Inhibition of calcium dependent protein kinase 1 (CDPK1) by pyrazolopyrimidine analogs decreases establishment and reoccurrence of central nervous system disease by *Toxoplasma gondii*.

Supporting Information

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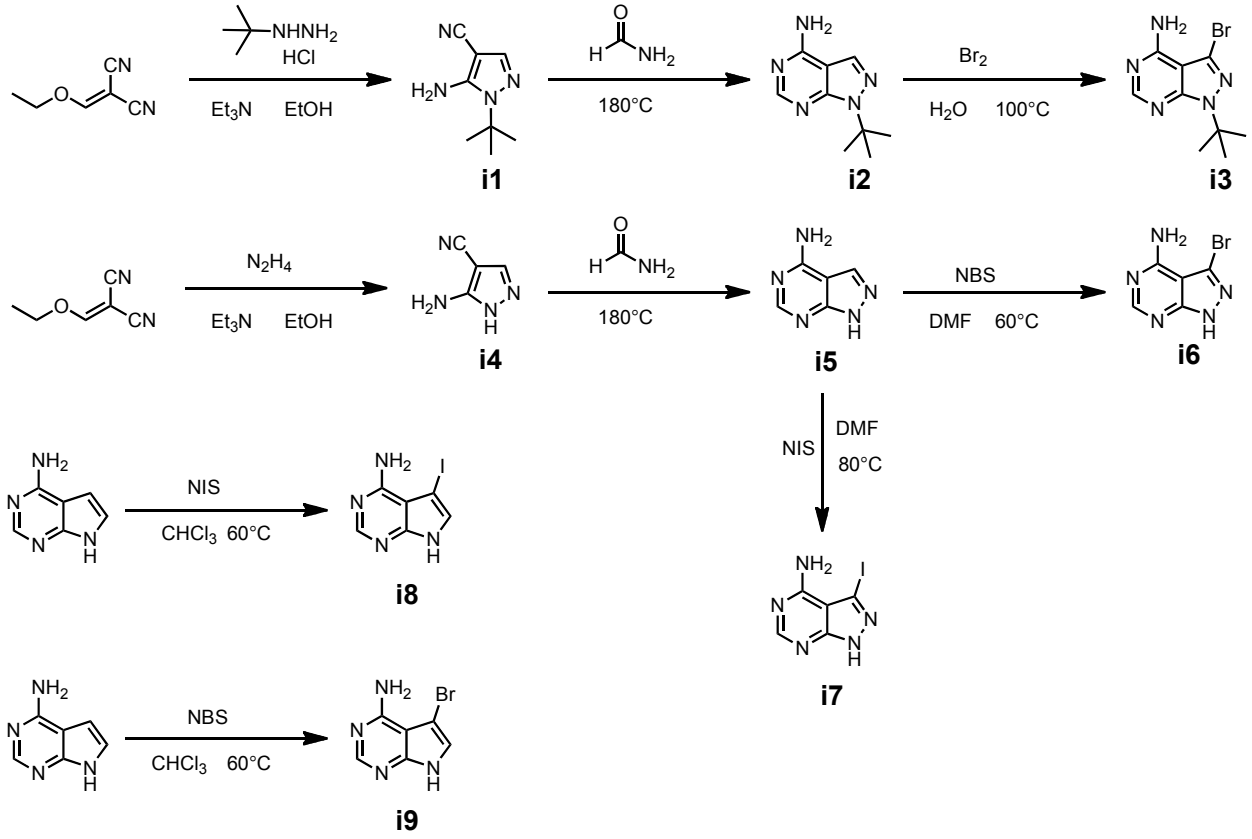
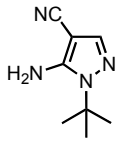
Synthesis and Characterization of Intermediate Compounds

Scaffold Synthesis



Generation of the pyrazolopyrimidine scaffold

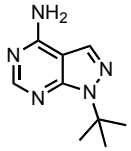
The synthesis of the pyrazolopyrimidine scaffold and methods for the diversification and utilization of this scaffold have been previously described [1–3].



i1

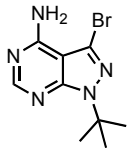
i1: 5-amino-1-(*tert*-butyl)-1*H*-pyrazole-4-carbonitrile. In a 250mL flame-dried argon purged round bottom flask, triethylamine (1.78g, 17.7mmol), and t-butyl hydrazine hydrochloride (1.56g, 12.5mmol) are dissolved in anhydrous ethanol (85mL). Ethoxymethylenemalononitrile (1.98g, 17.7mmol) is added slowly and reaction mixture is brought to reflux at 82°C for 3 hours. The solvent is removed *in vacuo* and 10% ethyl acetate / hexane is added (5mL) and the mixture is sonicated (or simply utilize recrystallization from 10% ethyl acetate / hexane). The resulting crystalline solid is

filtered, and washed with ether to yield **i1**. LC-MS (ES+) calcd for $C_8H_{12}N_4$ $(M+H)^+$ 165.11, found 165.05.



i2

i2:1-(*tert*-butyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Formamide (35mL) is added to intermediate **i1** (~2.75g) and the mixture is headed to 180°C for 3 hours. Upon cooling, the mixture is added to water and extracted with ethyl acetate using sodium bicarbonate to wash the organic followed by a careful water wash as to avoid emulsion and lastly a wash with saturated brine. The organic layer is dried *in vacuo* and is recrystallized from a small amount of ether to yield intermediate **i2**. LC-MS (ES+) calcd for $C_9H_{13}N_5$ $(M+H)^+$ 192.12, found 192.21.



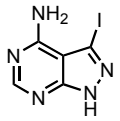
i3

i3: 3-bromo-1-(*tert*-butyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Intermediate **i2** (~300mgs) is suspended in water (7mL) and bromine (188uL, 2 equivalents) is added and the reaction mixture is stirred at room temperature for 1 hour followed by stirring at 100°C for 1 hour. Upon cooling, the precipitated product is separated by filtration and the filtrate is stirred in 5% aqueous sodium hydrogen sulfite (12mL) for 30 minutes and the solution is treated with 5mL of saturated aqueous sodium bicarbonate. The precipitate is separated by filtration, washed with water and dried to yield brominated intermediate **i3**. LC-MS (ES+) calcd for $C_9H_{12}BrN_5$ $(M+H)^+$ 270.03, found 270.55, 272.40.

Synthesis of pyrazolopyrimidines without t-butyl modification at N1 proceeds similarly although hydrazine is utilized instead of a derivatized hydrazine reagent to yield intermediate **i4** followed by similar procedures in generating intermediates **i2** and **i3** to yield intermediates **i5** and **i6** [4].

i6

i6: 3-bromo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. In a 250mL argon purged, flame-dried flask, 50mL DMF was added to dissolve 5g (37mmol) of starting material **i5**. NBS (6.5g, 36.5mmol) was added and the reaction heated to 60°C overnight. Upon cooling, and completion of reaction monitored by TLC, precipitate was filtered to yield intermediate **i6**. LC-MS (ES+) calcd for $C_5H_4BrN_5$ $(M+H)^+$ 213.97, found 213.76, 215.71.

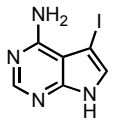


i7

i7: 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. In a 100mL argon purged, flame-dried flask, 30mL DMF was added to dissolve 5g (37mmol) of starting material **i5**. NIS (12.3g, 54.7mmol) was added and the reaction was heated to 80°C overnight. Upon cooling, and completion of reaction monitored by TLC, precipitate was filtered to yield intermediate **i7**. Water was added to filtrate and resulting precipitant was also filtered to yield a second batch of intermediate **i7**. LC-MS (ES+) calcd for $C_5H_4IN_5$ $(M+H)^+$ 261.95, found 261.87.

Generation of the pyrrolopyrimidine scaffold

The synthesis of the pyrrolopyrimidine scaffold proceeds by halogenation of advanced starting materials.



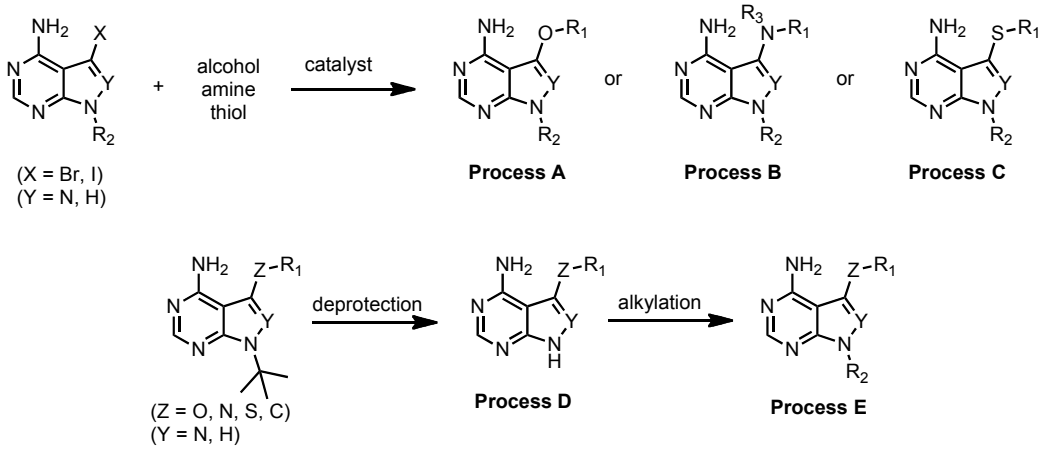
i8

i8: 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. In a 20mL scintillation vial, purchased pyrrolopyrimidine (1g, 7.45mmol) was dissolved in 7mL of chloroform. NIS (2.18g, 9.69mmol) was added and reaction was refluxed for 2 hours. Upon cooling, precipitate was filtered to yield intermediate **i8**. LC-MS (ES+) calcd for $C_6H_5IN_4$ $(M+H)^+$ 260.96, found 260.61.

i9

i9: 5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. In a 20mL scintillation vial, purchased pyrrolopyrimidine (2g, 14.9mmol) was dissolved in 7mL of chloroform. NBS (3.5g, 19.38mmol) was added and reaction was refluxed for 2 hours. Upon cooling, the reaction is concentrated *in vacuo*. As dibromination was observed in addition to desired intermediate I9, silica gel chromatography (DCM/MeOH) was required to obtain desired intermediate **i9.** LC-MS (ES+) calcd for $C_6H_5BrN_4$ $(M+H)^+$ 212.97, found 212.68, 214.68. **i9** can also be purchased commercially.

Strategy for Aryl Coupling



Addition of alcohol, amine or thiol to bromide or iodide pyrazolopyrimidine or pyrrolopyrimidine intermediate in the presence of catalyst generates ether (Process A), amine (Process B) and thioether (Process C) linked compounds respectively. Extensive summaries of copper [5] and palladium [6] catalyzed aryl couplings are available. Deprotection of t-butyl (Process D) followed by alkylation (Process E) generates advanced compounds with diversity at R_2. Preparation can also proceed with R_2 already installed followed by catalyst assisted coupling. General procedures for each example follow.

Process A

Synthesis proceeds by copper-catalyzed aryl coupling [7]. To an argon purged vial with magnetic stir bar is added intermediate **i3**, **i6**, or **i9** (1 eq.), alcohol (1.5 eq.), N,N-dimethylglycine (0.6 eq.), and cesium carbonate (2 eq.) in dry dioxane. After stirring for 5 minutes at room temperature, copper iodide is added (20mol%) and reaction is heated to 120°C and stirred overnight. Reaction completion is monitored by TLC and LC/MS.

After reaction is complete, water is added and compound is extracted into ethyl acetate and washed with water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated *in vacuo.* Purification occurs via silica flash chromatography with a hexanes – ethyl acetate, dichloromethane – methanol, or ethyl acetate - ethanol gradient. Product fractions are collected and concentrated to a solid. For final products, an additional purification step with RP-HPLC eluting with water + 0.1% formic acid and acetonitrile + 0.1% formic acid is conducted to ensure >95% purity.

