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ORL1 Receptor Ligands: Structure–Activity Relationships of 8-Cycloalkyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones

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Abstract—We have investigated 8-cycloalkyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones as ligands for the ORL1 receptor. These unsophisticated, achiral compounds show remarkable affinity for the ORL1 receptor. Optimizing for selectivity we show that the maximum of affinity and selectivity versus the other opioid receptors is achieved for 8-cyclodecyl-1-phenyl-1,3,8-triaza-spiro-[4.5]decan-4-one **2e** and 8-(*cis*-4-isopropyl-cyclohexyl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **2q**. The identified compounds (**2e**, **2q**) are more or less equipotent to the natural ligand itself, both in the binding assay and in the functional GTPγS assay.

Introduction

The 17-amino acid neuropeptide Orphanin FQ (OFQ, nociceptin) was identified in 1995 simultaneously by two groups of researchers.[1] OFQ is the endogenous agonist of the ORL1 receptor, a G-protein coupled receptor negatively coupled to adenylate cyclase, which itself was discovered in 1994 by homology cloning to the known opioid receptors.[2] In spite of obvious similarities of the OFQ peptide to opioid peptides like dynorphin A, OFQ binds selectively to its receptor and does not interact with the known opioid receptors.[3] Research on the physiological role of the OFQ receptor system has been hampered by the peptidic nature of the endogenous ligand. Still, by applying the peptide intracerebroventricularly or intrathecally, a role of the OFQ system in the pain response,[4] feeding behavior[5] and stress response[6] of animals has been established.

Recently, we have described a series of substituted 1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones (Scheme 1; **I**) as potent non-peptidic ORL1 receptor agonists.[7] Specifically, compound **1** combined high affinity at the ORL1 receptor with moderate selectivity versus μ and κ opioid receptors.

Unfortunately, highest affinity and best selectivity in this series of compounds was realized with chiral compounds which increases the number of synthesis steps and does not facilitate fast development of structure–activity relationships.

To redress this issue, we investigated and describe in this paper the synthesis and structure–activity relationship of a series of simple 8-cycloalkyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones **II**. This work culminated in the discovery of *cis*-8-(4-isopropyl-cyclohexyl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **2q**, a very potent ORL1 receptor agonist with selectivity versus μ and κ opioid receptors similar to **1**.

Chemistry

The 8-cycloalkyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones **2a–h** were synthesized from the corresponding commercially available ketones by condensation with 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one to afford enamines and subsequent reduction with sodium cyanoborohydride. 8-(4-alkyl-cyclohexyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones (R≠H) were synthesized by the same route, the only modification being the isolation of the respective *cis*- (**2o–s**) and *trans*-isomers (**2i–n**) by preparative HPLC (Scheme 2). To corroborate the assignment of isomers via ^{1}H NMR,[8] the most interesting compound in this series, **2q**, has also been made starting from *cis*-4-isopropyl-cyclohexylamine.[9]

Results and Discussion

The compounds described were evaluated in radioligand binding assays (Tables 1 and 2) using membranes

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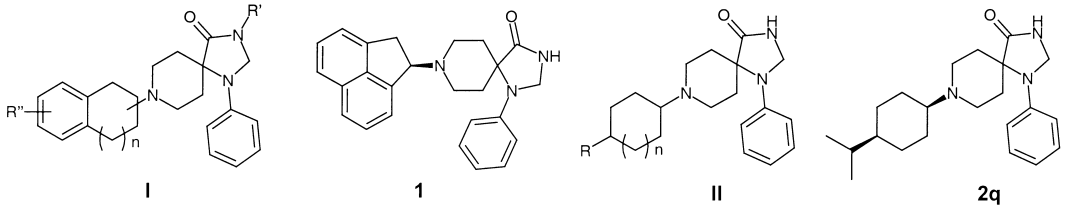
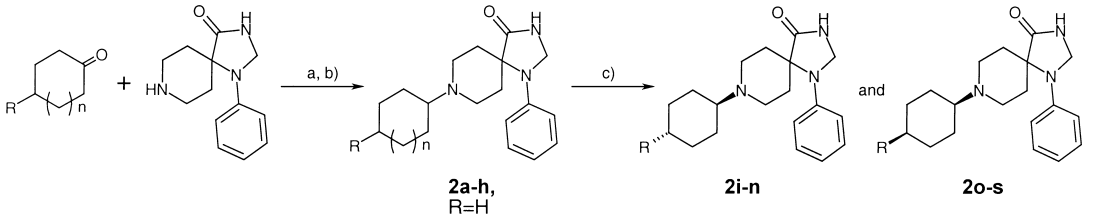


Scheme 1.

