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Discovery of GlyT1 inhibitors with improved pharmacokinetic properties

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ABSTRACT

Glycine transporter 1 (GlyT1) represents a novel target for the treatment of schizophrenia via the potentiation of glutamatergic NMDA receptors. The discovery of 4,4-disubstituted piperidine inhibitors of GlyT1 which exhibit improved pharmacokinetic properties, including oral bioavailability, is discussed.

Mounting evidence suggests that the long established dopamine hyperfunction model[1] for schizophrenia may inadequately account for the symptoms of this widespread disease.[2,3] Although both typical and atypical antipsychotics target dopamine receptors and address the positive symptoms of schizophrenia (hallucinations, paranoia, and other delusions), negative symptoms (blunted affect, withdrawal) and cognitive deficits are not satisfactorily addressed with these treatments. A growing body of evidence[4] indicates that hypofunction of *N*-methyl-D-aspartate (NMDA) glutamatergic receptors may contribute to the etiology of the disease, yet direct agonists of NMDA receptors are neurotoxic.[5] Glycine transporter 1 (GlyT1) has emerged as a promising alternative target due to existing reports of its potentiation of NMDA receptor activity by modulating the local concentrations of the NMDA co-agonist glycine.[6]

Recently, we disclosed potent and selective inhibitors of GlyT1 based on a 4,4-disubstituted piperidine lead structure. Exemplified by **1** and **2** (Fig. 1), these compounds exhibit potent (<10 nM), selective (versus GlyT2, taurine transporter) inhibition of GlyT1 and selectively elevate glycine levels in rat prefrontal cortex.[7,8]

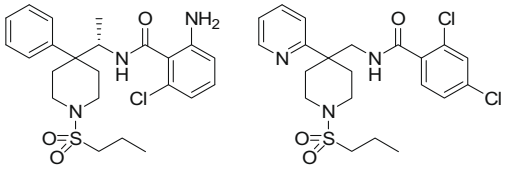

Figure 1. 4,4-Disubstituted piperidine GlyT1 inhibitors.

Furthermore, **1** significantly enhances prepulse inhibition in DBA/2J mice without impairing basal startle amplitude indicating an antipsychotic effect without sedation.[7]

While **1** and **2** were effective in animal models after being dosed subcutaneously, they exhibit poor pharmacokinetic properties including low bioavailability. In addition to optimizing these properties to enable oral dosing, a further objective was developing compounds which demonstrate high transporter occupancy in vivo. Herein we report the achievement of these goals employing an iterative analogue library approach.

A variety of strategies were pursued in order to improve the properties of **1** and **2**, including modification of the piperidine C4 substituent and substitution of the piperidine *N*-sulfonamide. Aryl group replacements were prepared according to the route described in Scheme 1a. Quenching the lithium anion of nitrile **3** with a variety of electrophiles (alkyl halides, epoxides), followed by Ra-

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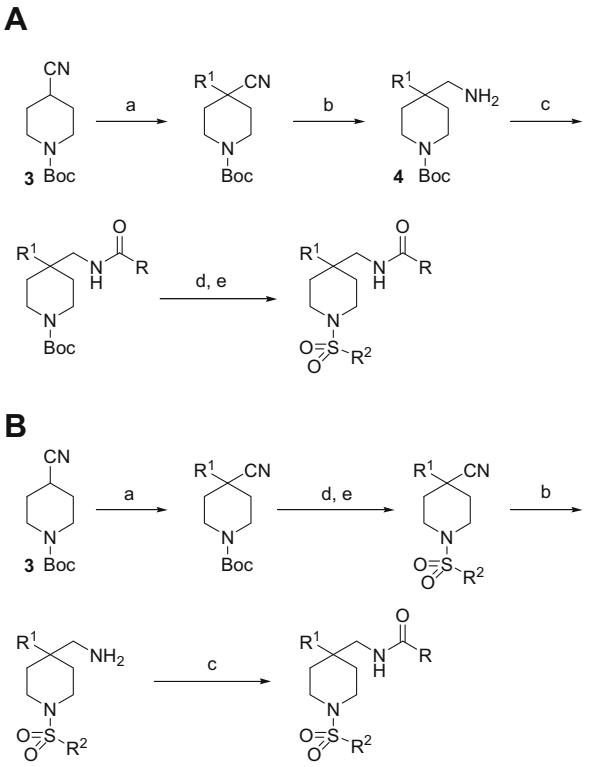
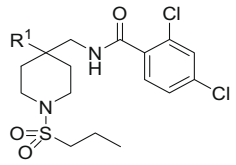
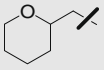
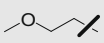
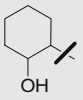
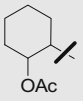


