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Design, synthesis and structure–activity relationship of simple bis-amides as potent inhibitors of GlyT1

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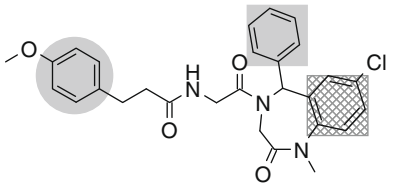
ABSTRACT

Several novel classes of potent and small amide-type inhibitors of glycine transport (GlyT1) were developed through sequential simplification of a benzodiazepinone-lead structure identified from a high-throughput screening. The most potent compounds of these structurally simple classes show low nanomolar inhibition at the GlyT1 target.

NMDA receptor hypofunction is suggested to be involved in the pathophysiology of schizophrenia.[1] The strongest evidence supporting this hypothesis is based on the observation that schizophrenic like symptoms can be induced in healthy subjects upon administration of NMDA blockers such as PCP.[2] Thus, therapeutic intervention aimed at increasing NMDA synaptic tone is expected to show beneficial effect in schizophrenic patients. As glycine is an obligatory co-agonist at the NMDA receptor complex,[3] one strategy to enhance NMDA receptor activity is to elevate extracellular levels of glycine in the local microenvironment of synaptic NMDA receptor. Glycine elevation can be achieved by inhibition of the glycine transporter 1 (GlyT1) which is co-expressed in the brain with the NMDA receptor and is responsible for glycine removal from the synaptic cleft.[4,5] Strong support for this approach in the treatment of schizophrenia comes from clinical studies where glycine[6] and D-serine[7] (co-agonists at the glycine site of NMDA receptor) and sarcosine[8] (a prototypical weak GlyT1 inhibitor) improved positive, negative and cognitive symptoms in schizophrenic patients, when added to conventional therapy. As a result, considerable efforts have been focused on the development of selective GlyT1 inhibitors.[9] The first examples reported were glycine or sarcosine derivatives.[10] More recently, a wide variety of non amino-acid GlyT1 inhibitors have been disclosed.[9b]We also recently reported on synthesis and optimization of non-sarcosine based spiropiperidine compounds.[11]

During our effort to discover and develop structurally different and selective GlyT1 inhibitors, a high throughput screening of the



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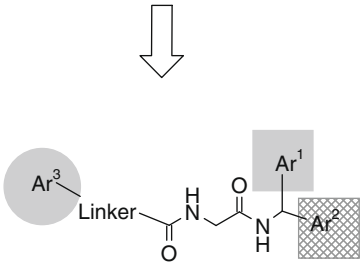

2

Scheme 1. Simplification of the high throughput screening hit **1** (IC_{50} human GlyT1 = 154 nM). Ring opening of the benzodiazepinone moiety led to the structurally simple and potent amides **2**.

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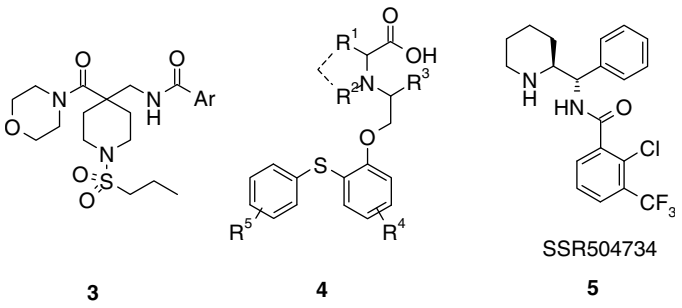
Roche compound collection led to several hits containing 5-phenyl-1,3,4,5-tetrahydro-benzo [e] [1,4]–diazepine-2-ones linked to N-acylated glycine side-chains. The structurally simplest derivative **1** already shows an IC_{50} of 154 nM for inhibition of GlyT1.

Compounds related to our high throughput screening hits have been described as tranquilizers, showing activities related to diazepam.[12] Therefore, this potential liability of the benzodiazepine moiety as well as the rather high molecular weight of 506 prompted us to search for structurally simpler derivatives of these hits. During this optimization process it became rapidly apparent that the benzodiazepinone moiety was not essential for GlyT1 activity. This part of the molecule could be replaced by a diarylmethylamine moiety which nicely mimicked both aromatic rings of the benzodiazepinone moiety. These modifications led to a series of highly active GlyT1 inhibitors of the general formula **2**[13] (Scheme 1).

