Supporting Information

SAR Studies of 5-Aminopyrazole-4-carboxamide Analogs as Potent and Selective Inhibitors of *Toxoplasma gondii* CDPK1

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**Chemical Synthesis.**

The synthesis of AC derivatives has been reported in the literature.\(^1-3\) Compounds were synthesized using an established route with slightly altered reaction conditions (Scheme 1). A quinoline aldehyde with varied substitutions was reacted with a hydrazine to give the hydrazone, which was then brominated by NBS. The reactive intermediate was then reacted with cyanoacetamide anion that was generated \textit{in situ} to afford the final product. The majority of the synthetic effort was spent on generating quinoline aldehydes and hydrazines with desired substitutions. All the compounds were purified via flash chromatography on silica gel. Further purification, if necessary, was performed via preparatory RP-HPLC to get the HPLC pure final products. Some of the compounds were recovered to the free base by washing with NaHCO\(_3\) and others were converted to their HCl salts.

Scheme 1.

\[
\begin{align*}
\text{R}_{2}\text{N}=\text{N}-\text{H} & \quad \text{HCl} \\
\text{Ar}_{1}\text{H} & \quad \text{DIPEA, EtOH, Microwave, 70 °C, 20 min} \\
\text{a} & \quad \text{NBS, DMF, 0 °C, 20 min} \\
\text{b} & \quad \text{Cyanoacetamide, NaH, DMF} \\
\end{align*}
\]

Reagents and conditions (a) DIPEA, EtOH, Microwave, 70 °C, 20 min; (b) NBS, DMF, 0 °C, 20 min; (c) Cyanoacetamide, NaH, DMF.

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. Microwave irradiation was performed on a CEM Discover System. Reaction progress was monitored by thin-layer chromatograph on silica gel containing an inert binder and a fluorescent indicator (activated at 254 nm) coated flexible sheet (J. T. Baker). Chromatography was performed using an automated flash chromatography system, eluting on pre-packed silica gel columns with CH\(_2\)Cl\(_2\)/MeOH gradient solvent system. The purification by preparative RP-HPLC was performed on Waters Xterra Prep RP18 OBD 5µM (19 mm x 50 mm), eluting with a CH\(_3\)CN/H\(_2\)O solvent system with 0.1% TFA. The purity of all final compounds was determined by analytical LCMS using an Onyx Monolithic C18 column (4.6 mm x 100 mm) (Phenomenex, Torrance, CA) and eluting with CH\(_3\)CN/H\(_2\)O solvent system (+0.1% TFA). The products were detected by UV at 220 nm. All compounds were determined to be >95% pure by this method. The mass spectra were recorded with an Ion Trap Mass Spectrometer (Agilent, Santa Clara, CA). NMR spectra were recorded on Bruker 300 or 500 MHz spectrometers at ambient temperature. Chemical shifts are reported in parts per million (δ) referenced to the internal standards (7.26 ppm for CDCl\(_3\), 3.34 ppm for CD\(_3\)OD and 2.50 ppm for (CD\(_3\))\(_2\)SO) and coupling constants in Hz.

**General Synthetic Procedure.**

Quinoline aldehyde (0.1 mmole, 1 eq), a corresponding hydrazine hydrochloride (0.11 mmole, 1.1 eq) and DIPEA (0.11 mmole, 1.1 eq) were dissolved in EtOH (2 mL) in a microwave tube,
purged with N₂, heated to 70 °C under microwave for 20 min. After evaporation of the solvent, the product hydrazone was used for the next step directly.

Hydrazone obtained above (0.1 mmole, 1eq) was dissolved in 2 mL DMF and cooled to 0 °C, then NBS (0.11 mmole, 1.1 eq) dissolved in 1 mL DMF was added drop wise, the reaction mixture was stirred at 0 °C for 20 min.

In another flask, NaH (0.3 mmole, 3 eq) was added to a solution of 2-cyanoacetamide (0.2 mmole, 2 eq) in DMF. After stirring for 20 min, the solution was added to the above bromo-hydrazone solution at 0 °C, and the resulted reaction mixture was stirred at r.t. overnight. The solvent was removed; the residue was extracted with CH₂Cl₂, dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude product was then purified via flash chromatography over silica, eluting with a CH₂Cl₂/MeOH gradient. Further purification, if necessary, was performed via preparatory RP-HPLC, eluting with H₂O/CH₃CN gradient (+0.1% TFA).

Characterization of all final compounds:

2: 5-amino-3-(7-ethoxyquinolin-3-yl)-1-isopropyl-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and isopropylhydrazine hydrochloride as starting materials following the General Procedure. ¹H NMR (500 MHz, CDCl₃) δ 9.08 (s, 1H), 8.44 (s, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.53 (s, 1H), 7.31 (d, J = 9.0 Hz, 1H), 5.45 (s, 2H), 5.21 (s, 2H), 4.39 – 4.30 (m, 1H), 4.26 (q, J = 6.9 Hz, 2H), 1.64 – 1.49 (m, 9H); MS (ESI) (M+H)⁺=340.5 ; HPLC analysis: 97.9% purity.

