Transformation of naltrexone into mesembrane and investigation of the binding properties of its intermediate derivatives to opioid receptors

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Abstract

We transformed naltrexone (**5**) with morphinan skeleton into mesembrane (**4**) belonging to the *Sceletium* alkaloids *via* key intermediate **6** to investigate the binding affinities of **4** and the key intermediate **6** derivatives to the opioid receptors. Among the tested compounds, **15′** with *cis*-fused hydroindole core bound to the opioid receptors with strong to moderate affinities. The observed difference of binding affinities among the tested compounds was reasonably explained by the conformational analyses of the compounds. The structure-activity relationship of the tested compounds like **15′** with hydroindole structure was completely different from the reported one of morphinan derivatives with hydroisoquinoline skeleton. Compound **15′** with a different structure from morphinans was expected to be a useful fundamental skeleton with a novel chemotype to provide characteristic opioid ligands.

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Introduction

G protein-coupled receptors (GPCRs) are one of the most valuable drug targets and more than 25% of the FDA-approved drugs interact with them.[1] In the past, it was thought that a substrate interacted with a GPCR one for one, and that an agonist exerted pharmacological responses *via* inherent pathway. However, recent investigations unveiled that actual phenomena would be more complicating. GPCRs do exist not only as monomeric receptor but also homo- or hetero-dimer and the dimeric receptors function in a living organism.[2] Concerning the opioid receptor, it is known heterodimerization between opioid receptor types such as δ opioid receptor (DOR) – κ opioid receptor (KOR), μ opioid receptor (MOR) – DOR, and MOR – KOR as well as heterodimerization between opioid receptors and non-opioid receptors such as MOR – cannabinoid CB1 receptor, DOR – chemokine CXCR2 receptor, and KOR – β_2 adrenergic receptor.[3] On the other hand, GPCRs are recently reported to engage effectors other than G proteins such as β-arrestins. Traditional agonists like endogenous agonists exert pharmacological responses *via* various effectors, whereas biased agonists selectively induced cell responses through an effector.[4] Moreover, GPCR splice variants, which are mutated receptors produced from a gene with more than one exon, were also investigated. A large series of splice variants of the MOR have already been identified.[5] With respect to these phenomena, that is, receptor dimerization, biased agonism, and splice variants, intense efforts were paid from the viewpoint of not only molecular biology, molecular pharmacology, but also development of specific ligands. For example, 6′-guanidinonaltrindole (6′-GNTI) and *N*-naphthoyl-β-naltrexamine (NNTA) were reported to be the DOR-KOR[6] and MOR-KOR[7] agonist, respectively. Herkinorin and TRV-130 were reportedly G protein-biased MOR agonists.[8] It is reported that amidino-TAPA produced antinociceptive effects *via* the MOR splice variants with which representative MOR agonist, morphine, did not interact.[9] Although some specific agonist were reported as mentioned above, the development of novel and unique ligands required for the further investigation of those phenomena were not sufficient. Moreover, there are almost no clue for designing such ligands. However, the structures of the ligands might play

an important role in discrimination of receptor dimers, effectors engaging with GPCRs (ligand bias), and/or receptor splice variants. Indeed, it is reported that ligand bias would stem from distinct receptor conformation which was stabilized by each agonist. Therefore, development of ligands with various chemotypes would be important to investigate receptor dimers, ligand bias, and/or receptor splice variants.

Sceletium tortuosum Zembrin®, which is a mixture of three *Sceletium* alkaloids[10] (mesembrenone (**1**) + mesembrenol (**2**) > 70%, mesembrine (**3**) <20%, Fig. 1), is recently reported to bind to the opioid receptors with very weak affinities (10-30% of the control bindings were remained).[11] Mesembrane (**4**)[12] is also a member of *Sceletium* alkaloids (Fig. 1). The basic nitrogen, phenyl ring, and phenolic hydroxy group are known to be essential pharmacophoric substituents to interact with the opioid receptors.[13] The *Sceletium* alkaloids indicated in Fig. 1 had these binding determinants although the phenolic hydroxy group was masked by methyl group. Moreover, it is interesting that both the *Sceletium* alkaloids and representative opioid ligand like naltrexone (**5**) have a common structure, phenethylamine motif indicated by red line (Fig. 1). However, naltrexone (**5**) has *trans*-fused hydroisoquinoline structure with a suspending phenyl ring fixed by both epoxy and methylene bridges, whereas the *Sceletium* alkaloids possesses *cis*-fused hydroindole skeleton with a freely rotatable phenyl ring. Therefore, we planned to transform from naltrexone (**5**) into *Sceletium* alkaloids like mesembrane (**4**) *via* a key intermediate **6** (Scheme 1), which has *cis*-fused hydroindole skeleton with suspending phenyl ring fixed by an epoxy bridge,[14] and evaluate the binding affinities of mesembrane (**4**) and key intermediate **6** derivatives to the opioid receptors. We chose mesembrane (**4**) as a target compound because among the *Sceletium* alkaloids **1**-**4** mesembrane (**4**) was the structurally simplest compound lacking the oxo or hydroxy group which was a potential substituent interacting with the opioid receptors. Herein, we report the transformation from naltrexone (**5**) into mesembrane (**4**) and opioid binding abilities of **4** and key intermediate **6** derivatives. The structure-activity relationship is also discussed based on conformational analyses of the tested compounds.

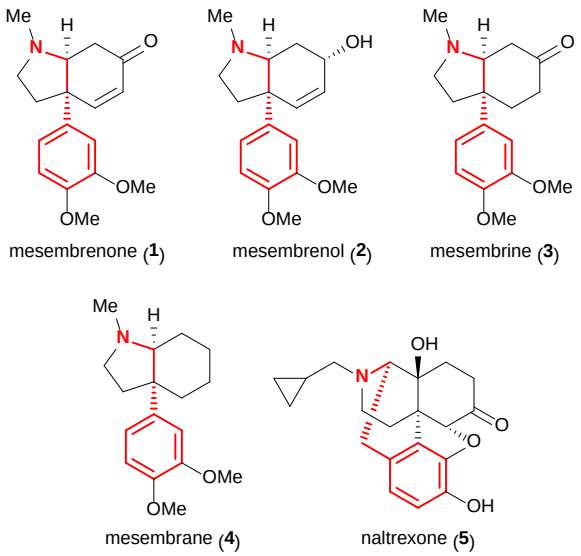
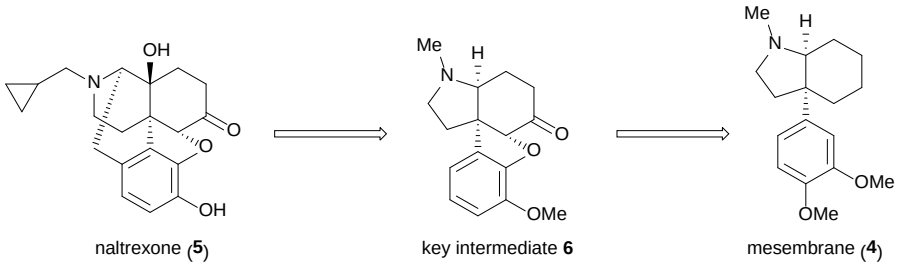


Fig. 1 Structures of *Sceletium* alkaloids **1** - **4** and naltrexone (**5**). The phenethylamine moieties were indicated by red lines.



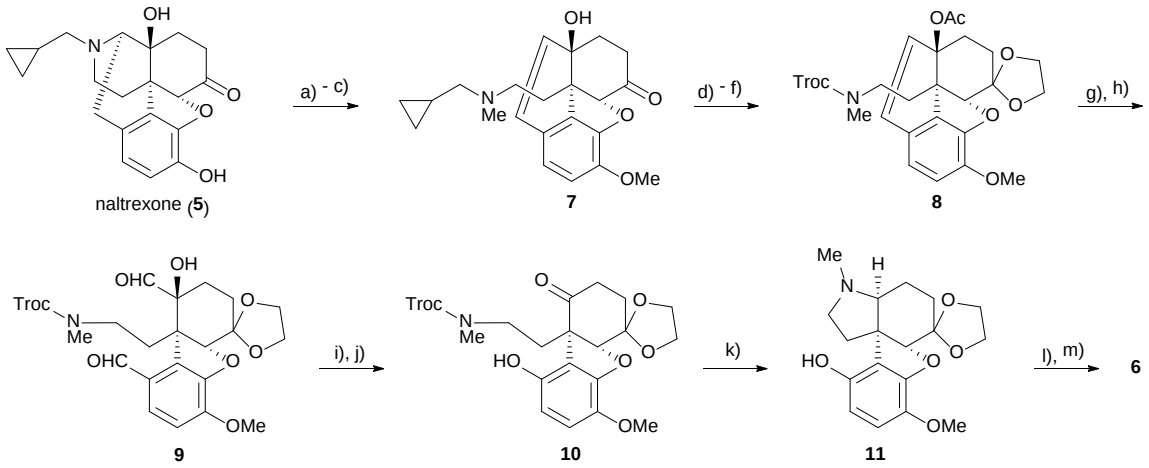
Scheme 1 Strategy of transformation from naltrexone (**5**) into mesembrane (**4**) *via* key intermediate **6**

