SAR of 4-Alkoxybenzoic Acid Inhibitors of the Trypanosome Alternative Oxidase

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Content:

1) Table S1
2) Experimental protocol for the synthesis of compounds 4–21. S2
3) Experimental protocols for drug sensitivity assays against
   \textit{T. b. brucei} and HEK cells S4
4) Experimental protocols for Ubiquinol oxidase/TAO inhibitory assay S24
   S25
Table S1. EC$_{50}$ values ($\mu$M) against Wild Type and Drug-resistant Strains of *T. b. brucei*, and Cytotoxicity against Human Cells (CC$_{50}$, $\mu$M).

<table>
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<tr>
<th>Cmpd</th>
<th><em>T. b. brucei</em> WT$^a$</th>
<th><em>T. b. brucei</em> WT (+ 5 mM glycerol)</th>
<th>RF$^b$</th>
<th><em>T. b. brucei</em> AQP1-3 KO$^c$</th>
<th>RF$^d$</th>
<th><em>T. b. brucei</em> B48$^e$</th>
<th>RF$^d$</th>
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*Notes:*
- ND: Not determined.
- NE/100: Not effective at 100 µM.
- SI: Selectivity Index.

EC$_{50}$ values (µM) against *T. b. brucei* and cytotoxicity against human cells (CC$_{50}$, µM).
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<td>21a</td>
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<td>1.22 ± 0.03</td>
<td>4.6</td>
<td>1.1 ± 0.15</td>
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*aTrypomastigotes of T. b. brucei s427 (n ≥ 4). **Resistance factor relative to WT without glycerol: RF = EC_{50} (in the presence of glycerol)/EC_{50} (without glycerol). *T. brucei strain from which all aquaporins were knocked out. †Resistance factor relative to WT. ‡T. b. brucei strain resistant to pentamidine, diminazene, and melaminophenyl arsenicals. §Human embryonic kidney cells (n = 3). ‡Selectivity index (SI) = CC_{50}/EC_{50} (T. brucei WT). †Not effective at 100 µM. ‡Not determined. ‡Salicylhydroxamic acid. ‡Pentamidine. ‡Diminazene. ‡Phenylarsine oxide.
2) Experimental protocol for the synthesis of compounds 4–21.

Chemistry. Anhydrous solvents were purchased to Aldrich/Fluka in SureSeal™ bottles and used as received. Thin Layer chromatography (TLC) was performed on silica gel 60 F254 aluminum TLC plates (MERCK). Chromatography was performed on silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, MERCK). LC-MS spectra were recorded on a WATERS apparatus integrated with a HPLC separation module (2695), PDA detector (2996) and Micromass ZQ spectrometer using electrospray ionization (ES⁺ or ES⁻). Analytical HPLC was performed with a SunFire C18-3.5 μm column (4.6 mm × 50 mm). Mobile phase A: CH₃CN + 0.08% formic acid and B: H₂O + 0.05% formic acid. UV detection was carried over 190 to 440 nm. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance-300, Varian Inova-400, Varian-Mercury-400, and Varian-system-500 spectrometers. Chemical shifts of the ¹H NMR spectra were referenced to tetramethylsilane (δ 0 ppm) for CDCl₃. Chemical shifts of the ¹³C NMR spectra were referenced to CDCl₃ (δ 77.16 ppm). Coupling constants J are expressed in hertz (Hz). Accurate mass was measured with an Agilent Technologies Q-TOF 6520 spectrometer using electrospray ionization. All of the biologically tested compounds were ≥ 95% pure by HPLC.

Synthesis of phosphonium salts 6–7 (a-f) and quinolinium salts 8c, 8d, and 8f

General procedure A. A Kimax tube was charged with the alcohol (1 equiv.), the phenol (2a-e, 1.2 equiv.), and triphenylphosphine (1.2 equiv.) in anhydrous THF. A solution of diisopropylazodicarboxylate (DIAD, 1.2 equiv.) in anhydrous THF was added and the reaction mixture was stirred at room temperature for 4–21 h. The solvent was removed under vacuum and the crude product was partitioned between EtOAc and HCl 0.1 M. The organic phase was washed successively with water (2×) and brine (2×). The organic phase was dried (MgSO₄) and the solvent was removed under vacuum. The crude product was purified by column chromatography using a gradient of EtOAc in hexanes.

General procedure B. A solution of triphenylphosphine (1.2 equiv.), the alcohol (1 equiv.) and DIAD (1.2 equiv.) in anhydrous THF was added dropwise to a cooled (ice-bath) solution of the phenol (2a-e, 1.2 equiv.) in anhydrous THF. The ice-bath was removed and the reaction mixture was stirred at room temperature for 2–20 h. The product was purified as described in procedure A.

Methyl 4-((10-bromodecyl)oxy)-2-hydroxybenzoate (4a)

![Structure of 4a]

A mixture of 2a (499 mg, 3.1 mmol), 1,10-dibromodecane (1.031 g, 3.4 mmol) and NaHCO₃ (254 mg, 3.0 mmol) in anhydrous acetonitrile (18 mL) was stirred at 65 °C under argon atmosphere for 1 week. The product was isolated by silica chromatography using Hexane/EtOAc: 100:0→40:1. Unreacted 1,10-dibromodecane eluted first (100% hexane)
followed respectively by 4a (Hex/EtOAc 40:1). Colorless solid (256 mg, 22 %). HPLC
(UV) > 95 %. 1H NMR (300 MHz, CDCl3) δ 10.89 (s, 1H), 7.65 (d, J = 9.5 Hz, 1H), 6.36 – 6.33 (m, 2H), 3.90 (t, J = 6.5 Hz, 2H), 3.85 (s, 3H), 3.34 (t, J = 6.8 Hz, 2H), 1.81-1.67 (m, 4H), 1.38 – 1.24 (m, 12H). 13C NMR (75 MHz, CDCl3) δ 170.6, 165.3, 163.9, 131.3, 108.1, 105.3, 101.2, 68.4, 52.1, 34.2, 32.9, 29.5, 29.5, 29.4, 29.1, 28.9, 28.3, 26.1. LRMS (ESI+) m/z 387.1 (M+H)+.

**Methyl 4-((14-bromotetradecyl)oxy)-2-hydroxybenzoate (5a)**

![Methyl 4-((14-bromotetradecyl)oxy)-2-hydroxybenzoate (5a)](image)

General procedure A using 2a (44.6 mg, 0.27 mmol), 14-bromotetradecan-1-ol (612 mg, 0.21 mmol) and PPh3 (68.3 mg, 0.26 mmol) in THF (3 mL), and a solution of DIAD (58.6 mg, 0.29 mmol) in THF (1.5 mL). The reaction mixture was stirred 20 h and the compound was purified by chromatography using hexane/EtOAc (100:0 → 60:1) to give 5a as colorless solid (52 mg, 56%). HPLC (UV) 96 %.

1H NMR (400 MHz, CDCl3) δ 10.94 (s, 1H, OH), 7.72 (d, J = 9.6 Hz, 1H, ArH), 6.46 – 6.39 (m, 2H, ArH), 3.97 (t, J = 6.6 Hz, 2H, OCH2), 3.91 (s, 3H, OCH3), 3.41 (t, J = 6.9 Hz, 2H, CH2Br), 1.91 – 1.81 (m, 2H), 1.82 – 1.72 (m, 2H), 1.48 – 1.38 (m, 20H). 13C NMR (101 MHz, CDCl3) δ 170.5, 165.4, 163.9, 131.3, 108.1, 105.3, 101.3, 68.4, 52.0, 34.2, 33.0, 29.73, 29.69, 29.66, 29.61, 29.57, 29.45, 29.1, 28.9, 28.3, 26.1. HRMS (ESI+) m/z 442.1721 (C22H35BrO4 requires 442.1719).

**Methyl 4-((14-bromotetradecyl)oxy)-2-fluorobenzoate (5b)**

![Methyl 4-((14-bromotetradecyl)oxy)-2-fluorobenzoate (5b)](image)

General procedure B using 14-bromotetradecan-1-ol (141 mg, 0.48 mmol) in THF (1 mL), and a solution of 2b (99 mg, 0.58 mmol), PPh3 (156 mg, 0.59 mmol), and DIAD (121 mg, 0.60 mmol) in THF (2 mL). The reaction mixture was stirred 3 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 60:1) to give 5b as colorless solid (139 mg, 65%). HPLC (UV) 95 %. 1H NMR (400 MHz, CDCl3) δ 7.88 (t, J = 8.7 Hz, 1H, ArH), 6.70 (dd, J = 2.4, 8.9 Hz, 1H, ArH), 6.61 (dd, J = 2.4, 12.8 Hz, 1H, ArH), 3.98 (t, J = 6.5 Hz, 2H, OCH2), 3.89 (s, 3H, OCH3), 3.40 (t, J = 6.9 Hz, 2H, CH2Br), 1.88 – 1.82 (m, 2H), 1.82 – 1.75 (m, 2H), 1.48 – 1.38 (m, 4H), 1.36 – 1.27 (m, 16H). 13C NMR (101 MHz, CDCl3) δ 164.9 (d, overlapping, J = 4.3 Hz), 164.3 (d, J = 11.5 Hz), 163.6 (d, J = 259.5 Hz), 133.5 (d, J = 2.8 Hz), 110.7 (d, J = 2.6 Hz), 110.6, 102.8 (d, J = 25.9 Hz), 68.8, 52.1, 34.2, 33.0, 29.74, 29.73, 29.69, 29.67 (m), 29.58, 29.45, 29.1, 28.9, 28.3, 26.1. HRMS (ESI+) m/z 444.1679 (C22H33BrO4 requires 444.1675).

**Methyl 4-((14-bromotetradecyl)oxy)-2-methylbenzoate (5c)**
General procedure B using 14-bromotetradecan-1-ol (168 mg, 0.58 mmol) in THF (0.5 mL), and a solution of 2c (112 mg, 0.67 mmol), PPh$_3$ (178 mg, 0.68 mmol) and DIAD (142 mg, 0.70 mmol) in THF (2 mL). The reaction mixture was stirred 4 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 45:1) to give 5c as colorless powder (162 mg, 64%). HPLC (UV) 95%.

**$^1$H NMR** (400 MHz, CDCl$_3$) $\delta$ 8.03 – 7.78 (m, 1H, ArH), 6.82 – 6.67 (m, 2H, ArH), 3.98 (t, $J$ = 6.6 Hz, 2H, OCH$_2$), 3.85 (s, 3H, OCH$_3$), 3.40 (t, $J$ = 6.9 Hz, 2H, CH$_2$Br), 2.59 (s, 3H, ArCH$_3$), 1.89 – 1.81 (m, 2H), 1.81 – 1.73 (m, 2H), 1.49 – 1.37 (m, 4H), 1.37 – 1.22 (m, 16H).

