High-Affinity, Non-Peptide Agonists for the ORL1 (Orphanin FQ/Nociceptin) Receptor

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Received July 23, 1999

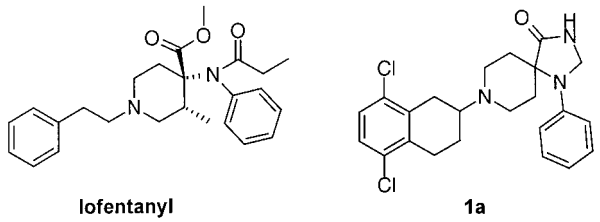
The discovery of 8-(5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one, **1a**, as a high-affinity ligand for the human ORL1 (orphanin FQ/nociceptin) receptor led to the synthesis of a series of optimized ligands. These compounds exhibit high affinity for the human ORL1 receptor, exhibit moderate to good selectivity versus opioid receptors, and behave as full agonists in biochemical assays. In this paper we present the synthesis, structure−activity relationship (SAR), and biochemical characterization of substituted 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones culminating in the discovery of 8-(5-methyl-1,2,3,4-tetrahydro-naphthalen-1-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **1p**, and 8-acenaphten-1-yl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **1q**, two high-affinity, potent ORL1 receptor agonists with good to moderate selectivity versus the other opioid receptors.

Introduction

The cloning of G-protein coupled receptors selective for δ, μ, and κ opioids led several groups[1] to the discovery of a fourth member of the family by homology cloning. The amino acid sequence of this new receptor, the ORL1 (OFQ/nociceptin) receptor, is 47% identical to the opioid receptors overall and 64% identical in the transmembrane domains. None of the established opioid ligands, however, with the exception of lofentanil,[2] a high-affinity μ opioid receptor agonist, have appreciable affinity for this new opioid receptor. Only after the discovery that the endogenous agonist is a 17 amino acid neuropeptide, named orphanin FQ or nociceptin,[3] has it been possible to investigate the pharmacological relevance of this new opioid-like receptor system. The isolation of orphanin FQ (nociceptin) has led to an explosion of biochemical, pharmacological, and physiological data on the OFQ/ORL1 receptor system.[4] The primary focus of research has been on the traditional opiate field of pain and analgesia,[5] but additionally cardiovascular,[6] memory,[7] and behavioral effects[8] have been observed. Interestingly it has been found that OFQ (nociceptin) also displays potent anxiolytic and antistress effects.[9]

Many exciting results have been generated with OFQ (nociceptin) and peptide analogues including the discovery of [Phe$^1\psi$(CH_2−NH)Gly^2]-NC(1−13)-NH_2, which has been characterized as an antagonist in the mouse vas deferens but, probably depending on receptor density, behaves like a partial agonist in other models.[10] The field of research still suffers from limitations inherent in the use of peptides, namely poor metabolic stability and the need for intravenous or even intrathecal or intracerebroventricular administration in animals. These limitations may be overcome by the identification of non-peptide agonists (and antagonists) suitable for intraperitoneal or oral administration. These compounds should be potent in vitro and in vivo and selective versus the other opioid receptors.

Scheme 1



Recently, we and groups from Banyu and Pfizer have independently discovered and patented the first non-peptide ligands for the ORL1 receptor.[11] Herein we describe the discovery of our series of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones as ORL1 receptor ligands. These new compounds as well as the compounds discovered by Banyu and Pfizer bear some family resemblance to lofentanil (Scheme 1) and the fentanyl series of opioid receptor ligands.[12]

We started with a high-throughput screening hit, 8-(5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **1a**, which is an unselective ORL1 receptor ligand, and developed high-affinity, potent ORL1 receptor ligands with fair to moderate selectivity against the other opioid receptors. In a GTPγS assay these compounds behave as agonists at the ORL1 receptor.

Chemistry

Naphthalen-2-one **7a** (Scheme 2) was prepared starting from the corresponding inden-1-one[13] **3** by ring expansion with ethyl diazoacetate followed by decarboxylation to naphthalen-1-one **4**. Subsequent reduction, elimination, and dihydroxylation furnished the diol **6**, which in turn was treated with *p*-toluenesulfonic acid to give **7a**. The 8-substituted 1-phenyl-1,3,8-triazaspiro-

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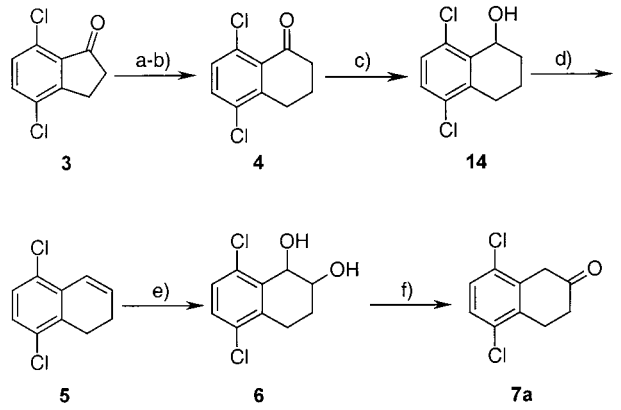
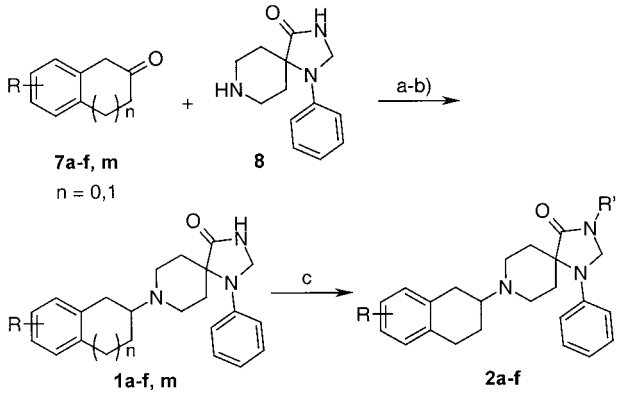
10.1021/jm991129q CCC: $19.00
Published on Web 03/17/2000

Scheme 2[a]



[a] Reagents and conditions: (a) $N_2CH_2CO_2Et$, $Et_3O^+BF_4^-$, CH_2Cl_2, 0 °C−rt, 37%; (b) H_2O, 240 °C, 17 bar, 75%; (c) $NaBH_4$, EtOH/H_2O, rt − reflux; (d) *p*-TsOH, toluene, reflux, 89% both steps; (e) *t*-BuOH, NMMO, OsO_4, rt, 88%; (f) *p*-TsOH, toluene, reflux, 22%.

Scheme 3[a]

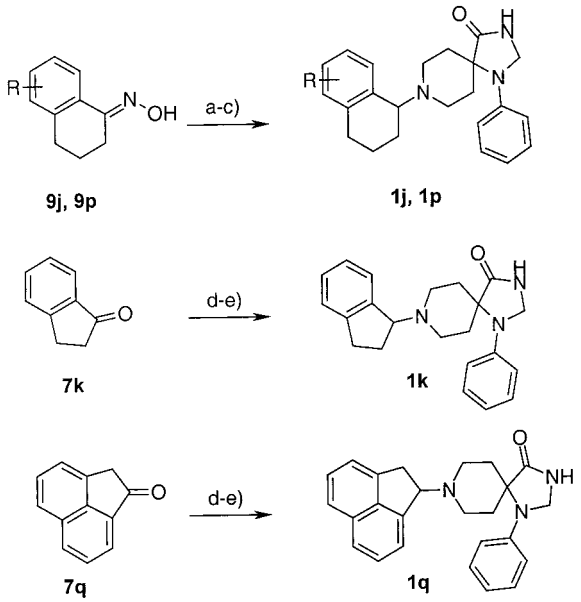


[a] Reagents and conditions: (a) MS 4 Å, toluene, reflux; (b) $NaBH_3CN$, THF/EtOH, rt; (c) NaH, DMF, 80°, then R′-X, rt.

[4.5]decan-4-ones **1a**−**f** and **1m** were synthesized from the corresponding ketones by condensation with commercially available 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **8** to enamines and subsequent reduction with sodium cyanoborohydride (Scheme 3). The two-step procedure worked in our hands somewhat better than the one-pot reductive alkylation of amines. While this works quite well for naphthalen-2-one and other sterically unencumbered substrates, 1-indanones and naphthalen-1-ones required somewhat more forceful conditions (Scheme 4). Reductive amination of 1-indanone or acenaphthenone to give **1k** and **1q** worked well in the presence of titanium(IV) isopropoxide,[14] while for the synthesis of **1j** and **1p** the ketones had to be activated as nitrimines which in turn were prepared from the corresponding oximes in situ.[15]

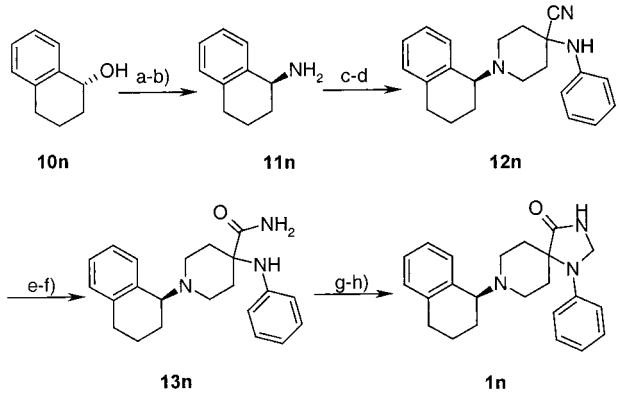
The (*R*)- and (*S*)-enantiomers (**1h**, **1g**) of 8-(5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **1a** could be obtained by fractional crystallization of the diastereomeric salts with chiral 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate. Since we were not able to identify a suitable chiral acid for resolution of **1j**, the corresponding enantiomers **1n** (*S*) and **1o** (*R*) were obtained by total synthesis, starting from commercially available chiral 1,2,3,4-tetrahydro-1-naphthols (Scheme 5).

