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Identification of a New Class of Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors Followed by a Structure–Activity Relationship Study

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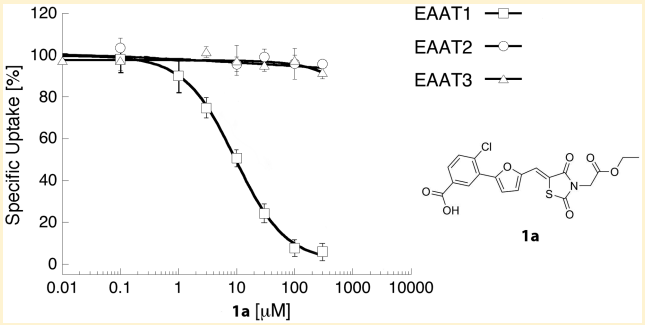
Supporting Information

ABSTRACT: Screening of a small compound library at the three excitatory amino acid transporter subtypes 1–3 (EAAT1–3) resulted in the identification of compound (*Z*)-4-chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)furan-2-yl)benzoic acid (**1a**) that exhibited a distinct preference as an inhibitor at EAAT1 (IC_{50} 20 μM) compared to EAAT2 and EAAT3 (IC_{50} > 300 μM). This prompted us to subject **1a** to an elaborate structure–activity relationship study through the purchase and synthesis and subsequent pharmacological characterization of a total of 36 analogues. Although this effort did not result in analogues with substantially improved inhibitory potencies at EAAT1 compared to that displayed by the hit, it provided a detailed insight into structural requirements for EAAT1 activity of this scaffold. The discovery of this new class of EAAT1-selective inhibitors not only supplements the currently available pharmacological tools in the EAAT field but also substantiates the notion that EAAT ligands not derived from α-amino acids hold considerable potential in terms of subtype-selective modulation of the transporters.



INTRODUCTION

(*S*)-Glutamate (*S*)-(Glu) is the major excitatory neurotransmitter in the CNS being involved in a plethora of physiological and pathophysiological processes. Dysfunctional glutamatergic neurotransmission is known to constitute a core component of a wide range of cognitive, psychiatric, and neurotoxic/neurodegenerative disorders. This fact makes glutamatergic targets highly interesting as drug targets. The synaptic reuptake of (*S*)-Glu following its release from presynaptic terminals into the synaptic cleft during neurotransmission is mediated by the family of excitatory amino acid transporters (EAATs).[1−6] The EAAT family consists of five transporters, termed EAAT1–5. The EAAT1,2 subtypes are predominantly expressed in glia cells, and the EAAT3–5 subtypes are predominantly expressed in neurons. The five subtypes are also characterized by distinct expression patterns in the CNS. EAAT2 is the major physiological subtype believed to account for >90% of total Glu uptake in the brain, and EAAT1 and EAAT3 are also widely expressed in the CNS. As for EAAT1, it is the major EAAT subtype in the cerebellum (where the highest density of the transporter is found in Bergmann glia cells), circumventribular organs, inner ear, and the retina, but the transport are also abundantly distributed in other CNS regions.[1] In contrast, EAAT4 and EAAT5 are expressed almost exclusively in cerebellum and retina, respectively.[1,2]

In contrast to the immense efforts made in terms of ligand development in the glutamate receptor field over the last couple of decades, the EAATs have been given considerably less attention as putative drug targets. Because of this, the selection of pharmacological tools for the transporters are rather limited and only a few ligands displaying true subtype-selectivity between the five EAATs have been identified. This has been an obstacle to explorations of the physiological functions and putative therapeutic potential of the respective subtypes.[2] Most of the ligand development in the EAAT field has been based on the scaffolds of the endogenous substrates for the transporters, (*S*)-Glu and (*S*/*R*)-aspartate (*S*/*R*)-(Asp) (Figure 1A). As exemplified in Figure 1B, these efforts have yielded a couple of ligands exhibiting distinct selectivity or preference for one EAAT subtype over others. The substrate binding sites in the EAATs are believed to be quite conserved, and this presents a challenge to the development of orthosteric subtype-selective ligands.[3,5,6] In the light of this, we have previously applied the strategy of identification of novel EAAT inhibitors by screening of compound libraries followed by medicinal chemistry

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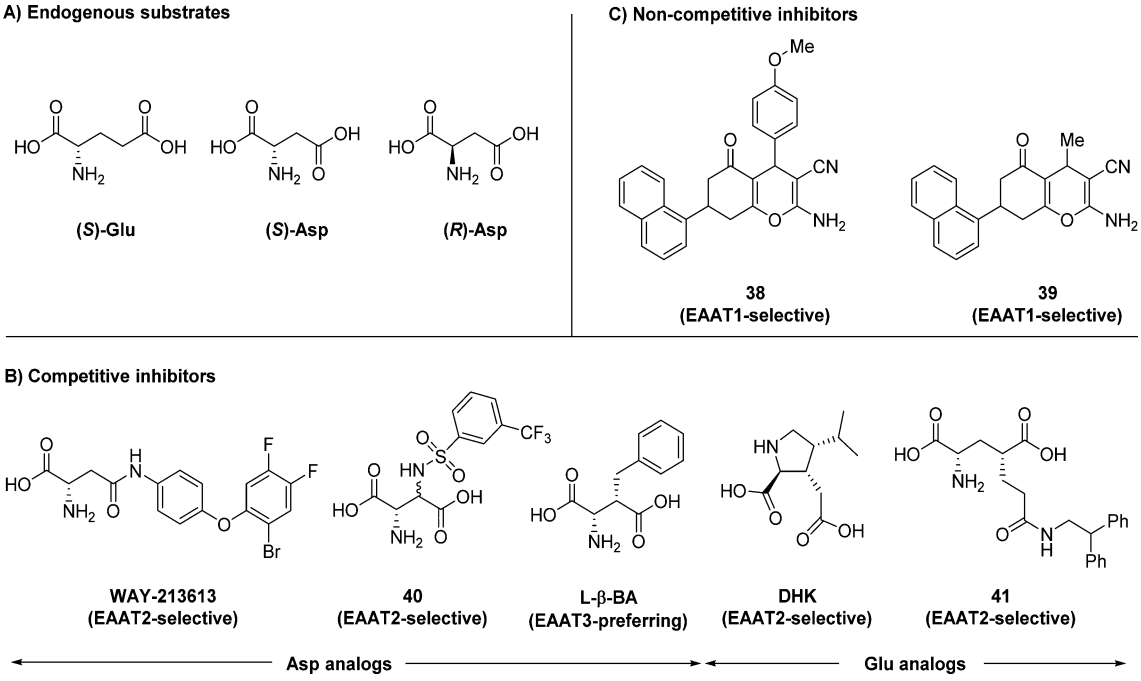

Figure 1. Chemical structures of the endogenous EAAT substrates (*S*)-Glu and (*S*/*R*)-Asp and reported subtype preferring/selective EAAT inhibitors: WAY213613,[13] (2*S*)-3-((3-(trifluoromethyl)phenyl)sulfonamido)aspartic acid (**40**),[14] L-*β*-BA,[15] DHK,[16,17] (2*S*,4*R*)-2-amino-4-(3-(2,2-diphenylethylamino)-3-oxopropyl)pentanedioic acid (**41**),[18] **38**,[10] and **39**.[8]

optimization. Compounds comprised in these library screenings are often structurally very different from *α*-amino acids, which leads to the assumption that hit compounds likely act through allosteric sites in the transporter. A successful outcome of this approach is discovery of the first class of EAAT1-selective inhibitors, represented by the analogues 2-amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-4*H*-chromene-3-carbonitrile (**38**, UCPH-101)[7] and 2-amino-5,6,7,8-tetrahydro-4-(4-methyl)-7-(naphthalen-1-yl)-5-oxo-4*H*-chromene-3-carbonitrile (**39**, UCPH-102)[8] (Figure 1C).[9,10] This inhibitor class was developed based on a hit compound identified in a screening of a commercial 3040-compound library at EAAT1−3,[9] and in subsequent studies the structure−activity-relationship (SAR) of this scaffold was explored in great detail.[9−12] In the present study, we report the discovery of a new class of selective EAAT1 inhibitors based on another hit compound from this screening and present an elaborate structure−activity relationship (SAR) study of this inhibitor class.

RESULTS AND DISCUSSION

From a library screening, compound **1a** was found to be a putative EAAT1-selective inhibitor.[7] When tested at a concentration of ∼100 *μ*M, **1a** almost completely inhibited the EAAT1-mediated uptake of [^{3}H]-D-Asp uptake in EAAT1-HEK293 cells, whereas it exhibited no significant effects on [^{3}H]-D-Asp uptake measured in EAAT2- and EAAT3-HEK293 cells in concomitant screenings. This apparent selective EAAT1-selectivity was subsequently verified by detailed characterization of the compound in the [^{3}H]-D-Asp uptake assay. **1a** was found to inhibit EAAT1-mediated uptake in a concentration-dependent manner exhibiting an IC_{50} value of 20 *μ*M, whereas it did not inhibit EAAT2- or EAAT3-mediated uptake at concentrations up to 300 *μ*M (Tables 1−2). These pharmacological properties were subsequently confirmed for a batch of **1a** synthesized by us, which exhibited IC_{50} values of 12 *μ*M, >300 *μ*M, and >300 *μ*M at EAAT1, EAAT2, and EAAT3, respectively (Figure 2, Tables 3−4). The fact that no significant inhibition of EAAT2,3 was observed for **1a** at 300 *μ*M suggests that the compound is at least 50-fold selective for EAAT1 over EAAT2,3 (Figure 2).

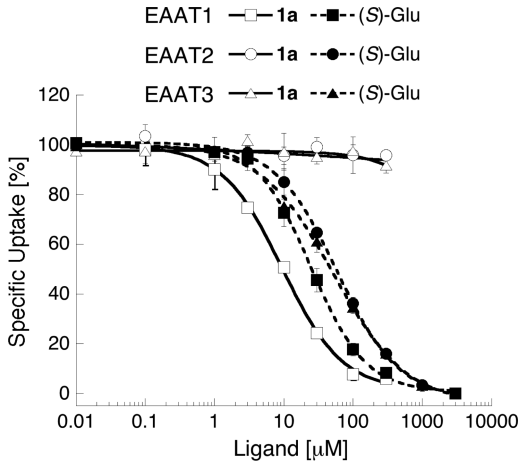

Figure 2. Concentration−inhibition curves for (*S*)-Glu and **1a** at EAAT1-, EAAT2-, and EAAT3-HEK293 cells in the [^{3}H]-D-Asp uptake assay. The figure depicts data from single representative experiments, and data are given as mean ± SD values (in % of specific uptake determined in the absence of test compound).

The interesting selectivity profile exhibited by **1a** prompted us to perform an elaborate SAR study on this compound scaffold, where a total of 36 analogues were either purchased from commercial sources or synthesized and subsequently tested on EAAT1−3 in a [^{3}H]-D-Asp uptake assay. A structural analysis of **1a** identifies five rotatable bounds, of which the aryl-

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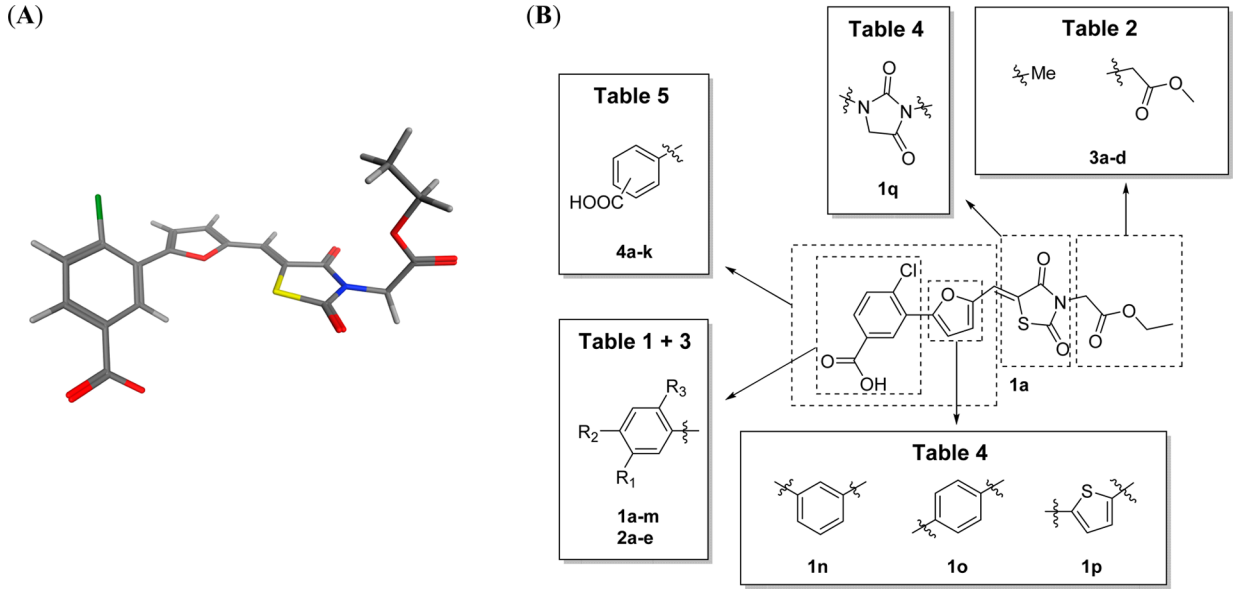


Figure 3. (A) Low-energy conformation of **1a**. (B) Overview of disconnections and chemical modifications of **1a**.

heteroaryl and the heteroaryl-alkenyl bonds are restrained by strong rotational barriers. Thus, **1a** is a highly conformationally locked structure with a well-defined low-energy conformation (Figure 3A). To explore the SAR of this compound class, we disconnected **1a** into synthetically feasible fragments: an ester moiety (purple), an aryl moiety (blue), a heteroaromatic moiety (red), and heterocycle (green) (Figure 3B).

