Supplemental material

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Supplemental materials and methods

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-(methylsulfonamido)propanoate (2a)

Protected methane sulfonamide (2a) was prepared according to the general procedure (step 1 (a)). mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 154.2 mg of pure product as a colorless oil (74.8%). ^{1}H NMR (400 MHz, chloroform-d) 5 5.35 (d, J = 36.6 Hz, 1H), 4.93 (t, J = 6.4 Hz, 1H), 4.28 (d, J = 6.5 Hz, 1H), 3.64 - 3.31 (m, 2H), 2.98 (s, 3H), 1.48 (d, J = 15.7 Hz, 18H). 2b was prepared from 2a (74.2mg, 0.219 mmol) according to the general deprotection procedure to form a final TFA salt, which was used as it is (21.4 mg, white solid, 33% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-(ethylsulfonamido)propanoate (3a)

Protected ethane sulfonamide (3a) was prepared according to the general procedure (step 1 (a)). 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 119.0 mg of pure product as a colorless oil (100% quantitative). ^{1}H NMR (400 MHz, chloroform-d) 5 5.52 - 5.33 (m, 1H), 4.83 (s, 1H), 4.26 (q, J = 5.6 Hz, 1H), 3.61 - 3.31 (m, 2H), 3.06 (q, J = 7.4 Hz, 2H), 1.48 (d, J = 15.9 Hz, 18H), 1.37 (t, J = 7.4 Hz, 3H). 3b was prepared from 3a (128.0 mg, 0.363 mmol) according to the general deprotection procedure to form a TFA salt (36.0 mg pale yellow fine powder, 32% yield).

Benzenesulfonyl chloride (4)

4 was prepared as shown in the general procedure DCM to yield a dark-brown liquid crude product, 547.0 mg (98% quantitative) which solidified when kept in the fridge. This was used without further purification to prepare 4a.

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-(phenylsulfonamido)propanoate (4a)

Protected benzene sulfonamide (4a) was prepared according to the general procedure (step 1 (a)). 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 134.0 mg of pure product as a cream white solid (99% yield). ^{1}H NMR (400 MHz, chloroform-d) 5 7.86 (dd, J = 7.3, 1.8 Hz, 2H), 7.63 - 7.55 (m, 1H), 7.52 (dd, J = 8.4, 6.6 Hz, 2H), 5.33 (d, J = 7.1 Hz, 1H), 5.25 (s, 1H), 4.19 (q, J = 6.1, 5.4 Hz, 1H), 3.50 - 3.12 (m, 2H), 1.44 (d, J = 16.5 Hz, 18H). 4b was prepared from 4a (90.0 mg, 0.225 mmol) according to the step 2 procedure to form a TFA salt (46.0 mg, white solid, 57% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((4-methylphenyl)sulfonamido)propanoate (5a)

Protected toluene sulfonamide (5a) was prepared according to the general procedure (step 1 (a)). 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 130.0 mg of pure product as a white solid (95% yield). ^{1}H NMR (400 MHz, chloroform-d) 5 7.71 - 7.62 (m, 2H), 7.29 - 7.21 (m, 2H), 5.25 (s, 1H), 5.04 (s, 1H), 4.30 - 3.91 (m, 1H), 3.23 (tt, J = 11.2, 5.5 Hz, 2H), 2.36 (s, 3H), 1.38 (d, J = 16.2 Hz, 18H). 5b was prepared according to the general deprotection procedure using 70.0 mg (0.168 mmol) of 5a to form a TFA salt (40.0 mg, white solid, 64% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((4-(trifluoromethoxy)phenyl)sulfonamido)propanoate (6a)

Protected 4-(trifluoromethoxy)benzene sulfonamide (6a) was prepared as 5a above. 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 160.0 mg of pure product as a colorless oil (98% yield). ^{1}H NMR (400 MHz, chloroform-d) 5 8.05 - 7.95 (m, 2H), 7.78 (d, J = 8.3 Hz, 2H), 5.60 (d, J = 6.2 Hz, 1H), 5.38 (d, J = 6.9 Hz, 1H), 4.27 - 4.08 (m, 1H), 3.50 - 3.20 (m, 2H), 1.44 (d, J = 19.7 Hz, 18H). 6b was prepared from 6a (90.0 mg, 0.186 mmol) according to the step 2 procedure to form a TFA salt (43.0 mg, light pink solid, 52% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((2-(trifluoromethoxy)phenyl)sulfonamido)propanoate (7a)

