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***In silico* Screening and Evaluation of *Plasmodium falciparum* Protein Kinase 5 (PK5) Inhibitors**

Amber L. Eubanks^{#a}, Marisha M. Perkins^{#a}, Kayla Sylvester^{#b}, Jack G. Ganley^a, Dora Posfai^b, Paul C. Sanschargin^c, Jiyong Hong^a [Prof], Piotr Sliz^c, and Emily R. Derbyshire^{a,b} [Prof]

^aDepartment of Chemistry, Duke University, 124 Science Drive, Durham, North Carolina 27708 (USA), emily.derbyshire@duke.edu

^bDepartment of Molecular Genetics and Microbiology, Duke University Medical Center, 213 Research Drive, Durham, North Carolina 27710 (USA)

^cDepartment of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 45 Shattuck Street, Boston, Massachusetts 02115 (USA)

[#] These authors contributed equally to this work.

Abstract

An *in silico* screen of 350,000 commercially available compounds was conducted with an unbiased approach to identify potential malaria inhibitors that bind to the *Plasmodium falciparum* protein kinase 5 (*PPK5*) ATP-binding site. *PPK5* is a cyclin-dependent kinase-like protein with high sequence similarity to human cyclin-dependent kinase 2 (*HsCDK2*), but its precise role in cell cycle regulation remains unclear. After two-dimensional fingerprinting of the top scoring compounds, 182 candidates were prioritized for biochemical testing based on their structural diversity. Evaluation of these compounds demonstrated that 135 bound to *PPK5* to a similar degree or better than known *PPK5* inhibitors, confirming that the library was enriched with *PPK5*-binding compounds. A previously reported triazolodiamine *HsCDK2* inhibitor and the screening hit 4-methylumbelliferone were each selected for an analog study. The results of this study highlight the difficult balance between optimization of *PPK5* affinity and binding selectivity for *PPK5* over its closest human homolog *HsCDK2*. Our approach enabled the discovery of several new *PPK5*-binding compounds from a modest screening campaign and revealed the first scaffold to have improved *PPK5/HsCDK2* selectivity. These steps are critical for the development of *PPK5*-targeting probes for functional studies and antimalarials with reduced risks of host toxicity.

Graphical Abstract

A virtual screen of 350,000 compounds identified several molecules with potential to inhibit *Plasmodium falciparum* protein kinase 5 (*PPK5*). Biochemical testing of predicted *PPK5*-binding compounds identified a 4-methylumbelliferone as able to bind the kinase and inhibit *P*.

Conflict of Interest

The authors declare no conflict of interest.

Supporting information for this article is given via a link at the end of the document.

falciparum blood stage parasites. Compound **2b** binds *PPK5* with a similar affinity as it binds to the structurally related human cyclin-dependent kinase 2, offering improved selectivity when compared to previously reported *PPK5* inhibitors.

Keywords

anti-*Plasmodium* activity; protein kinase 5; species selectivity; malaria; cyclin-dependent kinase 2

Malaria is a life-threatening disease that affects millions of people annually and resulted in over 400,000 deaths in 2016.^[1] Protozoan parasites from the *Plasmodium* genus infect human hepatocytes and erythrocytes to cause malaria, where *P. falciparum* is the species responsible for the largest number of deaths. Unfortunately, artemisinin-based combination therapies (ACT), the recommended treatment for *P. falciparum* infections, are losing efficacy due to the development of parasite drug resistance. This resistance to the first-line malaria treatment threatens disease control efforts and necessitates the development of new drugs with novel targets.^[2–3]

Protein kinases are attractive drug targets for several conditions including cancer, inflammatory diseases, infectious diseases, and others.^[4–7] The *P. falciparum* genome encodes for 86 protein kinases, many of which are thought to be essential based on genetic^[8–10] and chemical inhibition studies.^[11–12] Thus, the *Plasmodium* kinome remains a rich yet largely untapped area for novel drug target development.^[13–15] In contrast to human kinases, the biological functions of many *Plasmodium* kinases are unresolved due to the lack of molecular and chemical tools to probe mechanisms. Genetic methods to produce knockouts are often lethal and small molecule probes lack the selectivity necessary for cell-based studies.^[16]

