Supplementary data for the article:

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Supporting information-III

Reinvestigating Old Pharmacophores: Are 4-Aminoquinolines and Tetraoxanes Potential Two-Stage Antimalarials?

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Inhibition potential of antiplasmodials 23 and 25 against the hERG K+ channel.

The experiments are performed on an IonWorks™ HT instrument (Molecular Devices Corporation), which automatically performs electrophysiology measurements in 48 single cells simultaneously in a specialized 384-well plate (PatchPlate™). All cell suspensions, buffers and test compound solutions are at room temperature during the experiment. The cells used are Chinese hamster ovary (CHO) cells stably transfected with hERG (cell-line obtained from Cytomyx, UK). A single-cell suspension is prepared in extracellular solution (Dulbecco's phosphate buffered saline with calcium and magnesium pH 7-7.2) and aliquots added automatically to each well of a PatchPlate™. The cells are then positioned over a small hole at the bottom of each well by applying a vacuum beneath the plate to form an electrical seal. The vacuum is applied through a single compartment common to all wells which is filled with intracellular solution (buffered to pH 7.2 with HEPES). The resistance of each seal is measured via a common ground-electrode in the intracellular compartment and individual electrodes placed into each of the upper wells.
Electrical access to the cell is then achieved by circulating a perforating agent, amphotericin, underneath the PatchPlate™ and then measuring the pre-compound hERG current. An electrode is positioned in the extracellular compartment and a holding potential of -80 mV applied for 15 sec. The hERG channels are then activated by applying a depolarizing step to +40 mV for 5 sec and then clamped at -50 mV for 4 sec to elicit the hERG tail current, before returning to -80 mV for 0.3 s.

Test compound is then added automatically to the upper wells of the PatchPlate™ from a 96-well microtitre plate containing a range of concentrations of each compound. Solutions are prepared by diluting DMSO solutions of the test compound into extracellular (final DMSO concentration 0.25%). The compound **23** (**25**) is left in contact with the cells for 300 s before recording currents using the same voltage-step protocol as in the pre-compound scan. Quinidine, an established hERG inhibitor, is included as a positive control and buffer containing 0.25% DMSO is included as a negative control. The results for all compounds on the plate are rejected and the experiment repeated if the IC_{50} value for quinidine or the negative control results are outside quality-control limits. Each concentration is tested in 4 replicate wells on the PatchPlate™. However, only cells with a seal resistance greater than 50 MOhm and a pre-compound current of at least 0.1 nA are used to evaluate hERG blockade. Post-compound currents are then expressed as a percentage of pre-compound currents and plotted against concentration for each compound. Where concentration-dependent inhibition is observed, the data are fitted to the following equation and an IC_{50} value calculated:

$$y = y_{min} + \frac{y_{max} - y_{min}}{1+(x/x_{50})^{s}}$$

y = (post-compound current/pre-compound current) x 100; x = concentration; x_{50} = concentration required to inhibit current by 50% (IC_{50}) and s = slope of the graph.

Test Article	% Mean Inhibition						
	0 μM	0.008 μM	0.04 μM	0.2 μM	1 μM	5 μM	25 μM
25	0	5.45	-9.95	12.4	-4.78	8.54	N/A
23	0	4.93	1.74	-0.24	0.35	11.7	N/A

AMES mutagenicity screening of antiplasmodial 23.

Approximately ten million bacteria are exposed in triplicate to compound **23** (six concentrations), a negative control (vehicle) and a positive control for 90 minutes in medium containing a low concentration of histidine (sufficient for about 2 doublings.) The cultures are then diluted into indicator medium lacking histidine, and dispensed into 48 wells of a 384 well plate (micro-plate format, MPF). The plate is incubated for 48 h at 37 °C, and cells that have undergone a reversion will grow in a well, resulting in a color change in wells with growth. The number of wells showing growth are counted and compared to the vehicle control. An increase in the number of colonies of at least two-fold over baseline (mean + SD of the vehicle control) and a dose response indicates a positive response. An unpaired, one-sided Student's T-test is used to identify conditions that are significantly different from the vehicle control.
Where indicated, S9 fraction from the livers of Aroclor 1254-treated rats is included in the incubation at a final concentration of 4.5 %. An NADPH-regenerating system is also included to ensure a steady supply of reducing equivalents.
Strains used in this study:
S. typhimurium TA98: hisD3052, rfa, uvrB / pKM101; detects frame-shift mutations.
S. typhimurium TA100: hisG45, rfa, uvrB / pKM101; detects base-pair substitutions.