Select pyrimidinones inhibit the propagation of the malarial parasite, *Plasmodium falciparum*

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1. Introduction

*Plasmodium falciparum* malaria kills ~3000 people each day, mostly children in sub-Saharan Africa. Several anti-malarial agents are available that—in principle—could prevent this disease. Unfortunately, the use of some of these agents is compromised by parasite resistance, high cost, and/or dangerous or unpleasant side-effects. Also, some therapies are combinations of existing drugs (e.g., artemether-lumefantrine, dihydroartemisinin-piperaquine, or artemunate-amodiaquine) that depend on the inability of the parasite to become resistant to artemisinin derivatives. Therefore, continued efforts to identify novel compounds that kill *P. falciparum* are critical.

The *P. falciparum* genome encodes six Hsp70 and forty-three Hsp40 molecular chaperones. Hsp70 molecular chaperones couple the hydrolysis of ATP with the binding and release of polypeptide substrates and play vital roles in protein folding, degradation and transport. Hsp40s interact with Hsp70 through a conserved, four helix-bundle known as the ‘J domain’. J domain interactions enhance Hsp70 ATPase activity; similar to Hsp70s, many Hsp40s are also polypeptide-binding proteins. Hsp70s and Hsp40s can constitute a significant amount of total cellular protein, a percentage that rises when cells are stressed (hence the ‘heat shock protein’ nomenclature). There are several reasons why *P. falciparum* might require the function of Hsp70 and Hsp40 chaperones. First, this parasite, like other members of the Apicomplexa, contains several endo-membrane systems. Because Hsp70-Hsp40 pairs engineer protein transport across membranes and are essential for membrane integrity, each internal membrane might require its own set of chaperones. In support of this hypothesis, it was recently shown that a J domain-containing protein in *P. falciparum* (PF10_0381) is required for ‘knob’ formation, a structure that helps the presentation of PfEMP1 proteins on the red blood cell surface; this, in turn, leads to the binding of parasitized red blood cells to the vascular endothelium.

Second, the parasite is exposed to radically different environments during its life cycle. It is capable of thriving in the mosquito, in the host liver, and in the highly oxidizing environment of the red blood cell. Thus, Hsp70s and Hsp40s might be necessary to offset cellular stresses that are encountered during the *P. falciparum* life cycle. Third, *P. falciparum* exhibits sudden bursts of protein synthesis as it enters the trophozoite stage that marks the initiation of several rounds of intracellular division.
Molecular chaperones help retain newly synthesized polypeptides in soluble conformations and facilitate folding. For example, an inhibitor of the Hsp90 chaperone, which is required for folding select cellular proteins, was shown to inhibit the ‘ring’ to trophozoite transition. Finally, it appears that the parasite contains extensive chaperone networks that are involved in a multitude of cellular activities. Based on these data, P. falciparum viability should be exceptionally sensitive to Hsp70 inhibition. Indeed, parasite growth is inhibited by 15-deoxypergualin, a non-specific chaperone modulator that binds to Hsp70 and to Hsp90 with a Kd of ~5 μM.

We previously reported the synthesis and characterization of pyrimidinone-peptoid hybrid molecules that modulate Hsp70 activity in vitro and that, in some cases, prevent cancer cell proliferation. These data indicate that specific Hsp70 modulators can be identified and that at least a sub-group of these compounds is membrane-permeable, as evidenced by their activity in a cellular assay. To test whether molecules in this class also compromise P. falciparum replication, we screened a small collection of pyrimidinones and identified nine compounds that exhibited potent effects on parasite metabolism. Some of these compounds inhibited P. falciparum viability with similar potencies to some established antimarial drugs. We also developed new purification schemes for Hsp70 proteins from Homo sapiens and P. falciparum and compared the effects of these compounds on the ATPase activities of the human, yeast, and parasite chaperones. Together, our data support the continued investigation of pyrimidinones as antimalarial agents.

2. Results and discussion

To assess whether pyrimidinones inhibit P. falciparum growth, we examined the effects of 157 compounds in this class and related Biginelli and Ugi multicomponent condensation-derived compounds on the uptake of [3H]hypoxanthine into infected human erythrocytes. The hypoxanthine assay provides a rapid, quantifiable read-out of parasite viability, and the compounds assayed included several recently described agents, as well as precursors and structurally related analogs.

