Supporting Information for

8,8-Dialkyldihydroberberines with Potent Antiprotozoal Activity

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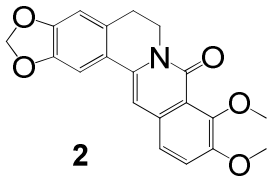
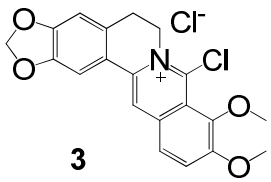
Characterization of intermediates and target compounds



2

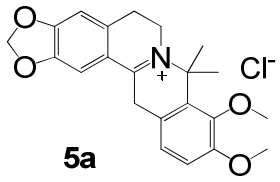
8-Oxoberberine (2).[1] A solution of berberine hemisulfate (3.0 g, 7.8 mmol) in 10 mL of water was added to a 100 mL solution of KOH (20% in water) and the mixture was heated to reflux overnight. The resulting yellow precipitate was collected by filtration. The filtrate was further washed with 0.1 N HCl (20 mL) and water (20 mL) and triturated with ethyl acetate (20 mL × 4). The aqueous phase was extracted with ethyl acetate (30 mL × 3) and the combined organic phase was dried over anhydrous sodium sulfate. Solvent was removed and the combined yellow solid was purified by column chromatography using $CHCl_3$/MeOH (50:1). 8-Oxoberberine **2** (1.51 g, 55% yield) was isolated as pale-yellow needles. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.87 (t, 2H, J = 5.7 Hz), 3.77 (s, 3H), 3.86 (s, 3H), 4.11 (t, 2H, J = 5.7 Hz), 6.07 (s, 2H), 6.91 (s, 1H), 7.09 (s, 1H), 7.40 (d, 1H, J = 9 Hz), 7.47 (s, 1H), 7.52 (d, 1H, J = 9 Hz).



3

8-Chloroberberine (3).[1] A suspension of **2** (1.2 g, 3.4 mmol) in $POCl_3$ (15 mL) was heated at 80 °C for 3 h. The mixture was cooled and the resulting orange crystalline precipitate was collected by filtration, washed with dichloromethane (3 × 5 mL) and dried under high vacuum overnight to provide crude compound **3** (1.23 g, 89% yield), which was stored at 4 °C under argon until further use. ^{1}H NMR (DMSO-d_6) δ 2.87 (t, 2H, J = 5.7 Hz), 3.76 (s, 3H), 3.86 (s, 3H), 4.11 (t, 2H, J = 5.7 Hz), 6.07 (s, 2H), 6.92 (s, 1H), 7.10 (s, 1H), 7.40 (d, 1H, J = 9 Hz), 7.49 (s, 1H), 7.53 (d, 1H, J = 9 Hz).

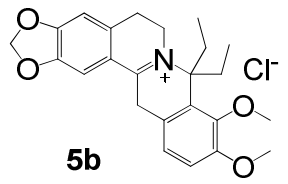
General procedure A - Synthesis of compounds 5a - 5c. Target compounds were prepared through minor modifications of the literature procedure.[1] The appropriate Grignard reagent (2-3 M in ether or in THF, 4.5-7 equiv.) was added dropwise to a suspension of **3** (1 equiv.) in anhydrous diethyl ether at 0 °C and the reaction mixture was heated to reflux for 2 h. This mixture was allowed to cool to room temperature, quenched with a saturated aqueous NH_4Cl solution at 0 °C, and extracted with CH_2Cl_2 (4 × 10 mL). The combined organic phases were brought to a basic pH with ammonium hydroxide, concentrated in vacuo, and the crude material was purified by silica gel column chromatography using hexanes/ethyl acetate (25:1 to 15:1). The solvent was removed under reduced pressure to give the 8,8-dialkyldihydroberberine as the free base, which was then dissolved in dry ethyl acetate. HCl gas was bubbled slowly into the solution containing the free base for about 1 min, then this solution was allowed to stir for 1 h. The resulting yellowish precipitate was filtered, washed with ethyl acetate, and dried under high vacuum to give pure target compounds **5a** - **5c**, which were stored under argon at 4 °C. The quaternary immonium cations were obtained rather than the tertiary enamine salts, consistent with previous literature reports in similar ring systems,[2, 3] as assessed by the disappearance of olefinic hydrogen signals (H-13) and the appearance of benzylic hydrogen signals in the ^{1}H NMR spectra.



