

## Supporting Information for

# Synthesis, Characterization and Biological Activity of Organometallic Derivatives of the Antimalarial Drug Mefloquine as New Antischistosomal Drug Candidates

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## Experimental section

### Materials

All chemicals were of reagent grade quality, obtained from commercial suppliers unless otherwise stated and used without further purification. Solvents were distilled or dried over molecular sieves prior to their use. All reactions described were performed under an atmosphere of dry nitrogen using standard Schlenk techniques and oven dried glassware unless otherwise specified. The manipulations of hygroscopic compounds were performed in an inert and dry atmosphere of nitrogen, using glove box techniques.

### Instrumentation and general methods

Evaporation of the solvents *in vacuo* was done with a rotary evaporator in a 40°C water bath. Thin layer chromatography (TLC) was performed using silica gel 60 F-254 (Merck) plates with detection of spots being achieved by exposure to UV light. <sup>1</sup>H, <sup>13</sup>C and <sup>19</sup>F NMR spectra were recorded in deuterated solvents on Bruker AV-400, AV-401 and AV-500 spectrometers at room temperature. Signal positions ( $\delta$ ) are given in parts per million from tetramethylsilane ( $\delta$  0) and were measured relative to the signal of the solvent (CDCl<sub>3</sub> :  $\delta$  7.26, <sup>1</sup>H NMR; CH<sub>3</sub>OD:  $\delta$  3.31, <sup>1</sup>H NMR; (CD<sub>3</sub>)<sub>2</sub>CO :  $\delta$  2.05, <sup>1</sup>H NMR; D<sub>2</sub>O :  $\delta$  4.79, <sup>1</sup>H NMR) Coupling constants (J values) are given in Hertz (Hz) and are reported to the nearest 0.1 Hz. <sup>1</sup>H NMR spectral data are tabulated in the order: multiplicity (*s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet), coupling constants, number of protons. Elemental microanalyses were performed on a LecoCHNS-932 elemental analyzer. ESI mass spectrometry was performed using a Bruker Esquire 6000 spectrometer. In the assignment of the mass spectra, the most intense peak is listed. UPLC-ESI-MS was performed on a Waters Acquity UPLC System coupled to a Bruker HCTTM, using an Acquity UPLC BEH C18 1.7  $\mu$ m (2.1 x 50 mm) as a reverse phase column with a flow rate of 0.6 mL min<sup>-1</sup>. The UV absorption was measured at 254 nm. The runs were performed with a linear gradient of A (acetonitrile (Sigma-Aldrich HPLC grade) and B (distilled water containing 0.02% TFA and 0.05% HCOOH): t = 0 – 0.5 min, 5% A; t = 4.0 min, 100% A; t = 5 min, 100% A. High-resolution mass spectrometry were performed on a Bruker ESQUIRE-LC quadrupole ion trap instrument (Bruker Daltonik GmbH, Bremen, Germany), equipped with a combined Hewlett-Packard Atmospheric Pressure Ion (API) source (Hewlett-Packard Co., Palo Alto, CA, USA). The solutions (about 0.1-1  $\mu$ mol/ml) were continuously introduced through the electrospray interface with a syringe infusion pump

(Cole-Parmer 74900-05, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA) at a flow rate of 5  $\mu\text{L min}^{-1}$ . The MS-conditions were: Nebulizer gas ( $\text{N}_2$ ) 15 psi, dry gas ( $\text{N}_2$ ) 7  $\text{min}^{-1}$ , dry temperature 300°C, capillary voltage 4000 V, end plate 3500 V, capillary exit 100 V, skimmer 30 V, and trap drive 70. The MS acquisitions were performed at normal resolution (0.6 u at half peak height), under ion charge control (ICC) conditions (10'000) in the mass range from  $m/z$  100 to 2000. To get representative mass spectra, 8 scans were averaged. Chiral high-performance liquid chromatography (HPLC) was performed with a *Daicel* CHIRALPAK IC column based on cellulose and immobilized on 5  $\mu\text{m}$  silica-gel at a flow rate of 16  $\text{mL min}^{-1}$  with a gradient of A (acetonitrile; Sigma-Aldrich HPLC-grade) and B (distilled water containing 0.1% TFA).

### **Syntheses and characterization**

**Preparation of [2,8-bis(trifluoromethyl)quinolin-4-yl] ferrocenemethanol (3).** To a stirred solution of 2,8-bis-(trifluoromethyl)-4-bromoquinoline (200 mg, 0.58 mmol, 1 equiv.) in anhydrous diethylether (5 mL) cooled at -78 °C was slowly added a 2.5 M solution of *n*-butyllithium in hexane (0.36 mL, 0.58 mmol, 1 equiv.) under nitrogen. The resulting mixture was stirred at -78 °C for 20 min. A solution of ferrocenecarboxaldehyde (**3a**, 124 mg, 0.58 mmol, 1 equiv.) in anhydrous diethylether (5 mL) was then dropwise added over 1 h and the mixture was stirred for an additional hour. The solution was progressively warmed up to room temperature and then quenched with water (10 mL). The layers were separated and the aqueous layer was washed with diethylether (2x25 mL). The combined organic layers were dried with  $\text{Na}_2\text{SO}_4$ , filtered and the solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography on  $\text{C}_{18}$  silica gel (hexane- $\text{Et}_2\text{O}$ - $\text{Et}_3\text{N}$  6:3:1) to afford **3** (266 mg, 0.56 mmol, 94% yield) as a red-orange oil.  $R_f$  (hexane- $\text{Et}_2\text{OAc}$  1:1) 0.21;  $^1\text{H NMR}$  (400 MHz,  $(\text{CD}_3)_2\text{CO}$ ):  $\delta$  = 8.81 (*d*,  $J$  = 8.6 Hz, 1H), 8.31 (*d*,  $J$  = 7.5 Hz, 1H), 8.17 (*s*, 1H), 7.91 (*dd*,  $J$  = 8.0, 8.0 Hz, 1H), 6.52 (*d*,  $J$  = 4.7 Hz, 1H), 5.09 (*d*,  $J$  = 4.8 Hz, 1H), 4.30-4.28 (*m*, 1H), 4.25-4.22 (*m*, 6H), 4.17-4.15 ppm (*m*, 2H);  $^{13}\text{C-NMR}$  (125 MHz,  $(\text{CD}_3)_2\text{CO}$ ):  $^m$  = 155.8, 148.7 (*q*,  $^2J$  = 35.0 Hz), 144.6, 130.9, 130.4, 130.3, 130.3, 130.2, 129.2 (*q*,  $^2J$  = 28.8 Hz), 128.2, 127.0 (*q*,  $^1J$  = 273.4 Hz), 122.6 (*q*,  $^1J$  = 274.7 Hz), 116.0, 93.0, 93.0, 69.8, 69.3, 69.2, 69.0, 68.7, 68.1, 67.7 ppm;  $^9\text{F-NMR}$  (470 MHz,  $(\text{CD}_3)_2\text{CO}$ ):  $^m$  = -60.9, -68.7 ppm; HRMS (ESI)  $m/z$  calcd for  $\text{C}_{22}\text{H}_{15}\text{F}_6\text{FeNO}$   $[\text{M}]^+$  479.0402, found 479.0400 (89); calcd for  $\text{C}_{22}\text{H}_{16}\text{F}_6\text{FeNO}$   $[\text{M}+\text{H}]^+$  480.0480, found 480.0465 (100). Elemental analysis: Calcd C 55.14%, H 3.16%, N 2.92%, Found C 55.23%, H 3.21%, N 2.82%.

