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Preparation of 3-spirocyclic indolin-2-ones as ligands for the ORL-1 receptor

Gilles C. Bignan,[a,*] Kathleen Battista,[a] Peter J. Connolly,[a] Michael J. Orsini,[a] Jingchun Liu,[a] Steven A. Middleton[a] and Allen B. Reitz[b]

[a]*Johnson & Johnson Pharmaceutical Research and Development, L.L.C, PO Box 300, 1000 Route 202, Raritan, NJ 08869, USA*
[b]*Johnson & Johnson Pharmaceutical Research and Development, L.L.C, PO Box 776, Welsh and McKean Rds., Spring House, PA 19477-0777, USA*

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Abstract—A novel series of indolin-2-ones having a spirocyclic piperidine ring at the 3-position was synthesized and found to bind with high affinity to the ORL-1 receptor. Structure–activity relationships at the piperidine nitrogen were investigated. Substitution on the phenyl ring and nitrogen atom of the indolin-2-one core generated several selective high-affinity ligands that were antagonists of the ORL-1 receptor.

Since its discovery a decade ago, remarkable progress has been made in the research on the biological significance and functions of the opioid receptor-like-1 (ORL-1) receptor and its endogenous peptide ligand, nociceptin [orphanin FQ (OFQ) or nociceptin/orphanin FQ (N/OFQ) peptide].[1] The ORL-1 receptor, also known as OP4 receptor, was first identified in 1994 as an orphan opioid receptor having close homology to the classical μ, κ, and δ opioid receptors. ORL-1 does not bind endogenous ligands of the other opioid receptors with high affinity, but instead favors the 17-amino acid peptide nociceptin.[2,3] Initial interest in ORL-1 led to a period of intense investigation and resulted in a number of significant reports on the biology of the receptor and ligand. Numerous studies have suggested that ORL-1 agonists may be clinically useful for treatment of stress/anxiety and in the treatment of opioid dependence and withdrawal.[4,5] Other accounts suggested that ORL-1 antagonists may be useful as analgesics and might enhance learning ability and memory.[6,7] Because of their potential therapeutic value, ORL-1 and its peptide ligand, nociceptin, have attracted the attention of many research groups interested in the development of non-peptidic small molecule agonists and antagonists. The interest in ORL-1 has resulted in an explosion of publications in the scientific and patent literature that has been summarized in several recent reviews.[8–10]

Here, we report the SAR of a new series of ORL-1 ligands. It is interesting to note that many reported small-molecule ORL-1 ligands incorporate a piperidine framework found in spiropiperidine, spirofused benzofuran, spiroindane/indene, benzimidazole/benzimidazolinone, and aryl piperidine structures, shown in Figure 1.[11–24] For each of the piperidine-containing scaffolds, extensive SAR work has been carried out around the substitution on the piperidine nitrogen. In general, certain substituents on these scaffolds, such as cyclooctylmethyl, naphthyl, and acenaphthenyl, have been found to confer high affinity for ORL-1.

Spiroindole pyrrolidines and piperidines have been investigated extensively in the literature and have been found to be key moieties in a variety of biologically active compounds.[11–26] Synthesis of the spiroindolinone core can be accomplished using different procedures. The classical route involves the conversion of indolin-2-one to spiroindolin-2-ones using bis(2-chloroethyl) methylamine under basic conditions (Scheme 1).[27] Deprotection of the methyl on the piperidine nitrogen requires a two-step procedure involving conversion to the trichloroethyl carbamate and cleavage with Zn. An alternative preparation of indolin-2-ones proceeding

* Corresponding author. Tel.: +1 908 704 4908; fax: +1 908 526 6469; e-mail: gbignan@prdus.jnj.com

