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Identification of *N*-(2-(azepan-1-yl)-2-phenylethyl)-benzenesulfonamides as novel inhibitors of GlyT1

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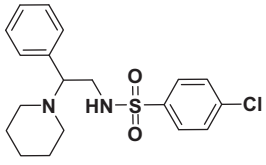
ABSTRACT

A novel series of glycine transporter 1 (GlyT1) inhibitors is described. Scoping of the heterocycle moiety of hit 4-chlorobenzenesulfonamide **1** led to replacement of the piperidine with an azepane for a modest increase in potency. Phenyl sulfonamides proved superior to alkyl and non-phenyl aromatic sulfonamides, while subsequent *ortho* substitution of the 2-(azepan-1-yl)-2-phenylethanamine aromatic ring yielded **39** (IC_{50} 37 nM, solubility 14 μM), the most potent GlyT1 inhibitor in this series. Favorable brain–plasma ratios were observed for select compounds in pharmacokinetic studies to evaluate CNS penetration.

Schizophrenia affects approximately 1% of the world's population; however, current therapies are associated with unwanted side affects and/or do not provide sufficient relief across all symptom domains.[1] Based on pre-clinical models and clinical data, the *N*-methyl-D-aspartate receptor (NMDAr) hypofunction hypothesis of schizophrenia correlates disease symptomology with glutamatergic neurotransmission dysfunction.[2] Hence, increasing NMDAr neurotransmission represents a promising approach for the development of antipsychotics. Direct NMDAr agonists and elevated levels of its endogenous ligand glutamate are known to be neurotoxic. In contrast, targeted elevation of synaptic glycine, an NMDAr coagonist, represents a viable opportunity for neurotransmission enhancement. Synaptic glycine levels are regulated by glycine transporter 1 (GlyT1), making the blockade of GlyT1-mediated glycine reuptake an attractive method for enhancing NMDAr neurotransmission. Considerable effort has been invested in discovering GlyT1 inhibitors and the area has been extensively reviewed.[2b,3] Herein, we describe a novel series of GlyT1 inhibitors as a potential therapy for schizophrenia.



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Our lead-finding strategy relied on a virtual screen to identify compounds that had pharmacophoric similarity with validated GlyT1 inhibitors from the literature. The computational screening model employed in-house developed 2D topological fingerprints. They encoded the distribution of various pharmacophore features (e.g., aromaticity, lipophilicity, H-bond donors and acceptors, positive and negative charges) up to distances of 12 bonds. Compounds that met our similarity criteria to literature inhibitors were clustered and a representative sample of members from each cluster was filtered for suitable CNS-drug like properties (e.g., molecular weight, lipophilicity) and the absence of toxic or reactive functional groups. A final set of 15,000 candidates was submitted for biological screening. These were tested in a GlyT1 uptake inhibition assay[4] at 10 μM.

The most potent hit was 4-chlorobenzenesulfonamide **1** (IC_{50} 1.4 ± 0.1 μM) which contains elements that are related to some reported GlyT1 inhibitors. For example aryl sulfonamides also appear in the 1,3-diaminopropanol series from GSK,[5] and a

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related benzyl linked amine comprises the basic region in SSR504734 from Sanofi.[2d] Intrigued by the potential for rapid structural modification, we embarked on a campaign to scope the structure–activity relationship of the phenethyl diamine scaffold of **1** with respect to GlyT1 inhibition.

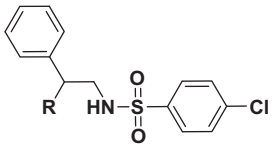
Compounds were prepared from commercially available 2-amino-2-phenylethanamines via sulfonylation or acylation. Where said phenylethanamines could not be purchased, they were obtained via a Strecker reaction between an amine and aldehyde followed by an alane reduction.[6,7] For example, as shown in Scheme 1, the aldehyde used to prepare compound **2** was derived from 4-mercaptobenzoic acid (**3**) by first alkylating with propyl iodide and then oxidizing with Oxone® to afford sulfone **4**. Reduction of the ester to the corresponding alcohol was followed by oxidation to give aldehyde **5**. A Strecker reaction between azepane and **5** provided the corresponding nitrile, which was reduced with alane to furnish amine **6**. Subsequent sulfonylation yielded **2**. If amide analogs of **2** were desired, they were obtained via acylation (ArCOCl, DIPEA, CH_2Cl_2) or amide coupling (ArCOOH, HOBt, TBTU, DIPEA, DMF).[8]

We started our investigation by evaluating the impact of changes to the piperidine moiety of **1** (Table 1). As demonstrated with morpholine **8** and piperazine **9**, attenuation of nitrogen basicity resulted in complete loss of activity, suggesting that a basic heterocyclic nitrogen is important for potent inhibition.

