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2-(Aminomethyl)-benzamide-based glycine transporter type-2 inhibitors

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Abstract—Structure–activity studies on benzamide **1** obtained from library screening led to the discovery of a novel series of potent and selective glycine transporter type-2 inhibitors.

1. Introduction

Neurotransmitters are chemicals, which execute the transfer of signals between neurons and target cells. After a neurotransmitter is released from an activated nerve terminal, it is actively transported back to the presynaptic nerve terminal by a specific reuptake transporter system.[1] This reuptake mechanism controls the precise concentration and duration of the neurotransmitter present in the synaptic space, and in turn controls the extent of stimulation of the target cell.

The amino acid glycine is a major neurotransmitter within the central nervous system (CNS) of vertebrates, functioning at both inhibitory and excitatory synapses. The synaptic levels of glycine are believed to be controlled by high affinity uptake systems (transporters). Two major classes of glycine transporters, $GlyT_1$ and $GlyT_2$,[2] have been cloned and expressed. These transporters are members of a large family of sodium/chloride-dependent transporters, which are composed of an oligomeric protein containing 12 hydrophobic membrane-spanning domains.[3]

The $GlyT_1$ transporter is closely associated with the excitatory system in which glycine acts as a co-agonist for the glutamate receptors.[4] The $GlyT_2$ transporter is closely associated with the inhibitory system mediated through the strychnine-sensitive glycine receptor (ssGlyR). The localization of the $GlyT_2$ protein parallels the distribution of the ssGlyR, suggesting that it controls the level of synaptically released glycine in the synaptic space associated with this receptor.[5] This inhibitory system is almost entirely confined to the spinal cord and hindbrain, where it participates in a variety of motor and sensory functions.

Inhibition of the $GlyT_2$ transporter by a drug molecule would be expected to increase the synaptic glycine level and in turn alter the ssGlyR function. In view of the evidence implicating glycine-mediated inhibition in muscle relaxation[6] and diminishing the transmission of pain-related information,[7] such compounds would be useful as muscle relaxants, anesthetic and analgesic agents.[8] This paper describes a novel class of $GlyT_2$ inhibitors.

2. Screening

A recombinant Chinese hamster ovary (CHO) cell line expressing human $GlyT_2$ transporter ($hGlyT_2$) was developed for the high-throughput screening (HTS) assay. This cell line produces a 5–6-fold increase in glycine uptake over null CHO cells and is not inhibited by

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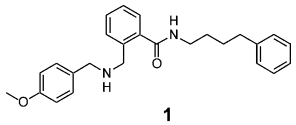
sarcosine at concentrations up to 1 mM. The effect of small molecule inhibitors of $GlyT_2$ was evaluated by measuring their ability to inhibit ^{3}H-glycine transport (uptake) in whole cells. Pharmacopeia's discovery libraries; representing approximately 2.1 million compounds (as of 1997), were screened in 96-well plate format. 2-Aminomethylbenzamide **1** ($IC_{50} \sim 1.8\,\mu M$) was selected as a starting point for the lead optimization program described below.

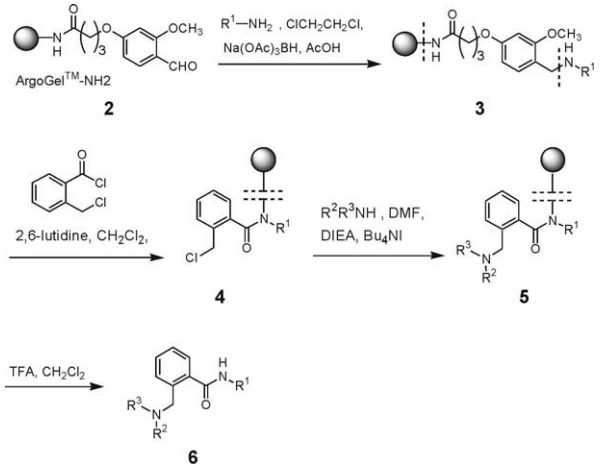


1

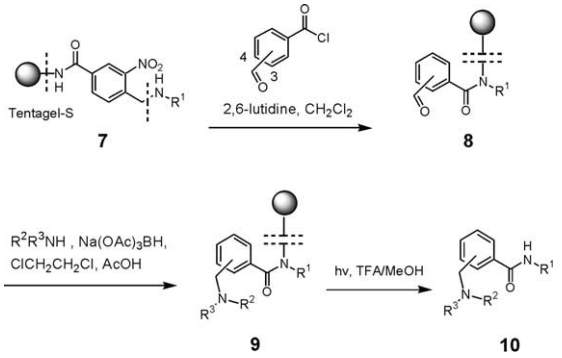
$hGlyT_2$: IC50 = 1.8 μM

3. Chemistry

Schemes 1 and 2 outline the preparation of the 2-aminomethylbenzamide analogues. The compounds were prepared on resin via acid labile (**2**) or photolabile (**7**) linkers. Acid labile resin **2** (Scheme 1) was reductively alkylated with an amine, which was then acylated with chloromethylbenzoyl chloride. Displacement of the chloride on resin **4** with a primary or a secondary amine affords the 2-aminomethylbenzamide analogues. The 3-aminomethylbenzamide and the 4-aminomethylbenzamide analogues were prepared via the photolabile resin



