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The discovery of potent glycine transporter type-2 inhibitors: Design and synthesis of phenoxymethylbenzamide derivatives

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ABSTRACT

We describe the discovery of phenoxymethylbenzamide derivatives as a novel class of glycine transporter type-2 (GlyT-2) inhibitors. We found hit compound **1** (human GlyT-2, IC_{50} = 4040 nM) in our library and converted its 1-(1-(naphthalen-2-ylmethyl)piperidin-4-yl)pyrrolidin-3-yl group to an 1-(*N*, *N*-dimethylaminopropyl)piperidyl group and its *tert*-butyl group to a trifluoromethyl group to obtain *N*-(1-(3-(dimethylamino)propyl)piperidin-4-yl)-4-((4-(trifluoromethyl)phenoxy)methyl)benzamide (**20**). Compound **20** showed good inhibitory activity against human GlyT-2 (IC_{50} = 15.3 nM) and exhibited anti-allodynia effects in a mouse neuropathic pain model.

Glycine and gamma-aminobutyric acid serve as major inhibitory neurotransmitters in the central nervous system. The extracellular concentration of glycine is regulated by glycine transporters (GlyTs) on the presynaptic terminals of glycinergic inhibitory neurons or glial cells adjacent to inhibitory synapses and excitatory synapses. Two GlyT subtypes, GlyT-1 and GlyT-2, have been identified. GlyT-1 is widely distributed in the central nervous system and predominantly expressed in glial cells near both excitatory and inhibitory neurons.[1] Recent studies of GlyT-1 have suggested its therapeutic potential for the treatment of schizophrenia and cognitive disorders based on the glutamatergic hypothesis of schizophrenia.[2] Some GlyT-1 inhibitors are now being investigated in clinical trials. Meanwhile, GlyT-2 is specifically distributed in the spinal cord and brainstem and localized in the presynaptic terminals of inhibitory glycinergic neurons.[1] Recent studies have further demonstrated that the GlyT-2 inhibitors (Fig. 1),[3] which are thought to increase extracellular glycine levels in the synaptic cleft in the spinal cord, ameliorate allodynia in an animal model of neuropathic pain.[4] GlyT-2 has received much attention as a target for the treatment of neuropathic pain.

Neuropathic pain syndrome is caused by injury to the nervous system, which can result from metabolic disorders, infection, or physical trauma. Examples include painful diabetic neuropathy, postherpetic neuralgia, complex regional pain syndrome, and chronic postoperative pain. However, the molecular mechanisms underlying these pain states remain unclear. Some anticonvulsants, antidepressants, antianxiety agents, and antiepileptics have been used to treat neuropathic pain; however, patients are not satisfied with the treatment provided by these agents because of poor efficacy and adverse reactions.[5] Consequently, compounds with a high analgesic efficacy and lower adverse effects are required.

Against this background, we have been interested in GlyT-2 inhibitors for treating neuropathic pain. Here we report the design and synthesis of novel GlyT-2 inhibitors which exhibited favorable efficacy in an animal model of neuropathic pain.

First, we screened our chemical library to find inhibitors of human GlyT-2 (hGlyT-2) recombinantly expressed in HEK293 cells.[6] We found a phenoxymethylbenzamide derivative (**1**; Fig. 1) with moderate inhibitory activity against hGlyT-2 (IC_{50} = 4010 nM). With an aim to improve the inhibitory activity against hGlyT-2, we explored the structural modifications of **1**.

We divided compound **1** into three components (left part, middle part, and right part), as shown in Figure 2. These three components can be connected by the following methods: coupling between phenol **2** and benzyl bromide **3**, followed by hydrolysis of ester **4**, provided benzoic acid **5**. Phenoxymethylbenzamide **7** was prepared by a condensation reaction between benzoic acid **5** and amine **6** (Scheme 1). The obtained compounds were converted into corresponding salts (hydrochloride or dihydrochloride) for

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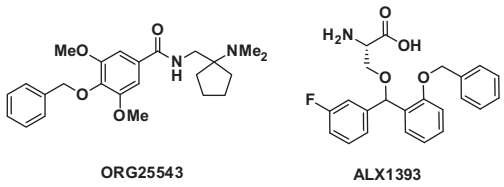


Figure 1. Representative GlyT-2 inhibitors.

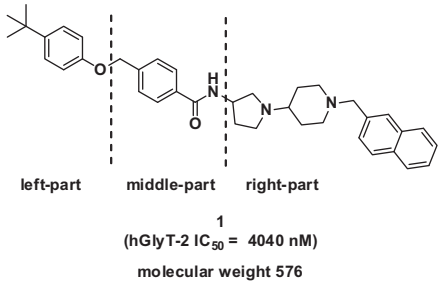


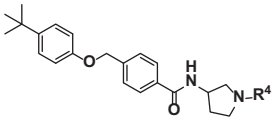
Figure 2. Compound discovered by a screening campaign.

