Supplementary material to:

R.M. Beteck, M. Isaacs, H.C. Hoppe and S.D. Khanye,

Synthesis, *in vitro* Cytotoxicity and Trypanocidal Evaluation of Novel 1,3,6-Substituted Non-fluoroquinolones

S. Afr. J. Chem., 2018, **71**, 188–195.

Synthesis, *in vitro* cytotoxicity and trypanocidal evaluation of novel 1,3,6-substituted non-fluoroquinolones.

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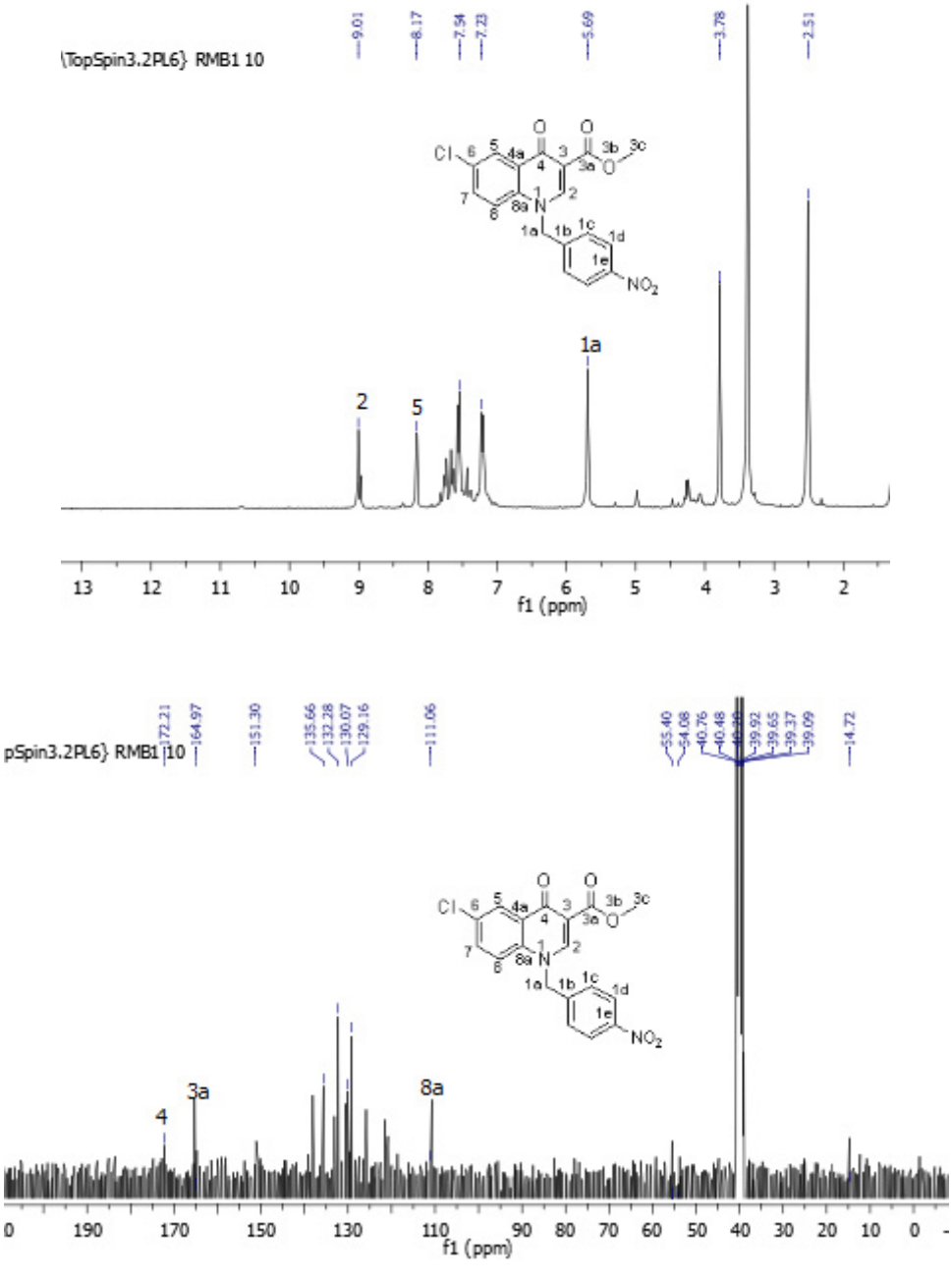
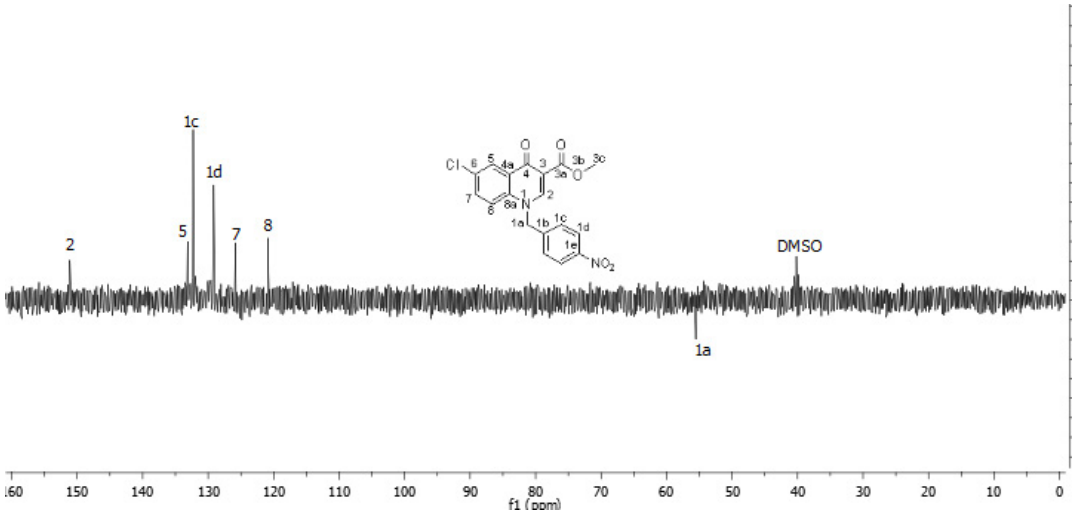
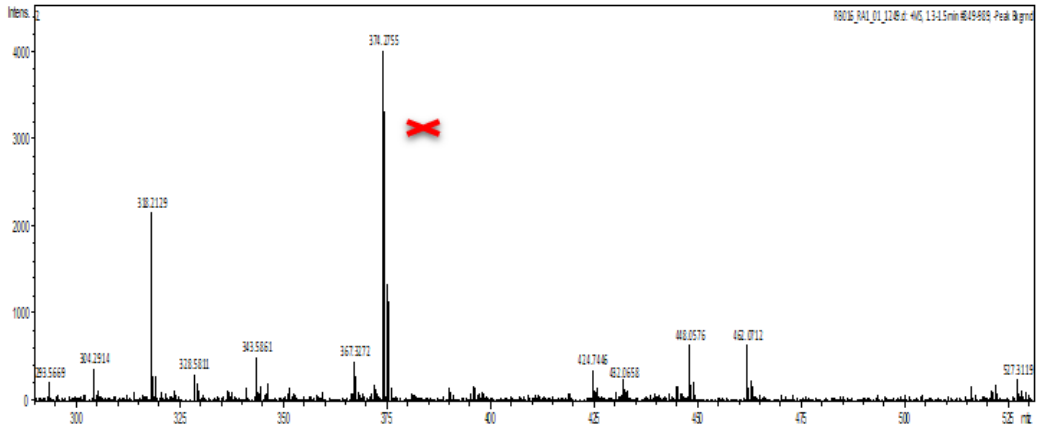
NMR (^{1}H, ^{13}C, DEPT135) AND MS SPECTRA AND BIOLOGICAL DATA OF COMPOUNDS

Compound 3a




1c
1d
2
5
7
8
DMSO
1a
f1 (ppm)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462.0712
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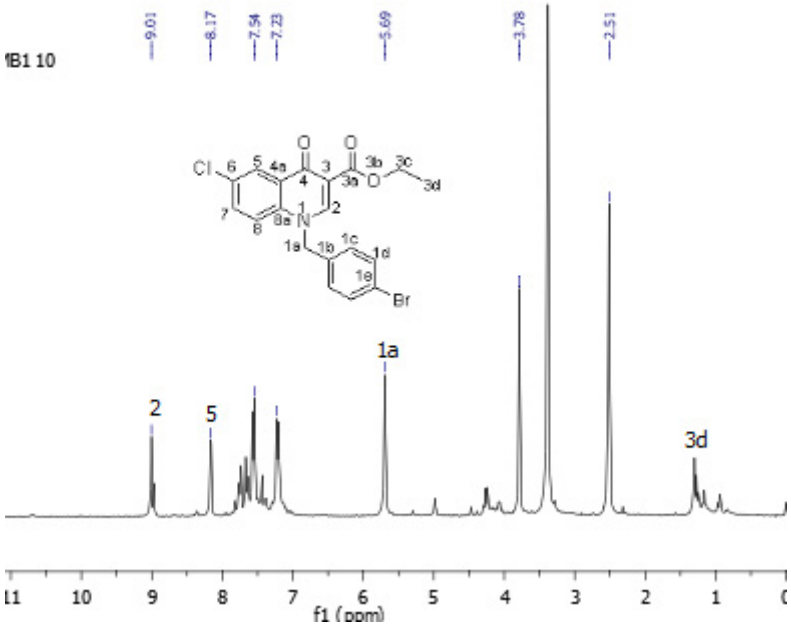
mlz

Compound 3b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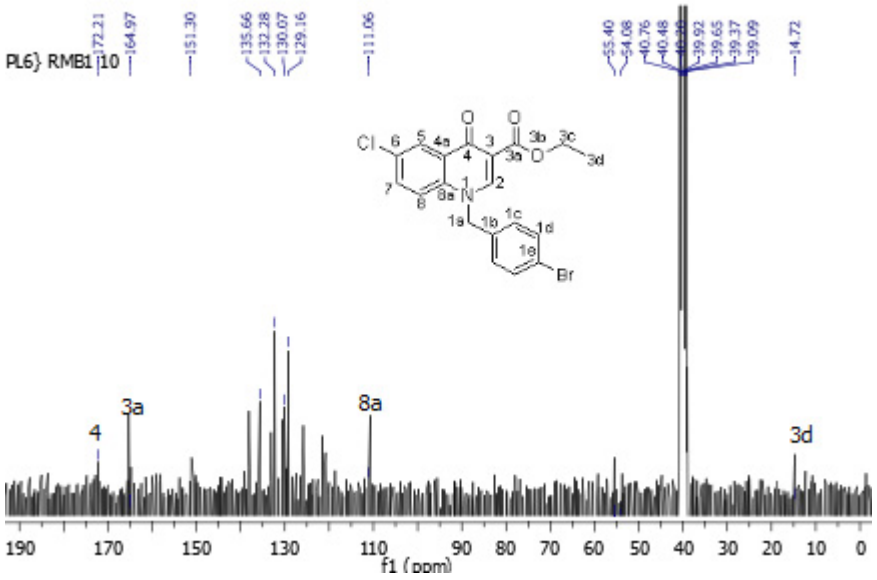
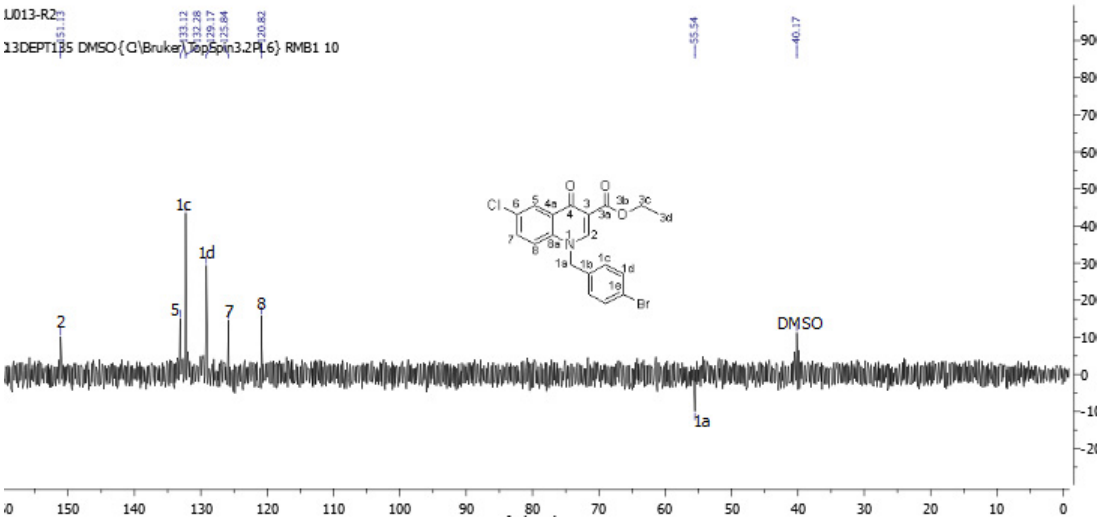
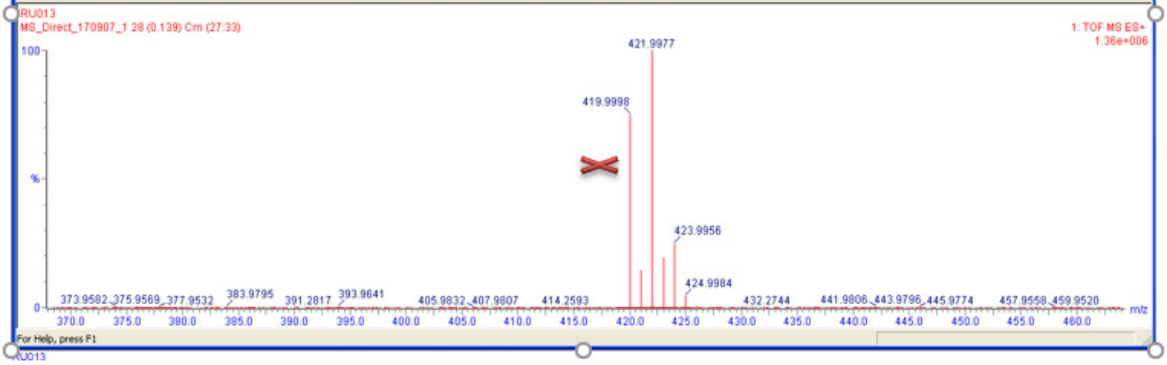
5.69
3.78
2.51
1a
2
5
3d
f1 (ppm)

