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Supporting Information

Peptide Structure Stabilization by Membrane Anchoring and its General Applicability to the Development of Potent Cell-Permeable Inhibitors

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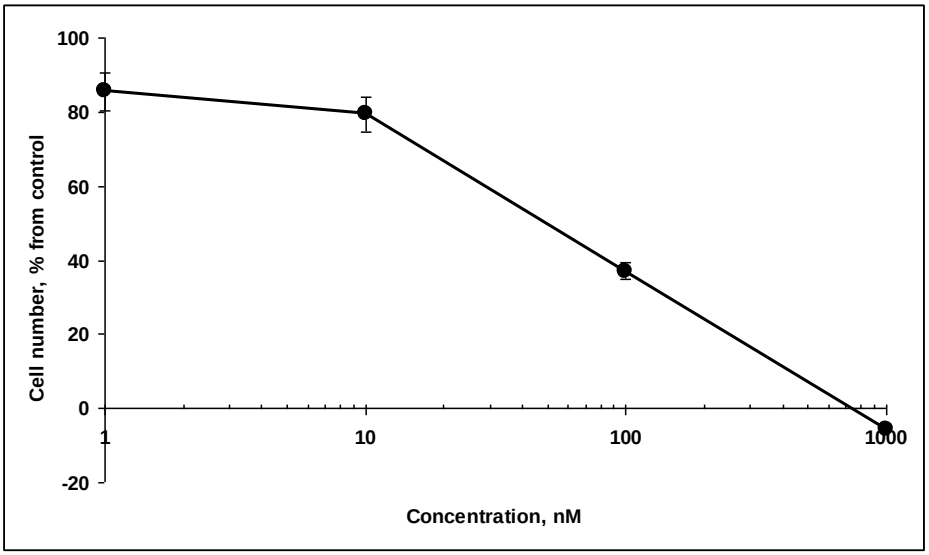
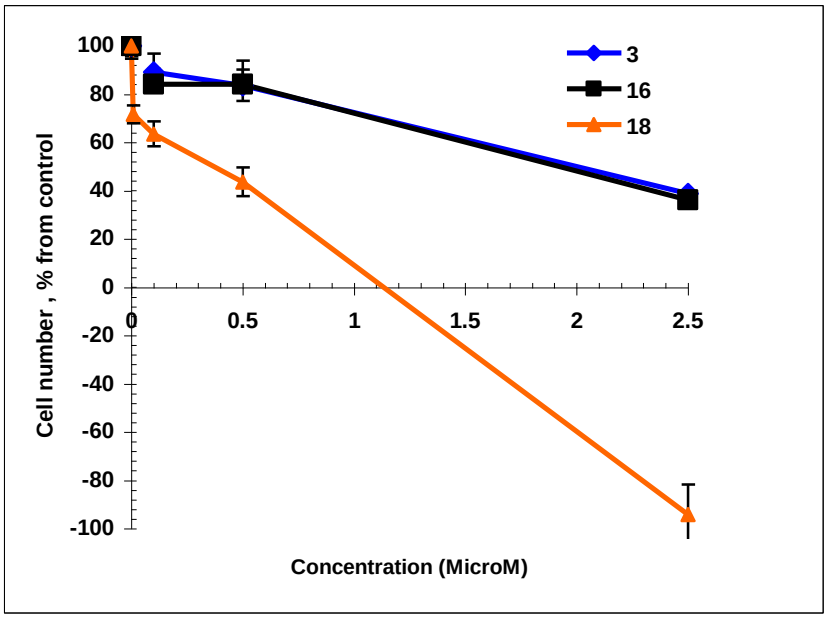
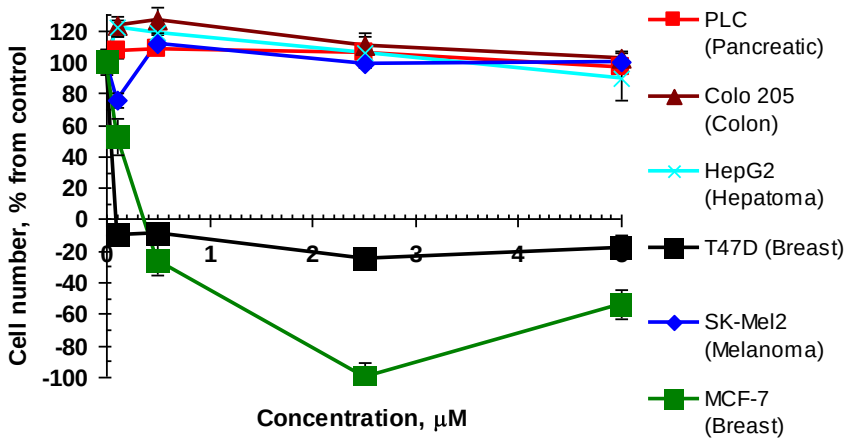


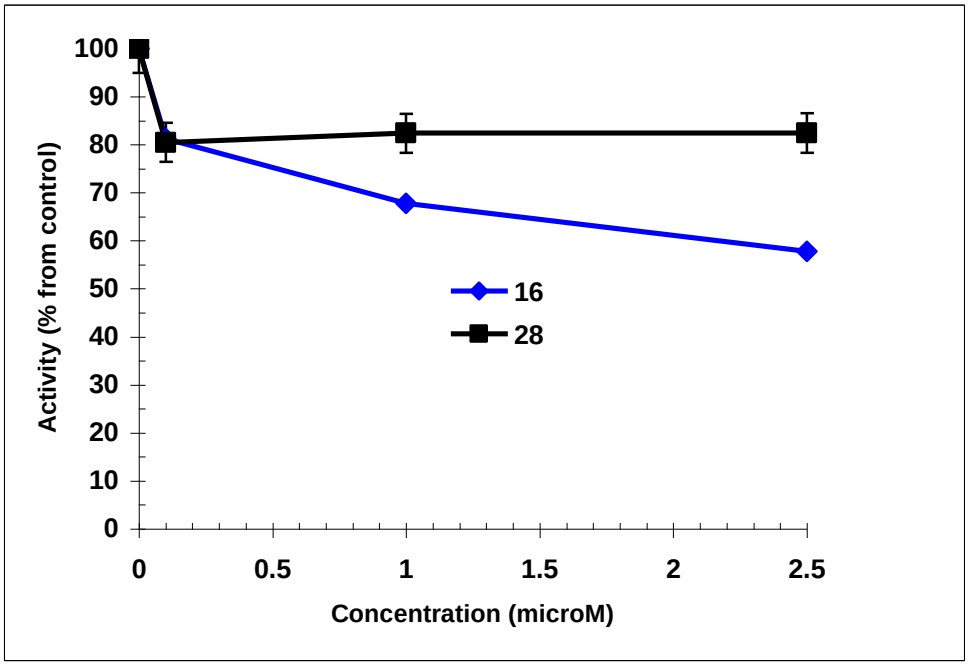
Supplementary Figure S1. Inhibition of SK-Mel2 melanoma cells growth by Hedgehog pathway inhibitor SMOi2-43 (Ac-(ε-Pal)-KLTYAWHTSFKAL-NH2). Cell number was determined with the help of MTT assay after 48 hours exposure to the compound. Negative values in cell number reflect cell death.



