Journal of
Medicinal
Chemistry

Volume 44, Number 17 August 16, 2001

Letters

The First Potent and Selective Inhibitors of the Glycine Transporter Type 2

Wilson L. Caulfield, Iain T. Collie, Rachel S. Dickins, Ola Epemolu, Ross McGuire, David R. Hill, Gillian McVey, J. Richard Morphy, Zoran Rankovic,* and Hardy Sundaram

Lead Discovery Unit, Organon Laboratories Ltd., Newhouse ML1 5SH, Scotland, U.K.

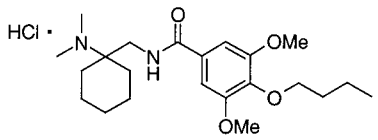
Received November 30, 2000

Introduction. Glycine is one of the major inhibitory neurotransmitters in the spinal cord and brain stem of vertebrates.[1] The inhibitory actions of glycine are mediated by the strychnine-sensitive glycine receptor (ssGlyR), a ligand-gated chloride channel distributed throughout the spinal cord and brain stem.[2] Glycine is also known to potentate the action of glutamate acting as an essential co-agonist on postsynaptic *N*-methyl-D-aspartate (NMDA) receptors.[3] Synaptic levels of glycine are believed to be controlled by high-affinity glycine transporters. These transporters are members of a large family of sodium/chloride-dependent transporters, which are composed of single oligomeric proteins containing 12 hydrophobic membrane-spanning domains.[4] Molecular cloning has revealed the existence of two major classes of glycine transporter subtypes: type 1 (GlyT-1) and type 2 (GlyT-2).[5] These have now been further divided into three subtypes of GlyT-1 (a, b, and c) and two splice variant versions of GlyT-2 (a and b).[5] Recent immunocytochemical studies showed that the GlyT-1 transporter has a wide distribution throughout the CNS whereas the GlyT-2 transporter has a similar distribution to ssGlyR, being confined to the spinal cord and brain stem.[6]

There is evidence that glycine-mediated inhibition produces muscle relaxation[7] and blockade of this inhibition produces convulsions.[8] Therefore, we postulated that modulators of endogenous levels of glycine might provide skeletal muscle relaxation. A significant amount of data has accumulated over recent years, indicating that glycine also has an important role in the modulation of nociceptive pathways.[9] Thus, it was anticipated that an increase in synaptic levels of endogenous glycine by a selective inhibition of the GlyT-2 transporter in the spinal cord may offer a unique approach for developing a novel muscle relaxant, anesthetic, and/or analgesic reagent, suitable for use during surgical anesthesia. Due to the discrete localization of both ssGlyR and the GlyT-2 transporter within the spinal cord and brain stem, a glycine modulator might not be expected to lead to serious CNS side effects that are characteristic for currently used μ-opioid analgesics.

Since testing of this hypothesis has been hampered by the lack of a suitable GlyT-2 inhibitor, we sought a potent and selective inhibitor of the transporter that would enable us to conduct proof-of-principle studies. It is interesting to note that a tricyclic antidepressant, amoxapine, has been recently shown to inhibit the GlyT-2a transporter with a 10-fold selectivity over the GlyT-1b transporter.[10] It was suggested that the sedative and psychomotor side effects associated with amoxapine might be related to its inhibitory activity at the GlyT-2 transporter. Unfortunately, a relatively low GlyT-2 transporter activity (IC_{50} 92 μM) and a high norepinephrine transporter activity preclude use of this drug in validation of our hypothesis.