The *Plasmodium* parasite within the human host first infects hepatocytes during the asymptomatic liver stage, which is requisite for the development of parasites that can infect erythrocytes to cause disease during the blood stage. Multi-stage inhibition of *Plasmodium* is desirable for drug development to reduce the risk of drug resistance. Additionally, dual stage inhibitors can be used to both prevent and treat malaria. Due to the broad importance

of many kinase families for cellular functions in other systems, kinases have the potential to be multi-stage drug targets for malaria.^[6–7, 17]

Protein structures can often be leveraged to facilitate inhibitor discovery, but only six *P. falciparum* protein kinases currently have elucidated crystal structures.^[18–19] Of these, *P. falciparum* protein kinase 5 (*PfPK5*), which has been crystallized at 1.9 Å resolution, is a putative cyclin-dependent kinase (CDK)-like protein^[18, 20–21] with the highest similarity in sequence and structure^[18] to *Homo sapiens* CDK2 (*HsCDK2*, 63% sequence identity and 74% similarity). Like *HsCDK2*, *PfPK5* may regulate cell division, however this role has not been established in the parasite.^[22] The high degree of homology to *HsCDK2* (Figure S1) makes studying *PfPK5* function with small molecules during infection difficult and all known *PfPK5* inhibitors exhibit poor selectivity.

Previous studies have leveraged the wealth of compounds with known *HsCDK2* affinity to discover *PfPK5*-binding compounds.^[18] This approach has been successful in identifying compounds with high nM affinity for *PfPK5*, but they often exhibit >100-fold selectivity for *HsCDK2*. For example, CDK1/2 Inhibitor III (**1a**) is a known *HsCDK2* inhibitor with an IC₅₀ of 0.5 nM.^[24] Previously, **1a** was found to bind to *PfPK5* with a *K_d* of 320 nM,^[11] but this affinity is offset by poor species selectivity. There have been limited efforts to modulate species selectivity of *PfPK5* inhibitors through a structure-activity relationship (SAR) approach. Therefore, to explore whether the selectivity of **1a** can be favorably adjusted towards *PfPK5*, we conducted a modest SAR study. Compound **1a** along with a strategically selected set of its structural analogs (**1b–1e**, Table 1) were synthesized^[24] for testing (see the Supporting Information for details). These compounds were selected from a previous SAR campaign of 16 compounds based on their varying inhibition of *HsCDK1*, a closely related kinase to *HsCDK2*, where potency decreased for **1b** (217-fold), **1c** (13-fold), **1d** (1,100-fold), and **1e** (75-fold) relative to **1a**. Of these compounds, we observed that the parent compound **1a** has the highest binding affinity to both *PfPK5* (*K_d*(app) 0.37 μM) and *HsCDK2* (*K_d*(app) 0.00039 μM), but it is 950-fold selective for *HsCDK2* (Figure 1A). This observation agrees with the reported activity of **1a** against *HsCDK1* as determined with a kinase inhibition assay.^[24] Among the analogs tested, changes in potency/affinity generally agreed between *HsCDK1*, *HsCDK2*, and *PfPK5* where **1c** was the next most active (Figure 1B) and **1d** was the least active (Figure S2). Compounds **1d** and **1e** did not bind to *PfPK5* up to 30 μM, therefore binding constants could not be determined. Due to a reduction in *HsCDK2* affinity for **1c** we observed increased selectivity for *PfPK5* — 180-fold selective for the human protein versus 950-fold for **1a**. However, while selectivity was improved, the analogs from this approach did not produce a compound selective for the parasite kinase or achieve desirable potency. These results demonstrate the challenges of designing selective *PfPK5* inhibitors using previously characterized CDK1/CDK2 inhibitors as starting points.

A target-based screening campaign is a previously unexplored approach to identify *PfPK5* inhibitors without bias for *HsCDK2* activity. To reduce both the cost and time of evaluating a large compound library, we first employed an *in silico* screening method to evaluate 350,000 accessible compounds for the ability to bind the *PfPK5* ATP-binding site. Such a strategy proved valuable for identifying selective inhibitors of *PfMRK*, a homolog of *HsCDK7*,^[23] and we predicted that it may also facilitate the discovery of *PfPK5* inhibitors

