Supporting Information for

Trioxolane-Mediated Delivery of Mefloquine Limits Brain Exposure in a Mouse Model of Malaria

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General Procedures: Reactions were magnetically stirred unless otherwise indicated. Air and/or moisture sensitive reactions were carried out under an argon atmosphere in oven-dried glassware using anhydrous solvents from commercial suppliers. Air and/or moisture sensitive reagents were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa. Reaction product solutions and chromatography fractions were concentrated by rotary evaporation at room temperature at 20 Torr then at 0.5 Torr unless otherwise indicated. Thin phase chromatography was performed on EMD precoated glass-backed silica gel 60 F-254 0.25 mm plate.

Materials: All chemical reagents and solvents used were purchased from Sigma-Aldrich or Fisher Scientific. Anhydrous dichloromethane and tetrahydrofuran (EMD Drisolv) were used without further purification. Alcohol **5**, the corresponding 4-nitrophenyl carbonate, and compound **6** were prepared as described previously (see below).

Preparation of 5, its 4-nitrophenyl carbonate, and compound 6: Fontaine, S. D.; Antonio G. DiPasquale; Renslo, A. R. Efficient and Stereocontrolled Synthesis of 1,2,4-Trioxolanes Useful for Ferrous Iron-Dependent Drug Delivery. *Org. Lett.*, **2014**, *16*, 5776-5779.

Instrumentation: ^{1}H NMR spectra were recorded on a Varian INOVA-400 400 MHz spectrometer. Chemical shifts are reported in δ units (ppm). NMR spectra were referenced relative to residual NMR solvent peaks. Coupling constants (J) are reported in hertz (Hz). Unless otherwise noted. Column chromatography was performed on Silicycle Sili-prep cartridges using a Biotage Isolera Four automated flash chromatography system. LC/MS and compound purity were determined using Waters Micromass ZQTM, equipped with Waters 2795 Separation Module and Waters 2996 Photodiode Array Detector. Separations were carried out with an XTerra® MS C18, 5µm, 4.6 x 50 mm column, at ambient temperature (unregulated) using a mobile phase of water-acetonitrile containing a constant 0.20 % formic acid. High resolution MS data was performed at the UCSF Mass Spectrometry Facility.

Synthetic Procedures

Preparation of dispiro[adamantane-2,2'-[1,3,5]trioxolane-4',1''-cyclohexane]-3''-yl 4-nitrophenyl carbonate. A 50-mL heat-gun dried, two-necked, round-bottomed flask equipped with a stirbar, argon inlet adapter and rubber septum was charged with alcohol **5** (0.500 g, 1.78 mmol, 1.0 equiv), dichloromethane (7 mL), *N,N*-diisopropylethylamine (0.932 mL, 5.35 mmol, 3.0 equiv), and 4-dimethylaminopyridine (0.218 g, 1.78 mmol, 1 equiv). The mixture was cooled to 0 °C while 4-nitrophenyl chloroformate (0.719 g, 3.57 mmol, 2 equiv) was added as a solid in two portions (some gas evolution observed). The reaction mixture was stirred at 0 °C for 15 min, allowed to warm to rt over 10 min, and stirred at rt for 10 min. The reaction mixture was

diluted with 30 mL of Et_2O, washed with 10 mL of 5% aq $KHSO_4$ solution, washed with five 10-mL portions of satd aq $NaHCO_3$ solution, washed with 25 mL of satd aq NaCl solution, dried over $MgSO_4$, filtered, and concentrated to afford a yellow/orange semi-solid. This material was dissolved in 25 mL of CH_2Cl_2 and deposited onto 5 g of silica gel. The free flowing powder was loaded atop a 80 g silica gel cartridge. Gradient elution (0-20% EtOAc/hexanes) afforded 0.747 g (94%) of the desired 4-nitrophenyl carbonate intermediate as pale yellow glassy oil: IR (neat) 2915, 2859, 1765, 1615, 1594, 1526, 1493, 1452, 1348, 1259, 1215, 1168, 1114, 1140, 1087, 1067, 1045, 1020, 980, 858, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 - 8.31 (m, 2 H), 7.36 - 7.41 (m, 2 H), 4.81 - 4.90 (m, 1 H), 4.92 - 5.00 (m, 1 H, minor *cis* diastereomer), 2.40 (ddt, *J*=12.9, 4.3, 1.9 Hz, 1 H), 2.33 - 2.36 (m, 1 H, minor *cis* diastereomer), 2.07 - 2.15 (m, 1 H), 1.64 - 2.03 (m, 18 H), 1.55 ppm (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 151.8, 145.5, 125.5, 121.9, 112.2, 108.5, 76.4, 39.8, 36.9, 36.6, 36.5, 35.1, 35.0, 34.9, 33.7, 30.3, 27.0, 26.6, 19.8; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{27}NO_8$: 445.1809; found 446.1789.

