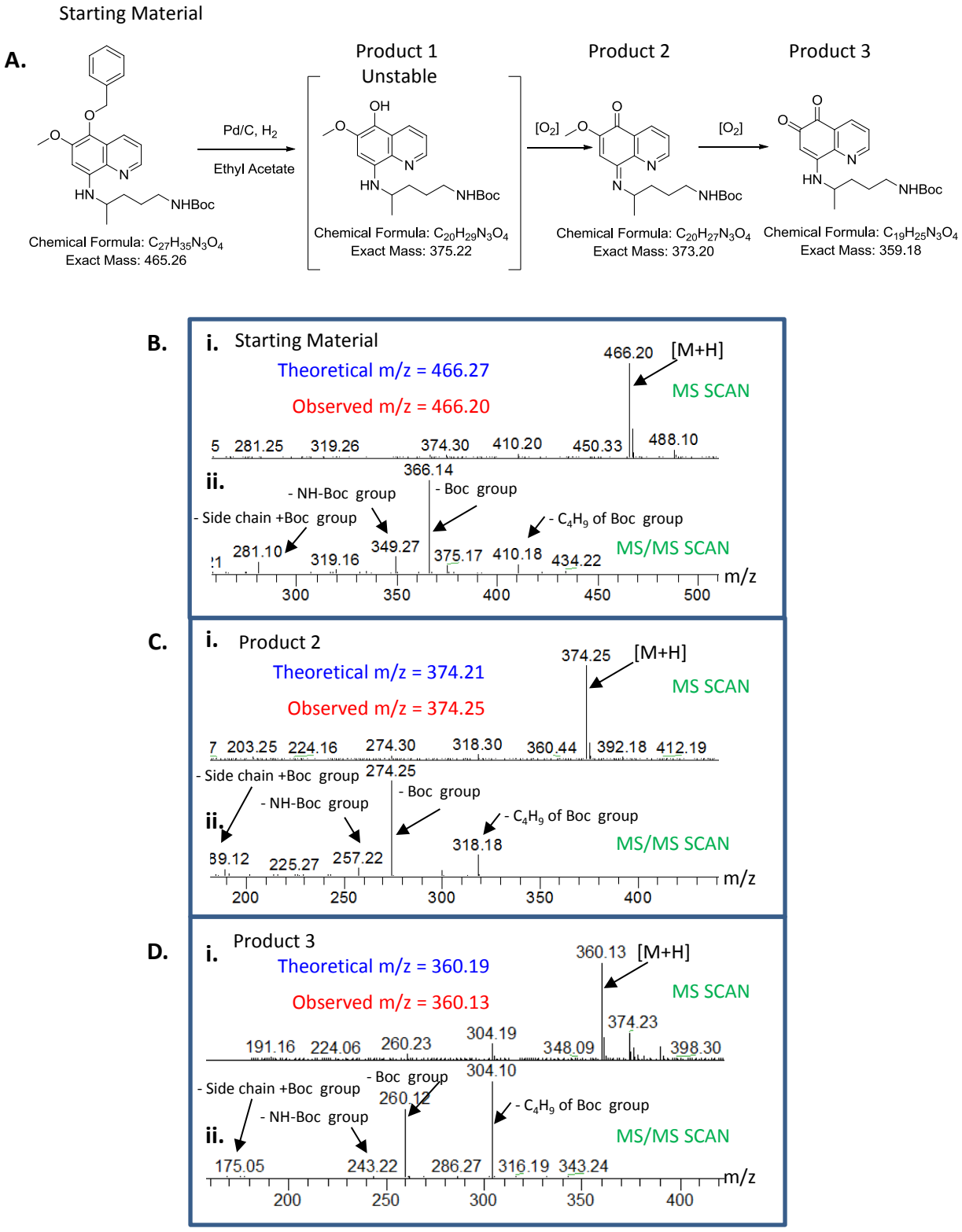
Supplemental Material

Figure legends

Supplemental Figure 1. Synthesis of 5-hydroxy-PQ and subsequent air oxidation to the 5,6-ortho-quinone. **A.** 5-OH-PQ was synthesized via palladium catalyzed hydrogenation of the 5-o-benzyl-PQ starting material in ethyl acetate for 120 minutes at 25 °C. The reaction mixture was characterized using NMR (300 MHz, $CDCl_3$) 8.76 (dd, *J* =4.8, 1.5Hz, 1H), 8.41 (dd, *J* =4.8, 1.8Hz, 1H), 7.59 (dd, *J* =4.8, 1.8Hz, 1H), 7.53 (br s, 1H), 5.95 (s, 1H), 4.58 (br s, 1H), 3.75-3.81 (m, 1H), 3.17 (dd, *J* =12.9, 6.6Hz, 2H), 1.60-1.77 (m, 4H), 1.45 (s, 9H), 1.38 (d, *J* =6.6Hz, 3H) and LC-MS analyses. Masses corresponding for the starting material (**B**), 6-methoxy-quinone imine (product 2, **C**), and 5,6-ortho-quinone (product 3, **D**) were observed. Intact mass spectra are indicated in i, and MS/MS fragmentation data in ii. The corresponding mass fragments in ii are labelled and mass differences are indicated from the parent ions. Purification of the shown reaction mixture using chromatography on silica gel yielded the 5,6-orthoquinone (product 3).

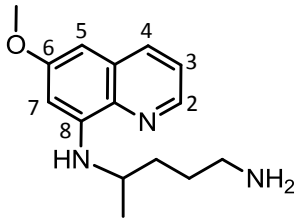
