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Optimisation of a series of potent, selective and orally bioavailable GlyT1 inhibitors

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ABSTRACT

A series of heterocyclic sulfonamides have been developed which are potent and selective inhibitors of hGlyT1. SAR studies to optimise the in vitro and in vivo properties are described. Optimisation of the central scaffold resulted in cyclohexane sulfones **28** and **29**, which have good PK properties and show promise for further development.

Glycine plays a major role as a neurotransmitter in the mammalian central nervous system (CNS). In the forebrain, glycine acts as a necessary co-agonist with glutamate at *N*-methyl-D-aspartate (NMDA) receptors, thereby modulating NMDA-dependent excitatory neurotransmission.[1–4] The concentration of glycine at the synapse is regulated primarily by re-uptake via glycine transporters, of which two, GlyT1 and GlyT2, have been characterized.[2–6] These belong to the Na^+/Cl^- dependent family, which includes γ-aminobutyric acid, taurine and proline transporters.[2] GlyT2, which is localised largely in the brainstem and cerebellum, is responsible for glycine uptake at glycinergic synapses. GlyT1, which is located throughout the brain and is predominantly expressed by glial cells, is involved in regulating glycine concentration in the vicinity of NMDA receptor-expressing synapses.[7] Hypofunction of NMDA receptors has been implicated in the pathophysiology of schizophrenia, with evidence coming from both pre-clinical models and clinical data.[8–13] For example, sarcosine, an endogenous inhibitor of GlyT1, has been shown to provide significant improvements in the positive, negative and cognitive symptoms of stable schizophrenics when used as an adjunct to standard antipsychotic therapy.[10] This suggests that the pharmacological manipulation of synaptic glycine concentration using a GlyT1 inhibitor may be a viable method of potentiating NMDA receptor function in vivo and hence ameliorating the negative symptoms of schizophrenia. In this communication we describe the development of a series of potent and selective inhibitors of GlyT1.[14]

Propyl sulfonamide **1**, a potent and selective GlyT1 inhibitor, evolved from 'hit-to-lead' optimisation of a compound identified from an HTS screen of the Merck sample collection.[14–16] This compound exhibited excellent in vivo occupancy when dosed *i.p.*, and microdialysis studies showed that it increased levels of glycine in both the hippocampus and cortex.[16,17] However, this compound was compromised by very low plasma exposures on *p.o.* dosing, which correlated with high turnover in rat liver microsomes. Preliminary studies indicated that oxidation of the propyl side chain was a significant metabolic pathway. Attempts to find a more metabolically stable replacement for the propyl sulfonamide were hindered by very tight SAR, which indicated a lack of tolerance to substitution on the chain or replacement with more bulky substituents. Intriguingly, compound **2**, in which the propyl chain was replaced with cyclopropyl methyl, retained good potency. This led us to speculate that replacement of the cyclopropylmethyl moiety with a small π-system, for example a five-membered aromatic heterocycle, might be tolerated and afford an improved metabolic profile. A diverse range of heterocyclic sulfonamides were synthesised by treatment of the piperidine intermediate with the appropriate sulfonyl chloride in the presence of base (Scheme 1) utilising a RAS strategy with commercially available sulfonyl

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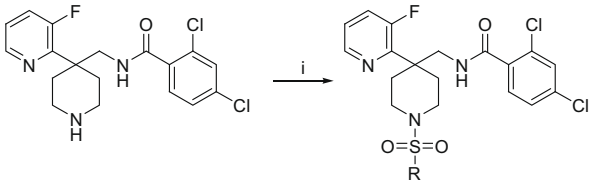
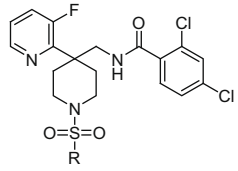
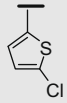
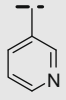
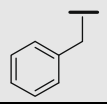


Scheme 1. Reagents and condition: (i) RSO_2Cl, $^iPr_2NEt$, DCM, rt.

