Identification of a New Chemical Class of Antimalarials

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The increasing spread of drug-resistant malaria strains underscores the need for new antimalarial agents with novel modes of action (MOAs). Here, we describe a compound representative of a new class of antimalarials. This molecule, ACT-213615, potently inhibits in vitro erythrocytic growth of all tested *Plasmodium falciparum* strains, irrespective of their drug resistance properties, with half-maximal inhibitory concentration (IC_{50}) values in the low single-digit nanomolar range. Like the clinically used artemisinins, the compound equally and very rapidly affects all 3 asexual erythrocytic parasite stages. In contrast, microarray studies suggest that the MOA of ACT-213615 is different from that of the artemisinins and other known antimalarials.

ACT-213615 is orally bioavailable in mice, exhibits activity in the murine *Plasmodium berghei* model and efficacy comparable to that of the reference drug chloroquine in the recently established *P. falciparum* SCID mouse model.

ACT-213615 represents a new class of potent antimalarials that merits further investigation for its clinical potential.

Malaria, caused by protozoan parasites of the genus *Plasmodium*, remains a major health problem. More than 240 million cases of malaria occur every year and the number of annual fatalities is estimated at over 800 000 [1]. The disease accounts for 20% of all childhood deaths in Africa [1]. In many temperate areas such as Western Europe or North America, malaria has been controlled or eliminated [2]. In contrast, economically less developed regions face 2 main problems fighting the disease: high-priced antimalarials [3] and the increasing drug resistance of the parasite [4–8]. Therefore, the need for new and affordable drugs is urgent and indisputable.

In 2007, the Bill and Melinda Gates Foundation unveiled an agenda with the overall goal of eradicating malaria [9]. This objective is pursued in conjunction with several other institutes such as the Roll Back Malaria partnership of the World Health Organization (www.rollbackmalaria.org) and one main nonprofit private public partnership, Medicines for Malaria Venture (MMV, www.mmv.org). Such strong partnerships were a boost for antimalarial research, leading to an encouraging global antimalarial portfolio [10–12] that currently contains more than 10 projects (preclinical to phase IV). Furthermore, a plenitude of chemical structures, potentially serving as starting points for new antimalarial lead substances, has been disclosed after extensive compound screenings [13–15]. Nevertheless, since 1996, not a single new chemical class of antimalarials has been registered [14], and the current global portfolio of antimalarial compounds in development relies largely on novel combinations—not novel compounds. Since 2007, there have been very few reports providing in-depth characterizations of new antimalarial chemotypes, stressing the necessity

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for new drugs with novel modes of action. Recent examples of such novel compounds are the imidazolopiperazines [16], the spiroindolone NITD609 [17], the synthetic peroxide OZ439 [18], or the dihydroorotate dehydrogenase inhibitors [19, 20].

In the quest for a new antimalarial compound, we initially based our activities on food-vacuolar plasmepsins (PMs) as drug targets [21, 22]. These efforts resulted in potent PM inhibitors that showed only moderate activity against in vitro–cultured *P. falciparum* parasites [23–25], consistent with concurrent reports demonstrating that the studied food-vacuolar PMs are not essential for parasite proliferation [26, 27]. Cell-based antimalarial screens were thus performed to find new lead structures independent of molecular targets. In a library with an assortment of aspartic protease inhibitors and compounds with undefined targets, novel piperazine-containing compounds were identified. These compounds were considerably more potent than the PM inhibitors and were further developed. Medicinal chemistry efforts led to improved potency of the piperazine-containing compounds with half-maximal inhibitory concentration (IC_{50}) values in the low nanomolar range. Herein we describe the in vitro and in vivo properties of one example of this new chemotype: ACT-213615. The compound showed a fast onset of action against all 3 asexual erythrocytic stages of the parasite in vitro and was effective against a panel of drug-resistant strains with IC_{50} values in the range of that of artesunate, one of the most potent clinically used antimalarials [28]. The molecular target of this novel compound remains unknown. However, microarray data from ACT-213615-treated in vitro cultures indicate a MOA distinct from that of several registered antimalarials. ACT-213615 was orally bioavailable in mice and showed activity in 2 murine models of malaria; importantly, in the *P. falciparum* severe combined immunodeficiency (SCID) mouse model [29], the activity of the compound was comparable to that of the reference drug chloroquine, emphasizing the potential of this class of compounds as new therapeutic agents for the treatment of malaria.

