Biochemical and Structural Characterization of Selective Allosteric Inhibitors of the
*Plasmodium falciparum* Drug Target, Prolyl-tRNA-synthetase.

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SUPPLEMENTAL INFORMATION:

Figure S1: Comparison of PfProRS gene constructs expressed and purified. Five constructs were cloned and expressed in *E. coli*. B3 (AA224 to 746) was insoluble; all other recombinant proteins were expressed and purified. Construct B4 (AA249-746) crystallized and diffracted and all solved structures were based off this construct. B2 (AA7-746) was the closest to full-length and retained full enzymatic activity, this recombinant protein was used in all subsequent screening and activity assays.

| Construct ID | Forward Primer sequence |
|--------------|-------------------------|
| PlfaA.18681.a.B1 | CTCACCACCACCACCACTATGAATATAATAATACAAATGGAGAAATCA |
| PlfaA.18681.a.B2 | CTCACCACCACCACCACTATGGGAGAAATCATTATCCCCCAA |
| PlfaA.18681.a.B3 | CTCACCACCACCACCACTATGAATCATATCAAGGATACCATTTTA |
| PlfaA.18681.a.B4 | CTCACCACCACCACCACTATGAATATTTTAGGTATCACTTCA |
| PlfaA.18681.a.B5 | CTCACCACCACCACCACTATGAATCATATCAAGGATACCATTTTA |

Table S1: Primer sequences used for the amplification of ProRS from *Plasmodium falciparum 3D7* cDNA. PCR amplicons were gel purified from DNA agarose and were treated with T7 polymerase in the presence of dCTP to prepare them for ligation-independent cloning into the BG1861 LIC-ready vector.
Supplemental Information

Figure S2: Dose response curve for halofuginone and N-Boc-halofuginone in the [³H]-L-proline aminoacylation incorporation assay (10 nM ProRS enzyme, 100 nM [³H]-L-proline, 100 μM ATP and 400 μg/ml yeast tRNA was incubated for 2 h at 25 °C; Z' = .67). Lines are fit to a Hill Slope = 1 and IC₅₀ for Halofuginone is calculated to be 0.011 μM and N-Boc-Halofuginone is >3 μM. Error bars indicate the standard deviation of triplicate samples for each experimental condition.

| Compound [μM] | 1250 | 625 | 313 | 156 | 78 | 39 | 19.5 | 9.8 | 4.9 | 2.5 |
|---------------|------|-----|-----|-----|----|----|------|-----|-----|-----|
| Pf TCMDC-124506 | 97.00 | 96.24 | 80.39 | 79.51 | 55.81 | 30.90 | 10.76 | 3.73 | -2.40 | -0.36 |
| Hs TCMDC-124506 | 0.04 | 0.12 | 0.26 | 0.82 | 0.84 | 0.26 | -0.08 | 0.01 | 0.28 | 0.30 |
| Compound [μM] | 750 | 375 | 187.50 | 93.75 | 46.88 | 23.44 | 11.72 | 5.86 | 2.93 | 1.46 |
| Pf ProRS glyburide | 102.06 | 94.04 | 87.00 | 81.37 | 60.01 | 39.68 | 23.76 | 12.40 | 6.24 | 1.82 |
| Hs ProRS glyburide | 36.10 | 15.06 | 4.93 | 1.41 | 0.36 | 0.12 | 0.01 | 0.29 | -0.04 | 0.03 |
| Pf ProRS telmisartan | 11.82 | 15.48 | 80.83 | 48.77 | 13.45 | 3.94 | 2.19 | -0.08 | -0.17 | -0.06 |
| Hs ProRS telmisartan | 0.58 | 0.25 | 4.23 | 0.37 | -0.02 | 0.00 | 0.01 | -0.10 | 0.04 | 0.05 |
| Pf ProRS flunixin meglumine | 99.81 | 87.10 | 62.03 | 30.73 | 11.40 | 2.17 | 1.63 | -0.41 | -0.70 | -0.28 |
| Hs ProRS flunixin meglumine | 1.37 | 0.43 | 0.03 | -0.02 | 0.27 | 0.52 | 0.37 | 0.17 | 0.20 | 0.19 |
| Compound [μM] | 500 | 250 | 125 | 62.50 | 31.25 | 15.63 | 7.81 | 3.91 | 1.95 | 0.98 |
| Pf ProRS diclazuril | 80.19 | 73.70 | 42.95 | 19.54 | 7.77 | 2.75 | 2.35 | -0.59 | -0.74 | -1.18 |
| Hs ProRS diclazuril | 9.51 | 5.06 | 0.68 | 0.07 | 0.40 | 0.09 | 0.42 | 0.28 | 0.08 | -0.02 |
| Compound [μM] | 30.00 | 10.00 | 3.33 | 1.46 | 0.37 | 0.12 |
| Pf Sj000368256 | 39.66 | 0.96 | 0.44 | -0.33 | -0.09 | -0.29 |
| Hs Sj000368256 | 0.41 | 0.08 | 0.06 | -0.15 | 0.01 | -0.10 |
| Compound [μM] | 25.00 | 12.50 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.20 | 0.10 | 0.05 |
| Pf ProRS halofuginone | 93.87 | 93.63 | 94.98 | 99.11 | 98.87 | 90.04 | 68.96 | 34.36 | 10.85 | 2.49 |
| Hs ProRS halofuginone | 89.21 | 85.67 | 74.26 | 64.10 | 46.54 | 24.70 | 9.43 | 1.95 | 0.21 | -0.14 |
| Compound [μM] | 22.50 | 7.50 | 2.50 | 0.83 | 0.28 | 0.09 | 0.03 |
| Pf TCMDC-135537 | 84.85 | 39.01 | 11.72 | 1.73 | -1.18 | -1.11 | -1.72 |
| Hs TCMDC-135537 | 0.55 | 0.28 | 0.00 | 0.80 | 0.03 | -0.49 | -0.20 |
| Pf TCMDC-135512 | 56.75 | 40.43 | 11.81 | 3.62 | 2.37 | 2.63 | 1.13 |
| Hs TCMDC-135512 | -0.20 | -0.42 | -0.12 | -0.24 | -0.52 | 0.03 | -0.42 |
| Pf TCMDC-125377 | 40.14 | 33.88 | 24.66 | 15.45 | 6.80 | 2.41 | 1.17 |
| Hs TCMDC-125377 | 0.03 | 0.20 | -0.37 | -0.91 | -0.22 | -0.10 | 0.18 |
| Compound [μM] | 2.50 | 1.88 | 0.94 | 0.47 | 0.23 | 0.12 | 0.06 | 0.03 |
| Pf ProRS MMV306025 | 72.85 | 29.99 | 28.43 | 21.78 | 4.50 | 0.62 | -0.04 | -0.55 |
| Hs ProRS MMV306025 | 21.64 | 14.93 | 4.10 | 0.67 | 0.10 | -0.07 | 0.30 |
### Table S2: Percentage inhibition of ProRS enzyme (*Pf* = *Plasmodium*, *Hs* = human) activity by ATP-depletion assay. Full inhibition (100) was equivalent to controls of no enzyme or no substrate and no inhibition (0) was equivalent to all reagents with 2% DMSO (no compound). Percentage inhibition values are the average of measurements in triplicate. The simplified molecular-input line-entry system (SMILES) formula for each compound is indicated in the lower portion of the table.
A. Na⁺

Figure S3 A. The effects of TCMDC-124506 (tested at 1 μM and 5 μM), DMSO (0.1% v/v; solvent control) and the spiroindolone KAE609 (50 nM) on the fluorescence of isolated 3D7 trophozoites loaded with the Na⁺-sensitive dye SBFI. An increase in fluorescence ratio corresponds to an increase in the intracellular [Na⁺] ([Na⁺]ᵢ); the fluorescence ratios corresponding to 130 mM Na⁺ and 0 mM Na⁺ are shown. TCMDC-124506 and KAE609, but not DMSO, gave rise to an increase in [Na⁺]ᵢ.

B. pH

The effects of various compounds on pHᵢ in isolated 3D7 trophozoites. DMSO (0.1% v/v; solvent control) did not increase pHᵢ, and on addition of the V-type H⁺ pump inhibitor concanamycin A (100 nM; red arrow) the pHᵢ decreased to below the pH of the extracellular solution. TCMDC-124506 (tested at 5 μM) and KAE609 (50 nM) caused an increase in pHᵢ and a reduction in the extent of the concanamycin A-induced acidification. In A and B, the traces shown are from a single experiment, and are representative of those obtained in two similar experiments.
**Figure S4** Competition of proline and ATP with TCMDC-124506 for P/ProRS demonstrates that both compete with TCMDC-124506.

