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Discovery of *N*-{[1-(propylsulfonyl)-4-pyridin-2-ylpiperidin-4-yl]methyl}-benzamides as novel, selective and potent GlyT1 inhibitors

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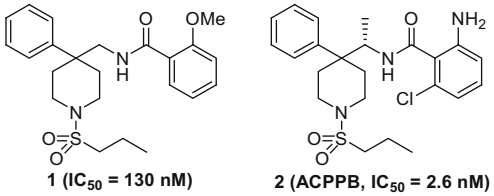
ABSTRACT

Employing an iterative analogue library approach, novel potent and selective glycine transporter 1 (GlyT1) inhibitors containing a 4-pyridin-2-ylpiperidine sulfonamide have been discovered. These inhibitors are devoid of time-dependent CYP inhibition activity and exhibit improved aqueous solubility versus the corresponding 4-phenylpiperidine analogues.

A growing body of evidence suggests that hypofunction of *N*-methyl-D-aspartate (NMDA) glutamatergic receptors may play an important role in the pathophysiological development of schizophrenia.[1] To test this NMDA hypofunction hypothesis of schizophrenia, compounds that activate NMDA receptors are needed. However, NMDA receptor agonists that target the glutamate binding site tend to be neurotoxic and lead to cell death.[2] An alternative approach is to modulate NMDA receptor activation indirectly, for example by increasing the levels of glycine, an endogenous, obligatory co-agonist with glutamate for NMDA receptor activation.[3] It is known that NMDA receptor activity can be enhanced by elevating the concentration of glycine in the local microenvironment of NMDA receptor-containing synapses.[4] Glycine levels in the brain are regulated by glycine transporter 1 (GlyT1) which appears to be largely expressed at glutamatergic synapses,[5] and inhibition of GlyT1 leads to increased local glycine concentrations and activation of synaptic NMDA receptors.[6] Therefore, increasing the activity of NMDA receptors by inhibition of GlyT1 has emerged as an attractive approach to test the NMDA hypofunction hypothesis and potentially provide a treatment of schizophrenia.[7] This approach is supported by clinical trials in which treatment of schizophrenic patients with glycine itself or sarcosine, a weak GlyT1 inhibitor, improved symptoms.[8] Because of the drawbacks with these compounds (weak GlyT1 inhibition, poor pharmacokinetics and brain penetration), numerous efforts have been reported to identify improved GlyT1 inhibitors.[9,10]

Recently, we reported the optimization of HTS lead **1** using a focused library approach which identified 2-amino-6-chloro-*N*-((4-phenyl-1-(propylsulfonyl)piperidin-4-yl)methyl)benzamide (ACPPB, **2**) as a novel potent (IC_{50} = 2.6 nM) and selective (vs glycine transporter 2 (GlyT2) and taurine transporter (TauT)) GlyT1 inhibitor based on a 4,4-disubstituted piperidine core structure (Fig. 1). Further in vivo evaluation demonstrated that **2** not only increases glycine levels in rat prefrontal cortex (PFC), but also



1 (IC_{50} = 130 nM) **2** (ACPPB, IC_{50} = 2.6 nM)

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

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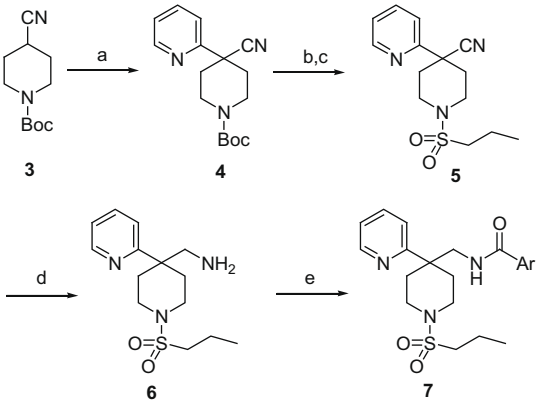
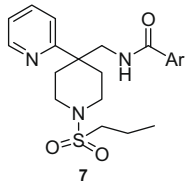


