Supporting Information

Discovery of an oral potent selective inhibitor of hematopoietic prostaglandin D synthase (HPGDS)

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General experimental details

All solvents were obtained from commercially available sources and used without further purification. Flash chromatography purifications were performed on prepacked cartridges on a Biotage system. RP-HPLC purification was performed on a Waters FractionLynx system, coupled to a Waters ZQ Mass spectrometer; ions are generated by ESI+. Purification of 1 ml of sample in DMSO is performed on Waters SunFire Prep C18 ODB 5um (19 x 50 mm) using 8 minutes Water-Methanol gradients containing 0.1% Formic Acid at a flow rate of 30mL/min. Alternatively purification of 1 ml of sample in

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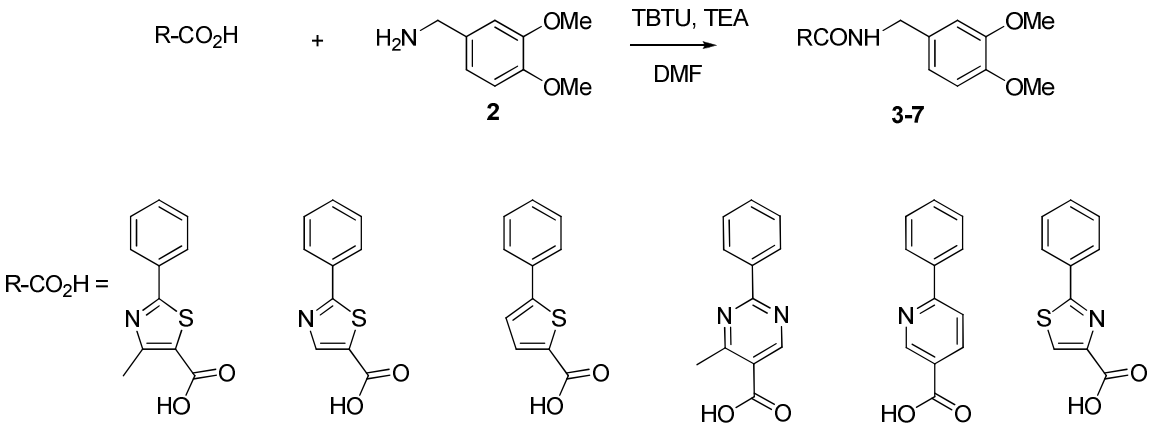
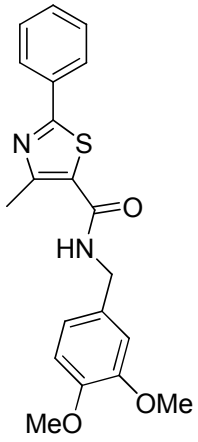
DMSO is performed on Waters XBridge MS C18 5um (19 x 50 mm) using 8 minutes Water-Methanol gradients containing 0.1% Ammonium Hydroxide at a flow rate of 30mL/min. Quality control was performed on Waters LC system multiplexed (4 way MUX) to a Waters LCT Premier Mass spectrometer in W mode; ions are generated by ESI+. Leucine-Enkephalin is used as the internal reference channel. 10ul of a 2 mM solution are injected on a Waters XTerra MS C18 5um (2.1 x 50mm) column using a 10 min Water-Acetonitrile gradient containing 0.005% Trifluoroacetic acid at a flow rate of 1 mL/min. Nuclear magnetic resonance spectra (1H NMR recorded at 400 Mhz, 19FNMR recorded at 376 MHz) were obtained on Bruker AMX spectrometers and are referenced in ppm. Purity of all compounds established by HPLC to be > 98% @ 215 nm using the above mentioned QC.

Experimental details for compounds 1,3 and 8-15, 17-19

The following compounds where prepared in accordance with the following general procedure. To the acid (0.2 mmol) in CH_3CN (4 mL) under N_2 was added 3,4-dimethoxybenzylamine (0.4 mmol), HATU (1H-1,2,3-Triazolo[4,5-b]pyridinium, 1-[bis(dimethylamino)methylene]-, 3-oxide, hexafluorophosphate(1-) (1:1) (0.26 mmol) and finally *N, N*-diisopropylethylamine (0.2 mL). The resulting mixture was stirred overnight. The crude reaction mixture was concentrated and purified by chromatography (silica, hexane/EtOAc (0-50%)) to provide the desired compounds in 70-80% yield. All corresponding acids are commercially available with the exception of 6-(3,5-difluorophenyl)nicotinic acid, which was prepared as described in US 200814659. Amines used are commercially available or were available in our in house library of compounds.