**A. IC50 of TCMDC-124506 as a function of proline**

![Graph showing the IC50 of TCMDC-124506 as a function of proline concentration.](image)

- **Parameter** | **Value** | **Std. Error** |
- --- | --- | --- |
- IC 50 | 87.96 | 4.10 |
- Slope factor | 2.35 | 0.27 |

| Parameter | Value | Std. Error |
| --- | --- | --- |
| IC 50 | 109.98 | 9.72 |
| Slope factor | 1.82 | 0.19 |

| Parameter | Value | Std. Error |
| --- | --- | --- |
| IC 50 | 127.56 | 14.20 |
| Slope factor | 3.73 | 1.10 |

| Parameter | Value | Std. Error |
| --- | --- | --- |
| IC 50 | 151.43 | 15.29 |
| Slope factor | 3.09 | 0.53 |

**B. Linear relationship with upward trend indicates competitive behavior of proline with TCMDC-124506**

Cheng-Prusoff equation for competitive inhibitor

\[
\text{IC}_{50} = K_i \left(1 + \frac{[S]}{K_m}\right)
\]

\[
\text{IC}_{50} = K_i + [S] \frac{K_i}{K_m}
\]

![Graph showing the linear relationship between TCMDC IC50 and proline concentration.](image)

**Correlation coefficient:** 0.9596

| Parameter | Value | Std. Error |
| --- | --- | --- |
| a (intercept) | 80.0227 | 4.9020 |
| b (gradient) | 0.3292 | 0.0682 |

For A/B

\[
\text{SD} = \sqrt{\frac{(a^2B^2 + b^2A^2)}{B^2}}
\]

- \(K_i = 80.0 \pm 4.9 \mu M\)
- \(K_m = 243 \pm 17 \mu M\)
C. TCMDC-124506 IC\textsubscript{50} as a function of ATP

![Graph showing ATP consumption as a function of TCMDC-124506 concentration]

| Parameter   | Value  | Std. Error |
|-------------|--------|------------|
| IC\textsubscript{50} | 74.36  | 5.31       |
| Slope factor | 1.75   | 0.11       |

| Parameter   | Value  | Std. Error |
|-------------|--------|------------|
| IC\textsubscript{50} | 102.94 | 22.21     |
| Slope factor | 1.90   | 0.32       |

| Parameter   | Value  | Std. Error |
|-------------|--------|------------|
| IC\textsubscript{50} | 145.08 | 2.73       |
| Slope factor | 4.62   | 0.81       |

D. Linear relationship with upward trend indicates TCMDC-124506 is competitive with ATP

![Graph showing linear relationship between ATP and TCMDC-124506 IC\textsubscript{50}]

Correlation coefficient: 0.9998

| Parameter | Value    | Std. Error |
|-----------|----------|------------|
| a (intercept) | 51.0230  | 1.5690     |
| b (gradient) | 7.8404   | 0.1464     |

For \( A/B \):

\[
SD = \sqrt{\frac{(a^2B^2 + b^2A^2)}{B^2}}
\]

\( K = 51.0 \pm 1.6 \text{ \(\mu\text{M}\)} \)

\( K'_m = 6.51 \pm 1.84 \text{ \(\mu\text{M}\)} \)

**Figure S4 legend:** The experiments were run as described for the ATP consumption assay in the Materials and Methods except that the proline or the ATP was varied and the IC\textsubscript{50} for TCMDC-124506 was determined for each condition.
Figure S5. General synthetic route for compounds (1)-(6)

Conditions: (a) General procedure A: NaH, THF, 40°C, 2h; (b) General procedure B: hydrazine, MeOH, 100°C, microwave, 1h; (c) General procedure C: isocyanate, THF, rt.

Figure S6. Synthetic route for compounds (12-17)

Conditions: (a) NBS, acetonitrile, 0°C, 1h, 73% yield; (b) General procedure E: boronic acid, palladium acetate, X-Phos, potassium phosphate, toluene/water, 95°C; (c) isocyanate, THF or Dioxane, rt or 100°C.
Figure S7. Synthetic route for compounds (19), (23), (25) and (26)

Conditions: (a) NaH, EtOAc, 0°C, 3h; (b) MeNHNH₂, EtOH, 80°C, 8h, 40% yield; (c) Boc₂O, DMAP, THF, 40°C, 1h, 64% yield; (d) NBS, AIBN, CCl₄, 80°C, 8h, 63% yield; (e) NaCN, DMF, 2h, r.t., 38% yield; (f) HCl,
Supplemental Information

EtOAc.; (g) General Procedure C, 30% yield; (h) NaOH, EtOH/H₂O, 80 °C; (i) BH₃, THF, THF; (l) General Procedure C; (m) NaOH, EtOH/H₂O, 6% yield; (n) MsCl, TEA, DCM, 0-10 °C; (o) Me₂NH.HCl, TEA, DCM, 11% yield; (p) morpholine, THF, 17% yield.

Figure S8. Synthetic route for compounds (20) and (22)

Conditions: (a) AcOK, KI, DMF, 80 °C, 2h; (b) K₂CO₃, MeOH, r.t., 16h; (c) KMnO₄, H₂O, 15 °C, 15h; NH₄Cl, DIPEA, HATU, DMF, DCM, r.t., 16h; (e) HCl, EtOAc; (f) 1-fluoro-4-isocyanatobenzene, THF; (g) TFAA/Py, DCM, 0 °C, 1h.
Figure S9. Synthetic route for compounds (21) and (24)

Conditions: (a) MnO₂, DCM, 2h; (b) diethyl (cyanomethyl)phosphonate, t-BuOK, THF, 2h; (c) Pd/C, H₂, MeOH, 2h; (d) HCl/EtOAc; (e) 1-fluoro-4-isocyanatobenzene, THF; (f) NaOH, EtOH, 70°C, 12h; (g) NH₄Cl, HOBt, EDCI, DIPEA, DMF.

Supplemental Experimental Procedures

Biology Methodology

*Plasmodium falciparum* screening

Assays against *P. falciparum* were conducted as previously described (Trager and Jensen 1976; Bennett et al., 2004; Snyder et al., 2007). Cultures of the widely-used malaria reference strain of chloroquine-sensitive *Plasmodium falciparum* strain 3D7 were maintained in a 5% suspension of human red blood cells (obtained from East of Scotland Blood Transfusion Service, Ninewells Hospital, Dundee) cultured in RPMI 1640 medium (pH 7.3) supplemented with 0.5% Albumax II (Gibco Life Technologies, San Diego, CA), 12 mM sodium bicarbonate, 0.2 mM hypoxanthine and 20 mg/L gentamicin at 37°C, in a humidified atmosphere of 1% O₂, 3% CO₂ with a balance of nitrogen. Growth inhibition was quantified using a fluorescence assay utilising the binding of SYBR green to double stranded DNA, which emits a fluorescent signal at 528 nm after excitation at
Supplemental Information

485nm (Plouffe et al., 2008). Mefloquine (potency range 30-60 nM) was used as a drug control to monitor the quality of the assay ($Z' > 0.6$ to 0.8, Signal to background ≥3, where $Z'$ is a measure of the discrimination between the positive and negative controls on a screen plate). Compound bioactivity was expressed as EC$_{50}$, the effective concentration of compound causing 50% inhibition of parasite growth.

**Thermal Melt Analysis**

Color fluorimetry experiments to measure changes in protein thermal stability were conducted as previously described (Vedadi et al., 2006). Serial dilutions of the purified recombinant protein samples were prepared in 96 well PCR plates (Bio-Rad HSP9655) in a buffer containing 100mM HEPES, 150mM NaCl, pH 7.5 and SYPRO Orange Dye (Life Technologies S-6650). Protein stability for protein samples containing the substrates ATP and L-Proline were compared with apo protein samples by measuring an increase in fluorescence as detected on an MJ Research DNA Engine Opticon 2 qPCR thermocycler from 20°C to 99°C at half degree increments.

**In vitro Cell Assay Data Analysis**

All data was processed using IDBS ActivityBase raw data was converted into per cent inhibition through linear regression by setting the high inhibition control to 100% and the no inhibition control as 0%. Quality control criteria for passing plates were as follows: $Z' > 0.5$, S:B> 3, %CV$_{(no inhibition control)} < 15$. The formula used to calculate $Z'$ is:

$$ 3 \times \left( \frac{\text{StDev}_{\text{high}} + \text{StDev}_{\text{low}}}{\text{ABS}(\text{Mean}_{\text{high}} - \text{Mean}_{\text{low}})} \right) $$

All EC$_{50}$ Curve fitting was undertaken using XLFit version 4.2 using Model 205 with the following 4 parametric equation:

$$ y = A + \frac{B - A}{1 + \left( \frac{C}{x} \right)^D} X + \frac{(B-A)}{(1+(C/x)^D)}, \text{where } A=\% \text{ inhibition at bottom, } B=\% \text{ inhibition at top, } C= $$

EC$_{50}$, D= slope, x= inhibitor concentration and y= % inhibition. If curve did not reach 100% of inhibition, B was fixed to 100 only when at least 50% of inhibition was reached.

**Aqueous solubility**

The aqueous solubility of the test compounds was measured using laser nephelometry, as described previously (Patterson et al., 2013). Compounds were subject to serial dilution from 10 mg/mL to 0.5 mg/mL in DMSO. An aliquot was then mixed with MilliQ water to obtain an aqueous dilution plate with a final concentration range of 100 – 5 μg/mL, with a final DMSO concentration of 1.0%. Triplicate aliquots were transferred to a flat bottomed polystyrene plate which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against the aqueous solubility (μg/mL). Assays were run in triplicate.

**Intrinsic Clearance (CLi) experiments**

The procedure was carried out as reported previously (Patterson et al., 2013). Test compound (0.5 μM) was incubated with female CD1 mouse liver microsomes (Xenotech LLC TM : 0.5 mg/mL 50 mM potassium phosphate buffer, pH 7.4) and the reaction started with addition of excess NADPH (8 mg/mL 50 mM potassium phosphate buffer, pH 7.4). Immediately, at time zero, then at 3, 6, 9, 15 and 30 min an aliquot (50 µL) of the incubation mixture was removed and mixed with acetonitrile (100 µL) to stop the reaction. Internal standard was added to all samples, the samples were centrifuged to sediment precipitated protein and the plates then sealed prior to UPLCMSMS analysis using a Quattro Premier XE (Waters Corporation, USA).

XLfit (IDBS, UK) was used to calculate the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each timepoint. The rate of intrinsic clearance (CLi) of each test compound was then calculated using the following calculation:

$$ \text{CLi (mL/min/g liver)} = k \times V \times \text{Microsomal protein yield} $$

Where V (mL/mg protein) is the incubation volume/mg protein added and microsomal protein yield is taken as 52.5mg protein per g liver. Verapamil (0.5 μM) was used as a positive control to confirm acceptable assay performance. Experiments were performed using a single time-course experiment.