Protected 2-(trifluoromethoxy)benzene sulfonamide (7a) was prepared as 5a. 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 163.0 mg of product as a colorless oil (100%, quantitative). ^{1}H NMR (400 MHz, chloroform-d) 5 8.01 (dd, J = 8.0, 1.8 Hz, 1H), 7.67 - 7.58 (m, 1H), 7.44 - 7.36 (m, 2H), 5.32 (s, 1H), 5.25 (t, J = 6.6 Hz, 1H), 4.37 - 4.12 (m, 1H), 3.59 - 3.19 (m, 2H), 1.45 (d, J = 22.1 Hz, 18H). 7b was prepared from 7a (90.0 mg, 0.186 mmol) as 6b above to form a TFA salt (50.0 mg, white solid, 62% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((4-fluorophenyl)sulfonamido)propanoate (8a)
Protected 4-fluorobenzene sulfonamide (8a) was prepared according to the general procedure. 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 140.0 mg of pure product as a white powder (99% yield). ^{1}H NMR (400 MHz, Chloroform-d) 5 7.92 - 7.82 (m, 2H), 7.23 - 7.14 (m, 2H), 5.33 (d, J = 6.6 Hz, 2H), 4.39 - 4.06 (m, 1H), 3.48 - 3.16 (m, 2H), 1.47 (s, 9H), 1.42 (s, 9H). 8b was prepared according to the general deprotection procedure using 92.0 mg (0.220 mmol) of 8a to form a TFA salt (50.0 mg, light pink powder, 61% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((2-fluorophenyl)sulfonamido)propanoate (9a)
Protected 2-fluorobenzene sulfonamide (9a) was prepared as 8a. 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 139.0 mg of pure white foamy crystals (98.5% yield). ^{1}H NMR (400 MHz, Chloroform-d) 5 7.88 (td, J = 7.5, 1.8 Hz, 1H), 7.64 - 7.52 (m, 1H), 7.33 - 7.25 (m, 1H), 7.20 (ddd, J = 10.3, 8.3, 1.1 Hz, 1H), 5.49 - 5.23 (m, 2H), 4.22 (p, J = 5.5, 5.1 Hz, 1H), 3.56 - 3.23 (m, 2H), 1.48 (s, 9H), 1.43 (s, 9H). 9b was prepared from 9a (92.0 mg, 0.186 mmol) as 8b to form a TFA salt (40.0 mg, white solid, 48% yield).

Tert-butyl (S)-3-((2-bromophenyl)sulfonamido)-2-((tert-butoxycarbonyl)amino)propanoate (10a)
Protected 2-bromobenzene sulfonamide (10a) was prepared according to the general procedure. 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield 155.0 mg of pure product (colorless oil), which solidified to a white solid when left under vacuum overnight (96% yield). ^{1}H NMR (400 MHz, chloroform-d) 5 8.11 (dd, J = 7.7, 1.9 Hz, 1H), 7.73 (dd, J = 7.7, 1.5 Hz, 1H), 7.44 (dtd, J = 21.1, 7.5, 1.6 Hz, 2H), 5.62 (d, J = 6.6 Hz, 1H), 5.46 - 5.20 (m, 1H), 4.44 - 3.98 (m, 1H), 3.54 - 3.06 (m, 2H), 1.48 (s, 9H), 1.43 (s, 9H). 10b was prepared from 10a (90.0 mg, 0.188 mmol) according to the step 2 procedure to form a TFA salt (63.0 mg, white solid, 77% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((2,4-dichlorophenyl)sulfonamido)propanoate (11a)
Protected 2,4-dichlorobenzene sulfonamide (11a) was prepared as 10a, 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield a white solid (155.0 mg, 98% yield). ^{1}H NMR (400 MHz, Chloroform-d) 5 8.01 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.40 (dd, J = 8.5, 2.0 Hz, 1H), 5.59 (d, J = 6.5 Hz, 1H), 5.40 - 5.16 (m, 1H), 4.20 (q, J = 5.4 Hz, 1H), 3.34 (ddd, J = 12.8, 5.8, 4.2 Hz, 1H), 3.26 (ddd, J = 12.5, 6.8, 5.1 Hz, 1H), 1.48 (s, 9H), 1.43 (s, 9H). 11b was prepared from 11a (90.0 mg, 0.192 mmol) as 10b to form a TFA salt (60.0 mg, light pinkish solid, 73% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((4-(trifluoromethyl)phenyl)sulfonamido)propanoate (12a)
Protected 2,4-dichlorobenzene sulfonamide (11a) was prepared as 10a. 100.0 mg (0.337 mmol) Boc-DAP-OtBu hydrochloride was used to yield a white solid (157.0 mg, 99% yield). ^{1}H NMR (400 MHz, Chloroform-d) 5 8.05 - 7.93 (m, 2H), 7.78 (d, J = 8.3 Hz, 2H), 5.61 (t, J = 6.3 Hz, 1H), 5.38 (d, J = 6.9 Hz, 1H), 4.39 - 4.07 (m, 1H), 3.57 - 3.20 (m, 2H), 1.46 (s, 9H), 1.41 (s, 9H). 12b was prepared from 12a (90.0 mg, 0.192 mmol) as 10b to form a TFA salt (50.0 mg, white-pinkish solid, 61% yield).

