Bioorganic & Medicinal Chemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

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

Kazuya Konoura [a], Hideaki Fujii [a,*], Satomi Imaide [a], Hiroaki Gouda [b], Shigeto Hirayama [a], Shuichi Hirono [c], Hiroshi Nagase [a,d]

[a] Department of Medicinal Chemistry, School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
[b] School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
[c] Department of Physical Chemistry for Drug Design, School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
[d] International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

ARTICLE INFO

Article history:
Received 18 November 2014
Revised 15 December 2014
Accepted 15 December 2014
Available online xxxx

Keywords:
Mesembrane
Opioid
Sceletium alkaloids
Conformational analysis

ABSTRACT

We transformed naltrexone (**5**) with the morphinan skeleton into mesembrane (**4**) belonging to the *Sceletium* alkaloids via key intermediate **6**, characterized by a *cis*-fused hydroindole skeleton with a suspended phenyl ring fixed by an epoxy bridge. We then investigated the binding affinities of **4** and the key intermediate **6** derivatives to the opioid receptors. Among the tested compounds, **15′**, with a *cis*-fused hydroindole core, bound to the three opioid receptor types with strong to moderate affinities. The observed differences of binding affinities among the tested compounds were reasonably explained by the conformational analyses of the compounds. The structure–activity relationship (SAR) of the tested compounds like **15′** with the hydroindole structure was completely different from the reported SAR of morphinan derivatives with the hydroisoquinoline skeleton. Compound **15′** with a structure that differs from the morphinans represents a useful fundamental skeleton with a novel chemotype that may contribute to the development of new opioid ligands.

1. Introduction

G protein-coupled receptors (GPCRs) are among the most valuable drug targets and more than 25% of the FDA-approved drugs interact with them.[1] Originally, a substrate was thought to interact with a GPCR one for one, and that an agonist exerted pharmacological responses via inherent pathways. However, recent investigations have revealed that the actual phenomena are more complicated. GPCRs exist not only as monomeric receptors but also as homo- or hetero-dimers and the dimeric receptors are the functional unit in a living organism.[2] Concerning the opioid receptor, heterodimerization is known to occur 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 have been 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.[4–6] Moreover, GPCR splice variants, which are mutated receptors produced from a gene with more than one exon, have also been investigated. A large series of splice variants of the MOR have already been identified.[7,8] These phenomena, that is, receptor dimerization, biased agonism, and splice variants have been the subjects of investigations involving molecular biology, molecular pharmacology, and the development of specific ligands. For example, 6′-guanidinonaltrindole (6′-GNTI) and *N*-naphthoyl-β-naltrexamine (NNTA) were reported to be DOR-KOR[9] and MOR-KOR[10] agonists, respectively. Herkinorin and TRV-130 were reported to be G protein-biased MOR agonists.[11,12] Amidino-TAPA produced antinociceptive effects via the MOR splice variants with which the representative MOR agonist, morphine, did not interact.[13] Although the aforementioned specific agonists have been reported, the development of novel and unique ligands is required for the further investigation. Moreover, there have been almost no structural guidelines for the design of 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 has been

* Corresponding author. Tel.: +81 3 5791 6372; fax: +81 3 3442 5707.
E-mail address: fujiih@pharm.kitasato-u.ac.jp (H. Fujii).

http://dx.doi.org/10.1016/j.bmc.2014.12.032
0968-0896/

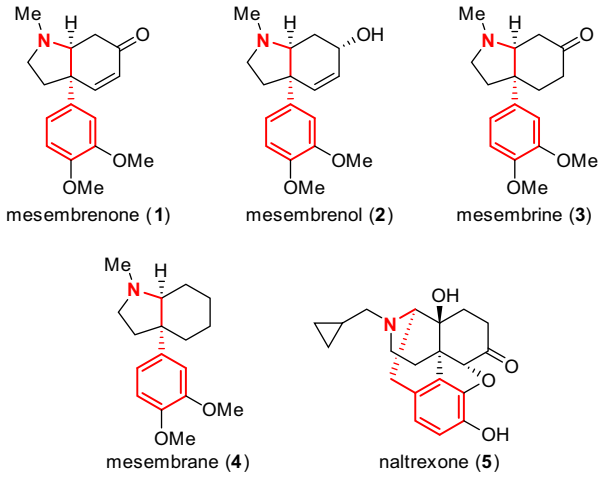


Figure 1. Structures of *Sceletium* alkaloids **1**–**4** and naltrexone (**5**). The phenethylamine moieties are indicated by red lines.

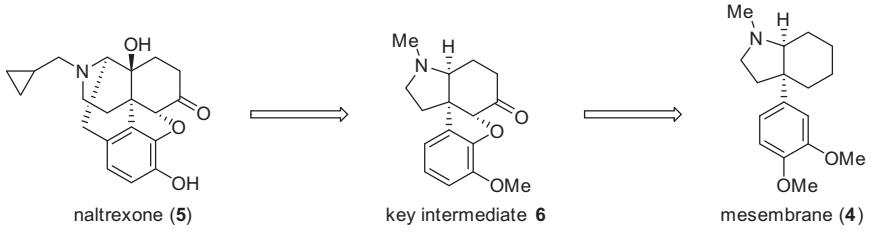
reported that ligand bias would stem from a distinct receptor conformation which is stabilized by each agonist. Therefore, development of ligands with various chemotypes would be important to investigate receptor dimers, ligand bias, and/or receptor splice variants.

