Supporting information

Identification, synthesis and biological evaluation of the major human metabolite of NLRP3 inflammasome inhibitor MCC950

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Contents

1. Instrumentation and Chemicals.

Unless otherwise stated, all the starting materials and reagents were purchased from commercial suppliers and used without any purification. Anhydrous organic solvents were purchased from Aldrich and used directly. Reaction progress was monitored by TLC using Merck silica gel 60 F-254 using UV light and/or suitable TLC stain solution for visualization. Compounds were purified by column chromatography using silica gel or using Grace Reveleris® chromatography system with either silica gel or reverse phase C-18 silica. All final products were obtained in >95% purity as determined by LC-MS using UV at 254 nm, ESI-MS and ELSD detection. Analytical LC-MS was performed on Agilent LC 1200 series with UV detection at 210 and 254 nm and a low resolution ESI using 0.05% formic acid in water (solvent A) and 0.05% formic acid in ACN (solvent B). High resolution mass spectrometry (HRMS) was performed on the Bruker MicroTOF mass spectrometer. NMR data was collected and calibrated in DMSO-d_6 and $CDCl_3$ at 298 K using Bruker Advance 600 MHz spectrometer. Chemical shifts were measured relative to TMS or the residual solvent signals (i.e. DMSO-d_6, and $CDCl_3$) as the internal reference. Data are presented as follows: chemical shift parts per million (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet), coupling constant (Hz) and integration. Optical rotation measured by JASCO P-1010 Polarimeter.

LC−MS and LC−MS/MS conditions for major metabolite identification and co-elution.

Analyses of MCC950 and major metabolites were carried out using a AB SCIEX Triple TOF 5600 system equipped with a Waters Atlantis T3, 2.1 × 50 mm, 5 µm column. The following mobile phase was used: solvent A = 0.1% formic acid/H_2O, solvent B = 0.1% formic acid/CH_3CN and the solvent gradient is as follows: 0.0–1.0 min, 2% B; 1.0–15.0 min, 40% B; 15.0–18.0 min, 80% B; 18.0–21.0 min, 80% B; 21.0–21.1 min, 98% B; 21.1–23.0 min, 98% B; 23.0–23.1 min, 2%; 23.1–32.0 min, wash; The flow rate was 0.18 mL/min, data were acquired from *m/z* 70−600 in positive and negative scan mode.

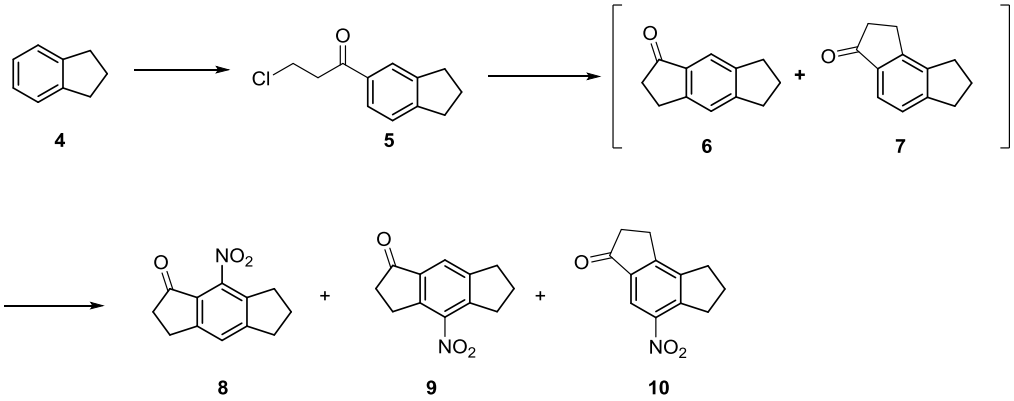
Co-elution of metabolite with synthetic standards (compounds **1**-**3**, figure 2) were carried out using a AB SCIEX 4000 QTRAP LC-MS/MS system equipped with a Waters Atlantis T3, 2.1 × 50 mm, 5 µm column. The following mobile phase was used: solvent A = 0.1% formic acid/H_2O, solvent B = 0.1% formic acid/CH_3CN and the solvent gradient is as follows: 0.0–0.5 min, 5% B;

0.5–7.0 min, 75% B; 7.0–8.0 min, 100% B; 8.0–12.0 min, 100% B; 12.0–12.1 min, 5% B; 12.1–17.0 min, wash. The flow rate was 0.25 mL/min.

Co-elution of metabolite with *R* and *S* isomers (2a and 2b, scheme 5) were carried out using a AB SCIEX 4000 QTRAP LC-MS/MS system equipped with a Phenomenex Lux® 3 µm Cellulose-2, 250 × 4.60 mm column. The following mobile phase was used: solvent A = 0.1% formic acid/H_2O, solvent B = 0.1% formic acid/CH_3CN. The flow rate was 0.4 mL/min, 30% B isocratic elution for 50 min.

2. Experimental procedures and characterization data for synthesized compounds.

Scheme



3-chloro-1-(2,3-dihydro-1H-inden-5-yl)propan-1-one ***(5)***

To indane (2.20 g, 18.6 mmol) in DCM (30 mL), was added 3-chloropropionyl chloride (2.60 g, 20.4 mmol) followed by $AlCl_3$ (2.98 g, 22.3 mmol) portion-wise at room temperature, the resulting reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured onto crushed ice/2 N HCl and extracted with DCM (2 × 100 mL), then the combined organic layer washed with brine (50 mL), dried over anhydrous magnesium sulfate, filtered and concentrated. The crude product was purified by column chromatography on silica gel, eluting with 15% Ethyl acetate (EtOAc) in hexane. The titled compound was obtained as pale yellow solid (3.3 g, 85%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.84 (s, 1H), 7.77 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 3.91 (t, *J* = 6.3 Hz, 2H), 3.51 (t, *J* = 6.3 Hz, 2H), 2.91 (t, *J* = 7.5 Hz, 4H), 2.07-2.02 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 197.1, 150.6, 144.9, 135.2,

126.9, 124.90, 124.3, 41.1, 40.1, 32.9, 32.4, 25.4. HRMS (ESI) *m/z* calculated for $C_{12}H_{14}ClO$ $[M+H]^+$, 209.0728; found 209.0730.

8-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one ***(8)*** *and 4-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one* ***(9)***

3-Chloro-1-(2,3-dihydro-1H-inden-5-yl)propan-1-one (3.00 g, 14.3 mmol) was added portion-wise to concd sulfuric acid (15 mL). The resulting reaction mixture was heated to 55–60 °C and stirred for 48 h. Formation of 3,5,6,7-tetrahydro-s-indacen-1(2H)-one (**6**) along with small amounts of regioisomer 1,6,7,8-tetrahydro-*s*-indacen-3(2H)-one (**7**) was confirmed by ^{1}H NMR spectroscopy upon isolating a small amount of product from the reaction mixture by quenching with ice cold water. The reaction mixture was cooled to 0 °C and a mixture of treated with concentrated nitric acid (1 mL) and concentrated sulfuric acid (1 mL) was added drop wise, with stirring for an additional 1 h at 0–5 °C. The reaction mixture was allowed to warm to room temperature then slowly added to a mixture of water (100 mL) and DCM (100 mL) with ice bath cooling. The organic layer was separated and the aqueous layer extracted with DCM (100 mL). The combined organic layers were washed with half-saturated brine (100 mL) and saturated aq. sodium bicarbonate (100 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated. The major isomer **8,** minor isomers **9** and **10** were separated by column chromatography on silica gel, eluting with 15-20% EtOAc in hexane provided the major isomer 8-nitro-3,5,6,7-tetrahydro-2H-*s*-indacen-1-one as pale yellow solid (1.30 g, 42%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45 (s, 1H), 3.14–3.11 (m, 2H), 3.05 (t, *J* = 7.2 Hz, 2H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.80–2.76 (m, 2H), 2.23–2.18 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$):δ 200.6, 156.0, 155.5, 136.8, 126.3, 124.9, 118.3, 37.0, 33.4, 29.6, 25.38, 25.36; HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_3$ $[M+H]^+$, 218.0812; found 218.0820.

