Structural Optimization of Foldamer-Dendrimer Conjugates as Multivalent Agents against the Toxic Effects of Amyloid Beta Oligomers

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Supplementary Information

Contents:

Supplementary materials and methods

Enzyme linked immunosorbent assay (ELISA)

Neutravidin coated microplates (PIERCE, Rockford, IL, USA; capacity: 60 pmol/well) were used for the measurements. The capture molecule was dissolved in 20 mM PBS (pH=7.4) to 10 μg ml^{-1} concentration, 100 μl capture molecule solution was pipetted in each well and incubated for 2 hours at room temperature. The plate was washed with 3 × 200 μl washing buffer (20 mM PBS, 1% BSA, 0.05% Tween 20, pH=7.4), and incubated with 100 μl amyloid solution under shaking overnight at 4 °C. The plate was washed with 3 × 200 μl TPBS (20 mM PBS, 0.05% Tween 20, pH= 7.4). The primary antibody 6E10 (Covance, Leeds, UK) was diluted with the washing buffer (1:10000 dilution) and 100 μl diluted primary antibody solution was pipetted to each well. The plate was incubated for 1 hour at room temperature and then washed with 3 × 200 μl TPBS. The secondary antibody Histols-M (Histopatology Ltd., Pécs, Hungary) was diluted with washing buffer (250 × dilution), 100 μl diluted secondary antibody solution was pipetted to each well and incubated for 1 hour at room temperature. The plate was washed again with 3 × 200 μl TPBS. Development was carried out with 100 μl 3,3',5,5'-tetramethylbenzidine solution (TMB, Cell Signaling Technology, Inc., Danvers, MA, USA) pipetted to each well and the absorbance was measured with a FLUOstar OPTIMA plate reader (BMG Labtech, Offenburg, Germany). Signals were recorded for approximately 1.5 hours, until the absorbance reached the plateau.

Supplementary figures and tables

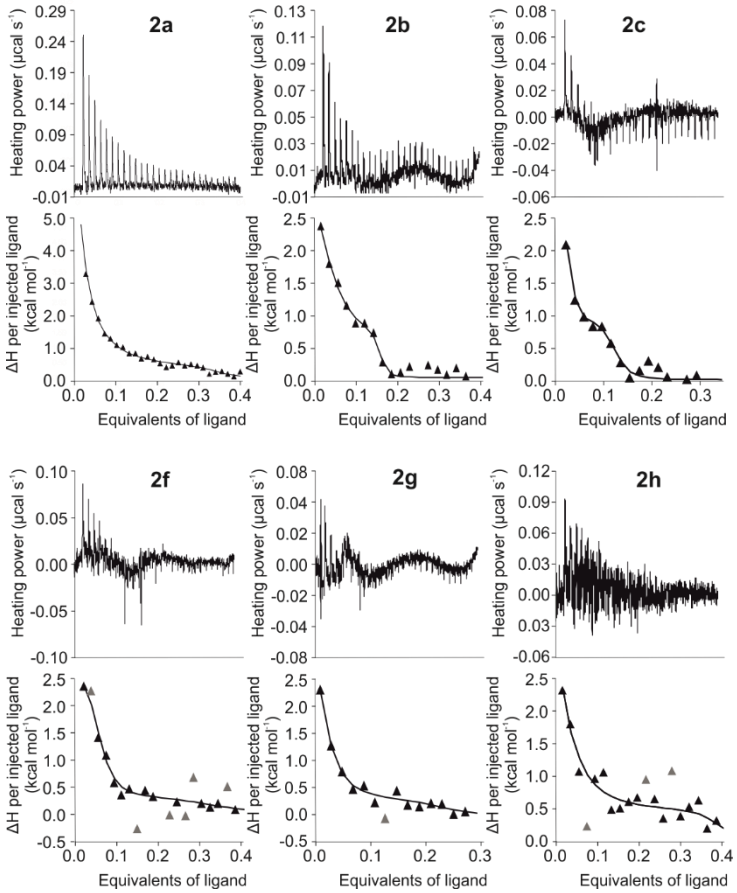


Figure S1. ITC titration data for 2a-2c and 2f-2h.
ITC titration raw data (upper) and integrated enthalpograms with fitted curves (lower).

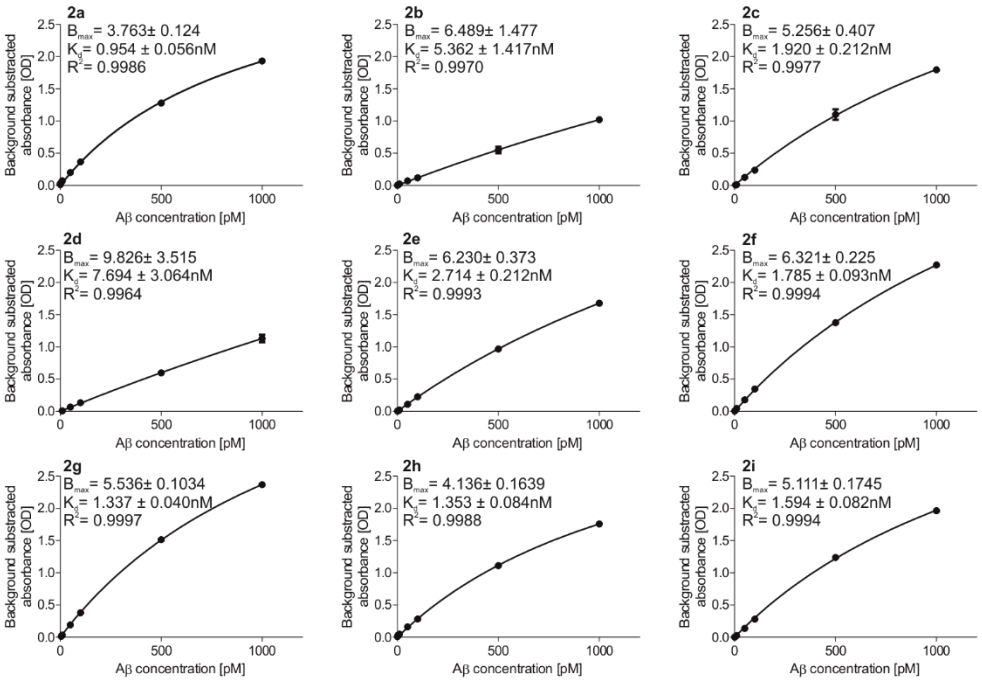


Figure S2. The results of the ELISA experiment of 2a-2i.

Absorbance values were corrected for background signal. Diagram of **2b-2i** contain the signal of the original conjugate **2a** (dashed line) to simplify comparison.

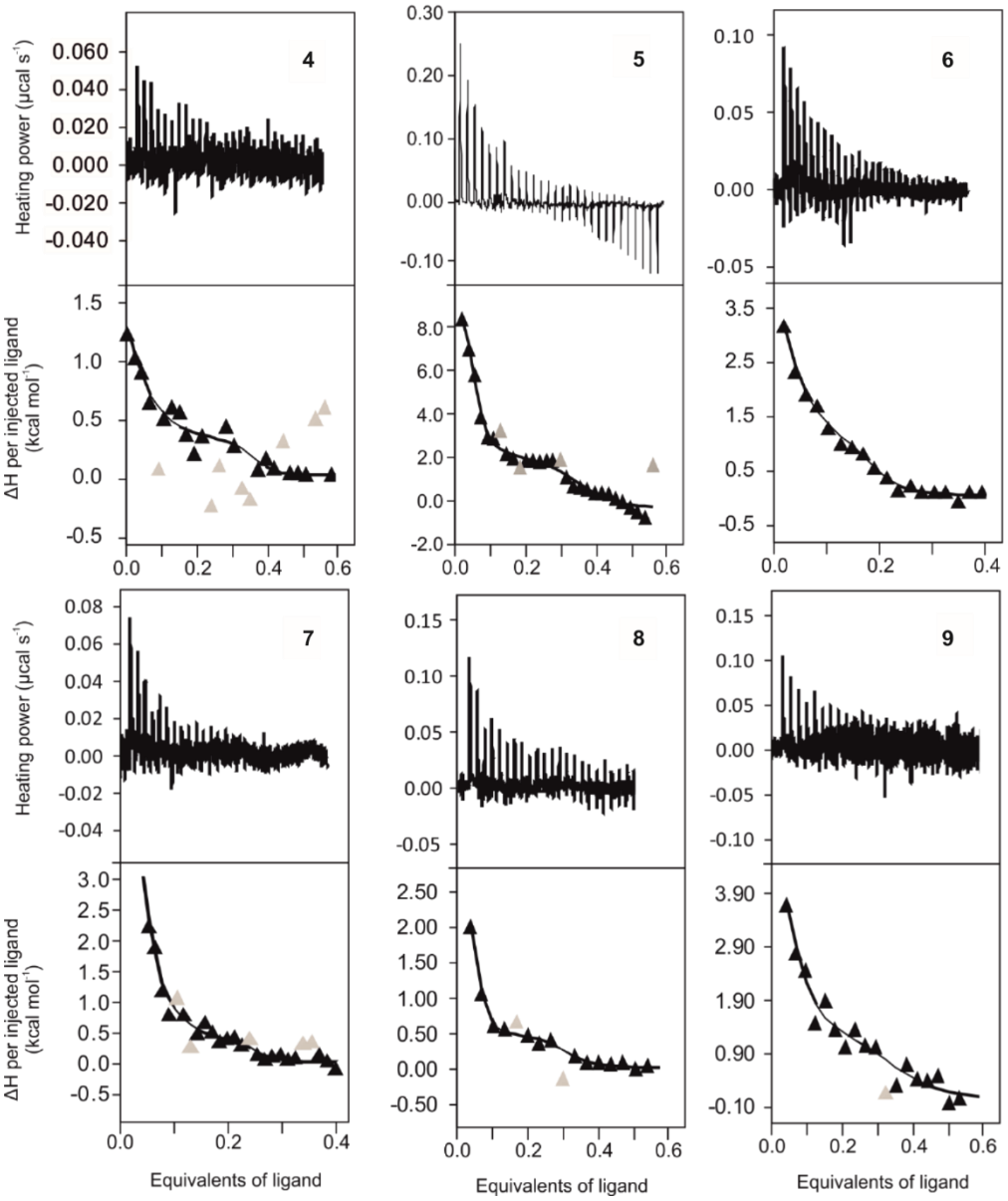


Figure S3. ITC enthalpogram for the titration of Aβ with different conjugate (4-9).
Data was fitted with the two independent site model (black)

Table S1. Details of the purification of compounds.

Compound	Column	Preparative HPLC gradient	calculated MW (g mol^{-1})
1	Luna C18	0-40% in 15 min, 40-65% in 80 min; 4 ml min^{-1}	1034.28
2a	Jupiter C4	20-35% in 20 min, 35-55% in 100 min; 4 ml min^{-1}	5497.60
2b	Jupiter C4	20-40% in 20 min, 40-60% in 80 min; 4 ml min^{-1}	5385.54
2c	Jupiter C4	15-35% in 20 min, 35-65% in 100 min; 4 ml min^{-1}	5553.70
2d	Jupiter C4	20-30% in 20 min, 30-60% in 100 min; 4 ml min^{-1}	5385.38
2e	Jupiter C4	15-45% in 90 min; 4 ml min^{-1}	5497.60
2f	Jupiter C4	20-50% in 90 min; 4 ml min^{-1}	5657.85
2g	Jupiter C4	20-40% in 20 min, 40-60% in 80 min; 4 ml min^{-1}	5657.85

2h	Jupiter C4	20-30% in 20 min, 30-60% in 100 min; 4 ml min^{-1}	5657.85
2i	Jupiter C4	20-60% in 120 min; 4 ml min^{-1}	5657.85
3	Jupiter C4	0-30% in 30 min, 30-50% in 90 min; 4 ml min^{-1}	2316.80
4	Jupiter C4	0-60% in 120 min; 4 ml min^{-1}	3999.81
5	Jupiter C4	0-60% in 120 min; 4 ml min^{-1}	5257.30
6	Jupiter C4	10-50% in 160 min; 4 ml min^{-1}	10 910.8
7	Jupiter C4	0-20% in 10 min, 20-40% in 80 min; 4 ml min^{-1}	5200.2
8	Jupiter C4	20-40% in 20 min, 40-60% in 80 min; 4 ml min^{-1}	5484.51
9	Jupiter C4	20-40% in 20 min, 40-60% in 80 min; 4 ml min^{-1}	3956.85

Phenomenex Luna C18 column: 250 x 10.00 mm, particle size: 10 micron, pore size: 100Å

Phenomenex Jupiter C4 column: 250 x 10.00 mm, particle size: 10 micron, pore size: 300Å

Table S2. Results of the *ex vivo* tissue viability test
Relative viability values compared to the control after treatment with Aβ oligomers in 10 μM concentration alone and in the presence of compounds **1, 3-6** in a 1:1 molar ratio (N=4)

