

Supporting Information

Synthesis of Rocaglamide Hydroxamates and Related Compounds as Eukaryotic Translation Inhibitors: Synthetic and Biological Studies

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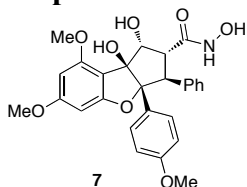
I. General Information

Proton NMR spectra were recorded at 400 or 500 MHz at ambient temperature with CDCl₃ or CD₃OD (Cambridge Isotope Laboratories, Inc.) as solvents. Data for ¹H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants in Hz. ¹³C NMR spectra were recorded at 100.0 or 125 MHz at ambient temperature with the same solvents unless otherwise stated. Chemical shifts are reported in parts per million relative to the deuterated solvents. All ¹³C NMR spectra were recorded with complete proton decoupling. Infrared spectra were recorded on a Nicolet Nexus 670 FT-IR spectrophotometer. High-resolution mass spectra were obtained in the Boston University Chemical Instrumentation Center using a Waters Q-TOF API-US mass spectrometer. Melting points were recorded on a Mel-Temp apparatus (Laboratory Devices). Analytical LC-MS was performed on a Waters Acquity UPLC (Ultra Performance Liquid Chromatography (Waters MassLynx Version 4.1) with a Binary solvent manager, SQ mass spectrometer, Water 2996 PDA (PhotoDiode Array) detector, and ELSD (Evaporative Light Scattering Detector). An Acquity UPLC BEH C₁₈ 1.7 μm column was used for analytical UPLC- MS. The method used for separation was gradient elution 10% to 99% acetonitrile in water over 2 minutes, then 99% acetonitrile in water for 1 minute. Retention times are reported. Purity of compounds are >95% pure as determined by UPLC-MS.

Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 200-400 mesh silica gel (Scientific Absorbents, Inc.). Yields refer to chromatographically and spectroscopically pure materials, unless otherwise stated. HPLC grade tetrahydrofuran, methylene chloride, diethyl ether, toluene, acetonitrile, and benzene were purchased from Fisher and VWR and were purified and dried by passing through a PURE SOLV[®] solvent purification system (Innovative Technology, Inc.). Other ACS grade solvents for chromatography were purchased from Clean Harbors. All other chemicals were purchased from Sigma Aldrich and used as received unless otherwise stated. Methyl rocaglate (**2**) and hydroxamate **3** were prepared according to the methods of Porco and coworkers.^{S1} Rocaglaic acid was prepared according to the methods of Porco and coworkers.^{S2} All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted.

II. Chemical Procedures And Compound Characterization

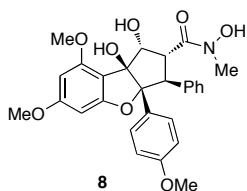
Preparation of amides



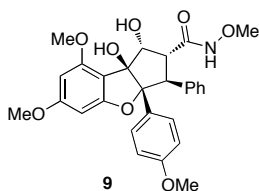
(+/-)-*N*-Hydroxyrocaglamide **7**: A round bottom flask was charged with (+/-)-rocaglaic acid (50 mg, 0.11 mmol), methylene chloride (2 mL), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (30.0 mg, 0.16 mmol), 1-hydroxybenzotriazole hydrate (20.8 mg, 0.14 mmol), hydroxylamine (34.5 mg, 0.52 mmol), and triethylamine (72.8 μ L, 0.52 mmol) and was stirred at room temperature for 12 h. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (gradient elution 2% to 20% methanol in methylene chloride) to afford compound **7** as a white solid (14 mg, 30%). ¹H NMR (500 MHz, CD₃OD) δ 7.16 (d, *J* = 9.1 Hz, 2H), 7.08 – 6.99 (m, 3H), 6.99 – 6.95 (m, 2H), 6.65 (d, *J* = 9.0 Hz, 2H), 6.31 (d, *J* = 1.9 Hz, 1H), 6.20 (d, *J* = 2.0 Hz, 1H), 4.77 (d, *J* = 6.1 Hz, 1H), 4.34 (d, *J* = 14.3 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.75 (dd, *J* = 14.2, 6.1 Hz, 1H), 3.69 (s, 3H); ¹³C NMR (101 MHz, CD₃OD) δ 169.19, 167.10, 163.16, 159.92, 159.87, 138.41, 136.04, 131.18, 130.77, 129.25, 128.80, 128.54, 127.51, 126.41, 114.89, 114.73, 113.01, 104.88, 102.47, 93.28, 90.42, 89.83, 77.63, 57.00, 56.39, 56.13, 55.29, 51.37; IR (film, cm⁻¹) 3600-3200, 2925, 2853, 1625, 1603, 1513, 1455, 1254, 1200, 1150, 1119; m.p. 233-234 °C; TLC *R*_f 0.2 (2% methanol/methylene chloride); UPLC *t*_R 1.34 min; HRMS calcd for [C₂₆H₂₇NO₈Na]⁺ requires *m/z* 516.1634; found 516.1613 (ES⁺).

^{S1} Roche, S. P.; Cencic, R.; Pelletier, J.; Porco, J. A., Jr. *Angew. Chem. Int. Ed.* **2010**, *49*, 6533-6538.

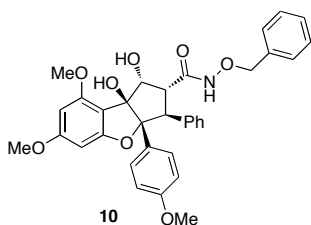
^{S2} Gerard, B.; Sangji, S.; O'Leary, D. J.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2006**, *128*, 7754-7755.



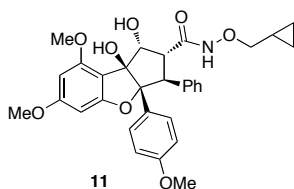
(+/-)-*N*-Hydroxy-*N*-methylrocaglamide **8**: A round bottom flask was charged with (+/-)-rocaglaic acid (20 mg, 0.04 mmol), methylene chloride (4 mL), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (12.0 mg, 0.06 mmol), 1-hydroxybenzotriazole hydrate (7 mg, 0.05 mmol), *N*-methylhydroxylamine (17 mg, 0.21 mmol), and triethylamine (23 μ L, 0.21 mmol) and was stirred at room temperature for 12 h. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (gradient elution 80% to 100% ethyl acetate in hexanes) to afford compound **8** as a white solid (10 mg, 49%). **¹H NMR** (500 MHz, CD₃OD) δ 7.14 (d, J = 8.3 Hz, 2H), 7.09 – 6.98 (m, 3H), 6.93 (d, J = 6.6 Hz, 2H), 6.65 (d, J = 8.3 Hz, 2H), 6.32 (d, J = 1.3 Hz, 1H), 6.20 (d, J = 1.3 Hz, 1H), 4.89 (d, J = 6.1 Hz, 1H), 4.30 (d, J = 14.2 Hz, 1H), 4.00 (dd, J = 14.2, 6.2 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.69 (s, 3H), 2.71 (s, 3H); **¹³C NMR** (126 MHz, CD₃OD) δ 171.54, 165.27, 162.21, 159.87, 159.32, 138.92, 130.17, 129.10, 128.55, 127.32, 113.22, 109.12, 102.69, 95.13, 93.10, 89.97, 80.56, 56.37, 56.10, 55.99, 55.44, 51.08, 39.37; **IR** (film, cm⁻¹) 3300-3100, 2924, 2853, 1625, 1610, 1508, 1445, 1238, 1211, 1148; **m.p.** 93-94 °C; **TLC** R_f 0.3 (80% ethyl acetate/hexanes); **UPLC** t_R 1.51 min; **HRMS** calcd for [C₂₈H₂₉NO₈H]⁺ requires m/z 508.1971; found 508.1982 (ES+).



(+/-)-*N*-methoxyrocaglamide **9**: A round bottom flask was charged with (+/-)-rocaglaic acid (27.0 mg, 0.06 mmol), methylene chloride (4 mL), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (16.2 mg, 0.08 mmol), 1-hydroxybenzotriazole hydrate (11.2 mg, 0.07 mmol), methoxylamine hydrochloride (23.6 mg, 0.28 mmol), and triethylamine (39.3 μ L, 0.28 mmol), and was stirred at room temperature for 12 h. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (gradient elution 50% to 100% ethyl acetate in hexanes) to afford compound **9** as a white solid (15 mg, 60%). **¹H NMR** (500 MHz, CD₃OD) δ 7.15 (d, J = 9.0 Hz, 2H), 7.09 – 6.99 (m, 3H), 6.94 (d, J = 6.9 Hz, 2H), 6.64 (d, J = 9.0 Hz, 2H), 6.32 (d, J = 1.9 Hz, 1H), 6.21 (d, J = 1.9 Hz, 1H), 4.79 (d, J = 6.2 Hz, 1H), 4.32 (d, J = 14.2 Hz, 1H), 3.87 (s, 1H), 3.85 (s, 1H), 3.72 (dd, J = 14.2, 6.2 Hz, 1H), 3.69 (s, 1H), 3.60 (s, 1H); **¹³C NMR** (126 MHz, CD₃OD) δ 169.36, 165.24, 162.20, 159.87, 159.28, 138.81, 130.21, 129.24, 129.20, 128.51, 127.35, 113.18, 109.37, 102.52, 95.17, 93.10, 90.07, 80.73, 64.31, 56.09, 56.03, 55.45; **IR** (film, cm⁻¹) 3600-3100, 2939, 2839, 1665, 1625, 1612, 1513, 1500, 1453, 1440, 1345, 1299, 1251, 1218, 1200, 1182, 1148, 1117, 1032, 814, 701, 641; **m.p.** 129-131 °C; **TLC** R_f 0.4 (100% ethyl acetate); **UPLC** t_R 1.36 min; **HRMS** calcd for [C₂₈H₂₉NO₈Na]⁺ requires m/z 530.1791; found 530.1772 (ES+).



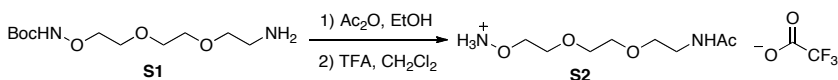
(+/-)-*N*-benzyloxycaglamide **10**: A round bottom flask was charged with (+/-)-rocaglaic acid (49.0 mg, 0.10 mmol), methylene chloride (4 mL), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (29 mg, 0.15 mmol), 1-hydroxybenzotriazole hydrate (18 mg, 0.13 mmol), benzylamine (38 mg, 0.30 mmol) and was stirred at room temperature for 12 h. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (100% ethyl acetate) to afford compound **10** as a white solid (55 mg, 85%). **¹H NMR** (500 MHz, CD₃CN, 60 °C) δ 7.50 (dd, *J* = 7.0, 1.4 Hz, 2H), 7.43 (br s, 1H), 7.17 – 6.97 (m, 2H), 6.79 (br s, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 6.19 (d, *J* = 7.8 Hz, 1H), 6.13 (d, *J* = 9.2 Hz, 2H), 5.97 (t, *J* = 5.0 Hz, 1H), 4.92 (d, *J* = 10.4 Hz, 2H), 4.64 (d, *J* = 14.5 Hz, 1H), 4.31 (dd, *J* = 14.5, 5.4 Hz, 2H), 3.94 (s, 3H), 3.42 (s, 3H), 3.37 (s, 3H), 1.98 (d, *J* = 1.1 Hz, 1H); **¹³C NMR** (101 MHz, CD₃CN) δ 171.67, 168.19, 166.27, 162.39, 159.27, 138.27, 136.98, 135.39, 130.94, 129.98, 129.46, 128.94, 128.30, 127.57, 125.32, 118.32, 114.86, 114.29, 112.74, 104.33, 102.74, 93.05, 89.91, 89.41, 79.00, 77.19, 60.96, 56.62, 55.94, 55.53, 52.02; **IR** (film, cm⁻¹) 3400-3200, 2923, 2848, 1631, 1453, 1411, 1260, 1117, 1017, 668; **m.p.** 172-173 °C; **TLC** *R_f* 0.3 (100% ethyl acetate); **UPLC** *t_R* 1.63 min; **HRMS** calcd for [C₃₄H₃₃NO₈H(-H₂O)]⁺ requires *m/z* 566.2179; found 566.2203 (ES+).



