Structure–activity relationships and inhibitory effects of various purine derivatives on the in vitro growth of Plasmodium falciparum

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Abstract

The development of novel chemotherapeutic agents has become an urgent task due to the development and rapid spread of drug resistance in Plasmodium falciparum, the protozoan parasite responsible for cerebral malaria. Cyclin-dependent kinases (CDKs) are essential for the regulation of the eukaryotic cell cycle, and several enzymes of this family have been identified in P. falciparum. In recent years, a number of purine-derived kinase inhibitors have been synthesised, some of which display selective activity against CDKs. This report describes a study in which various purine derivatives were screened for in vitro antimalarial activity. The erythrocytic asexual stages of the chloroquine-resistant P. falciparum strain (FCR-3) were cultivated in vitro in the presence of the various purines, and their effect on parasite proliferation was determined by the [3H]hypoxanthine incorporation assay. Our results show considerable variation in the sensitivity of P. falciparum to the different purines, as well as a general independence from their effect on purified starfish CDK1/cyclin B activity, which has been the standard assay used to identify CDK-specific inhibitors. Two subfamilies of purines with moderate to poor activity against CDK1/cyclin B activity showed submicromolar activity against P. falciparum. Structure–activity analysis indicates that certain structural features are associated with increased activity against P. falciparum. These features can be exploited to synthesise compounds with higher activity and specificity towards P. falciparum. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Plasmodium falciparum; Malaria; Cyclin-dependent kinases; Olomoucine; Roscovitine; Purvalanol; Kinase inhibitors

1. Introduction

Plasmodium falciparum is the causative agent of cerebral malaria and annually causes 1.5–2.7 million deaths throughout the world. Due to the development of resistance to chloroquine and more recently to mefloquine, there is an urgent need to find new chemotherapeutic agents with novel mechanisms of action [1,2]. Very little is known about the developmental regulation of the various stages of the parasite’s complex life cycle. Once sporozoites are injected into the human host, they invade hepatocytes and develop into merozoites which in turn invade erythrocytes, where further asexual multiplication occurs. The 42–48-hr intra-erythrocytic cycle is characterised by a succession of distinct morphological forms; the ring form is followed by the trophozoite which in turn forms a schizont. Upon the completion of nuclear divisions in the schizont, the erythrocyte ruptures to release 8–32 merozoites that infect new erythrocytes. After invasion, a fraction of parasites arrest their cell cycle and differentiate into male or female gametocytes, which are transmitted back to the mosquito vector [3]. This process is vastly different from the cell cycle events observed in higher eukaryotes. The eukaryotic cell cycle is controlled by a conserved group of enzymes known as the CDKs [4,5]. To date nine distinct CDKs (CDK1–CDK9) have been identified, each of which can associate with different cyclins, (A–K and T). CDK activity is regulated by the association of a cyclin to the kinase catalytic subunit, by site-specific

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Abbreviations: CDK, cyclin-dependent kinase; CDK1, cyclin-dependent kinase 1; and PIPK, Plasmodium falciparum protein kinase.
phosphorylation and dephosphorylation, and by interaction of the CDK/cyclin complex with CDK-inhibitory proteins such as p16 and p21 [4,5].

CDK/cyclin activity is frequently deregulated in human cancers, which stimulated interest in the chemical inhibition of these enzymes. A CDK1/cyclin B enzyme complex isolated from starfish oocytes has been used to identify CDK-specific inhibitory compounds, and some of these compounds are currently being evaluated as possible cancer therapeutic agents [6–9]. In this context, molecules based on the purine structure were tested for in vitro activity against purified CDKs and other kinases. Several compounds were found to have selective activity against CDKs. This group includes isopentenyladenine [10], olomoucine [11], roscovitine [12,13], and the purvalanol series of compounds [7,14]. Olomoucine showed selective activity against CDK1/cyclin B activity but with only moderate inhibition (IC\text{50}: 7 \text{\mu M}). Synthesis of derivatives of olomoucine resulted in the identification of roscovitine, which selectively inhibited a subset of CDKs when tested against 25 different kinases. Roscovitine inhibited CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E, and CDK5/p35 with IC\text{50} values of 0.65, 0.7, 0.7, and 0.2 \text{\mu M}, respectively, whereas the CDK4/cyclin D1 and CDK6/cyclin D2 complexes were not significantly inhibited by this molecule [12,13]. The purvalanol group of inhibitors was identified from combinatorial libraries of 2,6,9-substituted purines, and found to be more potent than roscovitine against the same subset of CDK/cyclin complexes [7]. Purvalanol A inhibits CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E, and CDK5/p35, with IC\text{50} values of 4, 70, 35, and 75 nM, respectively, and purvalanol B inhibits the same subset with IC\text{50} values of 6, 6, 9, and 6 nM, respectively. Although purvalanol B is more potent against the isolated enzyme assays, it has no significant inhibitory effects on several mammalian cell lines. This can be explained partially by the presence of an additional carboxyl group on the molecule, which presumably makes it less permeable to cell membranes [7].