3: 5-amino-3-(7-ethoxyquinolin-3-yl)-1-isobutyl-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and isobutylhydrazine hydrochloride as starting materials following the General Procedure. ¹H NMR (500 MHz, CDCl₃) δ 9.05 (s, 1H), 8.37 (s, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.53 (s, 1H), 7.30 (d, J = 8.8 Hz, 1H), 4.25 (q, J = 6.8 Hz, 2H), 3.78 (d, J = 7.4 Hz, 2H), 2.39 – 2.25 (m, 1H), 1.54 (t, J = 6.9 Hz, 3H), 1.03 (d, J = 6.6 Hz, 6H); MS (ESI) (M+H)⁺=354.6 ; HPLC analysis: 96.9% purity.
4: **5-amino-3-(7-ethoxyquinolin-3-yl)-1-neopentyl-1H-pyrazole-4-carboxamide.** This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and neopentylhydrazine hydrochloride as starting materials following the General Procedure. 1H NMR (300 MHz, CDCl3) δ 9.06 (d, J = 2.4 Hz, 1H), 8.39 (s, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.57 (s, 1H), 7.31 (dd, J 1= 9.0, 2.4 Hz, 1H), 5.48 (s, 2H), 5.21 (s, 2H), 4.25 (q, J = 7.0 Hz, 2H), 3.77 (s, 2H), 1.54 (t, J = 7.0 Hz, 3H), 1.10 (s, 9H); MS (ESI) (M+H)+=368.6; HPLC analysis: 95.0% purity.

5: **5-amino-3-(7-ethoxyquinolin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-pyrazole-4-carboxamide.** This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and (2,2,2-trifluoroethyl)hydrazine hydrochloride as starting materials following the General Procedure. 1H NMR (500 MHz, CDCl3) δ 9.03 (s, 1H), 8.32 (s, 1H), 7.78 (d, J = 9.0 Hz, 1H), 7.47 (s, 1H), 7.29 (dd, J = 9.0, 2.2 Hz, 1H), 5.65 (s, 2H), 5.23 (s, 2H), 4.65 (t, J = 6.9 Hz, 2H), 4.24 (q, J = 6.9 Hz, 2H), 1.54 (t, J = 6.9 Hz, 3H); MS (ESI) (M+H)+= 380.5; HPLC analysis: 96.8% purity.

6: **5-amino-1-(cyclopropylmethyl)-3-(7-ethoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide.** This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and (cyclopropylmethyl)hydrazine hydrochloride as starting materials following the General Procedure. 1H NMR (500 MHz, CDCl3) δ 9.06 (s, 1H), 8.37 (s, 1H), 7.79 (d, J = 9.0 Hz, 1H), 7.52 (s, 1H), 7.50 (d, J = 9.0, 2.3 Hz, 1H), 5.50 (s, 2H), 5.24 (s, 2H), 4.24 (q, J = 6.9 Hz, 2H), 3.93 (d, J = 6.6 Hz, 2H), 1.53 (t, J = 7.0 Hz, 3H), 1.31 (m, 1H), 0.69 (m, 2H), 0.46 (m, 2H); MS (ESI) (M+H)+= 352.6; HPLC analysis: 99.0% purity.
7: 1-isobutyl-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and cyclobutylhydrazine hydrochloride as starting materials following the General Procedure. 1H NMR (500 MHz, MeOD) δ 9.32 (s, 1H), 9.27 (s, 1H), 8.29 (d, J = 8.5 Hz, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.54 (s, 1H), 4.38 (q, J = 6.2 Hz, 2H), 2.71 (m, 2H), 2.46 (m, 2H), 1.92 (m, 2H), 1.56 (t, J = 6.2 Hz, 3H); MS (ESI) (M+H)+ = 352.4; HPLC analysis: 98.7% purity.

8: 5-amino-1-cyclopentyl-3-(7-ethoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and cyclopentylhydrazine hydrochloride as starting materials following the General Procedure. 1H NMR (500 MHz, MeOD) δ 9.28 (s, 1H), 9.24 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.61 (dd, J = 9.2, 2.1 Hz, 1H), 7.51 (s, 1H), 4.76-4.64 (m, 1H), 4.45-4.32 (q, J = 7.0 Hz, 2H), 2.23-2.06 (m, 4H), 2.03-1.90 (m, 2H), 1.85-1.70 (m, 2H), 1.57 (t, J = 7.0 Hz, 3H); MS (ESI) (M+H)+ = 366.3; HPLC analysis: 95.0% purity.

9: 5-amino-1-cyclohexyl-3-(7-ethoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and cyclohexylhydrazine hydrochloride as starting materials following the General Procedure. 1H NMR (500 MHz, CDCl3) δ 9.05 (s, 1H), 8.35 (s, 1H), 7.78 (d, J = 9.0 Hz, 1H), 7.52 (s, 1H), 7.28 (dd, J = 9.0, 2.3 Hz, 1H), 5.47 (s, 2H), 5.20 (s, 2H), 4.24 (q, J = 6.9 Hz, 2H), 3.96 – 3.82 (m, 1H), 2.10 – 1.92 (m, 6H), 1.53 (t, J = 6.9 Hz, 3H), 1.49 – 1.38 (m, 2H), 1.37 – 1.23 (m, 2H); MS (ESI) (M+H)+ = 380.5; HPLC analysis: 96.8% purity.
10: (2H buried in solvent): 5-amino-3-(7-ethoxyquinolin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and (tetrahydro-2H-pyran-4-yl)hydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, DMSO) δ 9.21 (s, 1H), 9.06 (s, 1H), 8.30 (d, $J = 9.1$ Hz, 1H), 7.65 (s, 1H), 7.56 (dd, $J = 9.1$, 1.9 Hz, 1H), 4.51-4.43 (m, 1H), 4.29 (q, $J = 6.9$ Hz, 2H), 4.03-3.96 (m, 2H), 2.10 – 1.97 (m, 2H), 1.86-1.78 (m, 2H), 1.46 (t, $J = 6.8$ Hz, 3H) ; MS (ESI) (M+H)$^+$= 382.4; HPLC analysis: 99.5% purity.

