Identification of a Novel Spiropiperidine Opioid Receptor-like 1 Antagonist Class by a Focused Library Approach Featuring 3D-Pharmacophore Similarity

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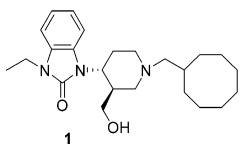
Abstract: A focused library approach identifying novel leads to develop a potent ORL1 antagonist is described. Beginning from a compound identified by random screening, an exploratory library that exhibited a diverse display of pharmacophores was designed. After evaluating ORL1 antagonistic activity, a highly focused library was designed based on 3D-pharmacophore similarity to known actives. A novel D-proline amide class was identified in this library and was found to possess potent ORL1 antagonistic activity.

The fourth opioid receptor, opioid receptor-like 1 (ORL1), was discovered in 1994 by homology cloning.[1] Subsequently, its endogenous agonist, a 17-amino acid peptide termed nociceptin or orphanin FQ (NC/OFQ), was identified.[2] Pharmacological studies using NC/OFQ and ORL1-deficient mice showed that the NC/OFQ-ORL1 system may have important roles in the regulation of pain response,[3] morphine tolerance,[4] learning and memory,[5] food intake,[6] anxiety,[7] the cardiovascular system,[8] locomotor activity,[9] and so on.[10]

These results prompted many pharmaceutical companies to identify potent and selective ORL1 agonists and antagonists. However, few structural classes were reported as ORL1 antagonists.[11] Therefore, additional structurally diverse ORL1 antagonists with distinct physicochemical properties and improved antagonistic activity from known ORL1 antagonists are required for better understanding of the physiological roles of ORL1 receptors and for examining the therapeutic potential of its antagonists.

As previously reported, we identified a small molecular ORL1 antagonist, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**1**),[12] and the search for structurally diverse ORL1 antagonists among our in-house chemical collection led to identification of spiropiperidine analogue **2**, which exhibited moderate but complete ORL1 antagonistic activity (IC_{50}: 8.3 nM in GTPγS assay).



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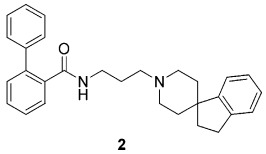

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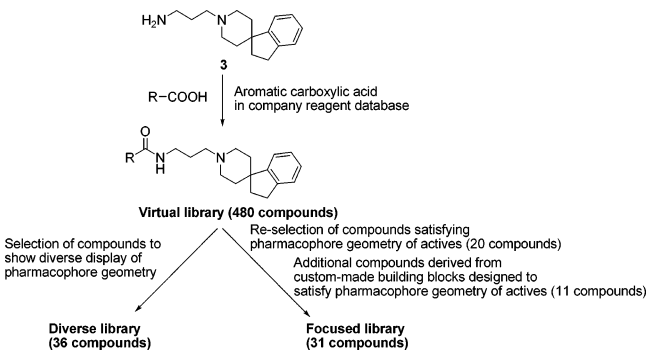
On the basis of preliminary SAR analysis of analogues in our chemical collection, an aromatic ring and cationic amine group in the spiropiperidine portion and an additional aromatic ring in the ortho position of terminal benzoic acid were predicted to be important pharmacophores for antagonistic activity in ORL1 receptors. We wanted to examine the relationship between binding activity and the optimal distance between the two pharmacophores by investigating a range of aromatic groups in the hydrophobic acyl part of **2**. In addition, introduction of hydrophilicity was attempted using this library approach in an effort to reduce the high hydrophobicity of **2** (log D >5).

