Non Peptidic Ligands at the Opioid Receptor Like-1 (ORL-1)

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Abstract: Opioid receptor like-1 (ORL-1) has recently been indicated as a potentially useful target for the treatment of a number of central disorders and several other diseases.

This review deals with non peptidic ligands at the ORL-1 receptor, focusing on their structural and binding properties. Agonism or antagonism evidenced from functional experiments is also commented. For some compounds, possible therapeutic applications are considered.

INTRODUCTION

Besides the three classical opioid receptors (μ, δ,and κ) a fourth receptor, opioid receptor like 1 (ORL-1), was identified in 1994 through cDNA expression cloning techniques [1]. This receptor is a member of the G-protein coupled receptor superfamily. It has a high degree of amino acid sequence homology to the classical opioid receptors, mainly in the putative transmembrane domains and cytoplasmic loops. In particular, Meunier [2] underlines the presence both in kappa receptors and ORL-1 of a highly acidic second exofacial loop. However, neither native opioid peptides nor synthetic opioid agents expressed significant affinity to ORL-1. This led to the search for a natural ligand, which was separately identified by two groups and named orphanin FQ (OFQ) [3] and nociceptin (NC) [4], respectively. It was found to be a heptadecapeptide, whose sequence was very similar to the opioid peptide dynorphine A (See Chart 1). Similarly to other neuropeptides, it was originated by a larger precursor, which was identified as a protein of 181 amino acids, named both prepro-orphaninFQ and prepronociceptin [5-7]. Besides NC, the precursor originates several peptides, the cleavage site being generally represented by pairs of the basic amino acids Lys-Arg. In particular, Ito and his group [8] have recently reported the identification of nocistatin, similar in length to nociceptin, but unable to bind the ORL-1 receptor. ORL-1 is negatively coupled to adenylate cyclase and calcium channels and positively coupled to inwardly rectifying K^+ channels. The receptor is widely distributed and nociceptin exerts several biological effects both in the central and peripheral nervous systems. Amongst others, its role in learning and memory, pain response, morphine tolerance, motor processes, neuroendocrine secretion, and cardiovascular system has been reported.

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

Chart 1.

Molecular models of the ORL-1 receptor have been built based on bovine [9] or frog [10] rhodopsin models. Modeling of the complexes with nociceptin and other opioids elucidated the mechanism of interaction with ligands [11]. For an exhaustive information on all details of ORL-1 receptor and nociceptin see previous reviews [12-18].

Though a lot of literature data are available on nociceptin and its analogues, the lack of selective ORL-1 antagonists has hampered a full understanding of the physiological roles of the NC/ORL-1 system. A potential antagonist at the ORL-1 was identified in the naloxonebenzoylhydrazone (NalBzOH) [19,20]. Unfortunately, this compound interacts also with κ-opioid receptors. The pseudopeptide [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]-NC(1-13)-$NH_2$, was reported by Calo' *et al.* [21] as a selective antagonist at ORL-1 in guinea pig ileum and vas deferens. However, the compound behaves as an agonist in controlling cAMP level in both CHO cells transfected with human ORL-1 and mouse N1E-115 neuroblastoma. Further studies from the same group have recently identified a related peptide as a pure antagonist both in vitro and in vivo, though less potent (pKi = 7.0; pK_B =

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6.3) [22]. Based on their results the Authors suggest that the chemical requirements for ORL-1 agonists are different from those of antagonists. However, the need still exists for non-peptide agonists and antagonists.

The observation that amongst many potent opioid ligands, only lofentanil (See Chart 2), a potent μ-agonist, showed a significant affinity for the human ORL-1 (Ki = 24 nM) [18] led to the use of its 4-anilinopiperidyl skeleton as a potentially useful substrate to develop novel more potent and selective non-peptidic ORL-1 ligands. This review focuses on such compounds, as useful tools to elucidate the physiological roles of the NC/ORL-1 system, as well as the therapeutic potential of ORL-1 agonists and/or antagonists. They are indicated as useful agents for the treatment of numerous pathologies, including pain [23], eating disorders [18], anxiety [24] and stress conditions [25], immune [26] and cardiovascular [27] system dysfunction, memory loss [28], several neurodegenerative processes [22], adult respiratory distress syndrome (ARDS) [17], and many others [14].

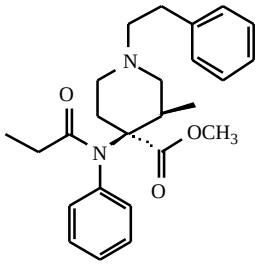


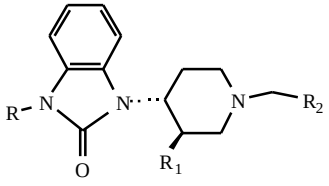
Chart 2. **Lofentanil**

DISCUSSION

Researchers from Banyu Pharmaceutical Company, in collaboration with Merck Research Laboratories, have recently reported [29,30] the structure of 1-(1-benzyl-4-piperidyl)-1,3-dihydro-2*H*-benzimidazol-2-one (**1a**), which was identified from their chemical library as a potential lead. In fact, it showed suitable affinity for ORL-1 receptors. However, poor selectivity over μ− and κ−receptors was also observed (See Table 1). Further studies to identify the structural requirements of this class for selective ORL-1 ligands led to a number of cyclooctyl analogs (**1b-e**), which displayed full antagonistic activity in the GTPγS assay. The most potent compound resulted by the simultaneous introduction of an ethyl group on the benzoimidazolinone nitrogen and of a hydroxymethyl group into the piperidine ring. Separation of the stereoisomers showed that 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**1d**, J-113397) showed excellent potency (IC_{50} = 2.3 nM) and antagonistic activity (IC_{50} = 5.6 nM), accompanied by a significant selectivity over μ, δ, and κ. The (3*S*,4*S*)-enantiomer (**1e**) was by approximately 400-fold a weaker ligand for the ORL-1 receptor (See Scheme 1 and Table 1). It should be noted that **1d** was found active in vivo when tested for antagonism against nociceptin produced hyperalgesia [31]. 1-Cyclooctyl-methyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3- dihydro-2*H*-benzimidazol-2-one, as a racemic mixture, was extensively tested in vitro also by an Italian group [32] and found to antagonize the nociceptin-induced inhibition of cAMP formation in cells expressing the recombinant human ORL-1 receptor (pA_2 = 7.52) and to displace [^{125}I]Tyr(14)nociceptin (pKi = 8.56). Many other effects were investigated, which led the Authors to conclude that it is a high-affinity, selective and competitive antagonist of the ORL-1 receptor.

