

Design, Synthesis and Biological Characterization of a Caspase 3/7 Selective Isatin Labeled with 2-[¹⁸F]fluoroethylazide

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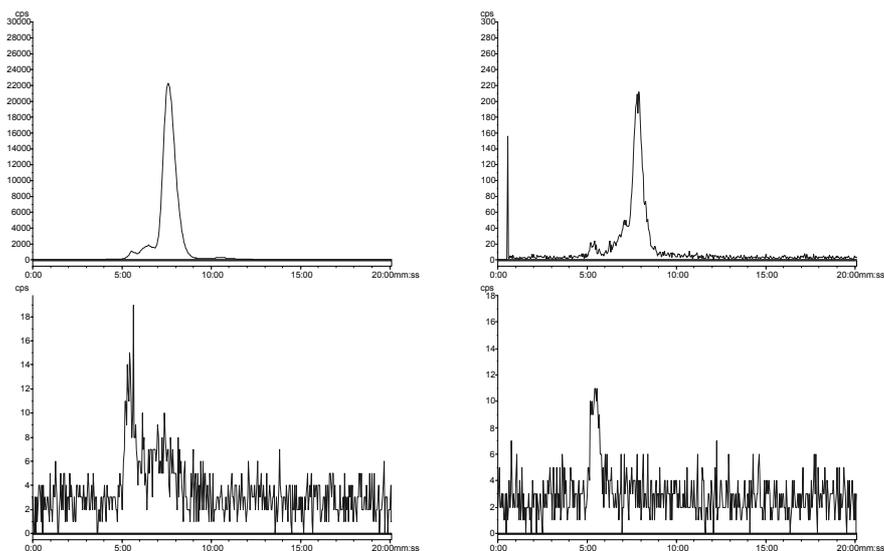


Figure S1. In vivo metabolism analysis of $[^{125}\text{I}]\mathbf{4}$ by radio-HPLC. Top row, left: $[^{125}\text{I}]\mathbf{4}$ control; right, plasma 2 mins sample; bottom row, left, plasma 10 mins sample; right, plasma 30 mins sample.

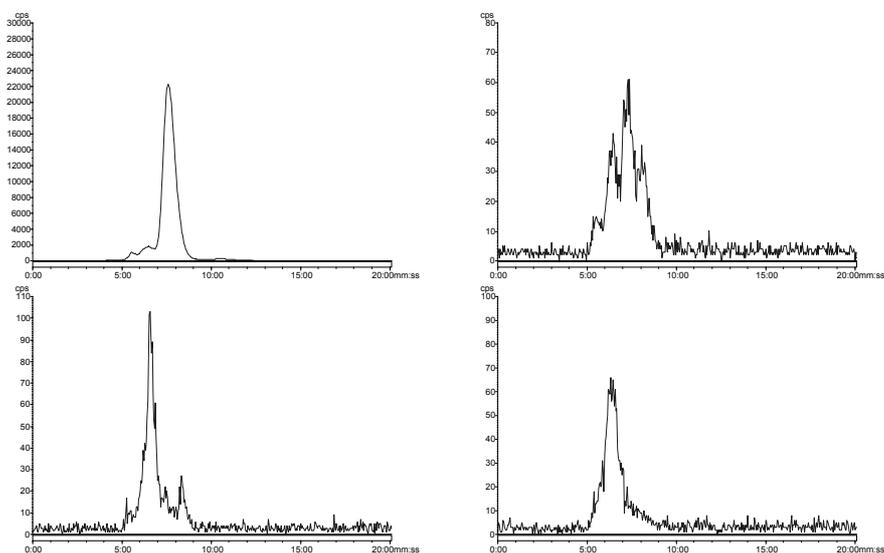


Figure S2. In vivo metabolism analysis of $[^{125}\text{I}]\mathbf{4}$ by radio-HPLC. Top row, left: $[^{125}\text{I}]\mathbf{4}$ control; right, liver 2 mins sample; bottom row, left, liver 10 mins sample; right, liver 30 mins sample.

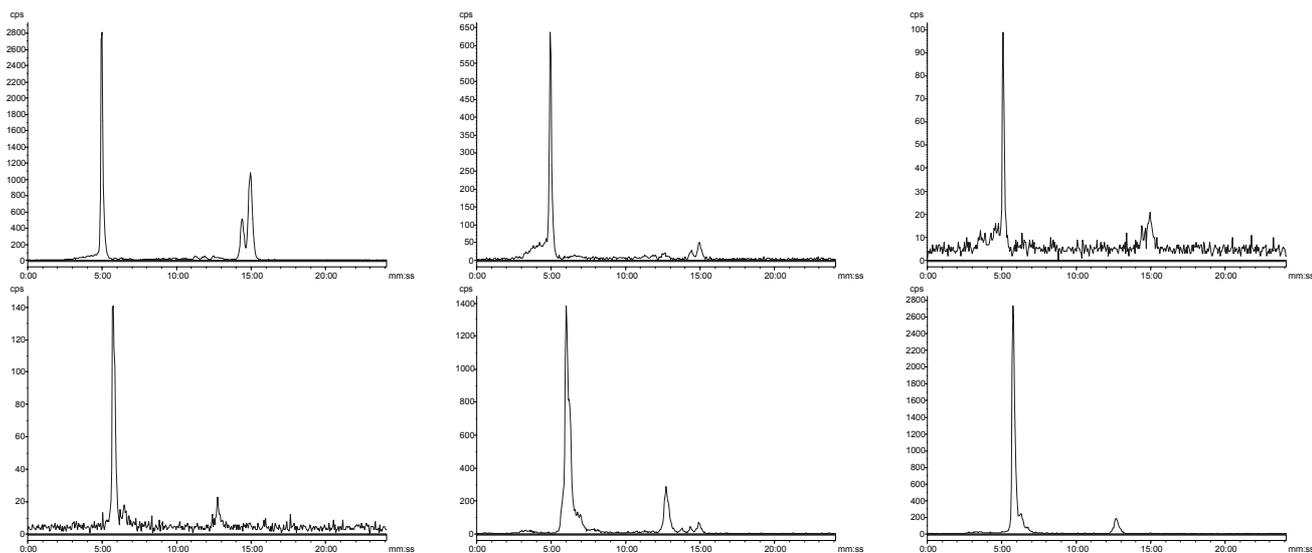


Figure S3. *In vivo* metabolism of [^{18}F]**15** assessed in liver and urine by radio-HPLC. Top row liver extracts, bottom row urine extracts, left to right 2, 15 and 60 minutes respectively.

Experimental Section

Chemistry. Reagents and solvents were purchased from Sigma-Aldrich (Gillingham, United Kingdom) and used without further purification. Potassium hydroxide and potassium carbonate were stored in a vacuum desiccator over phosphorus pentoxide. All reactions were carried out under argon unless otherwise stated. Petroleum ether refers to the fraction that is distilled between 40°C and 60°C. Automated flash chromatography was performed on a CombiFlash Companion machine (Companion Presearch Ltd.), using RediSep 4 g or 12 g normal phase silica cartridges (flow rate 12 mL/min or 26 mL/min). Manual flash chromatography was carried out using Davisil neutral silica (60 Å, 60-200 micron, Fisher Scientific, Loughborough, UK), solvent mixtures are quoted as volume/volume. ^1H NMR Spectra were obtained on a Bruker Avance 600 MHz NMR machine and spectra are referenced to residual solvent. Coupling constants (J) are given in Hertz (Hz). GC-MS data was acquired under electron ionization using an Agilent 6890N system. Mass

spectra were obtained in positive electrospray ionization mode on a WatersMicromass LCT Premier or a Finnigan MAT 900 XLT machine. Melting points were determined in capillary tubes on a Stuart Scientific SMP1 melting point apparatus and are uncorrected. Solvent mixtures for thin layer chromatography (TLC) are quoted as volume/volume and samples were developed on aluminium backed neutral silica plates (0.2 mm thickness) (Fluka, Seelze, Germany). Purity analysis for compounds **2**, **4**, **5**, **6**, **10b-10h**, **11b-11h**, **12**, **13**, **14**, **15** and **16** was evaluated by analytical HPLC; compounds **9b-9h** were analyzed by GC-MS with only a single peak observed in the GC trace. Tabulated values of purity can be found below and the purity of all compounds was greater than 95%.