The presence of the racemic mixture of **3** can be highlighted by chiral preparative HPLC at a flow rate of 16 mL/min with a gradient of A (Acetonitrile (Sigma-Aldrich, HPLC grade) and B (distilled water containing 0.1% TFA): t = 0 min A 40% + B 60%, t = 40 min A 35% + B 65%.

**Preparation of [2,8-bis(trifluoromethyl)quinolin-4-yl] ruthenocenemethanol (4).** To a stirred solution of 2,8-bis-(trifluoromethyl)-4-bromoquinoline (200 mg, 0.58 mmol, 1 equiv.) in anhydrous diethylether (5 mL) cooled at -78 °C was slowly added a 2.5 M solution of *n*-butyllithium in hexane (0.36 mL, 0.58 mmol, 1 equiv.) under nitrogen. The resulting mixture was stirred at -78 °C for 20 min. A solution of ruthenocenecarboxaldehyde (**4a**, 151 mg, 0.58 mmol, 1 equiv.) in anhydrous diethylether (5 mL) was then dropwise added over 1 h and the mixture was stirred for an additional hour. The solution was progressively warmed up to room temperature and then quenched with water (10 mL). The layers were separated and the aqueous layer was washed with diethylether (2x25 mL). The combined organic layers were dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was evaporated. The crude product was purified by flash chromatography (hexane-Et<sub>2</sub>O-Et<sub>3</sub>N 6:3:1) to afford **4** (236 mg, , 0.45 mmol, 78% yield) as a red-orange oil. R<sub>f</sub> (hexane-Et<sub>2</sub>OAc-Et<sub>3</sub>N 6:3:1) 0.21; <sup>1</sup>H NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>CO): δ = 8.75 (*d*, *J* = 8.1 Hz, 1H), 8.33 (*d*, *J* = 7.3 Hz, 1H), 8.21 (*s*, 1H), 7.94 (*dd*, *J* = 8.0, 8.0 Hz, 1H), 6.29-6.27 (*m*, 1H), 4.82 (*ddd*, *J* = 2.4, 1.2, 1.2 Hz, 1H), 4.57-4.53 (*m*, 6H), 4.49 (*ddd*, *J* = 2.4, 2.4, 1.1 Hz, 1H), 4.39 (*ddd*, *J* = 2.4, 1.1, 1.1 Hz, 1H), 4.13 ppm (*d*, *J* = 4.2 Hz, 1H); <sup>13</sup>C-NMR (126 MHz, (CD<sub>3</sub>)<sub>2</sub>CO): <sup>TM</sup> = 155.2, 148.5 (*q*, <sup>2</sup>*J* = 35.3 Hz), 144.3, 130.6, 130.3, 130.3, 130.3, 130.2, 129.1 (*q*, <sup>2</sup>*J* = 29.0 Hz), 128.3, 124.9 (*q*, <sup>1</sup>*J* = 273.4 Hz), 122.6 (*q*, <sup>1</sup>*J* = 274.7 Hz), 115.9, 98.3, 9 8.1, 71.9, 71.2, 71.1, 71.1, 71.0, 67.3, 67.3 ppm; <sup>9</sup>F-NMR (470 MHz, (CD<sub>3</sub>)<sub>2</sub>CO): <sup>TM</sup> = -60.8, -68.6 ppm; HRMS (ESI) *m/z* calcd for C<sub>22</sub>H<sub>15</sub>F<sub>6</sub>NORu [M + H]<sup>+</sup> 526.0174, found 526.0183. Elemental analysis: Calcd C 50.39%, H 2.88%, N 2.67%, Found C 50.72%, H 2.89%, N 2.61%.

The presence of the racemic mixture of **3** can be highlighted by chiral preparative HPLC at an isocratic flow rate of 16 mL/min with A (Acetonitrile (Sigma-Aldrich, HPLC grade) and B (distilled water containing 0.1% TFA): t = 0 min A 50% + B 50%, t = 30 min A 50% + B 50%.

## Biological assays

### *In vitro studies with S. mansoni*

Mefloquine was purchased from Sigma-Aldrich (Buchs, Switzerland). Culture medium components were obtained as follows: RPMI 1640 was purchased from Gibco (Basel, Switzerland), fetal calf serum (FCS) was obtained from Connectorate AG (Dietikon, Switzerland) and penicillin-streptomycin (10 000 units penicillin and 10 mg mL<sup>-1</sup> streptomycin) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich.

Adult worms were obtained as described by Keiser *et al.*<sup>1</sup> In brief, NMRI outbred mice were infected with 100 cercariae each and the infection was allowed to develop for 7 weeks, until worms reached the adult stage. The mice were then sacrificed, and the adult worms extracted from the mesenteric veins. The worms were kept in RPMI supplemented with 5% FCS and 1% penicillin/ streptomycin mixture at 37°C and 5% CO<sub>2</sub> until use.

In a 24-well plate, drugs were serially diluted in culture medium to obtain final assay concentrations of 6.66, 2.22 and 0.74 µM in 1600 µl total volume, using 2 wells per drug. Two additional wells served as control with culture medium and 0.1% DMSO. In each well, 3 adult schistosomes were placed, with an even number of worms from both sexes for each compound. The worms were incubated at 37°C and 5% CO<sub>2</sub> and evaluated microscopically at 72 hours using a viability scale of 0 to 3 (3 = totally vital, normally active, no morphological changes, 2 = slowed activity, first morphological changes and granularity visible, 1 = minimal activity, severe morphological changes and granularity, 0 = all worms dead, severe granularity). The viability scores were averaged across replicates and normalized to control-well viability scores using Microsoft Office Excel (2010). IC<sub>50</sub> values for the worm assays were computed using CompuSyn2® (ComboSyn Inc., 2007) by converting viability scores into effect scores for each drug concentration. To test if protein-binding affects the compound IC<sub>50</sub> values, we used the same procedure as above, with the exception that 45g/L bovine albumin (Sigma-Aldrich ; Buchs, Switzerland) was added to the assay and the concentrations of drug tested were 10, 5 and 2.5 µM.

### ***Cytotoxicity studies***

TIB-75 and HeLa cell lines were cultured in DMEM (Gibco, Life Technologies, USA) supplemented with 10% of fetal calf serum (Gibco). hTERT- immortalized retinal pigmented epithelial cells RPE-1 were cultured in DMEM/F-12 (Gibco) supplemented with 10% of fetal calf serum. Cell lines were complemented with 100 U/ml penicillin-streptomycin mixture (Gibco) and maintained in humidified atmosphere at 37°C and 5% of CO<sub>2</sub>.

Cytotoxicity of mefloquine and mefloquine derivatives was assessed by fluorometric cell viability assays using resazurin (ACROS Organics). HeLa, RPE-1 and TIB-75 cells were seeded in triplicates in 96 well plates at a density of 4000 cells per well in 100 µl, 24 h prior to treatment. Cells were then treated with increasing concentration of compounds for 48 h. Medium was then replaced by fresh complete medium containing resazurin (0.2 mg ml<sup>-1</sup> final concentration). After 4 h incubation at 37°C, fluorescence signal of resorufin product was read by SpectraMax M5 microplate reader (ex: 540 nm em: 590 nm). IC<sub>50</sub> values were calculated using GraphPad Prism software.