METHODS

Synthesis of ACT-213615

Please see the online edition of the *Journal of Infectious Diseases* for supplementary information.

In Vitro Antimalarial Activity

All *Plasmodium* strains were cultured according to Trager and Jensen [30] and are described at mr4.org (resistant strains: K1, TM90C2A, V1/S, W2, and 7G8, sensitive strains: D6, 3D7, and NF54). IC_{50} values were determined in vitro by measuring incorporation of the nucleic acid precursor [^{3}H]hypoxanthine [31]. In vitro time-, stage-, and concentration-dependent effects were assessed using pyrimethamine as a stage-specific and slow acting control, as described elsewhere [32].

Animal Experiments

In vivo efficacy studies in mice were conducted at the Swiss Tropical and Public Health Institute (Basel) according to the rules and regulations for the protection of animal rights ("Tierschutzverordnung") of the Swiss "Bundesamt für Veterinärwesen." They were approved by the veterinary office of Canton Basel-Stadt, Switzerland.

24-hour Schizont Maturation Assay (*P. berghei*)

In vitro (ex vivo) activity against *P. berghei* (GFP-transfected ANKA strain, donated by A. P. Waters and C. J. Janse, Leiden University, The Netherlands) was essentially measured as described elsewhere [31] with the following modifications: Heparinized blood of a *P. berghei*–infected and an uninfected (control) female NMRI mouse (final hematocrit of 2.5%) was used and exposed to compounds for 16 hours followed by 8 hours of [^{3}H]hypoxanthine incorporation.

Activity Against Other Parasitic Protozoa and Cytotoxicity Against L6 Cells

In vitro activity against *T. brucei rhodesiense*, *T. cruzi, Leishmania donovani,* and against L6 cells, a primary cell line derived from mammalian (rat) skeletal myoblasts, was determined as described elsewhere [33].

Pharmacokinetics

Bioavailability of ACT-213615 in *P. berghei*–infected female NMRI mice was measured after administration of a single oral dose of 100 mg/kg in 7% Tween80/3% ethanol. Time points of sample collections (50 µL whole blood): Group A ($n = 3$ mice): 0.25, 1, 4, and 24 hours; Group B ($n = 3$ mice): 0.5, 2, 8, and 48 hours. Measurement of plasma concentrations: see the online edition of *Journal of Infectious Diseases* for supplementary information.

In Vivo Antimalarial Activity

Efficacy of ACT-213615, artesunate and chloroquine (both from Sigma-Aldrich, Buchs, Switzerland) against *P. berghei* was studied as previously described [34] with the modification that mice ($n \geq 3$) were infected with a GFP-transfected *P. berghei* ANKA strain (see above) and parasitemia determined using standard flow cytometry techniques. Compounds were dissolved or suspended in 70/30 Tween80/ethanol (vol/vol) and diluted 10 times with water before dosing. With the single-dose regimen, blood was collected on day 3 (72 hours after infection). Samples for the triple- and quadruple-dose regimens were collected on day 4 postinfection. Animals were considered cured if there were no detectable parasites on day 30 postinfection.

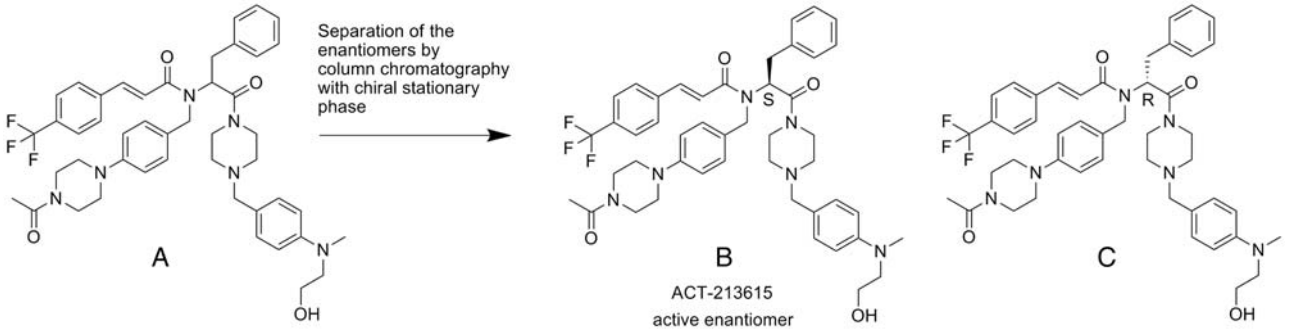

Figure 1. Structures of ACT-213615 (*B*) and its enantiomer (*C*). The compound is obtained by chiral separation of the racemic precursor (*A*). Molecular weight: 810.96 g/mole.