Sceletium tortuosum Zembrin®, which is a mixture of three *Sceletium* alkaloids[14] (mesembrenone (**1**) + mesembrenol (**2**) >70%, mesembrine (**3**) <20%, Fig. 1), was recently reported to bind to the opioid receptors with very weak affinities (10–30% of the control bindings remained).[15] Mesembrane (**4**)[16] is also a member of the *Sceletium* alkaloids (Fig. 1). The basic nitrogen, phenyl ring, and phenolic hydroxy group are known to be essential pharmacophores to interact with the opioid receptors.[17–19] The *Sceletium* alkaloids (Fig. 1) had these binding determinants although the phenolic hydroxy group was masked by the methyl group. Moreover, it is interesting that both the *Sceletium* alkaloids and a representative opioid ligand like naltrexone (**5**) have a common structure, the phenethylamine motif (indicated by red line, Fig. 1). However, naltrexone (**5**) has a *trans*-fused hydroisoquinoline structure with a suspended phenyl ring fixed by both epoxy and methylene bridges, whereas the *Sceletium* alkaloids possess a *cis*-fused hydroindole skeleton with a freely rotatable phenyl ring. Therefore, we proposed to transform naltrexone (**5**) into *Sceletium* alkaloids like mesembrane (**4**) via a key intermediate **6** (Scheme 1), which has *cis*-fused hydroindole skeleton with a suspended phenyl ring fixed by an epoxy bridge,[20] and to evaluate the binding affinities of mesembrane (**4**) and key intermediate **6** derivatives for 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 groups which were potential substituents to interact with the opioid receptors. Herein, we report the transformation from naltrexone (**5**) into mesembrane (**4**) and the binding abilities of **4** and key intermediate **6** derivatives to the opioid receptors. We also examine the structure–activity relationships (SARs) based on conformational analyses of the tested compounds.

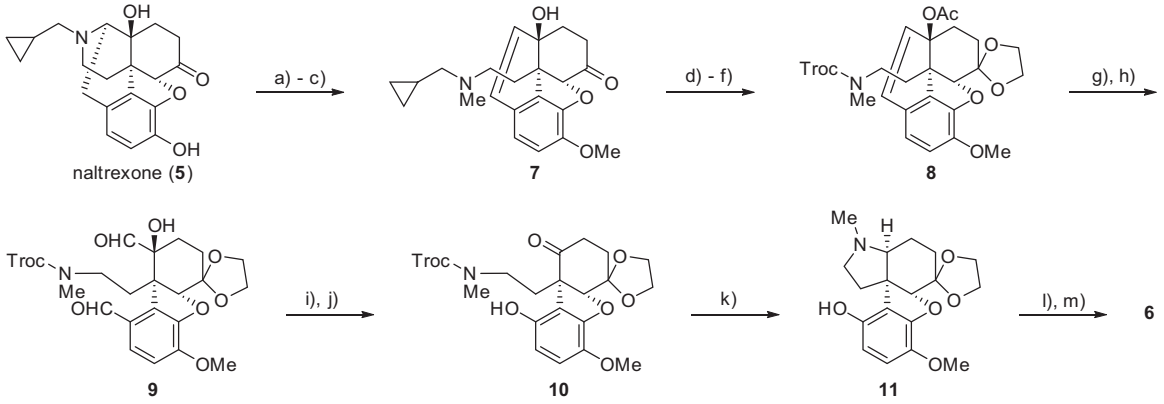
2. Results and discussion

2.1. Transformation from naltrexone (5) into mesembrane (4)

The key intermediate **6** was prepared from naltrexone (**5**) (Scheme 2). Naltrexone methyl ether, obtained by 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

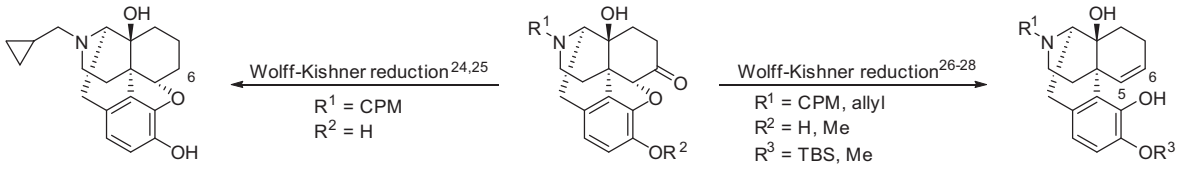


Scheme 1. Strategy of transformation from naltrexone (**5**) into mesembrane (**4**) via key intermediate **6**.



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 NaOH (aq), 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, (m) 1 M HCl, 79% from **11**.

Please cite this article in press as: Konoura, K.; et al. *Bioorg. Med. Chem.* (2015), http://dx.doi.org/10.1016/j.bmc.2014.12.032



Scheme 3. Wolff–Kishner reduction of naltrexone derivatives. CPM: cyclopropylmethyl.

