Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supplementary Information for

Inhibition of Mitochondrial Metabolism by (–)-Jerantinine A: Synthesis and Biological Studies in Triple-Negative Breast Cancer Cells

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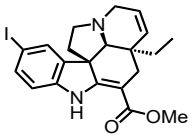
Syntheses of (–)-Jerantinine A, (–)-Melodinine P, and Derivatives

Materials and methods

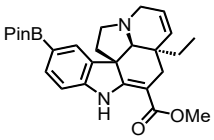
All commercially obtained reagents were used as received unless otherwise noted. A generous gift of 1Kg of (-)-tabersonine was donated by Dr Dong, Shanghai Institute of Organic Chemistry, China. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 pre-coated plates (0.25 mm). Visualization of the chromatogram was accomplished by UV, staining as necessary. All samples were purified using Biotage Selekt flash column chromatography. ^{19}F (Bruker-377 MHz), ^{1}H (Bruker-400 MHz), and ^{13}C NMR (Bruker-101 MHz) spectra were recorded using $CDCl_3$ as solvents. Data for ^{1}H are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz, and integration. When necessary, proton and carbon assignments were made by means of ^{1}H-^{1}H COSY, ^{1}H-^{13}C and HSQC. Mass spectra were obtained using a Q Exactive HF (Ionization source: HESI II) mass spectrometer at the Cold Spring Harbor Laboratory. IR were obtained using Perkin Elmer FT-IR spectrometer at the Cold Spring Harbor Laboratory.

(–)-15-Iodo-tabersonine (4)[1]



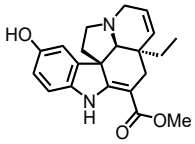
To a stirred solution of tabersonine (**TAB**, **3**) (2.93 g, 8.71 mmol) in TFA (90 mL) was added NIS (1.96 g, 8.71 mmol) in 4 portions over 30 min. The reaction was allowed to stir for 16 h. TFA was then evaporated and the residue was diluted with NaOH (1M *aq,* 100 mL) at 0 °C and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were then dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude material was dissolved in pentane (250 mL), filtered and concentrated *in vacuo* to give (–)-15-iodo-tabersonine (**4**) as a white solid (3.79 g, 94%). **m.p.**: 85 °C; $[\alpha]_D$ –119 (*c* 0.10, $CHCl_3$); $\mathbf{R_f}$ = 0.4 in 4:1 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 3359, 3022, 2959, 1671, 1131; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 7.43 (d, *J* = 1.8 Hz, 1H), 7.39 – 7.31 (m, 1H), 6.53 (d, *J* = 8.1 Hz, 1H), 5.71 (ddd, *J* = 10.0, 4.7, 1.5 Hz, 1H), 5.63 (dt, *J* = 9.9, 1.9 Hz, 1H), 3.69 (s, 3H), 3.38 (ddd, *J* = 16.0, 4.7, 1.5 Hz, 1H), 3.13 (dt, *J* = 16.0, 2.0 Hz, 1H), 3.02 – 2.93 (m, 1H), 2.62 (ddd, *J* = 10.7, 8.5, 4.8 Hz, 1H), 2.55 (s, 1H), 2.48 (dd, *J* = 15.2, 1.9 Hz, 1H), 2.33 (d, *J* = 15.1 Hz, 1H), 1.98 (td, *J* = 11.3, 6.5 Hz, 1H), 1.79 – 1.70 (m, 1H), 0.96 – 0.86 (m, 1H), 0.82 – 0.78 (m, 1H), 0.57 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.9, 165.5, 143.0, 140.7, 136.4, 132.8, 130.4, 124.9, 111.4, 92.9, 82.5, 70.1, 55.1, 51.1, 51.0, 50.4, 44.5, 41.2, 28.5, 27.1, 7.5; **HRMS (ESI$^+$)**: calculated for $C_{21}H_{24}IN_2O_2$ $[M+H]^+$: m/z = 463.0882, m/z found 463.0878.

(–)-15-BPin-tabersonine (5)[2]



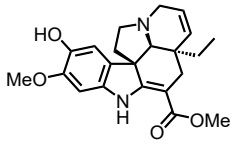
To a solution of (–)-15-iodo-tabersonine (**4**) (1.00 g, 2.16 mmol), B_2Pin_2 (1.40 g, 5.51 mmol) and KOAc (2.15 g, 21.60 mmol) in degassed DMF (20 mL) was added $PdCl_2(dppf)CH_2Cl_2$ (0.32 g, 0.39 mmol) under a positive flow of N_2. The resulting mixture was heated at 80 °C for 16 h (before returning to room temperature). The reaction mixture was then diluted with water (200 mL) and extracted with EtOAc (4 × 100 mL). The combined organic fractions were washed with H_2O (3 × 100 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (0-20% EtOAc in hexanes) to afford (–)-15-BPin-tabersonine (**5**) as a white solid (0.82 g, 82%). **m.p.**: 100-102 °C; $\mathbf{[\alpha]_D}$ –240 (*c* 0.010, $CHCl_3$); $\mathbf{R_f}$ = 0.4 in 2:1 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 2918, 1673; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.62 – 7.52 (m, 2H), 6.74 (d, *J* = 7.8 Hz, 1H), 5.71 (ddd, *J* = 9.9, 4.6, 1.5 Hz, 1H), 5.64 (dt, *J* = 9.9, 1.9 Hz, 1H), 3.70 (s, 3H), 3.39 (ddd, *J* = 15.8, 4.6, 1.5 Hz, 1H), 3.19 (dt, *J* = 15.9, 2.0 Hz, 1H), 2.95 (dd, *J* = 8.4, 6.2 Hz, 1H), 2.78 – 2.69 (m, 1H), 2.68 (s, 1H), 2.48 (dd, *J* = 15.1, 1.8 Hz, 1H), 2.37 (d, *J* = 15.1 Hz, 1H), 2.03 – 1.91 (m, 1H), 1.71 (dd, *J* = 11.5, 4.4 Hz, 1H), 1.27 (s, 12H), 0.95 – 0.86 (m, 1H), 0.77 (ddd, *J* = 9.8, 4.2, 2.6 Hz, 1H), 0.56 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.0, 166.5, 146.0, 137.4, 135.6, 133.0, 127.3, 125.0, 108.8, 92.9, 83.6, 69.7, 54.9, 51.1, 50.9, 50.6, 44.7, 41.5, 28.2, 26.8, 24.9, 24.9, 7.5; **HRMS (ESI$^+$)**: calculated for $C_{27}H_{36}BN_2O_4$ $[M+H]^+$: m/z = 463.2768, m/z found 463.2760.

(–)-Melodinine P (6)[2]



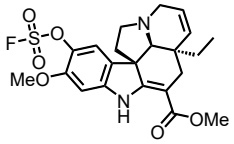
To a solution of (–)-15-BPin-tabersonine (**5**) (0.50 g, 1.08 mmol) and H_2O_2 (35% *aq*, 1.25 mL) in THF (10 mL) was added NaOH (1M *aq*, 3.00 mL) dropwise at 0 °C. The reaction was allowed to stir at 0 °C for a further 1 h. The reaction mixture was then neutralized to pH 7 using HCl (1M *aq*), washed with $Na_2S_2O_3$ (sat. *aq*) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (0-50% EtOAc in hexanes) to afford (–)-melodinine P (**6**) as a white solid (0.28 g, 74%). $[\alpha]_D$ –52.5 (*c* 0.008, $CHCl_3$); R_f = 0.6 in 1:2 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 2926, 1681; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 6.62 – 6.52 (m, 2H), 5.71 (ddd, *J* = 9.9, 4.7, 1.5 Hz, 1H), 5.64 (dt, *J* = 9.8, 1.8 Hz, 1H), 3.69 (s, 3H), 3.37 (ddd, *J* = 15.9, 4.7, 1.4 Hz, 1H), 3.09 (dt, *J* = 15.9, 1.9 Hz, 1H), 3.01 – 2.92 (m, 1H), 2.66 – 2.54 (m, 2H), 2.48 (dd, *J* = 15.1, 1.8 Hz, 1H), 2.34 (d, *J* = 15.0 Hz, 1H), 2.06 – 1.98 (m, 1H), 1.74 (ddd, *J* = 11.7, 4.8, 1.3 Hz, 1H), 0.92 (dt, *J* = 14.9, 7.5 Hz, 1H), 0.79 (dq, *J* = 14.3, 7.3 Hz, 1H), 0.57 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.2, 167.4, 150.4, 139.7, 136.8, 133.1, 124.8, 113.8, 110.1, 109.6, 91.3, 70.2, 55.5, 51.0, 51.0, 50.5, 44.4, 41.2, 28.6, 27.1, 7.5; **HRMS (ESI$^+$)**: calculated for $C_{21}H_{25}N_2O_3$ $[M+H]^+$: m/z = 353.1865, m/z found 353.1863.

(–)-Jerantinine A (1)[2]



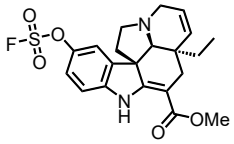
To a solution of (–)-melodinine P (**6**) (0.10 g, 0.26 mmol) in MeOH (2 mL) was added freshly prepared Ag_2O (0.61 g, 2.61 mmol) in one portion. The reaction was allowed to stir for 8 h in dark. The reaction mixture was filtered through Celite®, then concentrated *in vacuo.* The residue was taken up in CH_2Cl_2 (20 mL) was stirred with $Na_2S_2O_4$ (10% *aq*, 20 mL) for a further 15 min. The reaction mixture was further extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (0-50% EtOAc in hexanes) to afford (–)-jerantinine A (**1**) as an off-white solid (96 mg, 90%). $[\alpha]_D$ –70.9 (*c* 0.010, $CHCl_3$); R_f = 0.5 in 1:1 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 3548, 3379, 3035, 2965, 1669, 1605, 1110; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 6.81 (s, 1H), 6.38 (s, 1H), 5.71 (ddd, *J* = 9.9, 4.7, 1.5 Hz, 1H), 5.68 – 5.59 (m, 1H), 5.24 (s, 1H), 3.80 (s, 3H), 3.69 (s, 3H), 3.38 (ddd, *J* = 15.8, 4.7, 1.5 Hz, 1H), 3.08 (dt, *J* = 15.9, 1.9 Hz, 1H), 2.99 – 2.91 (m, 1H), 2.60 (ddd, *J* = 11.1, 8.4, 4.7 Hz, 1H), 2.52 (d, *J* = 1.8 Hz, 1H), 2.46 (dd, *J* = 15.0, 1.9 Hz, 1H), 2.34 (d, *J* = 15.0 Hz, 1H), 2.03 – 1.98 (m, 1H), 1.69 (ddd, *J* = 11.5, 4.8, 1.1 Hz, 1H), 0.99 – 0.84 (m, 1H), 0.79 (dt, *J* = 14.0, 7.2 Hz, 1H), 0.56 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.1, 167.9, 145.9, 139.9, 136.1, 133.1, 130.1, 124.9, 108.8, 94.6, 91.8, 70.2, 56.3, 55.3, 51.0, 50.9, 50.7, 44.4, 41.5, 28.3, 26.9, 7.5; **HRMS (ESI$^+$)**: calculated for $C_{22}H_{27}N_2O_4$ $[M+H]^+$: m/z = 383.1971, m/z found 383.1968.

(–)-Jerantinine A fluorosulfate (7)



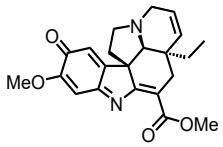
To a solution of (–)-jerantinine A (**1**) (22 mg, 0.057 mmol) in MeCN (5 mL) was added HMDS (17 µL, 0.086 mmol), and BTMG (2 µL, 0.011 mmol). To the above reaction mixture, SO_2F_2 gas was bubbled in for 5 min, and the resultant reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* to afford (–)-jerantinine A fluorosulfate (**7**) as a sticky solid (27 mg, 100%). **$[\alpha]_D$** –35.2 (*c* 0.007, $CHCl_3$); **R_f** = 0.7 in 1:1 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 3363, 2928, 1677; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.08 (s, 1H), 6.46 (s, 1H), 5.72 (ddd, *J* = 9.9, 4.8, 1.5 Hz, 1H), 5.62 (dt, *J* = 10.0, 1.9 Hz, 1H), 3.82 (s, 3H), 3.70 (s, 3H), 3.38 (ddd, *J* = 16.0, 4.8, 1.5 Hz, 1H), 3.13 (dt, *J* = 15.9, 2.0 Hz, 1H), 3.02 – 2.94 (m, 1H), 2.59 (ddd, *J* = 10.6, 8.6, 4.9 Hz, 1H), 2.55 – 2.41 (m, 2H), 2.33 (d, *J* = 15.2 Hz, 1H), 2.03 – 1.97 (m, 1H), 1.74 (ddd, *J* = 11.7, 5.0, 1.5 Hz, 1H), 0.91 (dt, *J* = 15.0, 7.6 Hz, 1H), 0.85 – 0.77 (m, 1H), 0.58 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.0, 166.0, 151.6, 144.0, 132.8, 129.8, 128.5, 125.0, 116.0, 95.4, 94.2, 70.2, 56.6, 54.9, 51.4, 51.1, 50.4, 44.6, 41.1, 28.8, 27.3, 7.6; **^{19}F NMR** (377 MHz, $CDCl_3$) δ 38.78; **HRMS (ESI$^+$)**: calculated for $C_{22}H_{26}FN_2O_6S$ $[M+H]^+$: m/z = 465.1495, m/z found 465.1498.

