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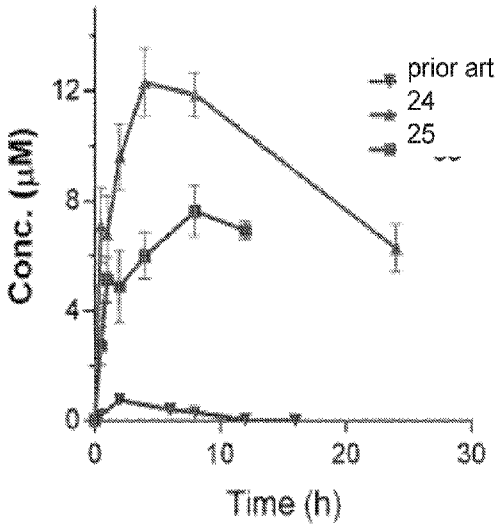
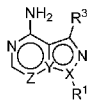
(54) Title: COMPOSITIONS AND METHODS FOR TREATING TOXOPLASMOSIS, CRYPTOSPORIDIOSIS AND OTHER APICOMPLEXAN PROTOZOAN RELATED DISEASES

Figure 1





(I)

(57) Abstract: The present disclosure is directed to compositions and methods for inhibiting either *Toxoplasma gondii (T. gondii)* calcium dependent protein kinases (*Tg*CDPKs) or *Cryptosporidium parvum (C. parvum)* and *Cryptosporidium hominus (C. hominus)* calcium dependent protein kinases (*Cp*CDPKs) using pyrazolopyrimidine and/or imidazo[1,5-α]pyrazine inhibitors, of the Formula (I), wherein the variables X, Y, Z, R^1, and R^3 are defined herein.

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COMPOSITIONS AND METHODS FOR TREATING TOXOPLASMOSIS, CRYPTOSPORIDIOSIS AND OTHER APICOMPLEXAN PROTOZOAN RELATED DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing dates of U.S. Provisional Patent Application Serial No. 62/107,746, filed January 26, 2015, and U.S. Provisional Patent Application Serial No. 62/131,539, filed March 11, 2015, each of which are hereby incorporated by reference in their entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant Nos. 5 R01 AI089441-02, P01 AI067921, R01 AI050506, R01 AI080625 and R01 GM086858, awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present disclosure is generally directed to compositions and methods for treating apicomplexan protozoan related disease, such as toxoplasmosis and cryptosporidiosis.

BACKGROUND OF THE INVENTION

The apicomplexan protozoans *Cryptosporidium parvum* and *Toxoplasma gondii* are ubiquitous parasites that infect humans and domesticated animals. Recently *C. hominus* was recognized to be distinct from *C. parvum*, and does not appear to infect domesticated animals, but rather appears limited to human infections. *C. parvum and C. hominus* are infectious parasites of major health concern in humans as they are a common cause of illness transmitted by water. *C. parvum* and *C. hominus* infections result in debilitating diarrhea that can be life-threatening in immunocompromised patients.

Recent studies have implicated *Cryptosporidium spp.* in around 15-20% of childhood diarrheal disease in the developing world. Currently, nitazoxanide is the only approved therapy for cryptosporidiosis but it is expensive and has not been shown to be effective in treating immunocompromised hosts. *T. gondii* may be the most common infectious eukaryotic parasite in humans, based on sero-surveys. Transmitted primarily through undercooked meat or accidental ingestion of cat feces, *T. gondii* infection presents major health concerns in immunocompromised hosts, where it causes toxoplasmic encephalitis, and in pregnancy, where it can result in severe birth defects or miscarriage.

Sulfadiazine and pyrimethamine are the current therapies for toxoplasmosis, but they can cause nephrotoxicity, rash, and additional complications in pregnancy. Thus, new

therapies for treating infections caused by both parasites are greatly needed. In *T. gondii*, calcium-regulated signaling is associated with a number of cellular functions such as secretion, gliding motility and host cell invasion. The proper control of intracellular calcium levels is important for host cell invasion and *T. gondii* use several mechanisms for the uptake and release of calcium. Furthermore, this organism contains specialized calcium-regulated signaling enzymes, including a unique family of calciumdependent protein kinases (CDPKs) which are present in plants, ciliates and green algae but not in animals. These kinases are believed to be mediators of secretion, invasion, and gliding motility. *T. gondii*, *C. parvum,* and *C. hominus* are highly related obligate intracellular parasites. While much less is known about the role of calcium signaling in *C.parvum* and *C. hominus*, it appears that many calcium-regulated signaling processes are conserved from *T. gondii* to *C. parvum*. *C. parvum* and *C. hominus* also possess CDPKs that are believed to play important roles in calcium-regulated processes and they are virtually identical in these two spp. Thus, inhibitors of *C. parvum* CDPKs would be expected to inhibit *C. hominus* CDPK.

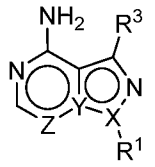
SUMMARY OF THE INVENTION

We have recognized that the roles that CDPKs play in calcium signaling in *T. gondii*, *C. parvum* and *C. hominus* make this family of kinases intriguing targets for the development of anti-parasitic agents. Previous studies have demonstrated that *Tg*CDPK1 plays an important role in *T. gondii* invasion of mammalian cells. Pharmacological agents that selectively inhibit the catalytic activity of *Tg*CDPK1 block parasitic invasion of human fibroblast cells. Furthermore, we have demonstrated that a unique sequence and structural variation in the ATP-binding cleft of *Tg*CDPK1 provides an opportunity to develop highly selective inhibitors of this kinase (the U.S. Patent Publication 2013/0018040). Specifically, *Tg*CDPK1 contains a glycine residue at the "gatekeeper" position which allows inhibitors to access a large hydrophobic pocket that is adjacent to the site of ATP binding (Hydrophobic Pocket II).

The present disclosure is generally directed to compositions and methods for the treatment of apicomplexan-related disorders, including but not limited to toxoplasmosis, caused by the infectious eukaryotic parasite *Toxoplasma gondii* (*T. gondii*), cryptosporidiosis, caused by the infectious eukaryotic parasites *Cryptosporidium parvum* (*C. parvum*) and *Cryptosporidium hominus* (*C. hominus*), malaria, caused by the eukaryotic parasites *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax (P. vivax)*, and *Plasmodium berghei* (*P. berghei*), neosporosis, caused by the eukaryotic parasite *Neospora caninum,*

sarcocystosis, caused by the eukaryotic parasite *Sarcocystis neuronae* and other species, besnoitiosis, (originally named globidiosis) caused by *Besnoitia besnoiti,* coccidiosis, caused by *Hammondia hammondi* and other *Hammondia spp.,* cystoisoporosis, caused by the eukaryotic parasitic species *Cystoisoporosis suis* and other species, babesiosis, caused by the eukaryotic parasites *Babesia microtii* and other species, and theileriosis, caused by the eukaryotic parasites *Theileria equi* and other species. Because all known human kinases possess larger residues (threonine, valine or a larger amino acid) at the gatekeeper position, the inventors have recognized that it is possible to gain selectivity by developing inhibitors that optimize interactions with the enlarged ATP-binding pocket of *Tg*CDPK1. In one embodiment, the present disclosure is directed to compositions and methods for inhibiting apicomplexan calcium dependent protein kinases, including but not limited to *T. gondii* calcium dependent protein kinases (*Tg*CDPKs), *C. parvum* and *C. hominus* calcium dependent protein kinases (*Cp*CDPKs), or *P. falciparum* and *P. berghei* calcium dependent protein kinase 4 (*Pf*CDPKs) using pyrazolopyrimidine inhibitors, or in another embodiment, imidazo[1,5-*a*]pyrazine inhibitors, both classes of compounds designed to be inactive against mammalian kinases.

In one aspect, the present disclosure provides compounds of the formula (I),



(I)

or a pharmaceutically acceptable salt thereof, wherein

X, Y, and Z are defined by either: (i) X is N, Y is C, and Z is N; or (ii) X is C, Y is N, and Z is C(H);

R^1 is C_{2-6} alkyl, C_{1-6} haloalkyl, -C_{1-6} alkyl-R^{12}, C_{3-8} cycloalkyl, heterocyclyl, heteroaryl, or aryl, wherein

the alkyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl groups are each optionally substituted with one or two R^{11} groups;

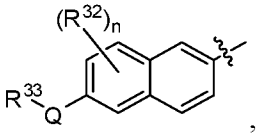
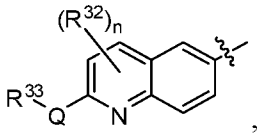
each R^{11} is independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, -NR_2, -C(O)R, -C(O)OR, -$C(O)NR_2$, -$S(O)_2NR_2$, or –$S(O)_2R$;

and

R^{12} is -OR, -SR, -NR_2, -C(O)R, -C(O)OR, -$C(O)NR_2$, -$S(O)_2R$, -OC(O)R, -OC(O)OR, -$OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, -$N(R)C(O)NR_2$, aryl, heteroaryl, C_{3-8} cycloalkyl, or heterocyclyl, wherein R^{12} is optionally substituted by one,

two, or three groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydoxyalkyl, -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$;

R^3 is one of the formulas,


, or

,

wherein

n is 0, 1, or 2;

Q is –O-, -S-, or $–N(R^Q)-$, wherein R^Q is hydrogen or C_{1-6} alkyl; and

R^{33} is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl,

each R^{32} is independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl;

and

each R is independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, heterocyclyl, aryl, arylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6} alkyl wherein the alkyl, aryl, arylalkyl, heteroaryl, and heteroarylalkyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^0$, $-SR^0$, $-N(R^0)_2$, $-C(O)R^0$, $-C(O)OR^0$, $-C(O)N(R^0)_2$, $-S(O)_2R^0$, $-OC(O)R^0$, $-OC(O)OR^0$, $-OC(O)N(R^0)_2$, $-N(R^0)C(O)R^0$, $-N(R^0)C(O)OR^0$, or $-N(R^0)C(O)N(R^0)_2$, wherein each R^0 is independently hydrogen or C_{1-6} alkyl.

In certain emdbodiments, the compound of the formula (I) is not:

1-(6-ethoxynaphthalen-2-yl)-3-isopropylimidazo[1,5-a]pyrazin-8-amine;

3-(6-isopropoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-propoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-methoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-methoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-isopropoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(piperidin-4-ylmethyl)-3-(6-propoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(benzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-butoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(allyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(2-chlorobenzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(3-chlorobenzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(4-chlorobenzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(benzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(allyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-butoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-isobutoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-isobutoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(2-chlorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(3-chlorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(2,5-dimethylbenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-(2-methylbenzyloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-(2-methyl-5-(trifluoromethyl)benzyloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(3-chloro-4-(2,2,2-trifluoroethyl)benzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(3-chloro-5-fluorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-(1-phenylethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(4-tert-butylbenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
1-isopropyl-3-(6-(pyridin-4-ylmethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(4-chlorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
6-(4-amino-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N,N-dimethylquinolin-2-amine;
3-tert-butyl-1-(6-ethoxynaphthalen-2-yl)imidazo[1,5-a]pyrazin-8-amine;
3-tert-butyl-1-(6-methoxynaphthalen-2-yl)imidazo[1,5-a]pyrazin-8-amine;
3-(6-ethoxynaphthalen-2-yl)-1-(1-ethylpiperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-ethoxynaphthalen-2-yl)-1-(piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine; or
3-(6-ethoxynaphthalen-2-yl)-1-(1-methylpiperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine.

In certain other emdbodiments, the compound of the formula (I) is not any one of compounds disclosed in International Patent Publication WO 2011/0094628 and/or the U.S. Patent Publication 2013/0018040, both incorporated herein by reference in their entirety.

In another aspect, the present disclosure provides methods for treating an apicomplexan protozoan related disease comprising providing to a patient in need of such treatment a therapeutically effective amount of either (i) a compound of the disclosure or (ii) a pharmaceutical composition comprising a compound of the disclosure and a pharmaceutically acceptable excipient, carrier, or diluent.

In another aspect, the present disclosure provides methods for treating toxoplasmosis, cryptosporidiosis, coccidiosis, and malaria comprising providing to a patient in need of such treatment a therapeutically effective amount of either (i) a compound of the disclosure or (ii) a pharmaceutical composition comprising a compound of the disclosure and a pharmaceutically acceptable excipient, carrier, or diluent.

In another aspect, the present disclosure provides methods for treating malaria comprising providing to a patient in need of such treatment a therapeutically effective amount of either (i) a compound of the disclosure or (ii) a pharmaceutical composition comprising a compound of the disclosure and a pharmaceutically acceptable excipient, carrier, or diluent.

In one aspect, the present disclosure provides methods for treating neosporosis, caused by the eukaryotic parasite *Neospora caninum,* sarcocystosis, caused by the eukaryotic parasite *Sarcocystis neuronae* and other species, besnoitiosis, caused by *Besnoitia besnoiti,* coccidiosis, caused by *Hammondia hammondi* and other *Hammondia spp.,* cystoisoporosis, caused by the eukaryotic parasitic species *Cystoisoporosis suis* and other species, babesiosis,

caused by the eukaryotic parasites *Babesia microtii* and other species, and theileriosis, caused by the eukaryotic parasites *Theileria equi* and other species comprising providing to a patient in need of such treatment a therapeutically effective amount of either (i) a compound of the disclosure or (ii) a pharmaceutical composition comprising a compound of the disclosure and a pharmaceutically acceptable excipient, carrier, or diluent. In some embodiments of this aspect, the compound is 3-(6-ethoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine or 1-(4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows **c**omparison of oral PK for compounds of Example **24** and **25** in mice. Both compounds were dosed at 10 mg/kg, PO.

Figure 2 shows multiple dose PK studies with compounds of Example **24** and **25**. The dosing regimens were simulated based on the disposition data following single oral doses to mice (lines) and the observed plasma concentrations (dots) compared to the simulation. (*top left panel*) **24** was dosed at 20 mg/kg on day 1 and then 5 mg/kg every 24 hours for 5 doses. (*bottom left panel*) **24** was dosed at 50 mg/kg every 48 hours for 5 doses. (*top right panel*) **25** was dosed at 20 mg/kg on day 1 and then 5 mg/kg every 24 hours for 5 doses. (*bottom right panel*) **25** was dosed at 20 mg/kg on day 1 and then 10 mg/kg every 48 hours for 5 doses. Each dot is a mean of 3 mice with standard deviation.

Figure 3 shows plasma concentration time curves for (**A**) compound of Example **24** (left) and of Example **25** (right) following IV and PO dosing to rats; and (**B**) compound of Example **24** following IV and PO dosing to dogs (left) and monkeys (right).

Figure 4 shows EC_{50} curves of inhibitors **24** (left) and **25** (right) for *T. gondii* over-expressing either wild type (wt) *Tg*CDPK1 (black circles) or a drug resistant G128M *Tg*CDPK1 mutant (gray squares). All experiments were performed in triplicate.

Figure 5 shows exposure to compound of Example **24** in multiple dose toxicity studies. The concentration profiles of **24** following 30 mg/kg and 100 mg/kg daily doses were simulated based on the single dose 10 mg/kg data. The mean observed concentrations (n=3) with standard deviation are plotted as triangles for 30 mg/kg dosing and circles for the 100 mg/kg dosing) with the simulation.

Figure 6 shows activity of compound of Example **24** against acute toxoplasmosis. Efficacy was evaluated by measurement of T. gondii in peritoneal fluid (top), spleen

(middle), and brain (bottom) in two experiments. Mice were treated daily for 5 days, beginning 2 days after IP infection with *T.gondii*. Mice were analyzed one day after the last dose. Peritoneal fluid was analyzed by fluorescent microscopy and spleen and brain tissue were analyzed by quantitative real-time PCR. Groups consisted of 4 mice. Bars represent the mean and the standard error of the mean. PEG = polyethylene glycol; mpk = mg/kg.

DETAILED DESCRIPTION OF THE INVENTION

Before the disclosed methods are described, it is to be understood that the aspects described herein are not limited to specific embodiments, or compositions, and as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and, unless specifically defined herein, is not intended to be limiting.

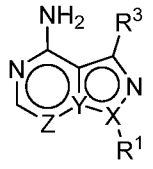
Throughout this specification, unless the context requires otherwise, the word "comprise" and "include" and variations (e.g., "comprises," "comprising," "includes," "including") will be understood to imply the inclusion of a stated component, feature, element, or step or group of components, features, elements or steps but not the exclusion of any other integer or step or group of integers or steps.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

The term "pharmaceutical composition" is used in its widest sense, encompassing all pharmaceutically applicable compositions containing at least one active substance, and optional carriers, adjuvants, constituents etc. The term "pharmaceutical composition" also encompasses a composition comprising the active substance in the form of derivative or pro-drug, such as pharmaceutically acceptable salts and esters. The manufacture of pharmaceutical compositions for different routes of administration falls within the capabilities of a person skilled in medicinal chemistry.

In view of the present disclosure, the methods described herein can be configured by the person of ordinary skill in the art to meet the desired need. In general, the disclosed methods provide improved compounds useful in the treatment of apicomplexan-related diseases.

In one aspect, the present disclosure provides compounds of the formula (I),



(I)

or a pharmaceutically acceptable salt thereof, wherein

X, Y, and Z are defined by either: (i) X is N, Y is C, and Z is N; or (ii) X is C, Y is N, and Z is C(H);

R^1 is C_{2-6} alkyl, C_{1-6} haloalkyl, -C_{1-6} alkyl-R^{12}, C_{3-8} cycloalkyl, heterocyclyl, heteroaryl, or aryl, wherein

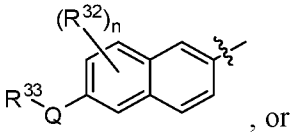
the alkyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl groups are each optionally substituted with one or two R^{11} groups;

each R^{11} is independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2NR_2$, or $-S(O)_2R$;

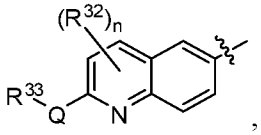
and

R^{12} is -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, $-N(R)C(O)NR_2$, aryl, heteroaryl, C_{3-8} cycloalkyl, or heterocyclyl, wherein R^{12} is optionally substituted by one, two, or three groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydoxyalkyl, -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$;

R^3 is one of the formulas,



, or



,

(R^3-a) **(R^3-b)**

wherein

n is 0, 1, or 2;

Q is –O-, -S-, or $-N(R^Q)-$, wherein R^Q is hydrogen or C_{1-6} alkyl; and

R^{33} is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$,

$-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl,

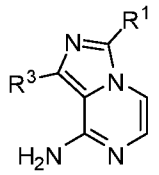
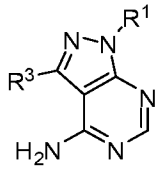
each R^{32} is independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl;

and

each R is independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, heterocyclyl, aryl, arylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6} alkyl wherein the alkyl, aryl, arylalkyl, heteroaryl, and heteroarylalkyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^0$, $-SR^0$, $-N(R^0)_2$, $-C(O)R^0$, $-C(O)OR^0$, $-C(O)N(R^0)_2$, $-S(O)_2R^0$, $-OC(O)R^0$, $-OC(O)OR^0$, $-OC(O)N(R^0)_2$, $-N(R^0)C(O)R^0$, $-N(R^0)C(O)OR^0$, or $-N(R^0)C(O)N(R^0)_2$, wherein each R^0 is independently hydrogen or C_{1-6} alkyl.

The disclosure further comprises subgenera of formula (I) in which the substituents are selected as any and all combinations of one or more of structural formula (I), n, Q, R^1, R^3, R^{32}, and R^{33} as defined herein, including without limitation, the following:

Structural Formula I is one of formulae (Ia) – (Ib):

	
(Ia)	**(Ib)**

R^1 is selected from one of the following groups (1a) – (1ii):

(1a) R^1 is C_{2-4} alkyl, $-C_{1-6}$ alkyl-R^{12}, C_{3-8} cycloalkyl, heterocyclyl, heteroaryl, or aryl, wherein the cycloalkyl, heterocyclyl, heteroaryl, and aryl groups are each optionally substituted with one or two R^{11} groups.

(1b) R^1 is C_{3-8} cycloalkyl, heterocyclyl, heteroaryl, or aryl, wherein the cycloalkyl, heterocyclyl, heteroaryl, and aryl groups are each optionally substituted with one or two R^{11} groups.

(1c) R^1 is C_{3-8} cycloalkyl or a monocyclic heterocyclyl optionally substituted with one R^{11} group.

(1d) R^1 is C_{3-8} cycloalkyl.

(1e) R^1 is monocyclic heterocyclyl optionally substituted with one R^{11} group.

(1f) R^1 is piperidinyl or tetrahydropyranyl, each optionally substituted with one R^{11} group.

(1g) R^1 is phenyl optionally substituted with one or two R^{11} groups.

(1h) R^1 is C_{2-6} alkyl optionally substituted with one or two R^{11} groups.

(1i) R^1 is C_{2-4} alkyl optionally substituted with one or two R^{11} groups.

(1j) R^1 is isopropyl or t-butyl.

(1k) R^1 is t-butyl.

(1l) R^1 is isopropyl.

(1m) R^1 is C_{2-6} alkyl or -C_{1-6} alkyl-R^{12}.

(1n) R^1 is -C_{1-6} alkyl-R^{12}.

(1o) R^1 is -C_{1-4} alkyl-R^{12}.

(1p) R^1 is –CH_2-R^{12}.

(1q) Any one of groups (1m) – (1p), wherein R^{12} is -OR, -C(O)OR, -C(O)NR_2, phenyl, monocyclic heteroaryl, C_{3-8} cycloalkyl, or monocyclic heterocyclyl, wherein the phenyl, heteroaryl, C_{3-8} cycloalkyl, and heterocyclyl groups are each optionally substituted by one, two, or three groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, -NR_2, -C(O)R, -C(O)OR,-C(O)NR_2, -$S(O)_2$R, -OC(O)R, -OC(O)OR, -OC(O)NR_2, -N(R)C(O)R, -N(R)C(O)OR, or -N(R)C(O)NR_2.

(1r) Any one of groups (1m) – (1p), R^{12} is -OR.

(1s) Any one of groups (1m) – (1p), R^{12} is -OH.

(1t) Any one of groups (1m) – (1p), wherein R^{12} is phenyl, monocyclic heteroaryl, C_{3-8} cycloalkyl, or monocyclic heterocyclyl, wherein the phenyl, heteroaryl, C_{3-8} cycloalkyl, and heterocyclyl groups are each optionally substituted by one or two groups that are each independently halogen, C_{1-6} alkyl, -C(O)R, -C(O)OR, -C(O)NR_2, -$S(O)_2$R, -OC(O)R, -OC(O)OR, -OC(O)NR_2, -N(R)C(O)R, -N(R)C(O)OR, or -N(R)C(O)NR_2.

(1u) Any one of groups (1m) – (1p), R^{12} is phenyl or monocyclic heterocyclyl, each optionally substituted by one, two, or three groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, -NR_2, -C(O)R, -C(O)OR, -C(O)NR_2, -$S(O)_2$R, -OC(O)R, -OC(O)OR, -OC(O)NR_2, -N(R)C(O)R, -N(R)C(O)OR, or -N(R)C(O)NR_2.

(1v) Any one of groups (1m) – (1p), R^{12} is monocyclic heterocyclyl optionally substituted by one, two, or three groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR,$-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$.

(1w) Any one of groups (1m) – (1p), wherein R^{12} is monocyclic heterocyclyl optionally substituted by one or two groups that are each independently halogen, C_{1-6} alkyl, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$.

(1x) Any one of groups (1m) – (1p), R^{12} is piperidinyl or tetrahydropyranyl, each optionally substituted by one or two groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$.

(1y) Any one of groups (1m) – (1p), wherein R^{12} is piperidinyl optionally substituted by one or two groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, $-NR_2$, -C(O)R, -C(O)OR,$-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$.

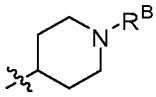
(1z) Any one of groups (1m) – (1p), wherein R^{12} is piperidinyl optionally substituted by one or two groups that are each independently C_{1-6} alkyl, $-C(O)R^A$, $-C(O)OR^A$, $-C(O)N(R^A)_2$, $-S(O)_2R^A$, $-OC(O)R^A$, $-OC(O)OR^A$, $-OC(O)N(R^A)_2$, $-N(R^A)C(O)R^A$, $-N(R^A)C(O)OR^A$, or $-N(R^A)C(O)N(R^A)_2$, wherein each R^A is independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, heterocyclyl, aryl, arylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6} alkyl.

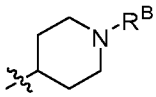
(1aa) Any one of groups (1m) – (1p), wherein R^{12} is piperidinyl optionally substituted by one or two groups that are each independently C_{1-6} alkyl, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$.

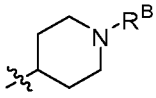
(1bb) Any one of groups (1m) – (1p), wherein R^{12} is piperidinyl optionally substituted by one or two groups that are each independently C_{1-6} alkyl, $-C(O)R^A$, $-C(O)OR^A$, $-C(O)N(R^A)_2$, $-S(O)_2R^A$, $-OC(O)R^A$, $-OC(O)OR^A$, $-OC(O)N(R^A)_2$, $-N(R^A)C(O)R^A$, $-N(R^A)C(O)OR^A$, or $-N(R^A)C(O)N(R^A)_2$, wherein each R^A is independently hydrogen or C_{1-6} alkyl.

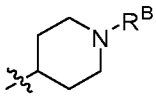
(1cc) Any one of groups (1m) – (1p), wherein R^{12} is piperidinyl optionally substituted by one or two groups that are each independently C_{1-6} alkyl, -C(O)R, -C(O)OR, $-C(O)NR_2$, or $-S(O)_2R$.

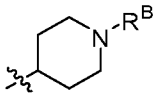
(1dd) Any one of groups (1m) – (1p), wherein R^{12} is piperidinyl optionally substituted by one or two groups that are each independently C_{1-6} alkyl, $-C(O)R^A$, or $-S(O)_2R^A$, wherein each R^A is independently hydrogen or C_{1-6} alkyl.

(1ee) Any one of groups (1m) – (1p), wherein R^{12} is  , wherein R^B is hydrogen, C_{1-6} alkyl, -C(O)R, -C(O)OR, $-C(O)NR_2$, $-S(O)_2R$, -OC(O)R, -OC(O)OR, $-OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or $-N(R)C(O)NR_2$.

(1ff) Any one of groups (1m) – (1p), wherein R^{12} is  , wherein R^B is hydrogen, C_{1-6} alkyl, $-C(O)R^A$, $-C(O)OR^A$, $-C(O)N(R^A)_2$, $-S(O)_2R^A$, $-OC(O)R^A$, $-OC(O)OR^A$, $-OC(O)N(R^A)_2$, $-N(R^A)C(O)R^A$, $-N(R^A)C(O)OR^A$, or $-N(R^A)C(O)N(R^A)_2$, wherein each R^A is independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, heterocyclyl, aryl, arylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6} alkyl.

