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Stepwise *in vitro* screening of MMV pathogen box compounds against *Plasmodium falciparum* to identify potent antimalarial candidates

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ABSTRACT

Development of resistance to deployed antimalarial drugs is inevitable and needs prompt and continuous discovery of novel candidate drugs. Therefore, the antimalarial activity of 125 compounds from the Medicine for Malaria Ventures (MMV) pathogen box was determined. Combining standard IC_{50} and normalised growth rate inhibition (GR_{50}) analyses, we found 16 and 22 compounds had higher potencies than CQ respectively. Seven compounds with relatively high potencies (low GR_{50} and IC_{50}) against *P. falciparum* 3D7 were further analysed. Three of these were tested on 10 natural *P. falciparum* isolates from The Gambia using our newly developed parasite survival rate assay (PSRA).

According to the IC_{50}, GR_{50} and PSRA analyses, compound MMV667494 was most potent and highly cytotoxic to parasites. MMV010576 was slow acting but more potent than dihydroartemisinin (DHA) 72 h after exposure. MMV634140 was potent against the laboratory-adapted 3D7 isolate, but 4 out of 10 natural Gambian isolates survived and replicated slowly despite 72 h of exposure to the compound, suggesting potential drug tolerance and risk of resistance development.

These results emphasise the usefulness of *in vitro* testing as a starting point for drug discovery. Improved approaches to data analyses and the use of natural isolates will facilitate the prioritisation of compounds for further clinical development.

1. Introduction

The constant threat of insecticide and drug resistance by both vectors and parasites, respectively, represents a major challenge for malaria control (World Health Organisation, 2020). *Plasmodium falciparum*, the deadliest malaria parasite, has developed resistance to almost all available antimalarial drugs, possibly contributing to the slow decline and eventually stalling of the malaria burden observed in the last 5 years (Blasco et al., 2017). Case management is heavily dependent on the efficacy of artemisinin-based combination treatments (ACTs), in which a rapidly acting artemisinin derivative (ART) is associated with a partner drug with a longer half-life. Indeed, ART rapidly kill parasites, reducing parasite load in infected individuals by 10,000-fold in 48 h (Nosten and White, 2007). However, ART are rapidly eliminated and thus should be administered as ACTs for 3 days to cover at least 3 to 4 parasite life cycles (Wells et al., 2015). Resistance to ART has recently emerged in Africa (Balikagala et al., 2021; Straimer et al., 2022; Uwimana et al., 2020). This emphasises the urgent need to develop new antimalarial drugs. As parasites that develop resistance to multiple drugs might be genetically more plastic and could rapidly evolve towards resistance to new chemical classes of candidate drugs, there is the need for even more diverse classes of drugs (Bloland, 2001).

The aim of this study was to explore the efficacy of 125 compounds with suggested antiplasmodial activities from the MMV pathogen box.

2. Materials and methods

2.1. Plasmodium falciparum culture

The laboratory-adapted, malaria reference isolate *P. falciparum*-3D7 was continuously cultured *in vitro* using standard incubation conditions of 90% N_2, 5% O_2, 5% CO_2 and 37 °C as previously described (Trager and Jensen, 1976). Parasites were cultured in leukocyte depleted O^+ human RBCs at 2% haematocrit and between 0.5% and 2%

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parasitaemia. The complete culture medium used constituted RPMI 1640 growth media (Sigma-Aldrich, UK) supplemented with 35 mM HEPES (Sigma, St. Louis, MO), 24 mM NaHCO3, 1 mg/l of hypoxanthine (Sigma), 5 μg/ml of gentamicin (Gibco-BRL) and 0.5% (w/v) Albumax II (Gibco-BRL). Thin blood smears from cultures were stained with Giemsa (1-part Giemsa: 9-parts 0.4% NaCl) for 10 min to determine parasite stage and density.

2.2. Natural malaria parasite collection and processing

Ethical clearance for this study was obtained from the Gambia Government/MRC Joint Ethics Committee (approval #1626). After obtaining a written informed consent, venous blood samples (3 mL) were collected from patients with confirmed clinical malaria attending Brikama Health Centre, West Coast Region, The Gambia. Following leukocyte depletion (Mbye et al., 2020), parasite density was determined by microscopy and flow cytometry.

2.3. MMV pathogen box compounds

The pathogen box compounds were obtained from MMV in sealed 96-well microtiter plates consisting of 400 compounds at a concentration of 10 mM dissolved in dimethyl sulfoxide (DMSO). Information about the pathogen box can be accessed via the MMV website (www.mmv.org/mmv-open/pathogen-box). Compounds were diluted to 1 mM in DMSO and aliquots made into 96-well microtiter plates for storage at −80 °C.

