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Review

The opioid-receptor-like 1 (ORL-1) as a potential target for new analgesics

Daniela Barlocco[a]*, Giorgio Cignarella[a], Giuseppe A.M Giardina[b], Lucio Toma[c]*

[a]Istituto di Chimica Farmaceutica e Tossicologica, Università di Milano, Viale Abruzzi 42, 20131 Milan, Italy
[b]Medicinal Chemistry Department, SmithKline Beecham S.p.A., Via Zambeletti, 20021 Baranzate di Bollate, Milan, Italy
[c]Dipartimento di Chimica Organica, Università di Pavia, Via Taramelli 10, 27100 Pavia, Italy

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Abstract – A new sequence, which encoded a novel G protein-coupled receptor, was disclosed by two different groups, using the nucleic acid probes based on the delta opioid receptor, first cloned in 1992. The new receptor, which Meunier called opioid-receptor-like 1 (ORL-1), was shown to share high homology with the opioid receptors and therefore thought to be a potential target for new analgesics. In this respect, the present review reports on the literature referring to ORL-1, to its natural ligand (nociceptin or orphanin FQ) and to several synthetic analogues recently described, both as agonists or antagonists at the receptor. © 2000 Éditions scientifiques et médicales Elsevier SAS

analgesia / opioid-receptor-like 1 (ORL-1) / nociceptin / orphanin FQ

Pain is a very complex and dynamic process involving multiple interrelated neurotransmitter/neuromodulator systems in the spinal cord, in ascending and descending spinal pathways, and at supraspinal sites. Qualitatively, pain can be defined in terms of its severity (mild, moderate and severe), temporality (acute and chronic), and etiology. Mechanistically, pain can be looked at in terms of hyperalgesia (an exaggerated response to a normally mildly painful stimulus), spontaneous (pain that occurs in the absence of external stimuli), and allodynia (pain that occurs in response to a normally non-noxious stimulus) [1].

For some types of pain efficient therapy is available (e.g. opioids for post-operative pain or non-steroidal anti-inflammatory drugs for inflammatory pain). However, the side-effects associated with these treatments makes it necessary to search for new approaches. Moreover, other situations are not adequately treated by the conventional drugs (e.g. neuropathic pain and other chronic status). Given the existence of several neurotransmitter/neuromodulators, it seems to be impossible to find an ideal analgesic for all types of pain. An intensive research effort over the past two decades, both by the academic community and the pharmaceutical industry, has led to the development of many different approaches which have been widely reviewed in the recent literature. See the perspective by Williams et al. [1] for an exhaustive review on: glutamate receptor, purinergic and ion channel modulators, GABA receptor, acetylcholine and alpha-2-adrenoceptor agonists, nitric oxide, cannabinoids, growth factors, vanilloids, cytokines and a number of other approaches.

This paper focuses on a recent hypothesis which considers the opioid-receptor-like 1 (ORL-1) as a potential target for new analgesics. Besides the mu and kappa opioid receptors, a new sequence was identified by different groups by using nucleic acid probes based on the delta opioid receptor which was cloned in 1992. This sequence, which encoded a novel G protein-coupled receptor, was named opioid-receptor-like 1 (ORL-1) by Mollereau et al. [2]. It shares as much sequence homology with the other opioid receptors as they share with each other ($\sim$ 60%), mainly in the putative transmembrane domains and cytoplasmic loops. In particular, Meunier [3] highlights a common physico-chemical feature between kappa receptors and ORL-1, namely the existence in both of them of a highly acidic second exofacial loop. However, when transfected into mammalian cells, ORL-1 did not bind opiates with significant

*Correspondence and reprints: Daniela.Barlocco@unimi.it toma@chifis.unipv.it

Nociceptin (orphanin FQ)	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln
Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln

Figure 1. Structure of nociceptin in comparison with that of dynorphin A.

