Supporting Information

Bridged Tetrahydroisoquinolines as selective NADPH oxidase 2 (Nox2) inhibitors

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EXPERIMENTAL METHODS

**General Chemistry Methods.** All moisture sensitive reactions were performed using syringe-septum techniques under an atmosphere of either dry N₂ or dry argon unless otherwise noted. All glassware used in moisture sensitive reactions was flame-dried or oven dried at 136 °C overnight and cooled in a desiccator prior to use. All chemicals were purchased from commercial suppliers and unless otherwise noted were used as received. All the microwave reactions were performed using a Biotage® Initiator microwave reactor. Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone under an argon atmosphere. Dry dichloromethane (CH₂Cl₂), dichloroethane (C₂H₄Cl₂), and ethyl acetate (EtOAc) were obtained by distillation over calcium hydride under an argon atmosphere. Deuterated chloroform (CDCl₃) was filtered through an alumina plug prior to use. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F₂₅₄ plates, 250 µm layer thickness) and visualized by using UV lamp (254 nm) and/or a potassium permanganate solution (3 g of KMnO₄ and 4 g of K₂CO₃ in 4 mL of 5% NaOH solution and 200 mL of H₂O). Flash column chromatography was performed with 40-63 µm silica gel (SiO₂, Silicycle). Infrared spectra were recorded on a Smiths IdentifyIR ATR spectrometer or a Perkin Elmer Spectrum 100 FT-IR spectrometer using the Universal ATR Sampling Accessory. All NMR data was collected at room temperature in CDCl₃ on a 300, 400 or 500 MHz Bruker instrument. Chemical shifts (δ) are reported in parts per million (ppm) with internal CHCl₃ (δ 7.26 ppm for ¹H and 77.23 ppm for ¹³C). ¹H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bm = broad multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, qd = quartet of doublets, sep = septet, app t = apparent triplet), integration, and coupling constant(s) (J) in Hertz (Hz). ¹³C NMR spectra were measured using a proton-decoupled pulse sequence. Final products were >95 % purity as analyzed by reverse-phase HPLC (ACCELA PDA detector, 1250 pump, Waters XTerra® MS 3.5 µm C-18 50 x 2 mm column, 0.5 mL/min, CH₃CN, H₂O and 0.1 % formic acid) with UV (210, 220 and 254 nm), ELSD (Agilent Technologies 385-ELSD, nebulizer 45 °C, evaporator 45 °C, N₂ flow 1.80 SLM), and MS detection using a Thermo Scientific Exactive Orbitrap LC-MS (HESI positive).
(±)-(1S,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)-naphthalene (7). To a stirred solution of 5-bromoisouquinoline (0.1 g, 0.5 mmol) in anhydrous THF (0.2 mL) was added methallylzinc bromide in THF (2 mL, 1.0 M) dropwise at 0 °C and the resulting solution was stirred for 10 min. The reaction mixture was warmed to room temperature, heated to 70 °C for 4.5 h, cooled to room temperature and further cooled to -10 °C. The reaction was quenched by dropwise addition of MeOH (0.1 mL). The reaction mixture was poured onto ice water (20 mL) and extracted with Et₂O (2 x 15 mL). The combined organic portion was dried (Na₂SO₄), concentrated, and the crude product was purified by chromatography on neutral Al₂O₃ (1:1, hexanes:EtOAc) followed by 7.93, CH₃OH:CH₂Cl₂) to give 7 (105 mg, 68%, 97% purity by ELSD) as a colorless oil: IR (neat) 3073, 2934, 1647, 1564, 1451, 1369, 1330, 1310, 1273, 1177, 1142, 1117, 1073, 965, 891, 822, 767, 742, 702, 688 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.43 (dd, J = 1.3, 7.8 Hz, 1 H), 7.10-7.00 (m, 2 H), 4.73 (s, 1 H), 4.55 (s, 1 H), 3.85 (t, J = 2.9 Hz, 1 H), 3.74 (ddd, J = 1.6, 5.2, 8.7 Hz, 1 H), 2.94 (s, 1 H), 1.86-1.79 (m, 2 H), 1.71 (s, 3 H), 1.34-1.18 (m, 5 H), 0.62 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 144.4, 142.2, 139.3, 129.9, 127.7, 123.7, 120.9, 113.1, 52.7, 49.7, 48.8, 42.5, 32.4, 30.9, 28.8, 22.4; HRMS (ESI) m/z calcd for C₂₇H₂₃BrN (M+H)+ 320.1008, found 320.1001.

(±)-(1S,4R,9S)-3,3-Dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano) - naphthalene (8). Isoquinoline (0.1 g, 0.8 mmol) was charged with a solution of methallylzinc bromide (2.50 mL, 1.12 M in THF, 2.47 mmol) at 0 °C and stirred for 10 min. After warming the reaction mixture to room temperature, it was heated to 70 °C for 1 h, then cooled to room temperature and further cooled to -10 °C. The reaction was quenched by dropwise addition of MeOH (0.2 mL). To this solution was added 6 N NaOH (20 mL) and the organic layer was separated, dried (Na₂SO₄), and purified by chromatography on neutral Al₂O₃ (1:1, hexanes:EtOAc...
followed by 7:93, CH$_2$OH:CH$_2$Cl$_2$) to give 8 (50 mg, 27%) as a colorless oil. The $^1$H NMR matched that of the previously reported compound.$^1$

