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Acylglycinamides as inhibitors of glycine transporter type 1

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ABSTRACT

A screening hit was used as the basis for the core structure of a new series of acylglycinamide GlyT-1 inhibitors. Investigation of the SAR around four areas of diversity used facile chemistry to prepare compounds quickly. By focussing on reducing the lipophilicity and improving the aqueous solubility in the series we were able to prepare a compound (**17e**) with a good level of activity at GlyT-1, selectivity over GlyT-2 and moderate oral bioavailability.

Molecular cloning has revealed the existence in mammalian brains of two classes of glycine transporters, termed GlyT-1 and GlyT-2. GlyT-1 is widely distributed throughput the CNS and is expressed on both neurones and glia.[1] Some expression of GlyT-1 is closely associated with *N*-methyl D-aspartate (NMDA) receptors,[2] which possess strychnine-insensitive glycine binding sites. GlyT-2, in contrast, is found predominantly in the brain stem and spinal cord, and its distribution corresponds closely to that of strychnine-sensitive inhibitory glycine receptors.[1] Another distinguishing feature of glycine transport mediated by GlyT-2 is that it is not inhibited by sarcosine, as is the case for glycine transport mediated by GlyT-1. It is thought that GlyT-1 has an overall role

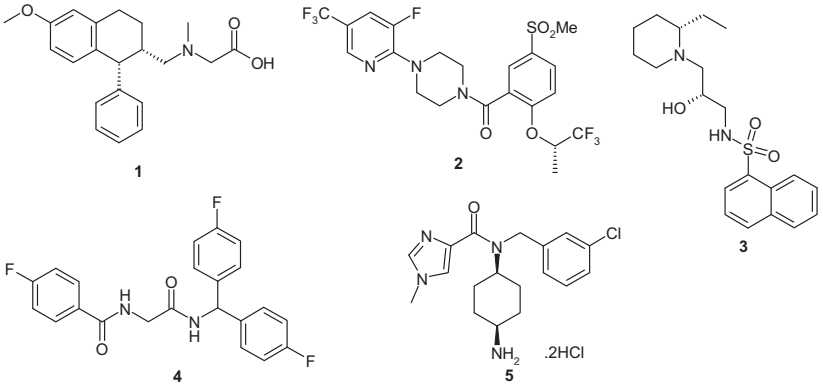

Figure 1. GlyT1 inhibitors.

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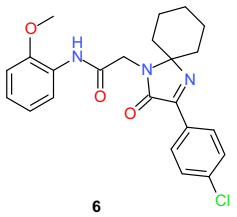


Figure 2. Example imidazolone.

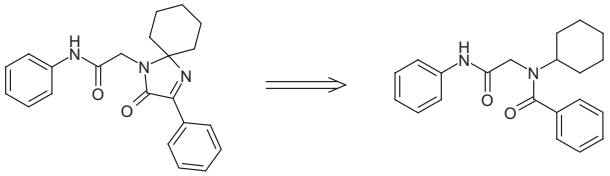


Figure 3. 'Ring-opened imidazolones'.

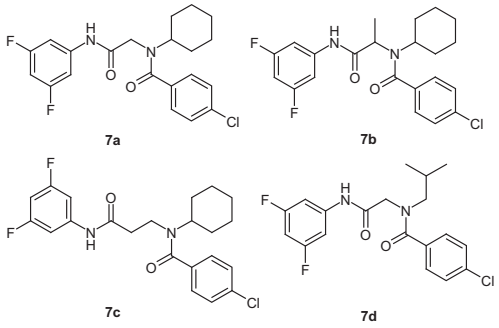


Figure 4. Acyglycinamide core variations.

Table 1
SAR for acylglycinamide variations

Compound	GlyT-1 pIC_{50}[12]	GlyT-2 pIC_{50}[14]
7a	6.2	5.0
7b	5.4	<4.6
7c	5.0	<4.6
7d	5.5	<4.6

in regulating glycine levels at both NMDA receptors and inhibitory glycine receptors,[3,4] whereas GlyT-2 has a more specific role in influencing glycine in glycinergic neurones.[1]

NMDA receptors are critically involved in memory and learning and, furthermore, decreased function of NMDA-mediated neurotransmission appears to underlie, or contribute to, the symptoms of schizophrenia.[5] Thus, agents that inhibit GlyT-1 and thereby increase glycine activation of NMDA receptors may be useful as novel anti-psychotics and anti-dementia agents.[6]

Table 2
SAR for amide replacements

Compound	GlyT-1 pIC_{50}[12]	GlyT-2 pIC_{50}[14]
8a	4.8	<4.6
8b	<4.6	<4.6
8c	5.6	4.9

The sarcosine derivative Org25935[7] (compound **1**) is in Phase 2 clinical trials, and R1678[8] (**2**) recently reported a positive outcome in a Phase 2 study on negative symptoms of schizophrenia. We have previously reported a non-sarcosine series of diaminopropanols (e.g., **3**[9]), and more recently compounds such as **4**[10] and **5**[11] have been reported (Fig. 1).

