

Supplemental Data for:

Development of highly selective pyrimidine-based aldosterone synthase (CYP11B2) inhibitors

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General protocols:

Recombinant CYP11B1 IC₅₀ determination:

The *in vitro* studies determined the IC₅₀ of the test compounds for inhibition in lysates of cells overexpressing human CYP11B1 and ADX/ADXR. A separate series of incubation mixtures was prepared with each test compound at final concentrations in the reaction ranging from 0.0024 μM to 10 μM. Each incubation mixture contained CYP11B1 cell lysate and the metabolic substrate deoxycorticosterone at experimentally determined Km concentrations (0.5 μM for deoxycorticosterone). Reaction control wells contained lysate, substrate, and a concentration of diluent (DMSO) equivalent to that in test compound wells. Standard inhibitor wells contained lysate, substrate, and the compound (fadrozole) substituted for test compound solutions. The reaction was initiated by addition of an enzyme cofactor source (NADPH-regenerating solution), and the mixtures were incubated at 30°C. After sixty minutes, incubation mixtures were quenched with acetonitrile containing the appropriate internal standard, mixed, and centrifuged. Supernatants were transferred to a new plate.

HPLC-MS/MS analysis was performed with electrospray ionization. The response (peak area) of corticosterone formed in incubation mixtures containing test compound was compared to the corresponding response, of an active control reaction containing only isozyme, cofactor, and substrate. Reaction conditions were such that substrate turnover was typically <20% at the time of termination.

Each product peak area was normalized to the enzyme control average. The IC₅₀ value for each test compound was determined using IDBS XLfit® 5.4 by fitting a 4-parameter logistic model to the dose response data.

Recombinant CYP11B2 IC₅₀ determination:

The *in vitro* studies determined the IC₅₀ of the test compounds for inhibition in lysates of cells overexpressing human CYP11B2 and ADX/ADXR. A separate series of incubation mixtures was prepared with each test compound at final concentrations in the reaction ranging from 0.0024 μM to 10 μM. Each incubation mixture contained CYP11B1 cell lysate and the metabolic substrate deoxycortisol, or human CYP11B2 cell lysate and the metabolic substrate deoxycorticosterone at experimentally determined Km concentrations (2 μM for deoxycortisol, and 0.18 μM for deoxycorticosterone). Reaction control wells contained lysate, substrate, and a percent of diluent (DMSO) to that in test compound wells. Inhibitor control wells contained lysate, substrate, and the standard inhibitor compound (fadrozole) substituted for test compound solutions. The reaction was initiated by addition of an enzyme cofactor source (NADPH-regenerating solution), and the mixtures were incubated at 30°C. After ninety minutes, incubation mixtures were quenched with acetonitrile containing the appropriate internal standard, mixed, and centrifuged. Supernatants were transferred to a new plate.

HPLC-MS/MS analysis was performed with electrospray ionization. For CYP11B2 reactions, the response (peak area) of aldosterone, 18-hydroxycorticosterone, and corticosterone were evaluated. Reaction conditions were such that substrate turnover was typically <20% at the time of termination.

Each product peak area was normalized to the enzyme control average. The IC₅₀ value for each test compound was determined using IDBS XLfit® 5.4 by fitting a 4-parameter logistic model to the dose response data.

Table 1 IC₅₀s with SD and N:

Compd	MBG	CYP11B2 IC ₅₀ (μM)	N for CYP11B2 IC ₅₀	CYP11B1 IC ₅₀ (μM)	N for CYP11B1 IC ₅₀	Selectivity ^a
3	1-1,2,3-triazole	1.064	1	9.072	1	8.5
4	2-oxadiazole	>10.0	1	>10.0	1	N.A.
5	4-pyrazole	0.782	1	1.668	1	2
6	2-thiazole	0.062 ± 0.73	6	4.523 ± 2.049	5	73
7	5-pyrimidine	0.040 ± 0.16	2	5.380 ± 1.653	3	136
8	2-pyrazine	0.541 ± 0.010	2	>10.0	2	>18
9	3-pyridazine	0.412 ± 0.015	2	>10.0	2	>24
10	4-pyridazine	0.330 ± 0.052	4	>10.0	4	>30

Table 2 IC₅₀s with SD and N:

Compd	R	CYP11B2 IC ₅₀ (μM)	N for CYP11B2 IC ₅₀	CYP11B1 IC ₅₀ (μM)	N for CYP11B1 IC ₅₀	S ^a	Microsome Stability ^b
12	-H	0.022 ± 0.009	2	2.267 ± 1.301	3	103	98
13	-Me	0.017	1	4.157	1	244	72
14	-Et	0.008 ± 0.001	2	3.330 ± 1.651	3	416	78
15	-iPr	0.005 ± 0.0003	2	1.907 ± 1.346	2	381	80
16	-cyclopropyl	0.172	1	>10.0	1	>58	
17	-CN	0.013	1	4.987	1	383	85
18	-NHMe	< 0.002	1	0.390	1	> 195	
19	-N(CH ₃) ₂	0.029	1	4.629	1	159	6
20	-NH (CH ₂) ₂ CF ₃	0.003 ± 0.0007	2	0.378 ± 0.004	2	126	55

Table 3 IC₅₀s with SD and N:

Compd	R	CYP11B2 IC ₅₀ (μM)	N for CYP11B2 IC ₅₀	CYP11B1 IC ₅₀ (μM)	N for CYP11B1 IC ₅₀	S ^a	Microsome Stability ^b
21	--CF ₂ H	0.018 ± 0.001	2	4.325 ± 0.556	2	240	106
22	-CF ₃	0.025 ± 0.010	5	4.771 ± 2.221	5	190	99
23	-CF ₂ CH ₃	0.050	1	5.723	1	114	

Table 4 IC₅₀s with SD and N:

Compd	R, X	CYP11B2 IC ₅₀ (μM)	N for CYP11B2 IC ₅₀	CYP11B1 IC ₅₀ (μM)	N for CYP11B1 IC ₅₀	S ^a	Microsome Stability ^b
24	-CF ₂ H, CH	0.009 ± 0.002	3	2.035 ± 0.497	4	226	84
25	-CF ₃ , CH	0.016 ± 0.004	2	4.171 ± 0.608	2	261	62
26	-CF ₂ H, N	0.029 ± 0.019	4	3.926 ± 1.447	4	135	101
27	-CF ₃ , N	0.024 ± 0.016	3	4.809 ± 2.414	3	200	87

Thermodynamic Solubility:

Thermodynamic solubility of test compounds in system solution (SS) at pH 7.4 was determined using a miniaturized shake flask method. This was accomplished by addition of buffer to pre-weighed test compounds and making 2.0-4.0 mg/mL stock followed by incubation at room temperature (24 ± 2° C) for 24 hours. The concentration of the analyte in the filtrate was determined using HPLC/LCMS-MS.

1. Materials:

System solution (SS) is a phosphate free buffer (pH 2.6) concentrate with low UV absorbance, was purchased from pION, Woburn, MA. DMSO (Lot # 038K0710, GC grade, 99.5% purity; UV cutoff 280 nm) was purchased from Sigma/Aldrich Chemical Company, St. Louis, MO, USA. Syringe filters 0.22 microns were procured from Advanced Micro devices Pvt. Ltd. India.

2. Equipment:

HPLC Shimadzu Prominence LC-20AD, SIL-HTc, CTO-20AC Japan, Orbital Shaker (Heidolph, Germany)

3. Method:**a. Preparation of 1X System Solution (SS)**

SS (phosphate free buffer) was supplied as 40X concentrate (pH 2.6). The concentrate was diluted 40-folds with Milli-Q water and pH was adjusted to 7.4 with 0.5 N NaOH.

b. Assay Conditions

- Test compound concentration:
 - **22** (3.77 mg/mL)
 - **27** (2.00 mg/mL)
- Incubation time: 24 hours

c. Solubility analysis procedure

Compounds were weighed into 10 mL glass tubes and appropriate volumes of pH buffer were added to prepare a final concentration of as mentioned above. All the glass tubes were shaken at 250 RPM on an orbital shaker at room temperature ($24 \pm 2^\circ\text{C}$) for 24 hrs. After incubation, the test solution was filtered through 0.22 micron syringe filters and the concentration of the analyte in the filtrate was determined using HPLC.