**General Method A: Reductive amination.**

(±)-(15S,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-(quinolin-4-ylmethyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11b). To a stirred solution of 7 (0.04 g, 0.1 mmol), 4-quinoline carboxaldehyde (22 mg, 0.14 mmol) and acetic acid (7.0 mL, 0.12 mmol) in dichloroethane (1.2 mL) was added sodium triacetoxyborohydride (53 mg, 0.25 mmol). The reaction mixture was stirred for 48 h under argon, diluted with CH$_2$Cl$_2$ (10 mL), and treated with a saturated solution of NaHCO$_3$ (10 mL). The organic layer was washed with H$_2$O (10 mL) and the combined organic layer was dried (Na$_2$SO$_4$) and concentrated. The crude product was purified by chromatography on SiO$_2$ (95:5, hexanes:EtOAc) to give 11b (0.04 g, 69%, 99% purity by ELSD) as a colorless oil: IR (neat) 3068, 2958, 1590, 1567, 1506, 1452, 1357, 1189, 908, 760, 731 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.85 (d, J = 4.3 Hz, 1 H), 8.21 (dd, J = 1.1, 8.5 Hz, 1 H), 8.12 (dd, J = 0.9, 8.5 Hz, 1 H), 7.69 (ddd, J = 1.4, 6.9, 8.3 Hz, 1 H), 7.52-7.48 (m, 2 H), 7.43 (dd, J = 0.9, 8.1 Hz, 1 H), 7.04 (app t, J = 7.6 Hz, 1 H), 6.88 (d, J = 7.2 Hz, 1 H), 4.78 (d, J = 4.8 Hz, 1 H), 4.46 (d, J = 14.9 Hz, 1 H), 4.23 (d, J = 15.0 Hz, 1 H), 3.54 (t, J = 2.5 Hz, 1 H), 3.38 (ddd, J = 1.7, 4.4, 9.0 Hz, 1 H), 3.05 (d, J = 1.5 Hz, 1 H), 2.12 (dd, J = 2.6, 13.7 Hz, 1 H), 1.79 (dd, J = 4.3, 14.3 Hz, 1 H), 1.82-1.67 (m, 1 H), 1.73 (s, 3 H), 1.41 (s, 3 H), 1.00 (dd, J = 2.5, 13.6 Hz, 1 H), 0.6 (s, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.6, 148.4, 145.9, 144.1, 142.5, 139.9, 130.3, 130.2, 129.3, 127.7, 127.6, 126.5, 124.0, 123.3, 121.3, 121.0, 113.2, 57.5, 55.8, 53.3, 49.4, 44.4, 35.2, 33.0, 31.0, 28.5, 22.9; HRMS (ESI) m/z calcd for C$_{27}$H$_{30}$O$_2$BrN$_2$ (M+H)$^+$ 461.1587, found 461.1581.

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(±)-(1S,4R,9S)-10-((1H-Indol-3-yl)methyl)-5-bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11c). According to General Method A, 7 (0.04 g, 0.1 mmol) was converted to pale yellow oily 11c (15 mg, 27%, 98% purity by ELSD): IR (neat) 3411, 2926, 1727, 1567, 1455, 1353, 1264, 1189, 1091, 1044, 1010, 930, 807, 772, 734 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.04 (s, 1 H), 7.71 (d, J = 8.0 Hz, 1 H), 7.37 (dd, J = 8.2, 12.8 Hz, 2 H), 7.21–7.15 (m, 2 H), 7.07 (t, J = 7.5 Hz, 1 H), 6.99 (t, J = 7.7 Hz, 1 H), 6.83 (d, J = 7.0 Hz, 1 H), 4.81 (d, J = 8.1 Hz, 2 H), 4.09 (s, 2 H), 3.75 (t, J = 2.7 Hz, 1 H), 3.26 (dd, J = 3.5, 9.9 Hz, 1 H), 3.04 (s, 1 H), 2.15 (dd, J = 2.4, 13.6 Hz, 1 H), 1.89 (dd, J = 3.5, 14.4 Hz, 1 H), 1.75-1.65 (m, 1 H), 1.69 (s, 3 H), 1.37 (s, 3 H), 0.92 (dd, J = 2.3, 13.6 Hz, 1 H), 0.57 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 144.8, 143.1, 140.3, 136.6, 130.0, 127.8, 127.5, 123.4, 123.2, 122.3, 121.6, 120.2, 119.6, 114.6, 113.0, 111.1, 57.1, 55.1, 49.3, 47.5, 44.5, 34.7, 33.0, 31.1, 29.9, 28.4, 22.9; HRMS (ESI) m/z calcd for C₂₅H₂₅BrN₂ (M+H)⁺ 449.1587, found 449.1575.

(±)-(1S,4R,9S)-10-(Benzo[d][1,3]dioxol-5-ylmethyl)-5-bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11d). According to General Method A, 7 (0.04 g, 0.1 mmol) was converted to colorless oily 11d (40.1 mg, 70%, 99% purity by ELSD): IR (neat) 2958, 2867, 1500, 1485, 1439, 1361, 1238, 1187, 1038, 928, 908, 771, 732 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.40 (dd, J = 7.4, 7.1 Hz, 1 H), 6.79 (dd, J = 1.5, 7.9 Hz, 1 H), 6.73 (d, J = 7.0 Hz, 1 H), 5.93 (dd, J = 1.4, 5.1 Hz, 2 H), 4.80 (s, 2 H), 3.78 (AB q, J = 13.4 Hz, 2 H), 3.57 (t, J = 2.5 Hz, 1 H), 3.19 (ddd, J = 1.7, 4.3, 9.2 Hz, 1 H), 2.99 (d, J = 1.7 Hz, 1 H), 2.05 (dd, J = 2.6, 13.5 Hz, 1 H), 1.78 (dd, J = 4.3, 14.2 Hz, 1 H), 1.71 (s, 3 H), 1.67-1.61 (m, 1 H), 1.33 (s, 3 H), 0.93 (dd, J = 2.5, 13.6 Hz, 1 H), 0.57 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 147.7, 146.5, 144.4, 142.7, 140.0, 134.1, 129.9, 127.5, 123.1, 121.6, 121.2, 112.8, 109.2, 107.8, 100.9, 56.5, 55.9, 54.8, 49.2, 44.4, 34.6, 32.8, 30.9, 28.2, 22.7; HRMS (ESI) m/z calcd for C₂₅H₂₅OBrN (M+H)⁺ 454.1376, found 454.1370.
General Method B: Reductive amination.