Scheme 1. Preparaation of compounds **3-7**





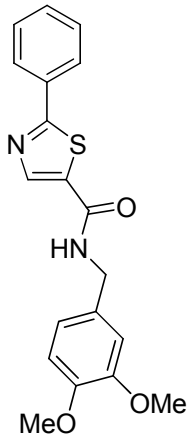
1

Chemical Formula: $C_{20}H_{20}N_2O_3S$

Exact Mass: 368.12

Molecular Weight: 368.45

N-(3,4-dimethoxybenzyl)-4-methyl-2-phenylthiazole-5-carboxamide (**1**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 2.37 (s, 3 H), 3.48 (s, 3 H), 3.49 (s, 3 H), 4.12 (d, *J*=6.06 Hz, 2 H), 6.57 - 6.62 (m, 1 H), 6.63 - 6.69 (m, 1 H), 6.70 (d, *J*=0.59 Hz, 1 H), 7.27 (dd, *J*=6.54, 0.49 Hz, 3 H), 7.69 (dd, *J*=6.45, 2.34 Hz, 2 H), 8.51 (t, *J*=5.96 Hz, 1 H)



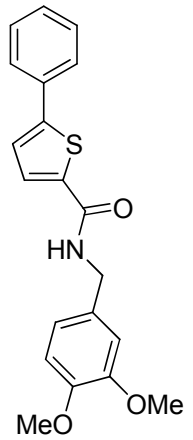
3

Chemical Formula: $C_{19}H_{18}N_2O_3S$

Exact Mass: 354.10

Molecular Weight: 354.42

2-Phenyl-thiazole-5-carboxylic acid 3,4-dimethoxy-benzylamide (**3**) ^{1}H NMR (400 MHz, Chloroform-*d*) δ ppm 3.89 (s, 3 H) 3.90 (s, 3 H) 4.59 (d, *J*=5.46 Hz, 2 H) 6.25 - 6.36 (m, 1 H) 6.81 - 6.97 (m, 3 H) 7.42 - 7.54 (m, 3 H) 7.92 - 8.02 (m, 2 H) 8.19 (s, 1 H)



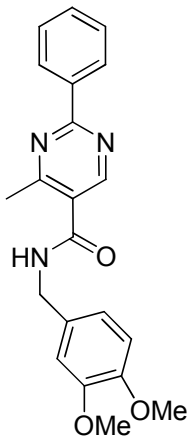
4

Chemical Formula: $C_{20}H_{19}NO_3S$

Exact Mass: 353.11

Molecular Weight: 353.43

5-Phenyl-thiophene-2-carboxylic acid 3,4-dimethoxy-benzylamide (**4**) ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.72 (s, 3 H) 3.74 (s, 3 H) 4.39 (d, *J*=5.86 Hz, 2 H) 6.79 - 6.99 (m, 3 H) 7.32 - 7.51 (m, 3 H) 7.54 (d, *J*=3.66 Hz, 1 H) 7.71 (d, *J*=7.32 Hz, 1 H) 7.80 (d, *J*=4.03 Hz, 1 H) 8.99 (t, *J*=5.86 Hz, 1 H)



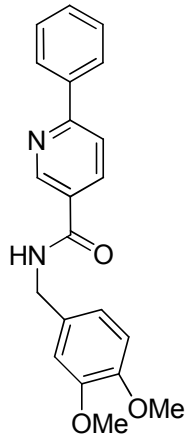
5

Chemical Formula: $C_{21}H_{21}N_3O_3$

Exact Mass: 363.16

Molecular Weight: 363.41

N-(3,4-dimethoxybenzyl)-4-methyl-2-phenylpyrimidine-5-carboxamide (**5**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 2.38 (s, 3 H), 3.50 (s, 3 H), 3.52 (s, 3 H), 4.19 (d, *J*=5.86 Hz, 2 H), 6.61 - 6.66 (m, 1 H), 6.67 - 6.70 (m, 1 H), 6.74 (d, *J*=0.59 Hz, 1 H), 7.25 - 7.34 (m, 3 H), 8.18 (ddd, *J*=4.69, 1.07, 0.88 Hz, 2 H), 8.60 (s, 1 H), 8.85 (t, *J*=5.86 Hz, 1 H)



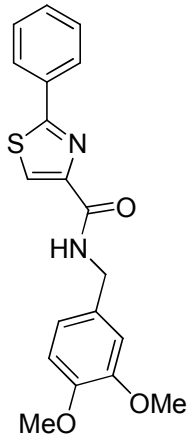
6

Chemical Formula: $C_{21}H_{20}N_2O_3$

Exact Mass: 348.15

Molecular Weight: 348.40

N-(3,4-dimethoxybenzyl)-6-phenylnicotinamide (**6**) ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.73 (s, 3 H) 3.75 (s, 3 H) 4.45 (d, *J*=5.80 Hz, 2 H) 6.82 - 6.95 (m, 2 H) 6.98 (d, *J*=1.37 Hz, 1 H) 7.45 - 7.56 (m, 3 H) 8.10 (d, *J*=8.19 Hz, 1 H) 8.16 (d, *J*=6.83 Hz, 2 H) 8.31 (dd, *J*=8.36, 2.22 Hz, 1 H) 9.13 (d, *J*=2.05 Hz, 1 H) 9.18 (t, *J*=5.80 Hz, 1 H)



