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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4321–4325

4-Substituted-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as a novel class of highly selective GlyT1 inhibitors with superior pharmacological and pharmacokinetic parameters

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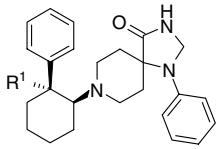
Received 7 April 2006; revised 17 May 2006; accepted 17 May 2006
Available online 9 June 2006

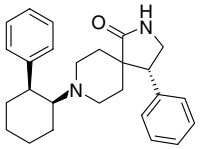
Abstract—A novel class of 4-substituted-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-ones have been discovered and developed as potent and selective GlyT1 inhibitors. The molecules are devoid of activity at the GlyT2 isoform and display excellent selectivities against the μ opioid receptor as well as the nociceptin/orphanin FQ peptide (NOP) receptor. These molecules also exhibit superior pharmacological and pharmacokinetic parameters, relative to all GlyT1 inhibitors of the spiropiperidine family, culminating in the identification of **16b** with an oral bioavailability of ~60%. In addition, a straightforward two-step procedure for the assembly of the target molecules is also presented.

Compelling evidence suggests that an impairment in NMDA neurotransmission is involved in the pathophysiology of schizophrenia.[1] This hypothesis originated from the observation that PCP, a recreational drug which has been demonstrated to be a potent and selective NMDA-R blocker, is able to reproduce, in healthy subjects, the positive, negative and cognitive symptoms of schizophrenia.[2] Thus, therapeutic intervention aimed at increasing NMDA synaptic tone is expected to show beneficial effect in schizophrenic patients. As glycine is known to act as a positive allosteric modulator of the NMDA receptor,[3] one strategy to enhance NMDA receptor activity is to elevate extracellular levels of glycine in the local microenvironment of the synaptic NMDA receptor. Glycine elevation can be achieved by inhibition of the glycine transporter 1 (GlyT1) which is co-expressed in the brain together with the NMDA receptor and is responsible for the maintenance of low extracellular glycine concentrations.[4,5] Strong support for this novel therapeutic approach comes from clinical studies where glycine[6] and sarcosine[7] (a prototypical weak GlyT1 inhibitor) displayed benefits to schizophrenic patients as an add-on to conventional therapy. As a result, considerable efforts have been focused on the development of selective GlyT1 inhibitors.[8]

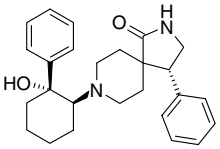
We have recently introduced a novel series of N-(2-arylcyclohexyl) substituted spiropiperidines **1a**,[9] **1b**,[10] **2**[11] and **3**[12] as a novel class of GlyT1 inhibitors which



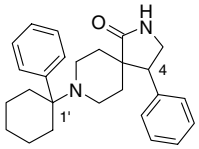
(1′*R* 2′*R* or 1′*S* 2′*S*)
1a R^1 = H
1b R^1 = OH



(1′*R* 2′*R* or 1′*S* 2′*S*)
2



(1′*R* 2′*R* or 1′*S* 2′*S*)
3



4

Keywords: GlyT1; GlyT2; NMDA; Schizophrenia; Transporter; Glycine; Spiropiperidine.
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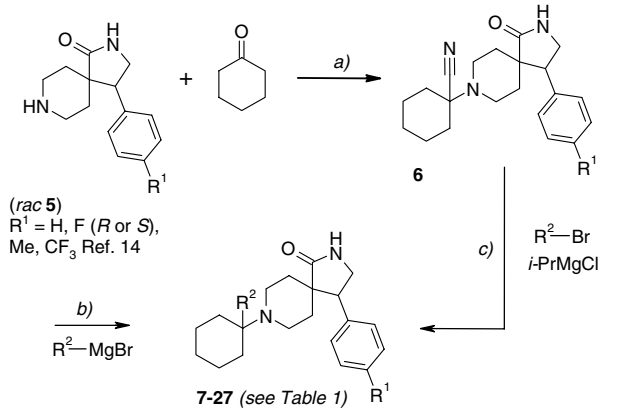
doi:10.1016/j.bmcl.2006.05.063

display excellent selectivity against the GlyT2 isoform. These series evolved through the stepwise incorporation of specific chemical mutations within the structures that allowed us to successfully address all relevant off-target liabilities with the accomplishment of excellent selectivities against the μ opioid receptor as well as the nociceptin/orphanin FQ peptide (NOP) receptor achieved for **1b**, **2** and **3**. The main deficiency within these triaza- and diazaspiropiperidine series was the relatively low microsomal stability which translated into non-optimal in vivo pharmacokinetic properties.