Process B

Compounds with amine linkages were contracted out for synthesis. Methods to achieve amine linkage to C3 of the pyrazolopyrimidine scaffold by palladium-assisted Buchwald coupling have been described [8–10]. Example conditions are as follows: To an argon purged vial with magnetic stir bar is added intermediate **i2**, amine (1.2eq), potassium *tert*-butoxide (2.7eq), and BrettPhosPd G3 (3.6mol%) in 1mL dioxane and the reaction is heated to 90°C and stirred overnight. Reaction completion is monitored by TLC and LC/MS. After reaction is complete, reaction is purified by RP-HPLC eluting with water 0.1% formic acid and acetonitrile + 0.1% formic acid, pooling fractions with >95% purity.

Process C

Different aryl couplings conditions are pursued dependent on X as bromide (intermediates **i3**, **i6**, or **i9**) or iodide (intermediates **i7**, **i8**).

For X as bromide, synthesis proceeds by palladium-catalyzed aryl coupling [11]. To an argon purged vial with magnetic stir bar is added intermediate **i3**, **i6**, or **i9** (1 eq.), N,N-diisopropylethylamine (2 eq.), $Pd(dba)_2$ (5mol%), XantPhos (5mol%) and thiol (1 eq.) in dioxane. Reaction is heated to 110°C overnight and then cooled to room temperature. Reaction completion is monitored by TLC and LC/MS. After reaction is complete, water is added and compound is extracted into ethyl acetate and washed with water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated *in vacuo.* Purification occurs via silica flash chromatography with a hexanes – ethyl acetate, dichloromethane – methanol, or ethyl acetate - ethanol gradient. Product fractions are collected and concentrated to a solid. For final products, an additional purification step with RP-HPLC eluting with water + 0.1% formic acid and acetonitrile + 0.1% formic acid is conducted to ensure >95% purity.

For X as iodide, synthesis proceeds by copper-catalyzed aryl coupling [12]. To an argon purged vial with magnetic stir bar is added intermediate **i7**, or **i8** (1 eq.), thiol (1 eq.), copper iodide (5mol%), potassium carbonate (2.5 eq.), and ethylene glycol (5eq) in isopropanol. Reaction is heated to 130°C for 1 hour and then cooled to room temperature for 1 hour. Reaction completion is monitored by TLC and LC/MS. After reaction is complete, reaction is concentrated *in vacuo*, water is added and compound is extracted into ethyl acetate and washed with water and brine. The organic layer is

dried over sodium sulfate, filtered and concentrated *in vacuo.* Purification occurs via silica flash chromatography with a hexanes – ethyl acetate, dichloromethane – methanol, or ethyl acetate - ethanol gradient. Product fractions are collected and concentrated to a solid. For final products, an additional purification step with RP-HPLC eluting with water + 0.1% formic acid and acetonitrile + 0.1% formic acid is conducted to ensure >95% purity.

Process D

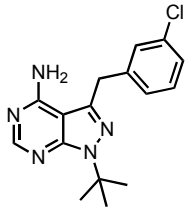
Synthesis proceeds by acid deprotection of t-butyl substituted pyrrolopyrimidine or pyrazolopyrimidine [13]. In a vial with magnetic stir bar is added t-butyl intermediate and a mixture of formic acid:hydrochloric acid (10:1), to dissolve the intermediate. The solution is refluxed for 2 hours, cooled and reaction completion is monitored by TLC and LC/MS. If reaction is not complete, the solution is brought again to reflux overnight or an additional part of hydrochloric acid is added and solution is refluxed for an additional 2 hours. Upon reaction completion, formic acid is removed *in vacuo*, water is added, and compound is extracted into ethyl acetate and washed with water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated *in vacuo.* Purification occurs via silica flash chromatography with a hexanes – ethyl acetate, dichloromethane – methanol, or ethyl acetate - ethanol gradient. Product fractions are collected and concentrated to a solid.

Process E

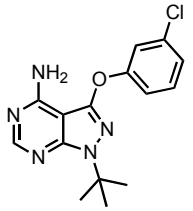
Synthesis proceeds by alkylation of unsubstituted pyrrolopyrimidine or pyrazolopyrimidine intermediate from *Process D* [14]. To an argon purged vial with magnetic stir bar is added *Process D* intermediate (1 eq.), R_2-mesylate (1.1 eq.) or R_2-halide (1.1 eq.), and cesium carbonate (2-3 eq.), in dry DMF. Reaction is heated to 80°C overnight followed by cooling. Reaction completion is monitored by TLC and LC/MS. After reaction is complete, reaction is concentrated *in vacuo*, water is added and compound is extracted into ethyl acetate and washed with water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification occurs via silica flash chromatography with a hexanes – ethyl acetate, dichloromethane – methanol, or ethyl acetate - ethanol gradient. Product fractions are collected and concentrated to a solid. For final products, an additional purification step with RP-HPLC eluting with water + 0.1% formic acid and acetonitrile + 0.1% formic acid is conducted to ensure >95% purity.

If R_2 is protected (eg. Boc), deprotection follows and purification occurs via silica flash chromatography with a hexanes – ethyl acetate, dichloromethane – methanol, or ethyl acetate - ethanol gradient. Product fractions are collected and concentrated to a solid. For final products, an additional purification step with RP-HPLC eluting with water + 0.1% formic acid and acetonitrile + 0.1% formic acid is conducted to ensure >95% purity.

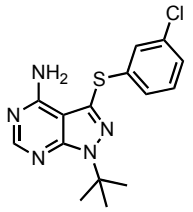
Synthesis and Characterization of Final Products



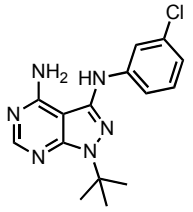
1: **1-(*tert*-butyl)-3-(3-chlorobenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine**
Compound synthesis is already described [15].



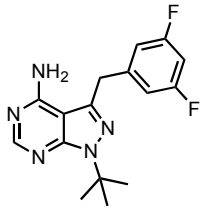
2: 1-(*tert*-butyl)-3-(3-chlorophenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine.
Compound is synthesized from **i3** using *Process A* with 3-chlorophenol as the alcohol. ESI-MS (ESI+) calcd for $C_{15}H_{16}ClN_5O$ $(M+H)^+$ 318.1043, found 318.1114. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.55 (t, J = 2.1 Hz, 1H), 7.47 – 7.36 (m, 2H), 7.23 (ddd, J = 7.7, 2.0, 1.3 Hz, 1H), 1.66 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.8, 156.6, 156.6, 153.6, 149.5, 133.8, 131.4, 124.2, 119.1, 117.2, 91.1, 60.0, 29.2.



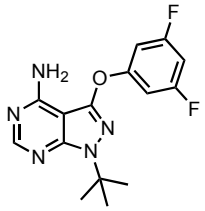
3: 1-(*tert*-butyl)-3-((3-chlorophenyl)thio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine.
Compound is synthesized from **i3** using *Process C* with 3-chlorobenznethiol as the thiol. ESI-MS (ESI+) calcd for $C_{15}H_{16}ClN_5S$ $(M+H)^+$ 334.0815, found 334.0884. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.38 – 7.26 (m, 3H), 7.09 (dt, J = 7.7, 1.4 Hz, 1H), 1.75 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.2, 156.0, 154.67, 138.1, 134.24, 131.6, 130.6, 127.1, 127.0, 126.3, 103.2, 61.3, 29.1.



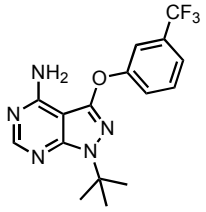
4: 1-(*tert*-butyl)-N^3-(3-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine. Compound is synthesized using *Process B* with 3-chloroaniline as the amine. ESI-MS (ESI+) calcd for $C_{15}H_{17}ClN_6$ $(M+H)^+$ 317.1203, found 317.1273. ^{1}H NMR ($CDCl_3$) δ 8.33 (1H), 7.20 – 7.17 (2H), 7.02 – 6.90 (2H), 6.78-6.76 (1H), 6.10 (1H), 5.28 (2H), 1.80 (9H).



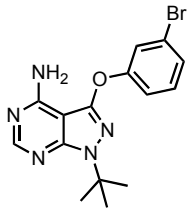
5: 1-(*tert*-butyl)-3-(3,5-difluorobenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound synthesis is already described [15].



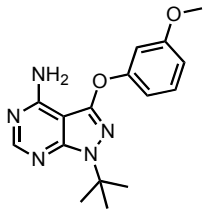
6: 1-(*tert*-butyl)-3-(3,5-difluorophenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i3** using *Process A* with 3,5-difluorophenol as the alcohol. ESI-MS (ESI+) calcd for $C_{15}H_{15}F_2N_5O$ $(M+H)^+$ 320.1245, found 320.1315. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.29 – 7.22 (m, 2H), 7.07 (d, J = 2.3 Hz, 1H), 1.67 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 161.9, 157.7, 157.4, 156.6, 153.5, 148.9, 102.9, 102.7, 91.0, 60.1, 29.2.



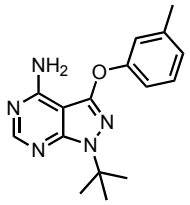
7: 1-(*tert*-butyl)-3-(3-(trifluoromethyl)phenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i3** using *Process A* with 3-(trifluoromethyl)phenol as the alcohol. ESI-MS (ESI+) calcd for $C_{16}H_{16}F_3N_5O$ $(M+H)^+$ 352.1307, found 352.1374. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.90 (t, J = 2.1 Hz, 1H), 7.77 (dd, J = 8.3, 2.4 Hz, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.57 – 7.51 (m, 1H), 1.66 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 157.9, 156.6, 155.9, 153.6, 149.5, 131.3, 130.7, 130.4, 122.6, 120.8, 115.9, 90.9, 60.0, 29.2.



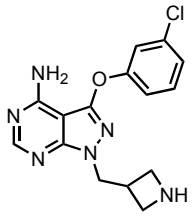
8: 3-(3-bromophenoxy)-1-(*tert*-butyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i3** using *Process A* with 3-bromophenol as the alcohol. ESI-MS (ESI+) calcd for $C_{15}H_{16}BrN_5O$ $(M+H)^+$ 362.0538, found 362.0607, 364.0587.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.72 – 7.67 (m, 1H), 7.48 – 7.33 (m, 3H), 1.66 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 157.8, 156.6, 156.6, 153.6, 149.5, 131.7, 127.1, 122.0, 121.9, 117.6, 91.0, 60.0, 29.2.



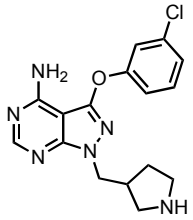
9: 1-(*tert*-butyl)-3-(3-methoxyphenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i3** using *Process A* with 3-methoxyphenol as the alcohol. ESI-MS (ESI+) calcd for $C_{16}H_{19}N_5O_2$ $(M+H)^+$ 314.1539, found 314.1607. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.29 (t, J = 8.2 Hz, 1H), 7.03 (t, J = 2.4 Hz, 1H), 6.93 (ddd, J = 8.2, 2.3, 0.8 Hz, 1H), 6.74 (ddd, J = 8.3, 2.5, 0.8 Hz, 1H), 3.76 (s, 3H), 1.66 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.7, 157.8, 157.1, 156.4, 153.6, 149.8, 130.5, 110.4, 110.0, 104.7, 59.9, 55.7, 29.3.



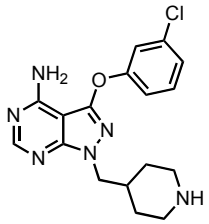
10: 1-(*tert*-butyl)-3-(*m*-tolyloxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i3** using *Process A* with 3-methylphenol (*m*-cresol) as the alcohol. ESI-MS (ESI+) calcd for $C_{16}H_{19}N_5O$ $(M+H)^+$ 298.1590, found 298.1659. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.28 (t, J = 7.9 Hz, 1H), 7.20 – 7.07 (m, 2H), 6.98 (d, J = 7.5 Hz, 1H), 2.32 (s, 3H), 1.65 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 157.81, 156.40, 156.04, 153.58, 150.03, 139.71, 129.76, 124.95, 119.18, 115.49, 91.22, 59.89, 29.25, 21.47.