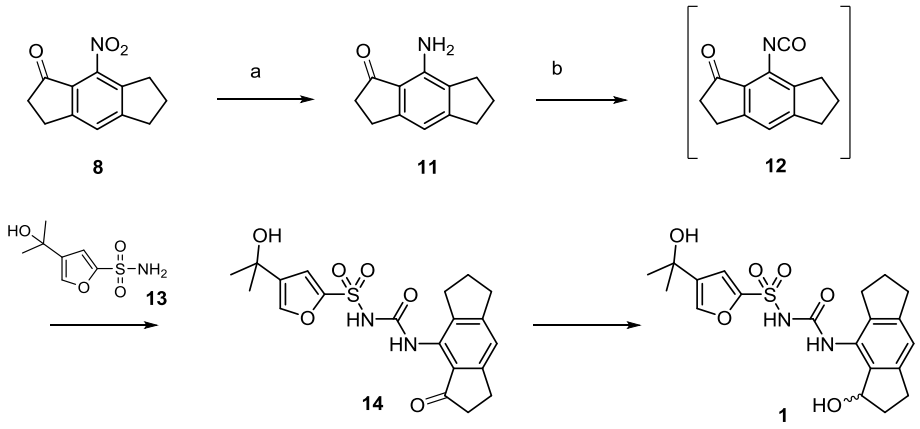
Minor isomers 4-nitro-3,5,6,7-tetrahydro-*s*-indacen-1(2H)-one (330 mg, 11%) pale yellow solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 (s, 1H), 3.51–3.49 (m, 2H), 3.41 (t, *J* = 7.5 Hz, 2H), 3.05 (t, *J* = 7.3 Hz, 2H), 2.80–2.77 (m, 2H), 2.26–2.20 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 204.6, 148.5, 148.5, 148.4, 138.8, 129.1, 123.8, 36.1, 34.1, 32.3, 26.2, 25.4; HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_3$ $[M+H]^+$, 218.0812; found 218.0814.

5-nitro-1,6,7,8-tetrahydro-*s*-indacen-3(2H)-one (160 mg, 5%) pale yellow solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.39 (s, 1H), 3.49 (t, *J* = 7.6 Hz, 2H), 3.13–3.10 (m, 2H), 3.02 (t, *J* = 7.6 Hz,

2H), 2.82–2.80 (m, 2H), 2.30–2.25 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 204.8, 155.2, 146.7, 145.7, 145.6, 136.8, 118.2, 36.61, 34.64, 30.3, 24.9, 24.7. HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_3$ $[M+H]^+$, 218.0812; found 218.0817.

The structures of all isomers were confirmed by single crystal X-ray crystallography. The single crystals for X-ray crystallography were grown by slow evaporation from MeOH solution.

Scheme



*8-amino-3,5,6,7-tetrahydro-s-indacen-1(2H)-one **(11)***

A solution of 8-nitro-3,5,6,7-tetrahydro-*s*-indacen-1(2H)-one **8** (200 mg, 0.92 mmol) in MeOH (5 mL) was degassed with nitrogen for 5 min, 10% Pd/C (20 mg, 10% wt/wt) was added and the mixture stirred under hydrogen atmosphere (1 atm) at room temperature for 2 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* to give 8-amino-3,5,6,7-tetrahydro-*s*-indacen-1(2H)-one as an off-white solid (160 mg, 93%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.49 (s, 1H), 6.34 (br s, 2H), 2.88–2.83 (m, 2H), 2.80 (t, J = 7.5 Hz, 2H), 2.62 (t, J = 7.4 Hz, 2H), 2.56 – 2.51 (m, 2H), 2.04–1.99 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 206.6, 155.4, 153.7, 144.2, 125.3, 118.6, 109.4, 36.8, 33.7, 28.6, 25.0, 24.9. HRMS (ESI) *m/z* calculated for $C_2H_{14}NO$ $[M+H]^+$ 188.1070, found 188.1077.

*8-isocyanato-3,5,6,7-tetrahydro-s-indacen-1(2H)-one **(12)***

To di-*t*-butyldicarbonate (163 mg, 0.74 mmol) in anhydrous CH_3CN (1 mL) was added DMAP (26.1 mg, 0.21 mmol) at rt, stirred for 5 min, a solution of amine intermediate **11** (100 mg, 0.53 mmol) in CH_3CN was added. The reaction mixture was stirred for 30 min at rt. The reaction mixture was used directly in the next step without workup.

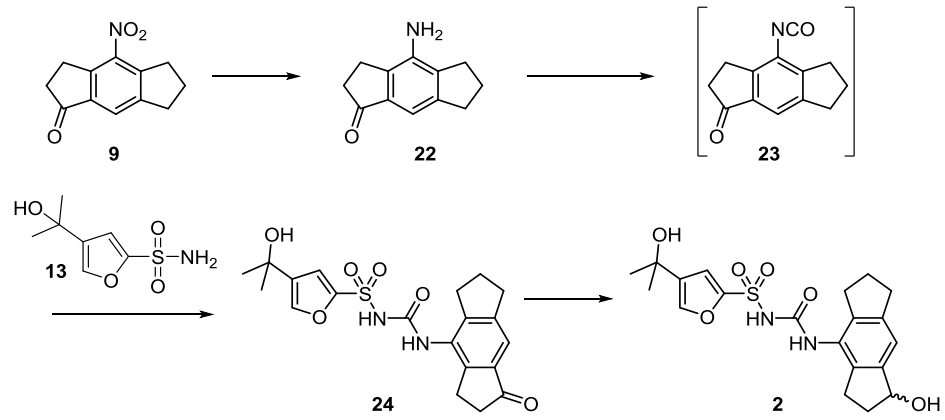
*4-(2-hydroxypropan-2-yl)-N-((3-oxo-1,2,3,5,6,7-hexahydro-s-indacen-4-yl) carbamoyl) furan-2-sulfonamide **(14)***

To 4-(2-hydroxypropan-2-yl) furan-2-sulfonamide intermediate (100 mg, 0.48 mmol) in anhydrous THF (1 mL) was added NaH (18.3 mg, 0.48 mmol) at 0 °C and stirred for 30 min at ambient temperature under nitrogen atmosphere. Again cooled to 0 °C, isocyanate **12** (previous step reaction mixture) was added and stirred at ambient temperature for 16 h. To the reaction mixture was added H_2O (0.5 mL), and the resulting solution was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. The titled compound was obtained as a white solid (150 mg, 67%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.79 (s, 1H), 7.37 (s, 1H), 6.94 (s, 1H), 6.61 (s, 1H), 4.92 (br s, 1H), 2.92 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 2.63–2.57 (m, 2H), 1.97–1.80 (m, 2H), 1.34 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 207.3, 157.1, 155.6, 155.0, 140.6, 137.7, 137.0, 136.1, 134.3, 123.5, 115.6,110.1, 66.9, 37.0, 33.8, 33.1, 31.5, 25.8, 24.8. HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_6S$ $[M+H]^+$ 419.1271, found 419.1291.

(±) *N-((3-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl) carbamoyl)-4-(2-hydroxypropan-2-yl) furan-2-sulfonamide **(1)***

To a solution of 4-(2-hydroxypropan-2-yl)-*N*-((3-oxo-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)furan-2-sulfonamide **14** (70 mg, 0.16 mmol) in MeOH (2 mL) was added $NaBH_4$ (63 mg, 1.67 mmol) at 0 °C under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with H_2O (2 mL), and the MeOH was removed *in vacuo*. The remaining aqueous layer was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. The titled compound was obtained as an off-white solid (60 mg, 86%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.73 (br s, 1H), 7.39 (s,1H), 6.81 (s, 1H), 6.60 (s, 1H), 5.63 (br s, 1H), 4.92 (br s, 1H), 4.87 (d, *J* = 6.0 Hz, 1H), 2.99–2.94 (m, 1H), 2.89–2.84 (m, 1H), 2.77 (t, *J* = 7.4 Hz, 2H), 2.61–2.52 (m, 2H), 2.07–2.03 (m, 1H), 2.01–1.87 (m, 3H), 1.35 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.5, 155.9, 144.9, 143.3, 138.3, 137.7, 136.8, 136.1, 133.0, 116.5, 109.8, 72.5, 67.0, 35.4, 33.1, 31.5, 31.0, 30.4, 25.5. HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_6S$ $[M-H]^+$ 419.1282, found 419.1263.

Scheme



*4-amino-3,5,6,7-tetrahydro-s-indacen-1(2H)-one **(22)***

A solution of 4-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one **9** (110 mg, 0.50 mmol) in MeOH (5 mL) was degassed with nitrogen for 5 min, added 10% Pd/C (11 mg, 10% wt/wt), stirred under hydrogen atmosphere (1 atm) at room temperature for ~2 h. The reaction mixture was filtered through a pad of Celite and the filtrate concentrated *in vacuo.* The titled compound was obtained as an off-white solid (75 mg, 80%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.72 (s, 1H), 5.13 (s, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.78–2.75 (m, 2H), 2.70 (t, J = 7.4 Hz, 2H), 2.60–2.56 (m, 2H), 2.05–2.00 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 206.9, 144.8, 141.8, 138.9, 136.8, 133.4, 106.1, 36.8, 32.7, 29.8, 25.3, 22.9. HRMS (ESI) *m/z* calculated for $C_{12}H_{14}NO$ $[M+H]^+$ 188.1070, found 188.1074.

