

A semi-automated luminescence based standard membrane feeding assay identifies novel small molecules that inhibit transmission of malaria parasites by mosquitoes

Martijn W. Vos^{1,†}, Will J. R. Stone^{2,†}, Karin M. Koolen¹, Geert-Jan van Gemert², Ben van Schaijk², Didier Leroy³, Robert W. Sauerwein^{1,2}, Teun Bousema², Koen J. Dechering^{1,*}

¹TropiQ Health Sciences, PO Box 9101, 6500 HB Nijmegen, The Netherlands

²Department of Medical Microbiology, Radboud University Nijmegen Medical Center, 6500 HB Nijmegen, The Netherlands

³Medicines for Malaria Venture, 20 Route de Pré-Bois, 1215 Geneva 15, Switzerland

[†]contributed equally

*corresponding author. E-mail: k.dechering@tropiq.nl. Tel. +31-6-51483935

SUPPLEMENTARY INFORMATION

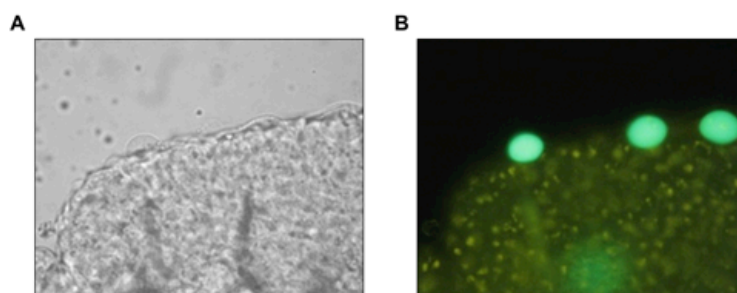


Figure S1. A. Brightfield micrograph of a section of mosquito midgut with 3 oocysts taken after mosquito dissection 6 days PI with NF54-HGL. **B.** Fluorescence micrograph of the same section of mosquito midgut with 3 oocysts taken after mosquito dissection 6 days PI with NF54-HGL.

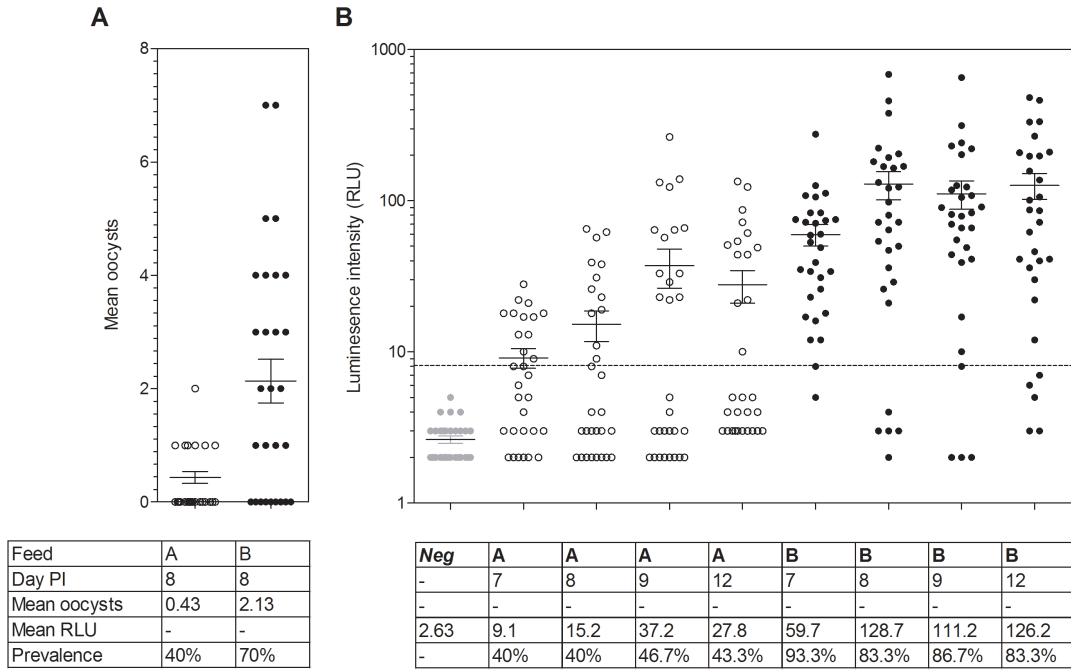


Figure S2. A. Oocyst intensity in individual mosquitoes from two groups of mosquitoes (*A* and *B*) infected with NF54-HGL, determined after dissection and standard midgut staining and microscopy at day 8 PI. **B.** Luminescence intensity in individual mosquitoes from the same two mosquito groups (*A* and *B*), assessed in separate mosquito samples removed from the primary mosquito storage cage at day 7, 8, 9 and 12 PI. The dashed line indicates the cut-off calculated statistically from the RLU values of all mosquitoes assayed in the current study (8.1 RLU). Prevalence estimates were based on this cut-off.

