benzylidenemalononitrile (50 mg, 0.324 mmol) was dissolved in absolute EtOH (9 mL). 5-Isopropylcyclohexane-1,3-dione (55 mg, 0.357 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 16 h before the solvent was removed in vacuo and the resulting colorless oil was purified by column chromatography (2 cm Ø, 50 mL SiO$_2$, eluent CH_2Cl_2:*n*-heptane:EtOAc 6:1:1) and the colorless band (R_f 0.2) was collected and the solvent was removed in vacuo giving the title compound as an off-white foam (96 mg, 0.311 mmol, 96 %). R_f (CH_2Cl_2/*n*-heptane/EtOAc 6:1:1) 0.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.16 (m, 5 H), 4.53 (s, 2 H), 4.44–4.39 (m, 1 H), 2.64–2.29 (m, 3 H), 2.13–2.03 (m, 1 H), 2.03–1.95 (m, 0.5 H), 1.94–1.81 (m, 0.5 H), 1.67–1.54 (m, 1 H), 0.98–0.89 ppm (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.6, 196.3, 163.6, 162.9, 157.7, 157.6, 143.3, 143.2, 128.7, 128.7, 127.7, 127.3, 127.3, 118.8, 115.0, 114.9, 63.7, 63.6, 41.5, 40.9, 40.1, 38.8, 35.8, 35.5, 31.9, 31.1, 30.9, 19.7, 19.6, 19.6, 19.5 ppm; LC–MS: $[M+H]^+$ calcd 309.15, $[M+H]^+$ found 309.1; HPLC: > 96 %, t_R = 1.68 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 219.3–222.5 °C (decomp.).

2-Amino-3-cyano-7-isobutyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene (15 e). 2-Benzylidenemalononitrile (28 mg, 0.182 mmol) was dissolved in absolute EtOH (3 mL) and 5-isobutylcyclohexanone (30 mg, 0.178 mmol) was dissolved herein before *N*-methylmorpholine (2.9 μL, 0.027 mmol) was added. The reaction mixture was stirred at RT for 16 h during which a precipitate was formed. This was filtered off giving a shiny white solid (7 mg, 0.027 mmol, 12 %). containing primarily two isomers of the product (estimated from ^{1}H NMR, 10:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.23 (m, 4 H), 7.23–7.15 (m, 1 H), 4.50 (s, 2 H), 4.46–4.38 (m, 1 H), 2.65–2.40 (m, 2 H), 2.32–2.11 (m, 2 H), 2.11–1.94 (m, 1 H), 1.73–1.57 (m, 1 H), 1.31–1.21 (m, 2 H), 0.96–0.77 ppm (m, 6 H). The filtrate was

evaporated giving an off-white solid which was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent 1% MeOH in CH_2Cl_2). The light-yellow band (R_f 0.3, 2% MeOH in CH_2Cl_2) was isolated and evaporated giving the title compound as an off-white solid (36 mg, 0.112 mmol, 63%, The total yield of the reaction is then 43 mg, 0.133 mmol, 75%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.32–7.23 (m, 3H), 7.23–7.17 (m, 2H), 4.51 (s, 2H), 4.43–4.39 (m, 1H), 2.63–2.53 (m, 1H), 2.49–2.12 (m, 3H), 2.08–1.98 (m, 1H), 1.70–1.60 (m, 1H), 1.31–1.20 (m, 2H), 0.91–0.85 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.2, 196.1, 163.1, 162.4, 157.7, 157.5, 143.3, 143.2, 128.8, 128.7, 127.8, 127.7, 127.3, 127.3, 118.8, 118.8, 115.2, 115.00, 63.8, 63.7, 45.0, 45.0, 43.9, 43.6, 35.7, 35.5, 33.8, 33.5, 31.2, 30.3, 29.8, 24.9, 24.8, 22.8, 22.7, 22.7, 22.6 ppm; LC–MS: $[M+H]^+$ calcd 323.18 $[M+H]^+$ found 323.1; HPLC: >95%, t_R = 1.86 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 147.0–154.8 °C (decomp.).

2-Amino-3-cyano-4-isobutyl-7-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (2 f). 2-Naphthaldehyde (45 mg, 0.288 mmol) and malononitrile (19 mg, 0.288 mmol) were dissolved in absolute EtOH (1.5 mL) before 5-(2-furyl)-cyclohexanone-1,3-dione (47 mmol, 0.264 mmol) and *N*-methylmorpholine (4.3 μL, 0.039 mmol) were added and the reaction mixture was stirred at RT for 16 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with ice cold absolute EtOH (2 mL) and dried giving the title compound as a white solid (88 mg, 0.230 mmol, 87%) which was slightly impure. The solid was triturated in boiling absolute EtOH (~35 mL) and the title compound was isolated as a white solid (42 mg, 0.110 mmol, 42%) containing almost exclusively two diastereomers (~94% evaluated by NMR). R_f (CH_2Cl_2/EtOH 10:1) 0.42; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.45 (s, 2H), 3.43–3.35 (m, 1H), 2.58–2.06 (m, 4H), 1.97–1.86 (m, 1H), 1.85–1.77 (m, 1H), 1.65–1.58 (m, 1H), 1.34 (t, J = 6.6 Hz, 2H), 0.99 (dd, J = 6.5, 4.8 Hz, 3H), 0.96–0.93 (m, 6H), 0.88 (d, J = 6.6 Hz, 2H), 0.85 ppm (d, J = 6.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.9, 164.3, 158.9, 119.8, 116.5, 47.0, 46.0, 41.5, 41.2, 40.1, 39.0, 32.0, 31.9, 31.1, 30.9, 27.6, 27.5, 24.9, 24.8, 24.0, 22.0, 22.0, 19.7, 19.7, 19.6, 19.6 ppm; LC–MS: $[M+H]^+$ calcd 289.19 $[M+H]^+$ found 289.1; HPLC: >98%, t_R = 1.51 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 250.2–253.6 °C (decomp.).

2-Amino-3-cyano-4-isobutyl-7-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (8 f). 2-Naphthaldehyde (45 mg, 0.288 mmol) and malononitrile (19 mg, 0.288 mmol) were dissolved in absolute EtOH (2 mL) before 5-(1-naphthyl)cyclohexane-1,3-dione (33 mg, 0.214 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 16 h and the resulting suspension was filtered. The white solid was washed with cold absolute EtOH (3 mL) and dried giving the title compound as a white solid (104 mg, 0.235 mmol, 82%). R_f (CH_2Cl_2/EtOH 10:1) 0.51; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.04 (d, J = 8.5 Hz, 0.6H), 7.97 (d, J = 8.0 Hz, 0.4H), 7.93–7.65 (m, 6H), 7.61–7.30 (m, 6H), 7.25–7.10 (m, 1H), 4.71 (s, 0.4H), 4.70 (s, 0.6H), 4.62 (s, 0.8H), 4.54 (s, 1.2H), 4.42–4.32 (m, 0.6H), 4.23–4.12 (m, 0.4H), 3.12–2.69 ppm (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 195.6, 195.4, 162.9, 161.9, 157.9, 157.5, 140.5, 140.3, 137.5, 137.4, 134.2, 133.6, 133.6, 133.0, 132.9, 130.9, 129.5, 129.4, 128.8, 128.6, 128.3, 128.2, 128.2, 128.1, 127.8, 127.8, 127.0, 126.8, 126.8, 126.7, 126.3, 126.2, 126.1, 126.0, 126.0, 125.9, 125.6, 125.6, 123.6, 122.9, 122.5, 122.5, 118.7, 115.4, 115.1, 63.8, 43.8, 43.1, 36.0, 35.9, 34.4, 34.1, 33.6, 33.4 ppm; LC–MS: $[M+H]^+$ calcd 443.18 $[M+H]^+$ found 443.1 HPLC: >98%, t_R = 2.05 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 206.9–219.4 °C (decomp.).