7

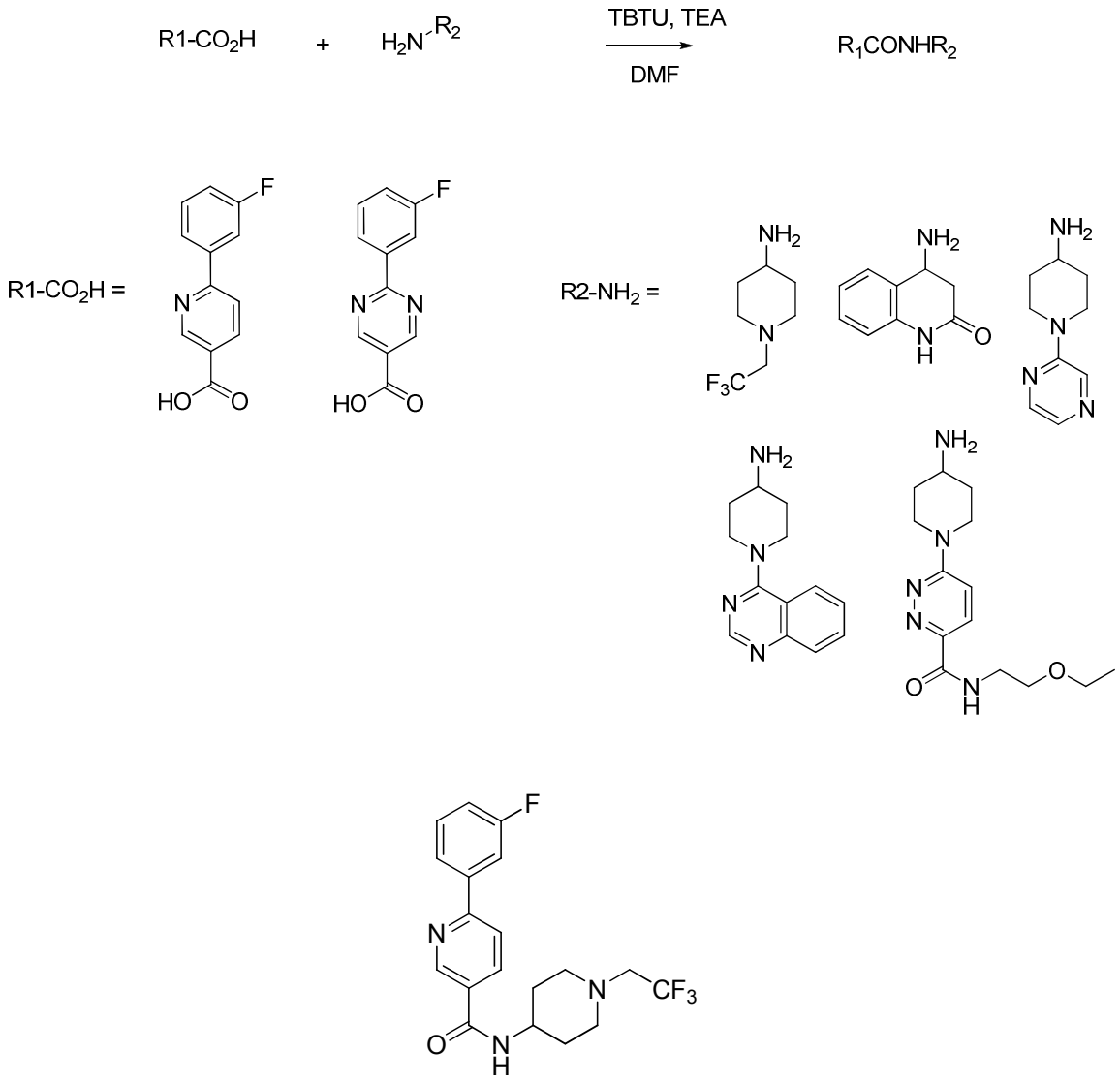
Chemical Formula: $C_{19}H_{18}N_2O_3S$

Exact Mass: 354.10

Molecular Weight: 354.42

N-(3,4-dimethoxybenzyl)-2-phenylthiazole-4-carboxamide (**7**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 3.49 (s, 3 H), 3.51 (s, 3 H), 4.20 (d, *J*=6.45 Hz, 2 H), 6.61 - 6.69 (m, 2 H), 6.76 (d, *J*=1.76 Hz, 1 H), 7.26 - 7.36 (m, 4 H), 7.79 - 7.88 (m, 2 H), 8.09 (s, 1 H), 8.75 (t, *J*=6.35 Hz, 1 H)

Scheme 2 Preparation of compounds **8-12**



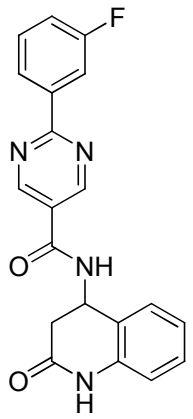
8

Chemical Formula: $C_{19}H_{19}F_4N_3O$

Exact Mass: 381.15

Molecular Weight: 381.37

6-(3-fluorophenyl)-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**8**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.60 (dq, J=11.84, 3.48 Hz, 2H), 1.81 (d, J = 10.62 Hz, 2H), 2.44 (t, J+11.16 Hz, 2H), 2.50 (br s, 2 H), 2.94 (d, J =11.53 Hz, 2 H), 3.74-3.87 (m, 1 H), 7.32 (dt, J = 8.37, 2.29 Hz, 1 H), 7.57 (q, 1 H), 7.96 (d, J = 10.43 Hz, 1 H), 8.01 (d, J = 7.69 Hz, 1 H), 8.14 (d, J= 8.24 Hz, 1 H), 8.28 (dd, J = 8.24, 2.20 Hz, 1 H), 8.49 (d, J = 7.50 Hz, 1 H), 9.07 (d, J = 1.83 Hz, 1H); ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -112.90 - -112.66 (m, 0 F), -68.12 (t, *J*=10.57 Hz, 3 F); MS (ES+) 382 (M+1); HRMS (TOF,ES+) calculated for $C_{19}H_{19}F_4N_3O$+H+: 382.1542; observed 382.1548.