**Ion homeostasis in the malaria parasite**

To measure ion concentrations inside the parasite, *P. falciparum* trophozoites (3D7 strain) were isolated from their host erythrocytes by brief exposure to saponin (0.05% w/v final concentration), then loaded with either the Na$^+$-sensitive dye SBFI (for measurements of intracellular [Na$^+$] ([Na$^+_i$])) (Spillman et al., 2013a) or the pH-sensitive dye BCECF (for measurements of intracellular pH ([pH$_i$])) (Saliba and Kirk, 1999). Fluorescence measurements and calibrations were performed at 37°C in the same manner described previously (Spillman et
Supplemental Information

al., 2013a; Lehane et al., 2014). To test TCMDC-124506, 1 μL of a DMSO stock of the compound was added to 1 mL of isolated parasites suspended in a saline solution (125 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 20 mM glucose, 25 mM HEPES; pH 7.10) at a density of ~ 3 × 10⁷ parasites mL⁻¹ to give the desired final concentration.

TCMDC-124506 gave rise to the ionic “signature” of PfATP4 inhibition that has been described previously for spirowindones (Spillman et al., 2013a; Spillman et al., 2013b) and other chemically diverse compounds (Lehane et al., 2014): it caused (i) an increase in the Na⁺ concentration inside the parasite (Figure S3A), (ii) a cytosolic alkalisation (Figure S3B), and (iii) a reduction in the extent of acidification seen following inhibition of the parasite’s V-type H⁺ pump with concanamycin A (Figure S3B).

Chemistry Experimental Section

Chemistry. General. Solvents and reagents were purchased from commercial suppliers and used without further purification. Dry solvents were purchased in sure sealed bottles stored over molecular sieves. Reactions using microwave irradiation were carried out in a Biotage Initiator microwave. Normal phase TLCs were carried out on pre-coated silica plates (Kieselgel 60 F(254), BDH) with visualisation via UV light (UV254/365 nm) and/or ninhydrin solution. Flash chromatography was performed using Combiflash Companion RF (Teledyne ISCO) and prepacked RediSep silica gel columns purchased from Teledyne ISCO. Mass-directed preparative HPLC separations were performed using a Waters HPLC (2545 binary gradient pumps, 515 HPLC make up pump, 2767 sample manager) connected to a Waters 2998 photodiode array and a Waters 3100 mass detector. Preparative HPLC separations were performed with a Gilson HPLC (321 pumps, 819 injection module, 215 liquid handler/injector) connected to a Gilson 155 UV/vis detector. On both instruments, HPLC chromatographic separations were conducted using Waters XBridge C18 columns, 19 x 100 mm, 5 μm particle size; using 0.1% ammonia in water or 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as mobile phase. ¹H NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (¹H at 500.1 MHz), or a Bruker Avance DPX 400 (¹H at 400 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broadened (br), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.1 Hz. Low resolution electrospray (ES) mass spectra were recorded on a BrukerDaltonics MicroTof mass spectrometer, run in positive mode. LC-MS analysis, HRMS analysis and chromatographic separation were conducted with a Bruker Daltonics MicrOTof mass spectrometer or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 μm particle size,) and the compounds were eluted with a gradient of 5 to 95% acetonitrile/water +0.1% Ammonia. All final compounds showed chemical purity ≥ 95% as determined by the UV chromatogram (190-450nm) obtained by LC-MS analysis and NMR. Unless otherwise stated herein reactions have not been optimized. Compounds 3 and 17 were purchased from Maybridge and tested without further purification after purity was determined to be ≥ 95% by the UV chromatogram (190–450nm) obtained by LC-MS analysis.

General procedure A. A mixture of ethyl 2,2,2-trifluoroacetate (1 g, 7.0 mmol) and the corresponding acetonitrile (1 eq) in dry THF (5 mL) was added to a stirred suspension of NaH (1.5 eq) in dry THF (5 mL) under nitrogen at 40°C over the course of 30 min. After stirring at 40°C for 2 hours, the reaction mixture was poured into water and acidified with HCl 3N and extracted with diethyl ether. The combined organic layers were dried over MgSO₄ and concentrated to give a crude material that was triturated using a mixture of CH₂Cl₂/petroleum ether and the solid was filtered to obtain a first fraction of the desired compound. The CH₂Cl₂/petroleum ethers filtrate was concentrated under reduced pressure to obtain a mixture of product and starting materials. This mixture was triturated again with CH₂Cl₂/petroleum ethers to yield a second fraction of the desired product.

General Procedure B. A solution of methylhydrazine (23 mg, 0.5 mmol) and the corresponding 3-oxo-butanenitrile (1 eq) in methanol (1 mL) was heated at 100°C for 1h under microwave irradiation. The solution was evaporated under reduced pressure and the resulting crude material purified by flash column chromatography using a silica cartridge eluting with 40% of ethyl acetate in heptane. The desired fractions were concentrated under reduced pressure to give the desired pyrazole as a solid.

General Procedure C. To a stirred solution of the desired pyrazol-5-amine (0.71 mmol) in THF (1 mL) the corresponding isocyanate (1.1 eq) was added and the reaction mixture was stirred at room temperature overnight. The reaction was concentrated to dryness and the crude material was purified by flash column
Supplemental Information

chromatography using a silica cartridge eluting with 10% of ethyl acetate in heptane to give the desired pyrazol-5-yl-urea.

TCMC-125506 (1). Prepared according to general procedure A followed by general procedures B and C (85 mg, 83%). 1H NMR (400 MHz, DMSO-d6) δ 7.43 - 7.26 (m, 6H), 7.10 (dd, J=8.9, 8.9 Hz, 2H), 3.79 (s, 3H). LC-MS (ESI) m/z 397 [M + H]+. High-resolution mass spectrum calculated for C18H14F5N4O [M+H]+ = 397.1082, observed for C18H14F5N4O [M+H]+ = 397.1090

4-(4-fluorophenyl)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-5-amine (2). Prepared according to general procedure A followed by general procedure B (12 mg, 9%). 1H NMR (400 MHz, DMSO-d6) δ 7.35 - 7.28 (m, 4H), 5.53 (s, 2H), 3.39 (s, 3H). LC-MS (ESI) m/z 260 [M + H]+. High-resolution mass spectrum calculated for C11H10F4N3 [M+H]+ = 260.0818, observed for C11H10F4N3 [M+H]+ = 260.0818

4-(4-(4-fluorophenyl)amino)-1-(trifluoromethyl)-1H-pyrazol-5-ylurea (4). Prepared according to general procedure A followed by general procedures B and C (63 mg, 39%). 1H NMR (500 MHz, DMSO-d6) δ 9.32 (1H, s), 8.41 (1H, s), 7.41 - 7.26 (6H, m), 7.12 (1H, d, J=7.8 Hz), 6.82 - 6.77 (1H, m), 3.80 (3H, s). LC-MS (ESI) m/z 397 [M + H]+. High-resolution mass spectrum calculated for C18H14F5N4O [M+H]+ = 397.1082, observed for C18H14F5N4O [M+H]+ = 397.1083

1-(3-fluorophenyl)-3-(4-(4-fluorophenyl)amino)-1-(trifluoromethyl)-1H-pyrazol-5-ylurea (6). Prepared according to general procedure A followed by general procedures B and C (138 mg, 73%). 1H NMR (500 MHz, DMSO-d6) δ 10.30 (s, 1H), 7.71 - 7.68 (m, 2H), 7.37 (dd, J=5.6, 8.5 Hz, 2H), 7.32 - 7.21 (m, 4H), 6.64 (s, 2H).

1-(4-fluorophenyl)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-5-ylurea (7). A solution of hydrazine hydrate (61 mg, 1.2 mmol, 1.4 eq) and 4,4,4-trifluoro-2-(4-fluorophenyl)-3-oxo-butanenitrile [7] (200 mg, 0.9 mmol) in acetic acid (1 mL) was stirred at room temperature overnight. The reaction mixture was then concentrated under reduced pressure and the residue portioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (3x). The organic layers were combined and dried over MgSO4 and concentrated under vacuum to give 4-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-amine (196 mg,0.9 mmol). 4-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-amine was then reacted with 1-fluoro-4-isocyanatobenzene following general procedure C to obtain 7 in 58% yield. 1H NMR (500 MHz, DMSO-d6) δ 10.30 (s, 1H), 7.71 - 7.68 (m, 2H), 7.37 (dd, J=5.6, 8.5 Hz, 2H), 7.32 - 7.21 (m, 4H), 6.64 (s, 2H).

1-(4-(4-fluorophenyl)-1-(2-hydroxyethyl)-3-(trifluoromethyl)-1H-pyrazol-5-ylurea (8). To a mixture of 4,4,4-trifluoro-2-(4-fluorophenyl)-3-oxo-butanenitrile (36 mg, 0.16 mmol) in acetic acid (0.5 mL), 2-hydradinoethanol (12 mg,0.16 mmol) was added and the reaction stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the crude taken onto next step which was performed following general procedure C to obtain 8 (7 mg, 9%). 1H NMR (400 MHz, DMSO-d6) δ 7.31 - 7.22 (m, 6H), 7.14 - 7.12 (m, 2H), 5.52 (s, 2H), 4.44 (m, 2H), 4.33 (m, 2H). LC-MS (ESI) m/z 427 [M + H]+. High-resolution mass spectrum calculated for C19H16F5N4O2 [M+H]+ = 427.1188, observed for C19H16F5N4O2 [M+H]+ = 427.1187