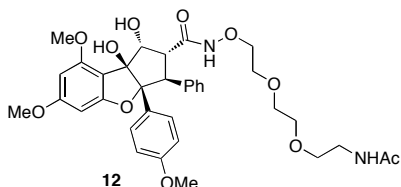
(+/-)-*N*-cyclopropylmethoxycaglamide **11**: A round bottom flask was charged with (+/-)-rocaglaic acid (25 mg, 0.05 mmol), methylene chloride (0.8 mL), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (15.0 mg, 0.08 mmol), 1-hydroxybenzotriazole hydrate (10.4 mg, 0.07 mmol), *O*-cyclopropylmethylhydroxylamine hydrochloride^{S3} (32.3 mg, 0.26 mmol), and triethylamine (36.4 μL, 0.26 mmol) and was stirred for 12 h at room temperature. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (100% ethyl acetate) to afford compound **11** as a white solid (6.0 mg, 21%). **¹H NMR** (500 MHz, CD₃OD) δ 7.13 (d, *J* = 8.8 Hz, 2H), 7.01 (m, 3H), 6.91 (d, *J* = 7.8 Hz, 2H), 6.63 (d, *J* = 8.8 Hz, 2H), 6.28 (d, *J* = 1.7 Hz, 1H), 6.17 (d, *J* = 1.6 Hz, 1H), 4.76 (d, *J* = 6.3 Hz, 1H), 4.60 (br s, 1H), 4.28 (d, *J* = 14.1 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.73 – 3.66 (dd, *J* = 14.1, 6.3 Hz, 1H), 3.66 (s, 3H), 3.60 – 3.46 (m, 2H), 0.90 (m, 1H), 0.37 (d, *J*

^{S3} Barrett, S. D.; Bridges, A. J.; Dudley, D. T.; Saltiel, A. R.; Fergus, J. H.; Flamme, A. M.; Delaney, A. M.; Kaufman, M.; LePage, S.; Leopold, W. R.; Przybranowski, S. A.; Sebolt-Leopold, J.; Van Becelaere, K.; Doherty, A. M.; Warmus, J. S.; Tecle, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6501-6504.

= 8.1 Hz, 2H), 0.07 (dd, J = 9.1, 4.8 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 178.93, 169.60, 165.24, 159.89, 138.81, 130.23, 129.28, 128.51, 127.38, 113.17, 106.42, 102.50, 95.09, 93.09, 90.07, 81.87, 80.77, 56.21, 56.09, 55.46, 10.03, 3.58, 3.30; IR (film, cm^{-1}) 3500-3200, 2924, 2852, 1622, 1611, 1513, 1465, 1251, 1200, 1148, 1117, 1032; m.p. 197-198 $^\circ\text{C}$; TLC R_f 0.1 (80% ethyl acetate/ hexanes); UPLC t_R 1.51 min; HRMS calcd for $[\text{C}_{31}\text{H}_{33}\text{NO}_8\text{H}]^+$ requires m/z 548.2284; found 548.2284 (ES+). (-)-**11** was prepared in the same way from (-)-rocaglaic acid. $[\alpha]_D^{20}$ = -40.8 (c 0.2, CH_3OH , at >98% ee).



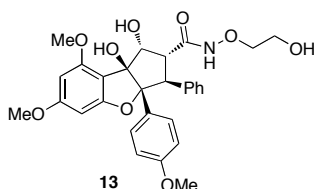
Acetate **S2**: Compound **S1** was prepared according to the methods of Jones and coworkers.^{S4} A round bottom flask was charged with amine **S1** (90 mg, 0.3 mmol), ethanol (400 μL), and acetic anhydride (32 μL , 0.3 mmol), and the reaction was stirred for 2 h. The reaction was concentrated to a yellow oil which was used without further purification. The oil was dissolved in methylene chloride (25 mL) and trifluoroacetic acid (2.5 mL, 32 mmol) was added dropwise. The reaction was stirred for 3 h, and then concentrated under a stream of air over 24 h, yielding compound **S2** as a crude oil (71 mg, quant over 2 steps) that was used in the next step without further purification. ^1H NMR (400 MHz, CDCl_3) δ 7.52 (s, 3H), 4.21 – 4.18 (m, 2H), 3.95 – 3.77 (m, 2H), 3.74 – 3.67 (m, 2H), 3.67 – 3.52 (m, 4H), 3.43 – 3.41 (m, 2H), 2.01 (s, 3H).



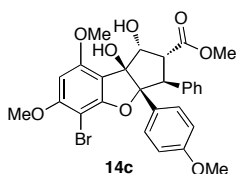
(+/-)-Hydroxamate **12**: A round bottom flask was charged with (+/-)-rocaglaic acid (20 mg, 0.04 mmol), methylene chloride (3 mL), *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (12.0 mg, 0.06 mmol), 1-hydroxybenzotriazole hydrate (8.3 mg, 0.05 mmol), a solution of amine **S2** (43 mg, 0.2 mmol) in methylene chloride (1 mL), and triethylamine (29 μL , 0.2 mmol) and was stirred for 12 h at room temperature. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (gradient elution 2% to 10% methanol in methylene chloride) to afford compound **12** as a white solid (15 mg, 53%). ^1H NMR (500 MHz, CD_3OD) δ 7.16 (d, J = 8.9 Hz, 2H), 7.11 – 6.99 (m, 3H), 6.95 (d, J = 6.3 Hz, 2H), 6.66 (d, J = 8.9 Hz, 2H), 6.32 (d, J = 1.9 Hz, 1H), 6.20 (d, J = 1.9 Hz, 1H), 4.80 (d, J = 6.2 Hz, 1H), 4.31 (d, J = 14.3 Hz, 1H), 3.94 – 3.88 (m, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.74 (dd, J = 14.3, 6.2 Hz, 1H), 3.69 (s, 3H), 3.62 – 3.56 (m, 5H), 3.53 (t, J = 5.5 Hz, 2H), 3.38 – 3.30 (m, 6H), 1.92 (s, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.43, 169.54, 165.25, 162.19, 159.89, 159.29, 130.23, 129.26, 129.22, 128.56, 127.42, 113.18, 102.49, 95.19, 93.11, 90.07, 80.71, 76.23, 71.46, 71.11, 70.48, 69.55, 56.10, 56.05, 55.46, 40.43, 22.54; IR (film, cm^{-1})

^{S4} Jones, D. S. Cockerill, K. A.; Gamino, C. A.; Hammaker, J. R.; Hayag, M. S.; Iverson, G. M.; Linnik, M. D.; McNeeley, P. A.; Tedder, M. E.; Ton-Nu, H.-T.; Victoria, E. J. *Bioconjugate Chem.* **2001**, 12, 1012-1020.

3500-3200, 2931, 2834, 1625, 1513, 1453, 1298, 1250, 1218, 1200, 1147, 1117, 812; **m.p.** 86-88 °C; **TLC** R_f 0.4 (10% methanol/ methylene chloride); **UPLC** t_R 1.29 min; **HRMS** calcd for $[C_{35}H_{42}N_2O_{11}Na]^+$ requires m/z 689.2686; found 689.2672 (ES+).

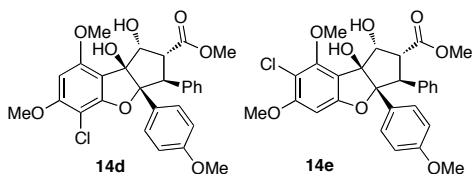


(+/-)-Hydroxamate **13**: A round bottom flask was charged with (+/-)-rocaglaic acid (25 mg, 0.05 mmol), methylene chloride (0.8 mL), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (15.0 mg, 0.08 mmol), 1-hydroxybenzotriazole hydrate (10.4 mg, 0.07 mmol), 2-(aminooxy)ethan-1-ol (20.1 mg, 0.26 mmol), and triethylamine (36.4 μ L, 0.26 mmol), and was stirred for 12 h at room temperature. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (80% ethyl acetate in hexanes) to afford compound **13** as a white solid (55 mg, 20%). **¹H NMR** (500 MHz, CD₃OD) δ 7.14 (d, J = 9.0 Hz, 2H), 7.08 – 6.97 (m, 3H), 6.92 (d, J = 7.3 Hz, 2H), 6.64 (d, J = 9.0 Hz, 2H), 6.30 (d, J = 2.0 Hz, 1H), 6.19 (d, J = 2.0 Hz, 1H), 4.79 (d, J = 6.3 Hz, 1H), 4.59 (br s, 1H), 4.28 (d, J = 14.3 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.82 – 3.77 (t, 2H), 3.73 (dd, J = 14.3, 6.3 Hz, 1H), 3.68 (s, 3H), 3.63 – 3.48 (m, 2H); **¹³C NMR** (126 MHz, CD₃OD) δ 170.23, 165.26, 162.17, 159.89, 159.26, 138.73, 130.23, 129.22, 129.18, 128.55, 127.43, 113.19, 109.37, 106.42, 102.47, 95.17, 93.12, 90.09, 80.71, 78.67, 60.14, 56.16, 56.10, 56.05, 55.46; **IR** (film, cm⁻¹) 3500-3200, 2926, 2854, 1618, 1610, 1513, 1453, 1250, 1200, 1148, 1117, 812; **m.p.** 178-179 °C; **TLC** R_f 0.1 (80% ethyl acetate/ hexanes); **UPLC** t_R 1.29 min; **HRMS** calcd for $[C_{29}H_{31}NO_9H]^+$ requires m/z 538.2077; found 538.2062 (ES+).



(+/-)-5-Bromo methyl rocaglate (**14c**): A round bottom flask was charged with *N*-bromosuccinimide (19.0 mg, 0.106 mmol), and a solution of (+/-)-methyl rocaglate (50 mg, 0.1 mmol) in tetrahydrofuran (1.24 mL) was added, and the reaction was stirred at room temperature for 50 minutes. The reaction was concentrated and purified by flash chromatography (gradient elution 30% to 75% ethyl acetate in hexanes) to afford compound **14c** as a colorless oil (21 mg, 40%). **¹H NMR** (500 MHz, CDCl₃) δ 7.13 (d, J = 8.8 Hz, 2H), 7.10 – 7.01 (m, 3H), 6.93 (d, J = 6.4 Hz, 2H), 6.67 (d, J = 8.9 Hz, 2H), 6.15 (s, 1H), 5.00 (dd, J = 6.5, 2.0 Hz, 1H), 4.33 (d, J = 14.2 Hz, 1H), 3.94 (d, J = 2.0 Hz, 1H), 3.91 (s, 3H), 3.91 (s, 3H), 3.69 (s, 3H), 3.65 (s, 3H), 3.56 (d, J = 1.6 Hz, 1H), 1.97 (s, 1H); **¹³C NMR** (126 MHz, CDCl₃) δ 170.68, 159.62, 158.98, 157.85, 156.19, 136.68, 129.02, 128.83, 127.93, 127.73, 126.74, 126.01, 113.88, 112.94, 109.16, 102.33, 94.55, 90.02, 86.27, 79.71, 56.97, 56.14, 55.26, 52.20, 50.36; **IR** (film, cm⁻¹) 3500-3350, 2950, 2842, 1742, 1611, 1513, 1496, 1453, 1435, 1420, 1346, 1298, 1250, 1208, 1179, 1133, 1096, 1032, 973, 864, 829, 791, 734, 699, 620; **TLC** R_f 0.2 (50% ethyl acetate/ hexanes);

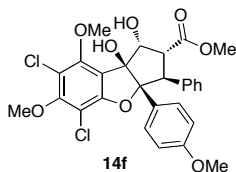
UPLC t_R 1.69 min; **HRMS** calcd for $[C_{28}H_{27}BrO_8Na]^+$ requires m/z 593.0787; found 593.0782 (ES+).



