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Discovery of *N*-(2-hydroxy-2-aryl-cyclohexyl) substituted spiropiperidines as GlyT1 antagonists with improved pharmacological profile

Simona M. Ceccarelli,* Emmanuel Pinard, Henri Stalder and Daniela Alberati

F. Hoffmann-La Roche Ltd, Pharmaceutical Division Basel, Discovery Chemistry, CH-4070 Basel, Switzerland

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Abstract—During SAR exploration of *N*-(2-aryl-cyclohexyl) substituted spiropiperidine as GlyT1 inhibitors, it was found that introduction of an hydroxy group in position 2 of the cyclohexyl residue considerably improves the pharmacological profile. In particular, reduction of the binding affinity at the nociceptin/orphanin FQ peptide and the μ opioid receptors was achieved.
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Enhancement of glutamate transmission, in particular via *N*-methyl-D-aspartate (NMDA) receptor activation, is postulated to produce both anti-psychotic and cognitive enhancing effects, thus constituting a potential therapeutic target for the treatment of schizophrenia, psychoses and cognitive impairment.[1] The amino acid glycine is known to act as a positive allosteric modulator and obligatory co-agonist with glutamate at the NMDA receptor complex.[2] Glycine transporters (GlyT) play an important role in the termination of post-synaptic glycinergic actions and maintenance of low extracellular glycine concentration by re-uptake of glycine into presynaptic nerve terminals or glial cells. In particular, GlyT1 is the only sodium chloride dependent glycine transporter in the forebrain, where it is co-expressed with the NMDA receptor. At this site, GlyT1 is thought to be responsible for control of extracellular level of glycine at the synapse.[3] Therefore, one strategy to enhance NMDA receptor synaptic function is to elevate the glycine concentration in the local microenvironment of synaptic NMDA receptors by inhibition of GlyT1.[4,5]

In the course of a program aimed at identifying new glycine transporter inhibitors for the treatment of schizophrenia, we discovered *N*-(2-aryl-cyclohexyl) substituted spiropiperidines of type **1** to be very potent inhibitors of GlyT1, showing good selectivity against the type 2 isoform.[6]

The spiropiperidine motif is a privileged structure in a variety of CNS active agents.[7] Indeed, compounds **1** and **2** and their derivatives showed consistently high level of binding to the μ opioid receptor (μ), with a resulting high risk of addiction and tolerance liabilities. Moreover, the *trans* diasteroisomer **2**, while showing comparable activity at the GlyT1 transporter, was also a potent inhibitor of the nociceptin/orphanin FQ receptor (NOP) (Table 1).[8] In the course of SAR exploration of the *N*-(2-aryl-cyclohexyl) needle, we discovered that the introduction of a hydroxy group in position 2 had a noticeable influence on affinity for the μ receptor. While the *trans* isomer **3** retains considerable activity at NOP, the *cis* isomer **4** shows only micromolar affinity towards the μ and the NOP receptors (Table 1). The most active enantiomer of **4** (obtained via chiral HPLC separation) shows an even improved pharmacological profile with respect to μ and NOP receptors.[9]

Rapid exploration of the substitution patterns at the two aryl rings of the *cis* isomer via parallel chemistry allowed identification of a number of active derivatives (Table 2), all sharing good pharmacological profile with respect to NOP and μ receptors. A number of different substituents in positions *meta* and *para* of the phenyl in position 2 of the cyclohexane ring are tolerated, while substitution in *ortho*, as in compound **10**, seems to be detrimental to selectivity vs the μ receptor. Also hetero-

Keywords: GlyT1; GlyT2; NMDA; Schizophrenia; Transporter; Spiropiperidine.
* Corresponding author. Tel.: +0041 061 6884437; fax: +0041 061 6886459; e-mail: simona_m.ceccarelli_grenz@roche.com

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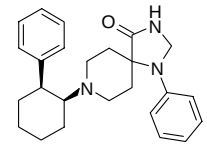
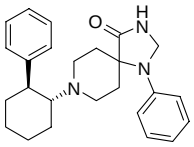
Table 1. In vitro inhibitory activity at the GlyT1 and the GlyT2 transporters and potency in inhibiting the NOP and μ receptors for compounds **1**–**4**