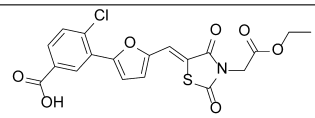
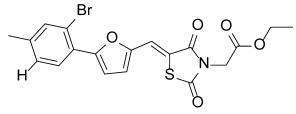
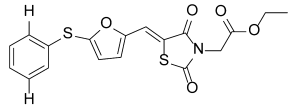
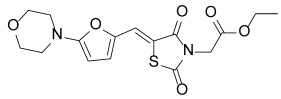
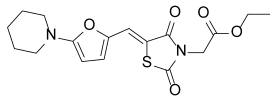
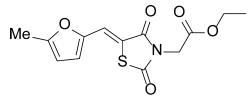
Pharmacological Characterization of 2a−2e. We first explored the influence of the chloro-carboxy substituted phenyl ring on the EAAT1 activity through the testing of the commercially available analogues **2a−2e** (Table 1). Perhaps not surprisingly, the dramatic structural changes introduced in analogues **2b−2e** resulted in complete loss of inhibitory activity at EAAT1 (and EAAT2,3). The inactivity of analogue **2a**, in which the phenyl ring substituent is retained but with a completely different substitution pattern compared to **1a**, was more informative. This suggested that the presence of a carboxylate group in the 4′-position and/or the presence of a substituent smaller than a bromo atom in the 2′-position was important for EAAT1 activity. The exact importance of these two substituents was investigated further in a subsequent part of the SAR study.

Pharmacological Characterization of 3a−d. We next turned to investigate the importance of the ester functionality by characterizing the commercially available analogues **3a−3d** at EAAT1−3 (Table 2). The ester functionality was found to be of key importance, and it was therefore decided to retain this functionality throughout the rest of the SAR study.

Synthesis and Pharmacological Characterization of 1b−m. We then proceeded with a detailed SAR study of the phenyl ring and its substituents. This work was commenced by resynthesis of **1a** by coupling of 3-bromo-4-chloro benzoic acid (**5**) with boronic acid **6** to give **7** in good conversion (Scheme 1). Crude **7** was therefore protected as its benzyl ester under standard conditions, and **8** was isolated in 65% over two steps. Condensation with **9**, readily obtained from **10**, gave the desired target compounds **11**, and after deprotection, **1a** in 58% yield.

Following the same strategy, (Scheme 1, step b) Pd-catalyzed coupling of 3-(5-formyl-2-furyl)-4-methoxybenzoic acid with boronic acid **6** gave the desired target compound **1d** in 24%. The synthesis of **1g** was achieved starting from 3-bromo-4-fluoro-benzoic acid which underwent Pd-catalyzed coupling with **6** (Scheme 1, step b) to give aldehyde **12** in 60%.

Table 1. Investigation of the Importance of the 2-Chloro-5-carboxyphenyl Functionality for EAAT1 Activity[b]

Compound	Chemical structure	EAAT1 IC_{50} (μM) [pIC_{50} ± SEM]
1a[a]		20 [4.70 ± 0.05]
2a[a]		>300 [<3.5]
2b[a]		>300 [<3.5]
2c[a]		>300 [<3.5]
2d[a]		>300 [<3.5]
2e[a]		>300 [<3.5]

[a]Compound obtained from commercial supplier. [b]None of the Compounds Displayed Inhibitory Activity at EAAT2,3 (IC_{50} > 300 μM)

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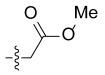
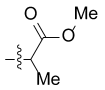
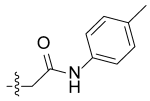
Table 2. Investigation of the Importance of the Ester Functionality for EAAT1 Activity (None of the Compounds Displayed Inhibitory Activity at EAAT2,3 (IC_{50} > 300 μM))

Compound		EAAT1 IC_{50} (μM) [pIC_{50} ± SEM]
3a [a]		23 [4.64 ± 0.07]
3b [a]		35 [4.46 ± 0.07]
3c [a]		>300 [<3.5]
3d [a]		>300 [<3.5]

[a]Compound obtained from commercial supplier.

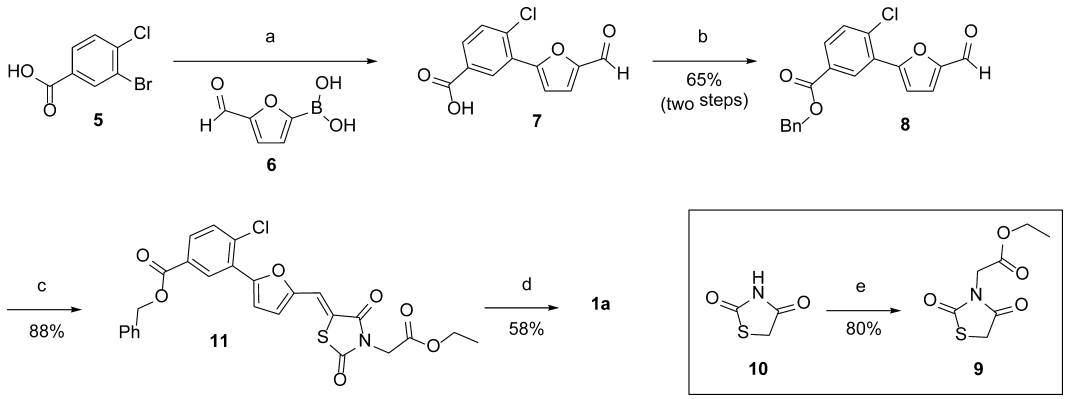
Condensation with **9** gave target compound **1g** in 37% yield. To access benzyloxy analogue **1e**, 4-(benzyloxy)-3-bromobenzoic acid (**14**) (Scheme 2) was prepared from **13** by benzylation of both the phenol and the carboxylic acid functionalities followed by saponification of the benzyl ester.[19] Coupling of **14** with **6** gave the desired product 4-(benzyloxy)-3-(5-formylfuran-2-yl)benzoic acid (**15**) in low yield as reported by others.[20] The synthesis of **1h** (Scheme 2) commenced by regioselective bromination of 3-amino-2-naphthoic acid (**16**) to give 3-amino-4-bromo-2-naphthoic acid (**17**) in 89%. Subsequent deamination by treatment with $NaNO_2$ in H_2SO_4 gave 4-bromo-2-naphthoic acid (**18**) in 91%. Coupling of bromine **18** with boronic acid **6** gave the desired aldehyde **19** in 11% yield. Despite the low yield, the reaction was not optimized further as condensation of **19** with **9** gave **1h** in quantities sufficient for spectral and pharmacological characterization. The synthesis of **1c** was carried out by condensation of **20** with **6** to give **1c** in 80% yield.

Pharmacological evaluation of **1a**–**1m** at EAAT1–3 (Table 3) revealed a number of interesting observations: Repositioning of the 2′-chlorine atom to the 4′-position (compound **1b**) led to complete loss of EAAT1 inhibitory activity. The same was observed when the carboxylate group was relocated from the 5′-position to the 4′-position (compound **1c**). Substitution of the chlorine for a methoxy group (compound **1d**) was well accepted, whereas the benzyloxy group introduced in this position in analogue **1e** evidently was too bulky (IC_{50} > 300 μM). Analogues with a methyl group (compound **1f**) or a fluorine atom (compound **1g**) in this position both exhibited ∼5-fold drop in inhibitory activity. Interestingly, a 1-naphthyl group (compound **1h**) was well accepted, which opens up for introduction of additional substitutions. Analogues **1i**–**1m** were all inactive, which further underlines the necessity of a substituent in the 2′-position and a carboxylate group in the 5′-position. Importantly, the size of the 2′-substituent is clearly essential for the EAAT1 inhibitory activity, as evidenced by the equipotent EAAT1 activity of the Cl- and MeO-analogues **1a** and **1d** compared to the inactivity of analogues with smaller (Me, F) or bigger (benzyloxy) substituents. It was decided to retain the 2′-chloro functionality throughout the rest of the SAR study.

Synthesis and Pharmacological Characterization of 1n–q. The next step was a SAR study of the importance of the furan ring and the alkene functionality for EAAT1 activity. In these analogues, the furan ring of **1a** was substituted with a phenyl (**1n** and **1o**) or a 2,5-thienyl (**1p**) ring systems and the alkene for an alkyl bond (**1q**). Synthesis of the meta-substituted phenyl analogue **1n** was accomplished by coupling of commercially available 3-bromo-4-chlorobenzoic acid with **6** to give **21**, which was then condensed with **9** to give the target compound **1n** in 55% yield. Analogously, the para-substituted analogue **1o** was prepared in 49% yield, while the 2,5-thienyl analogue **1p** was obtained in 50% yield from respective aldehydes. Synthesis of **1q** (Scheme 3) was achieved by first reducing aldehyde **8** to the alcohol **22**, which was then converted to chloride **23**. *N*-Alkylation of heterocycle **25** under standard conditions provided **24**, which after deprotection gave **1q** in 52%.

Interestingly, the meta-substituted phenyl analogue **1n** exhibited EAAT1 activity although it displayed slightly lower inhibitory potency compared to **1a**. On the other hand, the *para*-substituted phenyl analogue **1o** was completely inactive.

Scheme 1. Synthesis of 1a[a]



[a]Reagents and conditions: (a) $(PPh_3)_2PdCl_2$, Na_2CO_3 (aq), DME:EtOH 50 °C; (b) BnBr, K_2CO_3, DMF, rt; (c) **9**, piperidine, EtOH, reflux; (d) $FeCl_3$, DCM, rt; (e) ethyl 2-bromoacetate, NaH (60 w/w%), THF, reflux.

