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Structure−Activity Relationship Study of First Selective Inhibitor of Excitatory Amino Acid Transporter Subtype 1: 2-Amino-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (UCPH-101)

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The excitatory amino acid transporters (EAATs) are expressed throughout the central nervous system, where they are responsible for the reuptake of the excitatory neurotransmitter (*S*)-glutamate (Glu).[1] Recently, we have reported the discovery of the first subtype selective 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) (**1b**) and presented an introductory structure−activity relationship (SAR) study.[2] Here, we present a detailed SAR by the design, synthesis, and pharmacological evaluation of analogues **1g**−**1t**. By comparison of potencies of **1b**, **1h**, and **1i** versus **1j**, it is evident that potency is largely influenced by the chemical nature of the R^1 substituent. The study also demonstrates that any chemical change of the functional groups or a change to the parental scaffold results in the complete loss of inhibitory activity of the compounds at EAAT1. Finally, a bioavailability study of UCPH-101 determined the half-life to be 30 min in serum (rats) but also that it was not able to penetrate the blood−brain barrier to any significant degree.

Introduction

In the central nervous system (CNS[a]), the excitatory amino acid transporters (EAATs) are believed to be primarily responsible for the uptake of the excitatory neurotransmitter (*S*)-glutamate (Glu) into glial cells and neurons, hereby maintaining synaptic as well as extra-synaptic Glu concentrations below levels of neurotoxicity.[1] To this day, five EAAT subtypes have been identified and named EAAT1−5 in humans. For reasons of tradition, the rodent orthologues are named GLAST, GLT-1, EAAC1, EAAT4, and EAAT5. The five EAAT subtypes exhibit distinct expression patterns in the CNS: While EAAT1−3 (GLAST, GLT-1, EAAC1) are expressed throughout the CNS, the EAAT4,5 subtypes are expressed exclusively in Purkinje cells of the cerebellum and in the retina, respectively.[3] At the cellular level, EAAT1,2 are expressed predominantly in glia cells and astrocytes, whereas EAAT3,4 are expressed almost exclusively on neurons.[4] With respect to function, EAAT1−3 are high-capacity Glu transporters, while EAAT4,5 are considered to be low-capacity Glu transporters, functioning primarily as Glu-gated chloride channels.[4] Malfunction of the EAATs has been suggested to be an underlying factor in neurodegenerative diseases such as Alzheimer's,[5] Huntington's,[6] amyotrophic lateral sclerosis (ALS),[7] cerebral stroke,[8] and epilepsy.[1,9,10]

As a part of our medicinal chemistry research program within glutamatergic neurotransmission, we are interested in the development of subtype selective ligands for the EAATs.[2] Besides the therapeutic potential of such ligands, they are also highly interesting as pharmacological tools, enabling the explorations of the respective roles of the EAAT subtypes in physiology and in disease. Recently we reported the discovery of the first class of compounds acting as subtype selective inhibitors of EAAT1.[2] By screening of a 3040 compound library at the EAAT1−3, compound **1a** was found to selectively inhibit EAAT1 over EAAT2 and EAAT3, displaying inhibitory activities in low micromolar range at this subtype (IC_{50} = 4.7 μM, Table 1). An initial structure−activity relationship (SAR) study was subsequently carried out by variation of the R^1 and R^2 groups, and the compound 2-amino-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (UCPH-101, **1b**), which is now commercially available,[11] turned out to be the most potent analogue in the series (IC_{50} = 0.66 μM) (Figure 1). In the same study, it was also concluded that an aromatic ring was mandatory in the 7-position (R^1) of the parental skeleton and that an alkyl or aryl substituent in the 4-position (R^2) was essential for the inhibitory activity of the analogues at EAAT1. In this full paper, we present a comprehensive SAR study of this compound class as well as pharmacokinetic data for UCPH-101.

Results and Discussion

Building on the first reported SAR, a series of new analogues were designed in order to explore the importance of the

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[a]Abbreviations: BBB, blood−brain barrier; CNS, central nervous system; EAAT(s), excitatory amino acid transporter(s); HMBC, heteronuclear multiple bond coherence; HMQC, heteronuclear multiple quantum coherence; SAR, structure−activity relationship.

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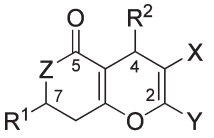
Table 1. Pharmacological Characterization of **1a**−**s** as Potential Inhibitors at hEAAT1 in a [^{3}H]-D-Aspartate Uptake Assay[a]



(1a-s)

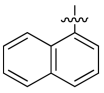
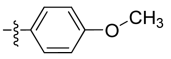
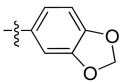
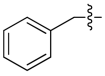
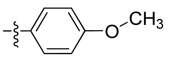
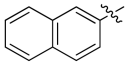
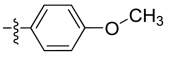
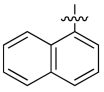
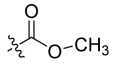
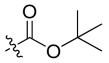
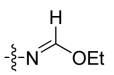
Entry	R^1	R^2	X	Y	Z	EAAT1
1a			-CN	$-NH_2$	$-CH_2-$	4.7 [5.32 ± 0.03]
1b, UCPH-101			-CN	$-NH_2$	$-CH_2-$	0.66 [6.18 ± 0.08]
1c			-CN	$-NH_2$	$-CH_2-$	9.2 [5.04 ± 0.05]
1d			-CN	$-NH_2$	$-CH_2-$	>300 [<3.5]
1e			-CN	$-NH_2$	$-CH_2-$	>300 [<3.5]
1f			-CN	$-NH_2$	$-CH_2-$	>300 [<3.5]
1g			-CN	$-NH_2$	$-CH_2-$	5.1 [5.32 ± 0.11]
1h			-CN	$-NH_2$	$-CH_2-$	3.2 [5.49 ± 0.04]
1i			-CN	$-NH_2$	$-CH_2-$	0.43 [6.47 ±0.21]
1j			-CN	$-NH_2$	$-CH_2-$	3.9 [5.48±0.20]
Syn- or *anti*-**1j**			-CN	$-NH_2$	$-CH_2-$	3.8 [5.50±0.18]
Syn- or *anti*-**1j**			-CN	$-NH_2$	$-CH_2-$	6.40 [5.20±0.08]
1k				$-NH_2$	$-CH_2-$	>300 [<3.5]
1l				$-NH_2$	$-CH_2-$	>75 [<3.8]
1m				$-NH_2$	$-CH_2-$	>100 [<4.0]
1n			-H	$-NH_2$	$-CH_2-$	>100 [<4.0]
1o			-CN		$-CH_2-$	>1000 [<3.0]
1p			-CN		$-CH_2-$	>100 [<4.0]
1q			-CN	-H	$-CH_2-$	>100 [<4.0]

Table 1. Continued

Entry	R^1	R^2	X	Y	Z	EAAT1
Syn-**1r** Lactam			-CN	$-NH_2$	-NH-	>300 [<3.5]
Anti-**1r** lactam			-CN	$-NH_2$	-NH-	>300 [<3.5]
Syn-**1s** lactone			-CN	$-NH_2$	-O-	>300 [<3.5]
Anti-**1s** lactone			-CN	$-NH_2$	-O-	>300 [<3.5]

[a] Data are given as IC_{50} values in μM with $pIC_{50} \pm$ SEM in brackets. All analogues were without inhibitory activity at EAAT2 and EAAT3 when tested at the highest possible concentration (75 μM for analogue **1l**, 100 μM for analogues **1a**, **1g**, **1i**, **1m**, **1n**, **1p**, **1q**, 300 μM for analogues **1b**−**f**, **1h**, **1k**, *syn*-**1r**, *anti*-**1r**, *syn*-**1s**, *anti*-**1s**, and 1000 μM for analogues **1j**, **1o**).

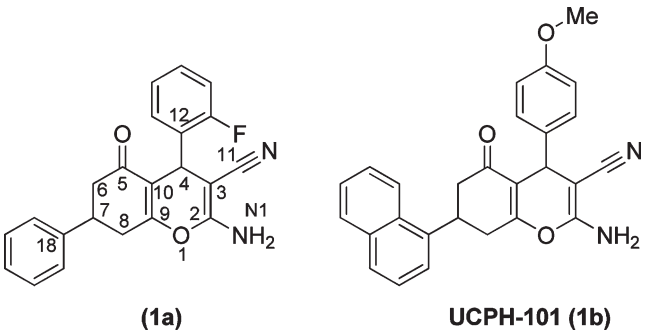


Figure 1. Lead structure **1a** identified from screening of a small compound library and **1b** (UCPH-101) as the most potent analogue from the initial SAR study.[2]

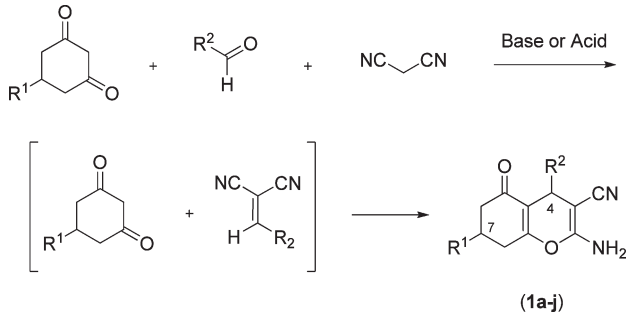
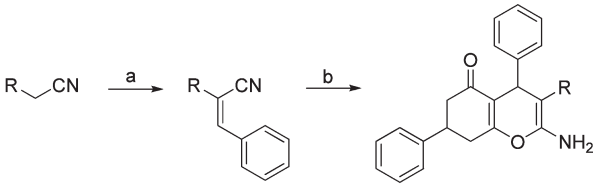


Figure 2. Generally used three component reaction of diketone, aldehyde, and malononitrile for the preparation of the 2-amino-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile parental skeleton with various substituents R^2 and R^1 in the 4- and 7-positions, respectively.

cyano-, amino-, and keto functionalities for the inhibitory activity at EAAT1. Furthermore, additional variations of the substituents in the R^1 and R^2 positions were performed.

