Near Infrared Fluorophore-tagged Chloroquine in *Plasmodium falciparum* Diagnostic Imaging

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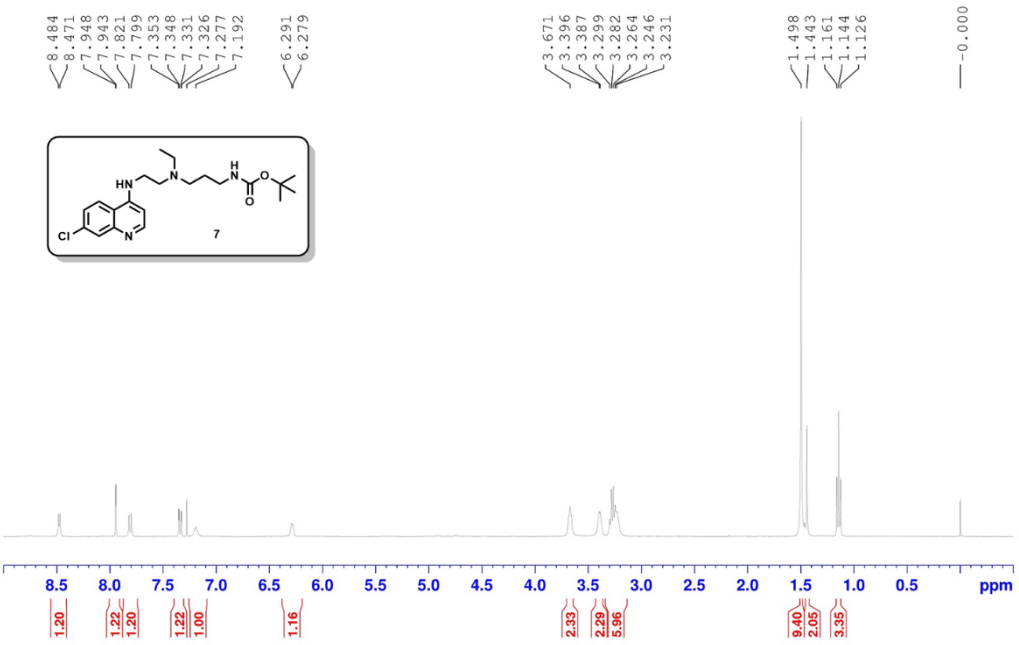
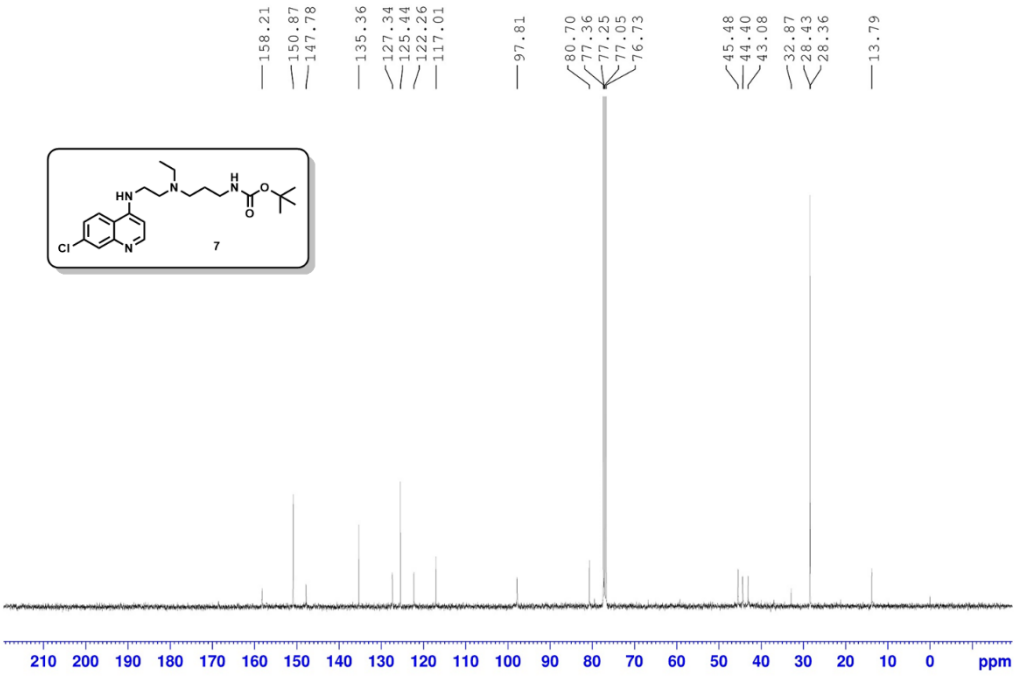
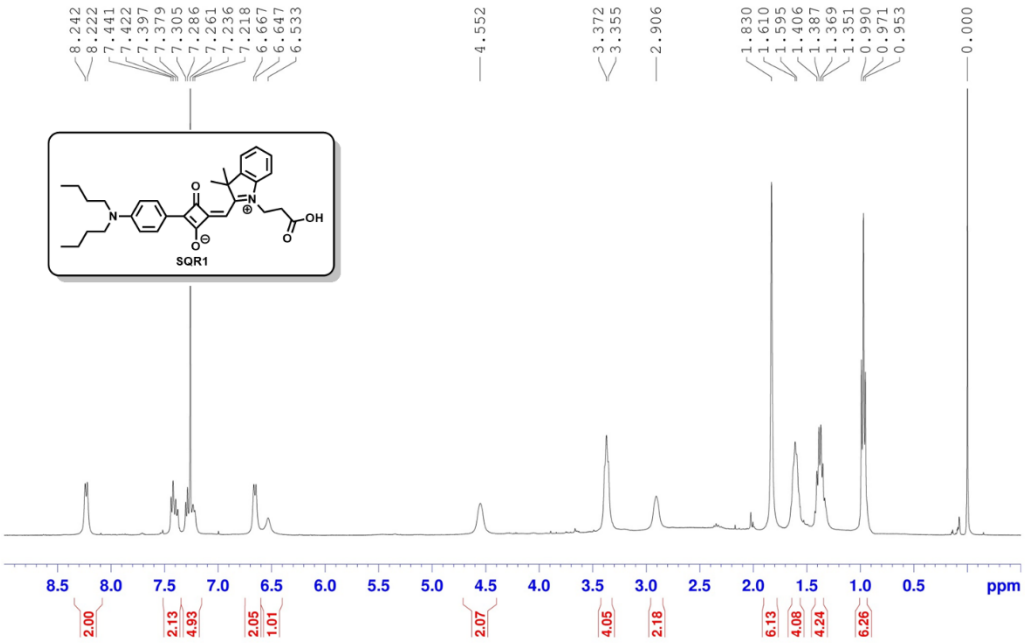