2.4. In vitro sensitivity assay

A primary screen of the 50% inhibitory concentration (IC_{50}) of 125 compounds with antiplasmodial activities was carried out. For each compound, a 5-fold serial dilution was done to obtain nine concentrations ranging between 20 μM and 0.05 nM. Each drug concentration was tested in triplicates against synchronised ring stages of *P. falciparum*-3D7 reference isolate. Parasites were synchronised twice with D-sorbitol to recover >80% rings prior to assay set-up. Briefly, infected RBC (iRBC) pellet was mixed 1:10 v/v with pre-warmed 5% sorbitol and incubated for 10 min at 37 °C. Following centrifugation at 1500 RPM for 5 min, the supernatant was removed and the pellet of mostly ring stages washed twice in incomplete RPMI medium. Each drug was tested for growth inhibition in 96-well flat-bottom microtiter plates using iRBCs at 0.5% parasitemia and 2% haematocrit. Parasites were cultured for at least 72 h to allow for a full growth and re-invasion cycle, using standard incubation conditions. Drug-free iRBCs cultured on the same plates were considered as negative controls (no drug inhibition), while 'kill-assays' containing 10 μM of DHA constituted positive control (complete inhibition). Following incubation, iRBC pellets were stained with 100 μL of SYBR Green I intercalating dye (diluted 1:5000 in lysis buffer containing 0.005% SDS). Nucleic acid fluorescence was measured at excitation and emission wavelengths of 485 nm and 538 nm respectively on a Fluoroskan Ascent multi-well plate reader (Thermo Scientific). Quinine (QN), chloroquine (CQ) and amodiaquine (AMD) were screened as standard antimalarial drugs.

Following initial screening, a subset of potent compounds were further assessed using more stringent concentrations. For MMV634140, MMV010576 and MMV085499, a 2-fold serial dilution series resulting in 10 concentrations ranging from 25 nM to 0.05 nM; for MMV024443, MMV023985 and MMV010545, a 3-fold serial dilution series to obtain 10 concentrations ranging from 25 nM to 0.001 nM; and for MMV667494, a 2 - fold serial dilution series of 8 concentrations ranging from 25 nM to 0.20 nM. To determine parasitemia after 72 h, iRBC pellets in each assay plate were resuspended with 100 μL/well of 1:10,000 dilutions of SYBR Green I DNA stain in phosphate-buffered saline (PBS) (Invitrogen, USA) and incubated in the dark for 20 min at room temperature. Stained cells were washed with incomplete medium, resuspended in 500 μL of PBS and 100,000 events for RBCs were acquired using BD Accuri™ C6 flow cytometer for increased accuracy.

2.5. Parasite survival rate assay (PSRA)

Ten isolates from malaria patients were assayed for growth inhibition following continuous exposure to 3-fold the previously determined *P. falciparum*-3D7 IC_{50} for selected compounds (Mbye et al., 2020). These include, lumefantrine (LUM) at 398 nM, dihydroartemisinin (DHA) at 8.1 nM, MMV667494 at 0.5 nM, MMV010576 at 5 nM, and MMV634140 at 2 nM. Briefly, 100 μL of iRBC suspension was incubated with 100 μL of the respective drugs at a final parasitaemia of 0.5% and 2% haematocrit for 24, 48 and 72 h using standard culture conditions as described above. At every 24 h, drugs were washed off and refreshed, and 50 μL of drug exposed isolates were washed and incubated with 100 μL of DDAO-SE (an amine-reactive fluorescent dye: 7-hydroxy-9H-(1, 3-dichloro-9,9-dimethylacridin-2-one) succinimidyl ester) labelled uninfected RBCs (uRBCs) for a further 48 h to allow for a full drug-free growth cycle. These were then counterstained with SYBR Green I as above. Stained cells were washed with incomplete medium, resuspended in PBS and 100,000 events of RBCs acquired using BD Accuri™ C6 flow cytometer. Drug survival for each isolate was estimated from parasite density in re-invaded DDAO-SE-stained RBCs (Mbye et al., 2020).

2.6. Statistical analysis

Fluorescent reads from Floroskan were analysed for IC_{50} determination of each compound using GraphPad Prism v7.0. All assays were carried out in triplicates and log transformed for normality. For each compound, a four-parameter non-linear regression curve for growth inhibition against drug concentration was fitted and IC_{50}, confidence interval and R^2 values for the fitted curves determined using the following formula:

$$IC_{50} = c\left(\frac{a - 50\%\ response}{50\%\ response - d}\right)^{\frac{1}{b}}$$

Where: a = the lower plateau or bottom of the curve.

b = the slope factor

c = the concentration corresponding to the response between a and d

d = the upper plateau or top of the curve (Sebaugh, 2011).