(+/-)-5-Chloro methyl rocaglate (**14d**) and (+/-)-7-chloro methyl rocaglate (**14e**): A round bottom flask was charged with *N*-chlorosuccinimide (14 mg, 0.10 mmol), cooled to -78 °C, and a solution of (+/-)-methyl rocaglate (50 mg, 0.1 mmol) in tetrahydrofuran (2 mL) was added. The reaction was stirred at -78 °C for 5 h. The reaction was concentrated and purified by flash chromatography (gradient elution 25% to 100% ethyl acetate in hexanes) to afford compound **14d** as a colorless oil (5 mg, 9%) and compound **14e** as a colorless oil (16 mg, 32%).

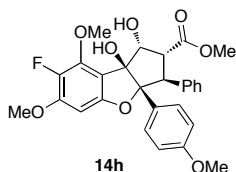
14d: 1H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, J = 8.8 Hz, 2H), 7.11 – 7.01 (m, 3H), 6.94 (d, J = 6.4 Hz, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.18 (s, 1H), 5.00 (dd, J = 6.4, 2.1 Hz, 1H), 4.36 (d, J = 14.1 Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.70 (s, 3H), 3.66 (s, 3H), 3.55 (d, J = 1.7 Hz, 1H), 2.77 (d, J = 0.5 Hz, 1H), 1.89 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.72, 159.01, 158.69, 156.62, 155.23, 136.67, 129.01, 127.95, 127.89, 126.76, 125.99, 112.96, 109.21, 102.67, 94.37, 90.03, 79.63, 77.62, 56.94, 56.17, 55.27, 52.22, 50.44, 29.69; **IR** (film, cm^{-1}) 3500-3400, 2924, 2850, 1711, 1613, 1514, 1435, 1350, 1253, 1210, 1181, 1136, 1101, 668; **TLC** R_f 0.2 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.67 min; **HRMS** calcd for $[C_{28}H_{27}ClO_8Na]^+$ requires m/z 549.1292; found 549.1282 (ES+).

14e: 1H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, J = 8.8 Hz, 2H), 7.09 – 7.00 (m, 3H), 6.90 (m, 2H), 6.69 (d, J = 8.6 Hz, 2H), 6.52 (s, 1H), 5.00 (d, J = 6.4 Hz, 1H), 4.31 (d, J = 14.1 Hz, 1H), 4.01 (s, 3H), 3.94 (s, 3H), 3.90 (dd, J = 14.1, 6.6 Hz, 1H), 3.85 (s, 1H), 3.71 (s, 3H), 3.65 (s, 3H), 1.81 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.61, 159.08, 159.04, 158.39, 153.99, 136.80, 128.99, 127.90, 127.80, 126.78, 126.01, 114.26, 113.20, 113.09, 109.04, 102.21, 94.24, 92.47, 79.17, 62.34, 56.82, 55.32, 55.27, 52.21, 50.64; **IR** (film, cm^{-1}) 3500-3350, 2922, 2850, 1744, 1612, 1514, 1438, 1410, 1298, 1252, 1201, 1167, 1103, 828, 736, 698, 668; **TLC** R_f 0.6 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.68 min; **HRMS** calcd for $[C_{28}H_{27}ClO_8Na]^+$ requires m/z 549.1292; found 549.1302 (ES+).

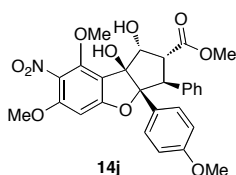


(+/-)-5,7-Dichloro methyl rocaglate (**14f**): A round bottom flask was charged with *N*-chlorosuccinimide (17 mg, 0.11 mmol) and a solution of (+/-)-methyl rocaglate (30 mg, 0.1 mmol) in tetrahydrofuran (0.5 mL) and was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (gradient elution 10% to 25% ethyl acetate in hexanes) to afford compound **14f** as a colorless oil (19 mg, 56%). 1H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 8.7 Hz, 2H), 7.00 – 6.98 (m, 3H), 6.84-6.82 (m, 2H), 6.61 (d, J = 8.7 Hz, 2H), 4.95 (d, J = 6.3 Hz, 1H), 4.25 (d, J = 14.2 Hz, 1H), 3.95 (s, 3H), 3.86 (s, 3H), 3.85 (dd, J = 14.2, 6.3 Hz, 1H), 3.63 (s, 3H), 3.59 (s, 3H), ^{13}C

NMR (126 MHz, CDCl₃) δ 170.52, 159.24, 155.61, 154.32, 152.09, 136.30, 128.93, 128.38, 128.17, 127.80, 126.93, 125.25, 119.16, 115.86, 113.20, 108.62, 102.68, 94.80, 79.27, 62.63, 61.11, 55.51, 55.29, 52.37, 50.41; **IR** (film, cm⁻¹) 3500-3300, 2925, 2850, 1742, 1666, 1606, 1514, 1463, 1409, 1352, 1265, 1252, 1211, 1182, 1116, 1081, 969, 831, 699; **TLC** *R_f* 0.8 (50% ethyl acetate/ hexanes); **UPLC** *t_R* 1.81 min; **HRMS** calcd for [C₂₈H₂₆Cl₂O₈Na]⁺ requires *m/z* 583.0902; found 583.0901 (ES+).

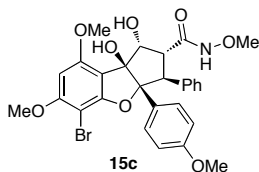


(+/-)-7-Fluoro methyl rocaglate (**14h**): A round bottom flask was charged with (+/-)-methyl rocaglate (15 mg, 0.03 mmol) and was dissolved in methanol (0.5 mL). Gold (III) chloride (1 mg, 0.003 mmol) and Selectfluor[®] (11 mg, 0.03 mmol) were added, and the suspension was stirred at room temperature for 5 h. The reaction was concentrated and purified by flash chromatography (gradient elution 25% to 100% ethyl acetate in hexanes) to afford compound **14h** as a colorless oil (7 mg, 40%). **¹H NMR** (400 MHz, CDCl₃) δ 7.10 (d, *J* = 9.0 Hz, 2H), 7.07-7.05 (m, 3H), 6.89-6.87 (m, 2H), 6.68 (d, *J* = 9.0 Hz, 2H), 6.44 (d, *J* = 5.8 Hz, 1H), 4.98 (d, *J* = 6.5 Hz, 1H), 4.30 (d, *J* = 14.2 Hz, 1H), 4.08 (s, 3H), 3.91 (s, 3H), 3.89 (dd, *J* = 14.2, 6.5 Hz, 1H), 3.71 (s, 3H), 3.69 (s, 1H), 3.65 (s, 3H), 1.82 (s, 1H); **¹³C NMR** (126 MHz, CDCl₃) δ 170.73, 158.96, 154.30, 152.08, 144.58, 141.44, 139.54, 136.84, 129.00, 127.91, 126.75, 126.20, 113.07, 112.96, 111.41, 101.82, 94.61, 91.53, 79.31, 62.05, 56.76, 55.25, 52.21, 50.53; **IR** (film, cm⁻¹) 3500-3400, 2922, 2849, 1743, 1629, 1611, 1514, 1481, 1444, 1299, 1251, 1200, 1182, 1140, 1113, 1031, 978, 810, 736, 700; **TLC** *R_f* 0.7 (50% ethyl acetate/ hexanes); **UPLC** *t_R* 1.62 min; **HRMS** calcd for [C₂₈H₂₇FO₈Na]⁺ requires *m/z* 533.1588; found 533.1585 (ES+).

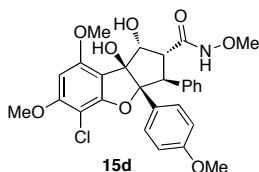


(+/-)-7-Nitro methyl rocaglate (**14j**): A round bottom flask was charged with (+/-)-methyl rocaglate (32.0 mg, 0.06 mmol) and copper dinitrate (9.4 mg, 0.05 mmol) and was cooled to 0 °C. Acetic anhydride (600 μ L) was added at 0 °C and the ice bath was removed and the reaction was stirred for 6 h. The reaction was quenched with water and extracted into ethyl acetate and the organic phase was concentrated. The crude product was purified by flash chromatography (gradient elution 20% to 100% ethyl acetate in hexanes) to afford compound **14j** as a beige solid (9 mg, 26%). **¹H NMR** (500 MHz, CDCl₃) δ 7.14 – 7.02 (m, 5H), 7.00 – 6.94 (m, 2H), 6.67 (d, *J* = 9.0 Hz, 2H), 6.49 (s, 1H), 4.91 (dd, *J* = 5.6, 2.6 Hz, 1H), 4.45 (d, *J* = 14.2 Hz, 1H), 4.06 (s, 3H), 3.98 (dd, *J* = 14.2, 5.4 Hz, 1H), 3.93 (s, 3H), 3.70 (s, 3H), 3.69 (s, 3H), 3.39 (d, *J* = 2.2 Hz, 1H), 1.83 (s, 1H); **¹³C NMR** (126 MHz, CDCl₃) δ 171.16, 161.59, 159.28, 155.57, 151.00, 136.47, 128.81, 128.05, 127.92, 126.90, 125.50, 113.28, 112.18, 103.02, 93.80, 91.34, 78.96, 63.62, 56.98, 55.81, 55.28, 52.44, 50.75; **IR** (film, cm⁻¹) 3500-3350, 2953, 2846, 1742, 1619, 1595, 1530, 1514, 1483, 1440, 1418, 1375, 1346, 1299, 1252, 1202, 1181, 1170, 1115, 1032, 911, 840, 733,

701, 671, 620; **m.p.** 193-194 °C; **TLC** R_f 0.7 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.66 min; **HRMS** calcd for $[C_{28}H_{27}NO_{10}Na]^+$ requires m/z 560.1533; found 560.1536 (ES+).

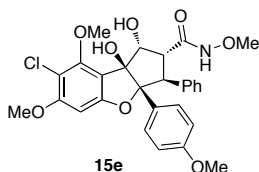


(+/-)-Hydroxamate **15c**: A round bottom flask was charged with ester **14c** (9 mg, 0.02 mmol), 1,4-dioxane (100 μ L), lithium hydroxide (2.0 mg, 0.07 mmol), and water (20 μ L), and was heated to 60 °C for 14 h. The reaction was cooled to room temperature, concentrated, and filtered through silica gel in 20% methanol in methylene chloride. The filtrate was transferred to a round bottom flask, concentrated, and dissolved in methylene chloride (750 μ L). *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (3.3 mg, 0.02 mmol), 1-hydroxybenzotriazole hydrate (2.3 mg, 0.01 mmol), methoxylamine hydrochloride (4.8 mg, 0.06 mmol), and triethylamine (8.0 μ L, 0.06 mmol) were added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (gradient elution 2% to 20% methanol in methylene chloride) to afford compound **15c** as a white solid (4 mg, 60%). **¹H NMR** (500 MHz, CD₃OD) δ 7.17 (d, J = 8.7 Hz, 2H), 7.11 – 6.95 (m, 5H), 6.64 (d, J = 8.9 Hz, 2H), 6.35 (s, 1H), 4.75 (d, J = 5.8 Hz, 1H), 4.59 (br s, 1H), 4.36 (d, J = 14.3 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.73 (dd, J = 14.3, 5.8 Hz, 1H), 3.68 (s, 3H), 3.60 (s, 3H); **¹³C NMR** (126 MHz, CD₃OD) δ 169.31, 160.47, 159.93, 159.01, 158.48, 138.65, 130.10, 128.94, 128.58, 127.37, 125.22, 116.63, 113.18, 103.61, 103.00, 91.04, 89.86, 80.62, 64.33, 62.48, 57.13, 56.30, 56.22, 55.42; **IR** (film, cm⁻¹) 3500-3350, 2920, 2849, 1665, 1612, 1514, 1463, 1347, 1252, 1210, 1181, 1133, 668; **m.p.** 204-205 °C; **TLC** R_f 0.6 (10% methanol/ methylene chloride); **UPLC** t_R 1.46 min; **HRMS** calcd for $[C_{28}H_{28}BrNO_8Na]^+$ requires m/z 608.0896; found 608.0884 (ES+).

