Supporting Information

Fused 3-Hydroxy-3-Trifluoromethylpyrazoles inhibit mutant huntingtin toxicity

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General Methods. Unless otherwise specified, all nuclear magnetic resonance spectra were recorded using a Varian Mercury Plus 400 MHz spectrometer equipped with a PFG ATB broadband probe. UPLC-MS analyses were run using an Acquity Waters UPLC equipped with a Waters SQD (ES ionization) and Waters Acquity PDA detector, using a column BEH C18 1.7 μm, 2.1 × 50 mm. Gradients were run using 0.05% formic acid water/acetonitrile 95/5 and acetronitrile with a gradient 95/5 to 100, flow: 1 mL/min over 10 min. Retention times were expressed in minutes. Temperature: 40 °C. UV detection at 215 and 254 nm. ESI+ detection in the 80–1000 m/z range. Preparative HPLC was run using a Waters 2767 system with a binary gradient module Waters 2525 pump and coupled to a Waters Micromass ZQ (ES) or Waters 2487 DAD, using a X-Bridge C18 5 μm, 19 mm × 150 mm, using a 0.1% formic acid/water and 0.1% formic acid/methanol flow: 17 mL/min. The purity of compounds submitted for screening were >95% as determined by integrating at 215 nm the peak area of the LC chromatograms, with no more than 5% single impurity. To further support the purity statement, all compounds were also analyzed at a different wavelengths (254 nm), and total ion current (TIC) chromatogram and NMR spectra were used to further substantiate results. All column chromatography was performed following small modifications of the original method of Still.[i] All TLC analyses were performed on silica gel (Merck 60 F254) and spots revealed by UV visualization at 254 nm and $KMnO_4$ or ninhydrin stain.

Section 1: Synthesis and characterization of compounds

Synthesis of intermediate 7

Synthesis of 2-(2,2,2-Trifluoro-acetyl)-cyclohexanone **7a**

To an ice-cooled suspension of sodium methoxide (27.5 g, 0.509 mol) in diisopropylether (500 mL) methyltrifluoroacetate (51.2 mL, 0.509 mol) in diisopropylether (50 mL) was added dropwise. Then a solution of cyclohexanone (52.8 mL, 0.509 mol) was added dropwise and the resulting mixture was allowed to reach room temperature continuing stirring for 20 hours. Then the mixture was filtered and a solid was collected and dried. The solid was suspended in diethyl ether (350 mL), kept under

mechanical stirring for 2 hours, and then filtered and dried under vacuum. The resulting white solid was suspended in ethyl acetate (350 mL) and a solution of hydrochloric acid (125 mL, 4M in dioxane) was added with stirring. Solvents were removed by evaporation under reduced pressure and the title compound was obtained as a clear liquid (47 g, 48%). 1H-NMR (400 MHz, CDCl3): δ ppm 1.69-1.75 (4H, m), 2.47-2.50 (4H, m), 15.05 (1H, s); 19F-NMR (376 MHz, CDCl3): δ ppm -73.90 (CF3).

Synthesis of 2-(2,2,2-Trifluoro-acetyl)-cycloheptanone **7b**

To a stirred suspension of sodium methoxide (1.44g, 26.74 mmol) and ethyltrifluoroacetate (3.18 mL). in dry diethyl ether (45 mL), cycloheptanone (3.15 mL, 26.74 mmol) were added dropwise at -10°C. The reaction was worm-up to room temperature and stirred overnight.

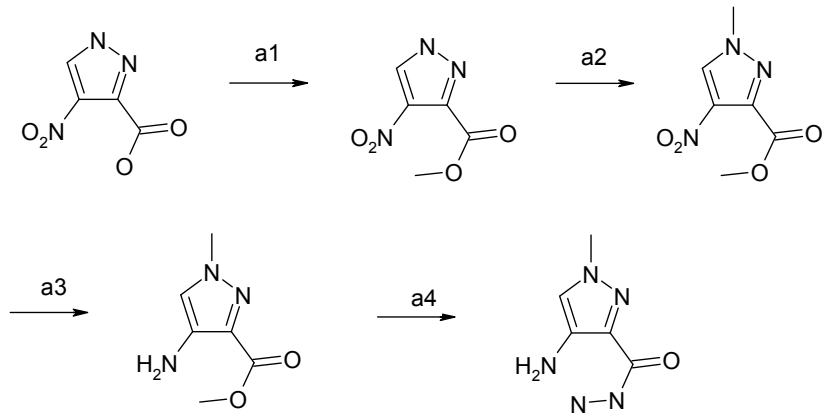
Then acetic acid glacial (2.0 mL) was added and the reaction mixture filtred throught a silica pad. Evaporation afford a crude product which was purified by flash chromatography (Biotage ® 40M, petroleum ether/ Ethyl acetate 99/1); obtained 2.554 g, 12.27 mmol, 54% yield. 1H-NMR (400 MHz, CDCl3): δ ppm 1.60-1.67 (2H, m), 1.67-1.80 (4H, m), 2.46-2.49 (2H, m), 2.65-2.68 (2H, m), 15.93 (1H, s); 19F-NMR (376 MHz, CDCl3): δ ppm -69.87 (CF3).

Synthesis of 2-(2,2,2-Trifluoro-acetyl)-cyclopyranone **7c**

To an ice-cooled suspension of sodium methoxide (5.4 g, 0.1mol) in diethyl ether (150 mL) methyltrifluoroacetate (14.2 g, 0.1 mol) in diisopropylether (20 mL) was added dropwise. Then a solution of tetrahydro-4H-pyran-4-one (10 g, 0.1 mol) was added dropwise and the resulting mixture was allowed to reach room temperature continuing stirring for 20 hours. Then the reaction mixture was quenched with 8 mL acetic acid and the resulting mixture filtered through a pad of silicagel and washed with 150 mL of diethyl ether. Volatiles were evaporated under reduced pressure and the oily residue was distilled on a Kughelhor apparatus. The product was isolated as a colourless liquid (14 g, 71%). 1H-NMR (400 MHz, CDCl3): δ ppm 2.62 (2H, m), 3.91 (2H, m), 4.52 (2H, m), 14.69 (1H, br s); 19F-NMR (376 MHz, CDCl3): δ ppm -74.69 (CF3).

Synthesis of acylhydrazides 11

Synthesis of 4-Amino-1-methyl-1H-pyrazole-3-carboxylic acid hydrazide



Step a1 - 4-Nitro-1H-pyrazole-3-carboxylic acid methyl ester

4-Nitro-1H-pyrazole-3-carboxylic acid (50 g, 318 mmol) was dissolved in methanol (800 mL) and cooled to 0°C. Thionyl chloride (30 mL) was added dropwise to the solution, keeping the temperature at 0°C. The reaction mixture was stirred at room temperature overnight, evaporated to dryness and then toluene (50 mL) was added and evaporated again. The resulting solid was slurried with pentane and filtered to yield the title compound as a white solid (54.9 g, quantitative yield). 1H-NMR (400 MHz, CDCl3): δ ppm 3.99 (3H, s), 7.19 (1H, s), 8.43 (1H, s).

Step a2 - 1-Methyl-4-nitro-1H-pyrazole-3-carboxylic acid methyl ester

To an ice-cooled suspension of 4-nitro-1H-pyrazole-3-carboxylic acid methyl ester (42 g, 248 mmol) in dry tetrahydrofuran (400 mL), sodium hydride (60% in oil, 12 g, 1.2 eq) was added portionwise and the resulting mixture stirred for 30 minutes. Methyl iodide (23.3 mL, 1.5 eq) was added to the mixture and the reaction was stirred at room temperature overnight. The reaction was quenched with water and the product extracted with ethyl acetate, dried over magnesium sulphate and evaporated to dryness under reduced pressure. The resulting orange solid was triturated with pentane, filtered and washed with

diethyl ether to yield the title compound. (37 g, 80%). 1H-NMR (400 MHz, CDCl3): δ ppm 3.99 (3H, s), 4.00 (3H, s), 8.13 (1H, s).

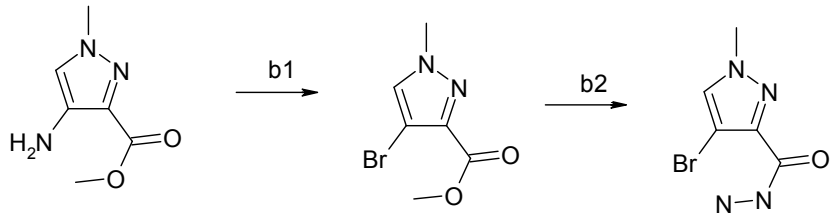
Step a3 - 4-Amino-1-methyl-1H-pyrazole-3-carboxylic acid methyl ester

Palladium on activated carbon (10%, 1 g) and 1-methyl-4-nitro-1H-pyrazole-3-carboxylic acid methyl ester (37 g, 200 mmol) were suspended in absolute ethanol (500 mL) in a 1 L autoclave. The reaction mixture was heated at 30°C under 20 bar hydrogen overnight. Catalyst was filtered off through celite and filtrate evaporated to dryness to afford the title compound (30 g, 96%). 1H-NMR (400 MHz, DMSO-d6): δ ppm 3.74 (3H, s), 3.75 (3H, s), 4.65 (2H, br s), 7.12 (1H, s).

