Supporting Information

Potent and selective inhibitors of CDPK1 from *T. gondii* and *C. parvum* based on a 5-aminopyrazole-4-carboxamide scaffold

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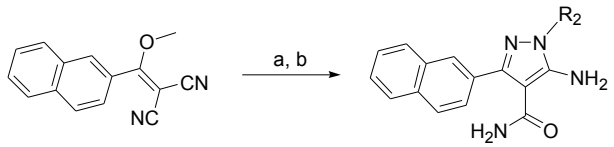
Synthetic Methods and Procedures

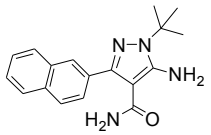
Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. The microwave irradiation was performed in a CEM Discover System. The final purity of all compounds was determined by analytical LCMS with Phenomenex Onyx Monolithic C18 column (4.6 mm x 100 mm). The products were detected by UV at 220 nm. All compounds were determined to be >95% pure by this method. The purification by preparative HPLC was performed on Waters Xterra Prep RP18 OBD 5µM (19 mm x 50 mm) with CH_3CN/H_2O and 0.1% TFA as eluent. The mass spectra were recorded with an Agilent Ion Trap Mass Spectrometer. NMR spectra were recorded on either a Bruker 500 MHz spectrometer or a Bruker 300 MHz spectrometer at ambient temperature. Inhibitors were synthesized through several different routes, as represented in **Schemes 1-9**. Syntheses of compounds **2**, **4**, **7** and **9** have been previously reported.[1, 2] All other syntheses and compound characterization data are presented below.

Scheme 1



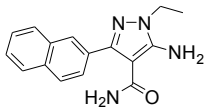
Reagents and conditions (a) R_1-$NHNH_2$, EtOH, microwave, 100°C, 30min; (b) NaOH, EtOH, microwave, 110°C, 10min.

General Procedure A (**1, 12-23**): A mixture of 2-(methoxy(naphthalene-7-yl)methylene)malononitrile (50 mg, 0.21 mmol) prepared according to the reference[1] and the appropriate alkyl hydrazine (1.2 equiv) were dissolved in ethanol (1ml) in an capped microwave tube. The reaction mixture was microwave irradiated at 100 °C for 30min. After cooling down, 0.2 ml of saturated aqueous NaOH was added *in situ* and the solution was microwave irradiated at 110 °C for 20min. After cooling down to 0 °C, concentrated HCl was added slowly to neutralize the solution. The solution was extracted with ethyl acetate, washed with water twice. The solvent was removed and the residue was dissolved in methanol and purified by preparative HPLC with an acetonitrile/water gradient with 0.1% TFA to yield the final products. Using this procedure, only one regioisomer as indicated in the scheme was isolated from the reaction.[3]



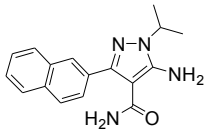
1

1 was synthesized using t-butyl hydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.05 (1 H, s), 7.92 (1 H, d, *J* 8.6), 7.88 (2 H, dd, *J* 8.7, 4.6), 7.65 (1 H, d, *J* 1.8), 7.56 – 7.48 (2 H, m), 5.72 (2 H, s), 1.70 (9 H, s). MS (ESI) $(M+H)^+$= 309.6.



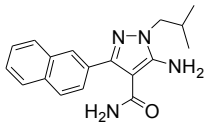
12

12 was synthesized using ethyl hydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.06 (1 H, d, *J* 1.2), 7.93 (1 H, d, *J* 8.4), 7.88 (2 H, dd, *J* 6.0, 3.4), 7.66 (1 H, dd, *J* 8.4, 1.6), 7.57 – 7.48 (2 H, m), 5.44 (2 H, s), 4.01 (2 H, q, *J* 7.3), 1.46 (3 H, t, *J* 7.3). MS (ESI) $(M+H)^+$= 281.8.



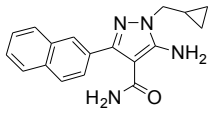
13

13 was synthesized using isopropylhydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.05 (1 H, s), 7.93 (1 H, d, *J* 8.5), 7.88 (2 H, dd, *J* 8.5, 5.2), 7.66 (1 H, d, *J* 3.4), 7.56 – 7.48 (2 H, m), 5.45 (2 H, s), 5.30 (2 H, s), 4.35 – 4.22 (1 H, m), 1.53 (6 H, d, *J* 6.6). MS (ESI) $(M+H)^+$= 295.7.



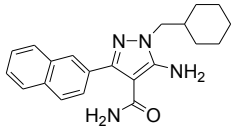
14

14 was synthesized using isobutylhydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.05 (1 H, s), 7.93 (1 H, d, *J* 8.4), 7.90 – 7.83 (2 H, m), 7.65 (1 H, dd, *J* 8.4, 1.7), 7.57 – 7.48 (2 H, m), 5.45 (2 H, s), 5.32 (2 H, s), 3.75 (2 H, d, *J* 7.4), 2.38 – 2.23 (1 H, m), 0.98 (6 H, m). MS (ESI) $(M+H)^+$= 309.7.