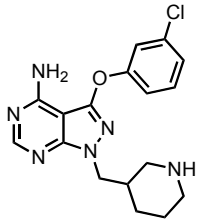
11: 1-(azetidin-3-ylmethyl)-3-(3-chlorophenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** followed by *Process D* and then *Process E* with 1-boc-3-(bromomethyl)azetidine as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with 1-boc-3-(bromomethyl)azetidine as the R_2-halide and coupling by *Process A* with 3-chlorophenol as the alcohol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{15}H_{15}ClN_6O$ $(M+H)^+$ 331.0996, found 331.1065. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.49 (t, J = 2.1 Hz, 1H), 7.40 – 7.31 (m, 2H), 7.20 (dt, J = 7.7, 1.6 Hz, 1H), 4.33 (d, J = 6.6 Hz, 2H), 3.94 – 3.59 (m, 4H), 3.11 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 156.7, 156.3, 156.0, 153.8, 152.5, 133.9, 131.5, 125.0, 119.9, 118.3, 89.7, 49.0, 47.7, 32.1.



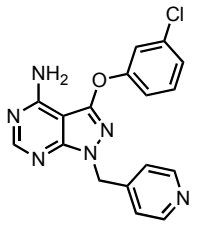
12: 3-(3-chlorophenoxy)-1-(pyrrolidin-3-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** followed by *Process D* and then *Process E* with tert-butyl 3-(bromomethyl)pyrrolidine-1-carboxylate as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with tert-butyl 3-(bromomethyl)pyrrolidine-1-carboxylate as the R_2-halide and coupling using *Process A* with 3-chlorophenol as the alcohol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{16}H_{17}ClN_6O$ $(M+H)^+$ 345.1152, found 345.1219. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.55 (t, *J* = 2.2 Hz, 1H), 7.51 – 7.35 (m, 2H), 7.28 (dt, *J* = 7.8, 1.5 Hz, 1H), 4.27 (qd, *J* = 14.1, 7.0 Hz, 2H), 3.22 (td, *J* = 11.2, 10.7, 6.2 Hz, 2H), 3.08 (dt, *J* = 11.4, 7.9 Hz, 1H), 2.95 (dd, *J* = 11.6, 7.9 Hz, 1H), 2.84 – 2.58 (m, 1H), 2.03 – 1.82 (m, 1H), 1.73 – 1.53 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 157.8, 157.7, 156.3, 154.2, 151.9, 133.9, 131.5, 124.7, 119.7, 118.0, 89.7, 47.9, 47.9, 44.9, 38.3, 28.0.



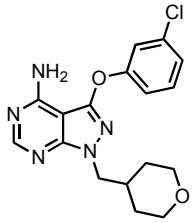
13: 3-(3-chlorophenoxy)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** followed by *Process D* and then *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively followed by coupling using *Process A* with 3-chlorophenol as the alcohol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{19}ClN_6O$ $(M+H)^+$ 359.1309, found 359.1375. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.46 (t, *J* = 2.2 Hz, 1H), 7.38 (t, *J* = 8.1 Hz, 1H), 7.31 (ddd, *J* = 8.3, 2.4, 1.1 Hz, 1H), 7.20 (dt, *J* = 8.0, 1.5 Hz, 1H), 4.04 (s, 2H), 3.15 (dt, *J* = 13.0, 3.5 Hz, 2H), 2.73 (td, *J* = 12.7, 3.0 Hz, 2H), 2.04 (dtt, *J* = 14.9, 7.1, 2.9 Hz, 1H), 1.64 – 1.49 (m, 2H), 1.28 (qd, *J* = 12.6, 4.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 157.8, 157.7, 156.3, 154.4, 151.7, 133.9, 131.5, 124.6, 119.6, 117.9, 89.6, 50.8, 43.2, 34.3, 26.6.



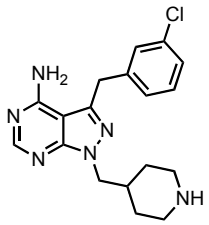
14: 3-(3-chlorophenoxy)-1-(piperidin-3-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** followed by *Process D* and then *Process E* with tert-butyl 3-(bromomethyl)piperidine-1-carboxylate as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with tert-butyl 3-(bromomethyl)piperidine-1-carboxylate as the R_2-halide followed by coupling using *Process A* with 3-chlorophenol as the alcohol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{19}ClN_6O$ $(M+H)^+$ 359.1309, found 359.1375. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.46 (t, *J* = 2.1 Hz, 1H), 7.43 – 7.30 (m, 2H), 7.20 (dt, *J* = 7.8, 1.6 Hz, 1H), 4.15 – 3.97 (m, 2H), 3.13 – 2.90 (m, 2H), 2.71 – 2.49 (m, 2H), 2.15 (td, *J* = 7.9, 4.2 Hz, 1H), 1.74 – 1.37 (m, 3H), 1.21 – 1.05 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 157.8, 157.7, 156.3, 154.4, 151.8, 133.9, 131.5, 124.7, 119.6, 118.0, 89.7, 48.9, 46.5, 43.8, 34.7, 26.2, 22.1.



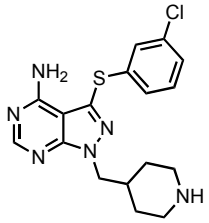
15: 3-(3-chlorophenoxy)-1-(pyridin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** followed by *Process D* and then *Process E* with 4-(Bromomethyl)pyridine as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with 4-(Bromomethyl)pyridine as the R_2-halide followed by coupling using *Process A* with 3-chlorophenol as the alcohol. ESI-MS (ESI+) calcd for $C_{17}H_{13}ClN_6O$ $(M+H)^+$ 353.0839, found 353.0908. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (d, *J* = 5.0 Hz, 2H), 8.15 (s, 1H), 7.45 (t, *J* = 2.1 Hz, 1H), 7.40 – 7.29 (m, 2H), 7.19 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.08 – 7.01 (m, 2H), 5.38 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 158.0, 157.8, 156.2, 154.6, 152.4, 150.3, 146.5, 133.9, 131.5, 124.8, 122.4, 119.7, 118.1, 89.9, 48.7.



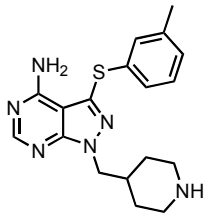
16: 3-(3-chlorophenoxy)-1-((tetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** followed by *Process D* and then *Process E* with 4-(Bromomethyl)tetrahydropyran as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with 4-(Bromomethyl)tetrahydropyran as the R_2-halide followed by coupling using *Process A* with 3-chlorophenol as the alcohol. ESI-MS (ESI+) calcd for Chemical Formula: $C_{17}H_{18}ClN_5O_2$ $(M+H)^+$ 360.1149, found 360.1219. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.50 – 7.26 (m, 3H), 7.19 (dt, *J* = 8.0, 1.4 Hz, 1H), 4.00 (d, *J* = 7.1 Hz, 2H), 3.73 (ddd, *J* = 11.5, 4.5, 1.9 Hz, 2H), 3.15 (td, *J* = 11.7, 2.1 Hz, 2H), 2.00 (ddq, *J* = 11.3, 7.8, 3.9 Hz, 1H), 1.32 (dd, *J* = 13.8, 3.3 Hz, 2H), 1.16 (qd, *J* = 12.0, 4.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 157.7, 157.6, 156.5, 154.3, 151.4, 133.9, 131.5, 124.5, 119.5, 117.8, 89.6, 66.9, 51.6, 35.6, 30.5.



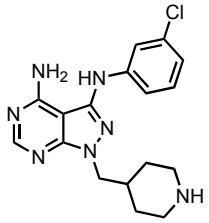
17: 3-(3-chlorobenzyl)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **1** followed by *Process D* and then *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{18}H_{21}ClN_6$ $(M+H)^+$ 357.1516, found 357.1583. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, *J* = 2.3 Hz, 1H), 7.28 – 7.21 (m, 2H), 7.19 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.16 – 7.09 (m, 1H), 4.30 (s, 2H), 4.11 (d, *J* = 7.0 Hz, 2H), 3.15 (dt, *J* = 13.0, 3.3 Hz, 2H), 2.74 (td, *J* = 12.7, 2.9 Hz, 2H), 2.12 (dtd, *J* = 15.4, 7.7, 4.5 Hz, 1H), 1.54 (dd, *J* = 14.2, 3.6 Hz, 2H), 1.39 – 1.20 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 158.5, 156.3, 155.0, 143.1, 142.0, 133.4, 130.7, 128.8, 127.6, 126.7, 98.6, 51.0, 43.3, 34.4, 33.1, 26.8.



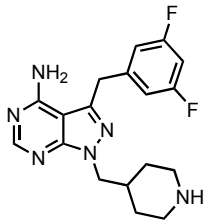
18: 3-((3-chlorophenyl)thio)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i7** using *Process C* with 3-chlorobenzenethiol as the thiol. Alkylation follows using *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Alternatively, alkylation of **i7** using *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively can precede coupling by *Process C* with 3-chlorobenzenethiol as the thiol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{19}ClN_6S$ $(M+H)^+$ 375.1080, found 375.1148. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (d, *J* = 11.3 Hz, 1H), 8.26 (s, 1H), 7.32 – 7.22 (m, 3H), 7.13 (dt, *J* = 7.6, 1.7 Hz, 1H), 4.26 (d, *J* = 7.0 Hz, 2H), 3.19 (d, *J* = 12.4 Hz, 2H), 2.87 – 2.71 (m, 2H), 2.18 (ddp, *J* = 11.0, 7.2, 3.6 Hz, 1H), 1.64 – 1.54 (m, 2H), 1.33 (qd, *J* = 12.5, 4.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 159.1, 156.9, 155.2, 154.7, 137.4, 134.3, 131.6, 127.7, 127.4, 128.0, 101.8, 51.8, 43.1, 39.9, 39.5, 34.3, 26.4.



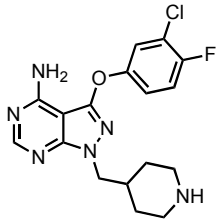
19: 1-(piperidin-4-ylmethyl)-3-(*m*-tolylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i7** using *Process C* with 3-methylbenzenethiol (*m*-thiocresol) as the thiol. Alkylation follows using *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Alternatively, alkylation of **i7** using *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively can precede coupling by *Process C* with 3-methylbenzenethiol (*m*-thiocresol) as the thiol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{18}H_{22}N_6S$ $(M+H)^+$ 355.1627, found 355.1693. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 7.09 – 6.89 (m, 2H), 4.21 (d, *J* = 6.6 Hz, 1H), 3.14 (s, 2H), 2.72 (d, *J* = 12.8 Hz, 2H), 2.16 (s, 3H), 1.54 (d, *J* = 12.7 Hz, 2H), 1.37 – 1.21 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 158.2, 157.1, 155.2, 139.4, 134.8, 134.1, 130.0, 128.8, 128.2, 125.5, 101.7, 51.7, 43.2, 34.6, 26.8, 21.3.



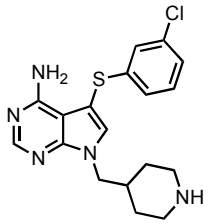
20: N^3-(3-chlorophenyl)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine. Compound is synthesized from **4** followed by *Process D* and then *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Alternatively, the compound can be synthesized by using an intermediate with N1 as -H (for example, intermediate **i6**) followed by alkylation (*Process E*) with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively followed by coupling using *Process B* with 3-chloroaniline as the amine. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{20}ClN_7$ $(M+H)^+$ 358.1469, found 358.1537. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.11 (s, 1H), 7.83 (t, *J* = 2.1 Hz, 1H), 7.62 (s, 2H), 7.58 (ddd, *J* = 8.3, 2.2, 0.9 Hz, 1H), 7.28 (t, *J* = 8.1 Hz, 1H), 6.89 (ddd, *J* = 7.9, 2.1, 0.9 Hz, 1H), 4.09 (d, *J* = 7.0 Hz, 2H), 3.14 – 3.02 (m, 2H), 2.64 (td, *J* = 12.4, 2.7 Hz, 2H), 2.08 (ddt, *J* = 11.4, 7.8, 3.8 Hz, 1H), 1.89 (s, 1H), 1.57 (d, *J* = 12.8 Hz, 2H), 1.27 (dd, *J* = 13.1, 3.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.9, 156.7, 153.9, 144.3, 142.7, 133.6, 130.7, 119.8, 116.3, 115.6, 90.9, 51.1, 44.4, 35.5, 28.4.