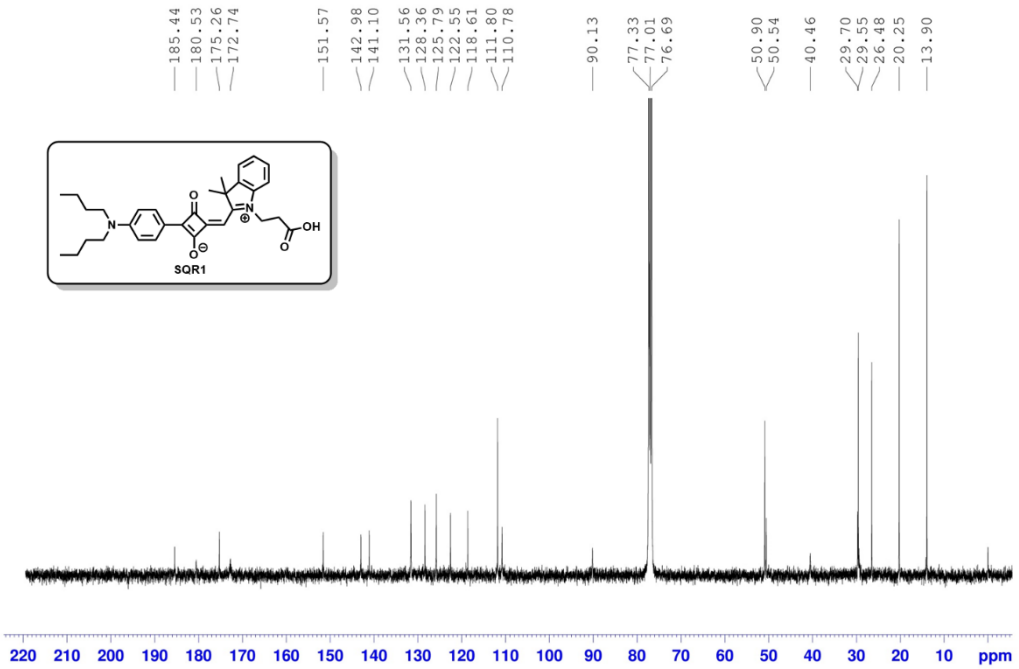
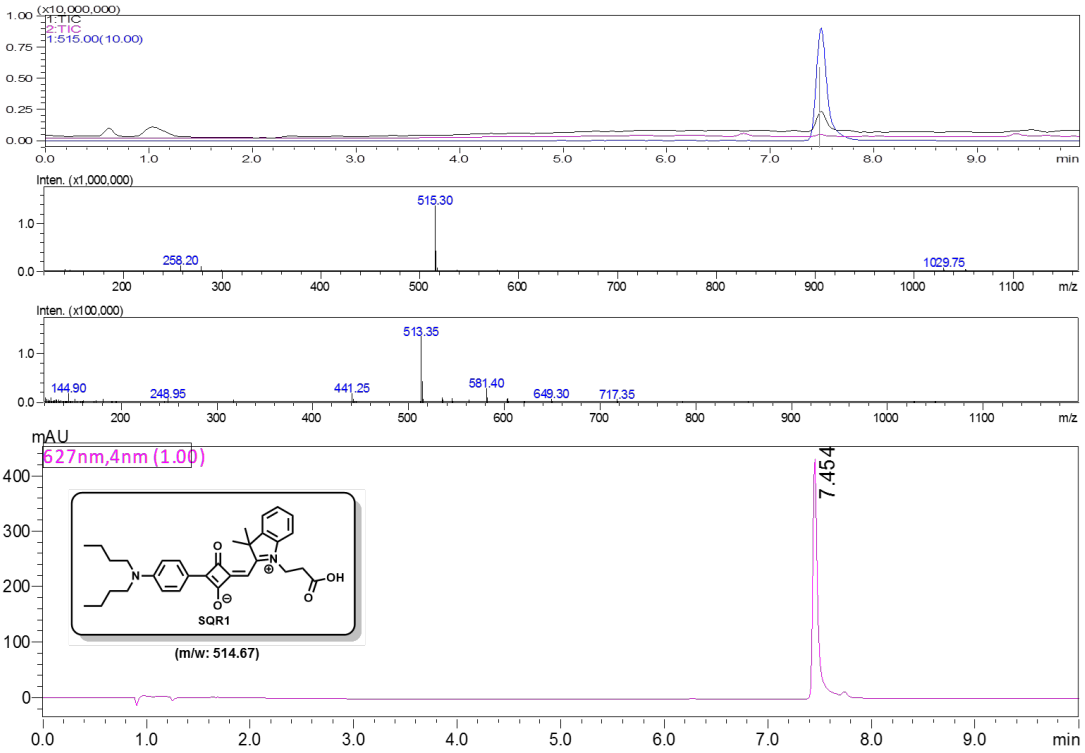
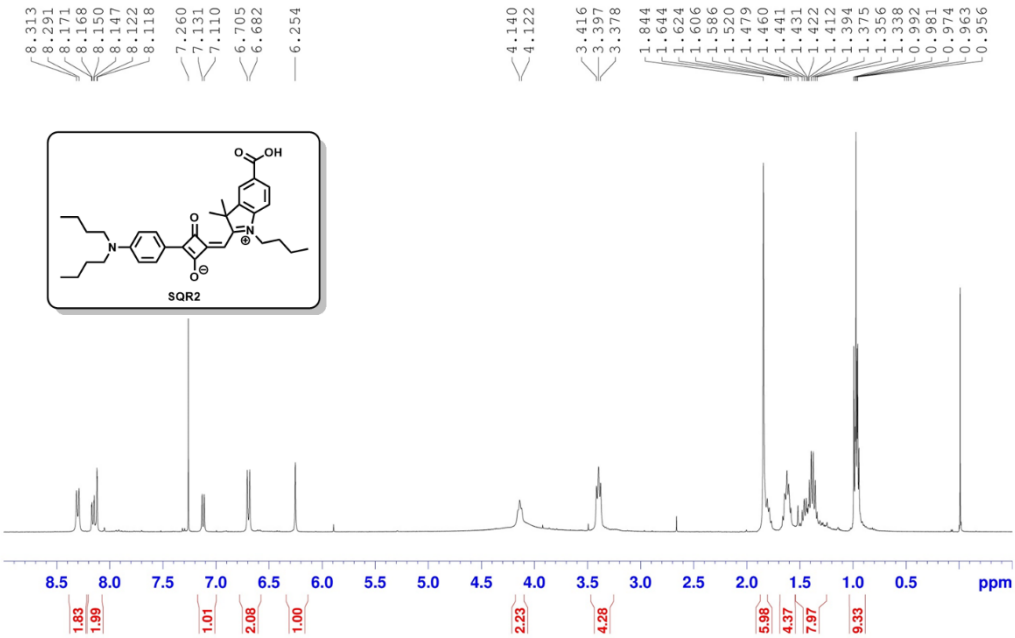

