SUPPORTING INFORMATION

β-Indolyloxy Functionalized Aspartate Analogs as Inhibitors of the Excitatory Amino Acid Transporters (EAATs)

Na Liu, Anders A. Jensen and Lennart Bunch*

Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

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EXPERIMENTAL SECTION

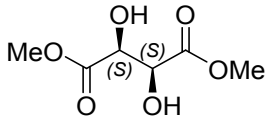
Chemistry: All reactions involving dry solvents or sensitive agents were performed under an argon atmosphere and glassware was dried prior to use. Commercially available chemicals were used without further purification. DCM, THF, and DMF were dried using a SG WATER solvent purification system (commercialized by Pure Process Technology). MeOH and Triethylamine were dried by standing over 3 Å molecular sieves for a minimum of 24 h. Reactions were monitored by analytical thin-layer chromatography (TLC, Merck silica gel 60 F_{254} aluminum sheets) or by UPLC-MS.

Flash chromatography was carried out using Merck silica gel 60A (40 − 63 μm). For dry column vacuum chromatography (DCVC), Merck silica gel 60 (15 − 40 μm) and a standard setup was used.

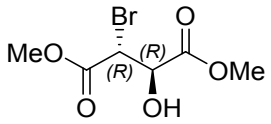
^{1}H NMR spectra were recorded at 400 or 600 MHz and ^{13}C NMR spectra at 101 or 151 MHz on a Bruker Avance III or Bruker Avance III HD, respectively. NMR data were obtained in $CDCl_3$ or DMSO-d_6 (purchased from Cambridge Isotope Laboratories, Inc.). Chemical shifts (δ) are reported in ppm relative to TMS.

Analytical HPLC was performed using an UltiMate HPLC system consisting of an LPG-3400A pump (1 mL/min), a WPS-3000SL autosampler, and a 3000 Diode Array Detector installed with a Gemini-NX C18 (250 × 4.60 mm, 3 μm) column. Solvent A: H_2O + 0.1% TFA; Solvent B: MeCN/H_2O (9:1) + 0.1% TFA. For HPLC control, data collection, and data handling, Chromeleon software v.6.80 was used. Preparative HPLC was carried out on an Ultimate HPLC system with an LPG-3200BX pump, a Rheodyne 9725i injector, a 10 mL loop, an MWD-3000SD detector (200, 210, 225, and 254 nm), and a Gemini-NX C18 (250 × 21.2 mm, 5 μm) column for preparative purifications. Solvent A: H_2O + 0.1% TFA; Solvent B: MeCN/H_2O (9:1) + 0.1% TFA.

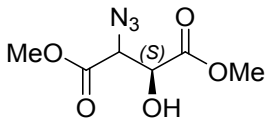
UPLC-MS spectra were recorded using an Acquity UPLC H-Class Waters series solvent delivery system equipped with an autoinjector coupled to an Acquity QDa and TUV detectors installed with an Acquity UPLCBECH C18 (50 × 2.1 mm,1.7 μm) column. Solvent A: H_2O/MeCN (95:5) + 0.1% HCO_2H; Solvent B: MeCN + 0.1% HCO_2H. For data collection and data handling, MassLynx software was used. Compounds were dried under high vacuum or freeze dried using a Holm & Halby, Heto LyoPro 6000 freeze drier. The purity of compounds submitted for pharmacological characterization was determined by HPLC to be > 95% until further notification.



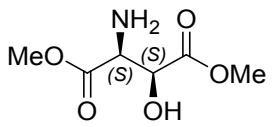
Dimethyl (2*S*,3*S*)-2,3-dihydroxysuccinate (3). To a solution of D-tartaric acid **2** (10.0 g, 66.6 mmol) in anhydrous methanol (30 mL) at 0 °C under an argon atmosphere, was slowly added thionyl chloride (25.0 mL, 346 mmol). After 1 h, the reaction mixture was heated to reflux for 3 h. Gaseous hydrogen chloride and methanol were removed under reduced pressure to give pale yellow oil (11.9 g, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.56 (s, 2H), 3.87 (s, 6H), 3.15 (brs, 2H).



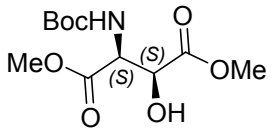
Dimethyl (2*R*,3*R*)-2-bromo-3-hydroxysuccinate (4). A solution of 33% HBr in acetic acid (56.0 mL) was added dropwise to **3** (11.9 g, 66.6 mmol) at 0 °C over 30 min. The reaction mixture was stirred at rt for overnight and then quenched with ice. The mixture was extracted with Et_2O, washed with water and brine, dried with $MgSO_4$. The concentrated crude oil was dissolved in anhydrous methanol (66 mL), and acetyl chloride (1.86 mL) was added cautiously. The mixture was heated under gentle reflux for 4 h. The product **4** (11.9 g, 74%) was obtained after concentration and used for next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 (dd, J = 1.67, 4.27 Hz, 1H), 4.67 (dd, J = 1.67, 4.27 Hz, 1H), 3.82 (dd, J = 1.53, 6.38 Hz, 6H), 2.82 (brs, 1H).



Dimethyl (3*S*)-2-azido-3-hydroxysuccinate (5a/5b). To a solution of **4** (6.47 g, 26.8 mmol) in DMF (25 mL) was added sodium azide (2.27 g, 34.9 mmol) under an argon atmosphere. The mixture was stirred at rt for overnight. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude was purified by flash column chromatography to afford **5** as a 5.6:1 mixture of L-*threo* **5a** and D-*erythro* **5b** diastereomers (3.70 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.77 (dd, J = 2.37, 5.79 Hz, 5.6H), 4.66 (dd, J = 2.83, 5.56 Hz, 1H), 4.34 (d, J = 2.86 Hz, 1H), 4.22 (d, J = 2.33 Hz, 5.6H), 3.88 (d, J = 2.36 Hz, 34H), 3.83 (d, J = 3.98 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 72.1, 72.0, 72.0, 64.5, 63.3, 53.5, 53.3, 53.2, 53.0.

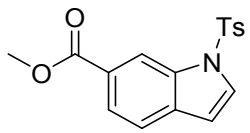


Dimethyl (2*S*,3*S*)-2-amino-3-hydroxysuccinate (6a). Diastereomers **5a/5b** (3.54 g, 17.4 mmol), 10% Pd/C (0.145 g) and methanol (30 mL) were put in a flask. The mixture was stirred under 1 atm hydrogen at 45 ^{o}C for 3 h, and filtered on celite to remove the solids, then evaporated to dryness. The crude was eluted carefully through flash column chromatography on a silica gel with DCM/MeOH to give title compound **6a** (1.12 g, 36%), and mixture of dimethyl (2*R*,3*S*)-2-amino-3-hydroxysuccinate (800 mg, 26%). The absolute configurations of **6a** was confirmed by comparing the ^{1}H NMR spectra of their derivatives of diethyl 2-(*N*-*tert*-butoxycarbonyl)-amino-3-hydroxysuccinate with those described in literature. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (d, *J* = 2.41 Hz, 1H), 3.90 (d, *J* = 2.41 Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 2.20 (s, 2.59H). LC-MS (*m/z*) calcd. for $C_6H_{12}NO_5$ $[M+H]^+$, 178.1; found, 178.1.



