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Synthesis and SAR of GlyT1 inhibitors derived from a series of *N*-((4-(morpholine-4-carbonyl)-1-(propylsulfonyl)piperidin-4-yl)-methyl)benzamides

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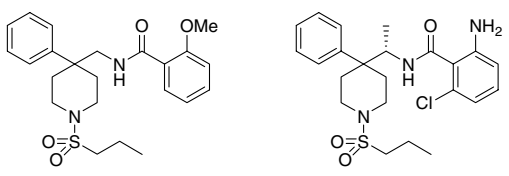
Abstract—This Letter describes the synthesis and SAR, developed through an iterative analog library approach, of potent and selective non-sarcosine-derived GlyT1 inhibitors.

Schizophrenia is a complex psychiatric disorder characterized by a combination of negative, positive, and cognitive symptoms that requires lifelong daily maintenance therapy.[1] Historically, schizophrenia has been managed clinically by treatment with dopamine D_2 antagonists (i.e., the dopamine hyperfunction hypothesis); however, this strategy only improves the positive symptoms of the disease—negative and cognitive symptoms remain.[2] Recent data suggest that *N*-methyl-D-aspartate (NMDA) receptor hypofunction is involved in the pathophysiology of schizophrenia, that is, the NMDA hypofunction (or glutamate dysfunction) hypothesis, as an alternative theory for the underlying causes of the disease. According to this hypothesis, any agent that can potentiate NMDA receptor currents has the potential to ameliorate the symptoms of schizophrenia.[3,4]

In the forebrain, glycine is a required co-agonist for the NMDA receptor and modulates NMDA-dependent excitatory neurotransmission; therefore, elevation of synaptic glycine levels should enhance NMDA receptor function.[5] In the CNS, glycine levels are tightly maintained by two transporters: GlyT1 and GlyT2.[6] GlyT1 expression in the neocortex mirrors NMDA receptor expression suggesting that GlyT1 is optimally positioned to modulate glycine levels near NMDA receptor-expressing synapses.[7] Hence, increasing the activity of NMDA receptors by inhibition of GlyT1 has emerged as an attractive target for drug discovery efforts. Early work in this arena focused on sarcosine-derived GlyT1 inhibitors which validated this approach in animal models, but suffered from poor pharmacokinetics (PK) and brain penetration.[1–4] Based on these results, numerous pharmaceutical companies have launched efforts to identify non-sarcosine-derived GlyT1 inhibitors, and reports are beginning to accrue.

Recently, we reported on the optimization of HTS lead **1** to afford **2**, a potent (GlyT1 IC_{50} = 2.6 nM) and selective (versus GlyT2 and TauT) GlyT1 inhibitor based on



1 (IC_{50} = 135 nM) **2** (IC_{50} = 2.6 nM)

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

Keywords: Schizophrenia; GlyT1; Inhibitor; Piperidine.
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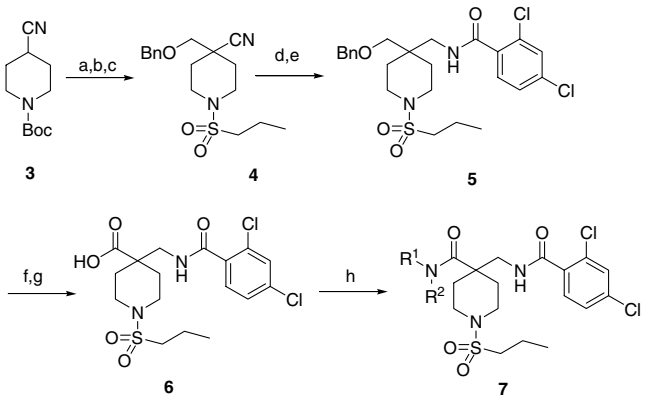


