Supporting Information

Organoselenium-Based Schiff Bases and Amidic Acid Derivatives as Promising Anticancer Agents Targeting Breast Cancer by Downregulating BCL-2: Design, Synthesis, and Biological Evaluation

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Methods and Materials

Melting points (MP) in ºC were recorded on the Gallenkamp instrument. The IR spectra (KBr, ύ cm^{-1}) were recorded at King Faisal University on a Mattson 5000 FTIR Spectrophotometer. The ^{1}H NMR (400 MHz) and the ^{13}C NMR (100.6 MHz) spectra were recorded at Martin-Luther-University, Halle, Germany, using a Varian VXR spectrometer employing the TMS internal reference and DMSO-d_6 and pyridine-d_6 solvents. The Mass spectra were measured at Martin Luther University, Halle, Germany, on an Agilent 6530 LC Q-TOF instrument.

Chemistry

4-Aminophenylselenocyanate (**2**) [1], bis(4-aminophenyl)diselenide (**3**) [2], methyl 2-amino-5-selenocyanatobenzoate (**6**) [3, 4], and dimethyl 5,5-diselanediylbis(2-aminobenzoate) (**7**) [3, 4] were synthesized as per the reported protocols.

4-Selenocyanatoaniline (2) [1]

Selenium dioxide (6 mmol) was added under stirring to a solution of malononitrile (3 mmol) in DMSO (15 mL). The mixture was stirred at room temperature for 15 min in order to obtain triselenium dicyanide. When the exothermic reaction had finished, aniline (5 mmol) was added. The mixture was stirred for 20 min. Water (150 mL) was added to the reaction mixture, and the resulting precipitate (4-selenocyanatobenzenamine) was filtered off, dried, and used without further purifications.

4-Aminophenylselenocyanate (**2**) is a yellow solid, melting point = 73–74 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.4 Hz, 2H, Ar-H), 6.64 (d, J = 8.4 Hz, 2H, Ar-H), 3.95 (s, 2H, NH_2).

Synthesis of 4,4'-diselanediyldianiline (3) [1]

Under argon, $NaBH_4$ (3 mmol) was added in small portions with caution to a solution of 4-selenocyanatobenzenamine (1 mmol) in absolute ethanol (40 mL). The mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the remaining residue was dissolved in dichloromethane, washed with water (350 mL). The organic layer was separated, dried with anhydrous Na_2SO_4, and removed under vacuum. The residue was purified by chromatography on silica gel (petroleum ether: ethyl acetate 4:2).

4,4′-Diselanediyldianiline (**2**) was obtained as pale-yellow crystals (82 % yield. Mp: 78–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (m, 4H, Ar-H), 6.5 (m, 4H, Ar-H), 3.7 ppm (s, 4H, NH_2).

Synthesis of methyl 2-amino-5-selenocyanatobenzoate (6) [3, 4]

Methyl 2-amino-5-selenocyanatobenzoate (**6**) was synthesized from the reaction of methyl 2-aminobenzoate with TSD prepared *in situ* from $CH_2(CN)_2$ and SeO_2 in 96% yield. Briefly, SeO_2 (30 mmol,

3300 mg) was added to $CH_2(CN)_2$ (15 mmol, 1000 mg) in 10 mL DMSO-D6, and the mixture was stirred for 20 min at RT. Next, the mixture was filtered off to get rid of any formed black selenium, and methyl 2-aminobenzoate (12.5 mmol, 1800 mg) was then added, and the reaction mixture was stirred for a further 2 hrs. Finally, adding 10 gm of ice terminated the reaction, and the formed precipitate was filtered, washed several times with H_2O and Na_2CO_3 solution, dried, and recrystallized from petroleum ether.

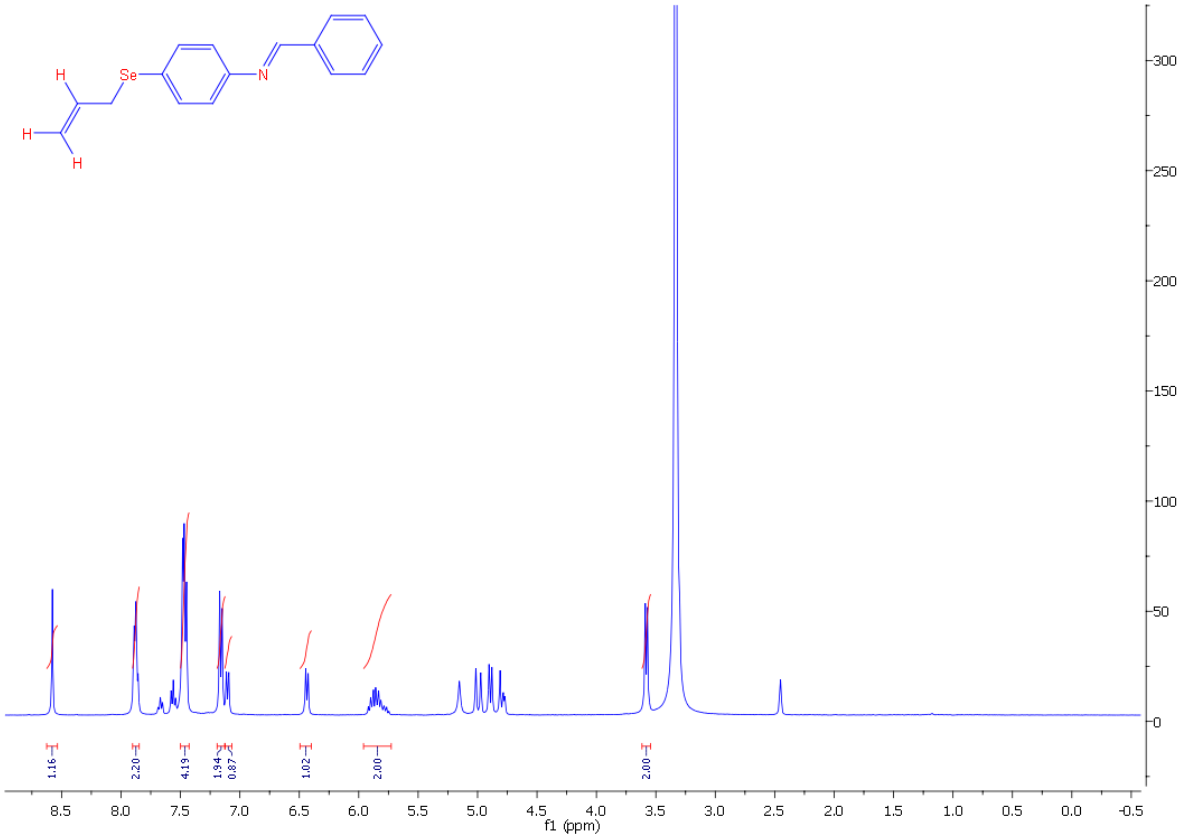
2-Amino-5-selenocyanatobnzoate was isolated as reddish solid; yield:3072 mg (96%); MP=118-119 ºC; Rf: 0.4 (petroleum ether/ ethyl acetate 4:2). IR(KBr): λ_{max}.cm $^{-1}$:3475, 3366, 2946, 2147, 1691. ^{1}H NMR (400 MHz, DMSO-D6-d_6) δ 8.02 (s, 1H, Ar-H), 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.08 (d, 1H, Ar-H), 6.84 (s, 2H, NH_2), 3.82 (s, 3H, OCH_3).^{13}C NMR (101 MHz, DMSO-D6-d_6) δ 166, 152, 140, 137, 118, 109, 105, 105, 51. MS (EI, 70 ev) *m/z* (%) = 259.35 (M+3H, 2.39),117 (29.02), 87 (26.6), 75 (2.70), 59 (100.0, base peak).

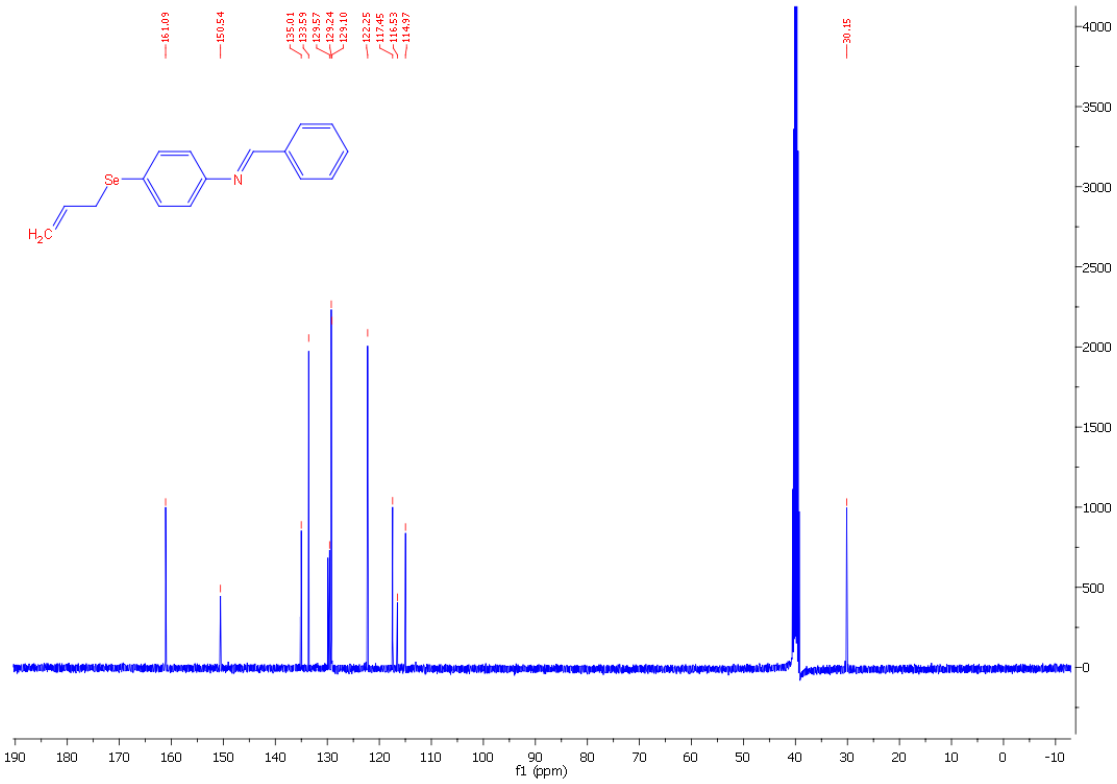
Synthesis of dimethyl 5,5-diselanediylbis(2-aminobenzoate) (7) [3, 4]

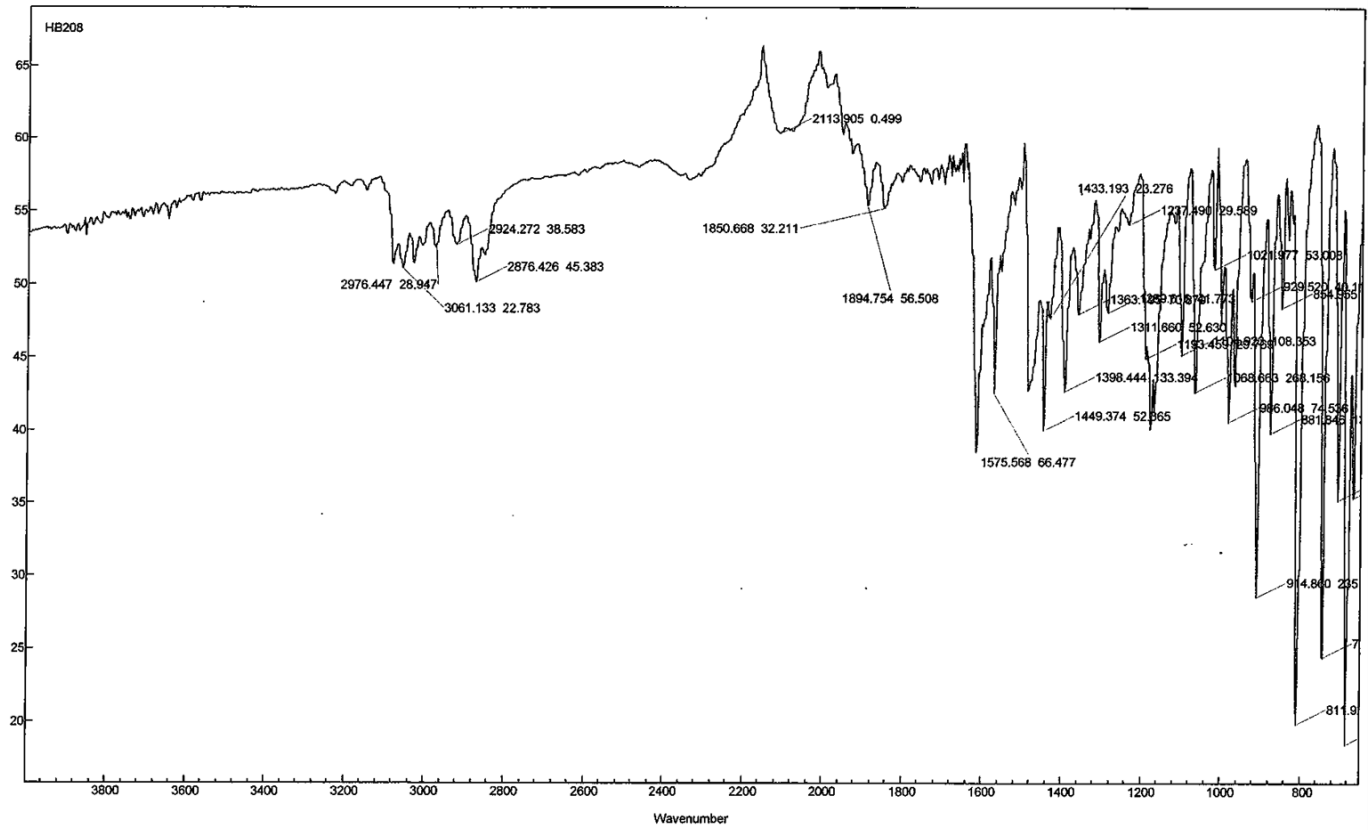
Dimethyl 5,5-diselanediylbis(2-aminobenzoate) (**7**) was synthesized from the reaction of **6** and sodium hydroxide in 92% yield. Briefly, compound **6** (4 mmol, 1000 mg) was dissolved in EtOH (20 mL), and then sodium hydroxide (4 mmol,160 mg) was added. The reaction mixture was stirred for 2 hours at room temperature, and the resulting precipitate was filtered, washed several times with H_2O, and recrystallized from chloroform.