Results and Discussion

Transformation from naltrexone (5) into mesembrane (4)

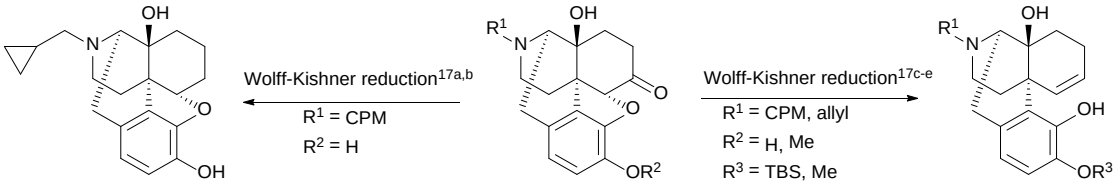
The key intermediate **6** was prepared from naltrexone (**5**) (Scheme 2). Naltrexone methyl ether, obtained by a methylation of the phenolic hydroxy group in naltrexone (**5**), was converted into olefin **7** under Hofmann elimination reaction conditions. The oxo and hydroxy groups in the obtained olefin **7** were sequentially protected, followed by treatment with 2,2,2-trichloroethyl chloroformate (Troc-Cl) to afford compound **8**. In the reaction of (cyclopropylmethy)methylamine **7** with Troc-Cl, the cyclopropylmethyl group was selectively dealkylated.[15] After ozonolysis of **8**, acetyl group was removed by methanolysis. The obtained dialdehyde **9** was oxidized by *m*-chloroperbenzoic acid

(*m*CPBA), followed by methanolysis of the obtained formate groups to give ketone **10**. Deprotection of Troc group in **10** and subsequent treatment with sodium cyanoborohydride ($NaBH_3CN$) provided compound **11**. The phenolic hydroxy group in **11** was removed by triflation and subsequent palladium catalyzed reduction, followed by deacetalization to afford the key intermediate **6**. The key intermediate **6** was determined to have *cis*-fused hydroindole structure by ROESY specta (Fig. S1).



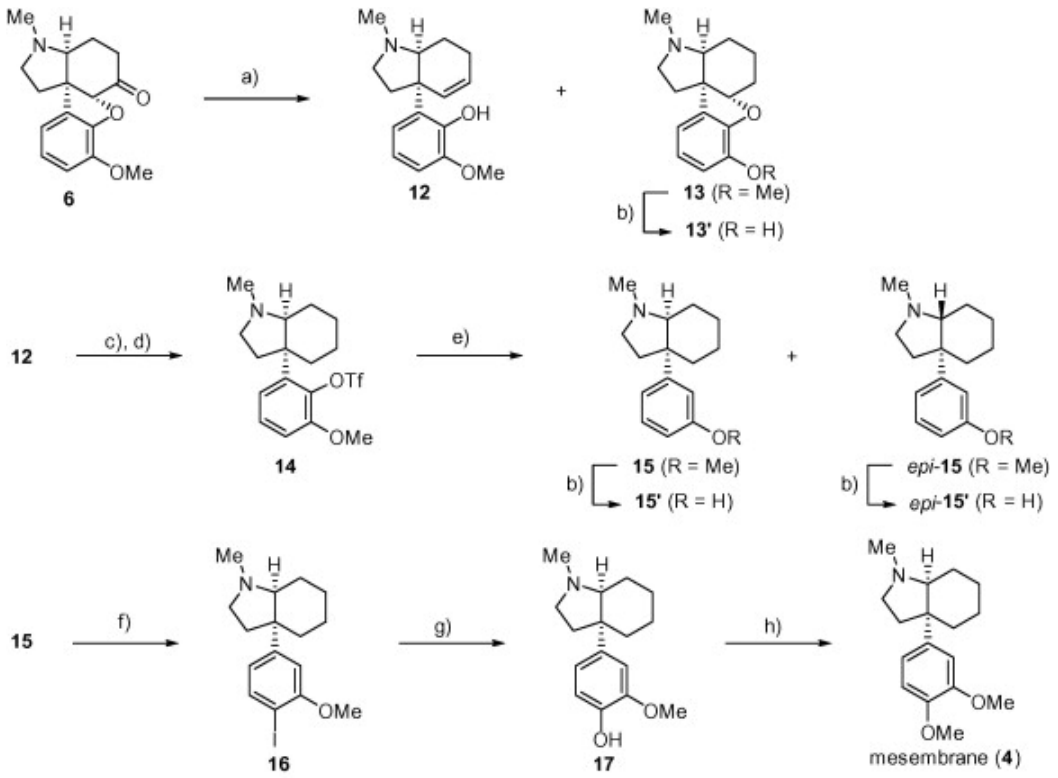
Scheme 2 Synthesis of the key intermediate **6**. Reagents and conditions: (a) MeI, K_2CO_3, DMF, (b) MeI, DMF, then concentrated, (c) 1 *M* NaOHaq, 1,4-dioxane, (d) ethylene glycol, TMSOTf, $CHCl_3$, (e) Ac_2O, (f) Troc-Cl, TEA, CH_2Cl_2, 72% from **5**, (g) O_3, CH_2Cl_2, then Me_2S, (h) TEA, MeOH, 73% from **8**, (i) *m*CPBA, CH_2Cl_2, (j) TEA, MeOH, 36% from **9**, (k) Zn, AcOH, filtered, then, $NaBH_3CN$, 92%, (l) Tf_2NPh, NaH, DME, then $PdCl_2(MeCN)_2$ (10 mol%), dppp (25 mol%), TEA, HCO_2H, (n) 1 *M* HCl, 79% from **11**

In morphinan derivatives, it is known that the presence or absence of the epoxy bridge sometimes critically affects the pharmacological properties of the compounds.[16] Therefore, from the viewpoint of the structure diversity, both hydroindole derivatives with and without the epoxy bridge were preferably prepared. Although Wolff-Kishner reduction is the general method converting an oxo group into methylene moiety, it is reported that Wolff-Kishner reduction of 4,5-epoxymorphinans provided two kind of products (scheme 3).[17] In hope that both compounds with and without the ethereal bond would be obtained, we attempted Wolff-Kishner reduction of the key intermediate **6**. As we expected, **12** and **13** were prepared in 73% and 25% yield, respectively (scheme 4).



Scheme 3 Wolff-Kishner reduction of naltrexone methyl ether. CPM: cyclopropylmethyl

Mesembrane (**4**) was prepared from compound **12** (Scheme 4). Catalytic hydrogenation of **12** and subsequent treatment with *N*-Phenylbis(trifluoromethanesulfonimide) (Tf_2NPh) afford triflate **14**. Palladium catalyzed reduction of **14** unexpectedly gave two products, **15** and *epi*-**15**. The configurations of these compounds were determined by nOe or ROESY spectra (Fig. 2). We are now investigating the reason why the epimerization occurred during the palladium catalyzed reduction of **14**. Selective iodination of **15** using trifluoroacetic acid (TFA) as a solvent successfully proceeded to afford **16**. The position of iodo group was determined by HMBC spectra (Fig. S2). After halogen-lithium exchange reaction of **16** with *n*-butyl lithium, treatment with triisopropyl borate ($B(O\textit{i}\text{-}Pr)_3$) and subsequently with sodium perborate tetrahydrate ($NaBO_3 \cdot 4H_2O$) yielded phenol **17** (39%) concomitantly with **15** (47%). The objective mesembrane (**4**) was obtained by methylation of phenolic hydroxy group in **17** with trimethylsilyldiazomethane ($TMSCHN_2$). The observed spectral data of prepared **4** were identical to the literature data.[18] For the binding assays, *O*-methyl groups in **13**, **15**, and *epi*-**15** were deprotected to give the corresponding **13′**, **15′**, and *epi*-**15′** (Scheme 4).