**$^{13}$C NMR** (101 MHz, CDCl$_3$) $\delta$ 167.7, 162.1, 143.2, 133.1, 121.6, 117.6, 111.5, 68.2, 51.6, 34.2, 33.0, 29.75, 29.74, 29.71, 29.69, 29.67, 29.58, 29.50, 29.3, 28.9, 28.3, 26.1, 22.5.

**Methyl 5-((14-bromotetradecyl)oxy)-2-hydroxybenzoate (5d)**

General procedure B using 2d (121 mg, 0.72 mmol) and 14-bromotetradecan-1-ol (174 mg, 0.60 mmol) in THF (2.5 mL). The reaction mixture was stirred 5 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 60:1) to give 5d as colorless solid (112 mg, 43%). HPLC (UV) > 95%.

**$^1$H NMR** (400 MHz, CDCl$_3$) $\delta$ 10.34 (s, 1H, OH), 7.29 (d, $J$ = 3.1 Hz, 1H, ArH), 7.08 (dd, $J$ = 3.1, 9.1 Hz, 1H, ArH), 6.90 (d, $J$ = 9.1 Hz, 1H, ArH), 3.94 (s, 3H, OCH$_3$), 3.91 (t, $J$ = 6.5 Hz, 2H, OCH$_2$), 3.40 (t, $J$ = 6.9 Hz, 2H, CH$_2$Br), 1.91 – 1.79 (m, 2H), 1.82 – 1.70 (m, 2H), 1.50 – 1.37 (m, 4H), 1.37 – 1.24 (m, 16H).

**$^{13}$C NMR** (400 MHz, CDCl$_3$) $\delta$ 170.5 (C=O), 156.1 (Ar-C$_2$), 151.7 (Ar-C$_5$), 124.7 (Ar-CH), 118.6 (Ar-CH), 113.0 (Ar-CH), 112.0 (Ar-C$_1$), 68.9 (OCH$_2$), 52.4 (OCH$_3$), 34.2 (CH$_2$Br), 33.0 (CH$_2$CH$_2$Br), 29.75 (CH$_2$), 29.73 (CH$_2$), 29.71 (CH$_2$), 29.70 (CH$_2$), 29.66 (CH$_2$), 29.57 (CH$_2$), 29.52 (CH$_2$), 29.4 (CH$_2$), 28.9 (CH$_2$CH$_2$O), 28.3 (CH$_2$), 26.2 (CH$_2$). HRMS (ESI$^+$) $m/z$ 442.1726 (C$_{22}$H$_{35}$BrO$_4$ requires 442.1719).

**Ethyl 4-((14-bromotetradecyl)oxy)-2-hydroxy-6-methylbenzoate (5e)**

General procedure A using 2e (280 mg, 1.43 mmol) and 14-bromotetradecan-1-ol (334 mg, 1.14 mmol) in THF (25 mL). The reaction mixture was stirred 4 h at room
temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 50:1) to give 5e as colorless solid (361 mg, 67 %). HPLC (UV) 95 %. ¹H NMR (400 MHz, CDCl₃) δ 11.84 (s, 1H, ArOH), 6.31 (d, J = 2.5 Hz, 1H, ArH), 6.28 (d, J = 2.5 Hz, 1H, ArH), 4.39 (q, J = 7.1 Hz, 2H, CH₂CH₂O), 3.95 (t, J = 6.6 Hz, 2H, ArOCH₂), 3.41 (t, J = 6.9 Hz, 2H, CH₂Br), 2.50 (s, 3H, ArCH₃), 1.85 (p, J = 7.0 Hz, 2H), 1.76 (p, J = 6.6 Hz, 2H), 1.47–1.37 (m, overlapping, 4H), 1.41 (t, overlapping, 3H, J = 7.1 Hz, CH₂CH₂), 1.34 – 1.27 (m, 16 H). ¹³C NMR (101 MHz, CDCl₃) δ 171.9, 165.7, 163.6, 143.2, 111.7, 105.3, 99.3, 68.2, 61.3, 34.2, 33.0, 29.73, 29.70, 29.67, 29.58, 29.46, 29.2, 28.9, 28.3, 26.1, 24.5, 22.1, 14.4. HRMS (ESI⁺) m/z 470.2010 (C₂₄H₃₉BrO₄ requires 470.2032).

**General procedure C.** A Kimax tube was charged with an equimolar amount of bromoalkane derivative (4a, 5a-e) and triphenylphosphine or quinoline in anhydrous acetonitrile. The tube was flushed with argon, stopped with a screwcap, and the reaction mixture was stirred at 80 °C for 5 to 10 days. The solvent was removed under vacuum and the product (6a, 7a-e) was isolated by recrystallization.

(10-(3-Hydroxy-4-(methoxycarbonyl)phenoxy)decyl)triphenylphosphonium bromide (6a)

General procedure C using 4a (53.1 mg, 0.14 mmol) and triphenylphosphine (38 mg, 0.14 mmol) in anhydrous acetonitrile (1 mL). The reaction was stirred for 7 days. The product was isolated as brownish gummy solid (77 mg, ~100%) by successive crystallizations from CH₂Cl₂/hexane and ethanol/hexane. HPLC (UV) 95 %. ¹H NMR (400 MHz, CDCl₃) δ 10.93 (s, 1H, OCH₃), 7.92 – 7.81 (m, 5H, ArH), 7.82 – 7.74 (m, 4H, ArH), 7.76 – 7.64 (m, 8H, ArH), 6.43 – 6.41 (m, 2H, ArH), 3.94 (t, J = 6.6 Hz, 2H, OCH₂), 3.90 (s, 3H, OCH₃), 3.92 - 3.83 (m, overlapping, 2H, CH₂P), 1.81 – 1.60 (m, 4H), 1.46 – 1.21 (m, 12H). ¹³C NMR (75 MHz, CDCl₃) δ 170.5, 165.2, 163.7, 135.1 (d, J = 3.2 Hz), 133.7 (d, J = 10.0 Hz), 133.3 (d, J = 10.8 Hz), 131.3, 130.6 (d, J = 12.5 Hz), 118.4 (d, J = 86.0 Hz), 107.9, 105.3, 101.2, 68.3, 52.0, 30.5 (d, J = 15.1 Hz), 29.4, 29.2, 29.1, 29.0, 25.9, 22.8 (d, J = 49.7 Hz), 22.7 (d, J = 4.1 Hz). HRMS (ESI⁺) m/z 569.2834 (C₃₆H₅₀O₄P requires 569.2821).

(14-(3-Hydroxy-4-(methoxycarbonyl)phenoxy)tetradecyl)triphenylphosphonium (7a)
General procedure C using 5a (14.8 mg, 0.033 mmol) and triphenylphosphine (9.8 mg, 0.037 mmol) in anhydrous acetonitrile (3 mL). The reaction was stirred for 14 days. The product was isolated as orangish gummy solid (8.7 mg, 42%) by crystallization from CH$_2$Cl$_2$/hexano. HPLC (UV) 95%. $^1$H NMR (400 MHz, CDCl$_3$) \( \delta \) 10.93 (s, 1H, OH), 7.88 – 7.67 (m, 16H, ArH), 6.42 (dd, \( J = 2.4, 4.6 \) Hz, 2H, ArH), 3.96 (t, \( J = 6.6 \) Hz, 2H, OCH$_2$), 3.90 (s, 3H, OCH$_3$), 3.98 – 3.80 (m, 2H, CH$_2$P), 1.77 (p, \( J = 6.9 \) Hz, 2H), 1.43 – 1.19 (m, 22H).

$^{13}$C NMR (75 MHz, CDCl$_3$) \( \delta \) 170.6, 165.4, 163.8, 135.1, 133.9 (d, \( J = 10.0 \) Hz), 131.3, 130.6 (d, \( J = 12.4 \) Hz), 118.6 (d, \( J = 86.0 \) Hz), 108.0, 105.3, 101.3, 68.4, 52.1, 30.6 (d, \( J = 15.6 \) Hz), 29.7, 29.4, 29.3, 29.12, 26.07, 23.0 (d, \( J = 49.1 \) Hz), 22.8 (d, \( J = 4.5 \) Hz). LRMS (ESI$^+$) \( m/z \) 626.2 (M+H$^+$).

(14-(3-Fluoro-4-(methoxycarbonyl)phenoxy)tetradecyl)triphenylphosphonium bromide (7b)

General procedure C using 5b (45.3 mg, 0.07 mmol) and triphenylphosphine (21.3 mg, 0.08 mmol) in anhydrous acetonitrile (1.5 mL). The reaction was stirred for 5 days. The product was isolated as yellowish gummy solid (29.4 mg, 65%) by crystallization from CH$_2$Cl$_2$/hexano. HPLC (UV) > 95%. $^1$H NMR (400 MHz, CDCl$_3$) \( \delta \) 7.86 – 7.75 (m, 10H, ArH), 7.70 – 7.66 (m, 6H, ArH), 6.67 (d, \( J = 8.9 \) Hz, 1H, ArH), 6.57 (d, \( J = 12.9 \) Hz, 1H, ArH), 3.95 (t, \( J = 6.6 \) Hz, 2H, OCH$_2$), 3.85 (s, 3H, OCH$_3$), 3.77 - 3.71 (m, 2H, CH$_2$P), 1.78 – 1.71 (m, 2H), 1.60 – 1.58 (m, 4H), 1.44 – 1.38 (m, 2H), 1.36 – 1.16 (m, 16H). $^{13}$C NMR (101 MHz, CDCl$_3$) \( \delta \) 164.8 (d, overlapping, \( J = 4.3 \) Hz), 164.3 (d, \( J = 11.7 \) Hz), 163.5 (d, \( J = 259.4 \) Hz), 135.1 (d, \( J = 3.1 \) Hz), 133.8 (d, \( J = 9.9 \) Hz), 133.4 (d, \( J = 2.8 \) Hz), 130.6 (d, \( J = 12.5 \) Hz), 118.5 (d, \( J = 85.8 \) Hz), 110.6 (d, \( J = 3 \) Hz), 110.5, 102.8 (d, \( J = 25.8 \) Hz), 68.8, 52.0, 30.5 (d, \( J = 15.6 \) Hz), 29.62, 29.57, 29.3, 29.0, 26.0, 22.9 (d, \( J = 49.7 \) Hz), 22.8 (d, \( J = 4.7 \) Hz). HRMS (ESI$^+$) \( m/z \) 627.3404 (C$_{40}$H$_{49}$FO$_3$P requires 627.3403).