Scheme 4[a]



[a] Reagents and conditions: (a) $NaNO_2$, H_2SO_4, Et_2O, rt; (b) 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, MS 4 Å, CH_3CN, rt; (c) $NaBH_3CN$, THF/EtOH, rt; (d) Ti(*i*-OPr)$_4$, 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, THF, rt; (e) $NaBH_3CN$, THF/EtOH, rt.

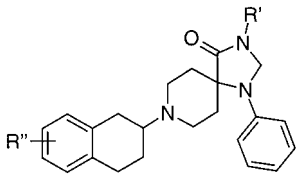
Scheme 5[a]



[a] Reagents and conditions: (a) DBU, DPPA, toluene, 0 °C − rt; (b) $LiAlH_4$, THF, rt, 63% both steps; (c) 1-methyl-1-ethyl-piperidinium iodide, K_2CO_3, EtOH, H_2O, reflux; (d) aniline, TMSCN, acetic acid, 76% both steps; (e) Ac_2O, HCO_2H, rt; (f) H_2O_2, *t*-BuOH, H_2O, NH_4OH, rt, 79% both steps; (g) $HCONH_2$, 200 °C; (h) $NaBH_4$, MeOH, rt, −60 °C, 23% both steps.

(*R*)-(−)-1,2,3,4-tetrahydro-1-naphthol **10n** was converted in good yield to the corresponding (*S*)-azide by treatment with diphenylphosphoryl azide (DPPA) and DBU,[16] and the azide in turn was reduced to the (*S*)-1,2,3,4-tetrahydro-1-naphthylamine **11n** with lithium aluminum hydride. The naphthylamine **11n** was then heated with 1-methyl-1-ethyl-piperidiniumiodide to furnish the corresponding piperidone in a double Hoffmann elimination, Michael addition sequence[17] and converted by a Strecker reaction to **12n**. Hydrolysis of **12n** to the corresponding amide **13n** proved to be relatively tedious, basic protocols leading to retro-Strecker reaction and acidic protocols to elimination of dihydro-naphthalene. This problem could be circumvented by formylating **12n** and subsequently hydrolyzing the nitrile with basic hydrogen peroxide to the amide (concomitantly cleaving the formamide bond). The procedure yielded **13n** in a fair yield. Compound **13n** was then cyclized by treat-

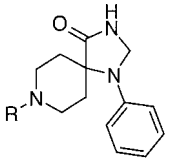
Table 1. Receptor Binding of 1-Phenyl-8-(1,2,3,4-tetrahydro-naphthalen-2-yl)-1,3,8-triazaspiro[4.5]decan-4-ones[a]



			K_i (± SEM) [nM]			
compd	R″	R′	hORL1	μ	κ	δ
1a	5,8-Cl_2	H	5.6 (1.1)	7.2 (1.7)	44.2 (8.4)	680 (160)
1b	5-Cl	H	2.9 (0.8)	8.9 (3.0)	20.7 (6.7)	450 (80)
1c	6-Cl	H	218 (55)	77 (19)	149 (41)	1800 (500)
1d	7-Cl	H	5.8 (1.4)	10.9 (1.6)	16.4 (4.5)	480 (150)
1e	8-Cl	H	7.3 (2.2)	16.6 (2.2)	48 (16)	1410 (420)
1f	H	H	6.3 (2.1)	15.1 (5.5)	47.1 (9.1)	620 (280)
1g (*S*)	5,8-Cl_2	H	2.8 (0.9)	5.9 (2.6)	40.1 (8.0)	415 (74)
1h (*R*)	5,8-Cl_2	H	20.7 (7.8)	8.4 (2.2)	47.3 (7.8)	587 (30)
2a	5,8-Cl_2	Me	3.3 (1.1)	2.6 (0.9)	17.0 (2.4)	313 (64)
2b	5,8-Cl_2	allyl	6.8 (2.0)	5.9 (1.8)	26.2 (9.4)	nd
2c	5,8-Cl_2	benzyl	15.3 (6.1)	19.2 (4.7)	52 (14)	146 (34)
2d	8-Cl	CH_2COPh	15.0 (1.5)	61 (23)	28.8 (7.5)	510 (190)
2e	8-Cl	CH_2CH_2OH	7.2 (0.9)	3.3 (1.0)	16.4 (2.4)	285 (58)
2f	8-Cl	CH_2OMe	8.5	7.5	17.5	240
lofentanil			24[b]	0.14[b]	5.5[b]	

[a] See Table 2. [b] Data given for lofentanil are literature values.[23]

Table 2. Receptor Binding of 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-ones Substituted with Aryl-cycloalkyl Derivatives on the Piperidine Nitrogen[a]



		K_i (± SEM) [nM]			
compd	R	hORL1	μ	κ	δ
1f	2-tetralinyl	6.3 (2.1)	15.1 (5.5)	47.1 (9.1)	620 (280)
1j	1-tetralinyl	2.1 (0.7)	12.8 (1.4)	10.7 (2.3)	480 (140)
1k	1-indanyl	0.7	3.4 (0.8)	5.3	540 (290)
1m	2-indanyl	2.5	26.0 (0.6)	161	710
1n (*S*)	1-tetralinyl	10.2 (3.7)	13.3 (4.6)	17 (13)	nd
1o (*R*)	1-tetralinyl	2.5 (1.3)	12.3 (2.6)	46.3 (5.2)	530 (160)
1p	5-Me-1-tetralinyl	1.4 (0.4)	31.7 (8.8)	44 (11)	460 (71)
1q	acenaphthenyl	0.52	5.9	26	250

[a] The data are the mean of two to three (± SEM) different binding experiments performed in triplicate. The K_d of the radioligands used were as follows: [^{3}H]-OFQ 70 pM for hORL1 receptors, [^{3}H]-naloxone 1.3 nM for hμ receptors and 2.8 nM for hκ receptors, [^{3}H][Ile^{5,6}]-deltorphin II 0.36 nM for hδ receptors.

ment with formamide at 200 °C to yield **1n** together with the corresponding unsaturated 1,3,8-triazaspiro-[4.5]dec-2-en-4-one. Reduction with sodium borohydride yielded pure **1n**.

A series of substituted amides (**2a**−**f**) was prepared by simple (and unoptimized) alkylation procedures in moderate yields (Scheme 3).

Results and Discussion

The compounds described were evaluated in radioligand binding assays as described in the experimental part using membranes expressing hORL1 receptors (permanent expression in HEK 293 cells) or membranes from BHK cells infected with Semliki Forest virus encoding the cDNAs for either hμ, hκ, or hδ receptors. Results are given as K_i values calculated according to Cheng and Prusoff[18] and compared to the values given for lofentanil in the literature.[23]

The lead compound, **1a** (Table 1 and Scheme 1), was discovered by high-throughput screening (HTS) screening of our compound libraries for hORL1 receptor ligands. Compound **1a** showed high affinity for the hORL1 receptor but had similar affinity for the hμ receptor and still significant affinity also for the hκ receptor. The compound was shown to be an agonist at the hORL1 receptor in GTPγS (Figure 1, EC_{50}(**1a**): 1.5 μM vs 63 nM for OFQ) and cyclase assays (data not shown). Being interested in the pharmacology of this new and exciting member of the opioid receptor family, we set out to improve the biochemical profile of the newly discovered ligand **1a** in a classical medicinal chemistry program. In this paper we report on the SAR of simple variations in the lipophilic substituent in the 8-position of the triazaspirodecanone (Tables 1 and 2) and the effects of different substitutions at the amide nitrogen (Table 1) on affinity and selectivity toward the members of the opioid receptor family. Our original lead

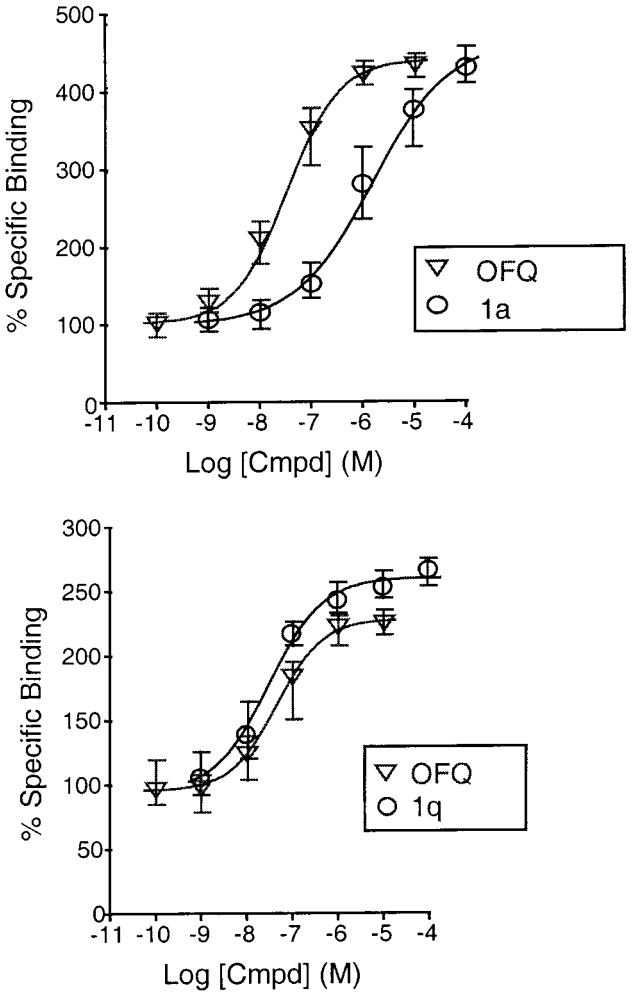


Figure 1. Stimulation of GTPγS binding by OFQ and **1a** or **1q**, respectively. Membranes were incubated with increasing concentrations (0.1 nM−100 μM) of agonists at 22 °C for 60 min. The results are representatives of two independent experiments performed in triplicates.