8.484
8.471
7.948
7.943
7.821
7.799
7.353
7.348
7.331
7.326
7.277
7.192
6.291
6.279
3.671
3.396
3.387
3.299
3.282
3.264
3.246
3.231
1.498
1.443
1.161
1.144
1.126
0.000
HN
N
H
N
O
O
Cl
N
7
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.20
1.22
1.20
1.22
1.00
1.16
2.33
2.29
5.96
9.40
2.05
3.35

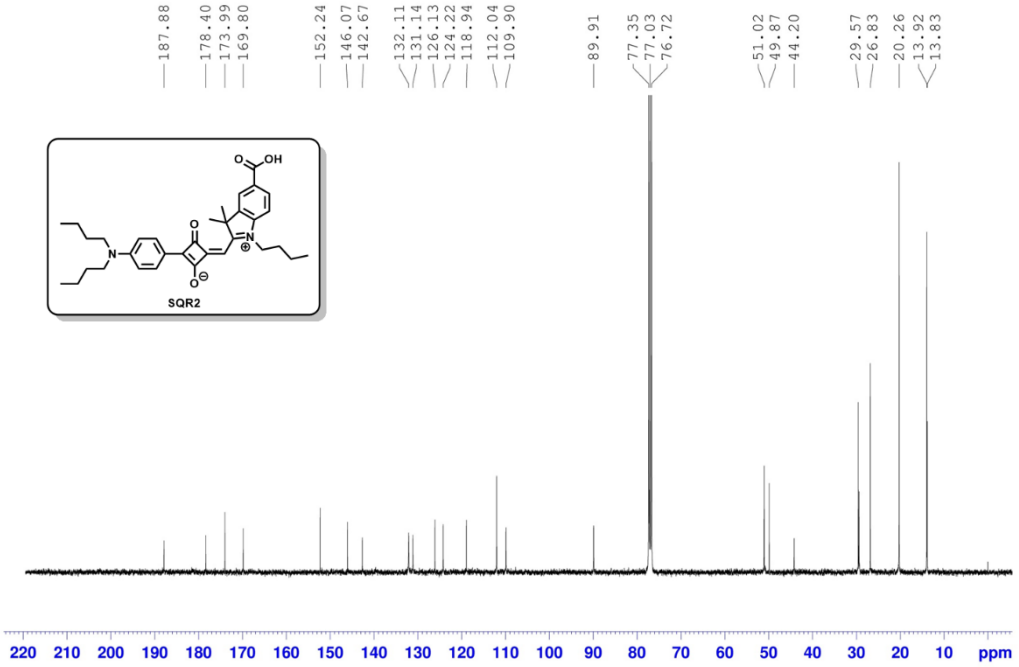

158.21
150.87
147.78
135.36
127.34
125.44
122.26
117.01
97.81
80.70
77.36
77.25
77.05
76.73
45.48
44.40
43.08
32.87
28.43
28.36
13.79
HN
N
H
N
O
O
Cl
N
7
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