(14-(4-(Methoxycarbonyl)-3-methylphenoxy)tetradecyl)triphenylphosphonium bromide (7c)
General procedure C using 5c (52.9 mg, 0.12 mmol) and triphenylphosphine (31.5 mg, 0.12 mmol) in anhydrous acetonitrile (2.5 mL). The reaction was stirred for 11 days. The product was isolated as yellowish gummy solid (78.9 mg, ~100%) by crystallization from CH$_2$Cl$_2$/hexane. HPLC (UV) 95%. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.81 (d, $J = 8.9$ Hz, 1H, ArH), 7.82 – 7.61 (m, 15H, ArH), 6.65 – 6.61 (m, 2H, ArH), 3.88 (t, $J = 6.5$ Hz, 2H, OCH$_2$), 3.74 (s, 3H, OCH$_3$), 3.63 - 3.55 (m, 2H, CH$_2$P), 2.48 (s, 3H, ArCH$_3$), 1.71 – 1.64 (m, 2H), 1.61 – 1.46 (m, 4H), 1.38 – 1.31 (m, 2H), 1.24 – 1.11 (m, 16H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.4, 161.8, 142.9, 135.0 (d, $J = 3.2$ Hz), 133.5 (d, $J = 9.8$ Hz), 132.8, 130.5 (d, $J = 12.4$ Hz), 121.2, 118.1 (d, $J = 85.7$ Hz), 117.3, 111.3, 67.9, 51.4, 30.9, 30.4 (d, $J = 15.5$ Hz), 29.4 (m), 29.2, 29.1, 29.0, 25.9, 22.7 (d, $J = 50.2$ Hz), 22.5 (d, $J = 4.7$ Hz), 22.2. HRMS (ESI$^+$) m/z 623.3663 (C$_{41}$H$_{52}$O$_3$P requires 623.3654).

(14-(4-hydroxy-3-(methoxycarbonyl)phenoxy)tetradecyl)triphenylphosphonium bromide (7d)

General procedure C using 5d (32 mg, 0.07 mmol) and triphenylphosphine (22.7 mg, 0.086 mmol) in anhydrous acetonitrile (1 mL). The reaction was stirred for 3 days. The product was isolated as colorless solid (2.4 mg, 5%) by successive crystallizations from CH$_2$Cl$_2$/hexane/EtOAc and EtOAc. HPLC (UV) > 95%. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.77 (td, $J = 1.6$, 7.5 Hz, 3H), 7.64 (td, $J = 3.4$, 7.8 Hz, 6H), 7.60 – 7.52 (m, 6H), 7.18 (d, $J = 3.1$ Hz, 1H), 6.95 (dd, $J = 3.1$, 9.1 Hz, 1H), 6.77 (d, $J = 9.1$ Hz, 1H), 3.83 (s, 3H, overlapp with solvent), 3.79 (t, $J = 6.6$ Hz, 2H), 3.13 – 3.05 (m, 2H), 1.69 – 1.59 (m, 2H), 1.54 (p, $J = 8.0$ Hz, 2H), 1.43 (p, $J = 7.3$ Hz, 2H), 1.36 – 1.28 (m, 2H), 1.25 – 1.10 (m, 16H). $^{31}$P NMR (162 MHz, CDCl$_3$) δ 28.5. $^{13}$C NMR (126 MHz, CDCl$_3$) δ 166.3, 155.5, 151.5, 135.5 (d, $J = 3.1$ Hz), 133.2 (d, $J = 9.8$ Hz), 130.7 (d, $J = 12.5$ Hz), 124.5, 118.2, 117.7 (d, $J = 86.4$ Hz), 113.0, 68.8, 52.2, 30.6 (d, $J = 15.6$ Hz), 29.6, 29.49, 29.46, 29.44, 29.43, 29.3 (d, $J = 17.9$ Hz), 29.2 (d, $J = 5.2$ Hz), 28.9, 25.9, 22.7 (d, $J = 51.2$ Hz), 22.5 (d, $J = 4.6$ Hz). HRMS (ESI$^+$) m/z 625.3423 (C$_{40}$H$_{50}$O$_4$P requires 625.3447).

(14-(4-(Ethoxycarbonyl)-3-hydroxy-5-methylphenoxy)tetradecyl)triphenylphosphonium bromide (7e)
General procedure C using 5e (56.8 mg, 0.12 mmol) and triphenylphosphine (39.6 mg, 0.15 mmol) in anhydrous acetonitrile (2 mL). The reaction was stirred for 6 days. The product was isolated as colorless gummy solid (65.2 mg, 83%) by crystallization from CH\(_2\)Cl\(_2\)/hexane. HPLC (UV) 95%. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 11.74 (s, 1H, ArOH), 7.77 – 7.71 (m, 10H, ArH), 7.67 – 7.62 (m, 5H, ArH), 6.20 (d, \(J = 2.6\) Hz, 2H, ArH), 4.30 (q, \(J = 7.0\) Hz, 2H, CH\(_3\)CH\(_2\)O), 3.85 (t, \(J = 6.6\) Hz, 2H, ArOCH\(_2\)), 3.63 – 3.51 (m, 2H, CH\(_2\)P), 2.42 (s, 3H, ArCH\(_3\)), 1.67 (p, \(J = 6.8\) Hz, 2H), 1.57 – 1.50 (m, 4H), 1.33 (t, \(J = 7.0\) Hz, 3H, CH\(_3\)CH\(_2\)O), 1.30 – 1.12 (m, 18H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 171.7, 165.4, 163.3, 143.0, 135.0 (d, \(J = 3.2\) Hz), 133.5 (d, \(J = 9.9\) Hz), 130.5 (d, \(J = 12.5\) Hz), 118.2 (d, \(J = 86\) Hz), 111.3, 105.0, 99.2, 67.9, 61.1, 30.4 (d, \(J = 15.3\) Hz), 29.4 (m), 29.2, 29.1, 28.9, 25.8, 24.3, 22.7 (d, \(J = 49.9\) Hz), 22.5 (d, \(J = 4.6\) Hz), 14.2. HRMS (ESI\(^+\)) \(m/z\) 653.3779 (C\(_{42}\)H\(_{54}\)O\(_4\)P requires 653.3760).

1-(14-(3-Fluoro-4-(methoxycarbonyl)phenoxy)tetradecyl)quinolin-1-ium (10b)

General procedure C using 5b (61.6 mg, 0.14 mmol) and quinoline (40.7 mg, 0.3 mmol) in anhydrous acetonitrile (2 mL). The reaction was stirred for 10 days. The product was isolated as colorless powder (24.6 mg, 36%) by successive crystallizations from CH\(_2\)Cl\(_2\)/hexane and CH\(_3\)CN. HPLC (UV) 96%. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.51 (d, \(J = 5.7\) Hz, 1H, ArH), 9.13 (d, \(J = 8.3\) Hz, 1H, ArH), 8.37 (t, \(J = 8.7\) Hz, 2H, ArH), 8.22 – 8.17 (m, 2H, ArH), 7.95 (t, \(J = 7.6\) Hz, 1H, ArH), 7.85 (t, \(J = 8.7\) Hz, 1H, ArH), 6.67 (dd, \(J = 2.5, 8.9\) Hz, 1H, ArH), 6.58 (dd, \(J = 2.5, 12.9\) Hz, 1H, ArH), 5.40 (t, \(J = 7.6\) Hz, 2H, CH\(_2\)N), 3.95 (t, \(J = 6.5\) Hz, 2H, OCH\(_2\)), 3.86 (s, 3H, OCH\(_3\)), 2.10 – 2.06 (m, 2H), 1.79 - 1.72 (m, 2H), 1.50 – 1.42 (m, 2H), 1.44 – 1.37 (m, 2H), 1.30 – 1.20 (m, 16H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 164.8 (d, \(J = 4.3\) Hz), 164.3 (d, \(J = 11.7\) Hz), 163.5 (d, \(J = 259.8\) Hz), 150.7, 147.2, 137.7, 136.0, 133.4 (d, \(J = 3.0\) Hz), 131.2, 130.2, 130.1, 122.7, 118.4, 110.7 (d, \(J = 3.4\) Hz), 102.7 (d, \(J = 25.8\) Hz), 68.8, 58.3, 52.1, 30.5, 29.62, 29.57, 29.52, 29.44, 29.35, 29.26, 29.0, 26.6, 26.0. HRMS (ESI\(^+\)) \(m/z\) 494.3077 (C\(_{31}\)H\(_{41}\)FNO\(_3\) requires 494.3071).

1-(14-(4-(Methoxycarbonyl)-3-methylphenoxy)tetradecyl)quinolin-1-ium bromide (10c)
General procedure C using 5c (75 mg, 0.17 mmol) and quinoline (86.1 mg, 0.67 mmol) in anhydrous acetonitrile (2.5 mL). The reaction was stirred for 11 days. The product was isolated as brown solid (54.9 mg, 66%) by successive crystallizations from CH$_3$CN and CH$_2$Cl$_2$/hexane. HPLC (UV) 95%. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.34 (d, $J = 5.4$ Hz, 1H, ArH), 9.17 (d, $J = 8.2$ Hz, 1H, ArH), 8.37 (d, 2H, $J = 7.6$ Hz, ArH), 8.16 (dd, $J = 6.9$, 6.1 Hz, 2H, ArH), 7.91 (t, $J = 7.6$ Hz, 1H, ArH), 7.83 (d, 1H, $J = 9.5$ Hz, ArH), 6.78 – 6.54 (m, 2H, ArH), 5.33 (t, $J = 7.5$ Hz, 2H, CH$_2$N), 3.90 (t, $J = 6.6$ Hz, 2H, OCH$_2$), 3.77 (s, 3H, OCH$_3$), 2.51 (s, 3H, ArCH$_3$), 2.03 (p, 2H, $J = 7.6$ Hz), 1.70 (p, 2H, $J = 6.7$ Hz), 1.45 – 1.32 (m, 4H), 1.28 – 1.15 (m, 16H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.5, 161.9, 150.3, 147.4, 142.9, 137.6, 136.0, 132.9, 131.2, 130.1, 130.0, 122.6, 121.3, 118.3, 117.4, 111.3, 68.0, 58.2, 51.5, 30.4, 29.5 (m), 29.32, 29.29, 29.14, 29.08, 26.5, 25.9, 22.3. HRMS (ESI$^+$) m/z 490.3320 (C$_{32}$H$_{44}$NO$_3$ requires 490.3321).

