Small Molecule Microarrays Enable the Identification of a Selective, Quadruplex-Binding Inhibitor of MYC Expression

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Supplementary Information

General Materials and Methods. Reactions were conducted using anhydrous solvents (passed through activated alumina columns). All commercially obtained reagents were used as received. Flash column chromatography was performed using normal phase silica gel (60 Å, 230-400 mesh, RediSep® Normal-phase Silica Flash Columns) on a CombiFlash® Rf 200i (Teledyne Isco Inc). Preparative HPLC was performed with a Waters® 2545 Binary Gradient Module equipped with a Waters® 2767 Sample Manager fraction collector and a Luna 10 μm C18 110 Å (75 x 30 mm) column obtained from Phenomenex, Inc. High-resolution LC/MS analyses were conducted on a Thermo-Fisher LTQ-Orbitrap-XL hybrid mass spectrometer system with an Ion MAX API electrospray ion source in positive ion mode. Analytical LC/MS was performed using a Shimadzu LCMS-2020 Single Quadrupole utilizing a Kinetex 2.6 μm C18 100 Å (2.1 x 50 mm) column obtained from Phenomenex Inc. Runs employed a gradient of 0→90% MeOH/0.1% aqueous formic acid over 4.5 min at a flow rate of 0.2 mL/min. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian and Bruker spectrometers (at 400 or 500 MHz or at 100 or 125 MHz) and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for ^{13}C NMR

spectra are reported in terms of chemical shift. Surface plasmon resonance analysis was performed at the ATRF (NCI-Frederick) using a Biacore T200 (GE Healthcare).

Experimental Procedures and Supplementary Data.

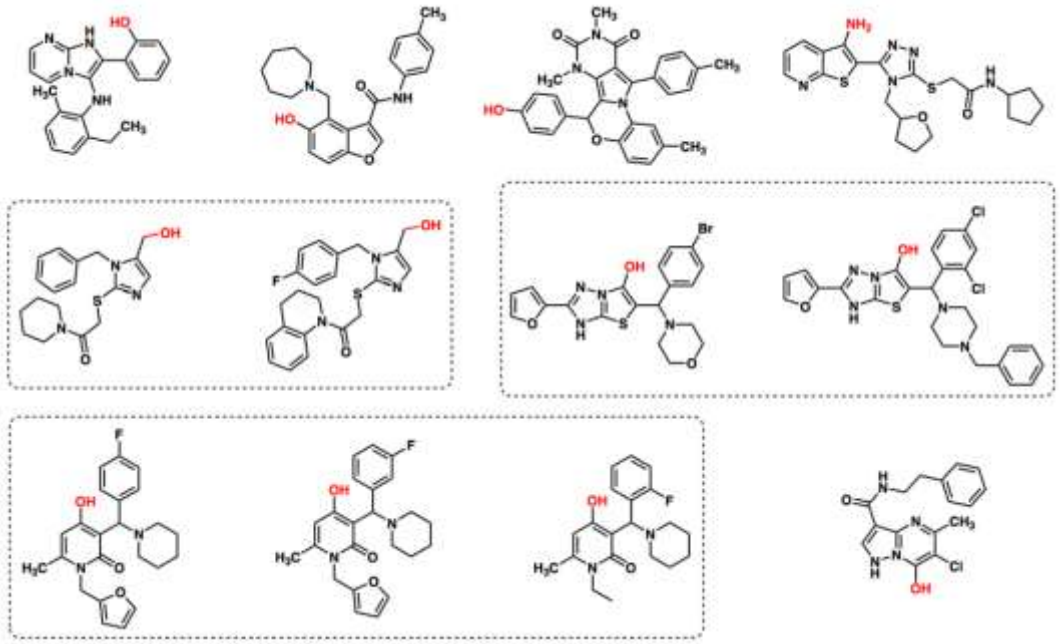


Figure S1. List of initial hits pursued from the SMM screen. Putative point of attachment to the array is indicated in red.

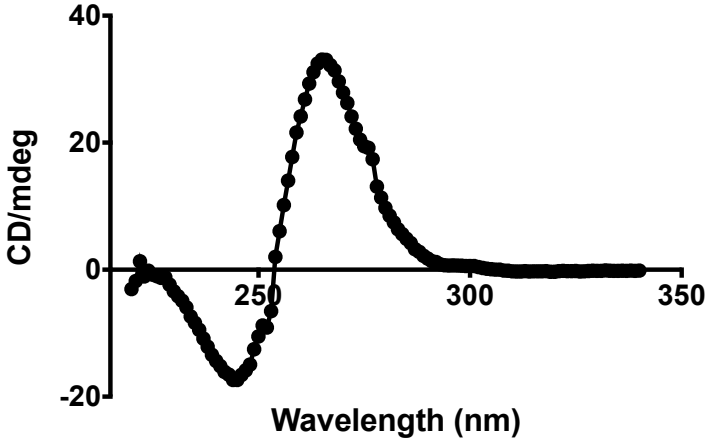


Figure S2. Circular dichroism spectrum of Pu22 quadruplex DNA. Observed maximum (262 nm) and minimum (244 nm) demonstrate formation of a properly folded parallel-stranded G-quadruplex structure.