(S)-tert-Butyl 2-(3-fluorophenoxymethyl)pyrrolidine-1-carboxylate (9c). The carboxylate **9c** was prepared according to the procedure for **9b** except using 3-fluorophenol. Chromatography (hexanes/ethyl acetate) afforded the title compound as a colorless oil (0.32 g, 54%). HRMS (ESI) = 296.1657 (M + H)⁺. Calcd. for C₁₆H₂₃FNO₃ 296.1656 ¹H NMR (600 MHz, CDCl₃) δ 7.20-7.15 (m, 1 H), 6.73-6.69 (m, 1 H), 6.65-6.59 (m, 2 H), 4.18-4.03 (m, 2 H), 3.97-3.74 (m, 1 H), 3.48-3.29 (m, 2 H), 2.05-1.79 (m, 4 H), 1.48 (s, 9 H). TLC (UV₂₅₄) R_f = 0.40 (3:1 hexanes/ethyl acetate).

(S)-tert-Butyl 2-(2,4-difluorophenoxymethyl)pyrrolidine-1-carboxylate (9d). The carboxylate **9d** was prepared according to the procedure for **9b** except using 2,4-difluorophenol. Chromatography (hexanes/ethyl acetate) afforded the title compound as a colorless oil (1.92 g, 58%). HRMS (ESI) = 314.1560 (M + H)⁺. Calcd. for C₁₆H₂₂F₂NO₃ 314.1562. ¹H NMR (600 MHz, CDCl₃) δ 7.08-6.76 (m, 3 H), 4.21-3.87

(m, 3 H), 3.47-3.32 (m, 2 H), 2.16-1.85 (m, 4 H), 1.48 (s, 9 H). TLC (UV₂₅₄) R_f = 0.51 (2:1 hexanes/ethyl acetate).

(S)-tert-Butyl 2-(3,5-difluorophenoxymethyl)pyrrolidine-1-carboxylate (9e). The carboxylate **9e** was prepared according to the procedure for **9b** except using 3,5-difluorophenol. Chromatography (hexanes/ethyl acetate) afforded the title compound as a colorless oil (0.31 g, 53%). HRMS (ESI) = 314.1563 (M + H)⁺. Calcd. for C₁₆H₂₂F₂NO₃ 314.1562. ¹H NMR (600 MHz, CDCl₃) δ 6.44-6.29 (m, 3 H), 4.11-3.98 (m, 2 H), 3.91-3.72 (m, 1 H), 3.42-3.24 (m, 2H), 1.99-1.79 (m, 4 H), 1.42 (s, 9 H). TLC (UV₂₅₄) R_f = 0.56 (2:1 hexanes/ethyl acetate).

(S)-tert-Butyl 2-(4-tetrahydropyranoxymethyl)pyrrolidine-1-carboxylate (9f). The carboxylate **9f** was prepared according to the procedure for **9b** except using 4-hydroxytetrahydropyran. Chromatography (ethyl acetate) afforded the title compound as a colorless oil (0.14 g, 25%). HRMS (ESI) = 286.2012 (M + H)⁺. Calcd. for C₁₅H₂₈NO₄ 286.2013. ¹H NMR (600 MHz, CDCl₃) δ 3.97-3.83 (m, 2 H), 3.64-3.23 (m, 9 H), 1.97-1.72 (m, 5 H), 1.60-1.52 (m, 2 H), 1.46 (s, 9 H). TLC (I₂) R_f = 0.62 (ethyl acetate).

(S)-5-(2-(3-Fluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (10c). The isatin **10c** was prepared according to the procedure for **10b** except using **9c** to give an orange solid (93 mg, 46%). Mp: 201-203°C. HRMS (ESI) = 405.0933 (M + H)⁺. Calcd. for C₁₉H₁₈FN₂O₅S 405.0920. ¹H NMR (600 MHz, CDCl₃) δ 8.14 (br, 1 H), 8.09-8.06 (m, 2 H), 7.21 (q, J = 7.2 Hz, 1 H), 7.03 (d, J = 7.8 Hz, 1 H), 6.67-6.62 (m, 2 H), 6.55 (dt, J = 10.8 Hz, J = 2.4 Hz, 1 H), 4.17 (dd, J = 9 Hz, J = 3 Hz, 1 H), 4.01-3.97 (m, 1 H), 3.94 (dd, J = 9 Hz, J = 7.2 Hz, 1 H), 3.54-3.50 (m, 1 H), 3.27-3.25 (m,

1 H), 2.08-1.96 (m, 2 H), 1.88-1.77 (m, 2 H). TLC (UV₂₅₄) R_f = 0.36 (2:1 ethyl acetate/hexanes). HPLC t_R = 8.27 min.

(S)-5-(2-(2,4-Difluorophenoxy)methyl)-pyrrolidine-1-sulfonyl)isatin (10d).

The isatin **10d** was prepared according to the procedure for **10b** except using **9d** to give an orange solid (0.86 g, 34%). Mp: 185-187°C. HRMS (ESI) = 423.0834 (M + H)⁺. Calcd. for C₁₉H₁₇F₂N₂O₅S 423.0826. ¹H NMR (600 MHz, CDCl₃) δ 8.08-8.06 (m, 2 H), 7.97 (br, 1 H), 7.03 (d, J = 9 Hz, 1 H), 6.97-6.92 (m, 1 H), 6.87-6.77 (m, 2 H), 4.21 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 4.03-3.97 (m, 2 H), 3.56-3.52 (m, 1 H), 3.23-3.17 (m, 1 H), 2.11-2.01 (m, 2 H), 1.88-1.75 (m, 2 H). TLC (UV₂₅₄) R_f = 0.46 (2:1 ethyl acetate/hexanes). HPLC t_R = 8.12 min.

(S)-5-(2-(3,5-Difluorophenoxy)methyl)-pyrrolidine-1-sulfonyl)isatin (10e). The isatin **10e** was prepared according to the procedure for **10b** except using **9e** to give an orange solid (112 mg, 53%). Mp: 196-198°C. HRMS (ESI) = 423.0834 (M + H)⁺. Calcd. for C₁₉H₁₇F₂N₂O₅S 423.0826. ¹H NMR (600 MHz, CDCl₃) δ 8.10-8.06 (m, 2 H), 8.04 (br, 1 H), 7.05 (d, J = 8.4 Hz), 6.45-6.38 (m, 3 H), 4.18 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 3.99-3.92 (m, 2 H), 3.56-3.49 (m, 1 H), 3.24-3.17 (m, 1 H), 2.04-1.92 (m, 2 H), 1.86-1.77 (m, 2 H). TLC (UV₂₅₄) R_f = 0.36 (2:1 ethyl acetate/hexanes). HPLC t_R = 9.18 min.

(S)-5-(2-(Tetrahydro-2H-pyran-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin

(10f). The isatin **10f** was prepared according to the procedure for **10b** except using **9f** to give an orange gum (63 mg, 32%). HRMS (ESI) = 395.1282 (M + H)⁺. Calcd. for C₁₈H₂₃N₂O₆S 395.1277. ¹H NMR (600 MHz, CDCl₃) δ 8.17 (br, 1 H), 8.10-8.08 (m, 2

H), 7.07 (d, $J = 8.4$ Hz, 1 H), 3.94-3.90 (m, 2 H), 3.77-3.73 (m, 1 H), 3.70 (dd, $J = 9.6$ Hz, $J = 3$ Hz, 1 H), 3.56-3.51 (septet, $J = 4.2$ Hz, 1 H), 3.48-3.44 (m, 3 H), 3.13-3.11 (m, 1 H), 2.05-1.87 (m, 4 H), 1.72-1.64 (m, 2 H), 1.60-1.52 (m, 2 H). TLC (UV₂₅₄) $R_f = 0.26$ (4:1 ethyl acetate/hexanes). HPLC $t_R = 2.65$ min.

(S)-5-(2-(Pyrimidin-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (10g). The isatin **10g** was prepared according to the procedure for **10b** except using **9g**.