Screening of Organon's compound collection, based on measuring the ability of test compounds to inhibit [^{3}H]-glycine uptake into CHO cells stably expressing the human GlyT-2 protein (hGlyT-2 assay),[11] led to identification of 4-butyloxy-3,5-dimethoxy-*N*-[(1-dimethylaminocyclohexyl)methyl]benzamide (**1**), a compound with an IC_{50} value of 214 nM at the hGlyT-2 and high selectivity over the hGlyT-1 transporter[12] (Table 1). The

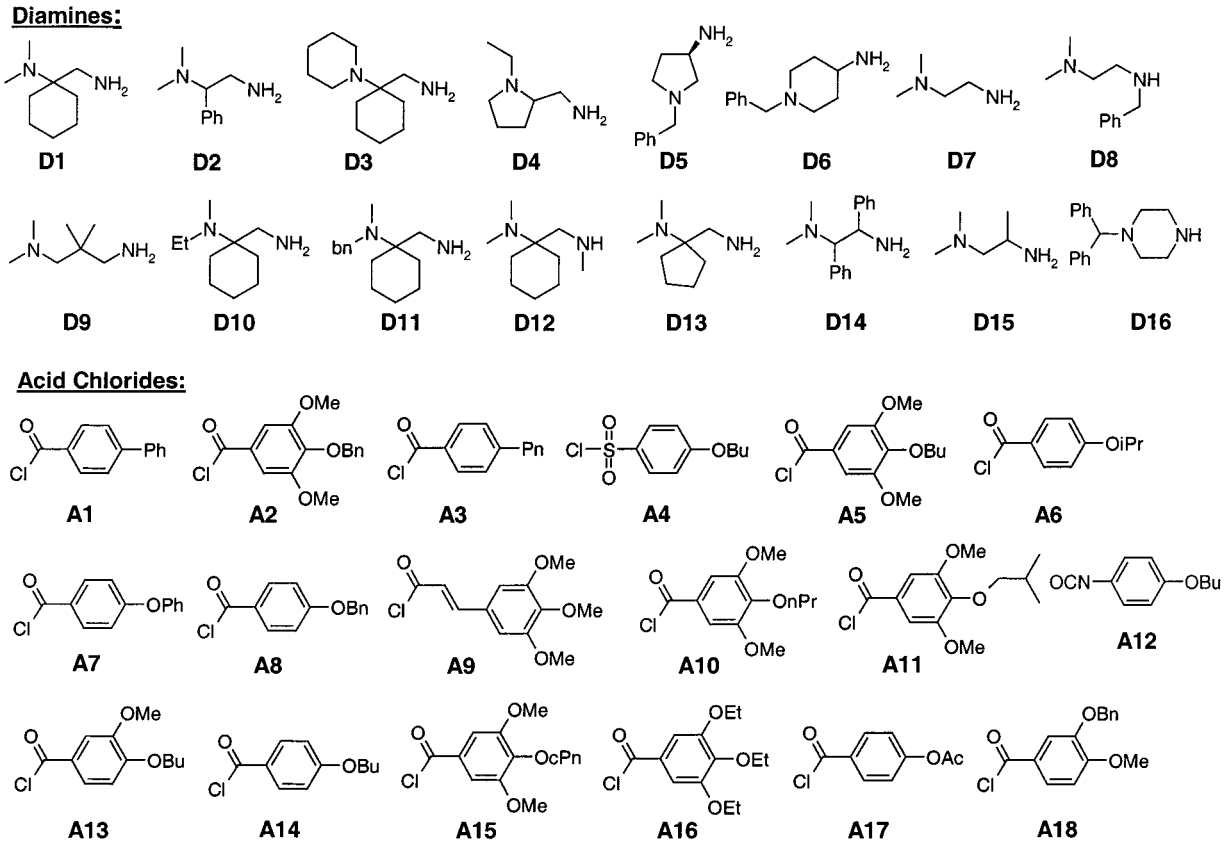


1

* To whom correspondence should be addressed. Tel: (+44) 1698 736157. Fax: (+44) 1698 736187. E-mail: z.rankovic@organon.nhe.akzonobel.nl.