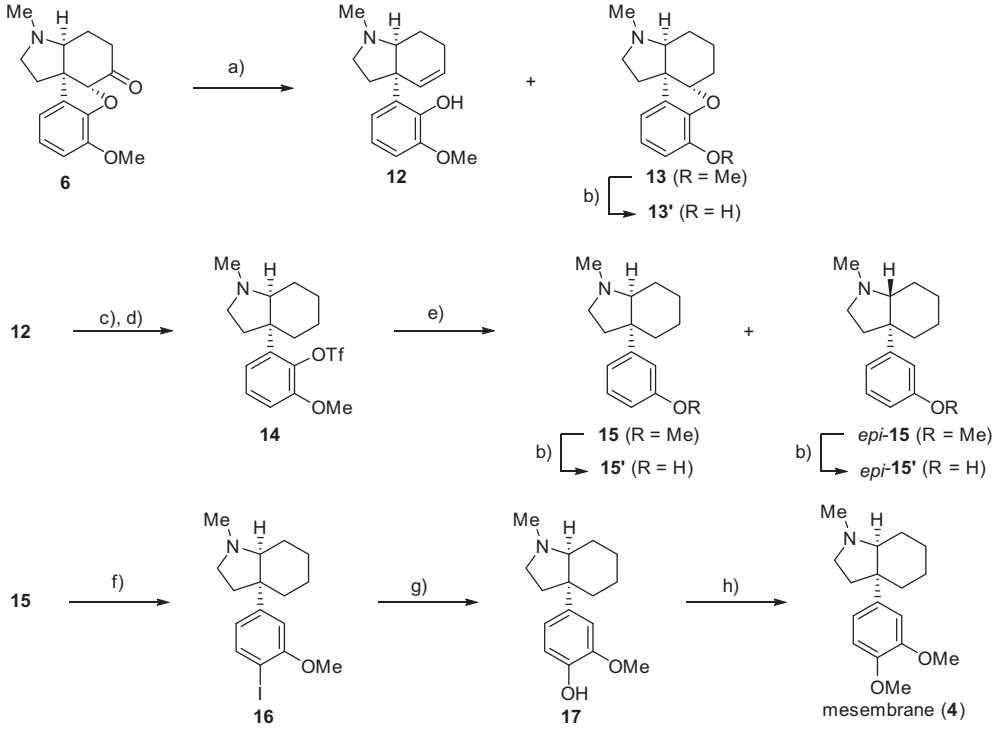
chloroformate (Troc-Cl) to afford compound **8**. In the reaction of (cyclopropylmethy)methylamine **7** with Troc-Cl, the cyclopropylmethyl group was selectively dealkylated.[21] After ozonolysis of **8**, the acetyl group was removed by methanolysis. The obtained dialdehyde **9** was oxidized by *m*-chloroperbenzoic acid (*m*CPBA), followed by methanolysis of the obtained formate groups to give ketone **10**. Deprotection of the 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 afforded the key intermediate **6**. The key intermediate **6** was determined to have *cis*-fused hydroindole structure by ROESY specta (Fig. S1).

In morphinan derivatives, the presence or absence of the epoxy bridge is known to critically affect the pharmacological properties of the compounds.[22,23] Therefore, from the viewpoint of the structural diversity, we attempted to prepare both hydroindole derivatives with and without the epoxy bridge. Although Wolff–Kishner reduction is the general method for converting an oxo group into a methylene moiety, the Wolff–Kishner reduction of 4,5-epoxymorphinans has been demonstrated to provide two kinds of products (Scheme 3).[24–29] In hope that both compounds with and without the ether bond would be obtained, we attempted the Wolff–Kishner reduction of the key intermediate **6**. As we expected,[29] compounds **12** and **13** were prepared in 73% and 25% yield, respectively (Scheme 4).

Mesembrane (**4**) was prepared from compound **12** (Scheme 4). Catalytic hydrogenation of **12** and subsequent treatment with *N*-phenylbis(trifluoromethanesulfonimide) (Tf_2NPh) afforded 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 presently investigating 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 the iodo group was determined by HMBC spectroscopy (Fig. S2). After the halogen-lithium exchange reaction of **16** with *n*-butyl lithium, treatment with triisopropyl borate ($B(Oi\text{-}Pr)_3$) followed by treatment 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 the phenolic hydroxy group in **17** with trimethylsilyldiazomethane ($TMSCHN_2$). The observed spectral data of prepared **4** were identical to the literature data.[30–32] 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).

2.2. 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 (Table 1 and S1). Although **13** displayed no binding



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%.

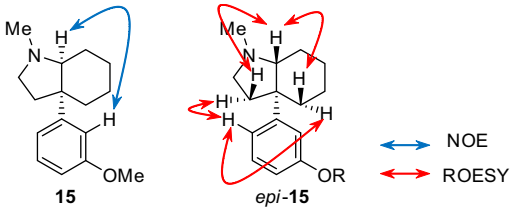


Figure 2. Observed NOE or ROESY spectra of **15** and *epi*-**15**.

