Pre-Clinical Characterization of the FAAH Inhibitor

JNJ-42165279

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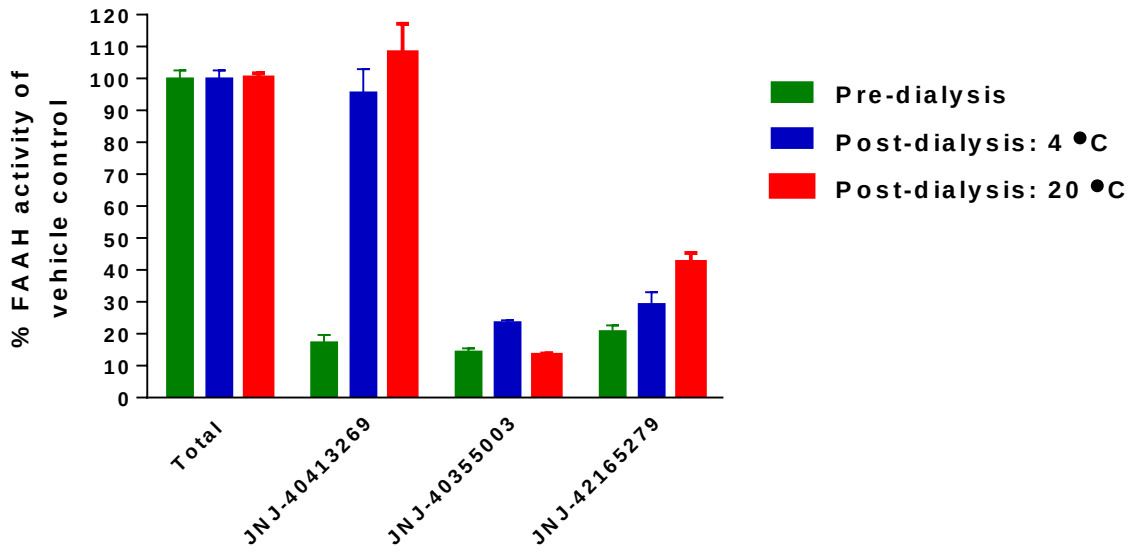
Supporting Information

Dialysis The reversibility of FAAH inhibition by **JNJ-42165279** was further assessed by a dialysis method using either human or rat recombinant FAAH expressed in SK-N-MC cells. Cell pellets were homogenized in FAAH buffer and a final concentration of test compound which gives approximately 70-80% enzyme inhibition under standard assay conditions was added to 3 mL of homogenate. 60 µL of this sample was assayed in triplicate in a FAAH activity assay and the remaining sample was injected into a dialysis cassette with a 10,000 MW cutoff membrane. The mixture was dialyzed against 1 L PBS on a stir plate for 18 hours at either 20 °C or 4 °C. The post-dialysis FAAH activity was determined by assaying 60 µL samples of the dialysis cassette contents in triplicate in the standard FAAH assay.

Figure 1S. Dialysis of **JNJ-42165279**



Pharmicokinetics

Table 1S. I.V. and P.O. PK of **JNJ-42165279** in the rat

I.V.[1] (2 mg/kg, N = 3)			
CL (mL/min/kg)	Vss (L/kg)	$T_{1/2}$ (h)	AUC_{inf} (h.µg/mL)
60 ± 9	2.5 ± 0.5	1.1 ± 0.5	0.6 ± 0.1
P.O.[2] (10 mg/kg, N = 3)			
C_{max} (µg/mL)	AUC_{inf} (h.µg/mL)	T_{max} (h)	%F
0.5 ± 0.06	0.92 ± 0.13	0.5 ± 0.0	32 ± 4

1) Dosed as an aqueous solution of the HCl salt; 2) dosed as an HPMC suspension.