Herein we wish to report on our recent efforts[13] to discover and develop a further structurally novel, potent and selective chemotype of GlyT1 inhibitors **4** which have inherently improved pharmacological and pharmacokinetic properties when compared to **1**–**3**.

The rationale for the design of this novel chemotype **4** of GlyT1 inhibitors with a goal to improve upon the pharmacokinetic properties when compared to **1**–**3** was derived from some proprietary in-house knowledge that the poor metabolic stability of this class of compounds was due to oxidative processes taking place resulting in cleavage of the N–C1′ bond. Therefore, we postulated that relocating the substituents from C2′ to the C1′ position, as shown in **4**, would offer some protection to the molecule from rapid oxidative clearance. More importantly, to explore this hypothesis, we also significantly simplified the stereocomplexity of the diazaspiropiperidine series moving from three stereocentres in **2** to only one stereocentre in **4** where we had already been able to ascertain the absolute configuration of our key diazaspiropiperidine building block **5**.[14]

The synthetic strategy to access the target molecules **4** relied on a straightforward two-step procedure involving a Bruylants amination reaction[15] as shown in Scheme 1. In our hands, in order to achieve, on a preparatively useful scale, useful yields we found it best to allow the first step of the sequence (Strecker-type reaction with TMSCN and AcOH) to proceed slowly (up to 5 days at rt) followed by isolation of the intermediate amino nitriles **6**. Encouraging the reaction to proceed to completion within a few hours, by heating, normally resulted in isolated yields of the desired product **6** of <40%. Thereafter, reaction of **6** with either a range of commercially available Grignard reagents at room temperature (step b) or through reaction with Grignard reagents formed in situ using *i*-PrMgCl (step c) normally afforded high yields of the final products **7**–**27**.

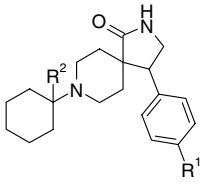


Scheme 1. Synthesis of compounds **7**–**27**. Reagents and conditions: (a) TMSCN, AcOH, rt, 3–5 days, 50–62%; (b) THF, rt, on, 73–94% or (c) THF, 0 °C to rt, 2 h, on, 10–75%.

As in part of our previously described work we specifically used *rac*-**5** in the synthesis strategy and preferred to separate the enantiomers routinely by chiral-phase HPLC (Chiralpak AD®). Table 1 shows the activity at GlyT1 for the racemate derivatives and also details the activity for individual enantiomers in the cases where we decided to separate them with the fastest eluting component designated a and the slowest eluting designated **b**.

For exploration of our preliminary SAR (Table 1), we initially focused on derivatives where R^1 = H and F since these substituents were found to be optimal in all of the previous series **1**–**3**. In general the SAR parallels what we earlier established for **1**–**3** with even the racemates, of nearly all derivatives, prepared showing nanomolar affinity as GlyT1 inhibitors. Without exception, strongly electron-withdrawing substituents such as CF_3

Table 1. In vitro inhibitory activity at the GlyT1 transporter for compounds **7**–**27**



Compound	R^1	R^2	GlyT1 EC_{50}[a] (μM)		
			rac	**a**	**b**
7	H	Ph		0.077	0.107
8	H	4-CF_3–Ph		1.212	6.969
9	H	4-CN–Ph		>30	16.00
10	H	4-CF_3O–Ph	4.563		
11	H	4-Cl–Ph		0.45	0.81
12	H	4-Me–Ph		0.284	0.480
13	H	3-F–Ph		0.423	0.684
14	H	3-MeO–Ph		5.61	26.231
15	H	3-Thienyl	0.682		
16	F	4-F–Ph	0.095	0.056	0.097
17	F	4-Me–Ph		1.474	0.419
18	F	3-F–Ph	0.393		
19	F	3-CN–Ph	>30		
20	F	3-CF_3O–Ph		16.728	21.519
21	F	3-Me–Ph	0.563	4.000	0.949
22	F	2-Me–Ph	0.350		
23	F	2-Thienyl		0.381	0.281
24	F	3-Thienyl		0.213	0.099
25	Me	Ph	0.139		
26	Me	4-F–Ph	0.073		
27	CF_3	4-F–Ph	0.433		