The library was designed as follows. First, a virtual library was constructed by combining amine **3** possessing spiropiperidine and *n*-propyl spacer group and all available aromatic COOH building blocks in the company reagent database. From this virtual library of 480 compounds, the diverse library (36 compounds)[13] was selected by visual inspection to exhibit a diverse 3D distribution of aromatic groups in the acyl region. To simplify understanding of the required pharmacophore

Scheme 1. Design of Libraries Focusing on Pharmacophore Similarity



Scheme 2. Preparation of Libraries

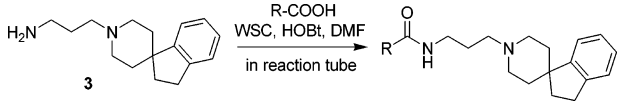
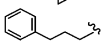


Table 1. Hit Compounds from the Diverse Library

cmpd	R	Binding affinity (IC_{50}, nM)[a]	cmpd	R	Binding affinity (IC_{50}, nM)[a]
4		>1000	**13**		>1000
5		730			
6		540	**14**		29
7		>1000			
8		350	**15**		>1000
9		>1000	**16**		>1000
10		>1000	**17**		>1000
11		580	**18**		>1000
12		7.5			

[a] Displacement of [^{125}I]Tyr^{14}-nocicpetin binding from the CHO cells stably expressing cloned human ORL1 receptors.

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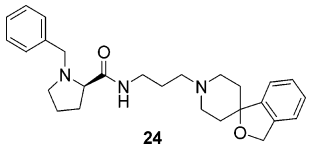
Table 2. Hit Compounds (purified) from Diverse and Focused Libraries

cmpd	R	Binding affinity IC_{50} (nM)				GTPγS	
		ORL1[a]	μ[b]	κ[c]	δ[d]	antagonism IC_{50} (nM)[e]	agonism EC_{50} (nM)[f]
19		8.2 ± 0.033	>10000	9600 ± 950	>10000	4.0 ± 0.20	>10000
20		83 ± 4.3	>10000	2300 ± 130	>10000	110 ± 17	>10000
21		12 ± 0.88	>10000	6900 ± 450	>10000	7.5 ± 0.29	>10000
22		47 ± 4.2	>10000	7900 ± 1200	>10000	42 ± 5.3	>10000
23		0.22 ± 0.025	4300 ± 780	950 ± 100	>10000	0.12 ± 0.001	>1000
2		10 ± 0.53	>10000	3700 ± 350	>10000	8.3 ± 0.76	>10000
1		2.4 ± 0.26	3400 ± 150	1600 ± 200	>10000	1.5 ± 0.20	>10000

[a] Displacement of [^{125}I]Tyr^{14}-nociceptin, [b] [^{3}H]diprenorphin, [c] [^{3}H]U-69593, [d] [^{3}H]naltrindole binding from CHO cells stably expressing cloned human ORL1, opioid μ-, opioid κ-, and opioid δ-receptors, respectively. [e] IC_{50} values on nociceptin-produced [^{35}S]GTPγS binding to ORL1-expressed in CHO cells. [f] EC_{50} values relative to the maximal [^{35}S]GTPγS binding produced by nociceptin in ORL1 expressed CHO cells. All values are means of three independent determinations performed in duplicate.

Table 3. In Vitro and in Vivo Profiles for ORL1 Antagonist **24**



24

	binding affinity lC_{50} (nM)				GTPγS			brain penetrability	
cmpd	ORL1[a]	μ[b]	κ[c]	δ[d]	antagonism IC_{50} (nM)[e]	agonism EC_{50} (nM)[f]	in vivo antagonism (% reversal)[g]	plasma (nM)[h]	brain levels (nmol/g brain)[h]
24	0.27 ± 0.038	6700 ± 4300	2500 ± 200	>10000	0.15 ± 0.0067	>1000	88 ± 9.7%	0.88	4.37

[a] Displacement of [^{125}I]Tyr^{14}-nociceptin, [b] [^{3}H]diprenorphin, [c] [^{3}H]U-69593, [d] [^{3}H]naltrindole binding from CHO cells stably expressing cloned human ORL1, opioid μ-, opioid κ-, and opioid δ-receptors, respectively. [e] IC_{50} values for nociceptin-produced [^{35}S]GTPγS binding to ORL1-expressed in CHO cells. [f] EC_{50} values relative to the maximal [^{35}S]GTPγS binding produced by nociceptin in ORL1-expressed CHO cells. [a−f] All values are means of three independent determinations performed in duplicate. [g] Data shows antagonistic activity of analogue **24** (10 mg/kg, sc) against the reduction in locomotor activity produced by an ORL1 agonist for 60 min in mice. Values are expressed as % reversal of the agonist response (mean ± SEM). [h] Plasma and blains of rats were collected 1 h after the drug administration (10 mg/kg, sc) and drug concentrations were measured (n = 3 mice/group).

geometry, only simple aromatic COOH building blocks without additional pharmacophores (i.e., H-bonding donor or acceptor) were selected. In addition, as the *n*-propyl spacer is a highly flexible structure, less flexible COOH building blocks were selected with higher priority.

