ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

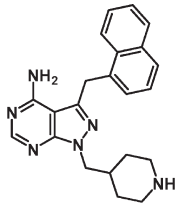
Discovery of Potent and Selective Inhibitors of CDPK1 from *C. parvum* and *T. gondii*

Ryan C. Murphy,[†,○] Kayode K. Ojo,[‡,○] Eric T. Larson,[§,∥] Alejandro Castellanos-Gonzalez,[#] B. Gayani K. Perera,[†] Katelyn R. Keyloun,[‡] Jessica E. Kim,[‡] Janhavi G. Bhandari,[†] Natascha R. Muller,[‡] Christophe L. M. J. Verlinde,[§,∥] A. Clinton White, Jr.,[#] Ethan A. Merritt,[§,∥] Wesley C. Van Voorhis,[*,‡,∥,⊥] and Dustin J. Maly[*,†]

[†]Department of Chemistry, [‡]Division of Allergy and Infectious Diseases, Department of Medicine, [§]Department of Biochemistry, [∥]Medical Structural Genomics of Pathogenic Protozoa Consortium, and [⊥]Department of Global Health, University of Washington, Seattle, Washington 98195, and [#]Infectious Disease Division, Department of Internal Medicine, University of Texas Medical Branch, Galveston, Texas 77555

ABSTRACT The protozoans *Cryptosporidium parvum* and *Toxoplasma gondii* are parasites of major health concern to humans. Both parasites contain a group of calcium-dependent protein kinases (CDPKs) which are found in plants and ciliates but not in humans or fungi. Here, we describe a series of potent inhibitors that target CDPK1 in *C. parvum* (*Cp*CDPK1) and *T. gondii* (*Tg*CDPK1). These inhibitors are highly selective for *Cp*CDPK1 and *Tg*CDPK1 over the mammalian kinases SRC and ABL. Furthermore, they are able to block an early stage of *C. parvum* invasion of HCT-8 host cells, which is similar to their effects on *T. gondii* invasion of human fibroblasts.

KEYWORDS Phosphorylation, protozoans, calcium-dependent protein kinases, *Cryptosporidium parvum*, *Toxoplasma gondii*, apicomplexan



IC_{50} (*Cp*CDPK1) = 9.0 nM
IC_{50} (*Tg*CDPK1) = 15 nM

The apicomplexan protozoans *Cryptosporidium parvum* and *Toxoplasma gondii* are ubiquitous parasites that infect humans and domesticated animals. *C. parvum* is a parasite of major health concern in humans as it is a common cause of illness transmitted by water.[1] *C. parvum* infection results in debilitating diarrhea that can be life-threatening in immunocompromised patients. Recent studies have implicated *C. parvum* in around 15−20% of childhood diarrheal disease in the developing world.[2,3] 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 serosurveys.[4] 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.

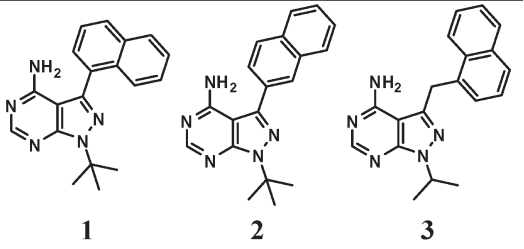
In *T. gondii*, calcium-regulated signaling is associated with a number of cellular functions such as secretion, gliding motility, and host cell invasion.[5,6] The proper control of intracellular calcium levels is important for host cell invasion, and *T. gondii* uses several mechanisms for the uptake and release of calcium. Furthermore, this organism contains specialized calcium-regulated signaling enzymes, including a unique family of calcium-dependent protein kinases (CDPKs), which are present in plants, ciliates, and green algae but not in animals.[7] These kinases are believed to be mediators of secretion, invasion, and gliding motility.[5,6,8] *T. gondii* and *C. parvum* are highly related obligate intracellular parasites. While much less is known about the role of calcium signaling in *C. parvum*, it appears that many calcium-regulated signaling processes are conserved between *T. gondii* to *C. parvum*.[9] *C. parvum* also possesses CDPKs that are believed to play important roles in calcium-regulated processes. The roles that CDPKs play in calcium signaling in *T. gondii* and *C. parvum* make this family of kinases intriguing targets for the development of antiparasitic agents. Previous studies have demonstrated that *Tg*CDPK1 plays an important role in *T. gondii* invasion of mammalian cells.[8] Pharmacological agents that selectively inhibit the catalytic activity of *Tg*CDPK1 block parasitic invasion of human fibroblast cells.[10,11] 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.[10] Specifically, *Tg*CDPK1 contains a glycine residue at the "gatekeeper" position, which allows inhibitors to access a large hydrophobic pocket

