Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5233–5238
Bioorganic & Medicinal Chemistry Letters

Discovery and SAR studies of novel GlyT1 inhibitors

Magnus W. Walter,[a,*] Beth J. Hoffman,[b,†] Kimberly Gordon,[b] Kirk Johnson,[b] Patrick Love,[b] Matthew Jones,[a] Teresa Man,[a] Lee Phebus,[b] Jon K. Reel,[b] Helene C. Rudyk,[a] Harlan Shannon,[b] Kjell Svensson,[b] Hong Yu,[b] Matthew J. Valli[b] and Warren J. Porter[b]

[a]*Lilly Research Laboratories, Eli Lilly and Company, Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK*
[b]*Lilly Research Centre, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA*

Received 2 April 2007; revised 22 June 2007; accepted 27 June 2007
Available online 30 June 2007

Abstract—Inhibition of the glycine transporter GlyT1 is a potential strategy for the treatment of schizophrenia. A novel series of GlyT1 inhibitors and their structure–activity relationships (SAR) are described. Members of this series are highly potent and selective transport inhibitors which are shown to elevate glycine levels in cerebrospinal fluid.

The amino acid glycine is a major neurotransmitter in the mammalian CNS with both inhibitory and excitatory actions.[1] It has also been shown to modulate excitatory neurotransmission as obligatory co-agonist with glutamate at NMDA receptors in the pre-frontal cortex. NMDA receptor hypofunction has been implicated in the etiology of schizophrenia and NMDA receptor activation constitutes a potential strategy for the development of novel schizophrenia treatments.[2] Since direct acting NMDA receptor agonists are epileptogenic and excitotoxic, indirect modulation of the NMDA receptor through its co-agonist (glycine) binding site has received considerable attention as a potentially non-toxic approach.[3] Glycine uptake is mediated by two glycine transporters, GlyT1 and GlyT2, which belong to the family of Na^+/Cl^--dependent neurotransmitter transporters.[4] Since NMDA receptors are co-localized with GlyT1, this transporter may be involved in the regulation of extracellular glycine concentrations at NMDA receptors. Modulation of extracellular glycine levels by glycine transporter inhibition therefore constitutes a novel strategy for the treatment of positive and negative symptoms of schizophrenia including its cognitive deficits.[5]

Figure 1 shows the structure of several reported GlyT1 inhibitors.[6–8] Although these compounds form a structurally heterogeneous group, a sarcosine (*N*-methyl glycine) substructure as in **1** is a known motif.[9] Piperazinyl acetic acids such as **2** are structurally related to sarcosines.[10] Other structural types without a carboxylate

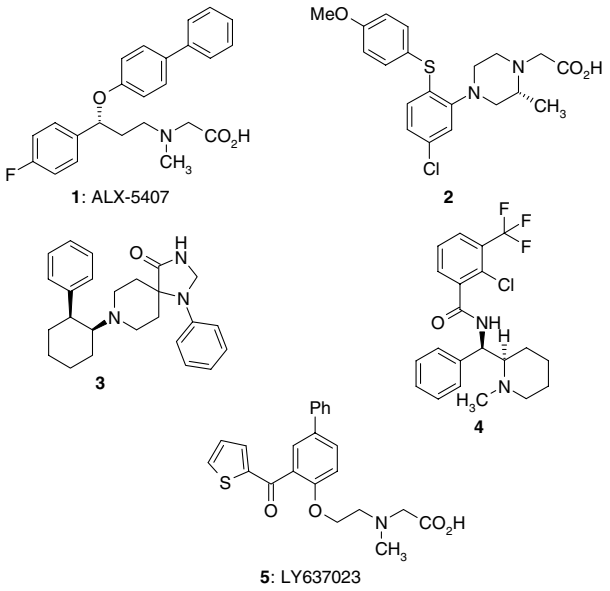


Figure 1. GlyT1 inhibitors.