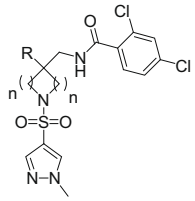
Table 1
Potency at GlyT1 and % microsomal turnover for compounds **1**–**10**



Compound	R	hGlyT1 IC_{50} (nM)[a]	% Turnover[b]		
			R	D	H
1		4.33	73	25	23
2		2	78	20	21
3		5.43	55	24	42
4		1.74	30	21	–
5		31%@3 μM	–	–	–
6		124.6	98	72	–
7		20%@3 μM	–	–	–
8		11.55	99	96	99
9		38%@3 μM	–	–	–
10		33%@3 μM	–	–	–

[a] See Ref. 15. For cases where <50% inhibition was achieved at 3 μM, values are expressed as %inhibition.
[b] Turnover of GlyT1 compounds (1 μM) in rat, dog and human liver microsomes. All incubations were carried out at 37 °C for 15 min. Protein concentration = 0.5 mg/ml; cosolvent = 0.99% MeCN + 0.01% DMSO.

Table 2
Potency at GlyT1 and % microsomal turnover for pyrazoles **11**–**13**

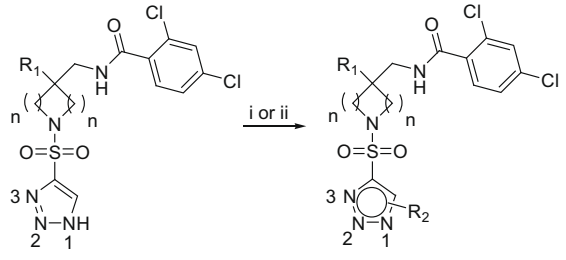


Compound	R^1	*n*	hGlyT1 IC_{50} (nM)[a]	% Turnover[b]		
				R	D	H
11		1	2.95	56	<5	25
12		2	10.35	5	38	10
13		1	3.62	17	70	21

[a,b] See Table 1.

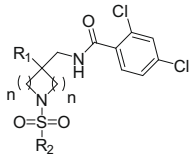
chlorides (Table 1).[15] Methyl imidazole **4**, which showed improved potency and microsomal turnover relative to compound **1**, emerged as a lead for further optimisation. Further substitution on the imidazole ring was not tolerated, as demonstrated by compound **5**. Methyl pyrazole **3** showed good potency but higher turnover in rat liver microsomes relative to compound **4**, although it was still an improvement over initial lead **1**. Although pyridine **8** was potent at the GlyT1 receptor, it was compromised by very high turnover in liver microsomes, which metabolism studies revealed was due to N-oxidation. Other six-membered aromatic systems were not tolerated (compounds **9** and **10**). Concurrently, we investigated azetidine as an alternative scaffold, which we anticipated might offer more steric tolerance to variation in the propyl sulfonamide side chain. The heterocyclic side chain modification gave potent compounds in this series (Table 2). In particular, the azetidine analogue of pyrazole **3**, compound **11**, was potent at the GlyT1 receptor, although turnover in rat liver microsomes remained high; metabolism studies indicated N-dealkylation to be a major route.

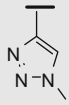
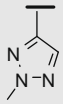
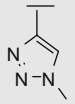
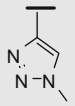
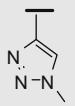
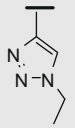
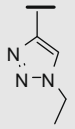
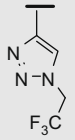
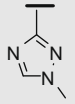
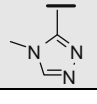
Replacement of the fluoropyridine moiety of piperidine **3** with a cyclopropyl methyl group gave **12**, with good potency at the GlyT1 receptor. Surprisingly, this compound displayed low turnover and no apparent N-dealkylation in rat liver microsomes, in sharp contrast to the 3-fluoropyridine analogue **3**. Combining these two changes resulted in the potent methyl pyrazole **13**. Microsomal turnover in rat was moderate but this compound possessed acceptable pharmacokinetics in rat (F = 48%; Clp = 46 ml/min/kg;



