Published in final edited form as:
Bioorg Med Chem Lett. 2014 February 15; 24(4): 1062–1066. doi:10.1016/j.bmcl.2014.01.011.

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

Douglas J. Sheffler[a,b,e], **Michael T. Nedelovych**[a,b], **Richard Williams**[b,g], **Stephen C. Turner**[f], **Brittany B. Duerk**[f], **Megan R. Robbins**[f], **Sataya B. Jadhav**[b], **Colleen M. Niswender**[a,b,c], **Carrie K. Jones**[a,b], **P. Jeffrey Conn**[a,b,c], **R. Nathan Daniels**[a,f,*], and **Craig W. Lindsley**[a,b,c,d,*]

[a]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[b]Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[c]Vanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA

[d]Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

[f]Department of Pharmaceutical Sciences, Lipscomb University, College of Pharmacy and Health Sciences, Nashville, TN 37024-3951, USA

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.

Keywords

GlyT1; Scaffold hopping; transporter; schizophrenia

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 clicnial trials,[8–14] we initiated scaffold hopping efforts to expediently develop novel GlyT1 inhibitors within a crowded intellectual property (IP)

*To whom correspondence should be addressed: craig.lindsley@vanderbilt.edu; nate.daniels@lipscomb.edu.
[e]Current Address: Apoptosis and Cell Death Research Program and Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037, USA
[g]Current Address: Centre for Cancer Research and Cell Biology, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK.

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**, respectivley, 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]

Based on work from our labs with $mGlu_1$ 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 bioisoteric 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 would represent a major strucutral change, eliminating the pendant cyclopropylmethyl moeity 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 benyl 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 piperdine **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 IC_{50} > 10 μM). 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 (IC_{50} > 30 μM). 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-triflouromethylbenzamide (**12b**) and the 2-chlorobenzamide (**12c**) were respectable, with GlyT1 IC_{50}s of 112 ± 6 nM and 115 ± 18 nM, respectively. The vast majority of other substitution patterns afforded a considerable loss in potency (GlyT1 IC_{50}s from 631 nM to 10 μM), as did a cyclohexyl amide congener **12l** (GlyT1 IC_{50} = 617 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 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 (f_u = 8.1%), clean CYP profile (IC_{50}s >10 μM), and reasonable microsomal stability (30% remaining at 90 minutes 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 encouraging, 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 μM), and one as a mixture (GlyT1 IC_{50} = 34 ± 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 ylid 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 $NaBH_4$ 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 μM). 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 inhbitors 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 Gringard 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 μM). Moreover, in the azetidine series **27**, the aryl/heteroaryl R_1 moieties could be replaced with aliphatic groups and retain potency (R_1 = *i*Pr, GlyT1 IC_{50} = 394 nM; R_1 = *n*-Pr, GlyT1 IC_{50} = 185 nM; R_1 = *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 [^{14}C]-glycine transport, and both were shown to be competitive with glycine, as well as selective versus GlyT2 (IC_{50} >30 μM). Initial evaluation in our *in vitro* DMPK assays demonstrated that **27c** was stable in fortified rat liver microsomes (75% parent remaining at 90 minutes), possessed a good unbound fraction in rat (f_u = 14%) and clean CYP profile (IC_{50}s >10 μM). 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.

Acknowledgments

This work was supported by the NIH/NIMH under a National Cooperative Drug Discovery and Development grant U01 MH08795. DJS is a recipient of a National Alliance for Research on Schizophrenia and Depression (NARSAD)–Dylan Tauber Young Investigator Award. Vanderbilt is a member of the MLPCN and houses the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development supported by U54 MH084659. The support of William K. Warren, Jr. who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.) is gratefully acknowledged.