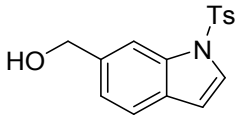
Dimethyl (2*S*,3*S*)-2-((tert-butoxycarbonyl)amino)-3-hydroxysuccinate (7). $(Boc)_2O$ (1.79 g, 8.22 mmol) was added to a solution of **6** (1.12 g, 6.32 mmol) and Et_3N (1.76 mL, 1.26 mmol) in DCM (40 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 10 h. Then the mixture was diluted with DCM, washed with 0.5 M HCl, sat. $NaHCO_3$ and brine, dried with $MgSO_4$. The concentrated crude was purified by flash column chromatography, to afford the title compound **7** as a clear oil (1.09 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 – 5.21 (m, 1H), 4.79 (dd, *J* = 6.09, 8.78 Hz, 1H), 4.68 (d, *J* = 5.49 Hz, 1H), 3.81 (dd, *J* = 1.57, 6.02 Hz, 6H), 3.17 (dd, *J* = 1.55, 5.88 Hz, 1H), 1.42 (d, *J* = 1.61 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 169.8, 155.2, 80.4, 71.1, 56.1, 53.2, 52.9, 28.2. LC-MS (*m/z*) calcd. for $C_{11}H_{19}NO_7$ $[M+H]^+$, 278.1; found, 178.1 (-Boc).

General procedure for synthesis and purification of methyl 1-tosyl-1*H*-indole-6-carboxylate analogs (General procedure A for 11a-d)



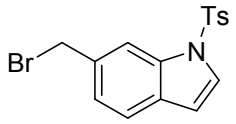
Methyl 1-tosyl-1*H*-indole-6-carboxylate (11a). To **8** (300 mg, 1.71 mmol) in THF (5 mL) at 0 °C was added NaH (53 mg, 2.22 mmol, 60% dispersion in oil), and the reaction mixture was allowed to stir for 10 min under an argon atmosphere. After TsCl (423 mg, 2.22 mmol) was added, the solution was stirred for 30 min. The reaction was quenched by water, extracted with EtOAc, washed with brine and dried over $MgSO_4$. The title product **11a** (552 mg, 98%) was obtained after concentration, which was used for next step directly. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.95 – 7.89 (m, 1H), 7.84 – 7.77 (m, 2H), 7.74 – 7.69 (m, 1H), 7.56 (d, *J* = 8.31 Hz, 1H), 7.24 (d, *J* = 8.25 Hz, 2H), 6.69 (d, *J* = 3.59 Hz, 1H), 3.96 (d, *J* = 1.57 Hz, 3H), 2.35 (s, 3H).

General procedure for synthesis and purification of (1-tosyl-1*H*-indol-6-yl)methanol analogs (General procedure B for 12a-d)



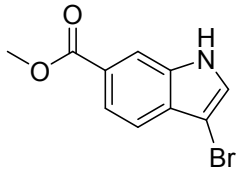
(1-Tosyl-1*H*-indol-6-yl)methanol (12a). $LiAlH_4$ (2.01 mL, 2.01 mmol, 1 M in THF) was added dropwise to a stirring solution of **11a** in THF (10 mL) at 0 °C under an argon atmosphere. The resulting solution was stirred at rt for 0.5 h. The reaction was then quenched by addition of H_2O, and the mixture was acidified with 0.5 M HCl and extracted with EtOAc. The combined organic extracts were washed with sat. Na_2CO_3 solution and brine, and dried over $MgSO_4$. The title product **12a** (435 mg, 86%) was obtained after concentration and used for next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.79 – 7.74 (m, 2H), 7.56 (d, *J* = 3.67 Hz, 1H), 7.51 (d, *J* = 8.07 Hz, 1H), 7.24 – 7.19 (m, 2H), 6.64 (d, *J* = 3.69 Hz, 1H), 4.80 (s, 2H), 2.34 (s, 3H).

General procedure for synthesis and purification of 6-(bromomethyl)-1-tosyl-1*H*-indole analogs (General procedure C for 13a-d)

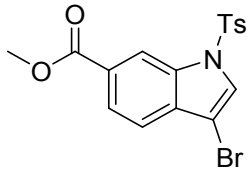


6-(Bromomethyl)-1-tosyl-1*H*-indole (13a). To **12a** (150 mg, 0.498 mmol) in THF (4.0 mL) at 0 °C were added LiBr (346 mg, 3.98 mmol) and Et_3N (0.278 mL, 1.99 mmol) under an argon atmosphere. The reaction was allowed to stir for 5 min, and then methanesulfonyl chloride (0.116 mL, 1.49 mmol) was added dropwise. The reaction mixture was allowed to warm to rt. After 4 h, the reaction was quenched by addition of sat. $NaHCO_3$, and extracted with EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. The title compound **13a** was

obtained by DCVC as a white solid (123 mg, 68%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.01 (m, 1H), 7.77 (d, *J* = 8.17 Hz, 2H), 7.57 (dd, *J* = 2.06, 3.66 Hz, 1H), 7.49 (t, *J* = 8.37 Hz, 1H), 7.27-7.25 (m, 1H), 7.24 – 7.21 (m, 2H), 6.63 (td, *J* = 0.79, 3.56 Hz, 1H), 4.69 (d, *J* = 28.75 Hz, 2H), 2.34 (s, 3H).

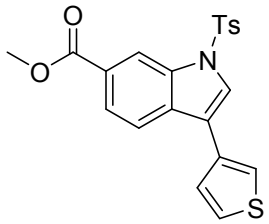


Methyl 3-bromo-1*H*-indole-6-carboxylate (9). *N*-Bromosuccinimide (NBS, 1.12 g, 6.28 mmol) in THF (10 mL) was added dropwise to a stirred solution of **8** (1.00 g, 5.71 mmol) in THF (30 mL) at -78 °C under an argon atmosphere. The reaction mixture was stirred for 2 h and allowed to warm to rt. Pyridine (0.57 mL) was added to the mixture and hexanes (10 mL), then filtered on celite. The combined filtrates were concentrated and purified by DCVC. The title compound **9** was obtained as a white solid (1.16 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.15 (dd, *J* = 0.69, 1.34 Hz, 1H), 7.89 (dd, *J* = 1.37, 8.43 Hz, 1H), 7.61 (dt, *J* = 0.76, 8.43 Hz, 1H), 7.39 (d, *J* = 2.62 Hz, 1H), 3.95 (s, 3H).

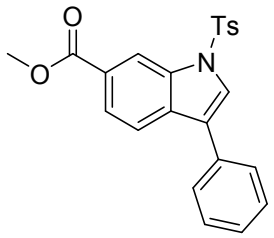


Methyl 3-bromo-1-tosyl-1*H*-indole-6-carboxylate (10). General procedure A. Yield from **9**: 3.67 g, 90%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.70 – 8.68 (m, 1H), 8.00 (dd, *J* = 1.38, 8.33 Hz, 1H), 7.82 – 7.79 (m, 2H), 7.75 (s, 1H), 7.53 (d, *J* = 8.24 Hz, 1H), 7.27 (d, *J* = 2.05 Hz, 2H), 3.98 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.8, 145.7, 134.6, 133.7, 133.2, 130.2, 127.7, 127.6, 127.0, 124.9, 119.9, 115.4, 99.1, 52.4, 21.6.

