Elucidation of fluorine’s impact on pKa and in vitro Pgp-mediated efflux for a series of PDE9 inhibitors

Kasper Fjelbye, a, b Mauro Marigo, a Rasmus Prætorius Clausen, b Erling B. Jørgensen, a Claus T. Christoffersen, a Karsten Juhl a, *

a H. Lundbeck A/S, Ottliavej 9, 2500 Valby, Danmark
b Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, 2 Universitetsparken, DK-2100 Copenhagen, Denmark

Supporting Information

Contents
General Methods ....................................................................................................................................................................... 2
MDCK-MDR1 assay ............................................................................................................................................................ 3
Determination of pK\textsubscript{a} values ................................................................................................................................................. 3
PDE9 inhibition assay ........................................................................................................................................................... 3
Syntheses .................................................................................................................................................................................. 3
General procedure for the reductive amination .................................................................................................................... 3
Spectre .................................................................................................................................................................................... 10
Reference List ......................................................................................................................................................................... 32
General Methods
The LCMS data were acquired using a Waters Acquity UPLC-MS consisting of a Waters Acquity system including column manager, binary solvent manager, sample organizer, PDA detector (operating at 254 nm), ELS detector, and TQ-MS equipped with APPI-source operating in positive ion mode. LC-conditions: The column was Acquity UPLC BEH C18 1.7 μm; 2.1 × 50 mm operating at 60 °C with 1.2 mL/min binary gradient consisting of H₂O+0.05% trifluoroacetic acid (TFA) (A) and MeCN+5% H₂O+0.05% TFA (B). Gradient: 0.00 min: 10% B; 1.00 min: 100% B; 1.01 min: 10% B; 1.15 min: 10% B. The retention times provided in the experimental section shall be compared to the total run time of 1.15 min. The HRMS data were acquired with a Bruker Daltonic MicroTOF using internal calibration with ESI in positive mode. NMR data were collected with a Bruker 600-Avance-III spectrometer equipped with a 5 mm TCI cryoprobe operating at 600 and 151 MHz for ¹H and ¹³C, respectively. ¹⁹F NMR spectra were recorded with a Bruker 500-Avance spectrometer equipped with a 5 mm QNP probe operating at 470.6 Hz, using CFCl₃ as reference. The solvents used for NMR were CDCl₃, with reference signals for CHCl₃ (δ = 7.26 ppm, ¹H) and (δ = 77.16 ppm, ¹³C), and DMSO-d₆ with the reference signals for residual DMSO (δ = 2.50 ppm, ¹H) and (δ = 39.51 ppm, ¹³C) using TMS as internal reference. The chemical shifts are provided in ppm and broad proton signals are labeled (br). Commercial reagents and solvents were purchased from Sigma-Aldrich. Wrt. = with regards to. The preparation of enantioenriched pyrrolidinyl carbaldehydes 4 and 5 is described in literature.¹
MDCK-MDR1 assay
The permeability of the test compounds was assessed in MDCK-MDRI cells that were cultured to confluency (4-6 days) in a 96 transwell plate. Test compounds were diluted with the transport buffer (HBSS + 1% BSA) to a concentration of 0.5 µM and applied to the apical or basolateral side of the cell monolayer. Permeation of the test compounds from A to B direction or B to A direction was determined in triplicate over a 60-minute incubation time at 37 °C and 5% CO₂ with a relative humidity of 95%. Test compounds were quantified by LC-MS/MS analysis based on the peaks area ratios of analyte/lS in both the receiver and donor wells of the transwell plate. The apparent permeability coefficient Papp (cm/s) was calculated using the equation: Papp = (dCr/dt) x Vr / (A x C0) Where dCr/dt is the cumulative concentration of compound in the receiver chamber as a function of time (gM/s); Vr is the solution volume in the receiver chamber (0.05 mL on the apical side; 0.25 mL on the basolateral side); A is the surface area for the transport, i.e. 0.0804 cm² for the area of the monolayer; C0 is the initial concentration in the donor chamber (µM). The final MDCK-MDR1 efflux ratio is then calculated Papp BA / Papp AB.

Determination of pKₐ values
The pKa values are determined by a series of three titrations on a dilution of 10µL 10µM compound stock at 25.0 ± 0.5 °C and ion strength of 0.16M using methanol as co-solvent. Methanol concentrations in the range 29 – 54% were used in the three titrations on each compound. The titrator is used in the mode with a diode-array-detector. This detector recorded the UV-Vis spectrum of the solution in-line, and thus the titration can be mapped via comparison to the spectra of the protonated and unprotonated species. The real aqueous pKₐ –value was determined by extrapolation to zero-methanol content using a Yasuda-Shedlovsky plot.