Step a4 - 4-Amino-1-methyl-1H-pyrazole-3-carboxylic acid hydrazide

Hydrazine monohydrate (25 mL) was carefully added to solid 4-amino-1-methyl-1H-pyrazole-3-carboxylic acid methyl ester (10 g, 64 mmol) and the resulting suspension was heated at 50°C for 4 hours. After cooling to room temperature hydrazine was removed by evaporation under reduced pressure and the residue was triturated with diethyl ether and filtered to afford the title compound as an off white solid (9.8 g, 98%). 1H-NMR (400 MHz, DMSO-d6): δ ppm 3.71 (3H, s), 4.23 (2H, br s), 4.56 (2H, br s), 7.07 (1H, s), 8.77 (1H, s).

Synthesis of 4-Bromo-1-methyl-1H-pyrazole-3-carboxylic acid hydrazide



Step b1 - 4-Bromo-1-methyl-1H-pyrazole-3-carboxylic acid methyl ester

To a suspension of 4-amino-1-methyl-1H-pyrazole-3-carboxylic acid methyl ester (2 g, 12.9 mmol) in tetrahydrofuran (20 mL) tert-butylnitrite (2.3 mL, 1.5 eq) was added and the resulting mixture stirred for

30 minutes at RT. Then a solution of copper(II)bromide (4.31g, 1.5 eq) in acetonitrile (5 mL) was added dropwise over 15 minutes. The resulting mixture was stirred at 60ºC for 6 hours. After cooling to room temperature the volatiles were evaporated under reduced pressure and the residue was purified by flash silica chromatography eluting with 30-40% ethyl acetate in pentane. Yield 2.7 g, 38.5%. 1H-NMR (400 MHz, CDCl3): δ ppm 3.94 (3H, s), 3.97 (3H, s), 7.48 (1H, s); m/z 219-221 (M+H)+; retention time = 4.20

Step b2 - 4-Bromo-1-methyl-1H-pyrazole-3-carboxylic acid hydrazide

The same procedure used in step a4 was employed using 4-bromo-1-methyl-1H-pyrazole-3-carboxylic acid methyl ester. Yield 1.6 g, 61%. 1H-NMR (400 MHz, DMSO-d6): δ ppm 3.85 (3H, s), 4.35 (2H, br s), 7.98 (1H, s), 9.23 (1H, s).

Synthesis of sulfonylhydrazides 13

General method from sulfonyl chlorides

A solution of the sulphonyl chloride (27 mmol) in tetrahydrofuran (50 mL) was dripped into a solution of hydrazine monohydrate (68 mmol) in tetrahydrofuran (50 mL) at 0°C with stirring. The reaction mixture was allowed to reach room temperature and was stirred for a further 30 minutes. Volatiles were evaporated under reduced pressure, the residue stirred in water, filtered and washed with n-hexane and diethyl ether to yield the required sulphonylhydrazide. Compounds obtained were used directly into the cyclisation step.

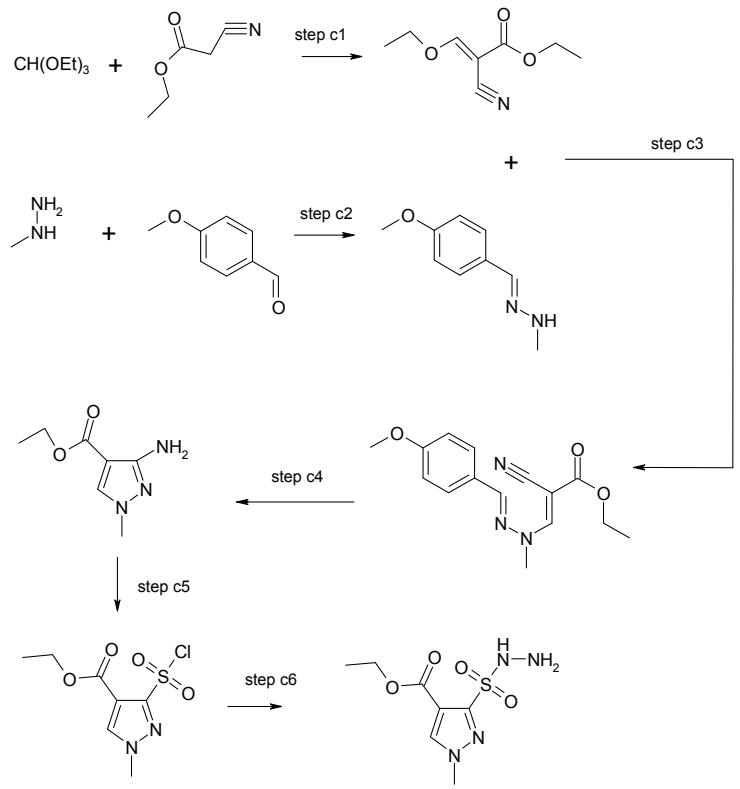
Synthesis of 1-Methyl-1H-imidazole-4-sulfonylhydrazide

Above mentioned general method was employed using commercially available 1-Methyl-1H-imidazole-4-sulfonyl chloride.

Synthesis of 5-Chloro-1,3-dimethyl-1H-pyrazole-4-sulfonylhydrazide

Above mentioned general method was employed using commercially available 5-Chloro-1,3-dimethyl-1H-pyrazole-4-sulfonyl chloride.

Synthesis of 3-hydrazinosulfonyl-1H-pyrazole-4-carboxylic acid ethyl ester



Step c1 - (E/Z)-2-Cyano-3-ethoxy-acrylic acid ethyl ester

Triethyl orthoformate (33.3 ml, 200 mmol) and ethyl cyanoacetate (21.3 ml, 200 mmol) were heated at reflux in acetic anhydride (80 mL) for 5 hours, cooled, evaporated and triturated with n-hexane yielding a pale yellow solid (21.98 g, 65%). This product was used without any further purification in the next step.

Step c2 - N-[1-(4-Methoxy-phenyl)-meth-(E)-ylidene]-N'-methyl-hydrazine

Para-anisaldehyde (27.2 g, 200 mmol) and methyl hydrazine (9.2 g, 200 mmol) were heated at reflux in ethanol (100 mL) for 5 hours, cooled, filtered, evaporated and triturated with n-hexane to yield the title compound (27.88 g, 85%). This product was used in the next step without any further purification.

Step c3 - (E/Z)-2-Cyano-3-{N'-[1-(4-methoxy-phenyl)-meth-(E)-ylidene]-N-methyl-hydrazino}-acrylic acid ethyl ester

(E/Z)-2-Cyano-3-ethoxy-acrylic acid ethyl ester (20.2 g, 120 mmol) and N-[1-(4-Methoxy-phenyl)-meth-(E)-ylidene]-N'-methyl-hydrazine (19.68 g, 120 mmol) were heated at reflux in toluene (300 mL) for 1 hour. After cooling the reaction mixture to room temperature a yellow precipitate formed, which was filtered and washed with diethyl ether yielding the title compound (23 g, 75%). This product was used in the next step without any further purification.

Step c4 - 3-Amino-1H-pyrazole-4-carboxylic acid ethyl ester

(E/Z)-2-Cyano-3-{N'-[1-(4-methoxy-phenyl)-meth-(E)-ylidene]-N-methyl-hydrazino}-acrylic acid ethyl ester (23 g, 89.5 mmol) was dissolved in ethanol (100 mL) and cHCl (6N, 15 mL) was added to the solution. The reaction mixture was heated at reflux for 1 hour, cooled, solvent were evaporated by reduced pressure and the residue was dissolved in methylene chloride (100 mL). The organic solution was washed with saturated sodium bicarbonate solution, dried, and evaporated. The residue was stirred in hot diethyl ether and a precipitate formed which was filtered and washed with diethyl ether to give the title compound (11.12 g, 74%). 1H-NMR (400 MHz, CDCl3): δ ppm 1.32 (3H, t, J = 7.1 Hz), 3.70 (3H, s), 4.26 (2H, q, J = 7.1 Hz), 4.39 (2H, br s), 7.55 (1H, s); m/z 170 (M+H)+; retention time = 3.18

