

# Supplementary Material

## **Characterization of the Second-Generation Covalent Fragment Library (CovLib Gen2): Thiol Reactivity Profiling and p53-Y220C Rescue**

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## Content

1	Purity Data.....	3
1.1	HPLC Method.....	3
1.2	Nuclear magnetic resonance (NMR) Method .....	3
1.3	Results of the Purity Determination.....	4
1.4	HPLC traces and NMR spectra.....	5
2	Calculated Molecular Properties.....	46
3	Turbidimetric Solubility Assessment .....	49
3.1	Spectra.....	49
4	DTNB Assay .....	75
4.1	Fit Results.....	75
4.2	Plots from the DTNB Assay .....	77
5	GSH Assay .....	84
5.1	Plots from the GSH Assay.....	84
6	Protein Sequences .....	91
7	DSF measurements.....	92
7.1	Results of the primary screening.....	92
7.2	Time Dependent DSF Measurements .....	94
7.3	Concentration Dependent DSF Measurements.....	95

## 1 Purity Data

### 1.1 HPLC Method

Purity was confirmed by high-performance liquid chromatography (HPLC) on an Ultimate 3000 HPLC- System with UV Detection (Thermo Fisher Scientific, Dreieich, Germany). The system was equipped with a ReproSil-XR 120 C18, 5  $\mu\text{m}$ , 150 mm x 4.6 mm column (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) with column temperature set to 25  $^{\circ}\text{C}$ . Mobile phase A was 10 mM phosphate buffer pH 2.3 and mobile phase B was MeOH. The flow rate was set to 0.5 mL per min with the following gradient: 0– 9 min: 10-85% B, 9–13 min: 85% B, 13–14 min: 85-10% B, 14– 18 min: 10% B. For analysis, the fragments were dissolved in ACN or MeOH. 5  $\mu\text{L}$  of the compound solution was injected on the column. The purity of the compounds was then calculated as the relative AUC of the main peak of the chromatogram at 218, 254, 280, or 350 nm depending on the absorbance properties of the individual fragment. Retention times, calculated purities and detection waves used for the evaluation are summarized in Table S 1.1.

### 1.2 Nuclear magnetic resonance (NMR) Method

Compounds were dissolved in DMSO- $d_6$  or  $\text{CDCl}_3$  and  $^1\text{H}$  spectra were acquired on a Bruker Avance III HD 400 MHz system. NMR spectra are reported as chemical shifts ( $\delta$ ) in parts per million (ppm) in relation to tetramethylsilane (TMS) and were calibrated using the residual peak of the used solvent. Coupling constants (J) are reported in units of hertz (Hz). The following abbreviations are used to describe multiplicities: s (singlet), d (doublet), q (quartet), and m (multiplet).

### 1.3 Results of the Purity Determination

Table S 1.1: Results of the purity determination. Compounds not meeting the purity criteria of  $\geq 90\%$  are colored in red

Compound	Retentiontime [min]	Detection wavelength [nm]	Relative Purity [%]
AA001	7.433	254	96.04
AA002	7.107	218	90.99
AA003	5.007	254	99.42
AA004	12.025	254	100.00
AA005	9.832	254	100.00
AA006	8.477	254	100.00
AA007	10.750	254	100.00
AA008	10.340	254	100.00
CA006	9.917	254	100.00
CA007	11.517	254	100.00
CA008	9.113	254	97.26
EO004	NMR <sup>a</sup>	NMR <sup>a</sup>	NMR <sup>a</sup>
EO005	10.398	218	91.18
EO006	NMR <sup>a</sup>	NMR <sup>a</sup>	NMR <sup>a</sup>
EO007	9.137	218	70.86
EO008	10.315	218	95.58
EO009	NMR <sup>a</sup>	NMR <sup>a</sup>	NMR <sup>a</sup>
EO010	10.698	254	91.32
SN013	8.915	218	98.31
SN014	9.855	254	100.00
SN015	7.265	254	99.55
SN016	11.332	254	100.00
SN017	11.043	254	94.42
SN018	4.235	254	100
SN019	9.717	254	91.92
SN020	6.042	254	99.22
SN021	10.805	254	98.65
SN022	9.660	254	100.00
SN023	8.047	254	100.00
SN024	5.405	254	96.44
SN025	9.388	254	100.00
SN026	8.553	254	100.00
SN027	8.990	254	99.05
SN028	4.630	218	96.20
SN029	11.473	254	100.00
SN030	9.268	254	100.00
SN031	13.423	254	97.13
SN032	12.34	254	98.75
SN033	11.795	254	97.47
SN034	9.972	254	84.55
SN035	10.510	254	100.00
SN036	12.300	254	100.00
SN037	8.968	254	98.56
SN038	8.078	254	99.14
SN039	11.155	254	96.57
SN040	11.907	254	94.65
SN041	11.795	254	99.03
SN042	9.088	254	98.36
SN043	5.857	254	100.00
SN044	9.723	254	97.08
SN045	8.817	254	100.00
SN046	9.36	254	100
SN047	7.032	254	100.00
SN048	11.922	254	92.24
SN049	12.258	254	96.03
SN050	9.225	254	99.02
SN051	6.452	280	96.64
SN052	9.897	254	100.00
SN053	9.980	218	98.54
SN054	10.025	254	98.96
SN055	11.305	254	99.06
SN056	8.4	254	97.6
SN057	11.245	254	96.87
SN058	9.182	254	99.35
SN059	10.198	254	97.72
SN060	11.300	254	100.00
SN061	9.762	254	97.47
SN062	10.463	254	99.42
SN063	8.228	254	98.90
SN064	6.020	254	99.66
SN065	10.052	254	95.75
SN066	8.598	254	58.78
SN067	10.565	350	100.00
SN068	9.913	254	95.65
SN069	11.500	254	95.62
SN070	11.503	254	100.00
SN071	9.087	254	97.26
SN072	9.473	350	100.00
VS005	11.383	254	100
VS006	NMR <sup>a</sup>	NMR <sup>a</sup>	NMR <sup>a</sup>
VS007	7.433	254	96.04

<sup>a</sup> The compound was not suitable for HPLC-UV analysis. <sup>1</sup>H-NMR was performed instead. The red color indicates that the determined purity is below 90%.

## 1.4 HPLC traces and NMR spectra

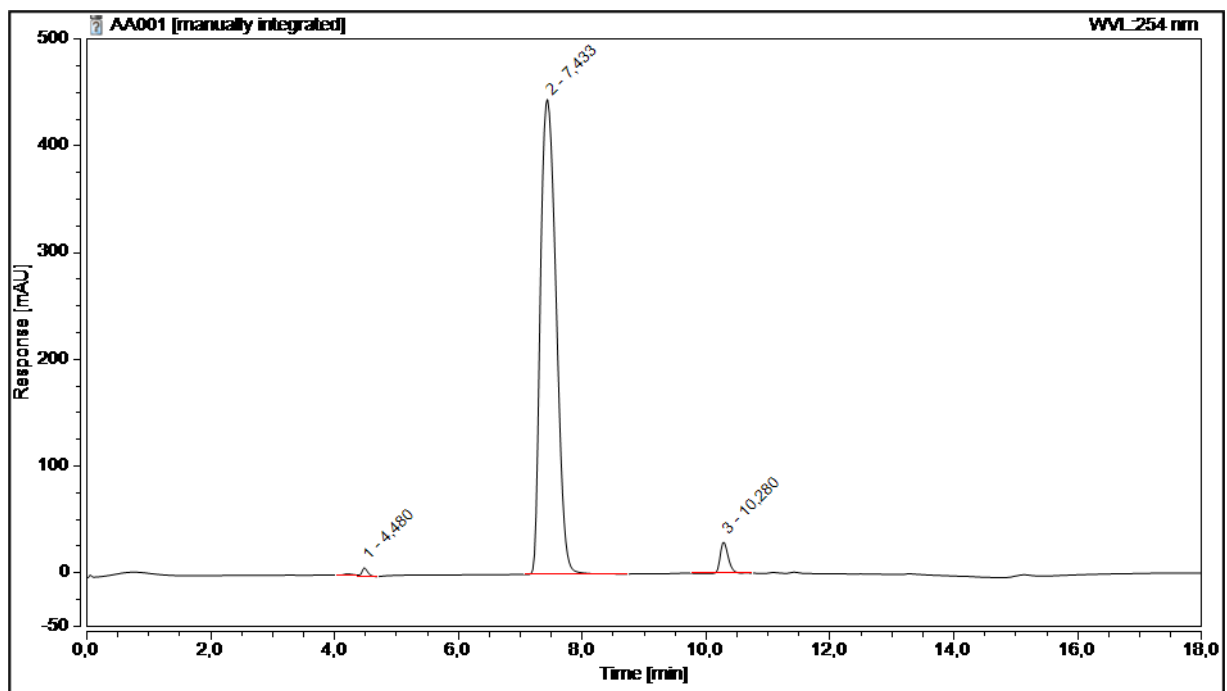


Figure S 1.1: HPLC chromatogram of AA001.

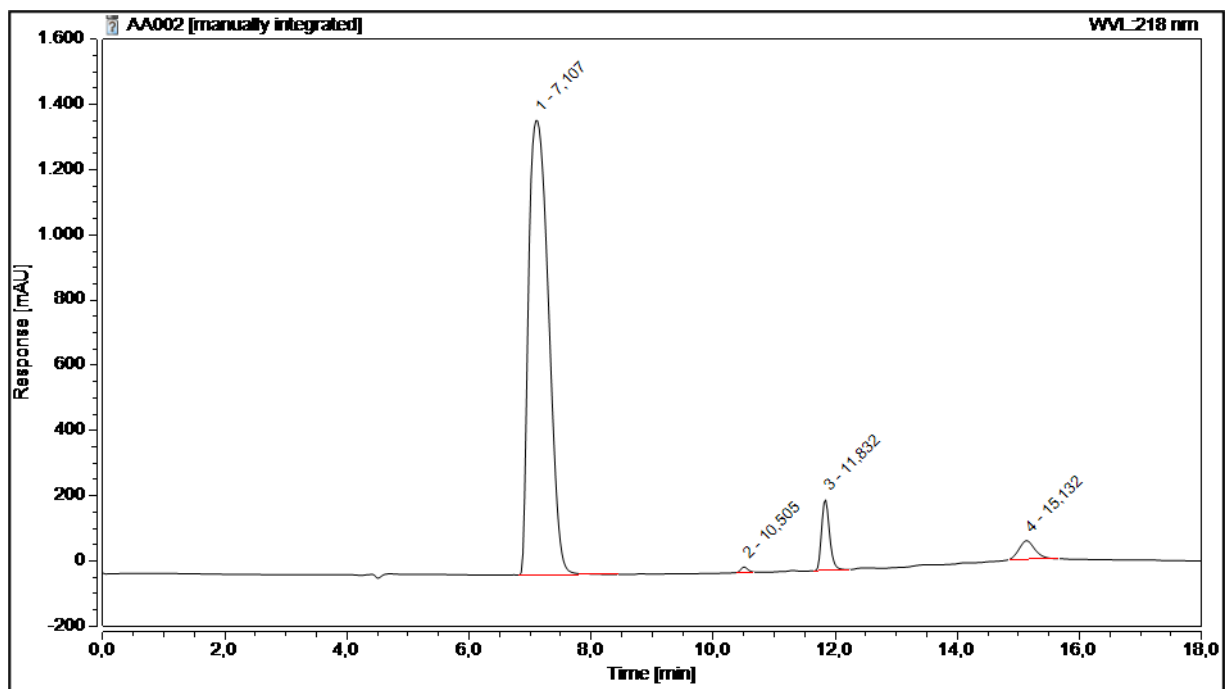


Figure S 1.2: HPLC chromatogram of AA002.

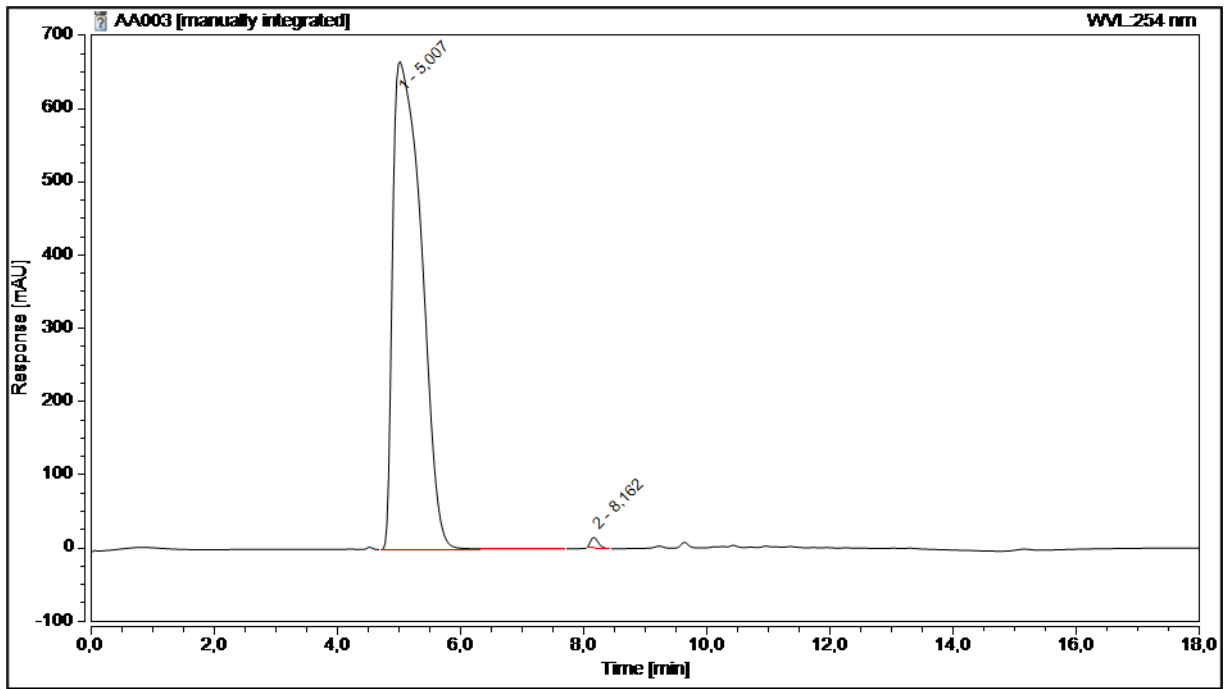


Figure S 1.3: HPLC chromatogram of AA003.

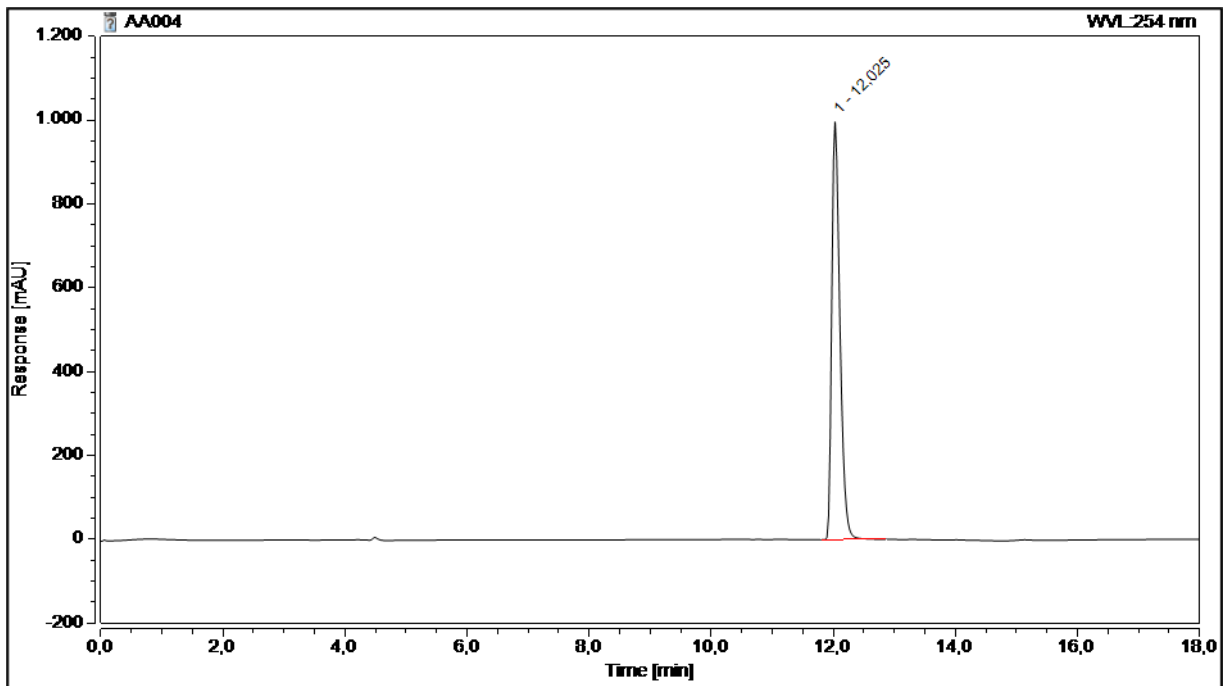


Figure S 1.4: HPLC chromatogram of AA004.

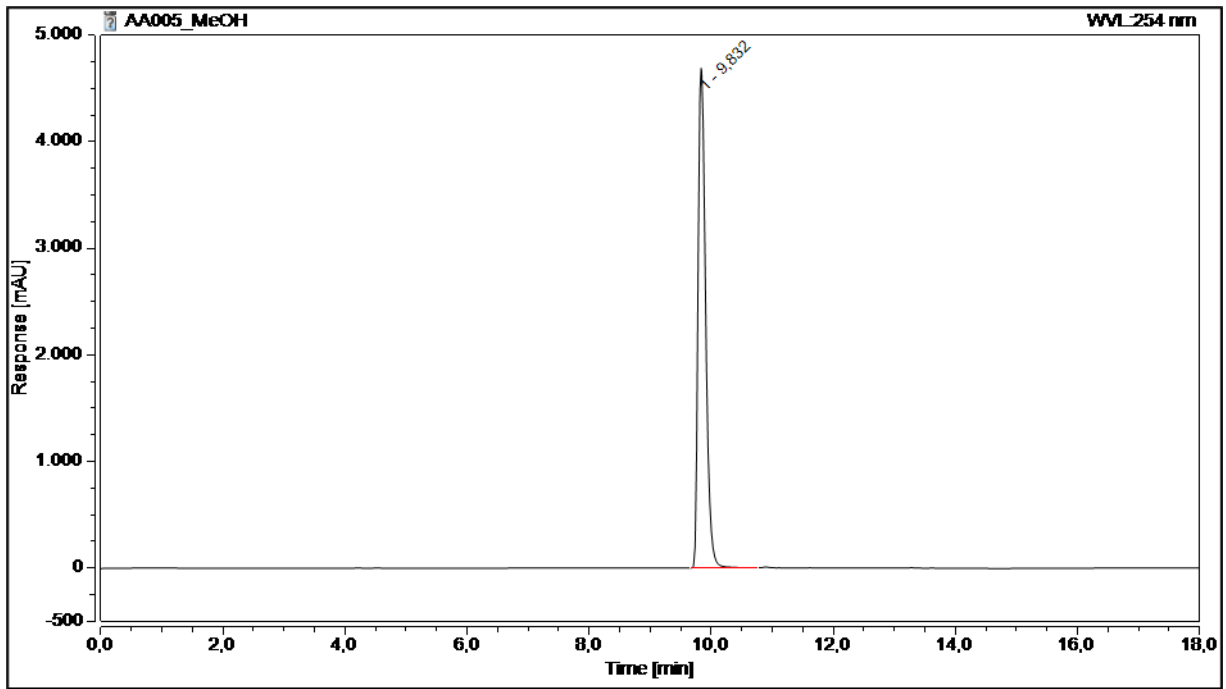


Figure S 1.5: HPLC chromatogram of AA005.

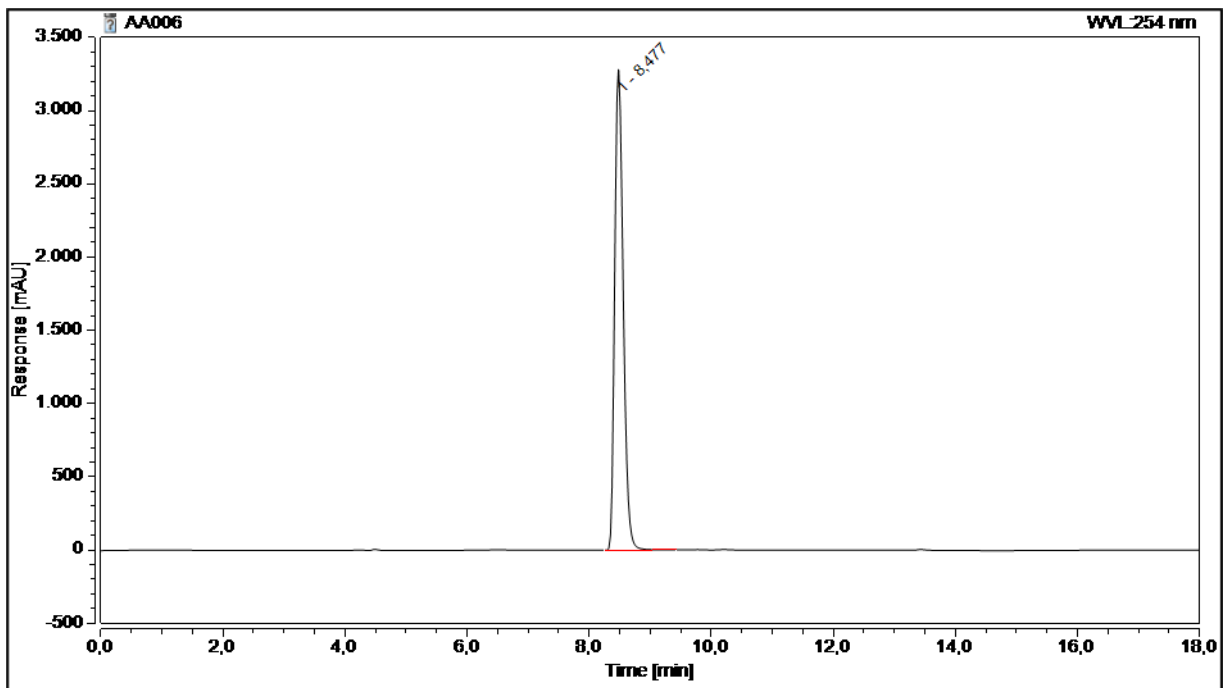


Figure S 1.6: HPLC chromatogram of AA006.

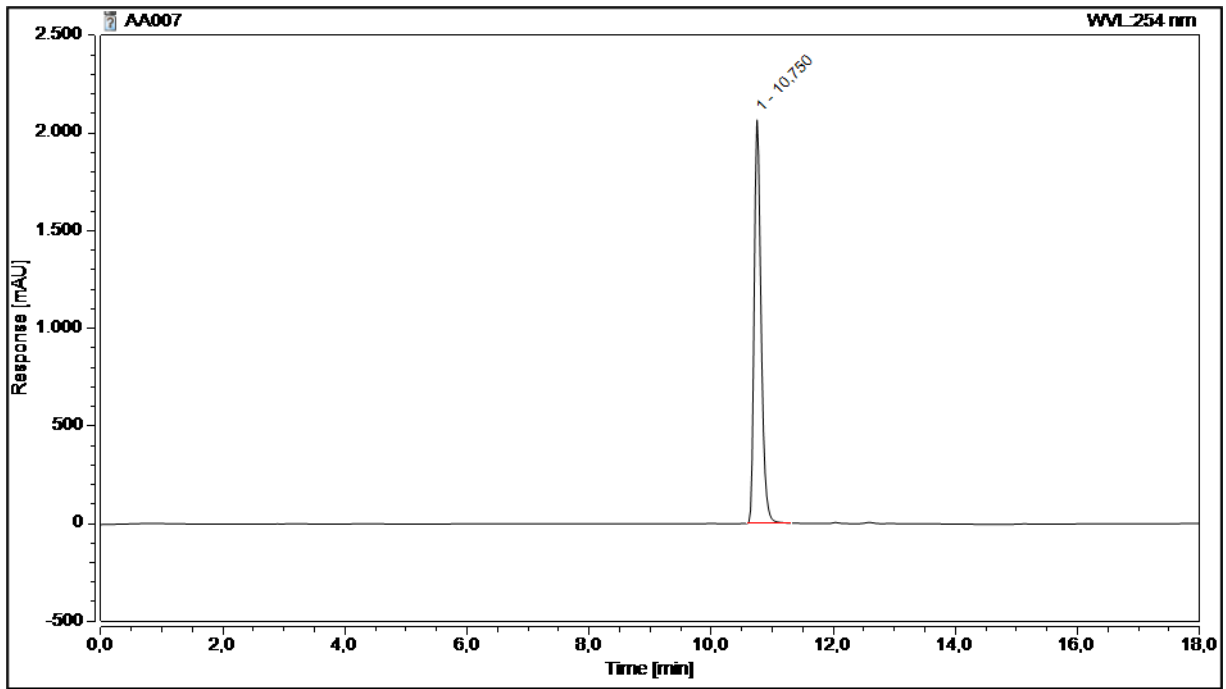


Figure S 1.7: HPLC chromatogram of AA007.

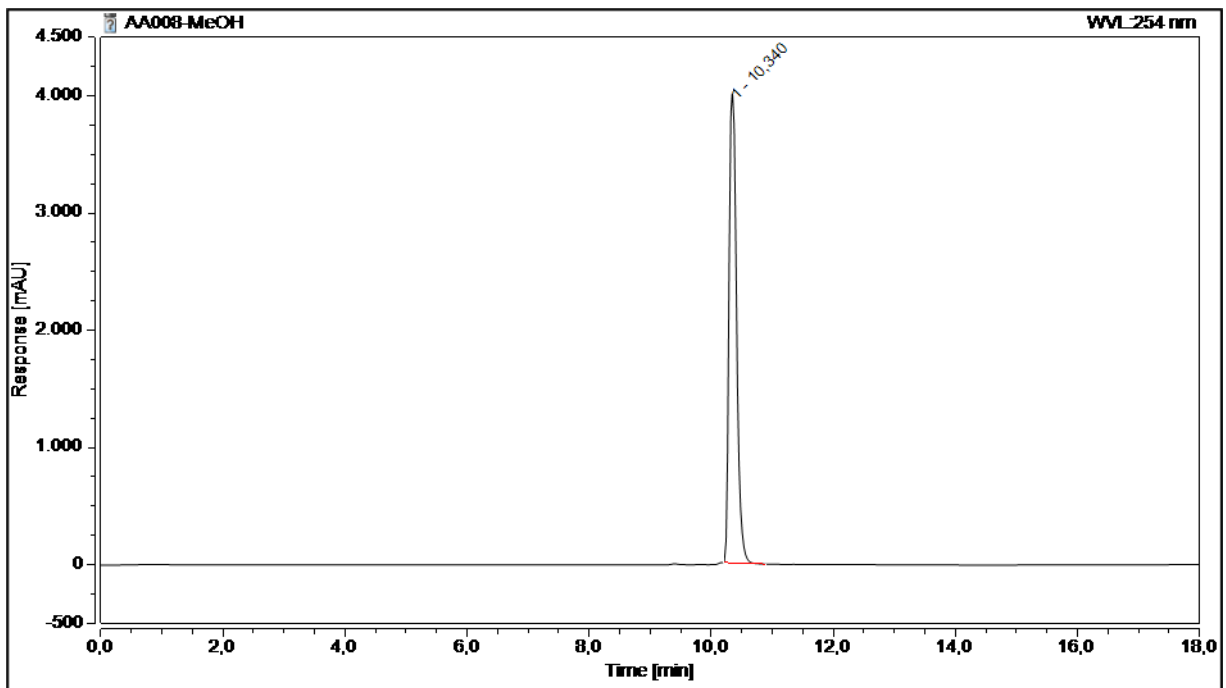


Figure S 1.8: HPLC chromatogram of AA008.

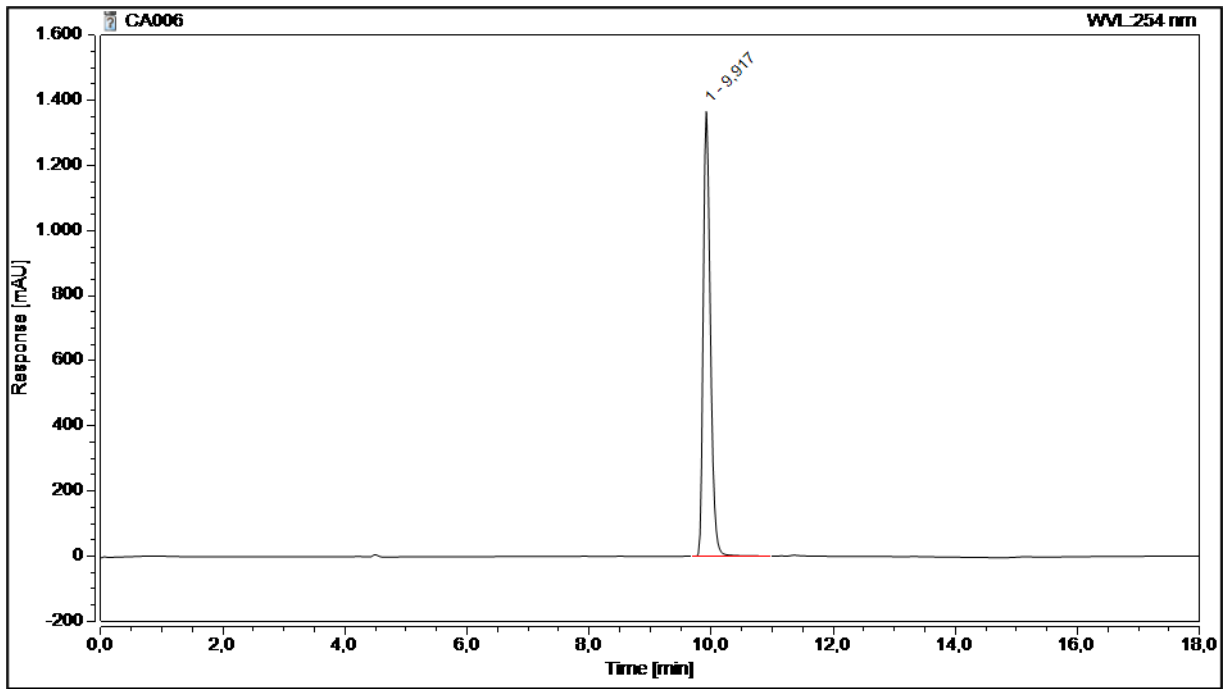


Figure S 1.9: HPLC chromatogram of CA006.

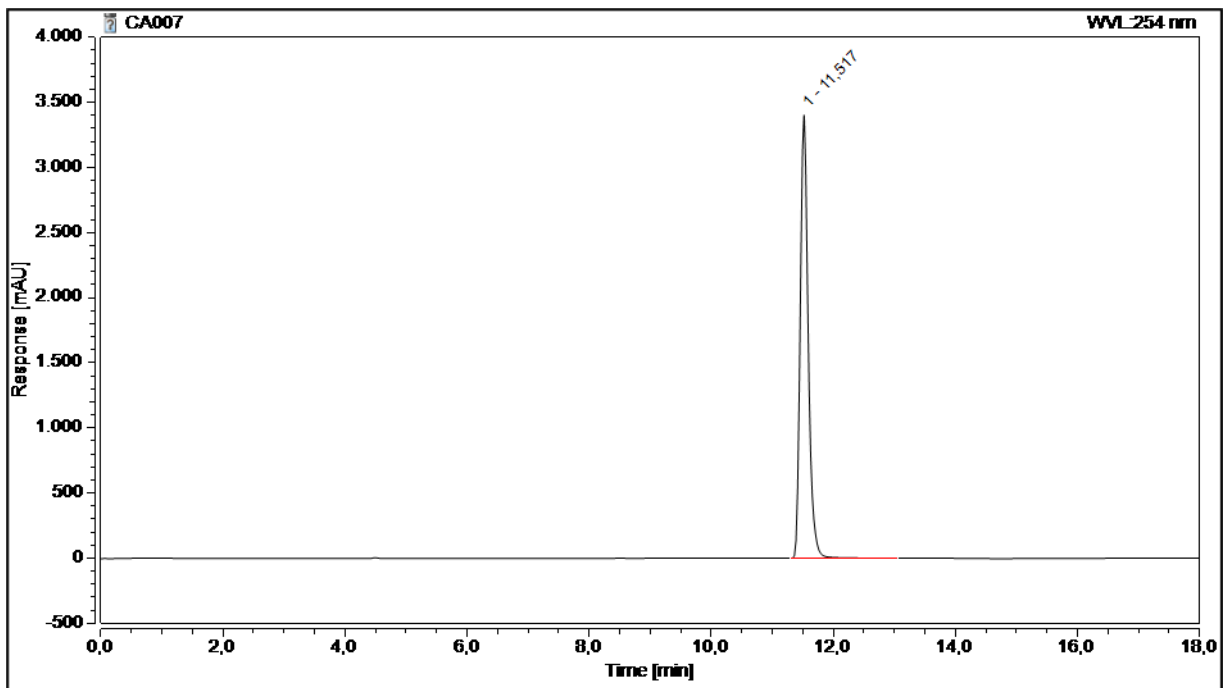


Figure S 1.10: HPLC chromatogram of CA007.

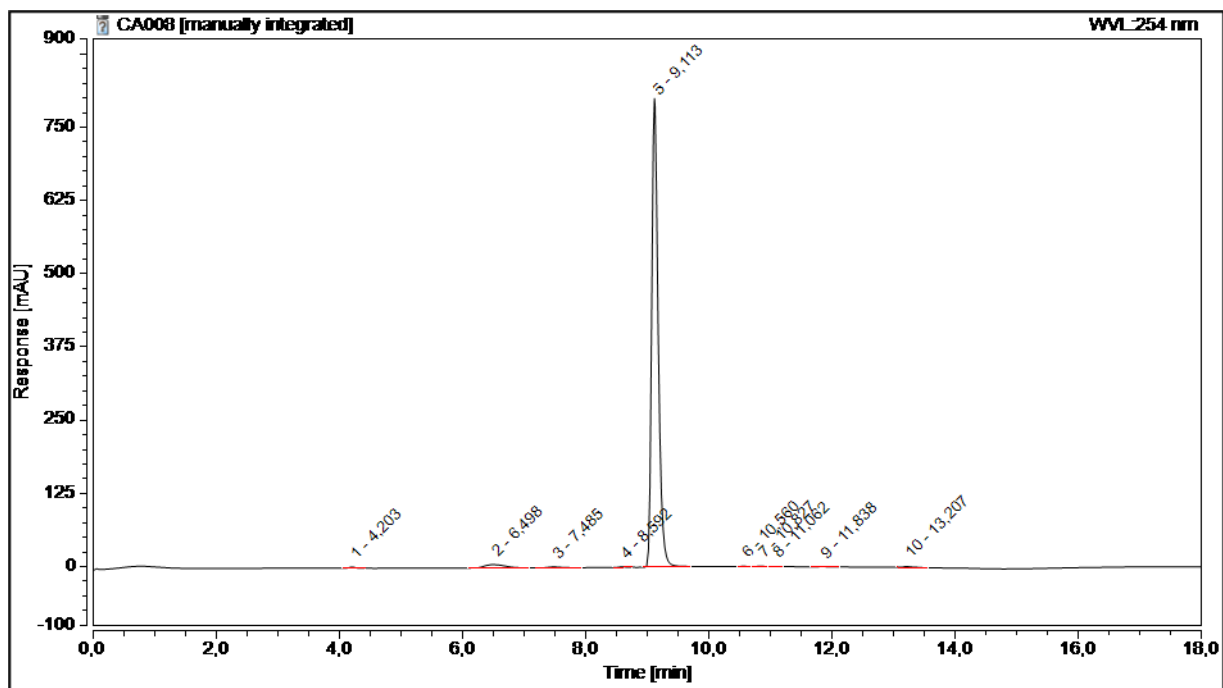


Figure S 1.11: HPLC chromatogram of CA008.

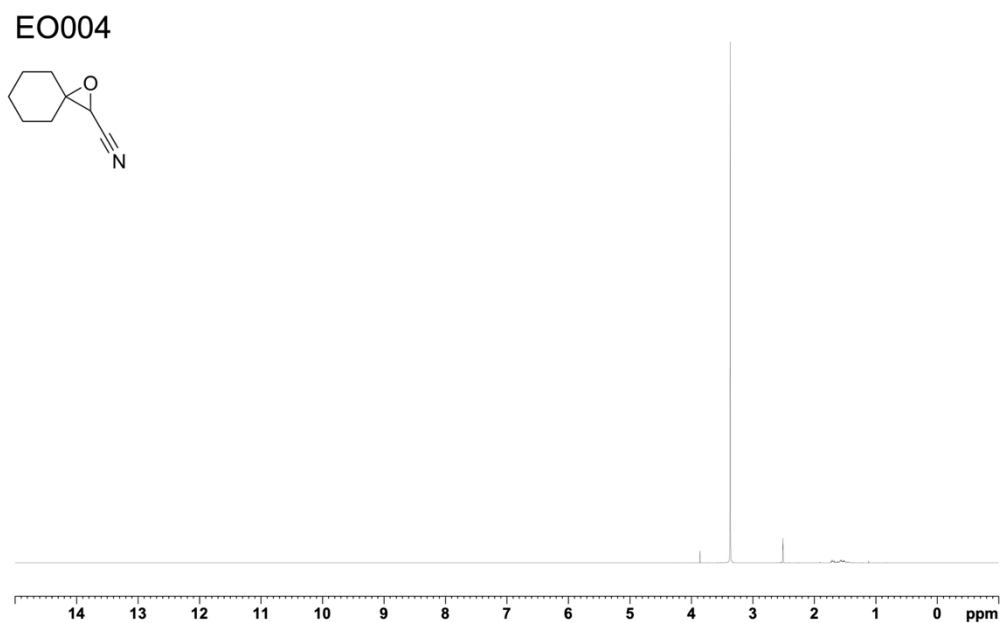


Figure S 1.12:  $^1\text{H-NMR}$  spectrum of EO004 in  $\text{DMSO-d}_6$ .  $\delta=3.86$  (s, 1H), 3.37 (s, water residual), 2.51 (m, DMSO residual), 1.73–1.42 (m, 10H).

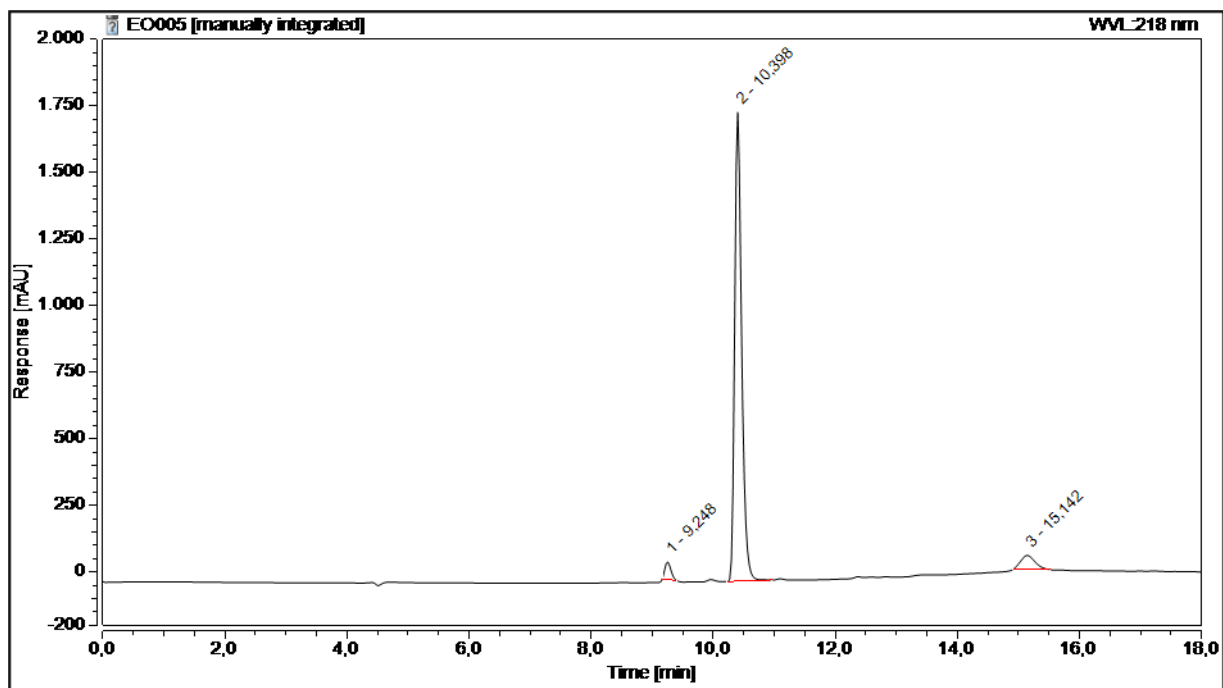


Figure S 1.13: HPLC chromatogram of EO005.

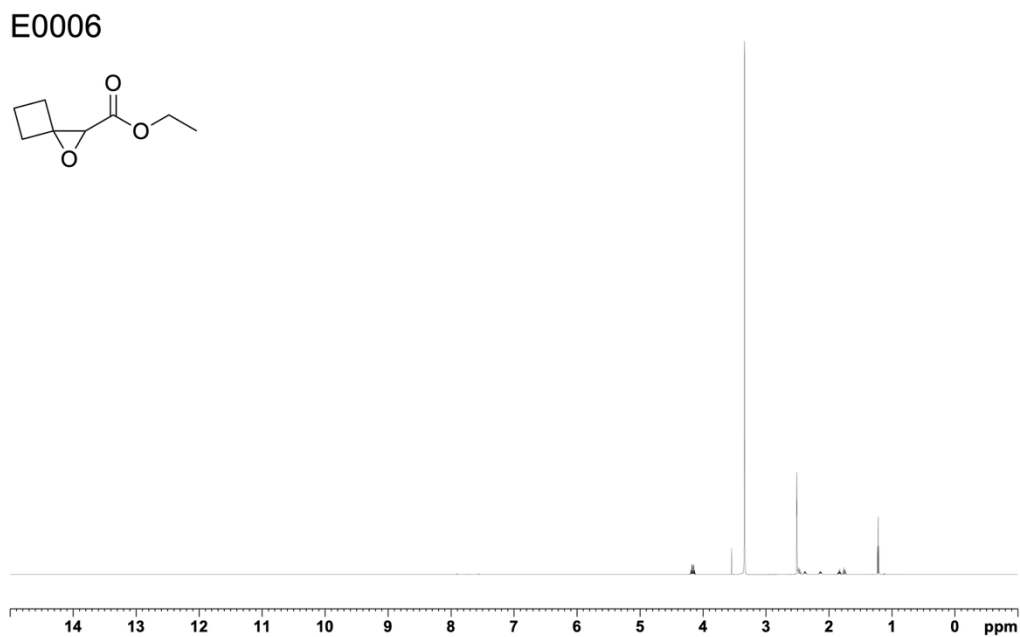


Figure S 1.14: <sup>1</sup>H-NMR spectrum of EO006 in DMSO-d<sub>6</sub>.  $\delta$ =4.19–4.13 (m, 2H), 3.54 (s, 1H), 3.34 (s, water residual), 2.51 (m, DMSO residual), 2.49–2.44 (m, 2H), 2.41–2.35 (m, 1H), 2.16–2.11 (m, 1H), 1.86–1.73 (m, 2H), 1.22 ppm (t, J=7.1 Hz, 3H).

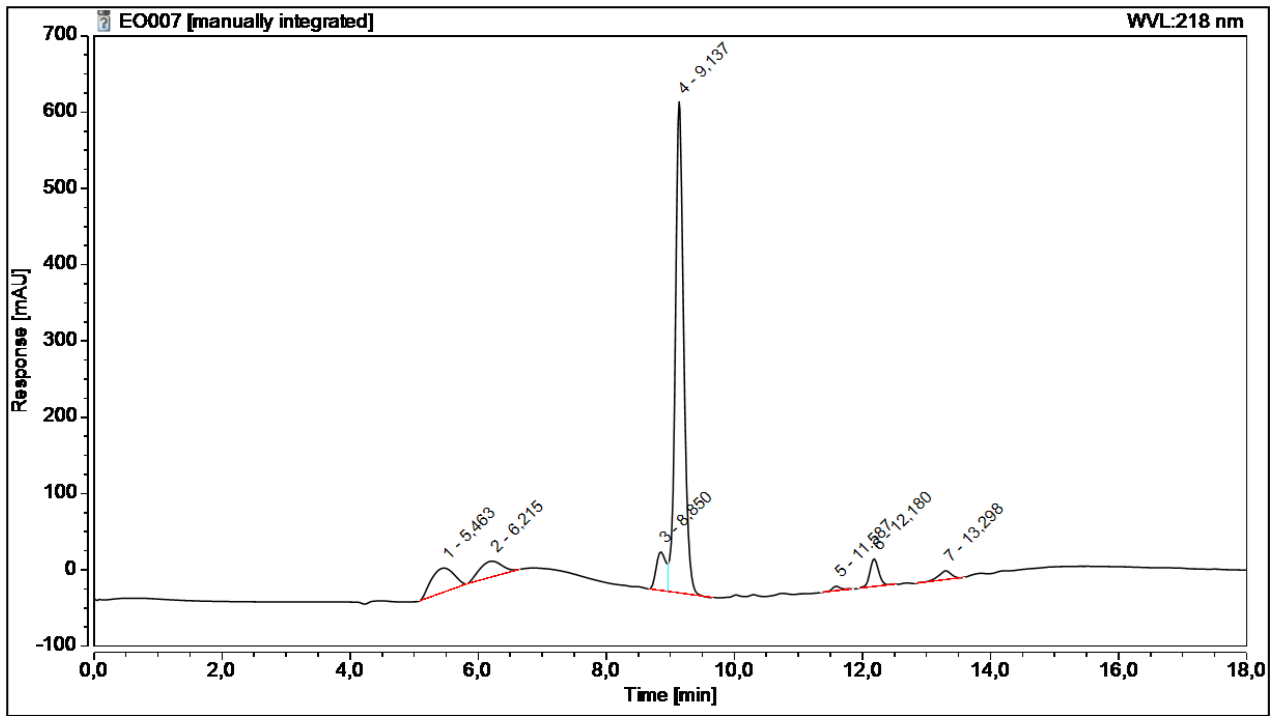


Figure S 1.15: HPLC chromatogramm of EO007.

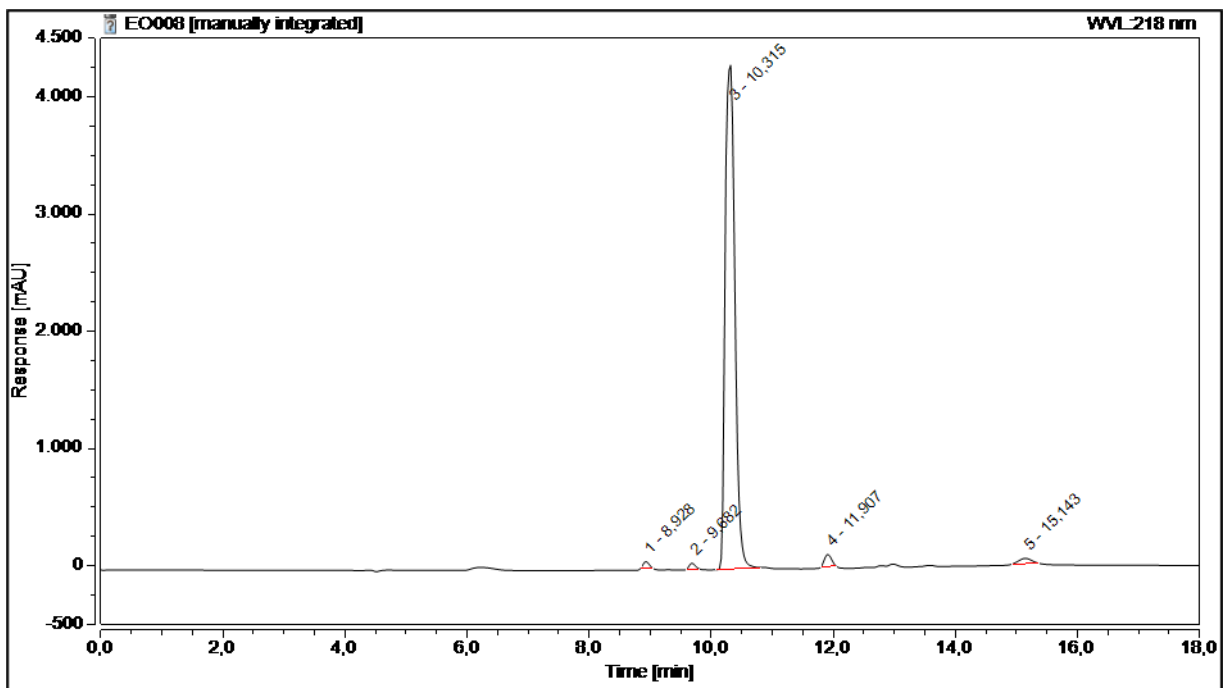


Figure S 1.16: HPLC chromatogramm of EO008.

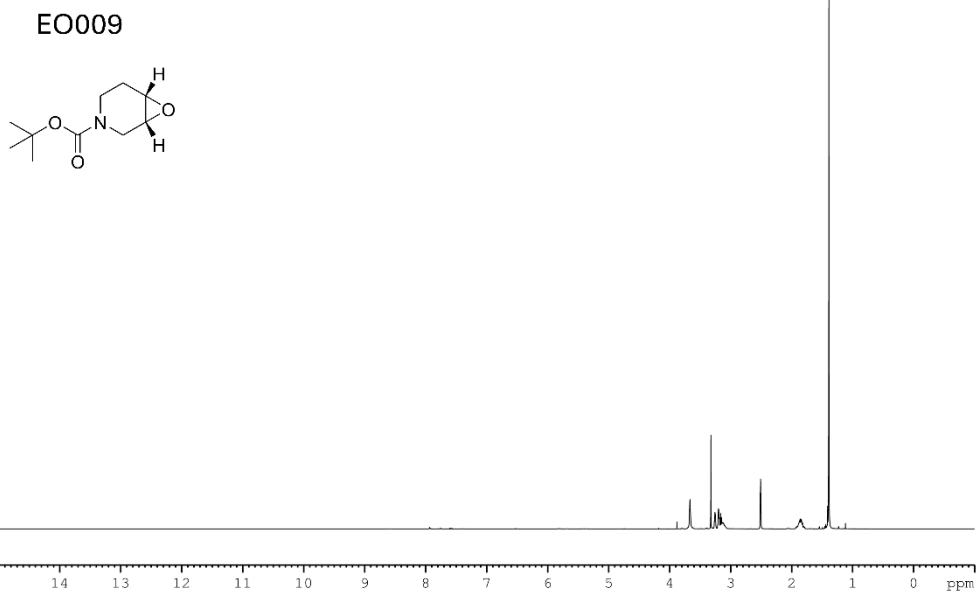


Figure S 1.17: <sup>1</sup>H-NMR spectrum of EO009 in CDCl<sub>3</sub>. δ=7.28 (s, CHCl<sub>3</sub> residual), 3.99 - 3.70 (m, 1H), 3.70 (m, 1H), 3.52 - 3.41 (m, 1H), 3.31 (m, 1H), 3.23 (s, 1H), 3.18 - 3.07 (m, 1H), 2.03 (m, 1H), 1.98 - 1.88 (m, 1H), 1.47 (s, 9H).

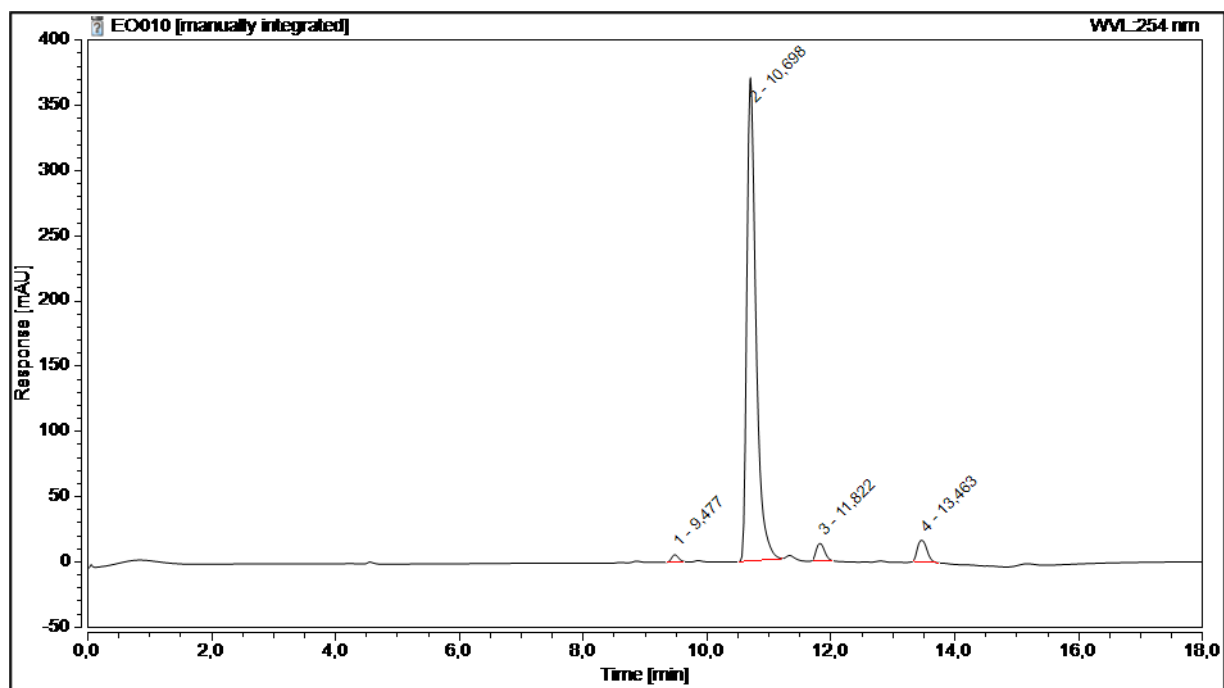


Figure S 1.18: HPLC chromatogram of EO010.