1a is highly substituted in the tetralinyl part as well as a racemate, so we first explored the importance of the chlorine substituents and stereochemistry of **1a** for binding affinity (Table 1).

As evident from the data of the monochloro and unsubstituted tetralinyls (**1b**−**e** and **1f**), all the receptors tolerate lipophilic substituents on the tetralinyl ring. With the exception of the 6-Cl compound **1c**, all monochloro-substituted tetralinyls are virtually equipotent to the original lead on all four receptors tested. The chloro substitution is tolerated, again with the exception of **1c**, but does not contribute to binding, since the molecule with the unsubstituted tetralinyl ring (**1f**) has a binding profile very similar to **1a**. Indeed, the first progress toward higher affinity and more selective ligands came from the resolution of **1a** into its enantiomers. The (*S*)-enantiomer **1g** is about 8 times more potent on hORL1 receptors than the (*R*)-enantiomer **1h**. Since the other three opioid receptors do not discriminate the enantiomers, both enantiomers being virtually equipotent at the μ, κ, and δ receptors, **1g** is about twice as selective as **1a** for hORL1 receptors.

With a small library of compounds we explored the relevance of substituents at the amide nitrogen. A small subset of all the compounds synthesized (**2a**−**f**, Table 1) may serve to illustrate the relatively weak effects one observes upon substitution at this position. Alkylating the amide with small substituents such as methyl and allyl (**2a**,**b**) leads only to a slight increase in affinity especially for the hμ and hκ receptors. This indicates that the amide proton does not participate in any type of H-bonding interaction with the hORL1 receptor, and the same is true for binding to the other opioid receptors. Increasing the substituent size as for **2c** and **2d** (benzyl and phenacyl) leads to a 3-fold loss of affinity for the hORL1 receptor, indicating some minor unfavorable interaction (probably steric). The opioid receptors in general, and the hORL1 receptor in particular, tolerate a wide range of functional groups at the amide position. Both polar groups like the amide itself or the hydroxyethyl amide (**1a**, **2e**) and lipophilic such groups as methyl, allyl, and methoxymethyl amides (**2a**−**b**, **2e**) are tolerated without strong effects on binding affinity. As both polar and small lipophilic groups have no significant impact on binding affinity, we can only speculate that the microenvironment in this part of the molecule does not change dramatically upon binding. The simplest explanation for this might be that this part of the ligand is exposed to the water layer.

Having discovered that substitution of the amide does not provide an easy handle to increase selectivity of the lead compound, we turned our attention back to variations of the lipophilic 8-substituent of **1a**. As evident from the data (Table 2), the 2-tetralinyl derivative **1f** is actually the least potent member of a family of tetralinyl and indanyl derivatives (**1j**−**m**).

The 1-indanyl derivative **1k** with a K_i of 700 pM is about 10-fold more potent for the hORL1 receptor than **1f**, but this gain in affinity toward hORL1 receptors is accompanied by a similar gain in affinity for the hμ and hκ receptors. Compounds **1j** and especially **1m**, with a K_i of 2.5 nM at the hORL1 receptor and a 10- and 70-fold lower affinity versus hμ and hκ receptors, respectively, present a more significant step toward our goal to develop selective hORL1 receptor ligands. Interestingly, in the pair of tetralinyls (**1f**, **1j**) and also in the pair of indanyls (**1k**, **1m**), it is always the 1-substituted derivative that has higher affinity for the ORL1 receptor (and the κ receptor). This may reflect the stability gain of gauche- (**1j**, **1k**) versus anti-conformers (**1f**, **1m**) for the 1-substituted compounds.

With a view to improve selectivity, the enantiomers of the 1-tetralinyl derivative **1j** were synthesized and tested. Surprisingly, this time it is the (*R*)-enantiomer **1o** that binds with higher affinity to hORL1 receptors. Indeed, this leads to a improvement in selectivity, especially toward κ receptors. The effect is, however, only small since the enantiomers differ only by a factor of 4 in affinity toward the ORL1 receptor, so that the better enantiomer **1o** was determined to be about equipotent to the racemate (the standard error being actually bigger than the expected improvement by a factor of 2).

Trying to understand the result that both the (*S*)-tetralin-2-yl derivative **1g** and the (*R*)-tetralin-1-yl **1o** are more potent than their respective enantiomers, we hypothesize that the complementary lipophilic binding pocket in the hORL1 receptor should be able to accommodate even bigger substituents. Indeed this seems to

Table 3. Stimulation of GTPγS Binding[a]

	ORL1		μ	
compd	EC_{50} [nM]	type	EC_{50} [nM]	type
1a	1500	full	310	full
1f	490	partial		
1j	87	full		
1k	63	full	7900	full
1m	500	partial		
1p	510	full	3700	full
1q	40	full	2500	full
OFQ	63	full		
DAMGO			39	full

[a] A compound was considered to be a full agonist when its ability to stimulate GTPγS binding was >75% in comparison to OFQ (ORL1) or DAMGO (μ). A partial agonist stimulated GTPγS binding with efficiency >25% but <75%.

be the case: both the 5-methyl-1-tetralinyl derivative **1p** and the acenaphtenyl derivative **1q**, which have an increased steric demand compared to **1j** and **1m**, respectively, are even more potent than their predecessors. Gratifyingly, this increase in potency was paralleled by an increase in selectivity. These new, high-affinity hORL1 receptor ligands are 10- (**1q**) to 20-fold (**1p**) selective toward hμ receptors in our in vitro binding assay. Selectivity toward hκ receptors is even better.

The efficacy of our compounds has been assayed primarily by their effect on stimulating GTPγS binding to membranes of HEK 293 cells overexpressing hORL1 receptors. Although this assay has in our hands a high interassay variability, it is suitable for miniaturization to a 96-well format. Our lead compound **1a** behaves as an agonist in this assay, despite its potency being 2 orders of magnitude lower than that of OFQ itself (Figure 1 and Table 3). Results in the GTPγS binding assay are given in Table 3. ORL1 receptor ligands such as **1k** and **1q** with sub-nanomolar affinity to the receptor had potencies similar to or even surpassing OFQ itself in the GTPγS assay. Potency expressed as EC_{50} for stimulation of GTPγS binding was 63 nM for **1k** and 40 nM for **1q** with intrinsic activity similar to that of OFQ as shown for **1q** (Figure 1). The compound **1p** was not as potent as expected in the GTPγS assay; consequently **1p** and **1q** were tested also in a cyclase assay head to head with OFQ (nociceptin). Both **1p** and **1q** are full agonists in this assay with EC_{50}'s comparable to OFQ (**1p**: 0.14 nM; **1q**: 0.28 nM; OFQ: 0.2 $\pm$ 0.1 nM).

We were aware that the 1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one core is a common substructure of antipyschotic compounds;[12] therefore, we have, in addition, assayed the affinity of a selection of the compounds described here toward dopamine and serotonin receptors. While our lead compound **1a** (ORL1: K_i = 5.6 nM) indeed shows some affinity for dopamine receptors (D_2: K_i = 32, $D_{4.4}$: K_i = 11 nM), selectivity toward dopamine receptors already improves for the 1-tetralinyl derivative **1j** (ORL1: 2.1, D_2: 84, D_3: 45, $D_{4.4}$: 1000 nM). The 1-indanyl **1k** has a selectivity profile very similar to **1j** (ORL1: 0.7, D_2: 56, D_3: 31, $D_{4.4}$: 470 nM). The best selectivity toward dopamine receptors was generally found for compounds which are also selective toward the opioid receptors, such as **1q** (ORL1: 0.52, D_2: 520, D_3: 1210, $D_{4.4}$: 350 nM). Affinities for D_1 and 5HT receptors ($5HT_{1D\alpha}$, $5HT_{2a}$, $5HT_{2c}$, $5HT_6$, $5HT_7$) are low for all these compounds (K_i > 1000 nM).