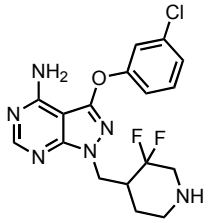
21: 3-(3,5-difluorobenzyl)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **5** followed by *Process D* and then *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{18}H_{20}F_2N_6$ $(M+H)^+$ 359.1718, found 359.1785. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.06 (tt, *J* = 9.4, 2.4 Hz, 1H), 7.00 – 6.91 (m, 1H), 4.39 (s, 2H), 4.14 (d, *J* = 7.1 Hz, 2H), 3.10 – 2.95 (m, 2H), 2.16 – 1.96 (m, 1H), 1.46 (d, *J* = 13.0 Hz, 2H), 1.38 (d, *J* = 1.9 Hz, 1H), 1.28 – 1.09 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 163.7, 161.7, 158.4, 156.3, 155.0, 142.3, 112.2, 112.0, 102.3, 98.5, 51.7, 44.7, 35.9, 33.1, 29.0.



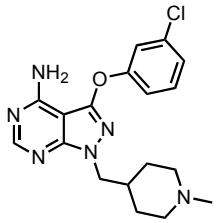
22: 3-(3-chloro-4-fluorophenoxy)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i3** using *Process A* with 3-chloro-4-fluorophenol as the alcohol followed by *Process D* and then *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively followed by coupling using *Process A* with 3-chloro-4-fluorophenol as the alcohol. Boc deprotection follows to obtain the final product. LC/MS (ES+) calcd for Chemical Formula: $C_{17}H_{18}ClFN_6O$ $(M+H)^+$ 377.1215, found 377.1282. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 2H), 8.19 (s, 1H), 7.79 – 7.71 (m, 1H), 7.53 – 7.44 (m, 2H), 4.08 (d, *J* = 6.9 Hz, 2H), 3.18 (dt, *J* = 11.5, 2.9 Hz, 2H), 2.77 (td, *J* = 12.6, 3.0 Hz, 2H), 2.10 (dt, *J* = 7.5, 3.7 Hz, 1H), 1.61 (dd, *J* = 14.2, 3.5 Hz, 2H), 1.44 (tt, *J* = 12.2, 6.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 157.8, 157.7, 154.4, 152.0, 151.7, 121.8, 120.0, 120.0, 117.9, 117.7, 89.3, 50.8, 42.9, 34.3, 26.3.



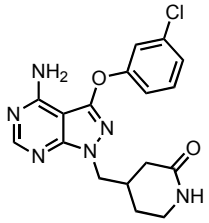
23: 5-((3-chlorophenyl)thio)-7-(piperidin-4-ylmethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. Compound is synthesized from **i8** using *Process C* with 3-chlorobenzenethiol as the thiol. Alkylation follows using *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively. Alternatively, alkylation of **i8** using *Process E* with tert-butyl 4-(bromomethyl)piperidine-1-carboxylate or tert-butyl 4-methylsulfonyloxymethyl-1-piperidinecarboxylate as the R_2-halide or R_2-mesylate respectively can precede coupling by *Process C* with 3-chlorobenzenethiol as the thiol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{18}H_{20}ClN_5S$ $(M+H)^+$ 374.1128, found 374.1197. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 2H), 8.09 (s, 1H), 7.67 (s, 1H), 7.23 (t, *J* = 7.9 Hz, 1H), 7.14 (dd, *J* = 7.7, 2.0 Hz, 1H), 7.01 (t, *J* = 1.9 Hz, 1H), 6.99 – 6.94 (m, 1H), 4.04 (d, *J* = 7.2 Hz, 2H), 3.06 (d, *J* = 12.1 Hz, 2H), 2.58 (t, *J* = 11.9 Hz, 2H), 2.03 (s, 1H), 1.46 (d, *J* = 13.0 Hz, 2H), 1.30 – 1.14 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 157.8, 153.1, 151.4, 141.9, 134.3, 133.8, 131.4, 126.0, 125.2, 124.6, 103.0, 96.8, 49.5, 43.7, 35.2, 27.6, 27.5.



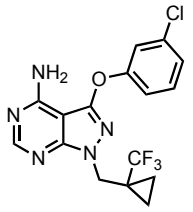
24: 3-(3-chlorophenoxy)-1-((3,3-difluoropiperidin-4-yl)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** using *Process D* followed by *Process E* with 1-boc-3,3-difluoro-4-(bromomethyl)piperidine or 1-boc-3,3-difluoro-4-((methylsulfonyloxy)methyl)piperidine as the R_2-halide or R_2-mesylate respectively. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with 1-boc-3,3-difluoro-4-(bromomethyl)piperidine or 1-boc-3,3-difluoro-4-((methylsulfonyloxy)methyl)piperidine as the R_2-halide or R_2-mesylate respectively followed by coupling using *Process A* with 3-chlorophenol as the alcohol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{17}ClF_2N_6O$ $(M+H)^+$ 395.1120, found 395.1187. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.48 – 7.26 (m, 3H), 7.19 (dd, *J* = 7.7, 1.9 Hz, 1H), 4.38 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.12 (dd, *J* = 14.1, 9.2 Hz, 1H), 3.14 – 2.96 (m, 1H), 2.88 – 2.63 (m, 2H), 1.31 (ddd, *J* = 28.8, 10.4, 4.0 Hz, 2H), 1.10 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 157.7, 157.7, 156.4, 154.6, 151.6, 133.9, 131.5, 124.6, 119.4, 117.7, 89.8, 46.2, 43.7, 28.3, 28.3, 9.2.



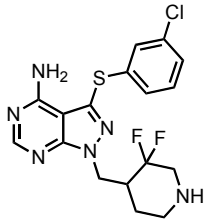
25: 3-(3-chlorophenoxy)-1-((1-methylpiperidin-4-yl)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Synthesis proceeds by reductive alkylation of 3-(3-chlorophenoxy)-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**13**). In a 1 dram vial with magnetic stir bar, **13** (25mg, 0.070mmol) was dissolved in methanol (200uL). A solution of formaldehyde (52uL of 37%, 0.70mmol) in 2% acetic acid was added to the reaction and was stirred for 10 minutes at room temperature. Sodium cyanoborohydride (21.9mg, 0.349mmol) was added and the reaction was stirred for 3 hours at room temperature. Purification by RP-HPLC using acetonitrile – water in the presence of 0.1% formic acid yields. ESI-MS (ESI+) calcd for $C_{18}H_{21}ClN_6O$ $(M+H)^+$ 373.1465, found 373.1533 ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, *J* = 3.9 Hz, 2H), 7.41 (t, *J* = 2.2 Hz, 1H), 7.35 (t, *J* = 8.1 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.21 – 7.14 (m, 1H), 3.97 (d, *J* = 7.1 Hz, 2H), 2.74 (dt, *J* = 12.2, 3.6 Hz, 2H), 2.14 (s, 3H), 2.01 – 1.84 (m, 2H), 1.84 – 1.62 (m, 1H), 1.45 – 1.33 (m, 2H), 1.24 – 1.09 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 164.6, 157.7, 156.4, 154.3, 151.5, 133.9, 131.5, 124.6, 119.5, 117.8, 89.6, 54.7, 51.3, 51.3, 45.6, 35.4, 29.0.



26: 4-((4-amino-3-(3-chlorophenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)methyl)piperidin-2-one. Compound is synthesized from **2** using *Process D* followed by *Process E* with *tert*-butyl 4-(bromomethyl)-2-oxopiperidine-1-carboxylate (prepared from 4-(hydroxymethyl)piperidin-2-one) as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with *tert*-butyl 4-(bromomethyl)-2-oxopiperidine-1-carboxylate (prepared from 4-(hydroxymethyl)piperidin-2-one) as the R_2-halide followed by coupling using *Process A* with 3-chlorophenol as the alcohol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{17}ClN_6O_2$ $(M+H)^+$ 373.1102, found 373.1170. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.45 (t, *J* = 2.2 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.31 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.22 – 7.17 (m, 1H), 4.05 (d, *J* = 7.0 Hz, 2H), 3.09 (ddt, *J* = 12.3, 6.2, 3.6 Hz, 1H), 2.98 (td, *J* = 11.4, 4.5 Hz, 1H), 2.32 – 2.18 (m, 1H), 2.01 (ddd, *J* = 17.3, 5.4, 1.5 Hz, 1H), 1.86 (dd, *J* = 17.3, 10.5 Hz, 1H), 1.58 (dd, *J* = 13.4, 3.8 Hz, 1H), 1.29 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.2, 157.7, 157.6, 156.3, 154.3, 151.7, 133.9, 131.6, 124.7, 119.5, 117.9, 89.6, 50.4, 35.3, 33.5, 28.7, 25.7.



27: 3-(3-chlorophenoxy)-1-((1-(trifluoromethyl)cyclopropyl)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **2** using *Process D* followed by *Process E* with 1-(Bromomethyl)-1-(trifluoromethyl)-cyclopropane as the R_2-halide. Alternatively, the compound can be synthesized by using intermediate **i6** followed by alkylation (*Process E*) with 1-(Bromomethyl)-1-(trifluoromethyl)-cyclopropane as the R_2-halide followed by coupling using *Process A* with 3-chlorophenol as the alcohol. ESI-MS (ESI+) calcd for Chemical Formula: $C_{16}H_{13}ClF_3N_5O$ $(M+H)^+$ 384.0761, found 384.0831. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.46 (t, *J* = 2.2 Hz, 1H), 7.39 (t, *J* = 8.1 Hz, 1H), 7.31 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.23 – 7.16 (m, 1H), 4.38 (s, 2H), 1.05 (d, *J* = 5.3 Hz, 2H), 0.96 (t, *J* = 3.5 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 157.8, 157.7, 156.3, 154.5, 151.4, 133.9, 131.5, 124.5, 119.2, 117.6, 89.8, 47.2, 8.5.



28: 3-((3-chlorophenyl)thio)-1-((3,3-difluoropiperidin-4-yl)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine. Compound is synthesized from **i7** using *Process C* with 3-chlorobenzenethiol as the thiol. Alkylation follows using *Process E* with 1-boc-3,3-difluoro-4-(bromomethyl)piperidine or 1-boc-3,3-difluoro-4-((methylsulfonyloxy)methyl)piperidine as the R_2-halide or R_2-mesylate respectively. Alternatively, alkylation of **i7** using *Process E* with 1-boc-3,3-difluoro-4-(bromomethyl)piperidine or 1-boc-3,3-difluoro-4-((methylsulfonyloxy)methyl)piperidine as the R_2-halide or R_2-mesylate respectively can precede coupling by *Process C* with 3-chlorobenzenethiol as the thiol. Boc deprotection follows to obtain the final product. ESI-MS (ESI+) calcd for $C_{17}H_{17}ClF_2N_6S$ $(M+H)^+$ 411.0892, found 411.0960. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 8.09 (s, 1H), 7.33 – 7.18 (m, 3H), 7.10 (dt, *J* = 7.6, 1.6 Hz, 1H), 4.61 (dd, *J* = 14.0, 5.3 Hz, 1H), 4.28 (dd, *J* = 14.0, 8.9 Hz, 1H), 2.96 (td, *J* = 12.6, 4.6 Hz, 1H), 2.83 – 2.51 (m, 3H), 1.30 (td, *J* = 10.7, 9.8, 3.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.1, 157.2, 157.2, 155.3, 137.8, 134.3, 133.1, 131.5, 127.3, 127.2, 126.6, 101.9, 45.4, 43.9.