(1gg) Any one of groups (1m) – (1p), wherein R^{12} is  , wherein R^B is hydrogen, C_{1-6} alkyl, $-C(O)R^A$, $-C(O)OR^A$, $-C(O)N(R^A)_2$, $-S(O)_2R^A$, $-OC(O)R^A$, $-OC(O)OR^A$, $-OC(O)N(R^A)_2$, $-N(R^A)C(O)R^A$, $-N(R^A)C(O)OR^A$, or $-N(R^A)C(O)N(R^A)_2$, wherein each R^A is independently hydrogen or C_{1-6} alkyl.

(1hh) Any one of groups (1m) – (1p), wherein R^{12} is  , wherein R^B is hydrogen, C_{1-6} alkyl, -C(O)R, -C(O)OR, $-C(O)NR_2$, or $-S(O)_2R$.

(1ii) Any one of groups (1m) – (1p), wherein R^{12} is  , wherein R^B is hydrogen, C_{1-6} alkyl, $-C(O)R^A$, or $-S(O)_2R^A$, wherein each R^A is independently hydrogen or C_{1-6} alkyl.

<u>R^3 is selected from one of the following groups (2a) – (2b):</u>

(2a) R^3 is group (**R^3-a**).

(2b) R^3 is group (**R^3-b**).

<u>Q is selected from one of the following groups (3a) – (3e):</u>

(3a) Q is –O- or $-N(R^Q)$-.

(3b) Q is –O- or –N(H)-.

(3c) Q is –O-.

(3d) Q is $-N(R^Q)-$.

(3e) Q is –N(H)-.

n and R^{32} are selected from one of the following groups (4a) – (4x):

(4a) n is 0.

(4b) n is 0 or 1 and R^{32} is as defined for formula (I).

(4c) n is 0 or 1 and R^{32} is halogen, cyano, nitro, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4d) n is 0 or 1 and R^{32} is halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4e) n is 0 or 1 and each R^{32} is $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4f) n is 0 or 1 and R^{32} is $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4g) n is 0 or 1 and R^{32} is $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, or $-S(O)_2R^{34}$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4h) n is as defined for formula (I) and each R^{32} is independently halogen, cyano, nitro, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4i) n is as defined for formula (I) and each R^{32} is independently halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4j) n is as defined for formula (I) and each R^{32} is independently $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4k) n is as defined for formula (I) and each R^{32} is independently $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4l) n is as defined for formula (I) and each R^{32} is independently $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, or $-S(O)_2R^{34}$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4m) n is 1 or 2 and each R^{32} is as defined for formula (I).

(4n) n is 1 or 2 and each R^{32} is independently halogen, cyano, nitro, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4o) n is 1 or 2 and each R^{32} is independently halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4p) n is 1 or 2 and each R^{32} is independently $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4q) n is 1 or 2 and each R^{32} is independently $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4r) n is 1 or 2 and each R^{32} is independently $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, or $-S(O)_2R^{34}$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4s) n is 1 and R^{32} is as defined for formula (I).

(4t) n is 1 and R^{32} is halogen, cyano, nitro, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4u) n is 1 and R^{32} is halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(4v) n is 1 and R^{32} is $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4w) n is 1 and R^{32} is $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

(4x) n is 1 and R^{32} is $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, or $-S(O)_2R^{34}$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl.

R^{33} is selected from one of the following groups (5a) – (5t):

(5a) R^{33} is C_{1-6} alkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5b) R^{33} is C_{1-6} alkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are each substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5c) R^{33} is C_{1-6} alkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are optionally substituted with one or two groups that are each independently halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(5d) R^{33} is C_{1-6} alkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are each substituted with one or two groups that are each independently halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(5e) R^{33} is C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the, cycloalkyl, and heterocyclyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5f) R^{33} is C_{3-8} cycloalkyl or (C_{3-8} cycloalkyl)C_{1-6} alkyl, wherein the cycloalkyl is opionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5g) R^{33} is C_{3-8} cycloalkyl, optionally substituted with one or two groups that are each independently halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(5h) R^{33} is C_{3-6} cycloalkyl, are each substituted with one or two groups that are each independently halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl.

(5i) R^{33} is C_{3-8} cycloalkyl, optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5j) R^{33} is C_{1-6} alkyl or C_{3-8} cycloalkyl, wherein the alkyl and cycloalkyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5k) R^{33} is C_{1-6} alkyl or C_{3-8} cycloalkyl, wherein the alkyl and cycloalkyl are optionally substituted with one or two groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5l) R^{33} is C_{1-6} alkyl or C_{3-8} cycloalkyl, wherein the alkyl and cycloalkyl are optionally substituted with one or two groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, or $-C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5m) R^{33} is C_{1-6} alkyl or C_{3-8} cycloalkyl, wherein the alkyl and cycloalkyl are optionally substituted with one or two groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, or $-C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

(5n) R^{33} is C_{1-6} alkyl or C_{3-8} cycloalkyl.

(5o) R^{33} is C_{1-4} alkyl or C_{3-6} cycloalkyl.

(5p) R^{33} is C_{1-4} alkyl, cyclopropyl, or cyclobutyl.

(5q) R^{33} is methyl, ethyl, propyl, isoproptl, butyl, isobutyl, tert-butyl, cyclopropyl, or cyclobutyl.

Particular embodiments of this aspect of the invention include compounds of any one of the formulae (I), (Ia), and (Ib), each as defined in each of the following rows, wherein each entry is a group number as defined above (e.g., (3c) refers to Q is –O-), a dash "-" indicates that the variable is as defined for formula (I) or defined according to any one of the applicable variable definitions **(1a)-(5q)** [*e.g.*, when R^1 is a dash, it can be either as defined for Formula (I) or any one of definitions **(1a)-(1ii)**]; and an "x" indicates that the variable is not applicable to the particular embodiment:

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-1	1h	2b	5a	4c	3a
(1)-2	1n	2b	5a	4c	3a
(1)-3	1p	2b	5a	4c	3a
(1)-4	1aa	2b	5a	4c	3a
(1)-5	1h	2b	5e	4c	3a

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-6	1n	2b	5e	4c	3a
(1)-7	1p	2b	5e	4c	3a
(1)-8	1aa	2b	5e	4c	3a
(1)-9	1h	2b	5i	4c	3a
(1)-10	1n	2b	5i	4c	3a

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-11	1p	2b	5i	4c	3a
(1)-12	1aa	2b	5i	4c	3a
(1)-13	1h	2b	5q	4c	3a
(1)-14	1n	2b	5q	4c	3a
(1)-15	1p	2b	5q	4c	3a
(1)-16	1aa	2b	5q	4c	3a
(1)-17	1h	2a	5a	4c	3a
(1)-18	1n	2a	5a	4c	3a
(1)-19	1p	2a	5a	4c	3a
(1)-20	1aa	2a	5a	4c	3a
(1)-21	1h	2a	5e	4c	3a
(1)-22	1n	2a	5e	4c	3a
(1)-23	1p	2a	5e	4c	3a
(1)-24	1aa	2a	5e	4c	3a
(1)-25	1h	2a	5i	4c	3a
(1)-26	1n	2a	5i	4c	3a
(1)-27	1p	2a	5i	4c	3a
(1)-28	1aa	2a	5i	4c	3a
(1)-29	1h	2a	5q	4c	3a
(1)-30	1n	2a	5q	4c	3a
(1)-31	1p	2a	5q	4c	3a
(1)-32	1aa	2a	5q	4c	3a
(1)-33	1h	2b	5a	4n	3a
(1)-34	1n	2b	5a	4n	3a
(1)-35	1p	2b	5a	4n	3a
(1)-36	1aa	2b	5a	4n	3a
(1)-37	1h	2b	5e	4n	3a
(1)-38	1n	2b	5e	4n	3a
(1)-39	1p	2b	5e	4n	3a
(1)-40	1aa	2b	5e	4n	3a

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-41	1h	2b	5i	4n	3a
(1)-42	1n	2b	5i	4n	3a
(1)-43	1p	2b	5i	4n	3a
(1)-44	1aa	2b	5i	4n	3a
(1)-45	1h	2b	5q	4n	3a
(1)-46	1n	2b	5q	4n	3a
(1)-47	1p	2b	5q	4n	3a
(1)-48	1aa	2b	5q	4n	3a
(1)-49	1h	2a	5a	4n	3a
(1)-50	1n	2a	5a	4n	3a
(1)-51	1p	2a	5a	4n	3a
(1)-52	1aa	2a	5a	4n	3a
(1)-53	1h	2a	5e	4n	3a
(1)-54	1n	2a	5e	4n	3a
(1)-55	1p	2a	5e	4n	3a
(1)-56	1aa	2a	5e	4n	3a
(1)-57	1h	2a	5i	4n	3a
(1)-58	1n	2a	5i	4n	3a
(1)-59	1p	2a	5i	4n	3a
(1)-60	1aa	2a	5i	4n	3a
(1)-61	1h	2a	5q	4n	3a
(1)-62	1n	2a	5q	4n	3a
(1)-63	1p	2a	5q	4n	3a
(1)-64	1aa	2a	5q	4n	3a
(1)-65	1h	2b	5a	4c	3c
(1)-66	1n	2b	5a	4c	3c
(1)-67	1p	2b	5a	4c	3c
(1)-68	1aa	2b	5a	4c	3c
(1)-69	1h	2b	5e	4c	3c
(1)-70	1n	2b	5e	4c	3c

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-71	1p	2b	5e	4c	3c
(1)-72	1aa	2b	5e	4c	3c
(1)-73	1h	2b	5i	4c	3c
(1)-74	1n	2b	5i	4c	3c
(1)-75	1p	2b	5i	4c	3c
(1)-76	1aa	2b	5i	4c	3c
(1)-77	1h	2b	5q	4c	3c
(1)-78	1n	2b	5q	4c	3c
(1)-79	1p	2b	5q	4c	3c
(1)-80	1aa	2b	5q	4c	3c
(1)-81	1h	2a	5a	4c	3c
(1)-82	1n	2a	5a	4c	3c
(1)-83	1p	2a	5a	4c	3c
(1)-84	1aa	2a	5a	4c	3c
(1)-85	1h	2a	5e	4c	3c
(1)-86	1n	2a	5e	4c	3c
(1)-87	1p	2a	5e	4c	3c
(1)-88	1aa	2a	5e	4c	3c
(1)-89	1h	2a	5i	4c	3c
(1)-90	1n	2a	5i	4c	3c
(1)-91	1p	2a	5i	4c	3c
(1)-92	1aa	2a	5i	4c	3c
(1)-93	1h	2a	5q	4c	3c
(1)-94	1n	2a	5q	4c	3c
(1)-95	1p	2a	5q	4c	3c
(1)-96	1aa	2a	5q	4c	3c
(1)-97	1h	2b	5a	4n	3c
(1)-98	1n	2b	5a	4n	3c
(1)-99	1p	2b	5a	4n	3c
(1)-100	1aa	2b	5a	4n	3c

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-101	1h	2b	5e	4n	3c
(1)-102	1n	2b	5e	4n	3c
(1)-103	1p	2b	5e	4n	3c
(1)-104	1aa	2b	5e	4n	3c
(1)-105	1h	2b	5i	4n	3c
(1)-106	1n	2b	5i	4n	3c
(1)-107	1p	2b	5i	4n	3c
(1)-108	1aa	2b	5i	4n	3c
(1)-109	1h	2b	5q	4n	3c
(1)-110	1n	2b	5q	4n	3c
(1)-111	1p	2b	5q	4n	3c
(1)-112	1aa	2b	5q	4n	3c
(1)-113	1h	2a	5a	4n	3c
(1)-114	1n	2a	5a	4n	3c
(1)-115	1p	2a	5a	4n	3c
(1)-116	1aa	2a	5a	4n	3c
(1)-117	1h	2a	5e	4n	3c
(1)-118	1n	2a	5e	4n	3c
(1)-119	1p	2a	5e	4n	3c
(1)-120	1aa	2a	5e	4n	3c
(1)-121	1h	2a	5i	4n	3c
(1)-122	1n	2a	5i	4n	3c
(1)-123	1p	2a	5i	4n	3c
(1)-124	1aa	2a	5i	4n	3c
(1)-125	1h	2a	5q	4n	3c
(1)-126	1n	2a	5q	4n	3c
(1)-127	1p	2a	5q	4n	3c
(1)-128	1aa	2a	5q	4n	3c
(1)-129	1h	2b	-	-	-
(1)-130	1n	2b	-	-	-

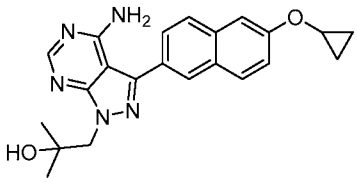
	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-131	1p	2b	-	-	-
(1)-132	1aa	2b	-	-	-
(1)-133	1h	2a	-	-	-
(1)-134	1n	2a	-	-	-
(1)-135	1p	2a	-	-	-
(1)-136	1aa	2a	-	-	-
(1)-137	1h	2b	-	-	3c
(1)-138	1n	2b	-	-	3c
(1)-139	1p	2b	-	-	3c
(1)-140	1aa	2b	-	-	3c
(1)-141	1h	2a	-	-	3c
(1)-142	1n	2a	-	-	3c
(1)-143	1p	2a	-	-	3c
(1)-144	1aa	2a	-	-	3c
(1)-145	1h	2b	-	-	3a
(1)-146	1n	2b	-	-	3a
(1)-147	1p	2b	-	-	3a
(1)-148	1aa	2b	-	-	3a
(1)-149	1h	2a	-	-	3a
(1)-150	1n	2a	-	-	3a
(1)-151	1p	2a	-	-	3a
(1)-152	1aa	2a	-	-	3a
(1)-153	-	-	5a	-	3a
(1)-154	-	-	5e	-	3a
(1)-155	-	-	5i	-	3a
(1)-156	-	-	5q	-	3a
(1)-157	-	-	5a	-	3c
(1)-158	-	-	5e	-	3a
(1)-159	-	-	5i	-	3a
(1)-160	-	-	5q	-	3a

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-161	-	2b	5a	-	3a
(1)-162	-	2b	5e	-	3a
(1)-163	-	2b	5i	-	3a
(1)-164	-	2b	5q	-	3a
(1)-165	-	2b	5a	-	3c
(1)-166	-	2b	5e	-	3a
(1)-167	-	2b	5i	-	3a
(1)-168	-	2b	5q	-	3a
(1)-169	-	2a	5a	-	3a
(1)-170	-	2a	5e	-	3a
(1)-171	-	2a	5i	-	3a
(1)-172	-	2a	5q	-	3a
(1)-173	-	2a	5a	-	3c
(1)-174	-	2a	5e	-	3a
(1)-175	-	2a	5i	-	3a
(1)-176	-	2a	5q	-	3a
(1)-177	-	2a	5k	-	3a
(1)-178	-	2b	5k	-	3a
(1)-179	-	2a	5k	-	3c
(1)-180	-	2b	5k	-	3c
(1)-181	-	2a	5m	-	3a
(1)-182	-	2b	5m	-	3a
(1)-183	-	2a	5m	-	3c
(1)-184	-	2b	5m	-	3c
(1)-185	-	2a	5o	-	3a
(1)-186	-	2b	5o	-	3a
(1)-187	-	2a	5o	-	3c
(1)-188	-	2b	5o	-	3c
(1)-189	-	2a	5q	-	3a
(1)-190	-	2b	5q	-	3a

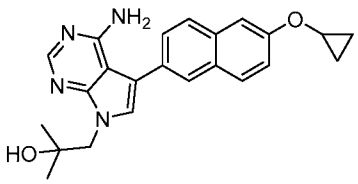
	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-191	-	2a	5q	-	3c
(1)-192	-	2b	5q	-	3c
(1)-193	-	2a	5k	4a	3a
(1)-194	-	2b	5k	4a	3a
(1)-195	-	2a	5k	4a	3c
(1)-196	-	2b	5k	4a	3c
(1)-197	-	2a	5m	4a	3a
(1)-198	-	2b	5m	4a	3a
(1)-199	-	2a	5m	4a	3c

	R^1	R^3	R^{33}	n & R^{32}	Q
(1)-200	-	2b	5m	4a	3c
(1)-201	-	2a	5o	4a	3a
(1)-202	-	2b	5o	4a	3a
(1)-203	-	2a	5o	4a	3c
(1)-204	-	2b	5o	4a	3c
(1)-205	-	2a	5q	4a	3a
(1)-206	-	2b	5q	4a	3a
(1)-207	-	2a	5q	4a	3c
(1)-208	-	2b	5q	4a	3c

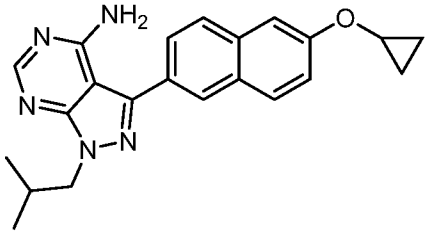
In some embodiments, the compound of the disclosure is selected from:



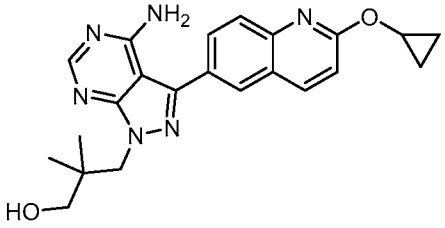
1-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;



1-(4-amino-5-(6-cyclopropoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol;

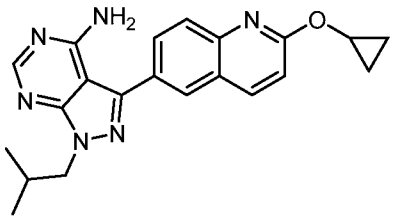


3-(6-cyclopropoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine



3-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol

and



3-(2-cyclopropoxyquinolin-6-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine.

One embodiment of the present disclosure provide a method of treating a subject in need of treatment for an apicomplexan-related disease comprising administering an effective amount of a compound of the disclosure or any embodiment thereof, that inhibits the activity of an apicomplexan calcium dependent protein kinase (CDPK).

Particular embodiments of the present disclosure provide a method of treating cryptosporidiosis in a subject comprising administering an effective amount of a compound of the disclosuure, that inhibits the activity of *Cryptosporidium parvum and C. hominus* calcium dependent protein kinase 1 (*Cp*CDPK1).

Other particular embodiments of the present disclosure provide a method of treating cryptosporidiosis in a subject comprising administering an effective amount of a compound of the disclosure, that inhibits the activity of *T. gondii*, calcium dependent protein kinase 1 (*Tg*CDPK1).

Other particular embodiments of the present disclosure provide a method of treating cryptosporidiosis in a subject comprising administering an effective amount of a compound of the disclosure, that inhibits the activity of *P. falciparum* or *P. berghei* calcium dependent protein kinase 4 (*Pf*CDPK4 or *Pb*CDPK4).

Optionally, the compound of any one the compounds of the dislcosure, can be administered in combination with a second agent, such as agents specific for use against the specific apicomplexan-related disorder being treated.

In one embodiment, the apicomplexan protozoan related disease is toxoplasmosis. As understood by one of ordinary skill in the art, toxoplasmosis can encompass a number of pathologies, including, but not limited to, encephalitis, retinitis, lymphadenopathy, disseminated disease, and hepatitis. Toxoplasmosis infects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family.

Cats are the definitive host for the *Toxoplasma* organism. Infection with this protozoan parasite is fairly common, but actual disease caused by this parasite is relatively

rare in cats. Cats can become infected by *Toxoplasma* by eating any of the three infective stages of the parasites. The most common route of infection is probably by ingestion of tissue cysts in infected prey or in other raw meat. Toxoplasma multiply in the small intestines and in approximately two to three weeks the oocysts are excreted in the infected cat's feces. In another example, cats may be treated prophylactically for toxoplasmosis (e.g, a gastrointestinal infection) provided by providing a therapeutically effective amount of compounds of the disclosure or to eliminate the chance that they would shed infectious *Toxoplasmodia* oocyts and infect their owners. In another embodiment, infected cats may be treated by providing a therapeutically effective amount of a compound of the disclosure to treat toxoplasmosis. As will be understood by those of skill in the art, similar prophylactic and therapeutic methods for limiting development of or treating toxoplasmosis can be used in any animal that can be infected by *Toxoplasma sp.*

Animals are infected by eating infected meat, by ingestion of feces of a cat that has itself recently been infected, or by transmission from mother to fetus. While cats are often blamed for spreading toxoplasmosis, contact with raw meat is a more significant source of human infections in many countries, and fecal contamination of hands is a greater risk factor. Infection has two stages (1) acute toxoplasmosis; and (2) latent toxoplasmosis. During acute toxoplasmosis, symptoms are often influenza-like: swollen lymph nodes, or muscle aches and pains that last for a month or more. Rarely, a patient with a fully functioning immune system may develop eye damage from toxoplasmosis. Young children (15 years old or younger) and immunocompromised patients, such as those with HIV/AIDS, those taking certain types of chemotherapy, or those who have recently received an organ transplant, may develop severe toxoplasmosis. In an embodiment, a young child can be 14 years old or younger; or 13 years old or younger; or 12 years old or younger; or 11 years old or younger; or 10 years old or younger; or 9 years old or younger; or 8 years old or younger; or 7 years old or younger; or 6 years old or younger; or 5 years old or younger; or 4 years old or younger; or 3 years old or younger; or 2 years old or younger; or 1 year old or younger. This can cause damage to the brain (encephalitis) or the eyes (necrotizing retinochoroiditis). Infants infected via placental transmission may be born with either of these problems, or with nasal malformations, although these complications are rare in newborns. In most immunocompetent patients, the infection enters a latent phase, during which only bradyzoites are present, forming cysts in nervous and muscle tissue. Most infants who are infected while in the womb have no symptoms at birth but may develop symptoms later in life. The most common current therapy for toxoplasmosis is sulfadiazine/pyrimethamine combination therapy, but therapy is often

limited by allergic reactions to the sulfa component, anemia and pancytopenia induced by blocking the folate pathway. When sulfadiazine cannot be used, clindamycin may be combined with pyrimethamine but most experts feel it does not work as well as sulfadiazine. Spiramycin has been used for toxoplasmosis during pregnancy but has issues with low efficacy and is no longer available in the United States. Thus few therapeutic alternatives are available.

In another embodiment, the apicomplexan protozoan related disease is cryptosporidiosis. Cryptosporidiosis is caused by infection with the single-celled parasite (not bacterium) *Cryptosporidium parvum.* This parasite is found in many mammals including lambs, calves, goat kids, piglets and humans. Research so far has shown two basic types, the bovine type which affects most species, and a second human type which causes disease in humans only. Outbreaks of human disease, where large numbers of people are affected, are usually water-borne and usually associated with the bovine type of cryptosporidium. Individual sporadic cases of cryptosporidiosis in humans are mostly (around 60%) associated with the human type of cryptosporidium.

Cryptosporidiosis affects the intestines of mammals and is typically an acute short-term infection. It is spread through the fecal-oral route, often through contaminated water; the main symptom is self-limiting diarrhea in people with intact immune systems. In immunocompromised individuals, such as HIV/AIDS patients, the symptoms are particularly severe and often fatal. *Cryptosporidium* is the organism most commonly isolated in HIV positive patients presenting with diarrhea. Cryptosporidiosis is one of the most common waterborne diseases and is found worldwide. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Infection is through contaminated material such as earth, water, uncooked or cross-contaminated food that has been in contact with the feces of an infected individual or animal. It is especially prevalent amongst those in regular contact with bodies of fresh water including recreational water such as swimming pools. Other potential sources include insufficiently treated or insufficiently filtered water water supplies, contaminated food, or exposure to feces. Symptoms appear from two to ten days after infection, and last for up to two weeks or more. In immunocompetent people, the disease can be asymptomatic or cause acute diarrhea or persistent diarrhea that can last for a few weeks. There is often stomach pain or cramping and a low fever. Other symptoms may include nausea, vomiting, malabsorption, and dehydration. Individuals who are asymptomatic (have no symptoms) are nevertheless infective. Immunocompromised people, as well as very

young or very old people, can develop a more severe form of cryptosporidiosis. When Cryptosporidium spreads beyond the intestine, as it can predominantly in patients with AIDS, it can reach the lungs, middle ear, pancreas, and stomach. Thus, one symptom is pain in the right upper quadrant. The parasite can infect the biliary tract, causing biliary cryptosporidiosis. This can result in cholecystitis and cholangitis. Current treatment is symptomatic, with fluid rehydration, electrolyte correction and management of any pain. Nitazoxanide has been FDA-approved for treatment of diarrhea caused by Cryptosporidium in people with healthy immune systems and is available by prescription, however it only shortens the duration of diarrhea by a couple of days. The effectiveness of nitazoxanide in immunosuppressed individuals is unclear and multiple trials have shown no benefit.

The inhibitors described herein may have use in other apicoplexa protozoan related diseases, such as sarcocystosis caused by *Sarcocystis neurona* which is the most common cause of equine protozoal myeloencephalitis (EPM) in horses in America and is transmitted by fecal contamination by opossums, neurosporosis caused by *Neurospora caninum* which causes epidemic abortions in cattle and sheep and is transmitted by canine species fecal contamination and verticle transmissions from mother to calf or lamb, cystoisosporosis caused by *Cystoisospora suis* and causes epidemic diarrhea in pigs and other species in humans and animals, besnoitiosis caused by *Besnoitia besnoiti* is found as a skin and systemic disease of cattle, coccidiosis caused by *Eimeria spp.*, cause infections and disease in poultry; which causes Babesiosis which is caused by *Babesia spp.* and results in a malaria-like disease, theileriosis a tick transmitted disease caused by *Theileria equi* and other species and causes a wasting disease in horses and other livestock, and malaria in humans and animals caused by *Plasmodium spp.* In general, calcium dependent protein kinases homologous to *Toxoplasma* and *Cryptosporidium* probably play important roles in the infection by these pathogens as we have examples of the above compounds that are active against the CDPKs of these apicoplexa protozoans and stop infection by these protozoans in vitro, and in the case of *S. neurona and N. caninum,* also evidence that therapy with these compounds successfully cure mouse infections with these protozoa.

Neospora caninum is a cyst-forming apicomplexan parasite. It is closely related to *Toxoplasma gondii*, but *N. caninum* exhibits distinct differences in transmission patterns, virulence, host specificity, immunogenetic aspects, and the pathology they induce. *N. caninum* represents one of the most important infectious causes of bovine abortion, stillbirth, and the birth of weak calves. The reservoir is the canid, such as dogs and wild foxes and wolves, and cattle are infected both by accidental ingestion of canine feces or by vertical

transmission from mother cow to calf. In addition, *N. caninum* causes neosporosis, a neuromuscular disease in dogs. Neosporosis has also been detected in a wide range of other species of livestock and wild animals.