Preparation of dispiro[adamantane-2,2'-[1,3,5]trioxolane-4',1''-cyclohexane]-3''-yl 2-{[2,8-bis(trifluoromethyl)quinolin-4-yl](hydroxy)methyl}piperidine-1-carboxylate (3). A 20-mL vial equipped with a stirbar and screw cap was charged with the nitrophenyl carbonate intermediate (0.045 g, 0.101 mmol, 1 equiv), mefloquine hydrochloride (0.045 mg, 0.108 mmol, 1.07 equiv), *N*,*N*-dimethylformamide (1 mL), and *N*,*N*-diisopropylethylamine (0.088 mL, 0.505 mmol, 5 equiv). The reaction mixture was stirred at rt for 5 min and then DMAP (0.012 g, 0.101 mmol, 1 equiv) was added and the reaction mixture was stirred at rt for 20 h. The reaction mixture was diluted with 20 mL of EtOAc and 20 mL of 1 M aq NaOH. The aqueous layer was separated and extracted with three 20-mL portions of EtOAc. The combined organic phases were washed with 30 mL of satd aq NaCl solution, dried over $MgSO_4$, filtered, and concentrated to afford a pale yellow oil. A solution of this material in 10 mL of CH_2Cl_2 was deposited onto 5 g of silica gel. The resulting free flowing powder was transferred to the top of a 12 g column of silica gel. Gradient elution (0-35% EtOAc/hexanes) afforded 0.046 g (67%) of the desired mefloquine conjugate **3** (four inseparable diastereomers) as a colorless, glassy oil: IR (neat) 2918, 2859, 1670, 1602, 1584, 1453, 1432, 1353, 1310, 1278, 1222, 1182, 1146, 1112, 1087, 1072, 1039, 1021, 929, 835, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (t, *J* = 9.7 Hz, 1 H), 8.11 (dd, *J* = 4.0, 7.1 Hz, 1 H), 8.07 - 8.04 (m, 1 H), 7.71 - 7.64 (m, 1 H), 5.80 (br. s., 1 H), 4.31

- 4.18 (m, 1 H), 4.01 - 3.86 (m, 1 H), 3.49 - 3.27 (m, 2 H), 2.27 - 2.11 (m, 1 H), 2.03 - 1.57 (m, 23 H), 1.56 - 1.39 (m, 3 H), 1.31 - 1.18 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 151.4, 151.2, 148.3 (q, J = 34.9 Hz), 148.2 (q, J = 34.9 Hz),143.8, 129.5, 129.2, 128.99, 128.94, 128.89, 128.4, 128.3, 127.8, 127.3, 126.9, 125.1, 123.7 (q, J = 278 Hz), 121.5 (q, J = 274.1 Hz), 117.4, 115.6, 112.1, 111.9, 108.8, 108.7, 72.2, 71.9, 71.9, 56.8, 42.1, 40.2, 40.0, 36.9, 36.54, 36.47, 35.8, 35.05, 34.97, 34.94, 34.93, 34.91, 34.89, 34.8, 34.2, 34.0, 31.0, 30.7, 27.1, 26.6, 24.2, 22.4, 19.9, 19.8, 19.73, 19.67; ^{19}F NMR (375 MHz, $CDCl_3$) δ -68.3, -60.7; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{34}H_{38}F_6N_2O_5$: 685.2707; found: 685.2693.

Experimental Procedures – In vitro antiplasmodial and blood stability studies

Plasmodium falciparum EC_{50} determinations

The growth inhibition assay for *P. falciparum* was conducted as described previously (Sijwali et al. PNAS, **2004**, *101*, 8721) with minor modifications. Briefly, *Plasmodium falciparum* strain W2 synchronized ring-stage parasites were cultured in human red blood cells in 96-well flat bottom culture plates at 37 ºC, adjusted to 1% parasitemia and 2% hematocrit under an atmosphere of 3% O_2, 5% CO_2, 91% N_2 in a final volume of 0.1 mL per well in RPMI-1640 media supplemented with 0.5% Albumax, 2 mM L-glutamine and 100 mM hypoxanthine in the presence of various concentrations of inhibitors. Tested compounds were serially diluted 1:3 in the range 10,000 – 4.6 nM (or 1,000-0.006 nM for more potent analogs), with a maximum DMSO concentration of 0.1%. Following 48 hours of incubation, the cells were fixed by adding 0.1 ml of 2% formaldehyde in phosphate buffered saline, pH = 7.4 (PBS). Parasite growth was evaluated by flow cytometry on a FACsort (Becton Dickinson) equipped with AMS-1 loader (Cytek Development) after staining with 1 nM of the DNA dye YOYO-1 (Molecular Probes) in 100 mM NH_4Cl, 0.1% Triton x-100 in 0.8% NaCl. Parasitemias were determined from dot plots (forward scatter vs. fluorescence) using CELLQUEST software (Becton Dickinson). EC_{50} values for growth inhibition were determined from plots of percentage control parasitemia over inhibitor concentration using GraphPad Prism software.

Plasma and Blood Stability Studies

Human whole blood was procured from the Australian Red Cross Blood Service, stored at 5 - 7°C, and used within one week of the collection date. An aliquot of whole blood was centrifuged (Heraeus, Multifuge 3 S-R; 4500 x g) for 10 min to obtain plasma. For each test compound, a 1 mg/mL stock solution was prepared in dimethyl sulfoxide (DMSO); this was subsequently diluted in a DMSO/acetonitrile/water mixture to prepare a spiking solution at a concentration of 10 μg/mL.

Aliquots (990 μL) of whole blood and plasma pre-equilibrated at 37°C were spiked with the spiking solution of each compound (10 μL) to a nominal plasma concentration of 100 ng/mL. The final DMSO and acetonitrile concentrations were 0.02 and 0.49% (v/v), respectively.

Aliquots of plasma (50 μL) and whole blood (120 μL) containing the test compounds were transferred into fresh microcentrifuge tubes and maintained at 37°C. At various time points over the 240 min incubation period, duplicate plasma samples were taken and immediately frozen in dry ice. At the same times, duplicate samples of whole blood were centrifuged (eppendorf, Mini Spin plus; 9500 x g) for 2 min and an aliquot (50 μL) of the plasma fraction from each sample was transferred into fresh microcentrifuge tubes and immediately frozen in dry ice. All plasma samples were stored frozen (-20°C) until analysis.

Concentrations of each test compound were determined by LC/MS on a Waters/Micromass Premier triple-quadrupole instrument. At each sample time, the percentage of test compound remaining in plasma and the plasma fraction of whole blood was calculated relative to the concentrations in the corresponding samples quenched at 2 min. The whole blood to plasma partitioning ratio (B/P ratio) was also calculated as the ratio of test compound concentration in plasma (as a surrogate for the whole blood concentration) to that in the plasma fraction of whole blood at 2 min. The complete data set for **1** and **3** at all time points is provided in the table below.