9

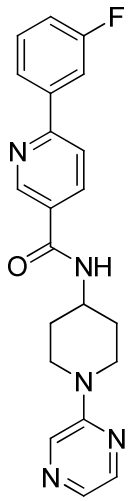
Chemical Formula: $C_{20}H_{15}FN_4O_2$

Exact Mass: 362.12

Molecular Weight: 362.36

2-(3-fluorophenyl)-N-(2-oxo-1,2,3,4-tetrahydroquinolin-4-yl)pyrimidine-5-carboxamide (**9**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 2.60 - 2.80 (m, *J*=17.16, 17.16, 16.85, 6.64 Hz, 1 H), 5.33 (q, *J*=7.55 Hz, 1 H), 6.85 - 6.98 (m, 3 H), 7.19 (t, *J*=7.72 Hz, 1 H), 7.26 (d, *J*=7.62 Hz, 1 H), 7.33 - 7.43 (m, 1 H), 7.51 - 7.60 (m, 1 H), 8.07 (ddd, *J*=9.77, 1.17, 0.78

Hz, 1 H), 8.23 (d, *J*=7.62 Hz, 1 H), 9.20 - 9.27 (m, 3 H), 10.24 (br. s., 1 H). ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -112.70 (dd, *J*=5.95, 3.30 Hz, 1 F).



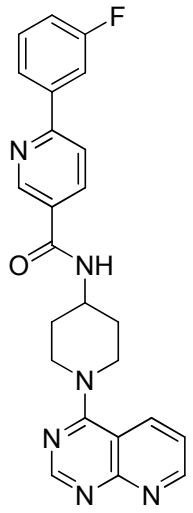
10

Chemical Formula: $C_{21}H_{20}FN_5O$

Exact Mass: 377.17

Molecular Weight: 377.41

6-(3-Fluorophenyl)-N-(1-(pyrazin-2-yl)piperidin-4-yl)nicotinamide (**10**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.32 - 1.49 (m, 2 H), 1.70 - 1.84 (m, 2 H), 2.92 (t, *J*=12.31 Hz, 2 H), 3.93 - 4.07 (m, 1 H), 4.22 (d, *J*=13.28 Hz, 2 H), 7.18 (t, *J*=8.20 Hz, 1 H), 7.42 (q, *J*=7.23 Hz, 1 H), 7.67 (s, 1 H), 7.77 - 7.90 (m, 2 H), 7.94 (s, 1 H), 8.00 (d, *J*=8.40 Hz, 1 H), 8.15 (d, *J*=8.20 Hz, 1 H), 8.23 (s, 1 H), 8.38 (d, *J*=7.62 Hz, 1 H), 8.94 (s, 1 H). ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -112.98 – -112.75 (m, 1 F)



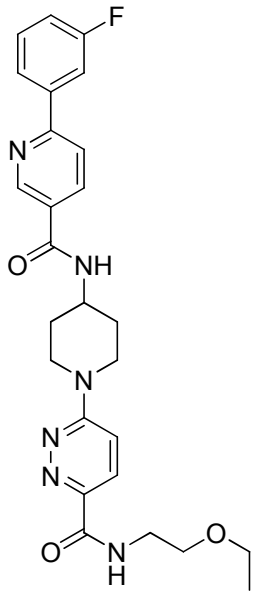
11

Chemical Formula: $C_{24}H_{21}FN_6O$

Exact Mass: 428.18

Molecular Weight: 428.46

6-(3-fluorophenyl)-N-(1-(pyrido[2,3-d]pyrimidin-4-yl)piperidin-4-yl)nicotinamide (**11**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.45 - 1.60 (m, 2 H), 1.75 - 1.83 (m, 2 H), 3.22 (d, *J*=0.59 Hz, 2 H), 3.95 - 4.05 (m, 1 H), 4.16 (d, *J*=13.48 Hz, 2 H), 7.04 - 7.14 (m, 1 H), 7.79 (dd, *J*=8.01, 0.78 Hz, 2 H), 7.92 (d, *J*=8.40 Hz, 1 H), 8.04 - 8.11 (m, 2 H), 8.21 (dt, *J*=8.25, 1.05 Hz, 1 H), 8.27 (dt, *J*=7.81, 1.07 Hz, 1 H), 8.39 (d, *J*=7.62 Hz, 1 H), 8.49 (s, 1 H), 8.72 (dd, *J*=4.20, 1.66 Hz, 1 H), 8.79 (dd, *J*=3.61, 1.07 Hz, 1 H), 8.87 (d, *J*=2.34 Hz, 1 H). ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -112.93 (dd, *J*=5.95, 3.30 Hz, 1 F).



