

S9 Table. Summary of resistance selection.

For ML901, Dd2-B2 parasites were subjected to pressure at 3 x IC₅₀. Mean IC₅₀ = 2.6 ± 0.05 nM. For ML471, Dd2-B2 parasites were subjected to a single-step selection at 10 x IC₅₀. Mean IC₅₀ and IC₉₀ values were 1.45 and 1.99 nM. DSM265, a *P. falciparum* dihydroorotate dehydrogenase inhibitor, was run as a control under the same pressure conditions. The Minimum Inoculum for Resistance (MIR) value was determined as 2.1 x 10⁵. For comparison, compounds exhibiting favourable MIR values include MMV1091186, a *P. falciparum* isoleucyl tRNA synthetase inhibitor, with an MIR value of >10⁸ [1], OSM-S-106, a *P. falciparum* asparagine tRNA synthetase inhibitor, with an MIR value of >2.4 x 10⁸ [2] and MP1-12, a *P. falciparum* proteasome inhibitor, with an MIR value of >6 x 10⁷ / < 2 x 10⁹) [3].

ML901		
Number of parasites in the selection	10 ⁷	10 ⁸
Day of recrudescence	12 (1/3), 14 (2/3)	14 (3/3)
IC ₅₀ fold shift (clones)	2-3 x IC ₅₀	3-6 x IC ₅₀
Minimum Inoculum for Resistance (MIR)	≤ 10 ⁷	

ML471	
Number of parasites in the selection	2x10 ⁵
Day of recrudescence	18 (29/96 wells)
IC ₅₀ shift	9-16 xIC ₅₀
Minimum Inoculum for Resistance (MIR)	7.1 x10 ⁵

References

1. Istvan ES, Guerra F, Abraham M, Huang KS, Rocamora F, Zhao H, et al. Cytoplasmic isoleucyl tRNA synthetase as an attractive multistage antimalarial drug target. *Science translational medicine*. 2023;15(686):eadc9249.
2. Xie SC, Wang Y, Morton CJ, Metcalfe RD, Dogovski C, Pasaje CFA, et al. Reaction hijacking inhibition of *Plasmodium falciparum* asparagine tRNA synthetase. *Nat Commun*. 2024;15(1):937.
3. Xie SC, Metcalfe RD, Mizutani H, Puhlovich T, Hanssen E, Morton CJ, et al. Design of proteasome inhibitors with oral efficacy in vivo against *Plasmodium falciparum* and selectivity over the human proteasome. *Proc Natl Acad Sci U S A*. 2021;118(39):e2107213118.