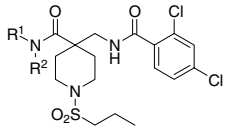
Scheme 1. Reagents and conditions: (a) KHMDS, THF, 25 °C, then $BnOCH_2Cl$, 82%; (b) 4 M HCl/dioxane, 100%; (c) *n*-$PrSO_2Cl$, *i*-Pr_2NEt, CH_2Cl_2, 0–25 °C, 95%; (d) H_2, Raney Ni; 2 M NH_3 in MeOH, 25 °C, 95%; (e) 2,4-diClPhCOCl, *i*-Pr_2NEt, CH_2Cl_2, 0–25 °C, 95%; (f) TMSI, AcCN, 25 °C, 95%; (g) cat. $RuCl_3/NaIO_4$, AcCN–CCl_4–H_2O, 88%; (h) NHR^1R^2, PS-DCC, HOBt, *i*-Pr_2NEt, CH_2Cl_2, 25 °C, 72–91%. All compounds purified by mass-guided HPLC.[9]

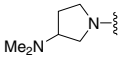
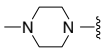
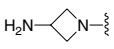
a 4,4-disubstituted piperidine scaffold (Fig. 1).[8] In vivo, **2** selectively increased glycine levels in the prefrontal cortex (340% of basal) and significantly enhanced prepulse inhibition (PPI) in DBA/2J mice, suggesting an antipsychotic profile. However, poor aqueous solubility, high log *P* (>3.5), and strong time-dependent CYP inhibition (TDI) prevented the further development of **2** and related analogs.[8]

In this Letter, we report the results of further lead optimization efforts, through an iterative analog library synthesis approach, of **2** to improve physical properties and eliminate CYP TDI. From past experience, we surmised that the 4-phenyl moiety in **2** might be the cause of the CYP TDI as well as contributing to the undesirable high log *P*. Early work in this series indicated that the propyl sulfonamide was required for maximal GlyT1 activity, but that a wide range of benzamides were tolerated.[8] Therefore, our initial libraries explored alternative, polar moieties in the 4-position while holding the propylsulfonamide and 2,4-dichlorobenzamide constant. For the scope of this Letter, replacement of the 4-phenyl moiety with polar 4-carboxamides will be discussed.

As illustrated in Scheme 1, commercially available *N*-Boc-4-cyanopiperdine **3** is deprotonated and the resulting anion trapped with $BnOCH_2Cl$, followed by deprotection of Boc and sulfonylation to provide analogs **4**. Raney Ni-catalyzed hydrogenation and subsequently acylation with 2,4-dichlorobenzoyl chloride provided analogs **5**.

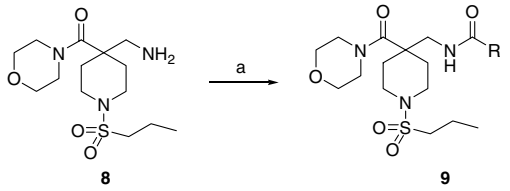
Table 1. Structures and activities of carboxamide analogs **7**



Compound	NR^1R^2	hGlyT1 IC_{50}[a] (nM)	rGlyT1 IC_{50}[a] (nM)	mGlyT1 IC_{50}[a] (nM)
7a		181	ND	ND
7b		517	ND	ND
7c		544	ND	ND
7d		472	ND	ND
7e		>5000	ND	ND
7f		69	ND	ND
7g		109	ND	ND
7h		2.4	3.2	7.6

All compounds >30,000 nM versus GlyT2 and TauT.

[a] Average of at least three measurements[11]; ND, not determined.

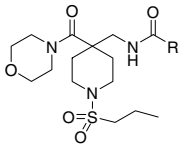


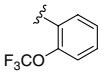
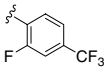
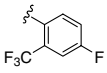
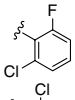
Scheme 2. Reagents and conditions: (a) RCOCl, *i*-Pr_2NEt, CH_2Cl_2, 0–25 °C, 95% or (i) RCOOH, PS-DCC, HOBt, DCM (ii) MP-carbonate, 70–88%. All compounds purified by mass-guided HPLC.[9]

Deprotection of the benzyl group and subsequent oxidation afforded the carboxylic acid **6** which was coupled with an amine to give amide **7**. Following this scheme, 50 analogs of **7** were synthesized and evaluated in our GlyT1 inhibition assay. Table 1 highlights selected compounds and data.

