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8-ACENAPHTHEN-1-YL-1-PHENYL-1,3,8-TRIAZA-SPIRO[4.5]DECAN-4-ONE DERIVATIVES AS ORPHANIN FQ RECEPTOR AGONISTS

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Abstract. A series of 8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives **1** was studied with respect to the binding affinity for the orphanin FQ (OFQ) and opioid (μ, κ, δ) receptors. The influence of stereochemistry as well as the substitution pattern of the phenyl-ring in position 1 on the affinity for the orphanin FQ receptor and selectivity to opioid (μ, κ, δ) receptors is discussed. The most interesting compound **1c** was tested for its anxiolytic-like properties *in vivo*.

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 (μ, κ, δ) receptors.[1] Orphanin FQ selectively binds to its own receptor (OFQR or ORL-1), which is a member of the G protein-coupled receptor superfamily.[2] The amino acid sequence of the OFQ receptor is 47% identical to the opioid (μ, κ, δ) 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.[3] As for the opioid receptors, OFQ receptor activation has been linked to the inhibition of adenylyl cyclase activity and/or modulation of neuronal K^+ and Ca^{2+} conductance.[4]

Orphanin FQ and its receptor are widely expressed throughout the central nervous system. Thus, 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.[5] Notably, 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 behavioral inhibition of animals being acutely exposed to anxiogenic environmental conditions.[6]

Recently we discovered (RS)-8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **1a** as being an OFQ receptor agonist with high affinity and moderate selectivity to the opioid (μ, κ, δ) receptors.[7] In this paper we discuss the influence of stereochemistry as well as the substitution pattern of the phenyl-ring in position 1 of this compound on the affinity and selectivity for OFQ and opioid (μ, κ, δ) receptors. Behavioral data obtained with compound **1c** are also presented.

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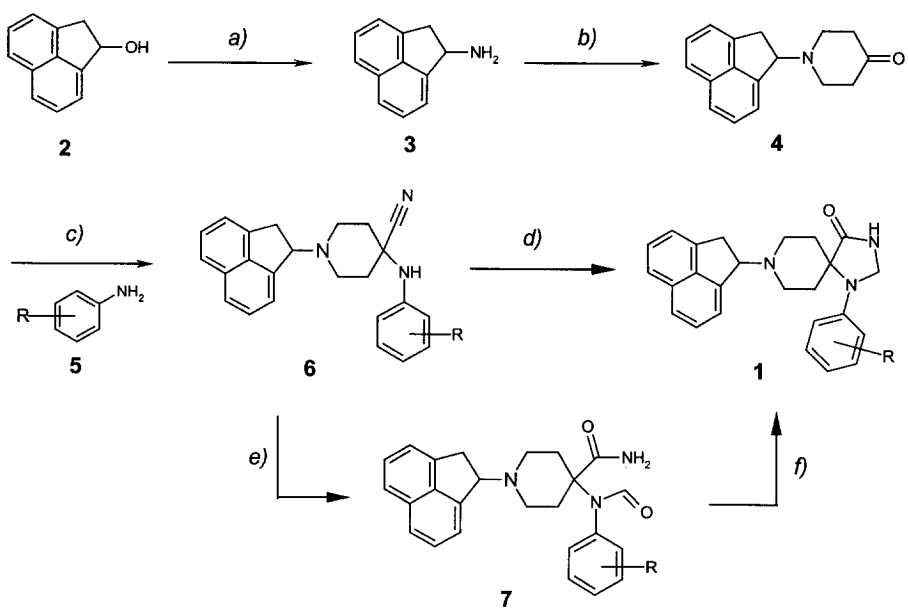
Chemistry

The 8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives **1a-o** were prepared according to scheme 1 starting from commercially available 1-acenaphthenol **2**, however, for the synthesis of the enantiomers **1b** and **1c** the starting materials are (R)- and (S)-1-acenaphthenol, respectively.[8]

Scheme 1



a) 1. $(PhO)_2P(O)N_3$, DBU, toluene, RT; 2. PtO_2/H_2, EtOH, RT. *b)* N-ethyl-N-methyl-4-oxo-piperidinium iodide, K_2CO_3, EtOH, reflux. *c)* TMSCN, AcOH, RT. *d)* 1. formic acid, Ac_2O, RT; 2. formamide, 200°C. *e)* 1. formic acid, Ac_2O, RT; 2. formic acid, acetic acid, RT. *f)* 1. triethyl orthoformiate, reflux; 2. $NaBH_4$, MeOH, RT.