2-(4-(4-fluorophenyl)-5-(3-(4-fluorophenyl)ureido)-3-(trifluoromethyl)-1H-pyrazol-1-yl)acetamide (9). A mixture of 4,4,4-trifluoro-2-(4-fluorophenyl)-3-oxo-butanenitrile (140 mg,0.6 mmol) and ethyl 2-hydradinoacetate hydrochloride (94 mg,0.6 mmol) in methanol (2 mL) was heated at 100°C for 1h under microwave irradiation. The solvent was evaporated and the crude was taken up in ethyl acetate and washed with a saturated aqueous solution of NaHCO3. The organic layer was dried over MgSO4 and solvents were evaporated under reduced pressure to obtain ethyl 2-[5-amino-4-(4-fluorophenyl)-3-(trifluoromethyl)pyrazol-1-yl]acetate (131 mg, 65.291% yield). Without further purification, ethyl 2-[5-amino-4-(4-fluorophenyl)-3-(trifluoromethyl)pyrazol-1-yl]acetate was then reacted with 1-fluoro-4-isocyanatobenzene following general procedure C to obtain ethyl 2-[4-(4-fluorophenyl)-5-[4-(trifluoromethyl)pyrazol-1-yl]acetate (99% yield) which was suspended in ammonium hydroxide solution (4 mL) and stirred at room temperature for 5h. The reaction was concentrated to dryness to obtain 9 (30 mg,
1-(4-fluorophenyl)-3-(1-methyl-5-(trifluoromethyl)-1H-pyrazol-5-yl)urea (10). To a stirred solution of commercially available 2-methyl-5-(trifluoromethyl)pyrazol-3-amine (150 mg, 0.9 mmol) in THF (1.5 mL), 1-fluoro-4-isocyanato-benzene (125 mg, 0.9 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction was concentrated to dryness and the crude material was purified by trituration with petroleum ether to obtain 10 as a white solid (213 mg, 73% yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 8.97 (s, 1H), 8.31 (s, 1H), 7.71 (s, 1H), 7.44 (s, 1H), 7.40 - 7.26 (m, 6H), 7.10 (dd, \(J=8.9, 8.9\) Hz, 2H), 4.84 (s, 2H). LC-MS (ESI) \(m/\ell\) 440 [M + H]\(^+\). High-resolution mass spectrum calculated for C19H15F5N5O2 [M+H]\(^+\) = 440.0856, observed for C19H10BrF4N4O [M+H]\(^+\) = 439.0050. 

4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine. To a solution of commercially available 2-methyl-5-(trifluoromethyl)pyrazol-3-amine (1.6 g, 10 mmol) in acetonitrile (5 mL), NBS (0.77 g, 4.4 mmol) was added at room temperature. The reaction was stirred at room temperature for 1h. Solvent was evaporated under reduced pressure, the residue was taken up in DCM (10 mL) and washed with water (5 mL). Solvents were removed and product was purified by column chromatography using a 24 g silica cartridge using heptane (A) and ethyl acetate (B) as eluents and the following gradient: 1 min hold 100%A, 18 min ramp to 30% B, 1 min hold 50%B. Fractions containing product were pooled together and solvents were removed under reduced pressure to obtain 11 as a white solid (730 mg, 84% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.79 - 7.69 (m, 2H), 7.06 (m, 2H), 3.86 (s, 3H). LC-MS (ESI) \(m/\ell\) 381 [M]+ and 383 [M + 2H]\(^+\). High-resolution mass spectrum calculated for C12H11F4N4O [M+H]\(^+\) = 380.9969, observed for C12H10BrF4N4O [M+H]\(^+\) = 380.9984.

General Procedure E. To a solution of 4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (1 eq), palladium acetate (0.05 eq), 2-dicyclohexylphosphino-2,4,6-triisopropylbiphenyl (X-Phos) (0.1 eq), and the corresponding boronic acid (1.5 eq) in toluene (3 mL) was added potassium phosphate (1.5 eq) in water (1 mL). The reaction was sealed and the mixture was stirred at 95°C overnight. Reaction crude was filtered through Celite (5g cartridge) and Celite washed with DCM (10 mL). Reaction was partitioned between DCM (10 mL) and brine (5 mL). The organic layer was dried over MgSO\(_4\). Solvents were evaporated under reduced pressure. The product was purified by column chromatography using silica cartridge (12 g) and heptane (A) and ethyl acetate (B) as eluents.

4-(3,4-difluorophenyl)-2-methyl-5-(trifluoromethyl)pyrazol-3-amine. Prepared according to general procedure E starting from 4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (200 mg, 0.8 mmol) to obtain the desired product (140 mg, 54% yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.23 - 7.11 (m, 2H), 7.06 - 7.03 (m, 1H), 3.75 - 3.70 (m, 5H). LC-MS (ESI) \(m/\ell\) 278 [M + H]\(^+\).

1-(4-(3,4-difluorophenyl)-1-methyl-3(trifluoromethyl)-1H-pyrazol-5-yl)-3-(4-fluorophenyl)urea (12). To a solution of the (4-(3,4-difluorophenyl)-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (140 mg, 0.4 mmol) in THF (10mL) at room temperature, 1-fluoro-4-isocyanato-benzene (183 mg, 1.3 mmol) was added and the reaction was stirred for two days at room temperature. The reaction mixture was concentrated to dryness under reduced pressure and the product was purified by preparative HPLC. Fractions containing product were pooled together and solvent was removed to obtain 12 as a white solid (55 mg, 28% yield). \(^1\)H NMR (500 MHz, MeOD) \(\delta\) 7.37 - 7.23 (m, 4H), 7.17 - 7.14 (m, 1H), 7.04 - 7.00 (m, 2H), 3.86 (s, 3H). LC-MS (ESI) \(m/\ell\) 415 [M + H]\(^+\). High-resolution mass spectrum calculated for C18H13F6N4O [M+H]\(^+\) = 415.0988, observed for C18H13F6N4O [M+H]\(^+\) = 415.0997.
Supplemental Information

[2-[5-amino-1-methyl-3-(trifluoromethyl)pyrazol-4-yl]phenyl]methanol. Prepared according to general procedure E starting from 4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (190 mg, 0.78 mmol) to obtain the desired product (211 mg, 65% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.54 (d, J=7.6 Hz, 1H), 7.45 - 7.35 (m, 2H), 7.26 - 7.24 (m, 1H), 4.58 - 4.48 (m, 2H), 3.80 (s, 3H), 3.69 (s, 2H). LC-MS (ESI) m/z 272 [M + H]⁺.

1-(4-fluorophenyl)-3-(4-(2-hydroxyethyl)phenyl)-1-methyl-3-(trifluoromethyl)1H-pyrazol-5-yl)urea

13) To a stirred solution of [2-[5-amino-1-methyl-3-(trifluoromethyl)pyrazol-4-yl]phenyl]methanol (138 mg, 0.51 mmol) in THF (10 mL) in an ice bath, 1-fluoro-4-isocyanato-benzene (70 mg, 0.51 mmol) was added and the reaction mixture was allowed to reach room temperature and stirred overnight. LCMS showed unreacted starting material. Further equivalent of 1-fluoro-4-isocyanato-benzene was added and the mixture was stirred overnight at room temperature. The reaction was concentrated to dryness under reduced pressure and the crude material was purified by preparative HPLC. Fractions containing product were pooled together and solvent was removed to obtain 13 as a white solid (20 mg, 9% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.55 (s, 1H), 7.45 - 7.40 (m, 2H), 7.32 - 7.29 (m, 1H), 7.13 - 7.04 (m, 2H), 6.98 - 6.94 (m, 2H), 6.54 (s, 1H), 4.51 (dd, J=3.6, 11.2 Hz, 1H), 4.41 (dd, J=6.3, 11.1 Hz, 1H), 3.93 (s, 3H), 2.04 - 2.04 (m, 1H). LC-MS (ESI) m/z 409 [M + H]⁺. High-resolution mass spectrum calculated for C19H17F4N4O2 [M+H]⁺ = 409.1291

4-[2-[2-[tert-butyl(dimethyl)silyl]oxyethyl]phenyl]-2-methyl-5-(trifluoromethyl)pyrazol-3-amine. Prepared according to general procedure E starting from 4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (225 mg, 0.9 mmol) to obtain the desired product (120 mg, 29% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.41 - 7.32 (m, 2H), 7.29 - 7.26 (m, 1H), 7.21 (d, J=7.4 Hz, 1H), 3.79 - 3.71 (m, 5H), 2.82 - 2.75 (m, 2H), 0.88 (s, 9H), -0.01 (d, J=7.2 Hz, 6H). LC-MS (ESI) m/z 400 [M + H]⁺.

1-[4-[2-[2-[tert-butyl(dimethyl)silyl]oxyethyl]phenyl]-2-methyl-5-(trifluoromethyl)pyrazol-3-yl]-3-(4-fluorophenyl)urea

To a solution of the 4-[2-[2-[tert-butyl(dimethyl)silyl]oxyethyl]phenyl]-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (120 mg, 0.3 mmol) in THF (10 mL) at room temperature, 1-fluoro-4-isocyanato-benzene (124 mg, 0.9 mmol) was added. The reaction was stirred overnight at room temperature. LCMS showed unreacted starting material. Further 1-fluoro-4-isocyanato-benzene (124 mg, 0.9mmol) was added and the reaction was stirred overnight. The reaction was concentrated to dryness under reduced pressure and the product was purified by preparative HPLC. Fractions containing product were pooled together and solvent was removed to obtain 14 as a white solid (20 mg, 12% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.38 - 7.36 (m, 2H), 7.28 - 7.24 (m, 1H), 7.18 (d, J=7.3 Hz, 1H), 7.07 (dd, J=4.7, 8.8 Hz, 2H), 6.96 (dd, J=8.5, 8.5 Hz, 2H), 6.50 (s, 1H), 6.41 (s, 1H), 3.93 (s, 3H), 3.77 - 3.66 (m, 2H), 2.75 - 2.62 (m, 2H), 0.85 (s, 9H), -0.01 (d, J=7.5 Hz, 6H). LC-MS (ESI) m/z 537 [M + H]⁺.