(±)-(15,4R,9S)-10-(3-Fluorobenzyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11a). To a stirred solution of 8 (16 mg, 0.070 mmol), 3-fluorobenzaldehyde (8.0 mL, 0.077 mmol) and acetic acid (4 mL, 0.07 mmol) in dichloroethane (0.7 mL) was added sodium triacetoxyborohydride (29 mg, 0.14 mmol). The reaction mixture was stirred overnight under argon and then diluted with CH₂Cl₂ (10 mL). The solution was treated with saturated solution of NaHCO₃ (10 mL), and the organic layer was washed with H₂O (10 mL) and concentrated under reduced pressure. The crude product was dissolved in THF-H₂O (10:1, 1 mL) and treated with NaBH₄ (10 mg). The solution was stirred for 3 h at room temperature, quenched with AcOH (1 drop) and diluted with EtOAc (10 mL). The solution was neutralized with a saturated solution of NaHCO₃ (5 mL) and washed with H₂O (5 mL). The combined organic layers were dried (Na₂SO₄), and the crude product was purified by chromatography on SiO₂ (9:1, hexanes:EtOAc) to give 11a (15 mg, 63%, 99 % purity by ELSD) as a colorless oil: IR (neat) 3073, 2927, 1647, 1615, 1485, 1446, 1359, 1253, 1127, 1069, 928, 890, 783, 754, 714, 684 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.30-7.20 (m, 3 H), 7.19-7.11 (m, 3 H), 7.04-7.01 (m, 1 H), 6.95 (dt, J = 2.6, 8.4 Hz, 1 H), 4.78 (s, 1 H), 4.66 (s, 1 H), 3.92 (AB q, J = 13.8 Hz, 2 H), 3.60 (t, J = 2.5 Hz, 1 H), 3.23 (ddd, J = 1.7, 4.3, 10.0 Hz, 1 H), 2.51 (br s, 1 H), 2.13 (dd, J = 2.6, 13.3 Hz, 1 H), 1.79 (dd, J = 4.2, 13.7 Hz, 1 H), 1.69 (s, 3 H), 1.63-1.57 (m, 2 H), 1.34 (s, 3 H), 1.01 (dd, J = 2.3, 13.3 Hz, 1 H), 0.57 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 164.2, 161.8, 143.5, 143.4, 143.2, 142.0, 139.7, 129.6, 129.5, 127.2, 126.1, 126.0, 124.1, 121.9, 115.5, 115.3, 113.8, 113.6, 112.4, 56.1, 56.0, 55.1, 49.6, 44.3, 35.1, 32.0, 31.9, 28.4, 22.4; HRMS (ESI) m/z calcd for C₂₄H₂₃FN (M)⁺ 350.2279, found 350.2266.
(±)-3-(15,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalen-10-yl)methyl)-5-methylisoxazole (11e). According to General Method B, 7 (25 mg, 0.12 mmol) was converted to pale yellow oily 11e (15 mg, 27%, 98% purity by ELSD): IR (neat) 3073, 2928, 1647, 1606, 1568, 1453, 1358, 1258, 1190, 1139, 1107, 1002, 957, 888, 797, 773, 716, 689 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.40 (d, J = 7.9 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 6.94 (d, J = 7.0 Hz, 1 H), 5.96 (s, 1 H), 4.79 (s, 2 H), 3.97 (d, J = 13.8 Hz, 1 H), 3.74 (d, J = 13.8 Hz, 1 H), 3.58 (t, J = 2.4 Hz, 1 H), 3.23-3.19 (m, 1 H), 2.97 (s, 1 H), 2.38 (s, 3 H), 2.10 (dd, J = 2.6, 13.8 Hz, 1 H), 1.77-1.62 (m, 2 H), 1.72 (s, 3 H), 1.31 (s, 3 H), 0.95 (dd, J = 2.2, 13.8 Hz, 1 H), 0.56 (s, 3 H); ¹³C NMR (125 MHz, CDCl₃) δ 169.5, 163.5, 144.0, 142.6, 139.9, 130.3, 127.7, 123.3, 121.5, 113.2, 101.9, 56.9, 55.9, 49.4, 47.4, 44.7, 34.7, 32.9, 31.0, 28.3, 22.8, 12.5; HRMS (ESI) m/z calcd for C₂₂H₂₈BrON₂ (M+H)⁺ 415.1380, found. 415.1373.