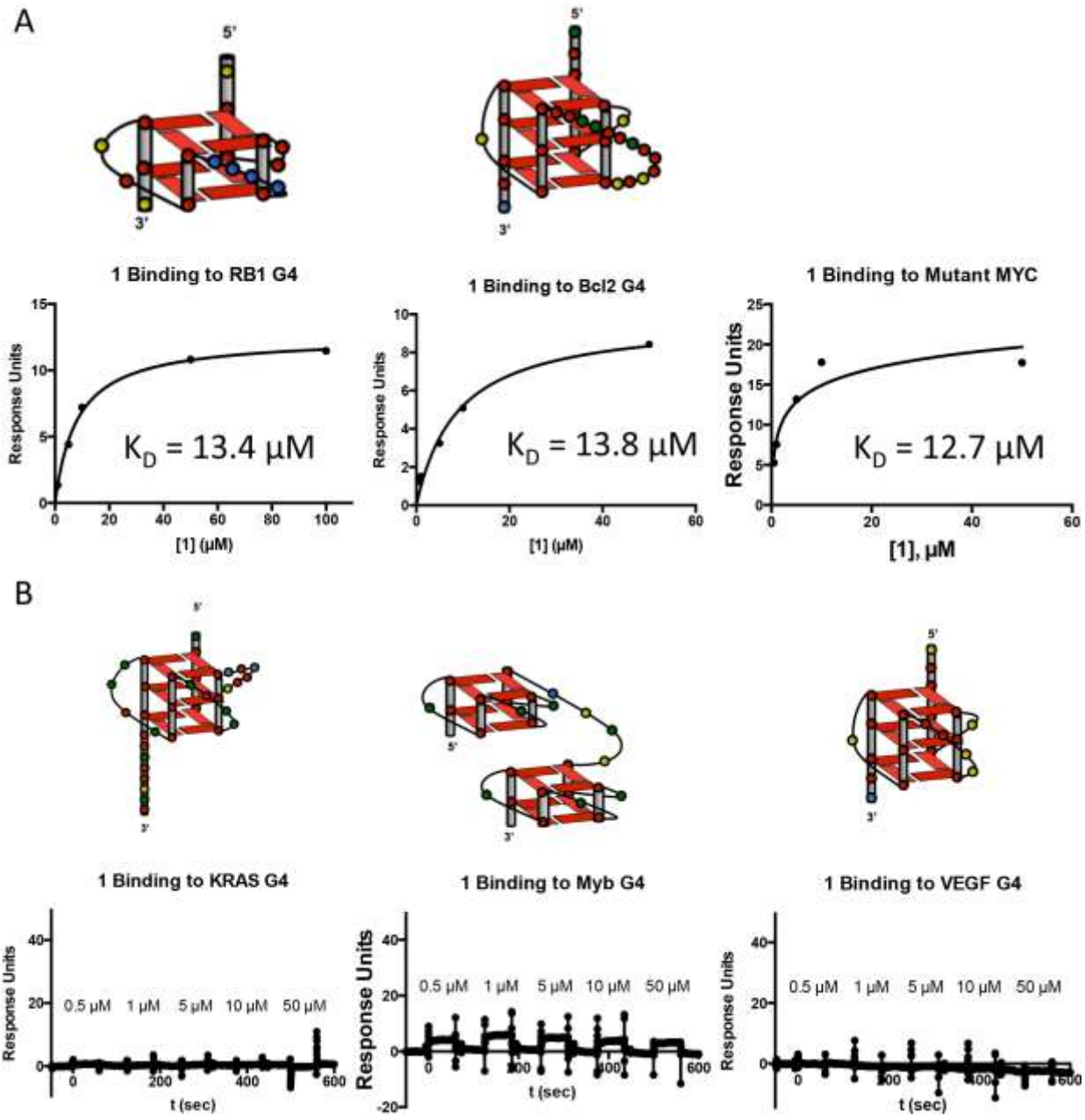


Figure S3. SPR analysis of **1** binding to various G-quadruplex and mutant sequences. Where binding is observed (A), the fitted K_D is shown, as for RB1, Bcl2, and a mutant sequence. Where no binding is observed (B), SPR sensorgrams are shown, as for KRAS, Myb, and VEGF. Cartoon schematics of each G4 are also provided. For each case, a biotinylated oligonucleotide was purchased, annealed in 100 mM KCl in PBS (pH 7.4) at 100 °C for 3 min and allowed to cool to room temperature. CD spectroscopy was used to confirm proper folding of each quadruplex before analysis.

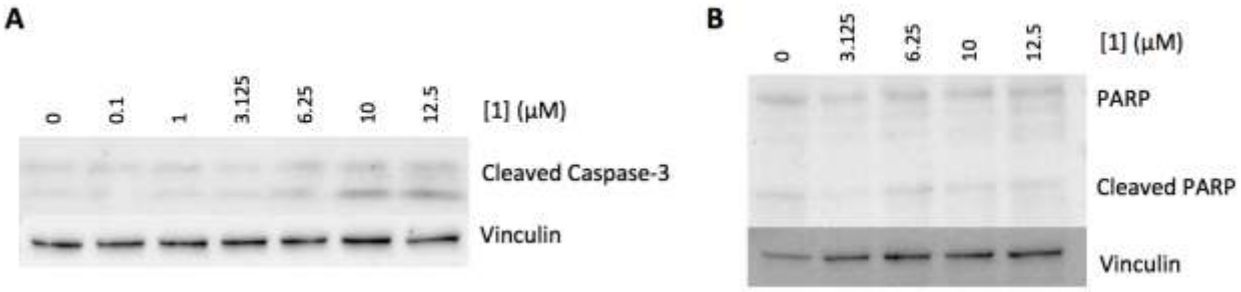


Figure S4. PARP Cleavage and Caspase Cleavage assays upon treatment with **1** (Western blot).

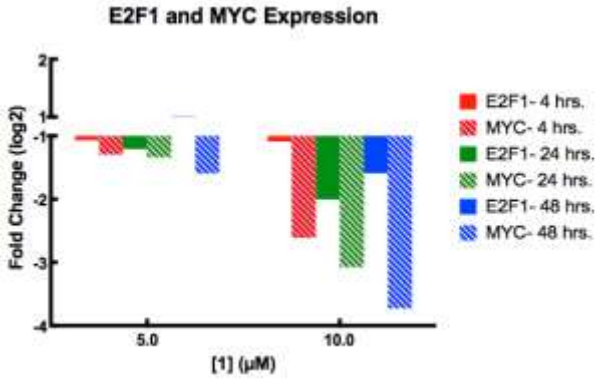


Figure S5. Analysis of MYC and E2F1 (a MYC target), by qPCR upon treatment with **1**.

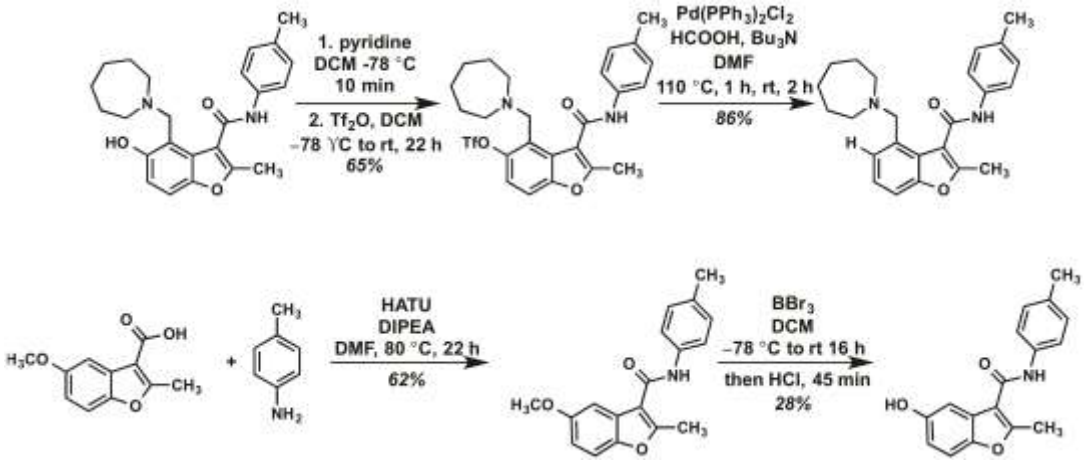
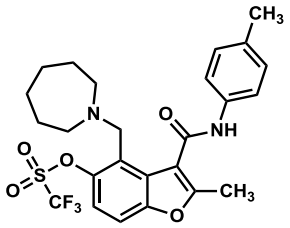
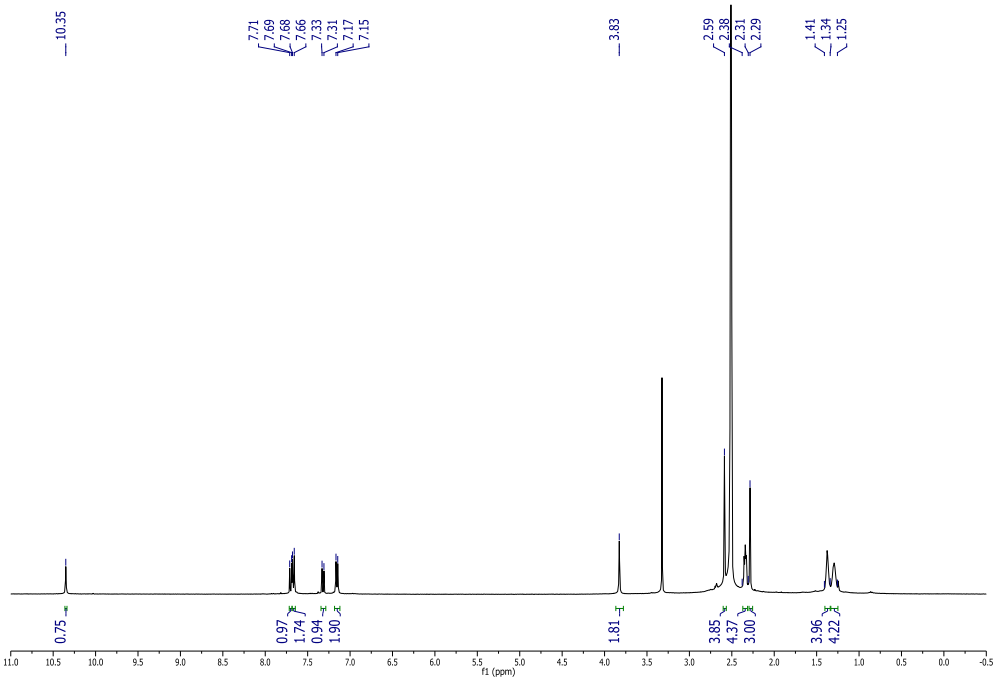