Step c5 - 3-Chlorosulfonyl-1H-pyrazole-4-carboxylic acid ethyl ester

3-Amino-1H-pyrazole-4-carboxylic acid ethyl ester (10 g, 64 mmol) was dissolved in cHCl (6N, 40 mL) at 30°C and then cooled at 5°C. A precipitate was formed. To this stirring suspension, a solution of sodium nitrite (4.7 g, 68 mmol) in water (10 mL) was added dropwise under the surface of the solution keeping the temperature below 5°C. After the addition was complete a solution was formed and this was stirred at 5-10°C for 1 hour and then filtered. A solution of sulphur dioxide was prepared bubbling the gas into acetic acid (64 mL) until the solution gained at least 20 grams in weight and then copper(II)chloride (3.2 g, 23 mmol) was add added. The solution containing the diazonium salt was dripped into the saturated sulphur dioxide solution under a nitrogen atmosphere. The resulting reaction

mixture was stirred for 10 minutes and then dichloromethane (100 mL) was added with stirring to dissolve solids, followed by water (200 mL). The organic layer was separated, washed with water, dried, passed through a short silica plug, evaporated under reduced pressure and the residue triturated with n-hexane to yield the title compound (7.46 g, 50%). Purity by LC was 81%; m/z 239-241 (M+H)+, 237-239 (M-H)-, retention time = 4.76

Step c6 - 3-Hydrazinosulfonyl-1H-pyrazole-4-carboxylic acid ethyl ester

A solution of 3-chlorosulfonyl-1H-pyrazole-4-carboxylic acid ethyl ester (6.5 g, 27 mmol) in tetrahydrofuran (50 mL) was dripped into a solution of hydrazine monohydrate (2.7 g, 68 mmol) in tetrahydrofuran (50 mL) at 0°C with stirring. The reaction mixture was allowed to reach room temperature and was stirred for a further 30 minutes. Volatiles were evaporated under reduced pressure, the residue stirred in water, filtered and washed with n-hexane and diethyl ether to yield the title compound (4.76 g, 75%). 1H-NMR (400 MHz, DMSO-d6): δ ppm 1.28 (3H, t, J = 7.1 Hz), 4.25 (1H, q, J = 7.1 Hz), 4.31 (2H, br s), 8.29 (1H, s), 8.50 (1H, s), 14.04 (1H, br s).

Synthesis of compounds 4 and 8

General method for the synthesis of compound 4 and 8

To an ice-cooled stirred suspension of 2-(2,2,2-trifluoro-acetyl)-cyclo derivative (10 mmol) in ethanol (20 mL) piperidine (11 mmol) was added followed by 3Å molecular sieves (1 g). This mixture was left stirring at 5-10°C for 30 minutes and then added to a suspension of carboxylic acid hydrazide or sulfonyl hydrazide derivative (11 mmol) in ethanol (5 mL) allowing the resulting mixture to warm to room temperature. Stirring was continued overnight at room temperature. The resulting mixture was evaporated and the residue purified by silicagel eluting with dichloromethane (20 mL) and dichloromethane/methanol (9:1). Some products were further purified by SCX cartridge extraction to yield the final product in >95% purity.

Synthesis of (4-Bromo-1-methyl-1H-pyrazol-3-yl)-(3-hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-methanone – **4b**

General procedure was employed using **7a** and 4-Bromo-1-methyl-1H-pyrazole-3-carboxylic acid hydrazide. Yield 0.22 g, 56%. 1H-NMR (400 MHz, CD3OD): δ ppm 1.43-1.31 (1H, m), 1.61-1.49 (1H, m), 1.78-1.67 (1H, m), 2.07-1.89 (3H, m), 2.25-2.34 (1H, m), 2.60-2.52 (1H, m), 3.31-3.27 (1H, m), 3.92 (3H, s), 7.75 (1H, s); 19F-NMR (376 MHz, CD3OD): δ ppm -80.88 (CF3); m/z 395-397 (M+H)+; retention time = 3.03

Synthesis of (3-Hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-pyridin-4-yl-methanone - **4c**

General procedure was employed using **7a** and Pyridine-4-carbohydrazide. Yield 0.023g, 15%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.33-1.55 (2H, m), 1.73 (1H, ddd, J = 25.8, 13.0, 3.3 Hz,), 1.95-2.10 (3H, m), 2.25 (1H, td, J = 13.5, 5.5 Hz), 2.61-2.66 (1H, m), 3.15 (1H, dd, J = 12.4, 6.2 Hz), 6.34 (1H, br s), 7.74-7.76 (2H, m), 8.75-8.76 (2H, m). 19F-NMR (376 MHz, CDCl3): δ ppm -82.21 (CF3); m/z 314 (M+H)+; retention time = 2.15

Synthesis of (3-Hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-(1H-pyrrol-2-yl)-methanone - **4d**

General procedure was employed using **7a** and 1H-Pyrrole-2-carbohydrazide. Yield 0.099g, 65%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.40-1.57 (2H, m), 1.71 (1H, qd, J = 12.9, 3.2 Hz), 1.96-2.12 (3H, m), 2.27 – 2.39 (1H, m), 2.73-2.79 (1H, m), 3.10 (1H, dd, J = 12.6, 6.1 Hz), 6.30-6.31 (1H, m), 6.74 (1H, s), 7.27-7.29 (1H, m). 19F-NMR (376 MHz, CDCl3): δ ppm -81.92 (CF3); m/z 302 (M+H)+; retention time = 3.53

Synthesis of (3-Hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-(6-methyl-pyridin-3-yl)-methanone - **4e**

General procedure was employed using **7a** and 6-Methyl-nicotinoyl-hydrazide. Yield 0.032g, 20%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.32-1.54 (2H, m), 1.73 (1H, ddd, J = 25.8, 13.0, 3.2 Hz), 1.94-2.08 (3H, m), 2.24 (1H, td J = 13.4, 5.4 Hz), 2.62 (3H, m), 3.14 (1H, dd, J = 12.4, 6.2 Hz), 6.55 (1H, s), 7.22 (1H, d, J = 8.1 Hz), 8.09 (1H, dd, J = 8.1, 2.3 Hz), 9.01 (1H, d, J = 2.1 Hz). 19F-NMR (376 MHz, CDCl3): δ ppm -81.92 (CF3); m/z 328 (M+H)+; retention time = 2.22

Synthesis of (2-Amino-thiazol-4-yl)-(3-hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-methanone - **4f**

General procedure was employed using **7a** and 2-Amino-thiazole-4-carboxylic acid hydrazide. Yield 0.87g, 30 %. 1H-NMR (400 MHz, DMSO): δ ppm 1.21-1.34 (1H, m), 1.45-1.65 (2H, m), 1.79-1.83 (2H, m), 1.93-1.98 (1H, m), 2.30-2.40 (1H, m), 2.57-2.61 (1H, m), 3.28-3.33 (1H, m), 7.83 (1H, s), 8.20(1H, br s), 8.20(1H, br s). 13C-NMR (100 MHz, DMSO-d6): δ ppm 23.3, 25.2, 25.6, 27.6, 51.7, 92.4 (q, J=33 Hz), 119.3, 122.6, 125.4, 155.2, 162.3, 169.2. 19F-NMR (376 MHz, DMSO): δ ppm -76.58 (CF_3, s); m/z 335 (M+H)+; retention time = 2.03

Synthesis of (4-Amino-1-methyl-1H-pyrazol-3-yl)-(3-hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-i Synthesis of ndazol-2-yl)-methanone – **4g**

General procedure was employed using **7a** and 4-amino-1-methyl-1H-pyrazole-3-carboxylic acid hydrazide. Yield 2.15 g, 65%. 1H-NMR (400 MHz, DMSO-d6): δ ppm 1.32-1.20 (1H, m), 1.66-1.45 (2H, m), 1.86-1.78 (2H, br m), 1.97-1.89 (1H, m), 2.32 (1H, dt, J = 14.2, 5.4 Hz), 2.54-2.51 (1H, m), 3.26 (1H, dd, J = 12.3 Hz), 3.75 (3H, s), 4.46 (2H, br s), 7.11 (1H, s), 7.87 (1H, s). 19F-NMR (376 MHz, DMSO-d6): δ ppm -77.50 (CF3); m/z 332 (M+H)+; retention time = 1.58

Synthesis of (2-Amino-oxazol-4-yl)-(3-hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-methanone - **4h**

General procedure was employed using **7a** and 2-Amino-oxazole-4-carboxylic acid hydrazide. Yield 0.060g, 9%.1H-NMR (400 MHz, DMSO-d6): δ ppm 1.20-1.31 (1H, m), 1.41-1.62 (2H, m), 1.76-1.82

(2H, m), 1.91-1.95 (1H, m), 2.30 (1H, td, J = 13.8, 5.3 Hz), 2.61 (1H, dd, J = 10.5 Hz), 3.21 (1H, dd, J = 11.7, 6.1 Hz), 6.80 (2H, s), 7.81 (1H, s), 8.05 (1H, s). 19F-NMR (376 MHz, DMSO-d6): δ ppm -76.36 (CF3); m/z 319 (M+H)+; retention time = 2.35