with improved species selectivity. Our initial docking studies (standard precision and extra precision) identified 400 structurally diverse potential inhibitors of *PPK5*. The top 182 compounds were chosen for further analysis based on structural diversity (Figure S3) and were obtained through the ICCB-Longwood Screening Facility (Harvard Medical School) for *in vitro* studies. Compounds were screened for binding to *PPK5* (Figure 2, Table S2) at 12 μM using an active-site-directed competition assay (KINOMEscan®). In this assay, protein bound to beads is released when test compounds bind to the ATP-binding site. After normalization to a DMSO control, the relative protein remaining on the beads is assessed. As controls, two known *PPK5* inhibitors, purvalanol A and indirubin-3'-monoxime, were included in the library as they were also predicted to bind *PPK5* based on the *in silico* analysis. The identification of known *PPK5*-binding compounds as candidates in the virtual screen supported the promise of the strategy to identify novel *PPK5* ligands. Additionally, the selection of these compounds for testing provided internal controls for the protein binding assay. After evaluation of the positive and negative controls, screening results were grouped into three categories; no/low-binding (100–95%, 44 compounds), moderate-binding (95–50%, 135 compounds, 74% hit rate), and high-binding (<50%, 3 compounds, 1.6% hit rate) (Figure 2).

Purvalanol A and indirubin-3'-monoxime are known to inhibit *PPK5* with IC_{50}s of 8 μM ^[18] and 6 μM ^[25], respectively. To the best of our knowledge these are among the most potent *PPK5* inhibitors reported; therefore, the identification of these controls within the moderate-binding group suggests many uncharacterized *PPK5*-binding compounds exist within this group. The high hit rate of 76% indicates that our *in silico* approach provided a library enriched with *PPK5*-binding compounds. While a wealth of potential *PPK5* inhibitors stemmed from the primary screen, we sought to prioritize our studies on a select number of compounds.

The 182 *PPK5*-binding candidates were counter-screened for *HsCDK2* binding at 12 μM using a similar active-site-directed competition assay (Table S2). *HsCDK2* was selected for this screen due to its high sequence similarity to *PPK5*. After normalization to the DMSO negative control, the percent bound to *HsCDK2* versus *PPK5* was plotted to reveal compounds that preferentially bind to either protein (Figure 3). This analysis highlights the challenge of discovering selective kinase inhibitors, as many compounds bound both proteins to a similar degree. However, four compounds displayed selectivity for *HsCDK2* and three, **2a**, **3a**, and **4a**, displayed selectivity for *PPK5*. Compounds **2a**, **3a**, and **4a** were selected for subsequent validation studies due to our interest in the selective targeting of *Plasmodium* parasites.

Screening actives were purchased and tested at additional concentrations (0–30 μM) to generate binding curves and determine apparent K_d values. Through this analysis, **3a** and **4a** (Figure S4) did not bind *PPK5* up to 30 μM . To probe this conflicting result, **3a** and **4a** from the original compound library plate were retested and it was observed that the binding observed in the primary screen was reproduced. This result indicates that the discrepancy in activity between the primary screen and follow up study is due to varying sample sources rather than a false discovery rate. It is not uncommon for compounds within screening libraries to degrade over time in DMSO, making validation studies critical. Unfortunately,

these experiments exhausted the original supply of **3a** and **4a** from the compound library plate, therefore no further evaluation of these compounds was pursued. Interestingly, **2a** (4-methylumbelliferone scaffold) showed 2.2-fold selective binding to *Pf*PK5 ($K_{d(app)}$ 5 μ M) over *Hs*CDK2 ($K_{d(app)}$ 11 μ M), as shown in Figure 4A. Compound **2a** binding to *Pf*PK5 and *Hs*CDK2 was evaluated with molecular docking (Figure S5), which suggested hydrogen bonds to the hydroxyl group on the 4-methylumbelliferone scaffold may stabilize binding. To further assess **2a**, five structural analogs (**2b–2f**, Table 1) were purchased for biochemical testing. The selection of analogs was based on 1) predicted interactions of **2a** to the *Pf*PK5 ATP-binding site (Figure S4), 2) structural similarity of >80% to **2a**, and 3) commercial availability. Among the analogs tested **2d–2f** did not bind *Pf*PK5 or *Hs*CDK2 up to 30 μ M, whereas **2b** and **2c** displayed dose-dependent binding to *Hs*CDK2 and *Pf*PK5 (Figure 4B). Compound **2b** bound to *Pf*PK5 ($K_{d(app)}$ 3.8 μ M) and *Hs*CDK2 ($K_{d(app)}$ 5.0 μ M) with similar potency while **2c** showed 2-fold selectivity for *Pf*PK5 ($K_{d(app)}$ 14 μ M) over *Hs*CDK2 ($K_{d(app)}$ 28 μ M). This difference in binding affinity is not considered parasite selective (>10-fold), similar to the parent compound **2a**, but it is significantly improved over the tested triazolo-diamine analogs (Figure 5). To the best of our knowledge **2a** and **2c** are the most *Pf*PK5 selective compounds reported to date.