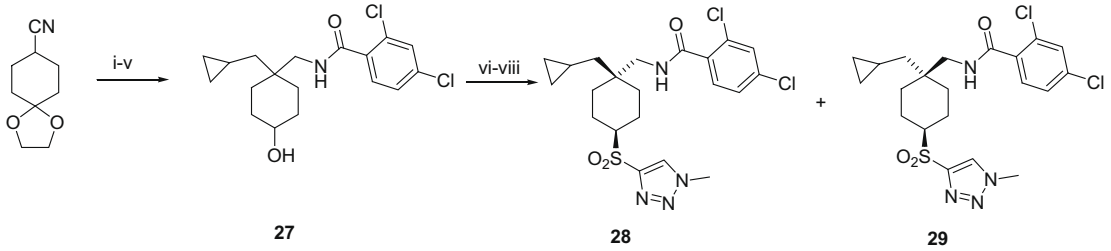
Scheme 2. Reagents and conditions: (i) *N*,*N*-dimethylformamide dimethyl acetal, toluene, reflux; (ii) NaH or K_2CO_3, R^2I, DMF, rt.

Table 3
Potency at GlyT1 and % microsomal turnover for triazoles **14–26**



Compound	R^1	R^2	*n*	hGlyT1 IC_{50} (nM)[a]	% Turnover[b]		
					R	D	H
14			1	0.1	88	24	32
15			1	131.7	–	–	–
16			1	184.9	–	–	–
17			1	870.8	–	–	–
18			2	.32	88	34	43
19			1	.31	15	5	22
20			2	.65	6	17	20
21			1	2.58	65	44	45
22			2	28.22	–	–	–
23			2	501.6	–	–	–
24			2	314.3	–	–	–
25			2	13%@3 μM	–	–	–
26			2	123.6	–	–	–

[a,b] See Table 1.



27 28 29

Scheme 3. Reagents and conditions: (i) KHMDS, toluene, cyclopropylmethyl bromide, −78 °C; (ii) Lithium aluminium hydride, Et_2O, −78 °C to rt; (iii) 2,4-dichlorobenzoyl chloride, $^iPr_2NEt$, DCM, 0 °C to rt; (iv) HCl, THF, rt; (v) $NaBH_4$, EtOH, rt; (vi) MsCl, pyridine, 0 °C; (vii) 1-methyl-1*H*-[1,2,3]triazole-4-thiol., $NaBH_4$, EtOH, 50 °C; (viii) oxone, acetone, water, reflux.

Table 4
Potency at GlyT1, microsomal turnover, rat pharmacokinetic properties and in vivo occupancy for compounds **28** and **29**

Compound	Stereochemistry	IC_{50} (nM)[a]	% Turnover[b]			Pharmacokinetics[c]			Occ_{50} (rat) (mg/kg)
			R	H	F (%)	$t_{1/2}$ (h)	Clp (ml/min/kg)	Vdss (l/kg)	
28	Sulfonyl and amide trans	1.89	23	28	59	1.8	16	2.3	2.2
29	Sulfonyl and amide cis	5.44	10	28	105	1.1	34	2.6	3.4

[a,b] See Table 1.
[c] See Ref. 18002E

$t_{½}$ = 2.4 h) with excellent occupancy (Occ_{50} = 3.8 mg/kg *p.o.*) in the mouse in vivo binding assay.[16]

Encouraged by these results, the analogous 1,2,4- and 1,2,3-triazoles were targeted. These compounds were accessed by sulfonamide formation as before to give the unsubstituted triazoles, which were subsequently methylated using *N*,*N*-dimethylformamide dimethyl acetal (Scheme 2).

