UV-triggered Affinity Capture Identifies Interactions between the Plasmodium falciparum Multidrug Resistance Protein 1 (PfMDR1) and Antimalarial Agents in Live Parasitized Cells

A representative of a new class of potent antimalarials with an unknown mode of action was recently described. To identify the molecular target of this class of antimalarials, we employed a photo-reactive affinity capture method to find parasite proteins specifically interacting with the capture compound in living parasitized cells. The capture reagent retained the antimalarial properties are mediated. Knowledge of the molecular target of a compound class is essential to develop agent-containing compound ACT-213615.

Recently, we reported the synthesis of a new chemical class of antimalarials, discovered in a cell-based anti-proliferative screen (1). ACT-213615, a representative of this class of compounds, was shown to inhibit erythrocytic Plasmodium falciparum growth in vitro with IC₅₀ values in the low single digit nanomolar range. In vivo, the compound exhibited activity comparable with that of the reference drug chloroquine in the P. falciparum SCID mouse model. Furthermore, ACT-213615 rapidly affected all three asexual erythrocytic parasite stages, in a fashion reminiscent of artemisinins, the fast-acting core components of World Health Organization-recommended combination therapies. This fast onset of action is a highly desirable feature for the development of a single dose antimalarial therapy.

Little is known about the mode of action of the ACT-213615 pharmacophore or the molecular interactions through which its antimalarial properties are mediated. Knowledge of the molecular target of a compound class is essential to develop biochemical assays to find novel follow-up compounds in high throughput screens and aids the establishment of structure-activity relationships during lead optimization. Understanding the mode of action also facilitates the identification of an appropriate companion drug during clinical development. Antimalarial therapies using combinations of drugs with disparate modes of action hinder Plasmodium parasites from developing multidrug resistance (2).

In this study, we sought to pinpoint the drug target within parasites by first identifying parasite candidate proteins that interact with ACT-213615. Conventional strategies to achieve this goal are often based on affinity capture methodology, using a derivative of the active molecule coupled to a solid matrix through a linker (3, 4). Cell lysates containing the desired target are exposed to the immobilized affinity capture reagent thereby allowing the target to bind. Thereafter, the affinity matrix is extensively washed. Bound proteins are eluted from the affinity matrix by denaturation or competition with a soluble high affinity ligand of the target. This method works well for soluble and stable proteins or protein complexes that have high affinity for the capture reagent and that stay active under the conditions of cellular lysis. However, some proteins, especially poly-

**Background:** We have previously identified potent novel antimalarial compounds with an unknown mode of action. **Results:** A photo-reactive affinity capture method was used to identify parasite proteins that interact with these antimalarials. **Conclusion:** ACT-213615 interacts with Plasmodium falciparum multidrug resistance protein 1 (PfMDR1). **Significance:** This photo-reactive affinity capture method can be generally used to identify drug targets in live cells.

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topic membrane proteins, can be rendered inactive by conditions of standard cellular lysis and solubilization. Because a large proportion of known drugs act through polytopic membrane proteins, the conventional strategy to identify interacting proteins is theorized to have a low chance of success.

To circumvent these problems, we incorporated a photo-reactive cross-linker into the structure of our affinity capture reagent (5). Activation of the UV-sensitive cross-linker in live parasites triggered covalent bond formation via reactive nitrenes with nearby molecular structures. These covalent bonds allowed the use of denaturing lysis conditions and stringent washes after immobilization, resulting in a significant reduction of background signals.

Here, we report our findings delineating drug target identification in live *P. falciparum*-infected cells using a pulldown method based on a photo-reactive affinity capture reagent. The capture reagent was designed, synthesized, and shown to retain the biological hallmarks of the parent molecule, namely potency and activity against all three asexual intra-erythrocytic parasite stages. By fluorescent imaging, this reagent accumulated within the parasite and was hardly detectable in the host erythrocytes. Pulldown experiments followed by mass spectrometry-based peptide sequencing and database queries identified several parasite proteins that specifically interacted with the capture reagent, the most notable of which was *P. falciparum* multidrug resistance protein 1 (*PfMDR1*). We demonstrate that the activity of ACT-213615 is dependent on the gene copy number of *PfMDR1*, and our results suggest a physical and functional interaction between ACT-213615 and *PfMDR1* in proliferating parasites. To our knowledge, this is the first report describing UV-triggered affinity-capture in live and intact cells to successfully identify a transmembrane protein interaction with drugs.

