A Novel Class of Cycloalkano[*b*]pyridines as Potent and Orally Active Opioid Receptor-like 1 Antagonists with Minimal Binding Affinity to the hERG K^+ Channel

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A series of compounds based on 7-{[4-(2-methylphenyl)piperidin-1-yl]methyl}-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-9-ol ((−)-**8b**), a potent and selective opioid receptor-like 1 (ORL1) antagonist, was prepared and evaluated using structure−activity relationship studies with the aim of removing its affinity to human ether-a-go-go related gene (hERG) K^+ channel. From these studies, **10l** was identified as an optimized structure with respect to ORL1 antagonist activity, and affinity to the hERG K^+channel. Furthermore, **10l** showed good in vivo antagonism with a wide therapeutic index in regards to adverse cardiovascular effects.

Introduction

A fourth opioid receptor, opioid receptor-like 1 (ORL1),[a] was discovered in 1994 based on its high degree of amino acid sequence homology to classical opioid receptors.[1] Despite this homology, this fourth member of the opioid receptor family did not bind classical opioids with appreciable affinity. Since the discovery of its endogenous agonist, a 17-amino acid peptide termed nociceptin or orphanin FQ (NC/OFQ) that was isolated from the brain,[2] the physiological roles of the NC/OFQ-ORL1 system have been the focus of intense research. This system may play important roles in pain regulation,[3] learning and memory,[4] food intake,[5] anxiety,[6] and cardiovascular system,[7] among others,[8] thus prompting many pharmaceutical companies to identify small molecules as potent and selective ORL1 agonists and antagonists. Numerous studies using these small molecules and antisense oligonucleotides as tools, as well as the use of ORL1-deficient mice, suggested that ORL1 antagonists may be of help in the management of pain, depression, dementia, and Parkinsonism.[9] Various classes of ORL1 antagonists have been reported, including quinoline (**1**, JTC-801),[10] phenylpiperidine (**2a**, SB612111),[11] spiropiperidines (**3**, **4**, and **5**),[12] benzimidazolone J-113397 (**6**),[13] and benzimidazole (**7**)[14] (Figure 1). Despite the extensive series of antagonists, however, no reports have described the human ether-a-go-go related gene (hERG) K^+channel binding affinity and pharmacokinetic profiles.

We have previously reported on the oral bioavailability of benzimidazole (**7**) and its safety in terms of cardiovascular side effects.[15] Furthermore, during the back-up program in the development of a new druggable and patentable pharmacophore as an ORL1 antagonist, we became interested in the structure of **2a** because of its highly rigid structure as well as its high

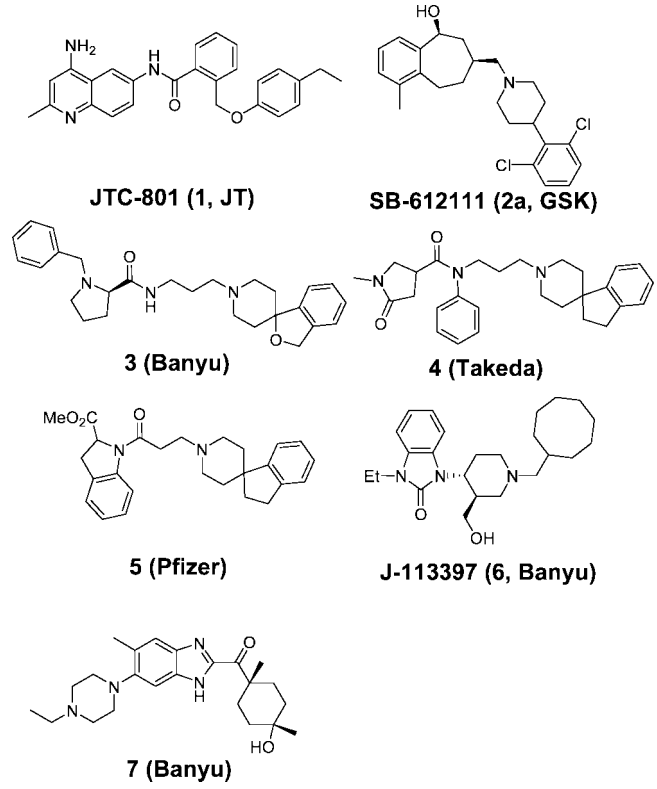

Figure 1. Reported ORL1 antagonists.

ORL1 antagonist activity.[11] We speculated that incorporating a nitrogen atom into the benzene ring of the benzosuberonyl moiety of **2a** would affect its physicochemical properties, in particular its hydrophilicity, leading to a new pharmacophore with a different biological profile.[16] An exploratory SAR study involving analogues of **2b** that incorporated a nitrogen atom in each position of the benzene ring (Figure 2) has revealed that the analogue with a nitrogen atom in the 1-position, 7-{[4-(2-methylphenyl)piperidine-1-yl]methyl}-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-9-ol ((−)-**8b**), is a potent ORL1 antagonist with moderate selectivity over hERG channel binding affinity (Table 1). In contrast, incorporation of a nitrogen atom into the other positions (2-, 3-, and 4-positions in Figure 2) resulted in

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[a] Abbreviations: ORL1, opioid receptor-like 1; hERG, human ether-a-go-go related gene; NC/OFQ, nociceptin/orphanin FQ; SAR, structure−activity relationship; TBS, *tert*-butyldimethylsilyl; TsCl, *p*-toluenesulfonyl chloride; DMAP, 4-dimethylaminopyridine; DIBAL, diisobutylaluminum hydride; NBS, *N*-bromoscuccinimide; TES, tetraethylsilyl; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; ACE-Cl, 1-chloroethylchloroformate; TFA, trifluoroacetic acid; DME, dimethoxyethane; dppf, 1,1′-bis(diphenylphosphino)-ferrocene; EDCI, 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; PK, pharmacokinetics; CHO, chinese hamster ovary; TMS, tetramethylsilane.

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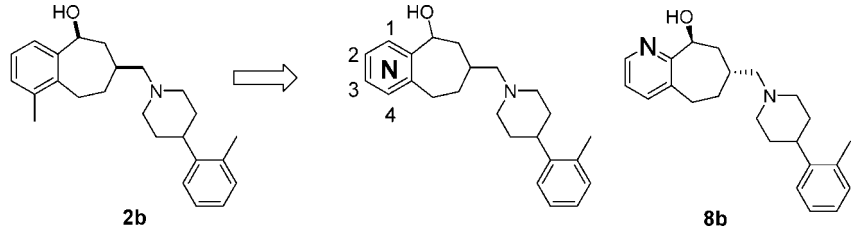
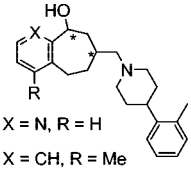


Figure 2. Incorporation of nitrogen atom in benzosuberonyl moiety.