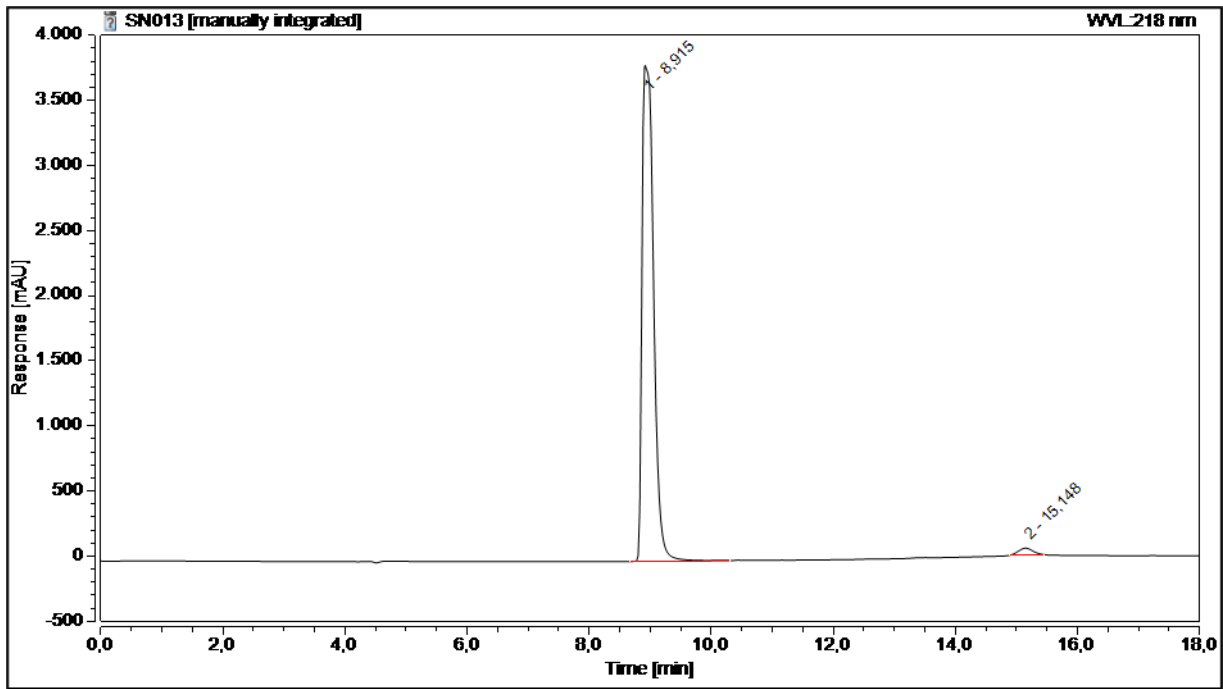


Figure S 1.19: HPLC chromatogramm of SN013.

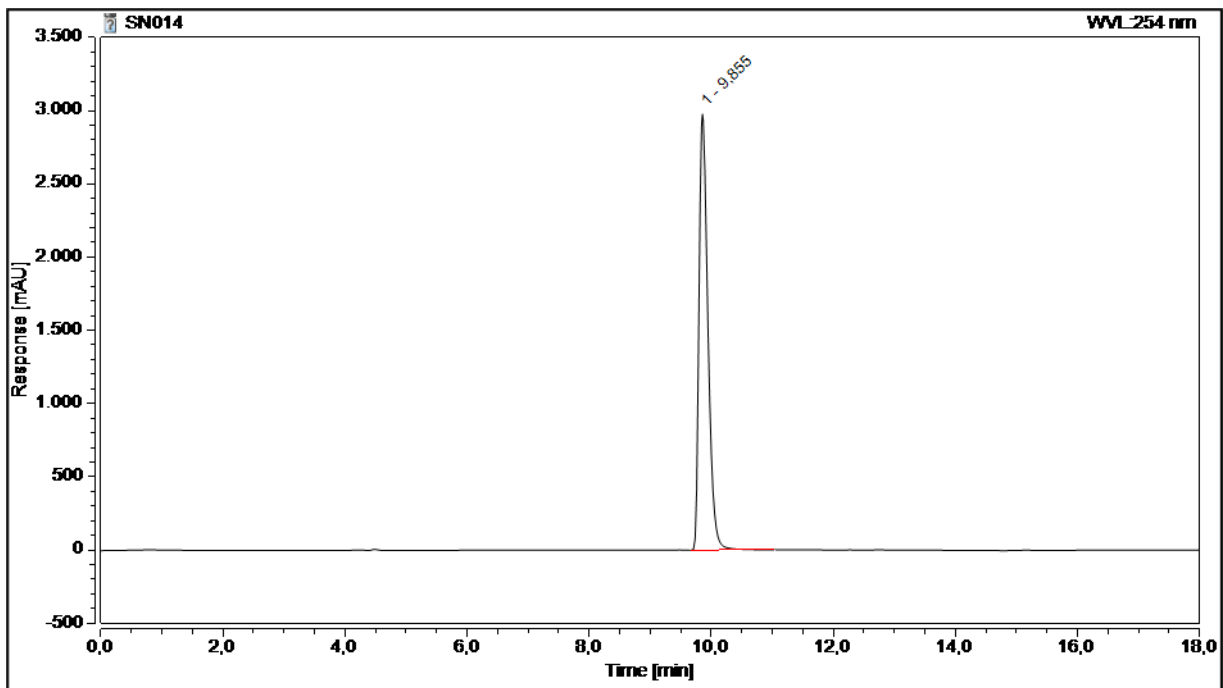


Figure S 1.20: HPLC chromatogramm of SN014.

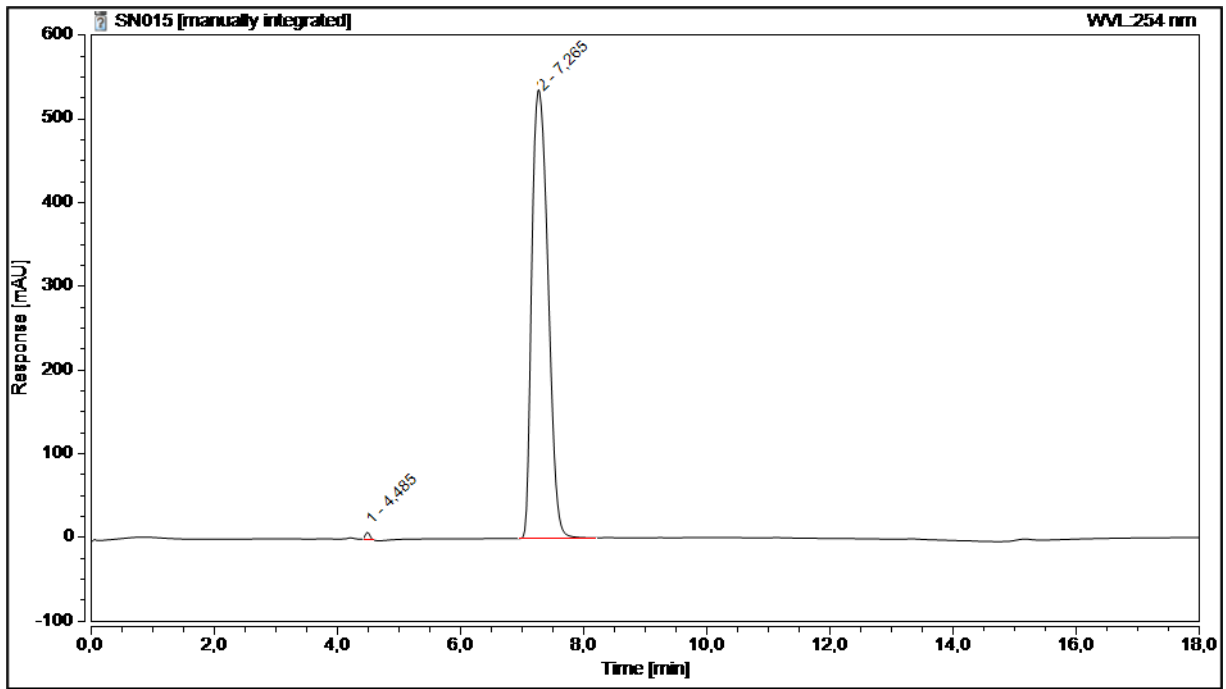


Figure S 1.21: HPLC chromatogramm of SN015.

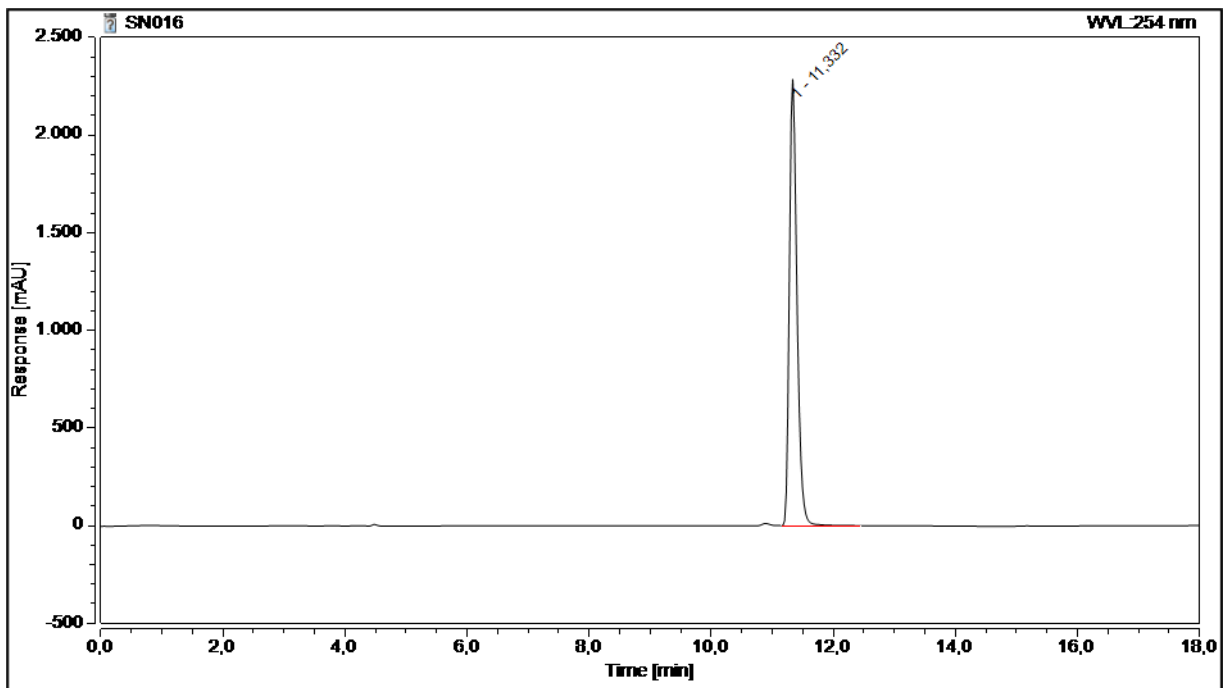


Figure S 1.22: HPLC chromatogramm of SN016.

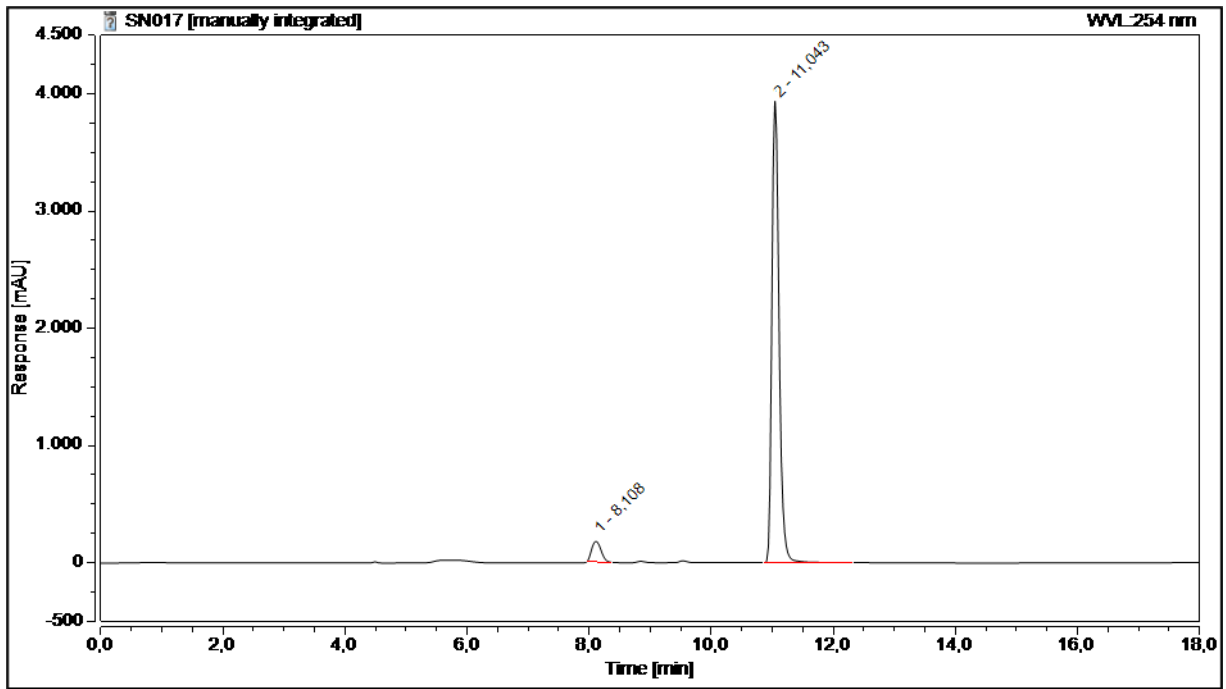


Figure S 1.23: HPLC chromatogramm of SN017.

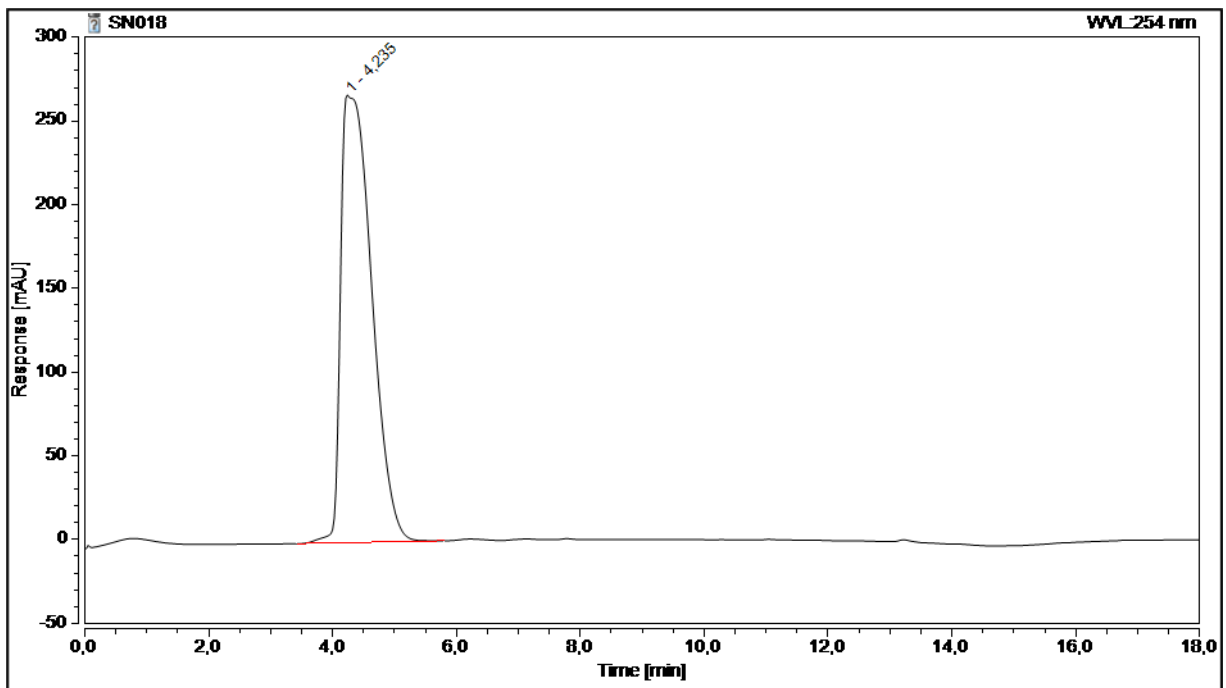


Figure S 1.24: HPLC chromatogramm of SN018.

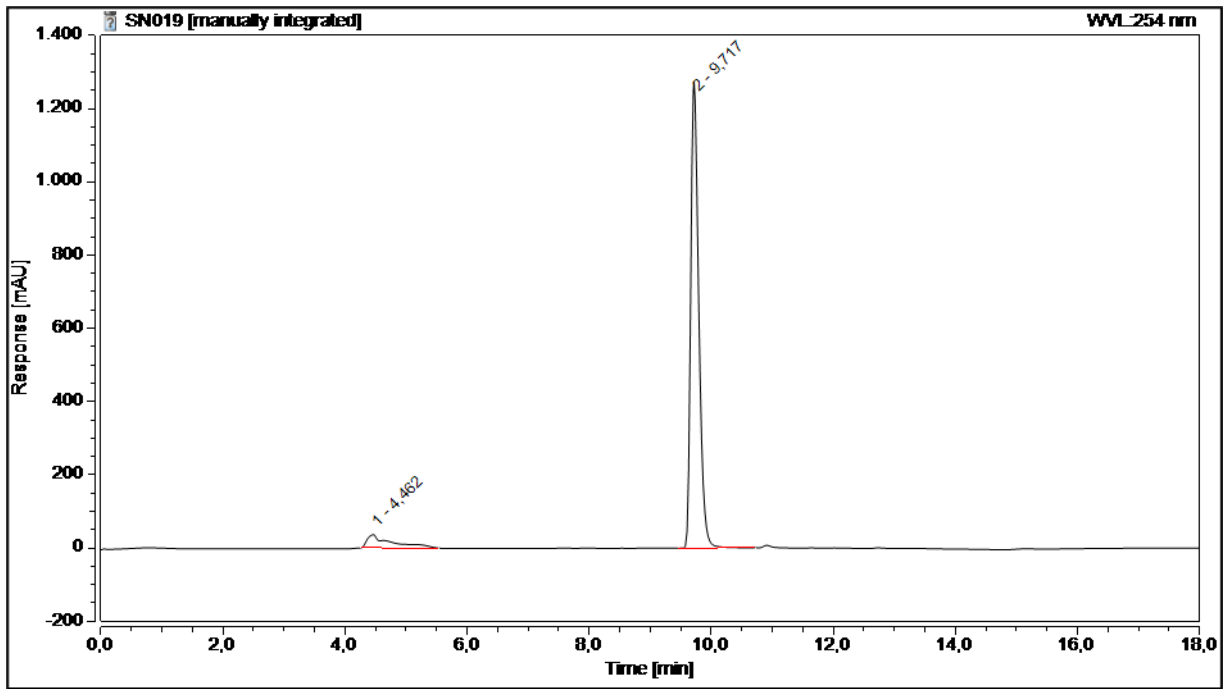


Figure S 1.25: HPLC chromatogramm of SN019.

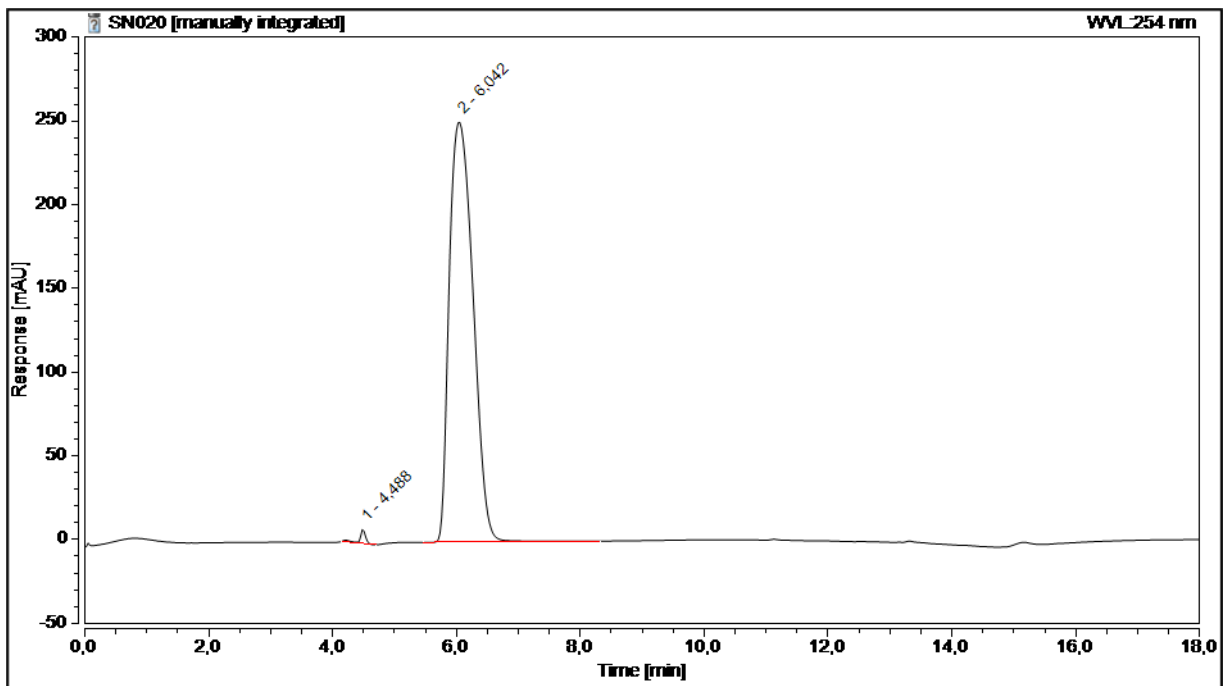


Figure S 1.26: HPLC chromatogramm of SN020.

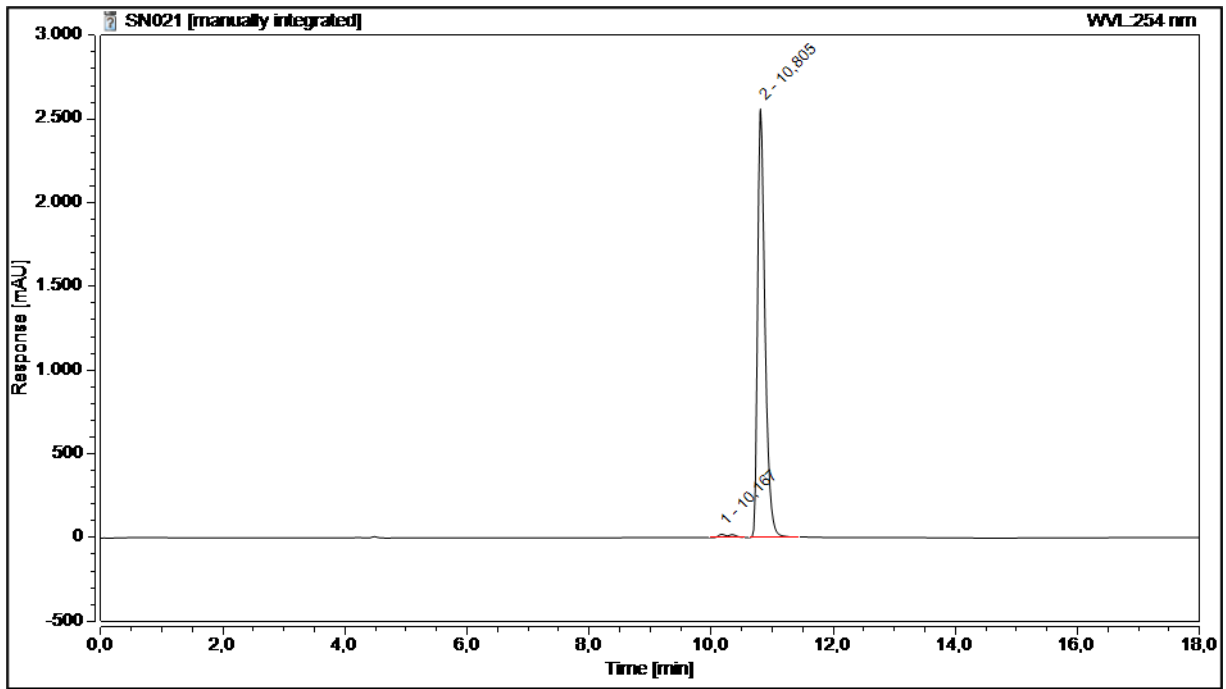


Figure S 1.27: HPLC chromatogramm of SN021.

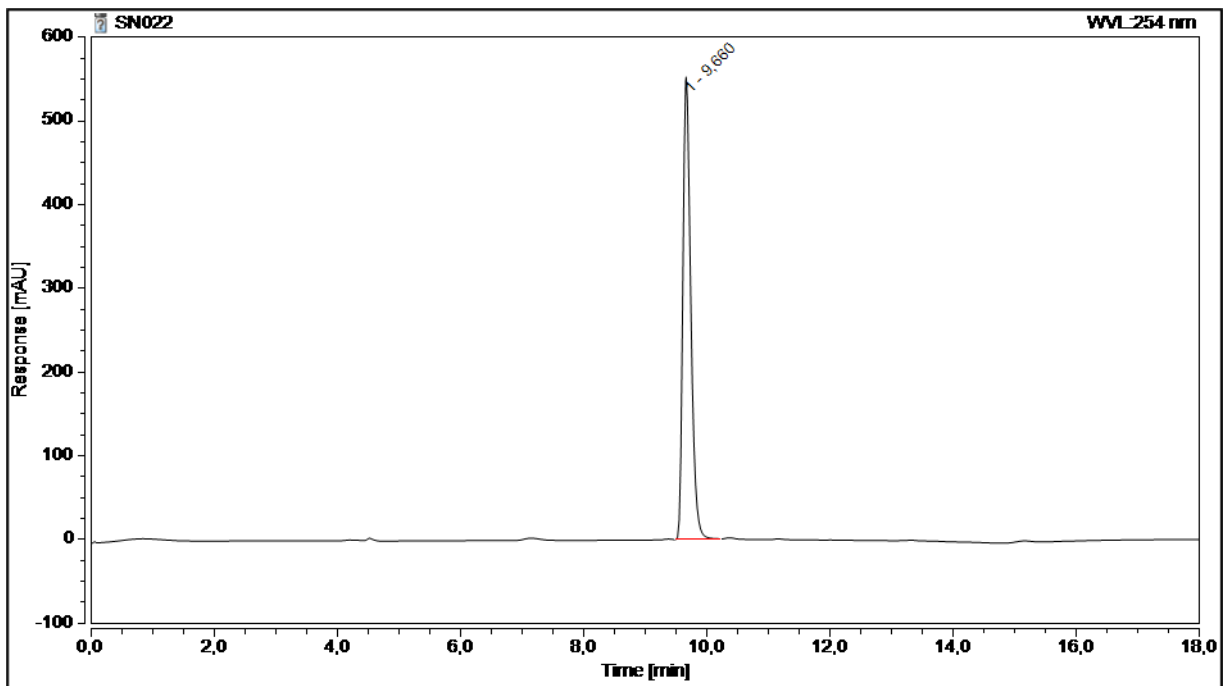


Figure S 1.28: HPLC chromatogramm of SN022.

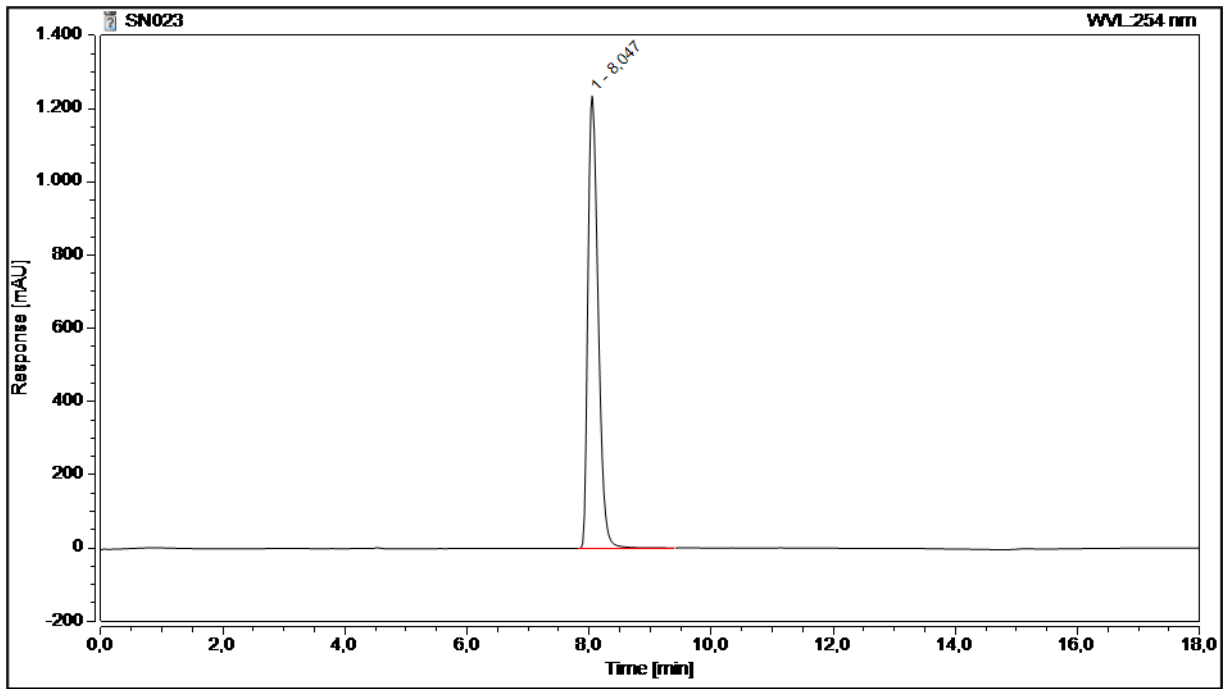


Figure S 1.29: HPLC chromatogramm of SN023.

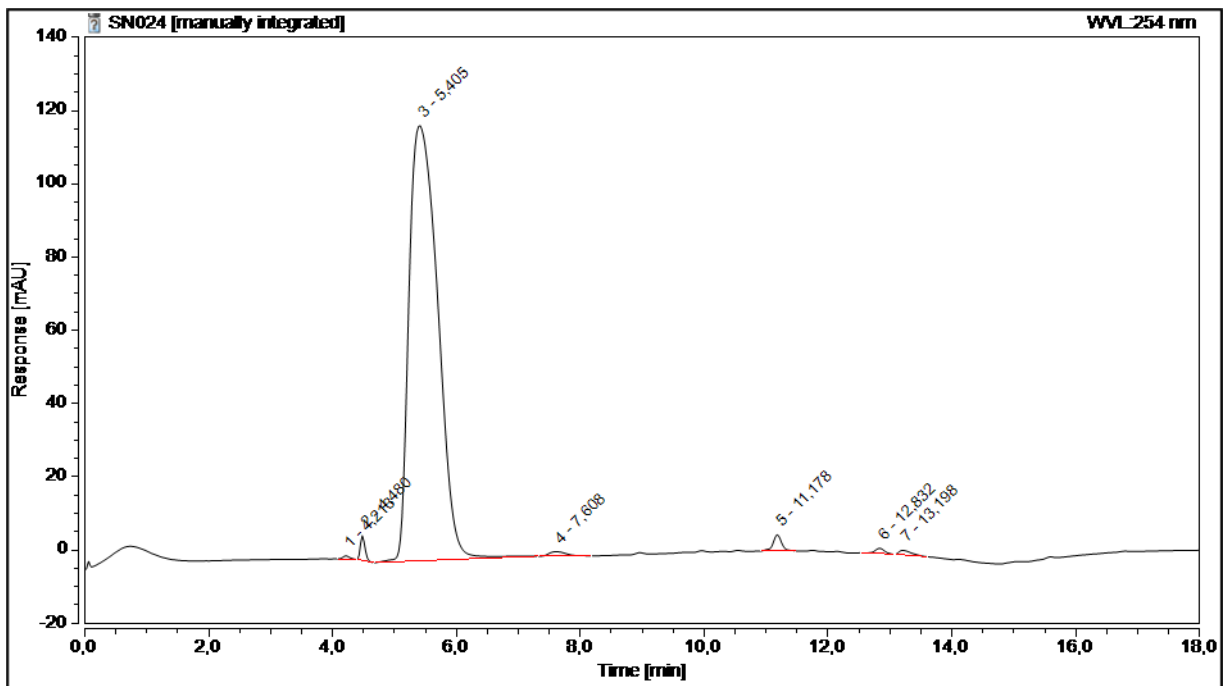


Figure S 1.30: HPLC chromatogramm of SN024.

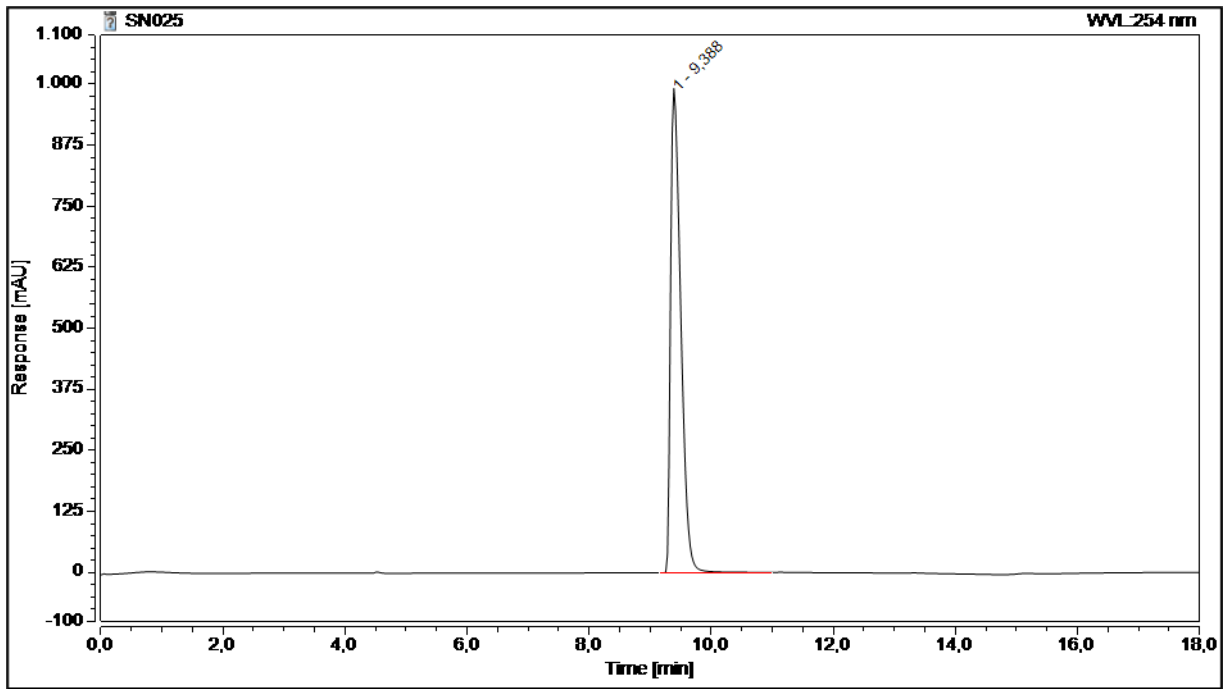


Figure S 1.31: HPLC chromatogramm of SN025.

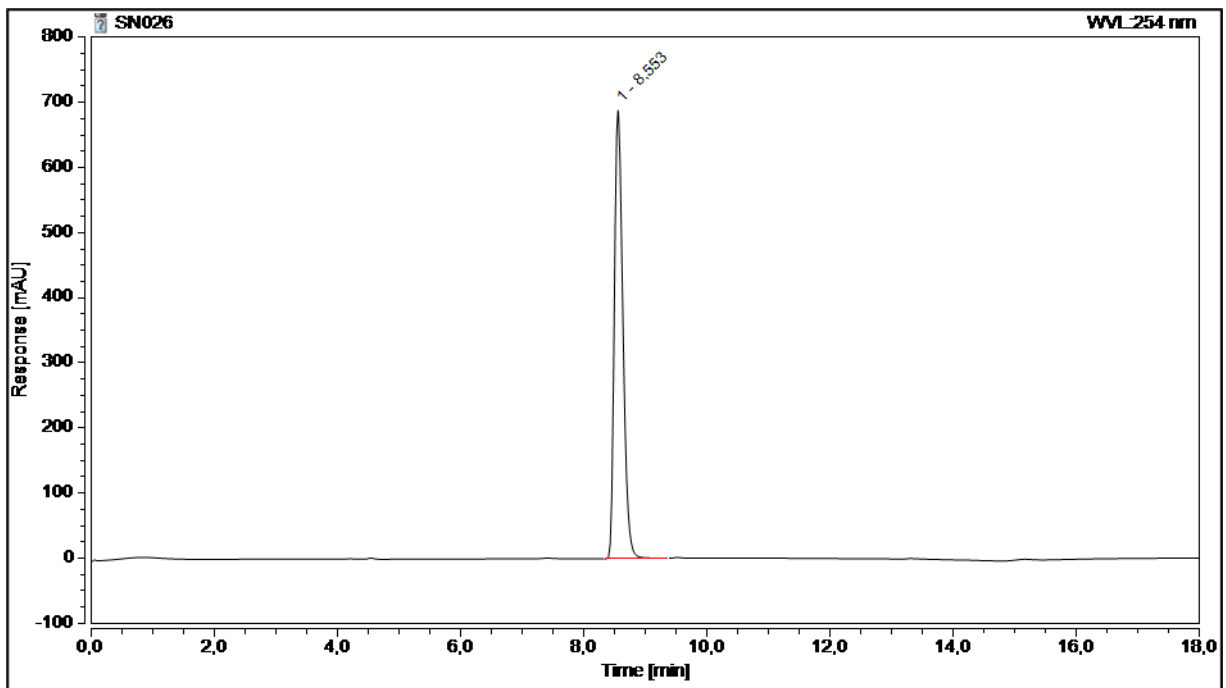


Figure S 1.32: HPLC chromatogramm of SN026.

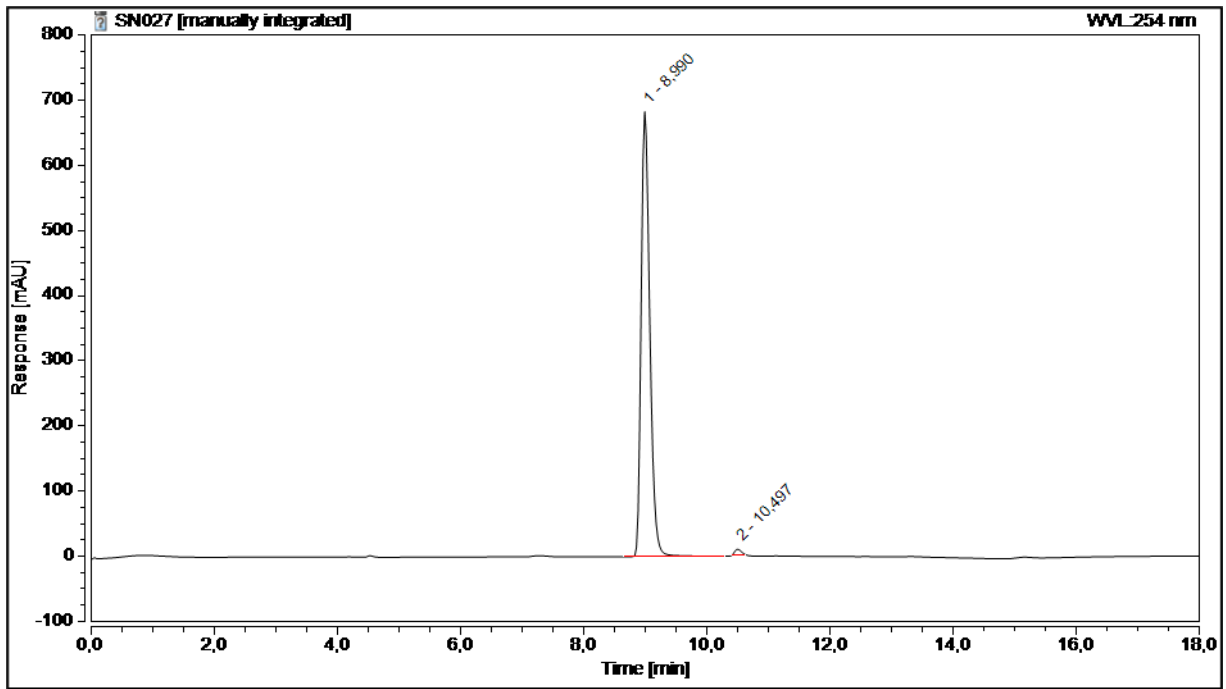


Figure S 1.33: HPLC chromatogramm of SN027.

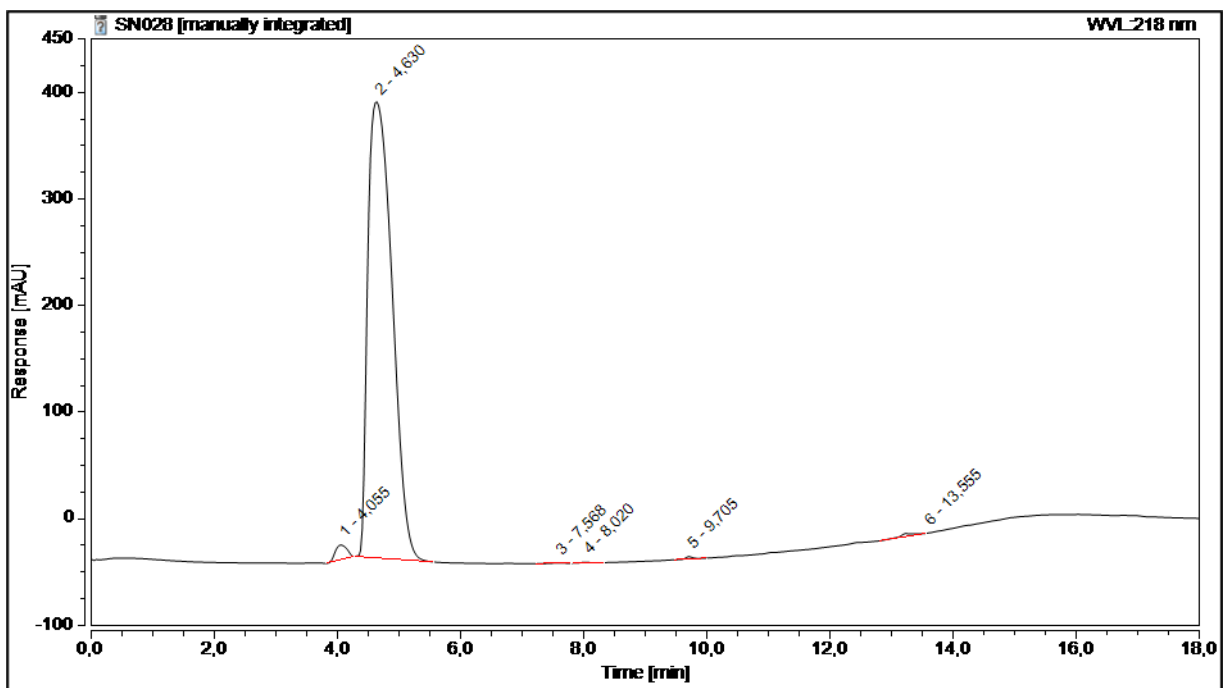


Figure S 1.34: HPLC chromatogramm of SN028.

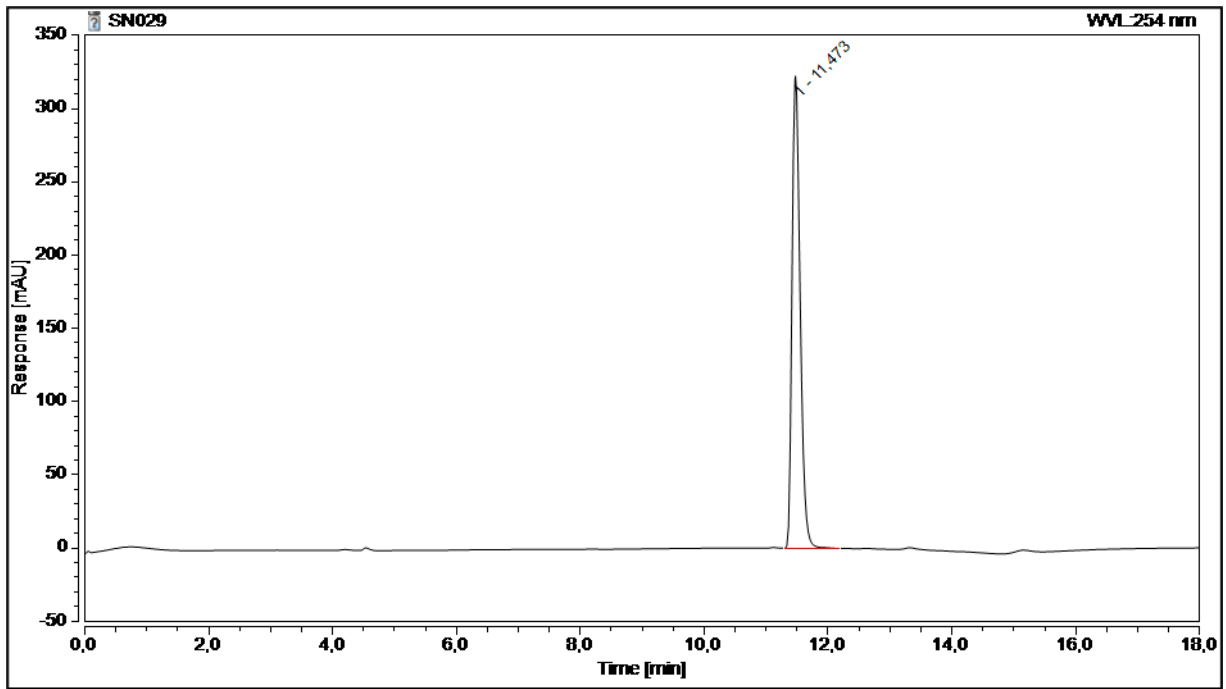


Figure S 1.35: HPLC chromatogramm of SN029.

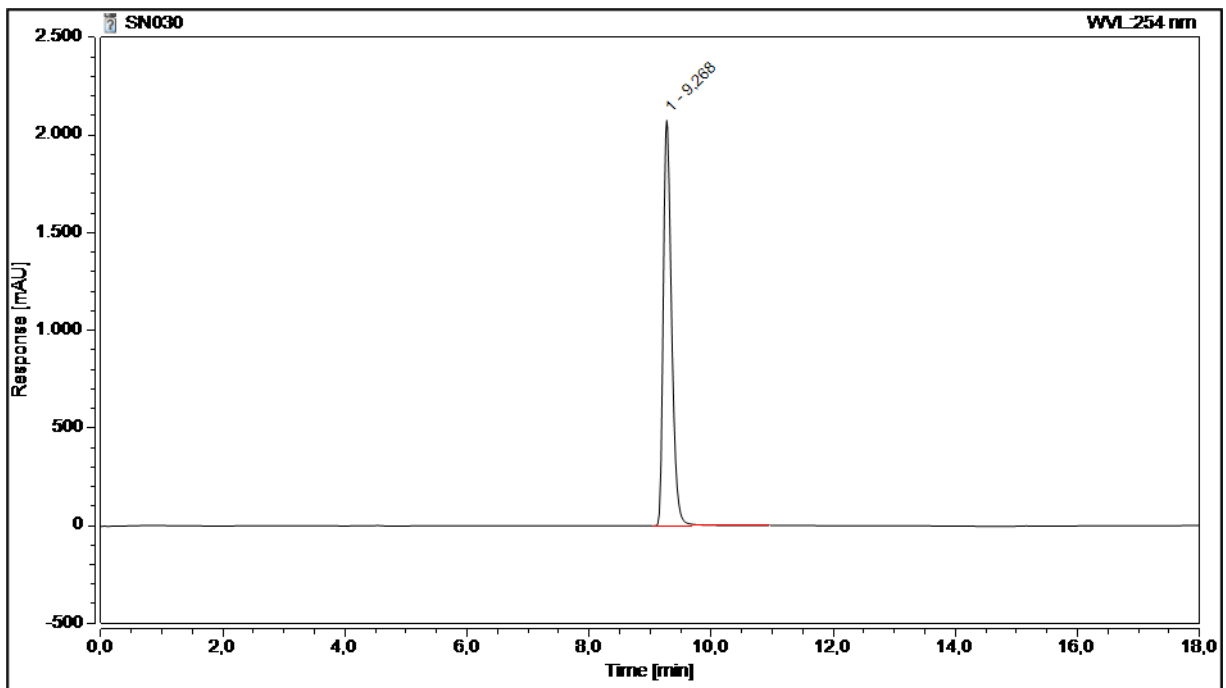


Figure S 1.36: HPLC chromatogramm of SN030.

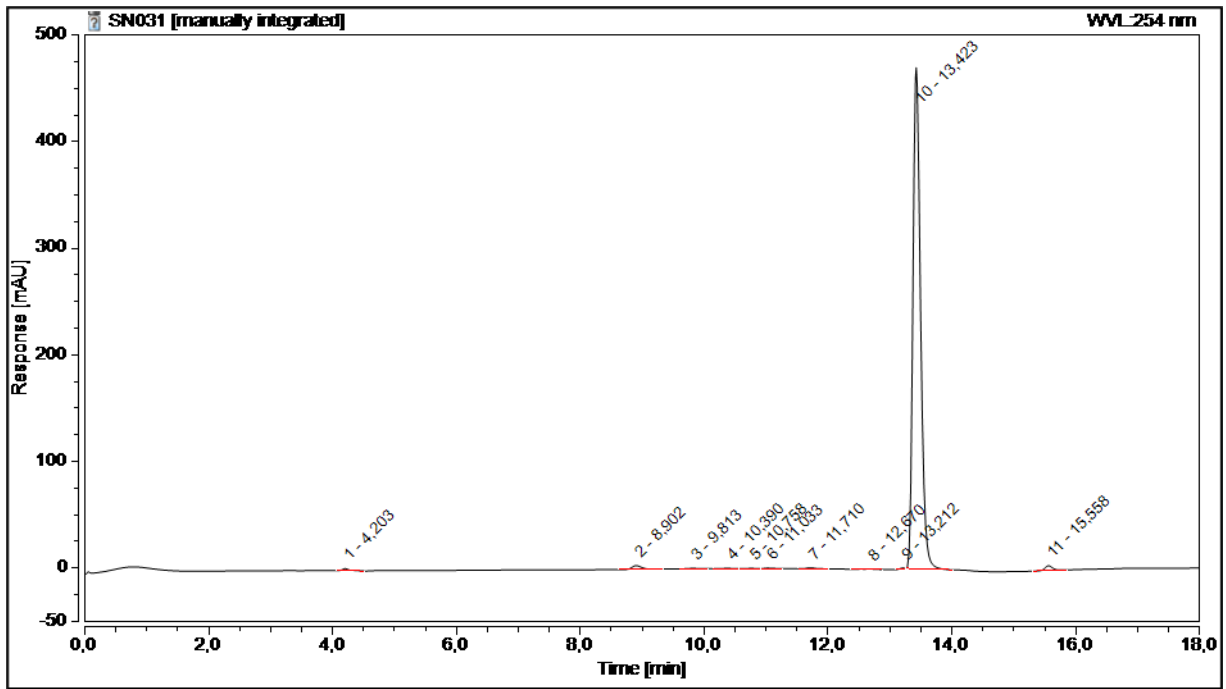


Figure S 1.37: HPLC chromatogramm of SN031.

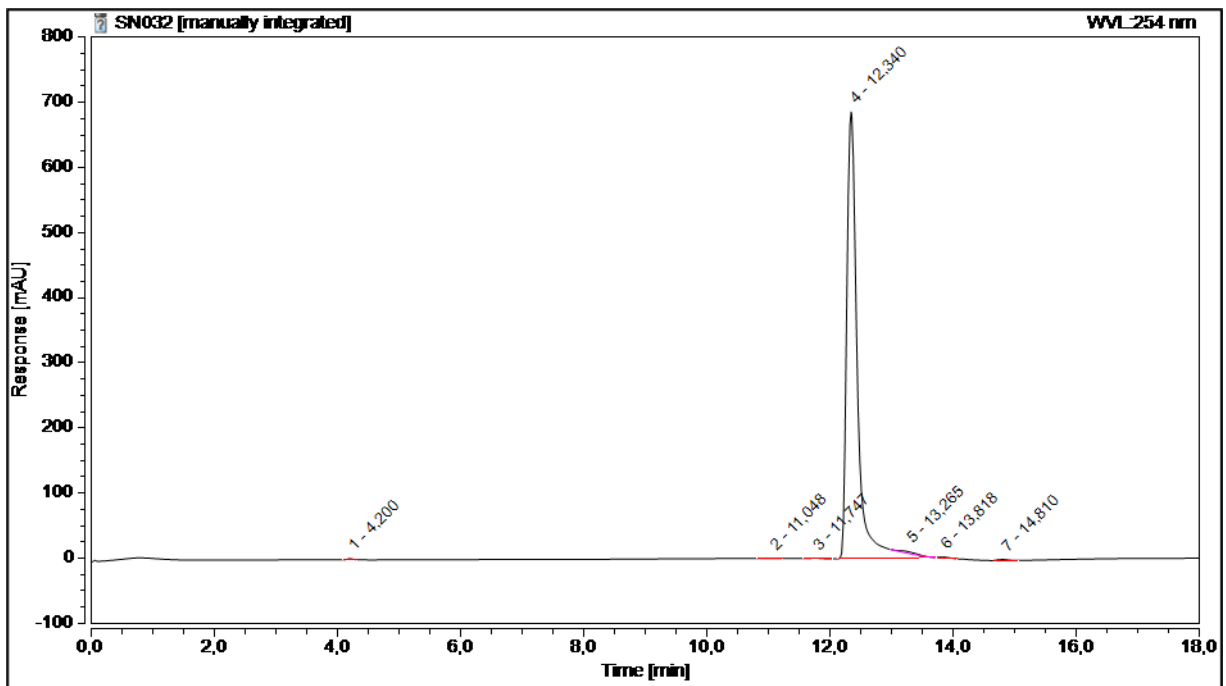


Figure S 1.38: HPLC chromatogramm of SN032.

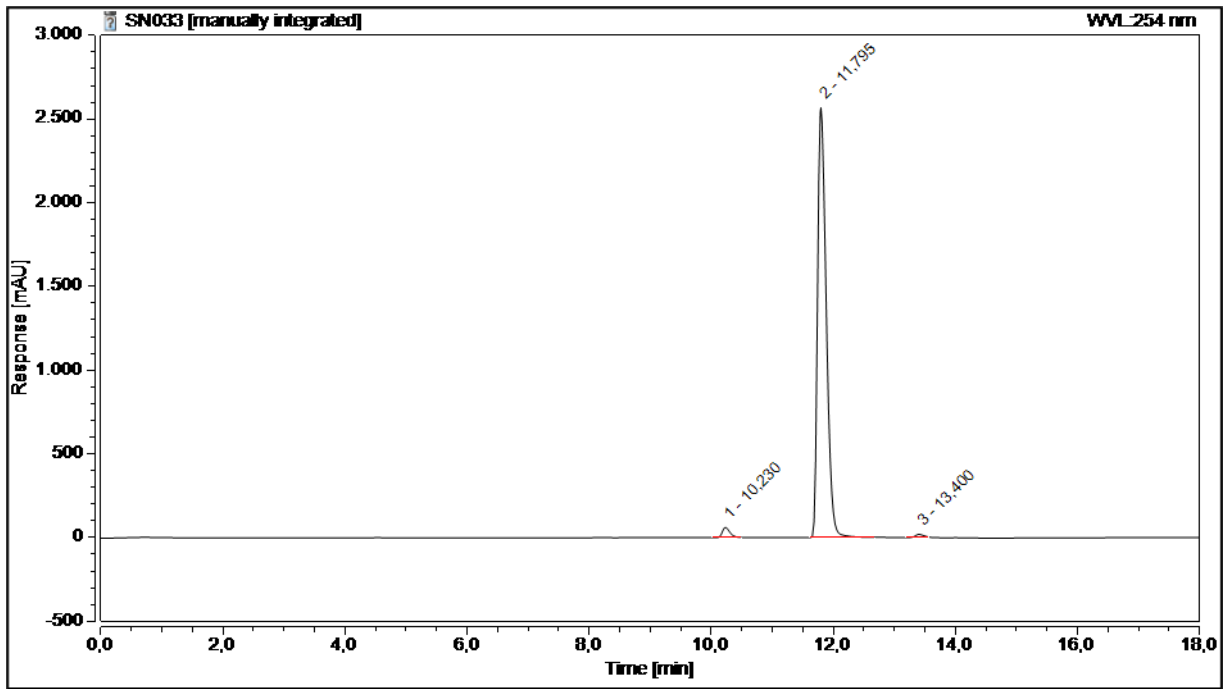


Figure S 1.39: HPLC chromatogramm of SN033.