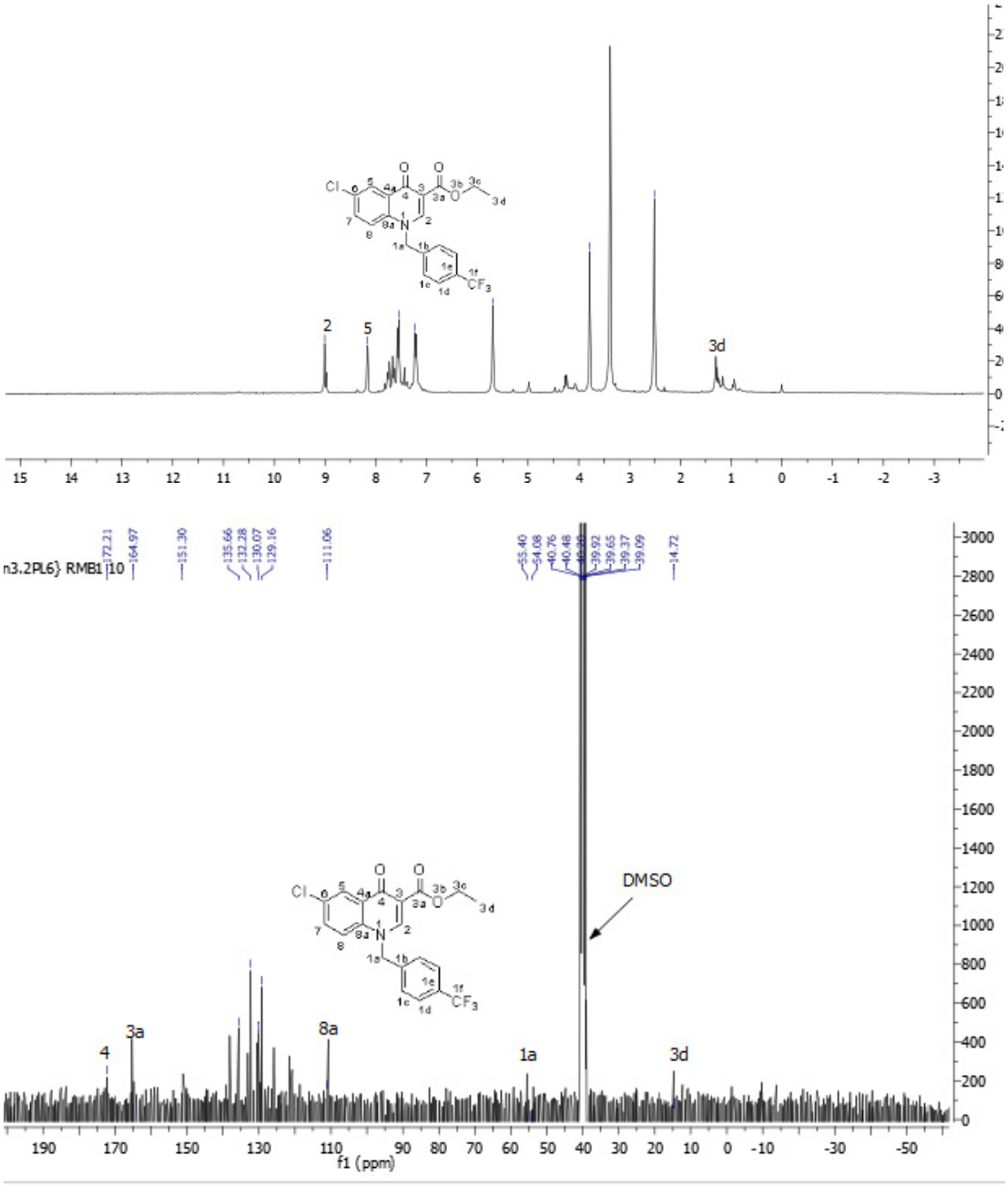


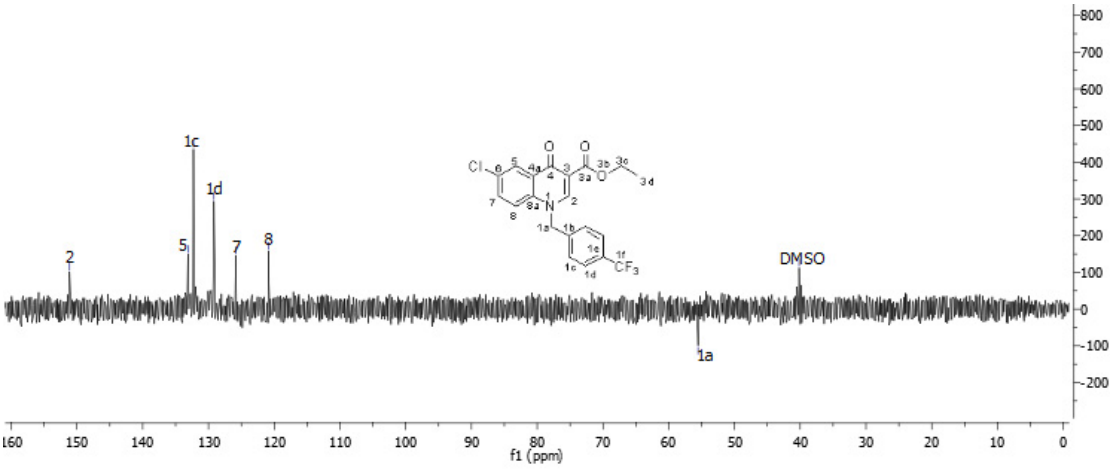


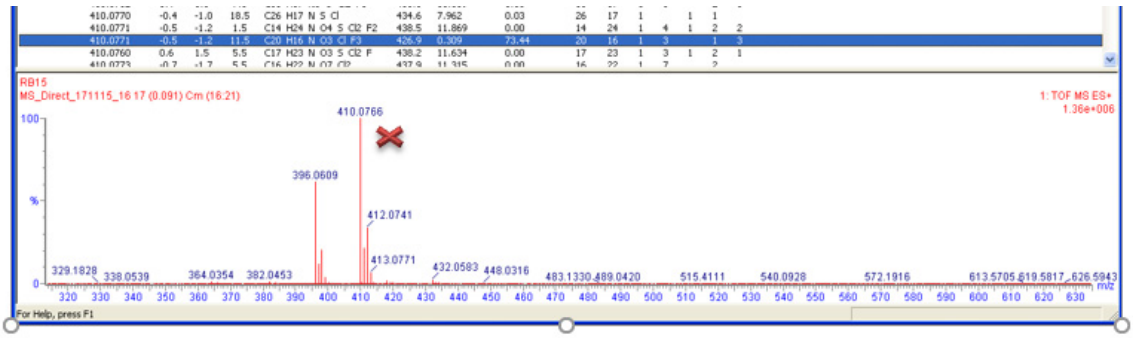
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Cl	Br
419.9998	420.0002	-0.4	-1.0	11.5	C19 H16 N O3 Cl Br	430.7	2.399	9.08	19	16	1	3	1	1
	420.0002	-0.4	-1.0	3.5	C8 H15 N5 O10 Br	440.3	12.042	0.00	8	15	5	10		1
	419.9992	0.6	1.4	18.5	C20 H6 N O10	454.0	25.711	0.00	20	6	1	10		
	420.0005	-0.7	-1.7	23.5	C21 H2 N5 O6	454.1	25.746	0.00	21	2	5	6		
	419.9984	1.4	3.3	16.5	C20 H11 N3 O3 Br	438.3	9.989	0.00	20	11	3	3		1
	419.9983	1.5	3.6	14.5	C15 H7 N5 O8 Cl	443.2	14.892	0.00	15	7	5	8	1	
	420.0021	-2.3	-5.5	-1.5	C7 H20 N3 O10 Cl Br	438.1	9.763	0.01	7	20	3	10	1	1
	420.0023	-2.5	-6.0	18.5	C20 H7 N3 O6 Cl	442.7	14.371	0.00	20	7	3	6	1	
	420.0024	-2.6	-6.2	20.5	C25 H11 N O Br	439.0	10.669	0.00	25	11	1	1		1



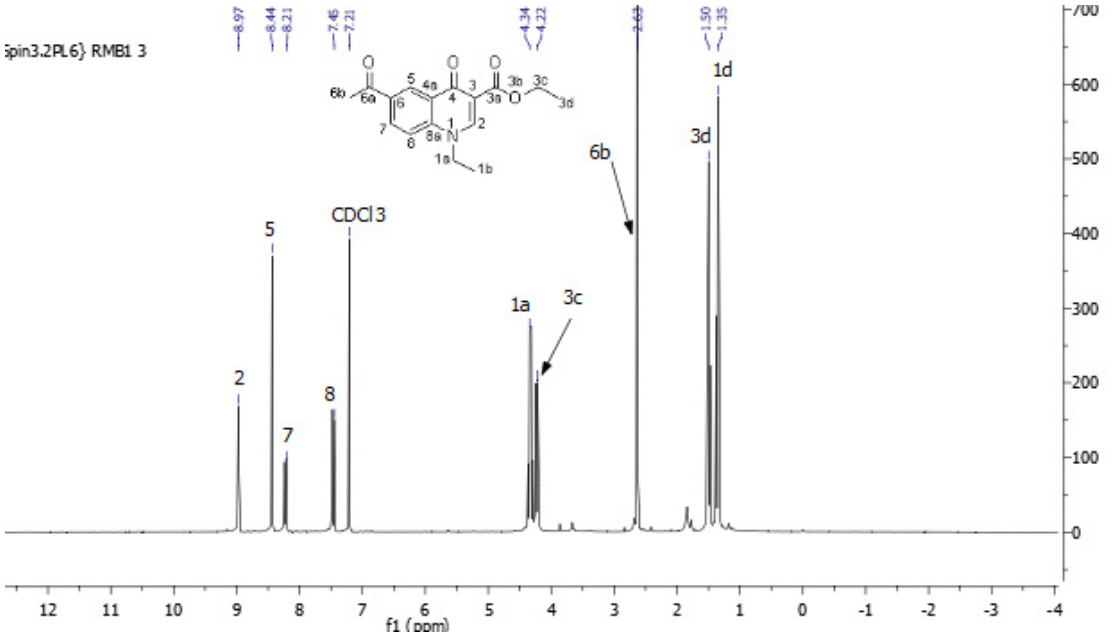
Compound 3c

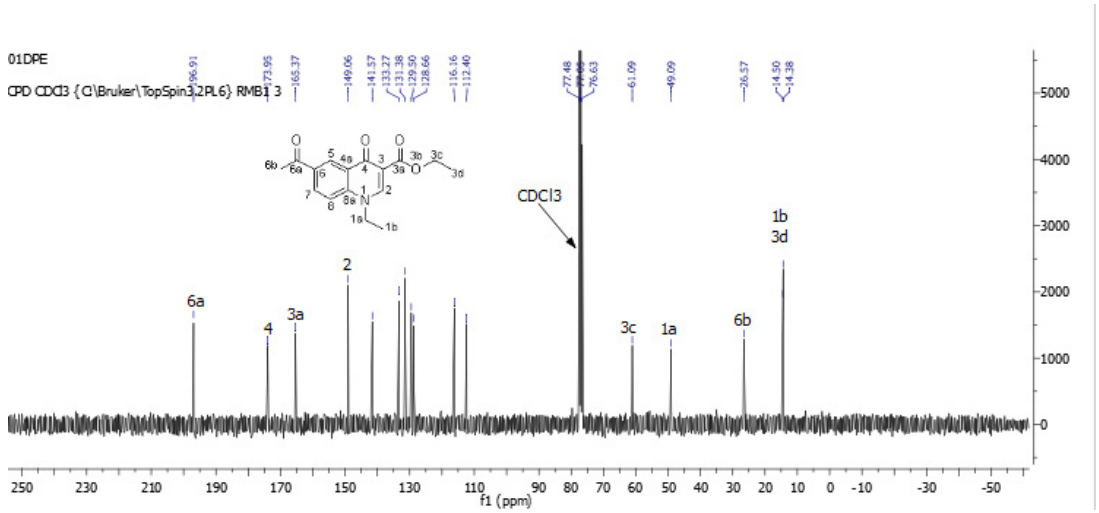

2
5
3d
n3.2PL6} RMB1 j10
172.21
164.97
151.30
135.66
132.28
130.07
129.16
111.06
55.40
54.08
40.76
40.48
39.92
39.65
39.37
39.09
14.72
DMSO
4
3a
8a
1a
3d
f1 (ppm)

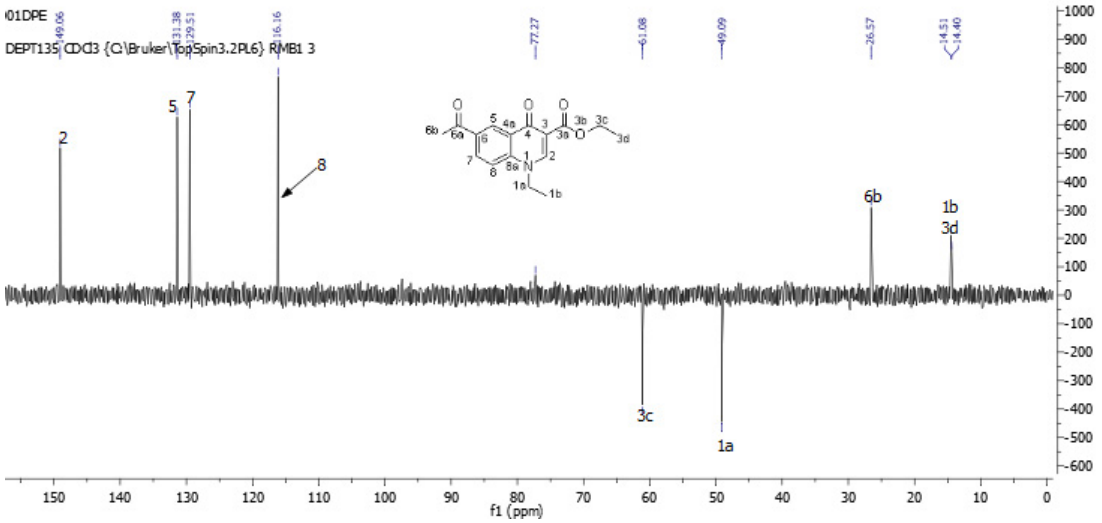

1c
1d
2
5
7
8
DMSO
1a
f1 (ppm)


RB15
MS_Direct_171115_16 17 (0.091) Cm (16:21)
1: TOF MS ES+
1.36e+006
410.0766
396.0609
412.0741
413.0771
432.0583
448.0316
329.1828
338.0539
364.0354
382.0453
483.1330 489.0420
515.4111
540.0928
572.1916
613.5705 619.5817 626.5943
m/z
For Help, press F1

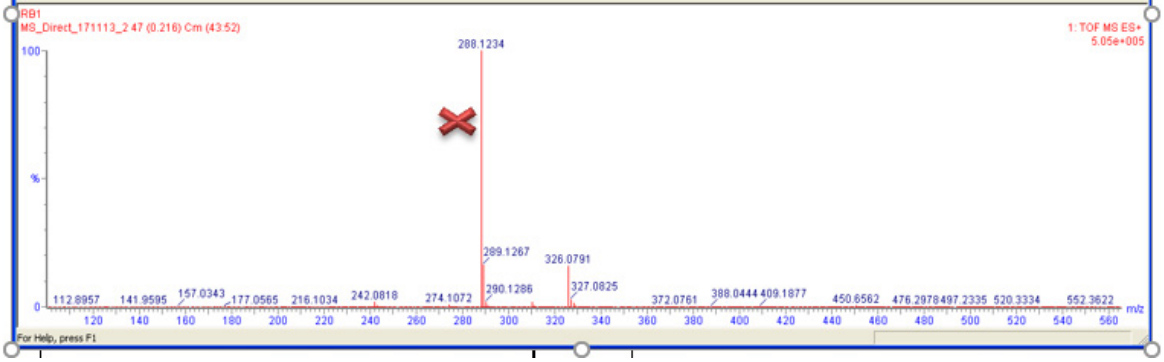
Compound 3d


8.97
8.44
8.21
7.45
7.21
4.34
4.22
2.63
1.50
1.35
1d
3d
6b
CDCl3
5
1a
3c
2
8
7
f1 (ppm)

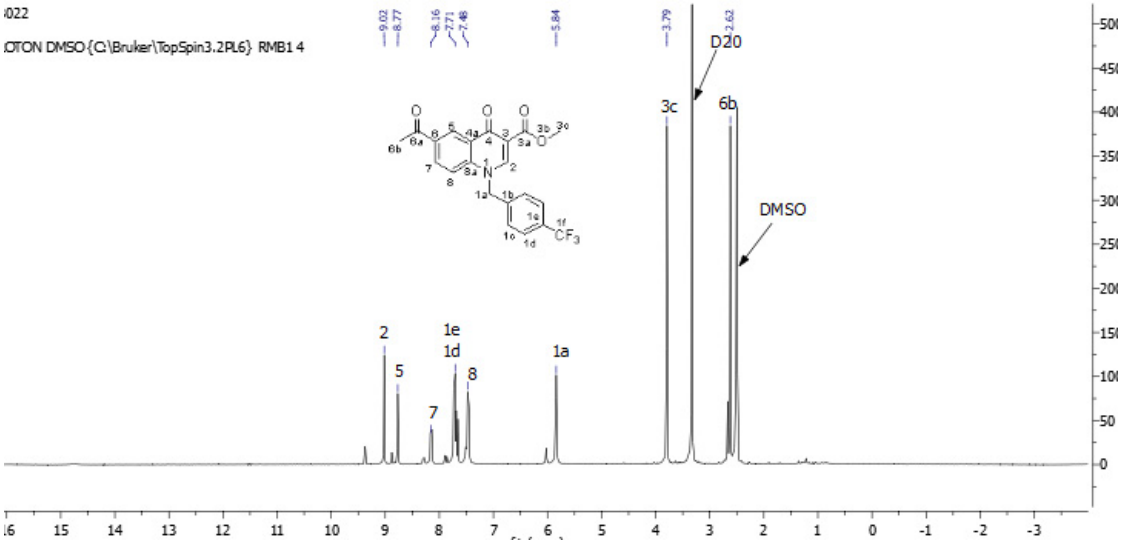


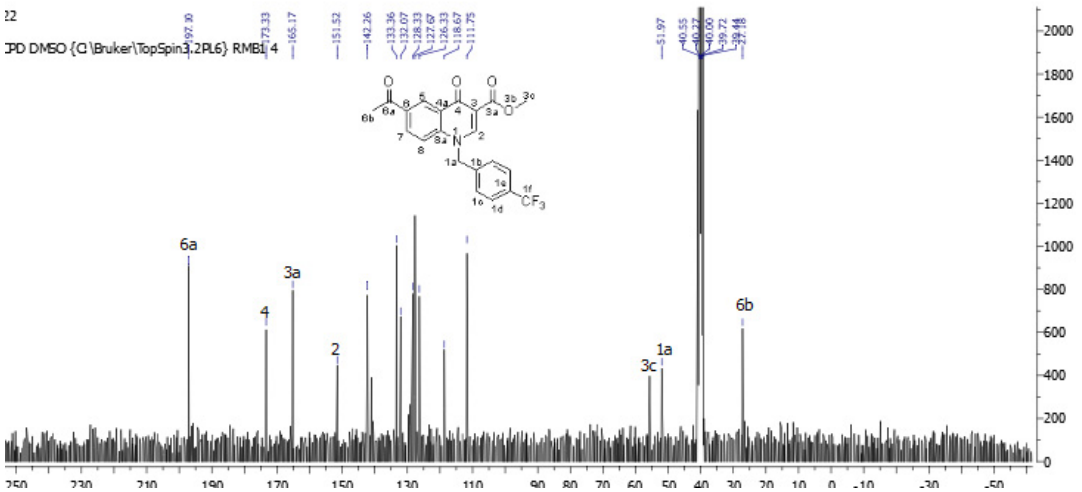


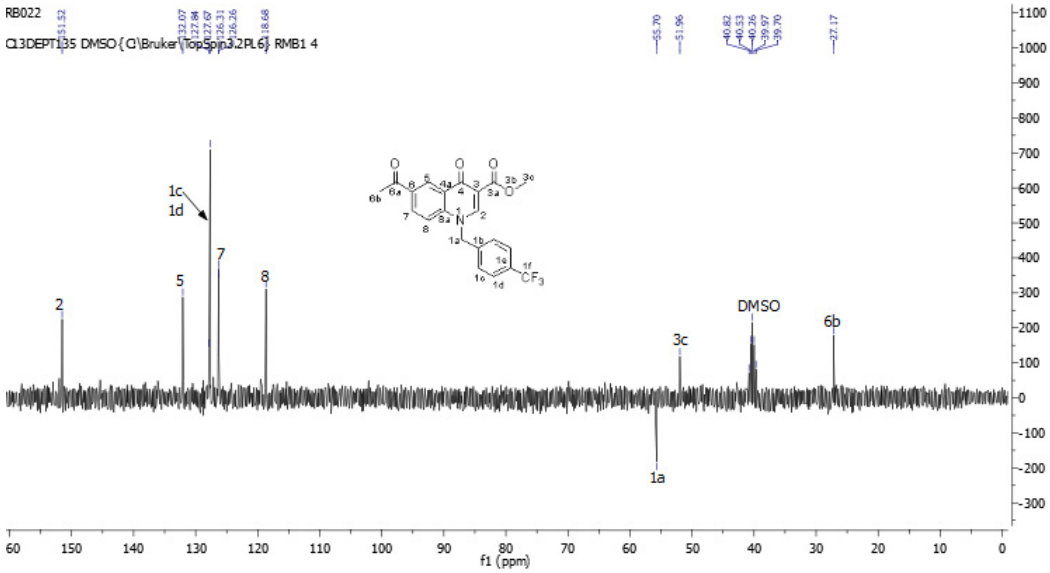
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
288.1234	288.1236	-0.2	-0.7	8.5	C16 H18 N O4	204.7	5.403	0.45	16	18	1	4
	288.1249	-1.5	-5.2	13.5	C17 H14 N5	208.3	8.974	0.01	17	14	5	
	288.1196	3.8	13.2	4.5	C11 H18 N3 O6	205.4	6.103	0.22	11	18	3	6
	288.1308	-7.4	-25.7	4.5	C10 H18 N5 O5	208.0	8.694	0.02	10	18	5	5
	288.1155	7.9	27.4	0.5	C6 H18 N5 O8	212.8	13.455	0.00	6	18	5	8
	288.1137	9.7	33.7	13.5	C18 H14 N3 O	210.2	10.914	0.00	18	14	3	1
	288.1348	-11.4	-39.6	8.5	C15 H18 N3 O3	205.0	5.701	0.33	15	18	3	3
	288.1097	13.7	47.5	9.5	C13 H14 N5 O3	199.3	0.010	98.96	13	14	5	3