Conclusion

We have succeeded in the identification of high-affinity, partially selective non-peptide agonists for the fourth member of the opioid receptor family, the ORL1 (OFQ/nociceptin) receptor. Systematic modification of our original lead, **1a**, resulted in the identification of compounds with improved affinity and high potency in the GTPγS and cyclase assays. Compounds **1p** and **1q** are moderately selective for OFQ versus the μ and κ receptors and have only low affinity toward δ receptors. These compounds will serve as starting points for further inroads into the development of truly selective non-peptide ORL1 receptor agonists and allow pharmacological characterization of the OFQ/ORL1-receptor system. Indeed **1q**, or more precisely one of its enantiomers, has already been shown to display anxiolytic-like properties in the elevated plus-maze procedure in rats.[19]

Experimental Section

Chemistry. General Procedures. Melting points were taken with a Büchi 535 melting point apparatus and are uncorrected. The ^{1}H spectra were recorded on a Bruker AC250 instrument in DMSO (unless noted otherwise). Low-resolution EI-MS spectra (EI: 70 eV) were recorded on a MS9 updated with a VG ZAB console, Finnigan data system SS300, with direct sample introduction. Microanalysis (C, H, N) were performed on a Heraeus Vario EL. NMR data are reported in parts per million (δ) relative to internal tetramethylsilane and are referenced to the deuterium lock signal from the sample solvent (DMSO-d_6 unless otherwise stated); coupling constants (J) are in hertz. All reactions were performed under argon. Drying of organic solutions was with Na_2SO_4, evaporation was in a rotary evaporator at 40 °C in vacuo as appropriate. For chromatography, Merck silica gel 60 (size 70−230 mesh) was used. Starting materials were high-grade commercial products unless stated otherwise.

5,8-Dichloro-3,4-dihydro-2*H*-naphthalen-1-one (4). To a solution of 4,7-dichloro-2,3-dihydro-1*H*-inden-1-one[13] (**3**, 20 mmol) in dichloromethane (80 mL) cooled to 0 °C was added triethyloxonium tetrafluoroborate (63 mmol). Diazoethyl acetate (37 mmol) was added drop by drop at a temperature below 3 °C with stirring. Stirring continued at 0 °C for 2 h and at room temperature overnight. The mixture was quenched with saturated sodium carbonate solution (100 mL) and extracted with dichloromethane (2 × 50 mL). Organic phases were pooled, and dried with $MgSO_4$, and the solvents were removed in vacuo. The residue (7.5 g, orange oil) was purified by chromatography on silica gel (ethyl acetate/hexane, 1:6) to yield 2.1 g (37%) of the mixture of keto- and enol-tautomers of 5,8-dichloro-1,2,3,4-tetrahydro-1-oxo-2-naphthalenecarboxylic acid ethyl ester as a reddish oil. A mixture of this oil and water (20 mL) was heated for 2.5 h to 240 °C (17 bar). After cooling, the mixture was partitioned between water and dichloromethane, organic phases were pooled and dried with $MgSO_4$, and the solvents were removed in vacuo. The residue (0.75 g, brown oil) was purified by chromatography on silica gel (ethyl acetate/hexane, 1:6) to yield 1.2 g (28%, both steps) of the title compound as a light brown oil which solidified on standing: mp 39−41 °C; ^{1}H NMR ($CDCl_3$) 2.15 (m, 2H, 3-CH_2), 2.69 (t, J = 6.6, 2H, 4-CH_2), 3.04 (t, J = 6.3, 2H, 2-CH_2), 7.28 (d, J = 8.6, 1H, 7-CH), 7.43 (d, J = 8.6, 1H, 6-CH); MS m/z 214, 216 $(M)^+$. Anal. Calcd ($C_{10}H_8Cl_2O$) C, H.

5,8-Dichloro-1,2,3,4-tetrahydro-naphthalen-1-ol (14). Sodium borohydride (2.7 mmol) was added to a solution of 5,8-dichloro-3,4-dihydro-2*H*-naphthalen-1-one (5.4 mmol) in ethanol/water (20 mL, 95%) with stirring. The mixture was stirred

for another hour at room temperature and for 0.5 h at reflux temperature. Ethanol was removed in vacuo and the residue was partitioned between water and dichloromethane. Organic phases were pooled and dried with Na_2SO_4, and the solvent was concentrated to yield 1.1 g (99%) of the title compound as beige solid: mp 99−105 °C; ^{1}H NMR ($CDCl_3$) 1.6−1.8 (m, 1H, 3-CH_2), 1.8−2.1 (m, 2H, 3-CH_2, 2-CH_2), 2.1−2.2 (m, 1H, 2-CH_2), 2.35 (bs, 1H, OH), 2.4−2.6 (m, 1H, 4-CH_2), 3.0 (dd, J = 18, J = 4.7, 1H, 4-CH_2), 5.07 (bs, 1H, CHOH), 7.15−7.30 (m, 2H, 6,7-CH); MS m/z 216, 218 $(M)^+$. Anal. Calcd ($C_{10}H_{10}Cl_2O$) C, H.

5,8-Dichloro-1,2-dihydro-naphthalene (5). A catalytic amount of *p*-toluenesulfonic acid was added to a solution of 5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-1-ol (5.1 mmol) in toluene (25 mL), and the mixture was boiled overnight with separation of water. The mixture was then washed with saturated sodium bicarbonate solution that in turn was extracted with toluene. Organic phases were pooled and dried with Na_2SO_4, and the solvents were removed in vacuo. 5,8-Dichloro-1,2-dihydro-naphthalene was isolated as brown oil (0.97 g, 96%) which was used without further purification in the next steps. ^{1}H NMR ($CDCl_3$) 2.34 (m, 2H, 3-CH_2), 2.91 (t, 2H, 4-CH_2), 6.22 (m, 1H, 2-CH), 6.84 ("d", 1H, 1-CH), 7.11 (s, 2H, 6,7-CH); MS m/z 198, 200 $(M)^+$.

5,8-Dichloro-1,2,3,4-tetrahydro-1,2-naphthalenediol (6). 5,8-Dichloro-1,2-dihydro-naphthalene (4.9 mmol) was dissolved in acetone (1 mL) and *tert*-butyl alcohol (1 mL). This solution was added to a preformed mixture of *N*-methylmorpholine *N*-oxide (5.4 mmol) and osmium tetroxide (0.25 mL of 2.5% solution) in water (4 mL), acetone (2.4 mL), and *tert*-butyl alcohol (0.7 mL). This mixture was then stirred for 20 h at room temperature and after that partitioned between brine (50 mL) and ethyl acetate (3 × 30 mL). Organic phases were washed with sodium hydrogensulfite (40 mL, 2%) and saturated sodium bicarbonate solution. Organic phases were pooled and dried with Na_2SO_4 and the solvents evaporated. The residue was boiled with hexane (20 mL) and yielded upon cooling the title compound (1.0 g, 88%) as a colorless solid: mp 148−149 °C; ^{1}H NMR ($CDCl_3$) 2.01 (m, 2H, 3-CH_2), 2.6−2.8 (m, 3H, 4-CH_2, OH(2×)), 3.15 (dt, J = 18, J = 4, 1H, 4-CH_2), 3.8 (m, 1H, 2-CH), 5.03 (bs, 1H, 1-CH), 7.27 (m, 2H, 6,7-CH); MS m/z 232, 234 $(M)^+$. Anal. Calcd ($C_{10}H_{10}Cl_2O_2$) C, H.

5,8-Dichloro-3,4-dihydro-1*H*-naphthalen-2-one (7a). *p*-Toluenesulfonic acid (150 mg) was added to a solution of 5,8-dichloro-1,2,3,4-tetrahydro-1,2-naphthalenediol (4.3 mmol) in toluene (30 mL). This mixture was boiled for 3 h with separation of water, cooled, and partitioned between saturated sodium bicarbonate solution and ethyl acetate. Organic phases were pooled and dried with $MgSO_4$ and the solvents evaporated. The brown oily residue (1.0 g) was purified by chromatography on silica gel (ethyl acetate/hexane, 1:7) to yield 0.2 g (22%) of the title compound as beige solid: mp 83−86 °C; ^{1}H NMR ($CDCl_3$) 2.61 (t, J = 6.9, 2H, 3-CH_2), 3.24 (t, J = 6.9, 2H, 4-CH_2), 3.66 (s, 2H 1-CH_2), 7.25 (s, 2H, 6,7-CH); MS m/z 214, 216 $(M)^+$. Anal. Calcd ($C_{10}H_8Cl_2O$) C, H.

Preparation of 8-Substituted 1-Phenyl-1,3,8-triazaspiro-[4.5]decan-4-ones. General Procedures. A mixture of the appropriately substituted 1,2,3,4-tetrahydro-naphthalen-2-one (**7a**−**f** or **7m**, 2.5 mmol),[20] 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (2.5 mmol), and molecular sieves (3 g, 4 Å) in toluene (50 mL) was boiled overnight. Molecular sieves were filtered off while hot and washed with toluene, and the filtrate was evaporated to dryness. The residue was dissolved in a mixture of THF and ethanol (9:1, 30 mL). Sodium cyanoborohydride (2.8 mmol) was added with stirring and the pH adjusted to 4.5 with HCl in ethanol. Stirring at room temperature was continued for 16 h. The mixture was poured into ice−water (100 g) and saturated potassium carbonate solution (50 mL) and extracted three times with dichloromethane (50 mL each). Organic phases were pooled, washed with brine, and dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography on silica gel (dichloromethane/methanol 2−5%) and crystallized as hydrochloride salt.