For biological assays, the imine on **2a** is undesirable due to its known reactivity. In buffer, imines can hydrolyze to produce a ketone or aldehyde, which we observed by LC-MS after incubating **2a** in buffer (pH 7.4) for 10 min and 60 min. Additionally, certain imines have been included as Pan Assay Interference Compounds (PAINS)^[26] and are removed from screening libraries. Compounds **2b** and **2c** were selected to evaluate the importance of the imine and examine if the hydrolysis product contributes to bioactivity. Compound **2b** is the aldehyde product of **2a**, while **2c** retains the imine, but includes an iodo- group that should hinder protein binding due to steric bulkiness. While we anticipated no binding of **2c**, only a 3.7-fold decrease in the *Pf*PK5 $K_{d(app)}$ was observed. Compounds **2d–2f**, which maintain the sterics of **2a** without the imine, did not bind to either kinase. These observations suggest the hydrolysis products of **2a** and **2c** bind to the ATP-binding site. In agreement with this prediction, molecular docking indicates that **2b** binds to the *Pf*PK5 and *Hs*CDK2 ATP-binding sites with a similar docking score as **2a** (Figure S5,S6). Unfortunately, a comparative computational study does not reveal a molecular basis for the varying selectivity observed between **1a** and **2a**, which highlights the need for future structural studies (Figure S7).

To assess species selectivity in a cellular context, the studied compounds were tested for activity against human hepatoma cell lines (HuH7 and HepG2) and *P. falciparum* Dd2 asexual blood stage parasites. None of the studied compounds significantly inhibited HepG2 cells at 20 μ M, but **1a** and **1c** were found to reduce HuH7 viability by >25% at 20 μ M (Figure S8). Further evaluation of this inhibition with a dose-response study revealed that **1a** and **1c** inhibited HuH7 viability with EC₅₀ values of 67 and 107 μ M, respectively. The varying inhibition activity between the two cells lines is likely due to differences in replication rates, where HuH7 cells double at a faster rate than HepG2 cells under our culture conditions.

Compounds were also tested for *anti-Plasmodium* activity. From an initial screen at 20 μ M, **2b**, **2e**, **2f**, and **1a–1e** were all found to inhibit blood stage parasites by >25% (Figure S9A). After a dose-response analysis, the parent compound **2a** was not active, but **2b**, **2e**, and **2f** had EC₅₀ values ranging between 10–30 μ M (Figure S9B). While these compounds did not exhibit liver cell toxicity, they selectively inhibited *Plasmodium* parasite growth. Evaluation of the triazolo-diamine derivatives revealed that **1a** was the most potent inhibitor (EC₅₀ 0.14 μ M), but both **1c** and **1e** also significantly reduced parasite load with EC₅₀ values of 0.38 μ M and 6.4 μ M, respectively (Figure S9C). Therefore, these series are selective for inhibiting *Plasmodium* over hepatoma cells. This observed species selectivity in cells may be due to the varying replication rates of *Plasmodium* and hepatocytes *in vitro*, which would make the parasites more susceptible to cell cycle inhibitors over the course of the 48-hour assay. Alternatively, the compounds may be binding to other targets in cells to influence parasite viability. The possibility of off-target binding would also explain the poor correlation observed between *Pf*PK5 binding and parasite inhibition. However, biophysical properties such as cell permeability, solubility, and pharmacodynamic/pharmacokinetic profiles would need to be optimized to further explore this trend.

*Pf*PK5 has been implicated as a *Plasmodium* cell cycle regulator, but identifying *Pf*PK5 inhibitors to probe its function has been challenging due to its homology to *Hs*CDK1 and *Hs*CDK2. Here we report an *in silico* strategy coupled with a high-throughput screen to identify *Pf*PK5-binding compounds. After prioritization of compounds by potency and selectivity for the parasite protein over *Hs*CDK2, we identified the most selective scaffold discovered to date. When compared to modification of known *Hs*CDK2 inhibitors, target-based *Pf*PK5 screening appears to be a more fruitful path towards developing selective compounds with reduced host toxicity. The continued optimization of scaffolds to obtain species selectivity will be critical to generate chemical probes for better elucidating the function of cyclin-dependent like kinases in *Plasmodium* parasites and possible drug leads.