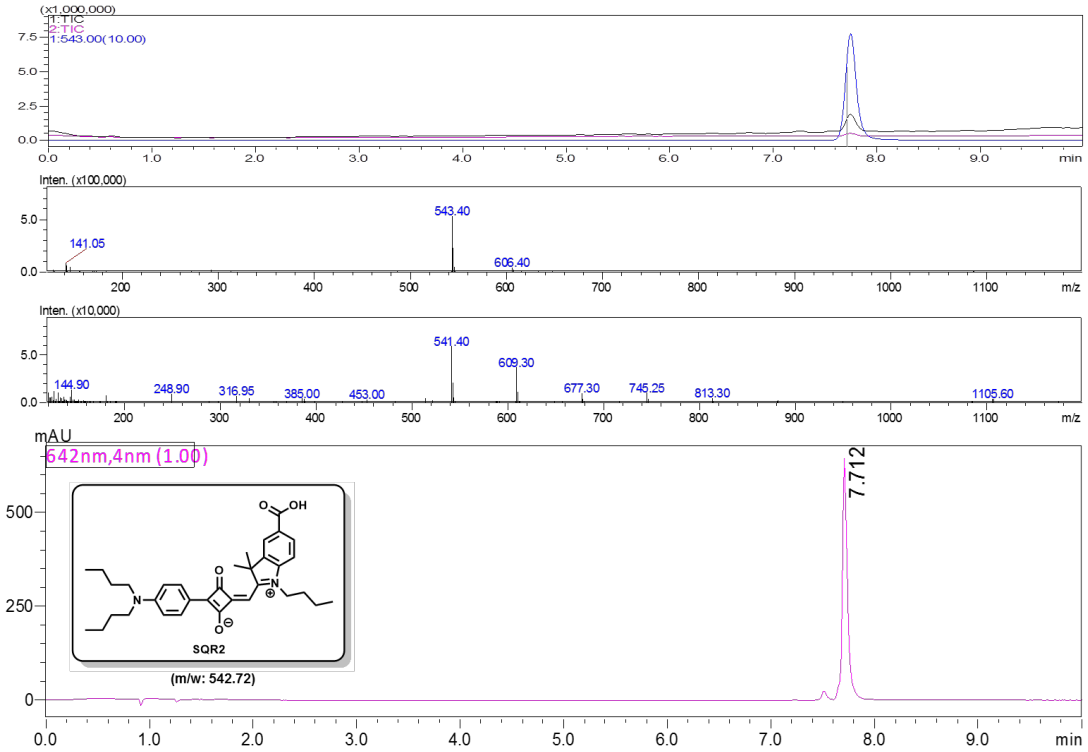

8.242
8.222
7.441
7.422
7.397
7.379
7.305
7.286
7.261
7.236
7.218
6.667
6.647
6.533
4.552
3.372
3.355
2.906
1.830
1.610
1.595
1.406
1.387
1.369
1.351
0.990
0.971
0.953
0.000
SQR1
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
2.13
4.93
2.05
1.01
2.07
4.05
2.18
6.13
4.08
4.24
6.26

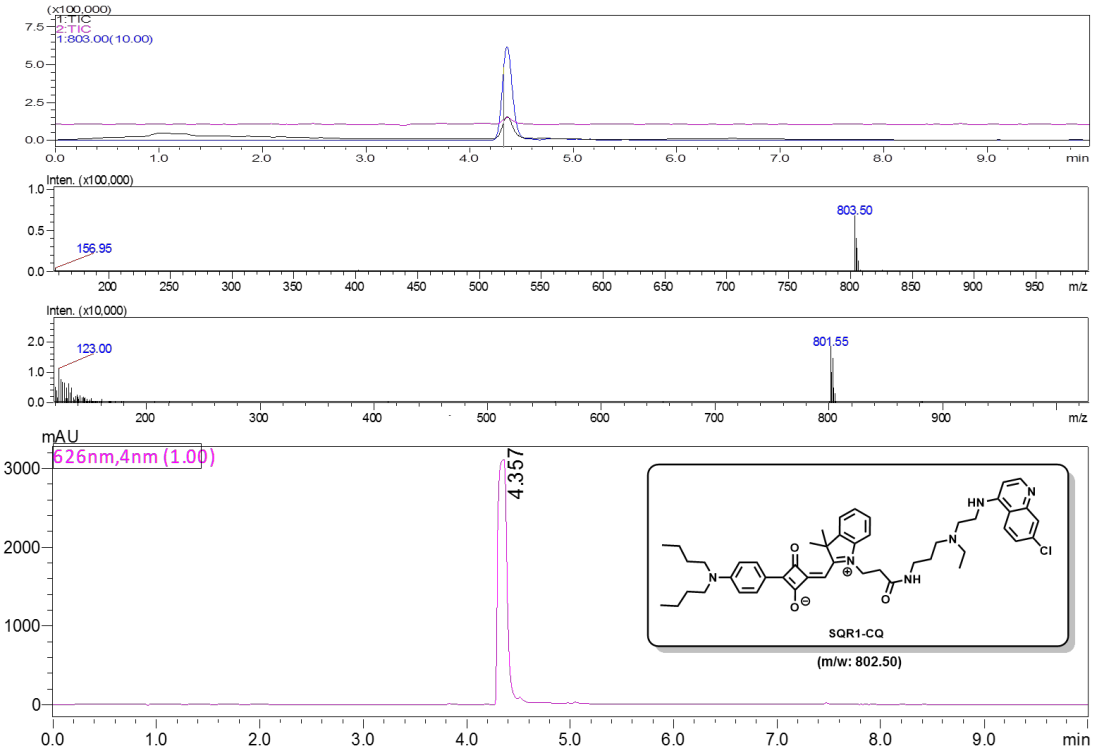

185.44
180.53
175.26
172.74
151.57
142.98
141.10
131.56
128.36
125.79
122.55
118.61
111.80
110.78
90.13
77.33
77.01
76.69
50.90
50.54
40.46
29.70
29.55
26.48
20.25
13.90
SQR1
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