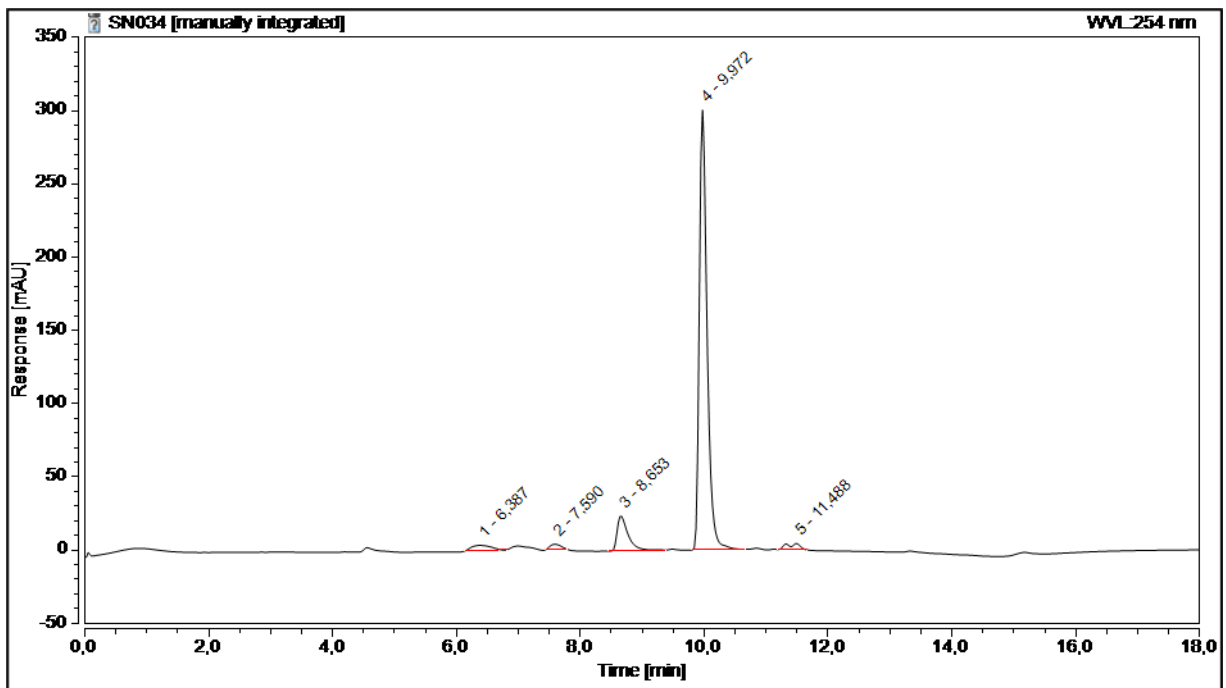


Figure S 1.40: HPLC chromatogramm of SN034.

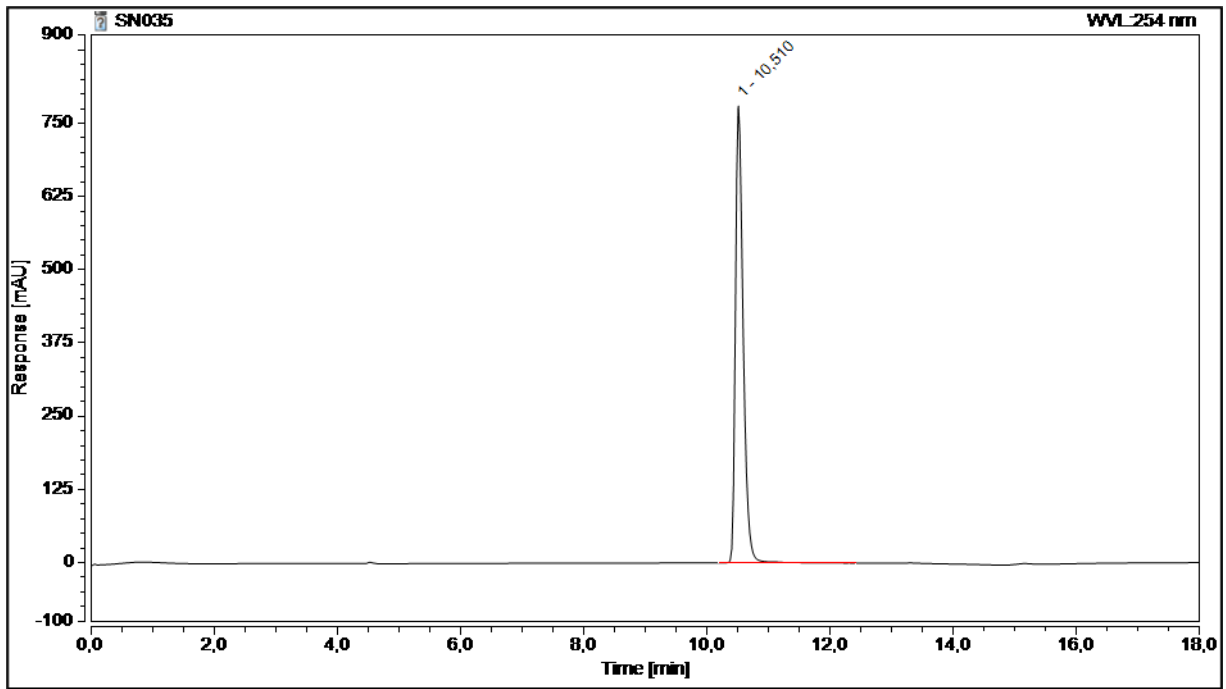


Figure S 1.41: HPLC chromatogramm of SN035.

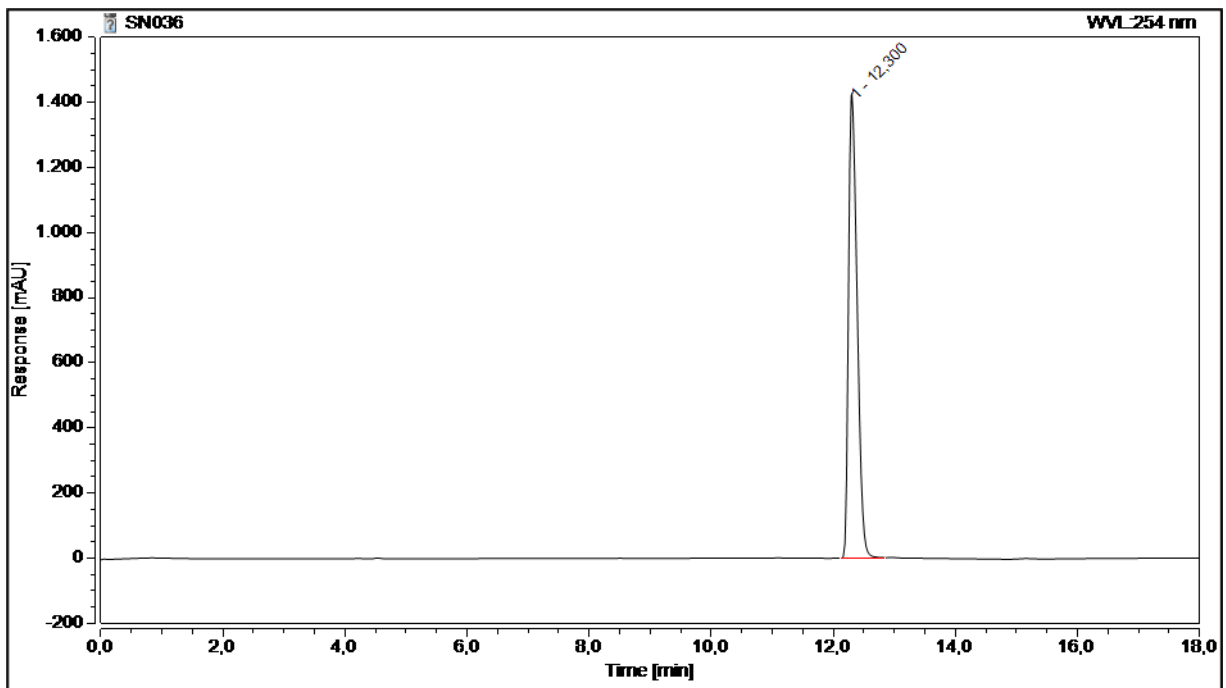


Figure S 1.42: HPLC chromatogramm of SN036.

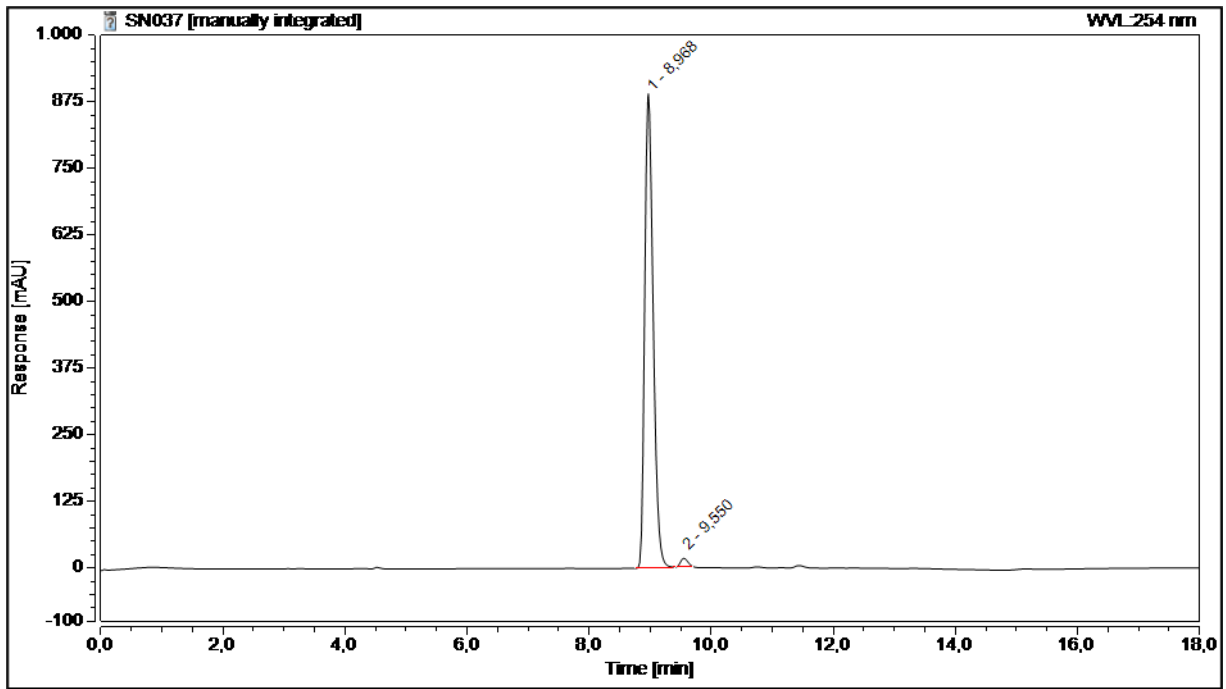


Figure S 1.43: HPLC chromatogram of SN037.

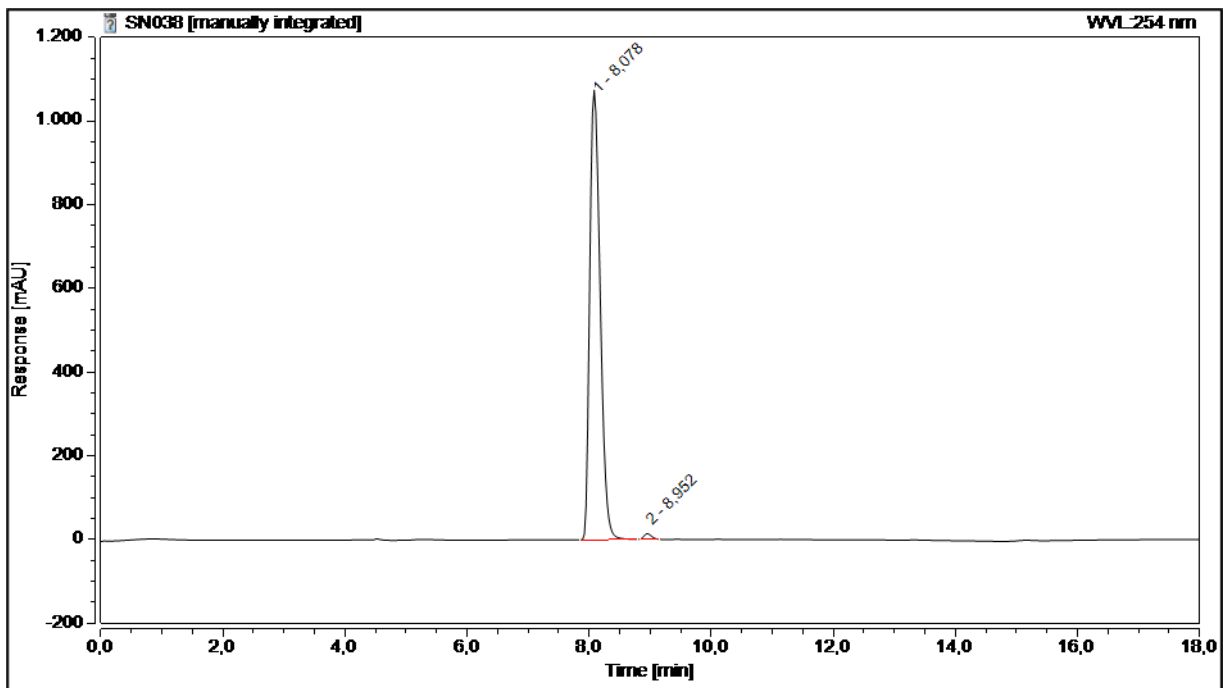


Figure S 1.44: HPLC chromatogram of SN038.

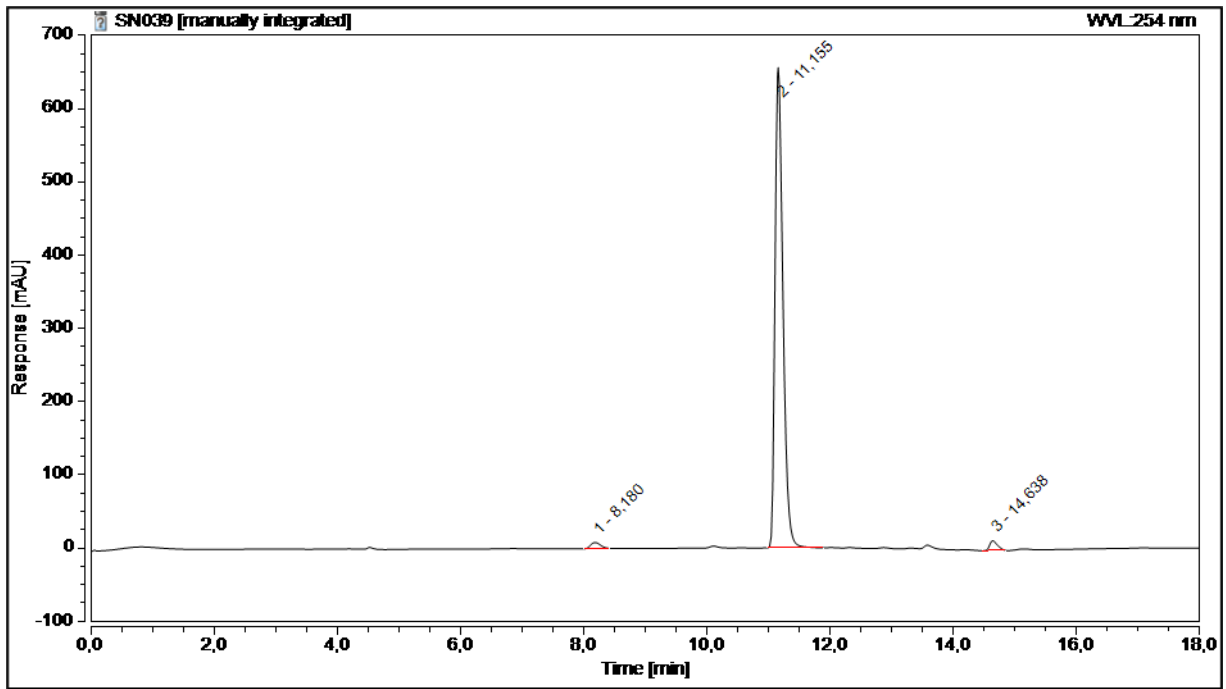


Figure S 1.45: HPLC chromatogramm of SN039.

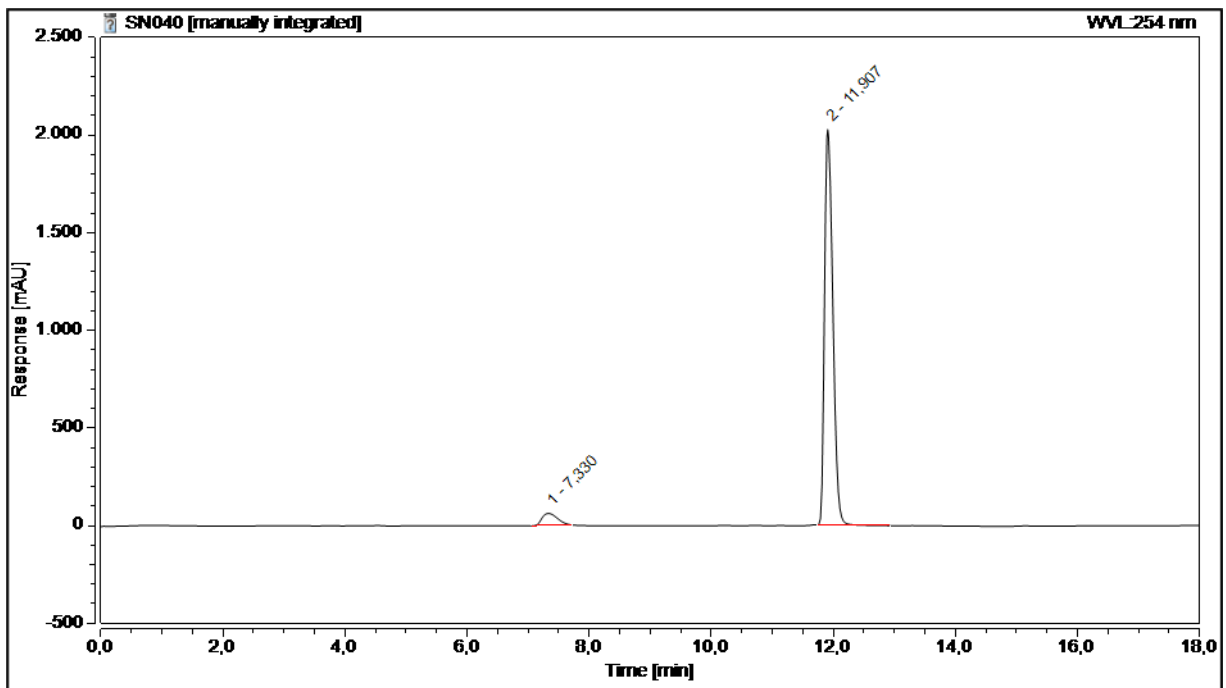


Figure S 1.46: HPLC chromatogramm of SN040.

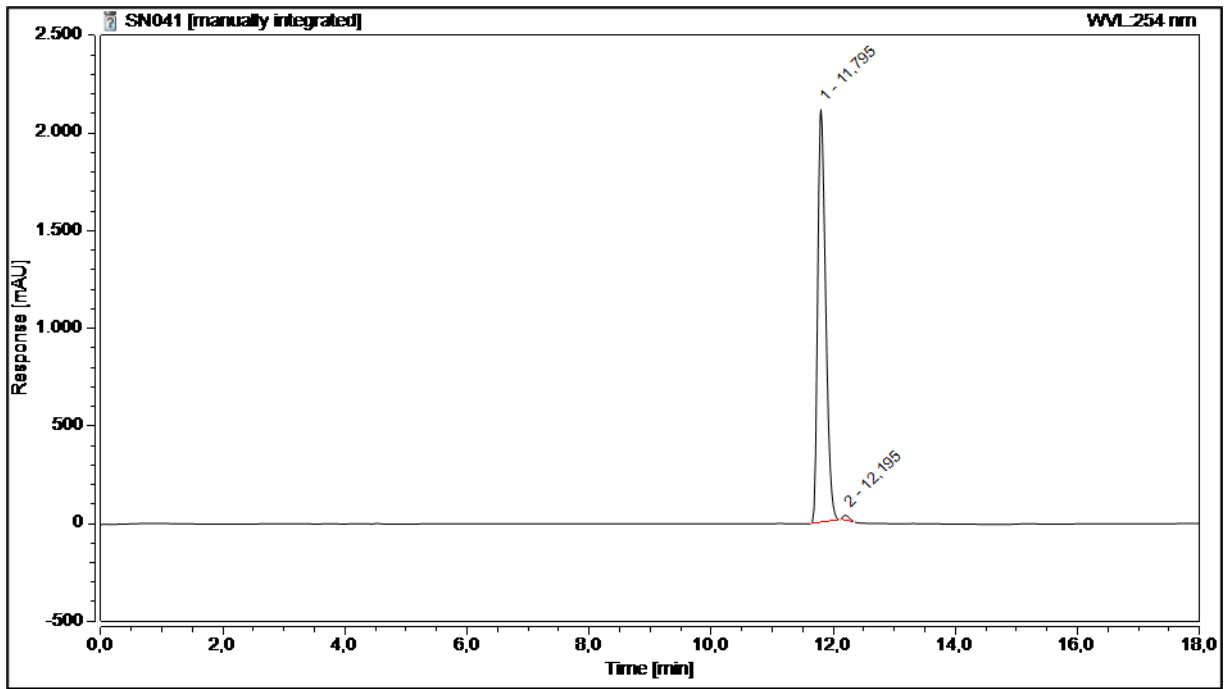


Figure S 1.47: HPLC chromatogramm of SN041.

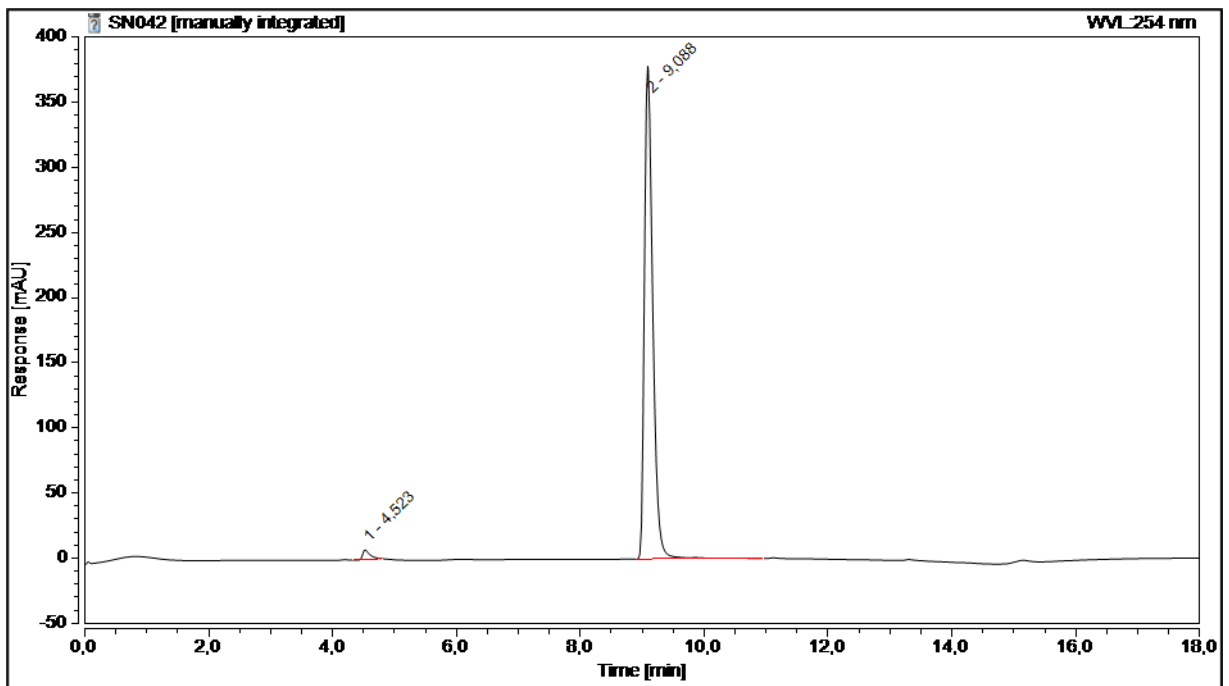


Figure S 1.48: HPLC chromatogramm of SN042.

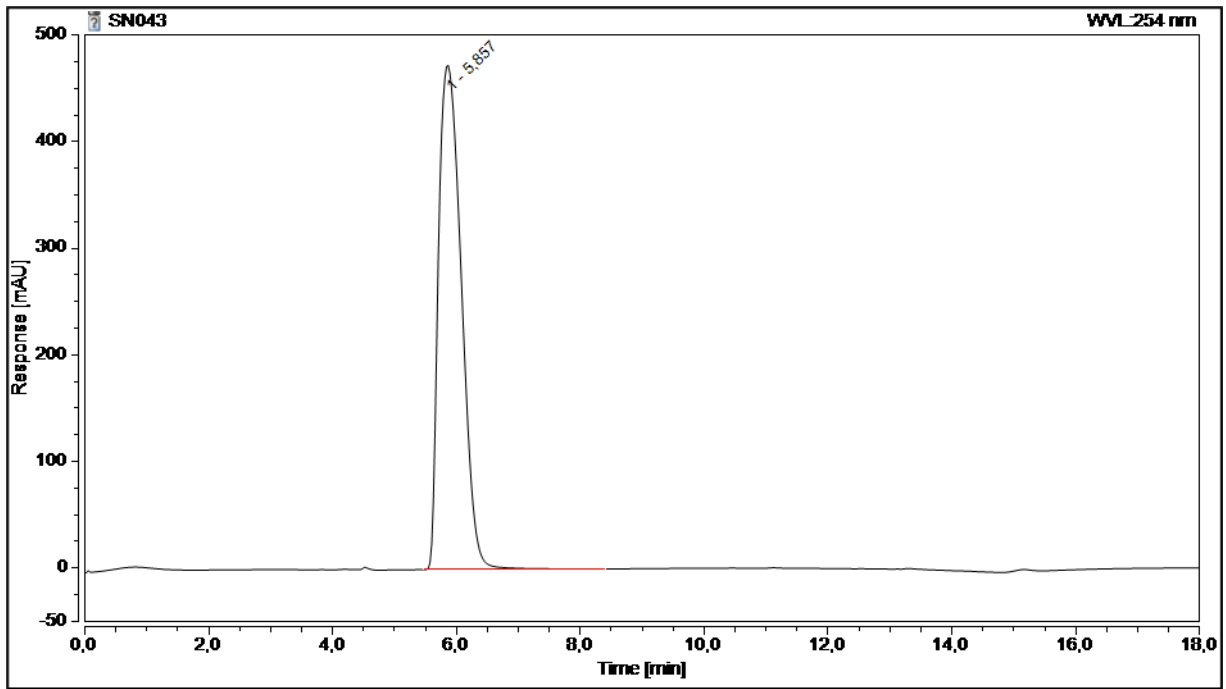


Figure S 1.49: HPLC chromatogramm of SN043.

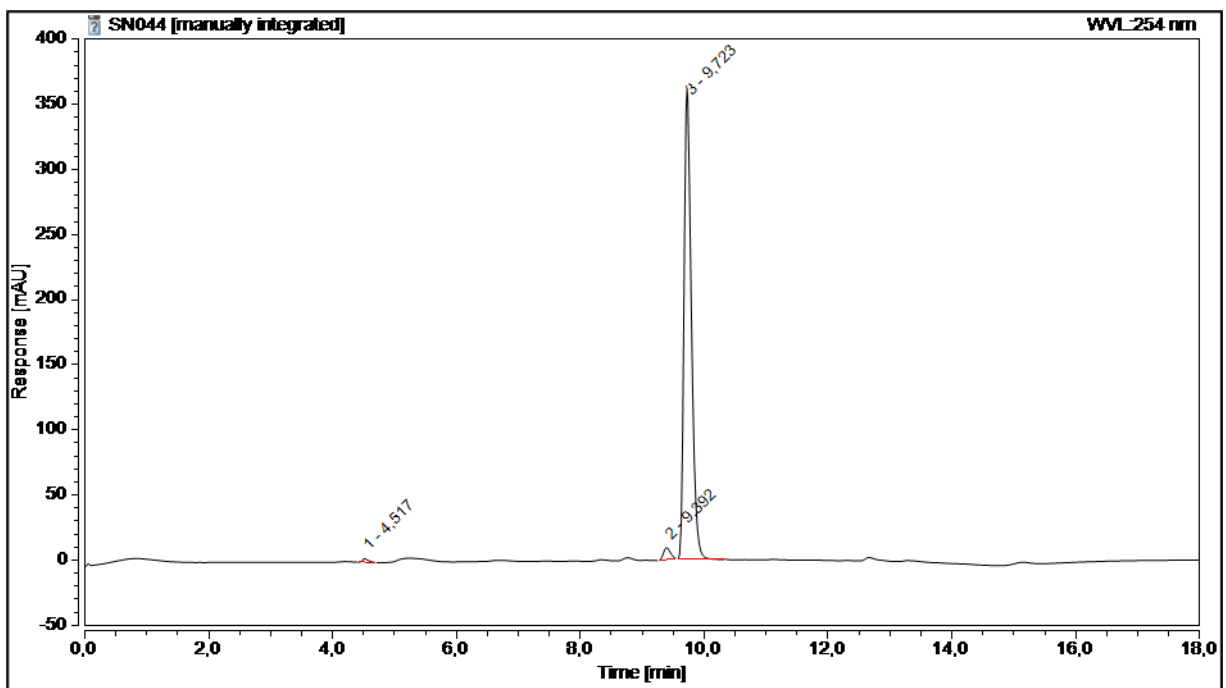


Figure S 1.50: HPLC chromatogramm of SN044.

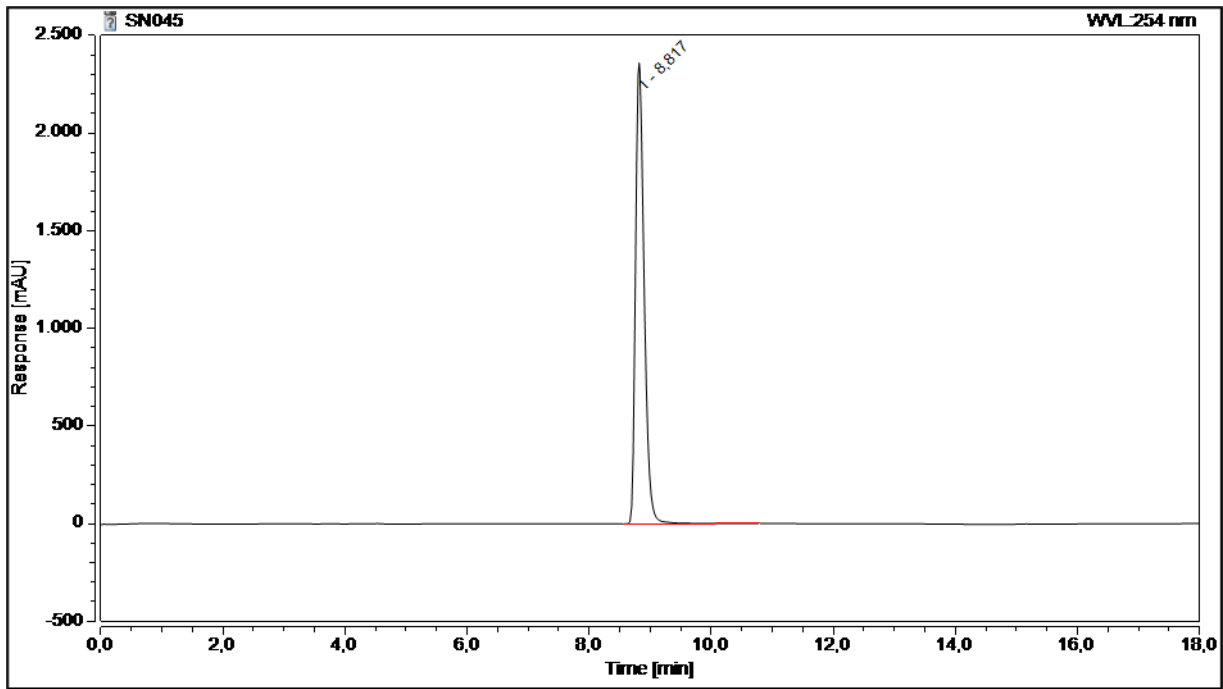


Figure S 1.51: HPLC chromatogramm of SN045.

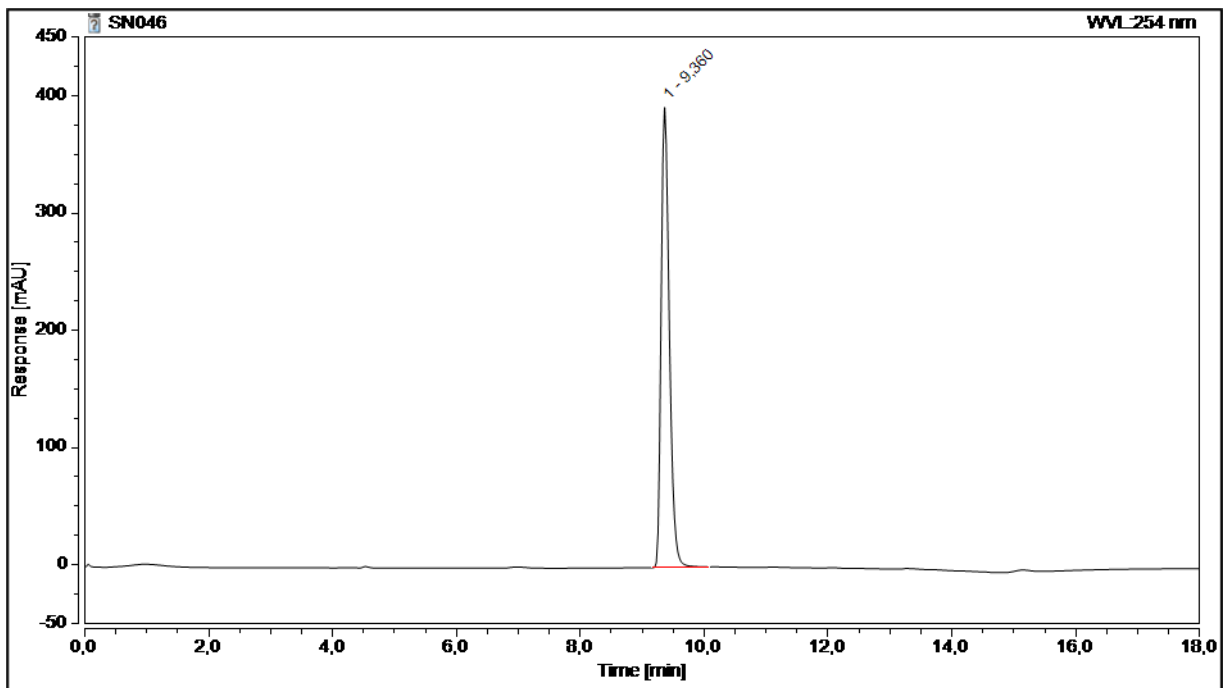


Figure S 1.52: HPLC chromatogramm of SN046.

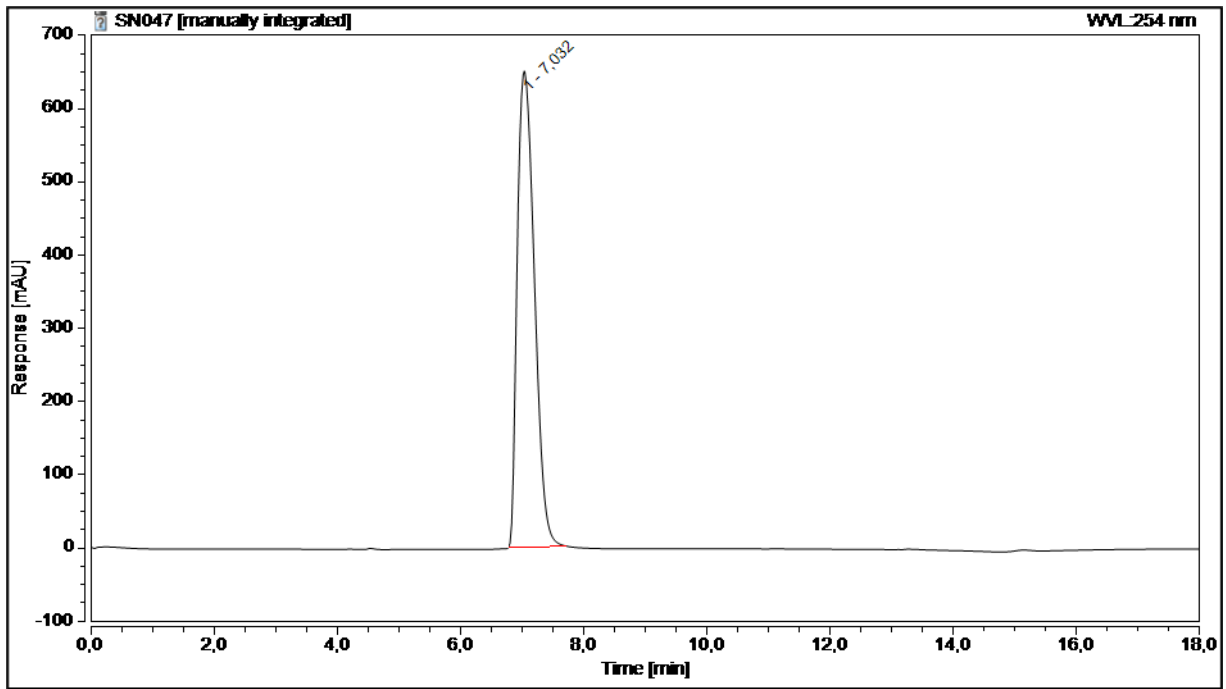


Figure S 1.53: HPLC chromatogramm of SN047.

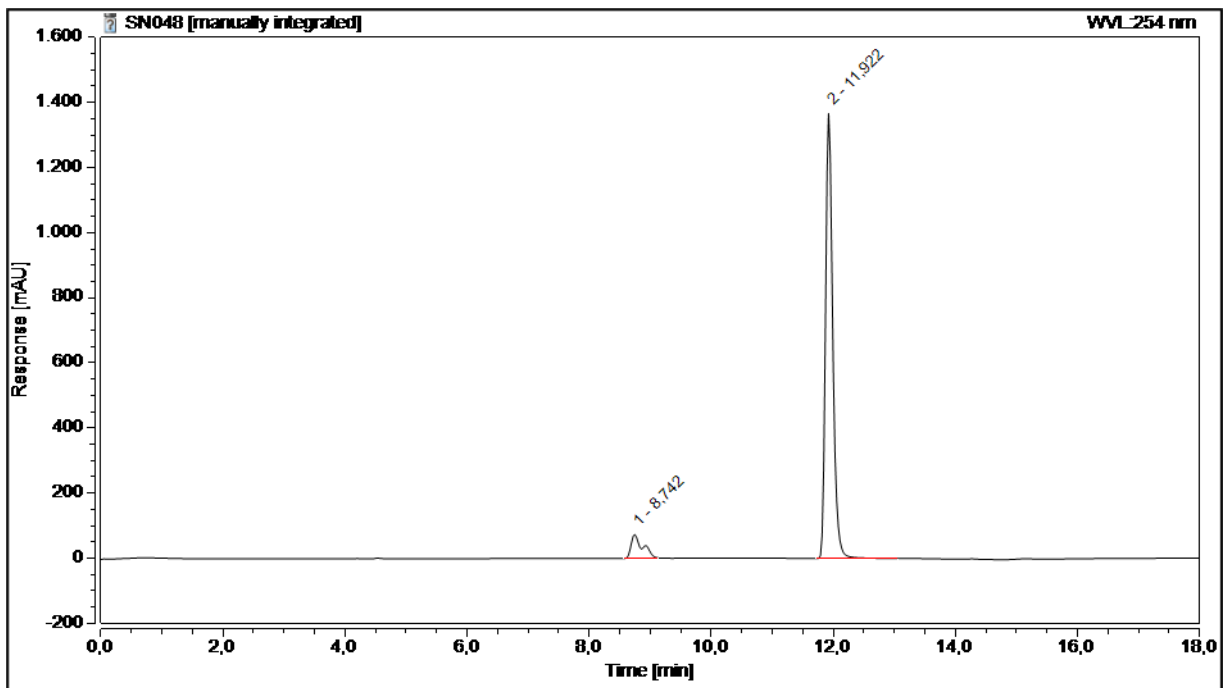


Figure S 1.54: HPLC chromatogramm of SN048.

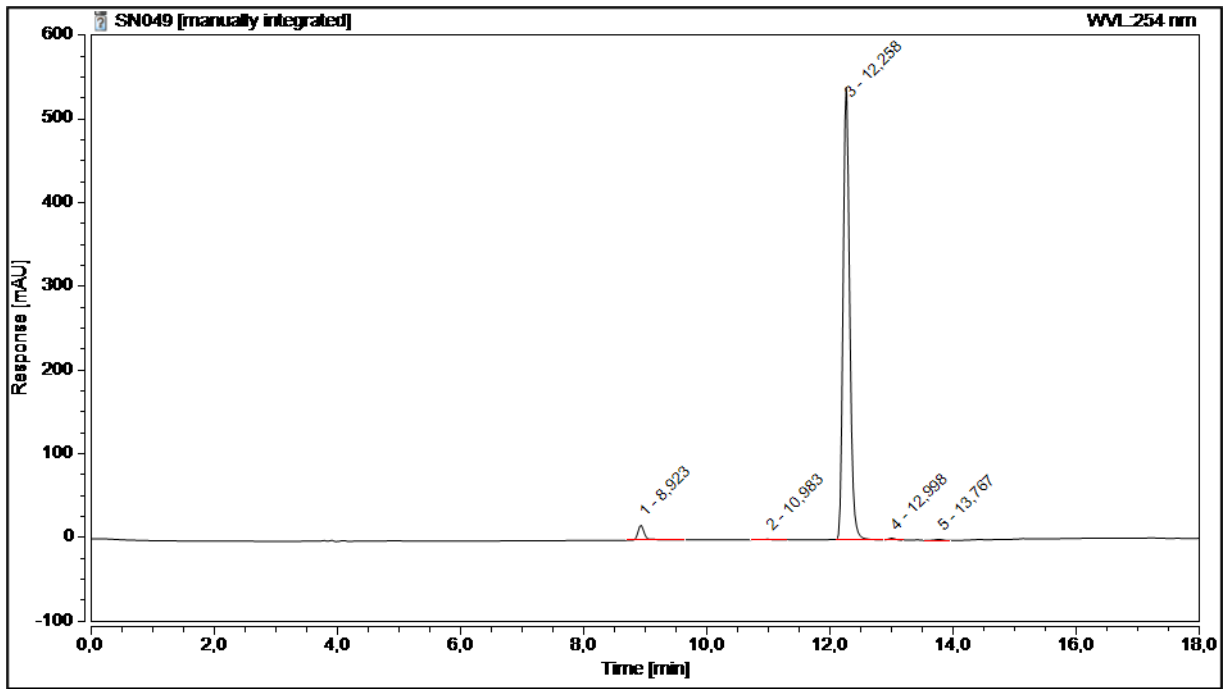


Figure S 1.55: HPLC chromatogramm of SN049.

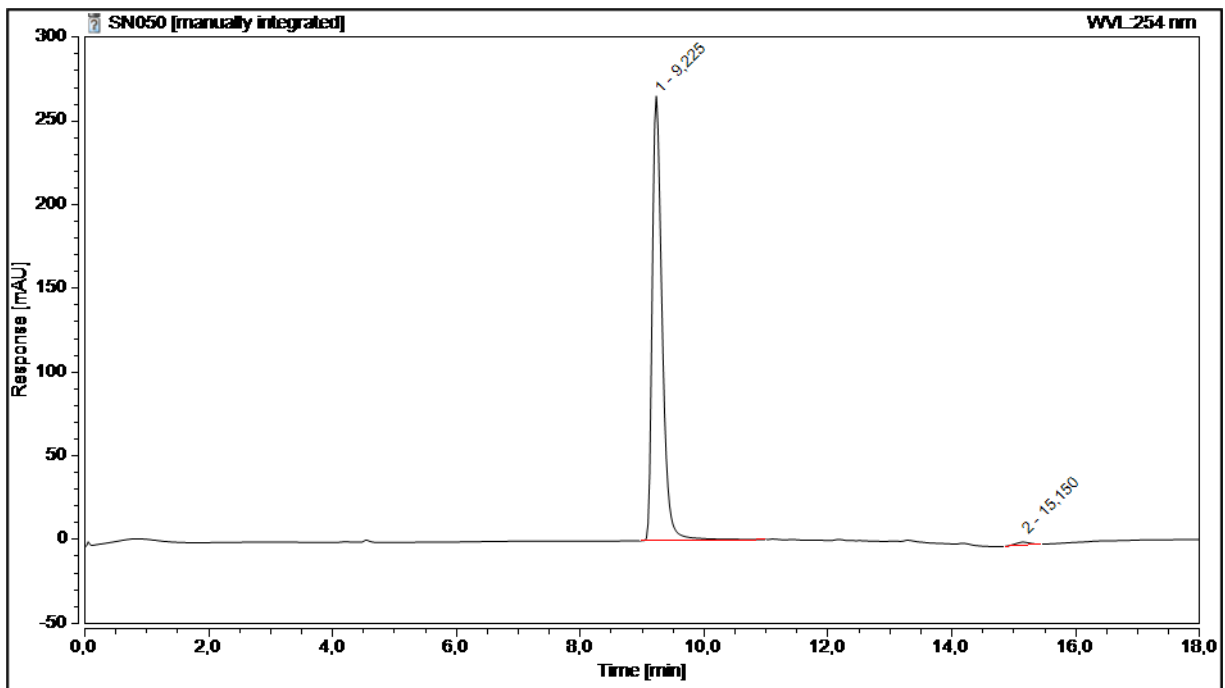


Figure S 1.56: HPLC chromatogramm of SN050.

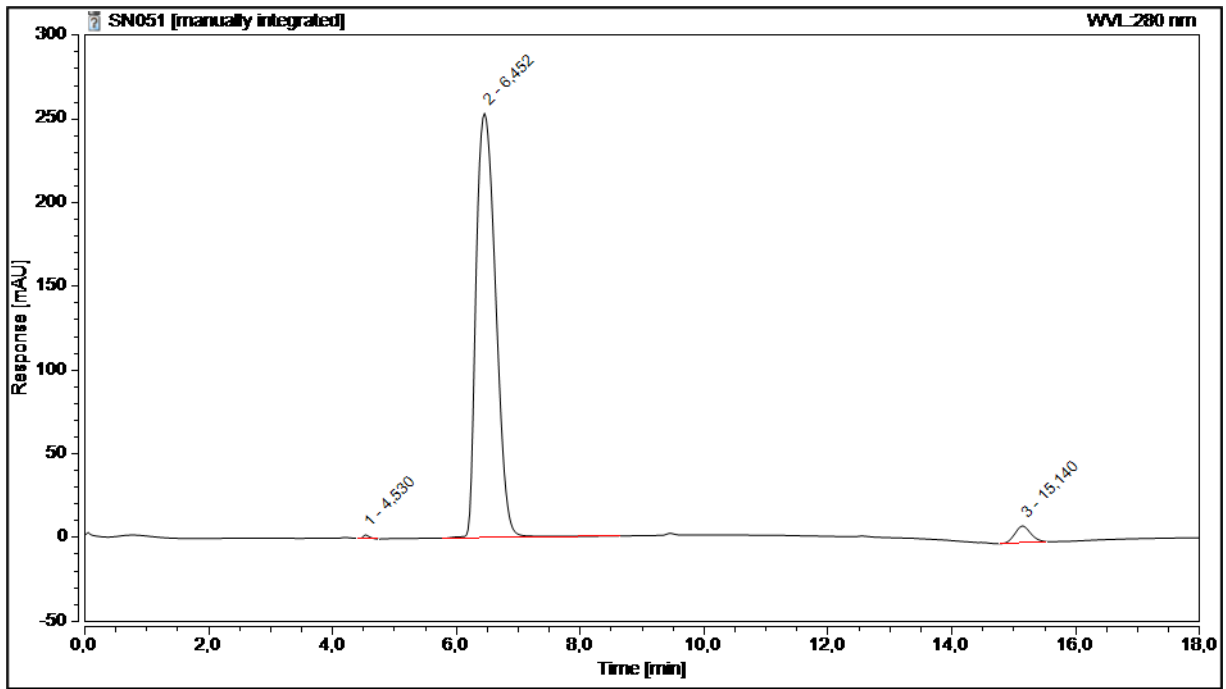


Figure S 1.57: HPLC chromatogramm of SN051.

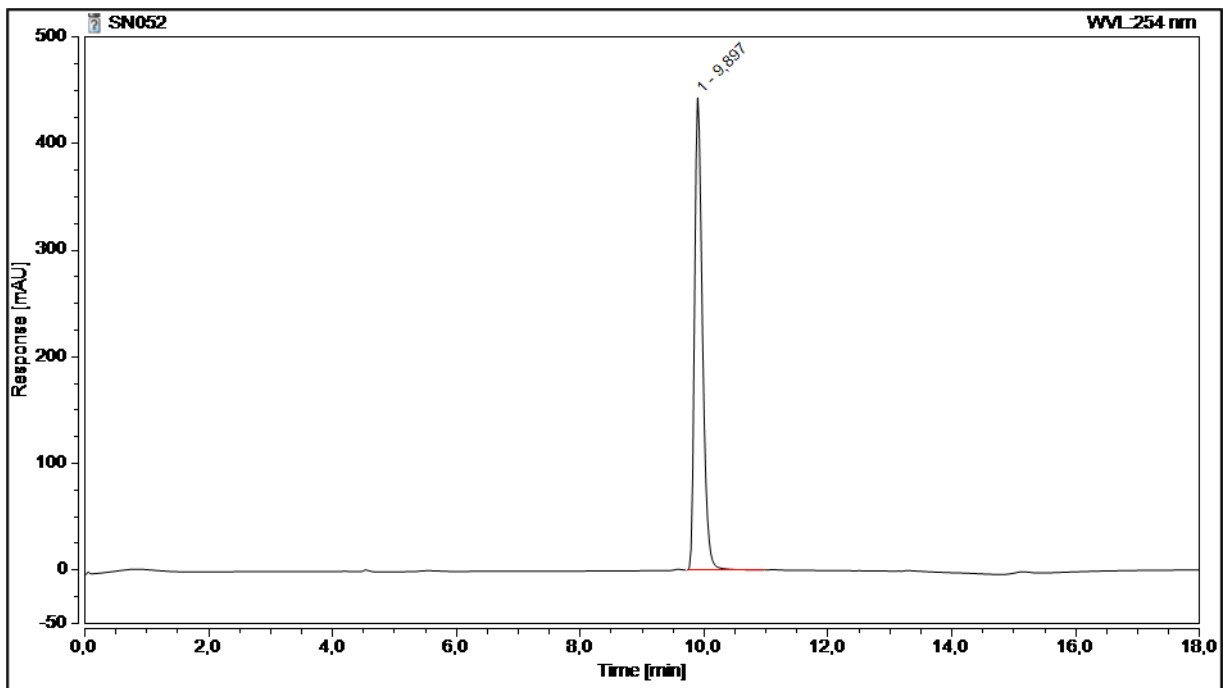


Figure S 1.58: HPLC chromatogramm of SN052.

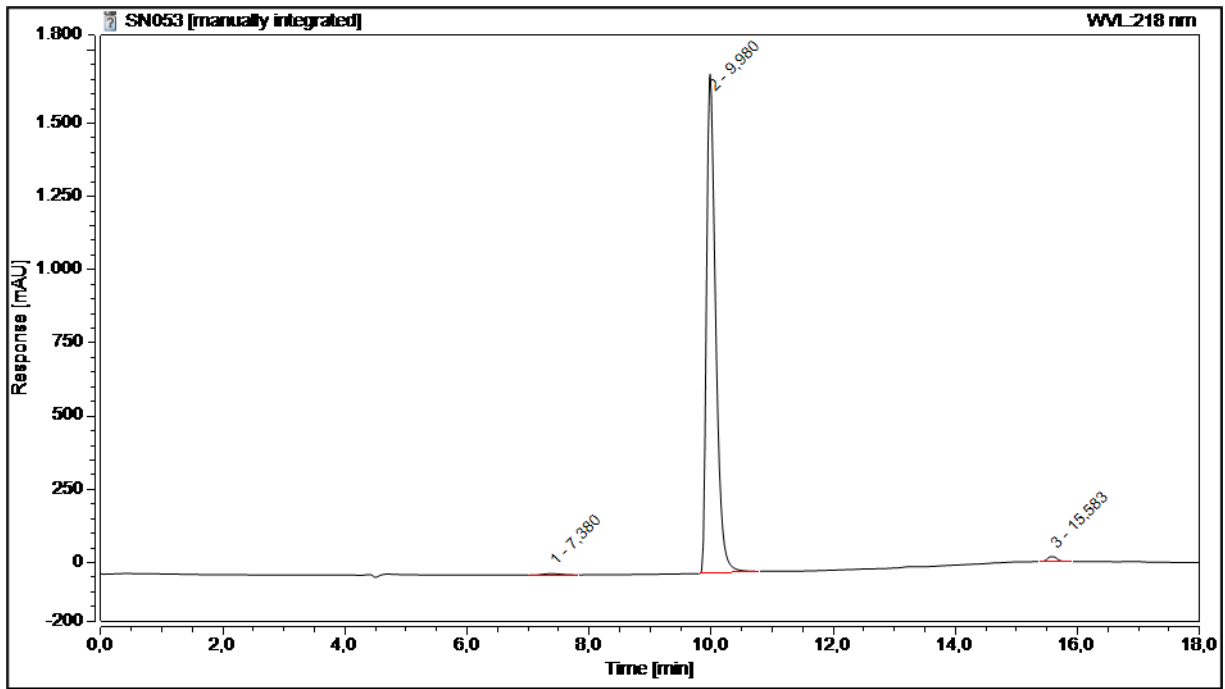


Figure S 1.59: HPLC chromatogramm of SN053.