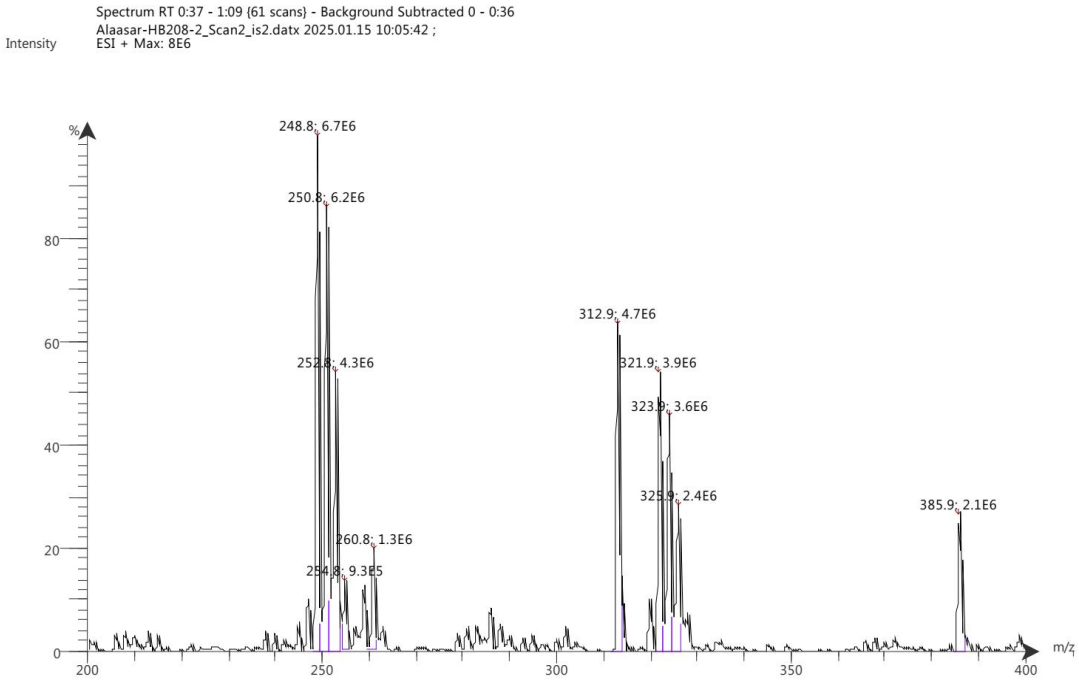
Dimethyl 5,5-diselanediylbis(2-aminobenzoate) was isolated as a yellow solid; yield: 1692.43 mg (92%); MP=138- 139 °C; Rf =0.5(petroleum ether/ ethyl acetate 4:3). IR(KBr): λ_{max}.cm^{-1}: 3455, 3344, 2931, 1684. ^{1}HNMR (400 MHz, DMSO-D6) δ7.70 (s,2H, Ar-H), 7.44(d, *J* = 8.6 Hz, 2H, Ar-H), 7.00(s, 4H,2NH_2), 6.77(d, *J* = 8.7 Hz, 2H, Ar-H), 3.74(s, 6H,2OCH_3). ^{13}CNMR (101 MHz, DMSO-D6-d_6) δ167, 151, 140, 138, 117, 113, 108, 51. MS (EI, 70 ev) *m/z* (%) = 460.15(M+H, 20.76), 459.15(M, 5.20) or 230(24.42), 119(9.45), 91(100.0, base peak),65(8.88).

N-(4-(Allylselanyl)phenyl)-1-phenylmethanimine (HB208)

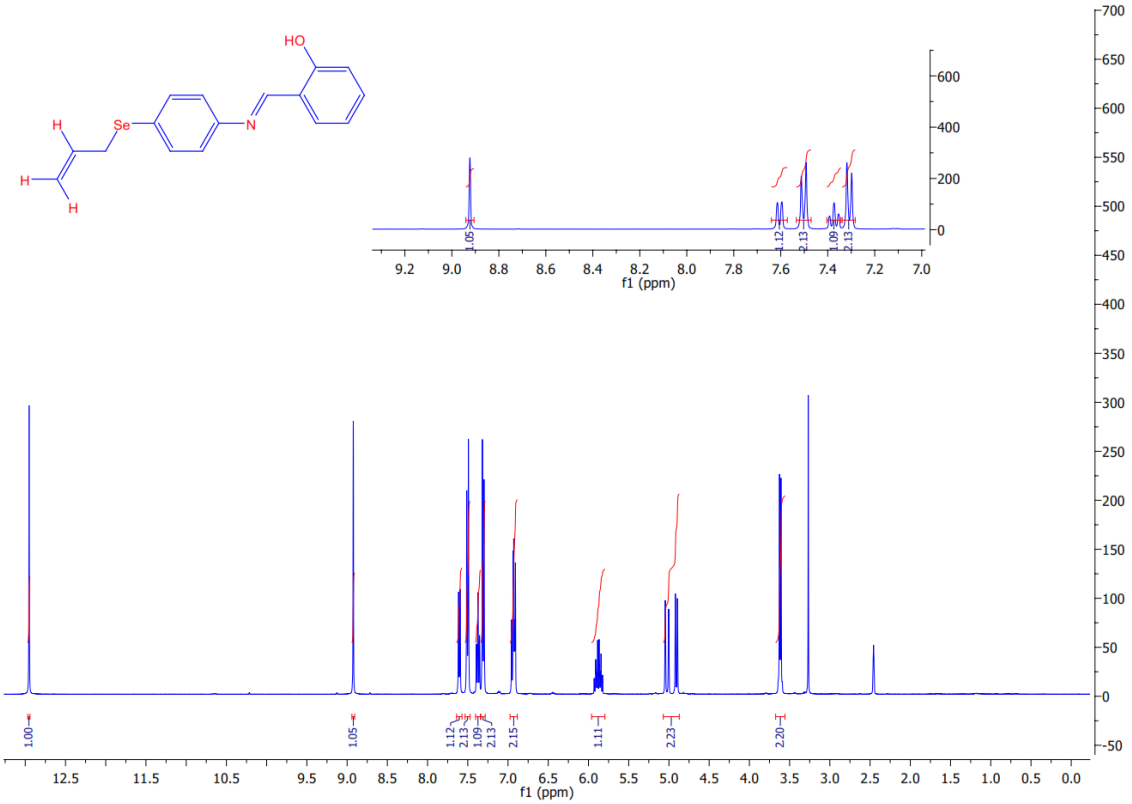


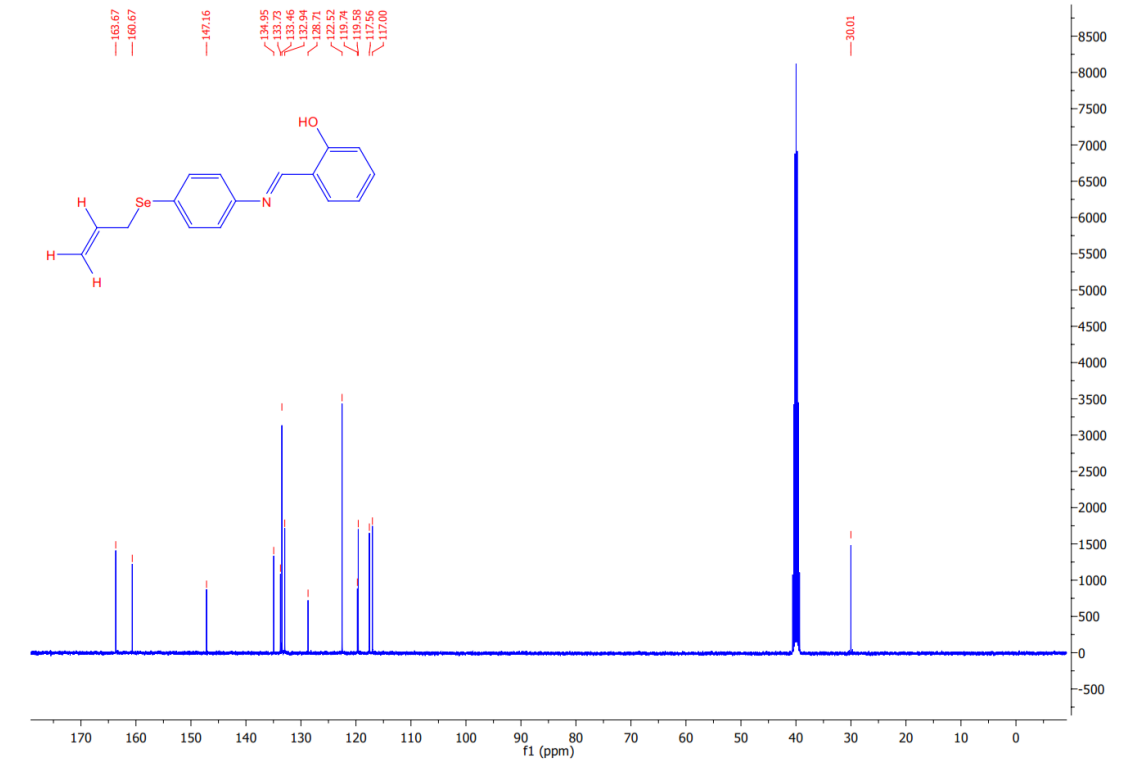


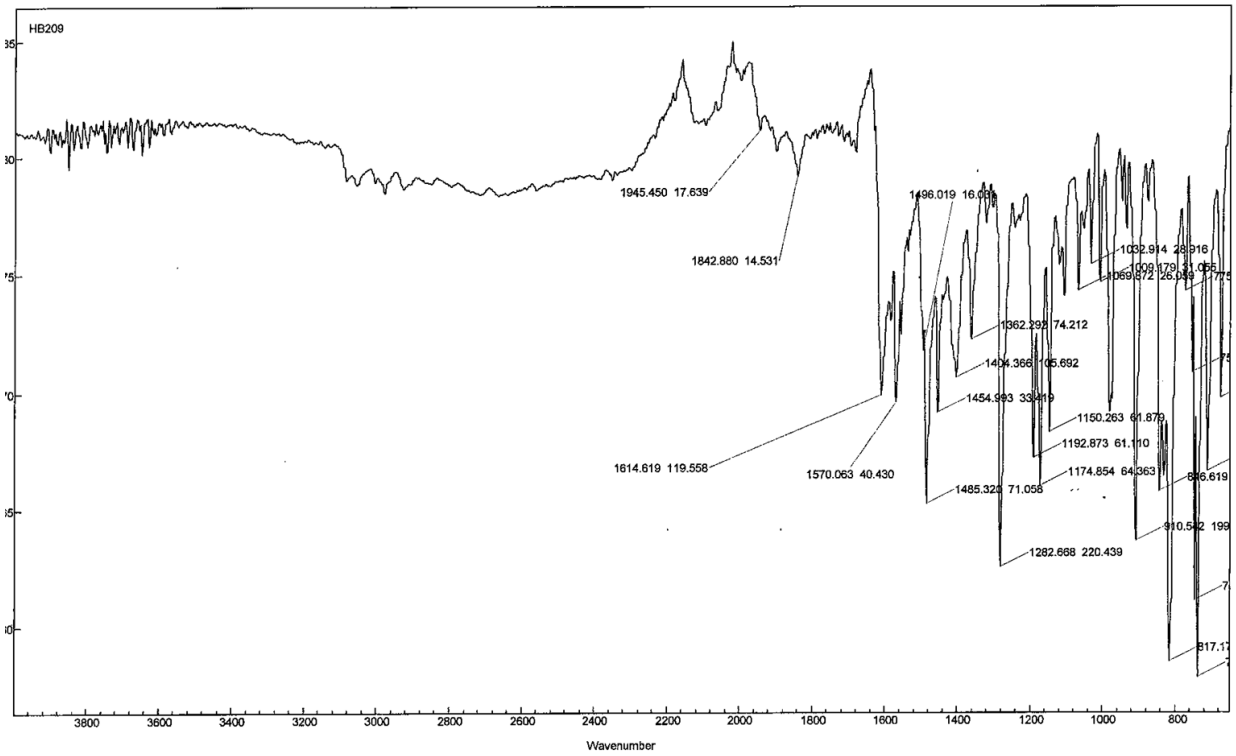

HB208
2113.905 0.499
1850.668 32.211
2924.272 38.583
2876.426 45.383
2976.447 28.947
3061.133 22.783
1894.754 56.508
1433.193 23.276
1237.490 29.589
1021.977 53.009
1363.139 39.108
1311.660 52.630
1398.444 33.394
1449.374 52.365
1575.568 66.477
914.860 235
811.9
Wavenumber

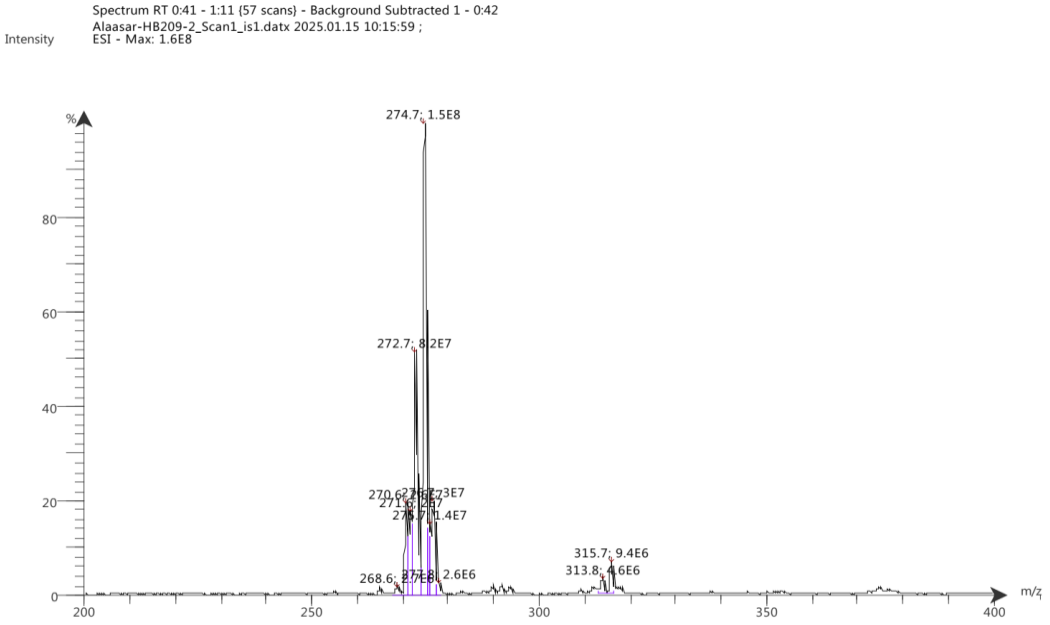

Spectrum RT 0:37 - 1:09 {61 scans} - Background Subtracted 0 - 0:36
Alaasar-HB208-2_Scan2_is2.datx 2025.01.15 10:05:42 ;
Intensity
ESI + Max: 8E6
%
248.8; 6.7E6
250.8; 6.2E6
312.9; 4.7E6
321.9; 3.9E6
323.9; 3.6E6
325.9; 2.4E6
385.9; 2.1E6
260.8; 1.3E6
254.8; 9.3E5
m/z

