Electronic Supplementary Information-

A new class of fluorinated 5-pyrrolidinylsulfonyl isatin caspase inhibitors for PET imaging of apoptosis

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1. Synthetic Chemistry

1.1. General methods

Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance DPX-400 spectrometer operating at 400.13 MHz for ^{1}H NMR and at 100.61 MHz for ^{13}C NMR. Low-resolution mass spectrometry (LR-MS) was performed on a Micromass ZQ quadrupole mass spectrometer, and high-resolution mass spectrometry (HR-MS) was performed at the University of Wollongong, Australia using a Bruker Daltonics BioApex-II 7T FTICR spectrometer equipped with an off-axis analytical electron spray ionisation source. Flash chromatography was performed on a Reveleris® Flash Chromatography System (Grace Davison Discovery Sciences, Rowville, Australia), fitted with an ELSD (isopropanol support) and dual-wavelength UV (254 and 280 nm) detectors; solvent systems, column sizes and flow-rates are described where appropriate. Microanalysis was performed by the Campbell Microanalytical Laboratory, Chemistry Department, University of Otago, Dunedin, New Zealand. HPLC purity analysis was performed using a Waters Empower 2 system with a Waters 600 pump, Waters in-line degasser AF, Waters temperature control module II, Waters 717 autosampler and Water 2996 PDA. Either an Alltech Alltima C18 (150 × 4.6 mm, 5 µm pore size) or Waters XTerraRP (150 × 4.6 mm, 5 µm pore size) analytical column was used, with absorbance measured at 210 nm or 254 nm. Samples were prepared as 1 mg/mL, with a 10 µL injection. Solvent conditions were as follows:

Entry	Time	Flow (mL/min)	%A (MeCN or MeOH)	%B (H_2O)	%C (100 mM NH_4HCO_3 pH 8 or 1% TFA)
1	0	1.00	10	80	10
2	20	1.00	90	0	10
3	21	1.00	10	80	10

Percentage chemical purity was calculated from the peak area under using Empower software (Waters). logP was determined using a Waters Empower 2 system with a Waters 600 pump, Waters in-line degasser AF, Waters 717 autosampler and Waters 2487 detector. A Waters XTerra RP18 (150 × 4.6 mm, 5 µm pore size) column was used, with methanol and potassium phosphate buffer (pH 7.5, 0.1 M) as mobile phase. The linear regression plot of retention time *vs*. logP for sample and standards (see table) was used to calculate the logP of unknown compounds.[1]

Standard	logP
aniline	0.98
benzene	2.13
bromobenzene	2.99
ethylbenzene	3.15
trimethylbenzene	3.58

1.2. Materials

Isatin-5-sulfonic acid sodium salt dihydrate, tetramethylene sulfone (sulfolane), phosphorous oxychloride, 4-fluorobenzyl bromide, 4-chlorobenzyl bromide, 4-iodobenzyl bromide, 4-

bromobenzyl bromide, 4-methoxybenzyl chloride, 4-nitrobenzyl bromide, potassium carbonate, sodium hydride (60% in mineral oil), potassium iodide, trifluoroacetic acid, chloroacetyl chloride, phosphorous pentachloride and benzylamine were purchased from Sigma Aldrich. (*S*)-2-Pyrrolidinemethanol, 2-nitrobenzyl bromide, 3-nitrobenzyl bromide and 4-hydroxy benzaldehyde were purchased from Alfa Aesar (BioScientific, Sydney, Australia). Triethylamine, benzyl bromide, 2-iodobenzyl bromide, 3-iodobenzyl bromide and Di-*tert*-butyl dicarbonate (Boc_2O) were purchased from Lancaster (Morecambe, UK). 1-Bromo-2-fluoroethane was purchased from Matrix Scientific. 2-Boc-hexahydropyrrolo[3,4-*c*]pyrrole (**27**) was purchased from Synnovator, Inc (Raleigh, NC, USA). Deuterated solvents were purchased from Cambridge Isotope Laboratories (Novachem, Collingwood, Australia). All commercial materials were used as received. Unless otherwise stated all solvents used were HPLC grade or were dried using the MBraun MB SPS-800 solvent purification system.

1.3. Synthesis of literature standards

(*S*)-1-(2-Fluoroethoxybenzyl)-5-(2-(phenoxymethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (**5**), (*S*)-1-(2-fluoroethoxybenzyl)-5-(2-(methoxymethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (**6**) and (*S*)-1-(2-methoxybenzyl)-5-(2-(methoxymethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (**7**) were synthesised according to the method of Kopka *et al*. and gave ^{1}H NMR data consistent with that reported in the literature.[2] These compounds were used in the enzyme assays for method validation.

1.4. Synthesis of fluoromethyl pyrrolidinylsulfonyl isatin series

1.4.1. (*S*)-*tert*-Butyl-2-[(hydroxy)methyl]pyrrolidine-1-carboxylate (9)

(*S*)-*tert*-Butyl-2-[(hydroxy)methyl]pyrrolidine-1-carboxylate (**9**) was synthesised using an adaptation of a published method[3] and gave ^{1}H NMR data consistent with that reported in the literature.[3, 4] (*S*)-2-Pyrrolidinemethanol (**8**, 7.60 g, 75.9 mmol, 1 eq.) and triethylamine (8.60 g, 85.0 mmol, 1.12 eq.) were dissolved in CH_2Cl_2 (100 mL) in an ice bath. Di-*tert*-butyl dicarbonate (Boc_2O, 17.1 g, 78.4 mmol, 1.03 eq.) was dissolved in CH_2Cl_2 (50 mL) and added dropwise over ~ 40 min. The solution was stirred at room temperature (RT) for 16 h. The solution was extracted with cold HCl (1 M, 100 mL), washed with a saturated solution of $NaHCO_3$ (40 mL), dried ($MgSO_4$) and evaporated to yield the *title compound* **9** as a yellow oil, which was recrystallised from hexane, to yield a white solid (11.2 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.73 (br s, 1H), 3.97 (m, 1H), 3.61 (m, 2H), 3.46-3.31 (m, 2H), 2.00 (m, 2H), 1.81 (m, 2H), 1.48 (s, 9H).

1.4.2. (*S*)-*tert*-Butyl-2-[(tosyloxy)methyl]pyrrolidine-1-carboxylate (10)

(*S*)-*tert*-Butyl-2-[(tosyloxy)methyl]pyrrolidine-1-carboxylate (**10**) was synthesised according to the method of Podichetty *et al*. and gave spectroscopic data consistent with that reported in the literature.[5, 6] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77 (d, J = 8.40 Hz, 2H), 7.34 (d, 2H, J = 6.00 Hz), 4.19-3.85 (m, 3H), 3.39-3.21 (m, 2H), 2.44 (s, 3H), 2.04-1.73 (m, 4H), 1.39 (br, 9H).

1.4.3. (*S*)-*tert*-Butyl 2-[(fluoro)methyl]pyrrolidine-1-carboxylate (11)

Tosylate (**10**) (14.0 g, 39.4 mmol) and Bu_4NF (50.0 mL, 1 M in THF, 50.0 mmol, 1.27 eq.) were combined in a round bottom flask and heated to reflux for 16 h. The THF was then removed under reduced pressure and the residue purified using automated flash chromatography (Grace 80 g silica column; 0-20% ethyl acetate/petroleum spirit gradient over 15 column volumes). The *title compound* **11** was obtained as a colourless oil (6.2 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.56-4.15 (m, 2H), 4.01-3.83 (m, 1H), 3.42-3.21 (m, 2H), 2.02-1.73 (m, 4H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 157.5, 83.6 (J = 175.4 Hz), 79.6, 56.5 (J = 22.7 Hz), 49.7 (J = 39.6 Hz), 28.5, 27.8 (J = 84.9 Hz), 23.5 (J = 97.8 Hz). HPLC Purity (MeOH/H_2O/0.1% TFA): 100%, 19.45 min. LRMS (ES^+, 40 V) *m/z* $[M + Na]^+$ $C_{10}H_{18}FNNaO_2$: calculated 226.12; found 226.06. HRMS (TOF MS AP^+) *m/z* $[M + Na]^+$ $C_{10}H_{18}FNNaO_2$: calculated 226.1219, found 226.1219.