Experimental Section

Compound **1a** (CDK 1/2 Inhibitor III) was tested for binding affinity to both *Pf*PK5 and *Hs*CDK2 as an initial study on selectivity and potency. Subsequently, analogs of **1a** (**1b–1e**) were synthesized according to a previously reported procedure.^[24] All compounds were confirmed to have >95% purity based on high-resolution mass spectrometry (Agilent LC-MS).

Molecular docking studies were conducted to predict new compounds that bind to *Pf*PK5. To begin, 350,000 readily accessible compounds were docked onto the ATP-binding site of *Pf*PK5 (PDB ID: 1V00) from a virtual library using Glide (Schrödinger),^[28–29] a high throughput virtual screening program. The library members were carefully selected to enable efficient biochemical testing of any screening active. Compounds were first docked using standard precision (SP) followed by extra precision (XP) mode to eliminate false positives. Finally, 2D fingerprint clustering was used to further eliminate false positives by estimating differences in sub-structural fragments of each compound. The top 182 predicted binders were selected based on docking scores and structural diversity. Screening compounds were then cherry picked from diverse libraries and obtained through the ICCB-

Longwood Screening Facility (Harvard Medical School) for biochemical testing. A target-based assay was used to test the ability of the 182 compounds to bind to *PPK5* and *HsCDK2* at 12 μ M. The assay conducted by DiscoverX employs an active site-directed competition binding assay using KINOMEscan® Technology.^[27] First, singleconcentration tests were completed with compounds from the ICCB-Longwood Screening Facility and then compounds that exhibited binding to *PPK5* at 12 μ M were purchased from ChemBridge Corporation for $K_{d(app)}$ determination with both *PPK5* and *HsCDK2* (DiscoverX). All compounds were >95% pure. Results were plotted using Excel for the initial biochemical test and GraphPad Prism for $K_{d(app)}$ determination where data were fit to a standard equation. Analogs of 4-methylumbelliferone (**2b–2e**, all >95% pure) were subsequently purchased from ChemDiv (**2b**) and ChemBridge Corporation (**2c–2e**) for further studies.

Purchased and synthesized compounds were tested for cytotoxicity in two different human liver cell lines (HuH7 and HepG2). Compounds (20 μ M) in triplicate were incubated with HepG2 (15,000 cells/well) or HuH7 (7,000 cells/well) cells in 384-well plates for 48 hrs in a standard tissue culture incubator at 37 °C. The final concentration of DMSO was 1% and the assay volume was 30 μ L. After 48 hrs, CellTiter-Glo (Promega) was added to each well to quantify ATP using an EnVision (Perkin Elmer). Dose-response curves were generated for any compound that exhibited significant cytotoxicity compared to the DMSO control. Compounds were also evaluated for *P. falciparum* Dd2 inhibition using a previously published protocol.^[30] Compounds (20 μ M) in duplicate were incubated with *P. falciparum* Dd2 in 384-well plates for 72 hrs with O-positive human blood in an atmosphere of 93% N₂, 4% CO₂, 3% O₂ at 37 °C. The final concentration of DMSO was 0.2% and the assay volume was 50 μ L. After 72 hrs, nuclei were stained with SYBR Green I (Invitrogen) and fluorescence was subsequently measured using an EnVision. The positive control was artesunate and the negative control was DMSO. Data were normalized to the DMSO control and any compound that inhibited *P. falciparum* viability 50% was selected for EC₅₀ determination (GraphPad Prism).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

- [1]. W. H. Organization, 2017, p. 196.
- [2]. Dondorp AM, Fairhurst RM, Slutsker L, Macarthur JR, Breman JG, Guerin PJ, Wellems TE, Ringwald P, Newman RD, Plowe CV, The New England journal of medicine 2011, 365, 1073–1075. [PubMed: 21992120]
- [3]. Fairhurst RM, Dondorp AM, Microbiology spectrum 2016,4.