These were further analysed for growth-corrected inhibition using GRmetrics Bioconductor package in R (https://bioconductor.org/packages/GRmetrics/) (Clark et al., 2017). GRmetrics, which include GR_{50}, GEC_{50} and comparable IC_{50} and GC_{50}, were obtained for 75 compounds with growth rates of >0.5, normalising growth against the no-drug controls and parasite density prior to assay set-up. Normalised GR inhibition values, GR(c), were calculated using the formula below as described by Hafner et al.,

$$GR(c) = 2^{\frac{k(c)}{k(0)}} - 1$$

Where: $k(c)$ = treated growth rate.

$k(0)$ = control growth rate.

The GR_{50} value is the concentration at which GR(c) = 0.5 (Hafner et al., 2016).

For the selected potent compounds re-analysed by flow cytometry, re-invaded RBC percentages from triplicate assays were also fitted with GraphPad Prism v7.0 and further analysed with growth corrected inhibition using the GRmetrics package as above.

Mann-Whitney U test was used to compare the medians of the distribution of IC_{50} and GR_{50} for the test drugs with the threshold of significance set at 0.05.

3. Results

3.1. Highly potent anti-plasmodial compounds identified from IC_{50} and GR_{50} analysis of primary in vitro sensitivity screening

The 125 compounds from the MMV pathogen box had a wide range of IC_{50} responses against the CQ-sensitive *P. falciparum* 3D7 laboratory-adapted reference isolate, between 0.02 nM for MMV687794 to 9702 nM for MMV011691, with a median of 804.40 nM (2.91 nM in log scale) (Supplementary Table 1, Fig. 1). The reference drugs with known anti-malarial properties had IC_{50} values of 41.72 nM for QN, 35.14 nM for CQ and 45.38 nM for AMD. A total of 16 compounds were more potent than CQ, with IC_{50}s ranging between 0.02 nM and 30.98 nM, and a median of 3.85 nM (Supplementary Table 1). The IC_{50}s of the remaining 109 compounds were higher than CQ, ranging between 36.39 nM and 9702 nM, with a median of 1197 nM. Using GRmetric analysis, 75 compounds were retained based on an R^2 cut-off of 0.7 for GRmetrics sigmoid non-linear fit of growth against drug concentration (Fig. 1, Supplementary Table 2). The GR_{50} values for these compounds ranged between 0.24 nM for MMV011511 and 6429.13 nM for MMV007625, with a median of 20 nM (2.32 nM in log scale) (Supplementary Table 2, Fig. 1). The GR_{50} for CQ and AMD were 42.87 nM and 52.44 nM, respectively. The GRmetrics fit for QN did not fit a sigmoid curve and GR_{50} was not calculated. Twenty-two compounds for which GR_{50} was determined from a sigmoid curve with an $r^2 > 0.9$ had a value equal to or less than that of CQ (Supplementary Table 2).

3.2. Re-evaluation of IC_{50} and GR_{50} of selected potent compounds

Seven candidate compounds with relatively low IC_{50}s from the initial screening were re-tested using more stringent compound concentrations, and more sensitive and specific re-invasion assays by flow cytometry. Both IC_{50} and GR_{50} were determined as above. Their IC_{50} values ranged between 0.01 nM and 2.02 nM, and the GR_{50} concentrations between 0.02 nM and 2.19 nM, with all seven compounds having negative GRmax values (Fig. 2, Table 1). Cytostatic and partially inhibitory compounds had positive GRmax values while cytotoxic compounds gave negative GRmax values. The median IC_{50} and GR_{50} for these compounds were 1.02 nM and 1.09 nM, respectively. Overall, the GR_{50} values were on average 1.09-fold higher than IC_{50} values.

