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

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Supplementary Information

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Figure S1 Observed ROESY spectra of 6

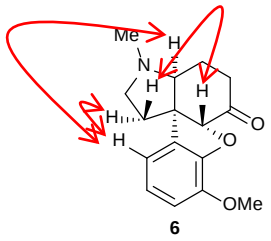

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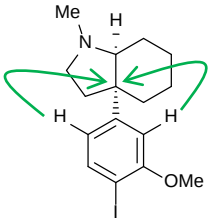
Figure S2 Observed HMBC spectra of 16



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Comparison of binding affinities between mesembrane and *Sceletium tortuosum* Zembrin®

According to Harvey *et al.*, the binding assays was carried out at Cerep in France (http://www.cerep.fr/cerep/users/pages/catalog/p_ProfileCatalogue.asp?profile=2118). As we cannot access to the web page indicated by the above URL, the assay protocol was not clear. However, Cerep shows the protocols of binding assays for the MOR, DOR, and KOR on view (MOR: http://www.cerep.fr/cerep/users/pages/catalog/Affiche_CondExp_Test.asp?test=118; DOR: http://www.cerep.fr/cerep/users/pages/catalog/Affiche_CondExp_Test.asp?test=114; KOR: http://www.cerep.fr/cerep/users/pages/catalog/Affiche_CondExp_Test.asp?test=1971).

Summary of our and Cerep's protocols was shown in Table S1 and S2. In the Cerep's protocol, the labeled ligands were used at two- or four-fold lower concentration compared with our protocol.

Table S1 Our protocol

	MOR	DOR	KOR
source	human recombinant	human recombinant	human recombinant
labeled ligand	[^{3}H] DAMGO	[^{3}H] DPDPE	[^{3}H] U-69,593
ligand concentration	2.0 nM	2.0 nM	2.0 nM
non specific	DAMGO (1.0 μM)	DPDPE (1.0 μM)	U-69,593 (1.0 μM)
reference	DAMGO (IC_{50}: 4.399 nM)	DPDPE (IC_{50}: 4.889 nM)	U-69,593 (IC_{50}: 2.671 nM)

Table S2 Cerep's protocol

	MOR	DOR	KOR
source	human recombinant	human recombinant	rat recombinant
labeled ligand	[^{3}H] DAMGO	[^{3}H] DADLE	[^{3}H] U-69,593
ligand concentration	0.5 nM	0.5 nM	1 nM
non specific	naloxone (10 μM)	naltrexone (10 μM)	naloxone (10 μM)
reference	DAMGO (IC_{50}: 0.537 nM)	DPDPE (IC_{50}: 1.8 nM)	U-50,488 (IC_{50}: 0.84 nM)

The binding abilities of the tested compounds at 10 μM (the highest concentration of the tested compounds) was shown by the not-replaced bindings of the labeled compounds (Table S3).

Table S3 The binding abilities of the tested compounds at 10 μM

Compound	Not-replaced binding (%)		
	MOR	DOR	KOR
mesembrane (**4**)	82.7	92.3	93.0
13′	40.4	39.8	12.1
15	82.8	97.8	59.4
15′	9.4	24.6	3.8
epi-**15′**	25.1	84.9	15.1

In the Cerep's protocol, the labeled ligands and the tested compound were used at lower and extremely higher concentration, respectively compared with our protocol. Both factors leads the values of both not-replacing binding and IC_{50} obtained by Cerep's protocol lower compared with the values obtained by our protocol.

It is difficult to compare the binding abilities between mesembrane and *Sceletium tortuosum* Zembrin®, but compound **13′**, which had the lowest binding affinities among the compounds with phenolic hydroxy group, would have higher affinities than did *Sceletium tortuosum* Zembrin®.

Conformational analyses

Conformational analyses of protonated compounds **13′**, **15′**, and *epi*-**15′** were carried out. The defined dihedral angles are indicated by red arrows (Fig. S3).

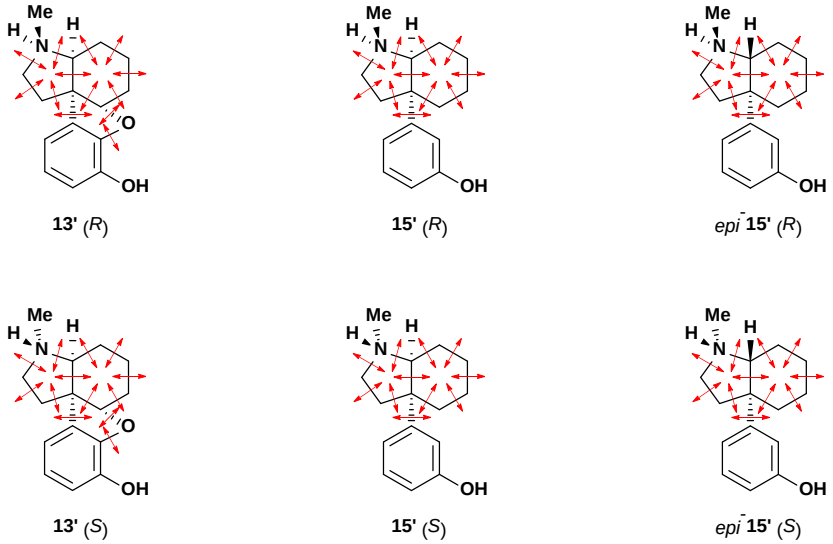

Figure S3 Chemical structures of protonated compounds **13′**, **15′**, and *epi*-**15′**. The red arrows indicates the defined dihedral angles.

The results of the conformational analyses were summarized in Table S4. The most stable conformer of these protonated compounds did not necessarily assume the *S*-configuration at the nitrogen. However, taking into account the conformers with higher energy, all the compounds adopted the *S*-configuration.

Table S4 Energy of the most stable conformer and the number of conformers within 2.5, 3.0, 4.0, 5.0, 7.5 and 10.0 kcal/mol of the most stable conformer.

	most stable conformer (kcal/mol)	< 2.5	< 3.0	< 4.0	< 5.0	< 7.5	< 10.0
13′ (*R*)	61.53	0	1	1	1	6	18
13′ (*S*)	58.55	3	4	7	12	26	36
15′ (*R*)	55.59	0	0	0	4	6	25
15′ (*S*)	50.75	8	9	12	15	47	92
epi-**15′** (*R*)	59.66	2	2	3	8	14	25
epi-**15′** (*S*)	65.65	0	0	0	0	2	5

Copies of NMR spectra