Keywords: Glycine transporter; GlyT1; GlyT2; Schizophrenia.
* Corresponding author. Tel.: +44 1276 484786; fax: +44 1276 483305; e-mail: magnus.walter@lilly.com
† Present address: Amgen Inc., Thousand Oaks, CA, USA.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.06.074

group have also been described, for example, **3**[11–14] and **4**.[15] Herein, we disclose the discovery and SAR studies of a novel class of GlyT1 inhibitors in which the sarcosine group is linked to a substituted biphenyl system via an ethanolamine linker (**5**).[16]

A high-throughput screening campaign of the Lilly corporate compound collection led to the discovery of **5** as potent and selective inhibitor of GlyT1 (IC_{50} = 45 nM, GlyT2 IC_{50} > 30,000 nM).

In addition to the characteristic *N*-methyl glycine moiety as acidic head group and the novel ethanolamine linker, **5** also contained a 2-acyl biphenyl system which was the focus of our initial SAR studies (Fig. 2). Herein we will report on the impact of changes in Domains 1 and 2 on glycine transporter binding affinity and selectivity.

Our synthetic strategy was based on the use of advanced intermediates from which the targets should be accessible in one or two steps using robust synthetic protocols amenable to a rapid parallel synthesis (RPS) approach (Fig. 3).

We envisaged the use of cross-coupling chemistry for the preparation of analogues containing a biphenyl system either *para* (Domain 2) or *ortho* (Domain 1) to the ethanolamine linker. A Weinreb amide intermediate could be used for the introduction of different acyl substituents (addition elimination).

To allow efficient exploration of a variety of aromatic substituents in Domain 2 of hit **5** we used *para*-bromo analogue **9** as late stage intermediate suitable for a rapid parallel synthesis approach (Scheme 1). Readily accessible phenol **8** was coupled with [(2-hydroxyethyl)-methylamino]acetic acid *tert*-butyl ester under modified Mitsunobu conditions using 1,1′-(azodicarbonyl)dipiperidine (ADDP) to give **9**.[17] Introduction of the *para*-aryl group was achieved following the Suzuki–Miyaura protocol.[18] Ester hydrolysis under acidic or basic conditions gave the final compounds. Both the coupling and the deprotection step were carried out in a parallel fashion.[19]

Replacement of the thiophene group in **5** by phenyl and cyclohexyl was achieved using the aforementioned ADDP-mediated coupling of the requisite phenol with the *tert*-butyl protected side chain. The phenol coupling partners were prepared following established literature procedures.[20]

Changes to the acyl group in Domain 1 required a variety of different chemical approaches (Scheme 2). Reduction of the carbonyl precursors, **23** and **24**, respectively, using triethylsilane and trifluoroacetic acid provided convenient access to the methylene linked phenyl and cyclohexyl analogues **25** and **26**. Attempted reduction of the carbonyl group in **5** to access **27** under similar

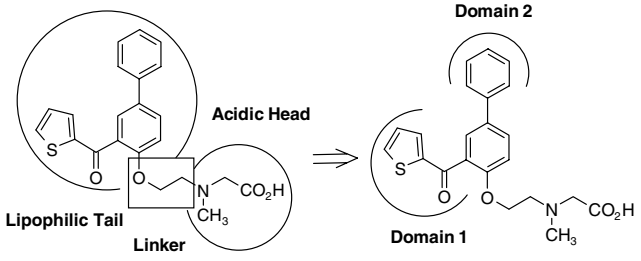


Figure 2. SAR Domains in HTS hit **5**.

