SUPPORTING INFORMATION

5-Hydroxyindole-2-Carboxylic Acid Amides: Novel Histamine-3 Receptor Inverse Agonists for the Treatment of Obesity.

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Content:

SYNTHESIS DESCRIPTION AND SPECTROSCOPIC DATA

GENERAL

Proton NMR's were obtained on a Bruker 300 or 400MHz instrument with chemical shifts (δ in ppm) reported relative to tetramethylsilane as an internal standard. NMR abbreviations: s, singlet; d, doublet; t, triplet; quad., quadruplet; quint., quintuplet ; sext., sextuplet; hept., heptuplet; mult., multiplet.

Elemental analyses were performed by Solvias AG (Mattenstrasse; Postfach; CH-4002 Basel). Column chromatography was carried out on silica gel 60 (32-60 mesh, 60Å) or on pre-packed columns (Isolute Flash Si). Electron impact ionization mass spectra were recorded on an SSQ 7000 (Finnigan-MAT) spectrometer. High Resolution Mass Spectra (HRMS) were recorded on a Nanospray Bruker Reflex spectrometer.

LC MS analytical conditions (chromatograms displayed displayed below):

Instrument: Finnigan LTQ (Thermo Fisher Scientific) + Agilent RRLC 1200

Column: Agilent Zorbax XDB C18 50 mmx2.1 mm, 1.8 µm

Solvent system: A: 10mM ammoniumacetate in water

B 10mM ammoniumacetate in acetonitrile 950 mL/ 50 mL water

Gradient: Initial 5% B (0.5 min) then 7.5 min 95% B (5 min)

Flow: 0.2 mL / min.

UV-Detector: DAD 190-400 nm step 2 nm

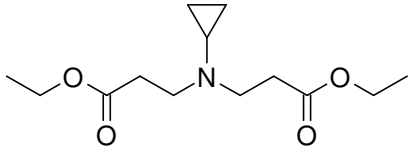
Interface: ESI

Sample solvent: in water/acetonitrile (8/2)

Injection volume: 2 µl

Scheme 2: Preparation of piperidines and piperazines

3-[Cyclopropyl-(2-ethoxycarbonyl-ethyl)-amino]-propionic acid ethyl ester (11)



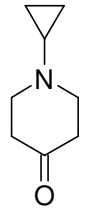
A mixture of ethyl acrylate (32.6 mL, 300 mmol, 2.0 eq.) and cyclopropylamine (**10**, 10.5 mL, 149 mmol) in absolute ethanol (45 mL) was stirred for 4 days at room temperature. Volatiles were evaporated in vacuo. Purification was performed by distillation under reduced pressure to afford the desired product as a colorless liquid (20.58 g, 54%).

Boiling point: 135 °C at 20 mBar.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (quad., *J* = 7 Hz, 4H); 2.91 (t, *J* = 7 Hz, 4H); 2.50 (t, *J* = 7 Hz, 4H); 1.73 (mult., 1H); 1.25 (t, *J* = 7 Hz, 6H); 0.50-0.35 (mult., 4H) ppm.

MS (EI) *m/e:* 258.2 $(M+H)^+$.

1-Cyclopropyl-piperidin-4-one



A solution of 3-[cyclopropyl-(2-ethoxycarbonyl-ethyl)-amino]-propionic acid ethyl ester (**11**, 10g, 39 mmol) in tetrahydrofuran (65 mL) was added dropwise to a suspension of sodium hydride (60% dispersion in oil, 2.33 g, 58 mmol, 1.5 eq.) in tetrahydrofuran (65 mL). Absolute ethanol was added (2.3 mL, 39 mmol, 1.0 eq.) and the resulting mixture was stirred 24h at reflux . The mixture was neutralized (pH: ca. 7) by addition of acetic acid and partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo, to afford a reddish oil (10.2 g).

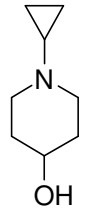
This oil was refluxed 5h with aqueous hydrochloric acid (18% w/w, 130 mL), and basified (pH: ca. 12) by addition of sodium hydroxide (ca. 31 g). The mixture was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by distillation under reduced pressure to afford the desired product as a colorless liquid (3.6 g, 67%).

Boiling point: 75°C at 20 mBar.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (t, *J* = 7 Hz, 4H); 2.42 (t, *J* = 7 Hz, 4H); 1.80-1.70 (mult., 1H); 0.55-0.47 (mult., 4H) ppm.

MS (EI) *m/e:* 140.2 $(M+H)^+$.

1-Cyclopropyl-piperidin-4-ol (12)

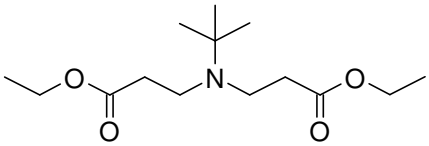


To a cold (ice bath) solution of 1-cyclopropyl-piperidin-4-one (1.5 g, 11 mmol) in ethanol (10 mL) was added sodium borohydride (306 mg, 8 mmol, 0.75 eq.). The mixture was stirred 3 days at room temperature and concentrated in vacuo. Ice water (20 mL) was added, followed by an aqueous solution of sodium hydroxide (28% w/w, 10 mL) and dichloromethane (20 mL). The mixture was stirred 2h at room temperature. After phase separation, the aqueous layer was extracted with dichloromethane. The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane : methanol : aqueous ammonia (90:9:1) as eluant to afford, after evaporation, the desired product as a colorless oil (1.44 g, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (hept., J = 6 Hz, 1H); 2.92-2.87 (mult., 2H); 2.33 (dt, J = 6 Hz, 12 Hz, 3 Hz, 2H); 1.89-1.83 (mult., 2H); 1.60-1.48 (mult., 4H); 0.46-0.37 (mult., 4H) ppm.

MS (EI) *m/e:* 142.2 $(M+H)^+$.

3-[tert-Butyl-(2-ethoxycarbonyl-ethyl)-amino]-propionic acid ethyl ester (14)



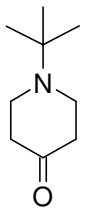
A mixture of *tert*-butylamine (**13**, 21.9 mL, 210 mmol) and ethyl acrylate (80 mL, 735 mmol, 3.5 eq.) was refluxed for 6 days. The volatile fractions (<100°C at 5 mBar) were removed by distillation under reduced pressure. The residue was purified by column chromatography on silica gel using cyclohexane/

ethyl acetate (4:1) as eluant to afford, after evaporation, the desired product as a light yellow oil (8.3 g, 14%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (quad., *J* = 7 Hz, 4H); 2.82 (t, *J* = 8 Hz, 4H); 2.43 (t, *J* = 8 Hz, 4H); 1.26 (t, *J* = 7 Hz, 6H); 1.059 (s, 9H) ppm.

MS (EI) *m/e:* 274.4 $(M+H)^+$.

1-tert-Butyl-piperidin-4-one



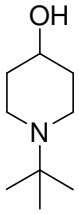
A solution of 3-[tert-butyl-(2-ethoxycarbonyl-ethyl)-amino]-propionic acid ethyl ester (**14**, 8.07 g, 30 mmol) in tetrahydrofuran (50 mL) was added dropwise to a suspension of sodium hydride (dispersion in oil, 60% w/w, 1.77 g, 44 mmol, 1.5 eq.) in tetrahydrofuran (50 mL). Absolute ethanol was added (1.72 mL, 30 mmol, 1.0 eq.) and the resulting mixture was stirred 24h at reflux . The mixture was neutralized (pH: ca. 8) by addition of acetic acid and partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo to afford a reddish oil (8.4 g).

This oil was refluxed 5h with hydrochloric acid (18% w/w, 100 mL) and basified (pH: ca. 12) by the addition of sodium hydroxide (ca. 23 g). The mixture was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane : methanol : aqueous ammonia (90:9:1) as eluant to afford, after evaporation, the desired product as a colorless oil (2.78 g, 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (t, *J* = 6 Hz, 4H); 2.43 (t, *J* = 6 Hz, 4H); 1.13 (s, 9H) ppm.

MS (EI) *m/e:* 156.4 $(M+H)^+$.

1-tert-Butyl-piperidin-4-ol (15)

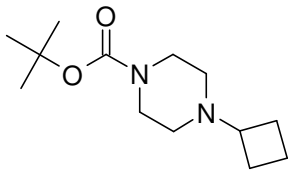


To a cold (ice bath) solution of 1-tert-butyl-piperidin-4-one (2.1 g, 14 mmol) in ethanol (15 mL) was added sodium borohydride (400 mg, 10 mmol, 0.75 eq.). The mixture was stirred 24h at room temperature and concentrated in vacuo. Ice water (28 mL) was added, followed by an aqueous solution of sodium hydroxide (40% w/w, 20 mL) and dichloromethane (28 mL). The mixture was stirred 2h at room temperature. The mixture was partitioned between water and dichloromethane and extracted with dichloromethane. The combined organic phases were washed with brined, dried over sodium sulfate, filtered and concentrated in vacuo to afford the desired product as a yellow oil (1.82 g, 86%). This oil was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67-3.60 (mult., 1H); 2.92-2.85 (mult., 2H); 2.26 (dt, *J* = 6 Hz, 12 Hz, 3 Hz, 2H); 1.95-1.87 (mult., 2H); 1.62-1.50 (mult., 2H); 1.07 (s, 9H) ppm.

MS (EI) *m/e:* 158.1 $(M+H)^+$.

4-Cyclobutyl-piperazine-1-carboxylic acid tert-butyl ester

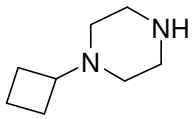


A solution of piperazine-1-carboxylic acid tert-butyl ester (**16**, 20 g, 105 mmol) and cyclobutanone (8.18 mL, 105 mmol, 1.0 eq.) in dichloromethane (200 mL) was stirred 30 min at room temperature. Sodium acetoxyborohydride (23 g, 105 mmol, 1.0 eq.) was added and the mixture stirred 16h at room temperature. The mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with a saturated aqueous solution of sodium hydrogenocarbonate, dried over sodium sulfate, filtered and concentrated in vacuo to afford the desired product as a yellow oil (25.95 g, 97%). This oil was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45-3.38 (mult., 4H); 2.71 (quint., J = 8 Hz, 1H); 2.28-2.20 (mult., 3H); 2.08-1.98 (mult., 2H); 1.92-1.78 (mult., 2H); 1.67-1.52 (mult., 2H); 1.41 (s, 9H) ppm.

MS (EI) *m/e:* 241.1 $(M+H)^+$.

1-Cyclobutyl-piperazine . 2HCl salt (17)



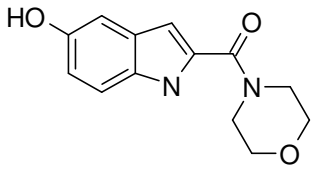
To a solution of 4-cyclobutyl-piperazine-1-carboxylic acid tert-butyl ester (25.95 g, 108 mmol) in dioxane (100 mL) was added dropwise a solution of hydrochloric acid in dioxane (4M, 108 mL, 432 mmol, 4.0 eq.). The brown mixture was stirred overnight at room temperature. The precipitate was filtered, washed with dioxane, and dried in vacuo to afford the desired product as a light brown solid (22.17 g, 96%). This solid was used in the next step without further purification.

^{1}H NMR (400 MHz, d^6-DMSO) δ 3.68-3.62 (mult., 1H); 3.57-3.25 (mult., 6H); 3.15-2.97 (mult., 1H); 2.38 (quint., *J* = 9 Hz, 2H); 2.20-2.09 (mult., 2H); 1.82-1.62 (mult., 2H) ppm.

MS (EI) *m/e:* 141.3 $(M+H)^+$.

Scheme 3: Preparation of di-substituted indoles from 5-hydroxy-indole-2-carboxylic acid

(5-Hydroxy-1H-indol-2-yl)-morpholin-4-yl-methanone (19)

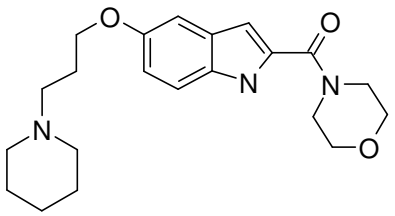


To a cold (ice bath) solution of 5-Hydroxy-1H-indole-2-carboxylic acid (**18**, 5.0 g, 28 mmol) in N,N-dimethylformamide (70 mL) were successively added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (10.85 g, 32 mmol, 1.15 eq.), morpholine (2.83 mL, 32 mmol, 1.15 eq.) and ethyl-diisopropyl-amine (24.6 mL, 141 mmol, 5.0 eq.). The mixture was stirred 16h at room temperature and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and evaporated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : methanol : aqueous ammonia (95:5:0.25 to 90:9:1) as eluant, to afford, after evaporation, the desired product as a colorless oil (5.97 g, 86%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H); 7.21 (d, *J* = 9 Hz, 1H); 6.87 (d, *J* = 2 Hz, 1H); 6.71 (dd, *J* = 9 Hz, 2 Hz, 1H); 6.61 (d, *J* = 2 Hz, 1H); 3.80-3.67 (mult., 4H); 3.67-3.60 (mult., 4H) ppm.

MS (EI) *m/e:* 247.4 $(M+H)^+$.

Morpholin-4-yl-[5-(3-piperidin-1-yl-propoxy)-1H-indol-2-yl]-methanone (20)



To a solution of (5-hydroxy-1H-indol-2-yl)-morpholin-4-yl-methanone (**19**, 1.2 g, 5 mmol), 1-piperidine-propanol (907 mg, 6 mmol, 1.3 eq.) and tri-n-butylphosphine (2.32 g, 10 mmol, 2.0 eq.) in tetrahydrofuran (100 mL) was slowly added 1,1'-(azodicarbonyl)-dipiperidine (2.48 g, 10 mmol, 2.0 eq.) within 15 min. The mixture was stirred overnight at room temperature. The resulting suspension was filtered. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : 2N ammonia in methanol (97:3 to 19:1) as eluant to afford, after evaporation, the desired product as a light yellow solid (514 mg, 28%).

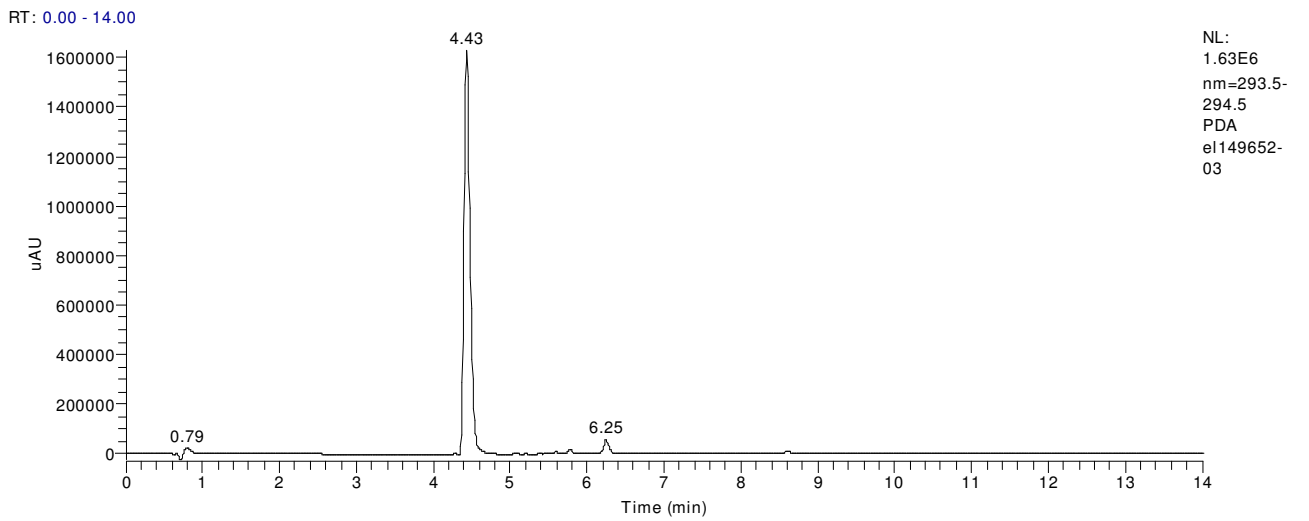
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (broad s, 1H); 7.30 (d, *J* = 9 Hz, 1H); 7.05 (d, *J* = 2 Hz, 1H); 6.96 (dd, *J* = 9 Hz, 2 Hz, 1H); 6.67 (d, *J* = 2 Hz, 1H); 4.05 (t, *J* = 6 Hz, 2H); 3.95-3.87 (mult., 4H); 3.79-3.72 (mult., 4H); 2.57-2.32 (mult., 6H); .2.01 (quint., *J* = 4 Hz, 2H); 1.67-1.37 (mult., 8H) ppm.

MS (EI) *m/e:* 372.1 $(M+H)^+$.

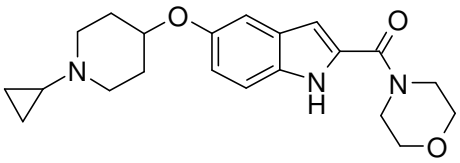
Anal. ($C_{21}H_{29}N_3O_3$) C, H, N. C: calcd, 67.90; found, 65.84. N: calcd, 11.31; found, 10.86.

HRMS $C_{21}H_{29}N_3O_3$: calcd, 372.22817; found, 372.22796 ($M+H^+$).

HPLC tracing:



[5-(1-Cyclopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-morpholin-4-yl-methanone (21)



To a cold (ice bath) solution of (5-hydroxy-1H-indol-2-yl)-morpholin-4-yl-methanone (**19**, 600 mg, 2.4 mmol), 1-cyclopropyl-piperidin-4-ol (**12**, 447 mg, 3.2 mmol, 1.3 eq.) and tributylphosphine (985 mg, 4.9 mmol, 2.0 eq.) in tetrahydrofuran (30 mL) was slowly added 1,1'-(azodicarbony)-dipiperidine (1.23 g, 4.87 mmol, 2.0 eq.) within 5 min. The mixture was stirred four days at room temperature. The mixture was concentrated *in vacuo*, stirred with dichloromethane / heptane 1:1 (15 mL), filtered, and the filtrate concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane : 2N ammonia in methanol (97:3) as eluant to afford, after evaporation, the desired product as a light yellow solid (130 mg, 14%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H); 7.31 (d, *J* = 9 Hz, 1H); 7.10 (d, *J* = 2 Hz, 1H); 6.98 (dd, *J* = 9Hz, 2 Hz, 1H); 6.67 (s, 1H); 4.27 (hept., *J* = 4 Hz, 1H); 3.96-3.83 (mult., 4H); 3.82-3.72 (mult., 4H);

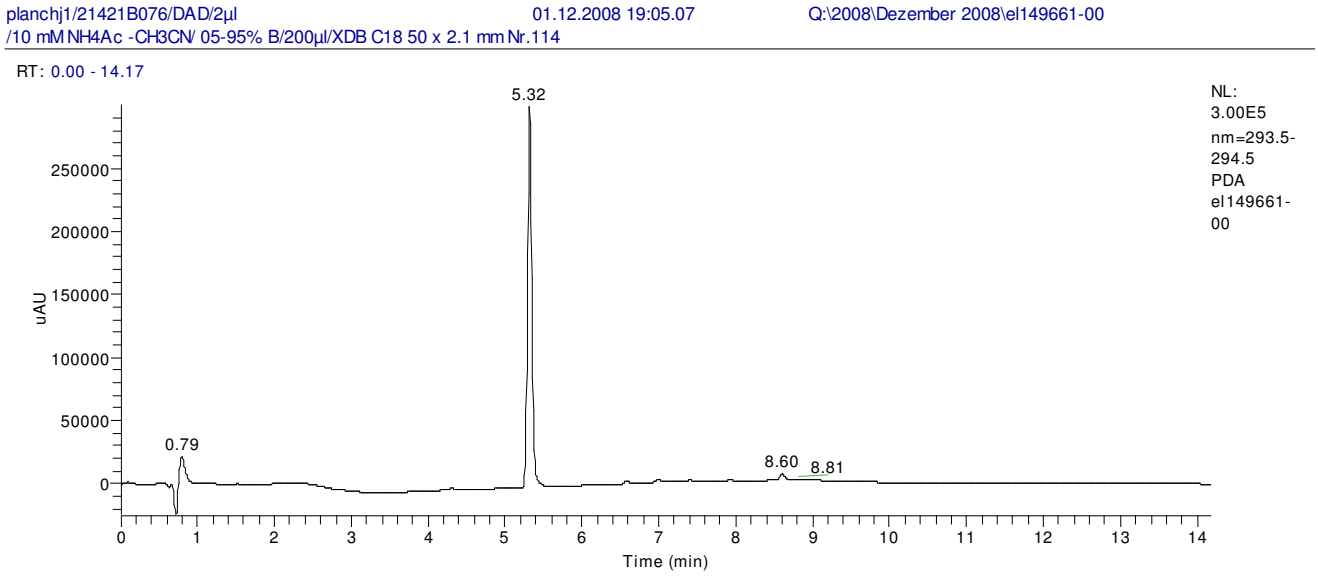
2.96-2.87 (mult., 2H); 2.51-2.40 (mult., 2H); 2.00-1.93 (mult., 2H); 1.83-1.72 (mult., 2H); 1.67-1.58 (mult., 1H); 0.49-0.38 (mult., 4H) ppm.