After testing the diverse library, the focused library was designed based on 3D-pharmacophore similarity to known actives. From the remainder of the virtual library, analogues that showed similar pharmacophore orientation to **2** and hit compounds from the diverse library were selected using a computational method (20 compounds).[13] In this case, aromatic COOH building blocks with heteroatoms were employed with higher priority in order to decrease the hydrophobicity of the molecule.

It should be noted that additional aromatic COOH building blocks were designed to show similar pharmacophore orientation to known actives and prepared by simple modification of commercially available reagents in order to enrich the structural variation of the original virtual library. Eleven aromatic COOH reagents[13] were thus prepared as custom-made building blocks and were added to the focused library.

Analogues were prepared by simple condensation reaction performed in parallel (Scheme 2), and all samples were tested by LC-MS and were confirmed to show more than 85% purity.

Library members were tested for their inhibitory effects on ligand binding to the human ORL1 receptors and on GTPγS binding to proteins using membrane fractions of CHO cells expressing ORL1. Binding affinities for ORL1 were determined by displacement of [^{125}I]Tyr^{14}-NC/OFQ, and agonist/antagonist activities were measured by the [^{35}S]GTPγS binding method.[14] Cross reactivity to other opioid receptors was also tested. Affinities for human μ-, κ-, and δ-receptors were assayed similarly to ORL1 using membrane fractions of CHO cells expressing in each receptor.

The results of representative library members are summarized in Table 1. From the diverse library, compounds **12** and **14** showed comparable binding activity (IC_{50} <100 nM) to **2**. On the basis of structure−activity relationship analysis of the diverse library set, it was clear that particular pharmacophore geometry in the acyl part was required for good affinity. The regio- and stereochemistry in the acyl part (compounds **12** vs **13** and **14** vs **15**) were important for ORL1 binding. Consequently, in the

focused library, analogues showing similar pharmacophore geometry to compounds **2**, **12**, and **14** were prioritized and tested.

Compounds with IC_{50} values of <100 nM from both the diverse and focused libraries were purified and subjected to further characterization. Binding activities for ORL1, μ-, κ-, and δ-receptors and functional activities for ORL1 receptors of purified compounds are summarized in Table 2.

All purified compounds showed potent ORL1 antagonistic activity and no agonistic activity. Among these, *N*-benzyl-D-proline analogue **23** showed substantially improved binding and antagonistic activity when compared with **2**. The stereochemistry of the proline structure was very important to potency (**23** vs **22**), and thus **23** was thought to successfully meet the required pharmacophore geometry by forming a stereospecific interaction with ORL1 receptor. Analogue **23** showed a 12.5-fold higher antagonistic activity than **1** and a more than 4000-fold higher selectivity over μ-, κ-, and δ-receptors. In addition, by introducing an additional *tert*-amino structure in the acyl moiety, **23** showed decreased hydrophobicity (log *D* of **23** and **2** were 2.7 and 3.1, respectively).

Analogue **23** showed novel structural features when compared with reported ORL1 antagonists, and thus we have identified a novel class of ORL1 antagonist with substantially improved antagonistic activity by searching chemotypes of random screening hit **2**.

In the course of our medicinal chemistry efforts to assess the potential of *N*-benzyl-D-proline derivatives as leads for CNS drugs, close analogue **24** with an isobenzofuran structure in the spiropiperidine portion was prepared.