Table 1
Binding affinities for the opioid receptors[a]

Compound	K_i (nM)		
	MOR[b]	DOR[c]	KOR[d]
Mesembrane (**4**)	9102	16,230	5800
Naltrexone (**5**)	0.27	12.3	0.70
13	ND[e]	ND[e]	ND[e]
13′	2151	2363	769.8
15	5533	19,130	4775
15′	90.0	1149	163.2
epi-**15**	10,650	42,380	48,940
epi-**15′**	494.7	6627	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.

affinities for any of the receptors, the compounds **13′**, **15′**, and *epi*-**15′** with a phenolic hydroxy group bound to the opioid receptors with sufficient binding affinities in the micromolar range, and **15′** and *epi*-**15′** both showed the same rank order of the binding abilities to the individual 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 the three opioid receptor types. Compounds **13**, **15**, and *epi*-**15** with a methoxy group exhibited lower binding abilities than did the corresponding compounds **13′**, **15′**, *epi*-**15′** with the phenolic hydroxy group. It is well-known that the phenolic hydroxy group is an important determinant for binding to the opioid receptor[17–19] and that morphinan derivatives with the phenolic hydroxy group like naltrexone (**5**) are stronger binders than the corresponding derivatives with the methoxy group. These results suggested that the tested compounds would bind to the opioid receptors with the same binding mode as observed for 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 moiety responsible for exerting opioid activities according to the message-address concept,[33,34] which is a useful guideline for the design of ligands selective for the opioid receptor types. Therefore, we predicted that compound **15′** would be a novel message structure with a skeleton distinct from morphinans to provide characteristic opioid ligands. However, 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 a freely rotatable phenyl ring have been reported to be lower or comparable to morphine (**20**) and nalorphine (**21**) (Fig. 3).[35] Although no binding affinities have been reported, the analgesic effects by **20** and codeine (**22**) with a *trans*-fused hydroisoquinoline structure were stronger than those of **23** and **24** possessing a *cis*-fused ring system (Fig. 3).[36] Interestingly, the SAR of the tested compounds with the hydroindole structure was completely different from that

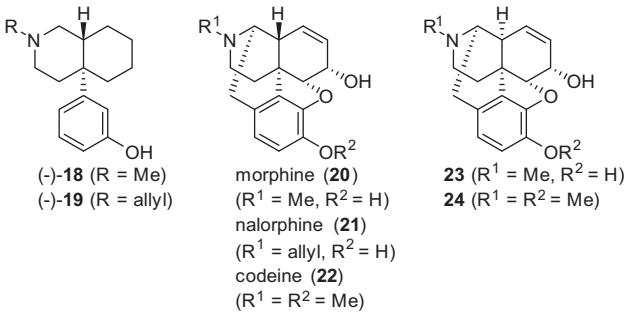


Figure 3. Structures of compounds (−)-**18**–**24**.

of morphinan derivatives with a hydroisoquinoline fundamental skeleton. The binding affinities of mesembrane (**4**) were extremely low as were those for the compounds **13**, **15**, *epi*-**15** with the methoxy group. The extremely low affinities may result from the absence of a phenolic hydroxy group in **4**. The lack of values for K_i in the report by Harvey et al. makes it difficult to compare the binding affinities of **4** with those of *Sceletium tortuosum* Zembrin®.[15] Concerning compound **4**, the non-displaced 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 a concentration of 750 μg/mL, which was calculated to be about 2.6 mM. Under these assessed conditions, the non-displaced bindings of the labeled compounds were about 10–30% (see the Supplementary data for more detailed discussion).[15]

2.3. Conformational analysis

To examine the differences in 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 data for details).[37] 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, respectively, at the protonated nitrogen atom. 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 the lower-energy conformers, but both configurations for each compound were observed within 10 kcal/mol of the most stable conformer (Table S4). 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.[38] Therefore, we paid attention to the conformers with *S*-configuration at the nitrogen atom, in which the *N*–H bond was directed axially. Figure 4 includes superimpositions of the low-energy conformers with *S*-configuration for **13′**, **15′**, and *epi*-**15′**, in which the phenol rings were superimposed. Although the spatial disposition of the nitrogen atom was tightly restricted for **13′**, those of **15′** and *epi*-**15′** were widely spread. The bound conformation of β-funaltrexamine (β-FNA, an irreversible MOR antagonist, Fig. 4G) in complex with the MOR[39] is also shown in Figure 4H and I. A comparison of Figure 4B and H indicated that the nitrogen atoms of many low-energy conformers of **15′** adopted a conformation which resembled that of the basic nitrogen of the bound conformer of β-FNA. The basic nitrogen is well-known to provide a pharmacophoric interaction with the

