A computational approach yields selective inhibitors of human excitatory amino acid transporter 2 (EAAT2)

Excitatory amino acid transporters (EAATs) represent a protein family that is an emerging drug target with great therapeutic potential for managing central nervous system disorders characterized by dysregulation of glutamatergic neurotransmission. As such, it is of significant interest to discover selective modulators of EAAT2 function. Here, we applied computational methods to identify specific EAAT2 inhibitors. Utilizing a homology model of human EAAT2, we identified a binding pocket at the interface of the transport and trimerization domain. We next conducted a high-throughput virtual screen against this site and identified a selective class of EAAT2 inhibitors that were tested in glutamate uptake and whole-cell electrophysiology assays. These compounds represent potentially useful pharmacological tools suitable for further exploration of the therapeutic potential of EAAT2 and may provide molecular insights into mechanisms of allosteric modulation for glutamate transporters.

Sodium-dependent glutamate transporters (SLC1A1–3, 6, or 7 or excitatory amino acid transporters EAAT1–5) play essential roles in the maintenance and regulation of glutamatergic neurotransmission (1–3) and have emerged as important targets for drug discovery (4). EAAT1–5 transporters rapidly bind and buffer glutamate, removing it from the synaptic cleft. This removal contributes to the termination of synaptic activity and to the clearance of potentially cytotoxic extracellular glutamate. EAAT1–5 transports the bound glutamate into cells where it can be recycled for use in subsequent synaptic events (3). Glutamate transporters can also influence membrane potential and cellular excitability, either as a result of the electrogenic cotransport of 3Na+/1H+ and coupled countertransport of 1K+/2 for each glutamate molecule transported or via the generation of an uncoupled chloride conductance (5). Importantly, major neuropsychiatric and neurological disorders such as schizophrenia, bipolar disorder, depression, and memory impairment are characterized by dysregulation of glutamatergic neurotransmission (6–8). Slowing glutamate uptake with selective EAAT inhibitors could improve transmission at glutamatergic synapses and provide therapeutic benefit under some conditions (1, 3, 9–13). Understanding the precise role of EAAT2 in health and disease requires identification of new selective modulators of EAAT2 function. However, to date the availability of pharmacological tools for EAAT2 is limited (14, 15). The aim of the present study, therefore, was to identify novel selective tools for the study of EAAT2.

Computational methods are routinely utilized in drug discovery to identify diverse sets of bioactive compounds for pharmacologically relevant targets (16–19). They can be largely classified as either structure-based or ligand-based techniques and have played a major role in the discovery and development of new drug molecules for over three decades. In this study, we applied structure-based tools to the discovery of novel modulators of the human excitatory amino acid transporter 2 (hEAAT2).

Much of what is known of the structure of glutamate transporters comes from crystal structures of the prokaryotic homolog from *Pyrococcus horikoshii* (GltPh). GltPh has been crystalized in an apo form, in complex with a substrate (L-aspartate), and in complex with the competitive inhibitor TBOA (20–22). These structures show that GltPh exists as a homotrimer, with each monomer of the trimeric structure consisting of two domains: a trimerization domain formed by transmembrane helices 1, 2, 4, and 5 and a transport domain formed by transmembrane helices 3, 6, 7, and 8 and two re-entrant loops (helical hairpins 1 and 2). The structures capture two distinct conformations, inward-facing and outward-facing, where individual transport domains undergo relocations ~15 Å normal to the membrane and provides substrate and ions alternating access to the extracellular (outward) and intracellular (inward) regions (23). Because interdomain interactions determine the transport rate of glutamate uptake (24), domain unlocking by disruption of interdomain interactions should modulate the movement of the transport domain and, hence, the glutamate transport rate.

Here, an hEAAT2 homology model built from GltPh was generated to identify novel allosteric site(s) and assist in the identification of selective hEAAT2 modulators. A virtual screen was completed as a component of the Janssen inventory, and our study led to the discovery of a novel and selective hEAAT2 inhibitor. To our knowledge, this is the first selective, allosteric hEAAT2 inhibitor described in the literature.

The authors declare that they have no conflicts of interest with the contents of this article.

