Supplementary data

Preparation of compounds **1** to **35**

All chemicals and solvents were obtained from commercial suppliers and used without further purification. Reactions were monitored by thin layer chromatography and / or liquid chromatography-mass spectrometry (LCMS). Flash column chromatography was performed on silica gel. NMR spectra were recorded on a Bruker DPX400 or DRX 400 spectrometer, and chemical shifts are reported in ppm relative to TMS. Compounds were prepared to ≥ 95% purity by LCMS, HPLC and / or NMR analysis. In the case of exploratory library synthesis, all compounds were analyzed by LCMS and representative examples were characterized by NMR. The synthesis of compound **1** is described elsewhere.[8(a)]

Compound 2: *N′*-[1-(Cyclohexylmethyl)-6-(2-morpholinoethoxy)-2-oxoindolin-3-ylidene]-2-phenylacetohydrazide

To a solution of 6-methoxyindoline-2,3-dione (268 mg, 1.51 mmol) in dimethyl formamide (7 ml) under nitrogen was added sodium hydride (47 mg, 1.96 mmol) and the mixture was stirred for 30 minutes. Bromomethyl cyclohexane (333 mg, 1.88 mmol) was added and the mixture was heated to 80°C for 1 hour. The reaction mixture was allowed to cool and then poured onto water and extracted with *t*-butyl methyl ether. The combined organic layers were dried ($MgSO_4$), filtered and evaporated and the resulting residue was triturated with di-isopropyl ether to afford 1-(cyclohexylmethyl)-6-methoxyindoline-2,3-dione (145 mg, 0.53 mmol).

A solution of 1-(cyclohexylmethyl)-6-methoxyindoline-2,3-dione (145 mg, 0.53 mmol) in dichloromethane (2 ml) was cooled to -78°C under nitrogen and boron tribromide (1M solution in dichloromethane;1.59 ml, 1.59 mmol) was added. The mixture was allowed to warm to -10°C and stirred for 1 hour and then poured onto ice water (50 ml) and extracted with diethyl ether (3 x 50 ml). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated to afford a brown gum, which was triturated with di-isopropyl ether to afford 1-(cyclohexylmethyl)-6-hydroxyindoline-2,3-dione as a light brown solid (80 mg, 0.31 mmol).

1-(Cyclohexylmethyl)-6-hydroxyindoline-2,3-dione (80 mg, 0.31 mmol) was dissolved in ethanol (5 ml) and phenylacetic hydrazide (64 mg, 0.43 mmol) was added. The mixture was heated to 60°C for 4 hours. The mixture was allowed to cool to room temperature and the resulting crystals were filtered off to afford *N′*-(1-(cyclohexylmethyl)-6-hydroxy-2-oxoindolin-3-ylidene)-2-phenylacetohydrazide as a mixture of *E* and *Z* isomers (56 mg, 0.14 mmol).

To a stirred solution of *N′*-(1-(cyclohexylmethyl)-6-hydroxy-2-oxoindolin-3-ylidene)-2-phenylacetohydrazide (20 mg, 0.05 mmol) in tetrahydrofuran (2 ml) under nitrogen was added *N*-(2-hydroxyethyl)morpholine (7.5 ul, 81 mg, 0.62 mmol) followed by triphenylphosphine (14 mg, 0.05 mmol) and diethyl azodicarboxylate (9 mg, 0.05 mmol). The mixture was stirred for 3 days at room temperature and then purified by flash column chromatography, eluting with 2% methanol in dichloromethane, to afford the required product as a mixture with triphenylphosphine oxide. The mixture was taken up in ethyl acetate and shaken with tertiary amine scavenger resin for 2 hours. The resin was washed with methanol (2x) and 1:1 dichloromethane / methanol (2x) and then eluted with 2M ammonia in methanol to afford **2** as a mixture of *E* and *Z* isomers (10 mg, 0.02 mmol). EIMS: m/z = 505.2 $[M+H]^+$.

Compound 10: 1-{[1-(Cyclohexyl)methyl-5-fluoro-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt

To a solution of 5-fluoroindole (1.0 g, 7.4 mmol) in dimethyl formamide (20 ml) was added sodium hydride (60% dispersion in mineral oil; 327 mg, 8.14 mmol). The mixture was stirred at room temperature for 10 minutes before the addition of bromomethylcyclohexane (1.3 ml, 9.3 mmol). The resulting mixture was stirred at room temperature for 15 hours. A further addition of sodium hydride (170 mg, 4.23 mmol) then bromomethylcyclohexane (0.65 ml, 4.65 mmol) was made and the reaction stirred for a further 15 hours.