MS (EI) *m/e*: 370.2 $(M+H)^+$.

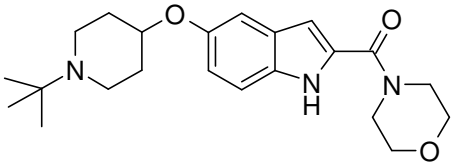
Anal. ($C_{21}H_{27}N_3O_3$) C, H, N. C: calcd, 68.27; found, 67.61.

HRMS $C_{21}H_{27}N_3O_3$: calcd, 370.21252; found, 370.21239 ($M+H^+$).

HPLC tracing:



[5-(1-tert-Butyl-piperidin-4-yloxy)-1H-indol-2-yl]-morpholin-4-yl-methanone (22)



To a cold (ice bath) solution of (5-hydroxy-1H-indol-2-yl)-morpholin-4-yl-methanone (**19**, 500 mg, 2 mmol), 1-tert-butyl-piperidin-4-ol (**12**, 479 mg, 3 mmol, 1.5 eq.) and tributylphosphine (1.18 mL, 4 mmol, 2.0 eq.) in tetrahydrofuran (20 mL) was slowly added 1,1'-(azodicarbonyl)-dipiperidine (1.035 g, 4 mmol, 2.0 eq.) within 20 min. The mixture was stirred four days at room temperature . The mixture

was concentrated *in vacuo*, stirred with diethyl ether, filtered, and the filtrate concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane : methanol: aqueous ammonia (95:5:0.25) as eluant, to afford, after evaporation, the desired product as an off-white solid (50 mg, 6%).

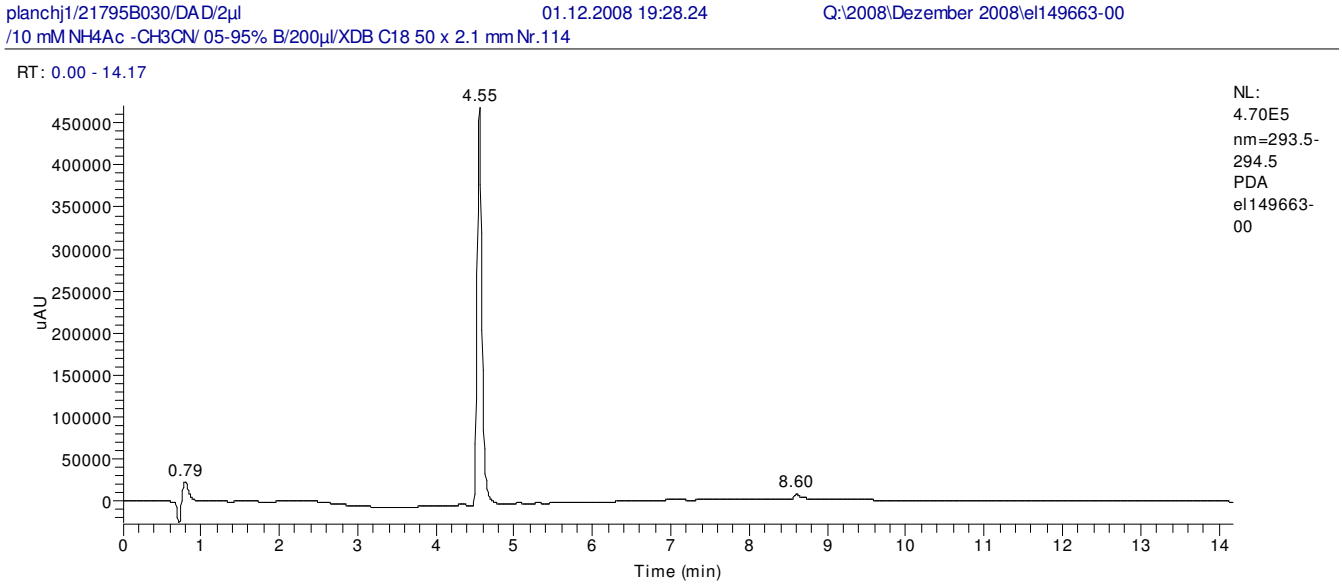
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H); 7.31 (d, J = 9 Hz, 1H); 7.10 (d, J = 2 Hz, 1H); 6.98 (dd, J = 9Hz, 2 Hz, 1H); 6.66 (d, J = 2 Hz, 1H); 4.24 (hept., J = 4 Hz, 1H); 3.96-3.87 (mult., 4H); 3.78-3.65 (mult., 4H); 2.94-2.86 (mult., 2H); 2.43-2.36 (mult., 2H); .2.07-1.96 (mult., 2H); 1.86-1.76 (mult., 2H); 1.09 (s, 9H) ppm.

MS (EI) *m/e*: 386.2 $(M+H)^+$.

Anal. ($C_{22}H_{31}N_3O_3$) C, H, N. C: calcd, 68.57; found, 67.47.

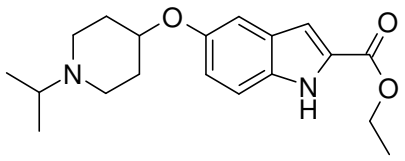
HRMS $C_{22}H_{31}N_3O_3$: calcd, 386.24382; found, 386.24366 ($M+H^+$).

HPLC tracing:



Scheme 4: Exploration of the 2,5- and 2,6-exit vectors

5-(1-Isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (23)

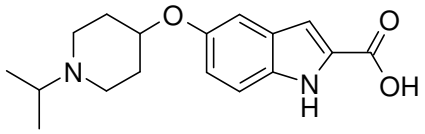


To a cold (ice bath) solution of 5-hydroxyindole-2-carboxylic acid ethyl ester (20 g, 97 mmol) and triphenylphosphine (30.7 g, 117 mmol, 1.2 eq.) in tetrahydrofuran (500 mL) was added dropwise a solution of di-tert-butyl azodicarboxylate (26.9 g, 117 mmol, 1.2 eq.) in tetrahydrofuran (100 mL). The mixture was stirred 48h at room temperature and concentrated in vacuo to afford a crude yellow oil (107 g). Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ ethyl acetate : methanol (19:1:0 then 9:1:0 then 19:0:1 then 9:0:1) as eluant, to afford, after evaporation, the desired product as a white solid (21.5 g, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8 Hz, 1H); 7.12 (dd, *J* = 8 Hz, 2Hz, 1H); 7.11 (d, *J* = 3 Hz, 1H); 7.00 (dd, *J* = 8 Hz, 2Hz, 1H); 4.00 (quad., *J* = 7 Hz, 2H); 4.30-4.20 (mult., 1H); 2.85-2.75 (mult., 2H); 2.75 (hept., *J* = 8 Hz, 1H); 2.39 (broad t, *J* = 10 Hz, 2H); 2.05-1.95 (mult., 2H); 1.90-1.80 (mult., 2H); 1.41 (t, *J* = 7 Hz, 3H); 1.06 (d, *J* = 8 Hz, 6H) ppm.

MS (EI) *m/e:* 331.2 $(M+H)^+$.

5-(1-Isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt, with lithium chloride.



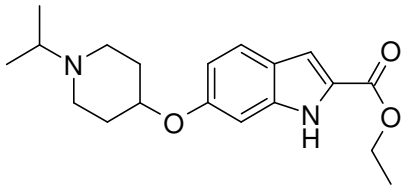
To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (**23**, 10.0 g, 30 mmol) in a mixture of tetrahydrofuran (130 mL), and methanol (27 mL) was added a solution of

lithium hydroxide monohydrate (1.40 g, 33 mmol, 1.1 eq.) in water (65 mL). The mixture was stirred 1h at reflux and concentrated in vacuo. The pH of the resulting suspension was adjusted to pH=2 with hydrochloric acid (2N). The solution was dried in vacuo to afford the desired product as a brown solid (11.6 g, quant.). This solid was used in the next step without further purification.

^{1}H NMR (300 MHz, d^6-DMSO) δ 11.67 (s, 1H); 7.35 (d, *J* = 9 Hz, 1H); 7.23 (broad s, 1H); 6.97 (d, *J* = 1 Hz, 1H); 7.00-6.90 (mult., 1H); 4.85-4.75 (mult., 1H); 3.46 (hept., *J* = 7 Hz, 1H); 3.25-3.15 (mult., 4H); 2.30-2.20 (mult., 2H); 2.10-2.00 (mult., 2H); 1.31 (d, *J* = 7 Hz, 6H) ppm.

MS (EI) *m/e:* 301.2 $(M-H)^-$.

6-(1-Isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (24)



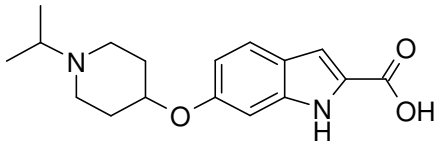
To a cold (ice bath) solution of 6-hydroxy-1H-indole-2-carboxylic acid ethyl ester[45] (1.0 g, 5 mmol, 1.0 eq.), 1-isopropyl-piperidin-4-ol (907 mg, 6 mmol, 1.3 eq.) and tributylphosphine (2.83 mL, 10 mmol, 2.0 eq.) in tetrahydrofuran (50 mL) was slowly added 1,1'-(azodicarbonyl)-dipiperidine (2.46 g, 10 mmol, 2.0 eq.) within 10 min. The mixture was stirred overnight at room temperature, filtered, and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : 2N ammonia in methanol (99:1 to 19:1) as eluant, to afford, after evaporation, the desired product as an off-white solid (260 mg, 15%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H); 7.53 (d, *J* = 9 Hz, 1H); 7.15 (d, *J* = 2 Hz, 1H); 6.86 (d, *J* = 2 Hz, 1H); 6.82 (dd, *J* = 9Hz, 2 Hz, 1H); 4.38 (quad., *J* = 7 Hz, 2H); 4.41-4.28 (mult., 1H); 2.86-2.70

(mult., 3H); 2.48-2.33 (mult., 2H); 2.12-1.98 (mult., 2H); 1.93-1.78 (mult., 2H); 1.40 (t, *J* = 7 Hz, 3H); 1.13-1.00 (mult., 6H) ppm.

MS (EI) *m/e:* 331.4 $(M+H)^+$.

6-(1-Isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt

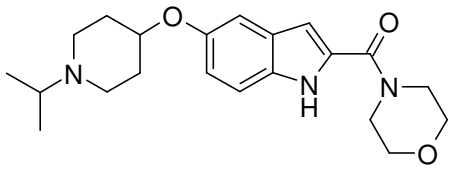


To a solution of 6-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (**24**, 8.5 g, 27 mmol) in tetrahydrofuran (60 mL), methanol (60 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.18 g, 28 mmol, 1.05 eq.). The mixture was refluxed 1h and concentrated in vacuo. Water was added to the residue, and the aqueous layer was washed with diethylether, and acidified (pH: ca. 2) with hydrochloric acid (4N). The mixture was concentrated in vacuo, and the residue was triturated with acetonitrile and filtered. The solid was washed with acetone and dried in vacuo to afford the desired product as a purple solid (9.0 g, 99%).

^{1}H NMR (400 MHz, *d*^{6}-DMSO) δ 7.55 (d, *J* = 8 Hz, 1H); 7.02 (d, *J* = 2 Hz, 1H); 6.95 (s, 1H); 6.81 (broad d, *J* = 9Hz, 1H); 4.87-4.57 (mult., 1H); 3.45 (hept., *J* = 7Hz, 1H); 3.40-3.02 (mult., 4H); 2.34-2.20 (mult., 2H); 2.12-2.01 (mult., 2H); 1.30 (d, *J* = 6Hz, 6H) ppm.

MS (EI) *m/e:* 303.1 $(M+H)^+$.

[5-(1-Isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-morpholin-4-yl-methanone (25)



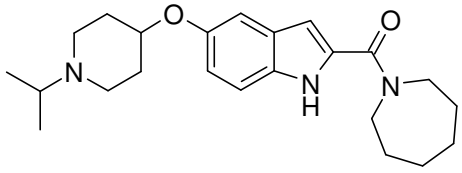
To a cold (ice bath) solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (9.8 g, 29 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (11.1 g, 35 mmol, 1.2 eq.) in N,N-dimethylformamide (100 mL) was added ethyl-diisopropyl-amine (29.9 mL, 174 mmol, 6.0 eq.) and morpholine (3.02 g, 35 mmol, 1.2 eq.). The mixture was stirred 2h at room temperature and partitioned between aqueous sodium carbonate solution (10%, 200 mL) and dichloromethane (150 mL). The aqueous layer was extrated with dichloromethane and the combined organic phases washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : 2N ammonia in methanol (98:2 to 92:8) as eluant, to afford, after evaporation, the desired product as an off-white solid (7.5 g, 76%).

^{1}H NMR (400 MHz, d^6-DMSO) δ 7.30 (d, J = 8 Hz, 1H); 7.09 (s, 1H); 6.85 (dd, J = 8 Hz, 2 Hz, 1H); 6.68 (d, J = 2 Hz, 1H); 4.31-4.17 (mult., 1H); 3.82-3.60 (mult., 8 H); 2.80-2.63 (mult., 3H); 2.38-3.22 (mult., 2H); 1.99-1.87 (mult., 2H); 1.67-1.51 (mult., 2H); 1.05-0.89 (mult., 6H) ppm.

MS (EI) *m/e:* 372.1 $(M+H)^+$.

Anal. ($C_{21}H_{29}N_3O_3$) C, H, N.

Azepan-1-yl-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (26)



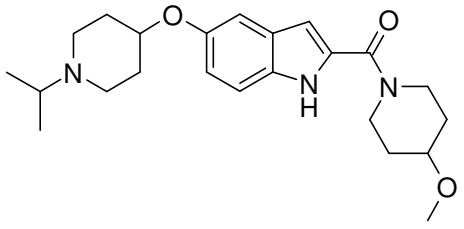
To a cold (ice bath) solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (3.90 g, 12 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (4.43 g, 14 mmol, 1.2 eq.) in N,N-dimethylformamide (60 mL) were added ethyl-diisopropyl-amine (11.9 mL, 69 mmol, 6.0 eq.) and azepane (1.37 g, 14 mmol, 1.2 eq.). The mixture was stirred six days at room temperature and partitioned between an aqueous solution of sodium carbonate (10%, 250 mL) and ethyl acetate (200 mL). The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : 2N ammonia in methanol (98:2 to 94:6) as eluant, to afford, after evaporation, the desired product as a light brown solid (2.95 g, 67%).

^{1}H NMR (400 MHz, d^6-DMSO) δ 7.29 (d, J = 8 Hz, 1H); 7.11 (d, J = 2 Hz, 1H); 6.83 (dd, J = 8 Hz, 2 Hz, 1H); 6.67 (d, J = 2 Hz, 1H); 4.21 (hept., J = 4 Hz, 1H); 3.87-3.73 (mult., 2H); 3.65-3.53 (mult., 2H); 2.74-2.62 (mult., 3H); 2.34-2.22 (mult., 2H); 1.97-1.87 (mult., 2H); 1.85-1.68 (mult., 4H); 1.65-1.49 (mult., 6H); 0.97 (d, J = 6 Hz, 6H) ppm.

MS (EI) *m/e:* 384.0 $(M+H)^+$.

Anal. ($C_{23}H_{33}N_3O_3$) C, H, N.

[5-(1-Isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-(4-methoxy-piperidin-1-yl)-methanone (27)



To a cold (ice bath) solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (1.2 g, 4 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium

tetrafluoroborate (1.36 g, 4 mmol, 1.2 eq.) in N,N-dimethylformamide (20 mL) were added ethyl-diisopropyl-amine (3.66 mL, 21 mmol, 6.0 eq.) and 4-methoxypiperidine (0.489 g, 4 mmol, 1.2 eq.). The mixture was stirred 2h at room temperature and partitioned between aqueous sodium carbonate solution (10%, 100 mL) and dichloromethane (100 mL). The aqueous layer was extracted with dichloromethane and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : 2N ammonia in methanol (98:2 to 92:8) as eluant to afford, after evaporation, the desired product as a light brown solid (1.15 g, 81%).

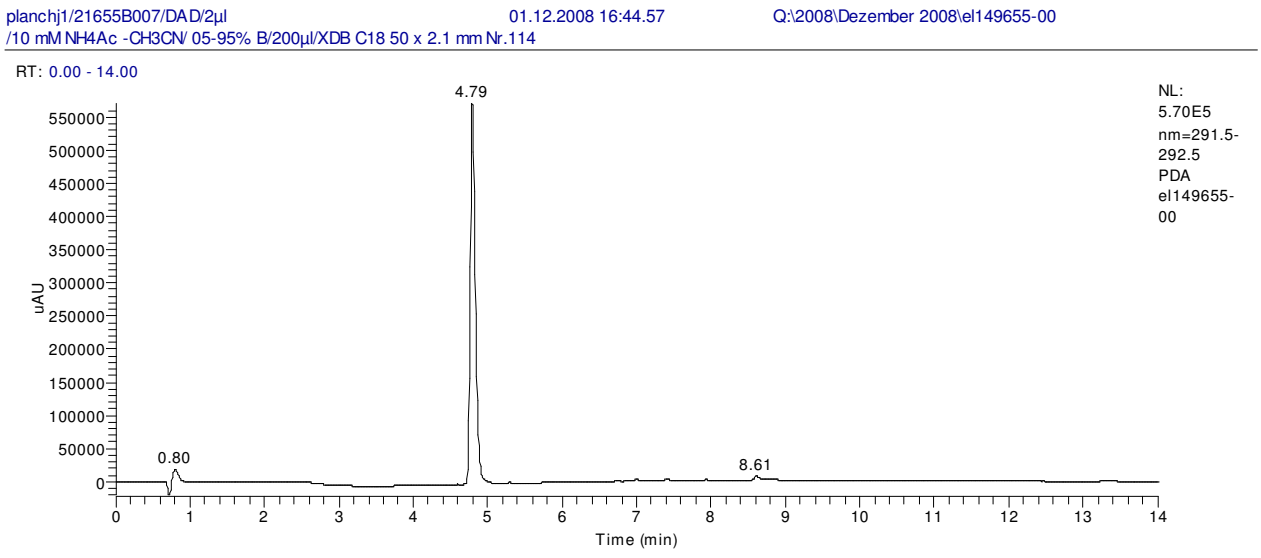
^{1}H NMR (400 MHz, d^6-DMSO) δ 7.28 (d, J = 8 Hz, 1H); 7.10 (s, 1H); 6.84 (d, J = 8 Hz, 1H); 6.64 (s, 1H); 4.32-4.16 (mult., 1H); 4.07-3.91 (mult., 2H); 3.53-3.35 (mult., 3H); 2.85-2.62 (mult., 3H); 2.40-2.19 (mult., 1H); 2.03-1.86 (mult., 4H); 1.70-1.42 (mult., 4H); 1.05-0.90 (mult., 6H) ppm.