4. ANALYSIS

A standard solution of 10 mM in DMSO stock was prepared which were further 100-folds diluted in acetonitrile: water (50:50) to get concentration of 100 μM . The filtrate samples obtained were diluted 100-folds in acetonitrile: water (50:50). Both the standard solution and filtrate samples were submitted for HPLC analysis.

5. DATA ANALYSIS

The test samples analyte areas were compared with the standard solution analyte area, calculation done using the following formula:

$$\frac{\text{Sample Area Ratio (Average)} \times \text{concentration of the Standard solution}}{\text{Standard area Ratio}}$$

Protein Binding:

Reference Materials:

Propranolol was evaluated in plasma from each species tested.

1. Materials

Monkey (Cynomolgus) Plasma, EDTA

Single-Use RED Plate with Inserts (product # 90006) was obtained from Thermo Scientific.

10X Phosphate Buffered Saline (PBS), Fisher (BP399)

2. Bioanalytical Method

Test samples were extracted from plasma by using a protein precipitation method. A working solution of VT-406 was used as the internal standard spiking solution. Extracts were analysed by HPLC-MS/MS using electrospray ionization.

3. Reagent Preparation

3.1. Phosphate Buffered Saline (PBS) Preparation

The PBS 10X stock solution was diluted 10-fold with purified water and then sterile filtered for a 1X PBS solution.

3.2. Plasma Internal Standard Working Solution

A working solution of VT-406 at 0.3125 μM in acetonitrile was used as the internal standard spiking solution.

4. Dialysis Mixture Preparation

Stock solutions were prepared from each compound received in DMSO:Acetonitrile (50:50, v/v) at a concentration of 20 mM. These solutions were further diluted to a 1 mM working solution in DMSO:Acetonitrile. Each 1 mM compound stock solution was further diluted to a 100 μM solution in 1X PBS. The 100 μM (1X PBS dilution) was diluted to 5 μM final concentration in appropriate species plasma.

5. Sample Analysis

5.1. Test Article Incubation

For all samples and control, 200 μ l of the 5 μ M spiked plasma samples were added to the sample chamber of the RED device insert, and 350 μ l of 1X PBS was added to the buffer chamber. The samples were incubated at 37°C (humidified) at approximately 150 rpm on a shaker for 6 hours.

5.2. Production of Matrix Equivalency

Following the incubation, equal volumes (50 μ L each) of post dialysis samples from both the plasma and the buffer chambers were removed and placed in a 96 well extraction plate. Equal volumes of fresh buffer or plasma were added, respectively, resulting in 1:1 (v/v) plasma:buffer for each sample.

5.3. Precipitation Method

Acetonitrile (150 μ L) was added to the 100 μ L plasma:buffer mixtures. The samples were vortexed for approximately 10 minutes and centrifuged at approximately 4,000 rpm at 4 °C for 10 minutes. The supernatant was transferred for LC-MS/MS analysis.

6. Data Analysis

HPLC-MS/MS data were acquired using the proprietary software application MassHunter Workstation Acquisition. Data were processed (integrated) using the software application MassHunter Quantitative Analysis for QQQ. Area response for each test article or control sample from the plasma chamber and buffer chamber was used for data calculations. Descriptive statistics were performed using Excel 2013.

To calculate the amount of protein-bound compound in plasma (after the matrices were made equivalent) the amount of total compound in the plasma chamber was compared with the amount of free compound that migrated into the buffer chamber. Per device instructions,

% Free = 100 x (Buffer chamber response/Plasma chamber response)

% Bound = 100 - % Free

Compound Stability in Cynomolgus Monkey Liver Microsomes:

Reference Materials

Propranolol was obtained from Sigma Aldrich. Monkey liver microsomes were obtained from Gibco. NADPH was obtained from Corning® Gentest™.

3.3. Test Method

Each sample was analyzed using LC/MS-MS with electrospray ionization with methods optimized for the detection of each compound. The percentage compound remaining at each time point (x) was determined by comparing the peak areas of the test compound at Tx with that at T0 minutes and was calculated as follows:

% remaining = [Area at Tx/Area at T0] \times 100%

3.4. Reagent Preparation

3.4.1. Phosphate Buffer Preparation

The KPO4 buffer was prepared by combining 500 mM solutions of the mono-basic and di-basic to yield a pH7.4 solution. That solution was diluted to 50 mM for use in the assay.