(±)-(15,4R,9S)-5-Bromo-10-(3-fluorobenzyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11f). According to General Method B, 7 (25 mg, 0.080 mmol) was converted to colorless oily 11f (13.2 mg, 39%, 98% purity by ELSD): IR (neat) 3074, 2928, 1648, 1614, 1590, 1568, 1485, 1449, 1358, 1253, 1189, 1139, 1123, 1070, 958, 924, 890, 772, 749, 713, 684 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.44 (dd, J = 1.0, 8.0 Hz, 1 H), 7.31-7.24 (m, 1 H), 7.15-7.04 (m, 3 H), 6.99-6.92 (m, 2 H), 4.82 (s, 2 H), 3.89 (s, 2 H), 3.56 (t, J = 2.5 Hz, 1 H), 3.24 (ddd, J = 1.7, 4.4, 8.9 Hz, 1 H), 3.02 (d, J = 1.6 Hz, 1 H), 2.10 (dd, J = 2.6, 13.5 Hz, 1 H), 1.80 (dd, J = 4.4, 14.1 Hz, 1 H), 1.74-1.65 (m, 1 H), 1.74 (s, 3 H), 1.37 (s, 3 H), 0.97 (dd, J = 2.4, 13.6 Hz, 1 H), 0.60 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 164.3, 161.8, 144.2, 143.0, 142.5, 139.9, 130.0, 129.7, 129.6, 127.5, 124.0, 123.1, 121.2, 115.4, 115.2, 113.9, 113.7, 112.9, 56.6, 55.6, 54.9, 49.2, 44.4, 34.6, 32.8, 30.8, 28.2, 22.7; HRMS (ESI) m/z calcd for C₂₄H₂₈BrFN (M+H)⁺ 428.1384, found 428.1371.
(±)-(15,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-pentyl-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11g). According to General Method B, 7 (0.04 g, 0.1 mmol) was converted to colorless oily 11g (35 mg, 72%, 99% purity by ELSD): IR (neat) 2928, 2860, 1648, 1568, 1453, 1360, 1275, 1189, 1138, 1102, 1060, 997, 958, 932, 891, 822, 768, 689 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.37 (dd, J = 2.8, 6.3 Hz, 1 H), 7.04-6.99 (m, 2 H), 4.79 (s, 2 H), 3.73 (t, J = 2.6 Hz, 1 H), 3.03-2.98 (m, 1 H), 2.92 (s, 1 H), 2.70-2.60 (m, 2 H), 2.01 (dd, J = 2.6, 13.6 Hz, 1 H), 1.86-1.82 (m, 1 H), 1.72 (s, 3 H), 1.61-1.43 (m, 3 H), 1.34-1.22 (m, 7 H), 0.93-0.85 (m, 1 H), 0.88 (t, J = 7.0 Hz, 3 H), 0.52 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 144.3, 142.9, 140.3, 130.3, 127.7, 123.3, 121.7, 112.8, 57.6, 56.4, 53.5, 48.8, 44.7, 34.7, 32.9, 31.1, 30.0, 29.3, 28.2, 23.0, 22.9, 14.3; HRMS (ESI) m/z calcd for C₃₂H₂₃BrN (M+H)⁺ 390.1791, found 390.1783.

(±)-(15,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-(thiophen-2-ylmethyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11h). According to General Method B, 7 (0.03 g, 0.09 mmol) was converted to colorless oily 11h (26.3 mg, 67%, 99% purity by ELSD): IR (neat) 3074, 2927, 1647, 1567, 1453, 1360, 1310, 1276, 1189, 1138, 1106, 957, 892, 823, 772, 753, 696 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.41 (dd, J = 1.0, 8.0 Hz, 1 H), 7.21 (dd, J = 1.5, 4.7 Hz, 1 H), 7.04 (dd, J = 7.3, 8.0 Hz, 1 H), 6.97-6.93 (m, 3 H), 4.81 (d, J = 5.2 Hz, 2 H), 4.15 (d, J = 14.0 Hz, 1 H), 3.97 (d, J = 14.0 Hz, 1 H), 3.71 (t, J = 2.6 Hz, 1 H), 3.22 (ddd, J = 1.8, 4.5, 8.7 Hz, 1 H), 2.99 (d, J = 1.7 Hz, 1 H), 2.05 (dd, J = 2.6, 13.6 Hz, 1 H), 1.81 (dd, J = 4.6, 14.0 Hz, 1 H), 1.73 (s, 3 H), 1.68 (dd, J = 8.7, 14.1 Hz, 1 H), 1.33 (s, 3 H), 0.95 (dd, J = 2.5, 13.7 Hz, 1 H), 0.58 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 145.0, 144.3, 142.7, 140.1, 130.2, 127.7, 126.5, 125.0, 123.2, 121.5, 113.1, 56.8, 55.0, 50.9, 49.5, 44.6, 34.8, 32.9, 31.0, 28.4, 23.0; HRMS (ESI) m/z calcd for C₃₂H₂₈BrNS (M)⁺ 416.1042, found 416.1036.
concentrated. The crude product was purified by chromatography on SiO₂.

The organic layer was washed with H₂O (2.2 mL) and Na₂CO₃ (27 mg, 0.25 mmol) in dioxane (0.7 mL) and Pd(PPh₃)₂Cl₂ (11 mg, 0.015 mmol) in dioxane (10 mL) was heated to 120 °C for 1 h in the microwave. After addition of EtOAc (20 mL) the mixture was washed with H₂O (10 mL) and brine (10 mL). The combined organic layers were dried (Na₂SO₄) and the crude residue was purified by chromatography on Al₂O₃ (1:1 to 1:2, hexanes:EtOAc) to give 12a (45 mg, 74%, 95% purity by ELSD) as a yellow oil:

IR (neat) 2929, 1647, 1548, 1458, 1372, 1274, 1195, 1133, 1078, 1031, 888, 772, 694 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.72-7.65 (m, 1 H), 7.52-7.47 (m, 1 H), 7.43 (s, 1 H), 7.31-7.26 (m, 1 H), 7.19-7.15 (m, 2 H), 4.71 (s, 1 H), 4.45 (s, 1 H), 3.97 (s, 1 H), 3.72 (dd, J = 4.4, 9.5 Hz, 1 H), 2.57 (s, 1 H), 1.88 (dd, J = 2.8, 12.9 Hz, 1 H), 1.80 (dd, J = 4.2, 13.6 Hz, 1 H), 1.71 (s, 3 H), 1.32-1.26 (m, 5 H), 0.61 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 142.5, 142.3, 141.0, 138.5, 136.9, 132.2, 131.5, 131.3, 130.1, 129.1, 127.4, 126.2, 121.8, 112.9, 52.8, 48.5, 47.2, 45.3, 42.6, 32.6, 32.0, 29.0, 22.3; HRMS (ESI) m/z calcd for C₂₃H₂₆NCl₂ (M+H)⁺ 346.1437, found 346.1428.