Changes to the size of the piperidine ring of **1** gave mixed results. Expansion from a six- to a seven-membered ring (**1** and **10**, respectively) was favored while conversion of **10** to the corresponding azabicyclo[3.2.2]nonane **11** resulted in a 10-fold loss of potency. Ring truncation (diethylamine **12**) or contraction (pyrrolidine **13**) also decreased in vitro glycine uptake inhibition by approximately 10-fold. These results suggest that there is a narrow SAR for the heterocycle moiety of the phenethyl diamine scaffold, and only certain ring sizes and substitutions (e.g., **7**) maintain or improve potency.

To determine what impact modifications might have on both potency and aqueous solubility, we examined alkyl, benzyl and alternative heterocyclic sulfonamides for comparison with simple phenyl analog **14** (Table 2). Initial data suggested that an aromatic moiety is required for activity. For example, while both compounds

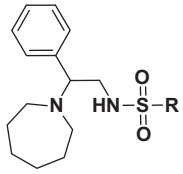
Table 1
Uptake inhibition and aqueous solubility data for **1** and **7–13**



Compd[a]	R	IC_{50} (μM)	Sol[b] (μM)
1	Piperidin-1-yl	1.4 ± 0.1	3
7	4-Me-piperidin-1-yl	1.4 ± 0.1	<1
8	Morpholin-4-yl	>20	16
9	4-Et-piperazin-1-yl	>20	240
10	Azepan-1-yl	0.46 ± 0.05	1
11	3-Azabicyclo[3.2.2]nonane-1-yl	13 ± 3	<1
12	Diethylamin-1-yl	10 ± 1	300
13	Pyrrolidin-1-yl	9.7 ± 0.7	47

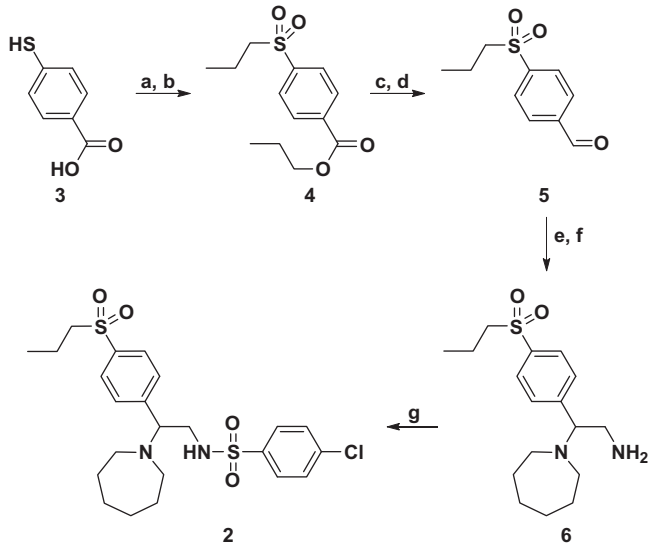
[a] Compounds are racemic.
[b] Equilibrium solubility (pH 7.4).[9]

Table 2
Uptake Inhibition and aqueous solubility data for **14–22**



Compd[a]	R	IC_{50} (μM)	Sol[b] (μM)
14	Phenyl	4 ± 1	67
15	Ethyl	>20	470
16	cPropyl	>20	371
17	Benzyl	14.8 ± 0.7	195
18	3,5-Cl-benzyl	1.5 ± 0.1	1
19	Pyridin-3-yl	>20	143
20	Quinolin-8-yl	4 ± 2	2
21	2-Br-thiophen-5-yl	0.93 to 0.9	9
22	3,5-Me-isoxazol-4-yl	8.3 ± 0.3	37

[a] Compounds are racemic.
[b] Equilibrium solubility (pH 7.4).[9]

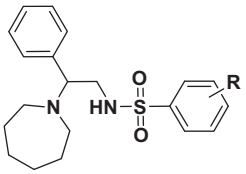


Scheme 1. Reagents: (a) Iodopropane, K_2CO_3, DMF, 34%; (b) Oxone®, DMF, 90%; (c) DIBAL-H, THF, 46%; (d) Dess-Martin periodinane, CH_2Cl_2, 96%; (e) azepane, HOAc, NaCN, 43%; (f) $LiAlH_4$, H_2SO_4, THF; Na_2SO_4. 10 H_2O, 51%; (g) 4-Cl-$PhSO_2Cl$, Et_3N, CH_2Cl_2, 68%.