1-(14-(4-(Ethoxycarbonyl)-3-hydroxy-5-methylphenoxy)tetradecyl)quinolin-1-ium bromide (10e)

General procedure C using 5e (174 mg, 0.37 mmol) and quinoline (58.3 mg, 0.45 mmol) in anhydrous acetonitrile (2 mL). The reaction was stirred for 10 days. The product was isolated as brownish solid (50.6 mg, 39%) by successive crystallizations from CH$_2$Cl$_2$/hexane and CH$_3$CN. HPLC (UV) > 95%. $^1$H NMR (400 MHz, CDCl$_3$) δ 11.80 (s, 1H, OH), 10.47 (d, $J = 5.7$ Hz, 1H), 9.13 (d, $J = 8.4$ Hz, 1H), 8.36 (t, $J = 7.5$ Hz, 2H), 8.21 - 8.16 (m, 2H), 7.94 (t, $J = 7.6$ Hz, 1H), 6.27 – 6.24 (m, 2H), 5.38 (t, $J = 7.6$ Hz, 2H, CH$_2$N), 4.35 (q, $J = 7.1$ Hz, 2H, CH$_3$CH$_2$O), 3.93 – 3.89 (m, 2H, OCH$_2$), 2.47 (s, 3H, ArCH$_3$), 2.14 – 1.99 (m, 2H), 1.85 – 1.70 (m, 4H), 1.54 – 1.42 (m, 2H), 1.38 (t, 3H, $J = 7.0$ Hz), 1.34 – 1.18 (m, 16H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.9, 165.6 (d), 163.5, 150.9, 147.1, 143.2 (d), 137.8, 136.0, 131.2, 130.2, 130.1, 122.8, 118.4, 111.6 (d), 105.2, 99.3 (d), 68.1, 61.3, 58.3, 34.2, 32.9, 30.5, 29.63, 29.61, 29.57, 29.52, 29.44, 29.40, 29.3, 29.1, 28.9, 28.3, 26.6, 26.0, 24.5, 14.3. HRMS (ESI$^+$) m/z 520.3427 (C$_{33}$H$_{46}$NO$_4$ requires 520.3427).

General procedure D. A Kimax tube was charged with a mixture of ester (0.02 mmol, 1 equiv.) and potassium carbonate powder (3 equiv.) in MeOH/H$_2$O solution (5/1, v/v; 2 mL). The reaction mixture was stopped with a screwcap and was stirred at 50 ºC for several days until complete hydrolysis. 0.1 N HCl (2 mL) and CH$_2$Cl$_2$ (3 mL) were added to the cooled reaction and the mixture was stirred at room temperature for a few minutes.
The organic phase was separated, dried (Na$_2$SO$_4$) and evaporated to give the pure crude carboxylic acid.

(10-(4-carboxy-3-hydroxyphenoxy)decyl)triphenylphosphonium bromide (8a)

General procedure D using 6a (13 mg, 0.02 mmol) and K$_2$CO$_3$ (8 mg, 0.06 mmol). The reaction mixture was stirred at 50 ºC for 67 h. The product was isolated as colorless gummy solid after workup (12 mg, ~100%). HPLC (UV) > 95 %. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.73 (ddp, $J$ = 2.7, 4.1, 6.6 Hz, 3H), 7.67 (d, $J$ = 8.7 Hz, 1H), 7.66 – 7.56 (m, 12H), 6.29 – 6.25 (m, 1H), 6.23 (dd, $J$ = 2.5, 8.7 Hz, 1H), 3.85 (t, $J$ = 6.4 Hz, 2H), 3.25 – 3.15 (m, 2H), 1.63 (p, $J$ = 6.6 Hz, 2H), 1.55 – 1.46 (m, 2H), 1.39 (p, $J$ = 7.3 Hz, 2H), 1.30 (p, $J$ = 7.1 Hz, 2H), 1.20 – 1.06 (m, 8H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 173.3, 163.6, 163.5, 135.3 (d, $J$ = 3.0 Hz), 133.4 (d, $J$ = 10.0 Hz), 133.1 (d, $J$ = 10.5 Hz), 130.6 (d, $J$ = 12.4 Hz), 130.5 (d, $J$ = 12.9 Hz), 118.1 (d, $J$ = 86.1 Hz), 106.3, 101.0, 67.9, 30.5 (d, $J$ = 15.7 Hz), 29.7, 29.1, 28.94, 28.88, 28.84, 28.78, 25.6, 22.4 (d, $J$ = 46.9 Hz). HRMS (ESI$^+$) m/z 555.2675 (C$_{35}$H$_{40}$O$_4$P requires 555.2664).

(14-(4-carboxy-3-hydroxyphenoxy)tetradecyl)triphenylphosphonium bromide (9a)

General procedure D using 7a (7 mg, 0.01 mmol) and K$_2$CO$_3$ (5 mg, 0.033 mmol). The reaction mixture was stirred at 50 ºC for 3 days. The product was isolated as greyish gummy solid after workup (5.3 mg, 77%). HPLC (UV) > 95 %. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.85 – 7.63 (m, 16H), 6.35 (d, $J$ = 2.5 Hz, 1H), 6.29 (dd, $J$ = 2.5. 8.7 Hz, 1H), 3.97 (t, $J$ = 6.2 Hz, 2H), 3.69 – 3.43 (m, 2H), 2.40 – 2.29 (m, 2H), 1.75 (p, $J$ = 6.6 Hz, 2H), 1.67 – 1.36 (m, 6H), 1.21 – 1.03 (m, 14H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 173.6, 163.9, 163.7, 135.1 (d, $J$ = 2.8 Hz), 133.7 (d, $J$ = 9.9 Hz), 132.2, 130.6 (d, $J$ = 12.4 Hz), 118.5 (d, $J$ = 85.8 Hz), 109.8, 106.2, 101.1, 67.6, 30.6 (d, $J$ = 15.7 Hz), 30.3, 29.6, 29.5 (d, $J$ = 7.9 Hz), 29.4 (d, $J$ = 12.0 Hz), 28.9, 28.6, 28.5, 27.9, 26.9, 25.2, 23.3, 22.3 (d, $J$ = 49.8 Hz). HRMS (ESI$^+$) m/z 611.3296 (C$_{39}$H$_{48}$O$_4$P requires 611.3290).

(14-(4-carboxy-3-fluorophenoxy)tetradecyl)triphenylphosphonium bromide (9b)
General procedure D using 7b (8 mg, 0.011 mmol) and K₂CO₃ (6.6 mg, 0.048 mmol). The reaction mixture was stirred at 50 °C for 67 h. The product was isolated as colorless gummy solid after workup (8 mg, ~100%). HPLC (UV) > 95%. ¹H NMR (500 MHz, CDCl₃) δ 7.84 (ddd, J = 2.6, 6.4, 8.9 Hz, 1H), 7.80 – 7.74 (m, 6H), 7.70 (ddd, J = 1.6, 5.1, 7.5 Hz, 3H), 7.62 (td, J = 3.3, 7.7 Hz, 6H), 6.61 (dd, J = 2.5, 9.0 Hz, 1H), 6.51 (dd, J = 2.7, 12.9 Hz, 1H), 3.95 – 3.91 (m, 2H), 3.73 – 3.67 (m, 2H), 1.70 (p, J = 6.7 Hz, 2H), 1.57 – 1.47 (m, 2H), 1.37 (p, J = 7.0 Hz, 2H), 1.28 – 1.02 (m, 16H).

¹³C NMR (126 MHz, CDCl₃) δ 166.7 (d, J = 5.7 Hz), 164.2 (d, J = 11.5 Hz), 163.9 (d, J = 259.9 Hz), 135.0 (d, J = 2.9 Hz), 134.1, 133.8 (d, J = 10.0 Hz), 130.5 (d, J = 12.6 Hz), 119.0 (d, J = 3.9 Hz), 118.4 (d, J = 3.8 Hz), 110.5, 102.8 (d, J = 25.8 Hz), 68.6 (d, J = 3.6 Hz), 30.6 (d, J = 15.8 Hz), 29.8, 29.7, 29.6, 29.5 – 29.4 (m), 29.40, 29.3 – 29.2 (m), 28.8 – 28.7 (m), 25.6 (d, J = 7.9 Hz), 22.8 (d, J = 4.3 Hz), 22.4 (d, J = 49.6 Hz). HRMS (ESI⁺) m/z 613.3240 (C₃₉H₄₇FOP requires 613.3247).

(14-(4-carboxy-3-methylphenoxy)tetradecyl)triphenylphosphonium bromide (9c)

General procedure D using 7c (12 mg, 0.017 mmol) and K₂CO₃ (8 mg, 0.058 mmol). The reaction mixture was stirred at 50 °C for 11 days. The product was isolated as colorless gummy solid after workup (5.4 mg, 46%). HPLC (UV) > 95 %. ¹H NMR (500 MHz, CDCl₃) δ 7.99 (d, J = 9.6 Hz, 1H), 7.84 (dd, J = 7.6, 12.6 Hz, 5H), 7.81 – 7.74 (m, 2H), 7.69 (td, J = 2.8, 7.2, 7.6 Hz, 6H), 7.59 – 7.43 (m, 2H), 6.76 – 6.69 (m, 2H), 4.01 (t, J = 6.4 Hz, 2H), 3.85 – 3.73 (m, 2H), 2.59 (s, 3H), 2.42 – 2.31 (m, 2H), 1.77 (p, J = 6.7 Hz, 2H), 1.68 – 1.11 (m, 20H). ¹³C NMR (126 MHz, CDCl₃) δ carboxylic C=O not observed due to long relaxation time, 162.3, 143.9, 135.0 (d, J = 3.1 Hz), 133.9 (d, J = 9.9 Hz), 132.3 (d, J = 9.9 Hz), 130.5 (d, J = 12.5 Hz), 128.7 (d, J = 12.3 Hz), 118.8 (d, J = 85.6 Hz), 117.7, 111.5, 68.0, 33.9, 30.6 (d, J = 15.8 Hz), 30.3, 29.7 (d, J = 6.7 Hz), 29.5 (t, J = 3.0 Hz), 29.45 – 29.41 (m), 29.3 (d, J = 3.7 Hz), 28.9, 26.9, 25.70, 25.0, 23.3, 22.8 (d, J = 18.3 Hz), 22.4 (d, J = 49.2 Hz). HRMS (ESI⁺) m/z 609.3514 (C₄₀H₅₀O₃P requires 609.3498).