1.4.4. 2,3-Dioxoindoline-5-sulfonyl chloride (12)

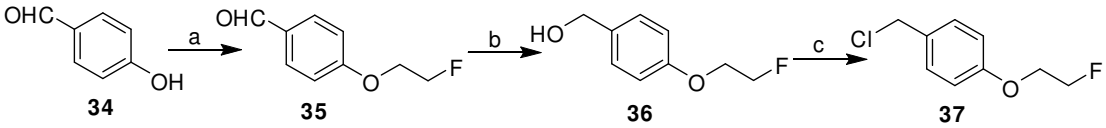
2,3-Dioxoindoline-5-sulfonyl chloride (**12**) was synthesised using a modified procedure from the literature.[7, 8] A mixture of isatin-5-sulfonic acid sodium salt dihydrate (10.0 g, 35.1 mmol, 1 eq.) was dissolved in tetramethylene sulfone (50 mL) to this was added phosphorous oxychloride (18.5 mL, 198 mmol, 5.6 eq.) was added dropwise. The mixture was heated at 60 °C for 3 h under a dry nitrogen atmosphere. The reaction was allowed to cool to RT and was added dropwise to a stirring slurry of crushed ice/CH_2Cl_2. After the ice had melted, the mixture was separated and the organic layer retained, from which the CH_2Cl_2 removed by evaporation. diluted with ethyl acetate (500 mL), washed with H_2O (10 × 200 mL), dried ($MgSO_4$) and evaporated to dryness to yield the *title compound* **12** as a bright yellow solid, which by NMR showed the presence of small amounts of residual tetramethylene sulfone. The material was used in the following steps without further purification (6.5 g, 75%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 11.12 (s, 1H), 7.81 (dd, 1H, J_1 = 8.09 Hz, J_2 = 1.57 Hz), 7.59 (d, 1H, J = 1.49 Hz), 6.87 (d, 1H, J = 8.06 Hz).

1.4.5. (*S*)-5-[1-(2-(Fluoromethyl)pyrrolidine-1-ylsulfonyl)1*H*-indole-2,3-dione (13)

The Boc-protected fluorinated pyrrolidine (**11**) (3.32 g, 16.35 mmol) was dissolved in CH_2Cl_2 (5 mL) and TFA (32.8 mmol, 2.5 mL) was added at RT. The solution was stirred for 16 h after which the TFA was removed under pressure to yield the crude trifluoroacetic acid salt which was immediately dissolved in chloroform/THF (30 mL) and then *N*,*N*-diisopropylethylamine (49 mmol, 6.33 g, 8.7 mL) and 2,3-dioxoindoline-5-sulfonyl chloride (**12**) (32.7 mmol, 8.03 g) were added. The solution was stirred at RT for 15 h; after which saturated aqueous NH_4Cl (50 mL) was added. The aqueous phase was separated from the organic phase and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give a crude green/orange oil that was purified using a Grace Reveleris medium pressure liquid chromatography system (Grace 40 g silica column; 10% ethyl acetate/petroleum spirit over 2 column volumes, increase to 65% over 8 column volumes, 65% over 6 column volumes). The *title compound* **13** was obtained as a yellow solid (5.1 g, 51%). ^{1}H NMR (400 MHz, d_6-

acetone): δ (ppm) 8.14 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.9 Hz), 7.96 (d, 1H, J = 1.8 Hz), 7.28 (d, 1H, J = 8.3 Hz), 4.51 (m, 2H), 3.89 (m, 1H), 3.43 (m, 1H), 3.23 (m, 1H), 1.90 (m, 2H), 1.77 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (100 MHz, d_6-acetone): δ (ppm) 205.1, 193.9, 123.5, 112.6, 85.2, 83.5, 84.4 (J = 170.7 Hz), 58.6 (J = 22.6 Hz), 49.0, 27.4 (J = 3.0 Hz), 23.5. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 97.68%, 14.92 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 99.30%, 17.88 min. LRMS (ES$^+$, 40 V) *m/z* [M + H]$^+$ $C_{13}H_{14}FN_2O_4S$: calculated 313.07; found 312.96. HRMS (TOF MS AP$^+$) *m/z* [M + H]$^+$ $C_{13}H_{14}FN_2O_4S$: calculated 313.0658, found 313.0668. Calc $C_{15}H_{17}FN_2O_5S$ C: 50.55, H: 4.81, N: 7.86; found C: 50.96, H: 4.46, N: 8.67.

1.4.6. 1-(Chloromethyl)-4-(2-fluoroethoxy)benzene (37)



34 **35** **36** **37**

Scheme 1. (a) K_2CO_3, 1-bromo-2-fluoroethane; (b) $NaBH_4$; (c) HCl.

1.4.6.1. 4-(2-Fluoroethoxy)benzaldehyde (35)

4-Hydroxybenzaldehyde (**34**, 1.00 g, 8.19 mmol) and K_2CO_3 (2.83 g, 20.5 mmol) were dissolved in dry DMF (40 mL) and heated at 80 °C for 10 min. 1-Bromo-2-fluoroethane (1.87 g, 1.10 mL, 14.7 mmol) was then added and the reaction mixture stirred at 80 °C for 4 h. H_2O (200 mL) was added and the mixture was extracted with 3 × 100 mL CH_2Cl_2, dried ($MgSO_4$), filtered and evaporated to yield the *title compound* **35** as a white powder (1.33 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.89 (s, 1H), 7.84 (dd, 2H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.03 (dd, 2H, J_1 = 1.8 Hz, J_2 = 8.8 Hz), 4.79 (dt, 2H, J_1 = 47.4 Hz, J_2 = 4.4 Hz), 4.30 (dt, 2H, J_1 = 27.4 Hz, J_2 = 4.4 Hz). The ^{1}H NMR spectral data was consistent with that previously reported in the literature.[9] LRMS (ES$^+$, 40 V) *m/z* [M + H]$^+$ $C_9H_{10}FO_2$: calculated 169.07; found 169.05.

1.4.6.2. 4-(2-Fluoroethoxyphenyl) methanol (36)

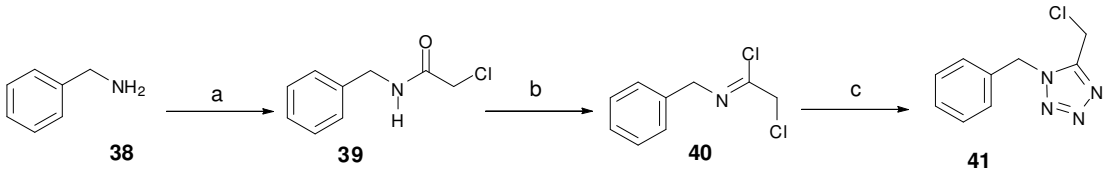
The benzaldehyde (**35**) (13.8 g, 82.1 mmol) was dissolved in dry EtOH (100 mL) at RT. $NaBH_4$ (1.55 g, 41.1 mmol) was added portion-wise over 30 min and the reaction mixture stirred at RT for 2 h. H_2O (100 mL) was added and the mixture was extracted with 2 × 150 mL $CHCl_3$, washed with brine (100 mL), dried ($MgSO_4$), filtered and evaporated to yield the *title compound* **36** as pale yellow crystals (12.7 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.17 (d, 2H, J = 7.2 Hz), 6.81 (d, 2H, J = 6.8 Hz), 4.65 (dd, 2H, J_1 = 47.6 Hz, J_2 = 4.4 Hz), 4.44 (s, 2H), 4.07 (dd, 2H, J_1 = 28.8 Hz, J_2 = 3.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 157.3, 133.5, 128.1, 114.0, 81.6 (J = 168.0 Hz), 66.7 (J = 20.0 Hz), 63.6 (J = 2.0 Hz). LRMS (ES$^-$, 40 V) *m/z* [M - H]$^-$ $C_9H_{10}FO_2$: calculated 169.07; found 169.09.