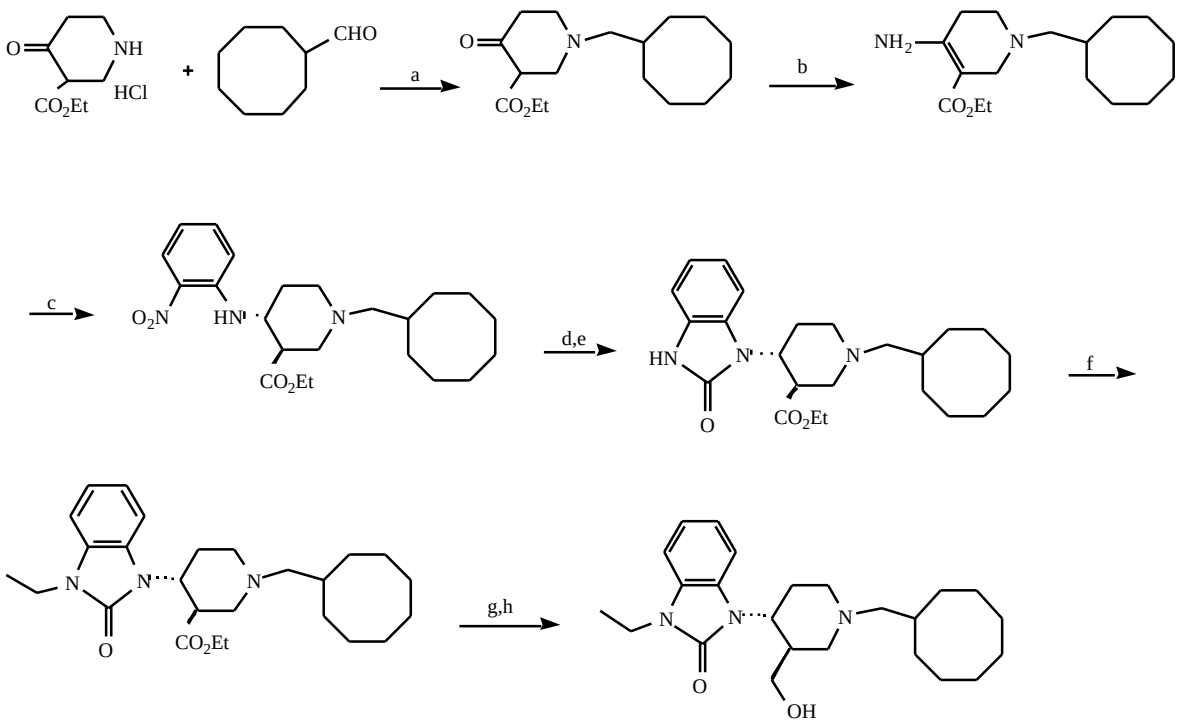
Parallel to this report, a group from Hoffmann-La Roche published a different series on ORL-1 ligands, namely 8-substituted-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones [33]. Similarly to the Banyu compounds, this class contains a piperidine ring and bears some resemblance to lofentanil.

Table 1. Structure and Biological Properties of Benzimidazolidinone Derivatives (1a-e)



				Binding IC_{50} nM[a]			GTPγS	
Compd	**R**	**R_1**	**R_2**	**ORL-1[b]**	**μ[c]**	**κ[d]**	**Antagonism IC_{50} (nM)[e]**	**Agonism EC_{50} (nM)[f]**
1a	H	H	Ph	200	1700	110	>10000	6900
1b	H	H	*c*-Oct	6.8	780	12	270	>10000
1c	Et	H	*c*-Oct	6.8	950	440	20	>10000
1d (J-113397) (3*R*,4*R*)	Et	CH_2OH	*c*-Oct	2.3	2200	1400	5.6	>10000
1e (J-112444) (3*S*,4*S*)	Et	CH_2OH	*c*-Oct	820	3300	2600	>10000	>10000

a) Assays made on CHO cells stably expressing cloned human ORL-1 receptor and μ−, κ−opioid receptors.
b) Displacement of [^{125}I]Tyr^{14}-nociceptin.
c) Displacement of [^{3}H]diprenorphin.
d) Displacement of [^{3}H]U-69593.
e) Values on nociceptin-produced [^{35}S]GTPγS binding to ORL-1 expressed in CHO cells.
f) Values relative to the maximal [^{35}S]GTPγS binding produced by nociceptin in ORL-1 expressed CHO cells.
All the values are the mean of three independent determinations performed in duplicate (See ref. 29).

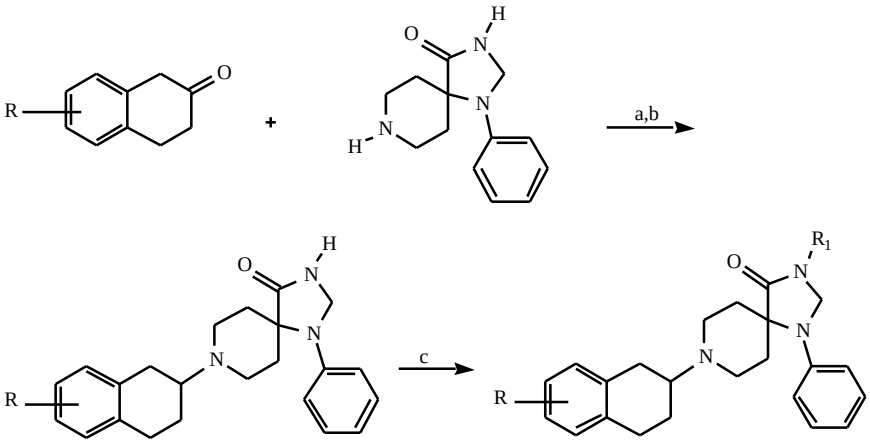


1d

(a) $NaB(OAc)_3H$, THF; (b) $AcONH_4$, MeOH; (c) 2-fluoronitrobenzene, $NaBH_3CN$, Na_2CO_3, n-BuOH, reflux; (d) H_2, Pd-C, MeOH: (e) carbonyldiimidazole, $CHCl_3$; (f) EtI, NaH, DMF; (g) $LiAlH_4$, THF; (h) optical resolution by CHIRALPAK AD, hexane/2-propanol/Et_2NH = 800/200/1.

