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Peptide and nonpeptide ligands for the nociceptin/orphanin FQ receptor ORL1: Research tools and potential therapeutic agents

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Abstract

The 17-amino acid neuropeptide nociceptin/Orphanin FQ (N/OFQ) was recently identified as the endogenous ligand for the opioid receptor-like (ORL1) receptor, a fourth member of the classical μ, δ, and κ opioid receptor family. Although ORL1 clearly belongs to the opioid receptor family, it does not bind classical opiates and the ORL1-N/OFQ system has pharmacological actions distinct from the opioid receptor system. This new ligand-receptor system has generated active interest in the opioid community because of its wide distribution and involvement in a myriad of neurological pathways. The past two years have witnessed tremendous advances in the design and discovery of very potent and selective peptide and nonpeptide agonist and antagonist ligands at ORL1. These discoveries have facilitated the understanding of the role of the ORL1-N/OFQ system in a variety of processes such as pain modulation, anxiety, food intake, learning, memory, neurotransmitter release, reward pathways, and tolerance development. The ORL1 receptor therefore represents a new molecular target for the design of novel agents for anxiety, analgesia, and drug addiction. Indeed, there is tremendous interest in the pharmaceutical industry in the development of nonpeptide ligands such as the potent ORL1 agonist, Ro 64–6198, as anxiolytics and the ORL1 antagonist JTC–801 as novel analgesics. This review presents an overview of the various peptide and nonpeptide ORL1 ligands with an emphasis on their potential therapeutic utility in various human disorders.

Keywords

ORL1; Nociceptin; Orphanin FQ; Nociceptin analog; ORL1 ligands; ORL1 agonist; ORL1 antagonist; Piperidines; Analgesia; Anxiety

Introduction

The discovery of a fourth member of the opioid receptor family, opioid receptor-like (ORL1) receptor (now also called nociceptin receptor, OP_4 receptor, NCR) in 1994 (Bunzow et al., 1994; Fukuda et al., 1994; Mollereau et al., 1994; Wang et al., 1994) gave rise to a new camp in opioid research, because although this new G-protein coupled receptor was clearly in the opioid family, it did not bind opiates with high affinity (Mollereau et al., 1994). A year later, two groups independently identified the endogenous ligand for this receptor, a 17-amino acid neuropeptide named nociceptin by one group (Meunier et al., 1995) and orphanin FQ (Reinscheid et al., 1995) by the other. We will henceforth refer to it as N/OFQ or nociceptin (NC).

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The ORL1 receptor and N/OFQ are widely distributed in the brain and central nervous system (CNS), as well as in the periphery (for reviews, see Mollereau and Mouledous, 2000; Bigoni et al., 1999; Meunier, 1997). Several studies have implicated roles for the N/OFQ-ORL1 system in pain, anxiety, learning, memory, food intake, diuresis, and drug addiction (for a detailed review, see Calo et al., 2000a, 2002a). In particular, N/OFQ was shown to have a potent anxiolytic effect in several rodent models of anxiety after intracerebroventricular (icv) infusion (Jenck et al., 1997; Griebel et al., 1999; Kyuhou and Gemba, 1999). N/OFQ inhibits the release of several neurotransmitters, including serotonin (Siniscalchi et al., 1999) and dopamine (Murphy et al., 1996; Schlicker and Morari, 2000), implicating this peptide in inhibition of reward pathways in drug addiction. Moreover, N/OFQ reduces the development of place preference to morphine (Murphy et al., 1999) in rats, and has been shown to inhibit dopamine release in the nucleus accumbens in rats, in response to iv morphine (Di Giannuario et al., 1999). This points to a potential utility of ORL1 agonists as anxiolytics and opiate/drug abuse treatments. Inhibition of tachykinergic bronchoconstriction by N/OFQ (Groneberg and Fischer, 2001) suggests a role for ORL1 agonists in asthma and cough treatment (Chung and Chang, 2002). On the other hand, N/OFQ's inhibitory effects on the memory process (Sandin et al., 1997; Manabe et al., 1998) and stimulation of food intake (Pomonis et al., 1996) suggest a role for ORL1 antagonists as nootropics and anorectics.

However, N/OFQ modulation of pain pathways is yet to be completely understood. Initial studies showed that N/OFQ had a pronociceptive effect when injected icv into mice (Meunier et al., 1995; Reinscheid et al., 1995). This was later shown to be due to inhibition of stress-induced analgesia (Mogil et al., 1996). Blockade of N/OFQ signalling by antisense oligonucleotides was shown to increase pain threshold (Meunier et al., 1995). Indeed, the newly reported ORL1 antagonist JTC-801 as well as the low affinity antagonists retronociceptin methyl ester and naloxone benzoylhydrazone all show analgesic effects (not reversible by naloxone), in animal models of pain (Yamada et al., 2002; Jinsmaa et al., 2000; Noda et al., 1998). Thus, ORL1 antagonists may represent a new class of analgesics.

From the myriad of modulatory actions of N/OFQ in several neurological pathways, it is clear that ORL1 represents an important new molecular target for the development of novel therapeutics for several neurological conditions, including nociception. This is also evident from the active interest of pharmaceutical companies in developing both agonist and antagonist nonpeptide ligands for the ORL1 receptor as potential drugs for various human disorders, as discussed below.

