Differential signaling properties at the kappa opioid receptor of 12-epi-salvinorin A and its analogues

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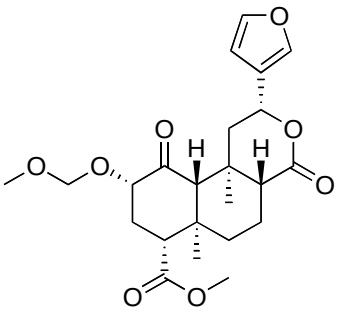
Supporting information

Experimental Section

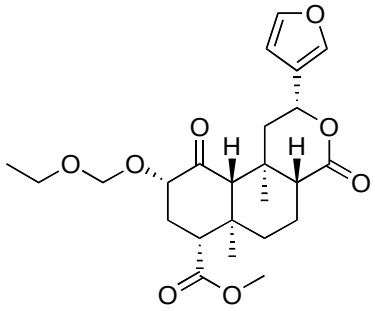
General

Reactions were carried out in flame-dried glassware under an argon atmosphere unless noted otherwise. Commercial reagents and solvents were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using either an ethanolic solution of vanillin and H_2SO_4 or an aqueous solution of ammonium molybdate, cerium sulfate, and H_2SO_4, and heat as developing agents. Products were purified using automated flash chromatography (50 μm silica gel), or manual flash chromatography (230–400 mesh silica gel). ^{1}H NMR and ^{13}C NMR chemical shifts are referenced to residual solvent peaks as internal standards: $CDCl_3$ (7.26 and 77 ppm) or CD_3OD (3.30 and 49 ppm).



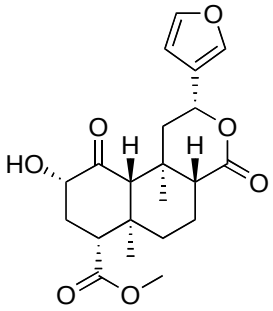
12-*epi*-Salvinorin B, methoxymethyl ether (2). 12-*epi*-Salvinorin B (11.6 mg, 0.0297 mmol) was dissolved in DMA (500 μL) under argon. To this solution was added NaI (17.8 mg, 0.119 mmol), diisopropylethylamine (28 μL, 0.16 mmol), and MOMCl (11 μL, 0.15 mmol). This solution was warmed to 80 ºC, and stirred for 18 hours. The mixture was then cooled to rt and diluted with EtOAc, washed with water and brine, and dried over $MgSO_4$. After evaporation of the solvent, flash chromatography (0% to 70% EtOAc in hexanes) gave MOM ether **2** as an off-

white solid. (7.7 mg, 60% yield); R_f 0.28 (98:2, CH_2Cl_2/MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (m, 1H), 7.41 (t, J = 1.5, 1H), 6.42 (m, 1H), 5.29 (dd, J = 6.0, 11.6, 1H), 4.71 (q, J = 7.0, 2H), 4.13 (m, 1H), 3.72 (s, 3H), 3.38 (s, 3H), 2.72 (dd, J = 3.3, 13.4, 1H), 2.46–2.18 (m, 5H), 2.05–1.68 (m, 4H), 1.58 (m, 1H), 1.39 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.8, 173.4, 172.1, 144.0, 140.0, 124.1, 109.0, 96.0, 78.1, 70.4, 66.4, 56.1, 54.0, 52.1, 47.7, 45.3, 42.4, 38.0, 35.5, 32.7, 21.4, 18.5, 16.4. HRMS(ESI) $[M+H]^+$ calcd for $C_{23}H_{31}O_8$: 435.2012, found: 435.2013.

