

## Synthesis of (1*S*,3*aS*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one, a potent and selective orphanin FQ (OFQ) receptor agonist with anxiolytic-like properties

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Received 18 February 2000; revised 7 April 2000; accepted 13 April 2000

**Abstract** – The development of 8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones **3** starting from (RS)-8-acenaphthen-1-yl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one **1** is reported. The synthesis and the binding affinities at human OFQ and opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors of the stereoisomers **3a–f** are described. In vitro the most selective compound, (1*S*,3*aS*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **3c**, was found to act as a full agonist at the OFQ receptor in the GTP $\gamma^{35}$ S binding test. It turned out to be selective versus a variety of other neurotransmitter systems. When tested in vivo following intraperitoneal injection, compound **3c** was found to decrease neophobia in a novel environment and to exhibit dose-dependent anxiolytic-like effects in the elevated plus-maze procedure, thus confirming the effects observed following intracerebroventricular infusion of the OFQ peptide in rat.  
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OFQ receptor / agonists / 1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-ones / stereoselective synthesis / anxiolytic activity

### 1. Introduction

Orphanin FQ (OFQ, nociceptin) is a recently discovered 17-amino acid neuropeptide that is structurally related to the opioid peptides but does not act on opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors [1, 2]. Orphanin FQ selectively binds to its own receptor (OFQ receptor or ORL-1), which is a member of the G protein-coupled receptor superfamily [3]. The amino acid sequence of the OFQ receptor is 47% identical to the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors overall, and is 64% identical in the transmembrane domains, however, when compared with classical opioid receptors, the OFQ receptor has low affinity for opioid ligands [4–5]. As for the opioid receptors, OFQ receptor activation has been linked to the inhibition of adenylyl cyclase activity and/or modulation of neuronal K<sup>+</sup> and Ca<sup>2+</sup> conductance [6–7].

Orphanin FQ and its receptor are widely expressed throughout the central nervous system. Orphanin FQ has been proposed to act as an anti-opioid peptide, but its

widespread sites of action in the brain suggest that it may have more general functions [8–10]. It has recently been shown that orphanin FQ plays an important role in higher brain functions and can act as an anxiolytic by attenuating the behavioural inhibition of animals being acutely exposed to anxiogenic environmental conditions [11].

Recently we reported on a series of 1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives [12–13] which show high affinity for the OFQ receptor. One of these compounds, (RS)-8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **1**, which represents a combination of 1- and 2-indanyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives [12–13], turned out to be an OFQ receptor full agonist with high affinity and moderate selectivity to the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors.

In this paper we report on the synthesis and pharmacology of 1*H*-phenalen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives **2** and **3a–f** (figure 1). Within this series of compounds we have identified full agonists of the OFQ receptor with high affinity and selectivity versus the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors. The most

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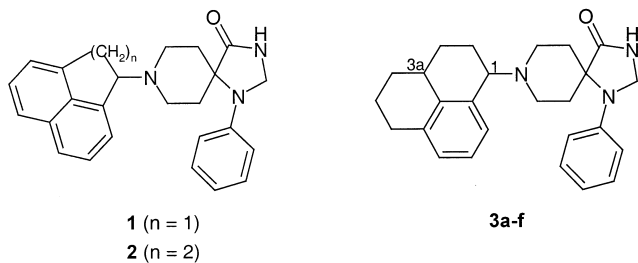


Figure 1. Structures of 1, 2 and 3.

selective compound, (1*S*,3*aS*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **3c**, was evaluated for its behavioural effects in rats with specific attention for effects on emotional reactivity and anxiolytic-like properties in the elevated plus-maze test in comparison to the classical anxiolytic alprazolam.

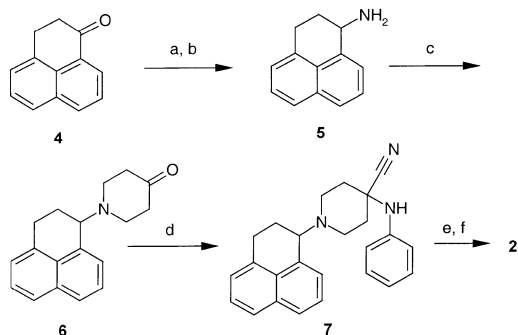
## 2. Chemistry

(*RS*)-8-(2,3-Dihydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **2** was prepared according to figure 2. 2,3-Dihydro-1*H*-phenalen-1-one **4** [14] was converted into the corresponding amine **5** by a two step procedure via the oxime, which was hydrogenated on Ra-Ni. For the introduction of the piperidin-4-one moiety a Hofmann-elimination-Michael-addition sequence [15] was used to give **6**. A Strecker-reaction with aniline and trimethylsilyl cyanide in acetic acid led to the anilino-nitrile **7**, which on treatment with formic acid and acetic acid anhydride and subsequent reaction of the crude product in formamide at 200 °C gave **2**.

The preparation of the 8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one diastereoisomers **3a-f** is shown in figure 3. A Stobbe-type condensation [16–17] of  $\alpha$ -tetralone and diethyl succinate with potassium *tert*-butoxide in *tert*-butanol and subsequent hydrolysis/decarboxylation in acetic acid/HCl yielded the  $\gamma$ ,  $\delta$ -unsaturated acid **9**, that contains 5% of the corresponding 3-(naphthalen-1-yl)-propionic acid as an impurity. The percentage of the impurity formed in the Stobbe-type condensation depends on the reaction time.

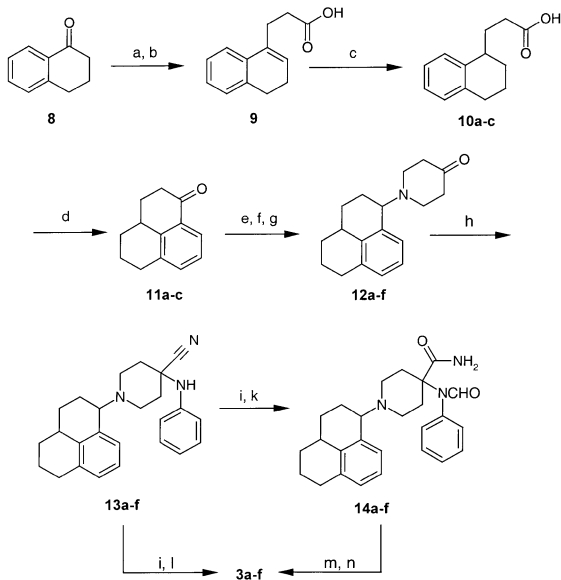
Hydrogenation of **9** with palladium on carbon as catalyst gave the racemic acid **10a**. The asymmetric hydrogenation of **9** in the presence of (*S*)- or (*R*)-6,6'-dimethoxybiphenyl-2,2'-diylbis(diphenylphosphine) (MeOBIPHEP)/Ru(OAc)<sub>2</sub> as catalyst [18] afforded **10b** (*S*) and **10c** (*R*), respectively, with 93–94% ee [19].

The acid **10a** can be transformed into the corresponding (*RS*)-2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-one **11a**



Reagents: a)  $\text{NH}_2\text{OH} \cdot \text{HCl}$ , NaOAc, MeOH-H<sub>2</sub>O-THF, 70 °C. b) Ra-Ni, H<sub>2</sub>, MeOH-NH<sub>3</sub>. c) N-ethyl-N-methyl-4-oxo-piperidinium iodide, K<sub>2</sub>CO<sub>3</sub>, EtOH, reflux. d) aniline, TMSCN, AcOH. e) formic acid, Ac<sub>2</sub>O, RT. f) formamide, 200 °C.

Figure 2. Synthesis of compound 2.



Reagents: a) diethyl succinate, KOtBu, tBuOH, reflux. b) AcOH, HCl, reflux. c) Pd-C, H<sub>2</sub> or (*R*)-/(*S*)-MeOBIPHEP/Ru(OAc)<sub>2</sub>, H<sub>2</sub>, MeOH. d) PPA, 130 °C or TFAA in TFA, RT. e)  $\text{NH}_2\text{OH} \cdot \text{HCl}$ , NaOAc, MeOH-H<sub>2</sub>O-THF, 70 °C. f) Ra-Ni, H<sub>2</sub>, MeOH-NH<sub>3</sub>. g) N-ethyl-N-methyl-4-oxo-piperidinium iodide, K<sub>2</sub>CO<sub>3</sub>, EtOH, reflux. h) aniline, TMSCN, AcOH. i) formic acid, Ac<sub>2</sub>O, RT. j) formic acid, acetic acid, RT. k) formic acid, acetic acid, RT. l) formamide, 200 °C. m) HCl(OEt)<sub>3</sub>, reflux. n) NaBH<sub>4</sub>, MeOH, RT.