PDE9 inhibition assay
The assay is performed in 60 µL samples containing a fixed amount of the PDE9 enzyme (sufficient to convert not more than 20% of the tritiated substrate), a buffer (50 mM HEPES pH 7.6; 10 mM MgCl₂; 0.02% Tween20), 0.1mg/ml BSA, and tritium labeled cGMP to a final concentration of 15 nM and varying amounts of inhibitors. Reactions are initiated by addition of ³H-cGMP, and reactions are allowed to proceed for 1 hour at room temperature before being terminated through mixing with 15 µL 8 mg/mL yttrium silicate SPA beads (Amersham). The beads are allowed to settle for 1 hour in the dark before the plates are counted in a Wallac 1450 Microbeta counter. The measured signal is converted to activity relative to an uninhibited control (100 %) and IC₅₀ values are calculated using the Xlfit (IDBS) extension to EXCEL.

Syntheses
General procedure for the reductive amination
To a stirred solution of 1, 2, 6 or 7 (1 equiv.) in MeOH (0.05M wrt. 1, 2, 6 or 7) was slowly added HCl (70 equiv., 4M in dioxane) at rt. The mixture was subsequently stirred 1.5 h followed by a removal of solvent at reduced pressure. To this material was then added 1,2-DCE (0.02M wrt. 1, 2, 6 or 7) and sodium acetate (3 equiv.) followed by the aldehyde (1.5 equiv.) and sodium triacetoxyborohydride (2.5 equiv.) after which the mixture was heated at 40 °C for 1.5 h. Saturated aq. NaHCO₃ was added and the aqueous phase extracted with CH₂Cl₂ after which the combined organic phases were dried over MgSO₄, filtered and concentrated in vacuo to provide a crude material that was purified using silica gel chromatography (85:10:5, EtOAc:MeOH:Et₃N) to provide 8-11.

tert-Butyl (3R,4R)-3-fluoro-4-methyl-3-(4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyrrolidine-1-carboxylate (1): To a mixture of 4 (1.00 g, 4.32 mmol) and 3 (1.09 g, 5.19 mmol) in THF (8.6 mL, 0.5M) was added Ti(OEt)₄ (7.89 g, 7.25 mL, 34.6 mmol) and the mixture was stirred for 24 h at 75 °C. Water was added followed by EtOAc and the dense mixture was passed through a plug of Celite. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over MgSO₄ and concentrated in vacuo to provide the crude aminal intermediate that was dried overnight at reduced pressure. To the dried residue was added CH₂Cl₂ (4 mL) and DMF
(29 mL, 4.32 mmol, 0.15M) and the resulting mixture was heated to 80 °C followed by the addition of I₂ (1.646 g, 6.49 mmol). After 30 min at 80 °C, the heating block was removed and the mixture allowed to cool for 5 min, followed by the addition of sat. aq. NaHCO₃ (15 mL) and 10% aq. Na₂S₂O₃ (35 mL). EtOAc was added and the phases separated, the aqueous phase was extracted with EtOAc (2 x 200 mL) and once with EtOAc/THF (150 mL, 1:1). The combined organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated in vacuo to provide a brown residue that was purified using silica gel chromatography to provide 1 (661 mg, 1.568 mmol, 36% yield) as a yellow oil.

Note: if heating is continued at 80 °C past 30 min in step 2, significant amounts of product will get Boc de-protected. It is then possible to add sat. aq. bicarb, 10% aq. sodium thiosulphate, EtOAc and re-install the Boc in situ using cat. 4-DMAP and Boc-anhydride and recover the material by extraction.

1H NMR (600 MHz, CDCl₃) δ 9.99 - 9.89 (m, 1H), 8.05 (2 x s, 1H), 4.83 - 4.74 (m, 1H), 4.17 - 4.11 (m, 2H), 4.08 - 3.81 (m, 3H), 3.63 - 3.55 (m, 2H), 3.28 - 3.22 (m, 1H), 2.44 - 2.29 (m, 2H), 1.93 - 1.85 (m, 2H), 1.50 - 1.45 (m, 9H), 1.45 - 1.43 (m, 1H), 1.06 - 1.03 (m, 3H). Mixture of conformers.