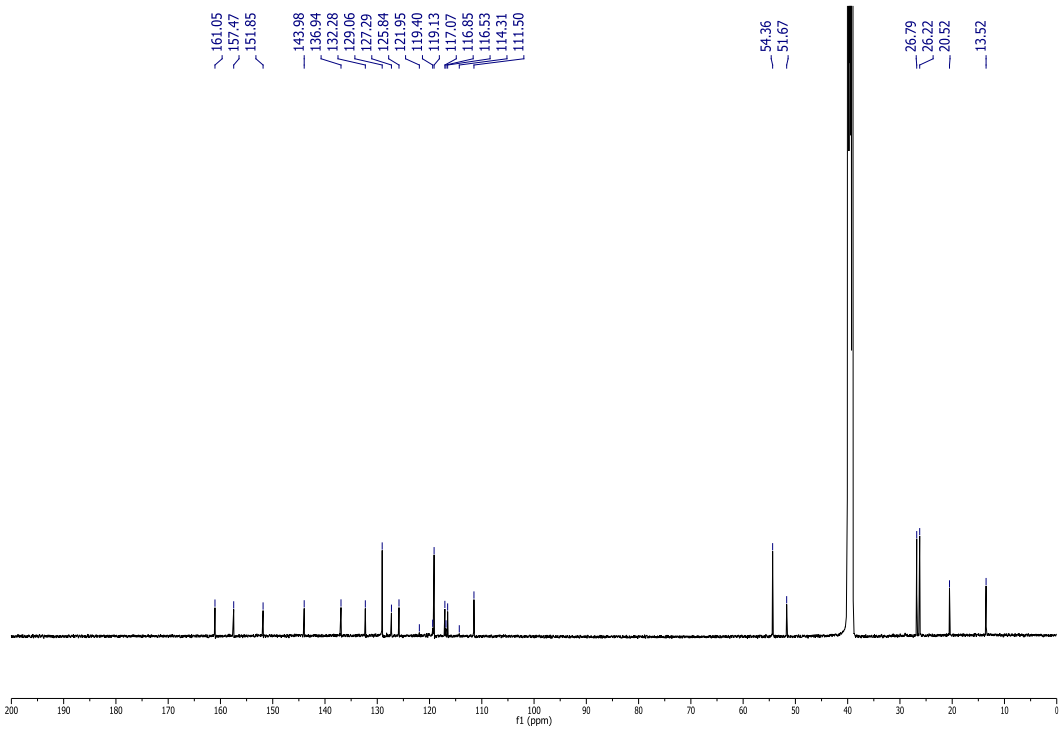
Figure S6. Preparation of compounds **2** and **3**.

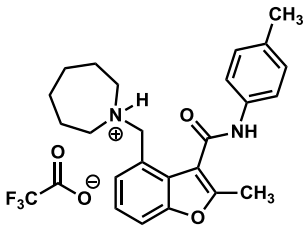
Procedures for Synthesis of Compounds 2, 3



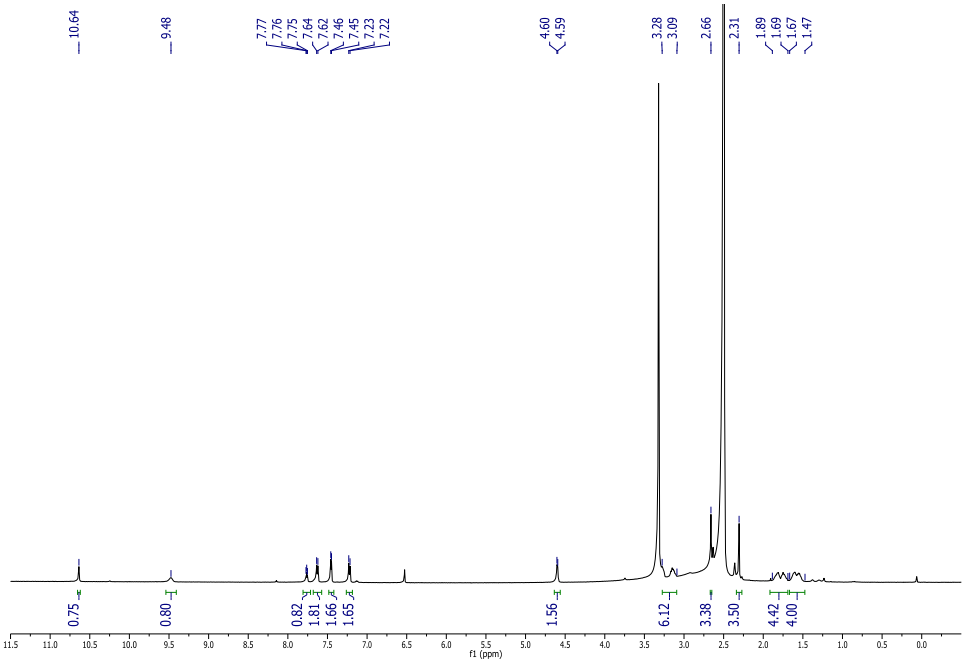
A solution of phenol (Otava 7018860558, 12 mg, 0.028 mmol) in DCM (0.28 mL) was cooled to −78 °C. Pyridine (4.5 μL, 0.056 mmol) was added and the solution was stirred for 10 min. At this point trifluoromethanesulfonic anhydride solution (1M in DCM, 34 μL, 0.034 mmol) was added slowly. The reaction was gradually warmed to rt overnight. After a total of 23 h, the solvent was removed in vacuo and the residue was purified by flash column chromatography (10-50% EtOAc/hexanes) to provide pure triflate (9.5 mg, 65%) as a white solid. R_f = 0.71 (33% EtOAc/ hexanes). ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.35 (s, 1H), 7.70 (d, *J* = 9.0 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 9.0 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 2H), 3.83 (s, 2H), 2.59 (s, 3H), 2.38-2.31 (m, 4H), 2.29 (s, 3H), 1.41-1.34 (m, 4H), 1.34-1.25 (m, 4H). ^{13}C NMR ($(CD_3)_2SO$, 125 MHz) δ 161.0, 157.5, 151.8, 144.0, 136.9, 132.3, 129.1, 127.3, 125.8, 119.1, 118.1 (q, J_{C-F} = 320 Hz), 117.1, 116.5, 111.5, 54.4, 51.7, 26.8, 26.2, 20.5, 13.5; HRMS (ESI) calculated for $C_{25}H_{28}N_2O_6S$ (MH^+) 541.1615, observed 541.1615.