3.5. Reaction Mixture Preparation

Previously prepared 20 mM stock solutions (50:50 acetonitrile:DMSO) of test compounds were used in this study. These solutions were further diluted in phosphate buffer to concentrations of 100 μ M. Human liver microsome suspensions (2.2 mg/mL) and NADPH (2.2X) were also prepared in phosphate buffer.

Reaction mixtures were prepared by combining aliquots of the components to achieve final concentrations as shown in Table 1. Mixing was affected by multiple aspirate-dispense cycles of the automated liquid-handling system. Reaction mixtures were prepared and incubated at 37 °C throughout the one-hour incubation period.

Table 1 Reaction Mixture Composition

Component	Liver Microsomes	Study Compound	NADPH (1X per supplier recommendation)
Reaction Mixture Concentration	1 mg/mL	1 μ M	1.3mM NADP ⁺ 3.3mM glucose-6-phosphate 0.4U/mL glucose-6-phosphate dehydrogenase 3.3mM magnesium chloride

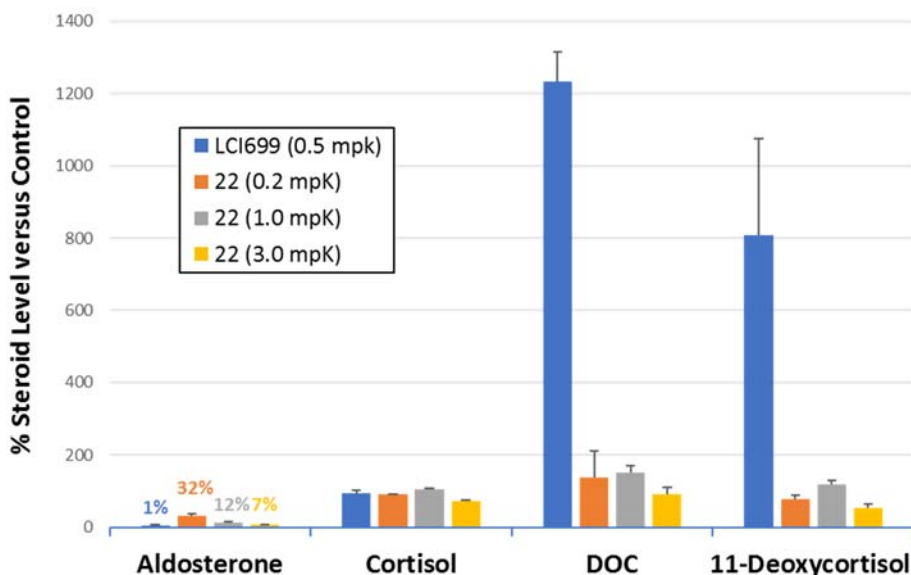
3.6. Sample Analysis

Samples were collected for analysis immediately after preparation of reaction mixtures (Time 0), and at subsequent intervals of 5, 20, 35 and 65 minutes. Samples were added to cold isopropyl alcohol to stop the reaction. After the last time point, the plates were heat sealed and stored at room temperature for 24 hours. The plates were then shaken for 5 minutes and centrifuged at 4000 rpm for 5 minutes. Aliquots of the resulting supernatants were transferred to a 96-well polypropylene plate that was heat-sealed for analysis. Analysis was by gradient HPLC with electrospray-ionization triple-quadrupole mass spectrometry in the positive ion mode.

4. RESULTS

The peak area response of each analyte (study compound) at each time point (Tx) was compared with area response at time=0 (T0) to calculate the percentage of each study compound remaining in the reaction mixture.

Cynomolgus Monkey ACTH Challenge Results:

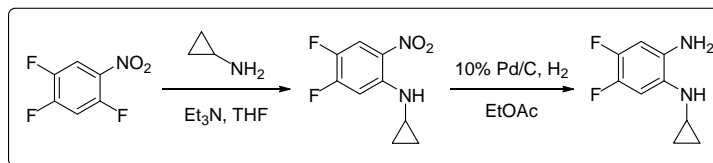


	% Control (Day 8 AUC/Day 1 AUC)			
	Aldosterone	Cortisol	DOC	11-Deoxycortisol
LCI699 (0.5 mpk)	1	94	1232	810
21 (0.2 mpK)	32	91	139	77
21 (1.0 mpK)	12	104	151	118
21 (3.0 mpK)	7	73	92	53