11: 5-amino-1-cycloheptyl-3-(7-ethoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and cycloheptylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.03 (s, 1H), 8.31 (s, 1H), 7.76 (d, $J = 8.9$ Hz, 1H), 7.48 (s, 1H), 7.26 (dd, $J = 8.9$, 1.8 Hz, 1H), 5.47 (s, 2H), 5.23 (s, 2H), 4.23 (q, $J = 6.9$ Hz, 2H), 4.14 – 4.02 (m, 1H), 2.21 – 2.03 (m, 4H), 1.94 – 1.83 (m, 2H), 1.74 – 1.61 (m, 4H), 1.60-1.48 (m, 5H) ; MS (ESI) (M+H)$^+$= 394.6; HPLC analysis: 99.3% purity.

12: 5-amino-1-(cyclohexylmethyl)-3-(7-ethoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and (cyclohexylmethyl)hydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.04 (s, 1H), 8.31 (d, $J = 1.8$ Hz, 1H), 7.77 (d, $J = 9.0$ Hz, 1H), 7.47 (d, $J = 2.0$ Hz, 1H), 7.26 (dd, $J = 9.0$, 2.0 Hz, 1H), 5.44 (s, 2H), 5.18 (s, 2H), 4.24 (q, $J = 7.0$ Hz, 2H), 3.80 (d, $J = 7.3$ Hz, 2H), 3.06-1.94 (m, 1H), 1.82-1.67 (m, 6H), 1.53 (t, $J = 7.0$ Hz, 3H), 1.32-1.24 (m, 2H), 1.014-1.01 (m, 2H) ; MS (ESI) (M+H)$^+$= 394.6; HPLC analysis: 97.2% purity.
13: 5-amino-3-(7-ethoxyquinolin-3-yl)-1-(1-hydroxypropan-2-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and 2-hydrazinylpropan-1-ol hydrochloride as starting materials following the General Procedure. \(^1\)H NMR (500 MHz, MeOD) \(\delta\) 9.31 (s, 1H), 9.26 (s, 1H), 8.29 (d, \(J = 9.1\) Hz, 1H), 7.62 (d, \(J = 9.1\) Hz, 1H), 7.54 (s, 1H), 4.55 – 4.44 (m, 1H), 4.38 (q, 6.9 Hz, 2H), 3.98 – 3.89 (m, 1H), 3.88 – 3.80 (m, 1H), 1.57 (t, \(J = 6.9\) Hz, 3H), 1.47 (d, \(J = 6.8\) Hz, 3H) ; MS (ESI) (M+H)^+ = 356.5; HPLC analysis: 99.0% purity.

14: 5-amino-3-(7-ethoxyquinolin-3-yl)-1-(2-hydroxy-2-methylpropyl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and 1-hydrazinyl-2-methylpropan-2-ol hydrochloride as starting materials following the General Procedure. \(^1\)H NMR (500 MHz, MeOD) \(\delta\) 9.30 (s, 1H), 9.25 (s, 1H), 8.29 (d, \(J = 8.9\) Hz, 1H), 7.61 (d, \(J = 8.9\) Hz, 1H), 7.52 (s, 1H), 4.37 (q, \(J = 6.8\) Hz, 2H), 4.06 (s, 2H), 1.56 (t, \(J = 6.8\) Hz, 3H), 1.31 (s, 6H) ; MS (ESI) (M+H)^+ = 370.6; HPLC analysis: 95.0% purity.

15: 5-amino-3-(7-ethoxyquinolin-3-yl)-1-(4-hydroxybutan-2-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carbaldehyde and 3-hydrazinylbutan-1-ol hydrochloride as starting materials following the General Procedure. \(^1\)H NMR (500 MHz, DMSO) \(\delta\) 9.20 (d, \(J = 1.8\) Hz, 1H), 9.04 (s, 1H), 8.29 (d, \(J = 9.1\) Hz, 1H), 7.62 (s, 1H), 7.55 (dd, \(J = 9.1, 1.8\) Hz, 1H), 6.67 (s, 2H), 4.56-4.50 (m, 1H), 4.29 (q, 6.9 Hz, 2H), 2.12-1.99 (m, 2H), 1.94-1.80 (m, 2H), 1.45 (t, \(J = 6.9\) Hz, 3H), 1.38 (d, \(J = 6.5\) Hz, 3H) ; MS (ESI) (M+H)^+ = 370.5; HPLC analysis: 97.3% purity.
16: 5-amino-3-(7-ethoxyquinolin-3-yl)-1-(3-hydroxy-2,2-dimethylpropyl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethoxyquinoline-3-carboxaldehyde and 3-hydrazinyl-2,2-dimethylpropan-1-ol hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.10 (s, 1H), 8.56 (d, $J = 9.7$ Hz, 1H), 7.87 (s, 1H), 7.71 (s, 1H), 7.36 (d, $J = 9.7$ Hz, 1H), 6.04 (s, 2H), 5.36 (s, 2H), 4.27 (q, $J = 6.9$ Hz, 2H), 3.89 (s, 2H), 3.33 (s, 2H), 1.54 (t, $J = 6.9$ Hz, 3H), 1.07 (s, 6H) ; MS (ESI) (M+H)$^+$= 384.6; HPLC analysis: 98.7% purity.

17: 5-amino-1-(tert-butyl)-3-(2-ethoxyquinolin-6-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-ethoxyquinoline-6-carboxaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.05 (d, $J = 8.4$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 1H), 7.94 (s, 1H), 7.82 (d, $J = 8.4$ Hz, 1H), 6.97 (d, $J = 8.8$ Hz, 1H), 5.42 (s, 2H), 4.61 (q, $J = 6.9$ Hz, 2H), 1.72 (s, 6H), 1.50 (t, $J = 6.9$ Hz, 3H) ; MS (ESI) (M+H)$^+$= 354.7; HPLC analysis: 95.4% purity.

