Supplementary Material

Second-generation piperazine derivatives as promising radiation countermeasures

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Supplementary Fig. 1. Proliferation of individual cell lines exposed to the compounds at 100 μM for 48 h. All data are an average of at least three independent measurements ± standard deviation.

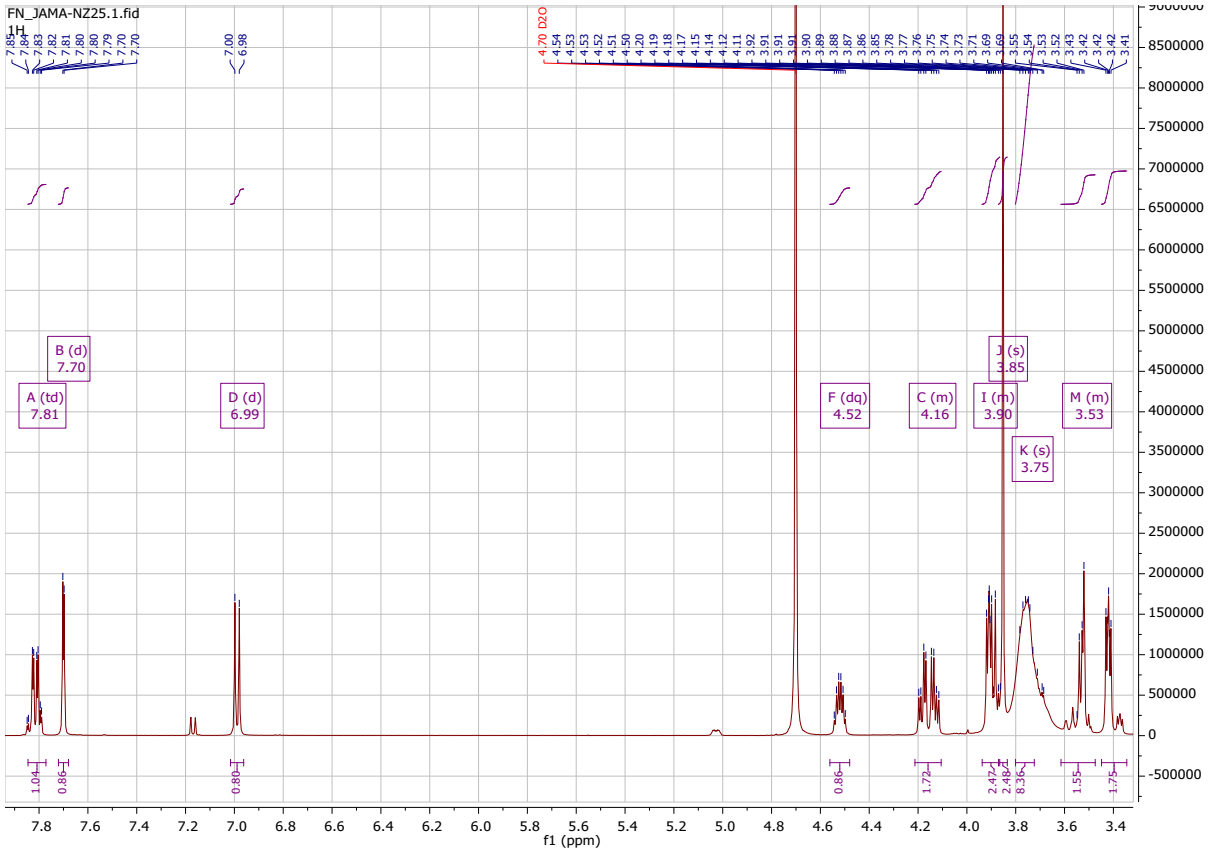
	Average viability (%)									
	WR2721	**WR1065**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Jurkat	10 ± 12	1 ± 0	91 ± 2	98 ± 3	92 ± 4	100 ± 3	85 ± 3	89 ± 5	2 ± 0	28 ± 4
MOLT-4	71 ± 21	3 ± 3	92 ± 8	90 ± 7	84 ± 11	102 ± 16	84 ± 8	89 ± 16	6 ± 5	41 ± 8
A549	5 ± 0	3 ± 0	112 ± 8	111 ± 11	106 ± 11	112 ± 14	109 ± 9	99 ± 11	6 ± 2	92 ± 12
HT-29	27 ± 3	20 ± 2	141 ± 42	103 ± 3	94 ± 9	99 ± 3	95 ± 6	85 ± 7	12 ± 2	33 ± 3
PANC-1	8 ± 4	3 ± 1	108 ± 3	104 ± 3	90 ± 4	97 ± 1	100 ± 3	94 ± 3	55 ± 6	101 ± 2
MCF-7	7 ± 2	2 ± 0	100 ± 9	97 ± 8	91 ± 12	102 ± 5	104 ± 11	86 ± 12	7 ± 7	41 ± 7
SAOS-2	9 ± 3	7 ± 4	101 ± 16	103 ± 9	92 ± 14	102 ± 6	89 ± 3	120 ± 26	15 ± 5	42 ± 5
MRC-5	8 ± 4	3 ± 1	111 ± 9	105 ± 1	103 ± 6	103 ± 2	98 ± 3	97 ± 1	0 ± 1	15 ± 10

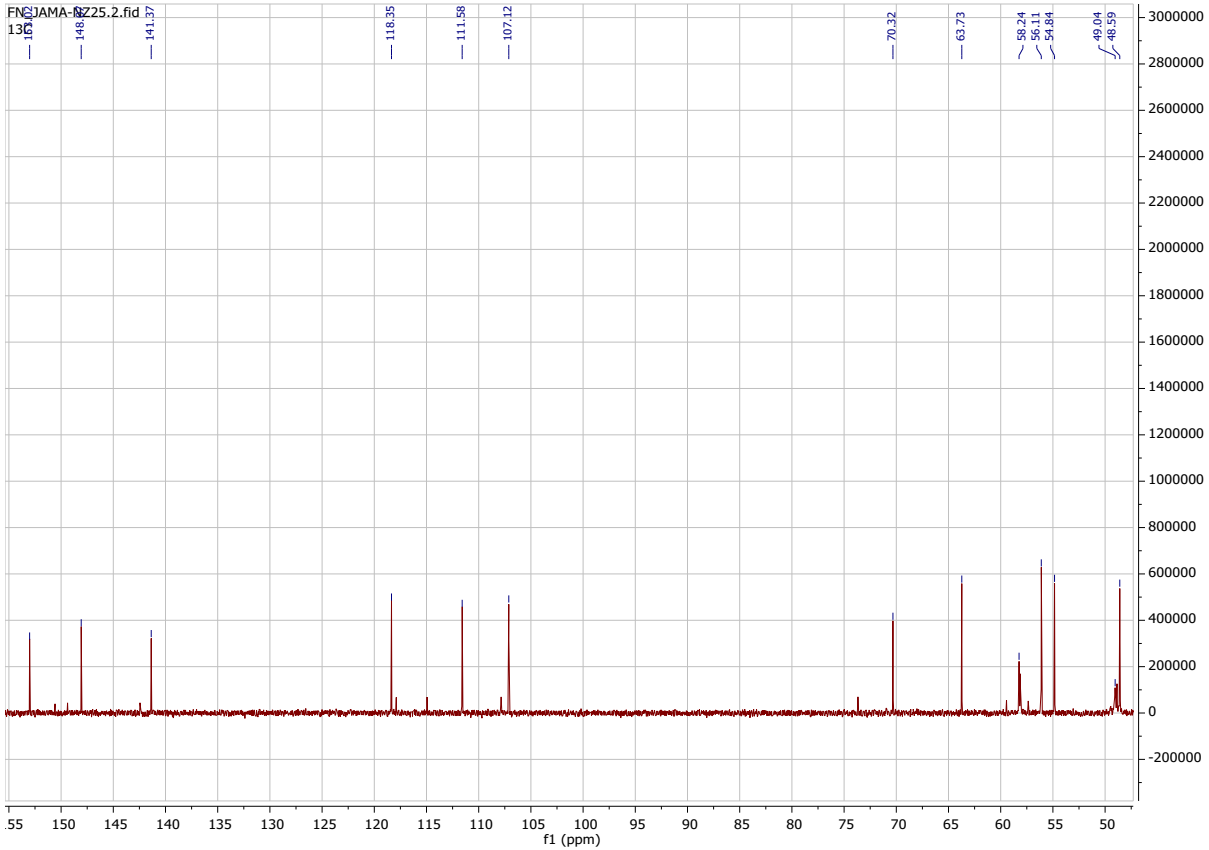
Supplementary Fig. 2. Viability in % of PBMCs after 48 h exposure to tested compounds. The viability of untreated control is 100%. All data are an average of at least three independent measurements ± standard deviation.