References

1. Langdon SR, Ertl P, Brown N. Mol Inf. 2010; 29:366.
2. Sun H, Tawa G, Wallqvist A. Drug Discov Today. 2012; 17:310. [PubMed: 22056715]
3. Martin YC, Muchmore S. QSAR Comb Sci. 2009; 28:797.
4. Xiang Z, Thompson AD, Brogan JT, Schulte ML, Mi D, Lewis LM, Yang L, Zhou B, Melancon BJ, Morrison R, Santomango T, Byers F, Brewer K, Aldrich JS, Yu H, Dawson ES, Li M, McManus O, Jones CK, Daniels JS, Conn PJ, Xie X, Weaver CD, Lindsley CW. ACS Chem Neurosci. 2011; 2:730. [PubMed: 22368764]
5. Lindsley CW, Shipe WD, Wolkenberg SE, Theberge CR, Williams DL Jr, Sur C, Kinney GG. Curr Topics in Med Chem. 2006; 8:771.
6. Menniti FS, Lindsley CW, Conn PJ, Pandit J, Zagouras P, Volkmann RA. Curr Topics in Med Chem. 2013; 13:26.
7. Olney JW, Newcomer JW, Farber NB. J Psychiatry Res. 1999; 33:523.
8. Coyle JT. Cell Mol Neurobiol. 2006; 26:365. [PubMed: 16773445]
9. Kinney GG, Sur C. Curr Neuropharmacology. 2005; 3:35.
10. Bridges TM, Williams R, Lindsley CW. Curr Opin Mol Ther. 2008; 10:591. [PubMed: 19051137]
11. Lindsley CW, Wolkenberg SE, Kinney GG. Curr Topics in Med Chem. 2006; 6:1883.
12. Wolkenberg SE, Sur C. Curr Topics in Med Chem. 2010; 10:170.
13. Hashimoto K. Curr Pharm Des. 2011; 17:112. [PubMed: 21355838]
14. For information on RG1678, please see: www.roche.com
15. Jones CK, Sheffler DJ, Williams R, Jadhav SB, Felts AS, Morrison RD, Niswender CM, Daniels JS, Conn PJ, Lindsley CW. Bioorg Med Chem Lett. in press.
16. Manka JT, Rodriguez AL, Venable DF, Morrison RD, Venable DF, Plumley HC, Blobaum AL, Daniels JS, Niswender CM, Conn PJ, Lindsley CW, Emmitte KA. Bioorg Med Chem Lett. 2013; 23:5091. [PubMed: 23932792]

17. Lindsley CW, Zhao Z, Leister WH, O'Brien JA, Lemiare W, Williams DL Jr, Chen TB, Chang RSL, Burno M, Jacobson MA, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Tsou NN, Duggan ME, Conn PJ, Hartman GD. Chem Med Chem. 2006; 1:807. [PubMed: 16902933]

18. Zhao Z, Leister WH, O'Brien JA, Lemiare W, Williams DL Jr, Jacobson MA, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Hartman GD, Lindsley CW, Wolkenberg SE. Bioorg Med Chem Lett. 2009; 19:1488. [PubMed: 19179073]

19. Wolkenberg SE, Zhao Z, Wisnoski DD, Leister WH, O'Brien JA, Lemiare W, Williams DL Jr, Jacobson MA, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Gibson C, Ma BK, Polsky-Fisher SL, Lindsley CW, Hartman GD. Bioorg Med Chem Lett. 2009; 19:1492. [PubMed: 19181525]

20. Lowe JA III, Hou X, Schmidt C, Tingley FD III, McHardy S, Kalman M, DeNinno S, Sanner M, Ward K, Lebel L, Tunucci D, Valnetine J. Bioorg Med Chem Lett. 2009; 19:2974. [PubMed: 19410451]

21. Beebe X, Darczak D, Henry RF, Vortherms T, Janis R, Namovic M, Donnelly-Roberts D, Kage KL, Surowy C, Milicic I, Niforatos W, Swensen A, Marsh KC, Wetter JM, Franklin P, Baker S, Zhong C, Simler G, Gomez E, Boyce-Rustay JM, Zhu CZ, Stewart AO, Jarvis MF, Scott VE. Bioorg Med Chem. 2012; 20:4128. [PubMed: 22626552]