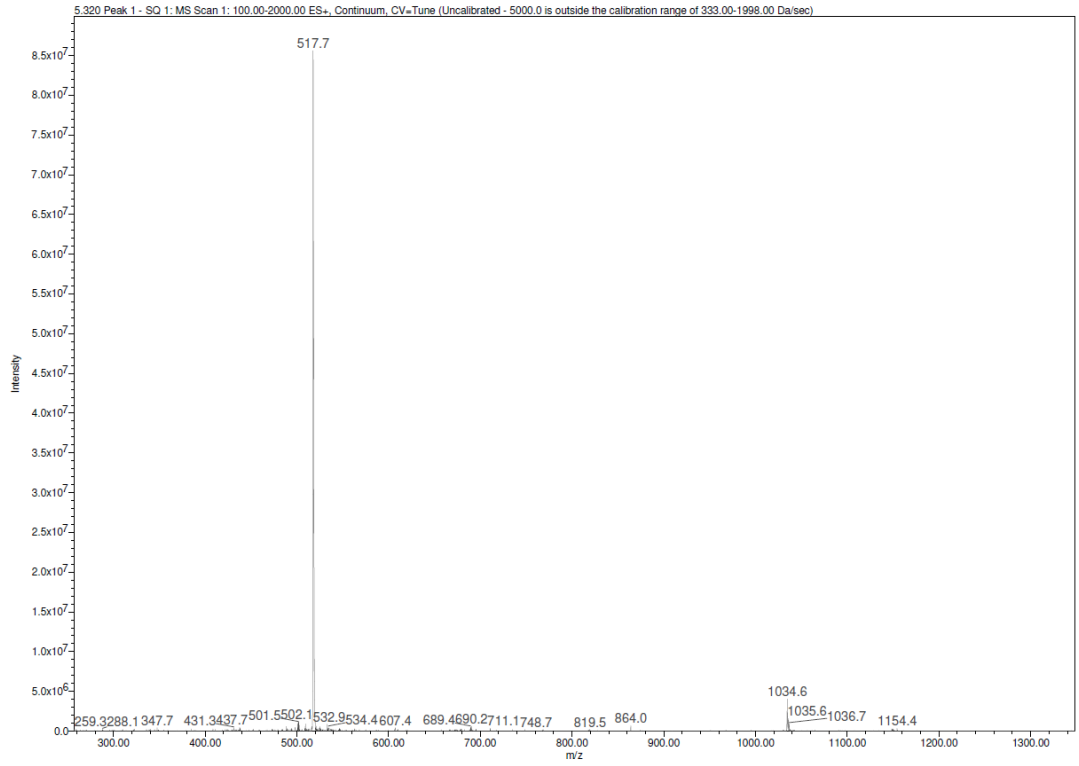
	% [(OD550-620 nm)/slices area mm^2] (mean ± SD)
control	100.0 ± 2.6
Aβ	43.2 ± 1.6
Aβ + **1**	51.6 ± 1.6
Aβ + **3**	55.6 ± 3.6
Aβ + **4**	74.1 ± 2.7
Aβ + **5**	85.5 ± 4.8
Aβ + **6**	89.3 ± 2.7

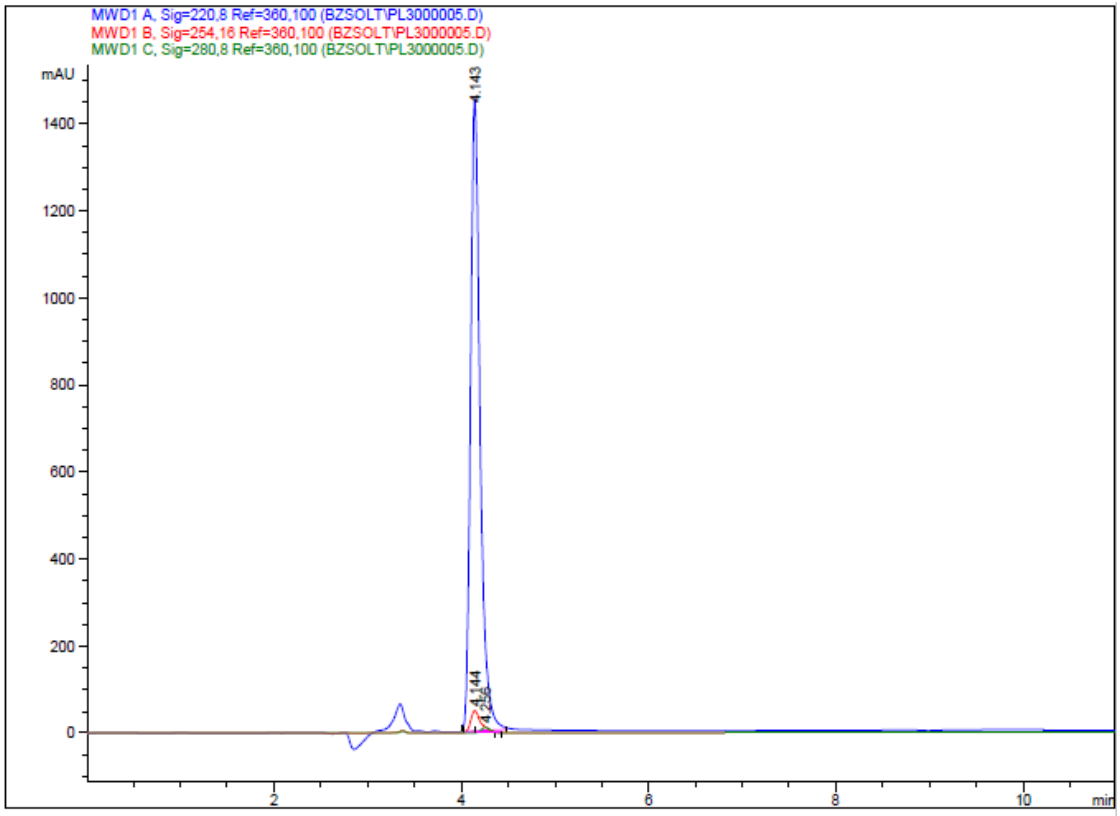
Table S3. Cell viability readouts based on impedance analysis.

	5 μM				10 μM			
	24 hours		48 hours		24 hours		48 hours	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
control	100.0	1.1	100.0	0.9	100.0	1.1	100.0	0.9
Aβ	85.1	2.6	84.0	2.0	85.1	2.6	84.0	2.0
Aβ + **1**	90.6	3.7	87.0	2.3	96.6	5.1	89.0	1.9
Aβ + **3**	85.4	2.9	85.8	3.2	78.9	2.4	79.7	1.0
Aβ + **4**	92.1	3.4	85.5	2.0	98.7	3.8	91.2	1.7
Aβ + **5**	98.0	4.5	98.6	3.4	102.2	4.8	102.1	4.0

Peptide and conjugate characterization data

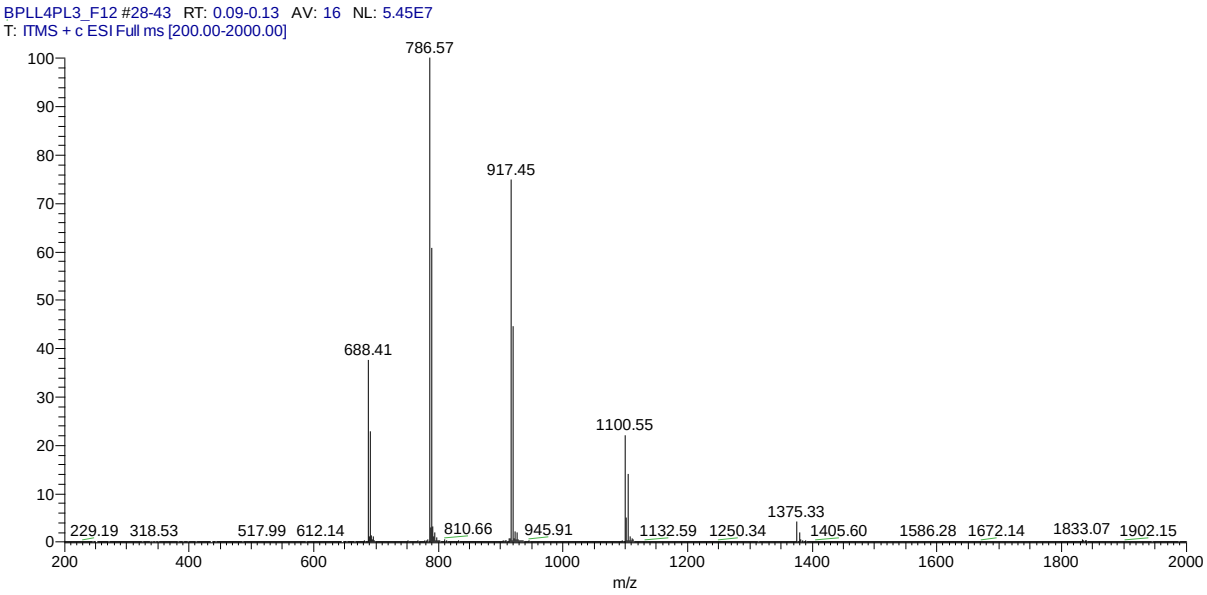
Compound **1**

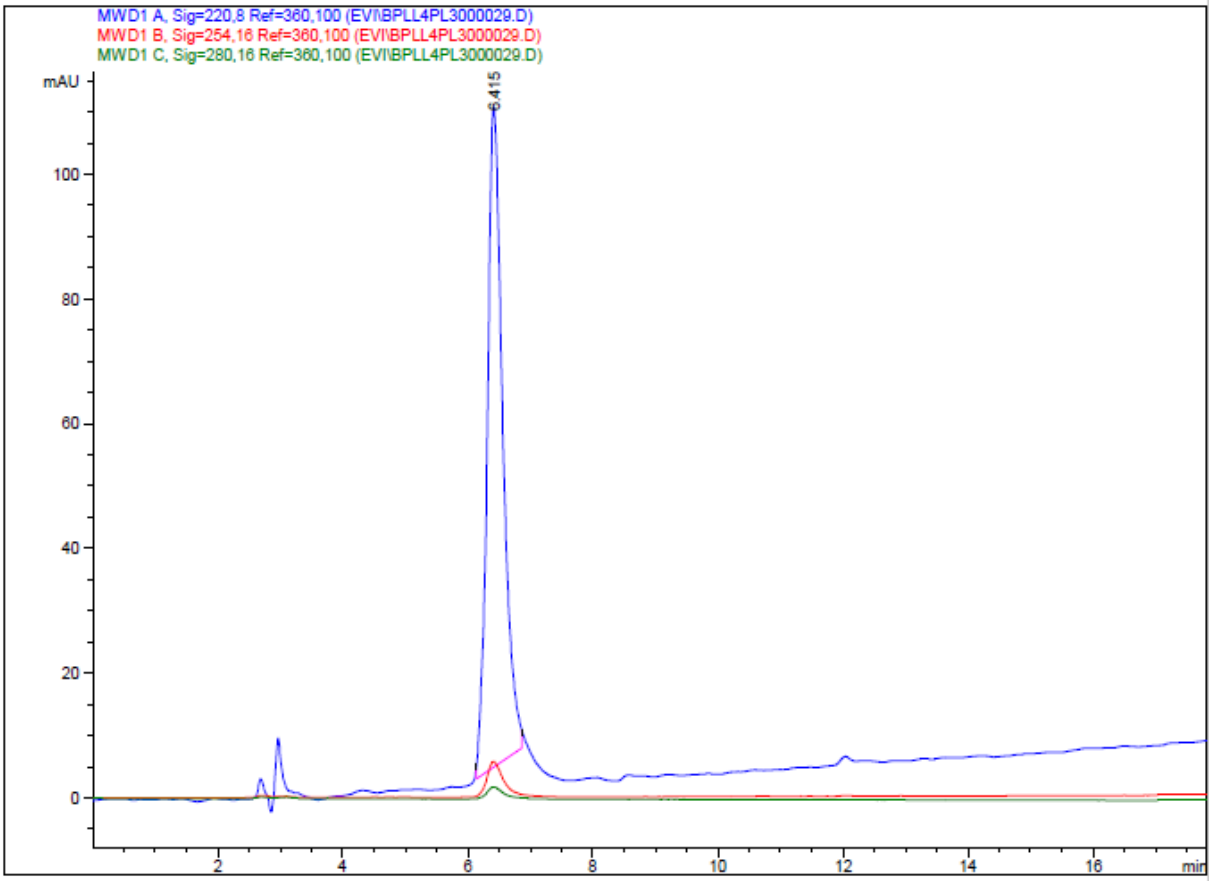




Column: Phenomenex Luna C18 (250 x 4.6 mm, particle size: 5 micron, pore size: 100Å); Gradient: 30-50% 20min 1.2 mL min^{-1}