12

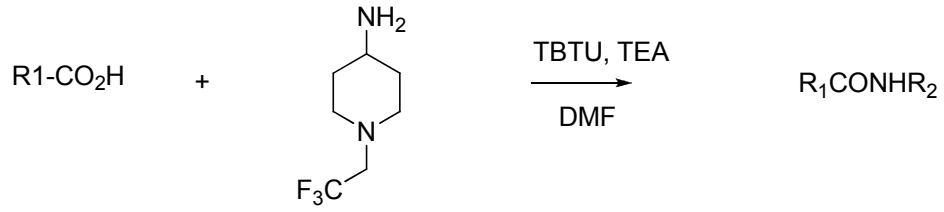
Chemical Formula: $C_{26}H_{29}FN_6O_3$

Exact Mass: 492.23

Molecular Weight: 492.55

N-(2-ethoxyethyl)-6-(4-(6-(3-fluorophenyl)nicotinamido)piperidin-1-yl)pyridazine-3-carboxamide (**12**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.00 (t, *J*=6.93 Hz, 3 H), 1.39 - 1.56 (m, 2 H), 1.85 (dd, *J*=14.06, 4.49 Hz, 2 H), 3.10 (t, *J*=11.92 Hz, 2 H), 3.30 - 3.45 (m, 6 H), 4.04 - 4.17 (m, 1 H), 4.39 (d, *J*=13.28 Hz, 2 H), 7.21 (ddd, *J*=8.55, 7.08, 1.37 Hz, 1 H), 7.31 (d, *J*=9.57 Hz, 1 H), 7.46 (td, *J*=8.16, 5.96 Hz, 1 H), 7.73 (d, *J*=9.38 Hz, 1 H), 7.81 - 7.93 (m, 2 H), 8.03 (d, *J*=8.40 Hz, 1 H), 8.18 (dt, *J*=8.40, 1.17 Hz, 1 H), 8.43 (d, *J*=7.62 Hz, 1 H), 8.59 (t, *J*=5.86 Hz, 1 H), 8.97 (br. s., 1 H). ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -112.82 (dd, *J*=5.95, 3.30 Hz, 1 F).

Scheme 3 Preparation of compounds **13-19**



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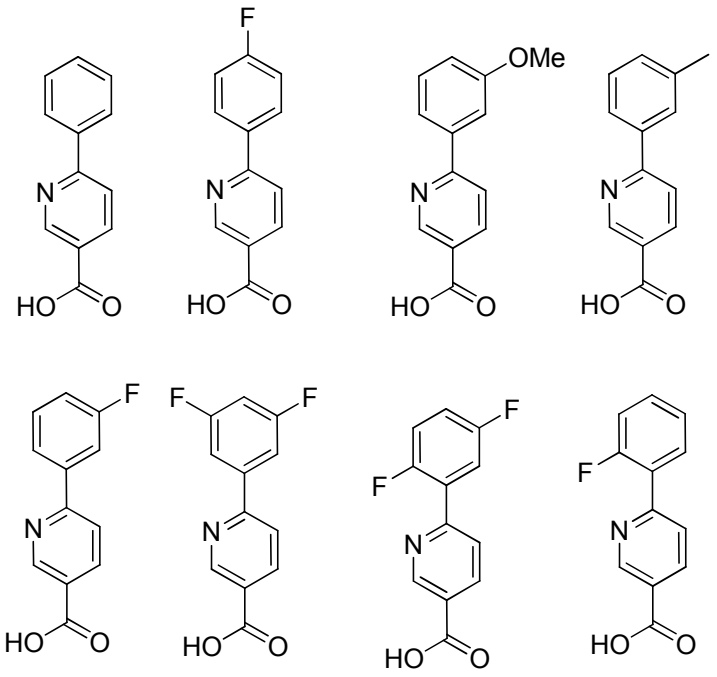
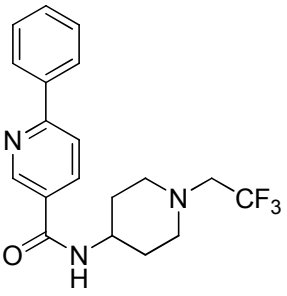




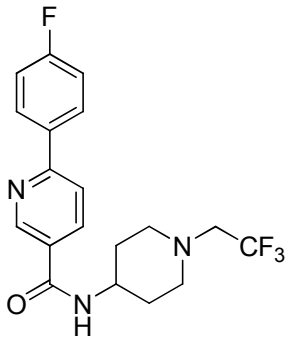
13

Chemical Formula: $C_{19}H_{20}F_3N_3O$

Exact Mass: 363.16

Molecular Weight: 363.38

6-phenyl-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**13**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.48 - 1.61 (m, 2 H), 1.71 - 1.80 (m, 2 H), 2.34 - 2.43 (m, 2 H), 2.89 (d, *J*=12.11 Hz, 2 H), 3.12 (q, *J*=10.16 Hz, 2 H), 3.69 - 3.81 (m, 1 H), 7.39 - 7.50 (m, 3 H), 8.02 (dd, *J*=8.30, 0.88 Hz, 1 H), 8.09 (dd, *J*=8.20, 1.56 Hz, 2 H), 8.21 (dd, *J*=8.40, 2.34 Hz, 1 H), 8.41 (d, *J*=7.81 Hz, 1 H), 9.01 (dd, *J*=2.44, 0.68 Hz, 1 H); ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -68.12 (t, *J*=10.57 Hz, 3 F); MS (ES+) 364 (M+1); HRMS (TOF, ES+, M+H) calculated for $C_{19}H_{20}F_3N_3O$: 364.1637; observed 364.1611.