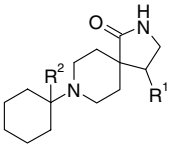
[a] Radiometric assay using [^{3}H]-glycine.[9]

and CN were not well tolerated as demonstrated by **8**, **9** and **19**. As we had observed in an earlier series, *m*- and *p*-CF_3O, as exemplified in **10** and **20**, was not a favoured substituent and neither was a *m*-OMe substituent, for example, **14**. Methyl substituents were well tolerated at all of the *p*-, *m*- and *o*-positions providing low nanomolar GlyT1 inhibitors for the racemates in **12**, **17**, **21** and **22**.

We also examined some small heterocyclic groups and pleasingly, the 2- and 3-thiophene substituents **15**, **23** and **24** provided very potent compounds. In the previous series, simple Ph, 4-Cl-phenyl 3-F-phenyl and 4-F-phenyl were optimal substituents based on their affinity as GlyT1 inhibitors, and this was found to be the case also as shown for **7**, **11**, **13**, **16**, **18**, **26** and **27**. In fact, some of these compounds showed good potency as their individual enantiomers as tabulated for **7a**,**b**, **11a**,**b**, **13a**,**b** and **16a**,**b**. We also briefly explored changes at R^1 by the introduction of Me (**25** and **26**) and CF_3 (**27**) groups which were also found to provide potent derivatives.

In order to further expand the SAR opportunities within this class, we examined the influence of more diverse changes to R^1 and R^2 detailed in Table 2. It was found, in analogy to our previous work,[10] that it was possible to replace the R^1 aryl group with an alkyl group. In this case the prototypical *n*-Pr group in combination with the 4-F-phenyl group provided the most potent derivative **29** even as a mixture of enantiomers. Separation of **28** into its enantiomers provided two compounds with an EC_{50} of 82 and 62 nM at GlyT1. In the previous series, the R^2 aryl group was essential for good inhibition at GlyT1. For this new series cyclic aliphatic units such as those shown in **33**–**35** and **40** were also shown to be inactive. However, replacement with an *i*-Pr group (**36** and **41**) provided the first examples of potent derivatives of this type within the diazaspiropiperidine class.

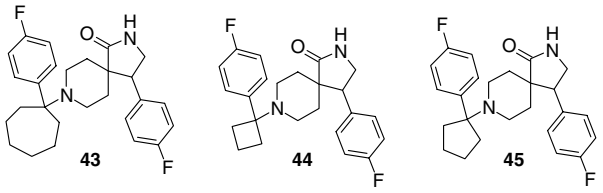
Table 2. In vitro inhibitory activity at the GlyT1 transporter for compounds **28**–**42**



Compound	R^1	R^2	GlyT1 EC_{50}[a] (μM) *rac*
28	*n*-Pr	Ph	0.161
29	*n*-Pr	4-F–Ph	0.093
30	*n*-Pr	2-Thienyl	0.229
31	*n*-Pr	2-Thienyl-5-Me	0.113
32	Ph	2-Thienyl	0.147
33	Ph	*c*-Hexyl	15.237
34	Ph	*c*-Pentyl	6.287
35	Ph	*c*-Pr	>30
36	Ph	*i*-Pr	0.970
37	Ph	$Me_2C{=}CH$	0.979
38	4-F–Ph	2-Thienyl	0.067
39	4-F–Ph	3-Thienyl	0.099
40	4-F–Ph	*c*-Pr	>30
41	4-F–Ph	*i*-Pr	0.787
42	4-F–Ph	Et	1.466

[a] Radiometric assay using [^{3}H]-glycine.[9]

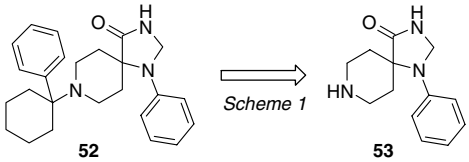
An additional structural modification examined was the replacement of the cyclohexyl unit of **4** as demonstrated in the preparation of the derivatives **43**–**45**. Interestingly, the cycloheptane unit was well tolerated with **43** showing a potency of 245 nM as an inhibitor at the GlyT1 transporter and was free of activity (>30 μM) at the GlyT2 isoform. However, in stark contrast the cyclobutane ring was not tolerated since **44** showed an EC_{50} > 30 μM. Unfortunately, it was not possible to prepare the corresponding cyclopentane **45** derivative following the same protocol as described in Scheme 1.