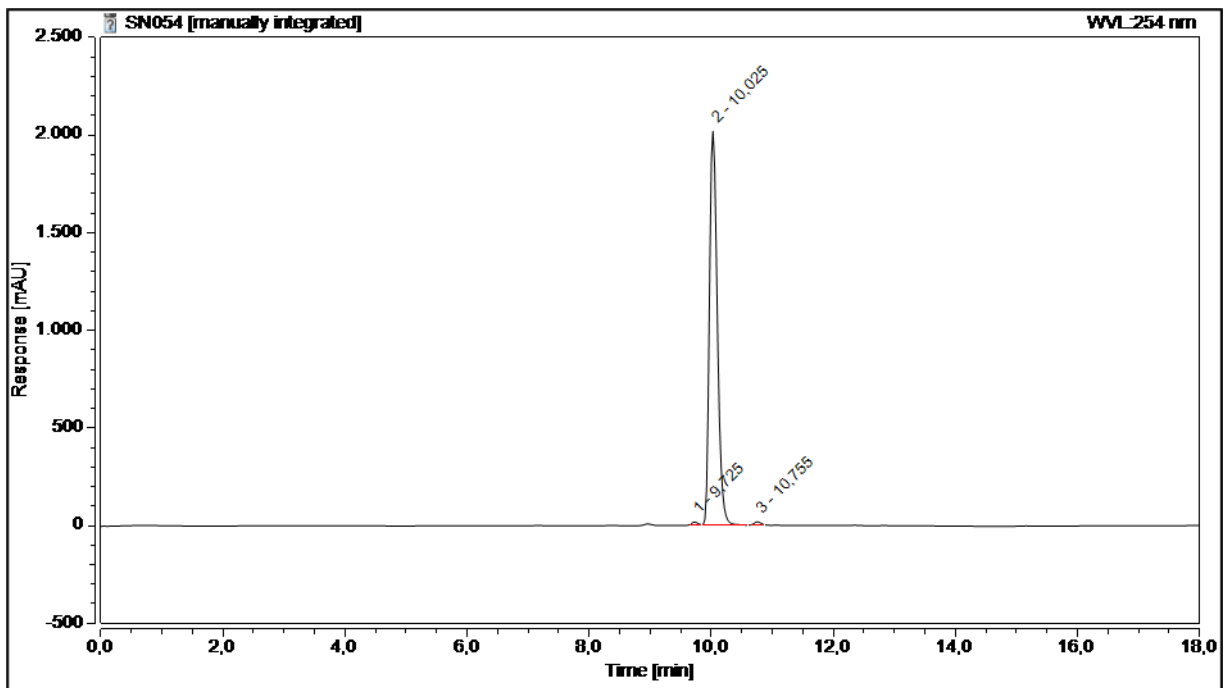


Figure S 1.60: HPLC chromatogramm of SN054.

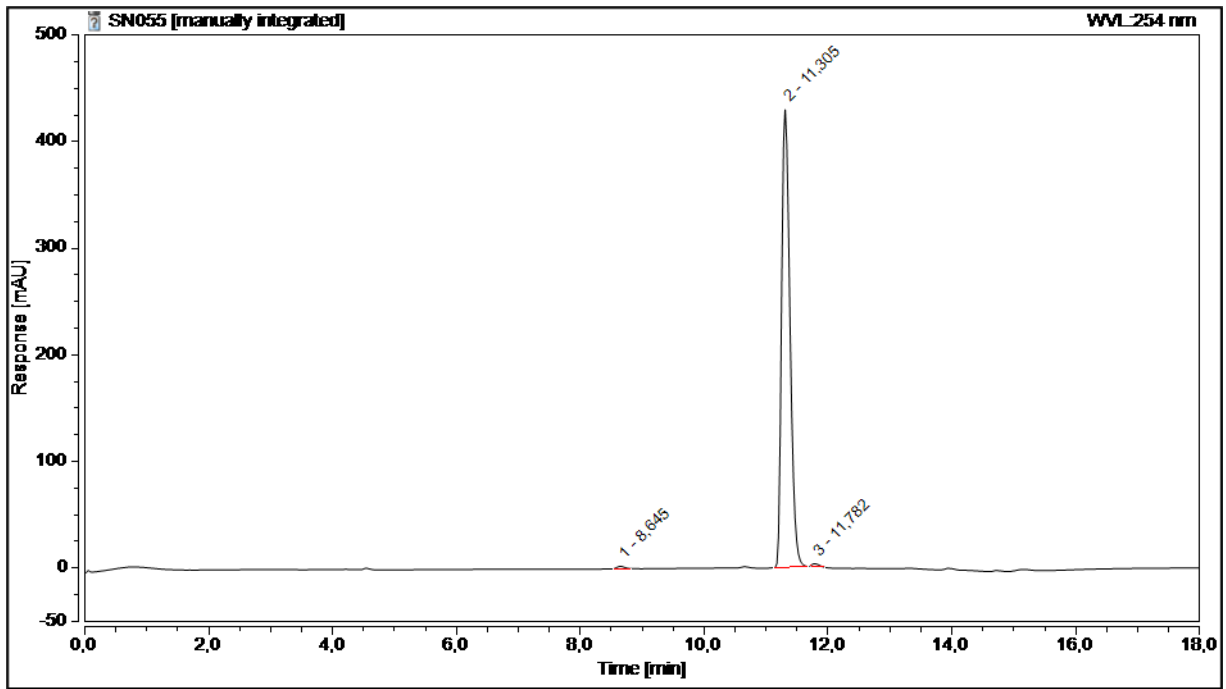


Figure S 1.61: HPLC chromatogramm of SN055.

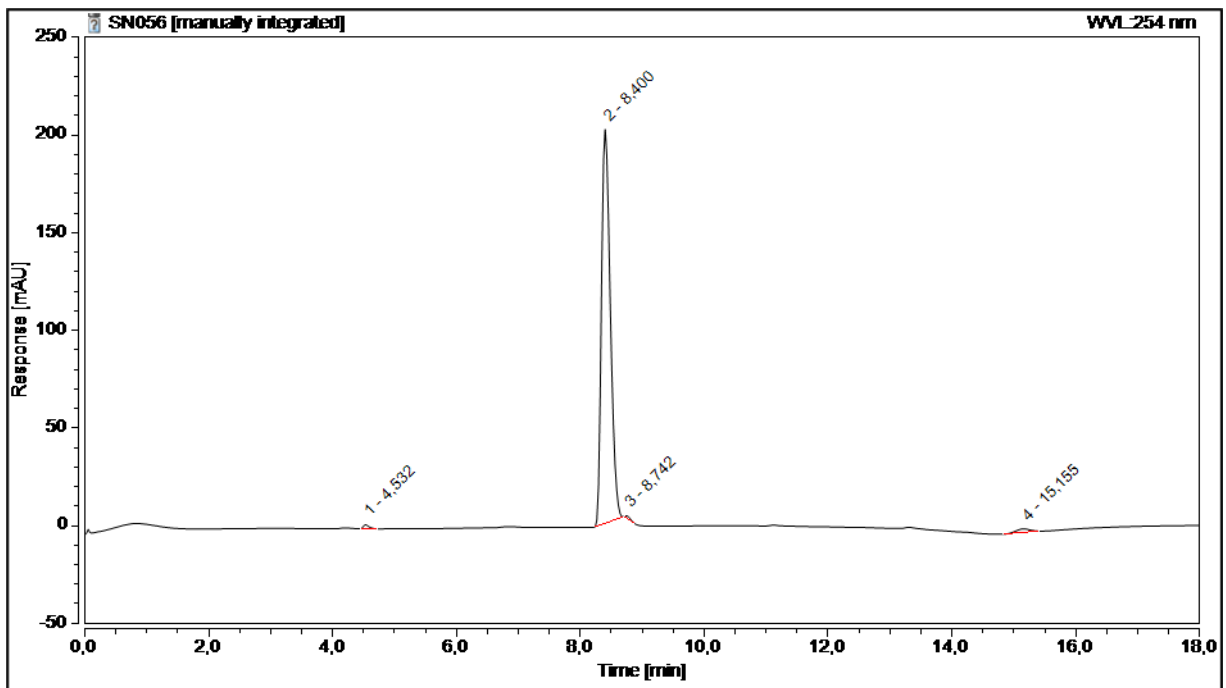


Figure S 1.62: HPLC chromatogramm of SN056.

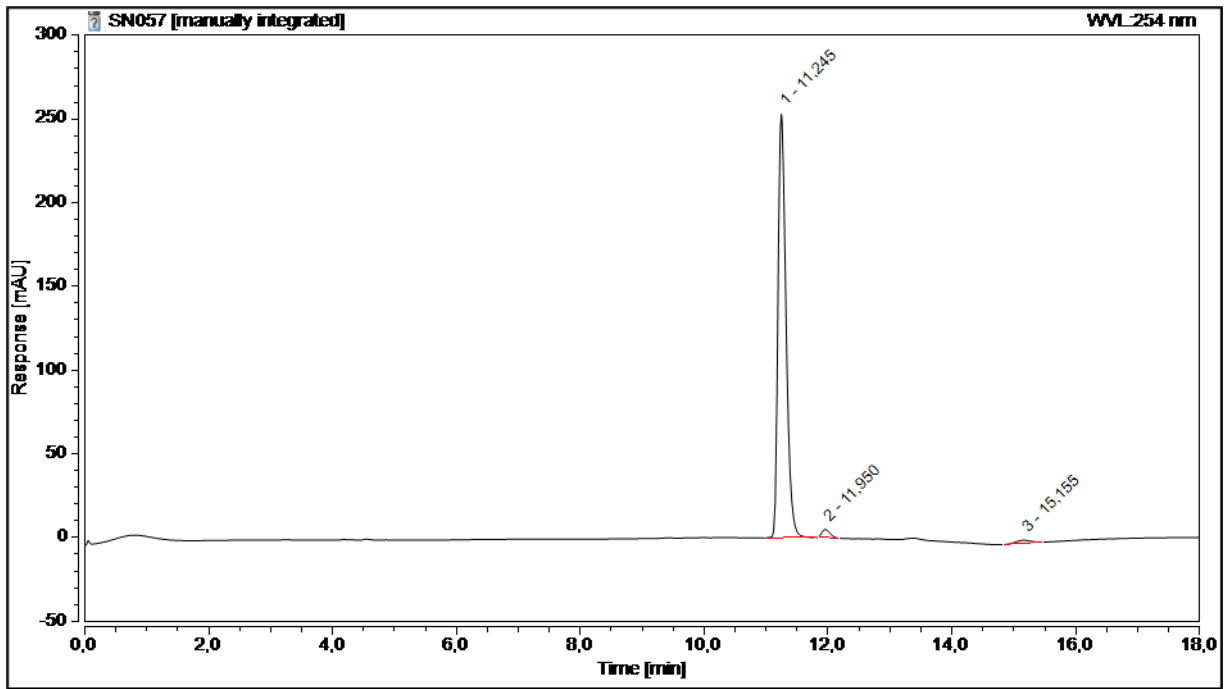


Figure S 1.63: HPLC chromatogramm of SN057.

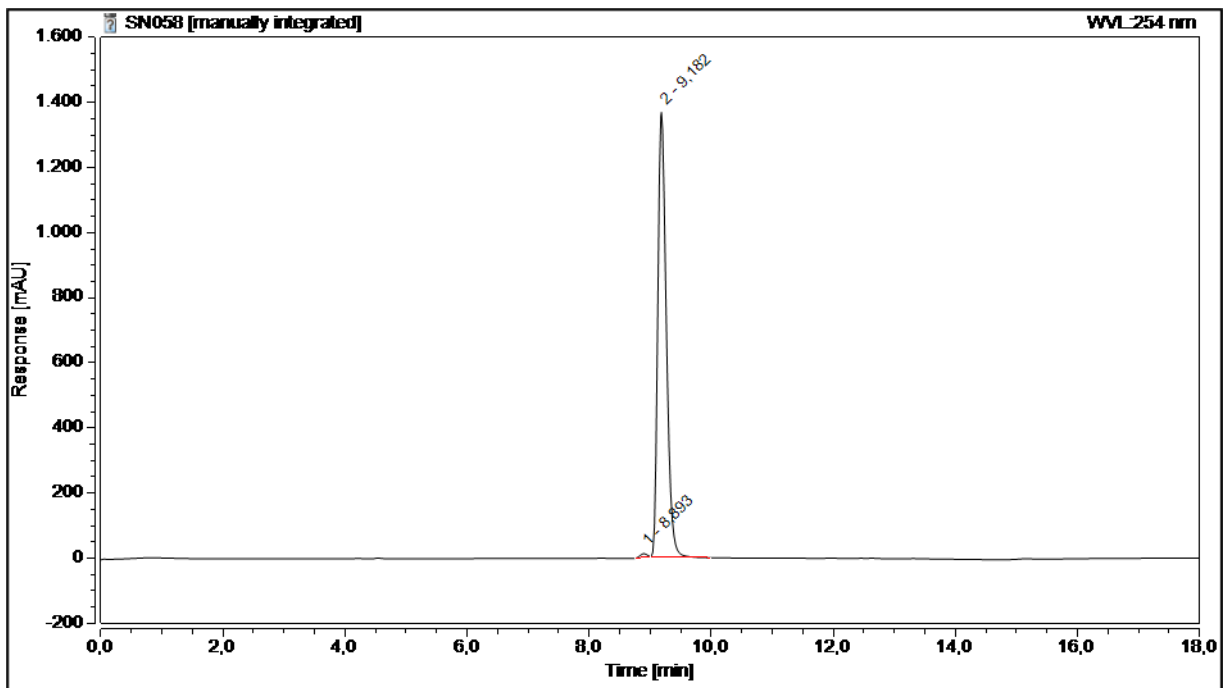


Figure S 1.64: HPLC chromatogramm of SN058.

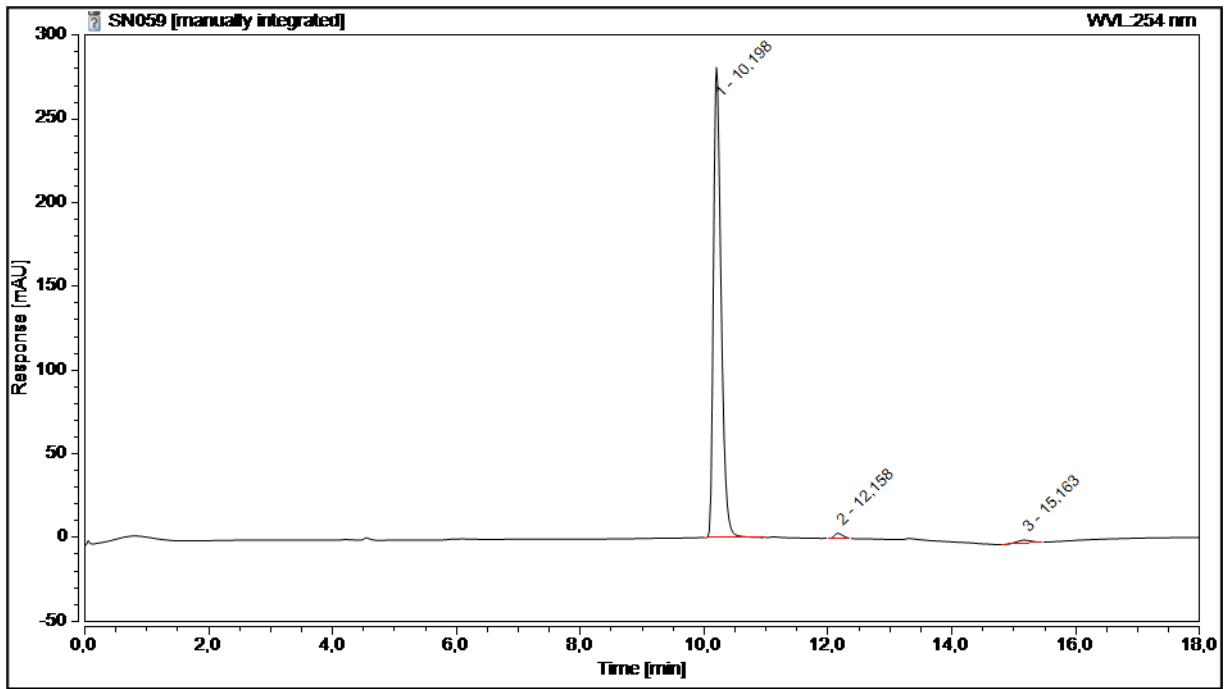


Figure S 1.65: HPLC chromatogramm of SN059.

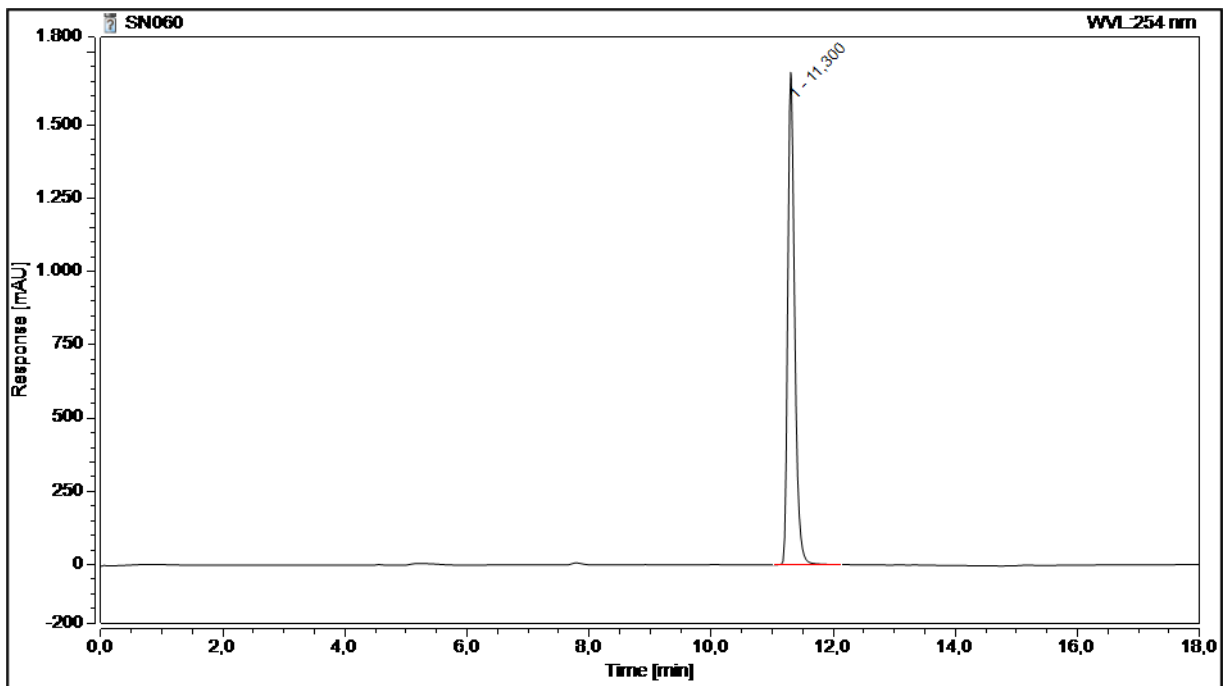


Figure S 1.66: HPLC chromatogramm of SN060.

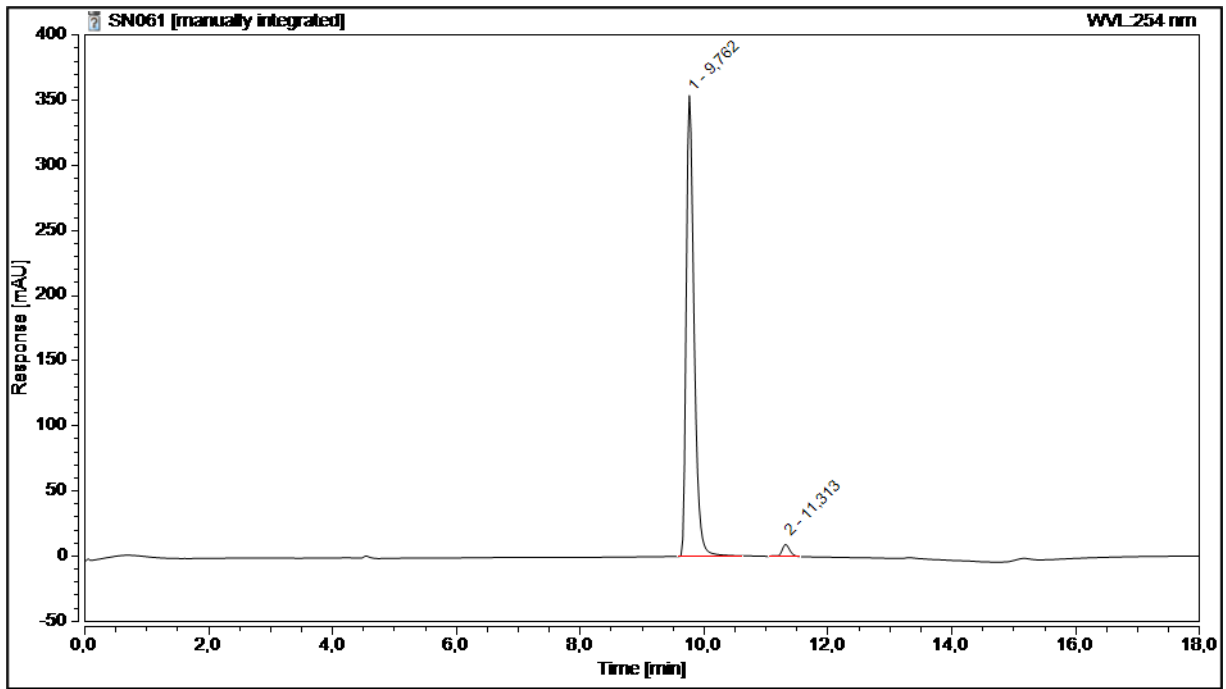


Figure S 1.67: HPLC chromatogramm of SN061.

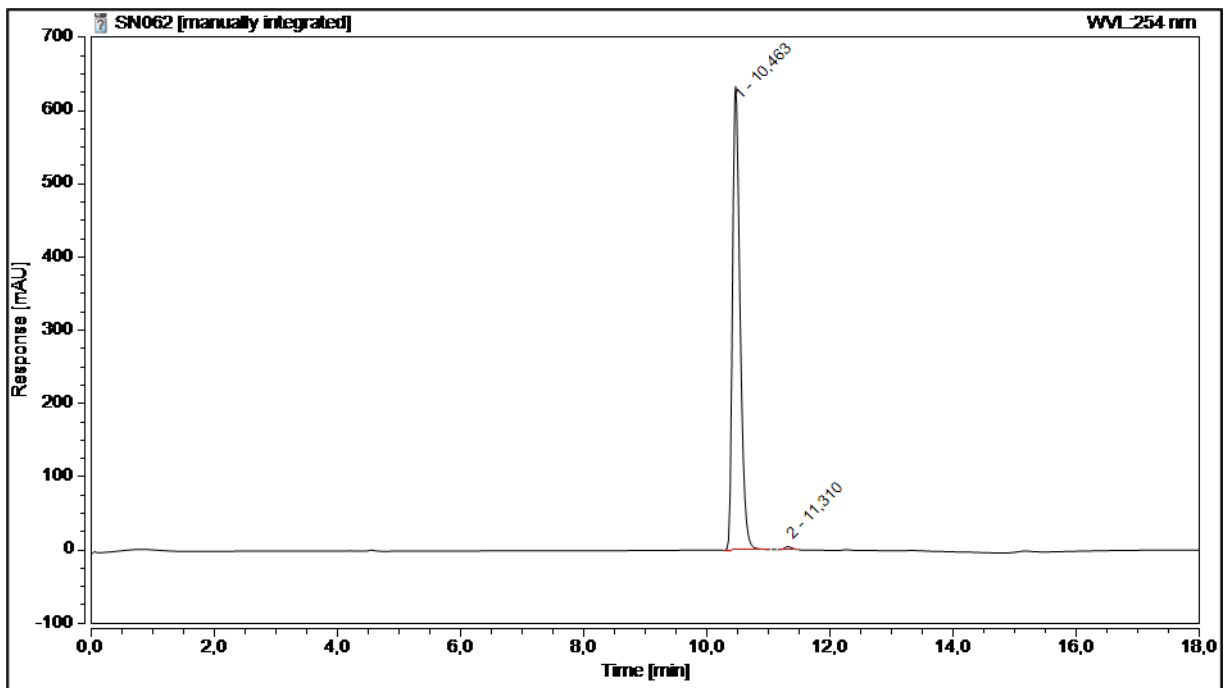


Figure S 1.68: HPLC chromatogramm of SN062.

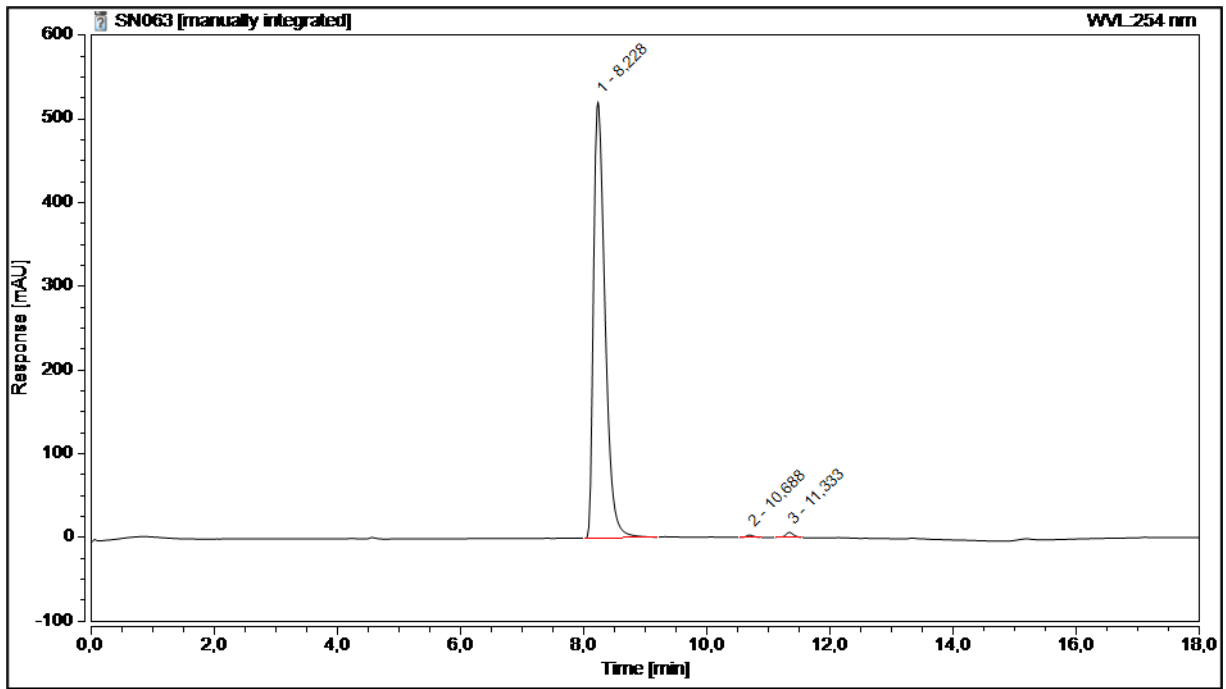


Figure S 1.69: HPLC chromatogramm of SN063.

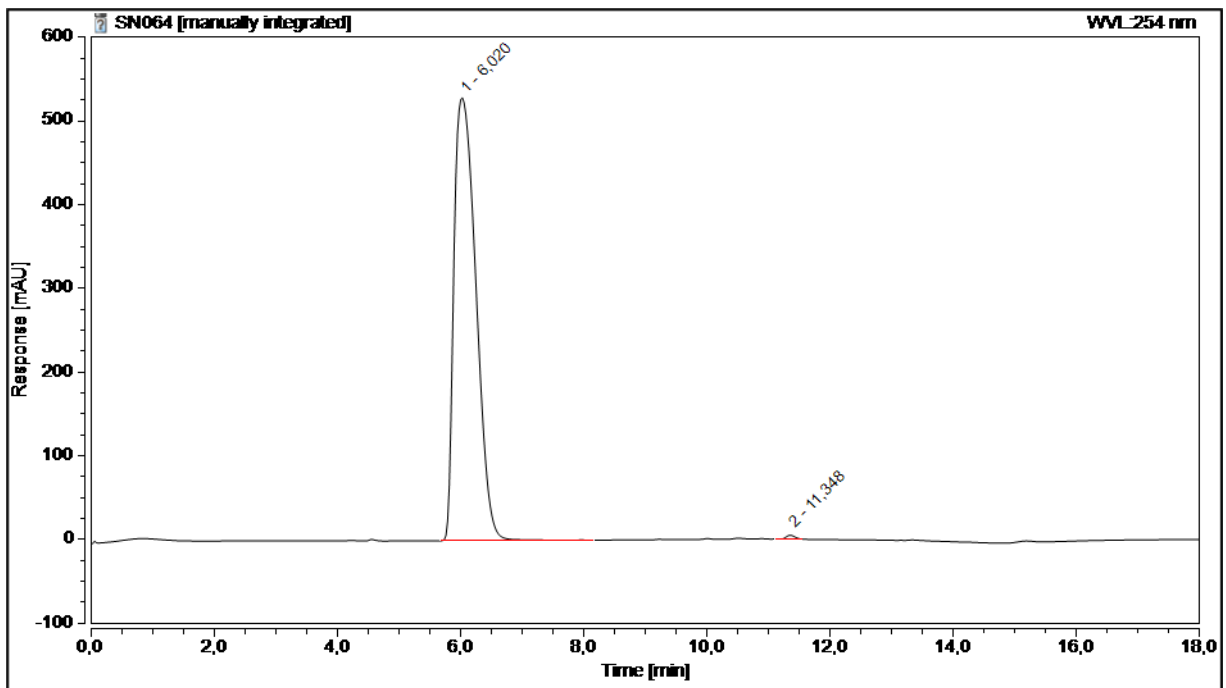


Figure S 1.70: HPLC chromatogramm of SN064.

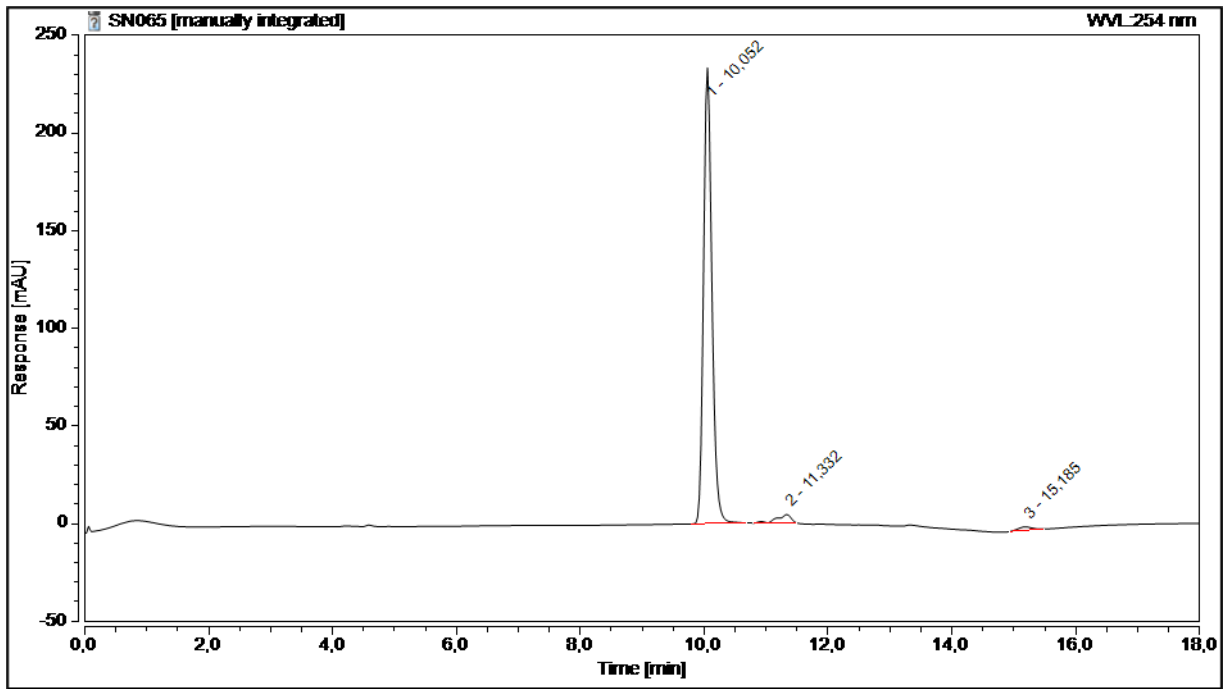


Figure S 1.71: HPLC chromatogramm of SN065.

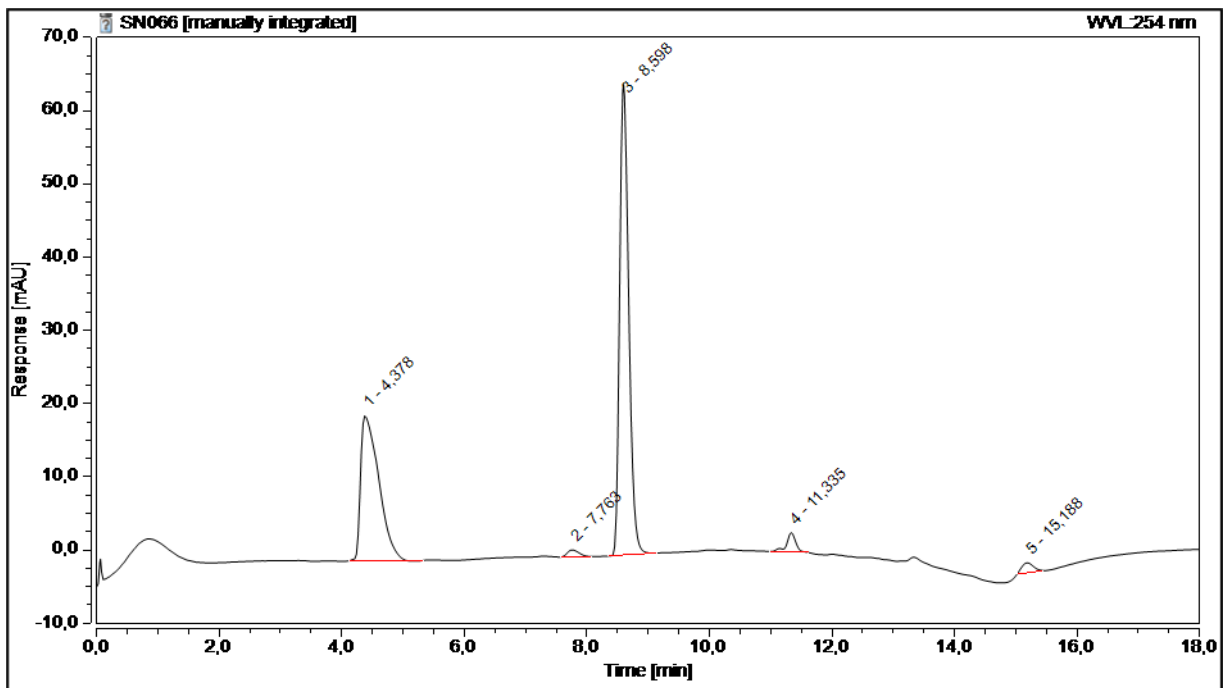


Figure S 1.72: HPLC chromatogramm of SN066.

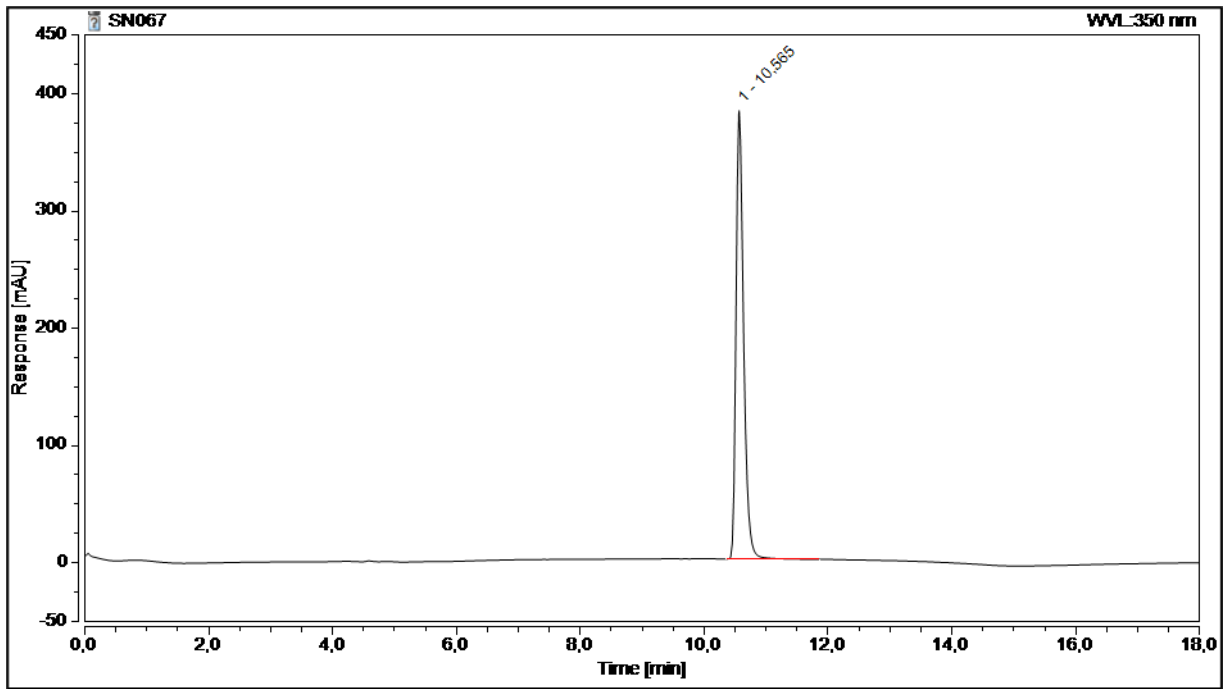


Figure S 1.73: HPLC chromatogramm of SN067.

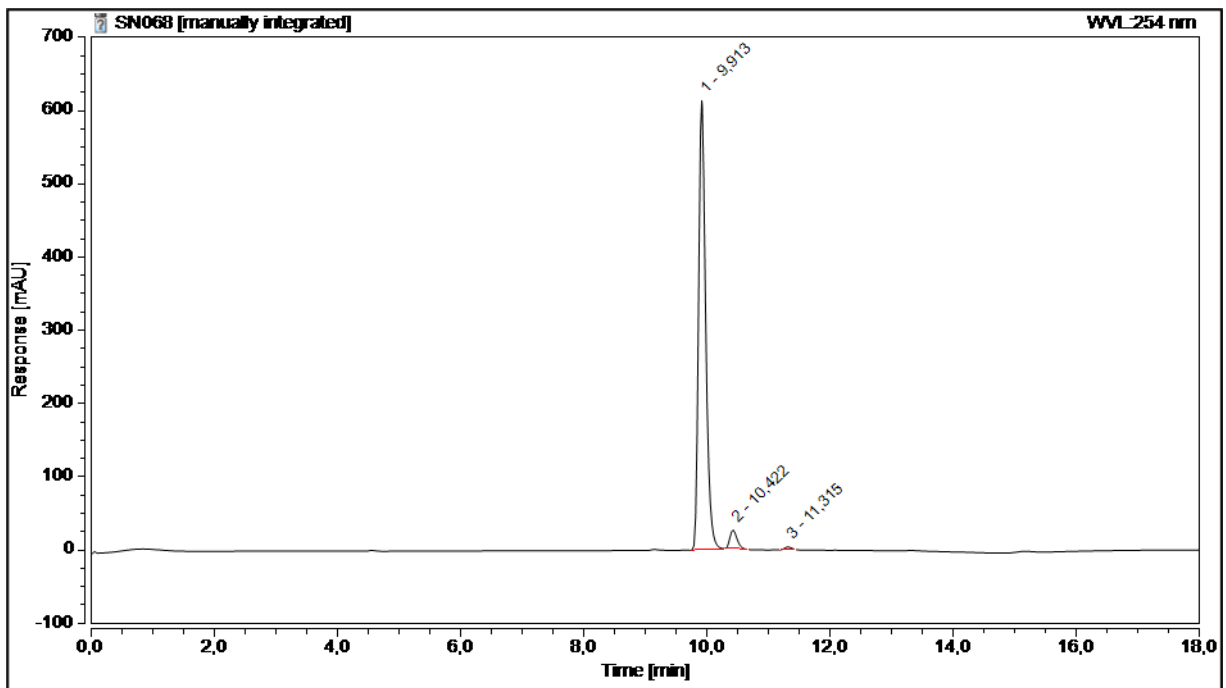


Figure S 1.74: HPLC chromatogramm of SN068.

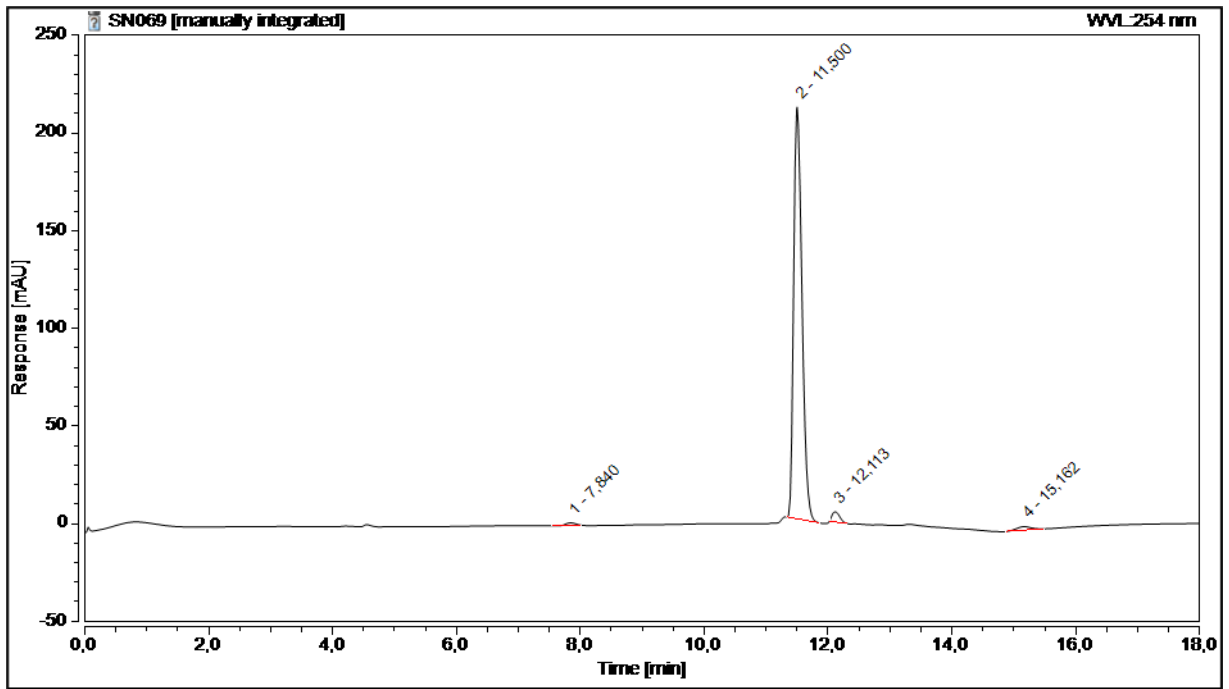


Figure S 1.75: HPLC chromatogramm of SN069.

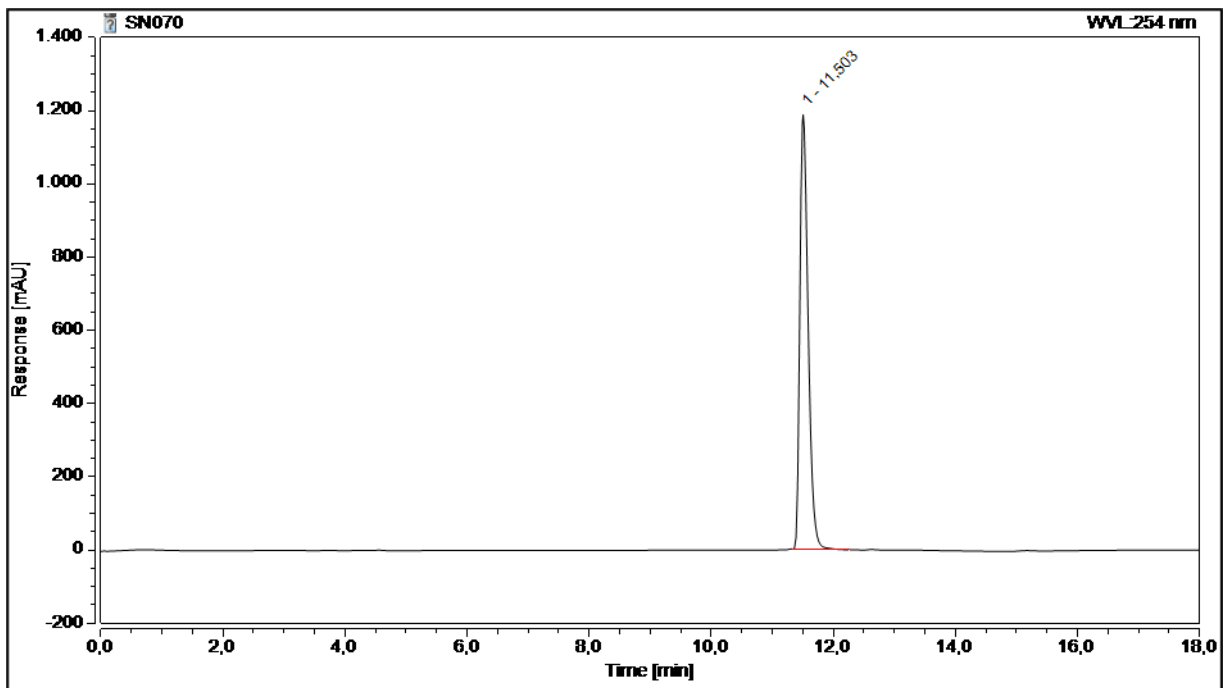


Figure S 1.76: HPLC chromatogramm of SN070.

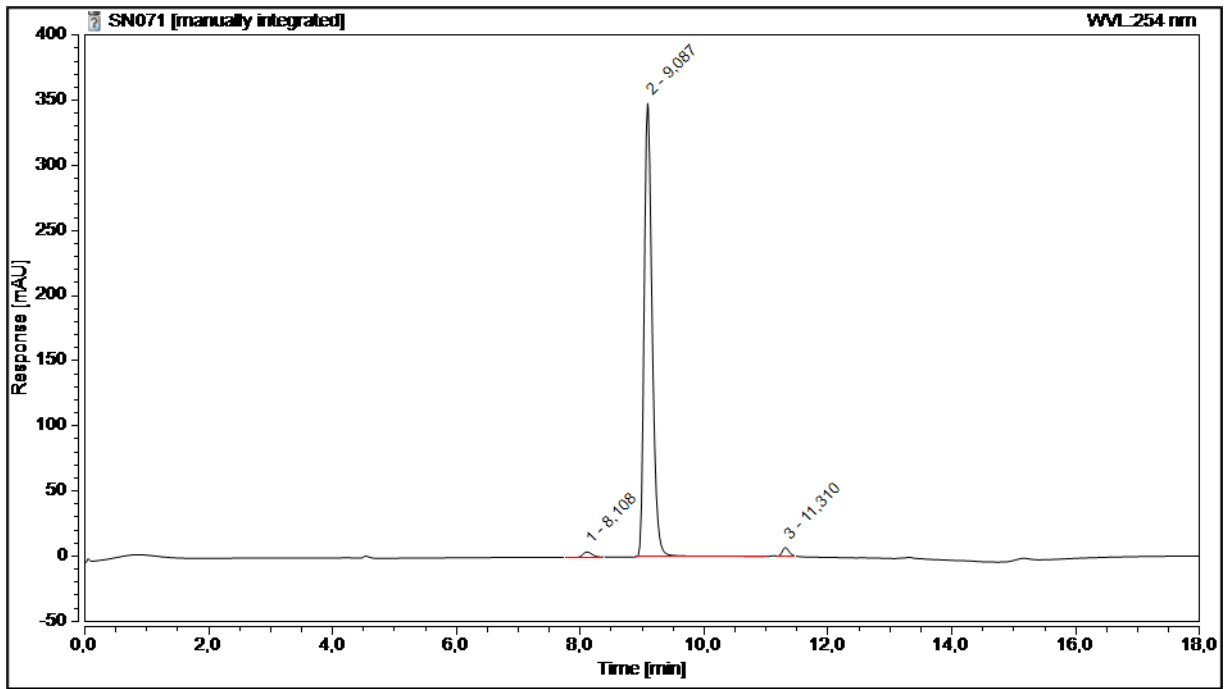


Figure S 1.77: HPLC chromatogramm of SN071.

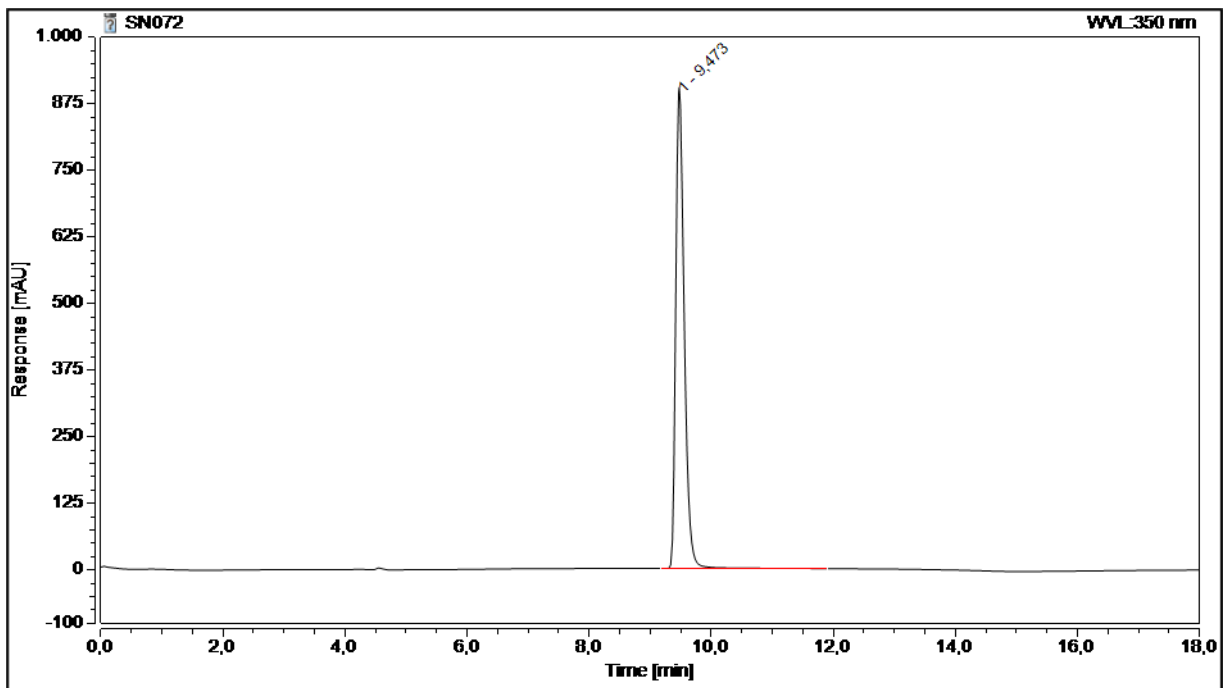


Figure S 1.78: HPLC chromatogramm of SN072.

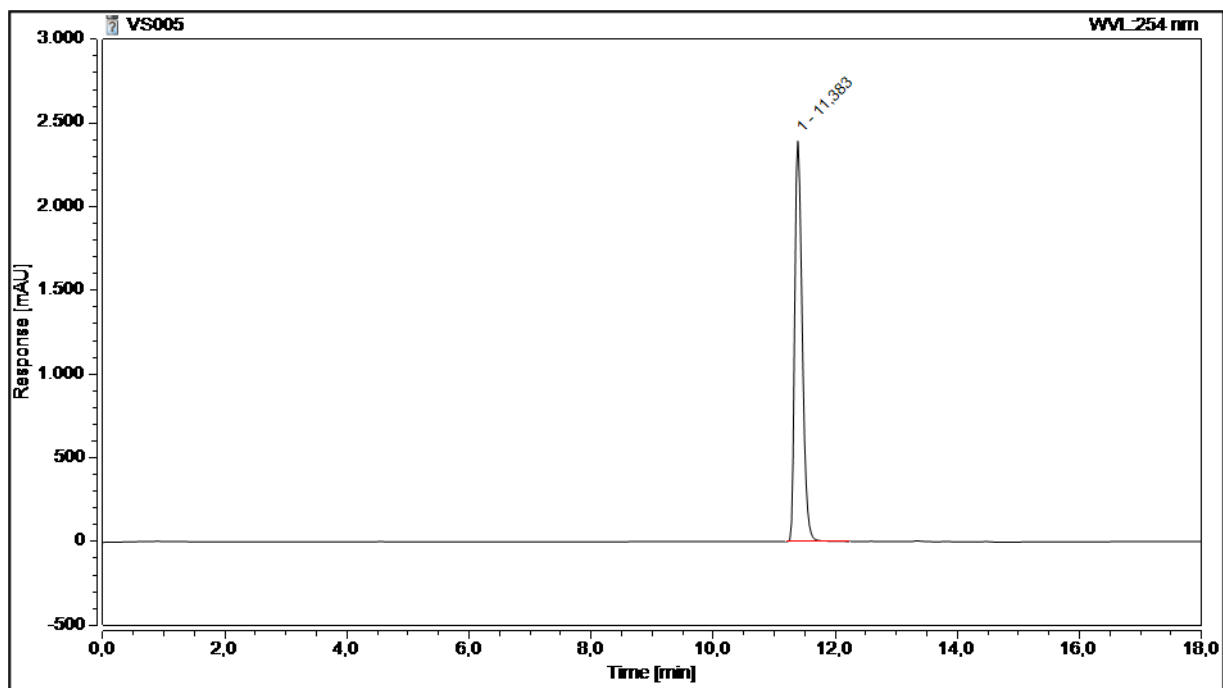


Figure S 1.79: HPLC chromatogram of VS005.