Table 1. Biological Data for Lead Compounds **8** and **2b**



	Binding assay	GTPγS		hERG binding	
compound	ORL1Ki (nM)[a]	antagonism IC_{50} (nM)[b]	agonism EC_{50} (nM)[c]	IC_{50} (nM)[d]	$logD_{7.4}$
(+)-**8a** (cis)	18 ± 3			310 ± 50	
(-)-**8a**	12 ± 0			220 ± 70	
(+)-**8b** (trans)	280 ± 24			1500 ± 310	
(-)-**8b**	4.5 ± 0.2	9.0 ± 1.7	>10000	2700 ± 340	3.1
2b (cis)	6.0 ± 0.1	10 ± 1.7	>10000	86[e]	4.5

[a] Displacement of [^{125}I]Tyr^{14}-nociceptin. [b] IC_{50} values on nociceptin-produced [^{35}S]GTPγS binding to ORL1-expressed in CHO cells. [c] EC_{50} values relative to the maximal [^{35}S]GTPγS binding produced by nociceptin in ORL1 expressed CHO cells. [d] Displacement binding assay of [^{35}S]-radiolabeled MK-499 in membranes derived from HEK 293 cells stably transfected with hERG gene and expressing the I_{Kr} channel protein. [e] When only two experiments were performed, the value is expressed as the mean of duplicate determinations, and the variances between determinations were less than ±10%.

significant decreases in the binding affinity to ORL1. On the basis of these results, further studies that involved derivatization of (−)-**8b** for the development of a novel class of ORL1 antagonist were initiated.

In this paper, we describe the development of a potent, selective, and orally active ORL1 antagonist, (−)-6-{[4-(2-chloro-4-fluorophenyl)-3-hydroxypiperidine-1-yl]methyl}-5,6,7,8-tetrahydroquinoline-8-ol (**10l**), which exhibited oral activity in an in vivo locomotor study with a wide therapeutic index with regards to adverse cardiovascular effects.

Chemistry

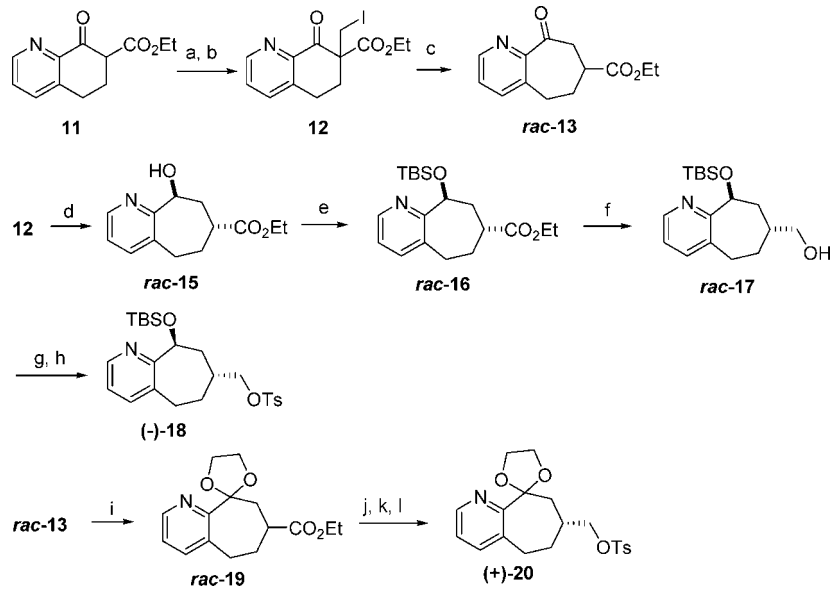
The six- and seven-membered key intermediates, ***rac*-13**, (−)-**18**, (+)-**20**, and (−)-**27**, were prepared as illustrated in Schemes 1 and 2. They were used for the preparation of the final products via simple alkylation or amidation with the corresponding secondary amines. As previously reported, the seven-membered intermediate ***rac*-13** was constructed via radical ring expansion reaction involving Bu_3SnH and 1,1′-azobis(cyclohexanecarbonitrile).[17] Highly stereoselective reduction was carried out using 1.7 mol equiv of Bu_3SnH to afford *trans*-alcohol **15**, which was protected using a *tert*-butyldimethylsilyl (TBS) group. Subsequently, the ester moiety was reduced using $LiAlH_4$ to provide primary alcohol **17**, which was converted into tosylate ***rac*-18**. The enantiomers of ***rac*-18** were separated by HPLC to afford the desired chiral tosylate (−)-**18**.[18] Similarly, chiral intermediate (+)-**20** was prepared using racemic ketal precursor ***rac*-19**, which was obtained by ketalization of ***rac*-13** with an excess amount of ethylene glycol.

For the synthesis of six-membered intermediate (−)-**27** (Scheme 2), the cyano group of 2-chloro-3-cyanopyridine (**21**) was subjected to reduction using diisobutylaluminum hydride (DIBAL) followed by Horner−Emmons reaction to afford cinnamic acid ester **22**. The chemoselective reduction of the double bond of **22** was successfully achieved using $NaBH_4$ in the presence of CuCl to give 2-pyridinepropanoic acid ester **23**, which was then subjected to Stille reaction using (1-ethoxyvinyl)tributyltin, then brominated using NBS to provide α-bromoketone **25**. The ketone group of **25** was reduced using BH_3-THF, then protected using a tetraethylsilyl (TES) group. Intramolecular cyclization was subsequently carried out using sodium hexamethyldisilazide as a base to exclusively yield the *trans*-product (ester **26**). Finally, under similar reaction conditions as described above, **26** was transformed into tosylate ***rac*-27**, which was readily separated by HPLC to provide chiral six-membered intermediate (−)-**27**. The absolute configuration of (−)-**27** was established from the results of a single-crystal X-ray structure determination of (−)-**10l**.[19]

The preparation of piperidines **30h** and **30i**, which possess a C-1 unit at the 3-position in the piperidine ring, is illustrated in Scheme 3. Conjugate addition of an *o*-tolyl group to arecoline (as free base) yielded the corresponding adduct as a mixture of diastereomers,[20] which were converted into *trans*-adduct **29** by treatment with potassium *tert*-butoxide. Deprotection of the *N*-methyl group by refluxing with 1-chloroethylchloroformate (ACE-Cl) in 1,2-dichloroethane then MeOH afforded secondary amine **30i**. Reduction of the ester group of **29** using $LiAlH_4$, subsequent mesylation in the presence of triethylamine, followed by further reduction using lithium triethylborohydride furnished 3-methyl derivative **31**. Secondary amine **30h** was obtained by treatment with ACE-Cl.