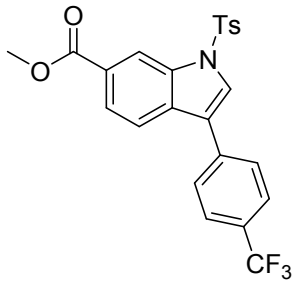
General procedure for synthesis and purification of methyl 3-substitution -1-tosyl-1*H*-indole-6-carboxylate analogs (General Procedure D for 11b-d)



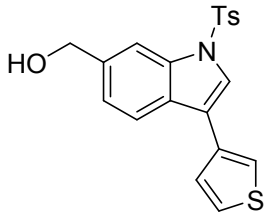
Methyl 3-(thiophen-3-yl)-1-tosyl-1*H*-indole-6-carboxylate (11b). A mixture of **10** (400 mg, 0.98 mmol), thiophen-3-ylboronic acid (251 mg, 1.96 mmol), Pd(dppf)$Cl_2 \cdot CH_2Cl_2$ (40 mg, 0.049 mmol), and Cs_2CO_3 (640 mg, 1.96 mmol) in dioxane/H_2O (4:1, 6.0 mL) were reacted at 110 °C for 15 min in microwave. The mixture was diluted with EtOAc, filtered on celite and evaporated to dryness. The crude was purified by DCVC to afford the product **11b** as a white solid (295 mg, 73%).^{1}H NMR (600 MHz, $CDCl_3$) δ 8.76 (d, *J* = 1.52 Hz, 1H), 8.02 (dd, *J* = 1.45, 8.34 Hz, 1H), 7.88 – 7.82 (m, 4H), 7.54 (dd, *J* = 1.28, 2.96 Hz, 1H), 7.48 (dd, *J* = 2.91, 4.97 Hz, 1H), 7.38 (dd, *J* = 1.28, 5.04 Hz, 1H), 7.27 – 7.26 (m, 2H), 4.01 (s, 3H), 2.37 (s, 3H).



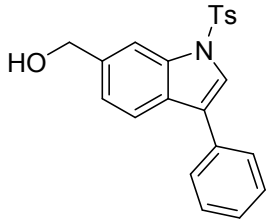
Methyl 3-phenyl-1-tosyl-1*H*-indole-6-carboxylate (11c). General procedure D. Yield from **10**: 162 mg, 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 1.45 Hz, 1H), 7.98 (dd, *J* = 1.51, 8.38 Hz, 1H), 7.89 – 7.78 (m, 4H), 7.62 – 7.56 (m, 2H), 7.51 – 7.44 (m, 2H), 7.42 – 7.36 (m, 1H), 7.27 – 7.26 (m, 2H), 3.98 (s, 3H), 2.35 (s, 3H).



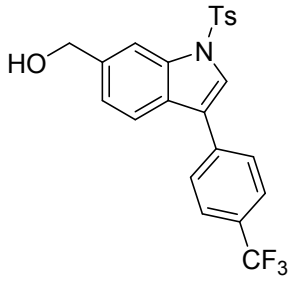
Methyl 1-tosyl-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-6-carboxylate (11d). General procedure D. Yield from **10**: 551 mg, 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 – 8.74 (m, 1H), 8.00 (dd, *J* = 1.44, 8.43 Hz, 1H), 7.89 (s, 1H), 7.88 – 7.84 (m, 2H), 7.78 (dd, *J* = 0.76, 8.29 Hz, 1H), 7.76 – 7.67 (m, 4H), 7.28 (d, *J* = 8.53 Hz, 2H), 3.99 (s, 3H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 145.6, 136.2, 134.9, 134.8, 132.2, 130.2, 128.1, 127.1, 127.0, 126.4, 126.0, 126.0, 124.9, 122.3, 119.9, 115.6, 52.4, 21.6.



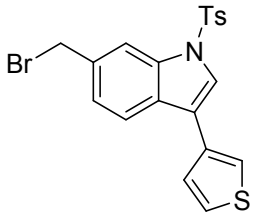
(3-(Thiophen-3-yl)-1-tosyl-1*H*-indol-6-yl)methanol (12b). General procedure B. Yield from **11b**: 230 mg, 84%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 – 8.03 (m, 1H), 7.82 – 7.79 (m, 2H), 7.78 (d, *J* = 8.29 Hz, 1H), 7.72 (s, 1H), 7.51 (dd, *J* = 1.29, 2.93 Hz, 1H), 7.44 (dd, *J* = 2.94, 4.99 Hz, 1H), 7.36 (dd, *J* = 1.30, 4.99 Hz, 1H), 7.33 (dd, *J* = 1.44, 8.16 Hz, 1H), 7.24 – 7.21 (m, 2H), 4.83 (s, 2H), 2.34 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.1, 138.2, 135.6, 135.2, 133.3, 130.0, 128.8, 127.0, 126.9, 126.2, 123.1, 122.9, 121.2, 120.6, 118.8, 112.3, 65.6, 21.6.



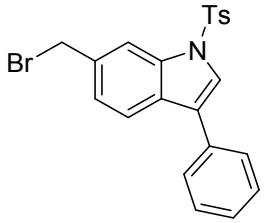
(3-Phenyl-1-tosyl-1*H*-indol-6-yl)methanol (12c). General procedure B. Yield from **11c**: 121 mg, 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.81 (d, *J* = 8.12 Hz, 2H), 7.76 (d, *J* = 8.19 Hz, 1H), 7.69 (s, 1H), 7.60 (d, *J* = 7.25 Hz, 2H), 7.46 (t, *J* = 7.51 Hz, 2H), 7.40 – 7.36 (m, 1H), 7.34 – 7.29 (m, 1H), 7.24 (d, *J* = 8.13 Hz, 2H), 4.83 (d, *J* = 5.30 Hz, 2H), 2.35 (s, 3H).



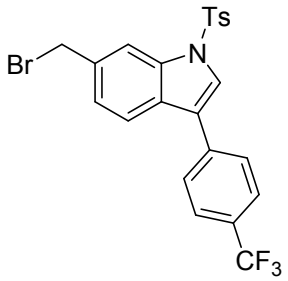
(1-Tosyl-3-(4-(trifluoromethyl)phenyl)-1*H*-indol-6-yl)methanol (12d). General procedure B. Yield from **11d**: 204 mg, 85%. ^{1}H NMR (600 MHz, DMSO-d_6) 8.24 (s, 1H), 8.03 (s, 1H), 7.98 (dd, *J* = 7.11, 8.81 Hz, 4H), 7.83 (dd, *J* = 6.07, 8.28 Hz, 3H), 7.41 (d, *J* = 8.19 Hz, 2H), 7.32 (dd, *J* = 1.46, 8.26 Hz, 1H), 5.38 (t, *J* = 5.84 Hz, 1H), 4.66 (d, *J* = 5.83 Hz, 2H), 2.33 (s, 3H). LC-MS (*m/z*) calcd. for $C_{23}H_{19}F_3NO_3S$ $[M+H]^+$, 446.1; found, 428.2 (-H_2O).



6-(Bromomethyl)-3-(thiophen-3-yl)-1-tosyl-1*H*-indole (13b). General procedure C. Yield from **12b**: 237 mg, 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 4.66 Hz, 1H), 7.80 (d, *J* = 8.07 Hz, 2H), 7.77 – 7.69 (m, 2H), 7.51 – 7.42 (m, 2H), 7.34 (dh, *J* = 1.23, 5.41 Hz, 2H), 7.26 – 7.23 (m, 2H), 4.71 (d, *J* = 29.89 Hz, 2H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.2, 135.1, 130.7, 128.7, 127.7, 127.3, 127.2, 125.5, 124.9, 122.3, 121.5, 114.4, 82.7, 47.0, 21.5. LC-MS (*m/z*) calcd. for $C_{20}H_{17}BrNO_2S_2$ $[M+H]^+$, 446.0; found, 366.1 (-HBr).