The impact of JAB75 (see Section 3) on [3H]hypoxanthine uptake is shown in Figure 1. In this and all other assays, we used the chloroquine (CQ)-resistant Dd2 clone and employed CQ as an internal control because CQ is known to inhibit Dd2 with an IC50 value of ~0.2 μM. In this experiment, the IC50 for JAB75 was calculated to be ~0.3 μM (Table 1, second column) and the IC50 for CQ was 0.19 μM (data not shown). From our initial analysis of 157 compounds, we identified nine molecules (Fig. 2; see Section 3) with IC50 values between 30 nM and 1.6 μM (Table 1, second column).

To ensure that the compounds were not generally cytotoxic, we also determined the 50% growth inhibitory concentrations (GI50) for each of these nine agents in two human cell lines, HepG2 hepatocellular carcinoma cells and WI-38 embryonic diploid lung cells, as previously described. Based on this analysis, all GI50 values in these cells were >10 μM, which is well above the concentration needed to inhibit P. falciparum growth (Table 1). As a control for this experiment, we found that the GI50 values for paclitaxel in HepG2 and WI-38 cells were 1.0 ± 0.6 nM and 13.7 ± 0.2 nM, respectively (data not shown).

We previously showed that a subset of pyrimidinones inhibit the activity of Hsp70. Therefore, we next assessed the effects of the nine compounds on Hsp70 ATPase activity. We first examined the ability of each pyrimidinone to modulate the ATP hydrolytic rate of a purified yeast Hsp70, Ssa1, as previously published. In addition, we wished to compare the effects of these agents on human and P. falciparum Hsp70. The three chaperones are 71–74% identical to one another at the amino acid level and, not surprisingly, Hsp70s from different species have been reported to substitute functionally for one another. For example, the growth of bacteria containing a mutation in the gene encoding DnaK, the Hsp70 homolog, can be rescued at high temperature by expression of a P. falciparum Hsp70. One might also envision, however, that a specific inhibitor could selectively target the P. falciparum chaperone but have no effect on Hsp70s from different species.

To compare the effects of the lead compounds on the ATPase activities of the Hsp70s, we first modified purification schemes for both the H. sapiens and P. falciparum chaperones (see Section 3). The peak fractions from the P. falciparum Hsp70 purification are shown Figure 3A, and the single-turnover ATPase activities of the enzyme in the absence and presence of a J domain chimera (see below) are shown in Figure 3B.

Next, each of the nine compounds was incubated with the P. falciparum, yeast and human chaperones, and steady-state ATPase assays were performed. The results presented in Table 1, columns 3–5, indicate that the compounds display a range of activities and...
ter the activities of each chaperone distinctly. For example, JAB75, MAL2-61, and MAL2-215 reduced the rate of ATP hydrolysis by the human enzyme by 30%, but had no effect or stimulated the activities of the parasite and yeast enzymes. In contrast, MAL2-39 compromised the ATPase activities of all three enzymes, but mainly HsHsp70. MAL3-39 inhibited all enzymes to a very similar extent. Perhaps most intriguing, DMT2264 affected the ATPase activity of HsHsp70. MAL3-39 inhibited all enzymes to a very similar extent. Promised the ATPase activities of all three enzymes, but mainly ties of the parasite and yeast enzymes. In contrast, MAL2-39 compromised the human enzyme by 30%, but had no effect or stimulated the activities of each pyrimidinone. We chose to use single-turnover conditions for this analysis because the level of J domain-mediated stimulation of Hsp70 ATPase activity is significantly greater for single-turnover assays as compared to steady-state assays (see, e.g., Fig. 3B). Furthermore, the effects of compounds on enzyme $k_{cat}$ values can specifically be monitored and the fold change measured. As shown in Table 2, these nine compounds exhibited a range of effects. First, relatively subtle changes were observed when the effects of the compounds were examined in the presence of the J domain protein. For example, a <50% decrease (in the presence of MAL2-213 or DMT2264) and a ~40% increase (in the presence of MAL2-61 or MAL3-39) was measured when the J domain was added into the assay in the presence of these chemicals (Table 2, ‘fold change PfHsp70 CAT’). In contrast, two of the compounds induced a potent ‘burst’ of ATP hydrolysis in the single-turnover assay in the absence of a J domain (‘fold change P’ Hsp70 $k_{cat}$'). Based on a fit of the data to a single exponential, MAL2-39 and MAL2-61 enhanced $P. falciparum$ Hsp70 ATPase activity by 7.2 and 6.5-fold, respectively. Each of the other compounds also enhanced ATP hydrolysis to varying extents, an effect that has been previously noted for certain other pyrimidinones. Because the cycle of Hsp70 ATP binding/hydrolysis is coupled with substrate binding and release, altered rates of endogenous or J domain-stimulated ATP hydrolysis will correspondingly alter the efficacy of substrate binding. Therefore, enhanced rates of ATP hydrolysis can lead to defects in the ability of Hsp70s to act as a molecular chaperone, especially under stress conditions. Moreover, recent data confirm that the individual chaperone cycles and conformations have been tailored to match the folding of specific substrates. In the future, it will be important to examine whether these agents affect the binding of known Hsp70 substrates. Moreover, it is critical to note that effects on ATPase activity in steady-state assays may result from alterations in any one of a number of steps in the hydrolytic cycle, including the $k_{cat}$, ATP binding, and inorganic phosphate and/or ADP release.

