MACHINE LEARNING-ENABLED DISCOVERY OF MULTISTAGE ANTIMALARIAL 1,4-NAPHTHOQUINONES WITH A *Pf*MFR3-ASSOCIATED RESISTANCE PROFILE

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Introduction: The continued spread of multidrug-resistant malaria demands new chemotypes with distinct mechanisms of action and activity throughout the parasite life cycle. **Objectives:** To discover and characterize novel multistage antiplasmodial compounds using a machine learning–enabled screening strategy and to elucidate their resistance determinants. **Methods:** A machine learning model was employed to prioritize compounds from a large chemical library, leading to the identification of a 1,4-naphthoquinone series. Selected hits were profiled across parasite developmental stages and evaluated for mitochondrial effects. Resistance was generated *in vitro* against compound *1*, followed by whole-genome sequencing and functional validation using CRISPR/Cas9. **Results:** The identified series exhibited potent nanomolar activity across parasite stages and retained efficacy against *P. falciparum* and *P. vivax* field isolates, including drug-resistant strains. Treatment resulted in mitochondrial membrane depolarization but occurred independently of canonical electron transport chain inhibition. Genomic analysis of resistant parasites revealed mutations in *Pf*MFR3, and genome editing confirmed its role as a resistance mediator. Importantly, compound *1* enhanced artemisinin responsiveness in artemisinin-resistant parasites, supporting its use in combination strategies. **Conclusion:** This work identifies a multistage-active 1,4-naphthoquinone scaffold associated with a *Pf*MFR3-linked resistance mechanism distinct from classical mitochondrial inhibitors. The findings reinforce the utility of AI-driven discovery frameworks in uncovering chemically and mechanistically novel antimalarial candidates.