Figure 3. Synthesis of compounds 3a-f.

**Table I.** Binding affinities (pKi  $\pm$  SEM) for human OFQ and opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors (number of experiments performed in triplicate) calculated according to Cheng and Prusoff [25].

Compound	pKi $\pm$ SEM			
	OFQ	$\mu$	$\kappa$	$\delta$
<b>1</b> (RS) [12]	9.28	8.23	7.59	6.61
<b>2</b> (RS)	8.95 $\pm$ 0.14 (5)	7.56 $\pm$ 0.18 (3)	7.05 $\pm$ 0.12 (3)	6.02 (2)
<b>3a</b> (1RS,3aRS)	9.29 $\pm$ 0.08 (5)	7.29 $\pm$ 0.09 (4)	6.98 $\pm$ 0.09 (3)	6.20 $\pm$ 0.14 (3)
<b>3b</b> (1RS,3aSR)	8.60 $\pm$ 0.02 (4)	7.47 $\pm$ 0.07 (4)	6.80 $\pm$ 0.08 (3)	6.00 (2)
<b>3c</b> (1S,3aS)	9.41 $\pm$ 0.06 (7)	7.33 $\pm$ 0.09 (7)	7.05 $\pm$ 0.07 (3)	5.86 $\pm$ 0.04 (3)
<b>3d</b> (1R,3aR)	7.91 (2)	7.2 (1)	6.7 (1)	6.2 (1)
<b>3e</b> (1S,3aR)	8.68 $\pm$ 0.11 (4)	7.05 $\pm$ 0.06 (3)	6.27 $\pm$ 0.06 (3)	5.92 $\pm$ 0.23 (3)
<b>3f</b> (1R,3aS)	8.76 $\pm$ 0.08 (4)	7.60 $\pm$ 0.10 (4)	6.84 $\pm$ 0.12 (3)	6.21 $\pm$ 0.05 (3)

with polyphosphoric acid at 130 °C, whereas the enantiomers (S)- and (R)-2,3,3a,4,5,6-hexahydro-1H-phenalen-1-ones **11b–c** [20] were prepared under milder conditions with trifluoroacetic acid anhydride in trifluoroacetic acid at room temperature from the corresponding acids **10b–c** [21–22].

Formation of the amine via the oxime and introduction of the piperidin-4-one moiety as described for the synthesis of **6** led to **12a–f**. No diastereoselectivity could be observed in the hydrogenation step, however, at this step it is possible to separate the diastereoisomers **12a/12b**, **12c/12e** and **12d/12f**, respectively, by column chromatography. The products of the Strecker-reaction are crystalline compounds, thus, at this step of the synthesis the pure diastereoisomers **13a–b** as well as the pure enantiomers **13c–f** were formed by crystallization.

It is possible to prepare **3a–f** from **13a–f** as described for the synthesis of **2**, but the yields are rather low. To get **3a–f** in good yields, it is necessary to prepare **14a–f** by reaction of **13a–f** with formic acid/acetic acid anhydride and subsequently with formic acid/acetic acid. Intramolecular ring-closure in triethyl orthoformate under reflux conditions and reduction of the crude product with NaBH<sub>4</sub> in methanol led to **3a–f**.

The absolute stereochemistry was determined by X-ray crystallography of **10b** [23], thus, the acid obtained by asymmetric hydrogenation of **9** in the presence of (S)-6,6'-dimethoxybiphenyl-2,2'-diylbis(diphenylphosphine) (MeOBIPHEP)/Ru(OAc)<sub>2</sub> as catalyst was crystallized as its (R)-1-phenylethylammonium salt from diethyl ether and turned out to have the (S)-configuration.

The relative stereochemistry was determined by X-ray crystallography of **13b** [24], which was crystallized from diethyl ether. It was found that **13b** is the (1RS,3aSR)-diastereoisomer, thus, the relative and absolute configu-

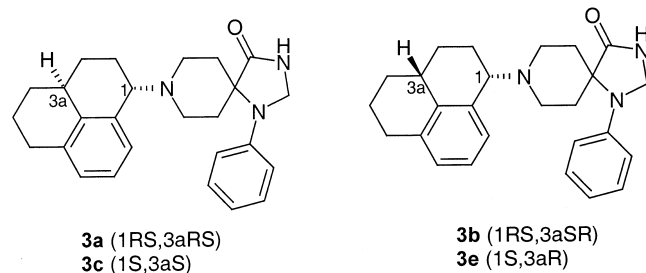
ration of the compounds **10b–c**, **11b–c**, **12c–f**, **13c–f**, **14c–f** and **3c–f**, respectively, could be defined.

### 3. Pharmacology

The affinities (pKi) of the compounds for human OFQ and opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors were determined from competition binding curves using [<sup>3</sup>H]-orphanin FQ and membranes prepared from permanently transfected HEK293 cells expressing hOFQ receptors as well as [<sup>3</sup>H]-naloxone ( $\mu$ ,  $\kappa$  receptors) and [<sup>3</sup>H]-deltorphan II ( $\delta$  receptors) and membranes prepared from BHK cells transiently expressing h $\mu$ , h $\kappa$  and h $\delta$  receptors. The results are shown in *table I*.

From our previous work it is known that a combination of 1- and 2-indanyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives gave the 8-acenaphthenyl-derivative **1**, an OFQ receptor agonist with high affinity, but moderate selectivity versus the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors [12, 13]. The 1H-phenalen-1-yl derivative **2**, a combination of the 1- and 2-tetralinyl series [12, 13] shows a better selectivity for the QFQ receptor compared to **1**. However, a real increase in selectivity was observed by introducing more stereochemical information into the molecule, which led to the 1-(2,3,3a,4,5,6)-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives **3a–f** (*figure 4*).

The two diastereoisomers **3a** (*trans*) and **3b** (*cis*) show a significant difference regarding their affinities for the OFQ receptor, whereas their affinities for the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors is comparable. Diastereoisomer **3a** with the higher affinity for the OFQ receptor is therefore the more selective derivative. The enantiomers of **3a** (**3c** and **3d**), again differ with respect to their affinities for the OFQ receptor ( $\Delta$ pKi = 1.5), whereas in the case of the enantiomers of **3b** (**3e** and **3f**) the affinities for the OFQ receptor



**Figure 4.** Structures of stereoisomers **3a**, **3c** and **3b**, **3e**.

are comparable. As in the case of the diastereoisomers, within the four enantiomers there is no major difference with respect to the affinities for the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors. Among the enantiomers (1S,3aS)-1-(2,3,3a,4,5,6)-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro [4, 5]decan-4-one **3c** shows the highest affinity for the OFQ receptor and therefore the best selectivity versus the opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors.

The compound **3c** was tested for its potency to stimulate  $\text{GTP}\gamma^{35}\text{S}$  binding [26, 27] using membranes prepared from 293s cells transfected with the human OFQ receptor and turned out to be a full agonist (figure 5).

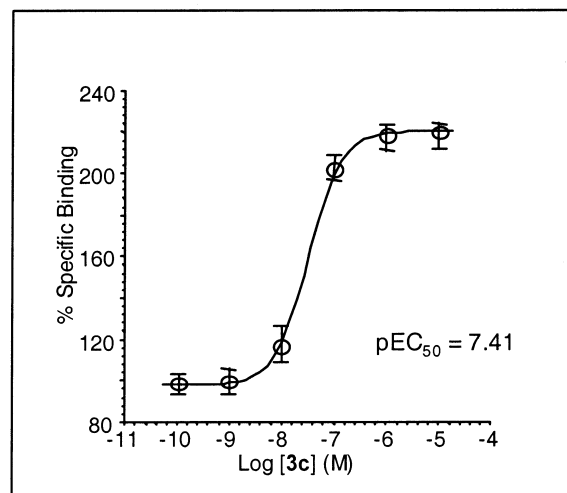
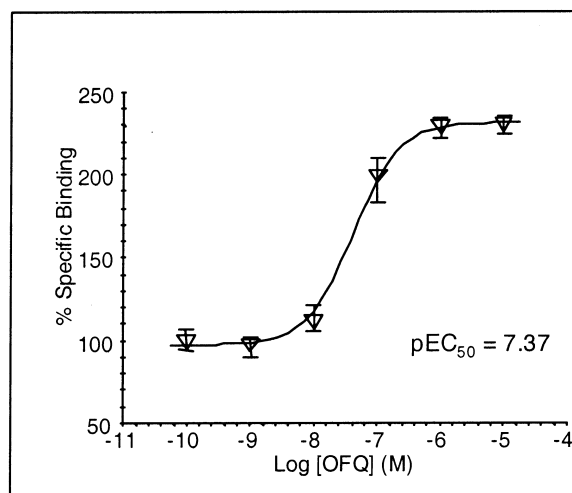
In addition, **3c** was found to have no significant ( $\text{pK}_i < 6.5$ ) affinity for a variety of other receptors like serotonin  $5\text{HT}_{1\text{D}\alpha}$ ,  $5\text{HT}_{2\text{A}}$ ,  $5\text{HT}_{2\text{C}}$ ,  $5\text{HT}_6$ ,  $5\text{HT}_7$ , dopamine D1, D3, D4, CRF1, CRF2 $\alpha$ , benzodiazepine, adenosine A1, A2a, A3, somatostatin, NPY, galanin, canna-

binoid, histamin H1, muscarin,  $\text{CCK}_A$  and central nicotine receptors. The compound was inactive on NE uptake, DA uptake, GABA uptake as well as calcium and potassium channels at concentrations up to  $1\text{ }\mu\text{M}$ , however, it showed some effects on histamine H2, sigma, dopamine D2 receptors and sodium-site 2 channels ( $\text{IC}_{50} \sim 1\text{ }\mu\text{M}$ ).