Supplemental Figure 2. Reference compounds utilized in Primaquine pharmacokinetic study. (**A**) The structures of Primaquine (PQ), carboxy-Primaquine (CPQ), 2, 3, and 4 hydroxylated Primaquine (OH-PQ) are shown. The marker for 5-hydroxylation (5,6-ortho-quinone) is also shown. The quinolone rings of Primaquine and the hydroxylated metabolites are numbered for reference. (**B**) Liquid chromatography and mass spectrometry parameters utilized for quantitation of PQ and the various metabolites. Shown for each molecule are the observed m/z ions, product ms/ms ions, cone voltages, collision energies, and electrospray polarities

utilized for quantitation. Additionally, the retention times for each molecule are also indicated. Separate standard curves and analyses of PK samples were conducted for each analyte.

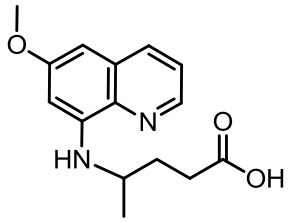


Supplemental Figure 1

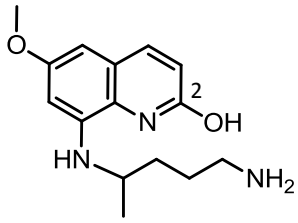
PQ

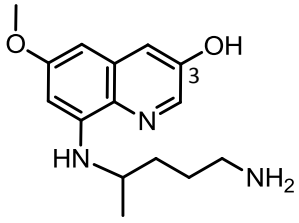


CPQ

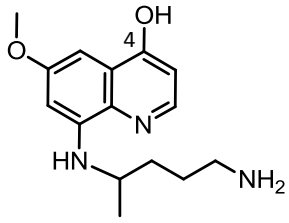


2-OH-PQ

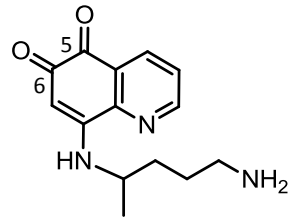




3-OH-PQ



4-OH-PQ



5,6-ortho-quinone

	Observed Parent m/z	Product m/z	Cone (v)	Collision (v)	ESI Mode	Retention Time	Plasma LLOQ (ng/mL)	Liver LLOQ (ng/mL)
PQ	260.26	175.02	22	22	Pos	2.10	2.5	2.5
CPQ	275.16	175.02	18	24	Pos	2.57	2.5	2.5
2-OH-PQ	276.13	69.08	8	24	Pos	2.00	10	50
3-OH-PQ	276.19	86.09	24	16	Pos	2.04	2.5	25
4-OH-PQ	276.19	191.17	38	16	Pos	1.87	25	25
5,6-o-quinone-PQ	260.20	147.08	26	30	Pos	1.80	50	50

Supplemental Figure 2