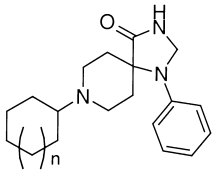


Scheme 2. (a) MS 4 Å, toluene, reflux; (b) $NaBH_3CN$, THF/EtOH, rt; (c) prep. HPLC.[13]

expressing rORL1 receptors, permanently expressed in HEK (human embryonic kidney) 293 cells, or membranes from BHK (baby hamster kidney) cells infected with Semliki Forest virus encoding the cDNAs for either μ, κ or δ receptors. Results are given as K_i values calculated according to Cheng and Prusoff.[10]

Table 1 details the effect of the size of the lipophilic cycloaliphatic substituent in 8-position on affinity in the receptor binding assays. Affinity for the rat ORL1 receptor increases with increasing ring size from cyclohexyl derivative **2a** with a K_i of 25 nM to cyclodecyl derivative **2e** (K_i of 82 pM) which is already equipotent to the endogenous ligand (OFQ) itself. The increase in affinity between these two members of the series parallels nicely the increasing number of C-atoms, affinity rising about one order of magnitude for every two sp^3-C-atoms. This is very much in line with the values expected for a favorable hydrophobic interaction of receptor and ligand.[11] For ring-sizes larger than cyclodecyl, as in **2f–g**, affinity for the ORL1 receptor decreases, pointing probably to unfavorable steric interactions. Affinity of **2a–h** for the other three opioid receptors follows very similar trends, the only major difference being a maximum of affinity at slightly larger ring-sizes. Specifically, it is the cycloundecyl derivative **2f** that shows highest affinity for μ, κ and δ receptors. Consequently, moderate selectivity in this series is achieved for the cyclononyl and cyclodecyl derivatives (**2d** and **2e**). These two compounds display about 10-fold selectivity for rORL1 receptors over μ and κ receptors. Selectivity towards δ opioid receptors (>200 fold for most of the compounds) is excellent, only the

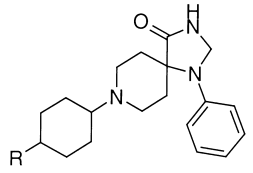
Table 1. Receptor binding of 1-phenyl-8-(cycloalkyl)-1,3,8-triaza-spiro[4.5]decan-4-ones[a]



Compound	*n*	K_i [nM] rORL-1	μ	κ	δ	Selectivity μ/rORL-1	κ/rORL-1
1		0.41	4.0	20	100	9.8	49
2a	1	25	158	100	nd	6.3	4.0
2b	2	4.7	51	12	>2000	11	2.5
2c	3	1.9	13	9.1	>200	7.0	4.8
2d	4	**0.24**	3.2	3.9	>200	**13**	**16**
2e	5	**0.082**	0.66	2.1	46	**8.2**	**26**
2f	6	**0.49**	**0.21**	**0.82**	**15**	0.4	1.7
2g	7	0.95	**0.28**	2.9	570	0.3	3.1
2h	10	600	nd	nd	nd	—	—

[a]See Table 2.

Table 2. Receptor binding of 1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones substituted with 4-substituted cyclohexyl derivatives on the piperidine nitrogen[a]



Compound	R	stereo	K_i [nM] rORL-1	μ	κ	δ	Selectivity μ/rORL-1	κ/rORL-1
2i	Me	*trans*	41	nd	nd	nd	—	—
2j	Pr	*trans*	52	nd	nd	nd	—	—
2k	*i*-Pr	*trans*	**4.6**	8.3	31	670	1.8	6.8
2m	*t*-Bu	*trans*	12	nd	nd	nd	—	—
2n	Chx	*trans*	320	nd	nd	nd	—	—
2o	Me	*cis*	7.1	64	57	>2000	9.1	8.0
2p	Pr	*cis*	2.0	7.3	57	>540	3.6	29
2q	*i*-Pr	*cis*	**0.079**	**3.2**	**26**	**242**	**40**	**330**
2r	*t*-Bu	*cis*	3.3	6.7	38	450	2.0	11
2s	Chx	*cis*	1.5	1.5	29	330	1.0	19