(±)-(15,4R,9S)-10-(Benzo[d][1,3]dioxol-5-ylmethyl)-5-(3,4-dichlorophenyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (12b). A solution of 11d (0.04 g, 0.09 mmol), 3,4-dichlorophenylboronic acid (33.5 mg, 0.170 mmol), Pd(PPh₃)₂Cl₂ (6 mg, 0.009 mmol) and Na₂CO₃ (27 mg, 0.25 mmol) in degassed dioxane (0.5 mL) and H₂O (0.15 mL) was heated to 120 °C for 1 h in the microwave and diluted with EtOAc (5 mL). The organic layer was washed with H₂O (10 mL), brine (10 mL), dried (Na₂SO₄), and concentrated. The crude product was purified by chromatography on SiO₂ (95:5, hexanes:EtOAc)
to give 12b (35.1 mg, 77%, 99% purity by ELSD) as a colorless oil: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.48 (d, $J$ = 8.3 Hz, 1 H), 7.42 (d, $J$ = 2.0 Hz, 1 H), 7.25 (t, $J$ = 7.3 Hz, 1 H), 7.18 (dd, $J$ = 2.0, 8.2 Hz, 1 H), 7.15 (dd, $J$ = 1.3, 7.8 Hz, 1 H), 7.04 (dd, $J$ = 1.1, 7.2 Hz, 1 H), 6.93 (d, $J$ = 1.4 Hz, 1 H), 6.84-6.77 (m, 2 H), 5.98, 5.97 (AB q, $J$ = 1.5 Hz, 2 H), 4.69 (s, 1 H), 4.44 (s, 1 H), 3.81, 3.80 (AB q, $J$ = 13.4 Hz, 2 H), 3.66 (t, $J$ = 2.6 Hz, 1 H), 3.24 (ddd, $J$ = 1.7, 4.5, 8.2 Hz, 1 H), 2.71 (d, $J$ = 1.4 Hz, 1 H), 2.14 (dd, $J$ = 2.5, 13.5 Hz, 1 H), 1.85 (dd, $J$ = 4.4, 15.4 Hz, 1 H), 1.71-1.64 (m, 1 H), 1.69 (s, 3 H), 1.31 (s, 3 H), 0.99 (dd, $J$ = 2.5, 13.6 Hz, 1 H), 0.56 (s, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 147.8, 146.6, 143.2, 143.0, 141.2, 138.5, 137.7, 134.3, 132.2, 131.5, 131.2, 130.0, 129.2, 127.6, 126.2, 122.4, 121.7, 111.6, 109.3, 108.0, 101.0, 56.3, 56.0, 54.6, 46.7, 44.3, 34.7, 33.2, 32.0, 28.6, 23.5; HRMS (ESI) m/z calcd for C$_{31}$H$_{32}$O$_2$NCl$_2$ (M+H)$^+$ 520.1805, found 520.1788.

![Chemical Structure](image-url)

($\pm$)(15,4R,9S)-5-(3,4-Dichlorophenyl)-3,3-dimethyl-9-(2-methylallyl)-10-pentyl-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (12c). A solution of 11g (34 mg, 0.090 mmol), 3,4-dichlorophenylboronic acid (33.2 mg, 0.170 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (6 mg, 0.009 mmol), and anhydrous sodium carbonate (26.7 mg, 0.250 mmol) in degassed dioxane (0.5 mL) and H$_2$O (0.15 mL) was heated to 120 °C for 1 h in the microwave and diluted with EtOAc (5 mL). The organic layer was washed with H$_2$O (10 mL), brine (10 mL), dried (Na$_2$SO$_4$), and concentrated. The crude product was purified by chromatography on SiO$_2$ (95:5, hexanes:EtOAc) to give 12c (21.2 mg, 53%, 99% purity by ELSD) as a colorless oil: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.46 (d, $J$ = 8.3 Hz, 1 H), 7.39 (d, $J$ = 2.0 Hz, 1 H), 7.29-7.25 (m, 1 H), 7.22-7.13 (m, 3 H), 4.68 (s, 1 H), 4.35 (s, 1 H), 3.87 (t, $J$ = 2.5 Hz, 1 H), 3.09-3.05 (m, 1 H), 2.80-2.62 (m, 3 H), 2.10 (dd, $J$ = 2.6, 13.5 Hz, 1 H), 1.90 (d, $J$ = 15.0 Hz, 1 H), 1.70-1.50 (m, 3 H), 1.68 (s, 3 H), 1.40-1.25 (m, 4 H), 1.26 (s, 3 H), 1.01 (dd, $J$ = 2.6, 13.5 Hz, 1 H), 0.93 (t, $J$ = 6.9 Hz, 1 H), 0.54 (s, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 143.2, 142.8, 141.2, 138.6, 137.7, 132.1, 131.5, 131.1, 130.0, 129.2, 127.7, 126.3, 122.7, 111.0, 57.2, 56.1, 53.2, 46.1, 43.9, 34.8, 33.2, 32.1, 30.0, 29.2, 28.3, 23.7, 22.8, 14.3; HRMS (ESI) m/z calcd for C$_{29}$H$_{38}$NCl$_2$ (M+H)$^+$ 456.2219, found 456.2205.
(±)-(15,4R,9S)-3,3-Dimethyl-9-(2-methylallyl)-5-(pyridin-3-yl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (12d). A solution of 7 (81 mg, 0.25 mmol), pyridine-3-boronic acid (62 mg, 0.50 mmol), and Pd(PPh₃)₂Cl₂ (18 mg, 0.025 mmol) in degassed dioxane (1.1 mL) was treated with a 2 M aqueous solution of Na₂CO₃ (0.4 mL) and heated to 120 °C for 1 h in a microwave. The reaction mixture was diluted with EtOAc (10 mL) and washed with H₂O (10 mL) and brine (10 mL). The combined organic layers were dried (Na₂SO₄), and the crude residue was purified by chromatography on SiO₂ (5:95, MeOH:CH₂Cl₂ with 0.1% Et₃N) to give 12d (19.5 mg, 24%, 97% purity by LC-MS) as a wax: IR (neat) 2928, 1463, 1437,1405, 1191, 1176, 773, 747, 715 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.63 (d, J = 1.2 Hz, 2 H), 7.68-7.65 (m, 1 H), 7.37 (dd, J = 7.2, 5.5 Hz, 1 H), 7.34-7.30 (m, 1 H), 7.28 (d, J = 1.3 Hz, 1 H), 7.22-7.17 (m, 2 H), 4.67 (s, 1 H), 4.40 (s, 1 H), 3.98 (s, 1 H), 3.72 (dd, J = 9.7, 4.3 Hz, 1 H), 2.57 (s, 1 H), 1.91-1.86 (m, 1 H), 1.79 (dd, J = 13.7, 4.2 Hz, 1 H), 1.67 (s, 3 H), 1.35-1.25 (m, 2 H), 1.27 (s, 3 H), 0.63 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 150.3, 148.4, 142.5, 142.3, 137.3, 137.2, 137.0, 136.6, 127.8, 126.3, 123.2, 122.0, 113.0, 52.8, 48.6, 47.2, 45.3, 42.6, 32.6, 32.0, 29.0, 23.0; HRMS (ESI) m/z calcd for C₂₂H₂₇N₂ (M+H)+ 319.2169, found 319.2161.