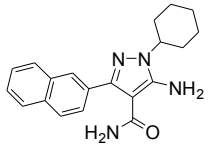
15

15 was synthesized using cyclopropylmethylhydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.06 (1 H, s), 7.94 (1 H, d, *J* 8.3), 7.91 – 7.86 (2 H, m), 7.66 (1 H, dd, *J* 8.4, 1.7), 7.56 – 7.51 (2 H, m), 5.48 (2 H, s), 3.91 (2 H, d, *J* 6.6), 1.36 – 1.23 (1 H, m), 0.66 (2 H, m), 0.47 – 0.40 (2 H, m). MS (ESI) $(M+H)^+$= 307.8.



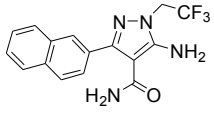
16

16 was synthesized using 1-(cyclohexylmethyl)hydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.04 (1 H, s), 7.98 – 7.82 (3 H, m), 7.64 (1 H, m), 7.59 – 7.47 (2 H, m), 3.77 (2 H, d, *J* 7.3), 2.09 – 1.90 (1 H, m), 1.72 (5 H, m), 1.23 (3 H, m), 1.03 (2 H, m). MS (ESI) $(M+H)^+$= 349.7.



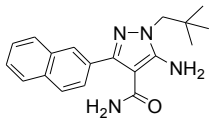
17

17 was synthesized using cyclohexylhydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.03 (1 H, s), 7.95 (1 H, d, *J* 8.4), 7.93 – 7.83 (2 H, m), 7.64 – 7.51 (3 H, m), 3.91 – 3.77 (1 H, m), 2.08 – 1.89 (6 H, m), 1.73 (1 H, m), 1.41 (2 H, m), 1.27 (1 H, m). MS (ESI) $(M+H)^+$= 335.7.



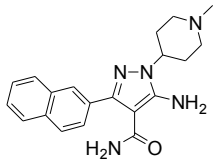
18

18 was synthesized using 2,2,2-trifluoromethylhydrazine following General Procedure A except using different hydrolytic condition. Hydrolysis of cyano group to amide was performed by using 30% H_2O_2 (0.2ml), NH_4OH (0.6ml) and ethanol (0.6ml) at room temperature for 5 days. H^1 NMR (500 MHz, $CDCl_3$) δ 8.06 (1 H, s), 7.95 (1 H, d, *J* 8.3), 7.89 (2 H, m), 7.64 (1 H, m), 7.60 – 7.50 (2 H, m), 5.57 (4 H, broad), 4.62 (2 H, m). MS (ESI) $(M+H)^+$= 335.7.



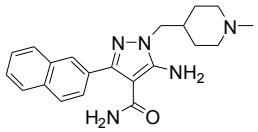
19

19 was synthesized using neopentylhydrazine following General Procedure A. H^1 NMR (500 MHz, $CDCl_3$) δ 8.13 (1 H, s), 7.99 (1 H, s), 7.92 (2 H, s), 7.70 – 7.64 (1 H, m), 7.59 (2 H, s), 3.99 (2 H, d, *J* 12.1), 1.14 (9 H, s). MS (ESI) $(M+H)^+$= 323.8.



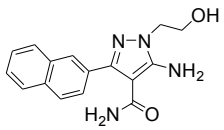
20

20 was synthesized using 1-(1-methylpiperidin-4-yl)hydrazine following General Procedure A. H^1 NMR (500 MHz, MeOD) δ 8.04 (1 H, s), 7.99 (1 H, d, *J* 8.6), 7.93 (2 H, m), 7.60 (1 H, dd, *J* 7.5, 1.5), 7.55 (2 H, d, *J* 8.3), 4.60 – 4.49 (1 H, m), 3.68 (2 H, m), 3.27 – 3.21 (2 H, m), 2.94 (3 H, s), 2.39 (2 H, m), 2.24 (2 H, m). MS (ESI) $(M+H)^+$= 350.6.



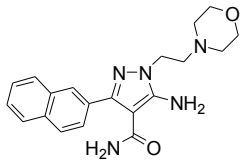
21

21 was synthesized using 1-methyl-4-(hydrazinylmethyl)piperidine following General Procedure A or General Procedure C by using **1** and 1-methyl-4-methanesulfonyloxymethylpiperidine. H^1 NMR (500 MHz, MeOD) δ 8.18 (1 H, s), 8.05 (1 H, d, *J* 8.6), 8.00 – 7.91 (2 H, m), 7.65 (1 H, d, *J* 8.9), 7.62 – 7.54 (2 H, m), 4.12 (2 H, d, *J* 7.0), 3.53 (2 H, d, *J* 13.1), 3.02 (2 H, t, *J* 12.0), 2.83 (3 H, s), 2.28 (1 H, m), 1.97 (2 H, d, *J* 12.1), 1.71 (2 H, m). MS (ESI) $(M+H)^+$= 364.6.



