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Discovery of *N*-(2-aryl-cyclohexyl) substituted spiropiperidines as a novel class of GlyT1 inhibitors

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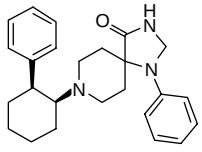
Abstract—Screening of the Roche compound library led to the identification of *cis*-*N*-(2-phenyl-cyclohexyl)-spiropiperidine **1** as structurally novel GlyT1 inhibitor. The SAR, which was developed in this series, resulted in the discovery of highly potent compounds displaying excellent selectivity against the GlyT2 isoform.

NMDA receptor hypofunction is suggested to be involved in the pathophysiology of schizophrenia.[1] The strongest evidence supporting this hypothesis is based on the observation that schizophrenic like symptoms can be induced in healthy subjects upon administration of NMDA blockers such as PCP.[2] Thus, therapeutic intervention aimed at increasing NMDA synaptic tone is expected to show beneficial effect in schizophrenic patients. As glycine is an obligatory co-agonist at the NMDA receptor complex,[3] one strategy to enhance NMDA receptor activity is to elevate extracellular levels of glycine in the local microenvironment of synaptic NMDA receptor. Glycine elevation can be achieved by inhibition of the glycine transporter 1 (GlyT1) which is co-expressed in the brain with the NMDA receptor and is responsible for glycine removal from the synaptic cleft.[4,5] Strong support for this approach in the treatment of schizophrenia comes from clinical studies where glycine[6] and D-serine[7] (co-agonists at the glycine site of NMDA receptor) and sarcosine[8] (a prototypical weak GlyT1 inhibitor) improved positive, negative and cognitive symptoms in schizophrenic patients, when added to conventional therapy. As a result, considerable efforts have been focused on the development of selective GlyT1 inhibitors.[9] The first examples reported were glycine or sarcosine derivatives.[10] More recently, a wide variety of nonamino-acid GlyT1 inhibitors have been disclosed.[9b]

We report here on our effort to discover and develop structurally novel, potent and selective GlyT1 inhibitors.[11a] To achieve this, screening of the Roche compound depository was performed employing a glycine uptake inhibition assay in cells transfected with human GlyT1.[11a] This screening campaign led to the identification of the *cis*-*N*-(2-phenyl-cyclohexyl)-spiropiperidine **1** as potent inhibitor of GlyT1 (26 nM), displaying more than 400-fold selectivity against the type 2 isoform.



cis, rac
1

In this paper, we report on the SAR we have obtained at the cyclohexyl moiety as well as around the aromatic rings. Selectivity against hGlyT2 transporter and affinity at opioid receptors are also discussed.

cis-*N*-(2-Aryl-cycloalkyl)-spiropiperidines **1**, **5**–**6**, **11**–**39** were prepared by following two complementary routes (Scheme 1). The first route, which was followed for exploring SAR around R^2, used the *cis*-piperidone **40** as a key intermediate. Compound **40** was obtained by reductive amination of the α-aryl-substituted cyclic ketone **41** with 1,4-dioxa-8-aza-spiro[4,5]decane followed by acidic cleavage of the ketal protective group. Importantly, this reductive amination provided exclusively the *cis*-isomer. Conversion of piperidone **40** into the final spiropiperidine compounds was achieved by following a well-known 4-step sequence:[12a,b] Strecker