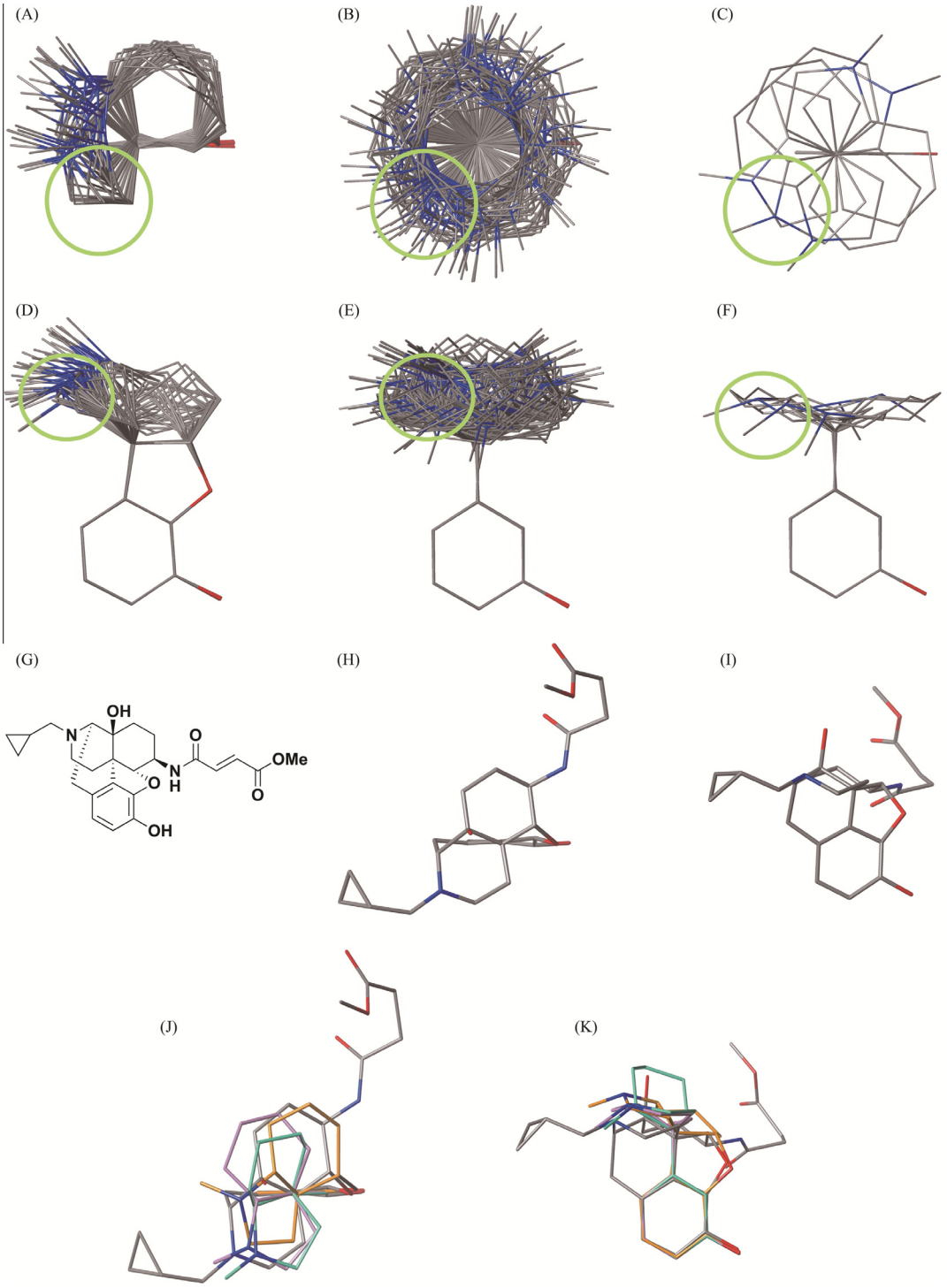


Figure 4. Results of conformational analyses of the protonated **13′**, **15′**, and *epi*-**15′**, and the structure of β-FNA. Structures within 10 kcal/mol of the most stable conformer were collected for the protonated forms of **13′**, **15′**, and *epi*-**15′**. In each figure, the green circles indicated the area around the position relative to the basic nitrogen adjacent to the phenol ring in β-FNA. (A) Top view of conformers of protonated **13′**; (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 drawn using the coordinates obtained in the X-ray crystallographic analyses of the β-FNA-MOR complex,[39] in which β-FNA bound to the MOR through a covalent bond provided by Michael addition of Lys233 in the MOR to 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 based on the location of the nitrogen atom that most closely resembled that of β-FNA. Compounds **13′**, **15′**, and *epi*-**15′** are indicated by orange, green, and light purple, respectively; (K) side view of superimpositions of **13′**, **15′**, *epi*-**15′**, and β-FNA.

opioid receptor.[17–19] These results suggested that **15′** could bind to the opioid receptors with a binding mode similar to that of the morphinans, thereby accounting for it having the strongest binding affinities among these three compounds. The compound *epi*-**15′** also had low-energy conformers with the basic nitrogen located in a favorable region (green circle), but as indicated by the much less congested model (Fig. 4C), the population of the adequate low-energy conformers was much lower than that of **15′**. These

observations reasonably suggested compound *epi*-**15**′ possessed poorer binding affinities than **15**′. Taken together, both observations on the most stable conformers of protonated **15**′ and *epi*-**15**′ (Table S4) and populations of the adequate low-energy conformers of **15**′ and *epi*-**15**′ sufficiently accounted for the difference in the binding affinities of these compounds. Furthermore, the most stable conformer of compound **13**′ had *S*-configuration at the protonated nitrogen atom, but it had almost no conformers having the basic nitrogen located in the proper space. Figure 4J, which pictures superimpositions of **13**′, **15**′, *epi*-**15**′, and β-FNA, clearly supported these observations. These phenomena resulted in the weakest binding affinities of **13**′ to the opioid receptors.