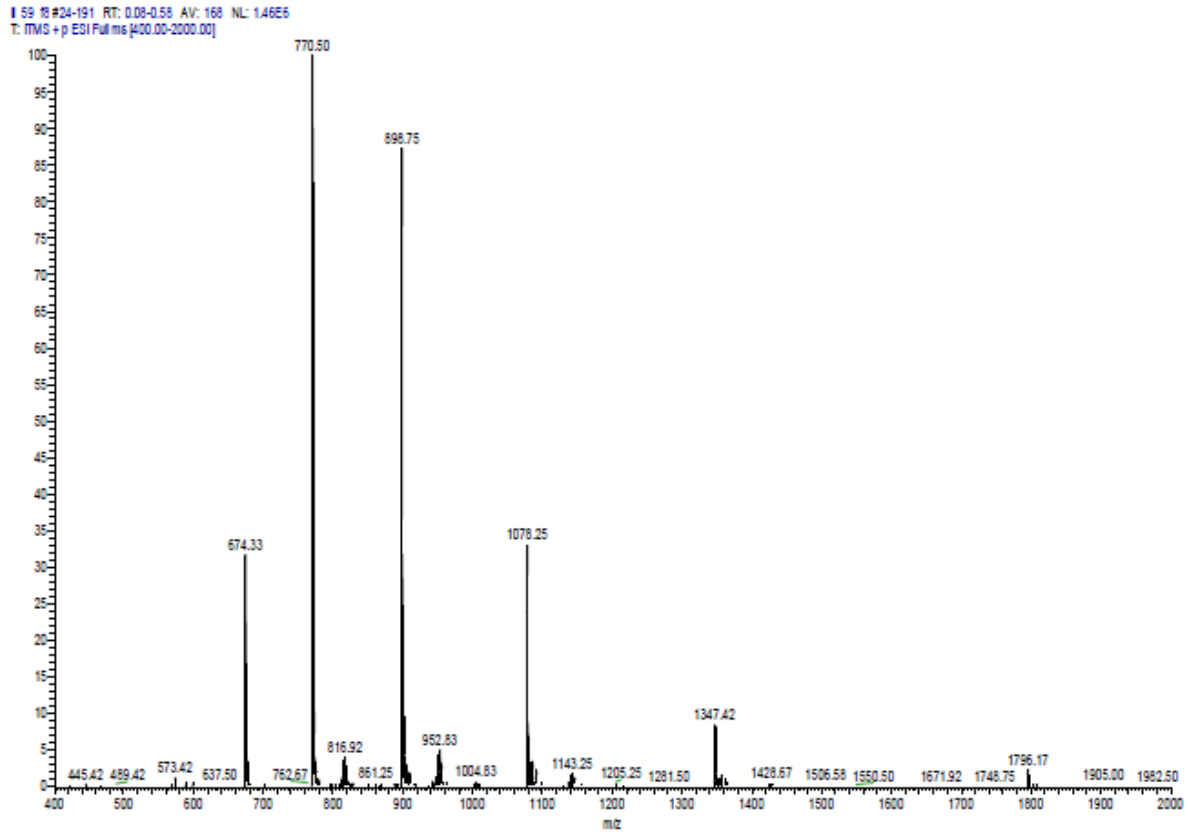
Compound **2a**

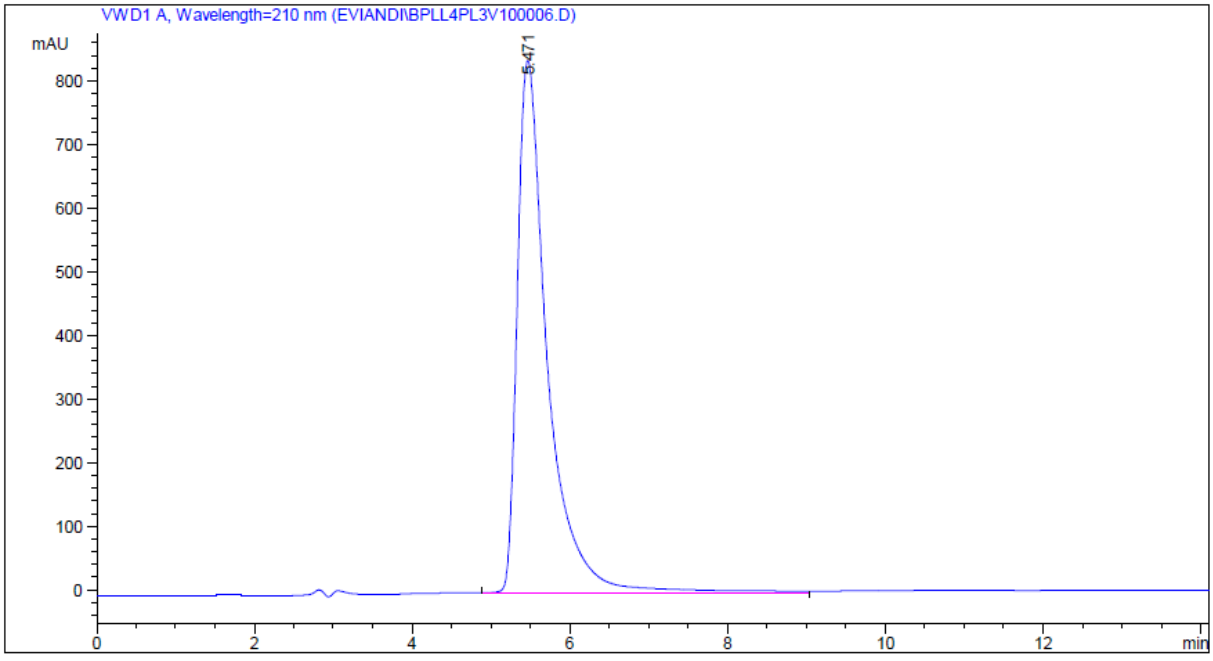




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 40-60% 20min 1.2 mL min^{-1}

Compound **2b**





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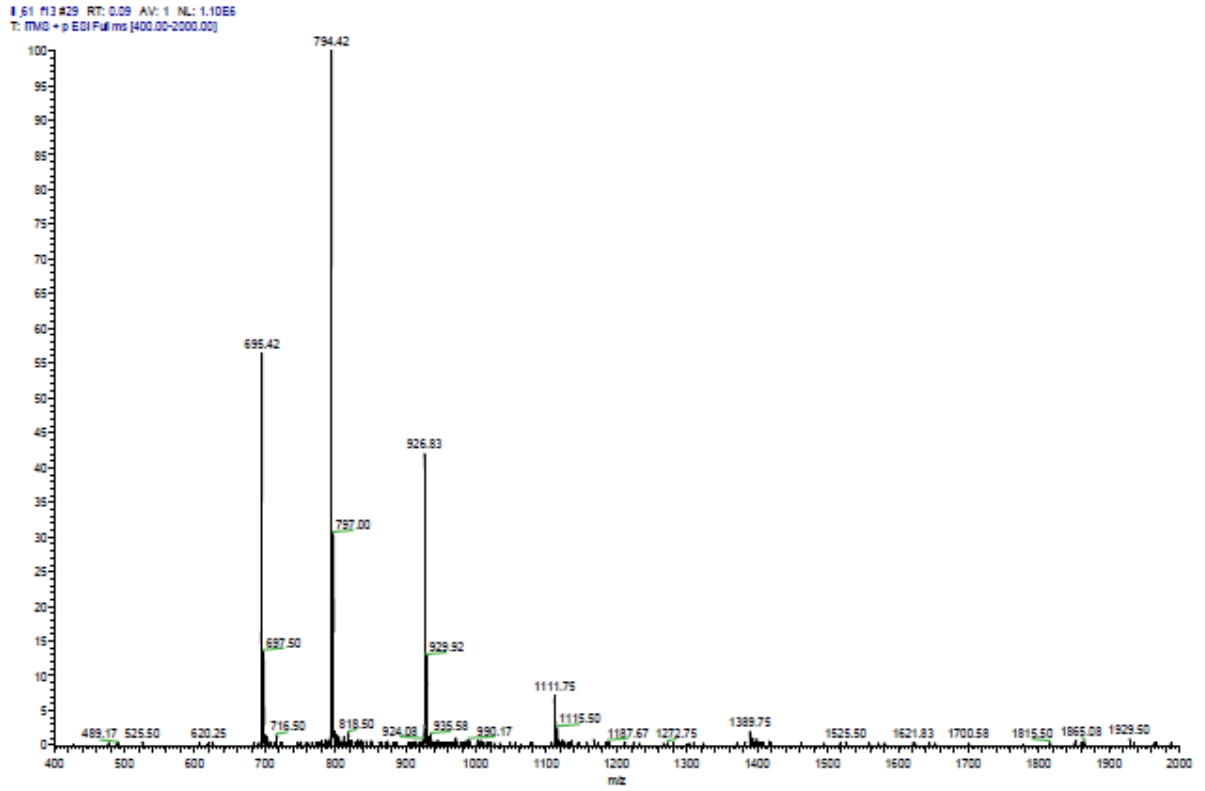
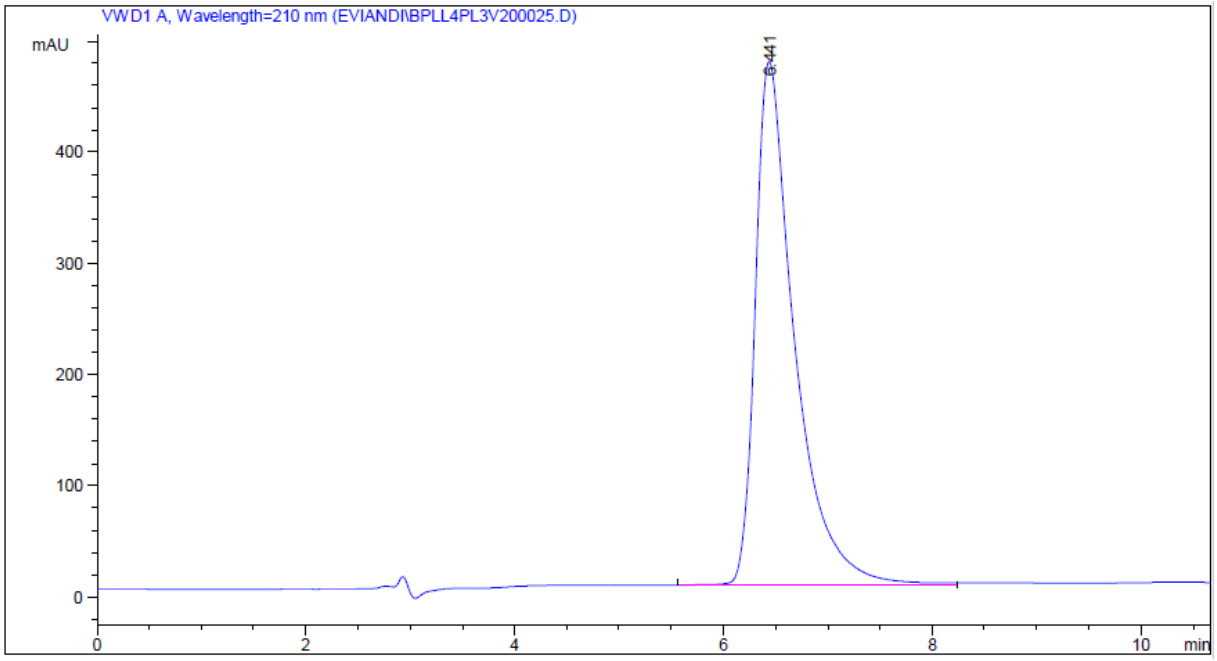
Compound **2c**