Plasmodium calcium dependent protein kinase 4 (CDPK4) is essential for exflaggelation of microgametes, sexual reproduction and infection of the mosquito host and is a potential drug target to block mosquito transmission. *Plasmodium* transmission-blocking compounds that act *via* inhibition of *Pf*CDPK4 have great promise in the armamentarium of malaria control. *Plasmodium* CDPK4 has a unique ATP binding site which renders CDPK4 differentially sensitive to bumped kinase inhibitors (BKIs). TgCDPK1 and *Cp*CDPK1 have ATP-binding pockets with an atypically small gatekeeper residue, glycine. *P. falciparum* CDPK4 (*Pf*CDPK4) has a serine residue at the gatekeeper position, smaller than any gatekeeper in mammalian kinases, and an overall ATP-binding pocket that is very similar to *Tg*CDPK1 and *Cp*CDPK1. BKIs inhibit *P. falciparum* CDPK4 (*Pf*CDPK4) and prevents the exflagellation of malaria microgametes. Administration of BKIs to mice stops the transmission of *P. berghei* to mosquitoes. Finally, addition of BKIs to blood containing *P. falciparum* gametocytes stops exflagellation of microgametocytes and blocks the infection of mosquitoes. BKIs are non-toxic, selective inhibitors that block malaria transmission to mosquitos, have favorable oral pharmacokinetic (PK) properties, and have a low likelihood of generating resistance.

Thus, other particular embodiments of the present disclosure provide a method for treating malaria comprising administering an effective amount of a compound of the disclosure that inhibits the activity of *Plasmodium falciparum* and *P. berghei* calcium dependent protein kinases. In one embodiment, the compound can be administered in combination with a second agent. In another embodiment, the subject has malaria, and the second agent is an anti-malarial therapeutic. The subject can be human. In further embodiments, the subject is a mammal other than a human, such as a cat or livestock (e.g., pigs, sheep, goats, cattle).

Pharmaceutical Compositions

In some embodiments, the method comprises the administration of BKI in a pharmaceutical composition having at least one pharmaceutically acceptable carrier, solvent, adjuvant or diluent.

The compounds described herein may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-

toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. The pharmaceutical compositions described herein may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques. In some cases such coatings may be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules, wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Formulations for oral use may also be presented as lozenges.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example

heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compositions disclosed herein may be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient. The daily dose can be administered in one to four doses per day. In the case of skin conditions, it may be preferable to apply a topical preparation of compounds of this invention to the affected area two to four times a day.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy. The compounds of the present invention may be administered alone or in combination with at least one additional therapeutic agent. The compounds of the present invention may be combined with one or more additional therapeutic agents simultaneously or sequentially.

Definitions

Unless the context clearly requires otherwise, throughout the description and the claims, the words ‘comprise’, ‘comprising’, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”. Words using the singular or plural number also include the

plural or singular number, respectively. Additionally, the words "herein," "above" and "below" and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of this application.

Terms used herein may be preceded and/or followed by a single dash, "-", or a double dash, "=", to indicate the bond order of the bond between the named substituent and its parent moiety; a single dash indicates a single bond and a double dash indicates a double bond. In the absence of a single or double dash it is understood that a single bond is formed between the substituent and its parent moiety; further, substituents are intended to be read "left to right" unless a dash indicates otherwise. For example, C_1-C_6alkoxycarbonyloxy and -OC(O)C_1-C_6alkyl indicate the same functionality; similarly arylalkyl and –alkylaryl indicate the same functionality.

The term "alkenyl" as used herein, means a straight or branched chain hydrocarbon containing from 2 to 10 carbons, unless otherwise specified, and containing at least one carbon-carbon double bond. Representative examples of alkenyl include, but are not limited to, ethenyl, 2-propenyl, 2-methyl-2-propenyl, 3-butenyl, 4-pentenyl, 5-hexenyl, 2-heptenyl, 2-methyl-1-heptenyl, 3-decenyl, and 3,7-dimethylocta-2,6-dienyl.

The term "alkyl" as used herein, means a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms, unless otherwise specified. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, and n-decyl. When an "alkyl" group is a linking group between two other moieties, then it may also be a straight or branched chain; examples include, but are not limited to -CH_2-, -CH_2CH_2-, -$CH_2CH_2CHC(CH_3)$-, -$CH_2CH(CH_2CH_3)CH_2$-.

The term "alkynyl" as used herein, means a straight or branched chain hydrocarbon group containing from 2 to 10 carbon atoms and containing at least one carbon-carbon triple bond. Representative examples of alkynyl include, but are not limited, to acetylenyl, 1-propynyl, 2-propynyl, 3-butynyl, 2-pentynyl, and 1-butynyl.

The term "aryl," as used herein, means a phenyl (*i.e.*, monocyclic aryl), a bicyclic ring system containing at least one phenyl ring or an aromatic bicyclic ring containing only carbon atoms in the aromatic bicyclic ring system or a multicyclic aryl ring system, provided that the bicyclic or multicyclic aryl ring system does not contain a heteroaryl ring when fully aromatic. The bicyclic aryl can be azulenyl, naphthyl, or a phenyl fused to a monocyclic cycloalkyl, a monocyclic cycloalkenyl, or a monocyclic heterocyclyl. The bicyclic aryl is

attached to the parent molecular moiety through any carbon atom contained within the phenyl portion of the bicyclic system, or any carbon atom with the napthyl or azulenyl ring. The fused monocyclic cycloalkyl or monocyclic heterocyclyl portions of the bicyclic aryl are optionally substituted with one or two oxo and/or thia groups. Representative examples of the bicyclic aryls include, but are not limited to, azulenyl, naphthyl, dihydroinden-1-yl, dihydroinden-2-yl, dihydroinden-3-yl, dihydroinden-4-yl, 2,3-dihydroindol-4-yl, 2,3-dihydroindol-5-yl, 2,3-dihydroindol-6-yl, 2,3-dihydroindol-7-yl, inden-1-yl, inden-2-yl, inden-3-yl, inden-4-yl, dihydronaphthalen-2-yl, dihydronaphthalen-3-yl, dihydronaphthalen-4-yl, dihydronaphthalen-1-yl, 5,6,7,8-tetrahydronaphthalen-1-yl, 5,6,7,8-tetrahydronaphthalen-2-yl, 2,3-dihydrobenzofuran-4-yl, 2,3-dihydrobenzofuran-5-yl, 2,3-dihydrobenzofuran-6-yl, 2,3-dihydrobenzofuran-7-yl, benzo[d][1,3]dioxol-4-yl, benzo[d][1,3]dioxol-5-yl, 2H-chromen-2-on-5-yl, 2H-chromen-2-on-6-yl, 2H-chromen-2-on-7-yl, 2H-chromen-2-on-8-yl, isoindoline-1,3-dion-4-yl, isoindoline-1,3-dion-5-yl, inden-1-on-4-yl, inden-1-on-5-yl, inden-1-on-6-yl, inden-1-on-7-yl, 2,3-dihydrobenzo[b][1,4]dioxan-5-yl, 2,3-dihydrobenzo[b][1,4]dioxan-6-yl, 2H-benzo[b][1,4]oxazin3(4H)-on-5-yl, 2H-benzo[b][1,4]oxazin3(4H)-on-6-yl, 2H-benzo[b][1,4]oxazin3(4H)-on-7-yl, 2H-benzo[b][1,4]oxazin3(4H)-on-8-yl, benzo[d]oxazin-2(3H)-on-5-yl, benzo[d]oxazin-2(3H)-on-6-yl, benzo[d]oxazin-2(3H)-on-7-yl, benzo[d]oxazin-2(3H)-on-8-yl, quinazolin-4(3H)-on-5-yl, quinazolin-4(3H)-on-6-yl, quinazolin-4(3H)-on-7-yl, quinazolin-4(3H)-on-8-yl, quinoxalin-2(1H)-on-5-yl, quinoxalin-2(1H)-on-6-yl, quinoxalin-2(1H)-on-7-yl, quinoxalin-2(1H)-on-8-yl, benzo[d]thiazol-2(3H)-on-4-yl, benzo[d]thiazol-2(3H)-on-5-yl, benzo[d]thiazol-2(3H)-on-6-yl, and benzo[d]thiazol-2(3H)-on-7-yl. In certain embodiments, the bicyclic aryl is (i) naphthyl or (ii) a phenyl ring fused to either a 5 or 6 membered monocyclic cycloalkyl, a 5 or 6 membered monocyclic cycloalkenyl, or a 5 or 6 membered monocyclic heterocyclyl, wherein the fused cycloalkyl, cycloalkenyl, and heterocyclyl groups are optionally substituted with one or two groups which are independently oxo or thia. Multicyclic aryl groups are a phenyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other ring systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl, provided that when the base ring is fused to a bicyclic cycloalkyl, bicyclic cycloalkenyl, or bicyclic heterocyclyl, then the base ring is fused to the base ring of the a bicyclic cycloalkyl,

bicyclic cycloalkenyl, or bicyclic heterocyclyl. The multicyclic aryl is attached to the parent molecular moiety through any carbon atom contained within the base ring. In certain embodiments, multicyclic aryl groups are a phenyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other ring systems independently selected from the group consisting of a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, and a monocyclic heterocyclyl, provided that when the base ring is fused to a bicyclic cycloalkyl, bicyclic cycloalkenyl, or bicyclic heterocyclyl, then the base ring is fused to the base ring of the a bicyclic cycloalkyl, bicyclic cycloalkenyl, or bicyclic heterocyclyl. Examples of multicyclic aryl groups include but are not limited to anthracen-9-yl and phenanthren-9-yl.

The term "arylalkyl" and "-alkylaryl" as used herein, means an aryl group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of arylalkyl include, but are not limited to, benzyl, 2-phenylethyl, 3-phenylpropyl, and 2-naphth-2-ylethyl.

The terms "cyano" and "nitrile" as used herein, mean a -CN group.

The term "cycloalkyl" as used herein, means a monocyclic, bicyclic, or a multicyclic cycloalkyl ring system. Monocyclic ring systems are cyclic hydrocarbon groups containing from 3 to 8 carbon atoms, where such groups can be saturated or unsaturated, but not aromatic. In certain embodiments, cycloalkyl groups are fully saturated. Examples of monocyclic cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl. Bicyclic cycloalkyl ring systems are bridged monocyclic rings or fused bicyclic rings. Bridged monocyclic rings contain a monocyclic cycloalkyl ring where two non-adjacent carbon atoms of the monocyclic ring are linked by an alkylene bridge of between one and three additional carbon atoms (*i.e.*, a bridging group of the form $-(CH_2)_w-$, where w is 1, 2, or 3). Representative examples of bicyclic ring systems include, but are not limited to, bicyclo[3.1.1]heptane, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, bicyclo[3.2.2]nonane, bicyclo[3.3.1]nonane, and bicyclo[4.2.1]nonane. Fused bicyclic cycloalkyl ring systems contain a monocyclic cycloalkyl ring fused to either a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocyclyl, or a monocyclic heteroaryl. The bridged or fused bicyclic cycloalkyl is attached to the parent molecular moiety through any carbon atom contained within the monocyclic cycloalkyl ring. Cycloalkyl groups are optionally substituted with one or two groups which are independently oxo or thia. In certain embodiments, the fused

bicyclic cycloalkyl is a 5 or 6 membered monocyclic cycloalkyl ring fused to either a phenyl ring, a 5 or 6 membered monocyclic cycloalkyl, a 5 or 6 membered monocyclic cycloalkenyl, a 5 or 6 membered monocyclic heterocyclyl, or a 5 or 6 membered monocyclic heteroaryl, wherein the fused bicyclic cycloalkyl is optionally substituted by one or two groups which are independently oxo or thia. Multicyclic cycloalkyl ring systems are a monocyclic cycloalkyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other rings systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl. The multicyclic cycloalkyl is attached to the parent molecular moiety through any carbon atom contained within the base ring. In certain embodiments, multicyclic cycloalkyl ring systems are a monocyclic cycloalkyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other rings systems independently selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, and a monocyclic heterocyclyl. Examples of multicyclic cycloalkyl groups include, but are not limited to tetradecahydrophenanthrenyl, perhydrophenothiazin-1-yl, and perhydrophenoxazin-1-yl.

"Cycloalkenyl" as used herein refers to a monocyclic, bicyclic, or a multicyclic cycloalkenyl ring system. Monocyclic ring systems are cyclic hydrocarbon groups containing from 3 to 8 carbon atoms, where such groups are unsaturated (*i.e.*, containing at least one annular carbon-carbon double bond), but not aromatic. Examples of monocyclic ring systems include cyclopentenyl and cyclohexenyl. Bicyclic cycloalkenyl rings are bridged monocyclic rings or fused bicyclic rings. Bridged monocyclic rings contain a monocyclic cycloalkenyl ring where two non-adjacent carbon atoms of the monocyclic ring are linked by an alkylene bridge of between one and three additional carbon atoms (*i.e.*, a bridging group of the form $-(CH_2)_w-$, where w is 1, 2, or 3). Representative examples of bicyclic cycloalkenyls include, but are not limited to, norbornenyl and bicyclo[2.2.2]oct-2-enyl. Fused bicyclic cycloalkenyl ring systems contain a monocyclic cycloalkenyl ring fused to either a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocyclyl, or a monocyclic heteroaryl. The bridged or fused bicyclic cycloalkenyl is attached to the parent molecular moiety through any carbon atom

contained within the monocyclic cycloalkenyl ring. Cycloalkenyl groups are optionally substituted with one or two groups which are independently oxo or thia. Multicyclic cycloalkenyl rings contain a monocyclic cycloalkenyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two rings systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl. The multicyclic cycloalkenyl is attached to the parent molecular moiety through any carbon atom contained within the base ring. In certain embodiments, multicyclic cycloalkenyl rings contain a monocyclic cycloalkenyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two rings systems independently selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, and a monocyclic heterocyclyl.

The term "halo" or "halogen" as used herein, means -Cl, -Br, -I or -F.

The term "haloalkyl" as used herein, means at least one halogen, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of haloalkyl include, but are not limited to, chloromethyl, 2-fluoroethyl, trifluoromethyl, pentafluoroethyl, and 2-chloro-3-fluoropentyl.

The term "heteroaryl," as used herein, means a monocyclic, bicyclic, or a multicyclic heteroaryl ring system. The monocyclic heteroaryl can be a 5 or 6 membered ring. The 5 membered ring consists of two double bonds and one, two, three or four nitrogen atoms and optionally one oxygen or sulfur atom. The 6 membered ring consists of three double bonds and one, two, three or four nitrogen atoms. The 5 or 6 membered heteroaryl is connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the heteroaryl. Representative examples of monocyclic heteroaryl include, but are not limited to, furyl, imidazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, oxazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, pyrazolyl, pyrrolyl, tetrazolyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, and triazinyl. The bicyclic heteroaryl consists of a monocyclic heteroaryl fused to a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocyclyl, or a monocyclic heteroaryl. The fused cycloalkyl or heterocyclyl portion of the bicyclic heteroaryl group is optionally substituted with one or two groups which are independently oxo or thia. When the bicyclic heteroaryl contains a fused cycloalkyl, cycloalkenyl, or

heterocyclyl ring, then the bicyclic heteroaryl group is connected to the parent molecular moiety through any carbon or nitrogen atom contained within the monocyclic heteroaryl portion of the bicyclic ring system. When the bicyclic heteroaryl is a monocyclic heteroaryl fused to a phenyl ring or a monocyclic heteroaryl, then the bicyclic heteroaryl group is connected to the parent molecular moiety through any carbon atom or nitrogen atom within the bicyclic ring system. Representative examples of bicyclic heteroaryl include, but are not limited to, benzimidazolyl, benzofuranyl, benzothienyl, benzoxadiazolyl, benzoxathiadiazolyl, benzothiazolyl, cinnolinyl, 5,6-dihydroquinolin-2-yl, 5,6-dihydroisoquinolin-1-yl, furopyridinyl, indazolyl, indolyl, isoquinolinyl, naphthyridinyl, quinolinyl, purinyl, 5,6,7,8-tetrahydroquinolin-2-yl, 5,6,7,8-tetrahydroquinolin-3-yl, 5,6,7,8-tetrahydroquinolin-4-yl, 5,6,7,8-tetrahydroisoquinolin-1-yl, thienopyridinyl, 4,5,6,7-tetrahydrobenzo[c][1,2,5]oxadiazolyl, and 6,7-dihydrobenzo[c][1,2,5]oxadiazol-4(5H)-onyl. In certain embodiments, the fused bicyclic heteroaryl is a 5 or 6 membered monocyclic heteroaryl ring fused to either a phenyl ring, a 5 or 6 membered monocyclic cycloalkyl, a 5 or 6 membered monocyclic cycloalkenyl, a 5 or 6 membered monocyclic heterocyclyl, or a 5 or 6 membered monocyclic heteroaryl, wherein the fused cycloalkyl, cycloalkenyl, and heterocyclyl groups are optionally substituted with one or two groups which are independently oxo or thia. The multicyclic heteroaryl group is a monocyclic heteroaryl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic heterocyclyl, a bicyclic cycloalkenyl, and a bicyclic cycloalkyl; or (ii) two ring systems selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic heterocyclyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic cycloalkyl. The multicyclic heteroaryl group is connected to the parent molecular moiety through any carbon atom or nitrogen atom contained within the base ring. In certain embodiments, multicyclic heteroaryl groups are a monocyclic heteroaryl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic heterocyclyl, a bicyclic cycloalkenyl, and a bicyclic cycloalkyl; or (ii) two ring systems selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic heterocyclyl, a monocyclic cycloalkenyl, and a monocyclic cycloalkyl. Examples of multicyclic heteroaryls include, but are not limited to 5H-[1,2,4]triazino[5,6-b]indol-5-yl, 2,3,4,9-tetrahydro-1H-carbazol-9-yl, 9H-pyrido[3,4-b]indol-9-yl, 9H-carbazol-9-yl, acridin-9-yl,

The term "heteroarylalkyl" and "-alkylheteroaryl" as used herein, means a heteroaryl, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of heteroarylalkyl include, but are not limited to, fur-3-ylmethyl, 1H-imidazol-2-ylmethyl, 1H-imidazol-4-ylmethyl, 1-(pyridin-4-yl)ethyl, pyridin-3-ylmethyl, pyridin-4-ylmethyl, pyrimidin-5-ylmethyl, 2-(pyrimidin-2-yl)propyl, thien-2-ylmethyl, and thien-3-ylmethyl.

The term "heterocyclyl" as used herein, means a monocyclic, bicyclic, or multicyclic heterocycle. The monocyclic heterocycle is a 3, 4, 5, 6 or 7 membered ring containing at least one heteroatom independently selected from the group consisting of O, N, and S where the ring is saturated or unsaturated, but not aromatic. The 3 or 4 membered ring contains 1 heteroatom selected from the group consisting of O, N and S. The 5 membered ring can contain zero or one double bond and one, two or three heteroatoms selected from the group consisting of O, N and S. The 6 or 7 membered ring contains zero, one or two double bonds and one, two or three heteroatoms selected from the group consisting of O, N and S. The monocyclic heterocycle is connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the monocyclic heterocycle. Representative examples of monocyclic heterocycle include, but are not limited to, azetidinyl, azepanyl, aziridinyl, diazepanyl, 1,3-dioxanyl, 1,3-dioxolanyl, 1,3-dithiolanyl, 1,3-dithianyl, imidazolinyl, imidazolidinyl, isothiazolinyl, isothiazolidinyl, isoxazolinyl, isoxazolidinyl, morpholinyl, oxadiazolinyl, oxadiazolidinyl, oxazolinyl, oxazolidinyl, piperazinyl, piperidinyl, pyranyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydrothienyl, thiadiazolinyl, thiadiazolidinyl, thiazolinyl, thiazolidinyl, thiomorpholinyl, 1,1-dioxidothiomorpholinyl (thiomorpholine sulfone), thiopyranyl, and trithianyl. The bicyclic heterocycle is a monocyclic heterocycle fused to either a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocycle, or a monocyclic heteroaryl. The bicyclic heterocycle is connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the monocyclic heterocycle portion of the bicyclic ring system. Representative examples of bicyclic heterocyclyls include, but are not limited to, 2,3-dihydrobenzofuran-2-yl, 2,3-dihydrobenzofuran-3-yl, indolin-1-yl, indolin-2-yl, indolin-3-yl, 2,3-dihydrobenzothien-2-yl, decahydroquinolinyl, decahydroisoquinolinyl, octahydro-1H-indolyl, and octahydrobenzofuranyl. Heterocyclyl groups are optionally substituted with one or two groups which are independently oxo or thia. In certain embodiments, the bicyclic heterocyclyl is a 5 or 6 membered monocyclic heterocyclyl ring fused to phenyl ring, a 5 or 6 membered monocyclic cycloalkyl, a 5 or 6

membered monocyclic cycloalkenyl, a 5 or 6 membered monocyclic heterocyclyl, or a 5 or 6 membered monocyclic heteroaryl, wherein the bicyclic heterocyclyl is optionally substituted by one or two groups which are independently oxo or thia. Multicyclic heterocyclyl ring systems are a monocyclic heterocyclyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other rings systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl. The multicyclic heterocyclyl is attached to the parent molecular moiety through any carbon atom or nitrogen atom contained within the base ring. In certain embodiments, multicyclic heterocyclyl ring systems are a monocyclic heterocyclyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other rings systems independently selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, and a monocyclic heterocyclyl. Examples of multicyclic heterocyclyl groups include, but are not limited to 10H-phenothiazin-10-yl, 9,10-dihydroacridin-9-yl, 9,10-dihydroacridin-10-yl, 10H-phenoxazin-10-yl, 10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl, 1,2,3,4-tetrahydropyrido[4,3-g]isoquinolin-2-yl, 12H-benzo[b]phenoxazin-12-yl, and dodecahydro-1H-carbazol-9-yl.

The term "nitro" as used herein, means a $-NO_2$ group.

The term "oxo" as used herein means a =O group.

The term "saturated" as used herein means the referenced chemical structure does not contain any multiple carbon-carbon bonds. For example, a saturated cycloalkyl group as defined herein includes cyclohexyl, cyclopropyl, and the like.

The term "thia" as used herein means a =S group.

The term "unsaturated" as used herein means the referenced chemical structure contains at least one multiple carbon-carbon bond, but is not aromatic. For example, an unsaturated cycloalkyl group as defined herein includes cyclohexenyl, cyclopentenyl, cyclohexadienyl, and the like.

The compounds of this invention may contain one or more asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. These compounds can be, for example, racemates, chiral non-racemic or diastereomers. In these situations, the single enantiomers, i.e., optically active forms, can be obtained by asymmetric synthesis or by

resolution of the racemates. Resolution of the racemates can be accomplished, for example, by conventional methods such as crystallization in the presence of a resolving agent; chromatography, using, for example a chiral HPLC column; or derivatizing the racemic mixture with a resolving reagent to generate diastereomers, separating the diastereomers via chromatography, and removing the resolving agent to generate the original compound in enantiomerically enriched form. Any of the above procedures can be repeated to increase the enantiomeric purity of a compound.

When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless otherwise specified, it is intended that the compounds include the cis, trans, Z- and E- configurations. Likewise, all tautomeric forms are also intended to be included.

As used herein, the phrase "pharmaceutically acceptable salt" refers to both pharmaceutically acceptable acid and base addition salts and solvates. Such pharmaceutically acceptable salts include salts of acids such as hydrochloric, phosphoric, hydrobromic, sulfuric, sulfinic, formic, toluenesulfonic, methanesulfonic, nitric, benzoic, citric, tartaric, maleic, hydroiodic, alkanoic such as acetic, HOOC-$(CH_2)_n$-COOH where n is 0-4, and the like. Non-toxic pharmaceutical base addition salts include salts of bases such as sodium, potassium, calcium, ammonium, and the like. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts.

As used herein, the term "subject", "individual," or "patient," used interchangeably, refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, birds, swine, horses, livestock (e.g., pigs, sheep, goats, cattle), primates or humans.

As used here, a subject "in need thereof" refers to a subject that has the disorder or disease to be treated or is predisposed to or otherwise at risk of developing the disease or disorder.

As used here, the terms "treatment" and "treating" means:

(i) inhibiting the progression the disease;

(ii) prophylactic use for example, preventing or limiting development of a disease, condition or disorder in an individual who may be predisposed or otherwise at risk to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease;

(iii) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder;

(iv) ameliorating the referenced disease state, for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing or improving the pathology and/or symptomatology) such as decreasing the severity of disease; or

(v) eliciting the referenced biological effect.

As used herein, the phrase "therapeutically effective amount" refers to the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response that is being sought in a tissue, system, animal, individual or human by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following: (1) preventing the disease; for example, preventing a disease, condition or disorder in an individual who may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease; (2) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder; and (3) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (*i.e.*, reversing the pathology and/or symptomatology) such as decreasing the severity of disease.

EXAMPLES

The methods of the disclosure are illustrated further by the following examples, which are not to be construed as limiting the disclosure in scope or spirit to the specific procedures and in them. It is understood that the nature of the substituents required for the desired target compound often determines the preferred method of synthesis.

General synthetic procedures

All chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. Reactions were monitored with thin-layer chromatography using silica gel 60 F254 coated glass plates (EM Sciences). Compound purification was performed with an IntelliFlash 280 automated flash chromatography system using pre-packed Varian Super Flash silica gel columns (hexanes/EtOAc or CH_2Cl_2/MeOH gradient solvent systems). A Varian Dynamax Microsorb 100-5 C_{18} column (250 mm x 21.4 mm), eluting with H_2O/CH_3CN or H_2O/ MeOH gradient solvent systems (+0.05% TFA) was used for preparatory HPLC purification. Products were detected by UV at λ=254 nm, with all final compounds displaying >95% purity. NMR spectra were recorded on Bruker 300 or 500

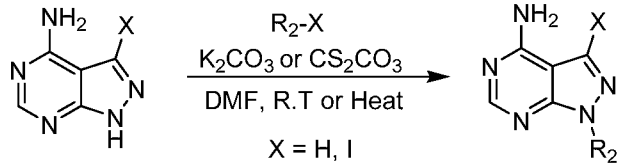
MHz spectrometers at ambient temperature. Chemical shifts are reported in parts per million (δ) and coupling constants in Hz. ^{1}H-NMR spectra were referenced to the residual solvent peaks as internal standards (7.26 ppm for $CDCl_3$, 2.50 ppm for d_6-DMSO, and 3.34 ppm for CD_3OD). Mass spectra were recorded with a Bruker Esquire Liquid Chromatograph - Ion Trap Mass Spectrometer.

In some examples and embodiments, the methods useful in synthesis of the inhibitors of disclosure have been previously described in International Patent Publication WO 2011/0094628 and the U.S. Patent Publication 2013/0018040, both incorporated herein by reference in their entirety.

Methods of Preparation

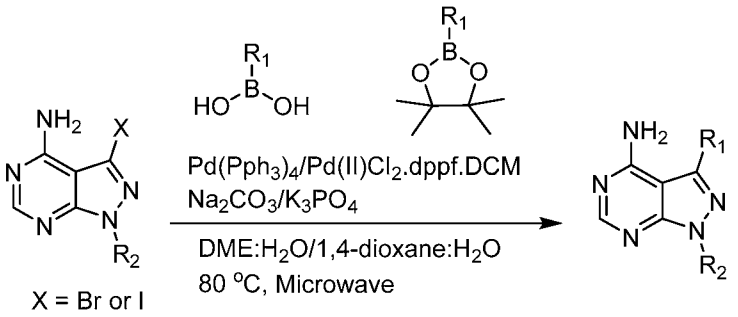
The exemplary synthetic routes described below can be used to generate derivatives that contain varying substituents at the 1- and 3-positions of the pyrazolopyrimidine or imidazopyrazine core.