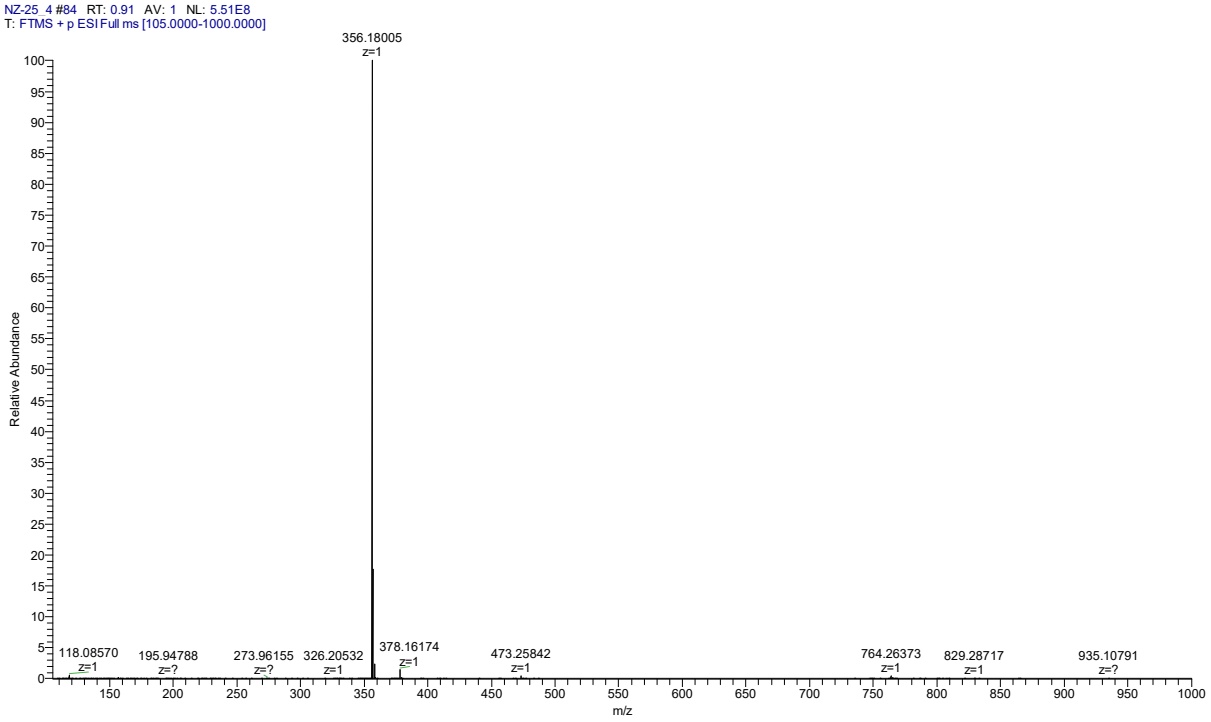
	Average viability (%)							
[μM]	**WR2721**	**WR1065**	**1**	**2**	**3**	**4**	**5**	**6**
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
10	93 ± 17	57 ± 39	114 ± 14	102 ± 12	98 ± 5	104 ± 18	121 ± 25	94 ± 12
20	75 ± 30	47 ± 34	110 ± 11	100 ± 11	95 ± 14	103 ± 26	99 ± 8	96 ± 5
50	37 ± 9	19 ± 17	111 ± 15	99 ± 6	90 ± 9	112 ± 30	111 ± 7	99 ± 4
100	33 ± 12	16 ± 14	101 ± 17	98 ± 8	92 ± 6	107 ± 30	102 ± 9	99 ± 7
200	35 ± 17	21 ± 8	102 ± 21	99 ± 11	92 ± 5	110 ± 33	107 ± 5	96 ± 6
500	37 ± 20	27 ± 9	97 ± 22	93 ± 3	92 ± 9	110 ± 28	79 ± 3	94 ± 7
1000	n/a	n/a	60 ± 10	78 ± 4	81 ± 11	105 ± 10	44 ± 11	51 ± 4
2000	n/a	n/a	n/a	n/a	n/a	53 ± 5	n/a	n/a
5000	n/a	n/a	n/a	n/a	n/a	39 ± 5	n/a	n/a

Supplementary Fig. 3. HRMS analysis of compounds **1 - 8**

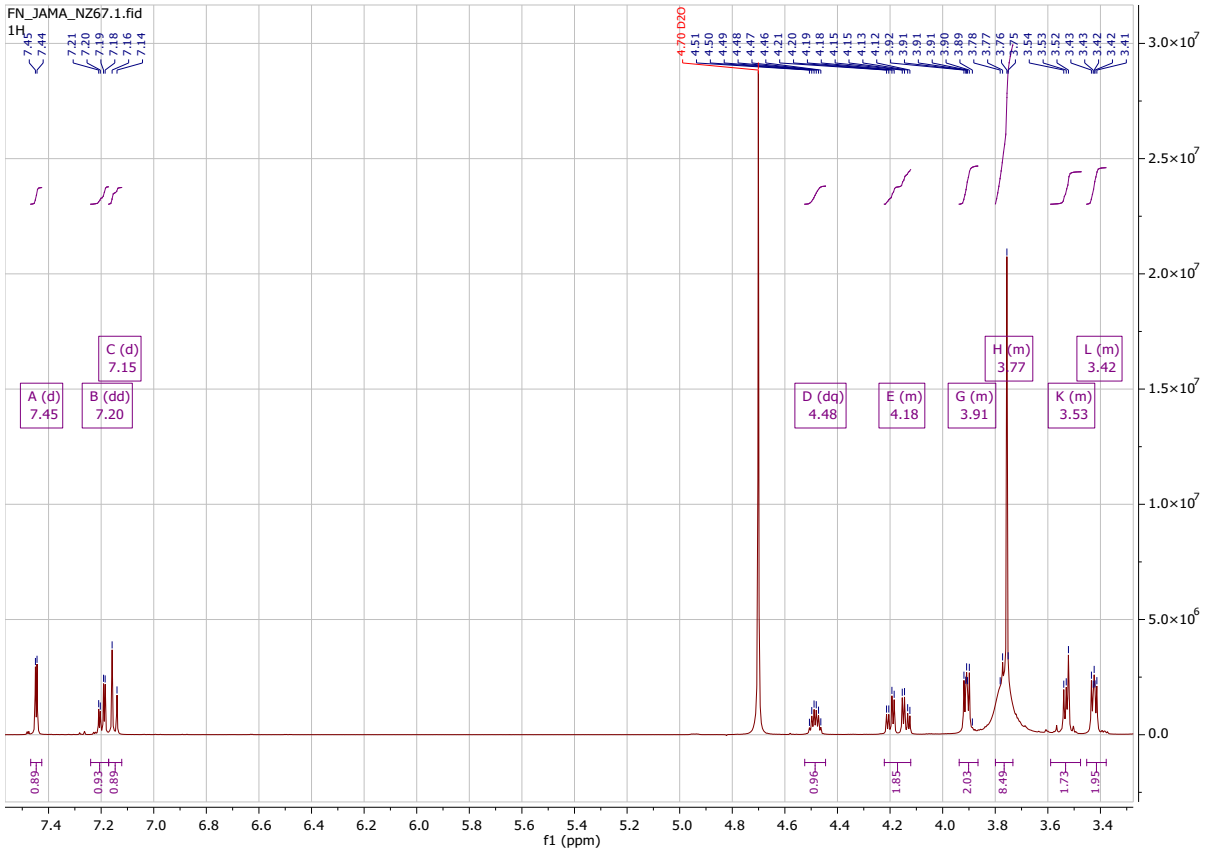
1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(2-methoxy-4-nitrophenoxy)propan-2-ol (**1**)

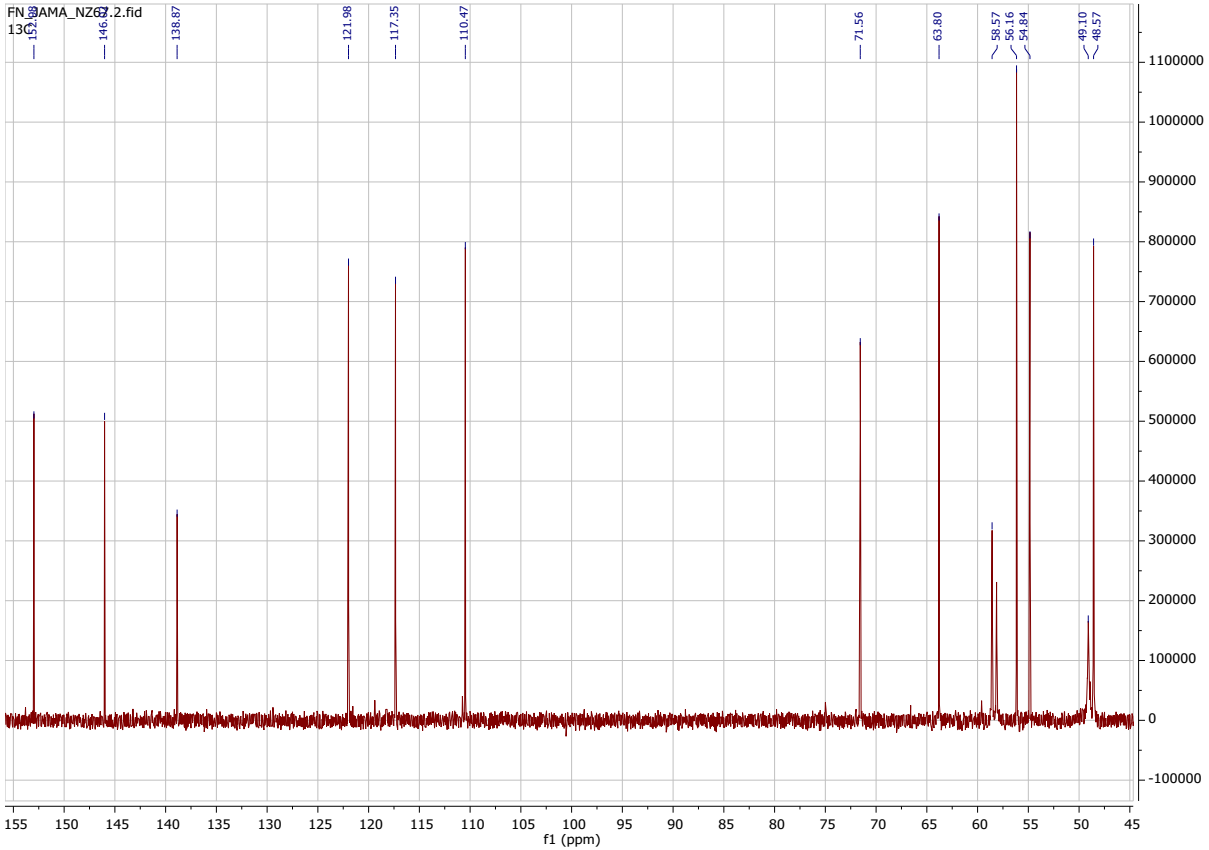


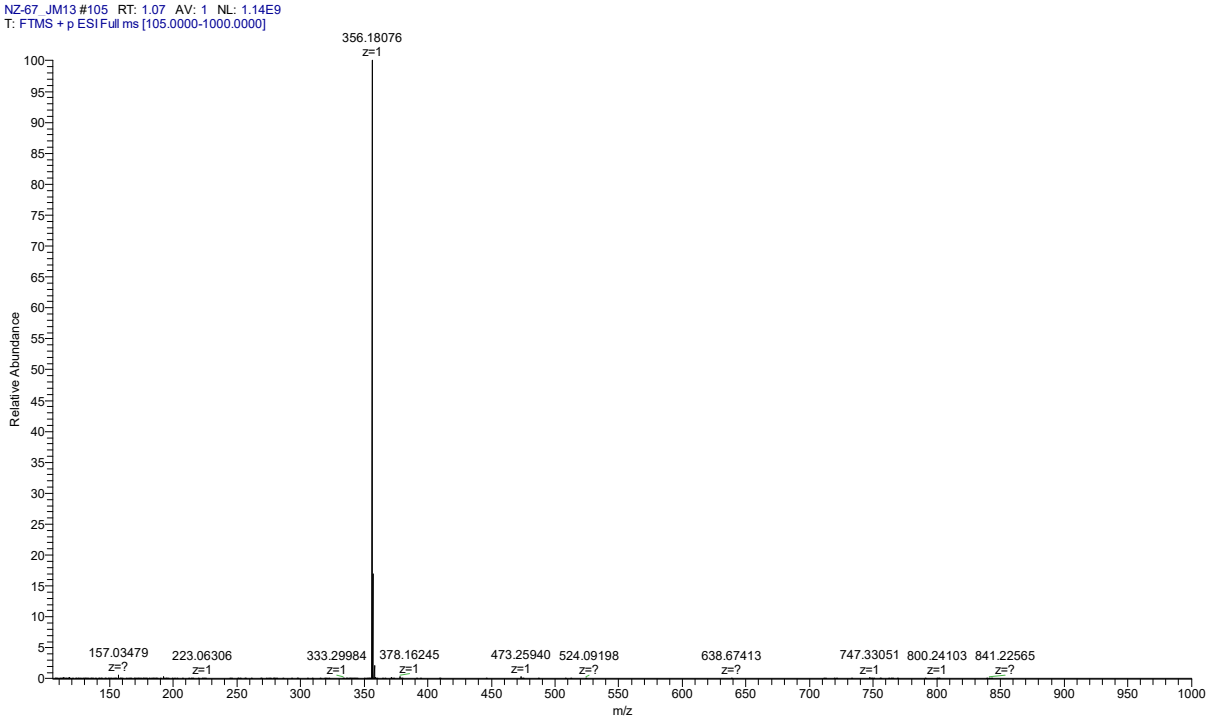

FNCJAMA-RZ25.2.fid
13C
151.37
148.82
141.37
118.35
111.58
107.12
70.32
63.73
58.24
56.11
54.84
49.04
48.59
3000000
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
.55
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
f1 (ppm)