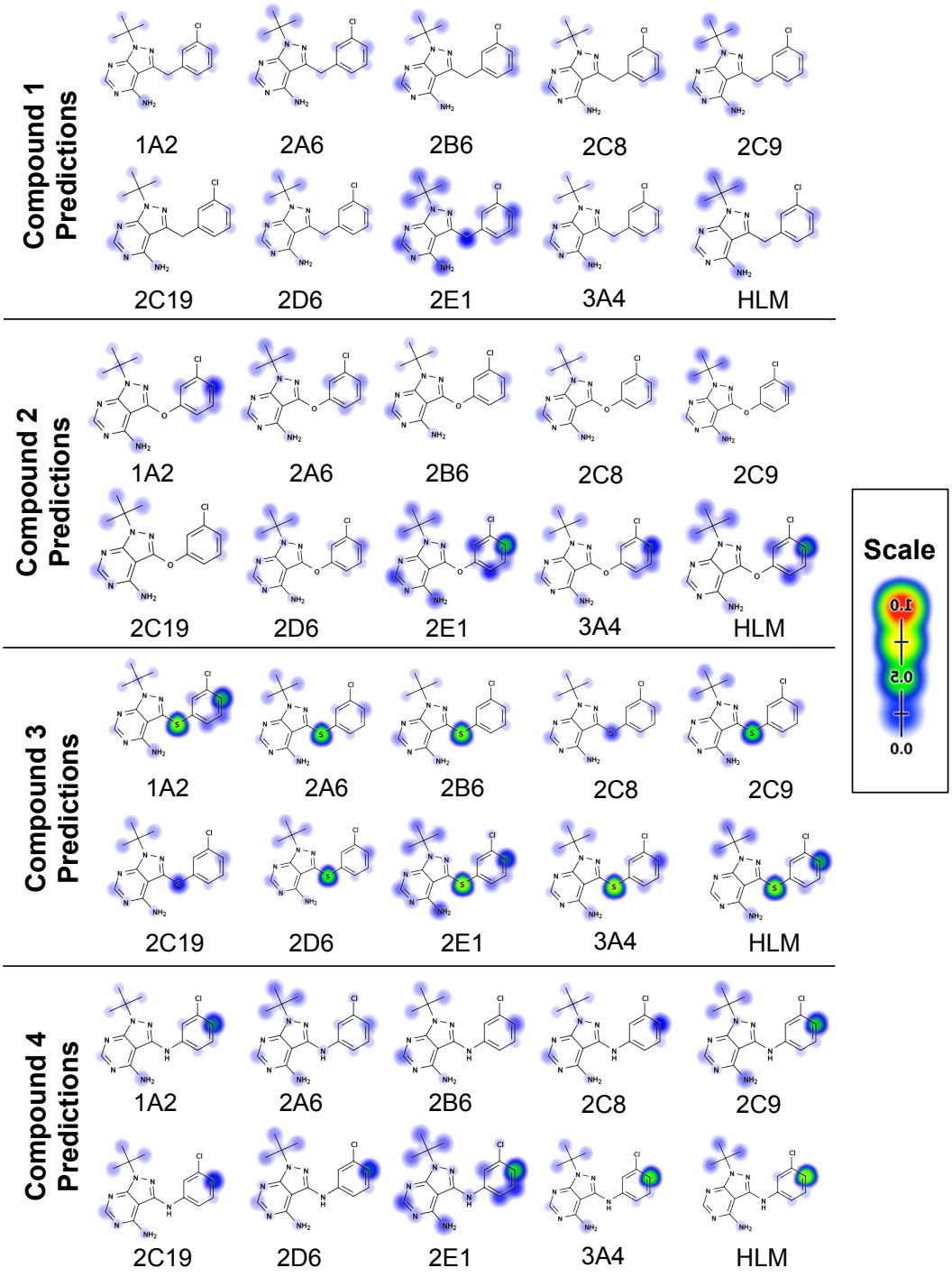


Figure S1 XenoSite Analysis. Results from XenoSite Cytochrome P450 Metabolism (v1.0)[16,17] analysis of compounds **1**, **2**, **3** and **4** predicts that the methylene linkage to C3 of the PP core, as in **1**, is subject to metabolism, particularly by CYP2E1. Metabolism at this position is less likely for ether and amine linkages as in **2** and **4** respectively. Thioether linkages such as in **3** are predicted to be highly metabolized by multiple CYP isozymes.

Table S1. Crystallographic Details and Refinement characteristics

PDB	4IHP	5W8R	5W80	5W9E	5W91
Ligand	2	1	13	3	4
Data Collection					
Space Group	P21	P21	P21	P21	P21
Cell Dimensions *a*, *b*, *c* (Å) α, β, γ (°)	 48.3, 73.2, 149.3 90.0, 90.0, 90.0	 48.0, 72.9, 65.5 90.0, 99.3, 90.0	 48.0, 72.8, 65.5 90.0, 99.5, 90.0	 48.0, 72.8, 64.6 90.0, 98.6, 90.0	 47.9, 72.2, 64.2 90.0, 98.7, 90.0
Refinement					
Resolution (Å)	29.55 - 2.27	48.40 - 2.20	48.33 – 2.00	48.01 – 2.44	47.65 – 2.30
R_{work} / R_{free}	0.23/ 0.25	0.20 / 0.25	0.19 / 0.24	0.21 / 0.25	0.25 / 0.31
No. atoms Protein Compound Water	 3553 22 89	 3584 22 146	 3600 25 249	 3498 22 70	 3440 21 105
Average B-factors	45.4	53.5	41.8	62.5	55.9
RMS deviations Bond lengths (Å) Bond Angles (°)	 0.01 1.39	 0.01 0.75	 0.01 0.81	 0.01 0.67	 0.01 0.82
Crystallization conditions	20% PEG3350, 0.2M potassium acetate, pH 7.5	25% PEG 3350, 0.2M sodium chloride, pH 7.5, 0.1M Hepes 7.5, 5% ethylene glycol, 5% glycerol	25% PEG8000, 0.2M sodium chloride, 0.1M Hepes, pH 7.5, 10 % glycerol	25% PEG8000, 0.2M sodium chloride, 0.1M Hepes, pH 7.5, 5% glycerol	25% PEG8000, 0.2M sodium chloride, 0.1M Hepes, pH 7.5, 10 % glycerol

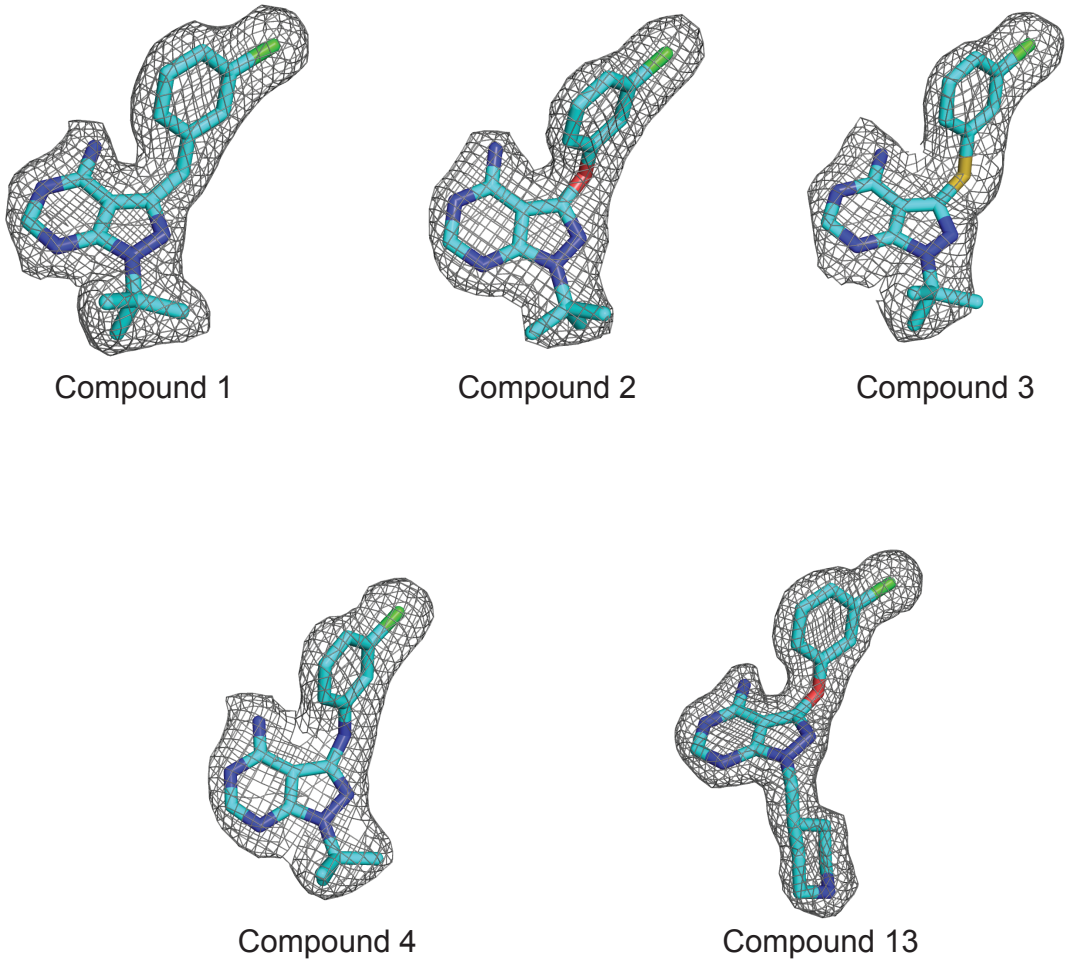


Figure S2 Crystallography Difference Maps. 2Fo-Fc omit maps (grey mesh, 1σ) of **1**, **2**, **3**, **4**, and **13**.

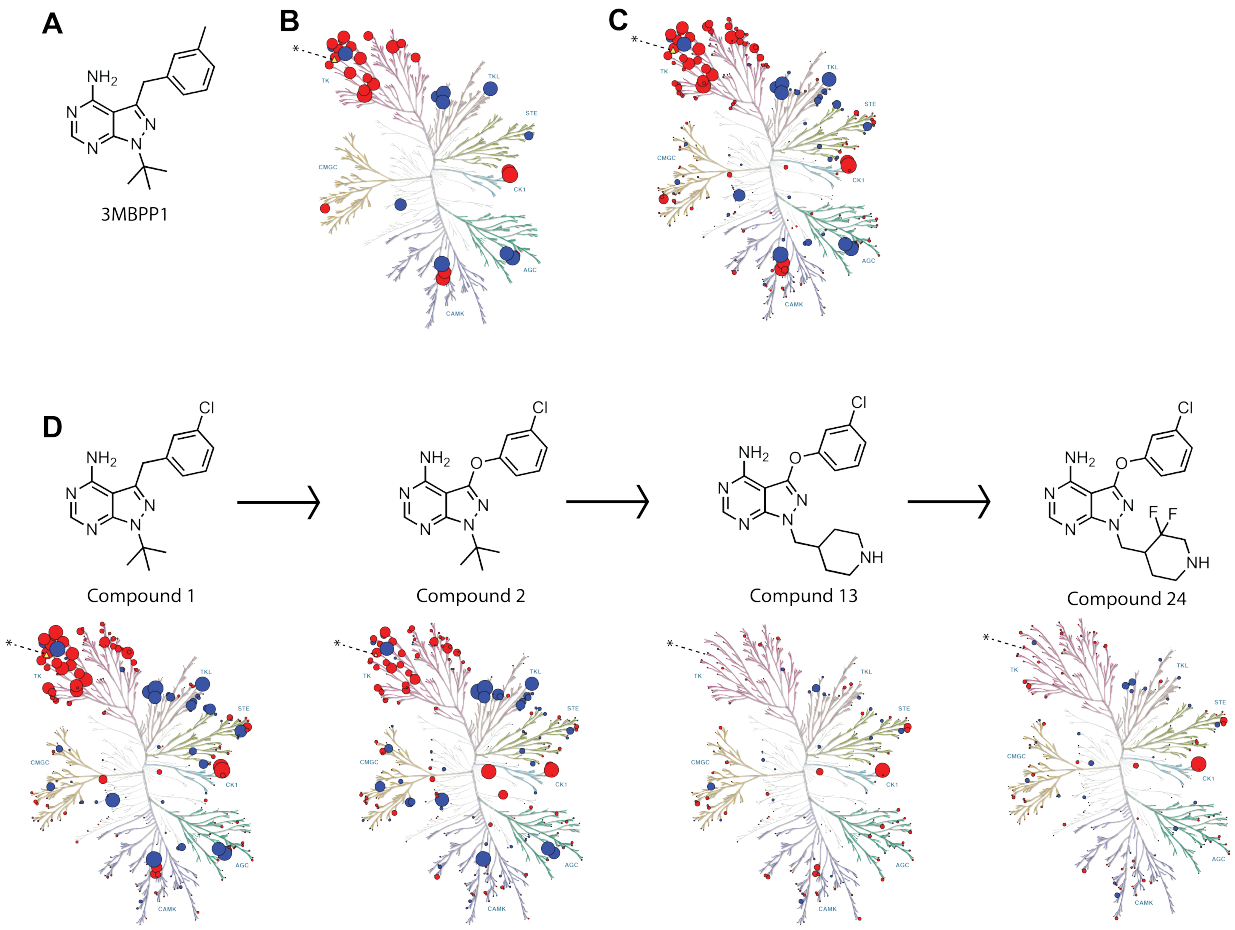


Figure S3. Kinome Profiling for compounds 3MBPP1, 1, 2, 13 and 24. (A) Structure of **3MBPP1**, a previously described TgCDPK1 inhibitor[15]. (B) Selectivity profile of **3MBPP1** for kinases that are inhibited by more than 50% or show greater than 50% tracer displacement. (C) Selectivity profile of **3MBPP1** for all kinases that are inhibited or show tracer displacement. (D) Selectivity profile of **1**, **2**, **13** and **24** for all kinases that are inhibited or show tracer displacement. For all trees, circle size reflects values obtained from ThermoFisher ACCESS kinase profiling for percent inhibition or displacement in the presence of compound and percentages are the mean of 2 data points. % inhibition value for Src is shown as a yellow triangle (emphasized by * and dotted line). Kinome tree images were generated using KinMap[18] and the kinase tree illustration is reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com). The full kinome profiling dataset is presented in supplementary tables S2-S4 (pages S26-S35).