(±)-(15,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)-naphthalene (13). A solution of 7 (37 mg, 0.11 mmol) in dry EtOAc (0.4 mL) was treated with 5% rhodium on carbon (37 mg) and stirred under a hydrogen atmosphere for 24 h. The catalyst was filtered through Celite and the solvent was removed in vacuo. The crude product was purified by chromatography on SiO₂ (100% EtOAc) to give 13 (34 mg, 91%, 99% purity by ELSD) as a yellow oil: IR (neat) 2954, 1564, 1452, 1385, 1366, 1326, 1180, 1116, 1066, 968, 758, 707, 687 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.43 (dd, J = 1.7, 7.3 Hz, 1 H), 7.09-7.01 (m, 2 H), 3.89 (br t, J = 2.9 Hz, 1 H), 3.62 (t, J = 7.0 Hz, 1 H), 2.95 (s, 1 H), 1.82 (dd, J = 2.7, 13.2 Hz, 1 H), 1.77-1.65 (m, 1 H), 1.30 (s, 3 H), 1.23 (dd, J = 3.0, 13.3 Hz, 1 H), 0.79 (d, J = 6.6 Hz, 3 H), 0.78 (d, J = 6.6 Hz, 3 H), 0.77 (dd, J = 6.8, 13.7 Hz, 1 H), 0.70-0.50 (m, 1 H), 0.58 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 150.3, 148.4, 142.5, 142.3, 137.3, 137.2, 137.0, 136.6, 127.8, 126.3, 123.2, 122.0, 113.0, 52.8, 48.6, 47.2, 45.3, 42.6, 32.6, 32.0, 29.0, 23.0; HRMS (ESI) m/z calcd for C₂₂H₂₇BrN₂ (M+Br)+ 371.1610, found 371.1613.
(±)-(15,4R,9S)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-(methylsulfonyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (14a). A solution of 76 (25 mg, 0.078 mmol) in dry CH₂Cl₂ (0.8 mL) was treated at 0 °C with triethylamine (22 µL, 0.16 mmol) and methanesulfonyl chloride (7 µL, 0.08 mmol), stirred for 24 h at room temperature, diluted with CH₂Cl₂ (3 mL), washed with H₂O (2 x 5 mL), dried (Na₂SO₄), and concentrated. The crude product was purified by chromatography on SiO₂ (10-20% EtOAc:hexanes) to give 14a (28.5 mg, 92%, 99% purity by ELSD) as a colorless wax: IR (neat) 3075, 2959, 1650, 1568, 1454, 1319, 1189, 1146, 1121, 1073, 1053, 963, 949, 927, 909, 891, 800, 769, 687, 662 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.53 (t, J = 4.7 Hz, 1 H), 7.16 (d, J = 4.4 Hz, 1 H), 4.89 (s, 1 H), 4.79 (s, 1 H), 4.74 (s, 1 H), 4.53 (d, J = 10.4 Hz, 1 H), 3.25 (s, 1 H), 2.92 (s, 3 H), 2.26 (d, J = 13.8 Hz, 1 H), 2.15 (dd, J = 3.1, 13.4 Hz, 1 H), 1.79 (s, 3 H), 1.51 (dd, J = 10.7, 13.7 Hz, 1 H), 1.38 (s, 3 H), 1.19 (d, J = 13.7 Hz, 1 H), 0.71 (s, 3 H); ¹³C NMR (125 MHz, CDCl₃) δ 141.0, 140.7, 139.6, 131.6, 128.4, 123.9, 121.8, 114.3, 54.6, 53.2, 49.3, 43.3, 42.7, 42.3, 31.6, 31.1, 28.3, 22.4; HRMS (ESI) m/z calcd for C₁₀H₇NO₃SBr (M+H)+ 285.9532, found 285.9526.