NZ-25_4 #84 RT: 0.91 AV: 1 NL: 5.51E8
T: FTMS + p ESI Full ms [105.0000-1000.0000]
356.18005
z=1
118.08570
z=1
195.94788
z=?
273.96155
z=?
326.20532
z=1
378.16174
z=1
473.25842
z=1
764.26373
z=1
829.28717
z=1
935.10791
z=?
Relative Abundance
m/z

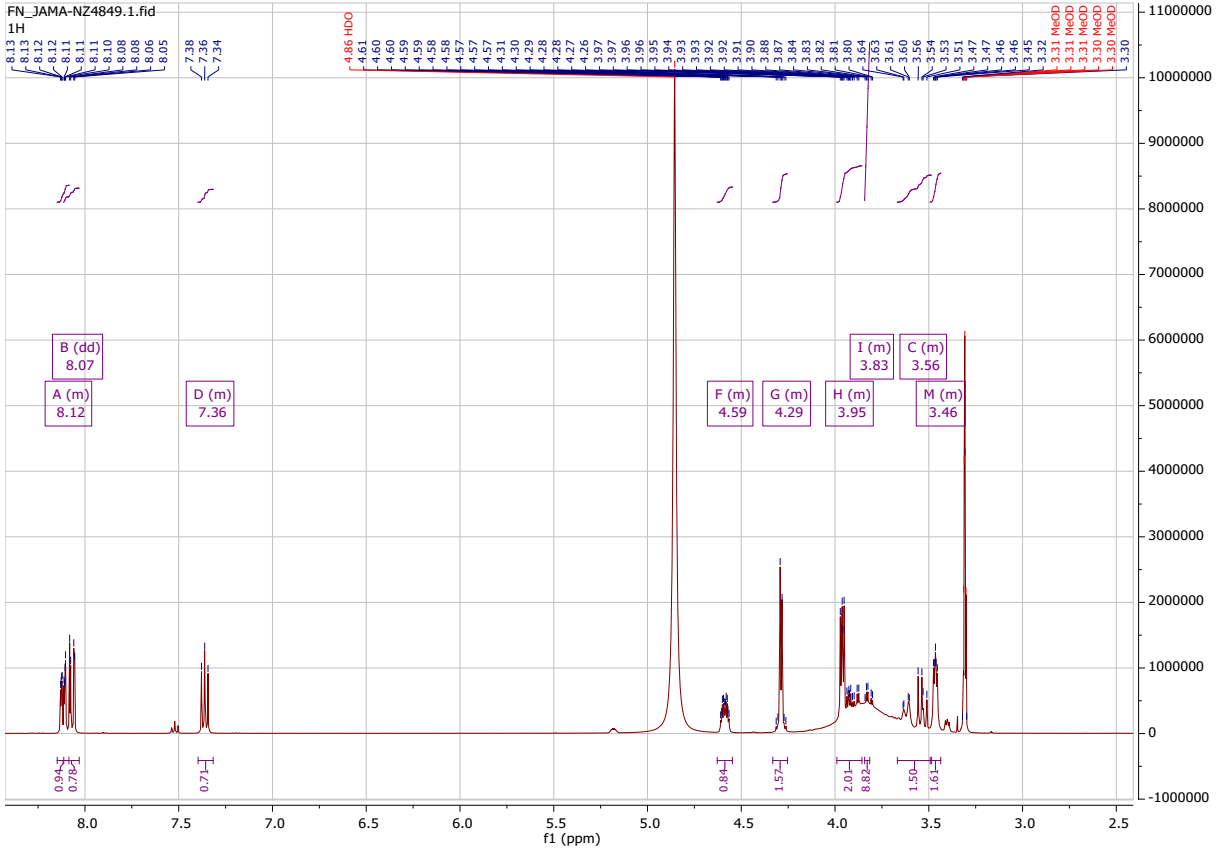
1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(4-methoxy-2-nitrophenoxy)propan-2-ol (**2**)

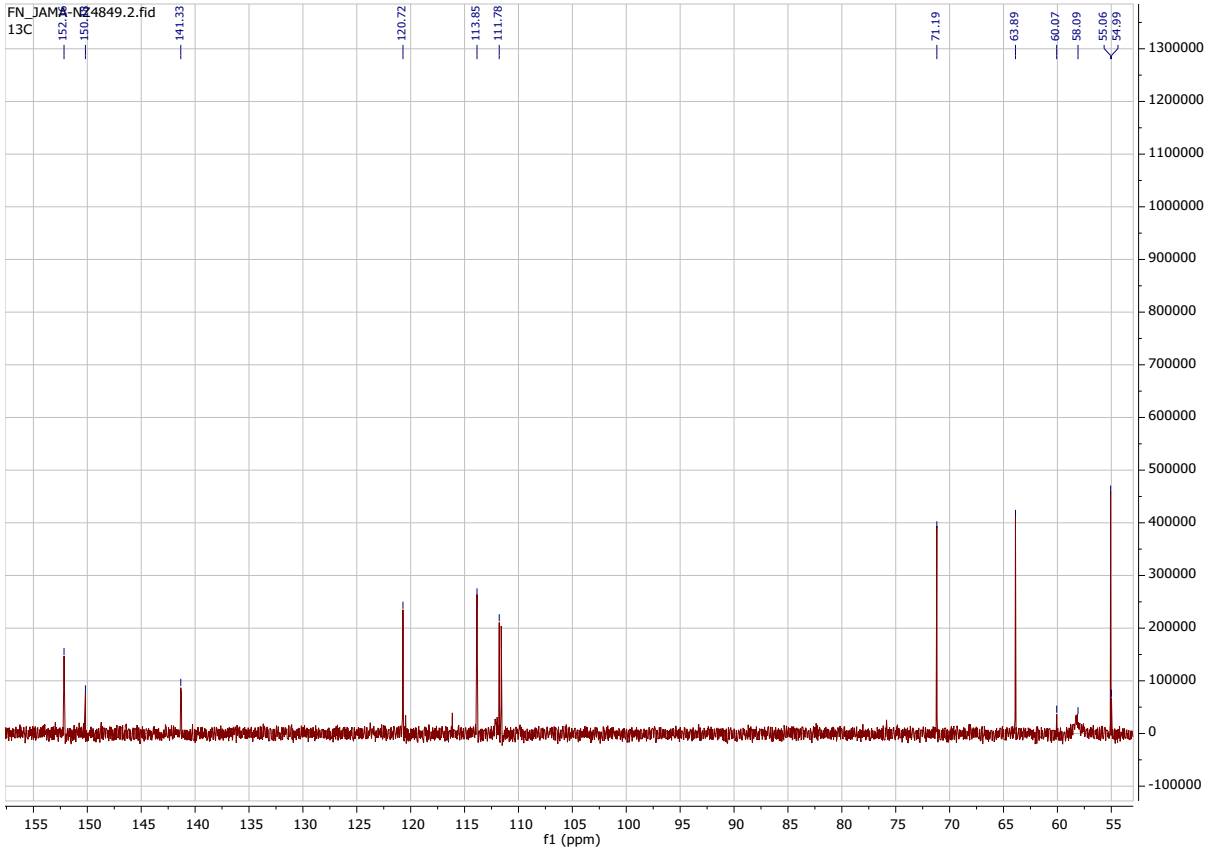


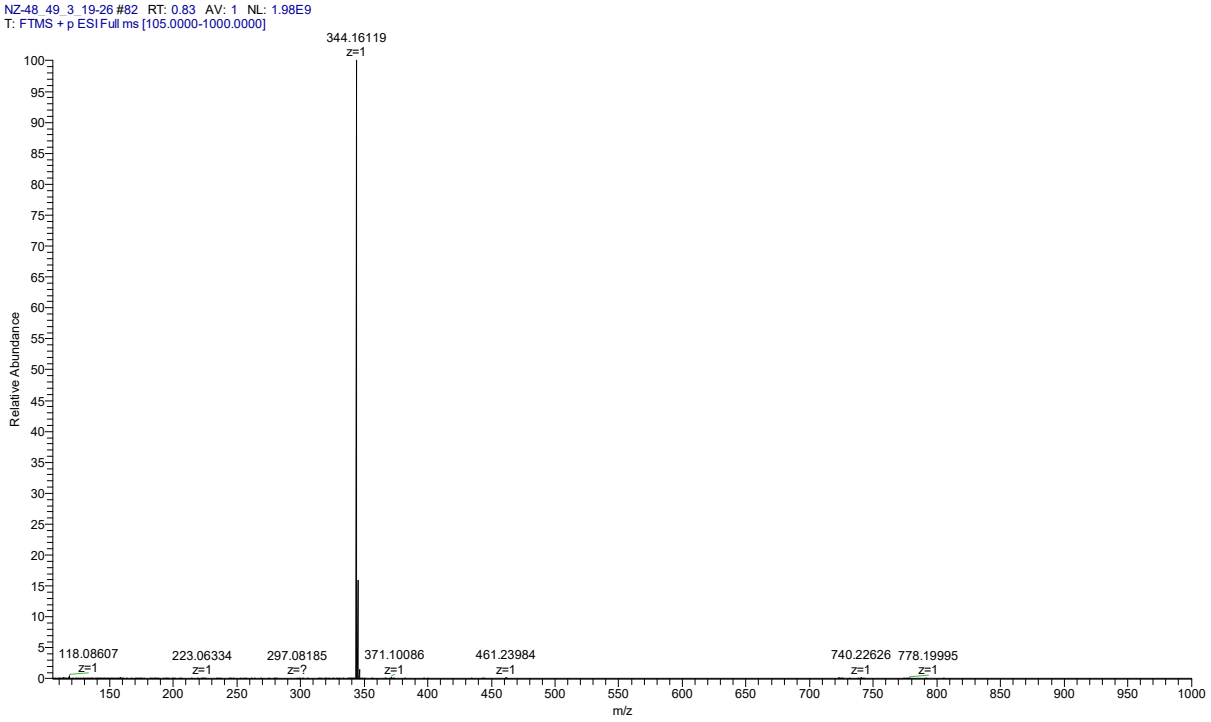

FN_BAMA_NZ62.2.fid
13C
151.88
146.27
138.87
121.98
117.35
110.47
71.56
63.80
58.57
56.16
54.84
49.10
48.57
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45
f1 (ppm)