Figure 2S. Brain and plasma levels of **JNJ-42165279** post 20 mg/kg p.o. dose. N = 3/time point

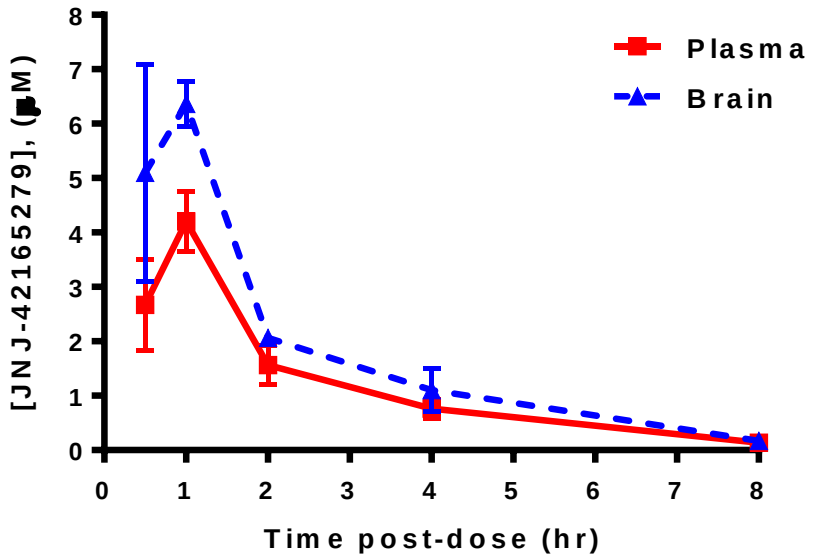


Table 2S. Metabolite distribution in pre-clinical species

Met.	Approx. R.T. (min)	Mod.	Obs. m/z	LM.	Hep.	In Vivo Plasma
M1	19	Unk.	652	Ms, R, D, Mk	-	-
M2	19.6	Unk.	342		R, Mk	R, Mk
M3	21.5	Unk.	350	R	R, Mk, H	-
M4	22.2	Ox de-Cl + GSH	698	R	-	R
M5	22.4	Unk.	349	--	-	R
M6	25.2	Unk.	359	all		
M7	26.7	Ox + gluc	603		-	D, Mk
M8	27.2	Ox	427	all	R, D, Mk, H	R, D, Mk
M9	27.6	di-Ox	443	-	H	-
M10	27.9	Ox	427	Ms, R	R, Mk, H	R, D, Mk
M11	28.5	Ox	427	all	R, Mk, H	R, D, Mk
M12	28.6	di-Ox	443	-	-	Mk
M13	30	di-Ox	443	all	-	D, Mk
M14	32.3	Ox	427	Ms, R, D,Mk	R, D, Mk, H	R, D, Mk

Metabolites of **JNJ-42165279** observed in liver microsomes, hepatocytes, and pharmacokinetic plasma samples. Met. = metabolite, Mod – modification, Obs. M/z – observed mass, L.M. – liver microsomes, Hep. – hepatocytes, Ms - mouse, R - rat, D - dog, Mk - monkey, H – human. Metabolite numbering is by order of elution from a HPLC column: **JNJ42165279** elutes between M13 and M14 (~30.7 min).

Figure 3S. Metabolite profile of **JNJ-42165279** in rat plasma from a pharmacokinetic study (10 mg/kg PO, 30 and 60 min samples pooled).

