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The discovery of a structurally novel class of inhibitors of the type 1 glycine transporter

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ABSTRACT

The type 1 glycine transporter plays an important in regulating homeostatic glycine levels in the brain that are relevant to the activation of the NMDA receptor by the excitatory neurotransmitter glutamate. We describe herein the structure–activity relationships (SAR) of a structurally novel class of GlyT1 inhibitors following on a lead derived from high throughput screening, which shows good selectivity for GlyT1 and potent activity in elevating CSF levels of glycine.

Glycine plays a key role in eukaryotic physiology as both an endogenous amino acid for protein synthesis and a neurotransmitter. In its role as a neurotransmitter, glycine binds to the NR1 subunit of the NMDA receptor, acting as an obligatory co-transmitter in modulating receptor sensitivity for the endogenous agonist glutamate. It also acts at strychnine-sensitive postsynaptic receptors in the spinal cord to control motor coordination. In both these roles, levels of glycine are controlled by glycine-uptake transporters, which consist of two subtypes, GlyT1 and GlyT2.[1] GlyT1 is found on glial cells in the brain and is widely distributed throughout the body, where it controls basal levels of glycine. GlyT2 is found primarily in the spinal cord and brainstem, where it controls levels of glycine following release by glycinergic neurons.

GlyT1 plays an important role not only throughout the body, but also in controlling glycine levels in the vicinity of NMDA synapses in order to prevent glycine levels from constantly saturating the glycine binding site on the NR1 subunit of the NMDA receptor. As a key player in mediating learning and memory, the NMDA receptor has long attracted attention as a possible target for novel therapeutic agents.[2] In particular, one theory of the etiology of schizophrenia hypothesizes that a deficit in NMDA-mediated neurotransmission underlies many of the disorder's defining symptoms.[3] For example, a deficiency in NMDA transmission may be responsible for the loss of executive functions such as decision-making ability, memory loss, and decreased affect (personality) that characterize the disease. Restoring NMDA function, however, is not a simple matter of providing more glutamate, which can be neurotoxic at high levels. As an alternative approach, elevating

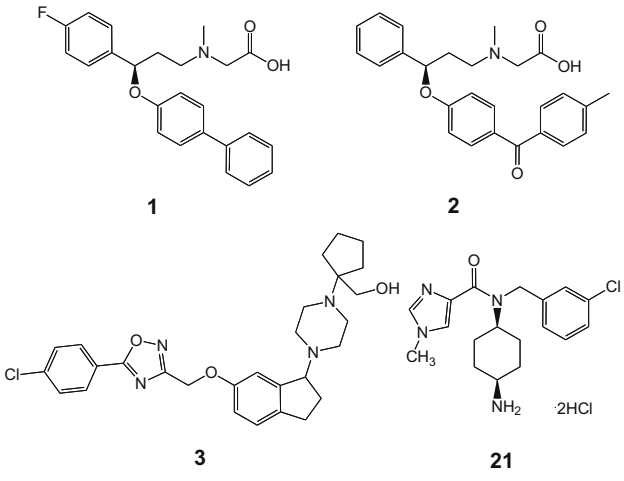

Figure 1. GlyT1 inhibitors discussed above.