2-Amino-3-cyano-7-methyl-4-(2-naphthyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (13 f). 2-Naphthaldehyde (45 mg, 0.288 mmol) and malononitrile (19 mg, 0.288 mmol) were dissolved in absolute EtOH (1.5 mL) before 5-methylcyclohexanone-1,3-dione (33 mmol, 0.261 mmol) and *N*-methylmorpholine (4.3 μL, 0.039 mmol) were added and the reaction mixture was stirred at RT for 16 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with ice cold absolute EtOH (2 mL) and dried giving the title compound as a white solid (77 mg, 0.233 mmol, 89%). R_f (CH_2Cl_2/EtOH 10:1) 0.40; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.78 (dd, J = 19.3, 8.1 Hz, 3H), 7.69 (d, J = 8.7 Hz, 1H), 7.47–7.39 (m, 2H), 7.34 (ddd, J = 15.5, 8.5, 1.8 Hz, 1H), 4.62–4.58 (m, 1H), 4.56 (d, J = 5.6 Hz, 2H), 2.68–2.56 (m, 1H), 2.49–2.28 (m, 2.5H), 2.28–2.18 (m, 1.5H), 2.10–2.03 (m, 1H), 1.09 ppm (dd, J = 8.4, 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.1, 196.0, 163.1, 162.3, 157.7, 157.6, 140.6, 140.5, 133.6, 132.9, 128.6, 128.6, 128.2, 127.7, 126.7, 126.6, 126.2, 125.9, 125.7, 125.7, 118.7, 115.1, 114.8, 63.7, 63.6, 45.2, 45.2, 35.9, 35.7, 35.2, 35.0, 28.5, 27.9, 20.9, 20.9 ppm; LC–MS: $[M+H]^+$ calcd 331.14, $[M+H]^+$ found 331.1; HPLC: >98%, t_R = 1.65 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 243.4–249.2 °C (decomp.).

2-Amino-3-cyano-7-isopropyl-4-(2-naphthyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (14 f). 2-Naphthaldehyde (45 mg, 0.288 mmol) and malononitrile (19 mg, 0.288 mmol) were dissolved in absolute EtOH (1.5 mL) before 5-isopropylcyclohexanone-1,3-dione (40 mg, 0.259 mmol) and *N*-methylmorpholine (4.3 μL, 0.039 mmol) were added and the reaction mixture was stirred at RT for 16 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with ice cold absolute EtOH (2 mL) and dried giving the title compound as a white solid (42 mg, 0.117 mmol, 45%). R_f (CH_2Cl_2/EtOH 10:1) 0.47; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.84–7.65 (m, 4H), 7.47–7.30 (m, 3H), 4.61–4.51 (m, 3H), 2.67–2.33 (m, 3H), 2.13–1.95 (m, 1.4H), 1.88 (dddd, J = 13.6, 11.1, 6.7, 4.3 Hz, 0.6H), 1.66–1.57 (m, 1H), 0.97–0.89 ppm (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.6, 196.4, 163.7, 162.9, 157.8, 157.6, 140.6, 140.4, 133.6, 132.9, 128.6, 128.6, 128.2, 127.7, 126.7, 126.7, 126.2, 125.9, 125.9, 125.8, 125.8, 118.7, 115.0, 114.8, 63.7, 41.5, 40.9, 40.1, 38.8, 36.0, 35.7, 32.0, 31.9, 31.1, 30.9, 19.7, 19.6, 19.6, 19.6 ppm; LC–MS: $[M+H]^+$ calcd 359.18, $[M+H]^+$ found 359.1; HPLC: >98%, t_R = 1.85 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 170.3–175.5 °C (decomp.).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-(2-furyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (2 g). 2-([1,1′-Biphenyl]-4-ylmethylene)-malononitrile (59 mg, 0.255 mmol) was dissolved in absolute EtOH (50 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(furan-2-yl)cyclohexane-1,3-dione (50 mg, 0.28 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred at RT for 20 h before the solvent was removed in vacuo giving an off-white solid which was recrystallized from absolute EtOH giving the title compound (1:1 mixture of diastereomers) as white shiny crystals (45 mg, 0.111 mmol, 43%). R_f (1:1 mixture of diastereomers, CH_2Cl_2:EtOAc 3:2) 0.6 and 0.7; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59–7.28 (m, 9H), 7.18–7.10 (m, 1H), 6.32 (dd, J = 3.3, 1.9 Hz, 0.5H), 6.26 (dd, J = 3.3, 1.9 Hz, 0.5H), 6.08 (dt, J = 3.3, 0.9 Hz, 0.5H), 5.95 (dt, J = 3.3, 0.9 Hz, 0.5H), 4.58–4.45 (m, 3H), 3.64–3.57 (m, 0.5H), 3.54–3.44 (m, 0.5H), 3.06–2.52 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 194.5, 194.5, 162.1, 161.3, 157.7, 157.4, 154.9, 154.7, 142.2, 142.1, 141.9, 141.9, 141.1, 141.0, 140.3, 140.1, 128.8, 128.8, 128.2, 128.0, 127.6, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 118.7, 118.7, 115.3, 114.9, 110.4, 110.4, 106.0, 105.3, 63.6, 63.5, 41.3, 41.0, 35.3, 32.1, 31.9, 31.9,