The reaction was quenched with 2-propanol (10 ml) and then concentrated. The resulting brown gum was partitioned between ethyl acetate (50 ml) and 5% sodium hydrogen carbonate solution (50 ml). The organic layer was washed with water (50 ml), dried over sodium sulfate and concentrated. The crude intermediate was then purified by flash chromatography using 95% dichloromethane, 5% methanol as eluent, to afford 1-(cyclohexylmethyl)-5-fluoroindole (1.26g, 5.45 mmol).

To a solution of 1-(cyclohexylmethyl)-5-fluoroindole (208mg, 0.9 mmol) in 1,1,2,2-tetrachloroethane (15 ml) at 0°C, was added oxalyl chloride (0.122 ml, 0.945 mmol) with stirring under a stream of nitrogen. The mixture was allowed to warm to room temperature over 1 hour, then heated to 120°C for a further 1.5 hours. The mixture was cooled to room temperature and triethylamine (0.138ml, 0.99mmol) was added. Stirring was continued for a further 10 minutes before the addition of *N*-ethylpiperazine (0.125ml, 0.99mmol). The mixture was stirred at room temperature for 15 hours and then partitioned between 0.4 M sodium hydroxide solution (10 ml) and dichloromethane (10ml). The organic layer was washed with water (10 ml), dried over Na_2SO_4 and concentrated. The resulting brown oil was purified by flash chromatography using 95% dichloromethane, 5% methanol as eluent to yield the title compound as the free base.

Hydrochloride salt formation was achieved by the addition of hydrogen chloride 2M solution in diethyl ether (3 ml) to a solution of the free base in diethyl ether (5 ml). The precipitate was filtered and dried. The solid was crystallised from diethyl ether and methanol to afford **10** (1:1 hydrochloric acid salt) as a crystalline solid (0.172 g, 0.42 mmol). ^{1}H NMR (400MHz, CD_3OD) δ_H 0.98-1.27 (2H, m), 1.17-1.27 (3H, m), 1.39 (3H, t, *J* 7.5), 1.59 (2H, d, *J* 13.0), 1.64-1.77 (3H, m), 1.83-1.93 (1H, m), 3.08-3.20 (2H, m), 3.24-3.33 (2H, m), 3.51 (2H, t, *J* 12.5), 3.63 (2H, d, *J* 11.0), 4.07 (2H, d, *J* 7.5), 4.58 (2H, d, *J* 13.5), 7.04 (1H , td, *J* 9.0, 2.5), 7.45 (1H, dd, *J* 9.5, 2.5), 7.47-7.51 (1H, m), 7.77 (1H, s).; EIMS: m/z = 372.0 $[M+H]^+$.

The procedure used for compound **10** was further used to prepare the following compounds:

Compound 8: 1-{[1-(Cyclohexyl)methyl-4-fluoro-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 4-fluoroindole. EIMS: m/z = 372.0 $[M+H]^+$.

Compound 11: 1-{[5-Chloro-1-(cyclohexyl)methyl-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 5-chloroindole. EIMS: m/z = 388.2 $[M+H]^+$.

Compound 13: 1-{[1-(Cyclohexyl)methyl-5-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 5-methoxyindole. EIMS: m/z = 384.2 $[M+H]^+$.

Compound 14: 1-{[1-(Cyclohexyl)methyl-6-fluoro-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 6-fluoroindole. EIMS: m/z = 372.0 $[M+H]^+$.

Compound 15: 1-{[6-Chloro-1-(cyclohexyl)methyl-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 6-chloroindole. EIMS: m/z = 388.5 $[M+H]^+$.

Compound 16: 1-{[6-Bromo-1-(cyclohexyl)methyl-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 6-bromoindole. EIMS: m/z = 432.4 $[M+H]^+$.

Compound 18: 1-{[1-(Cyclohexyl)methyl-6-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, maleic acid salt was obtained from 6-methoxyindole. EIMS: m/z = 384.5 $[M+H]^+$.

Compound 19: 1-{[1-(Cyclohexyl)methyl-7-fluoro-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 7-fluoroindole. EIMS: m/z = 372.0 $[M+H]^+$.

Compound 20: 1-{[7-Chloro-1-(cyclohexyl)methyl-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 7-chloroindole. EIMS: m/z = 388.0 $[M+H]^+$.

Compound 21: 1-{[7-Bromo-1-(cyclohexyl)methyl-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was obtained from 7-bromoindole. EIMS: m/z = 432.5 $[M+H]^+$.

Compound 27: 1-{[7-Methoxy-1-(2-morpholinoethyl)-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine was prepared using 7-methoxyindole instead of 5-fluoroindole and 4-(2-chloroethyl)morpholine instead of bromomethyl cyclohexane. EIMS: m/z = 401.2 $[M+H]^+$.

Compound 23: 1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, maleic acid salt

To a solution of 7-methoxyindole (3.5 g, 23.8 mmol) in dimethylformamide (35 ml) at 0°C was added trifluoroacetic anhydride (4.4 ml, 31.5 mmol) over 5 minutes. The mixture was stirred at room temperature for 1 h, then poured into water (200 ml). The resulting 7-methoxy-3-[(trifluoromethyl)carbonyl]indole precipitate was filtered off, washing with water and used directly in the next step.

