Neuropilin-1 peptide-like ligands with proline mimetics, tested using the improved chemiluminescence affinity detection method

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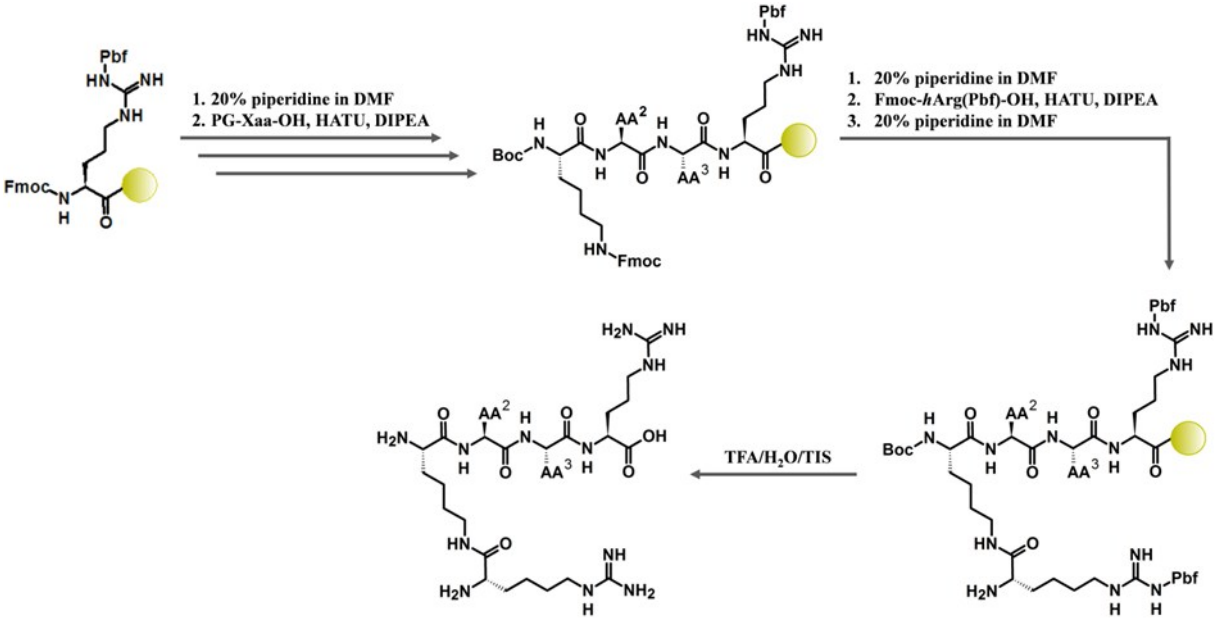
SUPPORTING INFORMATION

Contents

1. General scheme of the synthesis.



Scheme S1. General scheme of peptidomimetics synthesis on the solid support.

2. Analytical data. Purity of compounds (> 98%) was determined using RP-HPLC. Analysis of pure products was carried out by HPLC with a Prominence HPLC system (binary pump system LC-20AD and autosampler SIL-20AC HT coupled to a SPD-20A UV detector and LCMS-2020 quadrupole mass detector). Chromatographic separation was achieved on Phenomenex Jupiter Proteo C12 column (250 × 4.6 mm) at 35˚C. Mobile phases consisted of H_2O:TFA (99.95:0.05 v/v, phase A) and ACN : TFA (99.95:0.05 v/v, phase B) at a flow rate of 1.2 mL/min. Elution was performed with gradients as follows:

Method 1:

0 – 2 min 0%; 2 – 22 min 40%;

Method 2:

0 – 3 min 0%; 3 – 23 min 18%;

1 mg/ml solution of each compound was prepared in H_2O and 10 µL was injected. UV spectra were recorded at 190 nm. Purity of compounds was estimated using peak area.

High resolution mass spectra (HRMS) were recorded on a Shimadzu LCMS-IT-TOF with ESI ionization source. Flow rate of mobile phase (water : acetonitrile : formic acid 49.75:49.75:0.05 v/v/v) was set at 0.2 mL/min. 1 mg/ml solution of each compound was prepared in H_2O and 0.1 µL was injected.

The electrospray ionization (ESI) was operated in positive mode. Nitrogen was used as nebulizing gas, set at 1.5 ml/min and drying gas set at pressure 101 kPa. Desolvation line and

heat block temperature were set at 220°C. Needle voltage was set at +4.5 kV (positive mode) and -3.5kV (negative mode). Detector voltage was set to -1.76kV. Mass spectrometer was used in scan mode in range 250-2000 *m*/*z* (positive mode) and 100-1400 *m*/*z* (negative mode). Theoretical $[M+H]^+$ value and error was calculated using Formula Predictor tool integrated with spectrometer operating software.