NZ-67_JM13 #105 RT: 1.07 AV: 1 NL: 1.14E9
T: FTMS + p ESI Full ms [105.0000-1000.0000]
356.18076
z=1
157.03479
z=?
223.06306
z=1
333.29984
z=1
378.16245
z=1
473.25940
z=1
524.09198
z=?
638.67413
z=?
747.33051
z=1
800.24103
z=1
841.22565
z=?
Relative Abundance
m/z

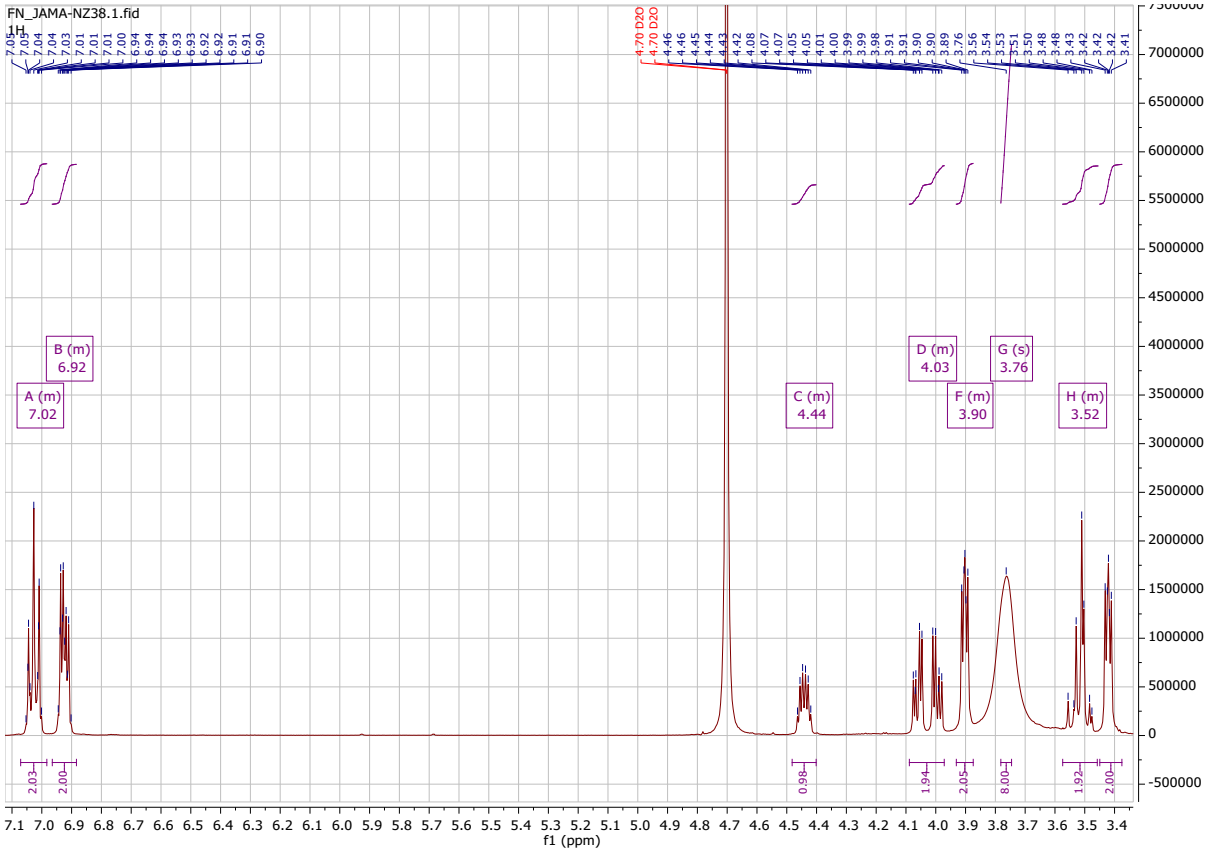
1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(2-fluoro-4-nitrophenoxy)propan-2-ol (**3**)

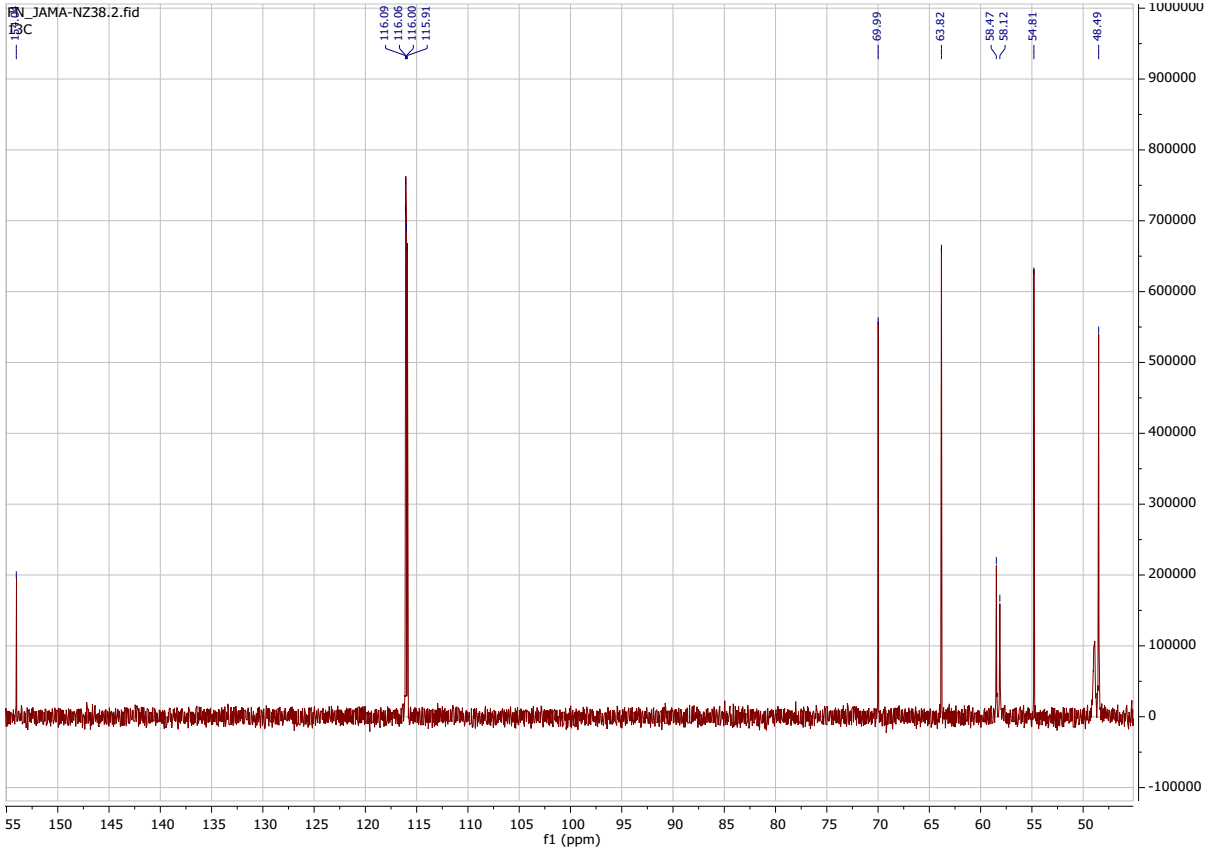


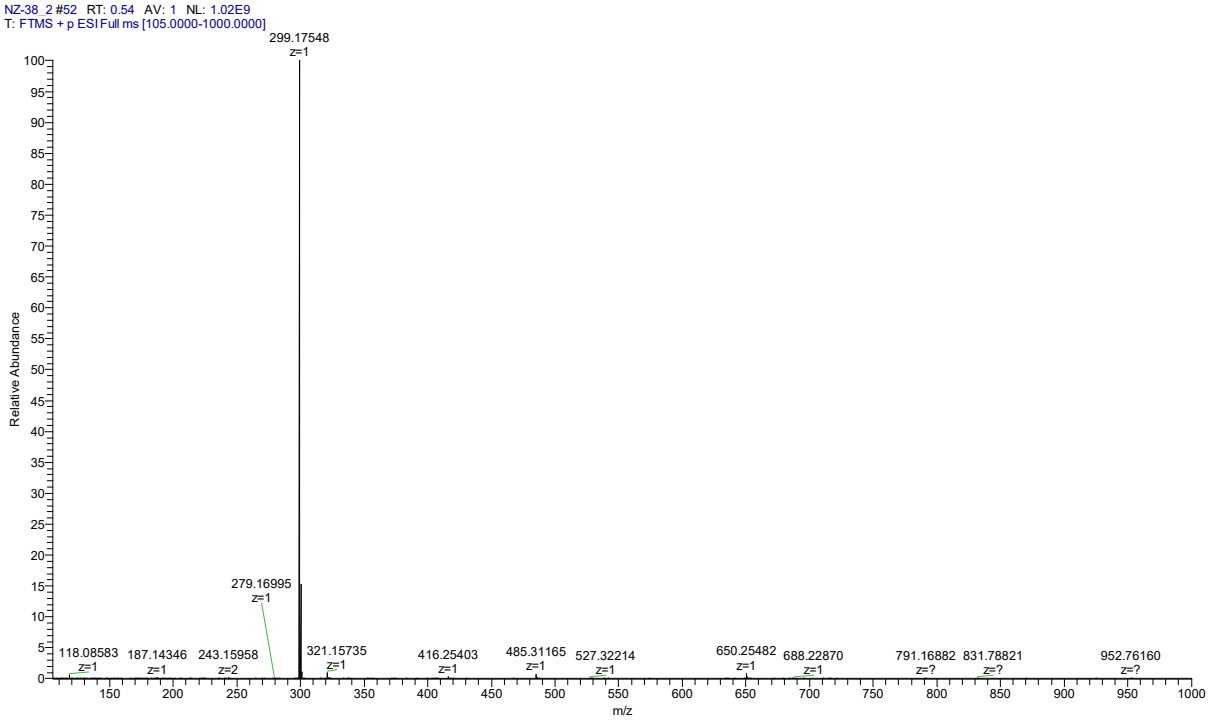

FN_JAM-N24849.2.fid
13C
152.18
150.22
141.33
120.72
113.85
111.78
71.19
63.89
60.07
58.09
55.06
54.99
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55
f1 (ppm)