(*RS*)-8-(5,8-Dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1a): (4.1 g, 47%) of a colorless solid from diethyl ether/ethanol (1:1); mp 290−293 °C; ^{1}H NMR 1.91 (m, 3H), 2.4 (b, 1H), 2.8 (m, 1H), 2.9−3.2 (m, 4H), 3.3−4.0 (m, 6H), 4.65 (s, 2H), 6.80 (t, 1H), 7.09 (d, 2H), 7.24 (t, 2H), 7.42 (s, 2H), 9.07 (s, 1H), 10.8 (bs, 1H); MS m/z 430.4 $(MH)^+$. Anal. Calcd ($C_{23}H_{25}Cl_2N_3O$·HCl) C, H, N.

(*RS*)-8-(5-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1b): (0.8 g, 74%) of a colorless solid from ethyl acetate/ethanol (1:1); mp 283−285 °C dec; ^{1}H NMR 1.90 (m, 3H), 2.4 (b, 1H), 2.7 (m, 1H), 3.0−3.9 (m, 10H), 4.64 (s, 2H, 2-CH_2), 6.78 (t, J = 7, 1H, *p*-ArH), 7.1−7.3 (m, 7H), 9.07 (s, 1H, 3-NH), 11.6 (bs, 1H, NH^+); MS m/z 396.4, 398.4 $(MH)^+$. Anal. Calcd ($C_{23}H_{26}ClN_3O$·HCl) C, H, N.

(*RS*)-8-(6-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1c): (0.44 g, 41%) of a colorless solid from chloroform/methanol (1:1); mp 288−290 °C; ^{1}H NMR 1.8−2.0 (m, 3H), 2.35 (m, 1H), 2.8−3.9 (m, 11H), 4.64 (s, 2H), 6.79 (t, 1H), 7.10 (d, 2H), 7.21 (m, 5H), 9.06 (s, 1H), 10.8 (bs, 1H); MS m/z 396.2, 398.2 $(MH)^+$. Anal. Calcd ($C_{23}H_{26}ClN_3O$·HCl) C, H, N.

(*RS*)-8-(7-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1d): (0.32 g, 30%) of a colorless solid from ethyl acetate/ethanol (8:1); mp 287−289 °C dec; ^{1}H NMR 1.8−2.0 (m, 3H), 2.35 (m, 1H), 2.7−3.8 (m, 11H), 4.64 (s, 2H, 2-CH_2), 6.77 (t, J = 7, 1H, *p*-ArH), 7.1−7.3 (m, 7H), 9.07 (s, 1H, 3-NH), 11.6 (bs, 1H, NH^+); MS m/z 396.2, 398.2 $(MH)^+$. Anal. Calcd ($C_{23}H_{26}ClN_3O$·HCl) C, H, N.

(*RS*)-8-(8-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1e): (0.59 g, 30%) of a colorless solid from ethanol; mp 286−289 °C; ^{1}H NMR 1.8−2.0 (m, 3H), 2.35 (m, 1H), 2.8−3.2 (m, 5H), 3.4−4.0 (m, 6H), 4.65 (s, 2H), 6.80 (t, 1H), 7.0−7.4 (m, 7H), 9.07 (s, 1H), 10.8 (bs, 1H); MS m/z 396.2, 398.2 $(MH)^+$. Anal. Calcd ($C_{23}H_{26}ClN_3O$·HCl) C, H, N.

(*RS*)-8-(1,2,3,4-Tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1f): (0.45 g, 46%) of a colorless solid from ethyl acetate/ethanol (10:1); mp >265 °C dec; ^{1}H NMR 1.8−2.0 (m, 3H), 2.35 (m, 1H), 2.8−4.0 (m, 11H), 4.64 (s, 2H), 6.78 (t, 1H), 7.1−7.3 (m, 8H), 9.06 (s, 1H), 11.4 (bs, 1H); MS m/z 362.3 $(MH)^+$. Anal. Calcd ($C_{23}H_{27}N_3O$·HCl) C, H, N.

8-Indan-2-yl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (1m): (0.60 g, 62%) of a colorless solid from ethyl acetate/ethanol (5:3); mp 270 °C dec; ^{1}H NMR 1.90 (d, J = 14, 2H, 6,10-CH_2, eq), 3.06 (td, J = 14, J = 4, 2H, 6,10-CH_2, ax), 3.3−3.7 (m, 8H, 7,9-CH_2, 1′,3′-CH_2), 4.12 (m, J = 7.5, 1H, 2′-CH), 4.64 (s, 2H, 2-CH_2), 6.78 (t, 1H, *p*-ArH), 7.0−7.3 (m, 8H, 4′,5′,6′,7′-ArH, *o*,*m*-ArH), 9.04 (s, 1H, 3-NH), 11.4 (bs, 1H, NH^+); MS m/z 348.4 $(MH)^+$. Anal. Calcd ($C_{22}H_{25}N_3O$·HCl) C, H, N.

(*S*)-8-(5,8-Dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1g). A hot solution of (*S*)-(+)-1.1′-binaphthyl-2,2′-diyl hydrogen phosphate (0.87 g, 2.5 mmol) in ethanol (40 mL) was added to a solution of **(1a)** (1.8 g, 4.2 mmol) in ethanol (30 mL). The salt was recrystallized 5 times from ethanol to afford **(1g)** (0.48 g) as the phosphate salt. The free base was liberated and subsequently crystallized as the hydrochloride salt from ethanol to provide a white crystalline solid: mp 273−275 °C; $[\alpha]^{20}_D$ −54.9 (*c* 0.9, CH_3OH); ^{1}H NMR 1.91 (m, 3H), 2.4 (b, 1H), 2.8 (m, 1H), 2.9−3.2 (m, 4H), 3.3−4.0 (m, 6H), 4.65 (s, 2H, 2-CH_2), 6.80 (t, J = 7, 1H, *p*-ArH), 7.09 (d, J = 8, 2H, *o*-ArH), 7.24 ("t", J = 7.5, 2H, *m*-ArH), 7.42 (s, 2H, 6′,7′-ArH), 9.07 (s, 1H, 3-NH), 10.8 (bs, 1H, NH^+). Anal. Calcd ($C_{23}H_{25}Cl_2N_3O$·HCl) C, H, N. Chiral HPLC on a Chiralpak AS column (hexane/0.4% triethylamine in hexane/ethanol (85:5:10), flow rate 1.5 mL/min) showed **1g** to have 93% ee. A typical retention time for the S-isomer **(1g)** was 7.1 min and for the R-isomer **(1h)** 6.3 min.

(*R*)-8-(5,8-Dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1h). A hot solution of (*R*)-(−)-1.1′-binaphthyl-2,2′-diyl hydrogen phosphate (0.66 g, 1.9 mmol) in ethanol (30 mL) was added to a solution of **(1a)** (0.82 g, 1.9 mmol, isolated from the mother liquors of **1g** in ethanol (20 mL). The salt was recrystallized 3 times from ethanol to afford **1h** (0.54 g) as the phosphate salt. The free base was liberated and subsequently crystallized as the hydrochloride salt from ethanol to provide a white crystalline solid: mp 274−276 °C; $[\alpha]^{20}_D$ +53.2 (*c* 0.9, CH_3OH); the absolute configuration was determined by X-ray crystallography. Anal. Calcd ($C_{23}H_{25}Cl_2N_3O$·HCl) C, H, N.

Preparation of 3-Substituted 8-(1,2,3,4-Tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones. General Procedure. The appropriately substituted 8-(1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one hydrochloride (0.4 mmol) was suspended in *N*,*N*-dimethylformamide (10 mL). Sodium hydride dispersion (1.5 mmol) was added, and the mixture was stirred for 30 min at room temperature and for 1 h at 80 °C. The mixture was cooled to room temperature, and the appropriate alkylating agent (1.5 mmol) was added. Stirring commenced at 80 °C till the educt was consumed as evidenced by TLC (dichloromethane/methanol, 9:1). The reaction mixture was poured into saturated sodium bicarbonate solution (100 mL) and extracted with ethyl acetate (3 × 50 mL). Organic phases were pooled, washed with brine (100 mL), and dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography on silica gel (dichloromethane/methanol 2−5%) and crystallized as hydrochloride salt.

(*RS*)-8-(5,8-Dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (2a). Compound **1a** and methyl iodide yielded a colorless solid (80 mg, 39%) from ethyl acetate: mp 256−259 °C dec; ^{1}H NMR 1.8−2.0 (m, 3H), 2.4 (m, 1H), 2.75 (m, 1H), 2.92 (s, 3H), 3.0−3.2 (m, 4H), 3.3−3.9 (m, 6H), 4.71 (s, 2H), 6.80 (t, 1H), 7.10 (d, 2H), 7.25 (t, 2H), 7.41 (s, 2H), 11.1 (bs, 1H); MS (high res) mass calcd for $C_{24}H_{27}Cl_2N_3O$ (MH^+) = 444.1609, obsd *m*/*z* at 444.1614, deviation from theoretical = 0.5 mmu.

(*RS*)-3-Allyl-8-(5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (2b). Compound **1a** and allyl bromide yielded a colorless solid (95 mg, 44%) from ethyl acetat: mp 231−235 °C dec; ^{1}H NMR 1.8−2.0 (m, 3H), 2.4 (m, 1H), 2.8 (m, 1H), 3.0−3.2 (m, 4H), 3.3−4.0 (m, 6H), 4.02 (d, *J* = 5, 2H, 1″-CH_2), 4.70 (s, 2H, 2-CH_2), 5.2−5.3 (m, 2H, 3″CH), 5.75−5.95 (m, 1H, 2″-CH), 6.82 (t, *J* = 7, 1H, *p*-ArH), 7.13 (d, *J* = 8, 2H, *o*-ArH), 7.22 (t, *J* = 7.5, 2H, *m*-ArH), 7.41 (s, 2H, 6′,7′-ArH), 11.2 (bs, 1H, NH^+); MS (high res) mass calcd for $C_{26}H_{29}Cl_2N_3O$ (MH^+) = 470.1766, obsd *m*/*z* at 470.1767, deviation from theoretical = 0.1 mmu.