Screening of the GSK compound collection[12] gave a number of hits containing an imidazolone moiety, for example, compound **6** (Fig. 2).

As part of the lead optimisation process we identified a set of ring-opened analogues—the acylglycinamides—as a possible alternative series (Fig. 3). This series has structural similarities to known bis-amides (e.g., **4**) but is differentiated by having a fully-substituted amide nitrogen. The facile chemistry required to construct the molecules allowed a large number of compounds to be prepared quickly and thus extensive structure–activity relationship (SAR) data could be obtained rapidly. In addition, it was anticipated that SAR data from this series might be used to inform further work in the imidazolone series, where more difficult chemistry slowed the preparation of large numbers of compounds.

The first investigations in this series concerned the acylglycinamide core. We treated a set of analogous amines with 4-chlorobenzoyl chloride[13] to give compounds **7a–d** (Fig. 4) and the results are summarised in Table 1. The simple cyclohexyl-glycinamide (compound **7a**) appeared to be the most potent core with a pIC_{50} of 6.2.

Next we investigated the effect of replacing one of the amides in the acylglycinamide, by treating the amine precursor of **7a** with 4-chlorobenzenesulfonyl chloride, 4-chlorobenzyl chloride and 4-chlorophenyl isocyanate to give sulfonamide **8a**, tertiary amine **8b** and urea **8c**, respectively (Fig. 5). All showed reduced activity compared to the diamide **6b** (Table 2).

Having established the optimum core structure we investigated the effect of replacing the 4-chlorophenyl moiety in compound **7a**. The synthetic route is shown in Scheme 1. By putting the diversity step at the end we were able to prepare a large number of compounds in parallel. R-groups were chosen, in part, for their potential to increase the polarity of the final compounds, as it was anticipated that this would improve the aqueous solubility.

Forty compounds were prepared in the first iteration and selected results are summarised in Table 3. From these results several conclusions were drawn: selectivity for GlyT-1 over GlyT-2 is highly substituent-dependent (compounds **7a** and **12a**); aromatic rings are preferred over aliphatic (compounds **12a, 12b**, **12c** and **12d**); small polar aromatic rings, though increasing solubility, greatly reduce the activity (compounds **12e** and **12f**); *para* substitution on phenyl rings is preferred over *meta* and *ortho*

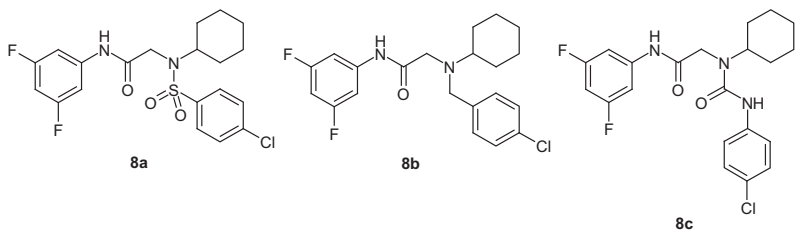
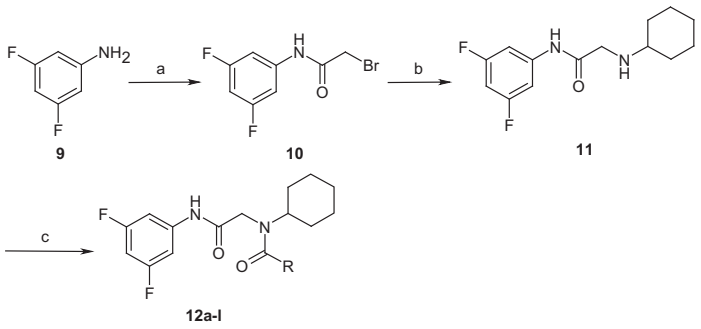
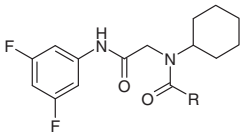


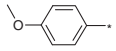
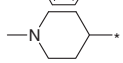
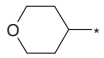
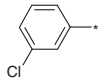
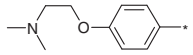
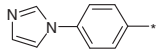
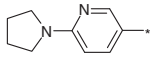
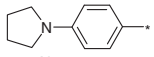
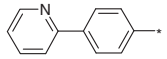
Figure 5. Amide replacements.