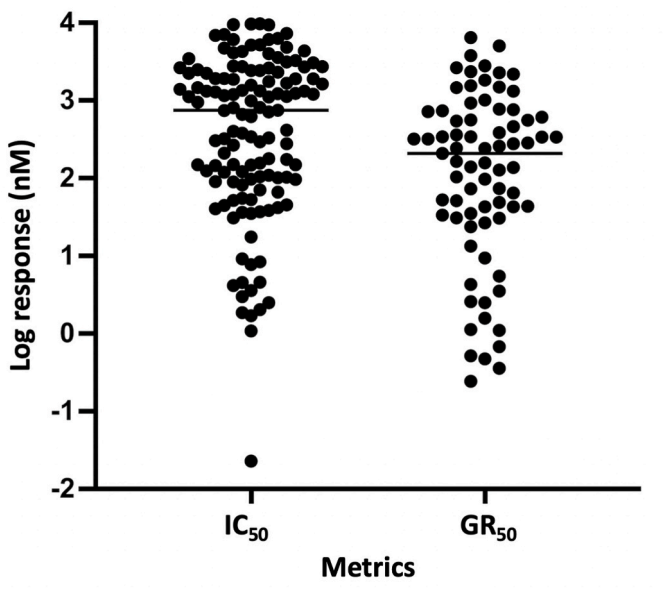

Fig. 1. Log10 IC_{50} for 125 compounds and GR_{50} for 75 compounds from the MMV pathogen box against *Plasmodium falciparum*-3D7 laboratory isolate.

However, there was a strong correlation between the IC_{50} and GR_{50} values ($R^2 = 0.9995$). The hill coefficients for the drugs ranged between 1.40 and 3.30, with MMV667494 having the slowest response and MMV085499 being the fastest.

3.3. Parasite survival patterns following exposure to potent compounds

Compounds MMV667494 and MMV634140 (Supplementary Fig. 1) with very low IC_{50}s, MMV010576 (Supplementary Fig. 1) with a relatively higher IC_{50}, and LUM and DHA were selected for PSRA. Their killing rates were determined on Gambian isolates with a growth rate of >1% in control cultures. Compound MMV667494 showed relatively higher potency at 6 h post-exposure (Fig. 3). Isolates BK19-1, BK19-3 and BK19-4 were relatively more sensitive to DHA and MMV634140 while BK19-2 and BK19-10 had similar sensitivity to all drugs tested. Isolate BK19-3 showed an increasing re-invasion trend despite exposure to LUM, comparable to the no-drug control. For most samples, peak parasitaemia was observed at 48 h post-exposure, corresponding to schizont rupture in the test assays (Fig. 3).

3.4. Parasite growth 72 h post-exposure

Re-invasion parasitaemia 72 h post-exposure was >0.2% in 3 isolates exposed to LUM and 2 exposed to DHA (Fig. 4a and b), 1 exposed to MMV01057 and 4 to MMV634140 (Fig. 4d and e). All isolates were sensitive to MMV667494, with a re-invasion parasitaemia of <0.2% (Fig. 4c). Overall, the highest re-invasion parasitaemia were observed with LUM exposure, with a mean re-invasion parasitaemia of 0.23%, followed by MMV634140, with a mean of 0.18% (Fig. 4f). Mean re-invasion parasitaemia was 0.14% for DHA, 0.06% for MMV667494, and 0.11% for MMV010576. Mean re-invasion parasitaemia was 1.48% for the no-drug control. Two isolates had higher tolerance to drugs; isolate BK19-1 had a re-invasion parasitaemia of >0.2% for LUM, MMV010576 and MMV634140, and isolate BK19-4 for LUM, DHA and MMV634140. Isolate BK19-3 had a re-invasion parasitaemia of >1% after exposure to LUM.

4. Discussion

The MMV pathogen box provides an excellent opportunity to identify lead compounds against several pathogens including malaria parasites (Medicines for Malaria Venture, 2022). The complexity of the malaria parasite life cycle is a major challenge for developing drugs with broad activity against all parasite ‘s stages, and to identify and characterise drug targets (Dans et al., 2020). The subset of 125 pathogen box anti-plasmodial compounds tested had varying levels of inhibitory activity against *P. falciparum* erythrocytic stage development. Such activity was measured with two metrics, IC_{50} and GR_{50}, that determine the mid-point of the effect of a compound against parasite growth. Parasite growth was determined either by bulk DNA estimation or by comparing the number of infected RBCs 72 h post-exposure of ring stage parasites with no-drug controls (Sebaugh, 2011). The first screening identified compounds that inhibited cell growth by 50% at low concentrations, i.e., with low IC_{50} values, and could be prioritised for further development. However, IC_{50} may vary by laboratory (Errington et al., 2014), because of variations in experimental conditions and differences in proliferation rates (Hafner et al., 2016). Therefore, we used an alternative GRmetrics analysis that corrects for cell division and assay conditions using the same assay set-up (Hafner et al., 2016). This approach uses the parasitemia in no-drug controls at the beginning and the end of the assay to normalise against growth; if parasites do not grow, the effect of the drug cannot be determined. The drug-free growth is a part of the standard IC_{50} assay, although parasitemia at the beginning of the assay is not factored into the calculations. As with every sensitivity test, GRmetrics assumes 100% parasite growth in the absence of drugs. The growth rate correction in GRmetrics could improve comparisons of results from different