5a

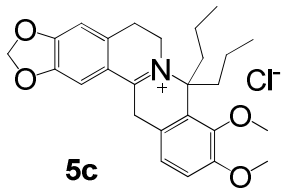
8,8-Dimethyldihydroberberine chloride salt (5a).[1] This compound was prepared according to general procedure A from **3** (0.32 g, 0.79 mmol) and MeMgBr (1.8 mL of a 3 M solution, 5.5 mmol), which gave 207.6 mg of **5a** (0.52 mmol, 66% yield over two steps), mp 167-169 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.92 (s, 6H), 3.06 (t, 2H, J = 6.3 Hz), 3.85 (s, 3H,), 3.90 (s, 3H), 4.15 (t, 2H, J = 6.3 Hz), 4.69 (s, 2H), 6.25 (s, 2H), 7.02 (d, 1H, J = 8.6 Hz), 7.18 (s, 1H), 7.20 (d, 1H, J = 8.6 Hz), 7.79 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 25.7, 26.1, 31.8, 45.6, 56.0, 60.4, 66.8, 102.9, 107.7, 108.7, 114.0, 117.1, 120.4,

122.3, 128.7, 135.9, 144.6, 147.3, 151.9, 153.5, 169.9. HRESIMS *m/z* calcd for $C_{22}H_{24}NO_4$ 366.1705; found 366.1699.



5b

8,8-Diethyldihydroberberine chloride salt (5b).[1] This compound was prepared according to general procedure A from **3** (0.4 g, 0.98 mmol) and EtMgBr (2.3 mL of a 3M solution, 6.89 mmol), which gave 250.5 mg of **5b** (0.58 mmol, 59% yield over two steps), mp = 164-166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.63 (t, 6H, *J* = 6.6 Hz), 2.35-2.40 (m, 2H), 2.68-2.74 (m, 2H), 3.26 (br s, 2H), 3.87 (s, 3H), 3.88 (s, 3H), 4.24 (brs, 2H), 4.73 (s, 2H), 6.13 (s, 2H), 6.86 (s, 1H), 7.01 (d, 1H, *J* = 8.4 Hz), 7.16 (d, 1H, *J* = 8.4 Hz), 7.67 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.0, 26.7, 29.4, 32.8, 45.5, 55.9, 60.4, 77.8, 103.1, 108.3, 108.9, 113.9, 118.3, 119.7, 123.1, 124.2, 136.5, 144.3, 148.5, 152.5, 155.3, 172.1. HRESIMS *m/z* calcd for $C_{24}H_{28}NO_4$ 394.2018; found 394.2019.

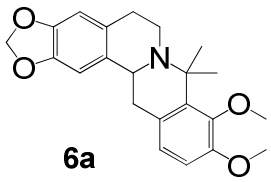


5c

8,8-Dipropyldihydroberberine chloride salt (5c).[1] This compound was prepared according to general procedure A from **3** (0.4 g, 0.98 mmol) and *n*-PrMgBr (2.3 mL, 2M in THF, 4.6 mmol), which gave 215.0 mg of **5c** (0.47 mmol, 48% yield over two steps), mp 155-157 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (t, 6H, *J* = 5.0 Hz), 0.88-0.96 (m, 4H), 2.14 (m, 2H), 2.66 (m, 2H), 3.28 (brs, 2H), 3.87 (s, 3H), 3.88 (s, 3H), 4.22 (brs, 2H), 4.76 (s, 2H), 6.12 (s, 2H), 6.94 (s, 1H), 6.99 (d, 1H, *J* = 7.1 Hz), 7.18 (d, 1H, *J* = 7.1 Hz), 7.73 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.7, 18.1, 27.0, 33.2, 38.6, 45.9, 55.9, 60.4, 76.3, 103.2,

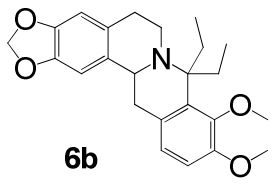
108.4, 109.3, 113.8, 117.8, 119.7, 123.3, 124.8, 136.3, 144.2, 148.4, 152.4, 155.2, 171.9. HRESIMS *m/z* calcd for $C_{26}H_{32}NO_4$ 422.233; found 422.234.

General procedure B - synthesis of compounds 6a and 6b. Target compounds were prepared through a slight modification of published procedures.[4, 5] To a solution of the crude 8,8-dialkyldihydroberberine free base (obtained from compound **3** as described in general procedure A) in methanol was added $NaBH_4$ portion wise at 0 °C. The reaction mixture was heated to reflux for 3 h and concentrated in vacuo. The resulting residue was triturated with Et_2O (4 × 30 mL), dried over anhydrous sodium sulfate and concentrated in vacuo. The solid residue was recrystallized from methanol to afford the desired 8,8-dialkylcanadines **6a** and **6b**.



