Novel Delta Opioid Receptor Agonist with Oxazatricyclodecane Structure

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Results of cAPM Assays

The functional activities of compounds **4** were also evaluated by cAMP assays (Table S1).

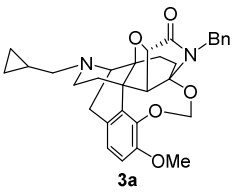
Table S1. Functional activities of **4a**, **c-f**, **h**, **i** for the opioid receptors assessed by cAMP assays[a]

Compd	MOR		DOR		KOR	
	EC_{50} (nM)	E_{max} (%)[b]	EC_{50} (nM)	E_{max} (%)[c]	EC_{50} (nM)	E_{max} (%)[d]
SNC80	ND[e]	ND[e]	13	100	NT[f]	NT[f]
4a	ND[e]	ND[e]	8.0	39	56	21
4c	421	102	204	89	ND[e]	ND[e]
4d	ND[e]	ND[e]	103	75	ND[e]	ND[e]
4e	ND[e]	ND[e]	14	27	ND[e]	ND[e]
4f	NT[f]	NT[f]	121	102	NT[f]	NT[f]
4h	ND[e]	ND[e]	1.7	78	76	41
4i	ND[e]	ND[e]	2.3	40	7.7	28

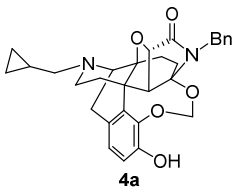
[a]cAMP assays were carried out in duplicate using human MOR, DOR, or KOR expressed CHO cells. [b]E_{max} was calculated as the % of the response obtained with DAMDO. [c]E_{max} was calculated as the % of the response obtained with SNC80. [d]E_{max} was calculated as the % of the response obtained with U-69,593. [e]Not determined. [f]Not tested.

Binding affinities of **3a** and **4a** for the opioid receptors

Binding assays were carried out in duplicate using human opioid receptor recombinant cell (CHO cell) membranes.



3a



4a

Table S2. Binding affinities of **3a** and **4a** for the opioid receptors.

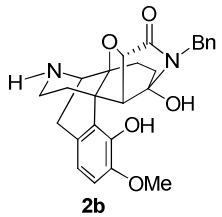
Compd	K_i (nM)			selectivity	
	MOR[a]	DOR[b]	KOR[c]	MOR/DOR	KOR/DOR
3a	1584	456.8	2565	3.5	5.6
4a	26.62	0.809	14.18	32.9	17.5

[a][^{3}H] DAMGO was used. [b][^{3}H] DPDPE was used. [c][^{3}H] U-69,593 was used.

Synthesis of Compounds

Melting points were measured on a Yanaco MT-5 melting point apparatus. Infrared (IR) spectra were measured on a JASCO FT/IR-460Plus. Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent Technologies Mercury-300 (300 MHz) or Agilent Technologies UNITY-400 (400 MHz) spectrometer. Chemical shifts were reported as δ values (ppm) related to tetramethylsilane (TMS). Mass spectra (MS) were measured on a JMSe700 MStation or JMS-T100LP instrument by applying an electron spray ionization (ESI) method. The progress of the reaction was determined on Merck Silica Gel Art. 5715. Column chromatographies were carried out using Kanto Silica Gel 60N. Preparative TLCs were performed using Merck Silica Gel Art. 5744.

(1*S*,3a*S*,5a*S*,6*R*,11b*R*,11c*R*)-3-Benzyl-3a,11-dihydroxy-10-methoxy-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaphtho[1,2-*e*]indol-2-one (2b)

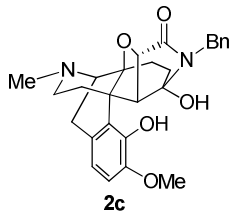


2b

Under an Ar atmosphere, to a solution of **2a** (787 mg, 1.6 mmol) in 1,1,2,2-tetrachloroethane (30 mL) were added potassium carbonate (865 mg, 6.3 mmol) and 2,2,2-trichloroethyl chloroformate (630 μL, 4.7 mmol) and stirred at 150 ˚C for 5 h. The reaction mixture was poured into distilled water and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The obtained residue was roughly purified by silica gel column chromatography. To a solution the obtained material in acetic acid (30 mL) was added zinc powder (3.1 g, 47 mmol) and stirred at rt for 17 h. The reaction mixture was filtered through a celite pad. After removing solvent under reduced pressure, distilled water was added the residue and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **2b** (563 mg) as a white solid in 80% yield.

IR (film, cm^{-1}): 3303, 2937, 1688, 1450, 1278, 754. HR-MS (ESI): Calcd for $C_{26}H_{29}N_2O_5$ $[M+H]^+$: 449.2077. Found: 449.2069. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88-1.03 (m, 1H), 1.22-1.44 (m, 3H), 1.46-1.66 (m, 2H), 2.53-2.76 (m, 2H), 3.05 (d, J = 18.0 Hz, 1H), 3.25-3.45 (m, 3H), 3.83 (s, 3H), 4.37 (d, J = 14.7 Hz, 1H), 4.57 (d, J = 14.7 Hz, 1H), 4.64 (d, J = 6.0 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 8.4 Hz, 1H), 7.14-7.29 (m, 3H), 7.41 (d, J = 6.6 Hz, 2H). three protons were not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.3, 30.7, 32.9, 34.4, 36.4, 42.1, 43.2, 50.8, 55.9, 56.1, 79.7, 81.2, 91.4, 109.5, 119.5, 126.6, 127.2, 128.3, 128.4, 129.5, 138.4, 141.2, 145.4, 170.4.

(1*S*,3a*S*,5a*S*,6*R*,11b*R*,11c*R*)-3-Benzyl-3a,11-dihydroxy-10-methoxy-14-methyl-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaphtho[1,2-*e*]indol-2-one (2c)

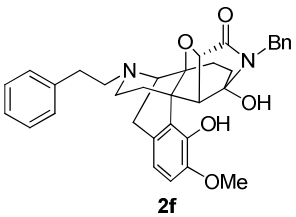


2c

Under an Ar atmosphere, to a solution of **2b** (448 mg, 1.0 mmol) in 1,2-dichloroethane (60 mL) were added paraformaldehyde (300 mg, 10 mmol), acetic acid (475 μL, 8.0 mmol), sodium triacetoxyborohydride (2.10 g, 10 mmol) and stirred at rt for 12 h. To the reaction mixture was added 12 M aqueous ammonia at 0 ˚C and stirred at rt for 30 min. The reaction mixture was poured into distilled water and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to quantitatively give **2c** (462 mg) as a white amorphous material.