[a]The data are the mean of two different binding experiments performed in triplicate. The K_d of the radioligands used, were as follows: [leucyl-^{3}H]-OFQ 100 pM for rOFQ receptors, [*N*-allyl-2-3-^{3}H]-naloxone 3.0 nM for r*mu* receptors and 4.5 nM for r*kappa* receptors, [Ile^{5,6}-^{3}H]-deltorphin 0.10 nM for r*delta* receptors.

cyclodecyl and cycloundecyl derivatives **2e** and **2f** show appreciable affinity for δ opioid receptors and consequently limited selectivity (**2e** 560 fold, **2f** 30 fold).

Medium-sized cycloalkyl rings like cyclononyl and cyclodecyl rings are conformationally flexible entities,[12] so it is conceivable to believe that different conformations may be responsible for binding to the different receptors. To explore further the lipophilic binding site of the ORL1 and opioid receptors, and perhaps to improve selectivity, we sought to limit the conformational space accessible to the cycloalkyl moiety. To that end we chose 4-alkyl-substituted cyclohexyl derivatives, mostly to avoid the introduction of stereogenic centers which would render the molecules chiral, and also because we learned early on that it is possible to separate the *cis*- and *trans*-isomers via preparative HPLC.[13]

In both the *trans*- and *cis*-series affinity for the ORL1 receptor peaks for the 4-isopropyl-cyclohexyl derivatives (**2k** and **2q** in Table 2), but interestingly it is always the *cis*-isomer, which is the more potent stereoisomer. The *cis*-4-isopropyl-cyclohexyl derivative **2q**, with a K_i of 79 pM, is about 40-fold more potent for ORL1 receptors than its *trans*-isomer **2k**. Analysis of the data for μ, κ and δ receptor binding reveals that steric and size requirements for high affinity binding to these receptors are much less stringent than the ones posed by the ORL1 receptor. Thus opioid receptor binding data for the *cis*- and *trans*-*i*-propyl derivatives (**2k**, **2**) are rather similar, the *cis* derivative being the more potent one only for μ and δ receptors and only by a factor of two.

When the steric demand of the ligands is increased as in the series of the *i*-propyl, *t*-butyl and cyclohexyl substituted derivatives (**2q**, **2r** and **2s**) this is virtually without effect on affinity in μ, κ and δ binding assays. The net result is that the more stringent conditions which the ORL1 receptor imposes on the substituent at the 8-position leads to a peak in selectivity for **2q**, which is about 40-fold selective for ORL1 receptors versus μ receptors and over 200-fold selective versus κ receptors. As shown by the NMR-data[8] **2q** adopts in $CDCl_3$ mainly the conformation where the basic nitrogen assumes an axial position on the cyclohexyl ring. This same conformer, the one with the basic nitrogen in the axial position, is according to NMR (data not shown) also preferred for the *cis*-*n*-propyl, *cis*-*t*-butyl and *cis*-cyclohexyl substituted compounds (**2p**, **2r** and **2s**). The relevance of this axial conformation for the binding event can probably only be assessed after an comprehensive reanalysis of all the available data on ORL1 receptor binding of 8-substituted-1-phenyl-1,3,8-triaza-spiro-[4.5]decan-4-ones.

The efficacy of the most interesting compounds (**2e** and **2**) has been assayed by their effect on stimulating GTPγS binding to membranes of HEK 293 cells overexpressing rORL1 receptors.

Both **2q** with a pEC_{50} of 7.42 and **2e** with a pEC_{50} of 7.9 (OFQ: $pEC_{50} = 7.2$) behave as agonists in this assay, confirming the earlier finding[7] that high affinity ligands in the 8-substituted-1-phenyl-1,3,8-triaza-spiro[4.5]-decan-4-ones series of compounds tend to be ORL1 receptor agonists.