MS (EI) *m/e:* 400.4 $(M+H)^+$.

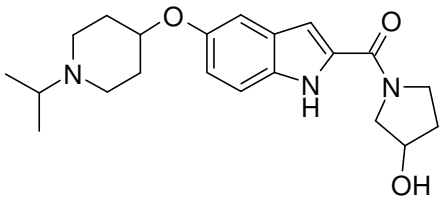
Anal. ($C_{23}H_{33}N_3O_3$) C, H, N. C: calcd, 69.14; found, 67.36. H: calcd, 8.33; found, 7.91.

HRMS $C_{23}H_{33}N_3O_3$: calcd, 400.25947; found, 400.25928 ($M+H^+$).

HPLC tracing:



Rac-(3-hydroxy-pyrrolidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1*H*-indol-2-yl]-methanone (28)



To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (3.90 g, 12 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (4.43 g, 14 mmol, 1.2 eq.) in N,N-dimethylformamide (60 mL) were added ethyl-diisopropyl-amine (11.9 mL, 69 mmol, 6.0 eq.) and (rac)-3-pyrrolidinol (1.20 g, 14 mmol, 1.2 eq.). The mixture was stirred 6 days at room temperature and partitioned between aqueous sodium carbonate solution (6% w/w, 400 mL) and ethyl acetate (400 mL). The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. The residue was triturated with ethyl acetate (30 mL) and filtered. The solid was washed with ethyl acetate and diethyl ether and dried in vacuo to afford the desired product as a light brown solid (3.6 g, 84%).

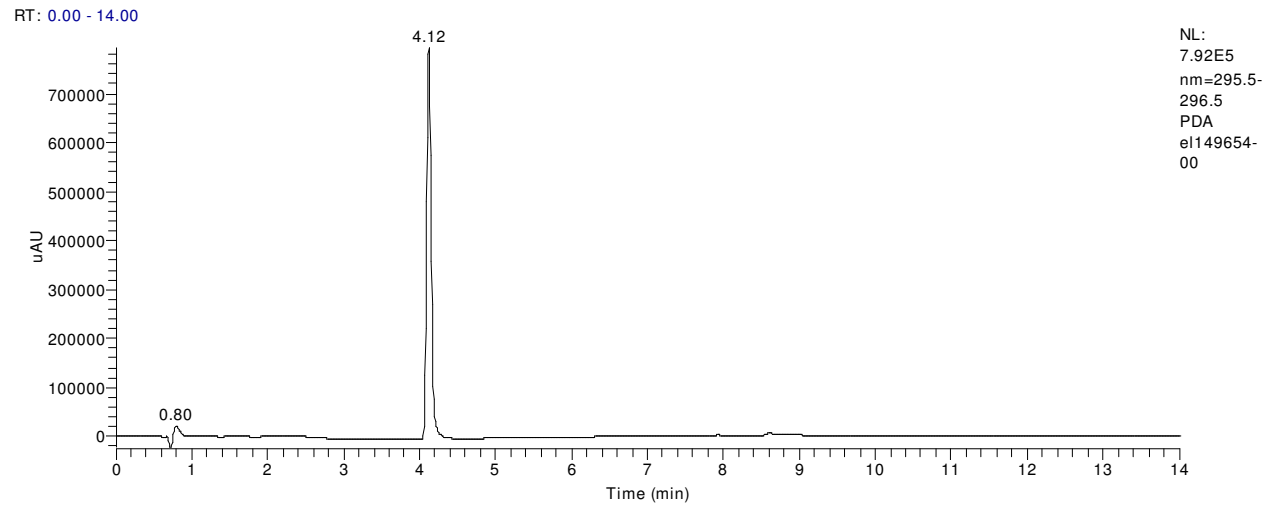
^{1}H NMR (400 MHz, d^6-DMSO) δ 7.32 (d, J = 8 Hz, 1H); 7.12 (s, 1H); 6.85 (dd, J = 8 Hz, 2Hz, 1H); 6.79 (s, 1H); 5.05-4.95 (mult., 1H); 4.43-4.17 (mult., 2H); 3.95-3.82 (mult., 3H); 3.70-3.45 (mult., 4H); 2.80-2.63 (mult., 3H); 2.37-2.23 (mult., 2H); 2.07-1.85 (mult., 4H); 1.68-1.52 (mult., 2H); 0.97 (d, J = 6Hz) ppm.

MS (EI) *m/e:* 371.9 $(M+H)^+$.

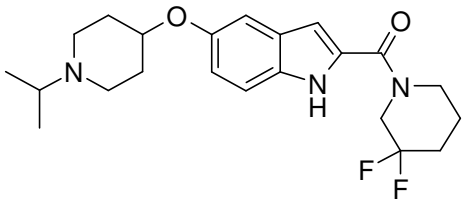
Anal. ($C_{21}H_{29}N_3O_3$) C, H, N. C: calcd, 67.90; found, 65.92.

HRMS $C_{21}H_{29}N_3O_3$: calcd, 372.22817; found, 372.22797 ($M+H^+$).

HPLC tracing:



(3,3-Difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1*H*-indol-2-yl]-methanone (29)



To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt, with lithium chloride (450 mg, 1.2 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (494 mg, 1.5 mmol, 1.25 eq.) in N,N-dimethylformamide (3 mL) were added ethyl-diisopropyl-amine (1.43 mL, 8 mmol, 6.0 eq.) and 3,3'-difluoropiperidine (0.245 g, 1.5 mmol, 1.25 eq.). The mixture was stirred overnight at room temperature and partitioned between saturated aqueous sodium hydrogenocarbonate solution and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : methanol: aqueous ammonia (98:2:0 to 95:5:0.25) as eluant to afford, after evaporation, the desired product as an off-white solid (350 mg, 73%).

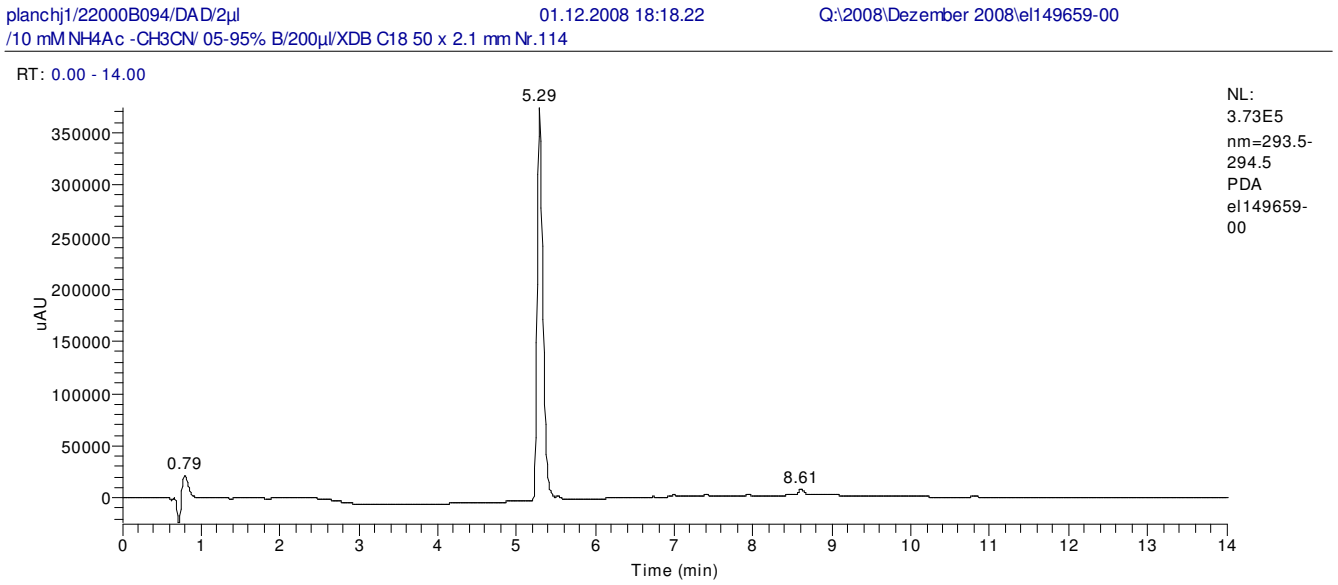
^{1}H NMR (400 MHz, d^6-DMSO) δ 8.90 (s broad, 1H); 7.30 (d, *J* = 8 Hz, 1H); 7.12 (d, *J* = 2 Hz, 1H); 6.98 (dd, *J* = 8 Hz, 2Hz, 1H); 6.74 (d, *J* = 2 Hz, 1H); 4.30-4.21 (mult., 1H); 4.09 (t, *J* = 10 Hz, 2H); 3.86-3.80 (mult., 2H); 2.87-2.70 (mult., 3H); 2.44-2.32 (mult., 2H); 2.20-1.98 (mult., 4H); 1.95-1.78 (mult., 4H); 1.07 (d, *J* = 6 Hz, 6H) ppm.

MS (EI) *m/e:* 406.2 $(M+H)^+$.

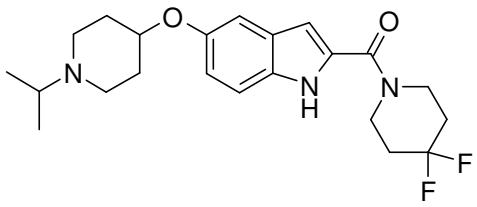
Anal. ($C_{22}H_{29}N_3O_2$) C, H, N. C: calcd, 65.17; found, 64.67.

HRMS $C_{22}H_{29}N_3O_2$: calcd, 406.23006; found, 406.22985 ($M+H^+$).

HPLC tracing:



(4,4-Difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (30)



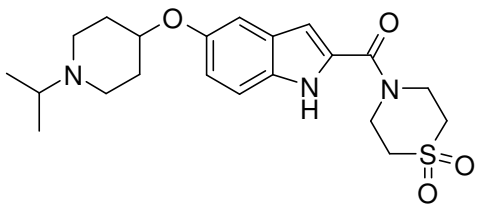
To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt, with lithium chloride (5.0 g, 13 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (5.3 g, 15.7 mmol, 1.2 eq.) in N,N-dimethylformamide (75 mL) were added ethyl-diisopropyl-amine (11.5 mL, 65.6 mmol, 5.0 eq.) and 4,4'-difluoropiperidine hydrochloride (2.48 g, 15.7 mmol, 1.2 eq.). The mixture was stirred overnight at room temperature and partitioned between saturated aqueous sodium hydrogenocarbonate solution (300 mL) and ethyl acetate (300 mL). The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water and brine, dried over sodium sulfate, filtered and concentrated to ca. 40 mL. Methyl-tert-butylether (40 mL) was added with stirring and the precipitate was filtered, washed with cold (5-10°C) methyl-tert-butylether and dried in vacuo to afford the desired product as a white solid (4.95 g, 93%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1H); 7.32 (d, *J* = 9 Hz, 1H); 7.11 (d, *J* = 2 Hz, 1H); 6.99 (dd, *J* = 9 Hz, 2 Hz, 1H); 6.69 (d, *J* = 2 Hz, 1H); 4.24 (hept., *J* = 4 Hz, 1H); 4.08-3.95 (mult., 4H); 3.87-3.70 (mult., 3H); 2.42-2.35 (mult., 2H); 2.19-1.97 (mult., 6H); 1.92-1.75 (mult., 2H); 1.06 (d, *J* = 7 Hz, 6H) ppm.

MS (EI) *m/e:* 406.2 $(M+H)^+$.

Anal. ($C_{22}H_{29}N_3O_2$) C, H, N.

(1,1-Dioxo-1λ^6-thiomorpholin-4-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1*H*-indol-2-yl]-methanone (31)

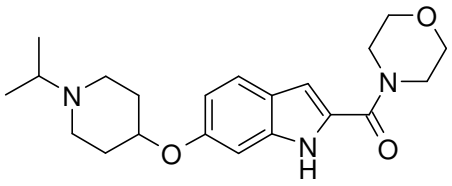


To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (188 mg, 0.55 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (198 mg, 0.67 mmol, 1.2 eq.) in N,N-dimethylformamide (5 mL) were added ethyl-diisopropyl-amine (0.325 mL, 2.77 mmol, 5.0 eq.) and thiomorpholine-1,1-dioxide (87 mg, 0.72 mmol, 1.3 eq.). The mixture was stirred overnight at room temperature and partitioned between saturated aqueous sodium hydrogenocarbonate solution and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water, brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane : 2N ammonia in methanol (97:3) as eluant, to afford, after evaporation, the desired product as an off-white solid (155 mg, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H); 7.33 (d, *J* = 9 Hz, 1H); 7.11 (d, *J* = 2 Hz, 1H); 7.01 (dd, *J* = 9 Hz, 2 Hz, 1H); 6.69 (d, *J* = 2 Hz, 1H); 4.44-4.17 (mult., 5H); 3.20-3.10 (mult., 4H); 2.90-2.71 (mult., 3H); 2.49-2.32 (mult., 2H); 2.14-1.96 (mult., 2H); 1.94-1.80 (mult., 2H); 1.08 (d, *J* = 4 Hz, 1H) ppm.

MS (EI) *m/e*: 420.1 $(M+H)^+$.

[6-(1-Isopropyl-piperidin-4-yloxy)-1*H*-indol-2-yl]-morpholin-4-yl-methanone (32)



To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (1.3 g, 3.84 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (1.604 g, 4.80 mmol, 1.25 eq.) in N,N-dimethylformamide (30 mL) were added ethyl-diisopropyl-amine (3.25 mL, 19 mmol, 5.0 eq.) and morpholine (0.42 mL, 4.80 mmol, 1.25 eq.). The mixture was stirred

overnight at room temperature and partitioned between saturated aqueous sodium hydrogenocarbonate solution and ethyl acetate. The aqueous layer was extrated with ethyl acetate and the combined organic phases were washed with water, brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : methanol : aqueous ammonia (95:5:0.25 to 90:9:1) as eluant to afford, after evaporation, the desired product as an off-white solid (1180 mg, 83%).

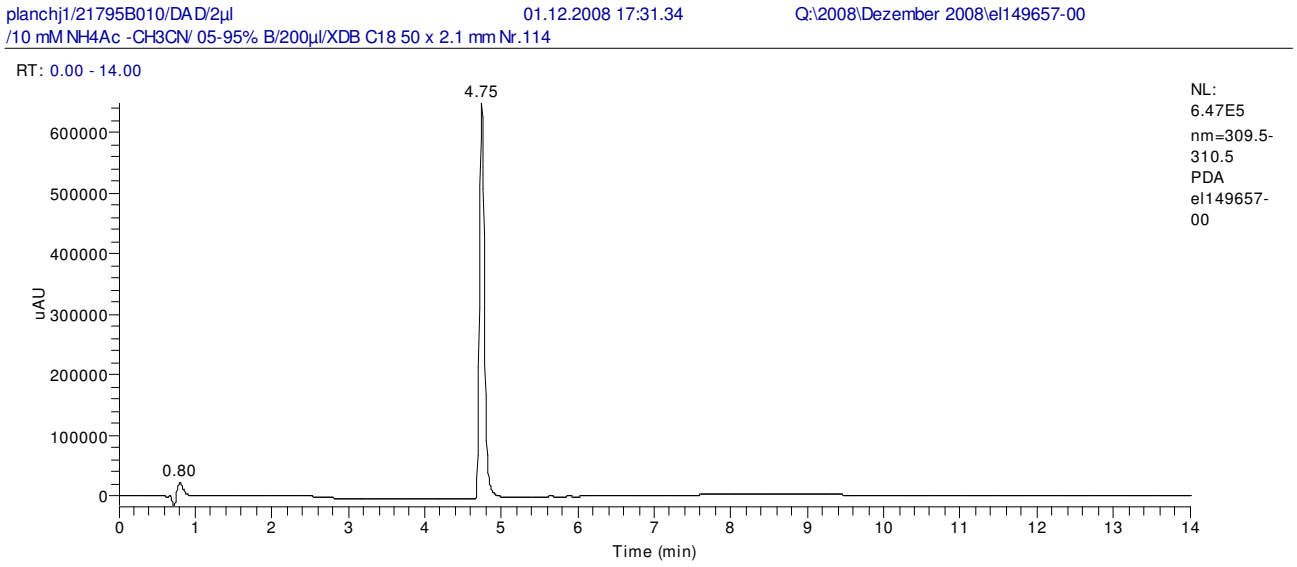
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H); 7.49 (d, *J* = 8 Hz, 1H); 6.88 (d, *J* = 2 Hz, 1H); 6.82 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.70 (dd, *J* = 2 Hz, 2 Hz, 1H); 4.37-4.28 (mult., 1H); 3.98-3.87 (mult., 4H); 3.80-3.73 (mult., 4H); 2.85-2.72 (mult., 3H); 2.47-2.36 (mult., 2H); 2.09-1.99 (mult., 2H); 1.90-1.80 (mult., 2H); 1.08 (d, *J* = 7 Hz, 1H) ppm.

MS (EI) *m/e:* 372.1 $(M+H)^+$.

Anal. ($C_{21}H_{29}N_3O_3$) C, H, N. C: calcd, 67.90; found, 67.04.

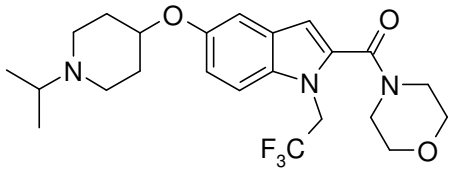
HRMS $C_{21}H_{29}N_3O_3$: calcd, 372.22817; found, 372.22799 ($M+H^+$).

HPLC tracing:



Scheme 5: Exploration of the N-indole substituent

[5-(1-Isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-morpholin-4-yl-methanone (33)



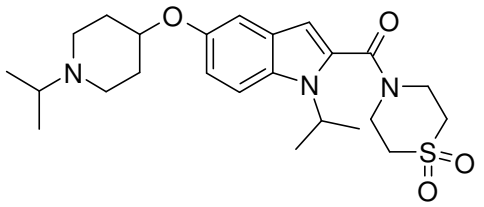
To a solution of [5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-morpholin-4-yl-methanone (**25**, 100 mg, 0.27 mmol) in N,N-dimethylformamide (1.0 mL) was added sodium hydride (55% w/w dispersion in oil, 13 mg, 0.30 mmol, 1.1 eq.). The mixture was stirred 30 min at 70°C, 2,2,2-trifluoroethyltrifluoromethanesulfonate (75 mg, 0.32 mmol, 1.2 eq.) was added and the mixture was stirred 24h at 70°C. The mixture was partitioned between water and ethyl acetate and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane/ methanol: aqueous ammonia (95:5:0.25) as eluant, to afford, after evaporation, the desired product as a light yellow solid (90 mg, 74%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.32 (mult., 2H); 7.09 (d, *J* = 2 Hz, 1H); 7.02 (dd, *J* = 9 Hz, 2Hz, 1H); 6.58 (s, 1H); 5.05 (quad., *J* = 9 Hz, 2H); 4.32-4.20 (mult., 1H); 3.84-3.59 (mult., 8H); 2.87-2.69 (mult., 3H); 2.45-2.32 (mult., 2H); 2.10-1.95 (mult., 2H); 1.92-1.76 (mult., 2H); 1.14-0.95 (mult., 6H) ppm.

MS (EI) *m/e:* 454.5 $(M+H)^+$.

Anal. ($C_{23}H_{30}F_3N_3O_4$) C, H, N.