IR (film, cm^{-1}): 3390, 2936, 1691, 1488, 1442, 1280, 708. HR-MS (ESI): Calcd for $C_{27}H_{31}N_2O_5$ $[M+H]^+$: 463.2233. Found: 463.2210. ^1H NMR (300 MHz, $CDCl_3$): δ 0.84-1.02 (m, 1H), 1.31-1.47 (m, 3H), 1.55-1.66 (m, 1H), 1.87 (dt, *J* = 5.1, 12.6 Hz, 1H), 2.13 (dt, *J* = 3.3, 12.3 Hz, 1H), 2.34-2.49 (m, 1H), 2.38 (s, 3H), 2.86 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.17 (d, *J* = 18.9 Hz, 1H), 3.23 (d, *J* = 6.0 Hz, 1H), 3.30 (d, *J* = 6.0 Hz, 1H), 3.82 (s, 3H), 4.38 (d, *J* = 14.4 Hz, 1H), 4.49 (d, *J* = 14.7 Hz, 1H), 4.67 (d, *J* = 6.0 Hz, 1H), 6.64-6.74 (m, 2H), 7.12-7.26 (m, 3H), 7.40 (d, *J* = 6.6 Hz, 2H). two protons were not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.6, 30.4, 30.9, 32.1, 41.9, 42.3, 43.1, 44.7, 55.4, 55.9, 57.7, 79.2, 82.2, 91.4, 109.2, 119.4, 126.4, 127.0, 128.1, 128.4, 129.6, 138.5, 140.8, 145.0, 170.3.

(1*S*,3a*S*,5a*S*,6*R*,11b*R*,11c*R*)-3-Benzyl-3a,11-dihydroxy-10-methoxy-14-(2-phenethyl)-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaphtho[1,2-*e*]indol-2-one (2f)

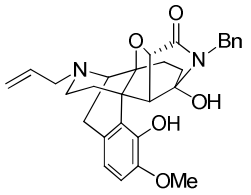


2f

White amorphous material. Yield: 74%. IR (film, cm^{-1}): 3389, 2934, 1685, 1488, 1279, 700. HR-MS (ESI): Calcd for $C_{34}H_{37}N_2O_5$ $[M+H]^+$: 553.2703. Found: 553.2684. ^1H NMR (300 MHz, $CDCl_3$): δ 0.84-1.00 (m, 1H), 1.33-1.49 (m, 3H), 1.62 (dd, *J* = 7.8, 14.4 Hz, 1H), 1.92 (dt, *J* = 5.1, 12.6 Hz, 1H), 2.19 (dt, *J* = 3.3, 12.3 Hz, 1H), 2.60 (dd, *J* = 4.2, 12.3 Hz, 1H), 2.68-2.86 (m, 4H), 2.91 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.16 (d, *J* = 18.6 Hz,

1H), 3.32 (d, *J* = 6.0 Hz, 1H), 3.45 (d, *J* = 6.0 Hz, 1H), 3.85 (s, 3H), 4.41 (d, *J* = 14.7 Hz, 1H), 4.51 (d, *J* = 14.7 Hz, 1H), 4.74 (d, *J* = 6.0 Hz, 1H), 6.64-6.75 (m, 2H), 7.14-7.32 (m, 8H), 7.38-7.45 (m, 2H). two protons were not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.9, 30.6, 31.2, 31.8, 34.5, 42.0, 43.1, 43.5, 55.6, 56.0, 57.3, 79.2, 82.2, 91.4, 109.2, 119.6, 125.9, 126.2, 127.1, 128.27, 128.28, 128.4, 128.7 129.9, 138.6, 140.4, 140.5, 144.7, 170.4.

(1*S*,3a*S*,5a*S*,6*R*,11b*R*,11c*R*)-14-Allyl-3-benzyl-3a,11-dihydroxy-10-methoxy-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaphtho[1,2-*e*]indol-2-one (2e)

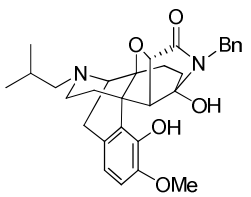


2e

Under an Ar atmosphere, to a solution of **2b** (224 mg, 0.50 mmol) in DMF (5 mL) were added sodium hydrogencarbonate (84.0 mg, 1.0 mmol) and allyl bromide (64.9 μL, 0.75 mmol), and stirred at rt for 1 h. The reaction was poured into distilled water and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **2e** (225 mg) as a white amorphous material in 92% yield.

IR (film, cm^{-1}): 3388, 2932, 1685, 1488, 1279. HR-MS (ESI): Calcd for $C_{29}H_{33}N_2O_5$ $[M+H]^+$: 489.2390. Found: 489.2380. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83-1.00 (m, 1H), 1.31-1.46 (m, 3H), 1.54-1.66 (m, 1H), 1.89 (dt, *J* = 5.1, 12.6 Hz, 1H), 2.12 (dt, *J* = 3.3, 12.3 Hz, 1H), 2.53 (dd, *J* = 5.2, 12.3 Hz, 1H), 2.85 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.07-3.27 (m, 3H), 3.30 (d, *J* = 6.0 Hz, 1H), 3.39 (d, *J* = 6.0 Hz, 1H), 3.85 (s, 3H), 4.40 (d, *J* = 14.4 Hz, 1H), 4.50 (d, *J* = 14.7 Hz, 1H), 4.72 (d, *J* = 5.7 Hz, 1H), 5.11 (dd, *J* = 1.5, 10.2 Hz, 1H), 5.18 (d, *J* = 1.5, 17.4 Hz, 1H), 5.84-6.00 (m, 1H), 6.66-6.74 (m, 2H), 7.14-7.29 (m, 3H), 7.37-7.44 (m, 2H). two protns were not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.3, 30.6, 31.1, 31.9, 42.0, 43.1, 43.2, 54.8, 55.5, 56.0, 58.3, 79.2, 82.2, 91.5, 109.2, 117.4, 119.6, 126.3, 127.1, 128.2, 128.4, 129.9, 136.0, 138.5, 140.5, 144.7, 170.4.

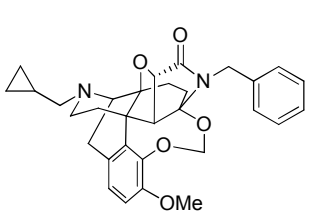
(1*S*,3a*S*,5a*S*,6*R*,11b*R*,11c*R*)-3-Benzyl-3a,11-dihydroxy-14-isobutyl-10-methoxy-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaththo[1,2-*e*]indol-2-one (2d)



