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Discovery and SAR of Org 24598—A Selective Glycine Uptake Inhibitor

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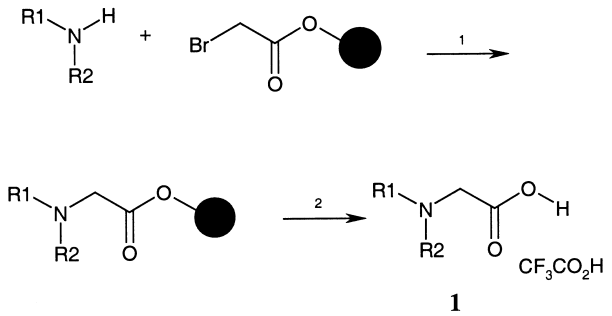
Abstract—The discovery of Org 24598, one of the first potent and selective inhibitors of the glycine transporter is discussed. In vitro structure–activity relationships (SARs) data for interaction of a ligand with this system is discussed.

Alteration of glycine levels in the mammalian central nervous system may influence inhibitory activity mediated by the strychnine-sensitive glycine receptor[1,2] or excitatory neurotransmission through the glycine receptor on the NMDA complex. ssGR's are located in the spinal cord and brainstem and are closely associated with the neuronal GlyT-2 transporter[3–5] whereas GlyT-1[4,6,7] is distributed more widely in the CNS and may play a role in controlling concentrations of the co-agonist glycine[8] in the vicinity of NMDA receptors.[9] This affords an opportunity for inhibition of the transporter to enhance NMDA receptor function through elevated concentrations of the co-agonist glycine. This mechanism may have relevance in addressing hypoglutamatergic function associated with psychosis.[10–12] We describe here the discovery of a series of selective GlyT-1b inhibitors, by application of solid-phase chemistry and library design. SAR relationships in the series are also described.

Chemistry

A library of some 1000 glycine derivatives **1**, derived from diverse commercially available primary and secondary amines, were synthesised on the solid support and tested as the trifluoroacetate salts. None of these showed significant inhibition of [^{3}H]-glycine uptake in the GlyT-1b assay (see Glycine Uptake Assay section).

A small focused library of *N*-substituted glycines from amines derived from known specific serotonin and mixed monoamine uptake inhibitors were synthesised by this method, and tested as the trifluoroacetate salts. Only one of these showed appreciable activity in the hGlyT-1b assay, the fluoxetine analogue Org 24461 (pIC_{50} 6.5) (Scheme 1).



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Scheme 1. Reagents and conditions: (1). Wang resin/2 M amine/DMSO or DMF, rt; (2) CF_3CO_2H, rt.

This series was chosen for further SAR studies. The different substituted fluoxetines were made by standard methods. The *N*-glycine derivatives **2** (Scheme 2) for this study were made by conventional solution-phase chemistry along lines suggested by the solid-phase method. Most of the compounds were tested as the lithium salts; we found that changing to the sodium or hydrochloride salts made no difference in the assay.

Glycine Uptake Assays and SAR

Glycine uptake assays were performed essentially as described by Morrow et al.[13] using CHO cells stably

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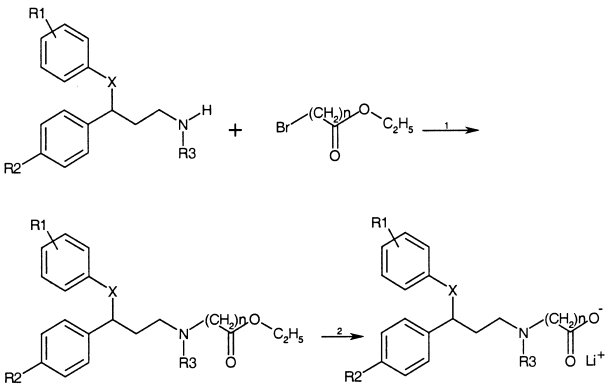


Scheme 2. Reagents and conditions: (1) K_2CO_3/DMF/Δ; (2) aq LiOH/EtOH, reflux.

