Novel Non-Peptide Nociceptin/Orphanin FQ Receptor Agonist, 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole: Design, Synthesis, and Structure−Activity Relationship of Oral Receptor Occupancy in the Brain for Orally Potent Antianxiety Drug[1,2]

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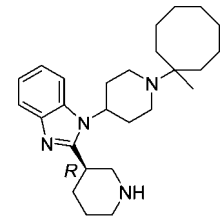
An endogenous heptadecapeptide, nociceptin/orphanin FQ (N/OFQ), and a G-protein-coupled receptor, N/OFQ peptide (NOP) receptor [or opioid-receptor-like-1 (ORL1) receptor], have been described in terms of its structure, distribution, and pharmacology. Thus, the N/OFQ and NOP receptor are located in the central nervous systems in humans, primates, and rodents, and are involved in the integration of the emotional components in the brain; e.g., N/OFQ displays anxiolytic activity in the brain. For identifying orally potent anxiolytic, drug-design studies were performed with a series of 1,2-disubstituted benzimidazole derivatives, which resulted in the identification of various chemotypes of highly potent NOP selective full agonists in vitro with high or moderate NOP receptor occupancy in the mice brain per os such as 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole **1** (MCOPPB), the most potent novel non-peptide NOP full agonist in vitro and an orally potent anxiolytic in the mice.

Introduction

Anxiety disorders[3−5] such as generalized anxiety disorder (GAD[a]),[6] social anxiety disorder (SAD),[7] post-traumatic stress disorder (PTSD),[8] panic disorder (PD),[9] specific phobias,[10] and obsessive-compulsive disorder (OCD),[11] caused by continuous or acute stress or fear, comprise the majority of all psychiatric disorders for a long period. For the acute treatment of anxiety, benzodiazepines remain the drug of choice because of their effectiveness and rapid onset of action, in spite of having several liabilities that limit their therapeutic utility,[12,13] namely, amnesia,[14] muscle relaxation,[15] daytime or driving-time sleepiness,[16,17] alcohol interaction,[15] and abuse potential by drug dependence.[18]

Meanwhile, an opioid receptor that was regarded as one of orphan G-protein-coupled receptors (GPCRs) and has high homology to classical opioid receptors (such as μ-, κ-, and δ-receptors) that belong to the superfamily of GPCRs, opioid-receptor-like-1 receptor (ORL1 receptor, or as the fourth opioid peptide receptor, OP4 receptor) has been described in terms of structure, distribution, and pharmacology, recently.[19−23] As well, the isolation of a heptadecapeptide, named nociceptin[24] (or orphanin FQ),[25] has subsequently been reported with its structure, location, and physiological roles with the receptor. Nowadays the ORL1 receptor is referred to as the N/OFQ peptide receptor, NOP receptor. The NOP receptor and N/OFQ are located at high levels in forebrain that includes cortical and limbic regions and in brainstem, e.g., cerebral cortex, hippocampus, hypothalamus, thalamus, amygdala, septum, nucleus accumbens, and locus coeruleus, which involve the integration of the emotional components of fear and stress in human, primate, and rodent.[26−30] Furthermore, N/OFQ[31−38] and other NOP receptor agonist[39−41] have demonstrated anxiolytic activities in some laboratory animals after intracerebroventricular (icv) or intraperitoneal (ip) administration. The aim of this study is to find orally potent, metabolically stable, and side effect free or minimized NOP receptor agonist with new chemotype as a new-class anxiolytic, together with differentiation from diazepam that is a representative of benzodiazepine-type anxiolytic.

Chart 1. Structure of Novel Orally Potent NOP Receptor Agonist, 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole **1** (MCOPPB)



MCOPPB, 1

Chemistry

The retrosynthesis of the NOP receptor agonist **1**, 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole (MCOPPB) (Chart 1), was outlined in Scheme 1. Thus, the optically pure (*R*)-enantiomer **1** was prepared from N-protected chiral (*R*)-nicopetic acid **2** and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3**.

First, the (*R*)-enantiomer of N-protected nicopetic acid **2** was synthesized as follows (Scheme 2). Thus, the amino portion of ethyl (*R*)-nipecotate **5**, prepared from commercially available

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[a] Abbreviations: GPCR, G-protein-coupled receptor; N/OFQ, nociceptin/orphanin FQ; [^{35}S]GTPγS, [^{35}S]guanosine 5′-γ-thiotriphosphate; HEK, human embryonic kidney; CHO, Chinese hamster ovary; GAD, generalized anxiety disorder; SAD, social anxiety disorder; PTSD, post-traumatic stress disorder; PD, panic disorder; OCD, obsessive-compulsive disorder; MCOPPB, 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole; LAH, lithium aluminium hydride; WSCI, water soluble carbodiimide, i.e., 1-ethyl-3-(3-dimethylaminopropylcarbodiimide) hydrochloride; ee, enantiomeric excess (or enantiomer excess); SAR, structure–activity relationship. The following are in accordance with Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR): N/OFQ peptide receptor, NOP receptor (or opioid-receptor-like-1 (ORL1) receptor); MOP receptor, μ-opioid receptor; KOP receptor, κ-opioid receptor; DOP receptor, δ-opioid receptor.

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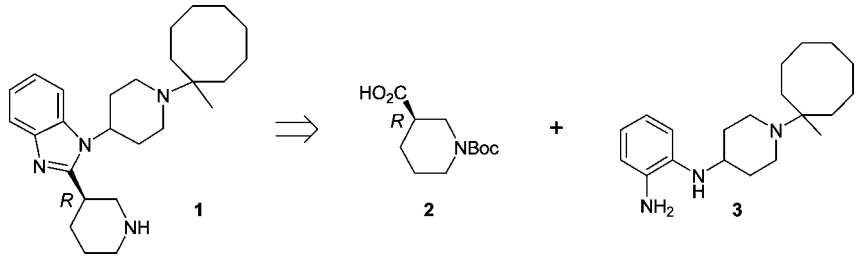
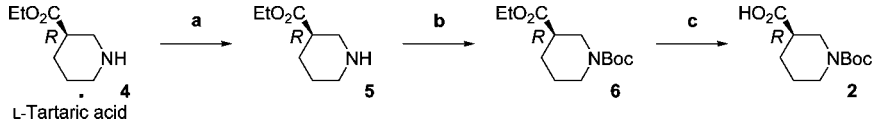
Scheme 1. Retrosynthesis of 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole **1** (MCOPPB) from **2** and **3**

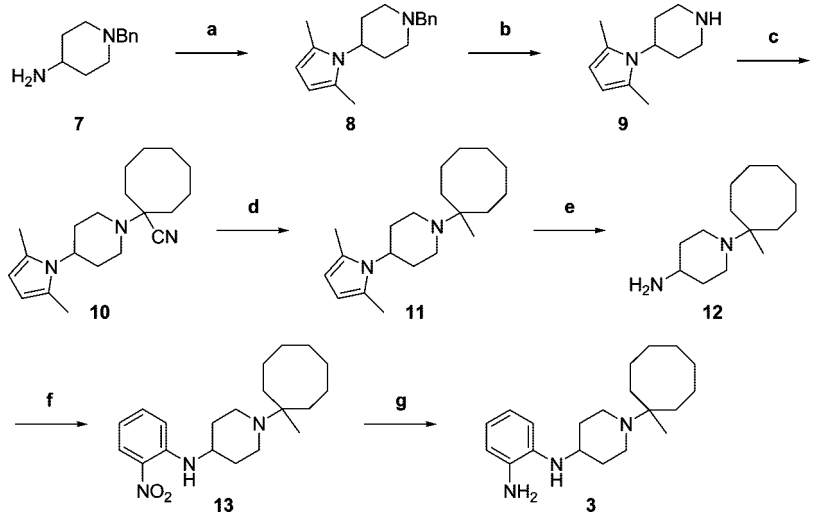


Scheme 2. Synthesis of (*R*)-*N*-Boc-Nipecotic Acid **2**[a]



[a] Reagents and conditions: (a) neutralization; (b) Boc_2O, CH_2Cl_2, 0 °C to room temp; (c) $LiOH/H_2O-MeOH/THF$, 0∼6 °C.

Scheme 3. Synthesis of *N*-[1-(1-Methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3**[a]



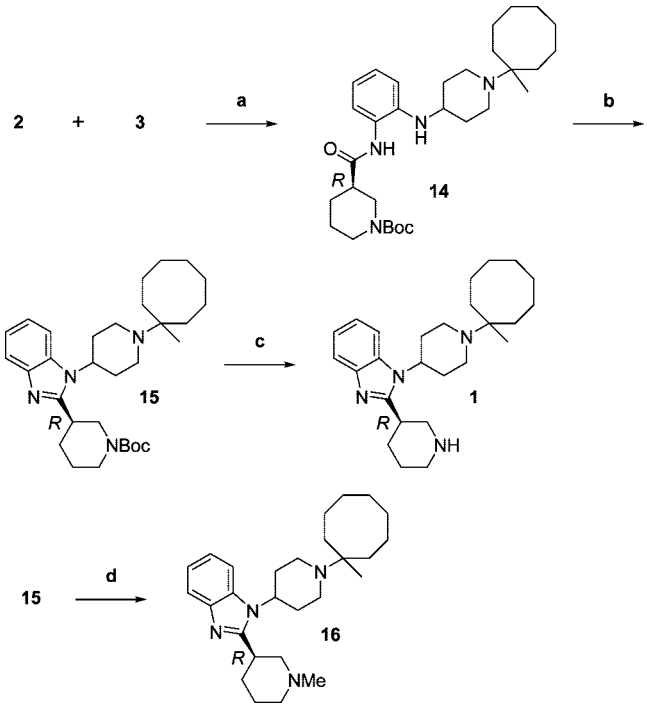
[a] Reagents and conditions: (a) 2,5-hexanedione, AcOH, toluene, reflux; (b) $Pd(OH)_2$, H_2 (4.5 atm), MeOH; (c) cyclooctanone, KCN, TsOH, H_2O, 45 °C; (d) MeMgBr/THF, CH_2Cl_2, −78 °C to room temp; (e) $NH_2OH\cdot HCl$, $EtOH/H_2O$, reflux; (f) 1-fluoro-2-nitrobenzene, K_2CO_3, CH_3CN, reflux; (g) Zn, NH_4Cl, $MeOH/H_2O$.

ethyl (*R*)-nipecoate L-tartrate **4** (Aldrich) by basic extraction, was protected with *tert*-butoxycarbonyl (Boc) group, followed by alkalic hydrolysis of its ester portion to give (*R*)-*N*-Boc-nipecotic acid **2**.[42,43]

N-[1-(1-Methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** was prepared as follows (Scheme 3). First, the primary amino group of commercially available 4-amino-1-benzylpiperidine **7** was protected as a pyrrole skeleton transformed by acidic condensation with 2,5-hexanedione to give 4-(1-benzylpiperidinyl)-2,5-dimethylpyrrole **8**[44] and then its benzyl group at the nitrogen portion of the piperidine ring was removed by hydrogenolysis with $Pd(OH)_2$ to afford 4-piperidinyl-2,5-dimethylpyrrole **9**. Second, the cyclooctylmethyl group was introduced onto nitrogen atom of the piperidine ring of the pyrrole derivative **9** by Strecker reaction with cyclooctanone and potassium cyanide followed by methylation with Grignard reagent[45,46] to give 4-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1-(1-methylcyclooctyl)piperidine **11**. Third, the pyrrole protective group of **11** was removed with hydroxylammonium to generate 1-(1-methylcyclooctyl)piperidin-4-amine **12**, which was introduced onto the 1-position of 1-fluoro-2-nitrobenzene by substitution reaction[47] to obtain 1-(1-methylcyclooctyl)-*N*-(2-nitrophenyl)piperidin-4-amine **13**. Then the nitro group of **13** was reduced to the amino group with zinc powder[48] to afford the corresponding 1,2-phenylenediamine derivative, e.g., *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3**.

By use of **2** and **3**, benzimidazole skeleton formation and the final conversion to compound **1** were consecutively performed as follows (Scheme 4). Thus, (*R*)-*N*-Boc-nipecotic acid **2** was amidated with *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** by water soluble carbodiimide, i.e., 1-ethyl-3-(3-dimethylaminopropylcarbodiimide) hydrochloride (WSCI) and 1-hydroxybenzotriazole (HOBT) method[42,43] to give *tert*-butyl (3*R*)-3-{[(2-{[1-(1-methylcyclooctyl)-4-piperidinyl]amino}phenyl)amino]carbonyl}-1-piperidinecarboxylate **14**. In succession, the resulting amide portion and the internal secondary amino portion of **14** were condensed with mild acidic dehydration in the presence of AcOH in toluene after reaction condition studies[49] to give *tert*-butyl (3*R*)-3-{1-[1-(1-methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate **15**. Finally, the *N*-Boc portion of the 2-(3-pipeidinyl)benzimidazole derivative **15** was deprotected by acidic condition, followed by neutralization to afford the requisite (3*R*)-*NH*-piperidine derivative, 1-[1-(1-methylcyclooc-

Scheme 4. Synthesis of 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole **1** and Its *N*-Me Derivative **16**[a]



[a] Reagents and conditions: (a) WSCI, HOBT, Et_3N, CH_2Cl_2, −30 °C to room temp; (b) AcOH, toluene, 100 °C; (c) 10% HCl in MeOH; (d) LAH, THF, room temp to reflux.

tyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole **1**, as the sole product as a solid. The analysis of chemical purity and enantiomeric excess (ee) [i.e., (*R**-enantiomer − *S**-enantiomer)/(*R**-enantiomer + *S**-enantiomer)] of compound **1** showed it to be 98% and 97% ee, respectively, which indicated that the total synthesis had no chemical and racemization problem.