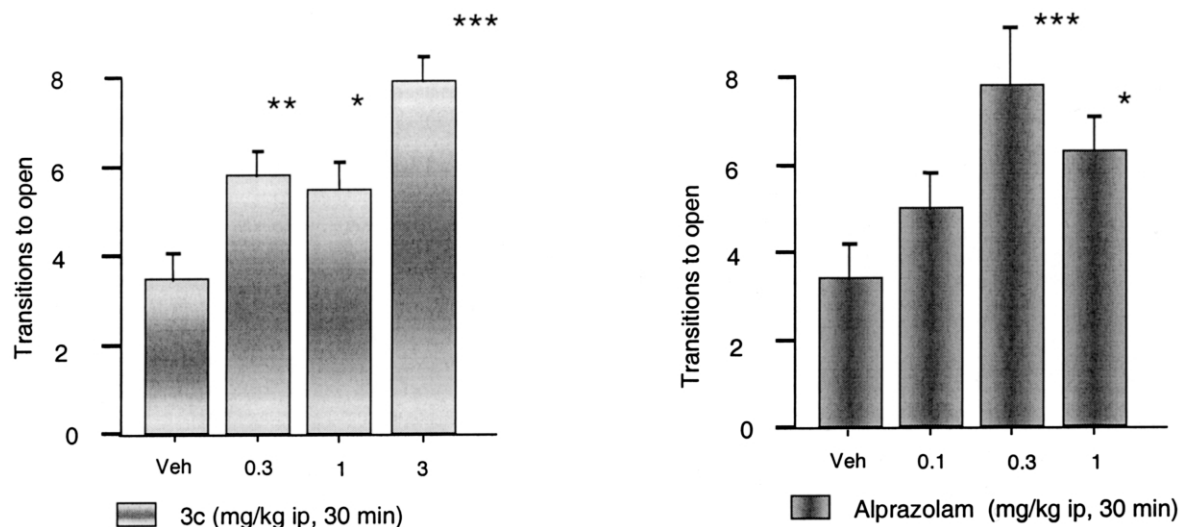
#### 4. Results and discussion

When tested in vivo following intraperitoneal administration in rats (0.3, 1, 3.2 mg/kg versus vehicle), **3c** was found to increase exploration in a novel environment (in a modified open field test, data not shown) and to elicit increased open arm activity in a standardized elevated plus-maze procedure (figure 6, number of transitions from closed to open arms). Increases were specifically observed in the open arm for transitions ( $F_{3,62} = 10.23$ ,  $P = 0.0001$ ), time spent ( $F_{3,62} = 9.82$ ,  $P = 0.0001$ ) and distance moved ( $F_{3,62} = 10.85$ ,  $P = 0.0001$ ) on those arms.

When compared to vehicle, the doses of 1 and 3.2 mg/kg were significantly active on all parameters ( $P < 0.05$  for Bonferroni-Dunn post-hoc test for multiple comparisons). The dose of 0.32 mg/kg was significant on transitions and distance moved on the open arms. No statistically significant effects were detected in closed arm activity ( $F_{3,62} = 0.795$ ,  $P > 0.05$  on transitions from closed to closed, and  $F_{3,62} = 1.106$ ,  $P > 0.05$  on distance moved in the closed arms). Forced motor performance



**Figure 5.** Effects of OFQ and **3c** on stimulation of  $\text{GTP}\gamma^{35}\text{S}$  binding. Membranes prepared from 293s cells transfected with human OFQ receptors were used.



**Figure 6.** Effect of **3c** in comparison to alprazolam in the elevated plus-maze test in rats (30 min pre-treatment for both drugs). This test is based on the natural aversion of rodents for open spaces and uses a maze with two open and two closed arms; the number of entries into open arms are indices of neophobic anxiety in animals. Anxiolytics increase and anxiogenics decrease these measures (see methods section).

also remained intact. Animals of all dose/vehicle groups performed to maximal scores on the traction test and their grip strength non-significantly varied between  $565 \pm 7.6$  g and  $548 \pm 7.9$  g under vehicle and 3.2 mg/kg i.p., respectively.

## 5. Conclusions

Compound **3c** was identified which exhibited high-affinity binding to the human OFQ receptor with good selectivity against the human opioid ( $\mu$ ,  $\kappa$ ,  $\delta$ ) receptors (> 100-fold). The compound was identified as an OFQ receptor full agonist in the  $\text{GTP}\gamma^{35}\text{S}$  assay. In a broad binding evaluation the compound showed no significant affinity for a variety of other receptors.

Following intraperitoneal injection, compound **3c** was found to decrease neophobia in a novel environment and to exhibit dose-dependent anxiolytic-like effects in the elevated plus-maze procedure, thus confirming the effects observed following intracerebroventricular infusion of the OFQ peptide in rat [11]. Increased exploration was very similar to that elicited by benzodiazepines in these novel environments and are interpreted as a decrease in emotional reactivity of animals treated with the drugs. Unlike psychostimulants however, **3c** and alprazolam did not non-specifically increase all parameters of locomotion but rather specifically decreased thymotaxis (wall-seeking tendency). The opioid receptor agonist morphine

was inactive when tested in the elevated plus-maze procedure in a dose range of 1–10 mg/kg i.p. (data not shown).

Agonists at OFQ receptors may offer interesting possibilities for the discovery of innovative anxiolytics and the role of OFQ in fear responses to stress opens novel avenues for exploring the pathophysiology of anxiety disorders. Agonists at OFQ receptors may also prove useful in the treatment of other stress-related psychiatric disorders such as depression or eating disorders. This innovative approach involving a novel peptidergic mechanism of neuromodulatory action may propose interesting possibilities for selective normalization of stress-related neuronal dysfunctions with limited side effects.

## 6. Experimental protocols

### 6.1. Chemistry

Reagent grade solvents were used without further purification. All reactions were performed under Argon. Evaporation means removal of the solvent by use of a Büchi rotary evaporator at 30–40 °C in vacuo. Melting points were determined in capillary tubes (Büchi B-540 apparatus) and are uncorrected. Column chromatography was carried out by using silica gel (230–400 mesh; Merck) and 0.3–1 bar pressure. Spectra were recorded with the following instruments:  $^1\text{H}$ -NMR ( $\delta$  values in

ppm relative to internal TMS): Bruker AS-250 (250 MHz) and WM-400 (400 MHz). MS: MS 9 updated with a Finnigan MAT data system SS 300. Microanalysis (C, H, N) were performed on a Heraeus Vario EL. Starting materials were high-grade commercial products unless stated otherwise.

**6.1.1. Preparation of (RS)-8-(2,3-dihydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **2****

**6.1.1.1. (RS)-2,3-Dihydro-1H-phenalen-1-ylamine **5****

A mixture of 2,3-dihydro-1H-phenalen-1-one **4** [14] (2.83 g, 15.5 mmol), hydroxylamine hydrochloride (1.73 g, 24.9 mmol) and water (14 mL) was stirred at 70 °C. Methanol (21 mL), THF (4.7 mL) and a solution of sodium acetate (3.19 g, 38.9 mmol) in water (9.4 mL) was added and stirring was continued for 85 min at 70 °C. Water (30 mL) was added, the mixture was stirred for 2 h at room temperature, the crude product was collected by filtration and subsequently hydrogenated on Ra/Ni in MeOH-NH<sub>3</sub> (200 mL, 3.5 N) over a period of 20 h at room temperature. The catalyst was filtered off, and the solution was evaporated to give **5** (2.47 g, 87%) as a light brown oil: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.70 (s, 2 H), 1.91–2.04 (m, 1 H), 2.17–2.28 (m, 1 H), 3.03–3.38 (m, 2 H), 4.28 (m<sub>c</sub>, 1 H), 7.26 (dd, *J* = 8.5, 1.5 Hz, 1 H), 7.35–7.54 (m, 3 H), 7.69 (dd, *J* = 8.5, 1.5 Hz, 2 H), 7.73 (dd, *J* = 8.5, 1.5 Hz, 1 H); MS (EI) *m/z* 183 (M<sup>+</sup>), 165 (100).