Table S2. CK1ε off-target inhibition by compounds 3MBPP1, 1, 2, 13 and 24.

	CK1ε IC_{50} (nM)				
[ATP] Tested	**3MBPP1**	**Compound 1**	**Compound 2**	**Compound 13**	**Compound 24**
Km_{app} (2 μM)	59.8	25.3	49.7	115	78.2
100 μM	838	303	450	745	673

Table S3. ThermoFisher Scientific Z'-LYTE Kinase Profiling

Kinase Tested	[ATP] Tested (μM)	3MBPP1 (1μM) % Inhibition (mean of 2 points)	Compound 1 (1μM) % Inhibition (mean of 2 points)	Compound 2 (1μM) % Inhibition (mean of 2 points)	Compound 13 (1μM) % Inhibition (mean of 2 points)	Compound 24 (1μM) % Inhibition (mean of 2 points)
ABL1 E255K	Km app	17	28	20	1	2
ABL1 F317I	Km app	18	27	16	4	3
ABL1 F317L	Km app	19	34	16	2	2
ABL1 G250E	Km app	25	38	26	10	9
ABL1 T315I	Km app	4	4	6	1	0
ABL1 Y253F	Km app	21	36	20	3	3
ABL1	Km app	21	35	31	1	8
ABL2 (Arg)	Km app	21	28	12	3	2
ACVR1B (ALK4)	Km app	14	16	11	6	1
ADRBK1 (GRK2)	Km app	5	5	0	1	1
ADRBK2 (GRK3)	Km app	2	-3	-1	-3	-2
AKT1 (PKB alpha)	Km app	4	2	2	2	4
AKT2 (PKB beta)	Km app	17	4	7	8	7
AKT3 (PKB gamma)	Km app	4	6	-2	-1	2
ALK	Km app	3	1	-2	-2	-2
AMPK (A1/B2/G2)	Km app	8	10	6	3	0
AMPK (A1/B2/G3)	Km app	9	9	0	1	1
AMPK (A2/B1/G2)	Km app	7	6	5	6	5
AMPK (A2/B1/G3)	Km app	6	5	2	4	3
AMPK (A2/B2/G3)	Km app	9	9	3	4	3
AMPK A1/B1/G1	Km app	5	8	5	-2	3
AMPK A2/B1/G1	Km app	5	7	10	10	7
AURKA (Aurora A)	Km app	8	2	25	5	13
AURKB (Aurora B)	Km app	11	4	4	6	3
AURKC (Aurora C)	Km app	9	11	-3	3	-6
AXL	Km app	8	8	5	0	1
BLK	Km app	21	12	5	6	2
BMX	Km app	38	48	32	3	29
BRAF V599E	100	26	34	14	8	10
BRAF	100	-3	0	-2	-3	-9
BRSK1 (SAD1)	Km app	9	8	5	11	11
BTK	Km app	33	41	22	6	13
CAMK1D (CaMKI delta)	Km app	6	6	3	3	2
CAMK1G (CAMKI gamma)	Km app	11	15	3	1	-1
CAMK2A (CaMKII alpha)	Km app	-1	1	0	1	2
CAMK2B (CaMKII beta)	Km app	5	9	-2	-3	1
CAMK2D (CaMKII delta)	Km app	13	7	7	13	14
CAMK4 (CaMKIV)	Km app	5	8	8	11	-3
CDC42 BPA (MRCKA)	Km app	7	10	2	0	5
CDC42 BPB (MRCKB)	Km app	4	6	1	1	-3
CDC42 BPG (MRCKG)	Km app	3	5	1	1	1
CDK1/cyclin B	Km app	5	5	4	3	4
CDK17/cyclin Y	Km app	11	11	4	3	3
CDK18/cyclin Y	Km app	7	10	5	3	3
CDK2/cyclin A	Km app	11	9	6	5	6
CDK5/p25	Km app	3	14	8	7	9
CDK5/p35	Km app	14	15	11	5	7
CDKL5	Km app	10	12	2	1	0
CHEK1 (CHK1)	Km app	5	3	5	3	-1
CHEK2 (CHK2)	Km app	8	3	5	4	11
CLK1	Km app	7	12	8	2	5
CLK2	Km app	13	19	38	10	14
CLK3	Km app	12	8	14	1	10
CSF1R (FMS)	Km app	11	10	16	0	13

CSK	Km app	26	26	3	-1	2
CSNK1A1 (CK1 alpha 1)	Km app	34	47	12	20	13
CSNK1A1L	Km app	16	29	3	8	3
CSNK1D (CK1 delta)	Km app	83	91	57	43	32
CSNK1E (CK1 epsilon) R178C	Km app	93	97	91	91	92
CSNK1E (CK1 epsilon)	Km app	92	93	86	86	89
CSNK1G1 (CK1 gamma 1)	Km app	-4	1	-3	10	2
CSNK1G2 (CK1 gamma 2)	Km app	14	7	3	2	4
CSNK1G3 (CK1 gamma 3)	Km app	3	9	1	2	5
CSNK2A1 (CK2 alpha 1)	Km app	2	1	-13	-6	-1
CSNK2A2 (CK2 alpha 2)	Km app	1	-5	0	-1	0
DAPK3 (ZIPK)	Km app	5	8	2	0	-2
DCAMKL1 (DCLK1)	Km app	3	4	0	-2	-4
DCAMKL2 (DCK2)	Km app	10	8	6	6	5
DNA-PK	Km app	9	2	5	3	8
DYRK1A	Km app	2	3	3	3	4
DYRK1B	Km app	12	5	1	3	4
DYRK3	Km app	16	4	5	0	7
DYRK4	Km app	5	4	1	1	0
EEF2K	Km app	1	-1	-3	3	-1
EGFR (ErbB1) C797S	Km app	61	81	51	3	-1
EGFR (ErbB1) G719C	Km app	62	77	38	0	1
EGFR (ErbB1) G719S	Km app	80	86	62	1	1
EGFR (ErbB1) L858R	Km app	37	72	35	0	8
EGFR (ErbB1) L861Q	Km app	50	78	43	0	5
EGFR (ErbB1) T790M C797S L858R	Km app	49	62	25	1	4
EGFR (ErbB1) T790M L858R	Km app	72	82	43	5	10
EGFR (ErbB1) T790M	Km app	66	74	29	-1	4
EGFR (ErbB1)	Km app	56	71	44	-1	11
EPHA1	Km app	73	81	61	12	14
EPHA2	Km app	54	59	40	2	6
EPHA4	Km app	60	68	36	3	6
EPHA5	Km app	64	70	40	1	0
EPHA8	Km app	48	65	41	-7	-6
EPHB1	Km app	52	60	40	4	7
EPHB2	Km app	72	84	62	4	11
EPHB3	Km app	61	69	48	6	6
EPHB4	Km app	73	75	43	2	5
ERBB2 (HER2)	Km app	27	24	8	2	0
ERBB4 (HER4)	Km app	43	36	20	-4	3
FER	Km app	20	30	17	12	16
FES (FPS)	Km app	12	9	4	1	-3
FGFR1	Km app	43	27	18	6	8
FGFR2 N549H	Km app	16	14	6	-5	0
FGFR2	Km app	26	26	10	-1	-2
FGFR3 K650E	Km app	21	22	10	10	-1
FGFR3 V555M	Km app	-1	3	10	-1	-3
FGFR3	Km app	2	3	3	-1	-4
FGFR4	Km app	23	18	12	3	-4
FGR	Km app	70	77	61	14	17
FLT1 (VEGFR1)	Km app	9	1	0	-3	1
FLT3 D835Y	Km app	12	10	13	8	8
FLT3	Km app	27	41	58	8	14
FLT4 (VEGFR3)	Km app	40	35	17	-5	-1
FRAP1 (mTOR)	Km app	6	4	4	0	13
FRK (PTK5)	Km app	66	73	43	-2	1
FYN	Km app	47	60	39	8	9
GRK4	Km app	-5	6	-1	-9	-7
GRK5	Km app	2	3	1	0	-1
GRK6	Km app	3	6	0	1	-1

GRK7	Km app	2	3	-1	3	-1
GSK3A (GSK3 alpha)	Km app	8	5	7	14	9
GSK3B (GSK3 beta)	Km app	11	4	5	3	7
HCK	Km app	24	33	26	7	11
HIPK1 (Myak)	Km app	6	2	0	-2	-1
HIPK2	Km app	5	4	3	3	3
HIPK3 (YAK1)	Km app	7	6	5	6	6
HIPK4	Km app	7	12	6	1	1
IGF1R	Km app	9	1	-1	-1	1
IKBKB (IKK beta)	Km app	10	3	2	2	3
IKBKE (IKK epsilon)	Km app	5	4	0	0	3
INSR	Km app	10	7	3	2	3
INSRR (IRR)	Km app	8	-2	-3	-2	1
IRAK4	Km app	5	-3	3	-1	-6
ITK	Km app	4	1	1	1	3
JAK1	Km app	0	0	1	-1	-1
JAK2 JH1 JH2 V617F	Km app	5	-8	2	-12	-5
JAK2 JH1 JH2	Km app	-2	4	0	-3	-2
JAK2	Km app	0	2	0	-5	-4
JAK3	Km app	0	1	-1	1	-3
KDR (VEGFR2)	Km app	52	30	22	3	7
KIT T670I	Km app	7	25	2	5	2
KIT V559D V654A	Km app	4	7	-3	-5	0
KIT V559D	Km app	12	8	3	1	0
KIT V560G	Km app	8	8	4	-2	-1
KIT	Km app	19	-1	3	3	7
KSR2	Km app	5	5	1	0	0
LCK	Km app	23	38	36	7	9
LTK (TYK1)	Km app	9	4	4	3	10
LYN A	Km app	50	55	29	11	13
LYN B	Km app	54	59	30	6	11
MAP2K1 (MEK1)	100	7	1	-1	-4	1
MAP2K2 (MEK2)	100	19	25	11	10	11
MAP2K6 (MKK6)	100	16	14	7	10	4
MAP3K19 (YSK4)	Km app	-3	3	0	0	-1
MAP3K8 (COT)	100	7	9	-4	-4	-7
MAP3K9 (MLK1)	Km app	8	1	6	-1	3
MAP4K2 (GCK)	Km app	10	7	8	4	4
MAP4K4 (HGK)	Km app	26	53	27	44	42
MAP4K5 (KHS1)	Km app	30	36	20	16	13
MAPK1 (ERK2)	Km app	4	1	0	-2	1
MAPK10 (JNK3)	100	9	2	3	1	-1
MAPK11 (p38 beta)	Km app	21	22	16	15	8
MAPK12 (p38 gamma)	Km app	10	6	6	5	7
MAPK13 (p38 delta)	Km app	6	-1	0	-1	1
MAPK14 (p38 alpha) Direct	Km app	57	17	14	30	20
MAPK14 (p38 alpha)	100	23	19	16	39	11
MAPK3 (ERK1)	Km app	10	16	8	8	4
MAPK7 (ERK5)	Km app	3	3	-2	-2	-1
MAPK8 (JNK1)	100	14	8	7	0	3
MAPK9 (JNK2)	100	11	-4	-3	-10	-6
MAPKAPK2	Km app	10	2	7	3	0
MAPKAPK3	Km app	3	4	2	2	1
MAPKAPK5 (PRAK)	Km app	7	3	3	3	5
MARK1 (MARK)	Km app	9	4	4	4	2
MARK2	Km app	5	4	2	3	0
MARK3	Km app	6	2	4	8	5
MARK4	Km app	7	8	1	1	-1
MATK (HYL)	Km app	10	11	1	2	-2
MELK	Km app	18	10	10	5	14
MERTK (cMER)	Km app	17	15	11	5	3