**EXPERIMENTAL PROCEDURES**

**Synthesis of Chemical Tools**—ACT-213615, its inactive enantiomer, ACT-186128, and des-acetyl ACT-186128 were synthesized as described previously (1). The affinity capture reagent ACT-460953 was produced by attaching des-acetyl ACT-186128 to the B1-NHS cross-linking and capturing tool from Caprotec Bioanalytics GmbH (Berlin, Germany), according to the manufacturer’s instructions. Phenylpiperazine instead of des-acetyl ACT-186128 was used to produce ACT-460953 by attaching des-acetyl reagent ACT-460954 (second negative control) at 37 °C for 2 h. Samples were pelleted, resuspended in 4 pellet volumes of 0.15% (v/v) saponin/PBS solution, incubated on ice for 8 min, centrifuged, washed in PBS, and resuspended in 1 ml of ice-cold PBS. Samples were then transferred to an ice-cold Petri dish cover and UV-irradiated as above. Irradiated samples were pelleted at 4 °C, resuspended in 50 µl of PBS by vortexing, and lysed in 1 ml of SDS lysis buffer (1% (w/v) SDS, 1× protease inhibitors (EDTA-free Protease Inhibitor Mixture Tablets, Roche Applied Science), 1 mM DTT in PBS) at room temperature for 10 min. For experiments in which infected cells were exposed to UV light prior to saponin lysis, the lysates were prepared as above, except that before UV irradiation, parasites were washed twice with culture medium, resuspended in PBS, transferred to the cover of a 96-well plate, and UV-irradiated. For the pulldown experiments, lysates were either processed directly or passed five times through a 0.6-mm needle. Lysates were then centrifuged, and 900 µl of supernatants were transferred to 200–300 µl of resuspended streptavidin beads (magnetic Dynabeads MyOne C1, Invitrogen). The suspension was incubated on a rotating wheel at room temperature for 1 h. Beads were consecutively washed with 1 ml of the following: (a) 1% (w/v) SDS in PBS; (b) Caprotec wash buffer, twice; (c) 1% (w/v) SDS in PBS, and (d) double distilled H2O. Proteins were eluted from the beads by incubation in 25 µl of 1.5× SDS loading buffer (150 mM Tris, pH 6.8, 3% (w/v) SDS, 7.6% (v/v) glycerol, 1.5% (v/v) β-mercaptoethanol, 0.06% (w/v) bromphenol blue) at 94 °C for 10 min. Any remaining beads were removed by centrifugation; 18 µl of supernatant was loaded on a polyacrylamide gel (4–12% (w/v) BisTris-polyacrylamide pre-cast gels, Invitrogen) and run for 75 min (30 mA, 150 V) using 1× MOPS as a running buffer. Gels were silver-stained (SilverQuest, Invitrogen), and areas that differed in the amount of protein (sample versus negative control) were cut out for mass spectrometry analysis.

**Mass Spectrometry**—In-gel digestion and LC-MS/MS (liquid chromatography-tandem mass spectrometry) analysis was performed as described previously (9). The LC-MS/MS data were searched using the SEQUEST search engine, version 3.3 (10), against the *P. falciparum* data bank (PlasmoDB version 5.5, July, 2008) and the NCBI human data bank (version June, 2010).
The precursor ion and fragment ion mass tolerances were set to 10 ppm and 0.6 Da, respectively. Two missed cleavages were allowed.

Functional Assays—IC$_{50}$ values of ACT-213615 against isogenic $P$. falciparum strains expressing one or two $pfmdr1$ copies were determined as described previously (11). Transport studies with PfENT using Xenopus laevis oocytes injected with mRNA encoding PfENT4, PfENT1, or PfENT1 were performed as described previously (12) except that oocytes were preincubated in transport buffer in the presence of 1 or 10 $\mu$M compound or solvent control for 15 min. Azido-biotin-chloroquine (AzBCQ) photolabeling of partially purified PfMDR1 protein was done as described previously (13). This method was adapted for ACT-460953 photolabeling by titrating the time of UV exposure and the concentration of photo-reactive probe. All other conditions were as reported previously (13). Specific labeling of PfMDR1 was observed at 40 $\mu$M probe after 10 min of UV irradiation, as described previously (12). Enzyme assays with up to 10 $\mu$M ACT-213615 were performed for glideosome-associated protein 50/secreted acid phosphatase as described previously (14) and for S-adenosylmethionine synthase as reported previously (15).