**6.1.1.2. (RS)-1-(2,3-Dihydro-1H-phenalen-1-yl)-piperidin-4-one **6****

To a stirred and boiling mixture of (RS)-2,3-dihydro-1H-phenalen-1-ylamine **5** (2.47 g, 13.5 mmol), potassium carbonate (1.86 g, 13.5 mmol) and ethanol (30 mL) was added dropwise over a period of 10 min a solution of N-ethyl-N-methyl-4-oxo-piperidinium iodide (5.0 g, 18.6 mmol) in water (9 mL). The reaction mixture was refluxed for 2 h, subsequently poured into water (200 mL) and 3 N NaOH solution was added (60 mL). The mixture was extracted with ethyl acetate (2 × 200 mL), the combined organic layers were washed with brine (150 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give 4.51 g of a dark brown oil, which was further purified by column chromatography (toluene/ethyl acetate, 5:1) to yield **6** (3.03 g, 84%) as a light orange oil: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.93 (ddd, *J* = 18.5, 12, 5 Hz, 1 H), 2.22–2.34 (m, 1 H), 2.53 (t, *J* = 6.5 Hz, 4 H), 2.76–2.91 (m, 2 H), 3.01–3.19 (m, 3 H), 3.29 (dt, *J* = 18.5, 5 Hz, 1 H), 4.25 (dd, *J* = 12, 5 Hz, 1 H), 7.24 (d, *J* = 7.5 Hz, 1 H), 7.38 (t, *J* = 7.5 Hz, 1 H), 7.48 (t, *J* = 7.5 Hz, 1 H), 7.70 (d, *J* = 7.5 Hz, 1 H),

7.75 (d, *J* = 7.5 Hz, 1 H), 7.84 (d, *J* = 7.5 Hz, 1 H); MS (EI) *m/z* 265 (M<sup>+</sup>), 165 (100), 152 (28), 100 (39).

**6.1.1.3. (RS)-1-(2,3-Dihydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **7****

To a stirred solution of (RS)-1-(2,3-dihydro-1H-phenalen-1-yl)-piperidin-4-one **6** (3.03 g, 11.4 mmol) in acetic acid (11 mL) was added at 0 °C aniline (1.20 mL, 12.6 mmol) and afterwards trimethylsilyl cyanide (1.43 mL, 11.4 mmol). The reaction mixture was stirred at room temperature for 4 h, poured into ice-water (150 mL) and ammonium hydroxide solution (80 mL) and extracted with dichloromethane (2 × 100 mL). The combined organic layers were washed with brine (80 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give **7** (4.06 g, 96%) as a light brown oil: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.88–2.17 (m, 3 H), 2.21–2.69 (m, 4 H), 2.80–3.19 (m, 4 H), 3.29 (m<sub>c</sub>, 1 H), 3.68 (s, 1 H), 4.12 (m<sub>c</sub>, 1 H), 6.88–6.96 (m, 3 H), 7.18–7.32 (m, 3 H), 7.33–7.50 (m, 2 H), 7.63–7.79 (m, 3 H); MS (FAB) *m/z* 368.2 (M + H<sup>+</sup>).

**6.1.1.4. (RS)-8-(2,3-Dihydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **2****

To a stirred solution of (RS)-1-(2,3-dihydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **6** (4.06 g, 11.0 mmol) in formic acid (24 mL) was added at 0 °C acetic acid anhydride (24 mL). The reaction mixture was stirred at room temperature for 41 h, poured into ice-sat. sodium hydrogen carbonate solution (400 mL) and extracted with dichloromethane (4 × 200 mL). The combined organic layers were washed with brine (300 mL), dried (MgSO<sub>4</sub>) and evaporated to give a brown oil, which was dissolved in formamide (50 mL) and heated at 200 °C for 2 h. The reaction mixture was poured into water (200 mL) and sat. sodium hydrogen carbonate solution (200 mL) was added. The crude product was collected by filtration, dissolved in dichloromethane (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give a brown foam (3.40 g), which was purified by column chromatography (1. dichloromethane/MeOH/NH<sub>4</sub>OH, 99:1:0.1 and 2. ethyl acetate) to yield, after formation of the hydrochloride from MeOH/HCl (15 mL) and diethyl ether (100 mL), **2** (0.53 g, 11%) as a pale brown solid: m.p. 251 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.76–1.98 (m, 2 H), 2.12–2.31 (m, 1 H), 2.72–3.09 (m, 3 H), 3.11–3.25 (m, 1 H), 3.27–3.48 (m, 2 H), 3.50–3.63 (m, 1 H), 3.75–3.99 (m, 2 H), 4.60 (s, 2 H), 4.94 (s, br, 1 H), 6.76 (t, *J* = 8 Hz, 1 H), 7.03 (d, *J* = 8 Hz, 2 H), 7.19 (t, *J* = 8 Hz, 2 H), 7.47–7.64 (m, 3 H), 7.89 (t, *J* = 7.5 Hz, 2 H), 8.05 (d, *J* = 7.5 Hz, 1 H), 9.05 (s, 1 H), 10.27 (s, br, 1 H); MS (FAB) *m/z* 398.3 (M + H<sup>+</sup>). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.2. Preparation of (1RS,3aRS)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride 3a**

**6.1.2.1. 3-(3,4-Dihydro-naphthalen-1-yl)-propionic acid 9**

A stirred mixture of  $\alpha$ -tetralone **8** (36.4 mL, 274 mmol), diethyl succinate (38.4 mL, 229 mmol), potassium *tert* butylate (46.9 g, 418 mmol) and *tert* butanol (200 mL) was heated under reflux conditions for 45 min, evaporated, poured into hydrochloric acid solution (8 N, 80 mL) and extracted with diethyl ether (2  $\times$  200 mL). The combined organic layers were washed with brine (150 mL), dried (MgSO<sub>4</sub>) and evaporated to give a brown oil (86.3 g), which was subsequently heated in a mixture of concentrated hydrochloric acid (200 mL) and acetic acid (200 mL) under reflux conditions for 3 h. The reaction mixture was evaporated, poured into water (500 mL) and extracted with diethyl ether (2  $\times$  400 mL). The combined organic layers were washed with sodium hydroxide solution (3 N, 120 mL) and water (2  $\times$  200 mL). The combined water phases were acidified with sulfuric acid (3 N, 220 mL), extracted with diethyl ether (2  $\times$  150 mL), the combined organic layers washed with water (2  $\times$  150 mL) and brine (150 mL), dried (MgSO<sub>4</sub>) and evaporated to give the crude product as a brown solid (39.2 g). Crystallization from ethyl acetate/hexane yielded **9** (10.5 g, 23%) as an off-white solid, that contains 5% of 3-(naphthalen-1-yl)-propionic acid as an impurity, and 28.4 g (61%) of crude product as a brown oil: m.p. 107 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.19–2.34 (m, 2 H), 2.58–2.67 (m, 2 H), 2.68–2.91 (m, 4 H), 5.91 (t, *J* = 4.5 Hz, 1 H), 7.08–7.29 (m, 4 H); MS (EI) *m/z* 202 (M<sup>+</sup>), 142 (70), 129 (100). Anal. (C<sub>13</sub>H<sub>14</sub>O<sub>2</sub>) C, H.

**6.1.2.2. (RS)-3-(1,2,3,4-Tetrahydro-naphthalen-1-yl)-propionic acid 10a**

3-(3,4-Dihydro-naphthalen-1-yl)-propionic acid **9** (28.4 g, 0.14 mol, crude product from B1) was hydrogenated on palladium (2.84 g, 5% on carbon) in methanol (250 mL) for 40 h at room temperature. The catalyst was filtered off and the solution was evaporated to give the crude product as a brown oil (25.7 g), which was purified by column chromatography (toluene/ethyl acetate, 3:1) to yield **10a** (21.4 g, 74%) as a white solid: m.p. 78 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.55–1.99 (m, 5 H), 2.01–2.19 (m, 1 H), 2.37–2.51 (m, 2 H), 2.64–2.92 (m, 3 H), 7.01–7.23 (m, 4 H); MS (EI) *m/z* 204 (M<sup>+</sup>), 144 (58), 131 (100). Anal. (C<sub>13</sub>H<sub>16</sub>O<sub>2</sub>) C, H.