MET (cMet) Y1235D	Km app	0	3	0	-1	-2
MET (cMet)	Km app	7	-10	6	-8	6
MET M1250T	Km app	7	10	1	4	7
MINK1	Km app	15	25	19	32	28
MKNK1 (MNK1)	Km app	4	2	3	-2	3
MST1R (RON)	Km app	2	4	-3	-1	-3
MST4	Km app	21	14	10	11	12
MUSK	Km app	12	17	16	4	0
MYLK2 (skMLCK)	Km app	1	-5	-2	-3	-3
NEK1	Km app	10	16	8	7	5
NEK2	Km app	5	8	3	6	3
NEK4	Km app	5	8	1	1	0
NEK6	Km app	12	6	7	8	4
NEK7	Km app	9	7	8	9	8
NEK9	Km app	5	5	3	8	1
NIM1K	Km app	7	6	2	2	1
NTRK1 (TRKA)	Km app	31	7	11	17	11
NTRK2 (TRKB)	Km app	55	43	27	4	5
NTRK3 (TRKC)	Km app	46	18	34	2	9
PAK1	Km app	11	15	12	5	13
PAK2 (PAK65)	Km app	9	9	5	8	7
PAK3	Km app	12	7	-12	3	0
PAK4	Km app	2	-2	0	-1	2
PAK6	Km app	9	15	10	13	10
PAK7 (KIAA1264)	Km app	9	4	4	4	3
PASK	Km app	13	8	14	11	7
PDGFRA (PDGFR alpha)	Km app	12	6	0	-1	2
PDGFRA D842V	Km app	9	15	8	-2	2
PDGFRA T674I	Km app	7	8	-6	-4	-9
PDGFRA V561D	Km app	35	37	22	7	7
PDGFRB (PDGFR beta)	Km app	6	10	-3	-6	-4
PDK1 Direct	Km app	16	-3	7	4	7
PDK1	100	7	-8	16	12	13
PEAK1	Km app	30	50	32	1	4
PHKG1	Km app	4	4	3	6	3
PHKG2	Km app	6	4	5	5	3
PIM1	Km app	6	5	7	6	7
PIM2	Km app	3	2	4	3	3
PIM3	Km app	17	14	6	7	3
PKN1 (PRK1)	Km app	7	5	12	11	6
PLK1	Km app	7	5	4	6	6
PLK2	Km app	5	5	1	2	1
PLK3	Km app	4	0	4	1	-4
PRKACA (PKA)	Km app	54	51	23	3	7
PRKCA (PKC alpha)	Km app	1	7	5	2	8
PRKCB1 (PKC beta I)	Km app	13	13	9	11	13
PRKCB2 (PKC beta II)	Km app	8	9	6	13	16
PRKCD (PKC delta)	Km app	8	9	4	5	2
PRKCE (PKC epsilon)	Km app	18	14	11	10	9
PRKCG (PKC gamma)	Km app	8	10	3	11	11
PRKCH (PKC eta)	Km app	7	17	15	15	15
PRKCI (PKC iota)	Km app	9	13	6	8	7
PRKCN (PKD3)	Km app	39	33	11	19	8
PRKCQ (PKC theta)	Km app	9	10	14	6	11
PRKCZ (PKC zeta)	Km app	7	13	1	2	6
PRKD1 (PKC mu)	Km app	80	54	10	36	13
PRKD2 (PKD2)	Km app	75	63	12	35	16
PRKG1	Km app	2	3	-1	0	2
PRKG2 (PKG2)	Km app	7	10	3	3	1
PRKX	Km app	9	8	2	5	4
PTK2 (FAK)	Km app	10	3	4	5	8

PTK2B (FAK2)	Km app	9	4	4	-1	-1
PTK6 (Brk)	Km app	88	75	46	1	7
RAF1 (cRAF) Y340D Y341D	100	18	19	6	0	5
RET A883F	Km app	63	59	35	-1	0
RET S891A	Km app	65	59	57	-2	6
RET V804E	Km app	16	22	0	-3	-3
RET V804L	Km app	8	11	14	2	3
RET Y791F	Km app	79	69	60	5	12
RET	Km app	76	62	52	5	13
ROCK1	Km app	3	4	4	6	4
ROCK2	Km app	-8	15	16	16	-10
ROS1	Km app	9	2	28	4	13
RPS6KA1 (RSK1)	Km app	9	3	9	8	12
RPS6KA2 (RSK3)	Km app	9	6	10	13	7
RPS6KA3 (RSK2)	Km app	6	1	2	1	3
RPS6KA4 (MSK2)	Km app	5	8	7	2	1
RPS6KA5 (MSK1)	Km app	10	1	5	6	7
RPS6KA6 (RSK4)	Km app	17	17	18	14	9
RPS6KB1 (p70S6K)	Km app	6	2	3	9	6
RPS6KB2 (p70S6Kb)	Km app	4	16	5	26	12
SBK1	Km app	3	5	-2	-1	-4
SGK (SGK1)	Km app	13	3	2	6	5
SGK2	Km app	6	8	2	1	1
SGKL (SGK3)	Km app	5	6	3	0	3
SNF1LK2	Km app	14	20	17	43	42
SRC N1	Km app	46	56	38	8	12
SRC	Km app	35	33	21	3	8
SRMS (Srm)	Km app	18	13	11	3	2
SRPK1	Km app	8	6	6	5	6
SRPK2	Km app	1	3	2	0	1
STK22B (TSSK2)	Km app	6	1	2	4	5
STK22D (TSSK1)	Km app	3	7	7	9	9
STK23 (MSSK1)	Km app	6	2	3	2	3
STK24 (MST3)	Km app	10	13	8	4	7
STK25 (YSK1)	Km app	10	15	9	10	10
STK3 (MST2)	Km app	9	11	9	3	8
STK4 (MST1)	Km app	4	9	4	5	2
SYK	Km app	7	11	4	3	1
TAOK2 (TAO1)	Km app	3	3	6	1	2
TBK1	Km app	4	7	0	-1	-1
TEK (TIE2) Y897S	Km app	2	5	5	3	-2
TEK (Tie2)	Km app	12	-1	16	1	6
TNK1	Km app	8	10	10	5	5
TXK	Km app	36	68	43	7	24
TYK2	Km app	7	9	2	3	-2
TYRO3 (RSE)	Km app	7	4	5	3	1
YES1	Km app	63	70	56	9	14
ZAP70	Km app	6	8	2	4	-1

Legend

< 40% Inhibition
40% - 80% Inhibition
≥ 80% Inhibition

Table S4. ThermoFisher Scientific Adapta Kinase Profiling

Kinase Tested	[ATP] Tested (μM)	3MBPP1 (1μM) % Inhibition (mean of 2 points)	Compound 1 (1μM) % Inhibition (mean of 2 points)	Compound 2 (1μM) % Inhibition (mean of 2 points)	Compound 13 (1μM) % Inhibition (mean of 2 points)	Compound 24 (1μM) % Inhibition (mean of 2 points)
CAMK1 (CaMK1)	10	-8	-11	9	0	-8
CDK4/cyclin D1	10	23	19	18	13	14
CDK4/cyclin D3	10	-11	-7	3	-10	-12
CDK6/cyclin D1	10	10	6	14	19	15
CDK7/cyclin H/MNAT1	Km app	-13	-3	-13	-9	-7
CDK9/cyclin T1	Km app	21	20	32	20	13
CHUK (IKK alpha)	Km app	6	6	6	4	4
DAPK1	Km app	27	15	31	25	26
GSG2 (Haspin)	Km app	25	30	87	41	32
IRAK1	Km app	-19	-10	-6	2	16
LRRK2 FL	Km app	1	-6	1	-6	-3
LRRK2 G2019S FL	Km app	3	4	6	1	0
LRRK2 G2019S	Km app	-7	-10	5	-3	-4
LRRK2 I2020T	Km app	-3	0	-1	-2	-5
LRRK2 R1441C	Km app	21	1	11	3	6
LRRK2	Km app	-1	3	9	0	-2
NUAK1 (ARK5)	Km app	10	-8	16	16	11
PI4K2A (PI4K2 alpha)	Km app	0	10	5	0	-5
PI4K2B (PI4K2 beta)	Km app	-7	-7	-12	-9	-9
PI4KA (PI4K alpha)	10	-11	-13	-8	-5	-15
PI4KB (PI4K beta)	Km app	18	18	15	12	17
PIK3C2A (PI3K-C2 alpha)	Km app	-2	-3	3	0	9
PIK3C2B (PI3K-C2 beta)	10	-1	-7	3	1	14
PIK3C2G (PI3K-C2 gamma)	Km app	0	3	16	11	9
PIK3C3 (hVPS34)	Km app	-2	-5	-2	-6	-2
PIK3CA E542K/PIK3R1 (p110 alpha E542K/p85 alpha)	10	7	0	7	8	8
PIK3CA E545K/PIK3R1 (p110 alpha E545K/p85 alpha)	Km app	-15	-5	2	-3	0
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	Km app	-4	-3	3	-10	2
PIK3CA/PIK3R3 (p110 alpha/p55 gamma)	Km app	-8	3	3	2	5
PIK3CB/PIK3R1 (p110 beta/p85 alpha)	Km app	-1	-6	-13	-8	6
PIK3CB/PIK3R2 (p110 beta/p85 beta)	Km app	5	4	0	4	-1
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	Km app	2	-2	15	9	8
PIK3CG (p110 gamma)	Km app	-4	7	5	10	7
PIP4K2A	10	-36	-7	-7	-22	-7
PIP5K1A	10	-6	8	18	20	23
PIP5K1B	10	-17	-8	3	-4	-4
PIP5K1C	10	0	18	37	10	21
SPHK1	Km app	-12	7	14	10	3
SPHK2	10	-8	0	-6	-1	6

Legend: same as Table S3.

Table S5. ThermoFisher Scientific LanthaScreen Kinase Profiling

Kinase Tested	3MBPP1 (1μM) % Displacement (mean of 2 points)	Compound 1 (1μM) % Displacement (mean of 2 points)	Compound 2 (1μM) % Displacement (mean of 2 points)	Compound 13 (1μM) % Displacement (mean of 2 points)	Compound 24 (1μM) % Displacement (mean of 2 points)
AAK1	1	0	8	3	4
ABL1 H396P	35	52	55	5	7
ABL1 M351T	35	52	56	3	2
ABL1 Q252H	33	55	49	-3	0
ACVR1 (ALK2) R206H	31	29	44	2	1
ACVR1 (ALK2)	20	20	36	0	2
ACVR2A	13	12	15	-4	-5
ACVR2B	34	45	42	-1	-2
ACVRL1 (ALK1)	32	26	41	6	-4
ADCK3	3	5	13	10	19
ALK C1156Y	8	-1	6	3	5
ALK F1174L	3	1	10	-3	1
ALK L1196M	2	4	8	1	1
ALK R1275Q	8	4	12	1	4
ALK T1151_L1152insT	2	3	3	1	1
AMPK (A1/B1/G2)	4	3	8	5	6
AMPK (A1/B1/G3)	-2	2	9	5	4
AMPK (A1/B2/G1)	-3	2	3	0	2
AMPK (A2/B2/G1)	-2	9	11	3	7
AMPK (A2/B2/G2)	0	-1	8	-8	8
ANKK1	0	3	8	13	4
AXL R499C	2	5	10	-3	1
BMPR1A (ALK3)	11	7	18	7	9
BMPR2	13	20	16	3	6
BRAF V599E	12	24	16	3	8
BRAF	12	26	14	-1	6
BRSK2	0	1	3	7	18
CAMK2G (CaMKII gamma)	-3	0	11	-4	7
CAMKK1 (CAMKKA)	-6	-6	-4	-7	0
CAMKK2 (CaMKK beta)	5	3	-1	-1	-6
CASK	5	10	15	14	3
CDC7/DBF4	13	10	23	11	32
CDK1/cyclin A2	11	-3	17	19	-3
CDK11 (Inactive)	7	19	42	1	19
CDK11/cyclin C	28	40	62	5	15
CDK13/cyclin K	7	8	15	14	6
CDK14 (PFTK1)/cyclin Y	-9	7	18	12	1
CDK16 (PCTK1)/cyclin Y	5	8	15	7	5
CDK2/cyclin A1	16	20	14	7	8
CDK2/cyclin E1	12	20	12	8	7
CDK2/cyclin O	19	43	20	13	12
CDK3/cyclin E1	2	7	8	6	2
CDK5 (Inactive)	-6	-1	0	-5	3
CDK8/cyclin C	9	13	54	3	7
CDK9 (Inactive)	19	16	28	15	19
CDK9/cyclin K	3	9	9	4	3
CLK4	19	40	49	8	14
DAPK2	7	11	3	1	4
DDR1	6	7	9	2	1