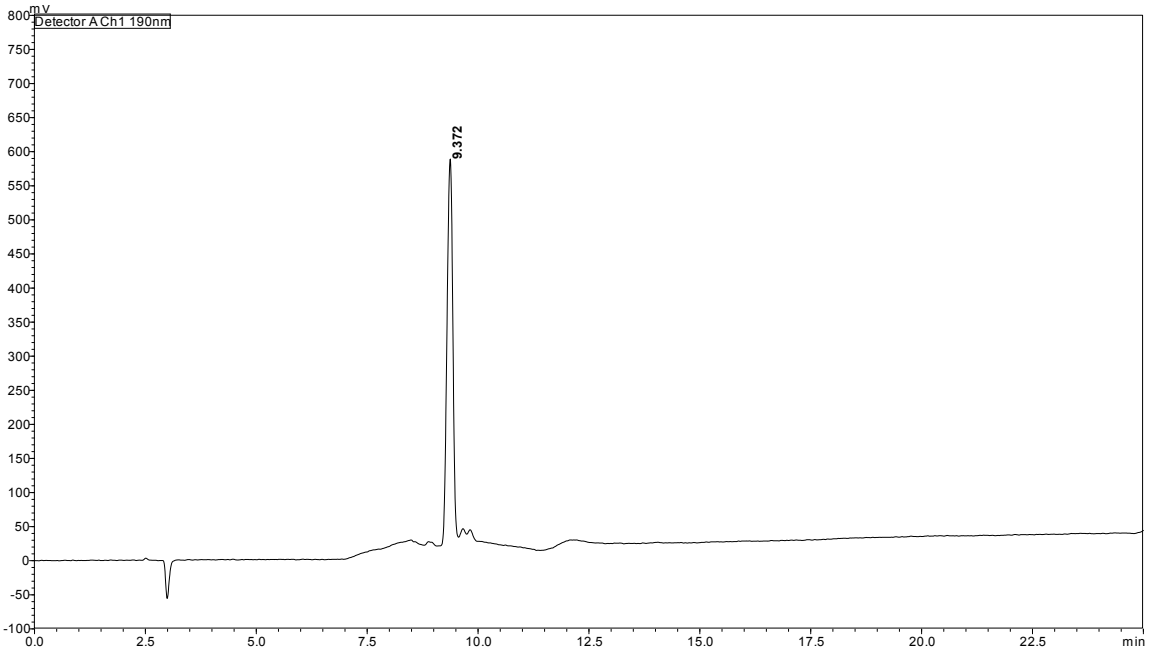
Table S1. HPLC analytical data of compounds **1**-**22**.

Compound	Yield	RT [min]	Molecular formula
1	49%	15.04*	$C_{40}H_{61}N_{11}O_9$
2	51%	11.63	$C_{29}H_{54}N_{12}O_6$
3	47%	9.59	$C_{28}H_{55}N_{13}O_6$
4	52%	10.44	$C_{27}H_{52}N_{12}O_6$
5	47%	8.99	$C_{29}H_{54}N_{12}O_8$
6	66%	10.12	$C_{27}H_{50}N_{12}O_6$
7	48%	10.23	$C_{29}H_{50}N_{12}O_6$
8	44%	21.81	$C_{37}H_{66}N_{12}O_6$
9	29%	4.95	$C_{29}H_{56}N_{14}O_6$
10	21%	22.75	$C_{39}H_{58}N_{12}O_6$
11	49%	5.98	$C_{28}H_{55}N_{13}O_7$
12	66%	8.71	$C_{27}H_{53}N_{13}O_6$
13	59%	9.37	$C_{28}H_{53}N_{13}O_6$
14	53%	14.28	$C_{32}H_{61}N_{13}O_6$
15	29%	5.02	$C_{28}H_{56}N_{14}O_6$
16	28%	14.56	$C_{33}H_{57}N_{13}O_6$
17	37%	9.26	$C_{27}H_{52}N_{12}O_7$
18	49%	9.77	$C_{26}H_{50}N_{12}O_6$
19	58%	9.85	$C_{27}H_{50}N_{12}O_6$
20	51%	15.47	$C_{31}H_{58}N_{12}O_6$
21	31%	8.75	$C_{27}H_{53}N_{13}O_6$
22	33%	15.91	$C_{32}H_{54}N_{12}O_6$