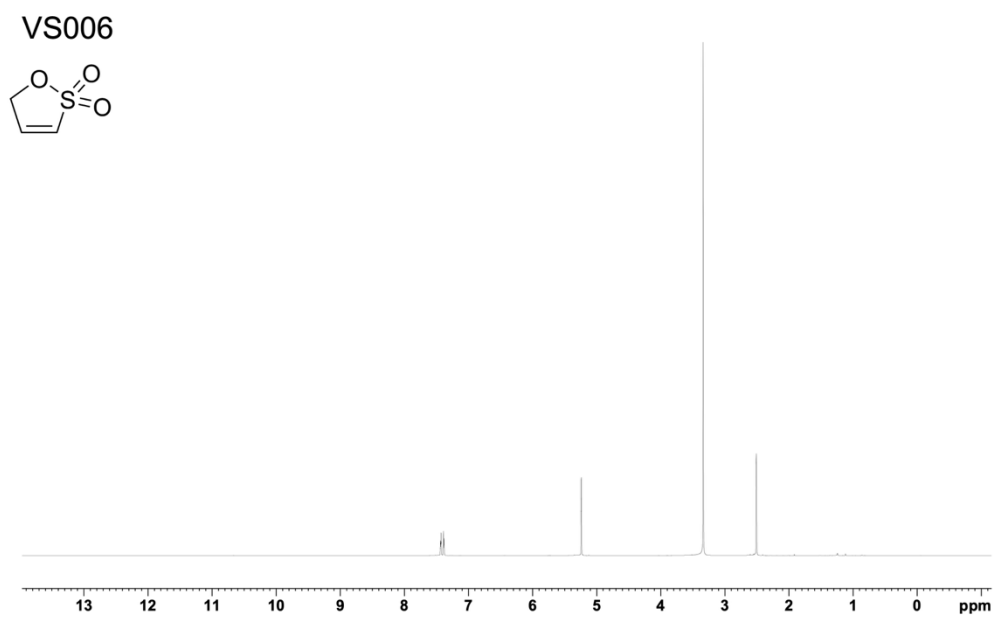


Figure S 1.80:  $^1\text{H-NMR}$  spectrum of VS006 in  $\text{DMSO-d}_6$ .  $\delta=7.44\text{--}7.42$  (m, 1H), 7.38 (d,  $J=6.7$  Hz, 1H) 5.24 ppm (s, 2H) 3.34 (s, water residual), 2.51 (m, DMSO residual).

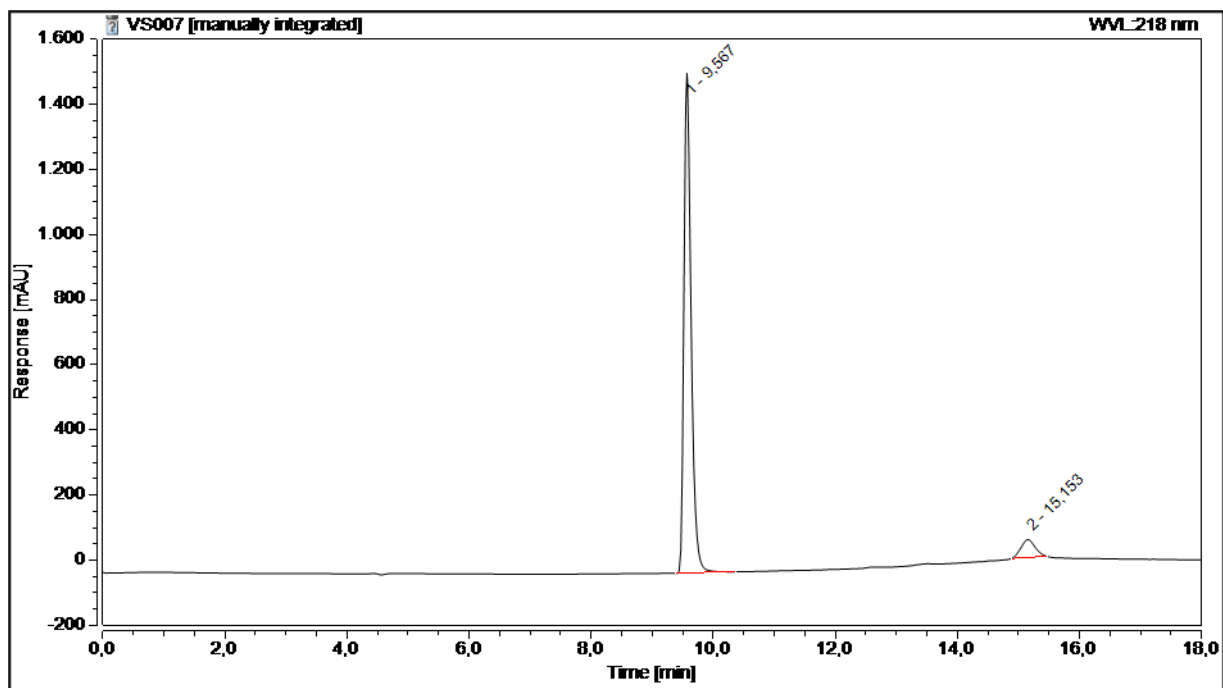


Figure S 1.81: HPLC chromatogram of VS007.

## 2 Calculated Molecular Properties

Table S 2.1: SMILES codes of the investigated compounds including CovLib Generation 1

Library	Name	SMILES
CovLib Gen2	AA001	<chem>BrC1=CNC(=O)C(=C)N1</chem>
CovLib Gen2	AA002	<chem>N1C(CC(CC1(C)C)NC(=O)C(=C)C)(C)C</chem>
CovLib Gen2	AA003	<chem>N(c1cc(ccc1)N)C(=O)C=C</chem>
CovLib Gen2	AA004	<chem>[S]1(=O)(=O)N([C@@H]2[C@]3(C1)C([C@H](C2)CC3)(C)C)C(=O)C=C</chem>
CovLib Gen2	AA005	<chem>N1c2c(cccc2)NC(=C)C1=O</chem>
CovLib Gen2	AA006	<chem>N(c1ccc(cc1)O)C(=O)C(=C)C</chem>
CovLib Gen2	AA007	<chem>[N+](=O)([O-])c1cc(ccc1)NC(=O)C=C</chem>
CovLib Gen2	AA008	<chem>N(c1cc(cc(c1)C(=O)O)C(=O)O)C(=O)C(=C)C</chem>
CovLib Gen1	CA001	<chem>[N-]1C(=O)CC(=C(C1=O)C#N)C</chem>
CovLib Gen1	CA002	<chem>N1(C(=O)CC(=C(C1=O)C#N)C)CCCN(C)C</chem>
CovLib Gen1	CA003	<chem>N1(C(=O)CC(=C(C1=O)C#N)C)CC(O)C</chem>
CovLib Gen1	CA004	<chem>N#C\C(=C(/C1CC1)\C)\C(=O)OC</chem>
CovLib Gen1	CA005	<chem>N2(CCOCC2)CCNC(=O)\C(=C\C=C\c1cccc1)\C#N</chem>
CovLib Gen2	CA006	<chem>[s]1cc(cc1)\C=C(/C(=O)N)\C#N</chem>
CovLib Gen2	CA007	<chem>[N+](=O)([O-])c1c(c(cc(c1)\C=C(\C(=O)N(CC)CC)/C#N)O)O</chem>
CovLib Gen2	CA008	<chem>Nc1ccc(cc1)\C=C(/C(=O)N)\C#N</chem>
CovLib Gen1	EO001	<chem>Fc1c(cccc1[C@@]3(O[C@@H]3C)C[n]2nnc2)F</chem>
CovLib Gen1	EO002	<chem>Fc1c(ccc(c1)F)C3(OC3)C[n]2ncnc2</chem>
CovLib Gen1	EO003	<chem>N3(C=C(C(=O)NC3=O)C)[C@@H]1O[C@@H]([C@@H]2O[C@@H]21)CO</chem>
CovLib Gen2	EO004	<chem>N#CC1OC21CCCC2</chem>
CovLib Gen2	EO005	<chem>O1C(C1)COc2c(cccc2)OC</chem>
CovLib Gen2	EO006	<chem>O1C2(C1C(=O)OCC)CCC2</chem>
CovLib Gen2	EO007	<chem>O1C(C1c2ccccc2)CO</chem>
CovLib Gen2	EO008	<chem>[S](=O)(=O)(OC[C@H]2OC2)c1cc(ccc1)[N+](=O)[O-]</chem>
CovLib Gen2	EO009	<chem>N1(C[C@H]2O[C@H]2CC1)C(=O)OC(C)(C)C</chem>
CovLib Gen2	EO010	<chem>O1C(C1C(=O)OC)c2c(cccc2)OC</chem>
CovLib Gen1	SN001	<chem>Fc1nc(c(cc1F)C#N)F</chem>
CovLib Gen1	SN002	<chem>Brc1[n](nc(n1)[N+](=O)[O-])CC#N</chem>
CovLib Gen1	SN003	<chem>[S](=O)(=O)(CC)c1[n](c(nn1)c2ccc(cc2)[N+](=O)[O-])C</chem>
CovLib Gen1	SN004	<chem>Brc1[n](nc(n1)[N+](=O)[O-])CCC(C)C</chem>
CovLib Gen1	SN005	<chem>Clc1n[n](cn1)c2ncccc2C#N</chem>
CovLib Gen1	SN006	<chem>Clc1nc(c(nc1C#N)C#N)N</chem>
CovLib Gen1	SN007	<chem>Brc1nc(c(nc1C#N)C#N)N</chem>
CovLib Gen1	SN008	<chem>FC(F)[n]1ncc(c1C)[S](=O)(=O)NCCC[n]2nc(c(c2)Cl)[N+](=O)[O-]</chem>
CovLib Gen1	SN009	<chem>N#CC1=CN=C(Cl)N=C1</chem>
CovLib Gen1	SN010	<chem>N#CC1=CN=C(Cl)N=C1N</chem>
CovLib Gen1	SN011	<chem>N#CC1=NN=C(Cl)C=C1</chem>
CovLib Gen1	SN012	<chem>N#CC1=NN=C(Cl)C(C)=C1</chem>
CovLib Gen2	SN013	<chem>Clc1ncc(cc1)C(=O)N2CCOCC2</chem>
CovLib Gen2	SN014	<chem>lc1cnc(nc1)Cl</chem>
CovLib Gen2	SN015	<chem>Clc1nc(ccc1C(=O)N)Cl</chem>
CovLib Gen2	SN016	<chem>Clc1nc(c(cc1)[N+](=O)[O-])OC</chem>
CovLib Gen2	SN017	<chem>Clc1ncc(cc1[N+](=O)[O-])C(=O)OC</chem>
CovLib Gen2	SN018	<chem>Brc1nnc(nc1)N</chem>
CovLib Gen2	SN019	<chem>Clc1nc2c(c(n1)Cl)COC2</chem>
CovLib Gen2	SN020	<chem>Clc1[nH]c(cn1)[N+](=O)[O-]</chem>
CovLib Gen2	SN021	<chem>[S](=O)(=O)(C)c1nc2c(cn1)CN(CC2)C(=O)OC(C)(C)C</chem>
CovLib Gen2	SN022	<chem>BrC1=NC(=C[n]2ncnc21)Br</chem>
CovLib Gen2	SN023	<chem>[S](=O)(=O)(CC)c1ncc(cc1)[N+](=O)[O-]</chem>
CovLib Gen2	SN024	<chem>Clc1nc([n+](c(c1)N)[O-])N</chem>
CovLib Gen2	SN025	<chem>Brc1[nH]c(c(n1)C#N)C#N</chem>
CovLib Gen2	SN026	<chem>lc1nc([nH]c1[N+](=O)[O-])C</chem>
CovLib Gen2	SN027	<chem>Brc1nc(c(cc1)O)[N+](=O)[O-]</chem>
CovLib Gen2	SN028	<chem>Clc1nc2c(c(n1)Cl)CNCC2.Cl</chem>
CovLib Gen2	SN029	<chem>Clc1nnc(cc1)C(C(=O)OCC)C#N</chem>

CovLib Gen2	SN030	Brc1cnc(c(c1)[N+](=O)[O-])C#N
CovLib Gen2	SN031	lc1c[n](nc1)c2ncnc(c2)Cl
CovLib Gen2	SN032	lc1c[n](nc1)c2nnc(cc2)Cl
CovLib Gen2	SN033	Clc1nnc(cc1)[n]2nc(cc2C)C
CovLib Gen2	SN034	Clc1cc(ncc1)C(=O)N(OC)C
CovLib Gen2	SN035	Clc1nc(ccc1[N+](=O)[O-])C
CovLib Gen2	SN036	FC(F)(F)C(=O)Nc1nc2[n](c1)N=C(C=C2)Cl
CovLib Gen2	SN037	Clc1nc2nc[nH]c2c(n1)Cl
CovLib Gen2	SN038	[S](=O)(=O)(C)c1nc(cc(n1)Cl)Cl
CovLib Gen2	SN039	Clc1nc(ncc1C(=O)OCC)C
CovLib Gen2	SN040	S(C)c1nc(c(cn1)Br)C(=O)OC
CovLib Gen2	SN041	Clc1nc(cnc1)[n]2nccc2
CovLib Gen2	SN042	Clc1ncc(nc1)C(=O)OC
CovLib Gen2	SN043	Clc1ncc[n+](c1)[O-]
CovLib Gen2	SN044	Clc1ncnc2[nH]cc(c21)C#N
CovLib Gen2	SN045	Clc1cnc(c(c1)[N+](=O)[O-])C#N
CovLib Gen2	SN046	C1C1=Nc2[n](ncc2C#N)C=C1
CovLib Gen2	SN047	Fc1c[n+](c(cc1)Br)[O-]
CovLib Gen2	SN048	Clc1nc(c(cn1)[N+](=O)[O-])NC(C)C
CovLib Gen2	SN049	S1Cc2ncnc(c2C1)Cl
CovLib Gen2	SN050	[s]1c(ncc1[N+](=O)[O-])Cl
CovLib Gen2	SN051	Brc1[nH]c(cn1)[N+](=O)[O-]
CovLib Gen2	SN052	Brc1ncccc1[N+](=O)[O-]
CovLib Gen2	SN053	Clc1ncnc2c1ccnc2
CovLib Gen2	SN054	Clc1nc(nc(c1C#N)Cl)N
CovLib Gen2	SN055	Clc1nc(c(cn1)C(=O)OCC)C
CovLib Gen2	SN056	Clc1nnc(cc1)C(=O)OC
CovLib Gen2	SN057	Clc1ncnc2[nH]cc(c21)C(=O)OCC
CovLib Gen2	SN058	[s]1c(nc(c1)C#N)Br
CovLib Gen2	SN059	Clc1ncnc2[nH]ccc21
CovLib Gen2	SN060	Clc1nc(nc(n1)N2CCOCC2)Cl
CovLib Gen2	SN061	Fc1ncc(cc1)C(=O)OC
CovLib Gen2	SN062	Brc1[nH]nc(c1[N+](=O)[O-])Br
CovLib Gen2	SN063	FC(F)(F)c1c[n+](c(cc1)Cl)[O-]
CovLib Gen2	SN064	Clc1ncc(cc1C(=O)N)[N+](=O)[O-]
CovLib Gen2	SN065	Clc1ncc(cc1Cl)C(=O)N2C(CCC2)C(=O)N
CovLib Gen2	SN066	[S]1(=O)(=O)CCc2nc(nc(c21)Cl)Cl
CovLib Gen2	SN067	S1c2[n](c(c(n2)Cl)[N+](=O)[O-])C=C1
CovLib Gen2	SN068	FC(F)(F)c1cnc(cc1)[S](=O)(=O)CC(=O)OC
CovLib Gen2	SN069	FC(F)(F)c1nc(ncc1C(=O)OC)Cl
CovLib Gen2	SN070	Clc1ncnc(c1)[n]2nccc2
CovLib Gen2	SN071	Brc1c([nH]nc1)C#N
CovLib Gen2	SN072	C1C1=N[n]2c(ncc2[N+](=O)[O-])C=C1
CovLib Gen1	VS001	[S](=O)(=O)(Cc1[o]ccc1)\C(=C\OCC)\C#N
CovLib Gen1	VS002	[S]1(=O)(=O)N(c2c([s]cc2)C(=O)\C\1=C\N(C)C)C
CovLib Gen1	VS003	Fc1cc2c(cc1)[S](=O)(=O)C=C2C
CovLib Gen1	VS004	[S]1(=O)(=O)c2c(cccc2)C(=C1)Sc3[nH]c(nn3)N
CovLib Gen2	VS005	[S](=O)(=O)(N2CCC(CC2)C(=O)O)\C=C1c1cccc1
CovLib Gen2	VS006	[S]1(=O)(=O)OCC=C1
CovLib Gen2	VS007	[S](=O)(=O)(c1cccc1)C=C

Table S 2.2: Calculated molecular properties of the investigated fragments

Name	Heavy Atoms	Molecular Weight [g/mol]	Hydrogen Bond Acceptors	Hydrogen Bond Donors	Rotatable Bonds	QLogPo/w	Polar Surface Area [Å <sup>2</sup> ]
AA001	9	189.011	3	2	1	0.648	55.038
AA002	16	224.345	3.5	2	2	2.23	41.271
AA003	12	162.191	3.5	2.5	3	1.069	63.072
AA004	18	269.358	5	0	1	1.63	62.948
AA005	12	160.175	3	2	1	1.405	53.958
AA006	13	177.202	3.25	2	3	1.646	57.36
AA007	14	192.174	3.5	1	3	1.536	81.59
AA008	18	249.223	6.5	3	4	0.888	135.402
CA006	12	178.208	4	2	4	0.324	75.236
CA007	22	305.29	7	2	9	0.645	138.305
CA008	14	187.201	5	3.5	5	-0.262	101.6
EO004	10	137.181	3.5	0	1	0.585	39.022
EO005	13	180.203	3.5	0	4	1.568	28.549
EO006	11	156.181	4	0	2	0.894	48.003
EO007	11	150.177	2.7	0	2	1.401	35.273
EO008	17	259.233	7	0	5	-0.265	104.296
EO009	14	199.249	4.5	0	1	1.581	45.797
EO010	15	208.213	4.75	0	2	1.48	56.762
SN013	15	226.662	5.7	0	1	1.046	50.496
SN014	8	240.431	2	0	0	1.741	25.23
SN015	11	191.016	3.5	2	1	0.695	64.264
SN016	12	188.57	2	0	2	1.806	61.235
SN017	14	216.58	4	0	2	0.706	94.492
SN018	8	174.987	3.5	2	1	-0.279	67.01
SN019	11	191.016	3.7	0	0	1.193	35.169
SN020	9	147.521	2.5	1	1	0.441	74.202
SN021	9	147.521	2.5	1	1	0.444	73.785
SN022	21	313.371	8.5	0	2	0.91	94.949
SN023	11	277.906	3.5	0	0	1.327	45.016
SN024	14	216.211	6	0	3	-0.385	94.674
SN025	10	160.563	3	4	2	-0.299	85.302
SN026	10	196.994	4.5	1	2	0.133	80.296
SN027	10	252.999	2.5	1	1	0.906	72.549
SN028	10	252.999	2.5	1	1	0.91	72.146
SN029	11	218.994	2.75	1	2	1.252	77.438
SN030	11	218.994	2.75	1	2	1.249	77.477
SN031	12	204.058	3.5	1	0	1.206	40.054
SN032	15	225.634	5.5	0	4	0.811	87.691
SN033	12	228.005	3.5	0	2	0.393	83.16
SN034	13	306.493	3	0	0	2.442	39.976
SN035	13	306.493	3	0	0	2.395	42.876
SN036	14	208.65	3	0	0	2.151	41.505
SN037	13	200.624	5.2	0	2	1.043	49.084
SN038	11	172.571	2	0	1	1.404	57.662
SN039	17	264.594	5	1	1	1.94	68.626
SN040	11	189.004	3.5	1	0	1.114	54.117
SN041	11	189.004	3.5	1	0	1.117	54.115
SN042	12	227.065	6	0	1	0.348	62.232
SN043	13	200.624	4	0	2	1.431	58.127
SN044	13	263.108	4	0	2	1.782	56.285
SN045	12	180.596	3.5	0	0	1.388	40.187
SN046	11	172.571	4	0	1	0.667	61.439
SN047	8	130.533	2	0	0	0.461	37.017
SN048	12	178.58	3.5	1	1	0.778	65.804
SN049	12	183.554	3.5	0	2	0.388	83.164
SN050	12	178.58	3.5	0	1	1.15	57.027
SN051	9	191.987	1	0	0	2.397	23.419
SN052	14	216.627	3.5	1	3	1.855	75.853
SN053	10	172.632	2.5	0	0	1.58	25.528
SN054	9	164.566	2.5	0	1	0.623	59.387
SN055	9	191.972	2.5	1	1	0.449	74.412
SN056	9	191.972	2.5	1	1	0.452	73.989
SN057	10	202.995	2	0	1	1.313	56.843
SN058	11	165.582	3.5	0	0	0.957	38.052
SN059	11	189.004	4	2	2	0.538	76.049
SN060	13	200.624	4	0	2	1.46	57.383
SN061	11	172.571	4	0	1	0.562	64.111
SN062	15	225.634	3	0	2	2.01	73.564
SN063	8	189.029	3	0	1	0.667	39.43
SN064	10	153.571	2	1	0	1.337	40.131
SN065	14	235.072	5.2	0	0	1.413	49.627
SN066	11	155.128	3	0	1	1.449	49.105
SN067	10	270.868	2	1	1	1.046	74.624
SN068	12	197.544	1	0	0	2.686	23.416
SN069	13	201.569	4.5	2	2	-0.013	109.457
SN070	18	288.133	6.5	2	2	0.643	91.277
SN071	13	239.076	6	0	0	0.134	64.812
SN072	12	203.603	2.5	0	1	1.387	59.691
VS005	18	283.222	7	0	3	0.739	81.594
VS006	15	240.569	4	0	1	1.733	55.906
VS007	12	180.596	3	0	0	1.195	40.006

### 3 Turbidimetric Solubility Assessment

#### 3.1 Spectra

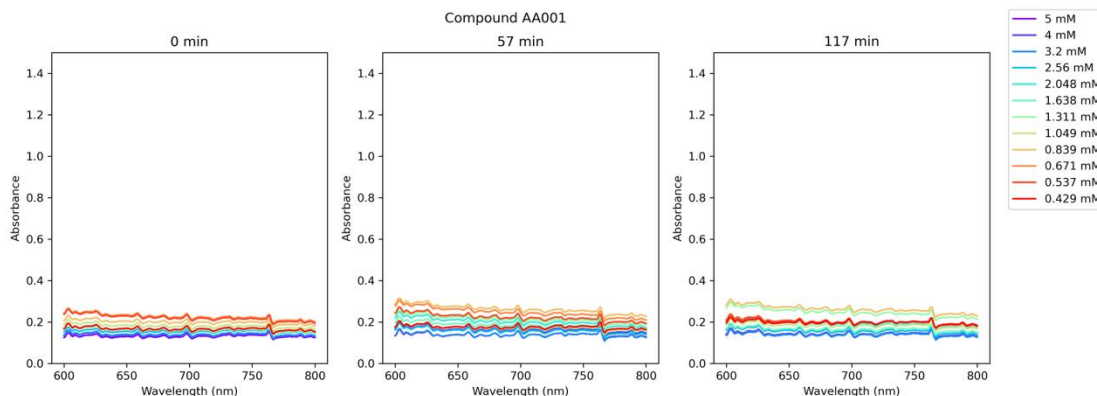


Figure S 3.1: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA001.

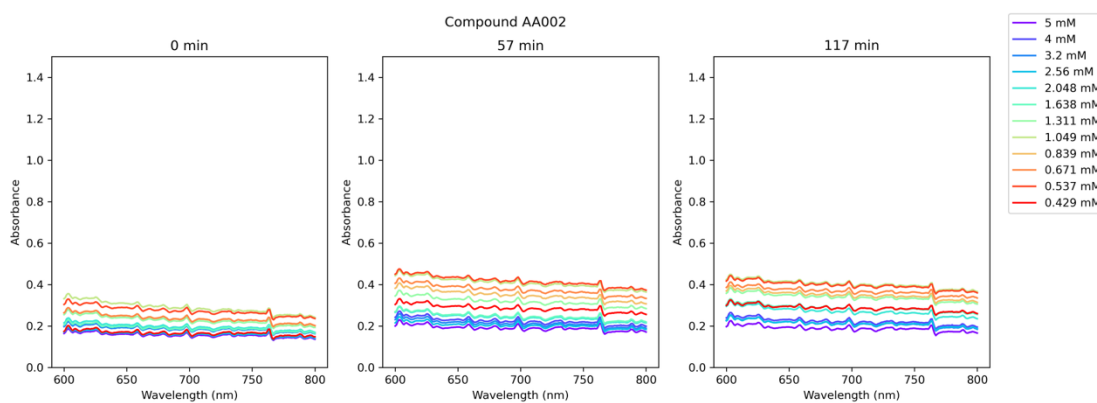


Figure S 3.2: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA002.

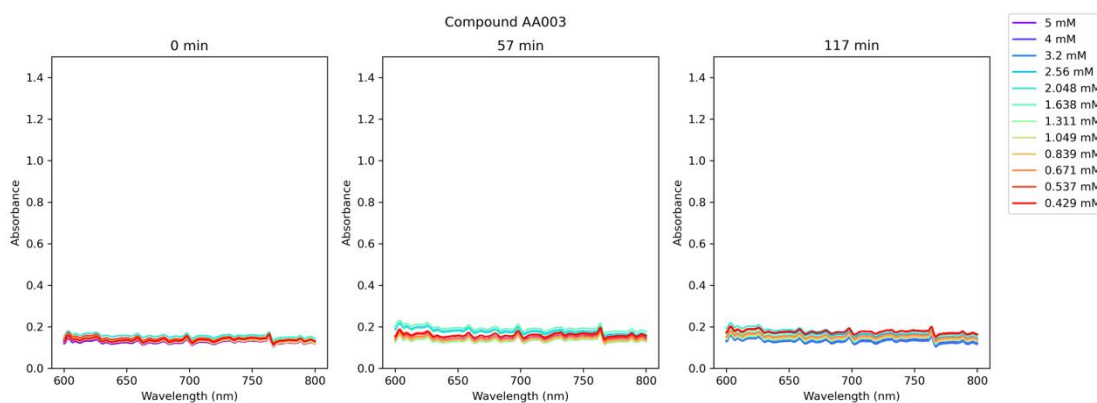


Figure S 3.3: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA003.

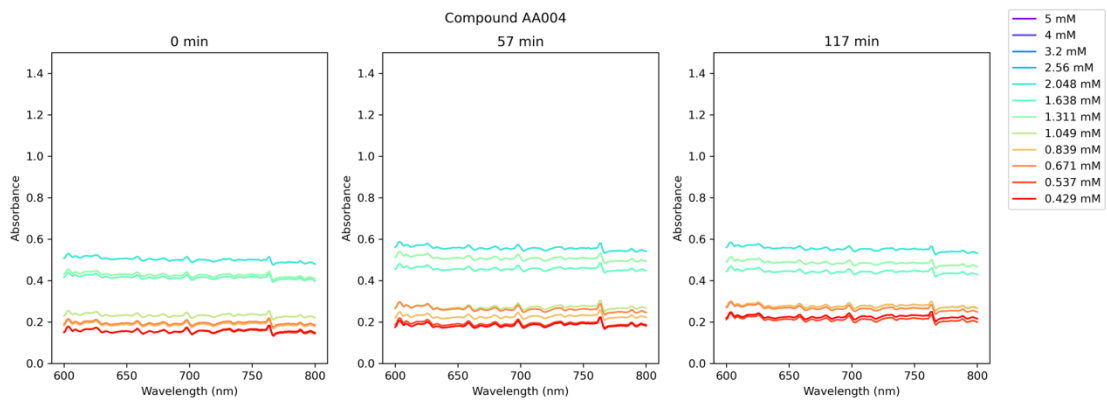


Figure S 3.4: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA004.

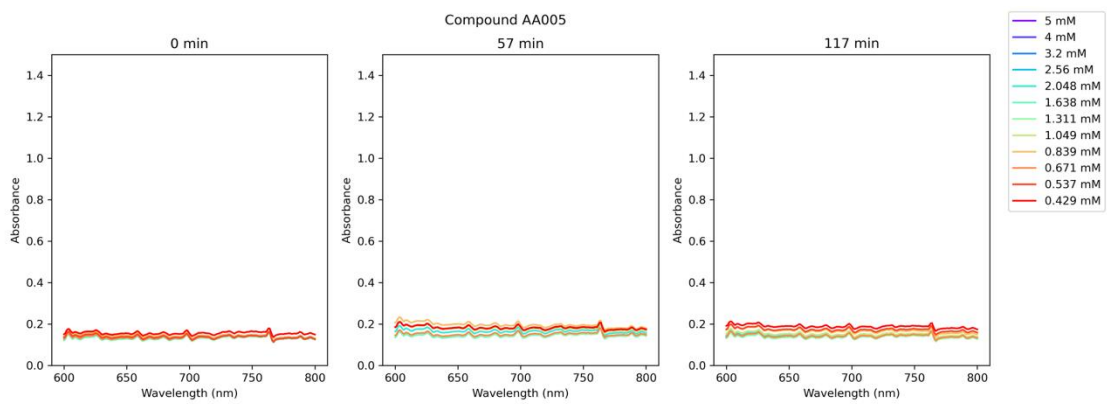


Figure S 3.5: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA005.

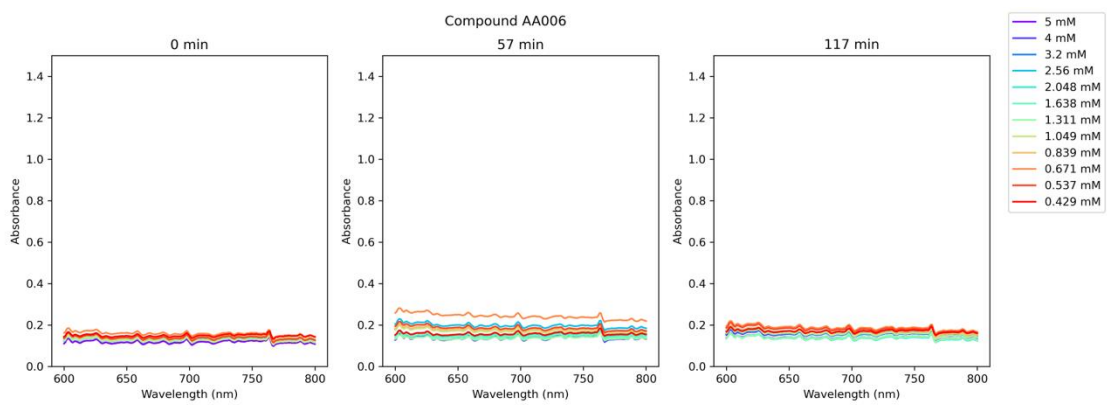


Figure S 3.6: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA006.

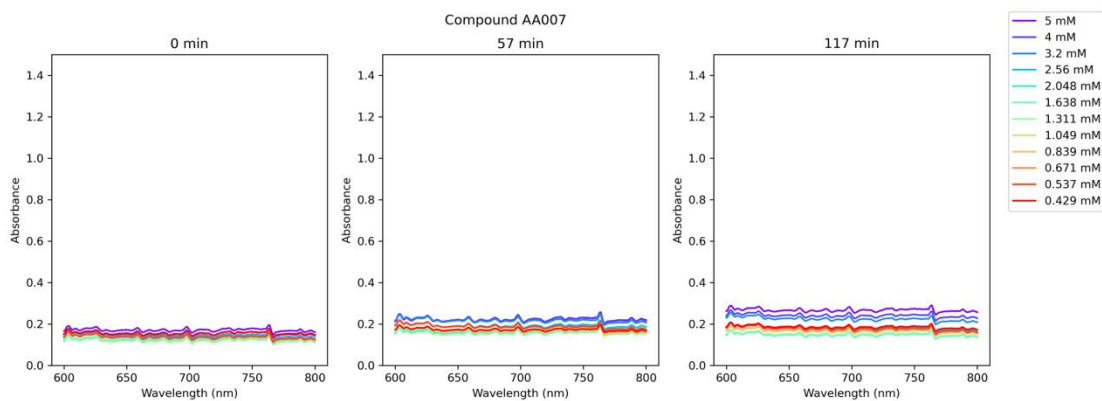


Figure S 3.7: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA007.

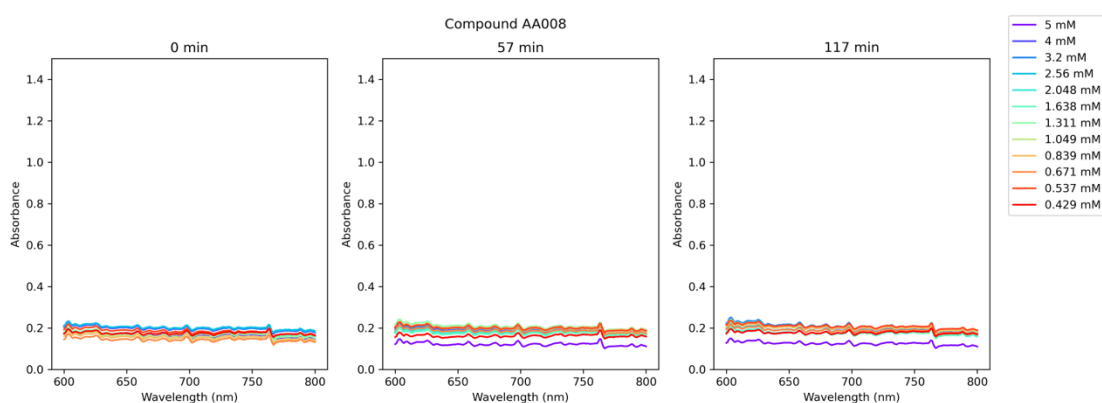


Figure S 3.8: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for AA008.

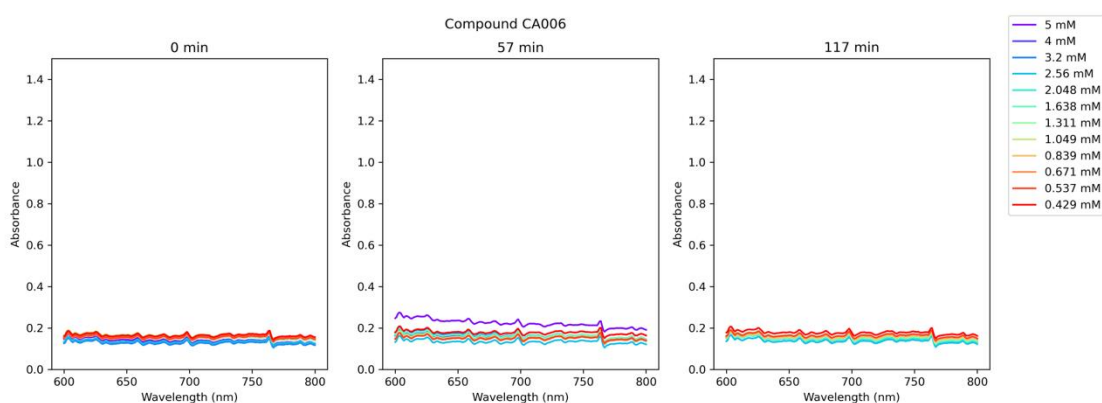


Figure S 3.9: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for CA006.

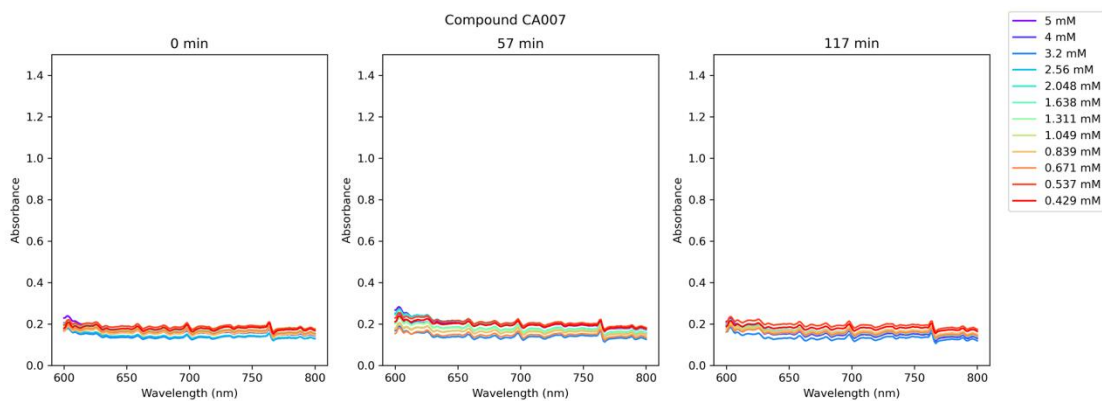


Figure S 3.10: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for CA007.

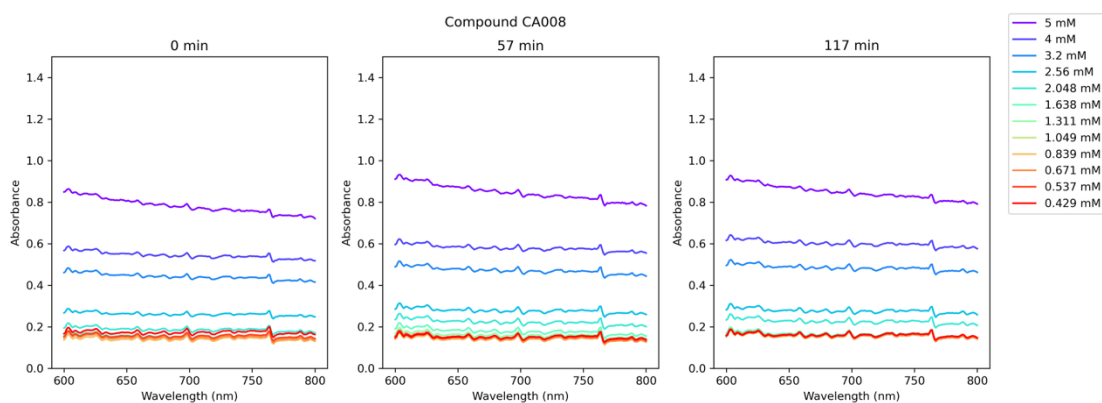


Figure S 3.11: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for CA008.

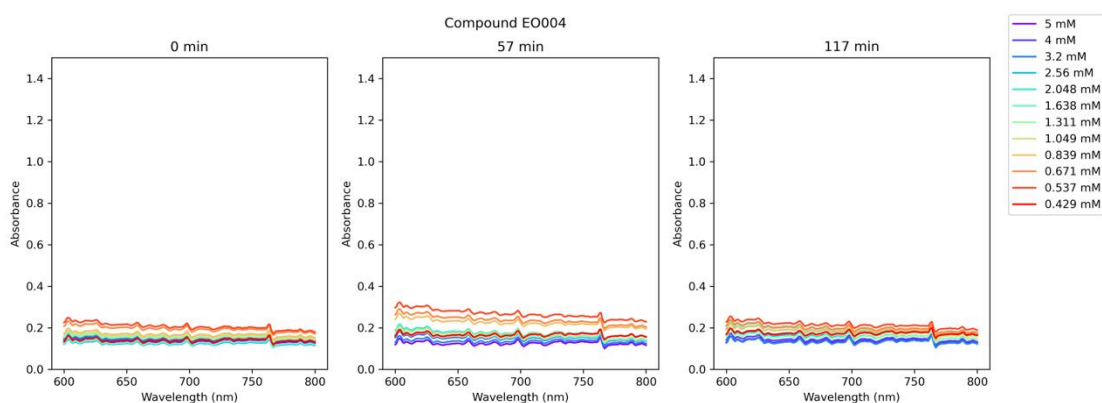


Figure S 3.12: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for EO004.

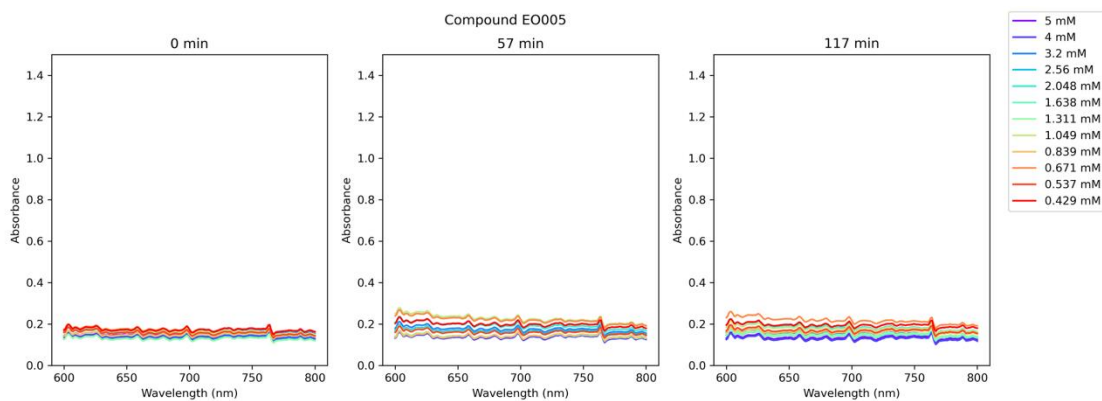


Figure S 3.13: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for EO005.

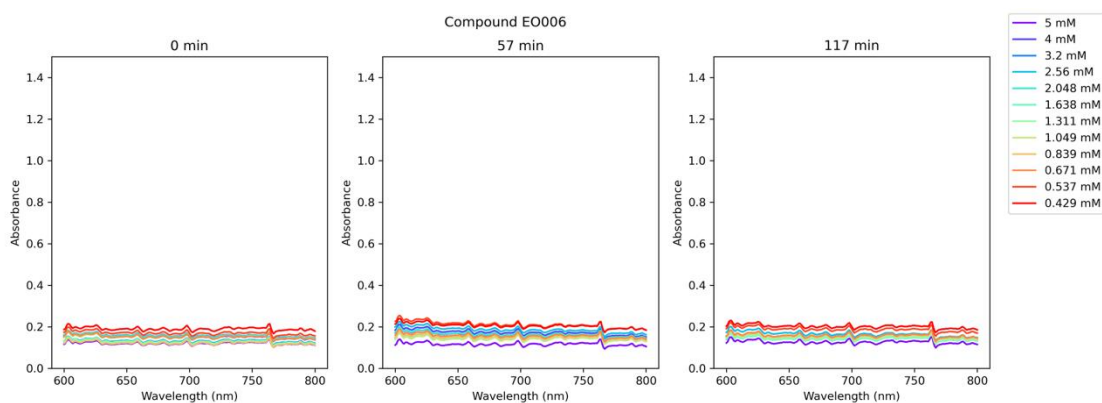


Figure S 3.14: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for EO006.

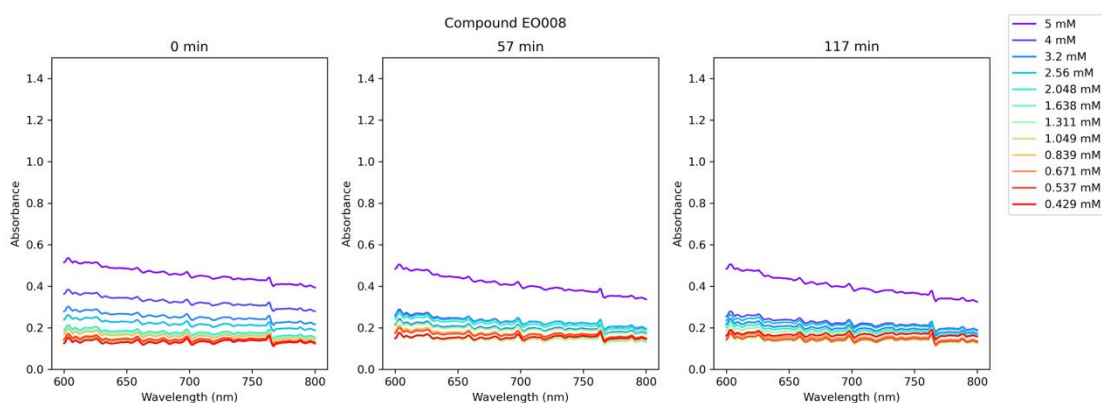


Figure S 3.15: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for EO008.

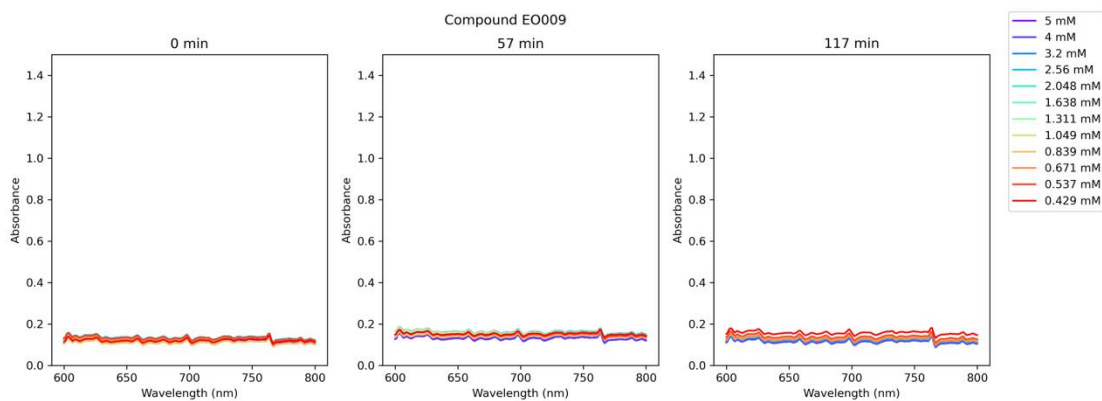


Figure S 3.16: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for EO009.

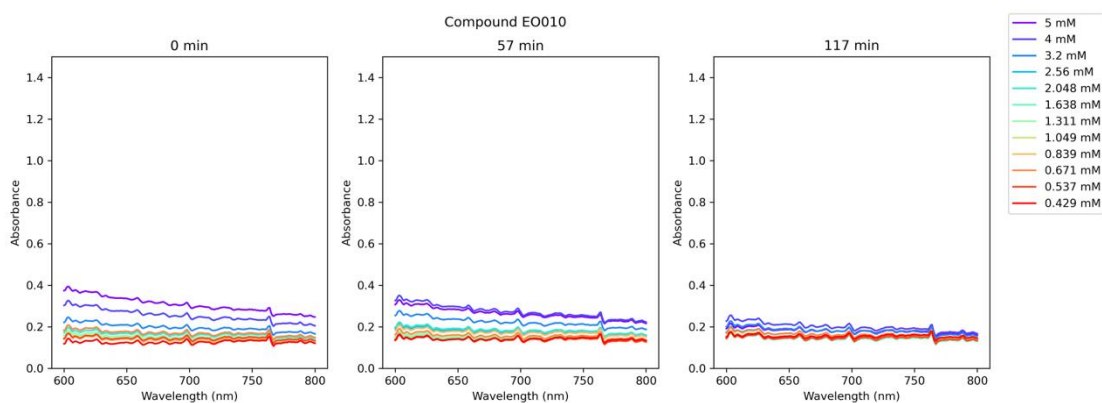


Figure S 3.17: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for EO010.

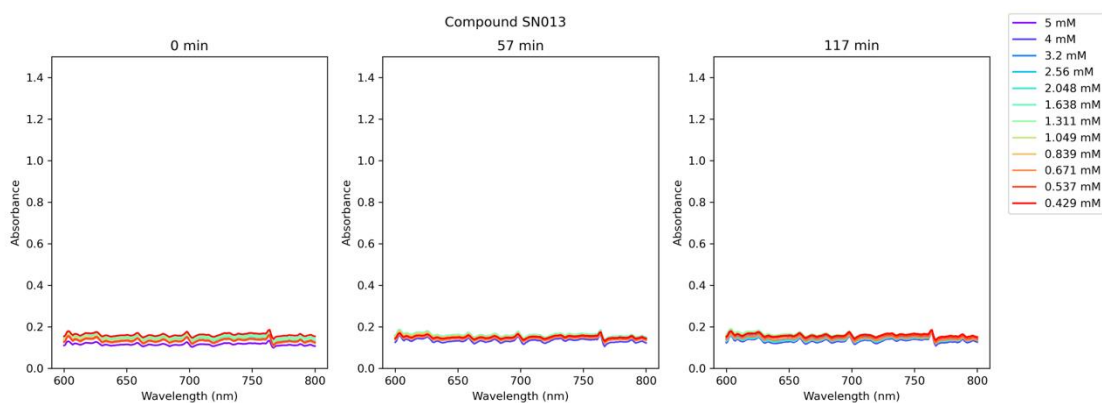


Figure S 3.18: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN013.

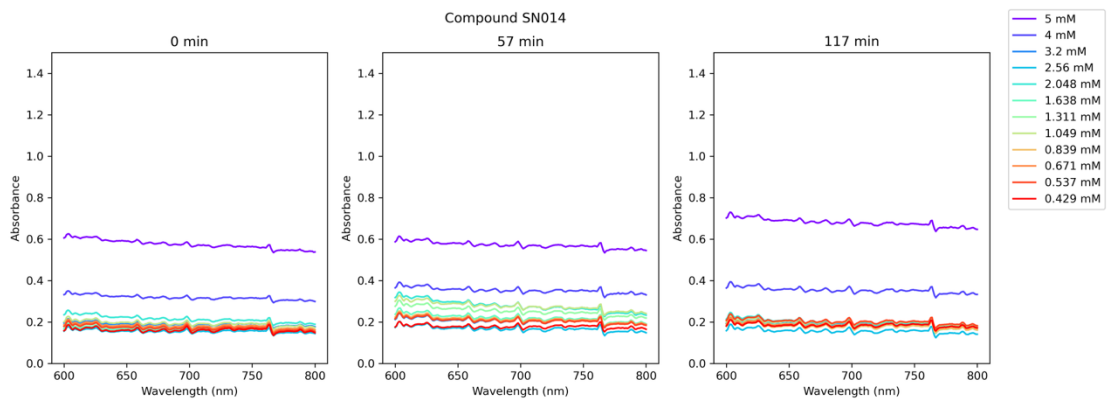


Figure S 3.19: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN014.

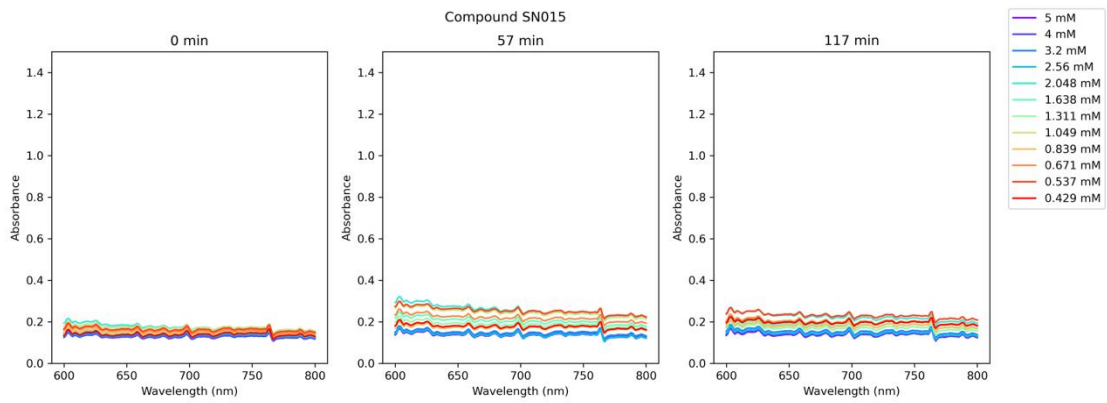


Figure S 3.20: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN014

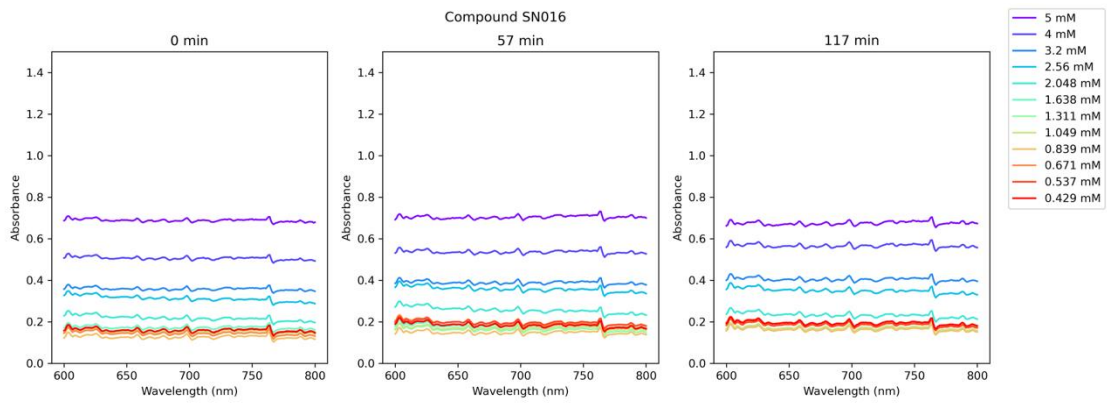


Figure S 3.21: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN016

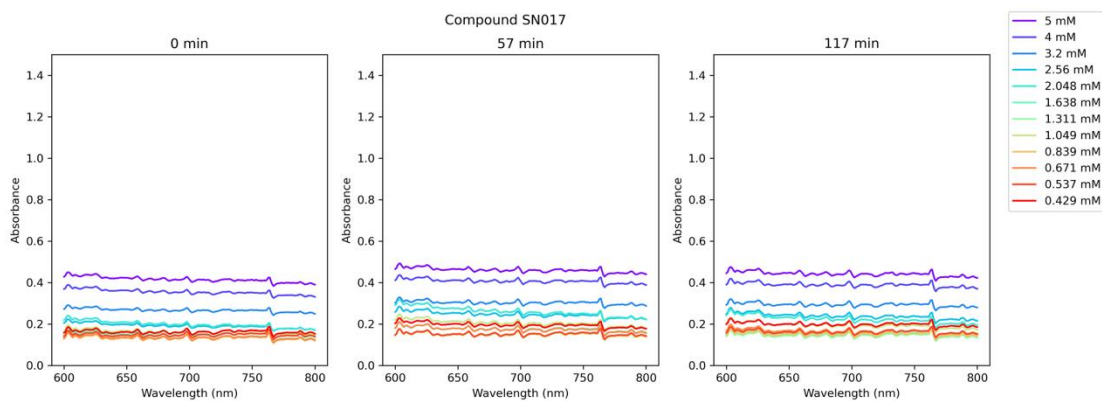


Figure S 3.22: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN017.