Over 80% of the analogs prepared were inactive with only a small number showing appreciable activity; however, the morpholino amide analog, **7h**, was a clear stand-out with low nanomolar inhibition of human, rat, and mouse GlyT1 (IC_{50}s of 2.4, 3.2, and 7.6 nM, respectively) with no inhibition of GlyT2 or TauT (taurine transporter). Unlike our previous 4-phenyl series of inhibitors, **1** and **2**, **7h** displayed good aqueous solubility (~18 mg/mL in saline), no CYP (3A4, 2C9, 2D6) inhibition, and no CYP 3A4 TDI. Compound **7h** displayed moderate pharmacokinetics

Table 2. Structures and activities of amide analogs **9**



Compound	R	hGlyT1 IC_{50}[a] (nM)	rGlyT1 IC_{50}[a] (nM)	mGlyT1 IC_{50}[a] (nM)
9a		>5000	ND	ND
9b		>5000	ND	ND
9c		264	ND	ND
9d		88.4	ND	ND
9e		12.6	10.1	28.5
9f		42.8	ND	ND
9g		67.8	ND	ND
9h		8.4	19.7	42.8
9i		12.5	22.1	57.3
9j		6.3	7.4	19.4
9k		131	ND	ND
9l		9.7	18.3	95.3

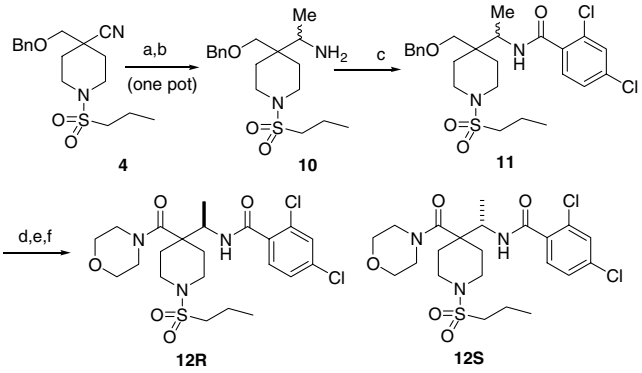
All compounds >30,000 nM versus GlyT2 and TauT.

[a] Average of at least three measurements[11]; ND, not determined.

(PK) in rats (Cl = 20.5 mL/min/kg, $t_{1/2}$ = 0.37 h, V_D = 0.38). In addition, the log *P* of **7h** was measured to be 1.4, marking a pronounced increase in polarity by incorporation of the morpholino amide moiety relative to **2**. As a consequence **7h** proved to be a mouse and human p-glycoprotein (P-gp) substrate (B − A/A − B ratios of 11.4 and 17.9, respectively); however, excellent passive permeability was maintained (Papp = 23.6×10^{-6} cm/s).[10] Despite overcoming several limitations of the 4-phenyl series (**1** and **2**), the P-gp data, predicting poor CNS exposure for **7h**, prevented further in vivo experiments from being conducted.

Encouraged by **7h**, additional libraries were prepared, according to Schemes 1 and 2, that examined alternative amide moieties while keeping the 4-morpholino amide functionality constant. In total, 96 compounds were synthesized and screened in our GlyT1 assay. Table 2 highlights selected compounds and data from this effort which generated a number of potent and selective (>30,000 nM versus GlyT2 and TauT) GlyT1 inhibitors with diverse benzamide moieties. In general, aliphatic amides, such as **9a** and **9b**, were inactive as were all heterocyclic amides (pyridyl, thienyl, etc., data not shown). Benzamides were uniformly active. For instance, unsubstituted phenyl, **9c**, had a GlyT1 IC_{50} of 264 nM which could be dramatically increased by the addition of a single halogen atom as in **9l**, possessing a 2-chloro substituent, with low nanomolar inhibition of human, rat, and mouse GlyT1 (IC_{50}s of 9.7, 18.3, and 95.3 nM, respectively). A wide-range of halogenation patterns about the phenyl ring of the benzamide were found to be tolerated. The most potent inhibitor from this library, **9j**, possessed a 2,4-dichloro-5-fluorobenzamide moiety and displayed low nanomolar inhibition of human, rat, and mouse GlyT1 (IC_{50}s of 6.3, 7.4, and 19.4 nM, respectively); however, this effort failed to identify a compound more potent than **7h**. Similar to **7h**, all analogs **9** displayed good aqueous solubility (10–25 mg/mL in saline), no CYP (3A4, 2C9, and 2D6) inhibition, no CYP 3A4 TDI, and displayed moderate PK in rats (Cl = 18–25 mL/min/kg, $t_{1/2}$ = 0.30–1.4 h, V_D = 0.30–0.52). Unfortunately, all of the analogs **9** were found to be mouse and human P-gp substrates (B − A/A − B ratios of 8.9–19 and 10.6–26.1, respectively) yet displayed excellent passive permeability (Papp > 22×10^{-6} cm/s).[10] In contrast, the 4-phenyl series, such as **1** and **2**, uniformly were not P-gp substrates.[8] This was surprising as analogs **9** did not incorporate additional hydrogen bond donors relative to the 4-phenyl series; however, the increase in P-gp susceptibility could be the result of increased polarity (log *P* of <1.4 for analogs **9** versus >3.0 for analogs **2**). The P-gp results did prove to be indicative of low CNS penetration for this series, as **7h** displayed low nanomolar total brain levels from brain:plasma studies in rats.