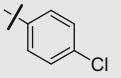
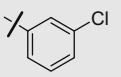
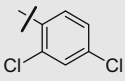
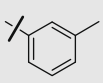
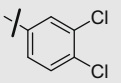
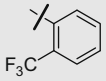
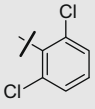
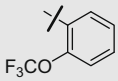
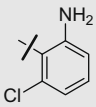
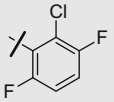
Scheme 1. Reagents and conditions: (a) KHMDS, THF, 25 °C, then 2-fluoropyridine; (b) 4 M HCl/dioxane; (c) *n*-$PrSO_2Cl$, *i*-Pr_2NEt, CH_2Cl_2, 0–25 °C; (d) H_2, Raney Ni; 2M NH_3 in MeOH, 25 °C; (e) i—ArCOCl, *i*-Pr_2NEt, CH_2Cl_2, 0–25 °C, or ii—$ArCO_2H$, PS-DCC, HOBt, *i*-Pr_2NEt, CH_2Cl_2, 25 °C, high throughput LC–MS purification.[12]

significantly enhances prepulse inhibition (PPI) in DBA/2J mice without impairing basal startle amplitude indicating an antipsychotic effect without sedation.[10] However, time-dependent CYP inhibition and poor water solubility prevented further development of **2**.

Table 1
Human GlyT1 inhibition of 2-pyridyl substitution at piperidine 4-position



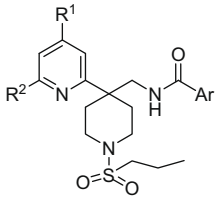
7

Compound	Ar	GlyT1 IC_{50}[a] (nM)	Compound	Ar	GlyT1 IC_{50}[a] (nM)
7a		107	**7h**		103
7b		49.4	**7i**		517
7c		19.5	**7j,CPyPB**		4.4
7d		833	**7k**		131
7e		31.3	**7l**		6.6
7f		19.4	**7m**		6.9
7g		11.9	**7n,CFPyPB**		5.7

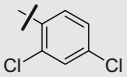
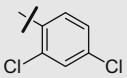
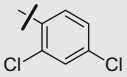
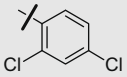
All compounds >30,000 nM versus GlyT2 and TauT.
[a] IC_{50} are average of at least 3 measurements.

In this letter, we report the results of our search for new selective and potent GlyT1 inhibitors with improved physical properties and diminished time-dependent CYP inhibition. Early SAR studies in the 4,4-disubstituted piperidine series suggested that a benzamide and an *n*-propyl sulfonyl group on the 1-nitrogen of the piperidine core are required for maximizing GlyT1 potency. Therefore, effort was focused on replacing the phenyl group on the C4 position of the piperidine core. Our first library simply replaced the C4-phenyl with a 2-pyridyl. As illustrated in Scheme 1, deprotonation of Boc-protected 4-cyanopiperidine **3** and subsequent reaction with 2-fluoropyridine generated **4**.[11] Deprotection of the Boc group, sulfonylation, and Raney Ni-catalyzed hydrogenation afforded the key intermediate amine **6**. Amide formation could be achieved by either direct acylation of amine **6** with an acid chloride

Table 2
Human GlyT1 inhibition of substituted 2-pyridyl substitution at piperidine 4-position



8--11

Compound	Ar	R^1	R^2	GlyT1 IC_{50}[a] (nM)
8a		CH_3	H	129
8b		CH_3	H	38.3
8c		CH_3	H	3.5
9a		H	CH_3	54.7
9b		H	CH_3	3.0
9c		H	CH_3	2.5
10a		H	OCH_3	48.3
10b		H	OCH_3	6.9
10c		H	OCH_3	7.4
11a		H		>5000
11b		H		482
11c		H		412

Compounds **8a–8c, 9a–9c**, and **10a–10c** >30,000 nM versus GlyT2 and TauT.
[a] IC_{50} are average of at least 3 measurements.

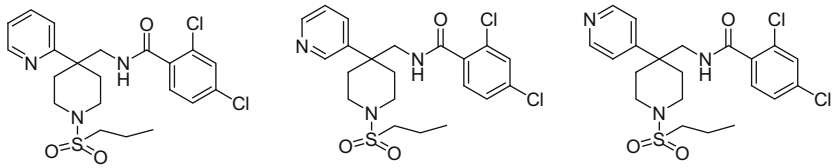


Figure 2. IC_{50} of 2-, 3- and 4-pyridyl in the same benzamide.

or coupling of **6** with a carboxylic acid, polymer-bound DCC (PS-DCC), HOBt, and *i*-Pr_2NEt. Accordingly, >200 benzamide analogues were synthesized in iterative focused libraries employing solution phase parallel synthesis. Selected compounds and data are summarized in Table 1.