1.4.6.3. 1-(Chloromethyl)-4-(2-fluoroethoxy)benzene (37)

The alcohol (**36**) (2.50 g, 14.7 mmol) was added to heptane (50 mL). 10 M HCl (19.1 mL, 191 mmol) was added and the reaction mixture shaken vigorously for 5 min and left to settle for another 5 min. The heptane layer was separated and the aqueous layer was extracted with 2 × 25 mL heptane. The combined organic extracts were dried ($MgSO_4$), filtered and evaporated to yield the *title compound* **37** as a colourless oil (2.31 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.33 (d, 2H, J = 8.4 Hz), 6.91 (d, 2H, J = 8.4 Hz), 4.76 (dt, 2H, J_1 = 47.6 Hz, J_2 = 4.4 Hz), 4.57 (s, 2H), 4.22 (dt, 2H, J_1 = 27.6 Hz, J_2 = 4.0 Hz). ^{13}C NMR (100

MHz, $CDCl_3$): δ (ppm) 158.5, 130.3, 130.1, 114.8, 81.8 (J = 170.0 Hz), 67.1 (J = 20.0 Hz), 46.1. LRMS (ES^-, 40 V) m/z $[M - Cl]^-$ $C_9H_{10}FO$: calculated 153.07, found 153.04.

1.4.7. Synthesis of alkylating agent 1-benzyl-5-(chloromethyl)-1*H*-tetrazole (41)



Scheme 2. (a) Chloroacetylchloride; (b) $POCl_5$; (c) HN_3.

1.4.7.1. *N*-Benzyl-2-chloroacetamide (39)

N-Benzyl-2-chloroacetamide was prepared according to the literature method of Touti *et al.*[10] Chloroacetylchloride (17 mL, 220 mmol) was added dropwise to a mechanically stirred solution of benzylamine (**38**, 48 mL, 440 mmol, 2 eq.) in toluene (350 mL) at 0 ºC resulting in the formation of a white precipitate. After 1 h the reaction was quenched with H_2O (600 mL). The precipitate was dissolved by the addition of ethyl acetate (500 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 × 250 mL). The organic fractions were combined, dried ($MgSO_4$), filtered and concentrated before being recrystallised from toluene to yield the *title compound* **39** as fine white crystals (27 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.30 (m, 5H), 6.89 (br s, 1H), 4.49 (m, 2H), 4.10 (s, 2H).

1.4.7.2. 1-benzyl-5-(chloromethyl)-1*H*-tetrazole (41)

Step 1) 1-benzyltetrazol-5ylchloromethane was prepared according to the literature method of Touti *et al.*[10] **39** (5.2 g, 28.3 mmol) was suspended in toluene (100 mL) that was cooled to 5 ºC with an ice bath and phosphorus pentachloride (6.5 g, 31.2 mmol) was added. The bath was removed and the reaction brought to RT. The yellow/orange solution was then heated to 60 ºC under reduced pressure in order to remove HCl and reduce the volume by half. Nitrogen gas was then bubbled through the remaining solution in order to displace any remaining HCl leaving a crude solution of *N*-benzyl-2-chloroacetimidoyl chloride (**40**), which was used without purification.

Step 2) Concentrated sulphuric acid (12.5 mL) was added dropwise to a stirred solution of sodium azide (4.25 g, 65 mmol) in H_2O (5 mL) overlaid with toluene (25 mL). The phases were separated and the aqueous layer extracted with toluene (2 × 10 mL). The combined organic extracts were dried over $MgSO_4$. This solution of HN_3 (CAUTION! HN_3 is highly toxic, use with extreme care) was then added dropwise to the solution of **40** prepared in the previous step and stirred overnight at RT in a well-ventilated fumehood. Nitrogen gas was bubbled through the solution for 1 h to drive off hydrazoic acid. Any remaining hydrazoic acid and toluene were then removed under reduced pressure. Ice was added to the brown crude oil and allowed to melt before being heated to reflux for 30 min then extracted with ethyl acetate (3 × 40 mL). The organic extracts were dried ($MgSO_4$) and concentrated to give a brown oil. This oil was purified by LPLC using a gradient of 18-87% ethyl acetate/petroleum spirit to yield the *title compound* **41** as a white solid (4.0 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.35 (m, 5H), 5.68 (s, 2H), 4.61 (s, 2H).

1.4.8. General procedure for the synthesis of *N*-alkylated isatin derivatives

(*S*)-5-[1-(2-(Fluoromethyl)pyrrolidine-1-ylsulfonyl)1*H*-indole-2,3-dione (**13**, ~100 mg, 320 µmol., 1 eq.) was dissolved in dry DMF (5-10 mL). Under a dry nitrogen atmosphere, K_2CO_3 (1.4 eq.) was added, causing the solution to become dark purple in colour. After 10 min the corresponding alkyl halide (2 eq.) and a catalytic quantity of KI (~ 5 mg) were added and the mixture stirred at 60 °C for 16 h, with the reaction becoming yellow/orange in colour over approximately 1 h. The mixture was acidified with HCl (1 M) and diluted with H_2O (25 mL). The product was extracted into ethyl acetate (50 mL) and washed with brine (2 × 50 mL). The organic layer was dried ($MgSO_4$), concentrated and purified using automated flash chromatography (Grace 4 g silica column; 10% ethyl acetate/petroleum spirit over 2 column volumes, increase to 50% over 12 column volumes, 50% over 6 column volumes).

(*S*)-1-(2-Fluoroethoxybenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (14)

14 was prepared using the general method as described in 1.4.8. **13** (58.0 mg, 186 µmol) was alkylated with **37** (49.0 mg, 260 µmol) to yield the *title compound* **14** as orange crystals (86.4 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.03 (d, 1H, J = 1.6 Hz), 7.98 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.28 (m, 2H), 6.92 (m, 3H), 4.91 (s, 2H), 4.75 (m, 2H), 4.46 (m, 2H), 4.21 (m, 2H), 3.80 (m, 1H), 3.43 (m, 1H), 3.12 (m, 1H), 1.94 (m, 2H), 1.72 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.8, 158.6, 157.9 (J = 28.0 Hz), 153.5, 137.4, 133.8, 128.6, 126.3, 124.5, 117.6, 114.7, 111.3, 84.4 (J = 173.0 Hz), 81.8 (J = 170.0 Hz), 67.2 (J = 20.0 Hz), 58.8 (J = 23.0 Hz), 49.3, 44.0, 28.2, 24.1. HPLC Purity (MeCN/H_2O/0.1% TFA): 96.86%, 20.31 min; (MeOH/H_2O/0.1% TFA): 86.35%, 21.81 min. LRMS (ES^-, 40 V) *m/z* [M - H]$^-$ $C_{22}H_{21}F_2N_2O_5S$: calculated 463.11; found 463.15. HRMS (TOF MS AP$^+$) *m/z* [M + H]$^+$ $C_{22}H_{23}H_2N_2O_5S$: calculated 465.1296; found 465.1306. Calc $C_{22}H_{22}F_2N_2O_5S$ C: 56.89, H: 4.77, N: 6.90; found C: 56.93, H: 5.14, N: 4.35.