Chromatography (ethyl acetate) gave an orange gum (51 mg, 27%). HRMS (ESI) = 389.025 (M + H)⁺. Calcd. for C₁₇H₁₇N₄O₅S 389.020. ¹H NMR (600 MHz, CDCl₃) δ 8.79 (s, 1 H), 8.45 (d, $J = 5.4$ Hz, 1 H), 8.12 (d, $J = 1.8$ Hz, 1 H), 8.09 (dd, $J = 8.4$ Hz, $J = 1.8$ Hz, 1 H), 7.95 (br, 1 H), 7.03 (d, $J = 8.4$ Hz, 1 H), 6.72 (d, $J = 5.4$ Hz, 1 H), 4.57 (dd, $J = 10.8$ Hz, $J = 4.8$ Hz, 1 H), 4.39 (dd, $J = 10.8$ Hz, $J = 7.2$ Hz, 1 H), 4.08-4.04 (m, 1 H), 3.53-3.46 (m, 1 H), 3.27-3.22 (m, 1 H), 2.01-1.93 (m, 2 H), 1.84-1.74 (m, 2 H). TLC (UV₂₅₄) $R_f = 0.49$ (9:1 ethyl acetate/methanol). HPLC $t_R = 1.90$ min.

(S)-5-(2-(2-Propynyloxymethyl)-pyrrolidine-1-sulfonyl)isatin (10h). The isatin **10h** was prepared according to the procedure for **10b** except using **9h** to give an orange gum (92 mg, 53%). HRMS (ESI) = 349.0867 (M + H)⁺. Calcd. for C₁₆H₁₇N₂O₅S 349.067. ¹H NMR (600 MHz, CDCl₃) δ 8.10-8.07 (m, 2 H), 7.84 (br, 1 H), 7.04 (d, $J = 8.4$ Hz, 1 H), 4.16 (s, 2 H), 3.83-3.79 (m, 1 H), 3.72 (dd, $J = 9.6$ Hz, $J = 3.6$ Hz, 1 H), 3.54 (dd, $J = 9.6$ Hz, $J = 3.6$ Hz, 1 H), 3.45-3.43 (m, 1 H), 3.19-3.15 (m, 1 H), 2.46 (t, $J = 2.4$ Hz, 1 H), 1.96-1.89 (m, 2 H), 1.74-1.67 (m, 2 H). TLC (UV₂₅₄) $R_f = 0.28$ (2:1 ethyl acetate/hexanes). HPLC $t_R = 2.93$ min.

(S)-1-(4-Fluorobenzyl)-5-(2-(3-fluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (11c). The isatin **11c** was prepared according to the procedure for **11b**

except using **10c** to give an orange gum (31 mg, 61%). HRMS (ESI) = 513.1298 (M + H)⁺. Calcd. for C₂₆H₂₃F₂N₂O₅S 513.1296. ¹H NMR (600 MHz, CDCl₃) δ 8.02 (d, *J* = 1.8 Hz, 1 H), 7.98 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.33-7.29 (m, 2 H), 7.18 (m, 1 H), 7.09-7.04 (m, 2 H), 6.86 (d, *J* = 8.4 Hz, 1 H), 6.66-6.62 (m, 2 H), 6.53 (dt, *J* = 10.8, *J* = 2.4 Hz, 1 H), 4.88 (s, 2 H), 4.15 (dd, *J* = 9.6 Hz, *J* = 3.6 Hz, 1 H), 3.98-3.88 (m, 2 H), 3.51-3.47 (m, 1 H), 3.23-3.18 (m, 1 H), 2.08-1.97 (m, 2 H), 1.84-1.72 (m, 2 H). TLC (UV₂₅₄) R_f = 0.64 (2:1 ethyl acetate/hexanes). HPLC t_R = 12.57 min.

(S)-1-(4-Fluorobenzyl)-5-(2-(2,4-difluorophenoxy)methyl)-pyrrolidine-1-sulfonylisatin (**11d**). The isatin **11d** was prepared according to the procedure for **11b** except using **10d** to give an orange gum (32 mg, 60%). HRMS (ESI) = 531.1204 (M + H)⁺. Calcd. for C₂₆H₂₂F₃N₂O₅S 531.1202. ¹H NMR (600 MHz, CDCl₃) δ 8.03 (d, *J* = 1.8 Hz, 1 H), 7.98 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.33-7.30 (m, 2 H), 7.09-7.06 (m, 2 H), 6.95-6.91 (m, 1 H), 6.88 (d, *J* = 8.4 Hz, 1 H), 6.81-6.76 (m, 2 H), 4.93 (d, *J* = 16.2 Hz, 1 H), 4.92 (d, *J* = 16.2 Hz, 1 H), 4.18 (dd, *J* = 9 Hz, *J* = 3 Hz, 1 H), 4.00-3.95 (m, 2 H), 3.51-3.49 (m, 1 H), 3.21-3.17 (m, 1 H), 2.09-1.98 (m, 2 H), 1.85-1.74 (m, 2 H). TLC (UV₂₅₄) R_f = 0.67 (2:1 ethyl acetate/hexanes). HPLC t_R = 12.50 min.

(S)-1-(4-Fluorobenzyl)-5-(2-(3,5-difluorophenoxy)methyl)-pyrrolidine-1-sulfonylisatin (**11e**). The isatin **11e** was prepared according to the procedure for **11b** except using **10e** to give an orange gum (31 mg, 58%). HRMS (ESI) = 531.1213 (M + H)⁺. Calcd. for C₂₆H₂₂F₃N₂O₅S 531.1202. ¹H NMR (600 MHz, CDCl₃) δ 8.04 (d, *J* = 1.8 Hz, 1 H), 7.99 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.34-7.29 (m, 2 H), 7.09-7.06 (m, 2 H), 6.90 (d, *J* = 8.4 Hz, 1 H), 6.44-6.38 (m, 3 H), 4.93 (s, 2 H), 4.18-4.15 (m, 1 H), 3.94-3.89 (m, 2 H), 3.51-3.48 (m, 1 H), 3.19-3.15 (m, 1 H), 2.03-1.93 (m, 2 H), 1.83-

1.73 (m, 2 H). TLC (UV₂₅₄) R_f = 0.64 (2:1 ethyl acetate/hexanes). HPLC t_R = 13.00 min.

(S)-1-(4-Fluorobenzyl)-5-(2-(tetrahydro-2H-pyran-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (11f). The isatin **11f** was prepared according to the procedure for **11b** except using **10f** to give an orange gum (26 mg, 52%). HRMS (ESI) = 503.1646 (M + H)⁺. Calcd. for C₂₅H₂₈FN₂O₆S 503.1652. ¹H NMR (600 MHz, CDCl₃) δ 8.06 (d, *J* = 1.8 Hz, 1 H), 8.01 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.34-7.31 (m, 2 H), 7.10-7.06 (m, 2 H), 6.91 (d, *J* = 8.4 Hz, 1 H), 4.87 (s, 2 H), 3.93-3.88 (m, 2 H), 3.73-3.70 (m, 1 H), 3.67 (dd, *J* = 9 Hz, *J* = 3 Hz, 1 H), 3.55-3.49 (m, 1 H), 3.47-3.41 (m, 4 H), 3.10-3.05 (m, 1 H), 1.97-1.85 (m, 4 H), 1.70-1.63 (m, 2 H), 1.59-1.54 (m, 2 H). TLC (UV₂₅₄) R_f = 0.55 (4:1 ethyl acetate/hexanes). HPLC t_R = 8.58 min.

(S)-1-(4-Fluorobenzyl)-5-(2-(pyrimidin-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (11g). The isatin **11g** was prepared according to the procedure for **11b** except using **10g** to give an orange gum (12 mg, 24%). HRMS (ESI) = 497.1287 (M + H)⁺. Calcd. for C₂₄H₂₂FN₄O₅S 497.1295. ¹H NMR (600 MHz, CDCl₃) δ 8.77 (s, 1 H), 8.43 (d, *J* = 5.4 Hz, 1 H), 8.08 (d, *J* = 1.8 Hz, 1 H), 8.01 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.34-7.32 (m, 2 H), 7.09-7.06 (m, 2 H), 6.89 (d, *J* = 8.4 Hz, 1 H), 6.70 (d, *J* = 5.4 Hz, 1 H), 4.90 (s, 2 H), 4.55 (dd, *J* = 10.8 Hz, *J* = 4.2 Hz, 1 H), 4.37 (dd, *J* = 10.8 Hz, *J* = 7.8 Hz, 1 H), 4.05-4.01 (m, 1 H), 3.50-3.46 (m, 1 H), 3.22-3.19 (m, 1 H), 1.98-1.87 (m, 2 H), 1.81-1.72 (m, 2 H). TLC (UV₂₅₄) R_f = 0.32 (2:1 ethyl acetate). HPLC t_R = 7.45 min.

