DISCOVERY BIOLOGY

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Open Source Drug Discovery
Anti-Plasmodial Activity Profiling of the Open Access MMV Pathogen Box

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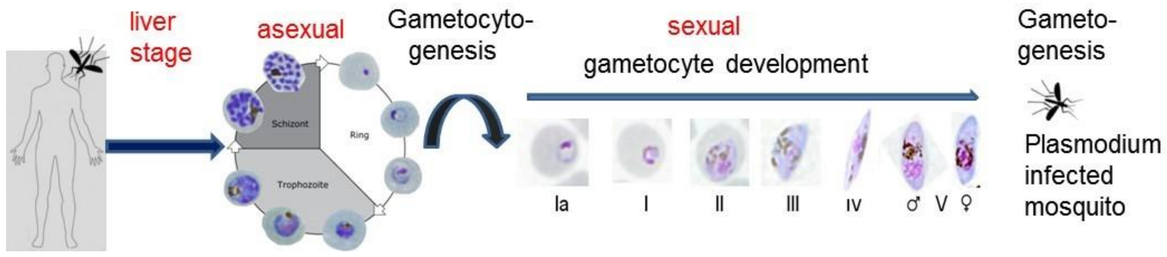
Introduction

Open source drug discovery utilises the world's research community to facilitate drug discovery. Experts across multiple disciplines contribute and openly discuss current data and future directions of what is in essence a worldwide drug discovery project. The MMV Pathogen Box is an open access set of 400 compounds with biological activity against numerous pathogens including Plasmodium, Mycobacterium and Kinetoplastid parasites (*Trypanosoma brucei* spp., *Leishmania* spp. and *Trypanosoma. cruzi*). In addition, a smaller number of compounds with activity against a range of other pathogens including Schistosoma, Toxoplasma, Cryptosporidium, Helminths and Dengue are included. The Pathogen Box contains 125 compounds within the "malaria set" , which we profiled for asexual and sexual biological activities using high content confocal imaging assays.

Plasmodium falciparum life cycle.



Infected mosquitoes transfer the parasites (sporozoites) to a human host through a blood meal. Sporozoites invade liver cells ultimately releasing daughter merozoites into the blood stream, which invade red blood cells (RBC). The majority of merozoites enter the asexual replication cycle responsible for the clinical symptoms of malaria. A small percentage are committed to sexual development through a process termed gametocytogenesis. Only fully developed mature stage gametocytes, taken up when a mosquito bites, are capable of withstanding the hostile environment within the mosquito gut. These undergo sexual replication resulting in new sporozoites which can again be transmitted to man during a mosquito blood meal.

Methods and Results

Asexual Assay. High content imaging and script analysis of 4',6-diamidino-2-phenylindole (DAPI) stained parasites[1]. The original assay measures changes to parasite numbers due to asexual replication (~ 5 fold increase per cycle). An optimised adaptation of the assay and script enables parasite age post RBC invasion to be determined based on parasite size and intensity of DAPI staining (Fig 2) in an automated fashion. The assay is referred to as the schizont maturation inhibition imaging assay (SMIIA).

Fig 2. Number of classified parasites vs parasite age post RBC invasion. The age of parasite post RBC invasion has a linear relationship with the number of parasites detected. As the parasite matures, increasing in size and fluorescent intensity, the more parasites are detected.

Average number ± SD for n=32.

Schizont Maturation Inhibition Imaging assay (SMIIA)

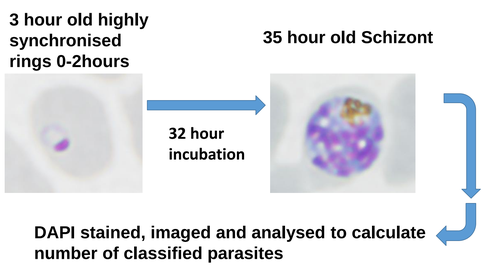


Fig 3. SMIIA concept. Compound is added to highly synchronous ring stage parasites and incubated for 32 hours. Parasites are stained with DAPI, images acquired and analysed for number of classified parasites. Percentage inhibition in relation to puromycin (active at all stage of parasite asexual lifecycle stages) is determined.

Fig 4. Reference compound IC_{50} values utilizing puromycin to normalize data. Pyronaridine and Artesunate have 100% Emax values, indicative of early ring stage parasite arrest (fast). Chloroquine has ~90% Emax corresponding with a slightly more mature ring to very early trophozoite (fast-moderate action), Halofantrine has mid-mature trophozoite (moderate-slow) and Pyrimethamine action exerted on very mature trophozoite (slow).