This article contains supporting data and Figs. S1–S10.

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3 The abbreviations used are: SLC, solute carrier; EAAT, excitatory amino acid transporter; hEAAT, human EAAT; GltPh, glutamate transporter *P. horikoshii*; HTVS, high-throughput virtual screening; PLB, propensity for ligand binding; TBOA, DL-threo-β-benzyloxyaspartic acid; PDB, Protein Data Bank; RMSD, root-mean-square deviation.

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**Results and discussion**

**Homology model generation**

The homology modeling tool Prime (25, 26) was used to generate two homology models built from GltPh crystal structures: one model in an inward-facing conformation (PDB code 4P19 (21) as template) and one in an outward-facing conformation (PDB code 1XFH (22) as template). Because of the low sequence identity between GltPh and hEAAT2 (30% sequence identity), the predicted sequence alignment from Prime required manual intervention. Data from a multiple sequence alignment generated by Yernool et al. (22) between GltPh, hEAAT3, and additional homologs was useful to guide the manual alignment as hEAAT2 and hEAAT3 have ~55% sequence identity. Additionally, there are many functionally important amino acids that are highly conserved across the SLC family and were used to guide the sequence alignment (the sequence alignment is provided in the Fig. S1).

The resulting models appeared to be robust; tertiary and secondary structures were maintained with small differences in flexible loop areas. Furthermore, the RMSD between the inward-facing model and 4P19 was found to be 0.38 Å, and the RMSD between the outward-facing model and 1XFH was 0.39 Å. Both models are provided in the Fig. S7. There is a large insertion in eukaryotic transporters between helices 4b and 4c (~50-residue insertion in hEAAT2) that was not modeled because it is very difficult to accurately predict the structure of these residues de novo. To assess the structural quality of the model, protein reports were generated within Maestro (27) to examine steric clashes, bond length deviations, etc. Based on this assessment, the models appear to be of good structural quality and suitable for structure-based applications (details provided in the supporting data).

During the study, hEAAT1 structures were solved in the outward conformation in complex with a substrate (L-aspartate), the competitive inhibitor (2S, 3S)-3-[3-[4-((trifluoromethyl)benzoylamino)benzyloxy]aspartate, and a novel allosteric inhibitor, UCPH101 (28). The structures were found to be very similar to GltPh. Additionally, there is good agreement between the hEAAT2 model and hEAAT1 crystal structure in the outward-facing conformation (PDB codes 5LLM (28), 5LLU (28), 5LM4 (28), and 5MJU (28)), provided in the Figs. S2 and S3; the RMSD between the model and 5LLM crystal structure is 0.52 Å. The insertion residues are not fully resolved in the hEAAT1 structures; hence, it is still unclear whether they take on any secondary structure and how they impact the overall fold.

**Pocket identification and evaluation**

The two hEAAT2 homology models (inward-facing and outward-facing conformations) were utilized to predict allosteric sites that could potentially modulate the transport rate of glutamate. A binding pocket evaluation using SiteMap (29, 30) identified a site at the interface of the trimerization and transport domains (site A, inward-facing conformation), located in a region previously identified as important for Parawixin 1 binding (15), shown in Fig. 1. The volume of the site is 956.8 Å³ and the propensity for ligand binding (PLB) score (31) was 3.93, the highest predicted index for a cavity on the model. The PLB is based off amino acid composition of the predicted site, and a validation study was found for 79% of their test data set; the site with the highest PLB index was indeed the true binding site (31). Additional sites were observed but 1) the PLB index was very low (<1), and 2) the PLB index did not appear to be in a region that would have a functional effect on the transporter. FTMap was also utilized to confirm that site A contained hot spots for ligand binding; 9 of the 16 probes used by FTMap mapped to this region.

A second pocket evaluation was conducted for the outward-facing homology model and identified two high-ranking sites. The top ranked site was the orthosteric binding site and has a volume of 932.4 Å³; the PLB index was 2.99. Identification of this site validated the use of SiteMap.