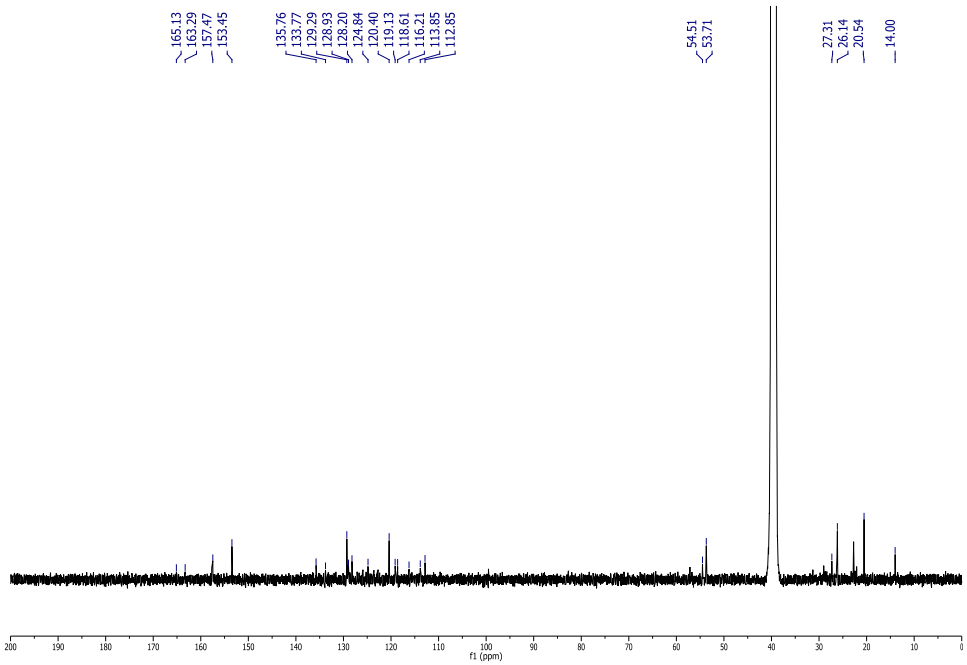

10.35
7.71
7.69
7.68
7.66
7.33
7.31
7.17
7.15
3.83
2.59
2.38
2.31
2.29
1.41
1.34
1.25
0.75
0.97
1.74
0.94
1.90
1.81
3.85
4.37
3.00
3.96
4.22
f1 (ppm)


161.05
157.47
151.85
143.98
136.94
132.28
129.06
127.29
125.84
121.95
119.40
119.13
117.07
116.85
116.53
114.31
111.50
54.36
51.67
26.79
26.22
20.52
13.52
f1 (ppm)



To a solution of triflate (3.1 mg, 0.0059 mmol) in DMF (60 μL, 0.098 M) was added formic acid (0.56 μL, 0.014 mmol), tributylamine (5 μL, 0.063 mmol), and $Pd(PPh_3)_2Cl_2$ (0.5 mg, 0.0007 mmol) successively under Ar. The solution was heated to 110 °C for 1 h at which point it was cooled to room temperature. The mixture was filtered through cotton and the filtrate was purified by HPLC (10-95% MeCN/H_2O containing 0.1% TFA in the running buffer) to provide **3** as a white solid (2.5 mg, 86%). ^{1}H NMR ($(CD_3)_2SO$, 500 MHz) δ 10.64 (s, 1H), 9.48 (brs, 1H), 7.76 (t, *J* = 4.5 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 2H), 7.45 (d, *J* = 4.6 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 1H), 4.60 (d, *J* = 5.3 Hz, 2H), 3.28-3.09 (m, 4H), 2.66 (s, 3H), 2.31 (s, 3H), 1.89-1.69 (m, 4H), 1.67-1.47 (m, 4H); ^{13}C NMR ($(CD_3)_2SO$, 125 MHz) δ 165.1, 163.3, 157.5, 153.5, 135.8, 133.8, 129.3, 128.9, 128.2, 124.8, 120.4, 119.1, 117.4 (q_{C-F}, *J* = 301.8 Hz), 113.8, 112.8, 54.5, 53.7, 27.3, 26.1, 20.5, 14.0; HRMS (ESI) calculated for $C_{24}H_{29}N_2O_2$ (MH^+) 377.2224, observed 377.2225.


10.64
9.48
7.77
7.76
7.75
7.64
7.62
7.46
7.45
7.23
7.22
4.60
4.59
3.28
3.09
2.66
2.31
1.89
1.69
1.67
1.47
0.75
0.80
0.82
1.81
1.66
1.65
1.56
6.12
3.38
3.50
4.42
4.00
f1 (ppm)


165.13
163.29
157.47
153.45
135.76
133.77
129.29
128.93
128.20
124.84
120.40
119.13
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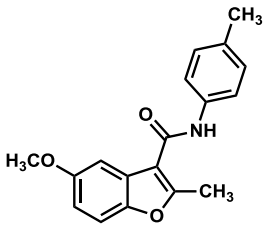
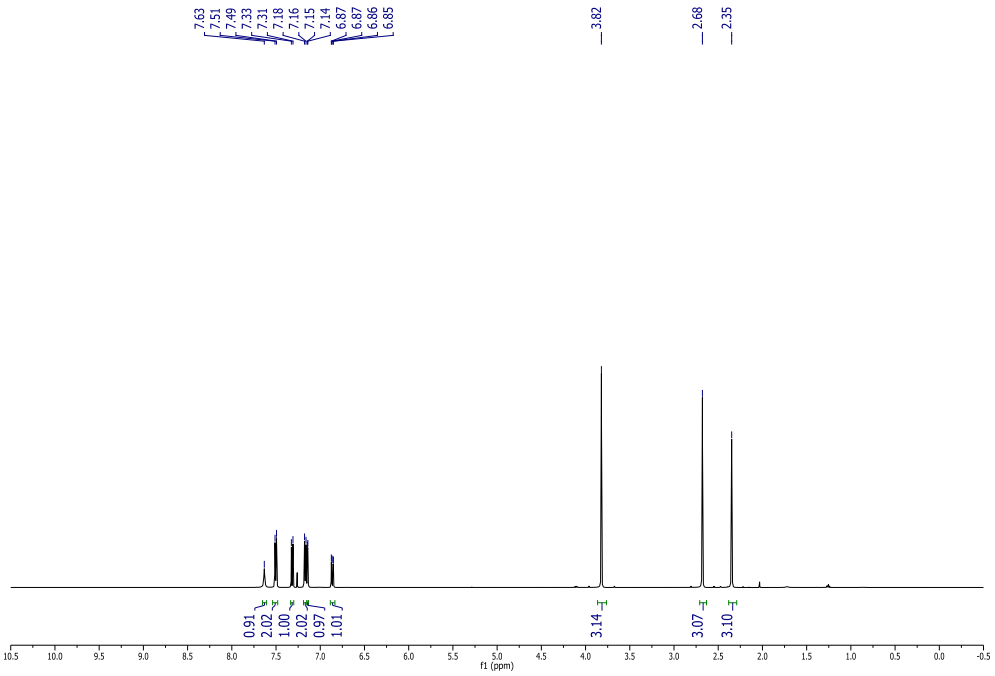
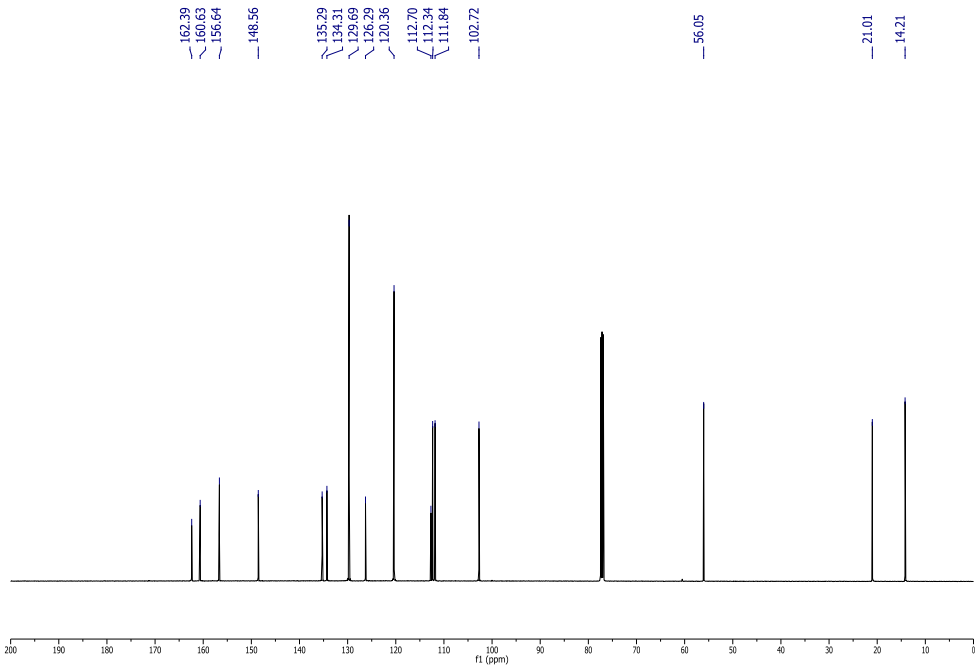
26.14
20.54
14.00
f1 (ppm)