(1,1-Dioxo-1λ^6-thiomorpholin-4-yl)-[1-isopropyl-5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (34)



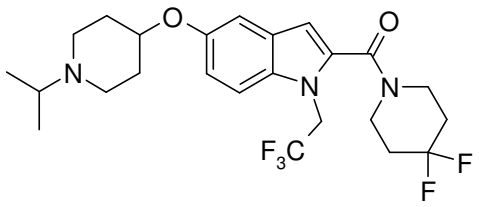
A mixture of (1,1-dioxo-1λ^6-thiomorpholin-4-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1*H*-indol-2-yl]-methanone (**31**, 100 mg, 0.24 mmol), cesium carbonate (156 mg, 0.48 mmol, 2.0 eq.) and isopropylmethanesulfonate (67 mg, 0.48 mmol, 2.0 eq.) in acetonitrile (4.0 mL) was stirred 22h at 95°C. The mixture was concentrated in vacuo and partitioned between water and methyl-tert-butylether. The aqueous layer was extracted with methyl-tert-butylether, the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of cyclohexane/ethyl acetate (19:1 to 1:1) as eluant to afford, after evaporation, the desired product as a pink solid (52 mg, 47%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8 Hz, 1H); 7.09 (d, *J* = 2 Hz, 1H); 6.94 (dd, *J* = 9 Hz, 2Hz, 1H); 6.45 (s, 1H); 4.75 (hept., *J* = 6 Hz, 1H); 4.30-4.20 (mult., 5H); 3.13-3.03 (mult., 4H); 2.86-2.70 (mult., 3H); 2.43-2.33 (mult., 2H); 2.08-1.99 (mult., 2H); 1.90-1.80 (mult., 2H); 1.62 (d, *J* = 6 Hz, 6H); 1.06 (d, *J* = 6 Hz, 6H) ppm.

MS (EI) *m/e:* 462.1 $(M+H)^+$.

Anal. ($C_{24}H_{35}N_3O_4S$) C, H, N, S.

(4,4-Difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone (35)

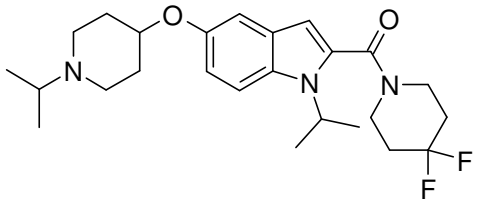


To a solution of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 118 mg, 0.29 mmol) in N,N-dimethylformamide (1.2 mL) was added sodium hydride (55% w/w dispersion in oil, 14 mg, 0.32 mmol, 1.1 eq.). The mixture was stirred 30 min at 70°C, 2,2,2-trifluoroethyltrifluoromethanesulfonate (81 mg, 0.35 mmol, 1.2 eq.) was added and the mixture was stirred 24h at 70°C. The mixture was partitioned between water and ethyl acetate and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane/ methanol: aqueous ammonia (95:5:0.25) as eluant, to afford, after evaporation, the desired product as a light yellow solid (125 mg, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 9 Hz, 1H); 7.10 (d, *J* = 2 Hz, 1H); 7.02 (dd, *J* = 9 Hz, 2Hz, 1H); 6.61 (s, 1H); 5.05 (quad., *J* = 9 Hz, 2H); 4.40-4.26 (mult., 1H); 3.97-3.34 (mult., 4H); 2.95-2.76 (mult., 3H); 2.60-2.38 (mult., 2H); 2.18-1.80 (mult., 8H); 1.18-1.02 (mult., 6H) ppm.

MS (EI) *m/e:* 488.2 $(M+H)^+$.

(4,4-Difluoro-piperidin-1-yl)-[1-isopropyl-5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (36)



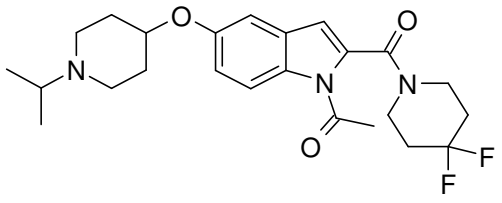
To a solution of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 1.5 g, 3.7 mmol) in N,N-dimethylformamide (18 mL) was added sodium hydride (50% w/w dispersion in oil, 178 mg, 4.1 mmol, 1.1 eq.). The mixture was stirred 50 min at 70°C before the addition of 2-bromopropane (0.55 mL, 4.4 mmol, 1.2 eq.). The mixture was stirred 18h at 70°C and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane : methanol (1:0 then 19:1) as eluant to afford, after evaporation, the desired product as a white solid (984 mg, 59%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 9 Hz, 1H); 7.09 (dd, *J* = 2 Hz, 1H); 6.92 (dd, *J* = 9 Hz, 2Hz, 1H); 6.40 (s, 1H); 4.73 (hept., *J* = 7 Hz, 1H); 4.30-4.20 (mult., 1H); 3.87-3.78 (mult., 4H); 2.85-2.75 (mult., 2H); 2.74 (hept., *J* = 7 Hz, 1H); 2.38 (broad t, *J* = 9 Hz, 2H); 2.08-1.95 (mult., 6H); 1.87-1.75 (mult., 2H); 1.62 (d, *J* = 7 Hz, 6H); 1.05 (d, *J* = 7 Hz, 6H) ppm.

MS (EI) *m/e:* 448.2 $(M+H)^+$.

Anal. ($C_{25}H_{35}F_2N_3O_2$) C, H, N.

1-[2-(4,4-Difluoro-piperidine-1-carbonyl)-5-(1-isopropyl-piperidin-4-yloxy)-indol-1-yl]-ethanone (37)



To a solution of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 100 mg, 0.25 mmol) in N,N-dimethylformamide (1.2 mL) was added sodium hydride

(55% w/w dispersion in oil, 13 mg, 0.3 mmol, 1.2 eq.). The mixture was stirred 30 min at room temperature and acetyl chloride (44 µL, 0.62 mmol, 2.5 eq.) was added, and the mixture was stirred 24h at room temperature. The mixture was partitioned between water and dichloromethane, and the aqueous layer was extracted with dichloromethane. The combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane/ methanol: aqueous ammonia (95:5:0.25) as eluant, to afford, after evaporation, the desired product as a yellow solid (97 mg, 77%).

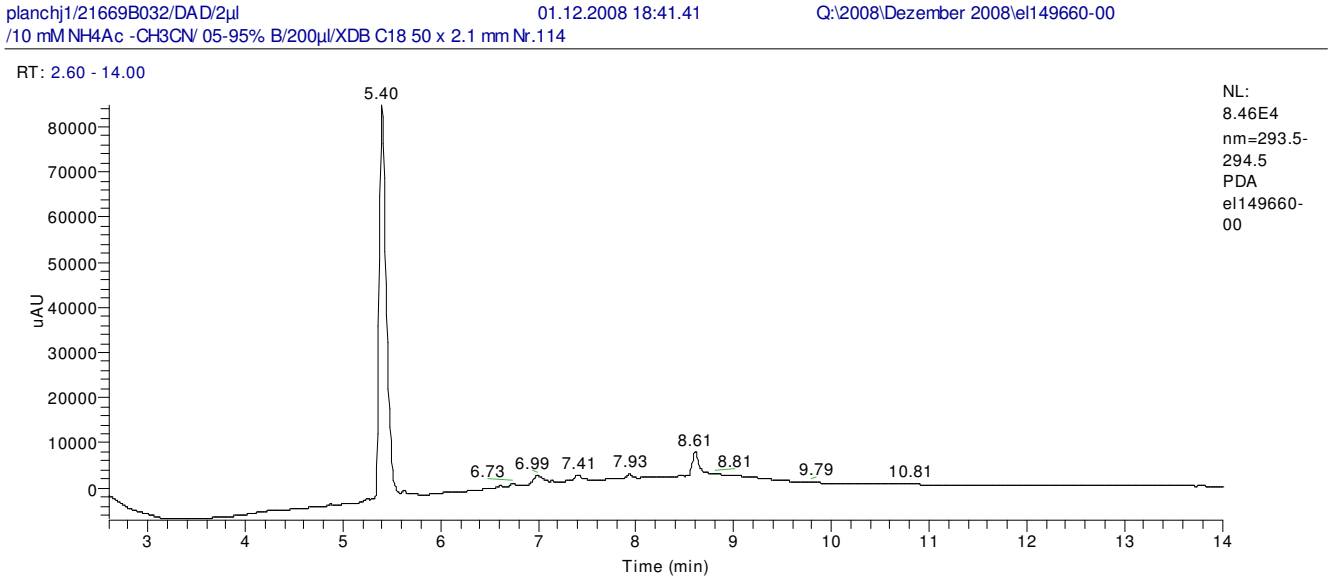
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8 Hz, 1H); 7.06 (d, *J* = 2 Hz, 1H); 7.00 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.60 (s, 1H); 4.37-4.28 (mult., 1H); 3.96-3.86 (mult., 2H); 3.63-3.56 (mult., 2H); 2.89-2.73 (mult., 3H); 2.69 (s, 3H); 2.50-2.36 (mult., 2H); 2.18-1.80 (mult., 9H); 1.08 (broad d, *J* = 6 Hz, 6H) ppm.

MS (EI) *m/e*: 448.1 $(M+H)^+$.

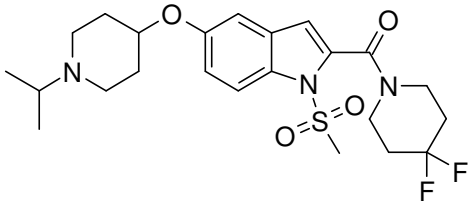
Anal. ($C_{24}H_{31}N_3O_3$) C, H, N. C: calcd, 64.41; found, 60.93. N: calcd, 9.39; found, 8.77.

HRMS $C_{24}H_{31}N_3O_3$: calcd, 448.24062; found, 448.24042 ($M+H^+$).

HPLC tracing:



(4,4-Difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1-methanesulfonyl-1*H*-indol-2-yl]-methanone (38)



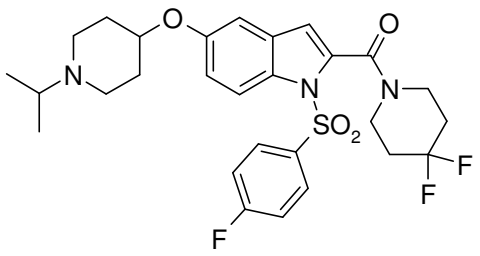
To a solution of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 100 mg, 0.25 mmol) in N,N-dimethylformamide (1.2 mL) was added sodium hydride (55% w/w dispersion in oil, 13 mg, 0.3 mmol, 1.2 eq.) and the mixture stirred 30 min at room temperature. Methanesulfonyl chloride (48 μL, 0.62 mmol, 2.5 eq.) was added and the mixture was stirred two days at room temperature. The mixture was partitioned between saturated aqueous sodium hydrogenocarbonate solution and ethyl acetate and the aqueous layer extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane/ methanol: aqueous ammonia (95:5:0.25) as eluant to afford, after evaporation, the desired product as a yellow solid (11 mg, 9%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8 Hz, 1H); 7.06 (d, *J* = 2 Hz, 1H); 7.02 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.59 (s, 1H); 4.45-4.29 (mult., 1H); 3.74-3.59 (mult., 2H); 3.39 (s, 3H); 2.91-2.74 (mult., 3H); 2.54-2.34 (mult., 2H); 2.18-2.00 (mult., 6H); 1.98-1.80 (mult., 2H); 1.20-1.02 (mult., 6H) ppm.

MS (EI) *m/e:* 484.3 $(M+H)^+$.

Anal. ($C_{23}H_{31}N_3O_4S$) C, H, N, S.

(4,4-Difluoro-piperidin-1-yl)-[1-(4-fluoro-benzenesulfonyl)-5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (39)



To a solution of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 200 mg, 0.49 mmol) in N,N-dimethylformamide (2.0 mL) was added sodium hydride (55% w/w dispersion in oil, 24 mg, 0.54 mmol, 1.1 eq.). The mixture was stirred 30 min at room temperature before the addition of 4-fluorobenzensulfonyl chloride (115 mg, 0.59 mmol, 1.2 eq.). The mixture was stirred 20h at 60°C. The mixture was partitioned between water and ethyl acetate and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane/ methanol (98:2) as eluant to afford, after evaporation, the desired product as a yellow solid (167 mg, 60%).

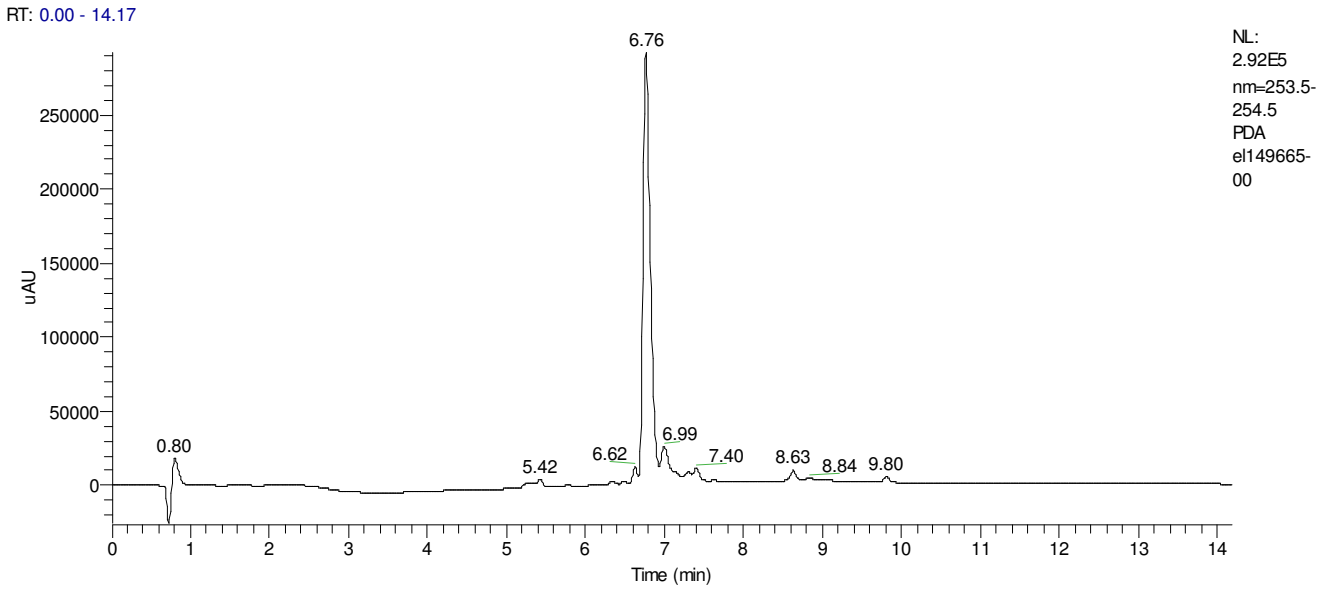
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (dd, *J* = 5 Hz, 2Hz, 2H); 7.90 (d, *J* = 8 Hz, 1H); 7.12 (t, *J* = 10 Hz, 2 H); 7.00-6.95 (mult., 2H); 6.61 (s, 1H); 4.51-4.33 (mult., 1H); 3.66-3.44 (mult., 4H); 3.08-2.86 (mult., 5H); 2.37-1.87 (mult., 8H); 1.34-1.10 (mult., 6H) ppm.

MS (EI) *m/e:* 564.4 $(M+H)^+$.

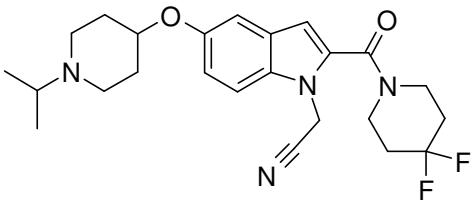
Anal. ($C_{28}H_{32}F_3N_3O_4S$) C, H, N. C: calcd, 59.67; found, 56.69. N: calcd, 7.46; found, 6.91.

HRMS $C_{28}H_{32}F_3N_3O_4S$: calcd, 564.21384; found, 564.21352 ($M+H^+$).

HPLC tracing:



[2-(4,4-Difluoro-piperidine-1-carbonyl)-5-(1-isopropyl-piperidin-4-yloxy)-indol-1-yl]-acetonitrile (40)



To a solution of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 200 mg, 0.49 mmol) in N,N-dimethylformamide (4.0 mL) was added sodium hydride (55% w/w dispersion in oil, 24 mg, 0.54 mmol, 1.1 eq.). The mixture was stirred 15 min at 70°C, bromo-acetonitrile (37 µL, 0.54 mmol, 1.1 eq.) was added and the mixture was stirred 5h at 70°C. The mixture was partitioned between saturated aqueous sodium hydrogenocarbonate solution and ethyl acetate and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol (95:5) as eluant, to afford, after evaporation, the desired product as a green solid (84 mg, 38%).

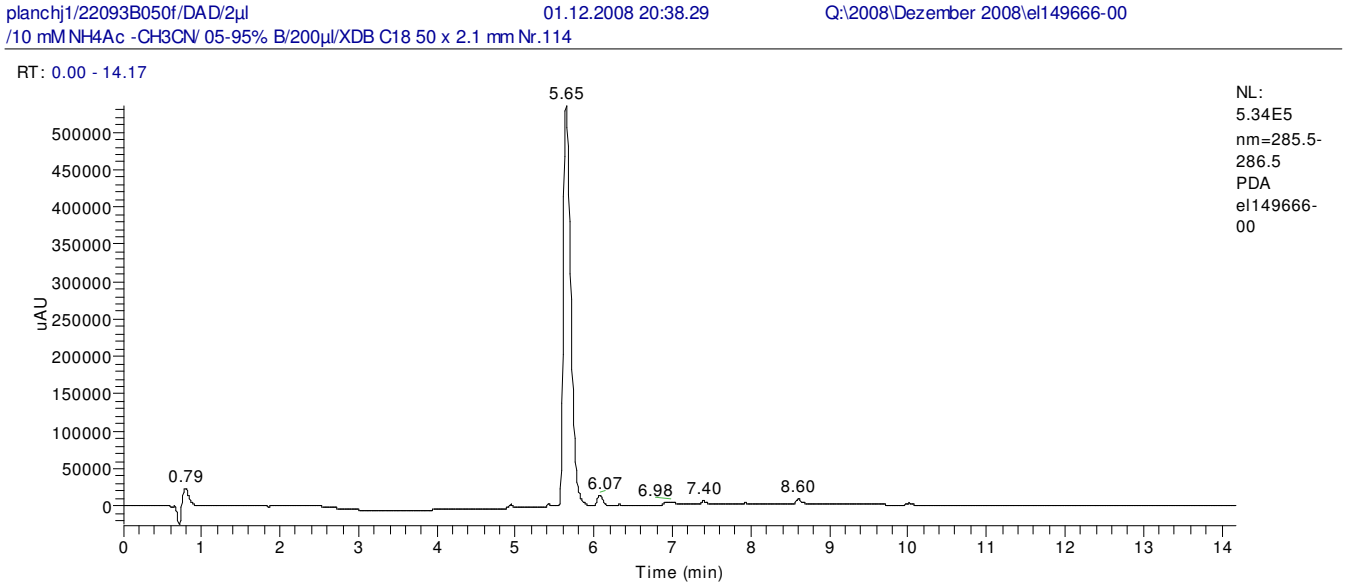
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8 Hz, 1H); 7.13 (d, *J* = 2 Hz, 1H); 7.08 (dd, *J* = 8 Hz, 2Hz, 1H); 6.67 (s, 1H); 4.38-4.28 (mult., 1H); 4.00-3.91 (mult., 4H); 2.92-2.78 (mult., 3H); 2.55-2.41 (mult., 2H); 2.16-2.04 (mult., 6H); 1.94-1.83 (mult., 2H); 1.11 (d, *J* = 6Hz, 6H) ppm.

MS (EI) *m/e:* 445.3 $(M+H)^+$.

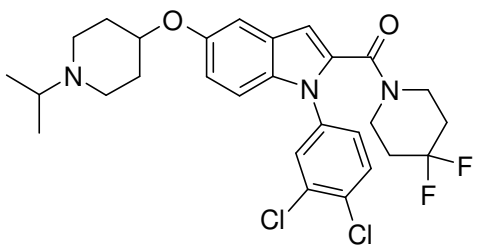
Anal. ($C_{24}H_{30}F_2N_4O_2$) C, H, N. C: calcd, 64.85; found, 63.84. N: calcd, 12.60; found, 12.14.

HRMS $C_{24}H_{30}F_2N_4O_2$: calcd, 445.24096; found, 445.24072 ($M+H^+$).