1-(4-fluorophenyl)-3-[4-[2-(hydroxyethyl)phenyl]-2-methyl-5-(trifluoromethyl)pyrazol-3-yl]urea

14) To a solution of 1-[4-[2-[2-[tert-butyl(dimethyl)silyl]oxyethyl]phenyl]-2-methyl-5-(trifluoromethyl)pyrazol-3-yl]-3-(4-fluorophenyl)urea (20 mg, 0.04 mmol) in anhydrous methanol (3 mL) in a bath ice, acetyl chloride (0.5 μL) was added. The reaction was allowed to reach room temperature and stirred for 3 hours. Solvents were removed and product was purified by preparative HPLC. Solvents were removed to obtain 14 as a white solid (10 mg, 63%). ¹H NMR (500 MHz, CDCl₃) δ 7.66 (s, 1H), 7.44 - 7.39 (m, 1H), 7.34 (d, J=7.3 Hz, 1H), 7.28 - 7.20 (m, 2H), 7.12 - 7.06 (m, 2H), 6.97 - 6.92 (m, 2H), 6.46 (s, 1H), 3.92 (s, 5H), 2.74 - 2.62 (m, 2H), 1.83 (s, 1H). LC-MS (ESI) m/z 423 [M + H]⁺. High-resolution mass spectrum calculated for C20H19F4N4O2 [M+H]⁺ = 423.1439, observed for C20H19F4N4O2 [M+H]⁺ = 423.1429

2-[3-[5-amino-1-methyl-3-(trifluoromethyl)pyrazol-4-yl]phenyl]ethanol. Prepared according to general procedure E starting from 4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (190 mg, 0.78 mmol) to obtain the desired product (86 mg, 36% yield). ¹H NMR (500 MHz, MeOD) δ 7.31 - 7.27 (m, 1H), 7.17 - 7.10 (m, 3H), 3.75 (t, J=6.9 Hz, 2H), 3.67 (s, 3H), 2.81 (t, J=6.9 Hz, 2H).

1-(4-fluorophenyl)-3-[4-[3-(2-hydroxyethyl)phenyl]-1-methyl-3-(trifluoromethyl)1H-pyrazol-5-yl]urea

15) To a solution of 2-[3-[5-amino-1-methyl-3-(trifluoromethyl)pyrazol-4-yl]phenyl]ethanol (80 mg, 0.3 mmol) in 1,4-Dioxane (4 mL) at room temperature, 1-fluoro-4-isocyanato-benzene (107 mg, 0.8 mmol) was added and the reaction was heated at 90 °C overnight. The reaction mixture was concentrated to dryness under reduced pressure and the product was purified by preparative HPLC and then by mass directed autopreparative HPLC. Fractions containing product were pooled together and solvent was removed to obtain 15 as a white solid (10 mg, 9% yield). ¹H NMR (500 MHz, MeOD) δ 7.36 - 7.31 (m, 3H), 7.25 - 7.18 (m, 3H), 7.04 - 6.99 (m, 2H), 3.87 (s, 3H), 3.73 (t, J=7.1 Hz, 2H), 2.82 (t, J=7.1 Hz, 2H). LC-MS (ESI) m/z 423 [M + H]⁺.
Supplemental Information

N-[[3-[5-amino-1-methyl-3-(trifluoromethyl)pyrazol-4-yl]phenyl]methyl]methanesulfonamide. Prepared according to general procedure E starting from 4-bromo-2-methyl-5-(trifluoromethyl)pyrazol-3-amine (225 mg, 0.9 mmol) to obtain the desired product (110 mg, 32% yield). 1H NMR (500 MHz, CDCl3) δ 7.28 -7.25 (m, 1H), 7.18 - 7.13 (m, 3H), 4.94 - 4.92 (m, 1H), 4.18 (d, J=6.1 Hz, 1H), 3.62 (broad s, 2H), 3.60 (s, 3H), 2.71 (s, 3H). LC-MS (ESI) m/z 349 [M + H]+.

N-(3-(5-(4-fluorophenyl)ureido)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)benzyl)methanesulfonamide (16). To a solution of the corresponding amine (4-fluorophenyl)urea (26 g, 127 mmol, 1 eq) in dry THF and the reaction stirred at room temperature overnight. Further three equivalents of 1-fluoro-4-isocyanato-benzene were added and the reaction was stirred overnight. The reaction was concentrated to dryness and the product was purified by preparative HPLC to obtain 16 as a white solid. 1H NMR (500 MHz, DMSO-d6) δ 9.08 (s, 1H), 8.34 (s, 1H), 7.61 - 7.58 (m, 1H), 7.45 - 7.30 (m, 4H), 7.24 (d, J=7.8 Hz, 1H), 7.12 - 7.08 (m, 2H), 4.16 (d, J=6.3 Hz, 2H), 3.80 (s, 3H), 2.80 (s, 3H). LC-MS (ESI) m/z 486 [M + H]+. High-resolution mass spectrum calculated for C20H20F4N5O3S [M+H]+ = 486.1217, observed for C20H20F4N5O3S [M+H]+ = 486.1201

1-(4-fluorophenyl)-3-(4-(fluorophenyl)-1-methyl-1H-pyrazol-5-yl)urea (18). A stirred solution of methylhydrazine (59 mg, 1.2 mmol) and 2-(4-fluorophenyl)-3-hydroxy-prop-2-enitrile (210 mg, 1.2 mmol) in ethanol (4 mL) was heated under reflux for 16h. After cooling to room temperature the reaction mixture was concentrated under vacuum and the crude material, which was a mixture of the two regioisomers, purified by flash column chromatography using a RediSep silica cartridge eluting with a mixture of CH2Cl2:MeOH 96:4. The separation was difficult and only a very small amount of pure desired isomer 1-(4-fluorophenyl)-1-methyl-pyrazol-3-amine (20 mg, 0.1 mmol, 8 % yield) was isolated. The remaining fractions were evaporated to give a mixture of regioisomers (122 mg) which was reacted in the next step. 1-fluoro-4-isocyanato-benzene (86 mg, 0.6 mmol) was added to a stirred solution of a mixture of 4-(4-fluorophenyl)-3-methyl-pyrazol-3-amine and 4-(4-fluorophenyl)-1-methyl-pyrazol-3-amine (120 mg, 0.6 mmol) in dry THF and the reaction stirred at room temperature overnight. After solvent removal under vacuum, the crude was washed several times with CH2Cl2: giving a small fraction of pure 1-(4-fluorophenyl)-3-(4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)urea (18.5 mg,0.05 mmol, 16.3% yield). 1H NMR (400 MHz, DMSO-d6) δ 9.12 (s, 1H), 8.28 (s, 1H), 7.77 (s, 1H), 7.56 (s, 2H), 7.49 (d, J=4.5 Hz, 2H), 7.22 (dd, J=8.1, 8.1 Hz, 2H), 7.11 (dd, J=7.9, 7.9 Hz, 2H), 3.69 (s, 3H). LC-MS (ESI) m/z 329 [M + H]+. High-resolution mass spectrum calculated for C17H15F2N4O [M+H]+ = 329.1208, observed for C17H15F2N4O [M+H]+ = 329.1218

2-(4-Fluorophenyl)-3-oxobutanenitrile. To a solution of compound 2-(4-fluorophenyl)acetonitrile (28 g, 207 mmol, 1 eq) in EtOAc (91 g, 1.0 mol, 5 eq) was added NaH (9.95 g, 60% in mineral oil, 249 mmol, 1.2 eq) at 0°C. The mixture was stirred at 0 °C for 3 hours. The reaction was quenched with H2O (100 mL) at 15°C and then adjusted to pH=5-6. The mixture was filtered and filter cake was dried to give 2-(4-fluorophenyl)-3-oxobutanenitrile as an off-white solid, which was used in next step without further purification. 1H NMR (400 MHz, CDCl3) δ 7.41-7.39 (m, 2H), 7.18-7.14 (m, 2H), 4.69(s, 1H), 2.31 (s, 3H). LC-MS (ESI) m/z 178 [M + H]+.

4-(4-Fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine. To a mixture of compound 2-(4-fluorophenyl)-3-oxobutanenitrile (62 g, 350 mmol, 1 eq) in EtOH (170 mL) was added MeNH2ClH2 (40 g, 350 mmol, 1 eq) in one portion at 10-25 °C to get a red solution. The mixture was stirred at 10-25 °C for 16 hours. TLC (PE:EtOAc=1:1) showed that reactant (Rf=0.7) was consumed and a new spot was formed (Rf=0.2). The mixture was concentrated and diluted with water (200 mL). The aqueous layer was extracted with EtOAc. The combined organic layers were dried and concentrated to get 4-(4-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine (52 g, 253 mmol, 72 % yield) as an off-white solid, which was used in next step without further purification. 1H NMR (400 MHz, CDCl3) δ 7.28-7.25 (m, 2H), 7.14-7.10 (m, 2H), 3.69 (s, 3H), 3.57 (s, 2H), 2.21 (s, 3H). LC-MS (ESI) m/z 206 [M + H]+.