Experimental procedures for compounds 13, 22, and 27:

Preparation of Compound 13

Scheme:



***N*-Cyclopropyl-4,5-difluoro-2-nitroaniline**

To 1,2,4-trifluoro-5-nitrobenzene (20 g, 112.99 mmol) in tetrahydrofuran (THF) (100 mL) under an inert atmosphere was added triethylamine (47.2 mL, 338.98 mmol) and cyclopropanamine (7 g, 124.29 mmol) drop wise at 0 °C. The reaction mixture was stirred at 60 °C for 2 h. After consumption of starting material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2 x 100 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 5% EtOAc/hexane) to afford *N*-cyclopropyl-4,5-difluoro-2-nitroaniline (11.8 g, 55.14 mmol, 48%) as yellow solid.

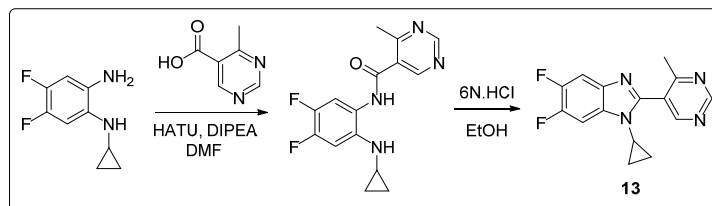
¹H NMR (500MHz, DMSO-*d*₆): δ 8.20-8.12 (m, 1H), 8.10 (s, 1H), 7.35-7.30 (m, 1H), 2.63-2.60 (m, 1H), 0.90-0.83 (m, 2H), 0.65-0.60 (m, 2H).

***N*¹-Cyclopropyl-4,5-difluorobenzene-1,2-diamine**

To a stirred solution of *N*-cyclopropyl-4,5-difluoro-2-nitroaniline (5 g, 23.36 mmol) in ethyl acetate (100 mL) under an inert atmosphere was added 10% Pd/C (50% wet, 2.5 g) at room temperature. The reaction mixture was stirred at room temperature under a hydrogen atmosphere (balloon pressure) for 5 h. After consumption of starting material (by TLC), the reaction mixture was filtered through a pad of celite and washed with methanol (15 mL) and EtOAc (10 mL). The filtrate was concentrated under reduced pressure to obtain *N*¹-cyclopropyl-4,5-difluorobenzene-1,2-diamine (4 g, crude) as brown viscous syrup which was used directly without further purification.

¹H NMR (400MHz, DMSO-*d*₆): δ 6.63-6.60 (m, 1H), 6.48-6.43 (m, 1H), 5.17 (br s, 1H), 4.60(brs, 2H), 2.32-2.28 (m, 1H), 0.70-0.66 (m, 2H), 0.39-0.35 (m, 2H)

Scheme:



***N*-(2-(Cyclopropylamino)-4,5-difluorophenyl)-4-methylpyrimidine-5-carboxamide**

To a stirred solution of *N*1-cyclopropyl-4,5-difluorobenzene-1,2-diamine (300 mg, 1.63 mmol) in DMF (5 mL) under an inert atmosphere was added 4-methylpyrimidine-5-carboxylic acid (309 mg, 1.95 mmol), HATU (745 g, 1.95 mmol) and ethyldiisopropylamine (1.16 mL, 6.52 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 16 h. After consumption of starting material (by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 2% MeOH/CH₂Cl₂) to afford *N*-(2-(cyclopropylamino)-4,5-difluorophenyl)-4-methylpyrimidine-5-carboxamide (150 mg, 0.50 mmol, 30%) as yellow solid.

¹H NMR (400 MHz, DMSO-*d*₆): δ 9.69 (s, 1H), 9.13 (s, 1H), 8.98 (s, 1H), 7.50-7.45 (m, 1H), 6.96-6.91 (m, 1H), 5.84 (s, 1H), 2.60 (s, 3H), 2.37-2.35 (m, 1H), 0.78-0.69 (m, 2H), 0.48-0.36 (m, 2H)

1-Cyclopropyl-5,6-difluoro-2-(4-methylpyrimidin-5-yl)-1H-benzo[*d*]imidazole (13): To a stirred solution of *N*-(2-(cyclopropylamino)-4,5-difluorophenyl)-4-methylpyrimidine-5-carboxamide (150 mg, 0.50 mmol) in EtOH (1 mL) under an inert atmosphere was added 6N HCl (3 mL) at room temperature. The reaction mixture was stirred at 80 °C for 45 min. After consumption of starting material (by TLC), the reaction mixture was diluted with saturated sodium carbonate solution (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 50%EtOAc/Hexane) which was further purified by preparative HPLC to afford 1-cyclopropyl-5,6-difluoro-2-(4-methylpyrimidin-5-yl)-1H-benzo[*d*]imidazole **13** (20 mg, 0.07 mmol, 14%) as an off white solid.