affinity. This observation led to the search of an endogenous neurotransmitter as the natural ligand for ORL-1. This neurohormone was subsequently identified almost simultaneously by two different groups [4, 5] who monitored it through the inhibition of forskolin-stimulated adenosine 3,5-monophosphate (cAMP) accumulation in ORL-1 transfected cells. It was found to be a heptadecapeptide with a sequence closely similar to the opioid peptide dynorphin A (*figure 1*). In this respect, it should be noted that, while all the endogenous peptides previously known have tyrosine as their N-terminal amino acid, it has phenylalanine [6]. Reinscheid et al. [4] named it orphanin FQ (OFQ) to denote its relation to an orphan receptor and to specify the fact that its first and last amino acids were phenylalanine and glutamine, respectively. Conversely, Meunier et al. [5] named it nociceptin (NC), to highlight its in vivo activity; in fact they found that after i.c.v. injection into mice it led to an increased reactivity to pain in the hot plate test. The same behaviour was reported by Reinscheid [4] in the tail-flick test. Being so similar to other neuropeptides, it was thought that nociceptin could be synthesized as part of a larger precursor. Several efforts in this direction eventually led to the cloning of a cDNA prepared from rat brain mRNA that encodes a protein of 181 amino acids [7–9] which is known both as a prepro-orphanin FQ (ppOFQ) and prepro-nociceptin. It contains several peptides, in addition to nociceptin, which are organized in a quite similar way to preprodynorphin and pro-opiomelanocortin. Pairs of the basic amino acids Lys-Arg are present, which represent a general cleavage site. It should be noted that quite recently Ito and his group [10] reported the identification of a second 17 amino acid peptide derived from the same precursor, which they named nocistatin. They stated that nocistatin does not bind to the ORL-1 but it binds with high affinity to the membrane of mouse brain and spinal cord. Its sequence was defined as Thr-Glu-Pro-Gly-Leu-Glu-Glu-Val-Gly-Glu-Ile-Glu-Gln-Lys-Gln-Leu-Gln. It was found to be able to inhibit nociceptin-evoked allodinya in the spinal cord in mice.

The wide distribution of the ORL-1 suggests that it could be involved in a variety of central processes. A review by Meunier [11] quotes in particular: learning and memory, attention and emotion, movement and motor processes, homeostasis, neuroendocrine secretion and several sensory perceptions. Though northern blot analysis did not clearly reveal ORL-1 transcripts outside the central nervous system, by the more sensitive reverse transcriptase polymerase chain reaction assay, Wang et al. [12] proved the presence of ORL-1 receptor mRNA in rat intestine and other organs. Moreover, the role of the ORL-1 in immune functions was reported [13–15]. As proven by several groups, activation of ORL-1 by nociceptin inhibits forskolin-stimulated cyclic AMP accumulation [15–17], activates K^+ channels [16] and inhibits voltage-dependent Ca^{2+} currents [16] in a way similar to that mediated by opioid receptors. However, as far as analgesia is concerned, several data were reported confirming production of hyperalgesia after i.c.v. injection of nociceptin both in the hot plate method and the tail flick assay. These effects were not reversed by naloxone [4, 5, 18]. In contrast, naloxone-sensitive analgesia was reported by Rossi et al. [19] after i.c.v. administration of nociceptin to mice. Antagonism of the supraspinal antinociception produced by opioid agonists has also been observed [20, 21]. Recently Ma et al. [16] reported that nociceptin stimulates the phosphorylation and activation of mitogen-activated protein kinase (MAPK), both through phospholipase C (PLC) and protein kinase C (PKC). This pathway differs from the classical opioid receptor-mediated MAPK activation and could be of importance in understanding several differences between nociceptin and other opioids. However, though MAPK is supposed to play a role in the development of opiate dependence and addiction, the biological significance of nociceptin induced activation of MAPK is not clear.

Intrathecal administration [22] of NC gave even more intriguing results. Reinscheid et al. report that, in mice, NC did not induce antinociception but caused paralysis [4]. The same results were published by Grisel et al. [23]. On the contrary, King et al. [24] found that intrathecal NC produced antinociception, which could be reversed by naltrexone. Furthermore, Okuda-Ashitaka et al. [25, 26] showed that NC was able to induce hyperalgesia and allodynia, though another group [27] came to the opposite conclusions. A further paper by Henderson et al. [28]

reports that intrathecal NC produced two distinct acute actions, namely an opioid-like antinociceptive effect and antagonism of morphine-induced antinociception. Tian et al. [29] reported that intrathecal administration of NC produces analgesia and potentiates the analgesic effects of morphine, while Yamamoto et al. [30] said that it attenuates the level of thermal hyperalgesia. Data on the peripheral analgesic effects of NC were recently discussed [31, 32].