NZ-48_49_3_19-26 #82 RT: 0.83 AV: 1 NL: 1.98E9
T: FTMS + p ESI Full ms [105.0000-1000.0000]
344.16119
z=1
118.08607
z=1
223.06334
z=1
297.08185
z=?
371.10086
z=1
461.23984
z=1
740.22626
z=1
778.19995
z=1
Relative Abundance
m/z

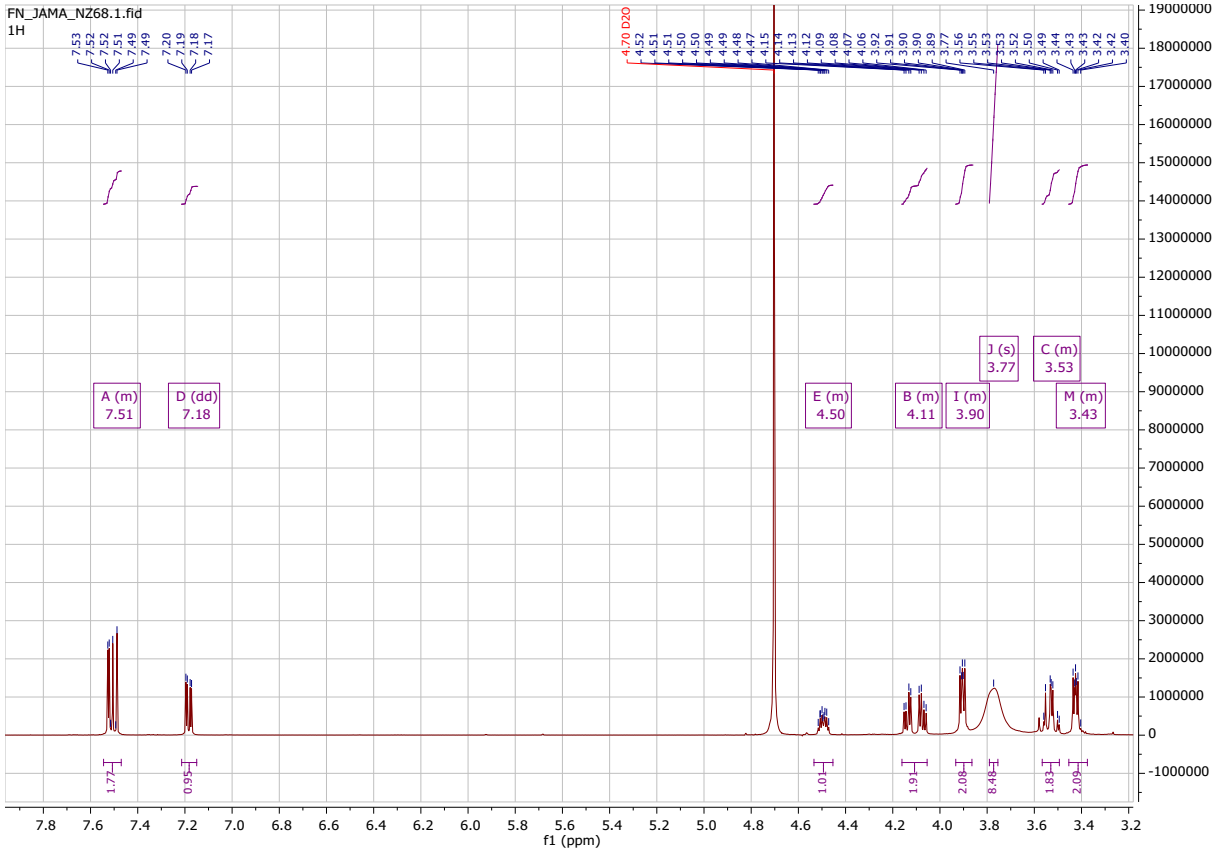
1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(4-fluorophenoxy)propan-2-ol (**4)**

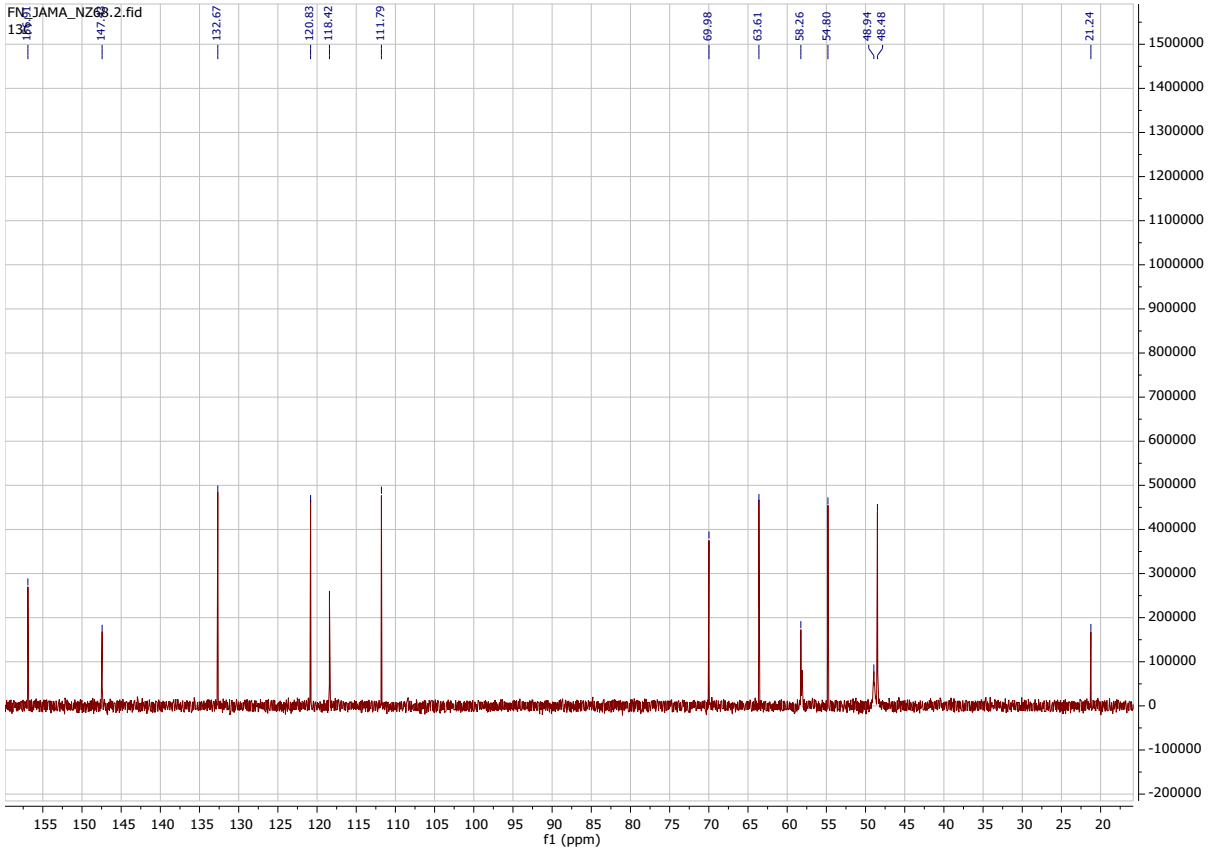


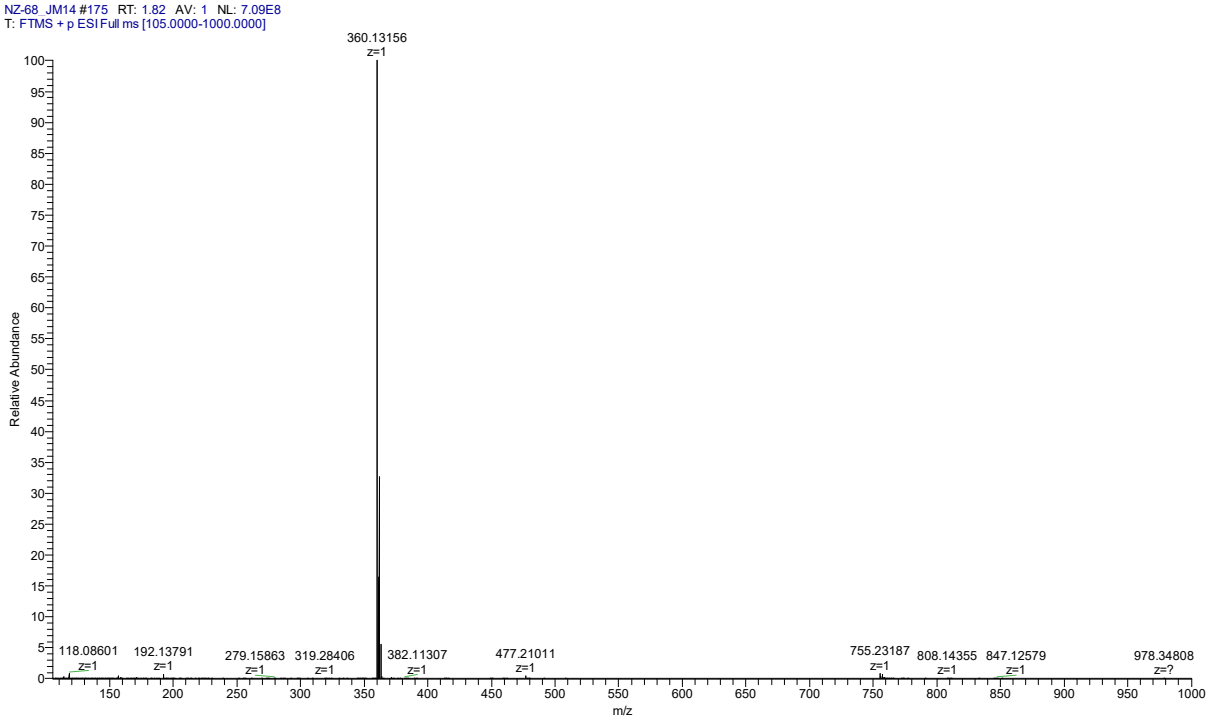

SN_JAMA-NZ38.2.fid
13C
116.09
116.06
116.00
115.91
69.99
63.82
58.47
58.12
54.81
48.49
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
55
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
f1 (ppm)