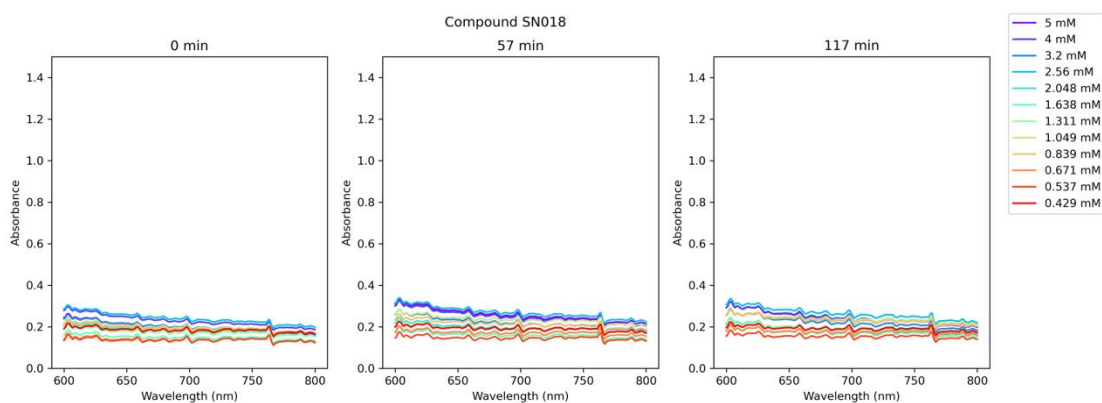


Figure S 3.23: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN018.

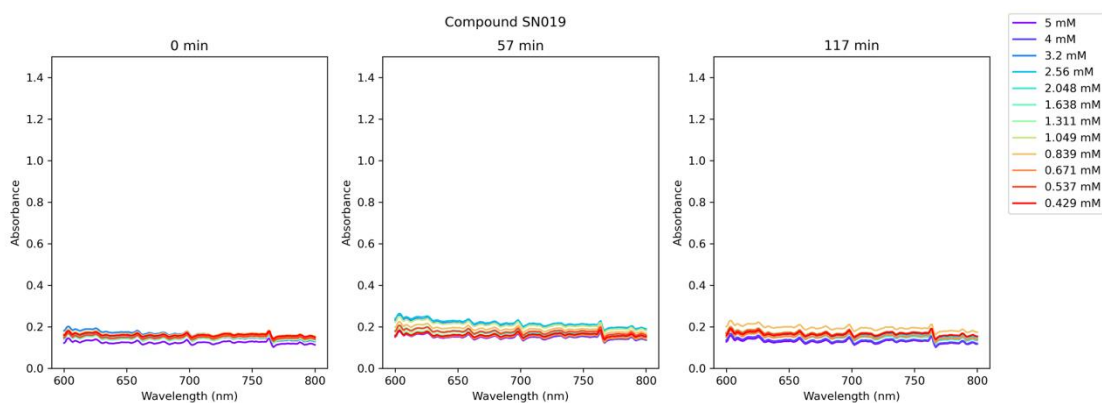


Figure S 3.24: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN019.

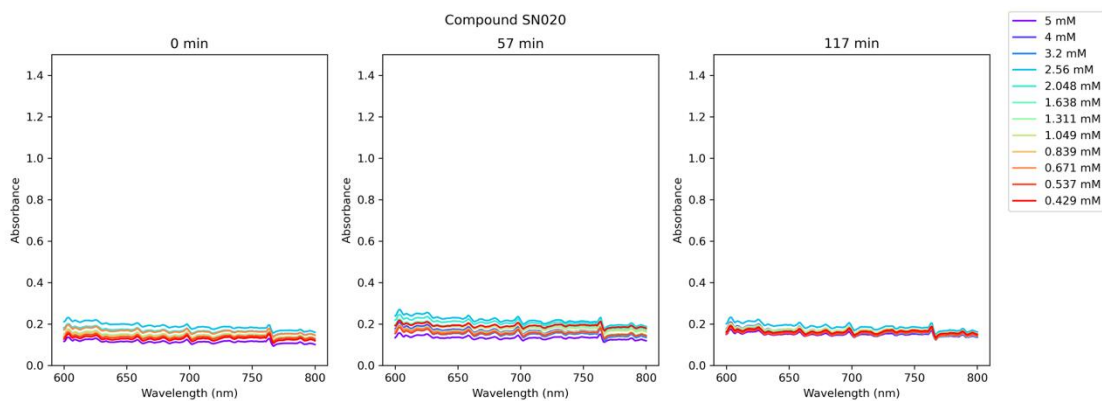


Figure S 3.25: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN020.

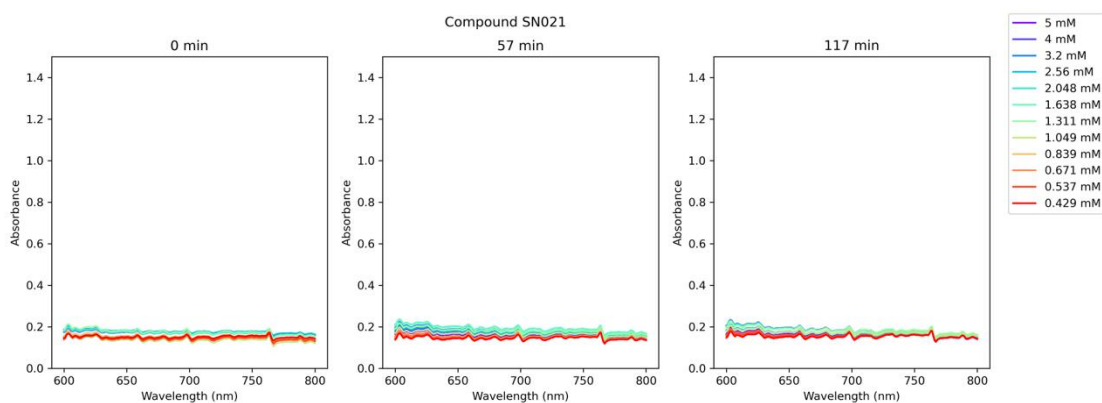


Figure S 3.26: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN021.

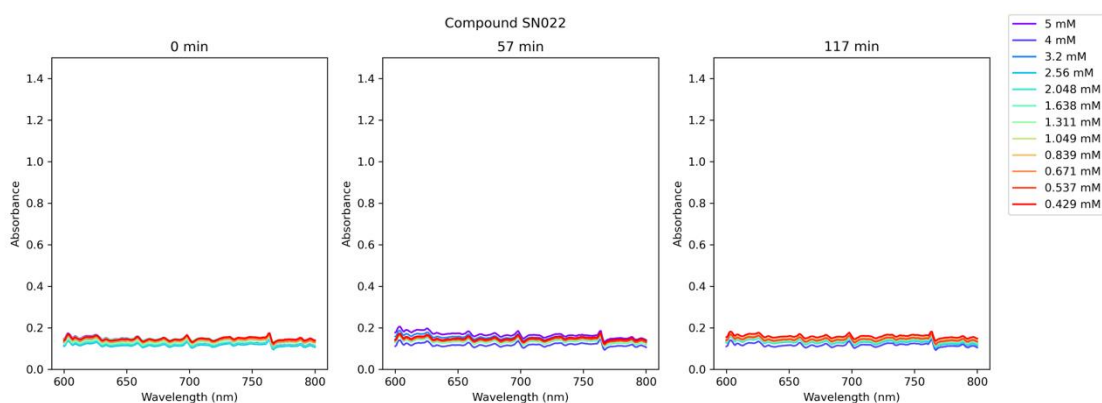


Figure S 3.27: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN022.

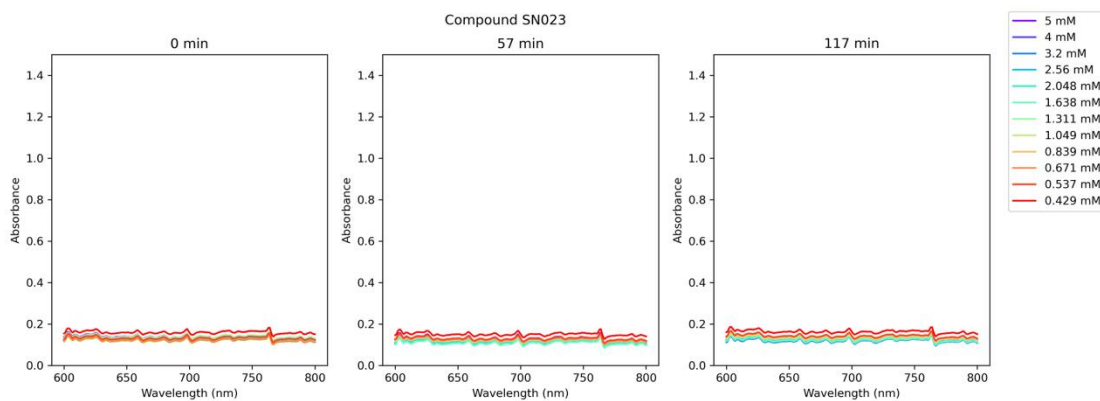


Figure S 3.28: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN023.

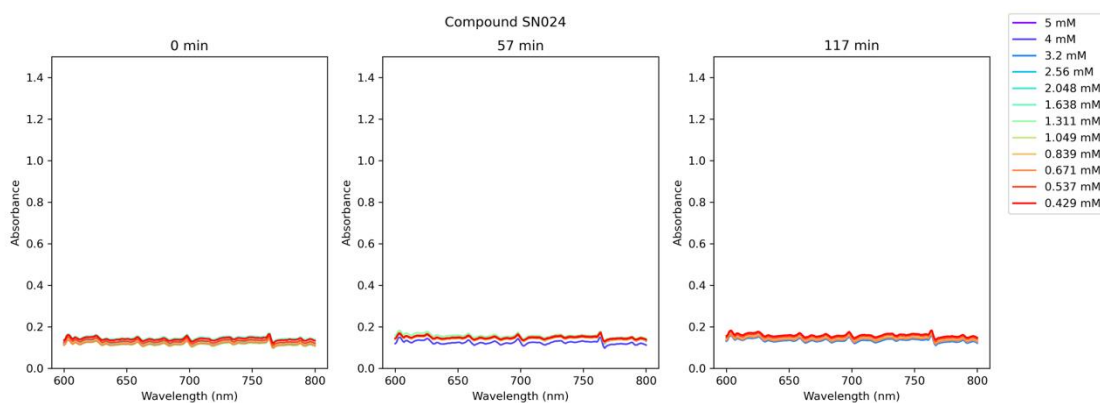


Figure S 3.29: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN024.

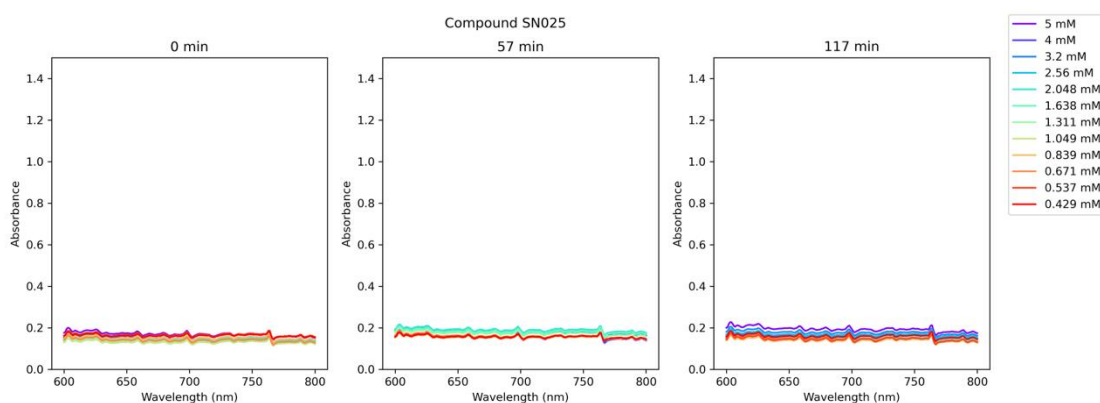


Figure S 3.30: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN025.

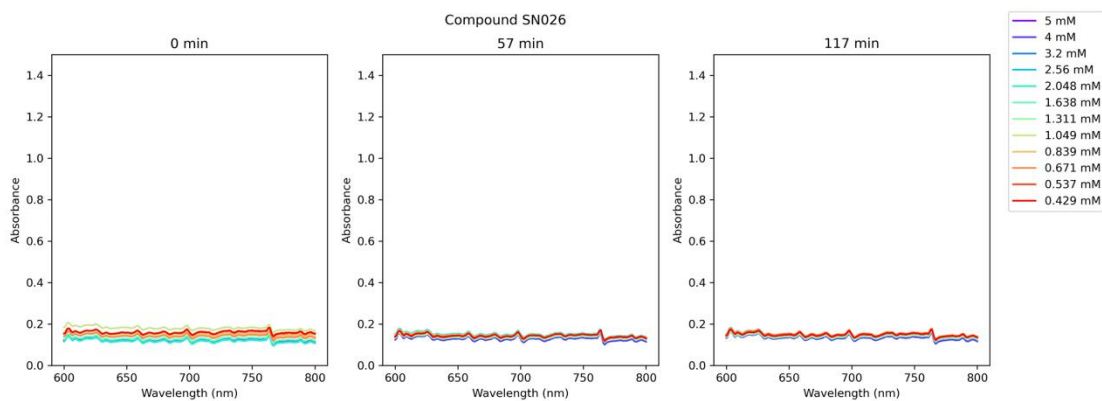


Figure S 3.31: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN026.

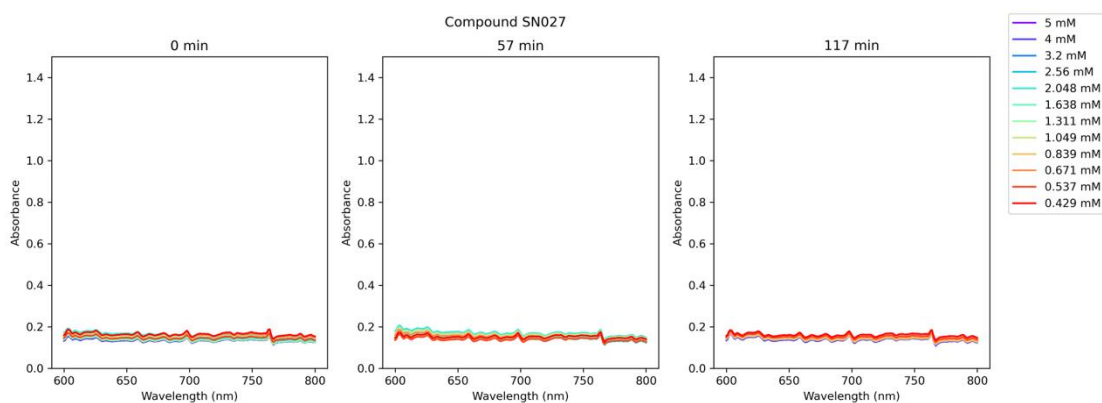


Figure S 3.32: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN027.

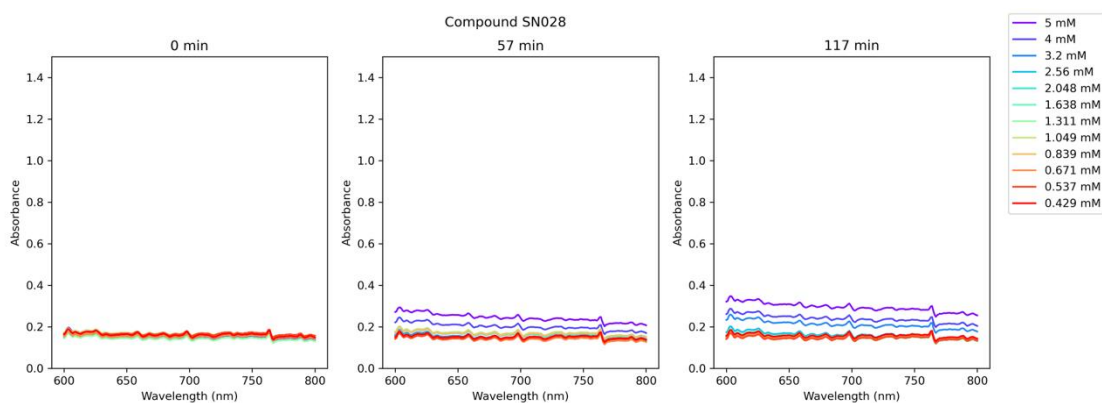


Figure S 3.33: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN028.

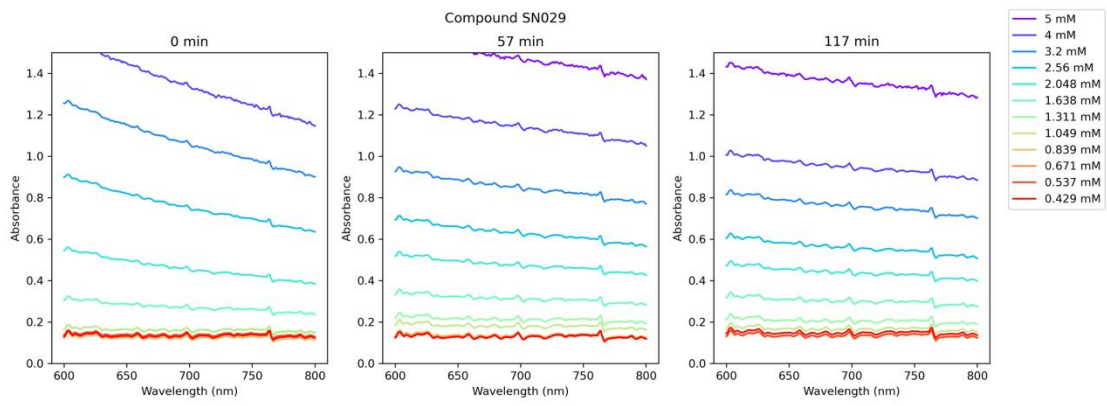


Figure S 3.34: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN029.

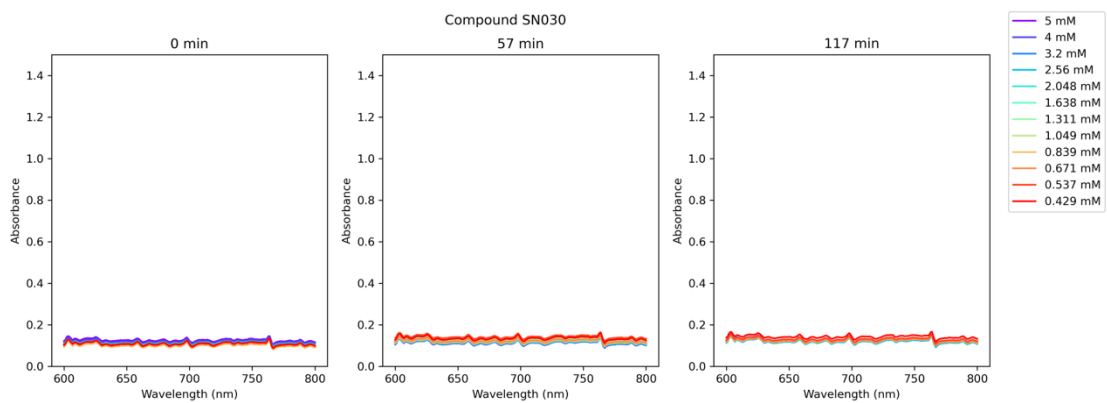


Figure S 3.35: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN030.

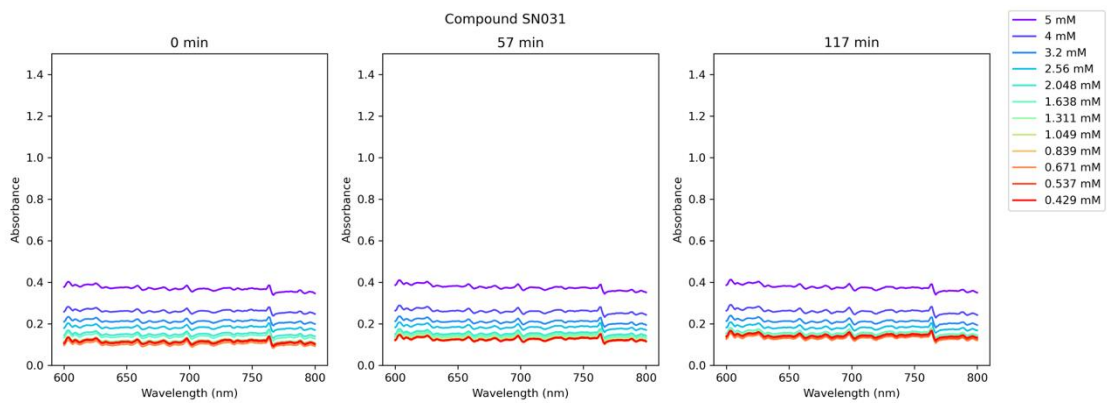


Figure S 3.36: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN031.

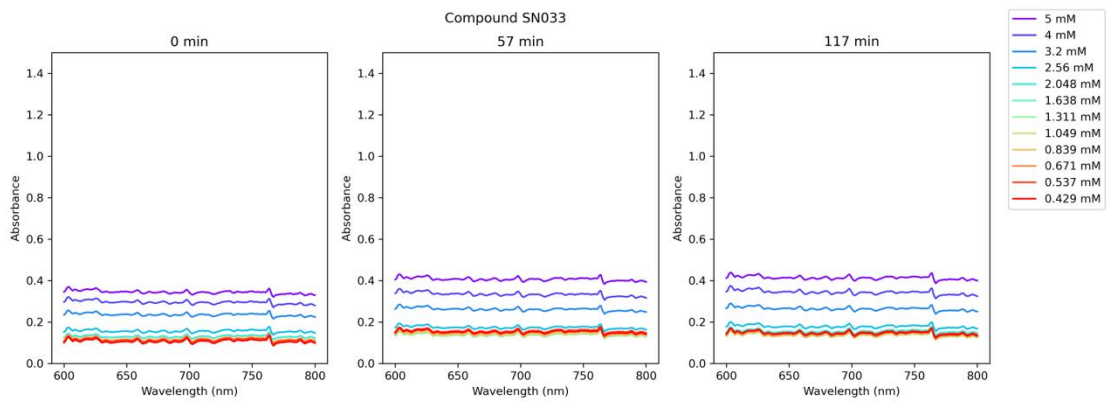


Figure S 3.37: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN033.

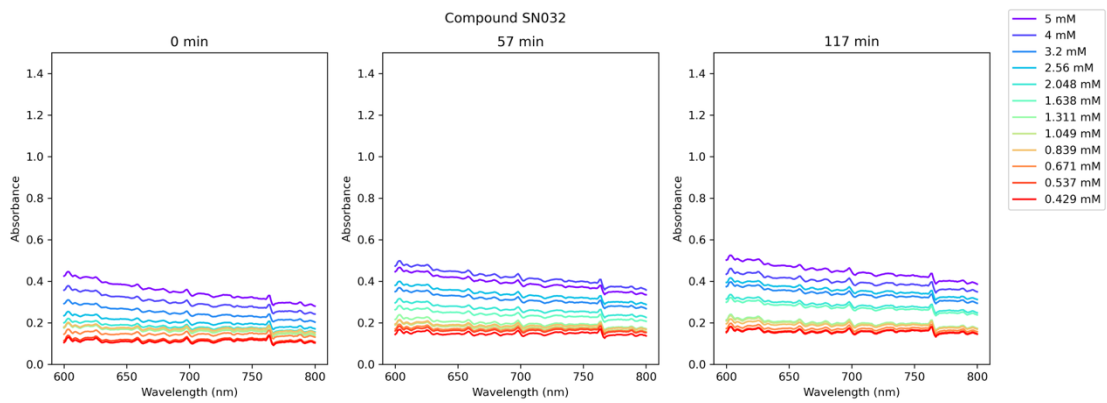


Figure S 3.38: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN032.

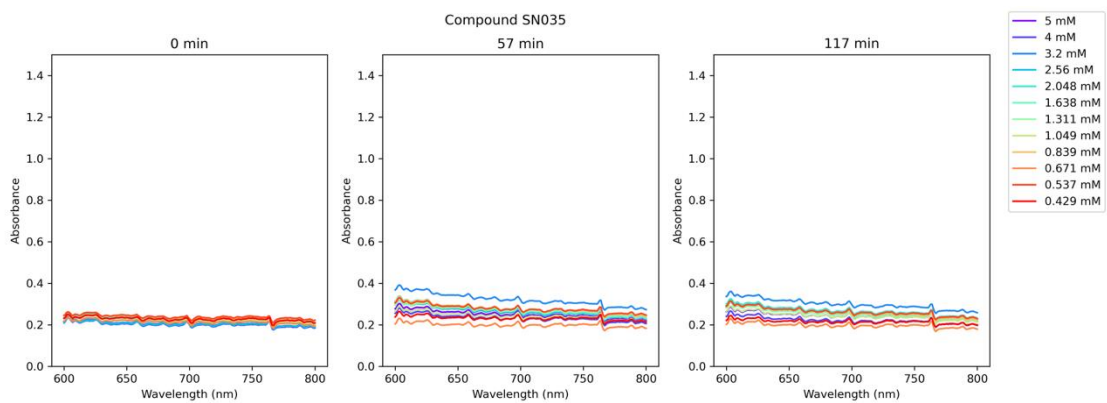


Figure S 3.39: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN035.

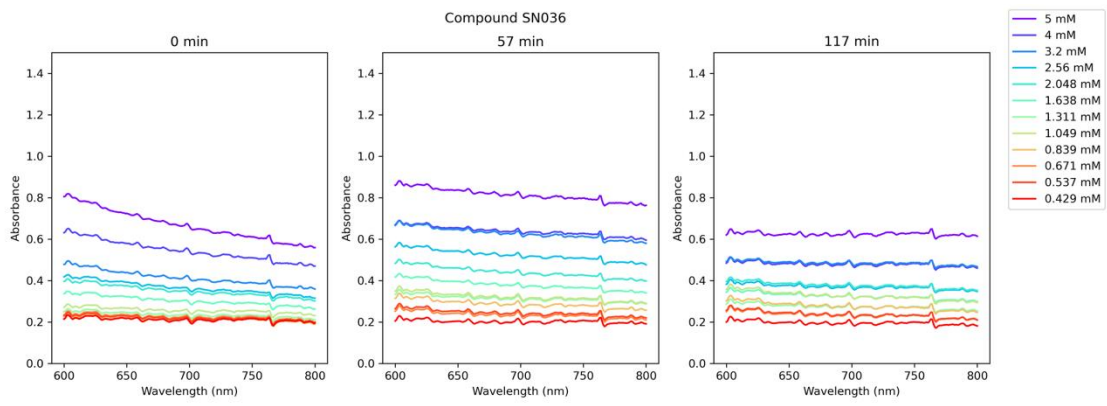


Figure S 3.40: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN036.

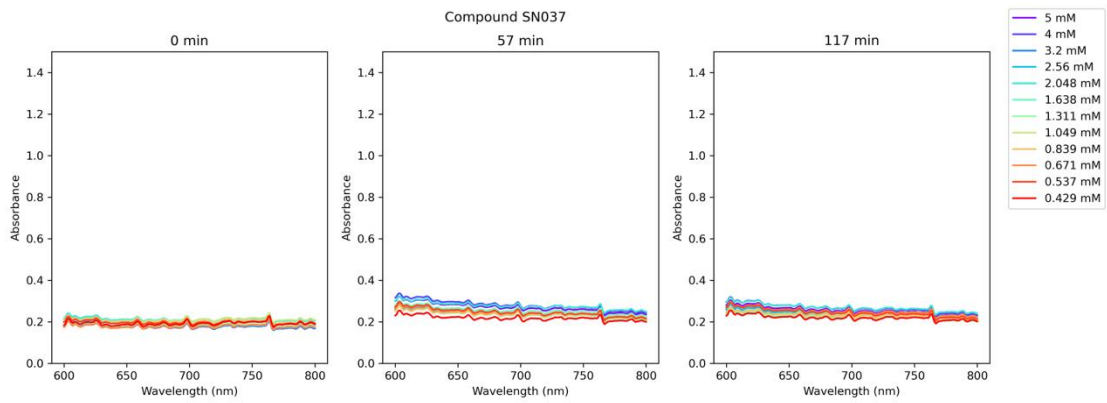


Figure S 3.41: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN037.

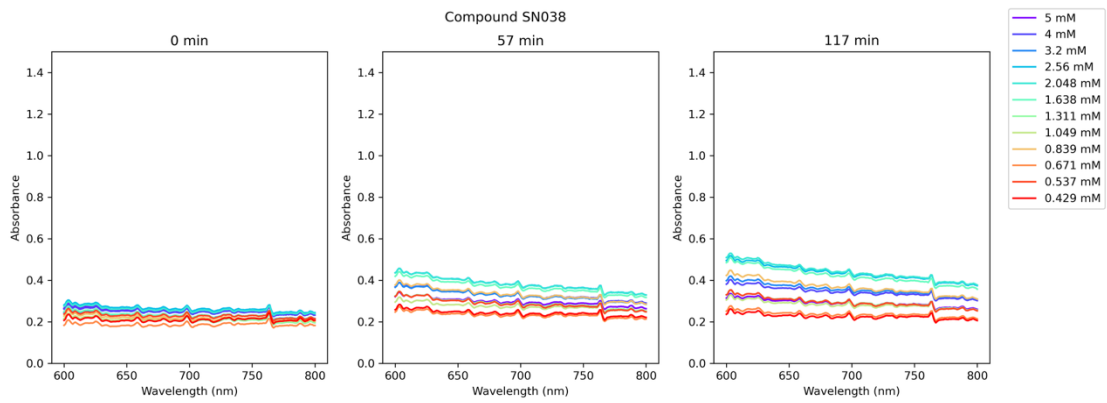


Figure S 3.42: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN038.

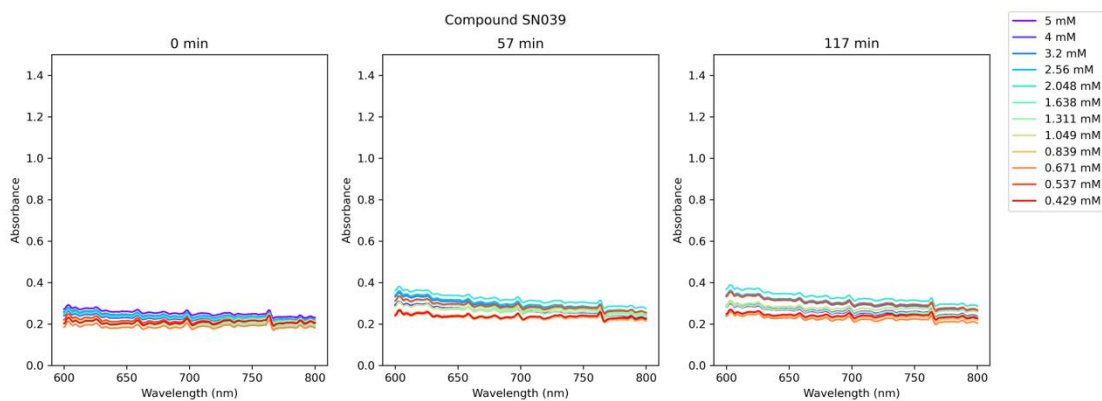


Figure S 3.43: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN039.

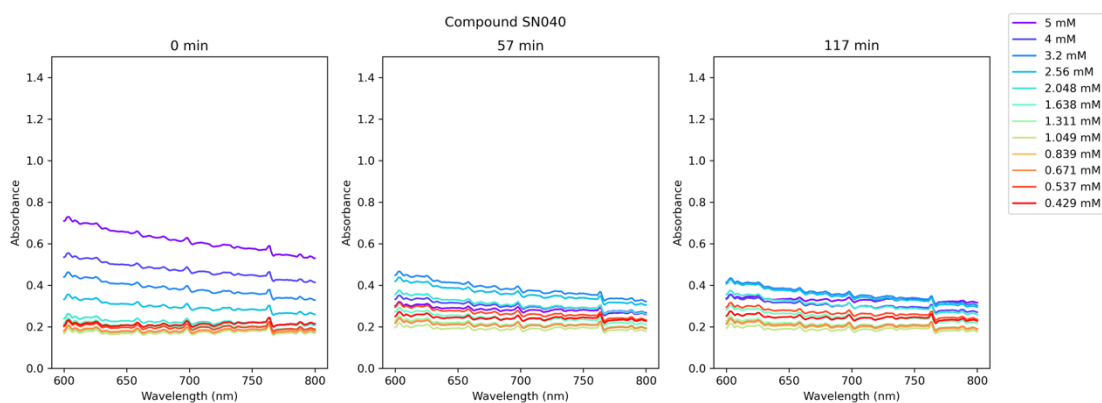


Figure S 3.44: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN040.

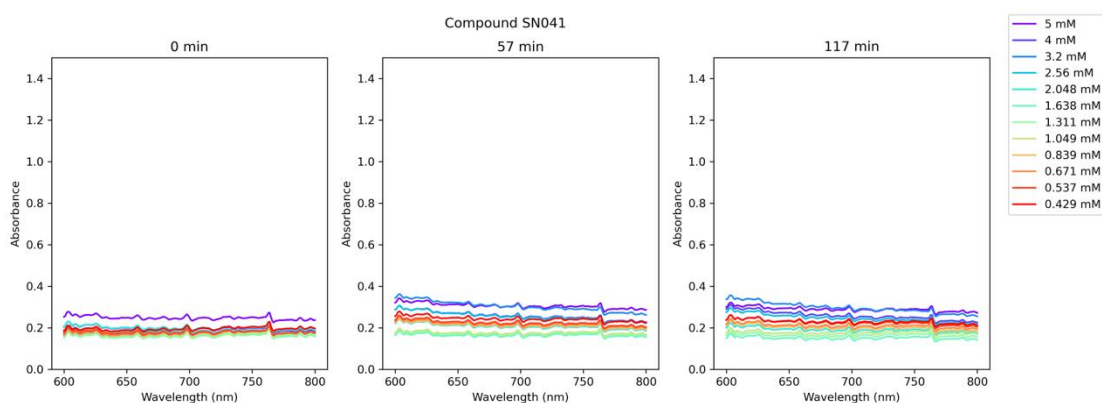


Figure S 3.45: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN041.

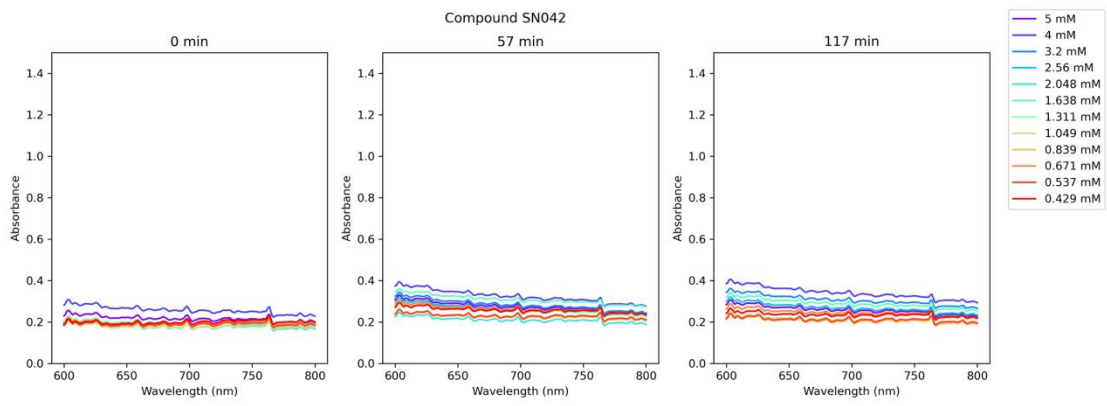


Figure S 3.46: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN042.

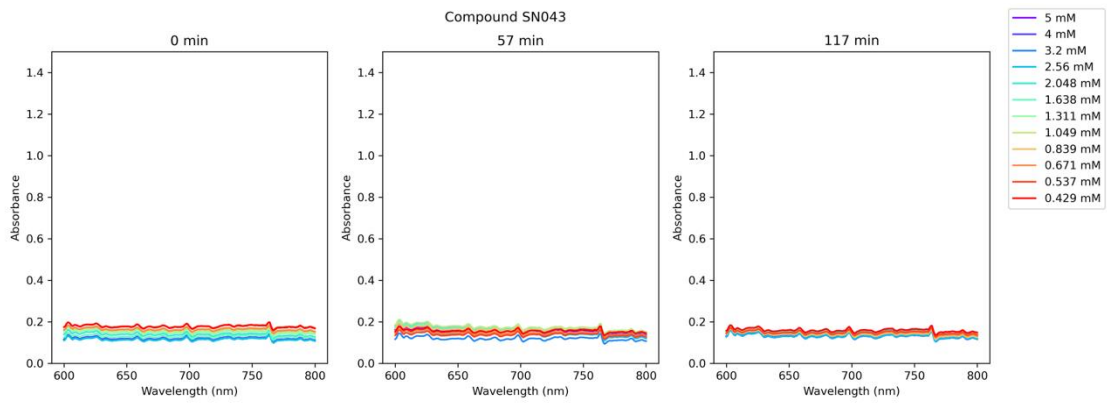


Figure S 3.47: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN043.

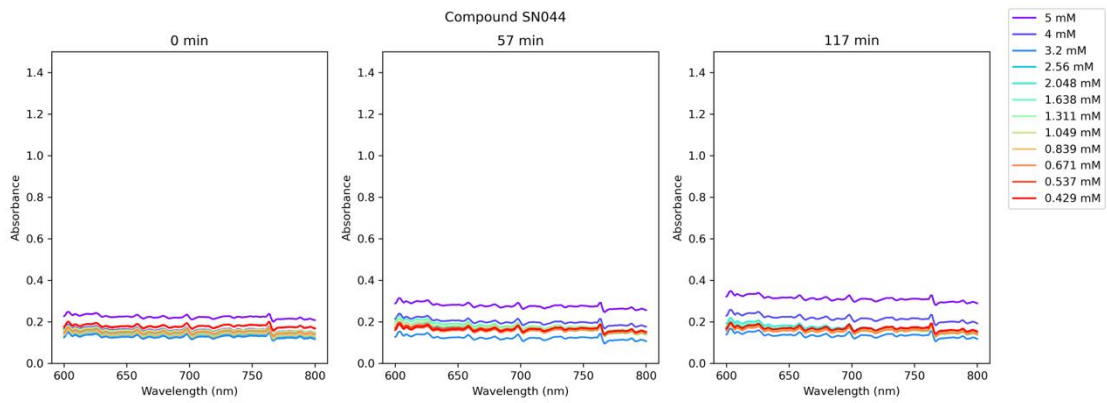


Figure S 3.48: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN044.

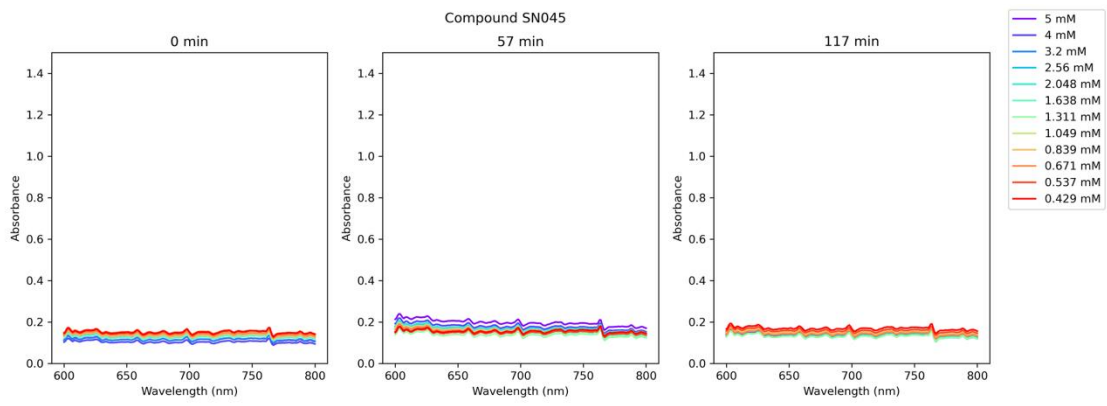


Figure S 3.49: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN045.

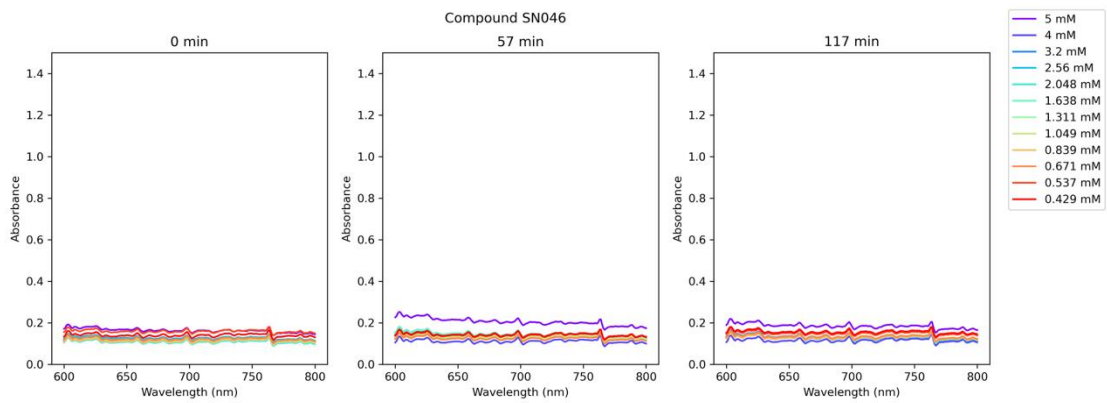


Figure S 3.50: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN046.

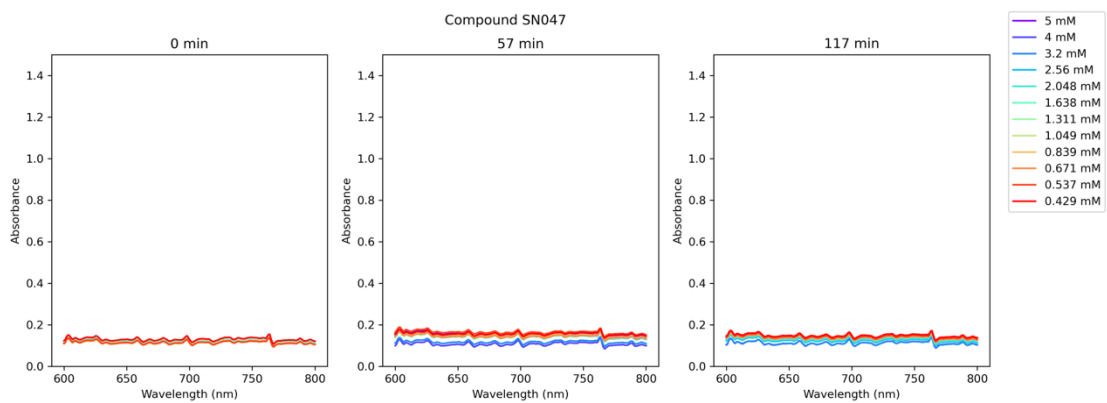


Figure S 3.51: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN047.

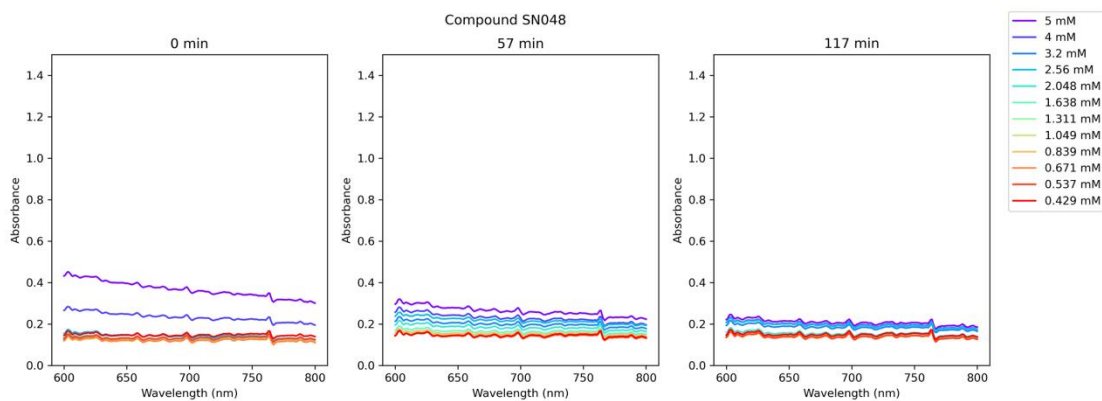


Figure S 3.52: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN048.

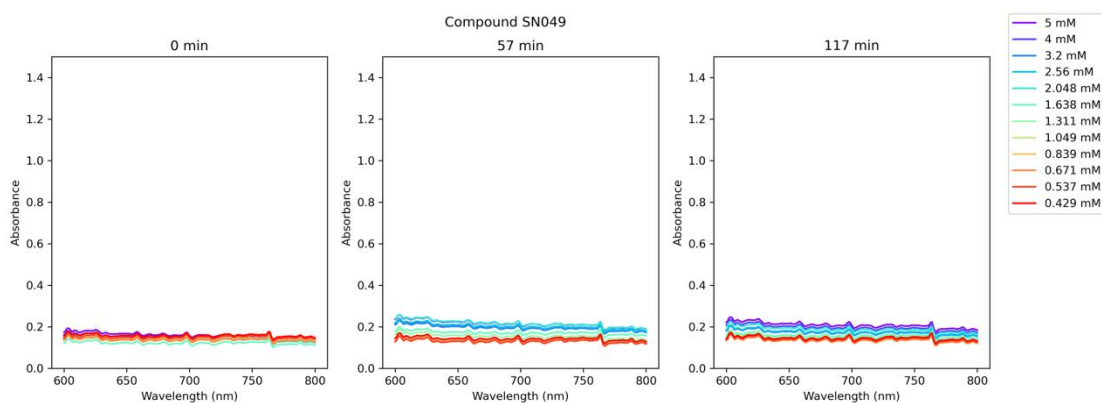


Figure S 3.53: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN049.

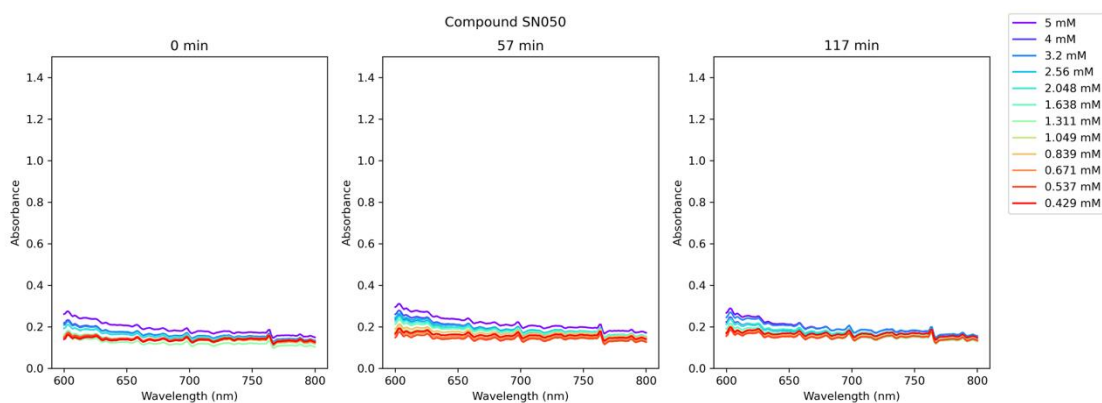


Figure S 3.54: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN050

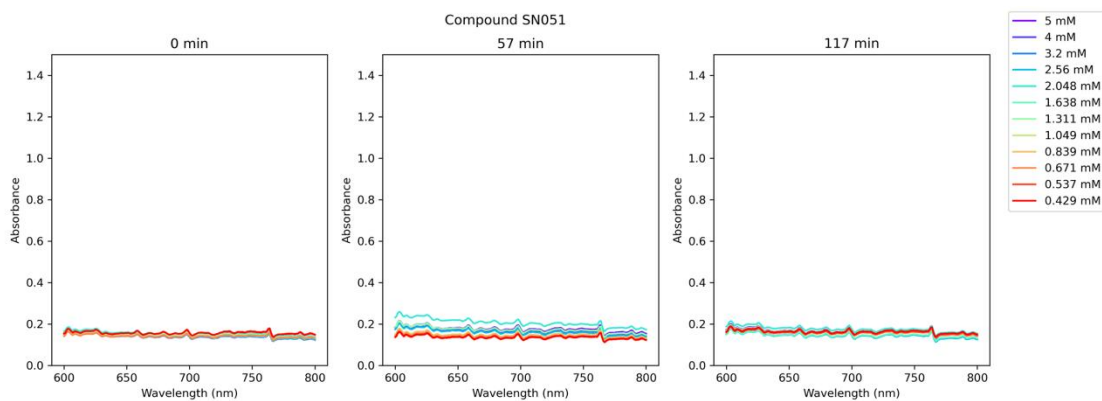


Figure S 3.55: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN051.

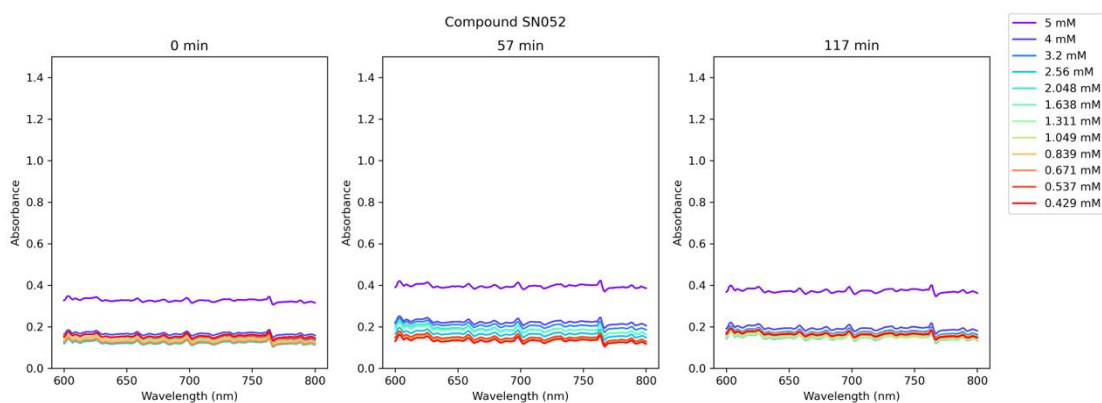


Figure S 3.56: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN052.

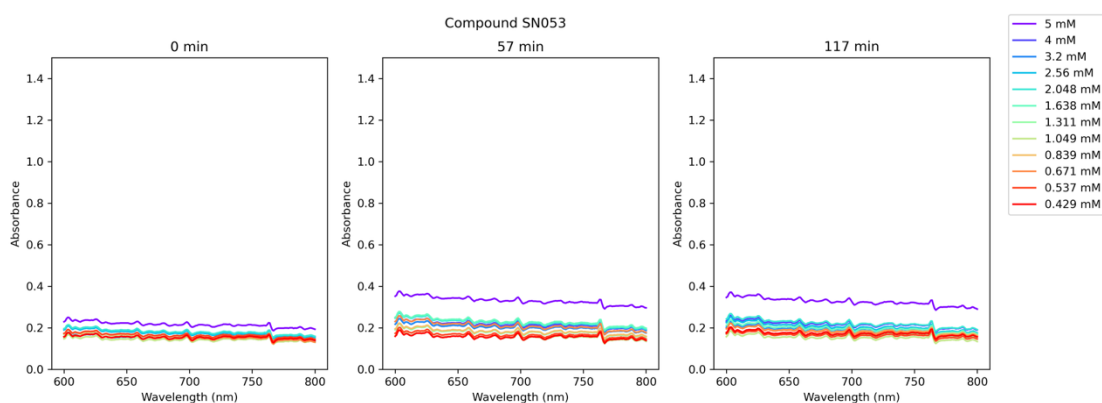


Figure S 3.57: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN053.

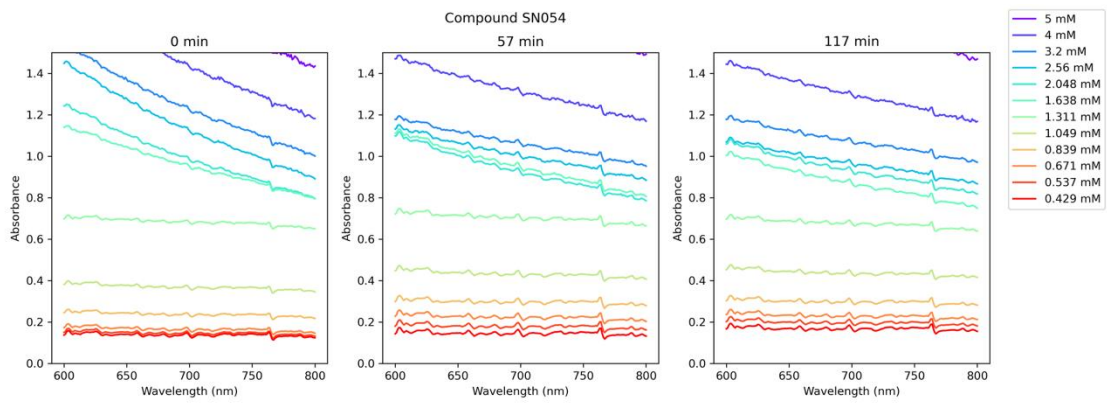


Figure S 3.58: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN054.

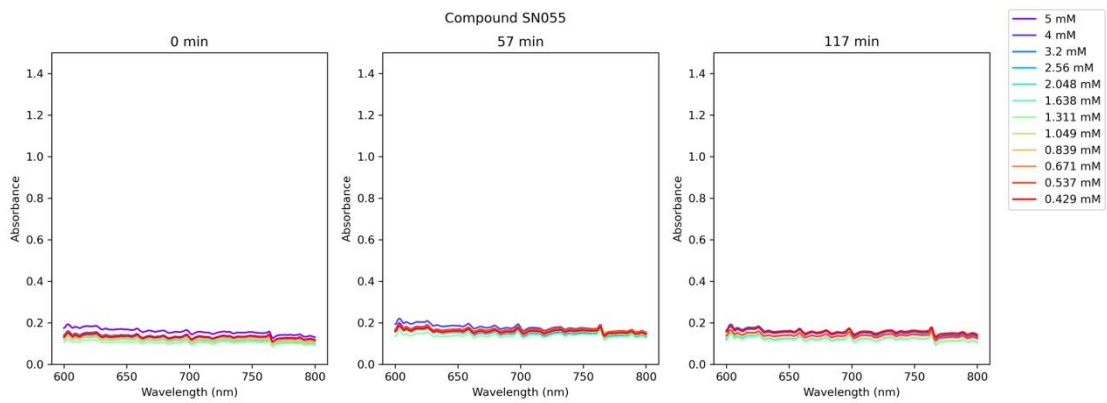


Figure S 3.59: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN055.