Received Date: May 5, 2010
Accepted Date: June 21, 2010
Published on Web Date: July 07, 2010

DOI: 10.1021/ml100096t | *ACS Med. Chem. Lett.* **2010**, *1*, 331–335

ACS Medicinal Chemistry Letters
pubs.acs.org/acsmedchemlett

a



	1	2	3
TgCDPK1 IC_{50} (nM)	1600 (600)	15 (4)	130 (40)
CpCDPK1 IC_{50} (nM)	2900 (400)	59 (12)	150 (20)

b

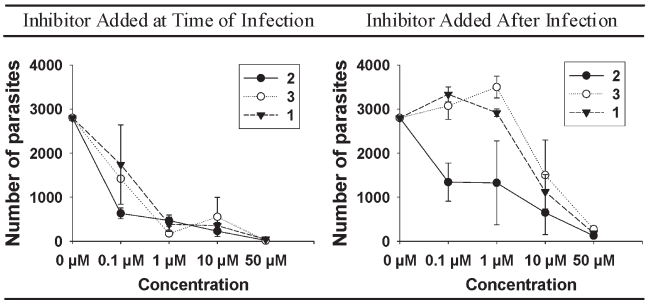


Figure 1. (a) *Tg*CDPK1 and *Cp*CDPK1 inhibitors **1**−**3**. IC_{50} values shown are the average of three assays ± SEM. (b) *C. parvum* parasite numbers (*Y*-axis) present after a 24 h infection of human intestinal cells (HCT-8) with *C. parvum* sporozoites (1:1 ratio HCT-8 cells:*C. parvum* sporozoites) in the presence of varying concentrations of inhibitors **1**−**3**. Cultures were exposed to each inhibitor at the time of infection (left graph). Cultures were exposed to each inhibitor 1 h after infection (right graph).

that is adjacent to the site of ATP binding (hydrophobic pocket II).[12] As all known human kinases possess larger residues (threonine, valine, or a larger amino acid) at the gatekeeper position, it is possible to gain selectivity by developing inhibitors that optimize interactions with the enlarged ATP-binding pocket of *Tg*CDPK1.[13,14] Indeed, Shokat and co-workers have shown that mutant mammalian kinases that contain an alanine or glycine residue at the gatekeeper position are uniquely sensitive to pyrazolopyrimidine inhibitors that contain bulky substituents at the 3-position (compounds **1**−**3**, Figure 1).[15−17]