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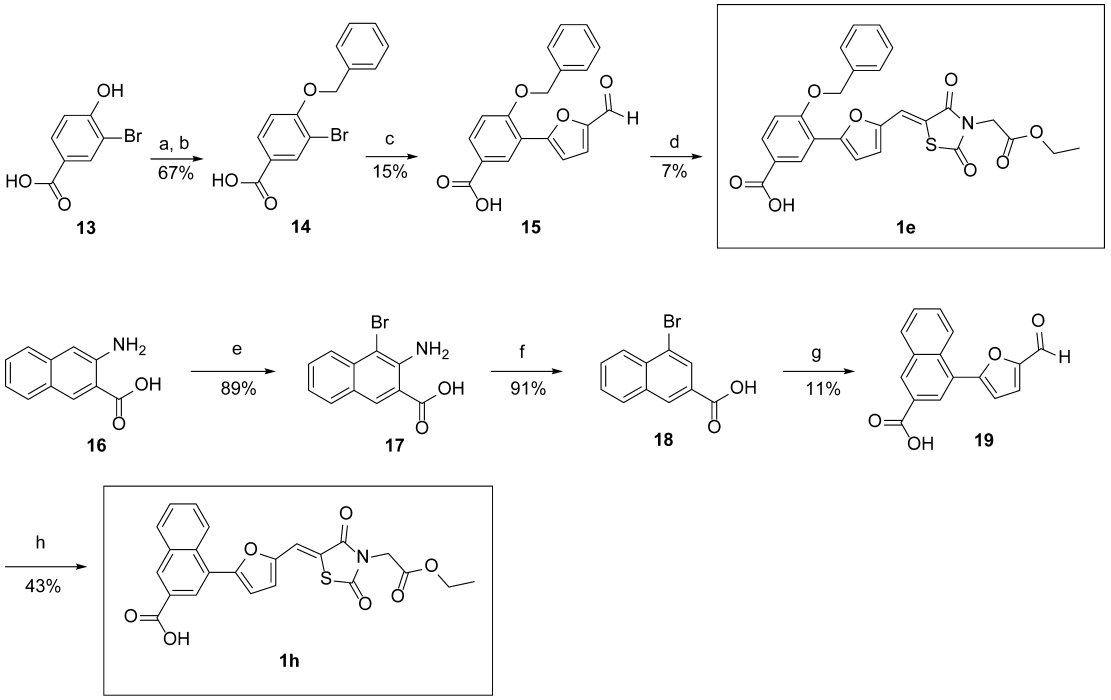
Scheme 2. Synthesis of *ortho*-Benzyloxy Analogue 1e and 1-Naphthyl Analogue 1h[a]



[a]Reagents and conditions: (a) benzyl bromide, anhydrous K_2CO_3, acetone, reflux; (b) 4 M NaOH (aq), MeOH, rt; (c) (5-formylfuran-2-yl)boronic acid (**6**), cat. $Pd(PPh_3)_4$, K_2CO_3, toluene:EtOH (7:3), 90 °C; (d) **9**, 0.1 equiv piperidine, 0.1 equiv AcOH, EtOH, 90 °C; (e) Br_2, $FeBr_3$, $CHCl_3$, 2 h, rt; (g) $NaNO_2$, H_2SO_4, AcOH, then Cu_2O, reflux; (g) **6**, cat. $Pd(PPh_3)_4$, K_2CO_3, toluene:EtOH (7:3), 90 °C. (h) **9**, 0.1 equiv piperidine, 0.1 equiv AcOH, EtOH, 90 °C.

The 2,5-thienyl analogue **1p** was also inactive, which is surprising given the similarity in substitution geometry compared to **1n** and **1a** (Table 4). The heterocyclic analogue **1q** displayed no inhibitory activity at EAAT1, which underlines the importance of the linear geometry of the molecule for transporter activity.

Synthesis and Pharmacological Characterization of 4b−4e. The limited success enhancing the potency of **1a** led us to explore more fundamental changes to the core skeleton. Having learned that the carboxylate and the alkene functionalities were important, we decided to synthesize truncated as well as conformationally relieved analogues of **1a** wherein only the ethyl 2-(2,4-dioxothiazolidin-3-yl)acetate (**9**) heterocycle was conserved. In total, 11 analogues **4b−4f** and **4g−4e**, respectively, were prepared by condensation of the corresponding aldehyde (Table 5) with **9** in accordance with previously applied conditions (Scheme 1). The pharmacological properties exhibited by these 11 analogues (Table 5) were however disappointing, as they were all very inactive at EAAT1−3.

CONCLUSION

We have identified a new class of EAAT1-selective inhibitors by screening of a commercially available compound library. While the SAR study presented herein of **1a** largely failed to to improve its EAAT1 inhibitory potency, it did disclose substantial insight into the pharmacophore elements which will be useful in future work within the field. More importantly, the EAAT1-selectivity exhibited by this inhibitor class substantiates the notion gained from the **38**/**39** inhibitor class, that subtype-selective EAAT modulators can emerge from scaffolds with no structural similarity to the α-amino acids. Considering the distinct chemical structure of **1a** compared to the EAAT substrates, it seems plausible that the compound analogously to **38**/**39** could mediate its EAAT1 inhibition through an allosteric site in the transporter.[21] However, it should be stressed that a definite conclusion as to whether **1a** and the other inhibitors in this class are indeed allosteric inhibitors or actually target the substrate-binding pocket in EAAT1 will have to await further experimental data. Finally, it is noteworthy that even though **1a** clearly is not nearly as potent and selective an EAAT1 inhibitor as **38**[7] or **39**,[8] the inhibitory potency and degree of selectivity exhibited by **1a** at EAAT1 are nevertheless roughly comparable to the properties displayed by DHK at EAAT2.[16] In view of the extensive use of this prototypic EAAT2 inhibitor as a pharmacological tool in studies of EAAT2 function in native tissues over the years, we propose that **1a** analogously could be a valuable tool in future explorations of the physiological roles mediated by and the therapeutic potential in EAAT1, be it as a supplement or alternative to **38**/**39**.

EXPERIMENTAL SECTION

Chemistry. All reactions involving anhydrous 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, Merck silica gel 60 F_{254} aluminum sheets). Flash chromatography was carried out using Merck silica gel 60A (35−70 μm), and dry column chromatography was carried out using Merck silica gel 60 (15−40 μm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz in $CDCl_3$ using $CHCl_3$ as internal standard unless otherwise noted. MS spectra were recorded using LC-MS performed using an Agilent 1200 solvent delivery system equipped with an autoinjector coupled to an Agilent 6400 triple quadrupole

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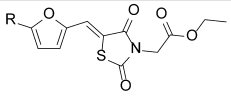
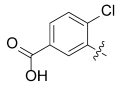
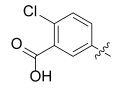
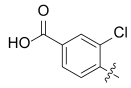
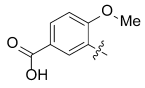
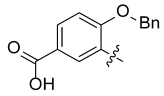
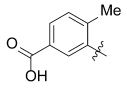
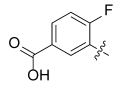
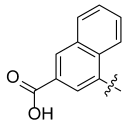
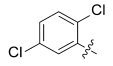
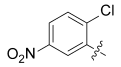
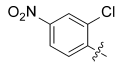
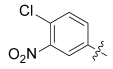
Table 3. Detailed SAR Study of the Phenyl Ring and Its Substitution Pattern for EAAT1 Activity (None of the Compounds Displayed Inhibitory Activity at EAAT2,3 (IC_{50} > 300 μM))

Compound		EAAT1 IC_{50} (μM) [pIC_{50} ± SEM]
1a		12 [4.95 ± 0.05]
1b[a]		>300 [<3.5]
1c		>300 [<3.5]
1d		12 [4.97 ± 0.11]
1e		>300 [<3.5]
1f[a]		~100[b] [~4.0]
1g		~100[b] [~4.0]
1h		38 [4.42 ± 0.04]
1i[a]		>300 [<3.5]
1j[a]		>300 [<3.5]
1k[a]		>300 [<3.5]
1l[a]		>300 [<3.5]
1m[a]		>300 [<3.5]

[a]Compound obtained from commercial supplier. [b]The concentration−inhibition curve for the compound was not complete within the tested concentration range, thus the IC_{50} value was estimated from the fitted curve.

mass spectrometer equipped with an electrospray ionization source. Gradients of 5% aqueous acetonitrile containing 0.05% formic acid (eluent A) and 95% aqueous acetonitrile containing 0.05% formic acid (eluent B) were employed. Analytical HPLC was performed using a Dionex UltiMate 3000 pump and photodiode array detector (200 and 210 nm, respectively) installed with an XTerra MS C_{18} 3.5 μm, 4.6 mm × 150 mm column, using a 5 → 95% MeCN gradient in H_2O containing 0.1% TFA. Melting points were measured using a MPA 100 Optimelt automatic melting point system. Optical rotation was measured using a PerkinElmer 241 polarimeter with Na lamp (589 nm). All commercial chemicals were used without further purification. The purity of all tested compounds was determined by HPLC to be >95%.

*(Z)-4-Chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)furan-2-yl)benzoic Acid (**1a**).* Under a nitrogen atmosphere, (*Z*)-benzyl 4-chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)furan-2-yl)benzoate (**11**) (0.100 g, 0.190 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (3.8 mL). $FeCl_3$ (0.124 g, 0.761 mmol, 4 equiv) was added during ice-cooling, and after 10 min of stirring at that temperature the mixture was left to come to rt for 2.5 h. The reaction was quenched by addition of H_2O (30 mL) and then extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried using anhydrous $MgSO_4$, filtered, and evaporated to give a brown solid (0.093 g). The crude product was purified by dry column vacuum chromatography (eluent: 100:1 CH_2Cl_2/AcOH) to give the title compound (0.048 g, 58%) as yellow solid. R_f (100:1 CH_2Cl_2/AcOH) 0.14. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.53 (d, 1H, J = 2.0 Hz), 7.94 (dd, 1H, J = 2.0 Hz, J = 8.3 Hz), 7.92 (s, 1H), 7.75 (d, 1H, J = 8.3 Hz), 7.52 (d, 1H, J = 3.8 Hz), 7.38 (d, 1H, J = 3.8 Hz), 4.50 (s, 2H), 4.18 (q, 2H, J = 7.3 Hz), 1.22 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.4, 166.7, 166.0, 164.6, 152.6, 149.0, 133.8, 131.6, 130.9, 130.4, 128.8, 127.1, 121.7, 119.7, 118.2, 115.0, 61.6, 42.2, 13.9. LC-MS [M + H^+] calcd for $C_{19}H_{15}ClNO_7S$, 436.03; found, 436.1. Elem. Anal. Calcd for $C_{19}H_{14}ClNO_7S$: C, 52.36; H, 3.24; N, 3.21; S, 7.36. Found: C, 52.28; H, 3.31; N, 3.20; S, 7.01. Melting point: 227.0−230.5 °C (1 °C/min) (decomposes).

*3-Chloro-4-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)2-furyl)benzoic Acid (**1c**).* 3-Chloro-4-(5-formyl-2-furyl)benzoic acid (**20**) (101 mg, 0.403 mmol) was suspended in abs EtOH (15 mL), and a solution of 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (81 mg, 0.399 mmol) in abs EtOH (3 mL) was added. After gentle heating and stirring for 10 min, piperidine (75 μL, 0.758 mmol) was added and the reaction mixture was heated to reflux and stirred at that temperature for 1 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (50 mL) and the resulting precipitate was filtered off, washed with H_2O (10 mL), and dried giving the title compound as a light-brown solid (140 mg, 0.321 mmol, 80%). R_f = 0 (*n*-heptane/EtOAc 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05−7.93 (m, 3H), 7.91 (s, 1H), 7.52 (d, J = 3.9 Hz, 1H), 7.39 (d, J = 3.9 Hz, 1H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.5, 166.7, 166.5, 164.6, 153.3, 149.1, 131.4, 129.3, 128.5, 128.4, 127.8, 121.8, 119.6, 118.1, 115.2, 61.6, 42.2, 14.0 (one signal missing or coinciding with others). LC-MS (Bruker): $[M + H]^+_{calcd}$ 436.02, $[M + H]^+_{found}$ 436.0. HPLC: >98%, retention time 2.39 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*3-(5-{[3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene]-methyl}-2-furyl)-4-methoxybenzoic Acid (**1d**).* 3-(5-Formyl-2-furyl)-4-methoxybenzoic acid (150 mg, 0.609 mmol) and 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (140 mg, 0.690 mmol) were suspended in abs EtOH (40 mL), and the reaction mixture was stirred for 5 min at rt under N_2 before piperidine (114 μL, 1.157 mmol) was added. The reaction mixture was then heated to reflux and stirred at that temperature for 5 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (15 mL) and H_2O (20 mL). The resulting dark-brown precipitate was filtered off, washed with H_2O (2 × 5 mL), and dried before being purified by column chromatography (2 cm Ø, 100 mL SiO_2, eluent CH_2Cl_2:EtOAc:AcOH, 100:10:1). The yellow band R_f = 0.31 was isolated, giving the title compound as an orange solid (64 mg, 0.148 mmol, 24%). R_f = 0.31 (CH_2Cl_2:EtOAc:AcOH, 100:10:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.48 (d, J = 2.1 Hz, 1H), 7.95 (dd, J = 8.6, 2.1 Hz, 1H), 7.88 (s, 1H), 7.34 (d, J = 3.7 Hz, 1H), 7.23 (d, J = 3.7 Hz, 1H), 7.21 (d, J = 8.6 Hz, 1H), 4.49 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 4.01 (s, 3H), 1.22 (t, J = 7.1 Hz, 5H). ^{13}C NMR (150 MHz, $CDCl_3$) (rotamers) δ: 167.6, 167.0, 166.7, 166.7, 166.5, 164.7, 162.6, 159.1,