(RS)-1-Acenaphthenol **2** was converted into the corresponding amine **3** by a two step procedure *via* the azide.[9] For the introduction of the piperidin-4-one moiety a Hofmann-elimination-Michael-addition sequence[10] was used. A Strecker-reaction with substituted anilines and trimethylsilyl cyanide in acetic acid led to the anilino-nitriles **6**, which on treatment with formic acid and acetic acid anhydride and subsequent reaction of the crude product in formamide at 200°C gave **1** in low yields (<20%).

However, better yields of **1** (>65%) were obtained by preparing **7** in a two step procedure by treatment of **6** with a mixture of formic acid and acetic acid anhydride and afterwards with acetic acid in formic acid.

Ring-closure in triethyl orthoformiate under reflux conditions and subsequent reduction of the crude product with sodium boronhydride gave **1**. The enantiomerically pure compounds **1b** and **1c** were prepared by use of the same reaction sequence starting from (R)- and (S)-1-acenaphthenol[8] with inversion of the configuration in the first step.

Pharmacology

The affinities (pKi) of the compounds **1a-o** for human OFQ and opioid (μ,κ,δ) receptors were determined from competition binding curves using [^{3}H]-orphanin FQ and membranes prepared from permanently transfected HEK293 cells expressing hOFQ receptors as well as [^{3}H]-naloxone (μ,κ receptors) and [^{3}H]-deltorphine (δ receptors) and membranes prepared from BHK cells transiently expressing hμ, hκ and hδ receptors. The results are shown in table 1 (the given pKi values are the mean values obtained from three experiments performed in triplicate).

Table 1. Binding affinities (pKi) for human OFQ and opioid (μ,κ,δ) receptors

compound	R	OFQ	μ	κ	δ
1a	H	9.2	8.2	7.6	6.6
1b (S)	H	8.7	7.9	7.6	< 6.3
1c (R)	H	9.6	8.4	7.7	7.0
1d	2-F	7.4	6.4	6.8	n.d.
1e	3-Cl	8.9	7.7	7.3	6.8
1f	3-Me	8.4	8.0	7.7	< 6.3
1g	3-OMe	7.8	7.4	n.d.	n.d.
1h	3-F	9.5	8.4	7.9	6.8
1i	3-Br	8.4	7.9	7.1	< 6.3
1j	3-CF_3	8.0	8.1	n.d.	n.d.
1k	4-Cl	8.8	7.5	7.1	< 6.3
1l	4-Me	8.3	7.3	6.7	< 6.3
1m	4-OMe	7.0	n.d.	n.d.	n.d.
1n	4-F	8.8	7.6	7.2	< 6.3
1o	3,5-diMe	8.0	7.7	n.d.	n.d.

It turned out that the (R)-enantiomer **1c** shows higher affinity for the OFQ receptor than the (S)-enantiomer **1b**. However, the affinity for the opioid (μ,κ,δ) receptor is also increased and therefore the selectivity is only slightly higher when compared to the racemate **1a**. The best position for substitution is

position 3 followed by position 4 whereas the 2-fluoro-derivative **1d** shows a substantial decrease in affinity at the OFQ receptor.