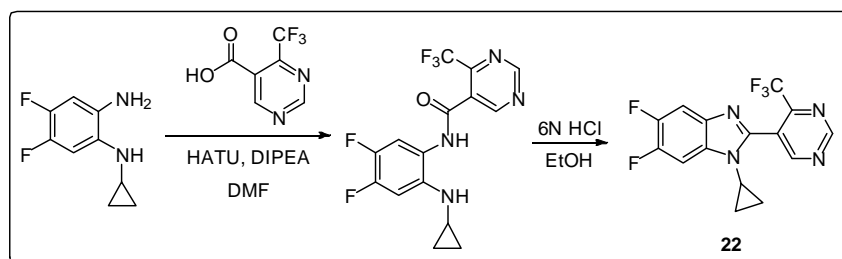
¹H NMR (400 MHz, CD₃OD): δ 9.20 (s, 1H), 8.96 (s, 1H), 7.72-7.68 (m, 1H), 7.62-7.51 (m, 1H), 3.60-3.54 (m, 1H), 2.58 (s, 3H), 1.11-1.01 (m, 2H), 0.77-0.66 (m, 2H)

LC-MS: *m/z* 287.1 [M+H]⁺ at 3.16 RT.

HPLC: 95.34%.

Preparation of Compound 22

Scheme:



***N*-(2-(Cyclopropylamino)-4,5-difluorophenyl)-4-(trifluoromethyl)pyrimidine-5-carboxamide**

To a stirred solution of *N*1-cyclopropyl-4,5-difluorobenzene-1,2-diamine (200 mg, 1.10 mmol) in DMF (4 mL) under an inert atmosphere was added 4-(trifluoromethyl)pyrimidine-5-carboxylic acid (208 mg, 1.1 mmol), HATU (486 mg, 1.28 mmol) and ethyldiisopropylamine (0.72 mL, 4.32 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. After consumption of starting material (by TLC), the reaction mixture was diluted with water (30 mL) and extracted with ether (2 x 40 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 20% EtOAc/hexane) to afford *N*-(2-(cyclopropylamino)-4,5-difluorophenyl)-4-(trifluoromethyl)pyrimidine-5-carboxamide (200 mg, 0.55 mmol, 34%) as a dark brown solid.

¹H NMR (500 MHz, DMSO-*d*₆): δ 10.00 (s, 1H), 9.57 (s, 1H), 9.51 (s, 1H), 7.42-7.38 (m, 1H), 6.99-6.95 (m, 1H), 5.70 (s, 1H), 2.41-2.37 (m, 1H), 0.79-0.72 (m, 2H), 0.47-0.38 (m, 2H)

1-Cyclopropyl-5,6-difluoro-2-(4-(trifluoromethyl)pyrimidin-5-yl)-1H-benzo[*d*]imidazole (22): To a stirred solution of *N*-(2-(cyclopropylamino)-4,5-difluorophenyl)-4-(trifluoromethyl)pyrimidine-5-carboxamide

(150 g, 1.50 mmol) in EtOH (3 mL) under an inert atmosphere was added 6N HCl (3 mL) at room temperature. The reaction mixture was stirred at 80 °C for 3 h. After consumption of starting material (by TLC), the reaction mixture was diluted with saturated sodium bicarbonate solution (10 mL) and extracted with EtOAc (2 x 10 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by preparative HPLC to afford 1-cyclopropyl-5,6-difluoro-2-(4-(trifluoromethyl)pyrimidin-5-yl)-1*H*-benzo[*d*]imidazole **22** (80 mg, 0.23 mmol, 56%) as an off white solid.