13C NMR (151 MHz, CDCl₃) δ 157.4, 154.9, 154.8, 154.7, 154.6, 154.2, 153.8, 151.1, 151.0, 135.1, 105.3, 101.8 (2 x d, J = 188.4 Hz), 80.3, 67.1, 56.8 (2 x d, J = 23.4 Hz), 54.4, 51.1, 50.6, 42.1 (2 x d, J = 20.9 Hz), 32.4, 32.3, 32.3, 32.2, 28.6. Mixture of conformers.

HRMS-ESI: m/z C₁₆H₂₁FN₅O₄ [MH⁺-tBu] calcd. 366.1572; found 366.1578.

tert-Butyl (3S,4S)-3-methyl-4-(4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyrrolidine-1-carboxylate (2): To a mixture of 5 (1.00 g, 4.32 mmol) and 3 (1.18 g, 5.63 mmol) in THF (9.4 mL, 0.5M) was added Ti(OEt)₄ (8.56 g, 7.86 mL, 37.5 mmol) and the mixture was stirred for 24 h at 75 °C. The reaction mixture was then poured into water (100 mL) and the resulting suspension was heavily stirred for 10 min after which EtOAc was added and the dense mixture was passed through a plug of Celite. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over MgSO₄ and concentrated in vacuo to provide a yellow residue that was taken up in CH₂Cl₂ (15 mL) and transferred to a round-bottom flask. The suspension was boiled and DMF (31 mL, 0.15M) was added while allowing the temperature to reach 80 °C followed by the addition of I₂ (1.785 g, 7.03 mmol). The reaction mixture was stirred for 20 min followed by the addition of sat. aq. NaHCO₃ (30 mL) and 10% sat. aq. Na₂S₂O₃. EtOAc was added and the phases were separated. The aqueous phase was extracted using EtOAc (2 x 150 mL) followed by EtOAc/THF (200 mL, 1:1). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated in vacuo to provide a crude residue that was further purified using silica gel chromatography to provide 2 (1.05 g, 2.60 mmol, 56% yield) as a yellow oil. The characterization data are in accordance with literature.

**tert-Butyl (3R,4R)-3-fluoro-4-methyl-3-(5-methyl-4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyrrolidine-1-carboxylate (6):** To a stirred solution of 1 (148 mg, 0.351 mmol) in DMF (540 µL 0.65M) was added NaH (15.5 mg, 0.386 mmol, 60%) and the resulting mixture was stirred for 30 min at 0 °C. Mel (75 mg, 33 µL, 0.53 mmol) was added and stirring was continued for 1.5 h. Water and NH₄Cl were added. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried over MgSO₄ and concentrated in vacuo. The crude material was purified using silica gel chromatography to provide 6 (20.0 mg, 0.046 mmol, 13% yield) as a colorless oil.
$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.08 (s, 1H), 4.77 - 4.70 (m, 1H), 4.27 - 4.08 (m, 3H), 3.88 - 3.74 (m, 2H), 3.63 - 3.54 (m, 2H), 3.24 - 3.18 (m, 1H), 2.46 - 2.31 (m, 2H), 1.96 - 1.90 (m, 2H), 1.51 - 1.44 (m, 9H), 1.18 (d, $J$ = 6.6 Hz, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 158.7, 154.0 ($J$ = 72.7 Hz), 153.7, 153.5, 149.0, 135.0, 105.0, 104.8, 102.9, 102.1 ($J$ = 126.9 Hz), 80.4 (2 peaks), 67.2 (2 peaks), 55.7 - 55.2 (m), 55.0, 54.8, 50.3, 49.7, 41.6 - 40.4 (m), 32.5, 32.2, 31.9 - 31.5 (m), 28.6, 10.2 (2 peaks). Mixture of conformers.

HRMS-ESI: $m/z$ C$_{17}$H$_{23}$FN$_{5}$O$_{4}$ [MH$^+$-tBu] calcd. 380.1729; found 380.1736.