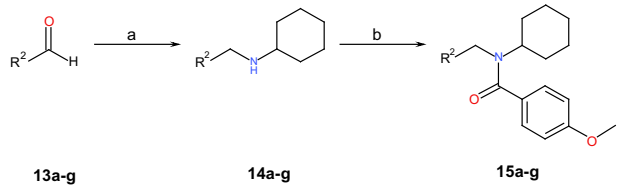


Scheme 1. Reagents and conditions: (a) Bromoacetyl bromide, $NaHCO_3$, DCM, 2 h, yield 88%; (b) cyclohexylamine, Et_3N, DCM, 4 h, yield 70%; (c) RCOCl, Et_3N, DMAP, THF, 100 °C, 10 min, microwave, yields 62–72%; or RCO_2H, HATU, Et_3N, MeCN, 100 °C, 10 min, microwave, yields 25–61%.

Table 3
SAR for substitution of the amide moiety

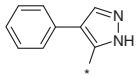
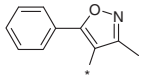
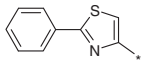
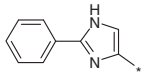
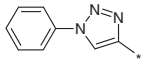
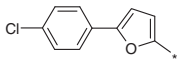
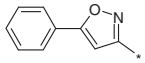


Compound	R	GlyT-1 pIC_{50}[12]	GlyT-2 pIC_{50}[14]	CHI log D[15]	Soln[16] (μg mL^{-1})
12a		6.3	<4.6	3.7	7
12b		<4.6	<4.6	1.8	140
12c		5.3	<4.6	3.0	127
12d		5.2	<4.7	N.D.	N.D.
12e		<4.6	<4.6	2.7	160
12f		<4.6	<4.6	3.0	163
12g		5.8	4.8	N.D.	N.D.
12h		4.7	4.7	N.D.	N.D.
12i		4.7	<4.6	2.4	199
12j		6.2	<4.6	2.7	32
12k		6.8	5.1	3.6	14
12l		7.1	5.3	4.5	5
12m		7.2	4.7	3.7	6



Scheme 2. Reagents and conditions: (a) Cyclohexylamine, sodium triacetoxyborohydride, DCM, 16 h, yields 70–100%; (b) 4-methoxybenzoyl chloride, Et_3N, DMAP, THF, 100 °C, 10 min, yields 52–75%.

Table 4
SAR of anilide replacements

Compound	R^2	GlyT-1 pIC_{50}[12]	GlyT-2 pIC_{50}[14]	CHI log D[15]	Soln[16] (μg mL^{-1})
16a		<4.6	<4.6	3.1	42
16b		4.8	<4.6	4.1	6
16c		5.1	4.8	4.6	2
16d		4.7	<5.2	2.9	20
16e		5.6	<4.6	3.5	28
16f		5.8	<4.6	5.0	<1
16g		6.4	<4.6	4.2	2

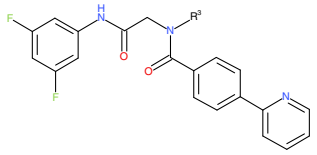
(compounds **7a, 12g** and **12h**); solubilising amine chain extensions are not tolerated (compound **12i**); 2-ring substituents (compounds **12j** and **12m**) offer the best compromise between Gly-T1 potency, selectivity and lipophilicity.[17]

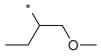
Compounds **12j** and **12m** were profiled further. They both showed high microsomal intrinsic clearance (CLi):[18] 1.2 mL min^{-1} g^{-1} (rat); 6.1 mL min^{-1} g^{-1} (human) and 15.4 mL min^{-1} g^{-1} (rat) and 5.2 mL min^{-1} g^{-1} (human), respectively. The solubilities in simulated gastric fluid (SGF; pH 1.2) were also measured, giving encouraging results of >1000 μg mL^{-1} and 507 μg mL^{-1}, respectively.

In an attempt to reduce flexibility of the molecules we also investigated replacing the anilide moiety with heterocycles. The process used for the syntheses is described in Scheme 2. Commercially-available heterocyclic aldehydes were subjected to a reductive amination step which was amenable to a parallel work-up procedure using SCX (solid-supported sulfonic acid) cartridges and the products required no further purification. Purification after step b by mass-directed HPLC gave the final compounds in >95% purity (by NMR). The results are shown in Table 4. 4-Methoxyphenyl was chosen as the amide substituent as it offered better GlyT-1 selectivity than 4-chlorophenyl (compounds **7a** and **12a**).