We believe that the structure of these bis-amide derivatives **2** is not related to other known mono-amide inhibitors **3** which carry *N*-sulfonylpiperidine moieties[14] nor to the aminoacid class of GlyT1 inhibitors[10,15] (for example compounds of the type **4**) which all bear free carboxylic acid moieties. They might show some structural similarity to the benzyl amide SSR504734[16] **5** which, however, contains a basic piperidine moiety and lacks the second amide bond (Scheme 2).



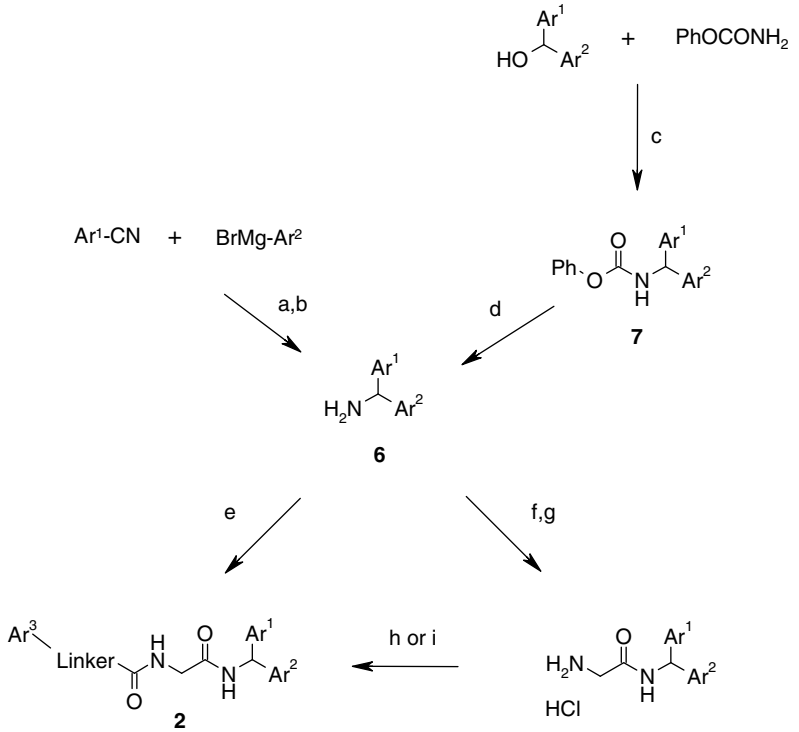
Scheme 2. Various classes of known GlyT1 inhibitors.

Synthesis of the compounds **2** described in this communication was performed by simple sequential amide coupling reactions (Scheme 3). Some of the required diarylmethylamines **6** are commercially available; others were prepared by reaction of aryl-Grignard reagents on aromatic nitriles followed by sodium borohydride reduction of the intermediate imine.[17] We also used the method of Laurent et al.,[18] involving acid catalyzed reaction of diarylmethanols with phenyl carbamate followed by cleavage of the intermediate **7** with lithium hydroxide.

We first investigated the influence of the *N*-acyl group of the glycine amide side chain and found that shorter linkers lead to better activities (**8**–**10**); simple *N*-benzoyl substituents were always superior to the 3-arylpropanoyl group present in the high-throughput hit (**1**). Electron withdrawing substituents on Ar_3(**11**–**13**) further improved the activity. The aromatic group Ar_3could also be replaced by small 5- or 6-membered heterocycles (**14**–**15**), whereas alkyl- or cycloalkyl derivatives were not tolerated (**16**–**17**). Most of the derivatives showed good to excellent selectivity against the Glyt2 isoform. Some characteristic examples are shown in Table 1.

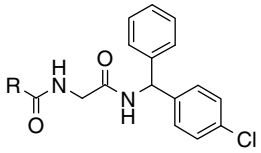
It is essential that both amide groups remain secondary; methylation of either one or the other NH led to inactive compounds.