NZ-38_2 #52 RT: 0.54 AV: 1 NL: 1.02E9
T: FTMS + p ESI Full ms [105.0000-1000.0000]
299.17548
z=1
279.16995
z=1
118.08583
z=1
187.14346
z=1
243.15958
z=2
321.15735
z=1
416.25403
z=1
485.31165
z=1
527.32214
z=1
650.25482
z=1
688.22870
z=1
791.16882
z=?
831.78821
z=?
952.76160
z=?
Relative Abundance
m/z

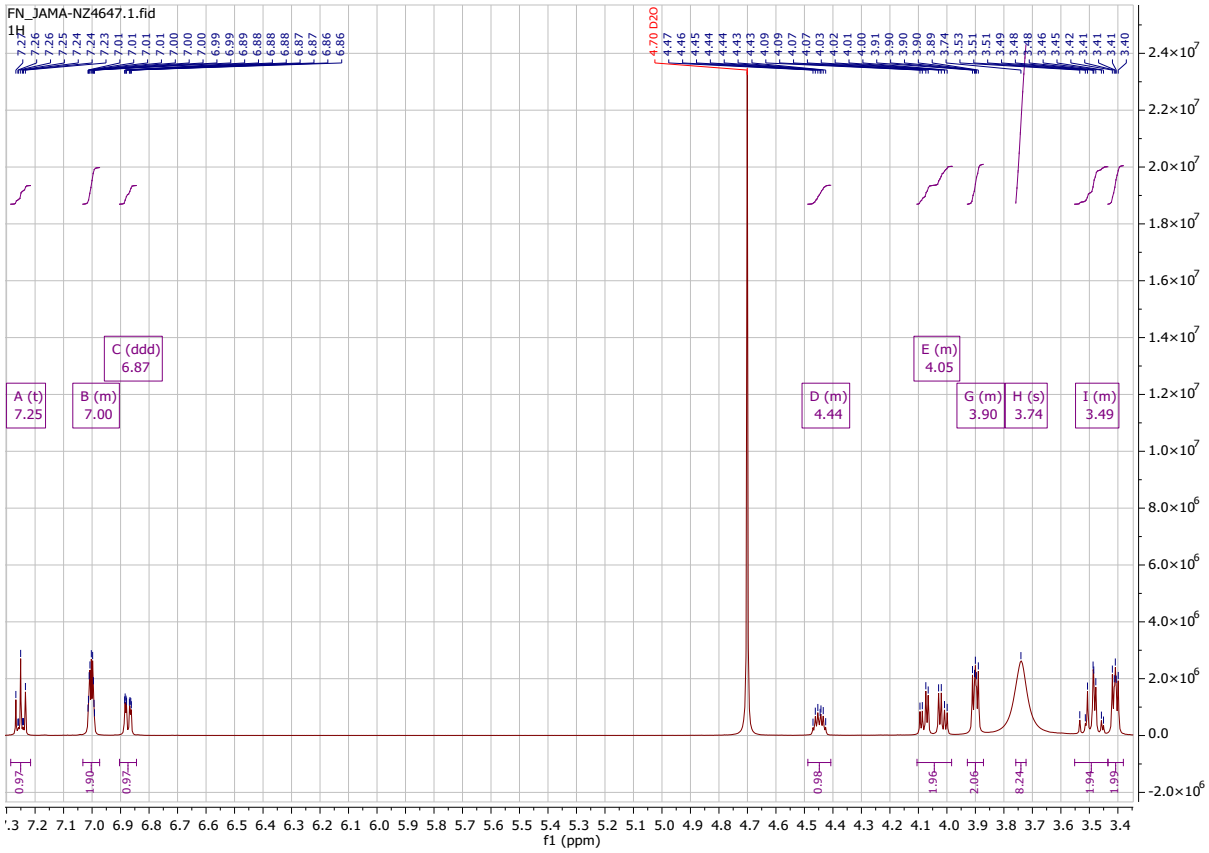
1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(4-chloro-3-nitrophenoxy)propan-2-ol (**5**)

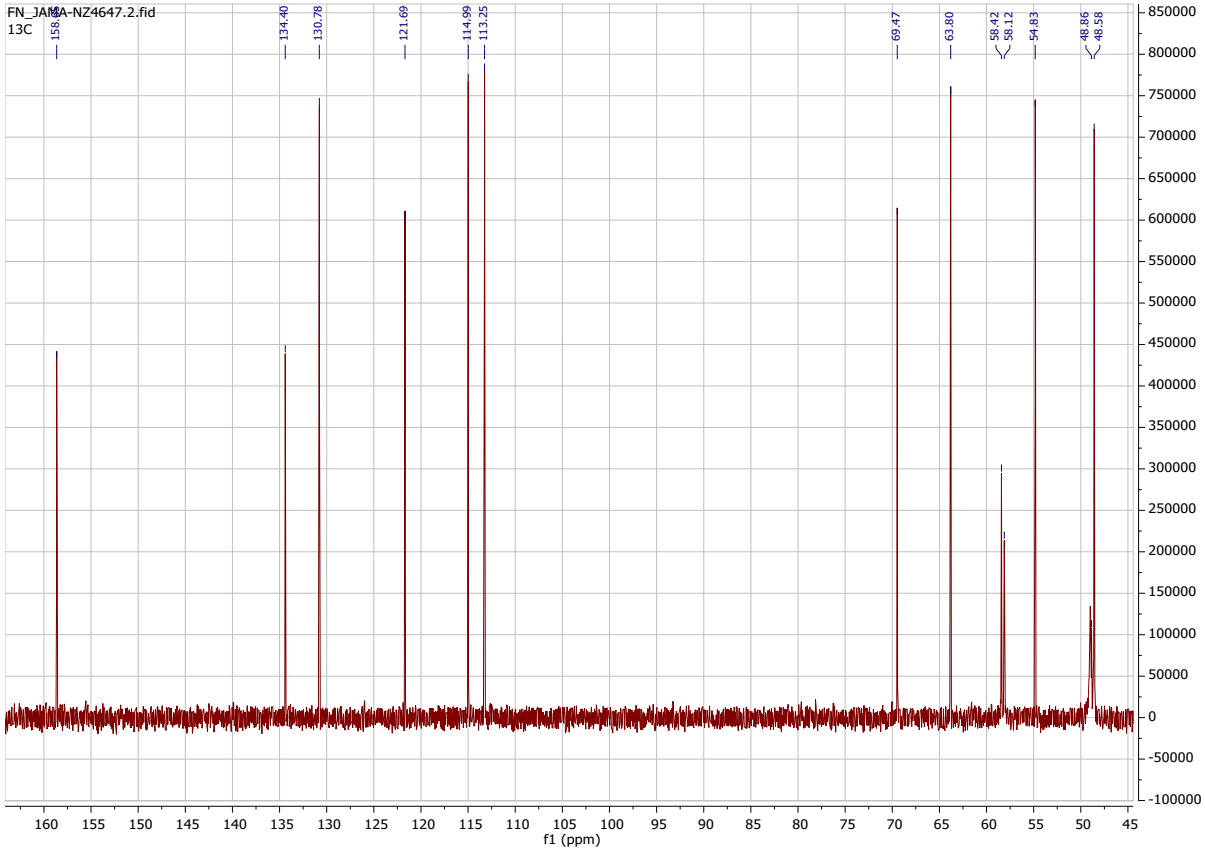


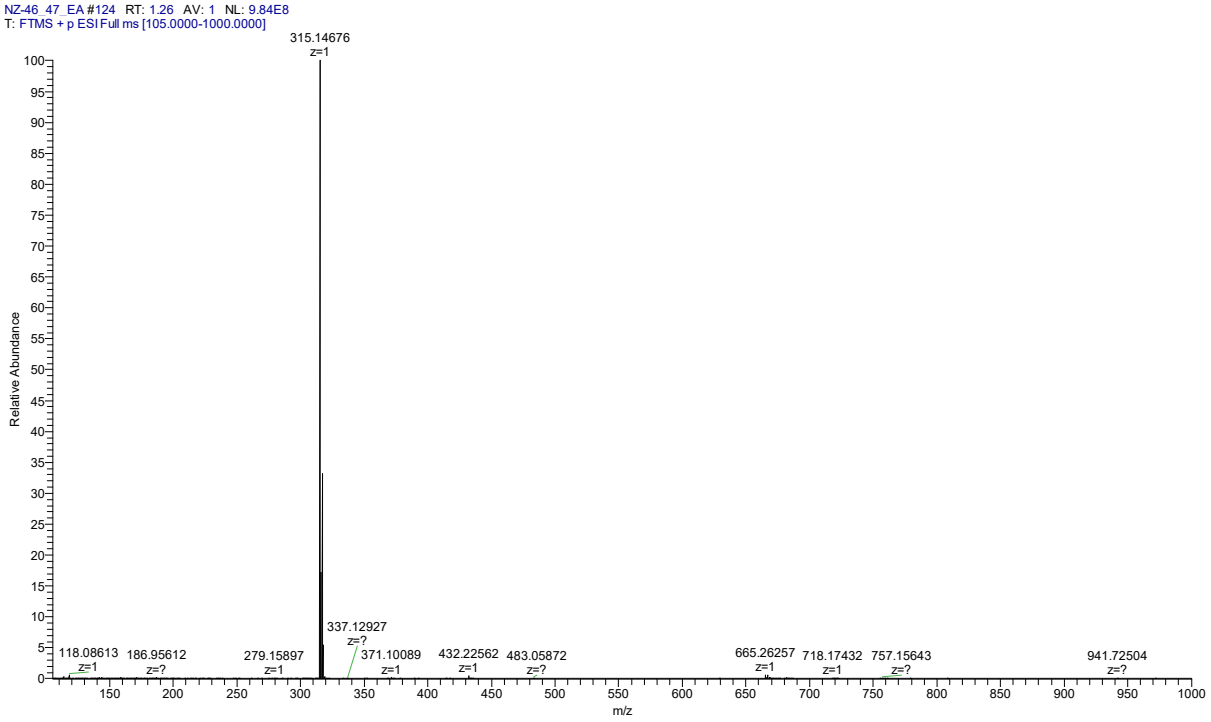

FN_JAMA_NZ68.2.fid
132.67
120.83
118.42
111.79
69.98
63.61
58.26
54.80
48.94
48.48
21.24
f1 (ppm)


NZ-68_JM14 #175 RT: 1.82 AV: 1 NL: 7.09E8
T: FTMS + p ESI Full ms [105.0000-1000.0000]
360.13156
z=1
118.08601
z=1
192.13791
z=1
279.15863
z=1
319.28406
z=1
382.11307
z=1
477.21011
z=1
755.23187
z=1
808.14355
z=1
847.12579
z=1
978.34808
z=?
Relative Abundance
m/z