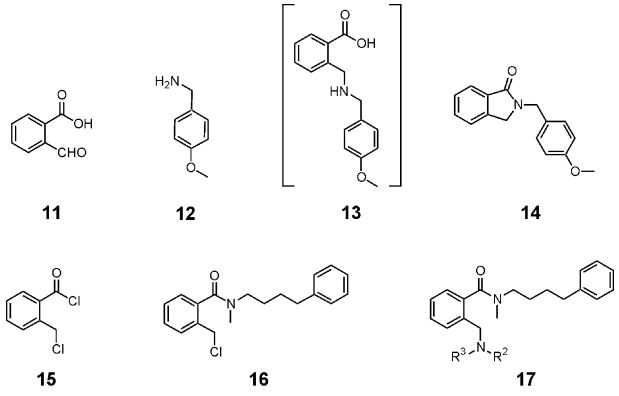
Scheme 1. Synthesis of 2-aminomethylbenzamide analogues via an acid labile linker.



Scheme 2. Synthesis of 3- and 4-aminomethylbenzamide analogues via a photolabile linker.

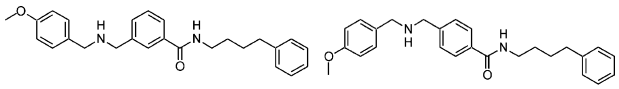
7. The resin (Scheme 2) was first acylated with 3- or 4-formylbenzoyl chloride and the resulting resin bound aldehyde **8** was reductively alkylated with an amine. In both cases, the final products were cleaved from the resin and purified.

Solution-phase syntheses of the benzamide analogues were attempted. Reductive amination of 2-formylbenzoic acid **11** with 4-methoxybenzylamine **12** gave a cyclization product **14** which is likely to be formed from a facile dehydration of intermediate **13**. This side reaction can be eliminated by using a tertiary amide **16**, which was prepared by reacting methyl-(4-phenylbutyl)-amine with chloromethylbenzoyl chloride **15**. The chloride in compound **16** can be displaced with various amines to afford tertiary benzamide analogues **17**.



4. Results and discussion

The optimal positional substitution of the benzamide core was first investigated. The *ortho*-analogue **1** is preferred over the *meta*- (**18**) and *para*- (**19**) substitutions.



18

$hGlyT_2$: IC50 > 10 μM

19

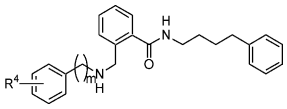
$hGlyT_2$: IC50 ~ 10 μM

With the optimal positional substitution established, analogues with modifications in the (4-methoxyphenylmethyl)amine domain were prepared (Table 1).

The 4-methoxyphenethylamino homologue **20** is about equal potent to **1**. However, the corresponding hydroxyl analogue **21** improved the potency by ~7-fold suggesting a hydrogen bond donor interaction may be important for **21**. The hydroxyl group cannot be replaced with an amino (**22**), a fluoro (**23**) or a trifluoromethyl (**22**) substituent, as these replacements resulted in a significant loss of potency. The ethyl tether between the 4-hydrophenyl and amino groups in compound **21** possesses the optimal chain length since both **25** ($n = 1$) and **26** ($n = 3$) are substantially weaker compounds. Similarly, a *para*-hydroxyl moiety is favored over the *meta*- (**27**) and the *ortho*- (**28**) substitution.

A methyl scan was performed in the lead structure **21**. The resulting tertiary amine analogue **29** retains

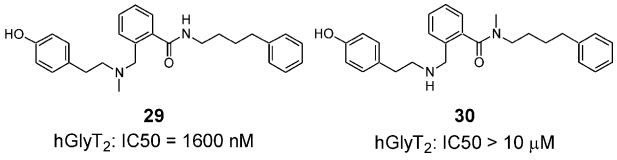
Table 1. Modifications of the (4-methoxyphenylmethyl)amino domain



Compd	*m*	R^4	$GlyT_2$ (IC_{50}, nM)[a]
1	1	4-MeO	1800
20	2	4-MeO	1200
21	2	4-HO	170
22	2	4-NH_2	4000
23	2	4-F	3400
24	2	4-CF_3	4100
25	1	4-HO	1100
26	3	4-HO	670
27	2	3-HO	330
28	2	2-HO	1200

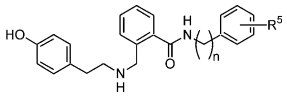
[a] Values are means of at least two experiments with standard deviation less than 20%.

micromolar potency, while the tertiary amide analogue **30** is inactive.