(*RS*)-3-Benzyl-8-(5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (2c). Compound **1a** and benzyl bromide yielded a colorless solid (150 mg, 63%) from ethyl acetate; mp 263−266 °C dec; ^{1}H NMR 1.8−2.0 (m, 3H), 2.4 (m, 1H), 2.8 (m, 1H), 3.0−3.2 (m, 4H), 3.3−4.0 (m, 6H), 4.60 (s, 2H), 4.66 (s, 2H), 6.80 (t, 1H), 7.10 (d, 2H), 7.22 (t, 2H), 7.3−7.4 (m, 5H), 7.41 (s, 2H), 11.2 (bs, 1H); MS *m*/*z* 520.3, 522.3 $(MH)^+$. Anal. Calcd ($C_{30}H_{31}Cl_2N_3O$·HCl) C, H, N.

(*RS*)-8-(8-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-3-(2-oxo-2-phenyl-ethyl)-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one Hydrochloride (2d). Compound **1e** and phenacyl bromide yielded a colorless solid (60 mg, 24%) from ethyl acetate; mp 272 °C; ^{1}H NMR 1.7−2.1 (m, 3H), 2.35 (m, 1H), 2.8−3.2 (m, 5H), 3.3−4.0 (m, 6H), 4.75 (s, 2H), 5.04 (s, 2H), 6.86 (t, 1H), 7.1−7.4 (m, 7H), 7.60 (t, 2H), 7.72 (t, 1H), 8.06 (d, 2H), 10.7 (bs, 1H); MS (high res) mass calcd for $C_{31}H_{32}ClN_3O_2$ (MH^+) = 514.2261; obsd *m*/*z* at 514.2258, deviation from theoretical = 0.3 mmu.

(*RS*)-8-(8-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-3-(2-hydroxy-ethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (2e). Compound **1e** and 2-chloroethoxy-trimethylsilane yielded a colorless solid (150 mg, 73%) from ethyl acetate—the trimethylsilyl protecting was removed quantitatively during the chromatography: mp >244 °C dec; ^{1}H NMR 1.8−2.1 (m, 3H), 2.35 (m, 1H), 2.8−3.2 (m, 5H), 3.3−4.0 (m, 10H), 4.78 (s, 2H), 4.95 (t, 1H), 6.83 (t, 1H), 7.1−7.4 (m, 7H), 11.1 (bs, 1H); MS (high res) mass calcd for $C_{25}H_{30}ClN_3O_2$ (MH^+) = 440.2105, obsd *m*/*z* at 440.2107, deviation from theoretical = 0.2 mmu.

(*RS*)-8-(8-Chloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-3-methoxymethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (2f). Compound **1e** and chloromethyl methyl ether yielded a colorless solid (110 mg, 54%) from ethyl acetate: mp >227−229 °C dec; ^{1}H NMR 1.8−2.1 (m, 3H), 2.35 (m, 1H), 2.8−3.2 (m, 5H), 3.29 (s, 3H), 3.3−4.0 (m, 6H), 4.80 (s, 2H), 4.81 (s, 2H), 6.86 (t, 1H), 7.1−7.4 (m, 7H), 11.1 (bs, 1H); MS (high res) mass calcd for $C_{25}H_{30}ClN_3O_2$ (MH^+) = 440.2105, obsd *m*/*z* at 440.2109, deviation from theoretical = 0.4 mmu.

(*RS*)-1-Phenyl-8-(1,2,3,4-tetrahydro-naphthalen-1-yl)-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1j). Sodium nitrite (75 mmol) in water (35 mL) was added to a solution of 3,4-dihydro-1(2*H*)-naphthalenone oxime (44 mmol) in diethyl ether (100 mL). Sulfuric acid (1 N, 75 mL) was added slowly with stirring at room temperature to this mixture. Stirring continued for 4 h after the addition was complete. The phases were separated, the water phase was extracted once with diethyl ether (100 mL), and organic phases were pooled, dried with Na_2SO_4, and concentrated. The red oily residue (7.6 g) was dissolved in acetonitrile (50 mL) and added slowly to a suspension of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (133 mmol) and molecular sieves (32 g, 4 Å) in acetonitrile (500 mL). The mixture was stirred at room temperature for 36 h, and molecular sieves were filtered off and washed with dichloromethane/methanol (9:1, 400 mL). The filtrate was concentrated and the residue dissolved in THF/ethanol (9:1, 500 mL). Sodium cyanoborohydride (32 mmol) was added with stirring and the pH adjusted to 4.5 with HCl in ethanol. Stirring at room temperature was continued for 2 h. The mixture was poured into ice−water (200 g) and saturated potassium carbonate solution (100 mL) and was extracted three times with dichloromethane (200 mL each). Organic phases were pooled, washed with brine, and dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography on silica gel (dichloromethane/methanol 2−4%) and decolorized with Norite to yield a beige solid (3.0 g, 19%) which was crystallized as the colorless hydrochloride salt from ethyl acetate/ethanol (2:3): mp 259 °C; ^{1}H NMR 1.67 (m, 1H), 1.86 (t, *J* = 14.7, 2H), 1.9−2.3 (m, 3H), 2.6−3.1 (m, 4H, 4′-CH_2, 7,9-CH_2, eq), 3.25 (m, 1H), 3.40 (m, 1H, 7,9-CH_2, eq), 3.7 (m, 1H, 7,9-CH_2, ax), 3.9 (m, 1H, 7,9-CH_2, ax), 4.62 (m, 2H, 2-CH_2), 4.86 (m, 1H, 1′-CH), 6.80 (t, *J* = 7.1, 1H, *p*-ArH), 7.11 (d, *J* = 8.1, 2H, *o*-ArH), 7.2−7.4 (m, 5H, 5′,6′,7′-ArH, *m*-ArH), 8.04 (m, 1H, 8′-ArH), 9.04 (s, 1H, 3-NH), 10.4 (bs, 1H, NH^+); MS *m*/*z* 362.2 $(MH)^+$. Anal. Calcd ($C_{23}H_{27}N_3O$·HCl) C, H, N.

(*RS*)-8-Indan-1-yl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1k). A solution of indan-1-one (113 mmol), 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (113 mmol), and titanium(IV) isopropoxide (142 mmol) in THF (100 mL) was stirred for 18 h at room temperature. The solvent was removed and the residue dissolved in THF/ethanol (1:2, 150 mL). Sodium cyanoborohydride (76 mmol) was added with stirring, and stirring at room temperature was continued for 20 h. Water (30 mL) was added, and the mixture was filtered through Celite. The filter cake was washed thoroughly with ethanol, the filtrate evaporated, and the residue purified by chromatography on silica gel (dichloromethane/methanol 2−10%) to yield a beige solid (17.7 g, 45%) which was crystallized as the colorless hydrochloride salt from ethyl acetate/ethanol (3:1): mp 270 °C; ^{1}H NMR 1.82 (d, *J* = 14.1, 2H, 2′-CH_2), 2.8−3.2 (m, 6H, 6,10-CH_2, 3′-CH_2), 3.3−3.7 (m, 3H, 7,9-CH_2, ax, eq), 3.8 (m, 1H, 7,9-CH_2, ax), 4.60 (s, 2H,

2-CH_2), 5.03 (m, 1H, 1′-CH), 6.77 (t, J = 7.3, 1H, *p*-ArH), 7.11 (d, J = 8.1, 2H, *o*-ArH), 7.19 (t, J = 7.2, 2H, m-ArH), 7.3−7.5 (m, 3H, 4′,5′,6′-ArH), 7.90 (d, 1H, 7′-ArH), 8.98 (s, 1H, 3-NH), 11.3 (bs, 1H, NH^+); MS *m*/*z* 348.4 $(MH)^+$. Anal. Calcd ($C_{22}H_{25}N_3O$·HCl) C, H, N.

(*S*)-(−)-1,2,3,4-Tetrahydro-1-naphthylamine Hydrochloride (11n). (*R*)-(−)-1,2,3,4-Tetrahydro-1-naphthol (**10n**, 35 mmol, $[\alpha]^{20}_D$ −33 (*c* 2.5, $CHCl_3$)) and diphenylphosphoryl azide (42 mmol) were dissolved in toluene (80 mL) and cooled to 0 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (42 mmol) dissolved in toluene (20 mL) was added slowly with cooling, and stirring was continued for 2 h at 0 °C and 16 h at room temperature. Filtration with ethyl acetate/hexane (1:6) through silica gel yielded a mixture of azide and 1,2-dihydro-naphthalene as colorless oil (5.9 g). This mixture was dissolved in THF (60 mL) and added slowly to a suspension of lithium aluminum hydride (42 mmol) in THF (50 mL) at room temperature. The reaction mixture was stirred for 1 h at room temperature and for another 1 h refluxed. After cooling, the mixture was hydrolyzed by subsequent careful addition of water (1.6 mL), sodium hydroxide solution (15%, 3.2 mL), and water (4.8 mL). The suspension was then aged and filtered through Celite. The filtrate was concentrated, dissolved in ethyl acetate (120 mL), and precipitated as the hydrochloride (4.9 g, 63%) after addition of hydrogen chloride in ethanol: mp 236−237 °C. Anal. Calcd ($C_{10}H_{13}N$·HCl) C, H, N. Chiral HPLC on a Crownpak CR(−) column (perchloric acid (pH 2)/acetonitrile (89:11), flow rate 0.9 mL/min) showed **11n** to have 92% ee. A typical retention time for the *S*-isomer **11n** was 8.1 min and for the *R*-isomer **(11o)** 6.7 min.