In a recent paper, Zaki and Evans [22] gave an exhaustive view of all the pharmacological effects reported on nociceptin. Though the authors anticipate that it is difficult to draw a conclusion on the role of NC, based on the available data, they recognize obvious functional differences between NC and endogenous opioids. They also suggest that a different distribution of the receptors, either within neurones or between neuronal populations, could explain the different effects of various ligands. The review also takes into consideration data on ORL-1 knockout animals and finds that, quite surprisingly, no differences in nociceptive threshold were noticed in mutant vs. wild type animals. The latter results were recently confirmed by another group [33].

Early attempts to define a structure–activity relationship with truncated analogues of NC were made by Dooley and Houghten [34] and Reinscheid et al. [35], who reported that the amino-terminal portion of the peptide is essential for high binding. Zhou et al. [36] examined a C terminal fragment NC-(8–17) together with nociceptin, and gave clear evidence that both compounds were able to modulate excitatory amino acid (EAA) induced currents. They suggested that NC-induced analgesia at the spinal level was probably due to this mechanism. Dooley et al. also reported [37] on 15 hexapeptides endowed with high affinity for ORL-1, which were identified from a combinatorial library containing more than 52 million different hexapeptides. The five most potent compounds, which show affinity for the ORL-1 in the nanomolar range, were also tested in three different assays, namely stimulation of ^{35}S-GTPγS binding and inhibition of forskolin-stimulated cAMP accumulation in Chinese hamster ovary (CHO) cells transfected with ORL-1 and inhibition of electrically induced contractions in the mouse vas deferens. All the compounds were found to act as partial agonists in the three assays. It should be noted that all the compounds are positively charged and therefore they could bind to the negatively charged second extracellular loop, which is thought to be a likely binding site for nociceptin. Another combinatorial approach was recently reported [38]. The authors screened a synthetic peptide library in binding studies towards the three opioid receptors and ORL-1. On these preliminary results they synthesized 66 individual compounds and tested them in the same binding assay. One of the new derivatives was shown to act as an antagonist for the ORL-l, while it was found to be an agonist at the opioid receptors.

Given the very strict resemblance between NC and dynorphin A, several attempts were made to elucidate a possible analogy in the interaction of these peptides with their receptor [39, 40]. In particular, attention has been devoted to the concept of the message and address division of dynorphin A. In a recent paper Meunier and his group [41] compared a number of nociceptin analogues and opioid agonists/antagonists for their ability to compete with the binding of ^{3}H-nociceptin in membrane preparations from CHO (ORL-1) cells. They report that replacing the N-terminal Phe residue of nociceptin by Tyr gave a compound nearly as potent as the parent peptide. The same result was previously reported by several different papers [42, 43]. However, the new peptides displayed higher affinity for the opioid receptors, mainly μ-receptors [43]. On the contrary, removing the N-terminal Phe reduced the affinity by about 2 000-fold. Stepwise cutting of the C-terminal amino acids (to nociceptin-(1–6)) resulted in a progressive loss of affinity [41]. However, no evident discontinuity was noticed in the affinity vs. peptide length relationships, which made it difficult to identify particularly important residues. In this respect it should be highlighted that Calo' et al. [44] indicated NC-(1–13)-NH_2 as the minimum active sequence, since NC-(1–12)-NH_2 was binding very poorly and NC-(1–9)-NH_2 was completely inactive. However Meunier et al. [41] noticed that the C-terminal nociceptin fragments 12–17 and 6–17 were fairly active (dissociation constants in the range of 20–50 nM; for nociceptin Ki = 0.13 nM). In contrast with these data, Reinscheid [35] had previously reported that nociceptin (10–17) was completely inactive both in binding and functional studies. A suggestion was made that these results could be interpreted on the basis of different conformations.