Tert-butyl (S)-3-([1,1′-biphenyl]-4-sulfonamido)-2-((tert-butoxycarbonyl)amino)propanoate (13a)
13a was prepared as 10a. 117.4 mg (0.396 mmol) Boc-DAP-OtBu hydrochloride was used to yield a white solid (173.0 mg, 92% yield). ^{1}H NMR (400 MHz, Chloroform-d) 5 7.97 - 7.87 (m, 2H), 7.78 - 7.68 (m, 2H), 7.65 - 7.58 (m, 2H), 7.54 - 7.46 (m, 2H), 7.46 - 7.38 (m, 1H), 5.35 (s, 1H), 5.17 (s, 1H), 4.33 - 4.14 (m, 1H), 3.36 (tdd, J = 12.6, 8.2, 5.1 Hz, 2H), 1.48 (s, 9H), 1.43 (s, 9H). 13b was prepared from 13a (72.0 mg, 0.151 mmol) according to general deprotection procedure but used thiophenol (46 pL, 0.453 mmol, 3 equivalent) instead of DL-dithiothreitol to form a TFA salt (72.0 mg, 0.151 mmol; white solid, 84% yield).

Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-((4′-fluoro-[1,1′-biphenyl])-4-sulfonamido)propanoate (14a)
14a was prepared as 13a. 120.2 mg (0.406 mmol) Boc-DAP-OtBu hydrochloride was used producing a white foamy solid (197.0 mg, 86% yield). ^{1}H NMR (400 MHz, Chloroform-d) 5 7.96 - 7.87 (m, 2H), 7.71 - 7.63 (m, 2H), 7.60 - 7.53 (m, 2H), 7.22 - 7.13 (m, 2H), 5.53 - 5.16 (m, 2H), 4.23 (p, J = 5.2 Hz, 1H), 3.58 - 3.18 (m, 2H), 1.47 (s, 9H), 1.42 (s, 9H). 14b was prepared from 14a (76.0 mg, 0.153 mmol) as 13b above to form a TFA salt (47.0 mg, white solid, 68% yield).

Di-tert-butyl (2R,4R)-4-(([1,1′-biphenyl]-4-ylsulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (15a)
Protected biphenyl sulfonic ester (15a) was prepared according to the general procedure. 100.0 mg (0.348 mmol) Boc-L-trans-4-hydroxyproline tert-butyl ester was used to yield a colorless oil (174.0 mg, 99% yield). ^{1}H NMR (400 MHz, chloroform-d) 5 7.96 (dd, J = 8.6, 2.4 Hz, 2H), 7.77 (dd, J = 8.4, 4.2 Hz, 2H), 7.66 - 7.56 (m, 2H), 7.55 - 7.40 (m, 3H), 5.08 (dqd, J = 10.2, 5.1, 3.4, 2.6 Hz, 1H), 4.28 (q, J = 8.2 Hz, 1H), 3.78 - 3.51 (m, 2H), 2.72 - 2.38 (m, 1H), 2.27 - 2.06 (m, 1H), 1.44 (d, J = 10.1 Hz, 18H). 15b was prepared from 15a (70.0 mg, 0.138 mmol) according to the general deprotection procedure, yielding a TFA salt (46.0 mg, light pinkish solid, 72% yield).