(–)-Melodinine P fluorosulfate (8)



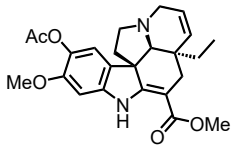
To a solution of (–)-melodinine P (**6**) (0.10 g, 0.28 mmol) in MeCN (25 mL) was added HMDS (65 µL, 0.31 mmol), and BTMG (11 µL, 0.06 mmol). To the above reaction mixture, SO_2F_2 gas was bubbled in for 30 min, and the resultant reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (30 mL), washed with H_2O (20 mL). The separated aqueous layer was further extracted EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (3 × 20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo.* Purification by flash column chromatography (0-20% EtOAc in hexanes) to afford (–)-melodinine P fluorosulfate (**8**) as a colorless oil (0.12 g, 100%). **$[\alpha]_D$** –44.8 (*c* 0.005, $CHCl_3$); **R_f** = 0.8 in 4:1 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 3366, 2963, 1679, 1615, 1475, 1448, 1268, 1231, 1117; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 7.20 (d, *J* = 3.0 Hz, 1H), 7.12 (dd, *J* = 8.5, 3.0 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 5.80 (ddd, *J* = 10.0, 5.2, 1.8 Hz, 1H), 5.70 (dt, *J* = 10.0, 1.8 Hz, 1H), 3.77 (s, 3H), 3.46 (dd, *J* = 16.0, 5.2 Hz, 1H), 3.21 (d, *J* = 16.0 Hz, 1H), 3.07 (t, *J* = 7.5 Hz, 1H), 2.72 – 2.66 (m, 2H), 2.56 (dd, *J* = 15.2, 1.9 Hz, 1H), 2.41 (d, *J* = 15.3 Hz, 1H), 2.12 – 2.04 (m, 1H), 1.87 – 1.82 (m, 1H), 1.02 – 0.86 (m, 2H), 0.65 (t, *J* = 7.5 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.7, 165.4, 144.0, 143.2, 140.0, 132.6, 124.8, 120.3, 114.9, 109.5, 94.0, 70.0, 55.2, 51.2, 51.0, 50.2, 44.5, 40.8, 28.8, 27.2, 7.5; **^{19}F NMR** (377 MHz, $CDCl_3$) δ 35.97; **HRMS (ESI$^+$)**: calculated for $C_{21}H_{24}FN_2O_5S$ $[M+H]^+$: m/z = 435.1384, m/z found 435.1379.

(–)-Jerantinine A iminoquinone (9)



To a solution of (–)-jerantinine A (**1**) (10 mg, 0.03 mmol) in $CDCl_3$ (2 mL) was added Ag_2O (60 mg, 0.26 mmol) at room temperature in one portion. The reaction was allowed to stir for 15 min in dark. The reaction mixture was filtered, and the filtrate concentrated *in vacuo* to afford (–)-jerantinine A iminoquinone (**9**) as a dark brown oil (10 mg, 100%). **$[\alpha]_D$** –36.1 (*c* 0.010, $CHCl_3$); **R_f** = 0.05 in 1:1 hexanes-ethyl acetate (degrades); **IR** (neat, ν, cm^{-1}): 2962, 2921, 1696, 1645, 1578, 1225, 1164; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.70 (s, 1H), 6.29 (s, 1H), 5.82 – 5.77 (m, 1H), 5.66 – 5.62 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.48 – 3.43 (m, 1H), 3.12 – 3.04 (m, 2H), 2.77 – 2.68 (m, 2H), 2.53 – 2.47 (m, 1H), 2.42 (s, 1H), 2.17 – 2.09 (m, 1H), 1.82 (dd, *J* = 11.9, 4.3 Hz, 1H), 0.98 – 0.85 (m, 2H), 0.73 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 181.1, 168.2, 167.4, 166.1, 158.1, 157.4, 132.1, 125.8, 120.5, 118.6, 104.3, 70.2, 56.5, 54.8, 52.5, 51.2, 50.8, 45.0, 43.0, 32.7, 27.3, 7.7; **HRMS (ESI$^+$)**: calculated for $C_{22}H_{25}N_2O_4$ $[M+H]^+$: m/z = 383.1809, m/z found 381.1808.

(–)-Jerantinine A acetate (2)[3]



A solution of (–)-jerantinine A (**1**) (0.11 g, 0.29 mmol) in Ac_2O-pyridine (1:1 *v/v*, 3 mL) was stirred at room temperature for 15 min. The reaction mixture was then diluted with water (10 mL), the pH adjusted to 10 with Na_2CO_3 (sat. *aq*, *ca.* 20 mL) and then extracted with CH_2Cl_2 (4 × 20 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (0-50% EtOAc in hexanes) to afford (–)-jerantinine A acetate (**2**) as an off-white solid (90 mg, 74%). **$[\alpha]_D$** –46.1 (*c* 0.004, $CHCl_3$); **R_f** = 0.5 in 1:1 hexanes-ethyl acetate; **IR** (neat, ν, cm^{-1}): 3364, 2962, 1762, 1675, 1614, 1494, 1456, 1368, 1262, 1156, 1109; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 6.92 (s, 1H), 6.50 (s, 1H), 5.79 – 5.75 (m, 1H), 5.69 (d, *J* = 10.1 Hz, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.44 – 3.40 (m, 1H), 3.16 (d, *J* = 15.9 Hz, 1H), 3.02 (t, *J* = 6.8 Hz, 1H), 2.66 – 2.61 (m, 2H), 2.54 (d, *J* = 15.0 Hz, 1H), 2.38 (d, *J* = 15.0 Hz, 1H), 2.30 (s, 3H), 2.06 – 2.01 (m, 1H), 1.84 (dd, *J* = 12.0, 3.5 Hz, 1H), 1.03 – 0.82 (m, 2H), 0.64 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.6, 169.1, 167.1, 151.2, 141.8, 133.5, 133.1, 129.6, 124.9, 116.5, 95.2, 92.9, 70.3, 56.3, 55.2, 51.2, 51.2, 50.5, 44.4, 41.2, 28.8, 27.3, 20.8, 7.6; **HRMS (ESI$^+$)**: calculated for $C_{24}H_{29}N_2O_5$ $[M+H]^+$: m/z = 425.2071, m/z found 425.2068.

Biological Evaluations of (–)-Jerantinine A, (–)-Melodinine P, and Derivatives

Cell culture

MDA-MB-231 breast cancer cells and MCF-10A human mammary epithelial cells were purchased from ATCC and cultured in a humidified incubator with 5% CO_2 at 37°C. MDA-MB-231 cells were cultured in RPMI-1640 plus 10% FBS. MCF-10A cells were cultured in DMEM/F12 medium supplemented with 5% horse serum, 20ng/ml epidermal growth factor, 0.5mg/ml hydrocortisone, 100 ng/ml cholera toxin, and 10 μg/ml insulin.

Cell proliferation assay

MDA-MB-231 breast cancer cells were seeded in 96-well black plates at a density of 2000 cells per well and then incubated with the indicated drug concentrations. The medium was changed every two to three days. On day 5, the viability was assessed using the CyQUANT proliferation assay (Thermo C35013), a fluorescence-based measurement of cellular DNA content, according to manufacture instruction.

Construction of oxidative stress reporter

Lentivirus-based oxidative stress reporter was described previously[4]. Briefly, the Nrf2 degron was conjugated to the N-terminus of green luciferase (CBG99) to indicate oxidative stress and constitutively-expressed red luciferase (PRE9) was used as an imaging internal control. Red fluorescence from mStrawberry expression was used for the positive selection of reporter gene-transduced cells. All of the gene cassettes were built into a single lentiviral vector (pBOB lentiviral backbone). Reporter genes (CBG99, PRE9 and mStrawberry) and NRF2 protein degron sequences were all synthesized from GenScript USA (New Jersey).

The generation of oxidative stress reporter expressing cells

The three lentiviral packaging plasmids (pMDL, pRSV-REV, pCMV-VSVG) and the lentiviral oxidative stress reporter plasmid (OxiLuc) were transfected into HEK293T packaging cells with Lipofectamine 3000 (Invitrogen, Waltham, MA; according to the manufacturer's recommended protocol) to produce OxiLuc lentivirus[5]. Lentiviral supernatant was then collected 48 hours and 72 hours later, aliquoted and stored at -80°C. MDA-MB-231 cells were seeded on a 6 well plate at a density of $3x10^5$ cells/well and transduced with 500 μL of OxiLuc lentiviral supernatant plus polybrene to a final concentration of 8 μg/mL. After expanding the transduced cells, FACS sorting was performed for red fluorescence and mStrawberry expression (BD-FACS Aria, BD Bioscience, Franklin Lakes, NJ) to select for MDA-MB-231 cells stably transduced with OxiLuc expression.

Oxidative stress imaging and drug treatment *in vitro*

Images of oxidative stress were taken with an IVIS Spectrum scanner (Perkin Elmer) as previously described[4]. $5x10^3$ MDA-MB231/OxiLuc cells were plated day -1 on a black-walled 96 well plate (Cellvis, Mountain View, CA). The next day, the cells were treated with escalating doses of MP, JA, and the fluorosulfate of both molecules for 24 hours. 24 hours after drug treatment, multiple bioluminescent images were acquired 2 minutes after adding 150 μg/mL of D-Luciferin (Goldbio, Saint Louis, MO), through a series of 20 nm bandpass optical filters, ranging from 520 to 660 nm. Using single-color CBG99 and PRE9 reference spectra, green and red emitted light was spectrally-unmixed using the Imaging Wizard in Living Imaging software 4.0 (Perkin Elmer). All *in vitro* images were acquired at field-of-view C and focused at 0.5 cm subject height. Photon flux from each well was quantified as photons/second/steradian/cm^2.

Metabolomics

MDA-MB-231 breast cancer cells were treated for 16 hours with vehicle (DMSO), 5 μM jerantinine A, or 5 μM jerantinine A fluorosulfate to perform untargeted metabolic profiling. A water solution containing 50% (*v/v*) methanol and 20% (*v/v*) acetonitrile was used to extract cell lysate. The supernatant was collected, transferred into glass vials, and stored at -80°C before being analyzed by mass spectrometry (MS). For the MS analysis, an Orbitrap Q Exactive HF (Thermo Fisher Scientific) with a HESI probe was used. Each polarity's mass was calibrated prior to analysis. Principal component analysis (PCA), clustered heatmap analysis, and metabolite set enrichment analysis were all performed using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca).

Table S1. Previously reported anticancer activity of (–)-jerantinine A (**1**).