Peptide ligands

N/OFQ is a heptadecapeptide (FGGFTGARKSARKLANQ) (Fig. 1) that has significant homology with dynorphin A (YGGFLRRIRPKLKWDNQ). The first four amino acids differ from the canonical opioid sequence only by the presence of Phe^1 instead of Tyr^1. This difference may be sufficient to prevent N/OFQ binding to opioid receptors. In fact, replacement of Phe^1 by Tyr^1 results in a peptide that also binds the opioid receptors (Varani et al., 1999). Amidation of the C-terminus (NC-NH_2) maintains full potency and activity. Early studies to determine the minimum active sequence showed that up to four C-terminal amino acids can be deleted without loss of activity. Although the free acid NC(1–13)OH loses receptor affinity and is considerably less potent, amidation of the C-terminus to give NC(1–13)NH_2 (Fig. 1) restores potency and agonist activity comparable to the parent NC(1–17)NH_2 (Dooley and Houghten, 1996; Reinscheid et al., 1996). C-terminal amidation protects from degradation by carboxypeptidases and is now a standard feature of most N/OFQ-based peptide ligands. The truncated peptide NC(1–13)NH_2 has been used as the structural basis for potent agonist as well as antagonist peptide ligands discovered thereafter.

However, Dooley et al.(1997) reported the identification of five hexapeptides (Fig. 1) with subnanomolar binding affinity for ORL1, using a combinatorial library approach. These highly charged peptides turned out to be partial agonists at ORL1 and did not possess any in vivo activity, presumably due to rapid degradation (L. Toll, personal communication).

Initial structure-activity studies on NC(1–13)NH_2 by Guerrini et al.(1997) determined that the Nterminal peptide FGGF is essential for activity and that Phe^4 and not Phe^1 appears to be important for receptor activation. Further studies by the same group on N-terminal modification resulted in the discovery of a purported ORL1 antagonist in which the Phe^1-Gly^2 amide bond was replaced with a pseudopeptide (CH_2-NH) bond (Calo et al., 1998). This peptide [$Phe^1\psi(CH_2NH)Gly^2$]NC(1–13)NH_2 (Fig. 1) was shown to be a selective competitive antagonist in the electrically stimulated guinea pig ileum and mouse vas deferens (Guerrini et al., 1998). This report set off a flurry of in vitro and in vivo assays (nicely summarized in Calo et al., 2000b) which showed, to some disappointment, that this peptide acted as an antagonist, partial agonist, or even full agonist, depending on the tissue preparation. Thus, while it showed different levels of partial agonist activity in [^{35}S]GTPγS assays in CHO cells transfected with human or mouse ORL1 (Burnside et al., 2000; Berger et al., 2000), it showed full agonist activity in several in vivo CNS assays (Xu et al., 1998; Grisel et al., 1998; Carpenter and Dickenson, 1998). Similar results were also observed with the partial agonist hexapeptide Ac-RYYRIK-NH_2 (Berger et al., 2000; Burnside et al., 2000). These observations gave rise to a notion that there might be species differences between the human and rodent ORL1 and that activity estimations in transfected cells can depend on the receptor number in low or high expressing clones (Burnside et al., 2000).

Further modifications of the N/OFQ N-terminus led to the design of [N-Phe^1]NC(1–13)NH_2 (Fig. 1) by transposition of the Phe^1 side chain from the α-carbon of Phe^1 to the N-terminal nitrogen (Guerrini et al., 2000). This peptide was the first pure ORL1 peptide antagonist; it had low potency (pA_2 values 6.0–6.4) (Calo et al., 2000c) but was devoid of any residual agonist activity. This modified N/OFQ peptide selectively antagonized the effects of N/OFQ in vitro in various isolated tissues and in CHO cells expressing the human recombinant ORL1 (Calo et al., 2000c; Hashimoto et al., 2000; Berger et al., 2000; Chiou et al., 2002a). In vivo, icv administration of this peptide inhibited the pronociceptive and antiopioid actions of N/OFQ (Rizzi et al., 2000; Calo et al., 2000c) and reversed the effects of N/OFQ on memory impairment (Redrobe et al., 2000), food intake (Polidori et al., 2000), and locomotor activity (Rizzi et al., 2001a). Importantly, a recent study by DiGiannuario and colleagues has shown that no tolerance develops to the antinociceptive action of this antagonist, unlike with opioid analgesics, suggesting that ORL1 antagonists can be developed as a novel class of analgesics (Di Giannuario et al., 2001). A similar lack of tolerance development was also observed with the antinociceptive effect of retronociceptin methyl ester, a nonselective ORL1 antagonist (Jinsmaa et al., 2000).

Modification of N/OFQ and NC(1–13)NH_2 has also produced peptide agonists more potent than N/ OFQ itself. Okada et al.(2000) reported the synthesis of [Arg^{14}, Lys^{15}]N/OFQ, which had 3-fold greater binding affinity than N/OFQ itself at human ORL1 and was 17 times more potent in the GTPγS functional assay. Interestingly, the [Arg-$Lys^{6–7}$] and [Arg-$Lys^{10–11}$] analogs exhibited weak activity. In in vivo experiments, this agonist was 30-fold more potent than N/OFQ in producing pronociceptive effects in the tail-withdrawal assay and produced longer-lasting effects compared to N/OFQ (Rizzi et al., 2002a).

Guerrini et al.(2001) focused their modification on the Phe^4 residue and found that para-substituted electron-withdrawing groups such as *p*-F and *p*-NO_2 increased binding affinity 5- and 3-fold, respectively. These agonist peptides were more potent than N/OFQ at recombinant hORL1 and at native ORL1 receptor sites as well as in isolated tissues (Bigoni

et al., 2002a; McDonald et al., 2002). These agonists also display longer duration of action in vivo in several assays, compared to N/OFQ (Rizzi et al., 2002b). These longer-lasting potent agonists will serve as useful tools to study the physiological roles of N/OFQ.