To a solution of 5-methoxy-2-methylbenzofuran-3-carboxylic acid (1.20 g, 5.80 mmol) in DMF (29 mL) was added HATU (5.50 g, 14.0 mmol), p-toluidine (2.50 g, 23.0 mmol), and DIPEA (1.5 mL. 8.7 mmol). The brown solution was heated to 80 °C under argon. After 22 h, the reaction was cooled to room temperature, after which it was diluted with EtOAc and washed with $NaHCO_3$ (sat., aq). The aqueous layer was extracted 2X with EtOAc and the combined organic layers were washed successively with NH_4Cl (sat., aq.), brine, and H_2O (3X). It was then dried over Na_2SO_4, filtered and concentrated to give a brown oil. Flash column chromatography (5-30% EtOAc/hexanes) afforded a fraction of pure amide in addition to a mixture of starting material and p-toluidine. The mixed fraction was repurified using the same conditions, and this process was repeated two additional times. In total 1.07 g (3.62 mmol, 62%) pure amide was obtained as a tan solid. R_f = 0.60 (25% EtOAc/ hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (brs, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.9 Hz, 1H), 7.17 (d, *J* = 8.2 Hz, 2H), 7.14 (d, *J* = 2.5 Hz, 1H), 6.86 (dd, *J* = 8.9, 2.5 Hz), 3.82 (s, 3H), 2.68 (s, 3H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.4, 160.6, 156.6, 148.6, 135.3, 134.3, 129.7, 126.3, 120.4, 112.7, 112.3, 111.8, 102.7, 56.0, 21.0, 14.2; HRMS (ESI) calculated for $C_{18}H_{18}NO_3$ (MH^+) 296.1281, observed 296.1285.


7.63
7.51
7.49
7.33
7.31
7.18
7.16
7.15
7.14
6.87
6.87
6.86
6.85
3.82
2.68
2.35
0.91
2.02
1.00
2.02
0.97
1.01
3.14
3.07
3.10
f1 (ppm)


162.39
160.63
156.64
148.56
135.29
134.31
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112.34
111.84
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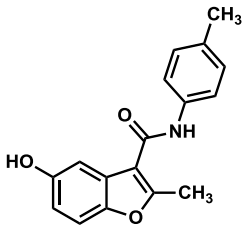
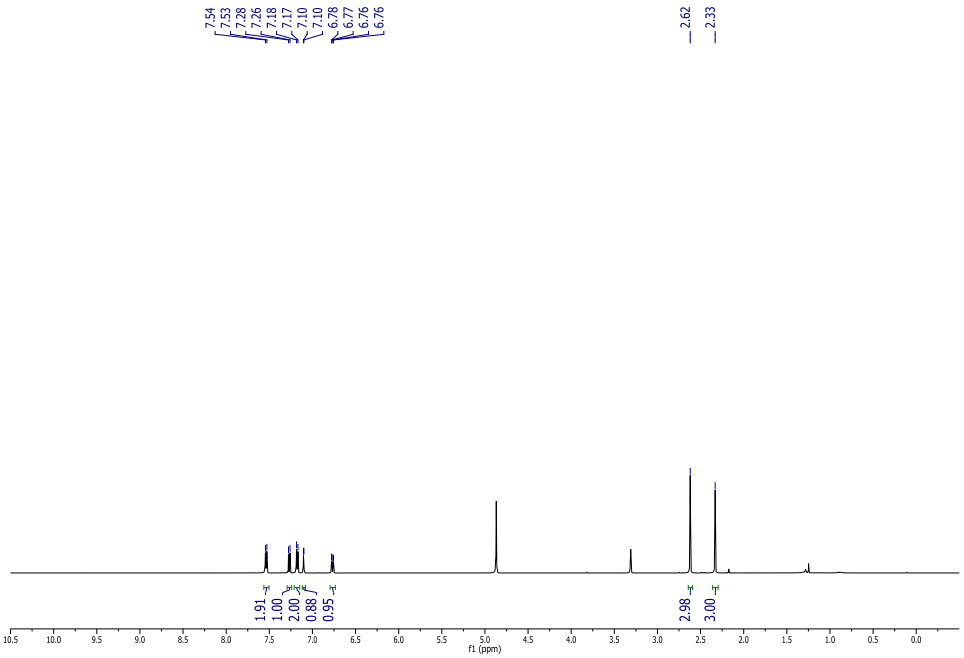
56.05
21.01
14.21
f1 (ppm)



A solution of methyl ether (703 mg, 2.38 mmol) in DCM (60 mL) was cooled to −78 °C. Then BBr_3 (1M in DCM, 11.9 mL) was added slowly. The solution was allowed to gradually warm to room temperature overnight. After a total of 22 h ~60 mL 1N HCl added and the resulting mixture was stirred vigorously for 45 min. At this point, the DCM was removed in vacuo and the aqueous mixture was extracted two times with EtOAc. The combined organic layers were washed with $NaHCO_3$ (sat., aq) followed by brine. It was then dried over Na_2SO_4, filtered, and concentrated to give a yellow solid. Flash column chromatography (0-15% MeOH/DCM) afforded **2** (189 mg, 28%) as a tan solid. R_f = 0.32 (5% MeOH/DCM); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.53 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.5 Hz, 1H), 2.62 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 165.1, 160.0, 155.0, 149.5, 137.1, 135.3, 130.3, 128.3, 122.0, 114.5, 113.9, 112.1, 106.1, 21.0, 13.9; HRMS (ESI) calculated for $C_{17}H_{16}NO_3$ (MH^+) 282.1125, observed 282.1124.