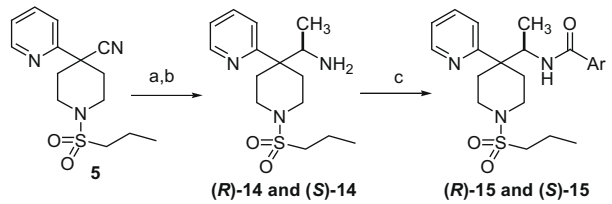
To our delight, the 2-pyridyl substituted compounds, exemplified by **7a–7n**, not only retain GlyT1 potency, but also maintain high selectivity versus GlyT2 and (TauT) even without the α-methyl group present in **2**. Table 1 reveals the GlyT1 inhibition SAR in the 2-pyridyl series. The GlyT1 potency is enhanced by an *ortho*-substitution on the phenyl ring of the benzamide moiety. This is clearly demonstrated by the 10× increase in potency from compound 7**a** to **7g**, where an *o*-chlorine is incorporated. Significantly, in this 2-pyridyl series, benzamides lacking the aniline present in **2** were identified which exhibit potency equivalent to **2** (compare **7j** and **7n** to **7m**). Further evaluation of **7j** (2,4-dichloro-*N*-((1-(propylsulfonyl)-4-(pyridin-2-yl)piperidin-4-yl)benzamide, CPyPB) and **7n**(3,6-dichloro-2-fluoro-*N*-((1-(propylsulfonyl)-4-(pyridine-2-yL)piperidin-4-yl)methyl)benzamide, CFPyPB) showed that they are superior to **1**: (1) Neither **7j** nor **7n** are P-gp substrates, a preferred for any compound to be developed as a CNS drug; (2) Neither **7j** nor **7n** are time-dependent CYP inhibitors which is an issue for **2**; and (3) both **7j** and **7n** have improved water solubility compared to **2** (**2** and its HCl salt are insoluble in water (<50 μg/mL); the HCl salt of **7n** and **7j** achieve water solubility of 570 μg/mL and 850 μg/mL, respectively). Moreover, **7n** exhibits effects in rodent microdialysis and pre-pulse inhibition studies equivalent to **2**.[13]

Following the same route illustrated in Scheme 1, different substituted 2-pyridyl libraries were prepared to investigate substituent effects at the 2-pyridyl ring. Selected compounds and GlyT1 inhibition data are presented in Table 2. In general, small substituents at the 4-position (4-Me, **8a–8c**) or 6-position (6-Me, **9a–9c**; 6-MeO, **10a–10c**) on the 2-pyridyl ring are well tolerated. In fact, in the cases of both 4-methyl and 6-methyl group substitution, GlyT1 potencies are slightly increased by the addition of a methyl group to the pyridyl ring (compare **8c** and **9c** with **7j**). However, a large, polar group such as morpholinyl, on the 6-position of the 2-pyridyl ring is not tolerated and dramatically decreases GlyT1 inhibition as illustrated by the comparison of **7j** and **11c**.

It should be mentioned that the nitrogen position at the pyridine ring is very important for GlyT1 potency based on our observations from libraries of 3- and 4-pyridyl substitution at the piperidine C4-position (These libraries were prepared following the same route shown in Scheme 1.). Replacement of 2-pyridyl in **7j** with 3- or 4-pyridyl leads to a significant potency loss as indicated in Figure 2.

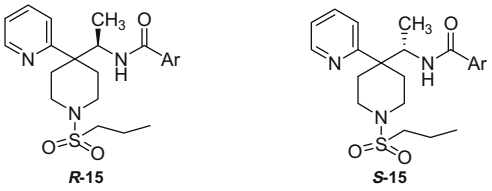
In an effort to evaluate the effects of a chiral methyl group α-position to the benzamide nitrogen, libraries **(*R*)-15** and **(*S*)-15** were prepared (Scheme 2). The chiral methyl group was introduced to **5** by methyl Grignard addition followed by $NaBH_4$ reduction of the intermediate imine.[14] The resulting racemic amines were separated by chiral HPLC to provide the enantiomerically pure **(*R*)-14** and **(*S*)-14**. Amide formation of pure **(*R*)-14** or **(*S*)-14** with 80 carboxylic acids or acid chlorides afforded the libraries **(*R*)-15** or **(*S*)-15**, respectively. Selected compounds and data are presented in Table 3. Two features stand out in this chiral α-methyl benzamide series. Firstly, this series displays highly enantioselective inhibition of GlyT1; all *S* isomers are much more potent then their *R* analogues. Secondly, the *S*-methyl analogues are equipotent to the analogues lacking an α-methyl group. This is in contrast to the 4-phenylpiperidine series where α-methyl substitution boosted potency up to 10-fold.[10]