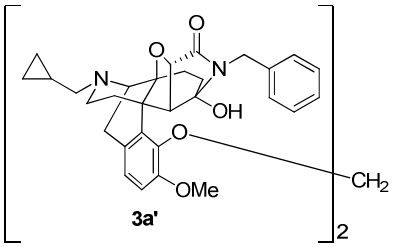
2d

White amorphous material. Yield: 48%. IR (film, cm^{-1}): 3393, 2953, 1684, 1488, 1279, 756. HR-MS (ESI): Calcd for $C_{30}H_{37}N_2O_5$ $[M+H]^+$: 505.2703. Found: 505.2711. ^1H NMR (300 MHz, $CDCl_3$): δ 0.82-0.98 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 1.24-1.41 (m, 3H), 1.50-1.80 (m, 2H), 1.86 (dt, *J* = 5.1, 12.6 Hz, 1H), 2.15-2.49 (m, 4H), 2.95 (dd, *J* = 6.0, 18.3 Hz, 1H), 3.12 (d, *J* = 18.6 Hz, 1H), 3.24-3.32 (m, 1H), 3.30 (d, *J* = 5.4 Hz, 1H), 3.83 (s, 3H), 4.36 (d, *J* = 14.7 Hz, 1H), 4.58 (d, *J* = 14.7 Hz, 1H), 4.66 (d, *J* = 5.7 Hz, 1H), 6.68 (d, *J* = 8.7 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 7.14-7.27 (m, 3H), 7.36-7.43 (m, 2H). two protons were not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 20.9, 26.4, 29.5, 30.6, 31.1, 41.9, 43.2, 43.3, 55.7, 55.9, 56.4, 63.4, 79.2, 82.3, 91.6, 109.1, 119.5, 126.3, 127.1, 128.2, 128.4, 130.1, 138.6, 140.6, 144.7, 170.8.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-7-(cycloprpylmethyl)-1-methoxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3a) and (1*S*,1'*S*,3a*S*,3'a*S*,5a*S*,5'a*S*,6*R*,6'*R*,11b*R*,11'b*R*,11c*R*,11'c*R*)-11,11'-(methylenbis(oxy))bis(3-benzyl-14-(cyclopropylmethyl)-3a-dihydroxy-10-methoxy-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaphtho[1,2-*e*]indol-2-one) (3a')



3a



3a'

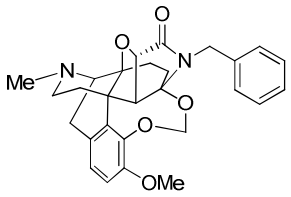
Under an Ar atmosphere, to a solution of **2a** (25.1 mg, 0.050 mmol) in DMF (100 mL) were added potassium carbonate (2.07 g, 15 mmol) and dibromomethane (520 μL, 7.2 mmol), and stirred at rt for 15 h. After removing the solvent under reduced pressure, to the residue was added saturated aqueous solution of sodium hydrogencarbonate and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by preparative TLC to give **3a** (16.8 mg) as a colorless oil in 66% yield and **3a'** (7.6 mg) as a colorless oil in 30 % yield.

3a: IR (film, cm^{-1}): 2925, 1705, 1491, 1093, 730. HR-MS (ESI): Calcd for $C_{31}H_{35}N_2O_5$ $[M+H]^+$: 515.2546. Found: 515.2556. ^1H NMR (300 MHz, $CDCl_3$): δ 0.06-0.19 (m, 2H), 0.44-0.63 (m, 2H), 0.90-1.12 (m, 2H), 1.27-1.41 (m, 2H), 1.46-1.65 (m, 2H), 1.93 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.22-2.40 (m, 2H), 2.68-2.86 (m, 3H), 3.18-3.35 (m, 1H), 3.51 (d, *J* = 6.0 Hz, 1H), 3.73-3.81 (m, 1H), 3.83 (s, 3H), 4.35 (d, *J* = 14.7 Hz, 1H), 4.46 (d, *J* = 14.7 Hz, 1H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.81 (d, *J* = 7.2 Hz, 1H), 5.74 (d, *J* = 7.2 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 1H), 7.16-7.30 (m, 3H), 7.34-7.44 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.1, 4.8, 8.9, 22.1, 26.9, 30.5, 32.8, 42.1, 42.7, 43.1, 54.5, 55.85, 55.91, 59.9, 78.9, 82.6, 95.0, 96.4, 111.5, 124.5, 127.2, 128.3, 128.4, 128.8, 134.9, 138.1, 144.4, 150.3, 170.5.

3a': IR (film, cm^{-1}): 3463, 2929, 1695, 1453, 1276. HR-MS (ESI): Calcd for $C_{61}H_{69}N_4O_{10}$ $[M+H]^+$: 1017.5014. Found: 1017.5027. ^1H NMR (300 MHz, $CDCl_3$): δ 0.03-0.17 (m, 4H), 0.42-0.60 (m, 4H), 0.72-1.01 (m, 4H),

1.17-1.51 (m, 6H), 1.54-1.71 (m, 2H), 1.90-2.36 (m, 6H), 2.57-2.75 (m, 4H), 2.83-2.98 (m, 2H), 3.11 (d, *J* = 18.6 Hz, 2H), 3.32 (d, *J* = 5.7 Hz, 2H), 3.53 (s, 6H), 3.72 (d, *J* = 5.7 Hz, 2H), 4.43 (d, *J* = 14.7 Hz, 2H), 4.47 (d, *J* = 14.7 Hz, 2H), 4.67 (s, 2H), 4.72 (d, *J* = 6.0 Hz, 2H), 5.51 (s, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 7.14-7.31 (m, 6H), 7.38-7.49 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.0, 4.5, 8.9, 27.1, 30.3, 31.1, 33.5, 41.9, 43.4, 43.5, 54.3, 55.0, 55.4, 59.8, 79.1, 82.1, 91.7, 99.4, 111.1, 124.4, 127.1, 128.1, 128.8, 129.7, 133.7, 138.6, 141.2, 149.7, 170.1.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-7-methyl-1-methoxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3c)

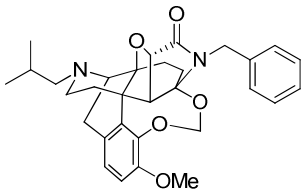


3c

Under an Ar atmosphere, to a suspension of bromochloromethane (2.27 mL, 340 mmol) and potassium carbonate (34.0 g, 680 mmol) in DMF (1000 mL) was added a solution of **2c** (187 mg, 0.41 mmol) in DMF (5 mL) at rt portion-wise (x 5) with 12 h intervals. After the final addition of the DMF solution of **2c**, the reaction mixture was stirred at rt for 12 h. After removing solvent under reduced pressure, the reaction mixture was poured into distilled water and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by preparative TLC to give **3c** (161 mg) as a white amorphous material in 84% yield.

IR (film, cm^{-1}): 2935, 1705, 1491, 1269. HR-MS (ESI): Calcd for $C_{28}H_{31}N_2O_5$ $[M+H]^+$: 475.2233. Found: 475.2216. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (dd, *J* = 5.7, 14.1 Hz, 1H), 1.27-1.42 (m, 2H), 1.46-1.64 (m, 2H), 1.90 (dt, *J* = 5.7, 12.9 Hz, 1H), 2.27 (dt, *J* = 3.6, 12.3 Hz, 1H), 2.41 (s, 3H), 2.50 (dd, *J* = 5.1, 12.3 Hz, 1H), 2.74 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.26 (d, *J* = 6.0 Hz, 1H), 3.33 (d, *J* = 18.3 Hz, 1H), 3.50 (d, *J* = 6.0 Hz, 1H), 3.83 (s, 3H), 4.36 (d, *J* = 14.4 Hz, 1H), 4.43 (d, *J* = 14.4 Hz, 1H), 4.72 (d, *J* = 6.0 Hz, 1H), 4.82 (d, *J* = 7.2 Hz, 1H), 5.74 (d, *J* = 7.5 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.16-7.29 (m, 3H), 7.39 (d, *J* = 6.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.0, 25.9, 30.3, 33.3, 41.3, 42.7, 43.3, 44.4, 55.8, 55.9, 57.9, 79.0, 82.7, 95.0, 96.3, 111.4, 124.5, 127.2, 128.3, 128.4, 128.8, 134.8, 138.0, 144.3, 150.2, 170.4.