General R_2 alkylation procedure:



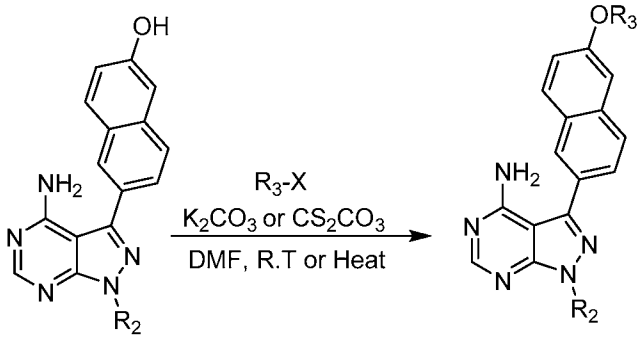
Pyrazolopyrimidine (1 equiv.), K_2CO_3, Cs_2CO_3 or K_2CO_3:NaH_2PO_4 (1.5-2 equiv.), and an alkylhalide (1.1 equiv.) or alkylmesylate (1.1 equiv.) were stirred in dry DMF at room temperature or 80 °C. The reaction was monitored by thin layer chromatography. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified *via* flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. If necessary, further purification was performed with preparatory RP-HPLC.

General Suzuki coupling procedure:



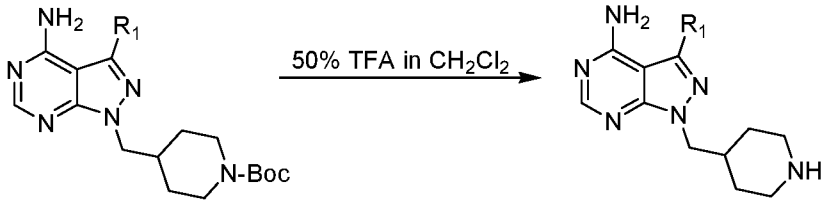
3-Iodopyrazolopyrimidines or 3-Bromopyrazolopyrimidines (1 equiv.), Na_2CO_3/K_3PO_4 (2-4 equiv.), $Pd(PPh_3)_4/Pd(II)Cl_2$dppf.DCM, (0.05 equiv.), and boronic acids or boronate pinacol esters (1-2 equiv.) were dissolved in a mixture of dimethoxyethane (1.5 mL) and water (0.5 mL) and then heated in a microwave at 80 °C for one hour. After cooling, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified *via* flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. If necessary, further purification was performed with preparatory RP-HPLC.

General naphthol alkylation procedure:



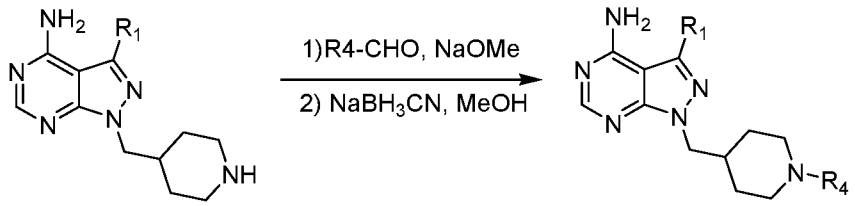
6-Hydroxy-2-naphthalene pyrazolopyrimidines (1 equiv.), K_2CO_3/CS_2CO_3 or (1.5-2 equiv.), and alkyl halides/epoxides (1.1 equiv.), NaH_2PO_4: K_2CO_3 (1:1 equiv.), were stirred in dry DMF at room temperature or 60-80°C and monitored by thin layer chromatography. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified *via* flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. If necessary, further purification was performed with preparatory RP-HPLC.

General boc-deprotection procedure:



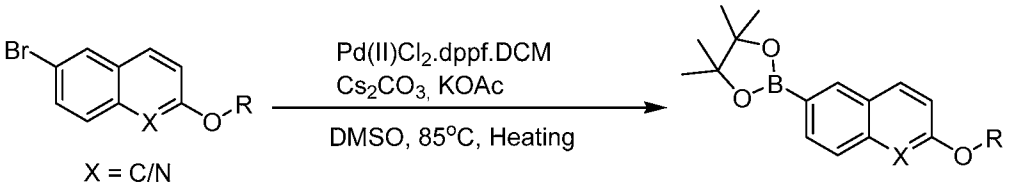
Boc-amine-containing pyrazolopyrimidines was stirred in a TFA/CH_2Cl_2 (1:1) mixture for ~3 h. The reaction was then concentrated and purified via preparatory RP-HPLC. After HPLC purification, the product was then re-concentrated from 1.25 M HCl in EtOH to afford the final, purified product as a bis-HCl salt.

General reductive amination procedure:



Deprotected pyrazolopyrimidines (1 equiv.) were dissolved in methanol and neutralized with sodium methoxide. A solution containing 2% acetic acid and an aldehyde or ketone (5-10 equiv.) was stirred at room temperature for 10 min. Sodium cyanoborohydride (5 equiv.) was then added and the reaction was stirred until reaching completion, as determined by thin layer chromatography (typically ~2 h). The reaction crude was then purified *via* preparatory RP-HPLC. After HPLC purification, the residue was dissolved in a small amount of 2 M HCl in methanol and, after concentration *in vacuo*, the final product was obtained as an HCl salt.

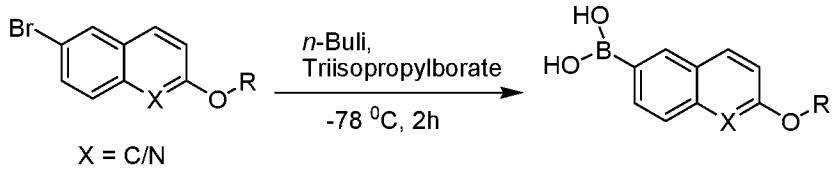
General pinacol ester formation procedure:



Alkylated naphthols or quinolones (1 equiv.), Cs_2CO_3 (1.5-2 equiv.), pinacolatodiborane (2.0 equiv.), Pd(II)Cl_2(dppf).DCM (0.05 equiv.), and KOAc (1 equiv.) in dry DMSO were heated at 85 °C for 5-8 h. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated

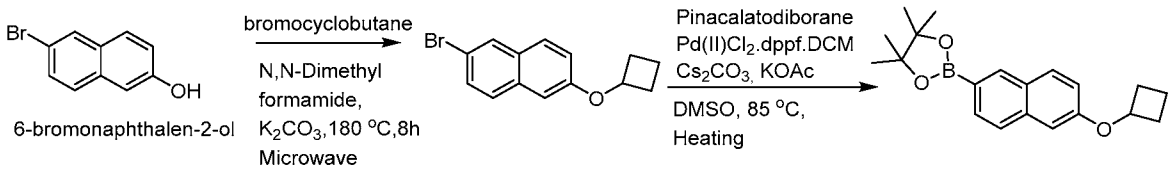
under reduced pressure. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient.

General procedure for boronylation using triisopropylborate:



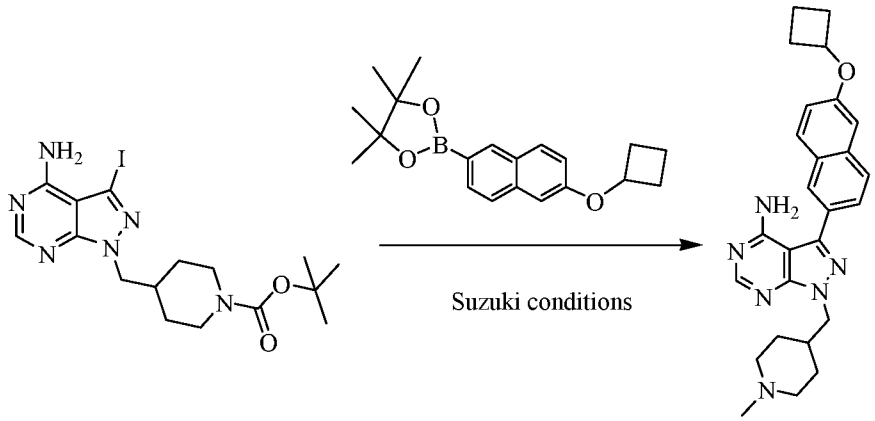
Aryl halides (1 equiv.) and triisopropylborate (1.5 equiv.) were dissolved in tetrahydrofuran:toluene (2:8), cooled to -78 °C, and *n*-Buli (1.7 equiv.) was added dropwise over 30-40 min. After addition, the reaction was stirred at -78 °C for 1 h. After 1 h, the reaction was allowed to warm to 0 °C and stirred for 15-25 min followed by addition of 2N HCl slowly. The organic layer was separated and concentrated *in vacuo* to afford the desired crude product as a white crystalline product or by collecting and washing with water the white crystalline solid that forms upon addition of 2N HCl.

Example 1: 3-(6-Cyclobutoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



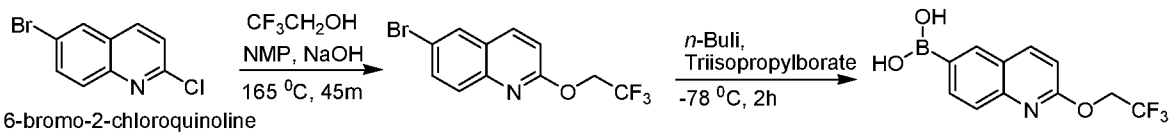
2-Bromo-6-cyclobutoxynaphthalene: 6-bromonaphthalene (700 mg, 3.1 mmol), K_2CO_3 (2.140 g, 15.5 mmol), and bromocyclobutane (1.75 mL, 18.6 mmol) in dry DMF were heated at 180 °C in a microwave for 8 h. After completion, ethyl acetate and water were added and the organic phase was separated. The aqueous phase was extracted with ethyl acetate (2x10mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient to afford 693 mg (80% yield) of pure product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.65-7.43 (m, 3H), 7.10 (dd, 1H), 6.94 (s, 1H), 4.74 (m, 1H), 2.58-2.45 (m, 2H), 2.30-2.13 (m, 2H), 1.95-1.69 (m, 2H); MS (ESI) 278.5 *m/z* [MH^+], $C_{14}H_{14}BrO$ requires 278.2.

2-(6-Cyclobutoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane: 2-Bromo-6-cyclobutoxynaphthalene and pinacolatodiborane were subjected to general pinacol ester formation procedure to afford the desired pure product (631 mg, 60% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.93-7.61 (m, 3H), 7.2-6.93 (m, 2H), 4.80 (m, 1H), 2.67-2.36 (m, 2H), 2.41-2.11 (m, 2H), 2.01-1.60 (m, 2H), 1.37 (s, 12H); MS (ESI) 325.1 *m/z* [MH^+], $C_{20}H_{26}BO_3$ requires 325.1.



2-(6-Cyclobutoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and tert-butyl 4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate were subjected to the general Suzuki coupling procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford 3-(6-cyclobutoxy-naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine. ^{1}H NMR (300 MHz, CD_3OD) δ 8.47 (s, 1H), 8.15 (s, 1H), 7.99 (d, J=8.3 Hz, 1H), 7.92 (d, J=9.5 Hz, 1H), 7.80 (d, J=8.9 Hz, 1H), 7.24 (m, 2H), 4.53 (d, J=6.6 Hz, 2H), 3.56 (m, 2H), 3.03 (m, 2H), 2.86 (s, 3H), 2.62 (m, 2H), 2.44 (m, 1H), 2.22 (m, 2H), 2.04-1.60 (m, 6H); MS (ESI) 443.4 m/z [MH+], $C_{26}H_{31}N_6O$ requires 443.2.

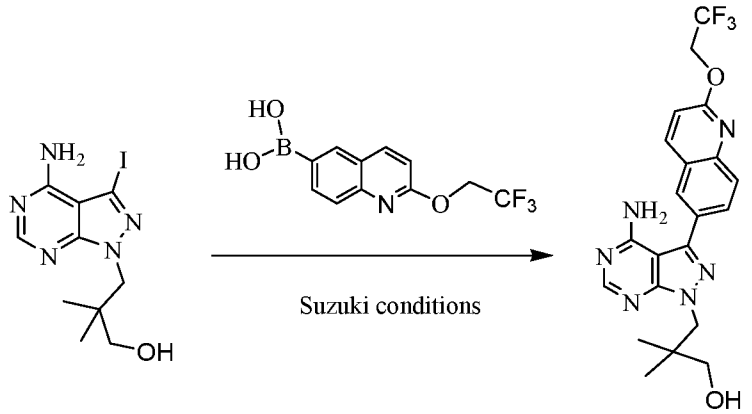
Example 2: 3-(4-Amino-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol



6-Bromo-2-(2,2,2-trifluoroethoxy)quinolone: 6-bromo-2-chloroquinoline (1.00 g, 4.1 mmol), CF_3CH_2OH (0.95 mL, 12.3 mmol), N-Methylmorpholine (12 mL), and NaOH (330

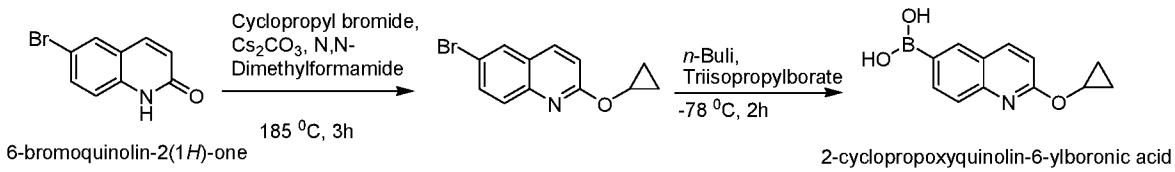
mg, 8.2 mmol) were taken in microwave tube and then heated at 165 °C for 45 min. After addition of water, the reaction mixture was extracted with ethyl acetate (3x20 mL). The organic layer was washed with brine, dried over sodium sulfate, and evaporated under reduced pressure. The crude compound was then taken to the next step without further purification.

Synthesis of 2-(2,2,2-Trifluoroethoxy)quinolin-6-ylboronic acid: 6-Bromo-2-(2,2,2-trifluoroethoxy)quinoline was subjected to the general procedure for boronylation using triisopropylborate to afford a white crystalline product (354mg, 80% yield,). ^{1}H NMR (300 MHz, CD_3OD) δ 6.91 (s, 1H), 6.82 (d, J=8.5 Hz, 1H,), 6.45 (s, 2H) 6.61 (d, J = 7.04 Hz, 1H), 3.5 (q, J = 8.7Hz, 2H,); MS (ESI) 272.4 *m/z* [MH+], $C_{11}H_{10}BF_3NO_3$ requires 272.2.



2-(2,2,2-Trifluoroethoxy)quinolin-6-ylboronic acid and 3-(4-amino-3-iodo-1H-pyrazolo-[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol were subjected to the general Suzuki coupling procedure in order to afford 3-(4-Amino-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol. ^{1}H NMR (300 MHz, CD_3OD) δ 8.40 (s, 1H), 8.17 (d, J=8.7 Hz, 1H), 8.07 (d, J=1.6 Hz, 1H), 8.01-7.95 (m, 2H), 7.12 (d, J=8.9 Hz, 1H), 4.98 (q, J=8.5 Hz, 2H), 4.35 (s, 2H), 3.15 (s, 2H), 1.05 (s, 6H); MS (ESI) 447.5 m/z [MH+], $C_{21}H_{21}F_3N_6O_2$ requires 447.4.

Example 3: 3-(2-Cyclopropoxyquinolin-6-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

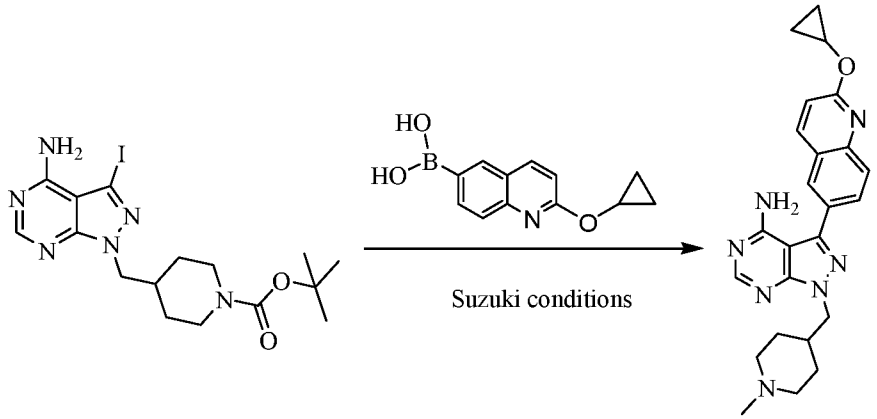


6-bromoquinolin-2(1*H*)-one

2-cyclopropoxyquinolin-6-ylboronic acid

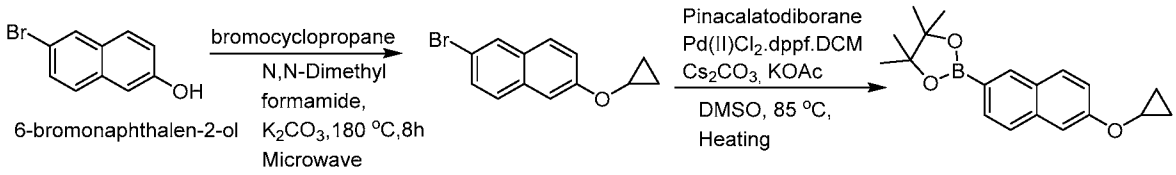
6-Bromo-2-cyclopropoxyquinoline: 6-Bromo-quinolin-2(1H)-one (1.00 g, 4.4 mmol, 1 equiv.), Cs_2CO_3 (5.08 g, 17.8 mmol), and bromocyclopropane (1.06 g, 13.3 mmol) in dry DMF (10 mL) were heated at 180 °C in a microwave for 3 h. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was further extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient to afford 0.235mg (20% yield) of pure product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.87-7.82 (m, 1H), 7.78-7.65 (m, 2H), 6.88 (d, J=8.91 Hz, 1H), 4.54-4.44 (m, 1H), 0.94-0.77 (m, 4H); MS (ESI) 265.5 m/z [MH^+], $C_{12}H_{11}BrNO$ requires 265.2.

2-Cyclopropoxyquinolin-6-ylboronic acid: 6-Bromo-2-cyclopropoxyquinoline (2.01 g, 7.95 mmol, 1 equiv.) and triisopropylborate (2.05 mg, 13.5 mmol, 1.69 equiv.) were subjected to general procedure for boronylation using triisopropylborate to afford the desired pure product (1.05 g, 80% yield); ^{1}H NMR (300 MHz, DMSO) δ 8.37-8.28 (m, 2H), 8.10-8.04 (m, 1H), 7.77 (d, J=8.5 Hz, 1H), 7.06 (d, J=8.9 Hz, 1H), 4.55-4.45 (m, 1H), 0.91-0.81 (m, 2H), 0.80-0.73 (m, 2H); MS (ESI) 230.2 *m/z* [MH^+], $C_{12}H_{13}BNO_3$ requires 230.2.



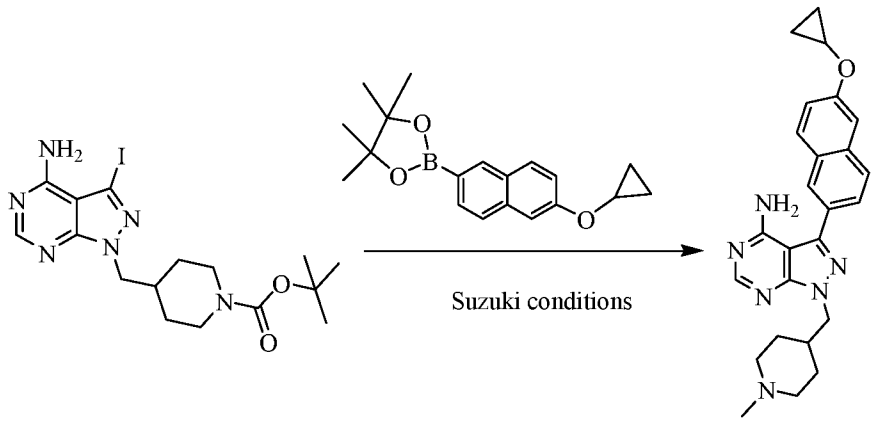
2-Cyclopropoxyquinolin-6-ylboronic acid and *tert*-butyl 4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate were subjected to the general Suzuki coupling procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford 3-(2-cyclopropoxyquinolin-6-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine. ^{1}H NMR (300 MHz, CD_3OD) *δ* 9.17 (s, 1H), 8.75-8.33 (m, 3H), 8.28-7.98 (m, 2H), 4.72 (m, 1H), 4.54 (d, *J*=6.0 Hz, 2H), 3.56 (m, 2H), 3.10 (m, 2H), 3.00 (s, 3H), 2.48 (m, 1H), 2.00 (m, 2H), 1.60 (m, 2H), 1.20-1.10 (m, 4H); MS (ESI) 430.5 *m/z* [MH+], $C_{24}H_{28}N_7O$ requires 430.6.

Example 4: 3-(6-Cyclopropoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



2-Bromo-6-cyclopropoxynaphthalene: 6-Bromonaphthalen-2-ol (3.00 g, 13.0 mmol), Cs_2CO_3 (1.29 g, 39.6 mmol) and bromocyclopropane (4.07 g, 39.0 mmol) were taken in a microwave tube and heated at 180 °C for 30 min. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was further extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient to afford 2.50 g (71% yield) of pure product. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.91 (m, 1H), 7.66-7.58 (dd, *J*=8.9, 4.6 Hz, 2H), 7.53-7.46 (dd, *J*=8.7, 1.9 Hz, 1H), 7.39 (d, *J*=2.1 Hz, 1H), 7.18-7.12 (dd, *J*=8.9, 2.3 Hz, 1H), 3.83 (m, 1H), 0.87-0.78 (m, 4H); MS (ESI): 264.2 *m/z* [MH+], $C_{13}H_{12}BrO$ requires 264.2.

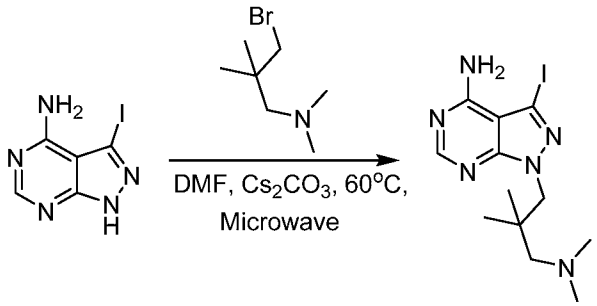
2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane: 2-Bromo-6-cyclopropoxynaphthalene was subjected to the general pinacol ester formation procedure to afford 1.01 g, (65% yield) of a white crystalline product. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.27 (s, 1H), 7.77 (m, 3H) 7.45 (d, , J=2.3 Hz, 1H), 7.14 (dd, J=8.9, 2.48 Hz, 1H), 3.88 (m, 1H), 1.40 (s, 12H), 0.87 (m, 2H), 0.82 (m, 2H); MS (ESI): 311.5 m/z [MH+], $C_{19}H_{24}BO_3$ requires 311.2.



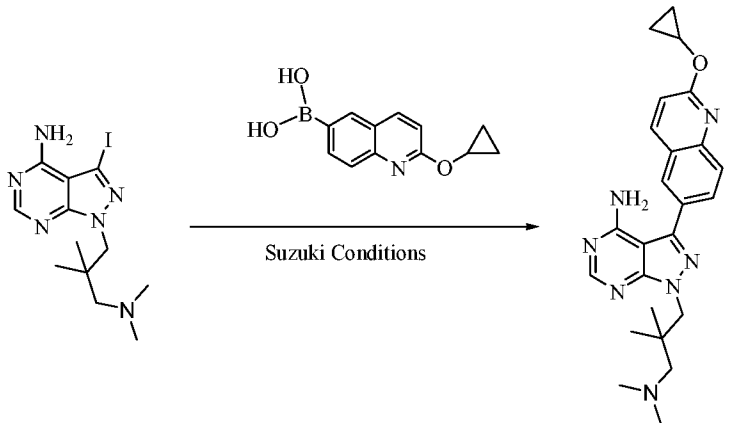
2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolaneand tert-butyl 4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate

were subjected to the general Suzuki coupling procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford 3-(6-cyclopropoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine. ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (s, 1H), 8.17 (s, 1H), 8.03 (d, J=8.5 Hz, 1H), 7.92 (d, J=9.1 Hz, 1H), 7.80 (d, J=8.5 Hz, 1H), 7.64 (d, J=2.0 Hz, 1H), 7.27 (dd, J=8.9, 1.8 Hz, 1H), 4.52 (d, J=6.0 Hz, 2H), 3.98 (m, 1H), 3.56 (m, 2H), 3.05 (m, 2H), 2.87 (s, 3H), 2.44 (m, 1H), 2.00 (m, 2H), 1.76 (m, 2H), 0.92 (m, 2H), 0.80 (m, 2H); MS (ESI) 429.5 *m/z* [MH+], $C_{25}H_{29}N_6O$ requires 429.6.

Example 5: 3-(2-Cyclopropoxyquinolin-6-yl)-1-(3-(dimethylamino)-2,2-dimethylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



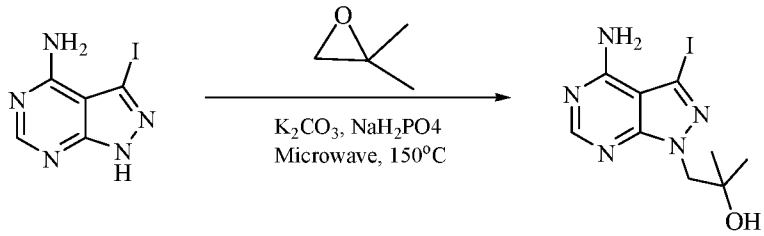
1-(3-(Dimethylamino)-2,2-dimethylpropyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine was generated with 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine and 3-bromo-N,N,2,2-tetramethylpropan-1-amine using the general R_2 alkylation procedure, to afford the product as pale yellow solid, (286 mg, 40% yield); ^{1}H NMR (300 MHz, MeOD4) δ 8.38 (s, 1H), 4.46 (s, 2H), 3.22 (s, 2H), 3.06 (s, 6H), 1.16 (s, 6H); MS (ESI) 375.2 [MH+], $C_{12}H_{20}IN_6$ requires 375.2.