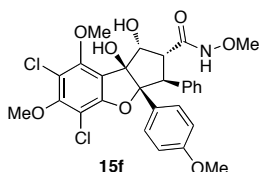


(+/-)-Hydroxamate **15d**: A round bottom flask was charged with ester **14d** (4 mg, 0.01 mmol), 1,4-dioxane (120 μ L), lithium hydroxide (2.0 mg, 0.07 mmol), and water (20 μ L), and was heated to 60 °C for 14 h. The reaction was cooled to room temperature, concentrated, and filtered through silica gel in 20% methanol in methylene chloride. The filtrate was transferred to a round bottom flask, concentrated, and dissolved in methylene chloride (500 μ L). *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (2.2 mg, 0.01 mmol), 1-hydroxybenzotriazole hydrate (1.6 mg, 0.01 mmol), methoxylamine hydrochloride (3.2 mg, 0.04 mmol), and triethylamine (6 μ L, 0.04 mmol) were added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (gradient elution 2% to 20% methanol in methylene chloride) to afford compound **15d** as a white solid (4 mg, quant.). **¹H NMR** (500 MHz, CD₃OD) δ 7.16 (d, J = 9.1 Hz, 2H), 7.12 – 6.93 (m, 5H), 6.65 (d, J = 9.0 Hz, 2H), 6.37 (s, 1H), 4.75 (d, J = 5.8 Hz, 1H), 4.37 (d, J = 14.2 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.73

(dd, $J = 14.3, 5.9$ Hz, 2H), 3.68 (s, 3H), 3.61 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 159.93, 159.50, 157.70, 157.59, 138.67, 130.07, 129.17, 128.93, 128.59, 127.39, 113.19, 107.98, 103.29, 95.81, 91.03, 80.53, 64.33, 57.10, 56.31, 56.22, 55.43; **IR** (film, cm^{-1}) 3400-3200, 2920, 2849, 1657, 1612, 1513, 1499, 1463, 1434, 1348, 1300, 1252, 1209, 1179, 1135, 1099, 1032, 873, 735, 698, 643; **m.p.** 172-173 °C; **TLC** R_f 0.8 (10% methanol/ methylene chloride); **UPLC** t_R 1.44 min; **HRMS** calcd for $[\text{C}_{28}\text{H}_{28}\text{ClNO}_8\text{H}]^+$ requires m/z 542.1582; found 542.1589 (ES+).

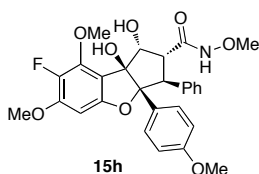


(+/-)-Hydroxamate **15e**: A round bottom flask was charged with ester **14e** (15 mg, 0.03 mmol), 1,4-dioxane (200 μL), lithium hydroxide (3.0 mg, 0.1 mmol), and water (40 μL), and was heated to 60 °C for 14 h. The reaction was cooled to room temperature, concentrated, and filtered through silica gel in 20% methanol in methylene chloride. The filtrate was transferred to a round bottom flask, concentrated, and dissolved in methylene chloride (1.8 mL). *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (8.2 mg, 0.04 mmol), 1-hydroxybenzotriazole hydrate (5.7 mg, 0.04 mmol), methoxylamine hydrochloride (12 mg, 0.14 mmol), and triethylamine (20 μL , 0.1 mmol) were added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (50% ethyl acetate in hexanes) to afford compound **15e** as a white solid (6 mg, 40%). ^1H NMR (500 MHz, CD_3OD) δ 7.17 (d, $J = 9.1$ Hz, 2H), 7.11 – 6.98 (m, 3H), 6.95 (d, $J = 6.3$ Hz, 2H), 6.67 (d, $J = 8.8$ Hz, 2H), 6.66 (s, 1H), 4.79 (d, $J = 6.0$ Hz, 1H), 4.32 (d, $J = 14.2$ Hz, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 3.72 (dd, $J = 14.2, 6.0$ Hz, 1H), 3.69 (s, 3H), 3.60 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 169.25, 160.03, 159.88, 155.74, 138.59, 130.17, 129.98, 129.21, 128.91, 128.55, 127.45, 115.64, 113.26, 109.23, 106.41, 102.66, 95.59, 92.96, 80.59, 64.33, 62.19, 61.24, 57.09, 56.34, 55.46; **IR** (film, cm^{-1}) 3450-3300, 2919, 2849, 1656, 1611, 1512, 1468, 1453, 1438, 1407, 1346, 1301, 1252, 1199, 1180, 1168, 1101, 1033, 1004, 970, 932, 834, 819, 778, 763, 735, 700, 664, 616; **m.p.** 219-220 °C; **TLC** R_f 0.6 (100% ethyl acetate); **UPLC** t_R 1.45 min; **HRMS** calcd for $[\text{C}_{28}\text{H}_{28}\text{ClNO}_8\text{H}]^+$ requires m/z 542.1582; found 542.1575 (ES+).

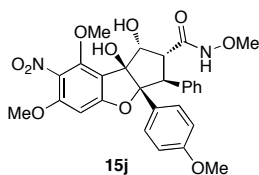


(+/-)-Hydroxamate **15f**: A round bottom flask was charged with ester **14f** (15 mg, 0.03 mmol), 1,4-dioxane (200 μL), lithium hydroxide (3.0 mg, 0.1 mmol), and water (40 μL), and was heated to 60 °C for 14 h. The reaction was cooled to room temperature, concentrated, and filtered through silica gel in 20% methanol in methylene chloride. The filtrate was transferred to a round bottom flask, concentrated, and dissolved in methylene chloride (1.8 mL). *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (7.9 mg, 0.04 mmol), 1-hydroxybenzotriazole hydrate (5.4 mg, 0.04 mmol), methoxylamine

hydrochloride (11 mg, 0.14 mmol), and triethylamine (20 μ L, 0.1 mmol) were added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (gradient elution 2% to 10% methanol in methylene chloride) to afford compound **15f** as a white solid (6 mg, 39%). **¹H NMR** (500 MHz, CD₃OD) δ 7.18 (d, J = 8.4 Hz, 2H), 7.11 – 7.02 (m, 3H), 7.00 (d, J = 7.2 Hz, 2H), 6.67 (d, J = 8.7 Hz, 2H), 4.76 (d, J = 5.4 Hz, 1H), 4.41 (d, J = 14.3 Hz, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 3.76 (dd, J = 14.3, 5.4 Hz, 1H), 3.69 (s, 3H), 3.61 (s, 3H); **¹³C NMR** (126 MHz, CD₃OD) δ 169.15, 160.12, 156.08, 155.99, 154.41, 138.29, 129.98, 129.20, 128.64, 128.48, 127.51, 120.57, 115.99, 113.36, 108.14, 106.46, 103.44, 96.27, 80.52, 64.34, 62.37, 61.29, 56.68, 55.47, 50.27; **IR** (film, cm⁻¹) 3500-3300, 2940, 1663, 1606, 1513, 1463, 1409, 1353, 1300, 1253, 1182, 1085, 1031, 966, 830, 747, 699, 668; **m.p.** 142-143 °C; **TLC** R_f 0.7 (10% methanol/ methylene chloride); **UPLC** t_R 1.60 min; **HRMS** calcd for [C₂₈H₂₇Cl₂NO₈H]⁺ requires m/z 576.1192; found 576.1186 (ES+).



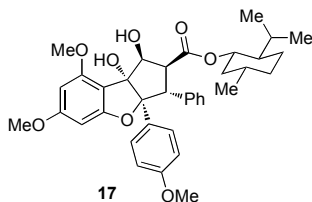
(+/-)-Hydroxamate **15h**: A round bottom flask was charged with ester **14h** (16 mg, 0.03 mmol), 1,4-dioxane (1 mL), lithium hydroxide (12 mg, 0.5 mmol), and water (300 μ L), and was heated to 60 °C for 14 h. The reaction was cooled to room temperature, concentrated, and filtered through silica gel in 20% methanol in methylene chloride. The filtrate was transferred to a round bottom flask, concentrated, and dissolved in methylene chloride (4 mL). *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (9 mg, 0.05 mmol), 1-hydroxybenzotriazole hydrate (6.4 mg, 0.04 mmol), methoxylamine hydrochloride (13 mg, 0.16 mmol), and triethylamine (22 μ L, 0.16 mmol) were added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (gradient elution 50% to 100% ethyl acetate in hexanes) to afford compound **15h** as a white solid (8 mg, 49%). **¹H NMR** (400 MHz, CD₃OD) δ 7.16 (d, J = 9.0 Hz, 2H), 7.10 – 6.99 (m, 3H), 6.98 – 6.88 (m, 2H), 6.66 (d, J = 8.9 Hz, 2H), 6.57 (d, J = 6.0 Hz, 1H), 4.76 (d, J = 6.0 Hz, 1H), 4.59 (br s, 1H), 4.30 (d, J = 14.2 Hz, 1H), 4.03 (d, J = 2.0 Hz, 3H), 3.92 (s, 3H), 3.72 (dd, J = 14.2, 6.0 Hz, 1H), 3.69 (s, 3H), 3.60 (s, 3H); **¹³C NMR** (126 MHz, CD₃OD) δ 186.90, 172.99, 169.33, 163.31, 159.93, 155.76, 152.77, 142.90, 141.02, 138.68, 130.18, 129.21, 128.53, 127.40, 113.20, 102.32, 95.91, 92.17, 80.55, 64.34, 62.04, 61.55, 57.06, 56.33, 55.44; **IR** (film, cm⁻¹) 3450-3300, 2924, 2851, 1657, 1627, 1610, 1511, 1479, 1443, 1429, 1301, 1252, 1200, 1179, 1146, 1105, 1067, 1035, 971, 945, 836, 809, 774, 738, 701, 668; **m.p.** 227-228 °C; **TLC** R_f 0.5 (75% ethyl acetate/ hexanes); **UPLC** t_R 1.39 min; **HRMS** calcd for [C₂₈H₂₈FNO₈H]⁺ requires m/z 526.1877; found 526.1875 (ES+).



(+/-)-Hydroxamate **15j**: A round bottom flask was charged with ester **14j** (6 mg, 0.01 mmol), 1,4-dioxane (90 μ L), lithium hydroxide (1.2 mg, 0.05 mmol), and water (20 μ L), and was heated to 60 $^{\circ}$ C for 14 h. The reaction was cooled to room temperature, concentrated, and filtered through silica gel in 20% methanol in methylene chloride. The filtrate was transferred to a round bottom flask, concentrated, and dissolved in methylene chloride (750 μ L). *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (3.3 mg, 0.02 mmol), 1-hydroxybenzotriazole hydrate (2.3 mg, 0.01 mmol), methoxylamine hydrochloride (4.8 mg, 0.06 mmol), and triethylamine (8 μ L, 0.06 mmol) were added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated and purified by flash chromatography (gradient elution 2% to 10% methanol in methylene chloride) to afford compound **15j** as a white solid (3 mg, 50%). **1 H NMR** (400 MHz, CD_3OD) δ 7.15 (d, J = 8.8 Hz, 2H), 7.11 – 6.96 (m, 5H), 6.66 (s, 1H), 6.65 (d, J = 8.5 Hz, 2H), 4.72 (d, J = 4.9 Hz, 1H), 4.46 (d, J = 14.2 Hz, 1H), 4.08 (s, 3H), 3.95 (s, 3H), 3.77 (dd, J = 14.1, 5.2 Hz, 1H), 3.68 (s, 3H), 3.62 (s, 3H); **13 C NMR** (101 MHz, CD_3OD) δ 162.93, 160.08, 155.93, 138.66, 130.07, 129.30, 128.94, 128.61, 128.31, 127.15, 126.68, 113.31, 111.56, 106.34, 105.57, 103.43, 91.68, 80.85, 57.34, 56.66, 55.44; **IR** (film, cm^{-1}) 3500–3350, 2923, 2850, 2509, 1620, 1514, 1441, 1254, 1202, 1112, 1019, 668; **m.p.** 218–219 $^{\circ}$ C; **TLC** R_f 0.6 (10% methanol/ methylene chloride); **UPLC** t_R 1.44 min; **HRMS** calcd for $[\text{C}_{28}\text{H}_{28}\text{N}_2\text{O}_{10}\text{H}]^+$ requires m/z 553.1822; found 553.1823 (ES+).