HPLC tracing:



[1-(3,4-Dichloro-phenyl)-5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-(4,4-difluoro-piperidin-1-yl)-methanone (41)



To a degassed (vacuum/nitrogen cycles) mixture of (4,4-difluoro-piperidin-1-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-methanone (**30**, 100 mg, 0.25 mmol), 3,4-dichlorophenylboronic acid (146 mg, 0.74 mmol, 3.0 eq.) and copper acetate (90 mg, 0.49 mmol, 2.0 eq.) in dichloromethane (5.0 mL) was added pyridine (80 µL, 1.0 mmol, 4 eq.). The mixture was stirred 3 days at room temperature and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol (98:2 to 95:5) as eluant to afford, after evaporation, the desired product as a yellow solid (110 mg, 81%).

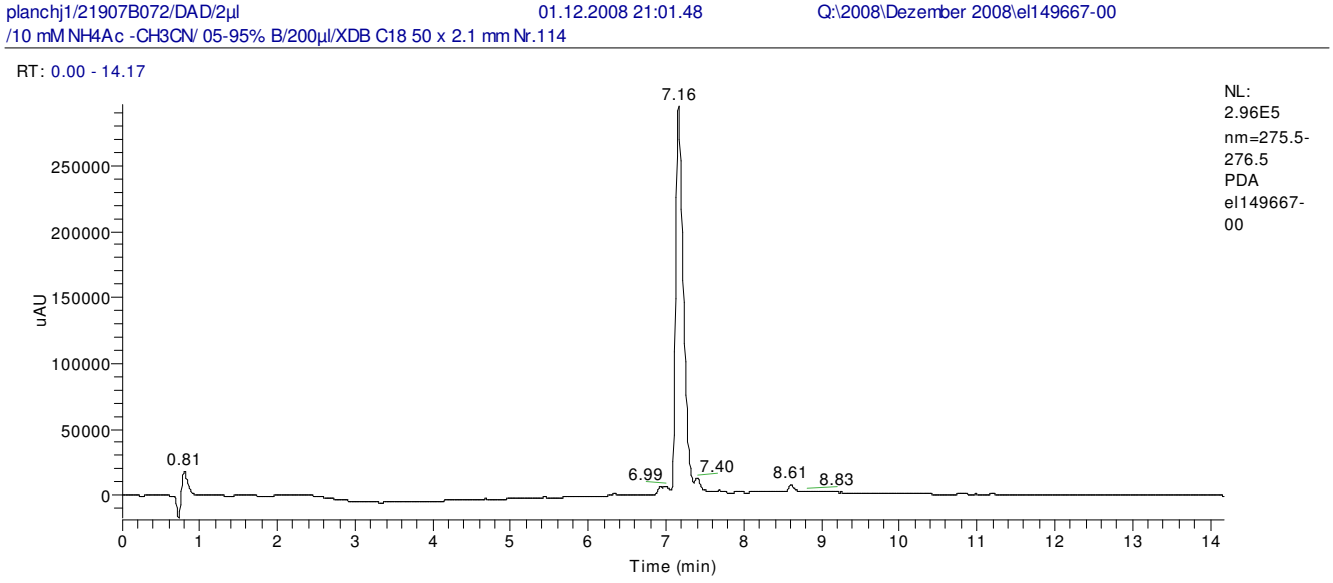
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8 Hz, 2H); 7.48 (d, *J* = 2 Hz, 1H); 7.24 (dd, *J* = 8 Hz, 1H); 7.19 (d, *J* = 8 Hz, 1H); 7.13 (d, *J* = 2 Hz, 1H); 6.95 (dd, *J* = 8 Hz, 2Hz, 1H); 6.73 (s, 1H); 4.37-4.29 (mult., 1H); 3.75-3.62 (mult., 4H); 2.93-2.82 (mult., 3H); 2.57-2.48 (mult., 2H); 2.16-2.05 (mult., 2H); 1.98-1.83 (mult., 6H); 1.10 (d, *J* = 6 Hz, 6H) ppm.

MS (EI) *m/e:* 550.2 $(M+H)^+$.

Anal. ($C_{28}H_{31}Cl_2F_3N_3O_2$) C, H, N. C: calcd, 61.09; found, 56.69. H: calcd, 5.68; found, 5.54.

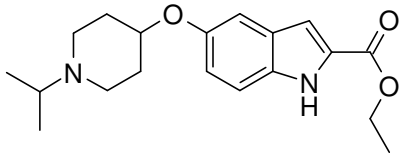
HRMS $C_{28}H_{31}Cl_2F_3N_3O_2$: calcd, 550.18342; found, 550.18309 ($M+H^+$).

HPLC tracing:



Scheme 6: Early stage incorporation of the 2,2,2-trifluoroethyl residue on the indole nitrogen

5-(1-Isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (23)

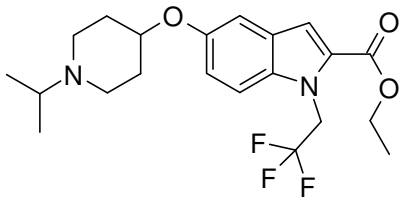


To a cold (ice water) solution of 5-hydroxyindole-2-carboxylic acid ethyl ester (20 g, 97 mmol), 1-isopropyl-piperidin-4-ol (16.73 g, 117 mmol, 1.2 eq.) and triphenylphosphine (30.68 g, 117 mmol, 1.2 eq.) in tetrahydrofuran (500 mL) was added dropwise a solution of di-tert-butyl azodicarboxylate (26.93 g, 117 mmol, 1.2 eq.) in tetrahydrofuran (100 mL). The mixture was stirred 2 days at room temperature and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol: aqueous ammonia (19:1:0 to 95:5:0.25) as eluant to afford, after evaporation, a colorless oil (40.26 g). This oil was dissolved in ethyl acetate and concentrated in vacuo to ca. 50 mL. Methyl-tert-butylether was added and the mixture was stirred at ca. 0-3°C (ice bath) then filtered and dried in vacuo to afford the desired product as a white solid (21.53 g, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s broad, 1H); 7.30 (d, *J* = 8 Hz, 1H); 7.15-7.10 (mult., 2H); 7.00 (dd, *J* = 8 Hz, 2Hz, 1H); 4.40 (quad., *J* = 7 Hz, 2H); 4.26 (hept., *J* = 4 Hz, 1H); 2.87-2.76 (mult., 2H); 2.75 (hept., *J* = 8 Hz, 1H); 2.42-2.33 (mult., 2H); 2.08-1.98 (mult., 2H); 1.87-1.77 (mult., 2H); 1.41 (t, *J* = 7 Hz, 3H); 1.06 (d, *J* = 6Hz, 6H) ppm.

MS (EI) *m/e:* 331.1 $(M+H)^+$.

5-(1-Isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indole-2-carboxylic acid ethyl ester



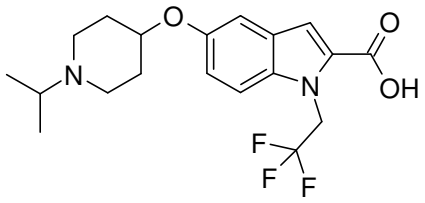
To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (**23**, 330 mg, 10 mmol) in N,N-dimethylformamide (4 mL) was added sodium hydride (dispersion in oil, 55% w/w, 48 mg, 11 mmol, 1.1 eq.) and the mixture was stirred 50 min at 70°C then 2,2,2-trifluoroethyl trifluoromethanesulfonate (279 mg, 12 mmol, 1.2 eq.) was added. The mixture was stirred at 70°C for 16h then cooled down and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water, brine, dried over sodium sulfate, filtered and concentrated in vacuo.

Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol: aqueous ammonia (1:0:0 to 95:5:0.25) as eluant to afford, after evaporation, the desired product as a yellow solid (461 mg, purity 89%, quant.). This solid was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8 Hz, 1H); 7.30 (s, 1H); 7.11 (d, *J* = 2Hz, 1H); 7.02 (dd, *J* = 8 Hz, 2Hz, 1H); 5.30 (quad., *J* = 8 Hz, 2H); 4.75-4.63 (mult., 1H); 4.39 (quad., *J* = 7 Hz, 2H); 3.57-3.45 (mult., 1H); 3.34-3.17 (mult., 4H); 2.52-2.40 (mult., 2H); 2.32-2.22 (mult., 2H); 1.49-1.38 (mult., 9H) ppm.

MS (EI) *m/e:* 413.2 $(M+H)^+$.

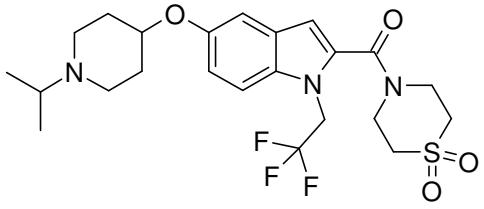
5-(1-Isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indole-2-carboxylic acid, hydrochloride acid salt, with lithium chloride



To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indole-2-carboxylic acid ethyl ester (3.9 g, 9.5 mmol) in tetrahydrofuran (30 mL), methanol (15 mL) and water (7 mL) was added lithium hydroxide monohydrate (460 mg, 11 mmol, 1.2 eq.). The mixture was refluxed for 16h then cooled down and concentrated in vacuo. The residue was acidified (pH=2) with hydrochloric acid (2N) and dried in vacuo to afford the desired product as an off-white solid (4.59 g, quant.) which was used in the next stept without further purification.

^{1}H NMR (400 MHz, d^6-DMSO) δ 12.1 (s broad, 1H); 7.61 (d, *J* = 8 Hz, 1H); 7.26 (d, *J* = 2Hz, 1H); 7.16 (s, 1H); 7.06 (dd, *J* = 8 Hz, 2Hz, 1H); 5.62 (quad., *J* = 8 Hz, 2H); 4.63-4.52 (mult., 1H); 3.42-2.94 (mult., 5H); 2.25-2.15 (mult., 2H); 2.05-1.93 (mult., 2H); 1.25 (d, *J* = 8 Hz, 6H) ppm.

(1,1-Dioxo-1λ^6-thiomorpholin-4-yl)-[5-(1-isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone (42)



To a solution of 5-(1-isopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indole-2-carboxylic acid, hydrochloride salt, with lithium chloride (950 mg, 2.05 mmol), thiomorpholine-1,1-dioxide (333 mg, 2.5 mmol, 1.2 eq.) and ethyl-diisopropyl-amine (1.8 mL; 10.2 mmol, 5.0 eq.) in N,N-dimethylformamide (10 mL) was added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (0.823 g, 2.5 mmol, 1.2 eq.). The mixture was stirred 24h at room temperature then

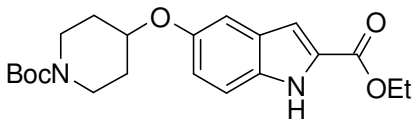
partitioned between an aqueous saturated solution of sodium hydrogenocarbonate and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water, brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by recrystallization in methanol to afford the desired product as a white solid (950 mg, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2Hz, 1H); 7.07 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.67 (s, 1H); 5.07 (quad., *J* = 8 Hz, 2H); 4.36-4.23 (mult., 3H); 3.13-3.03 (mult., 4H); 2.86-2.78 (mult., 2H); 2.75 (hept., *J* = 8 Hz, 1H); 2.43-2.35 (mult., 2H); 2.08-1.99 (mult., 2H); 1.89-1.78 (mult., 2H); 1.06 (d, *J* = 8Hz, 6H) ppm.

MS (EI) *m/e:* 502.2 $(M+H)^+$.

Anal. ($C_{23}H_{30}F_3N_3O_4S$) C, H, N, S.

5-(1-tert-Butoxycarbonyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (43)



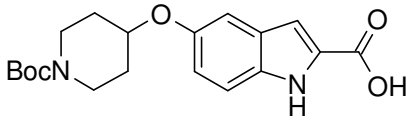
To a solution of ethyl 5-hydroxyindole-2-carboxylate (1.0 g, 4.9 mmol), 4-hydroxy-piperidine-1-carboxylic acid tert-butyl ester (1.02 g, 5.1 mmol, 1.04 eq.) and triphenylphosphine (1.6 g, 6.1 mmol, 1.25 eq.) in dichloromethane (10 mL) and dioxane (10 mL) was slowly added diisopropylazodicarboxylate (1.2 g, 5.9 mmol, 1.22 eq.). The mixture was stirred 3 days at room temperature then partitioned between dichloromethane and an aqueous saturated solution of sodium hydrogenocarbonate. The aqueous layer was extracted with dichloromethane and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of chloroform:

ethyl acetate (10:1 to 5:1) as eluant to afford, after evaporation, the desired product as an off-white solid (1.10 g, 58%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s broad, 1H); 7.32 (d, *J* = 8 Hz, 1H); 7.16-7.11 (mult., 2H); 7.00 (dd, *J* = 8 Hz, 2 Hz, 1H); 4.47-4.36 (mult., 3H); 3.79-3.69 (mult., 2H); 3.36-3.28 (mult., 2H); 2.00-1.89 (mult., 2H); 1.84-1.72 (mult., 2H); 1.47 (s, 9H); 1.41 (t, *J* = 8 Hz, 3H) ppm.

MS (EI) *m/e:* 389.4 $(M+H)^+$.

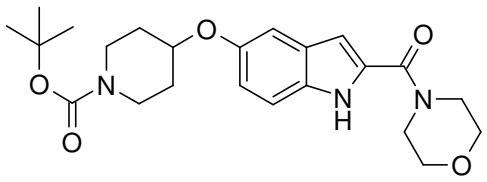
5-(1-tert-Butoxycarbonyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid hydrochloric acid salt, with lithium chloride



To a solution of 5-(1-tert-butoxycarbonyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid ethyl ester (**43**, 4.0 g, 10 mmol) in tetrahydrofuran (25 mL), water (12 mL) and methanol (6 mL) was added lithium hydroxide hydrate (480 mg, 11 mg, 1.1 eq.). The mixture was stirred at 80°C for 16h then the volatiles were removed in vacuo and the pH of the residue was adjusted to pH=1-2 by adding hydrochloric acid (2N). The resulting mixture was dried in vacuo to afford the desired product as a grey solid (4.35 g, 96%). This solid was used in the next step without further purification.

^{1}H NMR (400 MHz, *d*^{6}-DMSO) δ 11.6 (s broad, 1H); 7.33 (d, *J* = 8 Hz, 1H); 7.18 (d, *J* = 2 Hz, 2H); 6.97 (s, 1H); 6.92 (dd, *J* = 8 Hz, 2 Hz, 1H); 4.50-4.40 (mult., 1H); 3.72-3.61 (mult., 2H); 3.24-3.10 (mult., 2H); 1.95-1.84 (mult., 2H); 1.62-1.44 (mult., 2H); 1.41 (s, 9H) ppm.

4-[2-(Morpholine-4-carbonyl)-1H-indol-5-yloxy]-piperidine-1-carboxylic acid tert-butyl ester

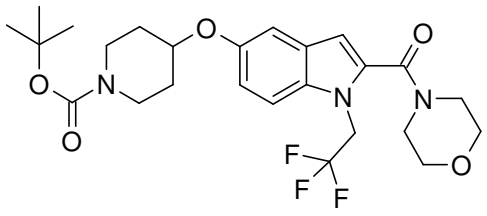


To a cold (ice bath) solution of 5-(1-tert-butoxycarbonyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid (5.0 g, 13.9 mmol) in tetrahydrofuran (46 mL) was slowly added 4-methylmorpholine (1.87 mL, 16.6 mmol, 1.2 eq.) and pivaloyl chloride (2.1 mL, 16.6 mmol, 1.2 eq.). The mixture was stirred 30 min at 0°C then morpholine (1.8 mL, 20.8 mmol, 1.5 eq.) was added. The mixture was stirred 4.5h at 0°C then partitioned between an aqueous solution of hydrochloric acid (1N, 50 mL) and ethyl acetate (100 mL). The organic layer was washed with a saturated aqueous solution of sodium hydrogenocarbonate (50 mL) and brine (200 mL) then dried over sodium sulfate, filtered and concentrated in vacuo. The residue was stirred at reflux with ethanol (12 mL) then cooled down to 0°C and after 1h was filtered. The solid was washed with cold ethanol and dried in vacuo to afford the desired product as a white solid (4.45 g, 75%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2Hz, 1H); 6.95 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.67 (d, *J* = 1Hz, 1H); 5.06 (hept., *J* = 3 Hz, 1H); 4.02-3.87 (mult., 4H); 3.82-3.68 (mult., 6H); 3.35-3.25 (mult., 2H); 1.97-1.87 (mult., 2H); 1.83-1.72 (mult., 2H); 1.47 (s, 9H) ppm.

MS (EI) *m/e*: 430.3 $(M+H)^+$.

4-[2-(Morpholine-4-carbonyl)-1-(2,2,2-trifluoro-ethyl)-1H-indol-5-yloxy]-piperidine-1-carboxylic acid tert-butyl ester

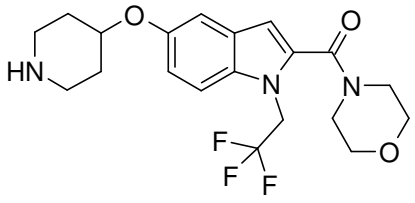


To a solution of 4-[2-(morpholine-4-carbonyl)-1H-indol-5-yloxy]-piperidine-1-carboxylic acid tert-butyl ester (1.55 g, 3.6 mmol) in N,N-dimethylformamide (15 mL) was added sodium hydride (dispersion in oil, 55% w/w, 173 mg, 4.0 mmol, 1.1 eq.). The mixture was stirred 45 min at 70°C then 2,2,2-trifluoroethyltrifluoromethane sulfonate (1.0 mL, 4.3 mmol, 1.2 eq.) was added and the mixture was stirred 20h at 70°C. The mixture was partitioned between ethyl acetate and an aqueous saturated solution of sodium hydrogenocarbonate. The aqueous layer was extracted with ethyl acetate. The combined organic phases were washed with an aqueous saturated solution of sodium hydrogenocarbonate and water then dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ ethyl acetate (98:2 to 90:10) as eluant to afford, after evaporation, the desired product as a light yellow solid (1.20 g, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2 Hz, 1H); 7.00 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.58 (s, 1H); 5.06 (quad., *J* = 9 Hz, 2 Hz); 4.43 (hept., *J* = 4 Hz, 1H); 3.86-3.62 (mult., 10H); 3.36-3.25 (mult., 2H); 1.98-1.83 (mult., 2H); 1.70-1.83 (mult., 2H); 1.47 (s, 9H) ppm.

MS (EI) *m/e:* 512.3 $(M+H)^+$.

Morpholin-4-yl-[5-(piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone (44)



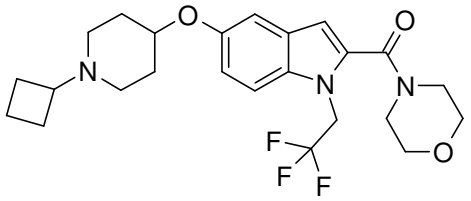
To a cold (ice bath) solution of 4-[2-(morpholine-4-carbonyl)-1-(2,2,2-trifluoro-ethyl)-1H-indol-5-yloxy]-piperidine-1-carboxylic acid tert-butyl ester (5.81 g, 11 mmol) in dichloromethane (40 mL) was added trifluoroacetic acid (8.7 mL, 114 mmol, 10 eq.). The mixture was stirred 1h at room temperature

and the volatiles were removed in vacuo. The residue was partitioned between a solution of potassium carbonate (30 g) in water (75 mL) and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo to afford the desired product as an off-white solid (4.3 g, 92%). This solid was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2 Hz, 1H); 7.02 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.58 (s, 1H); 5.06 (quad., *J* = 9 Hz, 2H); 4.33 (hept., *J* = 4 Hz, 1H); 3.87-3.62 (mult., 8H); 3.21-3.13 (mult., 2H); 2.77-2.67 (mult., 2H); 2.08-1.99 (mult., 2H); 1.75-1.62 (mult., 2H), 1.58 (broad s, 1H) ppm.