For the 1,2,3-triazoles, this method allowed access to the N2- and N3-methylated products but N1-methylation occurred with concomitant formylation at the 5-position. However, alkylation with methyl iodide in the presence of base gave the N1 and N2 isomers only, in approximately a 1:3 ratio. The *N*-ethyl analogues were similarly prepared. The 1,2,3-triazoles were more potent than the 1,2,4-triazoles; the N1-methylated isomers were consistently the most active, (see Table 3). For example, cyclopropyl methyl piperidine **18** showed excellent potency and occupancy in vivo (Occ_{50} < 1 mpk (mouse)). This compound had reasonable pharmacokinetic properties in rat, with moderate clearance (39 ml/min/kg), volume of distribution (2.2 l/kg) and bioavailability (27%) but a fairly short half-life (1.1 h).[18] Compound **18**, like all the cyclopropyl methyl triazole analogues, exhibited very high turnover in rat liver microsomes due to N-dealkylation. In an attempt to mitigate this pathway, the 2,2,2-trifluoroethyl analogue **23** was accessed by alkylation with 2,2,2-trifluoroethyl trifluoro-methanesulfonate. However, compound **23** showed poor potency compared with its methyl and ethyl analogues. Interestingly, high turnover was not seen with the corresponding fluoropyridine analogues. Thus, compound **20** retained excellent potency and showed good in vivo occupancy (Occ_{50} = 7.5 mg/kg (mouse)) with significantly decreased turnover in rat liver microsomes. However, this compound had a very short half-life (0.3 h), low bioavailability (3%) and high clearance in rat.[18] Routes of metabolism studies carried out on **20** indicated oxidation of the piperidine ring and no observed N-dealkylation. It is clear from our studies that in optimising the metabolic fate of the molecule, one needs to consider the gross structure and not simply the moiety undergoing metabolic modification; this is to be expected from the molecular recognition component of P450 mediated oxidation. To address the oxidative metabolism of the piperidine moiety of **18**, cyclohexane analogues **28** and **29** were prepared, as shown in Scheme 3. The sulfone moiety was constructed from the mesylate of alcohol **27** by displacement with the triazole thiolate anion to give two sulfide geometric isomers in a 1:1 ratio, which were subsequently oxidised to the sulfones **28** and **29**.[19] As shown in Table 4, both isomers showed excellent potency and occupancy in vivo with acceptable pharmacokinetics in rat. Turnover was low to moderate in both rat and human liver microsomes. SAR studies involving cyclohexane analogues will be the subject of a subsequent communication.

In conclusion, the replacement of the cyclopropylmethyl group of **2** with heterocycles gave a novel series of potent and selective GlyT1 inhibitors. Compounds such as **20** and **13** displayed excellent potency and in vivo occupancy upon oral dosing, with reduced metabolic turnover compared with the initial lead. Cyclohexane analogues **28** and **29** also showed improvements over the initial lead and benefited from a lack of oxidative metabolism on the central ring which translated into improved pharmacokinetic properties in rat.

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14. Selectivity over hGLYT2 appears to be inherent in this class of inhibitor. For example, compounds **3**, **4**, **12**, **13**, **18**, and **20** were all assayed at >10 μM against hGLYT2. See also Williams, J. B.; Mallorga, P. J.; Lemaire, W.; Williams, D. L.; Na, S.; Patel, S.; Conn, J. P.; Pettibone, D. J.; Austin, C.; Sur, C. *Anal. Biochem.* **2003**, *321*, 31.
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19(5), 1488–1491; (b) Wolkenberg, S. E.; Zhao, Z.; Wisnoski, D. D.; Leister, W. H.; O'Brien, J. A.; Lemaire, W.; Williams Jr., D. L.; Jacobson, M. A.; Sur, C.; Kinney, G. G.; Pettibone, D. J.; Tiller, P. R.; Smith, S.; Gibson; Ma, B. K.; Polsky-Fisher, S. L.; Lindsley, C. W.; Hartman, G. D. *Bioorg. Med. Chem. Lett.* **2009**, *19*(5), 1492–1495.

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