7.54
7.53
7.28
7.26
7.18
7.17
7.10
7.10
6.78
6.77
6.76
6.76
2.62
2.33
1.91
1.00
2.00
0.88
0.95
2.98
3.00
f1 (ppm)

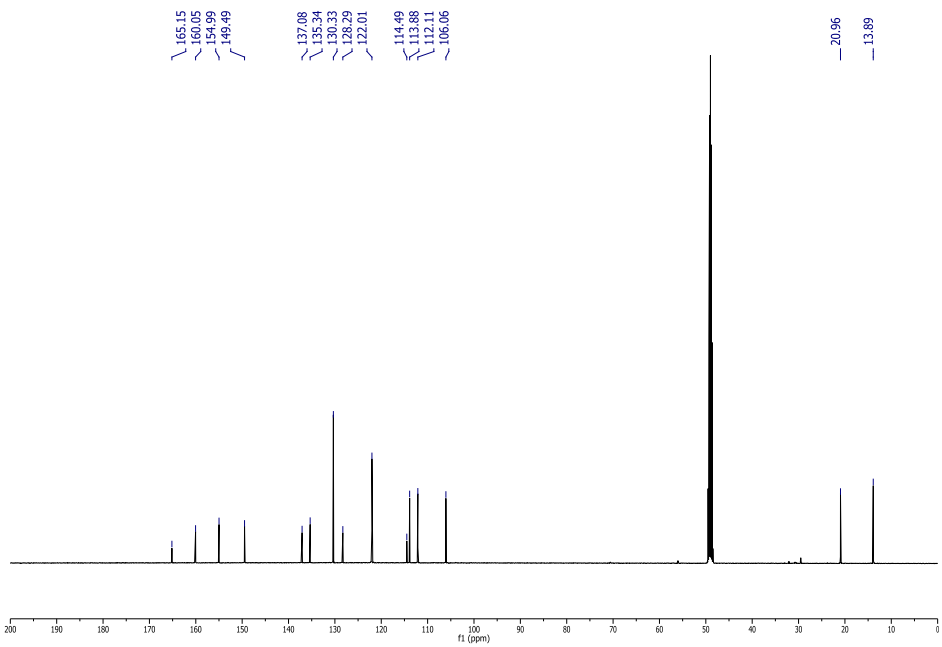
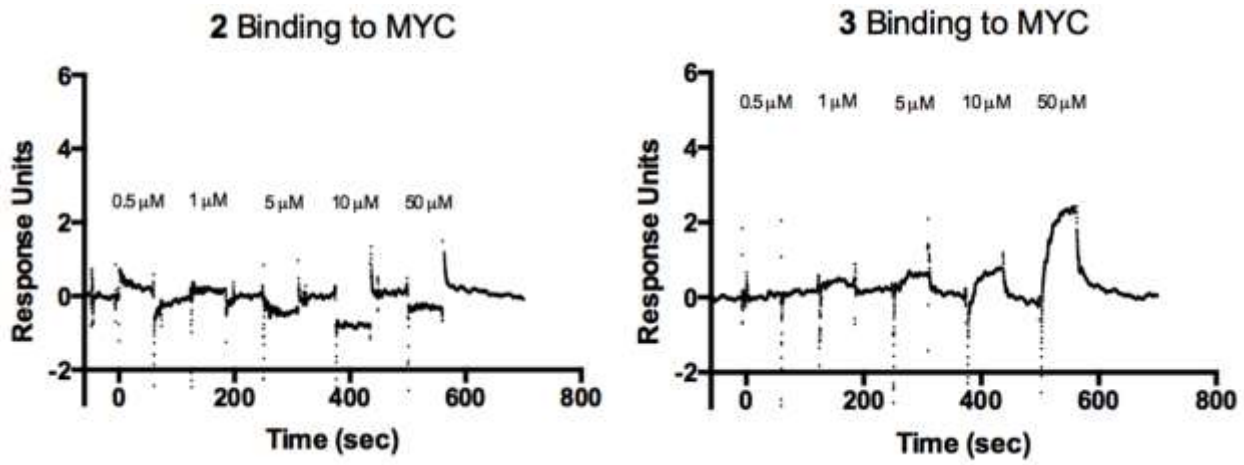

165.15
160.05
154.99
149.49
137.08
135.34
130.33
128.29
122.01
114.49
113.88
112.11
106.06
20.96
13.89
f1 (ppm)

Figure S7. SPR analysis of Compounds 2 and 3 binding to MYC G4 DNA



Compound 1 Stability Study

Compound **1** (10 μL of 1 mM DMSO solution) was added to 490 μL RPMI-1640 culture media. After 1 min, 25 h, 48 h, and 72 h, 100 μL of this solution was diluted into 100 μL acetonitrile. The mixture was centrifuged at 5500 rpm for 1 min and the supernatant was removed from the pellet. Another 800 μL MeCN was added to the supernatant and the mixture was centrifuged again at 5500 rpm for 1 min. The resulting supernatant was subjected to LC/MS (ESI^+) on an Agilent Technologies 1200 LC/MSD single quadrupole system, equipped with an in-line diode-array UV detector. The mass corresponding to **1** ($M+H^+ = 393$) was extracted and the chromatogram is shown. This mass persisted as a significant peak through 72 h. Masses of the putative hydrolyzed compound (see structure below; $M+H^+ = 312$) and o-quinone methide compound (see structure below; $M+H^+ = 294$) were extracted, and were found to be insignificant at all time points (see the chromatograms below).

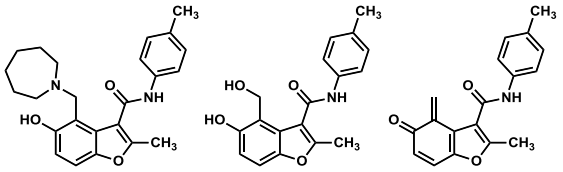
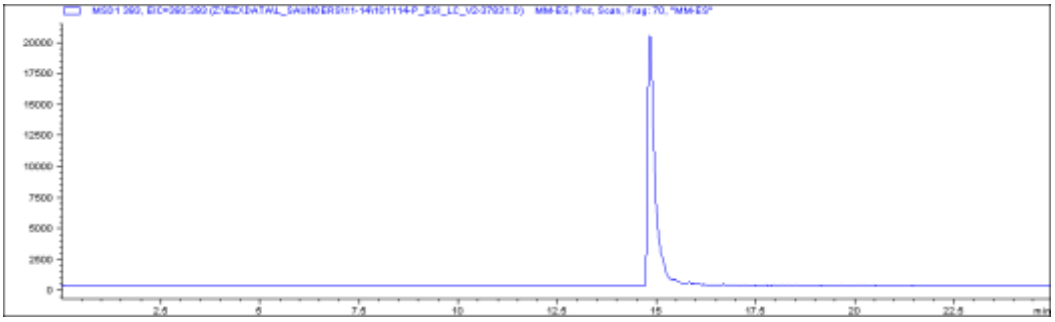