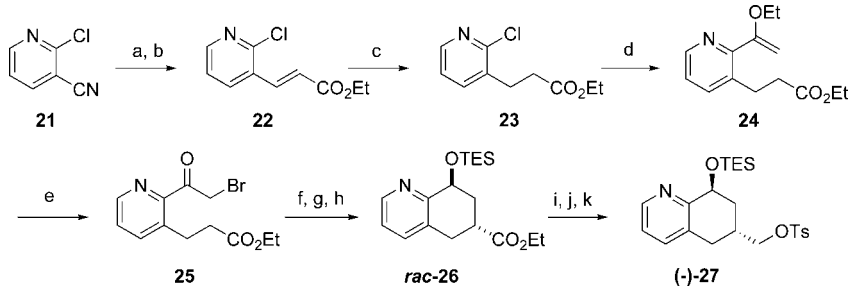
Piperidines **30j**, **30k**, and **30l**, which possess a hydroxyl group at the 3-position, were prepared as illustrated in Scheme 4. The *o*-tolyl-substituted dihydropyridines **33** and **34** were synthesized via addition reaction of the corresponding Grignard reagents to 1-benzylpiperidine-4-one, followed by dehydration using trifluoroacetic acid. Hydroboration of the dihydropyridines as a regioselective addition across the double bond, followed by the subsequent oxidative cleavage afforded *trans*-3-hydroxy-4-arylpiperidines **35** and **36**. Upon cleavage of the benzyl groups via hydrogenolysis in the presence of $Pd(OH)_2$, the resulting racemic secondary amines were separated using HPLC to yield chiral **30j** and **30k**. On the other hand, dihydropyridine **39** was prepared via Suzuki coupling reaction using boronic acid ester **38**.[21] In this case, the hydroboration reaction resulted in the mixture of 3- and 4-hydroxyl products (**40** and **41**). Upon removal of the *N*-*tert*-butoxycarbonyl group using 4N HCl in dioxane, the resulting mixture of regioisomeric as well as enantiomeric amines were separated by HPLC to afford chiral **30l**.

Scheme 1. Synthesis of Key Intermediates ***rac*-13**, **(−)-18**, and **(+)-20**[a]



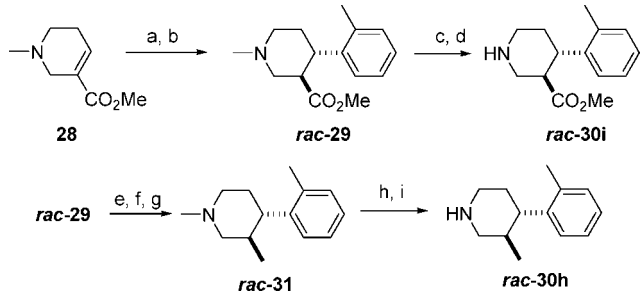
[a] Reagents: (a) $KHCO_3$, 30% aq HCHO, EtOH, 50 °C; (b) I_2, PPh_3, pyridine, CH_2Cl_2, 81% (from **11**); (c) Bu_3SnH (1.5 eq), 1,1′-azobis(cyclohexanecarbonitrile) (0.2 eq), toluene, reflux, 58%; (d) Bu_3SnH (1.7 eq), 1,1′-azobis(cyclohexanecarbonitrile) (0.2 eq), toluene, reflux, 58%; (e) TBSCl, imidazole, DMF, 0 °C to r.t., 87%; (f) $LiAlH_4$, THF, 0 °C to r.t., 94%; (g) TsCl, Et_3N, DMAP, $CHCl_3$, r.t.; (h) chiral separation by HPLC eluted with *n*-hexane/EtOH/Et_2NH (800/200/1), 47%; (i) ethyleneglycol, *p*-TsOH, toluene, reflux, 67%; (j) $LiAlH_4$, THF, 0 °C to r.t.; (k) TsCl, DMAP, Et_3N, THF, r.t., 51%; (l) chiral separation by HPLC eluted with *n*-hexane/EtOH/Et_2NH (800/200/1).

Scheme 2. Synthesis of Key Intermediate **(−)-27**[a]



[a] Reagents: (a) DIBAL, toluene, 0 °C; (b) triethyl phosphonoacetate, NaH, THF, 0 °C, 82%; (c) $NaBH_4$, CuCl, MeOH-H_2O, 0 °C to r.t., 77%; (d) (1-ethoxyvinyl)tributyltin, $Pd(PPh_3)_4$, DMF, 120 °C, 92%; (e) NBS, THF-H_2O, 69%; (f) BH_3-THF, THF, −18 °C, 68%; (g) TESCl, imidazole, DMF; (h) NaHMDS, THF, −18 °C, 51%; (i) $LiAlH_4$, THF, 0 °C to r.t., 82%; (j) chiral separation by HPLC eluted with *n*-hexane/^{i}PrOH (50/1)/0.1%Et_2NH; (k) TsCl, Et_3N, DMAP, $CHCl_3$, 50 °C, 97%.

Scheme 3[a]



[a] Reagents: (a) *o*-tolylMgBr, Et_2O, −40 to −20 °C; (b) *tert*-BuOK, THF, r.t., 31%; (c) ACE-Cl, 1,2-dichloroethane, reflux; (d) MeOH, reflux, 33% (from ***rac*-29**); (e) $LiAlH_4$, THF, 0 °C to r.t.; (f) MsCl, Et_3N, $CHCl_3$, 0 °C; (g) $LiBEt_3H$, THF, 0 °C to r.t., 24% (from ***rac*-29**); (h) ACE-Cl, 1,2-dichloroethane, reflux; (i) MeOH, reflux, 86%.

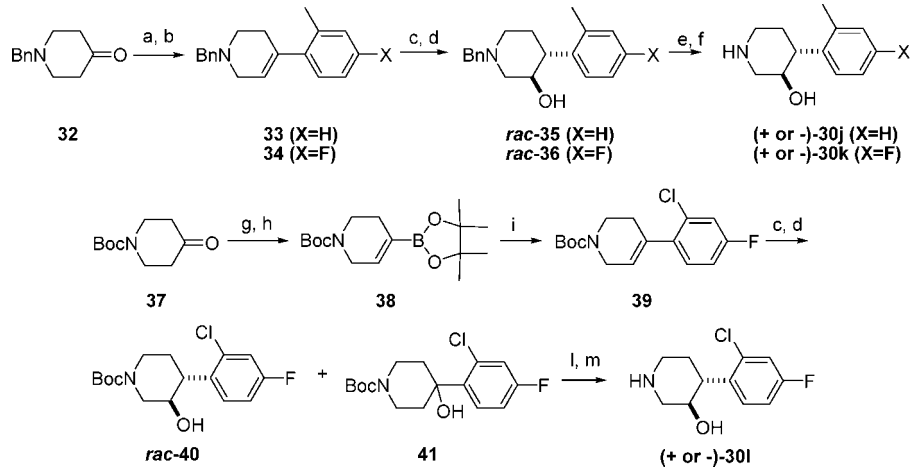
Stereoisomers (+)-, (−)-**8a** and (+)-, (−)-**8b** were synthesized as outlined in Scheme 5. The hydrolysis of ester **13** followed by amidation with 4-(*o*-tolyl)piperidine yielded amide **42**. Reduction of the two carbonyl moieties using $LiAlH_4$ provided the mixture of two diastereomers that were readily separated by column chromatography. These cis- and trans- racemates were separated by HPLC to afford optically pure analogues **8a** and **8b**.

General synthetic scheme for final products is illustrated in Scheme 6. In most cases (**8c**−**h**, **8j**−**l**, **9d**, **10b**, **10j**–**l**), the products were prepared by alkylation of the corresponding secondary amine with chiral tosylate intermediates **(−)-18**, **(+)-20**, or **(−)-27**, which proceeded smoothly by heating the mixture in the presence of Et_3N and NaI in DMF. Deprotection of the TBS or TES group using tetrabutylammonium fluoride provided the desired products in satisfactory yields. In the case of **8i**, piperidine-3-carboxylic acid ester was introduced in the same manner, followed by the reduction of ester **43**, then chiral separation using HPLC. In addition, treatment of ketal analogue **9d** with 4N HCl in dioxane and water at reflux temperatures gave ketone analogue **9a**, which was transformed into *tert*-alcohol analogues **9b** and **9c** by treating with methyl magnesium bromide.