Concerning the opioid ligands tested in this study [41] (amongst several others the agonists morphine, etorphine, ethylketocyclazocine, fentanyl, lofentanil, U-50488, dynorphin A, DAMGO, DTLET and the antagonists naloxone and diprenorphine) only lofentanil, dynorphin A and etorphine had Ki < 1 μM for the ORL-1 binding site. However, lofentanil was the most potent (Ki = 24 nM vs. 110 and 530 nM, respectively). It should be noted that fentanyl was completely inactive (*figure 2*). The allosteric inhibition by Na^+ previously observed in the binding of opioids to their receptors was confirmed for nociceptin. According to the authors this could probably anticipate that Asp in the putative second membrane-spanning

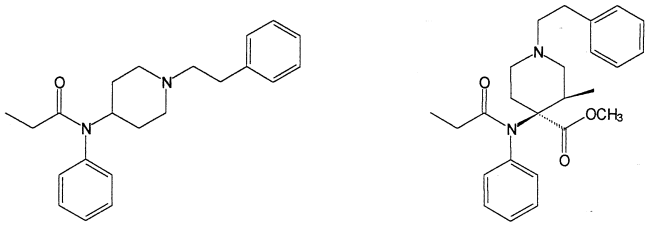


Figure 2. Structure of the opioid ligands fentanyl and lofentanil.

domain could be responsible for this effect in both receptors. The binding studies were paralleled by testing the ability of the compounds to inhibit forskolin-induced accumulation of cAMP in intact CHO (ORL-1) cells. Results showed that opioids with Ki > 1 μM were inactive in this functional assay. On the contrary lofentanil and etorphine acted as full agonists. A quite intriguing result was obtained with dynorphin A which, despite being shown to possess higher affinity for the ORL-1 compared to etorphine, was less efficient than the opiate in this test. This led the authors to suggest that dynorphin could be provided with antagonist properties for the receptor.

As far as the NC analogues are concerned, their agonist properties in this assay seemed to parallel their binding potency. These results give support to a previous study from Reinscheid et al. [4] who, in contrast to the results of Dooley and Houghten [34], concluded that the ORL-1 recognizes different parts of the nociceptin molecule with respect to dynorphin A. On the contrary, Dooley claimed that the nociceptin sequence could be divided into the same 'message' and 'address' moieties as dynorphin A, the N-terminal FGGF sequence representing the message and the highly basic C-terminal portion the address. However, it should be noted that, albeit both groups used a similar approach, including Ala scanning and systematic truncations of NC, Dooley and Houghten tested C-terminally amidated fragments, while Reinscheid and his group did not. However, all the data seem to confirm the importance of the basic core of NC both in receptor recognition and activation. Subsequent studies by the same group involving also ORL-1/kappa hybrid receptors (O–K) [45] showed a poor correlation between receptor binding affinity and biological activity for the majority of ligands acting at the receptor, which is consistent with the presence of distinct binding and transduction sites. Based on their studies, Meunier and his group suggest that distinct peptide–receptor interactions are responsible for the activation of ORL-1 vs. KOR-1 by nociceptin and dynorphin, respectively. In particular they believe that nociceptin interacts through its positively charged core with the negatively charged EL2 loop of the receptor, while dynorphin activates the KOR-1 receptor mainly through interactions of its N-terminal hydrophobic domain with the receptor hydrophobic binding site, which is probably located within the transmembrane helix bundle. They also suggest that the two peptides and the two receptors diverged primarily by co-ordinated inversion of their 'message' and 'address' domains, though they are not able to explain how this could happen.