Compounds **2** and **3** potently inhibit the catalytic activity of *Tg*CDPK1 and block *T. gondii* invasion of human fibroblasts (Figure 1a).[10] A homologue of *Tg*CDPK1 is also found in *C. parvum* (*Cp*CDPK1), but its role in parasitic invasion has yet to be elucidated. *Cp*CDPK1 and *Tg*CDPK1 share 70% sequence identity in the kinase catalytic domain, and both enzymes have a glycine residue at the gatekeeper position. Because pyrazolopyrimidines that contain bulky substituents at the 3-position are potent inhibitors of *Tg*CDPK1, we wished to determine if this class of compounds can serve as dual inhibitors of *Tg*CDPK1 and *Cp*CDPK1. First, the sensitivity of *Cp*CDPK1 to compounds **1**−**3** was determined with an in vitro activity assay (Figure 1a). Both *Tg*CDPK1 and *Cp*CDPK1 show similar levels of inhibition by these compounds, with analogue **2** as the most potent inhibitor. Next, the phenotypic response of *C. parvum* cells to **1**−**3** was tested by measuring the number of parasites present after 24 h of infection of human intestinal cells (HCT-8) with *C. parvum* sporozoites in the presence of these inhibitors. These assays were performed under two different sets of conditions: one in which the sporozoites were exposed to each inhibitor at the time of infection (Figure 1b, left graph) and a second assay in which the sporozoites were exposed to each inhibitor 1 h after infection (Figure 1b, right graph). Importantly, reduced levels of *C. parvum* sporozoites are observed with increasing concentrations of **1**−**3** under both sets of conditions, with analogue **2** demonstrating the most potent effect on parasite proliferation. The potencies of the pyrazolopyrimidine inhibitors were dramatically increased when they were added at the time of sporozoite infection rather than 1 h after *C. parvum* had been incubated with HCT-8 host cells. This suggests that these inhibitors affect an early stage of *C. parvum* host cell invasion, which is similar to their effects on *T. gondii* invasion. In all cases, the relative ability of each inhibitor to block *C. parvum* proliferation correlates with its in vitro activity against *Cp*CDPK1.

On the basis of the promising ability of pyrazolopyrimidine inhibitors to block *T. gondii* and *C. parvum* host cell invasion, we decided to further optimize compounds based on this scaffold as potent and selective dual inhibitors of *Tg*CDPK1 and *Cp*CDPK1. Inhibition of *Tg*CDPK1 and *Cp*CDPK1 was determined using a luminescent kinase assay (KinaseGlo).[10] Notably, both kinases were tested at the same ATP concentration, which allows direct comparison of inhibitor potencies due to these enzymes possessing similar K_m values for this cofactor.[18] To profile the sensitivity of CDPK1 to ATP-competitive kinase inhibitors, we tested the ability of a diverse panel of previously described kinase inhibitors to inhibit *Tg*CDPK1 (Supporting Information, Figure S1). While several compounds were weak inhibitors of *Tg*CDPK1, none were as potent as compounds **2** and **3**.

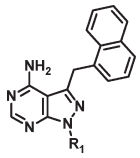
Encouraged by the similar potency of inhibitor **3** against *Tg*CDPK1 (IC_{50} = 150 ± 20 nM) and *Cp*CDPK1 (IC_{50} = 130 ± 40 nM), pyrazolopyrimidine analogues that contain a naphthylmethylene group at the 3-position and various alkyl substituents at the 1-position were tested for their ability to inhibit both kinases (Table 1). Derivatives containing smaller alkyl groups (**5a**−**c**) were found to have reduced potencies for both enzymes relative to **3**. Furthermore, a significant increase in potency was not observed for inhibitors that contain bulkier substituents at the 1-position (**5d**−**f**). However, the reduced potencies of compounds with larger substituents are not due to a size restriction in the ATP-binding pocket because derivatives that contain a basic piperidine group (derivatives **5g**−**i**) were found to be significantly more potent inhibitors of *Tg*CDPK1 and *Cp*CDPK1. Notably, compound **5h**, which contains a methylene linkage between the pyrazolopyrimidine core and the piperidine ring, has an 8- and 16-fold lower IC_{50} for *Cp*CDPK1 and *Tg*CDPK1 than parent compound **3**, respectively. Derivatization of **5g**−**i** with an acetyl (**5j**−**l**), ethyl (**5m**−**o**), or sulfonylmethyl (**5p**−**r**) group reduces the enhanced potency that the piperidine substituent

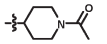
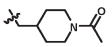
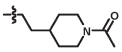
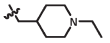
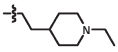
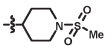
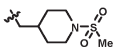
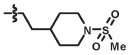
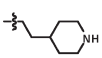
DOI: 10.1021/ml100096t | ACS Med. Chem. Lett. 2010, 1, 331–335

ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

Table 1. In Vitro Activities of **3** and **5a**−**r** against *Tg*CDPK1 and *Cp*CDPK1[a]



