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Design and synthesis of 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as a novel class of GlyT1 inhibitors: Achieving selectivity against the μ opioid and nociceptin/orphanin FQ peptide (NOP) receptors

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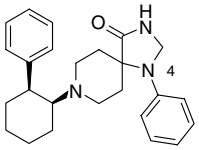
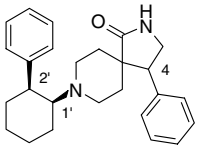
Abstract—A novel class of 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-ones have been discovered and developed as potent and selective GlyT1 inhibitors. The molecules are devoid of activity at the GlyT2 isoform and display excellent selectivities against the μ opioid receptor as well as the nociceptin/orphanin FQ peptide (NOP) receptor. A novel, straightforward and efficient synthetic strategy for the assembly of the target molecules is also presented.

NMDA receptor hypofunction is suggested to be involved in the pathophysiology of schizophrenia.[1] 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,[2] one strategy to achieve this is to inhibit GlyT1: a transporter known to be co-expressed in the brain with NMDA receptor and which is responsible for the selective reuptake of glycine into glial and neuronal cells.[3] Strong support for this approach comes from clinical studies where sarcosine, a low potency GlyT1 inhibitor, improved positive, negative and cognitive symptoms in schizophrenic patients, when added to risperidone.[4]

We have recently disclosed the discovery of N-(2-arylcyclohexyl) substituted spiropiperidines **1** 'triazaspiro derivatives' as a novel class of GlyT1 inhibitors, providing significant detail on the development of a robust SAR with the key achievement of excellent selectivity against the GlyT2 isoform.[5] The main liability identified of this novel class of GlyT1 inhibitors was the very low selectivity against the μ opioid receptor and the modest selectivity against the nociceptin/orphanin FQ peptide (NOP) receptor.



cis, rac
1



(1'*R* 2'*R* or 1'*S* 2'*S*)
2

Herein we wish to report on the pharmacological profile of another novel class of compounds **2** which we term diazaspiropiperidines, with a specific focus on comparing them to their triazaspiropiperidine predecessors **1**. Conceptually, the design of **2** can be considered as simply replacing the sp^2 4-nitrogen atom of the imidazolidinone ring in **1** with a sp^3 CH.[6]

Structural information gained from examination of the X-ray crystal structure of the parent triazaspiropiperidine **1** (Fig. 1) shows that the *N*-phenyl group lies in a

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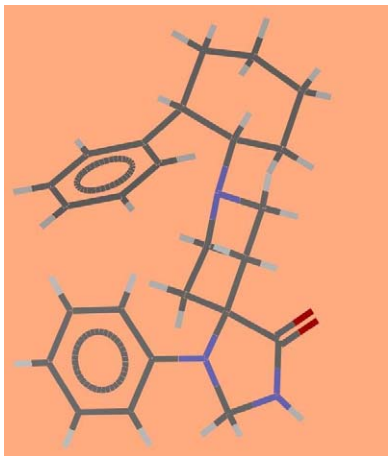


Figure 1. X-ray structure of **1**.

plane which is orthogonally oriented to the plane occupied by the piperidine moiety. Some privileged in-house knowledge suggested that this relative orientation of these two structural elements could in part explain the high potency of compound of type **1** at the opioid receptors. We reasoned that replacement of the sp^2 nitrogen atom at position 4 of the imidazolidinone ring with a sp^3 carbon would provide diazaspiropiperidines of type **2** with reduced activity at the opioid receptors since this chemical mutation is expected to induce a significant change in the orientation of the C-4 phenyl moiety (see later). Although this introduces an additional stereochemical complexity (compared to **1**) into the target molecules, we set out to challenge our hypothesis since reducing opioid activity was a key improvement necessary within this general class of molecules.