Synthesis of Analogues in 4- and 7-Positions. To further address the influence of the chemical nature of the substituents in the 4-/7-position, the four analogues **1g**−**j** were designed and prepared following a three-component reaction strategy (Figure 2) as reported for UCPH-101.[2] In general, an approximately 1:1 ratio of diastereomers was observed based on ^1H NMR analyses. By monitoring the three component reaction, it is observed that the aldehyde and the malononitrile components form an intermediate condensation product, which then reacts with the diketone to form the desired product. The compounds may also be synthesized in a two-step process, wherein the intermediate condensation product is first isolated and then reacted with the diketone component. The diketone components used in the synthesis of **1g** and **1i** were synthesized in accordance with literature.[2,12]

Scheme 1. Reagents and Conditions Used for the Synthesis of **1k**−**m** as Analogues of the Cyano Functionality[a]

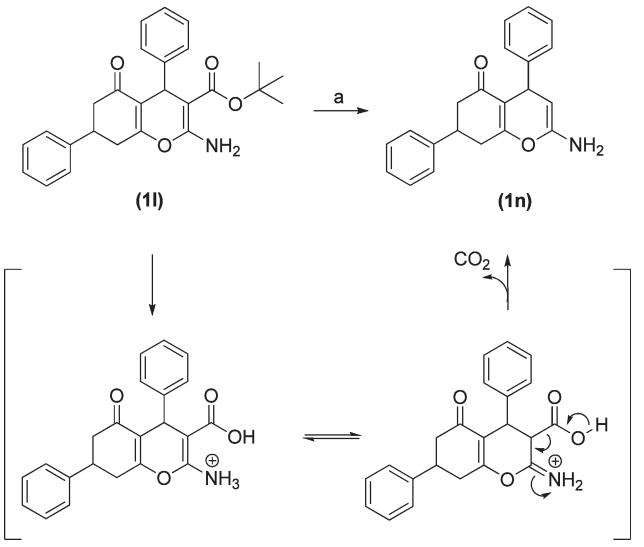


[a] Reagents and conditions: (a) For **3a**: benzaldehyde, piperidine, MeOH, reflux, 70 min, 93%; for **3b**: benzaldehyde, piperidine, *i*-PrOH, reflux, 70 min, 67%; for **3c**, benzaldehyde, piperidine, EtOH, rt., 3 h, 42%. (b) For **1k**,**l**: 5-phenylcyclohexane-1,3-dione, H_2O/EtOH, MW, 85 °C, 30 min, 67% and 72%, respectively; for **1m**: 5-phenylcyclohexane-1,3-dione, dioxane, piperidine, rt, overnight, 57%.

Synthesis of Analogues in the 3-Position (3-Cyano Group). To investigate the significance of the cyano group in the 3-position of parental skeleton and to explore the pharmacological effect of extending the ligand in this direction, esters and amide analogues **1k**−**m** were designed. The strategy for their synthesis seemed straightforward; exchange of the malononitrile component used in the three-component reaction with the corresponding 2-cyano analogues was believed to give access to the desired products. Despite several attempts, the three component reaction led to a condensation product between the aldehyde and diketone. However, performing the reaction in two steps by first synthesis and isolation of the condensation product (**3a**, **3b**, **3c**) of benzaldehyde and **2a**, **2b**, or **2c** followed by reaction with the diketone gave the amide and ester analogues in good yields (Scheme 1).

Elimination of the 3-cyano group will induce a significant change in the electronic distribution of the skeleton; thus

Scheme 2. Mechanism of Unexpected Decarboxylation of **1l** under Acidic Conditions to Give Analogue **1n**[a]

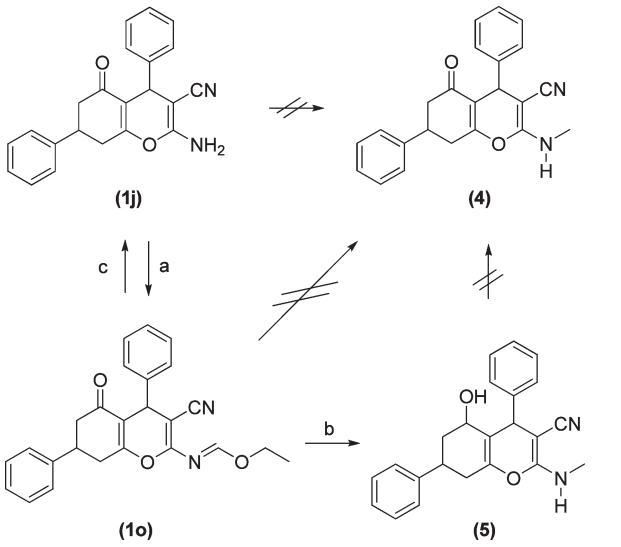


[a] Reagents and conditions: (a) HCl in dioxane, 50 °C, 25 h, 85%.

analogue **1n** is of interest to evaluate the impact of such a change for the inhibitory activity at EAAT1. On its synthesis, applying the strategy used for the synthesis of **1k**−**m** was not productive. However, in an attempt to synthesize the 3-carboxylic acid analogue (brackets Scheme 2), the treatment of **1l** with HCl in dioxane led to the expected cationic elimination of the *tert*-butyl group but also the immediate decarboxylation to give **1n**. (Scheme 2). Even at room temperature or with the use of different acids (TFA, acetic acid, dichloroacetic acid), these two sequential reactions could not be controlled as to produce the free 3-carboxylic acid analogue.

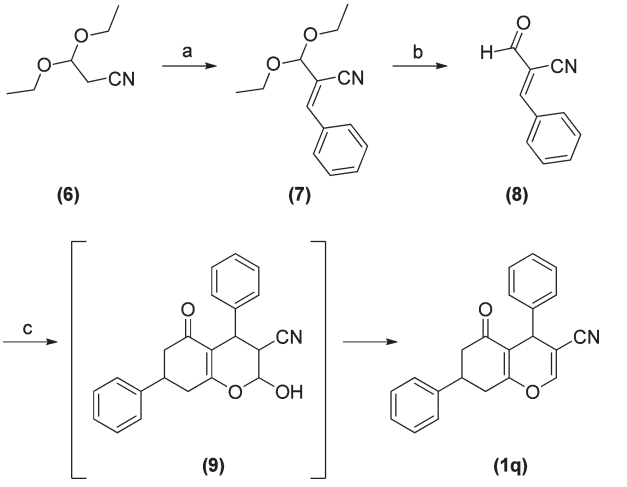
Synthesis of Analogues in the 2-Position (2-Amino Group). The 2-amino functionality is intriguing as it is in conjugation with the electron withdrawing 3-cyano group but also in competition with the electron donating ring oxygen. Given this analysis, the pK_a value of the 2-amine is comparable to an amide and thus *not* protonated under physiological conditions, making it capable of hydrogen bond donation and acceptation. Three analogues, **4**, **1p**, and **1q**, were designed to address this matter. To explore the possibility to expand the structure in direction of the amino group to obtain analogues of higher potency, the obvious choice of design is the *N*-methylated analogue (**4**). Different strategies were tried for synthesis of **4** (Scheme 3): Direct methylation of the amino group using dimethyl sulfate as the methylating agent with sodium hydride as base[13] did not lead to the desired product but rather a complex mixture of products including 5-methylated analogues. As a consequence, alternative strategies were explored. The tactic that seemed most promising was introduction of an ethoxymethylidene group (=$CHOCH_2CH_3$) on the amino functionality by reaction of **1j** with triethyl orthoformate to give **1o**. The subsiding reduction[14−16] to give **4** failed despite the fact that several reduction conditions ($NaBH_4$, $NaB(OAc)_3H$, $NaBH_3CN$, formic acid)[16,17] were tried. Either full reduction of the ethoxymethylidene group was not achieved or the reaction gave complex reactions mixtures. Interestingly, treating **1o** with formic acid yielded **1j**. Reduction of **1o** using $NaBH_4$ in ethanol at room temperature resulted in **5**, with the desired methylated amine, but also reduction of the 5-keto group to the alcohol as the main product. An LC-MS analysis of

Scheme 3. Synthesis of Analogues in the 2-Position[a]



[a] Reagents and conditions: (a) triethyl orthoformate, acetic anhydride, reflux, 3.5 h, 86%; (b) $NaBH_4$, EtOH, rt, 3 h, 34%; (c) formic acid, EtOH, rt to 60 °C, 2 days, quant.

Scheme 4. Synthetic Pathway for the Preparation of **1q**[a]



[a] Reagents and conditions: (a) benzaldehyde, NaOEt, EtOH, rt, 20 h; (b) 6N HCl, rt, 1 h, 40% over two steps; (c) 5-phenylcyclohexane-1,3-dione, triethylamine, CH_2Cl_2, rt, 2 h, then add DMAP, MsCl, rt., 4 days, 67%.

recrystallized **5** confirmed the structure. Reoxidation of the alcohol to give **4** was attempted by several methods (TPAP/NMO, Dess−Martin periodinane, MnO_2, DDQ, Swern),[18−22] however, without success.

The acetylated analogue also explores the possibility to extend the structure in the direction of the amino group. Treating **1j** with acetic anhydride gave the acetylated compound.[14]

Removal of the amino group will introduce major changes in the electronic distribution of the parental skeleton as well as deplete the possibility of ligand hydrogen donation and acceptance in this position. To synthesize **1q** by use of the three-component reaction (Scheme 4), the malononitrile component must be replaced with a component that can introduce a leaving group in the 2-position. On the basis of the observation made with the synthesis of the analogues in the 3-position, it was decided to synthesize

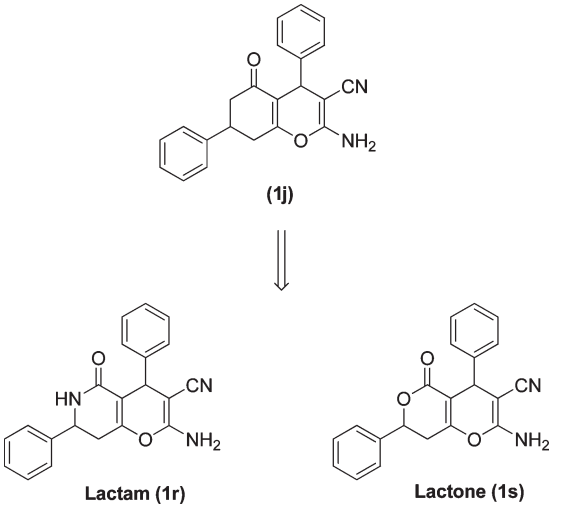


Figure 3. Chemical structure of new heterocyclic analogues of **1j**: lactam **1r** and lactone **1s**.