Results and Discussion

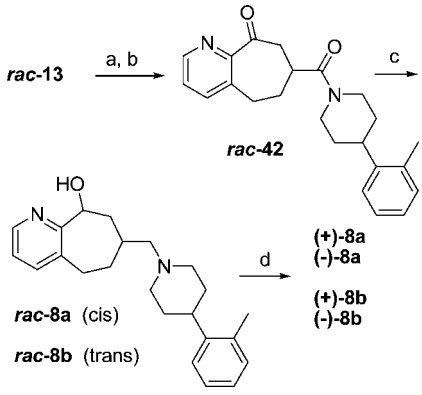
Initially, compounds were tested for their inhibitory activities on ligand binding to human ORL1 receptors and on GTPγS

Scheme 4[a]



[a] Reagents: (a) *o*-tolylMgBr/THF (for **33**), 2-bromo-5-fluorotoluene/*n*-BuLi/Et_2O (for **34**), 0 °C; (b) TFA, 80 °C, 72% (for **33**), 81% (for **34**); (c) $NaBH_4$, BF_3 etherate, r.t., DME; (d) 30% H_2O_2, 5N NaOH aq, 50 °C, 86% (for **35**), 49% (for **36**); (e) $Pd(OH)_2$, H_2 (1 atm), EtOH; (f) chiral separation by HPLC, *n*-hexane/EtOH/Et_2NH (800/200/1) (for **30j**), *n*-hexane/EtOH/Et_2NH (900/100/1) (for **30k**); (g) LiN^{i}Pr$_2$, Tf_2NPh, THF, −78 to 0 °C; (h) bis(pinacolate)diboron, $PdCl_2$(dppf), dppf, AcOK, dioxane, 80 °C, 98%; (i) 2-chloro-4-fluoroiodobenzene, $PdCl_2$(dppf), DMF, 80 °C, 38%; (l) 4N HCl-dioxane; (m) chiral separation by HPLC, *n*-hexane/EtOH/Et_2NH (900/100/1), 30% (from **39**).

Scheme 5. Synthesis of **8a** and **8b**[a]



[a] Reagents: (a) 6N aq HCl-dioxane, reflux; (b) 4-(*o*-tolyl)piperidine, EDCI, pyridine, r.t., 46%; (c) $LiAlH_4$, THF, 0 °C to reflux, 17% for ***rac*-8a**, 9% for ***rac*-8b**; (d) chiral separation by HPLC, *n*-hexane/^{i}PrOH/Et_2NH (900/100/1).

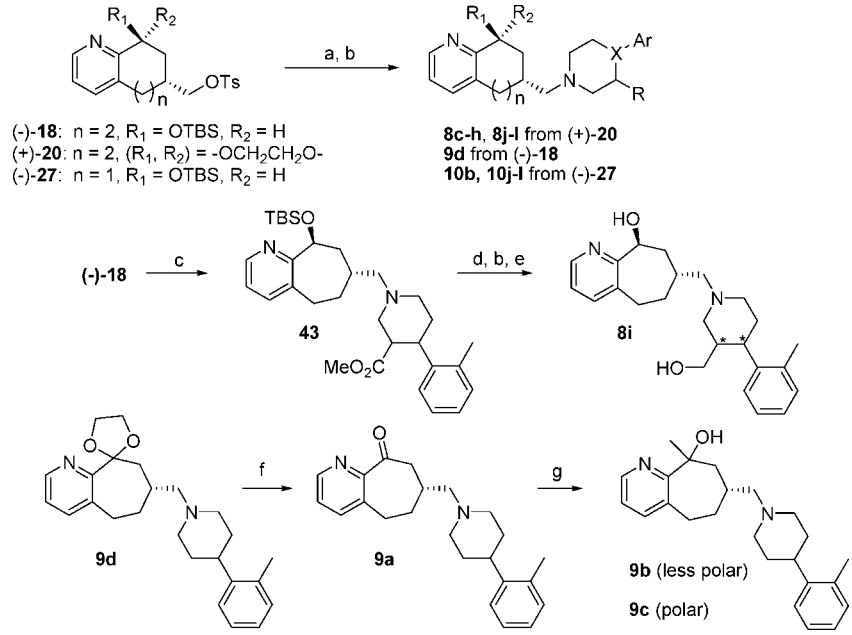
binding to proteins using membrane fractions of CHO cells that express ORL1. Binding affinities for ORL1 were determined by displacement of [^{125}I]Tyr^{14}-NC/OFQ, whereas antagonist activities were measured by the [^{35}S]GTPγS binding method.[22] For compounds with high binding affinity (K_i < 10 nM), their antagonist activity and binding affinity to the hERG K^+ channel were evaluated, unless otherwise noted. The binding affinity to the hERG K^+ channel was measured in the MK499 binding assay.[23] In all cases, optically pure compounds were used, and the values listed in the tables correspond to those of the more potent stereoisomer.

First, the binding affinities of the four stereoisomers ((+)-**8a**, (−)-**8a**, (+)-**8b**, and (−)-**8b**) to ORL1 and the hERG K^+ channel were compared (Table 1). Among these stereoisomers, (−)-**8b**, the enantiomer of the trans-isomer, exhibited the highest binding affinity to ORL1 (K_i 4.5 ± 0.2 nM) and a 600-fold selectivity over the hERG K^+ channel. The antagonist activity of (−)-**8b** to ORL1 was almost equal to that of benzene analogue **2b**.[24] Because (−)-**8b** exhibited a significantly higher binding affinity to ORL1 and a lower affinity to the hERG K^+ channel than its antipode (+)-**8b**, all subsequent analogues were prepared and evaluated as optically pure stereoisomers. It is important to note that, in the mouse locomotor activity tests, (−)-**8b** completely inhibited the ORL1 agonist-induced hypolocomotion at 3 mg/kg sc; in contrast, **2b** was not effective at 3 mg/kg sc,[25] despite comparable in vitro antagonist activity. In addition, the binding affinity to the hERG K^+ channel of (−)-**8b** was more than 30 times weaker than that of **2b**, which can be attributed to the increased hydrophilic character of **2b**, where the log $D_{7.4}$ values of (−)-**8b** and **2b** are 3.1 and 4.5, respectively.[26] These results prompted us to further optimize the structure of (−)-**8b** to overcome its moderate binding affinity to the hERG K^+ channel.

