Running title: Preclinical PK-PD of antimalarials

Pharmacokinetics-pharmacodynamics analysis of spiroindolone analogs and KAE609 in a murine malaria model

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Supplementary material

Table S1. *In vitro* PK properties of spiroindolone analogs

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Table S1: *In vitro* PK properties of spiroindolone analogs

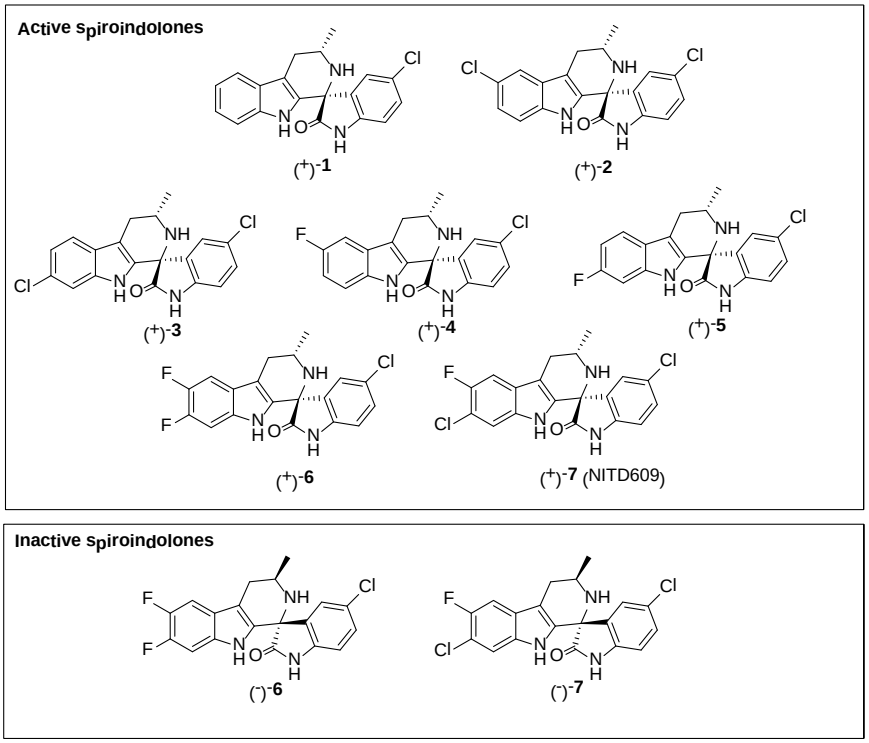
Compound	Solubility (pH 6.8, µM) [(a)]	PAMPA[(b)] %FA	Caco-2[(c)] P_{app}(A-B) 10^{-6} cm/sec	Hepatic CL_{int} in mouse liver microsomes[(d)] (µL/min/mg)	Mouse PPB[(e)] (%)	*RBC to Plasma Ratio*[(f)]
(+)-**1**	194	97	7.19	770	98.4	1.04
(+)-**2**	22	98	n.t	92	99.8	0.77
(+)-**3**	8	98	41.7	35	99.7	0.93
(+)-**4**	137	97	7.66	330	98.8	0.62
(+)-**5**	131	98	4.33	26	98.5	1.18
(+)-**6**	169	99	5.55	41	99.5	1.12
(+)-**7** (KAE609)	39	98	1.69	28	99.8	0.85
(-)-**6**	112	97	4.8	36	99.6	0.81
(-)-**7**	35	98	1.73	21	99.4	0.52

In vitro PK parameters like solubility, PAMPA, Caco-2 permeability, hepatic clearance, mouse plasma protein binding and RBC to plasma partitioning were determined in a medium

to high through put format using in-house standard assay protocols that are described in the literature.

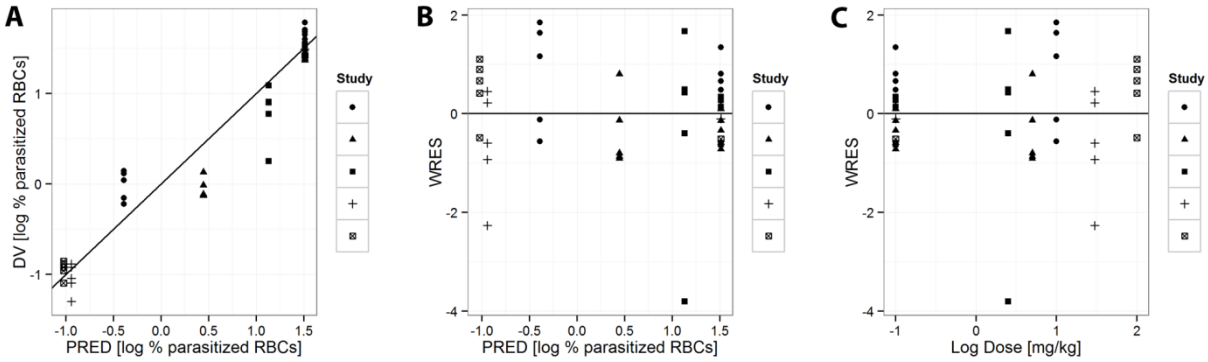
[(a)]HT-Equilibrium solubility at pH 6.8 (1); [(b)]Parallel Artificial Membrane Permeability Assay expressed in % fraction absorbed (FA) (2, 3); [(c)]Apparent permeability from apical to basolateral in Caco-2 cells expressed as P_{app} (A-B) 10^{-6} cm/sec (4, 5), n.t, not tested; [(d)]intrinsic clearance in mouse liver microsomes expressed as µL/min/mg protein, in the presence of liver enzymes (6); [(e)]Mouse plasma protein binding using rapid equilibrium dialysis method (7, 8), [(f)]RBC to plasma ratio, mean from 1 µM & 10 µM (9).