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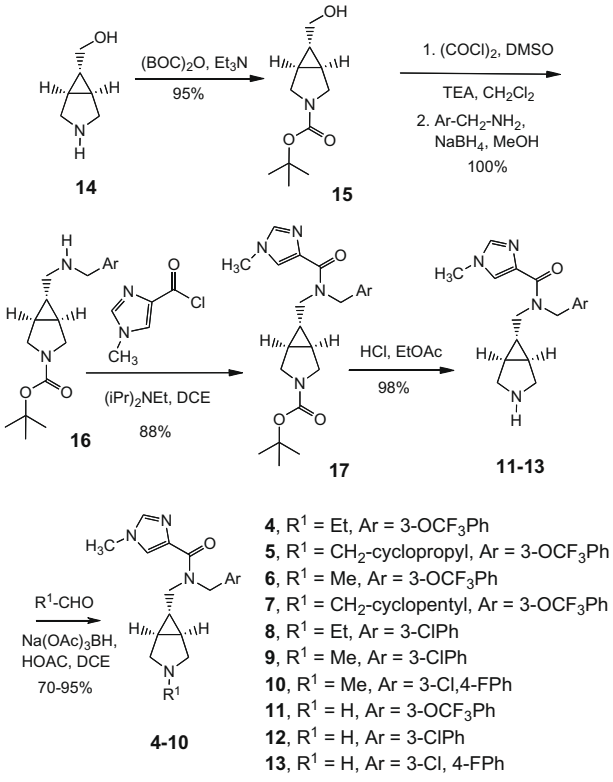
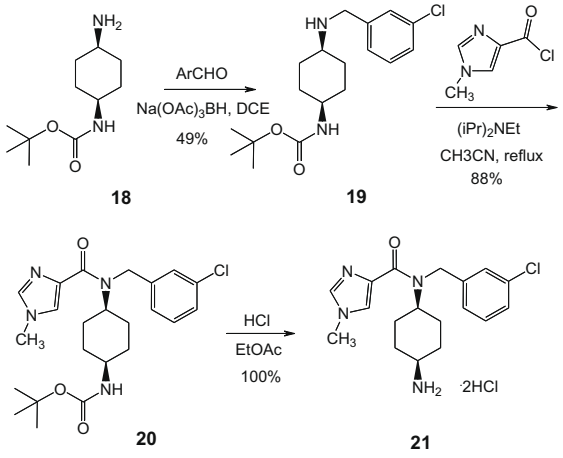
levels of glycine would augment endogenous glutamate transmission through NMDA receptors.[4] This elevation could be achieved through inhibition of GlyT1-mediated glycine uptake, and this mechanism forms the basis for our proposed approach to a novel antipsychotic agent described herein.

While the atypical antipsychotic agents, exemplified by risperidone, olanzapine, quetiapine, ziprasidone, and aripiprazole, have provided an important advance in therapy for schizophrenia, many opportunities remain for continued improvement. For a novel therapy to provide value, it should avoid the side-effect liabilities of the currently available classes of antipsychotics while addressing some of the symptom domains, such as negative and cognitive symptoms, poorly treated by these agents. GlyT1 inhibitors would fill this latter role by augmenting executive function and memory to address cognitive symptoms, as well as improving affect to address negative symptoms.

Several structurally diverse GlyT1 inhibitors have been described previously (Fig. 1).[5] The prototype GlyT1 inhibitor, (*R*)-*N*[3-(4′fluorophenyl)-3-(4′phenylphenoxy)propyl]-sarcosine, is designated ALX 5407, **1**.[6] NFPS, the racemic form of ALX 5407, has been shown to augment downstream effects of NMDA transmission, supporting the proposed role of GlyT1 in regulating NMDA transmission.[7] We recently reported an analogue of ALX 5407, (*R*)-*N*[3-phenyl-3-(4′-(4-toluoyl)phenoxy)-propyl]sarcosine ((*R*)-NPTS), **2**, as a suitable radioligand for a GlyT1 binding affinity assay.[8] In addition, we described more recently a series of indanyl piperazine and propylamine GlyT1 inhibitors based on a hit from high-throughput screening, exemplified by compound **3**.[9] Neither series, however, provided a compound suitable for advancement to development. Continued high-throughput screening using the radioligand version of **2** led to the discovery of compound **21**. We report herein our follow-up of this lead compound and the structure–activity relationships (SAR) for improving its permeability, as well as further structural modification for improving clearance.



Scheme 1. Preparation of compounds **4**–**17**.



Scheme 2. Preparation of HTS lead compound **21**.

The preparation of the compounds described in this work is shown in Schemes 1 and 2. In Scheme 1, following *t*-BOC protection of starting material **14**,[10] a series of straightforward steps leads to the final analogues for testing via oxidation, reductive amination, acylation, de-protection, and reductive amination. This chemistry lent itself to SAR elaboration using library design and production, and the analogues reported herein reflect that versatility. The synthesis of the HTS lead, compound **21**, is shown in Scheme 2 starting from the commercially available **18**, and follows the chemistry shown in Scheme 1.