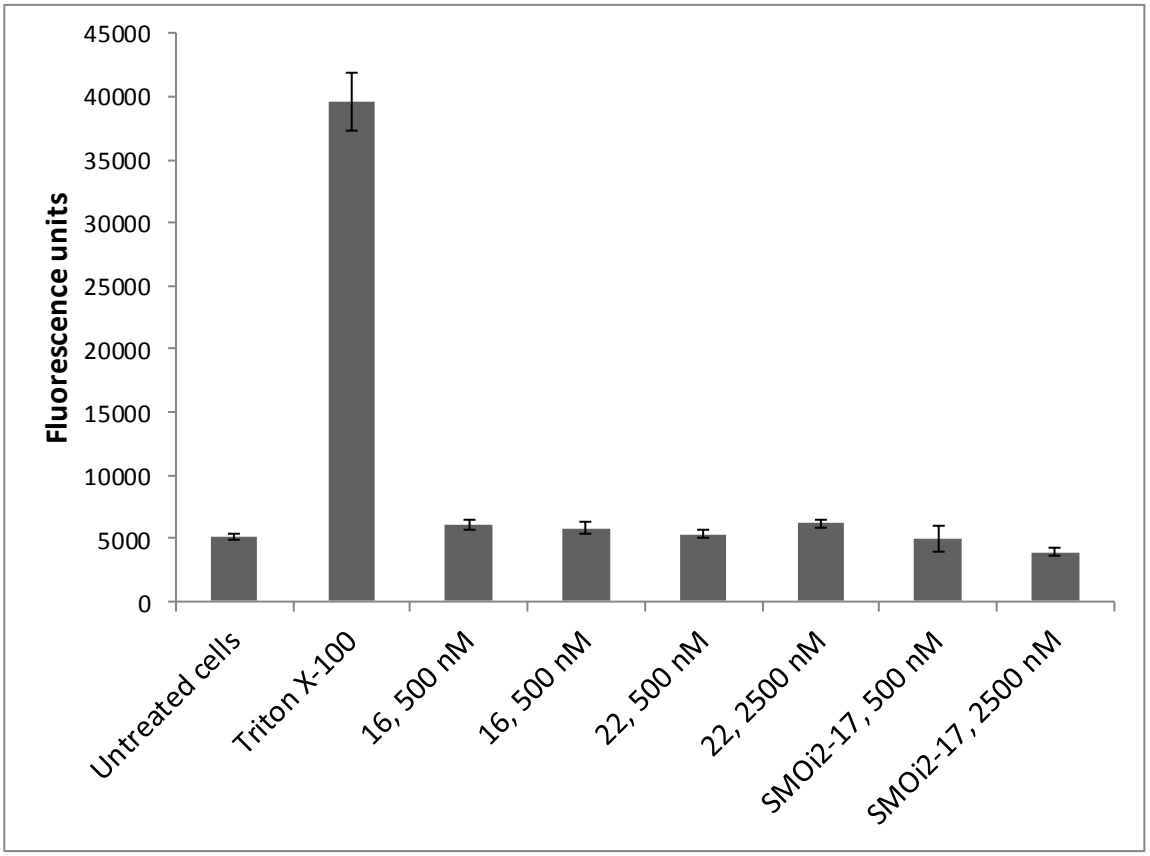
Supplementary Figure S2. Cytotoxic effects of IGF-1R JM analogs on MCF-7 cells grown in serum-free medium and stimulated to grow solely with human recombinant IGF-1 (10 μg/ml). The cells were exposed to compound for 48 hours. Relative number of live cells number was determined with the help of MTT assay



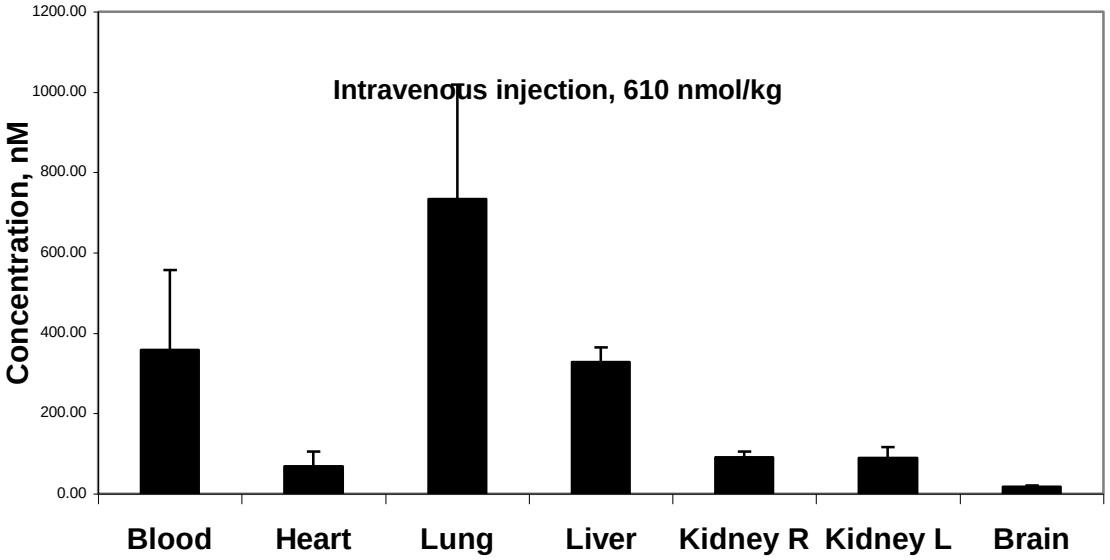
Supplementary Figure S3. Toxicity assay in multiple cancer cell lines showed that sensitivity to IGFR1 JM analog 16 correlated well with IGF1 expression levels. Toxicity was determined by MTT assay after 48 hours exposure to the compound.



Supplementary Supplementary Figure S4. Juxtamembrane domain analogs had low activity in inhibition of recombinant kinase domain of IFG1R in cell-free environment. Kinase activity was assayed with the help of OMNI recombinant kinase assay kit 4 (Biosource) that utilizes fluorogenic peptide substrate phosphorylated by IFG1R kinase. Recombinant human phosphorylated IGF1R kinase was a kind gift from Mrs. Carrie J. Saucedo (Molecular Target Development Program, NCI-Frederick).