18: 5-amino-3-(2-ethoxyquinolin-6-yl)-1-isopropyl-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-ethoxyquinoline-6-carboxaldehyde and isopropylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.89 (d, $J = 8.7$ Hz, 1H), 8.48 (d, $J = 8.9$ Hz, 1H), 8.26 (s, 1H), 8.05 (d, $J = 8.7$ Hz, 1H), 7.63 (d, $J = 8.9$ Hz, 1H), 4.76 (q, $J = 6.9$ Hz, 2H), 4.70-4.62 (m, 1H), 1.61 (t, $J = 6.9$ Hz, 3H), 1.53 (d, $J = 6.5$ Hz, 6H) ; MS (ESI) (M+H)$^+$= 340.5; HPLC analysis: 96.0% purity.
19: 5-amino-1-(cyclopropylmethyl)-3-(2-ethoxyquinolin-6-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-ethoxyquinoline-6-carbaldehyde and (cyclopropylmethyl)hydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) $\delta$ 8.67 (d, $J = 8.9$ Hz, 1H), 8.26 (s, 1H), 8.05 (d, $J = 8.4$ Hz, 1H), 7.99 (d, $J = 8.4$ Hz, 1H), 7.43 (d, $J = 8.9$ Hz, 1H), 7.39 (d, $J = 6.8$ Hz, 2H), 1.56 (t, $J = 6.9$ Hz, 3H), 1.42 – 1.36 (m, 1H), 0.68-0.60 (m, 2H), 0.54-0.44 (m, 2H); MS (ESI) (M+H)$^+$ = 352.6; HPLC analysis: 95.0% purity.

20: 5-amino-1-cyclohexyl-3-(2-ethoxyquinolin-6-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-ethoxyquinoline-6-carbaldehyde and cyclohexylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) $\delta$ 8.88 (d, $J = 9.1$ Hz, 1H), 8.38 (s, 1H), 8.15 (d, $J = 8.5$ Hz, 1H), 8.04 (d, $J = 8.5$ Hz, 1H), 7.63 (d, $J = 9.1$ Hz, 1H), 4.76 (q, $J = 7.0$ Hz, 4H), 4.36 – 4.28 (m, 1H), 2.08-2.01 (m, 2H), 2.01-1.94 (m, 2H), 1.93 – 1.85 (m, 2H), 1.83-1.74 (m, 2H), 1.55 (q, $J = 7.0$ Hz, 3H), 1.39 – 1.24 (m, 2H); MS (ESI) (M+H)$^+$ = 380.6; HPLC analysis: 97.1% purity.

21: 5-amino-1-cycloheptyl-3-(2-ethoxyquinolin-6-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-ethoxyquinoline-6-carbaldehyde and cycloheptylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) $\delta$ 8.31 (d, $J = 9.0$ Hz, 1H), 8.04 (d, $J = 1.8$ Hz, 1H), 7.93 (d, $J = 8.6$ Hz, 1H), 7.85 (dd, $J = 8.6$, 1.8 Hz, 1H), 7.11 (d, $J = 9.0$ Hz, 1H), 4.58 (q, $J = 7.1$ Hz, 2H), 4.41-4.26 (m, 1H), 2.17 – 1.96 (m, 6H), 1.95 – 1.81 (m, 2H), 1.81-1.71 (m, 2H), 1.69 – 1.59 (m, 4H), 1.49 (t, $J = 7.1$ Hz, 3H); MS (ESI) (M+H)$^+$ = 394.6; HPLC analysis: 96.2% purity.
22: 5-amino-3-(2-ethoxyquinolin-6-yl)-1-(3-hydroxy-2,2-dimethylpropyl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-ethoxyquinoline-6-carbaldehyde and 3-hydrazinyl-2,2-dimethylpropan-1-ol hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.01 (d, $J = 8.8$ Hz, 1H), 7.95 – 7.88 (m, 2H), 7.78 (d, $J = 8.5$ Hz, 1H), 6.95 (d, $J = 8.8$ Hz, 1H), 6.29-5.67 (br, 2H), 5.31 (s, 2H), 4.57 (q, $J = 7.0$ Hz, 2H), 3.86 (s, 2H), 3.33 (s, 2H), 1.48 (t, $J = 7.0$ Hz, 3H), 1.04 (s, 6H); MS (ESI) (M+H)$^+$ = 384.6; HPLC analysis: 96.3% purity.

23: 5-amino-1-(tert-butyl)-3-(7-fluoroquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-fluoroquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.36 (s, 1H), 8.87 (s, 1H), 8.18 – 8.09 (m, 1H), 8.06 (d, $J = 6.8$ Hz, 1H), 7.63 (t, $J = 6.8$ Hz, 1H), 6.17 (s, 2H), 1.69 (s, 9H); MS (ESI) (M+H)$^+$ = 328.5; HPLC analysis: 97.5% purity.

24: 5-amino-1-tert-butyl-3-(7-chloroquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-chloroquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.29 (s, 1H), 8.65 (s, 1H), 8.32 (s, 1H), 7.94 (d, $J = 8.3$ Hz, 1H), 7.69 (d, $J = 8.3$ Hz, 1H), 5.77 (s, 2H), 1.69 (s, 9H); MS (ESI) (M+H)$^+$ = 344.6; HPLC analysis: 97.4% purity.
25: 5-amino-1-tert-butyl-3-(7-(trifluoromethyl)quinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-(trifluoromethyl)quinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.32 (s, 1H), 8.57 (s, 1H), 8.54 (s, 1H), 8.07 (d, $J = 8.0$ Hz, 1H), 7.85 (d, $J = 8.0$ Hz, 1H), 5.72 (s, 2H), 1.71 (s, 9H); MS (ESI) (M+H)$^+$= 378.5; HPLC analysis: 95.0% purity.