(14-(4-carboxy-3-hydroxy-5-methylphenoxy)tetradecyl)triphenylphosphonium bromide (9e)
General procedure D using 7e (15.5 mg, 0.021 mmol), K$_2$CO$_3$ (8.8 mg, 0.063 mmol) and EtOH/H$_2$O solution (5/1, v/v; 1.2 mL). The reaction mixture was stirred at 50 °C for 7 days. The product was isolated as brownish gummy solid after workup (15 mg, ~100%). HPLC (UV) > 95%. $^1$H NMR (500 MHz, CDCl$_3$+CD$_3$OD) δ 7.78 (t, $J$ = 7.1 Hz, 3H), 7.70 – 7.61 (m, 13H), 6.22 – 6.16 (m, 2H), 3.87 (dt, $J$ = 3.3, 6.5 Hz, 2H), 3.70 – 3.18 (m, 6H), 2.47 (d, $J$ = 2.9 Hz, 3H), 1.73 – 1.63 (m, 2H), 1.53 – 1.43 (m, 2H), 1.36 (dt, $J$ = 3.8, 8.3 Hz, 2H), 1.26 – 1.14 (m, 16H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 173.9, 165.4, 163.0, 144.0, 135.3 (d, $J$ = 2.9 Hz), 133.4 (dd, $J$ = 1.8, 10.0 Hz), 130.6 (d, $J$ = 12.5 Hz), 118.1 (dd, $J$ = 2.9, 86.3 Hz), 110.9, 106.2, 99.0, 67.9, 30.5 (d, $J$ = 15.7 Hz), 29.7, 29.5, 29.43, 29.42, 29.3, 29.2, 29.0, 28.9, 25.8, 24.1, 22.6 (d, $J$ = 4.8 Hz), 22.5 (d, $J$ = 50.5 Hz). HRMS (ESI$^+$) m/z 625.3430 (C$_{40}$H$_{50}$O$_4$P requires 625.3447).

1-(14-(4-carboxy-3-methylenphenoxy)tetradecyl)quinolin-1-ium bromide (11c)

A mixture of 10c (9.2 mg, 0.016 mmol) in 48% aqueous HBr was stirred at 65 °C for 20 h. Then, AcOH (1 mL) was added to help dissolve the precipitate and the heating was resumed for 1.5 h. The crude reaction mixture was partitioned between CH$_2$Cl$_2$ and H$_2$O. The aqueous phase was extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried (Na$_2$SO$_4$), and evaporated to give a crude brown solid containing 11c and brominated by-products. Recrystallization from CH$_3$CN at 4 °C yielded 11c as off-white solid (3.8 mg, 42%). HPLC (UV) 95%. $^1$H NMR (400 MHz, CDCl$_3$+CD$_3$OD) δ 10.11 (brs, 1H), 8.93 (brs, 1H), 8.34 – 8.10 (m, 4H), 7.94 (dd, $J$ = 10.1, 5.5 Hz, 2H), 6.73 – 6.66 (m, 1H), 5.20 (brs, 2H), 3.96 (t, $J$ = 6.4 Hz, 2H), 2.56 (s, 3H), 2.34 – 1.99 (m, 8H), 1.74 (p, $J$ = 6.8 Hz, 2H), 1.60 – 1.10 (m, 14H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 169.9, 162.2, 149.8, 148.0, 143.4, 138.1, 136.6, 133.6, 131.5, 130.7, 130.6, 122.8, 121.8, 118.4, 117.7, 111.6, 68.3, 59.0, 33.9, 30.6, 29.73, 29.69, 29.59, 29.45, 29.33, 29.28, 26.9, 26.1, 22.4. HRMS (ESI$^+$) m/z 476.3169 (C$_{31}$H$_{42}$NO$_3$ requires 476.3165).

Synthesis of TAO inhibitors with geranyl and farnesyl tail

Note: the geranyl and farnesyl derivatives obtained as oil should be stored at 4 °C.

(2E,6E)-8-hydroxy-3,7-dimethylocta-2,6-dien-1-yl acetate (12)
A round-bottomed flask flushed with argon was charged with SeO₂ (115 mg, 1.04 mmol), salicylic acid (454 mg, 3.29 mmol) and anhydrous CH₂Cl₂ (38 mL). tBuOOH (12 mL, 124 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then, geranyl acetate (7 mL, 32.7 mmol) was added dropwise and the mixture was stirred at room temperature for 44 h. The solvent was removed under vacuum and the crude product was partitioned between EtOAc and 10% KOH. The organic phase was washed successively with 0.1 M HCl (1×) and brine (2×). The organic phase was dried (MgSO₄) and the solvent was removed under vacuum to give a crude oil which was purified by chromatography using hexane/EtOAc (7:1). Yellowish oil (3.01 g, 43%). HPLC (UV) > 90%. 1H NMR (400 MHz, CDCl₃) δ 5.38 – 5.31 (m, 2H), 4.57 (d, J = 7.0 Hz, 2H), 3.98 (s, 2H), 2.19 – 2.14 (m, 2H), 2.18 – 2.14 (m, 2H), 2.04 (s, 3H), 1.70 (s, 3H), 1.65 (s, 3H). LRMS (ESI⁺) m/z 213.4 (M+H)⁺.

(2E,6E,10E)-12-hydroxy-3,7,11-trimethyldodeca-2,6,10-trien-1-yl acetate (13)

A round-bottomed flask flushed with argon was charged with SeO₂ (106 mg, 0.95 mmol), salicylic acid (452 mg, 3.27 mmol) and anhydrous CH₂Cl₂ (55 mL). tBuOOH (11 mL, 114 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then, farnesyl acetate (7 mL, 32.7 mmol) was added dropwise and the mixture was stirred at room temperature for 3 days. The solvent was removed under vacuum and the crude product was partitioned between EtOAc and 10% KOH. The organic phase was washed successively with 0.1 M HCl and brine (2×). The organic phase was dried (MgSO₄) and the solvent was removed under vacuum to give a crude oil which was purified by silica chromatography using hexane/EtOAc (7:1). Yellowish oil (4.38 g, 52%). HPLC (UV) >80%. 1H NMR (300 MHz, CDCl₃) δ 5.40 – 5.31 (m, 2H), 5.09 (d, J = 6.3 Hz, 1H), 4.58 (d, J = 7.1 Hz, 2H), 3.99 (s, 1H), 2.11 – 2.01 (m, 11H), 1.70 – 1.60 (m, 9H), 1.65 (s, 3H). LRMS (ESI⁺) m/z 303.2 (M+Na)⁺.

(2E,6E)-8-bromo-3,7-dimethylocta-2,6-dien-1-yl acetate (14)

To a cooled (ice-bath) solution of 12 (210 mg, 0.98 mmol) in anhydrous CH₂Cl₂ (3 mL) was added PPh₃ (480 mg, 1.44 mmol) and CBr₄ (427 mg, 1.29 mmol). The reaction mixture was stirred at 4 °C for 1 day. The solvent was removed under vacuum and the crude product was dissolved in hexane. The solid precipitate was filtered off and the filtrate was evaporated under vacuum to give 14 as yellowish oil that was used without further purification (176 mg, 65%). HPLC (UV) >75%. 1H NMR (300 MHz, CDCl₃) δ 5.58 – 5.54 (m, 1H), 5.36 – 5.32 (m, 1H), 4.58 (d, J = 7.1 Hz, 2H), 3.96 (s, 2H), 2.20 – 2.08 (m, 4H), 2.06 (s, 3H), 1.75 (s, 3H), 1.70 (s, 3H). LRMS (ESI⁺) m/z 275.8 (M+H).
Ethyl 4-(((2E,6E,10E)-12-acetoxy-2,6,10-trimethyldeca-2,6,10-trien-1-yl)oxy)-2-hydroxy-6-methylbenzoate (15e)

General procedure B using 12 (285 mg, 1.38 mmol) in anhydrous THF (0.5 mL) and a solution of 2e (324 mg, 1.66 mmol), PPh3 (435 mg, 1.67 mmol) and a solution of DIAD (359 mg, 1.78 mmol) in anhydrous THF (4 mL). The reaction mixture was stirred at room temperature for 4 h and the crude product was purified by silica chromatography using hexane/EtOAc (100:0 → 25:1) to give 15e as colorless oil (169 mg, 27%). HPLC (UV) >95 %.

1H NMR (400 MHz, CDCl3) δ 11.81 (s, O\text{H}), 6.31 – 6.26 (m, 2H, 2×Ar\text{H}), 5.55 – 5.46 (m, 1H, C=C\text{H}), 5.38 – 5.28 (m, 1H, C=CH), 5.14 – 5.06 (m, 1H, C=C\text{H}), 4.58 (d, J = 7.1 Hz, 2H, CH\text{2}OAc), 4.40 – 4.35 (m, 4H, CH\text{3}CH\text{2}O, ArO\text{CH}\text{2}), 2.49 (s, 3H, Ar\text{CH}\text{3}), 2.18 – 2.08 (m, 4H, 2×CH\text{2}CH=), 2.05 – 1.98 (m, 7H, O\text{Ac}, 2×CH\text{2}C=), 1.69 (s, 3H, CH\text{3}), 1.67 (s, 3H, CH\text{3}), 1.59 (s, 3H, CH\text{3}), 1.40 (t, J = 7.1 Hz, 3H, CH\text{3}CH\text{2}O).

13C NMR (101 MHz, CDCl3) δ 171.9, 171.2, 165.6, 163.3, 143.1, 142.3, 134.9, 130.4, 129.3, 118.4, 111.8, 105.3, 99.7, 74.0, 59.3, 52.0, 39.6, 39.1, 26.4, 26.3, 24.5, 21.1, 16.6, 16.1, 14.4, 13.9. LRMS (ESI+) m/z 459.1 (M+H)+. HRMS (ESI+) m/z 468.2668 (C\text{27}H\text{38}O\text{6} requires 458.2668).

Methyl 4-(((2E,6E)-8-acetoxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-hydroxybenzoate (17a)

General procedure A using 12 (81.6 mg, 0.38 mmol), 2a (77.5 mg, 0.46 mmol) and Ph3P (119.4 mg, 0.46 mmol) in anhydrous THF (4 mL), and a solution of DIAD (84 mg, 0.48 mmol) in anhydrous THF (0.9 mL). The reaction mixture was stirred 17 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 30:1) to give 17a as colorless oil (68.3 mg, 49%). HPLC (UV) > 95%.