*4-isocyanato-3,5,6,7-tetrahydro-s-indacen-1(2H)-one **(23)***

To di-*t*-butyldicarbonate (81.6 mg, 0.37 mmol) in anhydrous CH_3CN (1 mL) was added DMAP (13.0 mg, 0.10 mmol) at rt, stirred for 5 min, a solution of 4-amino-3,5,6,7-tetrahydro-s-indacen-1(2H)-one **22** (50 mg, 0.26 mmol) in CH_3CN (1 mL) was added. The reaction mixture was stirred for 30 min at rt. The reaction mixture was used directly in the next step without workup.

*4-(2-hydroxypropan-2-yl)-N-((1-oxo-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl) furan-2-sulfonamide **(24)***

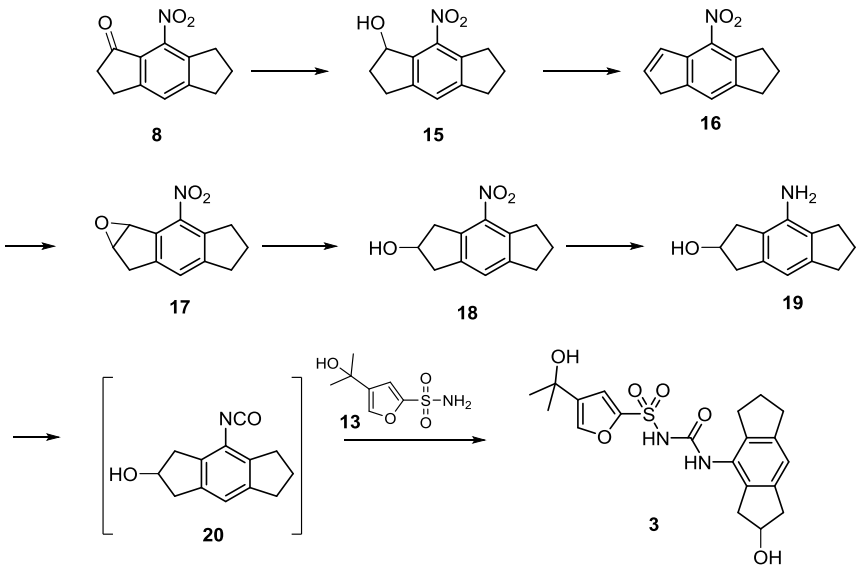
To 4-(2-hydroxypropan-2-yl) furan-2-sulfonamide (50 mg, 0.24 mmol) in anhydrous THF (1 mL) was added NaH (9.3 mg, 0.24 mmol) at 0 °C and stirred for 30 min at ambient temperature under nitrogen atmosphere. The suspension was again cooled to 0 °C, isocyanate **23** (previous

step reaction mixture) was added and the mixture stirred at ambient temperature for 16 h. To the reaction mixture was added H_2O (0.5 mL) and the resulting solution was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. The titled compound was obtained as an off-white solid (70 mg, 63%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.94 (br s, 1H), 7.38 (s, 1H), 7.17 (s, 1H), 6.60 (s, 1H), 4.92 (s, 1H), 2.93–2.90 (m, 2H), 2.85 (t, *J* = 7.4 Hz, 2H), 2.80 (t, *J* = 7.4 Hz, 2H), 2.55 – 2.52 (m, 2H), 2.00–1.94 (m, 2H), 1.35 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 205.9, 157.3, 155.6, 151.5, 149.5, 146.7, 144.3, 140.1, 137.0, 135.5, 113.3, 111.9, 66.4, 36.1, 32.0, 30.9, 24.9, 23.5. HRMS (ESI) *m/z* calculated for $C_{20}H_{21}N_2O_6S$ $[M-H]^+$ 417.1126, found 417.1113.

(±)*N-((1-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide* ***(2)***

To a solution of 4-(2-hydroxypropan-2-yl)-*N*-((1-oxo-1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)furan-2-sulfonamide **17** (50 mg, 0.11 mmol) in MeOH (2 mL) was added $NaBH_4$ (45 mg, 1.19 mmol) at 0 °C under nitrogen atmosphere, the resulting reaction mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with H_2O (1 mL), and the MeOH removed *in vacuo*. The remaining aqueous layer was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. The titled compound was obtained as an off-white solid (20 mg, 40%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.95 (s, 1H), 7.65 (s, 1H), 7.02 (s, 1H), 6.95 (s, 1H), 5.06 (d, *J* = 5.8 Hz, 1H), 5.04 (s, 1H), 4.91 (q, *J* = 6.3 Hz, 1H), 2.77 (t, *J* = 7.6 Hz, 2H), 2.63–2.54 (m, 3H), 2.46–2.40 (m, 1H), 2.24–2.19 (m, 1H), 1.90 (p, *J* = 6.8, 6.2 Hz, 2H), 1.68–1.62 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.4, 151.6, 145.8, 144.0, 142.7, 140.1, 138.2, 136.4, 135.9, 116.6, 112.0, 74.4, 66.5, 35.5, 32.5, 31.0, 30.3, 27.2, 25.1. HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_6S$ $[M-H]^+$ 419.1282, found 419.1265.

Scheme



8-nitro-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(15)***

To a solution of 8-nitro-3,5,6,7-tetrahydro-*s*-indacen-1(2H)-one **8** (0.73 g, 3.36 mmol) in MeOH (15 mL) was added $NaBH_4$ (0.63 g, 16.8 mmol) portion-wise at 0 °C under nitrogen atmosphere, the resulting reaction mixture was stirred at room temperature for 1 h. Solvent was removed *in vacuo*, to the residue was added H_2O (10 mL) and extracted with EtOAc (2 × 50 mL).The combined organic layers was washed with saturated aqueous NaCl (40 mL), dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with 15% EtOAc in hexane. The titled compound was obtained as an off-white solid (0.71 g, 97%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.42 (s, 1H), 5.45 (q, *J* = 6.1 Hz, 1H), 5.23 (d, *J* = 6.1 Hz, 1H), 3.15 (dt, *J* = 16.6, 8.2 Hz, 1H), 3.04–2.97 (m, 1H), 2.97–2.89 (m, 3H), 2.82–2.71 (m, 1H), 2.38–2.32(m, 1H), 2.15–2.06 (m, 1H), 2.06–1.95 (m, 1H), 1.95–1.82 (m, 1H).^{13}C NMR (150 MHz, DMSO-d_6): 148.0, 145.6, 137.9, 137.0, 125.68,125.67, 73.1, 35.9, 32.7, 32.0, 29.9, 25.5. HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_3$ $[M-H]^+$, 218.0823, found, 218.0814.

8-nitro-1,2,3,5-tetrahydro-s-indacene ***(16)***

To a solution of 8-nitro-1,2,3,5,6,7-hexahydro-*s*-indacen-1-ol **15** (0.70 g, 3.22 mmol) in anhydrous toluene (5 mL) was added activated 3 Å molecular sieves and *p*-TSA (122 mg, 0.64 mmol) and the mixture was heated to reflux under Dean-stark conditions for 2 h. The reaction

mixture was cooled to rt and diluted with EtOAc (50 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ solution (2 × 30 mL), saturated aqueous NaCl (30 mL), dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. Obtained solid was triturated with hexane. The titled compound was obtained as a light yellow solid (0.51 g, 80%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.53 (s, 1H), 7.49 (d, *J* = 6 Hz, 1H), 6.77 (dt, *J* = 5.7, 2.0 Hz, 1H), 3.45 (td, *J* = 2.0, 0.6 Hz, 2H), 3.35 (t, *J* = 6 Hz 2H), 3.01 (t, *J* = 6 Hz, 2H), 2.19–2.14 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 145.1, 144.0, 138.5, 138.2, 137.9, 130.9, 124.72, 124.71, 38.9, 33.6, 32.7, 25.3. HRMS (ESI) *m/z* calculated for $C_{12}H_{10}NO_2$ $[M-H]^+$, 200.0717, found, 200.0726.