Scheme 4 Synthesis of mesembrane (**4**) from **6**. Reagents and conditions: (a) $H_2NNH_2 \cdot H_2O$, KOH, ethylene glycol, **12**: 73%, **13**: 25%, (b) BBr_3, CH_2Cl_2, **13'**: 23%, **15'**: 62%, *epi*-**15'**: 87%, (c) H_2, Pd/C, AcOH, (d) Tf_2NPh, NaH, DME, 83% from **12**, (e) $Pd(MeCN)_2Cl_2$ (10 mol%), dppp (25 mol%), HCO_2H, TEA, DME, **15**: 40%, *epi*-**15**: 24%, (f) NIS, TFA, 85%, (g) *n*-BuLi, THF, then $B(Oi\text{-}Pr)_3$, $NaBO_3 \cdot 4H_2O$, **17**: 39%, **15**: 47%, (h) $TMSCHN_2$, MeOH, 81%

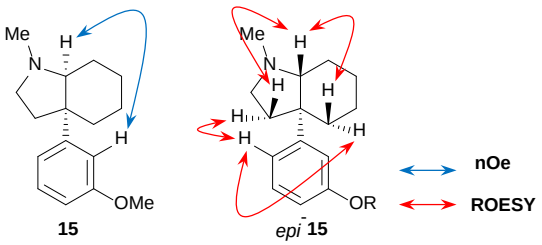


Fig. 2 Observed nOe or ROESY spectra of **15** and *epi*-**15**

Binding affinities for the opioid receptors

The binding abilities of mesembrane (**4**), **13**, **13′**, **15**, **15′**, *epi*-**15**, and *epi*-**15′** for the opioid receptors

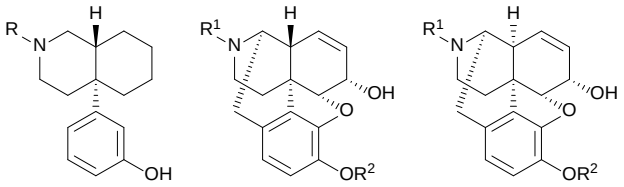
were evaluated by competitive binding assays (Tables 1 and S1). Although the binding affinities of **13′** were very weak, the compounds **13′**, **15′**, and *epi*-**15′** with phenolic hydroxy group bound to the opioid receptors with sufficient binding affinities, and **15′** and *epi*-**15′** showed the same rank order of the binding abilities to the opioid receptor types: MOR > KOR > DOR. Compound **15′** most strongly bound to the opioid receptors and its binding affinity for the MOR was highest (K_i = 90.0 nM) among for the three opioid receptor types. Compound **13**, **15**, *epi*-**15** with methoxy group exhibited lower binding abilities than did the corresponding compounds **13′**, **15′**, *epi*-**15′** with the phenolic hydroxy group, respectively. It is well-known that the phenolic hydroxy group is the important determinant for binding to the opioid receptor[13] and that morphinan derivatives with the phenolic hydroxy group like naltrexone (**5**) is stronger binder than the corresponding derivatives with the methoxy group. These results suggested that the tested compound would bind to the opioid receptors with the same binding mode as that of the morphinan derivatives. The binding affinities of **15′** were sufficient but lower than those of naltrexone (**5**), which was sometimes used as a message structure to provide novel opioid ligands. The message structure is the responsible moiety to exert opioid activities according to the message-address concept,[19] which is a useful guideline to design ligands selective for the opioid receptor types. Therefore, compound **15′** was expected to be used as a novel message structure with the skeleton distinct from morphinans to provide characteristic opioid ligands. Both the introduction of the epoxy bridge (compound **13′**) and *trans*-fusion of the hydroindole ring system (compound *epi*-**15′**) decreased the binding affinities. The binding affinities of (-)-**18** and (-)-**19** with freely rotatable phenyl ring were reported to be lower or comparable to morphine (**20**) and nalorphine (**21**).[20] Although no binding affinity was reported, the analgesic effects by **20** and codeine (**22**) with *trans*-fused hydroisoquinoline structure were known to be stronger than those of **23** and **24** possessing *cis*-fused ring system.[21] It is interesting that the structure-activity relationship of the tested compounds with hydroindole structure was completely different from that of morphinan derivatives with hydroisoquinoline fundamental skeleton. The binding affinities of mesembrane (**4**) were extremely

low as well as the compounds **13**, **15**, *epi*-**15** with methoxygroup. The extremely low affinities would partly result from the fact that **4** had no phenolic hydroxy group. It is difficult to compare the affinities of **4** with those of *Sceletium tortuosum* Zembrin® because Harvey *et al.* did not report the K_i values of *Sceletium tortuosum* Zembrin®.[11] Concerning compound **4**, the not-replaced bindings of the labeled compounds were 82.7%, 92.3%, and 93.0% for the MOR, DOR, and KOR, respectively (Table S3) at 10 μM of **4**, which was the highest concentration of the tested compounds in our binding assays. On the other hand, the affinities of *Sceletium tortuosum* Zembrin® were evaluated at 750 μg/mL, which was calculated to be about 2.6 mM. Under these assessed conditions, the not-replaced bindings of the labeled compounds were about 10-30% (see the supplementary information for more detailed discussion).[11]

Table 1 Binding affinities for the opioid receptors[a]

Compound	K_i (nM)		
	MOR[b]	DOR[c]	KOR[d]
mesembrane (**4**)	9,102	16,230	5,800
naltrexone (**5**)	0.27	12.3	0.70
13	ND[e]	ND[e]	ND[e]
13′	2,151	2,363	769.8
15	5,533	19,130	4,775
15′	90.0	1,149	163.2
epi-**15**	10,650	42,380	48,940
epi-**15′**	494.7	6,627	584.7

[a] Binding assays were carried out in duplicate using human MOR, DOR, or KOR recombinant cell (CHO) membranes. [b] [^{3}H] DAMGO was used. [c] [^{3}H] DPDPE was used. [d] [^{3}H] U-69,593 was used. *e* Not determined. The highest concentration of the tested compound was 10 μM.



(-)-**18** (R = Me)
(-)-**19** (R = allyl)

morphine (**20**)
(R^1 = Me, R^2 = H)
nalorphine (**21**)
(R^1 = allyl, R^2 = H)
codeine (**22**)
(R^1 = R^2 = Me)

23 (R^1 = Me, R^2 = H)
24 (R^1 = R^2 = Me)

Fig. 3 Structures of compounds (-)-**18 - 24**

Conformational analysis

To examine the difference of the binding affinities among the compounds **13′**, **15′**, and *epi*-**15′**, we carried out conformational analyses of these protonated compounds using Conformational Analyzer with Molecular Dynamics And Sampling (CAMDAS) 2.1 program (Fig. 4, see the supplementary information for details).[22] We considered two different stereoisomers with *R*- or *S*-configuration at the protonated nitrogen atom for each compound. Interestingly, the most stable conformations of **13′**, **15′**, and *epi*-**15′** were found to possess *S*-, *S*-, and *R*- configurations at the protonated nitrogen atom, respectively. As a result, we could more frequently see an isomer with *S*-configuration for **13′** and **15′** and one of *R*-configuration for *epi*-**15′** among lower-energy conformers, but both configurations for each compound were observed within 10 kcal/mol of the most stable conformer (Table 4S). The axial orientation of the N-H bond in the protonated compounds, was proposed to promote effective binding to the opioid receptor *via* the directional enforced ionic bond which was an ionic bond reinforced by the directional hydrogen bond.[23] Therefore, we paid attention to the conformers with *S*-configuration at the nitrogen atom, in which the N-H bond was directed to axial. Fig. 4 includes superimpositions of the low-energy conformers with *S*-configuration for **13′**, **15′**, and *epi*-**15′**, in which the phenol ring was superposed. Although the spatial disposition of nitrogen atom was tightly restricted for **13′**, those of **15′** and *epi*-**15′** were widely spread. The bound conformation of β-funaltrexamine (β-FNA, irreversible

MOR antagonist, Fig. 4 (G)) in the complex with MOR[24] was also shown in Figs. 4 (H) and 4 (I). From a comparison of Figs. 4 (B) and 4 (H), the nitrogen atoms of many low-energy conformers of **15′** adopted in a region indicated by green circle, in which the basic nitrogen of the bound conformer of β-FNA existed. The basic nitrogen is well-known to provide a pharmacophoric interaction with the opioid receptor.[13] These results suggested that **15′** could bind to the opioid receptors with the same binding mode as that of morphinans and give the strongest binding affinities among these three compounds. The compound *epi*-**15′** also had low-energy conformers with the basic nitrogen placing in a desired region (green circle), but the population of the adequate low-energy conformers was much less than that of **15′** (Fig. 4(C)). These observations reasonably suggested compound *epi*-**15′** possessed less binding affinities than **15′**. Contrarily, compound **13′**, which very weakly bound to the opioid receptors, had almost no conformers having the basic nitrogen locating in a proper space. Fig. 4(J), which pictured superimpositions of **13′**, **15′**, *epi*-**15′**, and β-FNA, clearly indicated these observation mentioned above.