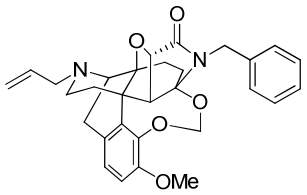
(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-7-isobutyl-1-methoxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3d)



3d

Colorless oil. Yield: 69%. IR (film, cm^{-1}): 2951, 1704, 1491, 1092, 754. HR-MS (ESI): Calcd for $C_{31}H_{37}N_2O_5$ $[M+H]^+$: 517.2702. Found: 517.2716. ^1H NMR (300 MHz, $CDCl_3$): δ 0.90 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.3 Hz, 3H), 0.94-1.04 (m, 1H), 1.17-1.57 (m, 4H), 1.61-1.78 (m, 1H), 1.87 (dt, *J* = 6.0, 12.6 Hz, 1H), 2.22-2.52 (m, 4H), 2.82 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.18-3.32 (m, 2H), 3.48 (d, *J* = 6.0 Hz, 1H), 3.82 (s, 3H), 4.32 (d, *J* = 14.7 Hz, 1H), 4.53 (d, *J* = 14.7 Hz, 1H), 4.68 (d, *J* = 6.0 Hz, 1H), 4.80 (d, *J* = 7.2 Hz, 1H), 5.73 (d, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.15-7.29 (m, 3H), 7.34-7.41 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 20.9, 22.1, 26.6, 29.3, 30.5, 32.3, 42.2, 42.6, 42.8, 55.9, 56.1, 56.4, 63.4, 78.9, 82.8, 95.1, 96.4, 111.3, 124.4, 127.2, 128.2, 128.3, 129.3, 135.0, 138.3, 144.4, 150.1, 170.9.

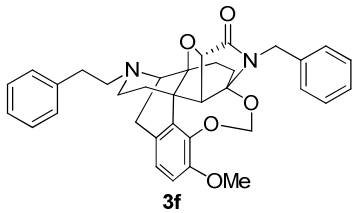
(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-Allyl-11-benzyl-1-mthoxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3e)



3e

White amorphous material. Yield: 87%. IR (film, cm^{-1}): 2926, 1705, 1491, 1093, 752. HR-MS (ESI): Calcd for $C_{30}H_{33}N_2O_5$ $[M+H]^+$: 501.2390. Found: 501.2387. ^1H NMR (300 MHz, $CDCl_3$): δ 0.98-1.10 (m, 1H), 1.23-1.63 (m, 4H), 1.90 (dt, *J* = 5.7, 12.9 Hz, 1H), 2.31 (dt, *J* = 3.3, 12.6 Hz, 1H), 2.57 (dd, *J* = 4.8, 12.6 Hz, 1H), 2.73 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.17 (dd, *J* = 7.5, 13.5 Hz, 1H), 3.22-3.36 (m, 2H), 3.42 (d, *J* = 6.0 Hz, 1H), 3.50 (d, *J* = 6.0 Hz, 1H), 3.83 (s, 3H), 4.35 (d, *J* = 14.7 Hz, 1H), 4.45 (d, *J* = 14.4 Hz, 1H), 4.73 (d, *J* = 6.6 Hz, 1H), 4.81 (d, *J* = 6.9 Hz, 1H), 5.14 (d, *J* = 10.2 Hz, 1H), 5.21 (dd, *J* = 1.5, 17.4 Hz, 1H), 5.74 (d, *J* = 7.2 Hz, 1H), 5.86-6.02 (m, 1H), 6.83 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 7.14-7.30 (m, 3H), 7.35-7.44 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.1, 26.8, 30.4, 32.8, 42.0, 42.7, 42.9, 54.7, 55.8, 55.9, 58.3, 78.9, 82.6, 95.0, 96.4, 111.4, 117.6, 124.4, 127.2, 128.28, 128.34, 128.8, 134.8, 135.8, 138.1, 144.4, 150.2, 170.5.

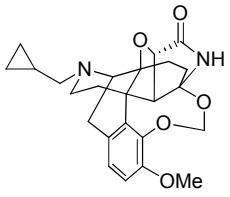
(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-1-methoxy-7-(2-phenethyl)-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3f)



3f

White amorphous material. Yield: 98%. IR (film, cm^{-1}): 2935, 1704, 1492, 1093, 701. HR-MS (ESI): Calcd for $C_{35}H_{37}N_2O_5$ $[M+H]^+$: 565.2703. Found: 565.2679. ^1H NMR (300 MHz, $CDCl_3$): δ 0.99-1.11 (m, 1H), 1.22-1.64 (m, 4H), 1.92 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.30-2.47 (m, 1H), 2.66 (dd, *J* = 4.8, 12.6 Hz, 1H), 2.72-2.93 (m, 5H), 3.34 (d, *J* = 18.3 Hz, 1H), 3.45-3.54 (m, 2H), 3.83 (s, 3H), 4.36 (d, *J* = 14.4 Hz, 1H), 4.46 (d, *J* = 14.4 Hz, 1H), 4.74 (d, *J* = 6.0 Hz, 1H), 4.81 (d, *J* = 7.5 Hz, 1H), 5.74 (d, *J* = 7.2 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 1H), 7.14-7.33 (m, 8H), 7.35-7.42 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.1, 27.6, 30.5, 32.5, 34.2, 42.0, 42.7, 42.9, 55.75, 55.81, 55.9, 57.3, 78.8, 82.6, 95.0, 96.4, 111.5, 124.5, 126.0, 127.2, 128.3, 128.6, 128.7, 134.6, 138.0, 140.1, 144.4, 150.3, 170.5.

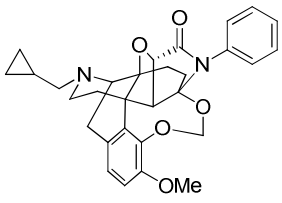
(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-(Cyclopropylmethyl)-1-methoxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3g)



3g

White amorphous material. Yield: 93%. IR (film, cm^{-1}): 3217, 2940, 1713, 1092, 754. HR-MS (ESI): Calcd for $C_{24}H_{29}N_2O_5$ $[M+H]^+$: 425.2077. Found: 425.2069. ^1H NMR (300 MHz, $CDCl_3$): δ 0.04-0.19 (m, 2H), 0.41-0.60 (m, 2H), 0.86-1.02 (m, 1H), 1.17-1.41 (m, 2H), 1.47-1.84 (m, 3H), 1.89 (dt, *J* = 5.7, 12.9 Hz, 1H), 2.16-2.48 (m, 2H), 2.61-2.89 (m, 3H), 3.28 (d, *J* = 18.6 Hz, 1H), 3.54 (d, *J* = 5.7 Hz, 1H), 3.78 (d, *J* = 6.0 Hz, 1H), 3.84 (s, 3H), 4.68 (d, *J* = 6.0 Hz, 1H), 4.77 (d, *J* = 7.2 Hz, 1H), 5.68 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 7.41 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 2.8, 4.8, 8.8, 25.4, 26.8, 30.8, 32.9, 42.2, 43.2, 54.3, 55.8, 56.2, 59.8, 79.6, 82.4, 91.9, 96.2, 111.4, 124.4, 128.8, 134.6, 144.3, 150.2, 172.7.