26: 5-amino-1-(tert-butyl)-3-(7-methylquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-methylquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.05 (d, $J = 2$ Hz, 1H), 8.31 (s, 1H), 7.90 (s, 1H), 7.75 (d, $J = 8.3$ Hz, 1H), 7.42 (d, $J = 8.3$ Hz, 1H), 5.70 (s, 2H), 5.18 (s, 2H), 2.58 (s, 3H), 1.69 (s, 9H); MS (ESI) (M+H)$^+$= 324.4; HPLC analysis: 95.0% purity.

27: 5-amino-1-(tert-butyl)-3-(7-ethylquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-ethylquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.12 (s, 1H), 8.41 (s, 1H), 8.03 (s, 1H), 7.83 (d, $J = 8.4$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 1H), 5.72 (s, 2H), 5.16 (s, 2H), 2.92 (q, $J = 7.5$ Hz, 2H), 1.72 (s, 9H), 1.39 (t, $J = 7.5$ Hz, 3H); MS (ESI) (M+H)$^+$= 338.5; HPLC analysis: 95.0% purity.
28: 5-amino-1-(tert-butyl)-3-(7-methoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-methoxyquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.21 (s, 1H), 8.87 (s, 1H), 7.99 (d, $J = 9.5$ Hz, 1H), 7.79 (s, 1H), 7.48 (d, $J = 9.5$ Hz, 1H), 6.49-5.98 (m, 2H), 4.05 (s, 3H), 1.68 (s, 9H); MS (ESI) (M+H)$^+$ = 340.2; HPLC analysis: 95.0% purity.

29: 5-amino-1-tert-butyl-3-(7-(trifluoromethoxy)quinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-(trifluoromethoxy)quinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.23 (s, 1H), 8.51 (s, 1H), 8.09 (s, 1H), 7.97 (d, $J = 8.9$ Hz, 1H), 7.52 (d, $J = 8.9$ Hz, 1H), 5.71 (s, 2H), 5.18 (s, 2H), 1.72 (s, 9H); MS (ESI) (M+H)$^+$ = 394.3; HPLC analysis: 97.2% purity.

30: 5-amino-1-(tert-butyl)-3-(7-isopropoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-isopropoxyquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.04 (s, 1H), 8.29 (s, 1H), 7.77 (d, $J = 8.9$ Hz, 1H), 7.48 (s, 1H), 7.24 (d, $J = 8.9$ Hz, 1H), 5.73 (s, 2H), 5.17 (s, 2H), 4.87-4.74 (m, 1H), 1.73 (s, 9H), 1.47 (d, $J = 6.0$ Hz, 6H); MS (ESI) (M+H)$^+$ = 368.6; HPLC analysis: 98.0% purity.
31: **5-amino-1-tert-butyl-3-(7-propoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide.** This compound was synthesized using 7-propoxyquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.24 (s, 1H), 8.84 (s, 1H), 7.98 (d, $J = 9.0$ Hz, 1H), 7.82 (s, 1H), 7.48 (d, $J = 9.0$ Hz, 1H), 5.89 (s, 1H), 4.20 (t, $J = 6.2$ Hz, 2H), 2.02-1.86 (m, 2H), 1.70 (s, 6H), 1.12 (t, $J = 7.4$ Hz, 3H) ; MS (ESI) (M+H)$^+$= 368.5; HPLC analysis: 95.7% purity.

32: **5-amino-1-(tert-butyl)-3-(7-(cyclopropylmethoxy)quinolin-3-yl)-1H-pyrazole-4-carboxamide.** This compound was synthesized using 7-(cyclopropylmethoxy)quinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.05 (s, 1H), 8.31 (s, 1H), 7.79 (s, 1H), 7.46 (s, 1H), 7.31 (d, $J = 8.9$ Hz, 1H), 5.72 (s, 2H), 5.17 (s, 2H), 4.01 (d, $J = 6.8$ Hz, 2H), 1.72 (s, 9H), 1.45-1.35 (m, 1H), 0.84-0.64 (m, 2H), 0.56-0.38 (m, 2H) ; MS (ESI) (M+H)$^+$= 380.4; HPLC analysis: 99.0% purity.

33: **5-amino-1-(tert-butyl)-3-(7-isobutoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide.** This compound was synthesized using 7-isobutoxyquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) $\delta$ 9.27 (s, 1H), 9.23 (s, 1H), 8.30 (d, $J = 9.2$ Hz, 1H), 7.63 (dd, $J = 9.2$, 1.8 Hz, 1H), 7.55 (s, 1H), 4.09 (d, $J = 6.4$ Hz, 2H), 2.35-2.16 (m, 1H), 1.72 (s, 9H), 1.15 (d, $J = 6.6$ Hz, 6H) ; MS (ESI) (M+H)$^+$= 382.4; HPLC analysis: 99.2% purity.
34: 5-amino-1-(tert-butyl)-3-(7-cyclopropoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-cyclopropoxyquinoline-6-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) δ 9.29 (s, 1H), 9.25 (s, 1H), 8.30 (d, $J = 9.0$ Hz, 1H), 7.86 (s, 1H), 7.63 (d, $J = 9.0$ Hz, 1H), 4.20-4.11 (m, 1H), 1.71 (s, 9H), 1.08-0.98 (m, 2H), 0.95-0.86 (m, 2H); MS (ESI) (M+H)$^+$= 366.5; HPLC analysis: 99.8% purity.