Another novel agonist peptide recently reported is cyclo [Cys^{10}, Cys^{14}] NC(1–14)NH_2, whose binding affinity is comparable to N/OFQ (Ambo et al., 2001). This cyclic peptide is the first conformationally restricted peptide with potent activity and may serve as a good template for studying the bioactive conformation of N/OFQ. The other conformationally restricted peptide that has affinity for ORL1 was peptide III-BTD (Becker et al., 1999) (Fig. 1) which was identified from a combinatorial library of h-turn constrained peptides. III-BTD has a very interesting profile of ORL1 antagonist activity coupled with classical opioid agonist activity in in vitro assays in isolated tissues (Bigoni et al., 2000a).

Combining the structure modification that led to potent, long-lasting agonist activity (Arg ^{14}, Lys ^{15}) and that which led to pure antagonism (N-Phe^1), Calo et al.(2002b) recently reported a potent, selective antagonist, [$NPhe^1$, Arg^{14}, Lys^{15}] NC-NH_2, also called UFP-101, whose potency is at least one order of magnitude greater than [$NPhe^1$]NC(1–13)NH_2. Furthermore, in the tail-withdrawal assay, UFP-101 not only inhibits the pronociceptive effect of N/OFQ but also elicited an antinociceptive effect *per se*, similar to that observed with the antagonist [$NPhe^1$]NC(1–13)NH_2 but longer lasting. This analgesic effect is also similar to that seen with the peptide antagonist retronociceptin methyl ester (Jinsmaa et al., 2000) and the nonpeptide quinoline antagonist JTC-801 (see below). This provides further evidence that ORL1 antagonists can be developed as analgesic agents.

In order to improve the stability and therapeutic utility of peptide ligands, a novel technology called structure inducing probes (SIP) (Larsen, 1999) was applied to the hexapeptide Ac-RYYRWK-NH_2 (Dooley et al., 1997), resulting in the design of the peptide Ac-RYYRWKKKKKKK-NH_2 (ZP 120) (Larsen, 2001). Like the parent peptide, ZP 120 behaves as a partial agonist at ORL1 receptors but produces a long-lasting effect in vivo (Rizzi et al., 2002c). ZP 120 was designed not to penetrate the CNS after iv administration for those indications in which ORL1 partial agonists produce selective renal diuretic and antinatriuretic effects but not cardiovascular effects (Kapusta et al., 2002a,b).

Although the design and pharmacological characterization of peptide ligands for ORL1 has facilitated great advances in elucidating the functional role of the N/OFQ-ORL1 system, the therapeutic utility of ORL1 ligands, particularly for neurological disorders, can only be realized with potent *nonpeptide* ligands, which are more likely to penetrate the CNS than peptides and can be more easily developed as drugs. Several pharmaceutical companies have discovered potent nonpeptide agonists and antagonists, as discussed below.

Nonpeptide ligands

Since the ORL1 receptor belongs to the opioid class of receptors, several groups have examined small-molecule opiate ligands for binding at ORL1. Kobayashi et al.(1997) reported that the j receptor ligands carbetapentane and rimcazole are low potency antagonists of ORL1-mediated N/OFQ effects on the G-protein activated, inwardly rectifying K^+ channels in *Xenopus* oocytes. Butour et al.(1997) tested the A-selective opiates lofentanil, an anilidopiperidine, and etorphine, an oripavine derivative (Fig. 2), and found that they not only have high affinity at hORL1 in CHO cells (lofentanil K_i = 24 nM; etorphine K_i = 0.53 μM) but also exhibit full agonist activity in cAMP inhibition assays in CHO cells. Interestingly, fentanyl, a close structural analog of lofentanil, has very low (K_i > 1 μm) affinity for ORL1. Hawkinson et al.(2000) also tested other anilidopiperidines, morphinans, and benzomorphan classes of opiate ligands and found them to be low affinity

agonists at ORL1. Our own results on the ORL1 affinities of various neuroleptics and opiates (Zaveri et al., 2001) revealed that the 5-HT partial agonist spiroxatrine, the neuroleptic pimozide, and the partial μ agonist buprenorphine (Fig. 2) had good affinity for ORL1 (K_i = 127 nM, 216 nM, and 112 nM, respectively) and could serve as useful leads for the development of ORL1-selective ligands. Indeed, the recently reported ORL1 antagonist J-113397 (Banyu) and Ro 64–6198 (Roche) bear close structural resemblance to pimozide and spiroxatrine, respectively, differing from these leads in the piperidine nitrogen substituent.

Another opiate that has served as a lead for the design of selective ORL1 ligands is the morphinan naloxonebenzoylhydrazone (NalBzoH) (Fig. 3). NalBzoH is a κ opioid agonist and a μ antagonist and has an antinociceptive effect in vivo (Gistrak et al., 1989). NalBzoH was shown to antagonize the effects of N/OFQ on cAMP accumulation in CHO cells and had a binding affinity of ~25 nM (Noda et al., 1998; Bigoni et al., 2002b). Like the ORL1 antagonists UFP-101 and JTC-801, NalBzoH not only blocks the pronociceptive effects of N/OFQ in vivo but also produces an antinociceptive effect *per se* (Noda et al., 1998). Interestingly, this antinociceptive effect is completely abolished in ORL1 knockout mice (Noda et al., 1998), suggesting that the ORL1 receptor plays a role in determining nociceptive threshold.

As discussed below, the above-mentioned nonselective opiate ligands have thus far provided useful leads for the design of selective ORL1 ligands. These nonpeptide ligands, both agonists and antagonists, can be broadly divided into five structural classes. Most of these ligands were first reported in the patent literature.