22. Lindsley, CW.; Conn, PJ.; Williams, R.; Sheffler, DJ. US. 8,211,933. 2012.

23. Davis TA, Danneman MW, Johnston JN. Chem Commun. 2012; 48:5578.

24. Lindsley, CW.; Conn, PJ.; Williams, R.; Jones, CK.; Sheffler, DJ. US. 8,207,155. 2012.

25. Lindsley, CW.; Conn, PJ.; Williams, R.; Jones, CK.; Sheffler, DJ. US. 8,436,019. 2013.

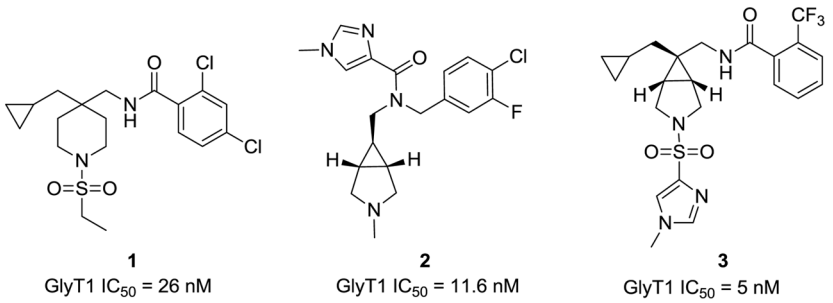


Figure 1.
Reported GlyT1 inhibitors **1** (Merck) and **2** (Pfizer), and the novel series **3** (VU0240391), derived from scaffold hopping.

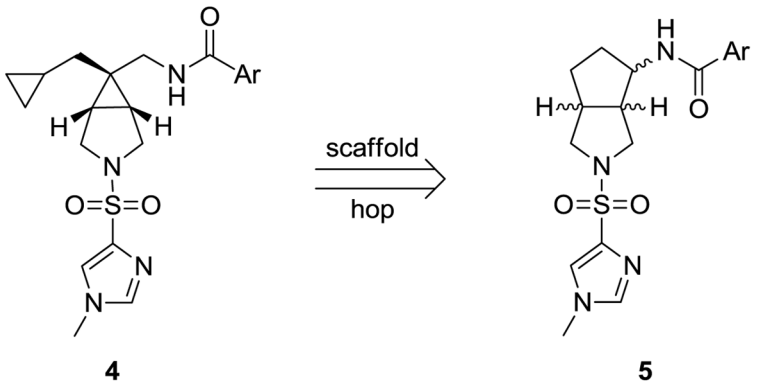


Figure 2.
Envisioned scaffold hopping from the novel series **4** to a [3.3.0]-core, an octahydrocyclopenta[*c*]pyrrole, **5**.

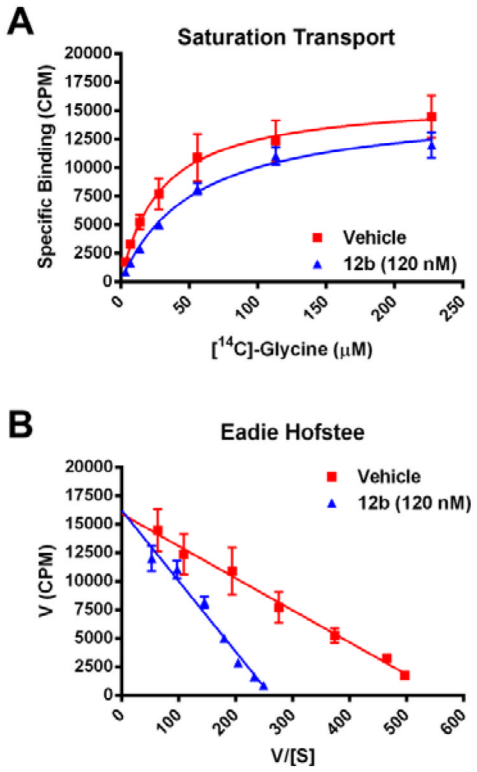
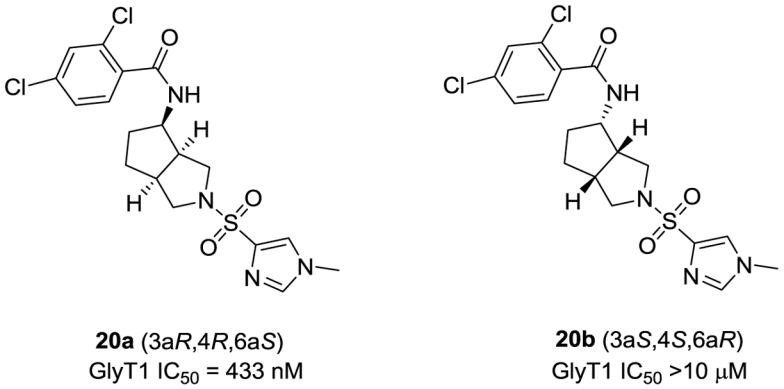