First, the effects of substituents at the benzylic position of the cycloheptanopyridine ring were investigated (Table 2). Decreased binding affinities to ORL1 for ketone **9a**, *tert*-alcohols **9b−c,** and ketal **9d** imply the importance of the secondary alcohol group at the 9-position for high binding affinity. Ring contraction of the cycloheptane ring to a cyclohexane ring (**10b**) resulted in merely a 2.4-fold decrease in the binding affinity to ORL1. In this case, the lipophilicity of **10b** (log $D_{7.4}$ = 2.0) was significantly lower than that of (−)-**8b** (log $D_{7.4}$ = 3.1), which could be important in optimizing the selectivity over the hERG K^+ channel. Accordingly, **10b** exhibited half the binding affinity to the hERG K^+ channel (IC_{50} = 4800 nM) than that of (−)-**8b** (IC_{50} = 2700 nM) (Table 4). Similar correlations between lipophilicity and binding affinity to the hERG K^+ channel were also observed for the SAR study of the piperidine moiety, as described below.

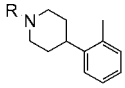
Next, SAR studies were carried out to investigate the effects of the 4-arylpiperidine moiety (Table 3). For **8d**, which possesses a chlorine atom instead of a methyl substituent at the 2-position of the benzene ring, the binding affinity to ORL1 was tolerable, whereas the affinity to the hERG K^+ channel was slightly enhanced. For **8c**, which possesses chlorine atoms at the 2- and 6-positions, the enhanced affinity to the hERG K^+ channel was remarkable and can be attributed to its higher lipophilicity. In contrast, the absence of any substituent at the 2-position (**8f**) significantly decreased not only the affinity to the hERG K^+ channel (IC_{50} = 11000 nM) but also to ORL1 (**8f**: K_i = 30 nM). Replacement of the piperidine with a piperazine ring (**8e**) decreased the binding affinity to ORL1 (**8e**: K_i = 20 nM). These results suggest that high binding affinity to ORL1 requires an

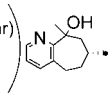
Scheme 6. Preparation of Final Products **8c−l**, **9a−d**, **10b**, and **10j−l**[a]



[a] Reagents: (a) secondary amines, Et_3N, NaI, DMF, 90 °C; (b) TBAF, THF, 50 °C; (c) *trans-rac* **30i**, Et_3N, NaI, DMF, 90 °C, 72%; (d) $LiAlH_4$, THF, 71%; (e) chiral separation by HPLC eluted with *n*-hexane/^{i}PrOH (17/3)/0.1% Et_2NH; (f) 4N HCl in dioxane−water, reflux, 58%; (g) MeMgBr, THF, 25% for **9b**, 52% for **9c**.

Table 2. Binding Affinity to ORL1 and Log $D_{7.4}$ Values of **9a−d** and **10b**



Compound	R	Binding assay: ORL1 K_i (nM)[a]	Log$D_{7.4}$[b]
(-)-8b		4.5 ± 0.2	3.1
9a		49 ± 7	1.3
9b (less polar)		24 ± 2	
9c (polar)		17 ± 1	2.9
9d		25 ± 1	3.1
10b		11 ± 0	2.0

[a] Displacement of [^{125}I]Tyr^{14}-nociceptin. [b] Determined by shake-flask method.

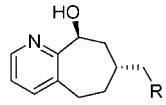
o-substituent, which presumably positions the 4-aryl ring at an optimal angle with respect to the piperidine ring. Replacing the 2-methyl group, however, with a bulkier substituent such as a phenyl ring or a more hydrophilic group resulted in a complete loss of potency (data not shown). For the 4-arylpiperidine moiety, *mono*-methyl or *mono*-chloro at the 2-position are optimal substituents in terms of the binding affinity to ORL1 and the selectivity to the hERG K^+ channel.

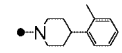
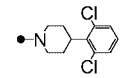
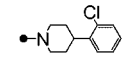
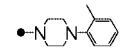
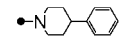
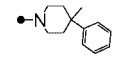
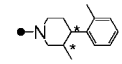
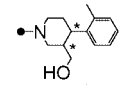
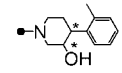
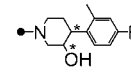
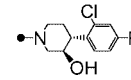
The effects of substituents at the piperidine ring were investigated by introducing a methyl group at the 3- or 4-position. The analogue with a methyl group at the 3-position (**8h**) was tolerable in terms of the binding affinity to ORL1—interestingly, however, its binding affinity to the hERG K^+ channel decreased 3-fold (IC_{50} = 9200 nM) without any loss of potency toward ORL1. Furthermore, incorporation of a hydroxymethyl group as a piperidinyl side chain (**8i**) resulted in a highly potent ORL1 antagonist with significantly improved selectivity over the hERG K^+ channel. Replacement of the methyl group of **8h** with a hydroxyl group (**8j**) substantially reduced the basicity of the piperidine-nitrogen atom, leading to the further decrease of the binding affinity to hERG.[26] Although the ORL1 antagonist activity of **8j** was halved, introduction of a fluorine atom into the 4-position of the phenyl ring (**8k** and **8l**) resulted in a 3- to 4-fold increase in the antagonist activity toward ORL1 (**8j** vs **8k**), along with a slight increase of the binding affinity to the hERG K^+ channel.

Because of the notable activity discovered in the 3,4-disubstituted piperidine analogues, these piperidines were combined with the six-membered ring scaffold to investigate their binding and physicochemical properties of **8j**, **8k**, and **8l** (Table 4). As was previously seen for cyclohexane analogue **10b**, the ORL1 binding for **10j** and **10k** decreased 5−6 fold as compared to the cycloheptane analogues. However, analogue **10l** exhibited good retention of its antagonist activity as well as binding affinity. Moreover, **10l** showed superior selectivity (>5000 fold) over the binding affinity to the hERG K^+ channel.

Having optimized the chemical structure of (–)-**8b** on the basis of decreasing the binding affinity for the hERG K^+ channel, we found that antagonists **8i**, **8j**, and **10l** possesses weak hERG binding affinities, with IC_{50} values of 20, 40, and >50 μM, respectively. Consequently, we found it necessary to determine their QTc intervals using isoflurane-anesthetized dogs (n = 1 or 3) to check the correlation between the in vitro hERG binding affinity and in vivo QTc interval (Table 5). Using a cumulative dosage of 7 mg/kg, **8i** exhibited moderate prolongation of the QTc interval (δQTc = 11.3%) at a peak plasma concentration of 21.8 μM. In contrast, **8j** and **10l** did not exhibit significant prolongation of the QTc interval at peak plasma levels of 17.9 and 29.8 μM, respectively. Because the plasma protein binding in dog were similar (64%, 62%, and 57%, respectively), optimization of the structural class that lack in vitro binding

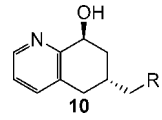
Table 3. Binding Affinity and Antagonist Activity to ORL1, Binding Affinity to hERG K^+ Channel, and Physicochemical Properties of Analogues **8**