3. Conclusion

We transformed naltrexone (**5**) with the morphinan skeleton into mesembrane (**4**) belonging to the *Sceletium* alkaloids via key intermediate **6** to investigate the binding affinities of **4** and derivatives of the key intermediate **6** to the opioid receptors. Compound **15**′, which had the *cis*-fused hydroindole skeleton, exhibited strong to moderate binding affinities for the MOR and KOR. Although compound *epi*-**15**′, which was a *trans*-fused congener of **15**′, also displayed moderate binding 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 greatly reduced binding. The SARs of the tested compounds **13**′, **15**′, and *epi*-**15**′ with hydroindole structures were completely different from that of morphinan derivatives with the hydroisoquinoline fundamental skeleton. The observed differences of binding affinities among the tested compounds were 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 structure that differed from the morphinans like naltrexone (**5**) is expected to be a useful fundamental structure with a novel chemotype to develop characteristic opioid ligands, which might provide useful ligands to investigate receptor dimers, ligand bias, and/or receptor splice variants. Synthesizing derivatives of **15**′ and evaluating their binding affinities and functional activities for the opioid receptors, including receptor dimers and ligand bias, are in progress.

4. Experimental

4.1. 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).

4.2. Synthesis

4.2.1. (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 the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into distilled 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) with stirring 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) with stirring at 80 °C for 4 h. The cooled reaction mixture was poured into distilled 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) with stirring at 50 °C for 4 h. The 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 the mixture was 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}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.39–1.50 (m, 1H), 1.55–1.67 (m, 2H), 1.87–1.98 (m, 0.35H), 1.98–2.24 (m, 1.65H), 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 = 9.8 Hz, 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.

Please cite this article in press as: Konoura, K.; et al. *Bioorg. Med. Chem.* (2015), http://dx.doi.org/10.1016/j.bmc.2014.12.032

4.2.2. 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 vigorous bubbling with N_2 gas for 30 min, Me_2S (25 mL, 338 mmol) was added to the reaction mixture at the same temperature, and then the reaction was 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) with stirring 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}. ^1H 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, 150.7, 153.9, 154.1, 191.2, 191.6.

4.2.3. 2,2,2-Trichloroethyl (2-((4a*R*,9b*S*)-9-hydroxy-6-methoxy-1-oxo-2,3,4a,9b-tetrahydro-1*H*-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 (27.4 g, 122 mmol) and the mixture was 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 the mixture was extracted with $CHCl_3$. The combined organic layers were concentrated under reduced pressure and to a solution of the residue in MeOH (322 mL) was added Et_3N (326 mL) with stirring 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}. ^1H 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, 213.5, 213.6.

4.2.4. (3a*S*,6a*R*,11b*R*)-8-Methoxy-3-methyl-1,2,3,3a,4,5-hexahydro-6a*H*-spiro[benzo-furo[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) with stirring at room temperature for 2 h. The reaction mixture was filtered through a Celite pad and to the obtained filtrate was added $NaBH_3CN$ (3.95 g, 62.9 mmol) at 0 °C, and then the mixture was 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, 1031 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.56–1.80 (m, 2H), 1.80–1.88 (m, 2H), 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, 2H), 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 °C (dec.).

4.2.5. (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 °C and the reaction mixture was stirred at the same temperature for 30 min. Tf_2NPh (3.12 g, 8.73 mmol) was added to the reaction mixture with stirring 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 the mixture was 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 with stirring at 80 °C for 10 h. Further HCO_2H (1.0 mL, 26.5 mmol) was added to the reaction mixture at room temperature with stirring at 80 °C for 4 h. The cooled reaction mixture was poured into distilled water and extracted with AcOEt. The combined organic layers were concentrated under reduced pressure. A solution of the residue in AcOEt was filtered through a short amine silica gel column (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 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 = 274. 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, 206.0. Mp: 122–124 °C.

4.2.6. 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 the mixture was stirred at 80 °C for 2 h. KOH (2.33 g, 41.5 mmol) was added to the reaction mixture at room temperature with stirring at 130 °C for 23 h. The 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 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.

Compound **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, 1257 cm^{-1}. ^1H 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.

Compound **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}. ^1H 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 Hz, 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.

Compound **13** hydrochloride: Anal. Calcd for $C_{16}H_{21}NO_2{\cdot}HCl{\cdot}0.2H_2O$: C, 60.54; H, 7.75; N, 4.41, found: C, 60.34; H, 7.57; N, 4.29. Mp: 201 °C (dec.).