ACT-213615 and chloroquine were tested in the murine *P. falciparum* model essentially as described elsewhere [29]. The only modification implemented was that daily human blood injections (0.75 mL) were administered by the intravenous route instead of the intraperitoneal route.

Microarray

See the online edition of the *Journal of Infectious Diseases* for supplementary information. Raw data of microarray: GEO database Accession No.GSE39485.

RESULTS

Discovery of ACT-213615

A diverse library of aspartic protease inhibitors and compounds with undefined targets was tested in search of antimalarial molecules. In a screen against in vitro–cultured *P. falciparum*, several classes of growth-inhibiting compounds were identified, including PM inhibitors [23, 24] and some very potent piperazine-containing compounds (not inhibiting PMs), which were chosen for optimization. Medicinal chemistry efforts led to improved and promising activity, both in vitro and in vivo, of the piperazine-containing compounds; one example, ACT-213615 (Figure 1), is characterized in this article. The new entity has stereospecific activity; ACT-213615 is the more active S-enantiomer (geometric mean ratio of IC_{50} values, R : S enantiomer = 49, 95% confidence interval: 37–66, n = 3, tested in vitro against 3D7 strain).

ACT-213615 Potently and Specifically Inhibits *P. falciparum* Growth in Vitro

The in vitro activity of ACT-213615 against a panel of chloroquine- and pyrimethamine-resistant and -sensitive *P. falciparum* strains was determined by means of hypoxanthine incorporation assays. ACT-213615 was found to very potently inhibit growth of all 7 tested strains (IC_{50} values from 0.4 to 2.7 nM) irrespective of the drug-sensitivity status of the parasites (Table 1).

Table 1. In Vitro Activity of ACT-213615 and Reference Antimalarials Against a Panel of Resistant and Sensitive *Plasmodium falciparum* Strains

			IC_{50}, mean ± SD (nM)			
Isolate	Origin	Resistance	CQ	PYR	AS	ACT-213615
NF54	Airport, NL	…	11 ± 2	18 ± 1	3.7 ± 0.5	1.0 ± 0.1
K1	Thailand	CQ, PYR	303 ± 37	10 138 ± 705	2.7 ± 0.4	0.46 ± 0.04
W2	Indochina	CQ, PYR	326 ± 38	13 923 ± 3525	2.4 ± 0.7	0.42 ± 0.09
7G8	Brazil	PYR	137 ± 21	10 484 ± 2574	1.8 ± 0.2	1.2 ± 0.2
TM90C2A	Thailand	CQ, PYR	174 ± 19	19 248 ± 3876	4.6 ± 1.7	2.7 ± 0.4
D6	Sierra Leone	…	16 ± 1	5.4 ± 1.3	7.1 ± 1.9	1.3 ± 0.2
V1/S	Vietnam	CQ, PYR	458 ± 66	21 936 ± 1072	3.2 ± 0.5	0.65 ± 0.12
Max			458	21 936	7.1	2.7
Min			11	5.4	1.8	0.42
Max/Min			42	4062	4	7

IC_{50} values were determined by [^{3}H]hypoxanthine incorporation. Data are the means ± SD of n = 3 independent experiments (each run in duplicate).
Max and Min depict the highest and lowest measured IC_{50} for each compound. The ratio (Max/Min) indicates the range of susceptibility to each compound.
Abbreviations: AS, artesunate; CQ, chloroquine; PYR, pyrimethamine.

Table 2. In Vitro Antiprotozoal and Cytotoxic Activities of ACT-213615 and Standard Controls

	IC_{50}, mean ± SD (nM)				
Experiment	*Plasmodium falciparum*, K1	*Trypanosoma brucei rhodesiense*	*Trypanosoma cruzi*	*Leishmania donovani*	Cytotoxicity, L6
ACT-213615	0.46 ± 0.04	2220 ± 396	5019 ± 1341	8693 ± 882	8854 ± 3068
Standard control	303 ± 37[a]	15 ± 3[b]	1223 ± 347[c]	402 ± 34[d]	15 ± 5[e]

Data are the means ± SD of n = 3 independent experiments.