RESULTS

Design and Validation of the Capture Compound—During lead optimization with ACT-213615, the structure-activity relationship indicated that the acetyl-piperazine moiety was amenable to chemical variations without significant loss of biological activity. Therefore, the acetyl group of the acetyl-piperazine in ACT-213615 was replaced by the B1-NHS moiety and sorting functionality to ACT-186128 did not significantly alter its biological activity.

We further investigated whether ACT-460953 retained two important hallmarks of ACT-213615 pharmacology, namely rapid onset of action and multistage activity in vitro (1). ACT-460953 showed a similar activity pattern to that of ACT-213615 with respect to both onset of action and activity against all asexual erythrocytic stages of the parasite (rings, trophozoites, and schizonts) (data not shown). Taken together, the capture tool had similar pharmacological hallmarks to the previously characterized antimalarial compound ACT-213615, implying that ACT-460953 interacts with the same targets as its precursor.

Fluorescent Imaging Using the Capture Compound—Fluorescent imaging was performed to investigate whether UV-reactive ACT-460953 can be cross-linked to interacting structures within intact and living $P$. falciparum cells. For this experiment, live 3D7 parasites were incubated with ACT-460953 or ACT-460954, followed by activation of the compounds with UV light. After washing, fixation, and blocking of the cells, the biotin moieties of the compounds were visualized using Alexa488-streptavidin. A parallel experiment without UV activation served as an additional negative control. Fluorescence was detected in the cytoplasm of parasites exposed to ACT-460953, irrespective of parasite stage. In contrast, only weak signals could be detected from ACT-460954-exposed parasites or from parasites that had not been UV-irradiated (Fig. 2). These results show that both the capture reagent as well as UV light penetrated the parasite and that cross-linking of ACT-460953 to

![Figure 1. Structure of the affinity-capture reagent ACT-460953. Highlighted in red is ACT-186128, a derivative of the previously described ACT-213615 (1).](image)
Validation of Target Candidates—In order to prioritize the target candidates for follow-up experiments, the following criteria were applied: (i) reproducibility and signal intensity of the pulldown results; (ii) candidate mRNA expression in all asexual P. falciparum blood stages (ACT-213615 activity is non–stage-specific (1)); (iii) literature evidence that the protein is essential for parasite survival; and (iv) availability of biochemical or cellular assays. Based on these criteria, five target candidates were chosen for follow-up experiments, namely PfMDR1, PfENT4, interacting molecules depended on UV irradiation. Furthermore, the capture compound specifically interacted with parasitic and not with erythrocytic structures. These data suggest that the chosen approach of UV-induced affinity cross-linking could lead to the identification of parasite proteins specifically interacting with the capture reagent.

Pulldown Experiments—UV-based pulldown experiments were performed with infected RBCs that were or were not saponin-lysed. P. falciparum 3D7 cultures were either preincubated with an excess of ACT-213615 as a competitor or not, then incubated with 100 nM ACT-460953 (~2× IC₅₀) or ACT-460954, UV-irradiated, lysed in SDS buffer, incubated with streptavidin beads, washed, and eluted with SDS loading buffer at 94 °C. Eluted proteins were separated on a polyacrylamide gel. Differentially silver-stained areas of the gels were cut out for mass spectrometry. When starting with non-saponin-lysed cultures (P. falciparum 3D7), the effect of this compound on [³H]adenine uptake into acidic phosphatase, and hexose transporter, glideosome-associated protein 50/secreted acidic phosphatase, and S-adenosylmethionine synthetase.

Multidrug Resistance Protein 1 (PfMDR1)—PfMDR1 mediates transport of and resistance to a number of antimalarials (16–20). The natural function of the transporter is unknown but is assumed to be essential for P. falciparum survival. In vitro susceptibility of P. falciparum to several antimalarials has been demonstrated to correlate with the gene copy number of pfmdr1 (11). We used isogenic lines of FCB that differed only in the pfmdr1 copy number to ascertain whether ACT-213615 potency was affected by pfmdr1 expression levels. The results show that the IC₅₀ value of both ACT-213615 and mefloquine are twice as high in the FCB parental line, containing two copies of pfmdr1, compared with the FCB mdr1 knockdown line that contains only one copy of pfmdr1. Values for ACT-213615 were 3.7 ± 0.5 nm for FCB and 1.8 ± 0.3 nm for the knockdown line (shown as means ± S.E.; n = 4 separate assays). This difference was significant as assessed using a nonparametric comparison (Mann-Whitney U test). This is in contrast to chloroquine, in which pfmdr1 copy number was not a factor in drug susceptibility (Table 1).