Supplementary Figure S5. Lipopeptide inhibitors do not cause cell lysis. Cell membrane integrity was evaluated with the help of Promega CytoTox-ONE^{TM} assay that measures lactate dehydrogenase (LDH) released from cells with damaged membranes. Fluorogenic LDH substrate was added to MCF-7 cells seeded in black 96 wells plates and exposed to indicated concentrations of compounds for 1 hour. After 10 min incubation with the substrate and addition of the stop solution, fluorescence was measured on BMG FluoStar plate reader using 560 nm excitation and 590 nm emission. Presented data is an average of six measurements. Triton X-100 was used as positive control that causes complete release of intracellular LDH.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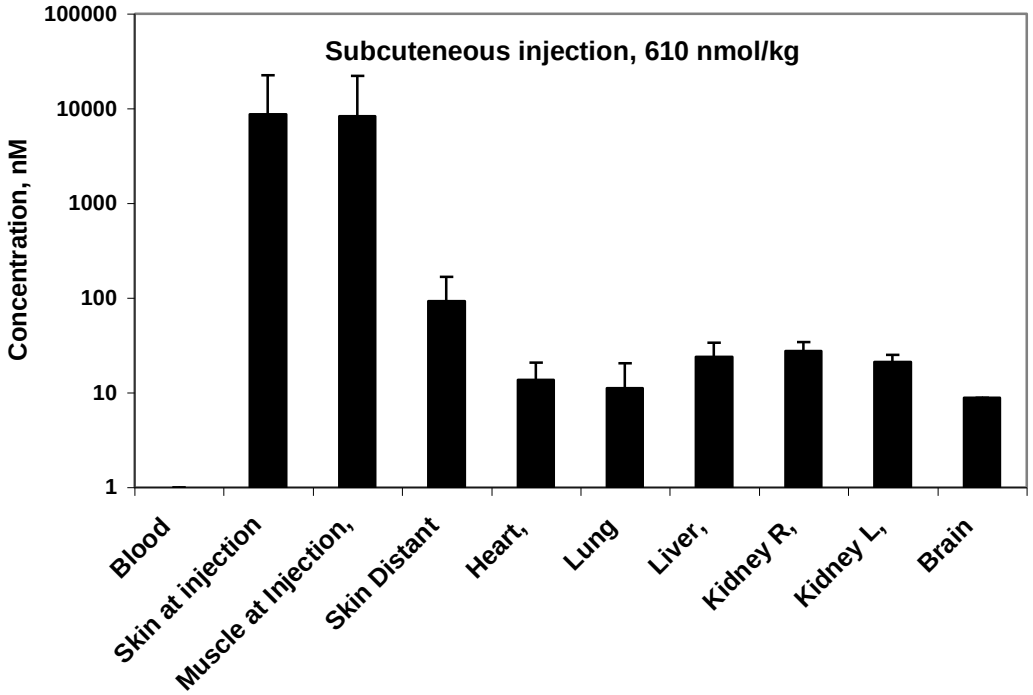
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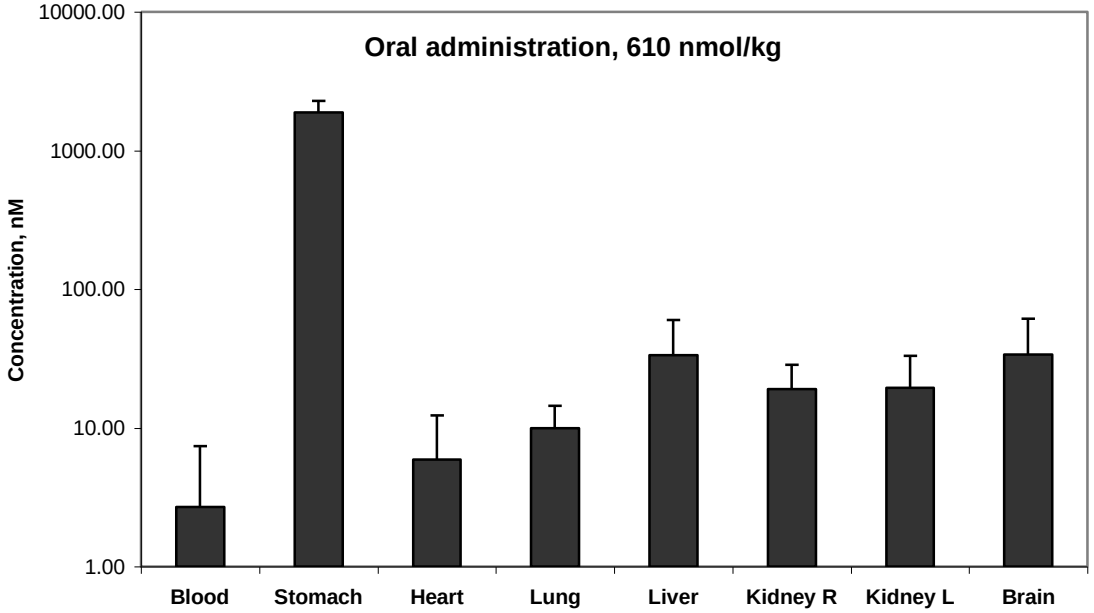

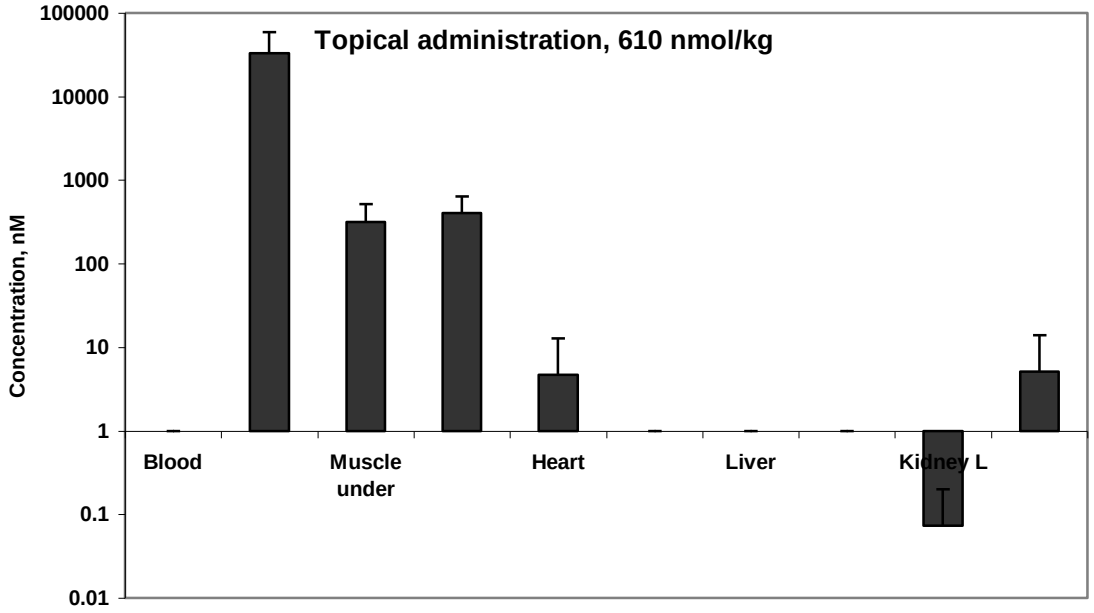

Supplementary Figure S6. Biodistribution of Hedgehog lipopeptide antagonist SMOi2-17 in mice. Distribution was evaluated with the help of ^{3}H-labeled compound upon injections of 2μCi per animal or 1.1 mg/kg (610 nmol/kg). The tritiated compound was generated by AmbiosLabs

by catalytic substitution of protons of hydrocarbon chains of the peptide (http://ambioslabs.com/label.php). Female wild type 8–12-wk-old BALB/c mice were provided by the Animal Production Area of the National Cancer Institute (NCI). Mice were sacrificed 30 min after administration. Organs were collected, tissue was homogenized with razor blades and radioactivity counted in scintillation counter. The data is an average for samples collected from groups of three animals.

NCI-Frederick is accredited by the American Association for the Accreditation of Laboratory Animal Care International and follows the Public Health Service Policy for the Care and Use of Laboratory Animals. Animal care was provided in accordance with the procedures outlined in the *Guide for Care and Use of Laboratory Animals* (National Research Council; 1996; National Academy Press; Washington, D.C.).

Table S1. Analytical data for compounds synthesized*.