Standard compounds: [a] Chloroquine.

[b] Melarsoprol.

[c] Benznidazole.

[d] Miltefosine.

[e] Podophyllotoxin.

In contrast, several strains showed considerable resistance against the control drugs chloroquine and pyrimethamine (IC_{50} values from 11 to 458 nM and 5.4 to 21936 nM, respectively). Notably, the activity of ACT-213615, and its lack of cross-resistance, was comparable to that of the highly potent clinically used comparator drug artesunate for every strain tested (IC_{50} values for artesunate: 1.8–7.1 nM) (Table 1).

The antiproliferative effect of ACT-213615 was observed to be *Plasmodium*-specific, that is, not caused by unspecific cytotoxicity, as demonstrated by an at least 750-fold lower activity against a panel of 3 different parasitic protozoa and mammalian L6 cells (Table 2).

ACT-213615 Is Fast Acting and Affects All Asexual Erythrocytic Stages of the Parasite in Vitro

To examine the onset of action of ACT-213615 and determine whether the compound shows parasite stage-specific activity, in vitro time-, stage-, and concentration-dependent effects of ACT-213615 were studied with synchronous cultures of *P. falciparum* 3D7. Growth of all 3 asexual erythrocytic stages of the parasite (rings, trophozoites, and schizonts) was quantified by hypoxanthine incorporation relative to untreated controls after incubation for 1, 6, 12, or 24 hours in presence of approximately 1, 10, and 100 times the IC_{50} of the compound. After each period of exposure, the compound was removed by extensive washing. ACT-213615 rapidly reduced parasite growth. Onset of action was detectable after 1 hour of compound exposure. Furthermore, ACT-213615 equally affected all asexual erythrocytic stages in a time- and concentration-dependent manner (Figure 2). A fast onset of action and the activity against all asexual erythrocytic parasite stages are rare and highly desirable features that are shared with the artemisinins [18, 28, 35, 36]. In contrast, pyrimethamine, for example, was reported to be stage-specific and relatively slow acting against cultured *P. falciparum* [36, 37].

ACT-213615 Is Efficacious in Mouse Models of Malaria

In order to investigate whether the promising in vitro activity of the compound translates into efficacy in vivo, ACT-213615 was tested in 2 different mouse models.

In vivo antimalarial activity of ACT-213615 was first studied in the *P. berghei* mouse model. Prior to using animals, in vitro (ex vivo) activity against the surrogate rodent parasite, *P. berghei*, was determined in a 24-hour schizont maturation assay, measuring hypoxanthine incorporation. ACT-213615 was more than 30 times less active against *P. berghei* than against *P. falciparum* NF54 (geometric mean ratio of IC_{50}

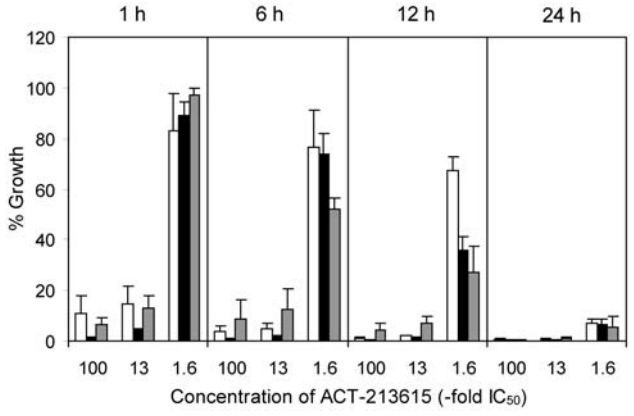

Figure 2. In vitro concentration- and stage-dependent effects of ACT-231615 on the growth of synchronous cultures of *Plasmodium falciparum* 3D7 determined by [^{3}H]hypoxanthine incorporation. Parasites were exposed to ACT-213615 for 1, 6, 12, or 24 h at the indicated concentration, expressed as folds of IC_{50}. After removal of the compound, parasites were incubated for 24 h in the presence of [^{3}H]hypoxanthine. Results are expressed as the percentage of growth of the respective development stage relative to an untreated control. White bars show ring stages, black bars show trophozoite stages, grey bars show schizont stages. Each bar represents the mean + SD of n = 3 independent experiments.

values, *P. berghei* : *P. falciparum* = 38, 95% confidence interval: 13–116, n = 3, both strains tested using a 24-hour assay).