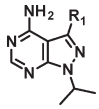
R_1 =	Compound Number	CpCDPK1 IC_{50} (nM)	TgCDPK1 IC_{50} (nM)	R_1 =	Compound Number	CpCDPK1 IC_{50} (nM)	TgCDPK1 IC_{50} (nM)
	**3**	150 (20)	130 (40)		**5j**	100 (10)	91 (26)
	**5a**	410 (90)	460 (240)		**5k**	360 (80)	210 (20)
	**5b**	130 (30)	990 (180)		**5l**	180 (60)	100 (10)
	**5c**	2000 (400)	1900 (100)		**5m**	50 (7)	36 (3)
	**5d**	140 (50)	31 (10)		**5n**	160 (30)	270 (30)
	**5e**	>3000	1800 (700)		**5o**	100 (10)	93 (4)
	**5f**	2400 (300)	1000 (300)		**5p**	75 (18)	54 (9)
	**5g**	56 (16)	59 (18)		**5q**	700 (240)	560 (10)
	**5h**	9.0 (0.5)	15 (5)		**5r**	200 (80)	120 (30)
	**5i**	34 (7)	52 (17)				

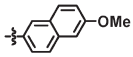
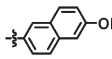
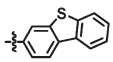
[a] Values shown are the averages of three assays ± SEMs.

confers, with only modified versions of piperidine **5g** showing similar inhibition as the parent compound. Notably, most compounds from this series are near equipotent inhibitors of both *Tg*CDPK1 and *Cp*CDPK1, demonstrating the overall similarity in this region of the ATP-binding pockets of both kinases.

To further probe the hydrophobic pocket adjacent to the gatekeeper residue (hydrophobic pocket II), a series of analogues that contain an isopropyl group at the 1-position and various aryl substituents at the 3-position were tested for their ability to inhibit both kinases (Table 2, compounds **7a**−**ab**). We felt that this series of compounds would provide insight into which substituents can be accommodated in this region. As shown in Table 2, a large number of aryl substituents are accommodated by both kinases. Almost all of the compounds from this series have an IC_{50} less than 1 μM, with several inhibitors demonstrating very high potency against both enzymes. Inhibitors that contain aryl rings that are monosubstituted with smaller substituents at the meta (**7b**, **7e**, **7g**, and **7ab**) or para positions (**7a**, **7d**, **7f**, and **7j**) are accommodated in the ATP-binding sites of both kinases but are more potent against *Tg*CDPK1 than *Cp*CDPK1. Analogues that contain meta- and para-substituted aryl rings (**7c**, **7i**, **7k,** and **7m**) are potent against both enzymes and show reduced selectivity for *Tg*CDPK1. Consistent with this observation, pyrazolopyrimidine derivatives that contain a 2-naphthyl (**7p**-**7r**) or quinoline group (**7t**) are potent inhibitors of both enzymes. However, bicyclic substituents that are

Table 2. In Vitro Activities of **7a**−**ab** against *Tg*CDPK1 and *Cp*CDPK1[a]



R_1 =	Compound Number	CpCDPK1 IC_{50} (nM)	TgCDPK1 IC_{50} (nM)	R_1 =	Compound Number	CpCDPK1 IC_{50} (nM)	TgCDPK1 IC_{50} (nM)
	**7a**	220 (70)	18 (4)		**7p**	10 (3)	5.0 (1.0)
	**7b**	120 (40)	20 (6)		**7q**	5.0 (1.0)	6.0 (1.0)
	**7c**	13 (3)	4.0 (1.0)		**7r**	12 (4)	5.0 (1.0)
	**7d**	210 (60)	48 (14)		**7s**	600 (100)	900 (210)
	**7e**	59 (18)	14 (5)		**7t**	20 (4)	24 (6)
	**7f**	87 (25)	13 (4)		**7u**	310 (20)	45 (14)
	**7g**	114 (33)	19 (9)		**7v**	990 (70)	180 (20)
	**7h**	22 (6)	9.0 (3.0)		**7w**	110 (10)	17 (6)
	**7i**	53 (9)	9.0 (2.0)		**7x**	>3000	>3000
	**7j**	410 (130)	40 (18)		**7y**	1400 (200)	320 (90)
	7k	170 (20)	37 (11)		**7z**	>3000	2200 (200)
	7l	93 (7)	420 (70)		**7aa**	1900 (400)	1100 (200)
	7m	17 (5)	5.0 (1.0)		**7ab**	120 (30)	56 (17)
	7n	2100 (200)	1500 (400)				
	7o	620 (130)	330 (80)				