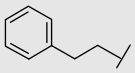
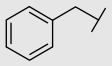
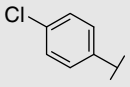
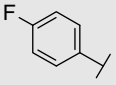
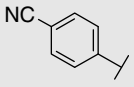
We then turned our attention to the structure–activity relationship around the diarylmethylamine moiety (see Table 2). It appeared that there was a large tolerance for substituents on both aromatic rings. 3-substituted derivatives were generally more potent than 4-substituted analogues and the latter were in turn much more potent than the 2-substituted compounds (**19**–**23**). Small electron withdrawing substituents like halides usually led to en-



Scheme 3. Synthesis of the GlyT1 inhibitors **2**. Reagents and conditions: (a) THF, reflux, 6 h; (b) MeOH, $NaBH_4$, rt, 1 h, 12–84% overall; (c) TFA, rt, 17 h, 41–85%; (d) LiOH, H_2O, CH_3CN, 50°, 3 h, 44–96%; (e) Ar^3-Linker-$CONHCH_2COOH$, TBTU, DIPEA, CH_3CN, rt, 1 h, 14–79%; (f) $tBuOCONHCH_2COOH$, TBTU, DIPEA, CH_3CN, rt, 1 h, 39–84%; (g) HCl gas in Et_2O, rt, 1 h, 84–100%; (h) Ar^3-Linker-COOH, TBTU, DIPEA, CH_3CN, rt, 1 h, 14–97%; (i) Ar^3-Linker-COCl, NEt_3, DCM, rt, 3 h, 12–97%.

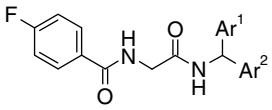
Table 1
In vitro inhibitory activity of **8**–**17** at GlyT1 and GlyT2[a]



Compound	R	GlyT1 EC_{50}[b] (μM)	GlyT2 EC_{50}[b] (μM)
8		4.79	29.2
9		1.80	n.d.
10		0.39	4.2
11		0.15	>30
12		0.088	14.8
13		0.23	19.1
14		0.20	>30
15		0.089	14.1
16		>10	n.d
17		>10	n.d.

[a] EC_{50} values are the average of at least two independent experiments.
[b] [^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1[19a] or hGlyT2[19b]cDNAs.

Table 2
In vitro inhibitory activity of **18**–**27** at GlyT1 and GlyT2[a]



Compounds	Ar^1	Ar^2	GlyT1 EC_{50}[b] (μM)	GlyT2 EC_{50}[b] (μM)
18	Ph–	Ph–	0.11	27
19	2-CH_3–C_6H_4–	Ph–	4.05	>30
20	3-CH_3–C_6H_4–	Ph–	0.056	9.57
21	4-CH_3–C_6H_4–	Ph–	0.30	n.d.
22	3-Cl–C_6H_4–	Ph–	0.033	7.52
23	4-Cl–C_6H_4–	Ph–	0.088	14.8
24	3-F–C_6H_4–	3-F–C_6H_4–	0.016	6.65
25	3-CF3–C_6H_4–	Ph–	0.025	n.d.
26	4-CH_3O–C_6H_4–	Ph-	1.10	11.4
27	4-CH_3O–C_6H_4–	4-CH_3O–C_6H_4–	6.63	23.1

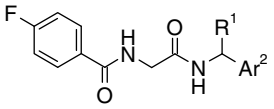
[a] EC_{50} values are the average of at least two independent experiments.
[b] [^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1[19a] or hGlyT2[19b]cDNAs.

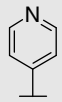
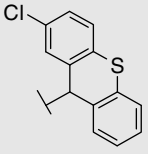
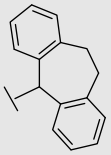
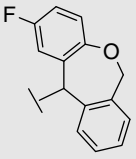
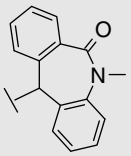
hanced activity against GlyT1 (**22**–**24**) whereas electron donating substituents tended to decrease the activity (**26**–**27**).

Replacement of an aromatic group by an alkyl substituent (**28**) or by simple heterocycles (**29**–**32**) led to poorly active derivatives. Bridging of the two aromatic groups to form various tricyclic derivatives (**33**–**36**) led to completely inactive compounds (Table 3).

When Ar^1 and Ar^2 are different, compounds of the type **2** are chiral. In order to clarify the influence of the absolute configuration, we isolated both enantiomers of the representative com-

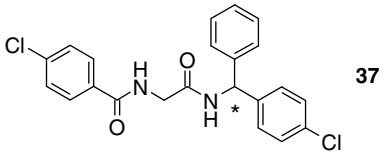
Table 3
In vitro inhibitory activity of **28**–**36** at GlyT1 and GlyT2[a]



Compounds	R^1	Ar^2	GlyT1 EC_{50}[b] (μM)
28	Et–	Ph–	4.10
29		Ph–	0.73
30		Ph–	1.27
31		Ph–	>10
32		Ph–	5.05
33			>10
34			>10
35			>10
36			>10

[a] EC_{50} values are the average of at least two independent experiments.
[b] [^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1[19a] or hGlyT2[19b]cDNAs.