### ***In vivo studies with S. mansoni***

Experiments with *S. mansoni*-infected mice were conducted at the Swiss Tropical and Public Health Institute (Basel, Switzerland) following approval by the veterinary authorities of the Canton Basel-Stadt (permission no. 2070). All experiments followed Swiss cantonal and national regulations (Article 455, Chapter 6 of the Swiss Animal Welfare Ordinance) on animal experimentation, as well as the Ethics Committee for Animal Experimentation of the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT) guidelines on animal experimentation.

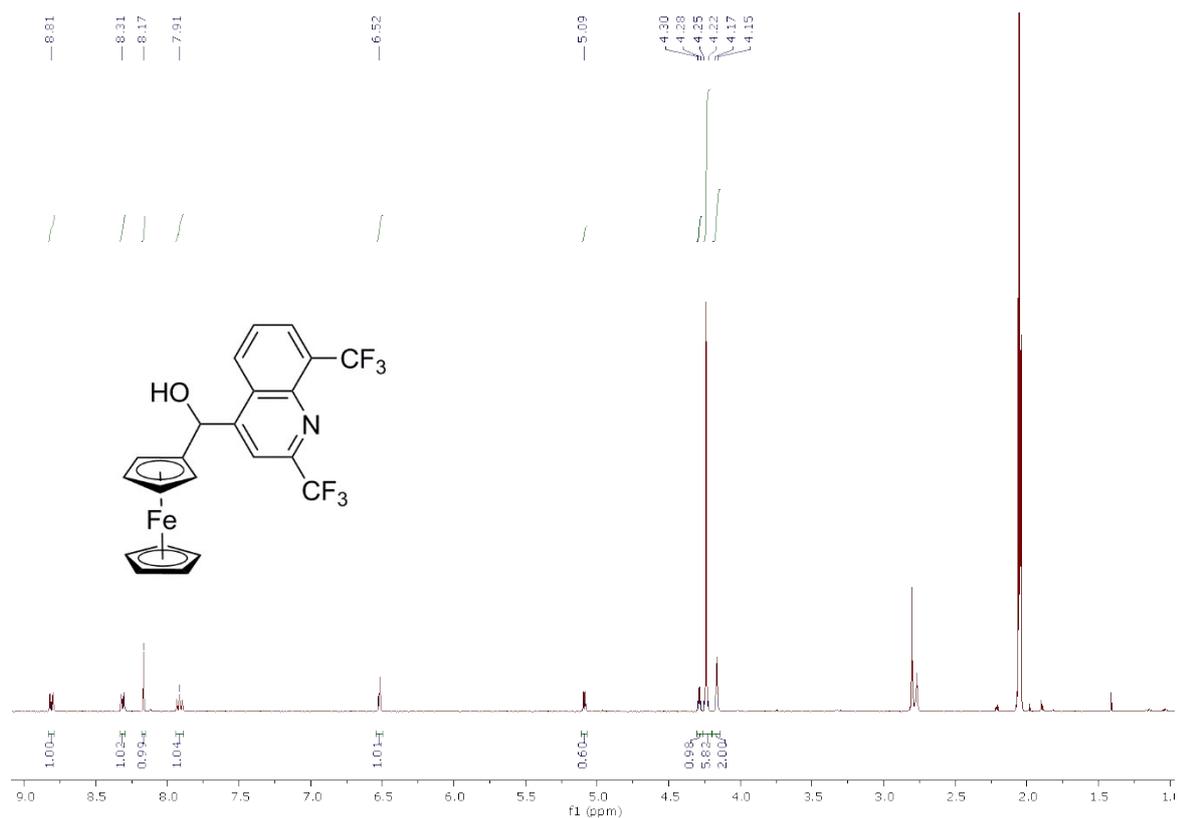
Female 3-week old NMRI mice were purchased from Charles River (Sulzfeld, Germany) and allowed to adapt under controlled conditions (temperature ca. 22 °C; humidity ca. 50 %; 12-hour light and dark cycle; free access to rodent diet and water) for one week before infection.

Mice were infected subcutaneously with approximately 100 *S. mansoni* cercariae, which were obtained by placing infected *Biomphalaria glabrata* snails in tap water under light for 4 hours and collecting the supernatant.<sup>47</sup> Seven weeks post-infection, 4 mice were assigned to each drug treatment (at a dose of 200 mg/kg), while 8 mice were left untreated to serve as controls. Compounds were prepared in a 70:30 Tween/EtOH mixture dissolved in dH<sub>2</sub>O (10%). Doses were adjusted to the mouse weight and were administered orally. Three weeks post-treatment, the mice were killed by the CO<sub>2</sub> method, dissected and the worms were sexed and counted. Mean worm burdens of treated mice were compared to the mean worm burden of untreated animals and worm burden reductions were calculated as follows:

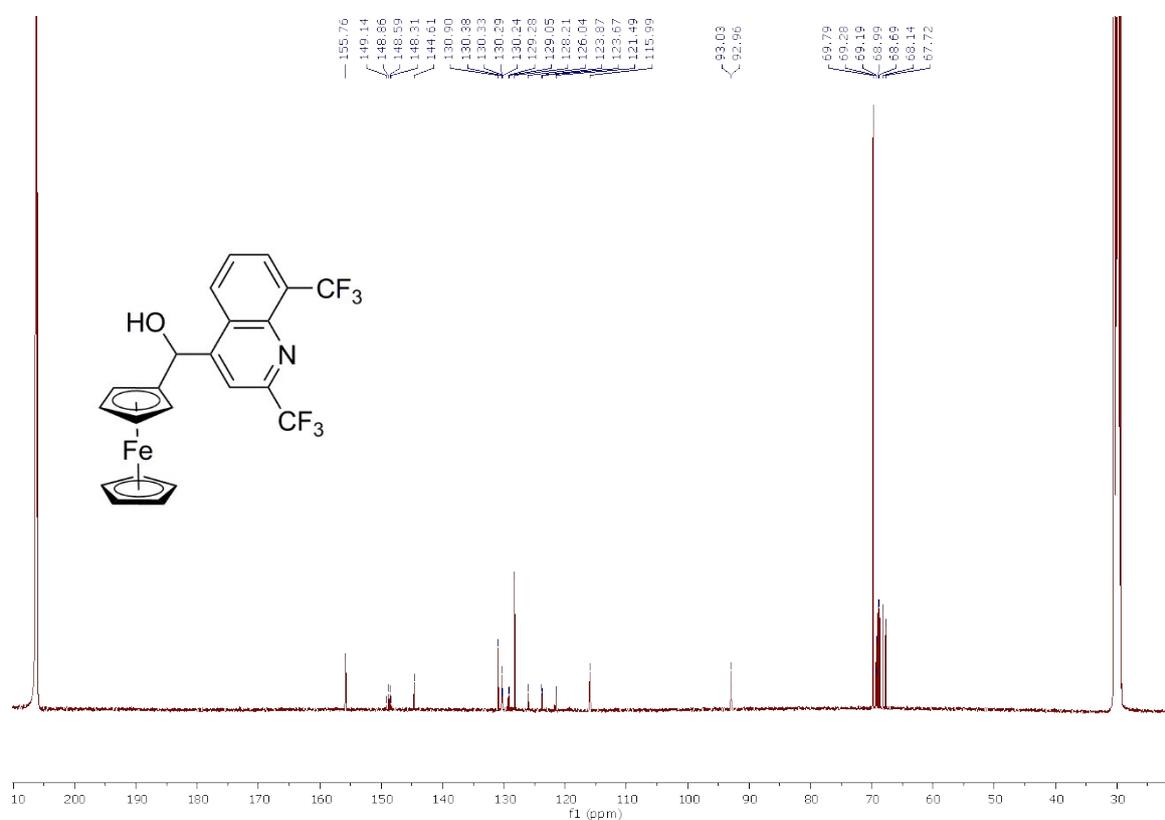
$$\text{WBR (\%)} = 100\% - (100\% / \text{WB}_{\text{control}} \times \text{WB}_{\text{treatment}})$$