31.7 ppm; LC–MS: $[M+H]^+$ calcd 409.15, $[M+H]^+$ found 409.1; HPLC: >98%, t_R = 2.13 + 2.19 min (1:1 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 191–193 °C (1 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-(furan-3-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (3 g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (28 mg, 0.122 mmol) was dissolved in absolute EtOH (40 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(furan-3-yl)cyclohexane-1,3-dione (24 mg, 0.135 mmol) and *N*-methylmorpholine (2 μL, 0.018 mmol) were added. The reaction mixture was stirred for 16 h during which the reaction mixture became turbid. The solvent was removed in vacuo and the resulting off-white precipitate was purified by gradient column chromatography (3 cm Ø, 200 mL SiO_2, eluent 0–3% MeOH in CH_2Cl_2) and the colorless band (R_f 0.6) was isolated and *n*-heptane (3 mL) was added before the solvent was removed in vacuo giving the title compound as a pale-pink solid (48 mg, 0.118 mmol, 96%). R_f (2% MeOH in CH_2Cl_2) 0.5; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58–7.50 (m, 3H), 7.49–7.45 (m, 1H), 7.45–7.37 (m, 3H), 7.36–7.28 (m, 2H), 7.21–7.15 (m, 2H), 6.32 (d, J = 1.9 Hz, 0.5H), 6.27 (d, J = 1.9 Hz, 0.5H), 4.58–4.46 (m, 3H), 3.49–3.39 (m, 0.5H), 3.37–3.26 (m, 0.5H), 2.94–2.63 (m, 3H), 2.60–2.40 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.4, 162.7, 161.9, 157.9, 143.7, 143.6, 142.3, 142.1, 141.0, 140.9, 140.2, 140.1, 138.8, 138.4, 128.8, 128.7, 128.1, 127.9, 127.5, 127.3, 127.2, 127.2, 127.1, 127.1, 126.4, 126.4, 119.2, 115.3, 115.0, 109.1, 108.9, 62.1, 43.4, 43.1, 35.3, 35.2, 34.0, 33.7, 29.6, 29.3 ppm; LC–MS: $[M+2H]^+$ calcd 410.16, $[M+2H]^+$ found 410.1; HPLC: >98%, t_R = 2.28 + 2.33 min (1:1 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 130.8–132.1 °C (1 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-5-oxo-7-(thiophen-3-yl)-5,6,7,8-tetrahydro-4*H*-chromene (4 g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (65 mg, 0.281 mmol) was dissolved in absolute EtOH (100 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(thiophen-3-yl)cyclohexane-1,3-dione (60 mg, 0.309 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 16 h during which it became turbid. The solvent was removed in vacuo and the resulting light brown precipitate was purified by gradient column chromatography (3 cm Ø, 200 mL SiO_2, eluent 0–3% MeOH in CH_2Cl_2) and the colorless band (R_f 0.5, 2% MeOH in CH_2Cl_2) was isolated and the solvent was removed in vacuo giving the title compound as light yellow crystals (116 mg, 0.274 mmol, 97%). R_f (2% MeOH in CH_2Cl_2) 0.5; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58–7.50 (m, 3H), 7.49–7.38 (m, 3H), 7.38–7.29 (m, 3H), 7.20–7.13 (m, 1H), 7.06–6.89 (m, 2H), 4.59–4.47 (m, 3H), 3.66–3.55 (m, 0.5H), 3.54–3.44 (m, 0.5H), 2.98–2.71 (m, 3H), 2.71–2.48 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.1, 195.0, 162.5, 157.7, 142.9, 142.7, 142.2, 141.9, 141.0, 140.4, 128.8, 128.8, 128.2, 128.0, 127.6, 127.5, 127.3, 127.3, 126.8, 126.7, 126.4, 126.1, 120.9, 120.4, 118.6, 115.4, 115.2, 63.8, 43.9, 43.6, 35.3, 35.3, 34.5, 34.3, 33.9, 33.8 ppm; LC–MS: $[M+2H]^+$ calcd 426.16, $[M+2H]^+$ found 426.0; HPLC: >98%, t_R = 2.36 + 2.41 min (1:1 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 126.2–127.9 °C (1 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-(thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (5 g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (56 mg, 0.255 mmol) was dissolved in absolute EtOH (50 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(furan-2-yl)cyclohexane-1,3-dione (**16**; 50 mg, 0.257 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 18 h after which the solvent was removed in vacuo to afford an off-white semisolid which was recrystallized from absolute EtOH to give the title compound (1:1 mixture of diastereomers) as white crystals (67 mg, 0.158 mmol, 62%). R_f (CH_2Cl_2/EtOAc 3:2, two spots, 1:1 mixture of diastereomers) 0.78–0.83; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59–7.50 (m, 3H), 7.48–7.38 (m, 3H), 7.38–7.29 (m, 2H), 7.24–7.18 (m, 1H), 7.19–7.12 (m, 1H), 6.97 (dd, J = 5.1, 3.5 Hz, 0.5H), 6.91 (dd, J = 5.1, 3.5 Hz, 0.5H), 6.88 (dt, J = 3.5, 1.1 Hz, 0.5H), 6.78 (dt, J = 3.5, 1.1 Hz, 0.5H), 4.60–4.47 (m, 3H), 3.84–3.75 (m, 0.5H), 3.69–3.63 (m, 0.5H), 3.04–2.56 ppm (m, 4H); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.68–7.53 (m, 3H), 7.52–7.27 (m, 5H), 7.12–6.87 (m, 4H), 4.28 (s, 0.5H), 4.26 (s, 0.5H), 4.03 (bs, 2H), 3.88–3.79 (m, 0.5H), 3.79–3.68 (m, 0.5H), 3.08–2.85 (m, 2H), 2.79–2.57 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 194.4, 194.2, 163.3, 162.6, 158.5, 158.3, 146.1, 143.8, 143.5, 140.0, 138.7, 138.5, 128.9, 127.9, 127.6, 127.3, 127.3, 127.0, 127.0, 126.8, 126.6, 126.6, 124.3, 124.1, 124.0, 119.7, 113.7, 113.4, 58.1, 58.1, 44.1, 43.7, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 35.2, 34.6, 34.3, 32.9 ppm; LC–MS: $[M+H]^+$ calcd 425.13, $[M+H]^+$ found 425.1 HPLC: >98%, t_R = 2.16 + 2.22 min, 1:1 mixture of diastereomers, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 126–127 °C (2 °C min^{-1}).

2-Amino-7-(benzo[*b*]thiophen-3-yl)-4-([1,1′-biphenyl]-4-yl)-3-cyano-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (6 g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (24 mg, 0.104 mmol) was dissolved in absolute EtOH (40 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(benzo[*b*]thiophen-3-yl)cyclohexane-1,3-dione (27 mg, 0.111 mmol) and *N*-methylmorpholine (2 μL, 0.018 mmol) were added. The reaction mixture was stirred for 16 h during which the reaction mixture became turbid. The solvent was decreased in vacuo to ~10 mL and the resulting white precipitate was filtered off and washed with cold MeOH (2×3 mL) giving the title compound as a white solid (42 mg, 0.089 mmol, 84%). R_f (2% MeOH in CH_2Cl_2) 0.6; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.93–7.85 (m, 1H), 7.84–7.71 (m, 1H), 7.61–7.48 (m, 4H), 7.47–7.30 (m, 6H), 7.25–7.14 (m, 1H), 6.88–6.84 (m, 1H), 4.61–4.45 (m, 3H), 4.01–3.90 (m, 0.5H), 3.87–3.75 (m, 0.5H), 3.14–2.84 (m, 3H), 2.83–2.63 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.2, 195.1, 162.6, 161.5, 157.7, 157.5, 142.2, 141.8, 141.1, 140.3, 137.7, 136.3, 128.9, 128.9, 128.3, 128.2, 127.7, 127.6, 127.4, 127.3, 127.3, 124.9, 124.9, 124.5, 124.4, 123.4, 123.4, 122.5, 121.5, 121.5, 121.2, 118.7, 115.4, 115.1, 63.5, 43.1, 42.6, 35.4, 35.4, 33.6, 33.0, 32.2, 32.1 ppm; LC–MS: $[M+H]^+$ calcd 475.15, $[M+H]^+$ found 475.1; HPLC: >98%, t_R = 2.49 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 153.1–154.7 °C (1 °C min^{-1}).

2-Amino-7-(benzo[*b*]thiophen-2-yl)-4-([1,1′-biphenyl]-4-yl)-3-cyano-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (7 g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (24 mg, 0.104 mmol) was dissolved in absolute EtOH (40 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(benzo[*b*]thiophen-2-yl)cyclohexane-1,3-dione (27 mg, 0.111 mmol) and *N*-methylmorpholine (2 μL, 0.018 mmol) were added. The reaction mixture was stirred for 24 h before additional 5-(benzo[*b*]thiophen-2-yl)cyclohexane-1,3-dione (1 mg, 0.004 mmol) and *N*-methylmorpholine (2 μL, 0.018 mmol) were added. The reaction mixture was stirred for further 16 h during which the reaction mixture became turbid. The solvent was decreased in vacuo to ~10 mL and the resulting white precipitate was filtered off and washed with cold MeOH (3× 3 mL) giving the title compound as a white solid (44 mg, 0.093 mmol, 88%). R_f (2% MeOH in CH_2Cl_2) 0.58–0.62 (two spots arising from two diastereomers); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83–7.20 (m, 12H), 7.12–6.88 (m, 2H), 4.60–4.47 (m, 3H), 3.90–3.81