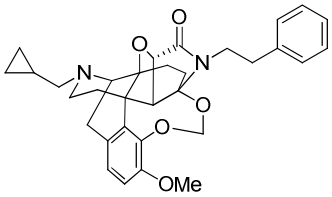
(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-(Cyclopropylmethyl)-1-methoxy-11-phenyl-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3h)



3h

Colorless oil. Yield: 73%. IR (film, cm^{-1}): 2950, 1714, 1492, 1043, 753. HR-MS (ESI): Calcd for $C_{30}H_{33}N_2O_5$ $[M+H]^+$: 501.2390. Found: 501.2412. ^1H NMR (300 MHz, $CDCl_3$): δ 0.05-0.20 (m, 2H), 0.42-0.62 (m, 2H), 0.82-1.03 (m, 1H), 1.11-1.44 (m, 3H), 1.66-1.85 (m, 2H), 1.98 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.23-2.41 (m, 2H), 2.72 (dd, *J* = 5.4, 12.6 Hz, 2H), 2.82 (dd, *J* = 6.6, 18.6 Hz, 1H), 3.28 (d, *J* = 18.6 Hz, 1H), 3.68 (d, *J* = 6.0 Hz, 1H), 3.80 (d, *J* = 6.9 Hz, 1H), 3.81 (s, 3H), 4.68 (d, *J* = 7.2 Hz, 1H), 4.85 (d, *J* = 6.0 Hz, 1H), 5.73 (d, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.24-7.41 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 2.9, 4.6, 9.0, 21.2, 27.1, 31.0, 32.8, 42.4, 43.1, 54.6, 55.4, 55.8, 59.8, 79.0, 82.6, 96.1, 96.3, 111.4, 124.4, 127.9, 128.5, 128.7, 129.0, 134.1, 134.8, 144.3, 150.2, 170.5.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-(Cyclopropylmethyl)-1-methoxy-11-(2-phenethyl)-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (3i)

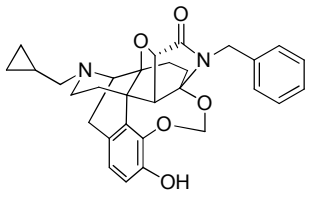


3i

Under an Ar atmosphere, to a solution of **3g** (118 mg, 0.28 mmol) in DMF (3 mL) was added sodium hydride (60% in oil, 55.7 mg, 1.4 mmol) and sttired at rt for 10 min. To the reaction mixture was added (2-bromoethyl)benzene (381 μL, 2.8 mmol) and stirred at rt for 1 h. The reaction mixture was poured into cold distilled water and extracted with chloroform and ethanol (3:1 mixture) and then chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **3i** (122 mg) as a colorless oil in 83% yield. IR (film, cm^{-1}): 2935, 1703, 1092, 752. HR-MS (ESI): Calcd for $C_{32}H_{37}N_2O_5$ $[M+H]^+$: 529.2703. Found:

529.2708. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04-0.17 (m, 2H), 0.42-0.60 (m, 2H), 0.86-1.04 (m, 1H), 1.18 (dd, *J* = 6.6, 14.4 Hz, 1H), 1.27-1.73 (m, 4H), 1.93 (dt, *J* = 5.4, 13.2 Hz, 1H), 2.18-2.37 (m, 2H), 2.60-3.15 (m, 6H), 3.27 (d, *J* = 17.4 Hz, 1H), 3.49 (d, *J* = 6.0 Hz, 1H), 3.52-3.66 (m, 1H), 3.75 (d, *J* = 5.4, 1H), 3.85 (s, 3H), 4.74 (d, *J* = 6.0 Hz, 1H), 4.79 (d, *J* = 7.2 Hz, 1H), 5.70 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 7.14-7.33 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.1, 4.7, 9.0, 21.9, 26.9, 30.6, 32.9, 35.3, 41.1, 42.2, 43.1, 54.5, 55.7, 55.9, 59.9, 79.0, 82.6, 95.1, 96.4, 111.5, 124.5, 126.2, 128.3, 128.8, 134.9, 139.3, 144.4, 150.3, 170.3.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-7-(cyclopropylmethyl)-1-hydroxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4a)



4a

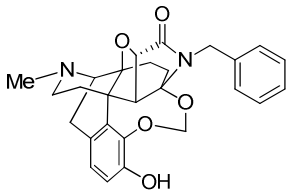
Under an Ar atmosphere, to a solution of **3a** (103 mg, 0.20 mmol) in dichloromethane (7 ml) was added 1.0 M solution of boron tribromide in dichloromethane (1.0 mL, 1.0 mmol) at 0 ˚C and stirred at rt for 30 min. To the reaction mixture was added saturated aqueous solution of potassium carbonate (7 mL) at 0 ˚C and stirred at rt for 1 h, and further added 12 M aqueous annmonia (7 mL) at 0 ˚C and stirred at rt for 1 h. The mixture was poured into distilled water and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **4a** (89.4 mg) as a colorless oil in 85% yield. The free base **4a** was converted into camphorsulfonate of **4a**.

IR (film, cm^{-1}): 3287, 2925, 1695, 1453, 1092, 1027. HR-MS (ESI): Calcd for $C_{30}H_{33}N_2O_5$ $[M+H]^+$: 501.2390. Found: 501.2382. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03-0.20 (m, 2H), 0.41-0.62 (m, 2H), 0.86-1.16 (m, 2H), 1.21-1.72 (m, 4H), 1.89 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.20-2.40 (m, 2H), 2.58-2.83 (m, 3H), 3.23 (d, *J* = 18.3 Hz, 1H), 3.46 (d, *J* = 5.7 Hz, 1H), 3.74 (d, *J* = 6.0 Hz, 1H), 4.36 (d, *J* = 14.7 Hz, 1H), 4.47 (d, *J* = 15.0 Hz, 1H), 4.72 (d, *J* = 6.0 Hz, 1H), 4.86 (d, *J* = 7.5 Hz, 1H), 5.78 (d, *J* = 7.2 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 7.13-7.29 (m, 3H), 7.38 (d, *J* = 6.9 Hz, 2H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.2, 4.6, 8.7, 22.9, 27.0, 30.4, 32.7, 41.9, 42.7, 43.0, 54.7, 55.7, 59.8, 78.9, 82.7, 95.2, 96.2, 116.1, 124.9, 127.2, 127.5, 128.2, 128.3, 133.7, 137.8, 143.0, 147.5, 170.8.