The damp solid was suspended in 4 M sodium hydroxide solution (140 ml) and heated to reflux with stirring for 1 hour. The mixture was cooled and washed twice with diethyl ether. The aqueous phase was then acidified to pH 1 using 5 M hydrochloric acid and the resulting fine precipitate was filtered off, washed with water and dried to afford 7-methoxyindole-3-carboxylic acid (3.6 g).

7-Methoxyindole-3-carboxylic acid (3.0 g, 16.6 mmol) was added portionwise to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 1.56 g, 39mmol) in dimethylformamide (75 ml). After 1 hour, bromomethylcyclohexane (5.7 g, 32.3 mmol) was added. The mixture was heated to 60°C with stirring for 1 hour. The mixture was diluted with water (250 ml) and washed with ethyl acetate and then diethyl ether. The aqueous phase was acidified to pH 1 using 5 M hydrochloric acid and the precipitate was filtered off. The crude product was recrystallised from ethyl acetate to afford 1-(cyclohexyl)methyl-7-methoxyindole-3-carboxylic acid (3.75 g) as a crystalline solid.

To a solution of 1-(cyclohexyl)methyl-7-methoxyindole-3-carboxylic acid (2.5 g, 8.8 mmol) in THF (30 ml) was added oxalyl chloride (4.5 g, 35.3 mmol), dropwise with stirring. The mixture was stirred at room temperature for 18 hours. The volatile components were evaporated under reduced pressure to afford 1-(cyclohexyl)methyl-7-methoxyindole-3-carbonyl chloride (2.7 g) as a crystalline solid.

To 1-(cyclohexyl)methyl-7-methoxyindole-3-carbonyl chloride (1.9 g, 6.2 mmol) was added a solution of *N*-ethylpiperazine (1.35 g, 11.8 mmol) in dichloromethane (60 ml). The mixture was stirred until the acid chloride dissolved. Triethylamine (3 ml, 21.5 mmol was added and the solution was stirred at room temperature for 18 hours. The reaction mixture was washed with water (2 x 50 ml), dried with sodium sulfate and evaporated to afford an oil. This was purified by flash chromatography eluting with 0-10% (v/v) methanol in dichloromethane to afford the title compound (free base) as a gum.

The free base was dissolved in diethyl ether (50 ml) and filtered into a stirred solution of maleic acid (0.83 g, 7.15 mmol) in ether (24 ml) and methanol (4 ml). The resulting mixture was stirred for 30 minutes and the solid filtered off. The solid was re-crystallised from methanol/diethyl ether to afford **23** (1:1 maleic acid salt) as a crystalline solid (2.7 g, 5.4 mmol). ^{1}H NMR (400MHz, CD_3OD) δ_H 0.99-1.08 (2H, m), 1.12-1.25 (3H, m), 1.36 (3H, t, *J* 7.5), 1.56 (2H, d, *J* 12.5), 1.63-1.74 (3H, m), 1.77-1.89 (1H, m), 3.22 (2H, q, *J* 7.5), 3.30-3.35 (4H, m), 3.95 (3H, s), 3.90-4.05 (4H, m), 4.25 (2H, d, *J* 7.0), 6.25 (2H, s, maleate) 6.76 (1H, d, *J* 7.5), 7.10 (1H, t, *J* 7.5), 7.26 (1H, d, *J* 7.5), 7.53 (1H, s); EIMS: m/z = 384.4 $[M+H]^+$.

The procedure used for compound **23** was further used to prepare the following compounds:

Compound 3: N-Benzyl-1-(cyclohexylmethyl)-1H-indole-3-

carboxamide was obtained using indole-3-carboxylic acid instead of 7-methoxyindole-3-carboxylic acid and benzylamine instead of *N*-ethylpiperazine. EIMS: m/z = 347.2 $[M+H]^+$.

Compound 4: 1-(Cyclohexylmethyl)-N-(2-(dimethylamino)ethyl)-1H-indole-3-carboxamide was obtained using indole-3-carboxylic acid instead of 7-methoxyindole-3-carboxylic acid and 2-(dimethylamino)ethylamine instead of *N*-ethylpiperazine. EIMS: m/z = 328.4 $[M+H]^+$.

Compound 5: 1-(Cyclohexylmethyl)-N-(2-(dimethylamino)ethyl)-N-methyl-1H-indole-3-carboxamide was obtained using indole-3-carboxylic acid instead of 7-methoxyindole-3-carboxylic acid and N,N,N'-trimethylethylenediamine instead of *N*-ethylpiperazine. EIMS: m/z = 342.0 $[M+H]^+$.