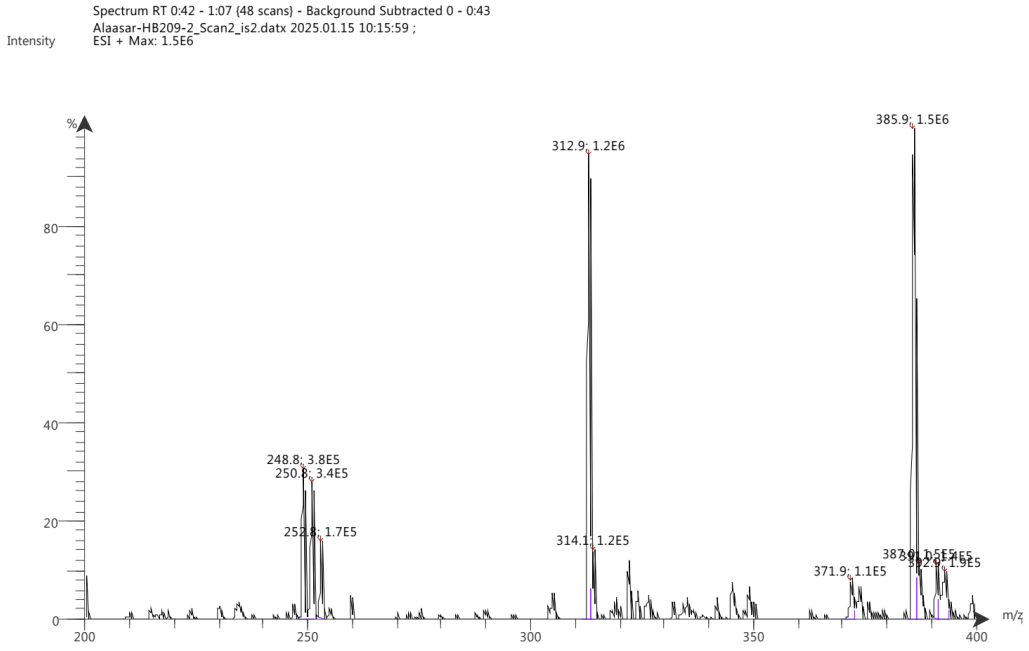
2-(((4-(Allylselanyl)phenyl)imino)methyl)phenol (HB209)



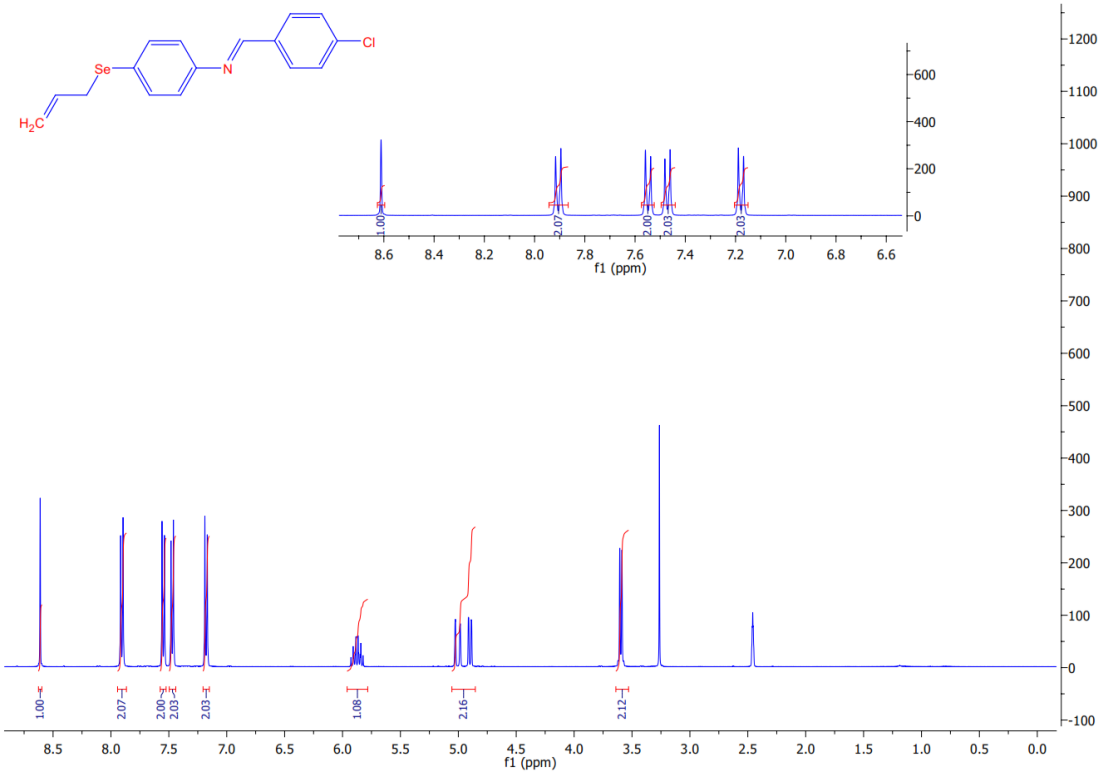


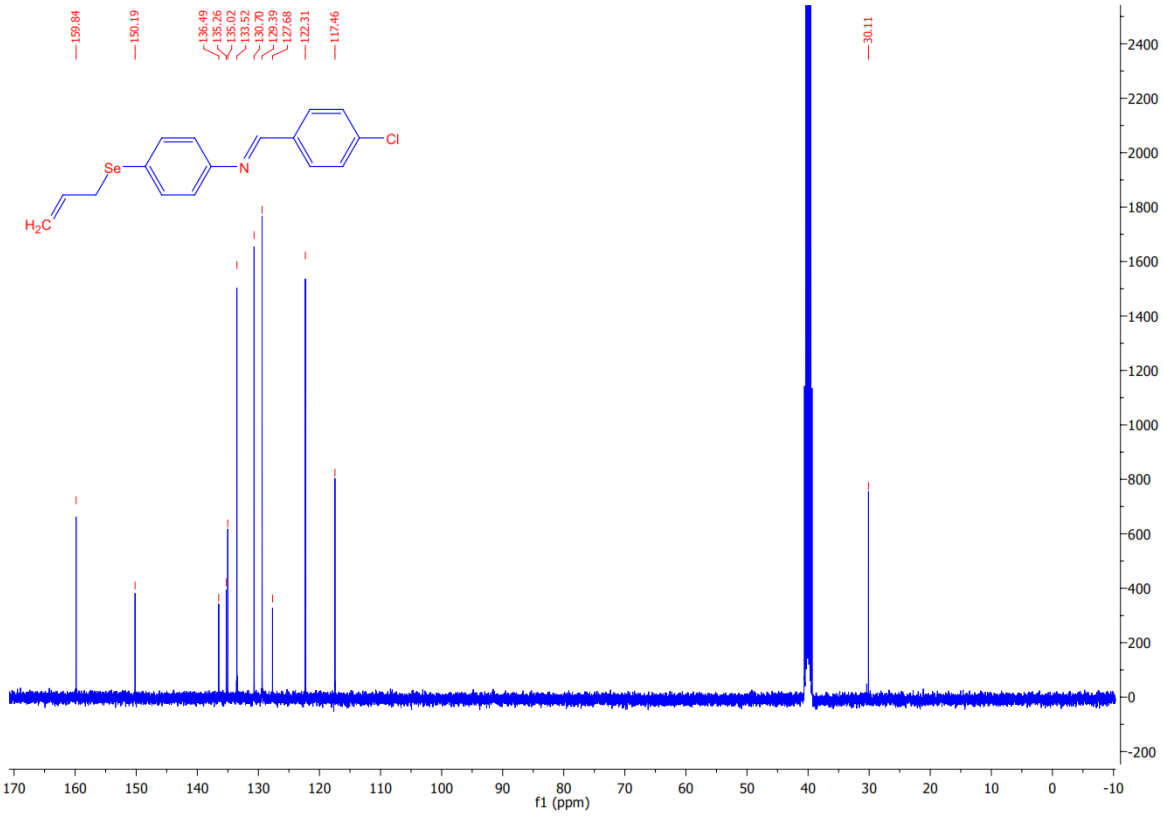

HB209
1945.450 17.639
1842.880 14.531
1614.619 119.558
1570.063 40.430
1362.292 74.212
1282.668 220.439
Wavenumber

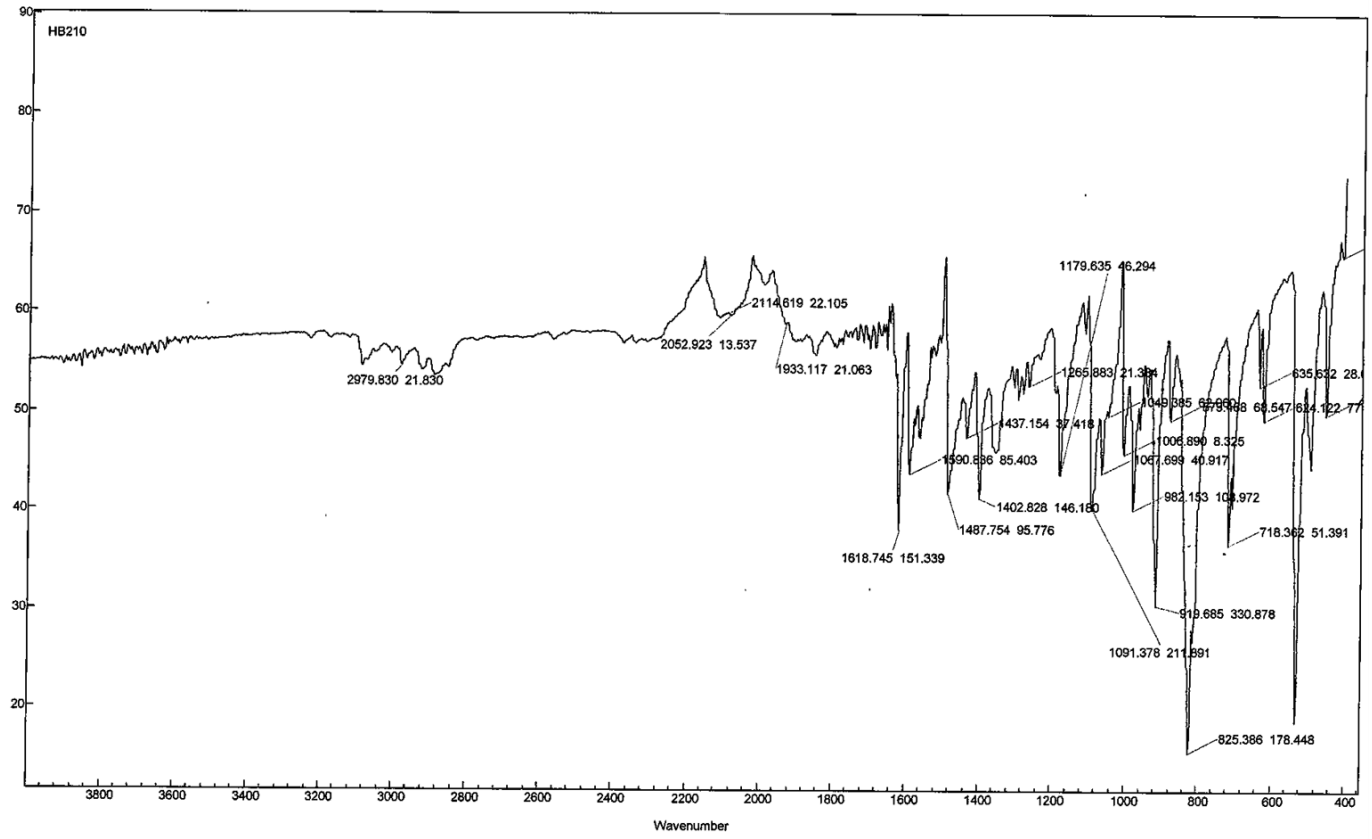

Spectrum RT 0:41 - 1:11 (57 scans) - Background Subtracted 1 - 0:42
Alaasar-HB209-2_Scan1_is1.datx 2025.01.15 10:15:59 ;
Intensity
ESI - Max: 1.6E8
%
274.7; 1.5E8
272.7; 8.2E7
315.7; 9.4E6
313.8; 4.6E6
m/z


Spectrum RT 0:42 - 1:07 (48 scans) - Background Subtracted 0 - 0:43
Alaasar-HB209-2_Scan2_is2.datx 2025.01.15 10:15:59 ;
Intensity
ESI + Max: 1.5E6
%
385.9; 1.5E6
312.9; 1.2E6
248.8; 3.8E5
250.8; 3.4E5
252.8; 1.7E5
314.1; 1.2E5
371.9; 1.1E5
387.9; 1.5E5
389.9; 1.9E5
m/z
0
20
40
60
80
200
250
300
350
400