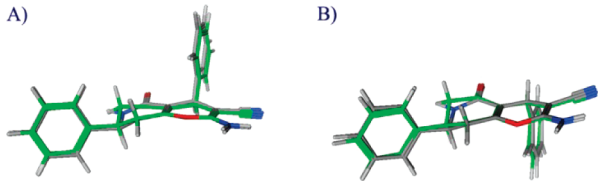


Figure 4. Three point superimposition of low-energy conformation of **1j** with lactam **1r**. (A) *syn*-Diastereomers: **1j** (green) and **1r** (type code). (B) *anti*-Diastereomers: **1j** (green) and **1r** (type code).

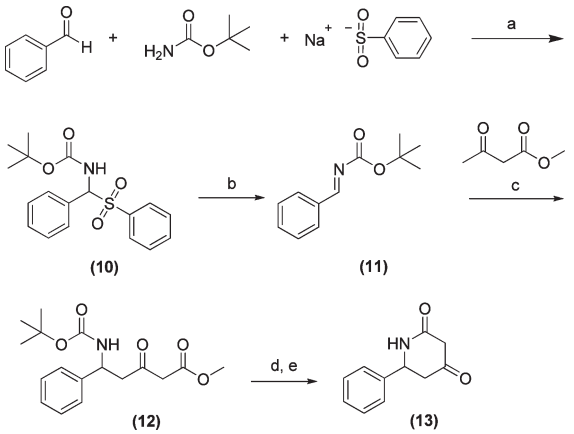
the intermediate product rather than forming it in situ to better be able to monitor and control the reaction (Scheme 4). 3,3-Diethoxypropionitrile (**6**) was reacted with benzaldehyde to form **7**, which then underwent hydrolysis to form **8**.[23] The initial strategy was that the reaction between **8** and 5-phenylcyclohexane-1,3-dione in the presence of catalytic amounts of base would produce condensation product **1q** simply due to the driving force for formation of the 2,3-conjugated system. However, these conditions only led to formation of the hemiacetal intermediate **9** (Scheme 4). To overcome this problem, conversion of the alcohol into the much better leaving group, the mesylate, by addition of DMAP and mesyl chloride in situ, cleanly gave **1q** in 67% yield.

Synthesis of Analogues in the 5,6-Position (Lactone and Lactam). To address the influence of the 5-oxo group on the selective EAAT1 inhibitory activity, we designed the corresponding lactam **1r** and lactone **1s** analogues by introduction of a nitrogen or an oxygen in the 6-position of **1j** (Figure 3).

An in silico study was conducted to assess the influence of the planned chemical changes. The study disclosed that **1r** and **1s** do not occupy a volume outside what is defined by **1j**. Furthermore, the low-energy conformations of the two new heterocyclic skeletons did not deviate considerably from the low energy conformation of **1j** (Figure 4, only data for the study of **1r** is shown).

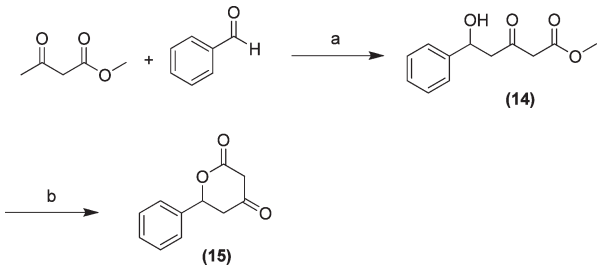
The two new diheteroatomic scaffolds found in **1r** and **1s** have not been reported earlier. Despite the at-first-glance chemical complexity, we believed they could be readily prepared following the same strategy used for the synthesis of **1a**−**j**, (Figure 2) only by substitution of the 1,3-cyclohexadione component for β-ketolactam **13** or β-ketolactone **15**.

Scheme 5. Synthetic Pathway towards β-Ketolactam **13**[a]



[a] Reagents and conditions: (a) formic acid, methanol/H_2O (1:2), rt, 3 days, 78%; (b) Cs_2CO_3, THF, 60 °C, 30 min 100%; (c) LDA, THF, −50 °C, then add **11**, 3 h, 80%; (d) TFA, dichloromethane, rt, 3 h; (e) $NaHCO_3$, dichloromethane, rt, 6 h HCl (pH = 3), 89%.

Scheme 6. Synthetic Pathway Towards β-Ketolactone **15**[a]



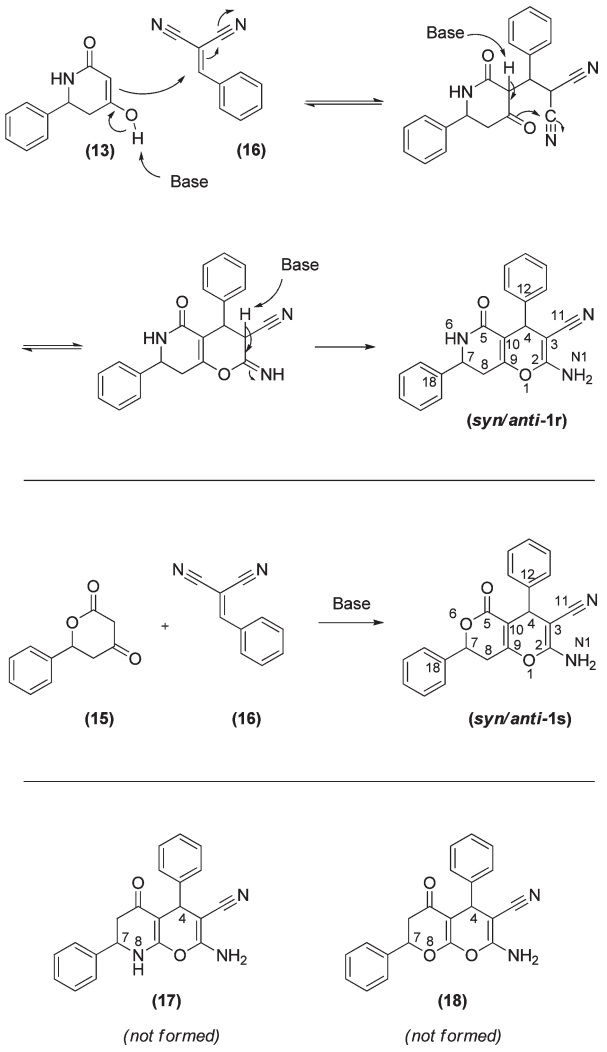
[a] Reagents and conditions: (a) NaH, *n*-BuLi, THF, rt −20 °C, 1.5 h, 57%; (b) KOH, rt, 30 min HCl (pH = 3), 90%.

The synthesis of β-ketolactam **13** commenced with the preparation of sulfone **10** from benzaldehyde, sodium benzenesulfinate, and *tert*-butyl carbamate (Scheme 5), which was subsequently converted to imine **11** in quantitative yield using cesium carbonate in THF.[24,25] Because of its susceptibility, the imine **11** was used immediately or stored in the freezer under a nitrogen atmosphere. The imine was reacted with 2.5 equiv of 1,3-dicarbonyl dianion to afford the corresponding δ-amino-β-keto ester **12** in a 80% yield.[26] Deprotection of the δ-amino-β-keto ester **12** with 5 equiv of TFA in dichloromethane removed the Boc-protecting group and gave the amine (not shown), which was reacted with saturated $NaHCO_3$ in dichloromethane to afford β-ketolactam **13** in 89% yield.[27,28]

β-Ketolactone **15** was synthesized by first trapping the dianion of methyl acetoacetate with benzaldehyde to give hydroxyketoester **14** (Scheme 6). Subsequent hydrolysis using potassium hydroxide followed by treatment with hydrochloric acid afforded the desired β-ketolactone **15** in 90% yield.[29,30]

With β-ketolactam **13** and β-ketolactone **15** in hand, the final step toward the synthesis of target compounds **1r** and **1s** was to be investigated. To better control this exploratory step, 2-benzylidenemalononitrile (**16**) was prepared first[31] rather than generating it in situ. Finally, conjugate addition of β-ketolactam **13** with the 2-benzylidene-malononitrile **16**[32] in absolute ethanol with a catalytic amount of piperidine for 30 min at room temperature afforded a mixture of two

Scheme 7. Synthetic Pathway and Mechanism for the Preparation of Lactam **1r**, Lactone **1s**, and Chemical Structures of 8-N/O Regioisomers **17** and **18** (Not Formed)[a]



[a] Reagents and conditions: Piperidine, abs ethanol, rt, 30 min 90% (*syn*/*anti*-**1r**, 2:3 ratio) and 91% (*syn*/*anti*-**1s**, 1:1 ratio).

compounds in 90% yield and in a ratio of 3:2. This mixture was readily separated by column chromatography. The same outcome was observed for the reaction of β-ketolactone **15** with **16** to give a mixture of two compounds in 91% yield with a ratio of 1:1 (Scheme 7). A mechanistic analysis of the formation of **1r** (Scheme 7) suggests that the two isolated compounds could be the respective *syn*/*anti* stereoisomers or the 8-regioisomer **17**.

X-ray Crystallography. To resolve the syn/antistereochemical configuration, a structural characterization of the four compounds by X-ray crystallography was carried out. To obtain high quality crystals, several solvents were tried out of which acetonitrile gave the best results. The four X-ray structures (Figure 5) revealed that the 6-hetero analogues **1r** and **1s** were formed and furthermore that the two products from each reaction were the *syn*- and *anti*-stereoisomers.

Because this is the first disclosure on these two heterocyclic skeletons, a detailed NMR study (heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC)) was conducted. In summary, the 2D NMR experiments HMQC and HMBC and their detailed analyses (see Experimental Section) highlight the

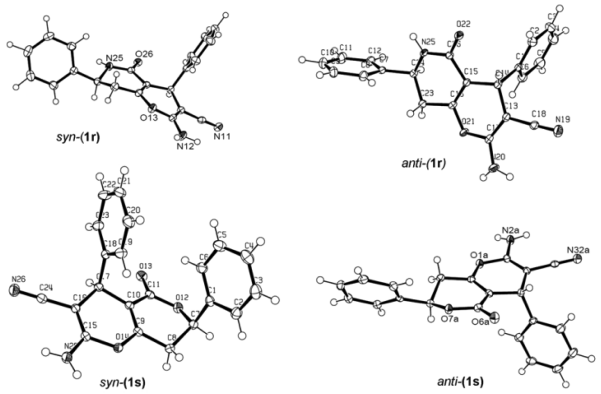


Figure 5. X-ray crystal structures of *syn*/*anti*-**1r** and *syn*/*anti*-**1s**; ellipsoids of the non-hydrogen atoms are represented at 50% probability. Hydrogen atoms are represented by spheres of arbitrary size.

similarity of the chemical shifts and coupling patterns for the four analogues. Furthermore, it is evident that the NMR experiments reported here could not be used as to unambiguously assign the *syn*/*anti* stereochemistry of **1r** and **1s** (see Supporting Information).