To further test a possible interaction with PfMDR1 protein, we probed whether active or inactive enantiomers of ACT-213615 inhibited ACT-460953 photolabeling of partially purified PfMDR1 protein. ACT-213615 inhibited photolabeling in a dose-dependent fashion, with 37-fold molar excess almost completely abolishing productive labeling under conditions of the assay (Fig. 3A, compare lane 2 with lane 1). In contrast, competition with the inactive enantiomer ACT-186128 showed significantly reduced dose-dependent inhibition (Fig. 3A, lanes 5, 6, 7 versus lane 1). Averaging results from three independent photolabeling experiments (Fig. 3B) showed that 37-fold molar excess of active ACT-213615 was twice as effective at competing ACT-460953 photolabeling, relative to the same fold molar excess of inactive enantiomer ACT-186128.

To assess whether the PfMDR1-binding sites for ACT-460953 and ACT-213615 overlap with the previously determined binding site of chloroquine and other quinolone-based drugs (13), we analyzed whether ACT-213615 and ACT-186128 also competed photolabeling of AzBCQ. 50% inhibition of AzBCQ photolabeling occurred at about 4-fold molar excess of ACT-213615 over the AzBCQ probe, whereas the inactive enantiomer did not show significant probe competition up to 6-fold molar excess over AzBCQ (data not shown). The potency of ACT-213615 for inhibiting AzBCQ photolabeling to PfMDR1 was similar to that of mefloquine, the most potent inhibitor of chloroquine probe binding to PfMDR1 yet measured (13).

Equilibrative Nucleoside Transporter 4 (PfENT4)—P. falciparum cannot synthesize purines de novo (21), and thus this parasite depends on salvaging these essential nutrients from its host (22). Purine transport has been shown to be mediated by PfENT1 (23, 24) and PfENT4 (12), each of which have differential specificities. These and other orthologs may represent potential drug targets. The interaction of ACT-213615 with equilibrative nucleoside transporters was studied by measuring the effect of this compound on [³H]adenine uptake into X. laevis oocytes expressing PfENT1, PfENT4, or the Plasmodium
**TABLE 1**

Susceptibility of *P. falciparum* to ACT-213615 correlates with the number of multidrug resistance protein 1 (*PfMDR1*) gene copies

| Exp. no. | ACT-213615 IC\textsubscript{50} (nM) | Mefloquine IC\textsubscript{50} (nM) | Chloroquine IC\textsubscript{50} (nM) |
|----------|----------------|----------------|----------------|
|          | Strain 2 | Strain 1 | Ratio 2:1 | Strain 2 | Strain 1 | Ratio 2:1 | Strain 2 | Strain 1 | Ratio 2:1 |
| 1        | 4.9      | 2.4      | 2         | 43.1     | 17.6     | 2.4       | 308.5     | 252.9     | 1.2 |
| 2        | 3.9      | 1.9      | 2.1       | 34.4     | 14.1     | 2.4       | 234.5     | 178.6     | 1.3 |
| 3        | 2.8      | 1.2      | 2.3       | 52.8     | 26.4     | 2.0       | 164.3     | 210       | 0.8 |
| 4        | 3.2      | 1.6      | 2         | 13.4     | 11       | 1.2       | 164.8     | 177.3     | 0.9 |
| Average  | 3.7      | 1.8      | 2.1       | 35.9     | 17.3     | 2         | 218       | 204.7     | 1.1 |
| S.E.     | 0.5      | 0.3      | 

* Indicates significant \( p \) value.

**FIGURE 3.** ACT-213615 physically interacts with partially purified *PfMDR1*. A, representative results of photolabeling of ACT-460952 (40 \( \mu \)M) to partially purified *PfMDR1* (0.25 \( \mu \)M) in vitro in the absence (lane 1) or presence of ACT-213615 (lanes 2–4; 1.5, 1.2, and 0.8 \( \mu \)M, respectively) or its inactive enantiomer ACT-186128 (lanes 5–7; 1.5, 1.2, and 0.8 \( \mu \)M, respectively). B, intensity of cross-linked bands was quantified by densitometry, and the results of three independent photolabeling reactions were averaged. Shown is labeling in the absence (control, left bar) versus in the presence of 1.5 \( \mu \)M ACT-213615 or ACT-186128. Error bars indicate standard deviation.