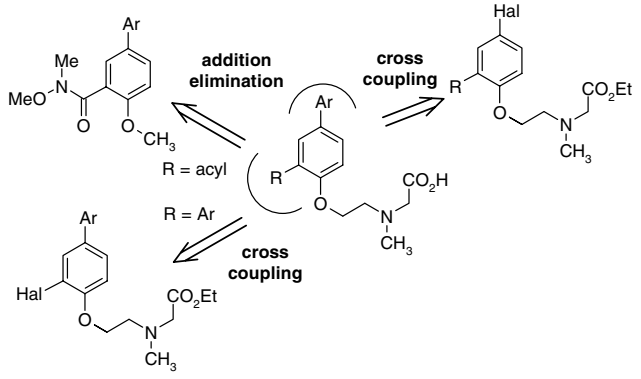
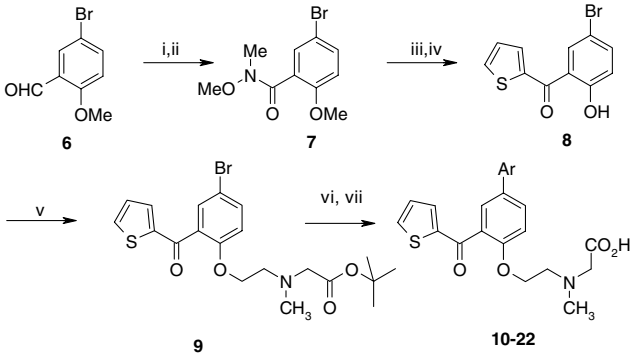
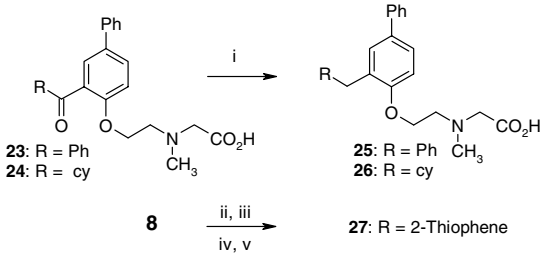


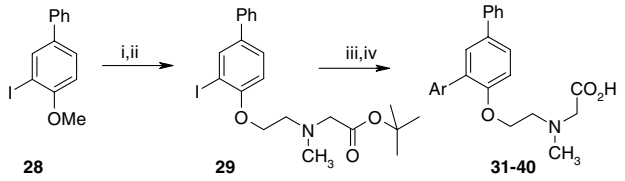
Figure 3. General synthetic approaches to analogues of **5**.



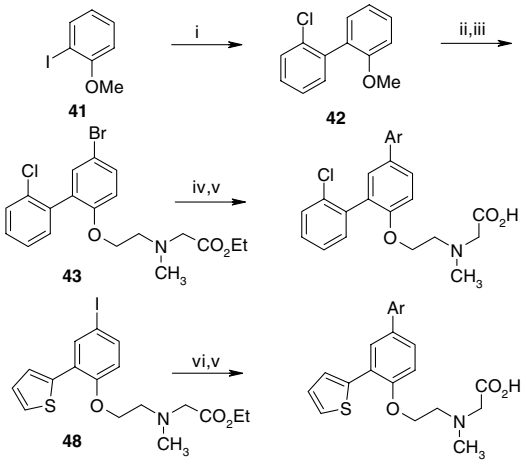
Scheme 1. Reagents and conditions: (i) $KMnO_4$, acetone/water, 40%; (ii) MeNHOMe·HCl, CDI, DCM, 87%; (iii) thiophen-2-yl magnesium bromide, THF, Et_2O, 72%; (iv) BBr_3, DCM, −78 °C, 88%; (v) [(2-hydroxyethyl)-methylamino]acetic acid *tert*-butyl ester, ADDP, PBu_3, THF, reflux, 75%; (vi) $ArB(OH)_2$, $P(cy)_3$, $Pd(OAc)_2$, KF, THF, reflux, (**5**: Ar = Ph: 77%); (vii) TFA, DCM, or NaOH/EtOH, reflux, (**5**: Ar = Ph: 85%).



Scheme 2. Reagents and conditions: (i) Et_3SiH, TFA, 87% (R = Ph); (ii) N_2H_4, ethylene glycol, NaOH, 120–140 °C, 71%; (iii) [(2-hydroxyethyl)-methylamino]acetic acid *tert*-butyl ester, ADDP, toluene, $P(Bu)_3$, 90 °C, 86%; (iv) $PhB(OH)_2$, $P(cy)_3$, $Pd(OAc)_2$, KF, THF, reflux, 98%; (v) TFA, DCM, 70%.