(±)-(15,4R,9S)-5-Bromo-10-((4-fluorophenyl)sulfonyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (14b). A solution of 7 (25 mg, 0.078 mmol) in dry CH₂Cl₂ (0.8 mL) was treated at 0 °C with triethylamine (22 µL, 0.16 mmol) and 4-fluorobenzenesulfonyl chloride (16 mg, 0.085 mmol), stirred overnight under argon at room temperature, diluted with CH₂Cl₂ (5 mL), washed with H₂O (10 mL), brine (10 mL), dried (Na₂SO₄), and concentrated. The crude product was purified by chromatography on SiO₂ (4:1,
hexanes:EtOAc to give 14b (25 mg, 67%, 99% purity by ELSD) as a colorless wax: IR (neat) 3075, 2962, 1648, 1592, 1493, 1455, 1349, 1234, 1153, 1090, 946, 908, 839, 769, 732, 682 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.89-7.85 (m, 2 H), 7.51 (dd, \(J = 1.2, 8.0\) Hz, 1 H), 7.21-7.16 (m, 2 H), 7.13 (t, \(J = 7.5\) Hz, 1 H), 7.09 (d, \(J = 7.3\) Hz, 1 H), 4.88 (br s, 1 H), 4.83 (app t, \(J = 2.5\) Hz, 1 H), 4.79 (s, 1 H), 4.37 (dt, \(J = 2.6, 11.0\) Hz, 1 H), 3.23 (d, \(J = 2.3\) Hz, 1 H), 2.33 (d, \(J = 14.2\) Hz, 1 H), 1.95 (dd, \(J = 3.3, 13.4\) Hz, 1 H), 1.78 (s, 3 H), 1.46 (dd, \(J = 11.1, 14.2\) Hz, 1 H), 1.11-1.06 (m, 1 H), 1.09 (s, 3 H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 166.2, 164.2, 141.2, 140.7, 139.5, 137.8, 131.7, 131.7, 130.0, 129.9, 128.4, 123.4, 121.9, 116.5, 116.4, 113.9, 54.9, 53.4, 49.2, 42.3, 41.4, 31.6, 31.1, 27.6, 22.6; HRMS (ESI) \(m/\ell\) calcd for C\(_{15}\)H\(_{10}\)NO\(_2\)SFB\(_r\) (M-C\(_8\)H\(_{15}\))\(^+\) 365.9594, found 365.9588.

**General Biology Methods**

**Cell lines**

All cell culture reagents were obtained from Invitrogen, unless indicated. COS-22 (COS-7 cells stably expressing human p22\(^{phox}\)) and COS-Nox2 (a.k.a. COS-phox) cells (COS-7 cells stably expressing human p22\(^{phox}\)) were kindly provided by Dr. Mary C. Dinauer (Indiana University, School of Medicine).\(^2\) COS-22 cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) with 4.5 g/L glucose, L-glutamine and sodium pyruvate containing 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin (complete media) supplemented with 1.8 mg/ml G418 (Calbiochem/EMB Bioscience, Gibbstown, NJ). COS-Nox2 cells were maintained in complete media supplemented with 1.8 mg/mL G418, 1 μg/mL puromycin (Sigma, St Louis, MO) and 0.2 mg/mL hygromycin B (Invitrogen, Carlsbad, CA). HEK-Nox5 cells were kindly provided by Dr. David Fulton (Georgia Health Sciences University) and maintained in complete media.

**Plasmid preparation, amplification, and purification**

Plasmids encoding full-length human cDNAs for Nox1 (pcDNA3.1-hNox1), NOXO1 (pcDNA3.1-hNOXO1), NOXA1 (pCMVsport 6-hNOXA1), p47\(^{phox}\) (pCMV-Tag4A-hp47) and Nox4 (pcDNA3-hNox4) were kindly provided by Dr. David Lambeth (Emory University, GA). Plasmids encoding Nox1, NOXO1, or NOXA1 were transformed and amplified into Escherichia coli strain TOP10 (Invitrogen, Carlsbad, CA). Plasmids were purified using a QIAfilter plasmid purification kit (QIAGEN Inc., Valencia, CA.). For human Nox4 expression, the BglII/NotI restriction fragment

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from the pcDNA3-hNox4 was subcloned into the plasmid pcDNA3.1/Hygro(−) (Invitrogen, Carlsbad, CA) to generate pcDNA3.1/Hygro-hNox4. The fragment sequence, in-frame insertion and orientation were validated by DNA sequencing after PCR amplification. pcDNA3.1/Hygro-hNox4 was amplified into Escherichia coli strain TOP10 and purified with a QIAfilter plasmid purification kit.

Transfection

Cell transfection was carried out using Lipofectamine LTX and Plus reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. COS-22 cells were transiently co-transfected with pcDNA 3.1-hNox1, pCMVsport 6-hNOXA1 and pcDNA3.1-hNoxO1 (COS-Nox1/NOXO1/NOXA1 cells) or with pcDNA3.1/Hygro-hNox4 (COS-Nox4 cells). Cells were used 24 hr after transfection. Adherent cells were harvested by incubating with 0.05% trypsin/EDTA for 5 min at 37 °C. Following addition of DMEM/10%FBS to neutralize the trypsin, the cells were pelleted by centrifugation at 1100 × g for 5 min at 4 °C and used for the experiments.