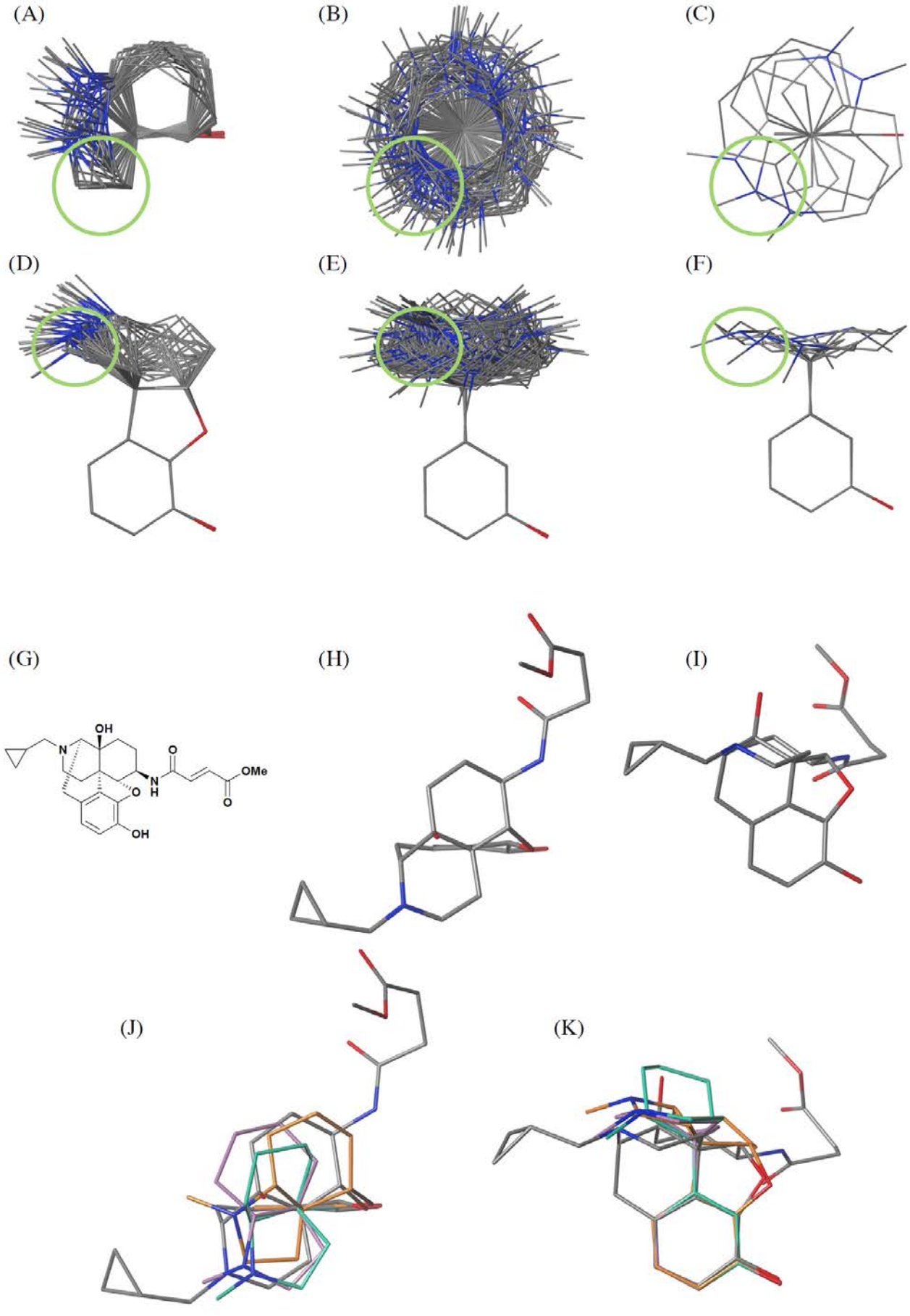

(A)
(B)
(C)
(D)
(E)
(F)
(G)
OH
N
O
N
H
OMe
O
O
OH
(H)
(I)
(J)
(K)

Fig. 4 Results of conformational analyses of the protonated **13′**, **15′**, and *epi*-**15′**, and structure of β-FNA. Structures within 10 kcal/mol of the most stable conformer were collected for the protonated **13′**, **15′**, and *epi*-**15′**. (A) top view of conformers of protonated **13′**. The green circles were indicated an area around the relative position of the basic nitrogen against the phenol ring in β-FNA. (B) top view of conformers of protonated **15′**, (C) top view of conformers of protonated *epi*-**15′**, (D) side view of conformers of protonated **13′**, (E) side view of conformers of protonated **15′**, (F) side view of conformers of protonated *epi*-**15′**, (G) structure of β-FNA, The structure of β-FNA was drew using the coordinates obtained in the X-ray crystallographic analyses of the β-FNA-MOR complex,[24] in which β-FNA bound to the MOR through a covalent bond provided by Michael addition of Lys 233 in the MOR with the α,β-unsaturated ester moiety in β-FNA. (H) top view of conformer of β-FNA, (I) side view of conformer of β-FNA. (J) top view of superimpositions of **13′**, **15′**, *epi*-**15′**, and β-FNA. The indicated conformer of each compound was selected whose nitrogen atom was located in the closest space to that of β-FNA. Compounds **13′**, **15′**, and *epi*-**15′** were indicated by orange, green, and light purple, respectively. (K) side view of superimpositions of **13′**, **15′**, *epi*-**15′**, and β-FNA.

Conclusion

We transformed naltrexone (**5**) with morphinan skeleton into mesembrane (**4**) belonging to the *Sceletium* alkaloids *via* key intermediate **6** to investigate the binding affinities of **4** and the key intermediate **6** derivatives to the opioid receptors. Compound **15′**, which had *cis*-fused hydroindole skeleton, exhibited strong to moderate binding affinities for the MOR and KOR. Although compound *epi*-**15′**, which was *trans*-fused congener of **15′**, also moderately bound to the MOR and KOR, the affinities of *epi*-**15′** were lower than those of **15′**. In contrast to compounds **15′** and *epi*-**15′**, compound **13′** showed almost no binding abilities. The structure-activity relationship of the tested compounds **13′**, **15′**, and *epi*-**15′** with hydroindole structure was completely different from that of morphinan derivatives with hydroisoquinoline fundamental skeleton. The observed difference of binding affinities among the tested compounds was reasonably explained by the conformational analyses and by the comparison of the relative relationship of the spacial locations between the protonated nitrogen and phenol ring among the tested compounds and β-FNA. Compound **15′** with a different structure from morphinans like naltrexone (**5**) was expected to be a useful message part with a novel chemotype to provide characteristic opioid ligands.

Experimaental

General information

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were determined on a Yanako MP-500P melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a JASCO FT/IR-460Plus. Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent Technologies VXR-400NMR for ^{1}H NMR and ^{13}C NMR. Chemical shifts were reported as δ values (ppm) referenced to the deuterated solvent ($CHCl_3$ or pyridine). Mass spectra (MS) were obtained on a JMS-AX505HA, JMS-700 MStation, or JMS-100LP instrument by applying an electrospray ionization (ESI) method. Elemental analyses were determined with a Yanako MT-5 and JM10 for carbon, hydrogen, and nitrogen. The progress of the reaction was determined on Merck Silica Gel Art. 5715 (TLC) visualized by exposure to ultraviolet light or with sodium phosphomolybdate-ethanol, anisaldehyde-sulfuric acid-ethanol, or ninhydrin-citric acid buffer-butanol stain. Column chromatographies were carried out using Kanto Silica Gel 60 N (60–230 μm), Fuji Silysia CHROMATOREX® PSQ 60B (60 μm), or Fuji Silysia CHROMATOREX® NH-DM2035 (60 μm).

Synthesis

(3a*R*,3a^{1}*S*,9a*R*)-5-methoxy-3a^{1}-(2-(methyl((2,2,2-trichloroethoxy)carbonyl)amino)ethyl)-1,2,3a,3a^{1}-tetrahydro-9a*H*-spiro[phenanthro[4,5-*bcd*]furan-3,2'-[1,3]dioxolan]-9a-yl acetate (8)

Under an Ar atmosphere, to a solution of naltrexone (**5**) hydrochloride (32.3 g, 85.5 mmol) in DMF (323 mL) were added K_2CO_3 (29.6 g, 214 mmol) and MeI (6.9 mL, 104 mmol), and stirred at room temperature for 3 h. The reaction mixture was poured into distillated water and extracted with Et_2O. The combined organic layers were washed with brine and concentrated under reduced pressure to give a solid material (32.82 g). The obtained material was used in the next reaction without purification.

Under an Ar atmosphere, to a solution of the obtained material (32.82 g) in DMF (324 mL) was added

MeI (58 mL, 874 mmol) and stirred at 130 °C for 7 h. After removing solvent under reduced pressure, to the residue were added 1,4-dioxane (320 mL) and 1 *M* aqueous solution of NaOH (325 mL) and stirred at 80 °C for 4 h. Cooled reaction mixture was poured into distillated water and extracted with $CHCl_3$. The combined organic layers were concentrated under reduced pressure to give crude product of **7** (31.97 g) as an oil. The obtained crude product was used in the next reaction without purification.

Under an Ar atmosphere, to a solution of the crude **7** (31.97 g) in $CHCl_3$ (636 mL) were added ethylene glycol (25.5 mL, 457 mmol) and TMSOTf (17.0 mL, 93.9 mmol), and stirred at 50 °C for 4 h. Cooled reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layers were concentrated under reduced pressure to give an oil (39.33 g). The obtained oil was used in the next reaction without purification.

Under an Ar atmosphere, a solution of the obtained oil (39.33 g) in Ac_2O (354 mL) was stirred at 80 °C for 4 h. The solvent was removed under reduced pressure and then further removed under azeotropic conditions with toluene. The solution of the residue in $CHCl_3$ was washed with saturated aqueous solution of $NaHCO_3$ and concentrated under reduced pressure to give an oil (42.24 g). The obtained oil was used in the next reaction without purification.

Under an Ar atmosphere, to a solution of the obtained oil (42.24 g) in CH_2Cl_2 (800 mL) were added Troc-Cl (23.6 mL, 171 mmol) and Et_3N (35.7 mL, 256 mmol) at 0 °C, and stirred at the same temperature for 5 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layers were concentrated under reduced pressure. The residue was purified by a silica gel column chromatography (hexane/AcOEt = 2/1) to give **8** (35.44 g, 61.4 mmol, 72% from **5** hydrochloride) as a colorless amorphous material.