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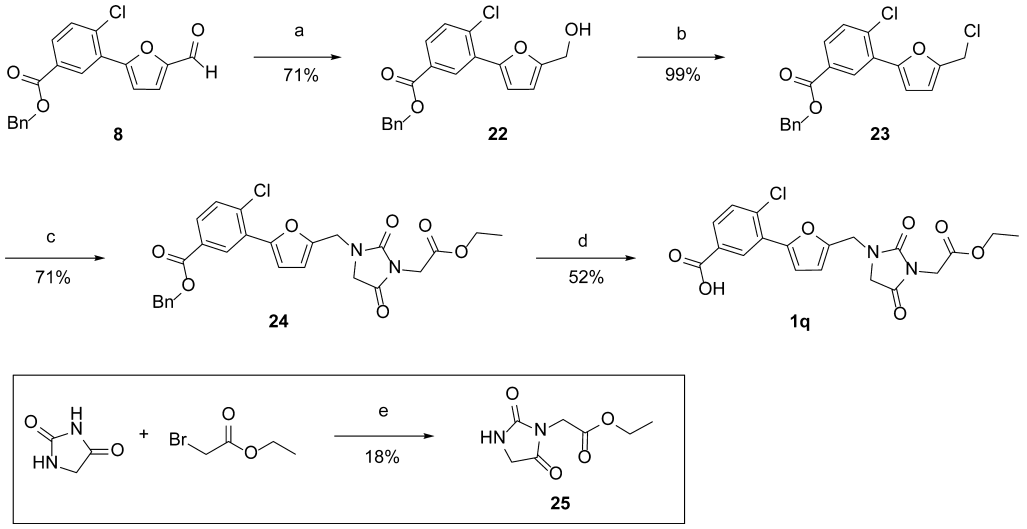
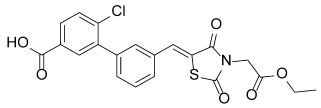
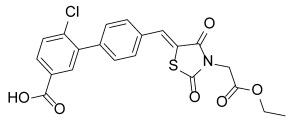
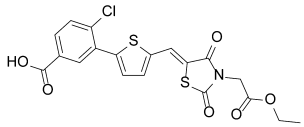
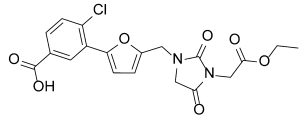
Scheme 3. Synthesis of Conformationally Relieved Analogue 1q[a]



[a]Reagents and conditions. (a) $NaBH_4$, THF, rt; (b) $SOCl_2$, pyridine, DCM; (c) NaH, DMF; (d) H_2 (g), Pd/C; (e) NaH, THF, 50 °C.

Table 4. SAR Study of the Influence of the Furan Ring and the Alkene Functionalities on EAAT1 Inhibitory Activity (None of the Compounds Displayed Inhibitory Activity at EAAT2,3 (IC_{50} > 300 μM))

Cmpd	Chemical structure	EAAT1 IC_{50} (μM) [$pIC_{50} \pm SEM$]
1n		45 [4.8 ± 0.33]
1o		>300 [<3.5]
1p		>300 [<3.5]
1q		>300 [<3.5]

153.3, 151.6, 148.7, 148.0, 131.6, 131.4, 129.6, 127.2, 124.5, 122.4, 121.2, 119.8, 117.4, 117.1, 116.8, 114.4, 114.3, 112.1, 112.0, 61.6, 56.3, 42.1, 13.9. LC-MS (Bruker): $[M + H]^+_{calcd}$ 432.07, $[M + H]^+_{found}$ 432.1. HPLC: >96%, retention time 2.45 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*(Z)-4-(Benzyloxy)-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)furan-2-yl)benzoic Acid (**1e**).* Synthesis was carried out as described for **1h**, with aldehyde **15** (36 mg, 0.11 mmol, 1.0 equiv), **9** (67 mg, 0.33 mmol, 3.0 equiv), piperidine (1 μL, 0.01 mmol, 0.1 equiv), AcOH (1 μL, 0.01 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 2 days. The precipitate was taken into acetone and filtered. The filtrate was concentrated under reduced pressure, and the obtained solid was recrystallized from EtOH which yielded the product as a yellow solid (4 mg, 7%). ^1H NMR (600 MHz, $CDCl_3$) δ: 8.71 (d, J = 2.1 Hz, 1H), 8.09 (dd, J = 8.7, 2.1 Hz, 1H), 7.70 (s, 1H), 7.49−7.38 (m, 5H), 7.14 (d, J = 8.7 Hz, 1H), 7.08 (d, J = 3.6 Hz, 1H), 6.87 (d, J = 3.7 Hz, 1H), 5.30 (s, 2H), 4.48 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, $CDCl_3$) δ: 168.4, 168.0, 166.5, 165.6, 159.2, 153.9, 148.5, 135.6, 132.3, 129.3, 129.1, 128.8, 128.0, 122.3, 121.0, 119.9, 118.7, 118.3, 114.5, 112.3, 71.3, 62.2, 42.2, 29.9, 14.3. LC-MS: $[M + H]^+_{calcd}$ 508.10, $[M + H]^+_{found}$ 508.1.

*4-Fluoro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)2-furyl)benzoic Acid (**1g**).* 4-Fluoro-3-(5-formyl-2-furyl)benzoic acid (**12**) (18 mg, 0.077 mmol) was suspended in abs EtOH (6 mL), and a solution of 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (16 mg, 0.0787 mmol) in abs EtOH (3 mL) was added followed by piperidine (15 μL, 0.151 mmol). The reaction mixture was heated to reflux under N_2 and stirred at that temperature for 2 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (20 mL). The resulting precipitate was filtered off, washed with H_2O (10 mL), and dried, giving the title compound as a brown solid (12 mg, 0.029 mmol, 37%). R_f = 0 (*n*-heptane/EtOAc 1:1). ^1H NMR (400 MHz, DMSO-d_6) δ: 13.32 (s, 1H), 8.49 (dd, J = 7.4, 2.2 Hz, 1H), 8.03 (ddd, J = 8.6, 4.9, 2.2 Hz, 1H), 7.94 (s, 1H), 7.54 (dd, J = 11.1, 8.7 Hz, 1H), 7.39 (d, J = 3.8 Hz, 1H), 7.25 (t, J = 3.6 Hz, 1H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.4, 166.7, 165.8, 164.6, 161.6, 159.9, 150.5, 149.1, 131.8, 131.8, 128.1, 127.6, 127.6, 122.1, 119.7, 117.9, 117.2, 117.1, 117.0, 117.0, 114.4, 114.3, 61.6, 42.2, 13.9. LC-MS: $[M + H]^+_{calcd}$ 420.06 $[M + H]^+_{found}$ 420.0. HPLC: >95%, retention time 1.96 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}. Melting point: decomposition.

*(Z)-4-(5-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)furan-2-yl)-2-naphthoic Acid (**1h**).* Piperidine (2 μL, 0.02 mmol, 0.1 equiv) and AcOH (1 μL, 0.02 mmol, 0.1 equiv) were added to a solution of **19** (45 mg, 0.17 mmol, 1.0 equiv) and **9** (35 mg, 0.17 mmol, 1.0 equiv) in EtOH (8 mL). The mixture was allowed to stir at reflux for 3 days, and the solvent was removed under reduced pressure. The crude product was purified by gradient flash chromatography (heptane:EtOAc, 1:0−2:1, with 0.5% of AcOH) on silica gel, which yielded the product as a yellow solid (33 mg, 43%). ^1H NMR (600 MHz, DMSO-d_6) δ: 13.32 (s, 1H), 8.72 (s, 1H), 8.44 (d, J = 8.6 Hz, 1H), 8.40 (d, J = 1.6 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.97 (s, 1H), 7.82 (ddd, J = 8.4, 6.8, 1.3 Hz, 1H), 7.74 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H), 7.47 (d, J = 3.7 Hz, 1H), 7.43 (d, J = 3.7 Hz, 1H), 4.49 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 167.5, 166.8, 166.7, 164.6, 156.3, 149.3, 133.1,

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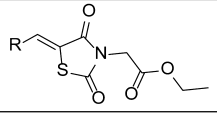
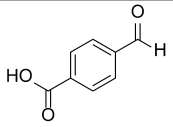
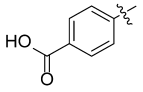
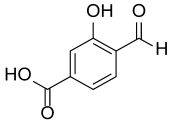
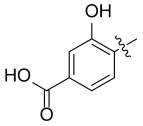
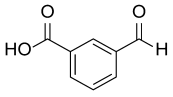
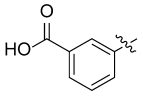
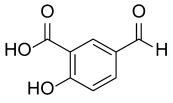
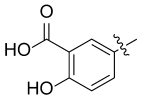
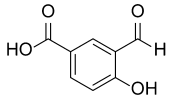
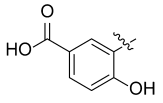
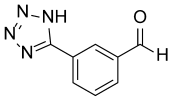
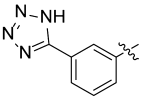
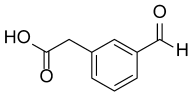
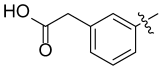
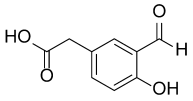
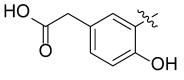
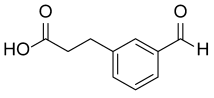
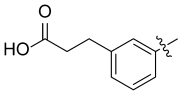
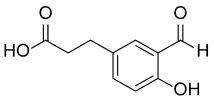
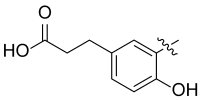
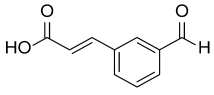
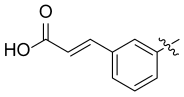
Table 5. SAR Study of Conformationally Relieved Analogues 4a−g Wherein the Ethyl 2-(2,4-Dioxothiazolidin-3-yl)acetate (9) Heterocycle Is Conserved from Lead Structure 1a[a]

	Reacting aldehyde	Product		EAAT1 IC_{50} (μM) [$pIC_{50} \pm SEM$]
26		4a		>300 [<3.5]
27		4b		>300 [<3.5]
28		4c		>300 [<3.5]
29		4d		>300 [<3.5]
30		4e		>300 [<3.5]
31		4f		>300 [<3.5]
32		4g		>300 [<3.5]
33		4h		>300 [<3.5]
34		4i		>300 [<3.5]
35		4j		>300 [<3.5]
36		4k		>300 [<3.5]

[a]None of the compounds **4a−g** displayed inhibitory activity at EAAT2,3 (IC_{50} > 300 μM).

132.1, 130.7, 130.5, 129.7, 127.8, 127.4, 126.6, 125.6, 124.9, 122.1, 120.0, 117.2, 114.0, 61.6, 42.1, 13.9. Melting point: 158.9−161.5 °C.

*6-Chloro-3′-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-[1,1′-biphenyl]-3-carboxylic Acid (**1n**).* 6-Chloro-3′-formyl-[1,1′-biphenyl]-3-carboxylic acid (**21**) (30 mg, 0.115 mmol) was suspended in abs EtOH (3 mL) and a solution of 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (23 mg, 0.115 mmol) in abs EtOH (3 mL) was added and the reaction mixture was stirred for 5 min at rt under N_2 before piperidine (21.6 μL, 0.219 mmol) was added. The reaction mixture was then heated to reflux and stirred at that temperature for 2 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (20 mL). The resulting yellow precipitate was filtered off, washed with H_2O (10 mL), and dried,