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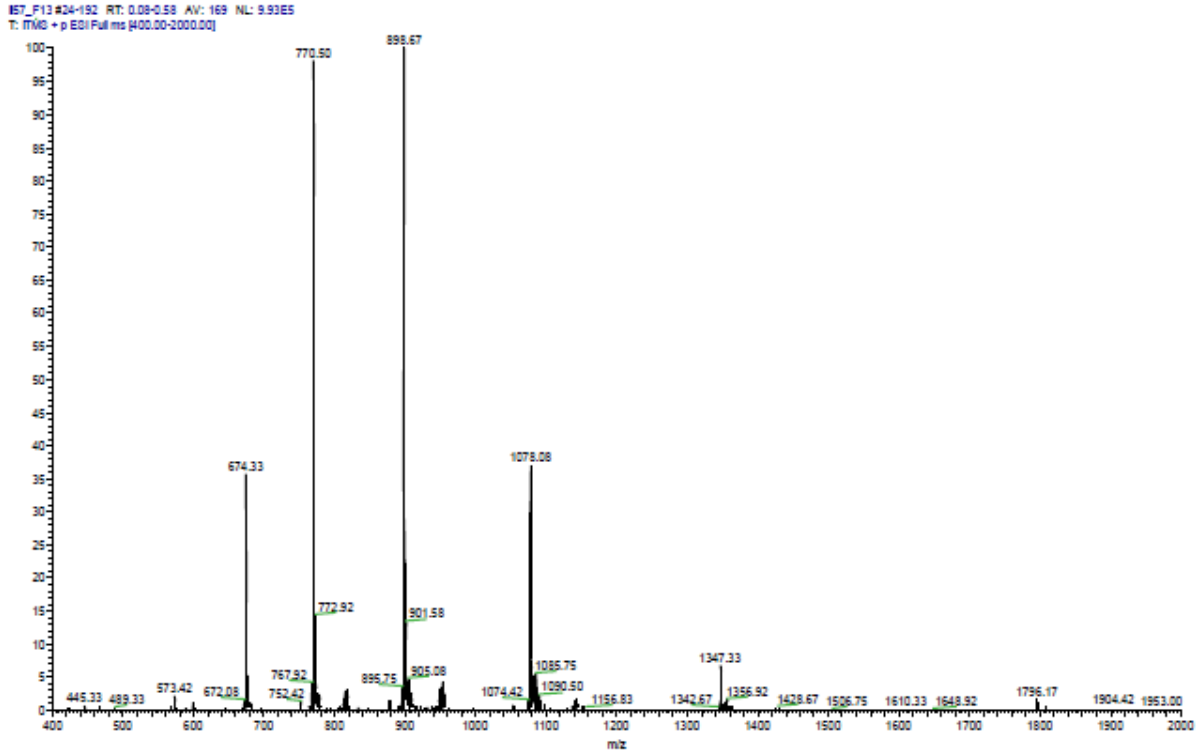
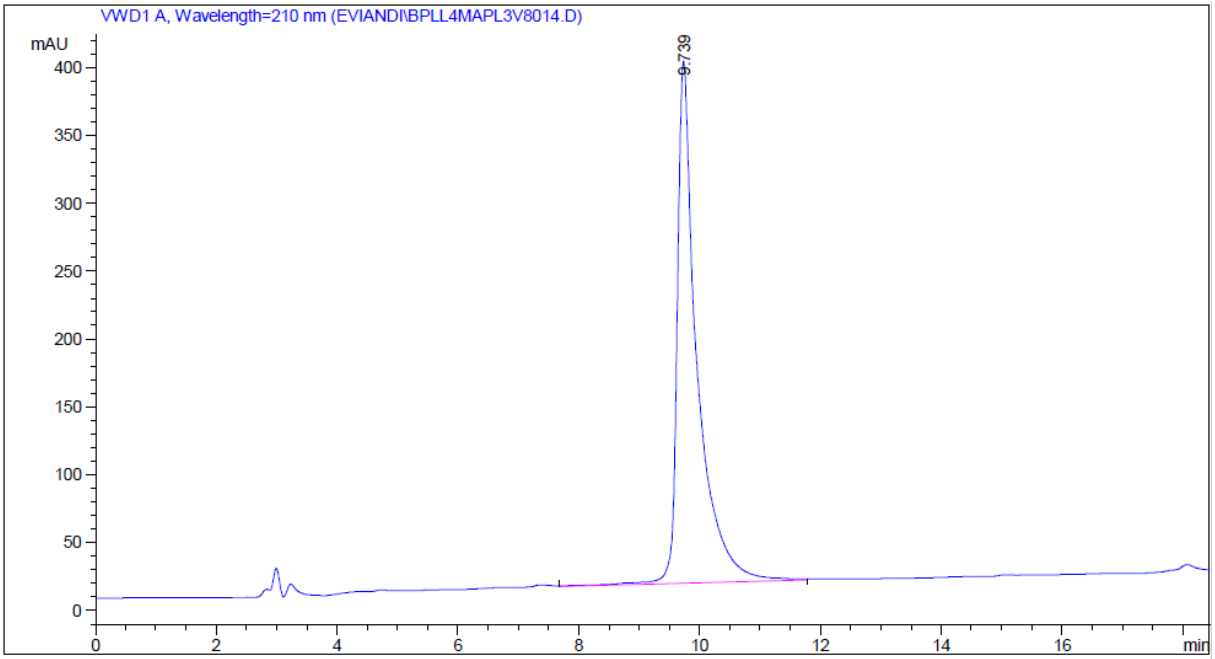
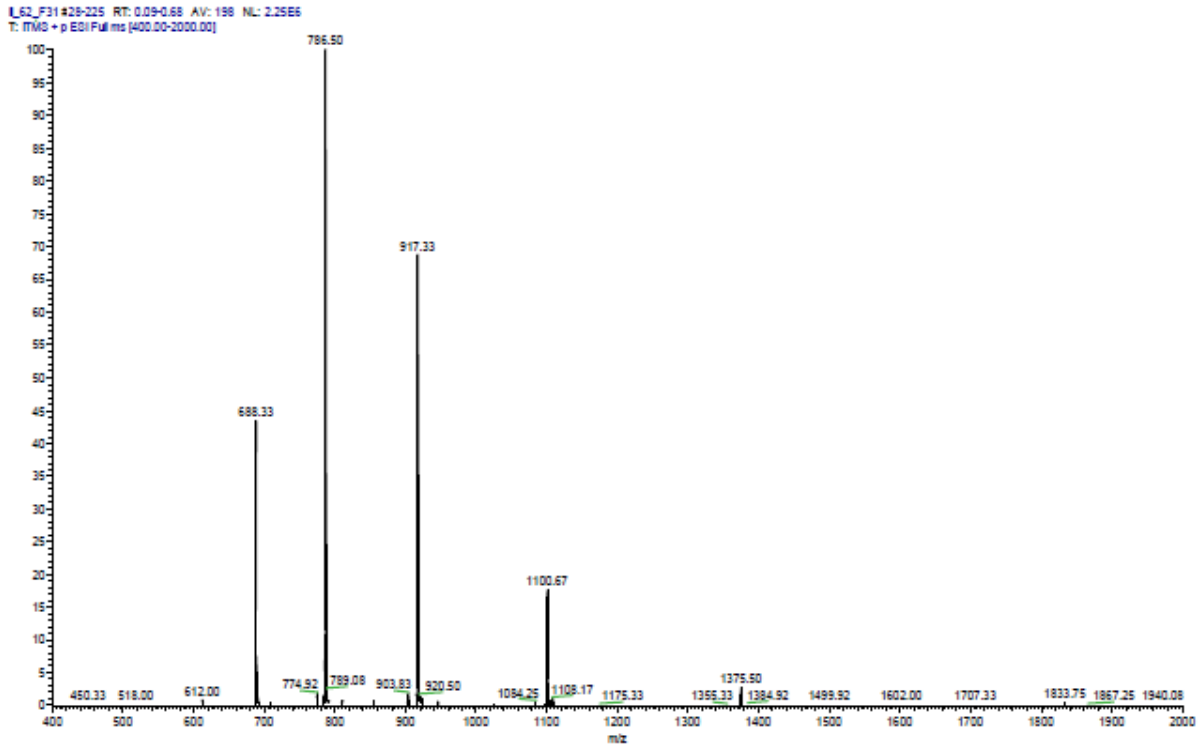
Compound **2d**

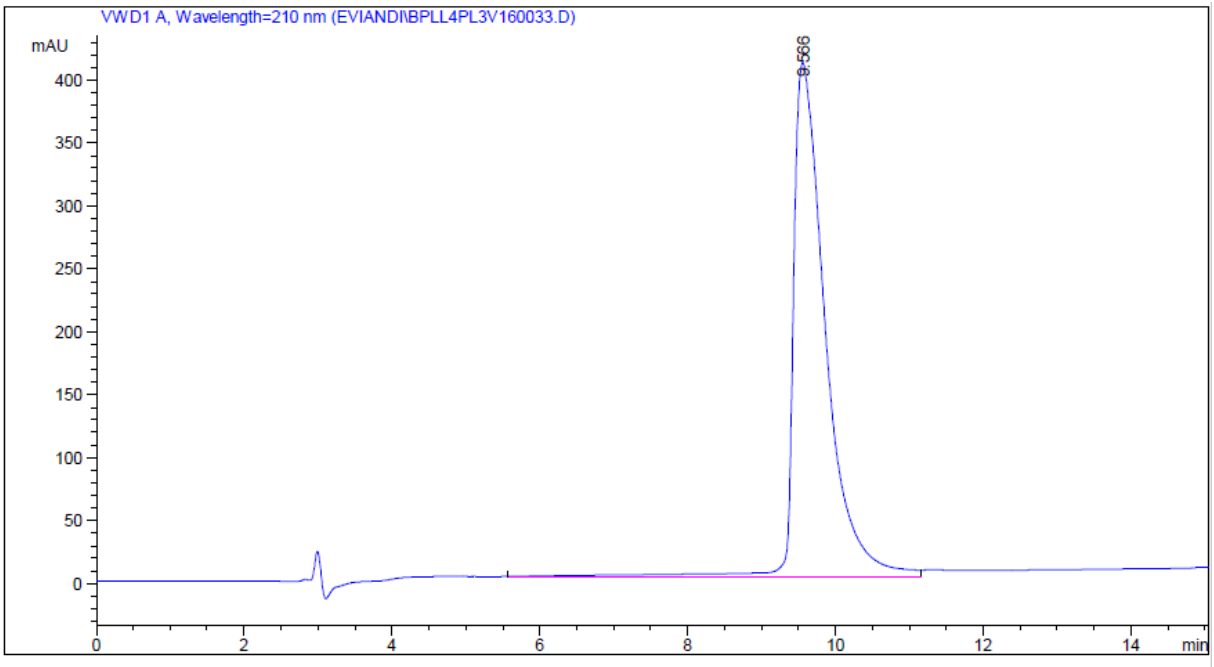




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 30-50% 20min 1.2 mL min^{-1}

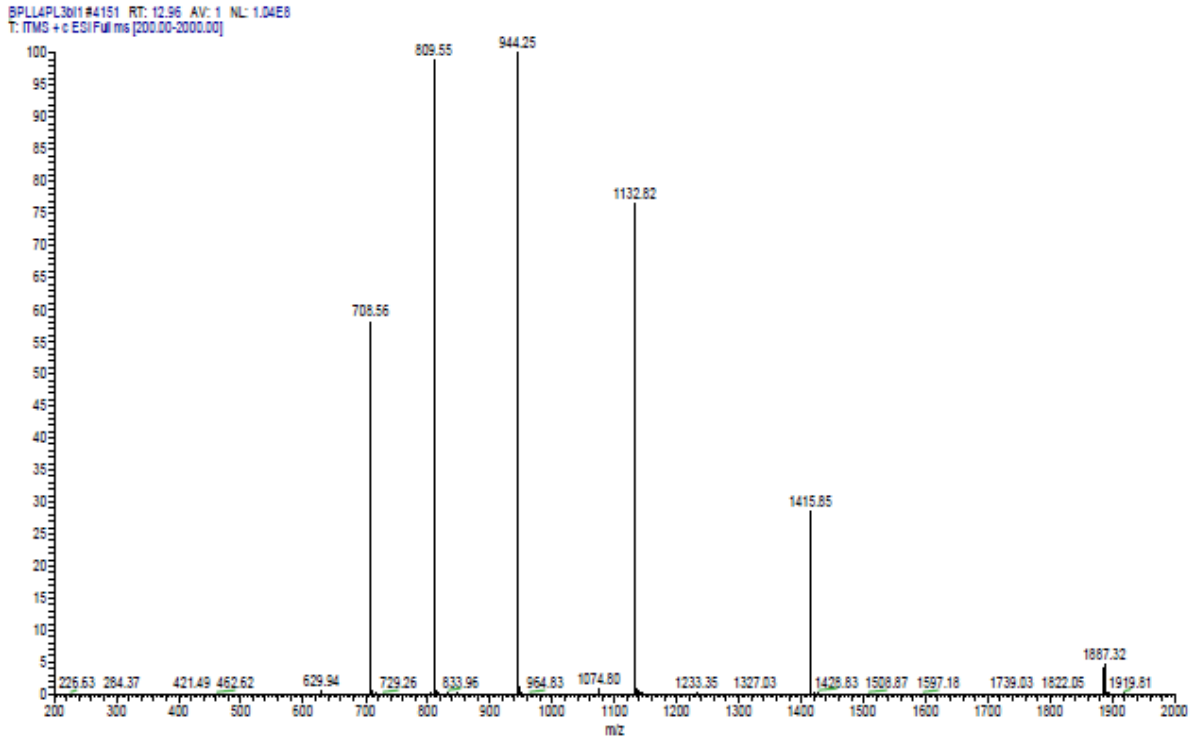
Compound **2e**

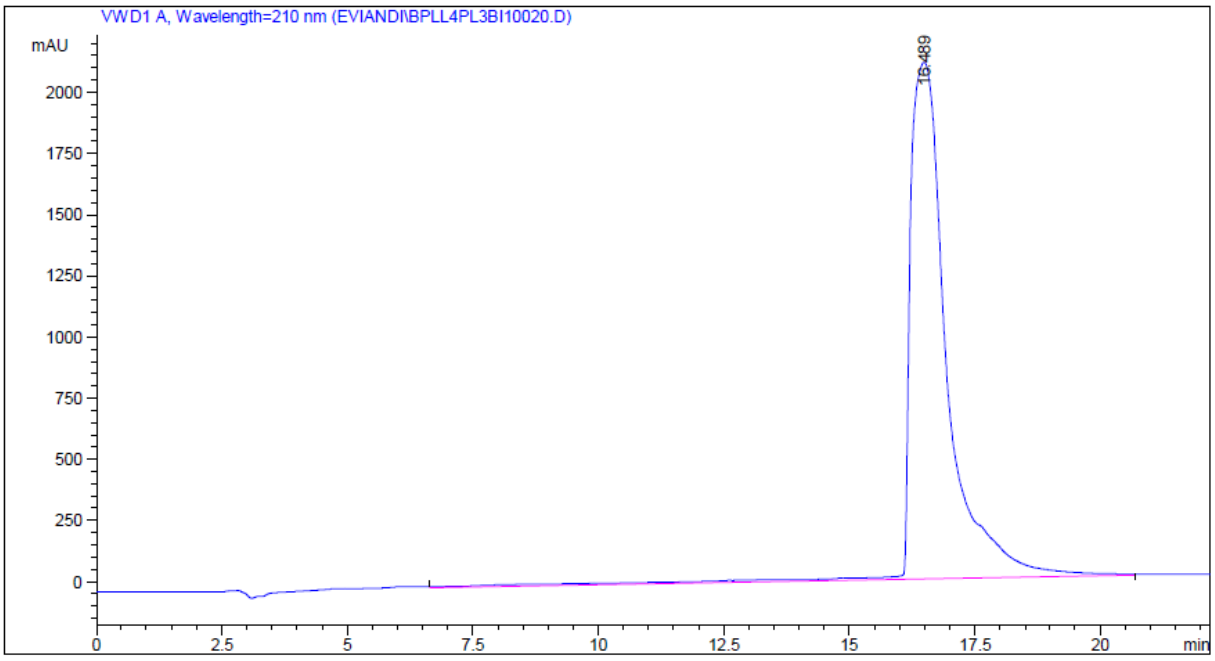




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 30-50% 20min 1.2 mL min^{-1}