(*S*)-1-(Benzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (15)

15 was prepared using the general method as described in 1.4.8. **13** (100 mg, 320 µmol) was alkylated with benzyl bromide (110 mg, 640 µmol) to yield the *title compound* **15** as a yellow/orange solid (74 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.05 (d, J = 1.74 Hz, 1H), 7.98 (dd, J_1 = 8.35 Hz, J_2 = 1.88 Hz, 1H), 7.36 (m, 5H), 6.93 (d, J = 8.40 Hz, 1H), 4.98 (s, 2H), 4.57-4.35 (m, 2H), 3.81 (m, 1H), 3.43 (m, 1H), 3.12 (m, 1H), 1.93 (m, 2H), 1.74 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.7, 157.8, 153.5, 137.4, 133.8, 133.7, 129.3, 128.6, 127.6, 124.5, 117.6, 111.3, 84.4 (J = 173.5 Hz), 58.8 (J = 23.4 Hz), 49.3, 44.5, 28.2 (J = 2.2 Hz), 24.2. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 96.20%, 19.78 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 94.98%, 21.68 min. LRMS (ES^+, 40 V) *m/z* [M + Na]$^+$ $C_{20}H_{19}FN_2NaO_4S$: calculated 425.09; found 424.90. HRMS (TOF MS AP$^+$) *m/z* [M + H]$^+$ $C_{20}H_{20}FN_2O_4S$: calculated 403.1128 found 403.1103. Calc $C_{20}H_{19}FN_2O_4S$ C: 59.69, H: 4.76, N: 6.96; found C: 59.81, H: 4.89, N: 6.72.

(*S*)-1-(4-Bromobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (16)

16 was prepared using the general method as described in 1.4.8. **13** (100 mg, 320 µmol) was alkylated with 4-bromobenzyl bromide (160 mg, 640 µmol) to yield the *title compound* **16** as a yellow/orange solid (38 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.07 (d, J = 1.61 Hz, 1H), 8.01 (dd, J_1 = 8.29 Hz, J_2 = 1.87 Hz, 1H), 7.52 (m, 2H), 7.23 (m, 2H), 6.90 (d, J = 8.37 Hz, 1H) 4.93 (s, 2H), 4.57-4.36 (m, 2H), 3.83 (m, 1H), 3.43 (m, 1H), 3.13 (m, 1H), 1.94 (m, 2H), 1.75 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.5, 157.8; 153.2, 137.5, 134.3, 132.8, 132.6, 129.3, 124.7, 117.7, 111.2, 84.5 (J = 175.8 Hz), 58.9 (J = 22.5 Hz), 49.4, 44.0, 28.3 J = 2.2 Hz), 24.29. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 95.67%, 20.04 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 98.44%, 22.61 min. LRMS (ES^+, 40 V) *m/z* $[M + H]^+$ $C_{20}H_{19}BrFN_2O_4S$: calculated 481.02; found 480.81. HRMS (TOF MS AP^+) *m/z* $[M + H]^+$ $C_{20}H_{19}BrFN_2O_4S$: calculated 481.0233; found 481.0251. Calc $C_{20}H_{18}BrFN_2O_4S$: C: 49.91, H: 3.77, N: 5.82; found C: 50.36, H: 3.77, N: 5.66.

(*S*)-1-(4-Chlorobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (17)

17 was prepared using the general method as described in 1.4.8. **13** (100 mg, 320 µmol) was alkylated with 4-chlorobenzyl bromide (132 mg, 640 µmol) to yield the *title compound* **17** as a yellow/orange solid (60 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.06 (d, J = 1.83 Hz, 1H), 8.01 (dd, J_1 = 8.35 Hz, J_2 = 1.87 Hz, 1H), 7.36 (m, 2H), 7.29 (m, 2H), 6.90 (d, J = 8.36 Hz, 1H) 4.94 (s, 2H), 4.57-4.36 (m, 2H), 3.82 (m, 1H), 3.43 (m, 1H), 3.14 (m, 1H), 1.95 (m, 2H), 1.77 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.6, 157.9, 153.3, 137.5, 134.3, 134.8, 134.2, 132.3, 129.7, 129.1, 124.7, 117.7, 111.3, 94.4, 84.5 (J = 175.5 Hz), 59.0 (J = 22.9 Hz), 49.4, 44.0, 28.3 (J = 2.6 Hz), 24.3. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 97.53%, 19.71 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 99.20%, 22.05 min. LRMS (ES^+, 40 V) *m/z* $[M + H]^+$ $C_{20}H_{19}ClFN_2O_4S$: calculated 437.07; found 436.86. HRMS (TOF MS AP^+) *m/z* $[M + H]^+$ $C_{20}H_{19}ClFN_2O_4S$: calculated 437.0738; found 437.0753. Calc $C_{20}H_{18}ClFN_2O_4S$ C: 54.98, H: 4.15, N: 6.41; found C: 55.17, H: 4.22, N: 6.16.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (18)

18 was prepared using the general method as described in 1.4.8 **13** (100 mg, 320 µmol) was alkylated with 4-fluorobenzyl bromide (121 mg, 640 µmol) to yield the *title compound* **18** as a yellow/orange solid (24 mg, 24%) ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.06 (d, J = 1.84 Hz, 1H), 8.01 (dd, J_1 = 8.29 Hz, J_2 = 1.90 Hz, 1H), 7.33 (m, 2H), 7.07 (m, 2H), 6.93 (d, J = 8.21 Hz, 1H) 4.94 (s, 2H), 4.56-4.35 (m, 2H), 3.82 (m, 1H), 3.43 (m, 1H), 3.13 (m, 1H), 1.94 (m, 2H), 1.74 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.6, 164.0, 161.5, 157.7, 153.2, 137.4, 134.0, 129.5 (J = 8.11 Hz), 124.6, 117.6, 116.4 (J = 22.0 Hz), 111.1, 84.4 (J = 176.0 Hz), 58.9 (J = 23.1 Hz), 49.3, 43.8, 28.2 (J = 2.6 Hz), 24.2. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 98.78%, 18.87 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 98.65%, 20.91 min. LRMS (ES^+, 20 V) *m/z* $[M + H]^+$ $C_{20}H_{19}F_2N_2O_4S$: calculated 421.10; found 420.88. HRMS (TOF MS AP^+) *m/z* $[M + H]^+$ $C_{20}H_{19}F_2N_2O_4S$ calculated: 421.1034; found 421.1044. Calc $C_{20}H_{18}F_2N_2O_4S$ C: 57.14, H: 4.32, N: 6.66; found C: 57.33, H: 4.40, N: 6.45.

(*S*)-1-(2-Iodobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (19)

19 was prepared using the general method as described in 1.4.8, with slight modification. **13** (100 mg, 320 µmol) was alkylated with 2-iodobenzyl bromide (141 mg, 640 µmol). Additional alkylating reagent (70 mg, 320 µmol) was added after 16 h as the reaction was still slightly purple in colour. Purification yielded the *title compound* **19** as a yellow/orange solid (45 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.09 (d, J = 1.75 Hz, 1H), 8.01 (dd, J_1 = 8.36 Hz, J_2 = 1.93 Hz, 1H), 7.93 (dd, J_1 = 1.10 Hz, J_2 = 7.99 Hz, 1H), 7.32 (dt, J_1 = 1.64 Hz, J_2 = 7.80 Hz, 1H), 7.11 (dd, J_1 = 1.42 Hz, J_2 = 7.85 Hz, 1H), 7.05 (m, 1H), 6.85 (d, J = 8.36 Hz, 1H) 5.05 (s, 2H); 4.60-4.33 (m, 2H), 3.84 (m, 1H), 3.43 (m, 1H), 3.15 (m, 1H), 1.94 (m, 2H), 1.75 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.7, 158.0, 153.4, 140.4, 137.7, 135.5, 134.3, 130.4, 129.4, 127.5, 124.8, 117.8, 112.1, 98.0, 84.5 (J = 173.7 Hz), 59.0 (J = 23.1 Hz), 49.5 (J = 16.6 Hz), 28.3 (J = 2.8 Hz), 24.4. HPLC Purity (MeCN/H_2O/0.1% TFA): 96.60%, 21.53 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 97.88%, 23.25 min. LRMS (ES^-, 40 V) m/z $[M + Cl]^-$ $C_{20}H_{18}ClFIN_2O_4S$: calculated 562.97; found 562.87. HRMS (TOF MS AP^+) m/z $[M + H]^+$ $C_{20}H_{19}FIN_2O_4S$: calculated 529.0096; found 529.0084. Calc $C_{20}H_{18}FIN_2O_4S$: C: 45.47, H: 3.43, N: 5.30; found C: 46.01, H: 3.38, N: 5.28.