In a next step, the compound was tested for its oral bioavailability in *P. berghei*–infected NMRI mice. Plasma concentrations of ACT-213615 (n = 3/time point) were measured by LC-MS/MS between 0.25 and 48 hours after a single oral dose of 100 mg/kg. ACT-213615 was found to be orally bioavailable: after 2 hours, a maximal plasma concentration of 9.6 ± 1.1 μg/mL was reached, which declined with a half-life of 5 hours (Figure S1). Exposure (AUC_0-∞) was 91.4 ± 12.7 μg/mL*h. These results indicate that exposure of ACT-213615 in *P. berghei*–infected mice should be sufficient to detect antimalarial efficacy in vivo, despite the moderate activity of the compound against *P. berghei* in vitro.

In vivo efficacy studies in the *P. berghei* mouse model were performed with 3 consecutive daily oral doses of 3, 10, 30, 100, 300, or 750 mg/kg of ACT-213615. In addition, 90% effective dose (ED_{90}) experiments were conducted applying single oral doses. A steep dose response was observed with an ED_{90} of 54.4 mg/kg, judged by the reduction of parasitemia compared to untreated controls. Furthermore, the compound caused dose-dependent prolongation of survival and cure at a dose of 3 × 750 mg/kg (Table S1). No acute toxicity was observed. Considering that ACT-213615 was about 38-fold more potent in vitro against *P. falciparum* than against *P. berghei* (see above), it was concluded from the results of the *P. berghei* mouse model that ACT-213615 should be highly active against *P. falciparum* in vivo.

The in vivo efficacy against *P. falciparum* was assessed in the SCID mouse *P. falciparum* model. This recently established model [29] uses SCID mice engrafted with human erythrocytes, offering the possibility to investigate the actual target parasite *P. falciparum* in vivo. Four consecutive daily oral doses of 2, 5, 10, and 20 mg/kg of ACT-213615 were administered. Quantification of parasitemia revealed a steep dose response and the compound was found to be very potent (ED_{90} = 8.4 mg/kg), comparable to the standard control chloroquine (ED_{90} = 6.4 mg/kg) (Table 3). Again, no acute toxicity was observed.

Table 3. In Vivo Activity of ACT-213615 and Chloroquine in the *Plasmodium falciparum* Mouse Model

Oral dose,[a] mg/kg	ACT-213615 activity,[b] %	Chloroquine activity,[b] %
4 × 2	8	68
4 × 5	64	86
4 × 10	94	94
4 × 20	95	Not tested

[a] *P. falciparum*-infected mice (mean of n = 3 animals); formulation: 7% Tween80/3% ethanol; animals were dosed once/day 3, 4, 5, and 6 days postinfection.

[b] Reduction of parasitemia compared to untreated controls. Assessed 96 h after the first treatment (4-day test by Peters). Parasitemia of untreated controls (means of n = 5 animals) before first treatment: 1.1%; after 96 h: 14.4%.

Mode of Action

As the new chemotype was discovered in a cellular screen, the mode of action (MOA) of ACT-213615 is unclear. To date, PM I, II, and IV could be ruled out as targets based on in vitro assays (IC_{50} values: 56 200, 45 400, and >100 000 nM, respectively, n = 1; see the online *Journal* for supplementary information). In order to gain more information about the MOA and a putative target, genome-wide expression changes induced by treatment with ACT-213615 were investigated in *P. falciparum* and compared to previously established changes in gene expression induced by 20 different compounds with antimalarial activity [38]. For this microarray study, highly synchronized 3D7 *P. falciparum* parasites were treated in vitro with an IC_{90} of ACT-213615 and control samples with DMSO only, starting at t_0 = 32 hours postinfection for 1, 2, 4, 6, and 8 hours.