Compound **2f**





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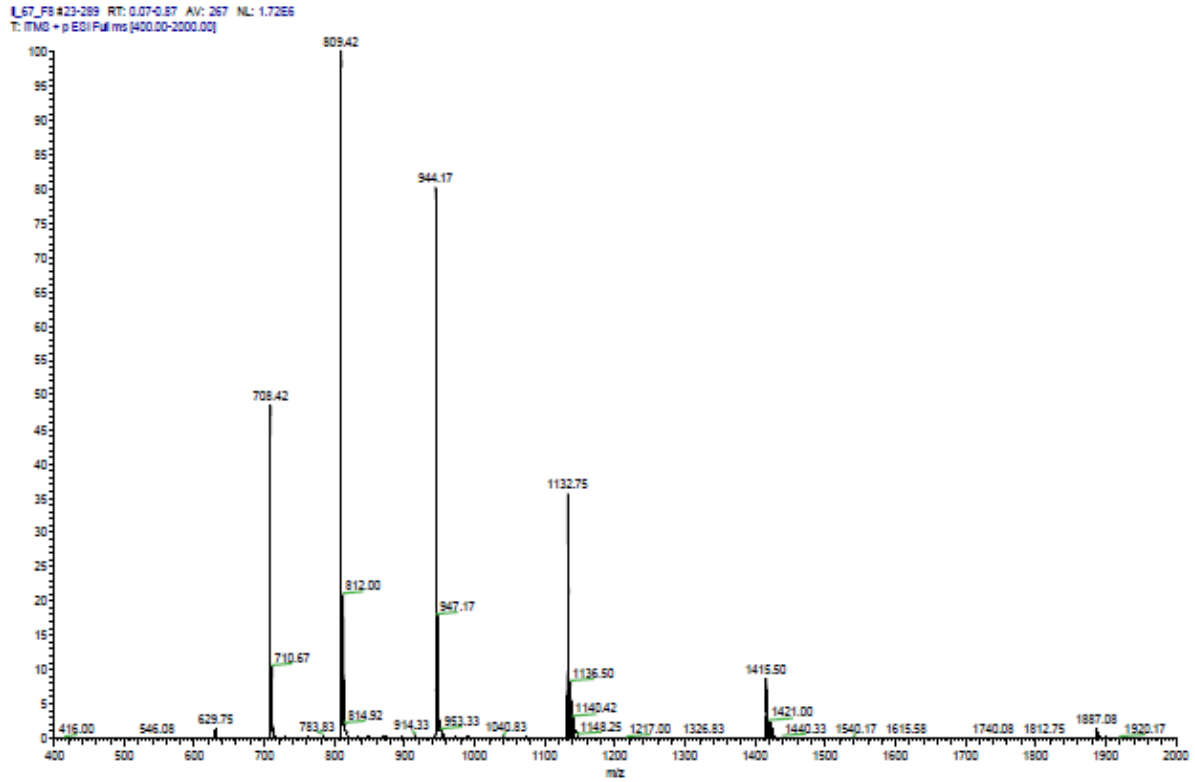
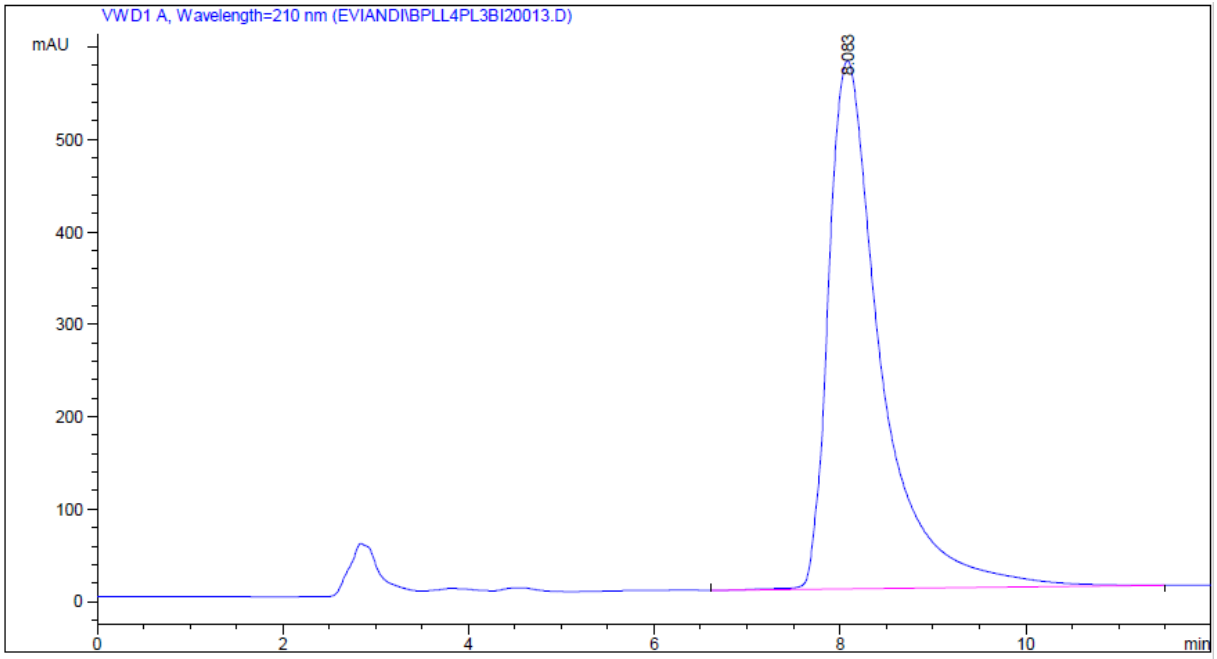
Compound **2g**





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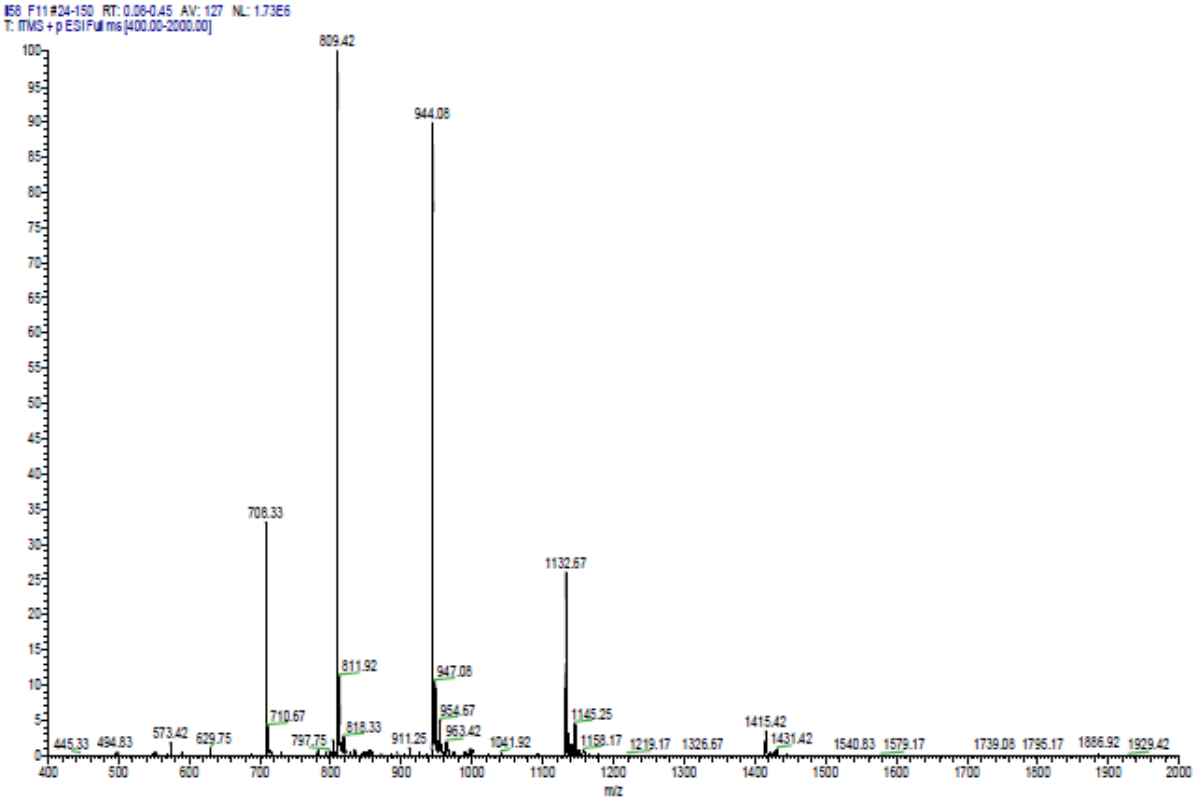
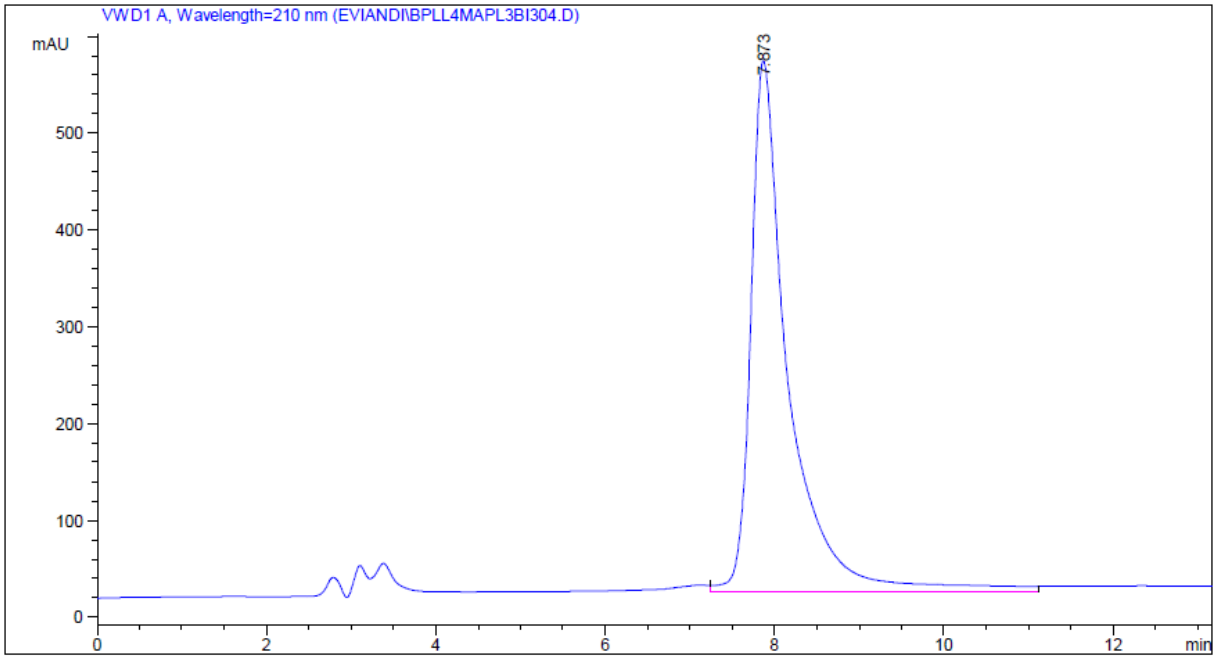
Compound **2h**