As well, compound **16**, the (3*R*)-*N*-Me piperidinyl analogue of the compound **1**, was prepared from the above (3*R*)-*N*-Boc piperidine derivative **15** by reduction of its *N*-Boc moiety with lithium aluminium hydride (LAH)[50] (Scheme 4).

On the other hand, compound **21**, the (3*S*)-enantiomer of the *NH*-piperidine derivative **1**, and compound **22**, the (3*S*)-enantiomer of the *N*-Me piperidine derivative **16**, were synthesized in the same preparation manners for their respective (3*R*)-enantiomers. Thus, utilizing (*S*)-*N*-Boc-nipecotic acid **18** prepared from commercially available (*S*)-nipecotic acid hydrochloride **17** (Digital Specialty Chemicals), and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3**, the corresponding 2-[(3*S*)-*N*-Boc-piperidinyl]benzimidazole derivative **20** was prepared by amidation, then deprotection of its *N*-Boc portion to obtain **21** and LAH reduction of the *N*-Boc portion to obtain **22** were conducted, respectively (Scheme 5).

Furthermore, various 2-aryl- or 2-bicycloarylbenzimidazole analogues **23**−**26** and **36** were prepared as shown below (Scheme 6). Thus, *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** was amidated with a variety of arylcarboxylic acids **27**−**30** or arylcarbonyl chloride **35**, followed by internal condensation with $POCl_3$ to afford the corresponding 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-aryl-1*H*-benzimidazole derivatives, e.g., 3′-$MeSO_2$-phenyl analogue **23**, 3′-Cl-4′-F-phenyl analogue **24**, 6′-indolyl analogue **25**, 2′-benzofuranyl analogue **26**, and 3′-CF_3-phenyl analogue **36**, respectively.[42,49,51]

As well, 2-arylbenzimidazole analogues bearing active-hydrogen of the functional groups at 3′-position of the aryl portion, e.g., 3′-($HOCH_2CH_2O$)-phenyl analogue **40** and 3′-(H_2NCH_2)-phenyl analogue **43**, were prepared as follows (Scheme 7). In the former case (**40**), the corresponding ethyl (3-formylphenoxy)acetate **38** for the construction of ($HOCH_2$-CH_2O)-phenyl portion was prepared from 3-hydroxybenzaldehyde **37** with ethyl 1-bromoacetate, which was converted into benzimidazole derivative **39** by oxidative condensation in the presence of $Pb(OAc)_4$[52] with the above 1-substituted 1,2-phenylenediamine **3**, followed by reduction of its ester group with LAH to afford 3′-($HOCH_2CH_2O$)-phenyl analogue **40**. In the latter case (**43**), the phenylenediamine derivative **3** was amidated with 3-cyanobenzoyl chloride **41**, followed by benzimidazole skeleton formation with $POCl_3$ to give **42**, whose nitrile portion on its phenyl core was reduced with LAH to afford 3′-(H_2NCH_2)-phenyl analogue **43**.[53]

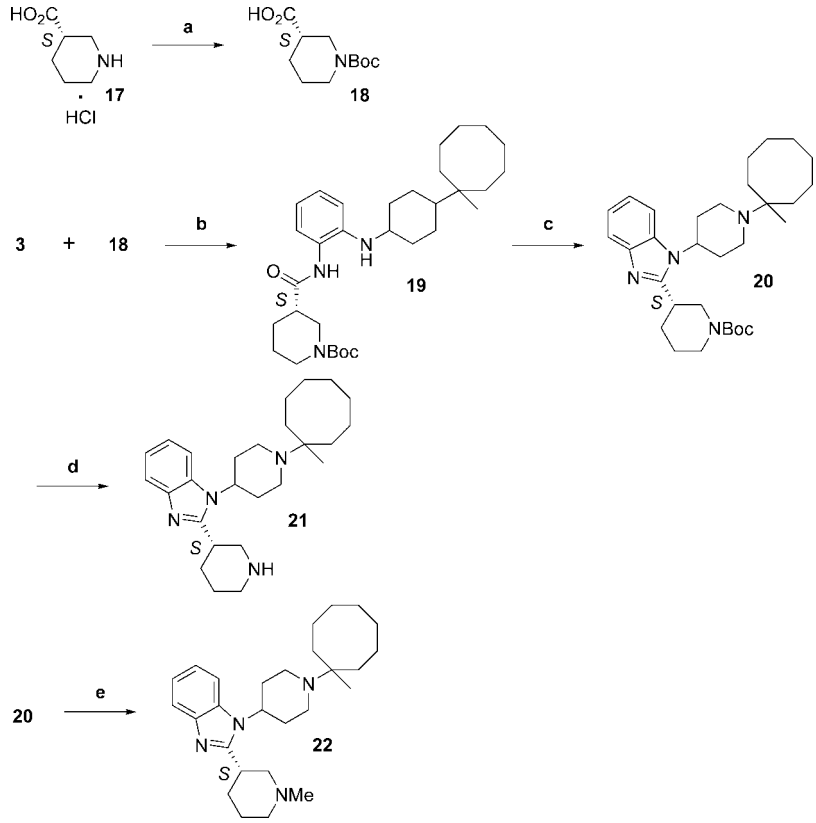
All of these 1,2-disubstituted benzimidazole derivatives **1**, **16**, **21**−**26**, **36**, **40**, and **43** were converted into tri- or dihydrochloride using HCl in MeOH. The purities of the hydrochlorides were confirmed by elementary analysis to be within ±0.3% of calculated values, respectively. These salts were used for SAR studies with pharmacological evaluations.

Results and Discussion

For structure−activity relationship (SAR) study of NOP receptor agonist in vitro, various types of 1,2-disubstituted benzimidazole derivatives were designed and synthesized for our in vitro criteria as NOP receptor agonists, i.e., the K_i value for the binding affinity to human NOP receptor (hNOP receptor) was less than 2 nM; the EC_{50} value for the functional activity of [^{35}S]GTPγS binding in response to the agonist–hNOP receptor binding was less than 10 nM; and the selectivities of the K_i and EC_{50} values for NOP receptor against other opioid receptors such as human μ-receptor (hMOP receptor), human κ-receptor (hKOP receptor), and human δ-receptor (hDOP receptor) were over 10-fold.

The biological assays in vitro were performed as follows. First, the binding affinities of the synthetic NOP agonists to hNOP receptors were measured by the displacement of tritium-labeled N/OFQ for the recombinant hNOP receptors expressed in human embryonic kidney (HEK)-293 cells. Second, the biological responses were determined by the induction of the binding of radiolabeled ligand [^{35}S]GTPγS to the α-unit of the G-protein due to the binding of the compounds to hNOP receptors in the HEK-293 cells.[54,55] As for the in vitro selectivity evaluation of the NOP agonists, binding affinities and biological functional activities for other opioid receptors were determined as well. Thus, the binding affinities of the NOP agonists to other human opioid receptors, i.e., μ-, κ-, and δ-receptors that expressed in Chinese hamster ovary (CHO)-K1 cells, HEK-293 cells, and CHO-K1 cells, respectively, were measured by the displacement of corresponding radiolabeled ligands such as [^{3}H]DAMGO for human μ-receptors, [^{3}H]enadoline for human κ-receptors, [^{3}H]DPDPE for human δ-receptors, respectively. And the functional activities were determined by the induction of [^{35}S]GTPγS binding stimulated by the binding of the compounds to the each opioid receptors (μ-, κ-, or δ-receptors) expressed in the above respective cell lines. In these function studies, the EC_{50} (potency) value was the concentration producing a half-maximal response of its own and the E_{max} (efficacy) value was the maximal response calculated as the percentage of the maximal response produced by each control such as N/OFQ, DAMGO, enadoline, and DPDPE.

Scheme 5. Synthesis of 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*S*)-3-piperidinyl]-1*H*-benzimidazole **21** and Its *N*-Me Derivative **22**[a]



[a] Reagents and conditions: (a) Boc_2O, Et_3N, MeOH, 0 °C to room temp; (b) WSCI, HOBT, Et_3N, CH_2Cl_2, −30 °C to room temp; (c) AcOH, toluene, 100 °C; (d) 10% HCl in MeOH; (e) LAH, THF, room temp to reflux.

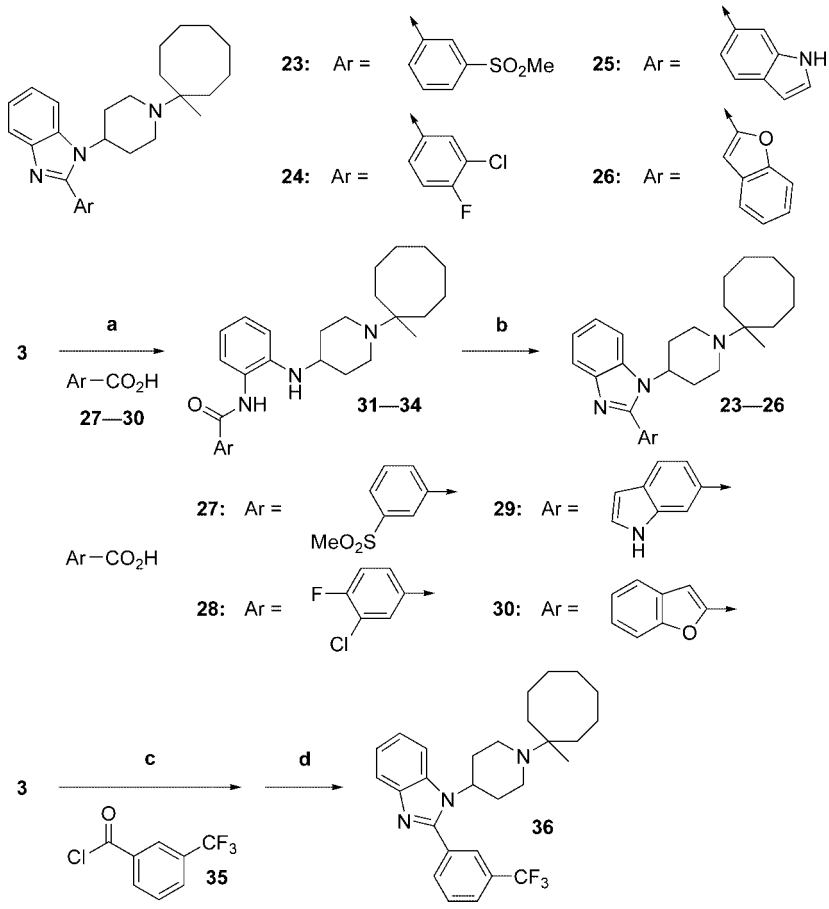
In general as summarized in Table 1, various 1,2-disubstituted benzimidazole analogues, e.g., most of the 1-[1-(1-alkylcyclooctyl)-4-piperidinyl]-2-substituted-1*H*-benzimidazole derivatives in the Table were satisfied for the above in vitro criteria. First, the K_i values of the compounds **1**, **16**, **21**–**26**, **36**, **40**, and **43** for the binding affinities to hNOP receptor were less than 2 nM, i.e., 0.0858−1.70 nM. Second, the EC_{50} values of the compounds **1**, **16**, **21**–**24**, **26**, **36**, **40**, and **43** for the activity of [^{35}S]GTPγS binding in response to the respective agonist–hNOP receptor binding were less than 10 nM, i.e., 0.39−5.7 nM, with greater maximal effects than that of N/OFQ. Furthermore, the selectivities of the binding (K_i) and function (EC_{50}) of the NOP agonists, including (3*R*)-enantiomers **1** and **16**, for hNOP receptor against other opioid receptors were greater than 10-fold except for (3*S*)-enantiomers **21** and **22** that were partially less selective (Tables 1 and 2). In the function studies, their maximal effects were low or partial to other opioid receptors except for the δ-receptor response with very low binding affinities (Table 1). Taken together, these were selective full NOP receptor agonists with comparative potency to N/OFQ, although their selectivities were not compatible to N/OFQ.[56]

From the viewpoint of SAR, these 1,2-disubstituted benzimidazole derivatives which possess a six-member ring such as the cycloalkylamino ring (**1**, **16**, **21**, and **22**), phenyl ring (**23**, **24**, **36**, **40**, and **43**), and bicyclic ring such as benzo-fuzed heteroaryl ring (**25** and **26**) were well tolerable as 2-substituents of the benzimidazole. In addition, the structural characteristics at the β-position on the six-member ring were significant and allowed for a variety of modifications of bulkiness and different chemotypes with diverse polarity, lipophilicity, basicity, and size for potent and selective NOP receptor agonists with the 1-(methylcyclooctyl-piperidinyl)benzimidazole skeleton, i.e., secondary amine (**1** and **21**) or tertiary amine portions (**16** and **22**) as part of the piperidine ring, and methanesulfonyl (**23**), halogen (**24**), trifluoromethyl (**36**), hydroxyethoxy (**40**), or aminomethyl (**43**) group as a substituent on phenyl core. In the 5′-indole case (**25**), NH-portion of the indole core is possible as a substituent at the β-position of phenyl core, like the above 2-aryl substituted benzimidazole analogues. Also, another bicycloaryl type, i.e., 2′-benzofuran skeleton (**26**), is allowable as a 2-substituent of benzoimidazole.