\textit{P. falciparum} homologues of several essential cell cycle genes have been identified [15,16]. The first kinase of this family to be identified was PfPK6 (\textit{Plasmodium falciparum} Protein Kinase 5), a putative homologue of CDK1 [17]. In vitro, PfPK6 kinase activity can be increased by three orders of magnitude by the addition of a newly described \textit{P. falciparum} cyclin homologue or by certain mammalian cyclins [18]. A second CDK-related kinase, PfPK6, shares structural properties with both CDK and mitogen-activated protein kinases and does not require cyclin binding for kinase activity \textit{in vitro} [19]. Pfcrk-1 (\textit{Plasmodium falciparum} \textit{cdc2}-related protein kinase) is a gametocyte-specific kinase most closely related to the p58\textsuperscript{GTA} subfamily found in vertebrates, isoforms of which are involved in cell differentiation or apoptosis [20]. Pfcrk (\textit{Plasmodium falciparum} MO15-related kinase) is a \textit{P. falciparum} CDK homologue that is most closely related to human CDK7, the kinase responsible for activation of CDK1 [21]. The \textit{P. falciparum} genome project has allowed the identification of additional CDK-related kinases: one of these tentatively called Pfcrk-3, shows maximal homology to a consensus sequence CDK1, from various species.\textsuperscript{1} Likewise, a gene encoding an enzyme with high homology to cyclin G-associated kinase is currently being characterised.\textsuperscript{2} The functions of these kinases in the life cycle of the parasite remain undetermined, mainly as a consequence of the difficulties associated with genetic manipulation of the parasite.

Despite being sufficiently conserved to allow their classification within the CDK family, the parasite's CDK-related kinases nevertheless display significant divergences when compared to the mammalian enzymes. In the catalytic domain, 40–60% of the residues are different between the mammalian and plasmodial enzymes, and the latter display unique structural features such as insertions or terminal extensions. Furthermore, there is evidence that the regulation of the activity of the \textit{P. falciparum} enzymes differ from that of mammalian CDKs: for instance, there is no known homologue of PIPK6 in mammalian cells, and PfPK5 is able to autophosphorylate in the presence of a cyclin, a peculiarity that has not, to our knowledge, been documented in the CDKs from higher eukaryotes [18]. Such considerations suggest that the enzymes from \textit{P. falciparum} may have different susceptibilities to small molecule kinase inhibitors.

Data obtained from inhibitor studies on recombinant active \textit{P. falciparum} enzymes showed high IC\text{50} values compared to that of the starfish CDK1/cyclin B assay. Purvalanol A (R) inhibits the activity of CDK1/cyclin B with an IC\text{50} value of 0.004 \text{\mu M} and the activity of the \textit{P. falciparum} enzyme PfPK5.p25 with an IC\text{50} value of 8 \text{\mu M} [18]. Similar discrepancies were evident when the IC\text{50} values of roscovitine and olomoucine on the kinase activity of PfPK6 were determined. The IC\text{50} values were found to be 30 \text{\mu M} for roscovitine and 180 \text{\mu M} for olomoucine, which is in discordance with their respective IC\text{50} values of 0.45 and 7 \text{\mu M} obtained for CDK1/cyclin B complex [19]. Since the \textit{in vivo} substrates, inhibitors, activators, and functions of these \textit{P. falciparum} kinase-related enzymes are still the object of active investigation, it was decided to determine the effect of purine-derived kinase inhibitors on the whole organism rather than on specific recombinant \textit{P. falciparum} enzymes.