22

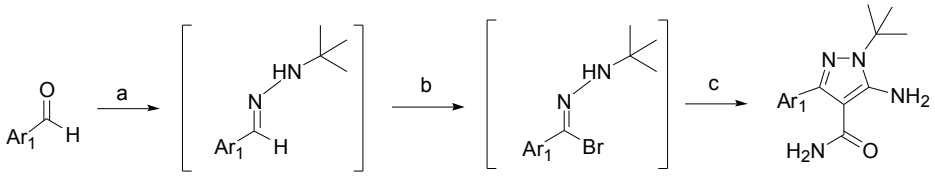
22 was synthesized using 2-hydrazinylethanol following General Procedure A. H^1 NMR (500 MHz, MeOD) δ 8.04 (1 H, s), 7.96 (1 H, d, *J* 8.5), 7.93 – 7.86 (2 H, m), 7.60 (1 H, m), 7.52 (2 H, m), 4.09 (2 H, t, *J* 5.3), 3.88 (2 H, t, *J* 5.2). MS (ESI) $(M+H)^+$= 297.5.



23

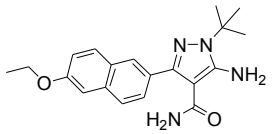
23 was synthesized using 4-(2-hydrazinoethyl)morpholine following General Procedure A. H^1 NMR (500 MHz, DMSO) δ 8.07 (1 H, s), 8.04 – 7.91 (3 H, m), 7.64 (1 H, m), 7.56 (2 H, dd, *J* 6.2, 3.3), 4.50 (2 H, t, *J* 6.8), 3.97 (2 H, m), 3.80 (2 H, m), 3.55 (2 H, t, *J* 6.7), 3.48 (2 H, m), 3.17 (2 H, m). MS (ESI) $(M+H)^+$= 366.6.

Scheme 2



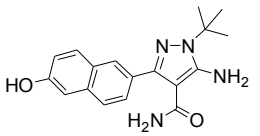
Reagents and conditions (a) t-butylhydrazine, DMF; (b) NBS, DMF, 0 °C; (c) EtONa, cyanoacetamide, EtOH.

General Procedure B (**3**, **24, 25, 27, 30, 34-39**): A mixture of the appropriate aromatic aldehyde (1.5 mmole) and powder t-butylhydrazine HCl salt (1.1 equiv) with DIPEA (1.1 equiv) were dissolved in 3ml of anhydrous DMF in a capped microwave tube. The mixture was microwave irradiated at 80 °C for 20 min or stirred at room temperature for 2h. After cooling down to 0 °C, to the solution was slowly added NBS (1.1 equiv) in 0.5ml DMF. The mixture was kept at 0 °C and stirred for 2h. Cyanoacetamide anion was generated by treatment of cyanoacetamide (1.1 equiv) in 3ml of anhydrous ethanol with 2.5 equiv of sodium ethoxide.[4] This mixture was mixed with the above bromohydrazone DMF solution. The reaction was stirred overnight at room temperature. After most solvents were removed, the residue was diluted with ethyl acetate and washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification via flash chromatography on silica gel eluted with MeOH –DCM and further with preparative HPLC gave the HPLC pure final products.



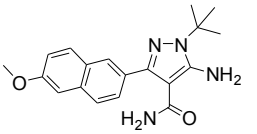
3

3 was synthesized using 6-ethoxynaphthalene-2-carboxaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 7.94 (1 H, s), 7.78 (2 H, dd, *J* 12.5, 8.7), 7.58 (1 H, dd, *J* 8.0, 1.1), 7.18 (1 H, dd, *J* 8.8, 2.5), 7.14 (1 H, d, *J* 2.4), 5.41 (2 H, s), 4.23 – 4.11 (2 H, m), 1.69 (9 H, s), 1.49 (3 H, t, *J* 5.8). MS (ESI) $(M+H)^+$= 353.6.



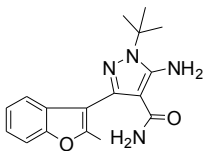
24

24 was synthesized using 6-hydroxynaphthalene-2-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 7.89 (1 H, s), 7.71 (2 H, d, *J* 8.1), 7.50 (1 H, m), 7.12 – 7.07 (2 H, m), 5.72 (2 H, s), 1.71 (28 H, s). MS (ESI) $(M+H)^+$= 325.6.



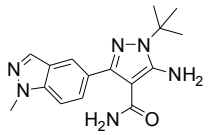
25

25 was synthesized using 6-methoxynaphthalene-2-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 7.96 (1 H, s), 7.79 (2 H, m), 7.60 (1 H, m), 7.18 (2 H, m), 3.94 (3 H, s), 1.69 (9 H, s). MS (ESI) $(M+H)^+$= 340.0.



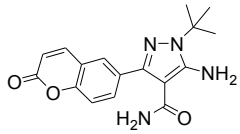
27

27 was synthesized using 2-methylbenzofuran-3-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 7.46 (1 H, d, *J* 8.1), 7.40 (1 H, d, *J* 7.3), 7.28 (1 H, t, *J* 7.1), 7.23 (1 H, t, *J* 7.0), 2.50 (3 H, s), 1.70 (9 H, s). MS (ESI) $(M+H)^+$= 313.7.



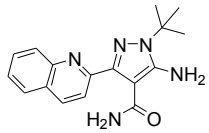
30

30 was synthesized using 1-methyl-1H-indazole-5-carbaldehyde following General Procedure B. B. H^1 NMR (500 MHz, DMSO) δ 8.72 (1 H, s), 7.57 (1 H, d, *J* 8.3), 7.45 (1 H, s), 7.39 (1 H, d, *J* 6.9), 3.65 (3 H, s), 1.58 (9 H, s). MS (ESI) $(M+H)^+$= 313.8.