(*R*)-(+)-1,2,3,4-Tetrahydro-1-naphthylamine Hydrochloride (11o). This compound was prepared from (*S*)-(+)-1,2,3,4-tetrahydro-1-naphthol (27 mmol, $[\alpha]^{20}_D$ +33 (*c* 2.5, $CHCl_3$)). Compound **11o** was isolated as the hydrochloride (3.0 g, 60%): mp 236−237 °C. Anal. Calcd ($C_{10}H_{13}N$·HCl) C, H, N. Chiral HPLC on a Crownpak CR(−) column (perchloric acid (pH 2)/acetonitrile (89:11), flow rate 0.9 mL/min) showed **11o** to have 98% ee.

(*S*)-4-Phenylamino-1-(1,2,3,4-tetrahydro-naphthalen-1-yl)-piperidine-4-carbonitrile (12n). To a boiling suspension of **11n** (26 mmol) and potassium carbonate (15 mmol) in ethanol (50 mL) was added 1-methyl-1-ethyl-4-oxopiperidinium iodide[16] (39 mmol) dissolved in water (17 mL). The reaction mixture was boiled for another hour, cooled, and diluted with water (50 mL). Ethanol was removed in vacuo and the residue was partitioned between sodium hydroxide solution (1 N, 100 mL) and ethyl acetate (3 × 100 mL). Organic phases were pooled, dried with Na_2SO_4, and concentrated. The residue was filtered with ethyl acetate/hexane (1:1) through silica gel to yield the intermediate 1-(1,2,3,4-tetrahydro-naphthalen-1-yl)-piperidin-4-one (5.3 g). To a solution of this raw material in acetic acid (20 mL) were added aniline (25 mmol) and trimethylsilyl cyanide (23 mmol) at room temperature. The mixture was stirred for 90 min after which it was poured onto a mixture of ice (100 g) and ammonia (25%, 60 mL) and extracted with dichloromethane (3 × 200 mL). Organic phases were pooled, dried with Na_2SO_4, and concentrated to yield a yellow solid. This solid was digested with diethyl ether to afford **12n** (5.8 g, 76%) as an off white solid, mp 152−153 °C: ^{1}H NMR ($CDCl_3$) 1.70 (m, 2H), 1.85 (m, 1H), 1.97 (m, 3H), 2.27 (m, 1H), 2.40 (m, 1H), 2.55 (m, 1H), 2.6−3.0 (m, 5H), 3.65 (s, 1H), 3.88 (m, 1H), 6.8−6.95 (m, 3H), 7.05−7.20 (m, 3H), 7.25 (t, 2H), 7.62 (m, 1H); MS *m*/*z* 332.2 $(MH)^+$. Anal. Calcd ($C_{22}H_{25}N_3$) C, H, N. Chiral HPLC on a Chiralcel OJ-R column (sodium perchlorate (0.5 M)/acetonitrile (30:70), flow rate 0.8 mL/min) showed **12n** to have 96% ee. A typical retention time for the *S*-isomer **12n** was 7.8 min and for the *R*-isomer **12o** 6.7 min.

(*R*)-4-Phenylamino-1-(1,2,3,4-tetrahydro-naphthalen-1-yl)-piperidine-4-carbonitrile (12o). This compound was prepared from **11o** (15 mmol). Compound **12o** (2.9 g, 58%) was isolated as an off white solid: mp 152−153 °C. Anal. Calcd ($C_{22}H_{25}N_3$) C, H, N. Chiral HPLC on a Chiralcel OJ-R column (sodium perchlorate (0.5 M)/acetonitrile (30:70), flow rate 0.8 mL/min) showed **12o** to have 98% ee.

(*S*)-4-Phenylamino-1-(1,2,3,4-tetrahydro-naphthalen-1-yl)-piperidine-4-carboxylic Acid Amide (13n). A mixture of acetic anhydride (30 mL), formic acid (30 mL), and **12n** (17 mmol) was stirred at room temperature for 3 d, poured onto ice−water (600 g), made basic with sodium hydroxide solution (28%), and extracted with dichloromethane (2 × 200 mL). Organic phases were pooled, dried with Na_2SO_4, and concentrated. The residue (6.2 g) was dissolved in *tert*-butyl alcohol (120 mL). Water (10 mL) and ammonia (25%, 10 mL) were added, and then hydrogen peroxide solution (33%, 20 mL) was slowly added at room temperature. The mixture was stirred 16 h, water (200 mL) was added, and the solvent was removed in vacuo. The residue was stirred with water (200 mL) and the precipitate collected and dried to afford **13n** (4.7 g, 79%) as an off white solid: mp 186−187 °C; ^{1}H NMR 1.61 (m, 2H), 1.9−2.8 (m, 12H), 3.74 (m, 1H), 5.47 (s, 1H), 6.5−6.6 (m, 3H), 7.95−7.20 (m, 7H), 7.60 (d, 1H); MS *m*/*z* 350.4 $(MH)^+$. Anal. Calcd ($C_{22}H_{27}N_3O$) C, H, N. Chiral HPLC on a Chiralcel OJ-R column (sodium perchlorate (0.5 M)/acetonitrile (65:35), flow rate 0.8 mL/min) showed **13n** to have >99.5% ee. A typical retention time for the *S*-isomer **13n** was 25.9 min and for the *R*-isomer **13o** 28.4 min.

(*R*)-4-Phenylamino-1-(1,2,3,4-tetrahydro-naphthalen-1-yl)-piperidine-4-carboxylic Acid Amide (13o). This compound was prepared from **12o** (8 mmol). Compound **13o** (2.1 g, 75%) was isolated as an colorless solid: mp 182−184 °C. Anal. Calcd ($C_{22}H_{27}N_3O$) C, H, N. Chiral HPLC on a Chiralcel OJ-R column (sodium perchlorate (0.5 M)/acetonitrile (65:35), flow rate 0.8 mL/min) showed **13o** to have >99.5% ee.

(*S*)-1-Phenyl-8-(1,2,3,4-tetrahydro-naphthalen-1-yl)-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1n). A suspension of **13n** (12 mmol) in formamide (65 mL) was heated for 2 h to 200 °C. The mixture was cooled, poured onto ice−water (800 g), and extracted with dichloromethane (3 × 400 mL). Organic phases were pooled, washed with brine, dried with Na_2SO_4, and concentrated. The residue (4.9 g) was dissolved in methanol (200 mL). Sodium borohydride (18 mmol) was added, and the mixture was stirred for 1 h at room temperature and for another hour at 60 °C. The solvent was removed in vacuo, and the residue was partitioned between ammonia (150 mL, 12%) and dichloromethane (3 × 150 mL). Organic phases were pooled and dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography on silica gel (ethyl acetate/hexane 1:1) and crystallized as the hydrochloride salt from ethyl acetate/ethanol to afford **1n** (1.0 g, 23%) as a colorless solid: mp 269−271 °C; $[\alpha]^{20}_D$ −30.4 (*c* 1, CH_3OH); ^{1}H NMR 1.67 (m, 1H), 1.86 (t, 2H), 1.9−2.3 (m, 3H), 2.6−3.1 (m, 4H), 3.25 (td, 1H), 3.3−3.5 (m, 1H), 3.7 (m, 1H), 3.9 (m, 1H), 4.61 (m, 2H), 4.86 (m, 1H), 6.79 (t, 1H), 7.11 (d, 2H), 7.2−7.4 (m, 5H), 8.13 (m, 1H), 9.04 (s, 1H), 10.5 (bs, 1H); MS *m*/*z* 362.2 $(MH)^+$. Anal. Calcd ($C_{23}H_{27}N_3O$·HCl) C, H, N. Chiral HPLC on a Chiralcel OC column (hexane/10% ethanol in hexane/0.4% triethylamine in hexane (40:40:20), flow rate 1.5 mL/min) showed **1n** to have >99.5% ee. A typical retention time for the *S*-isomer **1n** was 14.1 min and for the *R*-isomer **1o** 16.7 min.

(*R*)-1-Phenyl-8-(1,2,3,4-tetrahydro-naphthalen-1-yl)-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1o). This compound was prepared from **13o** (5 mmol). Compound **1o** (0.2 g, 11%) was isolated as an colorless solid: mp 269−270 °C. $[\alpha]^{20}_D$ +31.3 (*c* 1, CH_3OH); ^{1}H NMR 1.67 (m, 1H), 1.83 (t, 2H), 1.9−2.3 (m, 3H), 2.6−3.1 (m, 4H), 3.2−3.5 (m, 2H), 3.7 (m, 1H), 3.9 (m, 1H), 4.61 (m, 2H), 4.85 (m, 1H), 6.78 (t, 1H), 7.11 (d, 2H), 7.2−7.4 (m, 5H), 8.13 (m, 1H), 9.04 (s, 1H), 10.5 (bs, 1H); MS *m*/*z* 362.2 $(MH)^+$. Anal. Calcd ($C_{23}H_{27}N_3O$·HCl) C, H, N. Chiral HPLC on a Chiralcel OC column (hexane/10% ethanol in hexane/0.4% triethylamine in hexane (40:40:20), flow rate 1.5 mL/min) showed **1o** to have >99.5% ee.