Compound	Sequence	Calculated Molecular Weight	Actual Molecular Weight
1	Pal-HRKRNNSRLGNG-NH_2	1645.9	1645.8
2	Pal-HRKRNNSRLG-NH_2	1474.8	1474.6
3	Pal-HRKRNNSRLGNGVLYASVN-NH_2	2392.8	2392.5
4	Pal-HRKRNNSRLGNGVLYASVNPEYFSAA-NH_2	3158.6	3158.2
5	Pal-HRKRNNSRLGNGVLYASV-NH_2	2278.7	2277.8
6	Pal-VHRKRNNSRLGNGVLYASV-NH_2	2377.8	2378.0
7	Pal-HRKRNNSRLGNGVLYAS-NH_2	2179.6	2178.8
8	Pal-HRKRNNSRLGNGVLYA-NH_2	2092.5	2091.6
9	Pal-HRKRNNSRLGNGVLY-NH_2	2021.4	2021.6
10	Pal-HRKRNNSRLGNGVL-NH_2	1858.3	1858.2
11	Pal-HRKRNNSRLGNGV-NH_2	1746.1	1744.4
12	Pal-RKRNNSRLGNGVLYASVN-NH_2	2255.7	2255.2
13	Pal-KRNNSRLGNGVLYASVN-NH_2	2099.5	2100.8
14	Pal-RNNSRLGNGVLYASVN-NH_2	1971.3	1970.6
15	Pal-NNSRLGNGVLY-NH_2	1443.7	1443.2
16	Pal-RNNSRLGNGVLY-NH_2	1599.9	1599.4
17	Pal-NSRLGNGVLY-NH_2	1329.6	1329.2
18	Ac-YLVGNGLRSNNK-(ε-Pal)[All D]	1770.1	1770.4
22	Pal-YLVGNGLRSNNR-NH_2	1599.6	1599.4
23	Pal-HRKRNNSRLGNGVLYASVNP-NH_2	2489.1	2489.2
24	Pal-VFHRKRNNSRLGNGVLYASVN-NH_2	2639.1	2639.0
27	Pal-KRNNSRLGNGVLY-NH_2	1728.1	1727.4
IR-2	Pal-RQPDGPLGPLY-NH_2	1449.8	1450.0
SMOi2-43	Ac-(ε-Pal)-KLTYAWHTSFKAL-NH2	1845.2	1845.2
SMOi2-9	Ac-LTYAWHTSFKAL-NH2	1477.6	1477.3
SMOi2-27	Pal-C(Rhod)LTYAWHTSFKAL-NH2	2259.0	2258.8

*Molecular weight was determined by ion-spray mass spectrometry utilizing Agielent 1100 LC/MS

system.