Scheme 1.

Also in this case the model 8-(5,8-dichloro-1,2,3,4-tetrahydro-naphthalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one (**2a**) was a high-throughput screening hit. The compound was tested in radioligand binding assay using membranes expressing hORL-1 receptors (HEK 293 cells) or membranes from BHK cells infected with Semliki Forest virus encoding the cDNA for hμ, hδ, or hκ receptors. It was a potent but unselective ORL-1 receptor ligand (Ki = 5.6 and 7.2 nM, for ORL-1 and μ, respectively) and was shown to be an agonist at the hORL-1 receptor in GTPγS (EC_{50} = 63 nM). Based on these preliminary results, several analogs were then synthesized according to Scheme 2 and tested as reported above. In particular, variations at the 3-amidic and the 8-lipophilic substituents were considered. The data clearly indicate that the chlorine atoms do not contribute in special way to the binding, since the unsubstituted structure (**2f**) is as active as the 5,8-Cl_2-disubstituted model (**2a**); the only evident exception was the 6-Cl (**2c**), which was a much weaker ligand. On the contrary, the stereochemistry of **2a** proved to be extremely important, since resolution of the racemate demonstrated that the (*S*)-enatiomer (**2g**) is about 8 times more potent than the (*R*)-enantiomer (**2h**) on ORL-1, while the opioid receptors did not distinguish between the enantiomers. This resulted in a better selectivity for **2g**. When the nature of the amide nitrogen substituent was considered, it was clear that the amide proton does not participate in any type of H-bonding, since the presence of small alkyl groups as well as of small polar groups was well tolerated. Only an increased size of the substituent in this position led to a slight decrease in affinity, probably due to steric hindrance (See Scheme 2 and Table 2). In fact, the Authors suggest that this part of the molecule should be exposed to the water layer. Based on the evidence that the amide substitution was unable to increase the selectivity, the Authors went back to variations on the lipophilic substituent at position 8 of **2a**. Either different moieties, e.g. indanyl, and different points of attachment from position 2 to 1, were considered. Both 1-indanyl- (**2p**) and 1-tetralinyl- (**2o**) derivatives proved to be successful (See Table 3), showing high affinity and significant selectivity. The better properties of 1- vs 2-substitution in both series were interpreted as the consequence of a stability gain of the gauche conformers (**2o, 2p**) vs the anti conformers (**2f, 2q**). As previously evidenced for **2a**, also in this case the enantiomers of **2o** were separated. Quite surprisingly, the better compound had opposite stereochemistry, the (*R*)-enantiomer (**2s**) being about 4 fold more potent at ORL-1 than the (*S*)-isomer (**2r**). However, its selectivity was still very poor (Ki = 2.5 and 12.3 nM for ORL-1 and μ, respectively). Based on the assumption that altogether these results could suggest the existence in the ORL-1 receptor of a larger lipophilic binding pocket, the Authors synthesized two new derivatives, namely 5-methyl-1-tetralinyl (**2t**) and the acenaphthenyl derivative (**2u**), having a higher steric demand. Their hypothesis proved to be correct, both compounds being very potent and fairly selective (See Table 3).



2a: R = 5,8-Cl_2
2b: R = 5-Cl
2c: R = 6-Cl
2d: R = 7-Cl
2e: R = 8-Cl
2f: R = H
2g: R = 5,8-Cl_2 (S)
2h: R = 5,8-Cl_2 (R)

2i: R = 5,8-Cl_2; R_1 = Me
2j: R = 5,8-Cl_2; R_1 = allyl
2k: R = 5,8-Cl_2; R_1 = benzyl
2l: R = 8-Cl; R_1 = CH_2COPh
2m: R = 8-Cl; R_1 = CH_2CH_2OH
2n: R = 8-Cl; R_1 = CH_2OMe

(a) MS 4Å, toluene, reflux; (b) $NaBH_3CN$, THF/EtOH, rt; (c) NaH, DMF, 80°, then R_1-X, rt.

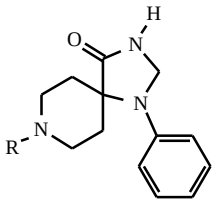
Scheme 2.

Table 2. Binding Affinity of Compounds 2a-n.[a]

Compd	hORL-1 Ki (± SEM) [nM]	μ Ki (± SEM) [nM]	κ (Ki ± SEM) [nM]	δ (Ki ± SEM) [nM]
2a	5.6 (1.1)	7.2 (1.7)	44.2 (8.4)	680 (160)
2b	2.9 (0.8)	8.9 (3.0)	20.7 (6.7)	450 (80)
2c	218 (55)	77 (19)	149 (41)	1800 (500)
2d	5.8 (1.4)	10.9 (1.6)	16.4 (4.5)	480 (150)
2e	7.3 (2.2)	16.6 (2.2)	48 (16)	1410 (420)
2f	6.3 (2.1)	15.1 (5.5)	47.1 (9.1)	620 (280)
2g (*S*)[c]	2.8 (0.9)	5.9 (2.6)	40.1 (8.0)	415 (74)
2h (*R*)[c]	20.7 (7.8)	8.4 (2.2)	47.3 (7.8)	587 (30)
2i	3.3 (1.1)	2.6 (0.9)	17.0 (2.4)	313 (64)
2j	6.8 (2.0)	5.9 (1.8)	26.2 (9.4)	n.d.[b]
2k	15.3 (6.1)	19.2 (4.7)	52 (14)	146 (34)
2l	15.0 (1.5)	61 (23)	28.8 (7.5)	510 (190)
2m	7.2 (0.9)	3.3 (1.0)	16.4 (2.4)	285 (58)
2n	8.5	7.5	17.5	240

a) The data are the mean of at least two different experiments performed in triplicate. The K_d of the radioligands were: [^{3}H]NC 70 pM for hORL-1 receptors, [^{3}H]naloxone 1.3 nM, and 2.8 nM for hμ and hκ receptors, respectively. [Ile^{5,6}-^{3}H]deltorphin 0.36 nM for δ receptors.
b) Not determined.