ACT-213615 altered the mRNA levels of 552 genes by at least 2-fold at ≥ 2 time points (Figure 3*A*). In total, 407 of these genes were up-regulated and 145 down-regulated. Functional enrichment analysis revealed statistical overrepresentation of several basic cellular and metabolic pathways among the up-regulated genes (Figure 3*B*). These include protein synthesis (ribosomal subunits and assembly factors) and post-translational modifications of proteins (N-myristoylation, S-acylation and prenylation). Furthermore, a major lipid metabolism pathway (phosphatidylethanolamine and phosphatidylserine metabolism) and a total of 18 protein kinases were found to be up-regulated by ACT-213615. On the other hand, treatment with the compound caused significant down-regulation of numerous components of the merozoite invasion machinery. Nonetheless, a specific MOA could not be derived from the above results. The gene expression data could, however, be used to differentiate the MOA of ACT-213615 from that of known antimalarial compounds. It was of particular interest to investigate whether the strikingly similar pharmacological effects of ACT-213615 and the artemisinins (see above) derive from a similar or a different MOA. Hu and coworkers [38] have recently characterized and profiled gene expression patterns induced by 20 diverse small molecular inhibitors and registered antimalarial drugs. The comparison of the transcriptional response induced by ACT-213615 to these 20 established profiles is summarized in Figure 3*C* and 3*D*. Hierarchical clustering analysis displayed as a principal coordinate plot (Figure 3*C*) or a dendrogram (Figure 3*D*) revealed 4 principal groups of the 20 chemical perturbations on the basis of similarities in induced expression patterns. ACT-213615 clustered with a subset of the perturbations including