transfected with hGlyT-1b or hGlyT-2. Cells were grown in 96-well microtitre plates (30,000 cells/well) for 24–48 h before removal of culture medium and addition of Hanks Balanced Salt Solution (HBSS), containing [^{3}H]-glycine (30 μM) and varying concentrations of test compounds. Plates were incubated at 37 °C for 10 min and uptake terminated by washing three times with ice-cold HBSS. After removal of excess HBSS, liquid scintillation cocktail was added to each well, prior to counting on a plate counter. Data were analysed using the GraphPad Prism analysis package and the sigmoid dose–response curve fitting option to produce pIC_{50} (the negative logarithm of the concentration of test compound producing 50% of uptake) values.

Tables 1–3 illustrate the pIC_{50} values for GlyT-1b where 2–5 independent experiments were performed in triplicate. Typical mean ± SEM values are shown in Table 3.

Table 1. SAR for compounds **2** with changes to α-amino acid part of the molecule ($R_2 = H$): racemates

Compound no.	R^1	R^3	X	*n*	GlyT-1b pIC_{50}
Org 24461	4-CF_3	CH_3	O	1	6.5
Org 24629	4-CF_3	CH_3	O	2	4.5
Org 24628	4-CF_3	CH_3	O	3	4.0
Org 24660	4-CF_3	C_2H_5	O	1	5.3
Org 24750	4-CF_3	CH_3	CH_2	1	6.0

Table 2. SAR for compounds **2** with changes to the aryl rings of the molecule: racemates

Compound no.	R^1	R^2	GlyT-1b pIC_{50}
Org 24461	4-CF_3	H	6.5
Org 24658	3-CF_3,4-Cl	H	6.4
Org 24668	3,4-Cl_2	H	6.3
Org 24667	4-Cl	H	5.9
Org 24642	4-CH_3	H	5.6
Org 24641	4-OCH_3	H	5.4
Org 24872	4-CH_3SO_2	H	4.0
Org 24730	2-Cl	H	4.9
Org 24520	2-OCH_3	H	4.7
Org 24747	3-CF_3	H	5.6
Org 24669	4-CF_3	4-Cl	7.0
Org 24645	4-CF_3	4-F	6.6
Org 24706	4-CF_3	4-OCH_3	6.3

The optical isomers of Org 24461 and two related potent compounds were separated and clear differences in activity were found. In general, the *R*-(−) isomer was more active than the *S*-(+) isomer (Table 3).

Table 3. SAR for compounds **2**: optical enantiomers

Compound no.	R^1	R^2	Isomer	GlyT-1b pIC_{50}
Org 24598	4-CF_3	H	*R*-(−)	6.90 ± 0.13
Org 24597	4-CF_3	H	*S*-(+)	5.58 ± 0.02
Org 24835	4-$CH(CH_3)_3$	H	*R*-(−)	6.35 ± 0.10
Org 24836	4-$CH(CH_3)_3$	H	*S*-(+)	4.93 ± 0.08
Org 24915	4-Ph	H	*R*-(−)	6.82 ± 0.04
Org 24914	4-Ph	H	*S*-(+)	6.01 ± 0.05

Org 24598 is a highly selective inhibitor of GlyT-1b (pIC_{50} 6.9) with negligible activity at hGlyT-2 (pIC_{50} < 4.0).

Affinity of Org 24598 at Glycine Receptors

Radioligand binding experiments to assess interaction with the strychnine-sensitive glycine receptor or the NMDA glycine co-agonist site were performed using rat spine and brain membranes and [^{3}H]-strychnine or [^{3}H]-MDL 105,519, respectively. Org 24598 showed negligible affinity ($pK_i < 4$) for both these glycine receptors.

Affinity of Org 24598 at Other Transporters and Receptors

Uptake assays for noradrenaline, dopamine, serotonin and GABA utilised synaptosomal preparations from rat brain and the corresponding [^{3}H]-labelled transmitter.

Potential for interaction with subtypes of dopamine, serotonin and noradrenaline receptors was assessed in radioligand binding experiments using heterologously expressed receptors or rat brain preparations. Org 24598 showed no appreciable affinity at these transporters and receptors (Table 4).