Analogue **24** showed comparable activity and selectivity as **23** and good brain permeability (Table 3). In addition, **24** showed statistically significant in vivo antagonistic activity against the reduction in locomotor activity produced by an ORL1 agonist (control; $n = 7$: 1844 ± 104 counts/1 h, agonist; $n = 7$: 187 ± 20counts/1 h, agonist + **24** (3 mg/kg); $n = 8$: 1619 ± 168 counts/1 h ($p < 0.05$ from agonist treated group), agonist + **24** (10 mg/kg); $n = 8$: 1647 ± 160 counts/1 h, ($p < 0.05$ from agonist treated group)).[13]

In conclusion, we discovered a novel class of ORL1 antagonist using a focused library approach starting from a moderately active hit compound found in our chemical collection. The *N*-benzyl-D-proline analogue showed significantly improved antagonistic activity when compared with other reported ORL1 antagonists and showed good brain penetrability and in vivo antagonistic activity. This newly identified class may serve as useful pharmacological tool in vivo to investigate the physiological roles of the NC/OFQ-ORL1 system as well as the therapeutic potential of ORL1 antagonists. In particular, its significantly improved potency, appropriate selectivity, and hydrophilicity would be suitable for development of a PET tracer to examine the in vivo pharmacodynamics of ORL1 antagonists. Further details of the SAR and pharmacological profiles of the analogues will be discussed in due course.

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Supporting Information Available: Building blocks for focused and diverse libraries, synthetic procedures, pharmacophore similarity search, and in vivo pharmacological experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Molleaau, C.; Parmentier, M.; Mailleux, P.; Butour, J.; Moisand, C.; Chalon, P.; Caput, D.; Vassart, G.; Meunier, J.-C. ORL-1, a novel member of the opioid receptor family Cloning, functional expression and localization. *FEBS Lett.* **1994**, *341*, 33−38. (b) Fukuda, K.; Kato, S.; Mori, K.; Nishi, M.; Takeshima, H.; Iwabe, N.; Miyata, T.; Houtani, T.; Sugimoto, T. cDNA cloning and regional distribution of a novel member of the opioid receptor family. *FEBS Lett.* **1994**, *343*, 42−46. (c) Chen, Y.; Fan, Y.; Liu, J.; Mestek, A.; Tian, M.; Kozak, C. A.; Yu, L. Molecular cloning, tissue distribution and chromosomal localization of a novel member of the opioid receptor gene family. *FEBS Lett.* **1994**, *347*, 279−283. (d) Pan, Y.-X.; Cheng, J.; Xu, J.; Rossi, G.; Jacobson, E.; Ryan-Moro, J.; Brooks, A. I.; Dean, G. E.; Standifer, K. M.; Pasternak, G. W. Cloning and functional characterization through antisense mapping of a k3-Related opioid receptor. *Mol. Pharmacol.* **1995**, *47*, 1180−1188.

(2) (a) Reinsceid, R. K.; Nothacker, H.-P.; Bourson, A.; Ardati, A.; Henningsen, R. A.; Bunzow, J. R.; Grady, D. K.; Langen, H.; Monsma, F. J., Jr.; Civelli, O. Orphanin FQ: a neuropeptide that activates an opioid like G protein-coupled receptor. *Science* **1995**, *270*, 792−794. (b) Meunier, J.-C.; Mollereau, C.; Toll, L.; Suaudeu, C.; Moisand, C.; Alvinerie, P.; Butour, J.-L.; Guillemot, J.-C.; Ferrara, P.; Monsarrat, B.; Mazarguil, H.; Vassart, G.; Parmentier, M.; Constentin, J.; Isolation and structure of the endogenous agonist of opioid receptor-like ORL-1 receptor. *Nature* **1995**, *377*, 532−535.

(3) Mogil, J. S.; Grisel, J. E.; Reinscheid, R. K.; Civelli, O.; Belknap, J. K.; Grandy, D. K. Orphanin FQ is a functional anti-opioid peptide. *Neuroscience (Oxford)* **1996**, *75*, 333−337.