[a] Values shown are the averages of three assays ± SEMs.

not planar show reduced potency (**7u**−**w**). In general, most inhibitors from this series are 2−12-fold selective for *Tg*CDPK1 over *Cp*CDPK1. However, several compounds (for example, compounds **7l**, **7q**, and **7t**) are equipotent or slightly selective for *Cp*CDPK1. This fact demonstrates that while the ATP-binding pockets of both kinases are very similar, they are not identical.

Key to the use of pyrazolopyrimidine-based kinase inhibitors as antiparasitic agents is the potential to selectively inhibit *Tg*CDPK1 and *Cp*CDPK1 over mammalian kinases. To determine the potency of these compounds against mammalian kinases, a subset of inhibitors were tested against the tyrosine kinases SRC and ABL. We felt that these two kinases would be a suitable counterscreen because they both contain the most permissive gatekeeper residue found

DOI: 10.1021/ml100096t | *ACS Med. Chem. Lett.* **2010**, *1*, 331–335

in mammalian kinases (threonine) and the pyrazolopyrimidine core was originally developed as a kinase inhibitor scaffold against SRC family kinases.[19] Most of the pyrazolopyrimidines tested have limited activity against SRC and ABL (Supporting Information, Table S1). Consistent with earlier studies, previously characterized "bumped" inhibitors **1** and **3** do not potently inhibit SRC or ABL kinase. Despite the increased activity of analogue **2** against *Tg*CDPK1 and *Cp*CDPK1, this compound only weakly inhibits SRC and ABL (IC_{50} > 1 μM). As expected, pyrazolopyrimidine inhibitors that contain smaller aryl groups at the 3-position (**7c** and **7e**) are less selective for both parasite kinases due to their increased potency against SRC and ABL. However, analogues that contain a substituted naphthyl (**7q** and **7r**) or quinoline (**7t**) substituent at the 3-position are much more selective due the increased size of these functional groups. Presumably, the larger threonine gatekeeper residues of SRC and ABL restrict access of these inhibitors to hydrophobic pocket II. While the substituent at the 3-position is the major contributor to inhibitor selectivity, substitution at the 1-position affects the potency of these compounds against *Tg*CDPK1 and *Cp*CDPK1 relative to SRC and ABL. For example, piperidine-containing analogues **5m** and **5p** are less selective for *Tg*CDPK1 and *Cp*CDPK1 than parent compound **1**. However, several analogues restore the high selectivity of this class of compounds, with secondary piperidine **5h** demonstrating over 1000-fold selectivity for the CDPKs. To test the overall toxicity of our pyrazolopyrimidines inhibitors, they were assayed for their ability to inhibit the growth of four human cell lines (Table S1): glioma derived (SF539), human lymphocyte (CRL8155), human promyelocytic leukemia (HL-60), and colorectal carcinoma (HC 2998). For all four cell lines tested, the inhibitors showed little or no growth inhibition at the highest concentration tested (10 μM).