Compound 6: 1-{[1-(Cyclohexylmethyl)-1*H*-indol-3-yl]carbonyl}-4-methylpiperazine was obtained using indole-3-carboxylic acid instead of 7-methoxyindole-3-carboxylic acid and *N*-methylpiperazine instead of *N*-ethylpiperazine. EIMS: m/z = 340.2 $[M+H]^+$.

Compound 7: 1-{[1-(Cyclohexylmethyl)-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine was obtained using indole-3-carboxylic acid instead of 7-methoxyindole-3-carboxylic acid and purified by HPLC. EIMS: m/z = 354.2 $[M+H]^+$.

Compound 9: 1-{[1-(Cyclohexylmethyl)-4-methyl-1*H*-indol-3-yl]carbonyl}-4-methylpiperazine was obtained using 4-methylindole instead of 7-methoxyindole and *N*-methylpiperazine instead of *N*-ethylpiperazine. EIMS: m/z = 354.2 $[M+H]^+$.

Compound 12: 1-{[1-(Cyclohexylmethyl)-5-methyl-1*H*-indol-3-yl]carbonyl}-4-methylpiperazine was obtained using 5-methylindole instead of 7-methoxyindole and *N*-methylpiperazine instead of *N*-ethylpiperazine. EIMS: m/z = 354.0 $[M+H]^+$.

Compound 17: 1-{[1-(Cyclohexylmethyl)-6-methyl-1*H*-indol-3-yl]carbonyl}-4-methylpiperazine was obtained using 6-methylindole instead of 7-methoxyindole and *N*-methylpiperazine instead of *N*-ethylpiperazine. EIMS: m/z = 354.4 $[M+H]^+$.

Compound 22: 1-{[1-(Cyclohexyl)methyl-7-methyl-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine was obtained using 7-methylindole instead of 7-methoxyindole. EIMS: m/z = 368.0 $[M+H]^+$.

Compound 24: 1-{[1-(Cyclopentyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt

Cyclopentanemethanol *p*-toluenesulfonate was prepared by the following method: To a solution of cyclopentanemethanol (2.0 g, 20.0 mmol) and pyridine (2.9 ml, 36.3 mmol) in dichloromethane (20 ml) was added *p*-toluenesulfonyl chloride (3.46 g, 18.1 mmol). The mixture was stirred at room temperature for 24 hours under nitrogen. The resulting mixture was washed with 2M hydrochloric acid and the aqueous layer separated and extracted with dichloromethane. The combined organics were dried over sodium sulfate and concentrated under reduced pressure to yield cyclopentanemethanol *p*-toluenesulfonate as a colourless oil (4.3 g, 17.0 mmol).

Compound **24** was prepared following the same method as compound **23**, using cyclopentanemethanol *p*-toluenesulfonate instead of bromomethylcyclohexane. ^{1}H NMR (400MHz, CD_3OD) δ_H 1.29-1.35 (2H, m), 1.38 (3H, t, *J* 7.5), 1.52-1.71 (6H, m), 2.39-2.49 (1H, m), 3.24 (2H, q, *J* 7.5), 3.05-3.35 (2H, br m), 3.35-3.70 (4H, br m), 3.95 (3H, s), 4.38 (2H, d, *J* 7.5), 4.40-4.65 (2H, br m), 6.79 (1H, d, *J* 7.5), 7.10 (1H, t, *J* 7.5), 7.27 (1H, d, *J* 7.5), 7.60 (1H, s); EIMS: m/z = 370.2 $[M+H]^+$.

Compound 25: 1-{[1-(cycloheptyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt was prepared using cycloheptanemethanol *p*-toluenesulfonate. EIMS: m/z = 398.2 $[M+H]^+$.

Compound 26: 1-{[1-Benzyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine, hydrochloride salt

To a stirred solution of 7-methoxyindole-3-carboxylic acid (5.1 g, 26.6 mmol) in tetrahydrofuran (50 ml), maintained at room temperature using a water bath, was added dropwise oxalyl chloride (10.0 g, 78.5 mmol). The reaction was stirred until LCMS (sample quenched with *N*-ethylpiperazine) indicated complete reaction. The solvent and excess oxalyl chloride were then removed under reduced pressure and the residue was dissolved in dichloromethane (50 ml) with stirring. *N*-Ethylpiperazine (9.0 g, 78.5 mmol) in dichloromethane (20 ml) was added dropwise and the reaction mixture was stirred under nitrogen for 18 hours. The resulting mixture was washed twice with water, dried over sodium sulfate, filtered and the solvent evaporated. The residue was purified by flash chromatography eluting with 0-25 % (v/v) methanol in dichloromethane to afford 1-{[7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine (4.24 g, 14.8 mmol).