Conclusion

Investigating 8-cycloalkyl-1-phenyl-1,3,8-triaza-spiro-[4.5]decan-4-ones as ligands for the ORL1 receptor we have shown that high affinity for the ORL1 receptor and moderate selectivity versus the other opioid receptors can be achieved with quite unsophisticated achiral cycloalkyl substituents, building on either the preference of the ORL1 receptor for slightly smaller lipophilic substituents or the improved affinity seen with 4-*cis*-substituted as opposed to 4-*trans*-substituted cyclohexyl derivatives. The identified compounds (**2e**, **2q**) are more or less equipotent to the natural ligand itself, both in the binding assay and in the functional GTPγS assay.

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8. **2k** (*trans*) ^1H NMR ($CDCl_3$): 0.85 (d, $J = 6.8$ Hz, 6H, CH_3), 0.9–1.1 (m, 3H), 1.1–1.5 (m, 3H), 1.6–1.85 (m, 4H, 6,10-CH_2 eq,), 1.92 (d, $J = 12$ Hz, 2H), 2.33 (tt, $J = 11$ Hz, $J = 3$ Hz, 1H,

1′-CH, ax), 2.59 (td, $J=13$ Hz, $J=5$ Hz, 2H, 6, 10-CH_2, ax), 2.78 (bd, $J=10$ Hz, 2H, 7,9-CH_2, eq), 3.04 (td, $J=12$ Hz, $J=4$ Hz, 2H, 7,9-CH_2, ax), 4.72 (s, 2H, 2-CH_2), 6.33 (bs, 1H, 3-NH), 6.87 (t, $J=7.6$ Hz, 1H, *p*-ArH), 6.94 (d, $J=8.1$ Hz, 2H, *o*-CH_2), 7.27 (t, $J=8.6$ Hz, 2H, *m*-CH_2); **2q** (*cis*) ^{1}H NMR ($CDCl_3$): 0.90 (d, $J=6.7$ Hz, 6H, CH_3), 1.1–1.2 (m, 1H), 1.25–1.85 (m, 11H), 2.38 (m, 1H, 1′-CH, eq), 2.63 (td, $J=14$ Hz, $J=5$ Hz, 2H, 7,9-CH_2, ax), 2.81 (td, $J=12$ Hz, $J=3$ Hz, 2H, 6,10-CH_2, ax), 2.85–2.95 (m, 2H, 7,9-CH_2, eq), 4.73 (s, 2H, 2-CH_2), 6.40 (bs, 1H, 3-NH), 6.85 (t, $J=7.2$, 1H, *p*-ArH), 6.92 (d, $J=8.0$ Hz, 2H, *o*-ArH), 7.26 (t, $J=8.0$ Hz, 2H, *m*-ArH).

9. *cis*-4-Isopropyl-cyclohexylamine was reacted with *N*-ethyl-*N*-methyl-4-oxo-piperidinium iodide (K_2CO_3, EtOH, reflux) and the product elaborated to **2q** (1. Aniline, TMSCN, acetic acid, 2. Ac_2O, HCO_2H, rt. 3. H_2O_2, *t*-BuOH, H_2O, NH_4OH, rt. 4. $HCONH_2$, 200 °C. 5. $NaBH_4$, MeOH, rt) in analogy to the method described in ref 7.

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13. Dynamax Microsorb Si 80-120-C5, UV Detection at 265 nm, analytical flow-rate 1.5 mL/min. 4-methyl-cyclohexylderivatives: 0.2% NEt_3/4% *i*-PrOH/hexane; R_t [min]: **2i**: 12.6; **2o**: 11.0. 4-*n*-propyl-cyclohexylderivatives: 0.2% NEt_3/3% *i*-PrOH/hexane; R_t [min]: **2j**: 14.2; **2p**: 12.9. 4-*i*-propyl-cyclohexylderivatives: 4% *i*-PrOH/hexane; R_t [min]: **2k**: 10.7; **2q**: 8.7. 4-cyclohexyl-cyclohexylderivatives: 4% *i*-PrOH/hexane; R_t [min]: **2n**: 9.9; **2s**: 8.44-*t*-butyl-cyclohexylderivatives (**2m** and **2r**): separation was achieved by conventional LC on silica gel (dichloromethane/methanol 2–5%).