MS (ESI): $[M+Na]^+$ m/z = 598.

HR-MS (ESI): $[M+Na]^+$ calcd for $C_{25}H_{28}Cl_3NNaO_8$: 598.07486, found: 598.07782.

IR (film): 1720, 1226, 1167, 1040 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ1.39-1.50 (m, 1H), 1.55-1.67 (m, 2H), 1.87-1.98 (m, 0.35H), 1.98-2.24

(m, 2H), 2.09 (s, 1.95H), 2.13 (s, 1.05H), 2.78-2.91 (m, 1.7H), 2.79 (s, 1.05H), 2.82 (s, 1.95H), 3.13-3.27 (m, 0.65H), 3.53 (ddd, *J* = 4.5, 13.0, 13.4 Hz, 0.65H), 3.79-3.94 (m, 2H), 3.88 (s, 3H), 4.04 (dd, *J* = 6.6, 13.0 Hz, 1H). 4.17-4.27 (m, 1H), 4.50 (d, *J* = 12.0 Hz, 0.65H), 4.69 (d, *J* = 12.0 Hz, 0.35H), 4.70 (d, *J* = 12.0 Hz, 0.35H), 4.72-4.82 (m, 1.65H), 6.31 (d, *J* = 9.8 Hz, 1H), 6.42 (d, *J* = 9.8Hz, 0.65H), 6.46 (d, *J* = 9.8 Hz, 0.35H), 6.58-6.74 (m, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.4, 26.9, 27.2, 27.4, 32.1, 32.5, 33.9, 34.9, 45.6, 46.2, 51.2, 56.2, 65.1, 66.56, 66.59, 74.86, 74.90, 86.5, 86.9, 95.0, 95.3, 95.5, 95.7, 107.8, 113.03, 113.08, 118.2, 118.3, 122.1, 122.2, 124.4, 124.5, 127.0, 127.5, 128.7, 128.9, 144.16, 144.19, 146.02, 146.05, 154.05, 154.10, 169.9, 170.2.

2,2,2-Trichloroethyl (2-((1*S*,4a*R*,9b*S*)-1,9-diformyl-1-hydroxy-6-methoxy-2,3,4a,9b-tetrahydro-1*H*-spiro[dibenzo[*b*,*d*]furan-4,2'-[1,3]dioxolane]-9b-yl)ethyl)(methyl)carbamate (9)

Ozone was bubbled into a solution of **8** (10.23 g, 17.7 mmol) in CH_2Cl_2 (1000 mL) at -78 ˚C for 1 h. After drastic bubbling of N_2 gas for 30 min, Me_2S (25 mL, 338 mmol) was added to the reaction mixture at the same temperature, and then stirred at room temperature for 12 h. After removing the solvent under reduced pressure, to a solution of the residue in MeOH (100 mL) was added Et_3N (100 mL) and stirred for 4 h. The residue obtained by removing the solvent under reduced pressure was purified by silica gel column chromatography ($CHCl_3$/AcOEt = 5/1) to give **9** (7.31 g, 12.9 mmol, 73%) as a colorless amorphous material.

MS (ESI): $[M+Na]^+$ m/z = 588.

HR-MS (ESI): $[M+Na]^+$ calcd for $C_{23}H_{26}Cl_3NNaO_9$: 588.05708, found: 588.05482.

IR (film): 2952, 1715, 1605, 1287, 757 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.75-1.92 (m, 2H), 2.00-2.18 (m, 1H), 2.23-2.49 (m, 2H), 2.74-3.16 (m, 5H), 3.28-3.43 (m, 1H), 3.91 (s, 3H), 4.04-4.22 (m, 4H), 4.42-4.76 (m, 3H), 4.93 (s, 0.4H), 4.95 (s, 0.6H), 6.90 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 0.6H), 7.44 (d, *J* = 8.5 Hz, 0.4H), 9.316 (s,

0.6H), 9.324 (s, 0.4H), 9.85 (s, 0.6H), 9.87 (s, 0.4H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.1, 26.7, 27.9, 28.0, 28.6, 28.7, 34.1, 34.8, 45.6, 46.3, 56.0, 56.2, 65.17, 65.21, 65.5, 74.8, 75.0, 78.8, 78.9, 85.8, 87.0, 95.7, 106.16, 106.23, 111.57, 111.63, 127.1, 127.3, 128.9, 129.0, 129.6, 129.67, 129.73, 148.2, 148.4, 1507., 153.9, 154.1, 191.2, 191.6.

2,2,2-Trichloroethyl (2-((4a*R*,9b*S*)-9-hydroxy-6-methoxy-1-oxo-2,3,4a,9b-tetrahydro-1H-spiro[dibenzo[*b*,*d*]furan-4,2'-[1,3]dioxolane]-9b-yl)ethyl)(methyl)carbamate (10)

Under an Ar atmosphere, to a solution of **9** (32.96 g, 58.3 mmol) in CH_2Cl_2 (662 mL) was added 77% *m*CPBA acid (27.4 g, 122 mmol) and refluxed with stirring for 4 h. To the cooled reaction mixture were added saturated aqueous solutions of $Na_2S_2O_3$ and $NaHCO_3$, and extracted with $CHCl_3$. After the combined organic layers was concentrated under reduced pressure, to a solution of the residue in MeOH (322 mL) was added Et_3N (326 mL) and stirred at room temperature for 6 h. The residue obtained by removing the solvent under reduced pressure was purified by silica gel column chromatography (hexane/AcOEt = 2/3) to give **10** (10.96 g, 20.9 mmol, 36%) as a colorless oil.

MS (ESI): $[M+Na]^+$ m/z = 546.

HR-MS (ESI): $[M+Na]^+$ calcd for $C_{21}H_{24}Cl_3NNaO_8$: 546.04652, found: 546.04505.

IR (film): 3303, 2952, 1715, 1505, 1163, 1033 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.97-2.07 (m, 1H), 2.08-2.38 (m, 3H), 2.66-2.82 (m, 2H), 2.87-3.08 (m, 4H), 3.36-3.49 (m, 1H), 3.79 (s, 3H), 3.86-4.16 (m, 3H), 4.20-4.31 (m, 1H), 4.58-4.88 (m, 3H), 6.40 (d, *J* = 8.8 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 1H), 7.70 (s, 0.45H), 7.82 (s, 0.55H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.3, 33.8, 34.4, 34.7, 35.5, 35.7, 44.9, 45.5, 56.67, 56.72, 61.7, 61.8, 65.7, 65.9, 66.2, 66.4, 75.1, 87.0, 87.8, 95.6, 106.3, 109.7, 109.8, 112.5, 112.9, 115.18, 115.24, 138.3, 138.4, 147.8, 148.1, 153.9, 154.3.

(3a*S*,6a*R*,11b*R*)-8-methoxy-3-methyl-1,2,3,3a,4,5-hexahydro-6a*H*-spiro[benzofuro[3,2-*d*]indole-

6,2'-[1,3]dioxolan]-11-ol (11)

Under an Ar atmosphere, to a solution of **10** (10.96 g, 20.9 mmol) in AcOH (218 mL) was added zinc powder (6.90 g, 105 mmol) and stirred at room temperature for 2 h. The reaction mixture was filtered through Cerite pad and to the obtained filtrate was added $NaBH_3CN$ (3.95 g, 62.9 mmol) at 0 ˚C, and then stirred at the same temperature for 17 h under an Ar atmosphere. After removing the solvent under reduced pressure, a solution of the residue in $CHCl_3$ was poured in a saturated aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. The residue obtained by concentration of the combined organic layers under reduced pressure was purified by silica gel (Fuji Silysia CHROMATOREX® NH-DM2035) column chromatography (toluene/$CHCl_3$ = 2/1) to give **11** (6.42 g, 19.3 mmol, 92%) as a colorless amorphous material.

MS (ESI): $[M+H]^+$ m/z = 334.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{18}H_{24}NO_5$: 334.16545, found: 334.16811.

IR (film): 2953, 2897. 2830, 1495, 1267, 1205, 1031cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.56-1.80 (m, 2H), 1.80-1.88 (m, 1H), 2.17-2.27 (m, 1H), 2.41 (s, 3H), 2.44-2.55 (m, 1H), 2.64-2.74 (m, 1H), 3.15-3.24 (m, 1H), 3.77 (s, 3H), 3.91-4.13 (m, 4H), 4.34 (s, 1H), 6.31 (d, *J* = 8.7 Hz, 1H), 6.61 (d, *J* = 8.7 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.2, 28.4, 35.6, 36.0, 50.2, 55.3, 56.6, 65.3, 65.8, 66.9, 88.6, 107.3, 108.7, 113.2, 123.4, 137.5, 146.9, 148.1.

Mp: 151 ºC (dec.).