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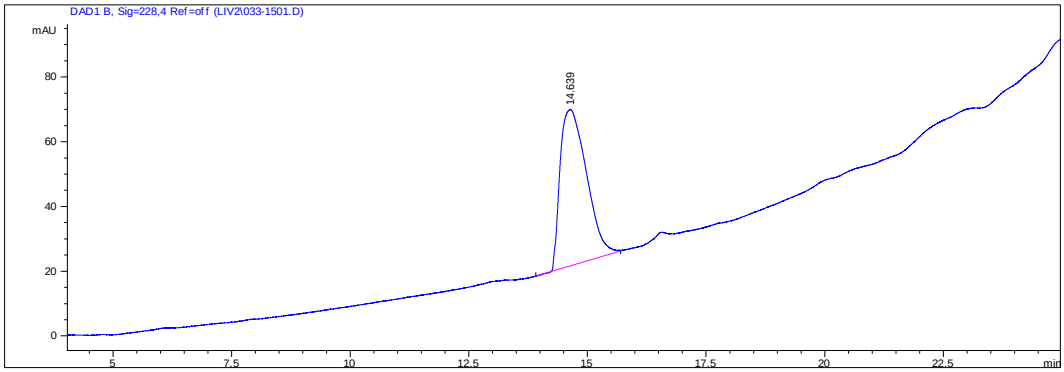
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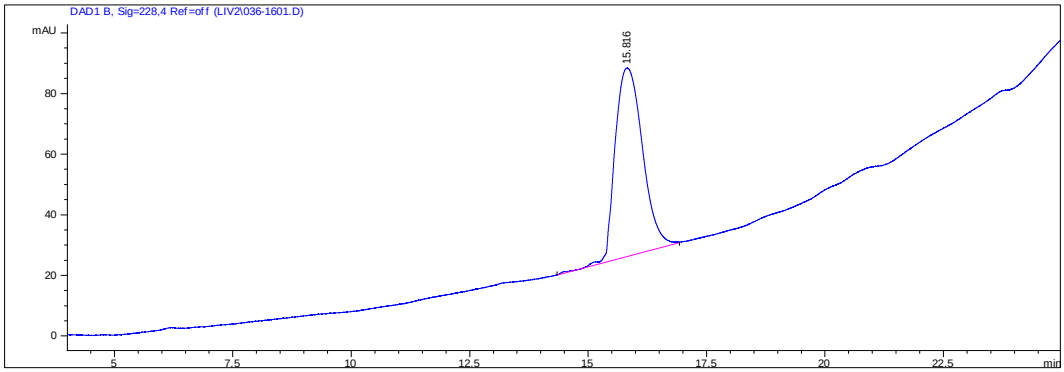
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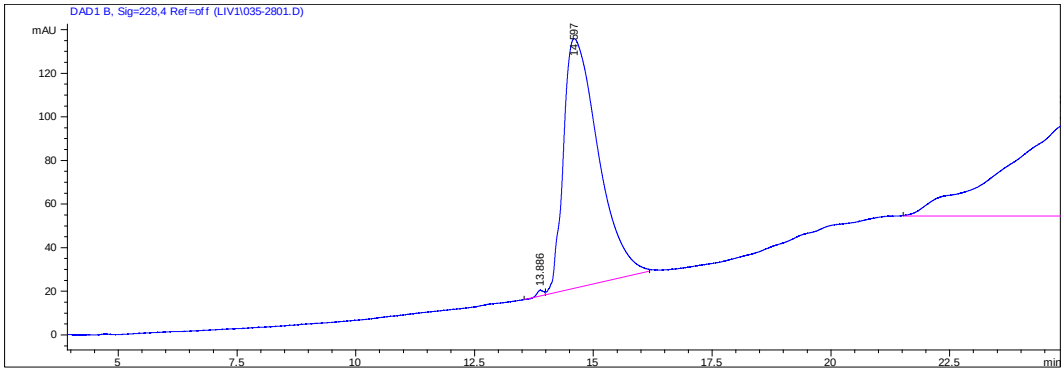
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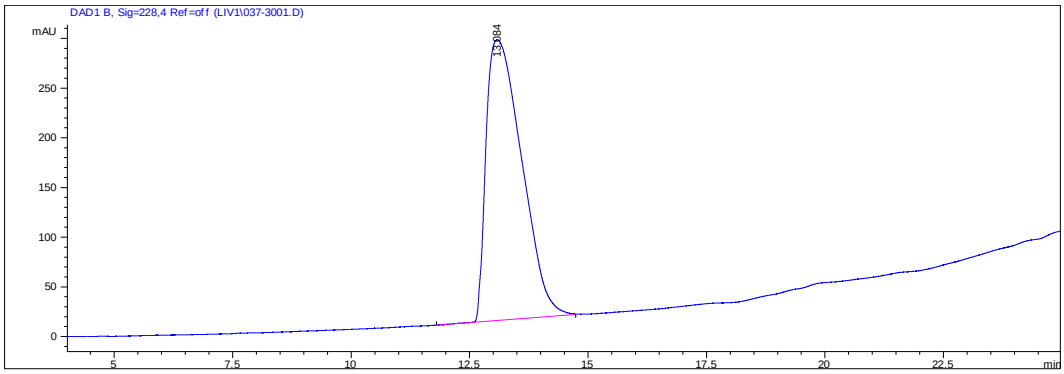
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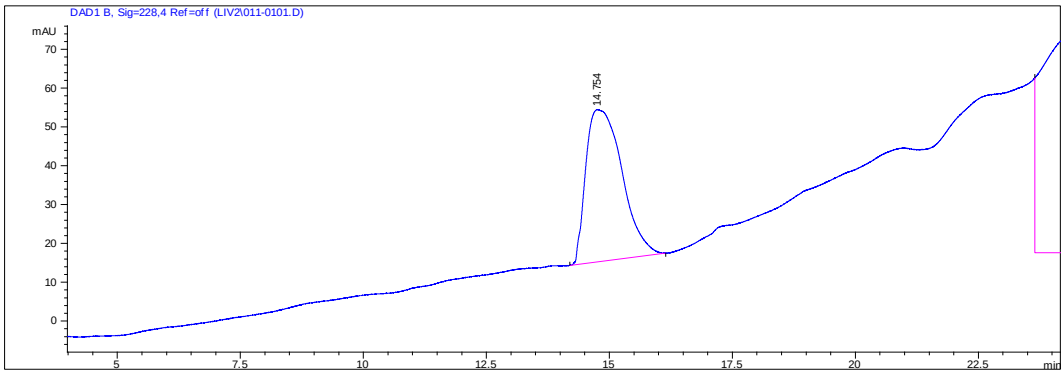
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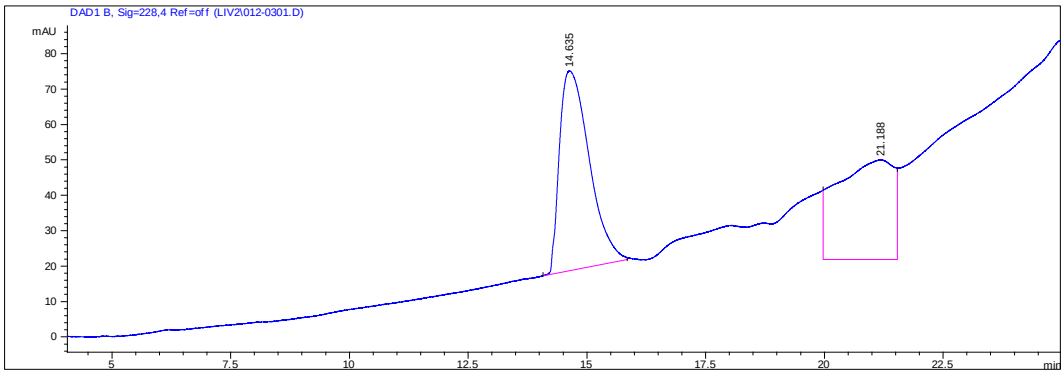
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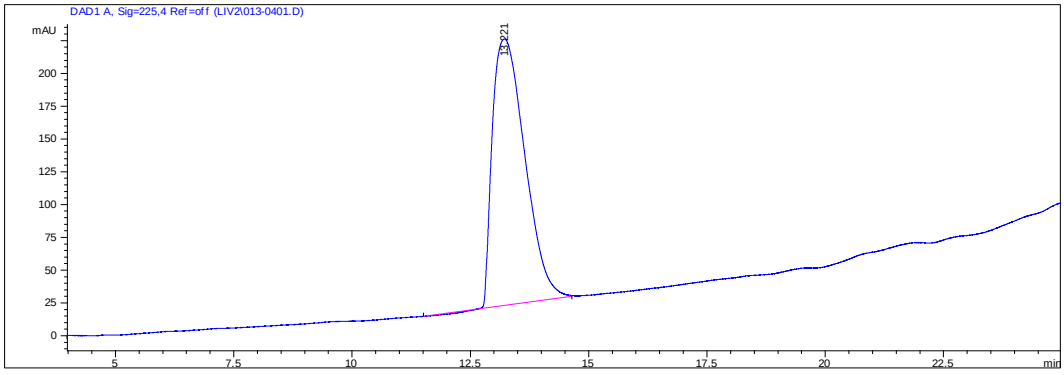
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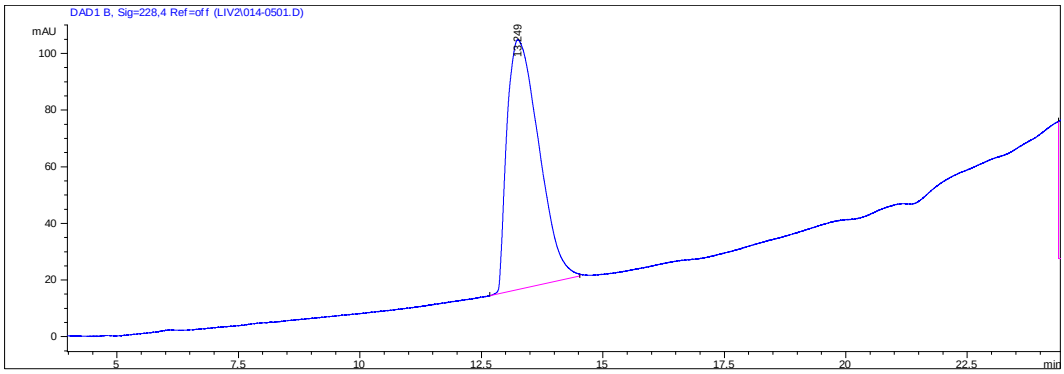