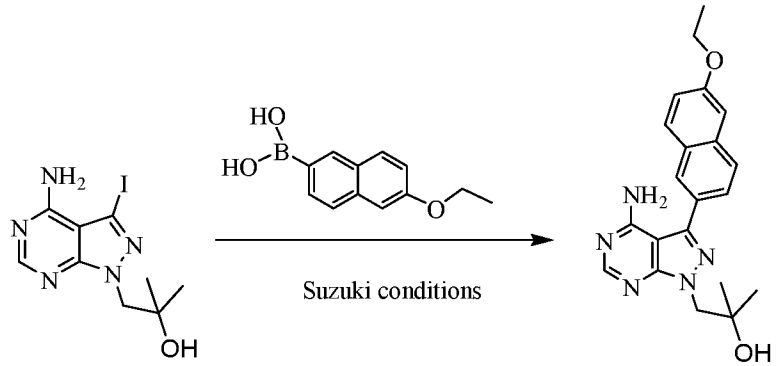
2-Cyclopropoxyquinolin-6-ylboronic acid and 1-(3-(dimethylamino)-2,2-dimethylpropyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine were subjected to the general

Suzuki coupling procedure to afford 3-(2-cyclopropoxyquinolin-6-yl)-1-(3-(dimethylamino)-2,2-dimethylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine. ^{1}H NMR (300 MHz, CD_3OD) δ 8.29 (s, 1H), 8.22 (d, J=8.9 Hz, 1H,), 8.11 (d, J=1.6 Hz, 1H) 8.01 (s, 1H), 8.00 (dd, J = 8.5, 1.5 Hz, 1H,), 7.07 (d, J=8.9 Hz, 1H), 4.46 (m, 1H), 4.36 (s, 2H), 2.46 (s, 2H), 2.44 (s, 6H), 1.03 (s, 6H), 0.93-0.82 (m, 4H). MS (ESI) 432.6 m/z [MH+], $C_{24}H_{30}N_7O$ requires 432.5.

Example 6: 1-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol



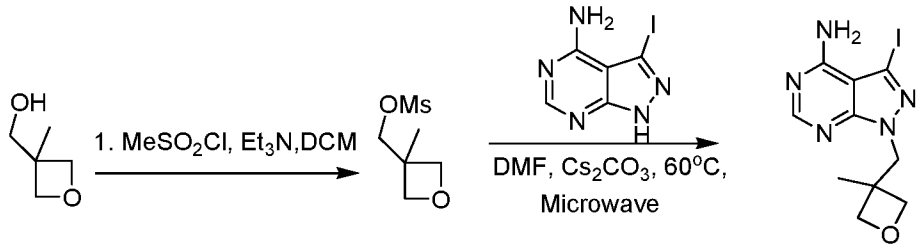
1-(4-Amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol was generating using the general R_2 alkylation procedure using 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (500 mg, 1.92 mmol), 2,2-dimethyloxirane (0.276 mg, 3.80 mmol), and K_2CO_3:NaH_2PO_4 (0.262 mg, 1.90 mmol) in 3 mL of a acetonitrile:water (8.5:1.5) mixture. The reaction was stirred at 150 °C for 3 h in a microwave, affording 1-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol (150 mg, yield, 23.4% yield) as a white solid after purification using a methanol/dichloromethane solvent gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.26 (s, 1H), 4.37 (s, 2H), 1.29 (s, 6H). MS (ESI) m/z 334.2 [MH^+], $C_9H_{13}IN_5O$ requires 334.1.



6-ethoxynaphthalen-2-ylboronic acid and 1-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol were subjected to the general Suzuki coupling procedure to afford compound 1-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol; ^{1}H NMR (300 MHz, CD_3OD) δ 8.26 (s, 1H), 8.09 (s,

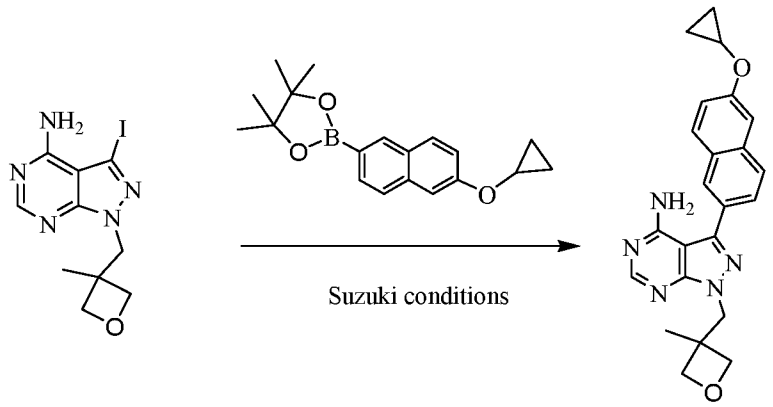
1H), 7.97-7.83 (m, 2H), 7.76 (dd, J=8.2, 1.4 Hz, 1H), 7.30 (d, J=2.0 Hz, 1H), 7.20 (dd, J=8.9, 1.4 Hz, 1H), 4.41 (s, 2H), 4.19 (q, *J*=7.0 Hz, 2H), 1.47 (t, *J*=6.8 Hz, 3H), 1.28 (s, 6H). MS (ESI) 378.2 *m/z* [MH+], $C_{21}H_{24}N_5O_2$ requires 378.1.

Example 7: 3-(6-cyclopropoxynaphthalen-2-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



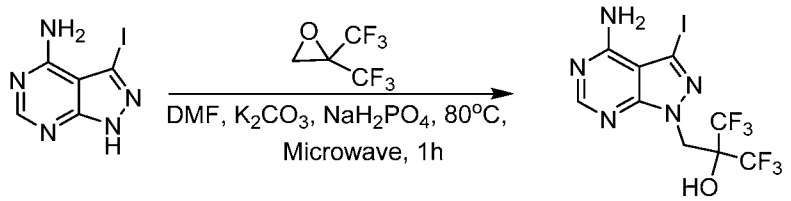
Methanesulfonyl chloride (7.54 mL, 97.9 mmol) was added slowly at 0 °C to a solution of (3-methyloxetan-3-yl)methanol (5.00 mg, 48.9 mmol) and triethylamine (13 mL, 97 mmol) in dichloromethane (20 mL). The reaction was stirred for 5 h at room temperature. After completion of the reaction, dichloromethane was removed by reduced pressure and the reaction was diluted with ethyl acetate. The ethyl acetate was washed with $NaHCO_3$ (25 mL), 1N HCl, brine (50 mL), dried over sodium sulfate, and concentrated. The crude product was subjected to flash chromatography using a hexane/ethyl acetate solvent gradient to afford pure 3-methyloxetan-3-yl)methyl methanesulfonate (4.40 g, 50% yield). ^{1}H NMR (301 MHz, $CDCl_3$) δ 4.70-4.12 (m, 6H), 3.07 (s, 3H), 1.39 (s, 3H); MS (ESI) 181.1 [MH+], $C_6H_{13}O_4S$ requires 181.0.

3-Iodo-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine was generated by subjecting 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.60 mmol) and (3-methyloxetan-3-yl)methyl methanesulfonate (83 mg, 0.46 mmol) to the general R_2 alkylation procedure (110 mg, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H), 5.94 (s, 2H), 4.79 (d, J=6.4 Hz, 2H), 4.56 (s, 2H), 4.41 (d, J=6.1 Hz, 2H), 1.28 (s, 3H); MS (ESI) m/z 346.2 [MH^+], $C_{10}H_{13}IN_5O$ requires 346.1.

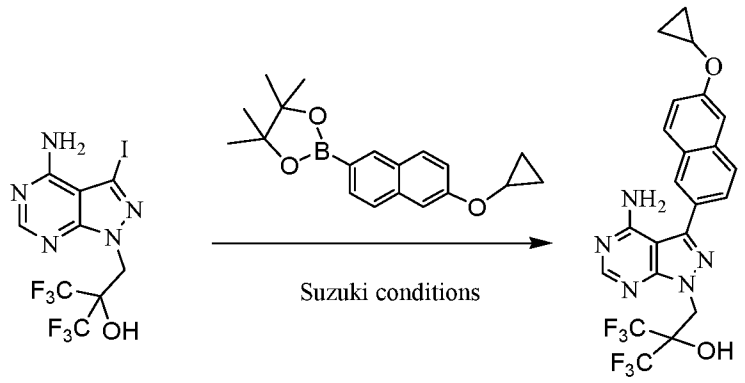


2-(6-cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 3-iodo-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine were subjected to the general Suzuki coupling procedure in order to afford 3-(6-cyclopropoxynaphthalen-2-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.08 (s, 1H), 7.93 (d, J=8.7 Hz, 1H), 7.83 (d, J=8.9 Hz, 1H), 7.77 (d, J=9.4 Hz, 1H), 7.52 (s, 1H), 7.24 (dd, J=10.0, 3.0 Hz, 1H), 5.68 (s, 2H), 4.90 (d, J=6.1 Hz, 2H), 4.64 (s, 2H), 4.45 (d, J=6.1 Hz, 2H), 3.91 (m, 1H), 1.36 (3, 3H), 0.96-0.83 (m, 4H); MS (ESI) 402.2 m/z [MH+], $C_{23}H_{24}N_5O_2$ requires 402.4.

Example 8: 2-((4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol

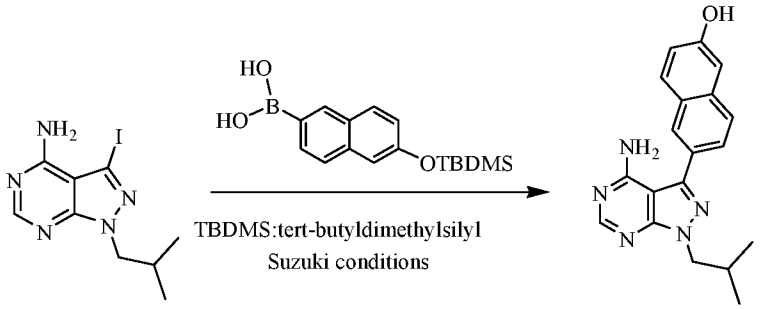


2-((4-Amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol was generated by subjecting 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (250 mg, 0.96 mmol), 2,2-bis(trifluoromethyl)oxirane (0.17 mL, 1.44 mmol), and K_2CO_3:NaH_2PO_4 (198 mg, 1.44 mmol) to the general R_2 alkylation procedure (126 mg, 30% yield). ^{1}H-NMR (301 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.02 (s, 1H), 6.33 (s, 2H); MS (ESI) *m/z* 442.1 [MH^+], $C_9H_6F_6IN_5O$ requires 442.2.

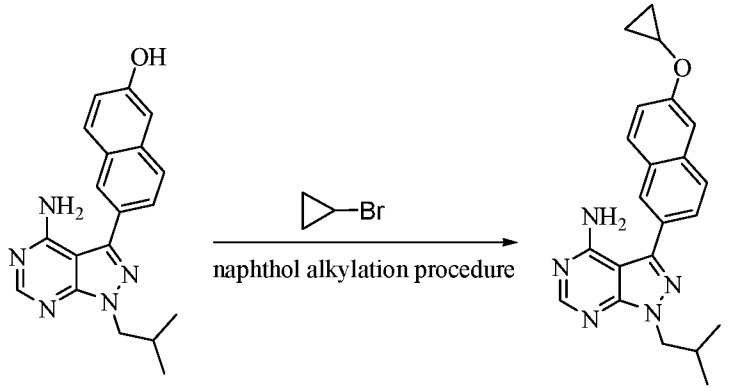


2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 2-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol were subjected to the general Suzuki coupling procedure in order to afford 2-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol. ^{1}H NMR (300 MHz, CD_3OD) δ 8.32 (s, 1H), 8.11 (s, 1H), 7.97 (d, J=8.5 Hz, 1H), 7.87 (d, J=9.1 Hz, 1H), 7.76 (dd, J=8.5, 1.6 Hz, 1H), 7.59 (d, J=2.0, 1H), 7.23 (dd, J=8.9, 2.2 Hz, 1H), 5.03 (s, 2H), 3.94 (m, 1H), 0.94-0.76 (m, 4H); MS (ESI) 498.2 *m/z* [MH+], $C_{22}H_{18}F_6N_5O_2$ requires 498.2.

Example 9: 3-(6-cyclopropoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine

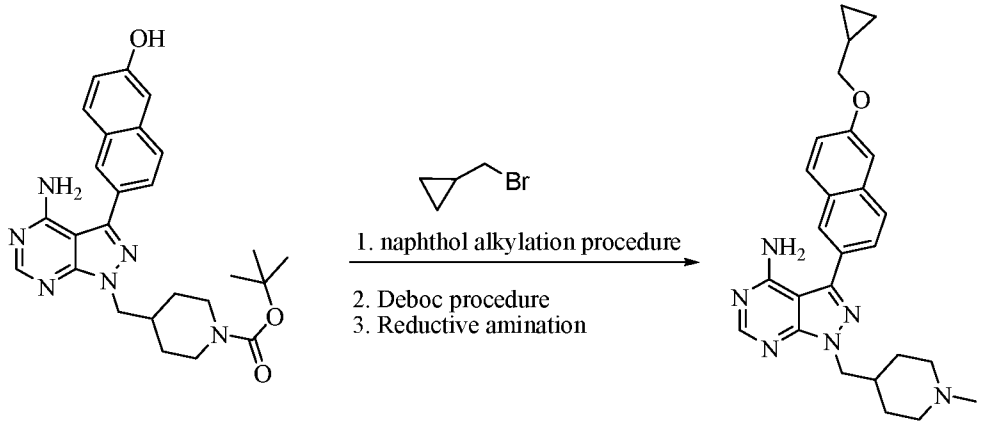


6-tert-butyldimethylsilyloxy-2-naphthaleneboronic acid and 3-iodo-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine[2] were subjected to the general Suzuki coupling procedure. The crude product was purified by silica gel using dichloromethane/methanol gradient (note: deprotection of the tert-butyldimethylsilyloxy protecting group was observed after purification). ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (s, 1H), 8.03 (s, 1H), 7.82 (m, 2H), 7.71 (m, 1H), 7.18 (m, 2H), 4.21 (d, J=4.2 Hz, 2H), 2.39 (m, 1H), 0.96 (d, J=6.5 Hz, 6H); MS (ESI) 334.4 *m/z* [MH+], $C_{19}H_{19}N_5O$ requires 334.2.



6-(4-amino-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-ol and bromocyclopropane were subjected to the general naphthol alkylation procedure in order to afford 3-(6-cyclopropoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine. ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (s, 1H), 8.10 (s, 1H), 7.98 (d, *J*=8.7 Hz, 1H), 7.89 (d, *J*=8.9 Hz, 1H), 7.76 (dd, *J*=8.5, 1.6 Hz, 1H), 7.61 (d, *J*=2.2 Hz, 1H), 7.25-7.18 (dd, *J*=8.9, 2.2 Hz, 1H), 4.24 (d, *J*=7.25 Hz, 2H), 3.95 (m, 1H), 2.38 (m, 1H), 0.97 (d, *J*=6.6 Hz, 6H), 0.90 (m, 2H), 0.79 (m, 2H); MS (ESI) 374.2 *m/z* [MH+], $C_{22}H_{24}N_5O$ requires 374.4.

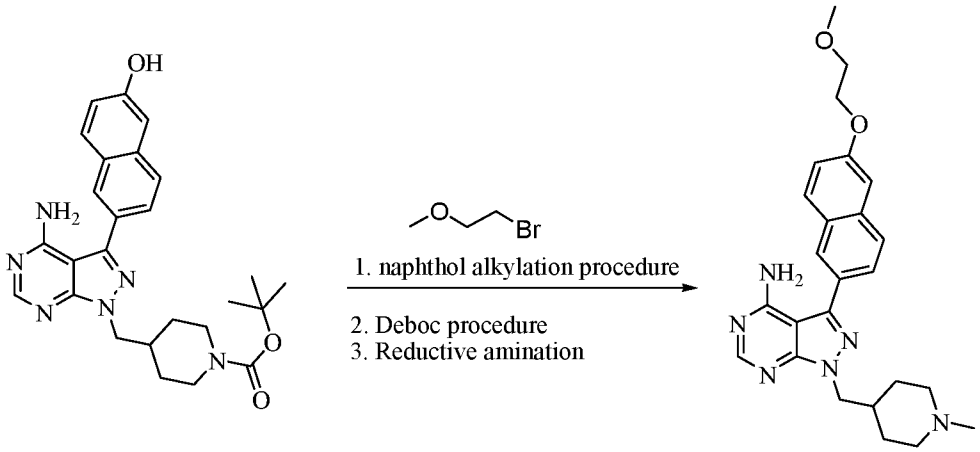
Example 10: 3-(6-(Cyclopropylmethoxy)naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



Tert-butyl4-((4-amino-3-(6-hydroxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate and (bromomethyl)cyclopropane were subjected to the general naphthol alkylation procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.51 (s, 1H), 8.17 (s, 1H), 8.01 (d, *J*=8.5 Hz, 1H), 7.93 (d, *J*=9.1 Hz, 1H), 7.81 (d, *J*=9.3 Hz, 1H), 7.36 (s, 1H), 7.30 (d, *J*=9.3 Hz, 1H), 4.56 (d, *J*=6.4 Hz, 2H), 4.03 (d, *J*=6.6 Hz, 2H), 3.57 (m, 2H), 3.06 (m, 2H), 2.88 (s, 3H), 2.45 (m, 1H), 2.02 (m, 2H), 1.77

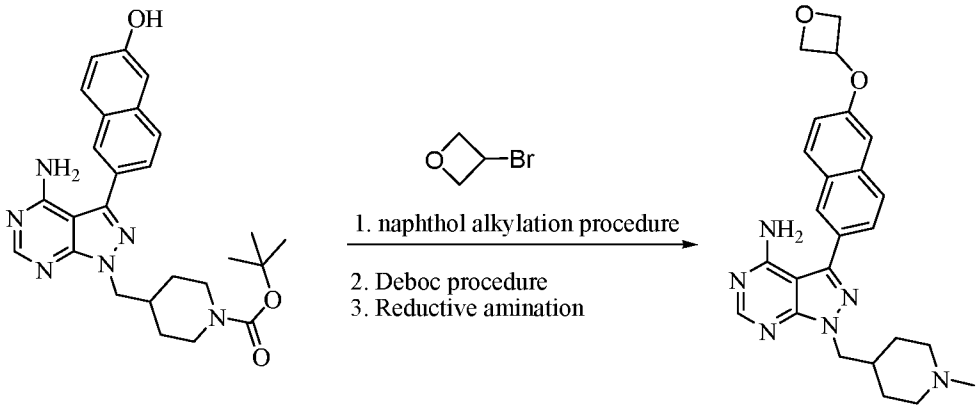
(m, 2H), 1.44 (m, 1H), 0.71 (m, 2H), 0.46 (m, 2H); MS (ESI) 443.5 *m/z* [MH+], $C_{26}H_{31}N_6O$ requires 443.5.

Example 11: 3-(6-(2-Methoxyethoxy)naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



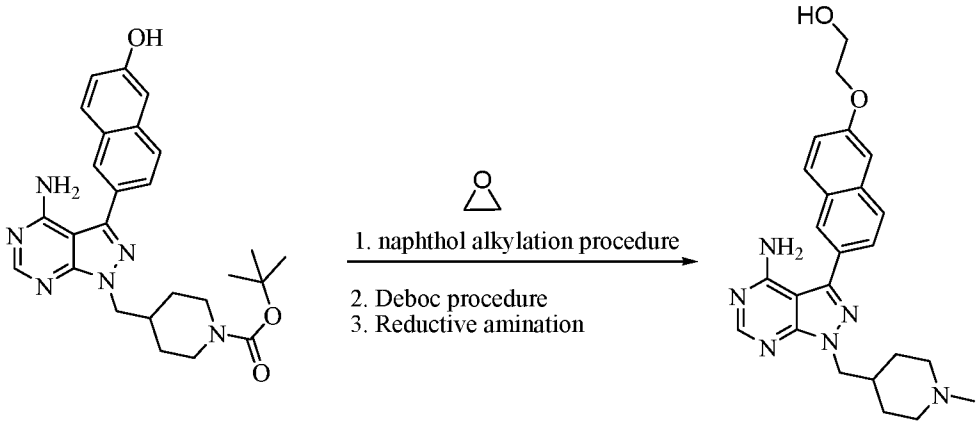
Tert-butyl4-((4-amino-3-(6-hydroxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate and bromomethoxyethane were subjected to the general naphthol alkylation procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.50 (s, 1H), 8.16 (s, 1H), 8.01 (d, *J*=7.8 Hz, 1H), 7.93 (d, *J*=8.5 Hz, 1H), 7.80 (d, *J*=8.3 Hz, 1H), 7.40 (s, 1H), 7.30 (d, *J*=7.8 Hz, 1H), 4.52 (d, *J*=4.2 Hz, 2H), 4.31 (m, 2H), 3.86 (m, 2H), 3.62 (m, 2H), 3.54 (s, 3H), 3.04 (m, 2H), 2.87 (s, 3H), 2.44 (m, 1H), 1.99 (m, 2H), 1.77(m, 2H); MS (ESI) 447.5 *m/z* [MH+], $C_{25}H_{31}N_6O_2$ requires 447.5.

Example 12: 1-((1-Methylpiperidin-4-yl)methyl)-3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



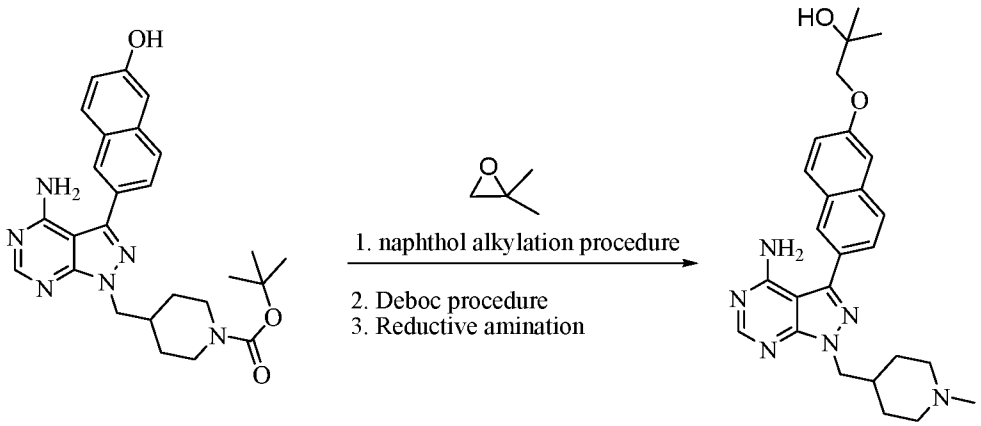
Tert-butyl4-((4-amino-3-(6-hydroxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate and 3-bromooxetane were subjected to the general naphthol alkylation procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (s, 1H), 8.18 (s, 1H), 8.06-7.92 (m, 2H), 7.82 (d, *J*=8.5 Hz, 1H), 7.51 (s, 1H), 7.36 (d, *J*=9.1 Hz, 1H), 4.81 (m, 1H), 4.53 (d, *J*=5.6 Hz, 2H), 3.99-3.84 (m, 4H), 3.57 (m, 2H), 3.05 (m, 2H), 2.86 (s, 3H), 2.44 (m, 1H), 2.00 (m, 2H), 1.74 (m, 2H); MS (ESI) 445.2 *m/z* [MH+], $C_{25}H_{29}N_6O_2$ requires 445.2.

Example 13: 2-(6-(4-Amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)ethanol



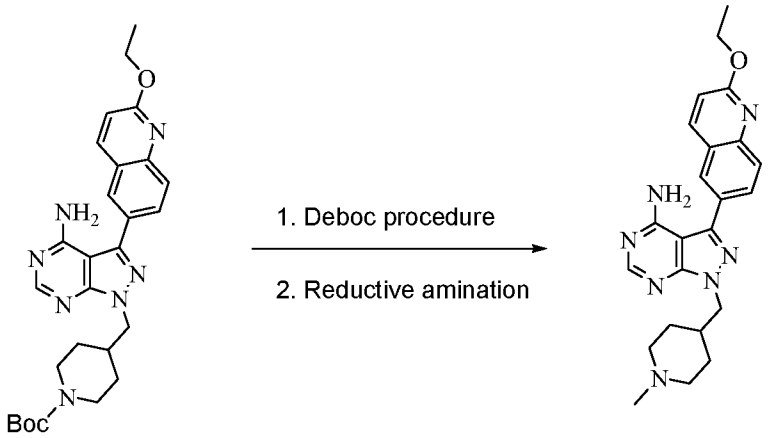
Tert-butyl4-((4-amino-3-(6-hydroxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate and oxirane were subjected to the general naphthol alkylation procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.50 (s, 1H), 8.17 (s, 1H), 8.02 (d, J=8.3 Hz, 1H), 7.94 (d, *J*=8.9 Hz, 1H), 7.80 (d, *J*=8.9 Hz, 1H), 7.41 (d, *J*=2.2 Hz, 1H), 7.34 (dd, *J*=8.7, 2.0 Hz, 1H), 4.53 (d, *J*=6.4 Hz, 2H), 4.25 (t, *J*=4.5 Hz, 2H), 3.99 (t, *J*=4.5 Hz, 2H), 3.56 (m, 2H), 3.04 (m, 2H), 2.86 (s, 3H), 2.44 (m, 1H), 2.00 (m, 2H), 1.72 (m, 2H); MS (ESI) 433.3 *m/z* [MH+], $C_{24}H_{29}N_6O_2$ requires 433.5.

Example 14: 1-(6-(4-Amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)-2-methylpropan-2-ol



Tert-butyl4-((4-amino-3-(6-hydroxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate and 2,2-dimethyloxirane were subjected to the general naphthol alkylation procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.48 (s, 1H), 8.17 (s, 1H), 8.02 (d, J=8.9 Hz, 1H), 7.94 (d, *J*=8.7 Hz, 1H), 7.79 (d, *J*=8.5 Hz, 1H), 7.43-7.32 (m, 2H), 4.53 (d, *J*=6.8 Hz, 2H), 3.98 (s, 2H), 3.56 (m, 2H), 3.04 (m, 2H), 2.87 (s, 3H), 2.44 (m, 1H), 2.00 (m, 2H), 1.77 (m, 2H), 1.41 (s, 6H); MS (ESI) 461.5 *m/z* [MH+], $C_{26}H_{33}N_6O_2$ requires 461.5.