(3a*S*,6a*R*,11b*R*)-8-methoxy-3-methyl-1,2,3,3a,4,5-hexahydrobenzofuro[3,2-*d*]indol-6(6a*H*)-one (6)

Under an Ar atmosphere, to a solution of **11** (1.92 g, 5.76 mmol) in DME (58 mL) was added NaH (55% in oil, 379 mg, 8.69 mmol) at 0 ºC and stirred at the same temperature for 30 min. Tf_2NPh (3.12 g, 8.73 mmol) was added to the reaction mixture and stirred at room temperature for 3 h. To the reaction

mixture were added Et_3N (9.7 mL, 69.6 mmol) and HCO_2H (1.75 mL, 46.4 mmol) at 0 °C, subsequently added dppp (600 mg, 1.45 mmol) and $Pd(MeCN)_2Cl_2$ (150 mg, 0.578 mmol) at room temperature, and stirred at 80 °C for 7 h. To the reaction mixture were added Et_3N (6.0 mL, 43.0 mmol) and HCO_2H (1.0 mL, 26.5 mmol) at room temperature and stirred at 80 °C for 10 h. Further HCO_2H (1.0 mL, 26.5 mmol) was added to the reaction mixture at room temperature and stirred at 80 °C for 4 h. Cooled reaction mixture was poured into distillated water and extracted with AcOEt. The combined organic layers were concentrated under reduced pressure. A solution of the residue in AcOEt was filtered through short amine silica gel (CHROMATOREX® NH-DM2035). The solvent was removed under reduced pressure to give an oil (1.80 g). The obtained oil was used in the next reaction without purification.

A solution of the obtained oil (1.80 g) in 1 *M* HCl (10 mL) was stirred at 100 °C for 5 h. After cooling, the reaction mixture was basified (pH 9) with a saturated aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. After concentration, the residue was purified by a silica gel column chromatography (hexane/AcOEt = 2/1) to give **6** (1.24 g, 4.54 mmol, 79% from **11**) as a colorless amorphous material.

MS (ESI): $[M+H]^+$ m/z = 273.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{20}NO_3$: 274.14432, found: 274.14220.

IR (film): 1719, 1491, 1459, 1292, 1201, 1126 cm^{-1}.

^1H NMR (pyridine-d_5, 600 MHz): δ 1.74-1.86 (m, 2H), 1.92 (ddd, *J* = 7.8, 8.9, 13.3 Hz, 1H), 2.10 (s, 3H), 2.15-2.18 (m, 1H), 2.21 (ddd, *J* = 3.1, 9.8, 13.3 Hz, 1H), 2.27 (ddd, *J* = 7.8, 9.1, 9.8 Hz, 1H), 2.44 (ddd, *J* = 1.9, 7.1, 17.5 Hz, 1H), 2.53 (ddd, *J* = 8.2, 11.4, 17.5 Hz, 1H), 3.04 (ddd, *J* = 3.1, 8.9, 9.1 Hz, 1H), 3.82 (s, 3H), 4.64 (s, 1H), 6.86 (d, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 7.05 (dd, *J* = 7.5, 8.0 Hz, 1H).

^{13}C NMR (pyridine-*d*5, 150 MHz): δ 20.0, 32.0, 36.2, 38.7, 54.6, 56.3, 60.4, 69.1, 93.8, 113.5, 115.9, 123.1, 132.9, 145.6, 148.6.

Mp: 122-124 ˚C.

2-methoxy-6-((3a*R*,7a*S*)-1-methyl-1,2,3,6,7,7a-hexahydro-3a*H*-indol-3a-yl)phenol (12) and (3a*S*,6a*S*,11b*R*)-8-methoxy-3-methyl-1,2,3,3a,4,5,6,6a-octahydrobenzofuro[3,2-*d*]indole (13)

Under an Ar atmosphere, to a solution of **6** (1.61 g, 5.90 mmol) in ethylene glycol (58 mL) was added $H_2NNH_2 \cdot H_2O$ (1.45 mL, 29.9 mmol), and stirred at 80 ^oC for 2 h. Potassium hydroxide (2.33 g, 41.5 mmol) was added to the reaction mixture at room temperature and stirred at 130 ^oC for 23 h. Cooled reaction mixture was poured into a saturated aqueous solution of NH_4Cl (2.37 g, 44.3 mmol) and extracted with $CHCl_3$. After concentration, the residue was purified by a silica gel column chromatography ($CHCl_3$/MeOH = 40/1 → 5/1 → 2/1) to give **12** (1.12 g, 4.32 mmol, 73%) as a colorless oil and **13** (379 mg, 1.46 mmol, 25%) as a colorless crystal.

12: MS (ESI): $[M+H]^+$ m/z = 260.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{22}NO_2$: 260.16505, found: 260.16360.

IR (film): 3521, 2935, 1469, 1257cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.50-1.62 (m, 1H), 1.63-1.72 (m, 1H), 1.80-1.98 (m, 2H), 2.07-2.18 (m, 1H), 2.46 (s, 3H), 2.57-2.63 (m, 1H), 2.99 (dd, *J* = 7.1, 7.3 Hz, 2H), 3.16 (dd, *J* = 4.2, 9.0 Hz, 1H), 3.87 (s, 3H), 5.89-5.97 (m, 2H), 6.67 (dd, *J* = 8.0, 8.0 Hz, 1H), 6.76 (dd, *J* = 1.5, 8.0 Hz, 1H), 6.86 (dd, *J* = 1.5, 8.0 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.8, 20.7, 36.8, 38.0, 48.4, 52.5, 55.8, 67.3, 109.3, 117.1, 119.7, 125.3, 133.97, 134.05, 145.7, 148.6.

13: MS (ESI): $[M+H]^+$ m/z = 260.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{22}NO_2$: 260.16505, found: 260.16545.

IR (film): 2936, 2778, 1493, 1459, 1292, 1201, 1027 cm^{-1}.

^{1}H NMR (pyridine-d_5, 400 MHz): δ 1.26-1.40 (m, 2H), 1.48-1.72 (m, 3H), 1.86 (ddd, *J* = 3.9, 9.6, 13.3 Hz, 1H), 1.92-2.04 (m, 2H), 2.18 (s, 3H), 2.35 (ddd, *J* = 7.7, 9.0, 9.6 Hz, 1H), 2.40-2.45 (m, 1H), 3.02 (ddd, *J* = 3.9, 8.8, 9.0, 1H), 3.80 (s, 3H), 4.57 (dd, *J* = 5.0, 6.9 Hz, 1H), 6.90-6.94 (m, 2H), 6.99 (dd, *J*

= 6.6, 8.6 Hz, 1H).

^{13}C NMR (pyridine-d_5, 100 MHz): δ 16.0, 22.1, 27.0, 37.8, 39.7, 54.2, 54.8, 56.2, 68.6, 89.7, 113.0, 116.0, 121.9, 136.6, 145.7, 148.2.

Mp: 72-73 ˚C.

13·hydrochlorode: Anal. Calcd for $C_{15}H_{19}NO_2$·HC·0.2H_2O: C, 63.13; H, 7.20; N, 4.91, found: C, 62.99; H, 7.03; N, 4.93.

Mp: 248 ˚C (dec.).

2-methoxy-6-((3a*S*,7a*S*)-1-methyloctahydro-3a*H*-indol-3a-yl)phenyl trifluoromethanesulfonate (14)

Under an Ar atmosphere, to a solution of **12** (111.6 mg, 430.9 μmol) in AcOH (2.3 mL) was added 10% Pd on carbon (33.7 mg), and after exchange of Ar into H_2 stirred at 80 ^{o}C for 21 h. Cooled reaction mixture was filtered on Cerite pad and washed with MeOH. After concentration of the filtrate, the residue was dissolved with $CHCl_3$. The obtained solution was poured into a saturated aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layers were concentrated under reduced pressure. A solution of the residue in hexane/AcOEt (1/3) was filtered through short amine silica gel (CHROMATOREX® NH-DM2035). The solvent was removed under reduced pressure to give dark brown solid (108.8 mg). The obtained solid was used in the next reaction without purification.

Under an Ar atmosphere, to a solution of the obtained solid (108.8 mg) in DME (2.0 mL) was added NaH (55% in oil, 56.0 mg, 1.28 mmol) at 0 ^{o}C and stirred at the same temperature for 20 min. To the reaction mixture was added Tf_2NPh (300 mg, 0.839 mmol) at 0 ^{o}C and stirred at the same temperature for 25 min, followed by addition of Tf_2NPh (150 mg, 0.420 mmol) with stirring at room temperature for 2 h. The reaction mixture was poured into 2 *M* aqueous solution of NaOH and extracted with AcOEt. After concentration, the residue was purified by a preparative TLC (AcOEt) to give **14** (140.4 mg, 0.357 mmol, 83% from **12**) as a colorless crystal.

MS (ESI): $[M+H]^+$ m/z = 394.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{17}H_{23}F_3NO_4S$: 394.12999, found: 394.12743.

IR (film): 2939, 1411, 1203, 1125, 883, 748 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 0.82-0.95 (m, 1H), 1.35-1.60 (m, 3H), 1.75-1.99 (m, 4H), 2.11-2.24 (m, 2H), 2.29 (s, 3H), 2.32-2.46 (m, 1H), 2.49-2.53 (m, 1H), 3.23 (ddd, *J* = 3.3, 9.2, 9.2 Hz, 1H), 3.86 (s, 3H), 6.91 (dd, *J* = 1.4, 8.2 Hz, 1H), 7.06 (dd, *J* = 1.4, 8.2 Hz, 1H), 7.23 (dd, *J* = 8.2, 8.2 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.6, 23.1, 24.0, 35.5, 38.0, 40.4, 46.7, 54.7, 55.6, 67.9, 110.7, 119.1 (ddd, J = 322.4 Hz, 1C), 120.8, 127.3, 138.6, 141.7, 150.9.