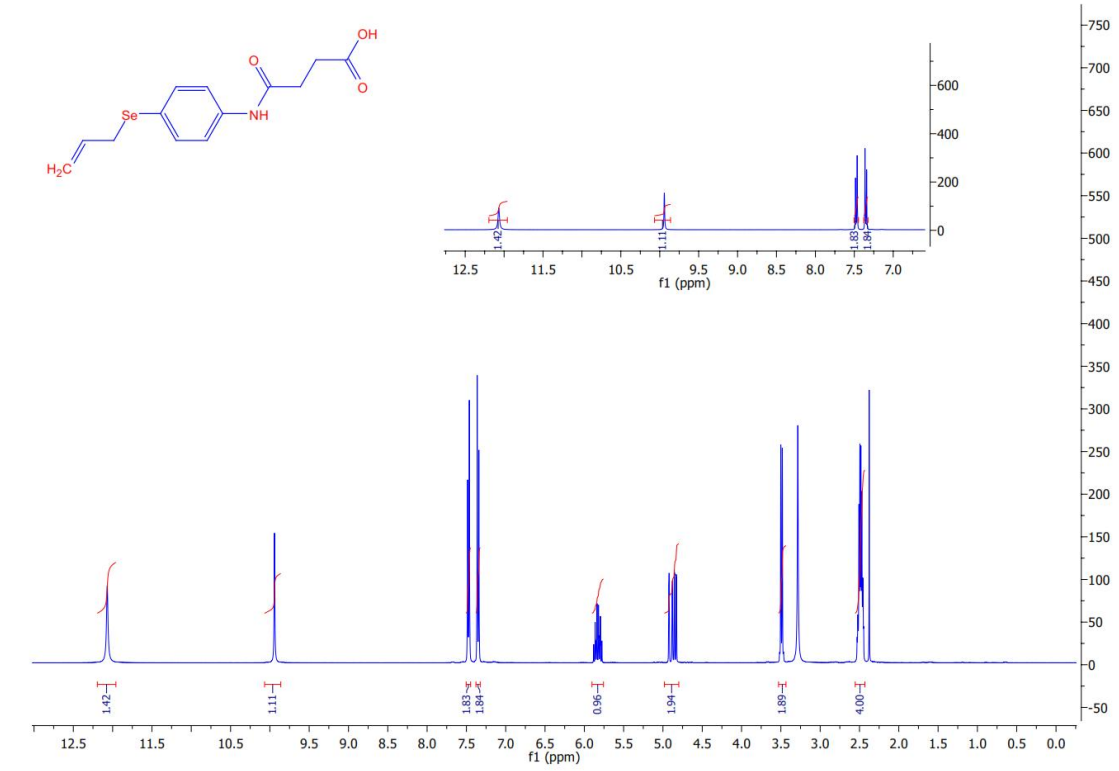
N-(4-(Allylselanyl)phenyl)-1-(4-chlorophenyl)methanimine (HB210)

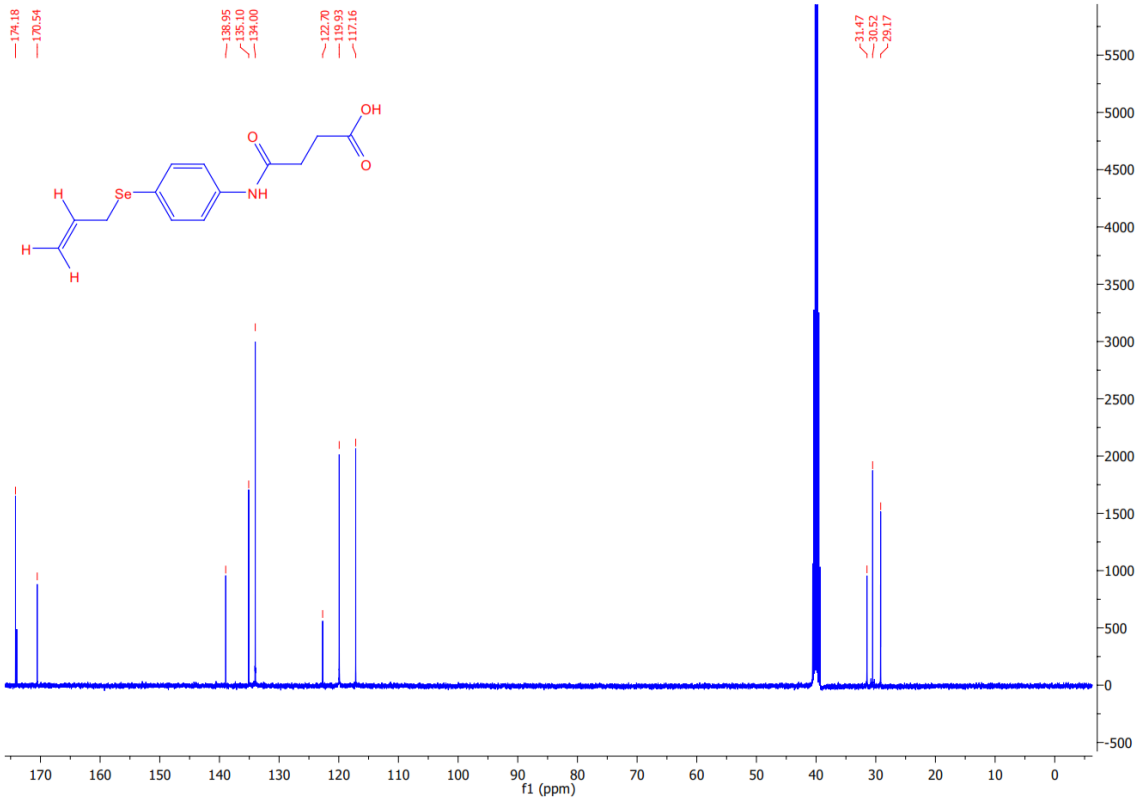


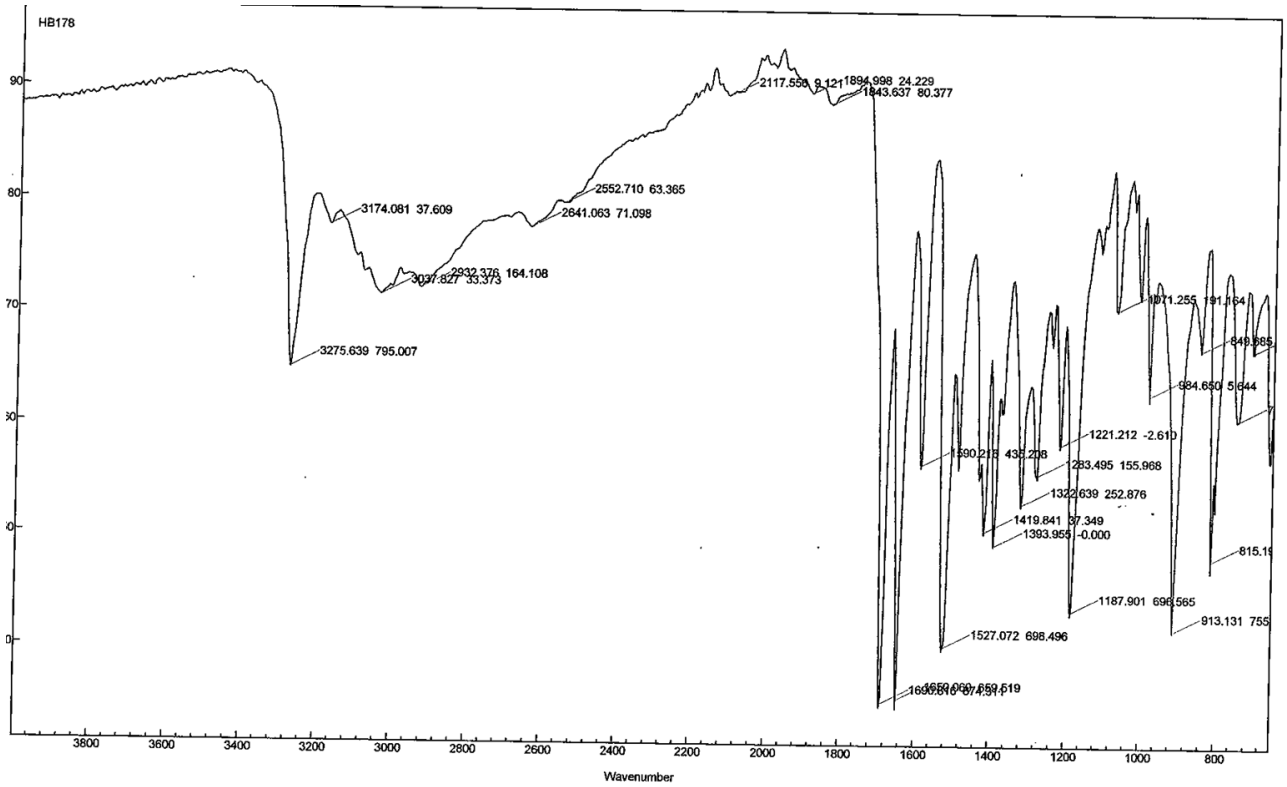


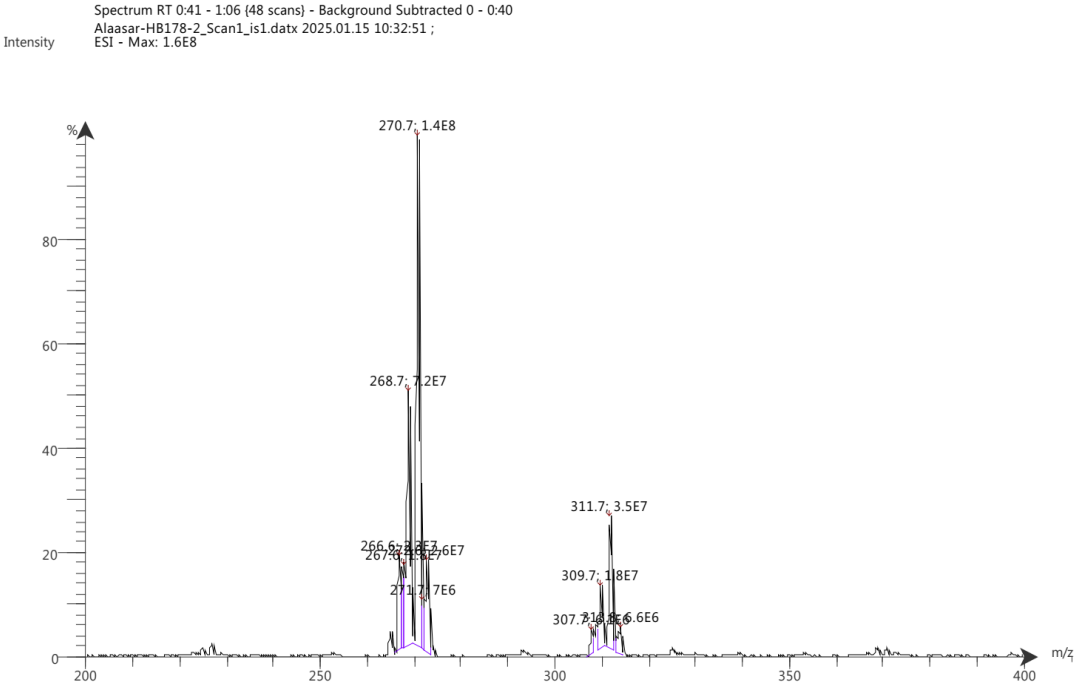

HB210
Wavenumber
2979.830 21.830
2052.923 13.537
2114.619 22.105
1933.117 21.063
1618.745 151.339
1487.754 95.776
1402.828 146.180
1437.154 37.418
1590.836 85.403
1179.635 46.294
1265.883 21.384
1049.385 67.960
1006.890 8.325
1067.699 40.917
982.153 103.972
718.362 51.391
919.685 330.878
1091.378 211.891
825.386 178.448
635.632 28.
679.988 68.547 624.122 77

4-((4-(Allylselanyl)phenyl)amino)-4-oxobutanoic acid (HB178)