An examination of the endogenous and J domain-stimulated ATPase activities of the yeast enzyme in the presence or absence of the compounds yielded quite different results (Table 2, ‘fold

Figure 2. Select pyrimidinones inhibit hypoxanthine uptake into $P. falciparum$-infected red blood cells. The depicted compounds inhibited $P. falciparum$ replication with IC$_{50}$ values of 30 nM–1.6 µM (see Table 1). The structures were drawn to maximize chemical similarity.
change Ssa1 $k_{\text{CAT}}$ and ‘fold change Ssa1 $k_{\text{CAT} + \text{Hlj1}}$’. We found that MAL2-215 exerted significant effects on both the endogenous and J domain-stimulated activity of Ssa1, but the compounds with the greatest impact on the *P. falciparum* enzyme (e.g., MAL2-39, MAL2-61, and MAL2-213) only modestly altered the activity of the yeast enzyme. Notably, the strongest effects of each pyrimidinone were observed when the activity of the human enzyme was examined in the presence of its partner, Hdj1. At this point, the substructure features that mediate these distinct phenomena remain to be elucidated. Our data nevertheless indicate that the continued examination of pyrimidinones with diverse Hsp70s will prove worthwhile, especially if a specific binding site on the chaperone for this class of modulators can be identified. Efforts toward this goal are underway.

It is important to note that the relative effects of the compounds on the endogenous or J domain-stimulated *P. falciparum* ATPase activities do not correlate with the IC50 values in the [3H]hypoxanthine uptake assay (compare Table 1 and Table 2). There are several explanations for this fact. First, it is possible that some compounds have secondary cellular targets, which may enhance the antimalarial effects. Second, some of the compounds may be metabolized when added to the infected erythrocytes to produce derivatives that may be more or less potent, depending on the nature of the modification. Third, the less potent agents in the [3H]hypoxanthine uptake assay might be actively excluded from
cells due to the action of multi-drug transporters or other gene products that are known to mediate drug resistance in this parasite. Assays with radiolabeled or fluorescently labeled compounds will help clarify this possibility by enabling measurements of compound accumulation in the parasite; however, these results are not yet available. Fourth, the bona fide Hsp70 that is a target of the active compounds may be any one of the other five Hsp70s that are encoded by the P. falciparum genome. It is striking how distinctly some compounds affect the activities of the yeast Ssa1 and human proteins and the P. falciparum Hsp70 utilized in this study (PfHsp70-1; PF08_0054) even though these proteins are >70% identical. Because Hsp70-1 and the mitochondrial Hsp70 in P. falciparum (PfHsp70-3; PF11_0351) are only 48% identical, the compounds are also expected to exhibit distinct effects on parasitic Hsp70s. The problem of identifying the target(s) of these compounds could, in principle, be rectified by the purification of each of the six P. falciparum Hsp70s and 43 P. falciparum J proteins so that each combination could be tested in ATPase assays in the presence of the modulators. A streamlined approach would be to prepare activated, affinity tagged derivatives of our novel P. falciparum inhibitors and then identify potential cellular target(s) using an unbiased screen. This experimental regimen would ideally isolate the ‘correct’ Hsp70 and/or Hsp40 chaperone target(s).

We note structural trends that relate to potency in the P. falciparum replication assay. Specifically, there are several common and distinct features amongst the identified inhibitors depicted in Figure 2. For example, all nine compounds share an ester pyrimidine core, substituted at C-4, and eight of the nine are alkylated at N1. Among the compounds, DMT3024, DMT2264, and MAL3-39 are more potent in the [3H]hypoxanthine uptake assay as established antimalarial agents and that are synthetically readily modified. Based on the success of our initial efforts, reported herein, we are confident that compounds with greater potency and improved pharmacological properties are within reach.

