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Novel GlyT1 inhibitor chemotypes by scaffold hopping. Part 2: Development of a [3.3.0]-based series and other piperidine bioisosteres

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ABSTRACT

This Letter describes the development and SAR of a novel series of GlyT1 inhibitors derived from a scaffold hopping approach, in lieu of an HTS campaign, which provided intellectual property position. Members within this new [3.3.0]-based series displayed excellent GlyT1 potency, selectivity, free fraction, and modest CNS penetration. Moreover, enantioselective GlyT1 inhibition was observed, within this novel series and a number of other piperidine bioisosteric cores.

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Scaffold hopping has emerged as an attractive approach to rapidly access new chemical space and enable fast-follower programs without the need for expensive and time-consuming HTS campaigns.^{1–4} As the negative symptom cluster in schizophrenia remains a critical unmet medical need,^{5–7} and GlyT1 inhibition has been shown to be affective toward negative symptoms in Phase II clinical trials,^{8–14} we initiated scaffold hopping efforts to expediently develop novel GlyT1 inhibitors within a crowded intellectual property (IP) space. In a recent Letter, we reported on our preliminary scaffold hopping exercise (Fig. 1) employing GlyT1 inhibitors from Merck and Pfizer, **1** and **2**, respectively, that generated a novel, patented series exemplified by **3**.¹⁵ Notably, **3** was a potent GlyT1 inhibitor with an exceptional DMPK profile, high CNS penetration and robust efficacy in preclinical models of schizophrenia.¹⁵

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Based on work from our labs with mGlu₁ NAMs, and the ability of [3.3.0] systems, such as the octahydropyrrolo[3,4-c]pyrrole, to effectively mimic piperazines,¹⁶ we focused our attention on the potential bioisosteric replacement of the [3.1.0] system of **2** and **3**, as well as the piperidine of **1**, with a [3.3.0] system, an octahydrocyclopenta[*c*]pyrrole, **5**, and effectively scaffold hop from analogs **4** (Fig. 2). If successful at maintaining GlyT1 inhibitory activity, this

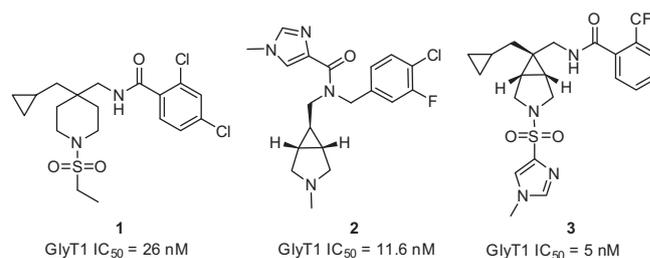


Figure 1. Reported GlyT1 inhibitors **1** (Merck) and **2** (Pfizer), and the novel series **3** (VU0240391), derived from scaffold hopping.

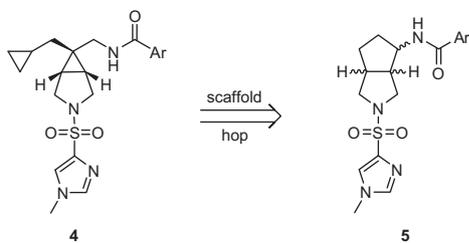
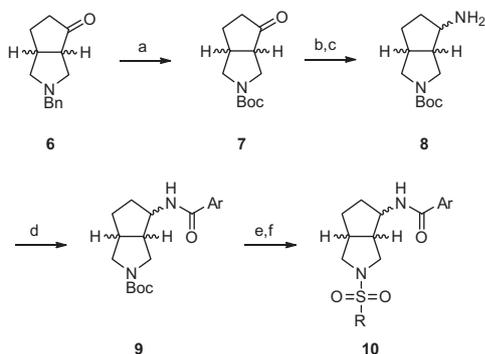


Figure 2. Envisioned scaffold hopping from the novel series **4** to a [3.3.0]-core, an octahydrocyclopenta[*c*]pyrrole, **5**.



Scheme 1. Reagents and conditions: (a) Boc_2O , $\text{Pd}(\text{OH})_2/\text{C}$, H_2 (50 psi), EtOH, rt; (b) NH_2OH , MeOH, 100°C ; (c) 'Raney' Ni, H_2 (50 psi), rt; (d) ArCOCl , DIEPA, CH_2Cl_2 , 0°C ; (e) 4 N HCl/dioxane, rt; (f) RSO_2Cl , DIEPA, CH_2Cl_2 , rt. Overall yields range from 10–34%.

would represent a major structural change, eliminating the pendant cyclopropylmethyl moiety while introducing an additional chiral center (providing an opportunity for enantioselective activity).