The second ranked site (site B, outward-facing conformation) was located on the opposite side of the transporter compared with the glutamate-binding pocket and at the interface of the trimerization and transport domains (Fig. 1). The volume of this site is 1278.0 Å³, and the PLB index was 1.53. FTMap also identified this site with all 16 probes being represented. An overlay of the two hEAAT2 conformations showed that the site is not present in the inward-facing conformation because it is occupied by the transport domain (provided in the Fig. S4). We hypothesized that binding to this region could poten-
tially result in negative allosteric modulation because compound binding would sterically block the conformational change of the transport domain that is necessary for glutamate uptake.

**HTVS led to NAM discovery**

Approximately 350,000 unique compounds, a component of the Janssen corporate library, were virtually screened using the two identified binding sites of the EAAT2 homology models (site A, inward-facing conformation; site B, outward-facing conformation). High-throughput virtual screening (HTVS) has been shown to be a promising and resource-efficient tool for hit identification (32–39) with a number of recent studies illustrating its successful application to drug discovery (40–45).

Because there was no available literature data for benchmarking, constraints were not utilized in either the site A or site B HTVS run. Various filters were applied to prioritize hits such as removing compounds with a molecular weight of <150 and reactive functionalities, quality control filters, and a GlideScore cutoff of −6.5 for site A and −7 for site B (because site A is smaller than site B, fewer compounds were scored). Additionally, post-docking hydrogen bond constraint filters were applied to ensure that at least one hydrogen bond was made to a residue in the predicted binding site. An additional filter based on availability was applied resulting in a high priority hit list of 2360. All 2360 compounds were tested for EAAT2 modulatory activity using a 384-well fluorescent membrane potential assay. Based on these results, 26 compounds were selected for follow-up in orthogonal assays of EAAT2 activity (glutamate uptake and whole-cell electrophysiology).

The compounds were tested at 25 μM, and only one compound, compound 1, significantly decreased EAAT2-mediated glutamate uptake as demonstrated by Fig. 2A (one-way analysis of variance; Dunnett test *, p < 0.0332). To confirm those data, we also assessed the potency and selectivity of compound 1 at hEAAT2 compared with the closely related hEAAT1. Compound 1 decreased hEAAT2-mediated glutamate uptake with an IC50 of 6.6 ± 0.6 μM (Fig. 2B) and was selective for hEAAT2 over hEAAT1 (EAAT1 IC50 value was >50 μM; Table 1) The inhibitory activity of compound 1 was further confirmed in whole-cell electrophysiology (Fig. 2D), considered a measure of direct binding; the inset shows inhibition of glutamate-induced current in a cell expressing hEAAT2. Inhibition was reversible upon washout of the compound.

The confirmed inhibitor, compound 1, was identified by allosteric site B on the outward-facing conformation of hEAAT2 and is provided in Table 1. A similarity search of compound 1 against the Janssen inventory identified 28 analogs available for experimental testing. Four of the 28 compounds: 1k, 1p, 1u, and 1v, exhibited significant hEAAT2 inhibitory activity, shown in Table 1. The molecular formula strings, an NMR analysis and a compound purity assessment for the compounds are provided in the Figs. S6, S8, and S9a–c, respectively. Additionally, the four analogs were tested against hEAAT1, and two were selective for hEAAT2. We performed mechanistic studies with those compounds. As shown in Fig. 2C, compound 1v decreased the maximal transport capacity of the EAAT2 transporter without affecting glutamate $K_m$ suggesting an allosteric noncompetitive
mode of action, further highlighted by the Eadie–Hofstee and Lineweaver–Burk analyses (provided in the Fig. S10).

Although the number of compounds tested is limited, some initial structure–activity relationship has emerged. Compounds with a single carbon linker between the amide and left-hand substituent were not active. However, substituents that are directly linked or with a two-atom linker (similar to compound 1) are active. The linker number does not appear to affect selectivity over hEAAT1. Cyclohexyl and benzyl groups are both tolerated as right-hand substituents of the benzoxazepine core. Interestingly, compounds with cyclohexyl are not selective for hEAAT2, whereas those with benzyl are selective. Both cyclohexyl compounds also have an ethyl rather than methyl ether, which could contribute to the loss of selectivity for hEAAT2 over hEAAT1. The ClogP for this series ranges from 3.55 to 4.81 (and AlogP from 3.54 to 4.28); as such, it is highly probable that the compounds are able to penetrate the membrane.