The rank of the orders of the binding affinities (K_i values) for the 2-substituents on benzimidazole portion is 3′-*NH*-piperidinyl (**1** and **21**) > 3′-*N*-Me-piperidinyl (**16** and **22**), 3′-($MeSO_2$)-phenyl (**23**), 3′-Cl-4′-F-phenyl (**24**), 3′-(H_2NCH_2)-phenyl (**43**) > 3′-($HOCH_2CH_2O$)-phenyl (**40**) > 2′-benzofuranyl (**26**) > 3′-CF_3-phenyl (**36**) > 6′-indolyl (**25**). In comparison, the order of the potencies of the biological response (EC_{50} values) is approximately the same as the above K_i values. To be more precise, there are highly significant positive correlations of the EC_{50} values of NOP agonists for [^{35}S]GTPγS binding to the respective K_i values of NOP agonists for inhibition of [^{3}H]N/OFQ binding as shown in Figure 1 ($r^2 = 0.984$, $p < 0.0001$), indicating that the binding of NOP agonist to NOP receptor is the primary mechanism of the G-protein activation response and the potency of the functional activity depends on the potency of the binding affinity for each analogue.

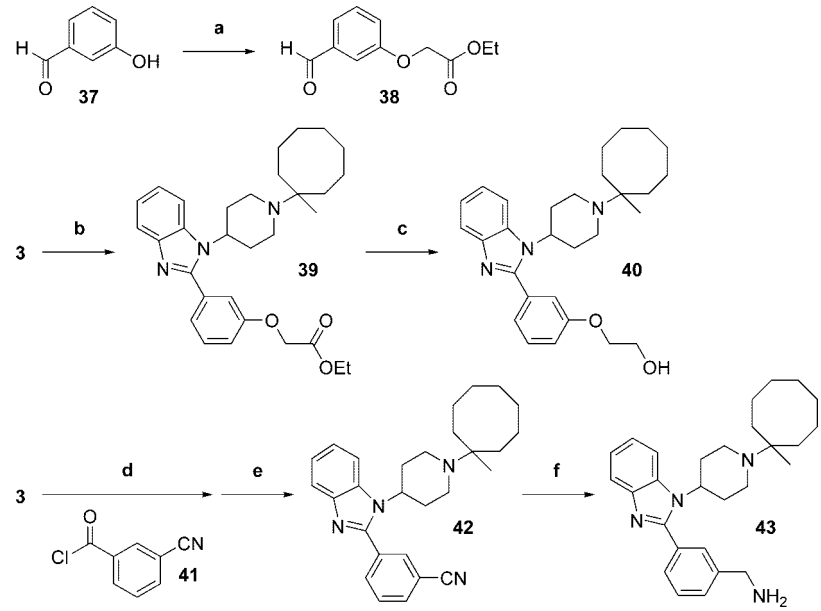
For clarifying SAR, the 2-piperidinylbenzimidazole analogues are particularly noteworthy. Comparison among the respective optical isomers of the piperidinyl analogues, (*R*)-*NH*-piperidine derivative **1** was more selective to NOP receptor over other opioid receptors (μ, κ, and δ) than the corresponding (*S*)-*NH*-

Scheme 6. Synthesis of 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-aryl-1*H*-benzimidazole Derivatives **23**−**26** and **36**[a]



[a] Reagents and conditions: (a) WSCI, THF, −20 °C to room temp; (b) $POCl_3$, reflux or 100 °C; (c) pyridine; (d) $POCl_3$, reflux.

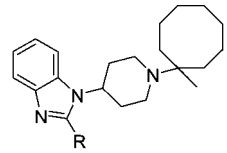
Scheme 7. Synthesis of Compounds **40** and **43**[a]



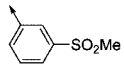
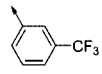
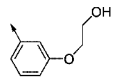
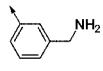
[a] Reagents and conditions: (a) ethyl 1-bromoacetate, K_2CO_3, CH_3CN, 70 °C; (b) (i) **38**, EtOH, reflux; (ii) $Pb(OAc)_4$, benzene, reflux; (c) LAH, THF, 0 °C to room temp; (d) pyridine; (e) $POCl_3$, reflux; (f) LAH, THF, 0 °C to room temp.

enantiomer **21**, and the (*R*)-*N*-Me form **16** was more NOP receptor selective than the (*S*)-*N*-Me form **22** over κ- and δ-receptors, as mentioned above generally. And the most superior NOP receptor agonist of this study, the (*R*)-*NH*-piperidine analogue **1**, was determined to have a K_i value of 0.0858 nM for hNOP receptor, whereas that of (*R*)-*N*-Me analogue **16** was 0.20 nM. Compared with the highly potent hNOP receptor binding, the binding affinities to other human opioid receptors of **1** were less potent; i.e., the K_i values were 1.052 nM (μ), 23.1 nM (κ), and greater than 667 nM (δ),

Table 1. Structure−Activity Relationships of in Vitro Binding Affinities and Functional Activities to Human Recombinant NOP Receptor and Other Opioid Receptors for 1,2-Disubstituted Benzimidazole Analogues[a]



1,2-Disubstituted Benzimidazole Analogues

Compounds		Binding affinity K_i (nM)[a]				Functional activity [^{35}S]GTPγS EC_{50} (nM)[b] E_{max} (%)			
No.	R	hNOP	μ	κ	δ	hNOP	μ	κ	δ
1		0.0858	1.052	23.1	>667	0.39 140%	34 30%	80 43%	4696 79%
16		0.20	3.7	25	>500	1.4 150%	67 32%	70 53%	2190 81%
21		0.10	0.59	9.2	168	0.5 147%	23 53%	8.4 45%	514 100%
22		0.24	11	10	110	1.6 145%	71 16%	13 65%	389 109%
23		0.15	6.3	NT[c]	NT	1.6 144%	288 31%	NT	NT
24		0.22	45	NT	NT	1.6 131%	759 21%	NT	NT
25		1.70	151	NT	NT	13 112%	8625 30%	NT	NT
26		0.53	102	NT	NT	3.1 133%	3673 50%	NT	NT
36		0.63	65	352	58	5.7 131%	>10000 15%	1578 28%	NT
40		0.30	11	43	9.5	2.6 113%	393 25%	48 32%	NT
43		0.18	20	72	140	1.3 135%	340 18%	154 27%	NT
	N/OFQ	0.39	>1000[d]	>1000[d]	>1000[d]	1.5 100%	NT	NT	NT

[a] K_i values for these compounds were measured by the displacement of [^{3}H]N/OFQ binding to hNOP receptors expressed in HEK-293 cells, [^{3}H]DAMGO binding to human μ-receptors expressed in CHO-K1 cells, [^{3}H]enadoline binding to human κ-receptors expressed in HEK-293 cells, and [^{3}H]DPDPE binding to human δ-receptors expressed in CHO-K1 cells. $K_i = IC_{50}/(1+[\text{radioligand}]/K_D)$. Radioligands: [^{3}H]N/OFQ as hNOP receptor agonist, concentration 0.4 nM, $K_D = 0.135$ nM; [^{3}H]DAMGO as μ-receptor agonist, concentration 1.0 nM, $K_D = 0.821$ nM; [^{3}H]enadoline as κ-receptor agonist, concentration 0.5 nM, $K_D = 0.683$ nM; [^{3}H]DPDPE as δ-receptor agonist, concentration 2.0 nM, $K_D = 2.00$ nM. [b] EC_{50} values for these compounds were determined by the induction of the binding of [^{35}S]GTPγS to α-unit of G-protein due to binding of the compounds to hNOP receptors expressed in HEK-293 cells, human μ-receptors expressed in CHO-K1 cells, human κ-receptors expressed in HEK-293 cells, and human δ-receptors expressed in CHO-K1 cells. E_{max} (efficacy) value was the maximal response calculated as the percentage of the maximal response produced by each control (N/OFQ, DAMGO, enadoline, and DPDPE). [c] NT: not tested. [d] Data from ref 56.

Table 2. K_i Values and Selectivity Profiles for Binding Affinities of (3*R*)-*NH*-Piperidine Analogue **1** and (3*R*)-*N*-Me-Piperidine Analogue **16** to Human NOP, μ-, κ-, and δ-Receptors[a]

	binding affinity K_i (nM)				selectivity ratio		
compd	hNOP	μ	κ	δ	μ/hNOP	κ/hNOP	δ/hNOP
1	0.0858	1.052	23.1	>667	12.26	269	>7770
16	0.20	3.7	25	>500	18.5	125	>2500

[a] The selectivities of NOP receptor agonists against μ-, κ-, or δ-receptor were calculated as the ratios of the K_i values for human μ-, κ-, or δ-receptor to the K_i values for hNOP receptor.

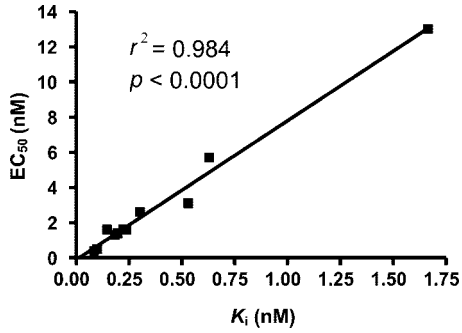


Figure 1. Correlation between K_i and EC_{50} for NOP receptor agonists: K_i values for binding affinity to hNOP receptor and EC_{50} values for [^{35}S]GTPγS binding in response to hNOP receptor binding.

respectively. Consequently, for the K_i values, the binding selectivities of **1** as NOP receptor agonist against other opioid receptors were 12.26-fold over μ-receptor, 269-fold over κ-receptor, and greater than 7770-fold over δ-receptor (Table 2). Furthermore, the functional activity of **1** for hNOP receptor was greater than other human opioid receptors, i.e., $EC_{50} = 0.39$ nM, $E_{max} = 140\%$ (NOP); $EC_{50} = 34$ nM, $E_{max} = 30\%$ (μ); $EC_{50} = 80$ nM, $E_{max} = 43\%$ (κ); $EC_{50} = 4596$ nM, $E_{max} = 79\%$ (δ), and the functional selectivities of the hNOP agonism against that of other human opioid receptors for EC_{50} values were 87-fold over μ-receptor, 205-fold over κ-receptor, and 12041-fold over δ-receptor.

Additionally, the binding affinity of NOP agonist **1** as well as the other NOP agonists described above is greater than that of 2-(3,5-dimethylpiperazin-1-yl)-1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazole (PCPB), a probe for NOP agonist study, which was very recently reported (Hirao et al., 2008),[57] whose K_i value for NOP binding affinity was 2.1 nM. In addition, the K_i values of PCPB for other opioids were 21 nM (μ), 15 nM (κ), and 29% inhibition at 1 μM (δ), and the functional activities of PCPB for opioid receptors were $EC_{50} = 6.0$ nM and $E_{max} = 188\%$ (NOP), $EC_{50} = 193$ nM and $E_{max} = 11\%$ (μ), $EC_{50} = 18$ nM and $E_{max} = 26\%$ (κ). Hence, PCPB was also a less selective NOP agonist than the selective NOP agonist **1**. As well, a 2-unsubstituted benzimidazole analogue, 1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazole, showed less selective NOP receptor binding over μ-receptor binding; i.e., the K_i values were 2.5 nM (NOP) and 11 nM (μ) (further data not shown).

Overall, the present series of 1,2-disubstituted benzimidazole analogues showed highly potent and selective hNOP binding affinities and functional activities as synthetic non-peptide NOP agonists. Significantly, NOP agonist **1** is the most potent full agonist among known 1,2-disubstituted benzimidazole derivative agonists for now.

Subsequently, SAR of the NOP agonist to identify appropriate structure was efficiently investigated for orally active anxiolytic.

Originally, the NOP receptor agonists in this study were rationally designed from the viewpoint of brain-active anxiolytic after oral administration, e.g., the well brain penetration through the blood−brain barrier and the druglikeness concept for oral activity. For example, to confirm essential factors for the purpose, appropriate physicochemical properties such as molecular size and bulkiness (molecular weight), shape, acceptable lipophilicity, basicity, and numbers of heteroatoms, hydrogen bond donors, hydrogen bond acceptors, and rotatable bonds were evaluated. And as a primary assessment for this study, NOP receptor occupancies of the NOP receptor agonists in the mice brain per os (po) were evaluated by their displacement of radioligand [^{3}H]N/OFQ from NOP receptors in the brain.

The flow of the procedure for the ex vivo NOP receptor binding is as follows. First, male ddY mice were deprived of water for 24 h and then given the NOP receptor agonist orally at 10 mg/kg. After 1 h, the brains were quickly removed in their entirety. The brain tissues except cerebellum and medulla were homogenized, and the resulting membranes were then used to test for the specific bindings of [^{3}H]N/OFQ to determine the occupation ratios of the NOP receptor agonists to NOP receptor in the brain.