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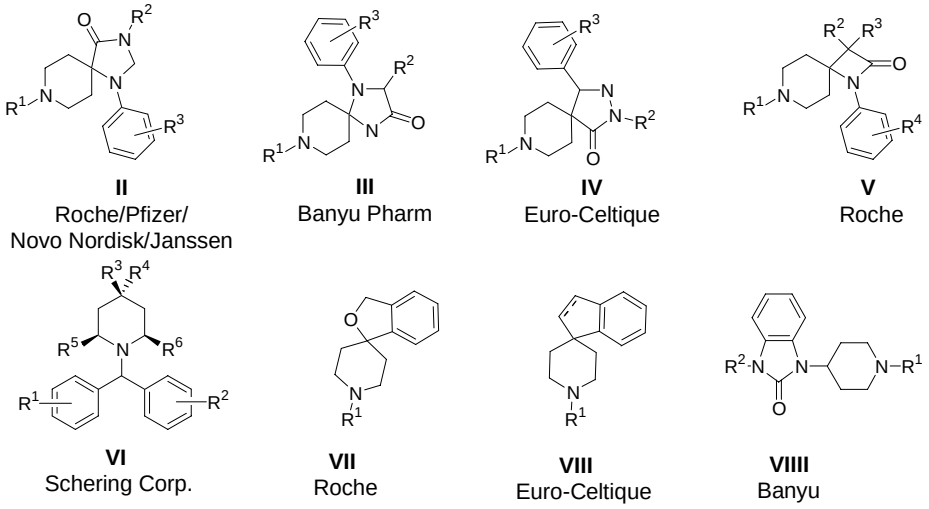
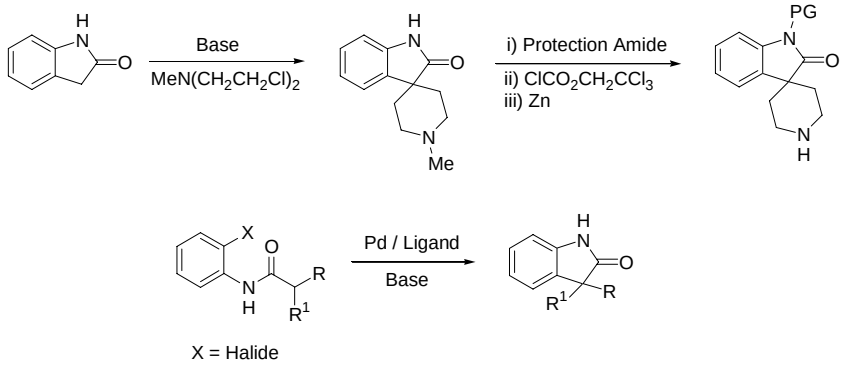


Figure 1. Piperidine-containing scaffolds that have been shown to exhibit ORL-1 receptor affinity.

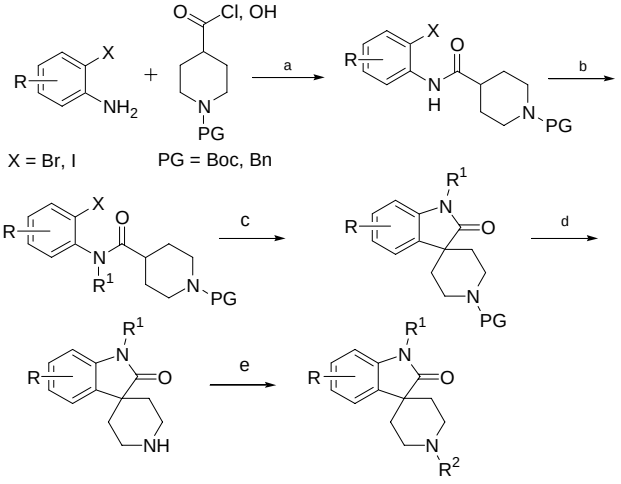


Scheme 1. Literature approaches to indoline-2-ones.

through an intramolecular amide α-arylation has been described by Hartwig and co-workers.[28,29] This methodology was used by others to scale up a key intermediate.[30]

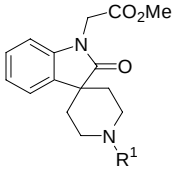
Our target molecules, spiroindolinone piperidines, were prepared using the Hartwig palladium chemistry. Described analogues were synthesized through a five-step sequence from commercially available or prepared substituted 2-bromo or 2-iodoanilines (Scheme 2). Substituents on the piperidine nitrogen were introduced at the last step through reductive amination, nucleophilic substitution, or amide coupling reactions.