Cell line	Description	Activity	Reference	Notes
KB/S	Keratin-forming tumor cell line HeLa	IC_{50} = 0.76 μg/mL (1.99 μM)	*J. Nat. Prod,* **2008**, *71*, 1591–1594	
KB/VJ300	Vincristine-resistant KB cells	IC_{50} = 0.66 μg/mL (1.73 μM)	*J. Nat. Prod,* **2008**, *71*, 1591–1594	
A549	Adenocarcinomic human alveolar basal epithelial cells	GI_{50} = 3.741 ± 0.65 μM	*Investig. New Drugs,* **2014**, *32*, 838–850	
HCT-116	Human colorectal carcinoma cell line	GI_{50} = 0.762± 0.13 μM	*Investig. New Drugs,* **2014**, *32*, 838–850	
HT-29	Human colorectal adenocarcinoma cell line with epithelial morphology	GI_{50} = 2.520 ± 0.28 μM	*Investig. New Drugs,* **2014**, *32*, 838–850	
MCF-7	Human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors	GI_{50} = 0.853 ± 0.09 μM	*Investig. New Drugs,* **2014**, *32*, 838–850	
MDA-MB-468	Human breast cancer cell line with upregulated EGF receptors	GI_{50} = 0.8503 ± 0.07 μM	*Investig. New Drugs,* **2014**, *32*, 838–850	
MRC-5	Human fetal lung fibroblast cells	GI_{50} = 1.723 ± 0.67 μM	*Investig. New Drugs,* **2014**, *32*, 838–850	
MCF-7	Human breast cancer with estrogen, progesterone, and glucocorticoid receptors	IC_{50} = 1.22 ± 0.15 μM	*Scientific Reports* **volume 7**, Article number: 42504 (2017)	Non-transformed cells were found to be significantly more resistant to **JA** treatment and apoptotic morphological changes were not noted in MCF-10A cell line.
T47D	Human breast	IC_{50} = 0.72 ± 0.18 μM	*Scientific Reports* **volume 7**,	

	cancer with significant resistance to estrogens and antiestrogens		Article number: 42504 (2017)	Rapid induction of apoptosis could imply endogenous proteins are targeted.
MDA-MB-468	Triple negative breast cancer (TNBC) cell line	IC_{50} = 0.86 ± 0.09 µM	*Scientific Reports* **volume 7**, Article number: 42504 (2017)	509 upregulated proteins, 429 down-regulated.
MCF-10A	Non-transformed human mammary epithelial cells bearing a Zinc-Finger Nuclease (ZFN) knock out modification	IC_{50} = >10 µM	*Scientific Reports* **volume 7**, Article number: 42504 (2017)	**JA** triggers dysregulation of SF3B1 and SF3B3 proteins, casing pre-mRNA splicing errors. This induced apoptosis.
U87MG	Human primary glioblastoma	IC_{50} = 0.62 ± 1.19 µg/mL (1.62 ± 3.11 µM) IC_{50} = 1.29 ± 1.26 µg/mL g-T3 combined with 0.16 µg/mL (0.42 µM) **JA**, CI = 0.67[x]	*Phytomedicine PHYMED 5217 (2017)*	A significant decrease in the toxicity to normal MRC5 cells was observed when **JA** was coadministered with g-T3.
MRC5	Normal fetal lung fibroblast cells	IC_{50} = 0.97 ± 0.11 µg/mL (2.54 ± 0.29 µM) IC_{50} = 6.55 ± 1.13 µg/mL g-T3 combined with 0.16 µg/mL (0.42 µM)		**JA** treatment resulted in cell cycle arrest at the G2/M phase while coadministering **JA** and g-T3 caused G0/G1 arrest. **JA** treatment caused disruption of microtubule networks and triggered FAas- and p53-induced apoptosis via death receptor and mitochondrial pathways.
HCT116	Human colorectal carcinoma	GI_{50} = 0.82 ± 0.07 µM	*Scientific Reports* **volume 8**, Article number: 10617 (2018)	**JA** induced cell cycle arrest at the G2/M phase.
MCF-7	Human breast cancer with estrogen, progesterone and glucocorticoid receptors	GI_{50} = 0.81 ± 0.07 µM	*Scientific Reports* **volume 8**, Article number: 10617 (2018)	**JA** prevents tubulin polymerization *in vitro*. **JA** also caused the formation of multipolar spindles, misaligned chromosomes, multicnucleation, and nuclear fragmentation.
MCF-7	Human breast cancer with	96 h of exposure, decrease in cell density	*Scientific Reports* **volume 8**, Article number: 10617 (2018)	

	estrogen, progesterone and glucocorticoid receptors	(3D culture)		Tubulin-jerantinine B acetate crystal structure obtained.
MDA-MB-231	Epithelial cell line isolated from the breast tissue of an adenocarcionma patient	96 h of exposure, decrease in cell density And a decrease in the invasive nature of this cell line (3D culture)	*Scientific Reports* **volume 8**, Article number: 10617 (2018)	
A549	Human lung adenocarcinoma	IC_{20} = 0.35 ± 0.32 µg/mL (0.92 ± 0.84 uM) IC_{50} = 1.42 ± 1.31 µg/mL (3.71 ± 3.43 uM) IC_{50} = 2.77 ± 1.09 µg/mL d-T3 combined with 0.35 µg/mL (0.92 µM) **JA**, CI = 0.47[x] Vinblastine (positive control) IC_{50} = 0.03 ± 1.45 µg/mL	*J. Herbmed. Pharmacol. 2019, 8, 333*	**JA** caused cell shrinkage in A549 cells due to microtubule disruption. **JA** induced caspase 8, 9, and 3, enzymatic activities. Combined treatment with d-T3 boosted caspase 9 and 3 activities (1.90- and 1.95-fold respectively).
MRC5	Normal fetal lung fibroblast cells	IC_{20} = 0.35 ± 0.32 µg/mL (0.92 ± 0.84 µM) IC_{50} = 0.97 ± 0.11 µg/mL (2.54 ± 0.29 µM) IC_{50} = 5.57 ± 1.22 µg/mL d-T3 combined with 0.35 µg/mL (0.92 µM) **JA**	*J. Herbmed. Pharmacol. 2019, 8, 333*	**JA** induced similar levels of toxicity to A549 and normal MRC5 when used alone. When combined with d-T3, 2-fold selective toxicity as observed.
PC3	Human prostate cancer	EC_{50} = 2.8 ± 0.7 µM (**JA**) EC_{50} = 20.9 ± 2.7 µM (**MP**) EC_{50} = 76.0 ± 10.1 µM (**TAB**)	*Org. Biomol. Chem.,* **2022**, *20*, 3988-3997	Antitumor activities enhanced with hydroxy/methoxy derivatizations on indole-ring.
SKBR-3 MDA-MB-231	TfR1+ breast cancers	GI_{50} = 0.02-0.15 µM (Apoferritin (AFt)-**JA-Ac**)	*ACS Omega*, **2022**, *7*, 21473−21482	AFt acts as a biocompatible vehicle for targeted delivery of **JA**-**Ac**, offering potential to minimize toxicity and enhance **JA-Ac** activity in TfR1-expressing tumors.

IC_{50} = half maximal inhibitory concentration.
GI_{50} = half maximal growth inhibition.
EC_{50} =half maximal effective concentration.
[x]CI = combination index, CI < 1 suggests synergistic effec

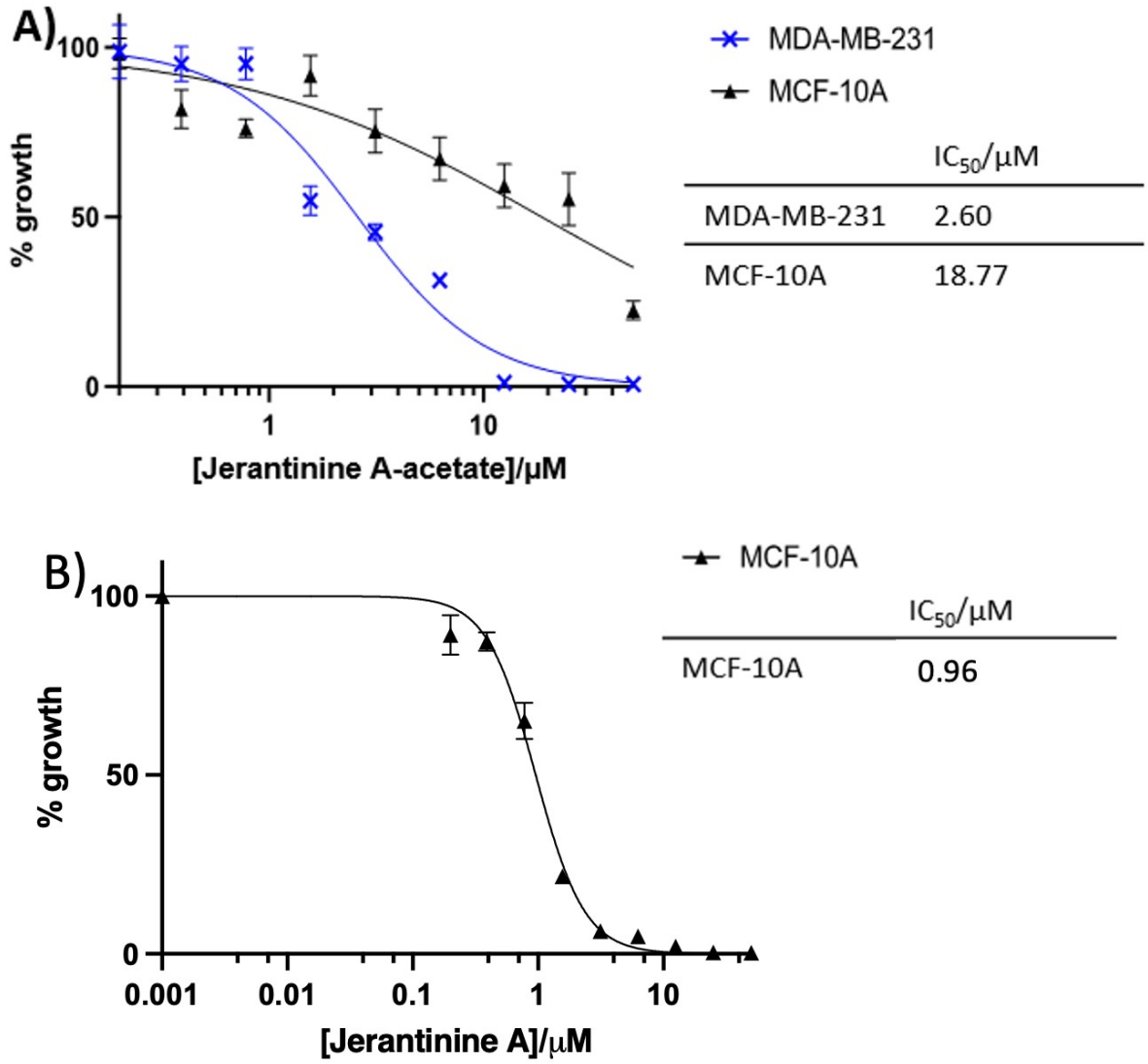


Figure S1. A) Growth inhibitory effect of (–)-jerantinine A acetate in MDA-MB-231 breast cancer cell line and non-transformed MCF-10A cell line. **B)** Growth inhibitory effect of (–)-jerantinine A in non-transformed MCF-10A cell line. Cell numbers were quantified by CyQUANT assay after 5 days treatment of indicated concentrations (error bars indicate SEM; n=6). IC_{50} values were calculated using GraphPad Prism.

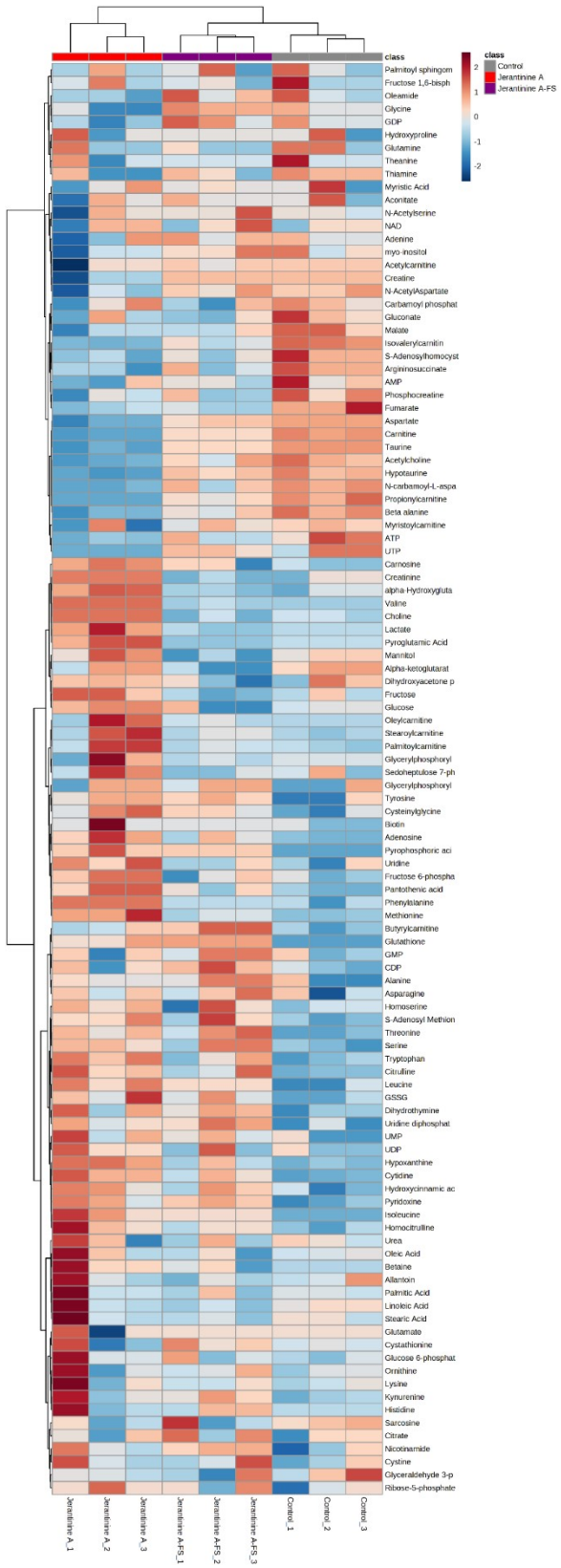


Figure S2. Clustered heatmap of all the normalized metabolites between groups of 5 μM (–)-jerantinine A, 5 μM (–)-jerantinine A fluorosulfate, and vehicle control.

