ABSTRACT

Plasmodium falciparum is responsible for ninety percent of malaria infections in sub-Saharan Africa, and the majority of malaria-related deaths, with antimalarial drugs failing at an alarming rate. To combat this deadly disease, not only are novel target-specific antimalarial drugs needed, but also a high-throughput method for the screening of compounds to replace standard methodologies.

A total of 112 novel compounds from three chemical classes were assessed for antimalarial activity against the chloroquine-sensitive 3D7 strain of *P. falciparum* using the [^{3}H]-hypoxanthine incorporation assay, and their haemolytic activity against healthy red blood cells determined. Lead compounds were examined for their effects on parasite morphology and parasitic development; as well as their pharmacological interactions when combined with standard antimalarial drugs. Antimalarial mechanisms of action were examined using the β-haematin inhibitory activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) free-radical scavenging, and ferrous iron chelating activity assays. Using the DNA probe dihydroethidium, flow cytometry as a high-throughput drug screening method was validated against the [^{3}H]-hypoxanthine incorporation assay and assessed for its ability to determine the stage-specific activity of the compounds.

Of the three classes of compounds, the metronidazole-thiosemicarbazone analogues were the most active, 75% of the compounds inhibited parasite growth at IC_{50} values below 10 μM, whilst also exhibiting no haemolytic activity. The most active of which, 4-(2-Chlorobenzyl)-1-(4-(2-(1-(2-hydroxyethyl)-5-nitro-1*H*-imidazol-2-yl)vinyl)benzylidene)-thio-semi-carbazide (compound Y-3) (IC_{50} value: 2.83 ± 0.20 μM) was also a potent inhibitor of β-haematin formation (IC_{50} value: 19.08 ± 2.37 μM), proving to be more active than chloroquine (IC_{50} value: 29.64 ± 3.35 μM). Similarly, metronidazole-thiosemicarbazones analogues were potent scavengers of the free-radical of DPPH$^\bullet$, with the activity of 1-(4-(2-(1-(2-Hydroxyethyl)-5-nitro-1*H*-imidazol-2-yl)vinyl)benzylidene)-4-benzyl-4-methyl-thio-semi-carbazide (compound Y-8) (IC_{50} value: 21.98 ± 0.56 μM), comparable to that of the standard, ascorbic acid (IC_{50} value: 19.31 ± 2.62 μM). When combined with quinine and dihydroartemisinin, compound Y-3 produced an additive pharmacological interaction.

Seventy one percent of the chloroquinoline-chalcones tested had IC_{50} values below 100 µM, with (E)-3-(2-chloro-7-methylquinoline-3yl)-1-(pyridine-2yl)prop-2-en-1-one (compound F-13) the most active (IC_{50} value: 31.31 ± 0.87 µM). None of the compounds displayed any notable activity in the antimalarial mechanisms of action tested for, whilst also resulting in no red blood cell toxicity. When combined with quinine, compound F-13 exhibited an additive interaction.

The nucleoside phosphonates, phosphonic acids and purine/pyrimidine derivatives exhibited disappointing antimalarial activity, with only 21% of the compounds inhibiting parasite growth with IC_{50} values below 100 µM, with compound DR-4850 (currently under patent) the most potent (IC_{50} value: 13.35 ± 0.38 µM). None of the compounds resulted in any red blood cell lysis, with the exception of compound DR-4914B (currently under patent) (50.20 ± 3.35% haemolysis at 100 µM). Some nucleoside derivatives were potent inhibitors of β-haematin formation, with Hexadecyloxypropyl uridin-5'-yl 2-([3*R*,4*R*]-3,4-dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (compound DR-4137) (IC_{50} value: 8.29 ± 1.11 µM) 3.6-fold more active than chloroquine, although this did not appear to be the primary antimalarial mechanism of action of this class of compounds. Combination studies with quinine produced an additive interaction, whilst combination studies with the nucleoside transporter inhibitor dipyridamole produced additive-antagonistic interactions.

In conclusion, this study examined a wide variety of compounds and identified lead compounds, which following structural modifications may produce potent antimalarial drugs.