2-nitro-1a,3,4,5,7,7a-hexahydro-s-indaceno[1,2-b]oxirene ***(17)***

To a solution of 8-nitro-1,2,3,5-tetrahydro-*s*-indacene **16** (0.35 g, 1.73 mmol) in anhydrous DCM (10 mL) was added *m*-CPBA (506 mg, 2.26 mmol) at 0 °C under nitrogen atmosphere; the resulting reaction mixture was stirred at ambient temperature for 16 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (5 mL) and $Na_2S_2O_3$ solution (5 mL), stirred for 15 min, and extracted with DCM (2 × 30 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with 10% EtOAc in hexane. The titled compound was obtained as an off-white solid (0.31 g, 82%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.49 (s, 1H), 4.72 (dd, *J* = 2.8, 1.0 Hz, 1H), 4.21 (t, *J* = 2.9 Hz, 1H), 3.24–3.10 (m, 4H), 2.95–2.91 (m, 1H), 2.10–2.05 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 148.2, 145.3, 137.03, 134.0, 127.3, 127.3 57.2, 56.9, 34.8, 32.4, 32.2, 24.7. HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_3$ $[M+H]^+$, 218.0812, found, 218.0817.

4-nitro-1,2,3,5,6,7-hexahydro-s-indacen-2-ol ***(18)***

To a solution of 2-nitro-1a,3,4,5,7,7a-hexahydro-s-indaceno[1,2-b]oxirene **17** (300 mg, 1.38 mmol) in anhydrous 1,2-dichloroethane (8 mL) was added ZnI_2 (661 mg, 2.07 mmol) and $NaCNBH_3$ (650 mg, 10.3 mmol) at ambient temperature. The resulting reaction mixture was heated to reflux for 4 h. The reaction mixture was cooled to room temperature, poured on to a solution of aqueous 6N HCl (20 mL), and extracted with DCM (3 × 30 mL), and the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with 20% EtOAc in hexane. The titled compound was obtained as an off-white solid (260 mg, 86%). ^{1}H

NMR (600 MHz, DMSO-d_6): δ 7.45 (s, 1H), 4.94 (d, *J* = 3.8 Hz, 1H), 4.54–4.51 (m, 1H), 3.33–3.26 (m, 1H), 3.20–3.06 (m, 3H), 3.06–2.99 (m, 1H), 2.91 (t, *J* = 7.6 Hz, 2H), 2.82–2.75 (m, 1H), 2.09–2.02 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 146.4, 144.2, 137.6, 135.6, 126.63, 126.65 71.2, 42.9, 42.2, 33.1, 32.6, 25.3. HRMS (ESI) *m/z* calculated for $C_{12}H_{14}NO_3$ [M-H_2O + H]$^+$, 202.0875, found, 202.0871.

4-amino-1,2,3,5,6,7-hexahydro-s-indacen-2-ol ***(19)***

A solution of 4-nitro-1,2,3,5,6,7-hexahydro-*s*-indacen-2-ol **18** (260 mg, 1.18 mmol) in MeOH (8 mL) was degassed with N_2 for 5 min, added 10% Pd/C (52 mg, 20% w/w). The resulting mixture was stirred under hydrogen atmosphere (1 atm) at ambient temperature for 16 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The titled compound was obtained as an off-white solid (155 mg, 69%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.30 (s, 1H), 4.74 (d, *J* = 6 Hz, 1H), 4.53 (s, 2H), 4.46–4.41 (m, 1H), 2.90 (dd, *J* = 15.5, 6.4 Hz, 1H), 2.80 (dd, *J* = 15.6, 6.5 Hz, 1H), 2.70 (t, *J* = 7.5 Hz, 2H), 2.61–2.55 (m, 3H), 2.46 (dd, *J* = 15.6, 4.3 Hz, 1H), 1.97–1.92 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 142.9, 140.5, 140.3, 125.3, 122.8, 109.0, 72.0, 42.8, 39.2, 33.1, 29.4, 25.3. HRMS (ESI) *m/z* calculated for $C_{12}H_{16}NO$ [M+H]$^+$, 190.1226, found, 190.1232.

4-isocyanato-1,2,3,5,6,7-hexahydro-s-indacen-2-ol ***(20)***

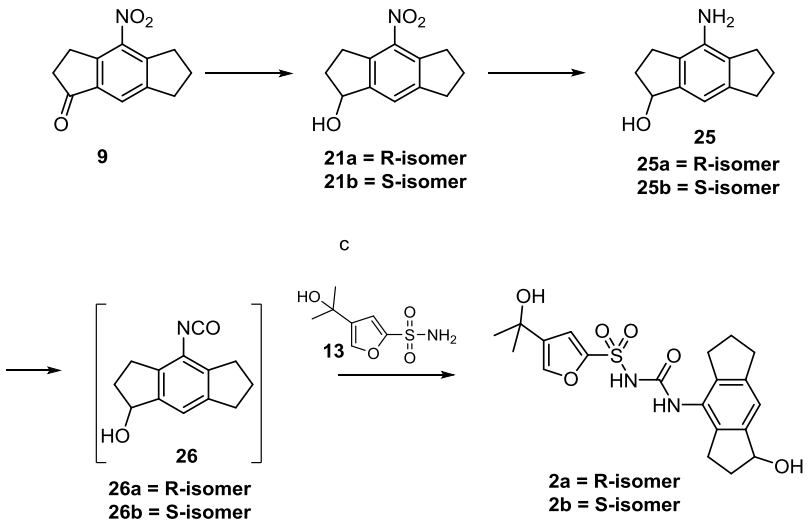
To di-*t*-butyldicarbonate (113 mg, 0.51 mmol) in anhydrous CH_3CN (1 mL) was added DMAP (18.0 mg, 0.14 mmol) at room temperature, stirred for 5 min, a solution of 4-amino-1,2,3,5,6,7-hexahydro-*s*-indacen-2-ol **19** (70 mg, 0.37 mmol) in CH_3CN (1 mL) was added. The reaction mixture was stirred for 30 min at rt. The reaction mixture was used directly in the next step without workup.

N-((2-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide ***(3)***

To 4-(2-hydroxypropan-2-yl) furan-2-sulfonamide **13** (70 mg, 0.34 mmol) in anhydrous THF (1 mL) was added NaH (13.4 mg, 0.34 mmol) at 0 °C and the mixture was stirred for 30 min at ambient temperature under a nitrogen atmosphere. Again cooled to 0 °C, isocyanate **20** (previous step reaction mixture) was added and stirred at ambient temperature for 16 h. To the reaction

mixture was added H_2O (0.5 mL), and the resulting solution was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. The titled compound was obtained as a white solid (60 mg, 39%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.97 (s, 1H), 7.69 (s, 1H), 7.07 (s, 1H), 6.87 (s, 1H), 5.07 (s, 1H), 4.76 (d, *J* = 4.0 Hz, 1H), 4.43–4.39 (m, 1H), 2.97 (dd, *J* = 15.8, 6.2 Hz, 1H), 2.83 (dd, *J* = 16.3, 6.2 Hz, 1H), 2.78 (t, *J* = 7.4 Hz, 2H), 2.68–2.56 (m, 3H), 2.49 (d, *J* = 8.2 Hz, 1H), 1.95–1.90 (m, 2H), 1.38 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.0, 156.1, 143.2, 140.9, 137.4, 137.1, 135.2, 125.0, 122.8, 118.1, 112.5, 71.8, 67.0, 42.6, 40.6, 33.0, 31.4, 30.7, 25.4. HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_6S$ $[M-H]^+$, 419.1282, found, 419.1298.

Scheme



(R)-(+)-4-nitro-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(21a)***

To a solution of (*S*)-(–)-2-Methyl-CBS-oxazaborolidine (12.7 mg, 0.04 mmol) in THF (1 mL) at 0 °C was added a 2.0 M solution of BH_3•Me_2S in THF (0.13 mL, 0.27 mmol). The reaction mixture was stirred for 15 min, then a solution of 4-nitro-3,5,6,7-tetrahydro-*s*-indacen-1(2H)-one **9** (50 mg, 0.23 mmol) was added. After 30 min, reaction mixture was quenched with MeOH (1 mL) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with 15% ethyl acetate in hexane. The titled compound was obtained as an off-white solid (42.0 mg, 84%, er = 96:4). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.52 (s, 1H), 5.42 (d, *J*

= 5.9 Hz, 1H), 5.06 (q, *J* = 6.4 Hz, 1H), 3.24–3.11 (m, 3H), 3.02–2.96 (m, 1H), 2.94 (t, *J* = 7.5 Hz, 2H), 2.40–2.35 (m, 1H), 2.13–2.04 (m, 2H), 1.86–1.80 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 148.5, 146.2, 142.5, 138.7, 136.0, 125.4, 73.5, 35.1, 32.6, 32.0, 29.4, 24.9. HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_2$ $[M-18+H]^+$, 202.0863; found 202.0863.