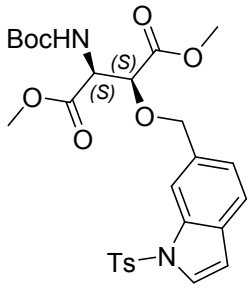
6-(Bromomethyl)-3-phenyl-1-tosyl-1*H*-indole (13c). General procedure C. Yield from **12c**: 150 mg, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.03 Hz, 1H), 8.11 (d, *J* = 1.65 Hz, 1H), 8.00 (d, *J* = 8.01 Hz, 2H), 7.82 (t, *J* = 8.76 Hz, 1H), 7.71 (t, *J* = 4.91 Hz, 2H), 7.50 (t, *J* = 7.54 Hz, 2H), 7.45 – 7.36 (m, 4H), 4.96 (d, *J* = 13.01 Hz, 2H), 2.32 (4, 3H). LC-MS (*m/z*) calcd. for $C_{22}H_{19}BrNO_2S$ $[M+H]^+$, 440.0; found, 360.1 (-HBr).



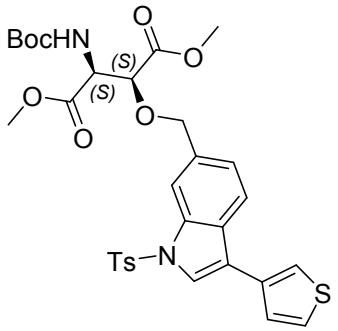
6-(Bromomethyl)-1-tosyl-3-(4-(trifluoromethyl)phenyl)-1*H*-indole (13d). General procedure C. Yield from **12d**: 314 mg, 61 %. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, *J* = 3.02 Hz, 1H), 8.17 (dd, *J* = 1.48, 11.86 Hz, 1H), 8.04 – 8.01 (m, 2H), 7.98 (dd, *J* = 2.59, 8.45 Hz, 2H), 7.89 (d, *J* = 8.26 Hz, 1H), 7.85 (dd, *J* = 5.50, 7.34 Hz, 2H), 7.45 (ddd, *J* = 1.53, 4.10, 8.24 Hz, 1H), 7.44 – 7.40 (m, 2H), 4.97 (d, *J* = 20.45 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 146.4, 136.9, 135.6, 135.1, 134.3, 130.9, 130.8, 128.8, 128.7, 128.5, 128.2, 127.5, 127.5, 126.4, 126.4, 126.3, 126.3,

126.3, 126.3, 126.2, 125.7, 121.7, 121.0, 114.5, 46.9, 21.5. LC-MS (*m/z*) calcd. for $C_{23}H_{18}BrF_3NO_2S$ $[M+H]^+$, 508.0; found, 428.1 (-HBr).

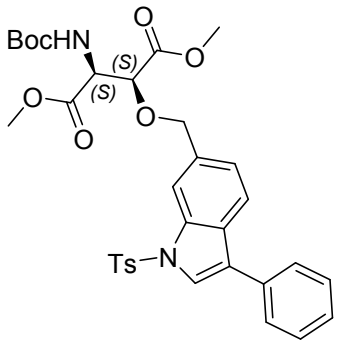
General procedure for synthesis and purification of dimethyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((1-tosyl-1*H*-indol-6-yl)methoxy)succinate analogs (General Procedure E for 14a-d)



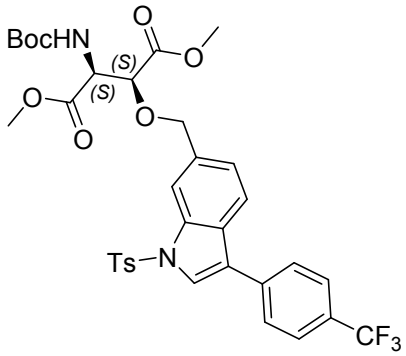
Dimethyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((1-tosyl-1*H*-indol-6-yl)methoxy)succinate (14a). NaH (60% in mineral oil, 32.5 mg, 0.813 mmol) was added to a cooled solution of **7** (225 mg, 0.813 mmol) in dry DMF (2.0 mL) at -15 °C under an argon atmosphere. Indole bromide (445 mg, 1.22 mmol) was added immediately afterwards and reacted for 4 h at -15 °C. The reaction mixture was then quenched with water and extracted with EtOAc. The collected organic phases were washed with water and brine, and dried over $MgSO_4$. The crude product was obtained after concentration and used for next step directly. LC-MS (m/z) calcd. for $C_{27}H_{33}N_2O_9S$ $[M+H]^+$, 561.2; found, 461.1 (-Boc), 284.1.



Dimethyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((3-(thiophen-3-yl)-1-tosyl-1*H*-indol-6-yl)methoxy)succinate (14b). General procedure E. LC-MS (m/z) calcd. for $C_{31}H_{35}N_2O_9S_2$ $[M+H]^+$, $[M+Na]^+$, 643.2, 665.3; found, 366.1, 543.1 (-Boc), 665.3.

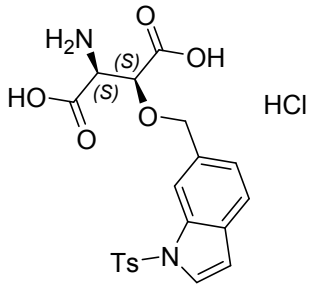


Dimethyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((3-phenyl-1-tosyl-1*H*-indol-6-yl)methoxy)succinate (14c). General procedure E. LC-MS (m/z) calcd. for $C_{33}H_{37}N_2O_9S$ $[M+H]^+$, $[M+Na]^+$, 637.2, 659.3; found, 360.1, 537.2 (-Boc), 659.3.



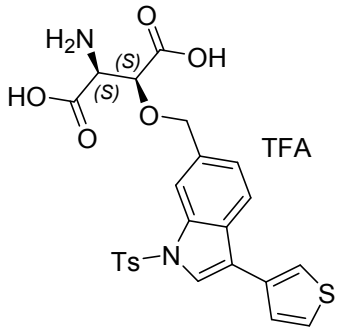
Dimethyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((1-tosyl-3-(4-(trifluoromethyl)phenyl)-1*H*-indol-6-yl)methoxy)succinate (14d). General procedure E. LC-MS (m/z) calcd. for $C_{34}H_{36}F_3N_2O_9S$ $[M+H]^+$, $[M+Na]^+$, 705.2, 727.2; found, 428.1, 605.2 (-Boc), 727.2.

General procedure for synthesis and purification of dimethyl (2*S*,3*S*)-2-amino-3-((1-tosyl-1*H*-indol-6-yl)methoxy)succinic acid analogs (General Procedure F for 15a-d)

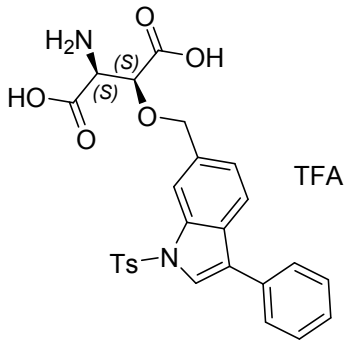


(2*S*,3*S*)-2-Amino-3-((1-tosyl-1*H*-indol-6-yl)methoxy)succinic acid (15a). To a solution of **14a** (0.589 mmol, based on Asp part) in dry DCM (6 mL), TFA (6 mL) was added slowly at 0 °C. The reaction mixture was stirred at rt for 2 h. The solvent was removed to provide deBoc product, which was directly used for the next step without purification.