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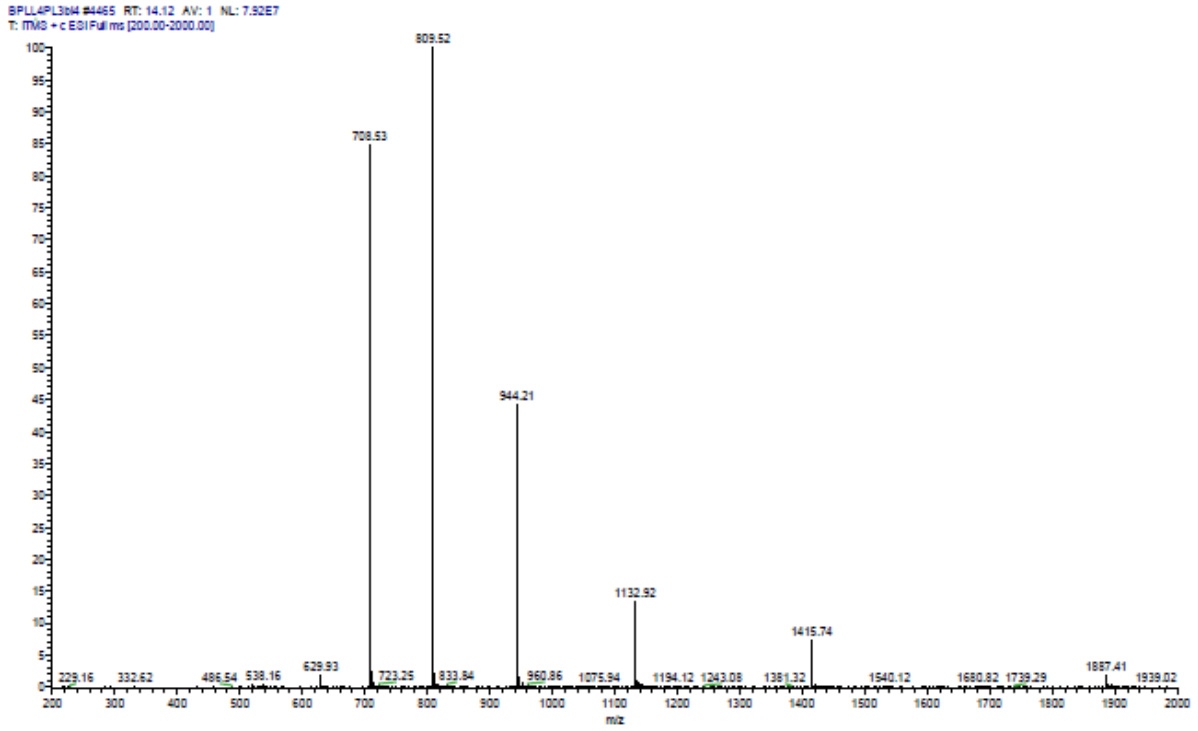
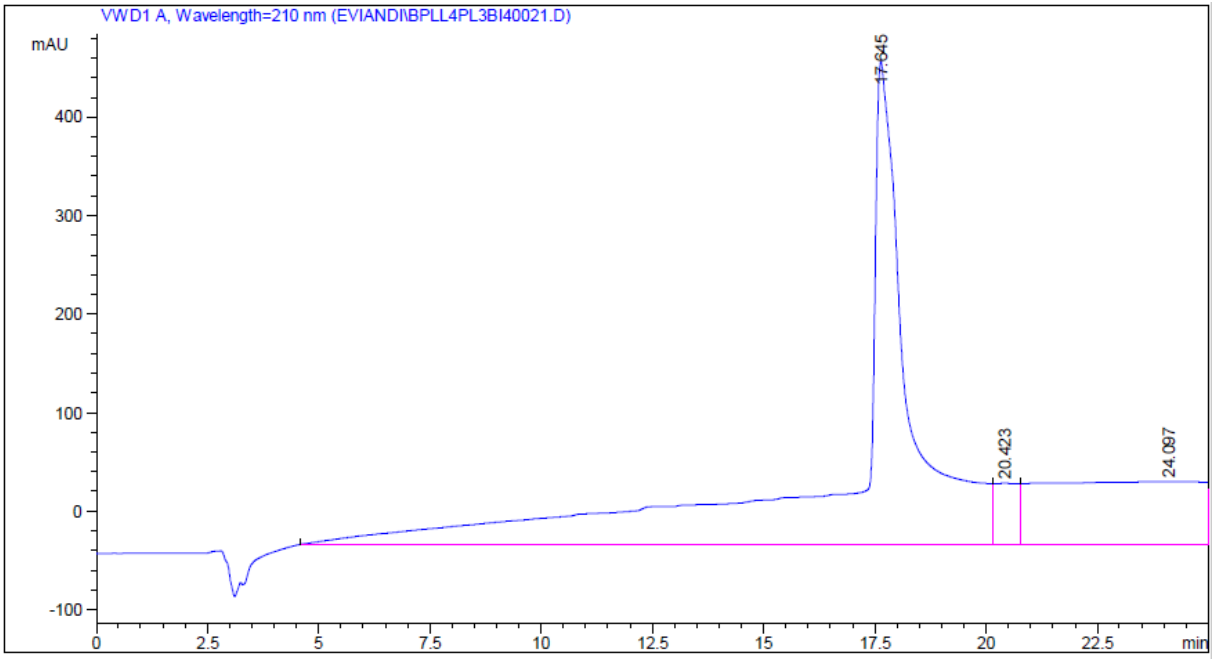
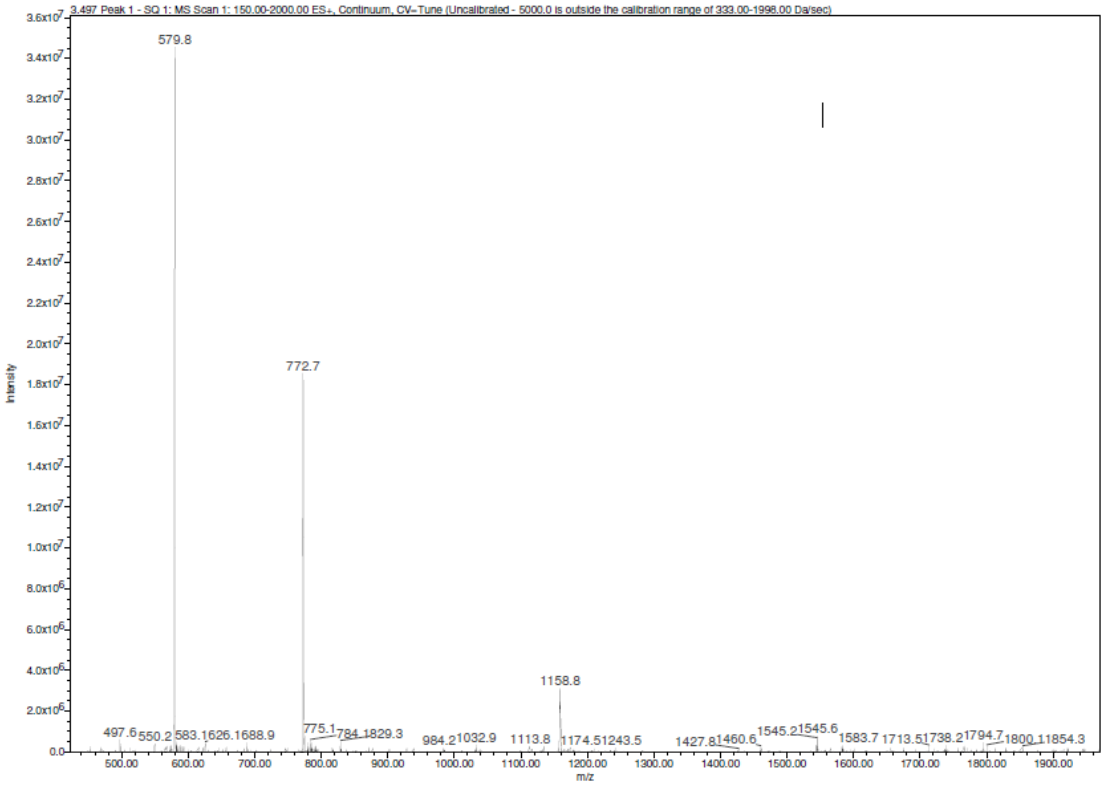
Compound **2i**

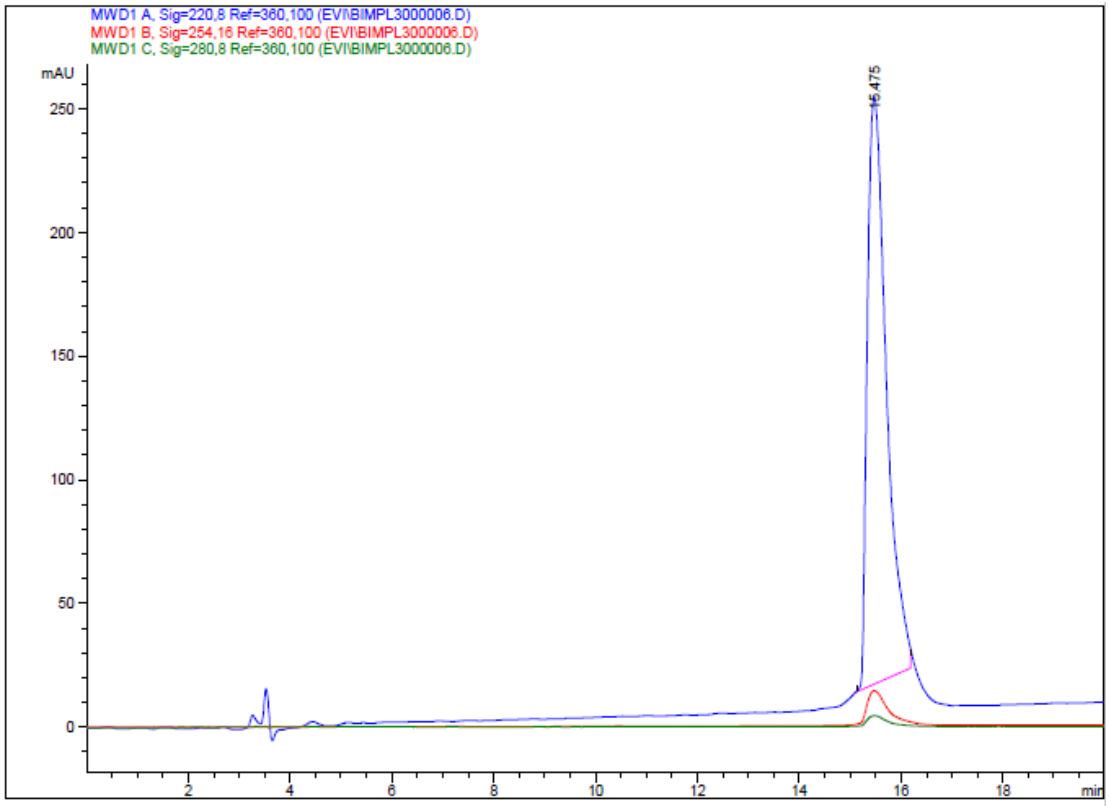




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 5-80% 25min 1.2 mL min^{-1}

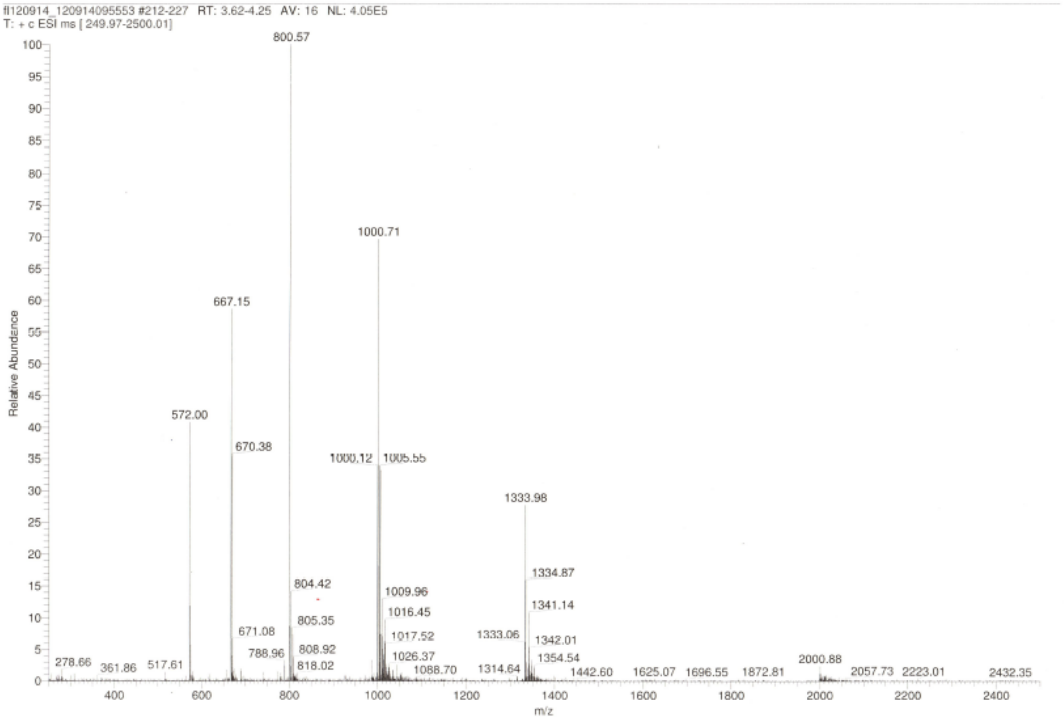
Compound **3**

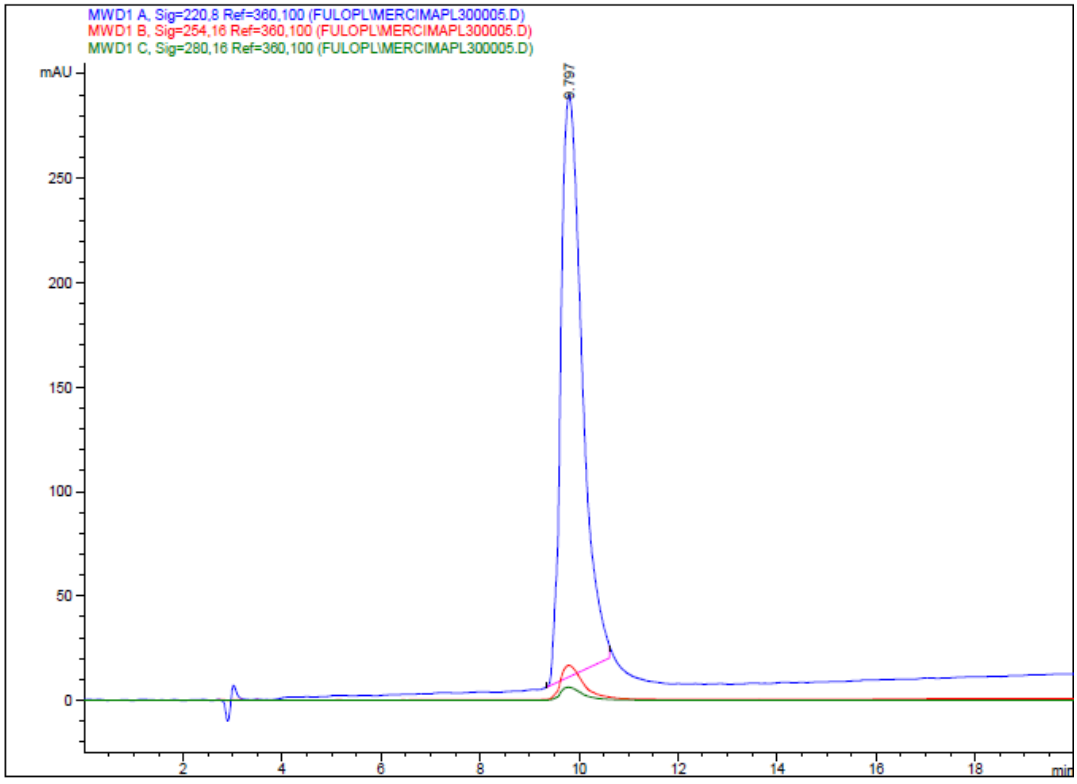




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 30-50% 20min 1.2 mL min^{-1}