Great attention to the address/message topic was devoted by the group of Salvadori et al. The solution conformation of NC was investigated by NMR techniques [46] and results were similar to that of dynorphin A in the N-terminal part containing the message domain, while the C-terminal part is more flexible than the corresponding address of dynorphin A. They investigated the nociceptin-1-(1–13) peptide amide [NC-(1–13)-NH_2], which was shown by the same authors to possess full biological activity [47], together with several analogues. All the experiments were done on the mouse vas deferens, which was previously confirmed as a two receptor system able to respond to both deltorphine and nociceptin [44]. To check the importance of the amidation of NC and its analogues, they tested NC-(1–17)-NH_2 and found that it was fully comparable to the naturally occurring NC. The same result had been reported by Dooley et al. [34] in binding studies to rat brain membranes. On the contrary, the potency of the NC-(1–13) fragment is strictly dependent on the C-terminal function. In fact, while the NC-(1–13)-NH_2 was found to be a full agonist equipotent to NC, the corresponding acid NC-(1–13)-OH was by two orders less potent. Further shortening of the sequence always led to very weak compounds. Several hypotheses have been made by the authors to interpret the different behaviour of the NC-(1–13)-NH_2 and NC-(1–13)-OH. The C-terminal amidation in truncated analogues could protect the peptide from enzyme degradation. In this respect, they argue that probably the loss of the C-terminal residue in the natural peptide is less detrimental than in its analogues. They also hypothesize that the metabolites which derive from NC could be more potent than those deriving from the truncated analogues. A further suggestion concerned a different spatial arrangement. In fact they hypothesize that the presence of an acidic group near Lys^{13}, which could be important to the interaction with the receptor, would surely lead to a 'neutralization' of this centre. This point could be further confirmed by the fact that passing from NC-(1–13)-NH_2 to NC-(1–12)-NH_2 with elimination of Lys^{13} causes almost the same loss in potency. The authors point out

that, contrary to what was seen with the μ and δ opioid receptors, the orphanin receptor can be occupied and activated only by a rather large sequence. This includes an N-terminal message site and a C-terminal cationic segment, which according to the authors could constitute the address, once more confirming that ORL-1 and δ-opioid receptors have different requirements for the occupancy by their ligands. Further results by the same group on analogues of the truncated ligand NC-(1–13)-NH_2 were reported [48]. In particular modifications were made on the N-terminal tetrapeptide Phe-Gly-Gly-Phe mainly in three directions: a) glycine residues were replaced by L- or D-Phe; b) the distance between Phe^1 and Phe^4 was altered in several ways; c) the peptide bond between Phe^1 and Gly^2 was replaced by CH_2–NH or Gly^2 was substituted by D-Ala. While paths a and b always led to either inactive or very weak compounds, substitution of Gly^2 by D-Ala^2 reduced the biological activity, though the binding wasn't greatly affected. The most interesting result came from the pseudopeptide [Phe^1ψ-(CH_2–NH)-Gly^2-]-NC-(1–13)-NH_2. This compound was prepared to protect NC-(1–13)-NH_2 from degradation by aminopeptidases, a rationale based on a recent report [49] which indicated the cleavage at the Phe^1-Gly^2 as the primary inactivation site of nociceptin. When tested in binding studies [50], it displayed a 13-fold reduced affinity for the ORL-1 but was unable to activate the receptor and proved to be a competitive antagonist in two different assays, namely electrically induced contractions of the guinea-pig ileum and mouse vas deferens [51]. This result was of great importance since it was the first example of a selective antagonist at the ORL-1 receptor, which would be very useful to clarify the role of endogenous nociceptin. In fact, only few reports of non-selective antagonists (carbetapentane, rimcazole, naloxone benzoylhydrazone) or partial agonists had appeared until then [51, 52].

Several groups devoted their attention to this new pseudopeptide. Butour et al. [53] found that it acts as an agonist in CHO cells expressing the human ORL-1 receptor, while Carpenter et al. [54] suggested it was an agonist at the spinal ORL-1. These findings could support the hypothesis of the heterogeneity of the ORL-1. Biochemical evidence of this topic was recently given in a paper by Mathis et al. [55]. A recent paper by Calo' et al. [56] demonstrated that this compound mimics NC both in causing hyperalgesia and in reversing opioid induced analgesia. These results clearly indicate that it is able to discriminate between NC action at the central and peripheral level. This in turn suggests that the NC functional sites which mediate the central action should be different from those responsible for the peripheral effects.