Di-tert-butyl (2R,4R)-4-(((4′-fluoro-[1,1′-biphenyl]-4-yl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (16a)
16a was prepared as 15a. 100.0 mg (0.348 mmol) Boc-L-trans-4-hydroxyproline tert-butyl ester was used to yield a colorless oil (140.0 mg, 77% yield). ^{1}H NMR (400 MHz, chloroform-d) 5 7.96 (dd, J = 8.5, 2.6 Hz, 2H), 7.72 (dd, J = 8.1, 4.0 Hz, 2H), 7.64 - 7.55 (m,

2H), 7.19 (t, J = 8.6 Hz, 2H), 5.23 - 4.98 (m, 1H), 4.28 (q, J = 7.6 Hz, 1H), 3.79 - 3.51 (m, 2H), 2.66 - 2.37 (m, 1H), 2.27 - 2.07 (m, 1H), 1.44 (d, J = 11.4 Hz, 18H). 16b was prepared from 16a (100.0 mg, 0.192 mmol) as 15a to form a light pink solid (70.0 mg, 76% yield).

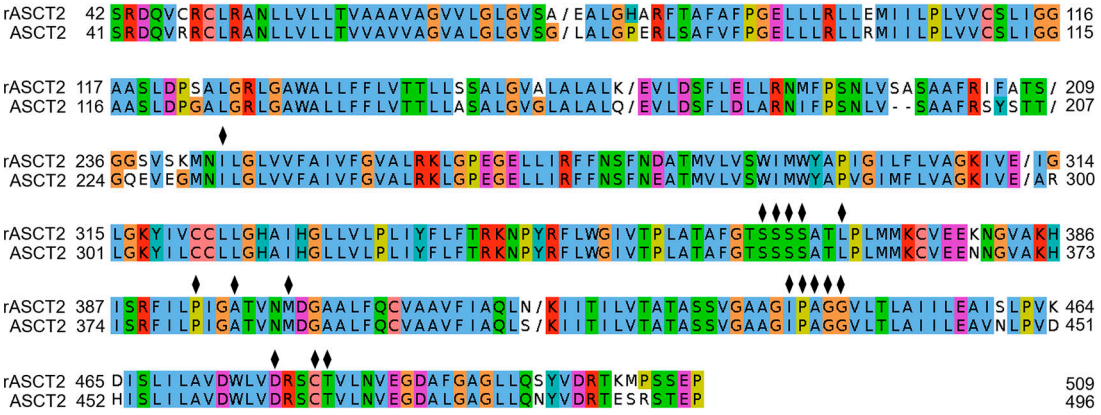

Figure S1. **Sequence alignment of the rASCT2 and hASCT2 homologues.** The modeled fraction of rASCT2 was aligned to the corresponding fraction of hASCT2 using MUltiple Sequence Comparison by Log-Expectation (MUSCLE) and visualized with a Jalview using the ClustalX color scheme. Protein segments that were not modeled due to gap regions in the alignment are represented by slashes (/).

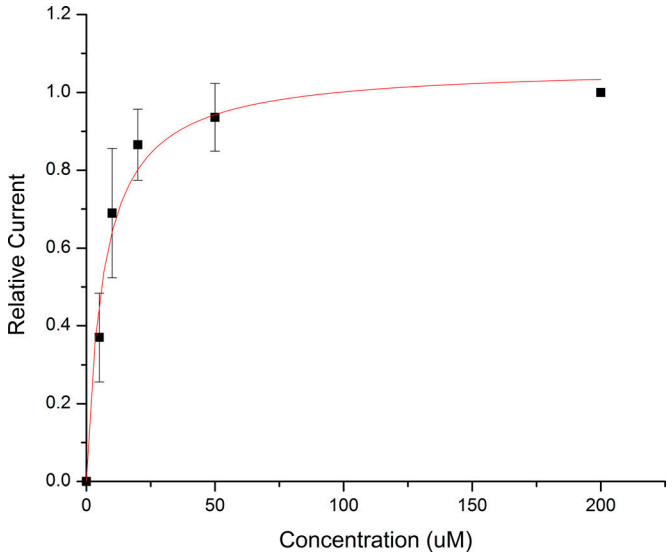

Figure S2. **Dose–response curve of current evoked by compound 16b in rat EAAT1.** The red line represents a fit with K_i = 7.0 ± 1.5 µM. The error bars represent ±SD.