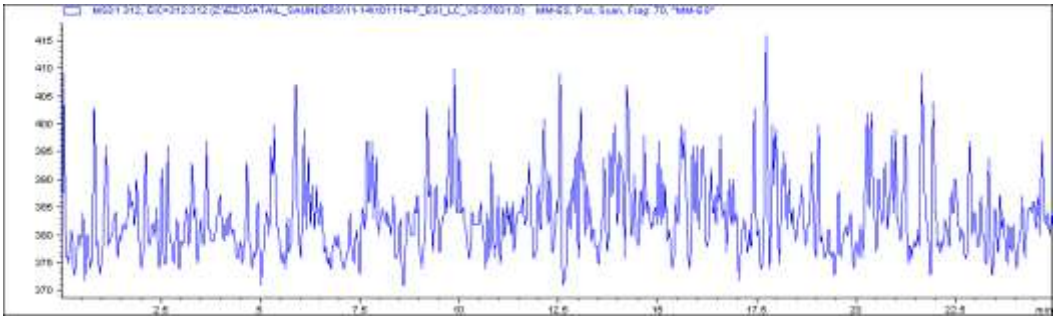
Figure S8. Stability study of compound **1**

T= 1 min

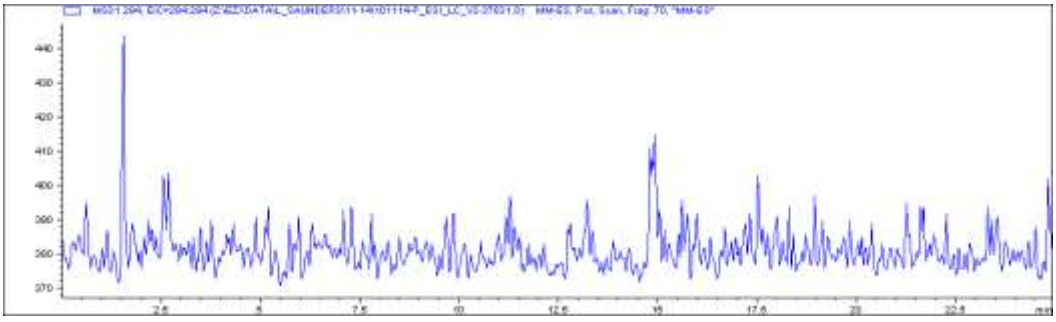
Extracted ion chromatogram for $M+H^+ = 393$



Extracted ion chromatogram for $M+H^+ = 312$

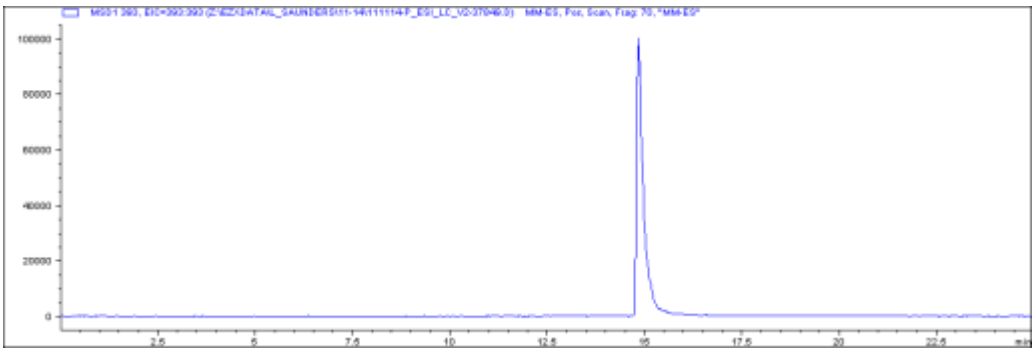


Extracted ion chromatogram for $M+H^+ = 294$

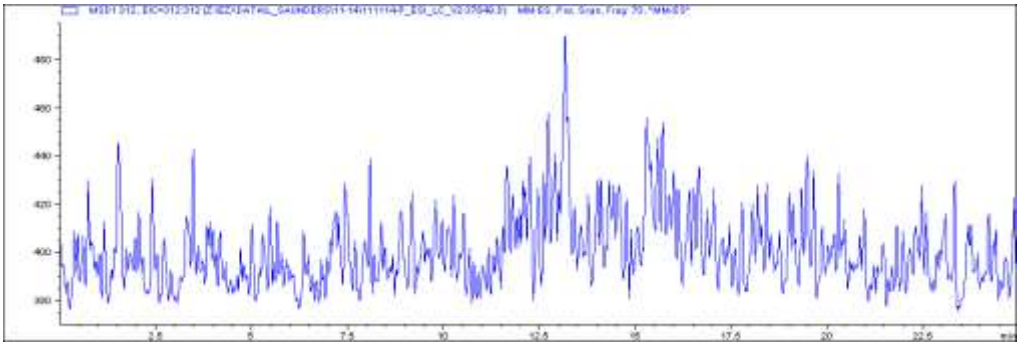


T= 25 h

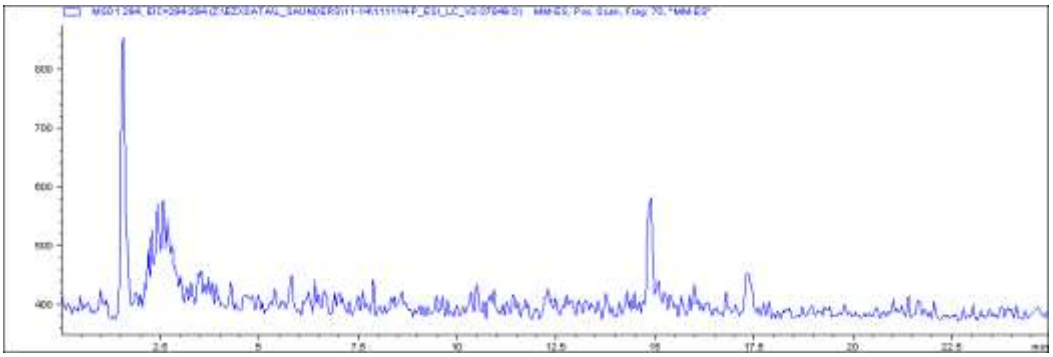
Extracted ion chromatogram for $M+H^+ = 393$