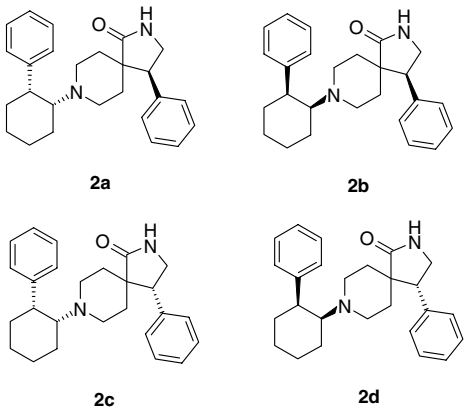
Pleasingly, the first derivative **2**, prepared and tested as a mixture of four diastereoisomers (1:1:1:1), which albeit less potent at the target GlyT1, displayed excellent selectivity against NOP and the μ opioid receptor (Table 1). Due to the potential superiority of this new diazaspiropiperidine series **3** in comparison to the triazaspiropiperidine series **1** (where low nanomolar activity was recorded for the *N*-aryl analogues at the μ opioid receptor)[5b] we therefore embarked upon a more detailed investigation to establish a preliminary SAR for this novel chemotype.

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

Compound	GlyT1 EC_{50}[a] (μM)	GlyT2 EC_{50}[a] (μM)	NOP IC_{50}[b] (μM)	μ IC_{50}[c] (μM)
1[5b]	0.026	12	6	0.15
2[d]	0.232	25	>10	3.92

[a] Radiometric assay using [^{3}H]-glycine.[7]
[b] Displacement of [^{3}H]-NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[8]
[c] Displacement of [^{3}H]-naloxone in membranes prepared from BHK cells transiently expressing hμ opioid receptors.[8]
[d] Mixture of four diastereoisomers (see Table 2).

As was already established for the triazaspiro series **1** we anticipated that the *cis*-series (i.e., *cis*-arrangement at the cyclohexane junction) would be the most favourable diastereoisomeric series to focus our efforts upon. This was quickly substantiated by the separation of the mixture obtained into its composite four enantiomers (1:1:1:1) by chiral-phase HPLC (Chiralpak AD®). The assignment of relative configuration was established by a combination of NMR spectrometry analyses, independent synthesis and X-ray crystallographic analyses. Surprisingly, the two most potent derivatives (**2b** and **2d**) are epimers and display opposite stereochemical configuration of the 4-phenyl group. The compounds are listed, in Table 2, with the fastest eluting component designated **2a** and the slowest eluting designated **2d**. The epimers **2a** and **2c** showed only very weak activity at GlyT1. In addition, **2b** and **2d** showed excellent selectivity against NOP and the μ opioid receptor, with **2b** also showing excellent selectivity against the GlyT2 isoform (Table 2). Unfortunately, it was unclear which was the most active enantiomer **2d** since two stereoisomers (*R*,*R*,*S* or *S*,*S*,*R*) were potentially the most potent from this limited dataset.



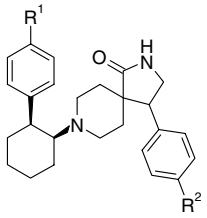
We believed that routine measurement of the affinity at GlyT1 of the (1:1:1:1) diastereoisomeric mixture made little sense, so where possible we endeavoured to separate the single isomers by the implementation of chiral-phase HPLC (Chiralpak AD®). As can be seen, at times this was not always possible and Table 3 shows

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

Compound	GlyT1 EC_{50}[a] (μM)	GlyT2 EC_{50}[a] (μM)	NOP IC_{50}[b] (μM)	μ IC_{50}[c] (μM)
2a	2.83	—	—	—
2b	0.246[d]	39.63	>10	>10
2c	2.88	—	—	5.48
2d	0.061	—	>10	3.49

[a] Radiometric assay using [^{3}H]-glycine.[7]
[b] Displacement of [^{3}H]-NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[8]
[c] Displacement of [^{3}H]-naloxone in membranes prepared from BHK cells transiently expressing hμ opioid receptors.[8]
[d] X-ray structure solved (*R*,*R*,*R* or *S*,*S*,*S*).