The in vitro assays used to characterize the compounds described have been reported previously.[8] The CSF glycine assay, which will be described in more detail in a subsequent report, takes advantage of the overflow of glycine into the CSF following GlyT1 inhibition in the brain.

Many previously described GlyT1 inhibitor series, such as those we have previously disclosed as exemplified by compounds **2** and **3**, suffer from drawbacks in their drug-like properties and consequent pharmacokinetics and safety profiles. In seeking a new GlyT1 inhibitor series, we prioritized drug-like properties as the starting point. We were therefore gratified when a high-throughput screen of our compound file revealed a new series of GlyT1 inhibitors exemplified by compound **21** (Table 1). In addition to its potent GlyT1 inhibitory potency, **21** exhibits potent in vivo activity in the CSF Glycine model. The excellent drug-like physical properties of this compound, in particular its low molecular weight and lipophilicity, were exactly what we had prioritized. In addition, its structure is very synthetically versatile, enabling rapid exploration of SAR at the amide, benzyl side chain, template, and amino portions of the molecule. Among the various surrogates for the 1,4-diaminocyclohexane template, we discovered that a [3.1.0] azabicyclic template provided good pharmacokinetic properties and improved in vivo activity in the CSF Glycine model (Table 1). This series, then, formed the basis for the remaining SAR investigation.

The first issue we addressed was the limited permeability in compound **21**, which is indicated by the low value of MDCK_AB_C in Table 1. This parameter measures the transit of a molecule across the cell membrane of MDCK cells, which are representative of many cell types, and thus gives a good idea of both gut and blood-brain barrier permeability. Increasing lipophilicity, as measured by cLog *P*, delivered improved permeability as expected, as shown in compounds **4** and **5**. Interestingly, further increases in lipophilicity, as illustrated in compound **7**, did not improve permeability. The improved permeability in compounds **4** and **5** comes at

Table 1
Data for GlyT1 inhibitors

Compd No.	GlyT1 K_i (nM)	GlyT2 IC50 (nM)	CSF Glycine ED200 (mg/kg)	hMic $t_{1/2}$ (min)	MDCK AB_C	HERG IC50 (nM)	cLog *P*	Mol. wt.
2	2.32 (2.08–2.57 $n = 15$)	>10,000	56.3 (25.6–124)	58	1.69	NT	3.8	417
3	(26.8 + 7.05)*	NT	NT	9.1	1.7	NT	5.42	509
4	2.24 (1.60–3.14 $n = 3$)	>10,000	1.8 (0.866–3.6)	21.6	12.1	2200	2.43	422
5	2.32 (1.84–2.92 $n = 8$)	>10,000	0.8	12.1	15.7	530	2.7	448
6	2.32 (1.49–3.60 $n = 3$)	>10,000	0.97	27.3	13.9	3800	1.9	408
7	3.82 (0.851–17.2 $n = 3$)	>10,000	4.7	11.2	7.55	460	3.82	476
8	4.58 (3.03–6.94 $n = 4$)	>10,000	2.9	63.1	12.4	NT	2.11	373
9	11.0 (0.511–236 $n = 2$)	>10,000	8.6	>120	14.1	NT	1.58	359
10	11.6 (7.34–18.3 $n = 7$)	>10,000	3.5 (1.79–6.88)	80	13.2	8500	1.73	377
11	15.2 (0.280–822 $n = 4$)	>10,000	>30	>85.7	1.7	NT	1.45	394
12	95.3 (31.4–289 $n = 3$)	NT	NT	>120	3.2	NT	1.14	345
13	109	>10,000	NT	>120	0.9	14,600	1.28	363
21	1.79 (1.59–2.01 $n = 68$)	1150	3.9 (2.17–7.41)	>120	0.5	65,600	1.75	347