In the 4-phenyl series, the introduction of a (*S*)-methyl group, as shown in **2**, improved intrinsic GlyT1 potency and, for a number of analogs with borderline mouse P-gp susceptibility (B − A/A − B ratios of ~5.6) eliminated the P-gp susceptibility (B − A/A − B ratios of 2.2).[8] According to Scheme 3, both the enantiopure (*R*)- and



Scheme 3. Reagents and conditions: (a) (i) MeMgBr, toluene, 25–40 °C, (ii) anhydrous MeOH; (b) $NaBH_4$, 0–25 °C, 63% (two steps); (c) 2,4-dichlorobenzoyl chloride, *i*-Pr_2NEt, 0–25 °C, 93%; (d) TMSI, AcCN, 25 °C, 95%; (e) cat. $RuCl_3/NaIO_4$, AcCN–CCl_4–H_2O, 84%; (f) (i) morpholine, PS-DCC, HOBt, *i*-Pr_2NEt, CH_2Cl_2, 25 °C, (ii) Chiral pack AD, 32% for **12R** and 37% for **12S**.

(*S*)-enantiomers of **7h** were prepared, **12R** and **12S**, respectively, and screened in our GlyT1 assay. To our surprise, both **12R** and **12S** were inactive (GlyT1 IC_{50} > 5000 nM); clearly, the SAR for the 4-morphoino amide series was distinct from the 4-phenyl series.

As the SAR for this series was distinct from our earlier work,[8] we next explored a multi-dimensional library approach (Fig. 2) wherein we simultaneously evaluated alternative sulfonamides, diverse benzamides, and alternative cappings for the eastern amino methyl group (2°- and 3°-amines, ureas, and sulfonamides), **13**. This effort afforded over 100 additional analogs that were evaluated in our GlyT1 assay. Only benzamide moieties were tolerated as capping agents for the eastern amino methyl group—basic amines, ureas, and sulfomamides possessed no GlyT1 inhibitory activity. Alternative sulfonamides for the piperidine amine maintained some GlyT1 inhibition, but at a loss of 90- to 500-fold. Based on these data and the lack of brain penetration for **7h** and related congeners, the 4-morpholino amide series was not developed further.

In summary, we have developed a novel series of potent and selective non-sarcosine-derived GlyT1 inhibitors. Compounds from this series, such as **7h**, addressed several of the liabilities of our early 4-phenyl series, depicted by **1** and **2**. Specifically, incorporation of the 4-morpholino amide moiety increases polarity (log *P* ~1.4), improves aqueous solubility (10–25 mg/mL in saline), and eliminates the CYP TDI which plagued the 4-phenyl series. However, while the increased

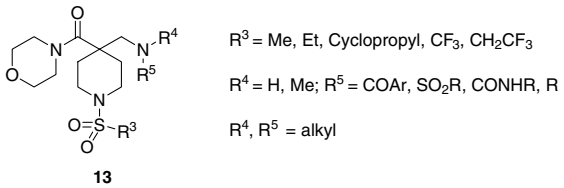


Figure 2. Multi-dimensional library generic **13**.

polarity of this series overcame several liabilities, it also engendered this series to be P-gp substrates and therefore not CNS penetrant. Further refinements to this general scaffold are in progress and additional SAR and biological studies will be reported in due course.

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