(m, 0.6H), 3.80–3.69 (m, 0.4H), 3.15–2.57 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 194.5, 194.5, 162.2, 161.2, 146.0, 145.8, 142.3, 141.8, 140.9, 140.8, 140.3, 139.9, 139.7, 139.5, 138.8, 128.8, 128.7, 128.7, 128.1, 127.9, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 124.7, 124.6, 124.5, 124.5, 123.5, 123.4, 122.4, 122.2, 121.3, 120.5, 119.1, 115.6, 115.2, 62.1, 62.0, 44.2, 43.5, 35.2, 35.1, 34.9, 34.5, 34.3 ppm; LC–MS: $[M+2H]^+$ calcd 476.16, $[M+H]^+$ found 476.1; HPLC: >98%, t_R = 2.46 + 2.57 min (0.6:0.4 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 205.4–205.2 °C (1 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-(1-naphthyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (8g). 2-([1,1′-biphenyl]-4-ylmethylene)malononitrile (60 mg, 0.261 mmol) was dissolved in absolute EtOH (60 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-(1-naphthyl)cyclohexane-1,3-dione (72 mg, 0.300 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 18 h after which a second portion of 5-(1-naphthyl)cyclohexane-1,3-dione (3 mg, 0.013 mmol) was added. After 1 h a precipitate was formed and additional 5-(1-naphthyl)cyclohexane-1,3-dione (4 mg, 0.017 mmol) was added. After a total of 66 h the solvent was decreased in vacuo to ~20 mL and the resulting pale-yellow suspension was filtered giving an off-white solid which was washed with cold absolute EtOH (3×1 mL). The white solid was recrystallized from absolute EtOH giving the title compound as white shiny crystals (52 mg, 0.111 mmol, 43%) containing 0.9 mol% EtOH. R_f (CH_2Cl_2/EtOAc 6:1) 0.6; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07–7.98 (m, 1H), 7.94–7.87 (m, 1H), 7.84–7.74 (m, 1H), 7.64–7.25 (m, 12H), 7.24–7.13 (m, 1H), 4.60–4.48 (m, 3H), 4.43–4.31 (m, 0.9H), 4.27–4.16 (m, 0.1H), 3.08–2.94 (m, 2H), 2.94–2.74 (m, 2H); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.31–8.19 (m, 1H), 8.02–7.91 (m, 1H), 7.89–7.80 (m, 1H), 7.71–7.33 (m, 11H), 7.26 (d, J = 8.3 Hz, 2H), 7.05 (bs, 2H), 4.51–4.40 (m, 1H), 4.38–4.31 (m, 1H), 3.21–2.64 ppm (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.3, 163.3, 158.5, 143.7, 140.0, 138.6, 138.3, 133.5, 130.6, 128.9, 127.9, 127.3, 127.3, 126.7, 126.6, 126.4, 125.7, 125.4, 123.4, 123.0, 119.8, 113.3, 58.0, 56.0, 42.9, 40.1, 39.9, 39.8, 39.7, 39.5, 39.3, 39.1, 38.9, 35.5, 33.3, 32.9, 18.5 ppm; LC–MS: $[M+H]^+$ calcd 469.19, $[M+H]^+$ found 469.1; HPLC: >98%, t_R = 2.31 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 130–223 °C (2 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-7-([1,1′-biphenyl]-2-yl)-3-cyano-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (9g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (42 mg, 0.180 mmol) was dissolved in absolute EtOH (50 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-([1,1′-biphenyl]-2-yl)cyclohexane-1,3-dione (49 mg, 0.185 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 16 h after which the solvent was removed in vacuo and the resulting foam was purified by gradient column chromatography (3 cm Ø, 200 mL SiO_2, eluent CH_2Cl_2:EtOAc, 1:0–6:1) giving the title compound as a pale-yellow powder (80 mg, 0.162 mmol, 90%). R_f (CH_2Cl_2/EtOAc 6:1, 1:1 mixture of diastereomers) 0.5 + 0.7; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.63–7.17 (m, 18H), 4.51 (s, 2H), 4.48–4.41 (m, 1H), 3.72–3.51 (m, 1H), 2.95–2.46 (m, 4H); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.67–7.26 (m, 15H), 7.28–7.08 (m, 3H), 7.05 (s, 1H), 6.99 (s, 1H), 4.25–4.19 (m, 1H), 3.62–3.50 (m, 0.5H), 3.49–3.38 (m, 0.5H), 3.19–2.99 (m, 1H), 2.84–2.44 (m, 2H), 2.30 ppm (ddd, J = 20.8, 16.3, 3.8 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = ^{13}C NMR (100 MHz, DMSO): δ = 194.9, 194.9, 163.8, 163.0, 158.3, 143.8, 143.6, 141.3, 140.0, 139.7, 139.6, 138.6, 130.1, 128.9, 128.9, 128.4, 128.3, 127.9, 127.8, 127.5, 127.2, 126.7, 126.6, 119.6, 113.3, 113.1, 58.1, 58.0, 54.9, 43.4, 35.3, 34.4, 33.9, 33.3 ppm; LC–MS: $[M+H]^+$ calcd 495.21, $[M+H]^+$ found 495.1; HPLC: >98%, t_R = 2.40 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 145.3–146.0 °C (1 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-7-([1,1′-biphenyl]-3-yl)-3-cyano-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (10g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (41 mg, 0.178 mmol) was dissolved in absolute EtOH (50 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-([1,1′-biphenyl]-3-yl)cyclohexane-1,3-dione (50 mg, 0.189 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 16 h after which the solvent was decreased in vacuo to ~20 mL and the resulting pale-yellow suspension was filtered giving an off-white solid which was washed with cold absolute EtOH (3×1 mL) giving the title compound as a white powder (76 mg, 0.154 mmol, 86%). R_f (CH_2Cl_2/EtOAc 6:1) 0.5; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.72–7.28 (m, 17H), 7.23–7.14 (m, 1H), 7.09 (s, 1H), 7.04 (s, 1H), 4.33–4.27 (m, 1H), 3.69–3.58 (m, 0.5H), 3.56–3.43 (m, 0.5H), 3.25–3.02 (m, 1H), 2.90–2.74 (m, 2H), 2.64–2.52 ppm (m, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.1, 195.1, 164.0, 163.2, 158.7, 158.5, 143.9, 143.7, 143.4, 143.3, 140.4, 140.1, 140.0, 139.9, 138.7, 138.5, 129.1, 129.1, 128.9, 128.9, 128.8, 127.9, 127.7, 127.4, 127.3, 127.2, 126.8, 126.8, 126.6, 126.6, 126.2, 126.0, 125.6, 125.4, 125.2, 119.7, 113.6, 113.5, 58.1, 43.3, 43.3, 37.6, 37.5, 35.3, 35.2, 33.8, 33.5 ppm; LC–MS: $[M+H]^+$ calcd 495.21, $[M+H]^+$ found 495.1; HPLC: >95%, t_R = 2.35 + 2.43 min (1:1 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 243.9–264.8 °C (decomp.).

2-Amino-4,7-di([1,1′-biphenyl]-4-yl)-3-cyano-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (11g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (42 mg, 0.180 mmol) was dissolved in absolute EtOH (50 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-([1,1′-biphenyl]-4-yl)cyclohexane-1,3-dione (50 mg, 0.189 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 16 h during which the reaction mixture became turbid. The solvent was decreased in vacuo to ~40 mL and the white precipitate was filtered off and washed with cold MeOH (20 mL) giving the title compound as a white solid (68 mg, 0.137 mmol, 76%). R_f (CH_2Cl_2:EtOAc 6:1, 1:1 mixture of diastereomers) 0.7 + 0.6; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.69–7.31 (m, 17H), 7.21–7.13 (m, 1H), 7.09 + 7.05 (2 × s, 2H), 4.31 (s, 0.5H), 4.29 (s, 0.5H), 3.67–3.55 (m, 0.5H), 3.53–3.38 (m, 0.5H), 3.18–2.97 (m, 1H), 2.91–2.69 (m, 2H), 2.62–2.45 ppm (m, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 195.0, 164.0, 163.1, 158.7, 158.5, 143.9, 143.6, 141.9, 141.7, 140.0, 139.9, 139.8, 138.8, 138.7, 138.6, 138.5, 128.9, 128.9, 127.9, 127.8, 127.6, 127.3, 127.3, 126.8, 126.8, 126.7, 126.6, 126.6, 126.6, 126.5, 119.7, 113.6, 58.1, 58.0, 43.3, 43.2, 40.2, 38.9, 37.1, 37.0, 35.2, 35.2, 33.7, 33.4 ppm; LC–MS: $[M+H]^+$ calcd 495.21, $[M+H]^+$ found 495.2; HPLC: >98%, t_R = 2.40 + 2.48 min (1:1 mixture of diastereomers), reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 256–257 °C (2 °C min^{-1}).