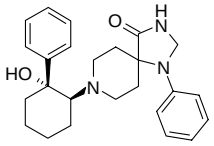
cis, rac

1



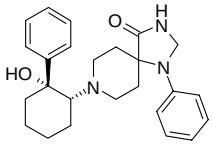
trans, rac

2



trans, rac

3



cis, rac

4

Compound	GlyT1 (EC_{50}, μM)[a]	GlyT2 (EC_{50}, μM)[a]	NOP (IC_{50}, μM)[b]	μ (IC_{50}, μM)[c]
1	0.026	12	6	0.15
2	0.073	10.3	0.31	0.54
3	0.056	26	0.13	1.6[d]
4	0.044	72	15	2.7[d]

[a] Radiometric assay using [^{3}H]glycine.[10]

[b] Displacement of [^{3}H]NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[11]

[c] Displacement of [^{3}H]naloxone in membranes prepared from BHK cells transiently expressing hμ receptors.[11]

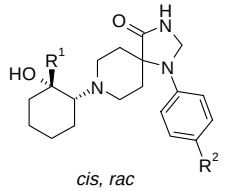
[d] Full (**3**) and partial (**4**) agonists as assessed in GTPγS binding assay in the same cell membranes, by comparison with DAMGO (full agonist) and morphine (partial agonist).

cycles are tolerated, with a preference for the 4-pyridine motif, as in **15**. Attempts to substitute the aryl ring with an alkyl chain (compounds **16**–**20**) met with no success. The SAR at the phenyl group in position 1 of the spiro systems parallels that of the derivatives lacking the hydroxy group, with 4-fluorophenyl derivatives **21** and **23** and 4-chloro derivative **22** showing the best activity. The selectivity profile of compound **21** was further explored by assessment of its activity at other glycine receptor binding sites present in brain, the glycine site of the NMDA receptor and the strychnine-sensitive glycine receptor, as well as towards a panel of 50 selected binding sites, ligand gated ion channels and other neurotransmitter transporters. In all cases, no sign of significant interaction was detected at concentrations below 10 μM, thus confirming that introduction of the hydroxy group in position 2 of the 2-aryl-cyclohexyl needle grants a clean pharmacological profile to this class of GlyT1 inhibitors.

The cyclohexyl ring of the 2-hydroxy-2-phenyl-cyclohexyl needle can also be substituted with a tetrahydropyran ring, as in compounds **25** and **26** with no loss in activity or selectivity (Table 3).

Synthesis of the *cis* isomer of 8-(2-hydroxy-2-phenyl-cyclohexyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one derivatives was performed as detailed in Scheme 1. Reaction of commercially available 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **27** with the epoxides **28** or **29** (derived from cyclohexene or dihydropyran) in refluxing ethanol affords the corresponding secondary alcohol

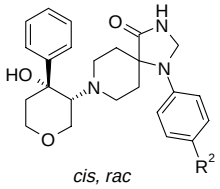
Table 2. In vitro inhibitory activity at the GlyT1 and the GlyT2 transporters and potency in inhibiting the NOP and μ receptors for compounds **5**–**24**



cis, rac

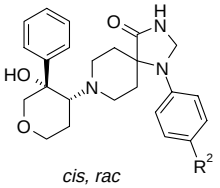
Compound	R^1	R^2	GlyT1 (EC_{50}, μM)	GlyT2 (EC_{50}, μM)	NOP (IC_{50}, μM)	μ (IC_{50}, μM)
5	4-MeO-Ph	H	0.261	67	7	2.5
6	4-Me-Ph	H	0.140	25	>10	3
7	4-Cl-Ph	H	0.080	16	1	3.6
8	3,4-Cl_2-Ph	H	0.173	6	10	3.7
9	4-F-Ph	H	0.040	57	1	4
10	2-Me-Ph	H	0.050	70	1	0.26
11	3-Cl-Ph	H	0.130	21	3.85	1.09
12	3-MeO-Ph	H	0.130	41	5.8	2.17
13	2-Py	H	0.130	>100	15	1.42
14	3-Py	H	0.110	—	13.4	1.8
15	4-Py	H	0.062	>100	>10	1
16	Me	H	9			
17	c-C_6H_{11}	H	17			
18	Bn	H	4.4			
19	*t*-Bu	H	29			
20	*i*-Pr	H	>3			
21	4-F-Ph	F	0.024	36	10	4
22	4-F-Ph	Cl	0.015	2.5	>10	2.2
23	4-Cl-Ph	F	0.024	19	10.4	2.4
24	4-Cl-Ph	MeO	0.099	25	>10	4.4