10.1021/jm0011272 CCC: $20.00
Published on Web 07/25/2001

Chart 1. Building Blocks Used in Synthesis of the Library[a]

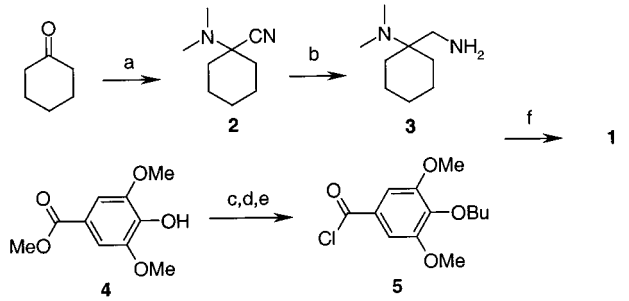


[a] Pn = pentyl; cPn = cyclopentyl.

IC_{50} value for glycine itself in this assay is approximately 100 μM.[11]

The compound's physicochemical parameters appeared to be consistent with CNS penetration:[13] cLogP 3.93 (free base); MW 429.0; polar surface area[14] 41.4; aqueous solubility 10 mg/mL. Thus, compound **1** was selected as a starting point for a "hit to lead" optimization program. In this communication we describe our efforts to further improve activity while maintaining the favorable selectivity profile and physicochemical properties of this hit.

Scheme 1[a]



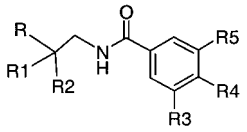
[a] (a) $Me_2H_2N^+Cl^-$, KCN(aq), 18 h; (b) H_2SO_4, $LiAlH_4$, THF, 0−20 °C, 18 h; (c) BuBr, K_2CO_3, acetophenone, 135 °C, 18 h; (d) KOH, MeOH, 65 °C; (e) $SOCl_2$, toluene, 110 °C; (f) Et_3N, THF.

Chemistry. Compound **1** was prepared following the procedure outlined in Scheme 1.[15] A standard Strecker reaction, involving cyclohexanone and dimethylamine hydrochloride, produced cyanoamine **2**. This was followed by a reduction of **2** with AlH_3, to obtain the corresponding diamine **3** in 57% overall yield. AlH_3 was generated in situ by treating a suspension of $LiAlH_4$ in THF with H_2SO_4 at 0 °C.[15] Attempts to reduce cyanoamine **2** with either $LiAlH_4$ or BH_3·THF produced complex mixtures containing only traces of the desired product, mainly due to a retro-Strecker reaction induced by these reagents. Acylation of diamine **3** with acid chloride **5**, obtained in three simple steps from a commercially available ester **4**, afforded **1** in 82% yield (Scheme 1).

To perform a rapid SAR exploration and optimization program around **1**, 16 diamines and 18 acid chlorides were coupled in a matrix fashion to produce a 2D library containing 288 amide analogues of compound **1** (Chart 1). In total 15 out of 34 building blocks were custom-made following the synthetic routes depicted in the Scheme 1. The presence of an internal tertiary amine group conveniently eliminated the need for an external base and, therefore, facilitated the reaction workup. The synthesis of the library was performed in four 96-well plates. After leaving the plates to stand overnight at room temperature, the solvent was removed under reduced pressure (GeneVac) to afford the desired products in quantities of 10−15 mg and of a purity sufficient for testing in the hGlyT-2 assay (>80% by LC−MS). The parent compound was resynthesized as an internal standard within the library and was found to exhibit similar activity (IC_{50} 218 nM) as compared to 214 nM for **1**.

Results and Discussion. Initially, several closely related analogues of **1** were selected from both internal and external sources for testing in the hGlyT-2 assay. This preliminary data indicated that, compared to the