SAR. The synthesized analogues **1g**−**q**, *syn*-**1r**, *anti*-**1r**, *syn*-**1s**, and *anti*-**1s** were characterized as ligands at EAAT1−3 in a [^{3}H]-D-aspartic acid uptake assay, and the results are presented in Table 1.[33]

On the comparison of UCPH-101, which comprises a 1-naphthyl group in the 7-position, a 5-fold decrease in inhibitory potency is observed for analogue **1h**, which holds a 2-naphthyl group in this position (IC_{50} values 0.66 μM vs 3.2 μM, respectively). Analogue **1i**, a structural hybrid of UCPH-101 and **1c**, is equipotent with UCPH-101 and underlines the importance of the R^1 group (7-position) being a 1-naphthyl moiety, while the chemical nature of R^2 group (4-position) is less important. However, a substituent in the R^2 position is mandatory for inhibitory activity, as **1d** shows no inhibitory activity in the concentration ranges tested (> 300 μM). The requirement of an aromatic group in R^1 was previously established by the observed loss of inhibitory activity of analogue **1e** (R^1 = Me, > 300 μM). It is interesting to see that inhibitory activity is reestablished, when a benzyl group is introduced as the R^1 substituent, as **1g** displays an IC_{50} value of 5.1 μM.

Analogues **1k**−**1n**, which address the importance of the cyano group in the 3-position, were all inactive at EAAT1,2,3. Hence, it appears from this SAR that the cyano functionality is mandatory for the inhibitory activity at EAAT1. The importance of the amino group is addressed by the two analogues **1o** and **1p**. Introduction of substituents on the 2-amino group leads to inactive compounds, and therefore it is concluded that there is no room for substituents in this direction. Removal of the amino (analogue **1q**) also results in the loss of activity. Thus, the amino group is clearly of crucial importance for the inhibitory activity at EAAT1 by its hydrogen bonding and accepting character.

The new heterocyclic analogues *syn*-**1r**, *anti*-**1r**, *syn*-**1s**, and *anti*-**1s** were all found to be without inhibitory activity at the EAAT1 subtype (IC_{50} > 300 μM), and no activity was observed for any of the compounds at neither EAAT2 nor EAAT3 (IC_{50} > 300 μM). Whereas the latter finding certainly was expected, the inactivity of the *syn*-**1r**, *anti*-**1r**, *syn*-**1s**, and *anti*-**1s** at EAAT1 is indeed in contrary to our expectations

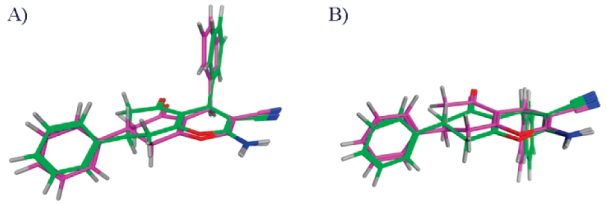
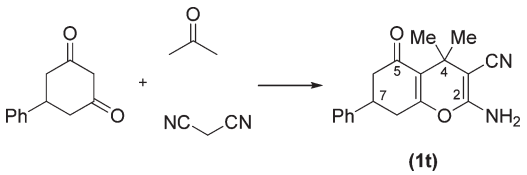


Figure 6. (A) Shows superimposition of *syn*-stereoisomer (4*S*,7*R*)-**1j** in green with the antistereoisomer (4*S*,7*S*)-**1j** in purple. (B) Shows superimposition of the *syn*-stereoisomer (4*R*,7*S*)-**1j** in green with the antistereoisomer (4*R*,7*R*)-**1j** in purple.

Scheme 8. Synthesis of 4,4-Dimethyl Analogue **1t**[a]



(**1t**)

[a] Inhibitory activity at EAAT1-3: > 300 μM.

based on the modeling study. The study concluded that there were no immediate concerns as to spacial clashes or loss of hydrogen bonding potential. However, the induced change in electronic distribution of the parental structure may be the underlying cause of the inactivity. Yet, further elucidation of this question is awaiting future studies.

Stereochemistry and 3D Pharmacophore. The parental skeleton contains the two stereogenic centers of C4 and C7 (R^1 and $R^2 \neq$ H), and the compounds in this series are generally obtained as an approximate 1:1 mixture of the two diastereomers as determined by ^{1}H NMR. Given the spacial orientation of the R^1 and R^2 groups relative to the parental skeleton, the two diastereomers may be designated the 4,7-*syn* enantiomers and 4,7-*anti* enantiomers. The only exemption from this is in the case R^1 is a 1-naphthyl group **1b** (UCPH-101) and **1i**, which gave a 9:1 mixture of the 4,7-*syn*/4,7-*anti* diastereomers. Despite extensive efforts using chiral HPLC, we have only been able to separate the *syn*/*anti* diastereomers of **1j** by flash chromatography. Both diastereomers, *syn*-**1j** and *anti*-**1j**, were found to be inhibitors of EAAT1 of comparable IC_{50} values (3.8 and 7.2 μM, Table 1), values which were also in concordance with the inhibitory potency of **1j** (3.9 μM, Table 1). To address this observation, we performed a modeling study commencing with the localization of the low-energy conformations of the four stereoisomers of **1j** by a stochastic conformational search (for details see Experimental Section). A subsequent superimposition of all four stereoisomers suggests that the stereochemistry at C7 is likely not important for inhibitory activity as the four R^1 groups (phenyl) occupies the same area of space (not shown). On the other hand, superimposition of the two pairs of diastereomers suggest that the stereochemistry at C4 is crucial as the R^2 group (phenyl) points either up (A, Figure 6) or down (B, Figure 6). This finding led us to hypothesize that the EAAT1 protein only recognizes one of the two pharmacophore models A and B (Figure 6).

To challenge this hypothesis, the 4,4-dimethyl analogue **1t** (Scheme 8) was designed and synthesized by the three component reaction by simple exchange of the aldehyde for a ketone (acetone). This analogue exhibited no inhibitory activity at any of the three EAAT subtypes (IC_{50} > 300 μM for EAAT1−3). This result allowed us to conclude that only one of the two pharmacophores A or B is descriptive for the ligands inhibitory activity at EAAT1. It remains to be elucidated which one, an objective that could be met by the enantioselective synthesis of the four stereoisomers of UCPH-101.

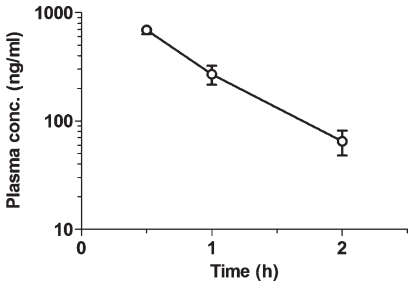


Figure 7. Plasma concentration−time profile of UCPH-101 in male Sprague−Dawley rats administered 10 mg/kg orally. Data points represent mean ± SEM of three individual animals. Brain levels not shown as these were below limit of quantification at all time points.

Bioavailability Study. Exposure studies with UCPH-101 in rats showed that high plasma concentrations could be obtained following an oral dose of 10 mg/kg (Figure 7). Following a rapid absorption, the compound was eliminated in a first-order fashion with a terminal half-life of approximately 30 min. Despite the fact that UCPH-101 was shown to be orally absorbed with high systemic exposure, the concentration in the brain was found to be below the limit of quantification (10 ng/g) at all evaluated time-points. This suggests that UCPH-101 possess limited brain penetration capabilities.

Conclusion

In conclusion, a series of analogues of UCPH-101 has been designed, synthesized, and characterized pharmacologically as EAAT inhibitors. On the basis of the functional properties of these analogues, the following SAR conclusions may be drawn: the R^1-substituent (7-position) must contain an aromatic group, whereas the R^2-substituent (4-position) must be an alkyl or aryl group. Interestingly, a 1-naphthyl group as the substituent in the 7-position as in analogue UCPH-101(**1b**) appears to be superior to a 2-naphthyl group, analogue **1h**, in terms of EAAT1 activity. Furthermore, the finding that **1i** (R^1 = 1-nathpthyl, R^2 = Me) is equipotent to UCPH-101 is evidence that the inhibitory potency of this compound class is mainly dependent on the chemical nature of the R^1-substituent over the R^2-substituent. In this connection, the fact that two substituents in the 4-position is clearly not allowed (compound **1t**), together with finding that *syn*-**1j** and *anti*-**1j** exhibit similar inhibitory activities, strongly suggest that only one of the two pharmacophore models described in Figure 6 is valid. Any of the chemical changes introduced to the scaffold (lactam **1r** and lactone **1s**) or in the form of changing the 2-amino and the 3-cyano groups resulted in loss of EAAT1 inhibitory activity. This SAR information will be valuable for future explorations of this first class of EAAT1 inhibitors. Finally, UCPH-101 was shown to be bioavailable in rats after oral administration with a plasma half-life of 30 min and furthermore the compound was found to be not able to penetrate the BBB. While this clearly limits the use of UCPH-101 for animal models using systemic administration, the compound is nevertheless highly valuable for a wide range of in vitro and ex vivo experiments.

Experimental Section

Chemistry. All reactions involving dry solvents or sensitive agents were performed under a nitrogen atmosphere, and glassware was dried prior to use. Solvents were dried according to standard procedures, and reactions were monitored by analytical thin-layer chromatography (TLC) analysis. TLC was carried out using Merck silica gel 60 F_{254} aluminum sheets. Flash chromatography was carried out using Merck silica gel 60A (35−70 μm). Dry column vacuum chromatography[34] was performed using Merck silica gel 60 (20−45 μm). ^{1}H NMR spectra were recorded on a 300 MHz Varian Mercury 300BB and ^{13}C NMR spectra on the 300 MHz Varian Gemini 2000BB. The 2D NMR spectra were recorded on a 600 MHz Bruker Avance spectrometer. MS spectra were recorded using LC-MS performed using an Agilent 1200 series solvent delivery system equipped with an autoinjector coupled to an Agilent 6400 series triple quadrupole mass spectrometer equipped with an electrospray ionization source. Gradients of 10% aqueous acetonitrile +0.05% formic acid (buffer A) and 90% aqueous acetonitrile +0.046% formic acid (buffer B) were employed. Melting points were measured with a MPA 100 Optimelt automatic melting point system. All purchased chemicals were used without further purification. Single crystals suitable for X-ray diffraction studies were grown from a solution in acetonitrile, and the data of *syn*/*anti*-**1r** and *syn*/*anti*-**1s** isomers were collected using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a κ CCD diffractometer with area detector. The purity of all tested compounds was determined by elementary analysis to be >95%.