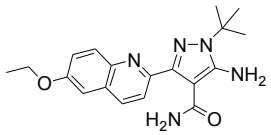
34

34 was synthesized using coumarin-6-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 7.73 (3 H, m), 7.41 (1 H, d, *J* 8.5), 6.48 (1 H, d, *J* 9.5), 5.70 (2 H, s), 5.13 (2 H, s), 1.68 (9 H, s). MS (ESI) $(M+H)^+$= 327.7.



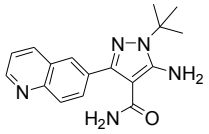
35

35 was synthesized using quinoline-2-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 8.39 (1 H, d, *J* 8.8), 8.17 (1 H, d, *J* 8.9), 7.95 (1 H, d, *J* 8.4), 7.82 (1 H, d, *J* 6.9), 7.74 – 7.66 (1 H, m), 7.57 – 7.50 (1 H, m), 6.08 (2 H, s), 1.73 (9 H, s). MS (ESI) $(M+H)^+$= 310.6.



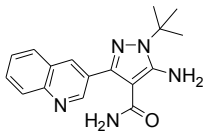
36

36 was synthesized using 6-ethoxyquinoline-2-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 8.33 (1 H, d, *J* 8.8), 8.05 (1 H, d, *J* 8.9), 7.84 (1 H, d, *J* 9.1), 7.34 (1 H, dd, *J* 9.1, 2.7), 7.08 (1 H, d, *J* 2.7), 6.04 (2 H, s), 4.17 (2 H, m), 1.72 (9 H, s), 1.50 (3 H, t, *J* 7.0). MS (ESI) $(M+H)^+$= 354.6.



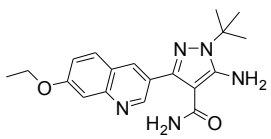
37

37 was synthesized using quinoline-6-carbaldehyde following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 8.95 (1 H, d, *J* 4.5), 8.18 (2 H, m), 8.05 (1 H, s), 7.91 (1 H, d, *J* 5.6), 7.50 – 7.40 (1 H, m), 5.73 (2 H, s), 1.68 (9 H, s). MS (ESI) $(M+H)^+$= 310.8.



38

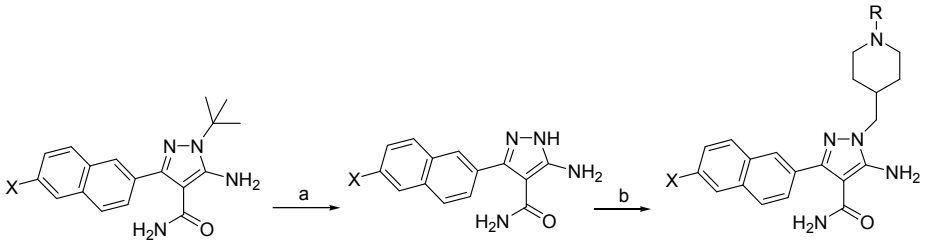
38 was synthesized using quinoline-3-carbaldehyde prepared according to the literature prodedure[5] following General Procedure B. H^1 NMR (500 MHz, DMSO) δ 9.01 (1 H, s), 8.44 (1 H, s), 8.06 (2 H, d, *J* 9.0), 7.84 – 7.74 (1 H, m), 7.68 – 7.60 (1 H, m), 6.23 (2 H, s), 1.61 (9 H, s). MS (ESI) $(M+H)^+$= 310.7.



39

39 was synthesized using 7-ethoxyquinoline-3-carbaldehyde prepared according to the literature prodedure[5] following General Procedure B. H^1 NMR (500 MHz, $CDCl_3$) δ 9.19 (1 H, s), 8.84 (1 H, s), 7.98 (1 H, d, *J* 13.2), 7.77 (1 H, s), 7.47 (1 H, d, *J* 10.5), 6.10 (2 H, s), 4.28 (2 H, m), 1.68 (9 H, s), 1.53 (3 H, t, *J* 6.9). MS (ESI) $(M+H)^+$= 354.5.

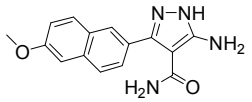
Scheme 3



1, 3, 25 **A** **6, 8, 10, 11**

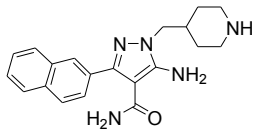
Reagents and conditions (a) concentrated H_2SO_4, 1 h; (b) 4-Methylsulfonyloxypiperidine, DMF, K_2CO_3, microwave, 90 °C, 30 min.

General Procedure C (**6, 8, 10, 11**): **1, 3** and **25** were treated with 1ml of concentrated sulfuric acid respectively for 1h at room temperature. After cooling down to 0 °C, solid NaOH was added in small portions until the solution became neutral or basic. The slurry mixture was slowly diluted with water and extracted with ethyl acetate twice. The organic extract was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure to give intermediate **A**. 4-Methylsulfonyloxypiperidine (0. 8mmol) and **A** (0.08mmol) in 1 ml of DMF containing K_2CO_3 (1.0mmol) was microwave irradiated at 90 °C for 30 min. After solvent was removed, the residues were extracted with ethyl acetate, washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was Boc deprotected if necessary before HPLC purification. The preparative HPLC purification can separate the isomers in N-1 and N-2 positions and the N-1position product was collected and confirmed by H^1 NMR in d6-DMSO.