(S)-1-(4-Fluorobenzyl)-5-(2-(2-propynyloxymethyl)-pyrrolidine-1-sulfonyl)isatin (11h). The isatin **11h** was prepared according to the procedure for **11b** except using **10h** to give an orange gum. Recrystallization from ethyl acetate/hexanes furnished the desired product as orange needles (93 mg, 58%). Mp: 115-117°C HRMS (ESI) = 457.1236 (M + H)⁺. Calcd. for C₂₃H₂₂FN₂O₅S 457.1233. ¹H NMR (600 MHz, CDCl₃) δ 8.07 (d, *J* = 1.8 Hz, 1 H), 8.01 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.43-7.31 (m, 2 H), 7.09-7.04 (m, 2 H), 6.90 (d, *J* = 8.4 Hz, 1 H), 4.91 (s, 2 H), 4.13 (d, *J* = 2.4 Hz, 2 H), 3.81-3.77 (m, 1 H), 3.69 (dd, *J* = 9.6 Hz, *J* = 4.2 Hz, 1 H), 3.51 (dd, *J* = 9 Hz, *J* = 7.2 Hz, 1 H), 3.43-3.40 (m, 1 H), 3.16-3.12 (m, 1 H), 2.43 (t, *J* = 2.4 Hz, 1 H), 1.94-1.87 (m, 2 H), 1.75-1.66 (m, 2 H). TLC (UV₂₅₄) R_f = 0.62 (2:1 ethyl acetate/hexanes). HPLC *t*_R = 8.80 min.

(S)-1-(2-Propynyl)-5-(2-(2,4-difluorophenoxy)methyl)-pyrrolidine-1-sulfonyl)isatin (13). The isatin **13** was prepared according to the procedure for **12** except using **10d** to give an orange solid (0.48 g, 70%). Mp: 101-103°C. HRMS (ESI) = 461.0976 (M + H)⁺. Calcd. for C₂₂H₁₉F₂N₂O₅S 461.0983. ¹H NMR (600 MHz, CDCl₃) δ 8.13 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 8.07 (d, *J* = 1.8 Hz, 1 H), 7.24 (d, *J* = 8.4 Hz, 1 H), 6.96-6.92 (m, 1 H), 6.85-6.78 (m, 2 H), 4.59 (dd, *J* = 18 Hz, *J* = 2.4 Hz, 1 H), 4.57 (dd, *J* = 18 Hz, *J* = 2.4 Hz, 1 H), 4.24-4.20 (m, 1 H), 4.03-3.98 (m, 2 H), 3.55-3.52 (m, 1 H), 3.26-3.21 (m, 1 H), 2.37 (t, *J* = 2.4 Hz, 1 H), 2.12-2.01 (m, 2 H), 1.88-1.76 (m, 2 H). TLC (UV₂₅₄) R_f = 0.63 (2:1 ethyl acetate/hexanes). HPLC *t*_R = 9.60 min.

(S)-1-((1-(2-Fluoroethyl)-1*H*-[1,2,3]-triazol-4-yl)methyl)-5-(2,4-difluorophenoxy)methyl)-pyrrolidine-1-sulfonyl)isatin (15). The isatin **15** was prepared according to the procedure for **14** except using **13** to give an orange gum. Recrystallization from ethyl acetate/hexanes gave an orange solid (94 mg, 57%). Mp:

130-131°C. HRMS (ESI) = 550.1381 (M + H)⁺. Calcd. for C₂₄H₂₃F₃N₅O₅S 550.1372.

¹H NMR (600 MHz, CDCl₃) δ 8.08 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 8.02 (d, *J* = 1.8 Hz, 1 H), 7.77 (s, 1 H), 7.52 (d, *J* = 8.4 Hz, 1 H), 6.95-6.91 (m, 1 H), 6.82-6.75 (m, 2 H), 5.05 (s, 2 H), 4.79 (dt, *J* = 46.2 Hz, *J* = 4.8 Hz, 2 H), 4.67 (dt, *J* = 26.4 Hz, *J* = 4.8 Hz, 2 H), 4.20 (dd, *J* = 9.6 Hz, *J* = 3 Hz, 1 H), 4.01-3.94 (m, 2 H), 3.53-3.50 (m, 1 H), 3.22-3.17 (m, 1 H), 2.10-1.98 (m, 2 H), 1.85-1.74 (m, 2 H). TLC (UV₂₅₄) R_f = 0.47 (ethyl acetate). HPLC *t*_R = 8.45 min.

(S)-1-(4-Fluorobenzyl)-5-(2-((1-(2-fluoroethyl)-1*H*-[1,2,3]-triazol-4-yl)methoxymethyl)-pyrrolidine-1-sulfonyl)isatin (16). The isatin **16** was prepared according to the procedure for **14** except using **11h** to give an orange gum (26 mg, 48%). HRMS (ESI) = 546.1632 (M + H)⁺. Calcd. for C₂₅H₂₆F₂N₅O₅S 546.1623. ¹H NMR (600 MHz, CDCl₃) δ 8.03 (d, *J* = 1.8 Hz, 1 H), 8.00 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.67 (s, 1 H), 7.35-7.33 (m, 2 H), 7.08-7.05 (m, 2 H), 6.90 (d, *J* = 8.4 Hz, 1 H), 4.95 (s, 2 H), 4.81 (dt, *J* = 46.8 Hz, *J* = 4.8 Hz, 2 H), 4.67 (dt, *J* = 26.4 Hz, *J* = 4.8 Hz, 2 H), 4.62 (d, *J* = 12 Hz, 1 H), 4.60 (d, *J* = 12 Hz, 1 H), 3.81-3.78 (m, 1 H), 3.65 (dd, *J* = 9.6 Hz, *J* = 4.2 Hz, 1 H), 3.51 (dd, *J* = 9.6 Hz, *J* = 7.2 Hz, 1 H), 3.40-3.37 (m, 1 H), 3.19-3.15 (m, 1 H), 1.92-1.87 (m, 2 H), 1.72-1.66 (m, 2 H). TLC (UV₂₅₄) R_f = 0.35 (ethyl acetate). HPLC *t*_R = 6.58 min.

Table S1. HPLC purity analysis of intermediate isatin sulfonamides.^a

Compound No.	Retention Time (mm:ss)	Purity (%)
10b	7.50	97
10c	8.16	98
10d	8.07	99
10e	9.11	98
10f	2.39	99
10g	1.54	98
10h	2.56	99
12	9.00	97
13	9.36	99

^aConditions: Phenomenex Luna 50 × 4.6 mm (3 μm) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 → 90% organic in 14 min; 90% organic for 4 min; 90 → 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

Table S2. HPLC purity analysis of final isatin sulfonamides.^a

Compound No.	Retention Time (mm:ss)	Purity (%)
2	12.00	97
4	14.07	98
5	12.08	98
6	6.50	98
11b	12.15	99
11c	12.34	98
11d	12.30	98
11e	13.00	99
11f	8.35	97
11g	7.27	99
11h	8.48	99
14	7.56	99
15	8.27	98
16	6.35	98

^aConditions: Phenomenex Luna 50 × 4.6 mm (3 μm) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 → 90% organic in 14 min; 90% organic for 4 min; 90 → 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