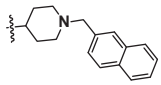
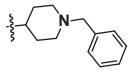
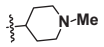
pharmacological tests as appropriate and were evaluated for inhibitory activity against hGlyT-2.

High molecular weight is typically disadvantageous for membrane permeability. Therefore, we made an attempt to simplify the structure of **1**, which had high molecular weight. First, we evaluated the impact of changes to the naphthylmethylpiperidine moiety of the right part of **1** (Table 1). Replacement of the naphthylmethyl group with a smaller benzyl group (**8**) or methyl group (**9**) improved the inhibitory activity against hGlyT-2 (IC_{50} = 516 nM and 250 nM, respectively). Here we recognized **9**, which had a low molecular weight, as a starting point. On the other hand, replacement of the naphthylmethylpiperidyl group with a cyclohexyl or isopropyl group did not seem to impact the inhibitory activities (**10** and **11**) by a great degree compared with replacement with a methyl group (**9**). From these results, we speculated that the nitrogen of piperidine was important for the interaction with hGlyT-2.

We next turned our attention to modifying the left part (Table 2). To clarify the substituent effects, we modified the substituents of the left-part phenyl group of **9**. First, we replaced the *tert*-butyl group with hydrogen (**11**); however, **11** exhibited decreased inhibitory activity. Next we introduced fluorine or chlorine as an electron-withdrawing substituent (**12** and **13**). Introduction of fluorine decreased the inhibitory activity, while the activity of the chlorine-substituted compound was comparable with that of **9**. Furthermore, introduction of a trifluoromethyl group gave the

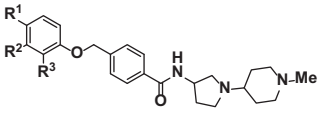
Table 1
In vitro inhibitory activity of phenoxymethylbenzamide derivatives against hGlyT-2: varying the R^4 substituent



Compound	R^4	hGlyT-2 IC_{50}[a] (nM)
1		4040
8		516
9		250
10		2200
11		2800

[a] IC_{50} values are the average of at least two independent experiments.

Table 2
In vitro inhibitory activity of phenoxymethylbenzamide derivatives against hGlyT-2: varying the R^1, R^2, and R^3 substituents

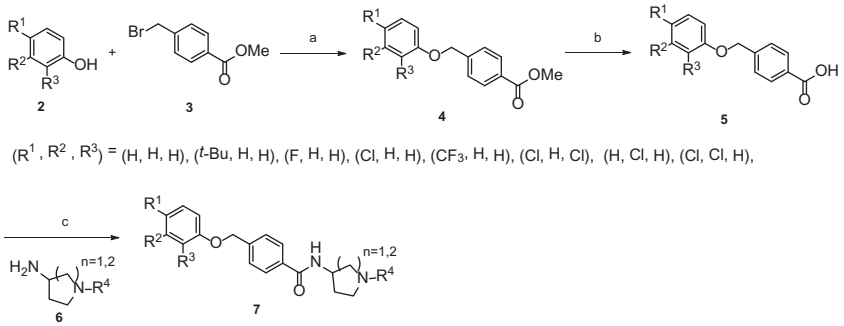


Compound	(R^1, R^2, R^3)	hGlyT-2 IC_{50}[a] (nM)
9	(*t*-Bu, H, H)	250
12	(H, H, H)	879
13	(F, H, H)	2320
14	(Cl, H, H)	440
15	(CF_3, H, H)	112
16	(Cl, H, Cl)	431
17	(H, Cl, H)	869
18	(Cl, Cl, H)	361

[a] IC_{50} values are the average of at least two independent experiments.

best activity among these groups (**15**). Moving the chlorine substituent from the 4-position to the 3-position of the phenyl group decreased the inhibitory activity (**17**). Furthermore, a substituent at the 2- or 3-position of the left-part phenyl group in **14** had no marked effect on inhibitory activity (**16** and **18**). These results show that the introduction at the 4-position of the phenyl group was favorable.

Next, each enantiomer of racemate **15** was prepared separately, and **15*S*** showed inhibitory activity twice that of **15*R*** (Table 3). **19**,



Scheme 1. Reagents and conditions: (a) K_2CO_3, dimethylformamide, rt, 72–99%; (b) 1 N NaOH aq, MeOH, THF, rt, 77–99%; (c) **6**, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 78–99%.