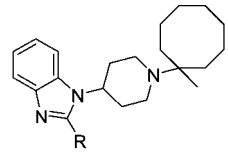
Through the design and synthetic study with various chemotypes, NOP receptor agonists that displayed high or moderate level NOP occupancies in the mice brain (10 mg/kg, po, 1 h) were identified. The results of the structure−NOP receptor occupancy relationships of the NOP receptor agonists for orally potent brain activity are illustrated in Table 3 with respective in vitro hNOP binding affinities and characteristic physicochemical properties.[58−60]

In general, various types of NOP receptor agonists were categorized by the level of oral NOP receptor occupancy in the mice brain (10 mg/kg, 1 h), i.e., to be greater than 50% as the high level, to be less than 50% and greater than 31% as the moderate level, and to be 31% or less as the low level. Therefore, from the viewpoint of structural feature, these analogues that have 1-[(cyclooctylmetyl)piperidinyl]benzimidazole moiety as a common portion were classified with the respective characteristic portion at 2-position of benzimidazole moiety for each molecule, e.g., 3′-*NH*-piperidinyl (**1**) and 3′-*N*-Me-piperidinyl (**16**) analogues in the high level category, 3′-$MeSO_2$-phenyl (**23**), 2′-benzofuranyl (**26**), and 3′-($HOCH_2CH_2O$)-phenyl (**40**) analogues in the moderate level category, and 3′-Cl-4′-F-phenyl (**24**), 6′-indolyl (**25**), 3′-CF_3-phenyl (**36**), and 3′-(H_2NCH_2)-phenyl (**43**) analogues in the low level category, while all these structural variations of the substituents at 3′-position of piperidinyl ring as well as at 3′-position of aryl ring were well allowable for the potent hNOP receptor binding affinity in vitro as described above.

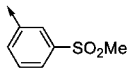
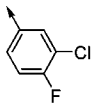
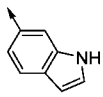
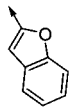
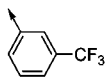
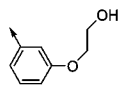
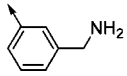
The relationships of the actual oral NOP receptor occupancy in the mice brain with in vitro hNOP binding affinities as well as physicochemical properties were significant as shown below.

First of all, it is noteworthy that the NOP receptor agonists that show high (**1** and **16**) or moderate (**23**, **26**, and **40**) NOP occupancy levels (10 mg/kg, po) have potent NOP binding affinities as a requirement, of which K_i values are greater than 0.53 nM in vitro. In contrast, there were no high or moderate NOP occupancy analogues of which K_i values are less than 0.6

Table 3. SAR of NOP Occupancies for 1,2-Disubstituted Benzimidazole Analogues: ex Vivo NOP Occupancies in the Mice Brain (po) versus in Vitro hNOP Binding Affinities and Physicochemical Properties[a]



1,2-Disubstituted Benzimidazole Analogues

Compounds		Ex vivo	In vivo	Physicochemical properties					
		NOP occupancy in mice brain[a] (10 mg/kg, po)	hNOP binding affinity	Size	TPSA	Lipophilicity	H bond donor	H bond acceptor	Rotational bond
No	R	%	K_i (nM)	MW	Å^2	ACDlogD$_{7.4}$[b]	Number	Number	Number
1		53 ± 5.9	0.0858	408.62	33.09	2.57	1	3	3
16		61 ± 0.9	0.20	422.65	24.30	3.57	0	3	3
23		34 ± 19.1	0.15	479.68	63.58	5.03	0	4	4
24		31 ± 7.7	0.22	454.02	21.06	7.49	0	2	3
25		−10 ± 9.7	1.70	440.62	36.85	6.18	1	2	3
26		34 ± 6.2	0.53	441.61	34.20	6.40	0	2	3
36		3.2 ± 8.5	0.63	469.59	21.06	7.38	0	2	3
40		38 ± 5.7	0.30	461.64	50.52	5.49	1	4	6
43		30 ± 4.8	0.18	430.63	47.08	7.38	2	3	4

[a] Data are expressed as inhibition of ex vivo specific binding of [^{3}H]N/OFQ in the mice brain for drug-treated mice versus vehicle-treated mice (mean ± SEM of three mice). The ex vivo binding was measured 1 h after oral administration of the NOP receptor agonists in male ddY mice (10 mg/kg, po, 0.1% methylcellulose). [b] Predicted by ACD/Laboratories 9.0.

nM in vitro. Therefore, it is essential for high or moderate NOP occupancy in the mice brain (10 mg/kg) of orally administered NOP receptor agonist to have highly potent intrinsic binding affinity to hNOP receptor in vitro; i.e., the K_i value is greater than 0.6 nM, at least.

On the other hand, the physicochemical properties for the NOP occupancy have been meaningful for drug design and succeeding optimization.

For example, the range of molecular weight is less than 480 and the range of TPSA is less than 64 Å, as a whole. Nowadays, these values are acceptable in many cases as the features for marketable oral drugs[58−60] and were useful for successful results as expected.

By comparision of NOP occupancy with lipophilicity, it is suggested that the appropriately lower lipohilic analogues were more suitable than higher lipophilic analogues for higher NOP occupancy per os. Specifically, for the high NOP occupancy analogues (53−61%) such as *NH* and *N*-Me piperidine derivatives (**1** and **16**), the lipophilicity values at pH 7.4 as ACDlogD calculated by ACD software ($ACDlogD_{7.4}$) were appropriately low, i.e., 2.57 and 3.57, respectively. And the moderate NOP occupancy aryl analogues (34−38%) such as 3′-$MeSO_2$-phenyl (**23**), 2′-benzofuranyl (**26**), and 3′-($HOCH_2CH_2O$)-phenyl (**40**) analogues had moderate $ACDlogD_{7.4}$ values, i.e., 5.03, 6.40, and 5.49, respectively, while the low NOP occupancy analogues (31% to no NOP occupancy) such as 3′-Cl-4′-F-phenyl (**24**), 3′-CF_3-phenyl (**36**), and 3′-(H_2NCH_2)-phenyl (**43**) analogues had much higher $ACDlogD_{7.4}$ values, i.e., 7.38−7.49. In the case of the 6′-indolyl analogue (**25**), no NOP occupancy in the mice brain per os was observed owing to its reduced NOP binding affinity in vitro, although the lipophilicity was a moderate $ACDlogD_{7.4}$ value, 6.18.

In addition, the range of the number of hydrogen bond donors of these NOP receptor agonists is 0−1 except for a primary amine analogue (**43**), whose number is 2, that showed low level NOP receptor occupancy.

In other physicochemical properties for the high or moderate NOP occupancy analogues, diverse characteristics such as numbers of hydrogen bond acceptors (2−4) and rotatable bonds (3−6), and basicity of functional group for 2-substituent of benzimidazole (neutral group, e.g., **23**, **26**, and **40**; basic group, e.g., **1** and **16**) were acceptable. As well, characteristic of ring skeleton for the 2-substituent of benzimidazole was variable; namely, both nonaromatic ring and aromatic ring were tolerable.

Conclusion

In the present strategy, brain-penetrative and oral-active potent NOP receptor agonists with requisite structures were efficiently developed and identified utilizing drug design and synthetic study with NOP occupancy evaluation in the mice brain after oral administration. Indeed, a novel non-peptide 1,2-disubstituted benzimidazole analogue **1**, namely, 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole (MCOPPB), demonstrated the most potent and selective NOP full agonism with high NOP occupancy in the mice brain per os.

As a succeeding step of this series of study, SAR and optimization for orally potent anxiolytic efficacy, long lasing metabolic stability, and little hHERG channel binding affinity with the above NOP agonists were investigated. As a result, the potent NOP agonist **1** was identified as a potential anxiolytic that demonstrated the most potent oral efficacy for the Vogel anticonflict test among the NOP agonists in our study, e.g., a significant Vogel anticonflict activity at 10 mg/kg (po, mice) as minimum effective dose with advantageous characteristics. These studies will be published elsewhere as a subsequent article.[1a,2] As well, on the basis of these facts, further pharmacological characterization of **1** as a new-class anxiolytic was performed for differentiation in terms of various potential side effects from diazepam that is a representative of classical benzodiazepine-type anxiolytic, which was already illustrated (Hayashi et al., 2006; Hirao et al., 2008).[1a,61]

Experimental Section

Chemistry. General Procedures. In general, reagents, solvents, reagents, and other chemicals were used as purchased without further purification unless noted otherwise. All reactions with air- or moisture-sensitive reactants and solvents were carried out under nitrogen atmosphere. Flash column chromatography (medium pressure liquid chromatography) purifications were carried out using Merck silica gel 60 (230−400 mesh ASTM). Preparative thin-layer chromatography (PTLC) purifications were carried out on Merck silica gel 60 F_{254} precoated glass plates at a thickness of 0.5 or 1.0 mm. The structures of all isolated compounds were ensured by NMR, IR, MS, or elementary analysis. Nuclear magnetic resonance (^1H and ^{13}C NMR) data were determined at 270 MHz on a JNM-LA 270 (JEOL) spectrometer, at 300 MHz on a JNM-LA300 (JEOL) spectrometer, or at 150 or 600 MHz on an AVANCE (Bruker) spectrometer. Chemical shifts are expressed in δ (ppm). ^1H NMR chemical shifts were determined relative to tetramethylsilane (TMS) as internal standard. ^{13}C NMR chemical shifts were determined relative to internal TMS at δ 0.0 or to the ^{13}C signal of solvent: $CDCl_3$ δ 77.04 or CD_3OD δ 49.8. NMR data are reported as follows: chemical shift, number of atoms, multiplicities (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broadened), and coupling constants. Distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) were measured for the determination of $^1H-^{13}C$ correlation, and the multiplicities by the proton interaction were expressed in ^{13}C NMR data. Infrared spectra were measured by an IR-470 (Shimadzu) infrared spectrometer. Low-resolution mass spectral data (EI) were obtained on an Automass 120 (JEOL) mass spectrometer. Low-resolution mass spectral data (ESI) were obtained on a Quattro II (Micromass) mass spectrometer Agilent 1100 HPLC system. Optical rotations were measured on a P-1020 (JASCO) polarimeter. The compounds **1**, **16**, **21**−**26**, **36**, **40**, and **43** were used for pharmacological evaluations after hydrochloride formation using HCl in MeOH, respectively.

Ethyl (*R*)-(1-*tert*-Butoxycarbonyl)-3-piperidinecarboxylate (6). Ethyl (*R*)-nipecotate L-tartrate {$[\alpha]_D$ +10 (*c* 5, H_2O), 5.04 g, 16.4 mmol} was dissolved in saturated aqueous $NaHCO_3$ (250 mL), then extracted with CH_2Cl_2 (300 mL × 10). The combined extracts were concentrated in vacuo. The residue was diluted with CH_2Cl_2 (200 mL), washed with saturated aqueous $NaHCO_3$ (80 mL), dried over anhydrous Na_2SO_4, filtered, then concentrated in vacuo. Subsequently, the resulting salt-free ethyl (*R*)-nipecotate **5** (2.62 g) was dissolved in CH_2Cl_2 (24 mL) under N_2, di-*tert*-butyl dicarbonate (3.58 g, 16.4 mmol) was added at 0 °C, and the mixture was stirred at the temperature for 15 min. Then the reaction mixture was warmed to room temperature, stirred for 18 h, and concentrated in vacuo to give 4.84 g of the title compound **6** as a colorless oil that including a small amount of CH_2Cl_2 on ^1H NMR analysis. This compound **6** was used for the next reaction without further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 4.14 (2H, q, J = 7.1 Hz), 4.34−2.76 (4H, m), 2.48−2.37 (1H, m), 2.09−2.00 (1H, m), 1.74−1.38 (3H, m), 1.46 (9H, s), 1.26 (3H, t, J = 7.1 Hz).

(*R*)-(1-*tert*-Butoxycarbonyl)-3-piperidinecarboxylic Acid (2). To a stirred solution of the above ethyl (*R*)-(1-*tert*-butoxycarbonyl)-3-piperidinecarboxylate **6** (4.84 g) in MeOH (24 mL)−THF (10 mL) was added dropwise 4 N LiOH (8.4 mL, 33.6 mmol)−H_2O (15.0 mL) solution at 0 °C under N_2. After being stirred below 6 °C for 17 h, the reaction mixture was concentrated on a rotary evaporator. To the residue was added H_2O (10 mL), and the mixture was cooled to 0 °C and acidified by adding 2 N HCl to adjust the

pH to 1. The mixture was extracted with CH_2Cl_2 (50 mL × 8). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give 3.57 g of the title compound **2** in 95% yield (two steps) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.23−4.03 (1H, m), 3.94−3.84 (1H, m), 3.14−2.97 (1H, m), 2.93−2.81 (1H, m), 2.57−2.43 (1H, m), 2.12−2.02 (1H, m), 1.78−1.40 (3H, m), 1.46 (9H, s).

1-Benzyl-4-(2,5-dimethyl-1*H*-pyrrol-1-yl)piperidine (8). In a 1 L flask with an azeotropic apparatus under N_2, a solution of 4-amino-1-benzylpiperidine (57.1 g, 300 mmol), 2,5-hexanedione (34.6 g, 303 mmol), and glacial acetic acid (4.5 mL) in dry toluene (400 mL) was heated under reflux conditions.[44] After no more aqueous layer was observed in a water trap (∼3 h), the reaction solution was cooled to room temperature. To the solution, hexane (300 mL), saturated aqueous $NaHCO_3$ (300 mL), and H_2O (100 mL) were added. The resulting slightly basic aqueous layer was separated, and the red-brown organic layer was washed with H_2O (100 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, then filtered. The filtrate was concentrated in vacuo to afford 82 g of the title compound **8** (crude), which became gradually solid including a small amount of toluene based on ^{1}H NMR analysis. This compound **8** was used for the next step without further purification. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.36−7.14 (5H, m), 5.74 (2H, s), 3.96−3.82 (1H, m), 3.55 (2H, s), 3.02 (2H, br d, J = 11.5 Hz), 2.30 (6H, s), 2.32−2.20 (2H, m), 2.08 (2H, td, J = 11.9 Hz, J = 1.9 Hz), 1.79 (2H, br d, J = 12.0 Hz).

