Identification of a selective sub-micromolar lead against *Plasmodium falciparum* based on structural optimization of pyridine scaffolds

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Introduction: Malaria remains a leading cause of global mortality, with *Plasmodium falciparum* resistance threatening current treatments. To address this, pyridine-containing heterocycles have emerged as key medicinal scaffolds with activity against the parasite.

Objective: To evaluate the antiplasmodial activity and structure–activity relationships (SAR) of N-(hydroxypyridinyl)pyridine-carboxamide derivatives designed as *P. falciparum* inhibitor candidates.

Methods: The N-(hydroxypyridinyl)pyridine-carboxamide derivatives bearing diverse substituents (bulky, electron-donating and electron-withdrawing groups) on the non-hydroxylated pyridine ring were screened against *P. falciparum* (3D7 strain) using the SYBR Green I assay. Compounds with IC_{50} < 10 µM were evaluated for cytotoxicity in HepG2 cells to determine selectivity indices (SI = CC_{50}/IC_{50}).

Results: SAR analysis showed that modifying the C-6 position significantly improved potency compared to **compound 1** (IC_{50} > 10 µM). Morpholine (**RMS09**) (1.6 ± 0.6 µM) and thiomorpholine substituents (**RMS10**) (3.6 ± 0.6 µM) showed comparable potencies. Similar trend was observed for piperazine (**RMS17**) (1.8 ± 0.2 µM), methylpiperazine (**RMS11**) (2.3 ± 0.7 µM), and homopiperazine (**RMS18**) (1.9 ± 0.2 µM) substituents. Small acyclic amines (**RMS15** and **RMS41**) maintained activity in the low micromolar range, whereas bulky dibutylamine (**RMS42**) (9 ± 1 µM) slightly reduced potency. **RMS40**, bearing a dimethylaminoethylpiperazinyl substituent, showed the highest potency (0.7 ± 0.1 µM), suggesting that bulky substituents on the piperazine group is favorable for antiplasmodial activity. The compounds exhibited low cytotoxicity (CC_{50} > 50 µM) on HepG2 cells.

Conclusion: SAR analysis shows that bulky piperazine substituents enhance activity. **RMS40** was the most potent, with sub-micromolar activity and high selectivity (SI > 71), demonstrating the potential of this series for antimalarial drug discovery.

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