Synthesis of (4-Ethylamino-1-methyl-1H-pyrazol-3-yl)-(3-hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-methanone - **4i**

To a solution of (4-amino-1-methyl-1H-pyrazol-3-yl)-(3-hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)-methanone **4g** (0.1 g, 0.3 mmol) in dichloromethane (anhydrous, 5 mL) molecular sieves (0.1 g) were added followed by acetaldehyde (17 µL, 0.3 mmol). The reaction mixture was left stirring for 3 hours, then sodium triacetoxyborohydride (0.064 g, 0.3 mmol) was added in one portion and stirring was continued overnight at room temperature. After addition of saturated sodium bicarbonate solution (5 mL), the organic phase was separated, dried and evaporated under reduced pressure. The residue was purified by preparative HPLC. Yield 0.038 g, 36%. 1H-NMR (400 MHz, DMSO-d6): δ ppm 1.13 (3H, t, J = 7.1 Hz), 1.35-1.17 (1H, m), 1.68-1.42 (2H, m), 1.88-1.75 (2H, m), 2.00-1.88 (1H, m), 2.32 (1H, dt, J = 13.6, 5.4 Hz), 2.56-2.51 (1H, m), 2.93 (2H, q, J = 7.0 Hz), 3.25 (1H, dd, J = 12.0 Hz), 3.78 (3H, s), 4.69 (1H, br s), 7.22 (1H, s), 7.82 (1H, s); 19F-NMR (376 MHz, DMSO-d6): δ ppm -77.25 (CF_3); m/z 360 (M+H)+; retention time = 2.22

Synthesis of (3-Hydroxy-3-trifluoromethyl-3a,4,5,6,7,8-hexahydro-3H-cycloheptapyrazol-2-yl)-(6-methyl-pyridin-3-yl)-methanone - **4j**

General procedure was employed using **7b** and 6-Methyl-nicotinoyl-hydrazide. Yield 0.08g, 47%. 1H-NMR (400 MHz, $CDCl_3$): δ ppm 1.40-1.57 (2H, m), 1.61-1.89 (5H, m), 2.03-2.11 (1H, m), 2.53 - 2.59 (1H, m), 2.62 (3H, s), 3.31-3.35 (1H, m), 6.61 (1H, br s), 7.22 (1H, d, J = 5.1 Hz), 8.09 (1H, dd, J = 8.1, 2.3 Hz), 9.02 (1H, d, J = 1.9 Hz). 19F-NMR (376 MHz, CDCl3): δ ppm -76.86 (CF3); m/z 342 (M+H)+; retention time = 3.3

Synthesis of (3-Hydroxy-3-trifluoromethyl-3a,4,5,6,7,8-hexahydro-3H-cycloheptapyrazol-2-yl)-pyridin-4-yl-methanone - **4k**

General procedure was employed using **7b** and Pyridine-4-carbohydrazide. Yield 0.039g, 24%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.40-1.57 (2H, m), 1.66-1.91 (5H, m), 2.07-2.11 (1H, m), 2.55-2.59 (2H, m), 3.32-3.35 (1H, m), 2.62 (3H, s), 3.31-3.35 (1H, m), 6.40 (1H, s), 7.68-7.71 (2H, m), 8.72-8.76 (1H, m). 19F-NMR (376 MHz, DMSO-d6): δ ppm -76.86 (CF3); m/z 328 (M+H)+; retention time = 3.27

Synthesis of (3-Hydroxy-3-trifluoromethyl-3a,4,5,6,7,8-hexahydro-3H-cycloheptapyrazol-2-yl)-(1H-pyrrol-2-yl)-methanone - **4l**

General procedure was employed using **7b** and 1H-Pyrrole-2-carbohydrazide. Yield 0.079g, 50%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.40-1.77 (4H, m), 1.79-1.91 (3H, m), 2.08-2.14 (1H, m), 2.61 - 2.75 (2H, m), 3.25-3.38 (1H, m), 6.29-6.31 (1H, m), 6.98-7.00 (1H, m), 7.26-7.28 (1H, m), 10.21 (1H, br s). 19F-NMR (376 MHz, CDCl3): δ ppm -82.93 (CF3); m/z 316 (M+H)+; retention time = 3.87

Synthesis of (5-Bromo-pyridin-3-yl)-(3-hydroxy-3-trifluoromethyl-3a,4,6,7-tetrahydro-3H-pyrano[4,3-c]pyrazol-2-yl)-methanone - **4m**

General procedure was employed using **7c** and 5-Bromo-nicotinic acid hydrazide XX. Yield 0.088g, 37%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.57-2.70 (2H, m), 3.34 - 3.44 (2H, m), 3.64-3.69 (1H, t), 4.25 - 4.32 (2H, m), 6.23 (1H, br s), 8.32 - 8.33 (1H, m), 8.79 - 8.80 (1H, m), 9.02 – 9.03 (1H, m). 19F-NMR (376 MHz, CDCl3): δ ppm -80.91 (CF3); m/z 394-396 (M+H)+; retention time = 2.93

Synthesis of (3-Hydroxy-3-trifluoromethyl-3a,4,6,7-tetrahydro-3H-pyrano[4,3-c]pyrazol-2-yl)-pyridin-3-yl-methanone - **4n**

General procedure was employed using **7c** and Nicotinic hydrazide. Yield 0.014g, 6%. 1H-NMR (400 MHz, CDCl3): δ ppm 2.55 - 2.70 (2H, m), 3.35-3.45 (1H, m), 3.65-3.71 (1H, t), 4.24 - 4.33 (1H, m),

6.29 (1H, s), 7.38 -7.41 (1H, m), 8.17 - 8.21 (1H, m), 8.73 - 8.75 (1H, m), 9.11 (1H, s). 19F-NMR (376 MHz, CDCl3): δ ppm -81.42 (CF3); m/z 316 (M+H)+; retention time = 1.68

Synthesis of 3-(3-Hydroxy-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-indazole-2-sulfonyl)-1H-pyrazole-4-carboxylic acid ethyl ester - **8a**

General procedure was employed using **7a** and 3-hydrazinosulfonyl-1H-pyrazole-4-carboxylic acid ethyl ester. Yield 0.055 g, 13%. 1H-NMR (400 MHz, DMSO-d6): δ ppm 1.27 (3H, t, J = 7.1 Hz), 1.42-1.30 (1H, m), 1.62-1.42 (1H, m), 1.99-1.62 (4H, m), 2.27 (1H, dt, J = 13.6 Hz), 2.49-2.40 (1H, m), 3.24 (1H, dd, J = 12.0 Hz), 4.23 (2H, dq, J = 7.1 Hz), 7.83 (1H,br s), 8.47 (1H, s); 19F-NMR (376 MHz, DMSO-d6): δ ppm -80.14 (CF3); m/z 411 (M+H)+; retention time = 2.97

Synthesis of 2-(1-Methyl-1H-imidazole-4-sulfonyl)-3-trifluoromethyl-3,3a,4,5,6,7-hexahydro-2H-indazol-3-ol - **8b**

General procedure was employed using **7a** and 1-Methyl-1H-imidazole-4-sulfonylhydrazide. Yield 0.017 g, 16%. 1H-NMR (400 MHz, CDCl3): δ ppm 1.10 - 1.20 (1H, m), 1.40 - 1.49 (1H, m), 1.54 – 1.63 (1H, m), 1.90 - 1.97 (2H, m), 2.11 - 2.19 (1H, m), 2.58 - 2.62 (1H, m), 3.09 – 3.14 (1H, m), 3.79 (3H, s), 7.54 (1H, br s), 7.59 (1H, br s). 19F-NMR (376 MHz, CDCl3): δ ppm -80.64 (CF3); m/z 353 (M+H)+; retention time = 2.42

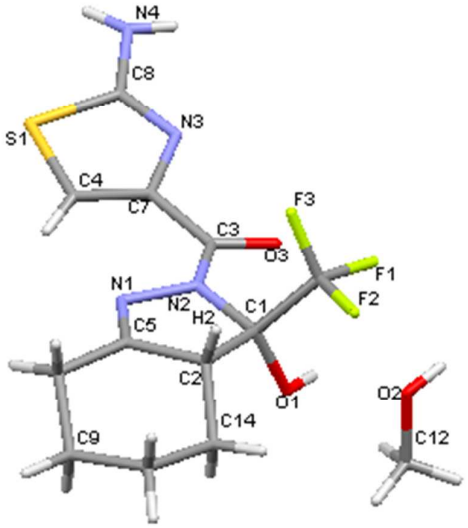
Synthesis of 2-(5-Chloro-1,3-dimethyl-1H-pyrazole-4-sulfonyl)-3-trifluoromethyl-2,3,3a,4,6,7-hexahydro-pyrano[4,3-c]pyrazol-3-ol - **8c**

General procedure was employed using **7c** and 5-Chloro-1,3-dimethyl-1H-pyrazole-4-sulfonylhydrazide. Yield 0.017 g, 16%. 1H-NMR (400 MHz, CDCl3): δ ppm 2.46 (3H, m), 2.51 - 2.65 (2H, m), 3.31 - 3.41 (2H, m), 3.56 – 3.62 (1H, t), 2.83 (3H, m), 4.17 - 4.24 (2H, m), 4.71 (1H, br s). 19F-NMR (376 MHz, CDCl3): δ ppm -80.91 (CF3); m/z 403 (M+H)+; retention time = 2.45

Cristal structure of compound 4f

Graphic representation of compound **4f** co-crystallized with a methanol molecule.