Scheme 1. Reagents and conditions: (a) LHMDS, THF, 25 °C, then electrophile R^1–X; (b) H_2, Raney Ni; (c) carboxylic acid, PS-DCC, HOBt, CH_2Cl_2, 25 °C; (d) 4 M HCl/dioxane; (e) sulfonyl chloride, *i*-Pr_2NEt, 25 °C.

Table 1
Piperidine 4-position replacements



Compound	R^1	GlyT1 IC_{50}, nM[a]	GlyT2 IC_{50}, nM[a]	TauT IC_{50}, nM[a]
5		2.9	>30,000	>30,000
6		26.5	>30,000	>30,000
7		2.8	>30,000	>30,000
8		17.7	nd[b]	nd
9		38.1	nd	nd
10		30.0	>30,000	>30,000

[a] Values are means of at least three experiments.
[b] Not determined.

ney Ni-catalyzed reduction provided mono-Boc protected diamine **4**. Acylation (PS-DCC, HOBt, or carboxylic acid chloride), acidic deprotection of the Boc group, and sulfonamide formation furnished the GlyT1 inhibitors listed in Table 1. Alternatively, the sequence could be modified (Scheme 1b) such that amide formation furnished the final products. Use of both routes enabled the facile generation of both amide and sulfonamide libraries; >300 analogues were prepared according to this procedure and selected data are presented in Table 1.[9]

Generally, replacement of the aryl group was well tolerated, with tetrahydropyran (**5**) and hydroxyl (**7**) functionality providing potency equivalent to **1** and **2**. These substitutions had no effect on selectivity versus GlyT2 and taurine transporter (TauT)—no activity was observed at micromolar concentrations. This level of selectivity (>30,000-fold) is notable among non-sarcosine derived GlyT1 inhibitors.[10] Despite the variety of structures incorporated at the piperidine C4 position and the removal of likely sites of metabolism (aromatic oxidation and *N*-oxide formation), compounds in Table 1 exhibited uniformly poor pharmacokinetic properties (dog Cl > 15 mL/min/kg, $t_{1/2}$ < 2 h).

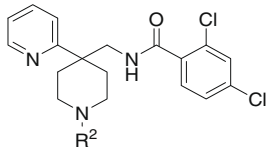
Evaluation of propylsulfonamide modifications was therefore undertaken; a focused sulfonamide library was prepared according to Scheme 1 and results are shown in Table 2. Metabolite profiling of **2** indicated that oxidation occurs on the propyl chain, and potential metabolite **11** lost 20-fold in potency. The corresponding fluoride (**12**), while only 2× less potent than **2**, demonstrated poor pharmacokinetics. Alkyl sulfonamides (**13–17**) were 10- to 50-fold less potent than **2**, with the truncation and extension of the straight alkyl chain by even a single methylene unit having a significant negative effect (**14** and **15**, respectively). Sulfamide (**18**) and trifluorinated alkyl chains (**19** and **20**) were poorly tolerated.

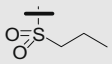
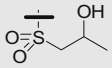
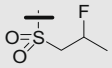
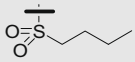
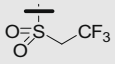
Although the truncated alkyl sulfonamides (**13**, **14**, and **16**) lose potency versus **1** and **2**, they constitute a breakthrough in terms of pharmacokinetic properties (Table 2). These compounds exhibit low clearance (<2 mL/min/kg) and long half lives (>7.5 h) in dogs.

