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5,5-Diaryl-2-amino-4-pentenoates as Novel, Potent, and Selective Glycine Transporter Type-2 Reuptake Inhibitors

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Abstract—A novel series of 5,5-diaryl-2-amino-4-pentenoates was synthesized and found to be potent and selective glycine transporter type-2 reuptake inhibitors.

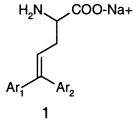
The amino acid glycine is a major neurotransmitter in the mammalian central nervous system (CNS) functioning at both inhibitory and excitatory synapses. High affinity membrane-associated glycine transporters typically mediate the rapid removal of glycine from the synaptic cleft, with uptake across a concentration gradient being thermodynamically coupled to transmembrane ion gradients (Na^+/Cl^--dependant transport).[1]

Two distinct glycine transporters, GlyT-1[2] and GlyT-2,[3] have been recently cloned and share 50% identity at both the nucleotide and amino acid levels. GlyT-1 (four isoforms: GlyT-1a, GlyT-1b, GlyT-1c, and GlyT-1d) is expressed in the hippocampal and cortical regions of the brain as well as in the spinal cord and brainstem. In contrast, GlyT-2 is expressed primarily in the spinal cord and cerebellum and is absent in the hippocampus and cortex.

Based on their patterns of expression, GlyT-1 is thought to co-localize with *N*-methyl-D-aspartate (NMDA) receptors where glycine functions in excitatory neurotransmission by modulating the actions of glutamate, the major excitatory neurotransmitter in the CNS. In other words, glycine acts as a co-agonist with glutamate at the NMDA receptor.[4] GlyT-2, on the other hand, is thought to co-localize with strychnine-sensitive glycine receptors, which mediates the inhibitory actions of glycine. The need for the precise regulation of glycine concentrations in these receptor systems implicates glycine transporters as potential therapeutic targets.

Compounds that selectively inhibit the glycine transporter GlyT-2 would thus be expected to alter receptor function, and therefore provide therapeutic benefit in a variety of disease states. For example, GlyT-2 inhibition can be used to increase the activity of the inhibitory neurons having strychnine-sensitive glycine receptors via increasing synaptic levels of glycine thus diminishing neuropathic pain transmission in the spinal cord. The intrathecal administration of glycine has been shown to alleviate neuropathic pain in rats.[5] This pain relief was blocked by the administration of strychnine but was not affected by the NMDA receptor channel blockers or NMDA glycine site antagonist. Moreover, enhancing inhibitory glycinergic transmission through strychnine-sensitive glycine receptors in the spinal cord can be used to decrease muscle hyperactivity, which in turn can be beneficial in treating diseases or conditions associated with increased muscle contraction, such as muscle spasticity and epilepsy.

This paper describes the synthesis and biological activity of a novel class of selective GlyT-2 reuptake inhibitors (**1**) demonstrating the significance of double bond geometry and amino-acid chirality in GlyT-2 activity.



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Compounds **1a**–**z** were synthesized as shown in Scheme 1. Benzophenophenone-imine glycine methyl ester **2** was treated with LDA followed by **3**[6] to give the allylated intermediate **4**. The versatile intermediate **4** was then subjected to the conditions of palladium catalyzed

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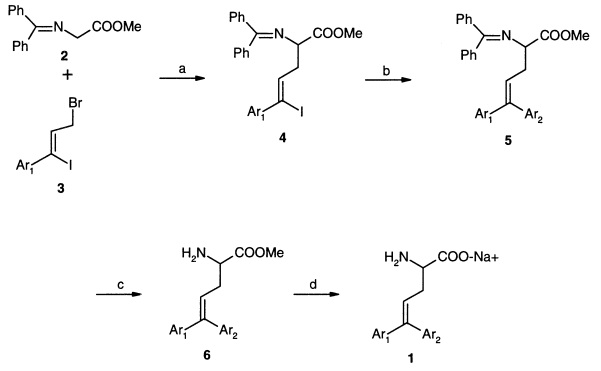
Suzuki cross-coupling to afford **5**. The benzophenone moiety was then removed by trifluoroacetic acid to give **6** followed by basic hydrolysis with sodium hydroxide in methanol to afford the sodium salts **1**.

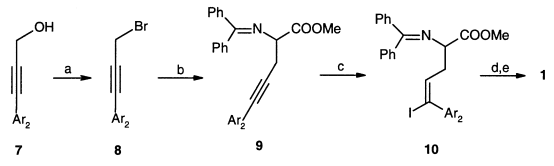
In the corresponding cases where the aryl substituent Ar_2 is fixed, the alternative synthesis is illustrated in Scheme 2. The propargyl alcohol **7**[6] was transformed into the corresponding propargyl bromide **8**, which was then utilized in the propargylation of **2** providing **9**. Regioselective hydrostannation[7] of **9** followed by tin–iodine exchange afforded the intermediate **10**, which was then subjected to Suzuki coupling, trifluoroacetic acid mediated deprotection followed by hydrolysis.