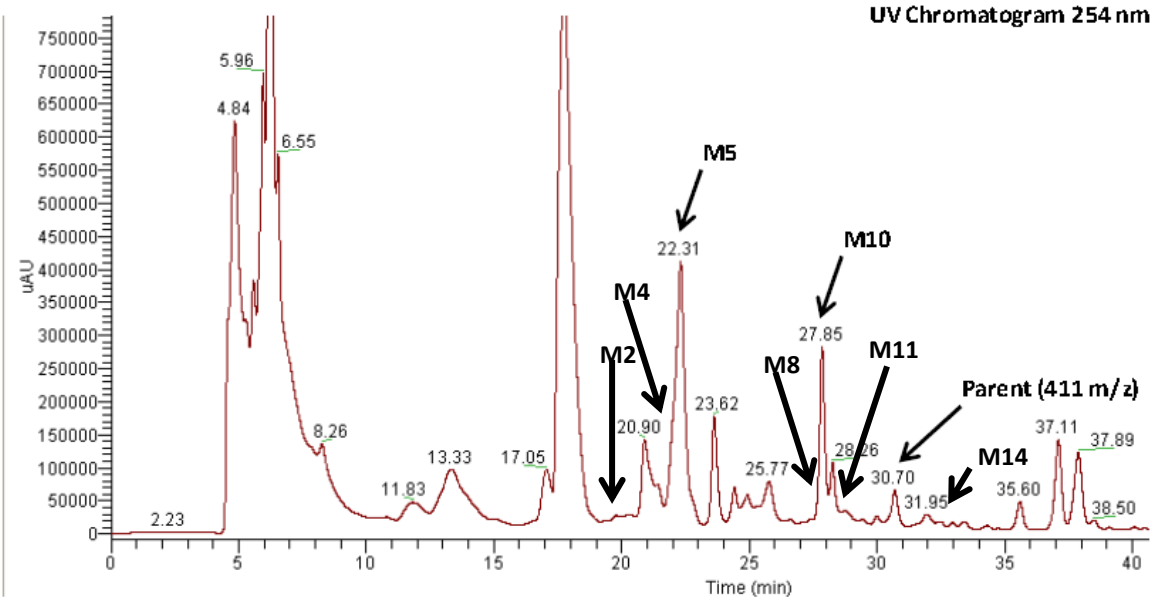


Figure 4S. Metabolite profile of **JNJ-42165279** in dog plasma from a pharmacokinetic study (50 mg/kg PO, 60 and 120 min samples pooled).