1H NMR (400 MHz, CDCl3) δ 10.93 (s, 1H, OH), 7.72 (d, J = 9.5 Hz, 1H, Ar\text{H}), 6.48 – 6.40 (m, 2H, Ar\text{H}), 5.56 – 5.47 (m, 1H, C=CH), 5.39 – 5.30 (m, 1H, C=CH), 4.58 (d, J = 7.1 Hz, 2H, CH\text{2}OAc), 4.40 (s, 2H, ArOCH\text{2}), 3.91 (s, 3H, O\text{Ac}), 2.27 – 2.14 (m, 2H, CH\text{2}CH\text{2}), 2.14 – 2.06 (m, 2H, CH\text{2}CH\text{2}), 2.05 (s, 3H, O\text{Ac}), 1.73 (s, 3H, CH\text{3}), 1.70 (s, 3H, CH\text{3}).

13C NMR (101 MHz, CDCl3) δ 171.9, 171.2, 165.6, 163.3, 143.1, 142.3, 134.9, 130.4, 129.3, 124.2, 118.4, 111.8, 105.3, 99.7, 74.0, 61.5, 61.3, 39.6, 39.1, 26.4, 26.3, 24.5, 21.1, 16.6, 16.1, 14.4, 13.9. LRMS (ESI+) m/z 459.1 (M+H)+. HRMS (ESI+) m/z 468.2668 (C\text{27}H\text{38}O\text{6} requires 458.2668).

Methyl 4-(((2E,6E)-8-acetoxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-fluorobenzoate (17b)
General procedure A using 12 (200.5 mg, 0.94 mmol), 2b (195.3 mg, 1.13 mmol), and Ph₃P (296 mg, 1.13 mmol) in anhydrous THF (10 mL), and a solution of DIAD (231 mg, 1.14 mmol) in anhydrous THF (5 mL). The reaction mixture was stirred 18 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 30:1) to give 17b as colorless oil (220 mg, 64%). HPLC (UV) 100%. ¹H NMR (400 MHz, CDCl₃) δ 7.87 (t, J = 8.7 Hz, 1H, ArH), 6.72 (dd, J = 2.4, 8.8 Hz, 1H, ArH), 6.63 (dd, J = 2.4, 12.8 Hz, 1H, ArH), 5.53 – 5.50 (m, 1H, C=CCH₂), 5.36 – 5.32 (m, 1H, C=CH₂), 4.58 (d, J = 7.1 Hz, 2H, CH₂OAc), 4.41 (s, 2H, ArOCH₂), 3.89 (s, 3H, OCH₃), 2.24 – 2.19 (m, 2H, CH₂CH₂), 2.12 – 2.08 (m, 2H, CH₂CH₂), 2.05 (s, 3H, OAc), 1.72 (s, 3H, CH₃), 1.71 (s, 3H, CH₃). ¹³C NMR (101 MHz, CDCl₃) δ 171.2, 164.9 (d, J = 4.2 Hz), 164.0 (d, J = 11.5 Hz), 163.5 (d, J = 259.8 Hz), 141.6, 133.5 (d, J = 2.8 Hz), 130.5, 129.1, 119.0, 111.0 (d, J = 2.9 Hz), 110.9 (d, J = 9.9 Hz), 103.2 (d, J = 25.9 Hz), 74.5, 61.4, 52.1, 39.0, 26.0, 21.2, 16.6, 13.9. LRMS (ESI⁺) m/z 387.2 (M+Na)⁺. HRMS (ESI⁺) m/z 364.1694 (C₂₀H₂₅FO₅ requires 364.1686).

Methyl 4-(((2E,6E)-8-acetoxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-methylbenzoate (17c)

General procedure B using 12 (193 mg, 0.91 mmol) in anhydrous THF (0.5 mL), and a solution of 2c (184 mg, 1.11 mmol), PPh₃ (307 mg, 1.17 mmol) and DIAD (228 mg, 1.13 mmol) in anhydrous THF (3 mL). The reaction mixture was stirred 4 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 25:1) to give 17c as colorless oil (223 mg, 68%). HPLC (UV) 100%. ¹H NMR (400 MHz, CDCl₃) δ 7.90 (d, J = 8.3 Hz, 1H, ArH), 6.77 – 6.69 (m, 2H, ArH), 5.65 – 5.42 (m, 1H, C=CH₂), 5.39 – 5.25 (m, 1H, C=CH), 4.58 (d, J = 7.1 Hz, 2H, CH₂OAc), 4.41 (s, 2H, ArOCH₂), 3.84 (s, 3H, OCH₃), 2.58 (s, 3H, ArCH₃), 2.28 – 2.15 (m, 2H, CH₂CH₂), 2.15 – 2.07 (m, 2H, CH₂CH₂), 2.04 (s, 3H, OAc), 1.72 (s, 3H, CH₃), 1.70 (s, 3H, CH₃). ¹³C NMR (400 MHz, CDCl₃) δ 171.2, 167.6, 161.8, 143.1, 141.7, 133.0, 131.1, 128.5, 121.8, 118.9, 117.9, 111.8, 73.9, 61.4, 51.6, 39.0, 26.0, 22.4, 21.2, 16.6, 14.0. LRMS (ESI⁺) m/z 361.1 (M+H)⁺. HRMS (ESI⁺) m/z 360.1943 (C₂₁H₂₈O₅ requires 360.1937).

Ethyl 4-(((2E,6E)-8-acetoxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-hydroxy-6-methylbenzoate (17e)
General procedure A using 12 (212 mg, 0.94 mmol), 2e (237 mg, 1.21 mmol), y Ph₃P (338 mg, 1.29 mmol) in anhydrous THF (10 mL), and a solution of DIAD (243 mg, 1.2 mmol) in anhydrous THF (5.3 mL). The reaction mixture was stirred 21 h at room temperature and the compound was purified by chromatography using hexane/EtOAc (100:0 → 25:1) to give 17e as colorless oil (195 mg, 52%). HPLC (UV) 100%. ¹H NMR (400 MHz, CDCl₃) δ 11.82 (s, 1H, OΗ), 6.32 (d, J = 2.6 Hz, 1H, ArΗ), 6.30 (dd, J = 1.0, 2.6 Hz, 1H, ArΗ), 5.52 – 5.37 (m, 1H, C=CΗ), 5.35 (tdd, J = 1.2, 2.7, 7.2 Hz, 1H, C=CH), 4.58 (d, J = 7.1 Hz, 2H, CH₂OAc), 4.39 (q, overlapping, J = 7.2 Hz, 4H, CH₃CH₂O), 4.37 (s, 2H, ArOCH₂), 2.50 (s, 3H, ArCH₃), 2.24 – 2.18 (m, 2H, CH₂CH₂), 2.12 – 2.05 (m, 2H, CH₂CH₂), 2.05 (s, 3H, OAc), 1.71 (s, 6H, 2×CH₃), 1.41 (t, J = 7.1 Hz, 3H, CH₂CH₃). ¹³C NMR (126 MHz, CDCl₃) δ 171.9, 171.3, 165.6, 163.3, 143.2, 141.8, 130.9, 128.6, 118.8, 111.9, 105.4, 99.7, 73.9, 61.5, 61.3, 39.0, 26.0, 24.6, 21.2, 16.6, 14.4, 14.0. LRMS (ESI⁺) m/z 413.2 (M+Na)⁺.

**General procedure E.** A Kimax tube was charged with a mixture of ester (0.05 mmol, 1 equiv.) and 1 M aqueous KOH (0.5 mL, 10 equiv.) in MeOH solution (0.5 mL). The reaction mixture was stopped with a screwcap and was stirred at 60 ºC until complete hydrolysis. The reaction was quenched with 1M aqueous HCl solution (0.6 mL) and partitioned between CH₂Cl₂ and water. The aqueous phase was further extracted with CH₂Cl₂ (1×) and the combined organic extracts were dried (Na₂SO₄) and evaporated to give the pure crude carboxylic acid.

2-hydroxy-4-(((2E,6E,10E)-12-hydroxy-2,6,10-trimethylldodeca-2,6,10-trien-1-yl)oxy)-6-methylbenzoic acid (16e)

General procedure using 15e (36 mg, 0.079 mmol) and ethanol as solvent. The reaction mixture was stirred for 26 h at 60 ºC and quenched with 1M aqueous HCl solution. Compound 16e was isolated as yellowish gummy solid by crystallization from EtOAc (18 mg, 50%). HPLC (UV): 95%. ¹H NMR (400 MHz, CDCl₃) δ 6.32 (s, 2H, ArΗ), 5.49 (t, J = 7.2, Hz, 1H, CH₂OH), 5.41 (t, J = 7.0 Hz, 1H, =CHCH₂), 5.10 (t, J = 6.7 Hz, 1H, =CHCH₂), 4.39 (s, 2H, ArOCH₂), 4.17 (d, J = 7.0 Hz, 2H, CH₂OH), 2.55 (s, 3H, ArCH₃), 2.2 – 1.9 (m, 8H, 2×CH₂CH₂), 1.70 (s, 3H, CH₃), 1.67 (s, 3H, CH₃), 1.59 (s, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ 175.5, 166.4, 164.2, 144.6, 140.2, 134.8, 130.2, 129.3, 124.5, 123.1, 112.2, 104.3, 99.8, 74.0, 59.6, 39.6, 39.1, 26.4, 26.3, 24.5, 16.4, 16.1, 14.0. HRMS (ESI⁺) m/z 388.2260 (C₂₃H₃₂O₅ requires 388.2250).
2-hydroxy-4-(((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)benzoic acid (18a)

General procedure E using 17a (19 mg, 0.052 mmol). The reaction mixture was stirred at 60 °C for 18 h and quenched with 1 M HCl. The precipitate was allowed to settle at the bottom of the tube overnight in the fridge. The tube was centrifuged at 7500 rpm for 5 min and the supernatant was discarded. The crude colorless solid was dissolved in CH₂Cl₂, washed with brine, and dried (Na₂SO₄). The solvent was evaporated to yield the pure acid as colorless amorphous solid (14.4 mg, 80%). HPLC (UV) > 95%.

1H NMR (500 MHz, CDCl₃/CD₃OD) δ 7.75 (d, J = 8.7 Hz, 1H), 6.44 (dd, J = 2.4, 8.7 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 5.50 (ddt, J = 1.4, 5.6, 8.5 Hz, 1H), 5.39 (tq, J = 1.4, 6.8 Hz, 1H), 4.39 (s, 2H), 4.14 (d, J = 6.9 Hz, 2H), 3.23 (br, 2H), 2.20 (q, J = 7.4 Hz, 2H), 2.07 (t, J = 7.6 Hz, 2H), 1.69 (d, J = 1.3 Hz, 3H), 1.66 (d, J = 1.3 Hz, 3H).