(R)-(+)-4-amino-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(25a)***

A solution of (*R*)-(+)-4-nitro-1,2,3,5,6,7-hexahydro-*s*-indacen-1-ol **21a** (35.0 mg, 0.15 mmol) in MeOH (10 mL) was degassed with nitrogen for 5 min, then added 10% Pd/C (7.0 mg, 20% wt/wt), stirred under hydrogen atmosphere (1 atm) at room temperature for about 2 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo.* The titled compound obtained as an off-white solid (28.0 mg, 93%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.43 (s, 1H), 4.87 (q, *J* = 5.9 Hz, 1H), 4.84 (d, *J* = 5.7 Hz, 1H), 4.54 (s, 2H), 2.74–2.68 (m, 2H), 2.67–2.62 (m, 1H), 2.57 (t, *J* = 7.3 Hz, 2H), 2.40–2.33 (m, 1H), 2.27–2.22 (m, 1H), 1.97–1.92 (m, 2H), 1.73–1.67 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 145.5, 142.6, 139.5, 125.8, 124.0, 108.1, 74.7, 35.5, 32.6, 28.9, 25.9, 25.0. HRMS (ESI) *m/z* calculated for $C_{12}H_{16}NO$ $[M+H]^+$, 190.1226; found 190.1232.

(R)-(+)-4-isocyanato-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(26a)***

To di-*t*-butyldicarbonate (40.3 mg, 0.18 mmol) in anhydrous CH_3CN (1 mL) was added DMAP (6.4 mg, 0.05 mmol) at room temperature, stirred for 5 min, a solution of amine intermediate **25a** (25.0 mg, 0.13 mmol) in THF (1 mL) was added. The reaction mixture was stirred for 30 min at room temperature. The reaction mixture was used directly in the next step without workup.

(R)-(+)-N-((1-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide ***(2a)***

To 4-(2-hydroxypropan-2-yl) furan-2-sulfonamide intermediate (25.0 mg, 0.12 mmol) in anhydrous THF (1 mL) was added NaH (4.86 mg, 0.12 mmol) at 0 °C and stirred for 30 min at ambient temperature under nitrogen atmosphere. Again cooled to 0 °C, isocyanate **26a** (previous step reaction mixture) was added and stirred at ambient temperature for 16 h. To the reaction

mixture was added H_2O (0.5 mL), and the resulting solution was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. Re-purified by semi prep HPLC using 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. The titled compound was obtained as an off-white solid (12.0 mg, 22%). δ 7.95 (s, 1H), 7.65 (s, 1H), 7.02 (s, 1H), 6.95 (s, 1H), 5.06 (d, *J* = 5.8 Hz, 1H), 5.04 (s, 1H), 4.91 (q, *J* = 6.3 Hz, 1H), 2.77 (t, *J* = 7.6 Hz, 2H), 2.63–2.54 (m, 3H), 2.46–2.40 (m, 1H), 2.24–2.19 (m, 1H), 1.90 (p, *J* = 6.8, 6.2 Hz, 2H), 1.68–1.62 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.5, 151.6, 145.7, 144.0, 142.8, 140.1, 138.2, 136.4, 135.9, 116.6, 112.0, 74.4, 66.5, 35.5, 32.5, 31.0, 30.3, 27.2, 25.1, HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_6S$ $[M-H]^+$, 419.1282; found 419.1260. Optical rotation [α] = +10.9° ± 2.8°.

(S)-(–)-4-nitro-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(21b)***

To a solution of (*R*)-(+)-2-Methyl-CBS-oxazaborolidine (25.5 mg, 0.09 mmol) in THF (1.5 mL) at 0 °C was added 2.0 M solution of borane di-methylsulfide in THF (0.27 mL, 0.55 mmol). The mixture was stirred for 15 min before adding a solution of 4-nitro-3,5,6,7-tetrahydro-*s*-indacen-1(2H)-one **9** (100 mg, 0.46 mmol). After 30 min the reaction was quenched with MeOH (1.5 mL) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with 15% ethyl acetate in hexane. The titled compound was obtained as an off-white solid (er = 80:20 ratio *S* and *R* isomer respectively). This was further purified by column chromatography using a chiral column with isocratic elution (35% 0.05% HCOOH in CH_3CN and 0.05% HCOOH in H_2O). The titled compound was obtained as an off-white solid (30.0 mg, 30%, er = 99:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.52 (s, 1H), 5.42 (d, *J* = 5.9 Hz, 1H), 5.06 (q, *J* = 6.4 Hz, 1H), 3.24–3.11 (m, 3H), 3.02–2.96 (m, 1H), 2.94 (t, *J* = 7.5 Hz, 2H), 2.40–2.35 (m, 1H), 2.13–2.04 (m, 2H), 1.86–1.80 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 148.5, 146.2, 142.5, 138.7, 136.0, 125.4, 73.5, 35.1, 32.6, 32.0, 29.4, 24.9. HRMS (ESI) *m/z* calculated for $C_{12}H_{12}NO_2$ $[M-18+H]^+$, 202.0863; found 202.0865.

(S)-(–)-4-amino-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(25b)***

A solution of (S)-*(–)*-4-nitro-1,2,3,5,6,7-hexahydro-s-indacen-1-ol **21b** (25.0 mg, 0.15 mmol) in MeOH (8 mL) was degassed with nitrogen for 5 min, 10% Pd/C (5.0 mg, 20% wt/wt) was added

and the reaction stirred under hydrogen atmosphere (1 atm) at room temperature until completion (approx. 2 h). The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* to give (S)-*(–)*-4-amino-1,2,3,5,6,7-hexahydro-*s*-indacen-1-ol as an off-white solid (20.0 mg, 95%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.43 (s, 1H), 4.87 (q, *J* = 5.9 Hz, 1H), 4.84 (d, *J* = 5.7 Hz, 1H), 4.54 (s, 2H), 2.74–2.68 (m, 2H), 2.67–2.62 (m, 1H), 2.57 (t, *J* = 7.3 Hz, 2H), 2.40–2.33 (m, 1H), 2.27–2.22 (m, 1H), 1.97–1.92 (m, 2H), 1.73–1.67 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 145.5, 142.6, 139.5, 125.8, 124.0, 108.1, 74.7, 35.5, 32.6, 28.9, 25.9, 25.0. HRMS (ESI) *m/z* calculated for $C_{12}H_{16}NO$ $[M+H]^+$, 190.1226; found 190.1234.

(S)-(–)-4-isocyanato-1,2,3,5,6,7-hexahydro-s-indacen-1-ol ***(26b)***

To di-*t*-butyldicarbonate (29.0 mg, 0.13 mmol) in anhydrous CH_3CN (1 mL) was added DMAP (4.6 mg, 0.03 mmol) at room temperature, stirred for 5 min, a solution of amine intermediate **25b** (18.0 mg, 0.09 mmol) in THF (1 mL) was added. The reaction mixture was stirred for 30 min at room temperature. The reaction mixture was used directly in the next step without workup.

(S)-(–)-N-((1-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide ***(2b)***

To 4-(2-hydroxypropan-2-yl) furan-2-sulfonamide intermediate (18.0 mg, 0.18 mmol) in anhydrous THF (1 mL) was added NaH (3.49 mg, 0.08 mmol) at 0 °C and stirred for 30 min at ambient temperature under nitrogen atmosphere. The solution was again cooled to 0 °C, isocyanate **26b** (previous step reaction mixture) was added and the mixture stirred at ambient temperature for 16 h. To the reaction mixture was added H_2O (0.5 mL), and the resulting solution was loaded directly onto a C18 column for purification using aqueous 10 mM $(NH_4)HCO_3$ solution and CH_3CN as mobile phase. Re-purified by semi prep HPLC using 0.05% HCOOH/H_2O and 0.05% HCOOH/ CH_3CN as mobile phase. Fractions were neutralized with 10 mM $(NH_4)HCO_3$ solution and freeze dried The titled compound was obtained as an off-white solid (3.0 mg, 7 %). ^{1}H NMR (600 MHz, DMSO-d_6): δ ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.65 (s, 1H), 7.02 (s, 1H), 6.95 (s, 1H), 5.06 (d, *J* = 5.8 Hz, 1H), 5.04 (s, 1H), 4.91 (q, *J* = 6.3 Hz, 1H), 2.77 (t, *J* = 7.6 Hz, 2H), 2.63–2.54 (m, 3H), 2.46–2.40 (m, 1H), 2.24–2.19 (m, 1H), 1.90 (p, *J* = 6.8, 6.2 Hz, 2H), 1.68–1.62 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (150 MHz,

DMSO-d_6): δ 159.4, 151.6, 145.8, 144.0, 142.7, 140.1, 138.2, 136.4, 135.9, 116.6, 112.0, 74.4, 66.5, 35.5, 32.5, 31.0, 30.3, 27.2, 25.1, HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_6S$ $[M-H]^+$, 419.1282; found 419.1264. Optical rotation [α] = –9.8° ± 0.9°.