Morphinan-based ligands

In 1998, a Pfizer patent reported a series of 6-substituted morphinan hydroxamic acids, **1–3** (Fig. 3), that were claimed to have ORL1 antagonist activity (IC_{50} < 50 nM) and agonist activity at the μ, δ, and κ opioid receptors (Ito, 1998). These compounds were expected to exhibit good analgesic activity, although no biological data were reported. In 1999, Seki et al.(1999) in collaboration with Toray Industries, Japan, reported that the morphinan κ agonist TRK-820 (Fig. 3) antagonized the effects of N/OFQ on cAMP accumulation of hORL1 in CHO cells and had a binding affinity of 380 nM at hORL1. TRK-820, a 6-N-methylamido morphinan, is structurally very similar to the Pfizer hydroxamic acids. Thus, the morphinan skeleton may provide a good lead for a unique profile of ORL1 antagonism coupled with opioid agonist activity for a novel class of analgesics.

Benzimidazopiperidines

The first nonpeptide pure ORL1 antagonist discovered was a benzimidazolinone, J-113397, reported by Kawamoto et al.(1999) and patented by Banyu Pharmaceutical Co. (Ozaki et al., 1998). The lead compound **4** (Fig. 4) identified by screening a chemical library, was a low affinity agonist at ORL1. Introduction of an α-methyl group onto the piperidine N-benzyl group and substituents on the pendant phenyl ring as in **5** increased ORL1 affinity dramatically, resulting in a nanomolar affinity ligand that was also a nonselective ORL1 agonist. Replacing the N-substituent with the large lipophilic cyclooctylmethyl group, as in **6**, transformed the compound to an antagonist, albeit with low selectivity. Introduction of an ethyl substituent on the benzimidazolinone nitrogen and a 3-hydroxymethyl group on the piperidine ring reduced affinity at other opioid receptors, resulting in a potent, highly selective pure antagonist at ORL1. J-113397, the (3R, 4R) isomer has 400-fold more binding affinity than its enantiomer. Compound **7**, a racemic analog, disclosed in another patent by Banyu (Ozaki et al., 2001) which exhaustively explored the benzimidazolinone 3-nitrogen,

was also reported to be as potent as the chiral J-113397 and had equivalent antagonist potency in the GTPγS assay (IC_{50} 5.8 nM).

The effects of J-113397 have been characterized in vitro at recombinant and native ORL1 receptors and in isolated tissues (Ozaki et al., 2000a,b; Bigoni et al., 2000b; Ichikawa et al., 2001; Hashiba et al., 2001; Chiou and Fan, 2002b). In in vivo assays, J-113397, administered subcutaneously (sc) at doses of 10–30 mg/kg, inhibited the hyperalgesia produced by icv N/OFQ. Surprisingly, J-113397 had no effect by itself on baseline tail flick latencies (Ozaki et al., 2000b), unlike the other ORL1 antagonists JTC-801 and UFP-101 (Yamada et al., 2002; Calo et al., 2002b). Another conflicting report also showed that intrathecal or icv administration of J-113397 increased agitation behavior in the rat formalin test (Yamamoto et al., 2001). Further studies are required to clarify the role of ORL1 antagonists in analgesia.

Pfizer has also patented a series of benzimidazolinones, eg. **8**, as ORL1 agonists. They are claimed as being selective for ORL1 versus the μ receptor (Ito et al., 1999). No biological data were reported. A patent application from EuroCeltique S. A. (Luxembourg) also claims benzimidazolinones such as **9**, however, the ORL1 affinity of **9** (Fig. 4) at hORL1 was reported to be 166 nM (Kyle et al., 2001).

Pfizer has also reported three series of benzimidazoles (exemplified by **10 a–b**) as ORL1 agonists, claimed to be useful for analgesia (Ito et al., 2001a,b; Ito, 2002). While their piperidine N-substituent was similar to that in the benzimidazolinone series, the primary site of modification was the 2-benzimidazole position, where a variety of open-chain and cyclic amines were explored. However, no biological data were reported.

Spiropiperidines

Around the same time as the Banyu report on the benzimidazolinones, Hoffmann La Roche disclosed a series of 1,3,8-triazaspiro[4,5]decan-4-ones, discovered through high throughput screening. The lead compound **11** (Fig. 5) was a potent but nonselective ligand at hORL1 and also a full agonist in functional assays (Adam et al., 1998; Rover et al., 2000a; Wichmann et al., 1999). Replacement of the 2-tetralinyl piperidine nitrogen susbtituent with the larger acenaphthyl group (to give Ro 65–6570) and the even bulkier hexahydrophenalenyl group (to give Ro 64–6198) (Wichmann et al., 2000) resulted in subnanomolar affinity ORL1 ligands, with >100-fold selectivity over the opioid receptors. Alicyclic substituents at the piperidine nitrogen such as cyclodecyl (as in **12**) and 4-isopropylcyclohexyl (as in **13**) are also reported to give highly potent and selective ORL1 agonists (Rover et al., 2000b).

Although Ro 65–6570 was found to show anxiolytic effects in an elevated plus-maze test (Wichmann et al., 1999), it was only 5 to 10-fold selective over μ receptors (Hashiba et al., 2001). Ro 64–6198, on the other hand, is far more selective and has shown an impressive anxiolytic profile comparable to benzodiazepines, in several in vivo anxiety paradigms (Jenck et al., 2000). As an agonist only slightly less potent than N/OFQ itself (Hashiba et al., 2002), Ro 64–6198 can potentially be used as a therapeutic in disorders where an ORL1 agonist may prove beneficial, such as anorexia (Ciccocioppo et al., 2002), anxiety (Jenck et al., 1997), and inhibition of reward pathways (Dautzenberg et al., 2001). However, Ro 64–6198 was found not to affect cocaine-induced conditioned place preference (Kotlinska et al., 2002). Moreover, at higher doses, Ro 64–6198 was found to have affinity for dopamine and σ receptors (Jenck et al., 2000; Rizzi et al., 2001b).