Table 3. In vitro inhibitory activity at the GlyT1 transporter for compounds **2**–**11**



Compound	R^1	R^2	GlyT1 EC_{50}[a] (μM)							
			a		**b**		**c**		**d**	
2	H	H	2.83		0.246[b]		2.88		0.061	
3	CF_3	F	2.33		0.953		0.129		0.091	
4	Me	H		0.672[c]				0.105[d]		
5	Me	F		4.78[c]				0.736[d]		
6	Me	3,4-Di-Cl		0.309[c]				3.641[d]		
7	Me	MeO	1.131			0.502[e]			16.64	
8	F	H	0.167			0.204[e]			0.048	
9	F	3,4-Di-Cl		0.232[c]			0.270		2.19	
10	F	MeO			0.249[f]				0.113	
11	F	F	1.064		1.296		0.036		0.043[b]	

[a] Radiometric assay using [^{3}H]-glycine.[7]
[b] X-ray structure solved.
[c] Mix of **a** and **b**.
[d] Mix of **c** and **d**.
[e] Mix of **b** and **c**.
[f] Mix of **a**–**c**.

the activity for the single isomers or the mixture of diastereoisomers where appropriate. The compounds are listed, in table, with the fastest eluting component designated **a** and the slowest eluting designated **d**. Note, that it was not possible to putatively assign stereochemistry to each isolated pure enantiomer, since there was no direct relationship between the order of elution and the relative stereochemistry. This is described in more detail below for **2a**–**d** and **11a**–**d**.

As can be seen in Table 3, in general, the SAR closely parallels that observed from the triazaspiro series **1**[5b] with the bis-4-fluoro-phenyl derivative **11** showing the best activity. In addition, from all of the substituents examined at least one active diastereoisomer in the nanomolar range was identified for each compound. Overall, the two most interesting pure enantiomers **11c** and **11d** [assigned as diastereoisomers (C-4 epimers) as they show non-identical NMR spectra] showed very high potency (36 and 43 nM) at the GlyT1 transporter and also exhibited high selectivity against the GlyT2 transporter. In addition, and in contrast to the triazaspiro series, **11c** and **11d** were also found to be completely inactive at the NOP receptor with concomitant good to excellent selectivities against the μ opioid receptor also demonstrated (Table 4).

We have recently reported a novel synthetic route to access a range of 4-substituted diazaspiropiperidine derivatives **12** in racemic form.[9] Our methods are based on a straightforward assembly strategy utilizing the construction of a quaternary centre via enolate addition

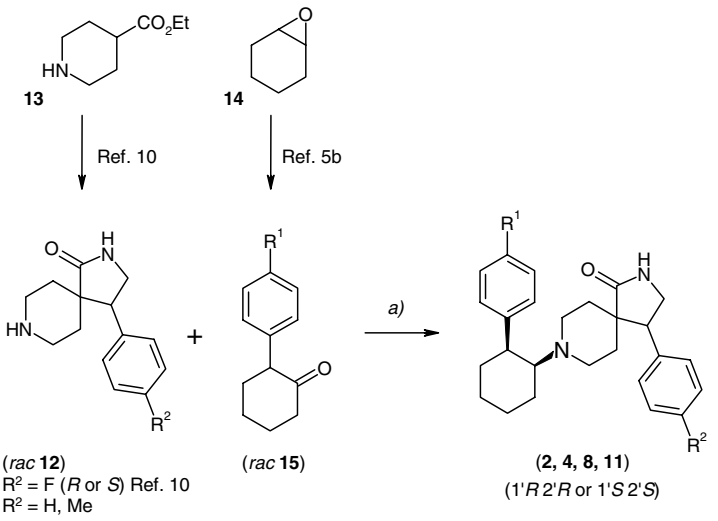
Table 4. In vitro inhibitory activity at the GlyT1 and the GlyT2 transporters and potency in inhibiting the NOP and μ receptors for compounds **11a**–**d**

Compound	GlyT1 EC_{50}[a] (μM)	GlyT2 EC_{50}[a] (μM)	NOP IC_{50}[b] (μM)	μ IC_{50}[c] (μM)
11a	1.064	>30	—	1.55
11b	1.296	8.36	—	>10
11c	0.036	39.95	>10	6.7
11d	0.043	16.68	>10	1.09