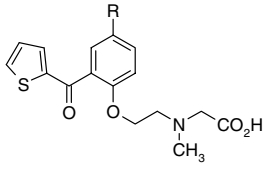
Scheme 3. Reagents and conditions: (i) NaSEt, DMF, reflux, 86%; (ii) [(2-hydroxyethyl)-methylamino]acetic acid *tert*-butyl ester, ADDP, toluene, $P(Bu)_3$, 90 °C, 86%; (iii) $ArB(OH)_2$, $P(cy)_3$, $Pd(OAc)_2$, KF, THF, reflux, 75% (**10**: Ar = 2-Cl–Ph); (iv) TFA, DCM.



Scheme 4. Reagents and conditions: (i) 2-Cl-$PhB(OH)_2$, S-Phos, $Pd(OAc)_2$, KF, THF, reflux, 96%; (ii) Br_2, $CHCl_3$, 57%; (iii) BBr_3, DCM, 69%; (iv) [(2-hydroxyethyl)-methylamino]acetic acid *tert*-butyl ester, ADDP, toluene, $P(Bu)_3$, 90 °C, 94%; (v) NaOH, EtOH, 70% (Ar = 5-benzo[1,3]dioxol-5-yl); (vi) $ArB(OH)_2$, $P(cy)_3$, $Pd(OAc)_2$, KF, THF, reflux, (**30**: Ar = Ph: 77%).

conditions led to decomposition of the starting material. Instead, Wolff–Kishner reduction of phenol **8** followed by ADDP-mediated coupling and Suzuki–Miyaura coupling gave **27**.

Table 1. Domain 2 SAR



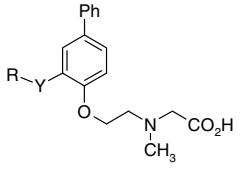
Compound	R	GlyT1 IC_{50} (nM)
5	Ph	45 ± 10
10	2-Cl–Ph	175 ± 18
11	3-Cl–Ph	26 ± 3
12	4-Cl–Ph	68 ± 10
13	2-Me–Ph	632 ± 76
14	3-Me–Ph	45 ± 11
15	4-Me–Ph	59 ± 14
16	3-OMe–Ph	104 ± 10
17	4-OMe–Ph	27 ± 8
18	3-Me,4-OMe–Ph	18 ± 0
19	3,4-Di-Me–Ph	76 ± 1
20	3,4-Di-OMe–Ph	834 ± 182
21	3,4-Methylene dioxy-Ph	19 ± 0
22	CF_3[16]	702 ± 43

In Domain 1, a biaryl system *ortho* to the ethanolamine linker was introduced by Pd-mediated cross-coupling methodology employing advanced aryliodide intermediate **29** and a variety of aryl and heteroaryl boronic acids (Scheme 3).

In Domain 2, analogues containing the preferred 2-chlorophenyl motif were obtained according to Scheme 4. Suzuki–Miyaura coupling of commercially available 2-iodo anisole **41** gave **42** followed by regioselective bromination, demethylation and ADDP-mediated coupling gave advanced intermediate **43** for the preparation of final targets by RPS methods. In the 2-thiophene series Domain 2 analogues were prepared in a similar fashion from advanced intermediate **48**.

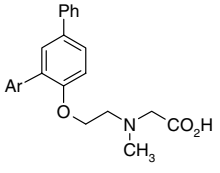
Glycine reuptake inhibition was determined in whole cell assays for both GlyT1 and GlyT2.[21] All members of this series had no significant activity at the GlyT2 (IC_{50} > 30μM). The effects of changes in Domain 2 while maintaining the thiophenacyl moiety in Domain 1 are summarized in Table 1. Both 2-chlorophenyl (**10**) and