showed marked improvement in solubility relative to their aromatic counterparts, ethyl (**15**) and cyclopropyl (**16**) sulfonamides were inactive. Several results indicated that large lipophilic aromatic groups are well tolerated. Thus, while only weak activity was observed for benzyl sulfonamide **17**, bis-chlorination (**18**) improved potency by 10-fold. Similarly, potency was retained in going from **14** to quinoline **20**. Thiophene **21**, with a large bromo substituent, was the most potent phenyl sulfonamide surrogate tested. In contrast, an attempt to increase solubility by conversion of the phenyl sulfonamide of **14** to a more polar pyridine analog (**19**) completely eliminated activity.

We concluded from the data of Table 2 that phenyl sulfonamides offer the best combination of potency and solubility, prompting us to aggressively investigate the scope of sulfonamide phenyl ring substitution (Table 3). In general, *meta* and *para*-substituted phenyl sulfonamides with large hydrophobic substituents were more active than sulfonamides with alternative substitution patterns or polar substituents. Supporting this hypothesis are *para*-substituted bromo and trifluoromethoxy analogs **34** and **35**, respectively, which are two of the more potent compounds of Table 3. In contrast, replacing chlorine with fluorine (e.g., **10** to **33** and **26** to **25**) improved solubility but reduced potency. Electron withdrawing cyano (**32** and **36**) and electron donating methoxy (**37**) moieties gave similar results, while sulfone **38** was completely

Table 3
Uptake inhibition and aqueous solubility data for **10** and **23–38**



Compd[a]	R	IC_{50} (μM)	Sol [b] (μM)
10	4-Cl	0.46 ± 0.05	1
23	2-F	15 ± 1	108
24	2-OCF_3	2.1 ± 0.7	10
25	2,4-diF	8 ± 1	19
26	2,4-diCl	1.8 ± 0.2	1
27	3-CF_3	0.83 ± 0.04	4
28	3-Cl	0.68 ± 0.02	7
29	3,5-diCl	0.391 ± 0.009	2
30	3,4-diF	1.0 ± 0.3	10
31	3-Cl, 4-F	0.6 ± 0.2	17
32	3-CN, 4-F	6 ± 2	14
33	4-F	2.9 ± 0.1	9
34	4-Br	0.39 ± 0.01	1
35	4-CF_3	0.17 ± 0.03	2
36	4-CN	5 ± 2	7
37	4-OMe	4.6 ± 0.9	30
38	4-SO_2Me	>20	4

[a] Compounds are racemic.
[b] Equilibrium solubility (pH 7.4).[9]

inactive. Hydrophobic substituents at the *ortho* position (**24**) retained only modest activity. Interestingly, compound **31**, with polar and non-polar moieties at the *para* and *meta* positions, respectively, represents a successful increase in solubility while maintaining activity at GlyT1 comparable to **10**.

We used 4-trifluoromethyl sulfonamide **35** as a platform to scope the impact of substitution of the phenyl ring of the 2-(azepan-1-yl)-2-phenylethanamine scaffold (Table 4). *Meta* substitution was only briefly explored and was not well-tolerated (data not shown). Electron withdrawing groups at the *para* position were weakly active. Of sulfones and sulfonamides **2** and **40–43**, only propylsulfone **2** retained any significant potency. Solubility was also low, which was likely due to the hydrophobic nature of the propyl side chain. Sulfonamide **42** was weakly active but approximately fourfold more potent than less lipophilic **43**, again suggesting that lipophilic electron withdrawing groups at the *para* position are favored.

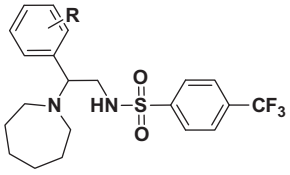
Ortho substitution had the greatest impact on in vitro potency, particularly when that substituent was a methoxy group (**39** and **46**). Not only was there more than a two- to fivefold increase in uptake inhibition, but a significant increase in solubility was also observed for **39** compared to **35** (Table 5). In contrast, an electron withdrawing group at the *ortho* position (**40**) decreased potency by two orders of magnitude. With regards to other alkoxy substituted analogs, dioxolane **44** was potent but less active than **39**, and had little to no aqueous solubility. Bis-substituted **45**, which could potentially capitalize on increased binding interactions via rotation of the phenyl ring, was weakly active.

No compounds in this series showed any appreciable activity at GlyT2 and can thus be considered GlyT1-selective. For **39** the GlyT2 IC_{50} was >20 μM.