Using the TM helical bundle bovine rhodopsin model of Herzyk and Hubbard [57] as a template, Topham et al. [58] built a molecular model of the human ORL-1 receptor (residues 50–276) and of the complex with nociceptin. An extended binding site for nociceptin has been characterized able to accommodate nociceptin-(1–13). The N-terminal FGGF tetrapeptide of nociceptin binds in a transmembrane region in a cavity formed by the TM helices 3, 5, 6 and 7 comprising two adjacent hydrophobic pockets for the two phenylalanine side-chains, while the protonated N-terminus interacts with the conserved aspartate residue (130) of the TM3 helix. This region of the receptor is highly conserved in the opioid receptor family and the topology of the FGGF binding site is in good accord with the mapping of analogous opioid binding pockets. Residues 5–7 of nociceptin bind at the EL2 extracellular loop in a largely non-conserved region in agreement with the fact that position 5 and 6 have been identified as the major determinants of ORL-1 and κ-opioid receptor (KOR-1) selectivity. Also the positively charged residues 8–13 can mainly interact with the acidic extracellular EL2 loop. The presence of charged arginine side-chains at positions 8 and 12 appears particularly critical for nociceptin affinity and activity as confirmed by the fact that the cationic hexapeptides NAc-RYYX_4WX_6-NH_2 that mimic the nociceptin-(8–13) core are potent agonists [37]. The critical role of the second extracellular loop in the activation of the ORL-1 receptor was confirmed [59] through generation and analysis of receptor chimeras between the ORL-1 and the KOR-1 receptors.

To explore the structural elements necessary for receptor recognition and activation, Okada et al. [60] synthesized a series of nociceptin analogues in which non-polar amino acid residues Gly^6, Ala^7, Ala^{11}, Leu^{14} and Ala^{15} were substituted, respectively, by Trp. The only analogue that retained full receptor binding activity was [Trp^{14}]nociceptin, that also exhibited an increased biological activity in the GTPγS assay. These results clearly indicate that the Ala residues, as well as the Gly^6, are indispensable for affinity. In contrast, Leu^{14} seems to tolerate substitution by other residues. Given the presence at position 12 and 13 of the basic amino acids Arg and Lys, which are believed to interact with the acidic extracellular EL2 loop [45, 47, 58], the authors suggest that Leu (or Trp) at position 14 could interact with the aromatic amino acids present at the C-terminal side of the same loop.

Quite recently a series of non-peptide mimetics of nociceptin were reported mainly in patent literature. Actually, only a few molecular skeletons have been

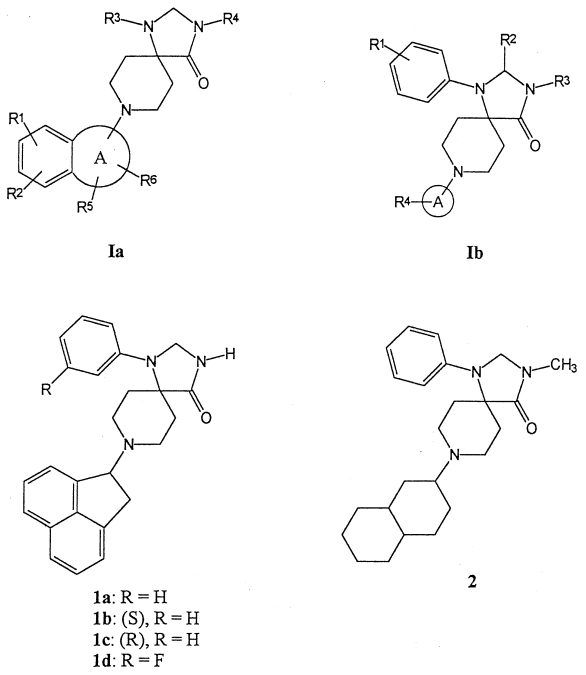


Figure 3. Structures of compounds **Ia**, **Ib**, **Ia–Id** and **2**.

proposed presenting the general structures reported in *figures 3–7*.