6a

8,8-Dimethylcanadine (6a).[5] This compound was prepared by conversion of **3** (103 mg, 0.25 mmol) to 8,8-dimethyldihydroberberine as described above, then by reduction of this crude free base according to general procedure B using $NaBH_4$ (48 mg, 1.27 mmol) to give 67.2 mg of **6a** (0.18 mmol, 72% yield from compound **3**), mp 136-137 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52 (s, 3H), 1.72 (s, 3H), 2.61-2.74 (m, 2H), 2.81-2.91 (m, 2H), 2.96 (dd, 1H, *J* = 3.6 Hz, 16 Hz), 3.19-3.24 (m, 1H), 3.86 (s, 3H), 3.91 (s, 3H), 4.07 (m, 1H), 5.91 (s, 2H), 6.59 (s, 1H), 6.68 (s, 1H), 6.80 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.2, 27.4, 31.1, 38.9, 41.1, 53.5, 56.0, 58.9, 60.8, 100.9, 106.6, 108.5, 111.1, 123.9, 128.1, 128.8, 132.9, 137.7, 145.8, 146.0, 147.3, 151.5. HRESIMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}NO_4$ 368.1862; found 368.1874.

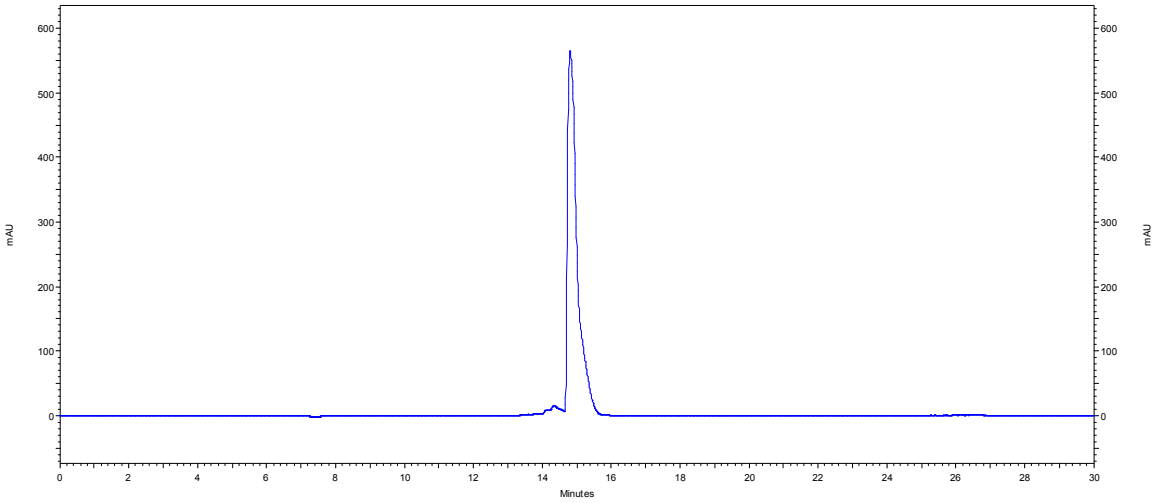


6b

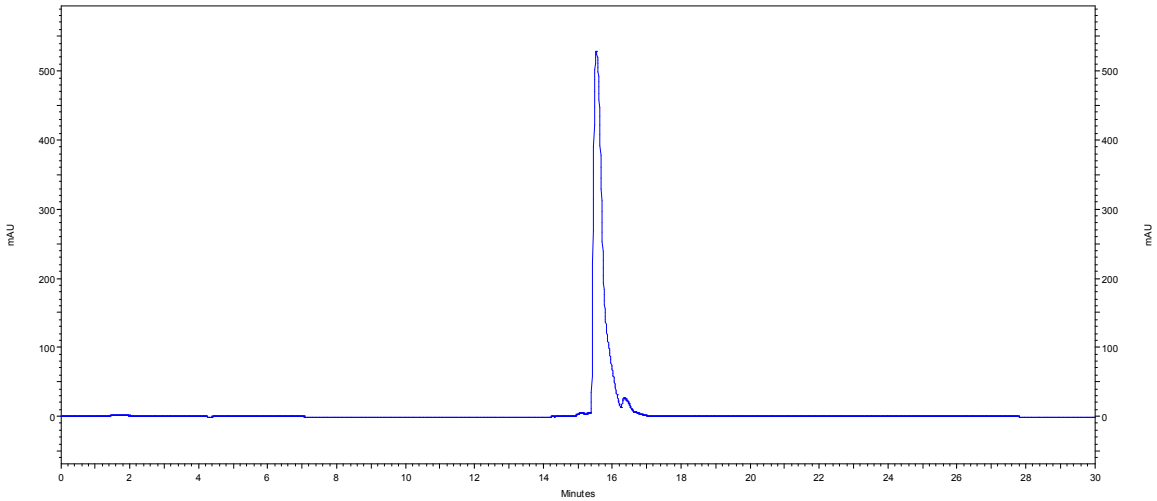