To determine CNS penetration of *N*-(2-(azepan-1-yl)-2-phenylethyl)-benzenesulfonamides, select compounds were evaluated in a cassette CNS exposure screen in male Sprague-Dawley rats. Compounds **39**, **47**, and **48** were selected based on their potencies and relative solubilities. Appreciable whole brain and plasma concentrations were observed one hour post subcutaneous injection. Brain–plasma ratios ranged from 1.6 to 2.1, indicating good partitioning across the blood–brain barrier and distribution into brain tissue.

In conclusion, starting from hit 4-chlorosulfonamide **1**, the azepane amine was identified as the only heterocyclic replacement more potent than a piperidine. Solubility was quickly identified as a physical property to be monitored and, if possible, improved. An analysis of various aromatic and non-aromatic sulfonamides identified phenyl sulfonamides as having the best balance between in vitro potency and solubility. Substitution of the *meta*- and/or *para*-positions of the phenyl sulfonamide with hydrophobic groups was preferred, with 4-trifluoromethyl sulfonamide **35** offering the most potent analog. Subsequent exploration of phenyl ring substitution of the 2-amino-2-phenylethanamine scaffold of **35** identified the 2-methoxy group (**39**) as a way to improve both uptake inhibition and solubility. To evaluate the CNS penetration of *N*-(2-(azepan-1-yl)-2-(2-methoxy-phenylethyl)-benzenesulfonamides, exemplar 4-trifluoromethyl, 4-chloro, and 3-chloro-4-fluoro-sulfonamides were analyzed via in vivo pharmacokinetic studies in Sprague-Dawley rats. All three compounds demonstrated good distribution into brain tissue and favorable brain–plasma ratios.

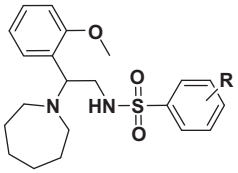
Table 4
Uptake inhibition and aqueous solubility for **2**, **35**, and **39–46**



Compd[a]	R	IC_{50} (μM)	Sol [b] (μM)
2	4-SO_2Pr	0.3 ± 0.2	1
35	H	0.17 ± 0.03	2
39	2-OMe	0.037 ± 0.006	14
40	2-SO_2Me	3.2 ± 0.8	5
41	4-SO_2Me	10 ± 2	3
42	4-$SO_2N(Me)_2$	2 ± 2	<1
43	4-SO_2NH_2	8.9 ± 0.7	9
44	2,3-(OCH_2O)	0.12 ± 0.07	<1
45	2,6-diOMe	16.4 ± 0.9	215
46	2-OMe, 4-F	0.07 ± 0.01	2

[a] Compounds are racemic.
[b] Equilibrium solubility (pH 7.4).[9]

Table 5
CNS exposure data for **39**, **47** and **48**[a]



Compd[b]	R	IC_{50} (μM)	Sol (μM)[c]	Brain (nmol/kg)	Plasma (nM)	Br/Pl[d]
39	4-CF_3	0.037 ± 0.006	14	154	72	2.1
47	4-Cl	0.15 ± 0.08	9	202	103	1.9
48	3-Cl, 4-F	0.3 ± 0.1	37	168	105	1.6

[a] Total plasma and whole brain concentrations at 1 h following a 2.5 μmol/kg subcutaneous dose.
[b] Compounds are racemic.
[c] Equilibrium solubility (pH 7.4).[9]
[d] Brain–plasma ratio; brain concentrations not corrected for 2–3% vascular contamination.