Table 2. Domain 1 SAR



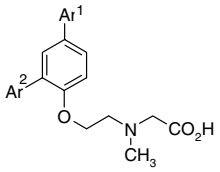
Compound	R	Y	GlyT1 IC_{50} (nM)
5	2-Thiophene	C=O	45 ± 10
27	2-Thiophene	CH_2	40 ± 1
30	2-Thiophene	Bond	70 ± 8
23	Phenyl	C=O	65 ± 6
25	Phenyl	CH_2	85 ± 9
31	Phenyl	Bond	229 ± 54
24	Cyclohexyl	C=O	70 ± 6
26	Cyclohexyl	CH_2	289 ± 139
53	Cyclohexyl[16]	Bond	251 ± 51

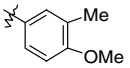
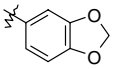
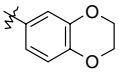
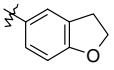
Table 3. Further Domain 1 SAR



Compound	Ar	GlyT1 IC_{50} (nM)
31	Ph	229 ± 54
32	2-Cl–Ph	53 ± 16
33	3-Cl–Ph	674 ± 0
34	4-Cl–Ph	965 ± 0
35	2-F–Ph	108 ± 0
36	2-Me–Ph	56 ± 0
37	2-OMe–Ph	351 ± 0
38	3-Thiophene	139 ± 13
39	2-Thiazole	957 ± 92
40	1-Naphthyl	496 ± 0

Table 4. Mix-and-match SAR



Compound	Ar_1	Ar_2	GlyT1 IC_{50} (nM)
44		2-Thiophene	161 ± 8
48		2-Cl–Ph	28 ± 6
45		2-Thiophene	34 ± 10
49		2-Cl–Ph	12 ± 3
46		2-Thiophene	25 ± 10
50		2-Cl–Ph	14 ± 1
47		2-Thiophene	17 ± 2
51		2-Cl–Ph	13 ± 3

2-methylphenyl (**13**) caused a significant decrease in activity, suggesting an important role of the biphenyl system orientation for maintaining potency. In general, electron-releasing groups appeared to be preferred as Domain 2 substituents. Combination of a 3-methyl and 4-methoxy group as in **18** resulted in increased potency. This motif was found to be preferred over 3,4-dialkyl substitution as in **19**. Interestingly, 3,4-dimethoxy analogue **20** was significantly less active, whereas the methylenedioxy analogue **21** was more potent than the original hit **5**. Replacement of Domain 2 by bulky non-aromatic groups as in **22** led to a significant drop in activity.

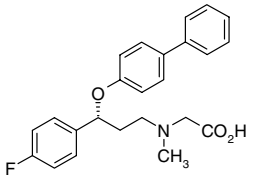
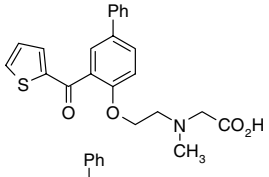
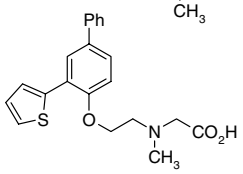
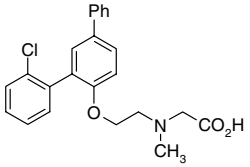
Our exploration of Domain 1 focused on the role of the carbonyl linker in **5** (Table 2). We found that replacement of this group by a methylene unit as in **27** or direct attachment of the thiophene ring to the central aromatic core as in **30** did not significantly impact potency. The thiophene ring in **5** could be replaced by phenyl (**23**) and cyclohexyl (**24**). In the case of phenyl analogues only a methylene linker was found to be equivalent in activity to the carbonyl group. A biphenyl system as in **31** led to a decrease in activity. Cyclohexyl analogues **26** and **53** without a carbonyl group linker were also significantly less active.

The effect of substituents in directly attached phenyl groups is detailed in Table 3.

ortho-Substitution as in **32** and **36** was found to improve potency. Introduction of a fluorine atom led to some loss of activity (**35**), a methoxy group (**37**) reduced activity even further. A limited exploration of heteroaromatic and fused systems as in **38–40** did not yield any compounds with greater activity.