We have previously reported crystal structures of inhibitors **2** and **3** bound to *Tg*CDPK1.[10] Similar to the structures of other pyrazolopyrimidines bound to tyrosine kinases, these inhibitors occupy the ATP-binding cleft of *Tg*CDPK1. The pyrazolopyrimidine scaffold superimposes with the purine ring of ATP, with the exocyclic amine and nitrogen at the 5-position forming hydrogen-bonding interactions with the hinge region. In addition, the glycine gatekeeper residue of *Tg*CDPK1 allows bulky substituents at the 3-position unobstructed access to hydrophobic pocket II. Replacing glycine with a larger amino acid at this position clearly creates a steric clash with the 2-naphthyl and 1-naphthylmethylene groups of inhibitors **2** and **3**, respectively.[10] On the basis of the similar sensitivities of *Tg*CDPK1 and *Cp*CDPK1 to pyrazolopyrimidines, we predicted that inhibitors of this class would bind in a similar orientation in both enzymes. The structure of compound **3** bound to *Cp*CDPK1 shows that this is indeed the case (Figure 2, top panel). Superposition of the active sites of *Tg*CDPK1 and *Cp*CDPK1 bound to **3** shows that the inhibitor has an identical mode of binding in both kinases. Furthermore, all of the residues surrounding the inhibitor are nearly superimposable, which is remarkable because *Tg*CDPK1 is in the calcium-free inactive conformation, while *Cp*CDPK1 is in the calcium-bound active conformation (Supporting Information, Figure S2). It is therefore clear that the inhibitors are able to

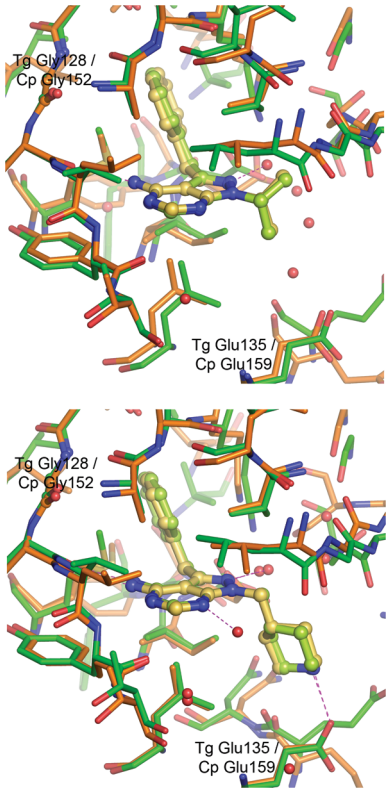


Figure 2. Superposition of the active sites of *Tg*CDPK1·**3** (green sticks and light green ball and sticks) and *Cp*CDPK1·**3** (orange sticks and light orange ball and sticks) complexes (PDB entries 3I7B[10] and 3NCG, respectively) (top). Overlay of the *Tg*CDPK1·**5h** and *Cp*CDPK1·**5h** complexes (PDB entries 3N51 and 3MWU). Only residues within 6 Å of the bound inhibitors are shown, and the unique glycine gatekeeper residue is shown as ball and sticks (bottom).

bind the ATP-binding site of CDPK1 whether it is active or not. While structures of both enzymes bound to **2** and **3** provided direct insight into how large substituents can be accommodated at the 3-position, we were interested in further exploring how substitution at the 1-position affects the potencies of these inhibitors. Structures of inhibitor **5h** bound to *Tg*CDPK1 and *Cp*CDPK1 show how increased potency can be obtained (Figure 2, bottom panel). While the pyrazolopyrimidine cores and 3-position substituents of inhibitors **3** and **5h** make identical contacts with both enzymes, the piperidine ring of **5h** forms a salt bridge with a glutamate residue that lines the ATP-binding cleft. Importantly, this interaction is present in the structures of **5h** bound to both *Tg*CDPK1 and *Cp*CDPK1. Alkylation, acetylation, or sulfonylation of the piperidine ring disrupts this interaction and accounts for the lower potency of compounds **5j**−**r**. Furthermore, the reduced potency of **5h** against SRC and ABL demonstrates that these kinases cannot form a similar interaction.