**tert-butyl (3S,4S)-3-methyl-4-(5-methyl-4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyrrolidine-1-carboxylate (7):** To a stirred solution of 2 (130 mg, 0.322 mmol) in DMF (496 µL, 0.322 mmol, 0.65M) was added NaH (12.9 mg, 0.322 mmol, 60%) and the resulting mixture was stirred for 30 min at 0°C. MeI (69 mg, 30 µL, 0.48 mmol) was added and stirring was continued for 1.5 h. Water and NH$_4$Cl were added. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried over MgSO$_4$ and concentrated in vacuo. The crude material was using silica gel chromatography to provide 7 (38 mg, 0.091 mmol, 28% yield) as a colorless oil.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.03 (s, 1H), 4.79 – 4.72 (m, 1H), 4.15 – 4.10 (m, 2H), 3.89 (ddd, $J$ = 47.8, 10.7, 7.7 Hz, 1H), 3.75 (ddd, $J$ = 33.7, 10.6, 7.4 Hz, 1H), 3.65 – 3.46 (m, 6H), 3.25 – 3.15 (m, 1H), 3.08 (ddd, $J$ = 22.7, 10.5, 8.4 Hz, 1H), 2.90 (ddd, $J$ = 12.7, 9.2, 6.4 Hz, 1H), 2.43 – 2.29 (m, 2H), 1.95 – 1.88 (m, 2H), 1.47 (2 x s, 9H), 1.14 – 1.11 (m, 3H). Mixture of conformers.

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 158.9, 158.6, 154.4, 149.9, 134.8, 104.3, 104.2, 79.8 (2C), 67.2 (2C), 67.1, 54.4, 54.2, 52.5, 52.0, 50.4, 50.2, 49.9, 49.1, 38.7, 37.6, 32.4, 32.3, 32.2 (2C), 30.3, 28.6, 17.1, 17.0. Mixture of conformers.

HRMS-ESI: $m/z$ C$_{21}$H$_{32}$N$_5$O$_4$ [MH$^+$] calcd. 418.2449; found 418.2454.

6-((3R,4R)-3-fluoro-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8a): Synthesized using the general procedure to provide 8a in 62% yield.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 10.07 (s, 1H), 8.78 (d, $J$ = 4.9 Hz, 2H), 8.09 (s, 1H), 7.23 (t, $J$ = 4.9 Hz, 1H), 4.91 – 4.84 (m, 1H), 4.22 (d, $J$ = 15.2 Hz, 1H), 4.13 (dd, $J$ = 11.8, 4.4 Hz, 2H), 4.08 (d, $J$ = 15.2 Hz, 1H), 3.91 (dd, $J$ = 28.4, 12.0 Hz, 1H), 3.65 – 3.60 (m, 2H), 3.44 (t, $J$ = 8.0 Hz, 1H), 3.18 – 3.08 (m, 1H), 3.02 – 2.92 (m, 1H), 2.76 (t, $J$ = 9.7 Hz, 1H), 2.41 – 2.30 (m, 2H), 1.92 – 1.87 (m, 2H), 1.10 – 1.07 (m, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 157.6, 157.5, 151.3, 135.1, 119.7, 105.1, 102.0 (d, $J$ = 190.8 Hz),67.1, 64.1 (d, $J$ = 23.8 Hz), 61.7, 60.0, 53.8, 43.5 (d, $J$ = 20.7 Hz), 32.4 (d, $J$ = 14.2 Hz), 10.3 (d, $J$ = 10.0 Hz).

$^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ -170.53.

HR MS-ESI: $m/z$ for C$_{20}$H$_{23}$FN$_7$O$_2$ [MH$^+$-tBu] calcd. 414.2449; found 414.2454.

6-((3R,4R)-3-fluoro-4-methyl-1-(pyridin-2-ylmethyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8b): Synthesized using the general procedure to provide 8b in 47% yield.
**H NMR (600 MHz, CDCl₃) δ 9.66 (br, 1H), 8.60 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 8.08 (s, 1H), 7.68 (dt, J = 7.6, 1.8 Hz, 1H), 7.39 (dt, J = 7.8, 1.0 Hz, 1H), 7.19 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 4.84 (tt, J = 11.7, 4.3 Hz, 1H), 4.16 - 4.12 (m, 2H), 4.00 (d, J = 13.7 Hz, 1H), 3.92 (d, J = 13.7 Hz, 1H), 3.71 (dd, J = 30.7, 12.1 Hz, 1H), 3.62 (tt, J = 11.8, 1.9 Hz, 2H), 3.31 - 3.27 (m, 1H), 3.17 - 3.09 (m, 1H), 2.99 - 2.90 (m, 1H), 2.70 (t, J = 9.1 Hz, 1H), 2.41 - 2.31 (m, 2H), 1.93 - 1.87 (m, 2H), 1.06 (dd, J = 7.0, 2.0 Hz, 3H).