35: 5-amino-1-(tert-butyl)-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-cyclopropoxyquinoline-6-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) δ 8.24 (d, $J = 8.9$ Hz, 1H), 8.00 (s, 1H), 7.96 (d, $J = 8.6$ Hz, 1H), 7.82 (dd, $J = 8.6$, 1.7 Hz, 1H), 7.06 (d, $J = 8.9$ Hz, 1H), 4.48 (td, $J = 6.2$, 3.1 Hz, 1H), 1.69 (s, 9H), 0.93-0.87 (m, 2H), 0.86-0.79 (m, 2H); MS (ESI) (M+H)$^+$= 366.4; HPLC analysis: 96.4% purity.

36: 5-amino-1-(tert-butyl)-3-(7-cyclobutoxyquinolin-3-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 7-chloroquinoline-3-carbaldehyde 7-cyclobutoxyquinoline-3-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) δ 9.27 (s, 1H), 9.22 (s, 1H), 8.29 (d, $J = 9.1$ Hz, 1H), 7.58 (dd, $J = 9.1$, 1.9 Hz, 1H), 7.41 (s, 1H), 5.08 – 4.99 (m, 1H), 2.73 – 2.59 (m, 1H), 2.38 – 2.25 (m, 2H), 2.06-1.95 (m, 1H), 1.94 – 1.82 (m, 1H), 1.71 (s, 9H); MS (ESI) (M+H)$^+$= 380.6; HPLC analysis: 99.0% purity.
37: 5-amino-1-(tert-butyl)-3-(2-cyclobutoxyquinolin-6-yl)-1H-pyrazole-4-carboxamide. This compound was synthesized using 2-cyclobutoxyquinoline-6-carbaldehyde and tert-butylhydrazine hydrochloride as starting materials following the General Procedure. $^1$H NMR (500 MHz, MeOD) δ 8.89 (d, $J = 9.1$ Hz, 1H), 8.34 (s, 1H), 8.17 (d, $J = 8.2$ Hz, 1H), 7.98 (d, $J = 8.2$ Hz, 1H), 7.52 (d, $J = 9.1$ Hz, 1H), 5.55 – 5.35 (m, 1H), 2.79-2.61 (m, 2H), 2.51-2.32 (m, 2H), 2.10-1.98 (m, 1H), 1.94-1.81 (m, 1H), 1.71 (s, 9H); MS (ESI) (M+H)$^+$ = 380.5; HPLC analysis: 98.3% purity.
**Solubility Test Procedures and Results.**

For the sample vial: 199 µL of pH 6.5 PBS buffer was added to the vial, then 1 µL of a 20 mM stock solution of compound in DMSO was added. The vial was capped and vigorously shaken to mix the sample thoroughly, and then the sample was incubated at 25 °C overnight. The vial was centrifuged at 25 °C for 40 min at 11800 rpm. 100 µL of supernatant was transferred to another vial and diluted with 100 µL CH₃CN. The two-fold diluted supernatants were analyzed by HPLC with UV detection.

For the standard calibration curve: 3 µL of 20 mM stock solution in DMSO was added to 57 µL of 50% CH₃CN in pH 6.5 PBS buffer solution to obtain the 1 mM solution. The 1 mM solution was diluted to 2 µM, 5 µM, 10 µM, 20 µM and 50 µM with 50% CH₃CN in pH 6.5 PBS buffer. The above solutions were analyzed by the HPLC/UV system (five-point standard calibration curve).

Solubility was calculated from the integrated peak area using the following equation.

\[
\text{Solubility (µM)} = \text{[concentration of two-fold diluted supernatant]} \times 2^4
\]

**Table S1: Solubility Results**

| Compound Number | Solubility pH=6.5 (µM) | Compound Number | Solubility pH=6.5(µM) |
|-----------------|------------------------|-----------------|------------------------|
| 1               | 26.0                   | 21              | 46.5                   |
| 2               | 99.0                   | 22              | >100                   |
| 6               | 26.5                   | 23              | >100                   |
| 7               | 10.9                   | 28              | >100                   |
| 8               | 98.7                   | 30              | >100                   |
| 9               | 34.3                   | 31              | 12.7                   |
| 11              | 8.5                    | 32              | 26.8                   |
| 16              | >100                   | 34              | 83.7                   |
| 17              | 83.5                   | 35              | 96.3                   |
| 18              | >100                   | 36              | 3.7                    |
| 19              | 10.9                   | 37              | 80.6                   |
| 20              | 88.9                   |                 |                        |

*a Values shown are the mean values of at least two experiments*
**TgCDPK1 Enzymatic Assay.**

Enzyme activity was determined by measuring ATP consumption in a coupled reaction for phosphate group incorporation to biotinylated Syntide-2 (BioSyntide-2) peptide substrate (Biotin-C6-PLARTLSVAGLPGKK) (American Peptide Company, Inc. Sunnyvale, CA) in the presence or absence of inhibitors. All assays were performed in a buffered solution containing 1 mM EGTA (pH 7.2), 10 mM MgCl$_2$, 20 mM HEPES pH 7.5 (KOH), 0.1% BSA and enzyme activation reagent containing 2 mM CaCl$_2$. The final reaction volume of 25 µL contained 20 µM BioSyntide-2, 2.1 nM TgCDPK1, with or without serial dilutions of inhibitors. The reaction was initiated with the addition of ATP at 10 µM final concentration. Internal positive and negative controls were included in each assay plate. Reaction mixture was incubated at 30 °C and 90 rpm for 90 min. Subsequent changes to initial ATP concentration were measured by a non-radioactive Kinaseglo® luciferase reagent (Promega, Madison, WI) as a luminescence readout using a MicroBeta2 multi-label plate reader (Perkin Elmer, Waltham, MA).
Human SRC Kinase Enzymatic Assay.