In conclusion, we have demonstrated that selective pyrazolopyrimidine kinase inhibitors are indeed potent inhibitors of *Cp*CDPK1 and that they are able to inhibit an early stage of *C. parvum* cell invasion. This class of compounds has similar effects on the ability of *T. gondii* to invade human fibroblast cells.[10,11] On the basis of these promising results, a

DOI: 10.1021/ml100096t | *ACS Med. Chem. Lett.* **2010**, *1*, 331–335

diverse panel of pyrazolopyrimidine analogues was generated, and their activities against *Cp*CDPK1 and *Tg*CDPK1 were determined. By exploiting a unique sequence and structural variation in the ATP-binding clefts of *Tg*CDPK1 and *Tg*CDPK1, potent dual inhibitors of these enzymes were obtained. Importantly, many of these inhibitors show minimal inhibition of the tyrosine kinases SRC and ABL and are not toxic to human cell lines. Future efforts will focus on increasing the potency and selectivity of these compounds and on optimizing their PK/ADME/Tox properties. Furthermore, optimized inhibitors will be used to investigate the role of *Cp*CDPK1 in *C. parvum* host cell invasion.

SUPPORTING INFORMATION AVAILABLE Three figures, two tables and details for synthetic procedures, analytical data, biological assays, and structural studies. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *To whom correspondence should be addressed. (W.C.V.V.) Tel: 206-543-2447. Fax: 206-616-4898. E-mail: wesley@u.washington.edu. (D.J.M.) Tel: 206-543-1653. Fax 206-685 7002. E-mail: maly@chem.washington.edu.

Author Contributions: ○These authors contributed equally to the manuscript.

Funding Sources: This work was funded by National Institute of Allergy and Infectious Diseases Grants R01AI080625 (W.C.V.V.) and R01AI067921 (C.L.M.J.V., E.A.M., and W.C.V.V.), National Institute of General Medical Sciences Grant R01GM086858 (D.J.M.), and financial support from G. and K. Pigotti.

ACKNOWLEDGMENT Dr. Raymond Hui (Structural Genomics Consortium, University of Toronto) kindly provided an expression construct for *C. parvum Cp*CDPK1. Portions of this research were carried out at the Stanford Synchrotron Radiation Light Source, a national user facility operated by Stanford University on behalf of the U.S. Department of Energy, Office of Basic Energy Sciences.

REFERENCES

(1) White, A. C. Cryptosporidiosis (*Cryptosporidium hominis*, *Cryptosporidium parvum*, and Other Species). In *Mandell, Douglas, & Bennett's Principles and Practice of Infectious Diseases*, 6th ed.; Mandell, G. L., Bennett, J. E., Dolin, R., Eds.; Churchill: Livingston, 2005; Chapter 280.

(2) Samie, A.; Bessong, P. O.; Obi, C. L.; Sevilleja, J. E.; Stroup, S.; Houpt, E.; Guerrant, R. L. Cryptosporidium species: Preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. *Exp. Parasitol.* **2006,** *114*, 314–322.

(3) Ajjampur, S. S.; Rajendran, P.; Ramani, S.; Banerjee, I.; Monica, B.; Sankaran, P.; Rosario, V.; Arumugam, R.; Sarkar, R.; Ward, H.; Kang, G. Closing the diarrhea diagnostic gap in Indian children by the application of molecular techniques. *J. Med. Microbiol.* **2008,** *57*, 1364–1368.

(4) Montoya, J. G.; Kovacs, J. A.; Remington, J. S. *Toxoplasma gondii*. In *Mandell, Douglas, & Bennett's Principles and Practice of Infectious Diseases*, 6th ed.; Mandell, G. L., Bennett, J. E., and Dolin, R., Eds.; Churchill: Livingston, 2005; Chapter 276.

(5) Nagamune, K.; Sibley, L. D. Comparative genomic and phylogenetic analyses of calcium ATPases and calcium-regulated proteins in the apicomplexa. *Mol. Biol. Evol.* **2006,** *23*, 1613–1627.

(6) Billker, O.; Lourido, S.; Sibley, L. D. Calcium-dependent signaling and kinases in apicomplexan parasites. *Cell Host Microbe* **2009,** *5*, 612–622.