**C NMR (151 MHz, CDCl₃) δ 158.3, 157.3, 157.1, 151.3, 149.6, 136.8, 135.1, 123.0, 122.5, 105.1, 102.6 (d, J = 189.3 Hz), 67.1, 64.7 (d, J = 23.7 Hz), 61.8, 59.9, 54.1, 43.7 (d, J = 20.6 Hz), 32.3 (d, J = 13.9 Hz), 10.26 (d, J = 10.1 Hz).

**F NMR (471 MHz, CDCl₃) δ -169.62.

HRMS-ESI: m/z C₂₁H₂₆FN₆O₂ [MH⁺] calcd. 413.2096; found 413.2102.

6-((3S,4S)-1-Isobutyl-4-methylpyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (9c): Synthesized using the general procedure to provide 9c in 33% yield.

**H NMR (600 MHz, CDCl₃) δ 8.03 (s, 1H), 4.83 (tt, J = 11.7, 4.3 Hz, 1H), 4.17 - 4.11 (m, 2H), 3.61 (tdd, J = 12.0, 7.3, 2.1 Hz, 2H), 3.45 - 3.39 (m, 1H), 3.14 - 3.07 (m, 1H), 2.89 - 2.78 (m, 1H), 2.54 - 2.45 (m, 1H), 2.45 - 2.27 (m, 4H), 1.94 - 1.89 (m, 2H), 1.81 (s, 2H), 1.58 (s, 2H), 1.21 (d, J = 6.9 Hz, 3H), 1.02 (t, J = 6.6 Hz, 6H).

HRMS-ESI: m/z C₁₉H₃₀N₅O₂ [MH⁺] calcd. 360.2394; found 360.2403.

6-((3R,4R)-3-Fluoro-1-isobutyl-4-methylpyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8c): Synthesized using the general procedure to provide 8c in 33% yield.

**H NMR (600 MHz, CDCl₃) δ 9.54 (s, 1H), 8.09 (s, 1H), 4.84 (tt, J = 11.6, 4.3 Hz, 1H), 4.16 - 4.11 (m, 2H), 3.60 (tt, J = 11.9, 2.1 Hz, 2H), 3.45 - 3.39 (m, 1H), 3.14 - 3.07 (m, 1H), 2.89 - 2.78 (m, 1H), 2.54 - 2.45 (m, 1H), 2.45 - 2.27 (m, 4H), 1.94 - 1.87 (m, 2H), 1.81 (s, 2H), 1.58 (s, 2H), 1.21 (d, J = 6.9 Hz, 3H), 1.02 (t, J = 6.6 Hz, 6H).

**F NMR (471 MHz, CDCl₃) δ -170.41.

HRMS-ESI: m/z C₁₉H₂₉FN₅O₂ [MH⁺] calcd. 378.2300; found 378.2306.

6-((3R,4R)-3-Fluoro-1-(4-fluorobenzyl)-4-methylpyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8d): Synthesized using the general procedure to provide 8d in 40% yield.

**H NMR (600 MHz, CDCl₃) δ 9.54 (s, 1H), 8.08 (s, 1H), 7.35 - 7.31 (m, 2H), 7.04 - 7.00 (m, 2H), 4.82 (tt, J = 11.6, 4.2 Hz, 1H), 4.17 - 4.13 (m, 2H), 3.81 - 3.71 (m, 2H), 3.64 - 3.58 (m, 3H), 3.20 - 3.16 (m, 1H), 3.04 (dd, J = 30.1, 12.0 Hz, 1H), 2.96 - 2.85 (m, 1H), 2.59 (t, J = 9.2 Hz, 1H), 2.42 - 2.33 (m, 2H), 1.94 - 1.88 (m, 2H), 1.06 (dd, J = 7.0, 2.1 Hz, 3H).

HRMS-ESI: m/z C₁₉H₂₉FN₅O₂ [MH⁺] calcd. 378.2306. 

S6
$^{13}$C NMR (151 MHz, CDCl$_3$) δ 163.1, 161.4, 157.3, 157.1, 135.1, 134.2, 130.3, 130.3, 115.5, 115.4, 105.1, 102.5 (d, J = 189.2 Hz), 67.1, 64.7 (d, J = 23.8 Hz), 59.7, 54.3, 43.6 (d, J = 20.6 Hz), 32.3 (d, J = 10.8 Hz), 10.4 (d, J = 10.3 Hz).

$^{19}$F NMR (471 MHz, CDCl$_3$) δ -115.74, -169.38.