### **Reference**

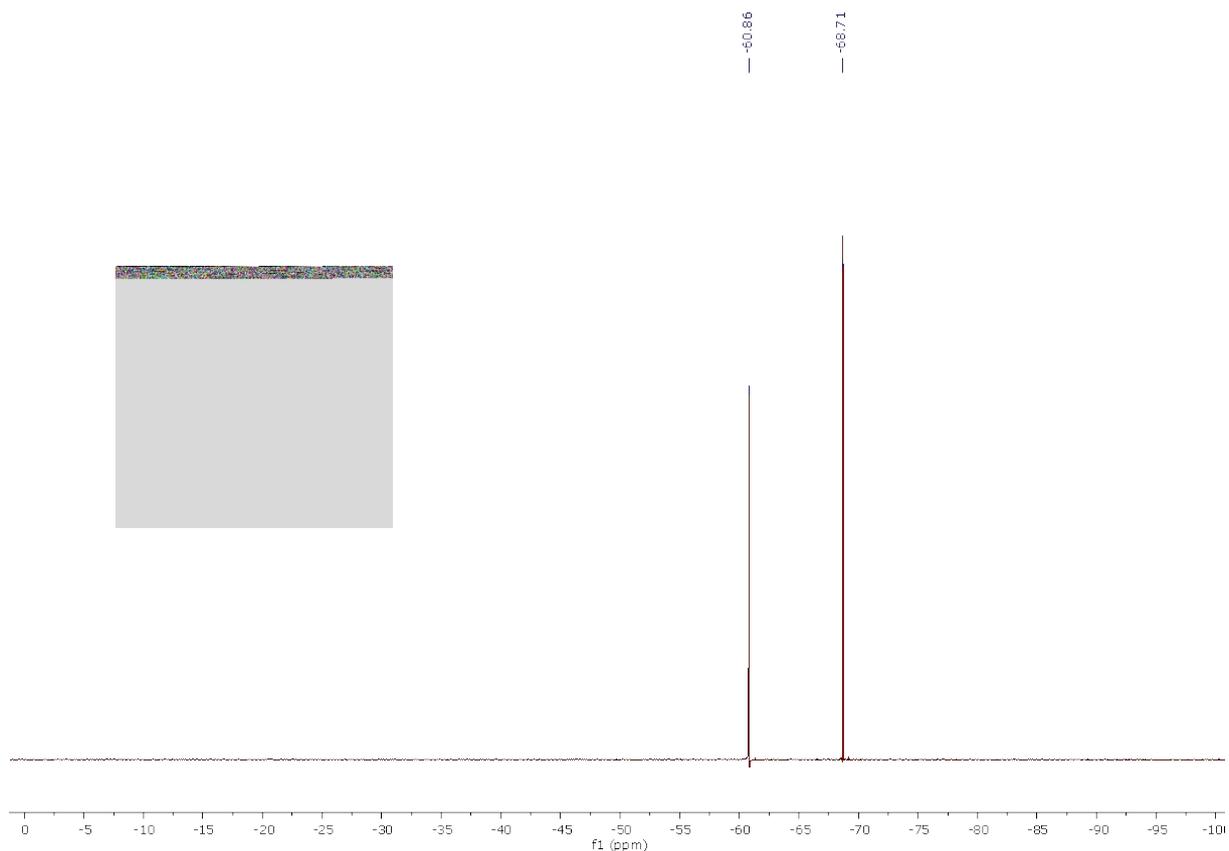
<sup>1</sup> Keiser, J. In Vitro and in Vivo Trematode Models for Chemotherapeutic Studies. *Parasitology* **2010**, 137 (3), 589–603.



**Figure S1.** <sup>1</sup>H-NMR spectrum of [2,8-bis(trifluoromethyl)quinolin-4-yl]ferrocenemethanol (**3**) in (CD<sub>3</sub>)<sub>2</sub>CO