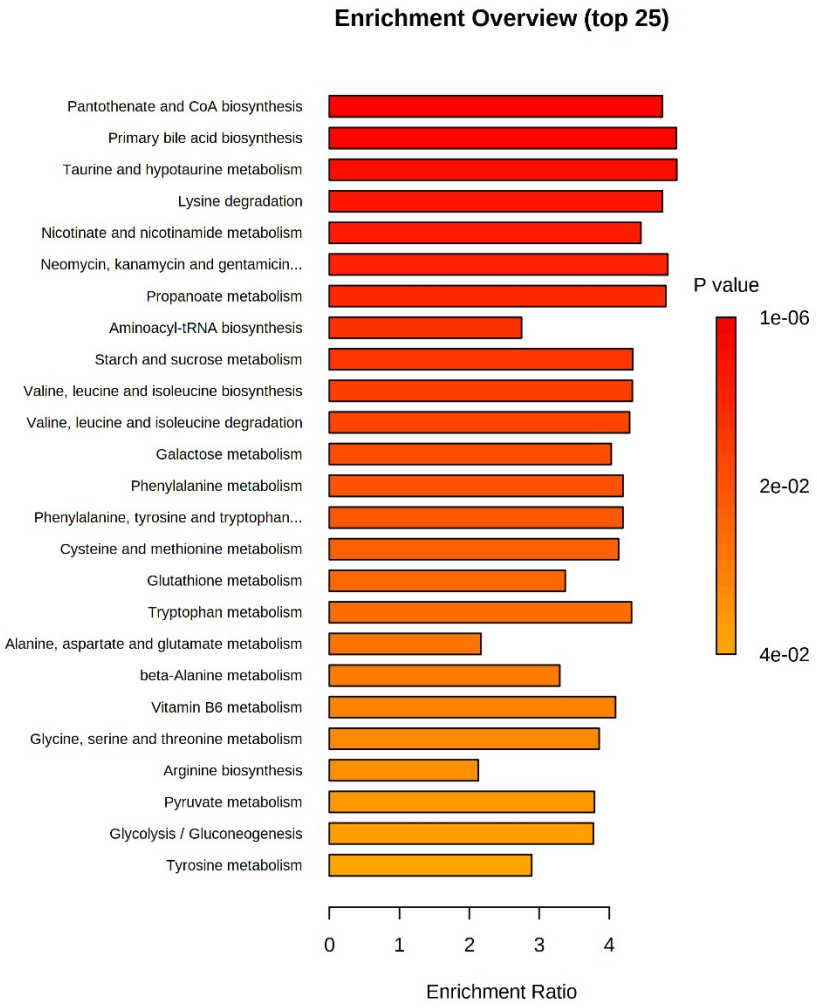


Figure S3. Overview of top 25 enriched metabolite sets between control and 5 μM (–)-jerantinine A**.**

Table S2. Metabolite set enrichment analysis of (–)-jerantinine A treated MDA-MB-231 cells.

	Total Cmpd	*Hits*	*Statistic Q*	*Expected Q*	*Raw p*	*Holm p*	*FDR*
Pantothenate and CoA biosynthesis	19	4	95.182	20	1.32E-06	6.98E-05	6.98E-05
Primary bile acid biosynthesis	46	2	99.25	20	1.30E-05	0.000674	0.000233
Taurine and hypotaurine metabolism	8	2	99.391	20	1.32E-05	0.000674	0.000233
Lysine degradation	25	2	95.174	20	3.99E-05	0.001996	0.000529
Nicotinate and nicotinamide metabolism	15	3	89.057	20	0.000283	0.01387	0.003001
Neomycin, kanamycin and gentamicin biosynthesis	2	2	96.793	20	0.00034	0.016316	0.003003
Propanoate metabolism	23	1	96.201	20	0.000548	0.025767	0.004151
Aminoacyl-tRNA biosynthesis	48	16	54.898	20	0.000848	0.039031	0.005214
Starch and sucrose metabolism	18	4	86.692	20	0.000885	0.03984	0.005214
Valine, leucine and isoleucine biosynthesis	8	4	86.591	20	0.001569	0.069044	0.007685
Valine, leucine and isoleucine degradation	40	3	85.788	20	0.001721	0.074009	0.007685
Galactose metabolism	27	5	80.62	20	0.00174	0.074009	0.007685
Phenylalanine metabolism	10	2	83.902	20	0.002494	0.10226	0.009442
Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	83.902	20	0.002494	0.10226	0.009442
Cysteine and methionine metabolism	33	5	82.732	20	0.004003	0.15612	0.013546
Glutathione metabolism	28	7	67.45	20	0.004089	0.15612	0.013546
Tryptophan metabolism	41	2	86.439	20	0.005108	0.189	0.015925
Alanine, aspartate and glutamate metabolism	28	12	43.366	20	0.006166	0.22197	0.018155
beta-Alanine metabolism	21	4	65.852	20	0.008721	0.30522	0.024326
Vitamin B6 metabolism	9	1	81.852	20	0.013184	0.44827	0.034939
Glycine, serine and threonine metabolism	33	7	77.151	20	0.015684	0.51756	0.039583
Arginine biosynthesis	14	10	42.523	20	0.020679	0.66174	0.049818
Pyruvate metabolism	22	2	75.84	20	0.02276	0.70555	0.052446
Glycolysis / Gluconeogenesis	26	4	75.495	20	0.024061	0.72183	0.053135
Tyrosine metabolism	42	2	57.879	20	0.035747	1	0.075785
Histidine metabolism	16	4	43.018	20	0.042103	1	0.085825
Amino sugar and nucleotide sugar metabolism	37	4	65.77	20	0.045227	1	0.08878
Fructose and mannose metabolism	20	3	64.827	20	0.047539	1	0.089984

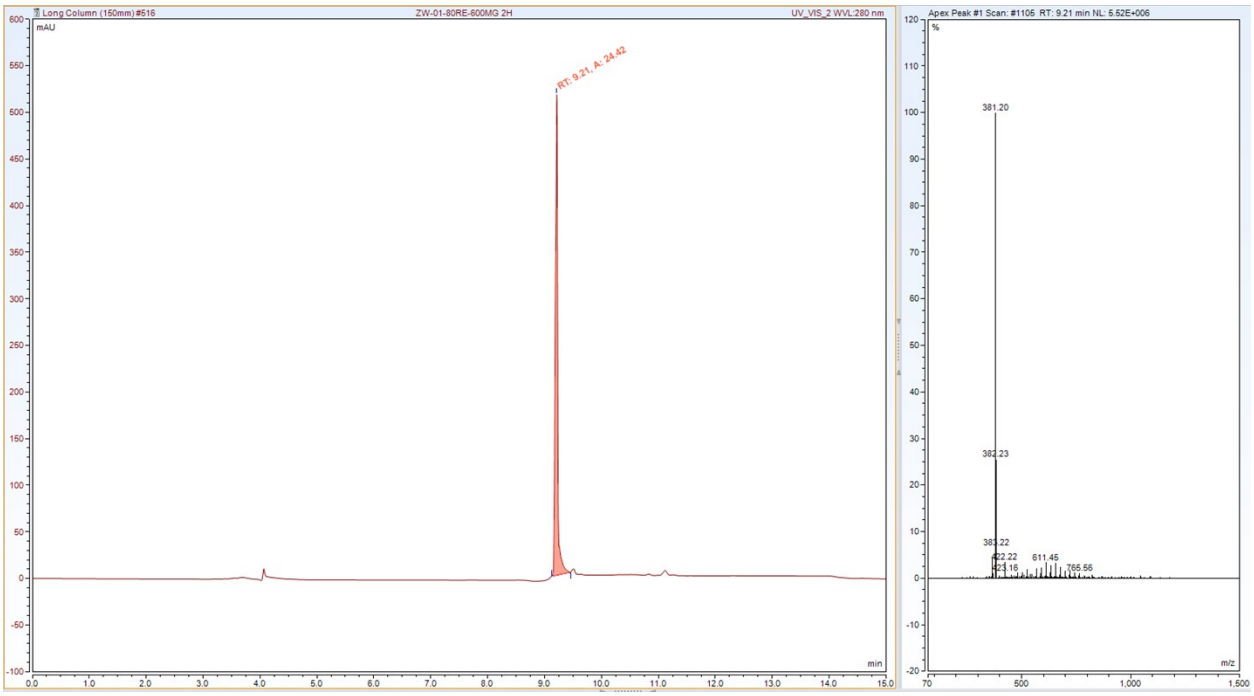
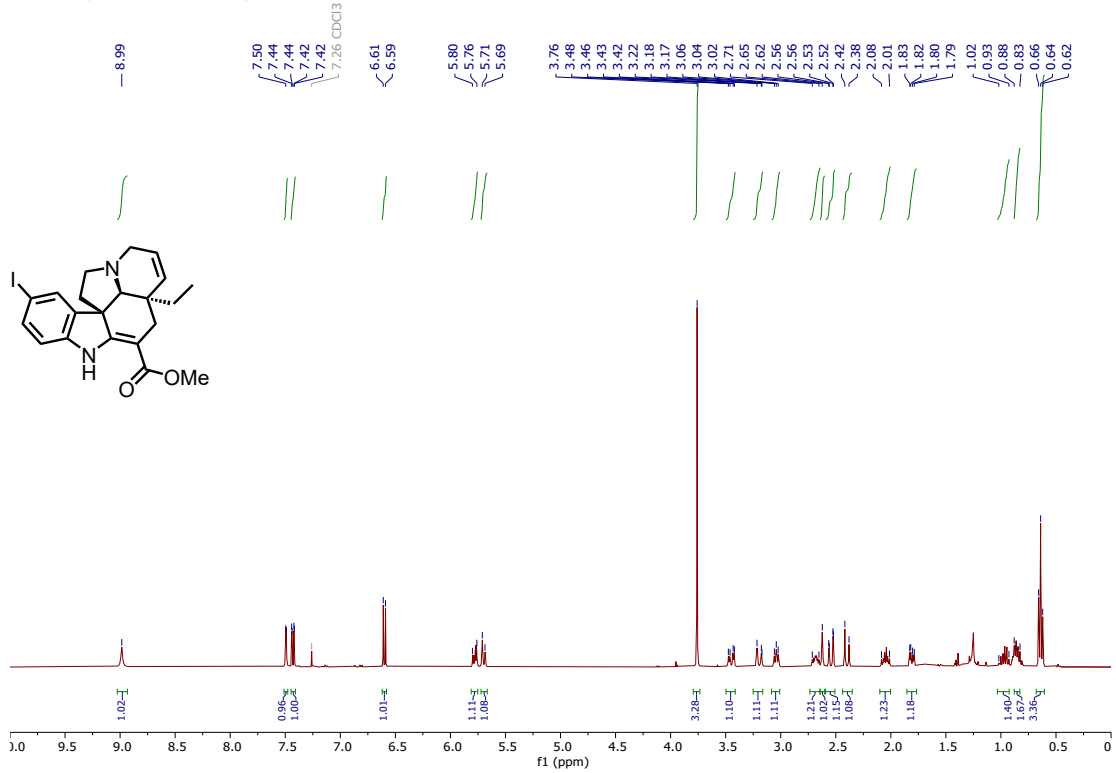


Figure S4. LC-MS chromatogram of oxidation of (–)-melodinine P using Ag_2O to (–)-jerantinine A iminoquinone**.**