(*S*)-1-(3-Iodobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (20)

20 was prepared using the general method as described in 1.4.8, with slight modification. **13** (100 mg, 320 µmol) was alkylated with 3-iodobenzyl bromide (132 mg, 640 µmol) to yield the *title compound* **20** as a yellow/orange solid (60 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.08 (d, J = 1.8 Hz, 1H), 8.02 (dd, J_1 = 8.4 Hz, J_2 = 2.2 Hz, 1H), 7.69 (m, 2H), 7.31 (d, J = 7.6 Hz, 1H), 7.124 (m, 1H), 6.919 (d, J = 8.3 Hz, 1H) 4.914 (s, 2H), 4.599-4.328 (m, 2H), 3.84 (m, 1H), 3.44 (m, 1H), 3.15 (m, 1H), 1.95 (m, 2H), 1.76 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.4, 157.7, 153.1, 137.8, 137.5, 136.1, 134.1, 131.0, 126.8, 124.6, 117.6, 111.2, 95.1, 84.4 (J = 174.6 Hz), 58.9 (J = 22.7 Hz), 49.3, 43.7, 28.2 (J = 2.6 Hz), 24.2. HPLC Purity (MeCN/H_2O/0.1% TFA): 99.46%, 21.47 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 96.87%, 23.04 min. LRMS (ES^-, 40 V) m/z $[M + Cl]^-$ $C_{20}H_{18}ClFIN_2O_4S$: calculated 562.97; found 561.91. HRMS (TOF MS AP^+) m/z $[M + Cl]^+$ $C_{20}H_{18}ClFIN_2O_4S$: calculated 562.9706; found 562.9716. Calc $C_{20}H_{18}FIN_2O_4S$: C: 45.47, H: 3.43, N: 5.30; found C: 45.93, H: 3.50, N: 5.19.

(*S*)-1-(4-Iodobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (21)

21 was prepared using the general method as described in 1.4.8. **13** (100 mg, 320 µmol) was alkylated with 4-iodobenzyl bromide (191 mg, 640 µmol) to yield the *title compound* **21** as a yellow/orange solid (30 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.05 (d, J = 1.68 Hz, 1H), 7.99 (dd, J_1 = 8.29 Hz, J_2 = 1.70 Hz, 1H), 7.73 (m, 2H), 7.09 (m, 2H), 6.89 (d, J = 8.34 Hz, 1H) 4.91 (s, 2H), 4.56-4.35 (m, 2H), 3.82 (m, 1H), 3.43 (m, 1H), 3.13 (m, 1H), 1.94 (m, 2H), 1.74 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.6, 157.9, 153.2, 138.6, 137.6, 134.3, 133.5, 129.5, 124.8, 117.7, 111.3, 94.4, 84.4 (J = 173.5 Hz), 59.0 (J = 22.9 Hz), 49.4, 44.1, 28.3 (J = 2.4 Hz), 24.3. HPLC Purity (MeCN/100 mM NH_4HCO_3 pH 8): 98.08%, 20.37 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 97.87%, 22.94 min. LRMS (ES^+, 40 V) m/z $[M + H]^+$ $C_{20}H_{19}FIN_2O_4S$: calculated 529.01; found 528.77. HRMS (TOF MS AP^+) m/z

$[M + H]^+$ $C_{20}H_{19}FIN_2O_4S$: calculated 529.0094; found 529.0106. Calc $C_{22}H_{22}FIN_2O_5S$: C: 46.16, H: 3.87, N: 4.89; found C: 46.30, H: 3.56, N: 5.06.

(*S*)-1-(4-Methoxybenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (22)

22 was prepared using the general method as described in 1.4.8. **13** (100 mg, 320 µmol) was alkylated with 4-methoxybenzyl chloride (60.8 mg, 448 µmol) to yield the *title compound* **22** as a yellow solid (108 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.03 (d, J = 1.79 Hz, 1H), 7.98 (dd, J_1 = 8.33 Hz, J_2 = 1.91 Hz, 1H), 7.28 (m, 2H), 6.96 (m, 1H), 6.89 (m, 2H), 4.91 (s, 2H), 4.57-4.35 (m, 2H), 3.82 (m, 1H), 3.78 (s, 3H), 3.43 (m, 1H), 3.11 (m, 1H), 1.93 (m, 2H), 1.73 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.9, 159.8, 157.8, 153.6, 137.3, 133.7, 129.1, 125.6, 124.5, 117.6, 114.7, 111.3, 84.4 (J = 171.7 Hz), 58.8 (J = 21.3 Hz), 55.4, 49.3, 44.1, 28.2 (J = 2.7 Hz), 24.2. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 99.35%, 18.52 min; (MeOH/H_2O/100 mM NH_4HCO_3 pH 8): 96.50%, 21.34 min. LRMS (ES^+, 40 V) *m/z* $[M + Na^+]$ $C_{21}H_{21}FN_2NaO_5S$: calculated 455.11; found 455.07. HRMS (TOF MS AP^+) *m/z* $[M + H^+]$ $C_{21}H_{22}FN_2O_5S$: calculated 433.1233; found 433.1234. Calc $C_{21}H_{21}FN_2O_5S$ C: 58.32, H: 4.89, N: 6.48; found C: 58.40, H: 4.90, N: 6.44.

(*S*)-1-(2-Nitrobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (23)

23 was prepared using the general method as described in 1.4.8 **13** (70.7 mg, 225 µmol) was alkylated with 2-nitrobenzyl bromide (98.0 mg, 454 µmol) with an exception for the purification conditions. Upon addition of ethyl acetate, the product became suspended in the organic layer, which was evaporated to dryness. The product was purified using automated flash chromatography (Grace 40 g C18 column, 5% H_2O (0.1% TFA)/MeCN for 6 column volumes, increase to 50% over 3 column volumes, 50% for 3 column volumes, increase to 100% over 3 column volumes, 100% for 3 column volumes). Fractions were lyophilised to yield the *title compound* **23** as a yellow/orange solid (40 mg, 40%). ^{1}H NMR (d_6-DMSO): δ (ppm) 8.23 (dd, J_1 = 1.34 Hz, J_2 = 8.06 Hz, 1H), 8.03 (dd, J_1 = 1.32 Hz, J_2 = 8.03 Hz, 1H), 7.93 (d, J = 1.88 Hz, 1H), 7.77 (m, 1H): 7.66 (m, 1H), 7.59 (m, 1H), 7.21 (d, J = 8.41 Hz, 1H), 5.32 (s, 2H), 4.54-4.34 (dd, J_1 = 4.60 Hz, J_2 = 47.12 Hz, 2H), 3.86 (m, 1H), 3.11 (m, 1H), 1.78 (m, 2H), 1.62 (m, 1H), 1.51 (m, 1H). ^{13}C NMR (100 MHz, d_6-DMSO): δ (ppm) 181.2, 159.2, 153.1, 147.5, 136.5, 134.3, 131.5, 130.7, 128.8, 128.0, 125.5, 122.8, 118.8, 111.9, 84.8 (J = 170.7 Hz), 58.6 (J = 21.6 Hz), 49.2, 41.9, 27.5 (J = 2.6 Hz), 23.8. HPLC Purity (MeCN/H_2O/0.1% TFA): 96.96%, 19.98 min; (MeOH/H_2O/0.1% TFA): 93.95%, 20.89 min. LRMS (ES^-, 40 V) *m/z* $[M + Cl]^-$ $C_{20}H_{18}ClFN_3O_6S$: calculated 482.06; found 481.98. HRMS (TOF MS AP^-) *m/z* $[M - H]^-$ $C_{20}H_{17}FN_3O_6S$: calculated 446.0822; found 446.0810. Calc $C_{20}H_{18}FN_3O_6S$: C: 53.69, H: 4.05, N: 9.39; found C: 53.40, H: 4.16, N: 9.13.