Compounds were evaluated for ORL-1 binding and opioid receptor binding selectivity in radioligand binding assays (Tables 1 and 2). IC_{50} values for binding to human recombinant ORL-1 were determined by measuring the ability of compound to compete with ^{125}I-Tyr^{14}-nociceptin for binding to membranes prepared from HEK-293 cells expressing ORL-1. IC_{50} values for binding to human recombinant opioid receptors were determined using membranes isolated from HEK-293 cells expressing the μ, κ, or δ opioid receptors. Labeled agonists specific for each opioid receptor were used as



Scheme 2. Reagents: (a) pyridine or HATU, DIPEA, THF 50–95%; (b) NaH, DMF or THF then R^1–X [X = Br, I, Cl], 60–90%; (c) 10% $Pd(dba)_2$, *rac*-BINAP, *t*-BuONa, dioxane, 60–85%; (d) TFA or 6N HCl (PG = Boc) or H_2, Pd/C (PG = Bn), 65–90%; (e) reductive amination [R^2–CHO, $NaBH(OAc)_3$] or nucleophilic substitution [base, R^2–X {X = Br or I}] or peptide coupling [HO_2C–R^2, EDCI or HATU, DIPEA, DMF].

Table 1. Receptor binding of spirocyclic indolin-2-one-1-acetic acid analogues



Compounds	R^1	ORL-1 IC_{50} (μM)
1	–CH_2-cyclopropyl	3.6
2	–CH_2-cyclohexyl	0.80
3	–CH_2-cycloheptyl	0.51
4	–CH_2-cyclooctyl	0.10
5	–CH_2-cyclododecane	1.12
6	*n*-Hexyl	0.82
7	2-Butyloctyl	10
8	4-Chlorobenzyl	0.71
9	1-Naphthalen-1-ylmethyl	0.47
10	1-Naphthalen-2-ylmethyl	0.25
11	4-(CF_3)benzyl	0.88
12	3-(CF_3)benzyl	0.14
13	4-Phenylbenzyl	10
14	1-Phenethyl	10
15	1-(2-Thiophen-3-yl-benzyl)	10
16	1-(1,2,3,4-Tetrahydronaphthalen-2-yl)	1.08
17	1-(Decahydro-naphthalen-2-yl)	0.90
18	Acenaphthenyl	0.35
19	1-(4-*t*-Butylcyclohexylmethyl)	0.10
20	–$COCH_2CH_2COPh$	>10
21	–CO–(4-methoxycyclohexyl)	>10

Values are means of three experiments.

competitors in the selectivity binding assays: ^{3}H-DAMGO for μ, ^{3}H-U69,593 for κ, and ^{3}H-DDPDE for δ. Functional (antagonist) activity was assayed using a HEK-293 cell line that overexpresses ORL-1 receptor together with the Gqi5 G protein (molecular devices) to make signaling through ORL-1 receptor detectable by calcium flux assay. The ability of compounds to inhibit calcium flux in cells challenged with 100 nM nociceptin was measured.

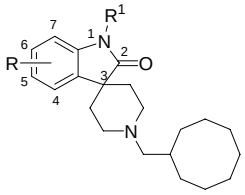
SAR studies around the spiroindolinone scaffold were carried out in two stages and are given in Tables 1 and 2. Table 1 details the effect of changing the substitution pattern on the piperidine nitrogen, while maintaining an acetic acid methyl ester substituent on the indolinone nitrogen. The size of the cycloaliphatic substituent on piperidine was crucial for affinity in the receptor binding assay. Optimal binding for ORL-1 was achieved with the cyclooctylmethyl substituent (**4**, IC_{50} = 0.1 μM). Binding affinity decreased significantly with extreme ring sizes, such as cyclopropylmethyl (**1**, IC_{50} = 3.6 μM) or cyclododecylmethyl (**5**, IC_{50} = 1.12 μM). Aliphatic analogues also had lower affinity for ORL-1, with a simple *n*-hexyl group (**6**) tolerated better than a branched C_{12} group (**7**). Aromatic substituents, such as substituted benzyl (**12**) or 1-naphthalen-2-ylmethyl (**10**) maintained affinity toward ORL-1 (IC_{50} = 0.14 and 0.25 μM, respectively).

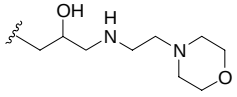
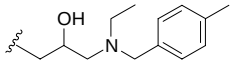
Partial or complete saturation of the naphthyl moiety (**16** and **17**) decreased the affinity for ORL-1. Introducing a large para substituent on the benzyl group (**13**) or increasing the spacer by one carbon (**14**) resulted in poor affinity for ORL-1, probably due to unfavorable steric interactions. Incorporation of an acenaphthenyl substituent (**18**), which conferred extremely potent ORL-1 affinity in other series,[12] did not improve ORL-1 potency in the spiroindolinone series (IC_{50} = 0.35 μM). Finally, attachment of the piperidine substituent via an amide linkage (**20** and **21**) resulted in a strong reduction of binding affinity to ORL-1. Favorable hydrophobic interaction of cyclooctylmethyl analogue **4** with the receptor suggests that other relatively compact hydrophobic substituents, such as 4-*t*-Bu-cyclohexyl, should also confer good affinity to ORL-1, as observed with analogue **19** (IC_{50} = 0.1 μM).

