

High throughput screening of a asexually naive small molecule library against late stage (IV-V) *Plasmodium falciparum* gametocytes.

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Aim: to identify compounds which kill late stage gametocytes and to subsequently profile them for activity against the remaining Inter-erythrocytic malaria parasite life cycle stages in man.

Method: Late stage gametocytes were produced in large scale and used within a high content imaging assay to identify compounds capable of killing gametocytes from a library of over 80,000 compounds. Active compounds were then profiled for activity against the asexual blood stage parasite plus early and mature gametocytes using image based assays.

Figure 1. Gametocytogenesis and gametogenesis

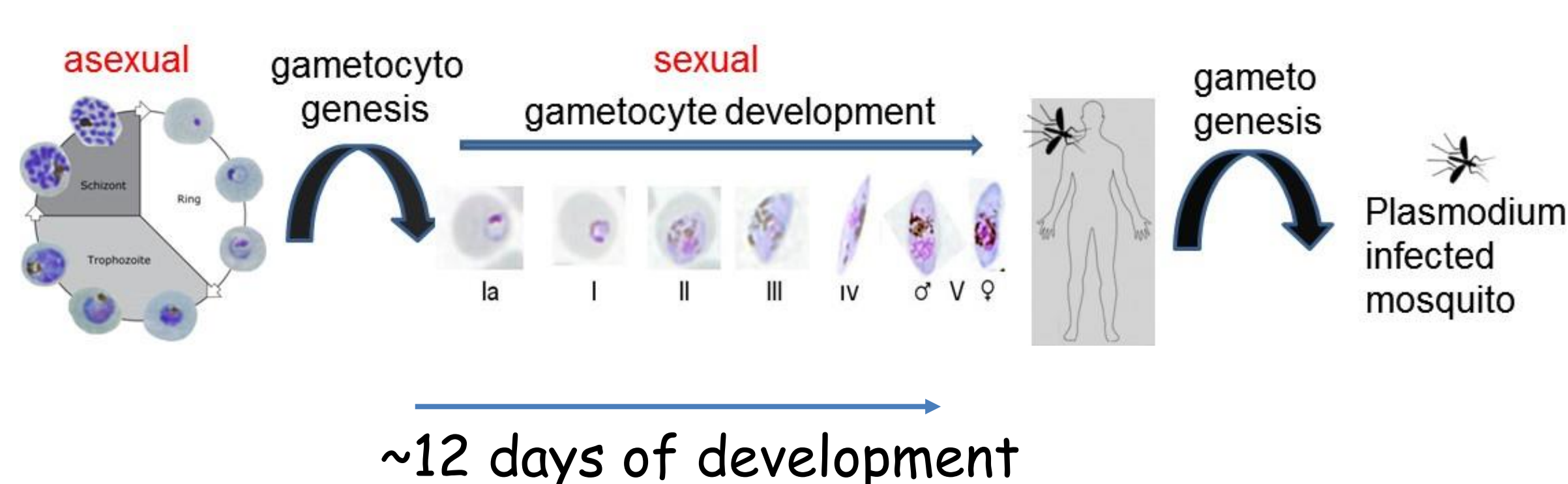


Figure 2. large scale gametocyte induction protocol

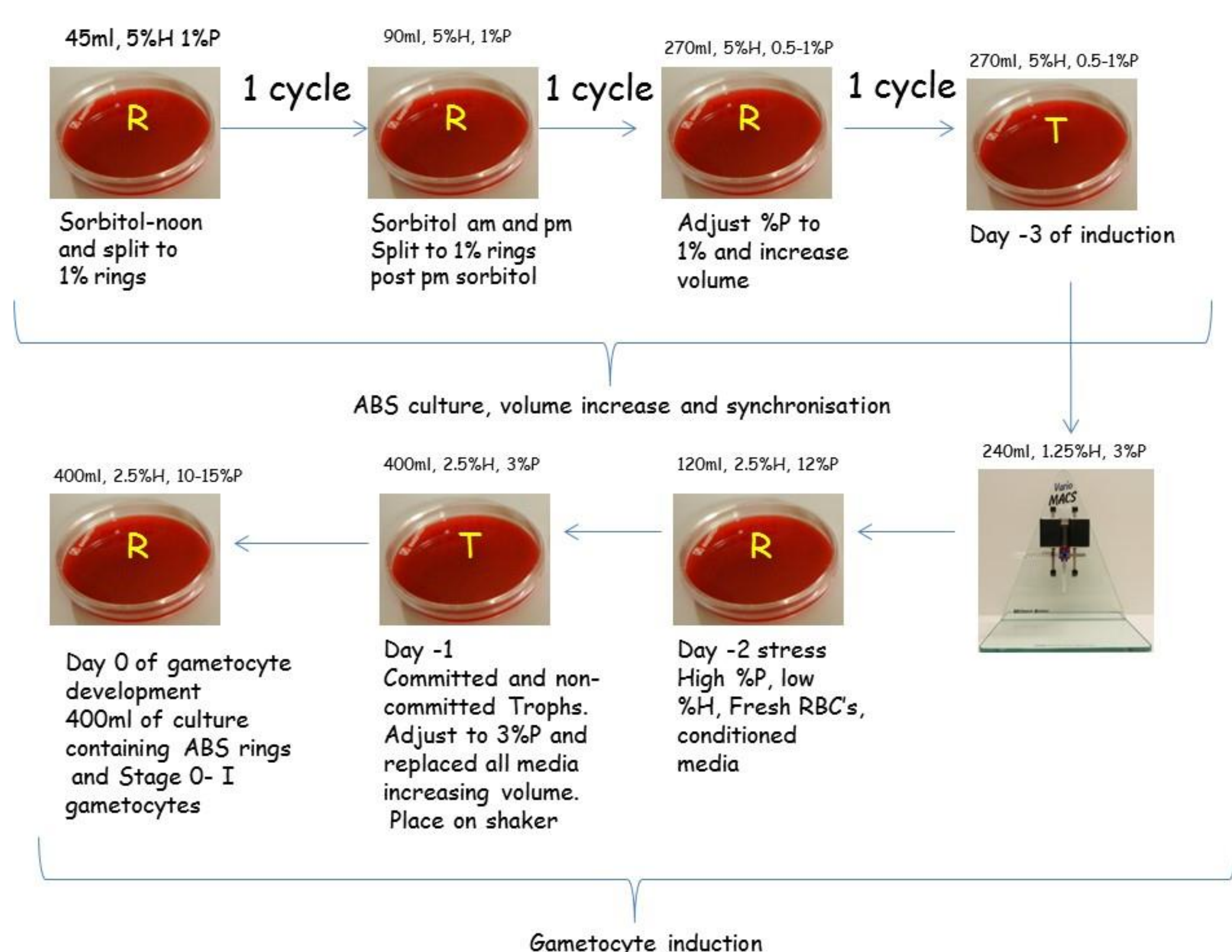
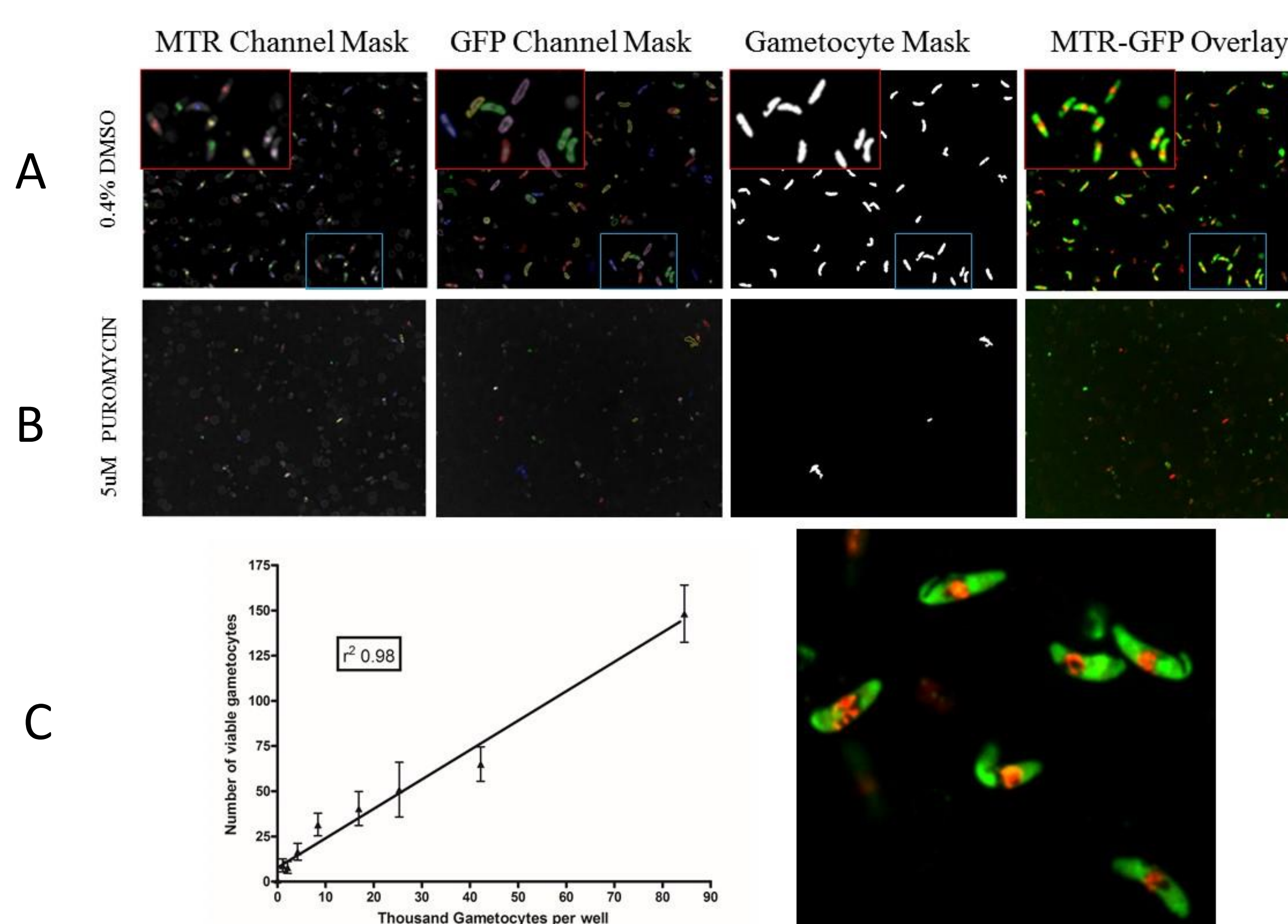
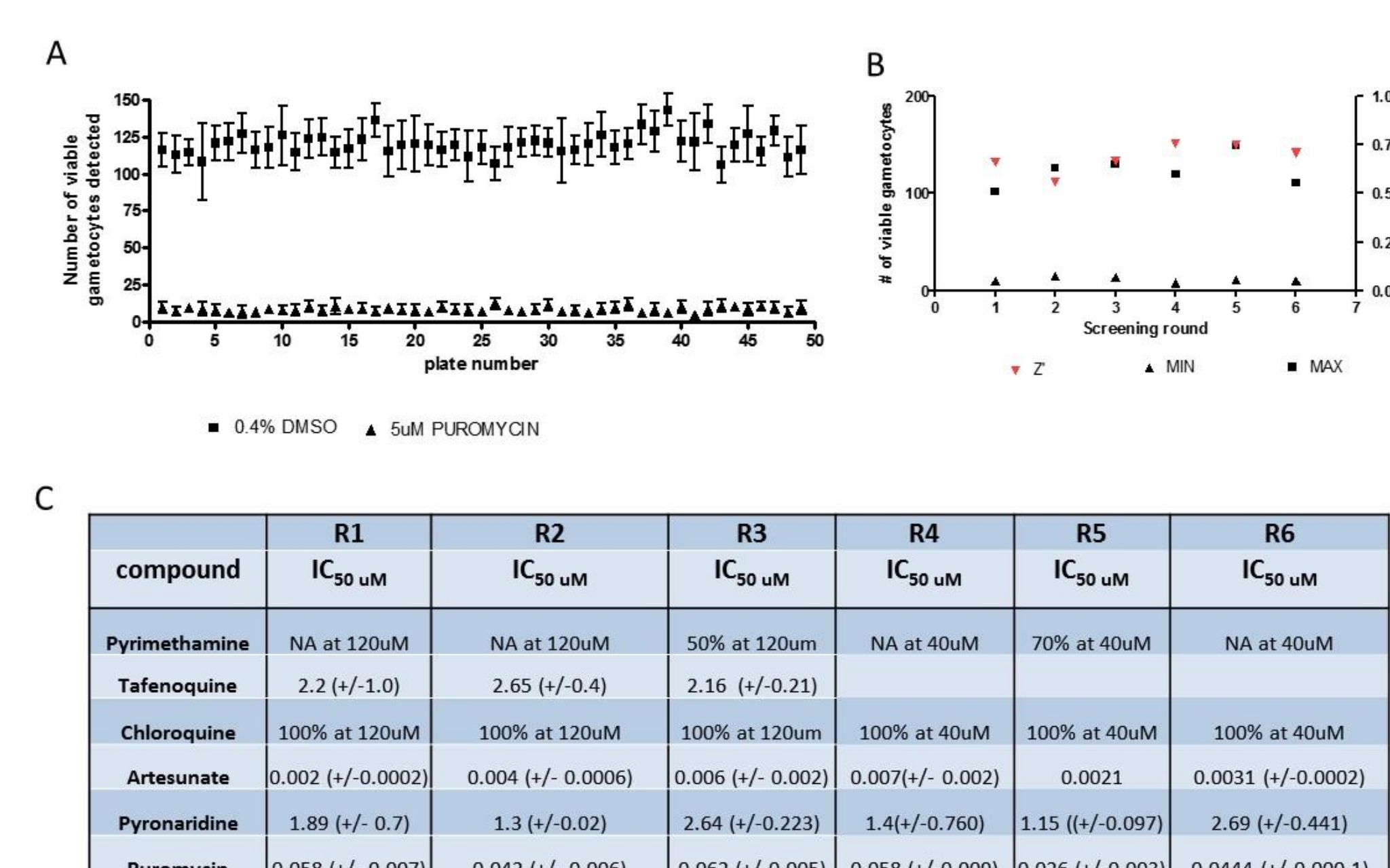