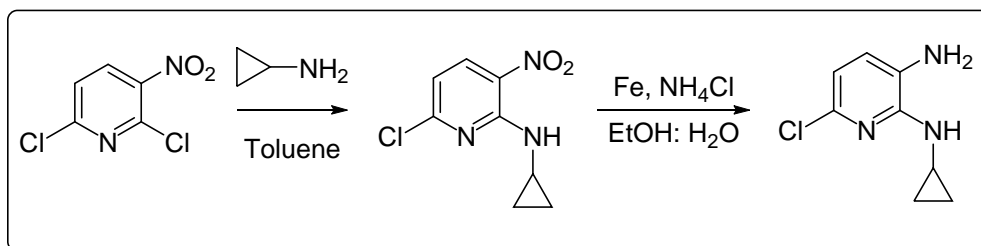
¹H NMR (400 MHz, CD₃OD): δ 9.59 (s, 1H), 9.33 (s, 1H), 7.74-7.69 (m, 1H), 7.64-7.60 (m, 1H), 3.53-3.39 (m, 1H), 1.09-0.96 (m, 2H), 0.81-0.66 (m, 2H)

LC-MS: *m/z* 340.9 [M+H]⁺ at 2.99 RT.

HPLC: 97.45%.

HRMS (EI): calculated for C₁₅H₁₀N₄F₅ (M+H) 341.0820, observed 341.0827.

Preparation of Compound 27



6-Chloro-N-cyclopropyl-3-nitropyridin-2-amine

To a stirred solution of 2,6-dichloro-3-nitropyridine (5 g, 26.04 mmol) in toluene (25 mL) under an inert atmosphere was added cyclopropylamine (3.7 mL, 52.08 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was warmed to room temperature and stirred for 2 h. After consumption of starting material (by TLC), the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (2 x 50 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 10% EtOAc/Hexane) to afford 6-chloro-N-cyclopropyl-3-nitropyridin-2-amine (4 g, 18.77 mmol, 72%) as a pale yellow solid.

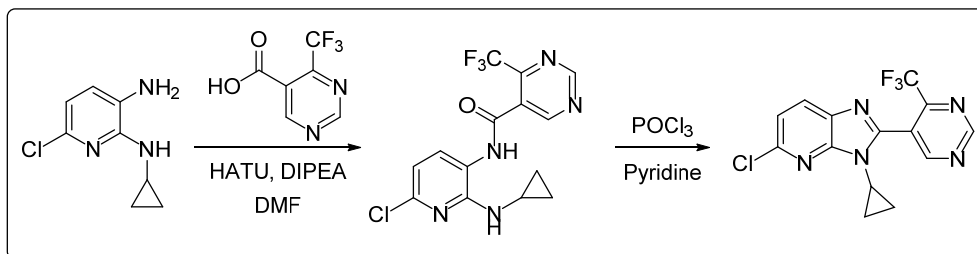
¹H NMR (500 MHz, CDCl₃): δ 8.34 (d, *J* = 8.7 Hz, 2H), 6.66 (d, *J* = 8.7 Hz, 1H), 3.10-3.05 (m, 1H), 0.97-0.93 (m, 2H), 0.67-0.64 (m, 2H)

6-Chloro-*N*²-cyclopropylpyridine-2,3-diamine

To a stirred solution of 6-chloro-*N*-cyclopropyl-3-nitropyridin-2-amine (1 g, 4.69 mmol) in EtOH: water (1:1, 10 mL) under an inert atmosphere was added iron (1.3 g, 23.47 mmol) and ammonium chloride (1.2 g, 23.47 mmol) at room temperature. The reaction mixture was heated to 80 °C for 1 h. After consumption of starting material (by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to obtain 6-chloro-*N*²-cyclopropylpyridine-2,3-diamine (700 mg, crude) as a pale yellow solid which was used directly without further purification.

LC-MS: *m/z* 183.9 [M+H]⁺ at 2.07 RT.