4a·CSA

Anal Calcd for $C_{30}H_{32}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 0.8H_2O$: C 64.29; H 6.69; N 3.75. Found: C 64.27; H 6.73; N 3.81. mp (dec): 208.0 ˚C.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-7-methyl-1-hydroxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4c)



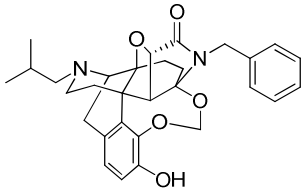
4c

Yellow oil. Yield: 76%. IR (film, cm^{-1}): 3231, 2936, 1693, 1092, 1026, 752. HR-MS (ESI): Calcd for $C_{27}H_{29}N_2O_5$ $[M+H]^+$: 461.2077. Found: 461.2073. ^1H NMR (300 Mz, $CDCl_3$): δ 1.02-1.16 (m, 1H), 1.33 (br d, *J* = 12.0 Hz, 1H), 1.39-1.71 (m, 3H), 1.87 (dt, *J* = 5.4, 12.6 Hz, 1H), 2.20-2.37 (m, 1H), 2.41 (s, 3H), 2.50 (dd, *J* = 4.8, 14.7 Hz, 1H), 2.75 (dd, *J* = 6.3, 18.6 Hz, 1H), 3.21-3.36 (m, 2H), 3.46 (d, *J* = 6.0 Hz, 1H), 4.37 (d, *J* = 14.7 Hz, 1H), 4.44 (d, *J* = 14.7 Hz, 1H), 4.70 (d, *J* = 6.0 Hz, 1H), 4.87 (d, *J* = 7.2 Hz, 1H), 5.77 (d, *J* = 7.5 Hz, 1H), 6.81-6.90 (m, 2H), 7.15-7.31 (m, 3H), 7.38 (d, *J* = 6.9 Hz, 2H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.8, 26.1, 30.2, 33.2, 41.1, 42.7, 43.1, 44.4, 55.6, 58.0, 79.0, 82.9, 95.1, 96.2, 116.1, 124.9, 127.2, 127.5, 128.3, 133.7, 137.7, 142.9, 147.6, 170.7.

4c·CSA

Anal Calcd for $C_{27}H_{28}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 2.3H_2O$: C 60.52; H 6.67; N 3.82. Found: C 60.56; H 6.41; N 4.11. mp (dec): 209.0 ˚C.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-1-hydroxy-7-isobutyl-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4d)



4d

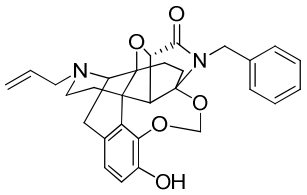
Colorless oil. Yield: 92%. IR (film, cm^{-1}): 3303, 2952, 1692, 1090, 1026, 753. HR-MS (ESI): Calcd for $C_{30}H_{35}N_2O_5$ $[M+H]^+$: 503.2546. Found: 503.2544. ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.97-1.12 (m, 1H), 1.14-1.24 (m, 1H), 1.35-1.77 (m, 4H), 1.84 (dt, *J* = 6.0, 12.6 Hz, 1H), 2.22-2.52 (m, 4H), 2.81 (dd, *J* = 6.3, 18.3 Hz, 1H), 3.22 (d, *J* = 18.3 Hz, 1H), 3.29 (d, *J* = 6.0 Hz, 1H), 3.43 (d, *J* = 6.0 Hz, 1H), 4.35 (d, *J* = 14.7 Hz, 1H), 4.51 (d, *J* = 15.0 Hz, 1H), 4.69 (d, *J* = 6.0 Hz, 1H), 4.85 (d, *J* = 7.2 Hz, 1H), 5.77 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 7.15-7.29 (m, 3H), 7.34-7.42 (m, 2H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 20.9, 21.9, 26.5, 29.1, 30.4, 32.4, 42.1, 42.6, 42.9, 55.8, 56.3, 63.3, 78.9, 82.9, 95.4, 96.2, 115.6, 124.9, 127.3, 128.2, 128.3, 128.5, 133.9,

138.0, 142.8, 147.0, 171.2.

4d·CSA

Anal Calcd for $C_{30}H_{34}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 1.2H_2O$: C 63.51; H 6.98; N 3.70. Found: C 63.54; H 6.94; N 3.89. mp (dec): 207.0 ˚C.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-Allyl-11-benzyl-1-hydroxy-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4e)



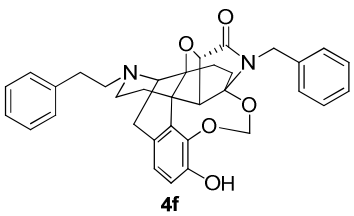
4e

Colorless oil. Yield: 89%. IR (film, cm^{-1}): 3278, 2927, 1693, 1092, 1027, 753. HR-MS (ESI): Calcd for $C_{29}H_{31}N_2O_5$ $[M+H]^+$: 487.2220. Found: 487.2233. ^1H NMR (300 MHz, $CDCl_3$): δ 1.01-1.14 (m, 1H), 1.22-1.35 (m, 1H), 1.38-1.69 (m, 3H), 1.87 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.30 (dt, *J* = 3.3, 12.3 Hz, 1H), 2.51-2.62 (m, 1H), 2.73 (dd, *J* = 6.6, 18.6 Hz, 1H), 3.10-3.32 (m, 3H), 3.42 (d, *J* = 6.0 Hz, 1H), 3.46 (d, *J* = 6.0 Hz, 1H), 4.37 (d, *J* = 14.7 Hz, 1H), 4.45 (d, *J* = 14.7 Hz, 1H), 4.74 (d, *J* = 6.0 Hz, 1H), 4.86 (d, *J* = 7.2 Hz, 1H), 5.13 (d, *J* = 10.2 Hz, 1H), 5.21 (dd, *J* = 1.2, 17.4 Hz, 1H), 5.78 (d, *J* = 7.2 Hz, 1H), 5.83-6.00 (m, 1H), 6.867 (d, *J* = 8.4 Hz, 1H) , 6.874 (d, *J* = 8.4 Hz, 1H), 7.16-7.28 (m, 3H), 7.34-7.42 (m, 2H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.9, 26.9, 30.3, 32.9, 41.9, 42.7, 42.8, 54.8, 55.6, 58.3, 78.9, 82.8, 95.2, 96.2, 115.9, 117.9, 124.9, 127.3, 127.9, 128.28, 128.32, 133.8, 135.6, 137.8, 142.8, 147.3, 170.8.