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (12g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (67 mg, 0.292 mmol) was dissolved in absolute EtOH (100 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before cyclohexane-1,3-dione (36 mg, 0.321 mmol) and *N*-methylmorpholine (5 μL, 0.045 mmol) were added. The reaction mixture was stirred for 16 h during which the reaction mixture became turbid. The solvent was decreased in vacuo to ~15 mL and the white precipitate was filtered off and washed with cold MeOH (4×3 mL) giving the title compound as a white solid (89 mg, 0.137 mmol, 89%). R_f (CH_2Cl_2/EtOAc 10:1) 0.4; ^{1}H NMR (400 MHz,

$CDCl_3$): δ = 7.57–7.48 (m, 4H), 7.45–7.37 (m, 2H), 7.35–7.28 (m, 3H), 4.53 (s, 2H), 4.50–4.46 (m, 1H), 2.71–2.51 (m, 2H), 2.50–2.29 (m, 2H), 2.15–1.94 ppm (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.1, 163.4, 157.6, 142.4, 141.1, 140.2, 128.8, 128.1, 127.6, 127.3, 127.2, 118.8, 115.4, 77.5, 77.4, 77.2, 76.8, 63.6, 37.0, 35.2, 27.2, 20.3 ppm; LC–MS: $[M+2H]^+$ calcd 344.15, $[M+H]^+$ found 344.1; HPLC: >98%, t_R = 1.94 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 239–240 °C (2 °C min^{-1}, melts after gradual decomposition).

2-Amino-4,([1,1′-biphenyl]-4-yl)-3-cyano-7-methyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (13g). 2-([1,1′-Biphenyl]-4-ylmethylene)-malononitrile (65 mg, 0.282 mmol) was dissolved in absolute EtOH (70 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-methylcyclohexane-1,3-dione (39 mg, 0.309 mmol) and *N*-methyl-morpholine (5 µL, 0.045 mmol) were added. The reaction mixture was stirred for 16 h before the solvent was removed in vacuo and the resulting white residue was purified by gradient column chromatography (3 cm Ø, 200 mL SiO_2, eluent 0–2% MeOH in CH_2Cl_2) giving the title compound as shiny white crystals (86 mg, 0.241 mmol, 86%). R_f (2% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56–7.47 (m, 4H), 7.44–7.38 (m, 2H), 7.35–7.26 (m, 3H), 4.52 (s, 1H), 4.51 (s, 1H), 4.48–4.45 (m, 1H), 2.66–2.55 (m, 1H), 2.50–2.24 (m, 3H), 2.18–2.06 (m, 1H), 1.11 (d, J = 6.1 Hz, 1.5H), 1.10 ppm (d, J = 6.1 Hz, 1.5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.2, 196.1, 163.1, 162.3, 157.7, 157.5, 142.4, 142.3, 141.1, 140.2, 140.2, 128.8, 128.1, 127.6, 127.6, 127.3, 118.8, 115.0, 114.8, 63.8, 63.7, 53.6, 45.3, 35.4, 35.2, 35.0, 28.5, 27.9, 20.9, 20.9 ppm; LC–MS: $[M+H]^+$ calcd 357.16 $[M+H]^+$ found 357.1; HPLC: >98%, t_R = 2.21 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 116.2–117.4 (1 °C min^{-1}).

2-Amino-4,([1,1′-biphenyl]-4-yl)-3-cyano-7-isopropyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (14g). 2-([1,1′-Biphenyl]-4-ylmethylene)malononitrile (135 mg, 0.590 mmol) was dissolved in absolute EtOH (70 mL) with gentle heating. The pale-yellow solution was allowed to cool to RT before 5-isopropylcyclohexane-1,3-dione (101 mg, 0.655 mmol) and *N*-methyl-morpholine (10 µL, 0.091 mmol) were added. The reaction mixture was stirred for 16 h before the solvent was removed in vacuo and the resulting white residue was purified by gradient column chromatography (3 cm Ø, 200 mL SiO_2, eluent CH_2Cl_2/EtOAc/*n*-heptane, 30:4:6–30:5:5) giving the title compound as a pale-yellow glass, which was further purified by gradient column chromatography (3 cm Ø, 200 mL SiO_2, eluent 0–2% MeOH in CH_2Cl_2) giving the title compound as a pale-yellow solid (135 mg, 0.34 mmol, 58%). R_f (CH_2Cl_2(EtOAc 6:1, 1:1 mixture of diastereomers) 0.4 + 0.5.^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59–7.45 (m, 4H), 7.45–7.38 (m, 2H), 7.35–7.26 (m, 3H), 4.54 + 4.53 (2 s, 2H), 4.47 (s, 1H), 2.68–2.31 (m, 3H), 2.15–2.05 (m, 1H), 2.04–1.83 (m, 1H), 1.67–1.58 (m, 1H), 0.98–0.90 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.7, 196.4, 163.7, 163.0, 157.8, 157.6, 142.4, 142.2, 141.1, 140.2, 140.2, 128.8, 128.1, 127.6, 127.5, 127.3, 127.2, 118.8, 115.0, 114.8, 63.6, 63.4, 41.5, 41.0, 40.1, 38.8, 35.5, 35.2, 32.0, 31.9, 31.1, 30.9, 29.2, 22.8, 19.7, 19.6, 19.6, 14.3 ppm; LC–MS: $[M+2H]^+$ calcd 386.20 $[M+2H]^+$ found 386.1; HPLC: >98%, t_R = 2.21 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 110–112 °C (2 °C min^{-1}).

Chiral preparative HPLC: **14g** was dissolved in the mobile phase (*n*-heptane/*i*PrOH 60:40) at a concentration of 16 mg mL^{-1} and filtered through a syringe filter prior to injection. Samples of 100–200 µL were injected at a flow rate of 5 mL min^{-1}. Fractions were collected manually and purity assessed by analytical HPLC on a Chiralpac IF column. 4.6 mm Ø, 25 cm 60:40 *n*-heptane/*i*PrOH, 1.0 mL min^{-1}. Only fractions of >98% purity were pooled and concentrated to give the pure stereoisomers. See the Supporting Information for chromatograms.

14g-A. Preparative HPLC t_R = 6.92 min. Purity >99% (analytical HPLC t_R = 6.79 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.56–7.53 (m, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.41 (t, J = 7.6 Hz, 2H), 7.34–7.30 (m, 1H), 7.28 (d, J = 8.1 Hz, 2H), 4.51 (s, 2H), 4.47 (s, 1H), 2.54–2.46 (m, 3H), 2.10 (dd, J = 15.6, 12.9 Hz, 1H), 2.01 (ddt, J = 12.8, 6.3, 3.4 Hz, 1H), 1.62 (h, J = 6.7 Hz, 1H), 0.94 ppm (dd, J = 12.3, 6.7 Hz, 6H); ^{1}H NMR (600 MHz, [D_6]ethanol/D_2O 1:1): δ = 7.62–7.59 (m, 2H), 7.55 (d, J = 8.3 Hz, 2H), 7.49 (t, J = 7.8 Hz, 2H), 7.39 (t, J = 7.4 Hz, 1H), 7.29 (d, J = 8.3 Hz, 2H), 4.36 (s, 1H), 2.72 (dd, J = 3.6, 1.8 Hz, 2H), 2.70–2.62 (m, 2H), 2.51 (dd, J = 16.2, 3.9 Hz, 1H), 2.20 (dd, J = 16.1, 11.7 Hz, 1H), 2.04 (d, J = 4.9 Hz, 1H), 1.66 (h, J = 6.7 Hz, 1H), 0.98 (d, J = 6.7 Hz, 3H), 0.94 ppm (d, J = 6.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.5, 162.8, 157.4, 142.0, 140.9, 140.1, 128.7, 128.0, 127.4, 127.1, 127.0, 118.6, 114.7, 63.4, 41.4, 39.9, 35.3, 31.9, 30.8, 29.7, 19.5 ppm.