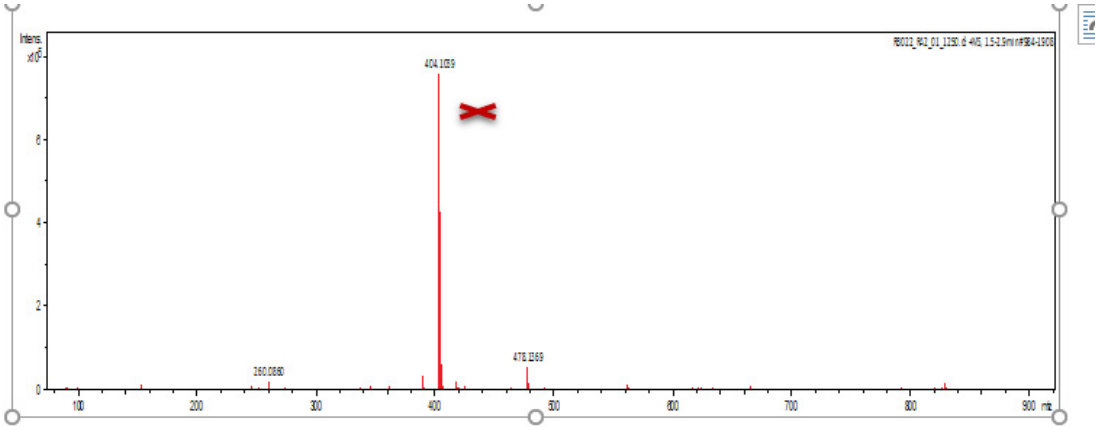


Compound 3e

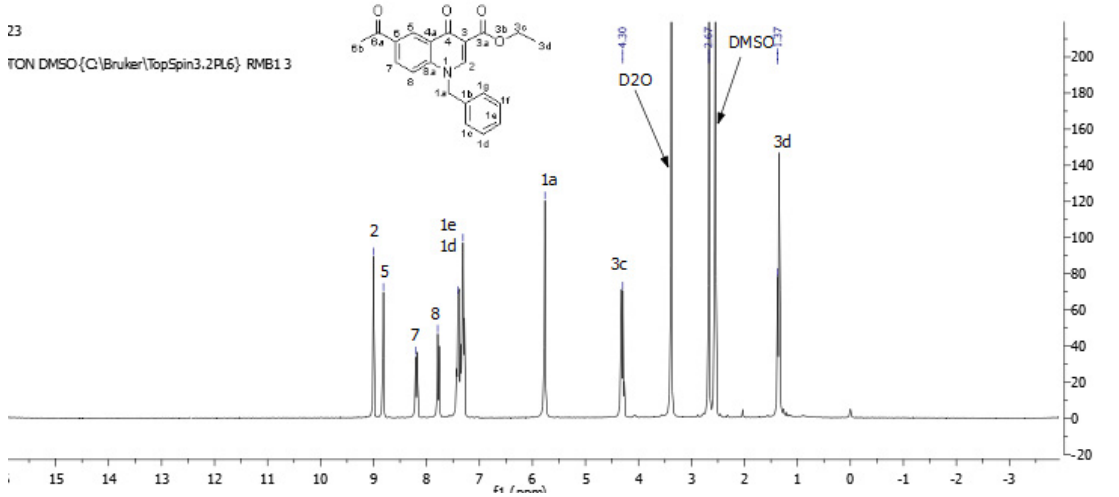

D2O
3c
6b
DMSO
2
5
1e
1d
7
8
1a

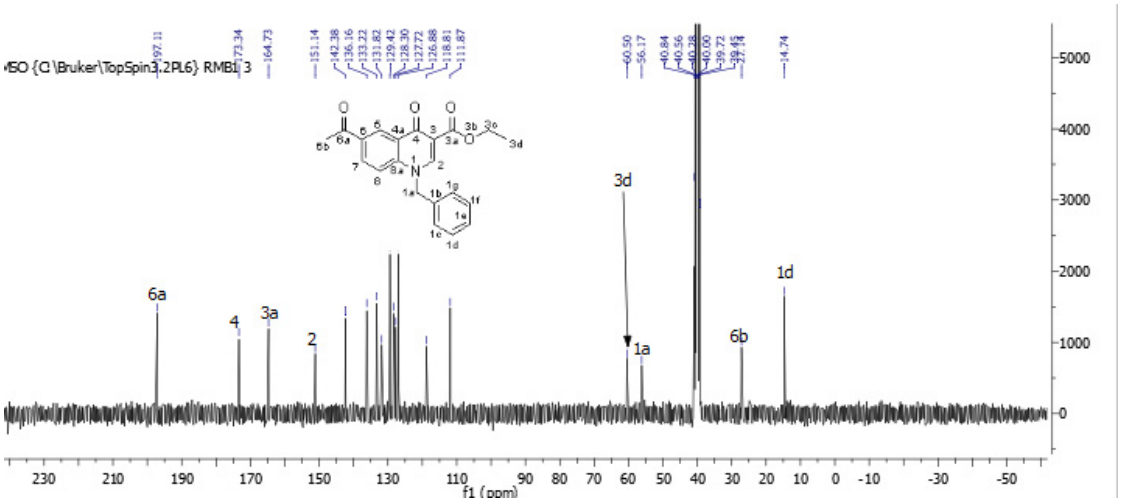

6a
3a
4
2
3c
1a
6b

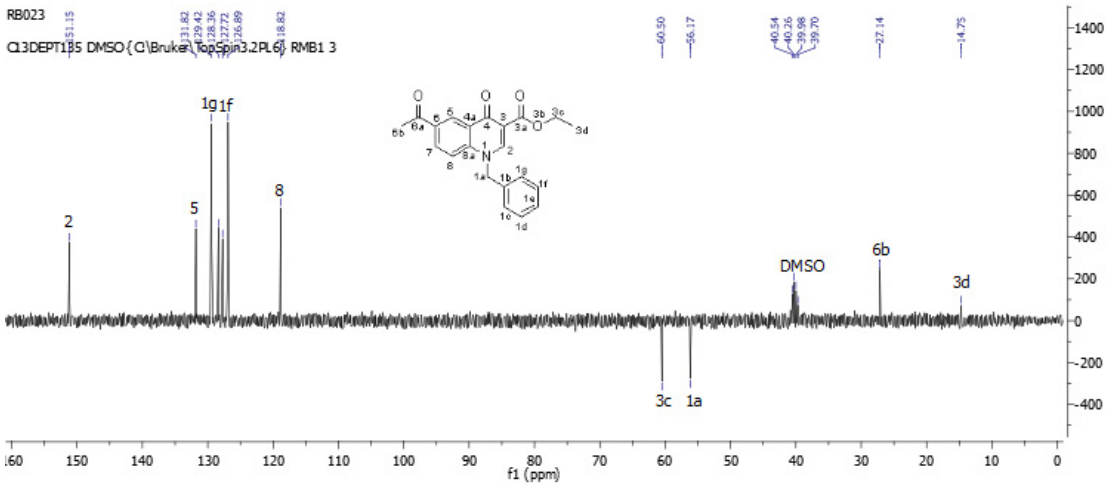

1c
1d
7
5
8
2
DMSO
3c
6b
1a
f1 (ppm)

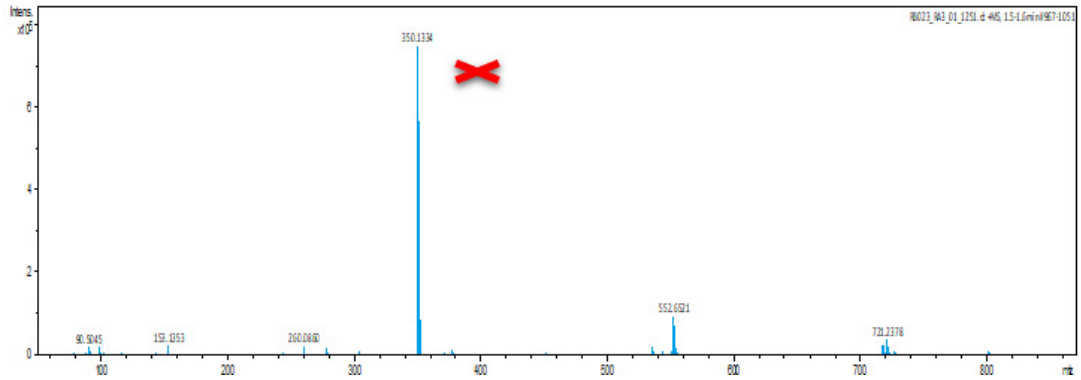

Intens.
404.1039
260.0860
478.1369
100
200
300
400
500
600
700
800
900 m/z

Compound 3f


23
TON DMSO{C:\Bruker\TopSpin3.2PL6} RMB1 3
D2O
DMSO
3d
1a
2
5
1e
1d
3c
8
7
f1 (ppm)


MSO {C:\Bruker\TopSpin3.2PL6} RMB1 3
3d
1d
6a
4
3a
2
1a
6b
f1 (ppm)


RB023
C13DEPT135 DMSO{C:\Bruker\TopSpin3.2PL6} RMB1 3
1g
1f
2
5
8
DMSO
6b
3d
3c
1a
f1 (ppm)


Intens.
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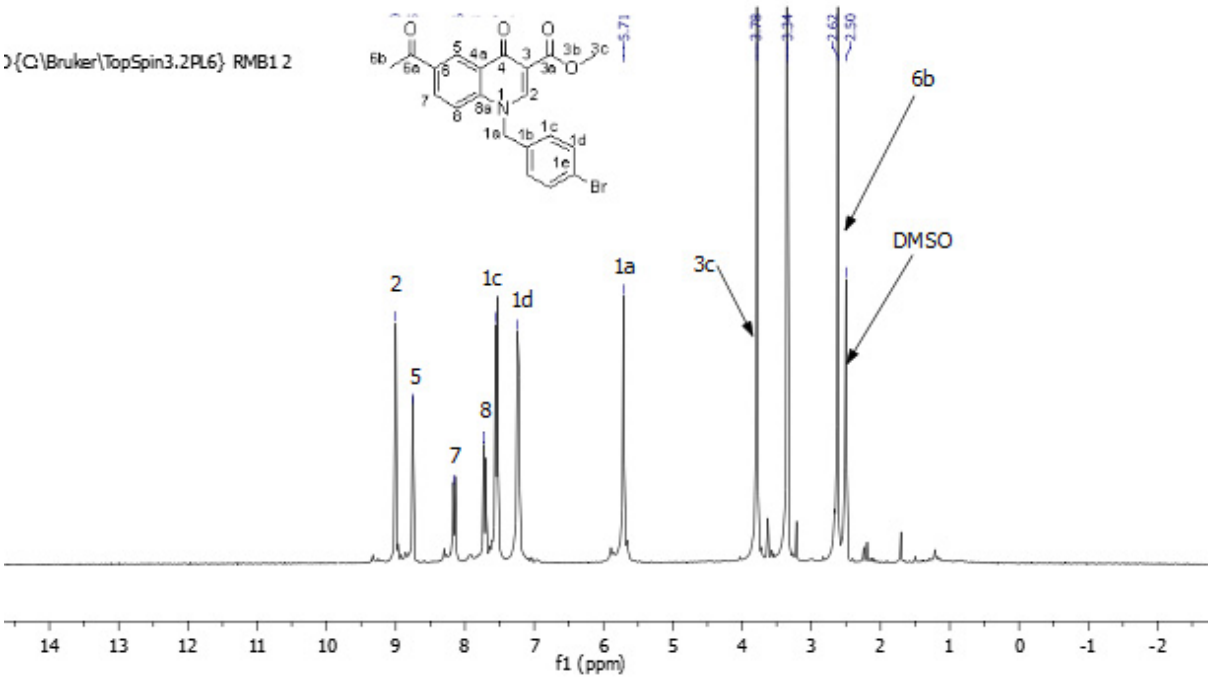
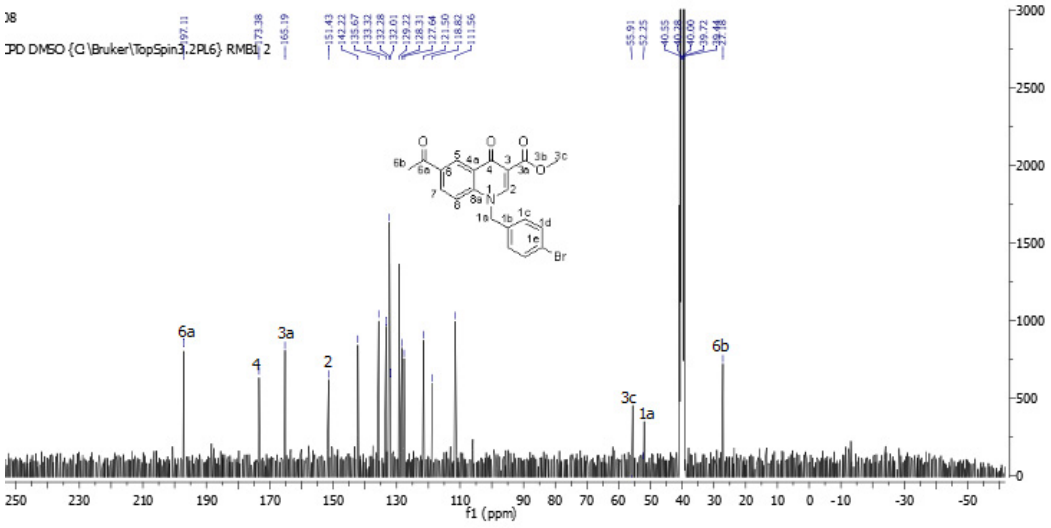
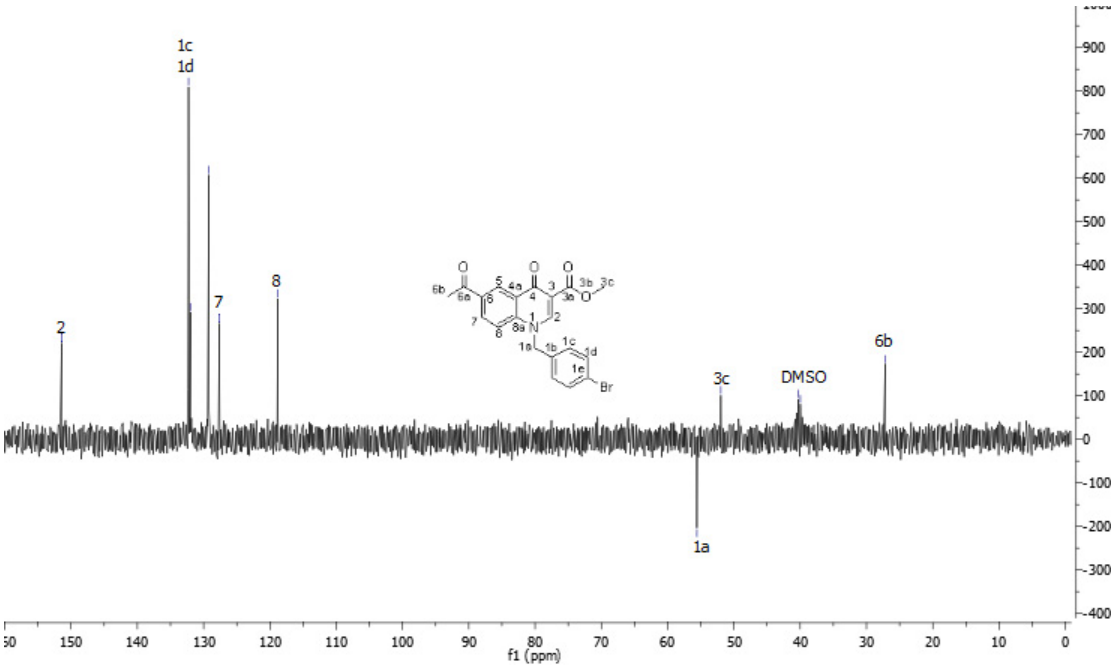
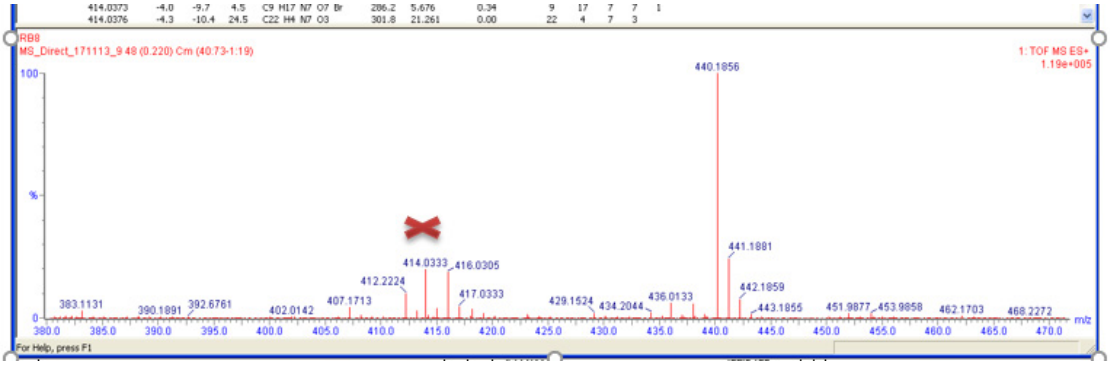
720.2378
m/z

Compound 3g


){C:\Bruker\TopSpin3.2PL6} RMB1 2
Br
6b
DMSO
3c
1a
2
1c
1d
5
8
7
f1 (ppm)


JPD DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 2
6a
3a
4
2
3c
1a
6b
f1 (ppm)