13C NMR (126 MHz, CDCl₃) δ 172.7, 165.3, 164.0, 139.1, 132.0, 130.7, 128.9, 123.8, 108.3, 105.3, 101.6, 74.2, 59.3, 38.9, 25.9, 16.3, 13.9. HRMS (ESI) m/z 306.1471 (C₁₇H₂₂O₅ requires 306.1467).

2-fluoro-4-(((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)benzoic acid (18b)

General procedure E using 17b (27 mg, 0.07 mmol). The reaction mixture was stirred at 60 °C for 5 h and quenched with 1 M HCl. The product was isolated as yellowish gummy solid after workup (19.5 mg, 86%). HPLC (UV) > 95%. 1H NMR (500 MHz, CDCl₃) δ 7.94 (t, J = 8.7 Hz, 1H), 6.74 (dd, J = 2.4, 8.9 Hz, 1H), 6.65 (dd, J = 2.4, 12.8 Hz, 1H), 5.53 (tq, J = 1.4, 7.1 Hz, 1H), 5.41 (tq, J = 1.4, 6.9 Hz, 1H), 4.42 (s, 2H), 4.17 (d, J = 6.9 Hz, 2H), 2.22 (q, J = 7.4 Hz, 2H), 2.09 (dd, J = 6.6, 8.7 Hz, 2H), 1.72 (d, J = 1.3 Hz, 3H), 1.68 (d, J = 1.3 Hz, 3H). 13C NMR (126 MHz, CDCl₃) δ 168.9 (d, J = 3.7 Hz), 164.8 (d, J = 11.7 Hz), 164.2 (d, J = 261.3 Hz), 139.2, 134.1 (d, J = 2.2 Hz), 130.3, 129.5, 123.8, 111.2 (d, J = 2.9 Hz), 109.9 (d, J = 9.1 Hz), 103.3 (d, J = 25.7 Hz), 74.7, 59.4, 38.9, 26.0, 16.4, 14.0. HRMS (ESI) m/z 308.1437 (C₁₇H₂₁FO₂ requires 308.1424).

4-(((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-methylbenzoic acid (18c)
General procedure E using 17e (18 mg, 0.05 mmol). The reaction mixture was stirred at 60 °C for 5 h and quenched with 1 M HCl. The product was isolated as greyish gummy solid after workup (15 mg, ~100%). HPLC (UV) > 95%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.06 – 8.02 (m, 1H), 6.78 (d, $J = 2.6$ Hz, 1H), 6.76 (dd, $J = 2.6$, 5.6 Hz, 1H), 5.53 (tq, $J = 1.4$, 7.1 Hz, 1H), 5.41 (tq, $J = 1.4$, 6.9 Hz, 1H), 4.44 – 4.41 (m, 2H), 4.16 (d, $J = 6.9$ Hz, 2H), 2.62 (s, 3H), 2.22 (q, $J = 7.4$ Hz, 2H), 2.09 (dd, $J = 6.4$, 8.8 Hz, 2H), 1.73 (d, $J = 1.3$ Hz, 3H), 1.68 (d, $J = 1.2$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 172.4, 162.5, 144.2, 139.3, 134.1, 130.9, 128.9, 123.8, 120.6, 118.1, 74.1, 59.5, 39.0, 26.1, 22.8, 16.4, 14.0. HRMS (ESI) $m/z$ 304.1675 (C$_{18}$H$_{24}$O$_4$ requires 304.1675).

2-hydroxy-4-(((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-6-methylbenzoic acid (18e)

General procedure E using 17e (16 mg, 0.046 mmol). The reaction mixture was stirred at 60 °C for 27 h and quenched with 1 M HCl. The precipitate was allowed to settle at the bottom of the tube overnight in the fridge. The tube was centrifuged at 7500 rpm for 5 min and the supernatant was discarded. The crude colorless solid was dissolved in CH$_2$Cl$_2$, washed with brine, and dried (Na$_2$SO$_4$). The solvent was evaporated to yield the pure acid as colorless amorphous solid (12.4 mg, 84%). HPLC (UV) > 95%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 6.31 (s, 2H), 5.57 – 5.45 (m, 1H), 5.40 (td, $J = 1.5$, 6.8 Hz, 1H), 4.38 (s, 2H), 4.14 (d, $J = 6.9$ Hz, 2H), 2.96 (br, 1H), 2.53 (s, 3H), 2.20 (q, $J = 7.4$ Hz, 2H), 2.08 (t, $J = 7.5$ Hz, 2H), 1.69 (s, 3H), 1.67 (s, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 174.5, 166.0, 163.7, 144.3, 139.1, 130.7, 128.8, 123.9, 112.1, 104.7, 99.5, 74.0, 59.3, 38.9, 25.9, 24.4, 16.3, 13.9. HRMS (ESI) $m/z$ 320.1626 (C$_{18}$H$_{24}$O$_5$ requires 320.1624).

Methyl 2-hydroxy-4-(((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)benzoate (19a)

A mixture of 17a (10.1 mg, 0.039 mmol) and K$_2$CO$_3$ (5.8 mg, 0.042 mmol) in MeOH (1.3 mL) was stirred at room temperature for 24 h. The solvent was removed under vacuum and the crude product was partitioned between EtOAc and water. The organic phase was washed with water, dried (MgSO$_4$), and evaporated to give 19a as yellowish
oil (12 mg, 98%). HPLC (UV) 100%. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.96 (s, 1H, ArOH), 7.72 (d, \(J = 9.5\) Hz, 1H, ArH), 6.46 – 6.43 (m, 2H, ArH), 5.53 – 5.50 (m, 1H, C=CH), 5.41 (td, 1H, \(J = 1.4, 7.0\) Hz, C=CH), 4.41 (s, 2H, ArOCH), 4.15 (d, \(J = 7.0\) Hz, 2H, CH\(_2\)OH), 3.91 (s, 3H, OCH\(_3\)), 2.22 – 2.19 (m, 2H, CH\(_2\)CH\(_2\)O), 1.68 (s, 3H, CH\(_3\)). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 170.6, 165.1, 163.7, 139.0, 131.3, 130.7, 129.0, 124.1, 108.4, 105.5, 101.7, 74.2, 59.5, 52.1, 39.0, 26.0, 16.4, 14.0. LRMS (ESI\(^+\)) \(m/z\) 321.25 (M+H). HRMS (ESI\(^+\)) \(m/z\) 320.1638 (C\(_{18}\)H\(_{24}\)O\(_5\) requires 320.1624).

Methyl 2-fluoro-4-((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)benzoate (19b)

A mixture of 17b (15 mg, 0.041 mmol) and K\(_2\)CO\(_3\) (6.4 mg, 0.045 mmol) in MeOH (2 mL) was stirred at room temperature for 2.5 h. The solvent was removed under vacuum and the crude product was partitioned between CH\(_2\)Cl\(_2\) and water. The organic phase was washed with brine, dried (Na\(_2\)SO\(_4\)), and evaporated to give a crude oil. Silica chromatography using hexane/EtOAc: 100/0 → 80/20 yielded 19b as colorless powder (11 mg, 83%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.88 (t, \(J = 8.7\) Hz, 1H), 6.72 (dd, \(J = 8.7, 2.5\) Hz, 1H), 6.64 (dd, \(J = 12.8, 2.5\) Hz, 1H), 5.52 (td, \(J = 6.3, 3.1\) Hz, 1H), 5.44 – 5.38 (m, 1H), 4.42 (s, 2H), 4.15 (d, \(J = 6.9\) Hz, 2H), 3.89 (s, 3H), 2.22 (q, \(J = 7.4\) Hz, 2H), 2.09 (t, \(J = 7.6\) Hz, 2H), 1.72 (s, 3H), 1.68 (s, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 164.9 (d, \(J = 4.1\) Hz), 164.0 (d, \(J = 11.4\) Hz), 163.5 (d, \(J = 260.0\) Hz), 139.1, 133.5 (d, \(J = 2.8\) Hz), 130.5, 129.4, 124.1, 111.1 (d, \(J = 3.0\) Hz), 110.9 (d, \(J = 10.0\) Hz), 103.2 (d, \(J = 26.0\) Hz), 74.6, 59.5, 52.1, 39.0, 26.1, 16.4, 14.0. HRMS (ESI\(^+\)) \(m/z\) 322.1587 (C\(_{18}\)H\(_{23}\)FO\(_4\) requires 322.1580).

Methyl 4-((2E,6E)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-methylbenzoate (19c)

A mixture of 17c (18 mg, 0.05 mmol) and K\(_2\)CO\(_3\) (7.6 mg, 0.055 mmol) in MeOH (2 mL) was stirred at room temperature for 2.5 h. The solvent was removed under vacuum and the crude product was partitioned between EtOAc and water. The organic phase was washed with brine, dried (Na\(_2\)SO\(_4\)), and evaporated to give a crude oil. Silica chromatography using hexane/EtOAc: 100/0 → 80/20 yielded 19c as colorless powder (10 mg, 63%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.91 (d, \(J = 8.2\) Hz, 1H), 6.77 – 6.71 (m, 2H), 5.58 – 5.48 (m, 1H), 5.45 – 5.35 (m, 1H), 4.41 (s, 2H), 4.15 (d, \(J = 6.9\) Hz, 2H), 3.85
(s, 3H), 2.59 (s, 3H), 2.22 (q, J = 7.3 Hz, 2H), 2.09 (t, J = 7.6 Hz, 2H), 1.73 (d, J = 1.0 Hz, 3H), 1.68 (d, J = 1.0 Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 167.7, 161.8, 143.1, 139.2, 133.0, 131.0, 128.8, 124.0, 121.8, 117.9, 111.8, 74.0, 59.5, 51.7, 39.0, 26.1, 22.4, 16.4, 14.0. HRMS (ESI\(^+\)) m/z 318.1825 (C\(_{19}\)H\(_{26}\)O\(_4\) requires 318.1831).

**Ethyl 2-hydroxy-4-(((2E,6E)-8-hydroxy-2,6-dimethyl-octa-2,6-dien-1-yl)oxy)-6-methylbenzoate (19e)**

![Chemical Structure](image)

A mixture of 17e (182 mg, 0.47 mmol) and K\(_2\)CO\(_3\) (64 mg, 0.47 mmol) in EtOH (10 mL) was stirred 40 h at 35 °C. The solvent was removed under vacuum and the crude product was partitioned between EtOAc and 0.1 M HCl. The organic phase was washed with water (2x), dried (MgSO\(_4\)), and evaporated to give 19e as yellowish oil (135 mg, 83%).