Mp: 88-90 ˚C.

(3a*S*,7a*S*)-3a-(3-methoxyphenyl)-1-methyloctahydro-1*H*-indole (15) and (3a*S*,7a*R*)-3a-(3-methoxyphenyl)-1-methyloctahydro-1*H*-indole (*epi*-15)

Under an Ar atmosphere, a solution of $Pd(MeCN)_2Cl_2$ (8.6 mg, 33.1 μmol) and dppp (32.0 mg, 77.6 μmol) in DME (0.5 mL) was stirred at room temperature for 1 h. To the reaction mixture were added a solution of **14** (111.4 mg, 308.4 μmol) in DME (1.8 mL), Et_3N (0.4 mL, 2.87 mmol), and HCO_2H (0.07 mL, 1.85 mmol), and stirred at 100 ºC for 37 h in a sealed tube. To the reaction mixture were added Et_3N (0.4 mL, 2.87 mmol) and HCO_2H (0.07 mL, 1.85 mmol) and further stirred at 100 ºC for 8 days. Cooled reaction mixture was poured into 2 *M* aqueous solution of NaOH and extracted with AcOEt. After removing the solvent, a solution of the residue in $CHCl_3$ was filtered through short amine silica gel (CHROMATOREX® NH-DM2035). The filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (AcOEt) to give **15** (30.2 mg, 0.123 mmol, 40%) as a colorless oil and *epi*-**15** (17.9 mg, 0.073 mmol, 24%) as a colorless oil.

15: MS (ESI): $[M+H]^+$ m/z = 246.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{24}NO$: 246.18579, found: 246.18405.

IR (film): 2933, 1607, 1581, 1448, 1246, 1053, 701 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.09-1.22 (m, 1H), 1.33-1.40 (m, 1H), 1.44-1.68 (m, 3H), 1.77-2.02 (m, 5H), 2.27-2.35 (m, 1H), 2.34 (s, 3H), 2.61-2.66 (m, 1H), 3.28 (ddd, *J* = 4.5, 9.8, 9.8 Hz, 1H), 3.81 (s, 3H), 6,73 (ddd, *J* = 0.7, 2.5, 8.0 Hz, 1H), 6.94 (dd, *J* = 2.1, 2.5 Hz, 1H), 6.97 (ddd, *J* = 0.7, 2.1, 8.3 Hz, 1H), 7.24 (dd, *J* = 8.0, 8.3 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.3, 22.8, 23.6, 35.9, 40.6, 40.8, 47.8, 54.3, 55.0, 68.5, 109.8, 113.7, 119.3, 128.9, 149.4, 159.3.

15·hydrochlorode: Anal. calcd for $C_{16}H_{23}NO{\cdot}HCl{\cdot}0.15H_2O$: C, 67.54; H, 8.61; N, 4.92, found: C67.44, ; H, 8.57; N, 4.91.

Mp: 224 ˚C (dec.).

epi-**15**: MS (ESI): $[M+H]^+$ m/z = 246.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{24}NO$: 246.18579, found: 246.18459.

IR (film): 2935, 1578, 1455, 1240, 1051, 709 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.12 (dddd, *J* = 3.8, 3.9, 13.3, 13.4 Hz, 1H), 1.25-1.35 (m, 1H), 1.37 (ddd, *J* = 3.5, 13.2, 13.3 Hz, 1H), 1.43-1.48 (m, 1H), 1.64-1.81 (m, 5H), 2.16-2.30 (m, 2H), 2.37 (s, 3H), 2.60-2.65 (m, 1H), 3.04 (dd, *J* = 8.4, 17.9 Hz, 1H), 3.79 (s, 3H), 6.70 (dd, *J* = 2.6, 8.1 Hz, 1H), 7.21 (dd, *J* = 7.9, 8.1 Hz, 1H), 7.37 (br d, *J* = 7.9 Hz, 1H), 7.52 (br s, 1H).

^{13}C NMR ($CDCl_3$, 150 MHz): δ 22.3, 24.1, 25.7, 38.1, 39.8, 41.6, 49.3, 53.2, 55.1, 109.6, 115.8, 121.2, 128.7, 147.4, 159.3.

epi-**15**·hydrochlorode: Anal. calcd for $C_{16}H_{23}NO{\cdot}HCl{\cdot}1.2H_2O$: C, 63.33; H, 8.77; N, 4.62, found: C, 63.25; H, 8.68; N, 4.38.

Mp. was not able to be measured due to high hygroscopicity.

(3a*S*,7a*S*)-3a-(4-iodo-3-methoxyphenyl)-1-methyloctahydro-1*H*-indole (16)

Under an Ar atmosphere, to a solution of **15** (125.1 mg, 510.6 μmol) in TFA (2.5 mL) was added NIS (114.7 mg, 509.8 μmol) at 0 ^{o}C and the reaction temperature was gradually raised to room temperature

with stirring for 24 h. After removing the solvent under reduced pressure, to the residue was added saturated aqueous solution of $Na_2S_2O_3$ (1 mL) and basified (pH 9) with 1 *M* aqueous solution of NaOH, and then extracted with $CHCl_3$. After concentration, the residue was purified by preparative TLC (chloroform/methanol = 20/1) to give **16** (160.6 mg, 433 mmol, 85%) as a dark brown oil.

MS (ESI): $[M+H]^+$ m/z = 372.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{23}INO$: 372.08243, found: 372.08208.

IR (film): 2933, 1461, 1393, 1045 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.06-1.19 (m, 1H), 1.33-1.44 (m, 1H), 1.45-1.66 (m, 3H), 1.75-2.02 (m, 5H), 2.28-2.37 (m, 1H), 2.35 (s, 3H), 2.60-2.64 (m, 1H), 3.30 (ddd, *J* = 4.7, 9.2, 9.2 Hz, 1H), 3.88 (s, 3H), 6.74 (dd, *J* = 2.1, 8.2 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.2, 22.8, 23.6, 36.0, 40.5, 40.7, 47.9, 54.2, 56.2, 68.4, 82.3, 110.2, 121.3, 138.7, 149.9, 157.8.

(3a*S*,7a*S*)-3a-(4-iodo-3-methoxyphenyl)-1-methyloctahydro-1*H*-indole (17)

Under an Ar atmosphere, to a solution of **16** (160.6 mg, 433 μmol) in THF (4.5 mL) was added a 2.65 *M* solution of *n*-BuLi in hexane (0.2 mL, 530 μmol) at -78 °C and stirred at the same temperature for 50 min. To the reaction mixture was added $B(O\textit{i}\text{-}Pr)_3$ (0.3 mL, 1.31 mmol) at -78 °C and the reaction temperature was gradually raised to room temperature with stirring for 19 h. To the reaction mixture were added 1 *M* aqueous solution of NaOH (2 mL) and $NaBO_3 \cdot 4H_2O$ (200 mg, 1.30 mmol). After stirring for 1 h, to the reaction mixture were added distillated water (2 mL), MeOH (0.5 mL), and $NaBO_3 \cdot 4H_2O$ (200 mg, 1.30 mmol), and then stirred for 2 h. The reaction mixture was poured into AcOEt and adjusted to pH 8 with NH_4Cl, and then extracted with AcOEt. After removing the solvent under reduced pressure, the residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to give **17** (44.4 mg, 0.170 μmol, 39%) as a colorless crystal and concomitantly with **15** (49.7 mg, 0.203 μmol, 47%).

MS (ESI): $[M+H]^+$ m/z = 262.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{16}H_{24}NO_2$: 262.18070, found: 262.18043.

IR (film): 2934, 1519, 1284, 1211, 1032 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.09-1.23 (m, 1H), 1.32-1.43 (m, 1H), 1.53-1.65 (m, 3H), 1.74-1.96 (m, 5H), 2.25-2.34 (m, 1H), 2.32 (s, 3H), 2.54-2.58 (m, 1H), 3.24 (ddd, *J* = 4.7, 9.1, 9.1 Hz, 1H), 3.89 (s, 3H), 5.37-5.60 (br, 1H), 6.83-6.90 (m, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4, 22.9, 23.6, 36.1, 40.6, 41.1, 47.5, 54.3, 55.9, 68.7, 109.7, 113.7, 119.6, 139.7, 143.2, 146.1.

Mp: 131 ˚C.

(3a*S*,7a*S*)-3a-(3,4-dimethoxyphenyl)-1-methyloctahydro-1*H*-indole (mesembrane) (4)

Under an Ar atmosphere, to a solution of **17** (44.4 mg, 0.170 μmol) in MeOH (1.35 mL) was added a 2 *M* solution of $TMSCHN_2$ in Et_2O (0.5 mL, 1.0 mmol) at 0 ^oC and stirred at the same temperature for 30 min, and then further stirred at room temperature for 40 min. To the reaction mixture was added AcOH/MeOH (1/1) at 0 ^oC until no foam was observed to be formed. After concentration, a solution of the residue in $CHCl_3$ was poured into a saturated aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. After removing the solvent under reduced pressure, the residue was purified by preparative TLC ($CHCl_3$/MeOH = 18/1) to give **4** (37.8 mg, 0.138 μmol, 81%) as a yellow oil.