1-{[7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethylpiperazine (250 mg, 0.87 mmol) was added portionwise to a stirred suspension of sodium hydride (60% dispersion in mineral oil; 106 mg, 2.6 mmol) in dimethylformamide (10 ml) under nitrogen and the mixture stirred for 1 hour. Benzyl bromide (155 mg, 0.91 mmol) was added and the mixture stirred at room temperature for three days. Water (5 ml) was added and the solvents evaporated under reduced pressure. The residue was purified by preparatory HPLC (Waters Xterra RP_8 30 mm x 100 mm column; 50-100% acetonitrile-water over a 25 minute gradient; 30 ml/min; 8mM ammonium bicarbonate buffer; detection by UV at 254 nm) followed by hydrochloride salt formation and re-crystallisation from ethyl acetate afforded **26** (129 mg, 0.31 mmol). EIMS: m/z = 378.2 $[M+H]^+$.

Preparation of intermediates for the methyl survey (compounds 28-35):

1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,5-dimethylpiperazine, hydrochloride salt

To a solution of 1-(cyclohexyl)methyl-7-methoxy-indole-3-carboxylic acid (0.25 g, 0.87 mmol, prepared following the method in Example **1**) and 2,6-dimethylpiperazine (0.12 g, 1.05 mmol) in dichloromethane (10 ml) was added diisopropylcarbodiimide (0.16 ml, 1.05 mmol) and 1-hydroxy benzotriazole (0.01 g, 0.09 mmol). The mixtures was stirred at room temperature for 18 hours. The mixture was washed with 5 M sodium hydroxide (2 x 10 ml), dried with magnesium sulfate and evaporated. The residue was purified by flash chromatography eluting with 5-10 % (v/v) methanol in dichloromethane to afford the title compound (free base) as a colourless oil. The free base (0.15 g) was dissolved in diethyl ether (3 ml) and treated dropwise with 2 M hydrochloric acid in diethyl ether (1 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (15 ml) and dried under reduced pressure to afford the title compound (1:1 hydrochloric acid salt) as a colourless solid (0.15 g, 0.36 mmol). ^{1}H NMR (400MHz, CD_3OD) δ_H 0.98-1.26 (5H, m) 1.32 (6H, d, *J* 6.5), 1.56 (2H, br d, *J* 12.0), 1.62-1.90 (4H, m), 3.06 (2H, dd, *J* 14.5, 11.5), 3.39–3.50 (2H, m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.5), 4.52 (2H, br d, *J* 13.5), 6.77 (1H, d, *J* 7.5), 7.1 (1H, t, *J* 8.0), 7.24 (1H, d, *J* 8.0), 7.54 (1H, s); EIMS: m/z 384.2 $[M+H]^+$.

The procedure described above was further used to prepare the following intermediates:

1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,3-dimethylpiperazine, hydrochloride salt was prepared using 1-(cyclohexyl)methyl-7-methoxy-indole-3-carboxylic acid and 2,2-dimethylpiperazine. ^{1}H NMR (400MHz, CD_3OD) δ_H 1.10-1.22 (5H, m), 1.38 (6H, s), 1.54-1.86 (6H, m), 3.31-3.34 (2H, m), 3.2 (2H, dd, *J* 14.5, 10.9), 3.81 (2H, s), 3.95 (3H, s), 3.96-3.99 (2H, m), 4.26 (2H, d, *J* 7.1), 6.76 (1H, d, *J* 7.5), 7.10 (1H, t, *J* 8.1), 7.24 (1H, d, *J* 8.0), 7.53 (1H, s). EIMS; m/z = 384.5 $[M+H]^+$.

(*S*)-1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3-methylpiperazine, hydrochloride salt was prepared using 1-(cyclohexyl)methyl-7-methoxy-indole-3-carboxylic acid and (*S*)-2-methylpiperazine. ^{1}H NMR (400MHz, CD_3OD) δ_H 1.01-1.23 (5H, m), 1.33 (3H, d, *J* 6.5), 1.52-1.87 (6H, m), 3.16-3.27 (2H, m), 3.38-3.51 (3H, m), 3.95 (3H, s), 4.27 (2H, d, *J* 7.0), 4.43 (2H, br d, *J* 14.3), 6.76 (1H, d, *J* 7.8), 7.10 (1H, t, *J* 7.9), 7.25 (1H, d, *J* 8.0), 7.54 (1H, s). EIMS; m/z = 370.0 $[M+H]^+$.

(*R*)-1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3-methylpiperazine, hydrochloride salt was prepared using 1-(cyclohexyl)methyl-7-methoxy-indole-3-carboxylic acid and (*R*)-2-methylpiperazine. ^{1}H NMR (400MHz, CD_3OD) δ_H 1.01-1.23 (5H, m), 1.33 (3H, d, *J* 6.5), 1.52-1.87 (6H, m), 3.16-3.27 (2H, m), 3.38-3.51 (3H, m), 3.95 (3H, s), 4.27 (2H, d, *J* 7.0), 4.43 (2H, br d, *J* 14.3), 6.76 (1H, d, *J* 7.8), 7.10 (1H, t, *J* 7.9), 7.25 (1H, d, *J* 8.0), 7.54 (1H, s). EIMS; m/z = 370.0 $[M+H]^+$.