1c
1d
2
7
8
3c
DMSO
6b
1a
f1 (ppm)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1.19e+005
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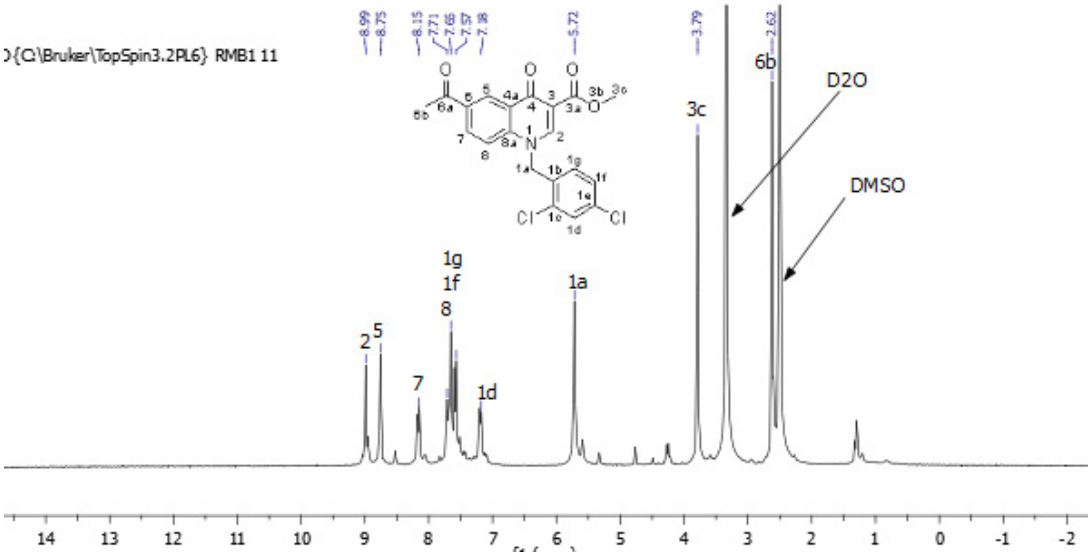
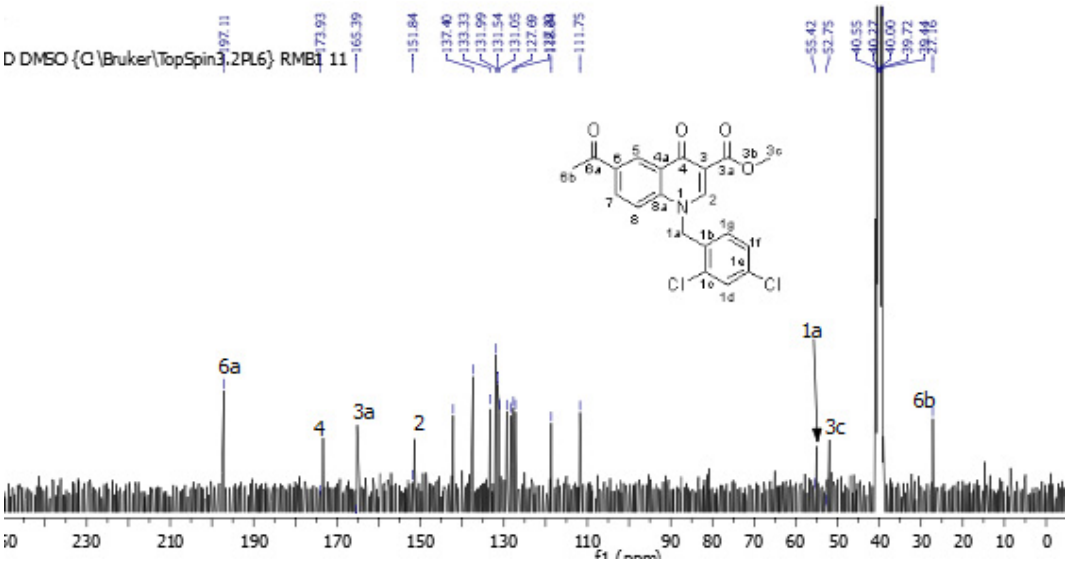
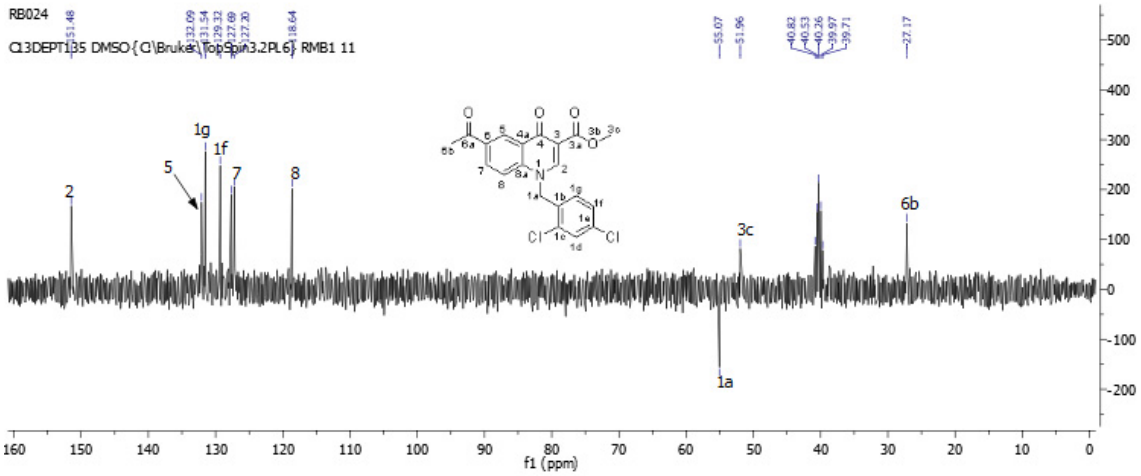
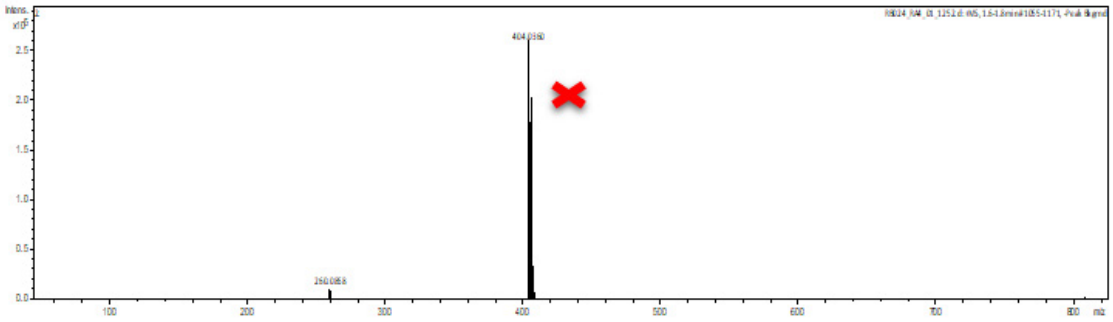
442.1859
414.0333
416.0305
412.2224
417.0333
383.1131
390.1891
392.6761
402.0142
407.1713
429.1524
434.2044
436.0133
443.1855
451.9877
453.9858
462.1703
468.2272
m/z

Compound 3h

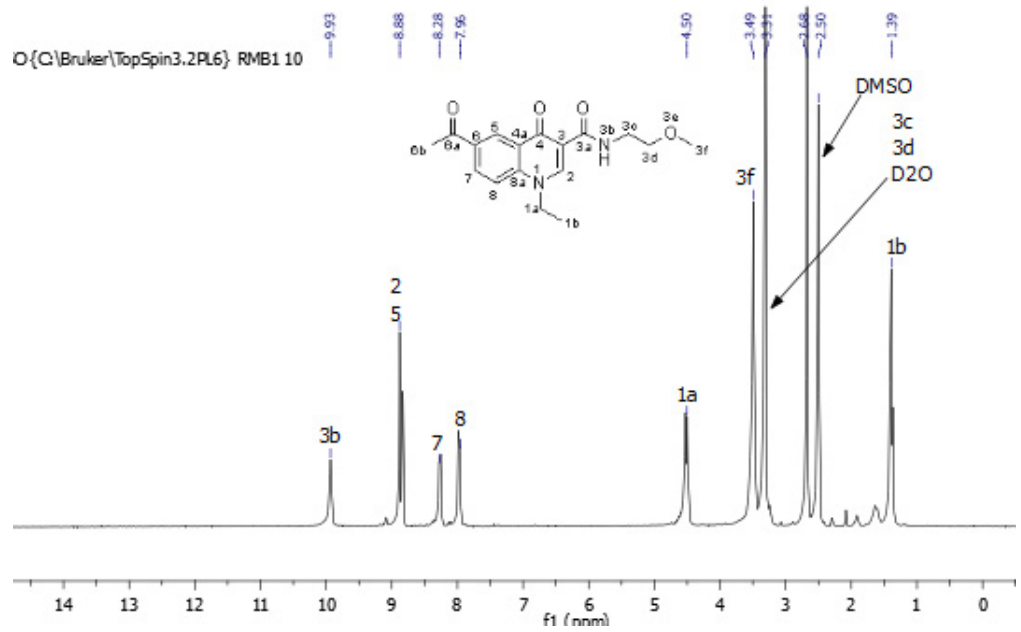

D2O
DMSO
6b
3c
1g
1f
8
2
5
7
1d
1a

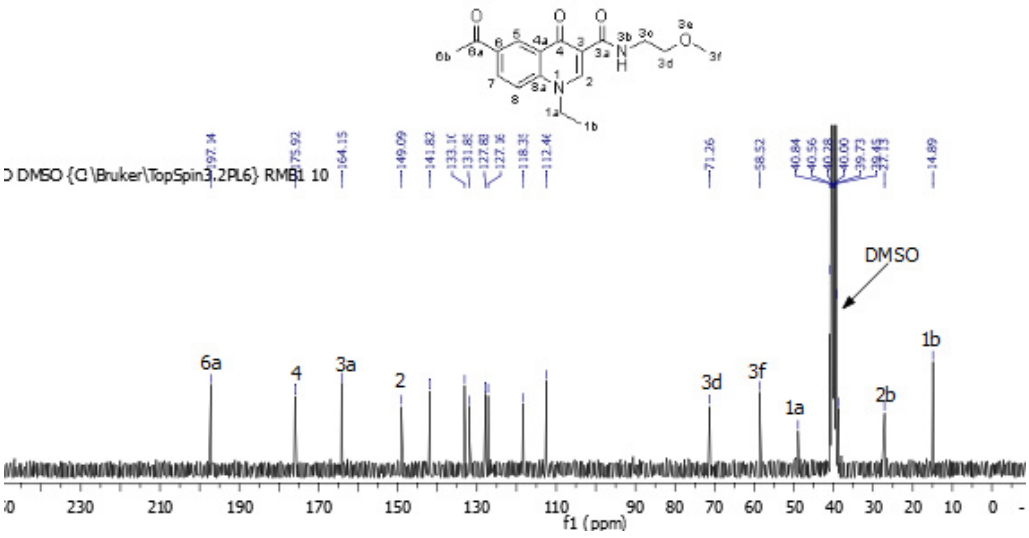

6a
4
3a
2
1a
3c
6b

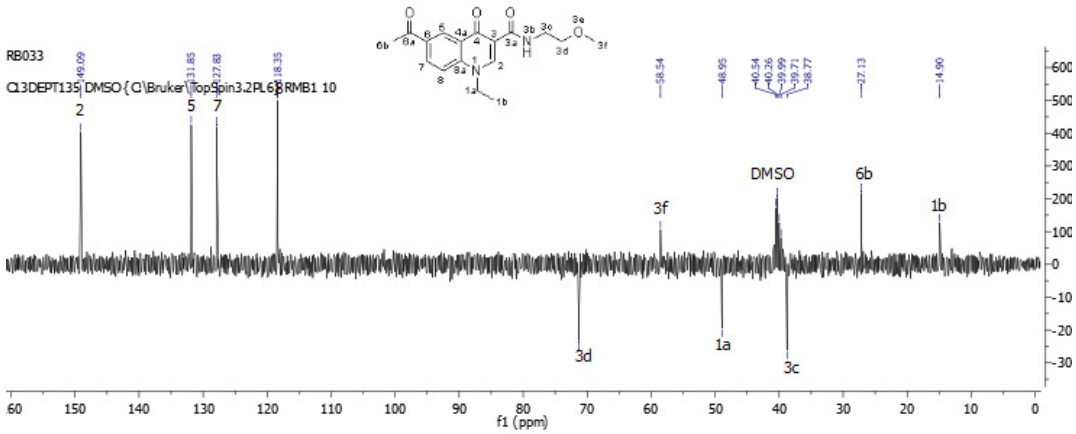

RB024
1g
1f
5
7
8
2
3c
6b
1a
f1 (ppm)



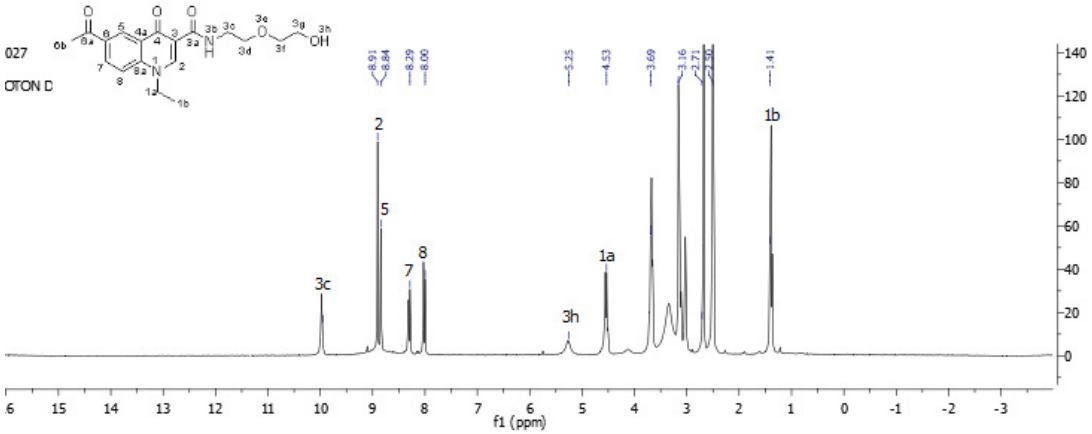
Compound 4a

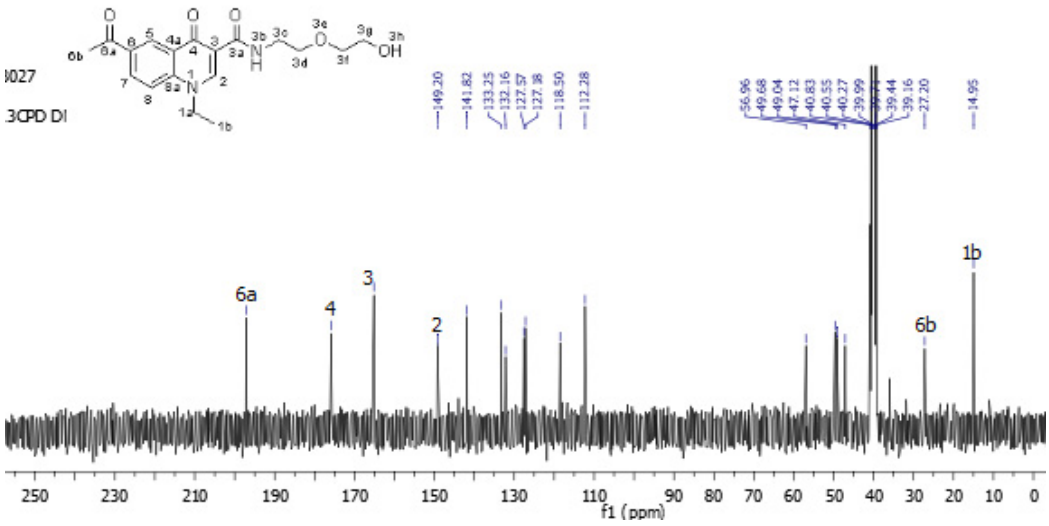

O{C:\Bruker\TopSpin3.2PL6} RMB1 10
9.93
8.88
8.28
7.96
4.50
3.49
3.31
2.68
2.50
1.39
DMSO
3c
3d
D2O
3f
1b
2
5
1a
3b
8
7
f1 (ppm)

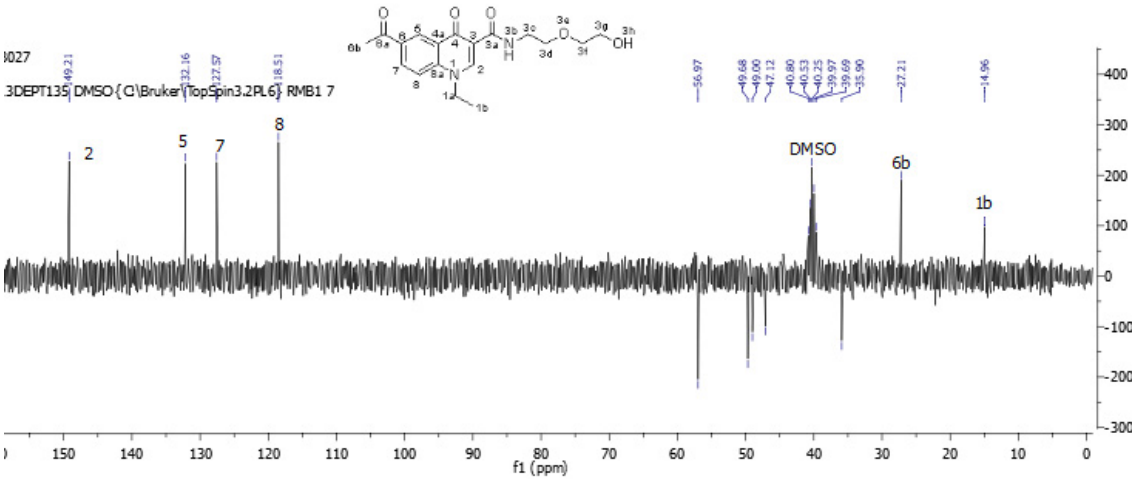

O DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 10
DMSO
6a
4
3a
2
3d
3f
1a
2b
1b
f1 (ppm)

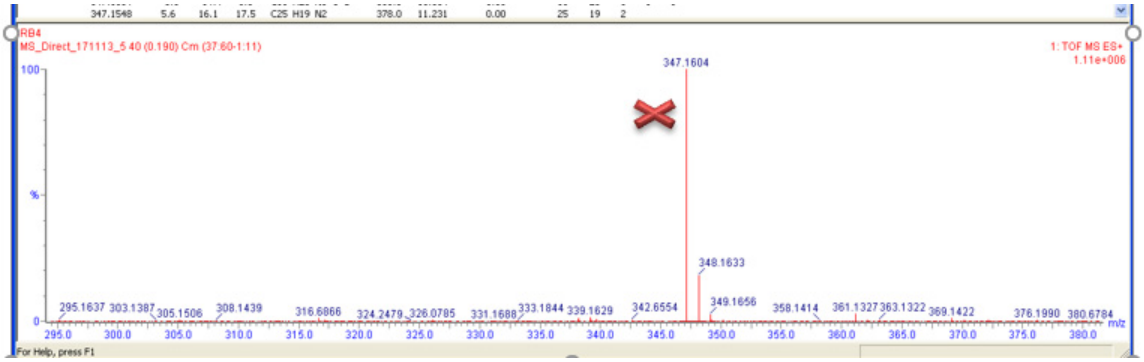

RB033
CL13DEPT135 DMSO {C:\Bruker\TopSpin3.2PL6}RMB1 10
2
5
7
DMSO
6b
1b
3f
3d
1a
3c
f1 (ppm)