- [4]. Cohen P, Nat Rev Drug Discov 2002, 1, 309–315. [PubMed: 12120282]
- [5]. Arslan MA, Kutuk O, Basaga H, Current cancer drug targets 2006, 6, 623–634. [PubMed: 17100568]
- [6]. Cohen P, Alessi DR, ACS chemical biology 2013, 8, 96–104. [PubMed: 23276252]
- [7]. Ward P, Equinet L, Packer J, Doerig C, BMC genomics 2004, 5, 79. [PubMed: 15479470]
- [8]. Solyakov L, Halbert J, Alam MM, Semblat JP, Dorin-Semblat D, Reininger L, Bottrill AR, Mistry S, Abdi A, Fennell C, Holland Z, Demarta C, Bouza Y, Sicard A, Nivez MP, Eschenlauer S, Lama T, Thomas DC, Sharma P, Agarwal S, Kern S, Pradel G, Graciotti M, Tobin AB, Doerig C, Nature communications 2011, 2, 565.
- [9]. Dorin-Semblat D, Quashie N, Halbert J, Sicard A, Doerig C, Peat E, Ranford-Cartwright L, Doerig C, Mol Microbiol 2007, 65, 1170–1180. [PubMed: 17651389]
- [10]. Holland Z, Prudent R, Reiser JB, Cochet C, Doerig C, Eukaryot Cell 2009, 8, 388–397. [PubMed: 19114502]
- [11]. Derbyshire ER, Zuzarte-Luis V, Magalhaes AD, Kato N, Sanschagrin PC, Wang J, Zhou W, Miduturu CV, Mazitschek R, Sliz P, Mota MM, Gray NS, Clardy J, Chembiochem : a European journal of chemical biology 2014, 15, 1920–1930. [PubMed: 25111632]
- [12]. Crowther GJ, Hillesland HK, Keyloun KR, Reid MC, Lafuente-Monasterio MJ, Ghidelli-Disse S, Leonard SE, He P, Jones JC, Krahn MM, Mo JS, Dasari KS, Fox AMW, Boesche M, El Bakkouri M, Rivas KL, Leroy D, Hui R, Drewes G, Maly DJ, Van Voorhis WC, Ojo KK, PloS one 2016, 11, e0149996. [PubMed: 26934697]
- [13]. Jirage D, Keenan SM, Waters NC, Infectious disorders drug targets 2010, 10, 134–146. [PubMed: 20334624]
- [14]. Lucet IS, Tobin A, Drewry D, Wilks AF, Doerig C, Future medicinal chemistry 2012, 4, 2295–2310. [PubMed: 23234552]
- [15]. Crowther GJ, Hillesland HK, Keyloun KR, Reid MC, Lafuente-Monasterio MJ, Ghidelli-Disse S, Leonard SE, He P, Jones JC, Krahn MM, Mo JS, Dasari KS, Fox AM, Boesche M, El Bakkouri M, Rivas KL, Leroy D, Hui R, Drewes G, Maly DJ, Van Voorhis WC, Ojo KK, PloS one 2016, 11, e0149996. [PubMed: 26934697]
- [16]. Greenbaum DC, Trends in pharmacological sciences 2008, 29, 51–56. [PubMed: 18199489]
- [17]. Doerig C, Billker O, Haystead T, Sharma P, Tobin AB, Waters NC, Trends Parasitol 2008, 24, 570–577. [PubMed: 18845480]
- [18]. Holton S, Merckx A, Burgess D, Doerig C, Noble M, Endicott J, Structure 2003, 11, 1329–1337. [PubMed: 14604523]
- [19]. Kim JJ, Flueck C, Franz E, Sanabria-Figueroa E, Thompson E, Lorenz R, Bertinetti D, Baker DA, Herberg FW, Kim C, PLoS pathogens 2015, 11, e1004639. [PubMed: 25646845]
- [20]. Graeser R, Wernli B, Franklin R, Kappes B, Molecular and Biochemical Parasitology 1996, 82, 37–49. [PubMed: 8943149]
- [21]. Ross-Macdonald PB, Graeser R, Kappes B, Franklin R, Williamson DH, European Journal of Biochemistry 1994, 220, 693–701. [PubMed: 8143724]
- [22]. Robbins JA, Absalon S, Strevia VA, Dvorin JD, mBio 2017, 8.
- [23]. Keenan SM, Geyer JA, Welsh WJ, Prigge ST, Waters NC, Combinatorial chemistry & high throughput screening 2005, 8, 27–38. [PubMed: 15720195]
- [24]. Lin R, Connolly PJ, Huang S, Wetter SK, Lu Y, Murray WV, Emanuel SL, Gruninger RH, Fuentes-Pesquera AR, Rugg CA, Middleton SA, Jolliffe LK, J Med Chem 2005, 48, 4208–4211. [PubMed: 15974571]
- [25]. Le Roch K, Sestier C, Dorin D, Waters N, Kappes B, Chakrabarti D, Meijer L, Doerig C, The Journal of biological chemistry 2000, 275, 8952–8958. [PubMed: 10722743]
- [26]. Baell JB, Holloway GA, Journal of medicinal chemistry 2010, 53, 2719–2740. [PubMed: 20131845]
- [27]. Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, Hocker M, Treiber DK, Zarrinkar PP, Nat Biotechnol 2011, 29, 1046–1051. [PubMed: 22037378]