Table 3. In vitro inhibitory activity at the GlyT1 and the GlyT2 transporters and potency in inhibiting the NOP and μ receptors for compounds **25**–**26**



cis, rac

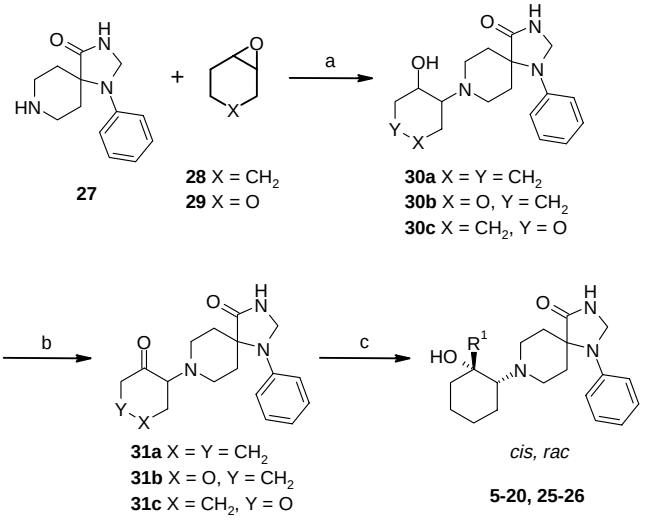
25



cis, rac

26

Compound	GlyT1 (EC_{50}, μM)	GlyT2 (EC_{50}, μM)	NOP (IC_{50}, μM)	μ (IC_{50}, μM)
25	0.058	35	>10	5.3
26	0.090	>100	>10	2.5

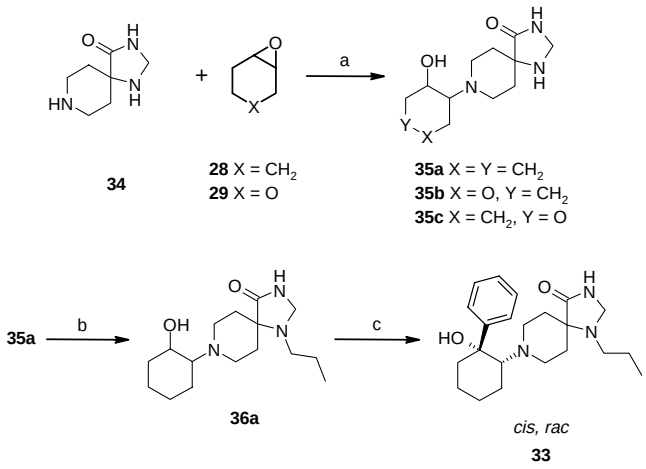


Scheme 1. Synthesis of compounds **5**–**20** and **25**–**26**. Reagents and conditions: (a) EtOH, reflux, 40–55%; (b) SO_3–pyridine complex, DMSO, TEA, DCM, 20–65%; (c) i—R^1Br, BuLi, THF, −78 °C; ii—**31a**–**c**, THF, −78 °C–rt, 20–70%.

30a,**b**, which can be easily oxidized to ketone **31a**,**b** with the SO_3–pyridine complex. Reaction of **31** with a variety of aryl-lithium reagents proceeded with very good stereoselectivity and was amenable to parallel chemistry, allowing easy access to compounds **5**–**20** and **25**. Compound **26** was obtained in an analogous way from the minor isomer **30c** deriving from epoxide opening of dihydropyrane-3,4-epoxide Scheme 2.[12]

The synthesis of compounds **21**–**24**, where the *N*(1)-phenyl is substituted, required construction of the substituted 1-aryl-1,3,8-triazaspiro[4.5]decan-4-ones starting from *N*-benzyl piperidin-4-one, in analogy to what was described in the preceding publication.[6a] These can then be employed in place of the unsubstituted analogue **27** as detailed in Scheme 1.