MS (EI) *m/e:* 412.1 $(M+H)^+$.

[5-(1-Cyclobutyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-morpholin-4-yl-methanone (45)



To a solution of morpholin-4-yl-[5-(piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone, trifluoroacetic acid salt (**44**, 200 mg, 0.38 mmol) and cyclobutanone (40 µL, 0.57 mmol, 1.5 eq.) in tetrahydrofuran (2 mL) was successively added acetic acid (70 µL, 1.14 mmol, 3.0 eq.) and a solution of sodium cyanoborohydride in tetrahydrofuran (1M, 0.57 mL, 0.57 mmol, 1.5 eq.). The mixture was stirred at 55°C for 16h and then concentrated in vacuo. The residue was partitioned between water and an aqueous solution of hydrochloric acid (1M) then washed with ethyl acetate. The aqueous layer was basified by adding a solution of sodium carbonate in water and the resulting mixture

was extracted with ethyl acetate. The combined organic phases were washed with brine then dried over sodium sulfate, filtered and concentrated in vacuo.

Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol (99:1 to 90:10) as eluant to afford, after evaporation, the desired product as an off-white solid (105 mg, 59%).

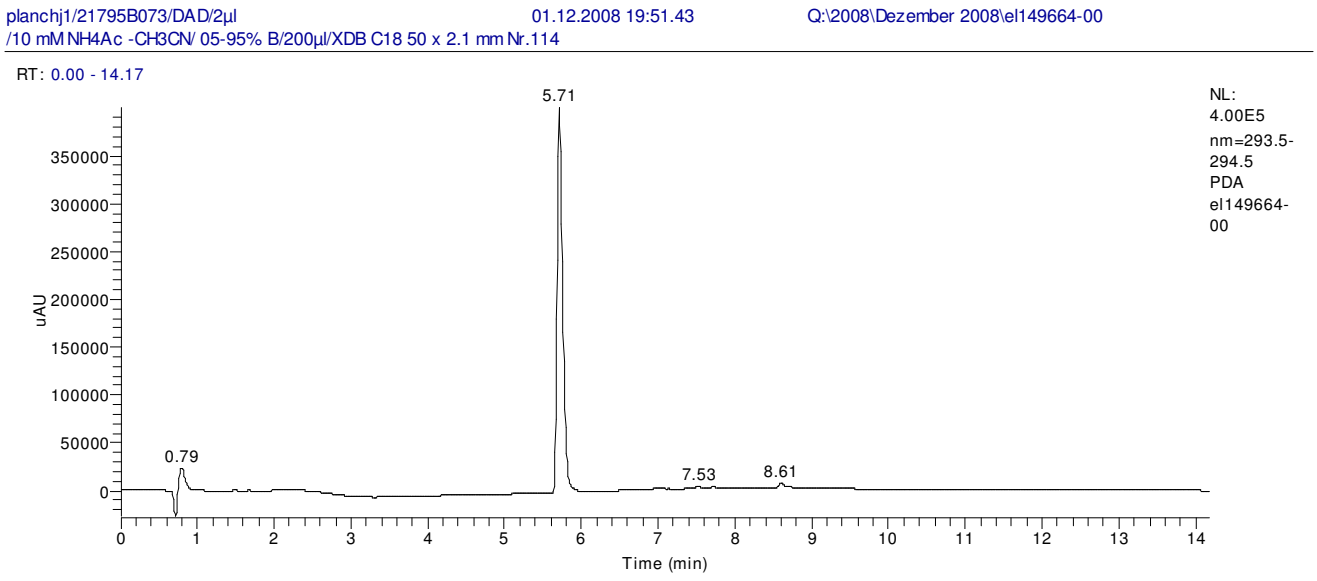
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2 Hz, 1H); 7.02 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.58 (s, 1H); 5.06 (quad., *J* = 9 Hz, 2H); 4.36-4.26 (mult., 1H); 3.86-3.62 (mult., 8H); 2.84-2.60 (mult., 3H); 2.30-2.15 (mult., 2H); 2.10-1.83 (mult., 9H); 1.78-1.62 (mult., 3H) ppm.

MS (EI) *m/e:* 466.2 $(M+H)^+$.

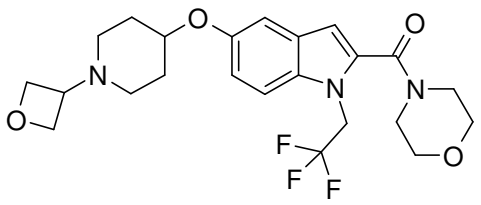
Anal. ($C_{24}H_{30}F_3N_3O_3$) C, H, N. C: calcd, 61.92; found, 61.16.

HRMS $C_{24}H_{30}F_3N_3O_3$: calcd, 466.23120; found, 466.23093 ($M+H^+$).

HPLC tracing:



Morpholin-4-yl-[5-(1-oxetan-3-yl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone (46)



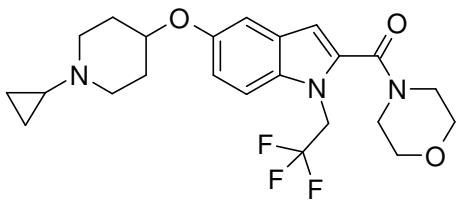
To a solution of morpholin-4-yl-[5-(piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone (**44**, 100 mg, 0.24 mmol) and oxetanone (156 mg, 0.48 mmol, 2.0 eq.) in tetrahydrofuran (3 mL) was added acetic acid (44 µL, 0.72 mmol, 3.0 eq.) and the mixture was stirred 1h at 55°C. Then sodium triacetoxyborohydride (106 mg, 0.48 mmol, 2.0 eq.) was added and the mixture was stirred 2h at 65°C then concentrated in vacuo. The residue was partitioned between water (5 mL) and saturated aqueous sodium hydrogenocarbonate solution. The organic layer was washed with water, dried over sodium carbonate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol (99:1 to 98:2) as eluant to afford, after evaporation, the desired product as a white solid (68 mg, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2Hz, 1H); 7.02 (dd, *J* = 8Hz, 2Hz, 1H); 6.58 (s, 1H); 5.06 (quad., *J* = 9 Hz, 2H); 4.69-4.59 (mult., 4H); 4.33 (hept., *J* = 4 Hz, 1H); 3.86-3.63 (mult., 8H); 3.52 (quint., *J* = 7 Hz, 1H); 2.63-2.52 (mult., 2H); 2.24-2.15 (mult., 2H); 2.06-1.98 (mult., 2H); 1.95-1.82 (mult., 2H) ppm.

MS (EI) *m/e:* 468.4 $(M+H)^+$.

Anal. ($C_{23}H_{28}F_3N_3O_4$) C, H, N.

[5-(1-Cyclopropyl-piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-morpholin-4-yl-methanone (47)



To a solution of morpholin-4-yl-[5-(piperidin-4-yloxy)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-methanone, trifluoroacetic acid salt (**44**, 600 mg, 1.14 mmol) and (1-ethoxycyclopropoxy)trimethylsilane (302 mg, 1.7 mmol, 1.5 eq.) in tetrahydrofuran (10 mL) were successively added acetic acid (0.2 mL, 3.42 mmol, 3.0 eq.) and a solution of sodium cyanoborohydride in tetrahydrofuran (1M, 1.7 mL, 1.7 mmol, 1.5 eq.). The mixture was stirred 20h at 55°C and concentrated in vacuo. The residue was partitioned between water (5 mL) and aqueous hydrochloric acid (1M, 1.5 mL) and washed with ethyl acetate. The aqueous layer was basified with aqueous sodium carbonate solution and the resulting mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using dichloromethane/ 2N ammonia solution in methanol (97:3) as eluant, to afford, after evaporation, the desired product as a white solid (230 mg, 45%).

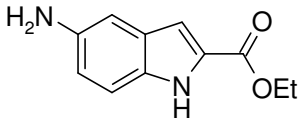
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8 Hz, 1H); 7.10 (d, *J* = 2 Hz, 1H); 7.02 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.58 (s, 1H); 5.06 (quad., *J* = 9 Hz, 2H); 4.29 (hept., *J* = 4 Hz, 1H); 3.86-3.62 (mult., 8H); 2.97-2.86 (mult., 2H); 2.53-2.40 (mult., 2H); 2.05-1.94 (mult., 2H); 1.86-1.76 (mult., 2H); 1.68-1.55 (mult., 1H); 0.50-0.37 (mult., 4H) ppm.

MS (EI) *m/e:* 452.2 $(M+H)^+$.

Anal. ($C_{23}H_{28}F_3N_3O_3$) C, H, N.

Scheme 7: Modulation of the N-piperidine substituent

5-Amino-1H-indole-2-carboxylic acid ethyl ester

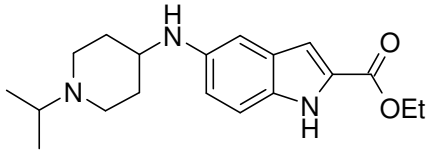


To a solution of ethyl-5-nitroindole-2-carboxylate (5.1 g, 21.8 mmol) in tetrahydrofuran (300 mL) was added platinum oxide hydrate (600 mg, 2.4 mmol, 0.11 eq.) and the suspension was flushed with hydrogen at atmospheric pressure. The mixture was vigorously stirred 2h at room temperature, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol (1:0 to 98:2) as eluant, to afford, after evaporation, the desired product as a yellow solid (4.17 g, 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s broad, 1H); 7.22 (d, *J* = 8 Hz, 1H); 7.02 (dd, *J* = 2 Hz, 1 Hz, 1H); 6.93 (d, *J* = 2 Hz, 1H); 6.79 (dd, *J* = 8 Hz, 2 Hz, 1H); 4.38 (quad., *J* = 7 Hz, 2H); 3.552 (broad s, 2H); 1.40 (t, *J* = 7 Hz, 3H) ppm.

MS (EI) *m/e:* 205.1 $(M+H)^+$.

5-(1-Isopropyl-piperidin-4-ylamino)-1H-indole-2-carboxylic acid ethyl ester (48)

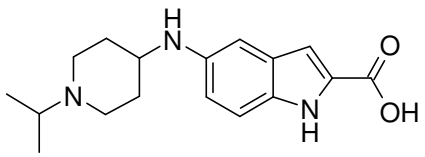


To a solution of 5-amino-1H-indole-2-carboxylic acid ethyl ester (4.1 g, 20.1 mmol) and 1-isopropyl-4-piperidone (2.84 g, 20.1 mmol, 1.0 eq.) in methanol (30 mL) was added titanium isopropoxide (7.4 mL, 24.1 mmol, 1.2 eq.). The orange mixture was stirred 3h at room temperature, cooled (ice bath) and sodium borohydride was added (456 mg, 12 mmol, 0.6 eq.) in 5 portions within 30 min. The mixture was stirred for 16h at room temperature and poured into ice water (330 mL). Ethyl acetate (450 mL) and aqueous sodium hydroxide solution (1N, 33 mL) were added to the stirred mixture. After separation of the phases, the aqueous layer was extracted with ethyl acetate, the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ 2N ammonia in methanol (95:5 to 93:7) as eluant, to afford, after evaporation, the desired product as a brown solid (5.64 g, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s broad, 1H); 7.21 (d, *J* = 8 Hz, 1H); 7.03 (s, 1H); 6.80 (d, *J* = 2 Hz, 1H); 6.73 (dd, *J* = 8 Hz, 2 Hz, 1H); 4.38 (quad., *J* = 7 Hz, 2H); 3.37-3.25 (mult., 2H); 2.93-2.84 (mult., 2H); 2.76 (hept., *J* = 7 Hz, 1H); 2.38-2.27 (mult., 2H); 2.16-2.06 (mult., 2H); 1.53-1.41 (mult., 2H); 1.40 (t, *J* = 7 Hz, 3H); 1.07 (d, *J* = 7 Hz, 6H) ppm.

MS (EI) *m/e:* 330.2 $(M+H)^+$.

1-[5-(1-Isopropyl-piperidin-4-ylamino)-1H-indol-2-yl]-ethanone, with 2 eq. hydrochloric acid



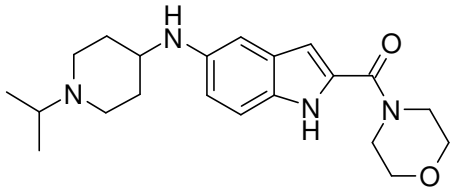
To a solution of 5-(1-isopropyl-piperidin-4-ylamino)-1H-indole-2-carboxylic acid ethyl ester (**48**, 5.4 g, 16.4 mmol) in tetrahydrofuran (70 mL), water (35 mL) and methanol (15 mL) was added lithium hydroxide hydrate (757 mg, 18 mmol, 1.1 eq.). The mixture was stirred 2h at 75°C and the volatiles

were removed in vacuo. The pH of the residue was adjusted to pH=1-2 by addition of aqueous hydrochloric acid (2N) and the solution was evaporated in vacuo to afford the desired product as a brown solid (7.16 g, quant.). This solid was used in the next step without further purification.

^{1}H NMR (400 MHz, d^6-DMSO) δ 7.87-7.62 (mult., 1H); 7.60-7.43 (mult., 1H); 7.39-7.24 (mult., 1H); 7.21-7.05 (broad s, 1H); 3.76-3.62 (mult., 1H); 3.62-3.20 (mult., 2H); 3.08-2.90 (mult., 2H); 2.29-2.06 (mult., 4H); 1.32-1.16 (mult., 6H) ppm.

MS (EI) *m/e:* 302.0 $(M+H)^+$.

[5-(1-Isopropyl-piperidin-4-ylamino)-1H-indol-2-yl]-morpholin-4-yl-methanone (49)



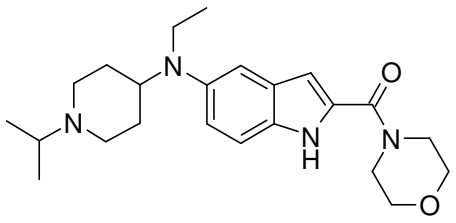
To a solution of 1-[5-(1-isopropyl-piperidin-4-ylamino)-1H-indol-2-yl]-ethanone, compound with 2 eq. hydrochloric acid (7.0 g, 21 mmol), morpholine (2.22 g, 25 mmol, 1.2 eq.) and ethyl-diisopropyl-amine (21.97 mL; 127 mmol, 6.0 eq.) in N,N-dimethylformamide (100 mL) was added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (8.19 g, 25 mmol, 1.2 eq.). The mixture was stirred 2h at room temperature and partitioned between aqueous sodium carbonate solution (10% w/w, 200 mL) and dichloromethane (150 mL). The aqueous layer was extracted with dichloromethane and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ 2N ammonia in methanol (98:2 to 92:8) as eluant, to afford, after evaporation, the desired product as an off-white solid (6.17 g, 78%).

^{1}H NMR (400 MHz, d^6-DMSO) δ 7.14 (d, *J* = 8 Hz, 1H); 6.65 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.62 (s, 1H); 6.54 (d, *J* = 2 Hz, 1H); 4.82 (d, *J* = 8 Hz, 1H); 3.82-3.60 (mult., 8H); 3.15-3.06 (mult., 1H); 2.80-2.13 (mult., 3H); 2.26-2.14 (mult., 2H); 1.97-1.87 (mult., 2H); 1.39-1.25 (mult., 2H); 0.96 (d, *J* = 6 Hz, 2H) ppm.

MS (EI) *m/e:* 371.3 $(M+H)^+$.

Anal. ($C_{21}H_{30}N_4O_2$) C, H, N.

{5-[Ethyl-(1-isopropyl-piperidin-4-yl)-amino]-1H-indol-2-yl}-morpholin-4-yl-methanone (50)



To a mixture of [5-(1-isopropyl-piperidin-4-ylamino)-1H-indol-2-yl]-morpholin-4-yl-methanone (**49**, 200 mg, 0.54 mmol) and potassium carbonate (93 mg, 0.67 mmol, 1.25 eq.) in N,N-dimethylformamide (2 mL) was added iodoethane (210 mg, 1.34 mmol, 2.5 eq.). The mixture was stirred 2h at 60°C and partitioned between aqueous sodium carbonate solution (10% w/w, 200 mL) and dichloromethane (150 mL). The aqueous layer was extracted with dichloromethane and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ 2N ammonia in methanol (98:2 to 94:6) as eluant to afford, after evaporation, the desired product as a light yellow solid (165 mg, 77%).

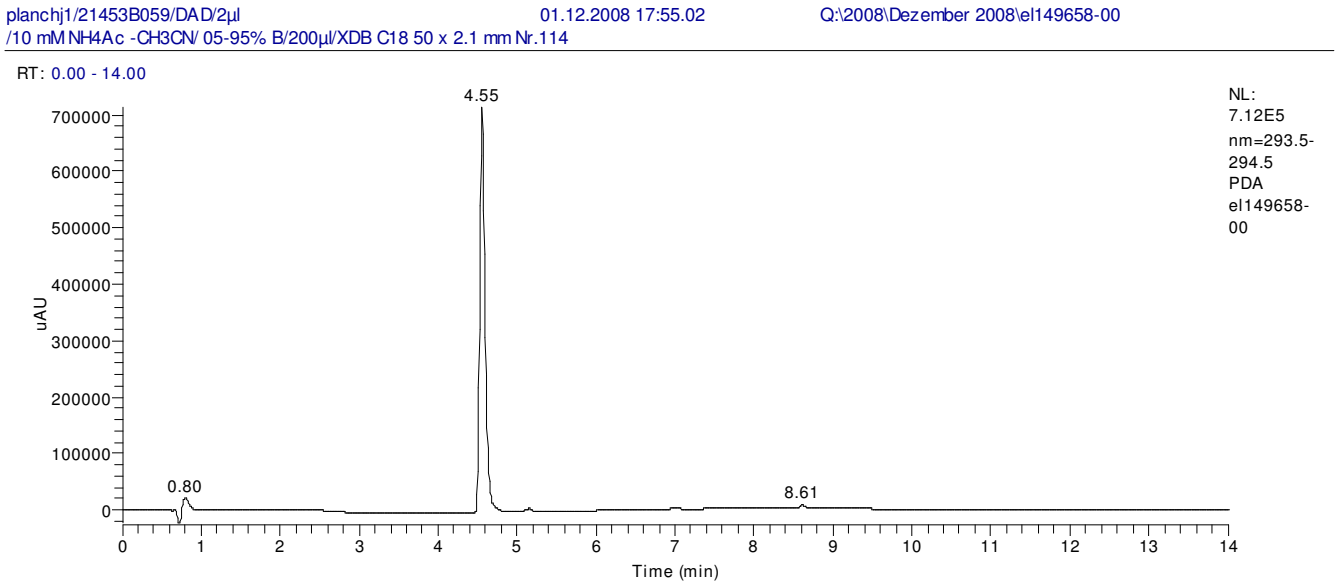
^{1}H NMR (400 MHz, d^6-DMSO) δ 7.27 (d, *J* = 8 Hz, 1H); 7.00 (broad s, 1H); 6.90 (broad d, *J* = 9 Hz, 1H); 6.54 (d, *J* = 2 Hz, 1H); 3.83-3.58 (mult., 8H); 3.27-3.11 (mult., 3H); 2.89-2.57 (mult., 3H); 2.22-2.05 (mult., 2H); 1.80-1.65 (mult., 2H); 1.54-1.40 (mult., 2H); 1.02-0.85 (mult., 9H) ppm.

MS (EI) *m/e:* 399.5 $(M+H)^+$.

Anal. ($C_{23}H_{34}N_4O_2$) C, H, N. C: calcd, 69.31; found, 67.81. N: calcd, 14.06; found, 13.34.

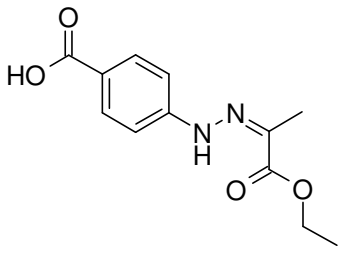
HRMS $C_{23}H_{34}N_4O_2$: calcd, 399.27545; found, 399.27529 ($M+H^+$).