Since the tertiary amide analogue **30** is devoid of any potency, a series of secondary amide compounds (Table 2) was prepared to explore the phenylbutyl moiety. Chain length homologues **31**, **32**, and **33** were prepared and shown to be inactive. Addition of a chloro group to the 3- or 4-position of the phenyl ring (**34** and **35**) increased the potency by approximately 2-fold at best over the parent compound **21**. Similarly, addition of a substituent (**37** to **40**) to the phenyl ring resulted in a subtle change in potency.

Table 2. Modifications of the phenylbutylamino domain



Compd	*n*	R^5	$GlyT_2$ (IC_{50}, nM)[a]
31	1	H	>5000
32	2	H	>5000
33	3	H	>5000
21	4	H	170
34	4	3-Cl	99
35	4	4-Cl	77
36	4	4-CH_3	86
37	4	4-CF_3	140
38	4	4-CH_3O	130
39	4	4-CF_3O	370
40	4	3,4-diCl	120
41	4	4-HO	2400

[a] Values are means of at least two experiments with standard deviation less than 20%.

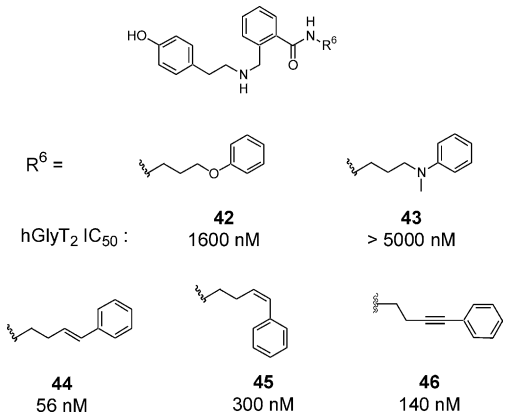


Figure 1. Modifications of the phenylbutylamino moiety. IC_{50} values are means of at least two experiments with standard deviation less than 20%.

The benzylic methylene group of the phenylbutylamine moiety was replaced with oxygen (**42**) and an *N*-methyl group (**43**) (Fig. 1). However, no improvement in potency was observed in either case. Removal of one rotatable bond in the phenylbutylamine moiety by a *trans*-alkene (**44**) increased the potency of the molecule by 3-fold in comparison to the **21**. In addition, the *trans*-isomer is more potent compared to the *cis*-isomer (**45**) or the alkyne analogue (**46**).

5. Selectivity

The objective of the project was to identify a selective and potent $hGlyT_2$ inhibitor for CNS applications. Compound **44** shows selective inhibition of $hGlyT_2$ over its subtype transporter $hGlyT_1$ (>100 μM) and the closely related receptor ssGlyR (>100 μM). However, good selectivity over other transporters and receptors present in the CNS is also required. Thus, the activity of the $GlyT_2$ inhibitors on dopamine transporter (DAT), norepinephrine (noradrenalin) transporter (NET) and 5-hydroxytryptamine transporter (5HTT) was monitored. Compound **44** exhibits low selectivity (Table 3) over the DAT (6-fold) and NET (3-fold), although reasonable selectivity over the 5HTT (38-fold) was observed.

Optimization of the selectivity of this compound was explored by using a series of constrained analogues (Fig. 2). We elected to constrain the 4-hydroxyphenethylamino moiety since the phenolic OH and basic amino groups were likely to be involved in binding to

Table 3. Selectivity of selected $hGlyT_2$ inhibitors

Compd	$hGlyT_2$ (IC_{50}, nM)[a]	Fold selectivity[b]		
		DAT/$GlyT_2$	NET/$GlyT_2$	5HTT/$GlyT_2$
44	56	6	3	38
47B	43	29	23	19
55B	30	85	84	>100
57B	33	>100	>100	>100

[a] Values are means of at least two experiments with standard deviation less than 20%.

[b] Fold selectivity was calculated by dividing the IC_{50} mean values of DAT, NET or 5HTT by the IC_{50} mean values of $hGlyT_2$.