Figure 3. Late stage gametocyte Imaging assay



A. DMSO negative control. B. 5µM puromycin control
C. Assay linearity relating gametocytes per well and classified gametocyte number

Figure 4. Assay performance

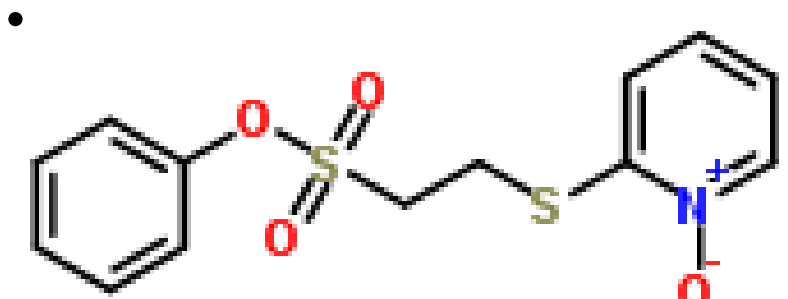


A. Example of in-plate controls for one single screening batch. B. Z' for six screening batches. C. reference compound data from screening campaign.

293 actives at greater than 70% inhibition at 2µM were identified from 80,309 compounds tested.

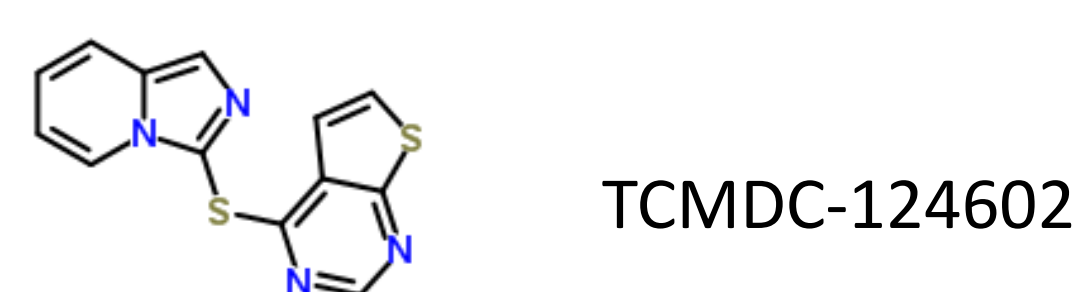
Results/outcome

Group 1.



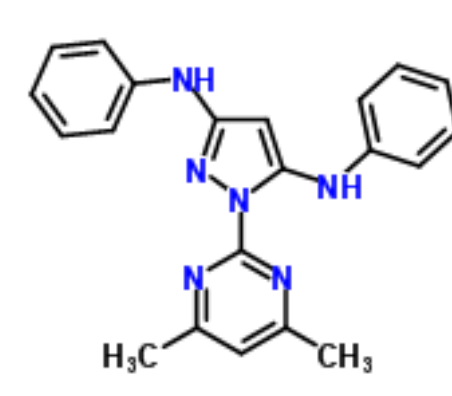
3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
23	43	52	23	28	1.2	4530.00

Group 2. Bis-heteroarylthioether



3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
141	140	377	332	2180	6.6	35% at 40µM

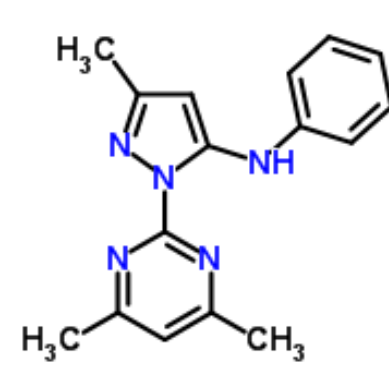
Group 3. Pyrazolopyrimidine



3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
1060	3640	1402	147	303	2.1	NA at 40µM

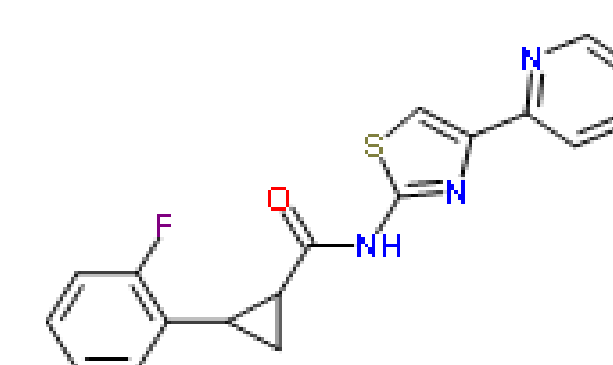
Gametocyte selective activity

Group 5. Pyrrolopyrimidine



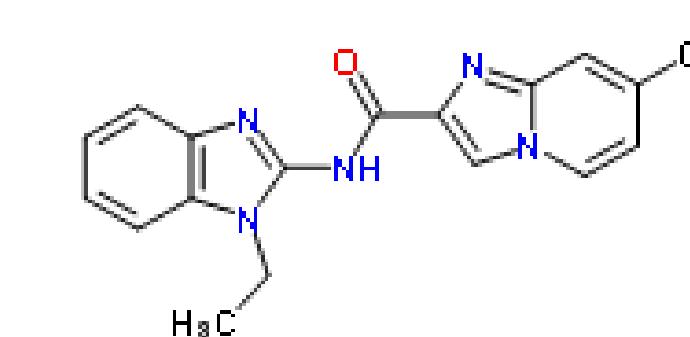
3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
1610	2480	731	59.8	413	6.9	NA at 40µM