Because cyclooctylmethyl analogue **4** exhibited the best ORL-1 receptor potency among those listed in Table 1, we kept the *N*-cyclooctylmethyl constant and varied substitution on the amide nitrogen and aryl ring (Table 2). Compound **4** showed modest selectivity toward μ and κ opioid receptors (35- and 6-fold, respectively). Substitution on the indolinone amide nitrogen with other alkyl (acetoxyethyl **22** or hydroxyethyl **24**) or benzyl (**28**) groups maintained the affinity for ORL-1 but resulted in a loss of selectivity toward μ and κ opioid receptors. Saponification of **4** gave the acid (**23**), which showed poor affinity for ORL-1. Bulky *N*-substituents, such as cyclohexylmethyl (**25**) or substituted aryl (**27**), decreased the affinity for ORL-1. Notably, the highest affinity for ORL-1 was achieved with a methyl substituent on the amide nitrogen, as shown by analogue **36** (IC_{50} = 0.032 μM).[31] Compound **36** exhibited a modest increase in selectivity versus μ and κ opioid receptors to 40- and 29-fold, respectively. Introducing chirality in the amide substituent generated compounds as potent as **4** toward ORL-1 (racemate derivatives **29**, **30**, and **31**) but with no selectivity versus κ opioid receptor. Compound **26**, which has an unsubstituted amide, showed reduced affinity toward ORL-1 (IC_{50} = 0.38 μM). Thus, the amide substitution pattern proved to be important to maintain good affinity toward ORL-1 and moderate selectivity over the other opioid receptors.

Next, a methyl substituent was maintained on the amide nitrogen, while the substitution pattern on the aromatic ring was altered. Introduction of an electron-donating moiety in the 6-position, such as methoxy or hydroxyl (**33** and **35**), resulted in an 85-fold decrease in binding affinity to ORL-1 compared to the corresponding unsubstituted compound **36**. Furthermore, increasing the size of the amide substituent produced an analogue (**32**) with poor affinity for ORL-1 and opioid receptors. However, a methyl group in the 6-position (**37**) was better tolerated than methoxy, nearly restoring ORL-1 binding affinity (IC_{50} = 0.15 μM) and maintaining selectivity versus the μ opioid receptor (43-fold) compared to **36**. Introducing a methyl (**38**) or an electron-withdrawing trifluoromethyl (**40**) in the 5-position decreased the affinity for ORL-1 (IC_{50} = 0.45 and 0.6 μM, respectively) compared to **36**. Interestingly, the 5-fluoro analogue

Table 2. Receptor binding of spirocyclic *N*-cyclooctylmethyl indoline-2-one analogues



Compounds	R	R^1	IC_{50} (μM)				% inhibition (100 nM)[a]
			ORL-1	μ IC_{50} (μM)	κ IC_{50}(μM)	δ IC_{50} (μM)	
4	H	$-CH_2CO_2Me$	0.10	3.55	0.64	>10	—[b]
22	H	$-CH_2CH_2OAc$	0.18	1.41	2.64	>10	—
23	H	$-CH_2CO_2H$	>10	>10	>10	>10	—
24	H	$-CH_2CH_2OH$	0.29	1.69	—	—	—
25	H	1-Cyclohexylmethyl	0.68	—	—	—	—
26	H	H	0.38	1.11	0.43	>10	45
27	H	4-(MeO)benzyl	1.31	—	—	—	—
28	H	Benzyl	0.12	0.96	1.1	>10	—
29	H	1-Oxiranylmethyl	0.083	0.84	0.48	>10	—
30	H		0.12	0.50	0.10	>10	—
31	H		0.32	0.62	0.22	>10	—
32	6-MeO	1-Cyclopropylmethyl	>10	>10	>10	>10	—
33	6-MeO	Me	2.4	1.61	1.22	>10	—
34	6-MeO	Et	2.75	1.46	—	>10	—
35	6-OH	Me	2.68	>10	0.12	0.30	—
36	**H**	**Me**	**0.032**	1.30	0.93	>10	None
37	6-Me	Me	0.15	6.47	2.38	8.8	48
38	5-Me	Me	0.45	4.28	0.54	9.4	—
39	**4-Me**	**Me**	**0.037**	0.48	0.56	>10	81
40	$5-CF_3$	Me	0.6	5.31	—	>10	—
41	**5-F**	**Me**	**0.023**	1.01	0.25	>10	66
42	5-*i*-Pr	*n*-Pr	>10	>10	4	5.7	—
43	5-*i*-Pr	Me	7.40	0.98	0.097	>10	—