(*S*)-1-(3-Nitrobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (24)

24 was prepared using the general method as described in 1.4.8. **13** (65.6 mg, 210 µmol) was alkylated with 3-nitrobenzyl bromide (98 mg, 450 µmol) to yield the *title compound* **24** as a yellow/orange solid (62 mg, 65%). ^{1}H NMR ($CDCl_3$): δ (ppm) 8.23 (m, 2H), 8.09 (m, 1H),

8.03 (dd, J_1 = 1.92 Hz, J_2 = 8.32 Hz, 1H), 7.71 (m, 1H): 7.61 (m, 1H), 6.93 (m, 1H), 5.08 (s, 2H), 4.59-4.33 (m, 2H), 3.83 (m, 1H), 3.43 (m, 1H), 3.14 (m, 1H), 1.94 (m, 2H), 1.75 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.2, 157.9, 152.9, 148.9, 137.7, 136.1, 134.7, 133.7, 130.7, 125.0, 123.9, 122.5, 117.8, 111.0, 84.3 (J = 177.3 Hz), 58.9 (J = 23.3 Hz), 49.3, 43.8, 28.2 (J = 2.5 Hz), 24.2. HPLC Purity (MeCN/H_2O/0.1% TFA): 99.76%, 19.85 min; (MeOH/H_2O/0.1% TFA): 99.42%, 20.86 min. LRMS (ES$^-$, 40 V) *m/z* [M + Cl]$^-$ $C_{20}H_{18}ClFN_3O_6S$: calculated 482.06; found 481.95. HRMS (TOF MS AP$^-$) *m/z* [M - H]$^+$ $C_{20}H_{17}FN_3O_6S$: calculated 446.0822; found 446.0805. Calc $C_{20}H_{18}FN_3O_6S$: C: 53.69, H: 4.05, N, 9.39; found C: 53.72, H: 4.15, N: 9.18.

(*S*)-1-(4-Nitrobenzyl)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (25)

25 was prepared using the general method as described in 1.4.8 **13** (100 mg, 320 µmol) was alkylated with 4-nitrobenzyl bromide (138 mg, 640 µmol) to yield the *title compound* **25** as a yellow/orange solid (40 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.28 (d, J = 8.6 Hz, 2H), 8.11 (s, 1H), 8.05 (d, 1H), 7.55 (d, 2H), 6.90 (d, J = 8.32 Hz, 1H) 5.10 (s, 2H), 4.58-4.40 (m, 2H), 3.87 (m, 1H), 3.46 (m, 1H), 3.17 (m, 1H), 1.97 (m, 2H), 1.78 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.0, 157.7, 152.7, 148.1, 140.9, 137.5, 134.6, 128.3, 124.8, 124.6, 117.7, 110.9, 84.3 (J = 173.4 Hz), 58.9 (J = 22.7 Hz), 49.3, 43.8, 28.2 (J = 2.5 Hz), 24.2. HPLC Purity (MeCN/H_2O/0.1% TFA): 99.17%, 19.74 min; (MeOH/H_2O/0.1% TFA): 97.61%, 21.20 min. LRMS (ES$^+$, 40 V) *m/z* [M + H]$^+$ $C_{20}H_{19}FN_3O_6S$: calculated 448.10; found 447.90. HRMS (TOF MS AP$^+$) *m/z* [M + H]$^+$ $C_{20}H_{19}FN_3O_6S$: calculated 448.0979; found 448.0970. Calc $C_{20}H_{18}FN_3O_6S$: C: 53.69, H, 4.05, N, 9.39; found C: 53.92, H:4.31, N: 8.92.

(*S*)-1-(1-Benzyl-5-methyl-1*H*-tetrazole)-5-(2-(fluoromethyl)pyrrolidin-1-ylsulfonyl)1*H*-indole-2,3-dione (26)

26 was prepared using the general method as described in 1.4.8 **13** (100.0 mg, 320 µmol) was alkylated with **41** (94 mg, 448 µmol) to yield the *title compound* **26** as a yellow/orange solid (84 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.01 (dd, J_1 = 8.40 Hz, J_2 = 1.94 Hz, 1H), 7.89 (d, J = 1.83 Hz, 1H), 7.32-7.16 (m, 5H), 7.01 (m, 1H), 5.70 (s, 2H), 5.23 (s, 2H), 4.59-4.33 (m, 2H), 3.81 (m, 1H), 3.42 (m, 1H), 3.12 (m, 1H), 1.95 (m, 2H), 1.75 (m, 2H). ^{13}C NMR (100 MHz, d_6-acetone): δ (ppm) 181.2, 158.2, 153.1, 150.7, 137.6, 134.6, 133.6, 129.4, 127.8, 123.9, 118.4, 112.4, 85.2 (J = 171.2 Hz), 59.4 (J = 22.7 Hz), 51.1, 49.7, 33.2, 28.2 (J = 2.8 Hz), 24.3. HPLC Purity (MeCN/H_2O/0.1% TFA): 97.13%, 18.57 min; (MeOH/H_2O/0.1% TFA): 95.89%, 20.44 min. LRMS (ES$^+$, 40 V) *m/z* [M + H]$^+$ $C_{23}H_{23}FN_5O_4S$: calculated 485.14; found 485.24. HRMS (TOF MS AP$^+$) *m/z* [M + H]$^+$ $C_{23}H_{23}FN_5O_4S$: calculated 485.1407; found 485.1407. Calc $C_{22}H_{21}FN_6O_4S$: C: 54.54, H: 4.37, N: 17.35; found C: 54.13, H: 4.41, N: 18.93.

1.4.9. Synthesis of bicyclic conformationally restricted diamine (30)

1.4.9.1. *tert*-Butyl 5-(2,3-dioxoindolin-5-ylsulfonyl)hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate (28)

Isatin sulfonyl chloride (**12**) (501 mg, 2.04 mmol) was dissolved in a mixture of $CHCl_3$/THF (1:1, 20 mL) at 0 °C. 2-Boc-hexahydropyrrolo[3,4-*c*]pyrrole (**27**, 476 mg, 2.24 mmol) and *N*,*N*-diisopropylethylamine (474 mg, 639 µL, 3.67 mmol) were dissolved in $CHCl_3$ (3 mL) and added portion-wise over 1 h at 0 °C. The reaction mixture was warmed to RT and stirred for 18 h before the solvent was removed by rotary evaporation. The residue was purified using automated flash chromatography (Grace 4 g silica column, petroleum spirit/ethyl acetate). The *title compound* **28** was obtained as an orange powder (534 mg, 62%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 11.51 (bs, 1H), 7.99 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.74 (s, 1H), 7.11 (d, 1H, J = 8.0 Hz), 3.28 (m, 4H), 2.98 (m, 4H), 2.77 (bs, 2H), 1.33 (s, 9H). ^{13}C NMR (d_6-DMSO): δ (ppm) 183.0, 159.5, 153.8, 153.4, 137.1, 129.4, 123.3, 118.1, 112.8, 78.5, 52.2, 49.6, 41.6, 28.2. HPLC Purity (MeCN/H_2O/0.1% TFA): 91.25%, 17.07 min; (MeOH/H_2O/0.1% TFA): 87.39%, 19.96 min. LRMS (ES$^-$, 40 V) *m/z* [M - H]$^-$ $C_{19}H_{22}N_3O_6S$: calculated 420.12; found 420.14. HRMS (TOF MS AP$^+$) *m/z* [M + Na]$^+$ $C_{19}H_{23}N_3NaO_6S$: calculated 444.1205; found 444.1211.