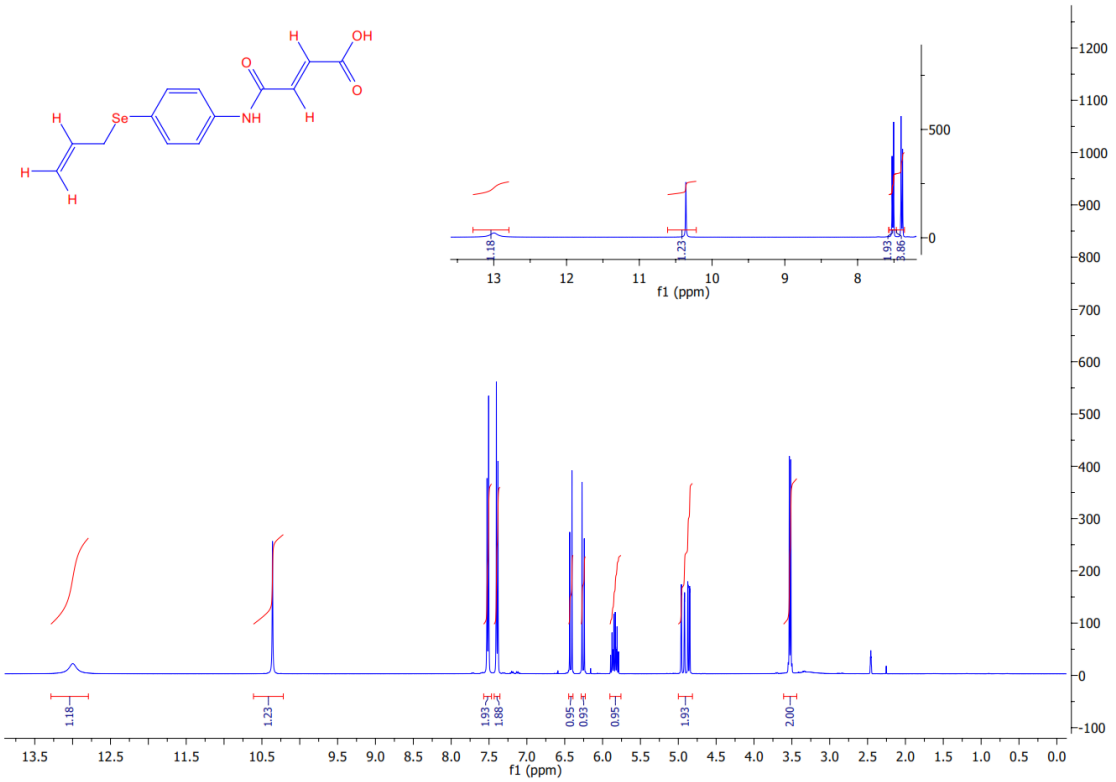


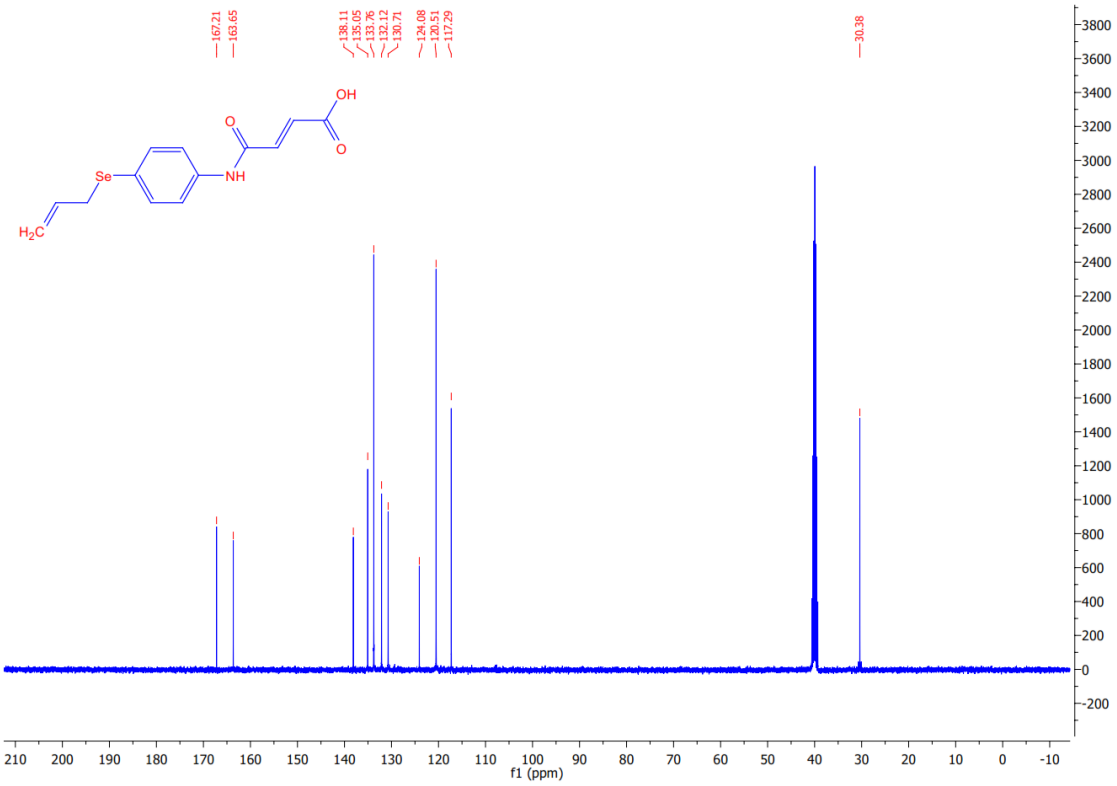


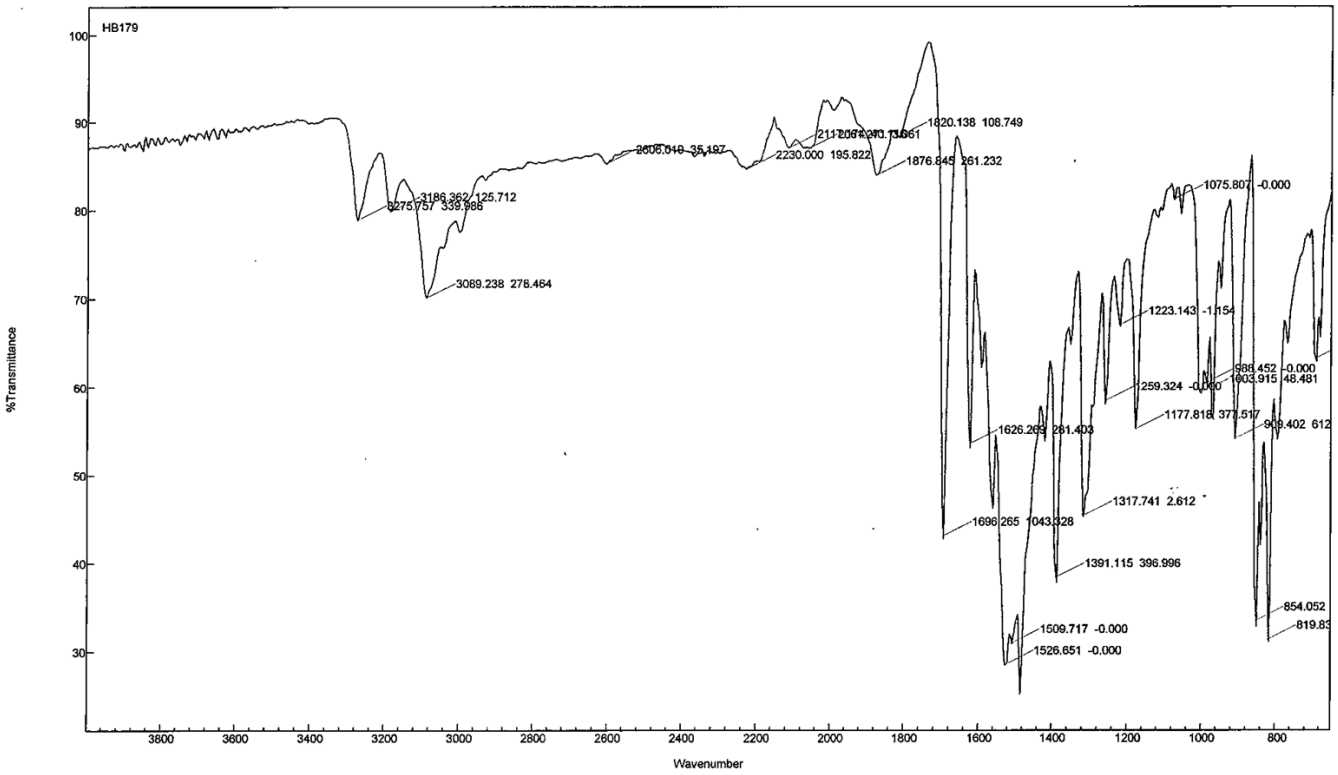

HB178
3275.639 795.007
3174.081 37.609
2932.376 164.108
2552.710 63.365
2641.063 71.098
2117.555 9.121
1894.998 24.229
1843.637 80.377
1221.212 -2.610
1187.901 696.565
1527.072 698.496
1419.841 37.349
1393.955 -0.000
913.131 755
984.650 5.644
Wavenumber

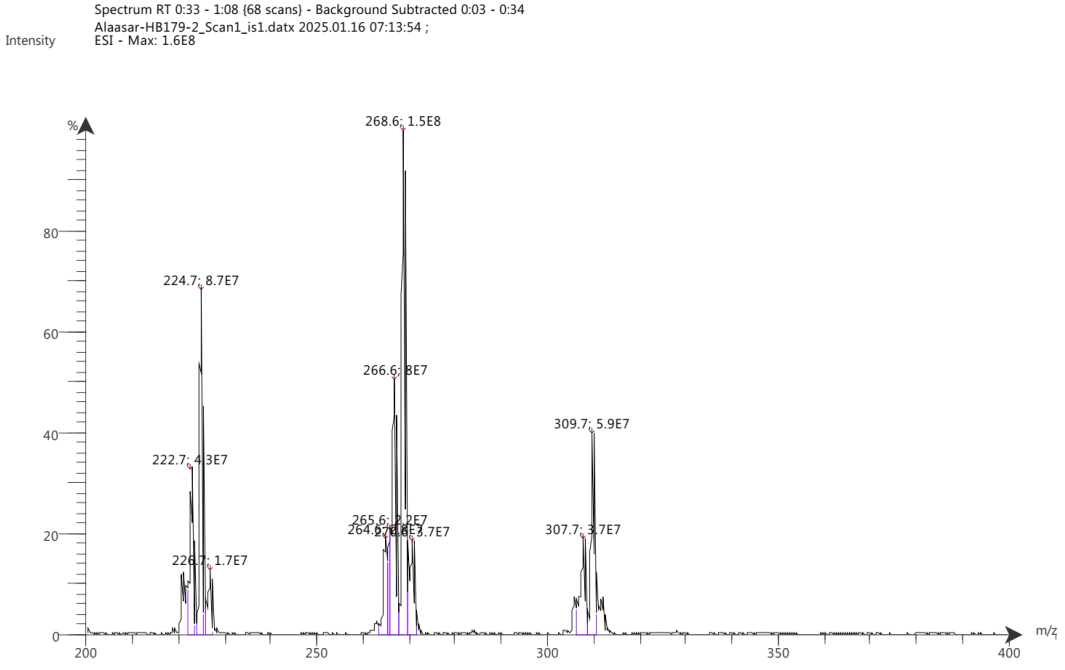

Spectrum RT 0:41 - 1:06 (48 scans) - Background Subtracted 0 - 0:40
Alaasar-HB178-2_Scan1_is1.datx 2025.01.15 10:32:51 ;
Intensity
ESI - Max: 1.6E8
%
270.7; 1.4E8
268.7; 7.2E7
311.7; 3.5E7
309.7; 1.8E7
271.7; 7.7E6
m/z

4-((4-(Allylselanyl)phenyl)amino)-4-oxobut-2-enoic acid (HB179)