Table 4. Affinity of Org 24598 at other transporters and receptors

Transporters	pIC_{50}	Receptors	pK_i
Noradrenaline	< 4	Adrenoreceptors (α_{1A}, α_{1B}, α_{2C}, β_1, β_2)	< 5
Dopamine	< 4	Dopamine (D_{2S}, D_4)	< 5
Serotonin	4.3	Serotonin ($5HT_{1A,2A,2C,6,7}$)	< 5
GABA	< 4		

Org 24598 was also screened for affinity at 56 potential biological targets, including ion channels, enzymes, peptide receptors and steroid receptors, by NovaScreen, but did not show any significant interaction.

Discussion

A library of some 1000 *N*-substituted glycines derived from diverse primary and secondary amines were

screened in an hGlyT-1b assay. None of the compounds showed appreciable activity.

Interestingly, an *N*-acetic acid compound (Org 24461), derived from the serotonin uptake inhibitor fluoxetine, proved to be active with pIC_{50} 6.5.

The Org 24461 series is highly sensitive to changes to the α-amino acid (glycine) part of the molecule (Table 1). The propionic and butyric acid homologues are inactive. Changing $N–CH_3$ to $N–C_2H_5$ loses activity. The oxygen atom of the phenoxy ring system contributes to binding but is not essential. Replacement with CH_2 reduces activity by 0.5 log unit.

Electronic, lipophilic and geometric factors are all involved in the interaction of these molecules with the transporter (Table 2).

Activity is associated with an electron-withdrawing, lipophilic group on the phenoxy ring:

$$4\text{-}CF_3 \sim 3\text{-}CF_3, 4\text{-}Cl \sim 3, 4\text{-}Cl_2 > 4\text{-}Cl > 4\text{-}CH_3$$

$$\sim OCH_3 > 4\text{-}CH_3SO_2$$

This region of the molecule is associated with a lipophilic cavity in the transporter which can accommodate bulky groups such as $4\text{-}CH(CH_3)_3$ and 4-Ph which contribute to binding.

Ortho substitution on the phenoxy ring greatly reduces binding, probably due to major steric/geometric effects related to how the di-aryl system binds to the transporter. *Meta* substitution on the phenoxy ring also reduces activity but not to the same extent.

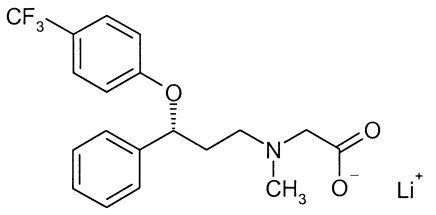
Modest increases in activity are associated with an electron-withdrawing group on the other phenyl ring. Substitution with an electron-donating group is accommodated with a slight reduction in activity.

Clear differences in activity are demonstrated by the optical isomers of these compounds with the GlyT-1b transporter. The *R*-(−) isomers are consistently more active than the *S*-(+) isomers (Table 3).

Conclusions

These findings, taken together, support the view that some of the most extremely important molecular properties (such as geometric factors) for inhibiting the GlyT-1 transporter are highly discriminating and, paradoxically, are not included in diversity calculations.

Org 24598, the *R*-(−) isomer of Org 24461, is a highly selective inhibitor of hGlyT-1b with negligible action at GlyT-2 and other transporters and receptors. The compound is approximately 600× more potent than sarcosine. The possible association of GlyT-1 and NMDA receptors may afford an opportunity for inhibition of the transporter to enhance NMDA receptor function through elevated concentrations of the co-agonist glycine. This mechanism may have relevance in addressing hypoglutamatergic function associated with psychosis.



Structure of Org 24598.
R-(−)-*N*-Methyl-*N*-[3-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine (1:1) lithium salt.

Acknowledgements

During the course of this work, some of the compounds in the series mentioned here, including Org 24598, were claimed for glycine uptake inhibition (GUI) activity in two patent applications in the name of Trophix Pharmaceuticals Inc. These are WO 9745115 (Dec 04, 1997) and WO 9745423 (Dec 04, 1997). To date, details of the method of discovery or of SARs in the series have not been published.

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