Compound 4b

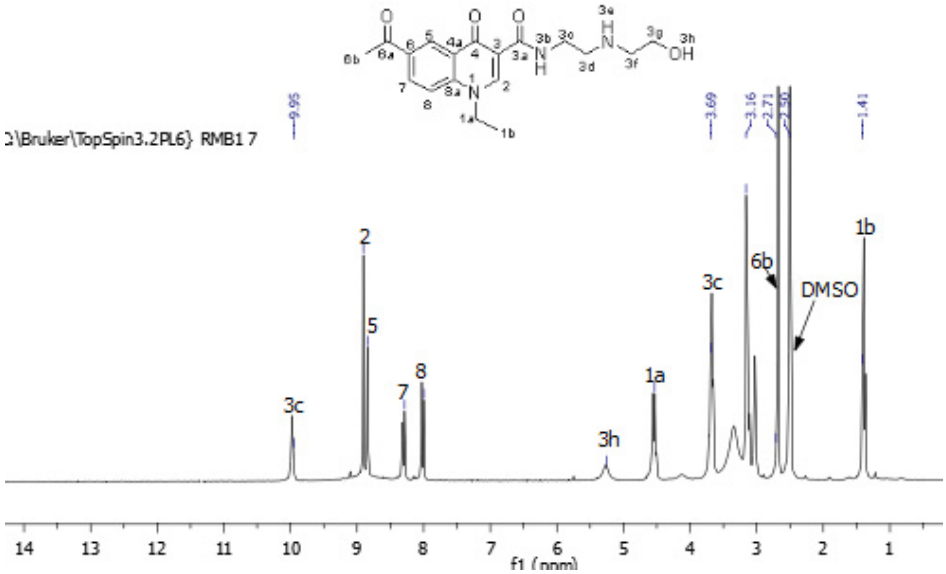

027
OTON D
2
5
8
7
3c
3h
1a
1b
f1 (ppm)

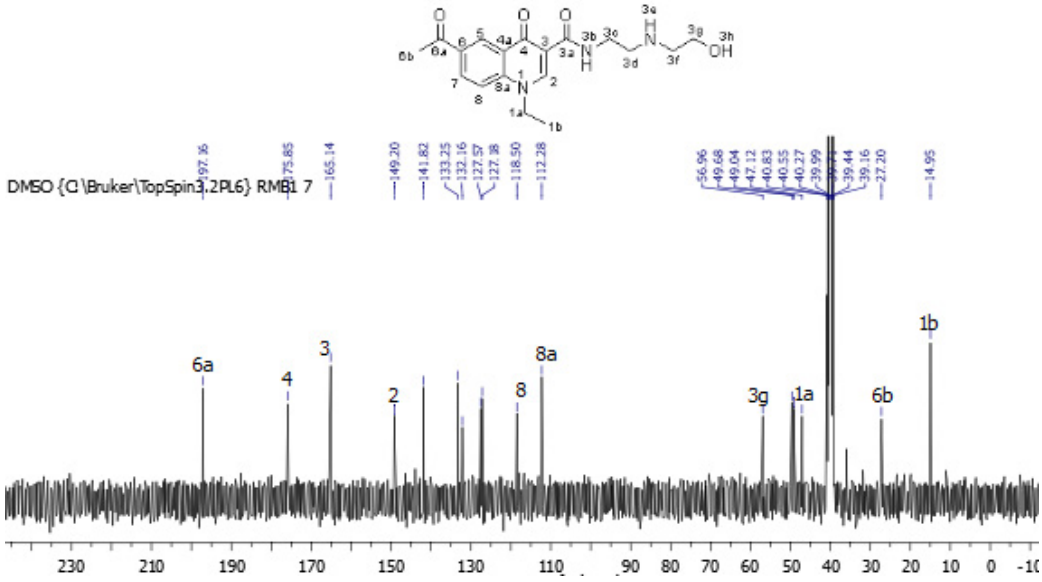

027
3CPD DI
6a
4
3
2
6b
1b
f1 (ppm)

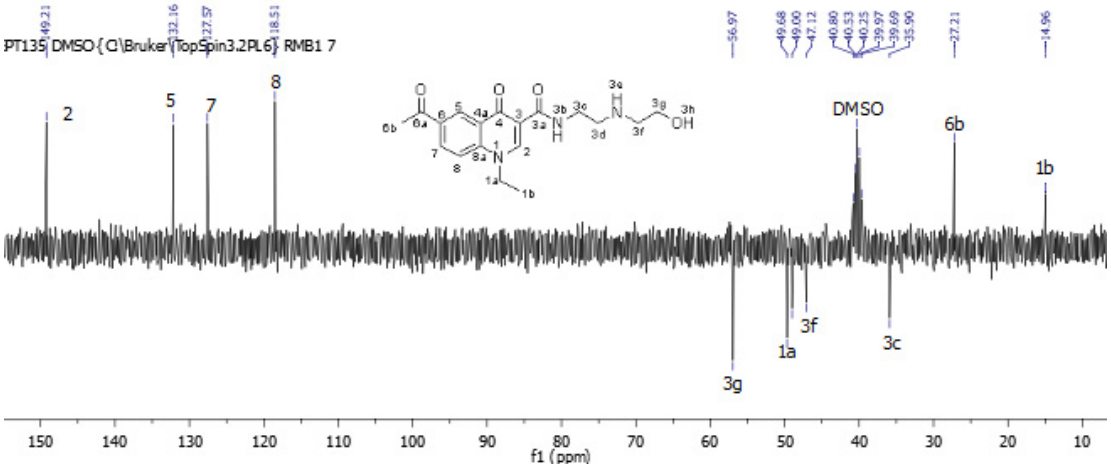

DMSO
f1 (ppm)

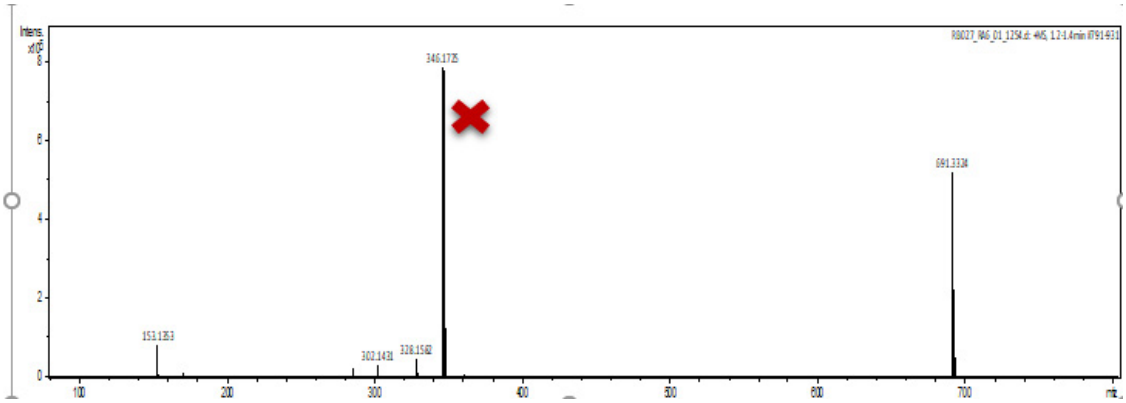

RB4
MS_Direct_171113_5 40 (0.190) Cm (37:60-1:11)
1: TOF MS ES+
347.1604
348.1633
m/z

COMPOUND 4c

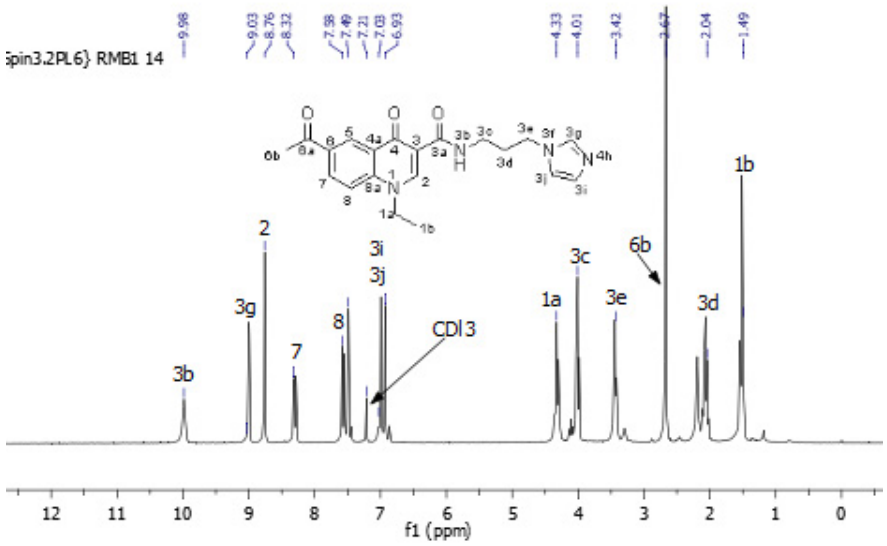

DMSO
f1 (ppm)

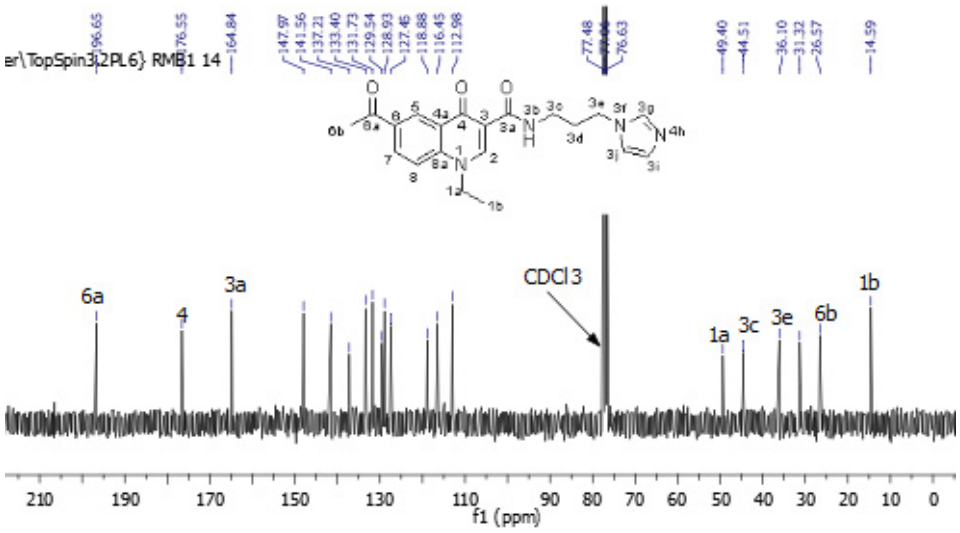

DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 7
6a
4
3
2
8
8a
3g
1a
6b
1b


PT135 DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 7
2
5
7
8
DMSO
6b
1b
3g
1a
3f
3c
f1 (ppm)


Intens.
346.1725
691.3334
151.1353
302.1438
328.1582
m/z

COMPOUND 4d


9.98
9.03
8.76
8.32
7.98
7.49
7.21
7.08
6.93
4.33
4.01
3.42
2.67
2.04
1.49
3b
3g
2
7
8
3i
3j
CDl3
1a
3c
3e
6b
3d
1b
f1 (ppm)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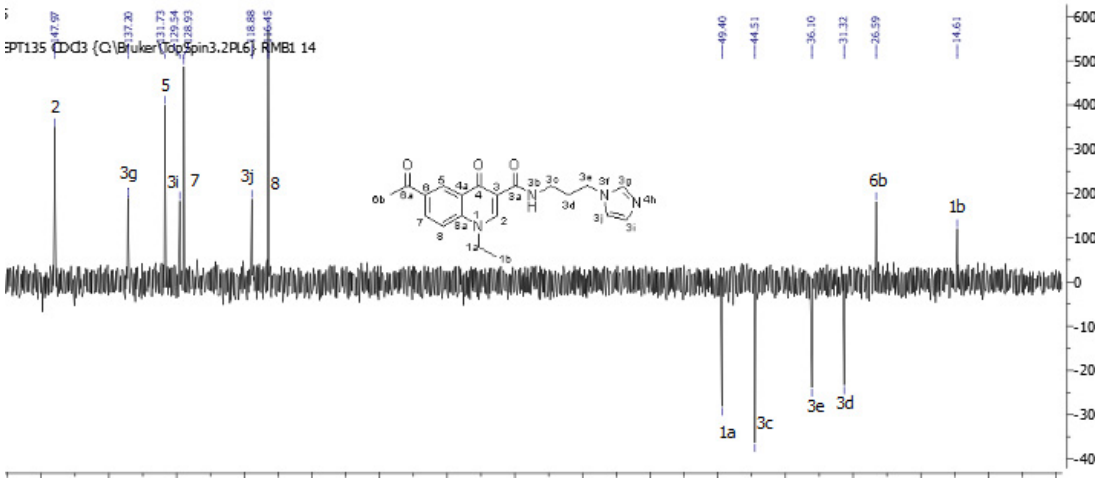
176.55
164.84
147.97
141.56
137.21
133.40
131.73
129.54
128.93
127.46
118.88
116.45
112.98
77.48
77.06
76.63
49.40
44.51
36.10
31.32
26.57
14.59
6a
4
3a
CDCl3
1a
3c
3e
6b
1b
f1 (ppm)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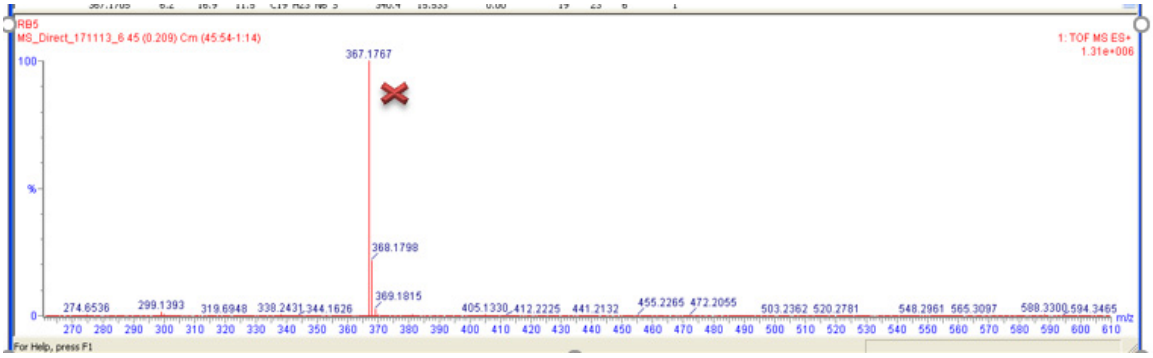
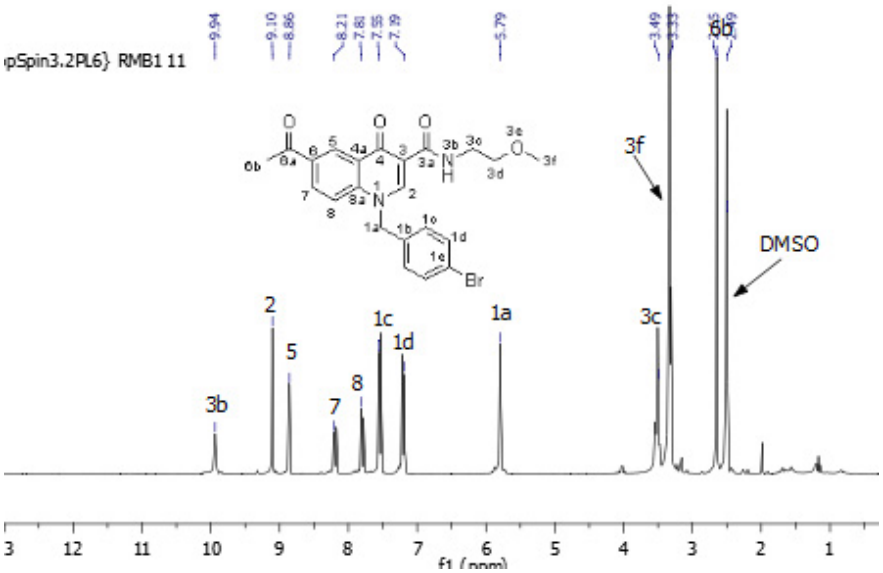
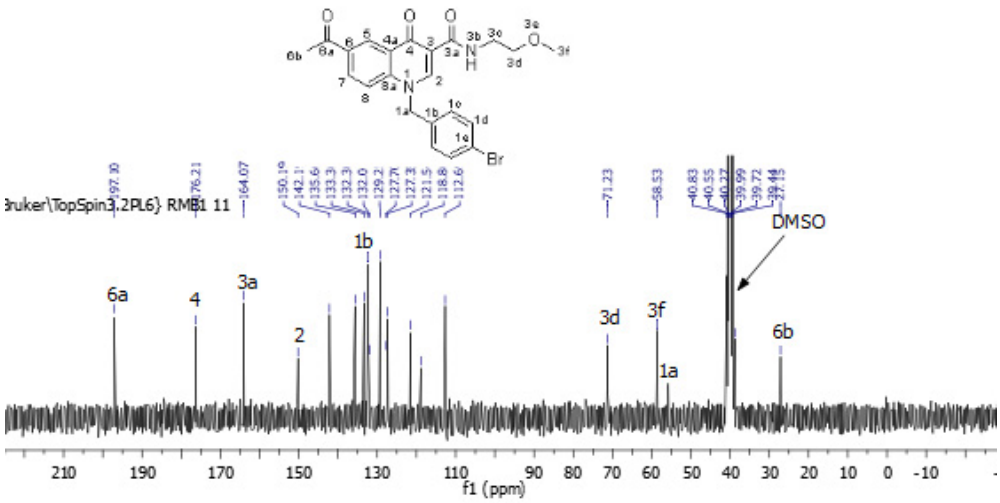
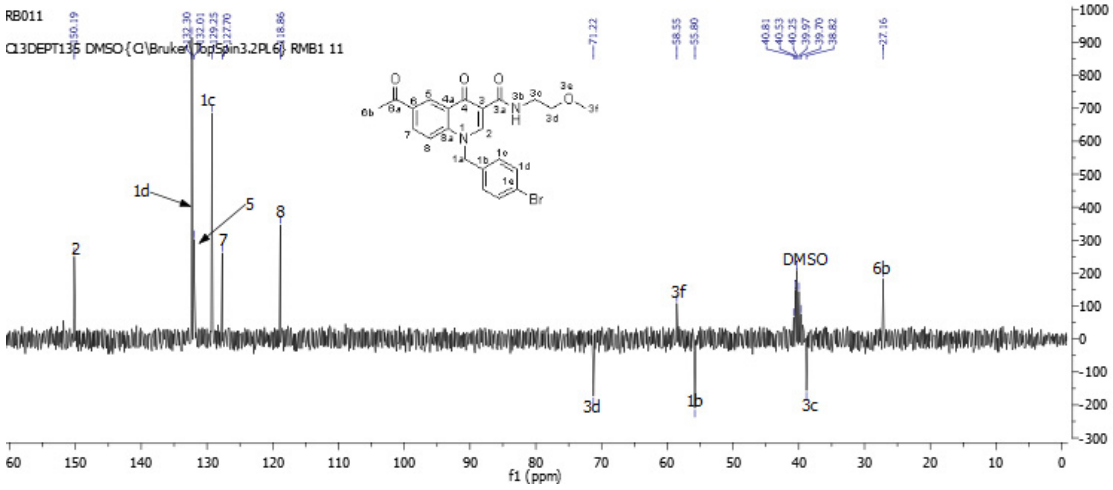
137.20
131.73
129.54
128.93
118.88
116.45
49.40
44.51
36.10
31.32
26.59
14.61
2
5
3g
3i
7
3j
8
6b
1b
1a
3c
3e
3d

