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Discovery of 4-substituted-8-(2-hydroxy-2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as a novel class of highly selective GlyT1 inhibitors with improved metabolic stability

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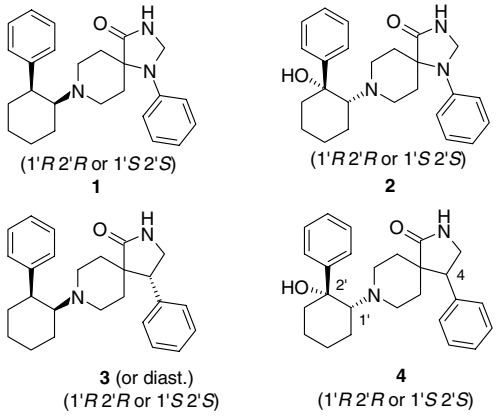
Abstract—A novel class of 4-aryl-8-(2-hydroxy-2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1- ones have been discovered and developed as potent and selective GlyT1 inhibitors. The molecules are devoid of activity at the GlyT2 isoform and display excellent selectivities against the μ-opioid receptor as well as the Nociceptin/Orphanin FQ peptide (NOP) receptor. In particular these novel compounds **4** as well as the 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one **3** show improved metabolic stability and pharmacokinetic profiles in rodents compared to previous triazaspiropiperidine series **1** and **2**. We have also identified within these diazaspiropiperidine series a key relationship between reducing basicity of the piperidine nitrogen and reducing hERG affinity.

GlyT1 is a selective transporter of the neurotransmitter glycine that is localized in the brain in close vicinity with the NMDA receptor for which glycine is an obligatory co-agonist.[1,2] In vitro experiments have recently shown that GlyT1 inhibition results in an elevation of glycine levels with a consequent enhancement of NMDA receptor activity.[3] This observation strongly suggests a role for GlyT1 inhibitors in the treatment of CNS disorders like schizophrenia where NMDA-R hypofunction is believed to be involved.[4] Additional support for this approach in the treatment of schizophrenia comes from clinical studies where sarcosine,[5] a prototypical weak GlyT1 inhibitor, improved positive, negative and cognitive symptoms in schizophrenic patients, when administered together with risperidone. As a result, considerable effort has been focused on the development of selective GlyT1 inhibitors.[6]

We have recently described the discovery of *N*-(2-aryl-cyclohexyl) substituted spiropiperidines **1**[7], **2**[8] and **3**[9] as a novel class of GlyT1 inhibitors which all display excellent selectivity against the GlyT2 isoform.



The main liability of **1** was identified early whereupon only very low selectivity against the μ-opioid receptor and the Nociceptin/Orphanin FQ peptide (NOP) receptor was achievable. However, we successfully addressed these issues in a focused programme culminating in the

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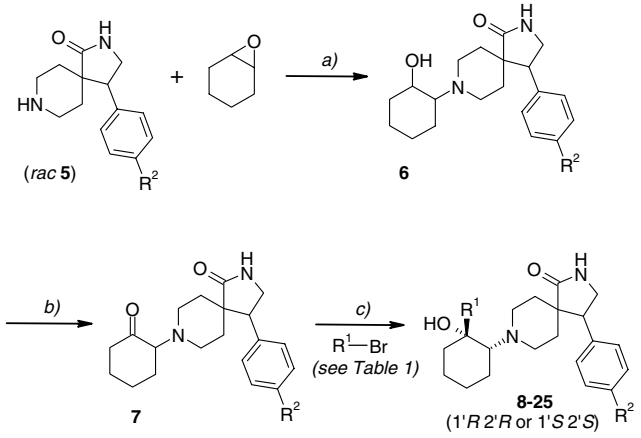
discovery of **2** and **3**. Since we had already demonstrated significant improvement towards our desired pharmacological properties within the triazaspiro series by performing the structural changes **1** to **2** (addition of a 2′-OH group) and also within the diazaspiro series **1**–**3** (replacement of the 4-N with CH group), herein we wish to report on our successful efforts to identify another new class of GlyT1 inhibitors **4** by the incorporation of both of the fore-mentioned chemical mutations into one series.[10]

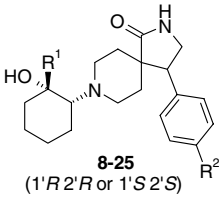


Scheme 1. Synthesis of compounds **8**–**25**. Reagents and conditions: (a) EtOH, reflux, 95–99%; (b) SO_3–pyridine complex, DMSO, TEA, DCM, rt, 58–76%; (c) i—R^1Br, BuLi, THF, −78 °C to rt, 45–90%.