4.2.7. 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, the reaction was stirred at 80 °C for 21 h. The cooled reaction mixture was filtered through a Celite 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 a short amine silica gel column (CHROMATOREX® NH-DM2035). The solvent was removed under reduced pressure to give a 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 °C, and the mixture was stirred at the same temperature for 20 min. To the reaction mixture was added Tf_2NPh (300 mg, 0.839 mmol) at 0 °C with stirring 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 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 (q, J = 322.4 Hz), 120.8, 127.3, 138.6, 141.7, 150.9. Mp: 88–90 °C.

4.2.8. (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 the mixture was stirred at 100 °C 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) with further stirring at 100 °C for 8 days. The 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 a short amine silica gel column (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.

Compound **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}. ^1H 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.

Compound **15** hydrochloride: Anal. Calcd for $C_{16}H_{23}NO{\cdot}HCl{\cdot}0.15H_2O$: C, 67.54; H, 8.61; N, 4.92, found: C, 67.44; 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}. ^1H 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–.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, 76.8, 109.6, 115.8, 121.2, 128.7, 147.4, 159.3.

epi-**15** hydrochloride: 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.

4.2.9. (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 °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 the mixture was extracted with $CHCl_3$. After concentration, the residue was purified by preparative TLC (chloroform/methanol = 20:1) to give **16** (160.6 mg, 433 μmol, 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–.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.

4.2.10. 2-methoxy-4-((3a*S*,7a*S*)-1-methyloctahydro-3a*H*-indol-3a-yl)phenol (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 the mixture was stirred at the same temperature for 50 min. To the reaction mixture was added B(O*i*-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 distilled water (2 mL), MeOH (0.5 mL), and $NaBO_3{\cdot}4H_2O$ (200 mg, 1.30 mmol), and then the mixture was 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, 170 μmol, 39%) as a colorless crystal and concomitantly with **15** (49.7 mg, 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}. ^{1}H 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.

4.2.11. (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, 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 °C and the mixture was 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 °C 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, 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}. ^{1}H 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.

Compound **4** hydrochloride: Anal. Calcd for $C_{17}H_{25}NO_2{\cdot}HCl{\cdot}0.9H_2O$: C, 62.24; H, 8.54; N, 4.27, found: C, 62.20; H, 8.42; N, 4.28. Mp: 195 °C (dec.).

4.2.12. 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 equiv) dropwise at −10 °C, and the mixture was 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 the mixture was extracted with $CHCl_3$. After removing the solvent under reduced pressure, the residue was purified by preparative TLC.

4.2.13. (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}. ^{1}H 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.

Compound **13′** hydrochloride: 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.).

4.2.14. 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}. ^{1}H 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.

Compound **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.).

4.2.15. 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}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.11 (dddd, 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 Hz, 1H), 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): δ 22.2, 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′** 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, 66.63; H, 8.24; N, 5.09. Mp: 227 °C (dec.).

4.3. 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.[40]

4.4. 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 configurations 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 1 fs. The lengths of covalent bonds were fixed with SHAKE algorithm through the MD.[41] 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.[42] 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 angle 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.

Supplementary data

Supplementary data (supplementary data (Figs. S1–S3, Tables S1–S4 and conformational analyses) and more discussion (comparison of binding affinities between mesembrane and *Sceletium tortuosum* Zembrin®)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.12.032.