Tert-Butyl-N-tert-butoxycarbonyl-N-[4-(4-fluorophenyl)-2,5-dimethyl-pyrazol-3-yl] carbamate. To a solution of 4-(4-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine (26 g, 127 mmol, 1 eq) in THF (260 mL) was added Boc2O (55 g, 253 mmol, 2 eq) drop-wise at 10-25 °C to get a black solution. The mixture was stirred at 40 °C for 1 hour. TLC (PE: EtOAc=1:1) showed that the reactant (Rf = 0.15) was consumed and a new spot was formed (Rf=0.80). The mixture was concentrated and diluted with water (200 mL). The aqueous layer was extracted with EtOAc. The combined organic layers were concentrated to give tert-butyl-N-tert-butoxycarbonyl-N-[4-(4-fluorophenyl)-2,5-dimethyl-pyrazol-3-yl] carbamate (33 g, 81% yield).
Supplemental Information

mmol, 64% yield) as an off-white solid. †H NMR (400 MHz, CDCl₃) δ 7.22-7.19 (m, 2H), 7.12-7.09 (m, 2H), 3.68 (s, 3H), 2.30 (s, 3H), 1.36 (s, 18H). LC-MS (ESI) m/z 406 [M + H]⁺.

tert-Butyl-N-[5-(bromomethyl)-4-(4-fluorophenyl)-2-methyl-pyrazol-3-yl]-N-tert-butoxycarbonyl-carbamate. The mixture of compound tert-butyl-N-[5-(bromomethyl)-4-(4-fluorophenyl)-2,5-dimethyl-pyrazol-3-yl] carbamate (16.50 g, 41 mmol, 1 eq) NBS (7.24 g, 41 mmol, 1 eq) and AIBN (1.34 g, 8 mmol, 0.2 eq) in CH₂Cl₂ (160 mL) was stirred under N₂ for 16 hours at 80 °C. TLC (PE:EtOAc=3:1) showed that reactant (Rf=0.3) was consumed and the desired spot (Rf=0.5) was formed. The mixture was filtered and the mother liquid was concentrated to give tert-butyl-N-[5-(bromomethyl)-4-(4-fluorophenyl)-2-methyl-pyrazol-3-yl]-N-tert-butoxycarbonyl-carbamate (12.50 g, 26 mmol, 63% yield) was obtained as a yellow oil. †H NMR (400 MHz, CDCl₃) δ 7.38-7.36 (m, 2H), 7.16-7.14 (m, 2H), 4.48 (s, 2H), 3.74 (s, 3H), 1.36 (s, 18H).

tert-Butyl (3-(cyanomethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate. The mixture of tert-butyl N-[5-(bromomethyl)-4-(4-fluorophenyl)-2-methyl-pyrazol-3-yl]-N-tert-butoxycarbonyl-carbamate (30.00 g, 62 mmol, 1 eq) and NaCN (3.04 g, 62 mmol, 1 eq) in DMF (300 mL) was stirred at 10-15°C for 2 hours. TLC (PE:EtOAc=3:1, Rf=0.60) showed the reaction was completed and a new spot was formed (Rf = 0.2). The mixture diluted with water (20 mL) and extracted with EA (3 x 50 mL). The aqueous layer was quenched with aq.NaClO (250 mL). The organic layer was stirred at 25 °C for 2 hours. LCMS showed the reaction was completed. The reaction mixture was concentrated and diluted with saturated NaHCO₃ aqueous layer (10 mL). The aqueous layer was extracted with EtOAc (20 mL). The organic layer was washed with brine (2 x 100 mL) and concentrated. The residue was purified by silica gel column to get tert-butyl (3-(cyanomethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (12.00 g, 24 mmol, 38% yield, 85% purity) as a yellow solid. †H NMR (400 MHz, CDCl₃) δ 7.25-7.23 (m, 2H), 7.16-7.14 (m, 2H), 5.95 (s, 1H), 3.81 (s, 3H), 3.66 (s, 2H), 1.46 (s, 9H). LC-MS (ESI) m/z 431 [M + H]⁺.

2-(5-Amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)acetonitrile. Tert-butyl (3-(cyanomethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)carbamate (400 mg, 0.93 mmol, 1 eq) was added to HCl/EtOAc (4 mol/L, 4 mL). The mixture was stirred at 25 °C for 2 hours. LCMS showed the reaction was completed. The reaction mixture was concentrated and diluted with saturated NaHCO₃ aqueous layer (10 mL). The aqueous layer was extracted with EtOAc (20 mL). The organic layer was washed with brine (10 mL), dried over Na₂SO₄ and concentrated to get 2-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)acetonitrile (200.00 mg, crude) as a yellow oil. LC-MS (ESI) m/z 231 [M + H]⁺.

1-(3-(Cyanomethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)-3-(4-fluorophenyl)urea (23). To a solution of 2-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)acetonitrile (200.00 mg, 0.869 mmol, 1.00 eq) in THF (5.00 mL) was added 1-fluoro-4-isocyanatobenzene (119.10 mg, 0.869 mmol, 1.00 eq). The mixture was stirred at 25 °C for 16 hours. LCMS showed the reaction was completed. The reaction mixture was concentrated and the residue was purified by Prep-HPLC (basic condition) to get 23 (87.00 mg, 0.237 umol, 4 steps 30% yield, 100% purity) as a white solid. †H NMR (400 MHz, DMSO-d₆) δ 9.05 (s, 1H), 8.26 (s, 1H), 7.42-7.37 (m, 4H), 7.30-7.27 (m, 2H), 7.13-7.10 (m, 2H), 4.01 (s, 2H), 3.69 (s, 3H). LC-MS (ESI) m/z 368 [M + H]⁺. High-resolution mass spectrum calculated for C₁₉H₁₆F₂N₅O [M+H]+ = 368.1317, observed for C₁₉H₁₆F₂N₅O [M+H]+ = 368.1306.

2-(5-((tert-Butyloxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)acetic acid. The reaction mixture of tert-butyl (3-(cyanomethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)carbamate (2.50 g, 6 mmol, 1 eq) and NaOH (929 mg, 23 mmol, 4 eq) in EtOH (25 mL) and H₂O (10 mL) was stirred at 70 °C for 8 hours. TLC (DCM:MeOH=10:1) showed the reaction was completed and two new desired spots were formed (Rf=0.1, Rf=0.35). The mixture was concentrated and diluted with water (20 mL). The aqueous layer was acidified with citric acid to pH=5 and extracted with EtOAc (3 x 30 mL). The combined organic layer was concentrated to get 2-(5-((tert-butyloxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)acetic acid (1 g, crude, a mixture of single Boc and free amine) as a yellow oil. LC-MS (ESI) m/z 250 [M + H]⁺ (RT 1.4min.) and m/z 350 [M + H]⁺ (RT 2.4min.).

tert-Butyl (4-(4-fluorophenyl)-3-(2-hydroxyethyl)-1-methyl-1H-pyrazol-5-yl)carbamate. To a solution of 2-(5-((tert-butyloxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)acetic acid (1.90 g, 5 mmol, 1.00 eq) in THF (20 mL) was added BH₃·THF (1 M, 21.76 mL, 4 eq) drop-wise at 0 °C to get a solution. The reaction mixture was stirred at 25 °C for 3 hours. The reaction mixture was quenched with MeOH (10 mL) and then concentrated to get crude product. The reaction mixture was concentrated to get tert-butyl (4-(4-fluorophenyl)-3-(2-hydroxyethyl)-1-methyl-1H-pyrazol-5-yl)carbamate (2 g, crude) as a light yellow oil, which was used in next step without purification.
2-(5-Amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)ethan-1-ol. To the solution of tert-butyl-(4-(4-fluorophenyl)-3-(2-hydroxyethyl)-1-methyl-1H-pyrazol-5-yl)carbamate (1.2 g, 3.6 mmol, 1 eq) in DCM (20 mL) was added EtOAc/HCl (5 g, 137 mmol, 39 eq) drop-wise at 10-25 °C to get a light yellow solution. The mixture was stirred at 10-25 °C for 16 hours. LCMS shows that reactant was consumed and the desired MS was detected. The reaction mixture was concentrated. The residue was dissolved in H2O (10 mL) and DCM (20 mL). HCl (2N) was added to adjust pH=3. The aqueous layer was separated and adjusted to pH=8 with saturated aqueous NaHCO3 solution. The aqueous layer was extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine (2 x 20ml) and concentrated to get 2-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)ethan-1-ol (600 mg, 71% yield, 74% purity) as a light yellow oil. 1H NMR (400 MHz, CDCl3) δ 7.26-7.22 (m, 2H), 7.14-7.10 (m, 2H), 3.86 (t, J=5.6 Hz, 2H), 3.78-3.75 (m, 1H), 3.70 (s, 3H), 3.62-3.60 (m, 2H), 2.78 (t, J=5.6 Hz, 2H). LC-MS (ESI) m/z 236 [M + H]+.

2-(4-(4-Fluorophenyl)-5-(3-(4-fluorophenyl)ureido)-1-methyl-1H-pyrazol-3-yl)ethyl (4-fluorophenyl)carbamate. The reaction mixture of 2-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)ethan-1-ol (70 mg, 0.3 mmol, 1 eq) and 1-fluoro-4-isocyanatobenzene (41 mg, 0.3 mmol, 1 eq) in THF (1 mL) was stirred at 10-25 °C for 16 hours. LCMS showed that reactant was consumed and the desired MS was detected. The reaction mixture was concentrated to get compound 2-(4-(4-fluorophenyl)-5-(3-(4-fluorophenyl)ureido)-1-methyl-1H-pyrazol-3-yl)ethyl (4-fluorophenyl)carbamate (120 mg, crude) as a light yellow oil, which was used in next step directly.

1-(4-Fluorophenyl)-3-(4-(4-fluorophenyl)-3-(2-hydroxyethyl)-1-methyl-1H-pyrazol-5-yl)urea (19). To the solution of 2-(4-(4-fluorophenyl)-5-(3-(4-fluorophenyl)ureido)-1-methyl-1H-pyrazol-3-yl)ethyl (4-fluorophenyl)carbamate (120 mg, 0.2 mmol, 1 eq) in EtOH (2 mL) was added the solution of NaOH/H2O (5 M, 235 µL, 5 eq) drop-wise at 10-15 °C to get a colorless solution. The reaction mixture was stirred at 70 °C for 3 hours. LCMS showed that reactant was consumed and the desired MS was detected. The reaction mixture was concentrated and the residue was purified with prep-HPLC to obtain 19 (6 mg, 0.02 mmol, 7% yield, 97% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 9.033 (s, 1H), 8.22 (s, 1H) 7.44-7.41 (m, 4H), 7.24 (m, 2H), 7.10 (m, 2H), 4.62 (t, J=5.2 Hz, 1H), 3.63-3.55 (m, 5H), 2.74-2.71 (m, 2H). LC-MS (ESI) m/z 373 [M + H]+. High-resolution mass spectrum calculated for C19H19F2N4O2 [M+H]+= 373.1471, observed for C19H19F2N4O2 [M+H]+= 373.1470

2-(4-(4-Fluorophenyl)-5-(2-(4-fluorophenyl)acetamido)-1-methyl-1H-pyrazol-3-yl)ethyl methanesulfonate. To the solution of 19 (750 mg, 2 mmol, 1 eq), TEA (611 mg, 6 mmol, 3 eq) in DCM (3 mL) was added MscI (254 mg, 2.2 mmol, 1.1 eq) portion wise below 10 °C under N2 to get a light yellow solution. The reaction mixture was stirred at 0 °C for 2 hours. LCMS showed that reactant was consumed and the desired MS was detected. The reaction mixture was concentrated to obtain 2-(4-(4-fluorophenyl)-5-(2-(4-fluorophenyl)acetamido)-1-methyl-1H-pyrazol-3-yl)ethyl methanesulfonate (900 mg, crude) as a light yellow solid. LC-MS (ESI) m/z 451 [M + H]+.