Compounds **Ia** [61] and **Ib** [62] from Hoffmann La Roche are based on the variously substituted bicyclic moiety of 1,3,8-triaza-spiro[4.5]decan-4-one (*figure 3*). They have been shown to possess valuable pharmacodynamic properties; they are agonists and/or antagonists at the ORL-1 receptor and have effects in animal models of psychiatric, neurological, and physiological disorders such as anxiety, stress disorders, depression, trauma, memory loss due to Alzheimer's disease or other dementias, epilepsy and convulsions, acute and/or chronic pain conditions, symptoms of withdrawal from drugs of addition, control of water balance, Na^+ excretion, arterial blood pressure disorders and eating disorders such as obesity. In the **Ia** group, A is a 4–7 saturated ring which may contain a heteroatom such as O or S and R groups are extremely variable to give more than 70 compounds. In particular, more detailed studies on the ORL-1 receptor agonist(RS)-8-acenaphten-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (**1a**) and on derivatives substituted on the phenyl ring that fall within the general formula **Ia**, have been recently published [63]. The affinities for the human ORL-1 and opioid (μ, κ, δ) receptors were determined from competition binding curves using the appropriate selective radioligands and receptor preparations. Compounds **1a** and **1d** showed the highest affinity

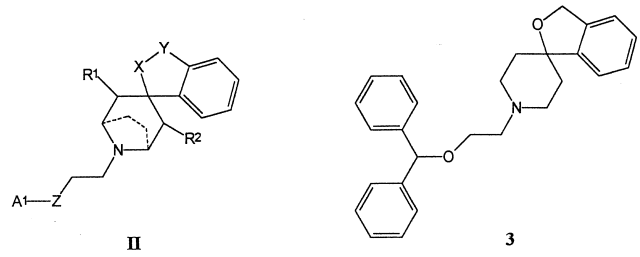
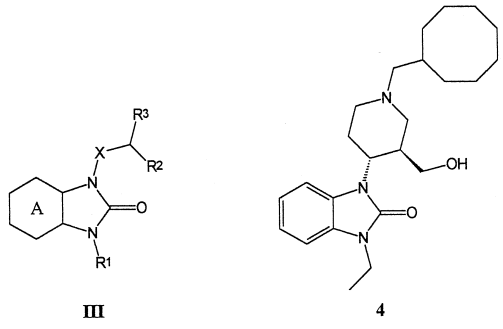


Figure 4. Structure of compounds **II** and **3**.

(pKi = 9.2 and 9.5, respectively) at the ORL-1 receptor but selectivity was poor. The (R)-enantiomer of **1a** (compound **1c)**, shows higher affinity to the ORL-1 receptor than its (S)-enantiomer **1b** (pKi = 9.6 vs. 8.7). 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 corresponding racemate (*figure 3*). Following intraperitoneal injection, the most interesting compound **1c** was found to decrease neophobia in a novel environment and to exhibit dose-dependent anxiolytic effects in the elevated plus-maze procedure. In the **Ib** group, A is a mono- or bi-cyclic saturated ring system and the R groups are very variable to give more than 100 compounds. Compounds of the general formula **Ib** show an affinity to the ORL-1 receptor, given as pKi, between 6.2 and 10.0, a representative compound being **2** that shows a pKi = 9.5 (*figure 3*).

A different spiro-skeleton is present in another series of antagonists (**II**) from Hoffmann La Roche [64], where X = O or CH_2 and Y = C(O), $(CH_2)_n$ or $N(CH_3)$, described as antagonists of the ORL-1-receptor. The affinity is in the range 6.7–8.2, a representative term being **3** that shows pKi = 7.9 (*figure 4*).

Compounds with a benzimidazolone nucleus (**III** and **IV**) are described in two patents from Banyu [65] and Pfizer [66], respectively. One hundred and thirty seven compounds of the general formula **III** were synthesized and characterized by MS and ^{1}H-NMR (*figure 5*). They are reported to be analgesics, antagonists against tolerance and dependence to narcotic analgesics typified by morphine, analgesic enhancers, agents for the treatment of obesity, drugs for ameliorating brain function, remedies for schizophrenia, remedies for Parkinson's disease, remedies for chorea, antidepressive drugs, remedies for diabetes insipidus, remedies for polyuria, or remedies for hypotension. In particular, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one **4** was discovered as a potent and selective ORL-1 antagonist [67]. It showed a high affinity for ORL-1 with an IC_{50} of 2.3 nM, full antagonistic