Preparation of hydroxamates (+)-9 and (-)-9

A round bottom flask was charged with (+/-)-rocaglaic acid (290 mg, 0.61 mmol), L-menthol (246 mg, 1.58 mmol), and *N,N'*-dicyclohexylcarbodiimide (216 mg, 1.05 mmol), and methylene chloride (2 mL) was added. A solution of 4-dimethylaminopyridine (6.5 mg, 0.053 mmol) in methylene chloride (300 μ L) was added dropwise to reaction, forming a white precipitate. The reaction was stirred at room temperature for 12 h, concentrated, and purified by flash column chromatography (gradient elution 15% to 40% ethyl acetate in hexanes) to afford ester **17** (99 mg, 27%) as a colorless oil eluting first and ester **16** (67 mg, 18%) as a colorless oil eluting second.

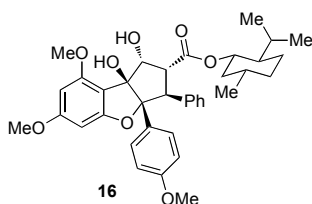


(+)-Rocaglaic acid-(L)-menthyl ester **17**: **1 H NMR** (500 MHz, CDCl_3) δ 7.13 (d, J = 9.5 Hz, 2H), 7.09 – 6.98 (m, 3H), 6.97 – 6.88 (m, 2H), 6.66 (d, J = 9.5 Hz, 2H), 6.28 (d, J = 1.9 Hz, 1H), 6.11 (d, J = 1.9 Hz, 1H), 4.95 (d, J = 6.2 Hz, 1H), 4.64 (td, J = 10.9, 4.1 Hz, 1H), 4.37 (d, J = 14.2 Hz, 1H), 3.88 (dd, J = 14.2, 6.2 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.69 (s, 3H), 3.46 (s, 1H), 1.95 (d, J = 12.2 Hz, 1H), 1.91 – 1.75 (m, 2H), 1.63 (d, J = 10.7 Hz, 2H), 1.49 – 1.14 (m, 3H), 1.08 – 0.75 (m, 8H), 0.58 (d, J = 6.9 Hz, 3H). **13 C**

NMR (126 MHz, CDCl₃) δ 170.08, 164.09, 161.20, 158.75, 157.36, 137.20, 129.04, 128.06, 127.71, 126.92, 126.50, 112.81, 107.54, 102.02, 94.08, 92.60, 89.38, 79.31, 77.41, 77.16, 76.91, 75.02, 55.87, 55.80, 55.41, 55.19, 50.89, 46.99, 40.69, 34.38, 31.49, 26.21, 23.30, 22.11, 20.95, 16.02; **IR** (film, cm⁻¹) 3500-3300, 2954, 2869, 1738, 1624, 1610, 1513, 1499, 1454, 1386, 1343, 1297, 1251, 1216, 1200, 1182, 1148, 1118, 1038, 984, 912, 812, 733, 698; **TLC** *R_f* 0.8 (50% ethyl acetate/ hexanes); [α]_D²⁰ = +26.6 (*c* 1.0, CDCl₃ at >98% ee); **HRMS** calcd for [C₃₇H₄₄O₈H]⁺ requires *m/z* 617.3114; found 617.3130 (ES+).

A round bottom flask was charged with menthyl ester **17** (67 mg, 0.11 mmol), powdered sodium hydroxide (28 mg, 0.70 mmol), dimethyl sulfoxide (4 mL), and water (250 μ L), and was heated to 60 °C for 3 h. The reaction was cooled to room temperature, acidified to pH =1 using 1N HCl, and extracted with ethyl ether (3 x 10 mL). The combined organic extracts were washed with 1N HCl (4 x 5 mL). The organic phase was dried with sodium sulfate and concentrated. Purified by flash column chromatography (2.5% methanol in methylene chloride), to yield (+)-rocaglaic acid (25 mg, 47%), as determined by optical rotation [α]_D²⁰ = +49.0 (*c* 1.0, CHCl₃, at >98% ee).^{S4}

A round bottom flask was charged with (+)-rocaglaic acid (23.0 mg, 0.05 mmol), methylene chloride (4 mL), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (14 mg, 0.07 mmol), 1-hydroxybenzotriazole hydrate (10 mg, 0.06 mmol), methoxylamine hydrochloride (20 mg, 0.24 mmol), and triethylamine (34 μ L, 0.24 mmol), and was stirred at room temperature for 12 h. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (gradient elution 50% to 100% ethyl acetate in hexanes) to afford compound (+)-**9** as white solid (15 mg, 60%). Spectral data as above for (+/-)-**9**; [α]_D²⁰ = +39.2 (*c* 0.5, CH₃OH, at >98% ee).



(-)-Rocaglaic acid-(L)-menthyl ester **16**: ¹H NMR (500 MHz, CDCl₃) δ 7.13 (d, *J* = 9.5 Hz, 2H), 7.09 – 6.99 (m, 3H), 6.93 – 6.88 (m, 2H), 6.65 (d, *J* = 9.5 Hz, 2H), 6.28 (d, *J* = 1.9 Hz, 1H), 6.12 (d, *J* = 1.9 Hz, 1H), 4.97 (d, *J* = 6.1 Hz, 1H), 4.58 (td, *J* = 10.9, 4.3 Hz, 1H), 4.34 (d, *J* = 14.3 Hz, 1H), 3.91 (dd, *J* = 14.3, 6.1 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.70 (s, 3H), 1.99 – 1.78 (m, 2H), 1.66 – 1.48 (m, 2H), 1.45 – 1.05 (m, 4H), 1.01 – 0.84 (m, 1H), 0.83 (m, 1H), 0.78 (d, *J* = 6.5 Hz, 3H), 0.58 (d, *J* = 6.9 Hz, 3H), 0.49 (d, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 170.20, 164.15, 161.20, 158.83, 157.35, 137.31, 129.09, 127.99, 127.81, 126.80, 126.52, 112.81, 107.69, 101.97, 93.90, 92.64, 89.39, 79.59, 77.41, 77.16, 76.91, 75.19, 55.90, 55.82, 55.35, 55.23, 51.34, 46.89, 40.76, 34.38, 31.49, 29.85, 25.27, 23.00, 22.09, 20.98, 15.74; **IR** (film, cm⁻¹) 3500-3200, 2953, 2926, 2852, 1716, 1624, 1610, 1514, 1499, 1454, 1386, 1343, 1250, 1217, 1200, 1181, 1148, 1118, 1032, 913, 827, 733, 698; **TLC** *R_f* 0.7 (50% ethyl acetate/ hexanes); [α]_D²⁰ =

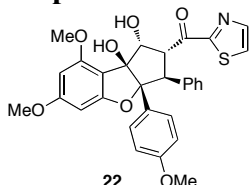
^{S4} Wang, S.-K.; Cheng, Y.-J.; Duh, C.-Y. *J. Nat. Prod.* **2001**, *64*, 92-94.

-43.6 (*c* 1.0, CDCl₃ at >98% ee); **HRMS** calcd for [C₃₇H₄₄O₈H]⁺ requires *m/z* 617.3114; found 617.3120 (ES+).

A round bottom flask was charged with menthyl ester **16** (67 mg, 0.11 mmol), powdered sodium hydroxide (28 mg, 0.70 mmol), dimethyl sulfoxide (4 mL) and water (250 μL), and was heated to 60 °C for 3 h. The reaction was cooled to room temperature, acidified to pH =1 using 1N HCl, and extracted with ethyl ether (3 x 10 mL). The combined organic extracts were washed with 1N HCl (4 x 5 mL). The organic phase was dried with sodium sulfate and concentrated. The crude product was purified by flash column chromatography (2.5% methanol in methylene chloride), to yield (-)-rocaglaic acid (30 mg, 57%) as determined by optical rotation [α]_D²⁰ = -46.6 (*c* 1.0, CHCl₃, at >98% ee).

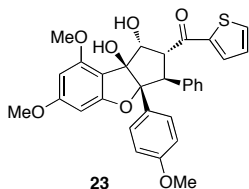
A round bottom flask was charged with (-)-rocaglaic acid (27.0 mg, 0.06 mmol), methylene chloride (4 mL), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (16 mg, 0.08 mmol), 1-hydroxybenzotriazole hydrate (11 mg, 0.07 mmol), methoxylamine hydrochloride (24 mg, 0.28 mmol), and triethylamine (39 μL, 0.28 mmol), and was stirred at room temperature for 12 h. The reaction was quenched with 1M HCl, concentrated, and purified by flash chromatography (gradient elution 50% to 100% ethyl acetate in hexanes) to afford compound (-)-**9** as white solid (14 mg, 49%). Spectral data as above for (+/-)-**9**; [α]_D²⁰ = -38.4 (*c* 0.5, CH₃OH, at >98% ee).

Preparation of rocaglaol-ketone derivatives

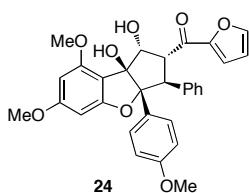


2-Thiazoyl ketone 22: In a flame dried round bottom flask thiazole (13 μL, 0.18 mmol) was dissolved in tetrahydrofuran (375 μL) and cooled to -30 °C. A solution of *n*-butyllithium (90 μL, 0.96 mmol; 2.1 M in hex) was added dropwise and the resulting orange solution was stirred at -30 °C for 30 minutes. A solution of hydroxamate **3** (30 mg, 0.06 mmol) in tetrahydrofuran (225 μL) was added and the reaction was stirred at -30 °C for 1 hour. The reaction was warmed to 0 °C over 10 minutes and was quenched with water at 0 °C and subsequently warmed to room temp. The reaction diluted with water and was extracted with methylene chloride (2 X 10 mL) and concentrated. The crude product was purified by flash column chromatography (gradient elution 30% to 75% ethyl acetate in hexanes) to afford compound **22** as a yellow solid (20 mg, 60%). **¹H NMR** (500 MHz, CDCl₃) δ 8.00 (s, 1H), 7.58 (d, *J* = 1.9 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 2H), 6.96-6.93 (m, 3H), 6.83-6.80 (m, 2H), 6.65 (d, *J* = 8.3 Hz, 2H), 6.23 (s, 1H), 6.02 (s, 1H), 5.36 (d, *J* = 6.9 Hz, 1H), 5.07 (dd, *J* = 14.0, 7.0 Hz, 1H), 4.42 (d, *J* = 14.0 Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.64 (s, 3H), 3.55 (s, 1H), 1.89 (s, 1H); **¹³C NMR** (126 MHz, CDCl₃) δ 189.15, 167.21, 164.16, 160.93, 158.84, 157.02, 144.81, 137.14, 129.15, 128.11, 127.78, 126.70, 126.60, 126.23, 112.94, 108.06, 102.30, 94.33, 92.80, 89.76, 80.85, 76.91, 55.85, 55.23, 54.06, 53.25; **IR** (film, cm⁻¹) 3500-3300, 2936, 2839, 2360, 2342, 2251, 1691, 1598, 1514, 1498, 1454, 1440, 1391, 1343, 1298, 1251, 1217, 1200, 1182, 1147, 1116, 1060, 1031, 996, 973, 910, 873, 813, 794, 729, 698, 648; **m.p.** 148-149

°C; **TLC** R_f 0.4 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.72 min; **HRMS** calcd for $[C_{30}H_{27}NO_7SH]^+$ requires m/z 546.1586; found 546.1606 (ES+).