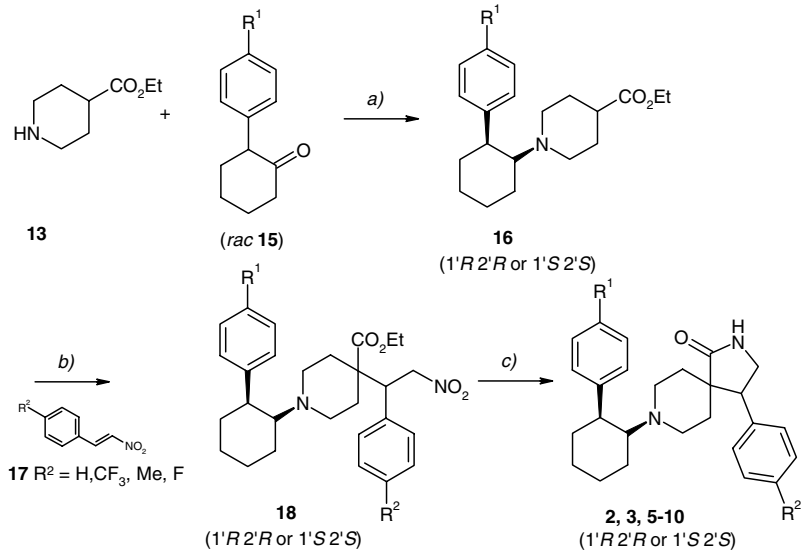
[a] Radiometric assay using [^{3}H]-glycine.[7]
[b] Displacement of [^{3}H]-NOP in membranes prepared from permanently transfected HEK293 cells expressing hNOP receptors.[8]
[c] Displacement of [^{3}H]-naloxone in membranes prepared from BHK cells transiently expressing hμ opioid receptors.[8]

chemistry from commercially available ethyl pipecolate **13** (Scheme 1). These novel building blocks were then transformed into the target molecules through correct choice of the most efficient synthetic pathways outlined below. We initially chose to prepare derivatives **2**, **4**, **8** and **11** as outlined in Scheme 1 where the direct reductive amination of the diazaspiropiperidine **12** with a range of 2-arylcyclohexanones **15**[5b] proceeded in acceptable yields to give selectively the desired *cis* products. The low yields obtained were mainly due to experimental difficulties dealing with the excess titanium residues in the reaction. As a result we sought a more efficient route for further exploration.

Derivatives **3** and **5**–**10** were initially prepared using a similar set of reaction conditions for the preparation



Scheme 1. Synthesis of 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one **2**, **4**, **8** and **11**. Reagents and conditions: (a) $Ti(O^iPr)_4$, PHMS, THF, rt, 17 h then $NaCN(BH_3)$, rt, 3 h, 17–40%.



Scheme 2. Synthesis of 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one **2**, **3** and **5**–**10**. Reagents and conditions: (a) cat *p*TsOH, toluene, reflux, Dean–Stark, 13 h then $NaBH(OAc)_3$, cat AcOH, 1,2-dichloroethane, rt, 38–48%; (b) LDA, −78 °C to −40 °C, THF, 1 h, **17**, 43–83%; (c) i—Ra-Ni, H_2 (60 bar), EtOH, 55 °C, 8 h then filtration, evaporation; ii—toluene, reflux, 4–5 h, 44–84%.

of the diazaspiropiperidine derivatives **12** in racemic form.[9] In this case, the piperidine **13** was efficiently condensed with the ketone **15** to give exclusively the *cis* piperidines **16** through reduction of the intermediate enamine. Enolisation of **16** using freshly prepared LDA, followed by quenching with a range of β-nitro alkenes **17**, afforded good to excellent yields of the Michael addition products **18**. Subsequent reductive cyclisation using pressurised hydrogen with Ra-Ni as catalyst followed by heating resulted in the formation of the target products **2**, **3** and **5**–**10** in good overall yields (Scheme 2).

As yet, we have not unequivocally elucidated the absolute stereochemical assignment of the structures **11a**–**d** during this work. However, we have ascertained their diastereoisomeric integrity (Fig. 2). Compounds **11a** and **11c** (and hence **11b** and **11d)** are diastereoisomeric (and enantiomeric) pairs by NMR assignment and as expected they have equal and opposite optical rotation. Again, as was earlier observed, the two most potent derivatives **11c** and **11d** are not epimers.