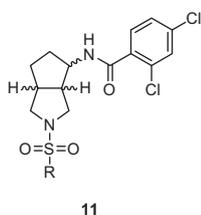
Synthetically, analogs **10** were initially prepared as racemates via a six step route that proceeded in ~22% overall yield

(Scheme 1). Commercial racemic, 90% *cis*-benzylhexahydrocyclopenta[*c*]pyrrol-4(2*H*)-one **6** was subjected to hydrogenation conditions to deprotect the benzyl moiety in the presence of Boc_2O to provide **7**. Conversion of the ketone to the oxime, followed by 'Raney' nickel reduction generated the racemic primary amine **8**, which was subsequently acylated with a variety of benzoyl chlorides to deliver analogs **9**. Finally, the Boc moiety was removed with HCl, and the secondary pyrrolidine nitrogen capped with various sulfonyl chlorides to afford analogs **10**.

Initially, we held the 2,4-dichlorobenzamide constant and surveyed a wide-range of sulfonamides in analogs **11** (Table 1). Unlike the piperidine **1** and [3.1.0] series **3**, few sulfonamide moieties were tolerated. Ethyl (**11a**) and propyl congeners (**11b**) that were very potent in the piperidine series **1**, afforded inactive compounds (GlyT1 $\text{IC}_{50} > 10\ \mu\text{M}$). Aryl and heteroaryl analogs, such as **11d–11f**, were also devoid of GlyT1 activity. Only the *N*-methyl imidazole (**11g**) and the *N*-methyl triazole (**11h**) derivative were active,¹⁵ both displayed low nanomolar potency (GlyT1 IC_{50} s of 25 nM and 15 nM, respectively) and were selective versus GlyT2 ($\text{IC}_{50} > 30\ \mu\text{M}$). Based on the disposition previously noted for the *N*-methyl imidazole sulfonamide in **3**, we prepared a second library held the *N*-methyl imidazole sulfonamide moiety constant, and surveyed a broader range of amides in analogs **12** (Table 2).

The SAR was far more shallow than in the case of **3**,¹⁵ with the 2,4-dichlorobenzamide (**11g/12a**) possessing optimal potency. Other analogs such as the 2-trifluoromethylbenzamide (**12b**) and the 2-chlorobenzamide (**12c**) were respectable, with GlyT1 IC_{50} s of $112 \pm 6\ \text{nM}$ and $115 \pm 18\ \text{nM}$, respectively. The vast majority of other substitution patterns afforded a considerable loss in potency (GlyT1 IC_{50} s from 631 nM to $10\ \mu\text{M}$), as did a cyclohexyl amide congener **12l** (GlyT1 $\text{IC}_{50} = 617\ \text{nM}$). To ensure that the major structural change in scaffold hopping from **1** to **3** to **12** did not alter the competitive mechanism of action of GlyT1 inhibition, we evaluated the affect of **12b** on enzyme kinetics of [^{14}C]-glycine transport. As shown in an Eadie–Hoffstee plot (Fig. 3), this [3.3.0] series, represented by **12b**, competitively inhibits the enzyme kinetics of [^{14}C]-glycine transport. Thus, this series is competitive

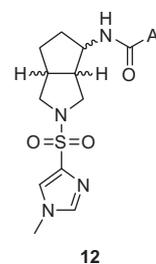
Table 1
Structures and activities of analogs **11**



Compound	R	GlyT1 IC_{50}^a (μM)	GlyT2 IC_{50}^a (μM)
11a		>10	>30
11b		>10	>30
11c		>10	>30
11d		>10	>30
11e		>10	>30
11f		>10	>30
11g		0.025	>30
11h		0.015	>30

^a IC_{50} s represent single determinations performed in duplicate.

Table 2
Structures and activities of analogs **12**



Compound	R	GlyT1 IC_{50}^a (μM)	GlyT2 IC_{50}^a (μM)
12a (11g)	2,4-diClPh	25	>30
12b	2-CF ₃ Ph	112 ^b	>30
12c	2-ClPh	115 ^b	>30
12d	2,4-diFPh	926	>30
12e	2,6-diFPh	631	>30
12f	2-FPh	1815	>30
12g	3-FPh	>10,000	>30
12h	4-FPh	2215	>30
12i	3,4-diFPh	1569	>30
12j	4-ClPh	1029	>30
12k	3,4-diClPh	891	>30
12l		617	>30

^a IC_{50} s represent single determinations performed in duplicate.