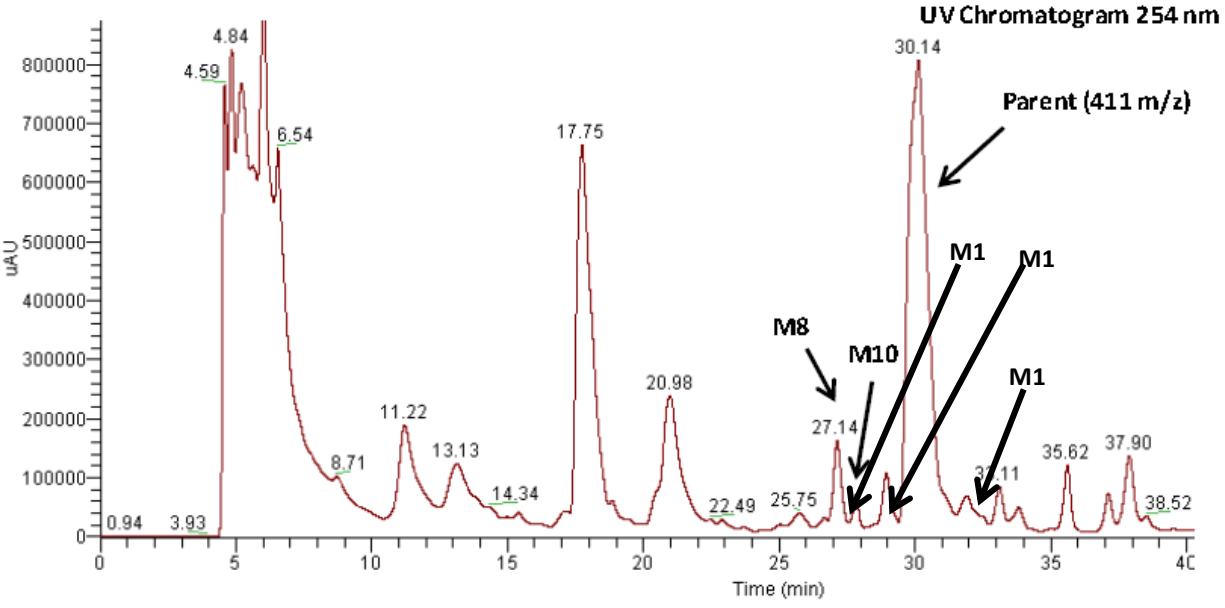
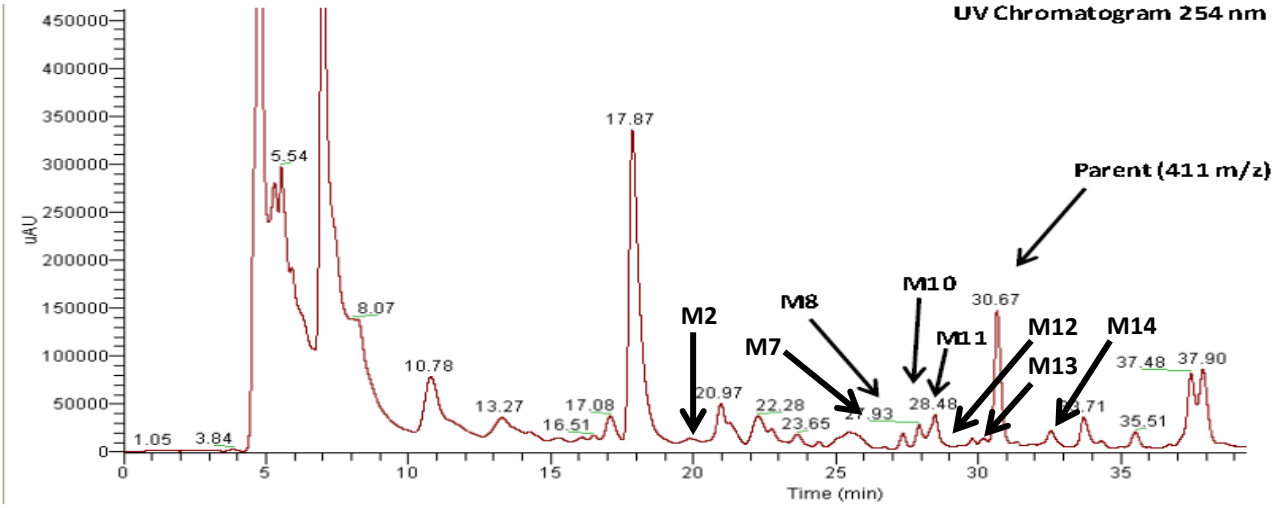


Figure 5S. Metabolite profile of **JNJ-42165279** in monkey plasma from a pharmacokinetic study (10 mg/kg PO, 30, 60, 120, and 240 min samples pooled).

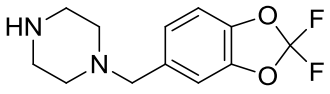


Chemistry

The following general experimental and analytical methods were used.

Reaction mixtures were stirred under a nitrogen atmosphere unless otherwise noted. Mass spectra were obtained using an Agilent series 1100 MSD using electrospray ionization (ESI) in positive mode. NMR spectra were obtained using Bruker models DPX400 and DPX600. Chemical names were generated using ChemDraw Ultra 6.0.2 (CambridgeSoft Corp., Cambridge, MA) or ACD/Name Version 9 (Advanced Chemistry Development, Toronto, Ontario, Canada).