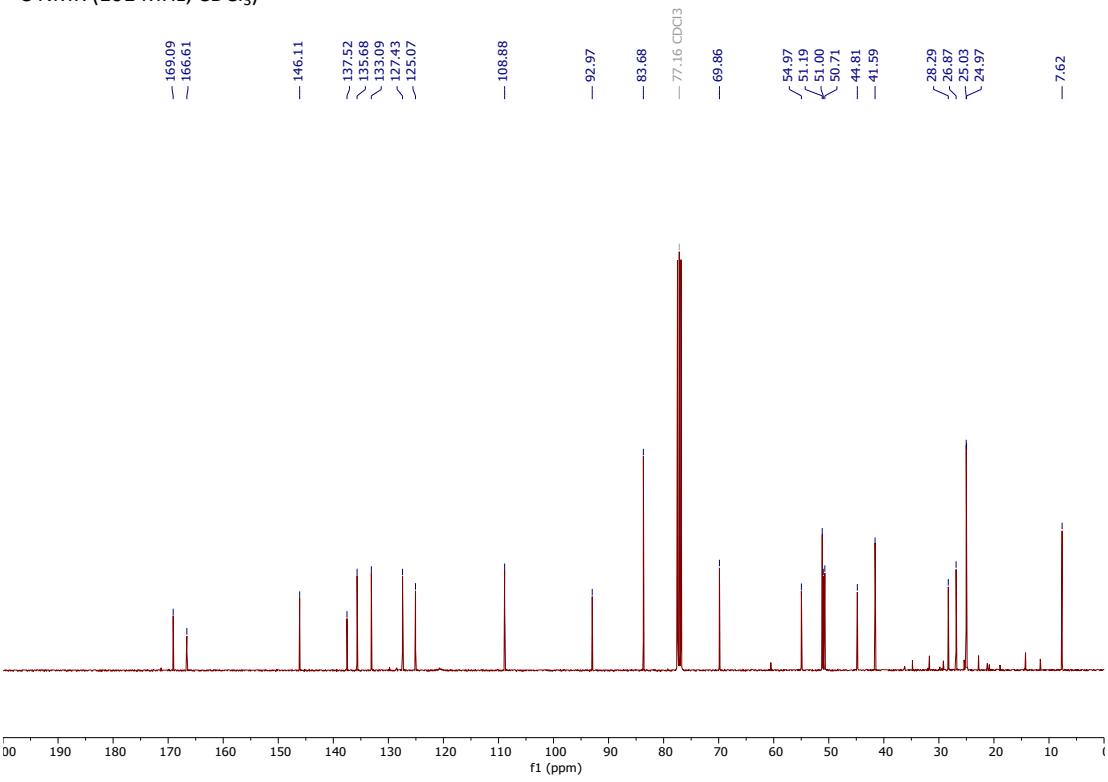
NMR spectra of synthesized compounds

(–)-15-Iodo-tabersonine (4)

^{1}H NMR (400 MHz, $CDCl_3$)

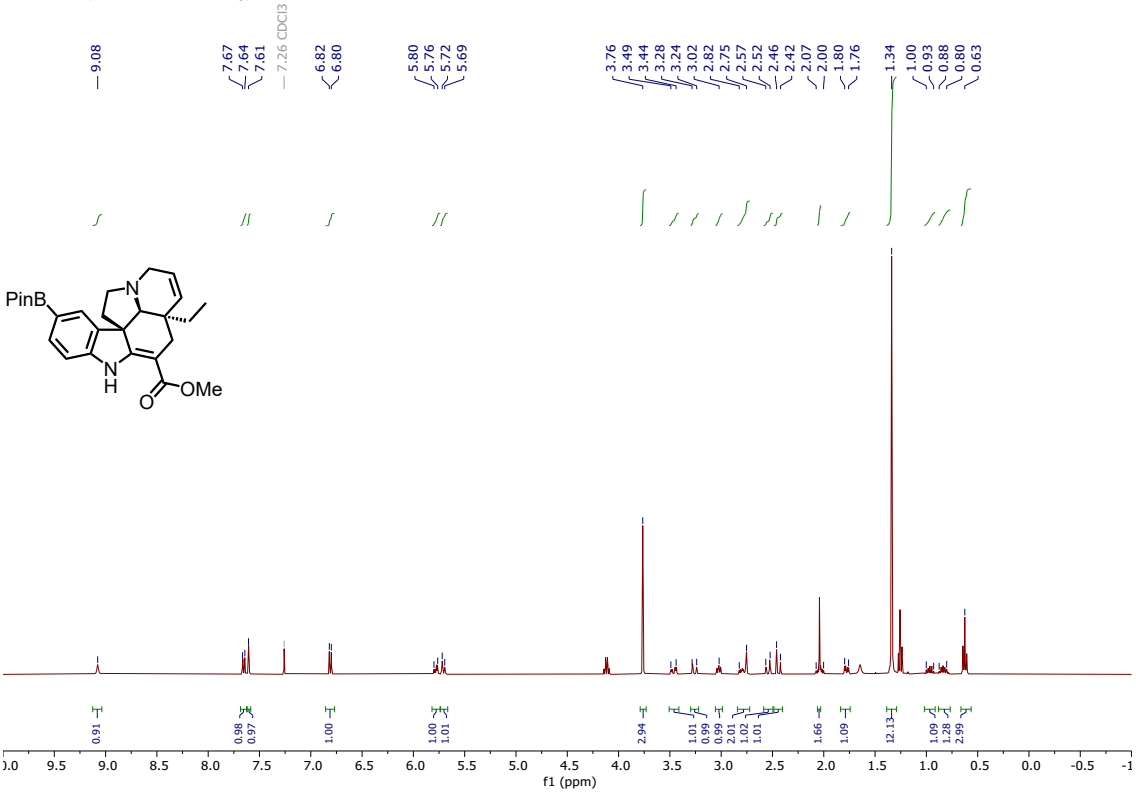


^{13}C NMR (101 MHz, $CDCl_3$)

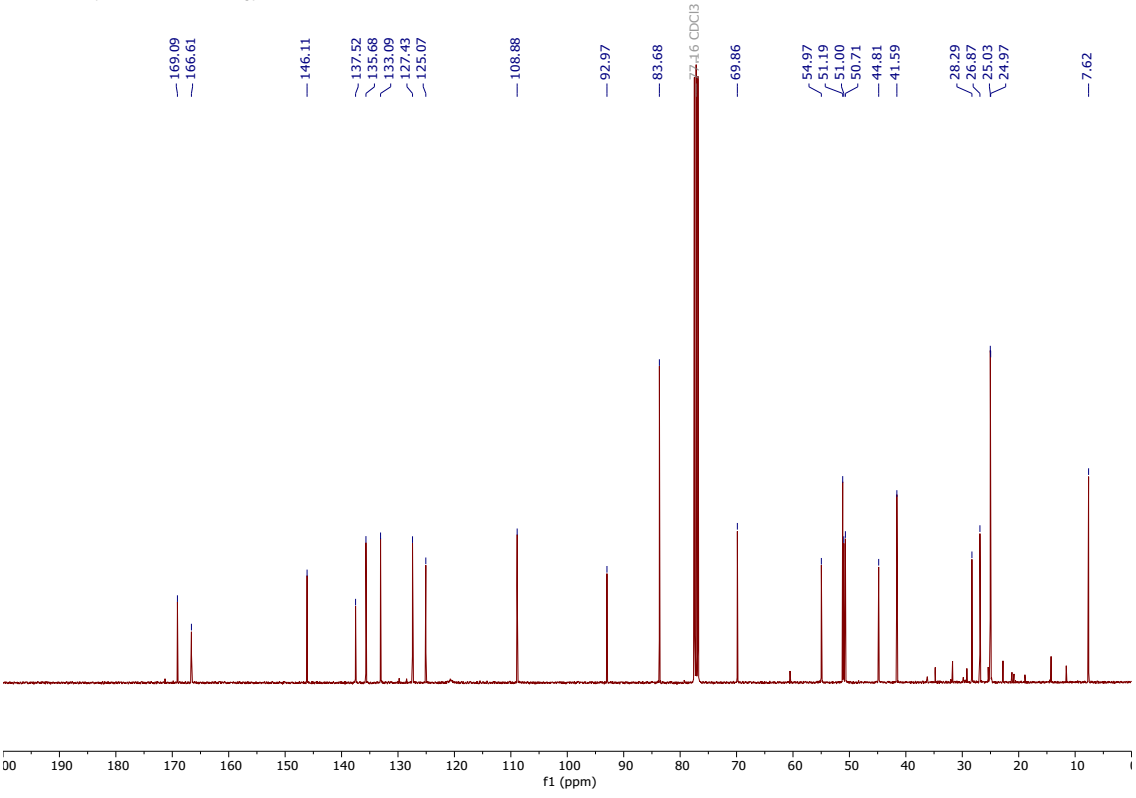


(–)-15-BPin-tabersonine (5)

^{1}H NMR (400 MHz, $CDCl_3$)

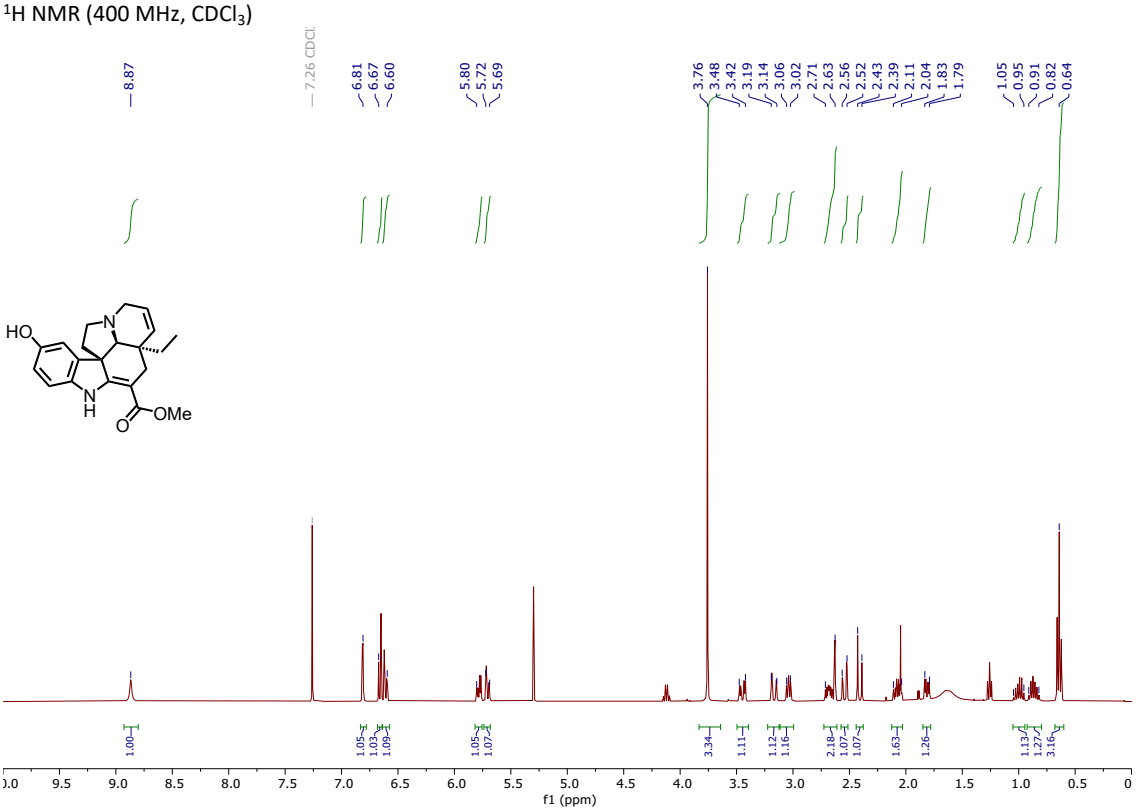


^{13}C NMR (101 MHz, $CDCl_3$)

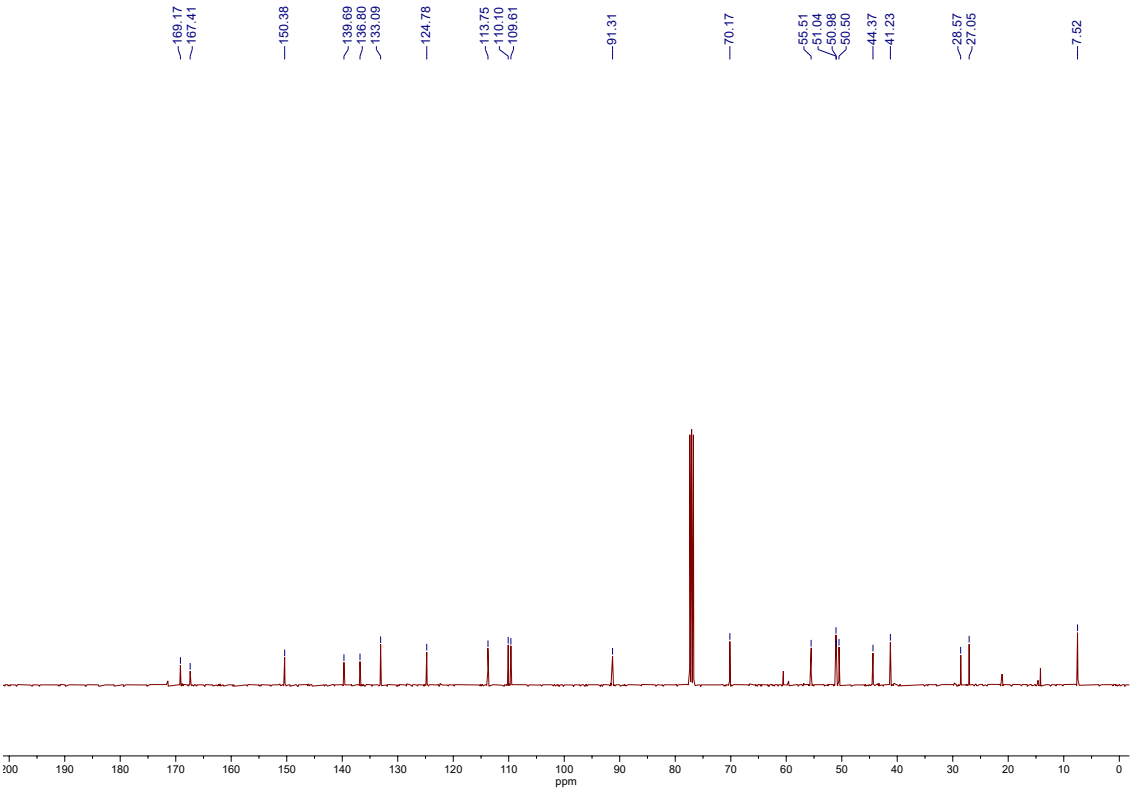


(–)-Melodinine P (6)