8,8-Diethylcanadine (6b). This compound was prepared by conversion of **3** (512 mg, 1.26 mmol) to 8,8-diethyldihydroberberine as described above, then by reduction of this crude free base using $NaBH_4$ (0.24 g, 6.3 mmol) to give 343.9 mg of **6b** (0.87 mmol, 69% yield from compound **3**), mp 165-166 °C (lit.[3] 168-169 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.55 (t, 3H, *J* = 7.3 Hz), 0.88 (t, 3H, *J* = 7.3 Hz), 1.73-1.85 (m, 1H), 1.98-2.24 (m, 2H), 2.37-2.50 (m, 1H), 2.58-2.73 (m, 3H), 2.83-2.99 (m, 2H), 3.27 (m, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 4.42 (br d, 1H, *J* = 10.8 Hz), 5.91 (s, 2H), 6.59 (s, 1H), 6.69 (s, 1H), 6.82 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.3, 12.5, 29.7, 31.6, 40.7, 41.3, 54.9, 56.0, 60.3, 65.7, 100.8, 106.6, 108.4, 110.8, 123.5, 129.4, 131.5, 133.3, 133.4, 145.6, 146.0, 146.9, 151.5. HRESIMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{29}NO_4$ 396.2175; found 396.2185.

HPLC chromatograms for target compounds

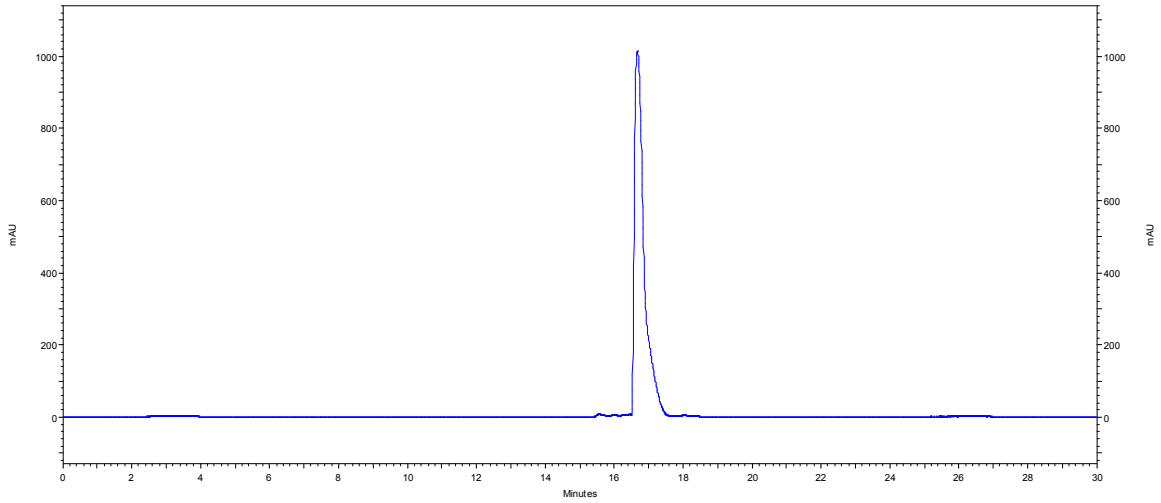
8,8-Dimethyldihydroberberine chloride (5a). Analysis performed on a Phenomenex® Gemini 5 µm C_{18} Axis packing reversed-phase column (250 × 4.1 mm), diode array detector at 358 nm, purity = 98%.