14g-B. Preparative HPLC t_R = 7.60 min. Purity >99% (analytical HPLC t_R = 7.47 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.56–7.53 (m, 2H), 7.51 (d, J = 8.3 Hz, 2H), 7.41 (t, J = 7.7 Hz, 2H), 7.36–7.30 (m, 3H), 4.52 (s, 2H), 4.47 (d, J = 1.3 Hz, 1H), 2.61 (ddd, J = 17.7, 4.5, 1.4 Hz, 1H), 2.51 (ddd, J = 16.7, 3.7, 1.4 Hz, 1H), 2.38 (ddd, J = 17.8, 11.6, 1.5 Hz, 1H), 2.10 (dd, J = 16.7, 13.5 Hz, 1H), 1.91 (dtt, J = 11.1, 8.4, 4.3 Hz, 1H), 1.62 (dd, J = 13.4, 6.7 Hz, 1H), 0.95 ppm (dd, J = 10.4, 6.8 Hz, 6H); ^{1}H NMR (600 MHz, [D_6]ethanol/D_2O 1:1): δ = 7.59 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 8.1 Hz, 2H), 7.50–7.45 (m, 2H), 7.42–7.35 (m, 2H), 7.31 (d, J = 8.2 Hz, 1H), 4.38 (s, 1H), 2.74 (dd, J = 17.7, 4.1 Hz, 1H), 2.57–2.51 (m, 1H), 2.48 (dd, J = 17.3, 3.4 Hz, 1H), 2.25 (dd, J = 11.1, 4.0 Hz, 1H), 1.91–1.83 (m, 1H), 1.72–1.65 (m, 1H), 0.99 (d, J = 6.8 Hz, 3H), 0.97 ppm (d, J = 6.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.4, 163.6, 157.7, 142.2, 140.9, 140.0, 128.6, 127.9, 127.4, 127.1, 127.0, 118.8, 114.8, 63.1, 40.8, 38.6, 35.0, 31.7, 30.9, 29.7, 19.4 ppm.

14g-C. Preparative HPLC t_R = 8.51 min. Purity 98.6% (contains 1.4% **14g-D**, analytical HPLC t_R = 8.31 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.55 (dd, J = 8.2, 3.8 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 7.41 (t, J = 7.7 Hz, 2H), 7.37–7.29 (m, 3H), 4.52 (s, 2H), 4.47 (s, 1H), 2.61 (dd, J = 17.2, 3.9 Hz, 1H), 2.51 (dd, J = 16.7, 2.6 Hz, 1H), 2.41–2.34 (m, 1H), 2.10 (dd, J = 16.7, 13.5 Hz, 1H), 1.95–1.88 (m, 1H), 1.62 (dd, J = 13.4, 6.7 Hz, 1H), 0.94 ppm (dd, J = 10.3, 6.8 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.3, 163.6, 157.6, 142.2, 140.9, 140.1, 128.7, 127.9, 127.4, 127.1, 127.0, 118.6, 114.8, 63.5, 40.8, 38.7, 35.0, 31.7, 30.9, 29.7, 19.4 ppm.

14g-D. Preparative HPLC t_R = 9.62 min. Purity >99% (analytical HPLC t_R = 9.41 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.54 (dd, J = 8.2, 1.1 Hz, 2H), 7.50 (d, J = 8.3 Hz, 2H), 7.41 (t, J = 7.7 Hz, 2H), 7.34–7.29 (m, 1H), 7.28 (d, J = 8.2 Hz, 2H), 4.51 (s, 2H), 4.47 (s, 1H), 2.55–2.45 (m, 3H), 2.15–2.06 (m, 1H), 2.04–1.96 (m, 1H), 1.66–1.59 (m, 1H), 0.94 ppm (dd, J = 12.3, 6.8 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.5, 162.8, 157.4, 142.0, 140.9, 140.0, 128.7, 128.0, 127.4, 127.1, 127.0, 118.6, 114.7, 63.4, 41.4, 39.9, 35.3, 31.9, 30.8, 29.7, 19.5 ppm.

Stereochemical stability

By HPLC: The stereoisomers **14g-A** and **14g-C** (~0.3 mg) were dissolved in 1 mL of EtOH/D_2O (1:1) plus a few drops of DMSO to keep the compounds in solution. Epimerization was monitored by analytical HPLC after 3 h, 24 h, and 48 h. Stereoisomer **14g-A** epimerized to **14g-B** at the following quantities: at 3 h: <2%, 24 h: 8%, 48 h: 18%. Stereoisomer **14g-C** epimerized to **14g-D** at the

following quantities: at 3 h: <1%, 24 h: 10%, 48 h: 21%. See the Supporting Information for chromatograms.

By ^{1}H NMR experiment with 14g-A: Stereoisomer **14g-A** was dissolved in [D$_6$]ethanol/D$_2$O (1:1) and spectra were collected at $t=0$, 24, 48 and 120 h. Epimerization was analyzed according to changes in the ratio of H4 signals of **14g-A**/**14g-B**.

2-Amino-4-([1,1′-biphenyl]-4-yl)-3-cyano-7-isobutyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene (15g). 2-([1,1′-Biphenyl]-4-ylmethylene)-malononitrile (39 mg, 0.169 mmol) was dissolved in absolute EtOH (20 mL) with gentle heating and the solution was left to cool to RT before 5-isobutylcyclohexanone (30 mg, 0.178 mmol) and *N*-methylmorpholine (2.9 μL, 0.027 mmol) were added. The reaction mixture was stirred at RT for 16 h during which the reaction mixture became yellow. The solvent was removed to give an off-white solid which was purified by column chromatography (2 cm Ø, 50 mL SiO$_2$, eluent 1% MeOH in CH$_2$Cl$_2$). The colorless band (R_f 0.3, 2% MeOH in CH$_2$Cl$_2$) was isolated and the solvent evaporated to give the title compound as an off-white solid (65 mg, 0.163 mmol, 96%). R_f (2% MeOH in CH$_2$Cl$_2$) 0.3; ^{1}H NMR (600 MHz, CDCl$_3$): $\delta=$ 7.58–7.48 (m, 4H), 7.41 (t, $J=7.5$ Hz, 2H), 7.35–7.26 (m, 3H), 4.56 (d, $J=7.4$ Hz, 2H), 4.49–4.44 (m, 1H), 2.65–2.53 (m, 1H), 2.51–2.44 (m, 1H), 2.44–2.37 (m, 0.5H), 2.35–2.24 (m, 1H), 2.23–2.14 (m, 0.5H), 2.10–2.00 (m, 1H), 1.29–1.23 (m, 3H), 0.92–0.86 ppm (m, 6H); ^{13}C NMR (150 MHz, CDCl$_3$): $\delta=$196.3, 196.2, 163.2, 162.6, 157.8, 157.6, 142.4, 142.3, 141.1, 141.1, 140.2, 140.2, 128.8, 128.2, 128.1, 127.6, 127.5, 127.3, 127.3, 127.2, 118.8, 118.8, 115.1, 114.9, 63.6, 63.5, 45.0, 44.7, 43.9, 43.6, 35.4, 35.3, 33.8, 33.5, 31.2, 30.4, 24.9, 24.8, 22.8, 22.8, 22.7, 22.6 ppm; LC–MS: $[M+H]^+$ calcd 299.20 $[M+H]^+$ found 299.2; HPLC: >98%, $t_R=$2.17 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 195.3–197.6 °C (decomp.).