Table 4 details the effect of combining preferred substituents from Domains 1 and 2. This approach led to the discovery of several compounds with significantly improved in vitro activity over the initial hit **5**. Incorpo-

Table 5. Glycine level increases in rat cerebrospinal fluid

Compound	Structure	K_i (nM)	CSF levels (ng/ml)	% Increase of glycine
1		7 ± 2.5	47 ± 1.35	204 ± 5.7
5		45 ± 10	15 ± 3.76	114 ± 12.8
30		70 ± 8	113 ± 5.91	196 ± 21.2
32		53 ± 16	25 ± 1.08	155 ± 4.2

Compounds were dosed subcutaneously at 30 mg/kg (values are derived from at least five separate measurements).

ration of alkoxy and alkyl substituents into five or six-membered aliphatic rings as in **45–47** and **49–51** was found to be particularly beneficial for in vitro activity.

In order to assess the ability of our compounds to selectively increase cortical glycine levels in an animal model, we measured their effect on glycine levels in rat cerebrospinal fluid (CSF) after subcutaneous administration (Table 5) at a dose of 30 mg/kg.[22] Animals were euthanized and 50–100 ml of CSF was sampled immediately after death from the cisterna magna.[23–25] The concentration of glycine and test compounds in the CSF was determined using an LC/MS method in positive electrospray mode (Table 5).[26]

Our initial hit molecule **5** showed no significant effect on CSF glycine levels. 2-Chloro-phenyl substituted analogue **32** had a weak effect on CSF glycine levels, but 2-thiophene substituted **30** proved to have a similar effect on glycine levels as ALX-5407 **1**. Corresponding to the increase in glycine levels compound levels of **30** measured in CSF were also significantly higher than those measured for **5**, **32**, and **1**.

Major SAR trends in a new class of GlyT1 inhibitors have been established. Optimization of in vitro potency led to the discovery of highly potent and selective GlyT1 inhibitors. The in vivo activity of selected members of this series was demonstrated by measurements of glycine levels in CSF. Further optimization of our lead molecules and activity after oral dosing will be disclosed in due course.

Acknowledgment

The authors thank Véronique Dehlinger for helpful discussions and suggestions.