4e·CSA

Anal Calcd for $C_{29}H_{30}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 1.1H_2O$: C 63.41; H 6.58; N 3.79. Found: C 63.34; H 6.53; N 3.90. mp (dec): 209.0 ˚C.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-11-Benzyl-1-hydroxy-7-(2-phenethyl)-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4f)



4f

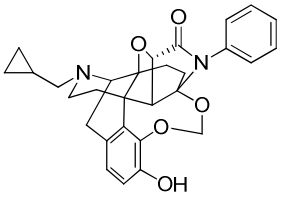
Colorless oil. Yield: 89%. IR (film, cm^{-1}): 3348, 2926, 1688, 1092, 1027, 750, 700. HR-MS (ESI): Calcd

for $C_{34}H_{35}N_2O_5$ $[M+H]^+$: 551.2546. Found: 551.2547. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01-1.16 (m, 1H), 1.22-1.35 (m, 1H), 1.39-1.71 (m, 3H), 1.88 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.27-2.44 (m, 1H), 2.63 (dd, *J* = 4.8, 12.3 Hz, 1H), 2.69-2.87 (m, 5H), 3.28 (d, *J* = 18.6 Hz, 1H), 3.47 (d, *J* = 5.7 Hz, 2H), 4.37 (d, *J* = 14.7 Hz, 1H), 4.46 (d, *J* = 14.7 Hz, 1H), 4.75 (d, *J* = 6.0 Hz, 1H), 4.87 (d, *J* = 7.2 Hz, 1H), 5.78 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 8.4, 1H), 7.14-7.30 (m, 8H), 7.36-7.41 (m, 2H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.9, 27.5, 30.4, 32.8, 34.2, 41.9, 42.7, 43.0, 55.6, 55.7, 57.2, 78.9, 82.8, 95.2, 96.2, 116.0, 124.9, 126.0, 127.3, 127.8, 128.25, 128.30, 128.7, 133.8, 137.8, 140.1, 142.9, 147.4, 170.8.

4f·CSA

Anal Calcd for $C_{34}H_{34}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 0.3H_2O$: C 67.04; H 6.47; N 3.55. Found: C 67.08; H 6.44; N 3.58. mp (dec): 255.0 ˚C.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-(Cyclopropylmethyl)-1-hydroxy-11-phenyl-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4h)



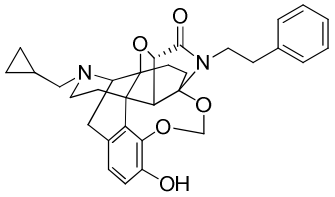
4h

Colorless oil. Yield: 89%. IR (film, cm^{-1}): 3303, 2925, 1702, 1039, 730. HR-MS (ESI): Calcd for $C_{29}H_{31}N_2O_5$ $[M+H]^+$: 487.2233. Found: 487.2237. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.06-0.18 (m, 2H), 0.44-0.60 (m, 2H), 0.87-1.01 (m, 1H), 1.13-1.27 (m, 1H), 1.29-1.49 (m, 2H), 1.70-1.84 (m, 2H), 1.96 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.24-2.40 (m, 2H), 2.62-2.77 (m, 2H), 2.82 (dd, *J* = 6.6, 18.6 Hz, 1H), 3.25 (d, *J* = 18.3 Hz, 1H), 3.63 (d, *J* = 5.7 Hz, 1H), 3.80 (d, *J* = 6.0 Hz, 1H), 4.71 (d, *J* = 7.2 Hz, 1H), 4.84 (d, *J* = 6.0 Hz, 1H), 5.75 (d, *J* = 7.5 Hz, 1H), 6.82-6.91 (m, 2H), 7.25-7.40 (m, 5H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.2, 4.7, 9.0, 21.1, 27.3, 30.9, 33.0, 42.3, 43.1, 54.8, 55.2, 59.9, 79.1, 82.7, 96.2, 96.3, 115.8, 125.0, 128.2, 128.7, 129.1, 133.9, 134.0, 142.8, 147.2, 170.7.

4h·CSA

Anal Calcd for $C_{29}H_{31}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 1.1H_2O$: C 63.41; H 6.58; N 3.79. Found: C 63.39; H 6.58; N 3.89. mp (dec): 229.0 ˚C.

(4b*R*,8*R*,8a*S*,9a*S*,11a*S*,11b*R*)-7-(Cyclopropylmethyl)-1-hydroxy-11-(2-phenethyl)-5,6,7,8,9a,11b-hexahydro-8a,11a-ethano-4,8-methano-9,12,14-trioxa-7,11-diazabenzo[*a*]benzo[4,5]cycloocta[1,2,3-*gh*]pentalen-10(11*H*)-one (4i)



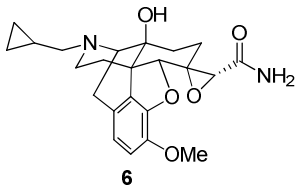
4i

Colorless oil. Yield: 61%. IR (film, cm^{-1}): 3230, 2927, 1687, 1092, 1027, 753. HR-MS (ESI): Calcd for $C_{31}H_{35}N_2O_5$ $[M+H]^+$: 515.2546. Found: 515.2526. ^1H NMR (300 MHz, $CDCl_3$): δ 0.05-0.19 (m, 2H), 0.41-0.61 (m, 2H), 0.82-1.03 (m, 1H), 1.12-1.36 (m, 2H), 1.46-1.80 (m, 3H), 1.91 (dt, *J* = 5.4, 12.9 Hz, 1H), 2.20-2.39 (m, 2H), 2.58-3.18 (m, 6H), 3.25 (d, *J* = 18.6 Hz, 1H), 3.45 (d, *J* = 6.0 Hz, 1H), 3.54-3.68 (m, 1H), 3.75 (d, *J* = 6.0, 1H), 4.73 (d, *J* = 6.0 Hz, 1H), 4.87 (d, *J* = 7.2 Hz, 1H), 5.78 (d, *J* = 7.2 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 7.13-7.32 (m, 5H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.2, 4.6, 8.8, 21.7, 27.1, 30.5, 32.9, 35.3, 41.2, 42.0, 43.1, 54.7, 55.4, 59.9, 79.0, 82.8, 95.4, 96.2, 115.8, 125.0, 126.3, 128.1, 128.4, 128.8, 133.8, 139.1, 142.8, 147.3, 170.6.

4i·CSA

Anal Calcd for $C_{31}H_{34}N_2O_5 \cdot C_{10}H_{16}O_4S \cdot 1.1H_2O$: C 64.23; H 6.86; N 3.65. Found: C 64.25; H 6.87; N 3.98. mp (dec): 187.0 ˚C.

(5*R*,6*S*,6'*R*,9*R*,13*S*,14*S*)-17-(cyclopropylmethyl)-4,5-epoxy-6,6'-epoxy-14-hydroxy-3-methoxy-6-methylmorphinan-6'-carboxyamide (6)

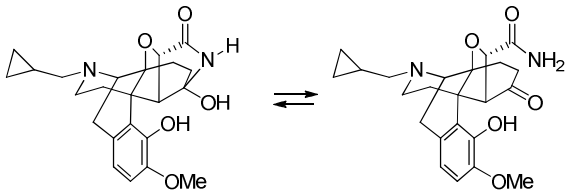


6

Under an Ar atmosphere, to a solution of **5** (2.0 g, 4.8 mmol) in EtOH (60 mL) was added 12 M aqueous ammonia solution (20 mL) and stirred at rt for 3 days. To the reaction mixture was added further 12 M aqueous ammonia solution (20 mL) and stirred at rt for 2 days. After removing a solvent under reduced pressure, the residue was poured into saturated aqueous solution of sodium hydrogen carbonate and extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **6** (1.36 g) as a white amorphous material in 73% yield.