1-(3-(2-(Dimethylamino)ethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)-3-(4-fluorophenyl)urea (25). To a solution of 2-(4-(4-fluorophenyl)-5-(2-(4-fluorophenyl)acetamido)-1-methyl-1H-pyrazol-3-yl)ethyl methanesulfonate (200 mg, 0.4 mmol, 1 eq) in DCM (2 mL) were added Me2NH.HCl (72 mg, 0.9 mmol, 2 eq) and TEA (112 mg, 1.1 mmol, 2.5 eq) at 0 °C to get a light yellow solution. The reaction mixture was stirred at 10-30 °C for 16 hours. LCMS showed that most of reactant was consumed and a major peak was observed. The reaction mixture was concentrated and the residue was purified with prep-HPLC to obtain 25 (19 mg, 0.05 mmol, 11% yield, 99% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 9.45 (s, 1H), 8.70 (s, 1H) 8.23 (s, 1H), 7.45-7.43 (m, 2H), 7.36-7.34 (m, 2H), 7.23 (m, 2H), 7.08 (m, 2H), 3.62 (s, 3H), 2.77-2.73 (m, 2H), 2.64-2.63 (m, 2H), 2.22 (s, 6H). LC-MS (ESI) m/z 400 [M + H]+. High-resolution mass spectrum calculated for C21H24F2N5O [M+H]+= 400.1943, observed for C21H24F2N5O [M+H]+= 400.1942

1-(4-Fluorophenyl)-3-(4-(4-fluorophenyl)-1-methyl-3-(2-morpholinoethyl)-1H-pyrazol-5-yl)urea (26). To the solution of 2-(4-(4-fluorophenyl)-5-(2-(4-fluorophenyl)acetamido)-1-methyl-1H-pyrazol-3-yl)ethyl methanesulfonate (200 mg, 0.4 mmol, 1 eq) in DCM (3 mL) was added morpholine (116 mg, 1.3 mmol, 3 eq) at 0°C to get a colorless solution. The reaction mixture was stirred at 30 °C for 16 hours. LCMS showed that most of reactant was consumed and a major peak was observed. The reaction mixture was concentrated and the residue was purified with prep-HPLC to get 26 (35 mg, 77 µmol, 17% yield, 97% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 9.28 (s, 1H), 8.50 (s, 1H), 8.20 (s, 1H), 7.43-7.41 (m, 2H), 7.35-7.33 (m, 2H), 7.23 (m, 2H), 7.08 (m, 2H), 3.62 (s, 3H) 3.52 (m, 4H), 2.72 (m, 4H), 2.50 (m, 2H), 2.33-2.32 (m, 4H). LC-MS (ESI) m/z 442 [M + H]+. High-resolution mass spectrum calculated for C23H26F2N5O2 [M+H]+= 442.2049, observed for C23H26F2N5O2 [M+H]+= 442.2054

Supplemental Information
Supplemental Information

(5-((tert-Butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)methyl acetate. To the solution of tert-butyl N-[5-(bromomethyl)-4-(4-fluorophenyl)-2-methyl-pyrazol-3-yl]-N-tert-butoxycarbonyl-carbamate (5 g, 10 mmol, 1 eq) in DMF (60 mL) were added AcOK (3 g, 31 mmol, 3 eq) and KI (171 mg, 1 mmol, 0.1 eq). The mixture was stirred at 80 °C for 15 hours. TLC (PE:EtOAc=3:1, Rf=0.3,0.1) and LCMS showed that single-Boc, Bi-Boc and free amine were observed on LCMS. The reaction mixture was partitioned between H2O (200 mL) and EtOAc (100 mL). The organic phase was washed with brine (50 mL), dried over Na2SO4 and concentrated. The residue was purified by column chromatography (SiO2, PE:EtOAc=20:1–10:1) to afford a mixture of (5-((tert-butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)methyl acetate and the corresponding di Boc protected compound (mixture, 2.75 g, 5.93 mmol, 57.49% yield) as a brown oil. LC-MS (ESI) m/z 364 [M + H]+ (RT= 0.801), m/z 464 [M + H]+′ (RT= 0.980).

tert-Butyl (4-(4-fluorophenyl)-3-(hydroxymethyl)-1-methyl-1H-pyrazol-5-yl)carbamate.

To the solution of a mixture of 5-((tert-butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)methyl acetate and the corresponding di Boc protected compound (2.2 g, 5 mmol, 1 eq) in MeOH (30 mL) was added K2CO3 (1.3 g, 10 mmol, 2 eq). The mixture was stirred at 80 °C for 2 hours. LCMS showed the reaction was completed. The reaction mixture was concentrated and diluted with H2O (30 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na2SO4 and concentrated to give a mixture of tert-butyl (4-(4-fluorophenyl)-3-(hydroxymethyl)-1-methyl-1H-pyrazol-5-yl)carbamate with the corresponding di-Boc protected and free amine (mixture, 1.5 g, crude) as a yellow oil.

5-((tert-Butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carboxylic acid. To a solution of a mixture of tert-butyl (4-(4-fluorophenyl)-3-(hydroxymethyl)-1-methyl-1H-pyrazol-5-yl)carbamate with the corresponding di-Boc protected and free amine (20 mg, 47 μmol, 1 eq) in H2O (2 mL) was added the solution of KMnO4 (15 mg, 95 μmol, 2 eq) in NaOH (2 mol/L, 1 mL). The mixture was stirred at 15 °C for 15 h. LCMS showed the reaction was completed. The reaction mixture was quenched by addition Na2SO4 aqueous solution (1 mL) at 15°C and adjusted pH=5 with HCl (2N). The aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were dried over Na2SO4 and concentrated to give a mixture of 5-((tert-butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carboxylic acid with the corresponding di-Boc protected and free amine (mixture, 15 mg, crude) as a yellow solid.

tert-Butyl (3-carbamoyl-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate.

To the solution of a mixture of 5-((tert-butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carboxylic acid with the corresponding di-Boc protected and free amine (701 mg, 2.2 mmol, 1 eq) and HCl (473 mg, 9 mmol, 4 eq) in DCM (15 mL) and DMF (3 mL) were added EDCI (551 mg, 3 mmol, 1.3 eq), DIPEA (1.7 g, 13 mmol, 6 eq). The mixture was stirred at 25 °C for 16 hours. TLC (DCM:MeOH = 10:1, Rf = 0.4) showed the reaction was completed. The mixture was washed with water (10 mL), brine (10 mL), dried over Na2SO4 and concentrated to afford a mixture of tert-butyl (3-carbamoyl-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate with the corresponding di-Boc protected and free amine (mixture, 520 mg, crude) as yellow oil which was used in next step without further purification.

5-Amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carboxamide. The reaction mixture of a mixture of tert-butyl (4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate with the corresponding di-Boc protected and free amine (520 mg, 1.6 mmol, 1 eq) in EtOAc/HCl (4 mol/L, 10 mL) was stirred at 15 °C for 15 hours. TLC (PE:EtOAc=0:1, Rf=0.15) and LCMS showed the reaction was completed. The reaction mixture was concentrated and adjusted to pH=8 with aqueous NaHCO3 solution. The aqueous layers were extracted with EtOAc (2 x 25 mL). The combined organic layers were dried over Na2SO4 and concentrated to give 5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carboxamide (250 mg, crude) as a yellow solid. LC-MS (ESI) m/z 235 [M + H]+.

4-(4-Fluorophenyl)-5-(3-(4-fluorophenyl)ureido)-1-methyl-1H-pyrazole-3-carboxamide (20). To the solution of 5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carboxamide (250 mg, 1.1 mmol, 1 eq) in THF (5 mL) was added 1-fluoro-4-isocyanatobenzene (145 mg, 1.1 mmol, 1 eq). The mixture was stirred at 15 °C for 15 hours. LCMS showed the reaction was completed. The mixture was concentrated. The residue was triturated with MeOH (2 mL) to afford (20) (90 mg, 0.2 mmol, 22% yield, 98% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 9.05 (s, 1H), 8.21 (s, 1H), 7.43-7.37 (m, 5H), 7.20-7.10 (m, 5H), 3.74 (s, 3H). LC-MS (ESI) m/z 372 [M + H]+. High-resolution mass spectrum calculated for C18H16F2N5O2 [M+H]+ = 372.1267, observed for C18H16F2N5O2 [M+H]+ = 372.1254.

tert-Butyl (3-cyano-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate.
Supplemental Information

To a solution of a mixture of tert-butyl (3-carbamoyl-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate with the corresponding di-Boc protected and free amine (330 mg, 1 mmol, 1 eq) and pyridine (234 mg, 3 mmol, 3 eq) in DCM (5 mL) was added TFAA (415 mg, 2 mmol, 2 eq) drop-wise at 0 °C. The mixture was stirred at 0 °C for 1 hour. TLC (PE:EtOAc = 1:1, Rf = 0.7) showed the reaction was completed. The mixture was quenched with saturated aqueous NaHCO₃ solution (5 mL) and extracted with DCM (10 mL). The organic layer was washed with brine (5 mL), dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by Prep-TLC (PE:EtOAc = 1:1) to afford tert-butyl (3-cyano-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (90 mg, 0.3 mmol, 28% yield, 95% purity) as a yellow oil. LC-MS (ESI) m/z 317 [M + H]+.