Scheme:



N-(6-Chloro-2-(cyclopropylamino)pyridin-3-yl)-4-(trifluoromethyl)pyrimidine-5-carboxamide

To a stirred solution of 6-chloro-*N*²-cyclopropylpyridine-2,3-diamine (500 mg, 2.73 mmol) in DMF (6 mL) under an inert atmosphere was added 4-(trifluoromethyl)pyrimidine-5-carboxylic acid (630 mg, 3.27 mmol), HATU (1.25 g, 3.27 mmol) and ethyldiisopropylamine (1.96 mL, 10.92 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. After consumption of starting material (by TLC), the reaction mixture was diluted with water (30 mL) and extracted with EtOAc (2 x 30 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to obtain *N*-(6-chloro-2-(cyclopropylamino)pyridin-3-yl)-4-(trifluoromethyl)pyrimidine-5-carboxamide (400 mg, 1.12 mmol, 41%) as a brown solid.

¹H NMR (400 MHz, DMSO-*d*₆): δ 10.00 (s, 1H), 9.58 (s, 1H), 9.52 (s, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.57 (s, 1H), 2.75-2.71 (m, 1H), 0.78-0.70 (m, 2H), 0.49-0.42 (m, 2H)

5-Chloro-3-cyclopropyl-2-(4-(trifluoromethyl)pyrimidin-5-yl)-3H-imidazo[4,5-*b*]pyridine

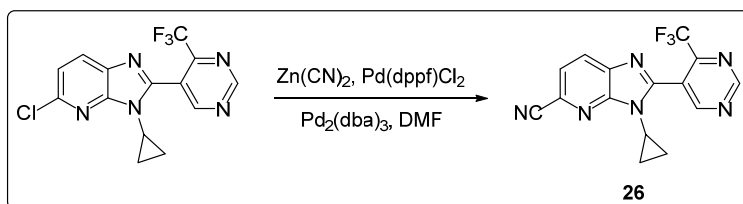
To a stirred solution of *N*-(6-chloro-2-(cyclopropylamino)pyridin-3-yl)-4-(trifluoromethyl)pyrimidine-5-carboxamide (200 mg, 0.56 mmol) in pyridine (0.4 mL) under an inert atmosphere was added phosphoryl chloride (0.3 mL, 2.80 mmol) at room temperature. The reaction mixture was heated to 120 °C and stirred for 16 h. After consumption of starting material (by TLC), the reaction mixture was basified with saturated sodium carbonate solution (30 mL) and extracted with EtOAc (2 x 30 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 25% EtOAc/hexane) to afford 5-chloro-3-cyclopropyl-2-(4-(trifluoromethyl)pyrimidin-5-yl)-3H-imidazo[4,5-*b*]pyridine (20 mg, 0.06 mmol, 10%) as a white solid.

¹H NMR (400 MHz, CD₃OD): δ 9.61 (s, 1H), 9.38 (s, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 3.44-3.39 (m, 1H), 1.08-1.00 (m, 2H), 0.95-0.90 (m, 2H)

LC-MS: *m/z* 340 [M+H]⁺ at 2.82 RT.

HPLC: 99.27%.

Scheme:



3-Cyclopropyl-2-(4-(trifluoromethyl)pyrimidin-5-yl)-3H-imidazo[4,5-*b*]pyridine-5-carbonitrile (27): To a stirred solution of 5-chloro-3-cyclopropyl-2-(4-(trifluoromethyl)pyrimidin-5-yl)-3H-imidazo[4,5-*b*]pyridine (70 mg, 0.21 mmol) in DMF (1 mL) at room temperature was added zinc cyanide (48.3 mg, 0.41 mmol) and the mixture was purged under argon for 10 min. Pd(dppf)Cl₂ (7.53 mg, 0.01 mmol) and Pd₂(dba)₃ (9.42 mg, 0.01 mmol) were added and the mixture was purged under argon for an additional 10 min. The reaction mixture was heated to 130-140 °C for 16 h in sealed tube and then cooled, diluted with

ice cold water (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: 20% EtOAc/hexane) to afford 3-cyclopropyl-2-(4-(trifluoromethyl)pyrimidin-5-yl)-3*H*-imidazo[4, 5-*b*]pyridine-5-carbonitrile (**27**) (26 mg, 0.08 mmol, 38%) as a pale yellow solid.

¹H NMR (400 MHz, CDCl₃): δ 9.60 (s, 1H), 9.13 (s, 1H), 8.21 (d, *J* = 8.3 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 1H), 3.39-3.31(m, 1H), 1.14-1.08 (m, 2H), 0.96-0.91 (m, 2H)

LC-MS: *m/z* 331 [M+H]⁺ at 2.33 RT

HPLC: 99.53%