Table 3. Structure and Binding Affinity of Compounds 2f-u



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

a) The data are the mean of at least two different experiments performed in triplicate. The K_d of the radioligands were: [^{3}H]NC 70 pM for hORL-1 receptors, [^{3}H]naloxone 1.3 nM, and 2.8 nM for hμ and hκ receptors, respectively. [Ile^{5,6}-^{3}H]deltorphin 0.36 nM for δ receptors.
b) Not determined.
c) Resolved enantiomers of **2o**.

The efficacy of the most interesting derivatives was initially checked by their ability to stimulate GTPγS binding to membranes of HEK 293 cells overexpressing hORL-1 receptors (See Table 4). In general, they were found less potent or similar to NC. The only exception was **2u**, slightly more potent than the model. All of them behaved as agonists. In a parallel cyclase assay, **2u** showed EC_{50} comparable to that of NC (0.28 and 0.2 nM, respectively). When tested *in vivo* (ip on rats), the compound was able to increase exploration in a novel environment and to exhibit dose-dependent anxiolytic effects in the elevated plus-maze procedure. Finally, the Authors highlighted that, though the 1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one moiety is a common substructure of antipsycotic drugs, **2u** did not show any significant affinity either for dopamine or serotonin receptors (D_2 : 520 nM; D_3 : 1210 nM; D_4 : 350 nM). Given its interesting profile, **2u** was submitted to enantiomeric separation. Moreover, the influence of the substitution pattern on its phenyl ring was investigated [34]. The (*R*)-enantiomer (**2w**) showed better affinity for the ORL-1 than the (*S*)-enantiomer (**2v**). However, its selectivity wasn't improved with respect to the racemate. As far as the phenyl ring substitution is concerned, position 3 proved to be the best. In particular, the 3-fluoro derivative (**2ue**, pKi = 9.5) had a slightly higher affinity for ORL-1 than the model (**2u**), but a similar pattern in selectivity versus the opioid receptors. On the contrary, the 2-fluoro substituted (**2ua**) significantly lost affinity for the receptor (See Table 5).

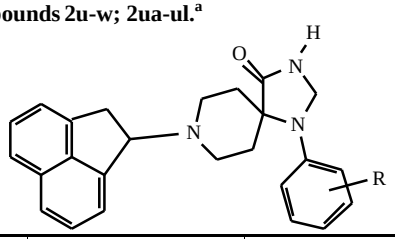
Table 4. Stimulation of GTPγS Binding by Compounds 2

	ORL-1	
Compd	**EC_{50} [nM]**	**type**
2a	1500	Full[a]
2f	490	Partial[b]
2o	87	Full
2p	63	Full
2q	500	Partial
2t	510	Full
2u	40	Full
NC	63	Full

a) Stimulation > 75% in comparison to NC.
b) Stimulation between 25% and 75%.

Since compound **2u** could be seen as a combination of 1- and 2-indanyl series, the Authors thought it worth synthesizing also the higher homolog (**3**), as a combination of the 1- and 2-tetralinyl series [35]. The compound was found to be more selective than **2u**, though the gain was very modest. However, a great increase in selectivity was observed when the 8-(2,3,3a,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives (**3a-f**), having an additional stereocenter, were synthesized. Once more, the ORL-1 proved to be less tolerant to stereochemical features than the opioid receptors. The *trans*-diastereoisomer (**3a**) was more potent at the ORL-1 than the

Table 5. Structure and Binding Affinities of Compounds 2u-w; 2ua-ul.[a]



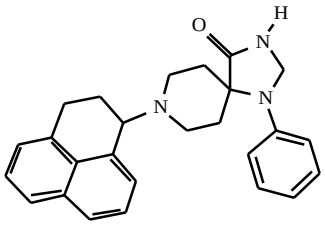
Compd	R	pKi ORL-1	pKi μ	pKi κ	pKi δ
2u	H	9.2	8.2	7.6	6.6
2v (*S*)[b]	H	8.7	7.9	7.6	<6.3
2w (*R*)[b]	H	9.6	8.4	7.7	7.0
2ua	2-F	7.4	6.4	6.8	n.d.[c]
2ub	3-Cl	8.9	7.7	7.3	6.8
2uc	3-Me	8.4	8.0	7.7	<6.3
2ud	3-OMe	7.8	7.4	n.d.	n.d.
2ue	3-F	9.5	8.4	7.9	6.8
2uf	3-Br	8.4	7.9	7.1	>6.3
2ug	3-CF_3	8.0	8.1	n.d.	n.d.
2uh	4-Cl	8.8	7.5	7.1	<6.3
2ui	4-Me	8.3	7.3	6.7	<6.3
2uj	4-OMe	7.0	n.d.	n.d.	n.d.
2uk	4-F	8.8	7.6	7.2	<6.3
2ul	3,5-Me_2	8.0	7.7	n.d.	n.d.

a) From competition binding curves using [^{3}H]-orphanin and membranes prepared from permanently transfected HEK293 cells expressing hOFQ receptors, and [^{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 the mean from three experiments performed in triplicate.
b) Resolved enantiomers of **2u**.
c) Not determined.