The following crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 928378.

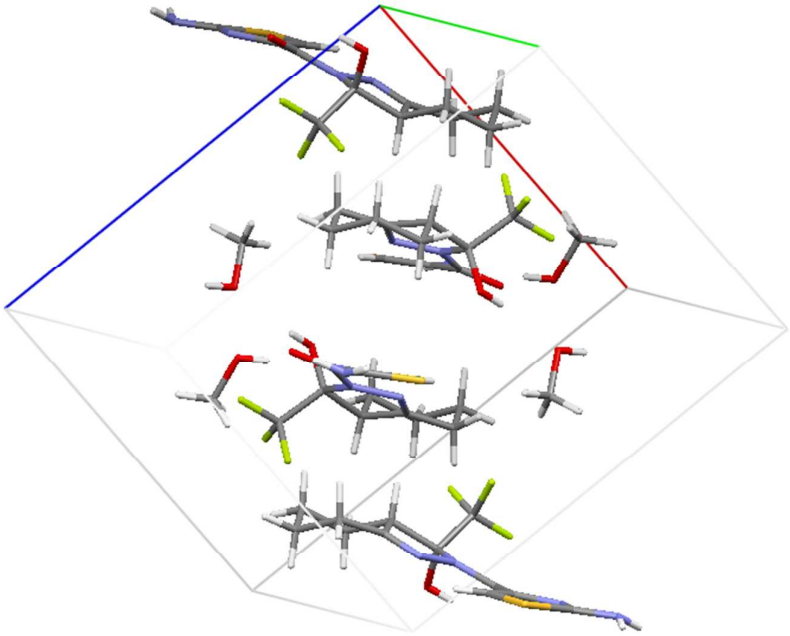


Unit cell parameters: a 11.1740(10) b 10.6050(10) c 13.8900(10) beta 97.920(10)

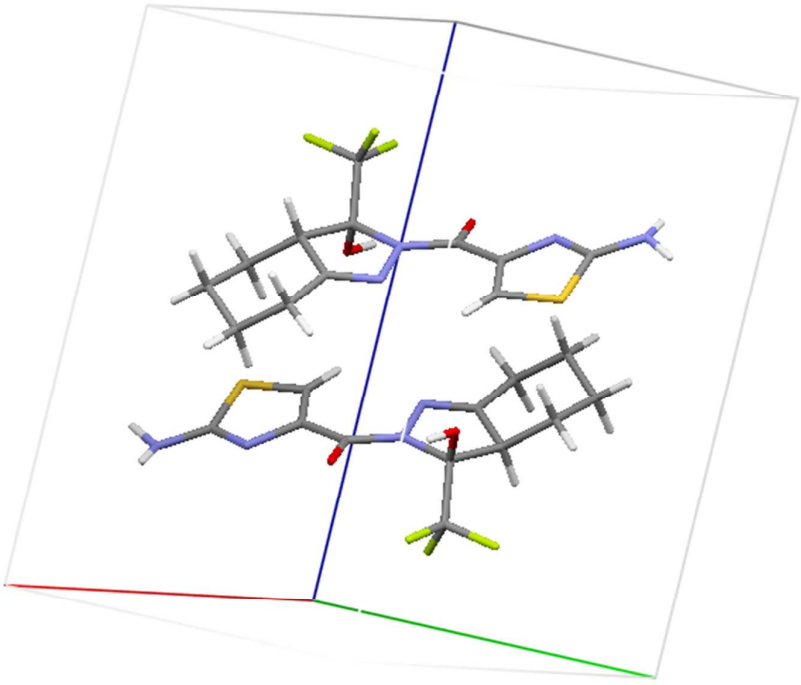
space group $P2_1/n$

Number of molecules per cell Z=4

Graphic representation of a cell packing.



Both enantiomers are present in the cell as shown in the following representation:



CEREP Panel on compound 4b

The mean values (2 repetitions) for the effects of **4b** tested at the concentration of 2.0E-05M.

Binding Assay	% inhibition of control specific binding	Enzyme Assay	% inhibition of control specific binding
A1 *(h)*	19	PDE1	16
A_{2A} *(h)*	12	PDE2 *(h)*	5
A_3 *(h)*	2	PDE3 *(h)*	-1
α_1 (non-selective)	6	PDE4 *(h)*	-4
α_2 (non-selective)	13	PDE5 *(h)*	2
β_1 *(h)*	3	Caspase-3 *(h)*	-3
β_2 *(h)*	-3	Caspase-6 *(h)*	25
AT_1 *(h)*	-26	Cathepsin D *(h)*	0
AT_2 *(h)*	0	Abl kinase *(h)*	-4
BZD (central)	9	Akt1/PKBα *(h)*	-3
B_1 *(h)*	-6	AMPKα	-3
B_2 *(h)*	-9	BMX kinase *(h)* (Etk)	3
CB_1 *(h)*	3	Brk *(h)*	-7
CB_2 *(h)*	3	CaMK2α *(h)*	-5
CCK_A *(h)* (CCK_1)	-5	CaMK4 *(h)*	-10
CCK_B *(h)* (CCK_2)	-10	CDC2/CDK1 *(h)* (cycB)	-5
CRF_1 *(h)*	-57	CDK2 *(h)* (cycE)	-7
D_1 *(h)*	-7	CHK1 *(h)*	3
D_{2S} *(h)*	3	CHK2 *(h)*	6
D_3 *(h)*	6	c-Met kinase *(h)*	-1
$D_{4.4}$ *(h)*	5	CSK *(h)*	-2
ET_A *(h)*	-1	EphB4 kinase *(h)*	0
ET_B *(h)*	-5	ERK_1 *(h)*	4
GABA (non-selective)	13	ERK_2 *(h)* ($P42^{mapk}$)	4
AMPA	3	FGFR2 kinase *(h)*	-5
Kainate	19	FGFR4 kinase *(h)*	-7
NMDA	4	FLT-1 kinase *(h)* (VEGFR1)	3
H_1 *(h)*	0	FLT-3 kinase *(h)*	5
H_2 *(h)*	5	Fyn kinase *(h)*	0
H_3 *(h)*	9	IGF1R kinase *(h)*	-14
I_1	-1	IRK *(h)* (InsR)	-3
I_2	4	JNK 2 *(h)*	-4
LTB_4 *(h)* (BLT_1)	0	KDR kinase *(h)* (VEGFR2)	-4
LTD_4 *(h)* ($CysLT_1$)	6	Lck kinase *(h)*	-1
MC_4 *(h)*	-10	Lyn A kinase *(h)*	-4
M (non-selective)	-9	MAPKAPK2 (h)	-2
NK_1 *(h)*	-3	MEK1/MAP2K1 *(h)*	-11
NK_2 *(h)*	2	p38α kinase *(h)*	5
NK_3 *(h)*	7	p38δ kinase *(h)*	-3
Y (non-selective)	2	p38γ kinase *(h)*	-2

N (neuronal) (α-BGTX-insensitive) (α4β2)	-6	PDGFRβ kinase *(h)*	9
Opioid (non-selective)	-2	PDK1 *(h)*	4
ORL1 *(h)* (NOP)	-5	PKA *(h)*	-2
PPARγ *(h)*	33	PKCα *(h)*	
PCP	1	PKCβ1 *(h)*	-5
EP_4 *(h)*	8	PKCβ2 *(h)*	0
IP *(h)* (PGI_2)	-3	PKCγ *(h)*	2
P2X	-17	Ret kinase *(h)*	-24
P2Y	11	ROCK1 *(h)*	-2
5-HT (non-selective)	-6	ROCK2 *(h)*	0
σ (non-selective)	2	RSK2 *(h)*	0
Glucocorticoid *(h)* (GR)	7	Src kinase *(h)*	-11
Estrogen *(h)* (ER)	-3	Syk *(h)*	0
Progesterone *(h)* (PR)	19	TRKA *(h)*	19
Androgen *(h)* (AR)	5	Acetylcholinesterase *(h)*	-1
TRH_1 *(h)*	4	COMT (Catechol- O-methyl transferase)	7
V_{1a} *(h)*	-3	GABA transaminase	-1
V_2 *(h)*	-6	MAO-A *(h)*	-9
Ca^{2+} channel (L, DHP site)	-19	MAO-B *(h)*	-1
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)	-8	PNMT (Phenylethanolamine- N-methyl transferase)	7
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	-7	Tyrosine hydroxylase	-11
K^+_{ATP} channel	9	ATPase (Na^+/K^+)	0
K^+_V channel	-6		
SK^+_{Ca} channel	-4		
Na^+ channel (site 2)	24		
Cl^- channel (GABA-gated)	14		
NE transporter *(h)*	6		
DA transporter *(h)*	12		
GABA transporter	5		
Choline transporter *(h)* (CHT1)	15		
5-HT transporter *(h)*	-13		