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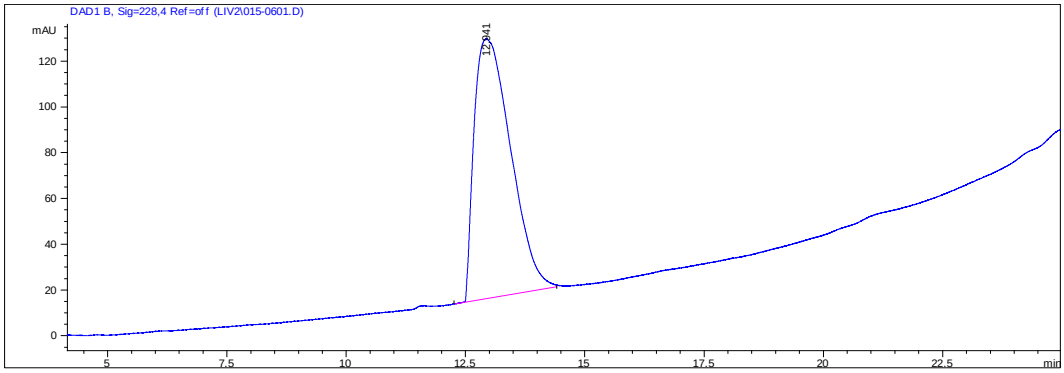
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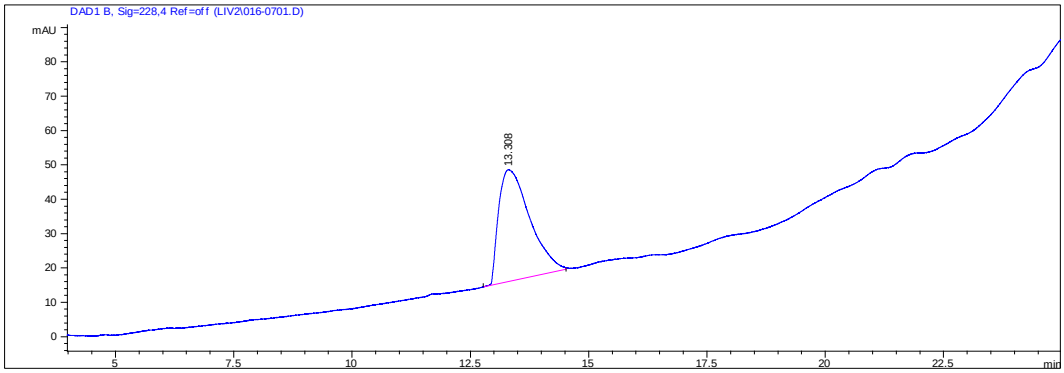
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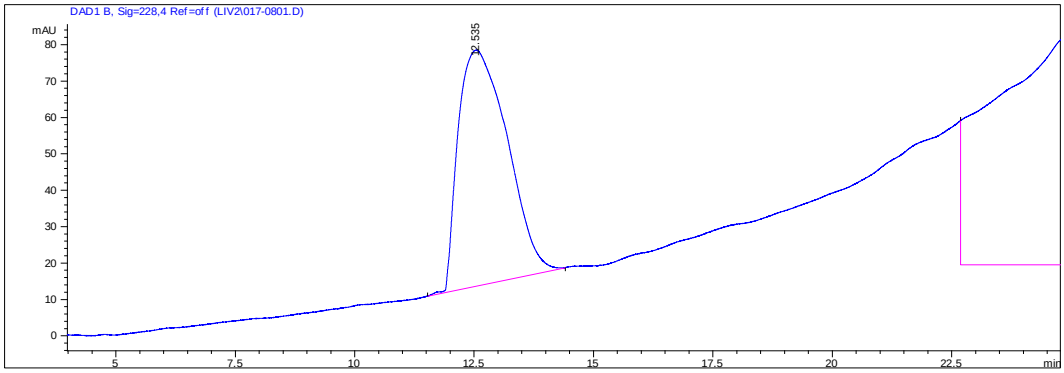
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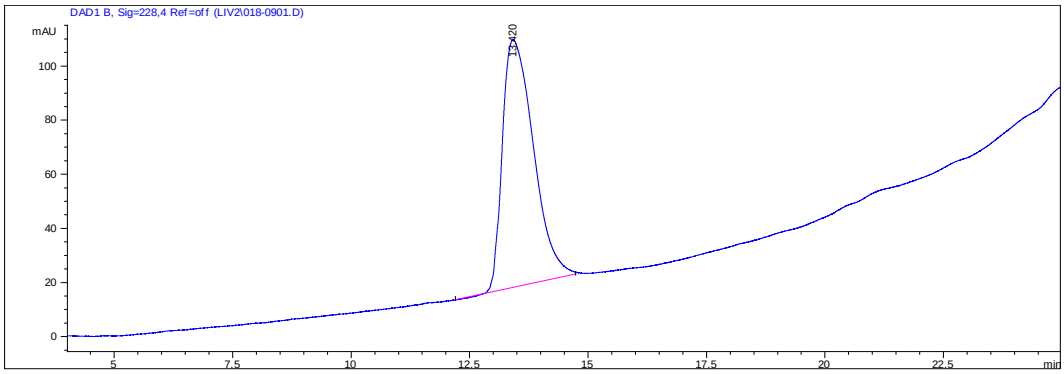
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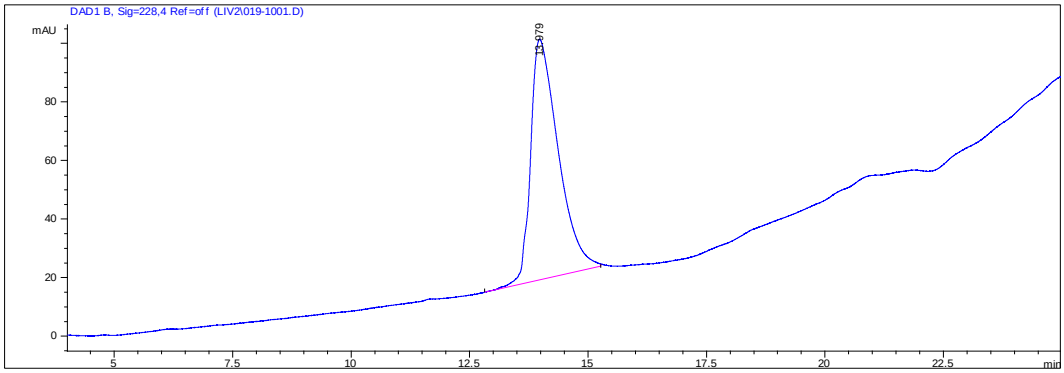
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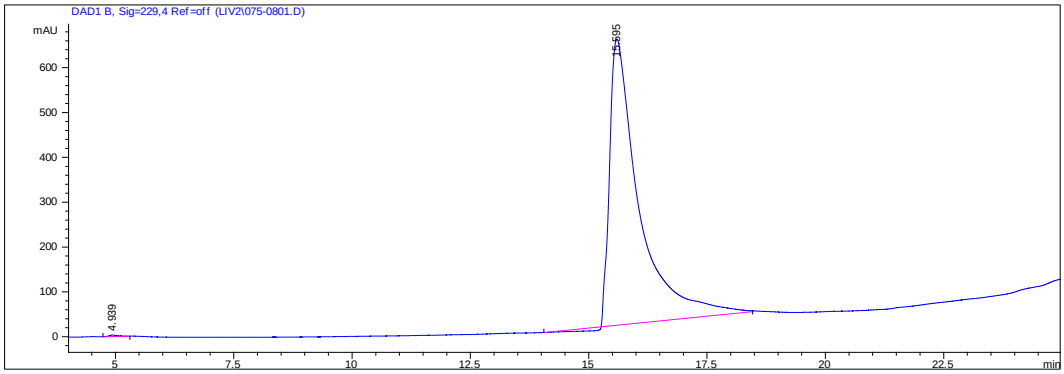
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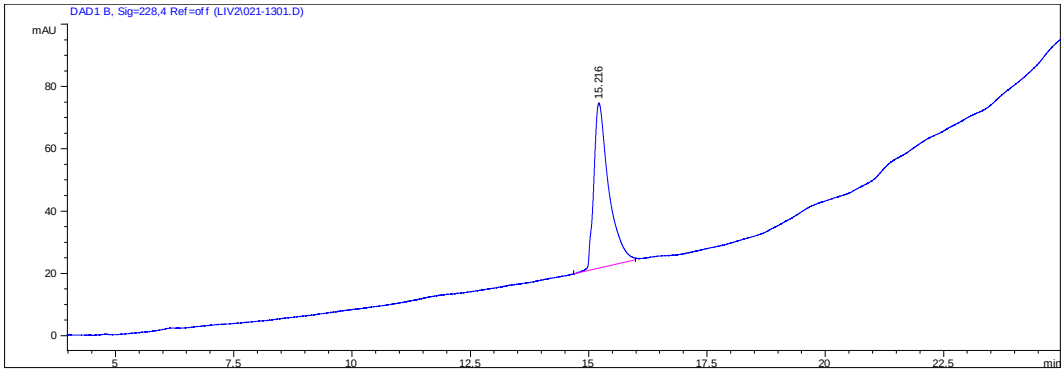
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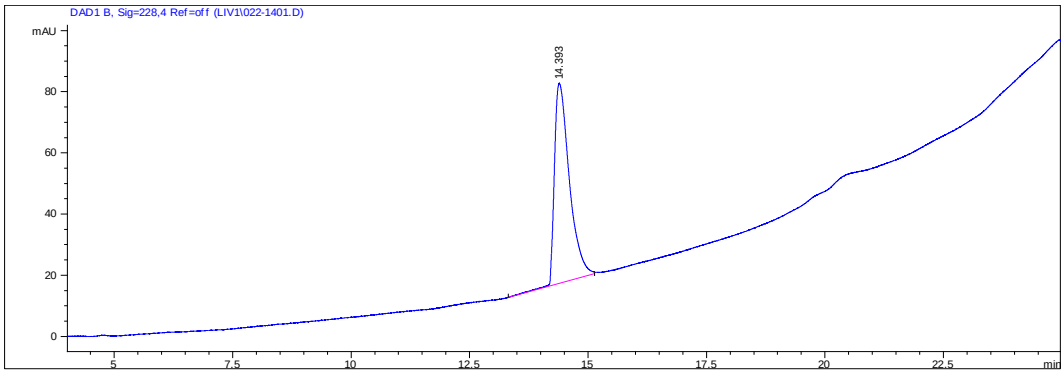