^b The average of four determinations performed in duplicate.

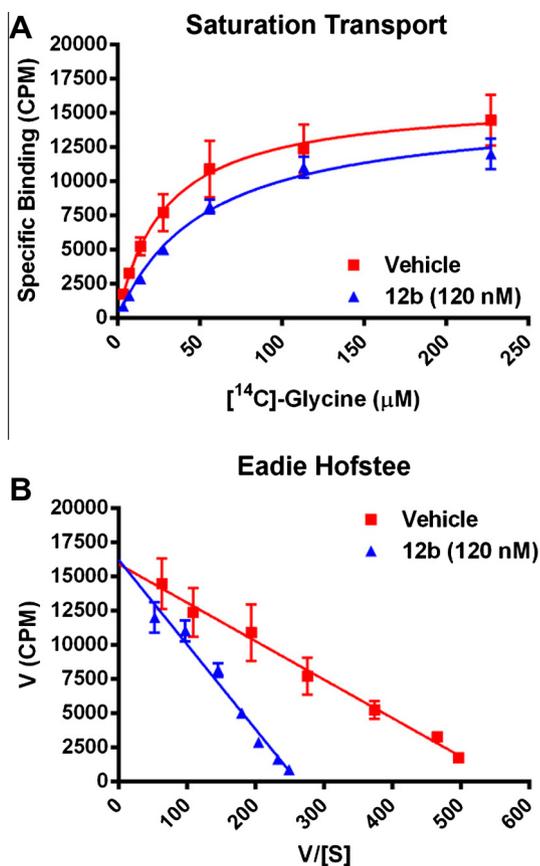


Figure 3. (A) Saturation [¹⁴C]-glycine transport in the presence of vehicle (red squares) or 120 nM **12b** (blue triangles). (B) An Eadie–Hofstee diagram for **12b** and [¹⁴C]-glycine.

with respect to glycine, in accordance with the known mechanism of action for **1–3**.^{15,17–20}

Racemic **12a**, the most potent of the [3.3.0]-series, possessed a favorable DMPK profile, with a good unbound fraction in rat

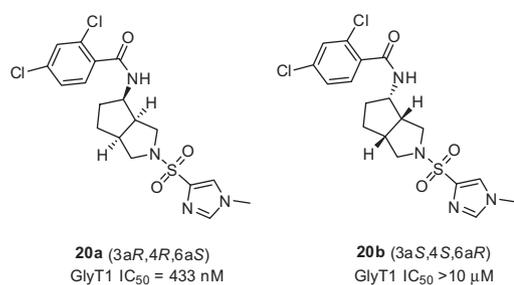
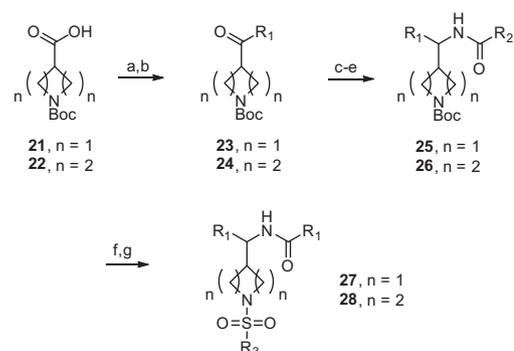
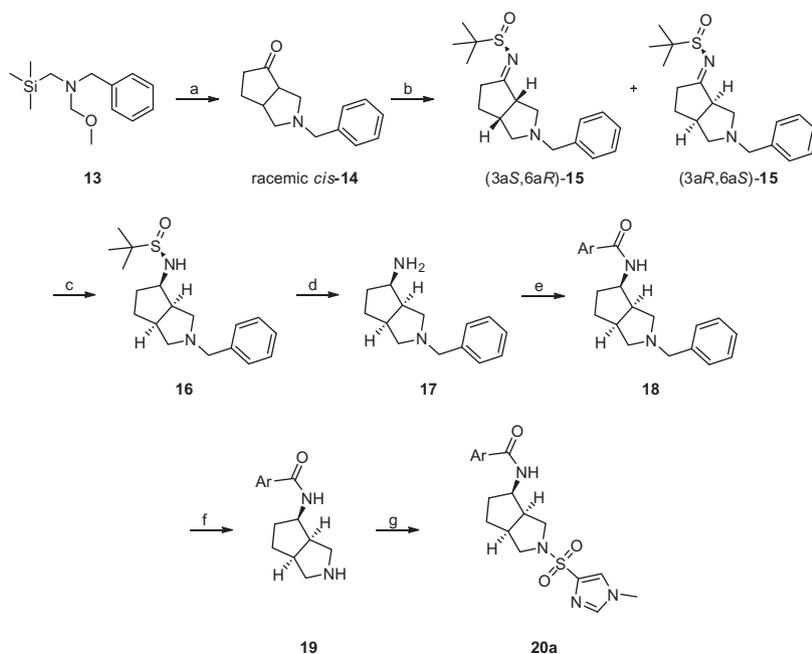