References and notes

1. Gether, U.; Andersen, P. H.; Larsson, O. M.; Schousboe, A. *Trends Pharmacol. Sci.* **2006**, *27*, 375.
2. Olney, J. W.; Newcomer, J. W.; Farber, N. B. *J. Psychiatry Res.* **1999**, *33*, 523.
3. Vandenberg, R. J.; Aubrey, K. R. *Expert Opin. Ther. Targets* **2001**, *5*, 507.
4. Betz, H.; Gomeza, J.; Armsen, W.; Scholze, P.; Eulenburg, V. *Biochem. Soc. Trans.* **2006**, *34*, 55.
5. Kinney, G. G.; Sur, C. *Curr. Neuropharmacol.* **2005**, *3*, 35.
6. Lindsley, C. W.; Wolkenberg, S. E.; Kinney, G. G. *Curr. Top. Med. Chem.* **2006**, *6*, 1883.
7. Lowe, J. A., III. *Expert Opin. Ther. Targets* **2005**, *15*, 1657.
8. Slassi, A.; Egle, I. *Expert Opin. Ther. Targets* **2004**, *14*, 201.
9. 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.; Klitenick, M. A. *Mol. Pharmacol.* **2001**, *60*, 1414.
10. Smith, G.; Ruhland, T.; Mikkelsen, G.; Andersen, K.; Christoffersen, C. T.; Alifrangis, L. H.; Mork, A.; Wren, S. P.; Harris, N.; Wyman, B. M.; Brandt, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4027.
11. Alberati, D.; Hainzl, D.; Jolidon, S.; Krafft, E. A.; Kurt, A.; Maier, A.; Pinard, E.; Thomas, A. W.; Zimmerli, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4311.
12. Alberati, D.; Ceccarelli, S. M.; Jolidon, S.; Krafft, E. A.; Kurt, A.; Maier, A.; Pinard, E.; Stalder, H.; Studer, D.; Thomas, A. W.; Zimmerli, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4305.
13. Ceccarelli, S. M.; Pinard, E.; Stalder, H.; Alberati, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 354.
14. Pinard, E., Alberati, D., Borroni, E., Ceccarelli, S., Fischer, H., Hainzl, D., Jolidon, S., Moreau, J. L., Stalder, H., Thomas, A. W. *Abstracts of Papers*, 231st ACS National Meeting, Atlanta, GA, United States, March 26–30, 2006; MEDI-407.
15. Dargazanli, G. et al. WO2838739.
16. Man, T. et al. WO2005100301.
17. Tsunoda, T.; Yamamiya, Y.; Ito, S. *Tetrahedron Lett.* **1993**, *34*, 1639.
18. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
19. The reactions were performed using Radley's Greenhouse Parallel Synthesizer.
20. Huang, Y.; Mahmood, K.; Mathis, C. A. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 949.
21. Compound IC_{50} values were determined using a whole cell transport assay formatted as a solid-scintillant-based assay using the Amersham 96-well Cytostar-T plate technology. Sixteen to 20 h prior to initiation of the assay, human medulloblastoma cells, BE(2)-C, stably expressing a human GlyT1a cDNA were trypsinized, counted, and plated at a cell density of 60,000 cells/well in culture medium (1:1 mixture of Eagle's minimum essential medium with non-essential amino acids and Ham's F12 medium, 90% and fetal bovine serum, 10%). After washing the cell layer with assay buffer (25 mM Hepes, 125 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5.6 mM, glucose, and 4 mM alanine), assay buffer and test compounds at various concentrations (0.1 nM–30 μM) were added to the cell layer. The uptake assay was initiated by addition of ^{14}C-glycine or ^{14}C-sarcosine (7.5 μM final) and allowed to proceed at 25 °C for 4–5 h prior to counting on a Wallac MicroBeta liquid scintillation spectrometer. Non-specific uptake was defined using 10 mM sarcosine (for ^{14}C-glycine) or 10 mM glycine (for ^{14}C-sarcosine). Each IC_{50} was obtained from at least two-independent experiments. The assay had a MSR of 2.5.
22. Compounds were dissolved in a 14% (2-hydroxypropyl)-β-cyclodextrin vehicle solution and administered to male Sprague–Dawley weighing 250–300 g in groups of 6–9 rats with an injection volume of 0.1 ml/100 g. CSF was sampled 1–24 h after compound administration. Animals were euthanized by CO_2 asphyxiation and, very shortly after death, a 27-gauge hypodermic needle was carefully inserted approximately 1 mm into the rat cisterna magna in order to withdraw 50–100 μl of CSF in a 1 ml syringe. Samples with any sign of blood contamination were discarded. CSF samples were immediately placed on ice. Analysis of CSF levels of test compounds was accomplished using an LC/MS in positive electrospray mode and ChemStation data analysis software (1100 Series, Agilent Technologies, Palo Alto, CA, USA). Three microliter injections of CSF samples were made onto a 5 micron Zorbax C-18 column (4.6 mm × 150 mm, Agilent Technologies) that was maintained at 30 °C. The mobile phase used was a mixture of acetonitrile:water: 0.1% formic acid at a flow rate of 0.5 ml/min. The mixture of acetonitrile:water was varied in order to have the analyte retention time stay within a

range of 3–4 min. Compounds eluting from the column were identified by their characteristic retention time and mass to charge (*m*/*z*) ratio, and quantified by comparison to a standard curve prepared in artificial CSF.

23. Frankmann, S. P. *Physiol. Behav.* **1986**, *37*, 489.
24. Huang, Y.-L.; Säljö, A.; Suneson, A.; Hansson, H.-A. *J. Neurosci. Methods* **1995**, *63*, 13.
25. Johnson, K. W.; Clemens-Smith, A.; Nomikos, G.; Davis, R.; Phebus, L.; Shannon, H.; Love, P.; Perry, K.; Katner, J.; Bymaster, F.; Yu, H.; Hoffman, B. J. *Ann. N. Y. Acad. Sci.* **2003**, *1003*, 412.