Compound 30: 1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,5-dimethyl-4-ethylpiperazine, hydrochloride salt

To a solution of 1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,5-dimethylpiperazine (0.7 g, 1.83 mmol) and potassium carbonate (0.3 g, 2.19 mmol) in dimethylformamide (5 ml) was added iodoethane (0.17 ml, 2.10 mmol). The mixture was heated to 50°C for 18 hours and diluted with water (20 ml). The suspension was then extracted with methyl *tert*-butyl ether (2 x 30 ml) and the combined organic layers were washed with water (3 x 20 ml), dried with magnesium sulfate and evaporated. The residue was purified by flash chromatography eluting with 5-10 % (v/v) methanol in dichloromethane to afford the title compound (free base) as a colourless oil. The free base (0.42 g) was dissolved in diethyl ether (10 ml) and treated dropwise with 2 M hydrochloric acid in diethyl ether (1 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (15 ml) and dried under reduced pressure to afford **30** (1:1 hydrochloric acid salt) as a white solid (0.35 g, 0.78 mmol). ^{1}H NMR (400MHz, CD_3OD) δ_H 0.98-1.23 (5H, m), 1.30 (3H, t, *J* 7.0), 1.39 (6H, d, *J* 7.0), 1.53-1.88 (6H, m), 3.22-3.35 (2H, m), 3.42–3.61 (4H, m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.0), 4.53 (2H, br d, *J* 13.0), 6.77 (1H, d, *J* 8.0), 7.10 (1H, t, *J* 8.0), 7.27 (1H, d, *J* 8.0), 7.57 (1H, s). EIMS: m/z 412.4 $[M+H]^+$.

The procedure used for compound **30** was further used to prepare the following compounds:

Compound 28: *(R)*-1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethyl-3-methylpiperazine, hydrochloride salt was prepared using *(R)*-1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3-methylpiperazine. ^{1}H NMR (400MHz, CD_3OD) δ_H 0.97-1.43 (11H, m), 1.56 (2H, br d, *J* 12.0), 1.64-1.89 (4H, m), 3.12-3.68 (7H, br m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.0), 4.50 (2H, br s), 6.77 (1H, d, *J* 8.0), 7.10 (1H, t, *J* 8.0), 7.26 (1H, d, *J* 8.0), 7.54 (1H, s). EIMS; m/z 398.2 $[M+H]^+$.

Compound 29: *(S)*-1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-4-ethyl-3-methylpiperazine, hydrochloride salt was prepared using *(S)*-1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3-methylpiperazine. ^{1}H NMR (400MHz, CD_3OD) δ_H 0.97-1.43 (11H, m), 1.56 (2H, br d, *J* 12.0), 1.64-1.89 (4H, m), 3.12-3.68 (7H, br m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.0), 4.50 (2H, br s), 6.77 (1H, d, *J* 8.0), 7.10 (1H, t, *J* 8.0), 7.26 (1H, d, *J* 8.0), 7.54 (1H, s). EIMS; m/z 398.2 $[M+H]^+$.

Compound 31: 1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,3-dimethyl-4-ethylpiperazine, hydrochloride salt was prepared using 1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,3-dimethylpiperazine and iodoethane. ^{1}H NMR (400MHz,

CD_3OD) δ_H 0.97-1.90 (20H, m), 2.82-3.69 (6H, br m), 3.95 (3H, s), 4.22-4.61 (4H, m), 6.77 (1H, d, *J* 7.9), 7.10 (1H, t, *J* 8.0), 7.25 (1H, d, *J* 8.1), 7.53 (1H, s). EIMS; m/z 412.4 $[M+H]^+$.

Compound 32: (*R*)-1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,4-dimethylpiperazine, hydrochloride salt was prepared using (*R*)-1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3-methylpiperazine and iodomethane. ^{1}H NMR (400MHz, CD_3OD) δ_H 0.97-1.89 (14H, m), 2.92 (3H, br s), 3.19-3.61 (5H, br m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.0), 4.49 (2H, m), 6.76 (1H, d, *J* 7.5), 7.10 (1H, t, *J* 8.0), 7.27 (1H, d, *J* 8.0), 7.54 (1H, s). EIMS; m/z 384.2 $[M+H]^+$.