1.4.9.2. *tert*-Butyl 5-(1-(4-(2-fluoroethoxy)benzyl)-2,3-dioxoindolin-5-ylsulfonyl)hexahydropyrrolo[3,4-c]pyrrole-2(1*H*)-carboxylate (29)

29 was prepared using the general method as described in 1.4.8. **28** (299 mg, 709 µmol) was alkylated with **37** (294 mg, 1.56 mmol) to yield the *title compound* **29** as a yellow solid (210 mg, 52%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 7.99 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.0 Hz), 7.79 (d, 1H, J = 2.0 Hz), 7.40 (d, 2H, J = 8.8 Hz), 7.21 (d, 1H, J = 8.4 Hz), 6.94 (d, 2H, J = 8.4 Hz), 4.89 (s, 2H), 4.73 (dt, 2H, J_1 = 47.6 Hz, J_2 = 4.0 Hz), 4.21 (dt, 2H, J_1 = 30.4 Hz, J_2 = 3.6 Hz), 3.27 (m, 4H), 2.97 (m, 4H), 2.76 (bs, 2H), 1.32 (s, 9H). ^{13}C NMR (d_6-DMSO): δ (ppm) 181.6, 158.5, 157.7, 153.3, 136.7, 130.5, 129.0, 127.3, 122.8, 118.2, 114.6, 111.6, 82.1 (J = 166.0 Hz), 78.5, 67.0 (J = 11.0 Hz), 52.2, 49.7, 46.2, 42.7, 28.1. HPLC Purity (MeCN/H_2O/0.1% TFA): 96.01%, 21.21 min; (MeOH/H_2O/0.1% TFA): 91.99%, 22.89 min. LRMS (ES$^+$, 40 V) *m/z* [M + Na]$^+$ calculated $C_{26}H_{32}FN_3NaO_7S$: 596.18; found 596.22. HRMS (TOF MS AP$^+$) *m/z* [M + Na]$^+$ $C_{26}H_{32}FN_3NaO_7S$: calculated 596.1843; found 596.1827.

1.4.9.3. 1-(4-(2-Fluoroethoxy)benzyl)-5-(hexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-ylsulfonyl)indoline-2,3-dione. TFA salt (30)

29 (146 mg, 255 µmol) was dissolved in dry CH_2Cl_2 (3.7 mL) at RT. TFA (750 µL) was added and the reaction mixture stirred at RT for 4 h before the solvent was removed. The residue was purified using automated flash chromatography (Grace 40 g C18 column, H_2O/MeCN). The *title compound* **30** was obtained as a dark yellow powder (68 mg, 45%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 7.97 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.8 Hz), 7.77 (d, 1H, J = 2.0 Hz), 7.41 (d, 2H, J = 8.8 Hz), 7.25 (d, 1H, J = 8.4 Hz), 6.95 (d, 2H, J = 8.8 Hz), 4.90 (s, 2H), 4.72 (dt, 2H, J_1 = 48.0 Hz, J_2 = 3.6 Hz), 4.21 (dt, 2H, J_1 = 30.4 Hz, J_2 = 4.0 Hz), 3.13 (m, 2H), 3.00 (m, 2H), 2.92 (m, 7H). ^{13}C NMR (d_6-DMSO): δ (ppm) 181.6, 158.4, 158.0, 153.5, 137.2, 129.2, 129.1, 127.3, 123.2, 118.3, 114.7, 111.7, 82.2 (J = 159.0 Hz), 67.1 (J = 18.1 Hz), 52.1, 49.9, 44.8, 42.7. HPLC Purity (MeCN/H_2O/0.1% TFA): 90.16%, 14.97 min.

LRMS (ES^+, 40 V) *m/z* $[M + H]^+$ $C_{23}H_{25}FN_3O_5S$: calculated 474.15; found 474.09. HRMS (TOF MS AP^+) *m/z* $[M + H]^+$ $C_{23}H_{25}FN_3O_5S$: calculated 474.1499; found 474.1489.

1.5. ^{18}F precursor synthesis

1.6. (*S*)-5-(2-(Hydroxymethyl)pyrrolidin-1-ylsulfonyl)1*H*-indol-2,3-dione (31)

31 was synthesised using a modified literature method.[11, 12] **12** (5.50 g, 22.5 mmol) was suspended in $CHCl_3$/THF (1:1, 30 mL) and chilled to 0 °C. **8** (2.30 g, 22.7 mmol) and *N*,*N*-diisopropylethylamine (4.38 g, 5.9 mL, 34 mmol) were dissolved in CH_2Cl_2 (30 mL) and added dropwise over 1 h, with the reaction turning a deep red colour immediately. After 1 h at RT, TFA (3 mL) was added. The reaction mixture was concentrated and purified in multiple runs using automated flash chromatography (Grace 40 g C18 column; 5% H_2O (0.1% TFA)/MeCN for 3-8 column volumes, increase to 20% over 3 column volumes, 20% for 4 column volumes, increase to 100% over 3 column volumes, 100% for 4 column volumes). Fractions containing product were lyophilised to yield the *title compound* **31** as a hygroscopic green solid (~ 2.0 g, 30%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 8.00 (dd, 1H, J_1 = 8.35 Hz, J_2 = 1.93 Hz), 7.75 (d, J = 1.99 Hz, 1H), 7.10 (d, J = 8.41 Hz, 1H), 4.84 (m, 1H), 3.51 (m, 2H), 3.04 (m, 2H), 1.78 (m, 2H), 1.48 (m, 2H). ^{13}C NMR (d_6-DMSO): δ (ppm) 183.1, 159.5, 136.7, 130.8, 123.0, 118.2, 112.8, 63.7, 61.1, 49.2, 27.7, 23.4. HPLC Purity (MeCN/H_2O/0.1% TFA): 100%, 11.18 min; (MeOH/H_2O/0.1% TFA): 99.11%, 13.70 min. LRMS (ES^-, 40 V) *m/z* $[M - H]^-$ $C_{13}H_{13}N2O_5S$: calculated 309.05; found 309.03. HRMS (TOF MS AP^-) *m/z* $[M - H]^-$ $C_{13}H_{13}N2O_5S$: calculated 309.0545; found 309.0532.

(*S*)-(1-(2,3-Dioxoindolin-5-ylsulfonyl)pyrrolidin-2-yl)methyl 4-methylbenzenesulfonate (32)

31 (360 mg, 1.15 mmol) was dissolved in pyridine (20 mL) at 0 °C, to which *p*-toluenesulfonyl chloride (320 mg, 2.15 mmol) was added. The reaction proceeded overnight, during which time the reaction came to RT. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with 1 M HCl (20 mL), H_2O (20 mL), dried ($MgSO_4$) and evaporated to yield a thick oil, which was purified using automated flash chromatography (Grace 40 g C18 column; 5% H_2O (0.1% TFA)/MeCN for 5-10 column volumes, increase to 25% over 2 column volumes, 25% for 2 column volumes, increase to 100% over 2 column volumes, 100% for 2 column volumes). Fractions containing product were evaporated under reduced pressure to remove the MeCN and then lyophilised to yield the *title compound* **32** a yellow solid (120 mg, 23 %). ^{1}H NMR (400 MHz, d_6-acetone): δ (ppm) 10.52 (br s, 1H), 8.07 (dd, J_1 = 8.06 Hz, J_2 = 2.00 Hz, 1H), 7.87 (m, 3H), 7.54 (d, J = 8.22 Hz, 2H), 7.26 (d, J = 8.20 Hz, 2H), 7.28 (d, J = 8.22 Hz, 2H), 4.25 (m, 1H), 4.09 (m, 1H), 3.83 (m, 1H), 3.39 (m, 1H), 3.18 (m, 1H), 2.50 (s, 3H), 2.23 (m, 1H), 1.91 (m, 1H), 1.62 (m, 2H). ^{13}C NMR (100 MHz, d_6-acetone): δ (ppm) 182.8, 158.8, 145.3, 137.3, 133.1, 131.7, 130.1, 127.9, 123.8, 118.2, 112.9, 71.6, 57.9, 49.4, 23.6, 20.7. HPLC Purity (MeCN/H_2O/1% TFA): 98.81%, 19.40 min; (MeOH/H_2O/0.1% TFA): 96.92%, 21.36 min. LRMS (ES^+, 40 V) *m/z* $[M + Na^+]$ $C_{20}H_{20}N_2NaO_7S_2$: calculated 487.07; found 486.96. HRMS (TOF MS AP^+) *m/z* $[M + H^+]$ $C_{20}H_{21}N_2O_7S_2$: calculated 463.0759; found 463.0541. Calc $C_{21}H_{21}NO_7S_2$ C: 54.41, H: 4.57, N: 3.02; found C: 53.33, H: 4.76, N: 5.65.