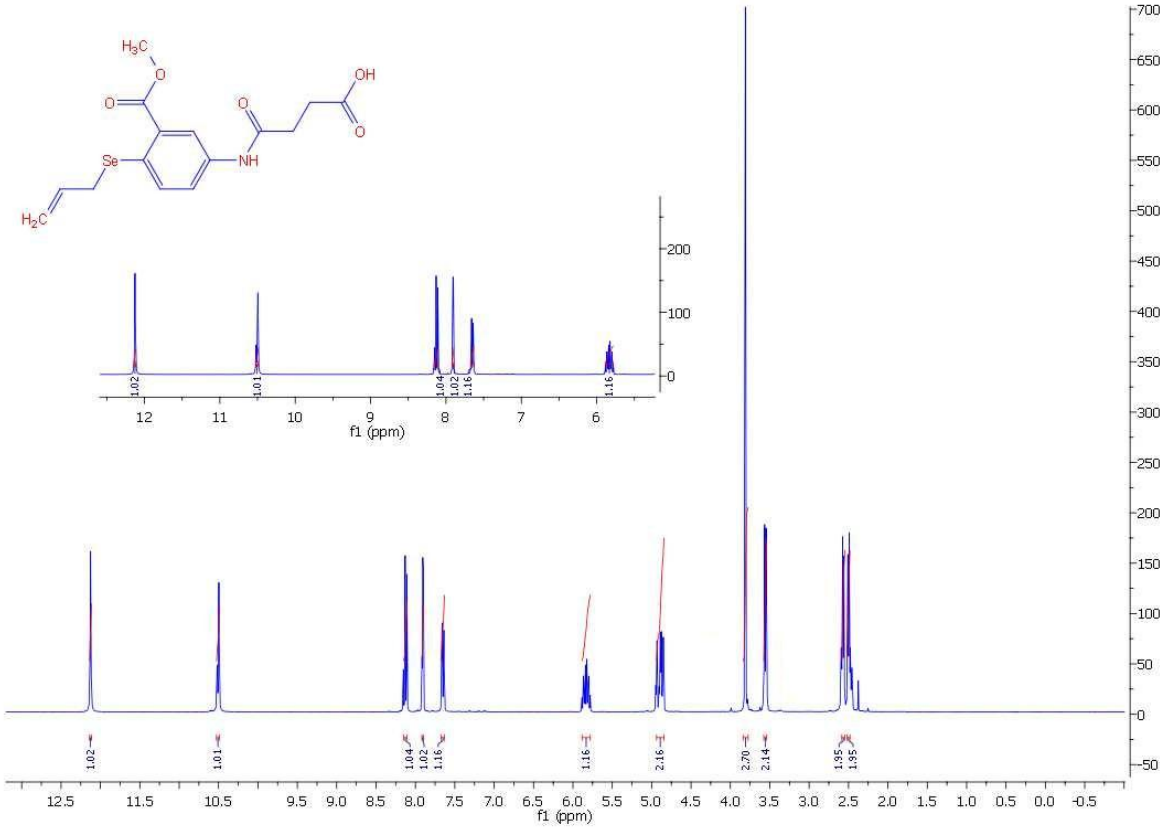


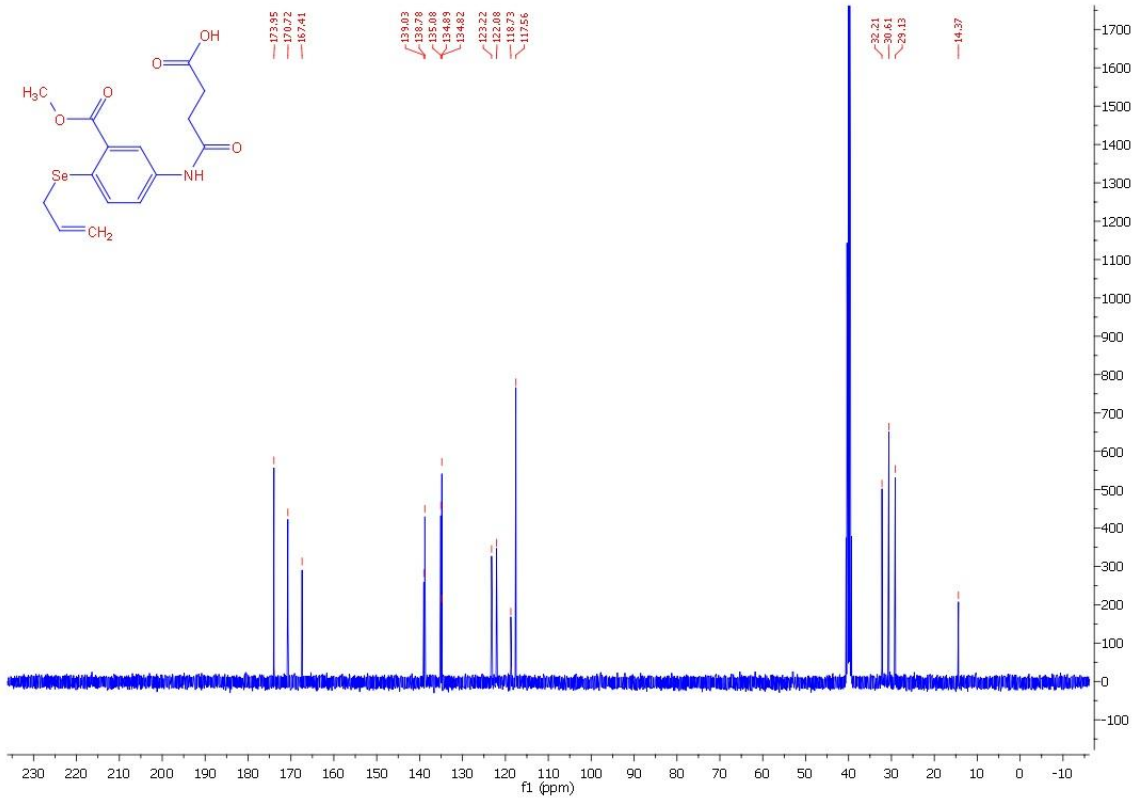


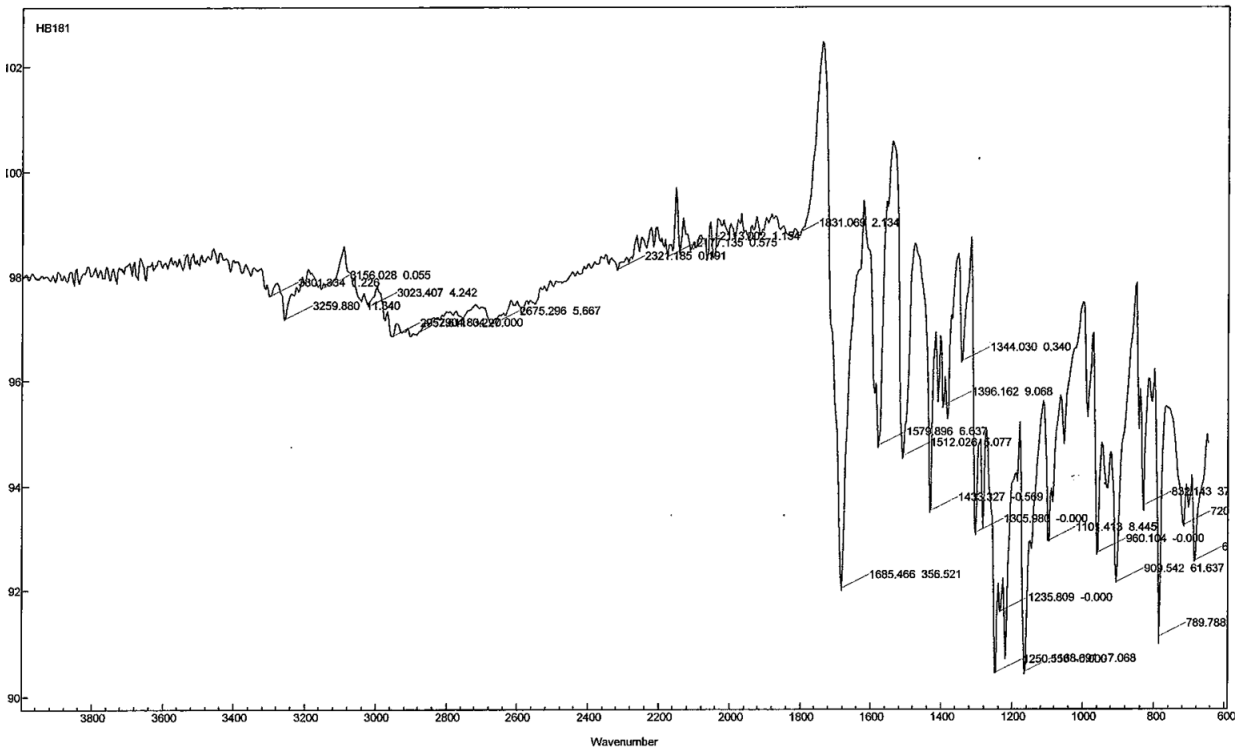

HB179
%Transmittance
Wavenumber
3089.238 278.464
1620.138 108.749
1876.645 261.232
1626.269 261.403
1696.265 1043.028
1317.741 2.612
1391.115 396.996
1509.717 -0.000
1526.651 -0.000
1075.807 -0.000
1223.143 -1.154
1177.818 377.517
854.052

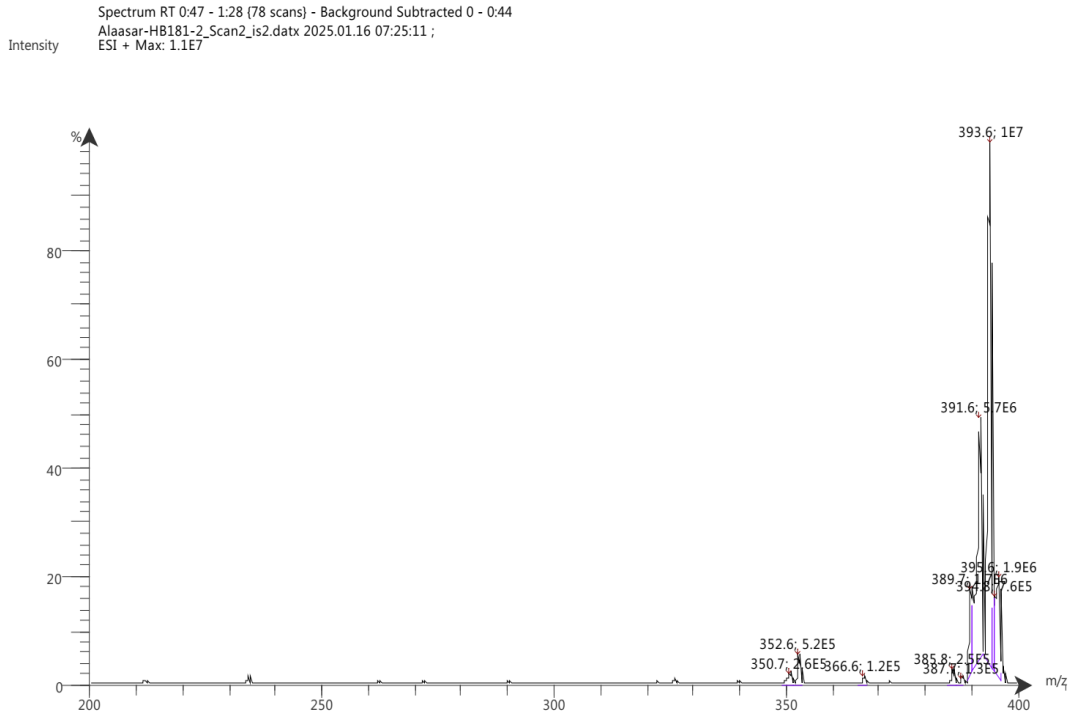

Spectrum RT 0:33 - 1:08 (68 scans) - Background Subtracted 0:03 - 0:34
Alaasar-HB179-2_Scan1_is1.datx 2025.01.16 07:13:54 ;
Intensity
ESI - Max: 1.6E8
%
m/z
268.6; 1.5E8
224.7; 8.7E7
266.6; 8E7
309.7; 5.9E7
222.7; 4.3E7
307.7; 3.7E7
226.7; 1.7E7

4-((4-(Allylselanyl)-3-(methoxycarbonyl)phenyl)amino)-4-oxobutanoic acid (HB181)

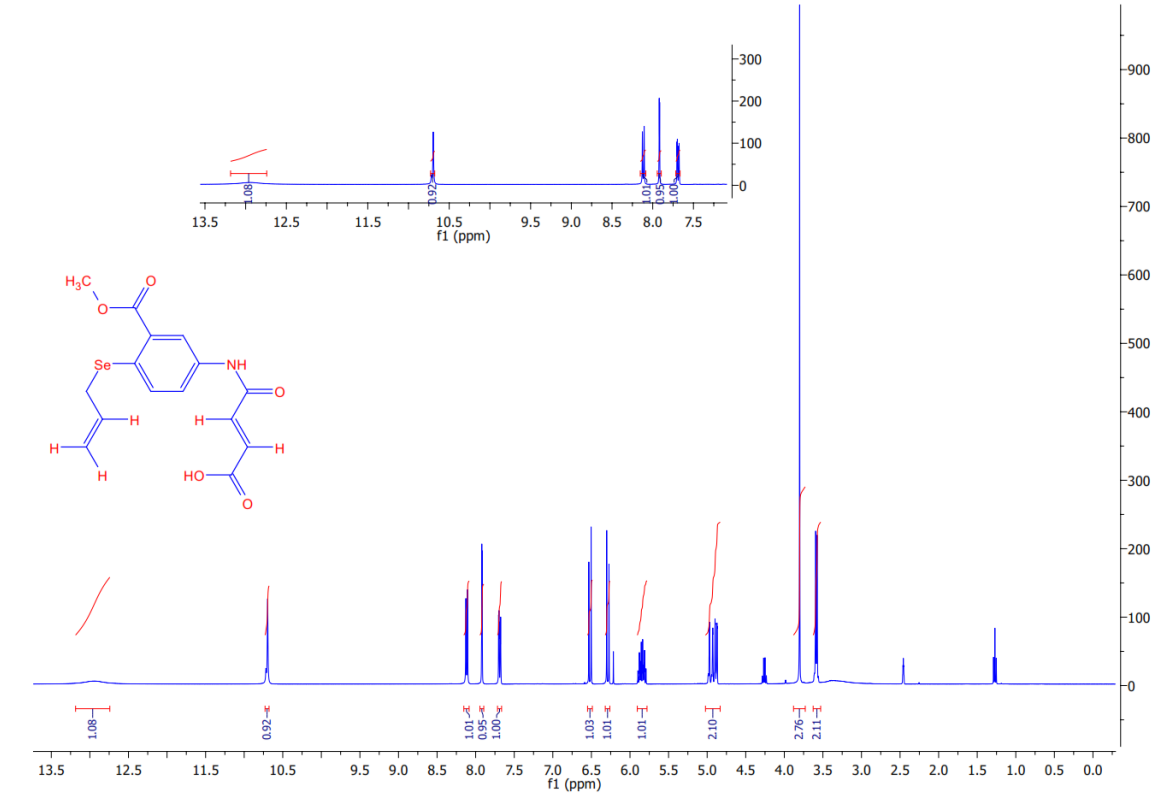


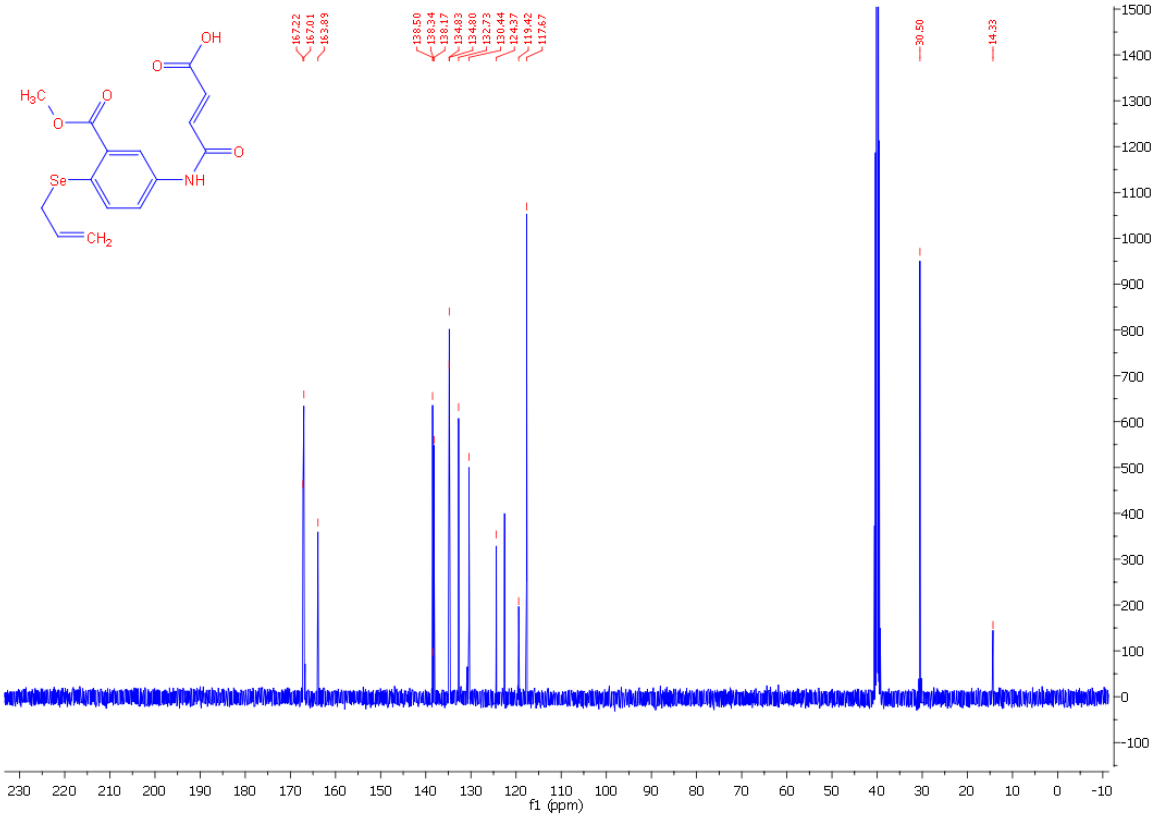


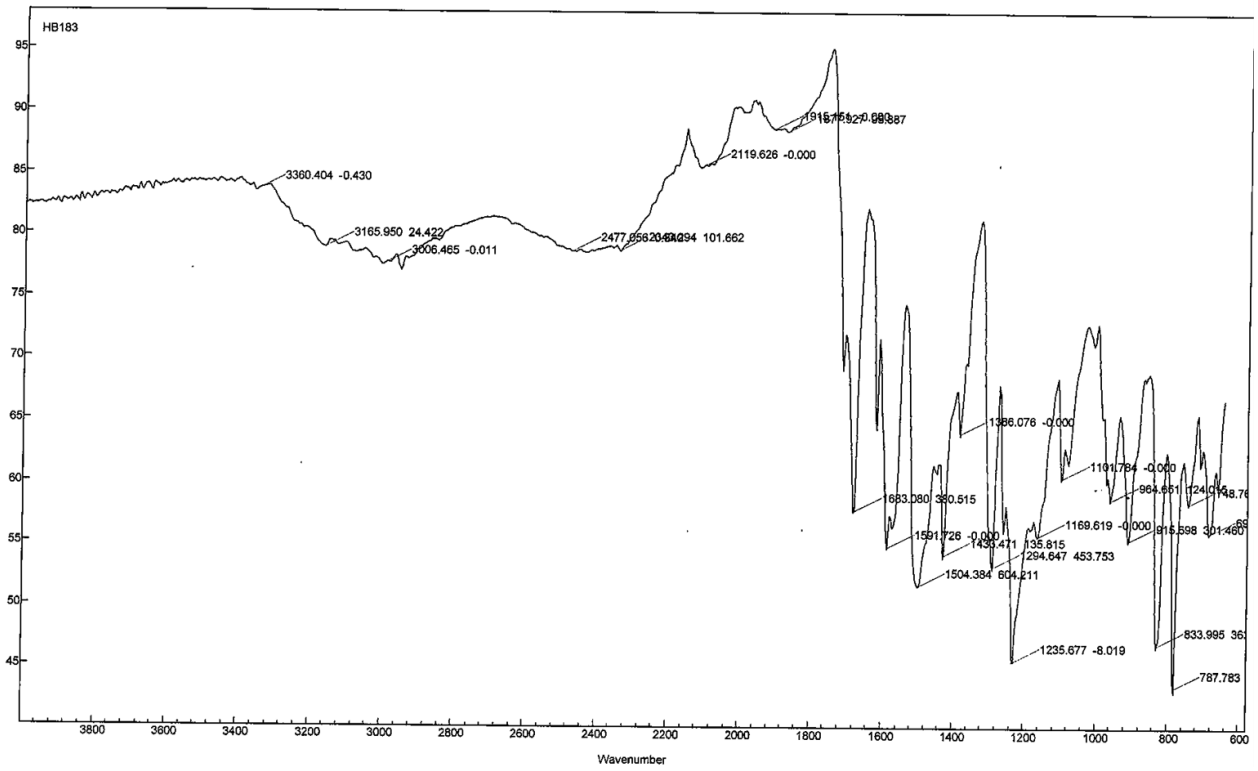

HB181
102
100
98
96
94
92
90
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
Wavenumber
3156.028 0.055
3023.407 4.242
3259.880 11.340
2675.296 5.667
2321.185 0.091
1831.069 2.134
1344.030 0.340
1396.162 9.068
1685.466 356.521
1235.809 -0.000
960.104 -0.000
909.542 61.637
789.788