2-Amino-3-cyano-5-oxo-4-(4-(naphthalen-1-yl)phenyl)-7-phenyl-5,6,7,8-tetrahydro-4*H*-chromene (1h). 4-(1-Naphthyl)-benzaldehyde (20 mg, 0.086 mmol) and malononitrile (7 mg, 0.106 mmol) were dissolved in absolute EtOH (2 mL). The mixture was stirred at RT for 5 min before 5-phenylcyclohexane-1,3-dione (18 mg, 0.096 mmol) and *N*-methylmorpholine (2 μL, 0.018 mmol) were added and the reaction mixture was stirred at RT for 16 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with cold absolute EtOH (1 mL) and dried to give the title compound as a white solid (30 mg, 0.064 mmol, 74%). R_f (2% MeOH in CH$_2$Cl$_2$) 0.6; ^{1}H NMR (400 MHz, CDCl$_3$): $\delta=$7.97–7.88 (m, 2H), 7.85 (d, $J=8.2$ Hz, 1H), 7.54–7.26 (m, 12H), 7.21 (d, $J=7.5$ Hz, 1H), 4.58 (t, $J=10.1$ Hz, 3H), 3.58–3.51 (m, 0.5H), 3.47–3.38 (m, 0.5H), 3.00–2.91 (m, 0.5H), 2.90–2.81 (m, 1.5H), 2.81–2.69 (m, 1.5H), 2.69–2.59 ppm (m, 0.5H); ^{13}C NMR (100 MHz, CDCl$_3$): $\delta=$195.4, 195.2, 162.8, 161.9, 157.8, 157.6, 142.2, 141.9, 141.8, 140.1, 139.8, 139.7, 133.9, 131.8, 130.5, 130.4, 129.2, 129.1, 128.3, 127.7, 127.6, 127.5, 127.1, 127.1, 126.9, 126.8, 126.4, 126.2, 126.1, 125.9, 125.5, 118.8, 115.4, 115.3, 63.9, 63.8, 44.0, 43.8, 38.7, 38.3, 35.5, 35.4, 34.8, 34.7 ppm; LC–MS: $[M+H]^+$ calcd 469.19, $[M+H]^+$ found; HPLC: >98%, $t_R=$2.21 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 148.3–208.8 °C (decomp.).

2-Amino-3-cyano-7-(2-furyl)-5-oxo-4-(4-(naphthalen-1-yl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene (2h). 4-(1-Naphthyl)-benzaldehyde (20 mg, 0.086 mmol) and malononitrile (7 mg, 0.106 mmol) were dissolved in absolute EtOH (2 mL). The mixture was stirred at RT for 5 min before 5-(2-furylcyclohexane-1,3-dione (17 mg, 0.095 mmol) and *N*-methylmorpholine (1.4 μL, 0.012 mmol) were added and the reaction mixture was stirred at RT for 16 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with cold absolute EtOH (0.5 mL) and dried to give the title compound as a white solid (26 mg, 0.567 mmol, 66%). R_f (2% MeOH in CH$_2$Cl$_2$, 2 diastereomers) 0.35+ 0.26; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=$8.00 (d, $J=8.0$ Hz, 1H), 7.94 (dd, $J=8.2$, 2.9 Hz, 1H), 7.80 (dd, $J=24.6$, 8.3 Hz, 1H), 7.62–7.48 (m, 4H), 7.43 (dd, $J=13.1$, 5.4 Hz, 2H), 7.35 (dd, $J=26.1$, 8.1 Hz, 2H), 7.08 (dd, $J=14.9$, 6.8 Hz, 3H), 6.39 (ddd, $J=32.6$, 3.1, 1.9 Hz, 1H), 6.17 (dd, $J=78.3$, 3.2 Hz, 1H), 4.33 (s, 0.5H), 4.29 (s, 0.5H), 3.70–3.65 (m, 0.5H), 3.63–3.56 (m, 0.5H), 3.11–2.84 (m, 2H), 2.79–2.58 ppm (m, 2H); ^{1}H NMR (400 MHz, CDCl$_3$): $\delta=$7.95–7.81 (m, 3H), 7.54–7.46 (m, 2H), 7.46–7.30 (m, 6H), 7.18 (d, $J=8.1$ Hz, 1H), 6.29 (ddd, $J=48.8$, 3.1, 1.9 Hz, 1H), 6.03 (dd, $J=82.4$, 3.2 Hz, 1H), 4.56 (dd, $J=25.5$, 13.1 Hz, 3H), 3.66–3.60 (m, 0.5H), 3.58–3.50 (m, 0.5H), 3.06–2.92 (m, 1.5H), 2.89–2.74 (m, 2H), 2.62 ppm (dd, $J=16.9$, 12.3 Hz, 0.5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=$194.4, 194.3, 163.4, 162.5, 158.6, 158.5, 155.5, 155.3, 143.7, 143.4, 142.0, 141.9, 139.2, 138.3, 138.1, 133.4, 130.8, 129.8, 129.6, 128.4, 127.6, 127.5, 127.3, 127.0, 126.9, 126.8, 126.4, 126.3, 125.9, 125.6, 125.2, 125.2, 119.8, 113.6, 113.4, 110.4, 110.4, 105.7, 105.2, 58.2, 58.2, 40.8, 40.4, 35.2, 35.1, 31.2, 31.1, 30.9, 30.7 ppm; ^{13}C NMR (100 MHz, CDCl$_3$): $\delta=$194.5, 162.1, 161.3, 157.7, 157.5, 154.9, 154.7, 142.1, 142.1, 142.0, 141.8, 140.2, 140.1, 139.8, 139.6, 133.9, 131.8, 131.7, 130.5, 130.4, 128.3, 127.7, 127.7, 127.5, 127.1, 127.0, 126.4, 126.2, 126.1, 125.9, 125.9, 125.5, 118.8, 118.7, 115.5, 115.2, 110.5, 110.4, 106.1, 105.3, 41.4, 41.0, 35.3, 35.3, 32.1, 32.0, 32.0, 31.7 ppm; LC–MS: $[M+H]^+$ calcd 459.17, $[M+H]^+$ found 459.1; HPLC: >98%, $t_R=$2.11 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 176.7–199.0 °C (decomp.).