- [28]. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS, J Med Chem 2004, 47, 1739–1749. [PubMed: 15027865]
- [29]. Shelley JC, Cholleti A, Frye LL, Greenwood JR, Timlin MR, Uchimaya M, J Comput Aided Mol Des 2007, 21, 681–691. [PubMed: 17899391]
- [30]. Plouffe D, Brinker A, McNamara C, Henson K, Kato N, Kuhen K, Nagle A, Adrian F, Matzen JT, Anderson P, Nam TG, Gray NS, Chatterjee A, Janes J, Yan SF, Trager R, Caldwell JS, Schultz PG, Zhou Y, Winzeler EA, Proc Natl Acad Sci U S A 2008, 105, 9059–9064. [PubMed: 18579783]

Figure 1. Binding of selected compounds to *PPK5* (red triangles) and *HsCDK2* (black circles). Structures are shown above plots. A) **1a** (CDK 1/2 Inhibitor III) and its most active analog B) **1c** are triazolo-diamines that exhibit selectivity to *HsCDK2*. Data are shown as the average $\pm$ SD and were fit to a standard dose response equation.

Figure 2.

Competition-based biochemical assay for *P/*PK5 binding. The ability of predicted compounds (circles) to bind to the *P/*PK5 ATP-binding site at 12 μ M was determined. Purvalanol A (blue circle) and indirubin-3'-monoxime (green circle) are known inhibitors. Compounds were grouped as high-binding, moderate-binding and no/low-binding. Controls are included in the moderate binding group (50–95%, indicated by grey dashed lines). Three compounds (**2a**, **3a**, and **4a**, red circles) are included in high binding group (>50%).

Figure 3. Relative binding to *Pf*PK5 and *Hs*CDK2 for top 182 scoring compounds (circles) from the *in silico* screen. The majority of the compounds display similar binding potency for *Pf*PK5 and *Hs*CDK2. The most potent *Pf*PK5-binding compounds are shown in red (**2a**, **3a**, and **4a**).

Figure 4. Binding of 4-methylumbelliferone compounds A) **2a** and B) **2b** to *PPK5* (red triangles) and *HsCDK2* (black circles). Structures are shown above plots. Data are shown as the average $\pm$ SD and were fit to a standard dose response equation.

Figure 5.

Fold selectivity of studied compounds for *Pfk5* and *HsCDK2* binding. Triazolo-diamine analogs (**1a–1c**) all display significant selectivity toward *HsCDK2* binding, while the 4-methylumbelliferone analogs (**2a–2c**) discovered via *in silico* screening displayed improved *Pfk5* selectivity. Fold selectivity was calculated as a ratio of the apparent dissociation constants, $K_{d(app)}$. Compounds with binding $>30 \mu\text{M}$ are not shown. Dashed red lines indicate ratios of -1 and 1 . Data for SNS-032 and flavopiridol are from previous reports. [11, 27]

Table 1.

Structures of the triazolo-diamine (**1**) and 4-methylumbelliferone (**2**) analogues examined for binding to *Pf*PK5 and *Hs*CDK2.^[a]

		<i>Hs</i> CDK2	<i>Pj</i> PK5
Compound	R ₁	R ₂	<i>K</i> _{d(app)} (μM)
1a		–	0.00039
1b		–	0.013
1c		–	0.013
1d		–	2.1
1e		–	0.13
2a		Me	11

1			2		
Compound	R ₁	R ₂	<i>Hs</i> CDK2	<i>Pf</i> PK5	
			<i>K</i> _{d(app)} (μM)	<i>K</i> _{d(app)} (μM)	
2b		Me	5	3.8	
2c		Me	28	14	
2d		Me	>30	>30	
2e		Me	>30	>30	
2f		Et	>30	>30	

^[a] Binding affinities determined in duplicate using the KINOMEScan® platform.