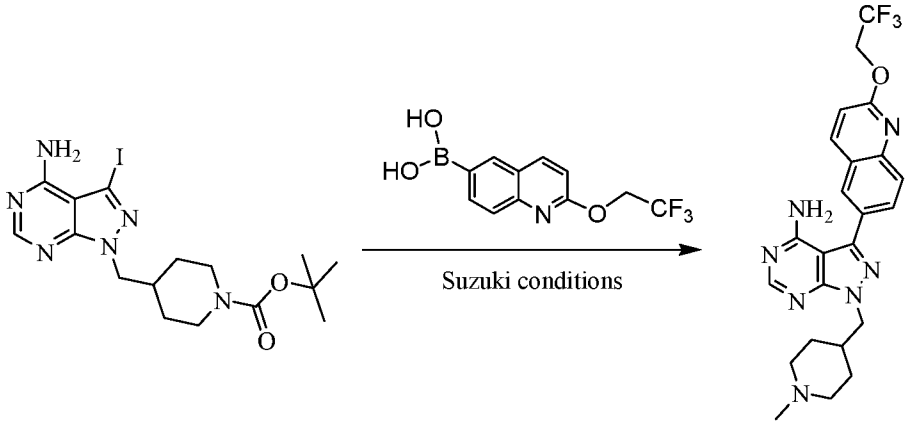
Example 15: 3-(2-Ethoxyquinolin-6-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



Tert-butyl4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate was subjected to the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.29 (s, 1H), 8.22 (d, J=8.2 Hz, 1H), 8.12 (s, 1H), 7.98 (m, 2H), 7.04 (d, J=8.5 Hz, 1H), 4.55 (q, J=6.8 Hz, 2H), 4.38 (d, *J*=4.3 Hz, 2H), 2.95 (m, 2H), 2.30 (s, 3H),

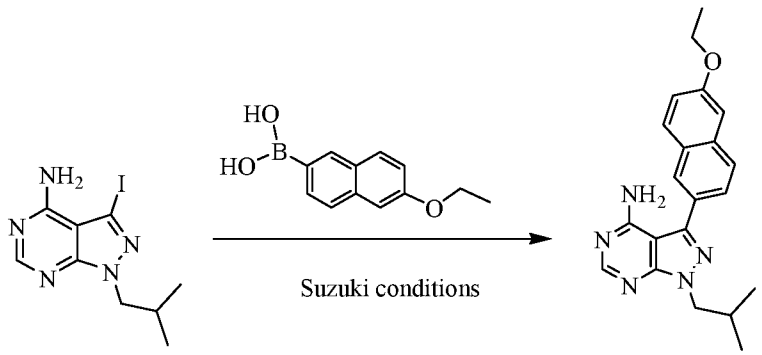
2.99 (m, 2H),1.67 (m, 2H), 1.48(m, 4H); MS (ESI) 418.3 *m/z* [MH+], $C_{23}H_{28}N_7O$ requires 418.5.

Example 16: **1-((1-Methylpiperidin-4-yl)methyl)-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine**



2-(2,2,2-Trifluoroethoxy)quinolin-6-ylboronic acid and tert-butyl 4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate were subjected to the general Suzuki coupling procedure followed by the general boc-deprotection procedure and general reductive amination procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (s, 1H), 8.39 (d, J=8.5 Hz, 1H), 8.24 (s, 1H), 8.04 (s, 2H), 7.18 (d, J=7.0 Hz, 1H), 5.09 (q, J=8.7 Hz, 2H), 4.52 (s, 2H), 3.54 (m, 2H), 3.04 (m, 2H), 2.84 (s, 3H), 2.45 (m, 1H), 1.98 (m, 2H), 1.75 (m, 2H); MS (ESI) 472.2 *m/z* [MH+], $C_{23}H_{25}F_3N_7O$ requires 472.5.

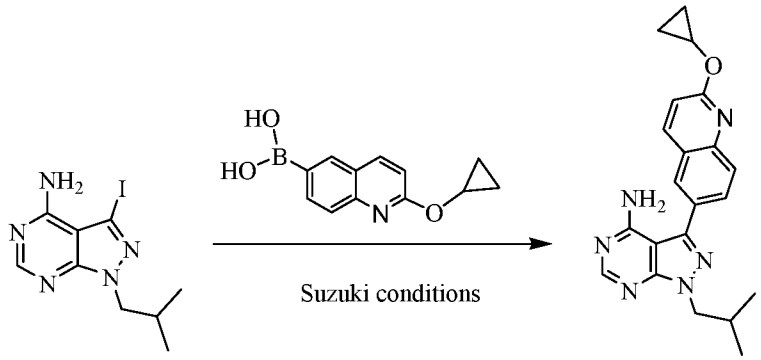
Example 17: 3-(6-ethoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine



6-ethoxynaphthalen-2-ylboronic acid and 3-iodo-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine were subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (s, 1H), 8.09 (s, 1H), 7.96 (d, J=8.1

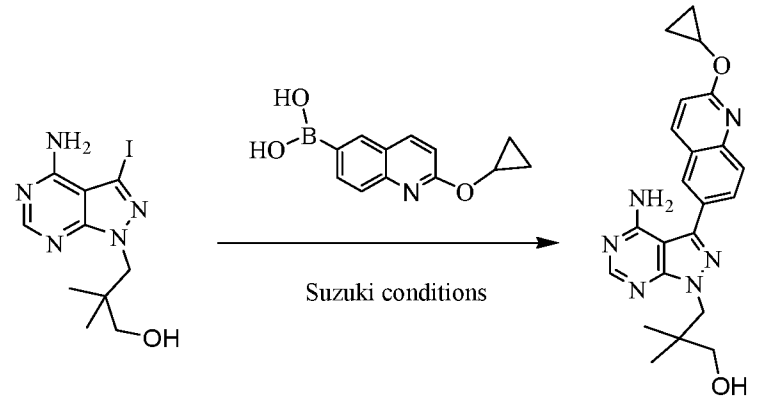
Hz, 1H), 7.89 (d, *J*=9.1 Hz, 1H), 7.75 (dd, *J*=8.2, 2.0 Hz, 1H), 7.32 (s, 1H), 7.22 (dd, *J*=9.1, 2.4 Hz, 1H), 4.28-4.17 (m, 4H), 2.39 (m, 1H), 1.48 (t, *J*=6.8 Hz, 3H), 0.96 (d, *J*=6.6 Hz, 6H); MS (ESI) 362.4 *m/z* [MH+], $C_{21}H_{24}N_5O$ requires 362.2.

Example 18: 3-(2-Cyclopropoxyquinolin-6-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine



2-Cyclopropoxyquinolin-6-ylboronic acid and 3-iodo-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine were subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.25 (s, 1H), 8.23 (d, *J*=8.9 Hz, 1H), 8.10 (s, 1H), 8.00-7.91 (m, 2H), 7.02 (d, *J*=8.7 Hz, 1H), 4.46 (s, 1H), 4.22 (d, *J*=7.4 Hz, 2H), 2.36 (m, 1H), 0.96 (d, *J*=6.6 Hz, 6H), 0.91-0.72 (m, 4H); MS (ESI) 375.4 *m/z* [MH+], $C_{21}H_{23}N_6O$ requires 375.4.

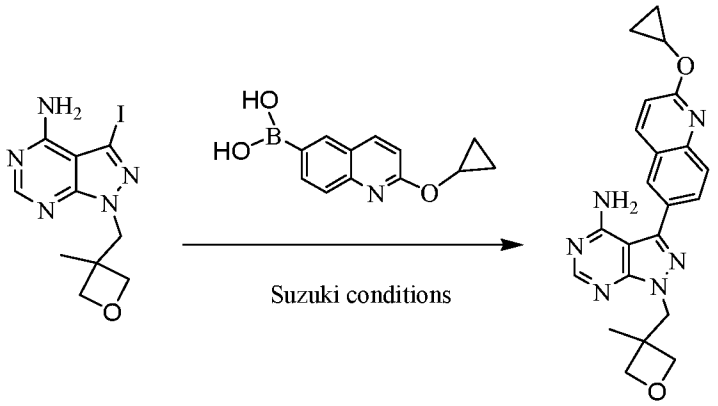
Example 19: 3-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol



2-Cyclopropoxyquinolin-6-ylboronic acid and previously reported 3-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol were subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) *δ*

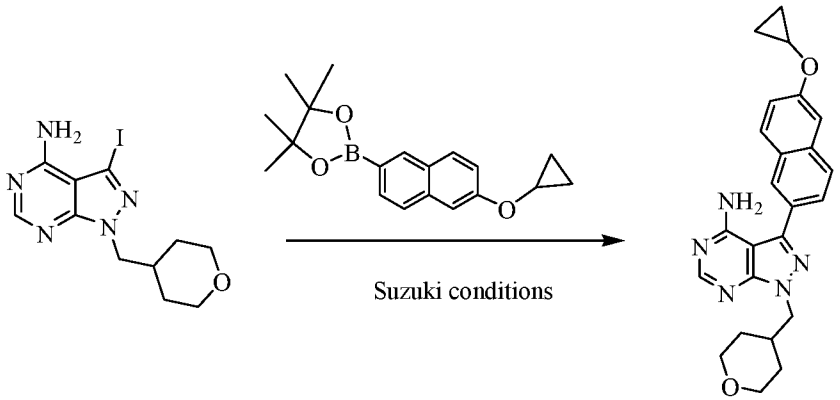
8.65 (s, 1H), 8.50 (s, 1H), 8.36 (m, 1H), 8.21-8.05 (m, 2H), 7.51 (d, *J*=8.3 Hz, 1H), 4.59 (s, 2H), 4.47 (s, 2H), 4.46 (m, 1H), 1.01 (s, 6H), 0.70 (m, 4H); MS (ESI) 405.2 *m/z* [MH+], $C_{22}H_{25}N_6O_2$ requires 405.4.

Example 20: 3-(2-Cyclopropoxyquinolin-6-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



2-Cyclopropoxyquinolin-6-ylboronic acid and 3-iodo-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine were subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.12-8.01 (m, 3H), 7.96 (d, *J*=8.3 Hz, 1H), 6.97 (d, *J*=8.0 Hz, 1H), 5.54 (s, 2H), 4.91 (d, *J*=6.1 Hz, 2H), 4.64 (s, 2H), 4.56 (m, 1H), 4.45 (d, *J*=6.1 Hz, 2H), 1.36 (s, 3H), 0.95-0.82 (m, 4H); MS (ESI) 403.2 *m/z* [MH+], $C_{22}H_{23}N_6O_2$ requires 403.4.

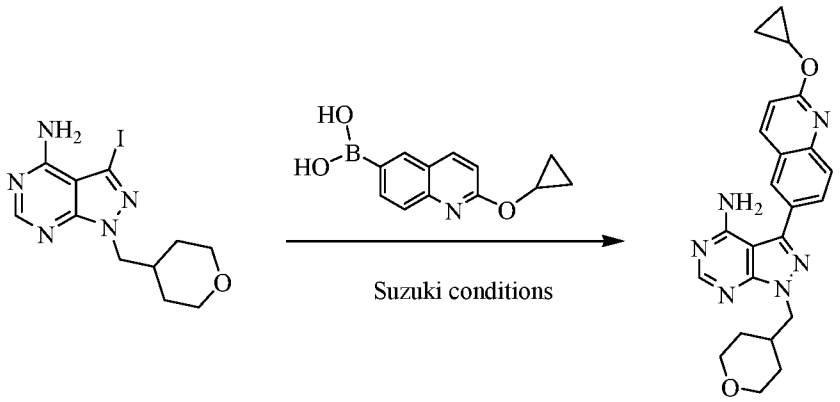
Example 21: 3-(6-Cyclopropoxynaphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 3-iodo-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine were

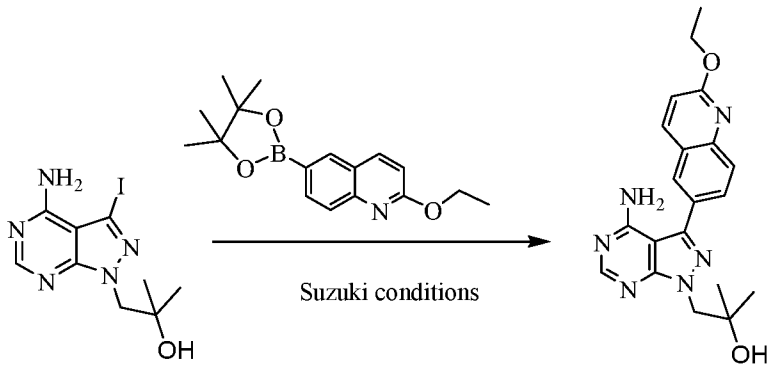
subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.47 (s, 1H), 8.16 (s, 1H), 8.03 (d, J=8.2 Hz, 1H), 7.92 (d, J=9.1 Hz, 1H), 7.78 (d, J=8.2 Hz, 1H), 7.64 (s, 1H), 7.27 (dd, J=9.1, 2.2 Hz, 1H) 4.45 (d, J=6.8 Hz, 2H,), 3.99 (m, 1H), 3.76 (m, 2H), 3.05 (m, 2H), 2.40 (m, 1H), 1.61 (m, 2H), 1.46 (m, 2H), 0.93-0.80 (m, 4H); MS (ESI) 416.4 *m/z* [MH+], $C_{24}H_{25}N_5O_2$ requires 416.4.

Example 22: 3-(2-Cyclopropoxyquinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine



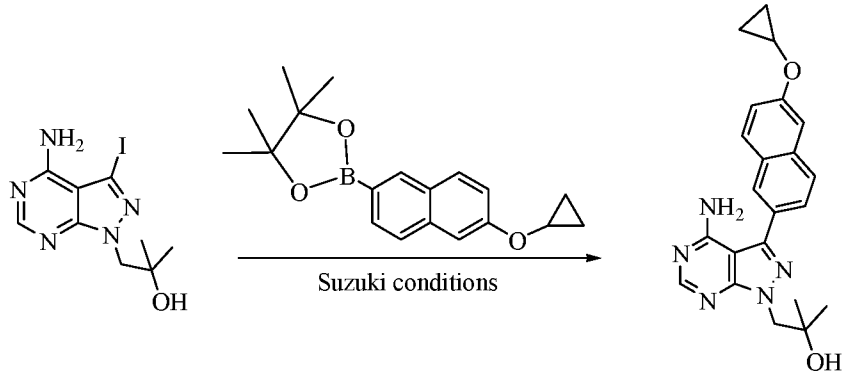
2-cyclopropoxyquinolin-6-ylboronic acid and 3-iodo-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine were subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.31 (s, 1H), 8.22 (d, J=8.9 Hz, 1H), 8.12 (d, J=1.8 Hz, 1H), 8.06 (d, J=8.7 Hz, 1H), 8.00 (dd, J=8.5, 1.2 Hz, 1H), 7.07 (d, J=8.9 Hz, 1H), 4.47 (m, 1H) 4.36 (d, J=7.2 Hz, 2H,), 3.98 (m, 2H), 3.42 (m, 2H), 2.34 (m, 1H), 1.59 (m, 2H), 1.49 (m, 2H), 0.93-0.83 (m, 4H); MS (ESI) 416.4 *m/z* [MH+], $C_{23}H_{24}N_6O_2$ requires 416.4.

Example 23: 1-(4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol



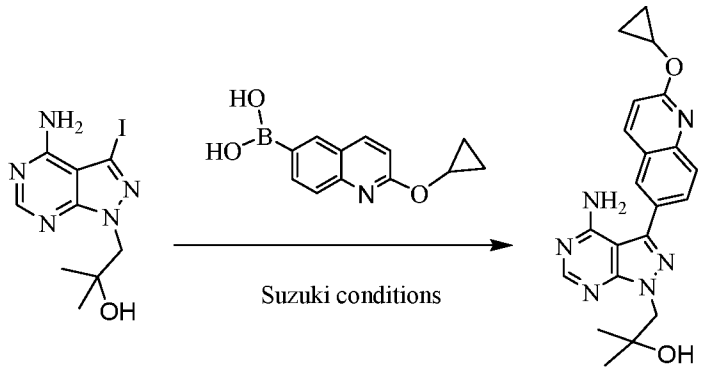
2-ethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and 1-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol were subjected to the general Suzuki coupling procedure to afford compound the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.69 (d, J=9.1 Hz, 1H), 8.45 (s, 1H), 8.34 (s, 1H), 8.17 (d, J=8.0 Hz, 1H), 8.04 (d, J=7.4 Hz, 1H), 7.45 (d, J=7.4 Hz, 1H), 4.67 (q, J=6.0 Hz, 2H), 4.50 (s, 2H), 1.54 (t, J=6.5 Hz, 3H), 1.31 (s, 6H). MS (ESI) 379.2 m/z [MH+], $C_{20}H_{23}N_6O_2$ requires 379.1.

Example 24: 1-(4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol



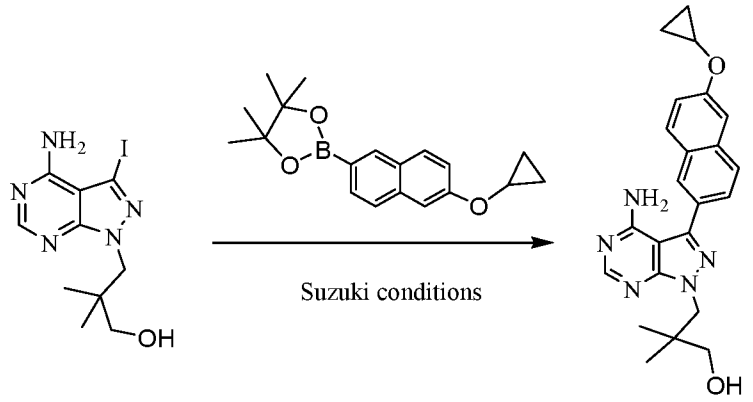
2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 1-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol were subjected to the general Suzuki coupling procedure to afford title compound the product; ^{1}H NMR (500 MHz, CD_3OD) δ 8.26 (s, 1H), 8.08 (s, 1H), 7.93 (d, J=8.4 Hz, 1H), 7.85 (d, J=8.81 Hz, 1H), 7.76 (d, J=8.4 Hz, 1H), 7.57 (s, 1H), 7.20 (dd, J=8.8, 1.8 Hz, 1H), 4.40 (s, 2H), 3.91 (m, 1H), 1.27 (s, 6H), 0.88 (m, 2H), 0.78 (m, 2H). MS (ESI) 390.2 m/z [MH+], $C_{22}H_{24}N_5O_2$ requires 390.1.

Example 25: 1-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol



2-Cyclopropoxyquinolin-6-ylboronic acid and 1-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol were subjected to the general Suzuki coupling procedure in order to afford the product. NMR (300 MHz, CD_3OD) δ 8.28 (s, 1H), 8.28-8.24 (d, J=8.9 Hz, 1H), 8.15 (t, J=1.4 Hz, 1H), 8.01 (d, J=1.2 Hz, 2H), 7.06 (d, J=8.7 Hz, 1H), 4.53-4.45 (m, 1H), 4.42 (s, 2H), 1.28 (s, 6H), 0.92-0.85 (m, 2H), 0.85-0.77 (m, 2H); MS (ESI) 391.1 m/z [MH^+], $C_{21}H_{23}N_6O_2$ requires 391.2.

Example 26: 3-(4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol



2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and previously reported 3-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol were subjected to the general Suzuki coupling procedure in order to afford the product. ^{1}H NMR (300 MHz, CD_3OD) δ 8.30 (s, 1H), 8.12 (s, 1H), 8.00 (d, J=8.7 Hz, 1H), 7.90 (d, J=8.9 Hz, 1H), 7.78 (dd, J=8.2, 1.5 Hz, 1H), 7.62 (d, J=2.4, 1H), 7.24 (dd, J=9.1, 2.4 Hz, 1H), 4.33 (s, 2H), 3.97 (m, 1H), 3.32 (s, 2H), 1.22 (s, 6H), 0.94-0.81 (m, 4H); MS (ESI) 404.5 m/z [MH+], $C_{23}H_{26}N_5O_2$ requires 404.4.

Examples 27-119

The following compounds were prepared by the methothes disclosed herein:

Ex. No.	Compound
27	3-(2-methoxyquinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
28	3-(2-(cyclopropylmethoxy)quinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
29	3-(6-(cyclopropylmethoxy)naphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
30	3-(6-cyclobutoxynaphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

31	3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
32	3-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol
33	1-(6-(4-amino-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)-2-methylpropan-2-ol
34	2-(6-(4-amino-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)ethanol
35	1-isobutyl-3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
36	1-isobutyl-3-(6-(2-methoxyethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
37	3-(4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol
38	3-(2-(2-methoxyethoxy)quinolin-6-yl)-1-neopentyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine
39	3-(2-cyclopropoxyquinolin-6-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
40	1-(azetidin-3-ylmethyl)-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
41	3-(2-cyclopropoxyquinolin-6-yl)-1-((1-methylazetidin-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
42	3-(2-(2-methoxyethoxy)quinolin-6-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
43	1-isobutyl-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
44	1-(piperidin-4-ylmethyl)-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
45	2-(3-(4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropoxy)ethanol
46	1-(4-amino-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol
47	3-(7-ethoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine
48	3-(7-ethoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
49	3-(6-ethoxynaphthalen-2-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
50	2-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-1-morpholinoethanone
51	2-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-1-morpholinoethanone
52	2-((4-amino-3-(6-(methoxymethyl)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol
53	2-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol
54	1-(3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrrolo[3,2-c]pyridin-1-yl)-2-methylpropan-2-ol

55	1-(4-amino-5-(2-cyclopropoxyquinolin-6-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol
56	4-(4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,3,3-trimethylbutan-2-ol
57	1-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclobutanol
58	1-(4-amino-5-(6-cyclopropoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol
59	1-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclobutanol
60	1-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclopentanol
61	1-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclopentanol
62	4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-tetrahydro-2H-pyran-4-ol
63	4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-tetrahydro-2H-pyran-4-ol
64	(3-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)oxetan-3-yl)methanol
65	(3-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)oxetan-3-yl)methanol
66	3-(6-cyclopropoxynaphthalen-2-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
67	3-(2-cyclopropoxyquinolin-6-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
68	methyl 3-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropanoate
69	methyl 3-(4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropanoate
70	4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol
71	4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol
72	methyl 2-((6-(4-amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)acetate
73	2-((6-(4-amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)acetonitrile
74	4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-4-carbonitrile
75	4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile
76	3-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropanoic acid
77	3-(6-cyclopropoxynaphthalen-2-yl)-1-(2-fluoro-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
78	4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol

79	4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol
80	4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol
81	4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol
82	4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol
83	4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile
84	4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol
85	4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol
86	4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-4-carbonitrile
87	4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile
88	2-(6-(4-amino-1-(2-hydroxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)acetonitrile
89	methyl 2-(6-(4-amino-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)quinolin-2-yloxy)acetate
90	3-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol
91	3-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylazetidin-3-ol
92	4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile
93	3-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol
94	3-(6-(difluoromethoxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
95	3-(6-(difluoromethoxy)naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
96	3-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol
97	3-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylazetidin-3-ol
98	4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol
99	4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol
100	4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-

	d]pyrimidin-1-yl)methyl)piperidine-4-carbonitrile
101	3-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol
102	4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile
103	3-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylazetidin-3-ol
104	1-(3-(6-ethoxynaphthalen-2-yl)-1-((1-(methylcarbamoyl)piperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-3-methylurea
105	4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-N-methylpiperidine-1-carboxamide
106	3-(2-ethoxyquinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine
107	3-(6-ethoxynaphthalen-2-yl)-1-(2-(piperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
108	3-(6-ethoxynaphthalen-2-yl)-1-(2-(1-methylpiperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
109	3-(6-ethoxynaphthalen-2-yl)-1-(2-(1-ethylpiperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
110	3-(6-ethoxynaphthalen-2-yl)-1-(2-(1-propylpiperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
111	1-(2-(1-(3-aminopropyl)piperidin-4-yl)ethyl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
112	3-(6-ethoxynaphthalen-2-yl)-1-(1-propylpiperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
113	1-(1-(3-aminopropyl)piperidin-4-yl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
114	1-(6-ethoxynaphthalen-2-yl)-3-(piperidin-4-ylmethyl)imidazo[1,5-a]pyrazin-8-amine
115	1-(6-ethoxynaphthalen-2-yl)-3-((1-methylpiperidin-4-yl)methyl)imidazo[1,5-a]pyrazin-8-amine
116	1-isopropyl-3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
117	1-((6-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)-2-methylpropan-2-ol
118	2-((6-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)ethan-1-ol
119	3-(2-ethoxyquinolin-6-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine.

Biological Example 1: *Tg*CDPK1 Enzymatic Inhibition Assay.

Inhibitors were evaluated in triplicate in eight-point dilutions (3-fold dilutions) during the enzymatic reactions. *Tg*CDPK1 enzymatic inhibition was determined with a coupled luciferase assay (Kinaseglo®). 2.1 nM *Tg*CDPK1 and 20 μM BioSyntide-2 (American Peptide Company, Inc. Sunnyvale, CA)) were incubated in 25 μL of buffer containing 1 mM EGTA (pH 7.2), 10 mM $MgCl_2$, 20 mM HEPES ,pH 7.5 (KOH), 0.1% BSA, and 2 mM

$CaCl_2$. The reaction was initiated with the addition of ATP at a 10 μM final concentration. After incubating at 30 °C for 90 min., changes in ATP concentration were determined by adding Kinaseglo® luciferase reagent (Promega, Madison, WI) and measuring luminescence with a MicroBeta2 multi-label plate reader (Perkin Elmer, Waltham, MA). Results were converted to percent inhibition, and IC_{50} values were calculated using nonlinear regression analysis in GraphPad Prism.

Biological Example 2: Src kinase enzymatic inhibition assay.

Inhibitors were evaluated in triplicate in eight-point dilutions (3-fold dilutions) during the enzymatic reactions. Chicken Src enzymatic inhibition was determined with a coupled luciferase assay (Kinaseglo®). 2 nM Src and 61 μM Src substrate peptide (GenScript, Piscataway, NJ) were incubated in 25 μL of buffer containing 40 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM DTT, and 0.1% BSA. The reaction was initiated with the addition of ATP at a 10 μM final concentration. After incubating at 30 °C for 90 min., changes in ATP concentration were determined by adding Kinaseglo® luciferase reagent (Promega, Madison, WI) and measuring luminescence with a MicroBeta2 multi-label plate reader (Perkin Elmer, Waltham, MA). Results were converted to percent inhibition, and IC50 values were calculated using nonlinear regression analysis in GraphPad Prism.

Biological Example 3: 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% CO_2 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.

Biological Example 4: *T. gondii* growth inhibition assay.

The *T. gondii* growth inhibition assay was performed according to a previously reported procedure. Briefly, a dilution series of an inhibitor (diluted in DMSO) was added to DMEM (final DMSO = 0.5%). *T. gondii* expressing a β-galactosidase reporter were added to the DMEM and incubated briefly before adding them to fibroblast monolayers in 96 well plates. Plates were visually inspected for evidence of cytotoxic effects on fibroblasts. After 44 h, β-galactosidase was assayed using chlorophenol red β-galactopyranose (Sigma-Aldrich, St. Louis, MO) as a substrate. Each dilution series of inhibitor was tested at least twice in triplicate. For assays with drug resistant *T. gondii*, the same procedure was followed except cell lines expressing HA-*Tg*CDPK1 or HA-Gly128Met *Tg*CDPK1 in a "wild type" background were tested.

Biological Example 5: *In vivo* efficacy against acute *T. gondii* infection in mice.

Infection and drug administration were performed as previously reported. Mice were infected with type 1 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 10 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 48 h after inoculation. 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 a 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. 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.

Biological Example 6: Pharmacokinetic analysis in mice and rats.

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. PK calculations were performed using Phoenix WinNonlin software (Pharsight).