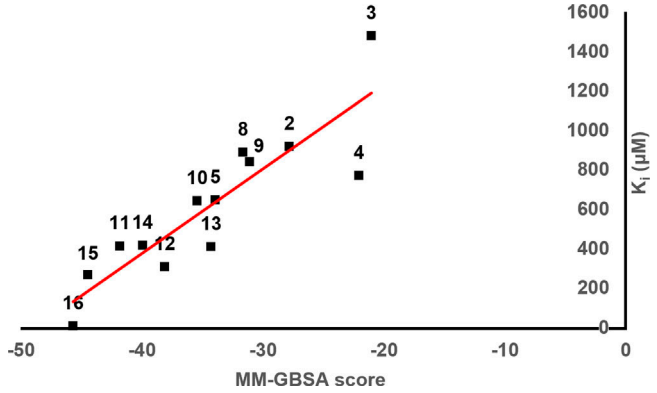

Figure S3. **Plot of K_i versus MM-GBSA score.** R^2 is 0.7848. Compound 1 was not included in the analysis.

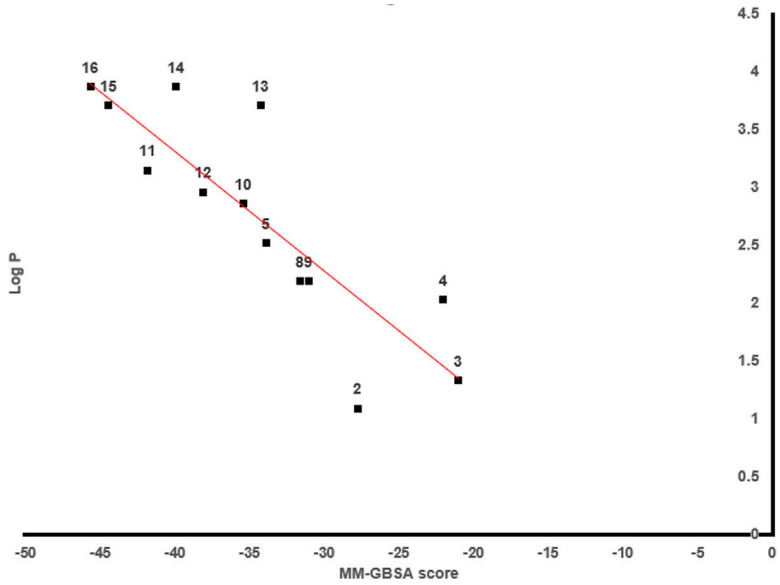

Figure S4. **Inhibitor side-chain hydrophobicity correlates with MM-GBSA score**. R^2 is 0.73, showing good correlation.

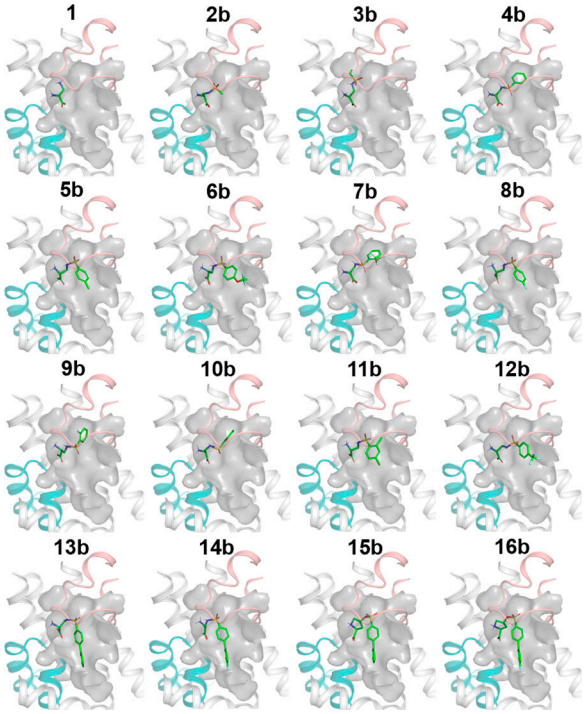

Figure S5. **Predicted binding poses of compounds 1b–16b in the ASCT2 substrate binding site.** The shape of the ASCT2 binding site is highlighted in dark gray. Ligands are shown in green sticks, HP1 is teal, and HP2 is salmon. Helices around the binding site are in light gray cartoon.