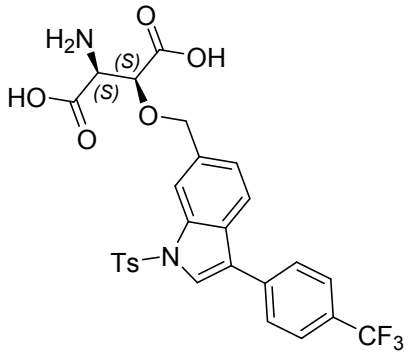
To a stirred solution of deBoc product in THF/H_2O (1:1, each 5 mL) was added LiOH·H_2O (198 mg, 47.1 mmol), and the reaction mixture was stirred at rt for 5 h. The reaction was washed with EtOAc (5 mL). The aqueous layer was purified by prep. HPLC (combined fractions were evaporated, 1 M HCl added and concentrated), and the title product **15a** was obtained as a HCl salt (30.2 mg, 8 % over 3 steps). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.41 (s, 2H), 7.95 (s, 1H), 7.91 – 7.87 (m, 2H), 7.79 (d, J = 3.67 Hz, 1H), 7.58 (d, J = 8.02 Hz, 1H), 7.39 (d, J = 8.15 Hz, 2H), 7.28 (dd, J = 1.37, 8.16 Hz, 1H), 6.83 (d, J = 3.66 Hz, 1H), 4.91 (d, J = 11.24 Hz, 1H), 4.67 (d, J = 11.22 Hz, 1H), 4.53 (d, J = 3.85 Hz, 1H), 4.32 (d, J = 3.86 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 170.2, 168.7, 145.9, 134.6, 134.5, 133.9, 130.7, 127.9, 127.2, 124.6, 121.8, 113.7, 109.8, 76.0, 73.7, 54.4, 21.5. LC-MS (m/z) calcd. for $C_{20}H_{21}N_2O_7S$ $[M+H]^+$, 433.1; found, 284.0, 433.1; calcd. for $C_{20}H_{19}N_2O_7S$ $[M-H]^-$, 431.1; found, 431.1.



(2*S*,3*S*)-2-Amino-3-((3-(thiophen-3-yl)-1-tosyl-1*H*-indol-6-yl)methoxy)succinic acid (15b). General procedure F. The aqueous layer was purified by prep. HPLC and combined fractions were evaporated. The product **15b** was obtained as a TFA salt. Yield from **7**: 12.3 mg, 6 % over 3 steps. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (s, 1H), 8.08 (s, 1H), 7.98 – 7.95 (m, 3H), 7.93 (d, J = 8.19 Hz, 1H), 7.69 (dd, J = 2.79, 4.99 Hz, 1H), 7.65 (dd, J = 1.37, 5.00 Hz, 1H), 7.42 (dd, J = 1.45, 8.09 Hz, 1H), 7.40 (d, J = 8.08 Hz, 2H), 4.95 (d, J = 10.75 Hz, 1H), 4.67 (d, J = 10.80 Hz, 1H), 4.36 (d, J = 6.79 Hz, 1H), 4.08 (d, J = 6.96 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 170.6, 168.9, 158.3, 158.1, 146.0, 135.1, 134.9, 134.4, 133.1, 130.8, 128.5, 127.7, 127.4, 127.2, 125.4, 124.5, 122.1, 120.9, 118.7, 114.3, 75.5, 73.6, 53.9, 21.5. LC-MS (m/z) calcd. for $C_{24}H_{23}N_2O_7S_2$ $[M+H]^+$, 515.1; found, 366.2, 515.2; calcd. for $C_{24}H_{21}N_2O_7S_2$ $[M-H]^-$, 513.1; found, 513.0.



(2*S*,3*S*)-2-Amino-3-((3-phenyl-1-tosyl-1*H*-indol-6-yl)methoxy)succinic acid (15c). General procedure F. The aqueous layer was purified by prep. HPLC and combined fractions were evaporated. The product **15c** was obtained as a TFA salt. Yield from **7**: 7.23 mg, 5% over 3 steps. Purity: 80%. Proton NMR showed product and impurities ratio is 1:0.25. The impurities didn't contain Asp fragment according to analyze ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR (600 MHz, DMSO-d_6) 8.09 (s, 1H), 8.08 (s, 1H), 8.01 – 7.98 (m, 2H), 7.80 (d, J = 8.16 Hz, 1H), 7.71 (dd, J = 1.83, 7.28 Hz, 2H), 7.50 (td, J = 1.86, 7.68 Hz, 2H), 7.40 (dd, J = 4.06, 8.36 Hz, 4H), 4.95 (d, J = 10.85 Hz, 1H), 4.68 (d, J = 10.85 Hz, 1H), 4.40 (s, 1H), 4.13 (s, 1H), 2.32 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 170.6, 168.8, 158.3, 158.1, 146.0, 135.2, 134.4, 132.8, 130.8, 129.5, 128.6, 128.2, 128.1, 127.4, 125.3, 124.5, 123.3, 120.5, 114.3, 75.6, 73.6, 54.0, 21.5. Impurities. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.24 (s, *0.52H*), 8.22 (d, J = 1.50 Hz, *0.41H*), 8.13 (s, *0.25H*), 8.05 – 8.03 (m, *0.53H*), 7.89 (d, J = 8.19 Hz, *0.24H*), 7.71 (dd, J = 1.83, 7.28 Hz, *0.74H*), 7.50 (td, J = 1.86, 7.68 Hz, *0.83H*), 7.44 (dd, J = 1.51, 8.27 Hz, *0.33H*), 7.40 (dd, J = 4.06, 8.36 Hz, *1.37H*), 4.24 (d, J = 4.98 Hz, *0.6H*) 2.33 (s, *0.75H*). ^{13}C NMR (151 MHz, DMSO) δ *146.2*, *135.3*, *134.8*, *132.5*, *131.4*, *130.7*, *129.5*, *128.9*, *128.2*, *127.5*, *125.5*, *125.0*, *123.3*, *121.0*, *114.8*, 114.3, *43.0*, *21.5*. UPLC-MS (m/z) calcd. for $C_{26}H_{25}N_2O_7S$ $[M+H]^+$, 509.1; found, 360.1, 509.1; calcd. for $C_{26}H_{23}N_2O_7S$ $[M-H]^-$, 507.1; found, 507.0.