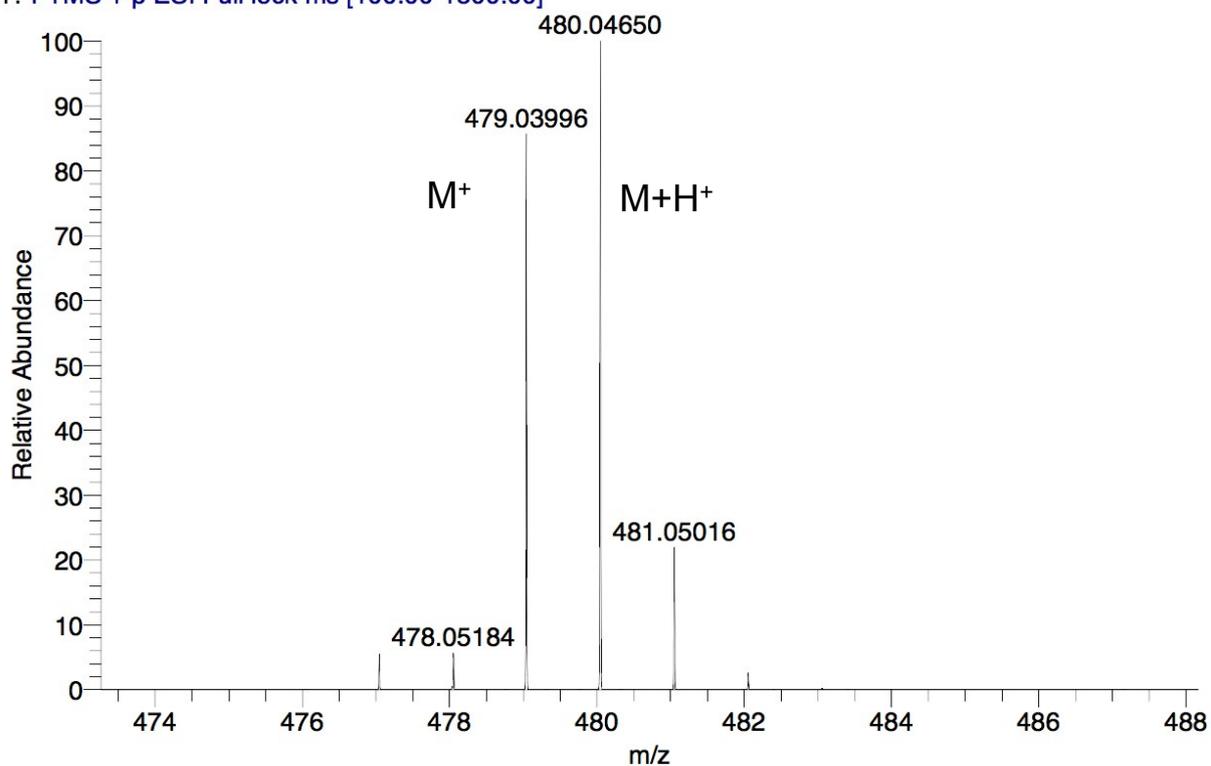


**Figure S2.** <sup>13</sup>C-NMR spectrum of **3** in (CD<sub>3</sub>)<sub>2</sub>CO

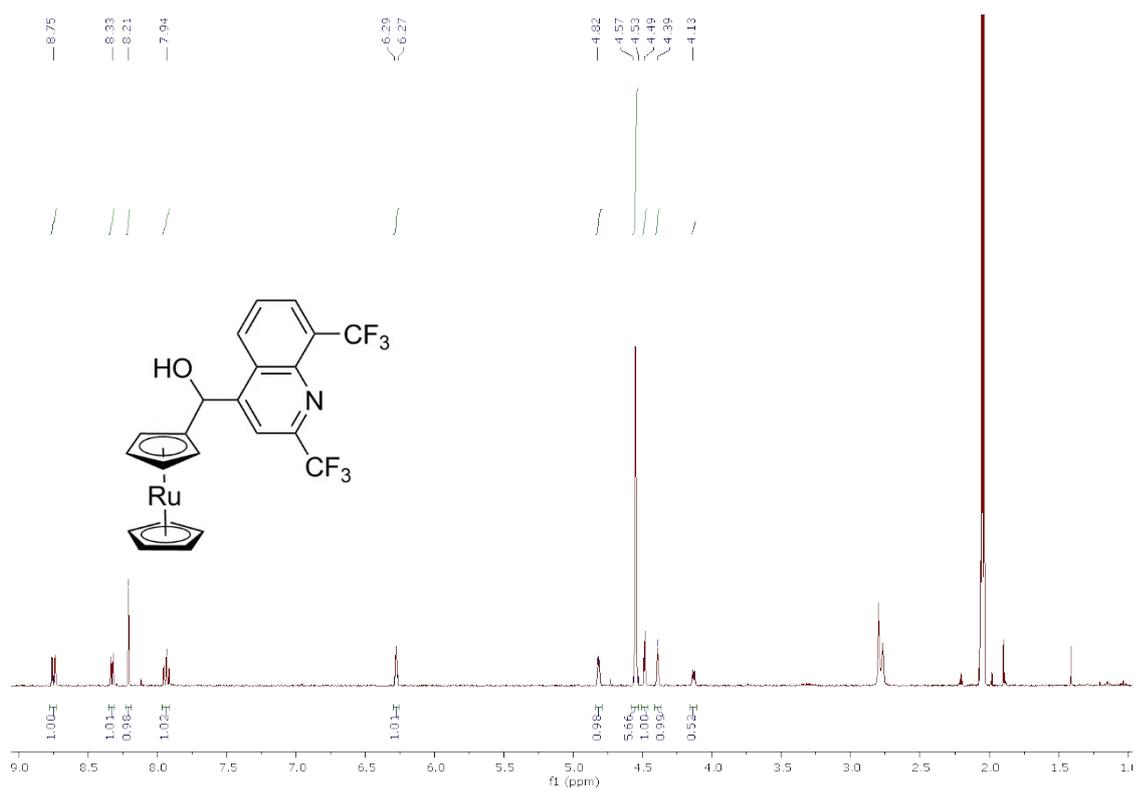


**Figure S3.**  $^{19}\text{F}$ -NMR spectrum of **3** in  $(\text{CD}_3)_2\text{CO}$

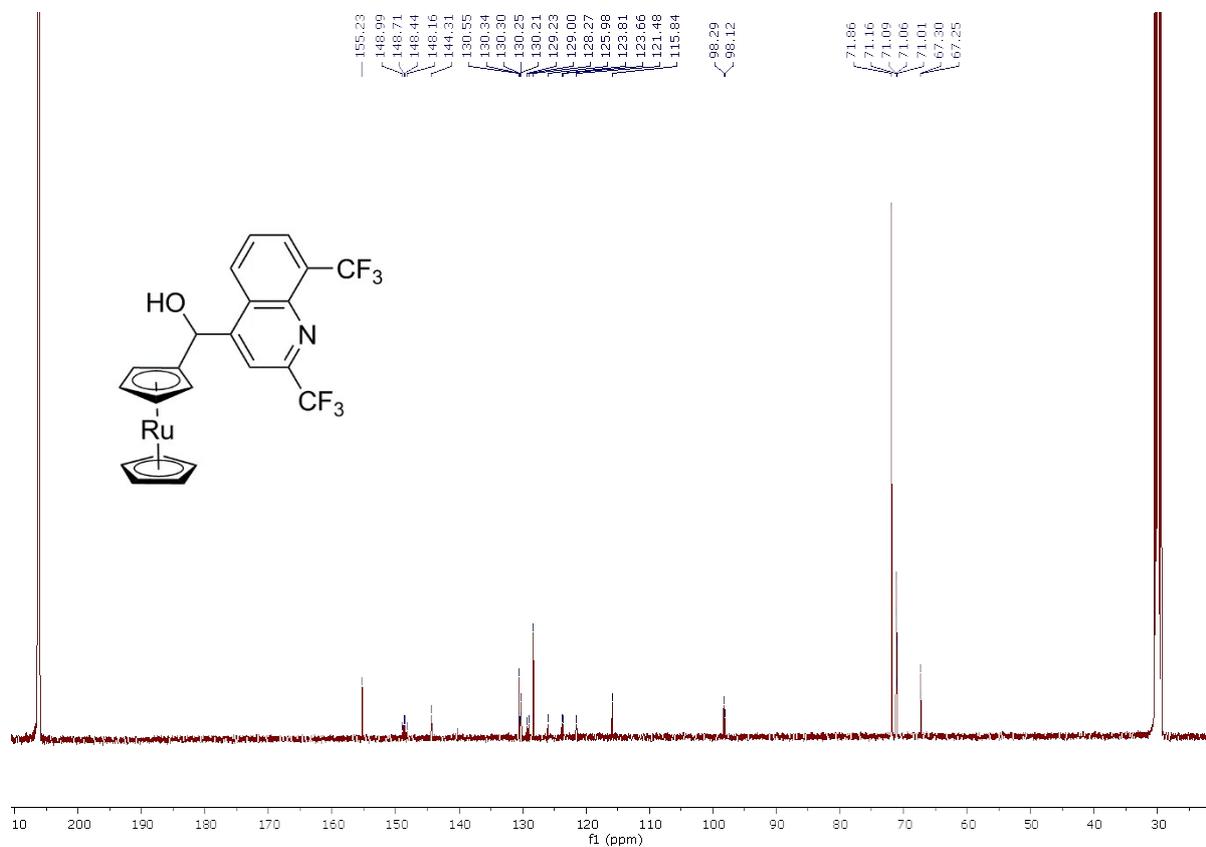
gaQEx2794 #37-49 RT: 0.38-0.50 AV: 7 SB: 24 0.07-0.29 , 0.71-0.98 NL: 1.25E8  
 T: FTMS + p ESI Full lock ms [100.00-1500.00]