The synthetic strategy to access the target molecules **4** closely follows our previous work and was designed to prepare a (1:1:1:1) mixture of four diastereoisomers with a 1,2-*cis* relationship (Scheme 1). In analogy, the synthesis commenced with an efficient opening of the commercially available cyclohexene epoxide, however this time, with the 4-substituted diazaspiropiperidine **5**[11] to afford the α-aminoalcohols **6** in excellent yield. Installation of the appropriately substituted aryl residue (R^1) with excellent stereoselectivity was efficiently achieved through a two-step process involving oxidation of **6** to the α-aminoketone **7** by SO_3–pyridine complex, followed by reaction with the appropriate aryl-lithium (R^1-Li) reagent formed in situ.

In order to aid interpretation of the biological results, where possible we endeavoured to separate the diastereoisomers by the implementation of chiral-phase HPLC (Chiralpak AD®) and did not routinely test the (1:1:1:1) mixture of four stereoisomers formed in the reaction sequence. This was not always possible and Table 1 shows the activity for the pure or the mixture of stereoisomers with the fastest eluting component designated **a** and the slowest eluting designated **d**.

Table 1. In vitro inhibitory activity at the GlyT1 transporter for compounds **8**–**25**



Compound	R^1	R^2	GlyT1 EC_{50}[a] (μM)			
			a	**b**	**c**	**d**
8	4-MeO–Ph	H		4.48[b]		
9	3-MeO–Ph	H		>30[b]		
10	4-MeO–Ph	F	3.300	1.181	1.128	0.158
11	3-MeO–Ph	F	0.158	2.599	0.127	5.301
12	4-F–Ph	H	0.129[d]		0.07	0.825
13	3-F–Ph	H	0.158	2.599	0.127	5.301
14	3-F–Ph	F	0.095	0.077	0.474	0.646
15	2-F–Ph	F	0.596	0.110	1.769	0.069
16	3-Cl–Ph	F	6.251	2.34	0.118	0.073
17	4-CN–Ph	F	1.953	11.33	>30[c]	
18	4-CF_3–Ph	F		1.313[b]		
19	4-$MeSO_2$–Ph	F		18.646[b]		
20	4-Me–Ph	F	0.42	>30	0.437	0.132
21	3-Me–Ph	F	2.638	10.71	>30[c]	
22	2-Me–Ph	F	0.596	0.110	17.690	0.069
23	4-*t*-Bu–Ph	F	>30	>30	>30[c]	
24	2-CF_3O–Ph	F	1.394[d]		0.646[c]	
25	4-Imidazole–Ph	F	7.345	>30	27.544	0.175

[a] Radiometric assay using [^{3}H]-glycine[8].
[b] Mix of **a**, **b**, **c** and **d**.
[c] Mix of **c** and **d**.
[d] Mix of **a** and **b**.

For exploration of our preliminary SAR (Table 1), we initially focused on derivatives where R^2 = H or F, since these were found to be optimal in the previous series **1**, **2** and **3**. Overall the SAR of this class of molecules **4** did not follow the same SAR pattern previously established with the series **1**–**3**. For example, although only tested as a mixture, compounds **8** and **9** bearing *p*-OMe and *m*-OMe groups, respectively, were surprisingly found to be inactive in sharp contrast to their potent congeners in the triazaspiropiperidine series **1** and **2** which had EC_{50} in the nanomolar range. Much improved activity was obtained for R^2 = F, with **10d** and **11a**,**c** also showing affinity in the nM range. We then explored the effects of the favoured halogen substituents at R^1 with compounds **12**–**16** each demonstrating a dramatic increase to low nanomolar affinity at GlyT1. Electron-withdrawing groups such as those evident in **17**–**19** provided only inactive compounds. Methyl substituents were also well tolerated at the *p*- and *o*-positions where **22** even provided three active diastereoisomers. Intriguingly, *m*-Me substitution as in **21** was not tolerated in this series.

Before moving further ahead with this novel diazaspiropiperidine class **4** it was essential to establish superiority over the previous series **1**–**3** and we therefore selected **12a**–**d** as a focus for further evaluation. Initially we set out to demonstrate that the introduction of an additional 2′-OH group together with an N to CH mutation would completely remove any off-target liabilities observed in **1**. Indeed, pleasingly these efforts were rewarded when we could demonstrate excellent selectivity over GlyT2, NOP and most importantly we had completely removed activity at the μ-opioid receptor as shown in Table 2.