**6.1.2.3. (RS)-2,3,3a,4,5,6-Hexahydro-phenalen-1-one 11a**

A stirred mixture of (RS)-3-(1,2,3,4-tetrahydro-naphthalen-1-yl)-propionic acid **10a** (21.4 g, 0.10 mol)

and polyphosphoric acid (220 g) was kept at 130 °C over a period of 1 h, subsequently poured into ice-water (1 L) and extracted with ethyl acetate (3  $\times$  1 L). The combined organic layers were washed with water (1 L), sat. sodium hydrogen carbonate solution (1 L), water (1 L) and brine (1 L), dried (MgSO<sub>4</sub>) and evaporated to give the crude product as a brown solid. Purification by column chromatography (ethyl acetate/hexane, 1:4) yielded **11a** (17.6 g, 94%) as brown oil, which crystallized upon standing: <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.01–1.22 (m, 1 H), 1.38–1.49 (m, 2 H), 2.20–2.39 (m, 1 H), 2.41–2.64 (m, 4 H), 6.94 (t, *J* = 7.5 Hz, 1 H), 7.01 (dd, *J* = 7.5, 1.5 Hz, 1 H), 7.59 (dd, *J* = 7.5, 1.5 Hz, 1 H); MS (EI) *m/z* 186 (M<sup>+</sup>), 158 (37), 144 (100), 129 (48), 115 (28).

**6.1.2.4. (1RS,3aRS)-1-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-piperidin-4-one 12a and (1RS,3aSR)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidin-4-one 12b**

The mixture of (1RS,3aRS)- and (1RS,3aSR)-2,3,3a,4,5,6-hexahydro-1H-phenalen-1-ylamine (10.5 g, 84% of a light yellow oil) was prepared by reaction of (RS)-2,3,3a,4,5,6-hexahydro-phenalen-1-one **11a** (12.5 g, 67.1 mmol) with hydroxylamine hydrochloride (7.90 g, 115 mmol) and subsequent hydrogenation of the oxime according to the procedure described for **5** (6.1.1.1.): <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.19–2.35 (m, 10 H), 2.44–2.93 (m, 3 H), 3.91–4.07 (m, 1 H), 6.91–7.38 (m, 3 H); MS (EI) *m/z* 187 (M<sup>+</sup>), 170 (100), 158 (27), 142 (40), 12 (17), 115 (16).

Reaction of a mixture of (1RS,3aRS)- and (1RS,3aSR)-2,3,3a,4,5,6-hexahydro-1H-phenalen-1-ylamine (10.5 g, 54.2 mmol) and N-ethyl-N-methyl-4-oxo-piperidinium iodide (20.0 g, 74.7 mmol) as described for **6** (6.1.1.2.) gave, after purification and separation of the diastereoisomers by column chromatography (ethyl acetate/toluene, 1:4), a mixture of **12a–b** (2.47 g, 17%) as a pale yellow oil, **12a** (3.65 g, 25%) as a colourless oil and **12b** (5.35 g, 36%) as a colourless oil: <sup>1</sup>H-NMR (CDCl<sub>3</sub>, **12a**)  $\delta$  1.19–1.51 (m, 2 H), 1.62–2.05 (m, 6 H), 2.34–2.64 (m, 5 H), 2.71–2.98 (m, 6 H), 4.09 (dd, *J* = 9.5, 5.5 Hz, 1 H), 6.98 (d, *J* = 7.5 Hz, 1 H), 7.12 (t, *J* = 7.5 Hz, 1 H), 7.60 (d, *J* = 7.5 Hz, 1 H); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, **12b**)  $\delta$  1.19–1.42 (m, 2 H), 1.62–2.08 (m, 6 H), 2.33–2.56 (m, 5 H), 2.71–2.99 (m, 6 H), 3.92 (dd, *J* = 6.5, 4.5 Hz, 1 H), 6.99 (d, *J* = 7.5 Hz, 1 H), 7.14 (t, *J* = 7.5 Hz, 1 H), 7.53 (d, *J* = 7.5 Hz, 1 H); MS (FAB) *m/z* 270.3 (M + H<sup>+</sup>).

**6.1.2.5. (1RS,3aRS)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidine-4-carbonitrile **13a****

Reaction of (1RS,3aRS)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidin-4-one **12a** (1.50 g, 5.57 mmol), aniline (0.56 mL) and trimethylsilyl cyanide (0.69 mL, 5.57 mmol) as described for **7** (6.1.1.3.) yielded **13a** (2.03 g, 98%) as a colourless oil, which crystallized upon standing: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.21–1.47 (m, 2 H), 1.69–2.11 (m, 8 H), 2.27 (m<sub>c</sub>, 1 H), 2.38 (m<sub>c</sub>, 1 H), 2.48–2.99 (m, 7 H), 3.64 (s, 1 H), 3.91 (dd, *J* = 9.5, 5.5 Hz, 1 H), 6.89–6.98 (m, 4 H), 7.09 (t, *J* = 7.5 Hz, 1 H), 7.21–7.29 (m, 2 H), 7.47 (d, *J* = 7.5 Hz, 1 H); MS (FAB) *m/z* 372.2 (M + H<sup>+</sup>).

**6.1.2.6. (1RS,3aRS)-4-(Formyl-phenylamino)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidine-4-carboxylic acid amide **14a****

To a stirred solution of (1RS,3aRS)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13a** (2.03 g, 5.46 mmol) in formic acid (20 mL) was added, dropwise at 0 °C over a period of 10 min, acetic acid anhydride (20 mL). The reaction mixture was stirred at room temperature for 3 h, evaporated and the crude product dissolved in formic acid (20 mL). Subsequently acetic acid (2 mL) was added, the solution was stirred at room temperature for 16 h, evaporated, poured into ice-sat. sodium hydrogen carbonate solution (70 mL) and extracted with dichloromethane (2 × 100 mL). The combined organic layers were washed with brine (70 mL) dried (MgSO<sub>4</sub>) and evaporated to give the crude product as a light brown oil, which was further purified by column chromatography (dichloromethane/methanol, 19:1) to give **14a** (2.15 g, 94%) as a pale yellow foam: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.15–1.39 (m, 2 H), 1.59–2.11 (m, 8 H), 2.27–2.65 (m, 7 H), 2.78 (dd, *J* = 7.5, 5.5 Hz, 2 H), 3.80 (dd, *J* = 9.5, 5.5 Hz, 1 H), 5.51 (br, 1 H), 6.92 (d, *J* = 7.5 Hz, 1 H), 7.04 (t, *J* = 7.5 Hz, 1 H), 7.15 (br, 1 H), 7.21–7.46 (m, 6 H), 8.24 (s, 1 H); MS (FAB) *m/z* 418.3 (M + H<sup>+</sup>).

**6.1.2.7. (1RS,3aRS)-8-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3a****

A stirred solution of (1RS,3aRS)-4-(formyl-phenylamino)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidine-4-carboxylic acid amide **14a** (2.15 g, 5.15 mmol) in triethyl orthoformate (70 mL) was heated under reflux conditions for 4 days, evaporated and dissolved in methanol (60 mL) and THF (30 mL). To the stirred solution was added, at room temperature, sodium boronhydride (0.39 g, 10.3 mmol) and the solution was

stirred for 3 h. The reaction mixture was evaporated, poured into ice-sodium hydroxide solution (3 N, 40 mL) and extracted with dichloromethane (2 × 80 mL). The combined organic layers were washed with brine (70 mL), dried (MgSO<sub>4</sub>) and evaporated to give a light brown foam. Further purification by column chromatography (1. dichloromethane/methanol/ammonium hydroxide, 150:10:1 and 2. ethyl acetate) and subsequent formation of the hydrochloride with methanol/hydrochloric acid (3 N, 5 mL) and diethyl ether (100 mL) yielded **3a** (1.08 g, 48%) as a light brown solid: m.p. 250 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.05–1.34 (m, 2 H), 1.59–2.04 (m, 7 H), 2.25–2.46 (m, 1 H), 2.62 (m<sub>c</sub>, 1 H), 2.71–2.93 (m, 4 H), 3.19–3.49 (m, 2 H), 3.61–3.97 (m, 2 H), 4.60 (m<sub>c</sub>, 2 H), 4.99 (m<sub>c</sub>, 1 H), 6.79 (t, *J* = 7.0 Hz, 1 H), 7.04–7.29 (m, 6 H), 7.90 (d, *J* = 7.0 Hz, 1 H), 9.01 (s, 1 H), 10.48 (s, br, 1 H); MS (FAB) *m/z* 402.3 (M + H<sup>+</sup>). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.3. Preparation of (1RS,3aSR)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3b****