Roche has also patented several series of spiropiperidines modified in the heterocyclic imidazoline portion, such as **14** (Fig. 5), which were claimed as agonists (Adam et al.,

2000a), and **15**, diazaspiro[3,5]nonane derivatives, also claimed as agonists (Adam et al., 2000b).

Further modification of the heterocyclic portion as well as the piperidine N-substituent has resulted in a series of spirofused benzofuranones, such as **16** and **17** (Fig. 5), patented by Roche and claimed as ORL1 'antagonists' (Adam et al., 2000c). No details on the biology of any of these patented compounds are reported.

Interestingly, Novo Nordisk has also reported on the synthesis and characterization of 1,3,8-triazaspirodecanones, similar to the Roche compounds, starting with spiroxatrine as their lead (Thomsen and Hohlweg, 2000; Hohlweg et al., 2001). Their best ligand, NNC 63–0532 (Fig. 6) had binding affinity of 6.3 nM against human ORL1 and only a 12-fold selectivity for ORL1.

Pfizer has also patented a series of triazaspirodecanones such as **18** (Fig. 6) that are claimed as ORL agonists, useful as analgesics. No further details were reported (Ito and Ohashi, 2000). Banyu, on the other hand, has claimed as ORL1 antagonists two series of triazaspirodecanones, exemplified by **19** and **20** (Fig. 6). The series represented by **19** (Kawamoto et al., 2000) are similar to the Roche and Pfizer ligands Ro 64–6198 and **18**, but ligands like **20** are isomeric compounds, with a subtly different spiro junction between the imidazolinone and piperidine rings (Satoh et al., 2000, 2001).

The unavailability of any pharmacological data on these compounds makes it difficult to define clearly the structural requirements for ORL1 affinity and selectivity.

Aryl piperidines

While the earlier ligands reported by Banyu and Roche contained spiro- and benzofused heterocycles in the 4-position of the central piperidine ring, Schering has recently reported a series of phenyl-piperidines, a new template, as ORL1 agonists, in a very broad patent that also encompassed the benzimidazolinones and triazaspirodecanones (Tulshian et al., 2001a). The ORL1 agonists **21** and **22** (Fig. 7) were claimed as being useful in the treatment of cough (Tulshian et al., 2001b).

Hoffman La Roche has also claimed a series of phenylpiperidines such as **23** as ORL1 ligands, although no details on the biology are reported (Cesura et al., 2000).

Smith Kline Beecham also patented a series of phenylpiperidines such as **24** as ORL1 ligands for several disorders related to the ORL1 receptor. The binding affinities for these compounds range from 1 to1000 nM (Barlocco et al., 2000).

4-Aminoquinolines

These are an entirely novel chemical class of ORL ligands recently disclosed by Japan Tobacco Inc. in a patent (Shinkai et al., 1999), the relevant details of which were also published in the literature (Shinkai et al., 2000). The optimized ligand, JTC-801 (Fig. 8), was obtained through an extensive structure-activity study of lead compound **25**, whose ORL affinity was 369 nM. SAR analysis also determined that the primary amino group in the 4-position of the quinoline, and the unsubstituted amide moiety, were important for binding. The 2-methyl group and the ortho-substituted phenethyl moiety were also optimum for binding affinity. Detailed pharmacological studies with JTC-801 were recently reported (Yamada et al., 2002). Its binding affinity for h ORL1 was 44.5 nM. It completely antagonized the inhibition of cAMP accumulation by N/OFQ. Furthermore, when administered in vivo orally or iv, at doses of 0.1–1 mg/kg, it antagonized N/OFQ-induced allodynia in mice and increased latency in the mouse hot plate test. These effects were not

inhibited by naloxone. JTC-801 was chosen as the clinical candidate for clinical trials for analgesia because of its oral bioavailability profile, which was more favorable than that of some other more potent analogs in this series. Intriguingly, the benzimidazolinone J-113397, also an ORL1 antagonist, has no analgesic effects *per se*, in similar models of nociception. This dichotomy in the effects on analgesia by the two structurally different ORL1 antagonists clearly requires further investigation.

Conclusions

Although, there is clearly an explosion of drug discovery efforts in the identification of small-molecule ORL1 ligands, the lack of availability of these ligands for detailed pharmacological studies has hampered the evaluation of the ORL1 receptor as a novel therapeutic target in several human disorders. Certainly, the ambiguities in the role of the ORL1-N/OFQ system in pain modulation and anxiety can be resolved by the availability and dissemination of pure agonists and antagonists discovered thus far.

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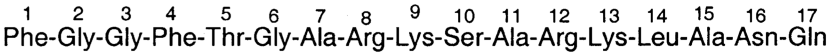
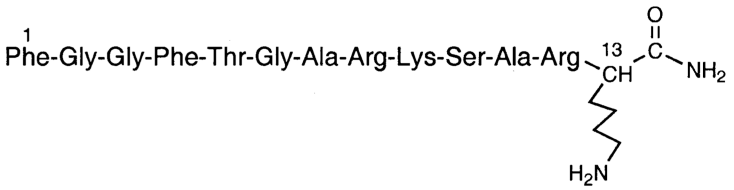
N/OFQ