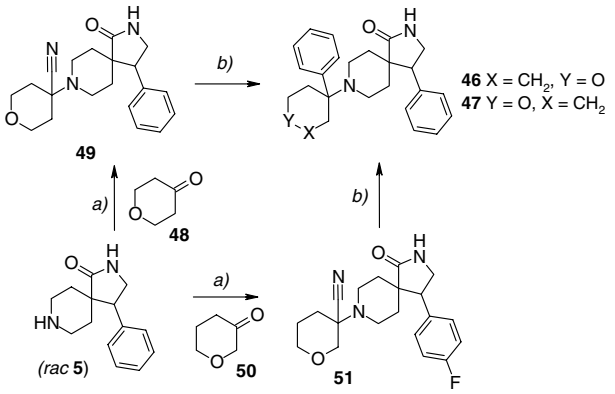


Following the strategy outlined below in Scheme 2 it was possible to prepare the pyrano-derivatives **46** as an enantiomeric mixture (GlyT1 EC_{50} = 1.419 μM) and **47** as a mixture of four diastereoisomers which we managed to separate (GlyT1 EC_{50} (μM) = a 3.661, b 7.290, c >30 and d 0.525).

With the difficulties we had previously encountered with **1a** it was essential to fully establish excellent levels of selectivity against the GlyT2 isoform as well as the μ opioid receptor and the nociceptin/orphanin FQ peptide (NOP) receptor. Table 3 nicely illustrates the levels of selectivity achieved against each of these receptors for the main molecules of interest. These results are particularly exceptional bearing in mind the remarkably high affinity (15 nM) towards the μ opioid receptor and the NOP receptor for the corresponding triazaspiropiperidine derivative **52** prepared in analogy to Scheme 1 using the commercially available 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **53**.

Next, we examined the effect on the metabolic stability in microsomes within this class since this was a key parameter which remained to be optimized. For example, the triazaspiropiperidine derivative **52** was a high clearance compound resulting in very low predicted maximum achievable bioavailability (MAB) of 6% and 16% in mouse and human, respectively. As can be seen Table 4 confirms our hypothesis since the structural





Scheme 2. Synthesis of compounds **46** and **47**. Reagents and conditions: (a) TMSCN, AcOH, rt, 1 day, 25–30%; (b) PhMgBr, THF, reflux, 1 h, 30–32%.

Table 3. In vitro inhibitory activity at the GlyT1 and the GlyT2 transporters and potency in inhibiting the NOP and μ receptors for compounds **7**, **11**–**13**, **16**–**18**, **21**, **22**, **28**, **29** and **52**

Compound	GlyT1 EC_{50}[a] (μM)	GlyT2 EC_{50}[a] (μM)	NOP IC_{50}[b] (μM)	μ IC_{50}[c] (μM)
7a	0.077	>30	>10	5.5
7b	0.107	>30	6.15	7.64
11a	0.45	>30	>10	19.8
11b	0.81	>30	>10	3.60
12a	0.284	>30	>10	9.12
12b	0.48	>30	>10	11.9
13a	0.423	>30	>10	8.04
13b	0.684	>30	>10	13.50
16	0.095	>30	>10	7.80
16a	0.056	>30	>10	>10
16b	0.097	>30	>10	7.40
18	0.393	>30	>10	7.18
21	0.563	>30	>10	7.83
21b	0.949	>30	>10	24.83
22	0.350	>30	>10	3.851
28	0.161	—	—	2.76
28a	0.082	—	—	2.73
28b	0.062	—	—	1.87
29	0.093	—	—	3.03
52	0.072	>30	0.015	0.015

[a] Radiometric assay using [^{3}H]-glycine.[9]
[b] Displacement of [^{3}H]-NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[16]
[c] Displacement of [^{3}H]-naloxone in membranes prepared from BHK cells transiently expressing hμ receptors.[16]

changes made in the development of **4** led to compounds with superior microsomal stability relative to all previous series. In close examination we can conclude that the 4-propyl-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro-[4.5]decan-1-one derivatives **28** and **29** displayed similar MAB values (60% and 47%, respectively, in human microsomes and 45% and 48%, respectively, in mouse microsomes), compared to their triazaspiropiperidine congeners.[10] However, the best improvements were observed in the 4-aryl-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one derivatives with the microsomal data for nearly all derivatives examined predicting MAB values generally superior to all our previous series. In particular, we were encouraged by the derivatives **7** and **16** where we had demonstrated levels of microsomal stability for both species (human and mouse) in the desired range.