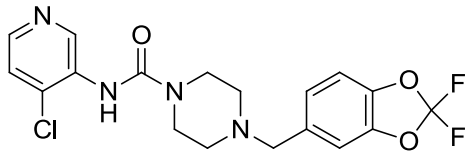
Intermediate: 1-((2,2-difluorobenzo[d][1,3]dioxol-5-yl)methyl)piperazine



A 2-L Erlenmeyer flask was charged with piperazine (185 g, 2.15 mol), 2,2-difluorobenzo[d][1,3]dioxole-5-carbaldehyde (100 g, 0.537 mol), and methanol (1.08 L). The resultant

mixture was stirred for 18 h at r.t. before passing the mixture through an H-Cube Midi™ (ThalesNano, Budapest, Hungary) utilizing the following reagents and conditions: 20% $Pd(OH)_2$/C MidiCart cartridge, 1 atm. H_2 (10% excess H_2 production), 70 °C, 6 mL/min flow-rate. The 2,2-difluorobenzo[d][1,3]dioxole-5-carbaldehyde was >90% consumed after the first pass through the H-Cube and completely consumed (determined via HPLC analysis) after two passes. The reaction mixture was then concentrated to dryness and the resultant residue treated with toluene (1.20 L) and stirred at r.t. for 18 h. The resultant white suspension was removed via filtration and then washed with toluene (200 mL). The combined filtrates were washed with water (2x 300 mL), dried over Na_2SO_4, filtered, and concentrated to dryness to give the product as a colorless oil. The crude product was dissolved in heptane (100 mL) in which it gradually crystallized at r.t. [Note: crystallization of subsequent batches of 1-((2,2-difluorobenzo[d][1,3]dioxol-5-yl)methyl)piperazine can be accelerated via the addition of seed crystals obtained from the 1st batch.] The suspension was cooled to 0 °C and isolated via filtration. Residual solvent was removed from the isolated white solid by heating in a vacuum oven at 50 °C for 24 h to yield the title compound (108 g, 78%). The heptane filtrate was concentrated to 20 mL and treated with seed crystals. The resultant mixture was stirred at r.t. overnight to yield an additional 6.7 g (5%) of title compound after drying in a vacuum oven, thus affording a combined yield of 115 g (83%). MS $(ESI)^+$: calcd for $C_{12}H_{14}F_2N_2O_2$ m/z 256.1, found 256.9 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.11 (d, J = 0.9 Hz, 1H), 6.99 (dd, J = 9.0, 0.9 Hz, 1H), 6.95 (d, J = 9.0 Hz, 1H), 3.45 (s, 2H), 2.92-2.83 (m, 4H), 2.39 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 143.9, 142.7, 134.7, 131.7 (t, J_{C-F} = 255.3 Hz), 123.9, 110.1, 108.8, 63.1, 54.4, 46.1.

JNJ-42165279: *N*-(4-chloropyridin-3-yl)-4-((2,2-difluorobenzo[*d*][1,3]dioxol-5-yl)methyl)piperazine-1-carboxamide

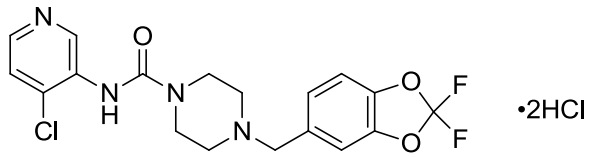


3-Amino-4-chloropyridine (35.0 g, 272 mmol) and toluene (740 mL) were added to 2-L 3-neck Morton flask under a nitrogen atmosphere and fitted with a thermocouple, mechanical stirrer, and addition funnel. The resultant brown mixture was cooled to 2 °C and treated with pyridine (25.3 mL, 310 mmol) followed by drop-wise addition of phenyl chloroformate (32.6 mL, 259 mmol) over the course of 30 minutes. The internal reaction temperature never exceeded 5 °C during the addition of phenyl chloroformate. The resultant mixture was stirred a further 7 h at 2 – 5 °C during which time a thick yellow suspension formed. A cooled solution of K_2CO_3 (53.6 g, 388 mmol) in water (216 mL) was added over 3 minutes (internal temperature reached 6 °C). Solid 1-((2,2-difluorobenzo[d][1,3]dioxol-5-yl)methyl)piperazine (66.3 g, 259 mmol) was then added to the mixture over the course of 1 minute, the mixture slowly warmed to r.t., and stirring continued for 15 h. Water (200 mL) was then added and the toluene phase isolated and subsequently washed with aqueous HCl (1.8 M, 600 mL). The aqueous phase was then extracted with toluene (2 x 300 mL) before diluting with methanol (600 mL) and cooling to 5 °C. The pH was adjusted to between 8 and 9 via addition of aqueous NaOH (50%, ~ 50 mL). The NaOH was added at such a rate that the internal temperature didn't exceed 17 °C. The resultant suspension was stirred at 5 °C for 2 h before isolating it via filtration. The solid thus obtained was washed with a mixture of methanol and water (1:1, 70 mL). The isolated solid was dried in a vacuum oven for 24 h at 50 °C to afford the title compound as a yellow green solid (73 g, 69%).