Extracted ion chromatogram for $M+H^+ = 312$

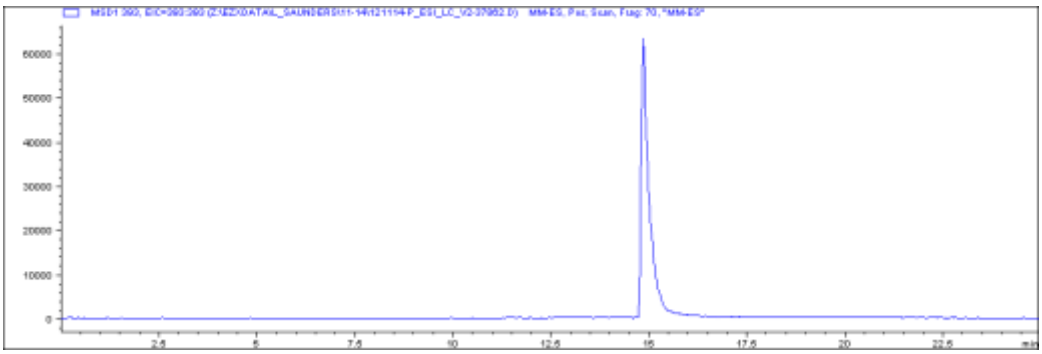


Extracted ion chromatogram for $M+H^+ = 294$

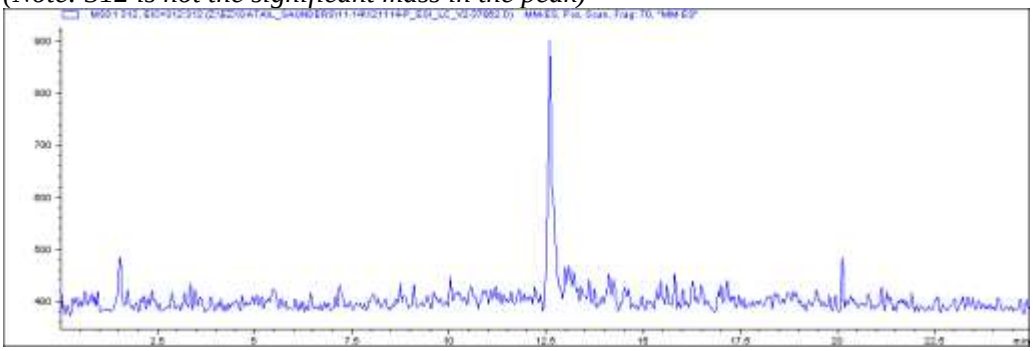


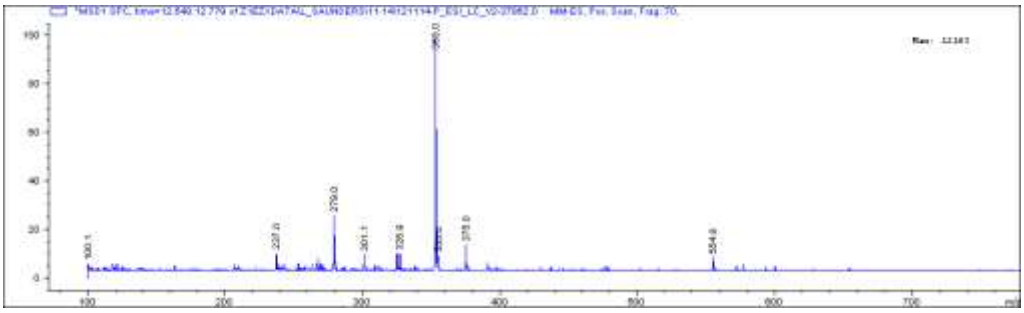
T= 48 h

Extracted ion chromatogram for $M+H^+ = 393$

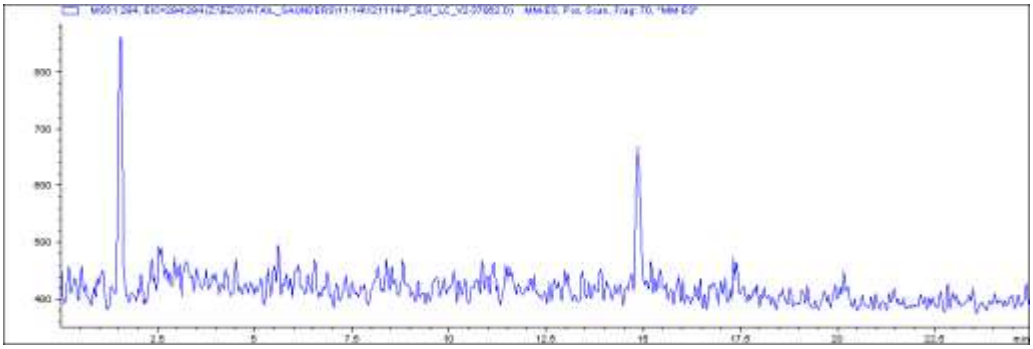


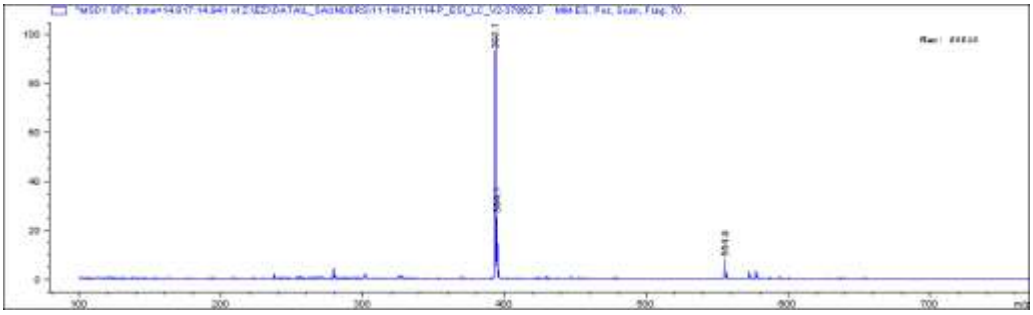
Extracted ion chromatogram for $M+H^+ = 312$
(Note: 312 is not the significant mass in the peak)





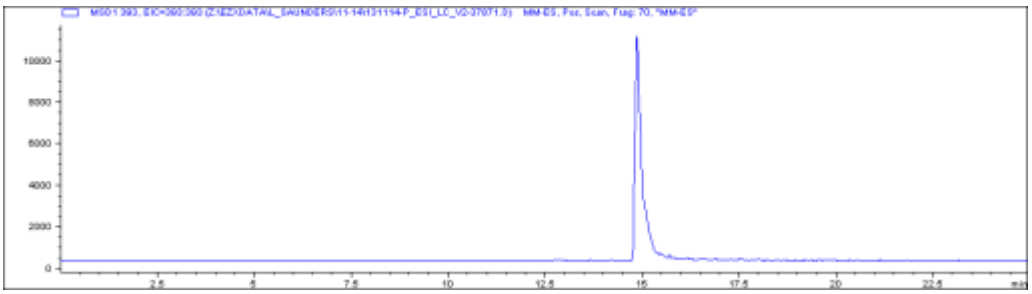
Extracted ion chromatogram for $M+H^+ = 294$
(Note: 294 is not the significant mass in the peak)



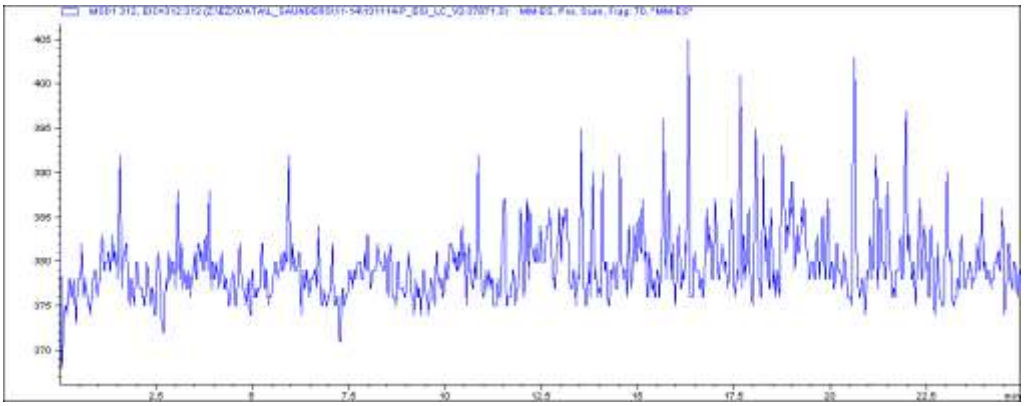


T= 72 h

Extracted ion chromatogram for $M+H^+ = 393$



Extracted ion chromatogram for $M+H^+ = 312$



Extracted ion chromatogram for $M+H^+ = 294$