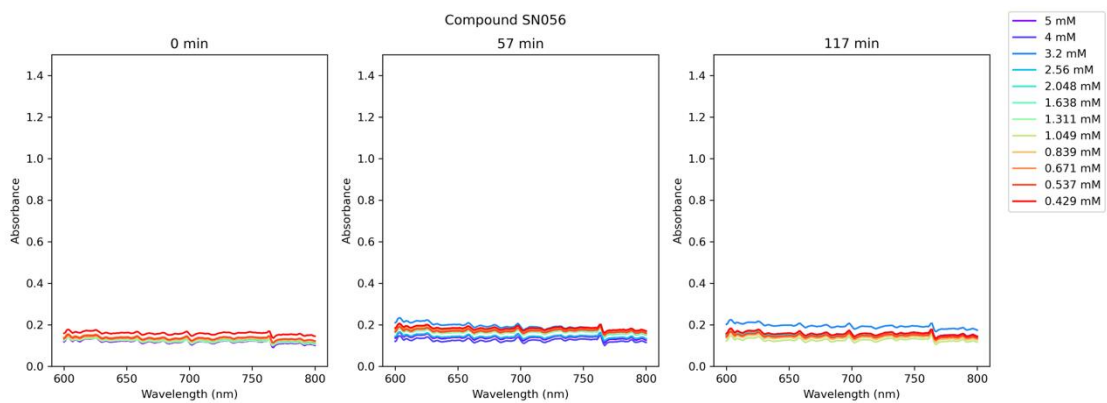


Figure S 3.60: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN056.

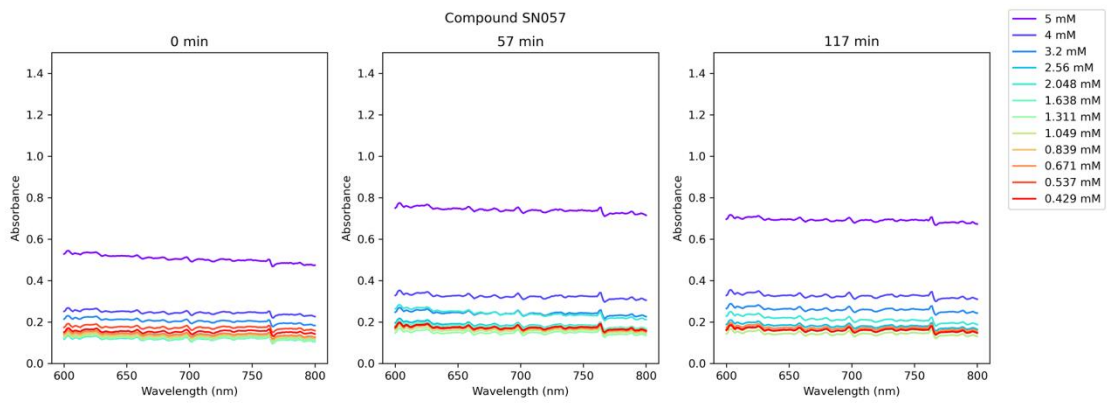


Figure S 3.61: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN057.

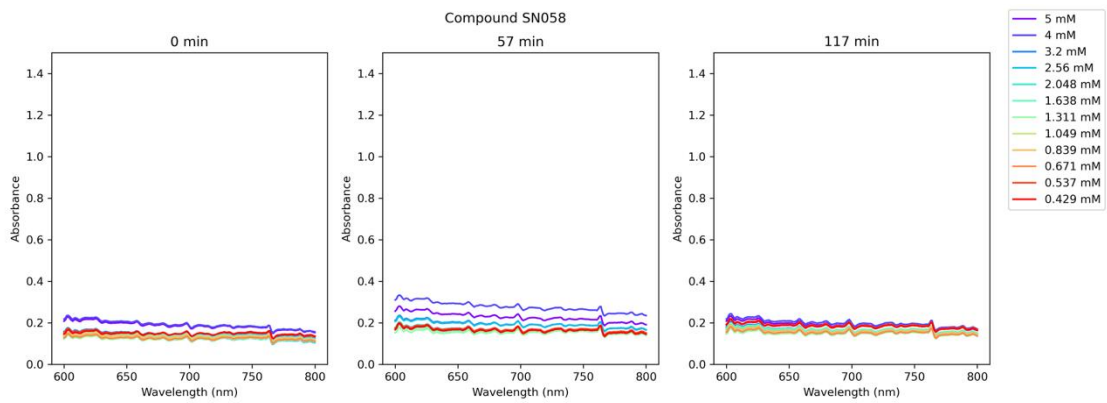


Figure S 3.62: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN058.

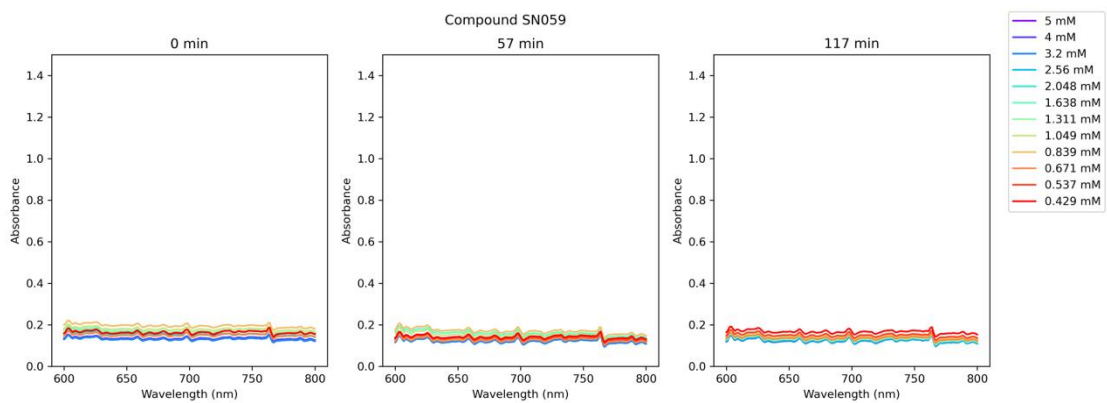


Figure S 3.63: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN059.

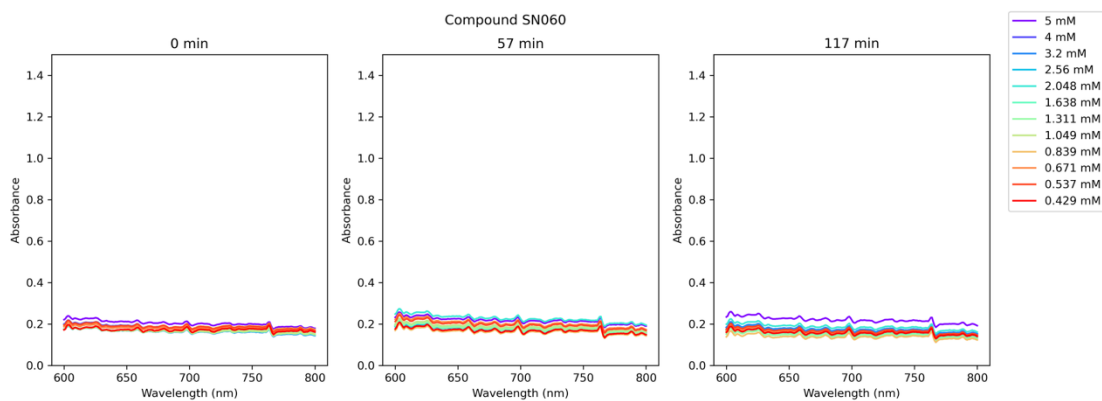


Figure S 3.64: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN060.

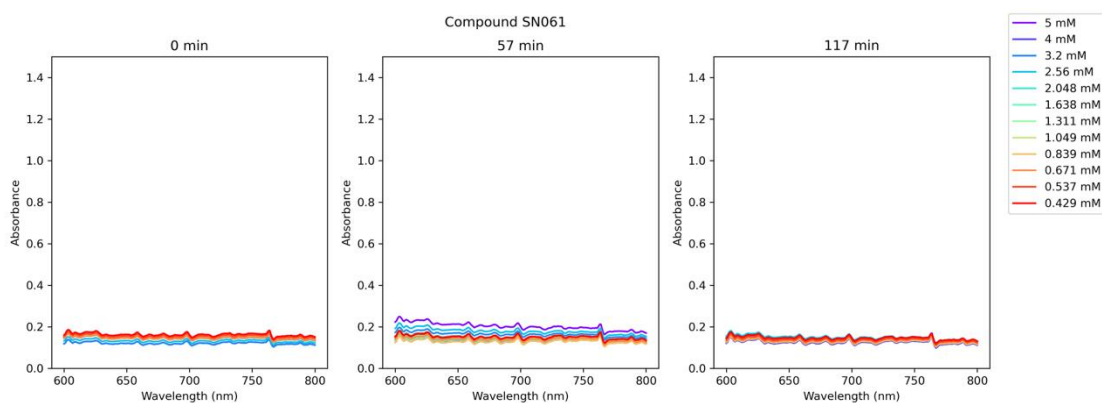


Figure S 3.65: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN061.

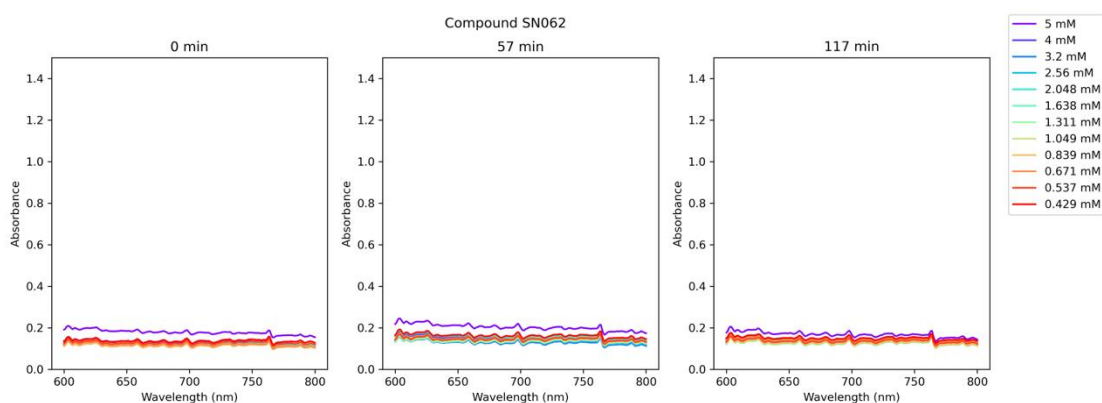


Figure S 3.66: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN062.

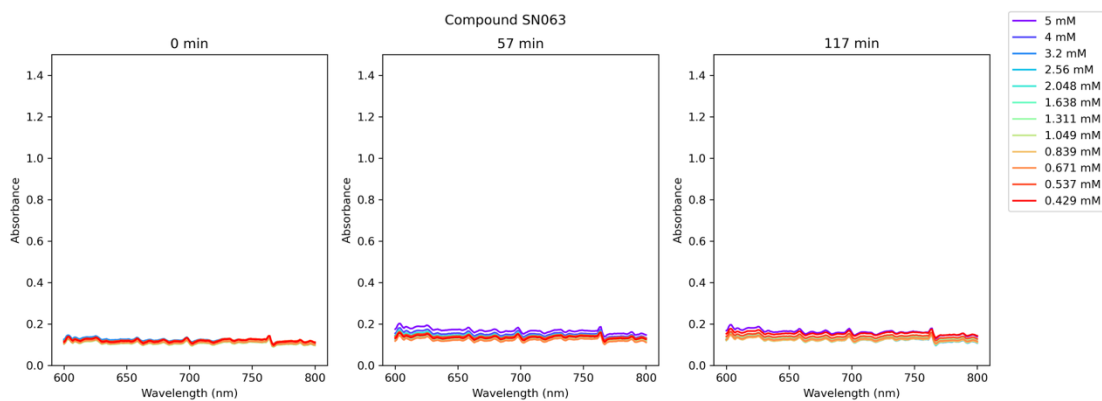


Figure S 3.67: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN063.

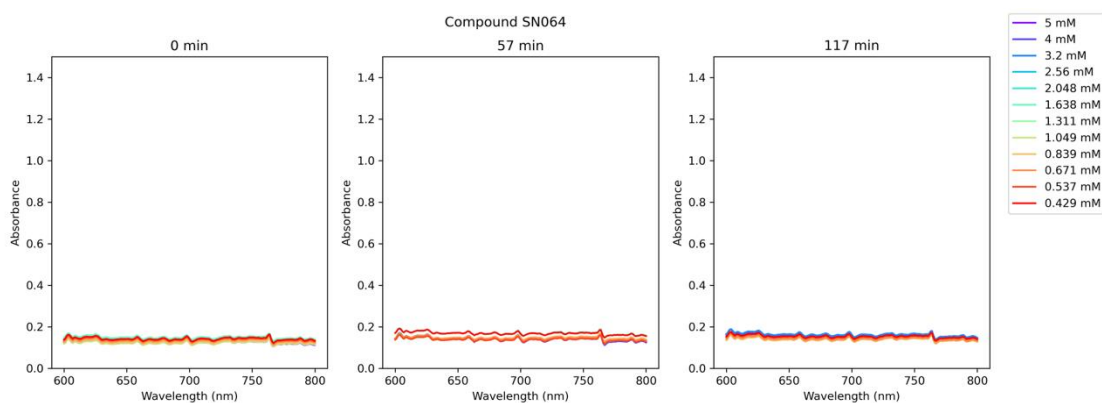


Figure S 3.68: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN064.

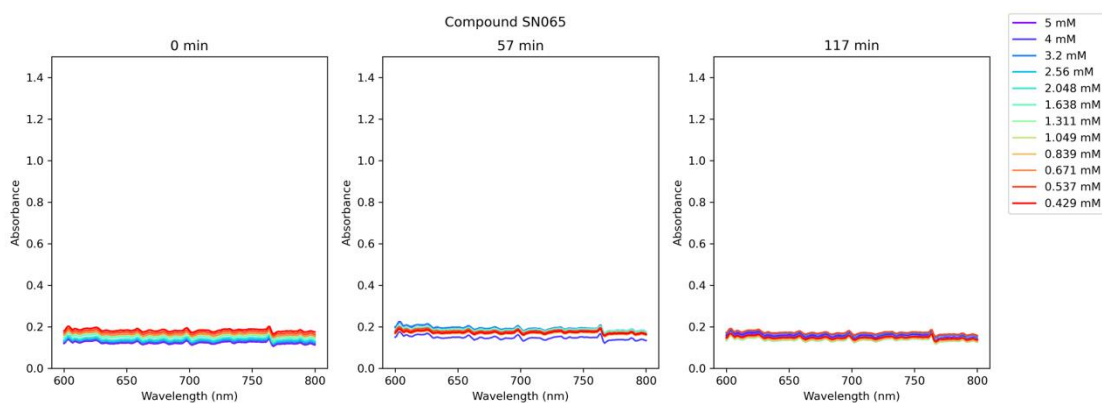


Figure S 3.69: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN066.

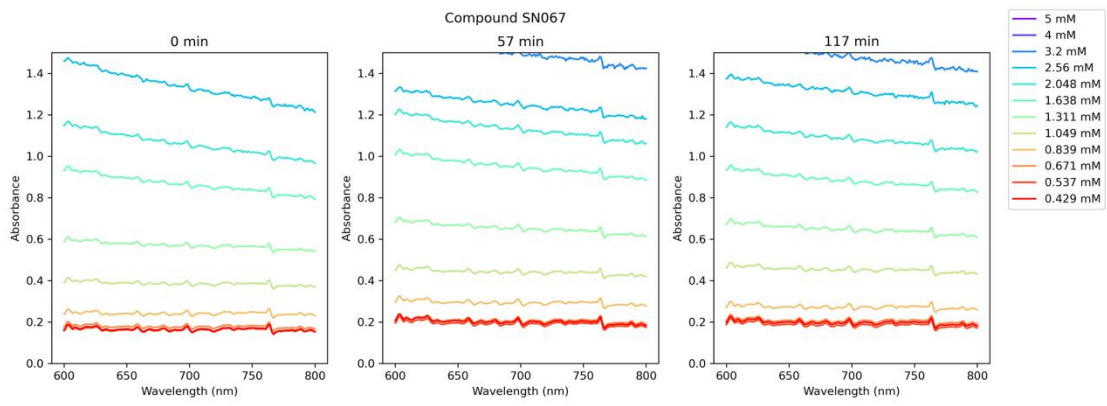


Figure S 3.70: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN067.

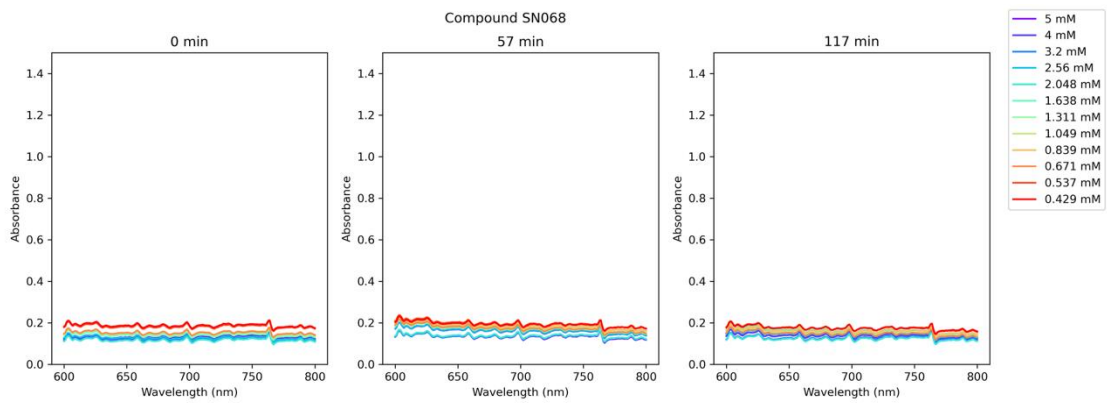


Figure S 3.71: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN068.

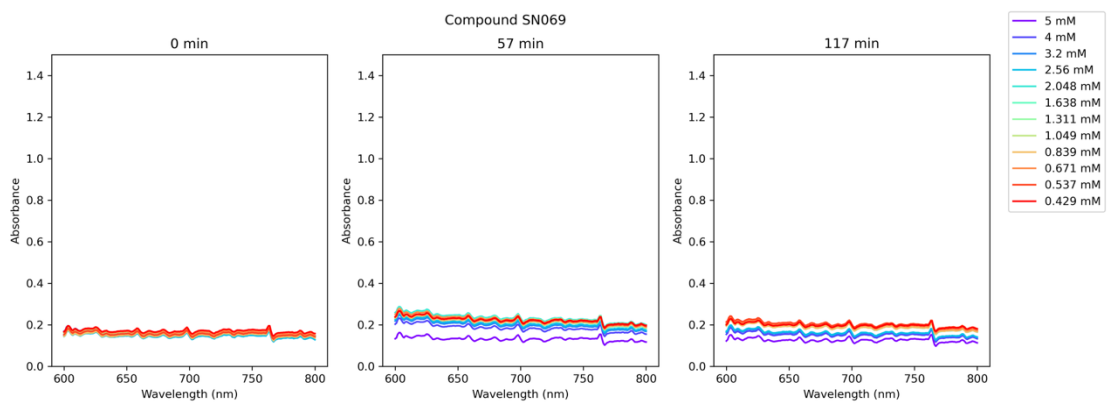


Figure S 3.72: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN069.

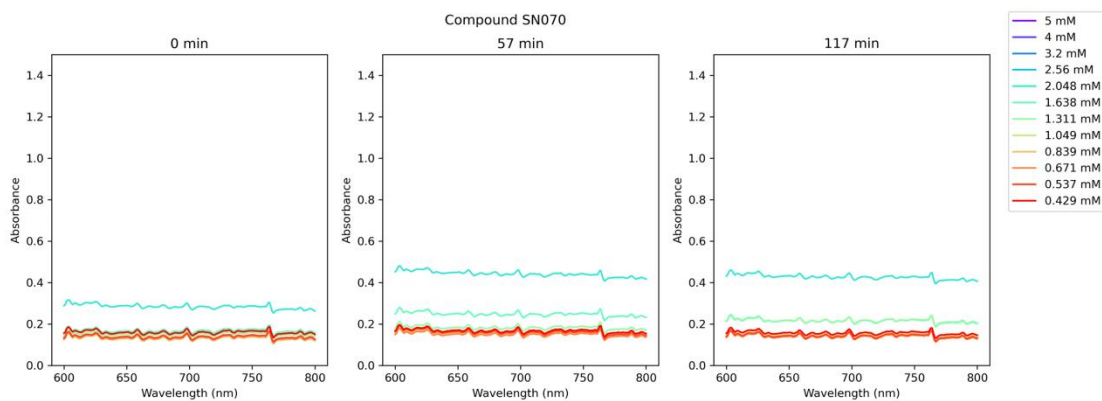


Figure S 3.73: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN070.

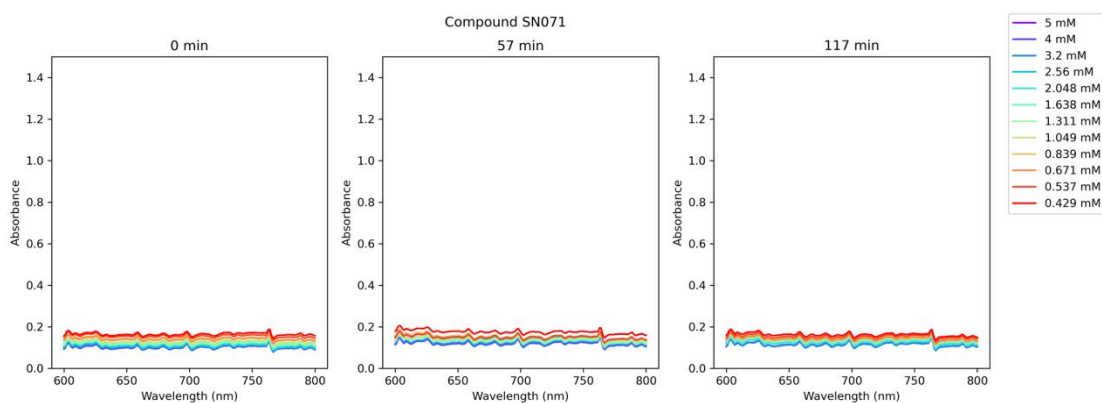


Figure S 3.74: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN071.

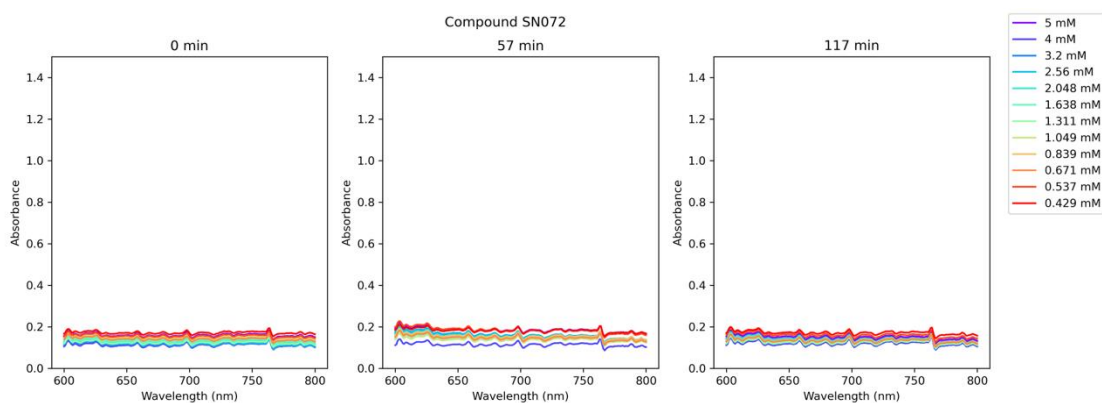


Figure S 3.75: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for SN072.

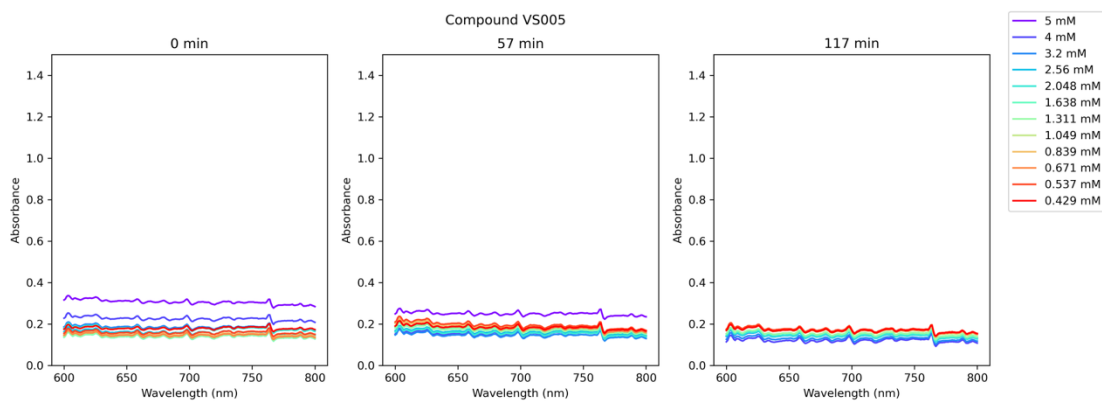


Figure S 3.76: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for VS005.

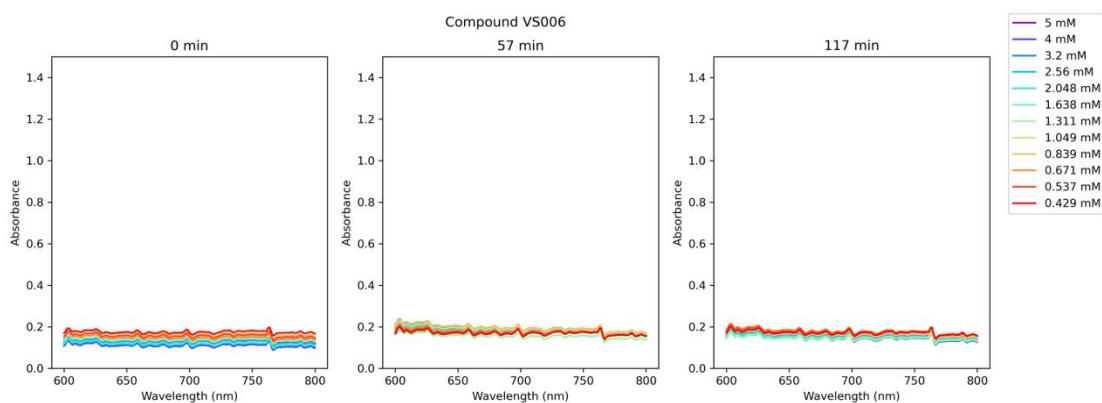


Figure S 3.77: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for VS006.

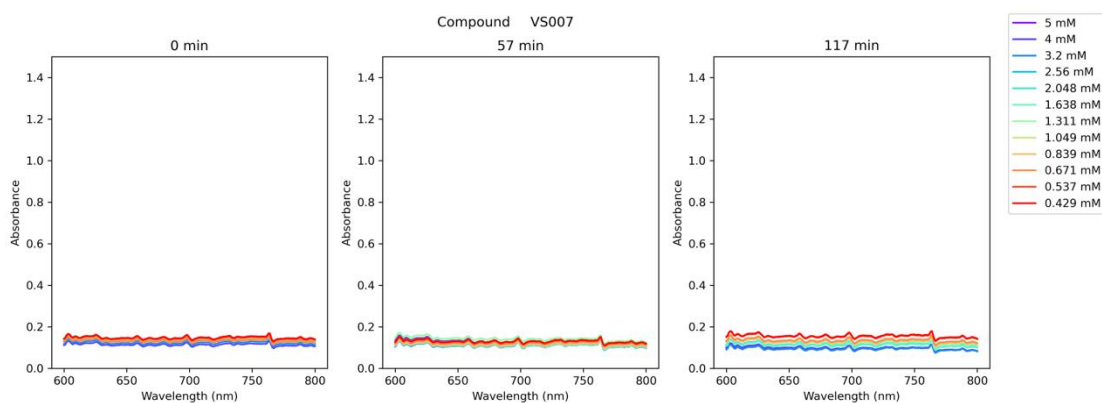


Figure S 3.78: Extinction spectra of the turbidimetric solubility assay after 0, 57, and 117 min for VS007.

## 4 DTNB Assay

### 4.1 Fit Results

Table S 4.1: Fit results of the DTNB Assay for AA001-SN035.

Compound	Measurement 1				Measurement 2				Measurement 3				Mean				
	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error	B0 [μM]	Error	A0 [μM]	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error	B0 [μM]	Error	A0 [μM]	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error	B0 [μM]	Error	A0 [μM]	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error
AA001	0.02871	0.00263	50.0	0.0	104.8	0.01065	0.00269	50.0	0.0	94.9	0.01425	0.00488	50.0	0.0	94.7	0.01787	0.00491
AA002	-0.00878	0.00181	50.0	0.0	109.2	0.01091	0.00123	50.0	0.0	97.8	-0.01216	0.00134	50.0	0.0	96.8	-0.00334	0.00086
AA003	0.01459	0.00234	50.0	0.0	103.9	-0.00159	0.00105	50.0	0.0	92.2	-0.01054	0.00161	50.0	0.0	94.9	0.00082	0.00101
AA004	0.04130	0.00315	50.0	0.0	102.0	0.01124	0.00112	50.0	0.0	94.9	0.01410	0.00130	50.0	0.0	0.0	0.00214	0.00120
AA005	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
AA006	0.00430	0.00163	50.0	0.0	103.0	0.01713	0.00114	50.0	0.0	93.7	0.00187	0.00151	50.0	0.0	100.0	0.00777	0.00083
AA007	0.00033	0.00093	50.0	0.0	99.4	0.02201	0.00222	50.0	0.0	101.1	-0.00705	0.00114	50.0	0.0	97.8	0.00510	0.00089
AA008	0.00000	0.00000	50.0	0.0	100.9	0.03155	0.00405	50.0	0.0	100.6	-0.01984	0.00156	50.0	0.0	99.1	0.00390	0.00145
CA006	-0.00066	0.00139	50.0	0.0	102.1	-0.00655	0.00179	50.0	0.0	97.8	-0.00769	0.00146	50.0	0.0	96.8	-0.00497	0.00090
CA007	-0.01513	0.00262	50.0	0.0	104.9	0.00817	0.00148	50.0	0.0	99.0	0.01229	0.00167	50.0	0.0	96.2	0.00178	0.00115
CA008	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
EO004	-0.01121	0.00229	50.0	0.0	103.4	0.01628	0.00138	50.0	0.0	97.6	-0.02992	0.00319	50.0	0.0	99.1	-0.00828	0.00139
EO005	0.02469	0.00206	50.0	0.0	104.5	0.00894	0.00199	50.0	0.0	100.6	0.00268	0.00177	50.0	0.0	100.6	0.01210	0.00112
EO006	-0.02040	0.00338	50.0		97.9	-0.01701	0.00324	50.0	0.0	101.3	-0.03499	0.00396	50.0	0.0	100.5	-0.02413	0.00204
EO007	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>
EO008	0.00411	0.00108	50.0	0.0	110.8	-0.00216	0.00121	50.0	0.0	98.4	0.00605	0.00179	50.0	0.0	102.0	0.00267	0.00081
EO009	-0.02232	0.00161	50.0	0.0	99.5	0.00229	0.00268	50.0	0.0	102.5	-0.04725	0.00423	50.0	0.0	106.1	-0.02243	0.00175
EO010	0.00878	0.00169	50.0	0.0	99.3	0.00986	0.00149	50.0	0.0	101.0	0.02008	0.00173	50.0	0.0	100.3	0.01291	0.00095
SN013	-0.01652	0.00210	50.0	0.0	101.5	-0.02043	0.00216	50.0	0.0	97.3	0.02698	0.00203	50.0	0.0	100.5	-0.00332	0.00121
SN014	0.01721	0.00390	50.0	0.0	110.5	-0.01414	0.00212	50.0	0.0	97.5	-0.01702	0.00284	50.0	0.0	101.5	-0.00465	0.00176
SN015	0.01177	0.00067	50.0	0.0	107.4	0.02929	0.00125	50.0	0.0	103.8	0.02032	0.00057	50.0	0.0	105.6	0.02481	0.00051
SN016	0.18717	0.01014	50.0	0.0	105.3	0.21411	0.00975	50.0	0.0	102.2	0.28556	0.01114	50.0	0.0	100.9	0.22895	0.00598
SN017	0.47423	0.00286	50.0	0.0	108.9	0.41466	0.00506	50.0	0.0	104.1	0.39638	0.01195	50.0	0.0	103.2	0.42842	0.00443
SN018	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN019	1.40327	0.03542	27.4	0.4	96.8	1.45550	0.05732	38.2	0.8	98.5	1.49397	0.03942	39.8	0.6	99.4	1.42939	0.02602
SN020	0.02923	0.00126	50.0	0.0	106.8	0.01420	0.00097	50.0	0.0	99.7	0.01166	0.00054	50.0	0.0	104.6	0.01836	0.00056
SN021	-0.02276	0.00509	50.0	0.0	108.3	-0.00891	0.00190	50.0	0.0	97.8	-0.02175	0.00315	50.0	0.0	101.2	-0.01781	0.00209
SN022	0.08469	0.00289	50.0	0.0	111.0	0.09007	0.00298	50.0	0.0	95.0	0.09613	0.00390	50.0	0.0	95.5	0.09030	0.00190
SN023	-0.00190	0.00101	50.0	0.0	94.6	0.00842	0.00143	50.0	0.0	95.1	-0.00568	0.00285	50.0	0.0	98.6	0.00028	0.00111
SN024	0.02142	0.00317	50.0	0.0	111.1	0.02247	0.00191	50.0	0.0	96.8	0.00902	0.00156	50.0	0.0	96.2	0.01764	0.00134
SN025	0.00011	0.00118	50.0	0.0	103.9	-0.00065	0.00070	50.0	0.0	96.0	-0.00426	0.00102	50.0	0.0	95.1	-0.00160	0.00057
SN026	0.03544	0.00212	50.0	0.0	106.0	0.05318	0.00314	50.0	0.0	104.7	0.02948	0.00190	50.0	0.0	101.5	0.03937	0.00141
SN027	0.11311	0.00192	50.0	0.0	104.1	0.08121	0.00190	50.0	0.0	98.9	-0.03366	0.00133	50.0	0.0	118.6	0.00190	0.00100
SN028	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN029	0.00994	0.00095	50.0	0.0	108.6	0.00827	0.00209	50.0	0.0	99.8	-0.00330	0.00060	50.0	0.0	101.0	0.00497	0.00079
SN030	0.03839	0.00415	50.0	0.0	115.2	0.01128	0.00339	50.0	0.0	99.4	0.01659	0.00333	50.0	0.0	100.0	0.02209	0.00210
SN031	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN032	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN033	0.02928	0.00136	50.0	0.0	113.4	0.01467	0.00130	50.0	0.0	105.2	0.03462	0.00211	50.0	0.0	103.3	0.02619	0.00094
SN034	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>
SN035	0.01106	0.00055	50.0	0.0	106.8	0.01544	0.00104	50.0	0.0	101.5	0.00845	0.00402	50.0	0.0	102.7	0.01165	0.00140

<sup>a</sup> 3.33 mM ACN stock not soluble. <sup>b</sup> The purity of the compound could not be confirmed.

Table S 4.2: Fit results of the DTNB Assay for SN036-VS007.

Compound	Measurement 1					Measurement 2					Measurement 3					Mean	
	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error	B0 [μM]	Error	A0 [μM]	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error	B0 [μM]	Error	A0 [μM]	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error	B0 [μM]	Error	A0 [μM]	$k_2$ [M <sup>-1</sup> s <sup>-1</sup> ]	Error
SN036	0.00012	0.00189	50.0	0.0	104.3	0.03665	0.00232	50.0	0.0	110.9	0.00911	0.00138	50.0	0.0	105.7	0.01529	0.00110
SN037	0.02408	0.00130	50.0	0.0	108.7	0.01422	0.00081	50.0	0.0	105.3	0.06520	0.00144	50.0	0.0	104.0	0.03450	0.00070
SN038	0.02771	0.00046	50.0	0.0	101.2	0.04480	0.00074	50.0	0.0	99.7	0.05543	0.00107	50.0	0.0	98.9	0.04265	0.00046
SN039	0.25290	0.00339	50.0	0.0	106.8	0.16800	0.00250	50.0	0.0	102.2	0.17292	0.00236	50.0	0.0	105.4	0.19794	0.00161
SN040	0.00738	0.00071	50.0	0.0	115.1	0.02013	0.00160	50.0	0.0	105.1	-0.04004	0.00262	50.0	0.0	105.7	-0.00418	0.00105
SN041	-0.00012	0.00145	50.0	0.0	111.6	0.00631	0.00074	50.0	0.0	100.8	-0.01304	0.00206	50.0	0.0	104.1	-0.00228	0.00088
SN042	0.00496	0.00176	50.0	0.0	101.1	0.02136	0.00095	50.0	0.0	107.2	0.05912	0.00103	50.0	0.0	108.7	0.02848	0.00075
SN043	0.02894	0.00150	50.0	0.0	107.7	0.01603	0.00109	50.0	0.0	106.3	0.02064	0.00102	50.0	0.0	105.5	0.02187	0.00071
SN044	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN045	0.03449	0.00064	50.0	0.0	101.5	0.04280	0.00063	50.0	0.0	99.6	0.05026	0.00076	50.0	0.0	98.6	0.04252	0.00039
SN046	0.00471	0.00070	50.0	0.0	100.4	0.06820	0.00130	50.0	0.0	98.3	0.02458	0.00119	50.0	0.0	97.1	0.03250	0.00063
SN047	0.02079	0.00109	50.0	0.0	108.3	0.00832	0.00073	50.0	0.0	103.5	0.00718	0.00052	50.0	0.0	102.4	0.00775	0.00030
SN048	0.20434	0.00279	50.0	0.0	97.7	0.19438	0.00232	50.0	0.0	94.8	0.24855	0.00192	50.0	0.0	94.5	0.21576	0.00137
SN049	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN050	1.99865	0.13690	26.2	0.0	93.2	2.36202	0.10761	28.2	0.0	93.0	2.74157	0.10621	26.7	0.0	88.6	2.36741	0.06799
SN051	0.00576	0.00082	50.0	0.0	96.1	0.00696	0.00047	50.0	0.0	91.1	0.02977	0.00147	50.0	0.0	91.0	0.01416	0.00058
SN052	0.02492	0.00121	50.0	0.0	94.3	0.01122	0.00045	50.0	0.0	94.2	0.01127	0.00049	50.0	0.0	91.6	0.01580	0.00046
SN053	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN054	0.14245	0.00364	50.0	0.0	86.8	0.12470	0.00328	50.0	0.0	81.5	0.09583	0.00305	50.0	0.0	83.5	0.12099	0.00192
SN055	0.01383	0.00129	50.0	0.0	86.7	0.01864	0.00086	50.0	0.0	87.7	0.01314	0.00093	50.0	0.0	84.7	0.01520	0.00060
SN056	0.01655	0.00140	50.0	0.0	109.6	0.01874	0.00078	50.0	0.0	104.1	0.01038	0.00055	50.0	0.0	100.5	0.01522	0.00056
SN057	0.03956	0.00141	50.0	0.0	82.4	0.01734	0.00117	50.0	0.0	81.2	0.01762	0.00095	50.0	0.0	81.7	0.02484	0.00069
SN058	0.00358	0.00059	50.0		101.8	-0.02176	0.00164	50.0	0.0	102.1	0.02480	0.00148	50.0	0.0	0.0	0.00221	0.00076
SN059	0.02614	0.00196	50.0	0.0	107.1	0.03584	0.00207	50.0	0.0	105.9	0.03532	0.00234	50.0	0.0	104.3	0.03243	0.00123
SN060	1.71617	0.01281	50.0	0.0	115.2	0.98475	0.02525	50.0	0.0	102.5	1.40847	0.00767	50.0	0.0	113.1	1.36980	0.00978
SN061	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN062	0.03175	0.00218	50.0	0.0	108.1	0.02201	0.00189	50.0	0.0	109.1	0.02654	0.00192	50.0	0.0	108.4	0.02677	0.00116
SN063	0.03789	0.00191	50.0		125.3	0.02670	0.00159	50.0		124.7	0.04190	0.00177	50.0		122.6	0.03550	0.00102
SN064	0.16945	0.00309	50.0	0.0	131.8	0.12725	0.00290	50.0	0.0	130.2	0.13592	0.00313	50.0	0.0	128.9	0.14421	0.00176
SN065	0.01316	0.00111	50.0	0.0	81.9	-0.00520	0.00164	50.0	0.0	78.9	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>
SN066	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>
SN067	0.04477	0.00105	50.0	0.0	76.3	0.02141	0.00068	50.0	0.0	77.5	0.02768	0.00086	50.0	0.0	76.8	0.03129	0.00051
SN068	-0.00606	0.00100	50.0	0.0	97.8	-0.01477	0.00113	50.0	0.0	98.2	-0.01267	0.00211	50.0	0.0	106.5	-0.01117	0.00086
SN069	6.77675	0.14173	19.2	0.1	93.8	6.46697	0.19235	26.2	0.2	92.0	8.33206	0.11152	28.3	0.1	94.5	7.19193	0.08789
SN070	0.03356	0.00189	50.0	0.0	123.0	0.02959	0.00189	50.0	0.0	122.8	0.03136	0.00201	50.0		121.4	0.03150	0.00111
SN071	0.01692	0.00107	50.0	0.0	80.2	0.02171	0.00130	50.0	0.0	75.5	0.01882	0.00091	50.0	0.0	79.8	0.01915	0.00064
SN072	-0.00203	0.00128	50.0	0.0	82.6	0.00060	0.00140	50.0	0.0	79.5	0.00807	0.00121	50.0	0.0	77.5	0.00221	0.00075
VS005	-0.01163	0.00066	50.0	0.0	98.5	0.00811	0.00121	50.0	0.0	100.8	-0.02060	0.00219	50.0	0.0	105.1	-0.00804	0.00086
VS006	0.01991	0.00239	50.0	0.0	101.5	0.01026	0.00200	50.0	0.0	101.2	-0.00067	0.00191	50.0	0.0	111.5	0.00983	0.00122
VS007	0.13415	0.00454	50.0	0.0	130.6	0.35883	0.00910	50.0	0.0	126.4	0.24701	0.00750	50.0	0.0	126.0	0.24666	0.00421

<sup>a</sup> 3.33 mM ACN stock not soluble. <sup>b</sup> The purity of the compound could not be confirmed. <sup>c</sup> one triplicate was not evaluable.

## 4.2 Plots from the DTNB Assay

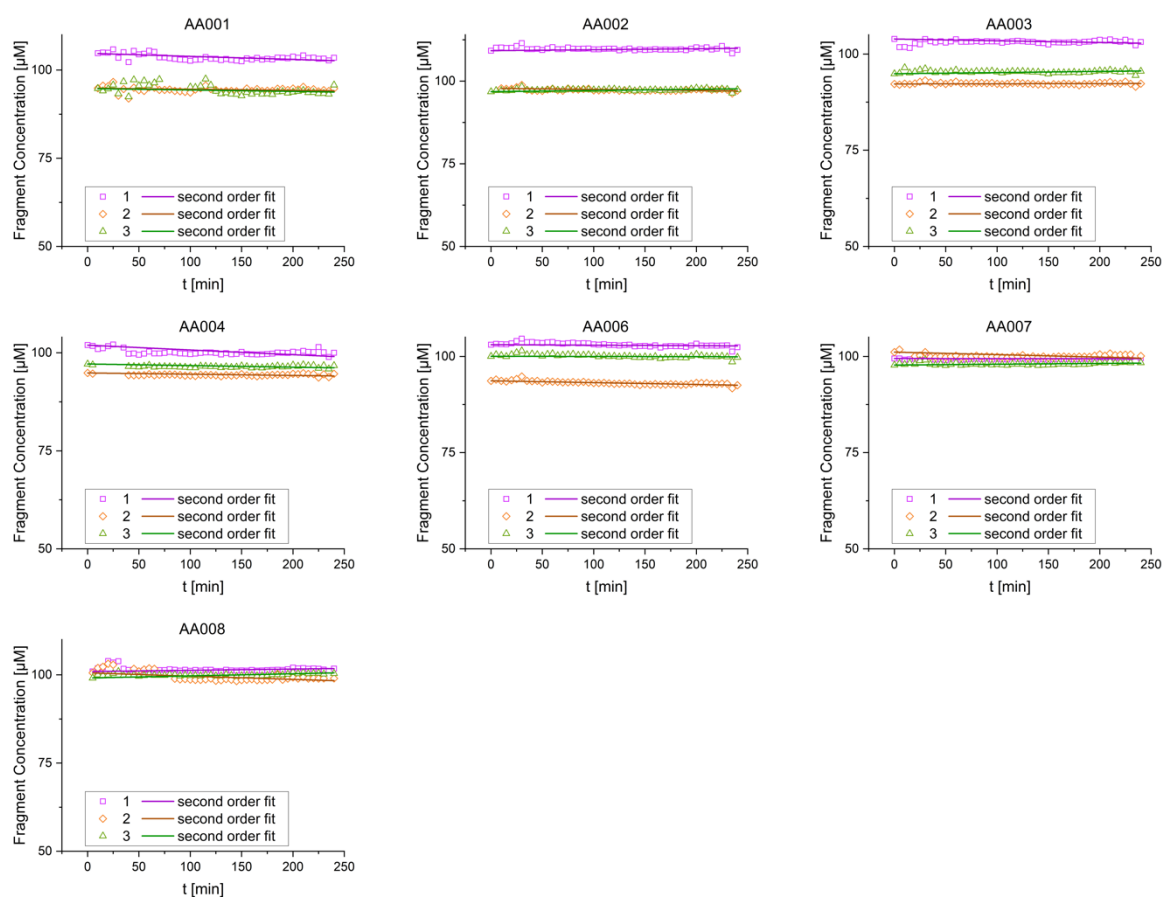


Figure S 4.1: Time-dependent depletion of the acrylamide fragments with DTNB.

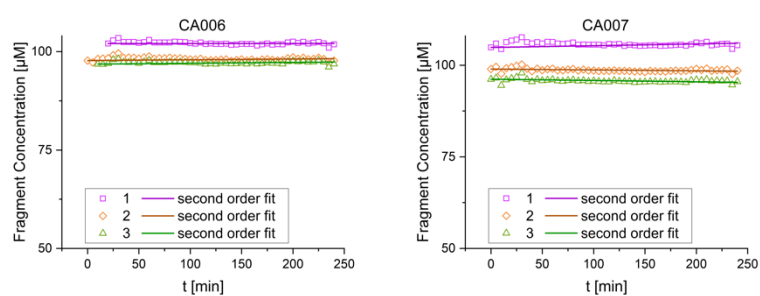


Figure S 4.2: Time-dependent depletion of the  $\alpha$ -cyano acrylamide fragments with DTNB.

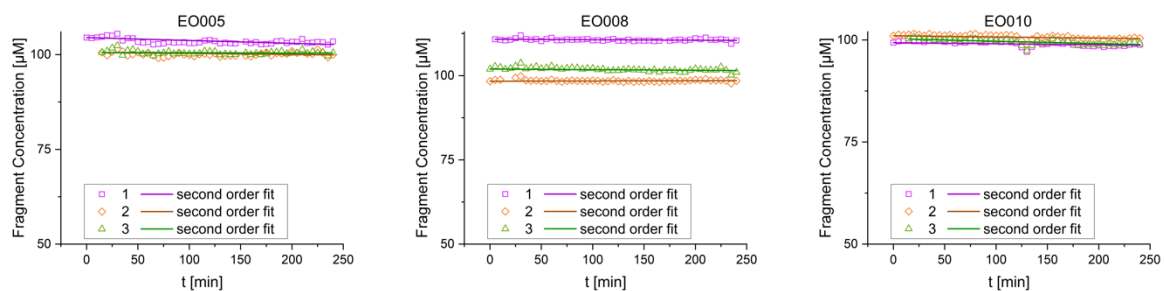


Figure S 4.3: Time-dependent depletion of the epoxide fragments with DTNB.

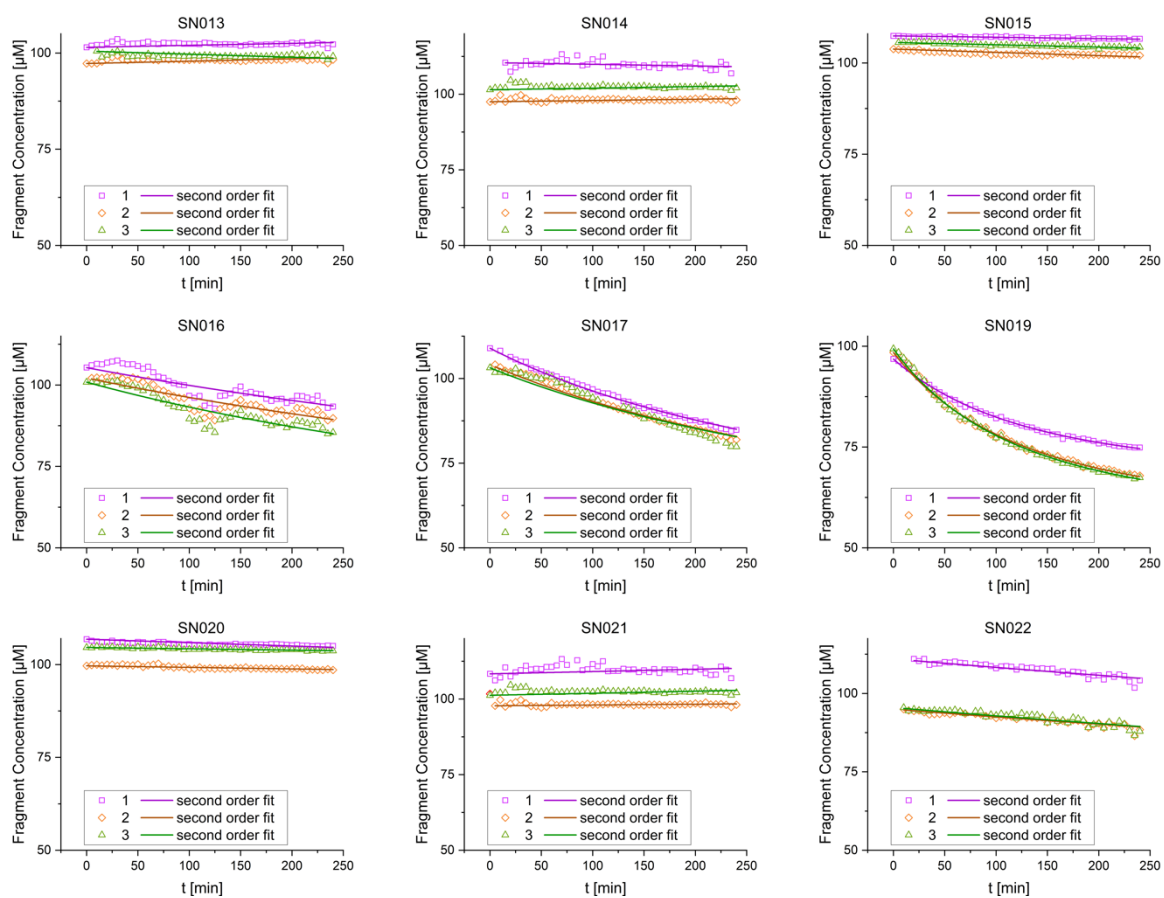


Figure S 4.4: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN013-SN022 with DTNB.

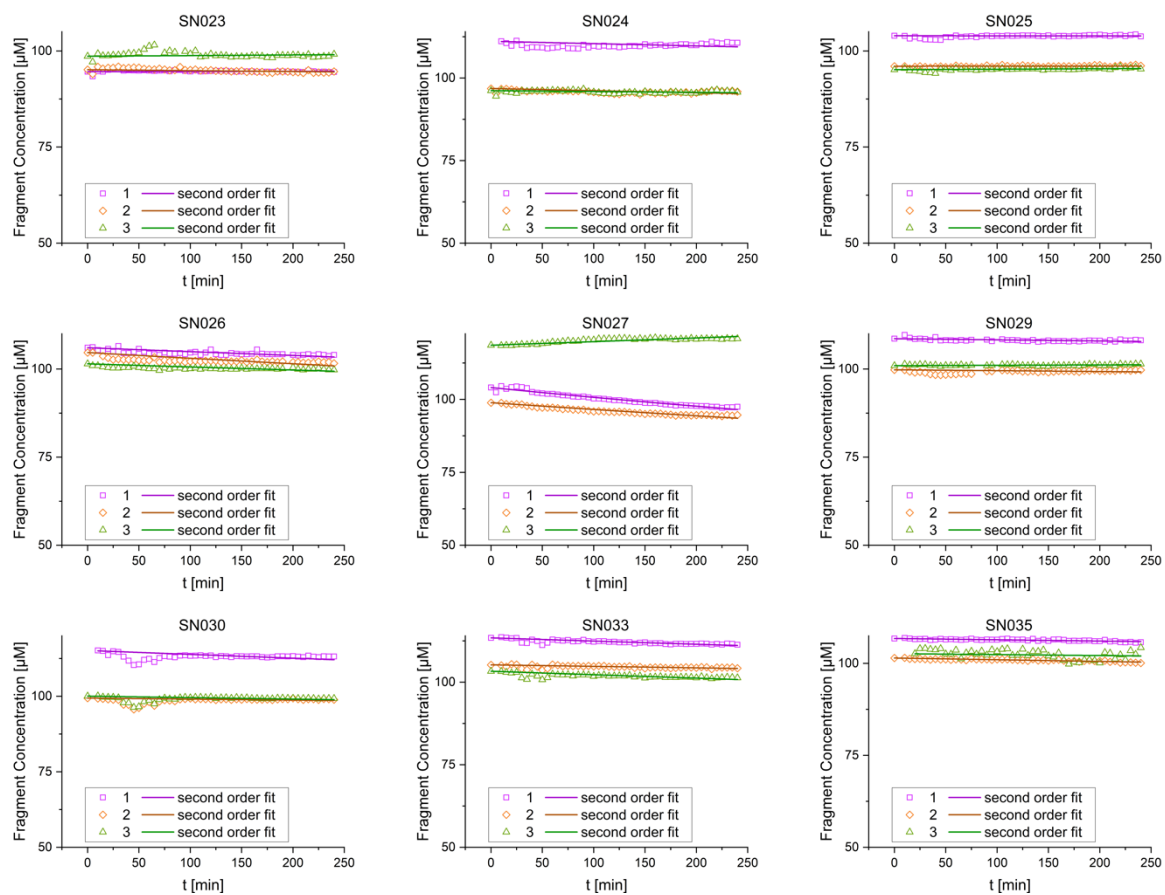


Figure S 4.5: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN023-SN035 with DTNB.