HPLC tracing:



Scheme 8: Fischer indole synthesis for the preparation of 2,5-bis-aminocarbonyl-indoles

4-{N'-[1-Ethoxycarbonyl-eth-(*E*)-ylidene]-hydrazino}-benzoic acid

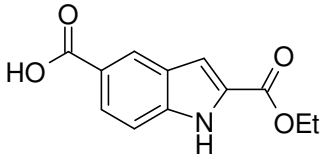


To a suspension of 4-hydrazinobenzoic acid (75 g, 493 mmol) in ethanol (850 mL) was added ethyl 2-oxopropionate (65.7 mL, 592 mmol, 1.2 eq.). The resulting mixture was heated at reflux for 18 h. After cooling to room temperature, the resulting suspension was filtered, washed twice with ethanol (100 mL) and once with diethyl ether. This afforded the desired product as a light brown solid (101.5 g; 82%)

^{1}H NMR (300 MHz, d^6-DMSO) δ 12.5 (broad, 1H); 10.16 (s, 1H); 7.84 (d, J = 9 Hz, 2H); 7.31 (d, J = 9 Hz, 2H); 4.21 (quad., J = 7 Hz, 2H); 2.11 (s, 3H); 1.28 (t, J = 7 Hz, 3H) ppm.

MS (Ion spray) *m/e*: 249.3 $(M-H)^-$.

1H-Indole-2,5-dicarboxylic acid 2-ethyl ester (51)

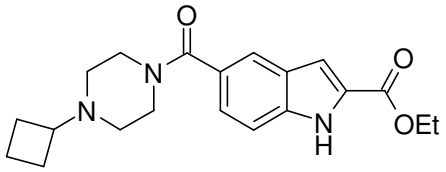


A mixture of 4-{N'-[1-ethoxycarbonyl-eth-(*E*)-ylidene]-hydrazino}-benzoic acid (2.4 g, 9.60 mmol) and polyphosphoric acid (5.0 g) was heated 20 min at 165 °C. The resulting black slurry was cooled to room temperature and partitioned between water and ethyl acetate. The aqueous layer was extracted seven times with ethyl acetate, and the combined organic phases were washed with water, brine, dried over magnesium sulfate, filtered and concentrated in vacuo to afford the desired product as a light brown solid (1.09 g, 44%), which was used for the next step without further purification.

^{1}H NMR (300 MHz, d^6-DMSO) δ 12.57 (broad s, 1H); 12.21 (s, 1H); 8.37 (s, 1H); 7.83 (d, J = 9 Hz, 1H); 7.49 (d, J = 9 Hz, 1H); 7.32 (s, 1H); 4.36 (q, J = 7 Hz, 2H); 1.34 (t, J = 4 Hz, 3H) ppm.

MS (EI) *m/e:* 234.2 $(M+H)^+$.

5-(4-Cyclobutyl-piperazine-1-carbonyl)-1H-indole-2-carboxylic acid ethyl ester

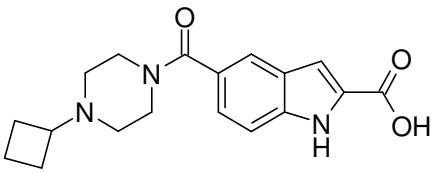


To a solution of 1H-indole-2,5-dicarboxylic acid 2-ethyl ester (**51**, 7.79 g, 33.4 mmol) in N,N-dimethylformamide (80 mL) was added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (16.10 g, 50 mmol, 1.5 eq.). The mixture was stirred 10 min at room temperature before the addition of 1-cyclobutyl-piperazine hydrochloride (8.90 g, 41.75 mmol, 1.25 eq.) and ethyl-diisopropyl-amine (39.76 ml, 233 mmol, 7 eq.). After stirring 1.5 h, the mixture was poured onto saturated aqueous sodium hydrogenocarbonate solution (300 mL) and extracted three times with ethyl acetate. The combined organic phases were washed three times with water and once with brine, dried over magnesium sulfate, filtered and evaporated to dryness. Purification was performed by silica gel column chromatography using a mixture of dichloromethane : methanol (9:1 v/v) as eluant to afford, after evaporation, a crude solid. This solid was suspended in tert-butyl methyl ether, stirred 30 min and filtered to afford the desired product as a light brown solid (7.42 g, 63%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (s, 1H); 7.78 (s, 1H); 7.38 (dd, J = 9 Hz, 2 Hz, 1H); 7.28-7.23 (mult., 1H); 4.42 (quad., J = 7 Hz, 2H); 3.72–3.49 (mult., 4H); 2.80-2.73 (mult., 1H); 2.40-2.30 (mult., 4H); 2.10-2.00 (mult., 2H); 1.92-1.82 (mult., 2H); 1.80-1.68 (mult., 2H); 1.42 (t, J = 7 Hz, 3H) ppm.

MS (Ion spray) *m/e*: 356.1 $(M+H)^+$.

5-(4-Cyclobutyl-piperazine-1-carbonyl)-1H-indole-2-carboxylic acid hydrochloric acid salt, with lithium chloride

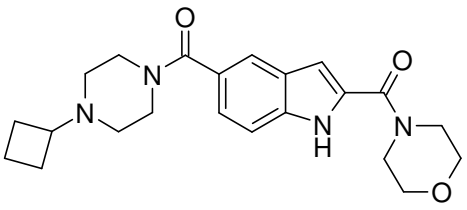


To a solution of 5-(4-cyclobutyl-piperazine-1-carbonyl)-1H-indole-2-carboxylic acid ethyl ester (1.0 g, 2.81 mmol) in tetrahydrofuran (38 mL) and water (19 mL) was added lithium hydroxide (86 mg, 2.5 mmol, 1.25 eq.). The mixture was stirred 2h at 75°C. The volatiles were removed in vacuo, the pH of the residue was adjusted to pH=1-2 with aqueous hydrochloric acid (3N) and the solution was evaporated in vacuo to afford the desired product as an orange solid (1.38 g, quant.). This solid was used in the next step without further purification.

^{1}H NMR (400 MHz, d^6-DMSO) δ 7.81 (s, 1H); 7.50 (d, *J* = 8 Hz, 1H); 7.34 (dd, *J* = 8 Hz, 2 Hz, 1H); 7.17 (d, *J* = 2 Hz, 1H); 4.45-3.80 (mult., 6H); 3.70-3.17 (mult., 4H); 2.95-2.78 (mult., 2H); 2.50-2.32 (mult., 2H); 2.18-2.05 (mult., 2H); 1.82-1.62 (mult., 2H) ppm.

MS (EI) *m/e:* 326.2 $(M-H)^-$.

[5-(4-Cyclobutyl-piperazine-1-carbonyl)-1H-indol-2-yl]-morpholin-4-yl-methanone (52)



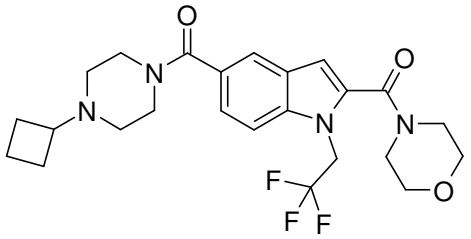
To a solution of 5-(4-cyclobutyl-piperazine-1-carbonyl)-1H-indole-2-carboxylic acid hydrochloric acid salt, with lithium chloride (1.36 g, 2.77 mmol), ethyl-diisopropyl-amine (2.42 mL, 13.9 mmol, 5 eq.) and morpholine (0.305 mL, 3.47 mmol, 1.25 eq.) in N,N-dimethylformamide (14 mL) was added 2-(1H-

benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (1.16 g, 3.47 mmol, 1.25 eq.). The mixture was stirred 3.5 h and partitioned between ethyl acetate and saturated aqueous sodium hydrogenocarbonate solution. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water and brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The residue was triturated with ethyl acetate (10 mL) and methyl-tert-butyl ether (50 mL) was added. The suspension was stirred 1h and filtered, and the solid was washed with methyl-tert-butyl ether to afford, after drying in vacuo, the desired product as a white solid (808 mg, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.50 (s, 1H); 7.74 (s, 1H); 7.43 (d, *J* = 8 Hz, 1H); 7.35 (dd, *J* = 8 Hz, 2 Hz, 1H); 6.80 (d, *J* = 2 Hz, 1H); 4.05-3.38 (mult., 12H); 2.75 (quint., *J* = 8 Hz, 1H); 2.50-2.17 (mult., 4H); 2.08-1.98 (mult., 2H); 1.95-1.83 (mult., 2H); 1.80-1.63 (mult., 2H) ppm.

MS (Ion spray) *m/e*: 397.1 $(M+H)^+$.

[5-(4-Cyclobutyl-piperazine-1-carbonyl)-1-(2,2,2-trifluoro-ethyl)-1H-indol-2-yl]-morpholin-4-yl-methanone (53)



To a solution of [5-(4-cyclobutyl-piperazine-1-carbonyl)-1H-indol-2-yl]-morpholin-4-yl-methanone (**52**, 780 mg, 1.97 mmol) in tetrahydrofuran (15 mL) was added sodium hydride (55% w/w dispersion in oil, 94 mg, 2.16 mmol, 1.1 eq.). The mixture was stirred 15 min at 75°C before the addition of 2,2,2-trifluoroethyl-trifluoromethane sulfonate (502 mg, 2.16 mmol, 1.1 eq.). The mixture was stirred 2 days at 75°C and partitioned between ethyl acetate and aqueous sodium hydrogenocarbonate solution (10% w/w). The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed

with water, brine, dried over sodium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ methanol (1:0 to 9:1) as eluant, to afford, after evaporation, the desired product as an off-white solid (718 mg, 76%).

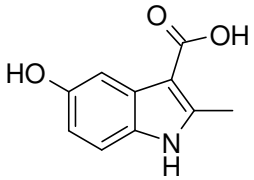
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H); 7.42 (s, 1H); 6.71 (s, 1H); 6.80 (d, *J* = 2 Hz, 1H); 5.11 (quad., *J* = 9 Hz, 2H); 3.93-3.40 (mult., 12H); 2.75 (quint., *J* = 8 Hz, 1H); 2.50-2.20 (mult., 4H); 2.10-1.98 (mult., 2H); 1.95-1.83 (mult., 2H); 1.78-1.65 (mult., 2H) ppm.

MS (EI) *m/e*: 479.2 $(M+H)^+$.

Anal. ($C_{24}H_{29}F_3N_4O_3$) C, H, N.

Scheme 9: preparation of 5-hydroxy-3-carboxamide indole

5-Hydroxy-2-methyl-1H-indole-3-carboxylic acid

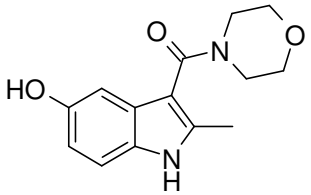


To a solution of ethyl-5-hydroxy-2-methylindole-3-carboxylate (27.6 g, 123 mmol) in tetrahydrofuran (500 mL) was added a solution of sodium hydroxide (27.87 g, 629 mmol, 5 eq.) in water (560 mL). This biphasic mixture was stirred 2 days at reflux and cooled to room temperature. The pH was adjusted to pH= 2 using aqueous hydrochloric acid (37% w/w, ca. 65 mL). The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. The residue was suspended in acetone/ diethyl ether (300 mL) and filtered. The solid was washed with diethyl ether and dried in vacuo to afford the desired product as a purple solid (18 g, 75%).

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.72 (s, 1H); 7.32 (d, *J* = 2 Hz, 1H); 7.10 (d, *J* = 8 Hz, 1H); 6.55 (dd, *J* = 8 Hz, 2 Hz, 1H); 2.58 (s, 3H) ppm.

MS (EI) *m/e:* 190.4 $(M-H)^-$.

(5-Hydroxy-2-methyl-1H-indol-3-yl)-morpholin-4-yl-methanone (54)

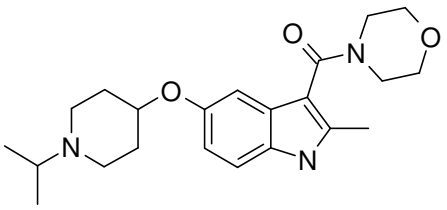


To a solution of 5-hydroxy-2-methyl-1H-indole-3-carboxylic acid (9.56 g, 50 mmol), ethyl-diisopropyl-amine (43.08 mL, 250 mmol, 5 eq.) and morpholine (5.23 g, 60 mmol, 1.20 eq.) in N,N-dimethylformamide (100 mL) was added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (19.26 g, 60 mmol, 1.20 eq.). The mixture was stirred 2 h at room temperature and partitioned between ethyl acetate and aqueous sodium carbonate solution (10% w/w). The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with water and brine, dried over magnesium sulfate, filtered and concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ 2N ammonia in methanol (98:2 to 96:4) as eluant, to afford, after evaporation, the desired product as an off-white solid (2.6 g, 20%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H); 7.09 (d, *J* = 8Hz, 1H); 6.75 (d, *J* = 2 Hz, 1H); 6.55 (dd, *J* = 8 Hz, 2 Hz, 1H); 3.67-3.58 (mult., 4H); 3.51-3.42 (mult., 4H); 2.37 (s, 3H) ppm.

MS (EI) *m/e*: 259.3 $(M-H)^-$.

[5-(1-Isopropyl-piperidin-4-yloxy)-2-methyl-1H-indol-3-yl]-morpholin-4-yl-methanone (55)



To a cold (ice bath) solution of (5-hydroxy-2-methyl-1H-indol-3-yl)-morpholin-4-yl-methanone (**54**, 2.6 g, 10 mmol), 1-isopropyl-piperidin-4-ol (1.86 g, 13 mmol, 1.3 eq.) and tri-n-butylphosphine (5.80 mL, 20 mmol, 2.0 eq.) in tetrahydrofuran (200 mL) was added 1,1'-(azodicarbonyl)dipiperidine (5.04 g, 20 mmol, 2.0 eq.) within 30 min. The mixture was stirred 3 days at room temperature, filtered and the filtrate concentrated in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ 2N ammonia in methanol (98:2 to 92:8) as eluant to afford, after evaporation, the desired product as an off-white solid (860 mg, 22%).

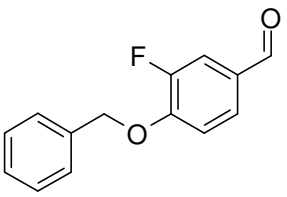
^{1}H NMR (400 MHz, d^6-DMSO) δ 7.19 (d, J = 8 Hz, 1H); 6.88 (s, 1H); 6.72 (d, J = 8 Hz, 1H); 4.27-4.15 (mult., 1H); 3.68-3.37 (mult., 8H); 2.80-2.62 (mult., 3H); 2.45-2.22 (mult., 5H); 2.95-2.82 (mult., 2H); 1.65-1.50 (mult., 2H); 0.97 (d, J = 6 Hz, 6H) ppm.

MS (EI) *m/e*: 386.4 $(M+H)^+$.

Anal. ($C_{22}H_{31}N_3O_3$) C, H, N.

Scheme 10: preparation of 6-hydroxy-2-carboxamide indole

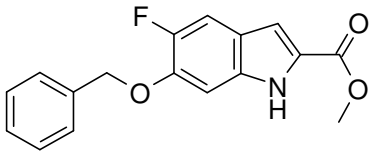
4-Benzyloxy-3-fluoro-benzaldehyde



To a solution of 3-fluoro-4-hydroxy-benzaldehyde (18.6 g, 133 mmol) in N,N-dimethylformamide (150 mL) were added potassium carbonate (22.02 g, 159 mmol, 1.2 eq.) and benzyl bromide (17.34 g, 146 mmol, 1.1 eq.). The mixture was stirred 2h at 55°C, cooled and filtered, and the solid was washed with N,N-dimethylformamide. The filtrate was concentrated in vacuo and partitioned between water and ethyl acetate, and the organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo. The crude solid was purified by precipitation in ethyl acetate : heptan (4:1 v/v) to afford, after drying in vacuo, the desired product as a light yellow solid (32.9 g, quant.). This solid was used without further purification in the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.86 (d, J = 2 Hz, 1H); 7.70-7.57 (mult., 2H); 7.47-7.35 (mult., 5H); 7.15-7.05 (mult., 1H); 5.24 (s, 2H) ppm.

6-Benzyloxy-5-fluoro-1H-indole-2-carboxylic acid methyl ester (56)



a) Preparation of the azido-acetic acid methyl ester

To a solution of methylbromoacetate (137.6 g, 899 mmol, 4.6 eq.) in toluene (250 mL) was added tetrabutylammonium hydrogen sulfate (6.10 g, 18 mmol, 0.09 eq.) and the mixture cooled to 5-10°C. A solution of sodium azide (61.4 g, 944 mmol, 4.9 eq.) and sodium carbonate (3.8 g, 36 mmol, 0.19 eq.) in water (150 mL) was added within 30 min. The mixture was stirred 3h at room temperature and the organic phase was separated and dried over sodium sulfate and filtered. The resulting solution of methyl azidoacetate was used in the next step

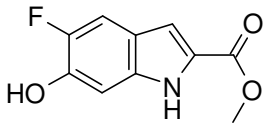
b) Reaction of azido-acetic acid methyl ester with 4-benzyloxy-3-fluoro-benzaldehyde

4-Benzyloxy-3-fluoro-benzaldehyde (44.2 g, 192 mmol) was dissolved in the solution of methyl azido-acetate in toluene (see above). The mixture was slowly added to a cold (-20°C) solution of sodium methoxide in methanol (3.2 M, 248 mL, 4.1 eq.) within 80 min. The mixture was stirred 3h at 0°C, filtered and the precipitate washed with small portions of methanol. The solid was then partitioned between a saturated aqueous solution of ammonium chloride (2.5 L) and ethyl acetate (2 L). The aqueous layer was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered and dried in vacuo, to afford 40.8 g of a yellow solid. The solid was suspended in p-xylene (500 mL), stirred 2h at reflux and concentrated to ca. 150 mL. The suspension was cooled to 0°C and filtered. The solid was washed with toluene (100 mL) and dried in vacuo to afford the desired product as a yellow solid (20.3 g, 35%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (broad s, 1H); 7.48 (d, *J* = 8 Hz, 2H); 7.42-7.30 (mult., 4H); 7.11 (dd, *J* = 2Hz, 1Hz, 1H); 6.92 (d, *J* = 7 Hz, 1H); 5.18 (s, 2H); 3.91 (s, 3H) ppm.

MS (EI) *m/e*: 300.3 $(M+H)^+$.

5-Fluoro-6-hydroxy-1H-indole-2-carboxylic acid methyl ester

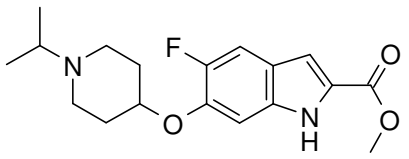


A mixture of 6-benzyloxy-5-fluoro-1H-indole-2-carboxylic acid methyl ester (**56**, 20.2 g, 67 mmol), and palladium on activated charcoal (10% Pd, 2.0 g, 1.9 mmol, 0.03 eq.) in ethyl acetate was flushed with hydrogen and vigourously stirred 2h and filtered on a Satorius filter. The filtrate was evaporated in vacuo and triturated with a solution of ethyl acetate and diethyl ether at 0°C, and filtered. The solid was washed with diethyl ether (20 mL) and dried in vacuo to afford the desired product as a white solid (10.95 g, 74%).

^{1}H NMR (400 MHz, d^6-DMSO) δ 7.36 (d, *J* = 8 Hz, 1H); 7.02 (d, *J* = 2 Hz, 1H); 6.96 (d, *J* = 8 Hz, 1H); 3.83 (s, 3H) ppm.

MS (EI) *m/e*: 208.1 $(M-H)^-$.