Keywords: GlyT1; GlyT2; NMDA; Schizophrenia; Transporter; Glycine; Spiropiperidine.
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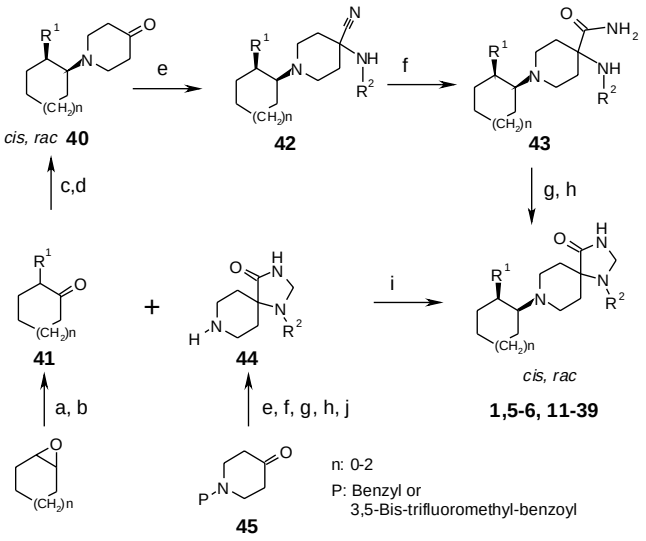
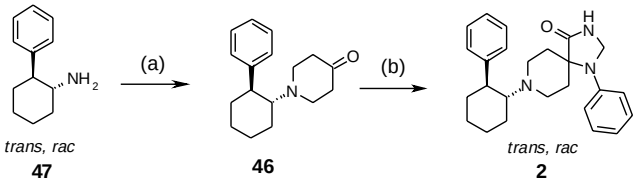


Scheme 1. Synthesis of *cis*-*N*-(2-aryl-cycloalkyl)-spiropiperidine **1**, **5**–**6**, **11**–**39**. Reagents and conditions: (a) R^1MgBr, $CuBr{\cdot}Me_2S$, THF, 0 °C, 40–80%; (b) Dess Martin periodinate, 0.1% H_2O, CH_2Cl_2, rt, 60–80%; (c) 1,4-dioxa-8-aza-spiro[4,5]decane, cat TsOH, toluene, reflux, Dean–Stark then $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane, rt, 70%; (d) 6 N HCl, MeOH, reflux, 70%; (e) R^2NH_2, KCN, H_2O, AcOH, rt; (f) 90% H_2SO_4, rt; (g) triethyl orthoformate, AcOH, toluene, reflux, 48 h or 20 min (microwave); (h) $NaBH_4$, MeOH, rt or H_2 (1 atm), Pd/C, AcOH, MeOH, 45 °C, 10–50% overall yield from **40**; (i) $Ti(OiPr)_4$, 105 °C then $NaBH_4$, EtOH, rt, 10–60%; (j) performed only when P is 3,5-bis-trifluoromethyl-benzoyl: LiOH, H_2O, MeOH, THF, rt, 80–90%.

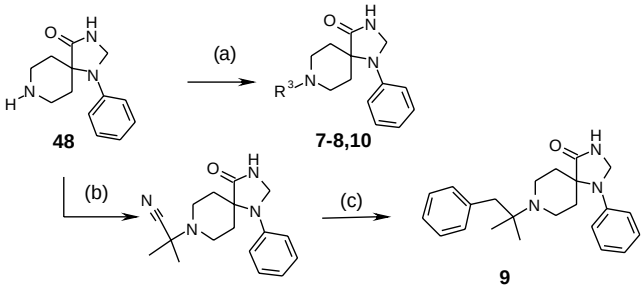
condensation with an aryl or alkyl amine, acidic hydrolysis of the resulting nitrile **42** into the amide **43**, ring closure of **43** in the presence of triethyl orthoformate followed by reduction of the double bond with sodium borohydride or hydrogen and Pd/C. Interestingly, the usually tedious thermal cyclisation step could be achieved efficiently with shorter reaction time in a microwave oven. The second route, especially suited for exploring variation around the left-hand aromatic group, involved a reductive amination of the already constructed spiropiperidine building block **44** with ketone **41**. This reaction worked best in our hands in the presence of titanium(IV) isopropoxide.[13] Again, this reductive amination was stereoselective leading exclusively to the *cis*-isomer. Spiropiperidine intermediate **44** was prepared from the *N*-protected-piperidone **45** using the same 4-step sequence described above followed by cleavage of the protective group.