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giving the title compound as a yellow solid (28 mg, 0.063 mmol, 55%). R_f = 0 (*n*-heptane/EtOAc 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.10 (s, 1H), 7.92−7.85 (m, 2H), 7.76−7.64 (m, 3H), 7.62−7.53 (m, 2H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.8, 166.7, 166.6, 164.8, 139.8, 137.7, 133.9, 132.8, 132.0, 131.7, 131.0, 130.1, 129.5, 129.3, 129.2, 121.2, 61.7, 42.3, 14.0. LC-MS (Bruker): $[M + H]^+_{calcd}$ 446.05 $[M + H]^+_{found}$ 446.0. HPLC: >95%, retention time 2.67 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*6-Chloro-4′-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-[1,1′-biphenyl]-3-carboxylic Acid (**1o**).* 6-Chloro-4′-formyl-[1,1′-biphenyl]-3-carboxylic acid (30 mg, 0.115 mmol) was suspended in abs EtOH (3 mL) and a solution of 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (23 mg, 0.115 mmol) in abs EtOH (3 mL) was added and the reaction mixture was stirred for 5 min at rt under N_2 before piperidine (21.6 μL, 0.219 mmol) was added. The reaction mixture was then heated to reflux and stirred at that temperature for 4 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (20 mL). The resulting yellow precipitate was filtered off, washed with H_2O (10 mL), and dried, giving the title compound as a yellow solid (28 mg, 0.063 mmol, 55%). R_f = 0 (*n*-heptane/EtOAc 1:1), 0.3 (CH_2Cl_2:EtOAc:AcOH, 100:10:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.08 (s, 1H), 7.98−7.90 (m, 2H), 7.82−7.76 (m, 2H), 7.72−7.64 (m, 3H), 4.52 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H), signal from COOH is missing. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.8, 166.7, 166.3, 164.9, 140.4, 138.6, 133.5, 132.4, 131.9, 130.3, 130.2, 121.1, 61.7, 42.3, 14.0, six Ar-C signals are missing or coinciding with others. LC-MS (Bruker): $[M + H]^+_{calcd}$ 446.05 $[M + H]^+_{found}$ 446.0. HPLC: >96%, retention time 2.70 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*4-Chloro-3-(5-{[3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene]methyl}thiophen-2-yl)benzoic Acid (**1p**).* 4-Chloro-3-(5-formylthiophen-2-yl)benzoic acid (17.6 mg, 0.065 mmol) was suspended in abs EtOH (2 mL) and a solution of 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (15 mg, 0.072 mmol) in abs EtOH (1 mL) was added and the reaction mixture was stirred for 5 min at rt under N_2 before piperidine (12 μL, 0.125 mmol) was added. The reaction mixture was then heated to reflux and stirred at that temperature for 2.5 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (20 mL). The resulting orange precipitate was filtered off, washed with H_2O (10 mL), and dried. The solid was subsequently redissolved in abs EtOH (2 mL), a solution of 3-(2-ethoxy-2-oxoethanyl)2,4-dioxothiazolidine (9 mg, 0.044 mmol) in abs EtOH (1 mL) was added, and the reaction mixture was stirred for 5 min at rt under N_2 before piperidine (20 μL, 0.202 mmol) was added. The reaction mixture was then heated to reflux and stirred at that temperature for 5 h. Thereafter, the reaction mixture was cooled to rt and quenched with satd aq NH_4Cl (5 mL) and H_2O (5 mL). The resulting orange precipitate was filtered off, washed with H_2O (5 mL), and dried, giving the title compound as an orange solid (15 mg, 0.033 mmol, 50%). R_f = 0.31 (CH_2Cl_2:EtOAc:AcOH, 100:10:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 13.44 (s, 1H), 8.32 (s, 1H), 8.17 (d, J = 2.1 Hz, 1H), 7.96 (dd, J = 8.3, 2.1 Hz, 1H), 7.83 (d, J = 4.0 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 3.9 Hz, 1H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.1, 166.5, 166.4, 165.0, 145.4, 138.5, 136.1, 135.9, 132.2, 131.9, 131.7, 131.2, 130.9, 130.1, 127.4, 119.0, 62.1, 42.9, 14.4. LC-MS: $[M + H]^+_{calcd}$ 452.00 $[M + H]^+_{found}$ 451.9. HPLC: >96%, retention time 2.08 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*4-Chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxoimidazolidin-1-yl)methyl)furan-2-yl)benzoic Acid (**1q**).* Under a nitrogen atmosphere, 10% Pd/C (0.136 g) was added to anhydrous THF (20 mL). H_2 was bobbled through the mixture, and it was allowed to stir under a H_2 atmosphere at rt for 20 min. Then a solution of benzyl 4-chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxoimidazolidin-1-yl)methyl)-furan-2-yl)benzoate (0.340 g, 0.665 mmol) in anhydrous THF (20 mL) was added, and the mixture was stirred at rt for 2 h. Then the H_2 atmosphere was exchanged with nitrogen, and the mixture was filtered through a plug of Celite, which was then washed with THF. The filtrate was evaporated to give slightly yellow oil (0.327 g). The crude product was purified by dry column vacuum chromatography (eluent: 2:8:0.1 EtOAc/CH_2Cl_2/AcOH) and coevaporation with toluene to give the title compound (0.145 g, 52%) as white solid. R_f (25:75:1 EtOAc/CH_2Cl_2/AcOH) 0.32. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.43 (d, 1H, J = 2.0 Hz), 7.85 (dd, 1H, J = 2.0 Hz, J = 8.3 Hz), 7.48 (d, 1H, J = 8.3 Hz), 7.05 (d, 1H, J = 3.5 Hz), 6.41 (d, 1H, J = 3.5 Hz), 4.63 (s, 2H), 4.22 (s, 2H), 4.15 (q, 2H, J = 7.3 Hz), 3.99 (s, 2H), 1.21 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.2, 167.0, 155.7, 149.7, 149.3, 135.7, 131.2, 129.7, 129.5, 129.1, 128.3, 112.7, 111.2, 62.0, 49.8, 39.9, 39.6, 14.1. LC-MS $[M + H^+]$ calcd for $C_{19}H_{18}ClN_2O_7$, 421.08; found, 421.1. Elem. Anal. Calcd for $C_{19}H_{17}ClN_2O_7 \cdot 0.05C_7H_8$: C, 54.63; H, 4.12; N, 6.58. Found: C, 54.72; H, 4.22; N, 6.65. Melting point: 120.1−123.8 °C (1 °C/min).

*(Z)-4-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)benzoic Acid (**4a**).* Synthesis was carried out as described for **1h**, with commercially available 4-formylbenzoic acid (100 mg, 0.67 mmol, 1 equiv), **9** (136 mg, 0.67 mmol, 1 equiv), piperidine (7 μL, 0.07 mmol, 0.1 equiv), AcOH (4 μL, 0.07 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 4 days. The precipitate was washed with EtOH and dried, which yielded the product as an off-white solid (98 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.24 (s, 1H), 8.06 (s, 1H), 8.10−7.75 (m, 4H), 4.51 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 167.1, 167.0, 166.9, 165.2, 137.1, 133.3, 132.7, 130.7, 130.5, 123.3, 62.2, 42.8, 14.4; Melting point: 200.9−205.1 °C.

*(Z)-4-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)-3-hydroxybenzoic Acid (**4b**).* Synthesis was carried out as described for **1h**, with commercially available 4-formyl-3-hydroxybenzoic acid (200 mg, 1.20 mmol, 1.0 equiv), **9** (244 mg, 1.20 mmol, 1.0 equiv), piperidine (12 μL, 0.12 mmol, 0.1 equiv), AcOH (7 μL, 0.12 mmol, 0.1 equiv), and EtOH (7 mL) and reflux for 4 days. Purified by gradient flash chromatography (heptane:EtOAc, 1:1−1:1.5, with 0.5% of AcOH) on silica gel, which yielded the product as a yellow solid (130 mg, 31%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.99 (s, 1H), 8.14 (s, 1H), 7.55−7.50 (m, 3H), 4.49 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 166.9, 166.7, 166.5, 165.0, 157.1, 134.0, 129.0, 128.3, 123.6, 121.5, 120.4, 116.5, 61.7, 42.2, 13.9. Melting point: 225.3−229.1 °C.

*(Z)-3-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)benzoic Acid (**4c**).* Synthesis was carried out as described for **1h**, with commercially available 3-formylbenzoic acid (28) (330 mg, 2.20 mmol, 1.0 equiv), **9** (447 mg, 2.20 mmol, 1.0 equiv), piperidine (22 μL, 0.22 mmol, 0.1 equiv), AcOH (13 μL, 0.22 mmol, 0.1 equiv), and EtOH (8 mL) and reflux for 16 h. The reaction mixture was allowed to cool to rt, and the precipitate was filtered out and recrystallized from EtOH, which yielded the product as a white solid (334 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.31 (s, 1H), 8.20 (t, J = 1.2 Hz, 1H), 8.10 (s, 1H), 8.05 (dt, J = 7.8, 1.2 Hz, 1H), 7.91 (dt, J = 7.9, 1.2 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 4.51 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.6, 166.5, 166.4, 164.7, 134.3, 133.2, 133.1, 131.8, 131.2, 130.4, 129.8, 121.7, 61.7, 42.3, 13.9. Melting point: 198.3−202.1 °C.

*(Z)-5-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)-2-hydroxybenzoic Acid (**4d**).* Synthesis was carried out as described for **1h**, with commercially available 5-formyl-2-hydroxybenzoic acid (100 mg, 0.60 mmol, 1.0 equiv), **1** (122 mg, 0.60 mmol, 1.0 equiv), piperidine (6 μL, 0.06 mmol, 0.1 equiv), AcOH (4 μL, 0.06 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 11 days. Purified by flash chromatography (heptane:EtOAc, 1:5, with 0.5% of AcOH) on silica gel, which yielded the product as a yellow solid (75 mg, 36%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.08 (d, J = 2.4 Hz, 1H), 7.97 (s, 1H), 7.77 (dd, J = 8.7, 2.4 Hz, 1H), 7.09 (d, J = 8.7 Hz, 1H), 4.48 (s, 2H), 4.17 (q, J = 7.1 Hz, 2H), 1.21 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 170.9, 166.7, 166.6, 165.0, 163.4, 137.0, 133.6, 132.9, 123.5, 118.5, 117.5, 114.9, 61.6, 42.2, 13.9. Melting point: 199.1−202.8 °C.

*(Z)-3-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)-4-hydroxybenzoic Acid (**4e**).* Synthesis was carried out as described for **1h**, with commercially available 3-formyl-4-hydrox-

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ybenzoic acid (250 mg, 1.50 mmol, 1.0 equiv), **9** (305 mg, 1.50 mmol, 1.0 equiv), piperidine (15 μL, 0.15 mmol, 0.1 equiv), AcOH (9 μL, 0.15 mmol, 0.1 equiv), and EtOH (8 mL) and reflux for 2 days. Purified by gradient flash chromatography (heptanes:EtOAc, 2:1−1:1, with 0.5% of AcOH) on silica gel, which yielded the product as a yellow solid (180 mg, 34%), ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.85 (s, 1H), 11.52 (s, 1H), 8.14 (s, 1H), 8.01 (d, J = 2.0 Hz, 1H), 7.91 (dd, J = 8.6, 2.1 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H), ^{13}C NMR (600 MHz, DMSO-d_6) δ: 166.7, 166.6, 166.4, 165.0, 161.1, 133.8, 130.3, 128.4, 122.1, 120.3, 119.6, 116.2, 61.6, 42.2, 13.9;, Melting point: 200.9−204.0 °C.

*(Z)-Ethyl 2-(5-(3-(1H-Tetrazol-5-yl)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate (**4f**).* Piperidine (10 μL, 0.10 mmol, 0.1 equiv) was added to a solution of 3-(1*H*-tetrazol-5-yl)benzaldehyde (**31**) (170 mg, 0.98 mmol, 1.0 equiv) and **9** (199 mg, 0.98 mmol, 1.0 equiv) in EtOH (4 mL). The mixture was allowed to stir at reflux for 18 h then cooled to 0 °C. Then 1 M HCl (5 mL) was added and the resulting precipitate was filtered off, washed with water (8 mL) and hepetanes (8 mL), and dried, which yielded the product as a white solid (162 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.28 (s, 1H), 8.14 (d, J = 7.7 Hz, 1H), 8.08 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 7.8 Hz, 1H), 4.52 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.6, 166.5, 164.7, 155.4, 133.8, 132.9, 132.4, 130.5, 128.9, 128.1, 125.6, 122.2, 61.7, 42.3, 13.9. Melting point: 220.5−223.8 °C.

*(Z)-2-(3-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl)acetic Acid (**4g**).* Synthesis was carried out as described for **1h**, with **32** (175 mg, 1.07 mmol, 1.0 equiv), **9** (217 mg, 1.07 mmol, 1.0 equiv), piperidine (10 μL, 0.11 mmol, 0.1 equiv), AcOH (6 μL, 0.11 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 5 days. Purified by flash chromatography (heptane:EtOAc, 1:1, with 0.5% of AcOH) on silica gel and recrystallized from MeOH, which yielded the product as a white solid (60 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.44 (s, 1H), 7.98 (s, 1H), 7.57−7.40 (m, 4H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 3.68 (s, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 172.8, 167.4, 167.1, 165.4, 136.9, 134.4, 133.2, 132.6, 131.5, 129.8, 129.1, 121.1, 62.1, 42.7, 40.7, 14.4. Melting point: 154.9−158.5 °C.