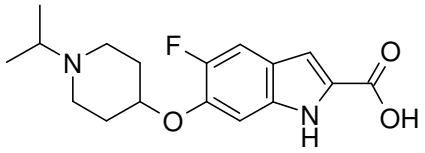
5-Fluoro-6-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid methyl ester (57)



To a cold (ice bath) solution of methyl 5-fluoro-6-hydroxy-1H-indole-2-carboxylate (3.85 g, 18 mmol), tri-n-butylphosphine (10.7 mL, 37 mmol, 2.0 eq.) and 1-isopropyl-3-piperidinol (3.43 g, 24 mmol, 1.3 eq.) in tetrahydrofuran (100 mL) was slowly added a solution of 1,1'-(azodicarbonyl)-dipiperidine (9.29 g, 37 mmol, 2.0 eq.) in tetrahydrofuran (100 mL). The mixture was stirred 22h at room temperature and filtered. The filtrate was concentrated in vacuo and the residue triturated in diethyl ether (200 mL). The resulting suspension was filtered and the solid was dried in vacuo. Purification was performed by column chromatography on silica gel using a gradient of dichloromethane/ 2N ammonia in methanol (98:2 to 92:8) as eluant, to afford, after evaporation, the desired product as an off-white solid (2.93 g, 48%).

MS (EI) *m/e*: 335.44 $(M+H)^+$.

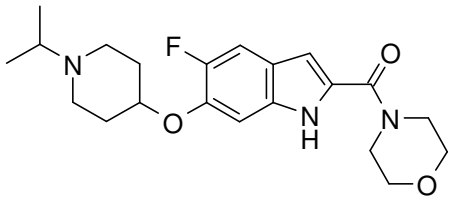
5-Fluoro-6-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt



To a solution of methyl 5-fluoro-6-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylate (**57**, 1.4 g, 4.2 mmol) in tetrahydrofuran (50 mL) and water (25 mL) was added lithium hydroxide monohydrate (193 mg, 4.6 mmol, 1.1 eq.). The mixture was stirred 1h at reflux. The volatiles were removed in vacuo and the pH of the residue was adjusted to pH= 2 with aqueous hydrochloric acid (2N). The mixture was evaporated in vacuo to afford the desired product as an off-white solid (1.6 g, quant.). This solid was used in the next step without further purification.

MS (EI) *m/e*: 321.4 $(M+H)^+$.

[5-Fluoro-6-(1-isopropyl-piperidin-4-yloxy)-1H-indol-2-yl]-morpholin-4-yl-methanone (58)



To a solution of 5-fluoro-6-(1-isopropyl-piperidin-4-yloxy)-1H-indole-2-carboxylic acid, hydrochloride salt (186 mg, 0.58 mmol) in N,N-dimethylformamide (10 mL) was added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (224 mg, 0.70 mmol, 1.2 eq.). The mixture was stirred 10 min at room temperature before the addition of morpholine (60 µL, 0.70 mmol, 1.2 eq.) and ethyl-diisopropyl-amine (0.6 mL, 3.48 mmol, 6 eq.). After stirring 17 h, the mixture was partitioned between ethyl acetate and aqueous sodium carbonate solution (10% w/w), the organic phase was separated and washed with brine, dried over sodium sulfate, filtered and evaporated. Purification was performed by silica gel column chromatography using a gradient of dichloromethane : 2N ammonia in methanol (98:2 to 94:6) as eluant, to afford, after evaporation, the desired product as a light brown solid (153 mg, 68%).

^{1}H NMR (300 MHz, d^6-DMSO) δ 7.37 (d, J = 12 Hz, 1H); 7.07 (d, J = 8 Hz, 1H); 6.73 (s, 1H); 4.30-4.23 (mult., 1H); 3.80-3.59 (mult., 8H); 2.76-2.64 (mult., 3H); 2.35-2.25 (mult., 2H); 2.00-1.88 (mult., 2H); 1.70-1.68 (mult., 2H); 0.97 (d, J = 7 Hz, 1H) ppm.

MS (Ion spray) *m/e*: 390.4 $(M+H)^+$.

Anal. ($C_{21}H_{28}N_3O_3$) C, H, N.

ELEMENTAL ANALYSIS DATA

Compound **20**
Formula: C21H29N3O3

Element	% theoretical	% experimental [a]
C	67.90	65.84
H	7.87	7.79
N	11.31	10.86
a: mean value from 2 measurements		

Compound **21**
Formula: C21H27N3O3

Element	% theoretical	% experimental [a]
C	68.27	67.61
H	7.37	7.12
N	11.37	11.26
a: mean value from 2 measurements		

Compound **22**
Formula: C22H31N3O3

Element	% theoretical	% experimental [a]
C	68.54	67.47
H	8.11	8.07
N	10.90	10.60
a: mean value from 2 measurements		

Compound **25**
Formula: C21H29N3O3

Element	% theoretical	% experimental [a]
C	67.90	67.85
H	7.87	7.89
N	11.31	11.27
a: mean value from 2 measurements		

Compound **26**
Formula: C23H33N3O2

Element	% theoretical	% experimental [a]
C	72.03	71.92
H	8.67	8.72
N	10.96	10.90
a: mean value from 2 measurements		

Compound **27**
Formula: C23H33N3O3

Element	% theoretical	% experimental [a]
C	69.14	67.36

H	8.33	7.91
N	10.52	10.25
a: mean value from 2 measurements		

Compound **28**
Formula: C21H29N3O3

Element	% theoretical	% experimental [a]
C	67.90	65.92
H	7.87	7.73
N	11.31	10.96
a: mean value from 2 measurements		

Compound **29**
Formula: C22H29N3O2

Element	% theoretical	% experimental [a]
C	65.17	64.67
H	7.21	6.99
N	10.36	10.30
a: mean value from 2 measurements		

Compound **30**
Formula: C22H29N3O2

Element	% theoretical	% experimental [a]
C	65.17	64.98
H	7.21	7.06
N	10.36	10.24
a: mean value from 2 measurements		

Compound **32**
Formula: C21H29N3O3

Element	% theoretical	% experimental [a]
C	67.90	67.04
H	7.87	7.68
N	11.31	11.10
a: mean value from 2 measurements		

Compound **37**
Formula: C24H31N3O3

Element	% theoretical	% experimental [a]
C	64.41	60.93
H	6.98	6.86
N	9.39	8.77
a: mean value from 2 measurements		

Compound **38**
Formula: C23H31N3O4S

Element	% theoretical	% experimental [a]
C	57.13	57.11
H	6.46	6.36
N	8.69	8.62
S	6.63	6.64
a: mean value from 2 measurements		

Compound **36**
Formula: C25H35F2N3O2

Element	% theoretical	% experimental [a]
C	67.09	66.90
H	7.88	7.69
N	9.39	9.30
a: mean value from 2 measurements		

Compound **39**
Formula: C28H32F3N3O4S

Element	% theoretical	% experimental [a]
C	59.67	56.69
H	5.72	5.54
N	7.46	6.91
S	5.69	5.61
a: mean value from 2 measurements		

Compound **33**
Formula: C23H30F3N3O3

Element	% theoretical	% experimental [a]
C	60.92	60.70
H	6.67	6.51
N	9.27	9.19
a: mean value from 2 measurements		

Compound **34**
Formula: C24H35N3O4S

Element	% theoretical	% experimental
C	62.45	62.07
H	7.64	7.41
N	9.10	9.15
S	6.95	6.94

Compound **40**
Formula: C24H30F2N4O2

Element	% theoretical	% experimental [a]
C	64.85	63.84
H	6.80	6.74

N	12.60	12.14
a: mean value from 2 measurements		

Compound **41**
Formula: C28H31Cl2F3N3O2

Element	% theoretical	% experimental [a]
C	61.09	58.59
H	5.68	5.39
N	7.63	7.00
a: mean value from 2 measurements		

Compound **42**
Formula: C23H30F3N3O4S

Element	% theoretical	% experimental [a]
C	55.08	54.80
H	6.03	5.92
N	8.38	8.34
S	6.39	6.32
a: mean value from 2 measurements		

Compound **45**
Formula: C24H30F3N3O3

Element	% theoretical	% experimental [a]
C	61.92	61.16
H	6.50	6.31
N	9.03	9.05
a: mean value from 2 measurements		

Compound **46**
Formula: C23H28F3N3O4

Element	% theoretical	% experimental
C	59.09	58.73
H	6.04	6.02
N	8.99	8.79

Compound **47**
Formula: C23H28F3N3O3

Element	% theoretical	% experimental [a]
C	61.19	60.84
H	6.25	6.07
N	9.31	9.21
a: mean value from 2 measurements		

Compound **49**
Formula: C21H30N4O2

Element	% theoretical	% experimental [a]
C	68.08	68.04
H	8.16	8.15
N	15.12	15.03
a: mean value from 2 measurements		

Compound **50**
Formula: C23H34N4O2

Element	% theoretical	% experimental
C	69.31	67.81
H	8.60	8.44
N	14.06	13.34

Compound **53**
Formula: C24H29F3N4O3

Element	% theoretical	% experimental
C	60.24	60.03
H	6.11	5.90
N	11.71	11.32

Compound **55**
Formula: C22H31N3O3

Element	% theoretical	% experimental
C	68.54	68.26
H	8.11	8.06
N	10.90	10.76

Compound **58**
Formula: C21H28N3O3

Element	% theoretical	% experimental [a]
C	64.76	64.60
H	7.25	7.09
N	10.79	10.67
a: mean value from 2 measurements		

IN VITRO ASSAYS

pKA MEASUREMENT BY PHOTOMETRIC TITRATION

Spectrophotometry is a powerful method of measuring pKa values provided the compound is water soluble to the extent of 10^{-6} M and contains chromophores in proximity to the ionization centers, such that the optical properties of the sample solution vary as a function of pH.

In the ProfilerSGA, an aqueous solution of sample is injected at constant flow rate into a flowing pH gradient. Changes in UV absorbance are monitored as a function of the pH gradient. The pKas are found and determined where the rate of change of absorbance is at a maximum. The pH gradient is established by proportionally mixing together two flowing buffer solutions containing 10%/vol methanol. The buffer solutions contain a mixture of weak acids and bases that do not absorb significantly in the UV above 240 nm.

LIPOPHILICITY DETERMINATION (LOG D)

The applied high-throughput (HT) method for the determination of the distribution coefficient HT-log D is based on microplate technique and derived from the conventional 'shake flask' method. The compound was distributed between water buffered at a specific pH and 1-octanol. The distribution coefficient was then calculated from the difference in concentration in the aqueous phase before and after partitioning and the ratio of the two phases. The "one phase-analysed" experiment starts with a pure dimethylsulfoxide solution of the compound of interest which was dispensed in aqueous buffer at a 0.5 mM concentration. A part of this solution is analyzed by measuring the UV absorption spectrum. The obtained optical density (reference) was equal to the concentration of the substance before partitioning. An exact amount of 1-octanol was added and the mixture incubated by quiet shaking (2 h). The emulsion was allowed to stand overnight to ensure that the partition equilibrium was reached. The next day, the phases were centrifuged at 3000 rpm for 10 min and the concentration of the substance in

the aqueous phase was determined again by measuring the UV absorption. The procedure above was carried out at four different octanol/water ratios, two with a large volume of octanol for hydrophilic compounds (log D < 1) and two with a low volume of octanol for the lipophilic compounds (log D > 1).

LYOPHILISATION SOLUBILITY ASSAY (LYSA):

Samples were prepared in duplicate from 10 mM dimethylsulfoxide stock solutions. After evaporation (1h) of dimethylsulfoxide with a centrifugal vacuum evaporator (Genevac Technologies), the compounds were dissolved in 0.05 M phosphate buffer (pH 6.5), stirred for one hour and shaken two hours. After one night, the solutions were filtered using a microtiter filter plate (Millipore MSDV N65) and the filtrate and its 1/10 dilution were analyzed by direct UV measurement or by HPLC-UV. In addition a four point calibration curve is prepared from the 10 mM stock solutions and used for the solubility determination of the compounds. The results are expressed in µg/ml. Starting from a 10 mM stock solution, the measurement range for MW 500 was 0- 666 µg/ml. In case the percentage of sample measured in solution after evaporation divided by the calculated maximum of sample amount was larger than 80% the solubility was reported as larger than this value.

hERG INHIBITION ASSAY

Measurement of the hERG current at the automated patch clamp system PatchXpress 7000A

Electrophysiological recordings of K^+ currents (IK_{hERG}) were conducted at room temperature (22-25°C) using Aviva Bioscience *Seal*Chip$_{16}$™ (Molecular Devices Corporation, Cat SealChip™16). CHO cells stably expressing hERG K^+ channels (Roche Palo Alto, USA) were added by the integrated Cavro robot to each well of the sealchip. Cells were held at a resting voltage of –80 mV and they were stimulated by a voltage pattern to activate hERG channels and conduct outward IK_{hERG} current at a stimulation frequency of 0.1 Hz. Cell health and membrane parameters (access resistance (Ra),

membrane resistance (Rm) and membrane capacitance (Cm)) were monitored on-line. After the cells have stabilized and the currents were steady, the amplitude and kinetics of IK_{hERG} were recorded under control conditions. Thereafter, the solution of the test compound in the extracellular buffer (NaCl 150 mM, KCl 4 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1 mM, HEPES 10 mM, pH 7.4 with NaOH, 300-310 mOsm) was directly added by the robot to each well at increasing concentrations. Currents were monitored continuously during the exposure to compounds.

Off-line analysis of the peak tail current was performed using DataXpress2 software (Molecular Devices Corporation, USA). The amplitude and kinetics of IK_{hERG} were recorded in each concentration of drug and they were compared to the control values (taken as 100%) to define fractional blocks. The hERG current was measured as the average current from 10 sweeps collected at the end of vehicle or compound addition. Data were expressed as mean±SEM. Concentration-response curves were fitted by non-linear regression analysis and the IC_{50} values were reported.

RAT PHARMACOKINETIC MEASUREMENT

In vivo studies

All *in vivo* studies were conducted in accordance with local regulations for animal treatment. Male Wistar rats were purchased from RCC-Füllinsdorf and were used for single dose pharmacokinetic studies. Animals were kept under standard laboratory conditions on a 12 hours light/dark cycle in controlled conditions (22°C, 55% humidity) with continuous access to dry pellet food and tap water. They were acclimatized for a minimum of 3 days before experiments were performed.

Plasma concentration-time profiles

At least three days before administration of the test compound, animals were operated after treatment with 0.05 mg/kg buprenorphin s.c. and anesthesia with ketamine and xylamine. A catheter was implanted into the right jugular vein. 5 and 24 hours after operation, the rats were treated with 1 mg/kg Meloxicam s.c.

For the intravenous administration (bolus), the solution containing test compound (~1 ml/kg) was injected through the jugular vein catheter. Following the injection, the catheter was washed twice by aspiration and reinjection of approx. 0.2-0.3 ml blood followed by 0.5 ml of physiologic saline solution.

For the oral administration, the formulated test compound (4 mL/kg) was given *per os* by gavage.

At designated time points over 24h, 0.3 ml blood samples were collected with a 1 ml syringe from the catheter implanted into the jugular vein, and then transferred to microcentrifuge tubes containing EDTA as anticoagulant. To maintain the blood volume, the corresponding volume of physiologic saline solution was injected after the collection of each blood sample. All blood samples were immediately centrifuged at 3000 G for 5 min at a temperature of 4°C. The plasma was transferred into micro-centrifuge tubes and stored frozen at -20°C until analysis.

Plasma samples were analysed for test compounds by a validated LC-MS/MS method.

Pharmacokinetic analysis

Plasma concentration versus time data were analysed by noncompartmental methods. The area under the plasma concentration-time curve (AUC_0^∞) and area under the moment curve ($AUMC_0^\infty$) were calculated by use of the linear trapezoidal rule, and log extrapolation to time infinity by adding C/β to AUC_0^t and $tC/\beta+C/\beta^2$ to $AUMC_0^t$, where C is the last predicted concentration at the last sampling time t. The slope of the terminal phase, β, was determined by log-linear regression of the last three to four data points, and the terminal half-life was calculated as $t_{½} = 0.693/\beta$. The plasma systemic clearance

(CL_P) was calculated by use of the relationship: $CL = Dose/AUC_0^\infty$. The volume of distribution at steady state (Vss) was calculated as: $Vss = CL \times AUMC_0^\infty / AUC_0^\infty$. Time to maximum plasma concentration (Tmax) and the maximum peak plasma concentration (Cmax) were determined directly from the plasma concentration-time profiles. Additionally, the oral bioavailability, F, was manually calculated from the plasma concentration data, estimated as:

$$F(\%)=[[AUC_{0\ po}^{\infty} / AUC_{0\ iv}^{\infty}] \times [Dose_{iv}/Dose_{po}] \times 100]$$

BEHAVIORAL SATIETY SEQUENCE

The aim of this study is to evaluate the effects of acute administration of compound **36** in the rat observational analysis of food intake (OAFI) assay. This test couples behavioural observations with measurement of food intake in non food-deprived rats. The assay examines the effects of the compound **36** on the four different components of the satiety sequence i.e. the characteristic sequence of behaviours (feeding followed by activity, grooming and resting) which normally occur when an animal eats until it is full. Animals are also closely observed during the assay for any other overt behavioural effects. The test can therefore be used to evaluate whether compound **36** is decreasing food intake in a specific manner, for example, by enhancing natural satiety (expressed as decreased feeding and increased resting) or by disrupting normal feeding behaviour in a non-specific manner, for example by inducing sedation or hyperactivity or any other motor effects which could interfere with food intake.

Animals

Thirty-two male Sprague-Dawley rats (weight range 200-250g) were ordered from Charles River, Margate, Kent (one set of 32 rats). The rats were individually housed in polypropylene cages with sawdust bedding at a temperature of 21±4°C and 55%±20% humidity. Animals were maintained on a reverse phase light-dark cycle (lights off for 8 h from 09:30 -17:30 h) during which time the room was

illuminated by red light. Animals had free access to a pelleted rat diet (HARLAN Teklad Global 2018 Diet) and tap water at all times. At approximately 09:30 each weekday morning each animal was presented with a glass jar containing wet mash (1 part powdered VRF1 diet and 1.5 parts tap water) for a 60 minute period. Animals were accustomed to these conditions for two weeks before experimentation.

Method

After having habituated to the presentation of wet mash, animals were allocated to one of four different treatment groups as described below. Experiments were performed over four days and results were pooled. Hence each treatment group contained eight animals. Allocation of animals to groups were such that two rats from each of the four treatment groups were tested on each day. The day prior to each test day, animals were weighed and dosed with the test compound orally by gavage 1 h prior to the onset of the dark cycle. At the time of dosing food was removed. Wet mash was presented 1 h post dose for 60 minutes. Subsequently, mash was replaced with food pellets. The next day this group of rats was weighed (to the nearest 0.1 g), dosed and placed in their home cages on a standard rack. Rats were receiving either vehicle or the test drug 1 h before the start of the behavioural observations by the oral route (po):-

Group	Treatment	n
A	Vehicle (Citric acid buffer) (po)	8
B	**36** 10mg/kg (po)	8
C	**36** 20mg/kg(po)	8
D	**36** 30mg/kg (po)	8

All food pellets were removed at the time of dosing. The cages were arranged to prevent interaction between animals and to ensure that they are evenly illuminated. The experimenter was unaware of the treatment received by each animal until after the test. Immediately before the start of the observation period, a jar containing wet mash was weighed (to the nearest 0.1g) and placed in the cage of the appropriate animals. Behaviours relevant to the satiety sequence – feeding (the acquisition and eating of food and the rare occasions when the animals take a drink), activity (including locomotor activity, rearing, sniffing and any other activities which could not be readily placed into one of the other categories), grooming (face and body washing and scratching) and resting (with eyes open or closed) - was scored for each animal using a time sampling procedure. Behaviours was scored twice a minute, in 5 min bins, for 60 min (i.e. the 8 animals tested on the same day will be observed in turn for 3-4 sec every 30 sec). The four behavioural categories are mutually exclusive. For each animal, the total score for each behavioural category for each 5 minute bin was calculated (maximum score 10). During the 60 min test period, the animals were also closely observed for any other overt behavioural effects, which could interfere with feeding behaviour. At the end of the test period, feeding jars were re-weighed and food intake over the test period will be calculated.