HPLC (UV) 100%. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 11.86 (s, 1H, OH), 6.32 – 6.30 (m, 2H, ArH), 5.52 – 5.49 (m, 1H, C=CH\(_2\)), 5.43 – 5.39 (m, 1H, C=CH\(_2\)), 4.42 – 4.36 (m, 4H, CH\(_3\)CH\(_2\)O, ArOCH\(_2\)), 4.14 (d, J = 6.9 Hz, 2H, CH\(_2\)OH), 2.50 (s, 3H, ArCH\(_3\)), 2.21 (q, J = 7.3 Hz, 2H, CH\(_2\)CH\(_2\)), 2.09 (t, J = 7.4 Hz, 2H, CH\(_2\)CH\(_2\)), 1.70 (d, J = 1.4 Hz, 3H, CH\(_3\)), 1.68 (d, J = 1.4 Hz, 3H, CH\(_3\)), 1.41 (t, J = 7.1 Hz, 3H, CH\(_2\)CH\(_3\)). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 171.9, 165.5, 163.3, 143.2, 139.0, 130.8, 128.8, 124.1, 112.0, 105.4, 99.7, 73.9, 61.3, 59.5, 39.0, 26.0, 24.5, 16.3, 14.4, 13.9. HRMS (ESI\(^+\)) m/z 348.1938 (C\(_{20}\)H\(_{28}\)O\(_5\) requires 348.1937).

**Ethyl 2-hydroxy-4-(((2E,6E)-8-acetoxy-2,6-dimethyl-octa-2,6-dien-1-yl)oxy)-6-methylbenzoate (20a)**

A mixture of 12 (371 mg, 1.35 mmol), K\(_2\)CO\(_3\) (183 mg, 1.32 mmol) and 2,4-dihydroxybenzoic acid (206 mg, 1.34 mmol) in anhydrous acetone (50 mL) was stirred at 60 °C for 23 h under argon atmosphere. The precipitate was filtered off and rinsed with acetone. The filtrate was evaporated under vacuum and the crude oil was purified by silica chromatography using hexane/EtOAc (100:0 → 10:1). Compound 20a eluted first (15:1) followed by 21a (10:1).

20a: Yellowish oil (116 mg, 32%). HPLC (UV) 95%. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 11.00 (s, 1H, OH), 7.72 (d, J = 8.6 Hz, 1H, ArH), 7.37 (br, 1H, OH), 6.41 (d, J = 2.3 Hz, 1H, ArH), 6.39 (dd, J = 2.3, 8.6 Hz, 1H, ArH), 5.52 – 5.49 (m, 1H, C=CH\(_2\)), 5.36 – 5.32 (m, 1H, C=CH\(_2\)), 4.67 (s, 2H, ArCOOCH\(_2\)), 4.59 (d, J = 7.2 Hz, 2H, CH\(_2\)OAc), 2.23 – 2.17 (m, 2H, CH\(_2\)CH\(_2\)), 2.15 – 2.07 (m, 2H, CH\(_2\)CH\(_2\)), 2.06 (s, 3H, OAc), 1.71 (s, 3H, CH\(_3\)), 1.70 (s, 3H, CH\(_3\)). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 172.2, 170.0, 165.5, 163.3, 143.2, 139.0, 130.8, 128.8, 124.1, 112.0, 105.4, 99.7, 73.9, 61.3, 59.5, 39.0, 26.0, 24.5, 16.3, 14.4, 13.9. HRMS (ESI\(^+\)) m/z 348.1938 (C\(_{20}\)H\(_{28}\)O\(_5\)S requires 348.1937).
(2E,6E)-8-acetoxy-2,6-dimethylocta-2,6-dien-1-yl 4-(((2E,6E)-8-acetoxy-2,6-dimethylocta-2,6-dien-1-yl)oxy)-2-hydroxybenzoate (21a)

Colorless oil (127 mg, 27%). HPLC (UV) 95%. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.96 (s, 1H, OH), 7.73 (d, $J$ = 9.5 Hz, 1H, ArH), 6.44 - 6.39 (m, 2H, ArH), 5.52 - 5.48 (m, 2H, C=C'H), 5.36 - 5.32 (m, 2H, C=C'H), 4.67 (d, $J$ = 3.6 Hz, 2H), 4.57 (d, $J$ = 7.1 Hz, 4H, 2xCH$_2$OAc), 4.38 (s, 2H), 2.26 - 2.07 (m, 4H, CH$_2$CH=), 2.03 (s, 6H, 2xOAc), 1.70 (m, 12H, 4xCH$_3$). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.2, 169.9, 165.0, 163.9, 141.61, 141.57, 131.2, 130.8, 130.3, 129.31, 129.30, 128.7, 118.88, 118.85, 108.2, 105.6, 101.7, 74.1, 70.5, 61.4, 38.95, 38.91, 26.02, 25.97, 21.1, 16.54, 16.52, 14.1, 13.9. LRMS (ESI$^+$) m/z 543.4 (M+H)$^+$. HRMS (ESI$^+$) m/z 542.2874 (C$_{31}$H$_{42}$O$_8$ requires 542.2880).
3) Experimental protocols for drug sensitivity assays against *T. b. brucei* and HEK cells

**Test organisms and culture media**

Three strains of bloodstream form (BSF) *Trypanosoma brucei* were used in this study to assess the trypanocidal activity of the new compounds: (i) A Wild type strain *T. b. brucei* Lister 427 (427-WT); (ii) A multi-drug resistant strain, B48, which was created from 427-WT after deletion of the *TbAT1* drug transporter followed by adaptation to increasing concentrations of pentamidine; (iii) A pentamidine-resistant aquaporin-null trypanosomes (*aqp1*-3 null), which was obtained by the knockout of all three aquaglyceroporin genes, hence were reported to display glycerol transport defects and respiratory-inhibitor sensitivity; the *aqp2* gene was specifically implicated in pentamidine-melarsoprol cross-resistance in field isolates of *T. b. gambiense*.

All *T. b. brucei* strains were used only as bloodstream trypomastigotes and cultured in standard HMI-9 medium, supplemented with 10% heat inactivated fetal bovine serum (FBS), 14 μL of β-mercaptoethanol, and 3.0 g of sodium hydrogen carbonate per liter of medium (pH 7.4). Parasites were cultured in vented flasks at 37 °C in a 5% CO₂ atmosphere and were passaged every third day.

**Drug sensitivity assays**

The drug susceptibilities of trypanosomes were determined using the resazurin-based assay as previously described. Briefly, the assays were performed in 96-well plates with 2 × 10⁴ cells/well. This involves adjusting cell density to the required concentration of 2 × 10⁵ cells/mL of which 100 μL was added to all of the wells in the plate having a 100 μl serially diluted test compound (200 μM top concentration) in HMI-9 medium + 10% Fetal Bovine Serum, then incubating trypanosomes and test compounds for a period of 48 hours at 37 °C and 5% CO₂ before adding 20 μl filter-sterilized resazurin solution, which was prepared by adding 25 mg resazurin sodium salt to 200 mL PBS. This was followed by a further 24 h of incubation. Well-known trypanocides including pentamidine isethionate and diminazene aceturate (both from Sigma-Aldrich) were used as positive control. Fluorescence from the 96-well plates was measured using a FLUOstar Optima (BMG Labtech, Durham, NC, USA) set at wavelengths of 544 nm for excitation, 590 for emission, and a gain of 1250. EC₅₀ values were calculated by non-linear regression using an equation for a sigmoidal dose-response curve with variable slope (GraphPad Prism 5.0, GraphPad Software Inc., San Diego, CA, USA).

**Cytotoxicity assay using human embryonic kidney (HEK) 293-T cells**

Toxicity of drugs to mammalian cells was carried out using Human Embryonic Kidney (HEK) Cells (strain 293T) according to a previously reported protocol. Briefly, HEK cells were grown in a culture containing 500 mL Dulbecco’s Modified Eagle’s Medium (DMEM) (Sigma), 50 mL New-born Calf Serum (NBCS) (Gibco), 5 mL Penicillin/Streptomycin (Gibco), and 5 mL L-Glutamine (200 mM, Gibco). The mammalian cells were incubated at 37 °C/5% CO₂ and were passaged when they reached
80 - 85% confluence in vented flasks. For the assay, cells were suspended at a density of $3 \times 10^5$ cells/mL, of which 100 µL was added to each well of a 96-well plate. The plate was incubated at 37 °C + 5% CO$_2$ for 24 hours to allow cell adhesion to the plate. Serial drug dilutions were prepared in a separate 96-well sterile plate and 100 µL was transferred to the corresponding wells containing the cells; phenylarsine oxide (PAO) was used as positive control. The plate was then incubated at 37 °C /5% CO$_2$ for an additional period of 30 h followed by the addition of 10 µL of resazurin solution (125 mg/L in PBS) and a final incubation at 37 °C /5% CO$_2$ for 24 hours. The plate was read in a FLUOstar OPTIMA fluorimeter at wavelengths 530 nm for excitation and 590 nm for emission. GraphPad Prism 5.0 was used for analyzing the generated data, which was plotted to a sigmoid curve with variable slope to determine EC$_{50}$ values. The selectivity index was calculated as EC$_{50}$ (HEK)/EC$_{50}$ (trypanosomes).

4) **Experimental protocols for Ubiquinol oxidase/TAO inhibitory assay**

Preparation of inner membrane-rich fraction, membrane solubilization, and purification of rTAO was performed exactly as described in our recent publication. The purified rTAO was used for the TAO inhibitory assay. In this method, the absorbance changes of ubiquinol-1 at 278 nm were recorded on a JASCO V-630 spectrophotometer (JASCO corporation, Tokyo, Japan) over 2 minutes. Reactions were initiated by the addition of 150 µM ubiquinol-1 ($\varepsilon_{278}=15,000 \text{ M}^{-1} \text{ cm}^{-1}$) after 2 min of pre-incubation at 25 °C in the presence of 150 ng rTAO in a total reaction volume of 500 µL 50 mM Tris–HCl buffer (pH 7.4). To determine the IC$_{50}$ values of test compounds, the reaction was initiated by the addition of a fixed concentration of ubiquinol-1 (150 µM), after 2 min pre-incubation (25 °C) in the presence of a fixed amounts of rTAO (150 ng) and varying concentrations of serially diluted test compounds, all in a 50 mM Tris–HCl (pH 7.4) buffer containing 0.05% (w/v) octaethylene glycol-monododecylether detergent. The data obtained were used to calculate the % inhibition of rTAO, which was plotted against the corresponding log of the test compound’s concentration (M) to generate a sigmoidal curve. EC$_{50}$ values were calculated by non-linear regression using an equation for a sigmoidal curve with variable slope (GraphPad Prism 6.0, GraphPad Software Inc., San Diego, CA, USA).
REFERENCES