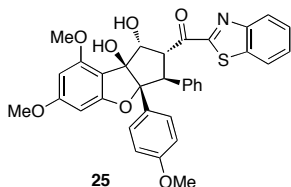


2-Thiophenyl ketone 23: In a flame dried round bottom flask thiophene (14 μ L, 0.18 mmol) was dissolved in tetrahydrofuran (375 μ L) and cooled to -30 °C. A solution of n-butyllithium (90 μ L, 0.96 mmol; 2.1 M in hex) was added dropwise and the solution was stirred at -30 °C for 30 minutes. A solution of hydroxamate **3** (30 mg, 0.06 mmol) in tetrahydrofuran (225 μ L) was added and the reaction was stirred at -30 °C for 1 hour. The reaction was warmed to 0 °C over 10 minutes, and was quenched with water at 0 °C and subsequently warmed to room temp. The reaction diluted with water and was extracted with methylene chloride (2 X 10 mL) and concentrated. The crude product was purified by flash column chromatography (gradient elution 30% to 50% ethyl acetate in hexanes) to afford compound **23** as an orange solid (11 mg, 40%), as well as recovered hydroxamate **3** (50%). **1H NMR** (500 MHz, $CDCl_3$) δ 7.98 (d, J = 3.6 Hz, 1H), 7.66 (d, J = 4.9 Hz, 1H), 7.22-7.19 (m, 3H), 7.03-7.01 (m, 3H), 6.89-6.87 (m, 2H), 6.71 (d, J = 8.3 Hz, 2H), 6.30 (s, 1H), 6.10 (s, 1H), 5.21 (d, J = 6.3 Hz, 1H), 4.68 (dd, J = 13.6, 6.3 Hz, 1H), 4.60 (d, J = 13.6 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.72 (s, 3H), 3.48 (s, 1H), 1.89 (s, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 188.11, 164.27, 161.21, 158.86, 157.18, 144.63, 137.18, 133.75, 131.54, 129.08, 128.32, 128.04, 127.83, 126.89, 126.54, 112.96, 107.47, 102.08, 94.39, 92.75, 89.62, 80.80, 55.86, 55.83, 55.25, 54.46, 54.11; **IR** (film, cm^{-1}) 3500-3300, 2935, 2839, 1667, 1623, 1514, 1499, 1454, 1415, 1391, 1251, 1218, 1200, 1148, 1117, 1061, 911, 813, 731, 699, 668; **m.p.** 198-199 °C; **TLC** R_f 0.5 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.72 min; **HRMS** calcd for $[C_{31}H_{28}O_7SNa]^+$ requires m/z 567.1453; found 567.1439 (ES+).

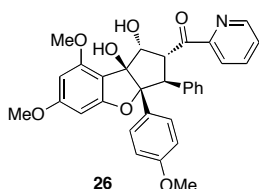


2-Furyl ketone 24: In a flame-dried round bottom flask, furan (28 μ L, 0.39 mmol) was dissolved in tetrahydrofuran (0.5 mL) and was cooled to 0 °C. A solution of n-butyllithium (175 μ L, 1.86 mmol, 2.1 M in hexanes) was added dropwise. The solution was stirred for 30 minutes at 0 °C and then a solution of hydroxamate **3** (40 mg, 0.08 mmol) in tetrahydrofuran (300 μ L) was added and was stirred at 0 °C for 1 hour. The reaction was quenched with water at 0 °C and was brought to room temperature. The reaction was concentrated and purified by flash column chromatography (gradient elution 20% to 60% ethyl acetate in hexanes) to afford compound **24** as a white solid (27 mg, 60%). **1H NMR** (500 MHz, $CDCl_3$) δ 7.66 (dd, J = 1.7, 0.7 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.20 (d, J = 9.0 Hz, 2H), 7.07 – 6.96 (m, 3H), 6.92 – 6.83 (m, 2H), 6.72 (d, J = 9.0 Hz, 2H), 6.58 (dd, J = 3.6, 1.7 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 6.10 (d, J = 2.0 Hz, 1H),

5.27 (dd, $J = 6.6, 1.1$ Hz, 1H), 4.69 (dd, $J = 13.8, 6.6$ Hz, 1H), 4.55 (d, $J = 13.9$ Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.72 (s, 3H), 3.49 (br s, 1H), 1.85 (br s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 184.95, 164.26, 161.21, 158.88, 157.21, 153.48, 146.18, 137.32, 129.17, 128.14, 127.82, 126.97, 126.55, 116.65, 112.97, 112.72, 107.72, 102.24, 94.47, 92.79, 89.69, 80.25, 55.93, 55.91, 55.89, 55.30, 54.10, 53.24; IR (film, cm^{-1}) 3550-3300, 2919, 2848, 2251, 1678, 1599, 1569, 1513, 1499, 1466, 1440, 1396, 1343, 1297, 1250, 1218, 1200, 1181, 1147, 1116, 1061, 1032, 994, 910, 874, 814, 730, 699, 647; **m.p.** 195-197 °C; **TLC** R_f 0.8 (100% ethyl acetate); **UPLC** t_R 1.62 min; **HRMS** calcd for $[\text{C}_{31}\text{H}_{28}\text{O}_8\text{Na}]^+$ requires m/z 551.1682; found 551.1697 (ES+).

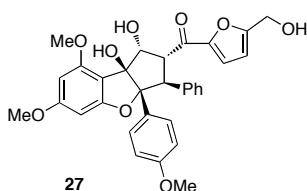


2-Benzothiazoyl ketone 25: In a flame-dried round bottom flask, benzothiazole (28 μL , 0.25 mmol) was dissolved in tetrahydrofuran (0.5 mL) and was cooled to -78 °C. A solution of n-butyllithium (128 μL , 1.36 mmol, 2.1 M in hexanes) was added dropwise. The solution was stirred for 30 minutes at -78 °C and then a solution of methyl rocaglate (**2**) (40 mg, 0.08 mmol) in tetrahydrofuran (320 μL) was added and was stirred at -78 °C for 1 hour. The reaction was quenched with water at -78 °C and was brought to room temperature. The reaction was concentrated and purified by flash column chromatography (gradient elution 10% to 50% ethyl acetate in hexanes) to afford compound **25** as a yellow solid (11 mg, 22%). Unreacted methyl rocaglate was also isolated (19.4 mg, 50%). ^1H NMR (400 MHz, CDCl_3) δ 8.31 (d, $J = 8.0$ Hz, 1H), 7.99 (d, $J = 8.2$ Hz, 1H), 7.73 – 7.55 (m, 2H), 7.35 (d, $J = 8.7$ Hz, 2H), 7.08 – 6.96 (m, 3H), 6.96 – 6.86 (m, 2H), 6.77 (d, $J = 8.7$ Hz, 2H), 6.30 (s, 1H), 6.09 (s, 1H), 4.96 (d, $J = 7.0$ Hz, 1H), 4.75 (dd, $J = 13.5, 7.1$ Hz, 1H), 4.03 (d, $J = 13.6$ Hz, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.63 (s, 1H), 2.00 (s, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 192.61, 167.28, 164.02, 160.08, 158.66, 158.07, 152.88, 137.14, 136.49, 129.09, 128.70, 128.39, 127.63, 126.76, 125.62, 122.72, 113.09, 112.92, 110.67, 102.04, 101.83, 92.72, 89.40, 85.83, 60.53, 58.05, 55.88, 55.81, 55.70, 55.25, 52.12, 51.90; IR (film, cm^{-1}) 3500-3300, 2934, 2839, 1681, 1600, 1515, 1500, 1454, 1439, 1298, 1251, 1218, 1200, 1147, 1115, 1031, 998, 813, 763, 731, 699; **m.p.** 118-119 °C; **TLC** R_f 0.5 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.93 min; **HRMS** calcd for $[\text{C}_{34}\text{H}_{29}\text{NO}_7\text{SNa}]^+$ requires m/z 618.1562; found 618.1558 (ES+).

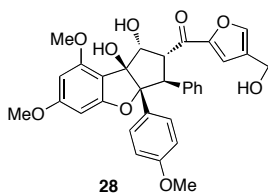


2-Pyridyl ketone 26: In a flame-dried round bottom flask, 2-bromopyridine (24 μL , 0.25 mmol) was dissolved in tetrahydrofuran (0.5 mL) and was cooled to -50 °C. A solution of n-butyllithium (128 μL , 1.36 mmol, 2.1 M in hexanes) was added dropwise. The resulting brown solution was stirred for 40 minutes at -50 °C and then a solution of

methyl rocaglate (**2**) (40 mg, 0.08 mmol) in tetrahydrofuran (320 μ L) was added and was stirred at -50 $^{\circ}$ C for 1 hour, then at 0 $^{\circ}$ C for 1 hour. The reaction was quenched with water at 0 $^{\circ}$ C and was brought to room temperature. The reaction was concentrated and purified by flash column chromatography (gradient elution 30% to 50% ethyl acetate in hexanes) to afford compound **26** as a colorless oil (13 mg, 29%). Unreacted methyl rocaglate was also isolated (11 mg, 29%). **^1H NMR** (500 MHz, CDCl_3) δ 8.80 (ddd, J = 4.9, 1.6, 0.8 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.92 (td, J = 7.7, 1.7 Hz, 1H), 7.61 (ddd, J = 7.6, 4.9, 1.3 Hz, 1H), 7.31 (d, J = 8.9 Hz, 2H), 7.12 (s, 1H), 7.04 – 6.96 (m, 2H), 6.86 – 6.78 (m, 2H), 6.73 (d, J = 9.0 Hz, 2H), 6.29 (d, J = 2.0 Hz, 1H), 6.07 (d, J = 2.0 Hz, 1H), 4.78 (d, J = 7.3 Hz, 1H), 4.64 (dd, J = 13.7, 7.4 Hz, 1H), 4.05 (d, J = 13.7 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H); **^{13}C NMR** (126 MHz, CDCl_3) δ 197.40, 163.82, 160.10, 158.48, 158.12, 153.01, 148.57, 138.45, 137.08, 128.71, 128.18, 128.04, 127.90, 127.76, 126.57, 123.59, 112.92, 111.05, 101.62, 92.63, 89.29, 85.92, 73.85, 57.44, 55.80, 55.71, 55.24, 51.55; **IR** (film, cm^{-1}) 3500-3300, 2931, 2839, 1696, 1600, 1515, 1501, 1465, 1440, 1345, 1299, 1251, 1218, 1200, 1183, 1148, 1116, 1052, 1000, 910, 813, 733, 699, 668, 648; **m.p.** 186-187 $^{\circ}$ C; **TLC** R_f 0.3 (50% ethyl acetate/ hexanes); **UPLC** t_R 1.80 min; **HRMS** calcd for $[\text{C}_{32}\text{H}_{29}\text{NO}_7\text{Na}]^+$ requires m/z 562.1842; found 562.1864 (ES+).