Human SRC enzyme activity was measured in a buffered medium containing 40 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, 1 mM MnCl₂, 1 mM DTT, and 0.1% BSA as previously described.⁵ The 25 µL final reaction mixture volume included 1.97 nM recombinant SRC enzyme, 61 µM SRC substrate peptide (sequence Ac-EIYGEFKKK GenScript, Piscataway, NJ) and 10 µM ATP per well. Similarly, the compound concentration to give 50% reduction in enzyme activity (IC₅₀) was determined via serial dilutions of compounds. The assay was allowed to proceed for 90 minutes at 30 °C. Enzyme inhibition was obtained indirectly by assessing changes in initial ATP concentration via luminescence with the non-radioactive Kinaseglo© luciferase reagent (Promega). Internal positive and negative controls were included in each reaction plate. Luminescence was read on a MicroBeta2® plate reader (PerkinElmer) after addition of Kinaseglo®.
**Human Cell Growth Inhibition Assay.**

CRL-8155 human lymphocytic cells (ATCC, WIL2-NS) were cultured in RPMI-1640 growth medium supplemented with 10% heat inactivated fetal bovine serum, 10 mM HEPES, 1 mM sodium pyruvate, and 1 mM L-glutamine. The Alamar Blue® assay (Invitrogen, Grand Island, NY), which measures general cellular metabolism, was used to quantify cell growth. Mid-log cells were seeded in 96-well flat-bottom plates (Corning, Corning, NY) at a density of 3x10^5 cells/mL containing test compounds at six final concentrations (40 µL, 20 µL, 10 µL, 5 µL, 2.5 µL, and 1.25 µL) in quadruplicate and grown at 37°C for 48 hours in a 5% CO₂ humidified incubator. A 1/10th volume of Alamar Blue® developing reagent was added to each well and incubated for an additional 3 hours and fluorescence was measured at the respective excitation and emission wavelengths of 560 nm and 590 nm in a FLx800 microplate reader (Biotek, Winooski, VT). Percent growth inhibition by test compounds was calculated based on DMSO vehicle and positive controls (50 µL quinacrine), which corresponded to 0% and 100% growth inhibition, respectively.
T. gondii Growth Inhibition Assay.

The procedure was performed as previously reported. In brief, a dilution series of the compound was mixed with extracellular T. gondii expressing a β-galactosidase reporter and incubated briefly before adding them to a fibroblast monolayer on 96 well plates. After 44 hours β-galactosidase was then assayed using chlorophenol red β-galactopyranose (Sigma-Aldrich, St. Louis, MO) as a substrate.
Pharmacokinetic Analysis in Mice.

For mouse oral PK studies, three female BALB/c mice (10 to 12 weeks old) were used in each group. Each group received a test compound at a dose of 10 mg/kg body weight dissolved in 3% ethanol/7% Tween 80/90% normal saline by oral gavage. Blood samples were taken at the designated time points by tail bleeding and centrifuged to obtain plasma. The samples were frozen at -20 °C. The test compounds were extracted from the plasma samples using acetonitrile/0.1% formic acid with an internal standard. A standard mix of all test compounds was prepared for comparison and quantification. The compounds were quantified by LC/MS analysis and pharmacokinetic parameters determined.
**Plasma Protein Binding.**

The test compound was incubated at 37 °C for four hours in human plasma. Following incubation each sample was split in half. One half continued to incubate at 37 °C for 90 minutes to represent whole plasma, the second half was centrifuged in an ultracentrifuge for 90 minutes at 37 °C. Plasma water was carefully removed by pipette from the centrifuged sample into new microfuge tubes. Both samples were stored at -20 °C. Samples were extracted with acetonitrile containing internal standard then analyzed by LC/MS. Plasma water concentrations were compared to whole plasma concentrations to calculate the proportion bound to plasma proteins.
Brain penetration experiment.

Mice were injected with test compounds (5 mg/kg IP) and sacrificed at the indicated times for collection of plasma and brain. Compound was dissolved in 0.4 mL of dosing solution (7% Tween 80, 3% ethanol, 5% DMSO, 0.9% saline) for IP injections. The brains were weighed and immediately frozen, then later homogenized in acetonitrile. Prior to animal studies, recovery of test compound was carried out by adding a known amount to a mouse brain in the test extraction solvent and performing the homogenization. Compound recovery was determined by liquid chromatography/tandem mass spectrometry analysis relative to a standard compound amount. Blood was taken from the same mice in heparinized capillary tubes for determination of compound concentration in plasma. The concentration of compound in the brain was obtained by dividing the moles of compound in the brain by the brain volume (obtained from the brain weight assuming 1 g is 1 mL) and correcting for the brain vasculature volume of 3% by weight.
In vivo Efficacy against Acute *T. gondii* Infection in Mice.

Infection and drug administration were performed as previously reported. Mice were infected with Type I RH strain *T. gondii* expressing a yellow fluorescent protein. *T. gondii* were harvested from human foreskin fibroblasts, passed through a 3-μm-pore filter, and 105 tachyzoites were inoculated in a volume of 100 μL of phosphate-buffered saline (PBS) intraperitoneally (i.p.) into 4 to 5 week-old, 25 g female CF-1 mice. The compounds were dissolved in polyethylene glycol (PEG) 400 and administered by oral gavage starting 48 h after inoculation and every 24 hours for a total of 5 doses, or in the case of compound 1, dosing was administered every day at 8 am and 5 pm with a higher dose in the evening as noted. Animals were sacrificed 24 hours after the final dose and analyzed for parasite levels. The control group received PEG 400 only. Groups consisted of 4 mice. After mice were euthanized on the eighth day, the brain and spleen were collected from the mice and peritoneal lavage was performed with 3 mL of PBS (pH 7.4).