Spiropiperidine systems of type **1** show suboptimal metabolic stability, which seems to be bound to the presence of the 2-phenyl-cyclohexyl ring system. The effect of 2-hydroxy substitution on in vitro microsomal metabolism was therefore of particular interest. Introduction

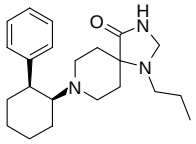


Scheme 2. Synthesis of *N*(1)-alkyl derivatives as, for example, **33**. Reagents and conditions: (a) EtOH, reflux, 40–55%; (b) propanal, $NaHB(OAc)_3$, 80%; (c) i—SO_3–pyridine complex, DMSO, TEA, DCM, 50%; ii—PhLi, THF, −78 °C–rt, 35%.

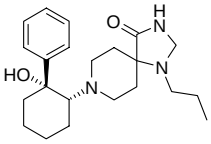
of the hydroxy group on the parent 8-(2-phenyl-cyclohexyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one did not have any significant effect on metabolic rate measured in mouse and human microsomes (Table 4). The corresponding *N*(1)-alkyl derivatives, on the contrary, appear to be considerably stabilized by the introduction of the hydroxy group in position 2. Microsomal clearance data of compound **33**, for example, predict a maximal achievable bioavailability (MAB) of up to 81% in mouse and 84% in human microsomes (Table 4).[13]

The synthesis of *N*(1)-alkyl derivatives as **33** was achieved in a way amenable to SAR exploration through the key building blocks **35**. These were obtained by simple reaction of the cyclohexene or dihydropyran epoxides **28** or **29** with 1,3,8-triazaspiro[4.5]decan-4-one **34**,[14] yielding the secondary alcohols **35a**–**c**. These can perform reductive amination at the *N*(1) nitrogen, as in the case of the reaction of **35a** with propanal to generate the *N*(1)-propyl derivative **36a**. Oxidation followed by reaction with phenyl lithium affords compound **33**. The two diversity vectors of such structures can be varied very easily by employing different aldehydes in the reductive amination step and various aryl

Table 4. Effects of the introduction of the 2-hydroxy group on microsomal metabolic stability of *N*(1)-aryl and *N*(1)-alkyl derivatives



32



33

Compound	GlyT1 (EC_{50}, μM)	Cl (mouse microsomes)[a]	Cl (human microsomes)[a]
1	0.026	—	68
21	0.024	102	92
32	0.45	10	25
33	0.40	7	5

[a] Cl, clearance, μL/min/mg protein.

lithium reagents in the final addition step. In this way, the SAR of selective and metabolically stable GlyT1 inhibitors can be extensively explored.

In conclusion, introduction of a hydroxy group in position 2 of the potent GlyT1 inhibitors 8-(2-aryl-cyclohexyl)-1-aryl-1,3,8-triazaspiro[4.5]decan-4-ones had a considerable influence on the pharmacological profile of such compounds, reducing, in particular, the affinity towards the μ and NOP receptors in the *cis* series. From the stereochemical point of view, the relative 1,2 orientation showing the optimal profile is inverted with respect to the non-hydroxy substituted derivatives. Synthetic access to such compounds was amenable to parallel synthesis, which allowed rapid exploration of the SAR and modulation of the physicochemical properties. In the *N*(1)-alkyl subset, introduction of the hydroxy group brings about a notable metabolic stabilization, paving the way for the identification of metabolically stable, potent and selective GlyT1 antagonists.

Acknowledgments

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10. Cells transfected with hGlyT-1b or hGlyT2 were seeded in 96-well culture plates. The cells were washed twice with uptake buffer (UB) and then incubated for 30 min at 22 °C with either (i) no potential competitor, (ii) 10 mM non-radioactive glycine, or (iii) a test compound. A solution was then immediately added containing [^{3}H]glycine 60 nM (11–16 Ci/mmol) and 25 μM non-radioactive glycine (hGlyt1) or [^{3}H]glycine 200 nM without cold glycine (hGlyt2). The cells were then incubated with gentle shaking for 30 min at 22–24 °C, after which the reaction was stopped by aspiration of the mixture and washing (three times) with ice-cold UB. The cells were lysed with scintillation liquid, shaken for 3 h and the radioactivity in the cells was counted using a scintillation counter.
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