^{1}H NMR (400 MHz, $CDCl_3$)

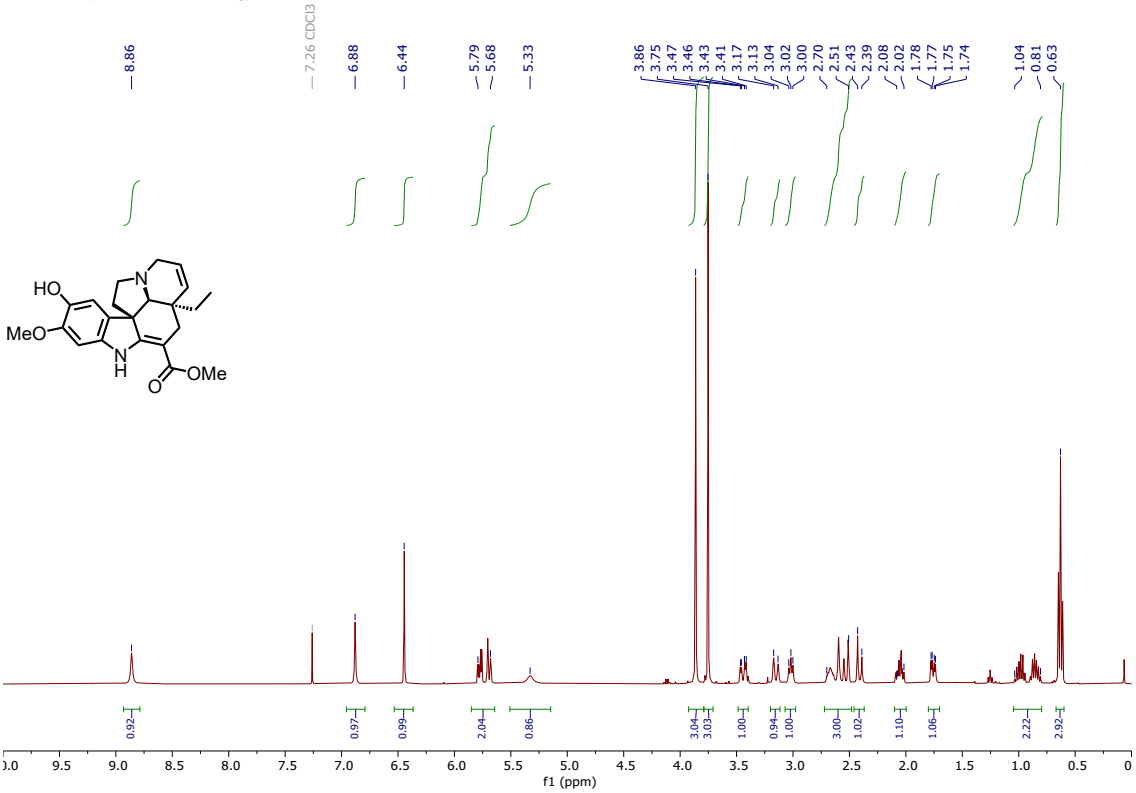


^{13}C NMR (101 MHz, $CDCl_3$)

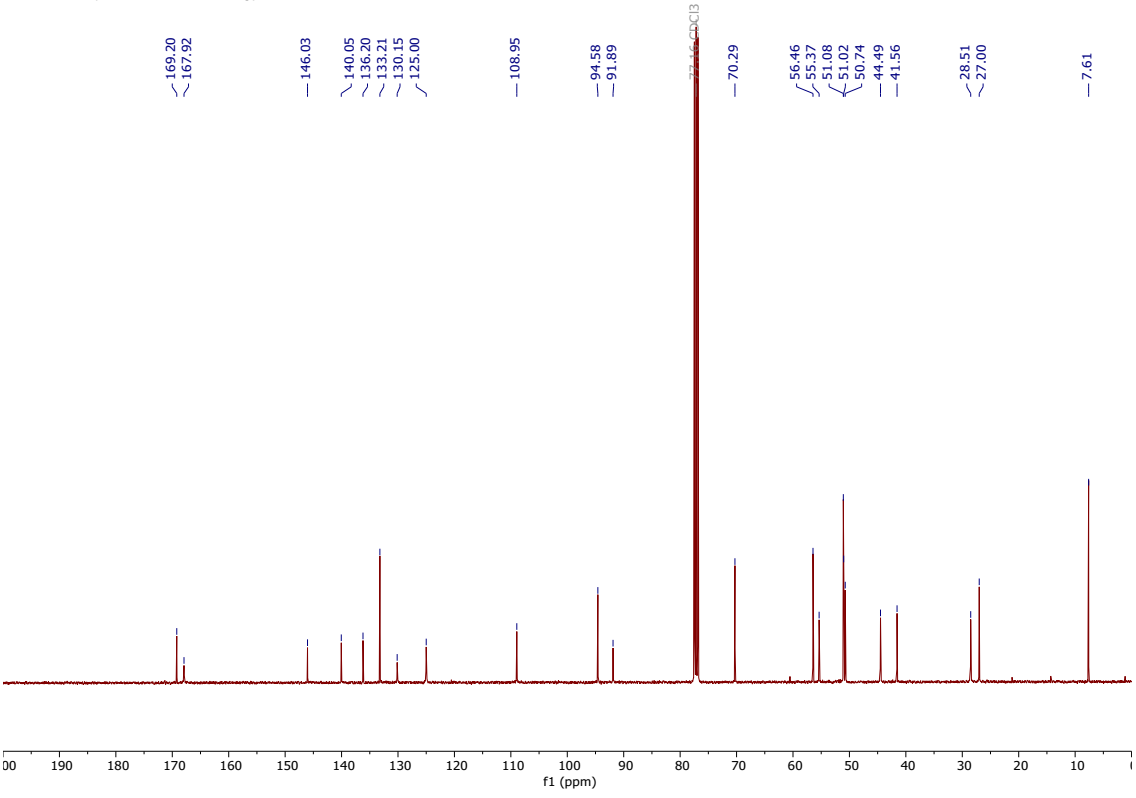


(–)-Jerantinine A (1)

^{1}H NMR (400 MHz, $CDCl_3$)

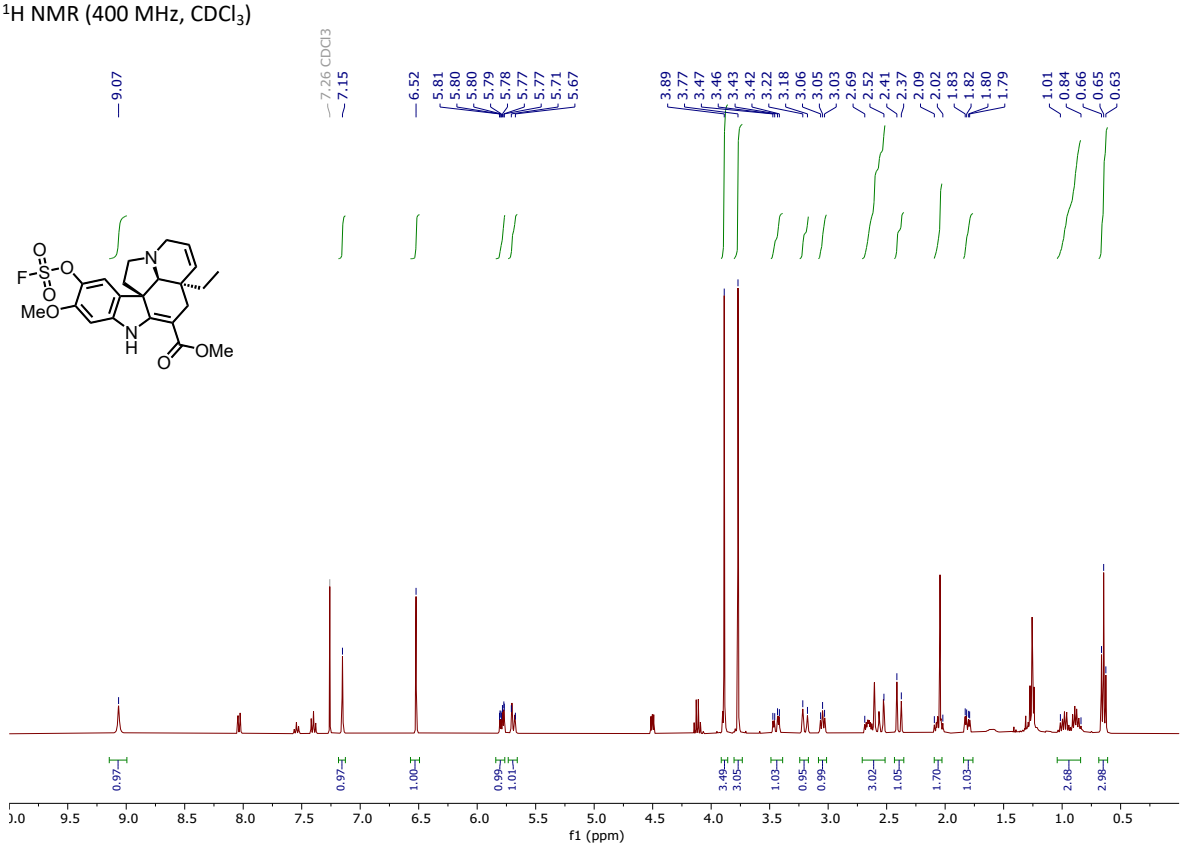


^{13}C NMR (101 MHz, $CDCl_3$)

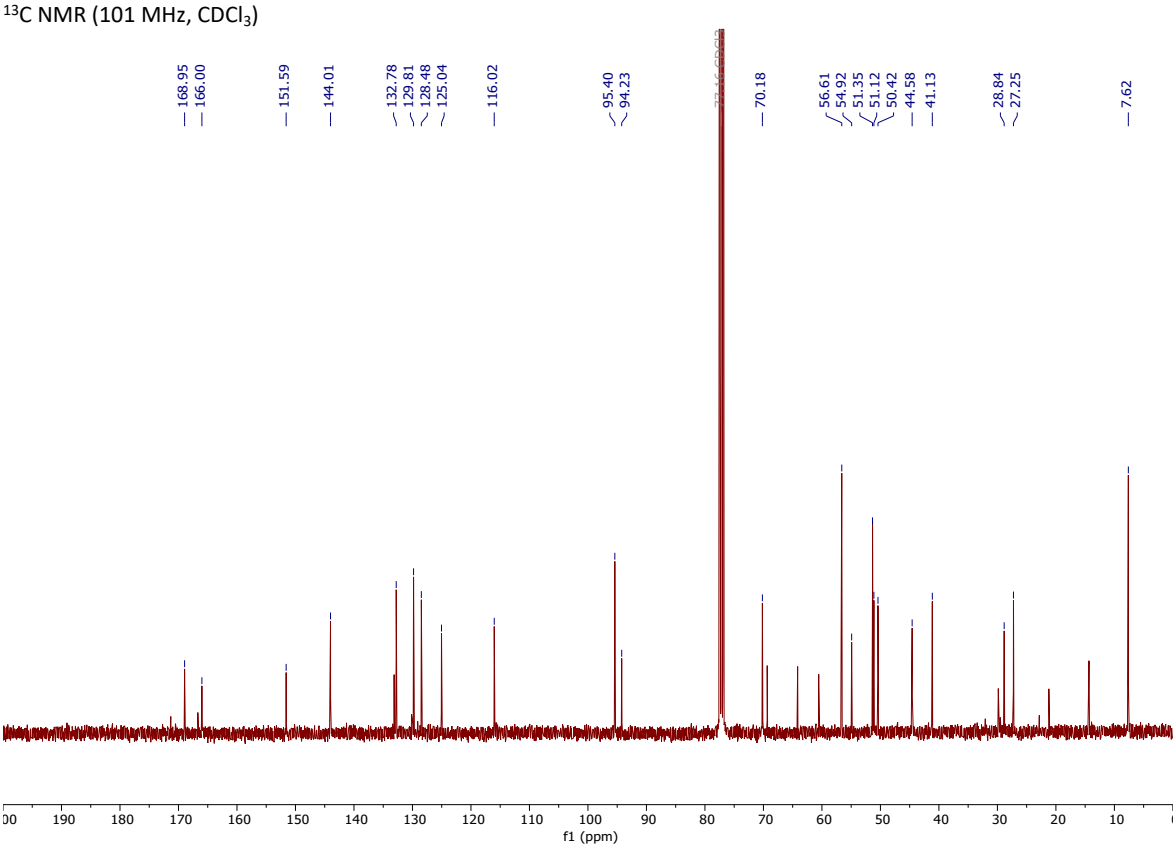


(–)-Jerantinine A fluorosulfate (7)