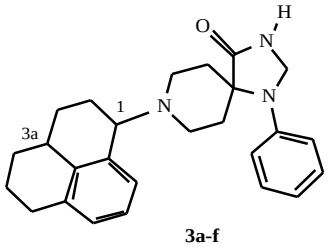
cis-analog (**3b**), while their affinity for the opioid receptors was fully comparable. Both compounds were resolved into their enantiomers. While in the case of **3a**, the (1*S*,3a*S* **3c**) was significantly more potent than the (1*R*,3a*R* **3d**) analog, for the *cis*-derivative, the enantiomers did not significantly differ (See Table 6). Compound **3c** was tested in functional assays and turned out to be a full agonist [24]. When administered i.p. to rodents, **3c** was found to decrease neophobia and to exhibit dose-dependent anxiolytic-like effects in a set of validated models of distinct types of anxiety states in the rat (elevated plus-maze, fear-potentiated startle, and operant conflict). It was found to have efficacy and potency comparable to alprazolam or diazepam. Contrary to classical benzodiazepine anxiolytic, **3c** lacked efficient anti-panic-like activity, anticonvulsant properties and effects on motor performance and cognitive function at doses ranging from 0.3 to 3 mg/kg. Higher doses (> 10 mg/kg i.p.) induced disruption in behaviour. In a broad binding evaluation on a series of receptor (e.g. serotonin $5HT_{1D\alpha}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_6$, $5HT_7$, dopamine D_1, D_3, D_4 and several others) the compound did not display any significant affinity (pKi < 6.5). On the contrary, it showed some effects on histamine H2, sigma, dopamine D2 receptors and sodium-site 2 channels (IC_{50} ~ 1 μM). Based on their results the Authors suggest that agonists at ORL-1 receptors could offer interesting possibilities for the discovery of innovative anxiolytics. They could also be useful in the treatment of other stress-related psychiatric disorders such as depression or eating disorders.

In a following paper [36], the same group expanded their studies on the 1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-ones (**4a-h**), focusing on a series of simple 8-cycloalkyl-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-ones, with the aim of avoiding chiral compounds, which do not allow a facile SAR development. The compounds were evaluated in the radioligand binding assay above reported. The data listed in Table 7 clearly show that affinity for the ORL-1 receptor increases by increasing the ring size from cyclohexyl (**4a**, Ki = 25 nM) to cyclodecyl (**4e**, Ki = 82 pM), which is equipotent to NC. Larger ring-sizes caused an evident decrease of activity, probably due to steric hindrance. Since the affinity for the opioid receptors follows a very similar trend, the only difference being a maximum of affinity at slightly larger ring-sizes, only the cyclononyl (**4d**) and the

Table 6. Stereoisomeric and Binding Properties of Compounds 3



3



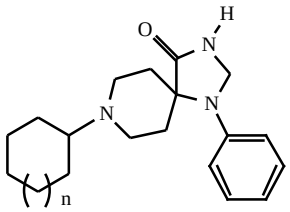
3a-f

Compd	ORL-1 pKi ± SEM	μ pKi ± SEM	κ pKi ± SEM	δ pKi ± SEM
3 (*RS*)	8.95 ± 0.14	7.56 ± 0.18	7.05 ± 0.12	6.02
3a (1*RS*,3a*RS*)[a]	9.29 ± 0.08	7.29 ± 0.09	6.98 ± 0.09	6.20 ± 0.14
3b (1*RS*,3a*SR*)[b]	8.60 ± 0.02	7.47 ± 0.07	6.80 ±0.08	6.00
3c (1*S*,3a*S*)[c]	9.41 ± 0.06	7.33 ± 0.09	7.05 ± 0.07	5.86 ± 0.04
3d (1*R*,3a*R*)[c]	7.91	7.2	6.7	6.2
3e (1*S*,3a*R*)[d]	8.68 ± 0.11	7.05 ± 0.06	6.27 ± 0.06	5.92 ± 0.23
3f (1*R*,3a*S*)[d]	8.76 ± 0.08	7.60 ± 0.10	6.84 ± 0.12	6.21 ± 0.05

a) *Trans* diasteroisomer as racemic mixture.
b) *Cis* diastereoisomer as racemic mixture.
c) Resolved enantiomers of **3a**.
d) Resolved enantiomers of **3b**.

Results are the mean of at least three experiments performed in triplicate on human ORL-1 and opioid receptors.

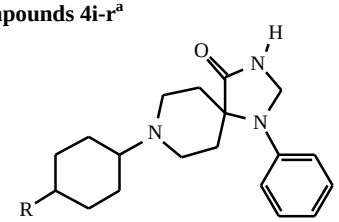
Table 7. Structure and Binding Affinities of Compounds 4a-h[a]



Compd	n	Ki rORL-1 (nM)	Ki μ (nM)	Ki κ (nM)	Ki δ (nM)
4a	1	25	158	100	n.d.[b]
4b	2	4.7	51	12	> 2000
4c	3	1.9	13	9.1	> 200
4d	4	0.24	3.2	3.9	> 200
4e	5	0.082	0.66	2.1	46
4f	6	0.49	0.21	0.82	15
4g	7	0.95	0.28	2.9	570
4h	10	600	n.d.	n.d.	n.d.

a) The data are the mean of at least two different experiments performed in triplicate. The K_d of the radioligands were: [^{3}H]NC 100 pM for hORL-1 receptors, [N-allyl-2-3-^{3}H]naloxone 3.0 nM, and 4.5 nM for rμ and rκ receptors, respectively. [Ile^{5,6}-^{3}H]deltorphin 0.10 nM for rδ receptors.
b) Not determined.