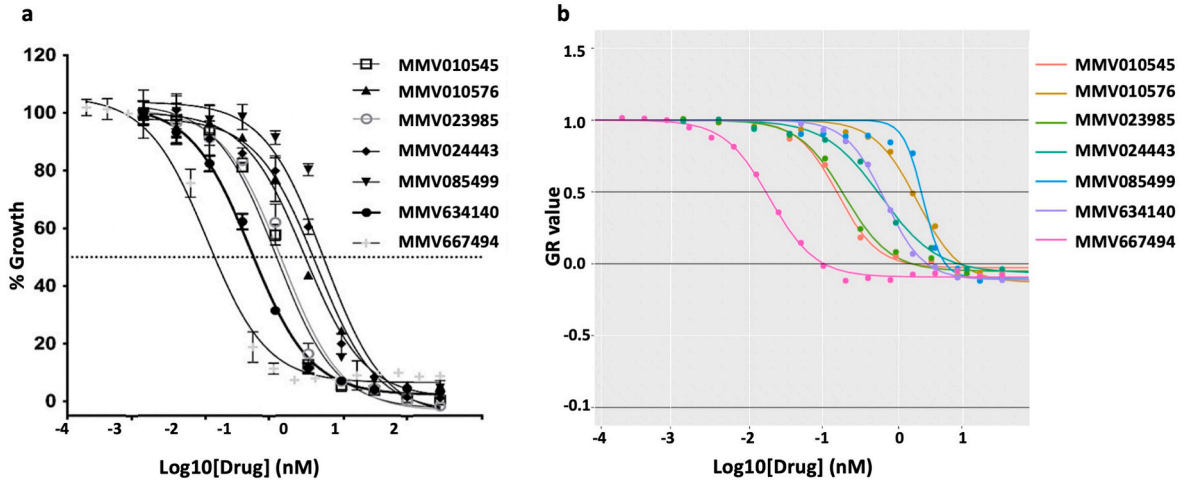

Fig. 2. Growth corrected responses of *P. falciparum* 3D7 isolate to seven potent antimalarial compounds. a) Sigmoid non-linear fit curves for IC_{50} determination for each compound. The error bars represent standard deviations for 3 replicates of each drug concentration tested. The dotted line shows the concentration at which 50% of the parasite population was inhibited by the compound. b) Growth inhibition curves for GR_{50} determination of selected compounds represented by separate lines.

Table 1
IC_{50} and GR_{50} values of seven compounds with high potencies against laboratory-adapted 3D7 isolate.

Compound	GR_{50} (nM)	GR_{max} (nM)	GEC_{50} (nM)	r^2_GR	pval_GR	IC_{50} (nM)	E_{max}	EC_{50} (nM)
MMV010545	0.98	−0.05	1.002	0.99	5.60E-10	0.92	0.13	0.81
MMV010576	1.55	−0.07	1.80	0.99	5.40E-08	1.39	0.12	1.24
MMV023985	1.09	−0.10	1.14	0.99	3.43E-09	1.02	0.11	0.90
MMV024443	2.19	−0.04	2.34	0.99	2.21E-09	2.02	0.14	1.75
MMV085499	2.04	−0.12	2.15	0.97	9.89E-07	1.89	0.11	1.74
MMV634140	0.58	−0.11	0.65	0.99	6.85E-12	0.52	0.11	0.45
MMV667494	0.02	−0.09	0.02	0.99	7.29E-21	0.01	0.12	0.01

All values are in log10 scale; GR50: the compound concentration at which the effect reaches 0.5; GRmax: the GR value measured at the highest compound concentration tested; GEC50: the compound concentration at which the drug has half-maximal effect (normalised); r2_GR: the coefficient of determination; pval_GR: the *P* value of the F−test comparing GR curve fits to data points; IC50: the 50% inhibitory concentration of the compound; Emax: the maximal effect at the highest compound concentration; EC50: the compound concentration at half-maximal effect.

laboratories and expand the uptake of *ex vivo/in vitro* surveillance.

The IC_{50} and GR_{50} analysis identified 16 and 22 compounds with similar or higher killing potencies than CQ respectively. It was not possible to estimate GR_{50} values for QN and some compounds in the MMV pathogen box because of either their low growth rates or the data not fitting a sigmoid curve, a limitation of this metric. GR_{50} can be estimated only if cells grow exponentially with a growth rate >0.5 (Brooks et al., 2019). Nonetheless, the strong correlation between GR_{50} and IC_{50} estimates are re-assuring and further strengthens the evidence on the potency of selected compounds. We further validated this approach by analysing a subset of the most potent compounds using higher resolution flow cytometry and cell invasion approaches, confirming the *in vitro* efficacy against the laboratory-adapted *P. falciparum* 3D7 isolate, a CQ sensitive parasite line.