Keto-furan **27:** In a flame-dried round bottom flask, 2-furanmethanol (34 μ L, 0.39 mmol) was dissolved in tetrahydrofuran (0.5 mL) and was cooled to -78 $^{\circ}$ C. A solution of *n*-butyllithium (328 μ L, 3.48 mmol, 2.5 M in hexanes) was added dropwise. The resulting yellow solution was stirred for 1 hour at -78 $^{\circ}$ C, then at 0 $^{\circ}$ C for 1 hour, and then a solution of hydroxamate **3** (40 mg, 0.08 mmol) in tetrahydrofuran (300 μ L) was added and was stirred at 0 $^{\circ}$ C for 1 hour. The reaction was quenched with water at room temperature. The reaction was concentrated and purified by flash column chromatography (gradient elution 20% to 60% ethyl acetate in hexanes) to afford compound **27** as a white solid (27 mg, 60%). **^1H NMR** (500 MHz, CDCl_3) δ 7.19 (d, J = 8.7 Hz, 2H), 7.06 – 6.96 (m, 3H), 6.93 – 6.84 (m, 2H), 6.72 (d, J = 8.8 Hz, 2H), 6.49 (d, J = 3.3 Hz, 1H), 6.30 (s, 1H), 6.11 (s, 1H), 5.23 (d, J = 6.3 Hz, 1H), 4.76 (s, 2H), 4.62 (dd, J = 13.8, 6.0 Hz, 1H), 4.57 (d, J = 13.8 Hz, 1H), 4.06 (t, J = 6.7 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.72 (s, 3H), 3.44 (s, 1H), 1.84 (s, 1H); **^{13}C NMR** (101 MHz, CDCl_3) δ 184.65, 164.23, 161.22, 158.82, 158.62, 157.20, 152.71, 137.25, 129.07, 128.08, 127.97, 127.78, 126.93, 126.48, 117.98, 112.93, 110.15, 107.43, 102.17, 94.47, 92.71, 89.58, 80.23, 57.80, 55.81, 55.22, 54.04, 53.23; **IR** (film, cm^{-1}) 3400-3300, 2932, 1664, 1611, 1514, 1453, 1392, 1345, 1298, 1250, 1201, 1148, 1117, 1068, 1032, 815, 700; **m.p.** 138-139 $^{\circ}$ C; **TLC** R_f 0.8 (100% ethyl acetate); **UPLC** t_R 1.47 min; **HRMS** calcd for $[\text{C}_{32}\text{H}_{30}\text{O}_9\text{Na}]^+$ requires m/z 581.1788; found 581.1782 (ES+).



Keto-furan **28:** In a flame-dried round bottom flask, 3-furanmethanol (25 μ L, 0.29 mmol) was dissolved in tetrahydrofuran (375 μ L) and was cooled to -78°C . A solution of *n*-butyllithium (245 μ L, 2.60 mmol, 2.5 M in hexanes) was added dropwise. The solution was stirred for 30 minutes at -78°C , then at 0°C for 30 minutes, and then a solution of hydroxamate **3** (30 mg, 0.06 mmol) in tetrahydrofuran (225 μ L) was added and was stirred at 0°C for 1 hour. The reaction was quenched with water at room temperature. The reaction was concentrated and purified by flash column chromatography (gradient elution 20% to 60% ethyl acetate in hexanes) to afford compound **28** as a white solid (17 mg, 50%). ^1H NMR (500 MHz, CDCl_3) δ 7.60 (d, $J = 1.7$ Hz, 1H), 7.21 (d, $J = 9.0$ Hz, 2H), 7.09 – 7.00 (m, 3H), 6.86 (m, 2H), 6.72 (d, $J = 9.0$ Hz, 2H), 6.57 (d, $J = 1.7$ Hz, 1H), 6.31 (d, $J = 2.0$ Hz, 1H), 6.11 (d, $J = 2.0$ Hz, 1H), 5.33 (dd, $J = 6.8, 1.6$ Hz, 1H), 4.77 (dd, $J = 13.9, 5.9$ Hz, 1H), 4.64 (m, 2H), 4.49 (d, $J = 14.0$ Hz, 1H), 3.98 – 3.90 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.73 (s, 3H), 3.56 (s, 1H), 2.01 (s, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 183.17, 165.47, 160.34, 156.93, 154.85, 150.69, 150.05, 145.13, 142.89, 142.37, 129.15, 127.97, 127.89, 126.57, 113.82, 112.87, 105.15, 102.06, 94.40, 92.89, 89.72, 80.11, 57.40, 55.90, 55.29, 53.79, 53.31; IR (film, cm^{-1}) 3500–3300, 2918, 2849, 1675, 1600, 1514, 1499, 1467, 1346, 1297, 1250, 1218, 1200, 1182, 1148, 1116, 1057, 910, 814, 732, 699, 668; **m.p.** 130–131 $^{\circ}\text{C}$; **TLC** R_f 0.8 (100% ethyl acetate); **UPLC** t_R 1.53 min; **HRMS** calcd for $[\text{C}_{32}\text{H}_{30}\text{O}_9\text{Na}]^+$ requires m/z 581.1788; found 581.1772 (ES+).

III. Biological Procedures

Cell Lines and Antibodies. The human Burkitt's lymphoma cell line BJAB was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and grown in suspension in RPMI-1640 medium (Wisent, St.-Bruno, Qc), supplemented with 10% fetal bovine serum and 100 U/ml penicillin/streptomycin.

Primary rabbit polyclonal anti-eIF4E antibody was from Cell Signaling (#9742) and mouse monoclonal anti-eIF4A antibody has been described previously.^{S5} Secondary antibodies were from Jackson Immuno Research (Burlington, ON).

In vitro transcriptions and translations. *In vitro* transcription and translation of the bicistronic mRNA construct FF/HCV/Ren used in this study has been reported previously.^{S6} Briefly, plasmid was linearized with *Bam*HI, followed by *in vitro* translation using SP6 RNA polymerase. The resulting mRNA was *in vitro* translated at an RNA concentration of 4ng/ μ L in extracts from rabbit reticulocyte lysates (RRL) from Promega according to the instructions of the manufacturer. *In vitro* translation reactions were performed in the presence of vehicle (0.5% DMSO) or the indicated concentrations of

^{S5} Edery, I.; Humbelin, M.; Darveau, A.; Lee, K. A.; Milburn, S.; Hershey, J. W.;

Trachsel, H.; Sonenberg, N. *J. Biol. Chem.* **1983**, 258, 11398–403.

^{S6} Novac, O.; Guenier, A. S.; Pelletier, J. *Nucleic Acids Res.* **2004**, 32, 902–915.

compound. Firefly (FF) and renilla (Ren) luciferase activities were measured on a Berthold Lumat LB 9507 Luminometer and values obtained were normalized against vehicle control, which was set at 1.

Ex vivo protein synthesis inhibition and determination of cell viability. To perform ³⁵S-Met labeling *ex vivo*, 60 000 HeLa cells/well or 250 000 BJAB cells/well were seeded in a 24-well plate. Cells were treated with either vehicle (0.5% DMSO) or the indicated concentrations of compounds for the given time points. For the last hour of treatment, medium was replaced by Met-free DMEM and 10% dialyzed serum. For the last 15 min, cells were incubated with ³⁵S-Met (150-220 μ Ci/ml) to label actively synthesizing proteins. Following labeling, cells were lysed in RIPA buffer (20 mM Tris_{7.5}, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% NP-40, 0.5% sodium desoxycholate, 0.1% SDS, 20 mM β -glycerophosphate, 10 mM NaF, 1 mM PMSF, 4 μ g/mL aprotinin, 2 μ g/mL leupeptin, 2 μ g/mL pepstatin) and protein precipitated with trichloroacetic acid (TCA). Radioactivity was quantitated by scintillation counting and values obtained normalized against total protein content. Protein content was measured using the Bio-Rad D_c Protein Assay (Bio-Rad Laboratories).

To determine cell viability, 20 000 BJAB cells/well were seeded in 96-well plates and treated with the indicated concentrations of compound for 72 h. At the end of treatment, propidium iodide (PI) at a final concentration of 5 μ g/mL was added. FACS analyses were performed using a Guava EasyCyte plus FACScan instrument from Millipore using Guava ExpressPro software.

Filter binding assay. Filter binding assays were performed as described previously.^{S7} Briefly, ³²P-labeled mRNA was incubated with recombinant eIF4AI and the indicated concentrations of compounds in 1x reaction buffer (25mM Tris_{7.5}, 100mM KCl, 1mM DTT, 5mM MgCl₂, 1mM ATP) for 10 min at 37 °C. Reactions were stopped by passing them through nitrocellulose filters (45 μ M HA Millipore) under vacuum, washing and drying the filters. The amount of RNA bound to protein was determined by scintillation counting.

m⁷ GTP affinity chromatography. Pulldown experiments of eIF4F were performed as described previously.^{S8} Ribosomal High Salt wash (RSW) was incubated for 1 h at 30 °C with either 0.5% DMSO or 50 μ M compound, followed by addition of 50 μ l of 50% m⁷ GTP-Sepharose beads (G.E. Healthcare). The beads were incubated for 2 h end over end at 4 °C, washed three times with LCB (20 mM HEPES_{7.5}, 100 mM KCl, 0.2 mM EDTA), followed by washing with LCB containing 1mM GTP. Bound eIF4F was eluted in LCB containing 1mM m⁷ GTP and Western blot analyses performed for the presence of eIF4A and eIF4E.

^{S7} Merrick, W. C.; Sonenberg, N. *Methods* **1997**, *11*, 333-42.

^{S8} Cencic, R.; Carrier, M.; Galicia-Vazquez, G.; Bordeleau, M. E.; Sukarieh, R.; Bourdeau, A.; Brem, B.; Teodoro, J. G.; Greger, H.; Tremblay, M. L.; Porco, J. A., Jr.; Pelletier, J. *PLoS ONE* **2009**, *4*, e5223.

Tumorigenesis Studies. Treatment studies were performed on 6-8 week old C57Bl/6 mice that had received 10^6 E μ -myc/(myr)Akt lymphoma cells intravenously. When tumors were palpable, mice were treated with silvestrol (0.2 mg/kg daily for 5 d), hydroxamate (-)-**9** (0.2 mg/kg daily for 5 d), or doxorubicin (once at 10 mg/kg), or combinations thereof. Compounds were administered in PEG 400/ 5.2% Tween 80 via intraperitoneal (i.p.) injection. For combination studies, mice were treated with silvestrol or hydroxamate (-)-**9** for five consecutive days, with doxorubicin being delivered once on the second day. Mice were monitored daily for tumor burden. Tumor-free survival was defined as the time between remission and reappearance of tumors. Data was analyzed using the log-rank (Mantel-Cox) test using SigmaStat software and is presented in Kaplan-Meier format.

To assess toxicity of hydroxamate (-)-**9**, 6-8 weeks old wild type female C57Bl/6 mice were treated for five consecutive days with either vehicle (PEG 400/ 5.2% Tween 80) or hydroxamate (-)-**9** (0.2 mg/kg). Two hours following the last treatment, bone marrow cell suspensions were isolated in PBS+ 2% FCS. Following removal of erythrocytes by lysis in ACK buffer (150 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA), cells were washed in PBS+ 2% serum and resuspended in 1 mL PBS+ 2% serum. The cells were blocked with purified anti-CD16/CD32 antibody (clone 93;eBioscience) for 5 min on ice, followed by labeling with fluorochrome conjugated substrate specific antibodies for 20 min on ice in the dark. Antibodies used were PE Rat anti-mouse CD11b (M1/70) (BD Pharmingen), a mature macrophages marker which recognizes CD11b expressed on macrophages, dendritic cells, granulocytes and precursors, NK cells; PE Rat anti-mouse CD4 [Clone: H129.19 (BD Pharmingen)], which recognizes CD4/TL3T4 expressed on most thymocytes and subpopulations, T-lymphocytes; PE Rat anti-mouse Ly-6G (1A8) (BD Pharmingen), selectively expressed on the majority of the largest cells, which are predominantly granulocytes; and PE anti-Hu/Mo CD45R (B220) [Clone: RA3-6B2 (eBioscience)], which recognizes a CD45R epitope expressed on B lymphoid cells at all stages from pro-B to mature B-cells, at lower expression levels also found on plasma cells on memory B-cells, as well as on NK cells. Data acquisition and analyses were performed on a FACSaria machine (BD Biosciences) using FlowJo (Treestar) software. Results are shown in Figure S1.