HRMS-ESI: m/z C$_{22}$H$_{26}$F$_2$N$_2$O$_2$ [MH$^+$] calcd. 430.2049; found 430.2056.

6-((3R,4R)-3-fluoro-4-methyl-1-((5-methylpyrazin-2-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8e): Synthesized using the general procedure to provide 8e in 35% yield.

$^1$H NMR (600 MHz, CDCl$_3$) δ 9.61 (s, 1H), 8.55 (d, J = 1.5 Hz, 1H), 8.44 - 8.43 (m, 1H), 8.08 (s, 1H), 4.87 - 4.80 (m, 1H), 4.16 - 4.11 (m, 2H), 4.01 (d, J = 13.8 Hz, 1H), 3.92 (d, J = 14.0 Hz, 1H), 3.74 (dd, J = 31.7, 12.1 Hz, 1H), 3.62 (tt, J = 12.0, 2.5 Hz, 2H), 3.30 - 3.26 (m, 1H), 3.13 (dd, J = 30.6, 12.2 Hz, 1H), 3.00 - 2.89 (m, 1H), 2.73 - 2.68 (m, 1H), 2.57 - 2.55 (m, 3H), 2.42 - 2.31 (m, 2H), 1.90 (dddt, J = 11.2, 6.7, 4.3, 2.1 Hz, 2H), 1.06 (dd, J = 7.0, 1.9 Hz, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) δ 157.3, 156.9 (d, J = 27.3 Hz), 152.7, 151.3 (2C), 150.4, 144.0, 143.6, 135.1, 105.1, 102.8 (d, J = 189.8 Hz), 67.1, 64.8 (d, J = 23.6 Hz), 59.9, 59.1, 54.1, 45.9, 43.7 (d, J = 20.7 Hz), 32.3 (d, J = 9.0 Hz), 21.4, 10.0 (d, J = 9.8 Hz).

$^{19}$F NMR (471 MHz, CDCl$_3$) δ -169.11.

HRMS-ESI: m/z C$_{21}$H$_{27}$FN$_{7}$O$_2$ [MH$^+$] calcd. 428.2205; found 428.2210.

6-((3R,4R)-3-fluoro-4-methyl-1-((5-methylpyrazin-2-yl)methyl)pyrrolidin-3-yl)-5-methyl-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (10e): Synthesized using the general procedure to provide 10e in 48% yield.

$^1$H NMR (600 MHz, CDCl$_3$) δ 8.51 - 8.50 (m, 1H), 8.41 - 8.40 (m, 1H), 8.06 (s, 1H), 4.76 (tt, J = 11.6, 4.2 Hz, 1H), 4.14 (dt, J = 10.7, 5.0 Hz, 2H), 3.96 (d, J = 13.7 Hz, 1H), 3.86 (d, J = 13.6 Hz, 1H), 3.69 - 3.64 (m, 1H), 3.62 (d, J = 2.8 Hz, 3H), 3.61 - 3.53 (m, 3H), 3.26 - 3.16 (m, 2H), 2.58 - 2.53 (m, 4H), 2.45 - 2.33 (m, 2H), 1.95 - 1.90 (m, 2H), 1.16 (dd, J = 6.8, 2.0 Hz, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) δ 158.9, 154.9 (d, J = 13.7 Hz), 152.7, 151.3 (2C), 150.4, 144.0, 143.9, 104.8, 103.7 (d, J = 186.5 Hz), 104.8, 103.7 (d, J = 25.8 Hz), 67.2 (2C), 64.7 (d, J = 25.0 Hz), 58.8 (d, J = 57.2 Hz), 54.8, 41.4, 41.3, 32.4 (d, J = 46.3 Hz), 31.7 (d, J = 11.2 Hz), 21.4, 10.1 (d, J = 10.3 Hz).

$^{19}$F NMR (471 MHz, CDCl$_3$) δ -157.63.

HRMS-ESI: m/z C$_{22}$H$_{26}$F$_2$N$_2$O$_2$ [MH$^+$] calcd. 442.2361; found 442.2368.

5-methyl-6-((3S,4S)-4-methyl-1-((5-methylpyrazin-2-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (11e): Synthesized using the general procedure to provide 11e in 55% yield.