Section 2: Assay protocols

CRE-LUC

T-Rex™-293 cells purchased from Life Technologies (Paisley, UK), were engineered to stably express as reporter gene luciferase under the control of a promoter containing the cAMP Responsive

Element (CRE) sequences (CRE-Luc) and, in an tetracycline- inducible manner, full-length mutant Huntingtin (HTT) with a stretch of 138 glutamine residues (T-Rex-Q138-CRE-RL1). Addition of doxycycline to these cells induces expression of mutant HTT leading to transcriptional dysregulation and, in consequence, reduction of expression of luciferase which is measured as a decrease of luminescence. Cells were maintained in DMEM (Invitrogen GIBCO, 21969-035) containing 10% Foetal Bovine Serum (FBS) Certified US Origin (Invitrogen GIBCO, 16000-044), 1% Glutamax (Cat 35050-038) and 1% Penicillin/Streptomycin (Cat 15140-122), with addition of 0.25 mg/ml hygromycin B (Invivogen, ant-hm-5), 5ug/ul of Blasticidin (Invivogen, ant-bl-1) and 50ug/ul of Zeocin (Invivogen, ant-zn-1). Cells were maintained at 37°C in a 95% air-5% carbon dioxide fully humidified environment, and used for up to 5 passages after thawing. T-Rex-Q138-CRE-RL1 cells were counted with a Neubauer chamber and 10,000 cells/100ul DMEM/10% FBS/well were transferred into the wells of a 96-well polylysine-coated plate (CulturPlate-96, white, sterile, with lid, 96 well Perkin Elmer, 6005688). Compounds (10mM stock solutions) to be screened were dissolved in DMSO (0.5% final DMSO concentration) and tested for activity at a single concentration of 20 micromolar followed by a concentration-response analysis for active compounds with concentrations ranging from 50 micromolar to 10 nanomolar. Compounds were tested under both, doxycycline-induced (1µg/ml) and doxycycline-uninduced conditions. Culture media with 0.5% DMSO (final concentration) was used as negative control. Plates were incubated for 72 h at 37°C in a 95% air-5% carbon dioxide atmosphere. After incubation, supernatant was replaced by 40ul DMEM w/o red phenol (Invitrogen GIBCO, 31053-028) supplemented with 0.5uM 3-isobutyl-1-methylxanthine (IBMX; SIGMA, I7018-100MG) and 1uM forskolin (SIGMA, F6886-10MG) final concentrations. Plates were then incubated for 5h at 37°C in a 95% air-5% carbon dioxide atmosphere followed by addition of 40 µl/well luciferase SteadyLite Plus™ substrate (Perkin Elmer®, 6016759) and incubation for 40 minutes at 37°C to develop luciferase activity. A luminescence plate reader (Mithras LB 940, Berthold) was used for measurement of luminescence intensity of the samples using a counting time of 0.1sec. In order to eliminate compounds with a non-specific activity ('false positives') all compounds found to be active in this assay

were also tested in recombinant T-RexTM-293 cells stably expressing CRE-luciferase (T-Rex-Zeo-Cre-Luc) but not mutant HTT under essentially identical experimental conditions.

Exon-1- Htt rat primary striatal neuron assay

Preparation of primary striatal neurons

Cultures were obtained after dissection of brain ganglionic eminence obtained from rat embryos at E15, after their removal from a pregnant CD Sprague Dawley rat humanely euthanized in accordance to law. Cells were mechanically dissociated in a solution of BSA (1 mg/mL) in DMX medium with a tip-flamed Pasteur pipette. The supernatant containing dissociated cells was removed after letting cell aggregates sediment to the bottom. Again, remaining cell aggregates were dissociated in a DMX/BSA solution, collected and added to the previous cell suspension. A 5% BSA-PBS solution was added slowly to the bottom of the tube containing the cell suspension, allowing the formation of a BSA gradient able remove any debris before plating. The suspension was centrifuge at 300g for 8 min at RT. Cells were resuspended in Neurobasal medium containing B27, 100 mU/mL Penicillin /Streptomycin, L-Glutamine (0.5 mM), KCl (15 mM), plated in poly-L-lysine (30-100 kD)-coated multi-well plates at a density of 150K cells/cm2 and placed in cell incubator set at 37°C with a 5% CO2 atmosphere.

Lentiviral production and infection of cultured striatal neurons

In vitro models of Huntington's disease were implemented using lentiviral (LV) vectors as described in Zala (Zala et al., 2005). These models involve the LV-mediated overexpression of N-terminal 171 amino acid fragments of wild-type Htt (with 18 glutamine repeats, 18Q) or mutant Htt (with 82 glutamine repeats, 82Q) under control of a tetracycline response element (TRE) promoter (TRE-htt171-18Q/82Q) in striatal neuronal cultures.

LVs were produced in human embryonic kidney 293T (HEK293T) cells as described previously {Zala, 2005 #7} {Perrin 2007}. Viral stocks were resuspended in PBS - 1% of BSA and matched for particle content to 1,5K ng/ml of p24 antigen as measured by ELISA (RETROtek; Gentaur).

For LV-mediated protein expression, cultures were infected 24h after seeding. At day 4, half of the medium was replaced with the fresh medium supplemented with/without testing compounds in 2X concentrations. Treatments with compounds were performed once a week thereafter by adding fresh medium with compounds at 1X concentrations. The strong promoter constructs htt-N171-82Q- but not htt-N171-18Q (high expression, 5-10 times endo¬ge¬nous) expo¬sed cells developed intracellular Htt inclusions, resulting in a polyQ-dependent cell death within 2-4 weeks in vitro.

Neuronal survival assessment by NeuN-positive cell counting

Primary cultured striatal neurons were fixed with 4% paraformaldehyde in PBS for 15 min at RT. After fixation, cells were washed with PBS and blocked with 10% normal goat serum (NGS, Invitrogen) in PBS containing 0.1% Triton X-100 (Sigma). An anti-NeuN monoclonal antibody (Chemicon) was diluted 1:500 in PBS with 5% NGS and 0.1% Triton X-100, and incubated with the cells overnight at 4°C. After appropriate PBS-washing, cells were incubated for 1h with goat-anti-mouse Cy3-conjugated antibody (1:1000 dilution, Invitrogen) in PBS with 1% NGS, followed by PBS washing and mounting on coverglass. Images were acquired employing an automated high-throughput imaging system BD Pathway 855 Bioimager (Becton Dickinson) using a 4x objective. Image processing and cell counting was performed using the ImageJ software. Thresholds for fluorescence intensity, size and circularity of fluorescent objects were set to uniform parameters for each experiment. Two-tailed Student's t-test was used for two-group comparisons.