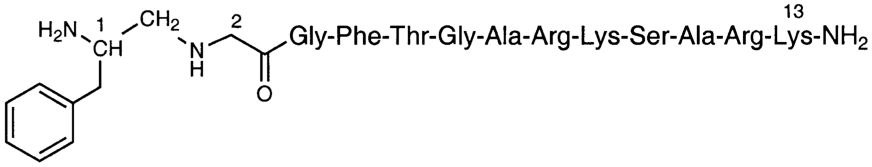


NC(1-13)NH_2



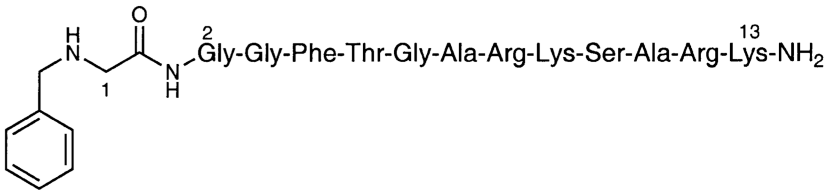
[$Phe^1\psi(CH2NH)Gly^2$] NC(1-13)NH2

(Partial agonist)



[N-Phe^1]NC(1-13)NH_2

(Pure antagonist)



Hexapeptides (Partial agonists)

Peptide	[^{3}H] N/OFQ Binding K_i (nM)
Ac-RYYRWR-NH_2	0.60 ± 0.05
Ac-RYYRWK-NH_2	0.71 ± 0.11
Ac-RYYRIK-NH_2	1.50 ± 0.11
Ac-RYYLWR-NH_2	1.47 ± 0.64
Ac-RYYKWK-NH_2	0.72 ± 0.21
N/OFQ	0.43 ± 0.05

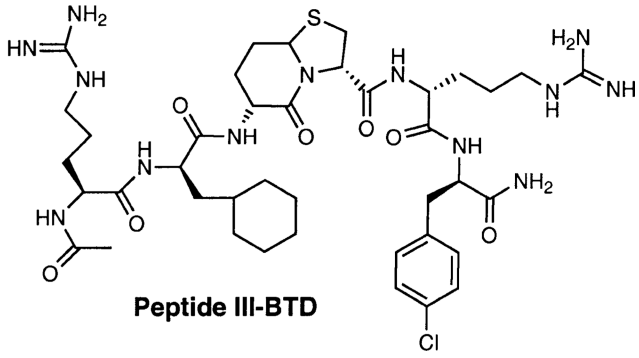


Fig. 1.
Structures of peptide ligands for the ORL1 receptor.

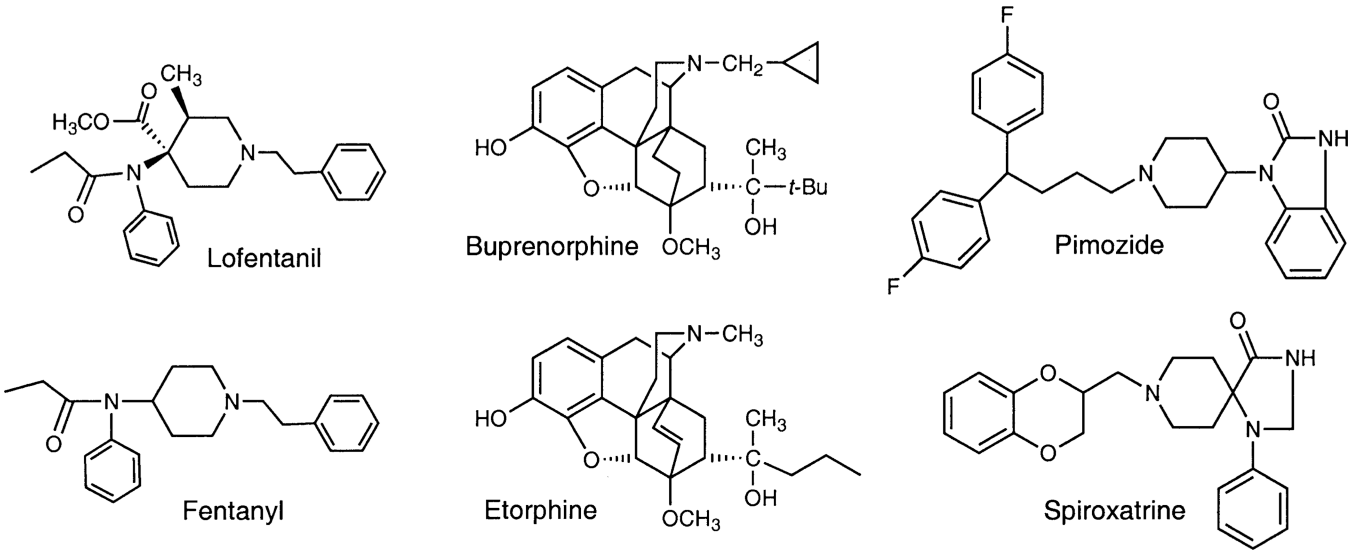
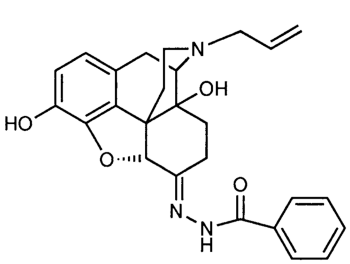
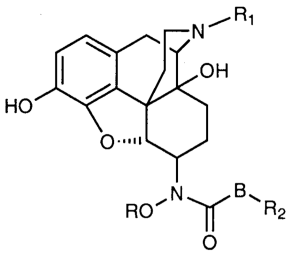


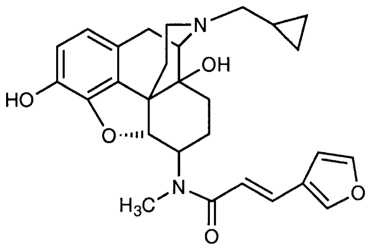
Fig. 2.
Structures of known opiates and neuroleptics that bind to the ORL1 receptor.