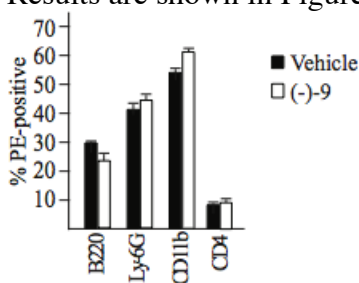


Figure S1. Hydroxamate (-)-**9** does not display general toxicity towards cells of the hematopoietic system. Female C57Bl/6 mice were treated with either vehicle (5.2% PEG 400/5.2% Tween-80) or 0.2 mg/kg hydroxamate (-)-**9** for five consecutive days followed by quantitative FACS analysis of the bone marrow cell populations. Results are the average of three experiments with the standard error of the mean shown.

IV. Pharmacological Studies

Performed by Dr. Layton H. Smith at the Sanford Burnham Pharmacology Core Facility, Orlando, FL

Solubility - Assay details:

- Diclofenac Na and Dipyridamole were used as standards. Diclofenac Na is highly soluble. Dipyridamole is poorly to moderately soluble.
- Standards and test compound stocks were made in 100% DMSO
- Assay concentration of standards: 500 μ M and test compound: 300 μ M
- Cosolvent used in the reference solution to suppress precipitation: 1-Propanol
- Assay DMSO final concentration: 1%

Table 1: Results for the controls and test compound

Compound	pH	Avg. Sol (μ g/mL)	SD Sol	Limit, μ g/mL*
Diclofenac.Na	5.0	101.1	16.2	157
	6.2	>157		157
	7.4	>157		157
Dipyridamole	5.0	59.0	3.4	250
	6.2	7.8	0.1	250
	7.4	4.8	0.1	250
(-)-9	5.0	>151		151
	6.2	>151		151
	7.4	>151		151

* Maximum concentration tested (assay concentration x MW).

Figure 1: Solubility result for (-)-9 (labeled as JAP-CR-1-3 in figure)

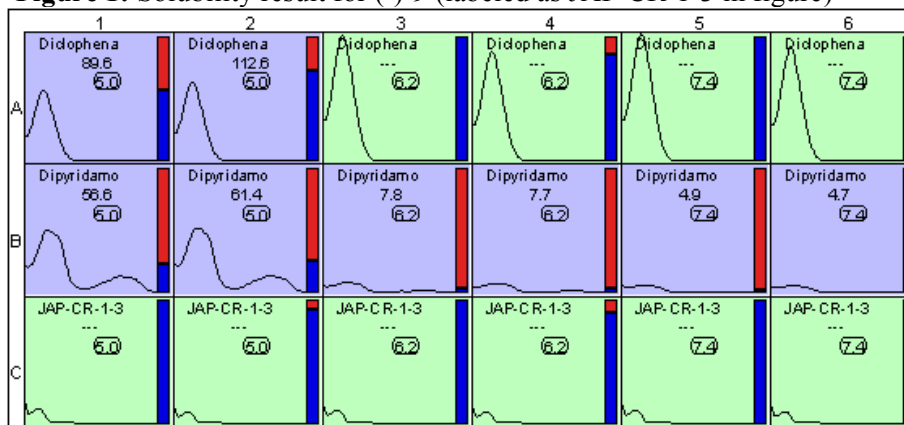


Fig 1. Graphic representation of the assay results. Each frame shows the name of the compound, the spectrum of the compound in the equilibrated aqueous buffer, the solubility (in μ g/mL), the pH value at which the assay was performed, a bicolor bar representing the distribution of the compound between the solution and the solid phases (red, amount of compound precipitated; blue, amount of compound in solution). If the frame is colored purple, precipitate formed; if colored green, no precipitate formed thus solubility could not be determined. Solubility measured was within 85% of the maximum concentration tested.

Permeability - Assay details:

- Verapamil HCl, Metoprolol, and Ranitidine were used as reference standards
- Verapamil HCl is considered highly permeable
- Metoprolol is considered moderately permeable
- Ranitidine is considered poorly permeable
- Permeation time: 30 min
- Moderate stirring (equivalent to 40 μm ABL, aqueous boundary layer, also known as the unstirred water layer)
- Donor buffer pH: 5.0, 6.2 and 7.4
- Double-Sink: pH gradients between donor and acceptor compartments; acceptor buffer contains chemical sink
- Assay DMSO final concentration : 0.5%

Table 2: Results for the controls and test compound

Compound, and Assay Conc in μM	MW	pH	Avg. Pe (10-6 cm/s)	SD Pe	-log Pe
Verapamil-HCl	491.1	5.0	194	4	3.7
		6.2	833	36	3.1
		7.4	1328	10	2.9
Metoprolol	684.8	5.0	8.5	0.6	5.1
		6.2	41	0	4.4
		7.4	254	7	3.6
Ranitidine	350.9	5.0	1.6		5.8
		6.2	1.5	0.1	5.8
		7.4	1.5	0.7	5.8
(-)-9	507.5	5.0	133	10	3.9
		6.2	120	9	3.9
		7.4	125	9	3.9

Figure 2: PAMPA result for (-)-9 (labeled as JAP-CR-1-3 in figure)

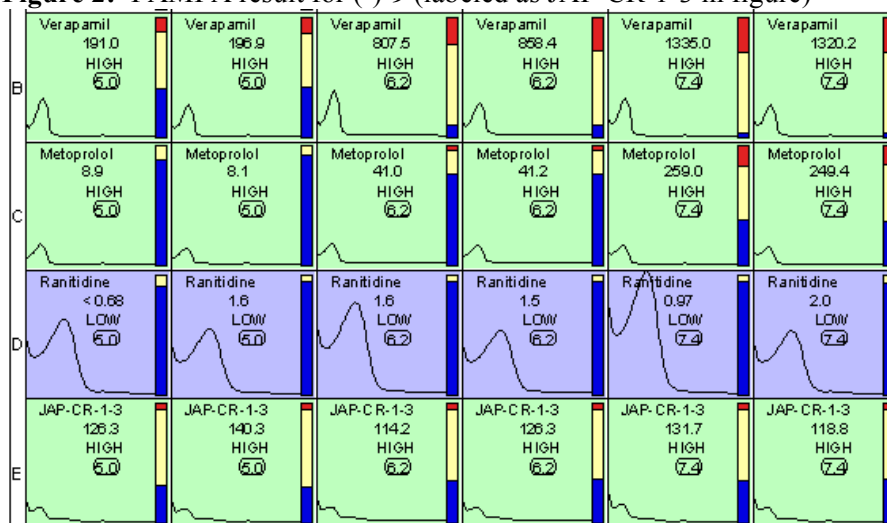


Fig 2. Graphic representation of the assay results. Each frame shows the name of the compound, the spectrum of the compound in the reference well, the effective permeability (Pe) and its ranking (high in green, low in purple), the pH value at which measurement was done, and a tricolor bar representing the distribution of the compounds at the end of the permeation time (blue, amount remaining in the donor well; red, amount permeated to the acceptor well; yellow, amount trapped in the membrane).

Hepatic Microsome Stability – Assay details:

- Human and Mouse Liver Microsomes: 0.5 mg/mL protein concentration
- NADPH Regenerating System: 1.55 mM NADP⁺, 1.33 mM glucose-6-phosphate, 1.33 mM Magnesium chloride, and 0.4 U/mL glucose-6 phosphate dehydrogenase
- Incubation Temperature: 37 °C
- Incubation Time: 60 min
- Standards: Verapamil-HCl and Testosterone, at 20 µM and 50 µM, respectively
- Test compound at 1 µM
- Assay DMSO final concentration: ≤ 0.5%
- Assay ACN final concentration: ≤ 1.2%

Matrix used in this study:

Cat No./Lot No.	H0630/0810063	M0090/0810141
Supplier	XenoTech	XenoTech
Gender	Mixed	Male
No. of Donors	50	1025

Table 3: Results for the controls and test compound

Compound	% Remaining at 60 min Human	% Remaining at 60 min Mouse
Verapamil-HCl	75.45	58.69
Testosterone	49.30	40.46
(-)-9	101.33	96.22

Plasma Stability – Assay details:

- Human Plasma in K₃ EDTA
- Procaine and Procainamide were used as standards. Procaine is highly unstable in human plasma, Procainamide is highly stable in human plasma.
- Assay concentrations of standards and test compound: 1 µM
- Incubation Time: 3 hrs
- Reaction pH: 7.4
- Assay DMSO final concentration: 2.5%

Matrix used in the study

Species	Human
Supplier	BioChemed Services
Gender	Mixed
Anticoagulant	K ₃ EDTA
Cat No./Lot No.	752PR-EK ₃ -PMG /BC052709K

Table 4: Results for the controls and test compound

Compound	% Remaining after 3 hrs
Procaine	0.50
Procainamide	100
(-)-9	100

Plasma Protein Binding – Assay details:

- Human Plasma in K₃ EDTA

- Propanolol and Metoprolol were used as standards. Propanolol is highly bound, Metoprolol is poorly bound
- Assay concentrations of standards and test compounds: 1 μ M and 10 μ M
- Incubation Time: 4 hrs
- Reaction pH: 7.4
- Assay DMSO final concentration: 1%

Matrix used in this study:

Species	Human
Supplier	BioChemed Services
Gender	Mixed
Anticoagulant	K ₃ EDTA
Cat No./Lot No.	752PR-EK3-PMG /BC052709K

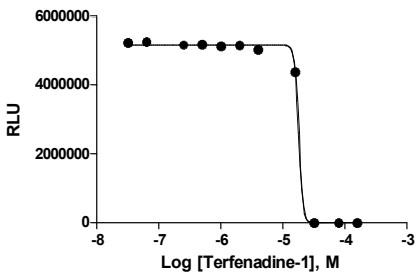
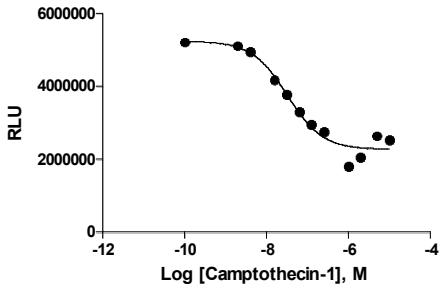
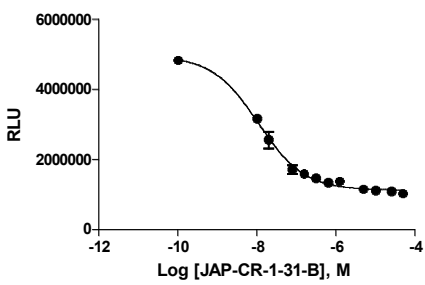
Table 5: Results for the controls and test compound

Compound	% Bound after 4 hrs 1 μ M/10 μ M
Propanolol	94.33/93.26
Metoprolol	9.68/21.30
(-)-9	84.10/82.21

Cytotoxicity – Assay details:

- Cells used: Fa2N-4, immortalized human hepatocytes
- Media used for Fa2N-4 cells: MFE Plating and MFE Support (with 1% Penicillin, Streptomycin, and Amphotercin mixture)
- Assay DMSO final concentration = 0.5%
- Treatment time: 24 hrs
- Camptothecin and Terfenadine were used as standards. Camptothecin is highly toxic and Terfenadine is highly non toxic.

Table 6: Results for the controls and tests compounds

Compound	Toxicity towards Fa2N-4 Immortalized Human Hepatocytes	LC ₅₀ (μM)
Terfenadine	 <p>Graph showing RLU (Y-axis, 0 to 6,000,000) versus Log [Terfenadine-1], M (X-axis, -8 to -3). The curve shows a sharp decrease in RLU starting around -5.5, reaching near zero by -4.5.</p>	18.1
Camptothecin	 <p>Graph showing RLU (Y-axis, 0 to 6,000,000) versus Log [Camptothecin-1], M (X-axis, -12 to -4). The curve shows a gradual decrease in RLU starting around -10, reaching near zero by -5.5.</p>	0.033
(-)-9 JAP-CR-1-31-B	 <p>Graph showing RLU (Y-axis, 0 to 6,000,000) versus Log [JAP-CR-1-31-B], M (X-axis, -12 to -4). The curve shows a gradual decrease in RLU starting around -10, reaching near zero by -5.5.</p>	0.011