5-Amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carbonitrile. tert-Butyl (3-cyano-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (90 mg, 0.3 mmol, 1 eq) was dissolved in HCl/EtOAc (3 mL) and the reaction mixture was stirred at 25 °C for 1 hour. TLC (PE:EtOAc = 1:1, Rf = 0.35) showed the reaction was completed. The reaction mixture was concentrated and diluted with EtOAc (5 mL). The organic layer was washed with saturated aqueous NaHCO₃ solution (5 mL), dried over anhydrous Na₂SO₄ and concentrated to get compound 5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carbonitrile (45 mg, 0.2 mmol, 73% yield) as a yellow solid.

1-(3-Cyano-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)-3-(4-fluorophenyl)urea (22). To the solution of 5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazole-3-carbonitrile (45 mg, 0.2 mmol, 1 eq) in THF (2.00 mL) was added 1-fluoro-4-isocyanatobenzene (34 mg, 0.25 mmol, 1.2 eq) and the reaction mixture was stirred at 25 °C for 16 hours. LCMS showed the reaction was completed. The reaction mixture was concentrated and the residue was purified by prep-HPLC (base condition) to afford 22 (15 mg, 0.04 mmol, 20% yield, >99% purity) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ 9.25 (s, 1H), 8.55 (s, 1H), 7.55-7.53 (m, 2H), 7.44-7.37 (m, 4H), 7.14-7.11 (m, 2H), 3.82 (s, 3H). LC-MS (ESI) m/z 354 [M + H]+. High-resolution mass spectrum calculated for C₁₈H₁₄F₂N₅O is [M+H]+= 354.1161, observed for C₁₈H₁₄F₂N₅O[d₆] is [M+H]+= 354.1175.

tert-Butyl (4-(4-fluorophenyl)-3-formyl-1-methyl-1H-pyrazol-5-yl)carbamate. The reaction mixture of tert-butyl (4-(4-fluorophenyl)-3-formyl-1-methyl-1H-pyrazol-5-yl)carbamate with the corresponding di-Boc protected and free amine (1 g, 3 mmol, 1 eq) and MnO₂ (27 g, 31 mmol, 10 eq) in DCM (10 mL) was stirred at 20 °C for 2 hours. TLC (PE:EtOAc = 2:1, Rf = 0.44) showed the reaction was completed and the desired MS was observed on LCMS. The mixture was filtered and the filtrate was concentrated to get tert-butyl (4-(4-fluorophenyl)-3-formyl-1-methyl-1H-pyrazol-5-yl)carbamate (700 mg, 2.2 mmol, 70% yield) as a yellow solid. LC-MS (ESI) m/z 320 [M + H]+.

tert-Butyl(E)-(3-(2-cyanovinyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate. The reaction mixture of tert-butyl (4-(4-fluorophenyl)-3-formyl-1-methyl-1H-pyrazol-5-yl)carbamate (650 mg, 2 mmol, 1 eq), i-ButOK (275 mg, 2.4 mmol, 1.2 eq) and diethyl (cyanomethyl)phosphonate (435 mg, 2.4 mmol, 1.2 eq) in THF (7.00 mL) was stirred at 20 °C for 2 hours. Desired MS was observed on LCMS. The mixture was concentrated and diluted with water (50 mL). The aqueous layer was extracted with EtOAc (3x 50 mL). The combined organic layers were concentrated and the residue was purified by column chromatography on silica gel (PE:EtOAc = 5:1 ~ 2:1) to get compound tert-butyl(E)-(3-(2-cyanovinyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (450 mg, E:Z mixture) as a yellow solid. The compound was used as a mixture in the next step. LC-MS (ESI) m/z 243 [M + H]+.

tert-Butyl (3-(2-cyanoethyl)-(E)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate. The reaction mixture of tert-butyl (E)-(3-(2-cyanoethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (450 mg, 1.3 mmol, 1 eq) and Pd/C (155 mg, 1.3 mmol, 1 eq) in MeOH (5 mL) was stirred under H₂ (15 psi) at 20 °C for 2 hour. TLC (PE:EtOAc = 2:1, Rf = 0.24) showed the reaction was completed. The mixture was filtered and the filtrate was concentrated to get tert-butyl (3-(2-cyanoethyl)-(4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (400 mg, 1.2 mmol, 89% yield, >99% purity) as a yellow solid, which was used in next step without further purification. LC-MS (ESI) m/z 345 [M + H]+.

3-(5-Amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanenitrile. tert-butyl (3-(2-cyanoethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (200 mg, 0.6 mmol, 1 eq) was added to HCl/EtOAc (4 mol/L, 2 mL) and the reaction mixture was stirred at 20 °C for 8 h. TLC (PE:EtOAc = 2:1, Rf = 0.35) showed the reaction was completed and the desired MS was observed on LCMS. The mixture was basified to pH=8 with saturated aqueous NaHCO₃ solution and extracted with EtOAc (3 x 10 mL). The combined organic layers were concentrated to get 3-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanenitrile (150 mg, crude) as a yellow oil, which was used in next step without further purification. LC-MS (ESI) m/z 245 [M + H]+.
Supplemental Information

1-(3-(2-Cyanoethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)-3-(4-fluorophenyl)urea (24). The reaction mixture of 3-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanenitrile (150 mg, 0.6 mmol, 1 eq) and 1-fluoro-4-isocyanatobenzene (101 mg, 0.7 mmol, 1.2 eq) in THF (2 mL) was stirred at 20 °C for 8 hours. TLC (DCM:MeOH=10:1, Rf=0.51) showed the reaction was completed and the desired MS was observed on LCMS. The mixture was concentrated and the residue was purified by Prep-HPLC to afford 24 (30 mg, 0.08 mmol, 13% yield, >99% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 9.06 (s, 1H), 8.28 (s, 1H), 7.43-7.42 (m, 2H), 7.37-7.36 (m, 2H), 7.27-7.25 (m, 2H), 7.11-7.09 (m, 2H), 3.66 (s, 3H), 2.81-2.79 (m, 2H), 2.67 (m, 2H). LC-MS (ESI) m/z 382 [M + H]^+. High-resolution mass spectrum calculated for C20H18F2N5O [M+H]^+= 382.1474, observed for C20H18F2N5O [M+H]^+= 382.1471

3-(5-((tert-Butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanoic acid. The reaction mixture of tert-butyl (3-(2-cyanoethyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (200 mg, 0.66 mmol, 1 eq) and NaOH (93 mg, 2.3 mmol, 4 eq) in H₂O (460 µL) and EtOH (2 mL) was stirred at 70 °C for 12 hours. TLC (PE:EtOAc=1:1, Rf=0.15) showed the reaction was completed and the desired MS was observed on LCMS. The mixture was diluted with water (10 mL) and adjusted pH=6 with HCl (2N, 2 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL). The organic layers were concentrated to get 3-(5-((tert-butoxycarbonyl)amino)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanoic acid (180 mg, crude) as a yellow oil, which was used in next step without further purification. LC-MS (ESI) m/z 364 [M + H]^+. 

tert-Butyl(3-(3-amino-3-oxopropyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate. The reaction mixture of tert-butyl (3-(3-amino-3-oxopropyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)carbamate (180 mg, 0.55 mmol, 1 eq), NH₄Cl (79 mg, 1.55 mmol, 3 eq), DIEA (224 mg, 1.7 mmol, 3.5 eq), EDCI (95 mg, 0.5 mmol, 1 eq), HOBT (67 mg, 0.55 mmol, 1 eq) and DMF (2 mL) was stirred at 20 °C for 8 hours. TLC (DCM:MeOH=10:1, Rf=0.51) showed the reaction was completed and the desired MS was observed on LCMS. The mixture was diluted with water (10 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were concentrated and the residue was purified by prep-TLC (DCM:MeOH=10:1) to get tert-butyl (3-(3-amino-3-oxopropyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (150 mg, crude) as a yellow oil. LC-MS (ESI) m/z 363 [M + H]^+

3-(5-Amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanamide. The reaction mixture of tert-butyl (3-(3-amino-3-oxopropyl)-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-5-yl)carbamate (150 mg, 0.4 mmol, 1 eq) in HCl/EtOAc (4 mol/L, 2 mL) was stirred at 20 °C for 0.5 h. The desired MS was observed on LCMS. The mixture was adjusted pH=8 with aqueous NaHCO₃ solution (10 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were concentrated to get 3-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanamide (100 mg, crude) as a yellow solid. LC-MS (ESI) m/z 263 [M + H]^+

3-(4-(4-Fluorophenyl)-5-(3-(4-fluorophenyl)ureido)-1-methyl-1H-pyrazol-3-yl)propanamide (21). The reaction mixture of 3-(5-amino-4-(4-fluorophenyl)-1-methyl-1H-pyrazol-3-yl)propanamide (100 mg, 0.4 mmol, 1 eq) and 1-fluoro-4-isocyanatobenzene (63 mg, 0.48 mmol, 1.2 eq) in THF (1 mL) was stirred at 20 °C for 8 hours. TLC (DCM:MeOH=10:1, Rf=0.51) showed the reaction was completed. The mixture was concentrated and the residue was purified by prep-HPLC to afford 21 (24 mg, 0.06 mmol, 15% yield, 94% purity) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.99 (s, 1H), 8.15 (s, 1H),7.43-7.42 (m, 2H), 7.36-7.35 (m, 2H), 7.34 (s, 1H), 7.26-7.24 (m, 2H), 7.12-7.09 (m, 2H), 6.75 (s, 1H), 3.63 (s, 3H), 2.76-2.73 (m, 2H), 2.42-2.38 (m, 2H). LC-MS (ESI) m/z 400 [M + H]^+. High-resolution mass spectrum calculated for C20H20F2N5O2 [M+H]^+= 400.158, observed for C20H20F2N5O2 [M+H]^+= 400.1574

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