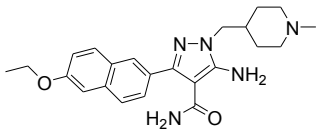
11

11 was synthesized from **25** following General Procedure C as one of the intermediate **A**. H^1 NMR (500 MHz, MeOD) δ 7.96 (1 H, s), 7.90 (1 H, d, *J* 8.5), 7.83 (1 H, d, *J* 9.0), 7.56 (1 H, m), 7.30 (1 H, t, *J* 3.7), 7.20 (1 H, m), 3.30 (3 H, s). MS (ESI) $(M+H)^+$= 283.6.



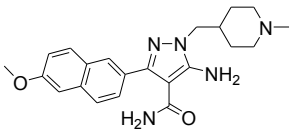
6

6 was synthesized using 1-Boc-4-Methanesulfonyloxymethyl-piperidine and **1** which was pre-treated with sulfuric acid following General Procedure C or using 4-(hydrazinylmethyl)-piperidine following General Procedure A. H^1 NMR (500 MHz, MeOD) δ 8.19 (1 H, s), 8.04 (1 H, d, *J* 8.4), 7.95 (2 H, m), 7.65 (1 H, d, *J* 8.4), 7.62 – 7.54 (2 H, m), 4.13 (2 H, d, *J* 7.0), 3.41 (2 H, d, *J* 13.6), 2.99 (2 H, m), 2.30 (1 H, s), 1.92 (2 H, d, *J* 12.6), 1.63 (2 H, m). MS (ESI) $(M+H)^+$= 350.5.



8

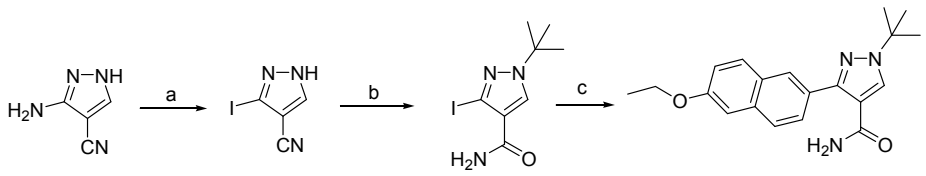
8 was synthesized using 1-methyl-4-methanesulfonyloxymethylpiperidine and **3** which was pre-treated with sulfuric acid following General Procedure C. H^1 NMR (500 MHz, MeOD) δ 8.09 (1 H, d, *J* 7.4), 7.93 (1 H, d, *J* 8.6), 7.86 (1 H, dd, *J* 8.4, 3.9), 7.60 (1 H, d, *J* 8.6), 7.30 (1 H, d, *J* 2.0), 7.21 (1 H, dd, *J* 8.6, 2.0), 4.27 – 4.10 (4 H, m), 3.71 (2 H, m), 3.06 (2 H, m), 2.99 – 2.86 (3 H, m), 2.83 (1 H, m), 2.26 (1 H, m), 1.99 (2 H, m), 1.72 (1 H, m), 1.44 (3 H, t, *J* 7.0). MS (ESI) $(M+H)^+$= 408.6.



10

10 was synthesized using compound **11** and 1-methyl-4-Methanesulfonyloxymethylpiperidine following General Procedure C. H^1 NMR (500 MHz, MeOD) δ 8.11 (1 H, s), 7.95 (1 H, d, *J* 8.5), 7.87 (1 H, m), 7.61 (1 H, d, *J* 8.5), 7.32 (1 H, d, *J* 2.2), 7.22 (1 H, m), 4.20 (2 H, m), 3.91 (3 H, s), 3.73 – 3.60 (1 H, m), 3.54 (1 H, m), 3.19 (1 H, m), 3.13 – 2.99 (1 H, m), 2.91 (3 H, s), 2.41 (1 H, m), 2.28 (1 H, m), 2.05 (2 H, m), 1.74 (1 H, m). MS (ESI) $(M+H)^+$= 394.5.

Scheme 4



B **C** **5**

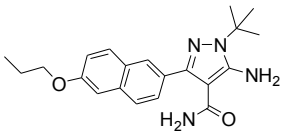
Reagents and conditions (a) $NaNO_2$, KI, HCl, H_2O; (b) ^tBuOH, H_2SO_4; (c) DME, H_2O, K_2CO_3, $Pd(PPh_3)_4$, (6-ethoxynaphthalen-2yl)boronic acid, microwave, 85°C, 20 min.

Synthetic Procedure for 5

Intermediate B: To a stirred suspension of 1H 3-amino-1H-pyrazole-4-carbonitrile (1.08 g, 10mmol) in concentrated HCl (13.0 ml) was added a solution of sodium nitrite (1.38 g, 20 mmol) in water (3.0 ml) over 5 min at 0 °C. To the resulting reaction mixture was added a solution of KI (4.1 g, 25 mmol) in water (7.0 ml) over 10 min. The reaction mixture was stirred for 5 min further, then extracted with ether (3 × 30 ml) and the combined organic extracts were washed with $Na_2S_2O_3$ (2 × 30ml), dried over Na_2SO_4 and concentrated under reduced pressure to give 1.63 g of **B** as light yellow solid, yield: 74.6 %. MS (ESI) $(M+H)^+$= 220.9.