Predicted binding mode of compound 1

Compound 1 was identified through a virtual screen of site B from the outward facing conformation. Fig. 3 shows the predicted binding modes that compound 1 could likely adopt. The poses are reasonable and consistent across varying parameters of the Glide docking program. However, although Trp-355 and Phe-348 are predicted to be important residues for binding in both poses, we have been unable to confirm the importance of these residues using site-directed mutagenesis (because single-point mutations, F348A and W355L did not reduce compound 1 activity; data not shown). Moreover, Fig. 3 shows the major differences between EAAT2 and EAAT1 in compound 1’s predicted EAAT2-binding site. Although Gly-329 in EAAT2 is a leucine in EAAT1 that would clash with one of the predicted binding modes of compound 1, mutating EAAT2 Gly-329 into leucine did not have any significant effect on compound 1 potency at inhibiting EAAT2-mediated glutamate uptake (not shown).

Because site B is a large, flexible pocket, it is possible that it might rearrange in an induced-fit manner to accommodate ligand binding, and a variation of these binding poses would likely be the actual binding mode. For example, there are many

| COMPOUND | EAAT2 IC50 | EAAT1 IC50 |
|----------|------------|------------|
| 1        | 6.6 ± 0.6 μM | >50        |
| 1K       | 7.4 ± 2.4 μM | 9.6 ± 7.3 μM |
| 1P       | 14.6 ± 6.0 μM | 10.4 ± 5.3 μM |
| 1U       | 5.2 ± 3.3 μM | >50        |
| 1V       | 3.6 ± 0.1 μM | >50        |
The homology modeling tool Prime (25, 26), from the Schrödinger suite of programs (27), was used to generate a model of the inward-facing conformation and outward-facing conformation of hEAAT2. A BLAST (46) search was conducted in the PDB (47) using the hEAAT2 sequence to determine which homologs were available in the public domain with structural information. The Glu transporter homolog from *P. horikoshii* (~30% sequence identity) crystal structure PDB code 4P19 (21) was utilized as the template for the inward-facing conformation, and PDB code 1XFH (22) for the outward-facing conformation, using default parameters. The crystal structures were first prepared using the Protein Preparation Wizard within Maestro (27) including adding hydrogens, filling in missing side chains, optimizing hydrogen bonds, and a restrained minimization of all protein atoms. Upon completion of the model-building calculations, the final models were optimized, and energy was minimized with a truncated-Newton energy minimization using OPLS 2000 all-atom force field (48).

Data from a multiple sequence alignment generated by Yernool et al. (22) between GltPh, hEAAT3, and additional homologs was used to guide the manual alignment because hEAAT2 and hEAAT3 have ~55% sequence identity. The sequence alignment is provided in the Fig. S1. A homology model of hEAAT2 was also generated using the hEAAT1 crystal structure PDB code 5LLM (28) (outward-facing conformation) again using the homology modeling tool Prime (25, 26) with default parameters and following the protocol discussed above. The amino acid sequences between EAAT1 and EAAT2 are 65% identical; hence, the alignment was straightforward.

**Pocket identification and evaluation**

SiteMap (29, 30), a grid-based method for quick calculation and comparison of pocket volumes, was used to assess the hEAAT2 inward- and outward-facing conformation homology models and to identify pockets amenable for small molecule binding. Default parameters were employed to analyze the pocket volume and chemical environment for both conformations. Additionally, FTMap (49) was utilized to detect hot spots, areas that have a high propensity for ligand binding, on the protein surface of both models again using default parameters.

**Ligand database preparation**

A virtual database was previously created from a portion of the Janssen corporate library using default LigPrep (27) parameters to generate tautomers, ionization states (using Epik (50, 51)), and stereoisomers. The final virtual data set contained 350,000 unique compounds. Because the library was prefilled, no compounds were flagged as PAINS (52). At the time of the study, there were no known hEAAT2 compounds for benchmarking.