Spectrum RT 0:47 - 1:28 {78 scans} - Background Subtracted 0 - 0:44
Alaasar-HB181-2_Scan2_is2.datx 2025.01.16 07:25:11 ;
Intensity
ESI + Max: 1.1E7
%
80
60
40
20
0
200
250
300
350
400
m/z
393.6; 1E7
391.6; 5.7E6
395.6; 1.9E6
352.6; 5.2E5
350.7; 2.6E5
366.6; 1.2E5
385.8; 2.5E5

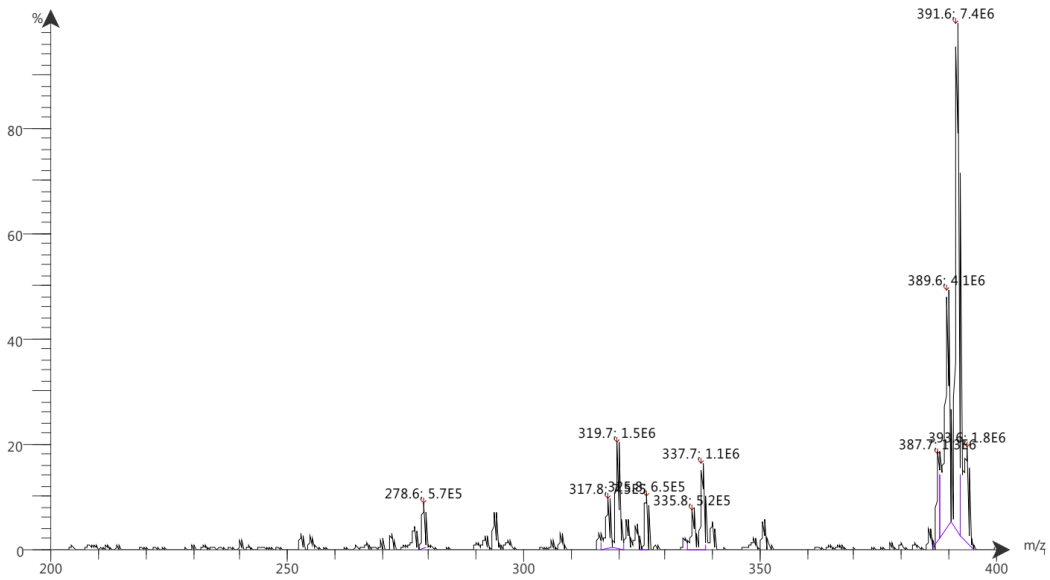
4-((4-(Allylselanyl)-3-(methoxycarbonyl)phenyl)amino)-4-oxobut-2-enoic acid (HB183)

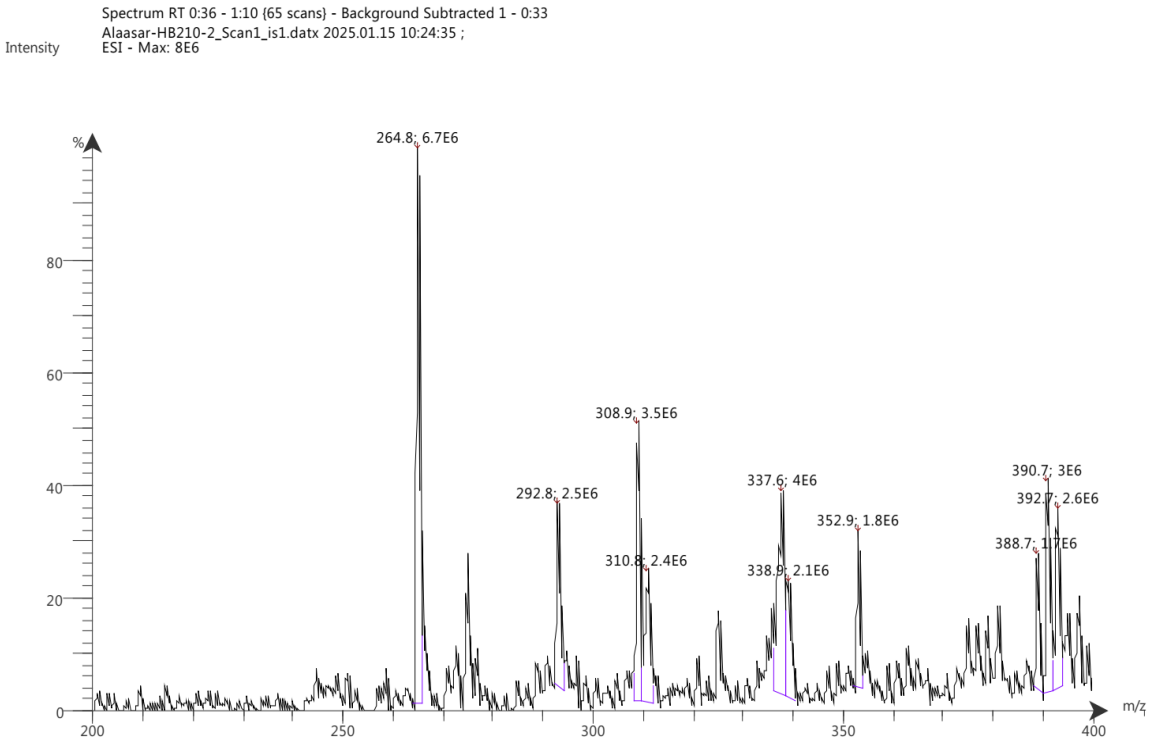




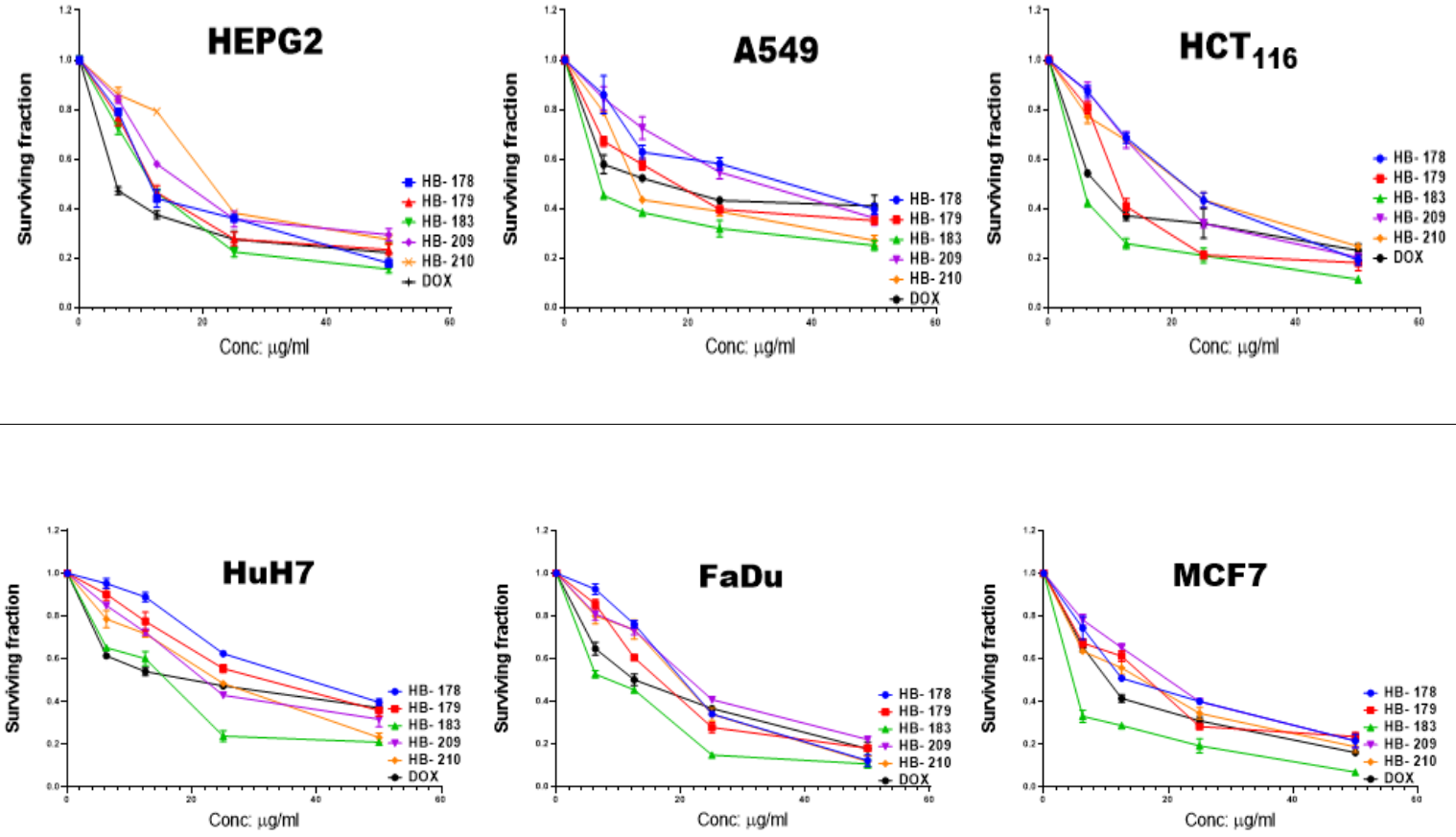


Spectrum RT 0:53 - 1:46 (100 scans) - Background Subtracted 0 - 0:51
Alaasar-HB183-2_Scan2_is2.datx 2025.01.16 07:36:58 ;
Intensity ESI + Max: 7.8E6

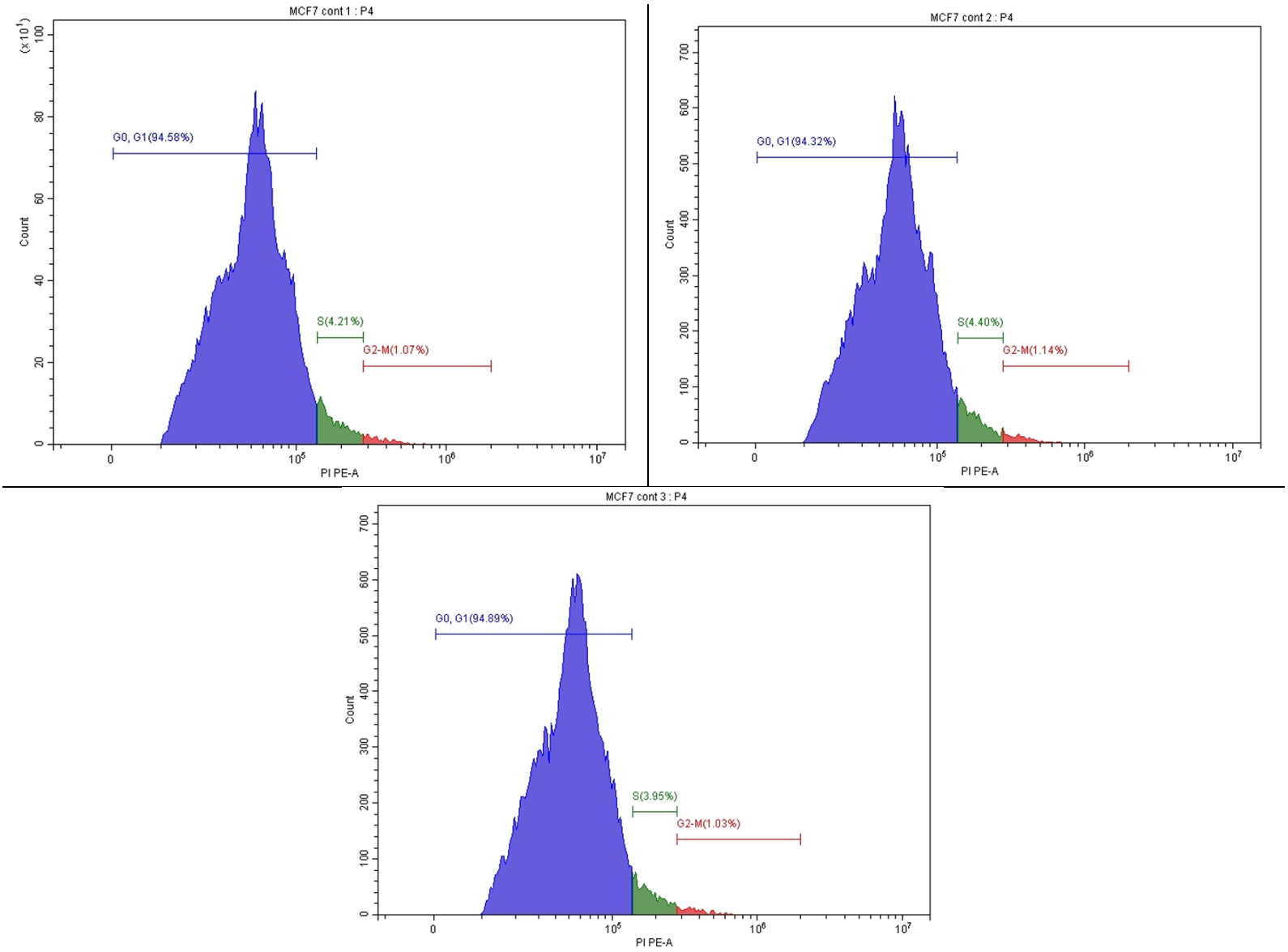



Spectrum RT 0:36 - 1:10 {65 scans} - Background Subtracted 1 - 0:33
Alaasar-HB210-2_Scan1_is1.datx 2025.01.15 10:24:35 ;
ESI - Max: 8E6
Intensity
%
264.8; 6.7E6
308.9; 3.5E6
292.8; 2.5E6
337.6; 4E6
352.9; 1.8E6
390.7; 3E6
392.7; 2.6E6
388.7; 1.7E6
310.8; 2.4E6
338.9; 2.1E6
0
20
40
60
80
200
250
300
350
400
m/z