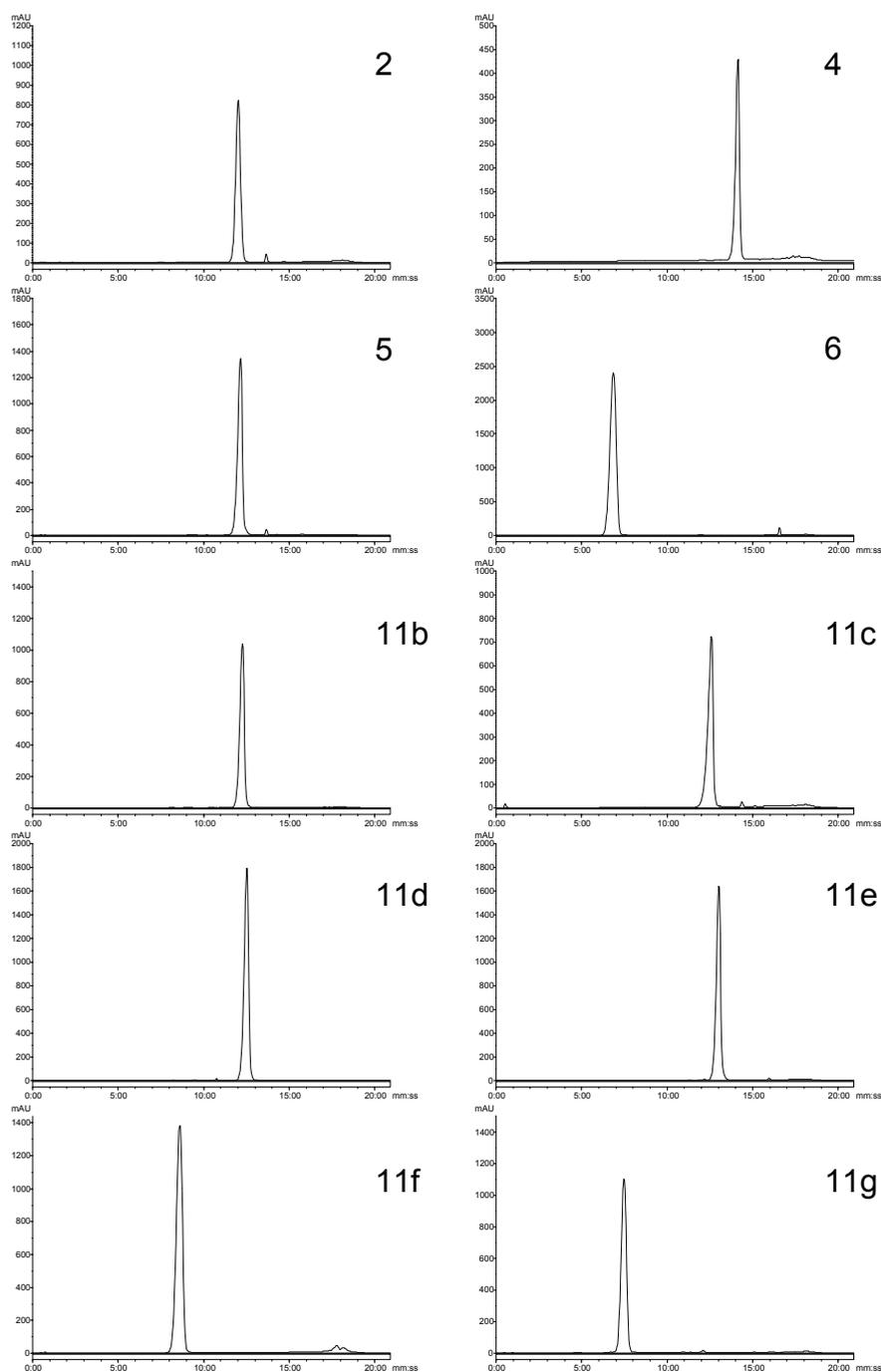


Figure S4. HPLC chromatograms for compounds **2-11g** screened for caspase affinity. Conditions: Phenomenex Luna 50×4.6 mm ($3 \mu\text{m}$) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 \rightarrow 90% organic in 14 min; 90% organic for 4 min; 90 \rightarrow 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

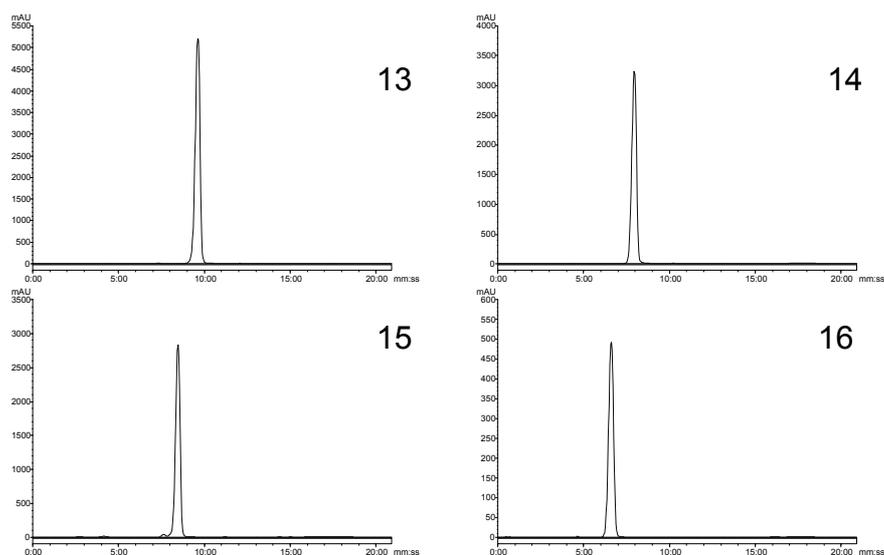
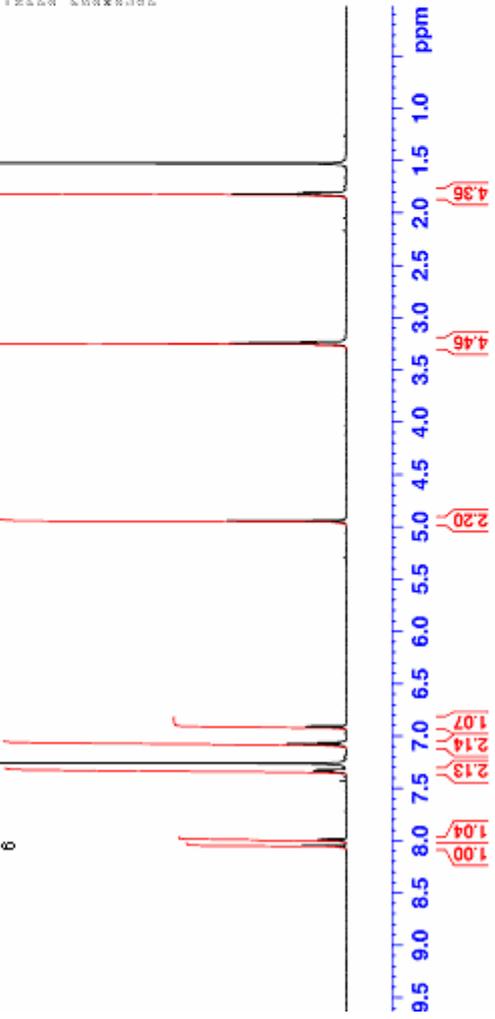
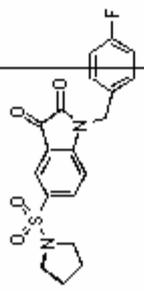


Figure S5. HPLC chromatograms for compounds **13-16** screened for caspase affinity. Conditions: Phenomenex Luna 50×4.6 mm ($3 \mu\text{m}$) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 \rightarrow 90% organic in 14 min; 90% organic for 4 min; 90 \rightarrow 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