Table 4. Microsome stability data for compounds **7**, **11**, **16**, **28**, **29**, **38**, **39** and **52**

Compound	CL_{int}[a] (mouse microsomes)	CL_{int}[a] (human microsomes)	MAB (%)	
			Mouse	Human
7	5	9	86	63
7a	0	8	100	67
7b	2	16	93	50
11a	10	114	76	12
16a	38	8	46	67
16b	1	3	98	84
28	39	10	45	60
28a	30	13	51	54
28b	34	18	48	47
29	24	14	57	53
38	80	9	28	64
39	39	4	45	78
52	470	82	6	16

[a] CL_{int}, intrinsic clearance (μL/min/mg protein).

Table 5. Selected pharmacokinetic parameters for compound **16b**

Compound	*F* (%)	CL[a]	$T_{0.5}$ (h)	V_{ss} (L/kg)	*B*/*P*
16b[b]	60	35	2.8	7.6	3.0

[a] CL, total clearance (mL/min/kg protein).
[b] Determined in mouse, *B*/*P*, brain to plasma ratio; V_{ss}, volume of distribution.

We then examined our most favoured derivative **16b**[17] in a single dose in vivo pharmacokinetic study as a representative of the 4-aryl-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one series (Table 5). As expected the excellent parameters obtained in vitro resulted in the highest achieved oral bioavailability (*F*) of 60% for all the spiropiperidine classes we have examined which was also concomitant with excellent levels of brain penetration.

Since the derivatives of type **4** have structural similarity with PCP we have also demonstrated that they do not have 'PCP-like' behaviour by examining **7**, **7a**, **7b** and **16b** in a displacement experiment with ^{3}H-MK801 in NMRI mouse forebrain membranes where an $IC_{50} > 30$ μM was obtained for each.

Moreover, derivatives from the 4-aryl-8-(1-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one class **4** have shown no significant inhibition of the hERG K channel as outlined in Table 6. Notably, we were able to diminish affinity towards the hERG K channel by incorporation of a β-oxygen atom as revealed in the structures **46** and **47** which closely follows the relationship[12b] we generated in the design of the GlyT-1 inhibitors **3** also free of hERG K channel activity.

In conclusion, our work in the spiropiperidine class has culminated in the discovery of a novel, potent and selective chemotype of GlyT1 inhibitors **4**. We stepwise designed the 4-substituted-8-(1-phenyl-cyclohexyl)-2,8-

Table 6. In vitro inhibitory activity at the hERG K channel for compounds **12c**, **27** and **28**

Compound	HERG IC_{50}[a] (μM)	pK_a[b]
16b	1.300	8.75
46	>11.000	7.1
47	>14.000	7.71

[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.

diaza-spiro[4.5]decan-1-one class to display excellent selectivities against the μ opioid receptor as well as the nociceptin/orphanin FQ peptide (NOP) receptor. In addition, this work has described in detail our strategy to improve upon the pharmacological properties and pharmacokinetic properties of the diazaspiropiperidine series by specific chemical mutations throughout the development of the class. Compound **16b** has demonstrated for the first time that GlyT1 inhibitors of the spiropiperidine family can achieve high oral bioavailability.

Acknowledgments

We thank Markus Bender, Serge Burner, Axel Maier, Eva Krafft, Thierry Meyer and Deborah Studer for additional technical assistance. Drs. Holger Fischer and Bjoern Wagner are also thanked for the measurement of pK_a values. Dr. Simona Ceccarelli is also thanked for helpful discussions during the preparation of the manuscript and Dr. Thomas Woltering is thanked for useful discussions at the onset of this work.

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17. Compound **16b** was prepared following the two-step Bruylants amination route established in Scheme 1 starting from (*R*)-**5**.[15]