3. Crystal structure data for compound 8, 9 and 10

Crystal structure for 8-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one (Compound 8)

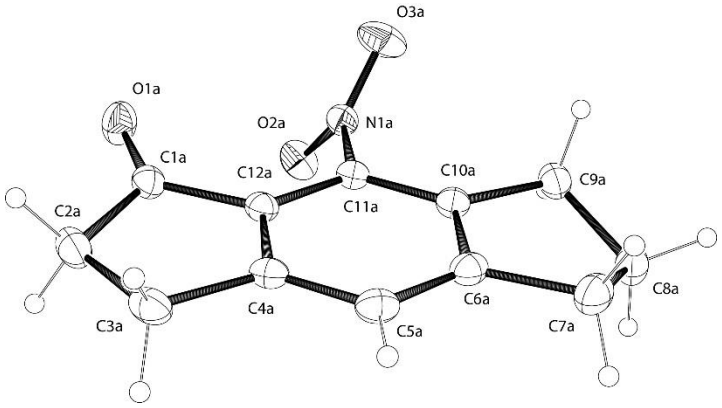


Table 1. Crystal data and structure refinement for 8-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one (Compound 8).

Identification code	1523ms-7760-25-spot3	
Empirical formula	$C_{12}H_{11}NO_3$	
Formula weight	217.22	
Temperature	190(2) K	
Wavelength	1.54184 Å	
Crystal system	Triclinic	
Space group	$P\ \bar{1}$	
Unit cell dimensions	a = 8.8200(6) Å	α = 93.133(5)°.
	b = 8.9621(6) Å	β = 94.496(5)°.
	c = 12.9701(6) Å	γ = 93.889(5)°.
Volume	1017.87(11) $Å^3$	
Z	4	
Density (calculated)	1.417 Mg/m^3	
Absorption coefficient	0.853 mm^{-1}	
F(000)	456	
Crystal size	0.3 x 0.1 x 0.05 mm^3	
Theta range for data collection	3.42 to 62.39°.	

Table 1. (Continued)

Index ranges	-10<=h<=10, -10<=k<=10, -14<=l<=11
Reflections collected	7183
Independent reflections	3209 [R(int) = 0.0322]
Completeness to theta = 62.39°	99.3 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1 and 0.86025
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3209 / 0 / 289
Goodness-of-fit on F^2	1.061
Final R indices [I>2sigma(I)]	R1 = 0.0425, wR2 = 0.1105
R indices (all data)	R1 = 0.0514, wR2 = 0.1172
Largest diff. peak and hole	0.223 and -0.231 e.$Å^{-3}$

Crystal structure for 4-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one (Compound 9)

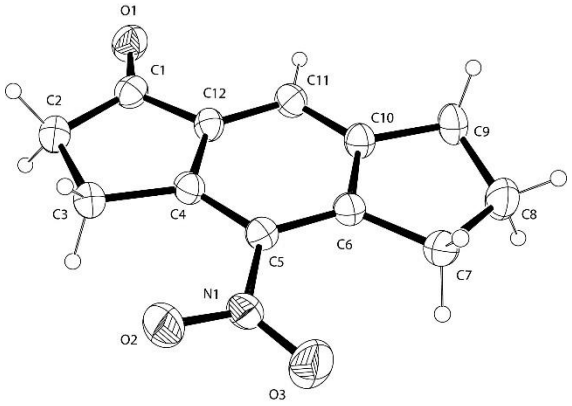


Table 2. Crystal data and structure refinement for 4-nitro-3,5,6,7-tetrahydro-s-indacen-1(2H)-one (Compound 9).

Identification code	1524ms-7760-25-spot1
Empirical formula	$C_{12}H_{11}N\ O_3$
Formula weight	217.22
Temperature	190(2) K
Wavelength	1.54184 Å

Table 2. (Continued)

Crystal system	Orthorhombic	
Space group	*P n* 2_1 *a*	
Unit cell dimensions	a = 7.292(1) Å	α = 90°.
	b = 8.974(2) Å	β = 90°.
	c = 15.007(2) Å	γ = 90°.
Volume	982.0(3) $Å^3$	
Z	4	
Density (calculated)	1.469 Mg/m^3	
Absorption coefficient	0.884 mm^{-1}	
F(000)	456	
Crystal size	0.4 x 0.05 x 0.05 mm^3	
Theta range for data collection	5.74 to 62.47°.	
Index ranges	-6<=h<=8, -10<=k<=10, -14<=l<=17	
Reflections collected	3300	
Independent reflections	831 [R(int) = 0.0540]	
Completeness to theta = 62.47°	98.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1 and 0.26955	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	831 / 1 / 145	
Goodness-of-fit on F^2	1.235	
Final R indices [I>2sigma(I)]	R1 = 0.0644, wR2 = 0.1671	
R indices (all data)	R1 = 0.0695, wR2 = 0.1746	
Absolute structure parameter	-10(10)	
Largest diff. peak and hole	0.247 and -0.206 $e.Å^{-3}$	

Crystal structure for 5-nitro-1,6,7,8-tetrahydro-as-indacen-3(2H)-one (Compound 10)

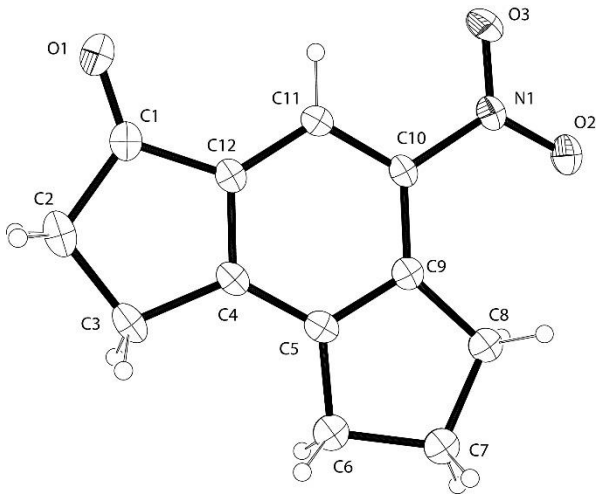


Table 3. Crystal data and structure refinement for 5-nitro-1,6,7,8-tetrahydro-s-indacen-3(2H)-one (Compound 10)

Identification code	1521ms-7760-25-spot2	
Empirical formula	C_{12} H_{11} N O_3	
Formula weight	217.22	
Temperature	190(2) K	
Wavelength	1.54184 Å	
Crystal system	Orthorhombic	
Space group	*P n m a*	
Unit cell dimensions	a = 9.4915(6) Å	α = 90°.
	b = 6.6626(6) Å	β = 90°.
	c = 15.7656(11) Å	γ = 90°.
Volume	996.99(13) $Å^3$	
Z	4	
Density (calculated)	1.447 Mg/m^3	

Table 3. (Continued)

Absorption coefficient	0.871 mm^{-1}
F(000)	456
Crystal size	0.25 x 0.15 x 0.08 mm^3
Theta range for data collection	5.44 to 62.24°.
Index ranges	-5<=h<=10, -6<=k<=7, -17<=l<=18
Reflections collected	2384
Independent reflections	867 [R(int) = 0.0283]
Completeness to theta = 62.24°	99.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1 and 0.78195
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	867 / 3 / 100
Goodness-of-fit on F^2	1.050
Final R indices [I>2sigma(I)]	R1 = 0.0508, wR2 = 0.1327
R indices (all data)	R1 = 0.0593, wR2 = 0.1438
Extinction coefficient	0.0031(12)
Largest diff. peak and hole	0.255 and -0.314 e.$Å^{-3}$

In the crystal structure of compound **8**, two molecules were found in the asymmetric unit both with significantly different conformations. Interestingly, the molecule shown in Figure 3 exhibits an unusual planar conformation of the five-membered ring that includes C7b, C8b and C9b, i.e. all methylene protons are eclipsed. The nitro group is twisted 77.2° away from the plane of the aromatic ring. The other molecule in the asymmetric unit exhibits an envelope conformer for this same ring and the nitro group is only twisted by 60° from the plane of the aromatic ring. In both cases the neighboring carbonyl O-atom is responsible for this twist. In the structure of compound **9** the nitro group is only twisted by 11.1° from the plane of the 6-membered ring and an envelope conformer is identified for the tri-methylene containing five-membered ring. The crystal structure of compound **10** finds the molecule located on a crystallographic mirror plane. However, the molecule is not planar and the tri-methylene carbon chain is disordered either side

of this plane defining a disordered envelope conformer with the central methylene carbon (C7) well removed from the mirror plane. The other five-membered ring bearing the carbonyl group is perfectly planar in compounds **8**, **9** and **10**.