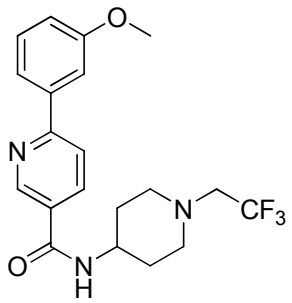
14

Chemical Formula: $C_{19}H_{19}F_4N_3O$

Exact Mass: 381.15

Molecular Weight: 381.37

6-(4-fluorophenyl)-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**14**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.43 - 1.62 (m, 2 H), 1.70 - 1.80 (m, 2 H), 2.32 - 2.43 (m, 2 H), 2.83 - 2.93 (m, 2 H), 3.06 - 3.17 (m, 2 H), 3.68 - 3.81 (m, 1 H), 7.23 - 7.34 (m, 2 H), 8.02 (d, *J*=8.40 Hz, 1 H), 8.11 - 8.24 (m, 3 H), 8.40 (dd, *J*=7.62, 0.39 Hz, 1 H), 9.00 (s, 1 H); 19F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -112.18 (dd, *J*=7.27, 3.30 Hz, 0 F), -68.12 (t, *J*=9.91 Hz, 3 F); MS(ES+) 382 (M+1); HRMS (TOF, ES+) calculated for $C_{19}H_{19}F_4N_3O$+H+: 382.1542; observed 382.1529.



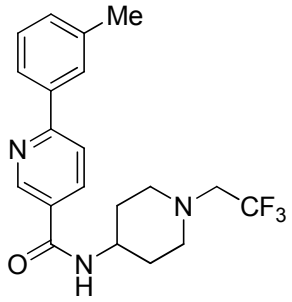
15

Chemical Formula: $C_{20}H_{22}F_3N_3O_2$

Exact Mass: 393.17

Molecular Weight: 393.40

6-(3-methoxyphenyl)-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**15**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.46 - 1.61 (m, 2 H), 1.70 - 1.80 (m, 2 H), 2.39 (t, *J*=11.72 Hz, 2 H), 2.84 - 2.92 (m, 2 H), 3.06 - 3.18 (m, *J*=10.22, 10.22, 10.22, 1.17, 0.98 Hz, 2 H), 3.69 - 3.76 (m, 1 H), 3.79 (s, 3 H), 6.94 - 7.03 (m, 1 H), 7.38 (t, 1 H), 7.60 - 7.70 (m, 2 H), 7.99 - 8.06 (m, 1 H), 8.16 - 8.23 (m, 1 H), 8.36 - 8.43 (m, 1 H), 9.00 (s, 1 H); ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -68.13 (t, *J*=9.91 Hz, 93 F); MS (ES+) 394 (M+1); HRMS (TOF, ES+) calculated for $C_{20}H_{22}F_3N_3O_2$+H+: 394.1742, observed 394.1742.



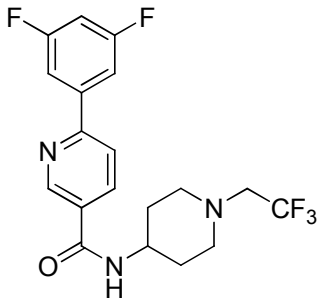
16

Chemical Formula: $C_{20}H_{22}F_3N_3O$

Exact Mass: 377.17

Molecular Weight: 377.40

6-*m*-Tolyl-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**16**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.46 - 1.62 (m, 2 H), 1.69 - 1.80 (m, 2 H), 2.32 - 2.42 (m, 5 H), 2.84 - 2.93 (m, 2 H), 3.12 (q, *J*=10.35 Hz, 2 H), 3.68 - 3.81 (m, 1 H), 7.21 - 7.26 (m, 1 H), 7.35 (t, *J*=7.72 Hz, 1 H), 7.87 (d, *J*=7.81 Hz, 1 H), 7.90 - 7.93 (m, 1 H), 8.00 (dd, *J*=8.30, 0.88 Hz, 1 H), 8.20 (dd, *J*=8.40, 2.34 Hz, 1 H), 8.40 (d, *J*=7.62 Hz, 1 H), 8.99 (dd, *J*=2.34, 0.78 Hz, 1 H); ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -68.13 (t, *J*=9.91 Hz, 3 F); MS (ES+) 378 (M+1); HRMS (TOF, ES+) calculated for $C_{20}H_{22}F_3N_3O$+H+: 378.1793; observed 378.1783.



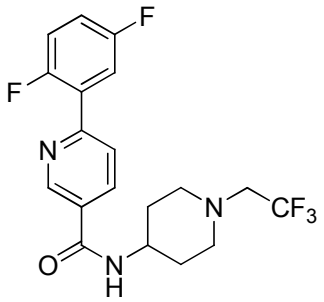
17

Chemical Formula: $C_{19}H_{18}F_5N_3O$

Exact Mass: 399.14

Molecular Weight: 399.36

6-(3,5-Difluorophenyl)-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**17**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.56-1.66 (m, 2 H), 1.76-1.87 (m, 2 H), 2.42-2.48 (m, 2 H), 2.95 (d, J = 11.0 Hz, 2 H), 3.11-3.23 (m, 2 H), 3.82 (br. s., 1 H), 7.36 (t, J = 10.1 Hz, 1 H), 7.88 (d, J = 7.0 Hz, 2 H), 8.19 (d, J = 8.1 Hz, 1 H), 8.30 (dd, J = 2.2, 8.4 Hz, 1 H), 8.47 (d, J = 7.0 Hz, 1 H), 9.08 (s, 1 H).