*(Z)-2-(3-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-4-hydroxyphenyl)acetic Acid (**4h**).* Synthesis was carried out as described for **1h**, with **33** (320 mg, 1.78 mmol, 1.0 equiv), **9** (362 mg, 1.78 mmol, 1.0 equiv), piperidine (18 μL, 0.18 mmol, 0.1 equiv), AcOH (10 μL, 0.18 mmol, 0.1 equiv), and EtOH (8 mL) and reflux for 5 days. Purified by flash chromatography (MeOH:EtOAc, 1:5, with 0.5% of AcOH) on silica gel and recrystallized from MeOH, which yielded the product as a yellow solid (149 mg, 23%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.32 (s, 1H), 10.58 (s, 1H), 8.14 (s, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.23 (dd, J = 8.4, 2.1 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 4.48 (s, 2H), 4.17 (q, J = 7.1 Hz, 2H), 3.54 (s, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 173.2, 167.7, 167.2, 165.6, 156.7, 134.5, 129.8, 129.7, 126.8, 119.8, 119.5, 116.6, 62.1, 42.6, 40.5, 14.4. Melting point: 204.3−208.6 °C.

*(Z)-4-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)-3-hydroxybenzoic Acid (**4i**).* Synthesis was carried out as described for **1h**, with aldehyde **34** (140 mg, 0.79 mmol, 1.0 equiv), **9** (161 mg, 0.79 mmol, 1.0 equiv), piperidine (8 μL, 0.08 mmol, 0.1 equiv), AcOH (5 μL, 0.08 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 3 days. Purified by gradient flash chromatography (heptane:EtOAc, 4:1−2.5:1, with 0.5% of AcOH) on silica gel, which yielded the product as an off-white solid (115 mg, 40%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.16 (s, 1H), 7.96 (s, 1H), 7.54−7.36 (m, 4H), 4.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 2.89 (t, J = 7.5 Hz, 2H), 2.59 (t, J = 7.5 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 173.6, 166.9, 166.7, 164.9, 142.2, 134.2, 132.8, 131.1, 130.1, 129.4, 128.0, 120.5, 61.7, 42.2, 34.8, 30.0, 13.9. Melting point: 148.2−152.1 °C.

*(Z)-3-(3-((3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-4-hydroxyphenyl)propanoic Acid (**4j**).* Synthesis was carried out as described for **1h**, with aldehyde **35** (100 mg, 0.51 mmol, 1.0 equiv), **9** (103 mg, 0.51 mmol, 1.0 equiv), piperidine (5 μL, 0.05 mmol, 0.1 equiv), AcOH (3 μL, 0.05 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 2 days. Purified by flash chromatography (heptane:EtOAc, 1:1, with 0.5% of AcOH) on silica gel and recrystallized from a mixture (1:1:0.01) of MeCN, H_2O and DMSO, which yielded the product as a yellow solid (84 mg, 43%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.14 (s, 1H), 10.47 (s, 1H), 8.13 (s, 1H), 7.23−6.89 (m, 3H), 4.48 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 2.78 (t, J = 7.4 Hz, 2H), 2.52 (t, J = 7.3 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 173.7, 167.3, 166.7, 165.1, 155.8, 133.0, 132.1, 129.5, 128.1, 119.4, 118.9, 116.2, 61.6, 42.1, 35.3, 29.3, 13.9. Melting point: 185.1−188.3 °C.

*(E)-3-(3-((Z)-(3-(2-Ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl)acrylic Acid (**4k**).* Synthesis was carried out as described for **1h**, with **36** (100 mg, 0.57 mmol, 1.0 equiv), **9** (115 mg, 0.57 mmol, 1.0 equiv), piperidine (6 μL, 0.06 mmol, 0.1 equiv), AcOH (4 μL, 0.06 mmol, 0.1 equiv), and EtOH (5 mL) and reflux for 3 days. Purified by flash chromatography (heptane:EtOAc, 1:1.25, with 0.5% of AcOH) on silica gel, which yielded the product as a white solid (101 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.51 (s, 1H), 8.03 (s, 1H), 7.96 (s, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.64 (d, J = 15.6 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 6.63 (d, J = 16.1 Hz, 1H), 4.51 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ: 167.4, 166.8, 166.6, 164.8, 142.5, 135.4, 133.5, 133.4, 130.7, 130.4, 130.1, 130.0, 121.4, 121.0, 61.7, 42.3, 13.9. Melting point: 172.6−175.1 °C.

*4-Chloro-3-(5-formylfuran-2-yl)benzoic Acid (**7**).* A flask was charged with (5-formylfuran-2-yl)boronic acid (5.95 g, 42.5 mmol, 2 equiv), 3-bromo-4-chlorobenzoic acid (4.96 g, 21.2 mmol, 1 equiv), and $(PPh_3)_2PdCl_2$ (0.75 g, 1.06 mmol, 0.05 equiv) and then evacuated and refilled with N_2 three times. DME (70 mL, degassed), EtOH (70 mL, degassed), and 2 M Na_2CO_3 (64 mL, 128 mmol, 6 equiv, degassed) were added, and the mixture was heated to 50 °C and stirred at that temperature for 20 h. The mixture was allowed to cool to rt and was then evaporated in vacuo. The residue was suspended in H_2O (700 mL) and filtered through a plug of Celite, which was then washed with H_2O (700 mL). The combined aqueous filtrates were acidified (pH 1−2) using 1 M HCl. The solids were filtered off, washed with H_2O, and freeze-dried to give off-white solid (5.4 g). The crude product was used in the next step without further purification.

*Benzyl 4-Choro-3-(5-formylfuran-2-yl)benzoate (**8**).* Crude 4-chloro-3-(5-formylfuran-2-yl)benzoic acid (**7**) (5.44 g, 21.2 mmol, 1 equiv) was dissolved in DMF (125 mL). K_2CO_3 (4.41 g, 31.8 mmol, 1.5 equiv) was added, followed by benzyl bromide (2.8 mL, 23.4 mmol, 1.1 equiv). The mixture was stirred at rt for 2.5 h, and then the DMF was reduced in vacuo. The residue was suspended in Et_2O (750 mL) and H_2O (750 mL). The phases were separated, and the aqueous phase was washed with H_2O (750 mL). Precipitation occurred in the organic phase, therefore additional Et_2O (250 mL) was added followed by EtOAc (750 mL). The organic phase was washed with H_2O (250 mL). The combined aqueous phases were then extracted with EtOAc (3 × 250 mL). The combined organic phases were dried using anhydrous $MgSO_4$, filtered, and evaporated to give orange solid (7.29 g). The crude product was purified by dry column vacuum chromatography (EtOAc/heptane) to give the title compound (4.70 g, 65%) as orange solid. R_f (10:48 EtOAc/petroleum ether (40−65 °C)) 0.54. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.73 (s, 1H), 8.64 (d, 1H, J = 2.0 Hz), 7.98 (dd, 1H, J = 2.2 Hz, J = 8.5 Hz), 7.55 (d, 1H, J = 8.5 Hz), 7.49−7.36 (m, 5H), 7.36 (d, 1H, J = 3.9 Hz), 7.31 (d, 1H, J = 3.6 Hz), 5.41 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.9, 165.1, 154.3, 152.1, 136.3, 135.7, 131.3, 130.9, 130.5, 129.5, 128.8, 128.6, 128.5, 128.1, 122.1, 113.9, 67.5. LC-MS [M + H$^+$] calcd for $C_{19}H_{14}ClO_4$, 341.06; found, 341.1. Melting point: 120.0−121.8 °C (1 °C/min).

*Ethyl 2-(2,4-Dioxothiazolidin-3-yl)acetate (**9**).* In an ice bath, NaH (60 w/w%, 2.22 g, 55.5 mmol, 1.3 equiv) was slowly added to a stirred solution of thiazolidine-2,4-dione (5.00 g, 42.7 mmol, 1.0 equiv) in anhydrous THF (25 mL). The stirring was continued at rt until the evolution of gas ceased. In an ice bath, ethyl 2-bromoacetate (7.00 mL, 64.0 mmol, 1.5 equiv) was added to the obtained solution. The reaction mixture was heated to reflux and stirred at that temperature

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for 5 h before the solvent was removed under reduced pressure. The residue was diluted with cold water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude was purified by flash chromatography (DCM) on silica gel, which yielded the product as a colorless oil (6.75 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.34 (s, 2H), 4.23 (q, J = 7.1 Hz, 2H), 4.03 (s, 2H), 1.29 (t, J = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 171.2, 170.8, 166.3, 62.3, 42.3, 34.0, 14.2.

*(Z)-Benzyl 4-Chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)furan-2-yl)benzoate (**11**).* 2,4-Dioxothiazolidine **9** (0.244 g, 1.2 mmol, 1 equiv) was dissolved in absolute EtOH (15 mL). Benzyl 4-chloro-3-(5-formylfuran-2-yl)benzoate (**8**) (0.41 g, 1.2 mmol, 1 equiv) and piperidine (119 μL, 1.2 mmol, 1 equiv) were added. The mixture was heated to reflux and stirred that temperature for 1.5 h and then allowed to cool to rt. The solids was filtered off and washed with cold EtOH. Drying in vacuum oven gave the title compound (0.55 g, 88%) as a yellow solid. R_f (1:5 EtOAc:heptanes) 0.30. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.64 (d, 1H, J = 2.2 Hz), 7.96 (dd, 1H, J = 2.2 Hz, J = 8.5 Hz), 7.70 (s, 1H), 7.55 (d, 1H, J = 8.5 Hz), 7.53−7.47 (m, 2H), 7.43−7.35 (m, 3H), 7.34 (d, 1H, J = 3.9 Hz), 6.95 (d, 1H, J = 3.9 Hz), 5.42 (s, 2H), 4.49 (s, 2H), 4.26 (q, 2H, J = 7.2 Hz), 1.32 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.0, 166.3, 165.4, 165.1, 153.4, 149.2, 135.7, 135.5, 131.4, 130.3, 129.63, 129.60, 128.9, 128.8, 128.7, 128.0, 120.3, 119.6, 114.9, 67.6, 62.4, 42.4, 14.5. LC-MS [M + H$^+$] calcd for $C_{26}H_{21}ClNO_7S$, 526.07; found, 526.1. Melting point: 177.8−178.4 °C (1 °C/min).

*4-Fluoro-3-(5-formyl-2-furyl)benzoic Acid (**12**).* 3-Bromo-4-fluorobenzoic acid (55 mg, 0.251 mmol), (5-formyl-2-furyl)boronic acid (71 mg, 0.508 mmol), and $Pd(PPh_3)_2Cl_2$ (9 mg, 0.013 mmol) were mixed in a vial, which was subsequently evacuated and backfilled with N_2 several times. DME (1 mL, degassed), aq Na_2CO_2 (2M, 1 mL, degassed), and EtOH (1 mL, degassed) were added and the two-phase reaction mixture was heated to 50 °C and stirred at that temperature for 17 h, after which it was cooled to rt and the solvent was removed in vacuo. The resulting black solid was resuspended in H_2O (50 mL) and filtered through a plug of Celite (3 cm Ø, 1.5 cm), and the Celite was washed through with additional H_2O (2 × 10 mL). The combined aq solution was extracted with EtOAc (3 × 20 mL) and CH_2Cl_2 (20 mL) before it was acidified with aq HCl (2M, app 10 mL) until pH ≈ 1. The resulting orange precipitate was filtered off, washed with H_2O (3 × 5 mL), and dried, giving the title compound as a yellow solid (35 mg, 0.149 mmol, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.37 (s, 1H), 9.70 (s, 1H), 8.45 (dd, J = 7.3, 2.3 Hz, 1H), 8.05 (ddd, J = 8.6, 5.0, 2.3 Hz, 1H), 7.70 (d, J = 3.8 Hz, 1H), 7.55 (dd, J = 11.0, 8.6 Hz, 1H), 7.21 (t, J = 3.8 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ: 179.5, 168.0, 164.8, 162.2, 153.6, 153.6, 153.5, 133.8, 133.7, 130.0, 130.0, 129.3, 125.1, 118.8, 118.7, 117.8, 117.6, 114.2, 114.1. LC-MS: $[M + H]^+_{calcd}$ 235.03 $[M + H]^+_{found}$ 235.0. HPLC: >98%, retention time 1.62 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*4-(Benzyloxy)-3-bromobenzoic Acid (**14**).* Benzyl bromide (157 mg, 0.92 mmol, 2.0 equiv) and anhydrous K_2CO_3 (159 mg, 1.15 mmol, 2.5 equiv) were added to a solution of 3-bromo-4-hydroxybenzoic acid (100 mg, 0.46 mmol, 1.0 equiv) in anhydrous acetone (10 mL). The mixture was allowed to stir at reflux for 16 h and filtered hot. The filtrate was concentrated under reduced pressure. Then 4 M aq NaOH (345 μL, 1.38 mmol, 3.0 equiv) was added to a solution of the obtained crude product in MeOH (8 mL), and the mixture was allowed to stir at rt for 16 h. The solvent was removed under reduced pressure, and the residue was acidified with 4 M HCl and extracted with EtOAc (3 × 15 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure, which yielded the product as a white solid (95 mg, 67% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, J = 2.1 Hz, 1H), 7.92 (dd, J = 8.6, 2.1 Hz, 1H), 7.51−7.33 (m, 5H), 7.31 (d, J = 8.7 Hz, 1H), 5.30 (s, 2H). Spectral data in agreement with reported by Kulkarni et al.[19]