Purines are structurally related to the natural substrate of kinases, ATP, and therefore attractive in terms of parent structures for rational drug design. Given the ubiquitous presence of purine-recognising enzymes, libraries of 2,6,9-trisubstituted purines may target a variety of enzymes involved in \textit{P. falciparum} proliferation and cell cycle control. Furthermore, purines represent an attractive scaffold for combinatorial chemistry, due to the ability to display diverse functionality at the 2, 6, 8, and 9 positions of the purine ring.

\textsuperscript{1} Le Roch K, Doerig C. Unpublished observations.

\textsuperscript{2} Harmse L, Doerig C. Unpublished observations.
In order to obtain a holistic picture of the effect of various purine derivatives on the parasite, we investigated the effect of purine derivatives of known CDK-selective and non-selective inhibitors on the survival of the chloroquine-resistant strain of *P. falciparum* (FCR-3). Information thus obtained can be useful to identify the specific target molecules of the purines in *P. falciparum*. Assessment of the inhibitory effect of CDK inhibitors will give an indication of the importance of purine target molecules for the proliferation of the parasite and can also be used to identify parasite-specific drug targets. The identification of compounds which display selective inhibition of parasite growth and the subsequent investigation of their molecular targets may provide information on the function of CDK-related enzymes in the life cycle of the parasites.

2. Materials and methods

2.1. Chemicals

All the purine compounds tested were prepared according to published methods [14, 21]. RPMI-1640 culture media were obtained from Highveld Biological, HEPES from Roche Biochemicals, d-sorbitol and d-glucose from Merck, and unlabelled hypoxanthine from Sigma, [3H]hypoxanthine, [γ-32P]-ATP, and ACS scintillation cocktail were obtained from Amersham, UK. GFB filtermats (Wallac) were obtained from SA Scientific.

2.2. Parasite culture

The chloroquine-resistant strain of *P. falciparum* (FCR-3) was obtained from Janet Freese of the Research Institute for Diseases in the Tropical Environment, Durban, South Africa. The parasites were cultured according to the method of Trager and Jensen [22]. Briefly, parasites were maintained in vitro in culture flasks in an erythrocyte suspension (5% haematocrit) in RPMI-1640 culture medium supplemented with 25 mM HEPES, 10 mM glucose, 0.32 mM hypoxanthine, and 10% (v/v) heat-inactivated human plasma. The growth medium was replaced daily and washed erythrocytes were added when the cultures were in the trophozoite stage. Cultures were synchronised with 5% d-sorbitol for 20 min at room temperature when the parasites were in the ring stage [23]. All experiments were carried out on synchronised cultures. The percentage parasitaemia and stages were assessed daily by microscopic examination of thin blood smears stained with Giemsa [22].

2.3. Hypoxanthine incorporation assay

The IC50 values of the CDK inhibitors were determined by the [3H]hypoxanthine incorporation assay [24]. Parasites were exposed to the agents for 76 hr, a period which permits near completion of two cycles of DNA synthesis. The longer exposure time (as opposed to 48 hrs) also permits evaluation of merozoite reinvasion of red blood cells. Briefly, parasites synchronised in the ring stage were plated in a 96-well plate at a 0.5% parasitaemia and 1% haematocrit in the presence of various inhibitor concentrations (0.01–100 μM). All assays were carried out using untreated parasites as controls. The purines were dissolved in DMSO at stock concentrations of 1 or 10 mM. Labelled [3H]hypoxanthine (0.5 μCi/well, 27.0 Ci/mmol) was added after 24 hrs and the cells were harvested on a GFB filtermat with a Titertek® cell harvester after 76 hrs of exposure. The filtermats were dried, transferred to plastic bags filled with scintillation cocktail (10 mL), and sealed. Incorporation of [3H]hypoxanthine into DNA was measured by liquid scintillation counting, and the IC50 values are averages of at least four experiments that were calculated from sigmoidal log dose–response curves using the Enzfitter® and Prizm® software packages.