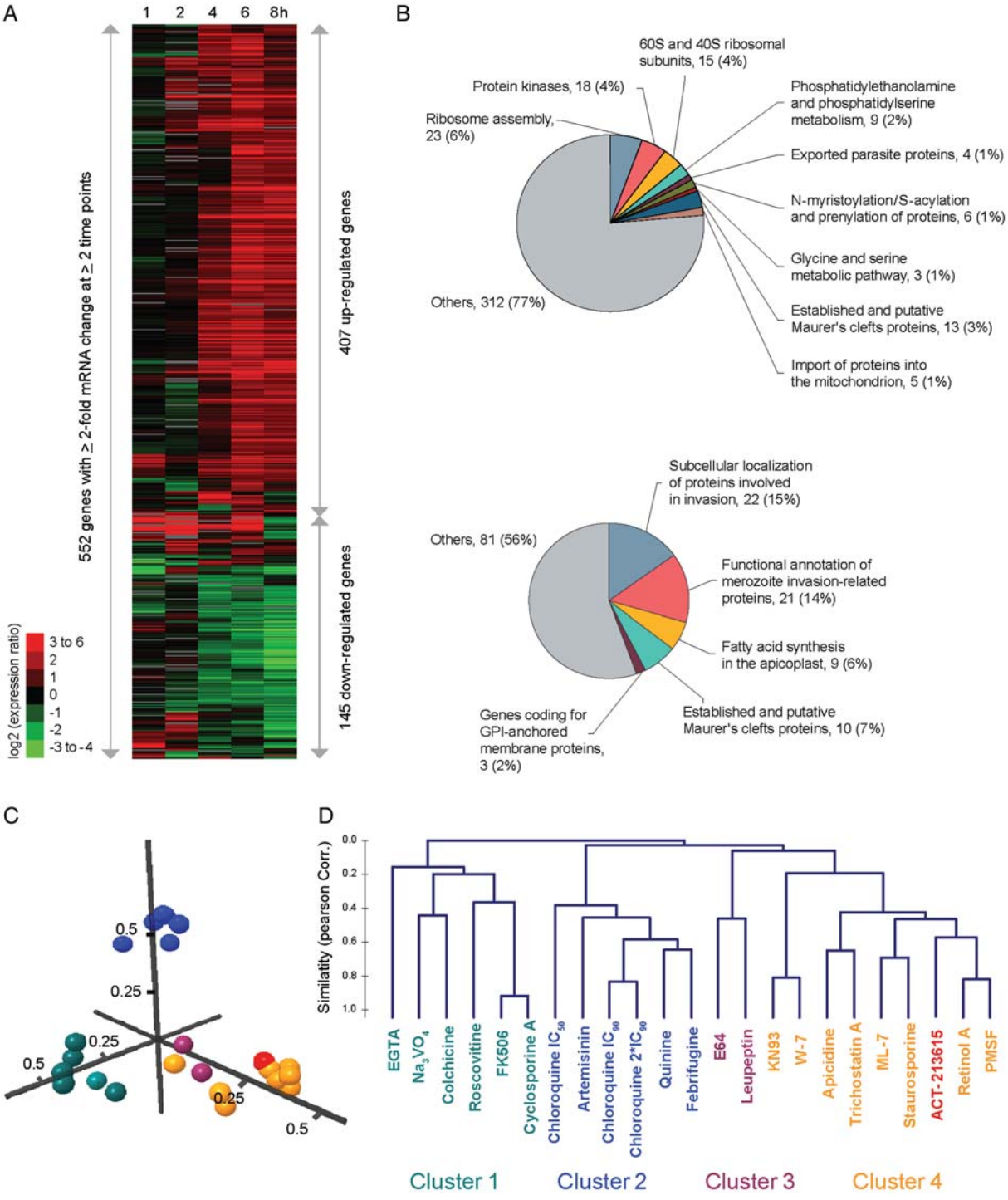

Figure 3. Analyses of genome-wide transcriptional response of *P. falciparum* parasites to treatment with ACT-213615. Highly synchronized 3D7 schizonts were treated with ACT-213615 (IC_{90}) and control samples with DMSO only. RNA was collected after 1, 2, 4, 6, and 8 h of treatment. *A*, The heat map represents genes found to be differentially expressed by at least 2-fold at more than one time point. *B*, The pie charts show significantly enriched pathways under treatment ($P < .05$) identified by functional enrichment analysis. Depicted is the fraction of differentially expressed genes that are within (*colored*) and not within (*gray*) the enriched functional pathways, the number and percentage of genes in each cluster are indicated. *C*, 3-dimensional principal coordinate plot in which distances between points indicate the degree of similarity between transcriptional profiles of individual antimalarial compounds. *D*, Dendrogram of hierarchical clustering of transcriptional responses to compounds revealed 4 distinct clusters. The color code is consistent in panels *C* and *D*.

generic protein kinase inhibitors such as staurosporine and ML-7 on one side and retinol A or the serine protease inhibitor PMSF on the other side. Importantly, ACT-213615 was not found in the same cluster as artemisinin (Figure 3*C* and 3*D*) despite the similar in vitro properties of these 2 compounds. In addition, the MOA of ACT-213615 also appeared to be different from that of chloroquine and quinine which clustered with artemisinin in the above analysis. Microarray data were validated using real-time quantitative PCR (qPCR) (Figure S2).

DISCUSSION

In a cell-based screen, we identified a new pharmacophore with potent antimalarial activity. Medicinal chemistry efforts led to the lead compound ACT-213615. In vitro, this compound inhibits erythrocytic *P. falciparum* growth of drug-sensitive and drug-resistant strains at single-digit nanomolar concentrations, comparable to the reference antimalarials chloroquine and artesunate. We have further demonstrated that ACT-213615 is highly effective in vivo against *P. falciparum* in the SCID mouse model (ED_{90} = 8.4 mg/kg), where its activity after oral dosing was comparable to that of chloroquine. These results suggest that ACT-213615 represents a class of molecules that has the potential to be further developed for clinical use against malaria.

The novel pharmacophore also showed activity in the *P. berghei* mouse model, including prolongation of survival and cure. The effective dose in the *P. berghei* model was considerably higher than in the *P. falciparum* mouse model (ED_{90} = 54.4 mg/kg). This difference in effectiveness between the 2 models can be explained by the approximate 38-fold difference in in vitro potency that was observed against the 2 parasite species. The result implies that testing compounds in vivo with the surrogate parasite *P. berghei* can in some cases lead to misguided elimination of compounds from drug discovery pipelines due to a perceived lack of efficacy, despite significant potency against *P. falciparum* in vitro.

Pharmacokinetic studies in *P. berghei*–infected NMRI mice revealed that ACT-213615 is orally bioavailable and has a short plasma half-life of 5 hours. A short plasma half-life suggests a low probability of resistance development in vivo, because the time of exposure to a sublethal concentration of the molecule would be short. Artemisinins, which are the backbone of antimalarial chemotherapy today [8], have short plasma half-lives as well [39], and a low propensity to develop resistance in vivo. However, reduced sensitivity to artesunate has recently been observed at the Thai/Cambodian border [6, 7]. In order to impede resistance development in vivo, the following strategies are recommended: Antimalarials should be developed as combinations of compounds that act through different MOAs [40], whereby molecules with a short half-life seem favorable and could be combined with compounds that display longer half-lives [41].