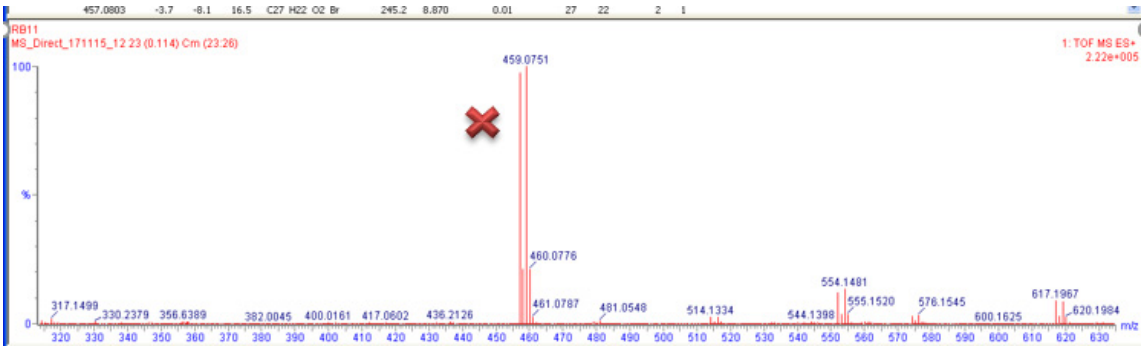

RB5
MS_Direct_171113_6 45 (0.209) Cm (45:54-1:14)
1: TOF MS ES+
1.31e+006
367.1767
368.1798
369.1815
For Help, press F1

Compound 4e


pSpin3.2PL6} RMB1 11
3f
DMSO
3b
2
5
7
8
1c
1d
1a
3c
6b
f1 (ppm)


ruker\TopSpin3.2PL6} RMB1 11
DMSO
6a
4
3a
2
1b
3d
3f
1a
6b
f1 (ppm)


RB011
C13DEPT135 DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 11
1c
1d
5
8
7
2
3f
DMSO
6b
3d
1b
3c
f1 (ppm)


RB11
MS_Direct_171115_12 23 (0.114) Cm (23:26)
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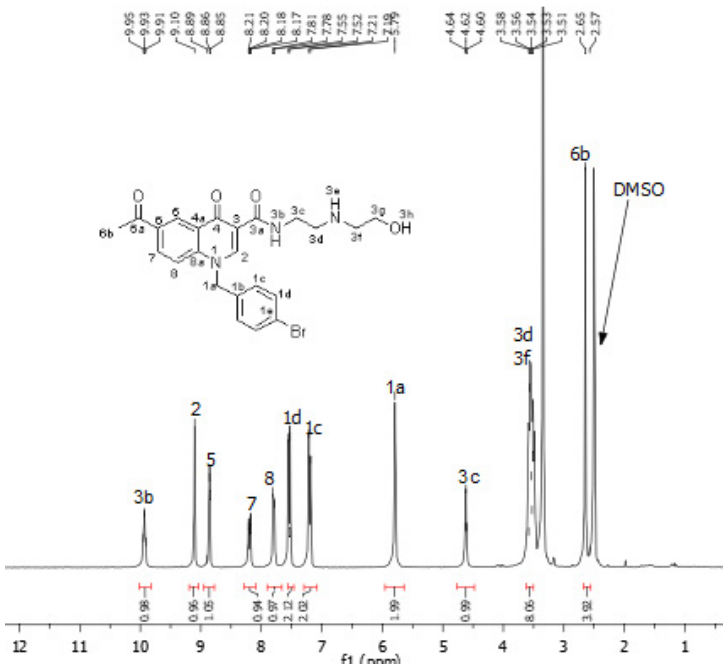
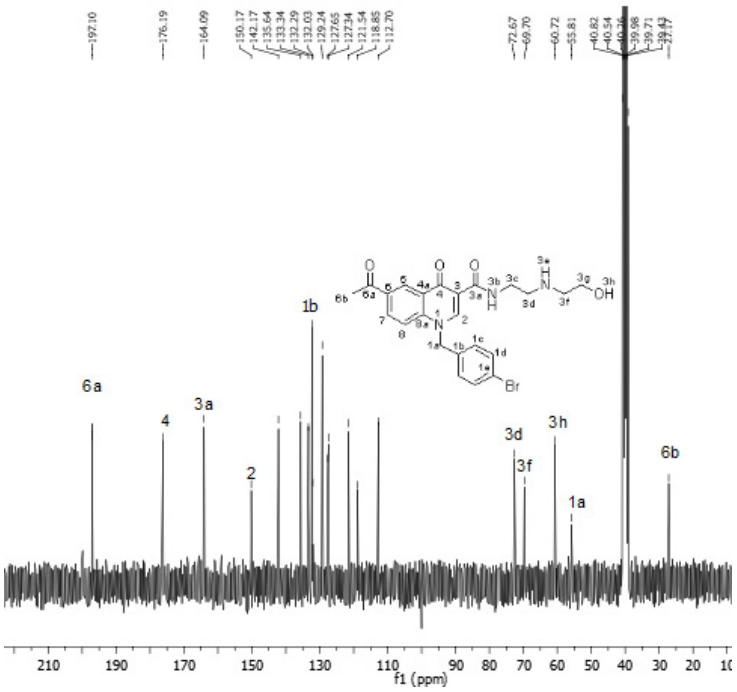
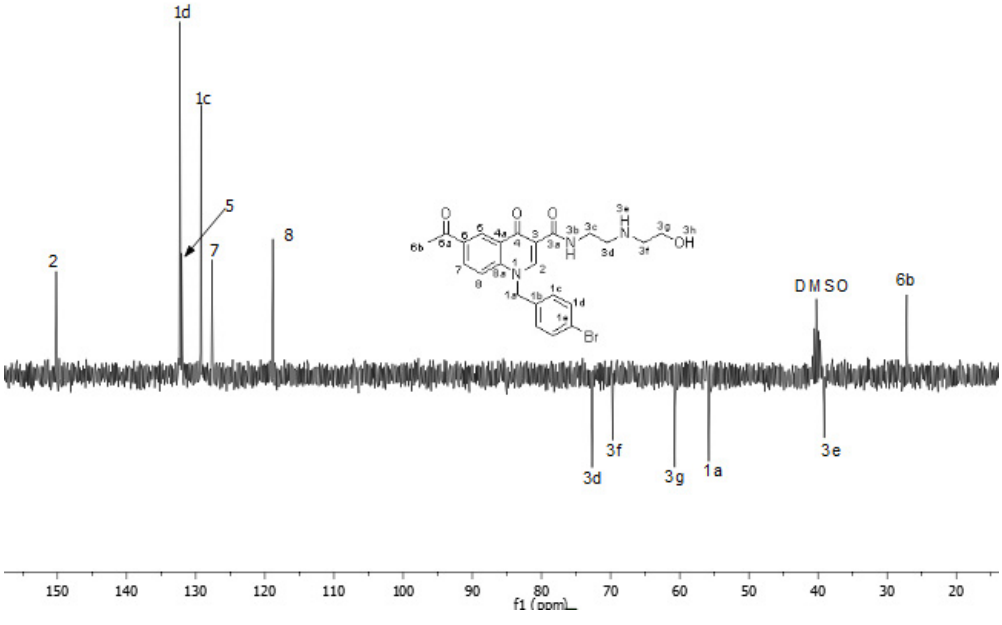
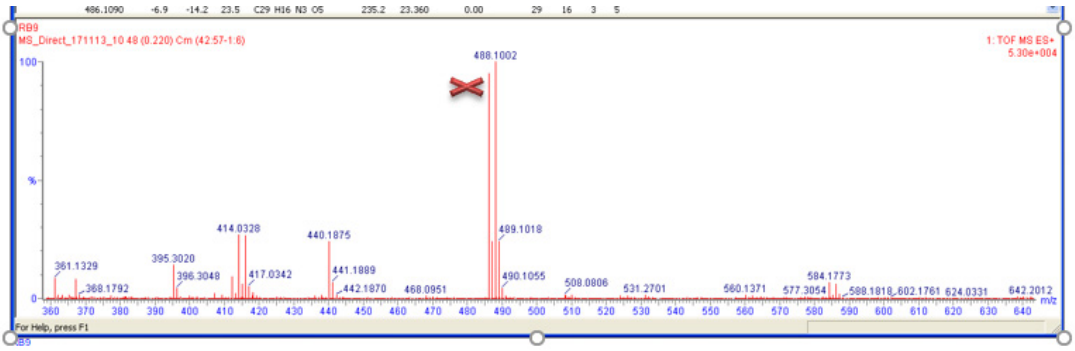
2.22e+005
459.0751
460.0776
461.0787
317.1499
330.2379
356.6389
382.0045
400.0161
417.0602
436.2126
481.0548
514.1334
544.1398
554.1481
555.1520
576.1545
600.1625
617.1967
620.1984
m/z

Compound 4f

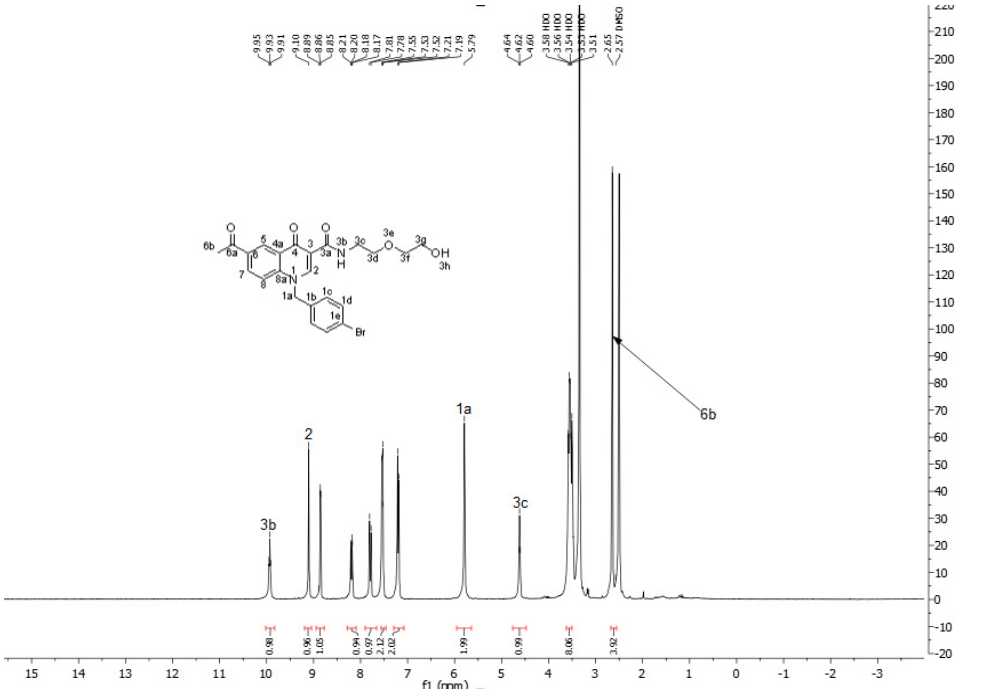

6b
DMSO
3d
3f
1a
2
1d
1c
5
8
7
3b
3c
f1 (ppm)

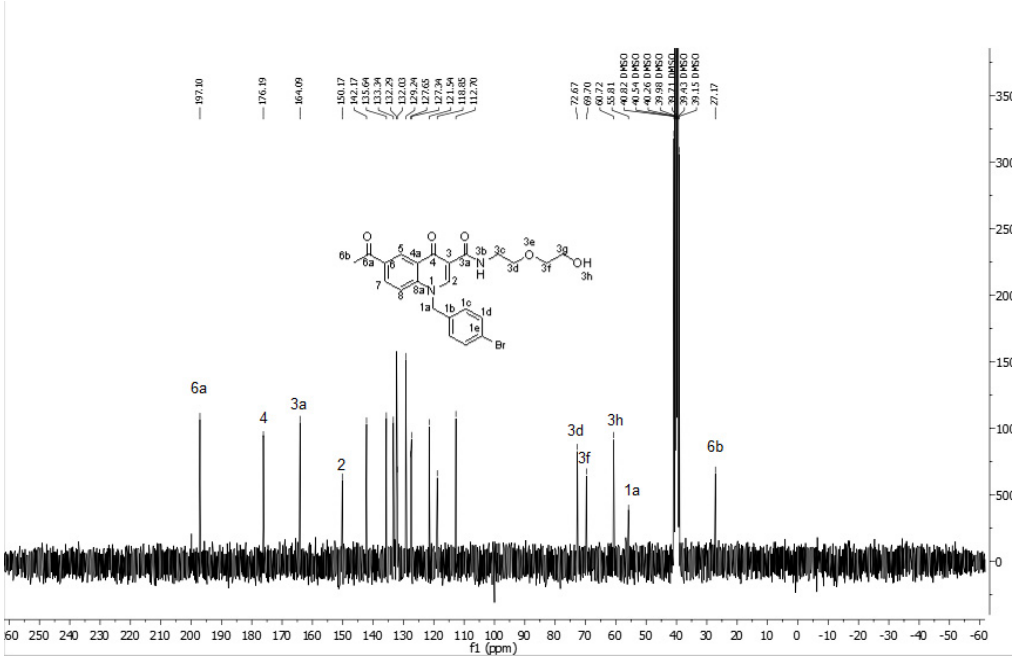

197.10
176.19
164.09
150.17
142.17
135.64
133.34
132.29
132.03
129.24
127.65
127.34
121.54
118.85
112.70
71.67
69.70
60.72
55.81
40.82
40.54
40.26
39.98
39.71
39.43
27.17
1b
6a
4
3a
2
3d
3f
3h
1a
6b
f1 (ppm)

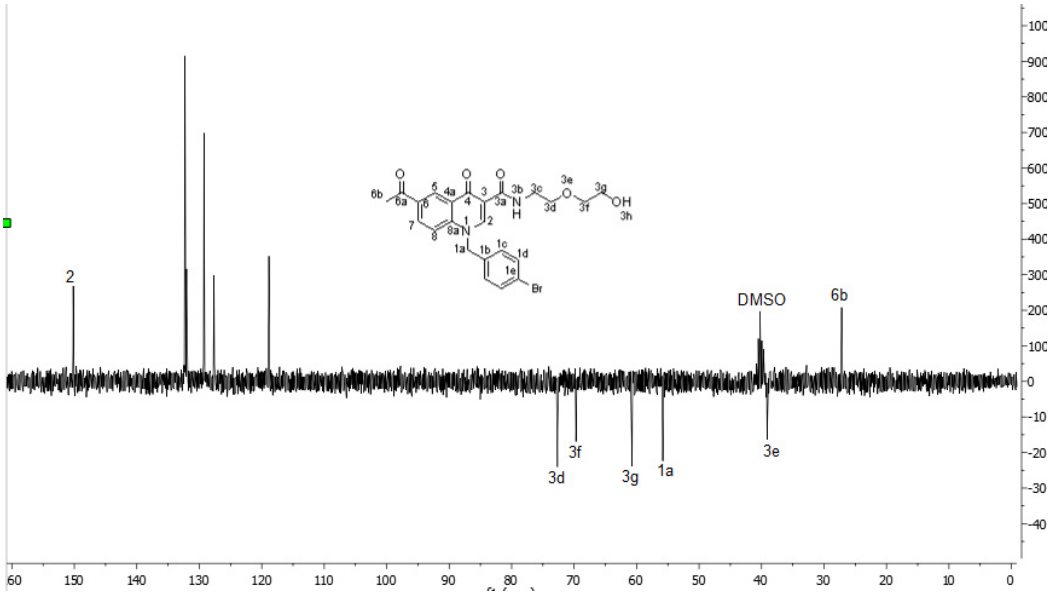

1d
1c
5
7
8
2
DMSO
6b
3d
3f
3g
1a
3e
f1 (ppm)

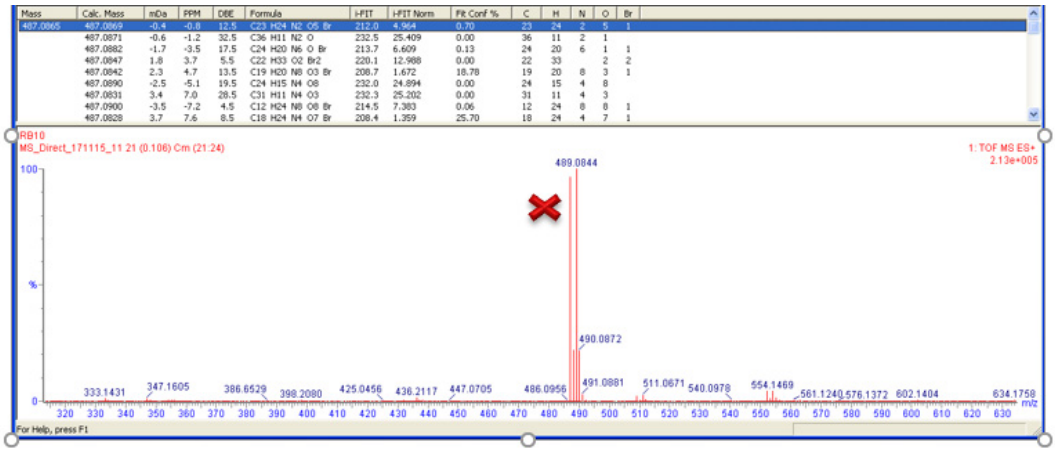

RB9
MS_Direct_171113_10 48 (0.220) Cm (42:57-1:6)
1: TOF MS ES+
5.30e+004
488.1002
489.1018
414.0328
440.1875
361.1329
395.3020
396.3048
417.0342
441.1889
442.1870
368.1792
468.0951
490.1055
508.0806
531.2701
560.1371
577.3054
584.1773
588.1818
602.1761
624.0331
642.2012
m/z