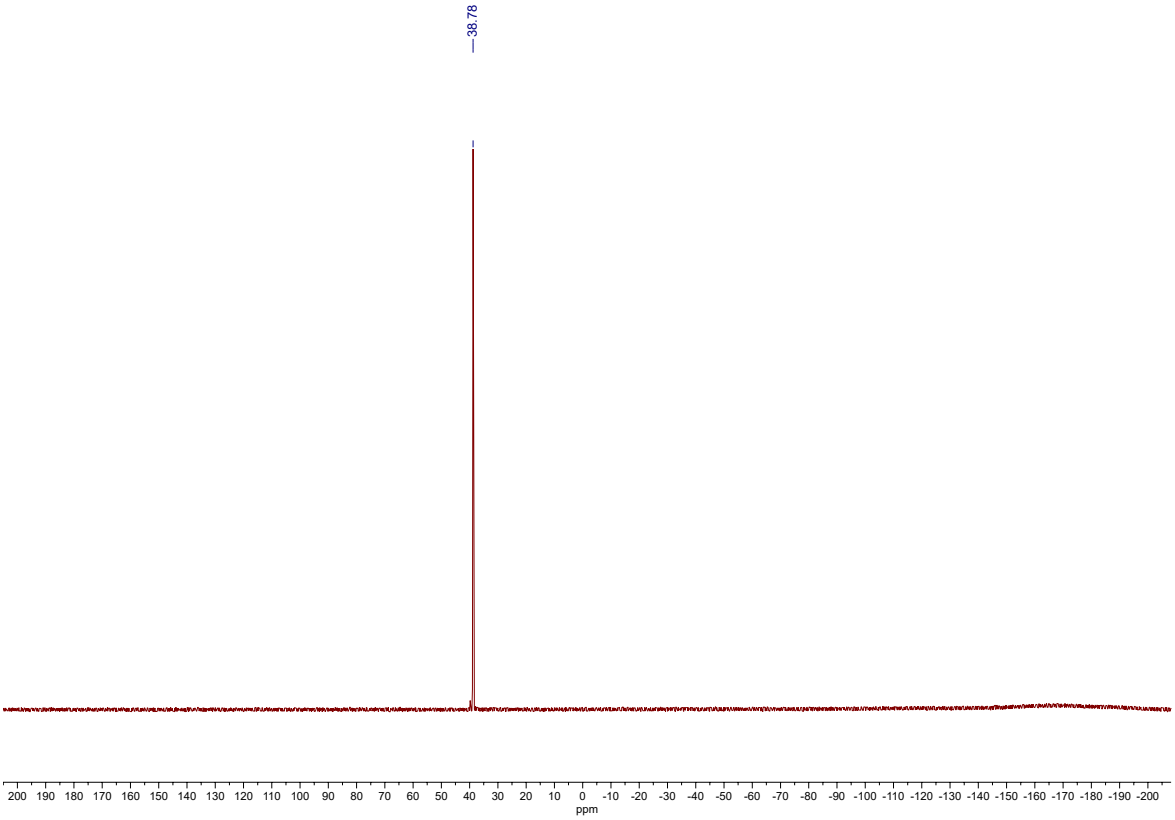
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, $CDCl_3$)

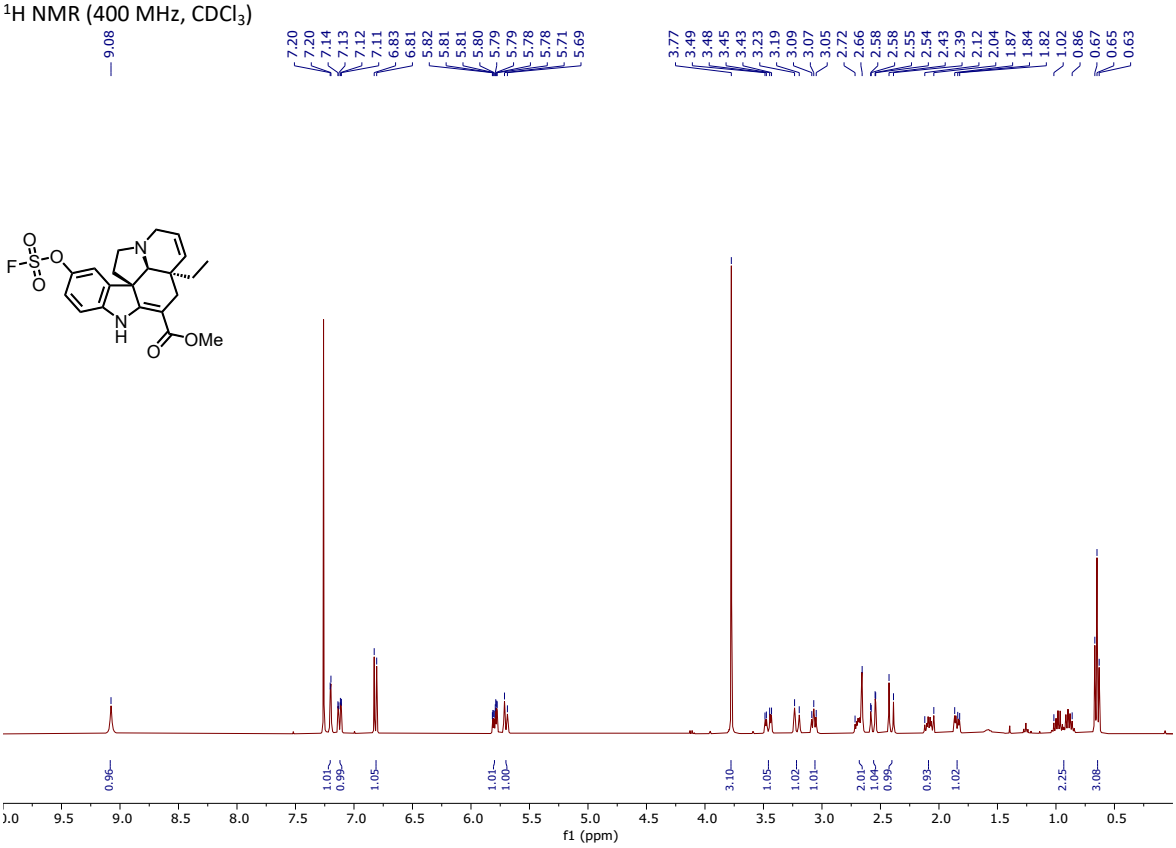


^{19}F NMR (377 MHz, $CDCl_3$)

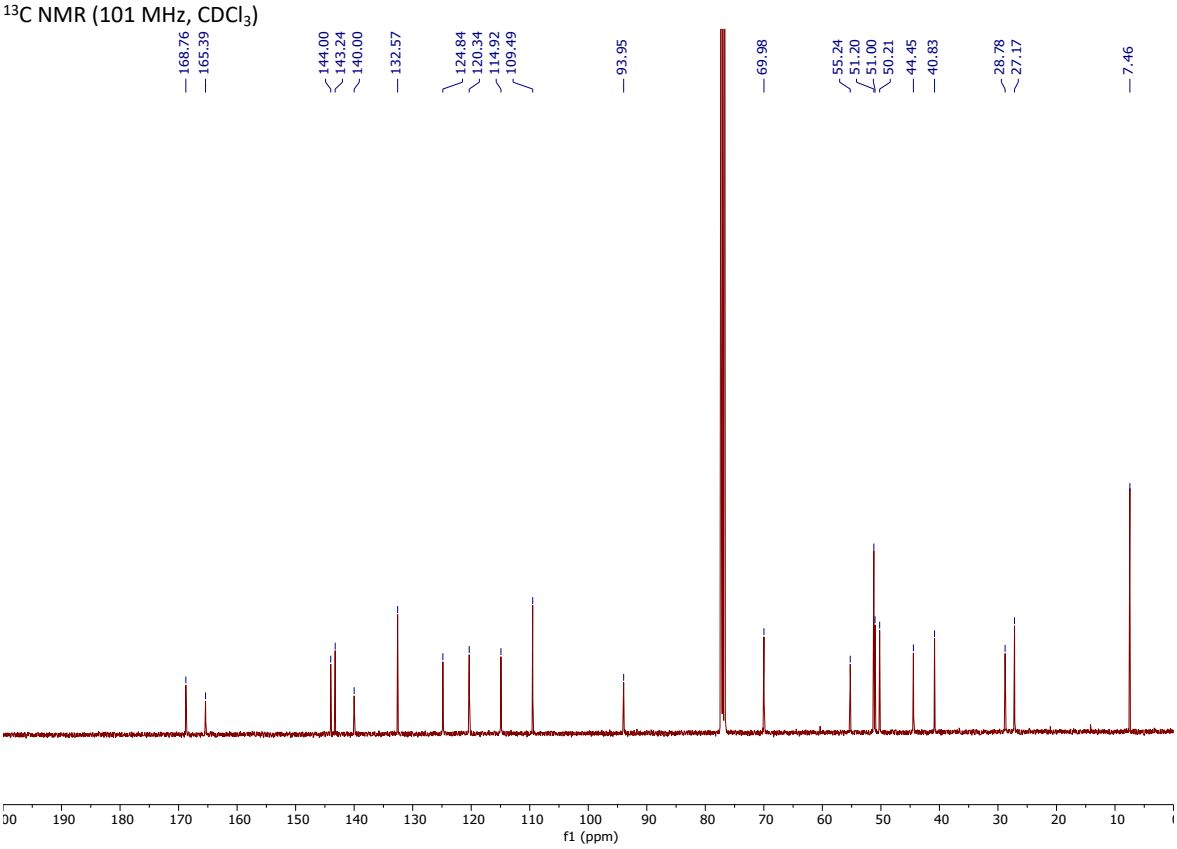


(–)-Melodinine P fluorosulfate (8)

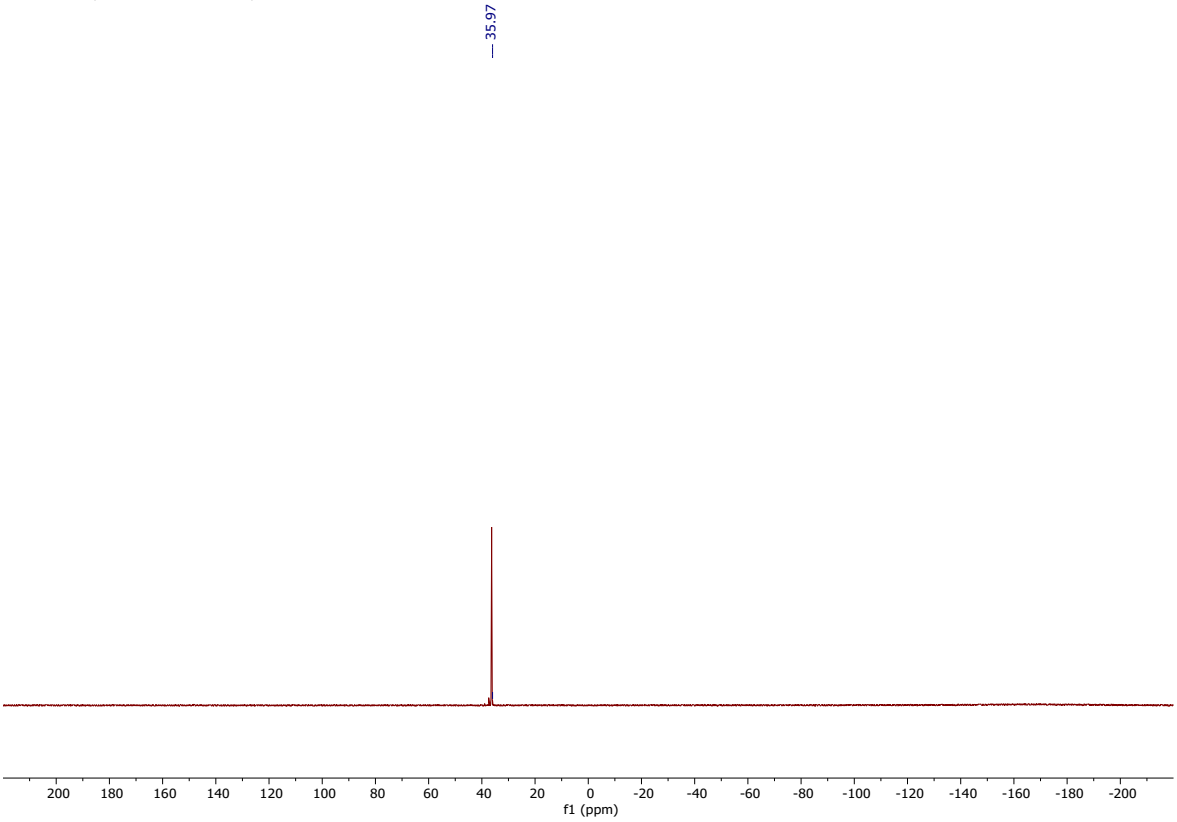
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, $CDCl_3$)