Sampling Time	% Remaining [a]		Apparent Blood/Plasma Ratio
	Plasma	Whole Blood	
Mefloquine (**1**)			
2 min	100	100	1.6
60 min	105	91	---
120 min	103	93	---
240 min	97	95	---
Compound **3**			
2 min	100	100	0.7
60 min	96	103	--
120 min	111	87	--
240 min	94	85	--

[a] Test compound remaining at the end of the specified incubation period expressed as a percentage of the samples quenched at 2 min.

Experimental Procedures – In vivo studies

***Plasmodium berghei* Mouse Malaria Model**

Female Swiss Webster Mice (average of 20 g body weight) were infected intraperitoneally with 10^6 *Plasmodium berghei*-infected erythrocytes collected from a previously infected mouse. Beginning 1 hour after inoculation the mice were treated once a day for four days by oral gavage with 100 uL of solution of test compound (see table below for quantities per dose) dissolved in 10% DMSO, 50% PEG 400, 8% 2-HP beta-cyclodextrin in water. There were five mice in each test arm.

Each test compound was administered at an equimolar daily dose as shown below. Negative controls were treated with vehicle only.

test compound	daily dose
mefloquine HCl	10 mg/kg
compound **6**	9.5 mg/kg
compound **3**	16.5 mg/kg
arterolane tosylate	13.6 mg/kg

Infections were monitored by daily microscopic evaluation of Giemsa-stained blood smears starting on day seven. Parasitemia were determined by counting the number of infected and uninfected erythrocytes. Body weight was measured over the course of the treatment. Mice were euthanized when parasitemia exceeded 50% or when weight loss of more than 15% occurred. Animal survival and morbidity were closely monitored for up to 28 days post-infection when the experiment was terminated.

***Plasmodium chabaudi* Mouse Malaria Model.**

Ten week old female Balb/c mice were inoculated with 10^6 *Plasmodium chabaudi* AJ parasites from an infected host. Four days after inoculation, when parasitemia reached 3-4% for all mice, test article was administered via intra-peritoneal infection in a vehicle prepared as follows. Test compounds at the appropriate dose were dissolved in a solution comprising 45% (vol/vol) polyethylene glycol (MW 400), 35% (vol/vol) propylene glycol, 10% (vol/vol) ethanol and 10% (wt/vol) 2-hydroxypropyl β-cyclodextrin. For dosing, an aliquot (360 μL) of the solution was

further diluted with 40 μL DMSO and 100 μL of the resulting solution was injected i.p. as a single bolus to each mouse. Three or four mice (*n*) were employed in each study arm as shown below.

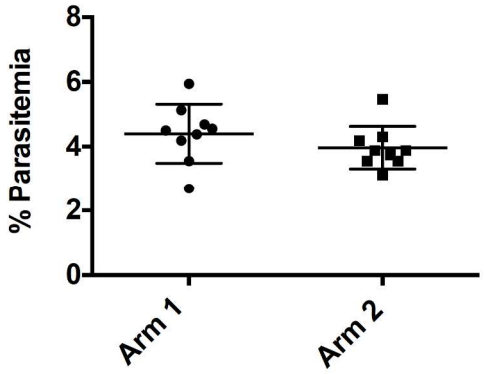
Arm 1: Vehicle only (*n* = 4)
Arm 2: Mefloquine HCl (**1**) 10 mg/kg (*n* = 3)
Arm 3: Compound **3** 8 mg/kg (*n* = 3)
Arm 4: Compound **3** 16.5 mg/kg (*n* = 3)
Arm 5: Compound **3** 50 mg/kg (*n* = 3)

Mice were evaluated for parasitemia daily by blood smears and FACS analysis. Mice were monitored daily for changes in appearance, activity, and weight. Mice in Arms **1** and **3** were euthanized on day 8 post-inoculation due to poor health and weight loss. All mice in Arms **2**, **4**, and **5** appeared healthy, maintained stable weight, and had undetectable parasitemia at the termination of the study on day 13 post inoculation.

Biodistribution Study of 1 and 3 in *P. chaubaudi* Infected Mice. The objective of this study was to evaluate the plasma and brain exposure of **1** and **3** following i.p. administration of either **1** (10 mg/kg) or **3** (50 mg/kg).

Arm 1: mefloquine **1** (10 mg/kg); LC/MS/MS analysis for **1** in brain and plasma
Arm 2: compound **3** (50 mg/kg); LC/MS/MS analysis for **1** and **3** in brain and plasma

Each study arm employed nine Balb/c mice. Mice were infected with *P. chaubaudi* parasites and test compounds were formulated and administered i.p. on day 4 post-inoculation, exactly as described above for the mouse efficacy study. Parasitemia prior to administration of test compounds was similar in the two arms, as shown below.



Plasma and brain samples were collected according the schedule below for both study arms. Blood samples (200 µL) were collected via retro-orbital bleed into tubes containing PBS with 30 units of heparin (approximately equal volume as blood) as anticoagulant. Plasma was isolated from blood samples via centrifugation at 2000 rpm for 10 minutes and transferred to a new tube where it was frozen and stored at -80 ^{o}C prior to analysis. For terminal brain samples, mice were anesthetized with 300 µL avertin, blood samples collected via retro-orbital bleeding, and tissues perfused via the left ventricle. The skull was cut and optical nerve severed. The brain was then placed in a 12-well plate containing 1 mL PBS and placed on ice. Brain samples were then dried carefully on paper and weighed, then frozen and stored at -80 ^{o}C prior to analysis.