2-Amino-3-cyano-7-methyl-5-oxo-4-(4-(naphthalen-1-yl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene (13h). 4-(1-Naphthyl)-benzaldehyde (20 mg, 0.086 mmol) and malononitrile (7 mg, 0.106 mmol) were dissolved in absolute EtOH (2 mL). The mixture was stirred at RT for 5 min before 5-methylcyclohexane-1,3-dione (12 mg, 0.095 mmol) and *N*-methylmorpholine (1.4 μL, 0.012 mmol) were added and the reaction mixture was stirred at RT for 16 h during which a white precipitate was formed. The suspension was filtered and the white solid was washed with cold absolute EtOH (0.5 mL) and dried to give the title compound as a white solid (27 mg, 0.066 mmol, 77%) containing a small amount of EtOH (4% *w/w*). R_f (2% MeOH in CH$_2$Cl$_2$) 0.35; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=$8.00 (d, $J=7.9$ Hz, 1H), 7.94 (d, $J=8.2$ Hz, 1H), 7.80 (dd, $J=8.3$, 5.3 Hz, 1H), 7.60–7.48 (m, 3H), 7.45–7.39 (m, 3H), 7.30 (dd, $J=12.5$, 8.1 Hz, 2H), 7.06 (d, $J=8.5$ Hz, 2H), 4.29 (d, $J=2.6$ Hz, 1H), 2.61 (s, 2H), 2.38 (s, 2H), 2.28–2.10 (m, 1H), 1.04 ppm (t, $J=5.8$ Hz, 3H); ^{1}H NMR (400 MHz, CDCl$_3$): $\delta=$7.94–7.87 (m, 2H), 7.83 (d, $J=8.2$ Hz, 1H), 7.53–7.45 (m, 2H), 7.44–7.37 (m, 4H), 7.34 (dd, $J=12.5$, 8.0 Hz, 2H), 4.56 (d, $J=3.9$ Hz, 2H), 4.53 (s, $J=5.7$ Hz, 0.5H), 4.52 (s, 0.5H), 2.70–2.56 (m, 1H), 2.54–2.27 (m, 3H), 2.20–2.07 (m, 1H), 1.15–1.10 ppm (m, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=$195.94, 195.90, 164.36, 163.56, 158.68, 158.57, 143.89, 143.86, 139.19, 138.19, 138.14, 133.43, 130.77, 129.81, 129.76, 128.35, 127.55, 127.20, 127.18, 126.88, 126.36, 125.91, 125.58, 125.23, 125.20, 119.88, 113.41, 113.22, 58.14, 58.07, 44.42, 44.39, 35.38, 35.15, 34.32, 33.96, 27.74, 27.37, 20.27 ppm; ^{13}C NMR (100 MHz, CDCl$_3$): $\delta=$196.3, 196.2, 163.2, 162.4, 157.8, 157.6, 142.3, 142.2, 140.1, 139.7, 133.9, 131.7, 130.5, 130.4, 128.3, 127.7, 127.6, 127.6, 127.1, 126.4, 126.1, 125.9, 125.5, 118.9, 114.9, 63.9, 58.7, 45.4, 45.2, 35.5, 35.3, 35.0, 28.6, 27.9, 21.0, 20.9 ppm; LC–MS: $[M+H]^+$ calcd 407.18, $[M+H]^+$ found 407.1; HPLC: >98%, $t_R=$2.07 min, reversed phase, H$_2$O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 212.5–216.7 °C (decomp.).

2-Amino-3-cyano-7-isopropyl-5-oxo-4-(4-(naphthalen-1-yl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene (14h). 4-(1-Naphthyl)-benzaldehyde (20 mg, 0.086 mmol) and malononitrile (7 mg, 0.106 mmol) were dissolved in absolute EtOH (2 mL). The mixture was stirred at RT for 5 min before 5-isopropylcyclohexane-1,3-dione (15 mg, 0.097 mmol) and *N*-methyl-morpholine (1.4 μL, 0.012 mmol) were added and the reaction mixture was stirred at RT for 16 h. The solvent was removed and the resulting yellow oil was purified by column chromatography (2 cm Ø, 50 mL SiO_2, eluent CH_2Cl_2/EtOAc 30:1) and the colorless band (R_f 0.34, 2% MeOH in CH_2Cl_2) was isolated and the solvent evaporated giving the title compound as a white solid (34 mg, 0.078 mmol, 91%) containing a small amount of EtOH (4% *w/w*). R_f (2% MeOH in CH_2Cl_2) 0.34; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.95–7.86 (m, 2H), 7.84 (d, J = 8.2 Hz, 1H), 7.53–7.31 (m, 8H), 4.57 (d, J = 3.3 Hz, 2H), 4.53 (s, 1H), 2.63 (dd, J = 17.8, 3.4 Hz, 0.5H), 2.59–2.46 (m, 2H), 2.39 (ddd, J = 17.8, 11.5, 1.3 Hz, 0.5H), 2.20–2.09 (m, 1H), 2.08–1.92 (m, 1H), 1.64 (dq, J = 13.4, 6.7 Hz, 1H), 0.99–0.93 ppm (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 196.8, 196.5, 163.8, 163.1, 157.8, 157.7, 142.3, 142.1, 140.1, 139.7, 139.6, 133.9, 131.7, 130.5, 130.4, 128.3, 127.7, 127.6, 127.6, 127.1, 127.1, 126.4, 126.1, 125.9, 125.5, 118.9, 118.9, 115.1, 114.9, 63.9, 63.6, 41.6, 41.0, 40.2, 38.8, 35.5, 35.3, 32.1, 31.9, 31.1, 31.0, 19.7, 19.6, 19.6 ppm; LC–MS: $[M+H]^+$ calcd 435.21, $[M+H]^+$ found 435.1; HPLC: >98%, t_R = 2.24 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 123.6–128.0 °C (decomp.).

5-(Thiophen-2-yl)cyclohexane-1,3-dione (16). Diethyl malonate (1.7 mL, 10.8 mmol) was dissolved in absolute EtOH (100 mL). Sodium ethoxide in EtOH (4.2 mL, 10.8 mmol) was added dropwise. The mixture was stirred at RT for 50 min, after which (*E*)-4-(thiophen-2-yl)but-3-en-2-one (1.50 g, 9.86 mmol) dissolved in absolute EtOH (100 mL) was added slowly over 20 min. The mixture was heated at reflux and stirred at reflux for 3.5 h after which it was allowed to cool to RT. The mixture was evaporated to dryness and then suspended in 2 M NaOH (20 mL). The suspension was heated at 90 °C and stirred 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 1–2. The mixture was heated at reflux and stirred at that temperature for 105 min. The mixture was cooled to RT and then extracted with EtOAc (150 mL and 100 mL). The combined organic phases were dried using anhydrous $MgSO_4$, filtered and evaporated to give a brown solid (2.18 g). The crude product was recrystallized from EtOAc/toluene to give the title compound (0.949 g, 50%) as an off-white solid. R_f (EtOAc/MeOH/AcOH 100:10:1) 0.71; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.20 (bs, 1H), 7.36 (dd, 1H, J = 1.8 Hz, J = 4.5 Hz), 6.99–6.94 (m, 2H), 5.27 (s, 1H), 3.67–3.58 (m, 1H), 2.67–2.51 ppm (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 147.2, 126.9, 123.73, 123.65, 103.7, 33.9 ppm; LC–MS: $[M+H]^+$ calcd for $C_{10}H_{11}O_2S$ 195.05, found 195.1; HPLC: >95%, t_R = 1.24 min, reversed phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}; mp: 145.3–147.9 °C (1 °C min^{-1}).

[^{3}H]D-Asp uptake assay. The [^{3}H]D-Asp uptake assay using stable EAAT1-HEK293, EAAT2-HEK293, and EAAT3-HEK293 cell lines was performed essentially as previously described.[16] Briefly, cells were split into poly-D-lysine-coated white 96-well plates (PerkinElmer, Boston, MA, USA); 16–24 h later the culture medium was aspirated, and cells were washed once with 100 μL assay buffer (Hank's buffered saline solution supplemented with 20 mM HEPES, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4). Assay buffer (50 μL) supplemented with 100 nM [^{3}H]D-Asp and various concentrations of test compound were then added to the wells, and the plate was incubated at 37 °C for 5 min. Nonspecific [^{3}H]D-Asp uptake was determined in wells with 3 mM L-glutamate. The assay mixtures were quickly removed from the wells, which were then washed with 2×100 μL ice-cold assay buffer, and 150 μL Microscint 20 scintillation fluid (PerkinElmer) was added to each well. The plate was shaken for 1 h and counted in a Wallac 1450 MicroBeta Trilux scintillation counter (GMI, Ramsey, MN, USA). The experiments were performed in duplicate at least three times for each ligand at each of the three cell lines.

In silico calculations. Log*P* values were calculated (cLog*P*) using the MOE 2014 software package (ChemComp Group). Standard setup was employed.

Abbreviations: EAAT1: excitatory amino acid transporter subtype 1; SAR: structure–activity relationship; UCPH-101: 2-amino-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile; UCPH-102: 2-amino-4-methyl-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile.

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Keywords: chromenes · excitatory amino acid transporters · glutamate · structure–activity relationships · three-component reactions

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