Group 6. Pyridylthiazole



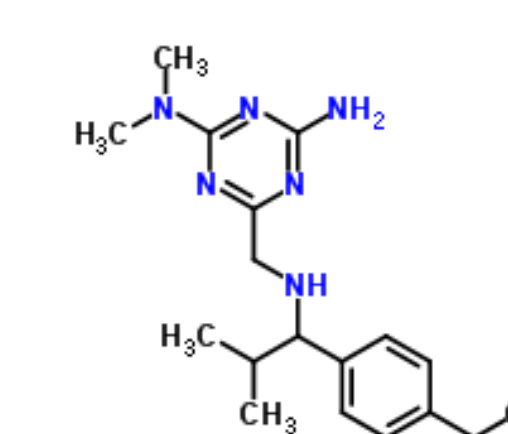
3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
657	1460	3399	244	708	2.9	54% at 40µM

Group 7. Amidobenzimidazole



3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
2660	3510	3185	272	1950	7.2	NA at 40µM

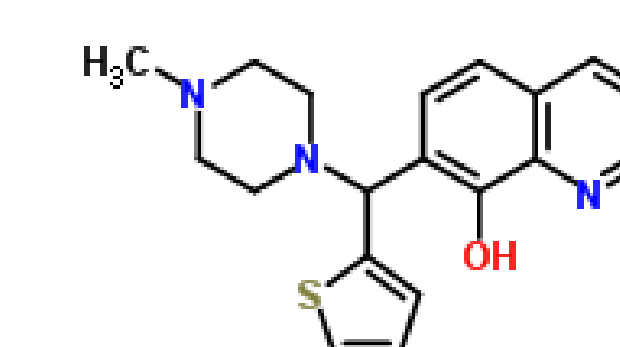
Group 8. Diaminotriazine



Red blood lysis seen in assays

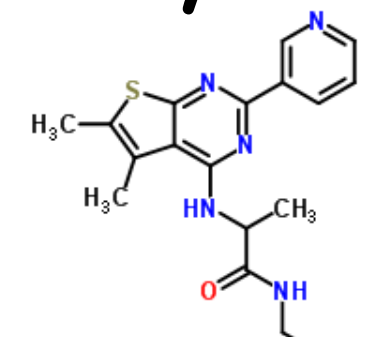
3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
266-sub maximal	Lysis	895	78.3	202	2.6	NA at 40µM

Group 9. Hydroxyquinoline



3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
865	940	3520	879	2900	3.3	74% at 40µM

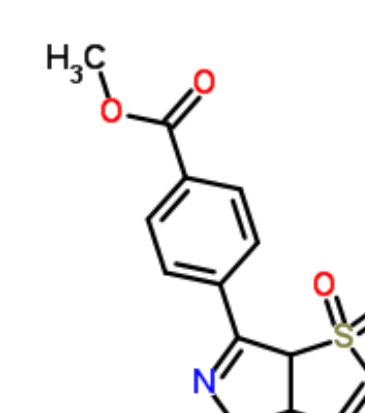
Group 4. Pyridylthienopyrimidine



Red blood cell lysis was seen within the assay's for these compounds

3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
1050	Lysis	2537	358	865	2.4	42% at 40µM

Group 10.



3D7 IC ₅₀	Dd2 IC ₅₀	ESG IC ₅₀	LSG IC ₅₀	MSG IC ₅₀	MSG/LSG	HEK IC ₅₀
nM	nM	nM	nM	nM	nM	nM
278	518	1061	261	1410	5.4	70% at 40µM

Acknowledgements
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