Naloxonebenzoylhydrazone



Pfizer morphinan hydroxamic acids

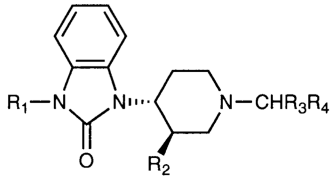


TRK-820

1	R=H	R_1=cyclopropylmethyl	R_2=dichlorophenyl	B= CH_2
2	R=Me	R_1=cyclopropylmethyl	R_2=dichlorophenyl	B= CH2
3	R=Me	R_1=allyl	R_2=furyl	B= CH=CH

Fig. 3.
Structures of the morphinan class of ORL1 ligands.

Banyu compounds



					Binding IC_{50} (nM)			GTPγS
	R_1	R_2	R_3	R_4	ORL1	μ	κ	Antagonism IC_{50} (nM)
4	H	H	H	Ph	200	1700	110	agonist
5	H	H	Me	2-Cl Ph	5.9	43	32	agonist
6	H	H	H	c-Oct	6.8	780	12	270
J-113397	Et	CH_2OH	H	c-Oct	2.3	2200	1400	5.6
7 (racemic)	CH_2CHF_2	CH_2OH	H	c-Oct	1.9	NA	NA	5.8

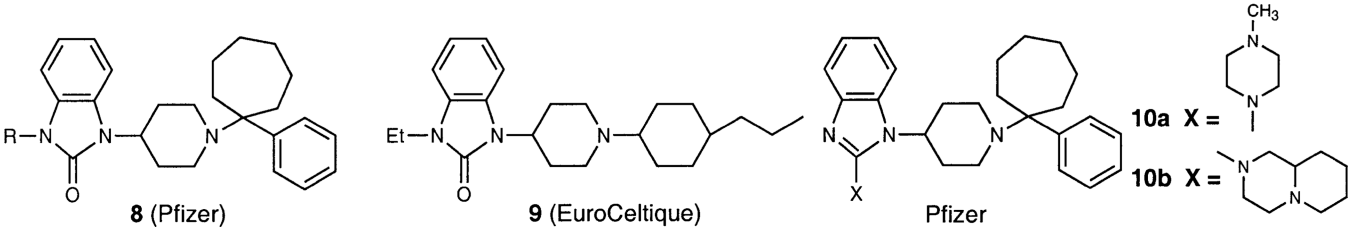
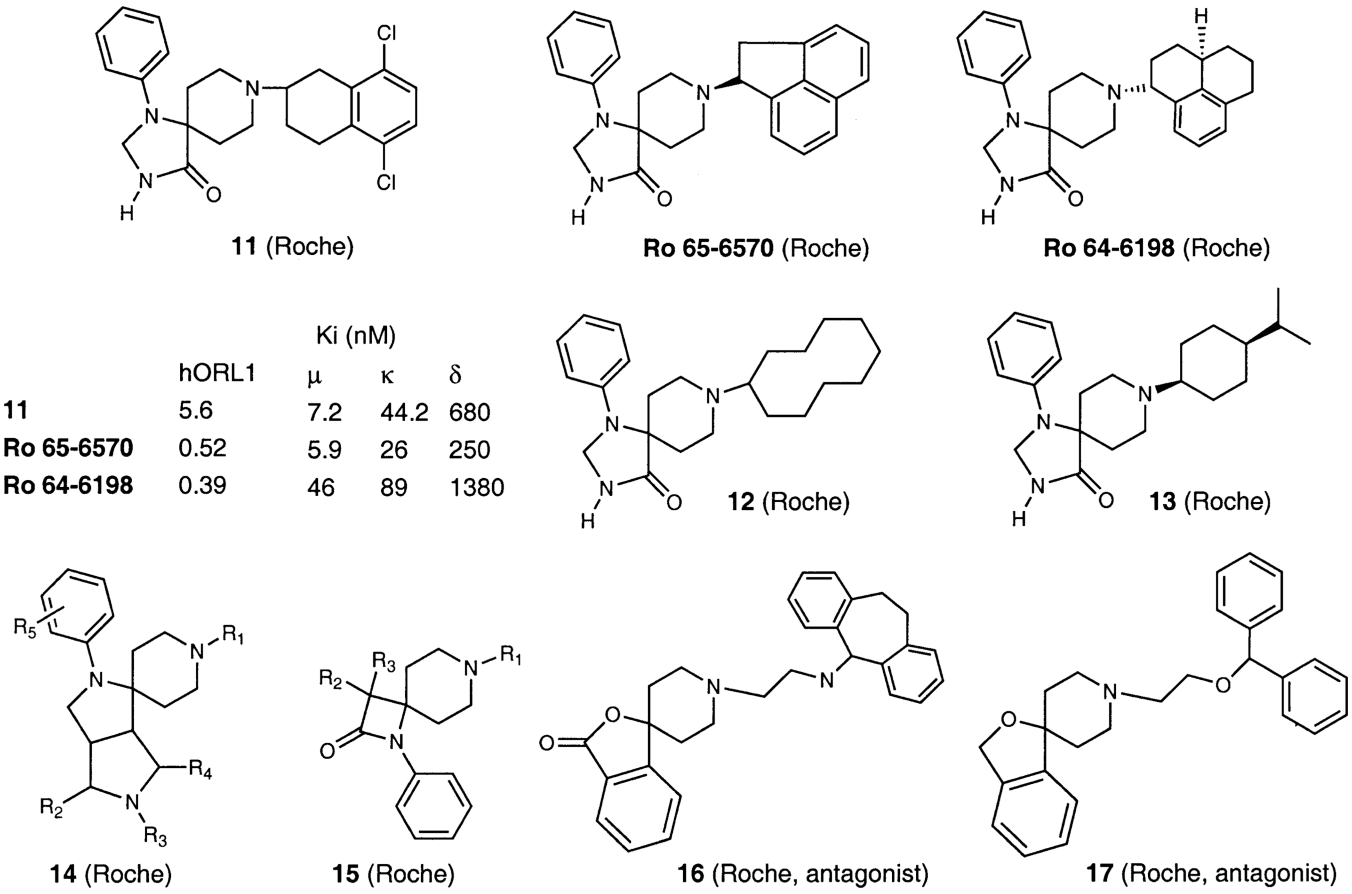


Fig. 4.
Structures of the benzimidazopiperidine class of ORL1 ligands.