	Time point (hr)								
Mouse	0	0.08	0.25	0.5	1	2	4	8	24
1		X		X					
2		X		X					
3		X		X					
4			X		X	X			
5			X		X	X			
6			X		X	X			
7							X	X	X
8							X	X	X
9							X	X	X

X plasma sample

X terminal plasma and brain sample

Plasma and brain samples from both study arms were analyzed for the presence of 1 (Arm 1) or 1 and 3 (Arm 2) at Integrated Analytical Solutions (Berkeley, CA) as described below.

Preparation of Calibration Standards Naïve EDTA mouse plasma and brain homogenate, prepared as a 1:1 (wt/vol) mixture of brain and water, were purchased from Bioreclamation, Inc. (Westbury, New York). Reference stock solutions (RSS) of **1** and **3** were each prepared at 1 mg/mL (freebase equivalent) in DMSO. Eight calibration standards containing both **1** and **3** were prepared in naïve plasma for quantitation. The top calibration standard (Standard 8) was prepared by diluting the 1-mg/mL RSS of **1** and **3** by a factor of 1 in 20 into naïve plasma (10 µL + 10 µL + 180 µL) to 50,000 ng/mL. The remaining standards were prepared by serial dilution of Standard 8. Three quality control (QC) samples, High, Mid and Low, were prepared by diluting the Standard 8.

Eight fortification standards containing both **1** and **3** were prepared in 50% MeOH for subsequent dilution into naïve brain homogenate. To prepare the top fortification standard (Fort-Standard 8), the 1-mg/mL RSS of **1** and **3** were diluted to 500,000 ng/mL. The remaining standards were prepared by serial dilution of Fort-Standard 8 in 50% MeOH. To prepare final calibration standards and QC samples in brain homogenate, a 4 µL aliquot of each fortification standard and fortification QC was added to 36 µL of naïve brain homogenate. This dilution yielded eight calibration standards that ranged from 15 to 50,000 ng/mL and three QC samples at 100, 1,000 and 10,000 ng/mL in brain homogenate.

Preparation and processing of study samples. A 350 µL aliquot of water was added to each brain sample and subsequently homogenized by bead□milling using a Bullet Blender (Next Advance, Inc., New York, NY). Calibration standards, QC samples and study samples were processed for LC/MS/MS analysis by precipitating 60 µL of each sample with three volumes of ice cold Internal Standard Solution (acetonitrile containing 50 ng/mL of dextromethorphan, 5 ng/mL diphenhydramine and 125 ng/mL diclofenac). The precipitated samples were centrifuged at 6100g for 30 minutes (or equivalent). Following centrifugation, an aliquot of each supernatant was transferred to an autosampler plate and diluted with five volumes of 0.2% formic acid in water.

LC/MS/MS analysis.

Processed samples were analyzed using the following LC/MS/MS conditions:

HPLC:	Shimadzu VP Series 10 System
Autosampler:	Shimadzu SIL-HTc at ambient temperature
Mobile Phase:	A-0.2% formic acid in water
	B-0.2% formic acid in methanol
Column:	2.1 x 10 mm Peeke Scientific Duragel G C18 guard cartridge
Injection Volume:	2 µL
Gradient:	5% B for 0.25 minutes, then 5-95% B in 1.0 minutes
Flow Rate:	0.8 mL/min
Mass Spectrometer:	Applied Biosystems/MDS SCIEX API 4000
Interface:	TurboIonSpray (ESI) at 400°C
Software:	Analyst v1.5
Polarity:	Positive Ion
Q1/Q3 Ions:	379.1/361.3 for mefloquine (**1**)
	702.4/390.1 for compound **3**
	256.2/167.2 for Diphenhydramine (I.S.)
	272.1/215.2 for Dextromethorphan (I.S.)

Arm 1 *- Plasma Sample Results*

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)
Mouse #1 Plasma 0.08 hr 10 mg/kg IP	137.
Mouse #2 Plasma 0.08 hr 10 mg/kg IP	188.
Mouse #3 Plasma 0.08 hr 10 mg/kg IP	172.
Mouse #4 Plasma 0.2 hr 10 mg/kg IP	204.
Mouse #5 Plasma 0.2 hr 10 mg/kg IP	193.
Mouse #6 Plasma 0.2 hr 10 mg/kg IP	160.
Mouse #1 Plasma 0.5 hr 10 mg/kg IP	152.

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)
Mouse #2 Plasma 0.5 hr 10 mg/kg IP	174.
Mouse #3 Plasma 0.5 hr 10 mg/kg IP	222
Mouse #4 Plasma 1 hr 10 mg/kg IP	164
Mouse #5 Plasma 1 hr 10 mg/kg IP	165
Mouse #6 Plasma 1 hr 10 mg/kg IP	205
Mouse #4 Plasma 2 hr 10 mg/kg IP	155
Mouse #5 Plasma 2 hr 10 mg/kg IP	203.
Mouse #6 Plasma 2 hr 10 mg/kg IP	124.
Mouse #7 Plasma 4 hr 10 mg/kg IP	170.
Mouse #8 Plasma 4 hr 10 mg/kg IP	140.
Mouse #9 Plasma 4 hr 10 mg/kg IP	208.
Mouse #7 Plasma 8 hr 10 mg/kg IP	179.
Mouse #8 Plasma 8 hr 10 mg/kg IP	191.
Mouse #9 Plasma 8 hr 10 mg/kg IP	167.
Mouse #7 Plasma 24 hr 10 mg/kg IP	133.
Mouse #8 Plasma 24 hr 10 mg/kg IP	177.
Mouse #9 Plasma 24 hr 10 mg/kg IP	261

Arm 1 *- Brain Sample Results*

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)	Mefloquine (**1**) Tissue Concentration (ng/g)†
Mouse #1 Brain 0.5 hr 10 mg/kg IP	168.	341
Mouse #2 Brain 0.5 hr 10 mg/kg IP	256.	498
Mouse #3 Brain 0.5 hr 10 mg/kg IP	276.	491
Mouse #4 Brain 2 hr 10 mg/kg IP	345.	792
Mouse #5 Brain 2 hr 10 mg/kg IP	315.	630
Mouse #6 Brain 2 hr 10 mg/kg IP	352.	749
Mouse #7 Brain 24 hr 10 mg/kg IP	1220.	2440

Mouse #8 Brain 24 hr 10 mg/kg IP	1740.	3263
Mouse #9 Brain 24 hr 10 mg/kg IP	1440.	2669

† Sample values were corrected for the addition of water (350µL) to facilitate homogenization.