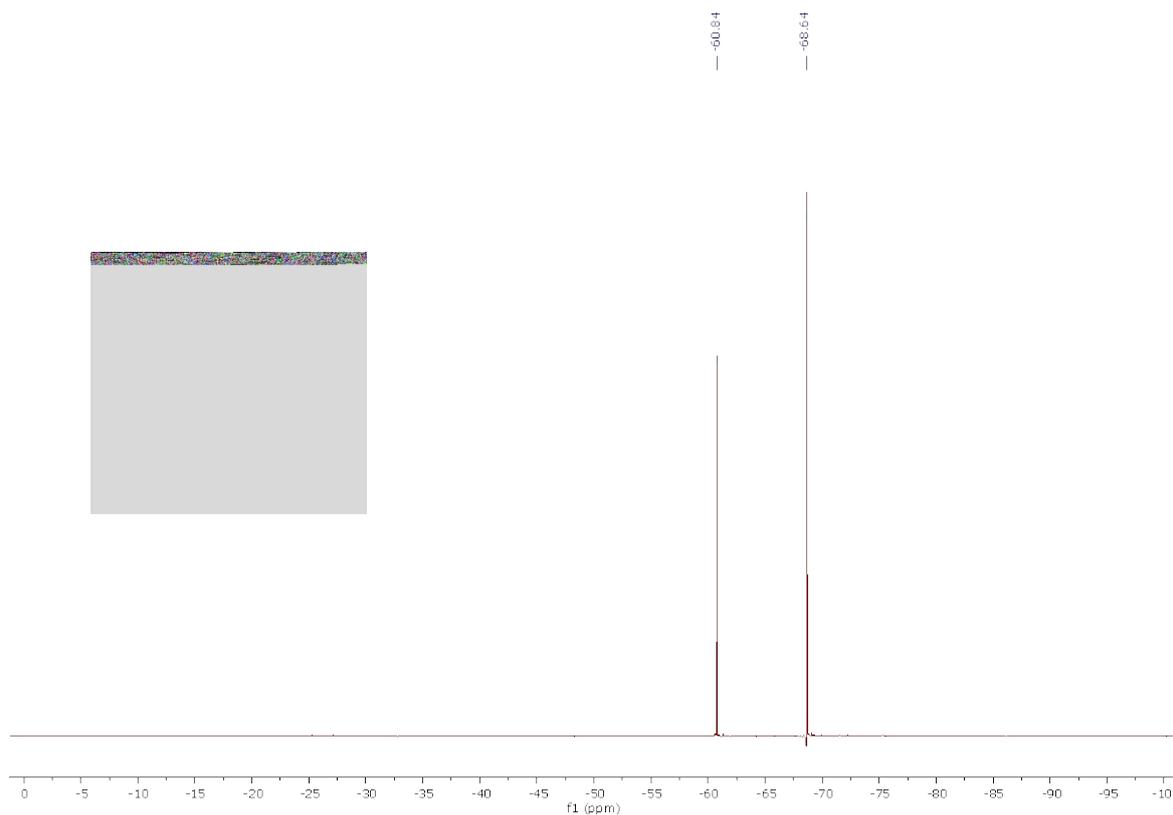
**Figure S4.** HRMS of **3**



**Figure S5.**  $^1\text{H-NMR}$  spectrum of [2,8-bis(trifluoromethyl)quinolin-4-yl] ruthenocenemethanol (**4**) in  $(\text{CD}_3)_2\text{CO}$