3. Experimental

3.1. Synthesis of compounds using in this study

The synthesis of MAL3-39 and MAL2-215, DMT3024, and DMT2264 has previously been reported. The following compounds were synthesized as described.

3.1.1. Synthesis of MAL2-29

TFA (50 μL) was added to a mixture of 6-ureidohexanoic acid (100 mg, 0.57 mmol), 3-nitrobenzaldehyde (73 mg, 0.48 mmol), and ethylacetate (61 μL, 0.48 mmol) in dichloroethane (3 mL), and the resulting mixture was heated to reflux under N2 for 12 h. Next, dichloroethane was removed in vacuo, and the residue was stirred in EtO, filtered, and washed with hexane/acetone to afford UPCLM00WMAL2-29 (128.0 mg, 53%) as a white solid. 1H NMR (300 MHz, DMSO-d6) δ 11.96 (s, 1H), 8.12 (d, 1H, J = 3.8 Hz), 8.06 (s, 1H), 7.65–7.63 (m, 2H), 5.26 (d, 1H, J = 3.6 Hz), 4.02 (q, 2H, J = 7.0 Hz), 3.87–3.83 (m, 1H), 3.48–3.42 (m, 1H), 3.33 (s, 1H), 2.49 (s, 3H), 2.09 (t, 2H, J = 7.2 Hz), 1.48–1.29 (m, 6H), 1.10 (t, 3H, J = 7.1 Hz); MS (API-ES) m/z (rel. intensity) 402.1 (MH+, 100%); purity determined by LC–UV at 254 nm: 94.9%.

3.1.2. Synthesis of MAL2-39

Benzyl acetoacetate (248 μL, 1.43 mmol) and para-anisaldehyde (175 μL, 1.43 mmol) were added to a solution of butyrylurea (200 mg, 1.72 mmol) in THF (3 mL). The solution was stirred for 10 min followed by the addition of concentrated HCl (two drops) and the resulting solution was stirred at ambient temperature for 48 h. The reaction mixture was concentrated to an oil, which was then purified by chromatography on SiO2 with hexane/EtOAc (2:1) and eluted with CHCl3 to afford UPCMLD00WMAL2-39 (502.9 mg, 86%). 1H NMR (300 MHz, CDCl3) δ 7.22–7.17 (m, 3H), 7.08–7.05 (m, 2H), 7.01 (dd, 2H, J = 8.7, 2.0 Hz), 6.65 (dd, 2H, J = 8.8, 2.1 Hz), 5.40 (d, 1H, J = 2.7 Hz), 5.22 (d, 1H, J = 2.7 Hz), 4.98 (d, 2H, J = 2.6 Hz), 3.82–3.75 (m, 1H), 3.70 (s, 3H), 3.53–3.47 (m, 1H), 2.45 (s, 3H), 1.49–1.43 (m, 2H), 1.26–1.19 (m, 2H), 0.84 (t, 3H, J = 7.2 Hz); MS (API-ES) m/z (rel. intensity) 409.1 (MH+, 100%); purity determined by LC–UV at 254 nm: 92.2%.

3.1.3. Synthesis of MAL2-61

Ethyl acetoacetate (141 μL, 1.11 mmol) and 2-formylphenyl-2-nitrobenzenesulfonate (341 mg, 1.11 mmol) were added to a solution of benzylurea (200 mg, 1.33 mmol) in THF (3 mL). The solution was stirred for 10 min followed by the addition of concentrated HCl (two drops) and the resulting solution was stirred at ambient temperature for 48 h. The reaction mixture was concentrated to a viscous oil, which was then purified by chromatography on SiO2 with hexane/EtOAc (2:1) and eluted with CHCl3 to afford UPCMLD00WMAL2-61 (488.3 mg, 80%) as a fine solid. 1H NMR (300 MHz, CDCl3) δ 7.22–7.17 (m, 3H), 7.08–7.05 (m, 2H), 7.01 (dd, 2H, J = 8.7, 2.0 Hz), 6.65 (dd, 2H, J = 8.8, 2.1 Hz), 5.40 (d, 1H, J = 2.7 Hz), 5.22 (d, 1H, J = 2.7 Hz), 4.98 (d, 2H, J = 2.6 Hz), 3.82–3.75 (m, 1H), 3.70 (s, 3H), 3.53–3.47 (m, 1H), 2.45 (s, 3H), 1.49–1.43 (m, 2H), 1.26–1.19 (m, 2H), 0.84 (t, 3H, J = 7.2 Hz); MS (API-ES) m/z (rel. intensity) 409.1 (MH+, 100%); purity determined by LC–UV at 254 nm: 83.8%.