In vitro, ACT-213615 showed a fast onset of action (detectable after 1 hour) and equal potency against all asexual erythrocytic parasite stages—qualities reminiscent of the highly potent and clinically used artemisinins [28, 35, 36]. Rapid effectiveness against all asexual erythrocytic stages is an essential characteristic of a new antimalarial, because it leads to a fast reduction of a mixed parasite population and, likewise, might reduce recrudescence as it helps prevent the escape of an otherwise unaffected stage during a short-time exposure. Efficacy against all asexual erythrocytic stages also represents a desirable attribute for combination therapies, because it quickly reduces the parasite load and complements a possible deficit of the combination partner with respect to stage-specificity. Finally, a fast onset of action potentially shortens treatment times.

The MOA of ACT-213615 currently remains unknown, but involvement of a specific target is suspected on the basis of enantiomer selectivity of the compound, evidence of parasite-specific activity, and absence of nonspecific cytotoxicity. In order to gain insight into cellular pathways affected in treated parasites, and to obtain information about the MOA and/or targets of the compound, a microarray study was performed assessing transcriptional changes induced by treatment with ACT-213615 in synchronized erythrocytic cultures of *P. falciparum*. It has previously been shown that perturbations induced by chloroquine [38, 42], antifolates [43], or certain protease inhibitors have only minimal effect on *Plasmodium* transcription, whereas other compounds such as protein kinase inhibitors induce high-amplitude mRNA changes [38]. Overall, however, all transcriptional responses, regardless of their amplitude, have some degree of biological relevance with functionally related genes being induced by particular perturbations. Functional enrichment analysis of deregulated genes in erythrocytic *P. falciparum* under treatment with ACT-213615 in vitro revealed an overrepresentation of several pathways. In the case of up-regulated genes, these pathways were, for example, associated with lipid metabolism and kinase-dependent signaling, providing starting points for specific MOA studies. Down-regulation, on the other hand, was predominantly observed for components required for merozoite invasion, suggesting that the parasite slows or halts its cell cycle progression under treatment with ACT-213615. Nevertheless, it was not possible to unambiguously identify a single likely target or pathway affected by ACT-213615.

Our microarray data, however, indicate that the MOA of ACT-213615 differs from that of several existing antimalarials, importantly artemisinin, chloroquine, and quinine. These drugs group together and are separated from ACT-213615 after hierarchical clustering analysis based on similarity in induced expression patterns. In contrast, ACT-213615

clustered with the protein kinase inhibitors staurosporine and ML7, with retinol A, and with the serine protease inhibitor PMSF. It could thus be speculated that ACT-213615 may also interfere with the largely unknown pathways of *P. falciparum* affected by those compounds. Additional studies to expand on these findings and to discover a molecular target of ACT-213615 are planned.

Artesunate and ACT-213615 share the fast onset of action and equal potency against all asexual erythrocytic parasite stages but differ in their MOA on the basis of the above hierarchical clustering analysis of the microarray data. This analysis indicates that chloroquine and quinine act differently from ACT-213615 as well. Among other currently used antimalarials, one can also rule out that stage-specific and slow-acting antimalarials, such as pyrimethamine [36, 37], share the MOA with ACT-213615. These observations demonstrate that ACT-213615 could potentially be combined with all of these above antimalarial drugs and that the combination would fulfill the recommendation that the 2 combination partners act independently [40].

In spite of recent and alarming reports of resistance [6, 7], artemisinin-based compounds remain the current mainstay of antimalarial treatment [8]. The excellent effectiveness of these molecules is largely attributable to their potency, their fast onset of action, and their activity against all asexual erythrocytic stages [18, 28, 35, 36]. These favorable properties of the artemisinins are shared by ACT-213615, together with the short plasma half-life [39]. Therefore, if the MOA of ACT-213615 is confirmed to be distinct from that of the peroxides, ACT-213615 or analogs of the same chemical class have the potential to be used as substitutes of the artemisinins in combination with compounds exhibiting longer plasma half-lives.

We conclude that ACT-213615 represents a potent new antimalarial chemotype that exhibits in vitro and in vivo activity comparable to chloroquine, appears to have a novel mode of action, and acts quickly and independently of asexual erythrocytic stages. Further research and development activities with this chemical class are warranted.

Supplementary Data

Supplementary materials are available at The *Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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