Compound[a]	R	Binding assay ORL1 K_i (nM)[b]	GTPγS antagonism IC_{50} (nM)[c]	hERG binding IC_{50} (nM)[d]	log$D_{7.4}$[f]	pKa[g]
(-)-**8b**		4.5±0.2	9.0±1.7	2700 ±340	3.1	(9.2)[h]
8c		0.47±0.07	1.6±0.2	940 ±220	>4.0	
8d		1.1±0.1	2.7±0.2	1500 ±170		
8e		20±2		2300 ±290		
8f		30±4		11000 ±1300		
8g		58±3		11000 ±1000		
8h	 (trans)	3.5±0.5	5.7±0.5	9200 ±1300	3.0	
8i	 (trans)	4.3±0.5	7.3±0.8	20000 ±2500	2.0	8.9 (8.7)[h]
8j	 (trans)	10±0	18±2	40000[e]	2.2	7.8 (8.2)[h]
8k	 (trans)	3.7±0.1	4.5±0.9	13000 ±1300	2.5	(8.2)[h]
8l	 (trans)	4.4±0.5	5.0±0.5	13000 ±1400	2.9	(7.8)[h]

[a] All of listed compounds were more potent enantiomers. [b] Displacement of [^{125}I]Tyr^{14}-nociceptin. [c] IC_{50} values on nociceptin-produced [^{35}S]GTPγS binding to ORL1 expressed in CHO cells. [d] Displacement binding assay of [^{35}S]-radiolabeled MK-499 in membranes derived from HEK 293 cells stably transfected with hERG gene and expressing the I_{Kr} channel protein. [e] When only two experiments were performed, the value is expressed as the mean of duplicate determinations, and the variances between determinations were less than ±10%. [f] Determined by shake-flask method. [g] Determined by pH-metric method using Sirius GLpKa-02 instrument. [h] Calculated pK_a values.

Table 4. Binding Affinity and Antagonist Activity to ORL1 and Binding Affinity to hERG K^+ Channel of a Series of Cyclohexano[*b*]pyridine Derivatives



10

Compound[a]	R	binding assay ORL1 K_i (nM)[b]	GTPγS antagonism IC_{50} (nM)[c]	hERG binding IC_{50} (nM)[d]	Log $D_{7.4}$[g]	pK_a[h]
10b	4-(*o*-tolyl)piperidine	11 ± 0		4800[e]	2.0	(9.0)[i]
10j	*trans*-3-hydroxy-4-(*o*-tolyl)piperidine	51 ± 2		>50000	1.4	
10k	*trans*-3-hydroxy-4-(4-fluoro-2-methylphenyl)piperidine	21 ± 2		NT[f]	1.9	
10l	*trans*-3-hydroxy-4-(4-fluoro-2-chlorophenyl)piperidine	11 ± 2	12 ± 2	>50000	2.3	7.4 (7.7)[i]

[a] All of listed compounds were more potent enantiomers. [b] Displacement of [^{125}I]Tyr^{14}-nociceptin. [c] IC50 values on nociceptin-produced [^{35}S]GTPγS binding to ORL1 expressed in CHO cells. [d] Displacement binding assay of [^{35}S]-radiolabeled MK-499 in membranes derived from HEK 293 cells stably transfected with hERG gene and expressing the I_{Kr} channel protein. [e] The value is expressed as the mean of duplicate determinations, and the variances between determinations were less than ± 10%. [f] Not tested. [g] Determined by shake-flask method. [h] Determined by pH-metric method using Sirius GLpKa-02 instrument. [i] Calculated pK_a values.

affinity to the hERG K^+ channel would help predict a decreased potential to cause QTc interval prolongation in vivo.

Because **8j** and **10l** were highly potent without significant prolongation of the QTc interval, we compared their in vitro metabolic stability using rat and human hepatocytes against their in vivo pharmacokinetic profiles. As shown in Table 6, **10l** exhibited higher metabolic stability than **8j** in both rat and human hepatocytes. In fact, the clearance value of **10l** in the rat PK study was less than that of **8j**. This metabolic stability in human hepatocytes and PK profile in rat indicates that **10l** may be a more favorable candidate than **8j**. Moreover, **10l** exhibited better oral bioavailability with a lower clearance value in dogs (F = 39%)

Table 5. QTc Interval after iv Cumulative Dosing of **8i**, **8j**, and **10l** in Isoflurane-Anesthetized Dogs

compound	hERG binding[a] IC_{50} (nM)	iv dose[b] (mg/kg)	plasma conc[c] (μM)	plasma protein binding[d] (%)	δQTc[e] (%)
8i[f]	20000	1	2.69	64 ± 2	−0.1
		2	7.62		+0.1
		7	21.8		+11.3
8j	40000	1	2.26 ± 0.19	62 ± 6	0.2 ± 1.6
		2	4.90 ± 1.27		0.2 ± 1.6
		7	17.9 ± 3.36		2.0 ± 1.6
10l	>50000	1	3.48 ± 1.34	57 ± 3	1.1 ± 1.3
		2	8.30 ± 2.84		0.9 ± 0.5
		7	29.8 ± 11.9		2.0 ± 4.4

[a] Displacement binding assay of [^{35}S]-radiolabeled MK-499 in membranes derived from HEK 293 cells stably transfected with hERG gene and expressing the I_{Kr} channel protein. [b] iv dosing (over 10 min) per each dose as solutions in saline; 2 mg/kg and 7 mg/kg iv dosing (over 10 min) were performed after 20 min from the prior administration. [c] Maximum plasma concentrations after each dosing. Data from $n = 3$ dogs. Values are mean from three experiments ± SD. [d] The values represents mean ± SD by equilibrium dialysis method. [e] QTc intervals were measured at 0, 10, and 20 min after dosing and recorded at each time as a percent change from baseline for each dog. [f] Data obtained by $n = 1$.

than in rats ($F = 13\%$). Furthermore, in vitro metabolic stability in dog and human hepatocytes indicated similar calculated clearance values (CL_h, u, int; mL/min/mg protein) (0.0075 and 0.0089, respectively), suggesting that **10l** may be orally available in humans.

With **10l** chosen as the lead candidate, its selectivity was evaluated using other opioid receptors (μ, κ, and δ), excellent selectivity was demonstrated, with binding affinity to μ, κ, and δ >10000 nM. Furthermore, its in vivo antagonist activity against reduction in locomotor activity produced by an ORL1 agonist was determined. As shown in Table 7 and Figure 3, when dosed in mice orally, **10l** exhibited in vivo antagonist activity in a dose-dependent manner—nearly complete in vivo antagonist activity was observed at 10 mg/kg po (control; $n = 6$: 1346 ± 128 counts/60 min, agonist; $n = 6$: 193 ± 36 counts/60 min, agonist + **10l** (10 mg/kg, po); $n = 6$: 1219 ± 176 counts/60 min ($p < 0.01$ from agonist treated group), this reflecting its excellent brain penetrability (brain/plasma ratio = 5.81 ± 0.86).

Conclusion

In conclusion, our efforts to optimize a novel class of cycloalkano[*b*]pyridines led us to modify the 3- and 4-positions of the piperidine portion of the lead (−)-**8b**. This has led to the discovery of (−)-6-{[4-(2-chloro-4-fluorophenyl)-3-hydroxypiperidine-1-yl]methyl}-5,6,7,8-tetrahydroquinoline-8-ol (**10l**), which possesses high ORL1 receptor antagonist activity ($K_i = 12$ nM) and excellent selectivity to binding affinity to the hERG K^+ channel, as measured in the MK499 binding assay (IC_{50} = >50000 nM), as well as to other opioid receptors (IC_{50} = >10000 nM for μ, κ, and δ). This lack of binding affinity to the hERG K^+ channel was manifested in a no-effect QTc prolongation using isoflurane-anesthetized dogs. In addition, **10l** was orally active in the in vivo model. To the best of our knowledge, this was the first report of an orally active ORL1 receptor antagonist without adverse cardiovascular effects.