Intermediate C: To a solution of **B** (110 mg, 0.5 mmol) in tert-butanol (5 ml) was added sulfuric acid (0.110 ml). The reaction mixture was heated at 100 °C for 3 h. After cooling down to 0 °C, solid NaOH was added in small portions until the solution became neutral or basic. The slurry mixture was slowly diluted with water and extracted with ethyl acetate twice. The organic extract was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification via flash chromatography on silica gel gave 50 mg of intermediate **C** as a white solid, yield: 34.1 %. MS (ESI) $(M+H)^+$= 294.8.

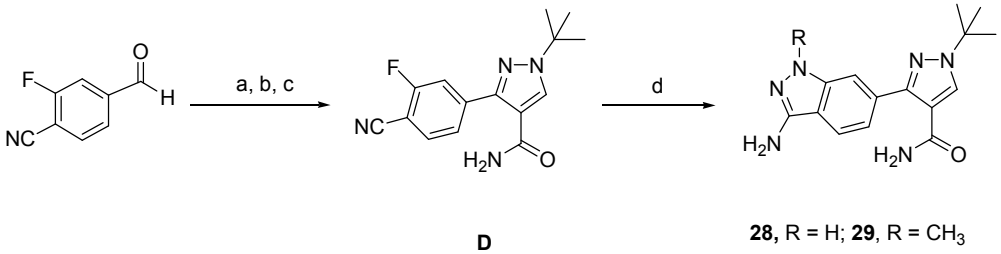
5: To a solution of **C** (20 mg, 0.068 mmol) in a mixture of DME:H_2O = 3:1 (4 ml) was added K_2CO_3 (28 mg, 0.2 mmol), $Pd(PPh_3)_4$ (8 mg, 0.007 mmol) and (6-ethoxynaphthalen-2yl)boronic acid (16 mg, 0.075 mmol). The mixture was microwave irradiated at 85 °C for 20 min. After cooling down to room temperature, ethyl acetate was added and organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification via flash chromatography on silica gel eluted with MeOH –DCM and further with preparative HPLC gave the HPLC pure final product. H^1 NMR (300 MHz, DMSO) δ 8.36 (1 H, s), 8.23 (1 H, s), 7.81 (3 H, m), 7.42 (1 H, s), 7.32 (1 H, s), 7.16 (1 H, d, *J* 8.9), 7.01 (1 H, s), 4.17 (2 H, q, *J* 7.1), 1.60 (9 H, s), 1.42 (3 H, t, *J* 6.9). MS (ESI) $(M+H)^+$= 338.8.



26

26: To a solution of **24** (20 mg) in DMF (0.5 ml) was added K_2CO_3 (20 equiv), 1-bromopropane (20 equiv). The mixture was microwave irradiated at 75 °C for 40 min. After cooling down to room temperature, ethyl acetate was added and the organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification via preparative HPLC gave the HPLC pure final product. H^1 NMR (500 MHz, $CDCl_3$) δ 7.97 (1 H, s), 7.80 (2 H, d, *J* 13.3), 7.57 (1 H, s), 7.20 (1 H, d, *J* 7.2), 7.15 (1 H, s), 4.05 (2 H, t, *J* 6.3), 1.88 (2 H, m), 1.78 (9 H, s), 1.09 (3 H, t, *J* 7.4). MS (ESI) $(M+H)^+$= 367.6.

Scheme 5



D **28,** R = H; **29**, R = CH_3

Reagents and conditions (a) t-butylhydrazine, DMF; (b) NBS, DMF, 0 °C; (c) EtONa, cyanoacetamide, EtOH; (d) R-$NHNH_2$, EtOH, heating

General Procedure D (**28, 29**):

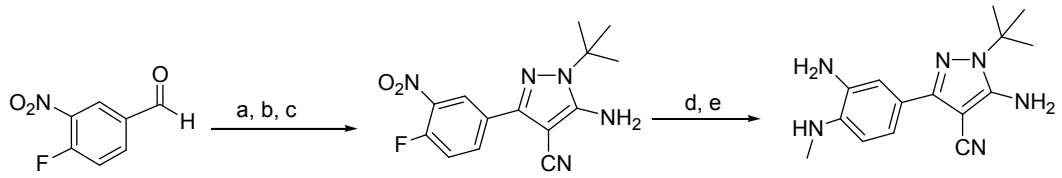
Intermediate D was synthesized using 2-fluoro-4-formylbenzonitrile following General Procedure B. H^1 NMR (500 MHz, MeOD) δ 7.81 (1 H, t, *J* 7.3), 7.59 (2 H, t, *J* 9.7), 1.67 (9 H, s). MS (ESI) $(M+H)^+$= 302.7.