The *trans*-isomer **2** was prepared from piperidone **46**, which was obtained from the known *trans*-2-phenylcyclohexylamine[14] using an efficient Hofmann-elimination–Michael-addition sequence[15] (Scheme 2).

Derivatives **7**–**8** and **10** bearing an acyclic R^3 group were prepared by reductive amination of commercially available spiropiperidine **48** with the required aldehydes (Scheme 3). The gem-dimethyl derivative **9** was obtained in 2 steps: Strecker condensation of **48** with acetone followed by nucleophilic displacement of the nitrile group by benzyl magnesium chloride.



Scheme 2. Synthesis of *trans*-*N*-(2-phenyl-cyclohexyl)-spiropiperidine **2**. Reagents and conditions: (a) 1-methyl-1-ethyl-4-oxo-piperidinium iodide, K_2CO_3, H_2O, EtOH, reflux, 50%; (b) see conditions of (e–h) in Scheme 1.



Scheme 3. Synthesis of spiropiperidines **7**–**10**. Reagents and conditions: (a) R^3CHO, $Ti(OiPr)_4$, 105 °C then $NaBH_4$, EtOH, rt, 60–70%; (b) acetone, TMSCN, AcOH, 35 °C, 18%; (c) benzylmagnesium chloride, THF, 70 °C, 24%.

First, the influence of the relative and absolute stereochemistry of the two vicinal chiral centres on GlyT1 activity and selectivity was investigated (Table 1). Interestingly, the *cis*- and *trans*-configurated compounds **1** and **2** showed comparably low nanomolar GlyT1 activity. Moreover, **1** and **2** exhibited an equally low activity at GlyT2 ($\geqslant$10 μM). Interestingly, in the *cis* stereomeric series, the (1*R*,2*R*)-enantiomer **3** demonstrated an extremely high activity at GlyT1 (4 nM) and two orders of magnitude potency higher than that of the (1*S*,2*S*)-enantiomer **4** (380 nM).[16] As a consequence, **3** showed a remarkably high selectivity against the GlyT2 transporter (>1500-fold).

Structural modifications were then directed towards the cyclohexyl system (Table 2). Replacement of the *cis*-cyclohexyl group by smaller (cyclopentyl, **5**) or larger (cycloheptyl, **6**) *cis*-aliphatic ring systems caused more than a 40-fold loss in GlyT1 activity. This sharp drop

Table 1. In vitro inhibitory activity of **1**–**4** at GlyT1 and GlyT2[a]

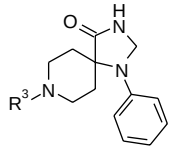
Compound	EC_{50}[b] (μM)		Selectivity[c]
	GlyT1	GlyT2	
1 (*cis*, *rac*)	0.026	12	461
2 (*trans*, *rac*)	0.073	10	137
3 (*cis*, 1*R*,2*R*)	0.004	7	1750
4 (*cis*, 1*S*,2*S*)	0.380	17	45

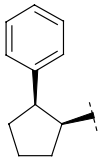
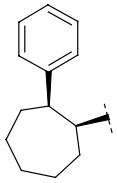
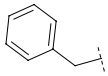
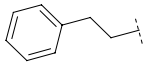
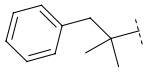
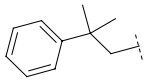
[a] EC_{50} values are the geometric mean of at least two experiments with <20% variance.

[b] [^{3}H]Glycine uptake inhibition assay in cells transfected with hGlyT1[11a] or hGlyT2[11b] cDNAs.

[c] Ratio of the EC_{50} (μM) values GlyT2/GlyT1.