$^1$H NMR (600 MHz, CDCl$_3$) δ 8.54 (d, J = 1.5 Hz, 1H), 8.40 (dd, J = 1.5, 0.6 Hz, 1H), 8.03 (s, 1H), 4.81 (tt, J = 13.7 Hz, 1H), 4.17 - 4.11 (m, 2H), 3.94 (d, J = 13.7 Hz, 1H), 3.80 (d, J = 13.8 Hz, 1H), 3.62 (tdd, J = 12.0, 3.7, 2.1 Hz, 2H), 3.57 (s, 3H), 3.21 (dd, J = 5.9, 3.0 Hz, 2H), 3.13 (p, J =
6.9 Hz, 1H), 3.06 (dd, $J = 7.8, 7.4$ Hz, 1H), 2.93 - 2.85 (m, 1H), 2.57 - 2.52 (m, 4H), 2.44 - 2.33 (m, 2H), 1.93 (ddt, $J = 13.0, 5.2, 2.6$ Hz, 2H), 1.15 (d, $J = 6.6$ Hz, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 160.6, 158.8, 152.5, 150.9, 150.0, 143.9, 143.7, 134.8, 104.1, 67.2 (2C), 61.3, 59.3, 59.0, 54.2, 50.8, 37.8, 32.4, 32.3, 30.5, 30.2, 21.4, 18.9.

HRMS-ESI: $m/z$ C$_{22}$H$_{30}$N$_7$O$_2$ [MH$^+$] calcd. 424.2455; found 424.2460.

6-((3R,4R)-3-fluoro-4-methyl-1-((2-methylpyrimidin-5-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8f): Synthesized using the general procedure to provide 8f in 75% yield.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.64 (s, 2H), 8.08 (s, 1H), 4.81 (tt, $J = 11.7, 4.2$ Hz, 1H), 4.17 - 4.13 (m, 2H), 3.76 - 3.68 (m, 2H), 3.63 - 3.59 (m, 3H), 3.55 - 3.45 (m, 1H), 3.21 - 3.16 (m, 1H), 3.06 (dd, $J = 30.3, 12.0$ Hz, 1H), 2.97 - 2.87 (m, 1H), 2.72 (s, 3H), 2.42 - 2.33 (m, 2H), 1.91 (dtt, $J = 13.1, 4.6, 1.9$ Hz, 2H), 1.06 (dd, $J = 7.0, 1.9$ Hz, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 167.6, 157.3, 157.2, 156.7 (d, $J = 27.2$ Hz), 151.2, 135.1, 128.2, 105.1, 102.8 (d, $J = 188.4$ Hz), 67.1 (d, $J = 23.7$ Hz), 59.6, 55.1, 54.4, 43.9, 43.7, 32.3, 10.11 (d, $J = 9.9$ Hz).

$^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ -168.80.

HRMS-ESI: $m/z$ C$_{21}$H$_{27}$FN$_7$O$_2$ [MH$^+$] calcd. 428.2205; found 428.2209.

6-((3R,4R)-3-fluoro-4-methyl-1-((2-methylpyrimidin-5-yl)methyl)pyrrolidin-3-yl)-5-methyl-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (10f): Synthesized using the general procedure to provide 10f in 48% yield.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.59 (s, 2H), 8.07 (s, 1H), 4.79 - 4.72 (m, 2H), 4.17 - 4.12 (m, 2H), 3.76 - 3.68 (m, 2H), 3.63 - 3.60 (m, 3H), 3.57 (s, 3H), 3.55 - 3.45 (m, 1H), 3.21 - 3.16 (m, 1H), 2.72 (s, 3H), 2.47 - 2.32 (m, 3H), 1.96 - 1.91 (m, 2H), 1.06 (dd, $J = 6.9, 2.1$ Hz, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 167.6, 158.9, 157.3, 154.8 (d, $J = 25.6$ Hz), 149.1, 134.9, 130.3, 128.2, 104.8, 103.5 (d, $J = 186.8$ Hz), 67.2 (2C), 64.6 (d, $J = 25.0$ Hz), 60.6, 58.3, 54.9, 54.8, 41.3 (d, $J = 20.8$ Hz), 32.4 (d, $J = 63.2$ Hz), 31.7 (d, $J = 11.2$ Hz), 25.9, 10.3 (d, $J = 10.5$ Hz).

$^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ -157.49.

HRMS-ESI: $m/z$ C$_{22}$H$_{29}$FN$_7$O$_2$ [MH$^+$] calcd. 442.2361; found 442.2368.