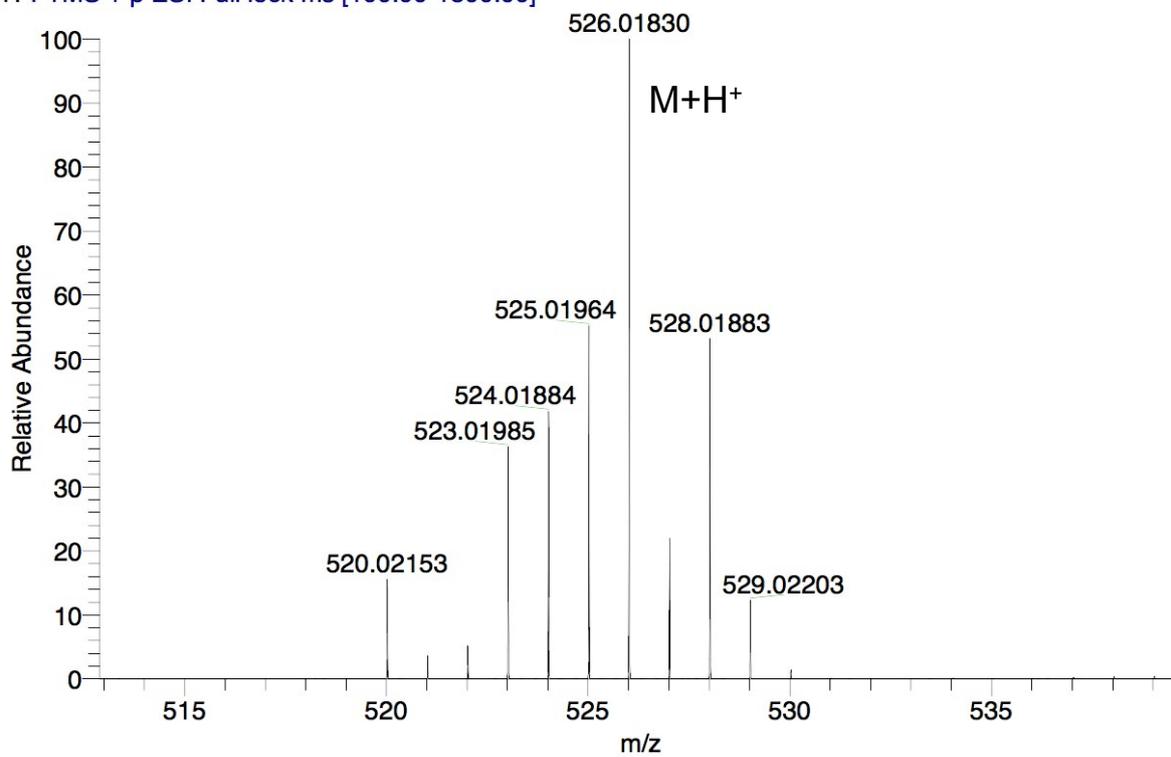


**Figure S6.**  $^{13}\text{C-NMR}$  spectrum of **4** in  $(\text{CD}_3)_2\text{CO}$

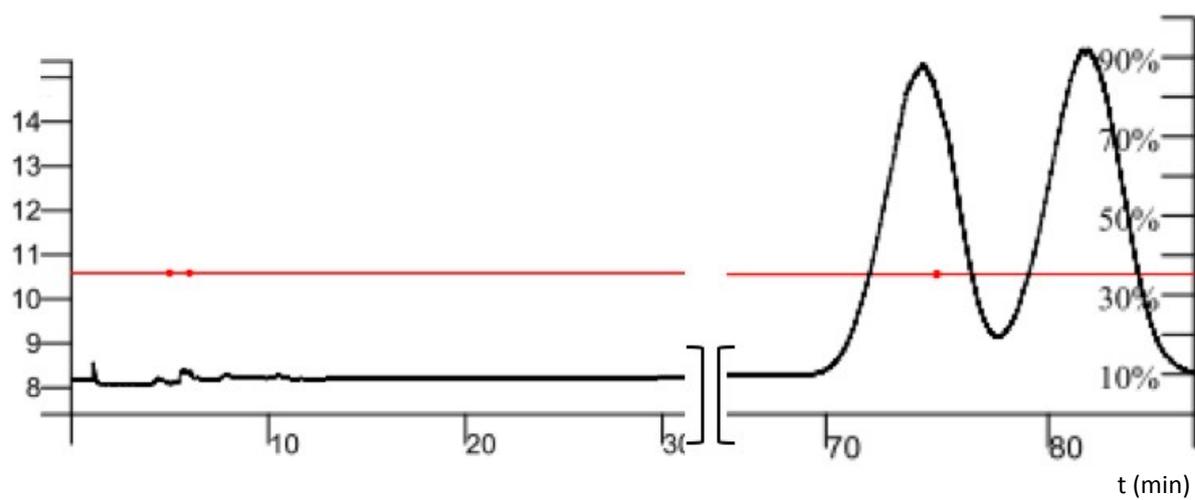


**Figure S7.**  $^{19}\text{F}$ -NMR spectrum of **4** in  $(\text{CD}_3)_2\text{CO}$

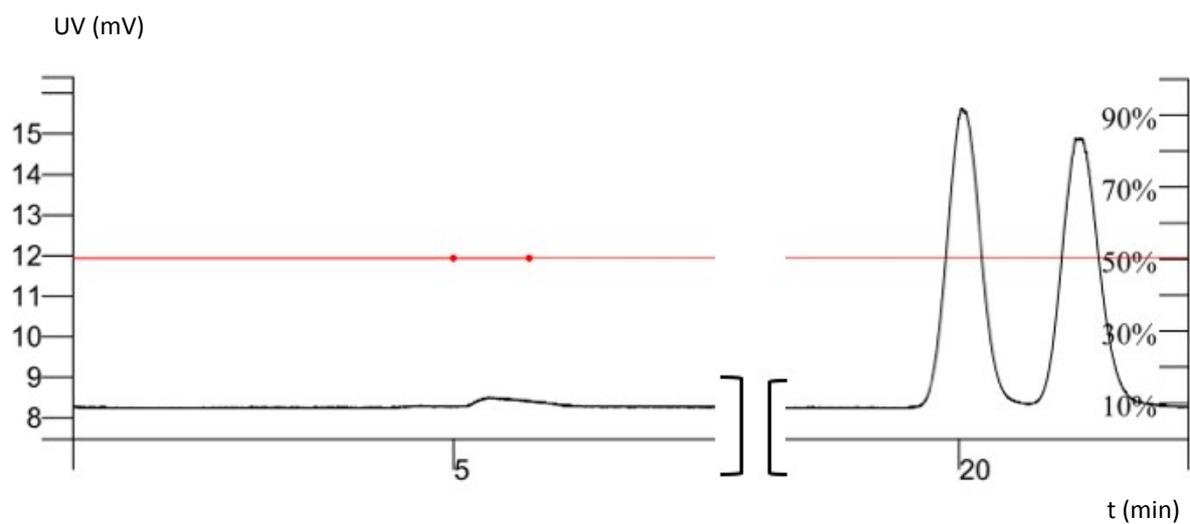
gaQEx2795 #35-55 RT: 0.35-0.54 AV: 11 SB: 26 0.07-0.29 , 0.71-0.98 NL: 1.60E8  
 T: FTMS + p ESI Full lock ms [100.00-1500.00]