Compounds **1a**–**z** were evaluated in vitro for their ability to block the reuptake of tritiated glycine in cells stably expressing the GlyT-1c and GlyT-2 transporters. The cells were incubated at 37 °C for 30 min with 10 different concentrations of each test compound in the presence of 50 nM tritiated glycine. Estimated IC_{50} values of each test compound were subsequently determined for both transporter subtypes.

Compound **1a**, which contains a 2,4-difluorophenyl group *trans* to the glycine moiety and the *cis* 4-isopropylphenyl substituent, had good potency at GlyT-2 ($IC_{50} = 0.330\,\mu M$) with good selectivity over the GlyT-1 transporter. With this early lead, the 2,4-difluorophenyl group was fixed and the *cis* aryl substituents were systematically explored. Increasing the steric bulk of the *para* substituent to phenyl and *tert* butyl resulted in a decrease in activity (see **1b** and **1d** in Table 1). The positional isomer **1c**, containing the (3-isopropylphenyl) also led to a decrease in GlyT-2 transporter potency. However, in the *cis* biphenyl series, the 3-biphenyl substituent **1g** ($IC_{50} = 1.340\,\mu M$) was more potent than the corresponding 2- and 4-biphenyl isomers.



Scheme 1. (a) LDA, THF, HMPA, −78 °C; (b) Ar_2-$B(OH)_2$, $Pd(PPh_3)_4$, DME/Na_2CO_3, 110 °C; (c) TFA, H_2O, CH_2Cl_2, rt; (d) 1 N NaOH, MeOH.



Scheme 2. (a) PBr_3, Et_2O, 0 °C; (b) **2**, LDA, THF, HMPA, −78 °C; (c) i. Bu_3SnH, $PdCl_2(PPh_3)_2$, THF; ii. I_2, CH_2Cl_2; iii. Ar_1- $B(OH)_2$, $Pd(PPh_3)_4$, DME/Na_2CO_3, 110 °C; (d) TFA, H_2O, CH_2Cl_2, rt; (d) 1 N NaOH, MeOH.

In contrast, the sterically less demanding substituents such as the 4-methylphenyl and the 4-ethylphenyl groups maintained reasonable potency at GlyT-2 transporter with IC_{50} values of 1.17 and 0.46 μM, respectively.

Despite the favorable nature of small substituent in the *para* position of the phenyl ring, electron-donating substituents such as the methoxy group provided ligands with reduced activity (**1k** with $IC_{50} = 2.03\,\mu M$). The electron-withdrawing group, exemplified by the 4-cyano substituent, was found to be inactive (**1l** with $IC_{50} > 10\,\mu M$). Unfortunately, *cis* substituted heterocyclic aromatic groups (**1m** and **1n**) had a negative effect on GlyT-2 transporter affinity giving rise to ligands with dramatically reduced activity.

Disubstitution in the 3- and 4-position, for example 3,4-diethylphenyl, **1h** ($IC_{50} = 0.66\,\mu M$) and 2-naphthyl, **1j** ($IC_{50} = 0.77\,\mu M$) showed good potency at the GlyT-2 transporter. With the 4-isopropylphenyl group as the optimal *cis* substituent, this group was kept fixed while several *trans* substituted phenyl groups were examined. Similar to the *cis* substitutions, *trans* bulky aryl *para* substituents such as phenyl, *tert*-butyl, and isopropyl (**1q**, **1u**, and **1o** in Table 1) resulted in reduced potency when compared to the *trans* 2,4-difluorophenyl group. Nevertheless, *trans* aryl groups such as 2-fluorophenyl **1t** ($IC_{50} = 0.29\,\mu M$) and phenyl **1s** ($IC_{50} = 0.4\,\mu M$) retained good GlyT-2 potency. Interestingly, the geometric isomer **1v** (isomer of **1a**) was found to be inactive ($IC_{50} > 10\,\mu M$) at both GlyT-1c and GlyT-2 suggesting that the geometric integrity of the double bond can be crucial for transporter activity. *trans*-Electron-donating substituted phenyls such as the 4-methoxyphenyl group provided ligands with slightly reduced activity (**1p** with $IC_{50} = 1.17\,\mu M$). Similarly, for the electron-withdrawing group, exemplified by the 4-cyano substituent, was found to also have reduced activity compared to **1a** (**1w** with $IC_{50} = 2.64\,\mu M$). The *trans* substituted heterocyclic aromatic analogues (**1x** and **1y**) showed significantly reduced GlyT-2 transporter affinity and dramatically reduced selectivity over GlyT-1c transporter.