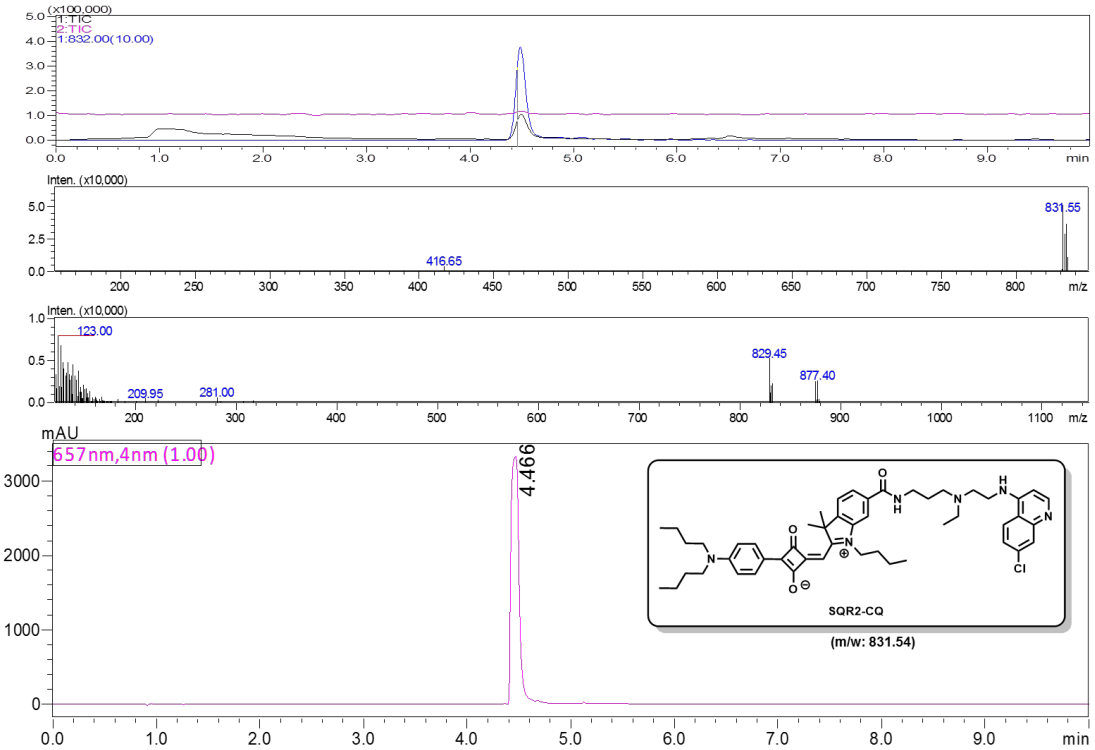

(x10,000,000)
1:TIC
2:TIC
1:515.00(10.00)
min
Inten. (x1,000,000)
515.30
258.20
1029.75
m/z
Inten. (x100,000)
513.35
144.90
248.95
441.25
581.40
649.30
717.35
m/z
mAU
627nm,4nm (1.00)
7.454
SQR1
(m/w: 514.67)
min


8.313
8.291
8.171
8.168
8.150
8.147
8.122
8.118
7.260
7.131
7.110
6.705
6.682
6.254
4.140
4.122
3.416
3.397
3.378
1.844
1.644
1.624
1.606
1.586
1.520
1.479
1.460
1.441
1.431
1.422
1.412
1.394
1.375
1.356
1.338
0.992
0.981
0.974
0.963
0.956
SQR2
ppm
1.83
1.99
1.01
2.08
1.00
2.23
4.28
5.98
4.37
7.97
9.33


187.88
178.40
173.99
169.80
152.24
146.07
142.67
132.11
131.14
126.13
124.22
118.94
112.04
109.90
89.91
77.35
77.03
76.72
51.02
49.87
44.20
29.57
26.83
20.26
13.92
13.83
SQR2
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


(x1,000,000)
1:TIC
2:TIC
1:543.00(10.00)
min
Inten. (x100,000)
543.40
141.05
606.40
m/z
Inten. (x10,000)
541.40
609.30
144.90
248.90
316.95
385.00
453.00
677.30
745.25
813.30
1105.60
m/z
mAU
542nm,4nm (1.00)
7.712
SQR2
(m/w: 542.72)
min


(x100,000)
1:TIC
2:TIC
1:803.00(10.00)
Inten. (x100,000)
803.50
156.95
Inten. (x10,000)
801.55
123.00
mAU
626nm,4nm (1.00)
4.357
SQR1-CQ
(m/w: 802.50)
min


(x100,000)
1:TIC
2:TIC
1:832.00(10.00)
Inten. (x10,000)
831.55
416.65
Inten. (x10,000)
123.00
829.45
877.40
209.95
281.00
mAU
657nm,4nm (1.00)
4.466
SQR2-CQ
(m/w: 831.54)
min