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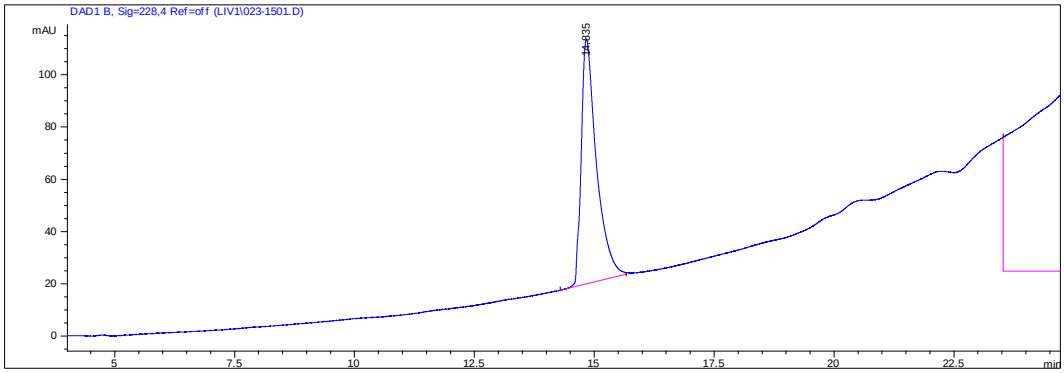
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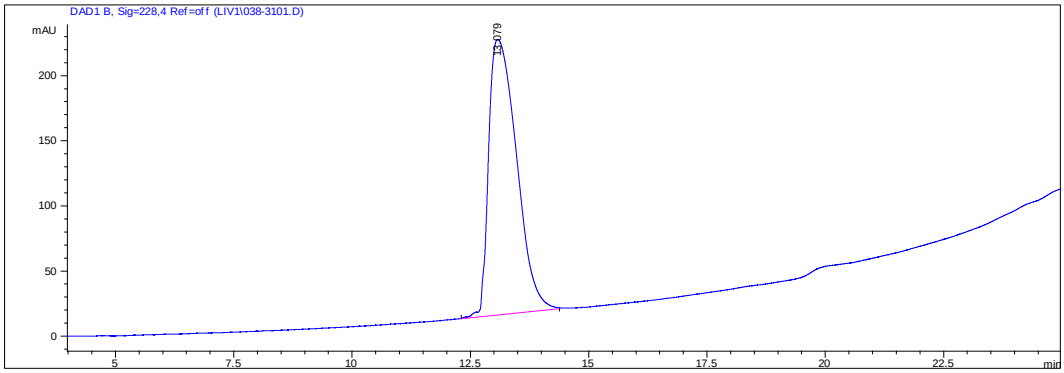
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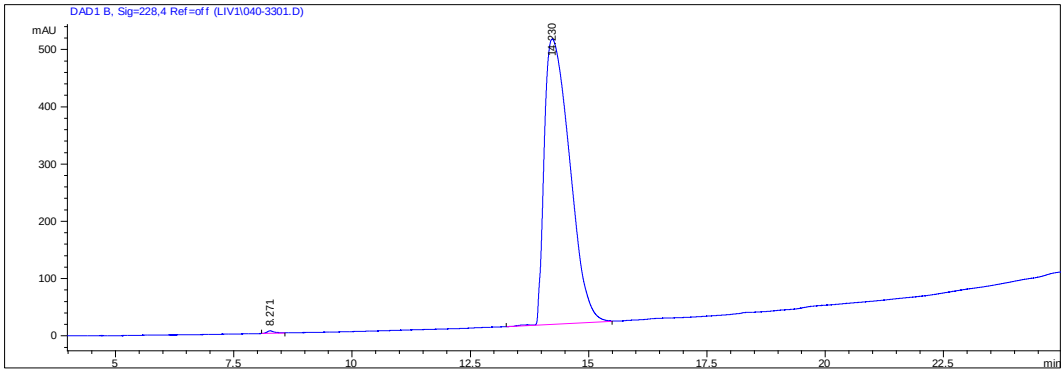
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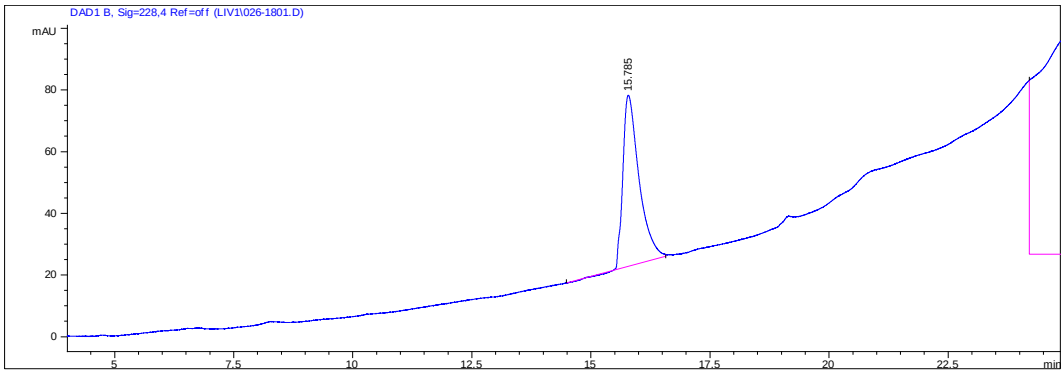
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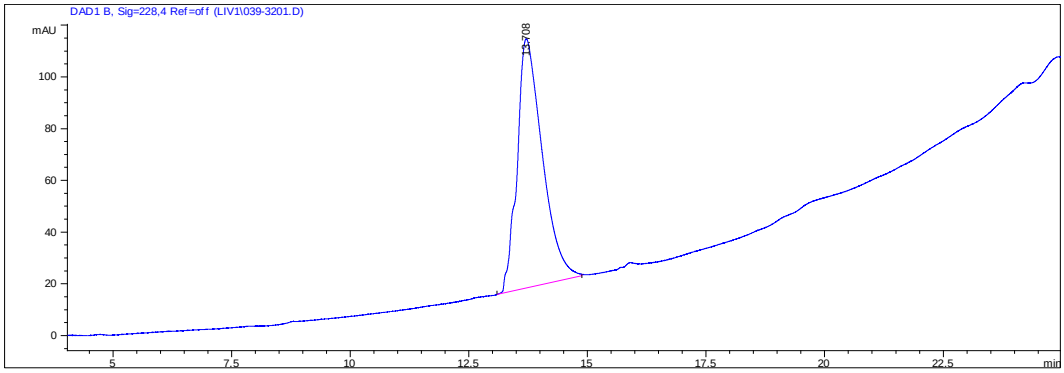
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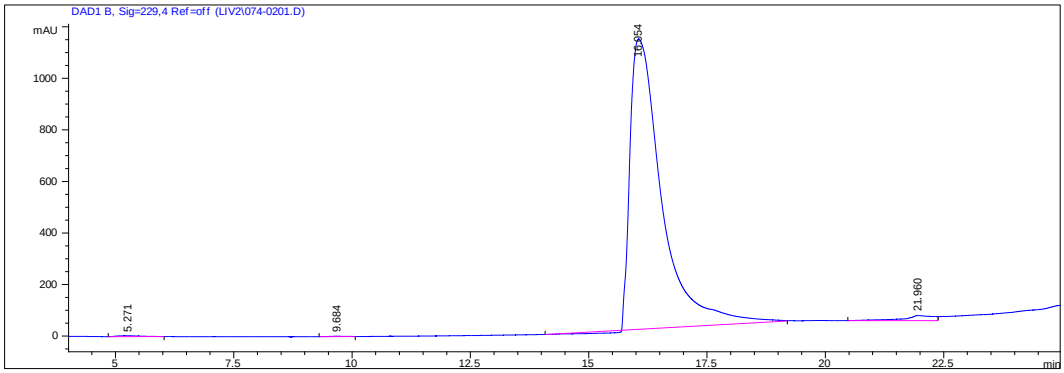
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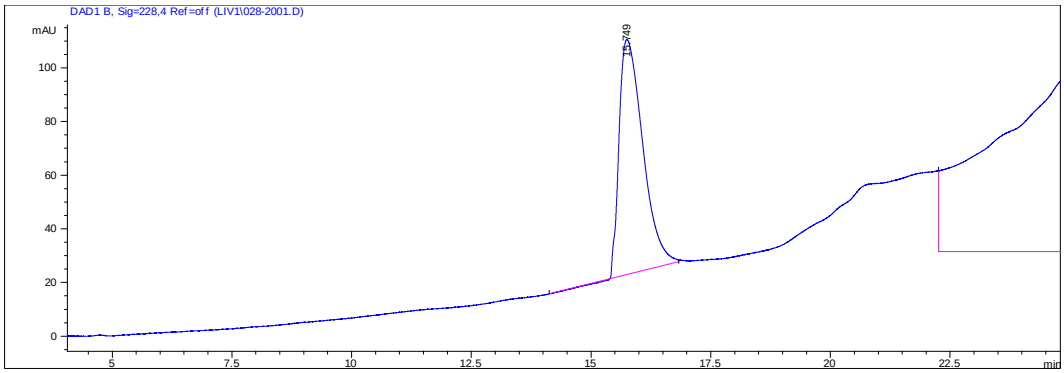