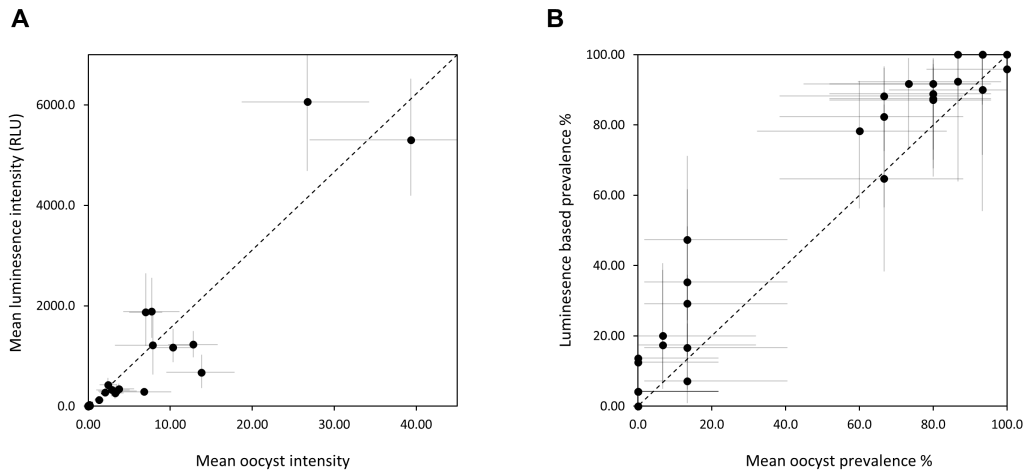


Figure S3. A. Relative oocyst and luminescence intensity among mosquitoes sampled from the same cages fed blood-meals without the addition of a test compound (16 feeds, 480 mosquitoes) or with the addition of DHA (16 feeds, 711 mosquitoes). **B.** Relative infection prevalence based on the observation of oocysts or calculated from luminescence intensity, among mosquitoes sampled from the same cages fed blood-meals without the addition of a test compound (16 feeds, 480 mosquitoes) or with the addition of DHA (16 feeds, 711 mosquitoes).

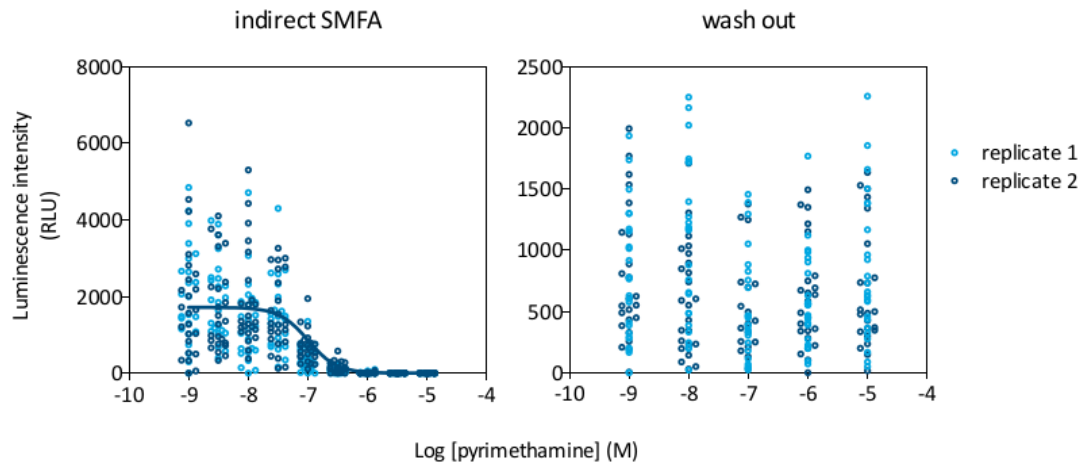


Figure S4. Efficacy of the washout protocol. The left panel shows the activity of pyrimethamine in the indirect SMFA as reported in Figure 2 of the main manuscript. The right panel shows the activity in the wash-out mode of the SMFA, where the compound was washed out prior to mosquito feeding.

primer	sequence 5'-3'
MWV 257	GTTAACGAAGTTCCTATACTATTGAAGAATAGGAACTTCACGCGTGCCGGCTATGGTACCGTACCTACTAACTGTTTTGAATCTC
MWV 259	CCGCGGTTTCATTATGCTAACATACATG
MWV 262	GCGCGGATTGGTAGTTGACTCATCTTG
MWV 267	CGAATTCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCCGGCATAGTATCTACATAACTGAAGCAAG
MWV 252	GGTACCACTAGTCCCGGGATCAACCGGTATGAGTAAAGGAGAAGAACITTTCACTGG
MWV 253	TTGAAGTTAACTTTGATTCCATTCTTTGTTGTCTG
MWV 198	TATCCCGGGGCTAGCCGCATAAATATCTGGTGAATAC
MWV 200	TATCGGATCCCTTTTGACTAGCCAATTTTC
MWV 268	AATGGCCCGAATTCGCGGCTACCTATTTGATGAATTAATACTACAC
MWV 271	TCTAGATTGTCGACAGGATTTATATAATATTTATGTAATACTACAATGGG
MWV 300 (P1)	TACATCAAATAACTCAGAGGGTAAC
MWV 301 (P2)	GTTTGTATATTTACCTTACATTTATCTCC

Table S1. List of DNA primer sequences as described in methods.