MS (ESI): $[M+H]^+$ m/z = 276.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{17}H_{26}NO_2$: 276.19635, found: 276.19884.

IR (film): 2931, 1520, 1463, 1254, 1147, 1029 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.08-1.22 (m, 1H), 1.32-1.42 (m, 1H), 1.43-1.51 (m, 1H), 1.51-1.66 (m, 2H), 1.75-2.00 (m, 5H), 2.27-2.36 (m, 1H), 2.34 (s, 3H), 2.60-2.64 (m, 1H), 3.28 (ddd, *J* = 4.8, 9.1, 9.2 Hz, 1H), 3.85 (br s, 3H), 3.87 (br s, 3H), 6.80 (d, J = 8.3 Hz, 1H), 6.85-6.92 (m, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4, 22.8, 23.6, 35.9, 40.7, 40.9, 47.5, 54.3, 55.8, 55.9, 68.7, 110.6,

110.7, 118.8, 140.1, 146.8, 148.6.

4·hydrochloride: Anal. calcd for $C_{17}H_{25}NO2 \cdot HCl \cdot 0.9H_2O$: C, 62.24; H, 8.54; N, 4.27, found: C, 62.20; H, 8.42; N, 4.28.

Mp: 195 ˚C (dec.).

Demethylation with BBr_3

(General procedure) Under an Ar atmosphere, to a solution of starting material in CH_2Cl_2 was added a 1 *M* solution of BBr_3 in CH_2Cl_2 (3 eq) dropwise at -10 ^oC and stirred at the same temperature for 1 h. To the reaction mixture was added 30% aqueous solution of NH_3 and the reaction temperature was raised to room temperature, and then extracted with $CHCl_3$. After removing the solvent under reduced pressure, the residue was purified by preparative TLC.

(3a*S*,6a*S*,11b*R*)-3-methyl-1,2,3,3a,4,5,6,6a-octahydrobenzofuro[3,2-*d*]indol-8-ol (13′)

A colorless crystal. 23% yield.

MS (ESI): $[M+H]^+$ m/z = 246.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{15}H_{20}NO_2$: 246.14940, found: 246.14767.

IR (film): 2937, 1611, 1465, 1232, 1176 cm^{-1}.

^1H NMR (pyridine-d_5, 400 MHz): δ 1.26-1.43 (m, 2H), 1.50-1.72 (m, 3H), 1.89 (ddd, *J* = 3.9, 9.2, 12.3 Hz, 1H), 1.94-2.03 (m, 2H), 2.18 (s, 3H), 2.35 (ddd, *J* = 7.7, 8.8, 9.2 Hz, 1H), 2.45 (dd, *J* = 3.5, 3.5 Hz, 1H), 3.03 (ddd, *J* = 3.9, 8.8, 8.8 Hz, 1H), 4.56 (dd, *J* = 4.9, 7.0 Hz, 1H), 6.85 (dd, *J* = 1.2, 7.4 Hz, 1H), 7.00 (dd, *J* = 7.4, 7.9 Hz, 1H), 7.17 (dd, *J* = 1.2, 7.9 Hz, 1H).

^{13}C NMR (pyridine-d_5, 100 MHz): δ 16.1, 22.1, 27.0, 37.9, 39.8, 54.4, 54.9, 68.6, 89.4, 114.1, 117.0, 122.2, 136.5, 143.8, 147.5.

Mp: 155 ˚C.

13′·hydrochlorode: Anal. calcd for $C_{15}H_{19}NO_2 \cdot HCl \cdot 0.2H_2O$: C, 63.13; H, 7.20; N, 4.91, found: C,

62.99; H, 7.03; N, 4.93.

Mp: 248 ˚C (dec.).

3-((3a*S*,7a*S*)-1-methyloctahydro-3a*H*-indol-3a-yl)phenol (15′)

A colorless crystal. 62% yield.

MS (ESI): $[M+H]^+$ m/z = 232.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{15}H_{22}NO$: 232.17014, found: 232.17026.

IR (film): 2933, 1583, 1449, 1241, 779, 701 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.09-1.23 (m, 1H), 1.33-1.51 (m, 2H), 1.53-1.72 (m, 2H), 1.82-2.00 (m, 5H), 2.29-2.39 (m, 1H), 2.36 (s, 3H), 2.66-2.71 (m, 1H), 3.29 (ddd, *J* = 4.9, 8.9, 9.3 Hz, 1H), 6.66 (br dd, *J* = 1.8, 8.0 Hz, 1H), 6.85-6.88 (m, 1H), 6.91 (br d, *J* = 7.9 Hz, 1H), 7.16 (dd, *J* = 7.9, 8.0 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4, 22.6, 23.5, 36.0, 40.6, 41.1, 47.8, 54.4, 68.9, 112.8, 114.4, 118.7, 129.1, 149.5, 156.1.

Mp: 141 ˚C.

15′·hydrochloride: Anal. calcd for $C_{15}H_{21}NO{\cdot}HCl{\cdot}0.15H_2O$: C, 66.60; H, 8.31; N, 5.18, found: C, 66.89; H, 8.27; N, 4.90.

Mp: 255 ˚C (dec.).

3-((3a*S*,7a*R*)-1-methyloctahydro-3a*H*-indol-3a-yl)phenol (*epi*-15′)

A colorless oil. 87% yield.

MS (ESI): $[M+H]^+$ m/z = 232.

HR-MS (ESI): $[M+H]^+$ calcd for $C_{15}H_{22}NO$: 232.17014, found: 232.16793.

IR (film): 2934, 1583, 1455, 1241, 756, 711 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.11 (ddd, *J* = 3.9, 3.9, 13.0, 13.2 Hz, 1H), 1.24-1.36 (m, 1H), 1.37

(ddd, *J* = 3.4, 12.9, 13.0), 1.41-1.49 (m, 1H), 1.64-1.82 (m, 5H), 2.17-2.24 (m, 1H), 2.29 (ddd, *J* = 2.7, 10.3, 10.3 Hz, 1H), 2.38 (s, 3H), 2.57-2.63 (m, 1H), 3.11 (ddd, *J* = 8.2, 8.2, 10.2 Hz, 1H), 6.63 (ddd, *J* = 1.0, 2.6, 7.9 Hz, 1H), 7.15 (dd, *J* = 7.9, 8.0 Hz, 1H), 7.22 (dd, *J* = 1.9, 8.0 Hz, 1H), 7.46 (dd, *J* = 1.0, 1.9 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ22.2, 24.0, 25.7, 38.4, 39.8, 42.2, 49.4, 53.3, 77.1, 112.3, 116.5, 120.3, 129.2, 146.9, 155.5.

epi-**15′**·hydrochlorode: Anal. calcd for $C_{15}H_{21}NO \cdot HCl \cdot 0.15H_2O$: C, 66.60; H, 8.31; N, 5.18, found: C, 66.63; H, 8.24; N, 5.09.

Mp: 227 ˚C (dec.).

Binding assay

The competitive binding assays were performed using human MOR, DOR, or KOR recombinant cell (CHO) membranes. [^{3}H]DAMGO (2 nM), [^{3}H]DPDPE (2 nM), and [^{3}H]U69,593 (2 nM) were used to label the MOR, DOR, and KOR, respectively. Nonspecific binding was measured in the presence of 1.0 μM unlabeled DAMGO, DPDPE or U-69,593. *K*i values were calculated according to the Cheng–Prusoff equation.[25]

Conformational analysis

SYBYL6.91 (Tripos, St Louis, MO, USA) was first employed to construct initial structures for compounds **13′**, **15′**, and *epi*-**15′**. We protonated the nitrogens of compounds **13′**, **15′**, and *epi*-**15′** to prepare two initial structures with different configuration at the nitrogen for each compound (Fig. S3). Then, the following calculations were performed for each initial structure using the CAMDAS 2.1 program. Ten molecular dynamic (MD) calculations were simultaneously performed using different

conformers generated randomly using the initial structure. Each of the MD calculations was carried out for 1000 ps with an integral time step of 1fs. The lengths of covalent bonds were fixed with SHAKE algorithm through the MD.[26] The temperature of the system was maintained at 1200 K in order to enhance the sampling efficiency. The Merck Molecular Force Field (MMFF) was used to evaluate the potential energy surface of the molecule.[27] To mimic the shield effects of solvent molecules on electrostatic interactions, the electrostatic potential term was neglected. Conformers were sampled at 100 step intervals, thus producing 10,000 conformations for each MD calculation. A total of 100,000 conformations were preclustered with a dihedral angles deviation threshold of ± 30˚. The dihedral angles used to cluster similar conformations are indicated for each compound by arrows in Fig. S3. Each of the conformers obtained after preclustering was then minimized until the root mean square (RMS) of the potential-energy gradient fell below 0.05 kcal mol^{-1} $Å^{-1}$. The minimized conformers were reclustered with a dihedral angle deviation threshold of ± 30˚, furnishing a final conformer set.

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