Values are means of three experiments. Compounds **29**, **30**, and **31** are racemic mixtures.

[a] % inhibition = antagonism in calcium flux functional assay in the presence of 100 nM nociceptin.

[b] '—' not determined.

(**41**)[31] was the most potent analogue for ORL-1 with an IC_{50} value of 0.023 μM. Compound **41** maintained selectivity versus μ opioid receptor (~44-fold) but displayed decreased selectivity versus κ opioid receptor (10-fold). Conversely, introducing a bulky isopropyl moiety in the 5-position (analogue **43**), while keeping the optimal methyl substituent on the amide, resulted in loss of affinity for ORL-1 and increased affinity toward the κ opioid receptor (IC_{50} = 0.097 μM). Finally, a methyl substituent in the 4-position (**39**)[31] was well-tolerated (ORL-1 IC_{50} = 0.037 μM).

None of the compounds tested in the functional assay (Table 2) functioned as ORL-1 agonists. However, compounds **26**, **37**, **39**, and **41** behaved as antagonists in the cellular calcium flux assay by inhibiting ORL-1 signaling induced by 100 nM nociceptin. Interestingly, compound **36**, which possesses reasonable affinity for ORL-1, did not display either agonist or antagonist activity. This may not be surprising because the nociceptin peptide possesses both 'address' and 'message' moieties that confer binding and signaling properties, respectively.[32] Thus, a compound may possess the molecular information required to bind but not to activate or inhibit the activation of the receptor.

In summary, we have developed a new spiroindolinone piperidine scaffold with sub-micromolar affinity for ORL-1. Using palladium-catalyzed intramolecular amide α-arylation reactions, we explored structure–activity relationships at different positions on the spirocyclic ring system. Several analogues bound ORL-1 receptor with moderate selectivity over the other opioid receptors. In particular, compounds **36**, **39**, and **41** displayed high affinity for ORL-1 (IC_{50} = 0.023–0.037 μM) and modest selectivity over μ and κ opioid receptors (40- to 45-fold). Most of the tested compounds behaved functionally as antagonists. These data suggest that selected candidates should be studied in different biological models of pain.

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31. Analytical data for compounds **36**, **39**, and **41**. Compounds were characterized by ^{1}H NMR and MS. Compound **36**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (1H, d, J = 7.1 Hz), 7.30–7.25 (1H, m), 7.07–7.02 (1H, m), 6.84 (1H, d, J = 7.7 Hz), 3.2 (3H, s), 2.91–2.84 (2H, m), 2.65–2.57 (2H, m), 2.25 (1H, d, J = 7.1 Hz), 2.03–1.94 (2H, m), 1.79–1.40 (15H, m), 1.30–1.20 (2H, m). MS (ES^+) m/z 341.2 $(M+H)^+$. Compound **39**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (1H, t, J = 7.7 Hz), 6.82 (1H, d, J = 7.7 Hz), 6.65 (1H, d, J = 7.7 Hz), 3.15 (3H, s), 2.98–2.84 (2H, m), 2.79–2.62 (2H, m), 2.55–2.42 (5H, m), 2.31–2.16 (2H, m), 1.82–1.39 (15H, m), 1.34–1.20 (2H, m). MS (ES^+) m/z 355.3 $(M+H)^+$. Compound **41**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.21 (1H, m), 7.01–6.97 (1H, m), 6.77–6.72 (1H, m), 3.19 (3H, s), 2.75–2.62 (2H, m), 2.42–2.29 (2H, m), 2.07–1.96 (2H, m), 1.76–1.68 (4H, m), 1.61–1.38 (15H, m). MS (ES^+) m/z 359.0 $(M+H)^+$.
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