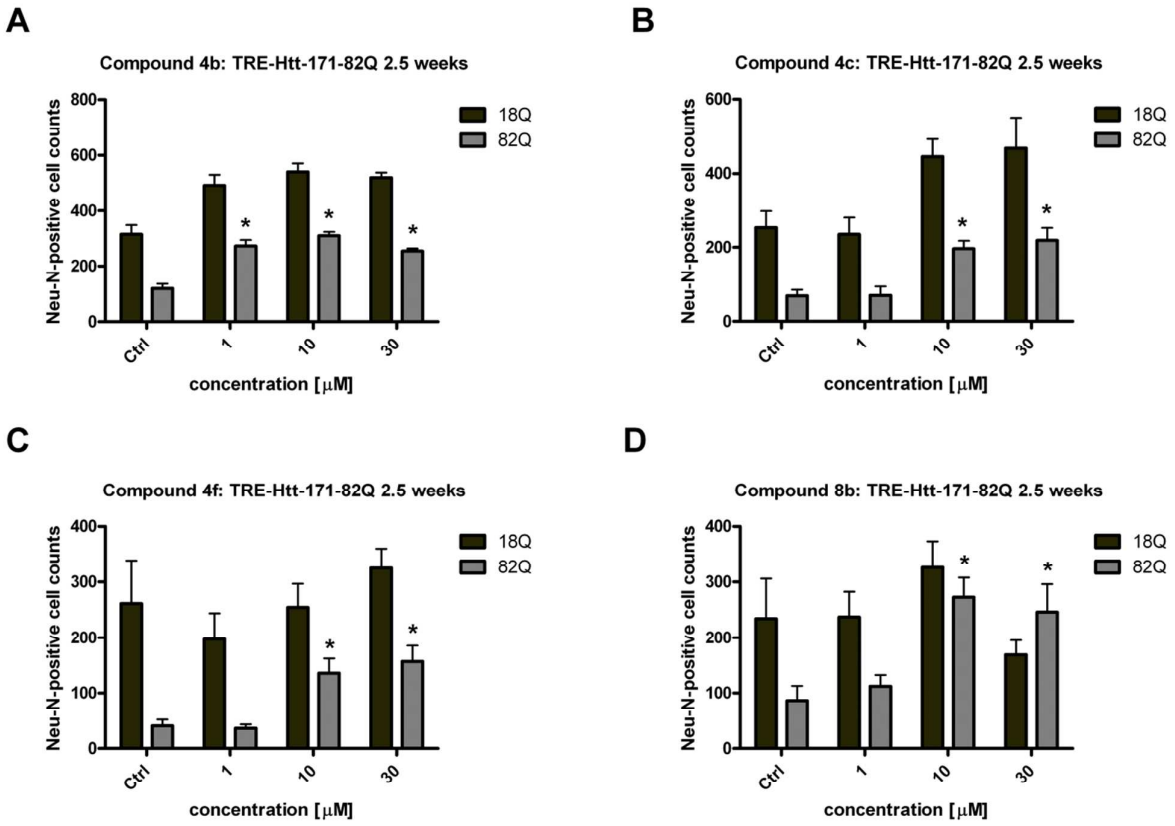


Figure. S1. Effect of compound 4b (A), 4c (B), 4f (C) and 8b (D) on LV-Htt (wt-18Q and mutant-82Q) infected cortical neurons. To note that all compounds were able to counteract neuronal death induced by mutated Htt (82Q). *$p<0.05$ Student's t-test vs 82Q Ctrl group.

PC12 ex1-mut-Htt expressing cell line assay

The cell system used was obtained from Rubinsztein's laboratory.[ii] Cells were the PC-12 7210 (Exon-1 mut74Q) (PC12-74Q) stably expressing the GFP tagged-Exon-1 fragment of the HD gene under control of tetracycline (Tet-on) system (inducible m-HTT). Construct includes a 74 CAG (PolyQ) repeat expansion which, once expressed, is toxic to the cells. Briefly, cells were seeded in a 96 wells Poly-D-Lysine (MW 70-150kD) (Sigma, MO, USA) pre-coated plate at a density of 45k cells/100mL medium/wells in DMEM containing 2% HS, 1% FBS, 100 mU/mL Penicillin /Streptomycin and 1% of Glutamax, then grown for 24h before the experiment in a cell incubator set at 10% CO2, 37°C. The day of the experiment the same medium was added to obtain a final concentration of serums of 0.66% HS,

0.33% FBS, as well as Doxycycline, or not, (1mg/mL), and contained SEN065 (to obtain the final concentrations described in the results) except in the control (CTR) groups. Final concentration of DMSO in all treatments and controls was 0.3%. 72h later, the cell death, induced by the m-HTT, was measured as level of lactic dehydrogenase (LDH) released from cells in the medium using a LDH-mix cytotoxicity test kit (Roche, Switzerland), absorbance was measured at 490 (reading) and 720 nm (blank) with a spectrophotometer (Benchmark Plus, BioRad, USA). LDH data were analyzed with Two-Way ANOVA followed by Tukey-Kramer test for multiple comparisons.

Section 3: Procedures for ADME and PK characterization

Solubility Assay

Standard and sample solutions were prepared from a 10 mM DMSO stock solution using an automated dilution procedure. For each compound, three solutions were prepared: one to be used as standard and the other two as test solutions. Standard: 250 μM standard solution in acetonitrile/buffer, with a final DMSO content of 2.5% (v/v). Test sample for pH 3.0: 250 μM sample solution in acetic acid 50 mM, pH = 3, with a final DMSO content of 2.5% (v/v). Test sample for pH 7.4: a 250 μM sample solution in ammonium acetate buffer 50 mM, pH = 7.4, with a final DMSO content of 2.5% (v/v). The 250 μM product suspensions/solutions in the aqueous buffers were prepared directly in Millipore MultiScreen-96 filter plates (0.4 μm PTCE membrane) and sealed. Plates were left for 24 h at room temperature under orbital shaking to achieve "pseudo-thermodynamic equilibrium" and to presaturate the membrane filter. Product suspensions/solutions were then filtered using centrifugation, diluted 1:2 with the same buffer solution, and analyzed by UPLC/UV/TOF-MS, using UV detection at 254 nm for quantitation. Solubility was calculated by comparing the sample and standard UV areas: $S = (A_{smp} \times FD \times C_{st})/A_{st}$, where S was the solubility of the compound (μM), A_{smp} was the UV area of the sample solution, FD was the dilution factor (2), C_{st} was the standard concentration (250 μM), and A_{st} was the UV area of the standard solution.

In Vitro Intrinsic Clearance

Test compounds were incubated separately at 1 μM concentration in 100 mM phosphate buffer (pH 7.4) and 1 mM EDTA with 0.2 mg/mL rat hepatic microsomal protein. The enzymatic reaction was initiated by addition of a NADPH regenerating system (final concentrations: 2 mM β-nicotinamide adenine dinucleotide phosphate (NADP) + 10 mM glucose-6-phosphate (G6P) + 0.4 U/mL glucose-6-phosphate dehydrogenase (G6PDH)). Reactions were terminated at regular time intervals (0–5–10–20–40 min) by adding an equal volume of acetonitrile. All incubations were performed in duplicate. Verapamil as positive control for the assay was incubated in parallel under the same conditions. Samples were analyzed by UPLC/TOF-MS. Quantitative data were automatically produced using the OpenLynx

software. Substrate depletion data (peak area at different time points) were fitted to a monoexponential decay model (eq 1), with a 1/*y* weighting, $C_{(t)} = C_0\ e^{-kt}$ where C_0 was the substrate concentration in the incubation media at time 0 and k was the terminal rate constant. Under the assumption that the concentration of 1 μM was far below the K_m of the test compound, the *in vitro* Cl_{int} was calculated by dividing the elimination constant (*K*) for the microsomal protein concentration (PMS), expressed in mg/μL, to obtain Cl_{int} in units of μL/min/mg protein: Cl_{int}= *K*/PMS = μL/min/mg protein. Compounds were defined as low, medium, or highly metabolized based on the *in vitro* Cl_{int} values: <3.4 low, 3.4–92.4 mediun, >92.4 high.

MDCK Cellular Permeability

Madin-Darby canine kidney cells were maintained in tissue culture flasks in EMEM with Glutamax added with 1% MEM, penicillin (100 U/mL), streptomycin (100 ug/mL), and 10% FBS. Five days before the permeability experiment, the cells were split and placed on permeable cell culture inserts (24-well Millipore) at a density of 250 000 cells/well. Transepithelial electrical resistance (TEER) was measured for each well before incubation using an EVOMX instrument (WPI) to ensure that the monolayer was confluent and the tight junctions intact. A TEER >70 Ohm × cm^2 was considered suitable for experimentation. Compounds (10 μM in HBSS-Hepes buffer) were added in duplicate to the donor chamber and buffer to the acceptor chamber (alternatively apical and basolateral) and incubated for 2 h at 37 °C under gentle agitation. Standards with a high (antipyrin), low (cimetidine), and medium (warfarin) permeability were incubated in the same plate under the same conditions. An aliquot (100 μL) from each well (both apical and basolateral) at time 0 and 120 min was filtered and analyzed by UPLC/TOF-MS. Following incubation, the cell monolayer was washed and incubated with Lucifer Yellow, a fluorescent probe with low permeability, to verify monolayer integrity after incubation. The UPLC separation was performed using a C-18 column (Acquity UPLC BEH C18, 1.7 μm, 2.1 mm × 50 mm, Waters). Samples were analyzed using an LTC premier TOF (Waters). The ESI positive W mode scan type was applied and the total ion current (TIC) scan range extended from 100 to 800 amu, with a scan time of 0.08 s. Acquisition was from 0.3 to 1.8 min. Quantitative data were automatically produced

using the OpenLynx software. The apparent permeability (Papp) in centimeters per seconds was calculated using the following equation in both directions (apical-to-basolateral and basolateral-to-apical): $P_{app} = dC \times V_r/dt \times A \times C_0$ (where V_r was the volume (mL) of the receiver chamber, A was the surface area of the cell culture insert and dt was time in seconds). The mass balance in both directions was estimated by the following equation: mass balance = (final donor mass + mass transferred) / initial donor mass. The efflux ratio was calculated by comparing $P_{app\ B\rightarrow A}$ with $P_{app\ A\rightarrow B}$. A high efflux ratio was an indication of the compound being a substrate for efflux transporters. The alert threshold was an efflux ratio >3.

Pharmacokinetics.