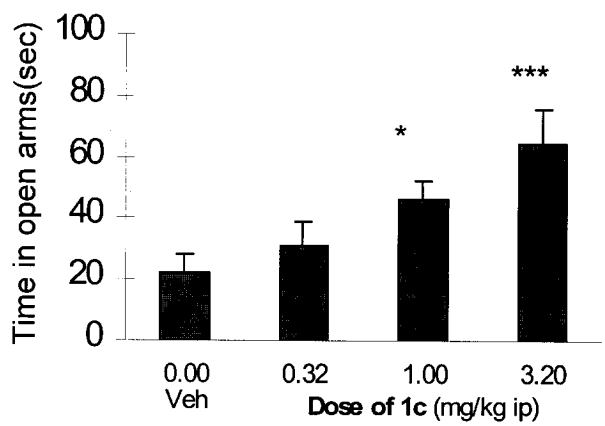
Fluoro- (**1h**, **1n**) and chloro-(**1e**, **1k**) substitution is well tolerated. However, sterically more demanding substituents like methoxy (**1g**, **1m**) or trifluoromethyl (**1j**) led to compounds with decreased affinities. The 3-fluoro derivative **1h** turned out to have higher affinity for the OFQ receptor than the unsubstituted compound **1a**, but the selectivity remains the same due to increased affinity to the opioid (μ,κ,δ) receptors.

Compound **1c** is the most interesting derivative in terms of affinity to the OFQ receptor and selectivity to the opioid (μ,κ,δ) receptors that came out of this approach. The compound was tested for its potency to stimulate GTPγ^{35}S binding[11] using membranes prepared from 293s cells transfected with the human OFQ receptor and turned out to be a full agonist (pEC_{50} = 7.4 with 116% of OFQ-responses).

In addition, **1c** was found to have no significant (pKi < 6.5) affinity for a variety of other receptors like serotonin $5HT_{1D\alpha}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_6$, $5HT_7$, dopamine D1, D2, D3, D4.4, CRF 1, 2α and benzodiazepine receptors.

When tested *in vivo* following intraperitoneal administration in rats (0.3, 1, 3.2 mg/kg *vs* vehicle), **1c** was found to increase exploration in a novel environment (modified openfield test) and to exhibit dose-dependent anxiolytic-like effects in the elevated plus-maze procedure in rats (Fig 1).

Figure 1: Effect of **1c** in the elevated plus-maze test in rats (30 min pretreatment).[6,12]



The opioid receptor agonist morphine was inactive in a dose range of 1–10 mg/kg ip under the experimental conditions used indicating that the *in vivo* effect of **1c** was not related to its opioid receptor

affinity. Spontaneous locomotion in the closed arms of the elevated plus maze and forced motor performance was not significantly affected by the drug treatment (Table 2).

Table 2: Effect of **1c** on motor functions in rats (30 min pretreatment).[13]

		vehicle	0.32 mg/kg ip	1 mg/kg ip	3.2 mg/kg ip
Spontaneous locomotion					
Distance moved (cm)	mean	**1305.94**	**1412.39**	**1465.79**	**1141.08**
in closed arms	sem	48.92	63.65	61.68	87.46
of the plus-maze			ns	ns	ns
Forced motor performance					
Grip strength (g)	mean	**544.25**	**516.25**	**521.63**	**479.00**
	sem	15.96	15.79	17.68	15.18
			ns	ns	ns
Traction test (score)	mean	**4.00**	**4.00**	**4.00**	**3.75**
	sem	0.00	0.00	0.00	0.14
			ns	ns	ns

In summary, we were able to show, that the stereochemistry as well as the substitution at the phenyl ring of (RS)-8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one **1a** has major influence on the affinity at the OFQ receptor and in the same direction on the affinity at the opioid (μ,κ,δ) receptors. Following intraperitoneal injection, the most interesting compound **1c** 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 orphaninFQ peptide in rats.[6] Agonists at OFQ receptors may offer interesting possibilities for discovery of innovative anxiolytics and for exploring the pathophysiology of stress-related psychiatric disorders.

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12) 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 time spent, the distance moved and the number of entries into open arms are indices of neophobic anxiety in animals. Anxiolytics increase and anxiogenics decrease these measures.

13) Distance moved in the closed arms of the plus-maze was recorded as measures of spontaneous general activity. Forced motor performance was evaluated in a traction test that consisted of forcing rats to grasp a horizontally strung wire and forelimb grip strength was quantitatively assessed using a digital strain gauge.