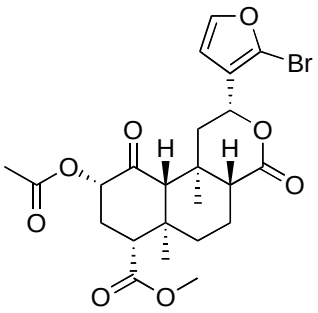


12-*epi*-Salvinorin B, ethoxymethyl ether (3). 12-*epi*-Salvinorin B (15 mg, 0.038 mmol) was dissolved in DMA (500 μL) under argon. To this solution was added NaI (23 mg, 0.15 mmol), diisopropylethylamine (37 μL, 0.21 mmol), and chloromethylethyl ether (16 μL, 0.19 mmol). This solution was warmed to 80 ºC, and stirred for 18 hours. The mixture was then cooled to rt and diluted with EtOAc, washed with water and brine, and dried over $MgSO_4$. After evaporation of the solvent, flash chromatography (0% to 70% EtOAc in hexanes) gave ethoxymethyl ether **3** as an off-white solid. (11.2 mg, 65% yield); R_f 0.28 (98:2, CH_2Cl_2/MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (m, 1H), 7.40 (t, J = 1.7 Hz, 1H), 6.41 (m, 1H), 5.29 (dd, J = 6.0, 11.6, 1H), 4.76 (q, J = 7.2, 2H), 4.16 (dd, J = 7.4, 12.2, 1H), 3.72 (s, 3H), 3.69–3.51 (m, 2H), 2.72 (dd, J = 3.3, 13.3, 1H), 2.46–2.27 (m, 4H), 2.20 (m, 1H), 2.05–1.68 (m, 4H), 1.57 (m, 1H), 1.38 (s, 3H), 1.18

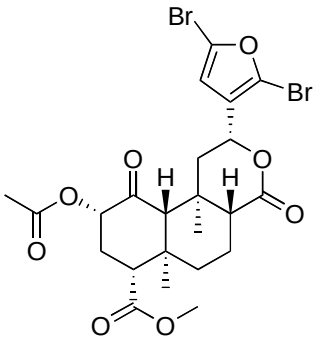
(t, J = 7.1, 2H), 1.06 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 173.4, 172.1, 144.0, 140.0, 124.2, 108.9, 94.6, 78.0, 70.5, 66.3, 64.1, 54.0, 52.1, 47.7, 45.2, 42.5, 38.0, 35.5, 32.7, 21.4, 18.5, 16.3, 15.3. HRMS(ESI) $[M+H]^+$ calcd for $C_{24}H_{33}O_8$: 449.2074, found: 449.2170.



12-*epi*-Salvinorin B (4). 12-*epi*-Salvinorin A (52 mg, 0.12 mmol) was dissolved in THF (3 mL) and H_2O_2 (3 mL). To this solution was added $NaHCO_3$ (202 mg, 2.4 mmol), and the reaction was stirred at rt for two hours. The mixture was then diluted with EtOAc, washed with brine, and dried over $MgSO_4$. After evaporation of the solvent, flash chromatography (0% to 5% MeOH in hexanes) gave alcohol **4** as an off-white solid. (27 mg, 58% yield); R_f 0.16 (98:2, CH_2Cl_2/MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (m, 1H), 7.41 (t, J = 1.6, 1H), 6.41 (m, 1H), 5.30 (dd, J = 6.1, 11.5, 1H), 4.21–3.97 (m, 1H), 3.72 (s, 3H), 2.73 (dd, J = 3.1, 13.5, 1H), 2.53–2.41 (m, 3H), 2.34 (dd, J = 11.6, 14.6, 1H), 2.18–1.93 (2H), 1.92–1.69 (m, 4H), 1.63 (m, 1H), 1.39 (s, 3H), 1.04 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.0, 173.3, 172.3, 144.1, 139.9, 124.1, 108.9, 74.6, 70.4, 65.6, 53.2, 52.2, 47.6, 43.1, 37.9, 34.5, 29.9, 21.5, 18.5, 16.4. HRMS(ESI) $[M+H]^+$ calcd for $C_{21}H_{27}O_7$: 391.1747, found: 391.1751.



16-Bromo-12-*epi*-salvinorin A (5). 12-*epi*-Salvinorin A (20 mg, 0.046 mmol) was dissolved in $CHCl_3$ (500 μL) under argon. To this solution was added NBS (6.0 mg, 0.051 mmol). The solution was stirred at rt for 20 hours. The mixture was then diluted with EtOAc, washed with 2M NaOH, saturated aqueous $NaHCO_3$, and saturated aqueous $Na_2S_2O_3$, and dried over $MgSO_4$. After evaporation of the solvent, flash chromatography (30% EtOAc in hexanes) gave bromide **5** as an off-white solid. (6.5 mg, 28% yield); R_f 0.32 (1:1, Hexanes/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 1.9, 1H), 6.49 (d, J = 2.0, 1H), 5.16 (m, 1H), 3.73 (s, 3H), 2.80 (m, 1H), 2.47 (m, 2H), 2.31 (m, 3H), 2.15 (s, 3H), 2.01–1.54 (m, 6H), 1.43 (s, 3H), 1.07 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.0, 173.1, 171.8, 170.2, 145.1, 122.2, 121.6, 111.3, 75.2, 70.2, 66.0, 53.6, 52.2, 47.4, 44.6, 42.6, 37.8, 35.4, 30.8, 21.5, 20.8, 18.5, 16.3.