3.1.4. Synthesis of MAL2-213

A stirred solution of dihydropyrimidinone (20 mg, 0.051 mmol) in CH2Cl2 (1 mL) containing DMAP (10 mg, 0.082 mmol) was treated with EDCI (11 mg, 0.056 mmol) followed by methyl 1-[(2-aminoethyl)-2-methyl-5-phenyl-1H-pyrole-3-carboxylate (15 mg, 0.051 mmol). The resulting solution was stirred/shaken at room temperature for 18 h. The mixture was washed with water, dried over MgSO4, and then purified by chromatography on SiO2 and eluted with hexane/EtOAc (3:1) and CHCl3/EtOAc (6:1) to afford UPCLM00WMAL2-213 (26 mg, 81%). 1H NMR (300 MHz, CDCl3) δ 8.18 (d, 2H, J = 6.8 Hz), 7.45–7.34 (m, 4H), 6.55 (s, 1H), 5.74 (br s, 1H), 5.54 (br s, 1H), 5.47 (d, 1H, J = 3.1 Hz), 4.16–4.09 (m, 4H), 3.80 (s, 3H), 3.79–3.73 (m, 1H), 3.63–3.60 (m, 1H), 3.30–3.23 (m, 2H), 2.62 (s, 3H), 2.55 (s, 3H), 1.99 (t, 2H, J = 6.5 Hz), 1.83–1.72.
4.12, 4.08 (AB, 2H, χH); MS (APCI) m/z (rel. intensity) 1285 (2MNa*, 60), 686 (100), 664 (40), 654 (MNa+, 40), 633 (MH+, 10); purity determined by 1H NMR: >95%.

3.1.5. Synthesis of JAB75

In the presence of polyphosphate ester (PPE, 300.0 mg), a solution of methyl 3-oxo-4-phenylbutanoate (384.0 mg, 2.0 mmol), 2-naphthaldehyde (312.0 mg, 2.0 mmol) and urea (180.0 mg, 3.0 mmol) in THF (40 mL) was heated at reflux for 15 h. The product was extracted into EtOAc, and the combined EtOAc extracts were dried (Na2SO4), concentrated, and purified by chromatography on SiO2 with hexane/EtOAc (3:1) to afford 330 mg (44%) of a mixture of JAB75 (H2, J = 7.1 Hz; MS (APCI) m/z (rel. intensity) 1285 (2MNa*, 60), 686 (100), 664 (40), 654 (MNa+, 40), 633 (MH+, 10); purity determined by 1H NMR: >95%.

3.2. Protein purifications

The yeast Hsp70, Ssa1, and a J domain-containing Hdj1-glutathione-S-transferase protein chimera were purified as previously described.19,33 Ydj1 was kindly provided by Dr. D. Cry (University of North Carolina School of Medicine), and Hdj1 was purchased from StressGen/Assay Designs. The purification of a hexahistidine-tagged version of Human Hsp70 was introduced into chemically competent Escherichia coli Rosetta 2 cells (Novagen), and transformed colonies were selected on LB with ampicillin and chloramphenicol. Overnight cultures were grown at 26°C and diluted 1:10 into 1 L of the same media, the cells were grown to mid-log phase, and IPTG was added before growth was continued for another 5 h at 26°C. Cells were harvested and lysed, and the extract was loaded on a nickel-chelating Sepharose column in lysis buffer (8 M urea, 300 mM NaCl, 10 mM Tris, pH 8.0, 10 mM imidazole, 1 mM PMSF, 2 mM leupeptin, 0.7 mM pepstatin A). The column was washed with 10 mL of low imidazole buffer (10 mM Tris, pH 8.0, 10 mM NaCl and 10 mM imidazole), followed by 20 mL imidazole buffer, and the bound proteins were eluted using a 10 M x 10 mL gradient of 25 mM to 500 mM imidazole. Samples from 1 mL fractions were analyzed by SDS–PAGE and peak fractions were pooled and dialyzed against 50 mM Tris, pH 7.4, containing 50 mM NaCl, 0.8 mM DTT, 2 mM MgCl2, and 5% glycerol. The dialysate was then subjected to a Q-Sepharose column using a preparative Superdex-200 gel filtration column equilibrated with TMC50 buffer (20 mM Tris, pH 7.2, containing 5 mM MgCl2 and 50 mM NaCl). Bound proteins were eluted with a step gradient of TMC50 and TMC100 (20 mM Tris, pH 7.2, containing 5 mM MgCl2 and 100 mM NaCl), and fractions containing Hsp70, as assayed by SDS-PAGE, were pooled and loaded onto an ATP-agarose column (Sigma-Aldrich), which was recirculated for 4 h. The column was washed with TMC100 and the bound Hsp70 was eluted with TMC100 containing 10% glycerol and 25 mM ATP, and then TMC100 containing glycerol, ATP, and 4 M NaCl. Fractions containing the majority of the Hsp70 protein, as assessed above, were pooled and dialyzed against 20 mM Tris, pH 7.2, containing 50 mM NaCl and 1 mM EDTA. The final dialysate was loaded onto a Q-Sepharose column (Sigma–Aldrich) equilibrated with TMC100, and the column was washed with TMC100 and the purified Hsp70 was eluted using a linear gradient of TMC100 to TMC400. The purified Hsp70 was dialyzed against 50 mM Tris, pH 7.4, containing 50 mM NaCl, 0.8 mM DTT, 2 mM MgCl2, and 5% glycerol, snap-frozen in liquid nitrogen, and stored at −80°C.