2-Amino-7-benzyl-4-(4-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1g). Equivalent molar amounts of 5-benzylcyclohexane-1,3-dione (0.10 g, 0.49 mmol) and 2-(4-methoxybenzylidene)malononitrile (0.90 g, 0.49 mmol) were dissolved in EtOH (2 mL). Piperidine (4 μL, 0.04 mmol) was added, and the mixture was heated to 50 °C and stirred overnight. The reaction mixture was evaporated and recrystallized from EtOH to afford the title compound (0.09 g; 47%) as a yellow crystalline solid. R_f 0.41(1:1 EtOAc/heptane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.38−7.18 (m, 3H), 7.18−7.04 (m, 4H), 6.87−6.75 (m, 2H), 4.48 (br s, 2H), 4.36 (s, 1H), 3.77 (s, 1.5H), 3.76 (s, 1.5H), 2.79−2.58 (m, 2H), 2.58−2.27 (m, 4H), 2.23−2.07 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 196.2, 164.4, 163.6, 159.2, 158.8, 139.9, 137.6, 129.9, 129.8, 129.3, 129.2, 129.1, 127.0, 120.7, 114.8, 114.7, 114.5, 59.3, 59.2, 55.9, 43.39, 43.32, 35.7, 35.5, 35.2, 35.0, 33.2, 32.6; mp 178.6−180.1 °C.

2-Amino-4-(4-methoxyphenyl)-7-(naphthalene-2-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1h). The product was prepared according to a procedure for the synthesis of UCPH-101 described previously by us.[2] The crude product was purified by recrystallization from EtOH (96%, 248 mL) to give the title compound (1.40 g, 79%) as a white powder. R_f 0.35 and 0.41 (3:1 ratio of diastereomers, 1:1 EtOAc/heptane).^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.90−7.66 (m, 4H), 7.60−7.42 (m, 3H), 7.14 (d, 1.5H, J = 8.5 Hz), 6.99 (s, 2H), 6.97 (d, 0.5H, J = 8.5 Hz), 6.86 (d, 1.5H, J = 8.5 Hz), 6.70 (d, 0.5H, J = 8.5 Hz), 4.20 (s, 0.75H), 4.18 (s, 0.25H), 3.72 (s, 2.25H), 3.67 (s, 0.75H), 3.60−3.45 (m, 1H), 3.19−2.98 (m, 1H), 2.93−2.70 (m, 2H), 2.67−2.50 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 195.7, 164.4, 163.4, 159.3, 159.1, 158.7, 158.6, 141.0, 140.9, 137.7, 137.3, 133.8, 133.7, 132.8, 132.7, 129.1, 129.0, 128.9, 128.5, 128.4, 128.3, 128.2, 127.0, 126.9, 126.7, 126.6, 126.5, 125.9, 125.8, 120.7, 114.7, 114.6, 114.4, 59.4, 59.3, 56.0, 55.9, 44.2, 44.1, 38.43, 38.37, 35.8, 35.6, 34.7, 34.3; mp 216.3−219.2 °C.

(*E*)-4-(Naphthalene-2-yl)but-3-en-2-one. To a suspension of sodium hydride 60% dispersion in mineral oil (0.572 g, 14.3 mmol) in dry diethyl ether (44 mL) was added a solution of diethyl 2-oxopropylphosphonate (2.8 mL, 14.6 mmol) in dry diethyl ether (10 mL). The solution was stirred for 1 h at room temperature, after which a solution of 2-naphthaldehyde (2.00, 12.8 mmol) in dry diethyl ether (10 mL) was added. After stirring for 46.5 h at room temperature, saturated $NH_4Cl_{(aq)}$ (20 mL) was added to the solution whereby all precipitated material dissolved. The aqueous layer was extracted with diethyl ether (3 × 20 mL), dried ($MgSO_4$), filtrated, and evaporated in vacuo to give a white solid. Purification by dry column vacuum chromatography (10:1 heptanes/EtOAc) afforded the title compound (2.0 g, 80%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.87−7.81 (m, 3H), 7.69−7.64 (m, 2H), 7.54−7.48 (m, 2H), 6.82 (d, 1H), 2.43 (s, 3H).

5-(Naphthalen-2-yl)cyclohexane-1,3-dione. To a 21 wt % solution of sodium ethoxide in denatured ethanol (8.34 mL, 22.3 mmol) was added diethyl malonate (3.4 mL, 22.3 mmol) followed by (*E*)-4-(naphthalene-2-yl)but-3-en-2-one (4.38 g, 22.3 mmol) under a nitrogen atmosphere. The solution was heated under reflux for 6 h, allowed to cool to room temperature overnight, and filtered to provide a crude solid. The filtrate was concentrated in vacuo to a gum which was suspended in water (30 mL) and washed with DCM (2 × 40 mL). The aqueous layer was concentrated in vacuo to a solid, which was then combined with the solid obtained above. The combined solid was treated with NaOH (16 mL, 2 N), and the resulting solution heated under reflux for 2 h. After cooling to room temperature, sulphuric acid (16 mL, 5 N) was added, and the suspension was heated under reflux for 4.5 h. After cooling to room temperature, filtration provided the crude product, which was triturated with toluene to give the title compound (4.07 g, 77%) as a white powder. ^{1}H NMR (300 MHz, CD_3OD) δ: 7.92−7.76 (m, 4H), 7.56−7.43 (m, 3H), 3.65−3.50 (m, 1H), 2.83 (dd, 2H, J = 17.0, 12.0 Hz), 2.68 (dd, 2H, J = 17.0, 5.0 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 141.0, 133.0, 131.9, 127.9, 127.5, 127.4, 126.0, 125.6, 125.5, 124.9, 103.6, 40.1, 39.8, 39.5, 39.2, 39.0; mp 208.2−211.3 °C.

2-Amino-4-methyl-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1i). 5-(Naphthalen-1-yl)cyclohexane-1,3-dione (50 mg, 0.20 mmol), malononitrile (14 mg, 0.20 mmol), and acetaldehyde (35 μL, 0.60 mmol) was stirred in absolute ethanol (3 mL) at room temperature for 5 min. *N*-Methylmorpholine (3 μL, 27 μmol) was added, and the reaction mixture was stirred for 5 h. The reaction mixture was concentrated with silica. The crude/silica mixture was purified by column chromatography on silica gel (eluent: 3:4 EtOAc/heptane). This afforded the title compound (62 mg, 90%) as colorless solid. R_f 0.30 (3:4 EtOAc/heptane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (dd, 1H, J = 13.5, 8.1 Hz), 7.91−7.86 (m, 1H), 7.78 (dd, 1H, J = 8.1, 3.0 Hz), 7.59−7.24 (m, 4H), 4.46 (s, 2H), 4.33−4.09 (m, 1H), 3.48−3.39 (m, 1H), 2.96−2.69 (m, 4H), 1.35 (d, 2H, J = 6.6 Hz), 1.26 (d, 1H, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 196.0, 162.8, 157.6, 137.4, 134.0, 130.8, 129.2, 127.9, 126.5, 125.9, 125.4, 122.8, 122.3, 118.8, 116.3, 43.6, 34.4, 33.4, 24.9, 22.9, 22.2; mp 204−206 °C.

2-Amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1j). Equimolar amounts of 5-phenylcyclohexane-1,3-dione (0.94 g, 5.0 mmol), benzaldehyde (0.55 mL, 5.0 mmol), and malononitrile (0.33 g, 5.0 mmol) were mixed together in H_2O/EtOH (1:1, 20 mL) and were allowed to react under microwave irradiation (reaction time, 30 min; temp, 85 °C; prestirring, 3 min; vial size, 10−20 mL; absorption level,: high; fixed hold time, on). The reaction mixture was allowed to cool to room temperature. The solvent was filtered off, and the solid obtained was washed with H_2O to give the title compound (1.28 g, 72%) as a white crystalline powder. R_f 0.16 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, DMSO-d_6) (∼1:1 ratio of diastereomers) δ: 7.39−7.12 (m, 10H), 7.11−6.96 (m, 2H), 4.22 (s, 0.5H), 4.21 (s, 0.5H), 3.58−3.46 (0.5H), 3.45−3.34 (m, 0.5H), 3.11−2.90 (m, 1H), 2.80−2.60 (m, 2H), 2.52−2.36 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 195.9, 164.8, 164.0, 159.4, 159.2, 145.6, 145.3, 143.54, 143.48, 129.4, 129.34, 129.25, 129.1, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 120.6, 114.4, 114.3, 59.1, 59.0, 44.24, 44.15, 38.5, 38.2, 36.5, 36.3, 34.6, 34.4; mp 178 °C (decomposes).

Methyl 2-Amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (1k). Equimolar amounts of 5-phenylcyclohexane-1,3-dione (0.94 g, 5.0 mmol) and (*E*)-methyl 2-cyano-3-phenylacrylate (**3a**) (0.94 g, 5.0 mmol) were mixed together in H_2O/EtOH (1:1, 20 mL) and were allowed to react under microwave irradiation (reaction time, 30 min; temp, 85 °C; prestirring, 3 min; vial size, 10−20 mL; absorption level, high; fixed hold time, on). The reaction mixture was allowed to cool to room temperature. The solvent was filtered off and the solid obtained was washed with H_2O to give a white powder. Recrystallization from absolute EtOH gave the title compound (1.25 g, 67%) as white crystals. R_f 0.33 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, DMSO-d_6) (∼1:1 ratio of diastereomers) δ: 7.57 (d, 2H, J = 7.6 Hz), 7.38−7.02 (m, 10H), 4.58 (s, 0.5H), 4.55 (s, 0.5H), 3.49 (s, 3H), 3.32−3.20 (m, 1H), 3.06−2.87 (m, 1H), 2.82−2.52 (m, 2H), 2.47−2.33 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 196.0, 169.2, 164.4, 163.5, 160.3, 160.2, 147.3, 146.9, 143.6, 129.4, 129.3, 128.8, 128.6, 128.5, 128.4, 127.9, 127.8, 127.6, 127.5, 126.8, 126.7, 117.6, 78.5, 78.3, 51.4, 44.3, 44.1, 38.8, 38.4, 34.5, 34.3, 34.1, 33.9; mp 189.2−189.8 °C (decomposes).