A 2-L 3-neck Morton flask equipped with a thermocouple, mechanical stirrer, and reflux condenser was charged with crude *N*-(4-chloropyridin-3-yl)-4-((2,2-difluorobenzo[*d*][1,3]dioxol-5-yl)methyl)piperazine-1-carboxamide (191 g, 465 mmol) and isopropyl acetate (705 mL). The resultant suspension was heated to 65 °C, treated with activated charcoal (11.2 g), and stirred for 1 h. The mixture was then heated to 75 °C and quickly filtered through a thin pad of Celite®. The filtrate was then slowly cooled to r.t. overnight before cooling further in an ice bath for 30 minutes. The resultant precipitate was isolated via filtration and washed with cold isopropyl acetate (40 mL) and dried in a vacuum oven at 50 °C for 72 h to afford the title compound as a slightly yellow solid (161 g, 84%). MS

ESI$^+$ calcd for $C_{18}H_{17}ClF_2N_4O_3$ m/z 410.1, found 411.1 $(M+H)^+$. anal. calcd for $C_{18}H_{17}ClF_2N_4O_3$: C, 52.63%; H, 4.17%; N, 13.64%. Found C, 52.73%; H, 4.15%; N, 13.62%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.36 (s, 1H), 8.19 (d, *J* = 5.2 Hz, 1H), 7.29 (dd, *J* = 5.3, 0.3 Hz, 1H), 7.13 (d, *J* = 0.9 Hz, 1H), 7.02 – 6.98 (m, 2H), 6.84 (s, 1H), 3.58 – 3.54 (m, 4H), 3.53 (s, 2H), 2.54 – 2.48 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 153.5, 144.0, 143.9, 143.3, 143.0, 134.1, 133.1, 131.66 (t, J_{C-F} = 155 Hz), 131.55, 123.9, 123.5, 110.0, 109.0, 62.3, 52.5, 44.2.

JNJ-42165279: *N*-(4-chloropyridin-3-yl)-4-((2,2-difluorobenzo[*d*][1,3]dioxol-5-yl)methyl)piperazine-1-carboxamide bis-hydrochloride



A solution consisting of *N*-(4-chloropyridin-3-yl)-4-((2,2-difluorobenzo[*d*][1,3]dioxol-5-yl)methyl)piperazine-1-carboxamide (5.0 g, 12 mmol) and ethanol (200 mL) was treated with saturated aqueous HCl (3.0 mL, 3.0 equiv.). The ethanol was then removed under reduced pressure and the resultant residue treated with ethanol (100 mL). The mixture was cooled to 0 °C and the white solid isolated by filtration. The solid was washed with cold ethanol (25 mL) and dried under vacuum to give the title compound as the bis-HCl salt (4.25 g, 72%). 1H NMR (400 MHz, DMSO-d_6) δ 11.61 (br. s, 1H), 8.98 (s, 1H), 8.65 (s, 1H), 8.37 (d, *J* = 5.4 Hz, 1H), 7.78 (d, *J* = 1.4 Hz, 1H), 7.69 (d, *J* = 5.4 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.46 (dd, *J* = 8.3, 1.5 Hz, 1H), 4.39 (s, 2H), 4.28-4.12 (m, 2H), 3.49-3.26 (m, 4H), 3.03 (s, 2H).