Second, a hexastidine (His6)-tagged form of human Hsp70 was constructed and purified, and significantly higher yields of protein were obtained. To this end, plasmid DNA pMSHSP (see above) was used as template in a polymerase chain reaction using oligonucleotide primers with the following sequences: ACGATCAGAAAGTTG ACCACCATCACATCACATTAG (forward) and GTCCACTTCTTCTGA TCCTGGGACCTGACCAAGACCCTGCTCTTCG (reverse), which replaced the six C-terminal amino acids with a His6 polypeptide tag at the C-terminus of the Hsp70 protein coding sequence. A ligation-independent mutagenesis protocol was used to design these primers and carry out the mutagenesis cloning.24 The sequence of the protein coding region of the recombinant plasmid was confirmed by DNA sequencing. For expression of recombinant Hsp70–His6, E. coli cultures containing the recombinant plasmid (pMSHSP- His6) were grown in LB containing 100 μg/ml ampicillin to O.D.600 = 0.8 at 37°C, and induced at 25°C for 18 h with 0.4 mM IPTG. Following centrifugation, the cell pellets were lysed by a combination of sonication (model W-220F, Branson) and passing the cells through a pressurized cell disruptor (Microfluidics) in 50 mM Tris, pH 8.0, containing 500 mM NaCl, 10% glycerol, and 20 mM imidazole. The Hsp70 was then purified at 4°C using a standard purification protocol recommended for use with Ni-NTA resin (QIAGEN), and the recombinant Hsp70–His6 was eluted from the resin with 100 mM Tris, pH 8.0, containing 200 mM imidazole and 500 mM NaCl. Next, the protein was further purified by size exclusion chromatography using a preparative Superdex-200 gel filtration column equilibrated in 50 mM Tris, pH 8.0, containing 500 mM NaCl. Fractions containing the purified protein were dialyzed overnight in 10 mM Tris, pH 8.0, at 4°C, and the dialyzed protein was pooled, aliquoted, and stored at −70°C.

3.3. In vitro and cell-based assays

Steady state and single turnover ATPase assays with the indicated Hsp70 preparations were performed as previously described.18,19 Compounds were assessed for their ability to inhibit the growth of P. falciparum asexual blood stages using [3H]hypoxanthine uptake assays, involving exposure of parasites to drug for 72 h and adding [3H]hypoxanthine for the last 24 h, as previously described.33 Each compound was screened at final concentrations of 1 μM and 5 μM. Compounds that initially appeared to inhibit [3H]hypoxanthine uptake by >50% at 5 μM and displayed at least as much inhibition at 1 μM were selected and re-screened. The group of nine compounds that passed this second test (Fig. 2) was then subjected to dose-response analyses. IC50 values were calculated using a linear regression analysis based on the linear portions of plotted values. The IC50 value for CQ was assessed as described.21
The effect of the compounds on the growth of HepG2 and WI-38 cells was assessed as previously described. GI50 values and were calculated relative to a DMSO (negative) control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.01.024.

References and notes