**High-throughput virtual screen**

The virtual data set of 350,000 unique compounds from the Janssen corporate library was docked into the two identified binding sites of the EAAT2 homology models (site A, inward-facing conformation; site B, outward-facing conformation) using Glide SP (53, 54) from the Schrödinger suite of programs (55). Default parameters were utilized for grid generation (site A grid center: 3.43, −4.62, 18.89; and site B: −9.63, 51.55, −49.39). Analysis of the HTVS results was performed using Pipeline Pilot (56). All scored compounds were filtered using the following criteria: GlideScore of −6.5 for site A and −7 for
site B, availability of the molecule for testing, in-house quality-control criteria, and filters for molecular weight of <150 and reactive functionalities. Additionally, post-docking hydrogen-bond constraint filters were applied to ensure that at least one hydrogen bond was made to a residue in the predicted binding site. Compounds in common between the screens were pooled, and duplicates were removed and clustered using proprietary clustering methodology (57), resulting in 2,360 hits.

**Cell culture and stable cell line generation**

The cDNA for hEAAT2 and hEAAT1 were subcloned into pcDNA4-TO (Invitrogen) using EcoRV and Xhol restriction sites. The HEK293-TRex cells were maintained at 37 °C in a humidified 5% CO2 incubator in culture medium (Dulbecco’s modified Eagle’s medium supplemented with penicillin (100 units/ml), streptomycin (100 mg/ml), and 10% tetracycline-free fetal bovine serum). For the stable expression of both human SLC1A2 (EAAT2) and SLC1A3 (EAAT1), the cells were transfected using FuGENE according to the protocol of the manufacturer (Promega, Madison, WI) and maintained for 2 weeks in selection medium containing 200 μg/ml Zeocin and 5 μg/ml Blasticidin. Antibiotic-resistant colonies were isolated and screened individually in the [14C]citrate uptake assay after overnight incubation with 1 μg/ml tetracycline. Noninduced cells were used as a negative control. Characterization of EAAT1 and EAAT2 stable cell lines provided in the Fig. S5.

**Glutamate uptake assay**

HEK293TRex-TO/hEAAT2 cells were plated and induced overnight with 1 μg/ml tetracycline at a density of 100,000 cells/well in PDL-pretreated 96-well plates (PerkinElmer). On the day of the experiment, the cells were washed twice with assay buffer (Hanks’ balanced salt solution supplemented with 20 mM HEPES, 2 mM CaCl2, 1 mM MgSO4, and 5 mM glucose, pH 7.4) before adding 100 μl of assay buffer. Inhibitors were added and incubated for 5 min at room temperature before addition of [14C]glutamic acid on ice for 5 min. Glutaraldehyde (0.5% final) was then added to block uptake. The experiments were performed in quadruplets three or four times for each compound. The data are presented as averages ± S.E., and statistical analysis, when presented, was performed as indicated using GraphPad Prism software version 6.02. Saturation experiments were performed to confirm the known Kd of glutamate at both EAAT2 and EAAT1 (supporting data S1 and not shown). WAY213613 (Tocris) was also used to confirm that the uptake measured was EAAT2-mediated.

**Whole cell electrophysiology**

HEK TREX cells stably expressing hEAAT2 were plated at ~10,000 cells/well on coverslips in 24-well plates and induced with 1 μg/ml tetracycline overnight. On the day of the experiment, glass coverslips were placed in a bath on the stage of an inverted microscope and perfused (~1 ml/min) with extracellular solution (149 mM NaCl, 4 mM KCl, 10 mM HEPES, 5 mM glucose, 2 mM CaCl2, 1 mM MgCl2, 311 mM, pH 7.4). Pipettes were filled with an intracellular solution of the following composition: 40 mM KCl, 100 mM KF, 10 mM EGTA, 10 mM HEPES, 2 mM MgCl2, pH 7.3–7.4, with KOH, ~290 mM (sucrose) and had a resistance of 2–4 MΩ. All recordings were made at room temperature (22–24 °C) using a Multiclamp 700A amplifier and pClamp 9 software (Axon Instruments). Current records were acquired at 2KHz and filtered at 1 KHz. The drugs were applied using an SF-77B Fast-Step Perfusion device (Warner Instruments). Transporter currents were elicited by 10 μM glutamate at holding potential of ~60 mV. Compounds were applied in the continued presence of glutamate.