As a result, of these encouraging results, we proceeded to explore the key liabilities identified in the previous series in much more detail. A key impediment in the general class of triazaspiropiperidines **1** and **2** has been the non-optimal metabolic stability. This was vastly improved upon in the triazaspiropiperidine series by replacement of the R^2 aryl residue with an alkyl substituent.[8]

Table 2. In vitro inhibitory activity at the GlyT1 and the GlyT2 transporters and potency in inhibiting the NOP and μ receptors for compounds **1**–**3**, **12a**–**d**

Compound	EC_{50}[a] (μM)		NOP IC_{50}[b] (μM)	μ IC_{50}[c] (μM)
	GlyT1	GlyT2		
1	0.026	12	6	0.15
2	0.044	72	15	2.7
3[d]	0.232	25	>10	3.92
12[d]	0.481	>30	>10	24
12ab	0.129	—	>10	>10
12c	0.07	>30	>10	>10
12d	0.825	—	>10	>10

[a] Radiometric assay using [^{3}H]-glycine.[8]
[b] Displacement of [^{3}H]NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[12]
[c] Displacement of [^{3}H]naloxone in membranes prepared from BHK cells transiently expressing hμ receptors.[12]
[d] Mixture of four diastereoisomers (see Table 1).

Table 3. Microsome stability data for compounds **1**, **12c**, **14b**, **15d**, **26**, and **27**

Compound	GlyT1 EC_{50} (μM)	CL_{int}[a]	
		Mouse microsomes	Human microsomes
1	0.026	240[b]	68
26	0.024	499[b]	78
27	0.061	36	23
15d	0.069	39	28
14b	0.077	16	11
12c	0.070	13	44

[a] CL_{int}, intrinsic, μL/min/mg protein.
[b] Rat data.

However, we endeavoured to retain the aryl moiety in the diazaspiropiperidine series and we were delighted to observe a significant improvement in metabolic stability of nearly all related derivatives in the diazaspiro series **3** and **4** compared to the triazaspiro series **1** and **2** (Table 3). For example, the predicted maximum achievable bioavailability (MAB), in human microsomes, for **1** was only ~18%, whereas effecting the N to CH replacement resulted in a significant improvement of the MAB to ~45% for **27**.

This trend was also observed in a single dose in vivo pharmacokinetic study in mice and rats for this series where a much lower in vivo clearance was recorded (Table 4). This resulted in an increase of oral bioavailability (*F*) from 8% to 27%. In addition, we had also achieved greater brain penetration for **27** compared to **1**.

In the 2′-OH series a significant improvement of MAB was also observed by examining the pair **26** and **12c** where an improvement in predicted MAB, in human microsomes, from 14% to ~55% was observed. In general, it was not possible to increase further the metabolic stability within these subseries of the diazaspiropiperidines **3** and **4** with all derivatives in Table 1 generally displaying comparable microsomal stability.

After achieving our desired in vitro pharmacological profile with suitable pharmacokinetic properties in vitro and in vivo, we then proceeded to further develop these

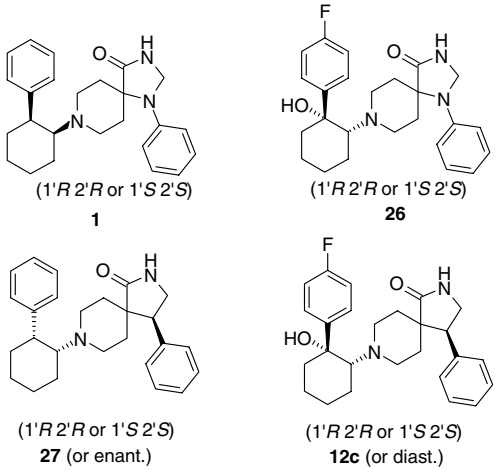


Table 4. Selected pharmacokinetic parameters for compounds **1** and **27**

Compound	*F* (%)	CL[a]	V_{ss} (L/kg)	B/P
1[b]	8	120	7.3	1.6
27[b]		63	4.3	5.2
27[c]	27	99	8.9	4.1

[a] CL, total clearance, mL/min/kg protein.
[b] Measured in rat.
[c] Measured in mouse, B/P = brain to plasma ratio; V_{ss} = volume of distribution.