Arm 2 *- Plasma Sample Results*

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)	959975 (**3**) Calculated Concentration (ng/mL)
Mouse #1 Plasma 0.08 hr 50 mg/kg IP	<15	852
Mouse #2 Plasma 0.08 hr 50 mg/kg IP	<15	817
Mouse #3 Plasma 0.08 hr 50 mg/kg IP	<15	813
Mouse #4 Plasma 0.2 hr 50 mg/kg IP	14.9	1150
Mouse #5 Plasma 0.2 hr 50 mg/kg IP	18.6	1070
Mouse #6 Plasma 0.2 hr 50 mg/kg IP	<15	1120
Mouse #1 Plasma 0.5 hr 50 mg/kg IP	16.1	1540
Mouse #2 Plasma 0.5 hr 50 mg/kg IP	<15	1280
Mouse #3 Plasma 0.5 hr 50 mg/kg IP	<15	1480
Mouse #4 Plasma 1 hr 50 mg/kg IP	17.6	1330
Mouse #5 Plasma 1 hr 50 mg/kg IP	16.5	817
Mouse #6 Plasma 1 hr 50 mg/kg IP	15.8	1260
Mouse #4 Plasma 2 hr 50 mg/kg IP	18.8	2130
Mouse #5 Plasma 2 hr 50 mg/kg IP	33.5	1520
Mouse #6 Plasma 2 hr 50 mg/kg IP	13.3	1060
Mouse #7 Plasma 4 hr 50 mg/kg IP	22.8	711
Mouse #8 Plasma 4 hr 50 mg/kg IP	30.7	925
Mouse #9 Plasma 4 hr 50 mg/kg IP	39.3	1140
Mouse #7 Plasma 8 hr 50 mg/kg IP	36.2	335
Mouse #8 Plasma 8 hr 50 mg/kg IP	41.8	371
Mouse #9 Plasma 8 hr 50 mg/kg IP	73.7	400
Mouse #7 Plasma 24 hr 50 mg/kg IP	112.	138

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)	959975 (**3**) Calculated Concentration (ng/mL)
Mouse #8 Plasma 24 hr 50 mg/kg IP	192.	162
Mouse #9 Plasma 24 hr 50 mg/kg IP	156.	154

Arm 2 *- Brain Sample Results*

Sample Name	Sample Type	Mefloquine Calculated Concentration (ng/mL)	Mefloquine Tissue Concentration (ng/g)†	959975 (**3**) Calculated Concentration (ng/mL)	959975 (**3**) Tissue Concentration (ng/g)†
Mouse #1 Brain 0.5 hr 50 mg/kg IP	Unknown	14.3	30	<15	NA
Mouse #2 Brain 0.5 hr 50 mg/kg IP	Unknown	19.1	35	<15	NA
Mouse #3 Brain 0.5 hr 50 mg/kg IP	Unknown	23.6	44	<15	NA
Mouse #4 Brain 2 hr 50 mg/kg IP	Unknown	44.9	87	<15	NA
Mouse #5 Brain 2 hr 50 mg/kg IP	Unknown	49.8	108	<15	NA
Mouse #6 Brain 2 hr 50 mg/kg IP	Unknown	43.7	82	<15	NA
Mouse #7 Brain 24 hr 50 mg/kg IP	Unknown	566	1132	<15	NA
Mouse #8 Brain 24 hr 50 mg/kg IP	Unknown	570	1045	<15	NA
Mouse #9 Brain 24 hr 50 mg/kg IP	Unknown	581	1116	<15	NA

† Sample values were corrected for the addition of water (350µL) to facilitate homogenization.

Biodistribution study of Compound 3 in uninfected animals. The purpose of this study was to evaluate the concentrations of **1** and **3** in plasma and brain following i.p. administration of compound **3** (50 mg/kg) to healthy, uninfected Balb/c mice. The study design, number of mice, compound formulation and administration, and sample collection and analysis were performed exactly as described above for the earlier studies with infected mice. Results of plasma and brain analysis are provided below.

Plasma Sample Results

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)	Compound **3** Calculated Concentration (ng/mL)
Mouse # 1 Plasma 0.08hr 50 mg/kg IP	4.08	1670
Mouse # 2 Plasma 0.08hr 50 mg/kg IP	7.91	2820
Mouse # 3 Plasma 0.08hr 50 mg/kg IP	6.38	2370
Mouse # 4 Plasma 0.25hr 50 mg/kg IP	13	3770
Mouse # 5 Plasma 0.25hr 50 mg/kg IP	8.09	3820
Mouse # 6 Plasma 0.25hr 50 mg/kg IP	7.91	3140
Mouse # 1 Plasma 0.5hr 50 mg/kg IP	4.45	2110
Mouse # 2 Plasma 0.5hr 50 mg/kg IP	6.8	3170
Mouse # 3 Plasma 0.5hr 50 mg/kg IP	6.15	1750
Mouse # 4 Plasma 1hr 50 mg/kg IP	17.4	2840
Mouse # 5 Plasma 1hr 50 mg/kg IP	17.5	4030
Mouse # 6 Plasma 1hr 50 mg/kg IP	18.2	3990
Mouse # 4 Plasma 2hr 50 mg/kg IP	20.8	2780
Mouse # 5 Plasma 2hr 50 mg/kg IP	14.1	2010
Mouse # 6 Plasma 2hr 50 mg/kg IP	18.5	2510
Mouse # 7 Plasma 4hr 50 mg/kg IP	18.1	1510
Mouse # 8 Plasma 4hr 50 mg/kg IP	26.9	2640
Mouse # 9 Plasma 4hr 50 mg/kg IP	30.2	2460
Mouse # 7 Plasma 8hr 50 mg/kg IP	50.3	969
Mouse # 8 Plasma 8hr 50 mg/kg IP	60.9	1100