Sexual (Gametocyte) Assays. *In vitro* induction of gametocytes is performed using a highly synchronous stress induction protocol[2]. On day 0 of gametocytogenesis the earliest form of gametocytes, rings, exist (Ring Stage Gametocytes = RSG) in addition to parasites which will continue on through asexual replication. The addition of NAG (N-acetyl-glucosamine) kills the asexual parasites but not the gametocytes. Through a 12 day period gametocytes continue to develop through distinct morphological stages, classified as either early (ESG) or late (LSG) stage gametocytes. Using a GFP gametocyte specific expressing transgenic parasite, plus a mitochondrial viability marker, the effect of Pathogen Box compounds were tested for their action against RSG, ESG (I-III) and LSG (IV-V)[3].

Pathogen Box Malaria Set
Asexual

Of the 125 compounds tested in the SMIIA, 27 had Emax values between 34 and 70%, whilst 52 were between 70-100%. Forty six compounds did not yield an Emax plateau and therefore IC_{50} values were not obtained. Of these 46 compounds, 15 demonstrated activity within the single replication assay, with a further 5 compounds demonstrating activity only within the second cycle of replication at levels enabling IC_{50} values determination.

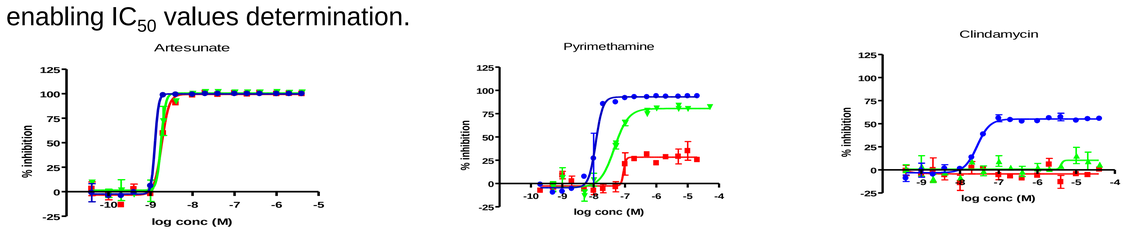


Fig 5. Asexual reference compound activity profile.

Red = SMIIA, Green= 1 cycle of replication, Blue = 2 cycles of replication.

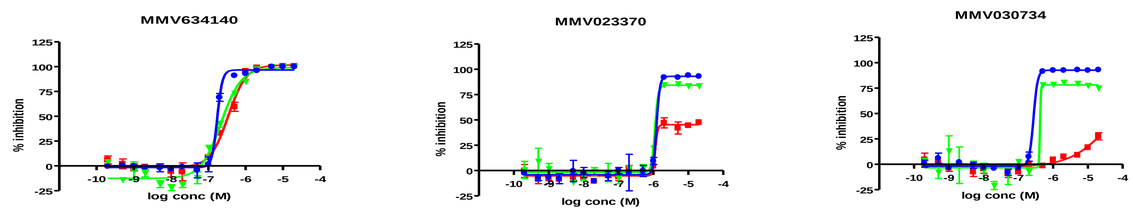


Fig 6. Exemplars of test compound profiles. MMV634140 is a quinolone 4-carboxamide, recently identified as inhibitors of the *Plasmodium* translational elongation factor 2 (*Pf*EF2)[4]

Relationship of Asexual and Gametocyte Activity Profiles.

Asexual classification	% active compounds RSG	% active compounds ESG	% active compounds LSG/MSG*
SMIIA Emax 34-69	60	26	7
SMIIA Emax 70-110	60	36	29
1st cycle active	46	33	0
2nd cycle active	0	0	0

Table 1. Relationship between asexual and gametocyte activity. The percentage of active compounds identified using SMIIA Emax % inhibition is comparable to those obtained for RSG, but diverge (reduction) from ESG through to LSG/MSG*.

The compounds active in the SMIIA with Emax >70% have increased numbers of compounds active against LSG. *(MSG data provided with Pathogen Box compounds)

Conclusion: Pathogen box contains anti-plasmodial compounds with diverse activity profiles. Not all asexually active compounds have activity against the youngest form of gametocytes i.e. RSG. Activity against the more mature gametocytes appears, from this limited evaluation, to be related to earlier onset of action against asexual parasites

References: 1. Duffy, Sandra, and Vicky M. Avery. *The American journal of tropical medicine and hygiene* 86.1 (2012): 84-92.. 2 Duffy, Sandra, et al. *Nature protocols* 11.5 (2016): 976-992. 3. Duffy, Sandra, and Vicky M. Avery. *Malaria journal* 12.1 (2013): 408. 4. Baragaña B, et al. *Journal of Medicinal Chemistry*. 2016 Nov 10; 59(21): 9672-9685.

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