In rats, test compound was administered to Sprague-Dawley jugular canulated rats (Charles River) by either oral gavage or IV injection followed by blood sampling from the jugular vein at designated time points. The oral dose was administered to each rat at 20 mg/kg for compound Ex. **24** and 5mg/kg for compound Ex. **25** at time = 0 in a 1 mL volume of dosing solution (7% Tween 80, 3% EtOH, 5% DMSO, 0.9% saline.) IV injections were administered at 5 mg/kg from time = 0 to 3 minutes in a 1 mL volume of dosing solution, and blood was sampled at the same time points via the jugular vein. Experiments were performed with groups of 2 rats each for the oral and IV dosing. Plasma was separated and extracted with acetonitrile and quantified by LC/MS analysis. PK calculations were performed using Phoenix WinNonlin software (Pharsight).

Biological Example 7: Distribution of compounds between mouse plasma and brain.

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.

5

Results

The following compounds were tested for inhibition *Tg*CDPK1:

Table 1

Ex. No.	TgCDPK1 IC50 (μM)
1	0.0025
2	0.0172
3	0.0016
4	0.0015
5	0.0045
6	0.0056
7	0.0021
8	0.0949
9	0.0060
10	0.0040
11	0.0155
12	0.0107
13	0.0463
14	0.0242
15	0.0061
16	0.0022
17	0.0008
18	0.0012
19	0.0013
20	0.0018
21	0.0024
22	0.0018
23	0.0033
24	0.0010
25	0.0020
26	0.0009
27	0.0212
29	0.0048
30	0.0026
31	0.0358

Ex. No.	TgCDPK1 IC50 (μM)
32	0.0012
33	0.0057
34	0.0058
35	0.0040
36	0.0017
37	0.0017
38	0.0038
39	0.0010
40	0.0023
41	0.0028
42	0.0080
43	0.0020
44	0.0014
45	1.1991
46	0.0183
47	0.0108
48	0.0191
49	0.0029
50	0.0429
51	0.0158
52	0.0842
53	0.0473
54	1.5500
55	0.0032
56	0.0708
57	0.0061
58	0.0091
59	0.0086
60	0.0127
61	0.0130

Ex. No.	TgCDPK1 IC50 (μM)
62	0.0192
63	0.0128
64	0.0036
65	0.0029
66	1.6629
67	0.4295
68	0.0097
69	0.0432
70	0.0233
71	0.0593
72	0.0496
73	0.0738
74	0.0027
75	0.0183
76	0.0194
77	0.0064
78	0.0007
79	0.0045
80	0.0231
81	0.0013
82	0.0007
83	0.0035
84	0.0040
85	0.0210
86	0.0015
87	0.1030
88	0.1124
89	1.4400
90	0.0051
91	0.0038

Ex. No.	TgCDPK1 IC50 (μM)
92	0.0055
93	0.0029
94	0.0019
95	0.0065
96	0.0078
97	0.0026
98	0.0075
99	0.0358
100	0.0025
101	0.0030
102	0.0182
103	0.0048
106	0.0020
107	0.0019
108	0.0022
109	0.0027
110	0.0030
111	0.0043
112	0.0043
113	0.0043
114	0.0100
115	0.0186
116	0.0061
117	0.0030
118	0.0062
119	0.0034

Based on the abilities of a number of inhibitors to potently block *T. gondii* proliferation in mammalian cells, while demonstrating little or no off-target toxicity or hERG inhibition, the solubility in water and pharmacokinetic (PK) properties were examined (Table 2). The solubility of selected inhibitors was determined at pH = 6.5. The initial PK profiles of these inhibitors were determined after a single 10mg/kg oral dose in three Balb/c mice, with sampling conducted at the time points indicated in the Experimental Section. As a reference, after a 10 mg/kg po dose, previsously reported inhibitor 3-(6-ethoxynaphthalen-2-yl)-1-((1-

methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (identified as compound **150** in US 2013/0018040, and hereafter) has a maximum concentration (C*max*) of 0.75 ±0.15 μM and total exposure (area under the plasma concentrations versus time curve, AUC) of 430 ±84 μM·min. For example Compounds of example **23-25**, which all contain a 2-methylpropan-2-ol at the R2 position, are highly soluble and **24** and **25** reach maximum serum concentrations (C*max*) >10-fold higher and total exposure >30-fold higher than 1-(4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol previsously reported compound **150** (Table 2 and Figure 1).

Table 2: Solubility and PK properties of potent and selective *Tg*CDPK1 inhibitors dosed at 10mg/kg PO to mice.

Ex. No.	**Solubility** (μM)	**T*max*** (min)	**C*max*** (μM)	**AUC*o->inf*** (μM.min)	**Half life** (min)	**clearance** (mL/min)
22	17	30	0.88±0.23	67±60	50±73	110
23	>100	50	5.2±1.0	850±411	114±115	0.2
24	>100	320	13±1	13700±1500	1190±510	0.2
25	>100	560	7.8±1.4	16600±4300	1110±400	0.3
26	6.2	40	0.39±0.16	31±8	40±5	6.7
27	8.0	30	3.9±1.1	3.9±1.1	41±21	21

Based on the PK parameters of **24** and **25** following single dose administration to mice, these compounds were evaluated for tolerability following escalated dosing. Both compounds were initially dosed at 10 mg/kg on day one. As no observable adverse effects were detected in mice dosed with either **24** or **25**, the next dose was increased to 50 mg/kg on day 4, followed by a final dose of 100 mg/kg on day 8. Mice showed no overt signs of toxicity or weight loss over the 10-day observation period. Next, the plasma protein binding of both compounds was evaluated. Both compounds were highly protein bound in mouse plasma with plasma protein binding of 96% for **24** and 88% for **25**. Based on the protein binding values the plasma concentrations required to maintain unbound plasma exposure above the *Tg*CDPK1 EC50 are 1.33 μM for **24** and 1.5 μM for **25**. Based on these target concentrations and the single dose PK data, a multiple dosing regimen with a loading dose of 20 mg/kg of **24** and **25** followed by a 5 mg/kg daily dose for five days was evaluated. Blood was collected at multiple time points to determine plasma concentrations over the course of treatment (Figure 2). Both compounds demonstrated excellent exposure through the 24-hour dosing interval with the trough concentrations remaining >1.5 μM throughout the study. The measured concentrations following multiple dosing were similar to those predicted from the

single dose PK studies in mice (Figure 3). Neither compound showed any evident signs of toxicity compared to mice dosed with vehicle only. Cardiac puncture blood collection was performed at the end of the study for complete blood count and serum biochemical profiles. All results were reported by Phoenix Central Laboratory to be within a normal range for species and age.

In a second multiple dose study compound **24** was administered at 50 mg/kg po every other day for 5 doses and compound **25** was administered with a 20 mg/kg loading dose followed by a 10 mg/kg maintenance dose every other day for 5 doses. The exposure to **25** with this dosing regimen was similar to the one observed with daily dosing of the lower dose and the plasma concentration at various time points were well predicted based on the single dose PK results. With compound **24** plasma concentrations remained >10 μM for the entire duration of the study (Figure 2) with maximum concentrations reaching 43 μM. The exposure to compound **24** following the last 50 mg/kg dose was higher than predicted from single dose studies with longer half-life suggesting possible saturation of metabolism at this dose.

Based on the favorable single and multiple dose PK and the lack of observable toxicity, compounds **24** and **25** were evaluated for tolerability and PK characteristics in rats following intravenous (IV) and PO dosing. Following IV administration to rats both **24** and **25** displayed biphasic kinetics with compound **25** reaching distribution equilibrium more rapidly than **24**. Compound **24** distributed approximately to total body water with a volume of distribution at steady state (Vss) of 0.9 L/kg. The distribution of **25** was more extensive with a Vss of 5.8 L/kg. Both compounds had very low systemic clearances in rats but, in contrast to mice, the clearance of **25** was 4-fold higher than that of **24**. The elimination half-lives of both compounds were acceptable for multiple dosing regimens, 9.6 and 13 hours for **24** and **25**, respectively (Figure 3).

Following oral administration to rats both compounds showed slow absorption with absorption phase continuing for 12 hours after oral dosing. The plasma concentrations over the 24-hour period after PO dosing exceeded those observed after IV dosing (Figure 3) suggesting that both **24** and **33** had essentially complete bioavailability in rats. The apparent bioavailability of these compounds was greatly improved compared to that of compound **150** (46%)

Because it is important that anti-toxoplasmosis therapies are able to prevent reactivation of parasites within tissue cysts, which largely reside in the central nervous system (CNS), the distribution of compounds **24** and **25** to the brain was next determined. To do this, the distribution of **24** and **25** into mouse brain (n=3) at one hr after intraperitoneal

dosing of 5 mg/kg was measured. The mean concentration of compound **24** at one hour in brain was 1.2 ±0.5 μM, a concentration well above the *Tg*CDPK1 EC_{50}. The corresponding plasma concentration of **24** was 4.1 ±1.1 μM resulting in a brain to plasma concentration ratio of **24** of 0.33 ±0.22. This brain penetration was comparable to compound **150** (0.31). In accordance with the larger distribution volume of compound **25**, it demonstrated a greater brain to plasma concentration ratio (1.65 ±0.84). Both the brain and plasma concentration of **25**, 1.90 ±0.24 μM and 1.23 ±0.76 μM, respectively, were above the *Tg*CDPK1 EC_{50}.

To demonstrate that *Tg*CDPK1 is the kinase target of these compounds in *T. gondii*, **24** and **25** were tested against parasitic cell lines overexpressing the Gly128Met *Tg*CDPK1 gatekeeper mutant or wildtype (wt) *Tg*CDPK1. Expression of the Gly128Met gatekeeper mutant of *Tg*CDPK1, but not wt*Tg*CDPK1, makes *T. gondii* highly resistant to PP-based inhibitors that contain 6-alkoxynaphthalen-2-yl groups at the R_3 position. Both **24** and **25** show a dramatic loss in potency against parasites overexpressing the Gly128Met gatekeeper mutant relative to the parent RH strain and to *T. gondii* overexpressing wild type *Tg*CDPK1 (Figure 4), which is consistent with *Tg*CDPK1 being the primary target through which these inhibitors exert their anti-parasitic effects. **24** and **25** were further profiled for any mammalian kinase off targets using a panel of 80 human kinases representing different subfamilies of the kinome tree with a fluorescence-based competition assay. 78 of the 80 mammalian kinases tested have an IC_{50} >1.5 μM (>1500-fold selective for *Tg*CDPK1) for **24**. For the two kinases–PKCv (PKD3) and MEK1–that have sub-micromolar IC50 values, compound **24** is a >120-fold and >900-fold less potent inhibitor than for *Tg*CDPK1, respectively. Compound **25** appears to be slightly more selective than compound **24**. Compound **25** demonstrated an IC50 value of greater than 5 μM (>2500-fold selective) for 79 of the kinases tested, with only PKCv (PKD3) demonstrating a submicromolar (IC_{50} = 0.280 μM; 140-fold selective) IC_{50} value.

The lack of toxicity in the initial mouse toxicity screens allowed us to move forward into large animal pharmacokinetic and tolerability profiling. Male calves (n=2 for each compound) were dosed orally at 10 mg/kg for compound **24** and 9.3 mg/kg for compound **25** and blood sampled up to 12 days after dosing (results not shown). Similar to rats, the absorption of both compounds was slow with maximum concentrations reached at 24 hours for compound **24** and 12 hours for compound **25**. The maximum plasma concentrations were similar for the two compounds, 7.9 μM and 9.8 μM, for compound **24** and **25**, respectively. However, the overall exposure to **25** was greater than that of **24** due to its lower oral clearance and longer half-life (Table 3). Yet, both compounds had very low oral clearances

and long systemic half-lives. Similar to rats, compound **25** had a higher apparent volume of distribution than compound **24** and the overall distribution characteristics were similar to those observed in rats.

Table 3: *In vivo* pharmacokinetic parameters of **24** and **25** in calves following PO administration of 10 mg/kg of **24** and 9.3 mg/kg of **25**. Data are shown as the mean and range between animals.

Ex.	*AUC (h*μmol/L)*	*CL/F (mL/hr/kg)*	*V/F (L/kg)*	*t1/2 (hr)*
24	588 (353-825)	52 (31-72)	1.5 (1.3-1.8)	23 (17-28)
25	849 (607-1091)	30 (22-39)	2.3 (1.4-3.2)	51 (46-56)

Potential further toxicity of compound **24** was examined in mice by testing two doses (30 mg/kg and 100 mg/kg PO) daily for five days, while observing mice for signs of toxicity and collecting blood samples. Both groups of mice remained active, well groomed, and appeared normal throughout the study. Upon necropsy, there were no gross abnormalities. Histology revealed mild focal inflammation in the spleen in two of three mice in the 30mg/kg group. The only abnormality seen in the 100mg/kg group was inflammatory infiltrate in the hepatic lobules of the liver in one of three mice. The concentrations following 30 mg/kg doses were slightly higher than those predicted from single dose PK data while the exposures following the 100 mg/kg doses were similar to those predicted from single 10 mg/kg dose data (Figure 5). Similar to the early multiple dosing experiments in mice, after the last 100 mg/kg dose the elimination of compound **24** was slower than predicted with considerable plasma concentrations persisting at 72 hours after the final dose.

Mice were then dosed with single PO doses of **24** ranging from 200 mg/kg to 1000 mg/kg. The lowest observable adverse effect level (LOAEL) was observed at 500 mg/kg and the no observable adverse effect level (NOAEL) was observed at 400 mg/kg. Mice had slightly ruffled fur and were less active than the control mice at 3 hours following the 500 mg/kg dose. The lowered activity persisted at 24 hours but was resolved by 30 hours. The lack of toxicity up to 500 mg/kg confirmed the safety of the compound for small animal efficacy studies.

The pharmacokinetics of **24** was further explored in dogs and monkeys following IV and PO administration. Similar to rats, **24** had a very low clearance in both species and a relatively long half-life (Table 4). The plasma concentrations time-profile following IV dosing was biphasic in both species with a similar extent of distribution in dogs and monkeys

as observed in rats and calves (Figure 3, panel B). The bioavailability of compound **24** was 66 ±17% in monkeys and 88 ±12% in dogs demonstrating good bioavailability in both species as predicted from rats.

Table 4: *In vivo* pharmacokinetic parameters of **24** following IV and PO administration of 1 mg/kg to dogs and monkeys (n=3 for each). Data is show as mean and standard deviation.

	*AUC (h*μ mol/L)*	*CL (mL/hr/kg)*	*Vss (L/kg)*	*t1/2 (hr)*
Dog				
IV	11200 ±1100	90 ±10	1.7 ±0.1	13.2
PO	9620 ±1350			
Monkey				
IV	8720 ±3400	130 ±50	1.8 ±0.3	9.6
PO	5730 ±1500			

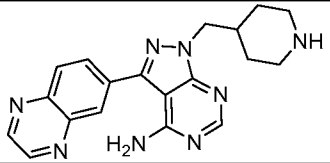
Example compound **24** was tested against a high inoculum of type 1 RH strain *T. gondii* to determine efficacy against fulminate toxoplasmosis in two experiments (Figure 6). Unlike type 2 *T.gondii* strains, type 1 strains do not typically form tissue cysts but rather cause death in 5-10 days depending on the size of the inoculum. Compound **24** was administered *via* oral gavage two days after infection and the burden of infection was measured 5 days after the initiation of treatment. Treatment with **24** at 20 mg/ kg for 5 days reduced the *T. gondii* in the peritoneal fluid below the limits of detection (less than 100 tachyzoites/mL). Treatment with **24** was highly effective at 20 mg/kg daily for 5 days in reducing infection in the spleen more than 99%, and infection in the brain 95%.The marked decrease in brain infection compared to controls coupled with the measurement of a sufficient brain concentrations of **24** suggest that this compound is active against actively replicating *T. gondii* in the brain, the target of current first-line anti-toxoplasma drugs. Previous mouse models of RH strain *T. gondii* in mice have detected *T. gondii* DNA in the brain after 2 days and recovery of *T. gondii* in culture at 4 days. In this study, decreases in brain infection may in part be due to efficacy against systemic infection. The favorable safety profile and brain permeability of **24** make it an attractive candidate to treat toxoplasmosis in pregnancy, as well as CNS toxoplasmosis.

Biological Example 8: *Sarcocystis neurona* calcium-dependent protein kinase 1 is targeted in selective therapeutic development for equine protozoal myeloencephalitis

Equine protozoal myeloencephalitis (EMP) is a common progressive, degenerative neurological disease of the central nervous system caused by the apicomplexan parasite Sarcocystis neurona. Widespread therapeutic failure or relapses even with long term use of available treatments and absence of viable vaccines underscore the need to validate molecular targets within the parasite for new drug development based on novel scaffolds with desirable therapeutic outcome. We recently identified and sequenced an equivalent CDPK homologue (*Sn*CDPK1) in *S. neurona* genome that has similar glycine "gatekeeper" residue. *Sn*CDPK1 and *Tg*CDPK1 have >85% amino acid identity. We have characterized BKIs for *in vitro* efficacy against *Sn*CDPK1 and *S. neurona* merozoites. Recombinant *Sn*CDPK1 was expressed, purified and screened against a selected group of BKIs previously shown to have low IC_{50}s against *Tg*CDPK1 and *T. gondii* tachyzoites. Growth assays with a yellow fluorescent protein-expressing clone of *S. neurona* demonstrated that parasite growth was inhibited by BKIs at nanomolar concentrations (Table 4).

BKI-CDPK1 binding confirmation was performed using *S. neurona* lysates in thermal shift assays using CDPK1-specific antibody. *Sn*CDPK1 was inhibited by low nM concentrations of BKIs. Analysis of *Sarcocystis* cell-inhibition data suggests that BKI interfered with an early step in *S. neurona* host cell invasion and egress processes. This presents molecular and phenotypic evidences that *Sn*CDPK1 could be targeted for rational drug development as *Tg*CDPK1 was previously validated for *T. gondii*. BKIs used in these assays have been chemically optimized with functional groups needed to improve potency, selectivity and pharmacokinetic properties. *In vivo* experiments were performed in murine model of infections using *Sarcocystis neurona* strain SN 37R. Experimental group treated with compound of Example **24** at a dose of 10 mg/kg/day/oral in drinking water having 0.5% Saccharine for 30 days showed no sign of disease relative to the control group 40 days after the end of treatment period.

Table 4. *In vitro* activities against *Sn*CDPK1.

Example No.	***Sn*CDPK1** IC_{50}(μM)	**STDEV** n=X	***S. neurona*** EC_{50} (μM)	**Thermal shift from DMSO**
24	0.0089	$0.0015^{n=4}$	0.042	4.1
25	0.0075	$0.0007^{n=2}$	0.128	ND
	>2	$0.3557^{n=3}$	>10	0

150	0.0059	$0.0005^{n=3}$	0.068	2.7

Biological Example 9: Treatment of non-pregnant and pregnant 248 Balb/c mice experimentally infected with the *N. caninum* isolate.

The materials and methods for assessing treatment of *N. caninum* in vitro, and treats infected mice and rids of *N. caninum* infection in brain and fetuses has been previsously described in Winzer et al. "*In vitro* and *in vivo* effects of the bumped kinase inhibitor 1294 in the related cyst-forming apicomplexans *Toxoplasma gondii* and *Neospora caninum*." Antimicrobial Agents and Chemotherapy 59(10):6361-74 (Oct 2015), incorporated herein by reference in its entirety.

Compound **150** is effective in treating *N. caninum* in vitro, and treats infected mice and rids of *N. caninum* infection in brain and fetuses. In addiotn, compound **24** has similar properties in that it is active in vitro against *N. caninum* cultures, and it treats pregnant mice removing *N. caninum* from their brains, their bodies, and their fetuses. A variety of other compounds have been found to be active against the presumed target, *N. caninum* calcium-dependent protein kinase 1 (cloned and expressed by our group and was also found to have a glycine gatekeeper residue), the compounds that are active against *T. gondii* CDPK1 are almost all uniformly active against *Nc*CDPK1, and are presumed active in the *in vitro* and *in vivo* models as well.

Biological Example 10: *Besnoitia besnoiti* activity

Besnoitiosis is a chronic and debilitating bovine disease caused by the apicomplexan *Besnoitia besnoiti*, a protozoan parasite belonging to the group of cyst-forming coccidians. There are currently no therapeutic remedies for this disease. Apicomplexan calcium dependent protein kinases, necessary for host cell invasion and egress, are promising targets for drug development because orthologs are absent in mammalian genomes. Unlike the mammalian host cell kinases, *Bb*CDPK1 has glycine at the entry to the ATP binding site (gatekeeper residue), which renders it sensitive to bumped kinase inhibitors (BKIs). The activity of two BKIs of the disclosure against *Besnoitia besnoiti* is provided in Table 5.

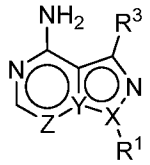
Table 5. *In vitro* activities against *Bb*CDPK1 and *Besnoitia besnoiti*.

Example	***Bb*CDPK1 IC_{50} (μM)**	***B. besnoiti* EC_{50} (μM)**	***B. besnoiti* EC_{100} (μM)**
150	0.004	0.045	2.580
24	0.005	0.097	3.590

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be incorporated within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated herein by reference for all purposes.

What is Claimed:

1. A compound of formula:



or a pharmaceutically acceptable salt thereof, wherein

X, Y, and Z are defined by either: (i) X is N, Y is C, and Z is N; or (ii) X is C, Y is N, and Z is C(H);

R^1 is C_{2-6} alkyl, C_{1-6} haloalkyl, -C_{1-6} alkyl-R^{12}, C_{3-8} cycloalkyl, heterocyclyl, heteroaryl, or aryl, wherein

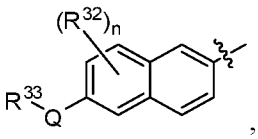
the alkyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl groups are each optionally substituted with one or two R^{11} groups;

each R^{11} is independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, -OR, -SR, -NR_2, -C(O)R, -C(O)OR, -$C(O)NR_2$, -$S(O)_2NR_2$, or –$S(O)_2R$;

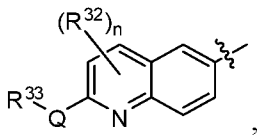
and

R^{12} is -OR, -SR, -NR_2, -C(O)R, -C(O)OR, -$C(O)NR_2$, -$S(O)_2R$, -OC(O)R, -OC(O)OR, -$OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, -$N(R)C(O)NR_2$, aryl, heteroaryl, C_{3-8} cycloalkyl, or heterocyclyl, wherein R^{12} is optionally substituted by one, two, or three groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydoxyalkyl, -OR, -SR, -NR_2, -C(O)R, -C(O)OR, -$C(O)NR_2$, -$S(O)_2R$, -OC(O)R, -OC(O)OR, -$OC(O)NR_2$, -N(R)C(O)R, -N(R)C(O)OR, or -$N(R)C(O)NR_2$;

R^3 is one of the formulas,



, or



,

wherein

n is 0, 1, or 2;

Q is –O-, -S-, or –$N(R^Q)$- , wherein R^Q is hydrogen or C_{1-6} alkyl; and

R^{33} is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, (C_{3-8} cycloalkyl)C_{1-6} alkyl, or heterocyclyl, wherein the alkyl, cycloalkyl, and heterocyclyl are optionally substituted with one, two, three, or four groups that are each independently

halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl,

each R^{32} is independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{34}$, $-SR^{34}$, $-N(R^{34})_2$, $-C(O)R^{34}$, $-C(O)OR^{34}$, $-C(O)N(R^{34})_2$, $-S(O)_2R^{34}$, $-OC(O)R^{34}$, $-OC(O)OR^{34}$, $-OC(O)N(R^{34})_2$, $-N(R^{34})C(O)R^{34}$, $-N(R^{34})C(O)OR^{34}$, or $-N(R^{34})C(O)N(R^{34})_2$, wherein each R^{34} is independently hydrogen or C_{1-6} alkyl; and

each R is independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, heterocyclyl, aryl, arylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6} alkyl wherein the alkyl, aryl, arylalkyl, heteroaryl, and heteroarylalkyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^0$, $-SR^0$, $-N(R^0)_2$, $-C(O)R^0$, $-C(O)OR^0$, $-C(O)N(R^0)_2$, $-S(O)_2R^0$, $-OC(O)R^0$, $-OC(O)OR^0$, $-OC(O)N(R^0)_2$, $-N(R^0)C(O)R^0$, $-N(R^0)C(O)OR^0$, or $-N(R^0)C(O)N(R^0)_2$, wherein each R^0 is independently hydrogen or C_{1-6} alkyl;

provided the compound is not:

1-(6-ethoxynaphthalen-2-yl)-3-isopropylimidazo[1,5-a]pyrazin-8-amine;

3-(6-isopropoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-propoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isopropyl-3-(6-methoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-methoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-isopropoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(piperidin-4-ylmethyl)-3-(6-propoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(benzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-butoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(allyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(2-chlorobenzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(3-chlorobenzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(4-chlorobenzyloxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(benzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(allyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-butoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-isobutoxynaphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-isobutoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(2-chlorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(3-chlorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(2,5-dimethylbenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
1-isopropyl-3-(6-(2-methylbenzyloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
1-isopropyl-3-(6-(2-methyl-5-(trifluoromethyl)benzyloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(3-chloro-4-(2,2,2-trifluoroethyl)benzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(3-chloro-5-fluorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
1-isopropyl-3-(6-(1-phenylethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(4-tert-butylbenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
1-isopropyl-3-(6-(pyridin-4-ylmethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(4-chlorobenzyloxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

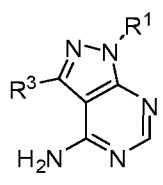
6-(4-amino-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N,N-dimethylquinolin-2-amine;
3-tert-butyl-1-(6-ethoxynaphthalen-2-yl)imidazo[1,5-a]pyrazin-8-amine;

3-tert-butyl-1-(6-methoxynaphthalen-2-yl)imidazo[1,5-a]pyrazin-8-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(1-ethylpiperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine; or

3-(6-ethoxynaphthalen-2-yl)-1-(1-methylpiperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine.

2. The compound of claim 1, which is of the formula 

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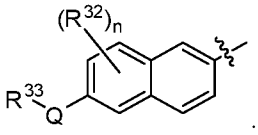
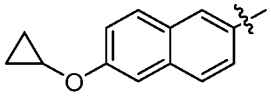
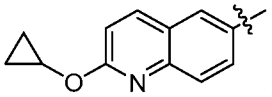
3. The compound of claim 1 or 2, wherein R^3 is of the formula  .

4. The compound of claim 4, wherein Q is –O–.

5. The compound of claim 4 or 5, wherein R^{33} is C_{1-6} alkyl or C_{3-8} cycloalkyl, wherein the alkyl and cycloalkyl are optionally substituted with one, two, three, or four groups that are each independently halogen, cyano, nitro, C_{1-6} alkyl, C_{1-6} haloalkyl, $-OR^{20}$, $-SR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2R^{20}$, $-OC(O)R^{20}$, $-OC(O)OR^{20}$, $-OC(O)N(R^{20})_2$, $-N(R^{20})C(O)R^{20}$, $-N(R^{20})C(O)OR^{20}$, or $-N(R^{20})C(O)N(R^{20})_2$, wherein each R^{20} is independently hydrogen or C_{1-6} alkyl.