The data cited in Table 2 were generated in a series of dog iv cassette experiments, which was an attractive strategy due to the number of compounds in the library for which pharmacokinetic evaluation was desired.[11] Although there are myriad potential complications associated with dosing in cassette format,[12] within this structural series we noted excellent agreement between iv clearance values determined via cassette and single dose experiments. This strong correlation led to a high degree of confidence in these data, and cassettes were followed with single dose experiments evaluating promising compounds. Based on the dramatic improvement in dog clearance afforded by the shortened alkyl sulfonamides, efforts were focused on enhancing potency with these groups in place.

Previous experience gained during the development of **1** suggested that incorporation of a chiral (*S*) methyl group alpha to the amide nitrogen could confer increases in potency up to 10-fold.[7] The methylated analogue of **14** was therefore prepared according to Scheme 2. Following incorporation of the 2-pyridyl group via a nucleophilic aromatic substitution reaction, the methyl group was installed through Grignard addition (MeMgBr, 25 °C) and reduction ($NaBH_4$) of the resulting imine. Following resolution using chiral chromatography, acylation of the amine, piperidine *N*-Boc deprotection, and sulfonamide formation provided optically pure **21**.[13] Gratifyingly, **21** exhibited improved GlyT1 potency (11 nM) while maintaining complete selectivity versus GlyT2 and TauT; this analogue was not a substrate for human or rat P-glycoprotein (PgP) and displayed excellent passive permeability (37×10^{-6} cm/s).[14] Moreover, **21** retained a favorable dog pharmacokinetic profile (see Fig. 3 and Table 3). Data contained in Fig. 3

Table 2
Propylsulfonamide replacements

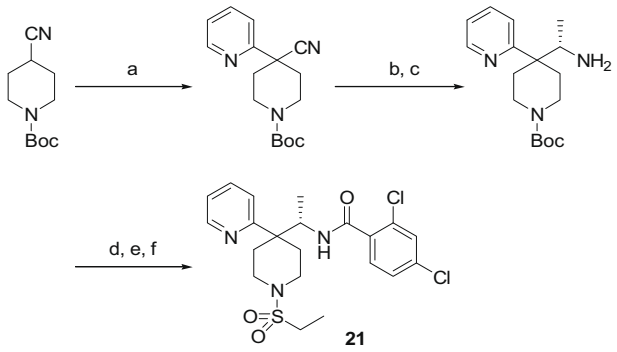


Compound	R^2	GlyT1	GlyT2	TauT	Dog PK[b]	
		IC_{50}, nM[a]	IC_{50}, nM[a]	IC_{50}, nM[a]	Cl[c]	$t_{1/2}$[d]
2		4.4	>30,000	>30,000	15	2.3
11		86	nd[e]	nd		
12		9.7	>30,000	>30,000		
13		300	>30,000	>30,000	1.0	8.4
14		57	>30,000	>30,000	1.4	8.0
15		120	nd	nd		
16		200	>30,000	>30,000	1.7	7.6
17		220	nd	nd		
18		77	nd	nd	11.8	1.9
19		>5000	nd	nd		
20		1070	nd	nd	2.5	4.9

[a] Values are means of at least three experiments.
[b] Determined in dog iv cassettes, 0.25 mg/kg—see text for a discussion.
[c] Clearance in mL/min/kg.
[d] Half life in hours.
[e] Not determined.

are derived from a dog iv cassette experiment and illustrate the utility of the approach. Simple visual inspection indicates the comparative stability of **21** and **22** (vide infra) versus **1**, and while iv clearance values were calculated, in general only a rank order of stabilities for compounds in cassettes was generated. Based on this order, compounds were prioritized for evaluation in single compound pharmacokinetic studies from which quantitative parameters were derived (Table 3). As noted previously, within this series excellent agreement of cassette and single-compound clearance rates was found.

Compound **21** was evaluated in a rat in vivo transporter occupancy assay via oral dosing using the previously described protocol.[15] A plasma Occ_{50} of 150 nM was measured, and this analogue represents the achievement of the desired profile with respect to in vitro potency and selectivity, pharmacokinetics, and in vivo occupancy.