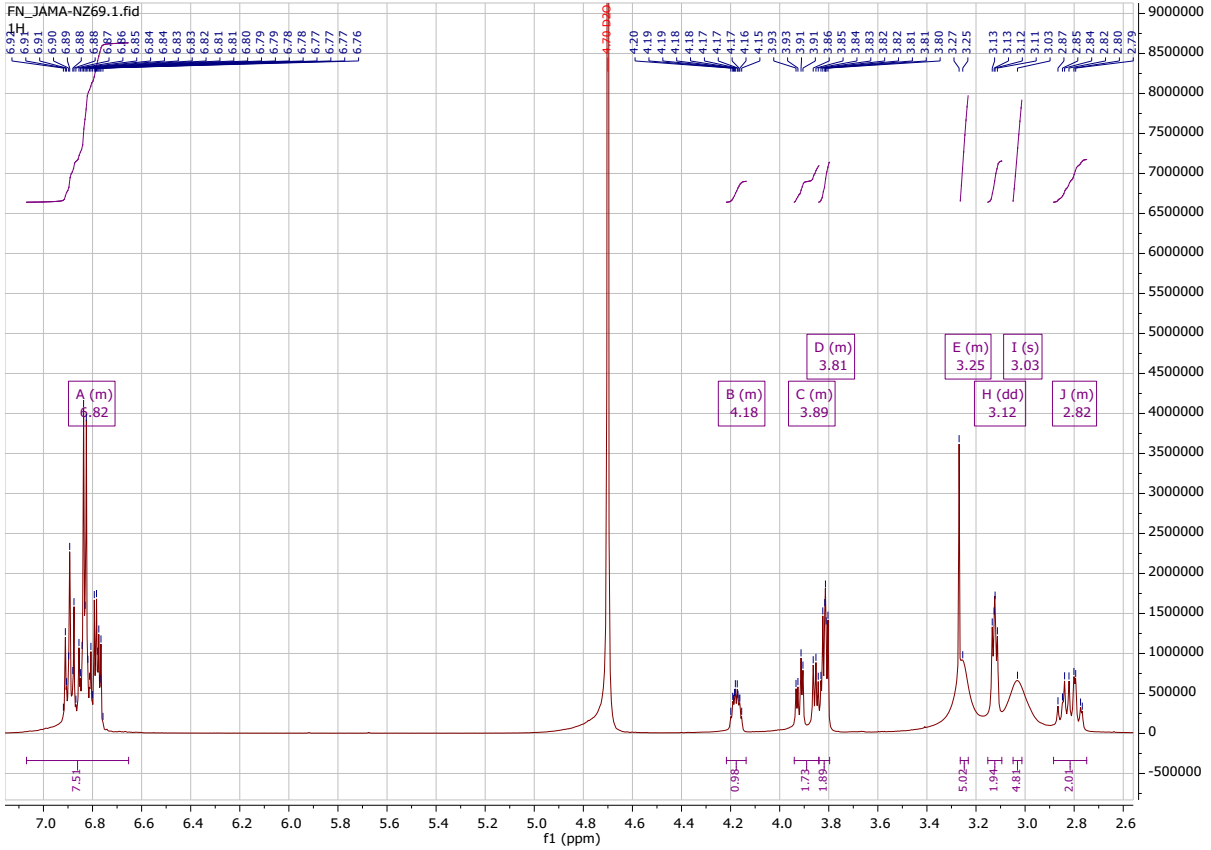
1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(3-chlorophenoxy)propan-2-ol (**6**)

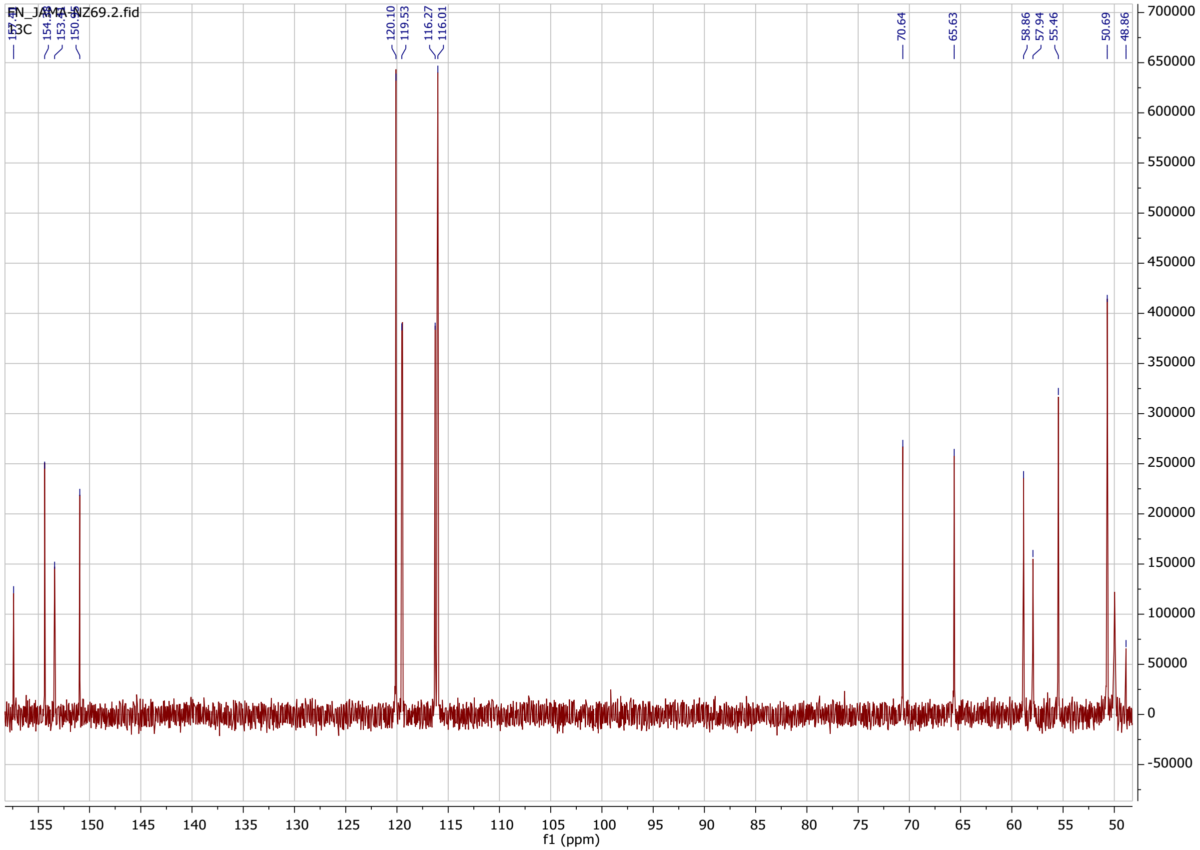



FN_JAMA-NZ4647.2.fid
13C
156.
134.40
130.78
121.69
114.99
113.25
69.47
63.80
58.42
58.12
54.83
48.86
48.58
850000
800000
750000
700000
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
-100000
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45
f1 (ppm)

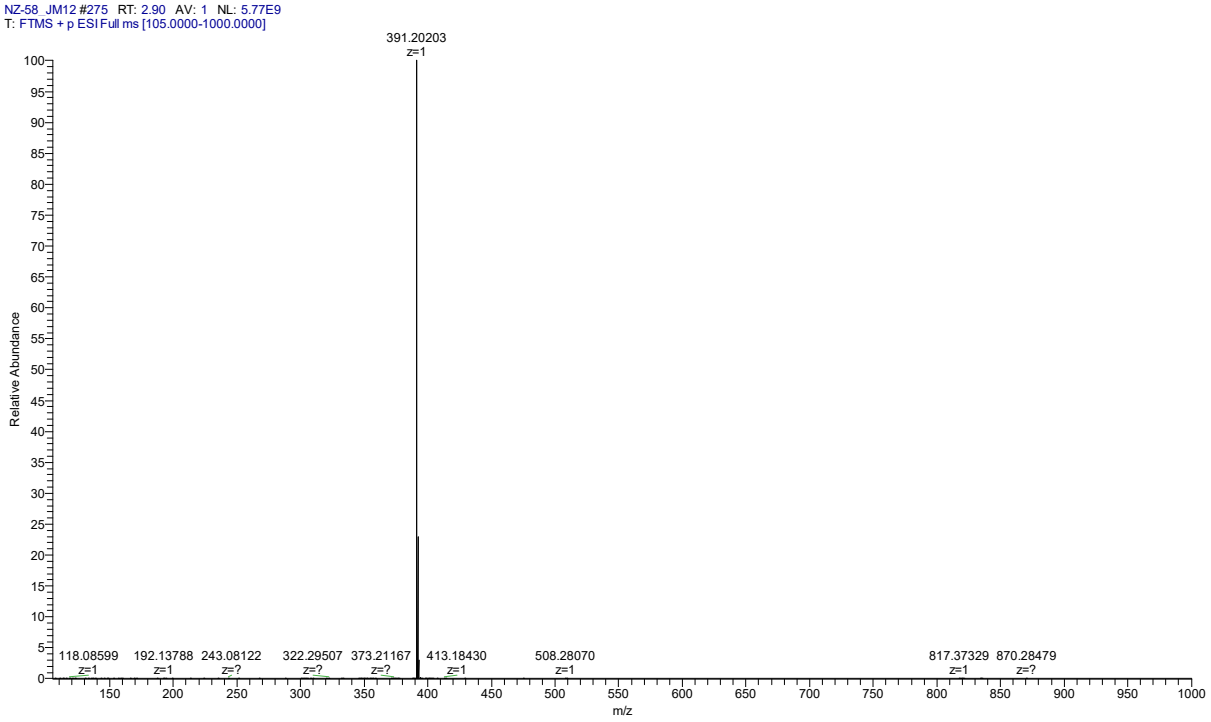

NZ-46_47_EA #124 RT: 1.26 AV: 1 NL: 9.84E8
T: FTMS + p ESI Full ms [105.0000-1000.0000]
315.14676
z=1
337.12927
z=?
118.08613
z=1
186.95612
z=?
279.15897
z=1
371.10089
z=1
432.22562
z=1
483.05872
z=?
665.26257
z=1
718.17432
z=1
757.15643
z=?
941.72504
z=?
Relative Abundance
m/z

1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(4-(4-fluorophenoxy)phenoxy)propan-2-ol (**7**)