		Ki (nM)		
	hORL1	μ	κ	δ
11	5.6	7.2	44.2	680
Ro 65-6570	0.52	5.9	26	250
Ro 64-6198	0.39	46	89	1380

Fig. 5.
Structures of the 1,3,8-triaza[4,5]spirodecanones and other spiropiperidine ORL1 ligands.

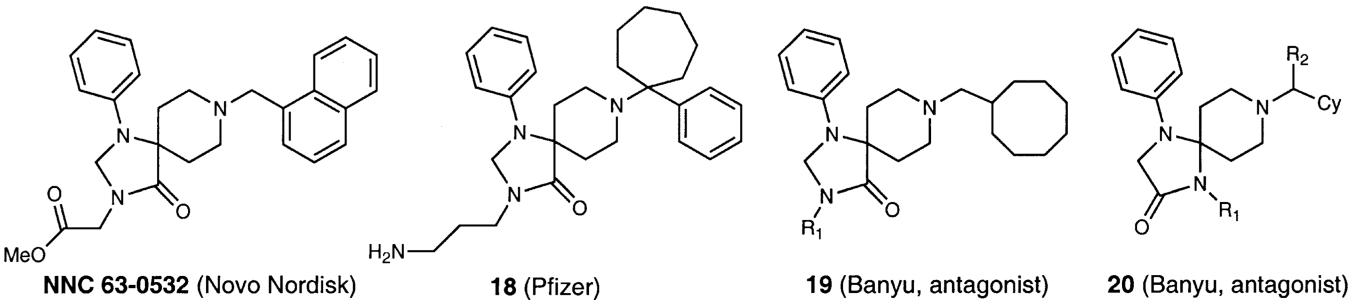


Fig. 6.
Structures of spiropiperidine ORL1 ligands.

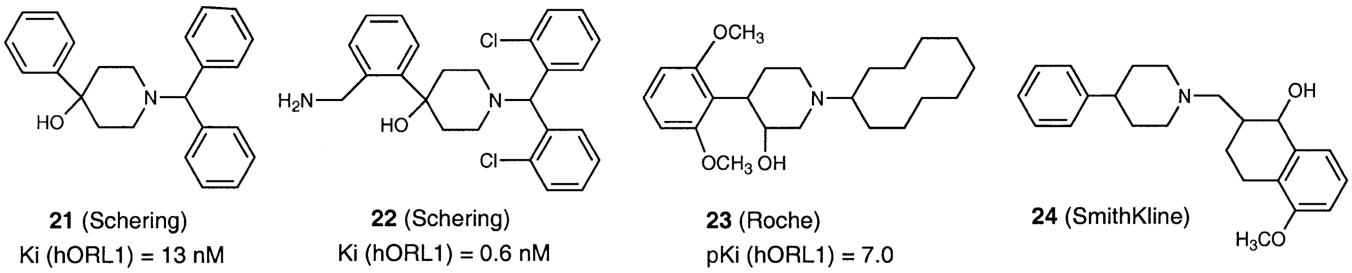


Fig. 7.
Structures of the arylpiperidine class of ORL1 ligands.

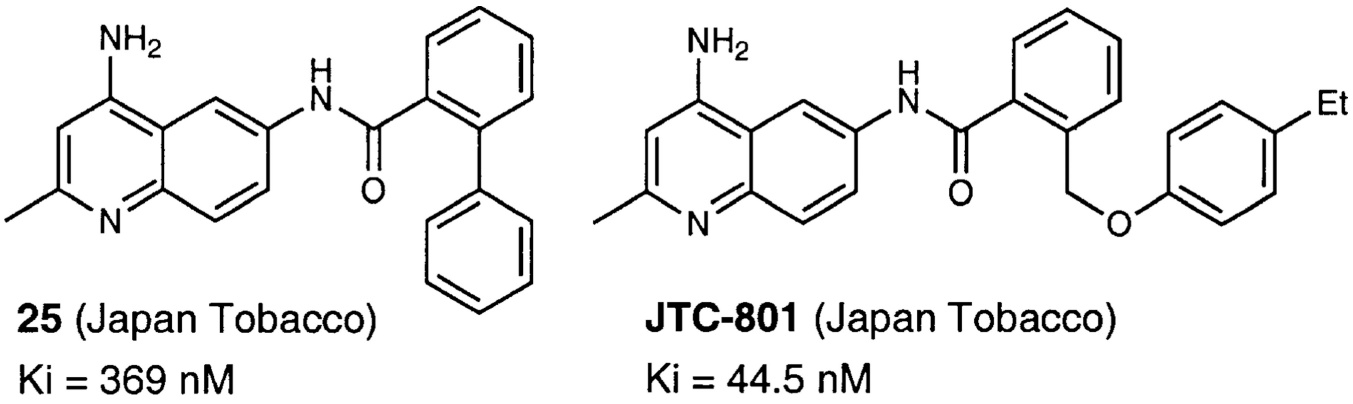


Fig. 8.
Structures of the aminoquinoline class of ORL1 ligands.