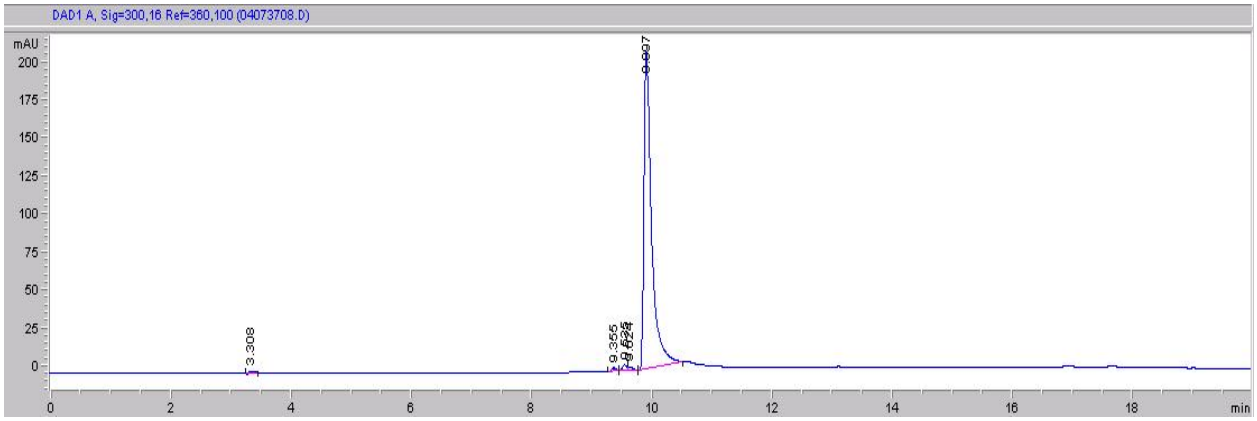
Compound 4g, HIT Compound



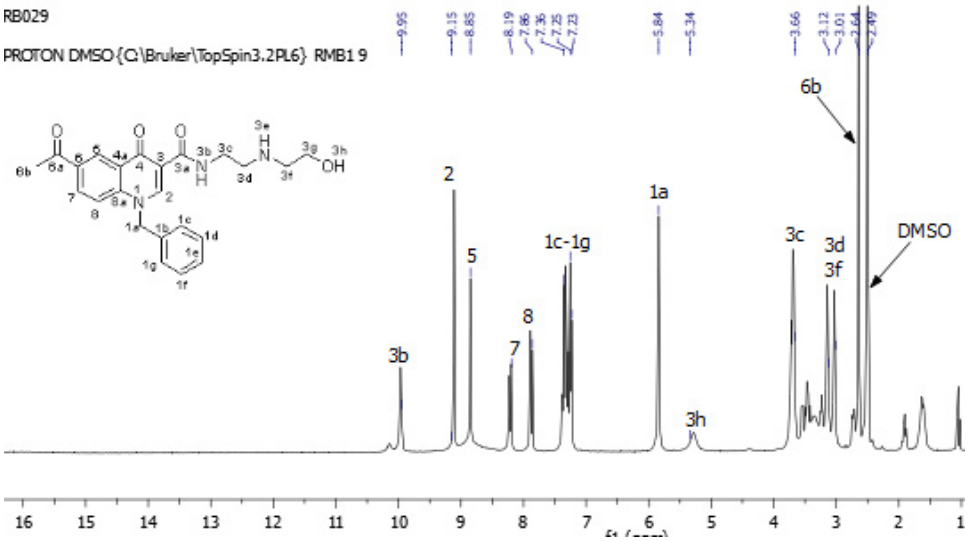


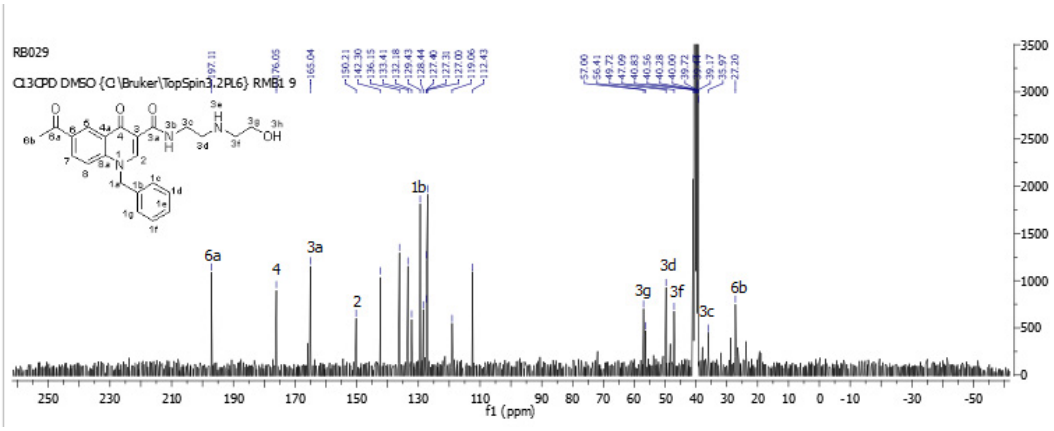

2
3d
3f
3g
1a
DMSO
3e
6b

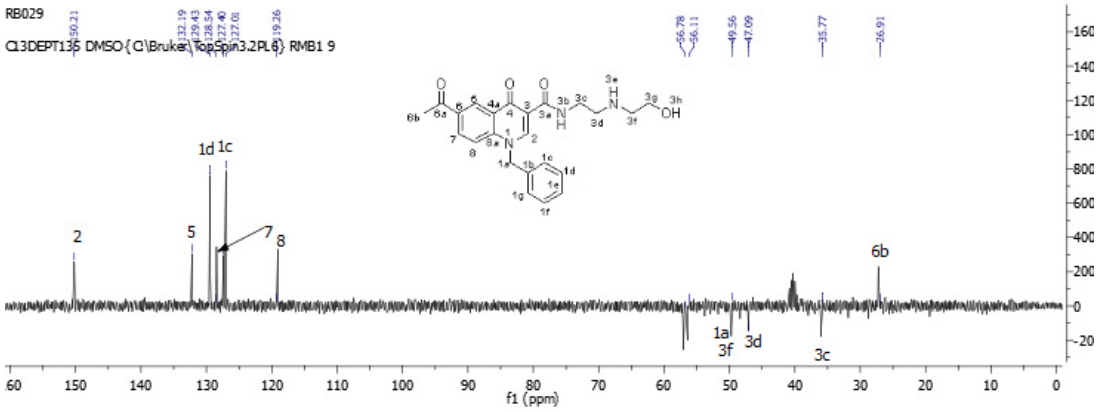

RB10
MS_Direct_171115_11 21 (0.106) Cm (21:24)
1: TOF MS ES+
2.13e+005
489.0844
490.0872
491.0881
333.1431
347.1605
386.6529
398.2080
425.0456
436.2117
447.0705
486.0956
511.0671
540.0978
554.1469
561.1240
576.1372
602.1404
634.1758
m/z
For Help, press F1

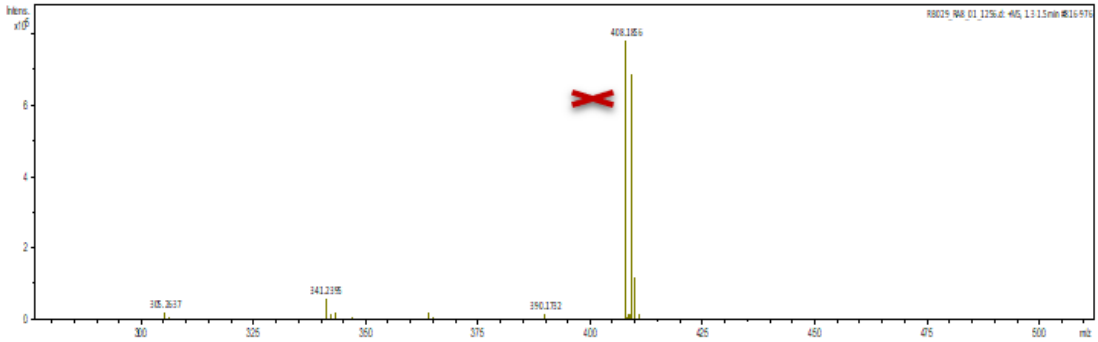

DAD1 A, Sig=300,16 Ref=360,100 (04073708.D)
mAU
9.997
3.308
9.355
9.624
min

Compound 4h

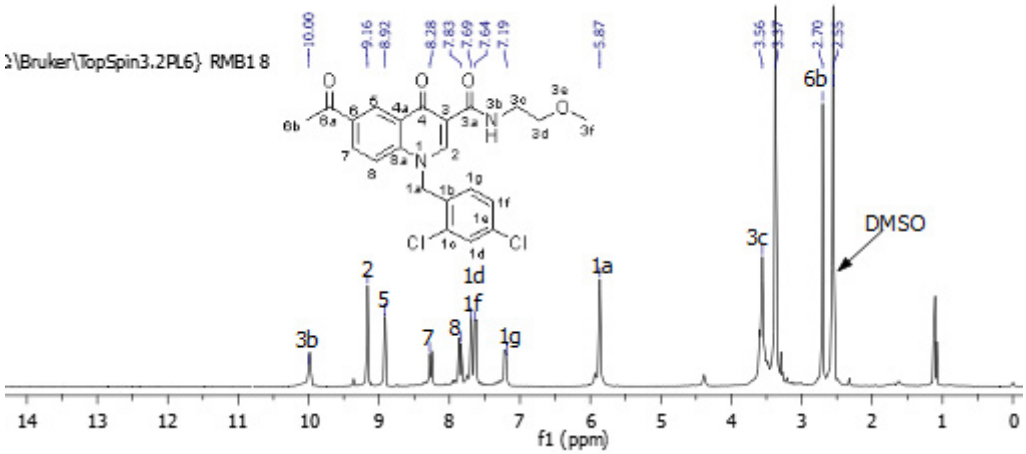

RB029
PROTON DMSO{C:\Bruker\TopSpin3.2PL6} RMB1 9
6b
2
1a
5
1c-1g
3c
3d
3f
DMSO
8
7
3b
3h

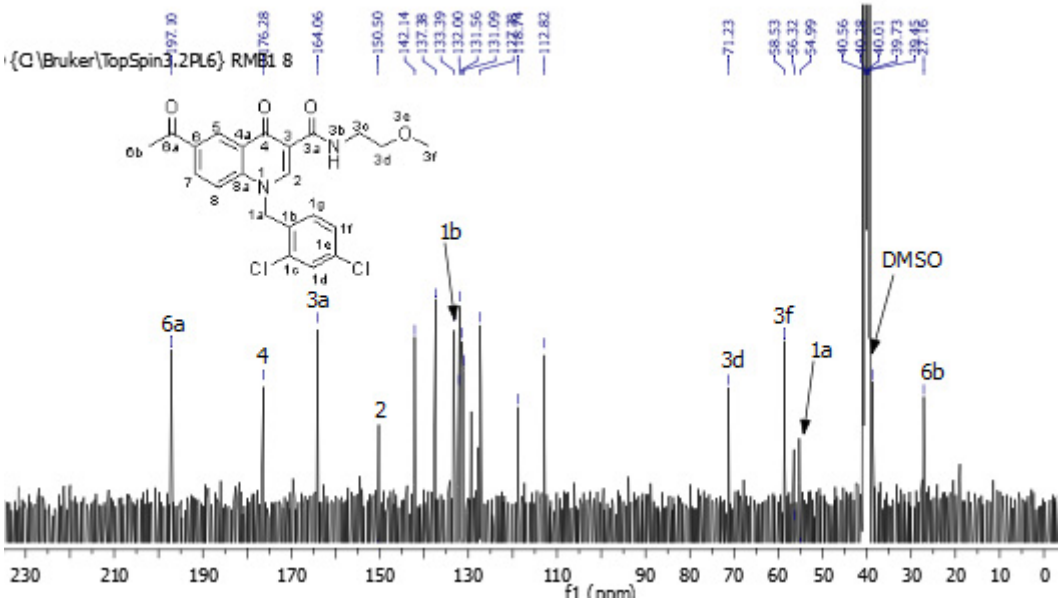

RB029
C13CPD DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 9
1b
6a
4
3a
2
3d
3g
3f
3c
6b
f1 (ppm)

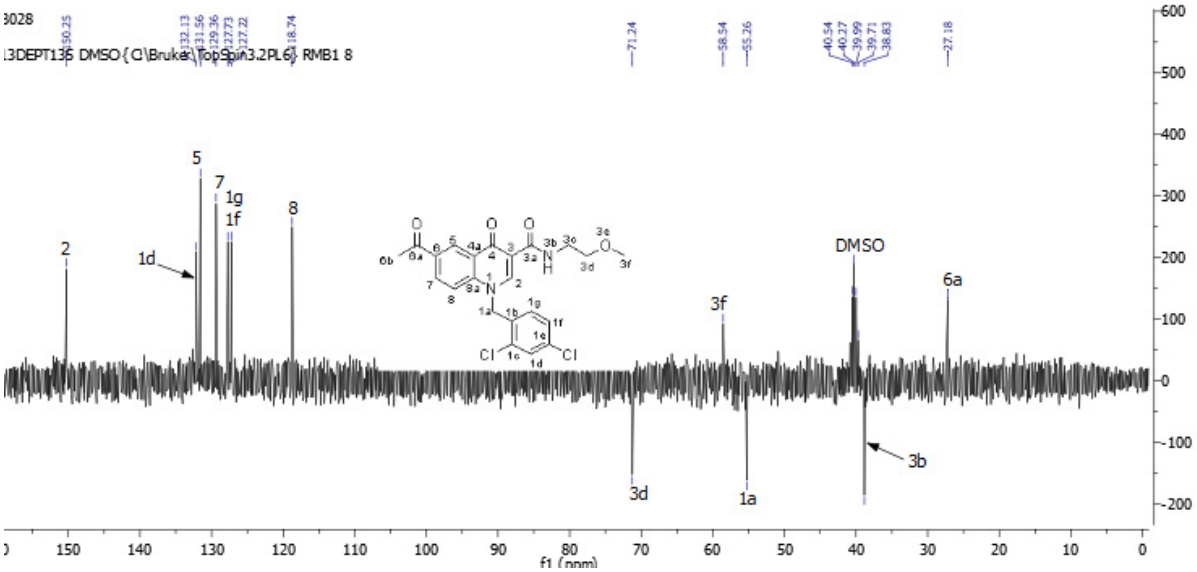

RB029
C13DEPT135 DMSO{C:\Bruker\TopSpin3.2PL6} RMB1 9
1d
1c
2
5
7
8
6b
1a
3f
3d
3c
f1 (ppm)

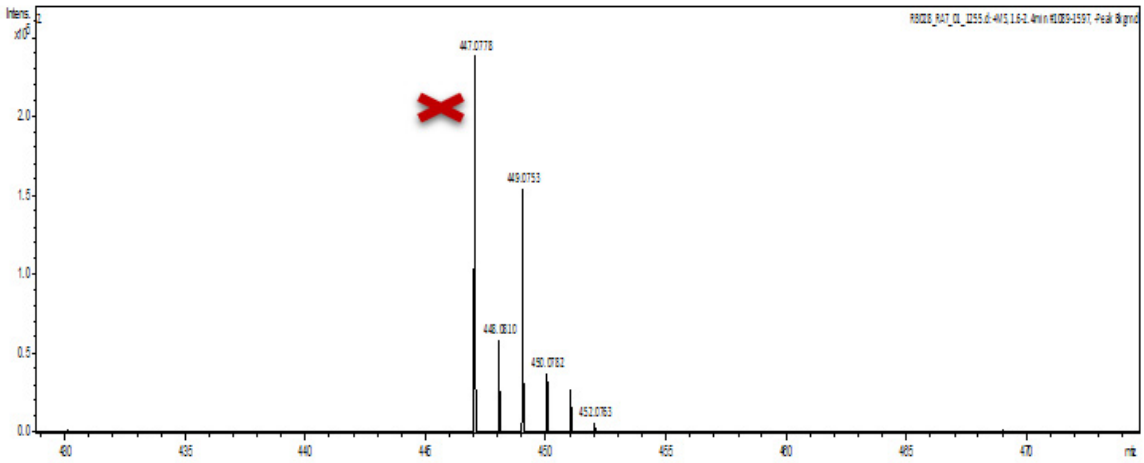


Compound 4i

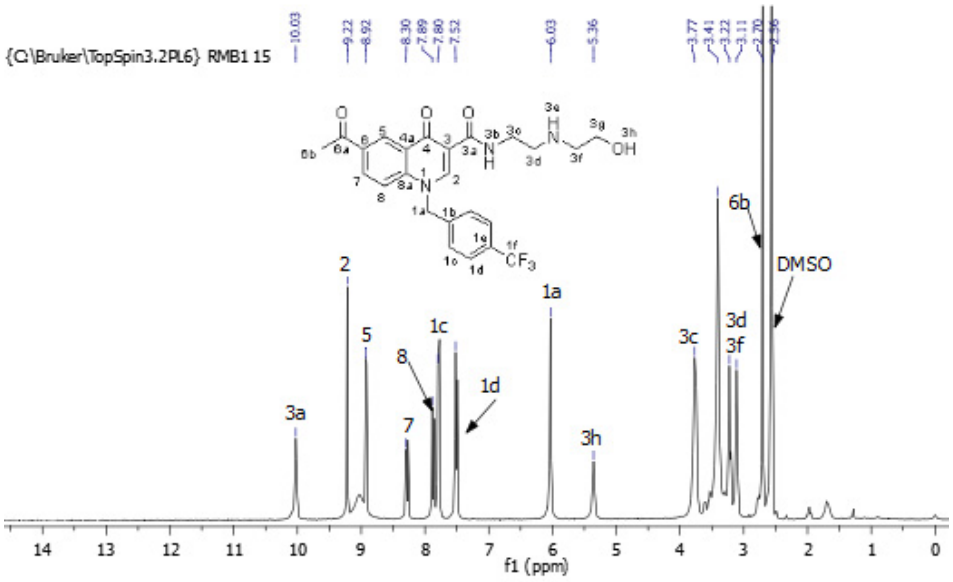

f1 (ppm)
DMSO
3b
2
5
7
8
1d
1f
1g
1a
3c
6b

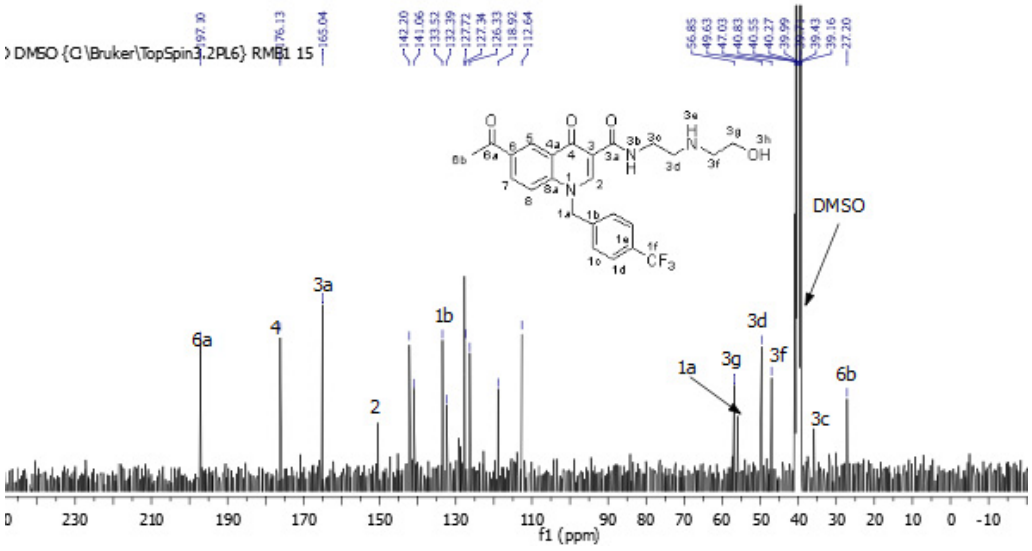

f1 (ppm)
DMSO
6a
4
3a
2
1b
3d
3f
1a
6b

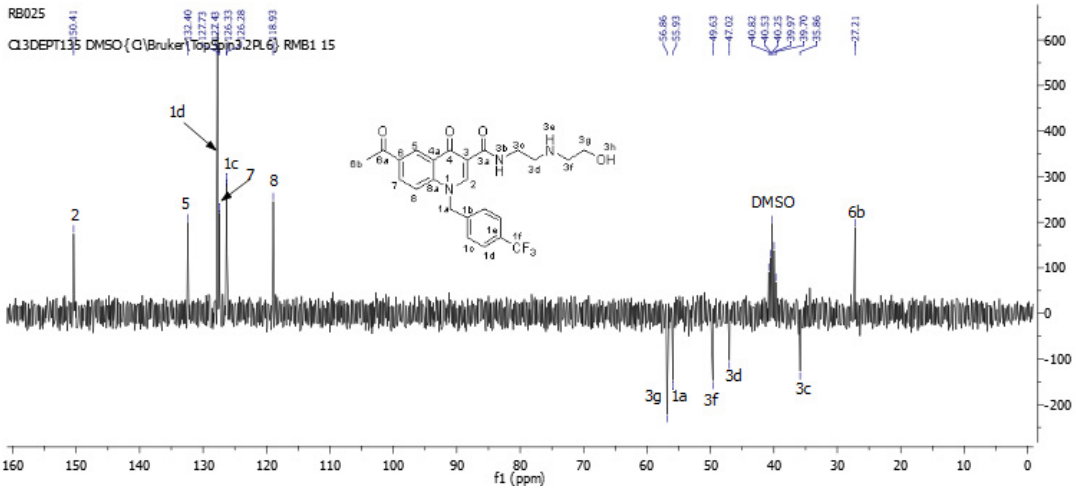

3028
13DEPT135 DMSO (C:\Bruker\TopSpin3.2PL6) RMB1 8
150.25
132.13
131.56
129.36
127.73
127.22
118.74
71.24
58.54
55.26
40.54
40.27
39.99
39.71
38.83
27.18
5
7
1g
1f
8
2
1d
3f
DMSO
6a
3d
1a
3b
f1 (ppm)