4-(2,5-Dimethyl-1*H*-pyrrol-1-yl)piperidine (9). In a 500 mL hydrogenation apparatus, to a solution of 1-benzyl-4-(2,5-dimethyl-1*H*-pyrrol-1-yl) piperidine **8** (30 g, crude) in MeOH (150 mL), $Pd(OH)_2$ (6 g, 20 wt%) was added. The resulting dark-brown mixture was stirred at room temperature under H_2 (4.5 atm) for 16 h. The resulting mixture was filtered through a Celite pad with MeOH washings (20 mL × 5). The filtrate was concentrated to give 18.5 g of the title compound **9** in 95% yield (two steps, crude) as an oil. This compound **9** was used for the next step without further purification. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.74 (2H, s), 4.06−3.90 (1H, m), 3.23 (2H, d, J = 11.9 Hz), 2.71 (2H, t, J = 12.2 Hz), 2.31 (6H, s), 2.22−2.04 (2H, m), 1.83 (2H, br d, J = 10 Hz), 1.61 (1H, br s).

1-[4-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1-piperidinyl]cyclooctanecarbonitrile (10). In a 300 mL flask with a reflux condenser under N_2, to a mixture of the above crude 4-(2,5-dimethyl-1*H*-pyrrol-1-yl)piperidine **9** (15.6 g, 87.5 mmol) in H_2O (30 mL), a solution of $TsOH \cdot H_2O$ (20 g, 105 mmol) in H_2O (20 mL) was added at room temperature. The reaction mixture was stirred and then turned red. This reaction was exothermic, and the internal temperature reached 33 °C. The resulting solution was maintained at that temperature with stirring for 10 min, then cyclooctanone (13.2 g, 105 mmol) was added. The resulting mixture was heated at 45 °C, a solution of KCN (11.4 g, 175 mmol) in H_2O (30 mL) was added, and the resulting mixture was stirred at the same temperature for 1 day. The resulting solid was collected by filtration and washed with H_2O (50 mL × 2). A mixture of the solid (∼23 g) and hexane (75 mL) was stirred in an ice-cold bath for 15 min to remove excess cyclooctanone from the solid. The resulting mixture was filtered, and the obtained cake was washed with hexane (15 mL × 2) and then dried in a vacuum oven (45 °C, 13 h) to give 19.8 g of the title compound **10** in 72% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.75 (2H, s), 4.04−3.88 (1H, m), 3.19 (2H, br d, J = 7.1 Hz), 2.31 (6H, s), 2,32−1.99 (10H, m), 1.75−1.43 (10H, m). MS (EI direct) m/z: M^+ 313. Anal. Calcd for $C_{20}H_{31}N_3$: C, 76.63; H, 9.97; N, 13.40. Found: C, 76.39; H, 9.89; N, 13.53.

4-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1-(1-methylcyclooctyl)piperidine (11). Typical Procedure. In a 30 mL flask with a septum under N_2, a solution of 1-[4-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1-piperidinyl]cyclooctanecarbonitrile **10** (313 mg, 0.998 mmol) in dry CH_2Cl_2 (3.1 mL) was cooled in a dry ice solmix bath. To this solution, 0.90 M MeMgBr in anhydrous THF (1.7 mL, 1.5 mmol) was added dropwise via syringe at −78 °C. The resulting mixture was stirred at that temperature for 30 min, then allowed to warm to room temperature gradually, and stirred at room temperature for 1.5 h. After the resulting solution was cooled to 0 °C, saturated aqueous NH_4Cl (5 mL) was added, and then the resulting mixture was stirred at the same temperature for 30 min. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined extracts were dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 15/1) to give 149 mg of the title compound **11** in 49% yield as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.74 (2H, s), 3.92−3.78 (1H, s), 3.08 (2H, br d, J = 6.8 Hz), 2.31 (6H, s), 2.23−2.05 (4H, m), 1.87−1.28 (16H, m), 0.84 (3H, s). MS (EI direct) m/z: M^+ 302.

1-(1-Methylcyclooctyl)piperidin-4-amine (12). Typical Procedure. In a 200 mL flask with a reflux condenser under N_2, a solution of 4-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1-(1-methylcyclooctyl)piperidine **11** (1.22 g, 4.03 mmol) and $NH_2OH \cdot HCl$ (2.2 g, 32 mmol) in EtOH (12 mL)−H_2O (6 mL) was heated under reflux conditions for 19 h, then cooled to room temperature. The resulting orange solution was poured into ice-cold 2 N NaOH (∼200 mL), and then CH_2Cl_2 (50 mL) was added. The organic layer was separated, and the basic aqueous layer was extracted with CH_2Cl_2 (50 mL × 2). The combined extracts were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo to give 0.86 g of the title compound **12** in 95% yield (crude) as an oil including a small amount of starting material **11** based on ^{1}H NMR. Compound **12**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.86 (2H, br d, J = 11.3 Hz), 2.65−2.50 (1H, m), 2.08 (2H, td, J = 11.3 Hz, J = 1.8 Hz), 1.86−1.17 (20H, m), 0.80 (3H, s).

1-(1-Methylcyclooctyl)-*N*-(2-nitrophenyl)piperidin-4-amine (13). To a solution of 1-(1-methylcyclooctyl)piperidin-4-amine **12** (3.46 g, 15.4 mmol) and 1-fluoro-2-nitrobenzene (2.39 g, 16.9 mmol) in dry CH_3CN (77 mL) was added anhydrous K_2CO_3 (2.77 g, 20.0 mmol) at room temperature under N_2. The resulting yellow suspension was stirred under reflux conditions using an oil bath (∼100 °C) overnight. After the mixture was cooled to room temperature, the solvent was evaporated. The residue was partitioned between CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (15 mL × 3). The combined extracts were washed with brine, dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo to give 5.75 g of orange oil, which was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH = 20/1) to give 4.95 g of the title compound **13** in 93% yield as an orange solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.17 (1H, dd, J = 8.6 Hz, J = 1.5 Hz), 8.11 (1H, br s), 7.40 (1H, ddd, J = 8.6 Hz, J = 7.1 Hz, J = 1.7 Hz), 6.86 (1H, br d, J = 8.6 Hz), 6.59 (1H, ddd, J = 8.6 Hz, J = 7.2 Hz, J = 1.2 Hz), 3.60−3.40 (1H, m), 2.97−2.85 (2H, m), 2.33−2.24 (2H, m), 2.10−2.00 (2H, m), 1.90−1.20 (16H, m), 0.84 (3H, s).

***N*-[1-(1-Methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine (3).** To a solution of the above 1-(1-methylcyclooctyl)-*N*-(2-nitrophenyl)piperidin-4-amine **13** (4.95 g, 14.3 mmol) in THF (128 mL) was added MeOH (41 mL), H_2O (12.8 mL), zinc powder (9.37 g, 143 mmol), and NH_4Cl (3.83 g, 71.6 mmol) at room temperature under N_2. The reaction mixture was stirred at that temperature, and the yellow color disappeared during 15 min. Then the reaction mixture was filtered through a Celite pad with THF washing, and the filtrate was concentrated in vacuo to give a mixture of a brown solid and aqueous solution. To the mixture was added saturated aqueous $NaHCO_3$ (50 mL), and then the mixture was extracted with CH_2Cl_2. The combined extracts were dried over anhydrous K_2CO_3, filtered, and concentrated to give 4.54 g of the title compound **3** in 100% yield as a dark-brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82−6.62 (4H, m), 3.40−3.10 (3H, m), 2.95−2.85 (2H, m), 2.30−2.15 (2H, m), 2.10−2.00 (2H, m), 1.90−1.20 (17H, m), 0.83 (3H, s).

***tert*-Butyl (3*R*)-3-{[(2-{[1-(1-Methylcyclooctyl)-4-piperidinyl]amino}phenyl)amino]carbonyl}-1-piperidinecarboxylate (14).** To a stirred solution of (*R*)-(1-*tert*-butoxycarbonyl)-3-piperidinecarboxylic acid **2** (756.6 mg, 3.30 mmol), *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (800 mg, 2.54

mmol), and 1-hydroxybenzotriazole (HOBT) (567.5 mg, 4.20 mmol) in dry CH_2Cl_2 (17 mL) was added Et_3N (1.1 mL, 7.9 mmol) followed by water soluble carbodiimide, i.e., 1-ethyl-3-(3-dimethylaminopropylcarbodiimide) hydrochloride (WSCI) (805.1 mg, 4.20 mmol) at −30 °C under N_2, and the reaction mixture was allowed to warm to room temperature. After being stirred at room temperature for 18 h, the reaction mixture was partitioned between CH_2Cl_2 (80 mL) and H_2O (80 mL). The organic layer was separated, washed with H_2O (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give 1.443 g of the title compound **14** as a brown solid (crude) including small amount of CH_2Cl_2 based on ^{1}H NMR, which was used for the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (1H, br s), 7.36−7.34 (1H, m), 7.12−7.05 (1H, m), 6.79−6.70 (2H, m), 4.07−1.20 (33H, m), 1.47 (9H, s), 0.83 (3H, s).

***tert*-Butyl (3*R*)-3-{1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate (15).** A solution of *tert*-butyl (3*R*)-3-{[(2-{[1-(1-methylcyclooctyl)-4-piperidinyl]amino}phenyl)amino]carbonyl}-1-piperidinecarboxylate **14** (1.443 g, crude) and glacial acetic acid (0.70 mL, 12.2 mmol) in dry toluene (70 mL) was stirred at 100 °C under N_2 for 16 h, cooled to room temperature, and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (50 mL), the mixture was cooled to 0 °C, and saturated aqueous $NaHCO_3$ (50 mL) was added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH = 32/1) to give 998.7 mg of the title compound **15** in 77% yield (two steps) as a pale-brown solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.76−7.71 (1H, m), 7.65−7.60 (1H, m), 7.25−7.19 (2H, m), 4.40−4.10 (3H, m), 3.25−2.70 (5H, m), 2.60−2.45 (2H, m), 2.35−2.20 (2H, m), 2.15−1.30 (20H, m), 1.47 (9H, s), 0.89 (3H, s).

1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole (1), MCOPPB. A mixture of *tert*-butyl (3*R*)-3-{1-[1-(1-methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate **15** (998.7 mg, 1.96 mmol) and 10% HCl/MeOH solution (100 mL) was stirred at room temperature under N_2 for 16 h, then concentrated in vacuo. To the residue was added CH_2Cl_2 (60 mL). The mixture was cooled to 0 °C and basified by adding saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (70 mL × 3). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH/25% aqueous ammonia = 120/10/1) to give 735.1 mg of the title compound **1** in 92% yield as a brown oil. HPLC analysis: (1) Chemical purity of 98% with no impurity over 0.5% (apparatus, Alliance 2690 with PDA detector, Waters; analytical column, Kromasil 5C4, 250 mm × 4.6 mm, 5 μm (particle size); eluent, CH_3CN/0.5% $HClO_4$ = 32/68 to 90/10 gradient; flow rate, 1.0 mL/min; column temperature, 40 °C; UV detection, 210 nm; retention time, 12 min. (2) Chiral analysis, 97% enantiomeric excess (ee), determined by HPLC employing a chiral stationary phase (apparatus, Alliance 2690 with PDA detector, Waters; analytical column, CHIRALCEL OG, 250 mm × 4.6 mm, Daicel Chemical Industries; eluent, hexane/EtOH/2-propanol/diethylamine = 95/2.5/2.5/0.1; flow rate, 1 mL/min; room temperature (27−28 °C); dissolving solvent, hexane/EtOH = 1:1; UV detection, 250 nm; retention time; peak of (*R*)-form, 12 min; peak of (*S*)-form, 10 min; (*R*)/(*S*) = 98.5:1.5. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75−7.71 (1H, m), 7.64−7.62 (1H, m), 7.23−7.18 (2H, m), 4.24−4.18 (1H, m), 3.26−3.05 (6H, m), 2.80 (1H, dt, J = 11.7 Hz, J = 2.64 Hz), 2.58−2.47 (2H, m), 2.29−2.21 (2H, m), 2.11−2.05 (1H, m), 2.05−1.92 (4H, m), 1.89−1.79 (3H, m), 1.77−1.51 (11H, m), 1.44−1.35 (2H, m), 0.89 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.9 (1C, s), 143.2 (1C, s), 133.5 (1C, s), 121.7 (1C, d), 121.5 (1C, d), 119.6 (1C, d), 112.2 (1C, d), 58.2 (1C, s), 55.0 (1C, d), 51.4 (1C, t), 46.5 (1C, t), 45.4 (2C, t), 36.0 (1C, d), 35.0 (2C, t), 31.6 (2C, t), 30.4 (1C, t), 28.4 (2C, t), 25.9 (1C, t), 25.0 (1C, t), 21.6 (2C, t), 20.6 (1C, q).

HCl Salt Formation. General Procedure. Compound **1** (730.7 mg) was dissolved in CH_2Cl_2 (30 mL) and excess equivalent of 10% HCl/MeOH solution (120 mL). The mixture was stirred at room temperature for 1 h and then concentrated in vacuo. The resulting white solid was collected and dried under vacuum at 50 °C to give 769.3 mg of the corresponding trihydrochloride as a white solid: $[\alpha]_D^{23}$ −4.0 (*c* 0.39, MeOH). IR (KBr): 3412, 2930, 2712, 1626, 1479, 1468, 1308, 1107, 1042, 1009, 856, 770 cm^{-1}. MS (ESI positive) *m*/*z*: $[M + H]^+$ 409. Anal. ($C_{26}H_{40}N_4 \cdot 3HCl \cdot 0.8H_2O$) C, H, N.