15,16-Dibromo-12-*epi*-salvinorin A (6). 12-*epi*-Salvinorin A (20 mg, 0.046 mmol) was dissolved in $CHCl_3$ (500 μL) under argon. To this solution was added NBS (12 mg, 0.10 mmol). The solution was stirred at rt for 20 hours. The mixture was then diluted with EtOAc, washed with 2M NaOH, saturated aqueous $NaHCO_3$, and saturated aqueous $Na_2S_2O_3$, and dried over $MgSO_4$. After evaporation of the solvent, flash chromatography (30% EtOAc in hexanes) gave bisbromide **6** as an off-white solid. (8.5 mg, 31% yield); R_f 0.38 (1:1, Hexanes/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.45 (s, 1H), 5.26–5.07 (m, 2H), 3.73 (s, 3H), 2.79 (m, 1H), 2.44 (m, 2H), 2.36–2.22 (m, 3H), 2.15 (s, 3H), 2.06–1.91 (m, 1H), 1.88–1.50 (m, 5H), 1.39 (s, 3H), 1.07 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.0, 172.7, 171.7, 170.2, 125.4, 123.5, 121.0, 113.0, 75.2, 69.8, 66.0, 53.6, 52.2, 47.4, 44.4, 42.6, 37.8, 35.4, 30.8, 21.4, 20.8, 18.5, 16.3. HRMS(ESI) $[M+H]^+$ calcd for $C_{23}H_{27}Br_2O_8$: 589.0067, found: 589.0065.

Drugs

The synthesized derivatives, salvA, and nalbuphine were all prepared as 10 mM stocks in DMSO. Vehicle concentrations (0.1% DMSO) were equalized between all dilutions of these drugs. Vehicle only wells are included in the DiscoveRx PathHunter assay.

Confocal Microscopy

Agonist-induced translocation of a GFP-tagged β-arrestin 2 (βarr2-GFP) to the KOPR was assessed using KOPR-βarr2eGFP-U2OS cells (a kind gift from Dr. Larry Barak, Duke University) plated on collagen coated glass confocal dishes (Matek Corp., Ashland, MA) as previously described.[1] The cells were serum deprived for up to 120 minutes prior to imaging in serum free MEM without phenol red (Invitrogen). Drug was then added at 10 μM and live cell images obtained by confocal microscopy (Olympus Fluoview 1000) at 10 minutes.[1–3]

DiscoveRx PathHunter™ Assay

Agonist-induced KOPR-βarrestin2 interactions were measured using the DiscoveRx PathHunter™ assay which utilizes a β-galactosidase enzyme fragment complementation approach. This assay utilized the PathHunter™ KOPR/βarrestin2 U2OS cell line (#93-0234C3) and the detection reagents provided by the manufacturer (DiscoveRx, Fremont, CA). The manufacturer's protocol was followed, which included a 90 min incubation of the agonist concentration curves prior to luminescence detection. Luminescence was recorded using a Synergy HT luminometer (BioTek, Winooski, VT).[2] The concentration response values were normalized to vehicle-treated cells prior to nonlinear regression analysis using GraphPad Prism software to derive EC_{50} values.

1. Barak, L. S.; Ferguson, S. S.; Zhang, J.; Caron, M. G. A beta-arrestin/green fluorescent protein biosensor for detecting G protein-coupled receptor activation. *J. Biol. Chem*. **1997**, *272(44)*, 27497–500.

2. Bohn, L. M.; McDonald, P. H. Seeking Ligand Bias: Assessing GPCR Coupling to Beta-Arrestins for Drug Discovery. *Drug Discov. Today Technol*. **2010**, *7(1)*, e37–e42.

3. Groer, C. E.; Tidgewell, K.; Moyer, R. A.; Harding, W. W.; Rothman, R. B.; Prisinzano, T. E.; Bohn, L. M. An opioid agonist that does not induce micro-opioid receptor--arrestin interactions or receptor internalization. *Mol. Pharmacol*. **2007**, *71(2)*, 549–57.