References and notes

1. Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. *Nat. Rev. Drug. Discov.* **2006**, *5*, 993.
2. Prinster, S. C.; Hague, C.; Hall, R. A. *Pharmacol. Rev.* **2005**, *57*, 289.
3. Rozenfeld, R.; Gomes, I.; Devi, L. A. In *The Opiate Receptors*; Pasternak, G. W., Ed., 2nd ed.; Humana Press: New York, 2010; pp 407–437.
4. Kenakin, T. *F1000 Biol. Rep.* **2009**, *1*, 87.
5. Luttrell, L. M. *Mol. Endocrinol.* **2014**, *28*, 281.
6. Correll, C. C.; McKittrick, B. A. *J. Med. Chem.* **2014**, *57*, 6887.
7. Pan, Y.-X.; Pasternak, G. W. In *The Opiate Receptors*; Pasternak, G. W., Ed., 2nd ed.; Humana Press: New York, 2010; pp 121–160.
8. Pasternak, G. W. *Neuropharmacology* **2014**, *76*, 198.
9. Waldhoer, M.; Fong, J.; Jones, R. M.; Lunzer, M. M.; Sharma, S. K.; Kostenis, E.; Portoghese, P. S.; Whistler, J. L. *Proc. Natl. Acad. Sci.* **2005**, *102*, 9050.
10. Yekkirala, A. S.; Lunzer, M. M.; McCurdy, C. R.; Powers, M. D.; Kalyuzhny, A. E.; Roerig, S. C.; Portoghese, P. S. *Proc. Natl. Acad. Sci.* **2011**, *108*, 5098.
11. Groer, C. E.; Tidgewell, K.; Moyer, R. A.; Harding, W. W.; Rothman, R. B.; Prisinzano, T. E.; Bohn, L. M. *Mol. Pharmacol.* **2007**, *71*, 549.
12. Chen, X. T.; Pitis, P.; Liu, G.; Yuan, C.; Gotchev, D.; Cowan, C. L.; Rominger, D. H.; Koblish, M.; DeWire, S. M.; Crombie, A. L.; Violin, J. D.; Yamashita, D. S. *J. Med. Chem.* **2013**, *56*, 8019.
13. Mizoguchi, H.; Watanabe, C.; Sakurada, T.; Sakurada, S. *Curr. Opin. Pharmacol.* **2012**, *12*, 87.
14. Jeffs, P. W. In *The Alkaloids*; Rodrigo, R. G. A., Ed.; Academic Press: New York, 1981; Vol. 19, pp 1–80. Chapter 1.
15. Harvey, A. L.; Young, L. C.; Viljoen, A. M.; Gericke, N. P. *J. Ethnopharmacol.* **2011**, *137*, 1124.
16. Capps, T. M.; Hargrave, K. D.; Jeffs, P. E.; McPhail, A. T. *J. Chem. Soc. Perkin II* **1977**, 1098.
17. Casy, A. F.; Beckett, A. H. *J. Pharm. Pharmacol.* **1954**, *6*, 986.
18. Beckett, A. H. *J. Pharm. Pharmacol.* **1956**, *8*, 848.
19. Fries, D. S. In *Foye's Principles of Medicinal Chemistry*; Lemke, T. L., Williams, D. A., Eds., 6th ed.; Lippincott Williams & Wilkins: Philadelphia, 2008; pp 652–678.
20. We reported that compounds with the fixed phenyl ring increased the binding ability to the opioid receptors compared with the compound with a freely rotatable phenyl ring. See Ref. 23.
21. Fujii, H.; Imaide, S.; Watanabe, A.; Nemoto, T.; Nagase, H. *Tetrahedron Lett.* **2008**, *49*, 6293.
22. Nagase, H.; Kawai, K.; Hayakawa, J.; Wakita, H.; Mizusuna, A.; Matsuura, H.; Tajima, C.; Takezawa, Y.; Endoh, T. *Chem. Pharm. Bull.* **1998**, *46*, 1695.
23. Nagase, H.; Osa, Y.; Nemoto, T.; Fujii, H.; Imai, M.; Nakamura, T.; Kanemasa, T.; Kato, A.; Gouda, H.; Hirono, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2792.
24. Wentland, M. P.; Lou, R.; Lu, Q.; Bu, Y.; Denhardt, C.; Jin, J.; Ganorkar, R.; VanAlstine, M. A.; Guo, C.; Cohen, D. J.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2289.
25. Qi, H. A.; Yakov, R.; Virendra, K. WO2009/132313, 2009.
26. Furrow, M. E.; Myers, A. G. *J. Am. Chem. Soc.* **2004**, *126*, 5436.
27. Wu, H.; Thatcher, L. N.; Bernard, D.; Parrish, D. A.; Deschamps, J. R.; Rice, K. C.; MacKerell, A. D., Jr.; Coop, A. *Org. Lett.* **2005**, *7*, 2531.
28. Yamamoto, N.; Fujii, H.; Imaide, S.; Hirayama, S.; Nemoto, T.; Inokoshi, J.; Tomoda, H.; Nagase, H. *J. Org. Chem.* **2011**, *76*, 2257.
29. Our attempts to conduct Wolff–Kishner reduction of some naltrexone derivatives sometimes provided both types of products, 6-methylene and 5,6-olefin derivatives. Unpublished results.
30. Pearson, W. H.; Szura, D. P.; Postich, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 1329.
31. Mori, M.; Kuroda, S.; Zhang, C.-S.; Sato, Y. *J. Org. Chem.* **1997**, *62*, 3263.
32. Klein, J. E. M. N.; Geoghegan, K.; Méral, N.; Evans, P. *Chem. Commun.* **2010**, 937.
33. Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 281.
34. Portoghese, P. S.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1990**, *33*, 1714.
35. Zimmerman, D. M.; Cantrell, B. E.; Swartzendruber, J. K.; Jones, N. D.; Mendelsohn, L. G.; David Leander, J.; Nickander, R. C. *J. Med. Chem.* **1988**, *31*, 555.
36. Kugita, H.; Takeda, M.; Inoue, H. *J. Med. Chem.* **1970**, *13*, 973.
37. Tsujishita, H.; Hirono, S. *J. Comput. Aided Mol. Des.* **1997**, *11*, 305.
38. Nagase, H.; Imaide, S.; Tomatsu, M.; Hirayama, S.; Nemoto, T.; Sato, N.; Nakajima, M.; Nakao, K.; Mochizuki, H.; Gouda, H.; Hirono, S.; Fujii, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3801.
39. Manglik, A.; Kruse, A. C.; Kobilka, T. S.; Thian, F. S.; Mathiesen, J. M.; Sunahara, R. K.; Pardo, L.; Weis, W. I.; Kobilka, B. K.; Granier, S. *Nature* **2012**, *485*, 321.
40. Chang, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
41. Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Chem.* **1977**, *23*, 327.
42. Halgren, H. *J. Am. Chem. Soc.* **1990**, *112*, 4710.