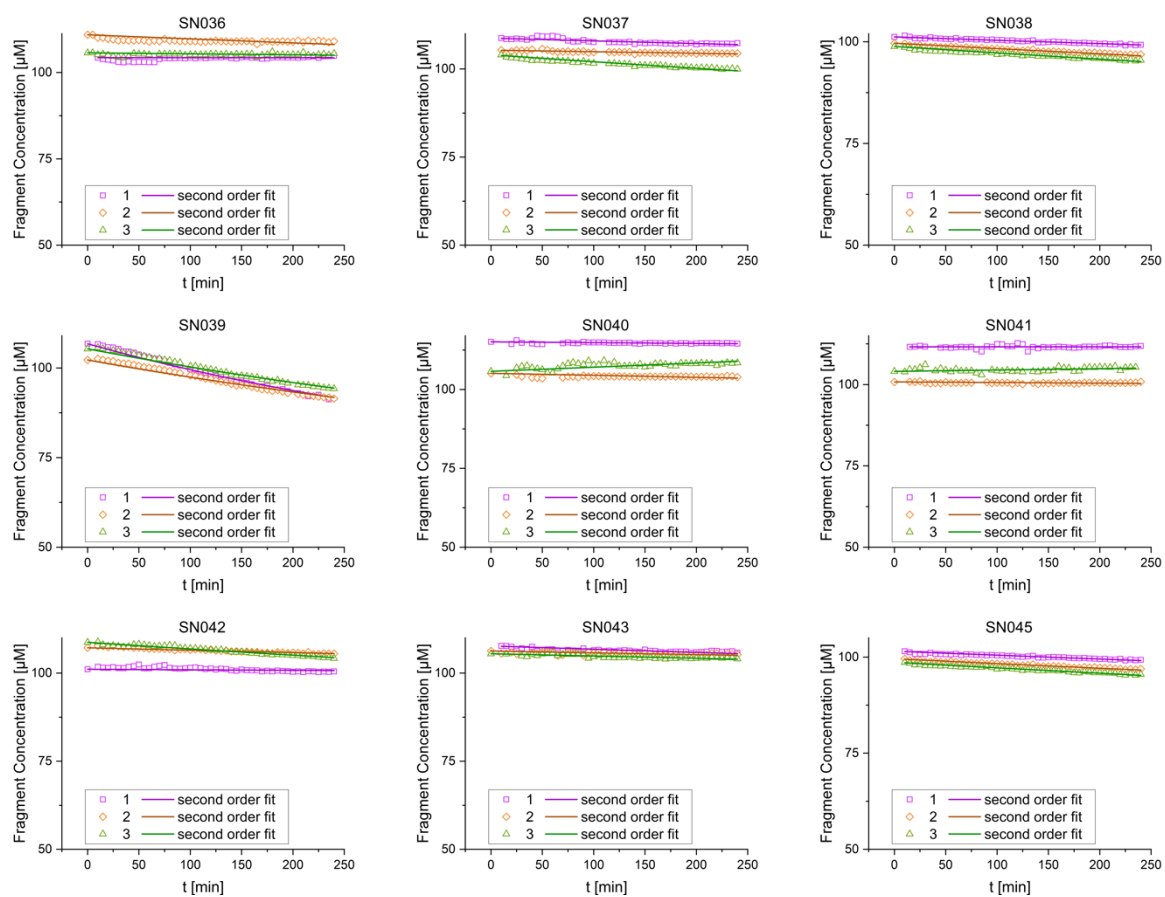


Figure S 4.6: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN036-SN045 with DTNB.

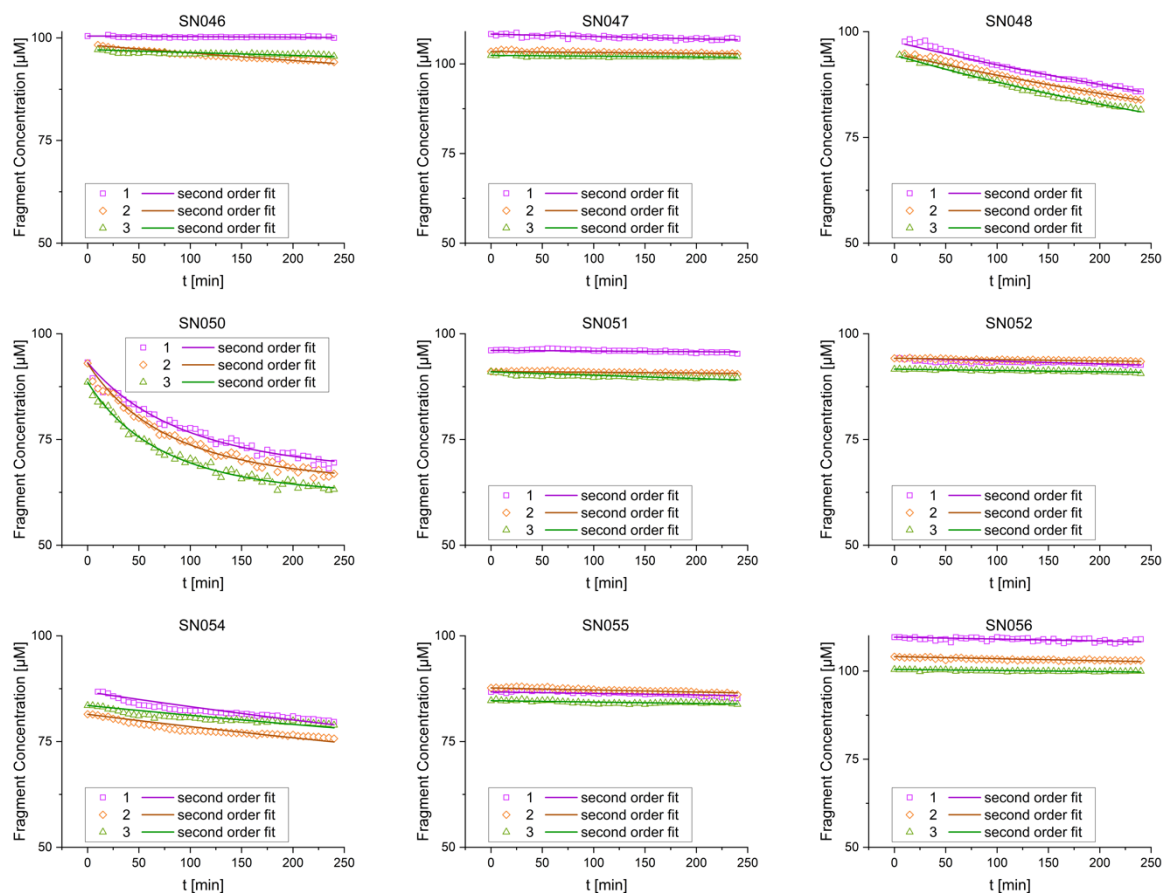


Figure S 4.7: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN046-SN056 with DTNB.

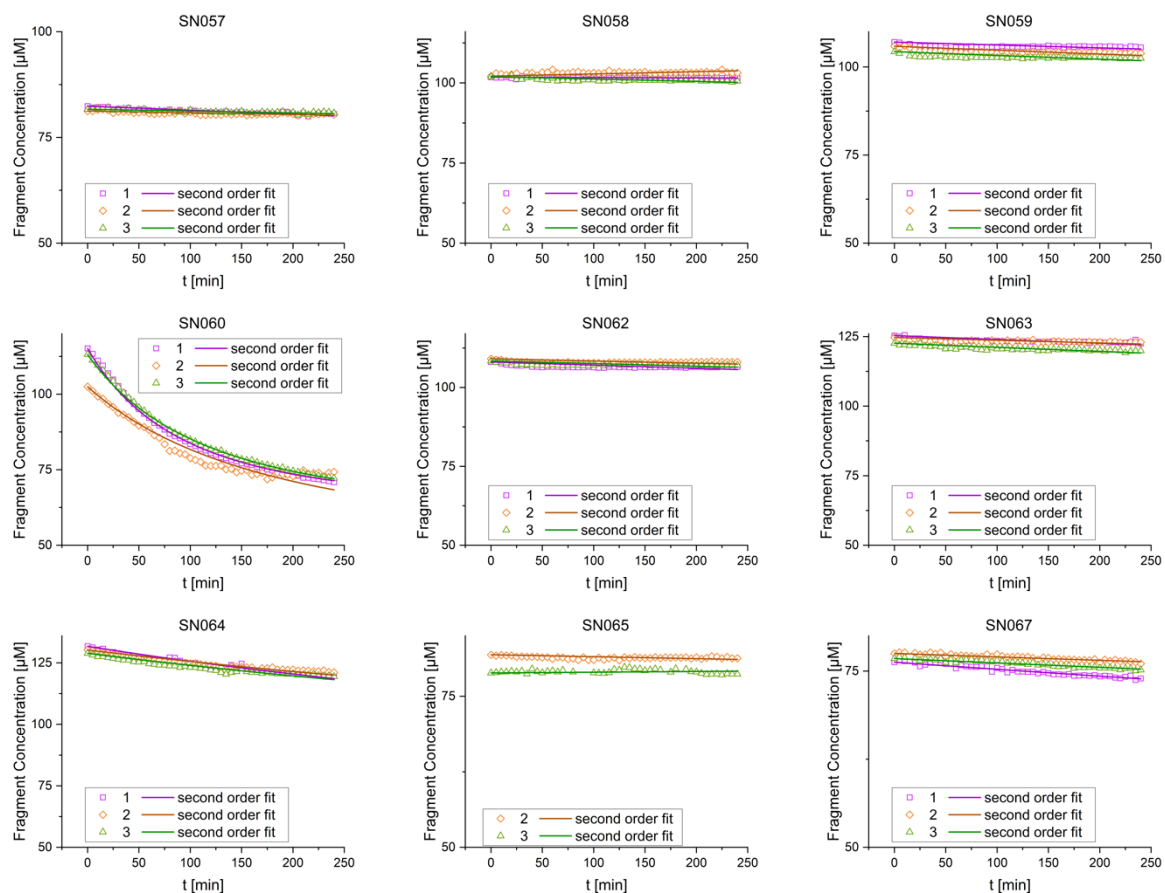


Figure S 4.8: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN057-SN067 with DTNB.

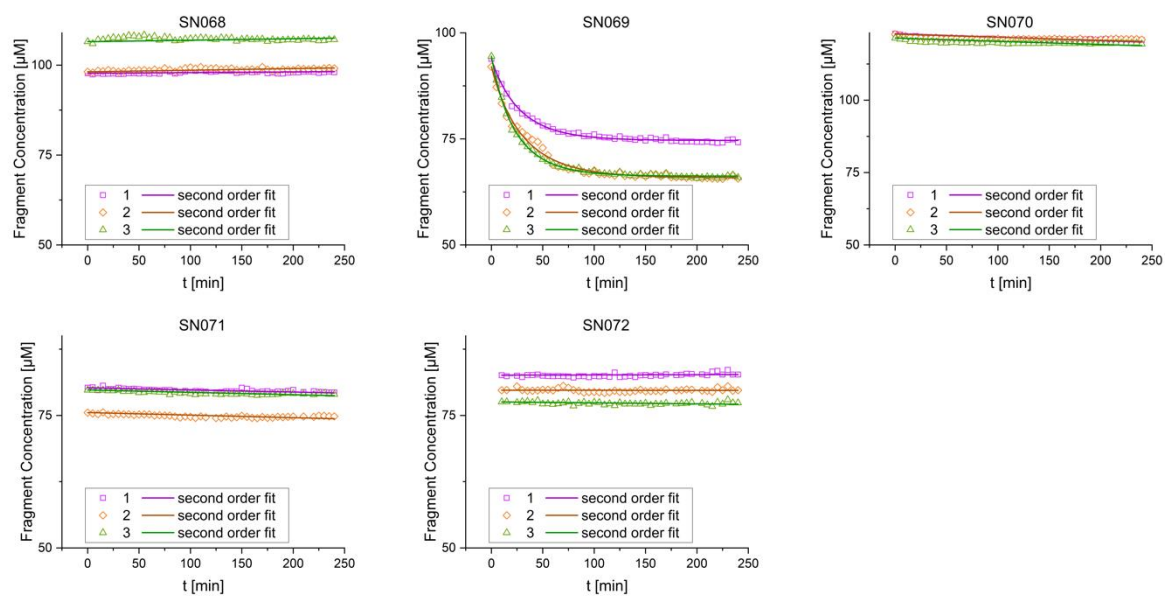


Figure S 4.9: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN067-SN072 with DTNB.

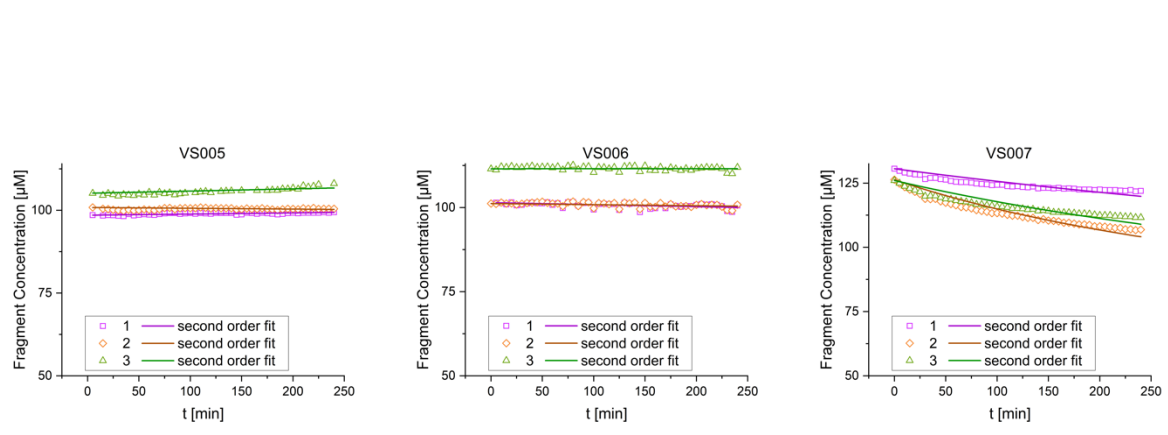


Figure S 4.10: Time-dependent depletion of the vinyl sulfone fragments with DTNB.

## 5 GSH Assay

### 5.1 Plots from the GSH Assay

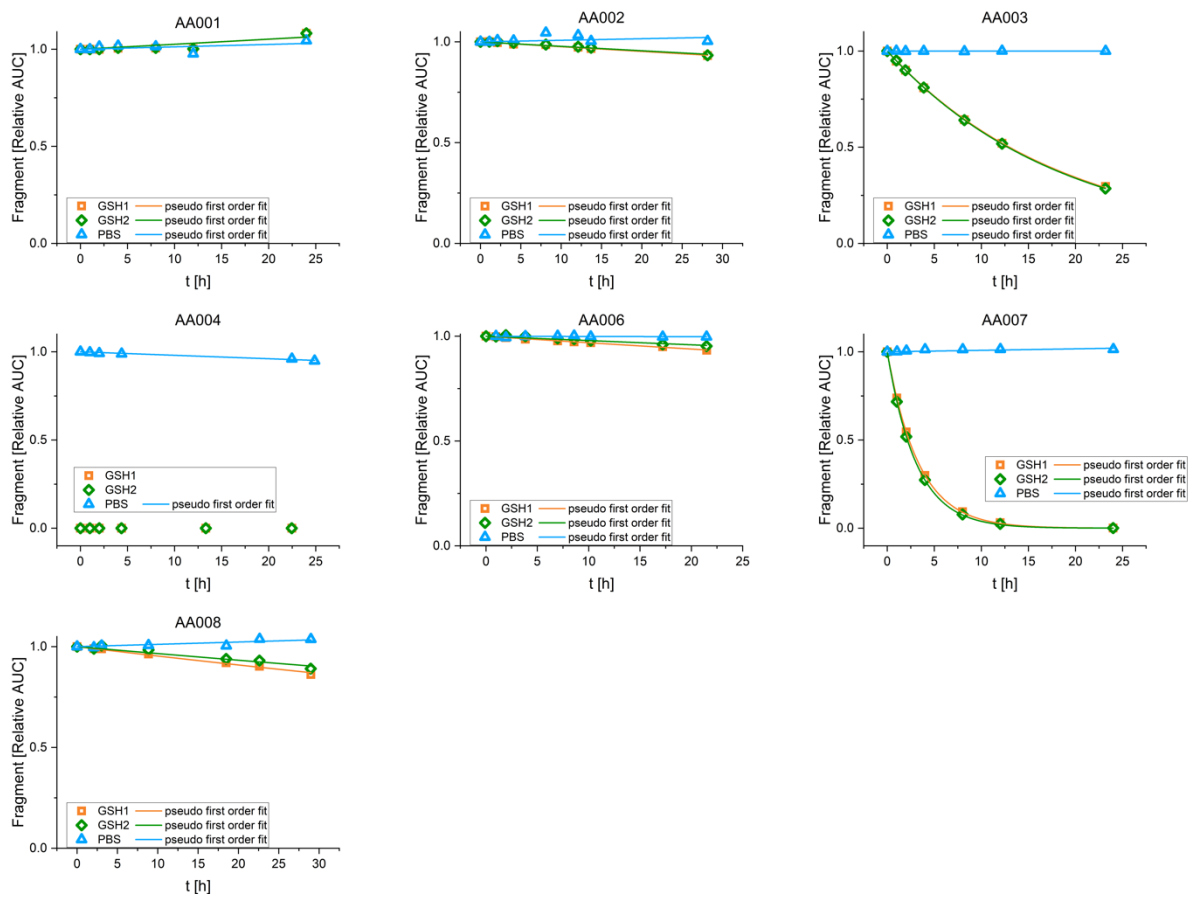


Figure S 5.1: Time-dependent depletion of the acrylamide fragments with GSH and reference measurement in PBS.

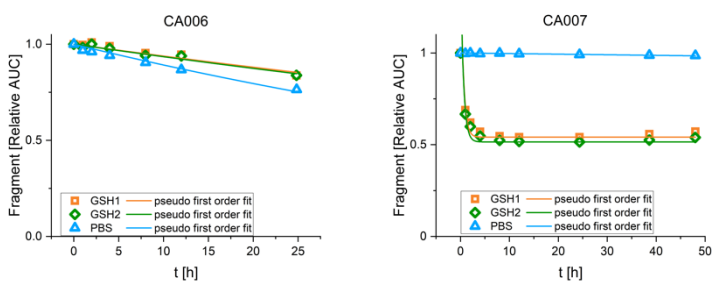


Figure S 5.2: Time-dependent depletion of the  $\alpha$ -cyano acrylamide fragments with GSH and reference measurement in PBS.

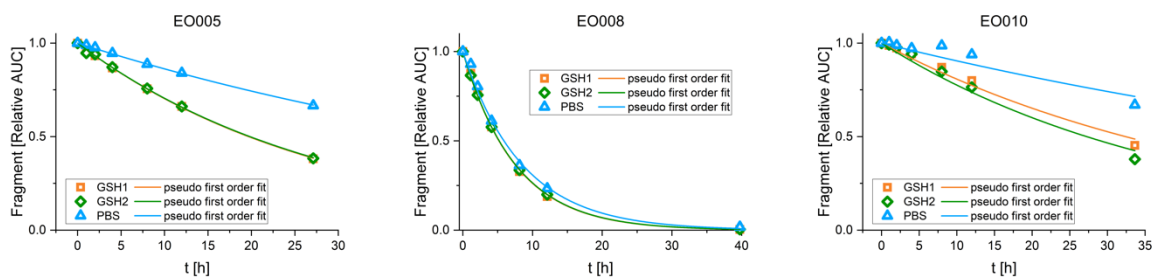


Figure S 5.3: Time-dependent depletion of the epoxide fragments with GSH and reference measurement in PBS.

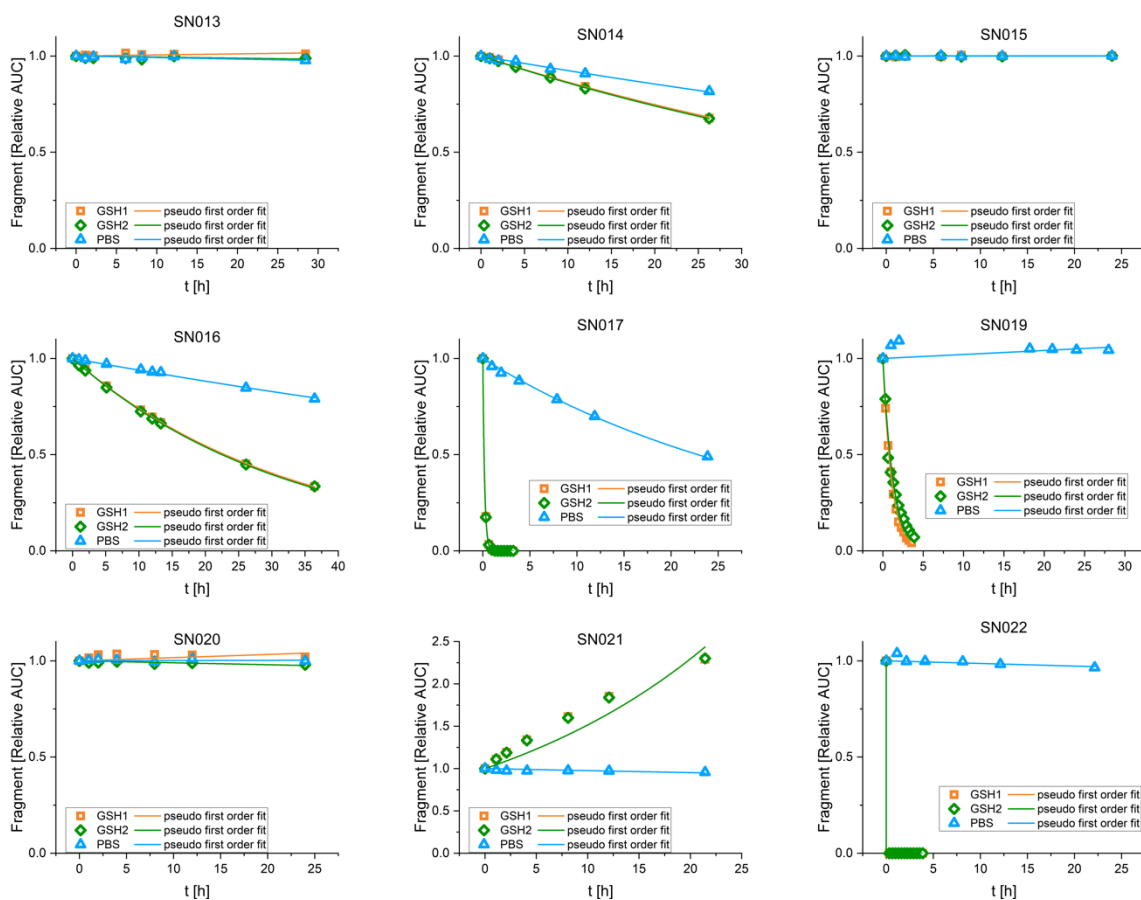


Figure S 5.4: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN013-SN022 with GSH and reference measurement in PBS.

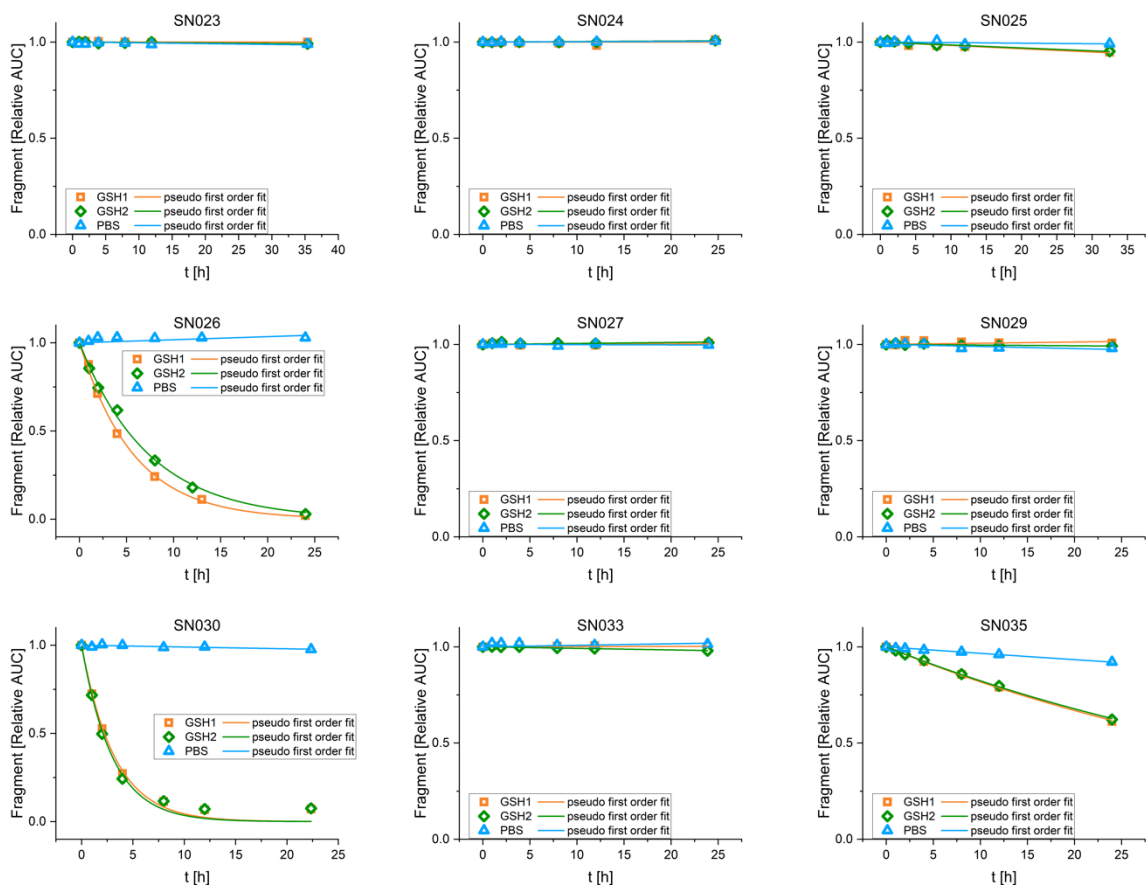


Figure S 5.5: Time-dependent depletion of the  $S_{Ar}$ -reactive heteroarenes SN023-SN035 with GSH and reference measurement in PBS.

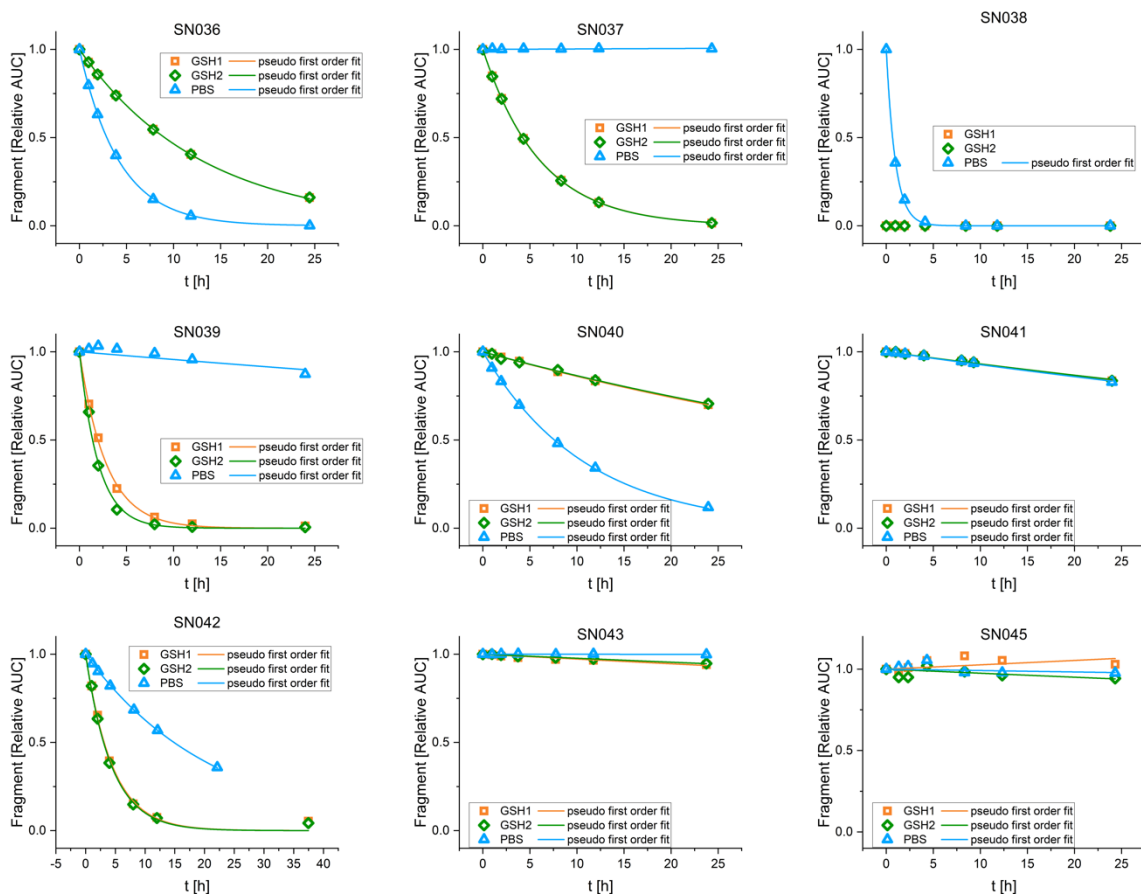


Figure S 5.6: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN036-SN045 with GSH and reference measurement in PBS.

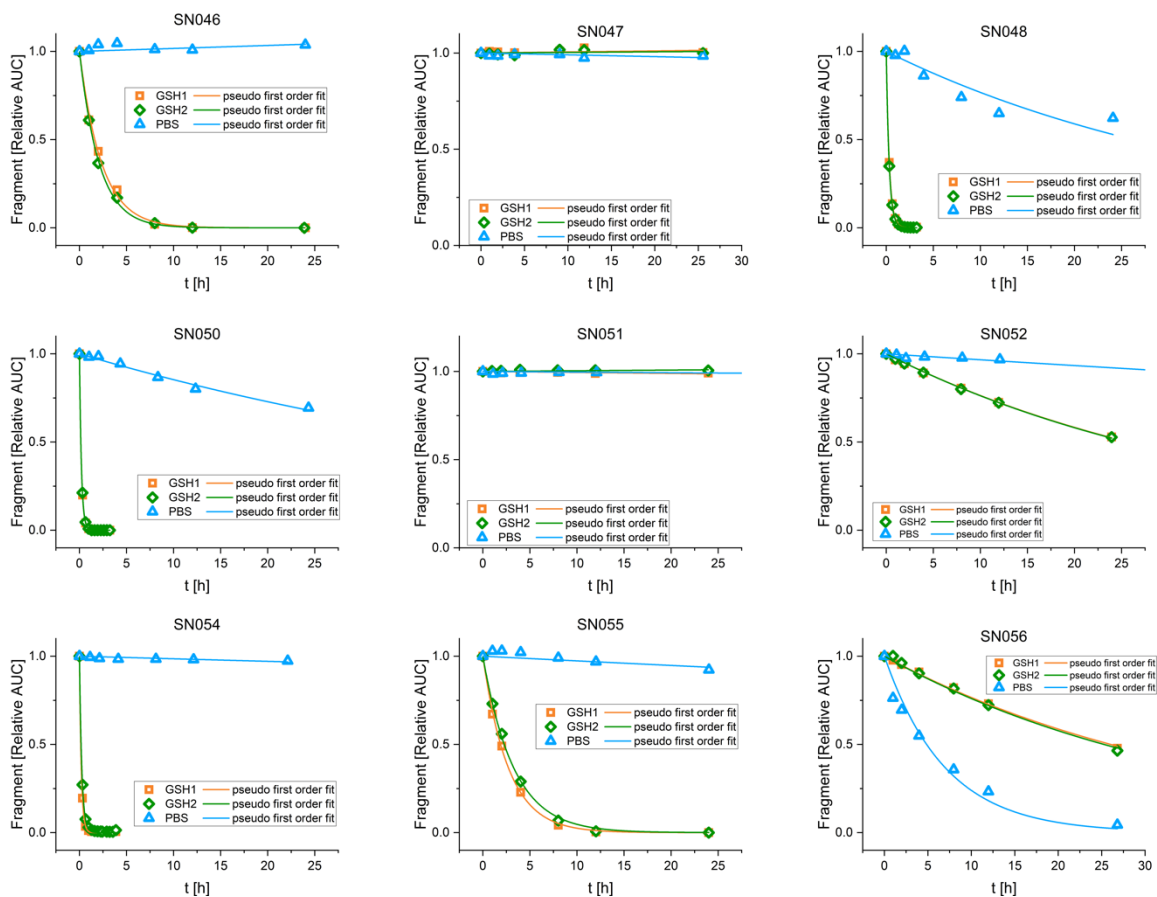


Figure S 5.7: Time-dependent depletion of the  $S_NAr$ -reactive heteroarenes SN046-SN056 with GSH and reference measurement in PBS.

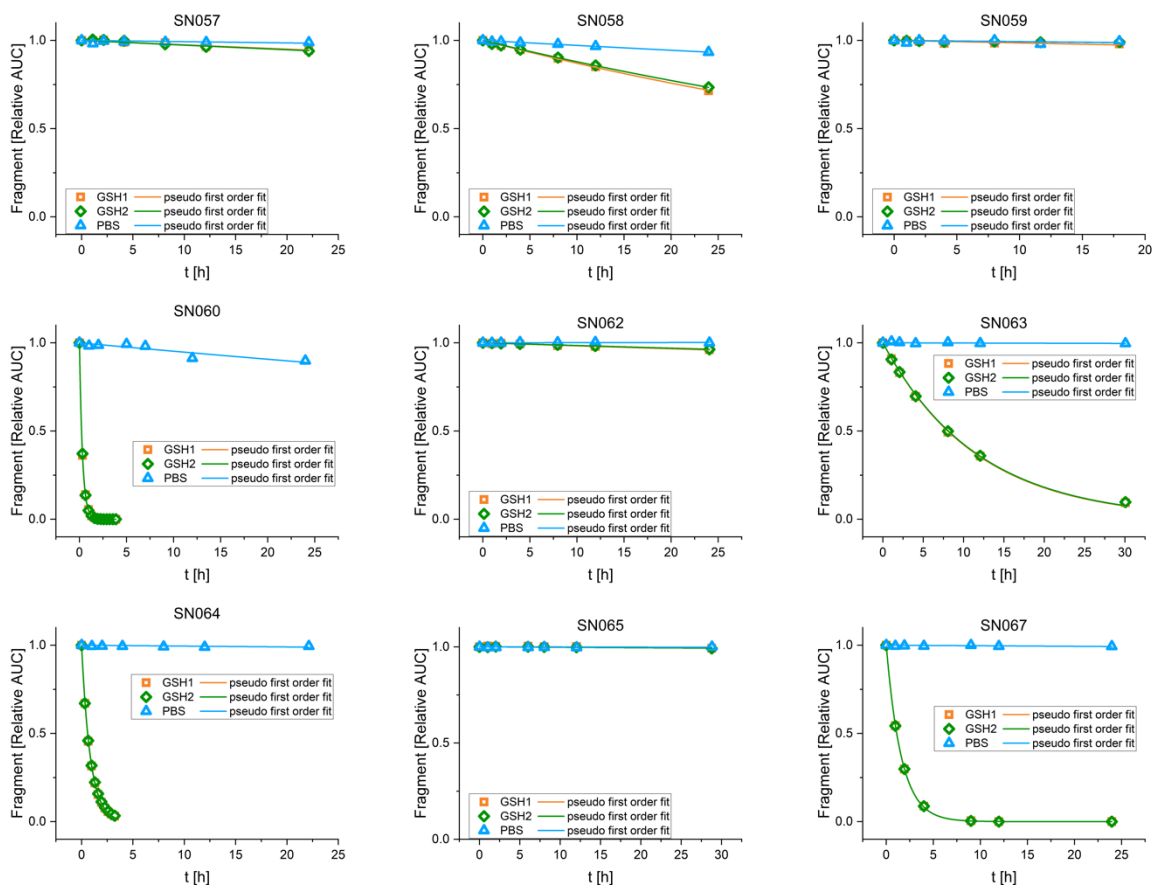


Figure S 5.8: Time-dependent depletion of the  $S_{Ar}$ -reactive heteroarenes SN057-SN067 with GSH and reference measurement in PBS.

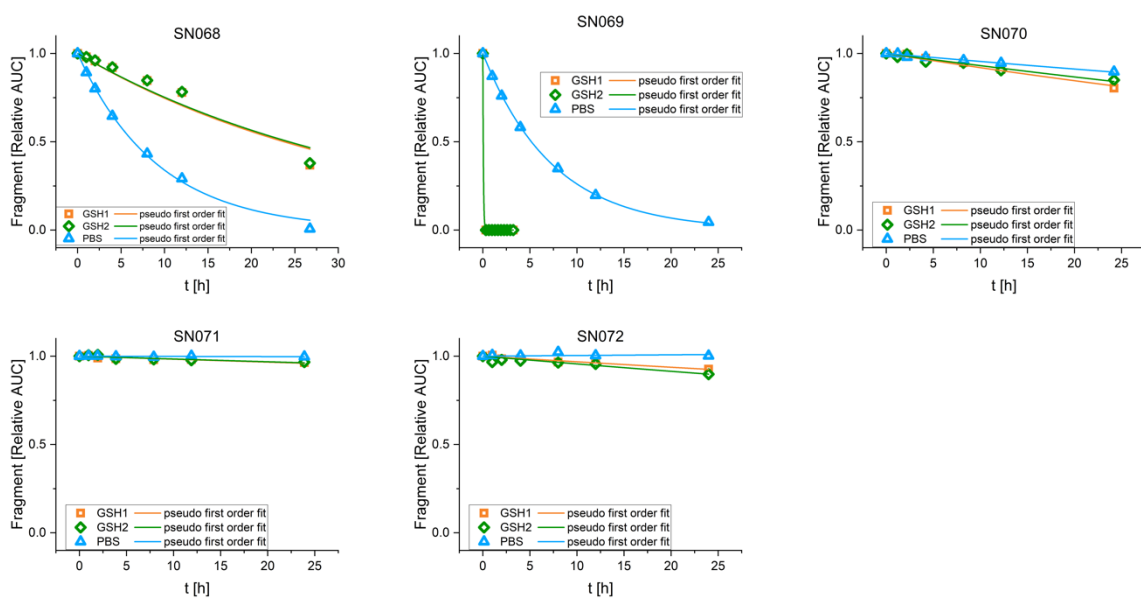


Figure S 5.9: Time-dependent depletion of the  $S_{Ar}$ -reactive heteroarenes SN068-SN072 with GSH and reference measurement in PBS.

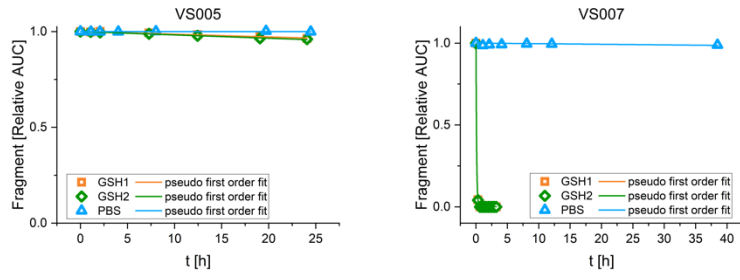


Figure S 5.10: Time-dependent depletion of the vinyl sulfone fragments with GSH and reference measurement in PBS.

## 6 Protein Sequences

Table S 6.1: All used protein sequences.

Protein	Sequence (N'-C')
T-p53C	GGSSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKLF CQLAKTCPVQLWVDSTPPPGRVVRAMAIYKQSQHMTEVRRCP HHERCSDSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHSVVPYE PPEVGSDCTTIHYNMICYSSCMGGMNRRPILTIITLEDSSGNLLG RDSFEVRVCACPGRDRRTEENLRKKGEPHHELPPGSTKRALP NNT
T-p53C-Y220C	SSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKLFCQL AKTCPVQLWVDSTPPPGRVVRAMAIYKQSQHMTEVRRCPHHE RCSDSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHSVVPCEPPE VGSDCTTIHYNMICYSSCMGGMNRRPILTIITLEDSSGNLLGRDS FEVRVCACPGRDRRTEENLRKKGEPHHELPPGSTKRALPNNT
T-p53C- Y220C-CL	GGSSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTSTYSPALNKLF CQLAKTCPVQLWVDSTPPPGRVVRAMAIYKQSQHMTEVRRCP HHERSSSDSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHSVVPCE PPEVGSDSTTIHYNMICYSSCMGGMNRRPILTIITLEDSSGNLLG RDSFEVRVSASPGRDRRTEENLRKKGEPHHELPPGSTKRALP NNT

## 7 DSF measurements

### 7.1 Results of the primary screening

Table S 7.1: DSF results of the primary screening for the fragments AA001-SN035.

Concentration [ $\mu\text{M}$ ]	250		250		1000		1000	
Incubation time [h]	4		24		4		24	
Compound	$\Delta T_m$ [°C]	Error	$\Delta T_m$ [°C]	Error	$\Delta T_m$ [°C]	Error	$\Delta T_m$ [°C]	Error
AA001	0.09	0.09	0.10	0.17	0.05	0.05	0.10	0.09
AA002	0.00	0.12	-0.55	0.12	0.05	0.05	0.30	0.00
AA003	0.00	0.12	-0.20	0.17	0.20	0.20	0.30	0.00
AA004	0.00	0.17	-0.40	0.12	-0.05	-0.05	-0.60	0.00
AA005	0.09	0.09	-0.05	0.41	0.00	0.00	0.40	0.09
AA006	0.15	0.12	0.05	0.12	0.20	0.20	0.15	0.15
AA007	-0.05	0.27	-0.20	0.17	0.05	0.09	0.15	0.00
AA008	0.09	0.24	-0.30	0.32	-0.05	-0.05	-0.10	0.09
CA006	-0.10	0.32	0.00	0.12	-0.10	0.09	-0.10	0.09
CA007	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
CA008	0.25	0.09	0.15	0.15	0.20	0.09	0.15	0.00
EO004	-0.20	0.17	-0.05	0.09	0.00	0.12	0.45	0.36
EO005	-0.20	0.17	-0.15	0.12	0.10	0.09	0.05	0.09
EO006	-0.15	0.12	-0.15	0.12	0.00	0.12	0.05	0.09
EO007	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>
EO008	-0.25	0.12	-0.25	0.12	-0.30	0.12	0.05	0.09
EO009	-0.10	0.12	-0.20	0.17	-0.05	0.17	0.05	0.09
EO010	-0.20	0.17	-0.05	0.09	-0.05	0.09	0.05	0.09
SN013	0.20	0.12	-0.05	0.17	0.00	0.12	0.05	0.09
SN014	0.10	0.17	-0.25	0.12	0.00	0.12	0.05	0.09
SN015	0.05	0.36	0.05	0.19	-0.20	0.44	0.15	0.00
SN016	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN017	-0.25	0.19	-0.40	0.12	-0.10	0.09	-0.75	0.54
SN018	0.10	0.27	0.10	0.41	0.05	0.09	0.15	0.00
SN019	0.35	0.12	0.65	0.12	0.75	0.12	1.95	0.26
SN020	-0.25	0.12	-0.50	0.09	-1.15	0.36	-0.75	0.91
SN021	-0.05	0.17	-0.10	0.19	-0.20	0.32	0.15	0.00
SN022	0.55	0.09	1.15	0.09	0.40	0.19	0.30	0.00
SN023	0.35	0.12	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN024	-0.10	0.17	-0.10	0.09	0.05	0.09	-0.05	0.12
SN025	0.10	0.17	0.05	0.09	0.05	0.09	0.25	0.19
SN026	0.10	0.23	0.00	0.15	-0.1	0.27	1.55	0.17
SN027	-0.15	0.15	0.00	0.00	-0.4	0.09	0.00	0.12
SN028	0.45	0.15	1.60	0.23	0.8	0.17	1.20	0.24
SN029	0.15	0.30	0.05	0.09	0.05	0.09	0.05	0.09
SN030	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN031	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>
SN032	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>	n.a. <sup>b</sup>
SN033	-0.20	0.17	n.a. <sup>a</sup>	n.a. <sup>a</sup>	-0.55	0.09	-0.30	0.36
SN034	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>
SN035	0.05	0.23	n.a. <sup>a</sup>	n.a. <sup>a</sup>	-0.5	0.12	-0.10	0.09

<sup>a</sup> no evaluable melting curves obtained. <sup>b</sup> not soluble under the used conditions. <sup>c</sup> The compound did not meet the purity criteria.

Table S 7.2: DSF results of the primary screening for the fragments SN036-VS007.

Concentration [ $\mu\text{M}$ ]	250		250		1000		1000	
Incubation time [h]	4		24		4		24	
Compound	$\Delta T_m$ [°C]	Error	$\Delta T_m$ [°C]	Error	$\Delta T_m$ [°C]	Error	$\Delta T_m$ [°C]	Error
SN036	-0.10	0.17	n.a. <sup>a</sup>	n.a. <sup>a</sup>	-0.1	0.17	-0.05	0.12
SN037	0.00	0.26	0.30	0.15	0.05	0.09	0.05	0.09
SN038	0.75	0.15	1.60	0.38	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN039	0.45	0.15	1.00	0.23	0.95	0.09	1.85	0.09
SN040	-0.10	0.17	0.25	1.54	-0.15	0.19	-0.10	0.09
SN041	-0.05	0.17	-0.20	0.09	-0.20	0.19	0.05	0.09
SN042	-0.05	0.17	n.a. <sup>a</sup>	n.a. <sup>a</sup>	0.45	0.12	1.50	0.36
SN043	-0.05	0.17	-0.50	0.48	-0.40	0.17	0.00	0.12
SN044	-0.05	0.17	n.a. <sup>a</sup>	n.a. <sup>a</sup>	-0.15	0.12	0.05	0.09
SN045	-0.50	0.62	0.45	0.26	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN046	0.15	0.21	0.45	0.00	0.40	0.12	1.25	0.27
SN047	0.05	0.27	-0.05	0.23	-0.15	0.12	0.05	0.09
SN048	0.05	0.17	1.60	0.09	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN049	0.05	0.09	-0.05	0.12	0.00	0.12	0.05	0.35
SN050	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN051	0.15	0.00	n.a. <sup>a</sup>	n.a. <sup>a</sup>	-0.05	0.09	0.00	0.30
SN052	0.00	0.00	-0.05	0.12	-0.10	0.12	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN053	0.00	0.15	-0.10	0.09	0.00	0.12	-0.35	0.17
SN054	2.60	0.23	3.80	0.17	3.30	0.12	4.45	0.27
SN055	0.30	0.15	0.10	0.12	0.90	0.12	1.40	0.17
SN056	0.10	0.09	0.00	0.12	0.00	0.12	0.25	0.17
SN057	-0.05	0.09	0.05	0.09	-0.30	0.19	-0.30	0.15
SN058	0.05	0.09	-0.10	0.09	-0.10	0.12	0.10	0.27
SN059	0.00	0.00	n.a. <sup>a</sup>	n.a. <sup>a</sup>	-0.15	0.12	0.05	0.27
SN060	1.65	0.00	2.70	0.44	1.75	0.17	2.90	0.17
SN061	0.20	0.23	-0.10	0.09	0.05	0.12	1.40	0.17
SN062	0.00	0.00	-0.10	0.09	-0.40	0.24	0.25	0.12
SN063	0.20	0.09	n.a. <sup>a</sup>	n.a. <sup>a</sup>	0.15	0.12	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN064	0.30	0.26	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>
SN065	0.05	0.09	-0.05	0.12	-0.25	0.12	-0.20	0.27
SN066	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>	n.a. <sup>c</sup>
SN067	-0.05	0.09	0.00	0.12	-0.12	0.12	-0.30	0.17
SN068	-0.30	0.24	-0.10	0.12	0.05	0.12	0.05	0.09
SN069	0.45	0.24	0.50	0.12	0.43	0.14	0.25	0.36
SN070	0.00	0.00	0.05	0.09	-0.10	0.12	0.10	0.27
SN071	0.05	0.09	0.05	0.09	-0.15	0.12	-0.10	0.17
SN072	0.10	0.09	0.05	0.09	-0.50	0.31	0.05	0.17
VS005	-0.15	0.12	-0.20	0.17	-0.35	0.09	0.00	0.12
VS006	-0.15	0.19	1.75	0.09	0.35	0.12	2.70	0.12
VS007	0.00	0.32	-0.20	0.09	1.65	0.19	1.65	0.12

<sup>a</sup> no evaluable melting curves obtained. <sup>c</sup> The compound did not meet the purity criteria.

## 7.2 Time Dependent DSF Measurements

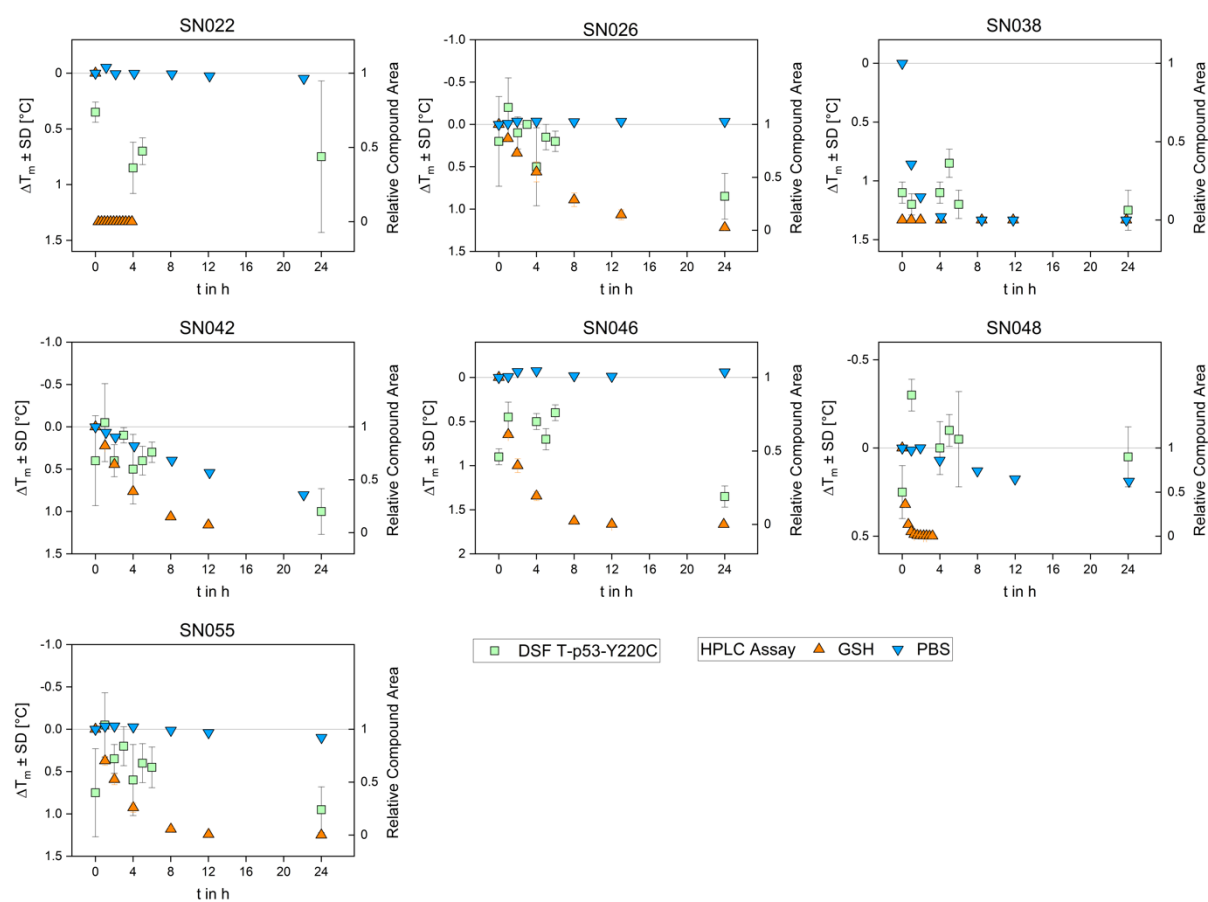


Figure S 7.1: Results for the time-dependent DSF measurement (1000  $\mu\text{M}$  compound concentration) and GSH reactivity assessment for the hit compounds. Thermal shifts obtained in the DSF assay are plotted on the left y-axis, while the relative compound area determined in the GSH assay is plotted on the right y-axis.

### 7.3 Concentration Dependent DSF Measurements

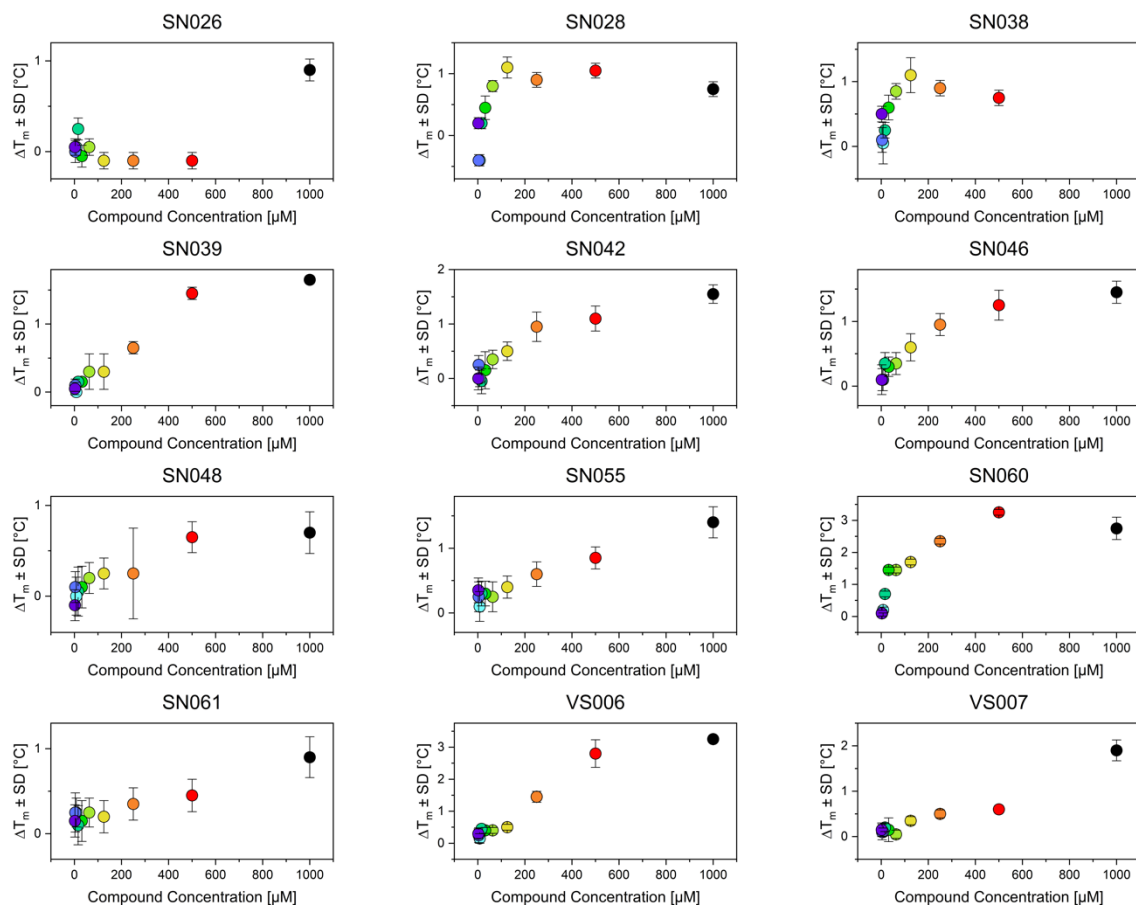


Figure S 7.2: Concentration-dependent DSF measurements for the hit compounds with 24 h incubation time.