**FIGURE 4.** ACT-213615 inhibits transport via *PfENT4* but not via *PfENT1*. A, effect of 1 and 10 \( \mu \)M compound on \( ^{3} \)H|adenine uptake into *PfENT4*-expressing oocytes as follows: untreated control (white bars), ACT-213615 (black bars), and inactive R-enantiomer of ACT-213615 (gray bars). B, effect of 10 \( \mu \)M ACT-213615 on \( ^{3} \)H|adenine uptake into oocytes expressing *PfENT4*, *PfENT4*, or *PfENT1*. Bars represent the mean uptake of eight oocytes, and error bars indicate standard deviations. * indicates that the mean uptake was significantly different from control (t test \( p \) value < 0.05).

ACT-213615 Interacts with *PfMDR1*

Some associated protein 50/secreted acidic phosphatase, and S-adenosylmethionine synthetase. No disruption of activity could be detected between ACT-213615 and any of these proteins (data not shown).

**DISCUSSION**

Resistance development threatens the efficacy of current antimalarial therapies (25–28). New antimalarial drugs are clearly required, and many research groups are working toward this goal. Interesting new molecules with antimalarial activity were recently identified using anti-proliferative assays based on *in vitro* *P. falciparum* growth in erythrocytes (29–33). Regrettably, anti-proliferative assays have the disadvantage of generally not providing any information about the mode of action or the molecular target of the new lead compound.
We recently described a new pharmacophore with potent antimalarial activity, which was identified in a cell-based anti-proliferative screen (1). Compounds with this pharmacophore were potent and affected all erythrocytic stages of the parasite with a fast onset of action. In vivo, in the P. falciparum SCID mouse model, ACT-213615, one of these compounds, was as active as chloroquine, underlining the potential of this novel pharmacophore for the development of a new antimalarial drug. Therefore, we wanted to identify parasite proteins that interact with ACT-213615 to increase our understanding about the mode of action, which may lead to molecular target identification.

Numerous early experiments using conventional affinity-capture methods like streptavidin beads in conjunction with biotin-labeled compounds or compounds directly linked to Sepharose beads did not lead to reproducible results. This was probably because these methods rely on lysed parasites, i.e. partially denatured cellular components. For this reason, a UV-dependent cross-linking approach was devised whereby living parasites are lysed only after the covalent coupling of the chemical probes with their target structures. We designed an affinity-capture reagent that contained both a photo-reactive cross-linking and a sorting functionality (5). The photo-reactive function was incorporated in close proximity to the pharmacophore, maximizing the chance that on formation of the highly reactive nitrene the photo-reactive moiety reacts with the binding partner and not a water molecule.

Our capture compound, ACT-460953, was shown to retain the anti-proliferative activity of its parent (the IC₅₀ of ACT-460953 was 34.1 ± 2.9 nM versus 14.9 ± 1.4 nM for ACT-186128) and also maintain activity against all three asexual erythrocytic stages of P. falciparum (data not shown). Therefore, it is highly likely that the mode of action and intracellular molecular interactions of ACT-460953 and its parent are similar.

ACT-460953 accumulated in a UV-dependent manner inside the P. falciparum parasite (Fig. 2). The staining appeared to be cytoplasmic but was indistinct. The imaging resolution and the signal intensity did not exclude specific labeling of the plasma membrane or internal parasite membranes. No comparable signal was observed in other regions of the parasite or in the surrounding RBCs. We therefore surmised that ACT-460953 could specifically pulldown covalently attached parasitic proteins after cellular lysis.

Pulldown experiments with intact infected RBCs, incubated with 2× IC₅₀ of ACT-460953 in the presence or absence of excess of ACT-213615, were subjected to mass spectrometry-based peptide sequencing. The only protein identified in intact infected RBCs was PfMDR1, a protein with 12 transmembrane segments located in the digestive vacuole membrane of P. falciparum. PfMDR1 mediates transport of small molecules, including antimalarials, between the digestive vacuole and the parasite cytoplasm (19). Although its function is unclear, PfMDR1 is assumed to be essential for parasite viability.