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**References**

1. Nakagawa, T., and Kaneko, S. (2013) SLC1 glutamate transporters and diseases: psychiatric diseases and pathological pain. *Curr. Mol. Pharmacol.* 6, 66–73 CrossRef Medline
2. Rives, M. L., Javitch, J. A., and Wickenden, A. D. (2017) Potentiating SLC transporter activity: emerging drug discovery opportunities. *Biochem. Pharmacol.* 135, 1–11 CrossRef Medline
3. Takahashi, K., Foster, J. B., and Lin, C. L. (2015) Glutamate transporter EAAT2: regulation, function, and potential as a therapeutic target for neurological and psychiatric disease. *Cell. Mol. Life Sci.* 72, 3489–3506 CrossRef Medline
4. Colas, C., Ung, P. M., and Schlessinger, A. (2016) SLC transporters: structure, function, and drug discovery. *Medchemcomm.* 7, 1089–1091 CrossRef Medline
5. Fairman, W. A., Vandenberg, R. J., Arriza, J. L., Kavanaugh, M. P., and Amara, S. G. (1995) An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* 375, 599–603 CrossRef Medline
6. Abdallah, C. G., Averill, C. L., Salas, R., Averill, L. A., Baldwin, P. R., Krytsal, J. H., Mathew, S. J., and Mathalon, D. H. (2017) Prefrontal connectivity and glutamate transmission: relevance to depression pathophysiology and ketamine treatment. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 2, 566–574 CrossRef Medline
7. Goff, D. C., and Coyle, J. T. (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am. J. Psychiatry* 158, 1367–1377 CrossRef Medline
8. Tang, Y. P., Shimizu, E., Dube, G. R., Rampon, C., Kerchner, G. A., Zhuo, M., Liu, G., and Tsien, J. Z. (1999) Genetic enhancement of learning and memory in mice. *Nature* 401, 63–69 CrossRef Medline
9. Behrens, P. F., Franz, P., Woodman, B., Lindenberg, K. S., and Landwehrmeyer, G. B. (2002) Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. *Brain* 125, 1908–1922 CrossRef Medline
10. Fontana, A. C. (2015) Current approaches to enhance glutamate transporter function and expression. *J. Neurochem.* 134, 982–1007 CrossRef Medline
11. Rajkowska, G., and Stockmeier, C. A. (2013) Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr. Drug Targets* 14, 1225–1236 CrossRef Medline
12. Reissner, K. J., and Kalivas, P. W. (2010) Using glutamate homeostasis as a target for treating addictive disorders. *Behav. Pharmacol.* 21, 514–522 CrossRef Medline
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53. Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T., Repasky, M. P., Knoll, E. H., Shelley, M., Perry, J. K., Shaw, D. E., Francis, P., and Shenkin, P. S. (2004) Glide: a new approach for rapid, accurate docking and scoring: 1. Method and assessment of docking accuracy. *J. Med. Chem.* 47, 1739–1749 CrossRef Medline

54. Halgren, T. A., Murphy, R. B., Friesner, R. A., Beard, H. S., Frye, L. L., Pollard, W. T., and Banks, J. L. (2004) Glide: a new approach for rapid, accurate docking and scoring: 2. Enrichment factors in database screening. *J. Med. Chem.* 47, 1750–1759 CrossRef Medline

55. Glide (2018) release 10.6, Schrödinger, LLC, New York

56. BIOVIA Pipeline Pilot, Accelrys, San Diego, CA

57. Hack, M. D., Rassokhin, D. N., Buyck, C., Seierstad, M., Skalkin, A., ten Holte, P., Jones, T. K., Mirzadegan, T., and Agrafiotis, D. K. (2011) Library enhancement through the wisdom of crowds. *J. Chem. Inf. Model.* 51, 3275–3286 CrossRef Medline