Table 4
In vitro inhibitory activity of the two enantiomers of **37** at GlyT1 and GlyT2[a]

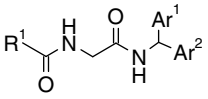


37

Compounds	Specific rotation[b]	GlyT1 EC_{50}[c] (μM)	GlyT2 EC_{50}[c] (μM)
37 (+)	+ 17.6°	0.30	28
37 (−)	−19.4°	0.28	5.9

[a] EC_{50} values are the average of at least two independent experiments.
[b] Measured in MeOH (c = 1; 589 nm, 20°).
[c] [^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1[19a] or hGlyT2[19b]cDNAs.

Table 5
Molecular properties and microsomal clearances of representative derivatives of the bis-amides **2**



	24	**25**	**13**	**8**
Membrane permeability[a][$\cdot 10^{-6}$ cm/s]	High Pe = 2.0	Medium Pe = 1.0	High Pe = 2.2	n.d.
Solubility[b] (μg/ml)	<1	<1	7	<1
Cl_{int}[c] (Mouse microsomes)	n.d.	19.3	8.6	926
Cl_{int}[c] (Human microsomes)	9.7	0	0	887

[a] Membrane permeability is measured in the PAMPA assay.[20]
[b] Aqueous solubility (μg/ml) measured in a lyophilisation solubility assay.[21]
[c] Cl_{int}: intrinsic clearance (μL/min/mg protein).

pound **37** via chromatographic separation of the racemate on a Chiralcell OD column (Daicel; elution: heptane/ethanol 85:15). Surprisingly, both enantiomers showed similar EC_{50}-values for inhibition of GlyT1, whereas the selectivity of the (+)-enantiomer versus inhibition of GlyT2 was somewhat better (Table 4).

Despite their high lipophilicity (Clog P = 3.8–4.7) and thus low aqueous solubility, these simple bis-amides display interesting molecular properties (Table 5). It is interesting to note that the most active compounds **24**, **25** and **13** show low microsomal clearances. This is in contrast to the less active derivative **8** containing a two carbon linker which is likely to be metabolically labile. However, no studies have been performed to identify potential metabolites of these inhibitors. Membrane permeability[20] is high to medium and most of the compounds show no or minor inhibition of CYP450 enzymes; all IC_{50}-values for 3A4, 2D6 and 2C9 are >50 μM, with the exception of compound **13** which has an IC_{50} of 4.9 and 14.7 μM against 2D6 and 2C9, respectively. We also tested the representative derivatives **20**, **22** and **24** for their activity at the $GABA_A$ receptor; all three compounds were inactive at 3.16 μM versus the benzodiazepine binding site of the rat $GABA_A\alpha1\beta3\gamma2$, $GABA_A\alpha2\beta3\gamma2$, $GABA_A$ $\alpha3\beta3\gamma2$ and $GABA_A$ $\alpha5\beta3\gamma2$ receptor subtypes.

In summary, we report here the discovery of a new and simple chemotype of GlyT1 inhibitors. Starting from a high throughput hit **1** containing a benzodiazepinone moiety we sequentially modified the original molecule to get structurally simple and potent bis-amides of the type **2** showing excellent metabolic stabilities. Further optimization of this new class of GlyT1 inhibitors will be reported in due course.

Acknowledgments

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19. (a) Ceccarelli, S. M.; Pinard, E.; Stalder, H. WO Patent 2005040166, 2005; *Chem. Abstr.* **2005**, *142*, 447121.; (b) Same conditions as described for the hGlyT1 assay (see Ref. 11a) with slight modifications: conc. [3H]-glycine: 200 nM, cold glycine not present.
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21. Compound initially in DMSO solution is lyophilised then dissolved in 0.05 M phosphate buffer (pH 6.5), stirred for 1 h and shaken for 2 h. After one night, the solution is filtered and the filtrate analyzed by direct UV measurement or by HPLC-UV.