1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-1-methyl-3-piperidinyl]-1*H*-benzimidazole (16). To a solution of *tert*-butyl (3*R*)-3-{1-[1-(1-methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate **15** (849.6 mg, 1.67 mmol) in anhydrous THF (25.0 mL) was added lithium aluminium hydride (LAH) (228.2 mg, 6.01 mmol) at room temperature under N_2. The reaction mixture was stirred at that temperature under N_2 for 1 h, then warmed up to reflux conditions and stirred for 2 h. The reaction mixture was cooled to room temperature, more LAH (90.0 mg, 2.37 mmol) was added, and the mixture was refluxed with stirring for 3 h. After cooling to 0 °C, AcOEt (10 mL) was added dropwise to the reaction mixture, stirred at 0 °C for 10 min, then stirred at room temperature for 20 min. The mixture was cooled to 0 °C, then H_2O (30.0 mL) was added dropwise. The mixture was stirred at 0 °C for 30 min, and stirred at room temperature for 30 min. The mixture was poured into saturated aqueous $NaHCO_3$ (5 mL), then extracted with CH_2Cl_2 (60 mL × 3). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH/25% aqueous ammonia = 100/10/2) to give the title product as a solid, which was repurified by flash column chromatography (silica gel, CH_2Cl_2/MeOH/25% aqueous ammonia = 1900/50/2.5) to give 517.5 mg of the title compound **16** in 73% yield as a slight brownish white solid. $[\alpha]_D^{23}$ −15 (*c* 0.13, MeOH). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75−7.73 (1H, m), 7.65−7.62 (1H, m), 7.23−7.18 (2H, m), 4.26−4.20 (1H, m), 3.21−3.14 (3H, m), 3.06−3.04 (2H, m), 2.94 (1H, d, J = 10.1 Hz), 2.56−2.44 (3H, m), 2.39 (3H, s), 2.26−2.21 (2H, m), 2.09−1.36 (23H, m). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.8 (1C, s), 143.2 (1C, s), 133.5 (1C, s), 121.7 (1C, d), 121.4 (1C, d), 119.6 (1C, d), 112.2 (1C, d), 60.2 (1C, t), 58.1 (1C, s), 55.7 (1C, t), 55.0 (1C, d), 46.5 (1C, q), 45.3 (2C, t), 35.7 (1C, d), 34.9 (2C, t), 31.6 (2C, t), 29.7 (1C, t), 28.4 (2C, t), 25.6 (1C, t), 25.0 (1C, t), 21.6 (2C, t), 20.5 (1C, q). According to the general procedure to form the HCl salt of **1** described above, compound **16** (490.0 mg) was converted into 554.3 mg of the corresponding trihydrochloride. IR (KBr): 3450, 2928, 2702, 1628, 1474, 1308, 1252, 1171, 1105, 968, 937, 854, 772 cm^{-1}. MS (ESI positive) *m*/*z*: $[M + H]^+$ 423. Anal. ($C_{27}H_{42}N_4 \cdot 3HCl \cdot H_2O$) C, H, N.

(*S*)-(1-*tert*-Butoxycarbonyl)-3-piperidinecarboxylic Acid (18). To a stirred solution of (*S*)-nipecotic acid hydrochloride {$[\alpha]_D$ +3.4 (*c* 1, H_2O), 596.2 mg, 3.60 mmol} in MeOH (17 mL) was added Et_3N (17 mL) followed by di-*tert*-butyl dicarbonate (942.8 mg, 4.32 mmol) at 0 °C under N_2. The reaction mixture was warmed to room temperature, stirred for 2 days, and concentrated in vacuo. The residue was diluted with CH_2Cl_2 (50 mL)−H_2O (10 mL), and the mixture was cooled to 0 °C, acidified by adding 2 N HCl to adjust to pH 3. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (60 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give 862.1 mg of the title compound **18** as a white solid in quantitative yield (crude). ^{1}H NMR of this compound was the same as that of (*R*)-enantiomer **2**.

***tert*-Butyl (3*S*)-3-{[(2-{[1-(1-Methylcyclooctyl)-4-piperidinyl]amino}-phenyl)amino]carbonyl}-1-piperidinecarboxylate (19).** According to the procedure for the corresponding (3*R*)-enantiomer, i.e., *tert*-butyl (3*R*)-3-{[(2-{[1-(1-methylcyclooctyl)-4-piperidinyl]amino}phenyl)amino]carbonyl}-1-piperidinecarboxylate **14**, 332.0 mg of the title compound **19** was prepared as a crude product,

including a small amount of CH_2Cl_2, from (*S*)-(1-*tert*-butoxycarbonyl)-3-piperidinecarboxylic acid **18** (95.4 mg, 0.416 mmol) and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (101.0 mg, 0.320 mmol). ^{1}H NMR of this compound **19** was the same as that of (*R*)-enantiomer **14**.

***tert*-Butyl (3*S*)-3-{1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate (20).** According to the procedure for the corresponding (3*R*)-enantiomer, i.e., *tert*-butyl (3*R*)-3-{1-[1-(1-methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate **15**, 132.0 mg of the title compound **20** was prepared in 78% yield (two steps) as a slight brownish white solid from the above *tert*-butyl (3*S*)-3-{[(2-{[1-(1-methylcyclooctyl)-4-piperidinyl]amino}phenyl)amino]carbonyl}-1-piperidinecarboxylate **19** (332.0 mg). ^{1}H NMR of this compound **20** was the same as that of (*R*)-enantiomer **15**.

1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*S*)-3-piperidinyl]-1*H*-benzimidazole (21). According to the procedure for the corresponding (3*R*)-enantiomer, i.e., 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-[(3*R*)-3-piperidinyl]-1*H*-benzimidazole **1**, 52.0 mg of the title compound **21** was prepared in 92% yield from *tert*-butyl (3*S*)-3-{1-[1-(1-methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate **20** (70.7 mg, 0.139 mmol). ^{1}H NMR of this compound was the same as that of (*R*)-enantiomer **1**. According to the general procedure to form HCl salt, compound **21** (52.0 mg) was converted into 63.3 mg of the corresponding trihydrochloride. HPLC analysis: 96% ee, determined by employing a chiral stationary phase (apparatus, Alliance 2690 with PDA detector, Waters; analytical column, CHIRALCEL OG, 250 mm × 4.6 mm, Daicel Chemical Industries; eluent, hexane/EtOH/2-propanol/diethylamine = 97/1.5/1.5/0.1; flow rate, 1 mL/min; room temperature; dissolving solvent, hexane/EtOH = 1:1; UV detection, 252 nm; retention time, peak of (*R*)-form, 22 min; peak of (*S*)-form, 15 min; (*R*)/(*S*) = 2:98. $[\alpha]_D^{24} = +5.3$ (*c* 0.53, MeOH). ^{13}C NMR (150 MHz, CD_3OD) δ 153.9 (1C, s), 135.1 (1C, s), 132.9 (1C, s), 128.2 (1C, d), 128.0 (1C, d), 117.2 (1C, d), 116.7 (1C, d), 73.7 (1C, s), 55.0 (1C, d), 48.3 (1C, t), 48.3 (1C, t), 48.2 (1C, t), 45.9 (1C, t), 33.9 (1C, d), 33.5 (1C, t), 33.4 (1C, t), 30.0 (1C, t), 29.9 (1C, t), 29.1 (2C, t), 28.6 (1C, t), 27.2 (1C, t), 24.8 (2C, t), 23.7 (1C, t), 23.1 (1C, q). IR (KBr) was the same spectrum as that of trihydrochloride of **1**. MS (ESI positive) *m*/*z*: $[M + H]^+$ 409. Anal. ($C_{26}H_{40}N_4 \cdot 3HCl \cdot 2.5H_2O$) C, H, N.

1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3*S*)-1-methyl-3-piperidinyl]-1*H*-benzimidazole (22). To a solution of *tert*-butyl (3*S*)-3-{1-[1-(1-methylcyclooctyl)-4-piperidinyl]-1*H*-benzimidazol-2-yl}-1-piperidinecarboxylate **20** (61.3 mg, 0.120 mmol) in anhydrous THF (3.0 mL) was added LAH (22.9 mg, 0.603 mmol) at room temperature under N_2. The reaction mixture was stirred for 30 min, then warmed up to reflux conditions, stirred for 5 h, and then cooled to 0 °C. AcOEt (3.0 mL) was added dropwise to the reaction mixture at 0 °C, stirred at 0 °C for 10 min, then stirred at room temperature for 20 min. After the mixture was cooled to 0 °C, H_2O (4.0 mL) was added dropwise. After being stirred at 0 °C for 1 h, the mixture was poured into saturated aqueous $NaHCO_3$ (5 mL), then the mixture was extracted with CH_2Cl_2 (25 mL × 3). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH/25% aqueous ammonia = 1000/100/8) to give 42.0 mg of the title compound **22** in 83% yield as a slight brownish white solid. ^{1}H NMR of this compound **22** was the same as that of (*R*)-enantiomer **16**. According to the general procedure to form HCl salt described above, compound **22** (42.0 mg) was converted into 47.4 mg of the corresponding trihydrochloride: $[\alpha]_D^{24}$ +4.6 (*c* 0.43, MeOH). ^{13}C NMR (150 MHz, CD_3OD) δ 153.2 (1C, s), 135.3 (1C, s), 132.9 (1C, s), 128.2 (1C, d), 128.0 (1C, d), 117.2 (1C, d), 116.8 (1C, d), 73.6 (1C, s), 57.8 (1C, t), 56.0 (1C, t), 55.0 (1C, d), 48.4 (1C, t), 48.3 (1C, t), 45.3 (1C, q), 34.9 (1C, d), 33.5 (1C, t), 33.4 (1C, t), 30.0 (1C, t), 29.9 (1C, t), 29.1 (2C, t), 27.4 (1C, t), 27.2 (1C, t), 24.8 (3C, t), 23.2 (1C, q). MS (ESI positive) *m*/*z*: $[M + H]^+$ 423. Anal. ($C_{27}H_{42}N_4 \cdot 3HCl \cdot 3.3H_2O$) C, H, N.

1-[1-(1-Methylcyclooctyl)piperidin-4-yl]-2-[3-(methylsulfonyl)phenyl]-1*H*-benzimidazole (23). To a stirred solution of 3-(methylsulfonyl)benzoic acid (84.1 mg, 0.420 mmol) and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (126.2 mg, 0.400 mmol) in anhydrous THF (3.5 mL) was added WSCI (99.7 mg, 0.520 mmol) at −20 °C under N_2. The reaction mixture was stirred at that temperature for 10 min, then stirred at 0 °C for 30 min, and then allowed to warm to room temperature. After being stirred at room temperature for 3 days, the reaction mixture was cooled to 0 °C, H_2O (3 mL) and saturated aqueous $NaHCO_3$ (3 mL) were added, then the mixture was extracted with CH_2Cl_2 (15 mL × 2). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Subsequently, the residue was mixed with $POCl_3$ (15 mL) under N_2, and the reaction mixture was stirred at 100 °C for 7 h, then cooled to room temperature, and then concentrated in vacuo. The residue was diluted with $CHCl_3$ (20 mL), cooled to 0 °C, H_2O (15 mL) was added, and the mixture was basified by adding 25% aqueous ammonia. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (15 mL × 3). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by PTLC (silica gel, CH_2Cl_2/MeOH = 20/1) to give 83.8 mg of the title compound **23** in 44% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.25−8.24 (1H, m), 8.13−8.09 (1H, m), 7.96−7.92 (1H, m), 7.85−7.73 (3H, m), 7.39−7.28 (2H, m), 4.29−4.12 (1H, m), 3.18−2.80 (5H, m), 2.59 (2H, dq, *J* = 11.9 Hz, *J* = 3.46 Hz), 2.09 (2H, t, *J* = 10.9 Hz), 1.94−1.33 (16H, m), 0.82 (3H, s). Compound **23** (83.8 mg) was converted into 86.9 mg of the corresponding dihydrochloride: ^{13}C NMR (150 MHz, CD_3OD) δ 152.2 (1C, s), 144.5 (1C, s), 137.1 (1C, d), 136.8 (1C, s), 133.5 (1C, s), 133.0 (1C, d), 133.0 (1C, d), 131.2 (1C, d), 128.2 (1C, s), 128.1 (1C, d), 127.9 (1C, d), 118.1 (1C, d), 117.0 (1C, d), 73.7 (1C, s), 56.2 (1C, d), 48.2 (2C, t), 45.1 (1C, q), 33.4 (2C, t), 29.9 (2C, t), 29.1 (2C, t), 27.1 (1C, t), 24.7 (2C, t), 22.9 (1C, q). IR (KBr): 3406, 2928, 2692, 1624, 1464, 1300, 1148, 966, 860, 777 cm^{-1}. MS (ESI positive) *m*/*z*: $[M + H]^+$ 480. Anal. ($C_{28}H_{37}N_3O_2S \cdot 2HCl \cdot 1.25H_2O$) C, H, N.