Table 2. In vitro inhibitory activity of **5**–**10** at GlyT1[a]



Compound	R^3	GlyT1 EC_{50}[b] (μM)
5	 *cis, rac*	1.1
6	 *cis, rac*	1.7
7		>10
8		>10
9		9.3
10		7.3

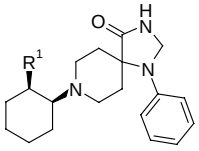
[a] EC_{50} values are the geometric mean of at least two experiments with <20% variance.
[b] [^{3}H]Glycine uptake inhibition assay in cells transfected with hGlyT1[11a] or hGlyT2[11b] cDNAs.

of activity suggests that the 6-membered ring system has the adequate size for establishing optimal lipophilic interaction with the GlyT1 transporter. Replacement of the cyclohexyl ring by noncyclic linkers was then considered with the aim of reducing the structural complexity of our compounds. However, these attempts were met with no success since the benzyl and phenethyl derivatives as well as the more rigid gem-dimethyl-substituted phenethyl analogues (**7**–**10**) were found to be much less active. In summary, all modifications considered around this part of the molecule were not tolerated and therefore the original *cis*-cyclohexyl system remained as the preferred template.

Next, exploration around the left-hand aromatic system was performed by keeping the *N*-cyclohexyl-spiropiperidine fixed (Table 3).

In the *para* position of this aromatic ring, the introduction of electron-withdrawing groups (**11**–**12**) was well tolerated giving compounds of similar activity to the original hit compound **1**. Substitution with strong

Table 3. In vitro inhibitory activity of **11**–**19** at GlyT1 and GlyT2[a]



Compound	R^1	EC_{50}[b] (μM)		Selectivity[c]
		GlyT1	GlyT2	
11	4-F-Ph	0.023	16	695
12	4-Cl-Ph	0.040	5	125
13	4-Me-Ph	0.085	15	176
14	4-MeO-Ph	0.610	24	39
15	3-Cl-Ph	0.130	2.6	20
16	3,4-Cl_2-Ph	0.067	6.8	101
17	3-CF_3,4-Cl-Ph	1.8	nd	
18	2-Me-Ph	0.510	13	25
19	2-Py	6.6	nd	

[a] EC_{50} values are the geometric mean of at least two experiments with <20% variance.
[b] [^{3}H]Glycine uptake inhibition assay in cells transfected with hGlyT1[11a] or hGlyT2[11b] cDNAs.
[c] Ratio of the EC_{50} (μM) values GlyT2/GlyT1.

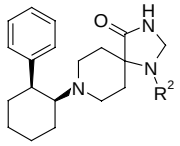
electron-donating groups (**14**) led to significant drop of GlyT1 inhibition. In the *meta* and *ortho* positions, a decrease of activity was also seen upon substitution (**15**, **17**–**18**). Replacement of the aryl group by the more polar 2-pyridyl group (**19**) led to a dramatic decrease of activity. Best selectivity against the GlyT2 transporter was achieved with the *para*-fluoro-phenyl-substituted compound **11** (nearly 700-fold).

By keeping the phenyl group fixed at the 2-position on the cyclohexyl ring, variation around the right-hand aromatic system was then explored (Table 4).

Introduction of electron-withdrawing or -donating groups at the *para* position of the aromatic ring was found to be well tolerated (**20**–**24**). Replacement of the aromatic group by aliphatic residues was then explored. The methyl derivative **25** was found to be completely inactive. However, gratifyingly, elongating the linear alkyl chain from 1 to 4 carbons resulted in a steep increase of potency. Excellent activity was indeed reached with the *N*-pentyl derivative **28** (34 nM). Addition of an extra methylene group then led to a slight decrease of activity (**29**). Introduction of cycloalkyl residues of various sizes provided weak compounds (**30**–**33**). However, insertion of 1- or 2-methylene units between the nitrogen atom and the cycloalkyl residue led to very potent derivatives as exemplified with the two cyclohexyl analogues **34** and **35** showing and EC_{50} of 65 and 25 nM, respectively. Interestingly, this high level of activity was retained by replacing the cyclohexyl group in **35** by a phenyl group (**37**). Introduction of polar functionalities in the alkyl chain however was found to be detrimental (**38**–**39**). Excellent selectivity against the GlyT2 transporter was obtained for the most potent derivatives (Table 4).