Table 1. In Vitro Activity of the Key Analogues



compd	R	R1	R2	R3	R4	R5	hGlyT-2[11] IC_{50} ± SEM (nM)[a]	hGlyT-1[12] IC_{50} (μM)[b]
1	Me_2N	$-CH_2-(CH_2)_3-CH_2-$		OMe	OBu	OMe	214 ± 35.2	>100
6	Me_2N	$-CH_2-(CH_2)_3-CH_2-$		OMe	OMe	OMe	>10000	ND
7	Me_2N	Ph	H	OMe	OBu	OMe	566 ± 178.5	ND
8	MeEtN	$-CH_2-(CH_2)_3-CH_2-$		OMe	OBu	OMe	1321 ± 507.0	ND
9	piperidine	$-CH_2-(CH_2)_3-CH_2-$		OMe	OBu	OMe	1563 ± 637.5	ND
10	Me_2N	$-CH_2-(CH_2)_3-CH_2-$		OMe	OBu	H	2563 ± 170.5	ND
11	Me_2N	$-CH_2-(CH_2)_3-CH_2-$		H	OBu	H	2816 ± 297.2	ND
12	Me_2N	$-CH_2-(CH_2)_3-CH_2-$		OMe	OPr	OMe	2313 ± 397.5	ND
13	Me_2N	$-CH_2-(CH_2)_3-CH_2-$		OMe	OBn	OMe	84 ± 3.0	>100
14	Me_2N	Me	Me	OMe	OBu	OMe	696 ± 205.1	ND
15	Me_2N	$-CH_2-(CH_2)_2-CH_2-$		OMe	OBu	OMe	77 ± 19.0	>100
16	Me_2N	$-CH_2-(CH_2)_2-CH_2-$		OMe	OBn	OMe	16 ± 1.9	>100

[a] Average of 3 determinations. [b] 1 measurement.

compound's aromatic region, the SAR around the cyclohexyl group of **1** appears to be more flexible. For example, replacement of the BuO by a MeO group in **6** abolished activity (IC_{50} >10 μM), whereas replacement of the cyclohexyl by a phenyl group in **7** reduced activity by 3-fold (IC_{50} 566 nM), as shown in Table 1. This preliminary SAR information was incorporated into the design of the 2D library aimed not only to produce more detailed SAR around **1** but also for purpose of hit optimization and hit explosion. The library included a range of "deletion" analogues (e.g. **D7** and **A13**) providing information on the minimum structural requirements for activity. Furthermore, since the importance of the BuO group has already been shown, a number of analogues with various replacements at this position, (e.g. **A2** and **A11**) are also included (hit optimization). Analogues designed to explore the bulk tolerance around the basic nitrogen as well as more diverse derivatives, containing the diamine moiety with varying degrees of steric constraint and various substitutions at the acid part of the molecule (hit explosion), were also included within the library (Chart 1).

The percent inhibition for each of the 288 amides in the hGlyT-2 assay was determined at 1 μM.[16] A total of 39 compounds (13% of the collection) showed >60% inhibition. These active compounds were derived predominately from 5 diamines (**D1**−**D3**, **D10**, and **D13**) and 10 acid chlorides (**A2**, **A3**, **A5**, **A7**, **A8**, **A10**, **A11**, and **A13**−**A15**). All "active" diamines contain at least one substituent at the carbon adjacent (α) to the tertiary amine functionality. Lack of α-substitution appeared to be detrimental for activity in the GlyT-2 assay (e.g. **D7**). Similarly, presence of β-substitution (e.g. **D14**) or a secondary amino group (e.g. **D12**) abolished GlyT2 activity. Products derived from acid chlorides with a *para*-alkyl or alkoxy group shorter than PrO (e.g. **A16** and **A18**) showed no activity in the assay, indicating the importance of hydrophobic interactions between this region of the ligand and the transporter binding site.