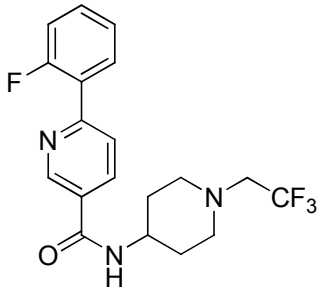
18

Chemical Formula: $C_{19}H_{18}F_5N_3O$

Exact Mass: 399.14

Molecular Weight: 399.36

6-(2,5-Difluorophenyl)-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**18**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.46 - 1.61 (m, 2 H), 1.70 - 1.81 (m, 2 H), 2.39 (t, *J*=11.43 Hz, 2 H), 2.89 (d, *J*=11.72 Hz, 2 H), 3.06 - 3.17 (m, 2 H), 3.69 - 3.81 (m, 1 H), 7.29 - 7.44 (m, 2 H), 7.67 - 7.76 (m, 1 H), 7.88 (d, *J*=8.40 Hz, 1 H), 8.25 (dt, *J*=8.20, 2.15 Hz, 1 H), 8.48 (d, 1 H), 9.05 (s, 1 H); ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -121.97 (d, *J*=9.25 Hz, 0 F), -118.16 (d, *J*=5.28 Hz, 0 F), -68.13 (t, *J*=10.57 Hz, 3 F)



19

Chemical Formula: $C_{19}H_{19}F_4N_3O$

Exact Mass: 381.15

Molecular Weight: 381.37

6-(2-Fluorophenyl)-N-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)nicotinamide (**19**). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.47 - 1.61 (m, 2 H), 1.70 - 1.81 (m, 2 H), 2.39 (ddd,

J=12.84, 11.48, 1.86 Hz, 2 H), 2.89 (d, *J*=12.11 Hz, 2 H), 3.12 (q, *J*=10.35 Hz, 2 H), 3.69 - 3.81 (m, 1 H), 7.26 - 7.36 (m, 2 H), 7.43 - 7.52 (m, 1 H), 7.83 (ddd, *J*=8.25, 2.20, 0.88 Hz, 1 H), 7.92 (td, *J*=7.86, 1.86 Hz, 1 H), 8.22 (dd, *J*=8.20, 2.34 Hz, 1 H), 8.45 (d, *J*=7.62 Hz, 1 H), 9.05 (dd, *J*=2.34, 0.78 Hz, 1 H); ^{19}F NMR (376 MHz, *DMSO-d*$_6$) δ ppm -117.11 - -116.76 (m, 0 F), -68.12 (t, *J*=10.57 Hz, 3 F).

Biological Experimental Methods

Fluorescence Intensity h-HPGDS TBA Enzyme Assay

Prostaglandin D Synthase (PGDS) converts the substrate prostaglandin H_2 (PGH_2) to prostablandin D_2. The depletion of PGH_2 was measured via an Fe(II) reduction of the remaining PGH_2 to malondialdehyde (MDA) and 12-HHT. The enzyme assay is based on the quantitative formation of a fluorescent complex from non fluorescent compounds MDA and 2-thiobarbituric acid (TBA), as described in US patent application US 2004/152148 by Lambalot.

The enzyme assay (31 uls) contained 100 mM Tris base pH 8.0, 100 uM $MgCl_2$, 0.1 mg/mL IgG Rabbit serum, 5.0 uM PGH_2 (Cayman, ethanol solution, #17020), 2.5 mM L-glutathione (Sigma; reduced form #G4251), 1:175,000 human recombinant H-PGDS (from 1 mg/mL), 0.5 % DMSO and inhibitor (varying concentration). Three uls of diluted inhibitor (dissolved in DMSO) was plated into a 384-well assay plate followed by a 25 uL addition of an enzyme solution containing h-HPGDS, Tris, MgCl2, igG and L-glutathione. After pre-incubation of inhibitor and enzyme solution of 10 minutes at room temperature, the reaction was initiated with a 3 uL addition of substrate solution in 10 mM HCl. The reaction was terminated after 42 seconds by the addition (3 uL) of stop buffer containing $FeCl_2$ and citric acid. After addition of 45.5 pis of TBA plates were heated for one hour in a 70 ^{o}C oven. Plates were cooled at room temperature overnight and read on a plate reader the next day with excitation @ 530 nM and emission @ 565 nM. IC_{50}'s of inhibitors were calculated with a 4-parameter fit using 11 inhibitor concentrations in duplicate with 3-fold serial dilutions. Controls on each plate included

no inhibitor (zero% effect) and an inhibitor 10-fold in excess of its IC_{50} (100% effect). The highest inhibitor concentration tested was typically 1 uM.

Rat Target Modulation Assay

Compound **8** dosed orally blocks PGD_2 production by the spleen. Male adult Sprague Dawley rats (~200g) received a single, oral dose of 1 mg/kg or 10mg/kg **8** in a 0.5% methylcellulose/0.1% Tween80. At various endpoints (0.5-36hrs), blood samples were collected to determine compound plasma levels, and then the animals were euthanized to harvest the spleens. The spleens were transferred into a solution of 100nM indomethacin in PBS on ice and macerated with a Polytron PT3100 to form a homogenate. The homogenate was centrifuged and the supernatant was removed and treated with equal amounts of MOX reagent to convert PGD_2 to the PGD_2-MOX derivative. The PGD_2-MOX levels were measured in the spleen supernatant using the Cayman PGD2-MOX EIA.