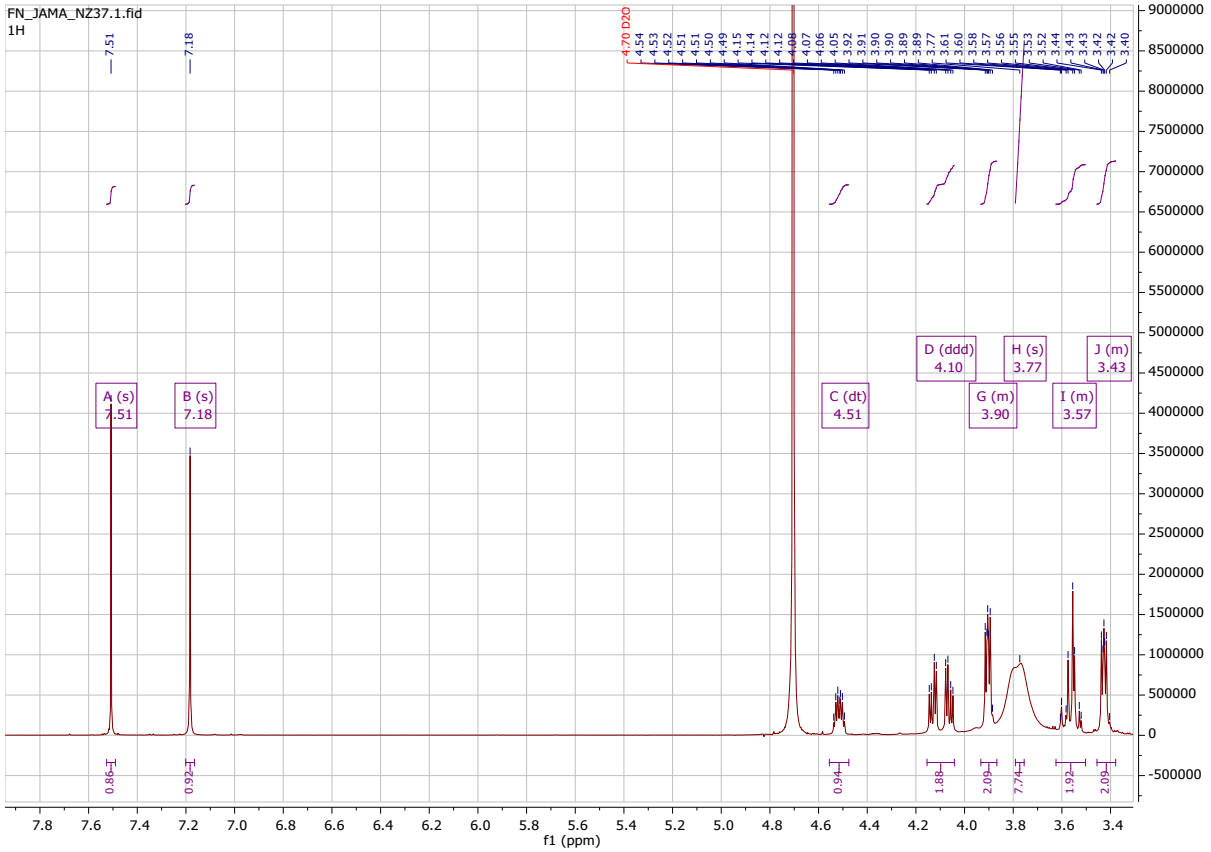


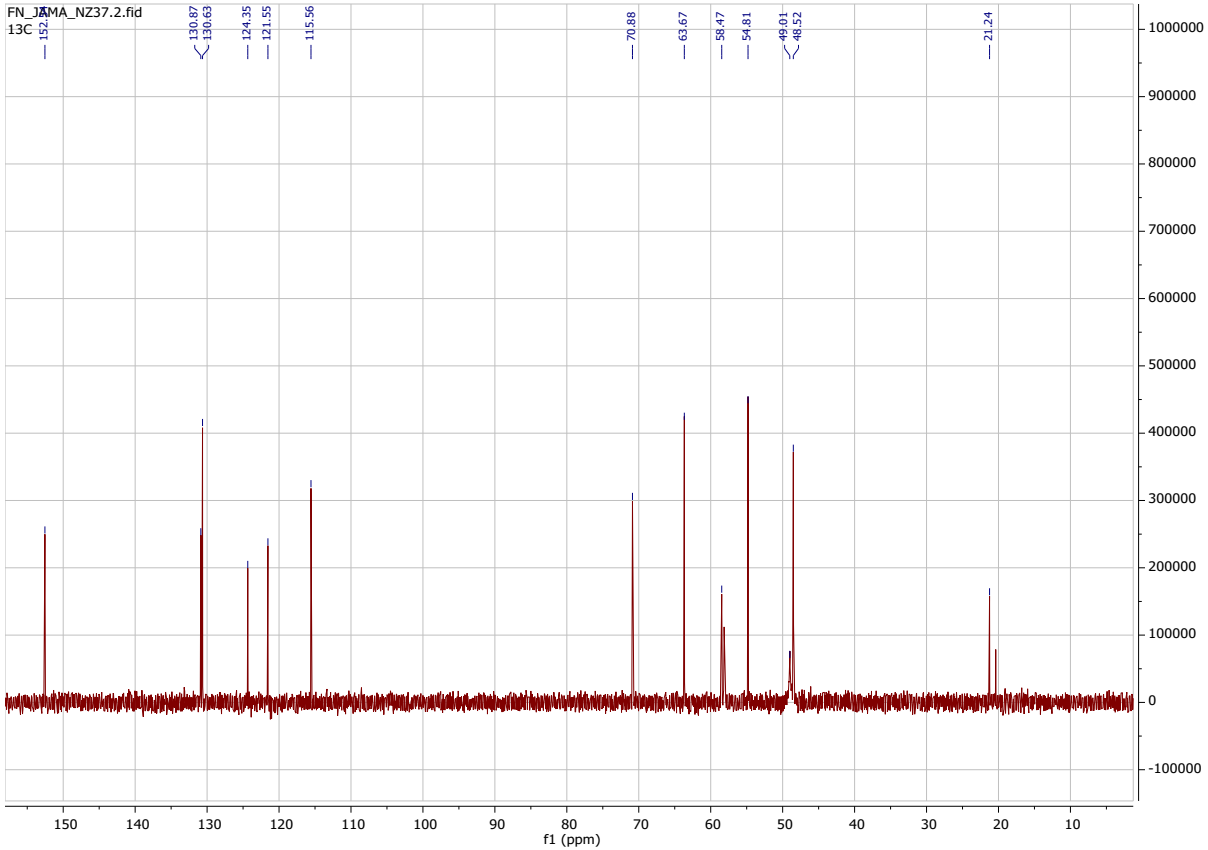


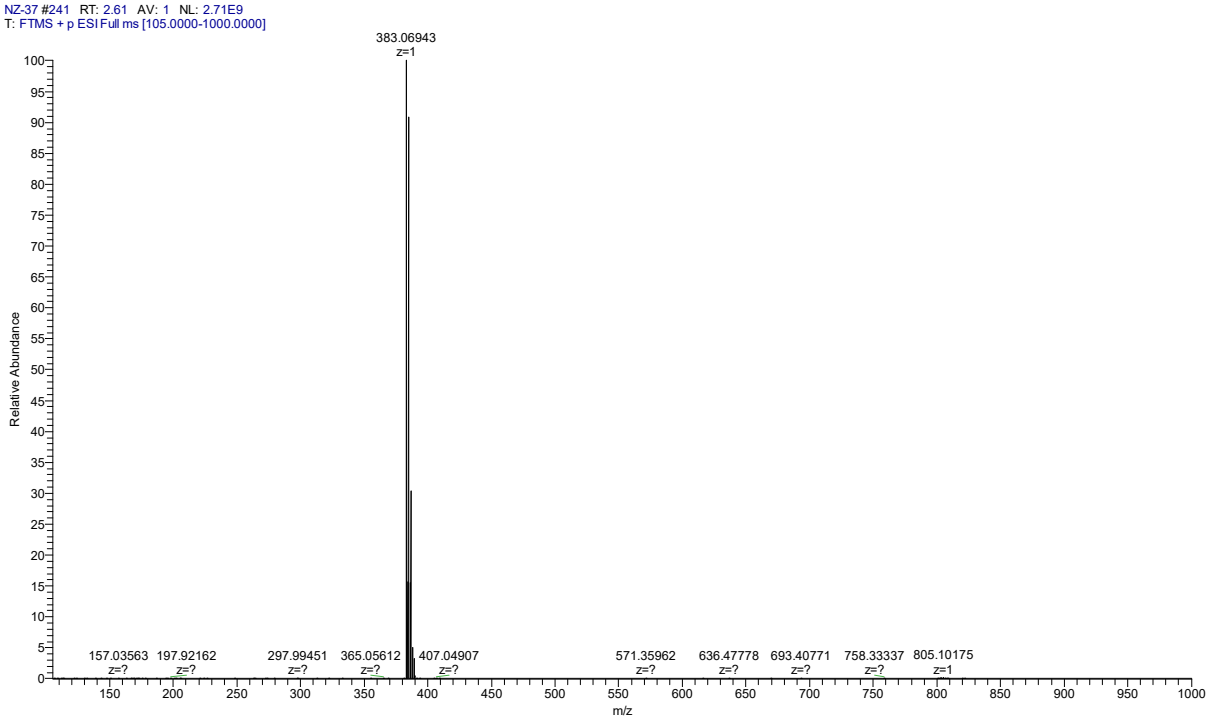
FN_JAMA-NZ69.2.fid
13C
120.10
119.53
116.27
116.01
70.64
65.63
58.86
57.94
55.46
50.69
48.86
f1 (ppm)


NZ-58_JM12 #275 RT: 2.90 AV: 1 NL: 5.77E9
T: FTMS + p ESI Full ms [105.0000-1000.0000]
391.20203
z=1
Relative Abundance
m/z
118.08599
z=1
192.13788
z=1
243.08122
z=?
322.29507
z=?
373.21167
z=?
413.18430
z=1
508.28070
z=1
817.37329
z=1
870.28479
z=?

1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-(2,4,5-trichlorophenoxy)propan-2-ol (**8**)




FN_JAMA_NZ37.2.fid
13C
152.
130.87
130.63
124.35
121.55
115.56
70.88
63.67
58.47
54.81
49.01
48.52
21.24
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


NZ-37 #241 RT: 2.61 AV: 1 NL: 2.71E9
T: FTMS + p ESI Full ms [105.0000-1000.0000]
383.06943
z=1
157.03563
z=?
197.92162
z=?
297.99451
z=?
365.05612
z=?
407.04907
z=?
571.35962
z=?
636.47778
z=?
693.40771
z=?
758.33337
z=?
805.10175
z=1
Relative Abundance
m/z