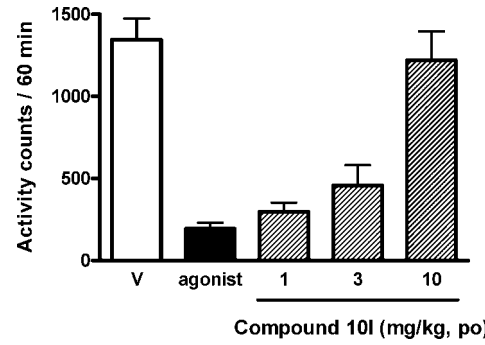


Figure 3. In vivo locomotor test by oral treatment of compound **10l** at 1, 3, and 10 mg/kg.

Experimental Section

General Information. All reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. Melting points were determined using a Yanako MP micromelting point apparatus and were not corrected. Optical rotations were determined on a JASCO P-1020 polarimeter. ^{1}H NMR spectra were recorded on a Varian MERCURYvx 400 spectrometer at 400 MHz or a Varian Gemini-300 spectrometer at 300 MHz or a Varian Gemini-200 spectrometer at 200 MHz and are referenced to residual solvent peaks (DMSO-d_6, δ 2.49 ppm; CD_3OD δ 3.30 ppm) or to an internal standard of tetramethylsilane (TMS, δ 0.00 ppm). Mass spectra were recorded with electron-spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on a Waters micromas ZQ, micromass Quattro II, or

Table 6. In Vitro Metabolic Stability in Rats and Human Hepatocytes and in Vivo Pharmacokinetic Profiles of **8j** and **10l**

	in vitro metabilic stability (%remaining)[a]		rat PK[d]					dog PK[e]				
			IV					IV				
no.	RH[b]	HH[c]	CLp (ml/min/kg)	$t_{1/2}$ (h)	$AUC_{0-\infty}$(μM·h)	Vd_{ss} (L/kg)	F (%)	CLp (ml/min/kg)	$t_{1/2}$ (h)	$AUC_{0-\infty}$(μM·h)	Vd_{ss} (L/kg)	F (%)
8j	6	62	91	0.9	1.6	6.3	11	NT				
10l	30	93	47	1.2	0.59	3.9	13	19	13.5	2.0	10.6	39

[a] Percent remaining after 30 min of incubation in extrapolated rat hepatocytes and cryopreserved human hepatocytes. [b] Rat hepatocytes. Values are expressed as the mean of duplicate determinations. [c] Human hepatocytes. Values are expressed as the mean of three experiments. [d] Pharmacokinetic study was conducted in fasted Sprague−Dawley rats ($n = 3$) dosed at 3 mg/kg (**8j**) and 0.65 mg/kg (**10l**) for intravenous dosing and dosed at 10 mg/kg (**8j**) and 3 mg/kg (**10l**) for oral dosing as a solution in water. [e] Pharmacokinetic study in fasted Beagle dogs ($n = 3$) dosed at 1 mg/kg for intravenous dosing and at 3 mg/kg for oral dosing.

Table 7. In Vitro and in Vivo Profiles for Compound **10l**

	binding assay	binding affinity IC_{50} (nM)				brain penetrability	
cmpd	ORL1[a] K_i (nM)	μ[a]	κ[a]	δ[a]	in vivo antagonism (% reversal)[b]	plasma (nM)[c]	brain levels (nmol/g brain)[c]
10l	11 ± 2	>10000	>10000	>10000	89	30 ± 7	172 ± 15

[a] Displacement of [^{125}I]Tyr^{14}-nociceptin, [^{3}H]diprenorphin, [^{3}H]U-69593, [^{3}H][D-Ala$_2$, D-Leu$_5$]enkephalin binding from CHO cells stably expressing cloned human ORL1, opioid μ-, opioid κ- and opioid δ-receptors, respectively. All values are means of three independent determinations performed in duplicate. [b] Data shows antagonist activity of analogue **10l** (10 mg/kg, po) against the reduction in locomotor activity produced by an ORL1 agonist for 60 min in mice ($n = 6$). Values are expressed as % reversal of the agonist response. [c] Plasma and brains of rats were collected at 1 h after the drug administration (10 mg/kg, po) and drug concentrations were measured ($n = 3$ mice/group).

micromass Q-Tof-2 instrument. Thin layer chromatography was performed using E-Merck Kieselgel 60 F_{254} plates (0.25 mm). Silica gel column chromatography was performed using Wakogel C-300 or an appropriately sized prepacked silica cartridge on a Biotage system. HPLC analysis of the final compounds was carried out using reversed phase HPLC (Zorbax Bonus RP 4.6 mm × 250 mm, s-3.5 um) with two diverse solvent systems: system A, compounds were eluted with a linear gradient of 10−90% B/A over 18 min at a flow rate of 1.0 mL/min, where solvent A was aqueous 10 mM potassium phosphate buffer and solvent B was MeOH; system B, compounds were eluted with a linear gradient of 10−90% B/A over 18 min at a flow rate of 1.0 mL/min, where solvent A was aqueous10 mM potassium phosphate buffer and solvent B was MeCN.

(−)-(7*R*,9*S*)-7-{[4-(2-Methylphenyl)piperidin-1-yl]methyl}-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-9-ol ((−)-8b). To a mixture of **42** (142 mg, 0.39 mmol) in THF (4.0 mL) was added $LiAlH_4$ (75 mg, 2.0 mmol) at 0 °C under a nitrogen atmosphere and the mixture was stirred at 70 °C for 2 h. The reaction mixture was quenched with the addition of $Na_2SO_4 \cdot 10H_2O$ and then $CHCl_3$ was added. The mixture was vigorously stirred at room temperature overnight, and the precipitates were filtered off. After the filtrate was concentrated under vacuum, the residue was purified using a preparative TLC ($CHCl_3$−MeOH = 95:5) to give ***rac*-8a** and ***rac*-8b** as a white powder. The racemic **8b** was optically resolved using chiral HPLC (CHIRALCEL OD, 2 cm × 25 cm, *n*-hexane/2-propanol (9:1) with 0.1% Et_2NH, flow rate = 15 mL/min) to give optically pure enantiomers of **8b**. Chiral analytical HPLC indicated that enantiomeric excess (ee) of each enantiomer was >99% (CHIRALCEL OD, 0.46 cm × 25 cm, *n*-hexane/2-propanol (9:1) with 0.1% Et_2NH, flow rate = 1.0 mL/min, t_R = 7.8 min. for (+)-**8b**, 14.7 min for (−)-**8b**. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.58−1.87 (m, 7H), 2.03−2.16 (m, 4H), 2.34 (s, 3H), 2.53−2.75 (m, 4H), 2.84−2.89 (m, 1H), 3.00−3.07 (m, 2H), 4.93−4.97 (m, 1H), 5.75 (brs, 1H), 7.08−7.28 (m, 5H), 7.43−7.46 (m, 1H), 8.36 (d, J = 4.9 Hz, 1H). MS (ESI) *m*/*z* 363 $(M + H)^+$. **(−)-8b** fumarate salt; Anal. ($C_{23}H_{30}N_2O_2 \cdot 1.05C_4H_4O_4$) C, H, N.