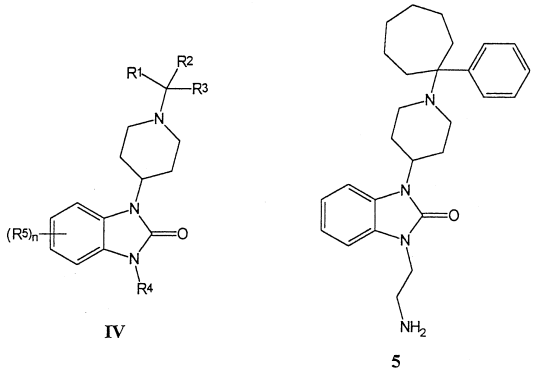


III 4

Figure 5. Structures of compounds **III** and **4**.

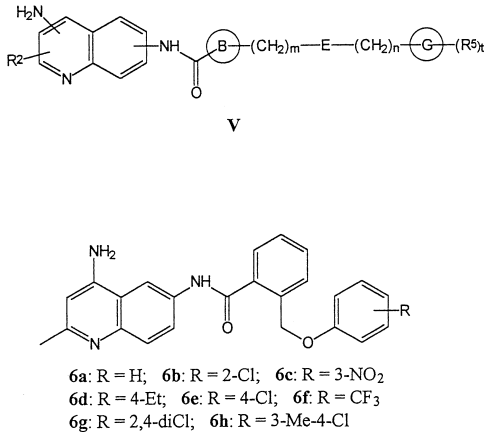
activity (IC_{50} = 5.6 nM) in the GTPγS assay and selectivity > 600-fold over μ, κ, and δ receptors. The effect of its (3S,4S)-enantiomer was approximately 400-fold weaker. About 90 compounds of the general formula **IV** were prepared, **5** being a representative term (*figure 6*). They are claimed to be ORL-1 receptor agonists and to be useful as analgesics in mammalian subjects. They showed good affinity for ORL-1 receptors, lower affinity for μ receptors and ED_{50} values in the range 0.02–1.0 mg/kg in the acetic acid writhing test in mice.

Finally, a Japan Tobacco Inc. patent [68] describes a series of N-quinolyl amides of the general formula **V**, that are ORL-1 binding agents and nociceptin antagonists, potentially useful as analgesics for treating serious pain such as post-operative pain. In particular, N-(4-amino-2-methyl-6-quinolyl)-2-(phenoxymethyl)benzamide **6a** and a series of mono- and disubstituted phenyl derivatives showed affinity at the ORL-1 receptor in the nanomolar range and a good selectivity. Substitution enhanced the activity and the selectivity independently from the nature of the substituent and the position of the substitution. In fact, **6a** showed Ki = 79 nM and a μ/ORL-1 ratio of 5 while, amongst others, the 2-chloro-derivative **6b** had



IV 5

Figure 6. Structures of compounds **IV** and **5**.



6a: R = H; **6b**: R = 2-Cl; **6c**: R = 3-NO_2
6d: R = 4-Et; **6e**: R = 4-Cl; **6f**: R = CF_3
6g: R = 2,4-diCl; **6h**: R = 3-Me-4-Cl

Figure 7. Structures of compounds **V** and **6a–6b**.

Ki = 15 nM and a μ/ORL-1 ratio of 28. Even better values were seen with the 3-nitro **6c** (9 nM, 43), the 4-ethyl **6d** (7 nM, 81), the 4-chloro **6e** (3 nM, 141), the 4-trifluoromethyl **6f** (4 nM, 98), the 2,4-dichloro **6g** (4 nM, 124), or the 3-methyl-4-chloro **6h** (4 nM, 186) derivatives (*figure 7*). Moreover, given orally to mice, they showed analgesic activity in the hot-plate test (for example, for **6d** the minimal effective dose was 1 mg/kg).

In conclusion, though many aspects still need to be clarified on ORL-1 and its natural ligand nociceptin, as well as on the agonists/antagonists which start to appear in the literature, the opioid-receptor-like 1 will surely be able to shed light on many points of pain perception and, maybe, to provide new powerful agents to treat it. A key aspect to succeed in this will be to identify potent and selective non-peptide agonists and antagonists able to serve as pharmacological tools for pre-clinical investigation.

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