6. The compound of claim 4 or 5, wherein R^{33} is C_{1-3} alkyl or C_{3-6} cycloalkyl.

7. The compound of claim 1 or 2, wherein R^3 is of the formula  or  .

8. The compound of any one of claims 1-7, wherein R^1 is C_{2-6} alkyl or $-C_{1-4}$ alkyl-R^{12}.

9. The compound of claim 8, wherein R^{12} is –OR or heterocyclyl, each optionally substituted.

10. The compound of claim 1, which is selected from the group consisting of:

3-(2-methoxyquinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(2-(cyclopropylmethoxy)quinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(cyclopropylmethoxy)naphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-cyclobutoxynaphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-cyclopropoxynaphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
3-(6-ethoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;
1-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;

3-(6-cyclopropoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol;

3-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol;

1-(6-(4-amino-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)-2-methylpropan-2-ol;

2-(6-(4-amino-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)ethanol;

1-isobutyl-3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-isobutyl-3-(6-(2-methoxyethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol;

1-(4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;

3-(2-(2-methoxyethoxy)quinolin-6-yl)-1-neopentyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol;

3-(2-cyclopropoxyquinolin-6-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(2-cyclopropoxyquinolin-6-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;

3-(2-cyclopropoxyquinolin-6-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(2-cyclopropoxyquinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(azetidin-3-ylmethyl)-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(2-cyclopropoxyquinolin-6-yl)-1-((1-methylazetidin-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(2-(2-methoxyethoxy)quinolin-6-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;

1-isobutyl-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(piperidin-4-ylmethyl)-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-((1-methylpiperidin-4-yl)methyl)-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

2-(3-(4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropoxy)ethanol;

1-(4-amino-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;

3-(4-amino-3-(2-(2,2,2-trifluoroethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol;

3-(2-cyclopropoxyquinolin-6-yl)-1-(3-(dimethylamino)-2,2-dimethylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(7-ethoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(7-ethoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-cyclopropoxynaphthalen-2-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(2-cyclopropoxyquinolin-6-yl)-1-((3-methyloxetan-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

2-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-1-morpholinoethanone;

2-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-1-morpholinoethanone;

2-((4-amino-3-(6-(methoxymethyl)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol;

2-((4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol;

2-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1,1,1,3,3,3-hexafluoropropan-2-ol;

1-(3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrrolo[3,2-c]pyridin-1-yl)-2-methylpropan-2-ol;

1-(4-amino-5-(2-cyclopropoxyquinolin-6-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol;

4-(4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,3,3-trimethylbutan-2-ol;

1-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclobutanol;

1-(4-amino-5-(6-cyclopropoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol;

1-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclobutanol;

1-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclopentanol;

1-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclopentanol;

4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-tetrahydro-2H-pyran-4-ol;

4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-tetrahydro-2H-pyran-4-ol;

(3-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)oxetan-3-yl)methanol;

(3-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)oxetan-3-yl)methanol;

3-(6-cyclopropoxynaphthalen-2-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(2-cyclopropoxyquinolin-6-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

methyl 3-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-

dimethylpropanoate;

methyl 3-(4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropanoate;

4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol;

4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol;

methyl 2-((6-(4-amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)acetate;

2-((6-(4-amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)acetonitrile;

4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-4-carbonitrile;

4-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile;

3-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropanoic acid;

3-(6-cyclopropoxynaphthalen-2-yl)-1-(2-fluoro-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol;

4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol;

4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol;

4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol;

4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol;

4-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile;

4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol;

4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol;

4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-4-carbonitrile;

4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile;

2-(6-(4-amino-1-(2-hydroxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)acetonitrile;

methyl 2-(6-(4-amino-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)quinolin-2-yloxy)acetate;

3-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol;

3-((4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylazetidin-3-ol;

4-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile;

3-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol;

3-(6-(difluoromethoxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(difluoromethoxy)naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol;

3-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylazetidin-3-ol;

4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidin-4-ol;

4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidin-4-ol;

4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-4-carbonitrile;

3-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-3-ol;

4-((4-amino-3-(6-(difluoromethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylpiperidine-4-carbonitrile;

3-((4-amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1-methylazetidin-3-ol;

1-(3-(6-ethoxynaphthalen-2-yl)-1-((1-(methylcarbamoyl)piperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-3-methylurea;

4-((4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-N-methylpiperidine-1-carboxamide;

3-(2-ethoxyquinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(2-(piperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(2-(1-methylpiperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(2-(1-ethylpiperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(2-(1-propylpiperidin-4-yl)ethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(2-(1-(3-aminopropyl)piperidin-4-yl)ethyl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-ethoxynaphthalen-2-yl)-1-(1-propylpiperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(1-(3-aminopropyl)piperidin-4-yl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-(6-ethoxynaphthalen-2-yl)-3-(piperidin-4-ylmethyl)imidazo[1,5-a]pyrazin-8-amine;

1-(6-ethoxynaphthalen-2-yl)-3-((1-methylpiperidin-4-yl)methyl)imidazo[1,5-a]pyrazin-8-amine;

1-isopropyl-3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-cyclopropoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-((6-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)-2-methylpropan-2-ol;

2-((6-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yl)oxy)ethan-1-ol;

3-(6-(cyclopropylmethoxy)naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-cyclobutoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

3-(6-(2-methoxyethoxy)naphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

1-((1-methylpiperidin-4-yl)methyl)-3-(6-(oxetan-3-yloxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine;

2-(6-(4-amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)ethanol;

1-(6-(4-amino-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-yloxy)-2-methylpropan-2-ol; and

3-(2-ethoxyquinolin-6-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine.

11. A method of treating an apicomplexan protozoan related disease comprising administering to a patient in need of such treatment a therapeutically effective amount of (i) a compound of any one of claims 1-10, or (ii) a pharmaceutical composition comprising a compound of any one of claims 1-10 and a pharmaceutically acceptable excipient, carrier, or diluent.

12. The method of claim 11, wherein the apicomplexan protozoan related disease is selected from toxoplasmosis, cryptosporidiosis, coccidiosis, malaria, neosporosis, sarcocystosis, besnoitiosis, coccidiosis, cystoisoporosis, babesiosis, and theileriosis.

13. The method of claim 11, wherein the apicomplexan protozoan related disease is selected from toxoplasmosis, cryptosporidiosis, coccidiosis, and malaria.

14. A method of treating malaria comprising administering to a patient in need of such treatment a therapeutically effective amount of (i) a compound of any one of claims 1-10, or (ii) a pharmaceutical composition comprising a compound of any one of claims 1-10 and a pharmaceutically acceptable excipient, carrier, or diluent.

15. The method of any one of claims 11-14, wherein the compound is administered in combination with a second therapeutically effective agent.

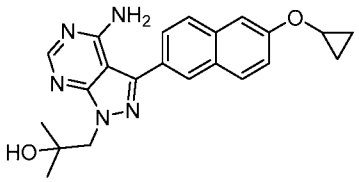
16. The method of any one of claims 11-15, wherein the patient is human.

17. The method of any one of claims 11-15, wherein the patient is livestock.

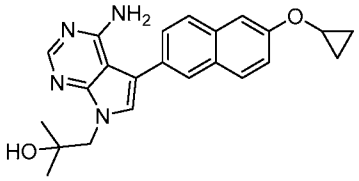
18. A method of inhibiting apicomplexan calcium dependent protein kinase, comprising administering a therapeutically effective amount of a compound of any one of claims 1-10.

19. The method of claim 18, wherein the apicomplexan calcium dependent protein kinases is selected from *T. gondii* calcium dependent protein kinases (*Tg*CDPKs), *C. parvum* and *C. hominus* calcium dependent protein kinases (*Cp*CDPKs), and *P. falciparum* and *P. berghei* calcium dependent protein kinase 4 (*Pf*CDPKs).

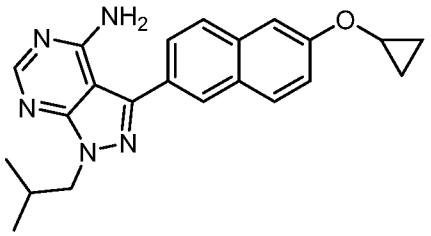
20. The method of any one of claims 11-19, wherein the compound is:



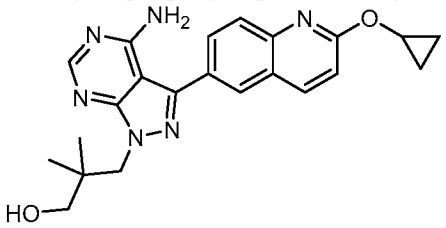
1-(4-amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol;



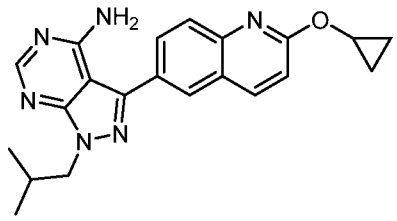
1-(4-amino-5-(6-cyclopropoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol;



3-(6-cyclopropoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine;



3-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol;

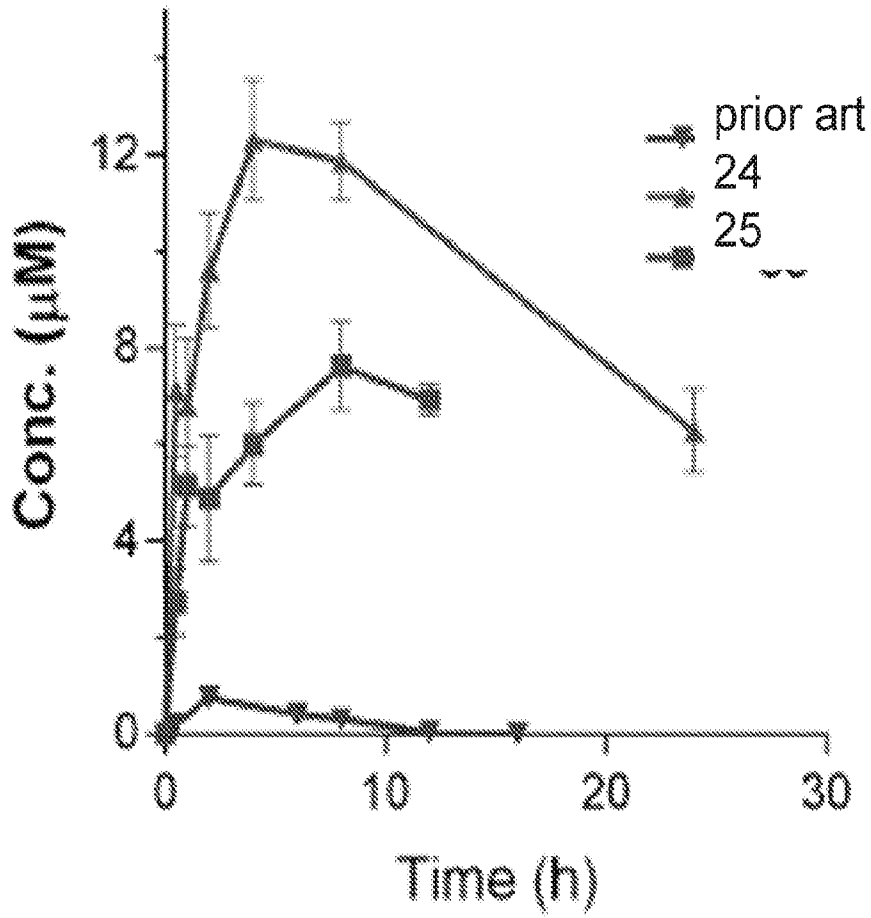


3-(2-cyclopropoxyquinolin-6-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine; and pharmaceutically acceptable salts thereof.

21. A method of treating neosporosis, sarcocystosis, besnoitiosis, coccidiosis, cystoisoporosis, babesiosis, or theileriosis, comprising administering to a patient in need of such treatment a therapeutically effective amount of (i) a compound of formula (I), or (ii) a pharmaceutical composition comprising a compound of formula (I) and a pharmaceutically acceptable excipient, carrier, or diluent.

22. The method of claim 21, wherein the compound is:
3-(6-ethoxynaphthalen-2-yl)-1-((1-methylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine; or
1-(4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol.

Figure 1



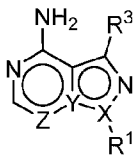


Figure 2

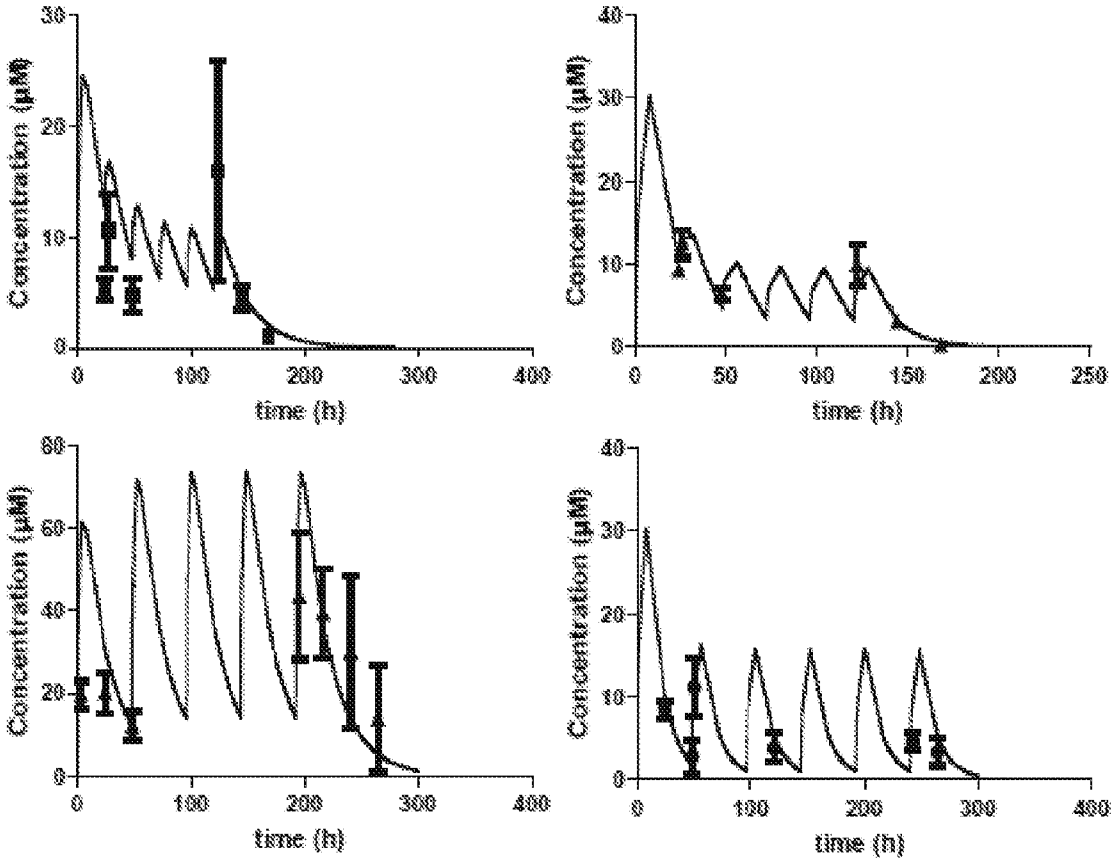

Concentration (μM)
time (h)
0
10
20
30
0
100
200
300
400
Concentration (μM)
time (h)
0
10
20
30
40
0
50
100
150
200
250
Concentration (μM)
time (h)
0
20
40
60
0
100
200
300
400
Concentration (μM)
time (h)
0
10
20
30
40
0
100
200
300
400

Figure 3

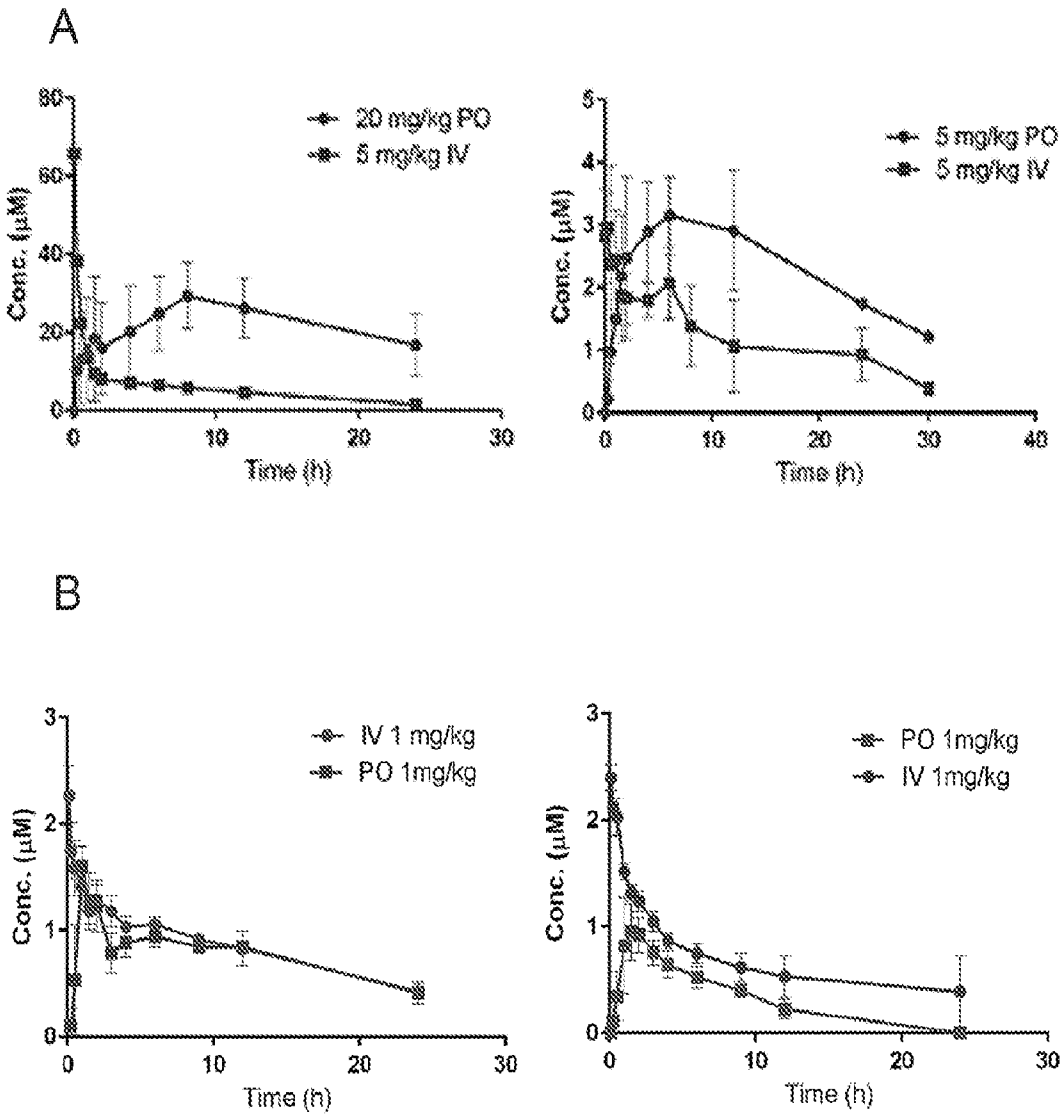

A
20 mg/kg PO
5 mg/kg IV
Conc. (μM)
Time (h)
5 mg/kg PO
5 mg/kg IV
Conc. (μM)
Time (h)
B
IV 1 mg/kg
PO 1mg/kg
Conc. (μM)
Time (h)
PO 1mg/kg
IV 1mg/kg
Conc. (μM)
Time (h)

Figure 4

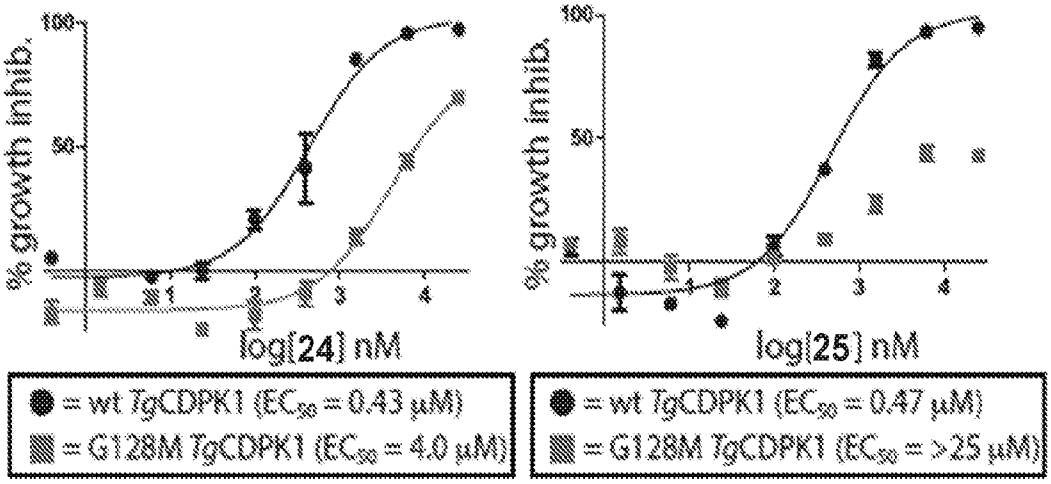


Figure 5

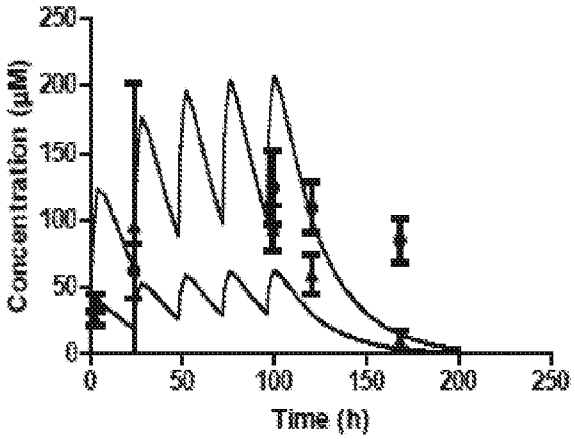
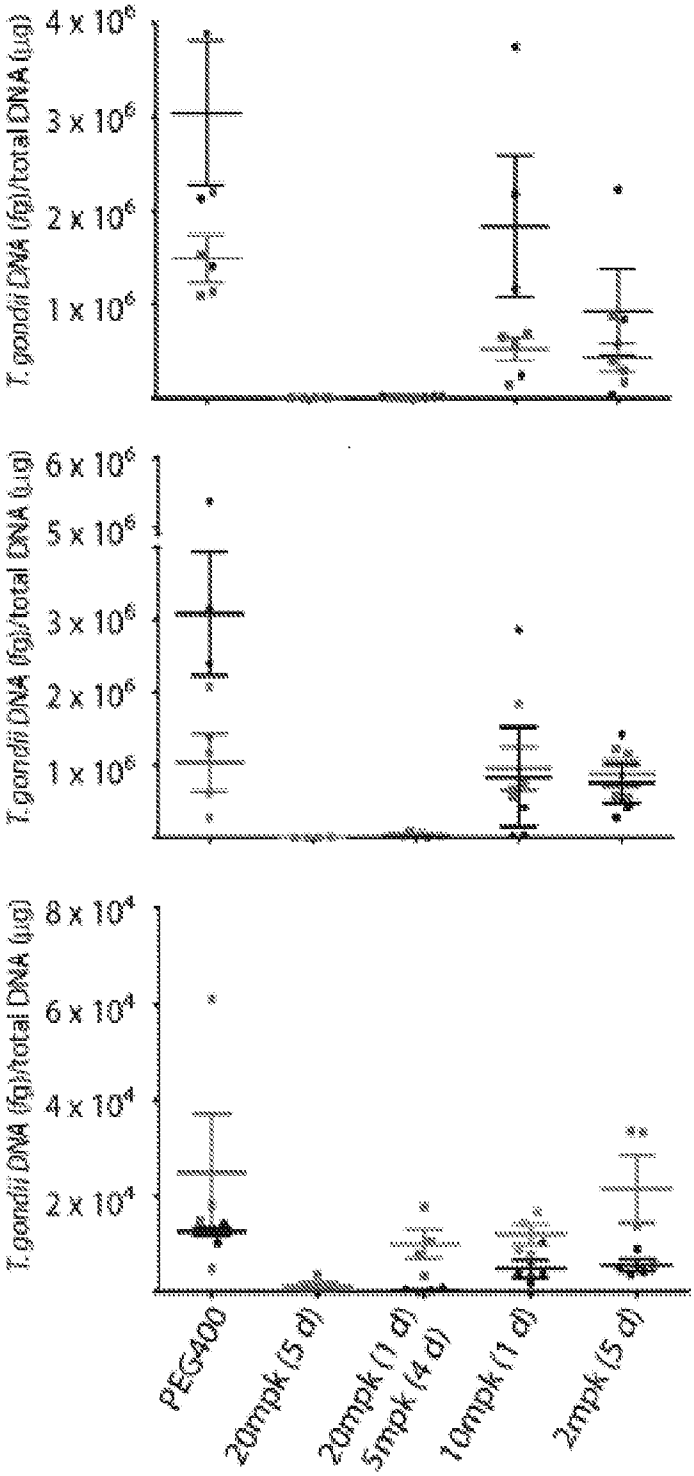


Figure 6


T. gondii DNA (fg)/total DNA (µg)
4 x 10^6
3 x 10^6
2 x 10^6
1 x 10^6
T. gondii DNA (fg)/total DNA (µg)
6 x 10^6
5 x 10^6
3 x 10^6
2 x 10^6
1 x 10^6
T. gondii DNA (fg)/total DNA (µg)
8 x 10^4
6 x 10^4
4 x 10^4
2 x 10^4
PEG400
20mpk (5 d)
20mpk (1 d)
5mpk (4 d)
10mpk (1 d)
2mpk (5 d)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/14996

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 33/06; A61K 31/44 (2016.01)
CPC - C07D 235/32, 401/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61P 33/06; A61K 31/44 (2016.01)
CPC: C07D 235/32, 401/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); ProQuest; Scifinder; Google/Google Scholar; KEYWORDS: toxoplasma, gondii, protein, kinase, cryptosporidium, pyrazolopyrimidine, apicomplexan, coccidiosis, malaria, sarcocystosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0018040 A1 (VAN VOORHIS, WC et al.) 17 January 2013; paragraphs [0036]-[0048], [0052], [0135], [0453], [0458], [0464]	1-2, 3/1-2, 4/3/1-2, 7/1-2, 10, 21-22
A	WO 2011/094628 A1 (UNIVERSITY OF WASHINGTON) 04 August 2011; entire document	1-2, 3/1-2, 4/3/1-2, 7/1-2, 10, 21-22
A	US 2006/0235031 A1 (ARNOLD, LD et al.) 19 October 2006; entire document	1-2, 3/1-2, 4/3/1-2, 7/1-2, 10, 21-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 March 2016 (04.03.2016)	11 APR 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/14996

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-6, 8-9, 11-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.