4. Biological Assays.

NLRP3 inhibition assay

To generate human monocyte derived macrophages (HMDM), human monocytes were isolated from buffy coat blood using Ficoll-Plaque Plus (GE Healthcare, Australia) and density centrifugation. CD14+ cell selection was performed using MACS magnetic beads (Miltenyl Biotec). Isolated CD14+ monocytes were differentiated in culture for 7 days with 10 ng/ml human CSF-1 (Miltenyl Biotec) in Iscove's modified Dulbecco's medium (IMDM) containing L-glutamine supplemented with 10% FBS and 1% penicillin/streptomycin (Life Technologies) as previously described by Croker *et al.*[1]

HMDM were seeded at 1×10^5 cells/well in 96-well TC plates. The following day cells were stimulated with 10 ng/ml LPS from Escherichia coli serotype 0111:B4 (Sigma Aldrich) for 3 h. The medium was removed and replaced with serum free medium (SFM) containing test compound, 30 min prior to NLRP3 stimulation. Cells were then stimulated with adenosine-5-triphosphate (ATP) disodium salt hydrate (Roche) at 5 mM for 1 h. Supernatants were then removed and stored at -20 °C till needed for assay.

Supernatants were analyzed for IL-1β using ELISA kits according to the manufacturer's instructions (BD Biosciences OptEIA™).

Metabolism by human liver microsomes.

MCC950 (5 μM) was combined with human liver microsomes (1 mg/mL), in phosphate buffer (pH 7.4, pre-heated at 37 °C for 20 mins), then NADPH (1 mM) was added to initiate the reaction. A 180 μL aliquot was immediately transferred to an Eppendorf tube (for a t = 0 sample) and quenched with 540 μL of precipitation solution (cold CH_3CN+IS), vortexed, and incubated at 4 °C for 15 min. The remainder of the reaction mixture was then transferred to the incubator and shaken at 37 °C at 150 rpm. Another 180 μL aliquot was processed at time t = 120 min and the above process repeated. The tubes were retrieved from the fridge, centrifuged at 14,000 x *g*

for 8 min and 180 µL of each supernatant were transferred to glass vial inserts for LC-MS/MS analysis.

Metabolism by recombinant cytochrome P450s

Recombinant P450s were co-expressed with human NADPH-cytochrome P450 reductase (hCPR) and the bacterial chaperones GroES and GroEL as outlined previously in Notley *et al*.[2] Bacterial cultures were harvested by centrifugation at 2200 x *g* for 10 min then resuspended in sonication buffer (100 mM potassium phosphate, 6 mM Mg acetate, 20% v/v glycerol, 0.1 mM DTT, pH 7.4) containing 2 µM leupeptin, 1 µM bestatin, and 0.04 U/ml aprotinin. Resuspended cells were disrupted by sonication and membranes were isolated by ultracentrifugation at 180,000 x *g* for 65 min then resuspended in TES buffer (50 mM Tris acetate, 250 mM sucrose, and 0.25 mM EDTA, pH 7.4). P450s were quantified by Fe(II).CO *vs* Fe(II) difference spectroscopy.[3] Bacterial membranes were used directly for analysis of MCC950 metabolism, using incubations containing 0.1 µM P450, with a total protein concentration of 1.2 µg/µL, in 100 mM potassium phosphate buffer at pH 7.4. MCC950 was added to final concentrations of 10 µM and 100 µM from a methanolic stock such that the final methanol concentration in incubations was 1 % v/v. Reactions were initiated with the addition of an NADPH generating system consisting of 10 mM glucose-6-phosphate, 250 µM NADP+ and 0.5 U/mL glucose-6-phosphate dehydrogenase and incubated with gentle agitation at 37 °C. After 120 min incubation, reactions were quenched and metabolites were extracted by addition of 2 x reaction volumes of CH_3CN. Extracts were prepared for LC-MS/MS analysis as described above for microsomal incubations.

Cytotoxicity assay.

The compounds were serially diluted in DMEM (Dulbecco's Modified Eagle Medium) with 10% FBS (Fetal Bovine Serum) in a 1 in 2 step decrease to give the two times concentrated range of 125 µM to 1.0 µM. A 20 µl aliquot of each dilution was added to a 384-well black wall clear bottom tissue culture plate in n = 2.

Medium only without cells served as negative control ("all cells are dead") and in addition a serial dilution of Tamoxifen (Sigma-Aldrich), starting at 200 µM final concentration, was included on each plate as a dose response control.

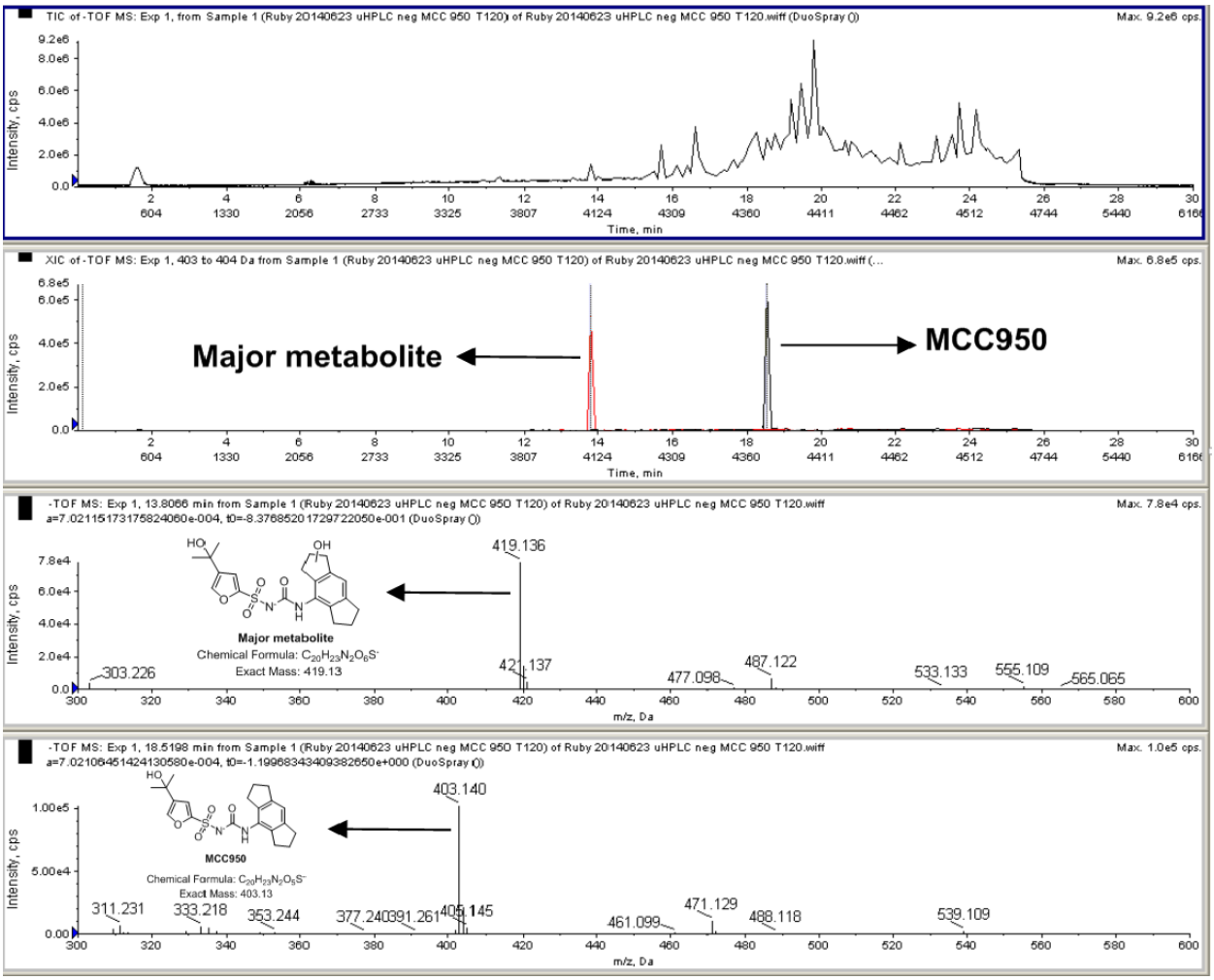
Hek293 and HepG2 cells were seeded into the plates containing the compounds, at 4000 and 5000 per well, respectively, in a volume of 20 µl in DMEM medium with 10% of FBS (Vtot = 40 µl), giving the final compound concentration range of 62.5 to 0.5 µM. The cells together with the compounds were incubated for 20 h at 37 °C, 5% CO_2.