In pulldown experiments with isolated parasites released from saponin-lysed RBCs, 20 candidate proteins were identified, including PfMDR1. This result confirmed the findings in intact parasitized RBCs and demonstrating increased sensitivity in detection (see supplemental Table 1).

To test for a specific physical interaction between PfMDR1 and ACT-213615, we probed the ability of active and inactive enantiomers of ACT-213615 to inhibit in vitro photolabeling of partially purified PfMDR1 with the capture reagent ACT-460953. Consistent with its antimalarial activity, ACT-213615 inhibited photolabeling of PfMDR1 in a dose-dependent manner, whereas the inactive enantiomer did not (Fig. 3). Furthermore, we found much stronger competition of ACT-213615 for both ACT-460953 and a known high affinity probe of PfMDR1, AzBCQ. The latter probe was previously used to determine relative affinity of PfMDR1 for various quinolone-based drugs, including chloroquine, quinine, and mefloquine (9). We found that ACT-213615, but not the inactive enantiomer, competed for probe binding to a degree similar to that previously found for mefloquine, making the active enantiomer of ACT-213615 a particularly effective competitor (not shown). We note that the concentration of ACT-213615 required to effectively compete for AzBCQ and ACT-460953 photolabeling was much higher than that needed to inhibit parasite proliferation. This shift is likely due to partitioning of the drug into the lipid phase of the proteoliposomal suspension in which the assay was performed and/or the fact that photolabeling was performed with PfMDR1 in de-energized proteoliposomal membranes. For many transporters, ligand affinity changes significantly in the presence of biologically relevant changes in transmembrane pH gradients or membrane electrical potential (34, 35). In any case, the implication of the photolabeling data is that ACT-213615 physically interacts with PfMDR1 and that the binding site at least partially overlaps with the previously determined binding site for chloroquine and other quinolone-based antimalarials.

To investigate the importance of PfMDR1 in ACT-213615 activity, we examined the anti-proliferative activity of ACT-213615 in the P. falciparum FCB line versus an isogenic pfmdr1 knockdown line tailored to have one less copy of pfmdr1. An increased level of pfmdr1 expression resulted in decreased susceptibility to mefloquine and ACT-213615 but not chloroquine (Table 1). This suggests a functional interaction between ACT-213615 and PfMDR1, in addition to the physical interaction shown above. Unfortunately, the nature of this interaction between ACT-213615 and PfMDR1 cannot be derived from the available data. One possibility is that ACT-213615 binds to PfMDR1 and interferes with its function. This interference could be through inhibition of an as yet unknown vital activity of PfMDR1 or by competition with the transport of essential endogenous molecules by PfMDR1. Additional experiments are needed to distinguish between these scenarios, but no direct PfMDR1 activity assays are available today.

Functional assays were also performed for PfENT4, hexose transporter, glideosome-associated protein 50/secreted acidic phosphatase, or S-adenosylmethionine synthetase. ACT-213615 did not reveal any effect in these assays, suggesting that the compound does not interfere with the activity of these proteins (data not shown). It remains possible that ACT-213615 binds to these parasite proteins without measurable consequence in the functional assays.
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Uptake assays, in which the transport of $[^3]$H]adenine into PfENT4-expressing X. laevis oocytes was assessed in the presence of ACT-213615 or its inactive enantiomer, demonstrated an inverse correlation between drug concentration and the amount of substrate transported (Fig. 4). This inhibition of uptake could be the result of binding of the drug to PfENT4 and inhibition of the conformational changes necessary for substrate transport. Given that ACT-213615 has potent anti-proliferative activity although its R-enantiomer does not, the enantiomer’s lack of selective inhibition of PfENT4-mediated transport implies that PfENT4 is not involved in the antimalarial activity of ACT-213615.

In summary, we have established a widely applicable UV-dependent affinity-capture method in intact cells and used this technique to identify proteins that interact with antimalarial compounds in P. falciparum. Functional tests for the identified proteins were used to confirm or rule out a role for the candidates in the action of ACT-213615. We have established both a physical and functional interaction between ACT-213615 and PfMDR1. It is not yet possible to differentiate whether PfMDR1 represents the actual molecular target of ACT-213615 anti-proliferative activity or whether it acts upstream of it. Future studies should shed light on this question.

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