IR (film, cm^{-1}): 3349, 2928, 1686, 1438, 1052, 750. HR-MS (ESI): Calcd for $C_{23}H_{29}N_2O_5$ $[M+H]^+$: 413.2077. Found: 413.2061. ^1H NMR (300 MHz, $CDCl_3$): δ 0.09-0.20 (m, 2H), 0.47-0.61 (m, 2H), 0.77-0.92 (m, 1H), 1.39 (td, *J* = 3.6, 14.4 Hz, 1H), 1.43-1.71 (m, 3H), 2.13 (dt, *J* = 3.6, 12.0 Hz, 1H), 2.22-2.44 (m, 4H), 2.57-2.71 (m, 2H), 3.06 (d, *J* = 18.9 Hz, 1H), 3.11 (d, *J* = 6.0 Hz, 1H), 3.61 (s, 1H), 3.87 (s, 3H), 4.73 (s, 1H), 5.72 (br d, *J* = 2.7 Hz, 1H), 6.12 (br d, *J* = 3.3 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H). a proton was not observed. ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.7, 3.9, 9.4, 21.3, 22.6, 28.6, 30.9, 44.0, 48.0, 56.8, 57.4, 59.1, 62.2, 62.8, 70.1, 85.6, 114.8, 118.8, 125.3, 130.3, 142.1, 145.7, 169.8.

An equilibrium mixture of (1*S*,3a*S*,5a*S*,6*R*,11b*R*,11c*R*)-14-(cyclopropylmethyl)-3a,11-dihydroxy-10-methoxy-1,3,3a,4,5,6,7,11c-octahydro-2*H*-6,11b-(iminoethano)-1,5a-epoxynaphtho[1,2-*e*]indol-2-one (2g) and (4*R*,4a*R*,10*R*,10a*S*,12*S*)-13-(cyclopropylmethyl)-5,6-dihydroxy-3-oxo-1,2,3,4,9,10-hexahydro-10,4a-(iminoethano)-10a,4-(epoxymethano)phenanthren-12-carboxyamide (7g)



2g ⇌ **7g**

According to the synthetic method of **2a**, an equilibrium mixture of **2g** and **7g** was quantitatively obtained from **6**.

IR (film, cm^{-1}): 3279, 2935, 1702, 1488, 1280, 755. HR-MS (ESI): Calcd for $C_{23}H_{29}N_2O_5$ $[M+H]^+$: 413.2077. Found: 413.2072. ^1H NMR (300 MHz, $CDCl_3$): δ 0.00-0.14 (m, 2H), 0.37-0.60 (m, 2H), 0.81-0.96 (m, 1H), 1.07-1.27 (m, 1H), 1.47-2.18 (m, 6H), 2.26 (dd, *J* = 7.2, 12.6 Hz, 1H), 2.51-2.72 (m, 2H), 2.77 (dd, *J* = 6.3, 18.6 Hz, 0.1H), 2.92 (dd, *J* = 6.3, 18.6 Hz, 0.9H), 3.10 (d, *J* = 18.6 Hz, 1H), 3.48 (d, *J* = 5.4 Hz, 0.9H), 3.69 (d, *J* = 6.0 Hz, 1H), 3.81 (s, 3H), 4.01 (d, *J* = 5.7 Hz, 0.1H), 4.65 (d, *J* = 5.7 Hz, 0.9H), 5.09 (d, *J* = 5.7 Hz, 0.1H), 5.24 (br s, 1.9H), 6.56-6.76 (m, 2H), 6.96 (br s, 0.2H), 7.17 (br s, 0.9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.1, 4.5, 8.9, 27.3, 31.5, 32.0, 33.1, 43.5, 43.6, 54.7, 55.6, 55.9, 59.8, 80.1, 82.1, 88.7, 109.4, 119.0, 126.7, 129.8, 141.5, 145.5, 172.6 (Major signals were shown).

Opioid Receptor Binding Assay

Mouse whole brain without cerebellum and guinea pig cerebellum membranes were prepared as described previously.[1] The MOR, DOR, or KOR binding assays were performed with [^3H] DAMGO, [^3H] DPDPE, or [^3H] U-69,593. Nonspecific binding was measured in the presence of 1 μM unlabeled DAMGO, DPDPE, or U-69,593. K_i values were calculated according to the Cheng-Prusoff equation.[2]

[^{35}S]GTPγS Binding Assay

Human MOR, DOR, or KOR recombinant cell (CHO) membranes, which were purchased from PerkinElmer, were incubated in 0.25 mL of assay buffer (50 mM Tris, 1 mM EGTA, 5 mM $MgCl_2$, 100 mM NaCl) with various concentrations of the tested compound, 30 μM GDP and 0.1 nM [^{35}S]GTPγS (PerkinElmer). Nonspecific binding was measured in the presence of 10 μM unlabeled GTPγS. DAMGO, SNC80, or U-69,593 was used as the standard MOR, DOR, or KOR agonist, respectively.

CellKey™ Assay

A high-throughput system, CellKey™ was designed to detect acute cellular responses in 96-well formats.[3,4] HEK293 cells stably expressing each of opioid receptors (MOR, DOR, KOR) were used for the assay. 4 x 10^4 of cells were seeded in each well of 96-well plate and a variety of drugs shown in Table 3 were applied for 5 min, and responses were monitored for up to 30 min. Peak impedance currents within 30 min were measured, and Emax was calculated as the % of the response obtained with each of specific against DAMGO, SNC-80 and (-)-U-50,488H, MOR, DOR and KOR, respectively.

cAMP Assay

Human MOR, DOR, or KOR expressing division-arrested cells (EZCells™) were purchased from ChanTest Corporation. Cells in suspension were stimulated for 30 min with the various concentrations of compound in the presence of 10 μM forskolin in HBSS containing 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA (pH 7.4). Following stimulation, cellular cAMP was detected by the additions of Eu-cAMP tracer and U*Light*-anti-cAMP provided with the LANCE *Ultra* cAMP assay kit (PerkinElmer). TR-FRET signal at 665 nm was measured by EnVision Multilabel Plate Reader (PerkinElmer). Data were analyzed by GraphPad Prism software ver.5.

Mouse Acetic Acid Writhing Assay

Each mouse was injected intraperitoneally (i.p.) with 0.6% acetic acid at a dose of 10 mL/kg 15 min after **4c** administration (s.c.). After a 10 min delay, the animals were observed for an additional 10 min, during which the number of abdominal constrictions was counted. Percent inhibition was calculated and compared with the number of writhing movements in the control group. NTI (10 mg/kg, s.c.) was administered s.c. 30 min before **4c** administration (s.c.). β-FNA (30 mg/kg, s.c.) or nor-BNI (10 mg/kg, s.c.) was administered s.c. 24 h before **4c** administration (s.c.).

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4. Scott, C. W.; Peters, M. F. Label-free whole-cell assays: ex-panding the scope of GPCR screening. *Drug Discov. Today* **2010**, *15*, 704-716.