Figure 4. Structures and activities of *cis*-(3a,6a)-enantiomers **20a** and **20b**.



Scheme 3. Reagents and conditions: (a) *N,O*-dimethylhydroxylamine, EDC, HOBt, DIPEA, DMF, rt; (b) Ar(Het)MgX or R₁MgX, THF, –78 °C; (c) NH₂OH, MeOH, 50 °C; (d) Raney Ni, H₂, (45 psi), MeOH; (e) RCOCl, DIEPA, CH₂Cl₂, rt; (f) 4 N HCl, dioxane, rt; (g) RSO₂Cl, DIEPA, CH₂Cl₂, rt.

(*f*_u = 8.1%), clean CYP profile (IC₅₀s > 10 μM), and reasonable microsomal stability (30% remaining at 90 min in fortified rat liver microsomes). An oral plasma:brain level (PBL) study with oral dosing (10 mg/kg p.o. in 0.5% methocellulose) of **12a** afforded a low Brain_{AUC}:Plasma_{AUC} of 0.15. This preliminary data was encourag-



Scheme 2. Reagents and conditions: (a) cyclopentenone, TFA, CH₂Cl₂, 0 °C, 16 h; (b) (*R*)-*tert*-butylsulfonamide, Ti(OEt)₄, THF, 0 °C, 16 h, chromatographic separation of diastereomers; (c) NaBH₄, MeOH, –78 °C to rt, 3 h; (d) 2 N HCl (aq), MeOH, rt, 16 h; (e) ArCOCl, CH₂Cl₂, rt, 16 h; (f) chloroethyl chloroformate, Et₃N, ClCH₂CH₂Cl, MeOH, rt, 20 h; (g) *N*-methyl imidazole sulfonyl chloride, Et₃N, CH₂Cl₂, rt, 12 h. Overall yields range 5–22%.

ing, and since **12a** was a racemate (90% *cis* at the bridgehead), and thus a mixture of **8** compounds, we then attempted to separate the mixture by chiral SFC. We were able to separate three peaks off the SFC, two as single species (GlyT1 IC_{50} s > 10 μ M), and one as a mixture (GlyT1 IC_{50} = 34 \pm 2 nM); however, we were unable to definitively assign the absolute stereochemistry. An enantioselective synthetic route (Scheme 2) was employed to quickly access the pure *cis*-(3a,6a)-enantiomers **20a** and **20b** (Fig. 4).²¹ Following the work of Beebe,²¹ azomethine ylide precursor **13** underwent a dipolar cycloaddition reaction with cyclopentenone to give the key racemic ketone **14**, with *cis*-stereochemistry at the ring junction. Enantiomeric resolution via the (*R*)-*tert*-butyl sulfonamide provided (3a*S*,6a*R*)-**15** and (3a*R*,6a*S*)-**15**, which were subsequently separated by silica gel chromatography, in accord with literature precedent.²¹ Scheme 2 shows the complete route to **20a**, employing (3a*R*,6a*S*)-**15**. Here, reduction with NaBH₄ delivered **16**, followed by deprotection under acidic conditions to the primary amine **17**. Acylation, removal of the benzyl protecting group and sulfonylation provided **20a**, the (3a*R*,4*R*,6a*S*) isomer. By employing (*S*)-*tert*-butyl sulfinamide, the other *cis*-(3a,6a)-enantiomers, (3a*R*,4*S*,6a*S*) and (3a*S*,4*R*,6a*R*) could not be accessed.²¹ For the isomers that could be obtained, enantiospecific inhibition was noted with **20a**, possessing a GlyT1 IC_{50} of 433 nM, while the other isomer **20b** was inactive (GlyT1 IC_{50} > 10 μ M). Interestingly, these analogs were weak to inactive relative to racemic **12a**, and suggests that the active isomer(s) are either the *trans*-(3a,6a)-isomers or the other *cis* congeners, and synthetic efforts to access both are underway. Thus, **12b**, derived from a scaffold-hopping exercise employing **1–3**, led to a novel [3.3.0]-based GlyT1 inhibitor with in vitro properties comparable to other advanced GlyT1 inhibitors in short order, and for which a U.S. patent was issued.²²