DDR2 N456S	80	61	73	34	26
DDR2 T654M	18	22	45	0	11
DDR2	4	7	9	2	2
DMPK	2	3	1	2	1
DYRK2	6	4	15	-7	14
EGFR (ErbB1) d746-750	83	96	92	12	34
EGFR (ErbB1) d747-749 A750P	68	97	78	20	15
EIF2AK2 (PKR)	-1	-8	9	5	18
EPHA3	51	61	32	-1	1
EPHA6	84	90	81	10	27
EPHA7	10	17	17	1	4
ERN1	3	-2	15	10	5
ERN2	-1	2	6	3	4
FGFR1 V561M	11	7	13	12	12
FGFR3 G697C	19	15	3	16	9
FGFR3 K650M	38	32	20	3	7
FLT3 ITD	6	0	31	-2	4
FYN A	26	41	26	-1	-7
GAK	70	84	80	16	3
GRK1	3	3	2	6	0
HUNK	91	92	89	12	10
ICK	3	4	7	4	8
IRAK3	69	79	92	18	42
KIT A829P	78	74	75	2	8
KIT D816H	71	69	58	9	2
KIT D816V	68	67	59	6	6
KIT D820E	8	6	5	1	2
KIT N822K	45	37	31	5	2
KIT T670E	3	0	3	7	2
KIT V559D T670I	5	6	5	4	3
KIT V654A	2	8	6	3	10
KIT Y823D	26	15	11	5	-9
LATS1	12	8	5	8	26
LATS2	-1	4	13	2	7
LIMK1	15	3	6	0	-1
LIMK2	1	3	3	3	4
MAP2K1 (MEK1) S218D S222D	15	32	17	4	2
MAP2K1 (MEK1)	19	26	17	-1	0
MAP2K2 (MEK2)	10	23	10	3	0
MAP2K3 (MEK3)	0	6	-1	-5	1
MAP2K4 (MEK4)	29	53	30	-3	4
MAP2K5 (MEK5)	27	43	32	5	8
MAP2K6 (MKK6) S207E T211E	20	36	23	17	15
MAP2K6 (MKK6)	5	15	5	10	4
MAP3K10 (MLK2)	5	4	8	2	0
MAP3K11 (MLK3)	2	-1	5	1	2
MAP3K14 (NIK)	-1	0	5	0	1
MAP3K2 (MEKK2)	14	42	16	1	-4
MAP3K3 (MEKK3)	49	63	22	0	1
MAP3K5 (ASK1)	1	-1	-4	2	0
MAP3K7/MAP3K7IP 1 (TAK1-TAB1)	16	17	21	10	6
MAP4K1 (HPK1)	6	13	5	0	2
MAP4K3 (GLK)	10	14	8	3	5
MAPK10 (JNK3)	33	43	39	0	0
MAPK15 (ERK7)	11	13	28	6	7
MAPK8 (JNK1)	4	20	19	6	4
MAPK9 (JNK2)	31	41	31	2	4

MASTL	2	-2	3	1	2
MERTK (cMER) A708S	10	1	13	5	1
MET D1228H	-4	-2	9	4	1
MKNK2 (MNK2)	-2	-1	8	-2	7
MLCK (MLCK2)	9	10	10	2	-2
MLK4	-3	2	6	7	5
MYLK (MLCK)	1	7	8	1	4
MYLK4	7	-3	9	12	19
MYO3A (MYO3 alpha)	6	9	6	3	7
MYO3B (MYO3 beta)	14	14	17	8	8
NEK8	0	3	4	3	-3
NLK	32	49	60	45	27
NUAK2	3	-2	12	13	7
OXSR1	5	2	16	4	7
PKMYT1	4	-1	5	2	12
PKN2 (PRK2)	-5	-2	5	7	5
PLK4	1	2	55	1	4
PRKACB (PRKAC beta)	86	85	71	9	11
PRKACG (PRKAC gamma)	81	79	80	-15	4
RAF1 (cRAF) Y340D Y341D	20	39	17	-1	5
RET G691S	79	77	64	5	12
RET M918T	63	58	57	-5	-1
RET V804M	9	6	16	6	2
RIPK2	82	90	92	46	41
RIPK3	82	75	70	-2	23
SIK1	6	13	13	3	4
SIK3	7	21	19	27	30
SLK	4	2	14	0	1
STK16 (PKL12)	5	7	-1	1	2
STK17A (DRAK1)	3	8	22	1	3
STK17B (DRAK2)	11	3	5	2	4
STK32B (YANK2)	40	45	18	6	6
STK32C (YANK3)	26	26	7	3	2
STK33	0	5	0	-2	-1
STK38 (NDR)	0	4	9	5	6
STK38L (NDR2)	2	3	11	2	7
STK39 (STLK3)	-3	-4	14	6	-2
TAOK1	9	15	22	8	3
TAOK3 (JIK)	4	4	7	-2	4
TEC	2	6	3	2	2
TEK (TIE2) R849W	-15	-9	6	-14	-4
TEK (TIE2) Y1108F	0	2	12	-10	2
TESK1	12	31	67	10	22
TESK2	32	49	36	-3	-3
TGFBR1 (ALK5)	84	86	80	-2	0
TGFBR2	41	60	54	7	17
TLK1	0	0	2	4	-1
TLK2	-2	-1	-1	3	-1
TNIK	51	72	43	34	30
TNK2 (ACK)	19	30	11	0	1
TTK	13	10	11	0	6
ULK1	-1	4	0	1	4
ULK2	5	4	2	4	5
ULK3	2	-11	5	4	-5
VRK2	2	13	17	10	7
WEE1	5	7	4	4	5

WNK1	-4	-4	-3	-10	-4
WNK2	0	-5	-9	-5	-4
WNK3	1	-2	3	1	-2
ZAK	1	2	5	-2	1

Legend

< 40% Displacement
40% - 80% Displacement
≥ 80% Displacement

References

(1) Bulawa, C. E.; Devit, M.; Elbaum, D. Modulation of Protein Trafficking. EP20080848219, 2010.

(2) Hanefeld, U.; Rees, C. W.; White, A. J. P.; Williams, D. J. One-Pot Synthesis of Tetrasubstituted Pyrazoles - Proof of Regiochemistry. *J. Am. Chem. Soc. Perkin Trans. 1* **1996**, *0* (13), 1545–1552.

(3) Schenone, S.; Radi, M.; Musumeci, F.; Brullo, C.; Botta, M. Biologically Driven Synthesis of Pyrazolo[3,4-D]pyrimidines As Protein Kinase Inhibitors: An Old Scaffold As a New Tool for Medicinal Chemistry and Chemical Biology Studies. *Chem. Rev.* **2014**, *114* (14), 7189–7238.

(4) Angiolini, M.; Buffa, L.; Menichincheri, M.; Motto, I.; Polucci, P.; Traquandi, G.; Zuccotto, F. Pyrrolo[2,3-D] Pyrimidine Derivatives, Process for Their Preparation and Their Use as Kinase Inhibitors. WO2014184069A1, 2014.

(5) Ley, S. V; Thomas, A. W. Modern Synthetic Methods for Copper-Mediated C(aryl)-O, C(aryl)-N, and C(aryl)-S Bond Formation. *Angew. Chemie Int. Ed.* **2003**, *42*, 5400–5449.

(6) Prim, D.; Campagne, J.; Andrioletti, B. Palladium-Catalysed Reactions of Aryl Halides with Soft, Non-Organometallic Nucleophiles. *Tetrahedron* **2002**, *58*, 2041–2075.

(7) Ma, D.; Cai, Q. N,N-Dimethyl Glycine-Promoted Ullmann Coupling Reaction of Phenols and Aryl Halides. *Org. Lett.* **2003**, *5* (21), 3799–3802.

(8) Fors, B. P.; Davis, N. R.; Buchwald, S. L. An Efficient Process for Pd-Catalyzed C-N Cross-Coupling Reactions of Aryl Iodides: Insight Into Controlling Factors. *J. Am. Chem. Soc.* **2009**, *131* (16), 5766–5768.

(9) Fors, B. P.; Watson, D. A.; Biscoe, M. R.; Buchwald, S. L. A Highly Active Catalyst for Pd-Catalyzed Amination Reactions: Cross-Coupling Reactions Using Aryl Mesylates and the Highly Selective Monoarylation of Primary Amines Using Aryl Chlorides. *J. Am. Chem. Soc.* **2008**, *130* (41), 13552–13554.

(10) Maiti, D.; Buchwald, S. L. Orthogonal Cu- and Pd-Based Catalyst Systems for the O- and N-Arylation of Aminophenols. *J. Am. Chem. Soc.* **2009**, *131* (47), 17423–17429.

(11) Itoh, T.; Mase, T. A General Palladium-Catalyzed Coupling of Aryl Bromides/Triflates and Thiols. *Org. Lett.* **2004**, *6* (24), 4587–4590.

(12) Kwong, F. Y.; Buchwald, S. L. A General, Efficient, and Inexpensive Catalyst System for the Coupling of Aryl Iodides and Thiols. *Org. Lett.* **2002**, *4* (20), 3517–3520.

(13) Apsel, B.; Blair, J. A.; Gonzalez, B.; Nazif, T. M.; Feldman, M. E.; Aizenstein, B.; Hoffman, R.; Williams, R. L.; Shokat, K. M.; Knight, Z. A. Targeted Polypharmacology: Discovery of Dual Inhibitors of Tyrosine and Phosphoinositide Kinases. *Nat. Chem. Biol.* **2008**, *4* (11), 691–699.

(14) Johnson, S. M.; Murphy, R. C.; Geiger, J. a; Derocher, A.; Zhang, Z.; Ojo, K.; Larson, E.; Perera, B. G. K.; Dale, E.; He, P.; Fox, A.; Mueller, N.; Merritt, E. a; Fan, E.; Reid, M.; Parsons, M.; Van Voorhis, W. C.; Maly, D. J. Development of Toxoplasma Gondii Calcium-Dependent Protein Kinase 1 (TgCDPK1) Inhibitors with Potent Anti-Toxoplasma Activity. *J. Med. Chem.* **2012**, *55* (5), 2416–2426.

(15) Lourido, S.; Zhang, C.; Lopez, M. S.; Tang, K.; Barks, J.; Wang, Q.; Wildman, S. A.; Shokat, K. M.; Sibley, L. D. Optimizing Small Molecule Inhibitors of Calcium-Dependent Protein Kinase 1 to Prevent Infection by Toxoplasma Gondii. *J. Med. Chem.* **2013**, *56* (7), 3068–3077.
(16) Zaretzki, J.; Matlock, M.; Swamidass, S. J. XenoSite: Accurately Predicting CYP-Mediated Sites of Metabolism with Neural Networks. *J. Chem. Inf. Model.* **2013**, *53*, 3373–3383.
(17) Boyle, N. M. O.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open Babel : An Open Chemical Toolbox. *J. Cheminform.* **2011**, *3*, 1–14.
(18) Eid, S.; Turk, S.; Volkamer, A.; Rippmann, F.; Fulle, S. KinMap : A Web-Based Tool for Interactive Navigation through Human Kinome Data. *BMC Bioinformatics* **2017**, 1–6.