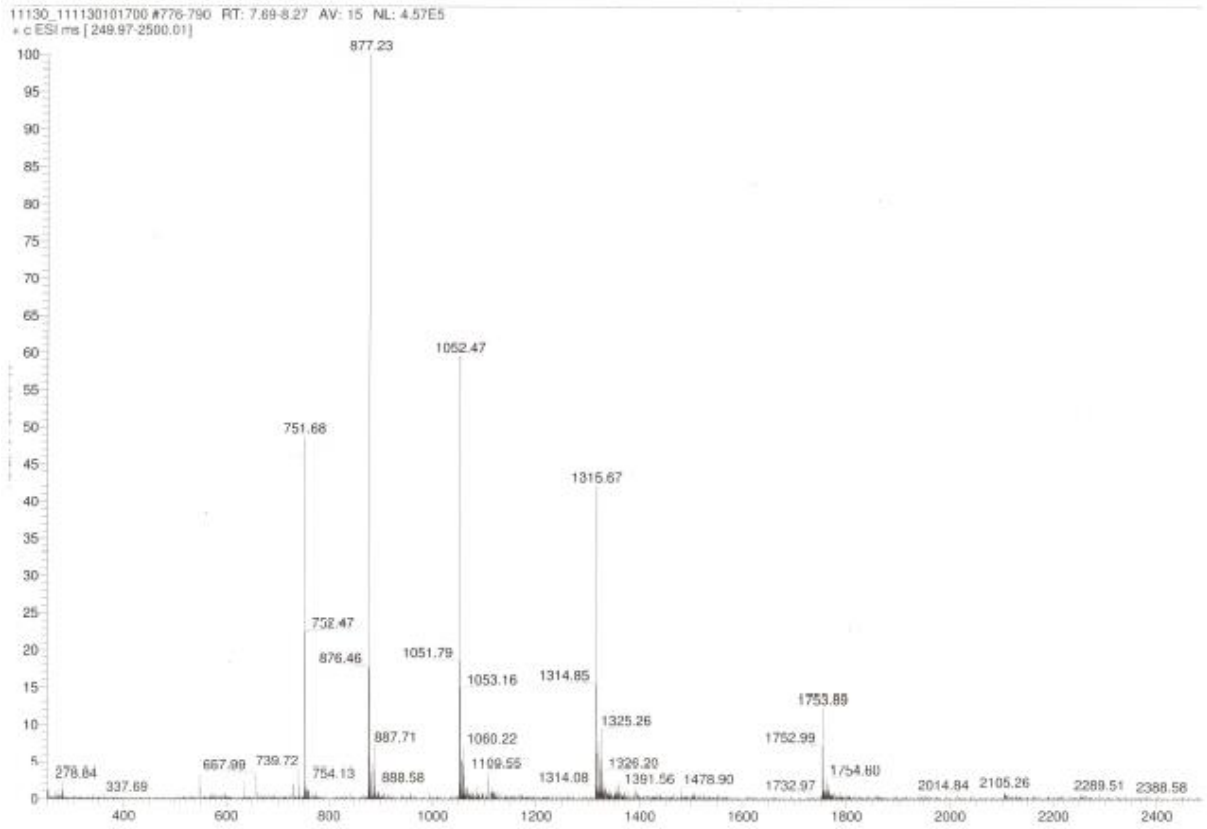
Compound **4**

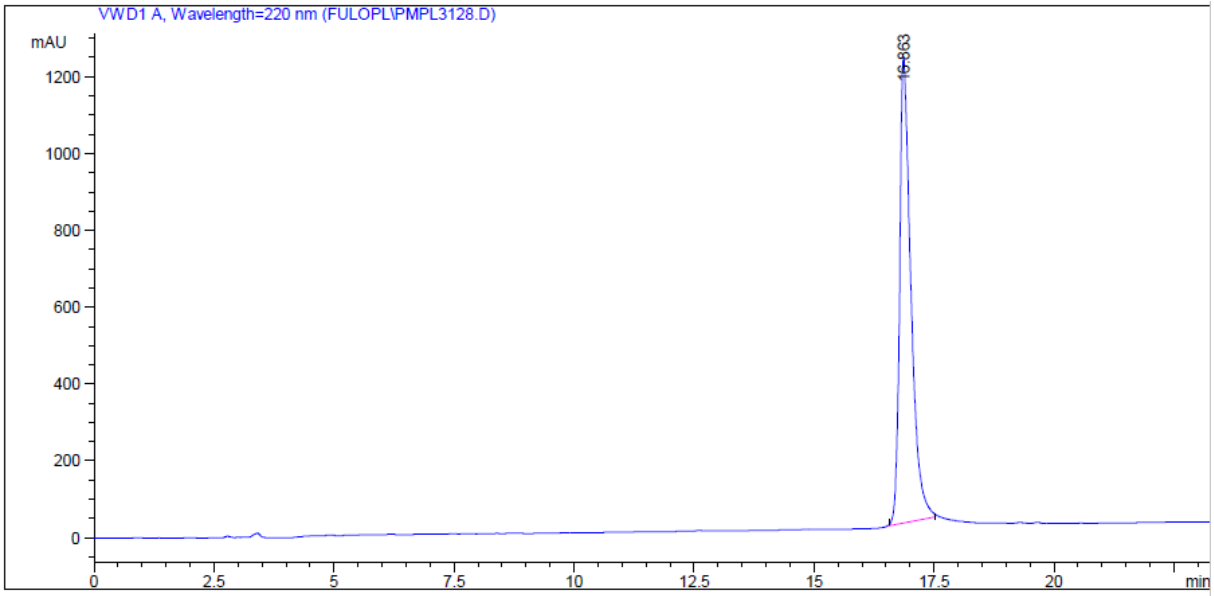




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 35-55% 20min 1.2 mL min^{-1}

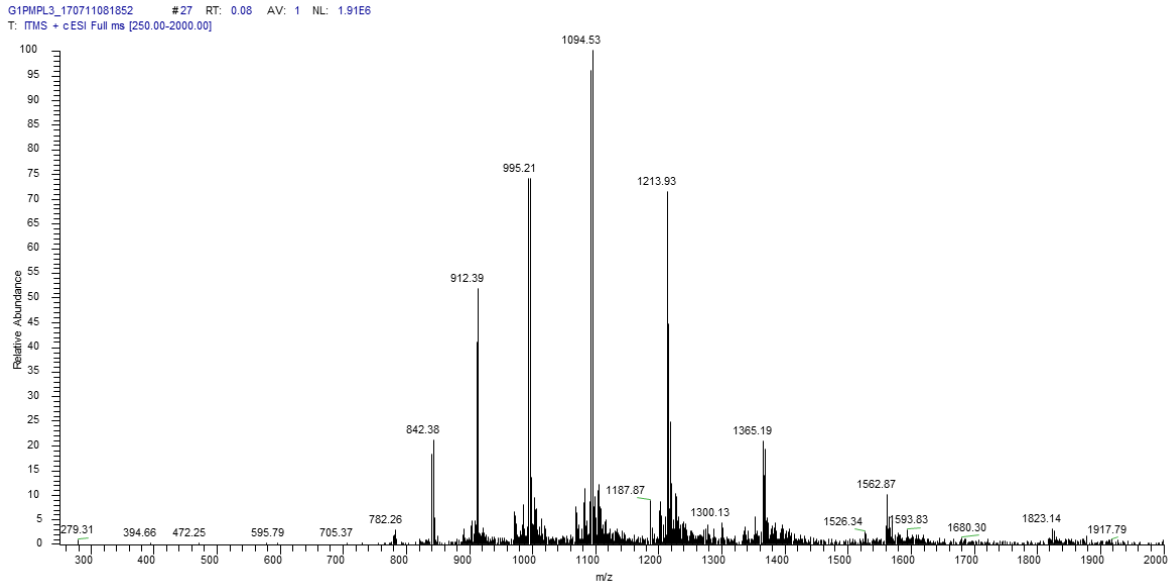
Compound **5**

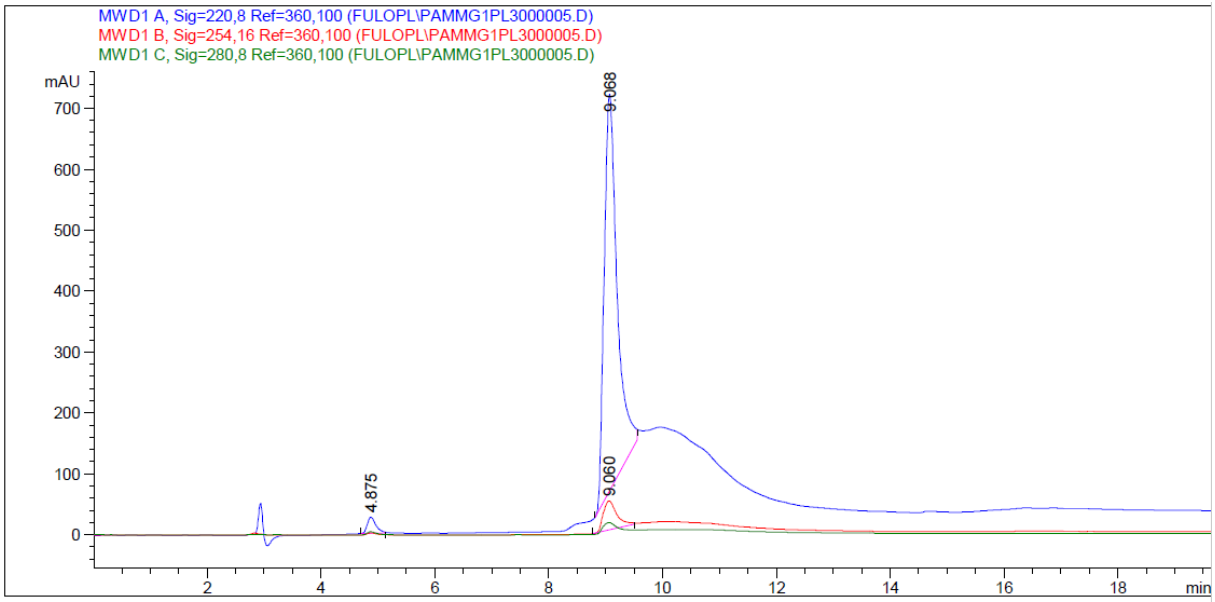




Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 5-80% 25min 1.2 mL min^{-1}