To further determine the effect of the chirality in this series of compounds on GlyT-2 potency, compounds **1a**, **1h**, and **1j** were resolved using a chiral column (Chirobiotic T^{TM}) of an analytical HPLC and the resolved components for each compound were separated and evaluated in vitro. For each racemic compound, only one enantiomer (the *S*-isomer, the eutomer) of the pair was active and the activity was about half that of the racemic mixture. The absolute stereochemistry (shown in Fig. 1) of the active

Table 1. In vitro glycine reuptake inhibitory activity of 5,5-diaryl-2-amino-4-pentenoates at the GlyT-2 and GlyT-1c transporters

Compound	A_1	A_2	GlyT-2 (IC_{50}, μM)[a]	GlyT-1c (IC_{50}, μM)[a]
1a	2,4-Difluorophenyl	4-Isopropylphenyl	0.33	>10
1b	2,4-Difluorophenyl	4-*tert*-Butylphenyl	1.74	>10
1c	2,4-Difluorophenyl	3-Isopropylphenyl	1.59	>10
1d	2,4-Difluorophenyl	4-Biphenyl	3.66	>10
1e	2,4-Difluorophenyl	4-Methylphenyl	1.17	9.56
1f	2,4-Difluorophenyl	2-Biphenyl	2.75	8.92
1g	2,4-Difluorophenyl	3-Biphenyl	1.34	>10
1h	2,4-Difluorophenyl	3,4-Diethylphenyl	0.66	>10
1i	2,4-Difluorophenyl	4-Ethylphenyl	0.46	>10
1j	2,4-Difluorophenyl	2-Naphthyl	0.77	>10
1k	2,4-Difluorophenyl	4-Methoxyphenyl	2.03	>10
1l	2,4-Difluorophenyl	4-Cyanophenyl	>10	>10
1m	2,4-Difluorophenyl	3-Thienyl	5.88	>10
1n	2,4-Difluorophenyl	3-Pyridyl	>10	>10
1o	4-Isopropylphenyl	4-Isopropylphenyl	2.03	>10
1p	4-Methoxyphenyl	4-Isopropylphenyl	1.16	>10
1q	4-Biphenyl	4-Isopropylphenyl	2.06	>10
1r	4-Biphenyl	2,4-Difluorophenyl	2.40	3.40
1s	Phenyl	4-Isopropylphenyl	0.40	8.34
1t	2-Fluorophenyl	4-Isopropylphenyl	0.29	8.02
1u	4-*tert*-Butylphenyl	4-Isopropylphenyl	5.47	>10
1v	4-Isopropylphenyl	2,4-Difluorophenyl	>10	>10
1w	4-Cyanophenyl	4-Isopropylphenyl	2.64	>10
1x	3-Thienyl	4-Isopropylphenyl	1.03	5.77
1y	3-Pyridyl	4-Isopropylphenyl	5.93	6.48
1z	2,4-Difluorophenyl	Phenyl	6.44	9.50

[a]IC_{50} values are given as the mean of at least two independent determinations performed in triplicate with less than 15% deviation.

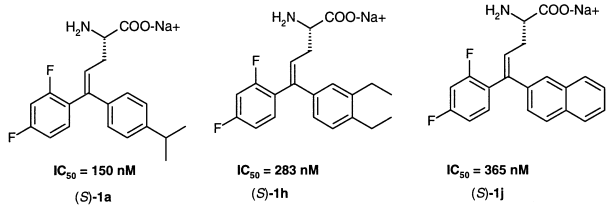


Figure 1.

enantiomers was confirmed using chemical correlation methods.[8]

In conclusion, a series of 5,5-diaryl-2-amino-4-pentenoates has been developed as a novel class of glycine reuptake inhibitors selective for the GlyT-2 transporter. Compounds **1a**, **1s**, and **1t** were the most potent reuptake inhibitors in the series. More specifically, the activity appears to reside in the *S*-enantiomer as exemplified by *S*-**1a**, *S*-**1h**, and *S*-**1j**. In addition, the geometric integrity of the double bond seems crucial for GlyT-2 transporter potency. The GlyT-2 selective compounds in this series are currently being further evaluated for their therapeutic potential.

References and Notes

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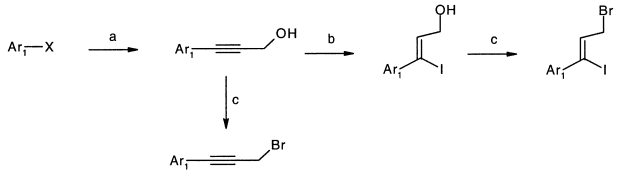

(a) Pd (PPh_3)$_4$, CuI, propargyl alcohol, Et_3N; (b) Red-Al, I_2, ether; (c) PBr_3, CH_2Cl_2, 0 °C.

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