*4-(Benzyloxy)-3-(5-formylfuran-2-yl)benzoic Acid (**15**).* Synthesis was carried out as described for **19**, with bromine **14** (280 mg, 0.91 mmol, 1.0 equiv), **6** (140 mg, 1.00 mmol, 1.1 equiv), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol, 0.05 equiv), 2 M aqueous K_2CO_3 solution (1.4 mL, 2.73 mmol, 3 equiv), and a mixture (7:3) of toluene and EtOH (10 mL) and reflux for 5 days. Purified by gradient flash chromatography (heptane:EtOAc, 1:0−2.5:1, with 0.5% of AcOH) on silica gel, which yielded the product as a yellow solid (45 mg, 15%). ^{1}H NMR (600 MHz, CD_3OD) δ: 9.63 (s, 1H), 8.70 (d, J = 2.2 Hz, 1H), 8.09 (dd, J = 8.7, 2.2 Hz, 1H), 7.58−7.40 (m, 5H), 7.47 (d, J = 3.8 Hz, 1H), 7.35 (d, J = 8.8 Hz, 1H), 7.14 (d, J = 3.7 Hz, 1H), 5.38 (s, 2H). Spectral data in agreement with earlier reported.[20]

*3-Amino-4-bromo-2-naphthoic Acid (**17**).* In a flask wrapped up with foil, $FeBr_3$ (1.36 g, 4.60 mmol, 0.1 equiv) was added to a well-stirred solution of 3-amino-2-naphthoic acid (**16**) (8.57 g, 45.8 mmol, 1.0 equiv) in $CHCl_3$ (220 mL). A solution of bromine (2.58 mL, 50.4 mmol, 1.1 equiv) in $CHCl_3$ (120 mL) was added dropwise. The reaction mixture was allowed to stir for 16 h at rt. The precipitate was collected and washed with $CHCl_3$ (200 mL). The crude was dissolved in 1 M aqueous NaOH (250 mL) and then acidified with AcOH. The precipitate was filtered out and dried, which yielded the product as a yellow solid (10.85 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.57 (s, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.61 (t, J = 7.9 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 169.2, 144.5, 135.2, 133.8, 130.9, 130.5, 125.9, 124.2, 123.0, 116.1, 103.7.

*4-Bromo-2-naphthoic Acid (**18**).* 3-Amino-4-bromo-2-naphthoic acid (**17**) (4.20 g, 15.8 mmol, 1.0 equiv) was added to AcOH (100 mL). The mixture was heated to boil then rapidly cooled to 15 °C. In an ice bath, the obtained suspension and $NaNO_2$ (1.33 g, 18.9 mmol, 1.2 equiv) was added to a mixture (1:1) of concentrated H_2SO_4 and AcOH (20 mL). The resulting mixture was allowed to stir for 2.5 h before it was poured into a well-stirred suspension of Cu_2O (6.33 g, 44.2 mmol, 2.8 equiv) in MeOH (90 mL) and allowed to stir at 60 °C for 1 h before it was cooled to rt. The obtained mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was diluted with Et_2O (100 mL) and extracted with saturated aqueous $NaHCO_3$ (3 × 250 mL). The combined aqueous phases were acidified with 4 M HCl. The precipitate was filtered off and dried, which yielded the product as a brown solid (3.62 g, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.37 (s, 1H), 8.67 (s, 1H), 8.25 (d, J = 1.5 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 8.20 (t, J = 8.4 Hz, 1H), 7.85 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 7.73 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H).

*4-(5-Formylfuran-2-yl)-2-naphthoic Acid (**19**).* 4-Bromo-2-naphthoic acid (**18**) (100 mg, 0.40 mmol, 1.0 equiv) was dissolved in a mixture (7:3) of toluene and EtOH (10 mL) and degassed with argon. $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 equiv) and (5-formylfuran-2-yl)boronic acid (**6**) (62 mg, 0.44 mmol, 1.1 equiv) were added to the obtained solution and degassed with argon for. Degassed 2 M aqueous K_2CO_3 solution (0.60 mL, 1.20 mmol, 3 equiv) was then added, and the mixture was allowed to stir at reflux for 4 days. The reaction mixture was allowed to cool to rt, then filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was acidified with 4 M HCl, then extracted with EtOAc (3 × 10 mL) and washed with water (2 × 20 mL) and brine (20 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by gradient flash chromatography (heptanes:EtOAc, 1:0−2:1, with 0.5% of AcOH) on silica gel, yielding the product as a yellow solid (12 mg, 11%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.79 (s, 1H), 8.76 (s, 1H), 8.49 (d, J = 8.6 Hz, 1H), 8.46 (d, J = 1.6 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.75 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.66 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H), 7.46 (d, J = 3.6 Hz, 1H), 7.02 (d, J = 3.6 Hz, 1H).

*3-Chloro-4-(5-formyl-2-furyl)benzoic Acid (**20**).* 4-Bromo-3-chlorobenzoic acid (600 mg, 2.55 mmol), (5-formyl-2-furyl)boronic acid (713 mg, 5.10 mmol), and $Pd(PPh_3)_2Cl_2$ (90 mg, 0.128 mmol) were mixed in a round-bottom flask which was subsequently evacuated and backfilled with N_2 four times. DME (11 mL, degassed), aq Na_2CO_2 (2M, 11 mL, degassed), and EtOH (11 mL, degassed) were added, and the reaction mixture was heated to 50 °C and stirred at that temperature for 19 h, after which it was cooled to rt and the solvent was removed in vacuo. The resulting black solid was resuspended in H_2O (100 mL) and filtered through a plug of Celite (4 cm Ø, 2 cm),

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and the Celite was washed through with H_2O (2 × 20 mL). The combined aq solution was extracted with EtOAc (3 × 50 mL) before it was acidified with aq HCl (1M, app 20 mL) until pH ≈ 1. The voluminous yellow precipitate was filtered off, washed with H_2O (3 × 10 mL), and dried, giving the title compound as a yellow solid (497 mg, 1.983 mmol, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.49 (s, 1H), 9.70 (s, 1H), 8.09−7.97 (m, 3H), 7.70 (d, J = 3.8 Hz, 1H), 7.51 (d, J = 3.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.6, 165.5, 153.1, 151.9, 132.5, 131.4, 130.5, 130.4, 129.2, 128.4, 124.1, 114.8. LC-MS: $[M + H]^+_{calcd}$ 251.01 $[M + H]^+_{found}$ 251.0. HPLC: >96%, retention time 1.66 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*6-Chloro-3′-formyl-[1,1′-biphenyl]-3-carboxylic Acid (**21**).* 3-Bromo-4-chlorobenzoic acid (99 mg, 0.420 mmol), (3-formylphenyl)-boronic acid (126 mg, 0.840 mmol), and $Pd(PPh_3)_2Cl_2$ (15 mg, 0.021 mmol) were mixed in a vial which was subsequently evacuated and backfilled with argon several times. DME (1.2 mL, degassed), aq Na_2CO_2 (2M, 1.3 mL, degassed), and abs EtOH (1.2 mL, degassed) was added, and the two-phase reaction mixture was heated to 50 °C and stirred at that temperature for 17 h. Thereafter, it was cooled to rt and the solvent was removed in vacuo. The resulting black solid was resuspended in H_2O (50 mL) and filtered through a plug of Celite (3 cm Ø, 2 cm), and the Celite was washed through with additional H_2O (2 × 10 mL). The combined aq solution was acidified with aq HCl (2M, app 10 mL) until pH ≈ 1. The resulting white precipitate was filtered off, washed with H_2O (3 × 10 mL), and dried, giving the title compound as a yellow solid (100 mg, 0.384 mmol, 91%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 13.32 (s, 1H), 10.09 (s, 1H), 8.02−7.94 (m, 4H), 7.86−7.81 (m, 1H), 7.78−7.71 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 193.0, 192.9, 166.2, 138.8, 138.7, 136.3, 136.3, 135.9, 135.2, 132.0, 130.4, 130.4, 130.3, 130.2, 129.3, 128.9. Rotamers of this compound gives double signals for some of the protons. LC-MS: $[M + H]^+_{calcd}$ 261.03 $[M + H]^+_{found}$ 261.0. HPLC: >94%, retention time 2.17 min, reverse phase, H_2O/MeCN/TFA gradient run, 4 mL min^{-1}.

*Benzyl 4-Chloro-3-(5-(hydroxymethyl)furan-2-yl)benzoate (**22**).* Under a nitrogen atmosphere, benzyl 4-chloro-3-(5-formylfuran-2-yl)benzoate (**8**) (0.638 g, 1.87 mmol, 1 equiv) was suspended in anhydrous MeOH (40 mL). $NaBH_4$ (0.215 g, 5.62 mmol, 3 equiv) was added, and the mixture was stirred at rt for 45 min. The reaction was quenched by addition of saturated $NaHCO_3$ (80 mL). MeOH was removed by evaporation in vacuo. The residue was partitioned between H_2O (200 mL) and EtOAc (200 mL), and the aqueous phase was extracted with EtOAc (2 × 200 mL). The combined organic phases were dried using anhydrous $MgSO_4$, filtered, and evaporated to give yellow oil (0.58 g). The crude product was purified by dry column vacuum chromatography (EtOAc/heptane) to give the title compound (0.46 g, 71%) as slightly yellow solid. R_f (3:7 EtOAc/heptane) 0.18. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.54 (d, 1H, J = 2.2 Hz), 7.85 (dd, 1H, J = 2.2 Hz, J = 8.3 Hz), 7.48 (d, 1H, J = 8.5 Hz), 7.48−7.32 (m, 5H), 7.11 (d, 1H, J = 3.6 Hz), 6.45 (d, 1H, J = 3.4 Hz), 5.40 (s, 2H), 4.70 (d, 2H, J = 6.3 Hz), 1.90 (t, 1H, J = 6.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.2, 156.9, 147.8, 136.5, 134.1, 132.1, 129.5, 129.4, 129.3, 129.2, 128.9, 128.7, 128.4, 113.5, 110.1, 67.3, 56.3. LC-MS [M + (−OH)] calcd for $C_{19}H_{14}ClO_3$, 325.06; found, 325.1. Melting point: 72.2−74.2 °C (1 °C/min).

*Benzyl 4-Chloro-3-(5-(chloromethyl)furan-2-yl)benzoate (**23**).* Under a nitrogen atmosphere, benzyl 4-chloro-3-(5-(hydroxymethyl)-furan-2-yl)benzoate (**22**) (1.00 g, 2.92 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (20 mL). The solution was cooled to approximately 0 °C. Pyridine (0.26 mL, 3.21 mmol, 1.1 equiv) was added, followed by thionyl chloride (1.3 mL, 17.2 mmol, 5.9 equiv). The mixture was stirred at rt for 2 h and was then diluted with CH_2Cl_2 (80 mL) and quenched by addition of H_2O (80 mL). The phases were separated, and the organic phase was washed with H_2O (3 × 80 mL), dried using anhydrous $MgSO_4$, filtered, and evaporated to give the title compound (1.05 g, quant) as slightly red solid. R_f (3:7 EtOAc/heptane) 0.60. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.56 (d, 1H, J = 2.2 Hz), 7.88 (dd, 1H, J = 2.2 Hz, J = 8.5 Hz), 7.49 (d, 1H, J = 8.3 Hz), 7.49−7.32 (m, 5H), 7.12 (d, 1H, J = 3.4 Hz), 6.52 (d, 1H, J = 3.4 Hz), 5.40 (s, 2H), 4.47 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.5, 150.3, 150.0, 135.9, 135.0, 131.1, 129.5, 129.2, 129.1, 128.8, 128.5, 128.4, 112.9, 112.1, 67.3, 37.7. LC-MS [M + H$^+$] calcd for $C_{19}H_{14}ClO_3$, 325.06; found, 325.1. Melting point: 87.5−89.2 °C (1 °C/min).