**6.1.3.1. (1RS,3aSR)-1-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13b****

Reaction of (1RS,3aSR)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidin-4-one **12b** (1.50 g, 5.57 mmol), aniline (0.56 mL) and trimethylsilyl cyanide (0.69 mL, 5.57 mmol) as described for **7** (6.1.1.3.) yielded **13b** (1.92 g, 93%) as a light yellow oil, which crystallized upon standing: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.17–1.41 (m, 2 H), 1.64–2.15 (m, 8 H), 2.29 (m<sub>c</sub>, 1 H), 2.31–2.53 (m, 2 H), 2.55 (m<sub>c</sub>, 1 H), 2.63–2.98 (m, 5 H), 3.62 (s, 1 H), 3.79 (dd, *J* = 6.5, 4.5 Hz, 1 H), 6.85–7.02 (m, 4 H), 7.09 (t, *J* = 7.5 Hz, 1 H), 7.21–7.29 (m, 2 H), 7.41 (d, *J* = 7.5 Hz, 1 H); MS (FAB) *m/z* 372.2 (M + H<sup>+</sup>).

**6.1.3.2. (1RS,3aSR)-4-(Formyl-phenylamino)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-piperidine-4-carboxylic acid amide **14b****

Reaction of (1RS,3aSR)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13b** (1.92 g, 5.17 mmol) as described for **14a** (6.1.2.6.) gave **14b** (2.03 g, 94%) as a white foam: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.13–1.38 (m, 2 H), 1.59–2.14 (m, 8 H), 2.32–2.59 (m, 6 H), 2.64 (m<sub>c</sub>, 1 H), 2.88 (dd, *J* = 7.5, 5.5 Hz, 2 H), 3.65 (dd, *J* = 6.5, 4.5 Hz, 1 H), 5.51 (br, 1 H), 6.94 (d, *J* = 7.5 Hz, 1 H), 7.08 (t, *J* = 7.5 Hz, 1 H), 7.15 (br, 1 H), 7.24–7.46 (m, 6 H), 8.24 (s, 1 H); MS (FAB) *m/z* 418.3 (M + H<sup>+</sup>).



**6.1.3.3. (1*R*S,3*a**S*)-8-(2,3,3*a*,4,5,6-Hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3b****

Transformation of (1*R*S,3*a**S*)-4-(formyl-phenyl-amino)-1-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-piperidine-4-carboxylic acid amide **14b** (2.03 g, 4.86 mmol) as described for **3a** (6.1.2.7.) yielded **3b** (0.98 g, 46%) as a white solid: m.p. 233 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.33–1.64 (m, 2 H), 1.72–2.19 (m, 7 H), 2.37–2.64 (m, 2 H), 2.80 (t, *J* = 6.0 Hz, 2 H), 2.92 (m, 1 H), 3.01–3.43 (m, 3 H), 3.73–4.02 (m, 2 H), 4.62–4.73 (m, 3 H), 6.78 (t, *J* = 7.0 Hz, 1 H), 7.07–7.29 (m, 6 H), 7.80 (d, *J* = 7.0 Hz, 1 H), 9.04 (s, 1 H), 10.41 (s, br, 1 H); MS (FAB) *m/z* 402.3 (M + H<sup>+</sup>). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.4. Preparation of (1*S*,3*a**S*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3c****

**6.1.4.1. (S)-3-(1,2,3,4-Tetrahydro-naphthalen-1-yl)-propionic acid **10b****

In a glove box (argon, < 1 ppm oxygen) a 185 mL stainless steel autoclave was charged with **9** (18.0 g, 89.0 mmol, containing 5% of the 3-(naphthalen-1-yl)-propionic acid as an impurity), a solution of (S)-MeOBIPHEP/Ru(OAc)<sub>2</sub> (713 mg, 0.89 mmol) in methanol (128 mL) and triethylamine (1.80 g, 17.8 mmol). After having been connected to a hydrogen source (99.9999% purity) the argon was replaced with hydrogen and then the hydrogenation was run at 20 °C under a constant pressure of 100 bar. After 114 h the conversion was complete. The dark hydrogenation mixture was evaporated, the residue was dissolved in sodium hydroxide solution (0.5 N, 200 mL) and extracted with dichloromethane (3 × 100 mL). Addition of hydrochloric acid 37% (ca. 250 mmol) to the aqueous phase until the pH was ca. 1 led to separation of **10b** as a dark oil, which was extracted into dichloromethane (3 × 100 mL). This organic phase was washed with water (2 × 100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to afford **10b** (17.8 g, 99%) as a light grey oil (94% ee) [19], which crystallized upon standing: [α]<sub>D</sub><sup>20</sup> = +6.75° (c = 0.32, CHCl<sub>3</sub>). Anal. (C<sub>13</sub>H<sub>16</sub>O<sub>2</sub>) C, H.

**6.1.4.2. (S)-2,3,3*a*,4,5,6-Hexahydro-phenalen-1-one **11b****

To a stirred solution of (S)-3-(1,2,3,4-tetrahydro-naphthalen-1-yl)-propionic acid **10b** (3.10 g, 15.2 mmol) in trifluoroacetic acid (30 mL) was added at 0 °C trifluoroacetic acid anhydride (3 mL). The reaction mixture was stirred at room temperature for 75 min, evaporated and the crude product purified by column chromatography

(hexane/ethyl acetate, 5:1) to yield **11b** (2.36 g, 84%) as a white solid: m.p. 59 °C; [α]<sub>D</sub><sup>20</sup> = –34.5° (c = 0.21, CHCl<sub>3</sub>).

**6.1.4.3. (1*S*,3*a**S*)-1-(2,3,3*a*,4,5,6-Hexahydro-1*H*-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13c****

Transformation of (S)-2,3,3*a*,4,5,6-hexahydro-phenalen-1-one **11b** as described for **13a** (6.1.2.4.–6.1.2.5.) gave, after crystallization from diethyl ether/hexane, **13c** as a light yellow solid (ee > 99%, HPLC): m.p. 152 °C; [α]<sub>D</sub><sup>20</sup> = +28.4° (c = 0.26, CHCl<sub>3</sub>).

**6.1.4.4. (1*S*,3*a**S*)-8-(2,3,3*a*,4,5,6-Hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3c****

Transformation of (1*S*,3*a**S*)-1-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13c** as described for **3a** (6.1.2.6.–6.1.2.7.) yielded **3c** as an off-white solid (ee > 98%, HPLC): m.p. = 262 °C, [α]<sub>D</sub><sup>20</sup> = –98.5° (c = 0.11, MeOH). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.5. Preparation of (1*R*,3*a**R*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3d****

**6.1.5.1. (R)-3-(1,2,3,4-Tetrahydro-naphthalen-1-yl)-propionic acid **10c****

The asymmetric hydrogenation of **9** (4.04 g, 20.0 mmol) in methanol (46 mL) in the presence of (R)-MeOBIPHEP/Ru(OAc)<sub>2</sub> (160 mg, 0.20 mmol) as catalyst under the same conditions as described for **10b** afforded **10c** (3.7 g, 92%) as a light grey oil (93% ee) [19], which crystallized upon standing: [α]<sub>D</sub><sup>20</sup> = –6.43° (c = 0.26, CHCl<sub>3</sub>). Anal. (C<sub>13</sub>H<sub>16</sub>O<sub>2</sub>) C, H.

**6.1.5.2. (R)-2,3,3*a*,4,5,6-Hexahydro-phenalen-1-one **11c****

Reaction of (R)-3-(1,2,3,4-tetrahydro-naphthalen-1-yl)-propionic acid **10c** (3.64 g, 17.8 mmol) as described for **11b** (6.1.4.2.) gave **11c** (3.13 g, 94%) as a light orange solid: m.p. 57 °C; [α]<sub>D</sub><sup>20</sup> = +35.1° (c = 0.30, CHCl<sub>3</sub>).

**6.1.5.3. (1*R*,3*a**R*)-1-(2,3,3*a*,4,5,6-Hexahydro-1*H*-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13d****

Transformation of (R)-2,3,3*a*,4,5,6-hexahydro-phenalen-1-one **11c** as described for **13a** (6.1.2.4.–6.1.2.5.) gave, after crystallization, from diethyl ether/hexane **13d** as a light yellow solid: m.p. 151 °C; [α]<sub>D</sub><sup>20</sup> = +29.2° (c = 0.31, CHCl<sub>3</sub>).