(*S*)-(1-(1-(4-Methoxybenzyl)-2,3-dioxoindolin-5-ylsulfonyl)pyrrolidin-2-yl)methyl 4-methylbenzenesulfonate (33)

33 was prepared using the general method as described in 1.4.8. **32** (91.7 mg, 197 µmol) was alkylated with 4-methoxybenzyl chloride (61.7 mg, 394 µmol) to yield the *title compound* **33** as orange crystals (59.9 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.95 (m, 2H), 7.79 (d, J = 8.32 Hz, 2H), 7.38 (d, J = 8.00 Hz, 2H), 7.23 (d, 2H), 6.96 (m, 1H), 6.90 (m, 2H), 4.91 (s, 2H), 4.18 (m, 1H), 3.95 (m, 1H), 3.79 (s, 3H), 3.75 (m, 1H), 3.39 (m, 1H), 3.04 (m, 1H), 2.47 (s, 3H), 1.88 (m, 2H), 1.69 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 181.6, 159.7, 157.7, 153.4, 145.1, 137.0, 132.7, 132.2, 129.90, 129.0, 127.8, 125.5, 124.4, 117.4, 114.6, 111.5, 70.8, 57.6, 55.5, 49.3, 44.0, 28.5, 23.7, 21.6. HPLC Purity (MeCN/H_2O/100 mM NH_4HCO_3 pH 8): 97.38%, 20.73 min. LRMS (ES$^+$, 40 V) *m/z* [M + H$^+$] $C_{28}H_{29}N_2O_8S_2$: calculated 585.14; found 585.01. HRMS (TOF MS AP$^+$) *m/z* [M + H$^+$] $C_{28}H_{29}N_2O_8S_2$: calculated 585.1365; found 585.1387. Calc $C_{28}H_{28}N_2O_8S_2$ C: 57.52, H: 4.83, N: 4.79; found C: 57.87, H: 5.19, N: 4.46.

2. Enzyme inhibition assay

Enzyme inhibition was conducted as per Chu *et al.*[6] with some modification to allow for differences in enzyme activation. Briefly, recombinant human caspase-1, -3, -6, -7 and -8, expressed in *E.coli,* were purchased from Calbiochem (San Diego, CA). The peptide specific fluorogenic substrates Ac-YVAD-AMC (caspase-1), Ac-DEVD-AMC (caspase-3, -7), and Ac-IETD-AMC (caspase-8) were purchased from Sigma-Aldrich (St. Louis, MO) while Ac-VEID-AMC (caspase-6) was purchased from Enzo Life Sciences (Farmingdale, NY). Enzymatic activity was detected by measuring the accumulation of the fluorescent product 7-amino-4-methylcoumarin (AMC). All reactions were performed at a volume of 210 µL at 37 °C with a final DMSO concentration of 5% under the following conditions:

- *Caspase-1*; 100 mM Na-HEPES (pH 7.4), 10% Sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 5 mM β-mercaptoethanol, 10 µM Ac-YVAD-AMC.
- *Caspase-3*; 20 mM Na-HEPES (pH 7.4), 10% Sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 10 µM Ac-DEVD-AMC.
- *Caspase-6*; 20 mM Na-HEPES (pH 7.4), 10% Sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 5 mM β-mercaptoethanol, 10 µM Ac-VEID-AMC.
- *Caspase-7*; 20 mM Na-HEPES (pH 7.4), 10% Sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 5 mM β-mercaptoethanol, 10 µM Ac-DEVD-AMC.
- *Caspase-8*; 20 mM Na-HEPES (pH 7.4), 10% Sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 10 µM Ac-IETD-AMC.

Peptide inhibitors Ac-DEVD-CHO (caspase-3, -7), Ac-VEID-CHO (caspase-6) and Ac-IETD-CHO (caspase-8) were purchased from Sigma Aldrich while Ac-YVAD-CHO (caspase-1) was purchased from Cayman Chemicals (Ann Arbor, MI). Inhibition curves of these peptides were run alongside the compounds being tested as a control for each assay (see table below). Compounds and peptides were dissolved and serially diluted in DMSO to obtain the desired concentrations. 10 µL was added to each well, to which was added 100 µL of

enzyme solution and incubated for 30 mins at 4 °C. 100 µL of substrate solution was added to each well and incubated for up to 2 h at 37 °C. All reactions were conducted in triplicate.

All assays were performed in 96 well plate format. The accumulation of AMC was measured using a Molecular Devices Spectramax M5 plate reader at excitation wavelength 350 nm and emission wavelength 450 nm. The IC_{50} values were determined by non-linear regression analysis using the one site fit model from GraphPad Prism. All reported IC_{50} values are the average of three or four separate assays except where results of >10000 nM were obtained. In this case only duplicates were reported.

	IC_{50} (nM)				
	Cas-1	Cas-3	Cas-6	Cas-7	Cas-8
Ac-YVAD-CHO	7.9 ± 1.8 (*n* = 18)	-	-	-	-
Ac-DEVD-CHO	-	5.3 ± 2.3 (*n* = 17)	-	5.8 ± 1.6 (*n* = 19)	-
Ac-VEID-CHO	-	-	28.4 ± 12.3 (*n* = 13)	-	-
Ac-IETD-CHO	-	-	-	-	2.8 ± 1.5 (*n* = 8)

3. Radiochemistry

3.1. Materials and methods

[^{18}F]HF was produced on a GE PETtrace cyclotron using the ^{18}O(p, n)^{18}F nuclear reaction (Cyclotek, Bundoora, Australia). [^{18}F]**22** was purified by HPLC using a Waters Empower 2 system with a Waters 515 pump, a Linear UVis 200 detector (λ = 254 nm) together with a Carroll and Ramsey model 105S gamma detector on a Phenomenex Luna C18 column (250 × 10 mm, 5 µm) at 2 mL/min with MeCN/H_2O/TFA (80:20:0.1, v/v) as the mobile phase. Radioactivity was measured using a Capintec R15C dose calibrator.

Radiochemical purity was determined by HPLC using a Waters Empower 2 system with a Waters 600 controller pump, a Waters 2389 UV/Vis detector (λ = 254 nm) together with an IN/US Systems β-Ram Model 4 Detector on a Waters X Select CSH column (150 × 4.6 mm column, 5 µm) at 1 mL/min, with the mobile phase composition as follows:

	Time	% H_2O	% MeOH	% Formic acid (1%)
1	0.01	80.0	10.0	10.0
2	1.00	80.0	10.0	10.0
3	8.00	0.0	90.0	10.0
4	18.00	0.0	90.0	10.0
5	18.10	80.0	10.0	10.0

(*S*)-1-(4-Methoxybenzyl)-5-(2-[^{18}F]fluoromethyl)pyrrolidin-1ylsulfonyl)1*H*-indole-2,3-dione ([^{18}F]22): General Method

An aqueous H[^{18}F] solution (200-400 MBq) was added to a 2.5 mL vial containing a solution of Kryptofix[2.2.2] (K_{222}, 2.6 mg, 26 µL in MeCN, 2 eq.) and K_2CO_3 (0.47 mg, 4.7 µL in H_2O, 1 eq.). The solvent was evaporated under a stream of N_2 at 100 °C under vacuum and the residue was azeotropically dried with 3 × 1 mL anhydrous MeCN. The precursor **33** (2 mg, 1 eq.) was dissolved in anhydrous MeCN (300 µL) and added to the dried K_{222}.KF complex before being heated at 100 °C for 20 min. The reaction mixture was then treated with HCl (1 mL, 4 M) and heated at 70 °C for 5 minutes. The crude reaction mixture was purified using HPLC to yield [^{18}F]**22** in 15 ± 0.2% radiochemical yield (n = 3). The identity of [^{18}F]**22** was confirmed by co-injection with the parent compound **22** on an analytical HPLC system utilising a different mobile phase.

4. References

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