(2*S*,3*S*)-2-Amino-3-((1-tosyl-3-(4-(trifluoromethyl)phenyl)-1*H*-indol-6-yl)methoxy)succinic acid (15d). General procedure F. The aqueous layer was purified by prep. HPLC and combined

fractions were evaporated. The product **15d** was obtained as a TFA salt. Yield from **7**: 8.95 mg, 3% over 3 steps. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.29 (s, 1H), 8.07 (s, 1H), 8.03 – 8.00 (m, 2H), 7.97 (d, J = 8.02 Hz, 2H), 7.85 (t, J = 8.14 Hz, 3H), 7.43 – 7.38 (m, 3H), 4.95 (d, J = 11.30 Hz, 1H), 4.71 (d, J = 11.26 Hz, 1H), 4.51 (d, J = 4.43 Hz, 1H), 4.27 (d, J = 4.44 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 170.3, 168.7, 158.4, 158.2, 158.0, 146.2, 137.1, 135.1, 134.8, 134.3, 130.9, 128.7, 128.3, 128.1, 128.1, 127.9, 127.5, 126.3, 126.2, 125.9, 125.7, 125.3, 123.9, 121.7, 120.5, 114.2, 75.9, 73.4, 54.3, 21.5. LC-MS (m/z) calcd. for $C_{27}H_{24}F_3N_2O_7S$ $[M+H]^+$, 577.1; found, 428.2, 577.3; calcd. for $C_{27}H_{22}F_3N_2O_7S$ $[M-H]^-$, 575.1; found, 575.0.

Pharmacology

Materials. Culture media, serum, antibiotics and buffers for cell culture and assays were obtained from Invitrogen (Paisley, UK) and Sigma (St. Louis, MO). [^{3}H]-$_D$-Asp was obtained from PerkinElmer (Boston, MA), Glu was purchased from Sigma, and $_{DL}$-TBOA was purchased from Tocris Cookson (Bristol, UK). The stable hEAAT1-, hEAAT2- and hEAAT3-HEK293 cell lines have been described previously,[1] and the stable rEAAT4-tsA201 cell line was a generous gift from Drs. Peter Kovermann and Christoph Fahlke and has been characterized pharmacologically in a previous study.[2]

Cell culture. All cell lines were cultured at 37 ºC in a humidified 5% CO_2 atmosphere. The hEAAT1-, hEAAT2- and hEAAT3-HEK293 cell lines were maintained in *Culture Medium I* (Dulbecco's Modified Eagle Medium Glutamax™-I (DMEM) supplemented with 5% dialyzed fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin and 1 mg/mL G-418), and the stable rEAAT4-tsA201 cell line was maintained in *Culture Medium II* (DMEM supplemented with 5% tetracycline-free fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.2 mg/mL hygromycin B and 10 µg/mL blasticidin).

[^{3}H]-$_D$-Asp uptake assay. The pharmacological characterization of various reference EAAT ligands and test compounds in the [^{3}H]-$_D$-Asp uptake assay was performed essentially as described previously.[1] The day before the assay, cells (5×10^4 cells/well) were split into poly-$_D$-lysine-coated white 96-well plates (PerkinElmer, Boston, MA) in *Culture Medium I* (hEAAT1-, hEAAT2- and hEAAT3-HEK293) or in *Culture Medium II* supplemented with 1 µg/mL tetracycline (rEAAT4-tsA201). After 16-24 h, 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 100 nM [^{3}H]-$_D$-Asp (PerkinElmer, Boston, MA) and various concentrations of test compounds were added to the wells, and the plate was incubated at 37 °C for 4 min. Nonspecific [^{3}H]-$_D$-Asp uptake/binding in the cells was determined in the presence of 3 mM Glu. The assay mixtures were quickly removed from the wells, and the cells were washed with 3 x 100 µL ice-cold assay buffer, after which 150 µL of Microscint^{20} scintillation fluid (PerkinElmer, Boston, MA) was added to each well. Then the plate was shaken for at least 1 h and counted in a TopCounter (PerkinElmer, Boston, MA). The experiments were performed in duplicate 3 times for each compound.

Modeling

All modeling was carried out in the Schrodinger Maestro program, Maestro Version 11.1.012, Release 2017-1, OPLS3 force field. Except for Figure 2b, images depicting overlay and proposed binding modes were generated using PyMOL, version 1.8.2.1.

The human EAAT1 amino acid sequence (homo sapiens, SLC1A3) was aligned with $EAAT1_{cryst}$ (pdb: 5mju), and the homology model was constructed using the SWISS-MODEL homology modeling function with a standard setup (ProMod3, PROMOD-II).[3] The hmEAAT1 protein was aligned with $EAAT1_{cryst}$ (pdb: 5mju) and merged with the ligand TFB-TBOA in Maestro. Then, it was prepared by Protein Preparation Wizard with a default setup. Compounds **15a**−**d**, $_{L}$-TBOA and TFB-TBOA were prepared by LigPrep and carried out by induced-fit docking under the default setup. For each ligand, up to 20 docking poses were generated and scored. The binding model of each compound was selected based on the overlay with TFB-TBOA and the IFDScore.[4]

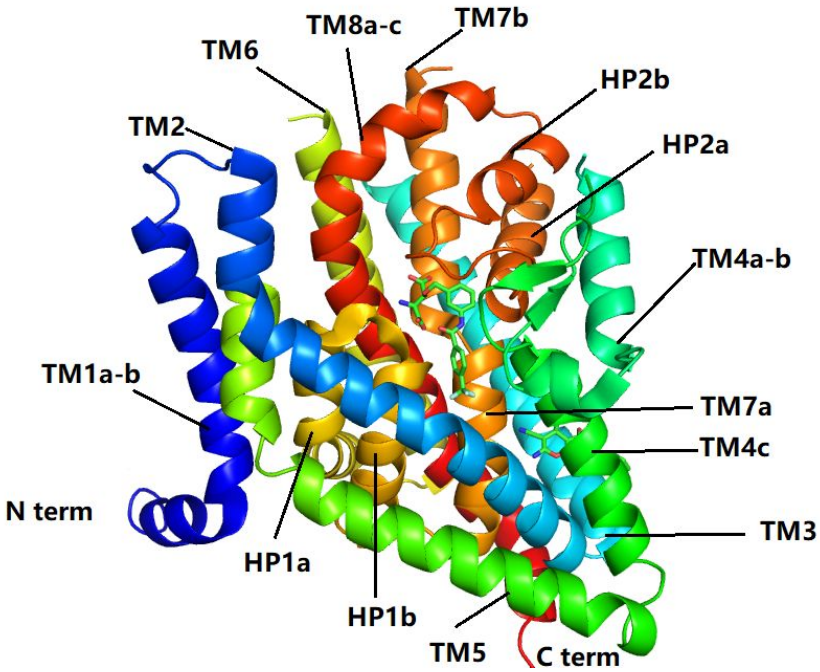


Figure S1. Overview of crystal structure of $EAAT1_{cryst}$ (pdb:5mju, depicted as rainbow cartoon) in complex with the competitive inhibitor TFB-TBOA and the allosteric inhibitor UCPH-101(depicted as green stick). Domain organization: a scaffold domain (ScaD), including transmembrane helices TM1–TM2 and TM4–TM5; and a transport domain (TranD), including TM3, TM6–TM8 and re-entrant helical loops 1–2 (HP1–HP2).[5]