NT—not tested; GlyT1 K_i values are given in nM units, with standard error of the mean and number of determinations indicated in parentheses. GlyT2 IC50 values are given in nM units, for inhibition of [^{3}H]-glycine uptake. CSF Glycine ED200 values represent the dose that doubles endogenous (baseline) levels of glycine in rat csf at a 90 min time point, following subcutaneous administration of a test compound, with 95% confidence limits in parentheses. hMic $t_{1/2}$—compound half-life in human microsomes given in minutes. MDCK_AB_C—apparent permeability through MDCK cell membranes in units of 10–6 cm/s, corrected for background, at an initial concentration of 2 μM. HERG IC50 values given in nM units for block of HERG current in patch clamp measurement in HEK-293 cells. cLog *P* and Mol. wt. (molecular weight).
* For compound **3**, GlyT1 IC50 value in nM units.

a cost, however, in that the projected human metabolic stability also decreases substantially. Table 1 indicates this metabolic stability in terms of the half-life of the compound when incubated in vitro with human microsomes under conditions for metabolic degradation by P450 enzymes. For example, compound **21** shows good microsomal stability with a hMic $t_{1/2}$ value of >120 min. Increasing lipophilicity to improve permeability resulted in greatly reduced hMic $t_{1/2}$ values for, for example, compounds **4** (21.6 min) and **5** (12.1 min). In general, we sought hMic $t_{1/2}$ values of greater than 60 min in order to provide good oral bioavailability. ADME in silico modeling suggested that maintaining the tertiary amine group in compounds **4** and **5** while reducing the molecular weight and cLog *P* would be favorable for reducing microsomal clearance. Although this balance of functionality and lipophilicity limits the range of potential compounds, adjusting the aromatic substituent on the benzyl side chain and using a small R group appended to the endocyclic nitrogen was found to afford the desired microsomal stability, as shown in compounds **8**, **9**, and **10**. Compound **10** in particular represents a favorable balance of potent GlyT1 inhibitory activity with good microsomal stability and permeability, resulting in potent activity in the CSF Glycine in vivo assay. It also shows that reduced lipophilicity decreases HERG activity (e.g., relative to the more lipophilic compound **5**), thus suggesting another reason for maintaining the proper balance of lipophilicity.

The GlyT1 inhibitor approach to schizophrenia tests the hypothesis that elevated synaptic levels of glycine will enhance NMDA receptor-mediated signaling and thereby ameliorate a major contributor to the symptomatic characteristics of the disease. The compounds described in the present work, in particular compound **10**, may prove applicable to testing this hypothesis clinically. Compound **10** provides a balance of in vitro and in vivo potency with favorable pharmacokinetic properties suggesting the potential for good oral bioavailability in humans.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.035.

References and notes

1. Betz, H.; Gomeza, J.; Armsen, W.; Scholze, P.; Eulenburg, V. *Biochem. Soc. Trans.* **2006**, *34*, 55.
2. Coyle, J. T. *Cell Mol. Neurobiol.* **2006**, *26*, 365.
3. Millan, M. J. *Psychopharmacology* **2005**, *179*, 30.
4. Kinney, G. G.; Sur, C. *Curr. Neuropharmacol.* **2005**, *3*, 35.
5. Slassi, A.; Egle, I. *Expert Opin. Ther. Patents* **2004**, *14*, 201.
6. (a) Atkinson, B. N.; Bell, S. C.; De Vivo, M.; Kowalski, L. R.; Lechner, S. M.; Ognyanov, V. I.; Tham, C.-S.; Tsai, C.; Jia, J.; Ashton, D.; Klitencik, M. A. *Mol. Pharmacol.* **2001**, *60*, 1414; (b) Aubrey, K. R.; Vandenberg, R. J. *Br. J. Pharmacol.* **2001**, *134*, 1429.
7. Bergeron, R.; Meyer, T. M.; Coyle, J. T.; Greene, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15730.
8. Lowe, J. A., III; Drozda, S. E.; Fisher, K.; Strick, C.; Lebel, L.; Schmidt, C.; Hiller, D.; Zandi, K. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1291.
9. Lowe, J.; Drozda, S.; Qian, W.; Peakman, M. C.; Liu, J.; Gibbs, J.; Harms, J.; Schmidt, C.; Fisher, K.; Strick, C.; Schmidt, A.; Vanase, M.; Lebel, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1675.
10. Brighty, K. E.; Castaldi, M. J. *Synth. Lett.* **1996**, *11*, 1097.