(*RS*)-8-(5-Methyl-1,2,3,4-tetrahydro-naphthalen-1-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Hydrochloride (1p). Sodium nitrite (33 mmol) in water (20 mL) was added to a solution of 3,4-dihydro-5-methyl-1(2*H*)-naphthale-

none oxime[21] (20 mmol) in diethyl ether (60 mL). Sulfuric acid (1 N, 35 mL) was added slowly with stirring at room temperature to this mixture. Stirring continued for 4 h after the addition was complete. The phases were separated, the water phase was extracted once with diethyl ether (60 mL), and organic phases were pooled, dried with Na_2SO_4, and concentrated. The orange-red, solid residue (3.6 g) was dissolved in acetonitrile (20 mL) and added slowly to a suspension of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (40 mmol) and molecular sieves (11 g, 4 Å) in acetonitrile (150 mL). The mixture was stirred at room temperature for 36 h, and molecular sieves were filtered off. The filtrate was concentrated, and the residue was dissolved in THF/ethanol (9:1, 500 mL). Sodium cyanoborohydride (20 mmol) was added with stirring and the pH adjusted to 4.5 with HCl in ethanol. Stirring at room temperature was continued for 16 h. The mixture was poured into ice−water (200 g) and saturated potassium carbonate solution (100 mL) and was extracted three times with dichloromethane (200 mL each). Organic phases were pooled, washed with brine, and dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography on silica gel (ethyl acetate/hexane 1:1) to yield a yellowish solid (1.5 g, 19%) which was crystallized as the colorless hydrochloride salt from ethyl acetate/ethanol (1:1): mp 259 °C; ^{1}H NMR 1.6−1.9 (m, 3H), 2.12 (m, 3H), 2.22 (s, 3H, CH_3), 2.6−3.5 (m, 6H), 3.7 (m, 1H, 7,9-CH_2, ax), 3.9 (m, 1H, 7,9-CH_2, ax), 4.61 (m, 2H, 2-CH_2), 4.78 (m, 1H, 1′-CH), 6.78 (t, J = 7.5, 1H, *p*-ArH), 7.11 (d, J = 7.6, 2H *o*-ArH), 7.2 (m, 4H, 6′,7′-ArH, *m*-ArH), 7.86 (m, 1H, 8′-ArH), 9.04 (s, 1H, 3-NH), 10.3 (bs, 1H, NH^+); MS *m*/*z* 376.3 $(MH)^+$. Anal. Calcd ($C_{24}H_{29}N_3O$·HCl) C, H, N.

(*RS*)-8-Acenaphten-1-yl-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one Hydrochloride (1q). Reaction of acenaphten-1-one (6.7 mmol), 1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one, and titanium(IV) isopropoxide (8.4 mmol) in THF (15 mL) and subsequent reduction with sodium cyanoborohydride (4.5 mmol) in EtOH (15 mL) as described for **1k** yielded, after column chromatography on silica gel (ethyl acetate/hexane 4:1), a pale brown foam (0.56 g, 22%) which was crystallized as the yellowish hydrochloride salt from diethyl ether/methanol (3:1): mp 263 °C; ^{1}H NMR 1.82 (d, J = 13.5, 2H), 2.89−3.25 (m, 3H), 3.29−3.97 (m, 5H), 4.59 (s, 2H, 2-CH_2), 5.67 (m, 1H, 1′-CH), 6.78 (t, J = 8.5, 1H, *p*-ArH), 7.11 (d, J = 8.5, 2H, *m*-ArH), 7.22 (t, J = 8.5, 2H, *o*-ArH), 7.50 (d, J = 7.5, 1H), 7.59 (t, J = 7.5, 1H), 7.69 (t, J = 7.5, 1H), 7.79 (d, J = 7.5, 1H), 7.95 (d, J = 7.5, 1H), 8.11 (d, J = 7.5, 1H), 8.99 (s, 1H, 3-NH), 10.74 (bs, 1H, NH^+); MS *m*/*z* 384.3 $(MH)^+$. Anal. Calcd ($C_{25}H_{25}N_3O$·HCl) C, H, N.

Biochemistry. Cell Culture and Membrane Preparation. The cDNAs encoding hμ, hκ and hδ receptors were subcloned into the pSFV2gen vector, and recombinant Semliki Forest Virus stocks were generated as described previously.[22] Baby hamster kidney (BHK) cells were infected and harvested for membrane preparation 24 h after infection. The cDNA encoding the hORL1 receptor was inserted into the pcDNA3 vector and stably transfected into human embryonic kidney 293 (HEK293) cells. One cell clone was selected for pharmacological characterization.

Membrane fractions were prepared by homogenization of cells followed by a 30 min centrifugation at 39000*g* using a Beckman JA-20 rotor. The resulting membrane pellet was resuspended in 50 mM Tris, pH 7.8, 1 mM EDTA, 6 mM $MgCl_2$ buffer at a concentration of $\sim 2 \times 10^7$ cells/mL and frozen at −80 °C.

Radioligands. The radioligands [^{3}H]-OFQ (specific activity 150 Ci/mmol), [^{3}H]-naloxone (specific activity 54.5 Ci/mmol), and [^{3}H][Ile^{5,6}]-deltorphin II (specific activity 72 Ci/mmol) were from Amersham (Little Chalfont, U.K.).

Radioligand Binding Assays. Competitive binding analysis was performed with membranes prepared from permanently transfected HEK293 cells expressing hORL1 receptors (20 μg of membrane protein) and 0.1 nM [^{3}H]-OFQ. Competitive binding displacement analyses for opioid receptors were performed with membranes prepared from BHK cells transiently expressing hμ, hκ or hδ receptors (10 μg of membrane protein each) and 1.5 nM (hμ) and 3 nM (hκ) [^{3}H]-naloxone or 0.3 nM [^{3}H][Ile^{5,6}]-deltorphin II (hδ).

Reactions were performed in 1 mL of binding buffer (50 mM Tris-HCl, pH 7.8; 1 mM EGTA; 5 mM $MgCl_2$; 0.1% BSA) for 60 min at 22 °C. At the end of the incubation, the samples were filtered through GF/C-filters (Amersham), which had been precoated (0.3% polyethyleneimine plus 0.1% BSA). Filters were then washed 3 times with 0.5 mL of cold wash buffer (50 mM Tris-HCl, pH 7.5). Finally, 60 μL of scintillation fluid (Micro Scint, Canberra Packard) was added, and samples were counted in a Top Counter (Packard). Nonspecific binding was defined in the presence of 1 μM unlabeled OFQ (hORL1 receptor), 10 μM naloxone (hμ and hκ receptors), or 10 μM deltorphin II (hδ receptors).

GTPγS Binding Assay. Agonist-mediated binding of GTPγS was investigated in 96-well plates using a scintillation proximity assay (SPA) and membranes prepared from cells expressing hOFQ, hμ, hκ, or hδ receptors. Binding was performed in 200 μL of 20 mM HEPES-buffer (pH 7.4, plus 6 mM $MgCl_2$ and 100 mM NaCl), supplemented with 20 μM GDP and 0.3 nM GTP[γ^{35}]S (1130 Ci/mmol). Twenty micrograms of membranes, 1 mg of wheatgerm agglutinin SPA beads (Amersham, Little Chalfont, U.K.), and either OFQ (10^{-5} M to 10^{-10} M) or synthetic compounds (10^{-4} M to 10^{-10} M) were added. Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS.

The reaction mixture was incubated on a shaker for 60 min at 22 °C and then centrifuged for 5 min at 1500 rpm in an Eppendorf 5403 centrifuge. Finally the plates were read in a Top counter (Packard).

cAMP Assay. The inhibition of forskolin-mediated cAMP accumulation by OFQ and synthetic OFQ agonists was determined in 96-well plates. Briefly, 20 000 cells were incubated in Krebs-Ringer-HEPES-buffered solution (KHR; 124 mM NaCl, 5 mM KCl, 1.25 mM $MgSO_4$, 1.5 mM $CaCl_2$, 1.25 mM KH_2PO_4, 25 mM HEPES, pH 7.4) in the presence of 100 μM Rolipram and 1 μM forskolin (both from Sigma) with increasing concentrations of agonists (10^{-11} to 10^{-7} M) for 15 min at 37 °C. Reactions were stopped by the addition of 0.12 mL of ice-cold ethanol, and mixtures were stored at −80 °C for at least 4 h. The cAMP content was determined from the supernatant using the Biotrak nonradioactive cAMP kit (Amersham, Little Chalfont, U.K.) according to the manufacturer's instructions.

Acknowledgment. The skillful assistance of B. Frei, R. Augsburger, P. Oberli, D. Wehrli, R. Henningsen, J. Higelin, C. Kuhn, G. Py, and I. Vogel is gratefully acknowledged. The authors thank W. Arnold, M. Hennig, W. Vetter, W. Meister, V. Meduna, and G. Oesterhelt for spectroscopic analysis, K. Lundstrom, P. Malherbe, and S. Hankel for cells expressing hOFQ and opioid receptors, and M. Graf and A. Sleight for binding data on dopamine and serotonin receptors.

Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM991129Q