A solution of **D** (20 mg) in ethanol (0.5 ml) and hydrazine monohydrate or methylhydrazine (60 µl) was microwave irradiated at 120 °C for 40 min. After cooling down to room temperature, ethyl acetate was added and the organic layer was washed with water three times, brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification via preparative HPLC gave the HPLC pure final product

28 was synthesized using hydrazine monohydrate following General Procedure D. H^1 NMR (500 MHz, DMSO) δ 7.72 (1 H, d, *J* 8.2), 7.26 (1 H, s), 6.98 (1 H, d, *J* 8.2), 6.29 (2 H, s), 5.39 (2 H, s), 1.55 (9 H, s). MS (ESI) $(M+H)^+$= 314.6.

29 was synthesized using methylhydrazine following General Procedure D. H^1 NMR (500 MHz, DMSO) δ 7.71 (1 H, d, *J* 8.2), 7.36 (1 H, s), 6.94 (1 H, d, *J* 8.2), 6.32 (2 H, s), 5.46 (2 H, s), 3.72 (3 H, s), 1.56 (9 H, s). MS (ESI) $(M+H)^+$= 328.6.

Scheme 6



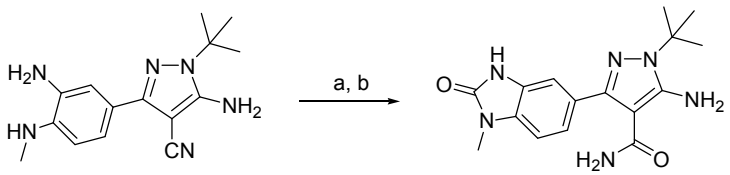
E **F**

Reagents and conditions (a) t-butylhydrazine, DMF; (b) NBS, DMF, 0 °C; (c) EtONa, malononitrile, EtOH; (d) CH_3NH_2; (e) Zn, $HCONH_2$.

Intermediate E was synthesized using 3-nitro-4-fluorobenzaldehyde following General Procedure B except using malononitrile instead of cyanoactamide. H^1 NMR (500 MHz, $CDCl_3$) δ 8.59 (1 H, m), 8.21 – 8.13 (1 H, m), 7.37 – 7.27 (1 H, m), 1.69 (9 H, s). MS (ESI) $(M+H)^+$= 304.8.

Intermediate F: A solution of **E** (160 mg, 0.53mmol) in ethanol (1.0 ml) and methylamine (4 ml, 33% wt in EtOH) was stirred at room temperature for 30 min. After solvents were removed, the residue was diluted with ethyl acetate. The organic extract was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in 5 ml of methanol. To the solution, ammonium formate (5 equiv) was added with stirring, then powder Zn (5 equiv) was added slowly with strong stirring. After 10 min, the solvent was removed and 20 ml of ethyl acetate was added. The organic extract was purified via flash chromatography on silica gel to obtain 96 mg of **F** in brownish solid. H^1 NMR (500 MHz, MeOD) δ 7.32 – 7.18 (2 H, m), 6.60 (1 H, d, *J* 8.2), 2.86 (3 H, s), 1.64 (9 H, s). MS (ESI) $(M+H)^+$= 285.6.

Scheme 7

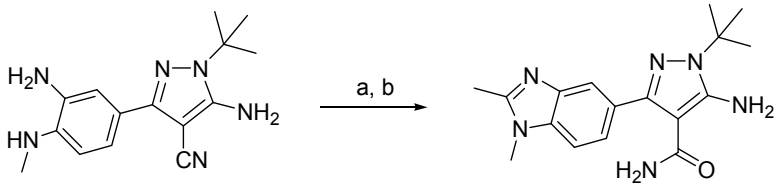


F **31**

Reagents and conditions (a) CDI, microwave, 70°C, 30min; (b) NaOH, EtOH, microwave, 110°C, 10min.

31: A solution of **F** (12 mg) in anhydrous THF (1.0 ml) was added 1,1'-carbonyldiimidazole (2.5 equiv). The mixture was microwave irradiated at 70 °C for 30 min. After cooling down to room temperature, ethyl acetate was added and the organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in 1 ml of ethanol, 0.2 ml of saturated aqueous NaOH was added and the solution was microwave irradiated at 110 °C for 20min. After cooling down to 0 °C, concentrated HCl was added slowly to neutralize the solution. The solution was extracted with ethyl acetate, washed with water twice. The solvent was removed and the residue was dissolved in methanol and purified by preparative HPLC with an acetonitrile/water gradient with 0.1% TFA to yield the final product. H^1 NMR (500 MHz, DMSO) δ 10.94 (1 H, s), 7.15 (2 H, m), 7.05 (1 H, s), 2.53 (3 H, s), 1.59 (9 H, s). MS (ESI) $(M+H)^+$= 329.5.

Scheme 8

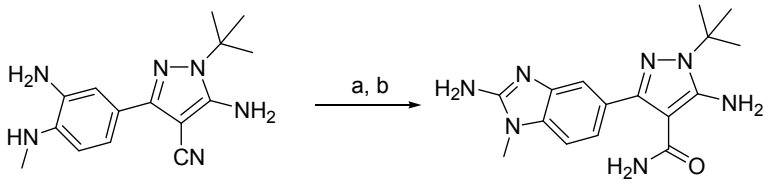


F **32**

Reagents and conditions (a) acetaldehyde, $NaHSO_3$, microwave, 70°C, 15min; (b) NaOH, EtOH, microwave, 110°C, 10min.