Laboratory-adapted strains may not be adequate to measure the potency of a compound as their genotype and physiology could be significantly different than circulating parasites. Therefore, the efficacy of three compounds was tested with the PSRA assay on 10 field isolates of *P. falciparum* from The Gambia (Mbye et al., 2020). Four of these isolates were sensitive to the three experimental compounds and known antimalarials. However, some isolates were able to develop to schizonts following 72 h of exposure to LUM and DHA, remaining viable and re-invading at least 0.2% of erythrocytes in culture. This is important as viable parasites were not expected following long exposures to fatal doses of potent drugs. The cut-off of 0.2% was based on the median re-invasion parasitaemia of 41 field isolates 72 h after drug exposure (Mbye et al., 2020). Two field isolates could be considered as tolerant to lumefantrine as re-invasion parasitaemia exceeded 0.5% after 72 h exposure. Lumefantrine resistance has not been fully characterised in natural malaria populations although increasing IC_{50} following the introduction of artemether-lumefantrine as first line treatment in The Gambia has been reported (Amambua-Ngwa et al., 2017). One of the lumefantrine tolerant isolates was also tolerant to MMV010576, while two showed relatively lower sensitivities to MMV634140. Although survival at 72 h after exposure to lethal lumefantrine concentrations may be due to several factors, it could also indicate evolution towards resistance. Artemether-lumefantrine is the most used ACT in Africa, hence any sign of lumefantrine tolerance warrants further careful investigations. As a result of resistance to CQ, The Gambia changed its antimalarial treatment policy, first to SP and then to artemether-lumefantrine, that has been used for over 10 years prior to this study (Dunyo et al., 2006) and thus could have selected for tolerant parasites (Amambua-Ngwa et al., 2017; Thu et al., 2017). Resistance to lumefantrine and other ACT partner drugs threaten the efficacies of these combinations, prompting the need for continuous development of new antimalarial compounds (Watson et al., 2022).

Compound MMV667494 had the highest killing potency amongst all other MMV compounds as it inhibited parasites at higher rates than DHA even at 6 h post-exposure. Given its high potency and the ability to inhibit multiple parasite stages, it could be further assessed for safety and pharmacokinetic properties towards antimalarial drug development (Baragana et al., 2016; Duffy et al., 2017; Patra et al., 2020). MMV667494 and MMV634140 were previously classified as quinoline-carboxamides, related to quinolines, possibly explaining the