Compound 33: (*S*)-1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,4-dimethylpiperazine, hydrochloride salt was prepared using (*S*)-1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3-methylpiperazine and iodomethane. ^{1}H NMR (400MHz, CD_3OD) δ_H 0.97-1.89 (14H, m), 2.92 (3H, br s), 3.19-3.61 (5H, br m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.0), 4.49 (2H, m), 6.76 (1H, d, *J* 7.5), 7.10 (1H, t, *J* 8.0), 7.27 (1H, d, *J* 8.0), 7.54 (1H, s). EIMS; m/z 384.2 $[M+H]^+$. Optical rotation $[\alpha]_D^{20}$ -25.4° at 2.44 mg/mL in MeOH.

Compound 34: 1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,4,5-trimethylpiperazine, hydrochloride salt was prepared using 1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,5-dimethylpiperazine and iodomethane. ^{1}H NMR (400MHz, CD_3OD) δ_H 0.97-1.89 (17H, m), 2.96 (3H, br s), 3.23-3.48 (4H, br m), 3.95 (3H, s), 4.26 (2H, d, *J* 7.0), 4.49 (2H, br d, *J* 12.0), 6.77 (1H, d, *J* 7.5), 7.10 (1H, t, *J* 8.0), 7.26 (1H, d, *J* 7.5), 7.54 (1H, s). EIMS; m/z 398.0 $[M+H]^+$.

Compound 35: 1-{[1-(Cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,3,4-trimethylpiperazine, hydrochloride salt was prepared using 1-{[1-(cyclohexyl)methyl-7-methoxy-1*H*-indol-3-yl]carbonyl}-3,3-dimethylpiperazine and iodomethane. ^{1}H NMR (400MHz, CD_3OD) δ_H 0.98-1.90 (17H, m), 2.85 (3H, s), 3.29-3.70 (4H, m), 3.95 (3H, s), 4.22-4.60 (4H, m), 6.77 (1H, d, *J* 7.7), 7.10 (1H, t, *J* 8.1), 7.25 (1H, d, *J* 8.2), 7.54 (1H, s). EIMS; m/z 398.2 $[M+H]^+$.

In-vitro determination of efficacy and potency at the human CB1 receptor expressed in CHO cells

Chinese Hamster Ovary (CHO) cells expressing the human CB1 receptor and a luciferase reporter gene under the control of Activator Protein-1 (AP-1) response elements were suspended in phenol red / serum free DMEM / F-12 nut mix containing penicillin / streptomycin (50U/50 □g/ml) and fungizone (1 □g/ml) and seeded into 96 well plates at a density of 3 x 10^4 cells per well (100 □l final volume). Cells were incubated overnight (approx. 18 hours at 37°C, 5% CO_2/95% air) prior to assay.

The test compound (10mM solution in DMSO) was diluted in F12 Nut Mix to give a range of stock solutions from 0.11 mM to 0.11 nM. The stock solutions (10□l) were added directly to the relevant wells. The plates were incubated at 37°C for 5 hours to allow agonist-induced expression of the luciferase enzyme. Under subdued light, LucLite substrate (Packard; reconstituted as per manufacturer's instructions; 100 □l) was added to each well. Plates were covered with Top Seal and then incubated at room temperature for 5 minutes before counting on the Packard TopCount (single photon counting, 0.01 minute count time, 5 minute count delay).

A "best-fit" curve was fitted by a minimum sum of squares method to the plot of counts per second (CPS) against compound concentration (M) to obtain an EC_{50} value.

In-vitro determination of binding affinity at the human CB1 and CB2 receptors

Binding assays were performed with 0.5nM ^{3}H-CP55940 (160Ci/mmol, Perkin Elmer NEN, USA) in 20mM HEPES buffer at pH 7.4 containing 5mM $MgCl_2$, 1mM EDTA and 0.3% w/v BSA in a final volume of 200µl.

Membranes prepared from Sf9 insect cells expressing the human CB1 or CB2 receptor and $G\alpha_{i3}\beta_1\gamma_2$ G-protein were purchased from Perkin Elmer Biosignal (Montreal, USA) and used a source of receptor protein. Binding reactions were initiated by the addition of 7µg and 1.4µg of CB1 and CB2 membrane, respectively, and incubated at room temperature for 70 minutes. Non Specific Binding was determined by 10µM WIN-55212. The reaction was terminated by rapid filtration using a Tomtec harvester onto 96 well Unifilter GF/C filter plates (Perkin Elmer, USA) pre-soaked with assay buffer. Filter plates were washed five times with 200µl ice cold wash buffer (20mM HEPES, 5mM $MgCl_2$, 1mM EDTA, 0.01% w/v BSA), oven dried and scintillated using 50µl Microscint-20 (Perkin Elmer, USA). The retained radioactivity was then determined using a Packard Topcount scintillation counter. A "best-fit" curve was fitted by a minimum sum of squares method to the plot of counts per minute (CPM) against compound concentration (M) to obtain an IC_{50} value. The IC_{50} was then converted to the negative logarithim of the inhibitor constant (pKi) according to the Cheng Prussof approximation.[1]

Determination of aqueous solubility

Aqueous solubility was determined using a variation of a shake-flask methodology. The test compound (1 mg) was accurately weighed into a small (2 ml) snap-lid plastic centrifuge tube. An amount of the test solution (250 µl) was added and the contents mixed (vortex, 1500 rpm, 24 ± 0.5 hours). After mixing, the contents of each tube were filtered under centrifugation (rcf 2872, 5 min) using a Whatman VectaSpin Micro centrifuge filter (PVDF membrane, 0.45 µm), and the pH measured using a micro pH electrode (Hamilton Minitrode). The filtrate was then diluted either 1:10 or 1:100 with DMSO depending upon the solubility of the compound.