***tert*-Butyl 2-Amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (1l).** Equimolar amounts of 5-phenylcyclohexane-1,3-dione (0.94 g, 5.0 mmol) and (*E*)-*tert*-butyl 2-cyano-3-phenylacrylate (**3b**)(1.15 g, 5.0 mmol) were mixed together in H_2O/EtOH (1:1, 20 mL) and were allowed to react under microwave irradiation (reaction time, 30 min; temp, 85 °C; prestirring, 3 min; vial size, 10−20 mL; absorption level, high; fixed hold time, on). The reaction mixture was allowed to cool to room temperature. The mixture was evaporated to give an off-white solid. Recrystallization from EtOH (absolute) gave the title compound (1.50 g, 72%) as white crystals. R_f 0.68 (2:8 EtOAc/toluene). ^{1}H NMR (75 MHz, DMSO-d_6) (∼1:1 ratio of diastereomers) δ: 7.53−7.44 (m, 2H), 7.37−7.02 (m, 10H), 4.49 (s, 0.5H), 4.47 (s, 0.5H), 3.56−3.44 (m, 0.5H), 3.29−3.21 (m, 0.5H), 3.04−2.87 (m, 1H), 2.80−2.53 (m, 2H), 2.47−2.33 (m, 1H), 1.27 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 196.1, 168.8, 164.0, 163.2, 159.8, 159.7, 147.6, 147.1, 143.6, 129.4, 129.3, 128.84, 128.79, 128.5, 128.3, 127.8, 127.6, 127.5, 126.63, 126.56, 117.3, 79.9, 79.7, 79.4, 44.3, 44.2, 38.8, 38.3, 34.8, 34.6, 34.5, 34.4, 28.9; mp 184.1−184.4 °C.

2-Amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxamide (1m). Under a nitrogen atmosphere, 5-phenylcyclohexane-1,3-dione (0.57 g, 3.0 mmol) and (*E*)-2-cyano-3-phenylacrylamide (**3c**) (0.52 g, 3.0 mmol) were suspended in dry dioxane (12 mL). Piperidine (39 μL, 0.39 mmol) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was allowed to stand without stirring overnight. The solvent was filtered off, and the solid obtained was washed with dioxane, which upon drying gave the title compound (0.62 g, 57%) as white powder. R_f 0.31 (4:6:0.5 EtOAc/heptane/AcOH). ^{1}H NMR (300 MHz, DMSO-d_6) (∼1:1 ratio of diastereomers) δ: 7.63 (br s, 1H), 7.60 (br s, 1H), 7.37−7.03 (m, 10H), 6.49 (br s, 2H), 4.62 (s, 0.5H), 4.60 (s, 0.5H), 3.54−3.44 (m, 0.5H), 3.28−3.15 (m, 0.5H), 2.99−2.86 (m, 1H), 2.78−2.33 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 195.9, 171.4, 164.1, 163.3, 158.4, 147.1, 146.6, 143.7, 129.34, 129.25, 128.8, 128.7, 128.5, 127.85, 127.81, 127.6, 127.5, 126.8, 126.7, 117.5, 80.3, 80.1, 44.4, 44.2, 38.8, 38.4, 34.6, 34.4, 34.2, 34.1; mp 186.3−186.8 °C (decomposes).

2-Amino-4,7-diphenyl-7,8-dihydro-4*H*-chromen-5(6*H*)-one (1n). Under a nitrogen atmosphere, *tert*-butyl 2-amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (**1l**) (0.50 g, 1.2 mmol) was dissolved in a solution of HCl in dioxane (4.0 M, 10 mL). The mixture was heated to 50 °C and then stirred at that temperature for 25 h. The reaction mixture was cooled to room temperature and evaporated to give an off-white solid which was triturated with ether. The crude product (0.32 g, 85%) was recrystallized from toluene to give the title compound (0.14 g, 37%, cocrystallized with 0.3 mol equiv toluene). R_f 0.13 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.22 (s, 0.33H), 10.20 (s, 0.66H), 7.44−7.00 (m, 10H), 4.18 (br t, 1H), 3.55−3.37 (m, 1H), 3.02−2.36 (m, 6H), 2,29 (from toluene). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 195.0, 194.7, 171.2, 171.0, 155.0, 154.7, 144.2, 144.1, 143.5, 143.4, 129.8, 129.42, 129.35, 129.3, 129.1, 127.9, 127.6, 127.5, 127.3, 126.2, 113.9, 113.8, 44.5, 44.0, 39.9, 39.4, 39.3, 35.0, 34.6, 34.5, 34.0, 22.0 (from toluene); mp > 180 °C (decomposes).

(*E*)-Ethyl *N*-3-Cyano-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-ylformimidate (1o). Under a nitrogen atmosphere, 2-amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1j**) (0.86 g, 2.5 mmol) was suspended in acetic anhydride (5.0 mL). Triethyl orthoformate was added, and the mixture was refluxed (150 °C) for 3.5 h. The reaction mixture was cooled to room temperature and then evaporated to give a light-brown solid. Recrystallization from ethanol gave the title compound (0.856 g; 86%) as off-white crystals. R_f 0.59 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, $CDCl_3$) (∼1:1 ratio of diastereomers) δ: 8.22 (s, 0.5H), 8.21 (s, 0.5H), 7.40−7.15 (m, 10H), 4.60 (s, 0.5H), 4.58 (s, 0.5H), 4.40 (q, 1H, J = 7.15 Hz), 4.39 (q, 1H, J = 7.15 Hz), 3.58−3.46 (m, 0.5H), 3.45−3.31 (m, 0.5H), 3.02−2.55 (m, 4H), 1.38 (t, 1.5H, J = 7.15 Hz), 1.37 (t, 1.5H, J = 7.15 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.6, 195.4, 163.6, 162.8, 159.6, 156.3, 156.1, 142.4, 142.1, 142.0, 129.4, 129.3, 129.2, 129.1, 128.3, 128.0, 127.9, 127.8, 127.7, 127.1, 127.0, 117.5, 114.5, 114.4, 84.6, 84.4, 64.9, 64.8, 44.2, 44.0, 39.0, 38.6, 38.1, 38.0, 35.2, 35.1, 14.3; mp 143.4−147.6 °C.

***N*-(3-Cyano-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)acetamide (1p).** Under a nitrogen atmosphere, 2-amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1j** (1.00 g, 2.92 mmol) was suspended in acetic anhydride (2.76 mL, 29.2 mmol) and the mixture was refluxed (150 °C) for 2 h. The reaction mixture was then cooled to room temperature and evaporated to give a light-brown oil. Purification of the crude product by dry column vacuum chromatography (eluent: 1:9 EtOAc/toluene) gave the title compound (0.095 g, 8%) as off-white crystals. R_f 0.24 and 0.31 (two pairs of diastereomers) (1:1 EtOAc/heptane). ^{1}H NMR (300 MHz, $CDCl_3$) (∼1:1 ratio of diastereomers) δ: 7.49−7.20 (m, 11H), 4.69 (s, 0.5H), 4.68 (s, 0.5H), 3.66−3.54 (m, 0.5H), 3.51−3.37 (m, 0.5H), 3.08−2.90 (m, 2H), 2.84−2.63 (m, 2H), 2.24 (s, 1.5H), 2.23 (s, 1.5H), 1.50 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 195.6, 195.5, 171.0, 165.1, 164.1, 151.0, 150.9, 143.03, 142.96, 141.8, 141.5, 129.4, 129.21, 129.17, 128.88, 128.85, 128.4, 128.3, 127.64, 127.61, 127.5, 115.2, 112.8, 112.7, 97.0, 96.9, 44.2, 38.3, 38.2, 38.0, 34.1, 33.8, 25.8; mp > 146 °C (decomposes).

5-oxo-4,7-Diphenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1q). Under a nitrogen atmosphere, (*E*)-2-formyl-3-phenylacrylonitrile (**8**) (0.10 g, 0.64 mmol) and 5-phenylcyclohexane-1,3-dione (0.12 g, 0.64 mmol) were suspended in dry CH_2Cl_2 (20 mL). Triethylamine (0.1 mL, 0.7 mmol) was added, and the mixture became clear. The reaction mixture was stirred for 2 h before DMAP (0.016 g, 0.127 mmol) and MsCl (50 μL, 0.64 mmol) were added. The mixture was stirred for 4 days and then washed with H_2O (20 mL × 2). The organic phase was dried using $MgSO_4$, filtered and evaporated to give an orange foamy solid (0.240 g). Purification by dry column vacuum chromatography (eluent, EtOAc/heptane; gradient, 0−25% EtOAc) gave the title compound (0.14 g, 67%) as white powder. R_f 0.51 (4:6 EtOAc/heptane). ^{1}H NMR (300 MHz, $CDCl_3$) (∼1:1 ratio of diastereomers) δ: 7.41−7.21 (m, 11H), 4.50 (s, 0.5H), 4.48 (s, 0.5H), 3.56−3.44 (m, 0.5H), 3.43−3.29 (m, 0.5H), 3.00−2.78 (m, 2H), 2.77−2.54 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.8, 195.6, 163.9, 163.0, 149.5, 149.3, 142.1, 142.0, 141.7, 141.4, 129.4, 129.3, 129.2, 128.5, 128.3, 128.2, 127.9, 127.8, 127.1, 127.0, 116.5, 114.0, 98.4, 98.2, 44.2, 44.1, 38.9, 38.5, 36.1, 36.0, 35.0, 34.9; mp 131.9−132.4 °C. LC-MS (m/z) calcd for $C_{22}H_{17}NO_2$ [M + H$^+$], 328.1; found, 328.1.