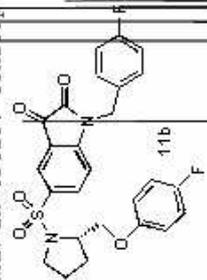
NMR in CDCl3: GSH8|rpt_rlxst



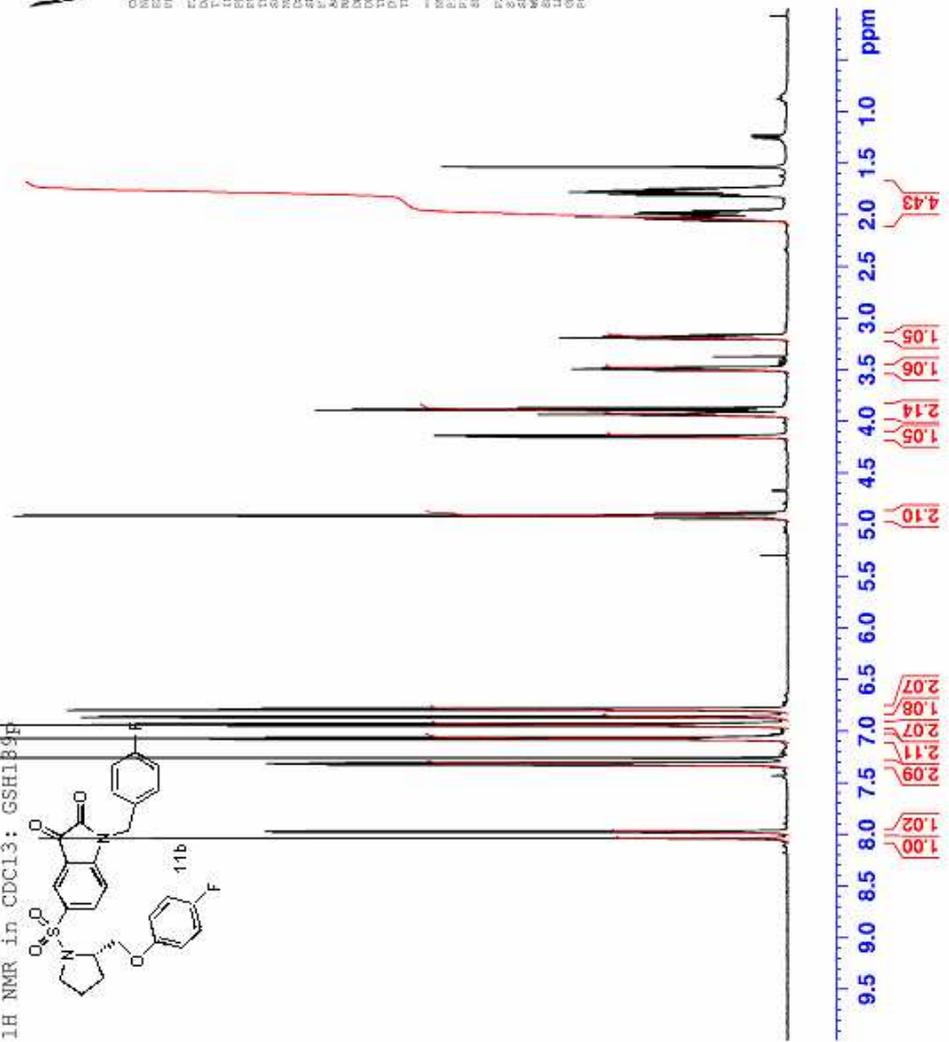
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D12: 0.0500
D13: 0.0500
D14: 0.0500
D15: 0.0500
D16: 0.0500
D17: 0.0500
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D19: 0.0500
D20: 0.0500
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WDW: EM
SSB: 0
GB: 0
PC: 1.100



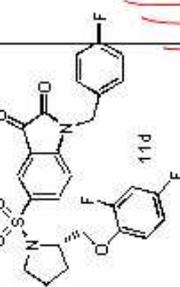
¹H NMR in CDCl₃: GSH184f



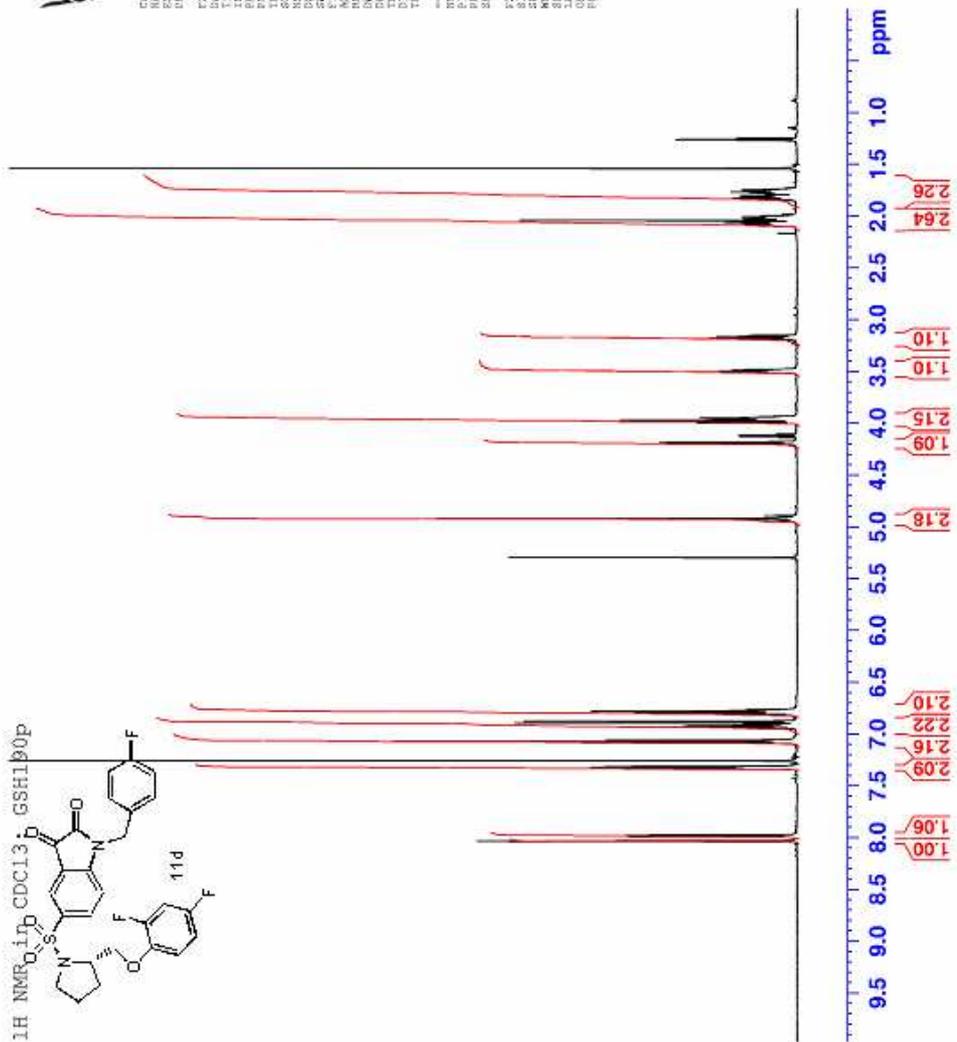
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 PULPROG: zgpg30
 SOLVENT: CDCl₃
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 DS: 4
 SWH: 12374.237 Hz
 FIDRES: 0.188864 Hz
 AQ: 2.617349 sec
 RG: 384
 DQ: 0.000000
 SFO: 400.000 MHz
 CF: 4.00 usec
 TC: 303.0 K
 TD: 1,000,000
 FIDRES: 0.188864 Hz
 F1: 7.50 usec
 F2: 4.00 usec
 F3: 4.00 usec
 F4: 4.00 usec
 F5: 4.00 usec
 F6: 4.00 usec
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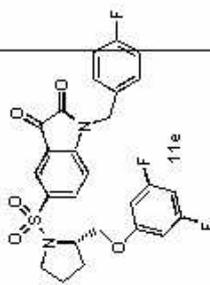
¹H NMR (400 MHz, CDCl₃) of GSH180p