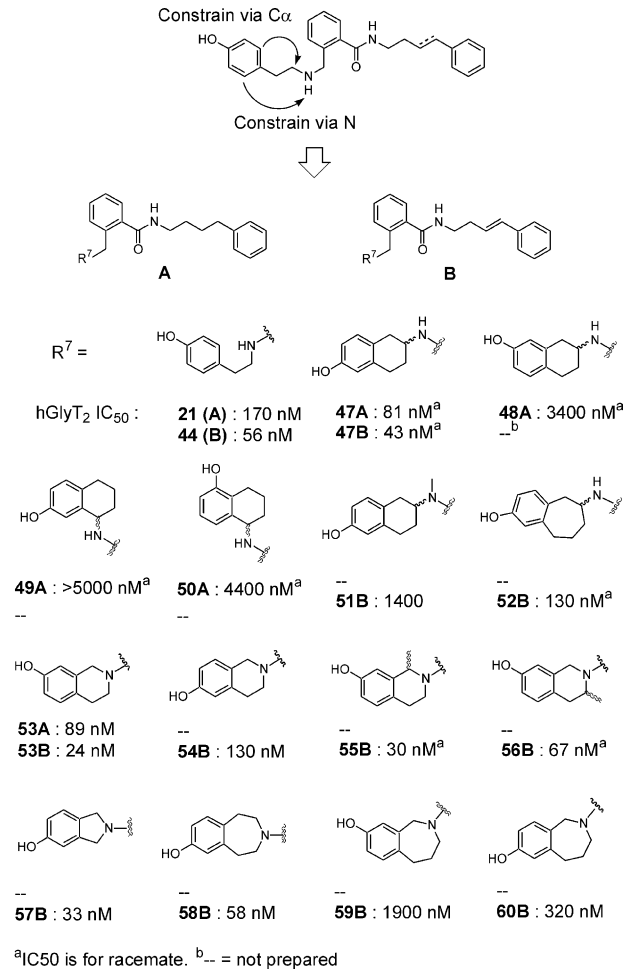


Figure 2. Constrained analogues with general structure **A** and **B**. Spatial arrangements between the amino and phenolic groups were explored by various cyclic structures. IC_{50} values are means of at least two experiments with standard deviation less than 20%.

the DAT and NET. Structures with different spatial arrangements between these two groups were expected to have diminished affinity to the DAT and NET. The compound was first constrained via the C_α carbon to give a series of aminotetrahydronaphthalenol analogues (**47A**, **47B**, **48A**, **49A**, **50A** and **51B**). Optimal arrangement for the hydroxyl and amino groups was established by **47B**. Changing the position of the hydroxyl group (**48A**), the amino group (**49A**) or both groups (**50A**) resulted in a significant loss in potency. The tertiary amine analogue (**51B**) retained micromolar potency consistent with the previously observed data for the phenethylamino analogue **29**.

The second approach we took was to constrain the phenyl ring via a tether to the basic amine group. This resulted in a series of tetrahydroisoquinoline analogues (**53A**–**56B**). Compound **53B** is more active than **54B**, and thus established the preferred position of the hydroxyl function. Addition of a 1-methyl substituent to the tetrahydroisoquinoline affords **55B** which is equipotent to **53B**. However, the 3-methyl analogue (**56B**) is about 3-fold less potent than **53B**. Optimal ring size was subsequently investigated. Thus, dihydroisoindole (**57B**) and tetrahydrobenzoazepine (**58B**, **59B** and **60B**) analogues were prepared. The dihydroisoindole analogue **57B**[9] was equipotent to tetrahydroisoquinoline analogues **53B** and **55B**. This analogue deviates from the SAR observed for the unconstrained series, in which a phenethylamine is favored.

Three cyclic analogues **47B**, **55B** and **57B** with IC_{50} <50 nM were tested for monoamine transporter selectivity (Table 3). They all exhibit better selectivity than the non-cyclic compound **44**. The exocyclic amino analogue (**47B**) was less selective in comparison to the endocyclic amino compounds (**55B** and **57B**). In fact, both **55B** and **57B** are highly selective towards $hGlyT_2$.

6. Conclusion

Chemical modifications of a $hGlyT_2$ hit (**1**) identified from the screening of compound libraries resulted in the discovery of potent and selective inhibitors for the human $GlyT_2$ transporter. The geometric arrangement of the hydroxyl and the amino functional groups in this chemical series was shown to be crucial for both potency and selectivity.

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9. Analytical data for a representative compound **57B** (HCl salt): ^{1}H NMR (CD_3OD) δ 7.75 (m, 1H), 7.60 (broad S, 3H), 7.50–7.10 (m, 6H), 6.75 (m, 2H), 6.40 (m, 2H), 4.50 (m, 2H), 4.38 (m, 4H), 3.60 (t, 2H), 2.55 (q, 2H); MS (ESI) m/z 399.0 $(M+H)^+$.