From this array the 3,5-disubstituted isoxazole **16g** was identified as having moderate potency at GlyT-1 but the lipophilicity was

Table 5
SAR of cyclohexyl replacements



Compound	R^3	GlyT-1 pIC_{50}[12]	CHI log D[15]	Soln[16] (μg mL^{-1})	CLi (rat/human) (mL min^{-1} g^{-1})
17a		7.0	3.3	12	4.9/1.3
17b		6.7	3.1	25	5.0/<0.5
17c		7.2	3.4	14	3.7/1.9
17d		5.9	3.0	42	1.8/<0.5
17e		7.1	2.7	56	2.0/2.9
17f		6.0	2.5	99	0.6/<0.5
17g		6.4	3.3	29	3.8/4.3
17h		6.9	3.5	7	4.8/2.0
17i		5.9	3.0	58	4.2/5.8
17j		6.0	N.D.	N.D.	3.0/1.5
17k		5.9	3.3	24	6.7/9.6
17l		5.9	2.5	102	3.2/3.9

still high and the compound had poor CLi (rat: 21.4 mL min^{-1} g^{-1} and human: 4.2 mL min^{-1} g^{-1}).

An alternative approach to improving the lipophilicity was to replace the cyclohexyl group with more polar moieties. Twelve compounds were prepared using the method of Scheme 1, with the appropriate amines replacing cyclohexylamine and 4-(2-pyridinyl)benzamide being retained as a consistent part of the molecules. The results are summarised in Table 5. All the compounds were inactive at GlyT-2.

Compounds **17e** (racemic mixture) and **17f** were judged to offer the best compromise between GlyT-1 potency, lipophilicity and in vitro clearance. Their pharmacokinetic properties were assessed in vivo (rat): both had estimated clearances of 70 mL min^{-1} kg^{-1} (i.e., 75% liver blood flow) and brain: blood ratios of 0.2. However, both compounds showed good oral absorption resulting in moderate oral bioavailabilities of 15% and 25%, respectively.

In summary, rational design of compounds based on a screening hit led to a new series of GlyT-1 inhibitors. Compounds **12j** and **12m** were identified as lipophilically efficient analogues with good GlyT-1 potency and selectivity. Further lead optimisation led to the identification of compound **17e** with improved lipophilic efficiency, in vitro developability and good oral absorption, but it suffered from high clearance in vivo. No further studies were performed to identify potential metabolites or reduce clearance in this series. However, four areas of diversity were explored, giving data which could be used to direct the work in the imidazolone series. This will be reported in a future communication.

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12. GlyT1 screening data was obtained using a [^{3}H]-glycine uptake scintillation proximity binding assay containing HEK293 cells expressing GlyT-1. Values are expressed as $pIC_{50} = -\log IC_{50}$, where IC_{50} is the half-maximal inhibitory concentration of the substance, and are the means of at least two experiments. Values given as 'less than' were below the sensitivity of the assay.
13. Work on the imidazolones had suggested that the 4-chlorophenyl and 3,5-difluoroanilide fragments were likely to give the compounds high potency at GlyT-1.
14. GlyT2 screening data was obtained using a [^{3}H]-glycine uptake scintillation proximity binding assay containing HEK293 cells expressing GlyT-2. Values given are means of at least two experiments. Values are expressed as $pIC_{50} = -\log IC_{50}$, where IC_{50} is the half-maximal inhibitory concentration of the substance. Values are expressed as $pIC_{50} = -\log IC_{50}$, where IC_{50} is the half-maximal inhibitory concentration of the substance, and are the means of at least two experiments. Values given as 'less than' were below the sensitivity of the assay.
15. A chromatographic method of measuring lipophilicity. See Valko, K.; Du, C. My.; Bevan, C.; Reynolds, D. P.; Abraham, M. H. *Curr. Med. Chem.* **2001**, *8*, 1137.
16. Aqueous solubility. Solubilities were determined from DMSO stock solutions using chemiluminescent nitrogen detection. See Bhattachar, S. N.; Wesley, J. A.; Seadeek, C. *J. Pharm. Biomed. Anal.* **2006**, *41*, 152.
17. A contributing factor to poor solubility and high clearance. See (a) Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881; and (b) Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817.
18. Values given are per gram of protein.