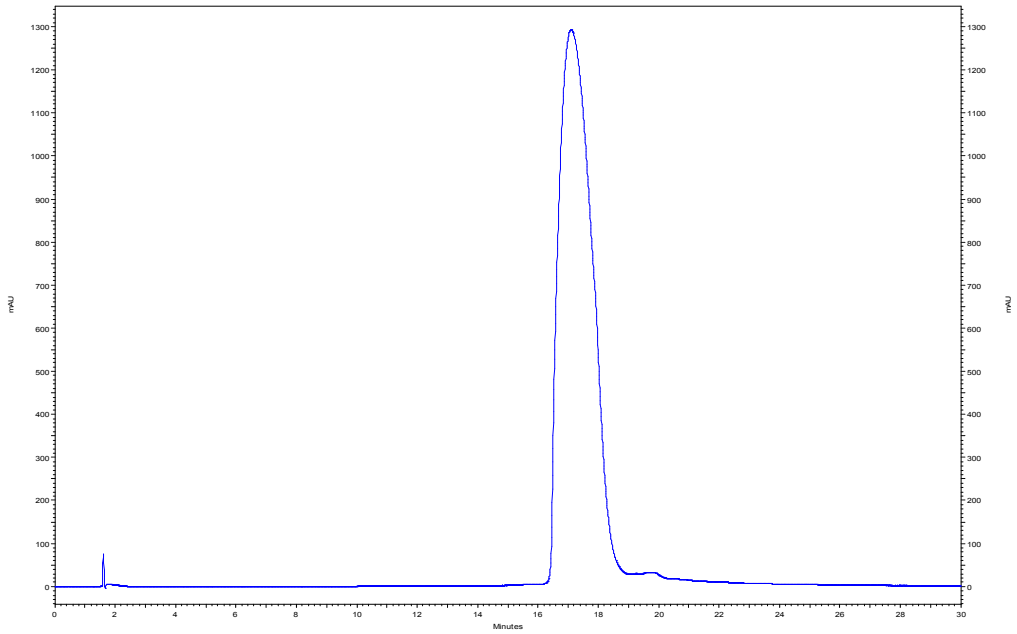
8,8-Diethyldihydroberberine chloride (5b). Analysis performed on a Phenomenex® Gemini 5 µm C_{18} Axis packing reversed-phase column (250 × 4.1 mm), diode array detector at 358 nm, purity = 96%.



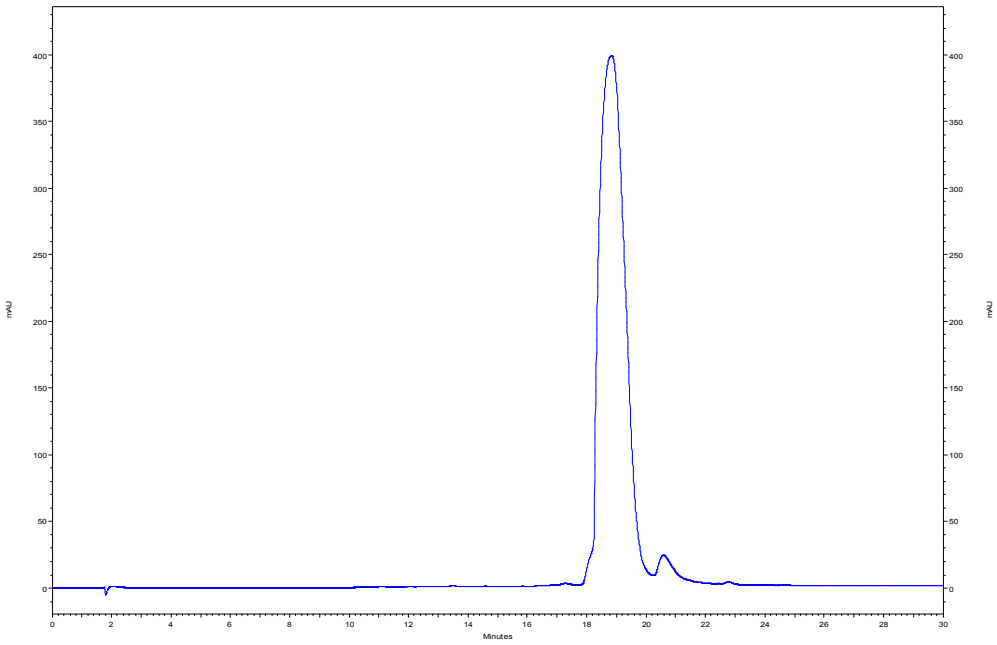
8,8-Dipropyldihydroberberine chloride (5c). Analysis performed on a Phenomenex® Gemini 5 µm C_{18} Axis packing reversed-phase column (250 × 4.1 mm), diode array detector at 358 nm, purity = >99%.



8,8-Dimethylcanadine (6a). Analysis performed using a Merck LichroCART Lichrospher 100 RP-18 10 µm reversed-phase column, diode array detector at 287 nm, purity = 98%.



8,8-Diethylcanadine (6b). Analysis performed using a Merck LichroCART Lichrospher 100 RP-18 10 μm reversed-phase column, diode array detector at 287 nm, purity = 95%.



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