Measurements of ROS generation in whole cells

L-012 chemiluminescence

COS-Nox2, COS-Nox1/NOXO1/NOXA1, HEK-Nox5 and COS-22 cells were re-plated in OPTIMEM into 384-well white micro-plates (Greiner-Bio One GmbH, Germany) at a density of 2.5 x 10^3 cells/well for COS-Nox2 and 5 x 10^4 cells/well for all others. The cells were incubated at 37 °C in PBS containing 400 μm L-012 for 10 min. Reaction was initiated by addition of 5 μM PMA in the case of Nox2 or 1 μM PMA/ 0.1 μM Ionomycin in the case of Nox5. Since Nox1 was constitutively active, Nox1-derived O_2•^- was assessed by comparison to non-transfected cells (Cos22). Luminescence was quantified over time using a Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader. The specificity of L-012 for O_2•^- was confirmed by the addition of SOD (150 U/mL).

Hydrogen peroxide (H_2O_2)-generating activity

H_2O_2-production was quantified in intact COS-Nox4 cells by Amplex® Red. Briefly, COS-Nox4 cells (5 x 10^4 cells/ml) were re-plated into 384-well plate in assay buffer (25 mM Hepes, pH 7.4, containing 0.12 M NaCl, 3 mM KCl, 1 mM MgCl_2). The cells were incubated in the presence or
absence of drugs at 37 °C in assay buffer supplemented with 0.1 mM Amplex® Red, and 0.32 U/ml of horse radish peroxidase (HRP) for 15 min. Fluorescence measurements were made using a Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader with a 530/25-excitation and a 590/35-emission filter. The reaction was monitored at 37 °C for 1 h. A standard curve of known H₂O₂ concentrations was included on each plate. Nox4 activity was obtained by calculating the rate of H₂O₂ production as RFU/min and then subtracting the equivalent value given by non-transfected COS-22 cells. Similarly, H₂O₂ production by Nox2 cells was also measured by Amplex® Red as described above but in the presence of 150 U/mL of SOD and the reaction was initiated by addition of 5 µM PMA. The rate of H₂O₂ production in this case was calculated as RFU/min after the subtraction of the equivalent value given by non-stimulated cells. Data are expressed as % of vehicle control.

Measurements of superoxide generation in cell-free preparations

**Cytochrome C assay**

COS-Nox2, COS-Nox1/NOXO1/NOXA1 and COS-22 cells were suspended to a concentration of 5 × 10⁷ cells/ml in ice-cold disruption buffer (8 mM potassium, sodium phosphate buffer pH 7.0, 131 mM NaCl, 340 mM sucrose, 2 mM Na₃HPO₄, 5 mM MgCl₂, 1mM EGTA, 1 mM EDTA and protease inhibitor cocktail).³⁴ The cells were lysed by freeze/thaw cycles (5 cycles), and passed through a 30-gauge needle 5 times to further lyse the cells. Cell disruption was confirmed by phase contrast microscopy. The cell lysate was centrifuged at 1000 × g for 10 min at 4 °C to remove unbroken cells, nuclei and debris. Throughout all procedures, extreme care was taken to maintain the lysate at a temperature close to 0 °C. Superoxide (O₂⁻) production was calculated from the initial linear rate (over 10 min) of SOD-inhibitable cytochrome c reduction quantified at 550 nm using the extinction coefficient of 21.1 mM⁻¹ cm⁻¹ (Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader). The oxidase assay buffer consisted of 65 mM sodium phosphate buffer (pH 7.0), 1 mM EGTA, 10 µM FAD, 1 mM MgCl₂, 2 mM NaN₃ and 0.2 mM cytochrome C.³ The components of the cell-free system were added in the following order: oxidase assay buffer, cell lysate (5 × 10⁶ cell equivalents/well) and drugs at a final concentration as shown on individual

graphs. The plates were placed on an orbital shaker to mix contents for 5 min at 120 movements/min at room temperature. LiDS, an established lipid activator of phagocyte cell-free system, was added at a concentration of 130 μM and O₂⁻⁻ production was initiated by the addition of 180 μM NADPH.

**Xanthine oxidase assay**
Bovine milk XO (grade 1, ammonium sulfate suspension; Sigma–Aldrich) was first desalted using PD-10 columns (Sephadex G-25, GE Healthcare Biosciences, Piscataway, NJ) equilibrated in PBS. XO activity was assayed by measuring H₂O₂ production using the Amplex® Red assay. Reaction mixture contained: 0.02 U/mL XO, 25 mM Hepes, pH 7.4, containing 0.12 M NaCl, 3 mM KCl, 1 mM MgCl₂, 0.1 mM Amplex® Red, and 0.32 U/mL of horse radish peroxidase (HRP), reaction was initiated by addition of 1 mM hypoxanthine. Fluorescence was quantified over time using a Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader. Data are expressed as % of vehicle control.

**Cytotoxicity assay**
The cytotoxic effect of compounds was tested using CytoTox-Glo kit (Promega, Madison, WI). This assay uses a luminogenic peptide substrate, the AAF-Glo™ Substrate, to measure dead-cell protease activity, which is released from cells that have lost membrane integrity. Cells were plated in 384-well plate at 2.5 x 10³ cells/well in OPTI-MEM, incubated for 15 min with the compounds at various concentrations and then carried out dead cell measurements according to manufacturer protocol. Fluorescence signals before and after the addition of digitonin was used to calculate the % of dead cells in each independent well. Data are expressed as % of live cells in each independent well (=100-%dead cells).

**Statistical analysis**
All data are expressed as means ± SEM. IC₅₀ values were determined using the GraphPad Prism software for non-linear regression three-parameters, with constrain values of 100 and 0 for top and bottom, respectively, and assumes a Hill slope of 1.
Cytotoxicity assessment of 7 and analogs
Xanthine oxidase (XO) assay data