2.4. CDK1/cyclin B kinase assay

CDK1/cyclin B was extracted from M-phase starfish oocytes (*Marthasterias glacialis*) in homogenisation buffer which consisted of 60 mM β-glycerophosphate, 15 mM p-nitrophenyl-phosphate, 25 mM 4-morpholine-propane sulfonic acid (pH 7.2), 15 mM EGTA, 15 mM MgCl2, 1 mM 1,4-dithiothreitol, 1 mM sodium vanadate, 1 mM NaF, 1 mM phenylphosphate, 10 μg/mL of leupeptin, 10 μg/mL of aprotinin, 10 μg/mL of soybean trypsin inhibitor and 100 μM benzamidine. It was purified by affinity chromatography on p9CKShs1-Sepharose beads, from which it was eluted by free p9CKShs1 (high-affinity CDK1-interacting protein) as previously described [6, 13]. The kinase activity was assayed in buffer C (homogenisation buffer with 5 mM EGTA, and without NaF and protease inhibitors), with 1 mg/mL of histone-H1, in the presence of various inhibitor concentrations (0.01–100 μM). All assays were carried out using untreated parasites as controls. The purines were dissolved in DMSO at stock concentrations of 1 or 10 mM. Labelled [3H]hypoxanthine (0.5 μCi/well, 27.0 Ci/mmol) was added after 24 hrs and the cells were harvested on a GFB filtermat with a Titertek® cell harvester after 76 hrs of exposure. The filtermats were dried, transferred to plastic bags filled with scintillation cocktail (10 mL), and sealed. Incorporation of [3H]hypoxanthine into DNA was measured by liquid scintillation counting, and the IC50 values are averages of at least four experiments that were calculated from sigmoidal log dose–response curves using the Enzfitter® and Prizm® software packages.

3. Results

3.1. Initial screening and selection of compounds

As a primary screen, we tested a selection of 565 compounds derived from known kinase inhibitors, most of which were purine derivatives. The effects of the compounds were evaluated using a final concentration of 2 μM,
from which a subset of 19 purines was selected for further study as a result of their relatively high activity against the parasite or their structural similarity to purines with high activity. Initially compounds were screened over a single (48 hrs) and a double (76 hrs) cycle of exposure. As some compounds had large discrepancies in their activity when the two exposure times were compared, it was decided to carry out the determination of their IC₅₀ values over a double cycle. With the exception of isopentenyladenine and compound 101, all these selected molecules were 2,6,9-tri-substituted purine derivatives with an amino group at C2 and a benzylamino or anilino group at C6 (Figs. 1, 2, 3, and 4). The dose–response curves, IC₅₀ values, and the relevant chemical structures are presented in Figs. 1, 2, 3, and 4.

3.2. Antimalarial activity of iso-olomoucine, olomoucine, roscovitine, and isopentenyladenine

Olomoucine, roscovitine, and isopentenyladenine display similar activity against P. falciparum (Fig. 1), with IC₅₀ values of 8.45, 5.35, and 8.34 μM, respectively. This is in sharp contrast with the effects of these compounds on iso-

3.3. Antimalarial activity of the purvalanol series of compounds

The purvalanol series of compounds showed a wide range of inhibitory activities against P. falciparum (Fig. 2). Molecules of this family are characterised by the presence of a chiral valinol substituent at C2. Within this family, the R and S isomers of purvalanol A (Fig. 2) displayed similar high levels of inhibitory activity against P. falciparum proliferation, with IC₅₀ values of 0.55 and 0.42 μM, respectively. In contrast, there was a 15-fold difference in activity between the two isomers on purified CDK1/cyclin B, with the R isomer being the most active with an IC₅₀ value of 0.03 μM compared to 0.5 μM of the S isomer. It is also important to note that the IC₅₀ value of purvalanol A (R) on recombinant PfPK5.p25 activity is 8 μM [8], which indicates that this enzyme is probably not the in vivo molecular target of purvalanol A (R) in P. falciparum.

Purvalanol B (95) contains a carboxyl group on the 3-chloroanilino C6 substituent of purvalanol A (Fig. 2), which makes it a very potent inhibitor when tested on the isolated CDK1/cyclin B enzyme system with an IC₅₀ value of 0.02 μM. Although it is a potent CDK inhibitor, it has a mediocre effect on P. falciparum with an IC₅₀ value of 7.07 μM. It is nevertheless noteworthy that this compound has no inhibitory effect on a large number of human cancer cell lines (IC₅₀ > 100 μM), presumably as a result of the hydrophilicity conferred by the carboxyl group and hence shows some specificity towards P. falciparum.³ The methylated derivative of purvalanol B (95M), which is inactive

³Gray N, unpublished observations.
in the kinase assay, is also less active against *P. falciparum* with an IC<sub>50</sub> value of 47.5 μM.