The assignment of the diastereoisomeric series was established by X-ray diffraction with the configuration assigned for **11d** as (*S*,*S*,*R* or *R*,*R*,*S*) (Fig. 3). The X-ray crystal structure of **11d** shows that the right-hand phenyl group has clearly a different relative orientation to the piperidine moiety compared to the original triazaspiropiperidine **1**. We believed that this conformational change is responsible for the much improved selectivity displayed in the new diazaspiropiperidine series and

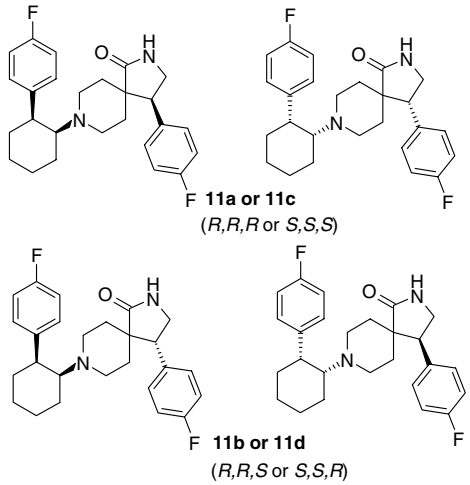


Figure 2. Assignment of relative stereochemistry for structures **11a**–**d**.

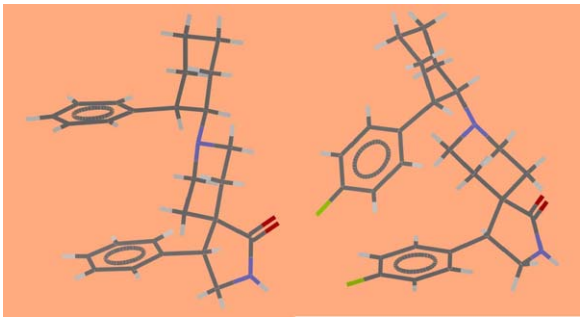


Figure 3. X-ray structures of **2b** and **11d**.

therefore fully supported our initial hypothesis for reducing μ opioid activity.

In a similar fashion, the diastereoisomeric pairs for **2** were established as **2b**,**2c** and **2a**–**d**. In a future experiment, it is planned to determine the most active enantiomeric series. This, in principle, could be achieved by repeating the synthetic sequence (outlined in Scheme 1 step a) this time employing the optically pure (*R*)-diazaspiropiperidine **12**[9] and then assigning the final product by co-eluting with its enantiopode **11a** or **11d** by chiral HPLC. The same can potentially be repeated with the (*S*)-diazaspiropiperidine **12**[9] followed by subsequent assignment with **11b** or **11c**.

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 gave rise to a novel and potent class of 4-substituted-8-(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 opioid and NOP receptors which we believe to be due to a conformational mismatch within their respective pharmacophoric spaces. A novel synthetic method was intentionally designed and developed to access the target compounds as a (1:1:1:1) mixture of four diastereoisomers with a *cis*-arrangement at the cyclohexane junction. This simplified somewhat the stereocomplexity by focusing only on two diastereoisomeric pairs. In general, the most active diastereoisomers within the 4-substituted-8-(2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one cannot yet be assigned. The subsequent paper will describe further pharmacological properties within this novel chemotype and we will also describe the development of another novel class of 4-substituted-8-(2-hydroxy-2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]decan-1-one as GlyT1 inhibitors.

Acknowledgments

We thank Marianne Rueher, Patrick Boisson and Serge Burner for additional technical assistance as well as Mr. André Alker and Dr. Armin Ruf for solving the X-ray structure of **1** and **11d**. Dr. Geo Adam is also thanked for helpful discussions at the onset of this work.

Supplementary data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers: compound **1**: CCDC608843, compound **2b**: CCDC608844 and compound **11d**: CCDC608842. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or deposit@ccdc.cam.ac.uk].

References and notes

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7. Cells transfected with hGlyT-1b or hGlyT2 were seeded in 96-well culture plates. The cells were washed twice with uptake buffer (UB), then incubated for 30 min at 22 °C with either: (i) no potential competitor, (ii) 10 mM non-radioactive glycine, (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 aspira-

tion of the mixture and washing (three times) with ice-cold UB. The cells were lysed with scintillation liquid, shaken 3 h and the radioactivity in the cells was counted using a scintillation counter. EC_{50} values are geometric means of at least two experiments with <20% variance.

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