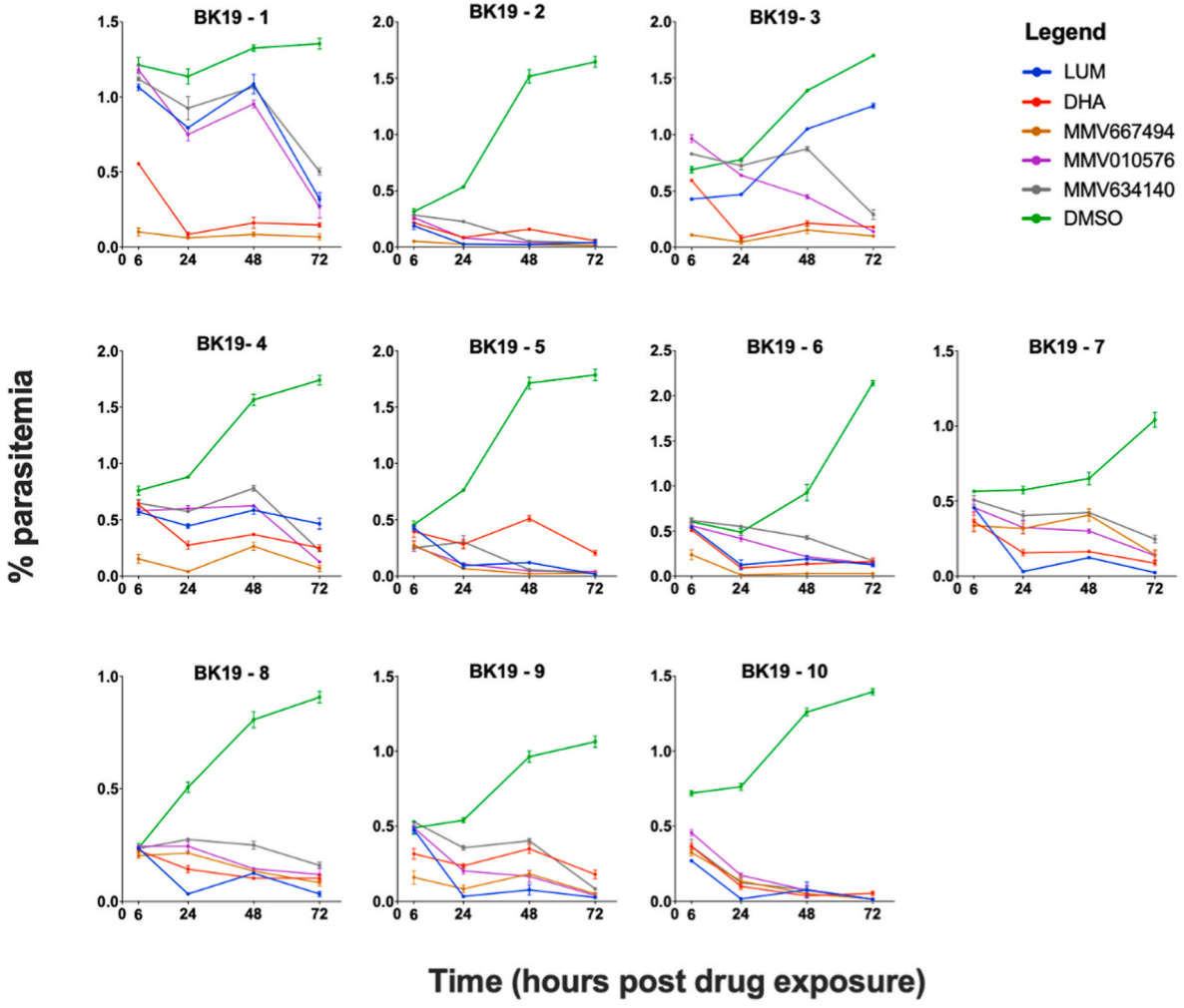

Fig. 3. Individual responses of 10 parasite samples following exposure to LUM, DHA, MMV667494, MMV010576, MMV634140 and a no-drug control (DMSO) at 6, 24, 48 and 72 h. The responses are shown in percentage parasitaemia and the error bars represent standard deviations of 3 individual replicates.

similar activity of MMV634140 and lumefantrine. Both compounds are thought to inhibit *P. falciparum* translational elongation factor 2 (*PfEF2*), required for protein synthesis (Baragana et al., 2016). The activities of these compounds against multiple stages of the *P. falciparum* life cycle, including the pre-erythrocytic, intra-erythrocytic, gametocyte and ookinete stages, have previously been confirmed (Baragana et al., 2016; Calit et al., 2018). In a recent screen of the pathogen box, MMV667494 and MMV634140 were also identified to be potent against tuberculosis, an indication of broad antipathogen activity (Calit et al., 2018).

MMV010576, one of our top hits, is a 2-amino pyridine derivative whose optimization resulted in the synthesis of the clinical candidate MMV390048 with inhibitory activity against phosphatidylinositol 4-kinase (PI4K) (Paquet et al., 2017). MMV010576, with structural similarity to some described kinase inhibitors, has been reported to effectively inhibit schizont maturation at sub-micromolar concentrations (Calit et al., 2018; Patra et al., 2020). PSRA analysis showed that MMV010576 is more potent than DHA 72 h after drug exposure which conforms with its characterisation as an egress inhibitor (Patra et al., 2020). Nevertheless, it seems to be a slow acting compound, with little activity 6 h after exposure. For ring stages within 6 h of exposure, we identified MMV085499 as a potent candidate. It is a 2-amino pyrazine also with PI4K activity that targets the parasite intra-erythrocytic stages (Duffy et al., 2017). In another recent screen of the pathogen box, MMV023985 and MMV010545 were classified as imidazopyridazines that inhibit *P. falciparum* calcium-dependent protein kinase 1 (PfCDPK1) and *P. falciparum* protein kinase 7 (PfPK7) (Duffy et al., 2017). MMV024443 which was also reported to have excellent egress inhibition activity, efficiently inhibited parasites at a relatively low concentration. This compound is an indole-2-carboxamide which has been shown to target *Pf*CDPK1, required for parasite invasion and development and is expressed throughout the intra-erythrocytic stages of the parasite (Patra et al., 2020).

Overall, the combination of IC_{50}-GR_{50} and PSRA analyses on both laboratory-adapted and wild isolates is a powerful approach that identified three highly potent compounds against *P. falciparum* isolates. This work opens opportunities to exploit these compounds for further development as antimalarial therapeutic agents. Based on their recent target identification, determining their pharmacokinetic and toxicity properties is essential for their progression through the drug development pipeline. The remaining compounds with hypothetical targets should be further explored to inform on their structure-activity relationship and improve their performance, pharmacokinetics, and toxicity.