5-methyl-6-((3S,4S)-4-methyl-1-((2-methylpyrimidin-5-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (11f): Synthesized using the general procedure to provide 11f in 75% yield.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.60 (s, 2H), 8.03 (s, 1H), 4.79 (tt, $J = 11.6, 4.2$ Hz, 1H), 4.17 - 4.12 (m, 2H), 3.72 (d, $J = 13.5$ Hz, 1H), 3.64 - 3.59 (m, 3H), 3.57 (s, 3H), 3.21 - 3.16 (m, 1H), 1.96 - 1.91 (m, 2H), 1.16 (d, $J = 6.8$ Hz, 3H).
$^{13}$C NMR (151 MHz, CDCl$_3$) δ 167.4, 160.6, 158.8, 157.3, 150.0, 134.8, 128.7, 104.2, 67.2 (2C), 61.1, 58.8, 54.8, 54.4, 50.7, 37.8, 32.4, 32.2, 30.5, 25.9, 19.3.

HRMS-ESI: $m/z$ C$_{22}$H$_{30}$N$_7$O$_2$ [MH$^+$] calcd. 424.2455; found 424.2452.
Spectra

Compound 1.

\[ \text{\textsuperscript{1}H NMR spectrum of 1 acquired in CDCl}_3. \]

\[ \text{APT spectrum of 1 acquired in CDCl}_3. \]
Compound 6.

\[ \text{\H NMR spectrum of 6 acquired in CDCl}_3. \]

\[ \text{\APT spectrum of 6 acquired in CDCl}_3. \]
Compound 7.

$\text{H NMR spectrum of 7 acquired in CDCl}_3.$

$\text{APT spectrum of 7 acquired in CDCl}_3.$
Compound 8a

1H NMR spectrum of 8a acquired in CDCl₃.

APT spectrum of 8a acquired in CDCl₃.
$^{19}$F NMR spectrum of 8a acquired in CDCl$_3$. 
Compound 8b.

$^1$H NMR spectrum of 8b acquired in CDCl$_3$.

APT spectrum of 8b acquired in CDCl$_3$. 
$^{19}$F NMR spectrum of 8b acquired in CDCl$_3$. 

---

S16
Compound 8c.

\[ \text{Me} \quad \text{HN} \quad \text{O} \quad \text{N} \quad \text{N} \quad \text{8c} \quad \text{Me} \quad \text{Me} \quad \text{Me} \]

$^1$H NMR spectrum of 8c acquired in CDCl$_3$.

APT spectrum of 8c acquired in CDCl$_3$. 
$^{19}$F NMR spectrum of 8c acquired in CDCl$_3$. 
Compound 9c.

$^1$H NMR spectrum of 9c acquired in CDCl$_3$. 
Compound 8d.

\[ \text{H NMR spectrum of 8d acquired in CDCl}_3. \]

\[ \text{APT spectrum of 8d acquired in CDCl}_3. \]
$^{19}$F NMR spectrum of 8d acquired in CDCl$_3$. 
Compound 8e.

\[ \begin{align*}
\text{H NMR spectrum of 8e acquired in CDCl}_3.
\end{align*} \]

\[ \begin{align*}
\text{APT spectrum of 8e acquired in CDCl}_3.
\end{align*} \]
$^19$F NMR spectrum of 8e acquired in CDCl$_3$. 
Compound 10e:

\[ \text{APT spectrum of } 10e \text{ acquired in CDCl}_3. \]

\[ \text{H NMR spectrum of } 10e \text{ acquired in CDCl}_3. \]
$^{19}$F NMR spectrum of 10e acquired in CDCl$_3$. 
Compound 11e:

\[
\begin{align*}
\text{1H NMR spectrum of 11e acquired in CDCl}_3.
\end{align*}
\]

\[
\begin{align*}
\text{APT spectrum of 11e acquired in CDCl}_3.
\end{align*}
\]
Compound 8f:

\[ \text{APT spectrum of } 8f \text{ acquired in CDCl}_3. \]

\[ \text{H NMR spectrum of } 8f \text{ acquired in CDCl}_3. \]
$^{19}$F NMR spectrum of 8f acquired in CDCl$_3$. 
Compound 10f:

$^{1}H$ NMR spectrum of 10f acquired in CDCl$_3$.

APT spectrum of 10f acquired in CDCl$_3$. 
$^{19}$F NMR spectrum of 10f acquired in CDCl₃.
Compound 11f.

$^1$H NMR spectrum of 11f acquired in CDCl$_3$.

APT spectrum of 11f acquired in CDCl$_3$. 
Reference List