IC_{50} values for the key analogues after purification (prep-LC−MS) are presented in Table 1. The obtained data indicated a very tight SAR around the Me_2N group. Even a slight increase in the size of substituents at the basic nitrogen in **8** and **9** resulted in a significant decrease of activity: IC_{50} 1321 and 1563 nM, respectively. The substitution pattern on the aromatic ring also appears to be important for activity. Deletion of one of the two MeO groups at the aromatic ring in **10** resulted in a significant reduction of the binding affinity (2563 nM), whereas only slight further reduction of activity followed removal of the remaining MeO group in compound **11** (2816 nM). This is probably due to both MeO groups being required to suppress free rotation of Ar−O−Bu bonds and therefore "freeze" the BuO group in a bioactive conformation. The BuO group itself is important for affinity perhaps due to hydrophobic interactions with the transporter binding site. Replacement of the BuO group by a PrO in **12** resulted in a significant reduction of activity (2313 nM). Interestingly, analogue **13** with the BuO replaced by a BnO group showed 3-fold increase of activity (83 nM) which could be attributed to the greater contact surface between the BnO group and the hydrophobic pocket within the transporter binding site. As the preliminary SAR indicated, the region around the cyclohexyl group appears to be more flexible to changes. Replacement of the cyclohexyl either by a phenyl (**7**) or a *gem*-dimethyl group (**14**) led to a ~3-fold drop of activity (566 and 696 nM, respectively), whereas a cyclopentyl analogue **15** showed a 3-fold increase of activity (77 nM). Effects of described structural changes on the GlyT-2 activity appeared to be additive, so that analogue **16**, containing both cyclopentyl and BnO groups, was identified as the most active compound in the library (IC_{50} 16 nM).[17] This compound appeared to be highly selective not only over the GlyT-1, where it showed no activity at concentrations of up to 100 μM, but also over 56 other common and relevant biological targets.[18] As indicated by its favorable physicochemical parameters (cLogP 3.97 (free base); MW 429.0; polar surface area[14] 52.7; aqueous solubility 10 mg/mL) compound **16** exhibits good blood−brain barrier penetration (logBB 0.6).[19] Since **16** also shows good metabolic stability in plasma and mouse hepatic microsomes (80% remaining after 30 min), the compound should prove to be a valuable tool that might help to define pharmacology of the GlyT-2 transporter.

Conclusion. In summary, high-throughput screening of Organon's compound collection provided compound **1**, an attractive drug-like GlyT-2 inhibitor suitable for high-throughput synthesis. A detailed study of the SAR

and rapid hit optimization were achieved through synthesis of a solution-phase 2D library. This led to identification of 4-benzyloxy-3,5-dimethoxy-*N*-[(1-dimethylaminocyclopentyl)methyl]benzamide (**16**), the first potent and selective GlyT-2 inhibitor. Further in vitro and in vivo evaluations of **16** and related analogues will be reported in due course.

Supporting Information Available: Detailed experimental procedures for the synthesis of compound **1** and the amide library, analytical data for all compounds presented in Table 1, and percent inhibition in the hGlyT-2 assay for each of the 288 compounds of the amide library. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Werman, R.; Davidoff, R. A.; Aprison, M. H. Evidence for glycine as the principle transmitter mediating postsynaptic inhybition in the spinal cord of the cat. *J. Gen. Physiol.* **1967**, *50*, 1093−1094.

(2) Langosch, D. M.; Becker, C.; Betz, H. The inhibitory glycine receptor: a ligand gated chloride channel for the central nervous system. *Eur. J. Biochem.* **1990**, *194*, 1−8.

(3) Johnson, J. W.; Ascher, P. Glycine potentiates the NMDA response in cultured mouse brain neurones. *Nature* **1987**, *325*, 529−531.

(4) Masson, J.; Sagne, C.; Hamon, M.; El Mestikawy, S. Neurotransmitter transporters in the central nervous system. *Pharmacol. Rev.* **1999**, *51*, 439−464.

(5) (a) Guastella, J.; Brecha, N.; Weigman, C.; Lester, H.; Davidson N. Cloning, expression, and localization of a rat brain high affinity glycine transporter. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7189−7193. (b) Liu, Q.-R.; Nelson, H.; Mandiyan, S.; Lopez-Corcuera, B.; Nelson, N. Cloning and expression of a glycine transporter from mouse brain. *FEBS Lett.* **1992**, *305*, 110−114. (c) Smith, K. E.; Borden, L. A.; Hartig, P. R.; Branchek, T.; Weinshank, R. L. Cloning and expression of a glycine transporter reveal colocalisation with NMDA receptors. *Neuron* **1992**, *8*, 927−937. (d) Ponce, J.; Poyatos, I.; Aragon, C.; Gimenez, C.; Zafra, F. Characterisation of the 5′ region of the rat brain glycine transporter GLYT2 gene: Identification of a novel isoform. *Neurosci. Lett.* **1998** *242*, 25−28.