2-(3-Chloro-4-fluorophenyl)-1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazole (24). To a stirred solution of 3-chloro-4-fluorobenzoic acid (110.0 mg, 0.630 mmol) and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (189.3 mg, 0.600 mmol) in anhydrous THF (4.0 mL) was added WSCI (149.5 mg, 0.780 mmol) at −20 °C under N_2. The reaction mixture was stirred at the temperature under N_2 for 10 min, then stirred at 0 °C for 30 min, and then allowed to room temperature. After being stirred at room temperature for 2 days, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (15 mL), then the mixture was extracted with CH_2Cl_2 (15 mL × 4). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Subsequently, to the residue was added $POCl_3$ (20 mL) at room temperature under N_2, and the reaction mixture was stirred under reflux conditions for 5 h, then allowed to room temperature, and concentrated in vacuo. The residue was cooled to 0 °C and diluted with $CHCl_3$ (30 mL). H_2O (20 mL) was added at 0 °C, then the mixture was basified by adding 25% aqueous ammonia at 0 °C. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (30 mL × 4). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by PTLC (silica gel, CH_2Cl_2/MeOH = 10/1) to give 155.6 mg of the title compound **24** in 57% yield as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.82−7.72 (3H, m), 7.52−7.46 (1H, m), 7.34−7.25 (3H, m), 4.30−4.20 (1H, m), 3.12 (2H, br d, *J* = 11.2 Hz), 2.65−2.51 (2H, m), 2.15−2.06 (2H, m), 1.92−1.30 (16H, m), 0.83 (3H, s). MS (ESI positive) *m*/*z*: $[M + H]^+$ 454. Compound **24** was converted into the corresponding trihydrochloride: ^{13}C NMR (150 MHz, CD_3OD) δ 162.7 (1C, s, J_{C-F} = 254.9 Hz, CF), 151.8 (1C, s), 136.7 (1C, s), 134.8 (1C, d), 133.4 (1C, s), 133.0 (1C, d, J_{C-F} = 8.5 Hz), 128.0 (1C, d), 127.8 (1C, d), 124.4 (1C, s, J_{C-F} = 18.4 Hz), 124.3 (1C, s), 120.1 (1C, d, J_{C-F} = 22.0 Hz), 118.0 (1C, d), 116.9 (1C, d), 73.7 (1C, s), 56.0 (1C, d), 48.1 (2C, t), 33.3 (2C, t), 29.9 (2C, t), 29.0 (2C, t),

27.1 (1C, t), 24.7 (2C, t), 22.9 (1C, q). IR (KBr): 3422, 2930, 1611, 1487, 1468, 1269, 1086, 1061, 764, 644 cm^{-1}. Anal. ($C_{27}H_{33}N_3FCl\cdot 2HCl\cdot 0.82H_2O$) C, H, N.

2-(1*H*-Indol-6-yl)-1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazole (25). To a stirred solution of 1*H*-indole-6-carboxylic acid (84.6 mg, 0.525 mmol) and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (157.8 mg, 0.500 mmol) in anhydrous THF (3.5 mL) was added WSCI (124.6 mg, 0.650 mmol) at −20 °C under N_2. The reaction mixture was stirred at that temperature under N_2 for 10 min, warmed to 0 °C, stirred for 30 min, and then allowed to warm to room temperature. After being stirred at room temperature for 3 days, the reaction mixture was cooled to 0 °C, H_2O (3 mL) and saturated aqueous $NaHCO_3$ (3 mL) were added, then the mixture was extracted with CH_2Cl_2 (15 mL × 2). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Subsequently, the residue was mixed with $POCl_3$ (15 mL) under N_2, and the reaction mixture was stirred at 100 °C for 7 h and then cooled to room temperature and concentrated in vacuo. The residue was diluted with $CHCl_3$ (20 mL), cooled to 0 °C, H_2O (15 mL) was added, and the mixture was basified by adding 25% aqueous ammonia. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (15 mL × 3). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by PTLC (silica gel, CH_2Cl_2/MeOH = 20/1) to give 98.0 mg of the title compound **25** in 44% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.59 (1H, br s), 8.01 (1H, s), 7.87−7.71 (3H, m), 7.32−7.23 (4H, m), 6.56−6.55 (1H, m), 4.59−4.46 (1H, m), 3.08 (2H, d, J = 11.4 Hz), 2.67−2.52 (2H, m), 2.10−1.30 (18H, m), 0.79 (3H, s). Compound **25** was converted into the corresponding dihydrochloride. IR (KBr): 3387, 2928, 1624, 1558, 1464, 1398, 1306, 1171, 1117, 943, 835, 762 cm^{-1}. MS (ESI positive) *m*/*z*: $[M + H]^+$ 441. Anal. ($C_{29}H_{36}N_4\cdot 2HCl\cdot 1.8H_2O$) C, H, N.

2-(1-Benzofuran-2-yl)-1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazole (26). To a stirred solution of 1-benzofuran-2-carboxylic acid (95.7 mg, 0.590 mmol) and *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (176.7 mg, 0.560 mmol) in anhydrous THF (4.0 mL) was added WSCI (139.6 mg, 0.728 mmol) at −20 °C under N_2, and the reaction mixture was stirred at the temperature for 20 min, then stirred at 0 °C for 1 h, and then allowed to warm to room temperature. After being stirred at room temperature for 5 days, the reaction mixture was suspended with $CHCl_3$ (2 mL) and saturated aqueous $NaHCO_3$ (1 mL). The mixture was filtered through Extrelut NT3 with $CHCl_3$ (10 mL), and the filtrate was concentrated in vacuo. Subsequently, the residue was mixed with $POCl_3$ (4.0 mL) under N_2, and the reaction mixture was stirred at 100 °C for 8 h in a sealed tube, and then cooled to room temperature, and concentrated in vacuo. The residue was diluted with $CHCl_3$ (4 mL), cooled to 0 °C, basified by adding 25% aqueous ammonia. The mixture was filtered through Extrelut NT3 with $CHCl_3$ (10 mL), then the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane/CH_2Cl_2/acetone = 7/1/1) followed by PTLC purification (silica gel, hexane/acetone = 5/1) to give 53.9 mg of the title compound **26** in 22% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88−7.82 (1H, m), 7.78−7.73 (1H, m), 7.72−7.69 (1H, m), 7.64−7.60 (1H, m), 7.43−7.29 (5H, m), 4.93−4.82 (1H, m), 3.19 (2H, d, J = 11.9 Hz), 2.60 (2 H, dq, J = 12.3 Hz, J = 3.66 Hz), 2.28−2.20 (2H, m), 2.04−1.33 (16H, m), 0.88 (3H, s). Compound **26** was converted into the corresponding dihydrochloride: ^{13}C NMR (150 MHz, CD_3OD) δ 158.2 (1C, s), 143.2 (1C, s), 140.1 (1C, s), 135.0 (1C, s), 133.3 (1C, s), 130.6 (1C, d), 129.4 (1C, s), 128.9 (1C, d), 128.4 (1C, d), 126.7 (1C, d), 125.2 (1C, d), 117.7 (1C, d), 117.6 (1C, d), 117.1 (1C, d), 114.0 (1C, d), 73.7 (1C, s), 56.9 (1C, d), 48.4 (2C, t), 33.4 (2C, t), 29.9 (2C, t), 28.8 (2C, t), 27.1 (1C, t), 24.8 (2C, t), 23.1 (1C, q). IR (KBr): 3406, 2924, 2654, 1612, 1466, 1443, 1344, 1306, 1259, 1229, 1192, 1144, 1109, 1076, 1009, 947, 889, 839, 752, 648, 615 cm^{-1}. MS (ESI positive) *m*/*z*: $[M + H]^+$ 442. Anal. ($C_{29}H_{35}N_3O\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

1-[1-(1-Methylcyclooctyl)piperidin-4-yl]-2-[3-(trifluoromethyl)phenyl]-1*H*-benzimidazole (36). To a solution of *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (250 mg, 0.792 mmol) in dry pyridine (3.5 mL) was added dropwise 3-(trifluoromethyl)benzoyl chloride (198 mg, 0.951 mmol) at room temperature under N_2. The resulting mixture was stirred at room temperature overnight. The volatile materials were removed in vacuo to give a black powder, which was dissolved in CH_2Cl_2 (8 mL), washed with saturated aqueous $NaHCO_3$, dried over anhydrous K_2CO_3, filtered, then concentrated in vacuo. Subsequently, to the residue was added $POCl_3$ (8 mL) under N_2, and the reaction mixture was stirred under reflux conditions for 4.5 h. After the mixture was cooled to room temperature, the volatile materials were removed in vacuo to give a dark-brown oil. The residue was dissolved in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo. The residue was purified by PTLC (silica gel, CH_2Cl_2/acetone = 12/1) to give 79.3 mg of the title compound **36** in 21% yield as a white powder. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.95 (1H, s), 7.84−7.64 (5H, m), 7.33−7.30 (2H, m), 4.32−4.17 (1H, m), 3.13 (2H, br d, J = 12 Hz), 2.66−1.30 (20H, m), 0.82 (3H, s). MS (EI direct) *m*/*z*: M^+ 469. Compound **36** was converted into the corresponding dihydrochloride: ^{13}C NMR (150 MHz, CD_3OD) δ 153.1 (1C, s), 139.4 (1C, s), 135.5 (1C, d), 134.0 (1C, s), 133.7 (1C, s, J_{C-F} = 33.3 Hz), 132.5 (1C, d), 130.4 (1C, d), 129.7 (1C, s), 128.9 (1C, d, J_{C-F} = 3.6 Hz), 127.1 (1C, d), 127.1 (1C, d), 125.9 (1C, s, J_{C-F} = 271.6 Hz, CF_3), 119.2 (1C, d), 116.3 (1C, d), 73.6 (1C, s), 55.6 (1C, d), 48.3 (2C, t), 33.4 (2C, t), 29.9 (2C, t), 29.1 (2C, t), 27.1 (1C, t), 24.7 (2C, t), 22.8 (1C, q). Anal. ($C_{28}H_{34}N_3F_3\cdot 2HCl\cdot 0.7H_2O$) C, H, N.

Ethyl (3-Formylphenoxy)acetate (38). To a solution of 3-hydroxybenzaldehyde (500 mg, 4.09 mmol) in dry CH_3CN (5 mL) was added anhydrous K_2CO_3 (679 mg, 4.91 mmol) followed by ethyl 1-bromoacetate (684 mg, 4.09 mmol) at room temperature under N_2. The reaction mixture was stirred at 70 °C for 4 h, cooled to room temperature, and then H_2O was added. The mixture was extracted with AcOEt (10 mL × 3). The combined extracts were washed with saturated aqueous $NaHCO_3$, dried over anhydrous K_2CO_3, filtered, and concentrated to give 1.21 g of pale-yellow oil. The residue was purified by flash column chromatography (hexane/AcOEt = 5:1) to give 890 mg of the title compound **38** in quantitative yield as a colorless oil including small amount of AcOEt based on ^{1}H NMR. ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.97 (1H, s), 7.53−7.44 (2H, m), 7.37 (1H, s), 7.27−7.22 (1H, m), 4.69 (2H, s), 4.29 (2H, q, J = 7.1 Hz), 1.33−1.25 (3H, m).

Ethyl (3-{1-[1-(1-Methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazol-2-yl}phenoxy)acetate (39). To a solution of ethyl (3-formylphenoxy)acetate **38** (198 mg, 0.951 mmol) in EtOH (6 mL) was added *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (300 mg, 0.951 mmol) at room temperature under N_2. The resulting solution was stirred under reflux conditions for 1 h. The volatile materials were removed in vacuo to give black oil, which was diluted with CH_2Cl_2 (10 mL). The organic layer was washed with saturated aqueous $NaHCO_3$, dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo. Subsequently, the residue was dissolved in dry benzene (6 mL), $Pb(OAc)_4$ (464 mg, 1.05 mmol) was added under N_2, then the mixture was stirred under reflux conditions for 1 h.[52] After the mixture was cooled, CH_2Cl_2 (10 mL) was added to the mixture, which was then washed with saturated aqueous $NaHCO_3$, dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH = 20/1) followed by PTLC purification (silica gel, CH_2Cl_2/MeOH = 20/1) to give 304.6 mg of the title compound **39** in 64% yield as a white powder. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.82−7.73 (2H, m), 7.43−7.07 (6H, m), 4.69 (2H, s), 4.31−4.23 (3H, m), 3.10 (2H, br d, J = 10 Hz), 2.60−1.27 (23H, m), 0.82 (3H, s).

2-(3-{1-[1-(1-Methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazol-2-yl}phenoxy)ethanol (40). To a solution of ethyl (3-{1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazol-2-yl}phenoxy)acetate **39** (304 mg, 0.604 mmol) in anhydrous THF (6 mL)

was added LAH (23.0 mg, 0.606 mmol) at 0 °C under N_2. The reaction mixture was stirred for 15 min at the temperature, then stirred at room temperature overnight. To the reaction mixture was added dropwise H_2O (23 μL), 15% aqueous NaOH (23 μL), and H_2O (23 μL × 3). The resulting mixture was diluted with THF (20 mL), filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH = 20/1) to give 228.4 mg of the title compound **40** in 82% yield as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.81−7.73 (2H, m), 7.43−7.07 (6H, m), 4.30 (1H, m), 4.18−4.14 (2H, m), 4.01−3.95 (2H, m), 3.15−3.03 (2H, m), 2.75−2.42 (2H, m), 2.18−1.30 (19H, m), 0.82 (3H, s). MS (EI direct) *m*/*z*: M^+ 461. Compound **40** was converted into the corresponding trihydrochloride: ^{13}C NMR (150 MHz, CD_3OD) δ 162.0 (1C, s), 153.4 (1C, s), 135.2 (1C, s), 133.1 (1C, s), 133.1 (1C, d), 128.4 (1C, d), 128.0 (1C, d), 126.9 (1C, s), 124.0 (1C, d), 121.1 (1C, d), 117.9 (1C, d), 117.2 (1C, d), 117.2 (1C, d), 73.7 (1C, s), 72.0 (1C, t), 62.4 (1C, t), 56.2 (1C, d), 48.2 (2C, t), 33.3 (2C, t), 29.9 (2C, t), 29.0 (2C, t), 27.1 (1C, t), 24.7 (2C, t), 22.9 (1C, q). IR (KBr): 3387, 2930, 2719, 1466, 1242, 1173, 1045, 955, 766, 694 cm^{-1}. Anal. ($C_{29}H_{39}N_3O_2 \cdot 2HCl \cdot 1.2H_2O$) C, H, N.