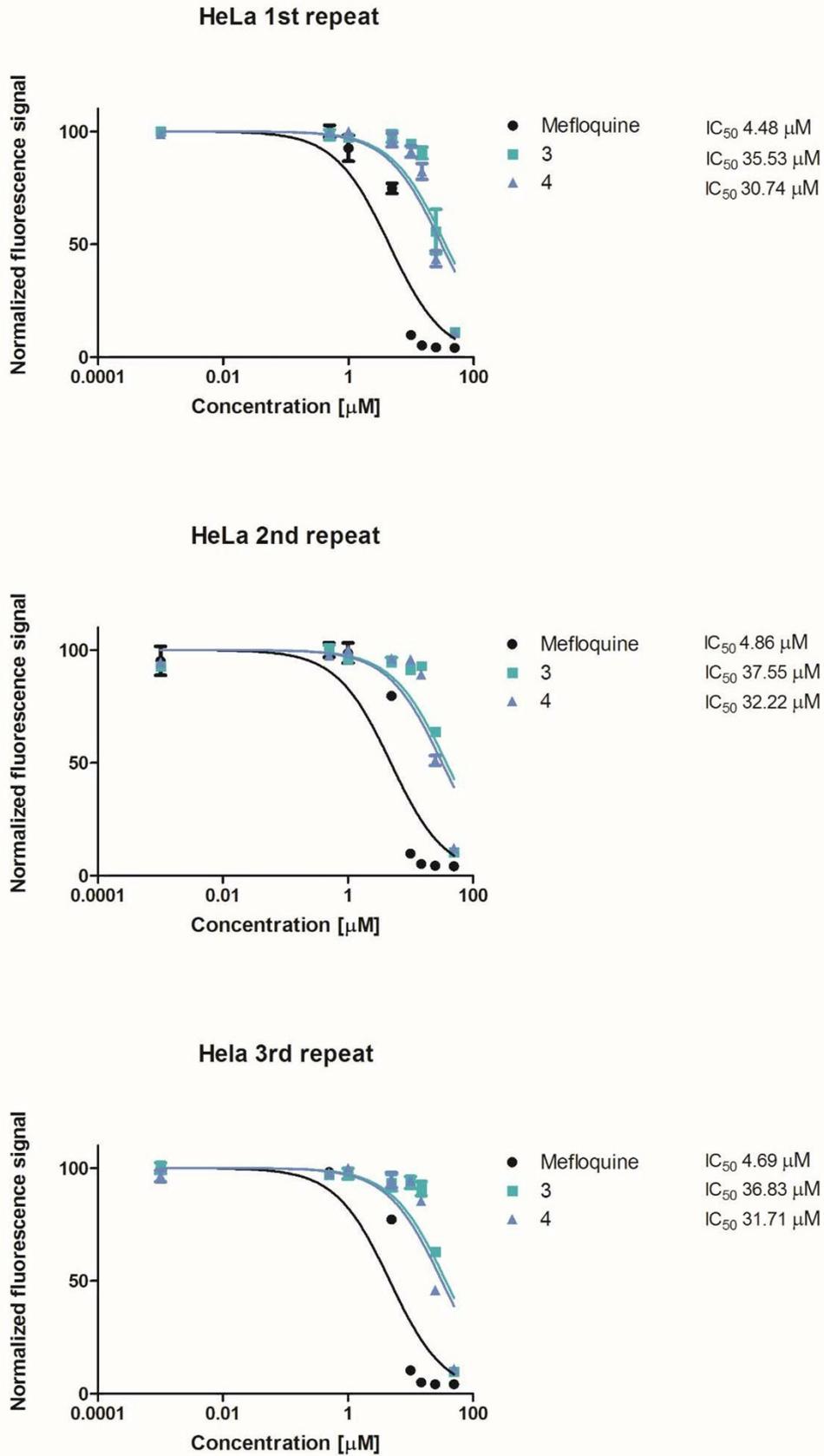
**Figure S8.** HRMS of **4**



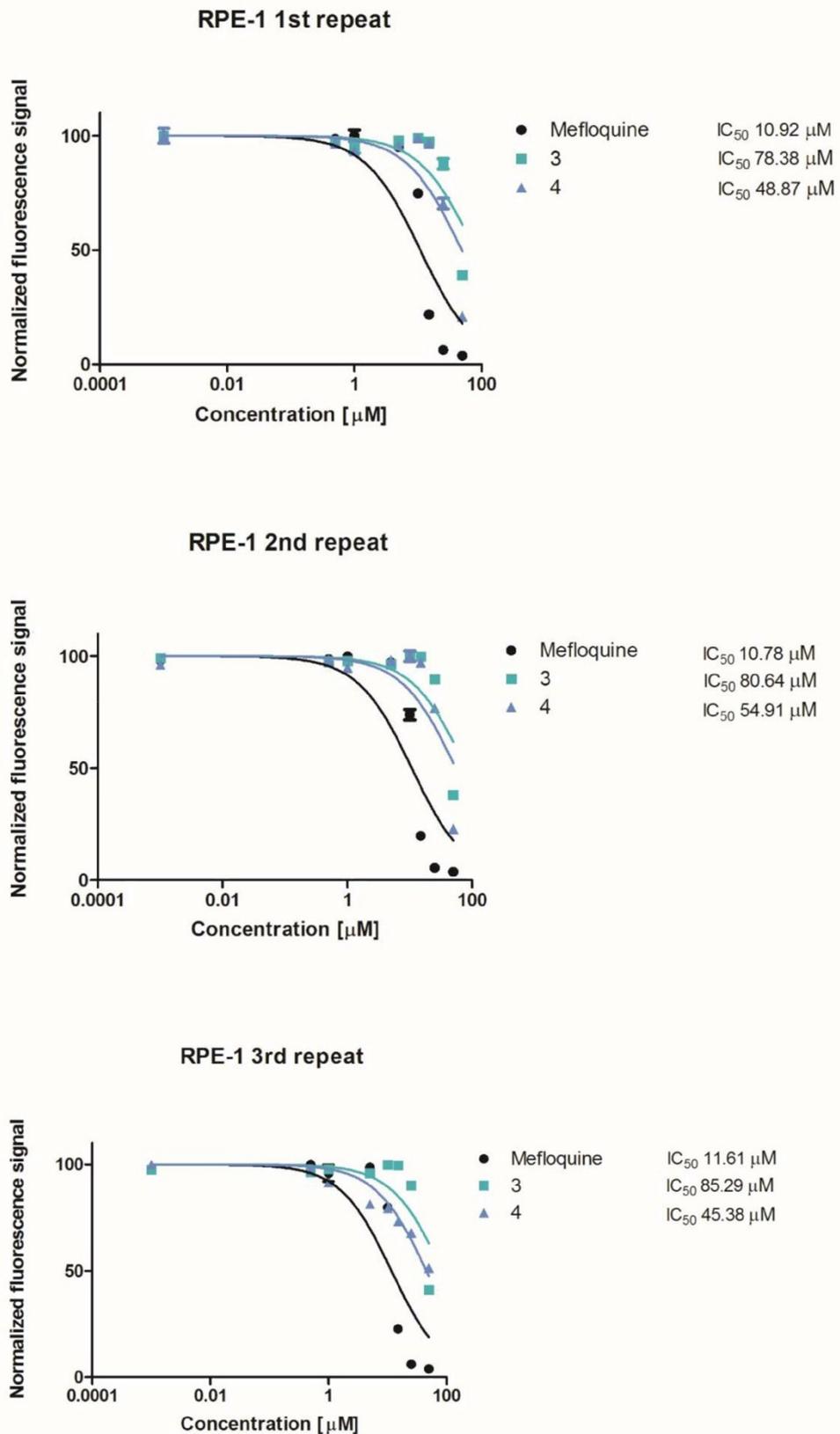
**Figure S9.** Chromatogram of **3** obtained by chiral HPLC



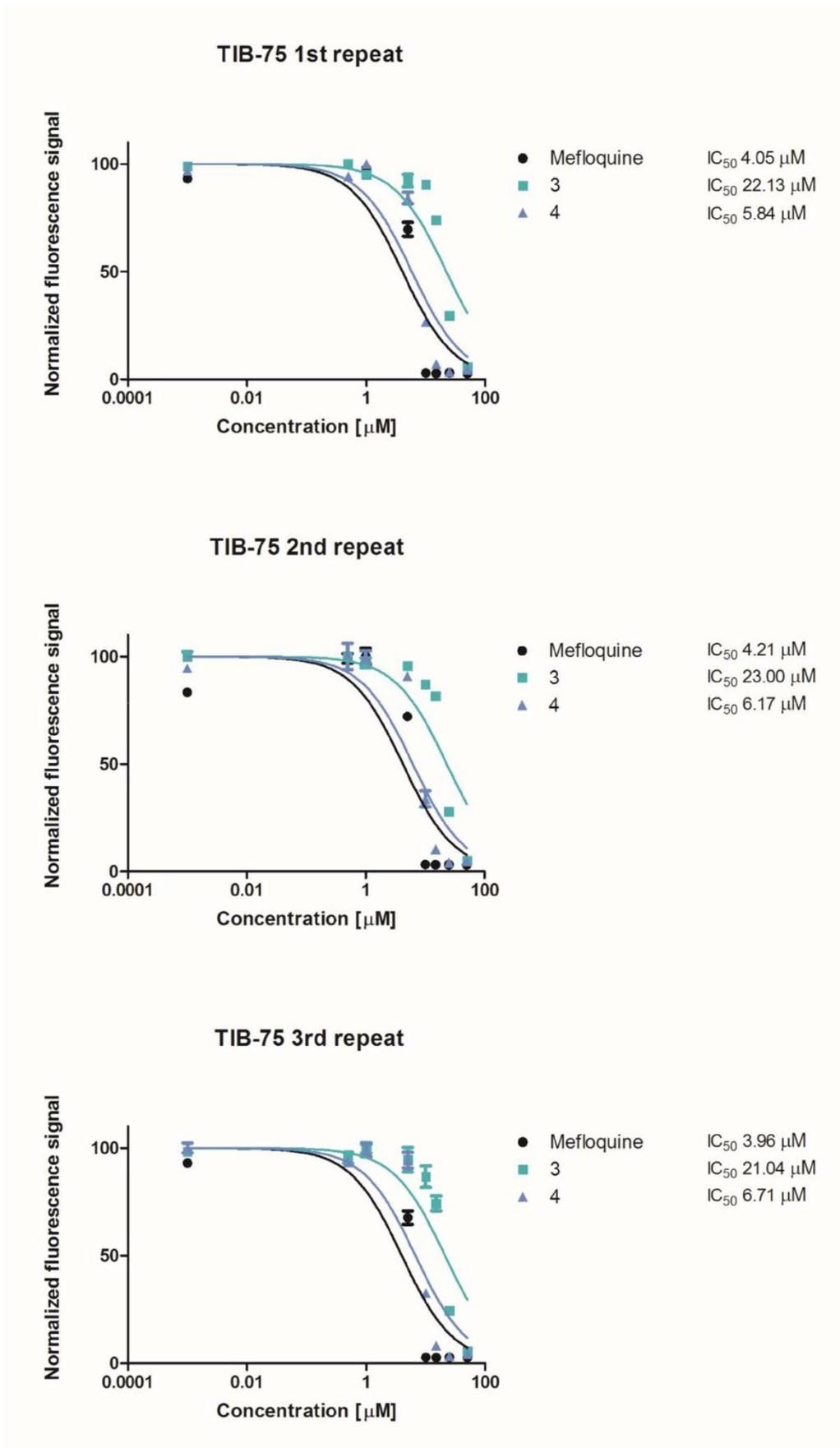
**Figure S10.** Chromatogram of **4** obtained by chiral HPLC



**Figure S11.** Cytotoxicity of 3, 4 and Mefloquine in HeLa cell line



**Figure S12.** Cytotoxicity of 3, 4 and Mefloquine in RPE-1 cell line



**Figure S13.** Cytotoxicity of 3, 4 and mefloquine in TIB-75 cell line