2-Amino-5-oxo-4,7-diphenyl-5,6,7,8-tetrahydro-4*H*-pyrano-[3,2-*c*]pyridine-3-carbonitrile (1r). 6-Phenylpiperidine-2,4-dione (**13**) (115 mg, 600 μmol) and 2-benzylidenemalononitrile (**16**) (94 mg,

600 μmol) were stirred in absolute ethanol (7 mL) in a sample vial at room temperature. Piperidine (12 μL, 120 μmol) was added, and the reaction mixture was stirred for 15 min. The reaction mixture was concentrated with silica gel in vacuum (using hot ethanol to dissolve) and purified by column chromatography in silica gel (eluent: 1:5 EtOAc/dichloromethane). This afforded compounds **1r** *syn*:*anti* stereoisomers (185 mg in a ratio of 2:3, 89%) as a colorless solid. Analytical data for *anti*-**1r**: R_f 0.53 (1:5 EtOAc/dichloromethane). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.63 (s, 1H), 7.41−7.16 (m, 10H), 6.92 (s, 2H), 4.72−4.65 (m, 1H), 4.28 (s, 1H), 2.75−2.70 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 35.0, 36.9, 53.7, 58.9, 108.0, 120.8, 127.4, 127.7, 127.8, 128.2, 128.6, 129.1, 129.2, 129.3, 141.9, 124.7, 155.0, 159.6, 166.2; mp 225−227 °C. LC-MS [M + H$^+$] calcd for $C_{11}H_{11}NO_2$, 344.1321; found, 344.1. Analytical data for *syn*-**1r**: R_f 0.42 (1:5 EtOAc/dichloromethane). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.88 (d, 1H, J = 3.9 Hz), 7.25−7.16 (m, 6H), 7.06−7.03 (m, 2H), 6.98−6.94 (m, 2H), 6.85 (s, 2H), 4.73−4.65 (m, 1H), 4.26 (s, 1H), 3.31−3.22 (m, 1H), 2.55 (dd, 1H, J = 17.1, 2.1). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.7, 36.9, 51.1, 58.7, 108.4, 120.7, 126.9, 127.0, 127.4, 128.0, 128.3, 128.4, 128.9, 129.0, 142.9, 145.0, 153.2, 159.4, 165.6; mp 226−228 °C. LC-MS [M + H$^+$] calcd for $C_{11}H_{11}NO_2$, 344.1321; found, 344.1.

2-Amino-5-oxo-4,7-diphenyl-4,5,7,8-tetrahydropyrano[4,3-*b*]-pyran-3-carbonitrile (1s). 6-Phenyldihydro-2*H*-pyran-2,4(3*H*)-dione (**15**) (57 mg, 0.3 mmol) and 2-benzylidenemalononitrile (**16**) (46 mg, 0.3 mmol) was stirred in absolute ethanol (2 mL) at room temperature for 10 min. Piperidine (6 μL, 60 μmol) was added, and the reaction mixture was stirred for 30 min. The precipitated solid was dissolved in hot ethanol and concentrated with silica. The crude/silica mixture was purified by column chromatography on silica gel (eluent: 1:10 EtOAc/dichloromethane). This afforded the title compound **1s** *syn*:*anti* stereoisomers (94 mg in a ratio of 1:1, 91%) as a colorless solid. Analytical data for *anti*-**1s**: R_f 0.38 (1:10 EtOAc/dichloromethane). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.55−7.27 (m, 10H), 7.19 (s, 2H), 5.62 (dd, 1H, J = 12.6, 3.6 Hz), 4.38 (d, 1H, J = 1.5 Hz), 3.32−3.21 (m, 1H), 2.88 (dd, 1H, J = 17.1, 3.9 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.0, 37.3, 58.8, 77.2, 105.2, 120.3, 127.5, 127.8, 128.4, 129.3, 129.5, 138.6, 144.6, 158.9, 159.8, 164.9, 175.0; mp 225−227 °C. LC-MS [M + H$^+$] calcd for $C_{11}H_{10}O_3$, 345.1161; found, 345.2. Analytical data for *syn*-**1s**: R_f 0.24 (1:10 EtOAc/dichloromethane). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.34 (s, 5H), 7.24−7.16 (m, 4H), 7.08 (s, 2H), 6.85 (d, 2H, J = 7.8 Hz), 5.73 (dd, 1H, J = 8.1, 5.1 Hz), 4.15 (s, 1H), 3.22 (dd, 1H J = 17.4, 8.5 Hz), 3.02 (dd, 1H, J = 17.4, 5.1 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 31.9, 37.6, 59.3, 76.6, 105.4, 120.2, 126.9, 127.5, 127.9, 129.1, 129.2, 129.3, 139.2, 144.8, 158.8, 159.5, 164.4; mp 222−224 °C. LC-MS [M + H$^+$] calcd for $C_{11}H_{10}O_3$, 345.1161; found, 345.1.

2-Amino-4,4-dimethyl-5-oxo-7-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1t). Acetone (0.22 mL, 3.0 mmol) was added to a mixture of 5-phenylcyclohexane-1,3-dione (0.58, 3.1 mmol) and malononitrile (0.20 g, 3.1 mmol) in 99.9% ethanol (6.0 mL). 4-Methylmorpholine (0.34 mL, 3.1 mmol) was added, and the mixture was stirred for 30 min, after which it was left to stand at room temperature for 28 h. The precipitate was filtered off and washed with ice-cold ethanol and ice-cold heptanes. Recrystallization from propan-2-ol gave the title compound (0.37 g, 42%) as white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.38−7.21 (m, 5H), 4.32 (br s, 2H), 3.36 (septet, 1H), 2.58−2.80 (m, 4H), 1.55 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (75.5 MHz, CDCl3) δ: 196.5, 161.5, 156.1, 142.0, 129.2, 127.6, 126.8, 118.7, 118.6, 70.3, 45.9, 38.5, 35.5, 32.2, 29.6, 28.7; mp 195.5−196.5 °C.

(*E*)-Methyl-2-cyano-3-phenylacrylate (3a). To benzaldehyde (2.1 mL, 20 mmol) dissolved in MeOH (25 mL) were added methyl 2-cyanoacetate (1.8 mL, 20 mmol) and piperidine (0.26 mL, 2.6 mmol). Upon addition of piperidine, the reaction mixture changed color from colorless to yellow. The reaction mixture was refluxed for 70 min and then allowed to cool to room temperature. Upon cooling, the product crystallized and the mixture was allowed to stand at room temperature for 2.5 h. The solvent was filtered off and the solid obtained was washed with ice-cold MeOH to give the title compound (3.47 g, 93%) as shiny white crystals. R_f 0.71 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.40 (s, 1H), 8.07−8.02 (m, 2H), 7.67−7.54 (m, 3H), 3.86 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2, 156.0, 134.3, 132.2, 131.7, 130.2, 116.4, 103.1, 54.2; mp 89.5−90.0 °C.

(*E*)-*tert*-Butyl-2-cyano-3-phenylacrylate (3b). To benzaldehyde (2.0 mL, 20 mmol) dissolved in *i*-PrOH (25 mL) were added *tert*-butyl 2-cyanoacetate (2.9 mL, 20 mmol) and piperidine (0.26 mL, 2.6 mmol). The reaction mixture was refluxed for 70 min and then allowed to cool to room temperature. The mixture was evaporated to give an yellow oil that solidifies on standing. Two successive recrystallizations from EtOH (absolute) gave the title compound (3.06 g, 67%) as white crystals. R_f 0.77 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.30 (s, 1H), 8.03−7.98 (m, 2H), 7.64−7.53 (m, 3H), 1.53 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 161.5, 155.3, 134.0, 132.2, 131.5, 130.1, 116.5, 104.8, 84.2, 28.4; mp 55.7−57.4 °C.

(*E*)-2-Cyano-3-phenylacrylamide (3c). To a suspension of 2-cyanoacetamide (1.7 g, 20 mmol) in EtOH (25 mL) were added benzaldehyde (2.0 mL, 20 mmol) and piperidine (0.26 mL, 2.6 mmol). The reaction mixture was allowed to stir at room temperature for 3 h. During stirring, the reaction mixture became clear and slightly yellow. The mixture was evaporated to give a yellow solid. Recrystallization from EtOH (absolute) gave the title compound (1.4 g, 42%) as slightly yellow crystals. R_f 0.15 (2:8 EtOAc/toluene). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.17 (s, 1H), 8.01−7.69 (m, 4H), 7.61−7.50 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.5, 151.5, 133.2, 132.8, 130.9, 130.1, 117.3, 107.5; mp 117.0−117.3 °C.

(*E*)-2-(Diethoxymethyl)-3-phenylacrylonitrile (7). Under a nitrogen atmosphere, benzaldehyde (3.1 mL, 30 mmol) and 3,3-diethoxypropionitrile (4.5 mL, 30 mmol) were mixed together and added to a solution of NaOEt in EtOH (11.2 mL, 21 wt %, 30 mmol). The mixture was stirred at room temperature for 1 h, and then additional dry EtOH (5 mL) was added and the mixture was stirred for further 20 h. The solvent was removed by evaporation in vacuo, and the yellow residue was partitioned between CH_2Cl_2 (600 mL) and H_2O (150 mL). The organic phase was evaporated to give an yellow oil (8.80 g). The crude product was used in the next step (**8**) without further purification. R_f 0.63 (2:8 EtOAc/toluene).

(*E*)-2-Formyl-3-phenylacrylonitrile (8). (*E*)-2-(Diethoxymethyl)-3-phenylacrylonitrile (**7**) (8.80 g, 30.0 mmol) was dissolved in 6N HCl (90 mL), and the mixture was stirred at room temperature for 1 h. The yellow precipitate was collected by filtration, washed with water, and then recrystallized from diethyl ether to give the title compound (1.90 g, 40% over two steps) as a yellow crystalline solid. R_f 0.52 (1:1 EtOAc/heptane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.59 (s, 1H), 8.04 (d, 2H, J = 7.7 Hz), 7.91 (s, 1H), 7.67−7.51 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 187.4, 159.6, 134.8, 131.8, 131.7, 130.0, 114.5, 112.8; mp 93.6−96.0 °C

***tert*-Butyl Phenyl(phenylsulfonyl)methylcarbamate (10).** *tert*-Butyl carbamate (2 g, 17 mmol) and sodium benzenesulfinate (5.6 g, 34 mmol) were stirred in a mixture of methanol (20 mL) and H_2O (40 mL) at room temperature under an nitrogen atmosphere. Benzaldehyde (3.4 mL, 34 mmol) and formic acid (1.2 mL, 34 mmol) were added to the mixture and stirred at room temperature for 3 days. The white solid material was filtered and washed several times with water and diethyl ether. This afforded the title compound (4.6 g, 78% yield) as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.92 (d, 2H, J = 7.2 Hz), 7.67−7.61 (m, 1H), 7.56−7.50 (m, 2H), 7.46−7.36 (m, 5H), 5.94 (d, 1H, J = 10.8 Hz), 5.84 (d, 1H, J = 10.8 Hz), 1.25 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.4, 74.3, 81.6, 129.1, 129.3, 129.4, 129.9, 130.2, 134.3, 137.3, 153.9; mp 177−178 °C. LC-MS [M + H$^+$] calcd for $C_{18}H_{21}NO_4S$, 348.1191; found, 348.1.