*Benzyl 4 Chloro-3-(5-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-1-yl)methyl)furan (**24**).* Under a nitrogen atmosphere, NaH (60% dispersion in mineral oil) (0.062 g, 1.523 mmol, 1.1 equiv) was suspended in anhydrous DMF (5 mL) and stirred at rt for 30 min. A solution of ethyl 2-(2,5-dioxoimidazolidin-1-yl)acetate (**25**) (0.260 g, 1.384 mmol, 1 equiv) in anhydrous DMF (5 mL) was added, and the mixture was stirred at rt for 30 min. A solution of benzyl 4-chloro-3-(5-(chloromethyl)furan-2-yl)benzoate (**23**) (0.500 g, 1.384 mmol, 1 equiv) in anhydrous DMF (5 mL) was added, and the mixture was stirred at rt for 19 h. The mixture was quenched by addition of H_2O (100 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 150 mL), diluted with H_2O (50 mL), and extracted with EtOAc (3 × 150 mL). The combined organic phases were dried using anhydrous $MgSO_4$, filtered, and evaporated to give yellow oil (0.734 g). The crude product was purified by dry column vacuum chromatography (EtOAc/heptane (0−30%)) to give the title compound (0.503 g, 71%) as yellow oil. R_f (1:1 EtOAc/heptane) 0.37. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.48 (d, 1H, J = 2.0 Hz), 7.91 (dd, 1H, J = 2.0 Hz, J = 8.3 Hz), 7.53 (d, 1H, J = 8.3 Hz), 7.51−7.35 (m, 5H), 7.11 (d, 1H, J = 3.5 Hz), 6.49 (d, 1H, 3.5 Hz), 5.42 (s, 2H), 4.70 (s, 2H), 4.28 (s, 2H), 4.24 (q, 2H, J = 7.3 Hz), 4.04 (s, 2H), 1.30 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.1, 167.0, 165.4, 155.6, 149.8, 149.2, 135.8, 135.0, 131.1, 129.3, 129.1, 129.0, 128.7, 128.4, 128.3, 112.5, 111.1, 67.1, 62.0, 49.8, 39.8, 39.6, 14.1. LC-MS [M + H$^+$] calcd for $C_{26}H_{24}ClN_2O_7$, 511.13; found, 511.2.

*Ethyl 2-(2,5-Dioxoimidazolidin-1-yl)acetate (**25**).* Under a nitrogen atmosphere, NaH (60% in mineral oil) (0.882 g, 22.0 mmol, 1.1 equiv) was suspended in anhydrous THF (90 mL). Hydantoin (2.00 g, 20.0 mmol, 1 equiv) was added, and the mixture was stirred at rt for 1 h. Ethyl 2-bromoacetate (2.25 mL, 20.0 mmol, 1 equiv) dissolved in anhydrous THF (8 mL) was added, and the mixture was heated to 50 °C and stirred at that temperature for 17.5 h. The mixture was allowed to cool to rt and was then quenched by addition of aqueous Na_2CO_3 (10%, 100 mL). The two phases were separated, and the aqueous phase was neutralized (pH 7) using concentrated aqueous HCl and then extracted with EtOAc (100 mL). The combined organic phases were dried using anhydrous $MgSO_4$, filtered, and evaporated to give off-white solid (2.45 g). The crude product was purified by dry column vacuum chromatography (EtOAc/heptane) to give the title compound (0.68 g, 18%) as white solid. R_f (1:1 EtOAc/heptane) 0.09. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.67 (br s, 1H), 4.26 (s, 2H), 4.23 (q, 2H, J = 7.2 Hz), 4.08 (d, 2H, 1.1 Hz), 1.30 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.8, 167.1, 157.6, 62.2, 46.9, 39.6, 14.4. LC-MS [M + H$^+$] calcd for $C_7H_{11}N_2O_4$, 187.07; found, 187.0. Melting point: 118.6−119.8 °C (1 °C/min).

*3-(1H-Tetrazol-5-yl)benzaldehyde (**31**).* Commercially available 3-formylbenzonitrile (50 mg, 0.38 mmol, 1.0 equiv) and TBAF·3H_2O (180 mg, 0.57 mmol, 1.5 equiv) were added to $TMSN_3$ (227 μL, 1.71 mmol, 4.5 equiv). The mixture was allowed to stir at 90 °C for 15 h. The reaction mixture was diluted with EtOAc (10 mL) and washed with 1 M HCl (3 × 5 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure, which yielded the product as a white solid (55 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.13 (s, 1H), 8.58 (t, J = 1.5 Hz, 1H), 8.37 (dt, J = 7.7, 1.3 Hz, 1H), 8.13 (dt, J = 7.7, 1.3 Hz, 1H), 7.86 (t, J = 7.7 Hz, 1H).

*2-(3-Formylphenyl)acetic Acid (**32**).* Benzoyl peroxide (7 mg, 0.03 mmol, 0.01 equiv) was added to a solution of commercially available 2-(3-methylphenyl)acetic acid (0.50 g, 3.33 mmol, 1.0 equiv) and *N*-bromosuccinimide (0.89 g, 5.00 mmol, 1.5 equiv) in $CHCl_3$ (20 mL). The mixture was heated to reflux and stirred at that temperature for 24 h before the solvent was removed under reduced pressure. The residue was dissolved in a mixture (1:1) of EtOH and H_2O (20 mL) and hexamethylenetetramine (1.26 g, 8.99 mmol, 2.7 equiv) was added, and the mixture was refluxed for for 4 h. Concentrated HCl (1.6 mL, 20.0 mmol, 6 equiv) was slowly added to the mixture at reflux. The

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mixture was allowed to stir at reflux for 30 min and then cooled to rt. DCM (10 mL) and H_2O (10 mL) were added, and the phases were separated. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was taken into saturated aqueous $NaHCO_3$ (10 mL) and washed with DCM (2 × 10 mL). The aqueous phase was acidified with 4 M HCl (8 mL) and extracted with DCM (3 × 20 mL). The organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure, which yielded the product as an off-white solid (275 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.02 (s, 1H), 7.84–7.50 (m, 4H), 3.76 (s, 2H).

*2-(3-Formyl-4-hydroxyphenyl)acetic Acid (**33**).* First, 1 M aqueous LiOH (30 mL, 30 mmol, 9.0 equiv) was added to a solution of **37** (660 mg, 3.40 mmol, 1 equiv) in THF (6 mL). The mixture was then allowed to stir at reflux for 15 h. The reaction mixture was acidified with 4 M HCl and extracted with EtOAc (3 × 40 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane:EtOAc, 1:1), which yielded the product as a white solid (254 mg, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.24 (s, 1H), 7.53 (d, J = 2.3 Hz, 1H), 7.40 (dd, J = 8.5, 2.4 Hz, 1H), 6.94 (d, J = 8.5 Hz, 1H), 3.53 (s, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 191.4, 172.7, 159.5, 137.6, 129.5, 126.1, 121.9, 117.2, 39.5.

*3-(3-Formylphenyl)propanoic Acid (**34**).* $NaBH_4$ (168 mg, 4.44 mmol, 3.0 equiv) was added portionwise over 5 min to a well-stirred solution of **36** (260 mg, 1.48 mmol, 1.0 equiv) and $NiCl_2{\cdot}6H_2O$ (352 mg, 1.48 mmol, 1.0 equiv) in MeOH (20 mL) at rt. The reaction mixture was allowed to stir at rt for 1 h and quenched with saturated aqueous $NH_3{\cdot}$HCl (10 mL). The mixture was filtered through a plug of Celite, and the filtrate was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane:EtOAc, 1:1, with 0.5% of AcOH) on silica gel, which yielded the product as a white solid (140 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.98 (s, 1H), 7.77 (t, J = 1.5 Hz, 1H), 7.73 (dt, J = 7.5, 1.5 Hz, 1H), 7.59 (dt, J = 7.5, 1.5 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 2.80 (t, J = 7.6 Hz, 2H), 2.51 (t, J = 7.6 Hz, 2H).

*3-(3-Formyl-4-hydroxyphenyl)propanoic Acid (**35**).* First 4 M NaOH (7.5 mL, 30 mmol, 10 equiv) was added to a solution of 3-(4-hydroxyphenyl)propanoic acid (500 mg, 3.01 mmol, 1.0 equiv) in $CHCl_3$ (10 mL). The mixture was then heated to reflux and stirred at that temperature for 6 h before it was acidified with 4 M HCl. The solvent was removed under reduced pressure, and the residue was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude was purified by flash chromatography (heptane:EtOAc, 1:1, with 0.5% of AcOH), which yielded a mixture (1:2) of the product and starting material (363 mg). The mixture was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.22 (s, 1H), 9.12 (s, 1H), 7.49 (d, J = 2.3 Hz, 1H), 7.39 (dd, J = 8.5, 2.4 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 2.77 (t, J = 7.5 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H).

*(E)-3-(3-Formylphenyl)acrylic Acid (**36**).* Commercially available isophthalaldehyde (1.0 g, 7.46 mmol, 1.0 equiv) and malonic acid (0.80 g, 7.46 mmol, 1.0 equiv) were added to a mixture of pyridine and EtOH (1:1, 20 mL). The mixture was heated to reflux and stirred at that temperature for 16 h. The reaction mixture was allowed to cool to rt and acidified with 6 M HCl (20 mL). The precipitate was filtered off and purified by flash chromatography (heptane:EtOAc, 1:1.25, with 0.5% of AcOH) on silica gel, which yielded the product as a white solid (0.75 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.52 (s, 1H), 10.04 (s, 1H), 8.22 (s, 1H), 8.03 (d, J = 7.7 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 15.9 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 6.66 (d, J = 16.1 Hz, 1H).

*Methyl 2-(3-Formyl-4-hydroxyphenyl)acetate (**37**).* Paraformaldehyde (1.90 g, 63.2 mmol, 7 equiv), $MgCl_2$ (1.72 g, 18.1 mmol, 2.0 equiv), and triethylamine (2.74 g, 27.1 mmol, 3.0 equiv) were added to a solution of methyl 2-(4-hydroxyphenyl)acetate (1.50 g, 9.03 mmol, 1.0 equiv) in MeCN (25 mL). The obtained yellow mixture was heated to reflux and stirred at that temperature for 2 h. The reaction mixture was acidified with 6 M HCl and concentrated under reduced pressure. The residue was extracted with diethyl ether (3 × 30 mL), and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (heptanes:EtOAc, 6:1), which yielded the product as a white solid (1.07 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.94 (s, 1H), 9.88 (s, 1H), 7.48 (d, J = 2.2 Hz, 1H), 7.44 (dd, J = 8.5, 2.3 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 3.71 (s, 3H), 3.62 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 196.5, 171.9, 160.9, 138.2, 134.2, 125.6, 120.6, 118.1, 52.3, 40.0.

[^{3}H]-D-Asp Uptake Assay. The screening of a commercial compound library consisting of 3040 compounds (Chembridge Corporation, San Diego, CA) at stable HEK293 cell lines expressing human EAAT1, EAAT2, and EAAT3 in a conventional [^{3}H]-D-Asp uptake assay has been described previously.[16] The pharmacological properties of the identified hit **1a**, and its analogues were subsequently characterized at the three cell lines in the assay essentially as described previously.[16] Briefly, the cell lines were maintained in culture medium [Dulbecco's Modified Eagle Medium (DMEM) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 5% dialyzed fetal bovine serum] supplemented with 1 mg/mL G-418 at 37 °C in a humidified 5% CO_2 incubator. The day before the assay, cells were split into poly-D-lysine-coated white 96-well plates (PerkinElmer, Boston, MA). Then 16–24 h later, the culture medium was aspirated and cells were washed twice with 100 μL of assay buffer (Hank's Buffered Saline Solution supplemented with 20 mM HEPES, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.4). Then 50 μL of assay buffer supplemented with 30 nM [^{3}H]-D-aspartate (PerkinElmer, Boston, MA) and various concentrations of test compounds were added to the wells, and the plate was incubated at 37 °C for 5 min. Nonspecific [^{3}H]-D-Asp uptake in the cells was determined in the presence of 3 mM (S)-Glu. The assay mixtures was quickly removed from the wells, which were then washed with 3 × 75 μL of ice-cold assay buffer, and 150 μL of Microscint20 scintillation fluid (PerkinElmer, Boston, MA) was added to each well. The plate was shaken for at least 1 h and counted in a TopCounter (PerkinElmer, Boston, MA). The experiments were performed in duplicate 3–4 times for each compound.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01058.

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DHK, dihydrokainic acid; CNS, central nercous system; EAAT, excitatory amino acid transporter; SAR, structure–activity-relationship; TBOA, threo-benzyloxy aspartate

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