Table S1. Sequence alignment of hEAAT1[6] with $EAAT1_{cryst}$ (pdb: 5mju)[5]. The engineered $EAAT1_{cryst}$ variant shares an overall ~75% sequence identity with the wild type EAAT1, and up to ~90% identity at the C-terminal core of the protein, where the transported substrate and coupled ions are known to bind.[5]

```
EAAT1   MTKSNGEEPKMGGRMERFQQGVRKRTLLAKKKVQNITKEDVKSYLFRNAFVL
5mju    MTKSNGEEPKMGGRMERFQQGVSKRTLLAKKKVQNITKEDVKSFLRRNALLL

EAAT1   LTVTAVIVGTILGFTLRPYRMSYREVKYFSFPGELLMRMLQMLVLPLIISSLVTG
5mju    LTVLAVILGVVLGFLLRPYPLSPREVKYFAFPGELLMRMLKMLILPLIVSSLITG

EAAT1   MAALDSKASGKMGMRAVVYYMTTTIIAVVIGIIIVIIIHPGKGTKENMHREGKIV
5mju    LASLDAKASGRLGMRAVVYYMSTTIIAVVLGIILVLIIHPGAASAAITASVGAAG

EAAT1   RVTAA-----------DAFLDLIRNMFPPNLVEACFKQFKTNYEKRSFKVPIQANETLVG
5mju    SAENAPSKEVLDCFLDLARNIFPSNLVSAAFRSYSTTYEERTI-------------------------

EAAT1   AVINNVSEAMETLTRITEELVPVPGSVNGVNALGLVVFSMCFGFVIGNMKEQGQ
5mju    ----------------------TGTRVK---VPVGQEVEGMNILGLVVFSMVFGFALGKMGEQGQ

EAAT1   ALREFFDSLNEAIMRLVAVIMWYAPVGILFLIAGKIVEMEDMGVIGGQLAMYTV
5mju    LLVDFFNSLNEATMKLVAIIMWYAPLGILFLIAGKIVEMEDLEVLGGQLGMYMV

EAAT1   TVIVGLLIHAVIVLPLLYFLVTRKNPWVFIGGLLQALITALGTSSSSATLPITFKCL
5mju    TVIVGLVIHGLIVLPLIYFLITRKNPFVFIAGILQALITALGTSSSSATLPITFKCL

EAAT1   EENNGVDKRVTRFVLPVGATINMDGTALYEALAAIFIAQVNNFELNFGQIITISIT
5mju    EENNGVDKRITRFVLPVGATINMDGTALYEAVAAIFIAQVNNYELDFGQIITISIT

EAAT1   ATAASIGAAGIPQGALVTMVIVLTSVGLPTDDITLIIAVDWFLDRLRTTTNVLGD
5mju    TAASIGAAGIPQAGLVTMVIVLTAVGLPTDDITLIIAVDWLLDRFRTMVNVLGDA

EAAT1   SLGAGIVEHLSRHELKNRDVEMGNSVIEENEMKKPYQLI AQDNETEKPIDSETKM
5mju    ALGAGIVEHLSRKELEKQDAELGNSVIEENEMKKPYQLI AQDNETEKPIDSETKM
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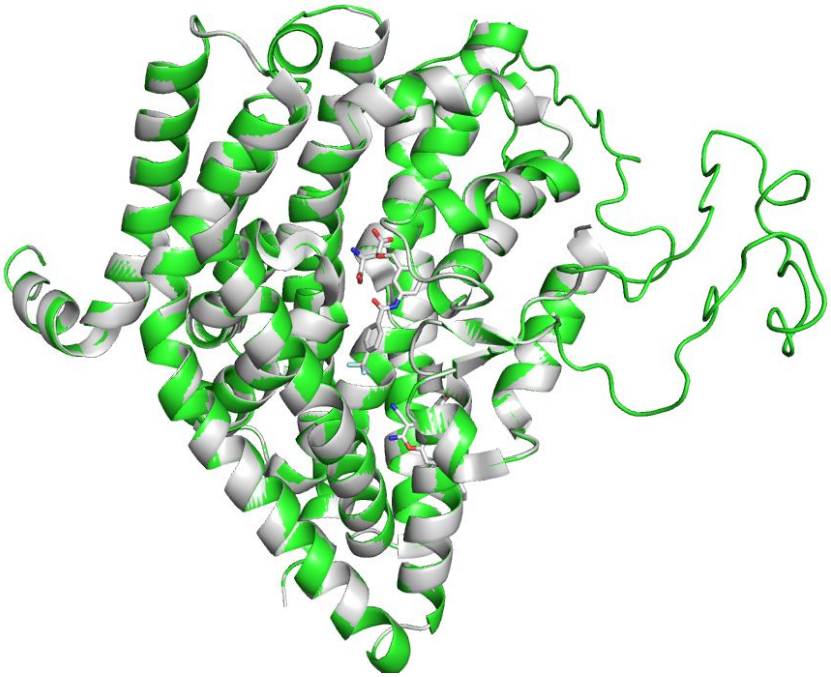


Figure S2. Structure overlay of EAAT1 homology model (hmEAAT1) structure (depicted as green) and thermostabilized $EAAT1_{cryst}$ mutant complex (pdb: 5mju, depicted as white), the competitive inhibitor TFB-TBOA and the allosteric inhibitor UCPH101 (depicted as white stick).

Table S2. Residues different from EAAT2,3 for the binding model of TFB-TBOA and **15a-d** into hmEAAT1.[a]

Compound	Transporter Isomers	Residues differences within 4Å							
TFB-TBOA	hmEAAT1	V96	A243	S363	S366	Q445	--	--	--
	hmEAAT2	I93	V242	A362	A365	S444	--	--	--
	hmEAAT3	I67	V212	S331	S334	Q413	--	--	--
15a	hmEAAT1	V96	A243	S363	S366	Q445	--	--	--
	hmEAAT2	I93	V242	A362	A365	S444	--	--	--
	hmEAAT3	I67	V212	S331	S334	Q413	--	--	--
15b-1	hmEAAT1	V96	A243	S363	S366	Q445	V241	I438	--
	hmEAAT2	I93	V242	A362	A365	S444	M240	V437	--
	hmEAAT3	I67	V212	S331	S334	Q413	I210	I406	--
15b-2	hmEAAT1	V96	A243	S363	S366	Q445	Q93	--	--
	hmEAAT2	I93	V242	A362	A365	S444	K90	--	--
	hmEAAT3	I67	V212	S331	S334	Q413	K64	--	--
15c	hmEAAT1	V96	A243	S363	S366	Q445	I438	--	--
	hmEAAT2	I93	V242	A362	A365	S444	V437	--	--