(4) Ueda, H.; Yamaguchi, T.; Tokuyama, S.; Inoue, M.; Nishi, M.; Takeshima, H. Partial loss of tolerance liability to morphine analgesia in mice lacking the nociceptin receptor gene. *Neuroscience Lett.* **1997**, *237*, 136−138.

(5) (a) Manabe, T.; Noda, Y.; Mamiya, T.; Katagiri, H.; Houtani, T.; Nishi, M.; Noda, T.; Takahashi, T.; Sugimoto, T.; Nabeshima, T.; Takeshima, H.; Facilitation of long-term potentiation and memory in mice lacking nociceptin receptors. *Nature (London)* **1998**, *394* (6693), 577−581. (b) Sandin, J.; Georgieva, J.; Schott, P. A.; Orgen, S. O.; Terenius, L. Nociceptin/Orphanin FQ microinjected into hippocampus impairs spinal learning in rats. *Eur. J. Neurosci.* **1997**, *9*, 194−197. (c) Yu, T. P.; Fein, J.; Phan, T.; Evans, C. J.; Xie, C.-W. Orphanin FQ inhibits synaptic transmission and long-term protection in rat hippocampus. *Hippocampus* **1997**, *7*, 88−94.

(6) Pomonis, J. D.; Billington, C. J.; Levine, A. S. Orphanin FQ, agonist of irphan opioid receptor ORL-1, stimulates feeding in rats. *NeuroReport* **1996**, *8*, 369−371.

(7) Jenck, F.; Moreau, J.-L.; Martin, J. R.; Kilpatrick, G. J.; Reinscheid, R. K.; Monsma, F. J., Jr.; Nothacker, H.-P.; Civelli, O. Orphanin FQ acts as an anxiolytic to attenuate behavioral response to stress. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14854−14858.

(8) (a) Champion, H. C.; Katwitz, P. J. Nociceptin, an endogenous ligand for ORL1 receptor, has novel hypotensive activity in the rat. *Life Sci.* **1997**, *60*, 241−245. (b) Gumusel, B.; Hao, Q.; Hyman, A.; Chang, J.-K.; Kapusta, D. R.; Lippton, H. Nociceptin: an endogenous agonist for central opioid like1 (ORL1) receptors possesses systemic vasorelaxant properties. *Life Sci.* **1997**, *60*, 141−145.

(9) Florin, S.; Suaudeau, C.; Meunier, J.-C.; Costentin, J. Nociceptin stimulates locomotion and exploratory behaviour in mice. *Eur. J. Pharmacol.* **1996**, *317*, 9−13.

(10) Champion, H. C.; Wang, R.; Hellstrom, W. J. G.; Kadowitz, P. J. Nociceptin, a novel endogenous ligand for ORL1 receptor, has potent erectile activity in the cat. *Am. J. Physiol.* **1997**, *273 (Endocrinol. Metab. 36)*, E214−E219.

(11) (a) Ronzoni, S.; Peretto, I and Giardina G. AM. Lead generation and lead optimization approaches in the discovery of selective, non-peptide ORL-1 receptor agonists and antagonists. *Exp. Opin. Ther. Pat.* **2001**, *11(4)*, 525−546. And the references sited therein. (b) Nurulain, Z. Peptide and nonpeptide ligands for the nociceptin/orphanin FQ receptor ORL1: Research tools and potential therapeutic agents. *Life Sci.* **2003**, *73*, 663−678.

(12) Kawamoto, H.; Ozaki, S.; Itoh, Y.; Miyaji, M.; Arai, S.; Nakashima, H.; Kato, T.; Ohta, H.; Iwasawa, Y. Discovery of the first potent and selective small molecule opioid receptor-like (ORL-1) antagonist: 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxy-methyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397). *J. Med. Chem.* **1999**, *42*, 5061−5063.

(13) See Supporting Information.

(14) Lazareno, S.; Birdsall, N. J. M. Pharmacological characterization of acetylcholine-stimulated [^{35}S]-GTPγS binding mediated by human muscarinic m1-m4 receptors: Antagonist studies. *Br. J. Pharmacol.* **1993**, *109*, 1120−1127.

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