Scheme 2. Reagents and conditions: (a) 2-fluoropyridine, LHMDS, THF, 25 °C; (b) i—MeMgBr, toluene, 25 °C; ii—$NaBH_4$, MeOH; (c) ChiralPak AD; (d) 2,4-dichlorobenzoylchloride, *i*-Pr_2NEt, 25 °C; (e) 4 M HCl/dioxane; (f) $EtSO_2Cl$, *i*-Pr_2NEt, 25 °C.

Table 3
Selected dog pharmacokinetic data

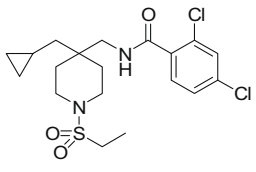
Compound	Cl (cass)[a]	Cl (single)[b]	$t_{1/2}$[b]	Vd_{ss}[b]	*F* (%)[b]
21	3.6	3.5	5.0	1.37	25
22	0.84	2.3	11.8	2.1	38

[a] Dosed as part of an iv cassette, 0.25 mg/kg; see text for a discussion.
[b] Dosed singly, 0.5 mg/kg iv; 1.0 mg/kg po; Cl in mL/min/kg; $t_{1/2}$ in h; Vd_{ss} in L/kg.

An alternative approach to improving pharmacokinetic profile while retaining high GlyT1 potency was also pursued. Libraries were prepared in which potency enhancing piperidine C4 substituents were combined with clearance lowering truncated alkyl sulfonamides. The result was the discovery of **22** (Fig. 2). Unlike the 4-pyridylpiperidine series, where truncation of the propylsulfonamide to an ethylsulfonamide results in a >10-fold decrease in potency (compare **2** and **14** in Table 2), within the 4-cyclopropylmethylpiperidine series the propyl and ethyl sulfonamides are equipotent (for **10** and **22** GlyT1 IC_{50} 30 and 26 nM, respectively).

In the 4-cyclopropylmethylpiperidine series incorporation of a chiral methyl group did not confer an increase in potency (data not shown). Despite these differences, **22** retains the favorable profile of the 4-pyridylpiperidine series. Compound **22** is not a substrate for human or rat PgP. Like **21**, compound **22** exhibits low clearance when dosed in either iv cassette (Fig. 3) or single format (iv and po, Table 3). Compound **22** was suitable for evaluation in the in vivo transporter occupancy assay and has a plasma Occ_{50} of 260 nM.

In conclusion, the pharmacokinetic profile of GlyT1 inhibitors within the 4,4-disubstituted piperidine series has been dramatically improved through modification of the sulfonamide alkyl chain. Accompanying losses in potency could be recovered through the installation of a chiral methyl group alpha to the amide nitrogen and through exchange of the 2-pyridyl group with a cyclopropylmethyl substituent. The improvements include an increase in bioavailability which allowed, for the first time, the measurement



22

Figure 2. Optimized GlyT1 inhibitor.

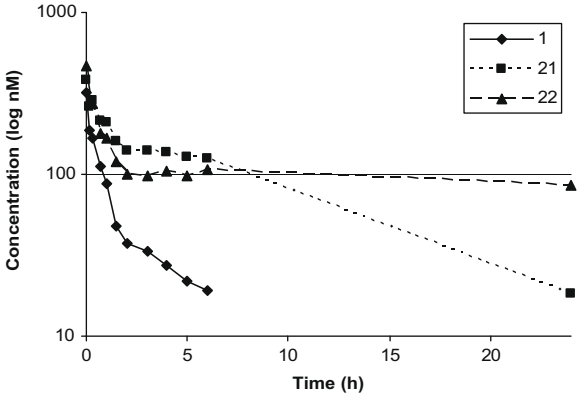


Figure 3. Dog iv cassette data; 0.25 mg/kg; see text for a discussion.

of in vivo transporter occupancy using oral dosing. Compounds **21** and **22** were identified which occupy brain GlyT1 in vivo at plasma concentrations <300 nM. The use of an iterative analogue library approach enabled the optimization of both potency and pharmacokinetic properties despite non-additive SAR. Key to the successful discovery of enhanced inhibitors was the extensive use of cassette dosing in dog pharmacokinetic experiments which, when combined with judicious follow up in single dose experiments, allowed the screening of hundreds of compounds for improved pharmacokinetics.

Acknowledgments

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