General Procedure for the Preparation of Compounds 8c−8l, 9d, 10b, and 10j−l. The corresponding chiral tosylate in DMF (0.2−0.5 M) was heated with the appropriate amine (1.5 equiv), sodium iodide (10 equiv), and Et_3N (5 equiv) at 90 °C overnight. After addition of water and EtOAc, the aqueous phase was washed twice with EtOAc. The combined organic layers were successively washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. After being purified by flush column chromatography, the product was stirred overnight at 50 °C with tetrabutylammonium fluoride (0.5 M in THF, 10 equiv). The mixture was diluted with EtOAc, washed with aq saturated $NaHCO_3$ three times and then brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by column chromatography (NH-SiO_2 eluted with *n*-hexane/EtOAc or $CHCl_3$/MeOH). The final compounds were isolated as a free base or fumaric acid salt or L-tartaric acid salt or HCl salt.

(−)-*trans*-3-(Hydroxymethyl)-1-{[(7*R*,9*S*)-9-hydroxy-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-7-yl]methyl}-4-(2-methylphenyl)piperidinium L-Tartarate (8i). ^1H NMR (CD_3OD, 200 MHz) δ: 1.23−1.70 (m, 2H), 1.83−2.20 (m, 3H), 2.20−2.36 (m, 1H), 2.35 (s, 3H), 2.41−2.68 (m, 1H), 2.68−3.27 (m, 8H), 3.27−3.47 (m, 2H), 3.63−3.90 (m, 2H), 4.46 (s, 2H), 4.93−5.12 (m, 1H), 7.00−7.33 (m, 5H),7.63 (d, J = 7.2 Hz, 1H), 8.26 (d, J = 4.8 Hz, 1H). Anal. ($C_{24}H_{32}N_2O_2 \cdot 1.0C_4H_6O_6 \cdot 1.8H_2O$) C, H, N.

(+)-*trans*-3-Hydroxy-1-{[(7*R*,9*S*)-9-hydroxy-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-7-yl]methyl}-4-(2-methylphenyl)piperidinium L-Tartarate (8j). ^1H NMR (CD_3OD, 400 MHz) δ: 1.22−1.38 (m, 1H), 1.52 (dd, J = 11.8 and 12.5 Hz, 1H), 1.85−2.27 (m, 3H), 2.35 (s, 3H), 2.71−2.82 (m, 3H), 2.89−3.07 (m, 4H), 3.30−3.37 (m, 1H), 3.55 (d, J = 12.5 Hz, 1H), 3.63 (dd, J = 3.3 and 11.4 Hz, 1H), 3.63 (dd, J = 3.3 and 11.4 Hz, 1H), 4.19 (dt, J = 4.4 and 10.3 Hz, 1H), 4.40 (s, 2H), 5.01 (d, J = 7.6 Hz, 1H), 7.05 (dd, J = 7.3 and 8.0 Hz, 1H), 7.08 (d, J = 8.0 Hz, 1H), 7.12 (dd, J = 7.2 and 7.3 Hz, 1H), 7.20 (dd, J = 5.1 and 7.0 Hz, 1H), 7.26 (d, J = 7.2 Hz, 1H), 7.55 (d, J = 7.0 Hz, 1H), 8.20 (d, J = 5.1 Hz, 1H). Anal. ($C_{23}H_{30}N_2O_2 \cdot 1.3C_4H_6O_6 \cdot 1.8H_2O$) C, H, N.

(−)-(3*R*,4*R*)-4-(2-Chloro-4-fluorophenyl)-3-hydroxy-1-{[(6*R*,8*S*)-8-hydroxy-5,6,7,8-tetrahydroquinolin-6-yl]methyl}piperidinium Chloride (10l). ^1H NMR (CD_3OD, 300 MHz) δ: 1.84−1.96 (m, 1H), 2.05−2.18 (m, 2H), 2.29−2.38 (m, 1H), 2.67−2.85 (m, 2H), 2.97−3.09 (m, 1H), 3.15−3.43 (m, 5H), 3.66−3.82 (m, 2H), 4.39−4.50 (m, 1H), 4.91−4.97 (m, 1H), 7.09−7.28 (m, 2H), 7.48−7.60 (m, 2H), 7.97 (d, J = 7.5 Hz, 1H), 8.53 (d, J = 4.8 Hz, 1H). Anal. ($C_{21}H_{24}ClFN_2O_2 \cdot HCl$) C, H, N.

Measurement of in Vitro Binding Assay to ORL1. Binding of [^{125}I][Tyr^{14}]nociceptin to membrane preparations is performed in 0.2 mL of 50 mM Hepes buffer (pH 7.4) containing 10 mM NaCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.025% Bacitracin, and 0.1% BSA. The membranes prepared from CHO cells expressing human ORL1 receptors are incubated at 37 °C for 60 min with [^{125}I][Tyr^{14}]nociceptin (approximately 50 pM) in the presence of various concentrations of the compound. Bound and free radioligands are separated by filtration using a unifilter plate GF/C-96 presoaked with 0.5% polyethylenimine. The remaining radioactivity on the filter is quantitated using a TopCount-HTS (PerkinElmer). Specific binding of [^{125}I][Tyr^{14}]nociceptin is defined as the difference between total binding and nonspecific binding in the presence of 1 μM nociceptin. IC_{50} values are calculated using GraphPad Prism (Ver.3.03). K_i values are determined from IC_{50} using the equation $K_i = IC_{50}/(1+[L]/K_d)$ [K_d value for nociceptin = 16 pM].

Measurement of in Vitro Antagonist Activity. [^{35}S]GTPγS binding assay to human ORL1 receptor is performed in 0.2 mL assay buffer (20 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4) containing 5 μM GDP. The membranes are incubated for 2.5 h at 25 °C with [^{35}S]GTPγS (approximately 200 pM) and 1.5 mg/assay wheat germ agglutinin-coated SPA beads in the presence of several concentration of compound and appropriate concentration of nociceptin (EC_{60}−EC_{80}: 10−20 nM). Nonspecific binding is determined in the presence of 10 μM unlabeled GTPγS. Membrane-bound radioactivity is quantitated using a TopCount-HTS (PerkinElmer). Specific binding of [^{35}S]GTPγS is determined as a difference between "control" (in the presence of appropriate concentration of nociceptin) and "baseline" (in the presence of DMSO instead of compound). IC_{50} values are calculated from % of specific binding in the presence of various concentration of the compound using GraphPad Prism 3.03.

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Supporting Information Available: A table listing the purity (area percent and retention time) based on HPLC analysis for all target compounds, a table listing the elemental analysis, melting point, MS $(M + H)^+$, and optical rotations for all target compounds, spectral data and experimental procedure for target compounds except for **(−)-8b**, **8i**, **8j**, and **10l**, experimental procedure and spectral data for synthetic intermediates, in vivo pharmacological experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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