Recently, structurally related *N*-(substituted-cyclohexyl) spiropiperidines have been described as Nociceptin/

Table 4. In vitro inhibitory activity of **20**–**39** at GlyT1 and GlyT2[a]



cis, rac

Compound	R^2	EC_{50}[b] (μM)		Selectivity[c]
		GlyT1	GlyT2	
20	4-F-Ph	0.024	23	958
21	4-Cl-Ph	0.027	2.9	107
22	4-CF_3-Ph	0.065	3.4	52
23	4-MeO-Ph	0.066	27	410
24	4-Me-Ph	0.055	9	164
25	Me	>30	nd	
26	Et	6.6	nd	
27	nPr	0.450	>100	>222
28	nPent	0.034	35	1029
29	nHex	0.049	24	490
30	cPr	3.3	nd	
31	cBu	1.5	>100	>67
32	cPent	0.63	>100	>159
33	cHex	0.75	11	15
34	CH_2-cHex	0.065	25	385
35	CH_2CH_2-cHex	0.025	15	600
36	CH_2-Ph	0.258	>100	>388
37	CH_2CH_2-Ph	0.025	18	720
38	CH_2CH_2-OMe	5.3	nd	
39		7.4	nd	

[a] EC_{50} values are the geometric mean of at least two experiments with <20% variance.
[b] [^{3}H]Glycine uptake inhibition assay in cells transfected with hGlyT1[11a] or hGlyT2[11b] cDNAs.
[c] Ratio of the EC_{50} (μM) values GlyT2/GlyT1.

Orphanin FQ peptide (NOP) receptor ligands.[12,17] Therefore, in vitro affinity of some of the most GlyT1 active compounds identified was determined at the NOP receptor as well as at the related μ opioid receptor to assess their potential side-effect liabilities (Table 5).

Interestingly, the *cis*-*N*-(2-aryl-cyclohexyl)-spiropiperidines (**1**, **3**, **21**, **28** and **37**) displayed low affinity for the NOP receptor. Disappointingly, however, these compounds showed a consistently higher level of binding to the μ opioid receptor. In particular, worst profile was achieved with the *N*-aryl analogues (**1**, **3** and **21**) which displayed nanomolar activity at this receptor. Moreover, the *trans*-diastereoisomer **2** exhibited an equally high activity at both the NOP and μ receptors.

Table 5. In vitro binding activities of **1**, **2**, **4**, **21**, **28** and **37** at the NOP and μ receptors[a]

Compound	GlyT1 EC_{50}[b] (μM)	NOP IC_{50}[c] (μM)	μ IC_{50}[d] (μM)
1	0.026	6	0.15
2	0.073	0.3	0.54
3	0.004	3.7	0.041
21	0.027	4.0	0.23
28	0.034	>10	2.2
37	0.025	>10	3.2

[a] EC_{50} values are the geometric mean of at least two experiments with <20% variance.
[b] [^{3}H]Glycine uptake inhibition assay in cells transfected with hGlyT1[11a] or hGlyT2[11b] cDNAs.
[c] Displacement of [^{3}H]NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[12b,c]
[d] Displacement of [^{3}H]naloxone in membranes prepared from BHK cells transiently expressing hμ receptors.[12b]

In summary, starting from hit compound **1**, an SAR was established which led to the identification of *cis*-*N*-(2-aryl-cyclohexyl)-substituted spiropiperidines as a novel class of highly potent GlyT1 inhibitors displaying excellent selectivity against the GlyT2 isoform. In this class, affinity at the μ opioid receptor has been identified as a key liability which requires optimization. In the next paper, results of our effort to address this issue will be reported.[18]

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16. (a) Enantiomers **3** and **4** were obtained by separation of racemic compound **1** on chiral-phase HPLC (Chiralpack AD); (b) Absolute configuration of enantiomer **4** has been determined by X-ray crystallography analysis of its (1*R*)-(−)-10-camphorsulfonic acid salt.
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