3-{1-[1-(1-Methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazol-2-yl}benzonitrile (42). To a mixture of *N*-[1-(1-methylcyclooctyl)-4-piperidinyl]-1,2-benzenediamine **3** (300 mg, 0.951 mmol) and 3-cyanobenzoyl chloride (189 mg, 1.14 mmol) was added dry pyridine (4 mL) at room temperature under N_2. The resulting mixture was stirred at room temperature overnight. The volatile materials were removed in vacuo. The residue was dissolved in CH_2Cl_2 (25 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), dried over anhydrous K_2CO_3, filtered, then concentrated in vacuo. Subsequently, the resulting solid was dissolved in $POCl_3$ (10 mL) under N_2, and the reaction mixture was stirred under reflux conditions for 4 h. After the mixture was cooled to room temperature, the volatile materials were removed in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous K_2CO_3, filtered, then concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH = 60/1 to 30/1) to give 186.2 mg of the title compound **42** in 46% yield as a white powder. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.95 (1H, s), 7.90−7.63 (5H, m), 7.35−7.30 (2H, m), 4.27−4.12 (1H, m), 3.14 (2H, br d, J = 12 Hz), 2.70−2.52 (2H, m), 2.14−1.31 (18H, m), 0.83 (3H, s).

1-(3-{1-[1-(1-Methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazol-2-yl}phenyl)methanamine (43). To 3-{1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1*H*-benzimidazol-2-yl}benzonitrile **42** (136 mg, 0.319 mmol) in anhydrous THF (1.6 mL) was added LAH (12.0 mg, 0.316 mmol) at 0 °C under N_2. The reaction mixture was stirred for 5 min at that temperature, then stirred at room temperature overnight. To the reaction mixture was added dropwise saturated aqueous NH_4Cl, then the mixture was extracted with CH_2Cl_2 (10 mL × 3). The combined extracts were dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo. The residue was purified by PTLC (silica gel, CH_2Cl_2/MeOH/acetone = 1/1/1) to give 73.6 mg as a pale-yellow oil. The residue was recrystallized from CH_2Cl_2−hexane to afford 35.2 mg of the title compound **43** in 26% yield as a white powder. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.85−7.73 (2H, m), 7.67 (1H, s), 7.52−7.25 (5H, m), 4.42−4.28 (1H, m), 3.97 (2H, s), 3.10 (2H, br d, J = 10.8 Hz), 2.67−2.49 (2H, m), 2.16−1.23 (20H, m), 0.81 (3H, s). Compound **43** was converted into the corresponding trihydrochloride: ^{13}C NMR (150 MHz, CD_3OD) δ 153.8 (1C, s), 139.0 (1C, s), 136.8 (1C, s), 134.3 (1C, d), 134.0 (1C, s), 132.6 (1C, d), 132.5 (1C, d), 132.2 (1C, d), 129.1 (1C, s), 127.1 (1C, d), 127.0 (1C, d), 118.8 (1C, d), 116.4 (1C, d), 73.6 (1C, s), 55.5 (1C, d), 48.3 (2C, t), 44.7 (1C, q), 33.4 (2C, t), 29.9 (2C, t), 29.2 (2C, t), 27.2 (1C, t), 24.7 (2C, t), 23.1 (1C, q). IR (KBr): 3427, 2930, 2926, 2748, 2363, 773, 700 cm^{-1}. Anal. ($C_{28}H_{38}N_4 \cdot 3HCl \cdot 3.6H_2O$) C, H, N.

Biology. General. All animal experiments were conducted according to the guideline of animal care and use, and all procedures were approved by the Animal Ethics Committee in Pfizer Global Research & Development Nagoya Laboratories. The animal work was also approved by the Pfizer Institutional Animal Care and Use Committee (IACUC).

In Vitro Characterization of NOP Receptor Agonists.[54] In vitro studies on NOP receptor binding affinities and functional efficacies ([^{35}S]GTPγS assays) as well as on other opioid (μ-, κ-, and δ-) receptors binding affinities and functional efficacies were conducted.

Materials. The human NOP receptor transfected human embryonic kidney (HEK)-293 cell membranes, the human μ-receptor transfected Chinese hamster ovary (CHO)-K1 cell membranes, the human κ-receptor transfected HEK-293 cell membranes, and the human δ-receptor transfected CHO-K1 cell membranes were purchased from Receptor Biology Inc. [^{3}H]N/OFQ (150 Ci/mmol), [^{3}H]enadoline (45.0 Ci/mmol), [^{35}S]GTPγS (1060-1150 Ci/mmol), and wheatgerm agglutinin (WGA)-scintillation proximity assay (SPA) beads were obtained from Amersham Pharmacia Biotech K.K. [^{3}H]DAMGO (54.0 Ci/mmol) and [^{3}H]DPDPE (45.0 Ci/mmol) were provided from NEN Life Science Products Inc. N/OFQ was from Peptide Institute Inc. DAMGO and DPDPE were from Sigma Chemical.

Evaluation of Receptor Binding Affinities to NOP Receptor and Other Opioid (μ-, κ-, and δ-) Receptors. All competitive displacement analyses (IC_{50} and K_i) for the NOP receptor as well as the μ-, κ-, and δ-receptors were performed in duplicate in a 96-well plate using a scintillation proximity assay (SPA). After the reaction, the assay plate was centrifuged at 1000 rpm for 1 min, then the radioactivity was measured by a 1450 MicroBeta (Wallac) liquid scintillation counter. IC_{50} values were calculated by nonlinear regression with the software GraphPad Prism, version 4.0 (GraphPad Software, Inc., San Diego, CA). K_i value was calculated by the following equation: $K_i = IC_{50}/(1 + [L]/K_D)$, where [L] is the radiolabeled ligand concentration and K_D is the dissociation constant.[55]

NOP Receptor Binding Assay. Methods. The NOP membranes (8.3 μg) were incubated for 45 min at 22 °C with 0.4 nM [^{3}H]N/OFQ, 1.0 mg of WGA-SPA beads, and six different concentrations of compounds (10^{-11}−10^{-5} M, 10-fold) in a final volume of 0.2 mL of 50 mM HEPES buffer, pH 7.4, containing 10 mM $MgCl_2$ and 1 mM EDTA. Nonspecific binding was determined by the addition of 1 μM unlabeled N/OFQ. Under these conditions, approximately 900 cpm of total binding were obtained, of which 30 cpm were nonspecific.

***μ*-Receptor Binding Assay. Methods.** The μ-membranes (18 μg) were incubated for 45 min at 25 °C with 1.0 nM [^{3}H]DAMGO, 1.0 mg of WGA-SPA beads, and six different concentrations of compounds (10-fold) in a final volume of 0.2 mL of 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM $MgCl_2$. Nonspecific binding was determined by the addition of 1 μM of unlabeled DAMGO. Under these conditions, approximately 240 cpm of total binding were obtained, of which 23 cpm were nonspecific.

***κ*-Receptor Binding Assay. Methods.** The κ-membranes (13 μg) were incubated for 45 min at 22 °C with 0.5 nM [^{3}H]enadoline, 1.0 mg of WGA-SPA beads, and six different concentrations of compounds (10-fold) in a final volume of 0.2 mL of 50 mM Tris-HCl buffer, pH7.4, containing 10 mM $MgCl_2$ and 1 mM EDTA. Nonspecific binding was determined by the addition of 1 μM unlabeled enadoline. Under these conditions, approximately 270 cpm of total binding were obtained, of which 15 cpm were nonspecific.

***δ*-Receptor Binding Assay. Methods.** The δ-membranes (18 μg) were incubated for 45 min at 22 °C with 2.0 nM [^{3}H]DPDPE, 1.0 mg of WGA-SPA beads, and six different concentrations of compounds (10-fold) in a final volume of 0.2 mL of 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM $MgCl_2$. Nonspecific binding was determined by the addition of 1 μM unlabeled DPDPE. Under these conditions, approximately 800 cpm of total binding were obtained, of which 70 cpm were nonspecific.

Evaluation of Functional Activity: [^{35}S]GTPγS Binding Assays. Methods. Agonist stimulated binding of [^{35}S]GTPγS was investigated according to the method of SPA G-protein-coupled

receptor assay provided by Amersham Pharmacia Biotech with slight modification. Each human NOP, μ-, κ-, and δ-receptor expressing cell membrane was suspended in assay buffer (20 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 5 μM GDP, 1 mM DTT, pH 7.4). The membranes (the same amount as for their respective receptor binding assays) were incubated for 30 min at 22 °C with 0.4 nM [^{35}S]GTPγS, 1.5 mg WGA-SPA beads, and various concentrations of compounds in duplicate in a 0.2 mL total volume. After the reaction, the assay plate was centrifuged at 1000 rpm for 2 min, and then the radioactivity was measured by a 1450 MicroBeta (Wallac) liquid scintillation counter. Nonspecific binding was assessed with 10 μM of unlabeled GTPγS. Agonist stimulated binding was determined as the difference between total binding in the presence of compound and basal binding determined in the absence of compound. EC_{50} (potency) value was the concentration producing a half-maximal response of its own. E_{max} (efficacy) value was the maximal response calculated as the percentage of the maximal response produced by each control (N/OFQ, DAMGO, enadoline, and DPDPE). All the binding data were analyzed by nonlinear regression with the software GraphPad Prism, version 4.0 (GraphPad Software, Inc., San Diego, CA).

Evaluation of NOP Occupancy of NOP Receptor Agonists. Methods. For drug administration, male ddY mice (5−6 week, Nippon SLC) were used. Mice were deprived of water for 24 h prior to drug administration. Each compound was suspended in 0.1% methylcellulose solution at a volume of 0.1 mL/10 g body weight. At 1 h after compound administration at a dose of 10 mg/kg per os, the whole brain was quickly removed. The cerebellum and medulla of each brain were discarded, and the remainder was frozen in −80 °C before tissue preparation. For tissue preparation, the brain tissues were homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 10 mM $MgCl_2$, and 320 mM sucrose (buffer A) with a Polytron homogenizer, and each homogenate was centrifuged at 1000 rpm (HIMAC CR5DL, Hitachi) for 10 min. The supernatant was recovered, and the pellet was resuspended in ice-cold buffer A and centrifuged again. The supernatant for two centrifugations was combined and centrifuged at 38000 × *g* for 30 min. The pellet was resuspended in ice-cold 50 mM Tris-HCl buffer (pH 7.4) and centrifuged again at 38000 × g for 30 min. The pellet was finally suspended in ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 320 mM sucrose and used in [^{3}H]N/OFQ binding assay. All steps were performed at 4 °C. Protein concentration was measured according to the method of Bradford with bovine serum albumin as a standard. The membranes (160 μg of protein) prepared from mice brain were incubated with 0.3 nM [^{3}H]N/OFQ in a total volume of 0.2 mL of 50 mM HEPES buffer (pH 7.4) containing 1 mM EDTA and 10 mM $MgCl_2$ for 30 min at 25 °C. The reaction was terminated by rapid filtration (cell harvester) through Whatman GF/B glass fiber filters. Radioactivity on the filtrate was measured by a β-plate counter. Specific binding of [^{3}H]N/OFQ was determined from the difference between counts in the absence and presence of 1 μM N/OFQ. All assays were conducted in triplicate, and the values are the mean ± SEM.

Physicochemistry. The lipophilicity values of NOP receptor agonists were estimated as values of $ACDlogD_{7.4}$, the octanol−water distribution coefficient for ionizable compounds at pH 7.4, calculated by ACD software, ACD/Laboratories 9.0 (Advanced Chemistry Development, Inc., Ontario, Canada).

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Supporting Information Available: Results from elemental analysis of compounds **1**, **16**, **21**−**26**, **36**, **40**, and **43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(1) (a) The first original report of this study in terms of drug design, SAR, and pharmacological evaluation of orally potent anxiolytic was including in the following: Hayashi, S.; Hirao, A.; Imai, A.; Sugie, Y.; Nakata, E.; Ohashi, K.; Kato, A.; Yamada, Y.; Toide, K. Novel Potent ORL1 Receptor Agonists as Anti-Anxiety Drug and Analgesic Drug. *The 25th Medicinal Chemistry Symposium Abstracts*, 25th Medicinal Chemistry Symposium, Nagoya, Japan, Nov 29−Dec 1, 2006; The Pharmaceutical Society of Japan, Division of Medicinal Chemistry: Tokyo, Japan, 2006; 2P-49, pp 262−263. (b) The present article is also the first original report of the synthetic study of MCOPPB and its analogues herein. (c) The details of a study of NOP receptor agonist as analgesic with a different series of analogues including those in ref 1a will be reported (Hayashi, S.; et al.).

(2) (a) The details of the SAR study of the NOP agonists in this article for potent anxiolytic activity, little hERG binding affinity, and metabolic stability will be reported as a subsequent article. (Hayashi, S.; et al.). (b) See also ref 61.

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