Table 5. In vitro inhibitory activity at the hERG K channel for compounds **12c**, **27** and **28**

Compound	hERG IC_{50}[a] (μM)	pK_a[b]
27	1.400	8.85
12c	1.800	8.54
28	>24.000	6.89

[a] Inhibition of hERG K channel determined by whole-cell patch-clamp experiments on a transfected CHO cell line.
[b] Determined by potentiometric titration in a MeOH/water mixture at rt with the GLpKa instrument from Sirius Analytical Instruments.

molecules. However, we were concerned with the potential liability for blocking the hERG K channel where overlapping pharmacophoric elements within our spiropiperidine class of molecules were known to exist. When tested, as expected, low micromolar activity was observed for **27** (Table 5) at the hERG K channel and we believed that we could reduce these effects by modulation of basicity of these compounds. Indeed, the introduction of an additional β-oxygen relative to a nitrogen atom is known to reduce its basicity and as expected **12c** and congeners show a slightly reduced effect at the hERG K channel in line with the reduction of basicity of the piperidine nitrogen. In order to reduce even further the effects at the hERG K channel, we introduced a second β-oxygen atom, this time within the cyclohexyl ring, as shown in the tetrahydropyran compound **28**, which gratifyingly had the desired outcome of completely removing activity at the hERG K channel.

The synthesis of **28** was efficiently achieved as outlined in Scheme 2. The 3,4-epoxytetrahydropyran **30** was prepared according to literature precedent,[13] and then reacted with **29** to give a mixture of regioisomeric α-aminoalcohols **31** and **32**. The major product in the reaction was the desired diastereoisomer **32** which was then separated by chromatography on silica gel, oxidized to the α-aminoketone **33** in good yield by SO_3–pyridine complex. Subsequent reaction with phenyl lithium afforded the final product **28** in 59% yield. Pleasingly, compound **28** apart from showing no activity at the hERG K channel also demonstrated good affinity (91 nM) at the GlyT1 transporter and was inactive at the GlyT2 transporter.

In conclusion, replacement of the sp^2 nitrogen atom at the position 4 of the imidazolidinone ring in our original series **1** with a sp^3 carbon followed by introduction of a 2′-OH group resulted in a novel and potent class of 4-aryl-8-(2-hydroxy-2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]- decan-1-one GlyT1 inhibitors. This diazaspiropiperidine series display, as we had anticipated, high selectivities against the μ- and NOP-opioid receptors. In general, the diazaspiropiperidine series are superior to the triazaspiropiperidine series where a significant increase in microsomal stability was achievable and related into an improved PK profile in vivo in mouse and rat. We have also identified a key relationship between reducing basicity of the piperidine nitrogen and reducing hERG affinity which allowed us to design derivatives such as **28** free of activity at the hERG K channel. The subsequent paper will describe additional pharmacological improvements within the diazaspiropiperidine family of GlyT1 inhibitors in the development of another novel class of 4-substituted-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one GlyT1 inhibitors.

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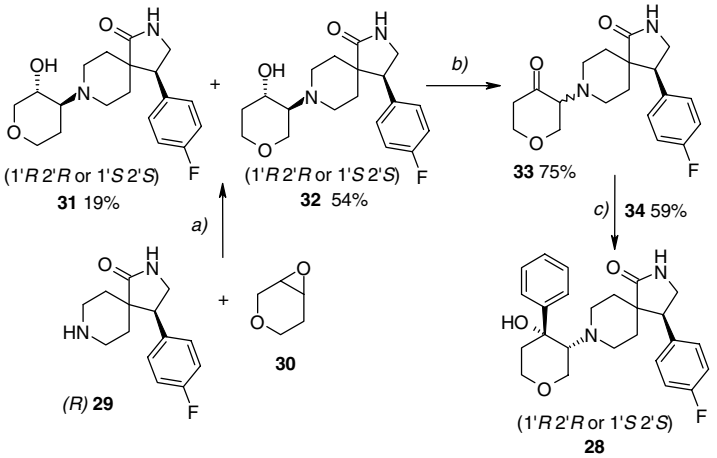

Scheme 2. Synthesis of compound **28**. Reagents and conditions: (a) EtOH, reflux, 73%; (b) SO_3–pyridine complex, DMSO, TEA, DCM, rt, 75%; (c) PhLi, THF, −78 °C to rt, 59%.

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