Compound **6**





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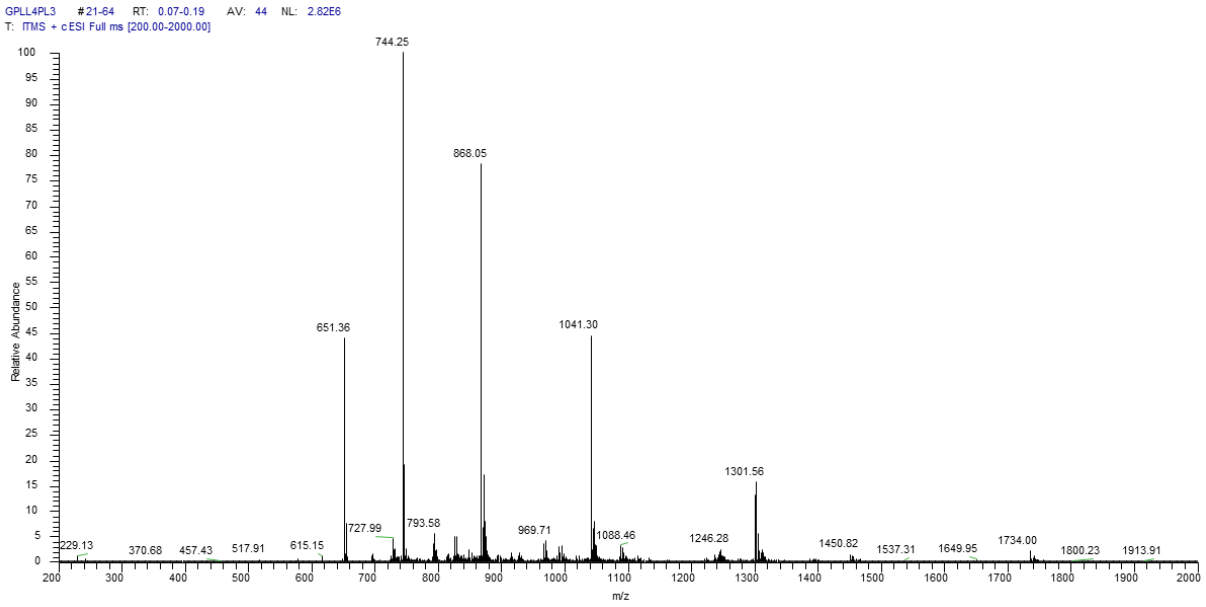
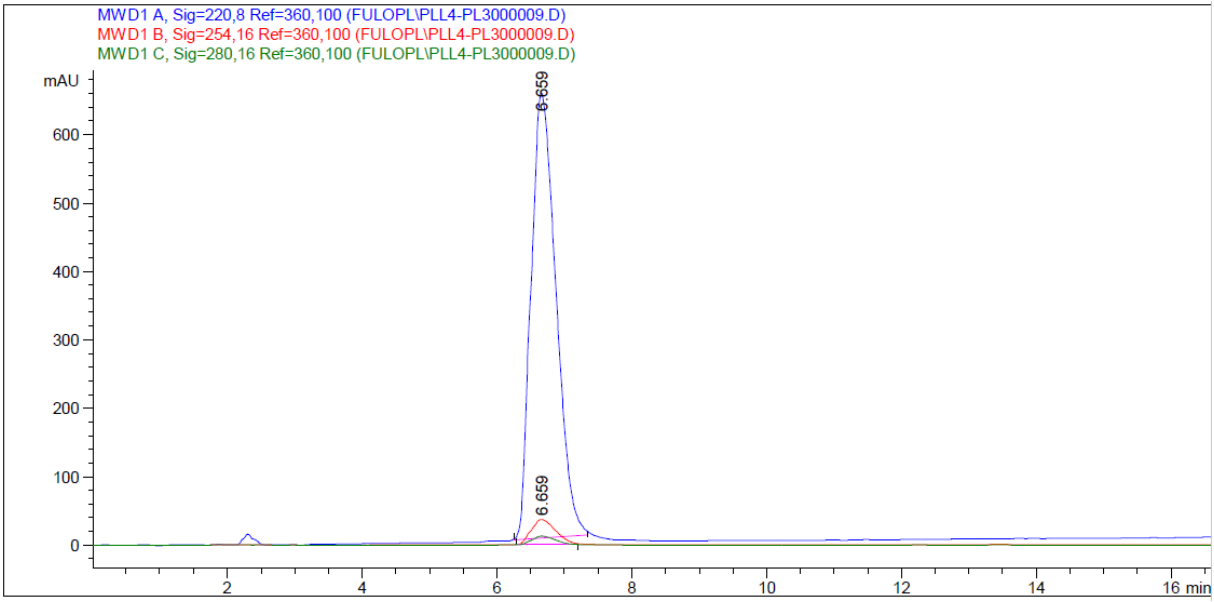
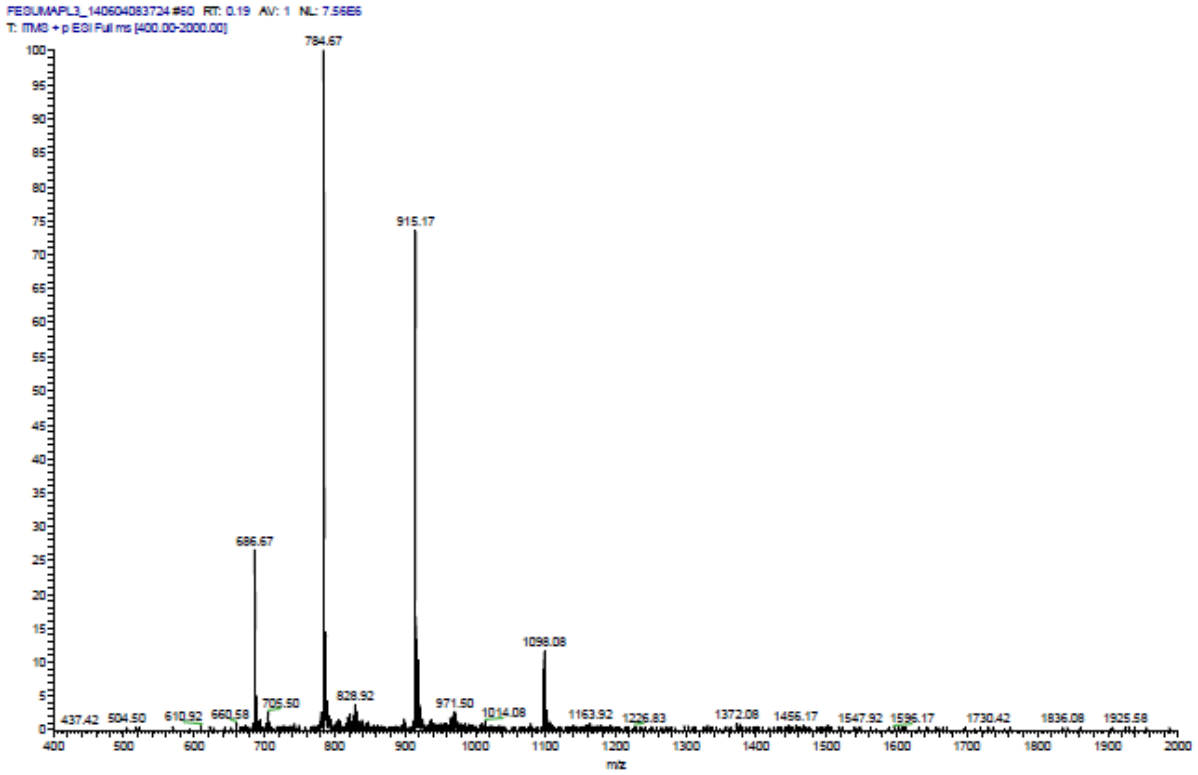
Compound **7**





Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 40-60% 20min 1.2 mL min^{-1}

Compound **8**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915.17
686.67
1098.08
828.92
705.50
971.50
1014.08
437.42
504.50
610.92
660.58
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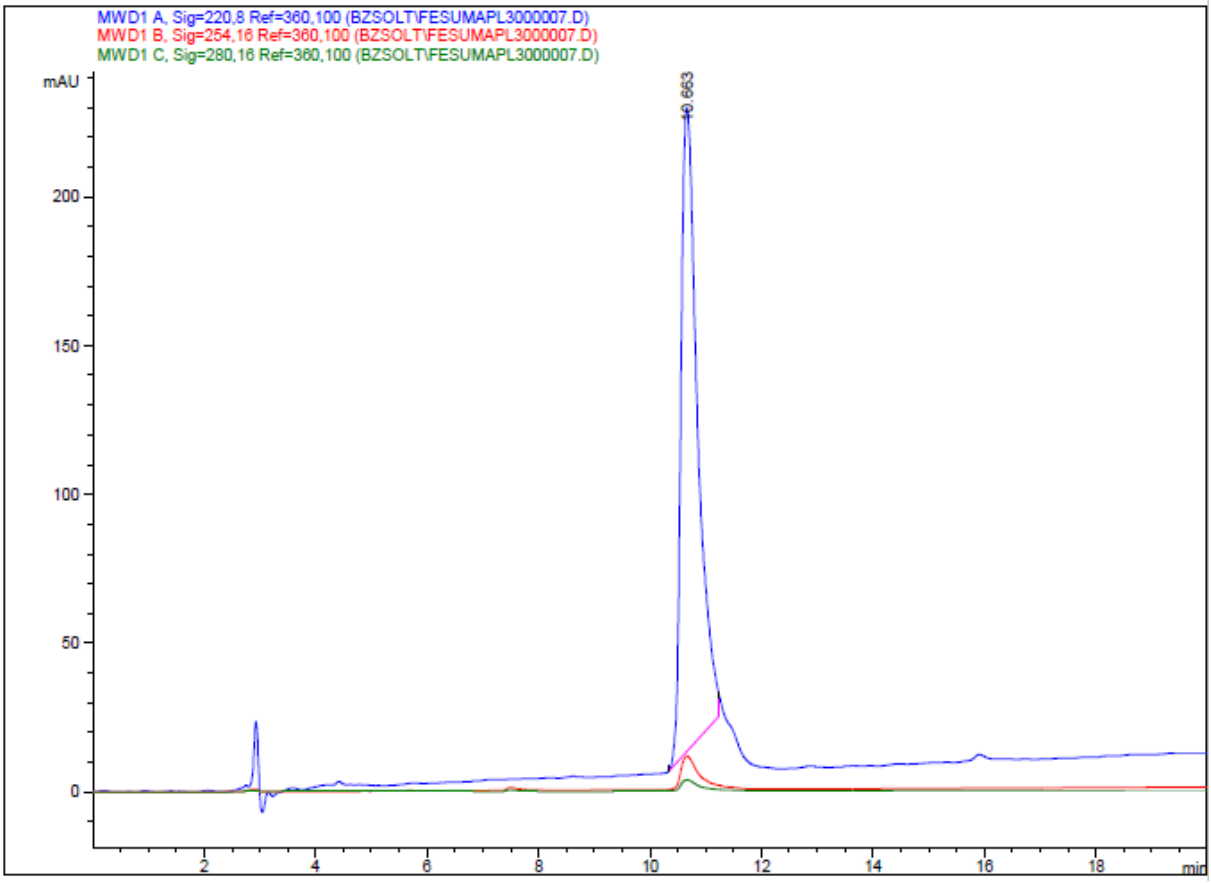
1595.17
1730.42
1836.08
1925.58
m/z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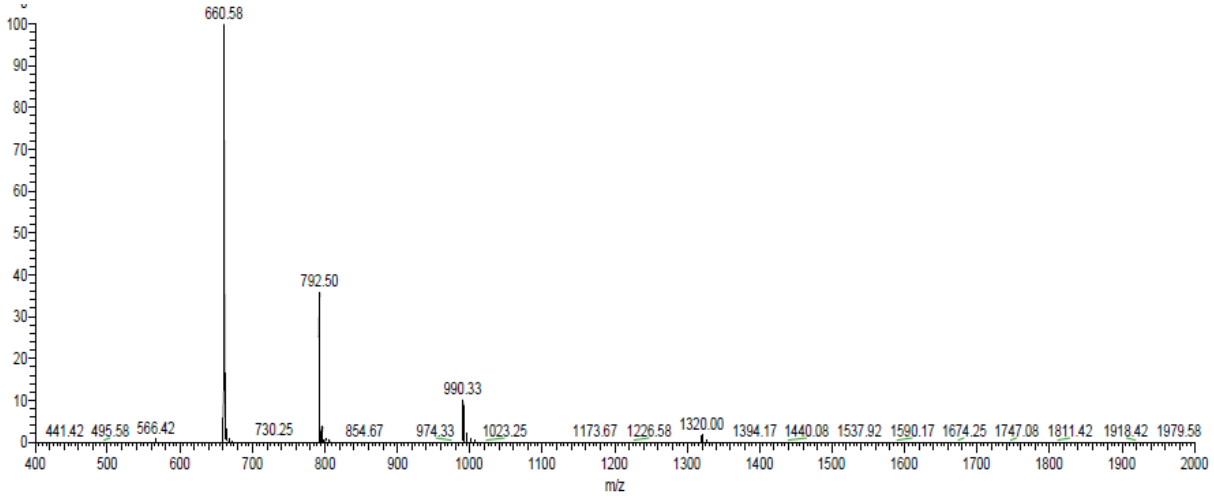
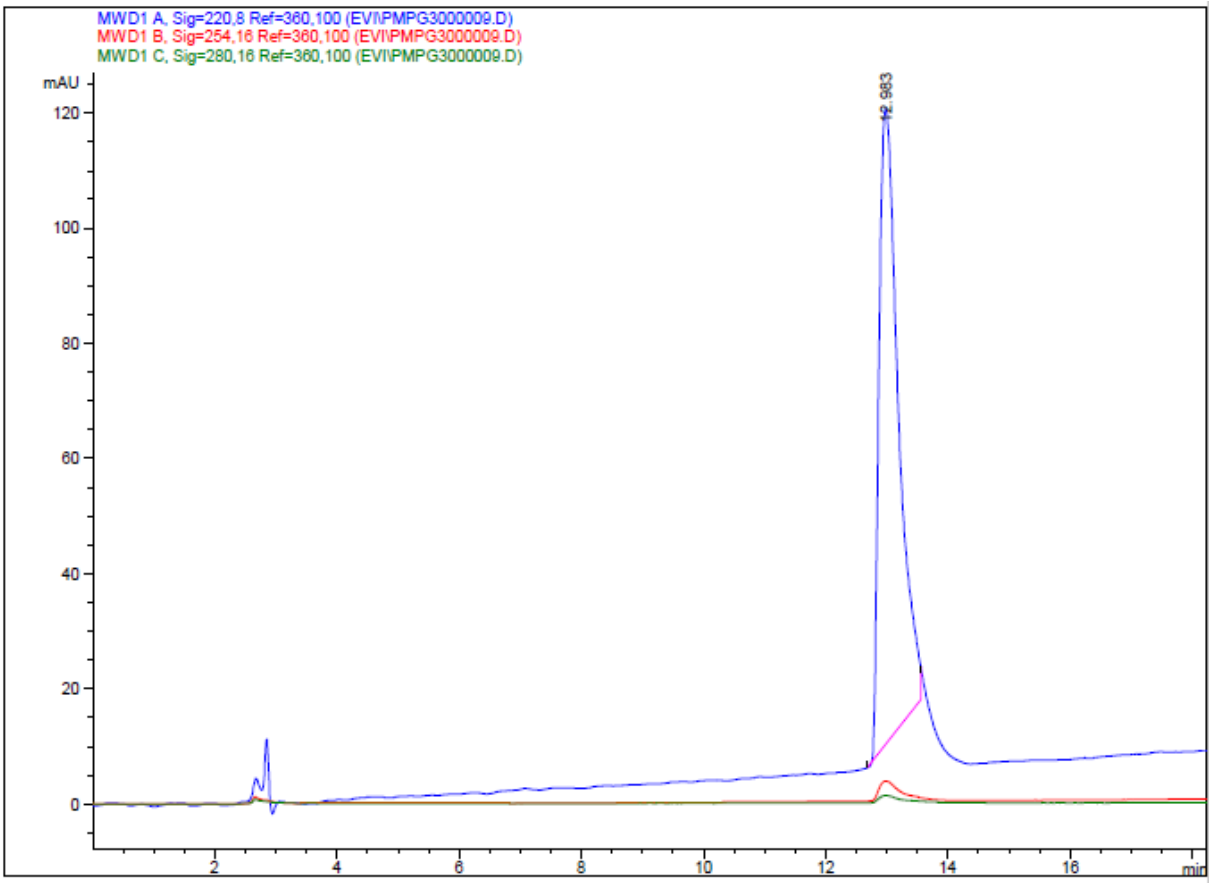
MWD1 B, Sig=254,16 Ref=360,100 (BZSOLT\FESUMAPL3000007.D)
MWD1 C, Sig=280,16 Ref=360,100 (BZSOLT\FESUMAPL3000007.D)
mAU
10.663
min

Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 35-55% 20min 1.2 mL min^{-1}

Compound **9**





Column: Phenomenex Jupiter C4 (250 x 4.6 mm, particle size: 5 micron, pore size: 300Å); Gradient: 45-65% 20min 1.2 mL min^{-1}