An amino substituent on the chloroanilino group of purvalanol A (forming compound 97) does not significantly increase the activity against *P. falciparum* compared to the carboxylated derivative (compound 95), decreasing the IC<sub>50</sub> value to 5.85 μM, which is in contrast to the high activity against the isolated CDK1/cyclin B system (IC<sub>50</sub>: 0.03 μM). Methylation of 97 (97M) abolishes the strong inhibitory effect against isolated CDK1/cyclin B, and also decreases its antimalarial activity (IC<sub>50</sub>: 11.04 μM). In conclusion, further substitutions of the chloroanilino substituent of purvalanol with either amino or carboxyl groups has a detrimental effect on the antimalarial activity of these compounds.

### 3.4. Selective inhibitory activity of compounds 99 and 101 against *P. falciparum*

The two purine derivatives, compounds 99 and 101, share common substitutions on C2 (3-trifluorobenzylamino) and C6 (4-aminobenzylamino), as seen in Fig. 3. These compounds show little effect against purified CDK1/cyclin B, with IC<sub>50</sub> values of 25 and 42 μM, respectively, while they are active against *P. falciparum* at submicromolar concentrations, with IC<sub>50</sub> values of 0.83 and 0.63 μM, re-
spectively (Fig. 3). Our data do not allow us to attribute the activity of these compounds to either one of the two substitutions on the C2 or C6 groups. However, considering the structure–activity profiles of the compounds discussed above (the olomoucine and purvalanol families), it seems likely that the increased activity is related to the C2 trifluoro-methyl-benzylamino substituent.

3.5. Antimalarial activity of R and S phenyl substitutions on the C2-hydroxyethyl substituent

Compounds 59 and 66 are enantiomers with bulky phenyl substitutions on the chiral carbon of the C2 hydroxyethylamino substituent (Fig. 4). Compound 59 is active against *P. falciparum* with an average IC\(_{50}\) value of 0.53 \(\mu M\), whereas compound 66 shows no activity against the parasite at the highest concentration tested (10 \(\mu M\)). Both compounds show submicromolar activity against the CDK1/cyclin B complex with compound 66 being the most active with an IC\(_{50}\) value of 0.4 and 0.8 \(\mu M\) for compound 59. This indicates that the spatial orientation of the phenyl ring has a major effect on the antimalarial activity but not against the isolated enzyme, and provides evidence that the parasite target is probably not identical in structure to the CDK1/cyclin B enzyme system. This is in contrast with the effect observed for the R and S isomer of purvalanol A, where the substitution on the same chiral carbon is the aliphatic isopropyl group of the amino acid valine, which displays similar antimalarial activities.

3.6. The effect of halogen substitutions on C6 on antimalarial activity

Compounds 43, 51, and 52 are structurally very similar, differing only in the substitutions on C6 (Fig. 4). Nevertheless, we observed a considerable variation of the IC\(_{50}\) values of these compounds on *P. falciparum* (IC\(_{50}\) values of 0.56 and 7.10 \(\mu M\) for compound 51 and 52, respectively). This is in contrast with the submicromolar IC\(_{50}\) values of 0.42 and 0.22 \(\mu M\) for 51 and 52, respectively, for these compounds on the CDK1/cyclin B system. Compound 43 has an IC\(_{50}\) value of 1.12 \(\mu M\) when tested against *P. falciparum*, which is almost four times lower than that of the CDK1/cyclin B system of 4.3 \(\mu M\). This is higher than that of compound 51 but much lower than that of its isomer, compound 52 (IC\(_{50}\): 7.1 \(\mu M\)). Methylation of compound 52 reduces the antimalarial activity to an IC\(_{50}\) value of 32.3 \(\mu M\), which is consistent with its lack of activity against the CDK1/cyclin B enzyme system.

3.7. Antimalarial activity of compound 40

This compound has a unique structure with bulky substitutions on the C2 and C6 rings (Fig. 4). It has a submicromolar IC\(_{50}\) value of 0.54 \(\mu M\) which compares well with that of the other purines tested. The IC\(_{50}\) value of this compound against *P. falciparum* is 10-fold lower than its IC\(_{50}\) value against the CDK1/cyclin B enzyme system of 5 \(\mu M\). Interestingly, this compound also has antifungal activity.  