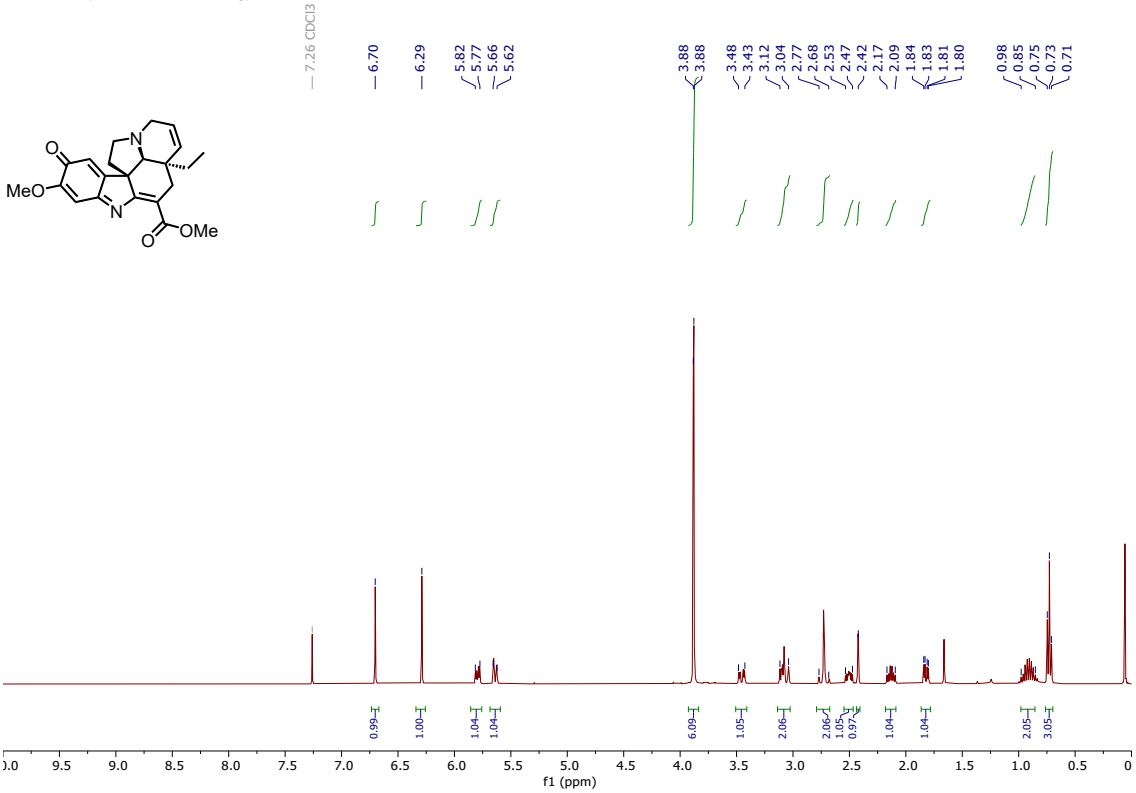


^{19}F NMR (377 MHz, $CDCl_3$)

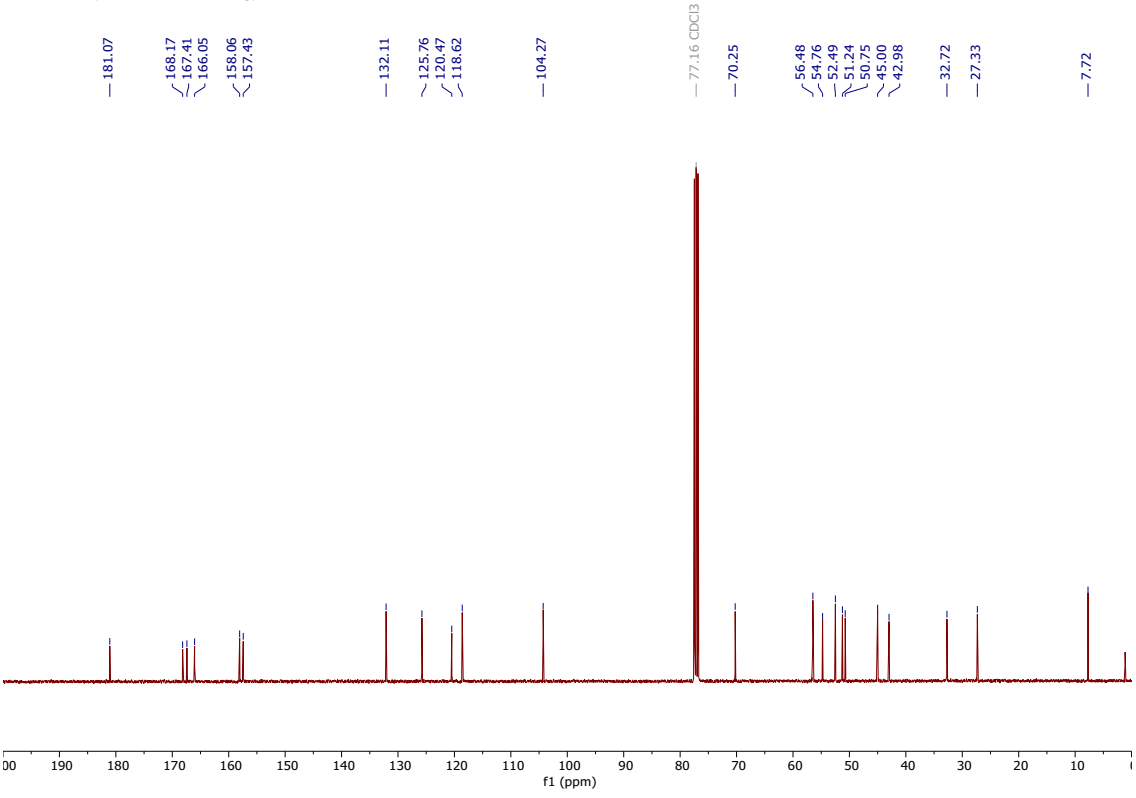


(–)-Jerantinine A iminoquinone (9)

^{1}H NMR (400 MHz, $CDCl_3$)

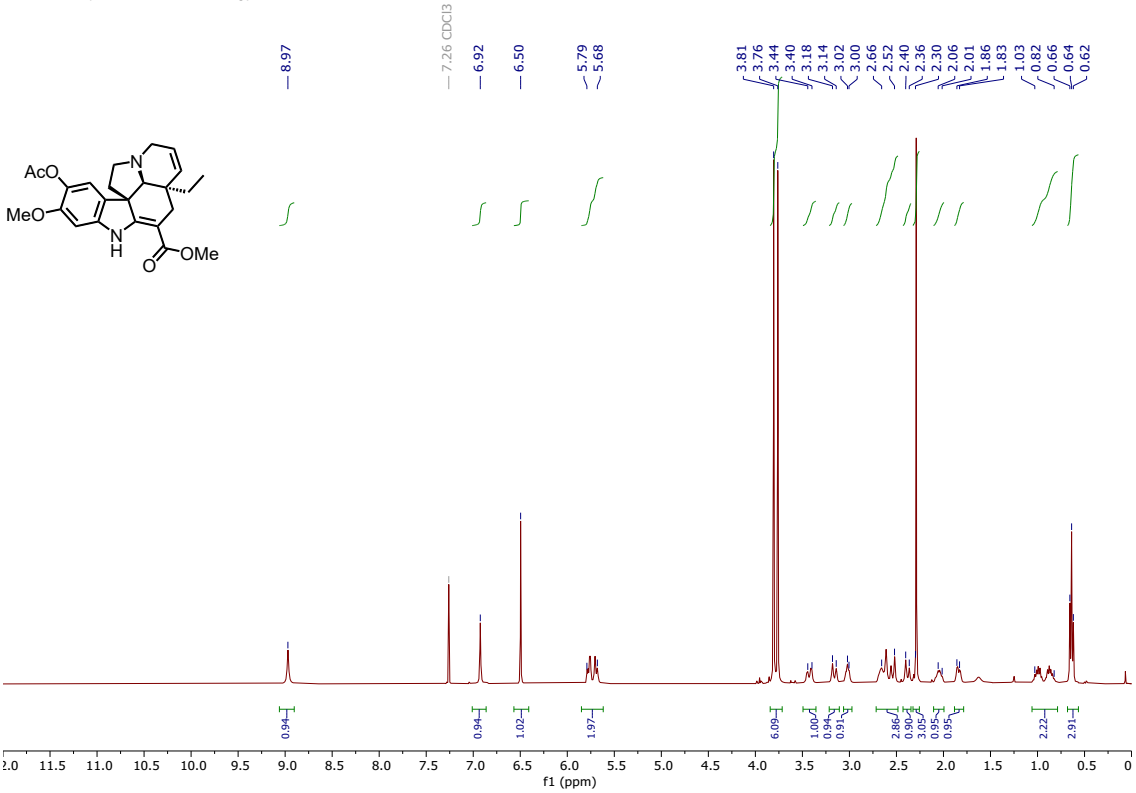


^{13}C NMR (101 MHz, $CDCl_3$)

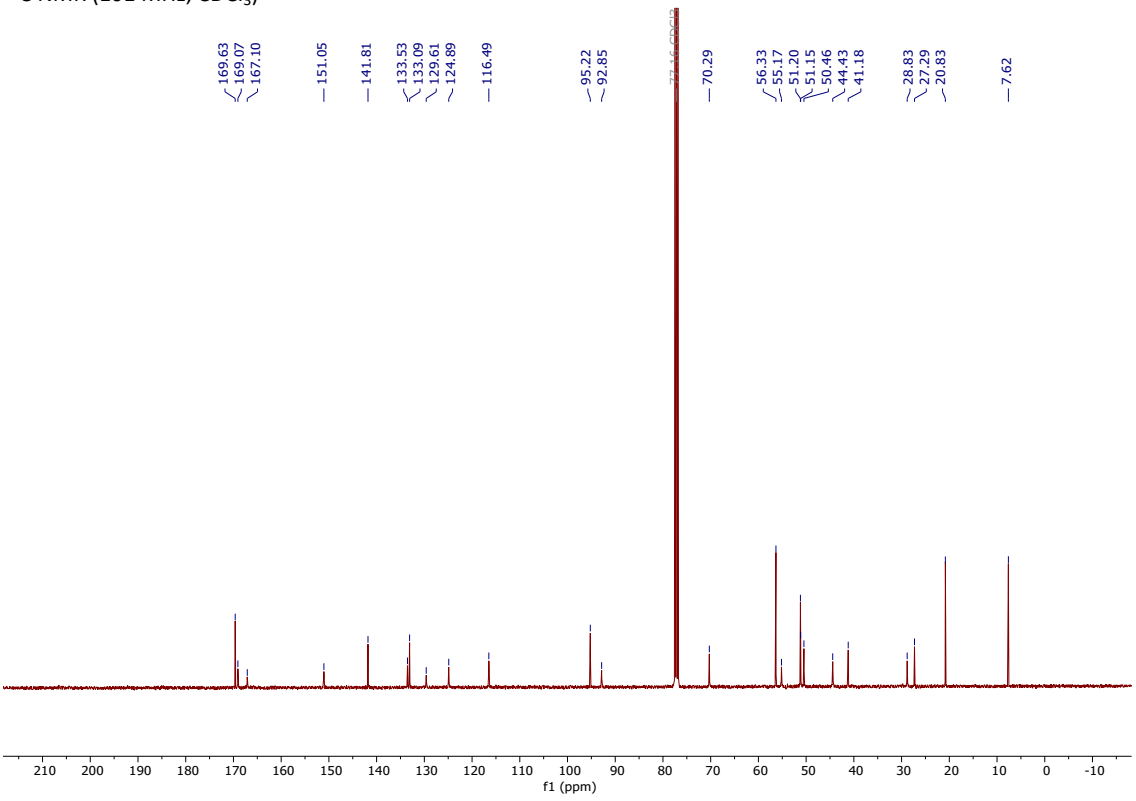


(–)-Jerantinine A acetate (2)

^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, $CDCl_3$)



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