Male CD1 mice (body weight 20–25 g) were administered with compounds as a single dose of 5 mg/kg *po* and *iv* at time 0 as a solution in 0.9% v/v PEG400 in saline solution (volume 10 mL/kg). In life phase of experiments was conducted at Avogadro-France. Bioanalytical and pharmakinetic analysis was performed in house as described: levels in plasma were determined over a time period of 6 h in the *po* study and in brain at 0, 0.5, 1, and 3 h. The concentration of compound in plasma was measured by high performance liquid chromatography in combination with mass spectrometry (LC-API-MS/MS –Applied Biosystems) with a limit of detection of 1 ng/mL in plasma. Plasma samples were prepared by protein precipitation with acetonitrile containing 250 ng/mL of internal standard, centrifugation and analysis of the supernatant by LC-MS/MS.

Table S1. CD1 mouse PK data of selected compounds

Compound	**4c**	**4f**	**8b**
	IV – 5mg/Kg		
C_0 (μM)	18.7	15.4	14.2
$AUC_{0\text{-}t(last)}$ (μMh)	8.2	20.6	2.6
CL (mL/min/kg)	30.8	13.3	93.4
V_{ss} (L/kg)	1.55	0.58	0.5
f_e (%)	2.5 ± 0.6	4 ± 2	0.28 ± 0.06
$T_{1/2,z}$ (h)	0.58	0.51	0.1
	PO – 5mg/Kg		
C_{max} (μM)	2.7	7.6	0.04
t_{max} (h)	0.25	0.25	0.25
$AUC_{0\text{-}t(last)}$ (μMh)	3.1	18.9	0.01
F (%)	38.1	91.9	0.5

Section 4: Procedures for R6/2 mouse model study

in life treatment and experiments

R6/2 transgenic mice carrying the N-terminal region of a mutant human Huntingtin gene (Mangiarini et al., 1996) were used in this study. Mice were bred in by crossing ovarian transplanted females on a CBAxC57BL/6 background (Jackson Laboratories) with CBAxC57BL/6 F1 wild-type males. All in life treatment and experiments were performed at a specialized CRO (Psychogenics, Tarrytown, NY). Behavioral tests were performed at 3.5-4, 6, 8, 10 and 12 weeks of age. Mice were identified before weaning by real-time PCR of tail snips. In mutant mice, the CAG repeat length was analyzed by ABI 377sequencer (Mangiarini et al., 1996). Only mice with at least 100 CAG repeats were included in the study. The average CAG repeat length in this study was 108.9±0.5. A battery of behavioural tests (Rotarod, Open field, and Grip streght) was performed at 3.5-4, 6, 8, 10 and 12 weeks of age during the light phase of the diurnal cycle. The baseline testing was performed at 3.5-4 weeks of age. Treatments were started at 4.5 weeks of age after mice had been tested at 3.5-4 weeks to establish baseline behavioral performance for all of the animals. Mice received daily (QD) oral gavage (PO, 10 ml/kg) of

4f (3, 10 or 30 mg/kg) or its vehicle (0.5% hydroxypropylmethylcellulose Methocel K4M Premium in sterile water; 0.5% HPMC).

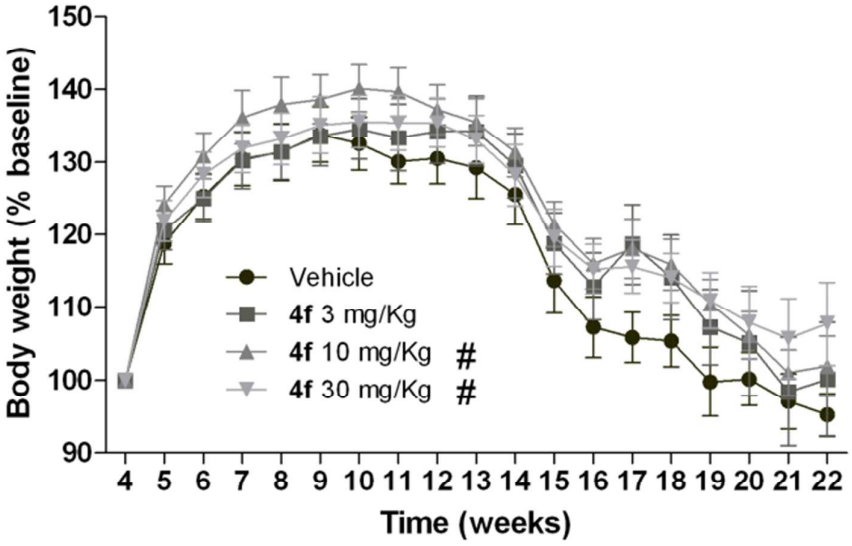


Figure S2. Effects of **4f** treatment at increasing doses on body weigth of R6/2 animals. #p< 0.05 vs vehicle group (Two-Way ANOVA, Tukey's post-hoc test).

Brain morphological analysis.

A satellite group of animals were sacrificed at 12 weeks of age for brain morphological analysis using both MRI and histological techniques. Animals were transcardially perfused with ice-cold paraformaldehyde solution (4% in phosphate-buffer, pH 7.4) and cryoprotected in 18% sucrose solution, Ex-vivo brains were imaged by a 7 Tesla MRI system (Bruker Pharmascan 70/16, Ettlingen, Germany) in order to evaluate brain atrophy through the volume measurement of lateral ventricles. Before ex-vivo MRI, specimens were placed into MR-compatible tubes filled with Fluorinert FC-77 (Sigma, USA), which is a MR invisible liquid for susceptibility matching also preventing brain dehydration and then placed in the MRI instrument. Ex-vivo MRI was performed by a fast T2-weighetd spin echo sequence with parameters as follows: Echo Time (TE) 35ms, Repetition Time (TR) 3319.2 ms, slice thickness 0.5 mm, matrix dimension 256x256 pixels, Echo Train Length (ETL) 8, spatial resolution 62 μm and 170 averages for a total acquisition time of about 5 Hours.[iii] Lateral ventricles volumes were segmented with

a semi-automatic free form segmentation algorithm[iv] using Matlab (The Mathworks Inc.,Natick, USA). Ventricle volumes were calculated as integral among all identified regions of interest and then normalized in respect to the whole brain volume (ventricle/brain ratio) on a per subject basis (Fig. S3). One-Way ANOVA was applied to evaluate group mean differences with a significance level of alpha=0.05. Tukey's post-hoc test was used for following pairwise multiple comparisons. Statistical analysis showed a significant difference in the ventricle/brain ratio measures of C57BL6 positive control group in respect to all other experimental groups with a p-value < 0.05. No statistical difference was observed in the ventricle/brain ratio between treated and study protocol control group.

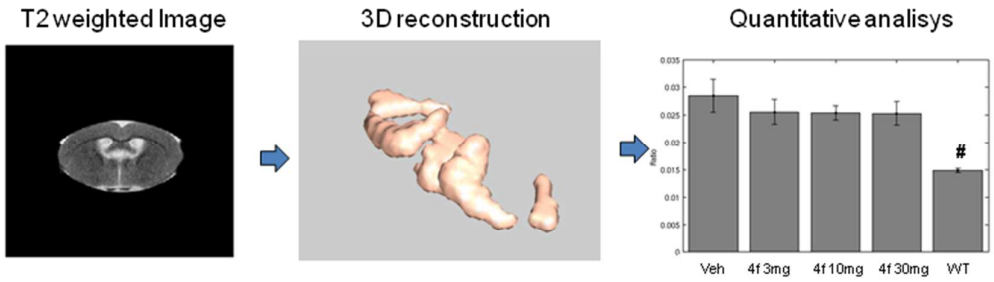


Figure S3. Ventricles' volume analysis by ex-vivo MRI. Images are representative of brain acquisition and 3D ventricular reconstruction, the graph shows the mean of the ventricle/brain ratio of each group of animals # P<0.05 vs R6/2 veh (Anova and Tukey's post-hoc test).

Once the brains were removed from MRI instrument, they were returned in sucrose for at least 1 night and sliced at the freezing microtome (30 µm thickness). For each brain 4 series of the whole brain were mounted directly in a slide to performe Nissl staining obtaining 1 slice every 300 µm in each slide. These slices were used to evaluate ventricle's area. In particular, a set of 7 representative slices from the whole brain sectioned were selected, acquired at 4x magnification by the video camera connected to the microscope (Nikon Eclipse 90i) and then the ventricular area was manually defined, but automatically calculated by the software (see Figure 3 in the paper). Moreover a set of 6 slices every 300µm was taken for immunohistochemistry on free-floating section to quantify Htt aggregates in the striatum with EM48 (MAB5374, Millipore) as primary antibody. Two images (20X) per striatum of each mouse were

acquired and a macro able to detect the aggregates was applied in the selected area. No significant effects of **4f** treatment on aggregate numbers and mean area (data not shown).

References

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