**6.1.5.4. (1R,3aR)-8-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3d****

Transformation of (1R,3aR)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13d** as described for **3a** (6.1.2.6.–6.1.2.7.) yielded **3d** as a light brown solid: m.p. = 253 °C,  $[\alpha]_D^{20} = +94.6^\circ$  (c = 0.10, MeOH). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.6. Preparation of (1S,3aR)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3e****

**6.1.6.1. (1S,3aR)-1-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13e****

Transformation of (R)-2,3,3a,4,5,6-hexahydro-phenalen-1-one **11c** as described for **13a** (6.1.2.4.–6.1.2.5.) gave, after crystallization from diethyl ether/hexane, **13e** as a white solid: m.p. 142 °C;  $[\alpha]_D^{20} = +50.4^\circ$  (c = 0.28, CHCl<sub>3</sub>).

**6.1.6.2. (1S,3aR)-8-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3e****

Transformation of (1S,3aR)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13e** as described for **3a** (6.1.2.6.–6.1.2.7.) yielded **3e** as an off-white solid: m.p. = 239 °C,  $[\alpha]_D^{20} = -60.7^\circ$  (c = 0.10, MeOH). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.7. Preparation of (1R,3aS)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3f****

**6.1.7.1. (1R,3aS)-1-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13f****

Transformation of (S)-2,3,3a,4,5,6-hexahydro-phenalen-1-one **11b** as described for **13a** (6.1.2.4.–6.1.2.5.) gave, after crystallization from diethyl ether/hexane, **13f** as a white solid (ee > 99%, HPLC): m.p. 141 °C;  $[\alpha]_D^{20} = -50.4^\circ$  (c = 0.27, CHCl<sub>3</sub>).

**6.1.7.2. (1R,3aS)-8-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride **3f****

Transformation of (1R,3aS)-1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile **13f** as described for **3a** (6.1.2.6.–6.1.2.7.) yielded **3f** as an off-white solid: m.p. = 240 °C,

$[\alpha]_D^{20} = +58.8^\circ$  (c = 0.10, MeOH). Anal. (C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**6.1.8. X-ray crystallography**

**6.1.8.1. Crystal data for **10b** (figure 7)**

(S)-3-(1,2,3,4-Tetrahydro-naphthalen-1-yl)-propionic acid as (R)-(+)-1-phenylethylammonium salt: C<sub>21</sub>H<sub>27</sub>NO<sub>2</sub> [C<sub>13</sub>H<sub>16</sub>O<sub>2</sub> (1:1) C<sub>8</sub>H<sub>11</sub>N], M = 325.44, monoclinic, space group P2(1), a = 11.992 (3) Å, b = 6.198 (2) Å, c = 12.709 (3) Å, β = 101.50 (5)°, T = 180 K, Z = 2; 1 612 unique reflections measured on a Siemens P3 diffractometer with graphite-monochromated Mo-Kα radiation, which were used in all calculations, structure solved by direct methods and expanded using Fourier techniques and the program SHELX-97 [28]. All non-hydrogen atoms were refined anisotropically using full matrix least-squares refinement without restraints, final R-factor 0.0803 and wR-factor 0.1490 (all data). The structure has been deposited at the Cambridge Crystallographic Data Centre, deposition number CCDC 133359.

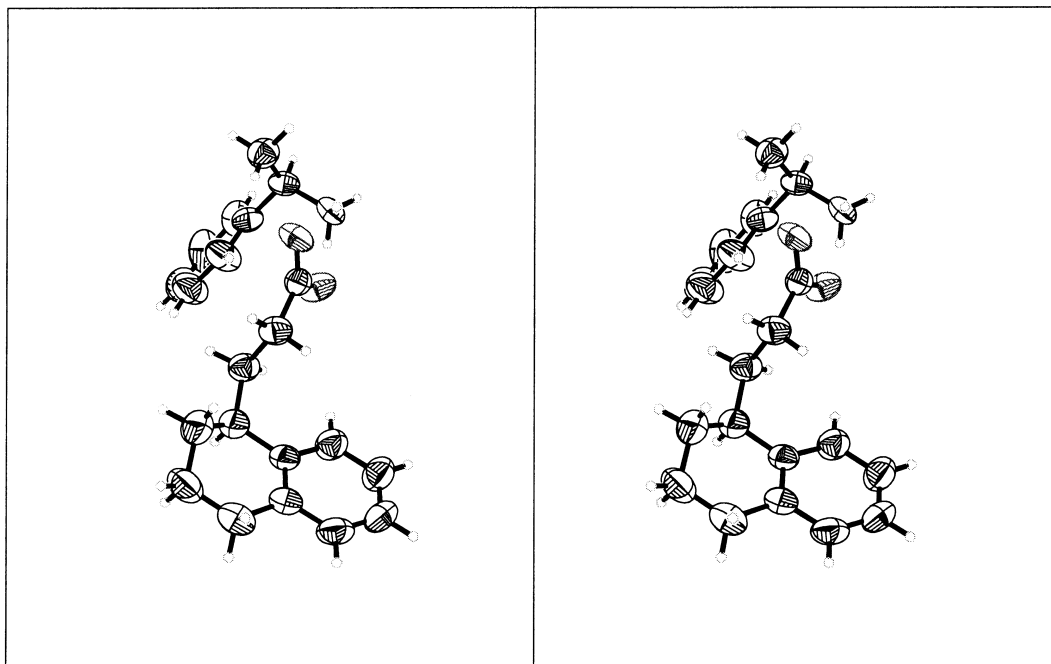
**6.1.8.2. Crystal data for **13b** (figure 8)**

(1R,3aSR)-1-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-4-phenylamino-piperidine-4-carbonitrile: C<sub>25</sub>H<sub>29</sub>N<sub>3</sub>, M = 371.51, monoclinic, space group P2(1)/c, a = 18.419 (4) Å, b = 8.3060 (17) Å, c = 14.076 (3) Å, β = 111.18 (3)°, T = 180 K, Z = 4; 3 246 reflections measured on a Siemens P3 diffractometer with graphite-monochromated Mo-Kα radiation, 2 610 unique (Rint = 0.0284) which were used in all calculations, structure solved by direct methods and expanded using Fourier techniques and the program SHELX-97 [28]. All non-hydrogen atoms were refined anisotropically using full matrix least-squares refinement without restraints, final R-factor 0.1087 and wR-factor 0.1387 (all data). The structure has been deposited at the Cambridge Crystallographic Data Centre, deposition number CCDC 133360.

**6.2. Pharmacology**

**6.2.1. Cell culture and membrane preparation**

The cDNAs encoding human μ opioid receptor (hμR), human κ opioid receptor (hκR) and human δ opioid receptor (hδR) were subcloned into the pSFV2gen vector and recombinant Semliki Forest Virus stocks were generated as described previously [29]. Baby hamster kidney (BHK) cells were infected and harvested for membrane preparation 24 h after infection. The cDNA encoding human OFQ receptor (hOFQR) was inserted into the pcDNA3 vector and stably transfected into human embryonic kidney 293 (HEK293) cells. One cell clone was selected for pharmacological characterization.



**Figure 7.** Crystal structure of **10b**.

Membrane fractions were prepared by homogenization of cells followed by a 30 min centrifugation at 39 000 g using a Beckman JA-20 rotor. The resulting membrane pellet was suspended in 50 mM Tris, pH 7.8, 1 mM EDTA, 6 mM MgCl<sub>2</sub> buffer at a concentration of  $\sim 2 \times 10^7$  cells/mL and frozen at  $-80^\circ\text{C}$ .

#### 6.2.2. Radioligands

The radioligands [leucyl-<sup>3</sup>H]-OFQ (specific activity 150 Ci/mmol), [N-allyl-2-3-<sup>3</sup>H]-naloxone (specific activity 54.5 Ci/mmol) and [Ile<sup>5,6</sup>-<sup>3</sup>H]-deltorphin II {[<sup>3</sup>H]Tyr-D-Ala-[<sup>3</sup>H]Phe-Glu-Ile-Ile-Gly-NH<sub>2</sub>} (specific activity 72 Ci/mmol) were purchased from Amersham (Little Chalfont, UK).