Figure 3.

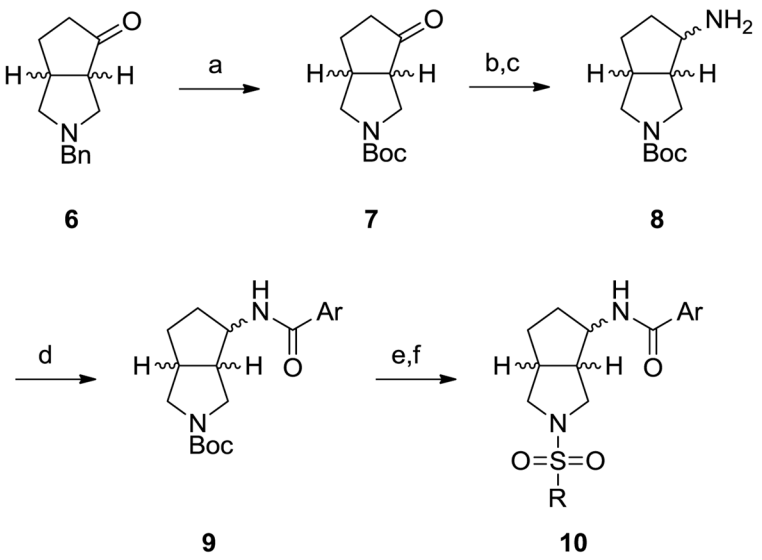
(A) Saturation [^{14}C]-glycine transport in the presence of vehicle (red squares) or 120 nM **12b** (blue triangles). (B) An Eadie-Hoffstee diagram for **12b** and [^{14}C]-glycine.



20a (3a*R*,4*R*,6a*S*)
GlyT1 IC_{50} = 433 nM

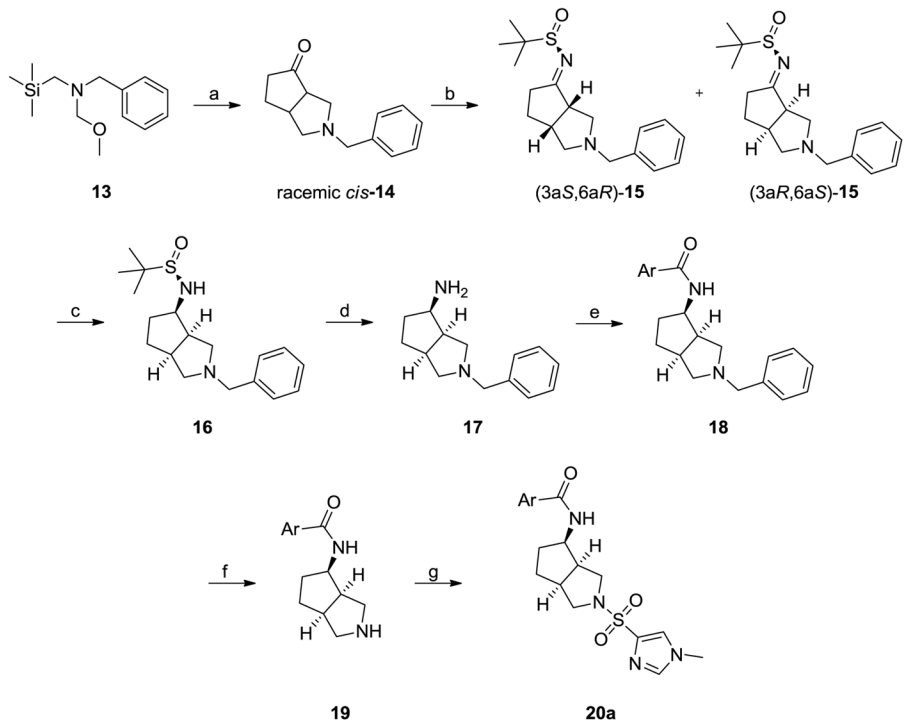
20b (3a*S*,4*S*,6a*R*)
GlyT1 IC_{50} >10 μM