After the incubation, 5 µl of 100 µM Resazurin (Sigma-Aldrich) dissolved in PBS was added to each well (final concentration was approx. 11 µM). The plates were then incubated for 3 h at 37 °C, 5% CO_2. The fluorescence intensity was read using the TECAN Infinite M1000 PRO with excitation/emission of 560/590 nm.

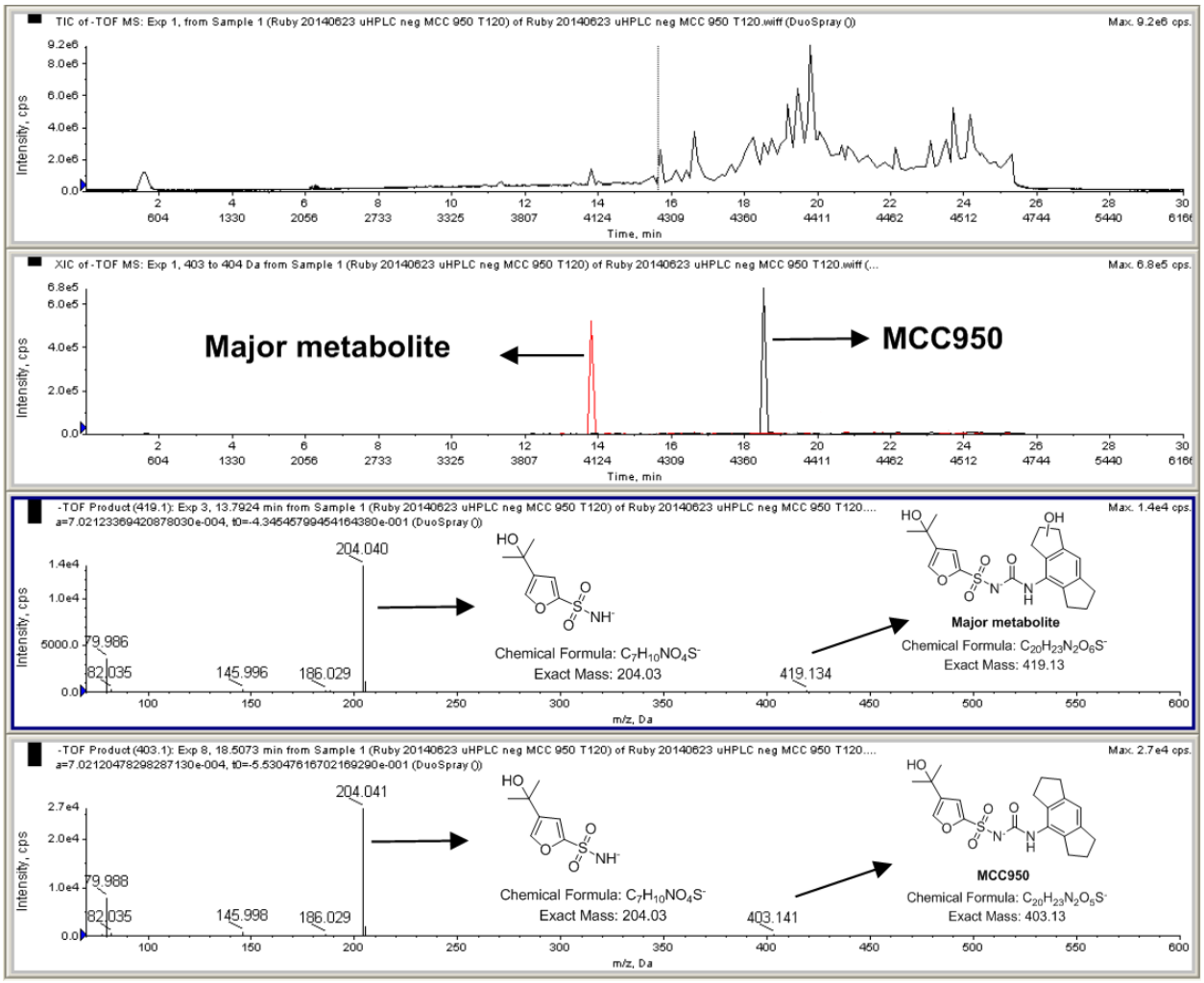
The data was analyzed by GraphPad Prism software. Results are presented as the average percentage of the controls using the following equation: Cell survival % = (FISample – FINegative/FIUntreated –FINegative)*100.

5. LC-MS and LC-MS/MS data of MCC950 and major metabolite

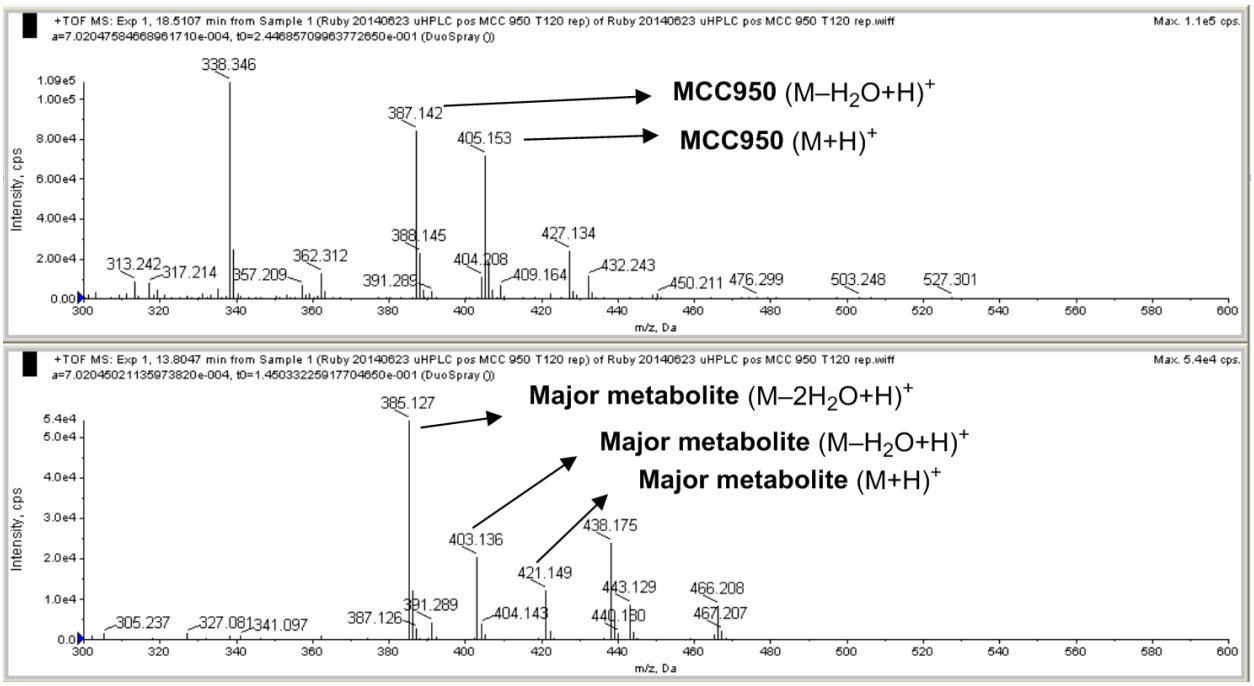
(–)-QTOF-ESI-LC-MS spectra of MCC950 and major metabolite



(–)-QTOF-ESI-LC-MS/MS spectra of MCC950 and major metabolite



(+)-QTOF-ESI-LC-MS spectra of MCC950 and major metabolite



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