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)	Compound **3** Calculated Concentration (ng/mL)
Mouse # 9 Plasma 8hr 50 mg/kg IP	64.9	1310
Mouse # 7 Plasma 24hr 50 mg/kg IP	76.2	303
Mouse # 8 Plasma 24hr 50 mg/kg IP	119	452
Mouse # 9 Plasma 24hr 50 mg/kg IP	121	431

Brain Sample Results

Sample Name	Mefloquine (**1**) Calculated Concentration (ng/mL)	Mefloquine (**1**) Tissue Concentration (ng/g)†	Compound **3** Calculated Concentration (ng/mL)	Compound **3** Tissue Concentration (ng/g)†
Mouse # 1 Brain 0.5hr 50 mg/kg IP	9.64	19.28	22.2	44.4
Mouse # 2 Brain 0.5hr 50 mg/kg IP	13.6	27.2	16.9	33.8
Mouse # 3 Brain 0.5hr 50 mg/kg IP	14.1	28.2	20.6	41.2
Mouse # 4 Brain 2hr 50 mg/kg IP	42.7	85.4	10.1	20.2
Mouse # 5 Brain 2hr 50 mg/kg IP	45.8	91.6	3.22	6.44
Mouse # 6 Brain 2hr 50 mg/kg IP	49.6	99.2	4.15	8.3
Mouse # 7 Brain 24hr 50 mg/kg IP	522	1044	< 3	
Mouse # 8 Brain 24hr 50 mg/kg IP	610	1220	< 3	
Mouse # 9 Brain 24hr 50 mg/kg IP	706	1412	< 3	

† Sample values were corrected for the addition of water (1:1) to facilitate homogenization.