4. Discussion

This study indicates that, as expected, some purine-based kinase inhibitors are able to interfere with parasite growth. We identified several compounds that were capable of inhibiting chloroquine-resistant parasites with IC\(_{50}\) values in the submicromolar range. Furthermore, in most cases, the IC\(_{50}\) values of the selected compounds on the parasite growth do not correlate with those of the higher eukaryotic CDK1/cyclin B enzyme. These findings are encouraging with regard to the identification of a parasite-specific inhibitor. It is clear, however, that any given compound based on a purine nucleus has a very large number of potential targets in a mammalian cell. The human genome encodes approximately two thousand kinases and countless additional purine-binding proteins. It is therefore essential to determine the effects of any potentially useful compound on human cells.

Variable toxic effects of the purine derivatives were observed using two different cell lines (data not shown). When the effect of roscovitine and olomoucine were eval-
uated using a tetrazolium-based assay on two cell lines, namely Graham embryonic epithelium cells and Giant Cell Tumour lung cells, there were significant differences between the two lines with Graham cells being more sensitive. This aspect of the study is hindered, though, by the fact that CDKs and cyclins are frequently over- or under-expressed in cancer cell lines, which decreases the relevance of the information obtained from cell culture toxicity studies. Ultimately, promising compounds will have to be evaluated using intact organisms in acute and chronic toxicity studies.

Since the $I_{50}$ values of the compounds tested are considerably higher than the $I_{50}$ values of chloroquine (176 nM), halofantrine (0.8 nM), mefloquine (15.4 nM), pyrimethamine (149 nM), and quinine (141 nM), it is clear that none of the purines tested have immediate potential as therapeutic agents on the erythrocytic stage of the *P. falciparum* and do need further development. The study did, however, provide some indication of specific structural features of the purine derivatives that show increased activity against the parasite. The data indicate that various substitutions on C2 of the purine ring can make substantial differences to the antimalarial activity. The size and spatial orientation of substitutions on the chiral carbon of the hydroxethyl amino group of C2 of the purine ring is of particular importance. A bulky aromatic ring in the R orientation abrogates the antimalarial effect, whereas the S orientation results in an antimalarial effect comparable to the most efficient *P. falciparum* purine inhibitors. This is in contrast with the effect of the same two compounds on the isolated enzyme system, where the S isomer is twice as efficient in inhibiting activity as the R isomer. This indicates that the structure of the target of the compounds in the parasite is different to that of the CDK1/cyclin B enzyme tested. Isopropyl substitution on the same chiral carbon in the R and S orientation does not have a major impact on the antimalarial activity, which is very similar to that of compound 59. This is in contrast with the effect of these two compounds on the isolated enzyme, where the R isomer is approximately 15 times more active than the S isomer with a low $I_{50}$ value of 35 nM. This information can now be used to develop compounds that are selectively more active against *P. falciparum*.

It has been shown that some of the parasite CDK-related kinases are expressed specifically in gametocytes. It is possible that CDKs expressed during the asexual cycle are required for differentiation into gametocytes or for the further stages of sexual development. Therefore, inhibitors of *P. falciparum* CDK-related kinases may well prevent gametocyte formation or maturation, and hence interfere with the sexual cycle of the parasite, and thereby represent potential transmission blocking agents. However, results obtained in this study indicate that roscovitine and olomoucine stimulate gametocyte formation in the FCR-3 strain in culture and have no inhibitory effect on gametocyte differentiation and development when the 3D7-strain is induced to form gametocytes (data not shown).

Although the direct effect of most of the compounds screened in this assay has not been tested on the cloned recombinant CDK homologous enzymes of *P. falciparum*, the limited data available indicate that recombinant enzyme systems must be used with caution in high throughput screening since many active compounds can be overlooked by using selective targets. Our results also clearly indicate that $I_{50}$ values obtained from the classical CDK1/cyclin B assay are not accurate predictors of antimalarial activity.

In conclusion, these results are particularly encouraging in that they illustrate how a simple screening approach utilising intact organisms can allow the detection of compounds that are much more active on the parasite than on individual enzyme systems. However, the actual targets of this subfamily of purines in *P. falciparum* remain to be identified.

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