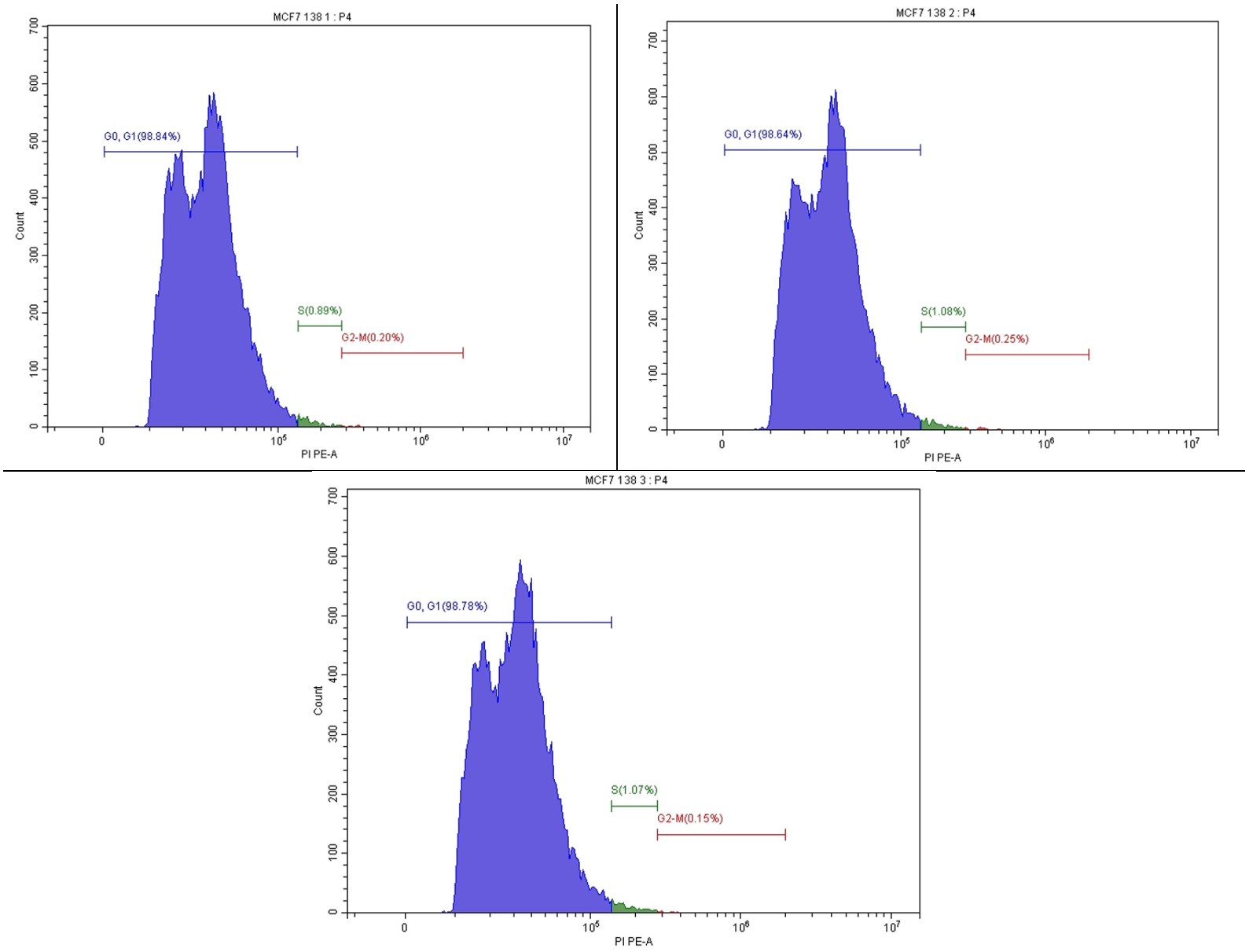
Biological Evaluation



Detailed representation of the IC_{50} of each tested cell line with the evaluated compound.

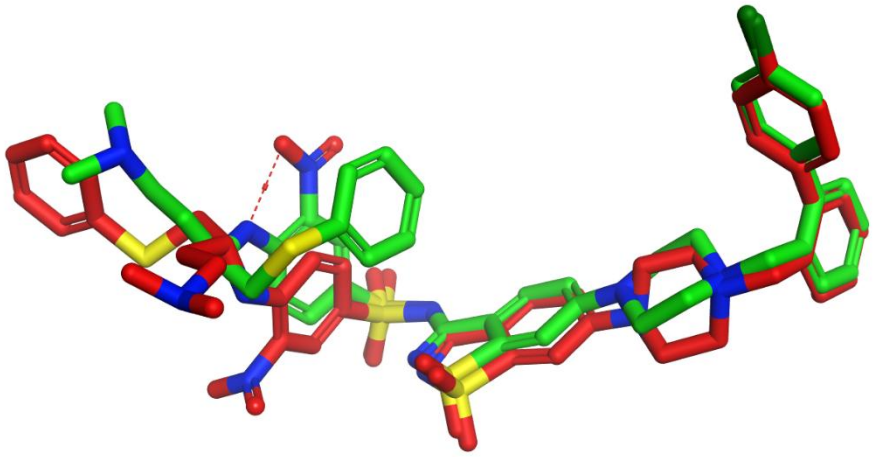


The triplicate experiments of the cell cycle analysis histogram of the untreated control MCF7 cancer cell line.



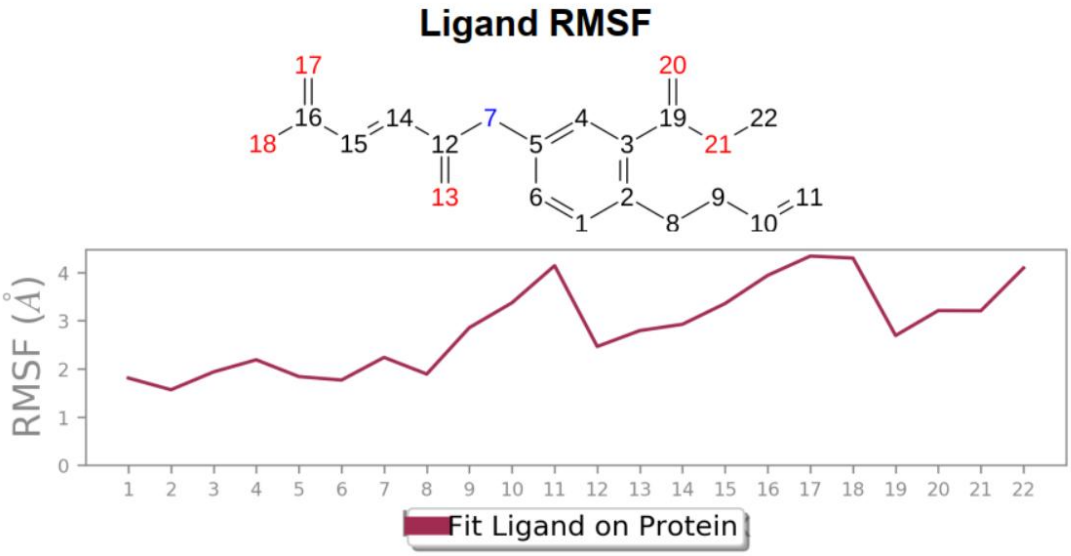
The triplicate experiments of the cell cycle analysis histogram of the HB183-treated MCF7 cancer cell line.

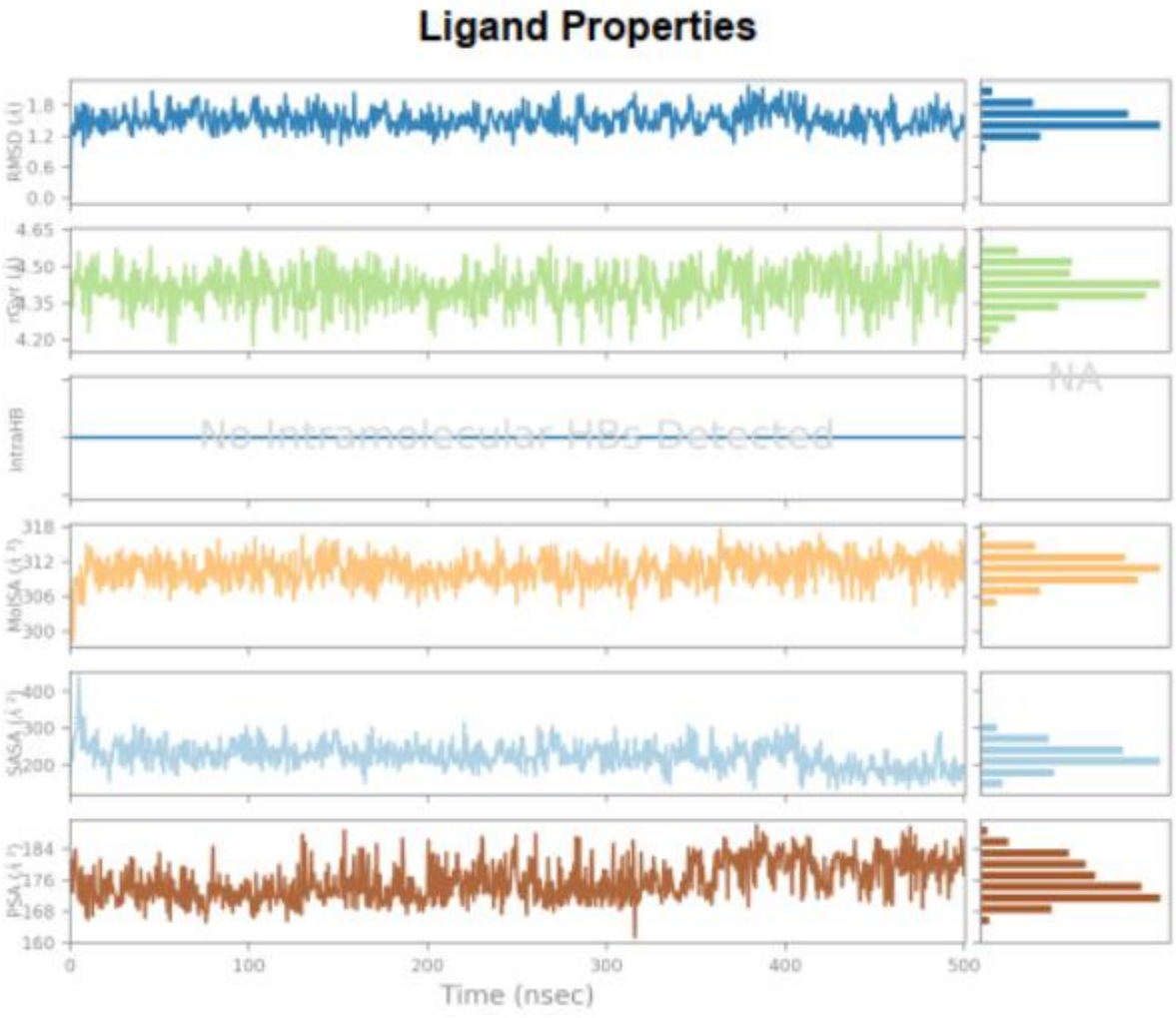
Docking Validation



3D Superimposition of the redocked co-crystal (green) of the BCL-2 receptor (PDB ID: 4IEH) over the native one (red) with an RMSD value of 1.68 Å.

Molecular Dynamics Simulation





Molecular dynamics (MD) simulation

The molecular dynamics simulation was carried out using the Desmond simulation package of Schrödinger LLC.[5-7] The NPγT ensemble with the temperature 300 K and a pressure of 1.01 bar was applied in all runs. The simulation length was 500 ns with a relaxation time of 1 ps. The OPLS4 force field parameters were used in all simulations.[8] The cutoff radius in Coulomb interactions was 9.0 Å. The orthorhombic periodic box boundaries were set 10 Å away from the protein atoms. The water molecules were explicitly described using the transferable intermolecular potential with the three-point (TIP3P) model.[9] Salt concentration was set to 0.15 M NaCl and was built using the System Builder utility of Desmond. The Martyna−Tuckerman−Klein chain coupling scheme with a coupling constant of 2.0 ps was used for the pressure control, and the Nosé−Hoover chain coupling scheme for the temperature control.[10, 11] Nonbonded forces were calculated using a RESPA integrator where the short-range forces were updated every step, and the long-range forces were updated every three steps. The trajectories were saved at 300 ps intervals for analysis. The behavior and interactions between the ligands and protein were analyzed using the Simulation Interaction Diagram tool implemented in the Desmond MD package. The stability of MD simulations was monitored by looking at the RMSD of the ligand and protein atom positions as a function of simulation time.

MD trajectory analysis and prime MM-GBSA calculations

The simulation interactions diagram panel of the Maestro software was used to monitor interactions contribution in the ligand-protein stability. The molecular mechanics generalized born/solvent accessibility (MM – GBSA) was performed to calculate the ligand binding free energies and ligand strain energies for docked compounds over the last 50 ns with thermal_mmgbsa.py python script provided by Schrodinger which takes a Desmond trajectory file, splits it into individual snapshots, runs the MM-GBSA calculations on each frame, and outputs the average computed binding energy.

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