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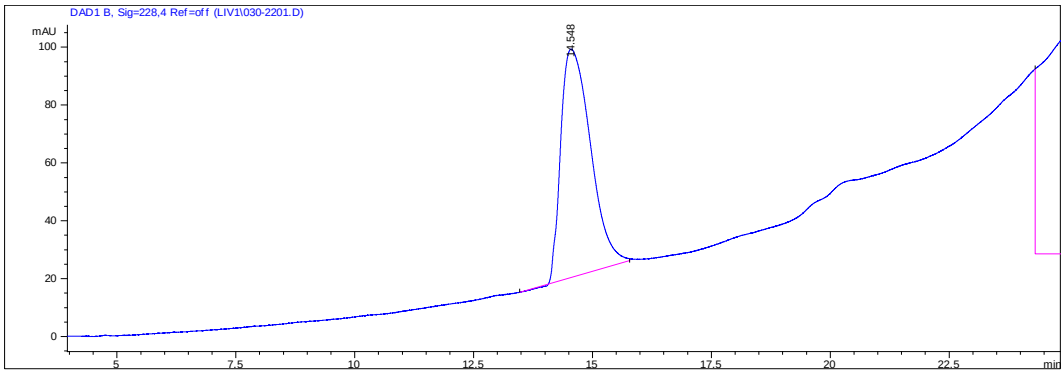
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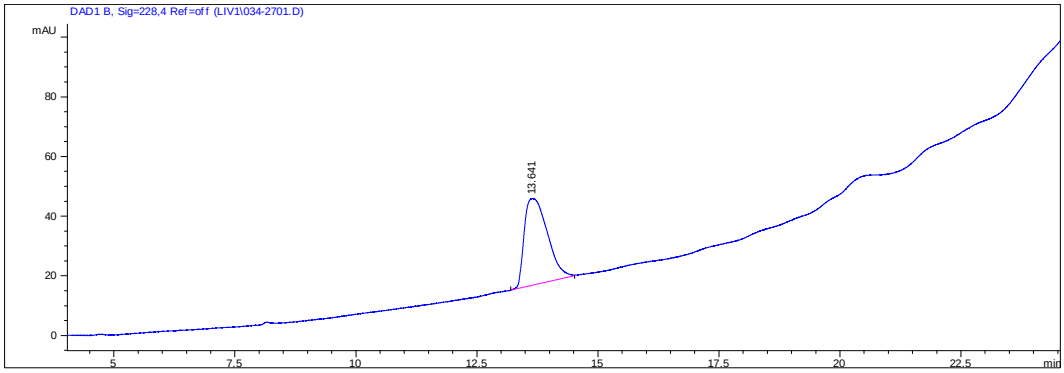
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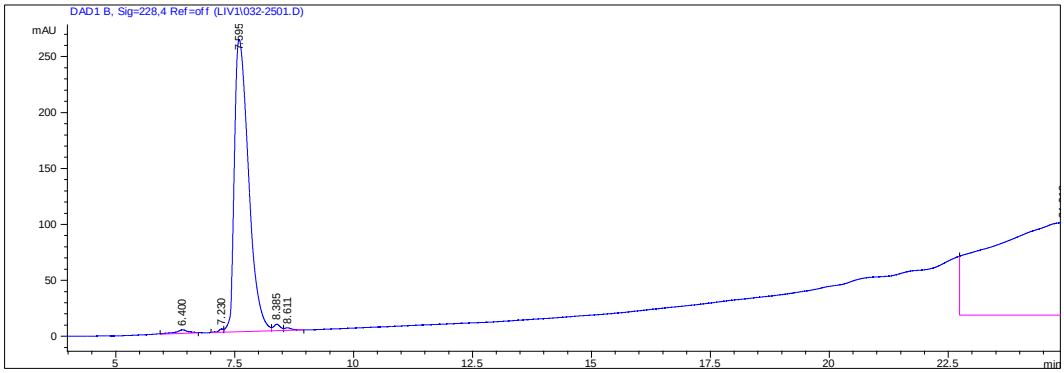
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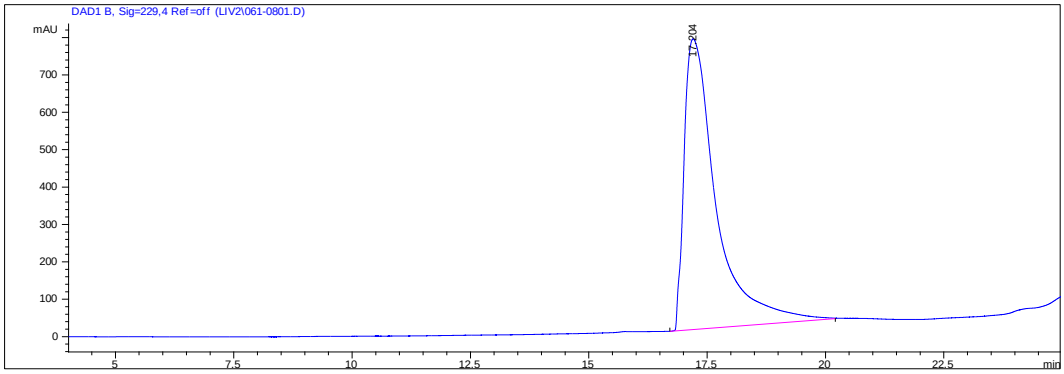
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Figure S7. **HPLC analysis of the key compounds**. Chromatography was performed on Microsorb-MW 300A C8 column (Varian) in 0-100% 20 min gradient of 0.1 % (v/v) trifluoroacetic acid in water/acetonitrile containing 0.1% trifluoroacetic acid, flow rate 1 ml/min. Peptides were detected by UV monitoring at 228, 256 and 280 nm.