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



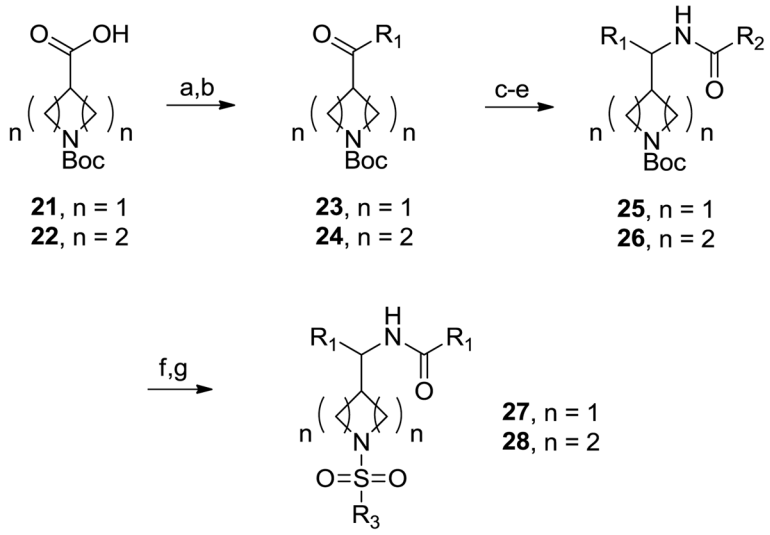
Scheme 1.

Reagents and conditions. (a) Boc_2O, $Pd(OH)_2/C$, 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%.



Scheme 2.

Reagents and conditions. (a) cyclopentenone, TFA, CH_2Cl_2, 0 °C, 16h; (b) (*R*)-*tert*-butylsulfinamide, $Ti(OEt)_4$, THF, 0 °C, 16 h, chromatographic separation of diastereomers; (c) $NaBH_4$, MeOH, −78 °C to rt, 3 h; (d) 2 N HCl (aq), MeOH, rt, 16 h; (e) ArCOCl, CH_2Cl_2, rt, 16 h; (f) chloroethyl chloroformate, Et_3N, $ClCH_2CH_2Cl$, MeOH, rt, 20 h; (g) *N*-methyl imidazole sulfonyl chloride, Et_3N, CH_2Cl_2, rt, 12 h. Overall yields range 5–22%.

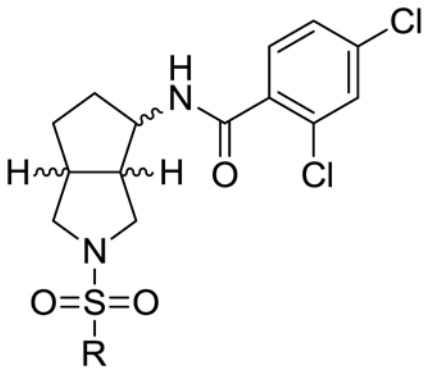


Scheme 3.