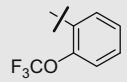
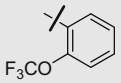
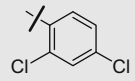
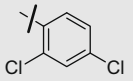
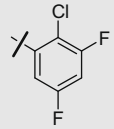
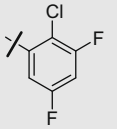
To explore how more dramatic structural changes affect GlyT1 activity, the planar 2-pyridyl group in the compound **7** series was replaced with the nonplanar 2-(1-methylpiperidinyl) moiety which resulted in a bispiperidine library **18** (48 analogues were prepared). The chemistry employed to incorporate the 2-(*N*-methylpiperidyl) group into library **18** is shown in Scheme 3. Although the potency decreases slightly (Table 4) from the 2-pyridyl to the



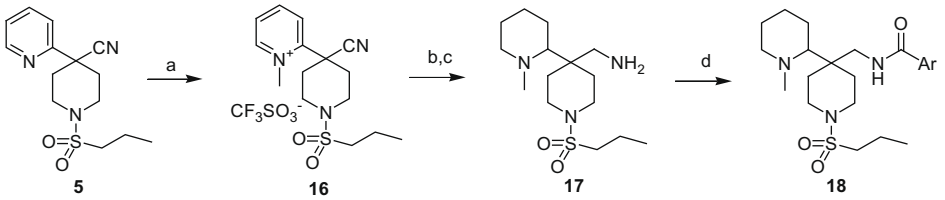
Scheme 2. Reagents and conditions: (a) i—MeMgBr, toluene, 25 °C; ii—$NaBH_4$, MeOH; (b) ChiralPak AD; (c) i—ArCOCl, *i*-Pr_2NEt, CH_2Cl_2, 0– 25 °C, or ii—$ArCO_2H$, PS-DCC, HOBt, *i*-Pr_2NEt, CH_2Cl_2, 25 °C, high throughput LC–MS purification.[12]

Table 3
Human GlyT1 activity of substituted 2-pyridyl substitution at piperidine 4-position



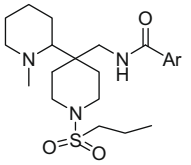
Compound	Ar	GlyT1 IC_{50}[a] (nM)	Compound	Ar	GlyT1 IC_{50}[a] (nM)
(*R*)-15a		2592	**(*S*)-15a**		17.7
(*R*)-15b		661	**(*S*)-15b**		3.0
(*R*)-15c		576	**(*S*)-15c**		4.0

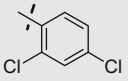
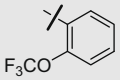
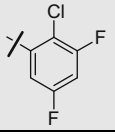
Compounds **(*S*)-15a–c** >30,000 nM versus GlyT2 and TauT.
The *R* and *S* configurations were determined by X-ray analysis of the Morsher amide of amine **(*S*)-14**.
[a] IC_{50} are average of at least 3 measurements.



Scheme 3. Reagents and conditions: (a) CF_3SO_3Me, 0–25 °C; (b) $MPBH_4$, MeOH 25 °C; (c) H_2, Raney Ni; 2 M NH_3 in MeOH, 25 °C; (d) i—ArCOCl, *i*-Pr_2NEt, CH_2Cl_2, 0–25 °C, or ii—$ArCO_2H$, PS-DCC, HOBt, *i*-Pr_2NEt, CH_2Cl_2, 25 °C, high throughput LC–MS purification.[12]

Table 4
Human GlyT1 activity of *N*-methylpiperidine substitution at piperidine 4-position



Compound	Ar	GlyT1 IC_{50}[a] (nM)	Compound	Ar	GlyT1 IC_{50}[a] (nM)
18a		3314	**18d**		58.9
18b		148	**18e**		9.5
18c		21.2	**18f**		43.1

[a] IC_{50} are average of at least 3 measurements.

2-(*N*-methylpiperidinyl) substitution at the central piperidine C4 position, the addition of a more basic amine is anticipated to improve physical properties within this series.

In summary, by modification of the piperidine C4-substituent of lead compounds **1** and **2**, we have discovered two series of potent and selective GlyT1 inhibitors through a library approach. New compounds **7j** and **7n** from 2-pyridyl library not only improved physical properties (e.g. greater water solubility) but also overcame undesirable time-dependent CYP inhibition. Further results within this series will be disclosed in due course.

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