Table 8. Structure and Binding Affinities of Compounds 4i-r[a]



Compd	R	stereo	Ki rORL-1 (nM)	Ki μ (nM)	Ki κ (nM)	Ki δ (nM)
4i	Me	*trans*	41	n.d.[b]	n.d.	n.d.
4j	Me	*cis*	7.1	64	57	> 2000
4k	Pr	*trans*	52	n.d.	n.d.	n.d.
4l	Pr	*cis*	2.0	7.3	57	> 540
4m	*i*-Pr	*trans*	4.6	8.3	31	670
4n	*i*-Pr	*cis*	0.079	3.2	26	242
4o	*t*-Bu	*trans*	12	n.d.	n.d.	n.d.
4p	*t*-Bu	*cis*	3.3	6.7	38	450
4q	C-Hx	*trans*	320	n.d.	n.d.	n.d.
4r	C-Hx	*cis*	1.5	1.5	29	330

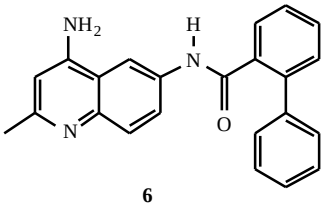
a) The data are the mean of two different experiments performed in triplicate. The K_d of the radioligands were: [^{3}H]NC 100 pM for hORL-1 receptors, [N-allyl-2-3-^{3}H]naloxone 3.0 nM, and 4.5 nM for rμ and rκ receptors, respectively. [Ile^{5,6}-^{3}H]deltorphin 0.10 nM for rδ receptors.
b) Not determined.

cyclodecyl (**4e**) showed a moderate selectivity. Given the conformational flexibility of the medium size cycloalkyl rings, and in order to explore the hypothesized lipophilic binding site of the ORL-1 receptor, the Authors prepared several compounds having a 4-alkylsubstituted cyclohexyl moiety. This led to a *trans* and a *cis* series, which were easily separated by preparative HPLC. In both series, the best substituent proved to be the 4-isopropylcyclohexyl (**4m, 4n**). However, the *cis*-compounds always resulted more potent than the corresponding *trans* (Ki's = 0.079 and 4.6 nM, respectively). Selectivity of this class is moderate, only **4n** being able to fairly differentiate the μ−opioid receptor. (See Table 8). Conformational analysis, made by NMR studies, shows that in $CDCl_3$ **4n** adopts a conformation where the basic nitrogen assumes an axial position on the cyclohexyl ring. Like the compounds previously discussed, also these derivatives proved to be agonists at the ORL-1 receptor, when tested in a GTPγS binding assay. Compound **4n** (pEC_{50} = 7.42) was fully comparable to nociceptin (pEC_{50} = 7.2).

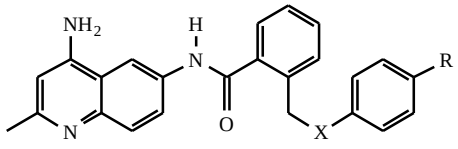
The 1,3,8-triazaspiro[4.5]decan-4-one moiety was also investigated by a group at Novonordisk [37], who based their studies on the observation that the 5-HT_{1A} spiroxatrine (See Chart 3) was a moderate (Ki = 118 nM) agonist at the human ORL-1 receptor. Chemical modifications on the compound led to (8-naphthalen-1-ylmethyl-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl)-acetic acid methyl ester (**5**, NNC 63-0532), which had remarkably improved affinity for the human recombinant ORL-1 receptor (Ki = 7.3 ± 0.9 nM) and good selectivity. In fact, when profiled in a commercial screen for activity at 75 different receptors, ion-channels and transporters, at a concentration of 1 μM, it did not display significant affinity for the majority of them. Only moderate affinity was seen for the μ-opioid (140 nM), κ-opioid (405 nM) and for dopamine D_2, D_3, and D_4 receptors. In functional assays, the compound proved to be an agonist at the ORL-1 receptor (EC_{50} > 305 nM), a much weaker agonist at the μ-opioid receptor (EC_{50} > 10 μM) and an antagonist or weak partial agonist at dopamine D_{2S} (IC_{50} = 2380 nM). It should also be noted that the compound has a high log P value (4.32), which allows it to cross the blood brain barrier.

Non piperidine-based compounds showing affinity for the ORL-1 receptor were reported by Shinkai et al. [38], having the N-(4-amino-2-methylquinolin-6-yl)-2-phenylbenzamide (**6**) as a model. This compound, during a random screening, was found to block the 51.7% of the binding of NC to the ORL-1 receptor at a concentration of 10 μM. Structure-activity relationships studies based on a receptor-binding assay with HeLa cells overexpressing the human ORL-1 receptor, led to the synthesis of several analogs, progressively modified at the substituents of the benzamide phenyl ring, the terminal ring and the quinoline moiety, as well as at the amido group. In particular, removal of the phenyl group at the *ortho*-position of the benzamide caused loss of activity, while its substitution with a benzyl group did not affect it. On the contrary, the presence of a two-atom linker between the terminal benzene and the benzamide ring significantly improved the binding of this class (See Table 9 for the most representative derivatives and Scheme 3 for the

Table 9. Structure and Binding Affinity of Compounds 6.[a]