#### 6.2.3. Radioligand binding assays

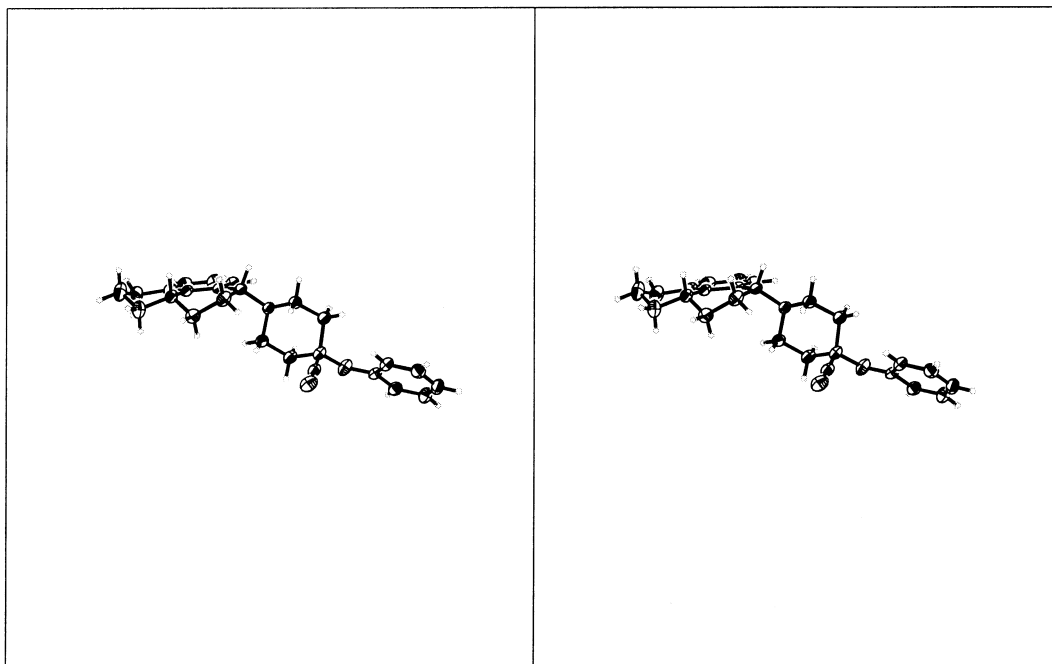
Competitive binding displacement analysis was performed with membranes prepared from permanently transfected HEK293 cells expressing hOFQR (20 µg membrane protein) and 0.1 nM [leucyl-<sup>3</sup>H]-OFQ. Competitive binding displacement analyses for opioid receptors were performed with membranes prepared from BHK cells transiently expressing hµR, hκR or hδR (10 µg membrane protein each) and 1.5 nM (hµR) and 3 nM (hκR) [N-allyl-2-3-<sup>3</sup>H]-naloxone or 0.3 nM [Ile<sup>5,6</sup>-<sup>3</sup>H]-deltorphin II (hδR). The K<sub>D</sub> values are: OFQ  $7 \times 10^{-11}$  M

(OFQR), naloxone  $1.3 \times 10^{-9}$  M (µR), naloxone  $2.8 \times 10^{-9}$  M (κR) and deltorphin II  $0.36 \times 10^{-9}$  M (δR).

Reactions were performed in 1 mL binding buffer (50 mM Tris-HCl, pH 7.8; 1 mM EGTA; 5 mM MgCl<sub>2</sub>; 0.1% BSA) for 60 min at 22 °C. At the end of the incubation, the reaction mix was filtered through GF/C-filters (Amersham), which had been pre-coated (0.3% polyethyleneimine plus 0.1% BSA). Filters were washed three times with 0.5 mL cold wash buffer (50 mM Tris-HCl, pH 7.5). Finally, 60 µL scintillation fluid (Micro Scint, Canberra Packard) was added and samples were counted in a Top Counter (Packard). Non-specific binding was defined in the presence of 1 µM unlabelled OFQ (hOFQR), 10 µM naloxone (hµR and hκR) or 10 µM deltorphin II (hδR).

#### 6.2.4. GTPγ<sup>35</sup>S binding assay

Agonist-mediated binding of GTPγ<sup>35</sup>S was investigated in 96-well plates using a scintillation proximity assay (SPA) and membranes prepared from cells expressing hOFQR, hµR, hκR or hδR. Binding was performed in 200 µL 20 mM HEPES-buffer (pH 7.4, plus 6 mM MgCl<sub>2</sub> and 100 mM NaCl), supplemented with 20 µM GDP, 10 µM cold GTPγS and 0.3 nM GTPγ<sup>35</sup>S (1 130 Ci/mmol). Twenty micrograms of membrane, 1 mg wheat-germ agglutinin SPA beads (Amersham, Little Chalfont,



**Figure 8.** Crystal structure of **13b**.

UK) and either OFQ ( $10^{-5}$ – $10^{-10}$  M) or synthetic compounds ( $10^{-4}$ – $10^{-10}$  M) were added.

The reaction mixture was incubated on a shaker for 60 min at 22 °C and then centrifuged for 5 min at 1 500 rpm in an Eppendorf 5403 centrifuge. Finally the plates were read in a Top Counter (Packard).

#### 6.2.5. *Elevated plus-maze test*

This test is based on the natural aversion of rodents for open spaces and heights and uses an elevated maze with two open and two closed arms; exposure to an open maze alley leads to an approach-avoidance conflict that is considerably stronger than that evoked by exposure to a closed maze alley. The time spent and the number of entries into open arms are indices of neophobic anxiety in animals [30]. Anxiolytics increase and anxiogenics decrease these two measures. The elevated plus-maze is based on spontaneous behaviour and thus does not necessitate training or use of appetitive behaviours induced by food or water deprivation [31].

Male Sprague-Dawley rats weighing 110–200 g and housed with ad libitum access to food and water on a 12 h light/12 h dark cycle (6 am–6 pm) light were used. The apparatus was 50 cm above the floor and consisted of two open arms (50 × 10 cm) perpendicular to two closed arms (50 × 10 × 50 cm high) extending from an open central area (10 × 10 cm). The maze was positioned in a closed,

white environment in a sound-proof observation room and the light intensity on the central platform was 225 lux. All parts of the apparatus were made of grey PVC plastic. The floor of the maze was thoroughly cleaned after each test trial. Thirty minutes after i.p. injection, rats were placed in the centre of the plus-maze facing one of the closed arms and observed via a closed-circuit TV camera by an observer located in an adjacent room. Locomotor behaviour in the maze was recorded for 5 min using an automated image analysis system (Ethovision, Noldus Information Technology). The number of entries, the distance and the total time spent in the open arms were measured. Total distance moved, distance and number of closed arm entries were also recorded as measures of spontaneous general activity.

Forced motor performance was subsequently evaluated in a traction test that consisted of forcing rats to grasp a horizontally strung wire (20 cm above the bench level, 2 mm in diameter, 20 cm long) with their fore paws. Various items were scored: the grasp reflex (score 1), body weight support (score 2), climb reflex (score 3) and escape (score 4); group scores were calculated by averaging individual scores. The maximum of two permissible readings was recorded. Fore limb grip strength was then quantitatively assessed using a digital strain gauge.

Animals held by the tail grasped a triangular bar (2 mm diameter, 5 cm wide) and were gently pulled away from the bar with a smooth steady pull until they released the triangle. The strain gauge remains fixed at its maximum deflection, three readings were taken for each animal and the maximum of three permissible readings was recorded as fore limb grip strength (in grams). Individual strengths were averaged over each dose group. Total duration of these tests was 1 min.

The experimental procedures used received approval based on international guidelines and adherence to Swiss federal regulations and guidelines on animal experimentation provided by the Swiss Academy of Sciences (1995).

Compound **3c** was freshly prepared in 0.3% v/v Tween 80 in physiological saline (NaCl 0.9%) and ultrasonified under normal laboratory conditions. It was injected intraperitoneally in a volume of 5 mL/kg body weight. Three doses (0.32, 1 and 3.2 mg/kg i.p.) administered versus vehicle were counterbalanced over four testing days. Sixteen animals were tested every day between 7 and 12 am. Dose-effects were assessed by single factor analysis of variance (ANOVA). Groups of 16 animals per dose were used. Where indicated by significant F-values, further a posteriori comparisons of individual dose versus placebo effects were performed using Bonferroni/Dunn statistics and a 5% significance level.

## Acknowledgements

We would like to express our thanks to Arthur Meili, Philipp Oberli, Sandra Müller, Daniel Wehrli, Patrick Biry and Jacqueline Higelin for their skilful technical assistance, Drs Wolf Arnold, Walter Huber, Stephan Müller, Mr Walter Meister, Roger Rueher, Vladimir Meduna and Gottfried Oesterhelt for spectroscopic determinations and analysis as well as Drs Kenneth Lundstrom and Parichehr Malherbe for membranes expressing human OFQ and opioid receptors.

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