Figure S1. Structure of spiroindolone analogs.



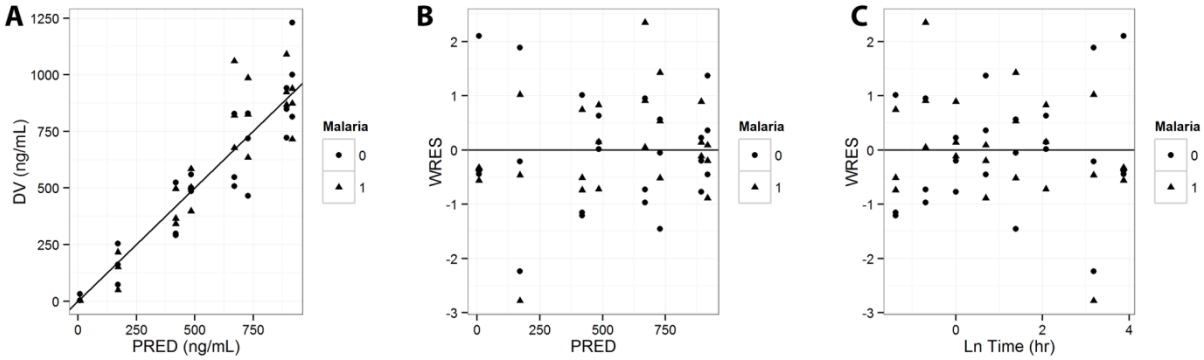
Active spiroindolones have the 1R, 3S configuration while inactive spiroindolones have the 1S, 3R configuration (10).

Figure S2. Goodness-of-fit plots for dose response relationship



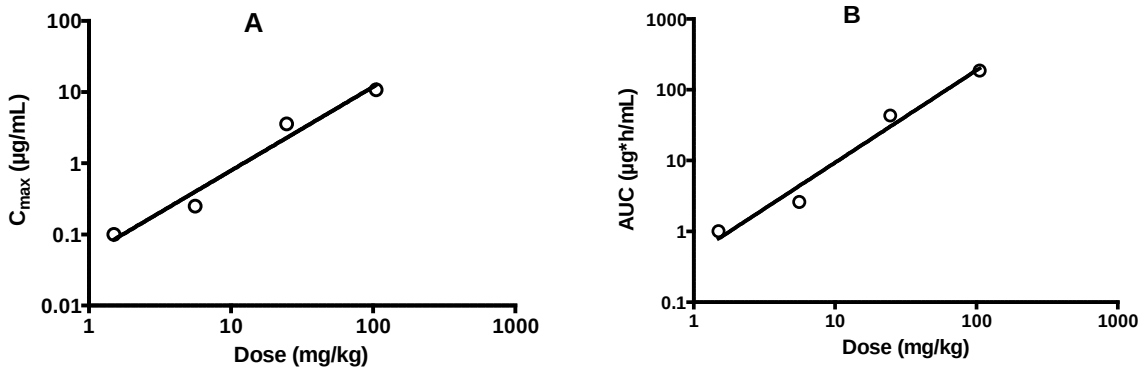
Goodness-of-fit plots for a representative compound [(+)-6] (A) observed data (DV) vs populations predictions (PRED) (B) weighted residuals (WRES) vs PRED (C) WRES vs Dose

Figure S3. Goodness-of-fit plots for pharmacokinetic modeling of KAE609



Goodness-of-fit plots for KAE609 pharmacokinetic modeling (A) observed data (DV) vs populations predictions (PRED) (B) weighted residuals (WRES) vs PRED (C) WRES vs Time

Figure S4. Dose proportionality for KAE609 in CD-1 mouse



Dose linearity tests on C_{max} and AUC_{0-24} were carried out by the regression of log-transformed data (power regression model) (11, 12). Doses (1.5 – 105 mg/kg) and PK parameters (C_{max} and AUC_{0-24}) were log-transformed, and correlation coefficient (R^2), slope, and 95 % confidence intervals of slope were calculated using the GraphPad Prism version 5.02 for windows (GraphPad software, San Diego, California USA). The system was considered to be linear when $R^2 \sim 1$, Slope 95 % $CI_{lower} \geq 0.8$, and $CI_{upper} \leq 1.25$ (11, 12). (A) Dose vs C_{max} ($R^2 = 0.97$, Slope = 1.18, 95 % $CI_{lower} = 0.52$ and $CI_{upper} = 1.83$); (B) Dose vs AUC_{0-24} ($R^2 = 0.97$, Slope = 1.30, 95 % $CI_{lower} = 0.65$ and $CI_{upper} = 1.96$). Based on the above observations, KAE609 shows nonlinear behavior in CD-1 mouse between the doses studied.

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