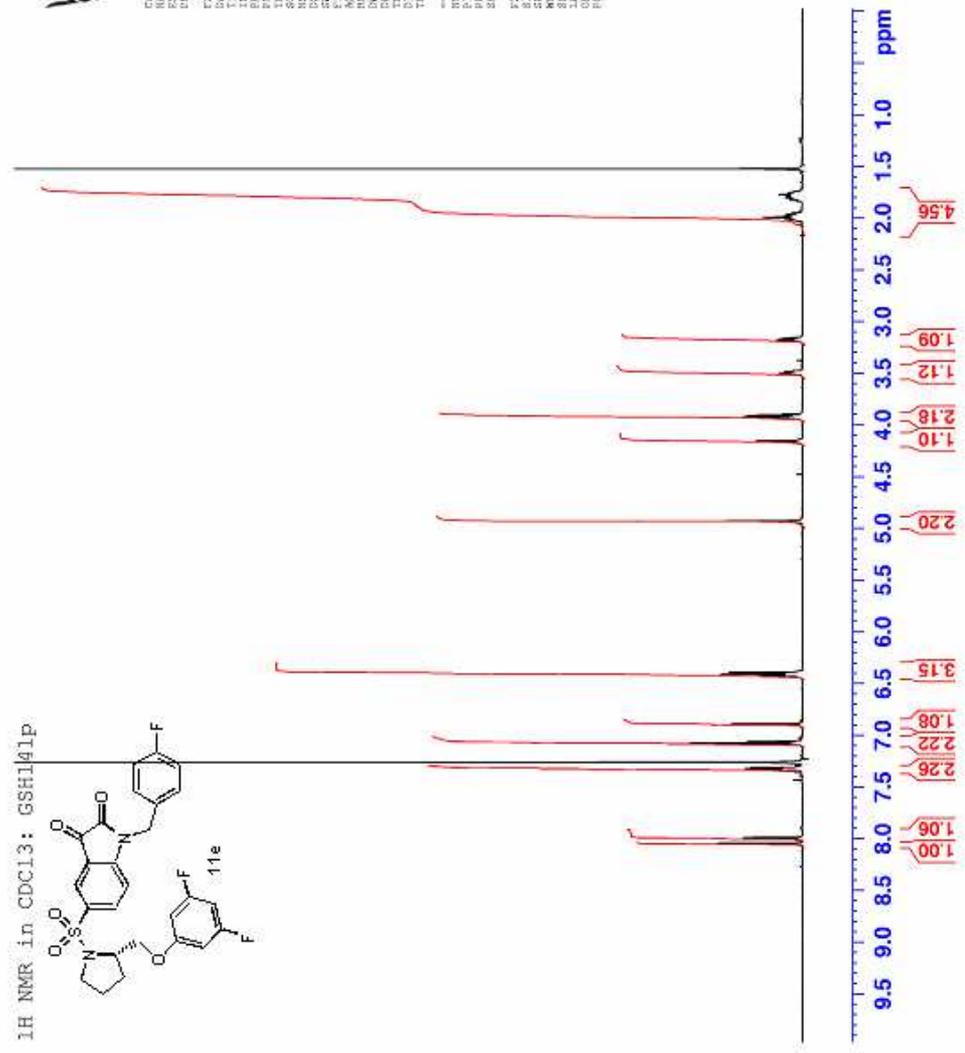
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 Processor: 3 pm TKI 10000
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 Solvent: CDCl₃
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 SWH: 12376.287 Hz
 FIDRES: 0.168868 Hz
 AQ: 0.183225 sec
 IN: 222.5 sec
 DM: 60.400 usec
 DE: 8.00 usec
 D3: 3.00 usec
 TDO: 1.0000000 sec
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 P1: 7.50 usec
 PL1: 0.00 dB
 SFO1: 400.1337000 MHz
 F2 - Processing parameters
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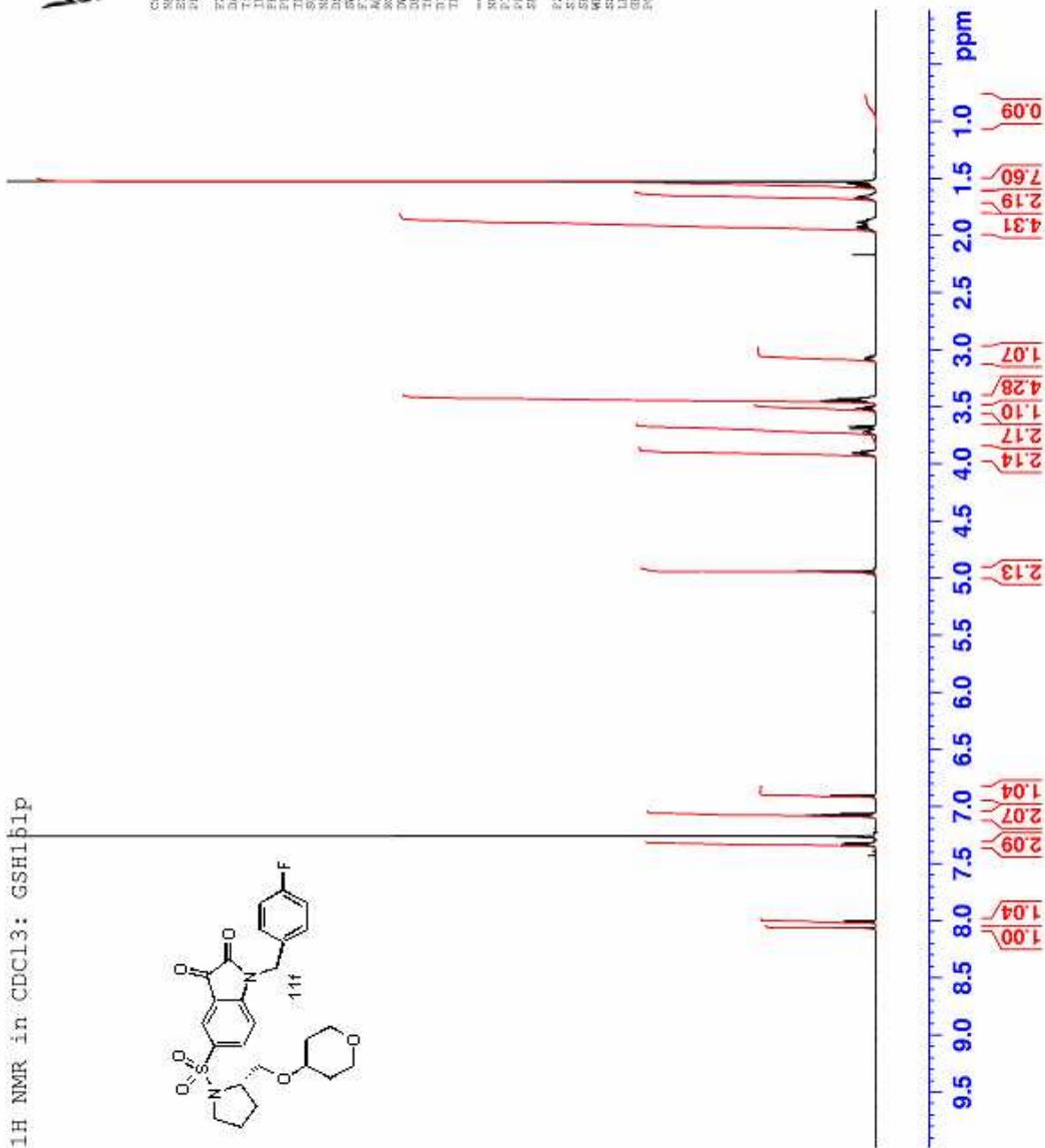
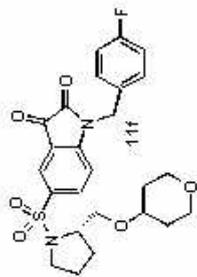
¹H NMR in CDCl₃: GSH101p



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AQ: 6.556
RG: 327.5
SOLVENT: CDCl₃
NS: 16
DS: 4
SWH: 12376.237 Hz
FIDRES: 0.168845 Hz
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RG: 327.5
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DR: 0.000000
DC: 503.0 K
TE: 1.000000000 sec
TD: 1
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PL1: 0.00 dB
SFO1: 600.137060 MHz
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SSB: 0
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GB: 0
PC: 1.00