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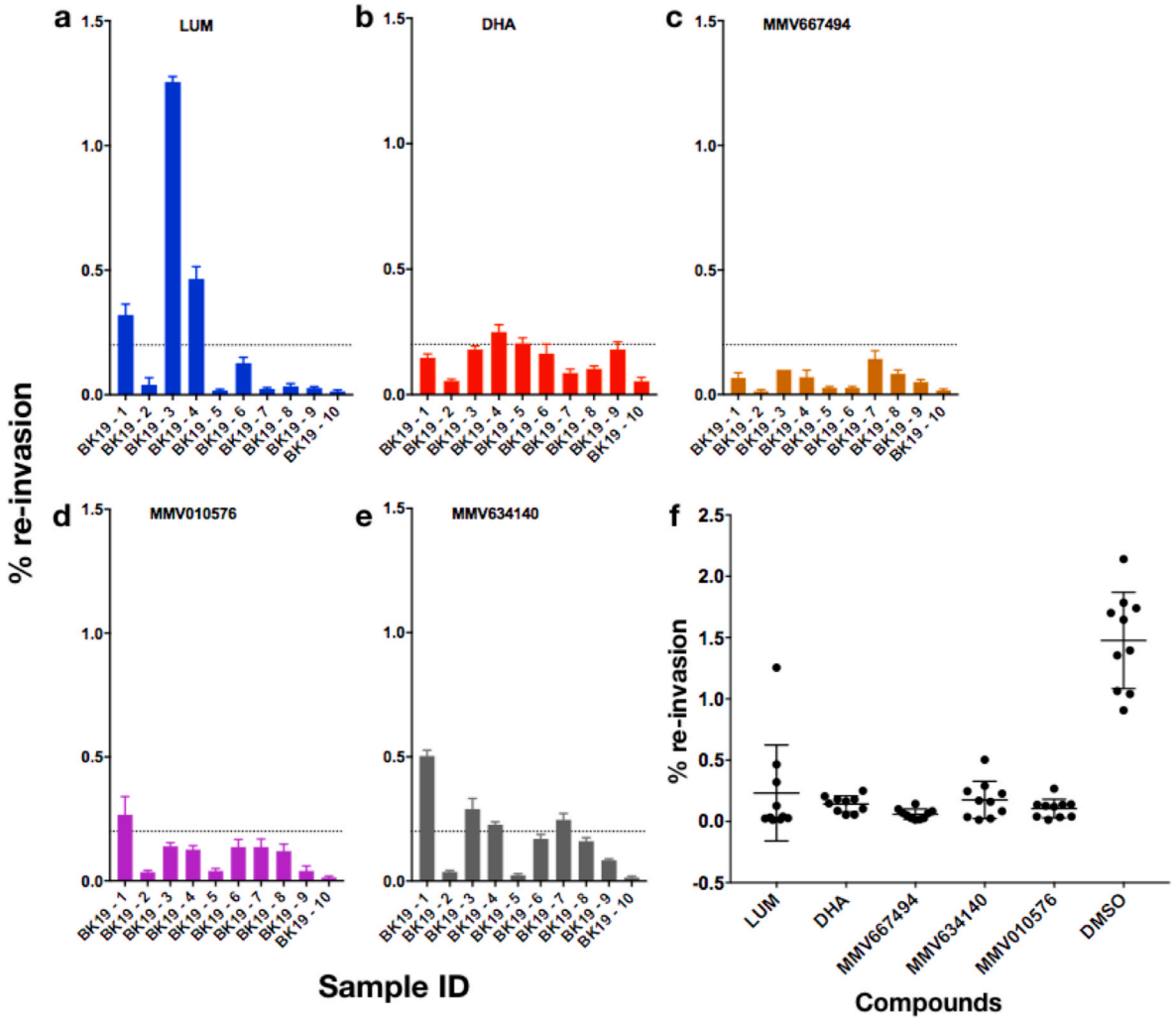

Fig. 4. Percentage re-invasion parasitaemia of 10 *P. falciparum* field isolates analysed against (a) LUM, (b) DHA, (c) MMV667494, (d) MMV634140, and (e) MMV010576 at 72 h post exposure. (f) Responses of 10 field isolates following 72 h exposure to drugs, test compounds and no-drug control (DMSO). The error bars represent standard deviations from 3 individual replicates.

studies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpddr.2023.05.005.

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