32: To a solution of **F** (12 mg) in THF (1.0 ml) was added acetaldehyde (6 equiv) and sodium bisulfite (6 equiv). The mixture was microwave irradiated at 70 °C for 15 min. After cooling down to room temperature, ethyl acetate was added and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in 1 ml of ethanol, 0.2 ml of saturated aqueous NaOH was added and the solution was microwave irradiated at 110 °C for 20min. After cooling down to 0 °C, concentrated HCl was added slowly to neutralize the solution. The solution was extracted with ethyl acetate, washed with water twice. The solvent was removed and the residue was dissolved in methanol and purified by preparative HPLC with an acetonitrile/water gradient with 0.1% TFA to yield the final product. H^1 NMR (500 MHz, $CDCl_3$) δ 8.22 (1 H, s), 7.81 (1 H, dd, *J* 8.4, 1.5), 7.27 (1 H, d, *J* 8.4), 4.45 (2 H, s), 3.71 (3 H, s),2.61 (3 H, s), 1.67(9 H,s) . MS (ESI) $(M+H)^+$= 327.7.

Scheme 9



F **33**

Reagents and conditions (a) EtOH, BrCN; (b) NaOH, EtOH, microwave, 110°C, 10min.

33: To a solution of **F** (12 mg) in anhydrous ethanol (0.5 ml) was added BrCN (2.5 equiv). The mixture was stirred at room temperature overnight. Ethyl acetate was added and washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in 1 ml of ethanol, 0.2 ml of saturated aqueous NaOH was added and the solution was microwave irradiated at 110 °C for 20min. After cooling down to 0 °C, concentrated HCl was added slowly to neutralize the solution. The solution was extracted with ethyl acetate, washed with water twice. The solvent was removed and the residue was dissolved in methanol and purified by preparative HPLC with an acetonitrile/water gradient with 0.1% TFA to yield the final product. H^1 NMR (500 MHz, MeOD) δ 7.67 – 7.60 (2 H, m), 7.55 (1 H, d, *J* 7.2), 3.72 (3 H, s), 3.32 (2 H, s), 1.72 (9 H, s). MS (ESI) $(M+H)^+$= 328.6.

Table S1. Crystallographic data and refinement statistics for CDPK1+cmpd 35

PDB entry	**4m84**
Space Group	**$P2_1$**
Unit Cell (a b c Å)	**48.12 73.18 66.01**
(α β γ °)	**90.0 99.8 90.0**
Resolution (Å)	**48.7-2.00 (2.05-2.00)**
Total unique reflections	**28948 (1692)**
Replicate cc(1/2)	**0.998 (0.393)**
Redundancy	**3.7 (3.1)**
Completeness (%)	**95 (68)**
Refinement resolution (Å)	**48.7-2.00**
R /R_{free}	**0.198 / 0.257**
RMSD bonds (Å)	**0.012**
RMSD angles (°)	**1.43**
Protein atoms	**3739**
Non-protein atoms	**109**
TLS groups	**8**
Mean B_{eq} protein atoms ($Å^2$)	**49.5**
Mean B_{eq} ligand atoms ($Å^2$)	**68.2**

Table S2. Selectivity of compound 39 for *Cp*CDPK1 over 20 representative kinases.

		Compound 39						
	Kinase	**IC_{50}, µM**	**K_i, µM**	**pK_i**				
	CDPK1	0.0003	0.0008	9.52				
	Prkcn	1.11	0.427	6.37				
	**Kdr	2.46	0.569	6.24				
	**EGFR	>10	1.67	5.78				
	CAMK1D^	>10	2.48	5.61				
	*MAP3K10	>10	3.06	5.51				
	CAMKK2^	>10	3.1	5.51				
	MEK1	>10	3.29	5.48				
	**BRAF	>10	3.37	5.47				
	**Rock1	>10	3.59	5.44				
	**FLT1	>10	3.67	5.44				
	p38 alpha	>10	3.92	5.41				
	**AUR1	>10	4.05	5.39				
	Ck1alpha1	>10	4.12	5.39				
	ACVR1	>10	4.32	5.36				
	*CAMK2A^	>10	4.32	5.36				
	*JAK3	>10	4.79	5.32				
	**Akt1	>10	5.01	5.30				
	Zipk^	>10	5.41	5.27				
	*ALK	>10	5.79	5.24				
	**Erk2	>10	6.6	5.18				
****/***	Denotes Kinases(**) or close relatives(*) whose inhibition causes cardiotoxicity[6]							
^	Denotes human kinases that are most closely related to CDPK1.							

Supporting Information References:

(1) Murphy, R. C.; Ojo, K. K.; Larson, E. T.; Castellanos-Gonzalez, A.; Perera, B. G. K.; Keyloun, K. R.; Kim, J. E.; Bhandari, J. G.; Muller, N. R.; Verlinde, C. L. M. J.; White, A. C.; Merritt, E. A.; Van Voorhis, W. C.; Maly, D. J. Discovery of Potent and Selective Inhibitors of CDPK1 from C. parvum and T. gondii. *ACS Medicinal Chemistry Letters* **2010**, *1* (7), 331-335.

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