In parallel, we were also preparing and evaluating other piperidine bioisosteres and modifications to **1–3** to further access additional novel intellectual property (IP) space. Modeling work suggested that 4-position homologated piperidines, as well as 3-position homologated azetidines overlapped favorably with

1–3, **12** and **20**. Thus, chemistry was quickly developed to access these cores (Scheme 3). Starting from commercially available *N*-Boc-azetidine-3-carboxylic acid **21** or *N*-Boc-piperidine-4-carboxylic acid **22**, conversion to the Weinreb amide and treatment with an aryl, heteroaryl or aliphatic Grignard reagent provided **23** and **24**, respectively. Condensation with hydroxylamine, reduction and acylation afforded amides **25** and **26**. Finally, removal of the Boc moiety and sulfonylation of the secondary amine led to putative, racemic GlyT1 inhibitor series **27** and **28**.

As shown in Table 3, the homologated azetidine-based analogs **27** were uniformly more potent than the corresponding homologated piperidine-based analogs **28**, affording GlyT1 inhibitors with low nanomolar potency. While the 2,4-dichlorobenzamide was the most potent congener, other benzamides displayed a wide range of GlyT1 potency (GlyT1 IC_{50} s from 80 nM to 7 μ M). Moreover, in the azetidine series **27**, the aryl/heteroaryl R₁ moieties could be replaced with aliphatic groups and retain potency (R₁ = *i*Pr, GlyT1 IC_{50} = 394 nM; R₁ = *n*-Pr, GlyT1 IC_{50} = 185 nM; R₁ = *cyc*Pr, GlyT1 IC_{50} = 253 nM), whereas the corresponding analogs in the piperidine series **28** were inactive.

Representative members from both **27** and **28** were evaluated for their effect on enzyme kinetics of [¹⁴C]-glycine transport, and both were shown to be competitive with glycine, as well as selective versus GlyT2 (IC_{50} > 30 μ M). Initial evaluation in our in vitro DMPK assays demonstrated that **27c** was stable in fortified rat liver microsomes (75% parent remaining at 90 min), possessed a good unbound fraction in rat (f_u = 14%) and clean CYP profile (IC_{50} s > 10 μ M). An oral plasma:brain level (PBL) study with oral dosing (10 mg/kg p.o. in 0.5% methocellulose) of **27c** afforded a low Brain_{AUC}:Plasma_{AUC} of 0.11. SCF separation of the **27c** enantiomers led to the isolation of the two pure enantiomers, and one was quite active (IC_{50} = 39 nM) while the other proved much weaker (IC_{50} = 900 nM). In consultation with the Johnston group, they developed an asymmetric synthesis of **27c**, via chiral proton catalysis of a secondary nitroalkane addition to an azomethine, and we were able to elucidate that the potent enantiomer had the (*S*)-configuration.²³ Overall, the low brain:plasma ratios of these series, **11**, **12**, **27** and **28** diminished enthusiasm; however, the scaffold hopping strategy again secured robust IP position for both the **27** and **28** series of GlyT1 inhibitors.^{24,25}

In summary, we were able to successfully further scaffold hop from **3**, originally derived at from a scaffold hopping exercise from **1** and **2**, and develop three new series for which US patents were granted without the need for an HTS campaign. This was critical, as the time required to perform a SPA-based HTS campaign and identify/optimize the hits would have required far more time and uncertain IP position in a highly crowded and competitive space. These new series retained the potency and selectivity of the advanced compounds from which they were derived, but did suffer from only modest CNS exposure. Finally, all of these new series displayed enantioselective inhibition of the GlyT1 transporter. Further refinements are in progress and will be reported in due course.

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Table 3
Structures and activities of analogs **27** and **28**

Compound	R1	R2	R3	GlyT1 IC_{50} ^a (μ M)
27a		2,4-diClPh		627
28a		2,4-diClPh		1500
27b		2,4-diClPh		39
28b		2,4-diClPh		201
27c		2,4-diClPh		68
28c		2,4-diClPh		46
27d		2,4-diClPh		40
28d		2,4-diClPh		374

^a IC_{50} s represent single determinations performed in triplicate.

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