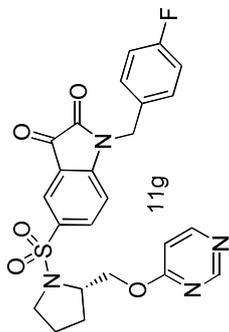


¹H NMR in CDCl₃: GSH151p

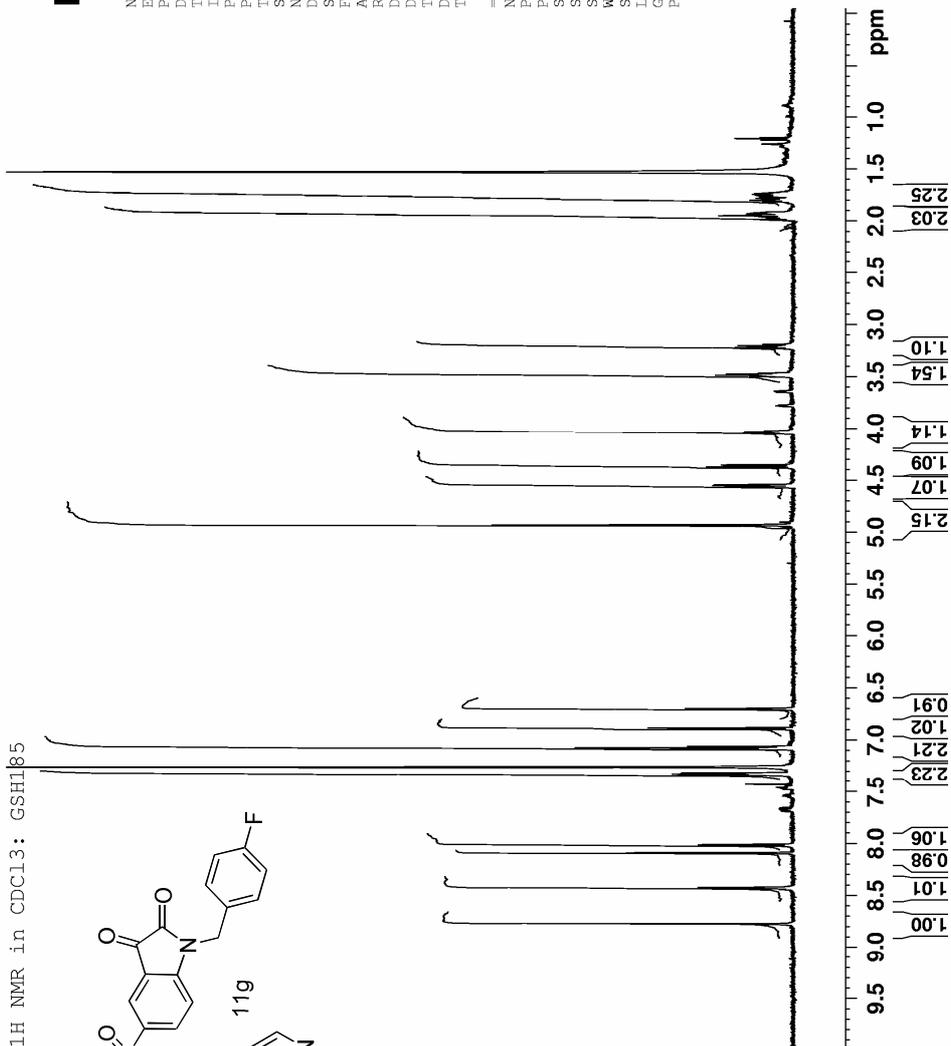


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PROCNO: 1
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Time 15:38
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PULPROG: zgpg30
TD: 65536
SOLVENT: CDCl3
NS: 2
DS: 2
SWH: 12376.237 Hz
FIDRES: 0.18866 Hz
AQ: 2.54768 sec
RG: 382
WV: 40.400 MHz
DE: 6.00 uM
TE: 303.0 K
D1: 1.00000000 sec
D10: 1
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NUC1: 13C
P1: 18
PL: 0.00
PR: 7.50 uM
SFO1: 101.6261200 MHz
ZPG1: 600.1337000 XPR
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SI: 32768
SF: 600.1300350 MHz
WDW: EM
SSB: 0
LB: 0.30 Hz
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PC: 1.00

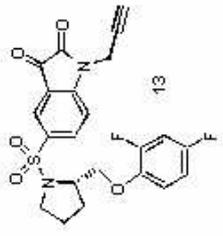
¹H NMR in CDCl₃: GSH185



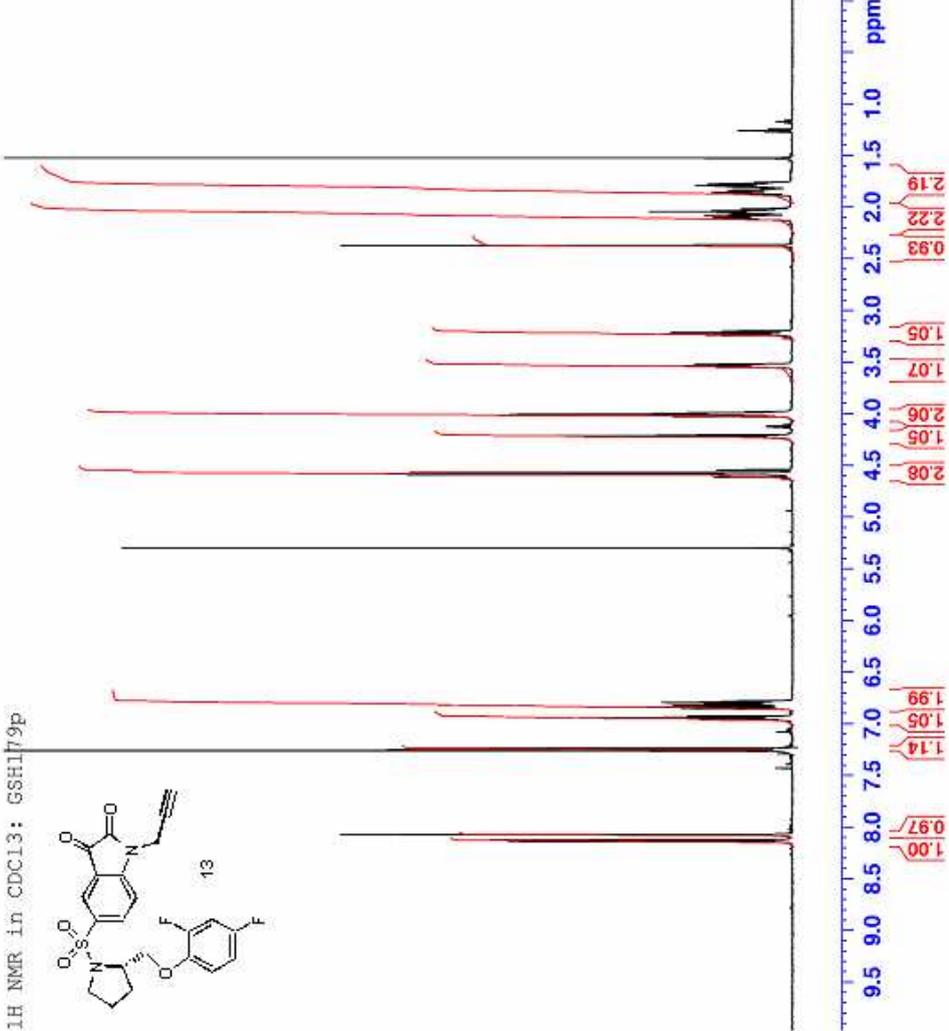
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PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 12376.237 Hz
FIDRES 0.188846 Hz
AQ 2.6477449 sec
RG 812.7
DW 40.400 usec
DE 6.50 usec
TE 303.0 K
D1 1.0000000 sec
TD0 1
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NUC1 ¹H
P1 7.50 usec
PL1 6.00 dB
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SI 32768
SF 600.1300163 MHz
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SSB 0
LB 0.30 Hz
GB 0
PC 1.00



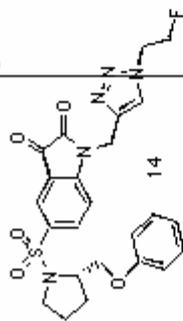
¹H NMR in CDCl₃: GSH179p



Experiment Parameters
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 File: 09_03_15_07_01
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 PRGHD0: 3 mm TKL 1H-13
 INSTRUM: zgpg30
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 F2: 101.6261262 MHz
 SFO1: 400.1460000 MHz
 SFO2: 101.6261262 MHz
 AQ: 2.4077558 sec
 AS: 455.1
 AV: 60.000 usec
 DE: 302.5 K
 TE: 302.5 K
 TD: 1,000,000,000 sec
 T0: 1
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 NUC1: 13C
 P1: 7.00 usec
 PL1: 0.00 dB
 SFO1: 600.1327060 MHz
 F2 - Processing Parameters
 SI: 32768
 SF: 600.1300159 MHz
 RG: 655
 ACQ: 0
 PC: 0
 PD: 0
 PR: 0
 SC: 0
 SS: 0
 ST: 0
 TD: 1,000,000,000 sec



¹H NMR in CDCl₃: GSH145p



Current Data Parameters
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EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
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Time 13:48
INSTRUM spect
PROBHD 5 mm TXI 1H-13
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 12376.237 Hz
FIDRES 0.188846 Hz
AQ 2.6277449 sec
RG 574.7
EW 60.400 usec
DE 6.50 usec
TE 303.0 K
D1 1.00000000 sec
TDO 1

===== CHANNEL f1 =====
NUC1 1H
P1 7.50 usec
PL1 6.00 dB
SFO1 600.1337060 MHz

F2 - Processing parameters
SI 32768
SF 600.1300159 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00





NAME gs26.12.08
 EXPNO 8
 PROCNO 1
 Date_ 20080226
 Time_ 12.34
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 PULPROG zg30
 ID 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 12376.237 Hz
 FIDRES 0.188846 Hz
 AQ 2.6477449 sec
 RG 574.7
 DW 40.400 usec
 DE 6.50 usec
 TE 303.0 K
 D1 1.0000000 sec
 TDO 1
 ===== CHANNEL f1 =====
 NUC1 1H
 P1 7.50 usec
 PL1 6.00 dB
 SFO1 600.1337060 MHz
 SI 32768
 SF 600.1300160 MHz
 WDW EM
 SSB 0
 GB 0
 PC 1.00

¹H NMR in CDCl₃: GSH189