The amount of compound in solution for the diluted filtrates was then determined by High Performance Liquid Chromatography (HPLC). Analysis was performed on an Agilent 1200 HPLC system, equipped with a CTC Analytics

HTS PAL autosampler and a Photodiode Array UV/visible detector. A Zorbax Eclipse XDB-C18 column (4.6 x 50 mm, 1.8 µm, particle size, Agilent Technologies) was used for the separation. The column temperature was 60°C. Mobile phase A and B consisted of 0.1% trifluoroacetic acid in 95/5 (v/v) water/acetonitrile and 0.1% trifluoroacetic acid in 95/5 acetonitrile/water (v/v), respectively. The flow rate was 2.0 ml/min, and the injection volume was 5 µl. A gradient elution was used to elute the test compounds from the HPLC column: The mobile phase composition was initially 95% A, 5% B. This was held for 0.3 min and then linearly increased to 5% A, 95% B over two minutes, and held at 95% B for a further 0.5 minutes. The total run time was 2.8 min. A detection wavelength of 230 nm was used for all compounds. Peak areas from analysis of the diluted filtrates were quantified by comparison to a calibration line prepared by injecting a series of calibration standards, made by diluting a stock solution of the test compound (1 mg/ml in DMSO) with DMSO to give solutions from 1 to 100 µg/ml. Solubilities were determined in duplicate for each test compound in phosphate buffered saline (PBS) pH 7.4 (0.05 M phosphate, 0.15 M chloride) and 0.01 M citrate buffer, pH 5, and average values reported.

Determination of Tail Flick Latency in Mice

Male ICR mice weighing 20-34 g were trained to sit still in a tail flick apparatus (Ugo Basile, Italy) whilst tail flick latency was measured. The tail was exposed to a focused beam of radiant heat at a point approximately 2.5 cm from the tip. Tail flick latency was defined as the interval between the appliance of the thermal stimulus and withdrawal of the tail. A 12 second cut-off was employed to prevent tissue damage. Four groups of eight mice were treated with vehicle or one of three doses of the test compound, administered intravenously (vehicle: 10% Tween 80 in saline 9 g/l; injection volume 10 ml/kg). Tail flick latency was measured before administration of the test compound and at 20, 40 and 60 minutes after compound administration. Data were plotted as mean ± s.e.m. and expressed as % maximum possible effect (%MPE).

$$\%MPE = \frac{\text{Post-drug latency} - \text{baseline latency}}{\text{Cut-off latency} - \text{baseline latency}} \times 100$$

The time of maximum effect for each mouse in the two top dose groups was determined and these values averaged to calculate the mean time of maximum effect. For analytical purposes Tmax was defined as the time point closest to this averaged value. Tmax data were then compared between groups using the Kruskal-Wallis one-way analysis of variance, a non- parametric statistical test. If statistical significance ($P < 0.05$) was observed with this test, the vehicle group and each of the treatment groups were compared using the non-parametric Dunn's test (Unistat 5.0 software).

Inhibition of nociceptive behavior following formalin injection in mice

Male ICR mice weighing 20-31 g were habituated to their test environment for 1-2 hours on the day prior to the experiment and for 1 hour prior to drug administration on the day of the experiment. Groups of eight mice were treated with test compound or vehicle, administered intravenously as above 15 minutes before subplantar injection of 20 µl of 3% formalin solution into the dorsal surface of one hind paw. Mice were then placed in observation boxes on a glass shelf situated above a video camera. The total time in seconds spent licking the paw between 0 and 5 minutes after formalin injection (Phase 1) and between 20 and 30 minutes after formalin injection (Phase 2) was recorded and the percent inhibition of licking for each mouse, compared to the mean time spent licking for the vehicle treated animals, was calculated. The mean and s.e.m. for each treatment group was calculated and compared between groups using the Kruskal-Wallis one-way analysis of variance. If statistical significance ($P < 0.05$) was observed with this test, the vehicle group and each of the treatment groups was compared using the Dunn's test.

Reference

1 Y. Cheng and W. H. Prusoff, *Biochem. Pharmacol.*, 1973, **22**, 3099–3108.