(7) Doerig, C.; Billker, O.; Pratt, D.; Endicott, J. Protein kinases as targets for antimalarial intervention: Kinomics, structure-based design, transmission-blockade, and targeting host cell enzymes. *Biophys. Biochim. Acta, Proteins Proteomics* **2005,** *1754*, 132–150.

(8) Kieschnick, H.; Wakefield, T.; Narducci, C. A.; Beckers, C. *Toxoplasma gondii* attachment to host cells is regulated by a calmodulin-like domain protein kinase. *J. Biol. Chem.* **2001,** *276*, 12369–12377.

(9) Chen, X. M.; O'Hara, S. P.; Huang, B. Q.; Nelson, J. B.; Lin, J. J. C.; Zhu, G.; Ward, H. D.; LaRusso, N. F. Apical Organelle discharge by *Cryptosporidium parvum* is temperature, cytoskeleton, and intracellular calcium dependent and required for host cell invasion. *Infect. Immun.* **2004,** *72*, 6806–16.

(10) Ojo, K. K.; Larson, E. T.; Keyloun, K. R.; Castaneda, L. J.; DeRocher, A. E.; Inampudi, K. K.; Kim, J. E.; Arakaki, T.; Murphy, R. C.; Zhang, L.; Napuli, A. J.; Maly, D. J.; Verlinde, C. L. M. J.; Buckner, F. S.; Parsons, M.; Hol, W. G. J.; MerrittE. A.; Van Voorhis, W. C. *Toxoplasma gondii* calcium-dependent protein kinase 1 is a target for selective kinase inhibitors. *Nat. Struct. Mol. Biol.* **2010,** *17*, 602–607.

(11) Sugi, T.; Kato, K.; Kobayashi, K.; Watanabe, S.; Kurokawa, H.; Gong, H.; Pandey, K.; Takemae, H.; Akashi, H. Use of the kinase inhibitor analog 1NM-PP1 reveals a role for *Toxoplasma gondii* CDPK1 in the invasion step. *Eukaryotic Cell* **2010,** *9*, 667–670.

(12) Liao, J. J. Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors. *J. Med. Chem.* **2007,** *50*, 409–424.

(13) Zhang, C.; Kenski, D. M.; Paulson, J. L.; Bonshtien, A.; Sessa, G.; Cross, J. V.; Templeton, D. J.; Shokat, K. M. A second-site suppressor strategy for chemical genetic analysis of diverse protein kinases. *Nat. Methods* **2005,** *2*, 435–441.

(14) Cohen, M. S.; Zhang, C.; Shokat, K. M.; Taunton, J. Structural bioinformatics-based design of selective, irreversible kinase inhibitors. *Science* **2005,** *308*, 1318–1321.

(15) Bishop, A. C.; Ubersax, J. A.; Petsch, D. T.; Matheos, D. P.; Gray, N. S.; Blethrow, J.; Shimizu, E.; Tsien, J. Z.; Schultz, P. G.; Rose, M. D.; Wood, J. L.; Morgan, D. O.; Shokat, K. M. A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* **2000,** *407*, 395–401.

(16) Bishop, A. C.; Shah, K.; Liu, Y.; Witucki, L.; Kung, C.; Shokat, K. M. Design of allele-specific inhibitors to probe protein kinase signaling. *Curr. Biol.* **1998,** *8*, 257–266.

(17) Bishop, A. C.; Kung, C.; Shah, K.; Witucki, L.; Shokat, K. M.; Liu, Y. Generation of monospecific nanomolar tyrosine kinase inhibitors via a chemical genetic approach. *J. Am. Chem. Soc.* **1999,** *121*, 627–631.

(18) *Cp*CDPK1's and *Tg*CDPK1's K_m values for ATP are 9.0 and 10 μM, respectively.

(19) Hanke, J. H.; Gardner, J. P.; Dow, R. L.; Changelian, P. L.; Brissette, W. H.; Weringer, E. J.; Pollok, B. A.; Connelly, P. A. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. *J. Biol. Chem.* **1996,** *271*, 695–701.

DOI: 10.1021/ml100096t | *ACS Med. Chem. Lett.* **2010,** *1*, 331–335