Acknowledgments

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References and notes

1. (a) Conn, P. J.; Tamminga, C.; Schoepp, D. D.; Lindsley, C. *Mol. Interventions* **2008**, *8*, 99; (b) Vandenberg, R. J.; Aubrey, K. R. *Expert Opin. Ther. Targets* **2001**, *5*, 507.
2. (a) Roberts, B. M.; Shaffer, C. L.; Seymour, P. A.; Schmidt, C. J.; Williams, G. V.; Castner, S. A. *Neuroreport* **2010**, *21*, 390; (b) Javitt, D. C. *Curr. Opin. Drug Discov. Devel.* **2009**, *12*, 468; (c) Gozzi, A.; Herdon, H.; Schwarz, A.; Bertani, S.; Crestan, V.; Turrini, G.; Bifone, A. *Psychopharmacology* **2008**, *201*, 273; (d) Depoortere, R.; Dargazanli, G.; Estenne-Bouhtou, G.; Coste, A.; Lanneau, C.; Desvignes, C.; Poncelet, M.; Heaulme, M.; Santucci, V.; Decobert, M.; Cudennec, A.; Voltz, C.; Boulay, D.; Terranova, J. P.; Stemmelin, J.; Roger, P.; Marabout, B.; Sevrin, M.; Vige, X.; Biton, B.; Steinberg, R.; Francon, D.; Alonso, R.; Avenet, P.; Oury-Donat, F.; Perrault, G.; Griebel, G.; George, P.; Soubrie, P.; Scatton, B. *Neuropsychopharmacology* **2005**, *30*, 1963.
3. (a) Wolkenberg, S. E.; Sur, C. *Curr. Top. Med. Chem.* **2010**, *10*, 170; (b) *Glycine Transporters and Their Inhibitors*; Gilfillan, R.; Kerr, J.; Walker, G.; Wishart, G. in Topics in Medicinal Chemistry: Glycine Transporters and Their Inhibitors, Springer-Verlag: Berlin, **2009**; *4*, 223; (c) Bridges, T. M.; Williams, R.; Lindsley, C. W. *Curr. Opin. Mol. Ther.* **2008**, *10*, 591; (d) Hashimoto, K. *Cent. Nerv. Syst. Agents Med. Chem.* **2007**, *7*, 177; (e) Harsing, L. G., Jr.; Juranyi, Z.; Gacsalyi, I.; Tapolcsanyi, P.; Czompa, A.; Matyus, P. *Curr. Med. Chem.* **2006**, *13*, 1017; (f) Lindsley, C. W.; Wolkenberg, S. E.; Kinney, G. G. *Curr. Top. Med. Chem.* **2006**, *6*, 1883; (g) Lowe, J. A., III. *Expert Opin. Ther. Pat.* **2005**, *15*, 1657; (h) Slassi, A.; Egle, I. *Expert Opin. Ther. Pat.* **2004**, *14*, 201.
4. *GlyT1 uptake inhibition assay:* The GlyT1 SPA assay measures [^{3}H] glycine uptake by CHO cells expressing human recombinant Glycine transporter 1 (GlyT1). Assay: To the wells of an OptiPlate, 2 μL of DMSO containing a test compound are spotted. This was followed by addition of 98 μL of cell suspension (~1 million/mL final). After incubating cells with compound for ~15 min, 100 μL of the SPA (200 μg/well final) and isotope mixture (30 nM isotope with 10 μM cold glycine, final) was added to initiate the glycine uptake. At 2 h, the plate was read on a TopCount to quantify SPA counts. Results: IC_{50}s are reported as the geomean and their corresponding standard deviation based on an *n* of 2, 4, or 6.
5. Rahman, S. S.; Coulton, S.; Herdon, H. J.; Joiner, G. F.; Jin, J.; Porter, R. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1741.
6. *General procedure for the Strecker reaction:* Acetic acid (1.1 equiv) was added to a mixture of benzaldehyde (1 equiv) in amine (12 equiv). Sodium cyanide (1.1 equiv) in 2 mL of water was added, and the resulting solution was stirred at room temperature until complete based on thin-layer chromatography (TLC) and/or LCMS analysis. The reaction was diluted with water, extracted with ethyl acetate (3×), and the combined organic layers were dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by flash column chromatography (SiO_2, ethyl acetate in hexanes) to afford the desired nitrile. Sluggish Strecker reactions or those with significant precipitate were warmed gently at 40 °C during stirring to achieve homogeneity.
7. *General procedure for alane reduction:* To a solution of sulfuric acid (2 equiv) in tetrahydrofuran (0.2 M) at 0 °C was added 2.0 M lithium aluminum hydride in tetrahydrofuran (4 equiv) rapidly dropwise and the resulting cloudy mixture was maintained at 0 °C for 30 min. The nitrile (1 equiv) was then added in tetrahydrofuran (0.2 M) via cannula, and the reaction was warmed to room temperature. After another 30 min, the reaction was cooled to 0 °C, and excess sodium sulfate decahydrate was added, which was accompanied by an exotherm. The resulting white mixture was diluted with ethyl acetate and stirred for 10 min before being filtered. The filtrate was concentrated and the resulting amine was carried on to the next step without further purification.
8. When sulfonamides were compared with their amide analogs, solubility improved; however, a 2- to 10-fold loss of potency was consistently observed for GlyT1 uptake inhibition (data not shown).
9. Alelyunas, Y. W.; Liu, R.; Pelosi-Kilby, L.; Shen, C. *Eur. J. Pharm. Sci.* **2009**, *37*, 172.