	hmEAAT3	I67	V212	S331	S334	Q413	I406	--	--
15d	hmEAAT1	V96	A243	S363	S366	Q445	Q93	V241	V247
	hmEAAT2	I93	V242	A362	A365	S444	K90	M240	I246
	hmEAAT3	I67	V212	S331	S334	Q413	K64	I210	I216

[a]The residues are found within 4Å around the ligand. The newly obtaining different residues are shaded for the binding model of **15a-d** in comparison with the binding model of TFB-TBOA.

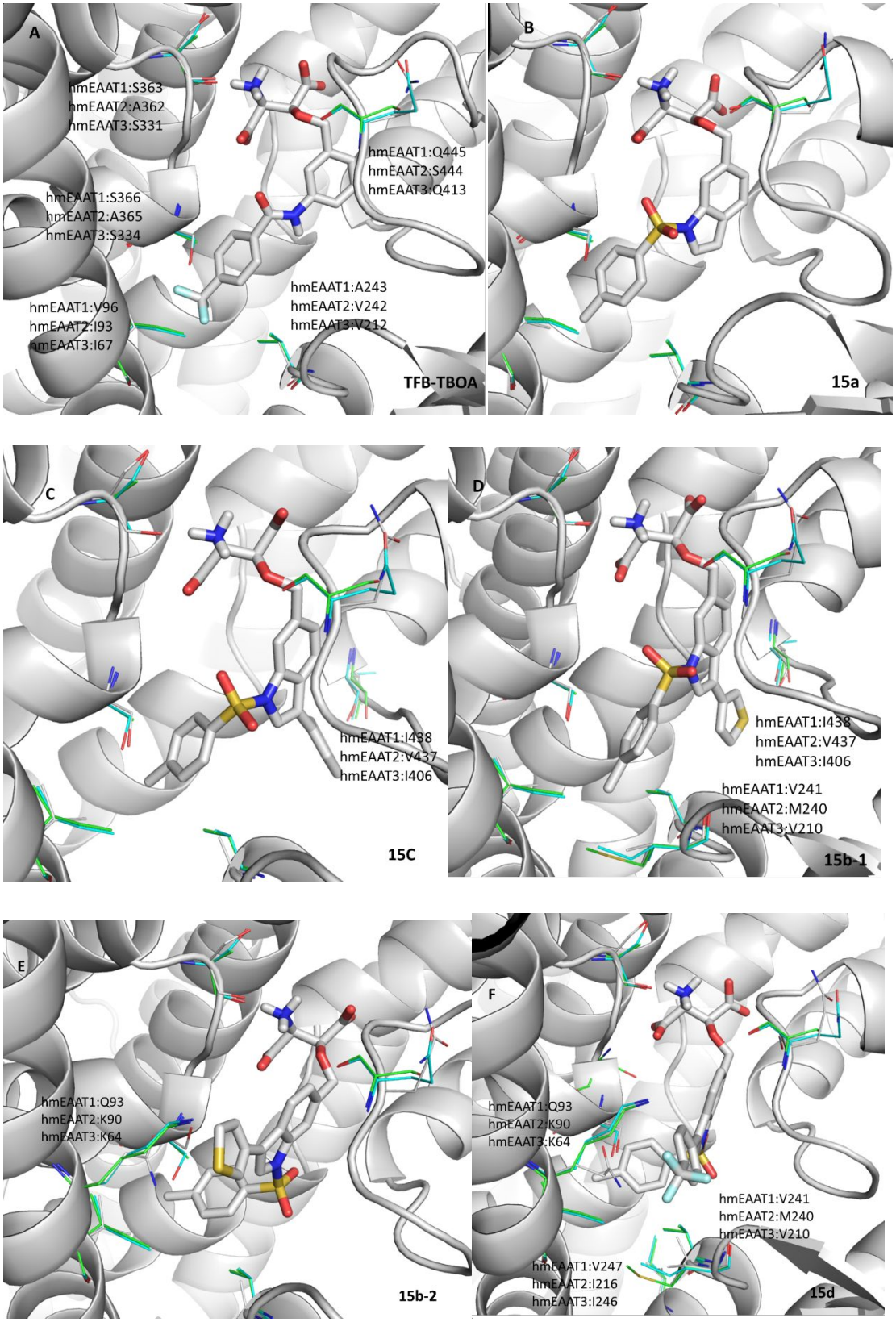


Figure S3. Overlay of different residues within 4 Å around TFB-TBOA and **15a**−**d** (white stick) into hmEAAT1 (white cartoon) by comparison with hmEAAT2,3 (structure not shown). Residues of hmEAAT1, hmEAAT2 and hmEAAT3 were depicted as white line, green line and cyan line,

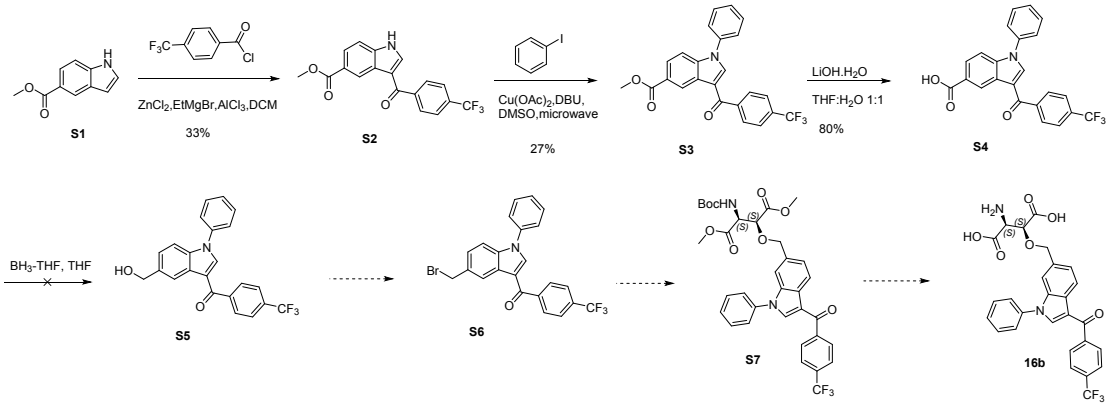
respectively. (A). Different residues were displayed and labeled in the binding model of TFB-TBOA; (B-D). Different residues were displayed and only the newly obtaining different residues were labeled for the binding model of **15a-d** in comparison with the binding model of TFB-TBOA.

Table S3. Induced-fit docking scores[7] for L-TBOA, TFB-TBOA, **16a** and **15a−d**[a,b]

Compound	Compound Pose[c]	Docking Score	Glide Gscore	Glide Emodel	Primary Energy	IFDScore[d]
L-TBOA	pose 1	-7.621	-7.621	-68.296	-17254.5	-870.346
	pose_3	-6.982	-6.982	-58.325	-17256.7	-869.82
TFB-TBOA	pose_1	-10.04	-10.04	-107.212	-17303.7	-875.226
	pose_3	-9.723	-9.723	-105.652	-17297.5	-874.6
16a	pose_1	-10.582	-10.582	-118.256	-17301.6	-875.661
	pose_7	-9.729	-9.729	-109.652	-17292	-874.329
15a	pose_1	-10.309	-10.309	-120.66	-17315.5	-876.086
	pose_9	-8.563	-8.563	-99.232	-17315.6	-874.345
15b	pose_1	-10.695	-10.695	-119.827	-17338.7	-877.628
	pose_4	-9.702	-9.702	-117.041	-17339.2	-876.66
	pose_14	-9.526	-9.526	-118.775	-17319	-875.477
15c	pose_1	-10.559	-10.559	-120.901	-17335.2	-877.317
	pose_5	-9.627	-9.627	-115.557	-17334	-876.325
15d	pose_1	-11.259	-11.259	-121.648	-17355.9	-879.056

[a] Full induced-fit docking poses and scores of each compound can be found from the Maestro file. [b]Potential Energy-OPLS3: -1127.076, Tautomer Probability: 1, Ionization Penalty: 0.0042, State Penalty: 0, Tot Q: -2. [c] Pose 1 of each compound is the first recommended one of the 20 output poses. Other pose(s) of each compound is the one with the best overlay with the reference ligand TFB-TBOA. [d]IFDScore = 1.0*GlideScore + 0.05*PrimeEnergy.

Scheme S1. Synthesis route for analog **16b**



The selective reduction of carboxylic acid **S5** to give the corresponding alcohol **S4** was attempted with borane-THF complex.[8] However, the reaction failed and gave a complex product mixture. In this regard, others have reported that reduction of 3-formalyindoles led to a mixture of 3-methylindole, dimeric products and higher polymeric products with diborane.[9]

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