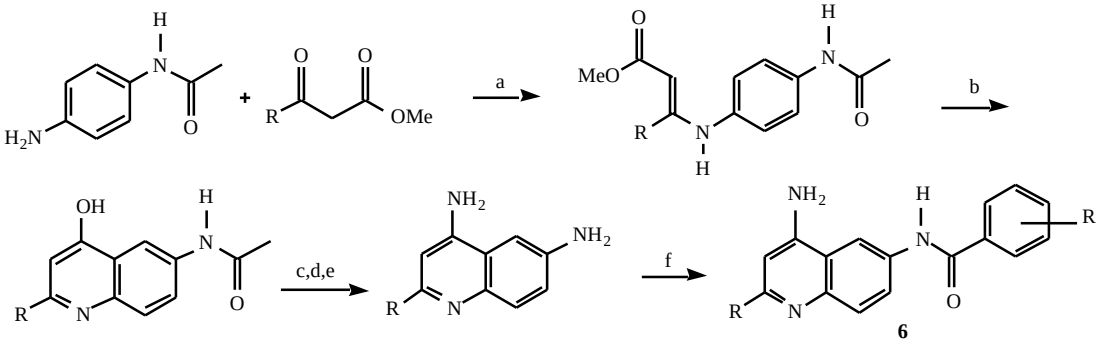
6



6a-k

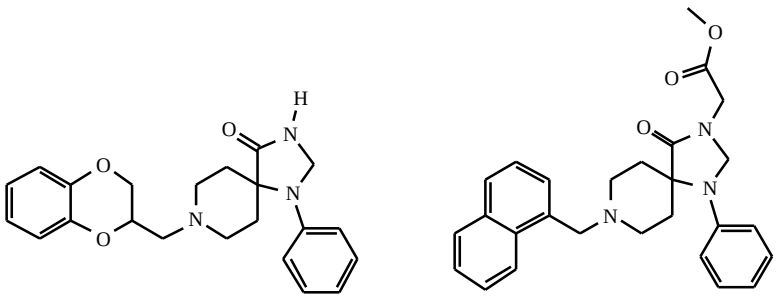
Compd	X	R	Ki (nM)[a]
6a	CH_2	H	79.9 ± 10.1
6b	O	H	50.7 ± 7.3
6c	O	CH_3	7.0 ± 1.4
6d	O	C_2H_5	8.2 ± 0.3
6e	O	OCH_3	11.8 ± 1.2
6f	O	CF_3	1.8 ± 0.2
6g	O	NO_2	2.3 ± 0.4
6h	O	Br	2.6 ± 0.3
6i	O	Cl	2.2 ± 0.3
6j	O	NH_2	82.4 ± 6.5
6k	O	OH	46.7 ± 4.9

a) Displacement of [^{3}H]nociceptin (0.5 nM) binding from human ORL-1 receptors expressed in HeLa cells. Data are given as mean ± SE of at least three experiments.



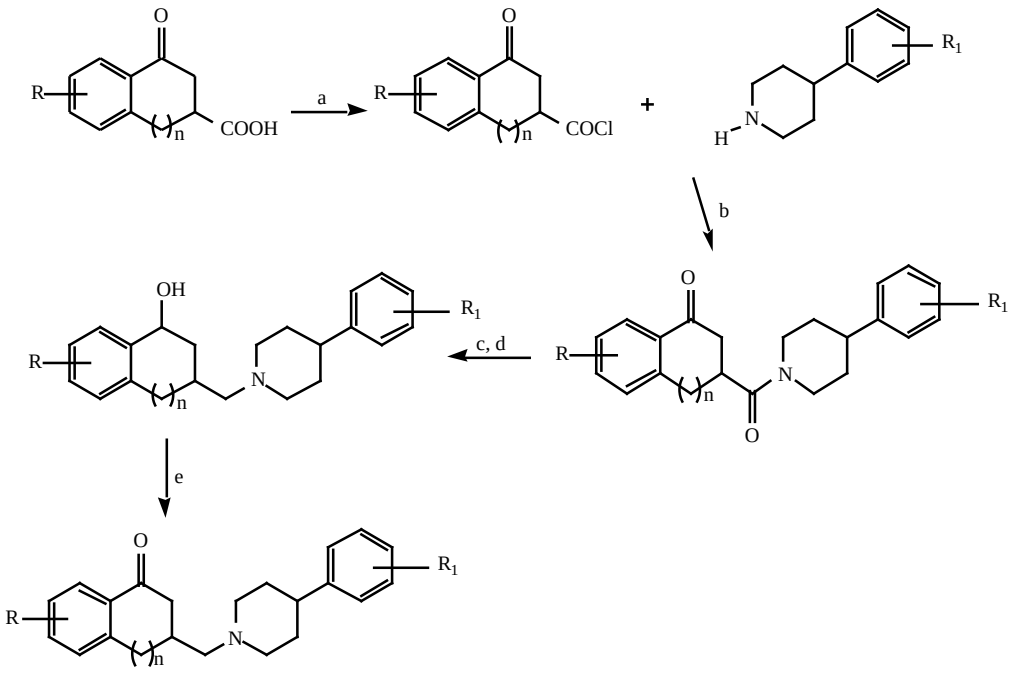
(a) Methanol, reflux; (b) Dowtherm A, 280 °C; (c) Me_2SO_4, toluene, reflux; (d) $AcONH_4$, 135 °C; (e) HCl; (f) benzoyl chlorides.

Scheme 3.



Spiroxatrine **5**

Chart 3.



(a) $SOCl_2$, 0 °C; (b) NEt_3, 0 °C; (c) $LiAlH_4$, Et_2O, 0 °C; (d) NaOH, water; (e) CrO_3, H_2SO_4.

Scheme 4.

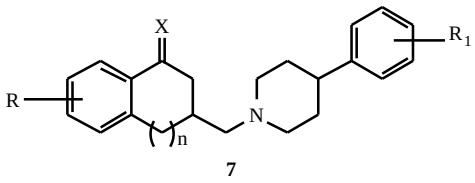
general synthetic approach). In particular, compounds **6a** and **6b** were selected for further investigation. First, moving of the phenethyl group of **6a** was studied, but the *ortho* position proved to be specific. Then, introduction of various substituents on the terminal phenyl ring of **6b** was examined. The best result was obtained with compound **6c**, which became the new lead. Several different groups (ethyl, methoxy, trifluoromethyl, nitro, bromo, or chloro) were introduced instead of the methyl group in *para* position of **6c**. All the compounds (**6d-i**) showed significant binding affinity, independently from the electronic properties of the substituent, although the effect of the electron-withdrawing groups was stronger than the electron-donating groups. However, introduction of either an amino (**6j**) or a hydroxy group (**6k**) at the *para* position decreased the affinity, though by different degrees. These results indicated that lipophilic, but not hydrophilic, substituents at this position played an important role in the enhancement of binding. In addition, the position of the substituent proved to be important, and its moving to the *meta* or *ortho* position resulted detrimental.

The SAR on the quinoline ring showed that both the primary 4-amino- and the 2-methyl- groups are essential for the activity. Insertion of an isoquinoline instead of a quinoline moiety led to a weaker but still active derivative, thus indicating that the nitrogen atom is not directly involved in binding. Finally, no substitution at the amide-nitrogen nor its inversion were tolerated.

Compound **6d**, though not the most active in the binding assay, was chosen for further *in vivo* studies, because it showed the best bioavailability. It did not inhibit forskolin-stimulated cyclic AMP accumulation in human ORL-1 receptor-expressing HeLa cells, but it prevented nociceptin-induced inhibition of cyclic AMP accumulation, thus indicating that it possesses full antagonistic activity. Oral administration of **6d** decreases allodinya induced by intrathecal injection of nociceptin. The analgesic activity of **6d** was verified on the hot-plate test (mice) and the biphasic paw-licking response induced by formalin injection in rats. In both cases, the analgesic effect was not reversed by naloxone, thus ruling out the involvement of the opioid receptors. In fact, the selectivity of **6d** for ORL-1 over μ, κ, and δ receptors is about 12.5, 129, and 1055-fold.