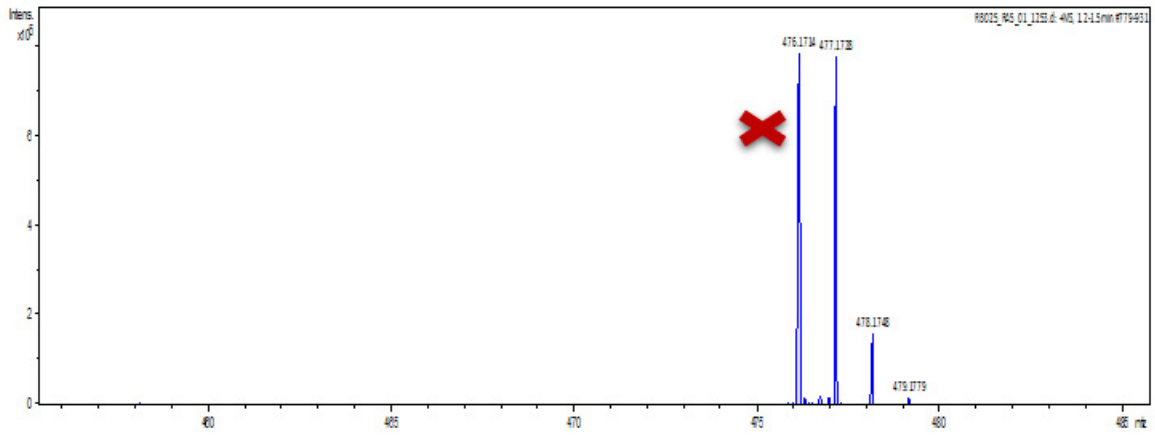

Intens.
x10⁵
447.0778
449.0753
448.0810
450.0782
451.0763
m/z

Compound 4J


{C:\Bruker\TopSpin3.2PL6} RMB1 15
DMSO
f1 (ppm)


DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 15
DMSO
f1 (ppm)


RB025
C13DEPT135 DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 15
DMSO
f1 (ppm)



BIOLOGICAL DATA

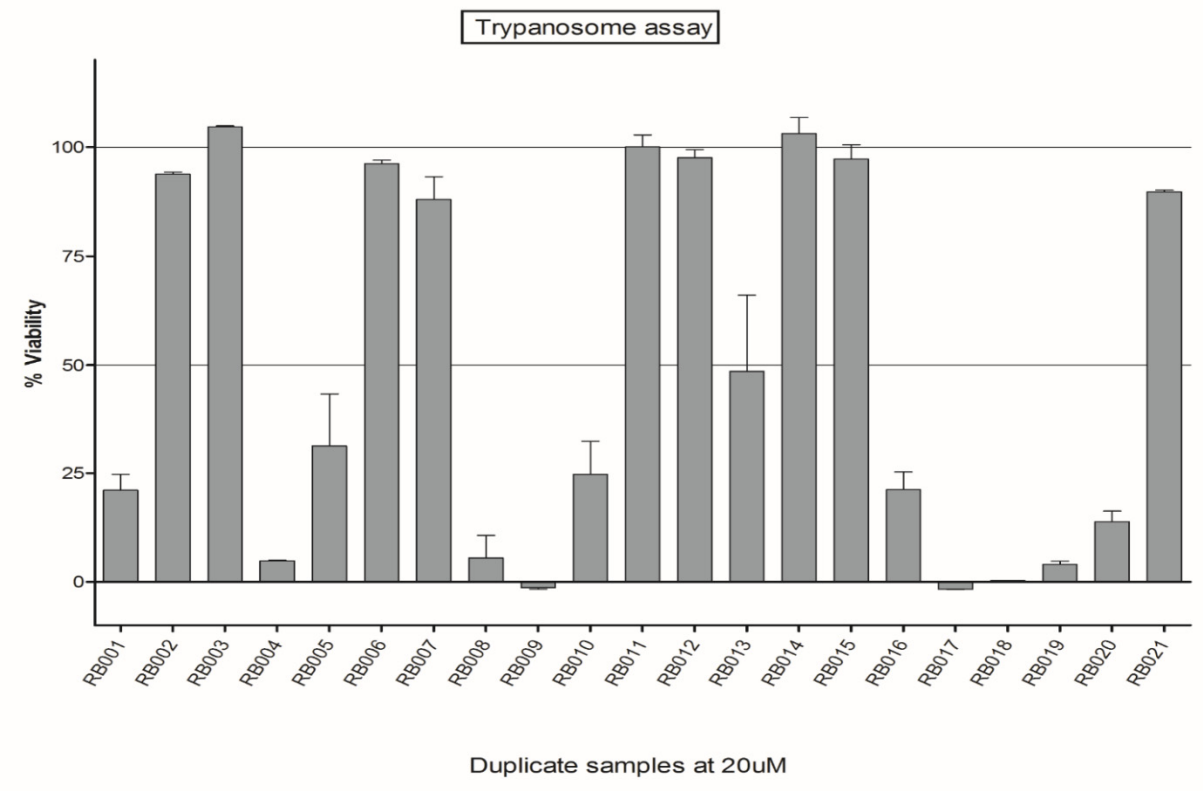


Figure S11a: Compounds inhibitory potential against *T.b. brucei* parasites at 20µM.

Compounds were added to *in vitro* cultures of *T.b. brucei* in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of parasites surviving drug exposure were determined by adding resazurin. Reduction of resazurin to resorufin by living cells was quantified in a multiwell fluorescence plate reader (Exc_{560}/Em_{590}). The results are expressed as parasite % viability relative to untreated controls.

Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. Only compounds exhibiting less than 20% parasite viability were considered for IC_{50} determination.

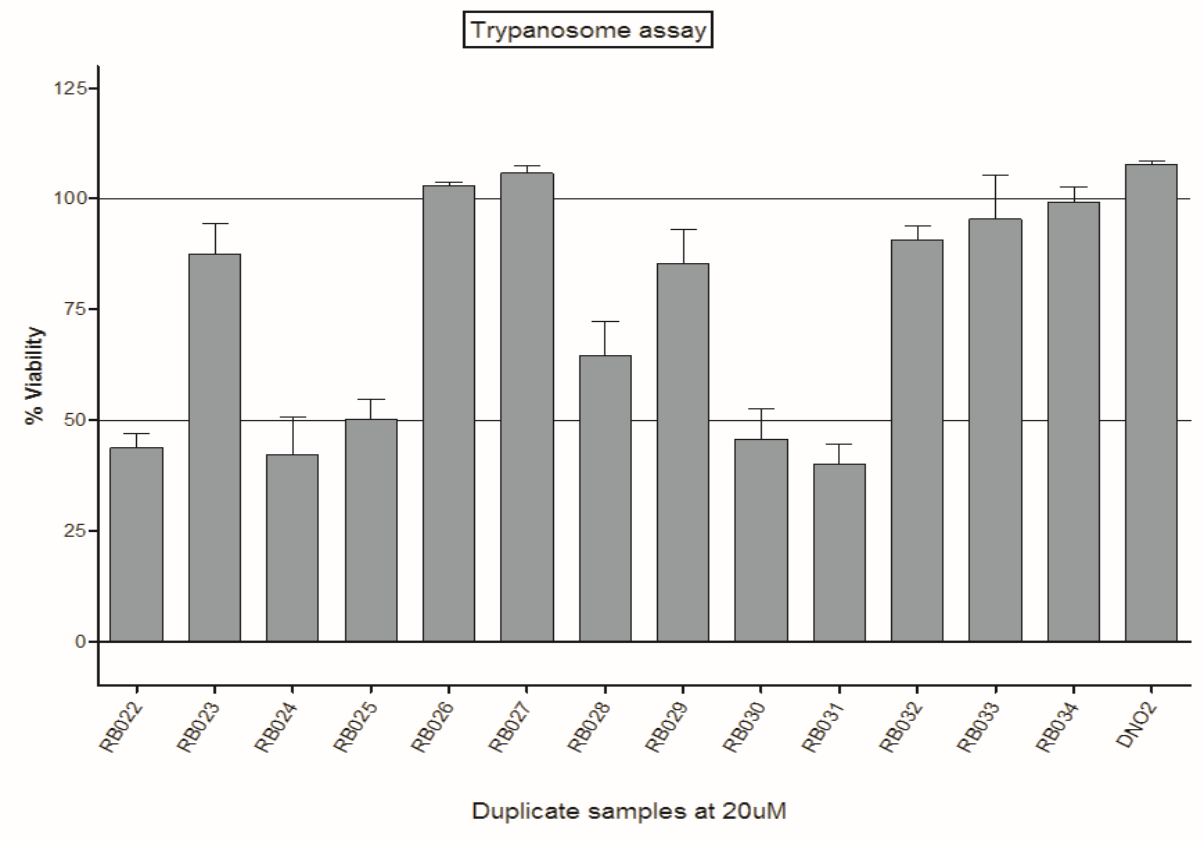


Figure S11b: Compounds inhibitory potential against *T.b. brucei* parasites at 20µM.

Compounds were added to *in vitro* cultures of *T.b. brucei* in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of parasites surviving drug exposure were determined by adding resazurin. Reduction of resazurin to resorufin by living cells was quantified in a multiwell fluorescence plate reader (Exc_{560}/Em_{590}). The results are expressed as parasite % viability relative to untreated controls. Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. Only compounds exhibiting less than 20% parasite viability were considered for IC_{50} determination.

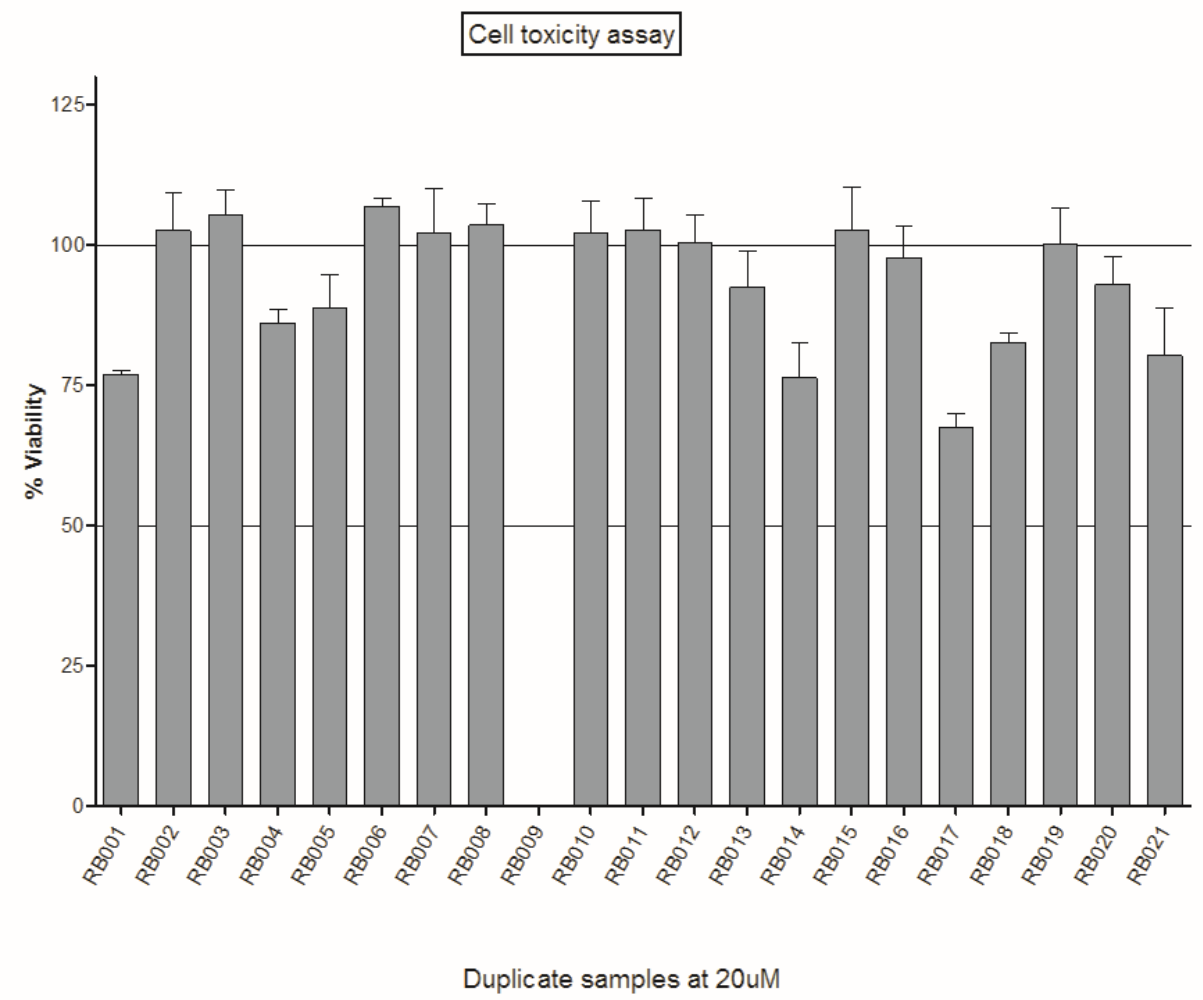


Figure SI2a: Compound cytotoxicity against HeLa cells at 20µM.

Compounds were added to *in vitro* cultures of HeLa (human cervix adenocarcinoma) cells in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of cells surviving drug exposure are determined by adding resazurin, which was reduced to resorufin by living cells. Resorufin was quantified in a multiwell fluorescence plate reader (Exc_{560}/Em_{590}). The results are express as cell % viability. Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. With the exception of compound **RB009**, this series shows no extensive cytotoxicity against HeLa cells.

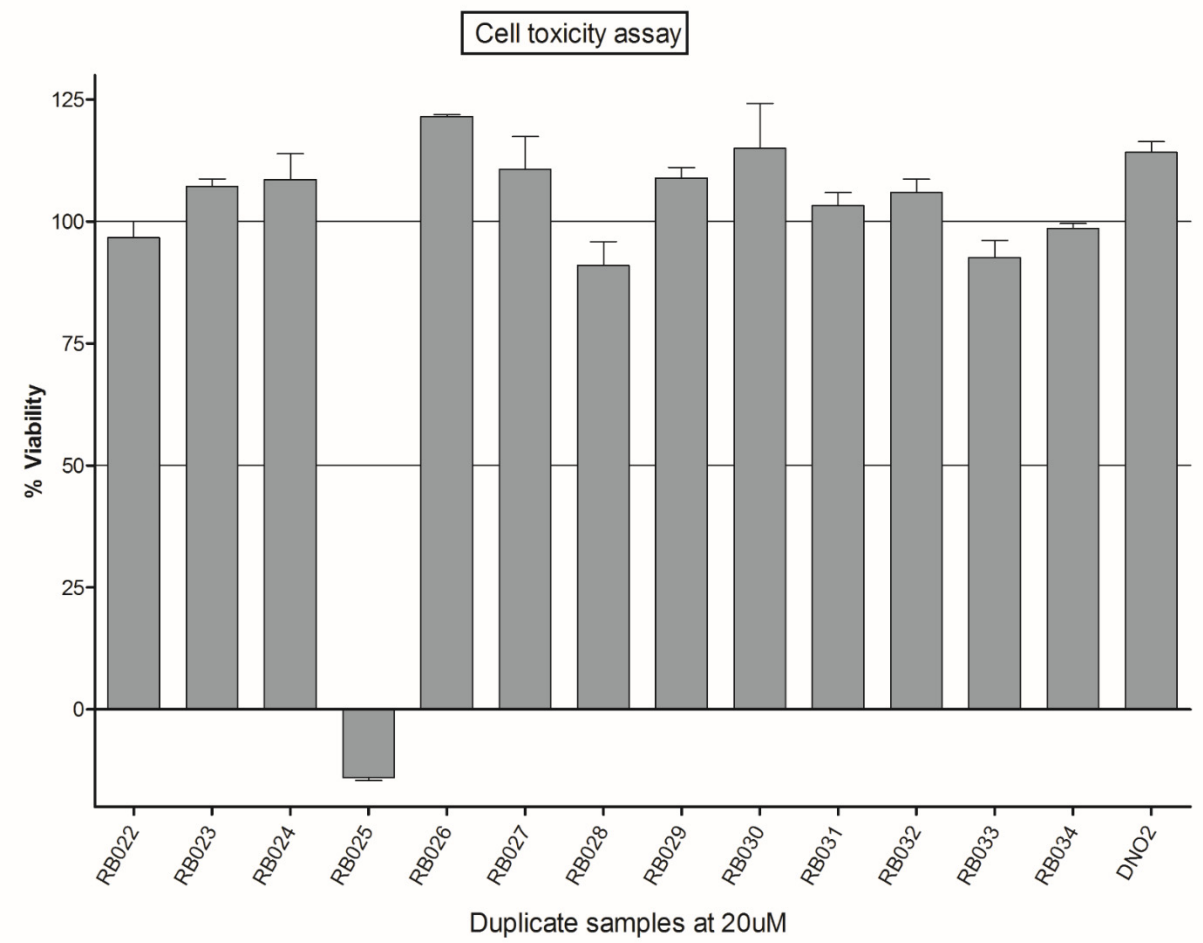


Figure SI2b: Compound cytotoxicity against HeLa cells at 20µM.

Compounds were added to *in vitro* cultures of HeLa (human cervix adenocarcinoma) cells in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of cells surviving drug exposure are determined by adding resazurin which was reduced to resorufin by living cells. Resorufin is was quantified in a multiwell fluorescence plate reader (Exc_{560}/Em_{590}). The results are express as cell % viability. Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. With the exception of compound **RB025**, this series shows no cytotoxicity against HeLa cells.