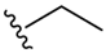
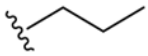
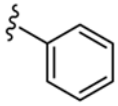
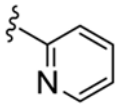
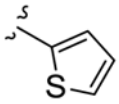
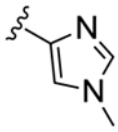
Reagents and conditions. (a) *N*,*O*-dimethylhydroxylamine, EDC, HOBt, DIPEA, DMF, rt; (b) Ar(Het)MgX or R_1MgX, THF, −78 °C; (c) NH_2OH, MeOH, 50 °C; (d) Raney Ni, H_2, (45 psi), MeOH; (e) RCOCl, DIEPA, CH_2Cl_2, rt; (f) 4 N HCl, dioxane, rt; (g) RSO_2Cl, DIEPA, CH_2Cl_2, rt.

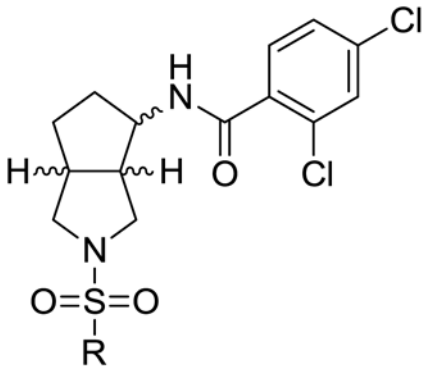
Table 1

Structures and activities of analogs **11**.

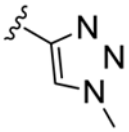


11

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



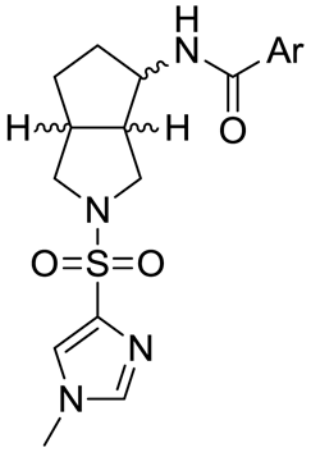
11

Compound	R	GlyT1 IC_{50} (μM)[a]	GlyT2 IC_{50} (μM)[a]
11h		0.015	>30

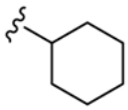
[a] IC_{50}s represent single determinations performed in duplicate

Table 2

Structures and activities of analogs **12**.



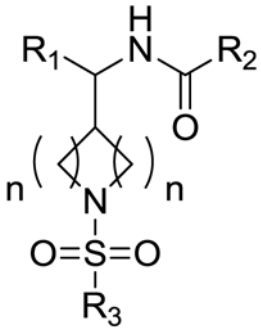
12

Compound	Ar	GlyT1 IC_{50} (nM)[a]	GlyT2 IC_{50} (μM)[a]
12a (11g)	2,4-diClPh	25	>30
12b	2-CF_3Ph	112*	>30
12c	2-ClPh	115*	>30
12d	2,4-diFPh	926	>30
12e	2,6-diFPh	631	>30
12f	2-FPh	1,815	>30
12g	3-FPh	>10,000	>30
12h	4-FPh	2,215	>30
12i	3,4-diFPh	1569	>30
12j	4-ClPh	1,029	>30
12k	3,4-diClPh	891	>30
12l		617	>30

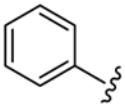
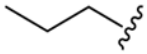
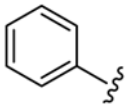
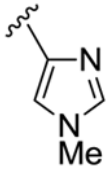
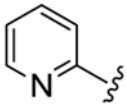
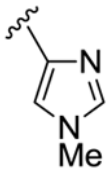
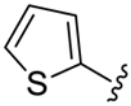
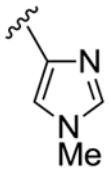
[a] IC_{50}s represent single determinations performed in duplicate or *the average of four determinations performed in duplicate

Table 3

Structures and activities of analogs **27** and **28**.



27, n = 1
28, n = 2

Cmpd	R1	R2	R3	GlyT1 IC_{50} (nM)[a]
27a **28a**		2,4-diClPh		627 1,500
27b **28b**		2,4-diClPh		39 201
27c **28c**		2,4-diClPh		68 46
27d **28d**		2,4-diClPh		40 374

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