Researchers from SmithKline Beecham in collaboration with the University of Milan [39] have recently patented a series of compounds of general formula **7**, which are said to be either agonists or antagonists at the ORL-1 receptor. Their synthetic approach and some of the most representative compounds are depicted in Scheme 4 and Chart 4, respectively. As constantly seen for ORL-1 agents, also for this series the affinity was greatly affected by stereochemical factors, when the substrate presented chiral atoms and/or multiple bonds. Therefore, enantiomers and geometrical isomers were isolated when possible and tested in comparison with their racemates and diastereoisomeric mixtures. As already seen for other ORL-1 ligands, normally one enantiomer proved to be more potent at the ORL-1 receptor than the other. On the contrary, the opioid receptors were less influenced by steric demands, what resulted in a better ORL-1/opioid receptor selectivity. Their affinity at the ORL-1 receptor ranged from 1 to 1000 nM. [^{3}H]-Nociceptin was used as radioligand.

Several other patents [40-46] have recently appeared, dealing with the ORL-1 non-peptidic agonists and/or



7

X =O, H, OH; R = H, Me, OMe, F, Cl,Br;
R_1 = H, Me, OH, OMe, F, Cl; n = 1,2

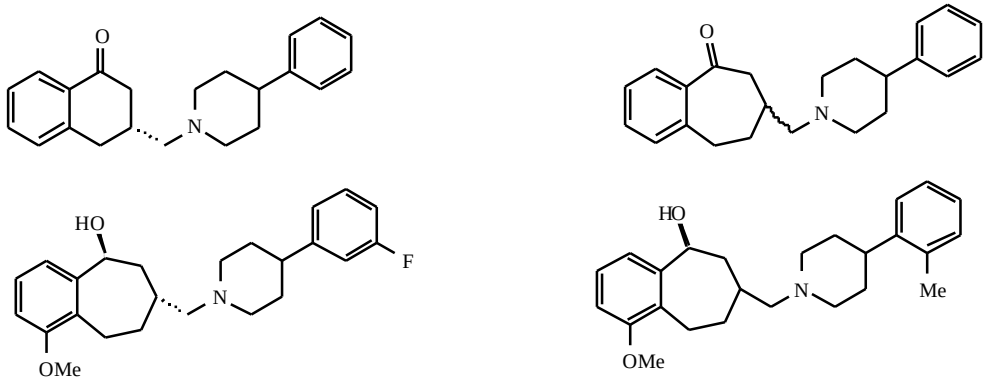


Chart 4.

antagonists. A selection of some representative structures is reported in Chart 5.

Finally, it should be noted that a few opioid ligands (e.g. etorphine, Mr 2266, TRK-820) were reported to have some activity on the ORL-1 receptor. However, it should be added that the concentrations required are extremely high (micromolar range) with respect to those which are effective on the opioid receptors (nanomolar range). The only exception seems to be buprenorphine, which was indicated as a full agonist of the ORL-1 receptor in several studies [47,48]. It showed pEC_{50} values ranging from 7.40 [49] to 8.07 [50].

CONCLUSION

In conclusion, the new non-peptidic ligands at the ORL-1 receptor will be of great utility to better understand the physiological roles of the nociceptin/ORL-1 system and to produce new pharmacologically active compounds. In particular, evidences seem to emerge that the ORL-1 agents could be not only potential new analgesics, but also able to treat several other CNS disorders, e.g. anxiety and depression. Moreover, the treatment of many pathologies (amongst them, motor processes, neuroendocrine secretion, and cardiovascular system) could take advantage from the

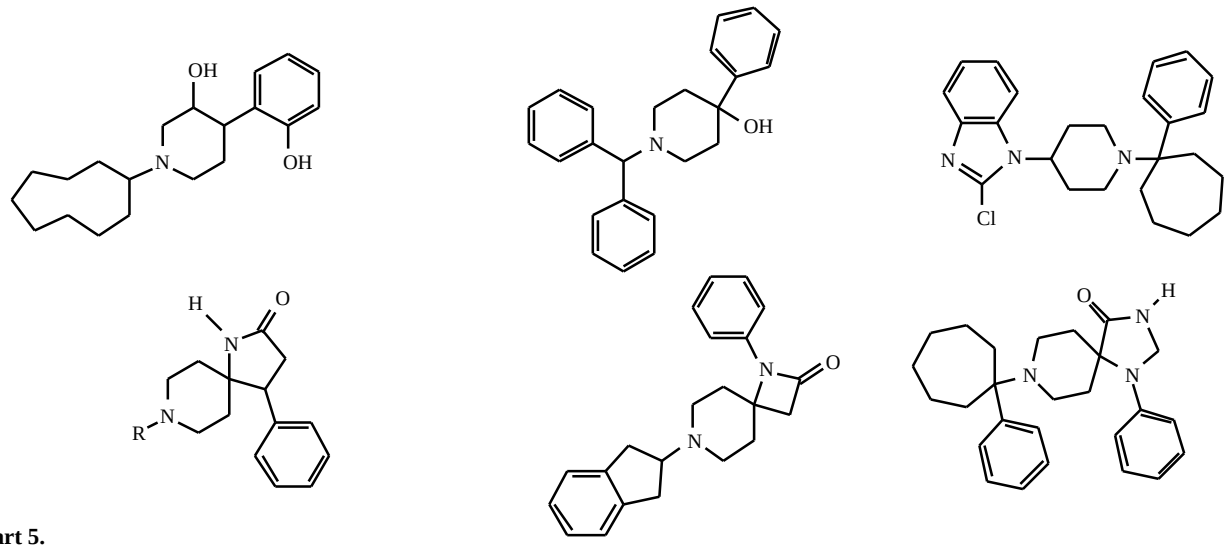


Chart 5.

availability of orally active, ORL-1 selective agonists and/or antagonists.

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