Supplementary Figures

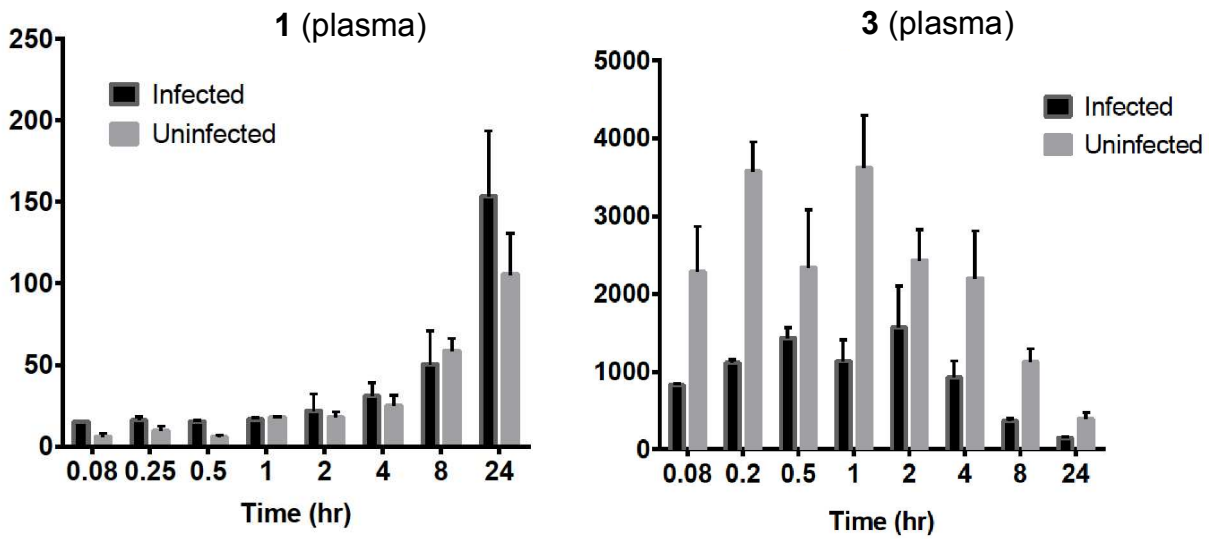
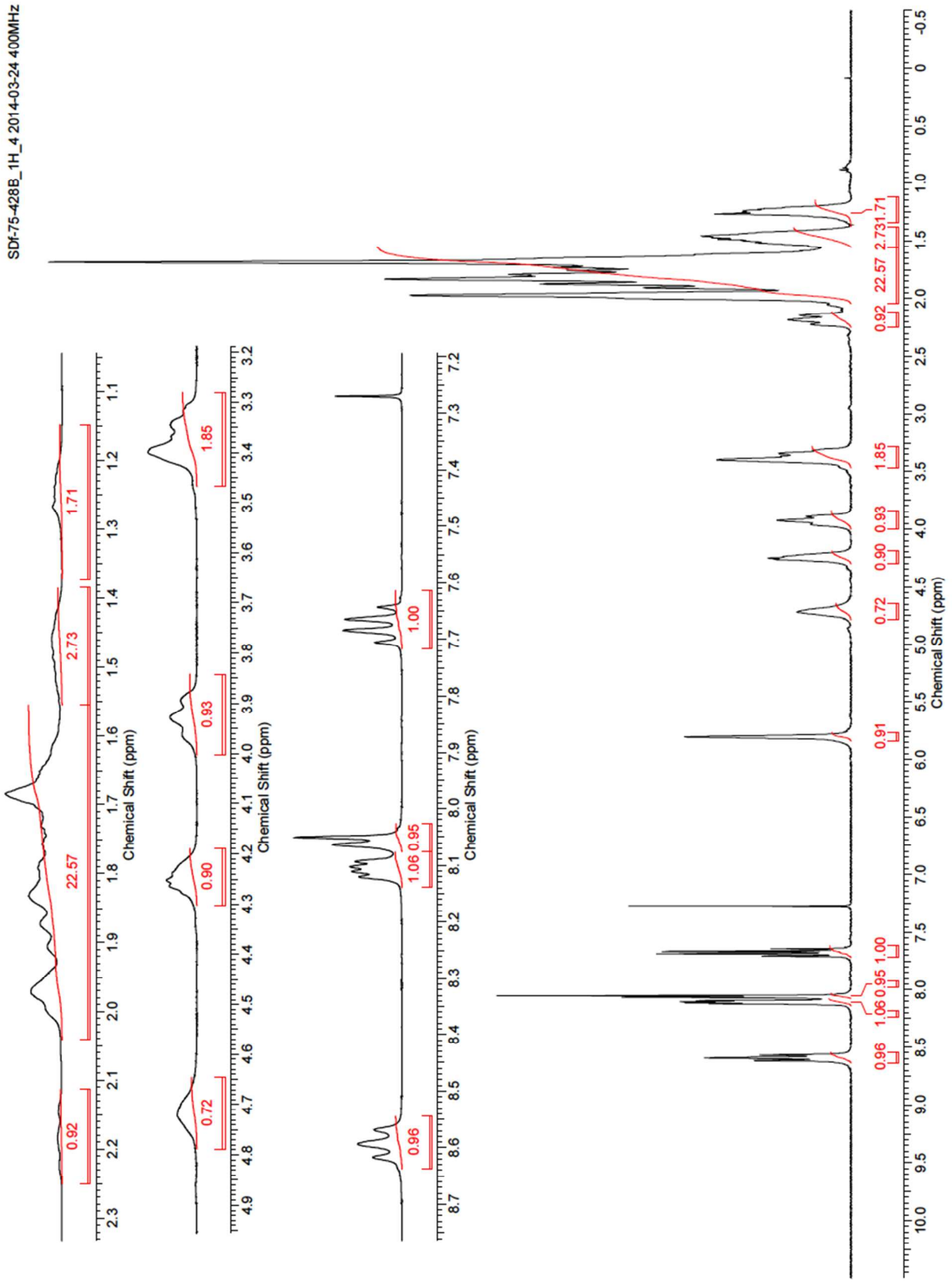
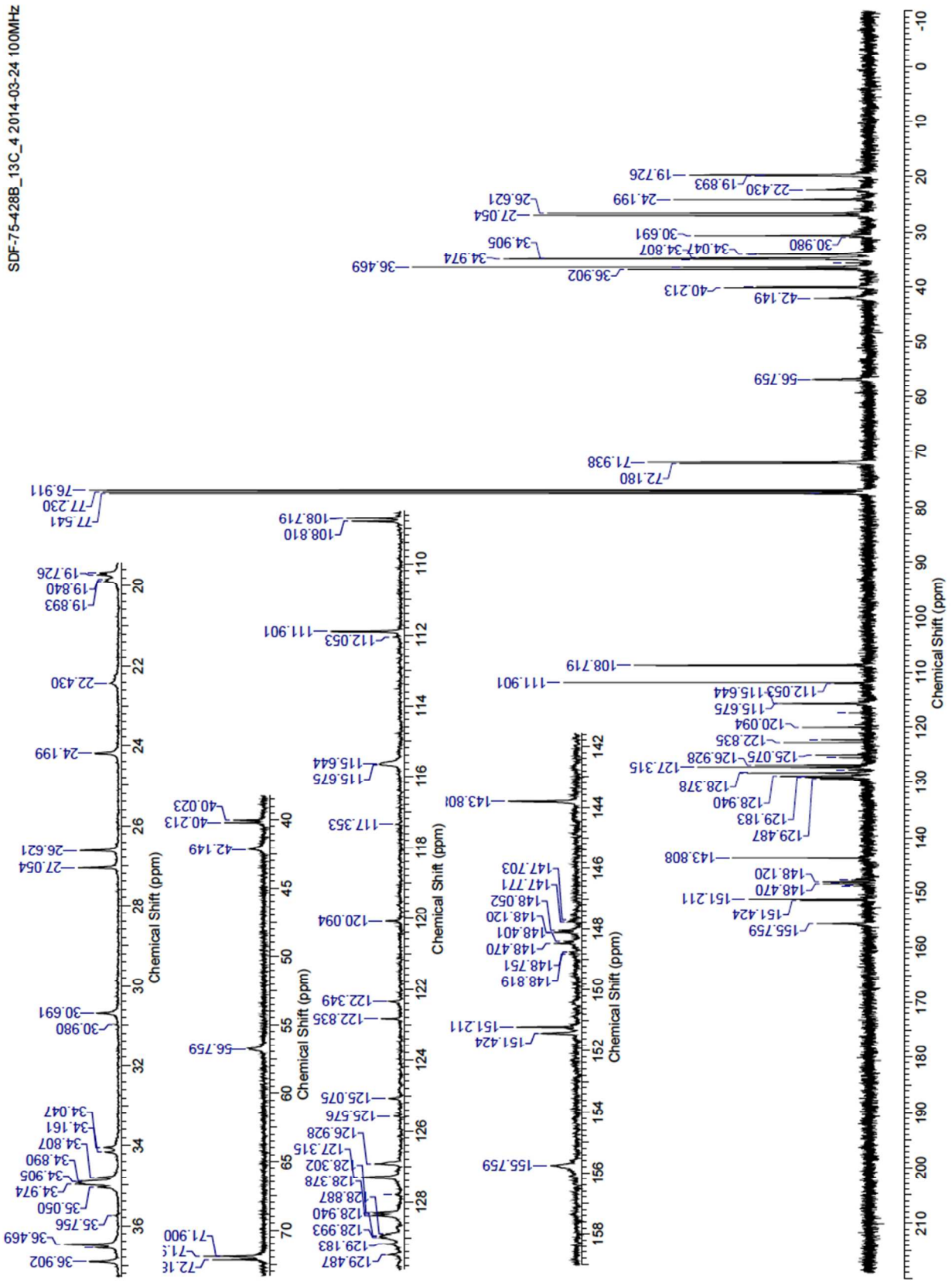
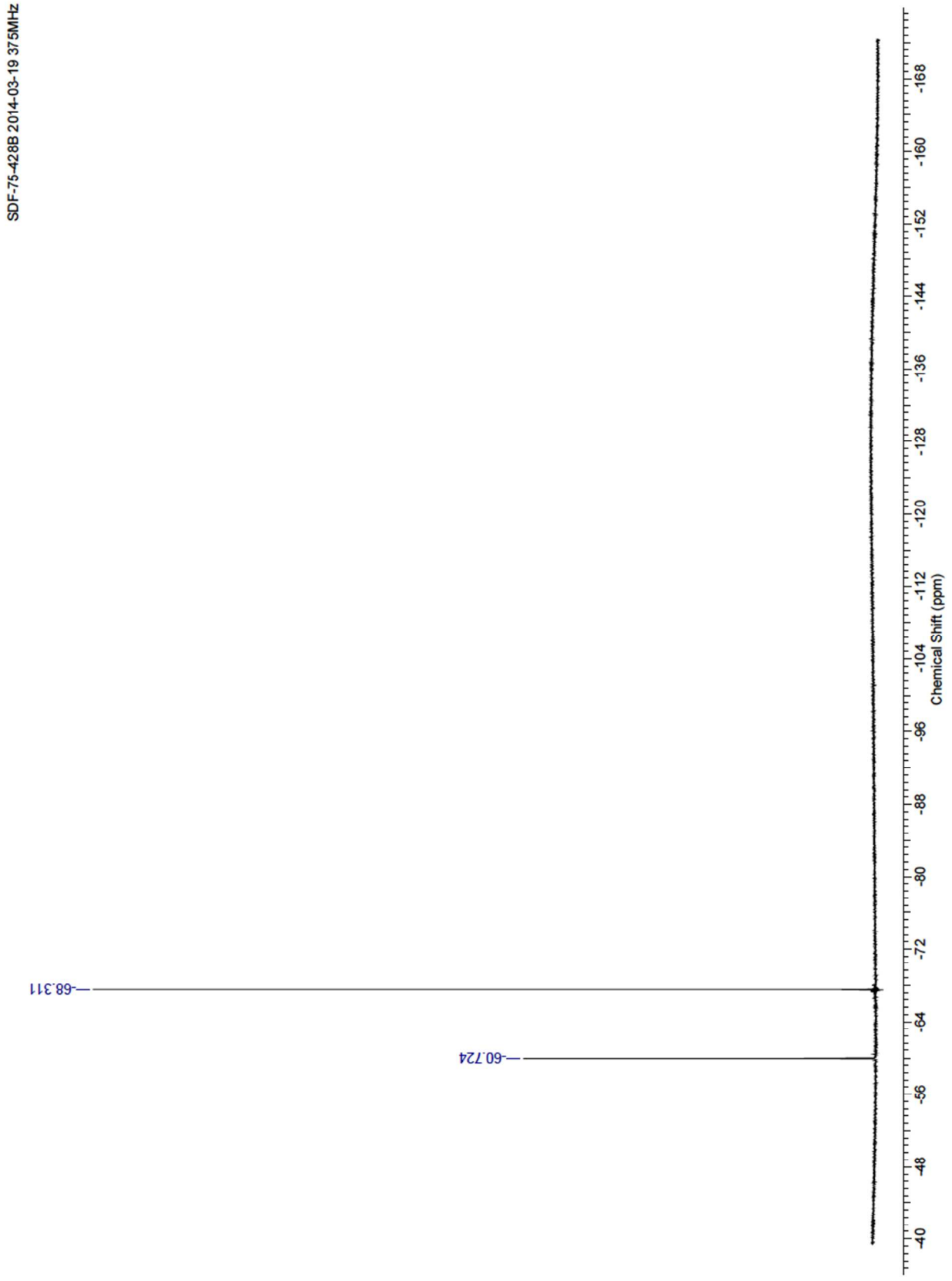


Figure S1. Plasma concentrations in ng/mL (y-axis) of mefloquine (**1**, left panel) and its trioxolane conjugate (**3**, right panel) at eight time points following i.p. administration of **3** at 50 mg/kg in either infected or uninfected mice.

Scans of ^{1}HNMR, ^{13}CNMR, and ^{17}FNMR spectra for Compound 3


SDf-75-428B_1H_4 2014-03-24 400MHz
Chemical Shift (ppm)


SDF-75-428B_13C_4 2014-03-24 100MHz
Chemical Shift (ppm)


SDF-75-428B 2014-03-19 375MHz
-68.311
-60.724
-40
-48
-56
-64
-72
-80
-88
-96
-104
-112
-120
-128
-136
-144
-152
-160
-168
Chemical Shift (ppm)