In vivo efficacy was evaluated with quantitative real-time PCR for *T. gondii* DNA from the brain and spleen, and quantification of peritoneal *T. gondii* infection as previously described. A sample of 10 μL of peritoneal lavage fluid was examined in a hemocytometer using fluorescence microscopy (excitation/emission 480/535 nm). Yellow-fluorescent tachyzoites were quantified per mL of fluid. After the mice were euthanized, the entire brain and spleen were collected and homogenized. DNA was isolated with a DNA purification kit (Qiagen, Germantown, MD). 300 ng of total DNA from the brain homogenate and 200 ng of total DNA from the spleen homogenate were analyzed per mouse. A standard curve was generated from DNA purified from *T. gondii* tachyzoites in 10-fold dilutions from 160 ng to 1.6 fg of DNA. Quantitative real-time PCR was performed in duplicate using an 7300 Real-Time PCR System (Applied Biosystems, Grand Island, NY) with iTaq SYBR GREEN PCR Supermix and primers for the *T. gondii* 529-bp repeat element (sense 5’-AGG AGA GAT ATC AGG ACT GTA G-3’ and anti-sense 5’-GCG TCG TCT CGT CTA GAT CG-3’). Results were quantified as *T. gondii* DNA per total DNA. Analysis was performed with GraphPad Prism 5.0 software. This protocol was approved by the Institutional Animal Care and Use Committee of the Portland Veterans Administration Medical Center.
Crystallographic data and refinement statistics

|                          | TgCDPK + (1)               | TgCDPK + (37)               |
|--------------------------|-----------------------------|-----------------------------|
| **PDB entry**            | 4ONA                       | 4YJN                       |
| **Space Group**          | P2₁                         | P2₁                         |
| **Unit Cell** (a b c Å)  | 48.27 72.63 67.20           | 48.30 72.64 65.71           |
| (α β γ °)                | 90.0 102.36 90.0            | 90.0 99.72 90.0             |
| **Resolution (Å)**       | 48.7-2.40 (2.46-2.40)       | 77-2.60 (2.72-2.60)         |
| **Total unique reflections** | 17101 (2498)               | 12536 (1548)                |
| **Replicate cc(1/2)**    | 0.995 (0.601)               | 0.984 (0.402)               |
| **Redundancy**           | 6.1 (6.2)                   | 5.8 (5.5)                   |
| **Completeness (%)**     | 96 (97)                     | 91 (92)                     |
| **Refinement resolution (Å)** | 48.7-2.40               | 65-2.60                     |
| **R / Rfree**            | 0.188 / 0.249               | 0.215 / 0.249               |
| **RMSD bonds (Å)**       | 0.012                       | 0.013                       |
| **RMSD angles (°)**      | 1.44                        | 1.60                        |
| **Protein atoms**        | 3727                        | 3707                        |
| **Non-protein atoms**    | 69                          | 57                          |
| **TLS groups**           | 6                           | 6                           |
| **Mean Beq protein atoms (Å²)** | 51.5               | 51.0                        |
| **Mean Beq ligand atoms (Å²)** | 41.8                 | 38.2                        |
| **Local Ligand Density (LLDF)** | -0.47               | 0.36                        |
Binding inhibition of compounds 34 and 35 against 20 representative human kinases.

**Table S3. List of IC₅₀s.**

| Kinase      | Compound 34 IC₅₀, µM | Compound 35 IC₅₀, µM |
|-------------|-----------------------|-----------------------|
| RIPK2       | 1.71                  | 0.20                  |
| Prkcn       | >10                   | 0.22                  |
| **Kdr**     | 1.09                  | 0.45                  |
| **EGFR**    | 1.03                  | >10                   |
| MEK1        | 8.23                  | 2.38                  |
| Ck1alpha1   | 2.91                  | >10                   |
| **FLT1**    | >10                   | 8.38                  |
| CAMK1D^     | >10                   | >10                   |
| *MAP3K10    | >10                   | >10                   |
| CAMKK2^     | >10                   | >10                   |
| **BRAF**    | >10                   | >10                   |
| **Rock1**   | >10                   | >10                   |
| p38 alpha   | >10                   | >10                   |
| **AUR1**    | >10                   | >10                   |
| ACVR1       | >10                   | >10                   |
| *CAMK2A^    | >10                   | >10                   |
| *JAK3       | >10                   | >10                   |
| **Akt1**    | >10                   | >10                   |
| Zipk^       | >10                   | >10                   |
| *ALK        | >10                   | >10                   |
| **Erk2**    | >10                   | >10                   |

**/* Denotes Kinases(**) or close relatives(*) whose inhibition causes cardiotoxicity.

^ Denotes human kinases that are most closely related to CDPK1.
Study of parasite growth inhibition using three *T. gondii* cell lines.

Figure S1.

Effects of compound 34 and 35 on the parental *T. gondii* cell line (blue), as compared to parasite lines overexpressing wild type (WT) CDPK1 (red) or its gatekeeper mutant G128M (green). All parasite lines express a β-galactosidase reporter. Briefly, various concentrations of compounds are mixed with the parasites, that are then added to fibroblast monolayers and grown for two days. The level of β-galactosidase is measured spectrophotometrically and the means of triplicate values are plotted as described.6
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