(*E*)-*tert*-Butyl Benzylidenecarbamate (11). *tert*-Butyl phenyl-(phenylsulfonyl)-methylcarbamate (**10**) (1 g, 2.9 mmol) and

Cs_2CO_3 (2.36 g, 7.25 mmol) were stirred in dry THF (30 mL) at 60 °C under an nitrogen atmosphere for 30 min. The mixture was cooled to room temperature and filtered through Celite. The filtrate was concentrated to give the title compound (590 mg, 100%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.88 (s, 1H), 7.93−7.90 (m, 2H), 7.57−7.54 (m, 1H), 7.49−7.45 (m, 2H), 1.59 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.0, 82.2, 128.7, 130.0, 133.4, 129.8, 162.6, 169.8.

Methyl 5-(*tert*-Butoxycarbonylamino)-3-oxo-5-phenylpentanoate (12). Lithium diisopropylamide (14.4 mL, 28.8 mmol) was stirred in dry THF (50 mL) at −78 °C under an nitrogen atmosphere. A solution of methyl acetoacetate (1.55 mL, 14.4 mmol) in dry THF (10 mL) was added with a syringes pump to the reaction mixture over a period of 3 h. (*E*)-*tert*-butyl benzylidenecarbamate (**11**) (993 mg, 4.84 mmol) in dry THF (10 mL) was added to the reaction mixture at −50 °C and stirred for 30 min. NH_4Cl (10 mL) was added at −50 °C, and the crude reaction was extracted with EtOAc (3 × 30 mL) and the combined organic phases were washed with H_2O (15 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent: 1:10 EtOAc/dichloromethane). This afforded the title compound (1.23 g, 80%) as colorless solid. R_f 0.33 (1:10 EtOAc/dichloromethane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36−7.24 (m, 5H), 5.32 (br s, 1H), 5.10 (d, 1H, J = 6 Hz,), 3.68 (s, 3H), 3.40 (s, 2H), 3.00−3.21 (m, 2H), 1.41 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.8, 49.8, 51.6, 52.9, 80.2, 126.6, 126.7, 127.9, 128.0, 129.1, 155.5, 167.7, 201.2; mp 95−97 °C. LC-MS [M + H$^+$] calcd for $C_{17}H_{23}NO_5$, 322.1567; found, 322.1.

6-Phenylpiperidine-2,4-dione (13). Methyl-5-(*tert*-butoxycarbonylamino)-3-oxo-5-phenylpentanoate (**12**) (250 mg, 0.78 mmol) was stirred in dichloromethane (10 mL) at 0 °C under a nitrogen atmosphere. Trifluoroacetic acid (0.3 mL, 3.89 mmol) was added and stirred at room temperature for 3 h. The reaction mixture was concentrated and redissolved in dichloromethane (6 mL) and $NaHCO_3$ (4 mL) and stirred at room temperature for 6 h. The crude reaction was quenched with concentrated HCl (pH ≈ 3) and extracted with EtOAc (3 × 20 mL), and the combined organic phases were washed with H_2O (15 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$. After concentration in vacuo, the crude product was crystallized with EtOAc to afford the title compound (131 mg, 89%) as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.27−7.44 (m, 5H), 6.37 (br s, 1H), 4.80 (octet, 1H, J = 9.0, 4.5, 1.5 Hz), 3.37 (s, 2H), 2.89 (dd, 1H, J = 17.1, 4.5 Hz), 2.75 (dd, 1H, J = 16.2, 9.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 38.8, 47.4, 53.2, 126.1, 129.1, 129.6, 139.2, 168.7, 202.5; mp 167−169 °C. LC-MS [M + H$^+$] calcd for $C_{11}H_{11}NO_2$, 190.0790; found, 190.1.

Methyl 5-Hydroxy-3-oxo-5-phenylpentanoate (14). Methyl acetoacetate (1.2 mL, 15 mmol) was added to a suspension of NaH (720 mg of a 60% dispersion in mineral oil, 18 mmol) in THF (8 mL) at room temperature and stirred for 30 min under a nitrogen atmosphere. The reaction mixture was cooled down to −20 °C, and *n*-BuLi (11.3 mL of a 1.6 M solution in THF, 18 mmol) was added and stirred for 30 min. Benzaldehyde (1.82 mL, 18 mmol) was added and stirred for an additional 30 min. The reaction mixture was quenched with saturated NH_4Cl (30 mL) and extracted with EtOAc (3 × 30 mL), and the combined organic phases were washed with H_2O (20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent: 1:10 EtOAc/dichloromethane). This afforded the title compound (1.8 g, 57%) as yellow oil. R_f 0.23 (1:10 EtOAc/dichloromethane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36−7.24 (m, 5H), 5.2−5.14 (m, 1H), 3.72 (s, 3H), 3.49 (s, 2H), 3.04−2.86 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 49.6, 51.6, 52.5, 69.8, 125.6, 127.8, 128.6, 142.4, 167.4, 202.6; mp 95−97 °C. LC-MS [M + H$^+$] calcd for $C_{12}H_{14}O_4$, 223.0892; found, 223.1.

6-Phenyldihydro-2*H*-pyran-2,4(3*H*)-dione (15). Methyl 5-hydroxy-3-oxo-5-phenylpentanoate (950 mg, 4.3 mmol) was stirred in a solution of 0.2 M KOH (8 mL) for 30 min at room temperature. HCl (4 M) was slowly added (pH ≈ 2), and the precipitated solid was filtered and dried to afford the title compound (730 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46−7.35 (m, 5H), 5.69 (dd, 1H, J = 9.3, 3.6 Hz), 3.67 (d, 1H, J = 19 Hz), 3.49 (d, 1H, J = 19 Hz), 2.99−2.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 45.5, 47.4, 76.9, 126.3, 129.5, 129.7, 136.8, 167.2, 199.7; mp 125−127 °C. LC-MS [M + H$^+$] calcd for $C_{11}H_{10}O_3$, 191.0630; found, 191.1.

2-Benzylidenemalononitrile (16). Benzaldehyde (1 mL, 9.85 mmol), malononitrile (651 mg, 9.85 mmol), and anhydrous zinc chloride (134 mg, 0.98 mmol) were stirred at 100 °C under nitrogen atmosphere for 15 min. The reaction mixture was cooled to room temperature and treated with a solution of absolute alcohol to obtain a white solid, which was filtered and dried to afford the title compound (1.42 g, 93%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.91−7.87 (m, 1H), 7.76 (s, 1H), 7.65−7.59 (m, 1H), 7.55−7.49 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 82.8, 112.5, 113.7, 129.6, 130.7, 130.9, 134.6, 159.9; mp 85−86 °C. LC-MS [M + H$^+$] calcd for $C_{10}H_6N_2$, 155.0531; found, 155.1.

Crystallographic Data. For collection and analysis, please see Supporting Information. CCDC 775129, 775130, 775131, and 775132 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk.

In Silico Study. The modeling study was performed using the software package MOE (Molecular Operating Environment, Chemical Computing Group, 2010) using the built-in mmff94x forcefield and the GB/SA continuum solvent model. General procedure: The compound of interest was submitted to a stochastic conformational search (standard setup) to determine its low-energy conformation. Superimposition of selected low-energy conformations was done using the built-in function by fitting the three atoms amino groups and the $C^{1\prime}$-carbons of the phenyl rings.

Pharmacology. Cell culture of the EAAT-HEK293 cell lines and the [^{3}H]-D-Asp uptake assay were performed essentially as previously described.[33] The experimental procedures are described in detail in Supporting Information.

Pharmacokinetics. Animals. All experimental animals were allowed to acclimatize at the animal facilities for a minimum of 5 days before experimentation. The animals were kept under a 12 h day/night cycle (lights on at 6:00 a.m.) in a temperature (21 ± 2 °C) and humidity (60 ± 10%) controlled environment. Rats were kept in groups of two rats per cage with free access to rat chow and tap water, ad libitum. All experiments were carried out in accordance with the ethical rules of the Danish Committee and Use of Laboratory Animals.

Exposure Studies and Bioanalysis. Exposure of UCPH-101 in plasma and brain was evaluated in male Sprague−Dawley rats (225 g at arrival). The test compound was formulated in 100% PEG400 and dosed orally (5 mL/kg). Three groups of rats (n = 3/group) received a dose of 10 mg/kg, and plasma and brain were taken at 0.5, 1, and 2 h, respectively, following drug administration. Under isoflurane anesthesia, cardiac blood was obtained in EDTA-coated tubes and centrifuged for 10 min at 4 °C, after which plasma was drawn off. Following decapitation, the brain was removed and brain homogenate was prepared by homogenizing the whole brain with 70% acetonitrile (1:4 v/v) followed by centrifugation and collection of the supernatant. Plasma and brain supernatant samples were frozen at −80 °C until analysis. Concentrations of UCPH-101 were determined in plasma and brain homogenate using UltraPerformance LC chromatography (UPLC) followed by MS/MS detection. Then 150 μL of internal standard (5 ng/mL in acetonitrile containing 0.1% ammonium hydroxide) was added to 25 μL of calibration standards, QC samples, and test samples. After centrifugation (6200*g*, 4 °C, 20 min), 100 μL of supernatant from each sample was transferred to a new plate and mixed with 100 μL of water

with 0.1% ammonium hydroxide, and the samples were placed in the autosampler. Ten μL was injected into the UPLC. The mobile phase consisted of water/acetonitrile with 0.1% ammonium hydroxide pumped as a gradient through an analytical column (Acquity UPLC BEH Phenyl 1.7 μM, 2.1 mm $\times$ 30 mm, Waters, MA). Detection was performed using a Sciex-API 4000 MS (Applied Biosystems, The Netherlands) using electro spray with positive ionization mode with a parent > daughter molecular mass of 423.1 > 357.2 AMU. Retention time was 0.72 min. The lower limit of quantification was 5.0 ng/mL in plasma and 10 ng/g in brain (peak $S/N > 6$).

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Supporting Information Available: X-ray data and 2D NMR data (HMQC and HMBC) of **1r** and **1s**. This material is available free of charge via the Internet at http://pubs.acs.org.

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