In vivo-Assay-Anti-Asthmatic Effects in a Sheep Model of Asthma

A validated model of A. suum antigen-induced asthmatic response in conscious sheep was used to evaluate the pharmacokinetic properties, inhibition of antigen-induced early and late airway bronco constriction in the lung by compound (Abraham, W.M., Am. Rev. Respir Dis. 1991; 143:787-796).

Sheep used in these studies exhibit both early and late airway responses to inhalation challenge with A. suum antigen as well as antigen-induced airway hyper-responsiveness to inhaled carbachol. An aerosol of A. suum extract generated using a disposable medical nebulizer (Raindrop, Puritan Bennett) was delivered in at a total volume of 500 mL and a rate of 20 breaths/min.

Initial modeling of the pharmacokinetics of compound **8** in sheep defined the concentration and volume of the dosing solution predicted to maintain a steady state plasma concentration of compound **8** over 0-8h post challenge. One hour prior to Ascaris challenge, compound **8** was administered as bolus iv loading dose (delivered in <5min) combined with the start of constant infusion of compound **8**. The loading dose combined with the constant infusion achieved targeted steady state plasma levels 60 min later, that is at the time of Ascaris challenge, and was maintained through 8hr post challenge at

which point the infusion was stopped. Vehicle volume was held constant (0.3ml/kg for the loading dose; 0.11ml/kg/h for the infusion) was held constant and the concentration of the dosing solution was varied to achieve a range of plasma concentrations. Baseline and post-drug measurements of specific lung resistance (RL) were obtained immediately prior to challenge (t = 30 min) with A. suum antigen, immediately after challenge and then hourly from 1 to 6 h post challenge and on the half-hour from 6.5, 5-8 h post challenge. Measurements of RL were obtained 24 h post challenge, followed by the 24 h post-challenge dose-response curve for carbachol.

To determine the effect of the compound **8** on antigen-induced airway hyper-responsiveness, measurements of RL were repeated immediately after inhalation of buffer and after each administration of 10 breaths of increasing concentrations of carbachol solution (0.25%, 0.5%, 1.0%, 2.0% and 4.0% wt/vol). To assess airway responsiveness, the cumulative carbachol dose in breath units (BU) that increased RL 400% over the post-buffer value (i.e. PC400) were calculated from the dose response curve. One breath unit is defined as one breath of a 1% wt/vol carbachol solution. Measurements of PC400 obtained after antigen were compared with those obtained before antigen challenge to determeine if antigen challenge induced airway hyper-responsiveness. The airway response (0-2 h after allergen challenge) was not reduced but the late airway response (4-8 h after antigen challenge) was inhibited by approximately 60%.

X-ray Crystallography Methods

Full length human H-PGDS was expressed with an amino terminal 6-histidine purification tag and a thrombin cleavage site and was purified by standard chromatography. The tag was removed during the purification. Crystallization was performed at 20°C by combining protein 1:1 with reservoir solution [100mM Tris (pH 8.5), 5mM GSH, 28-34% PEG 3350, and 20-200mM $MgCl_2$] and suspended over 500μL of reservoir solution. Crystals were harvested into a solution of 100mM Tris (pH 8.5), 50mM $MgCl_2$, 5mM GSH, 5mM DTT, and 45% PEG 3350. Ligand was added to this solution to a final concentration of 10mM, and the crystals were incubated for 1-14 days prior to plunging them into liquid nitrogen for data collection. Data were collected on a

MARCCD 165 (Rayonix Inc.) on a Micromax 007 rotating anode x-ray source (Rigaku Inc.). Data were scaled using HKL2000 [1]. The structure was determined by molecular replacement using Amore [2] and a prior internal structure as the search model. Refinement (Supplementary Table 1) was performed using Refmac [3]. Molecular graphics figures were prepared with PyMol and POV-Ray (www.pymol.org; www.povray.org).

Supplementary Table 1. Crystallographic data and refinement statistics

PDB accession code	3KXO
Data collection statistics	
Space group	$P2_1$
Resolution (Å)	20-2.1 (2.17-2.1)[a]
Observed reflections	65718
Unique reflections	22,810
Completeness (%)	98.8 (99.3)
Mean I/σ_I	18.2 (4.9)
R_{sym}%	6.3 (19.0)[b]
Refinement statistics	
Resolution (Å)	20.0 – 2.1
No. protein + ligand atoms	3,371
No. solvent atoms	395
R (%), Rfree (%)	16.4, 22.2
Wilson B ($Å^2$), Refined <B> ($Å^2$)	23.8, 28.8[c]
Rmsd ideal bond lengths (Å)	0.007
Bond angles (°)	1.057
Ramachandran plot statistics	
Most favored regions (%)	94.2
Disallowed regions (%)	0

[a] Highest resolution bin. [b] $R_{sym} = \Sigma(|I_i - \langle I \rangle|)/\Sigma I_i$. All reflections with $I/\sigma_I < -3.0$ eliminated from scaling. [c] For protein atoms.

References

1. Otwinowski, Z.; Minor, W., *Processing of X-ray Diffraction Data Collected in Oscillation Mode*. 1997; Vol. 276A, p 307-326.
2. Navaza, J., AMoRe: an Automated package for Molecular Replacement. *Acta Crystallogr D Biol Crystallogr* **1994,** D50, 157-163.

3. Murshudov, G. N.; Vagin, A. A.; Dodson, E. J., Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr* **1997,** 53, (Pt 3), 240-55.