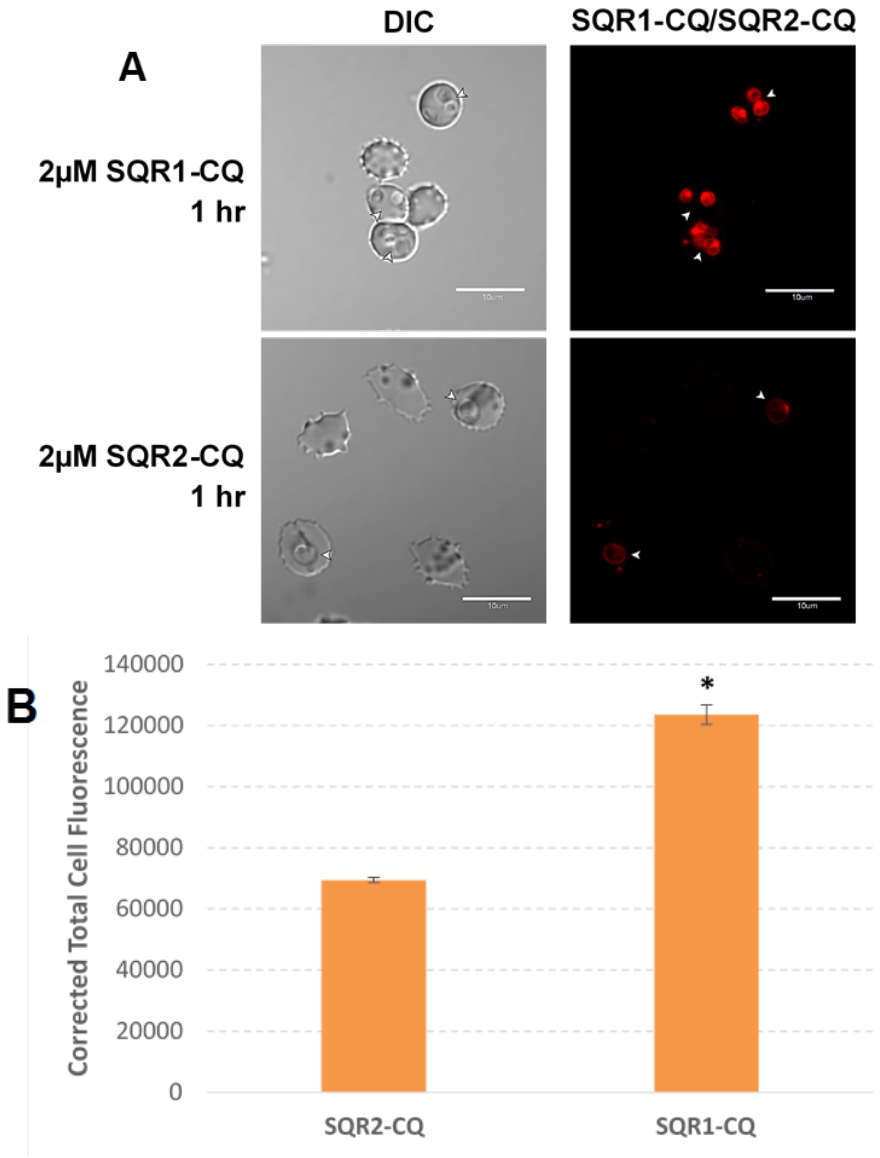


Figure S1. Comparison between **SQR1-CQ** and **SQR2-CQ**. (**A**) P. falciparum cultures were synchronised and labelled with either **SQR1-CQ** or **SQR2-CQ** and analysed via confocal microscopy. Representative confocal images of P. falciparum trophozoites labelled with either **SQR1-CQ** or **SQR2-CQ**, obtained at identical exposure and gain settings. We observe **SQR1-CQ** consistently exhibits higher fluorescence than **SQR2-CQ** (arrowheads). Both **SQR1-CQ** and **SQR2-CQ** were observed to sequester within cytoplasmic space of the parasites and not in RBCs. Surrounding unparasitized RBCs were not observed to retain **SQR1-CQ** or **SQR2-CQ**. Bar = 10 μm. (**B**) Analysis of corrected total cell fluorescence (CTCF) of trophozoites labelled with either **SQR1-CQ** or **SQR21-CQ**. Bar chart of mean CTCF from at least 3 ROI with error bars denoting standard deviation. * denotes statistical significance (p-value <0.01) compared to mean CTCF of **SQR2-CQ**.

After successful linkage of candidate squaraine compounds to CQ, **SQR1-CQ** and **SQR2-CQ** were assessed in their efficacy in fluorescence labelling of P. falciparum parasites. Both **SQR1-CQ** and **SQR2-CQ** were capable of specifically labelling only parasite cells and not the housing RBCs (Figure S1). We also did not detect **SQR1-CQ** and **SQR2-CQ** in unparasitized RBCs. Between **SQR1-CQ** and **SQR2-CQ**, we consistently observed that SQR1-CQ exhibited a stronger fluorescence signal from parasite cells than **SQR2-CQ**. We focused on further assessment of only **SQR1-CQ** for the remainder of the study.