(6) (a) Zafra, F.; Aragon, C.; Gimenez, C. Molecular Biology of Glycinergic Neurotransmission. *Mol. Neurobiol.* **1997**, *14*, 117−142. (b) Jursky, F.; Nelson, N. Localisation of GlyT-2 reveals correlation with the distribution of glycine receptor. *J. Neurochem.* **1995**, *64*, 1026−1033. (c) Zafra, F.; Aragon, C.; Olivares, L.; Danbolt, N. C.; Gimenez, C.; Storm-Mathisen, J. Glycine transporters are differentially expressed among CNS cells. *J. Neurosci.* **1995**, *15*, 3952−3969.

(7) (a) Simpson, R. K.; Robertson, C. S.; Goodman, J. C. The role of glycine in pain and spasticity. In *Spinal Cord Monitoring*, 2nd ed.; Stalberg, E., Sharma, H. S., Ollson, Y., Eds.; Springer: New York, 1998; pp 75−106. (b) Truong, D. D.; DeYebenes, J. G.; Pezzoli, G.; Jackson-Lewis, V.; Fahn, S. Glycine involvement in DDT induced myoclonus. *Movement Dis.* **1988**, *3*, 77−87.

(8) Becker, C. M. Convulsants acting at the Inhibitory Glycine Receptor. *Handbk. Exp. Pharmacol.* **1992**, *102*, 539−575.

(9) (a) Simpson, R. K. J.; Gondo, M.; Robertson, C. S.; Goodman, J. C. Reduction in the mechanociceptive response by intrathecal administration of glycine and related compounds. *Neurochem. Res.* **1996**, *21*, 1221−1226. (b) Sorkin, L. S.; Westlund, K. N.; Sluka, K. A.; Dougherty, P. M.; Willis, W. D. Neural changes in acute arthritis in monkeys. IV. Time-course of amino acid release into the lumbar dorsal horn. *Brain Res. Rev.* **1992**, *17*, 39−50.

(10) Nunez, E.; Lopez-Corcuera, B.; Vazquez, J.; Gimenez, C.; Aragon, C. Differential effects of the tricyclic antidepressant amoxapine on glycine uptake mediated by the recombinant GlyT-1 and GlyT2 glycine transporters. *Br. J. Pharmacol.* **2000**, *129*, 200−206.

(11) Morrow, J. A.; Collie, I. T.; Dunbar, D. R.; Walker, G. B.; Shahid, M.; Hill D. R. Molecular Cloning and Functional Expression of the Human Glycine Transporter GlyT-2 and Chromosomal Localisation of the gene in the Human Genome. *FEBS Lett.* **1998**, *439*, 334−340.

(12) Glycine uptake assays were performed as described by Morrow et al.[11] using CHO cells stable transfected with hGlyT-1b. A full protocol is described in the Supporting Information.

(13) Lipinski, C. A. Experimental and computational approaches to estimated solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.

(14) Camenisch, G.; van de Waterbeemd, H. Estimation of Caco-2 cell permeability using calculated molecular descriptors. *Quant. Struct.-Act. Relat.* **1996**, *15*, 480−490.

(15) Yang, D.; Bremont, B.; Shen, S.; Kefi, S.; Langlois, M. Serotoninergic properties of new conformationally restricted benzamides. *Eur. J. Med. Chem.* **1996**, *31*, 231−239.

(16) The percent inhibition for each of the 288 amides in the hGlyT2 assay is presented in Table 3 of the Supporting Information.

(17) Compounds with IC_{50} <100 nM were also tested for their selectivity in the GlyT-1 assay and were found to be inactive at 10 μM concentration.

(18) Compound **16** was screened across 56 selected NovaScreen targets at three concentrations: 0.1, 1, 10 μM. Limited affinity was observed only in serotonin 5-HT_{2A} binding assay (25% inhibition at 100 nM). For more details, see Table 4 of the Supporting Information.

(19) logBB = log(concentration in brain/concentration in blood).

JM0011272