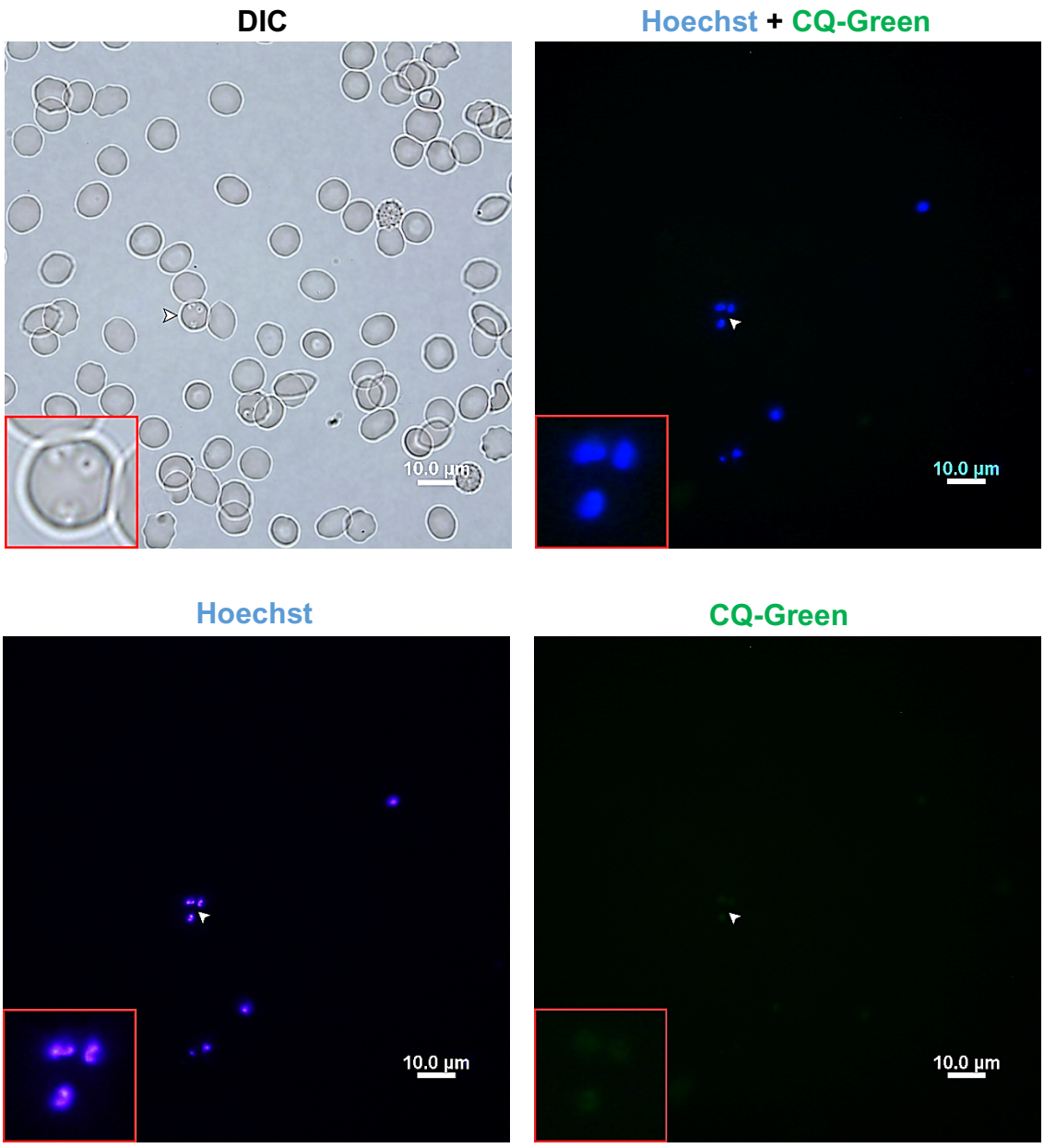


Figure S2. CQ-Green labelling of 3D7 ring trophozoites. Live 3D7 ring-form trophozoites were co-labelled with Hoechst 33342 and 2µM CQ-Green and analyzed via confocal microscopy. 3D7 rings were unable to be labelled vibrantly by CQ-Green, despite an extended incubation duration of 2 hours. Faint fluorescence signature of CQ-Green was not distinctly discernible in Hoechst-CQ-Green composite images. Insets of ring trophozoites indicated by arrowheads were magnified at further 5×. Bar = 10 µm.