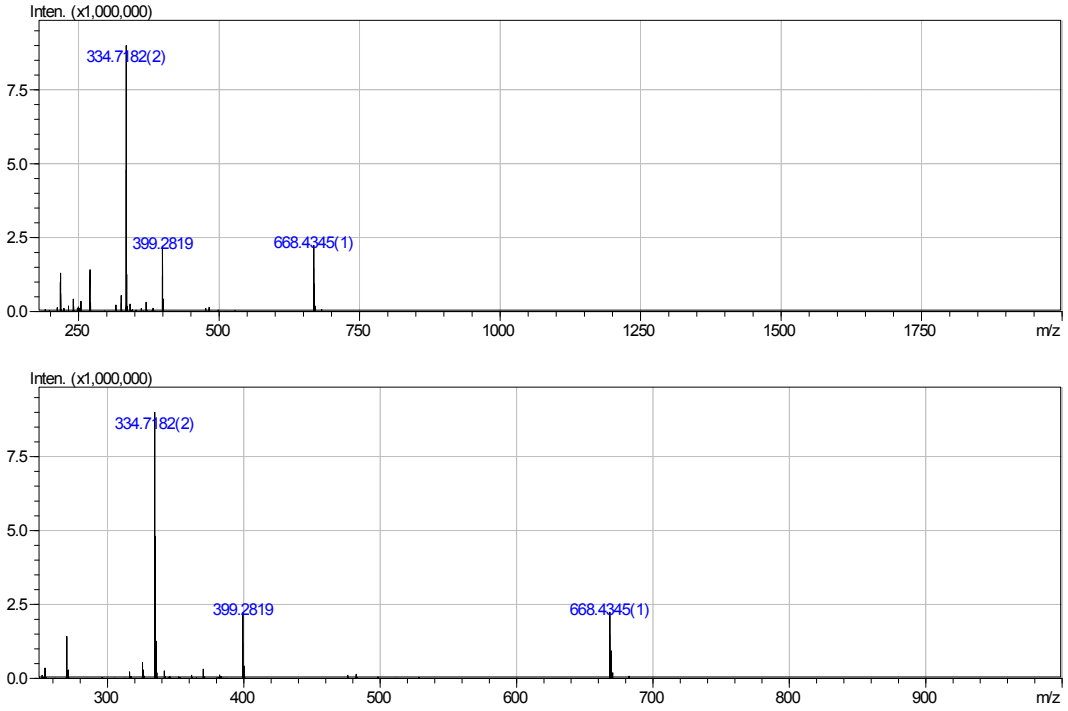
* Compound was analysed using method 1. The rest of compounds were analysed using method 2.

Table S2. HRMS analytical data of compounds **1**-**22**.

Compound	Molecular formula	$[M+H]^+$ calculated	$[M+H]^+$ found	Error [ppm]	$[M+2H]^{2+}$ calculated	$[M+2H]^{2+}$ found	Error [ppm]	$[M-H]^-$ calculated	$[M-H]^-$ found	Error [ppm]
1	$C_{40}H_{61}N_{11}O_9$	840.4726	-	-	420.7400	420.7404	1	838.4581	838.4605	2.9
2	$C_{29}H_{54}N_{12}O_6$	667.4362	667.4373	1.6	334.2217	334.2211	-1.8	665.4217	665.4248	4.7
3	$C_{28}H_{55}N_{13}O_6$	670.4471	670.4477	0.9	335.7272	335.7272	0	668.4326	668.4334	1.2
4	$C_{27}H_{52}N_{12}O_6$	641.4206	641.4207	0.2	321.2139	321.2157	5.6	639.4060	639.4053	-1.1
5	$C_{29}H_{54}N_{12}O_8$	699.4260	699.4273	1.9	350.2167	350.2158	2.6	697.4115	697.4135	2.9
6	$C_{27}H_{50}N_{12}O_6$	639.4049	639.4078	4.5	320.2061	320.2056	-1.6	637.3904	637.3900	-0.6
7	$C_{29}H_{50}N_{12}O_6$	663.4049	663.4066	2.6	332.2061	332.2058	-0.9	661.3904	661.3929	3.8
8	$C_{37}H_{66}N_{12}O_6$	775.5301	775.5331	3.9	388.2687	388.2685	0.5	773.5156	773.5177	2.7
9	$C_{29}H_{56}N_{14}O_6$	697.4580	697.4595	2.2	349.2326	349.2319	-2.0	695.4434	695.4428	-0.9
10	$C_{39}H_{58}N_{12}O_6$	791.4675	791.4695	1.1	396.2375	396.2379	1.0	789.4582	789.4585	0.4
11	$C_{28}H_{55}N_{13}O_7$	686.4420	686.4440	2.9	343.7246	343.7246	0	684.4275	684.4279	0.6
12	$C_{27}H_{53}N_{13}O_6$	656.4315	656.4342	4.1	328.7194	328.7198	1.2	654.4169	654.4188	2.9
13	$C_{28}H_{53}N_{13}O_6$	668.4315	668.4345	4.5	334.7194	334.7182	-3.6	666.4169	666.4183	2.1
14	$C_{32}H_{61}N_{13}O_6$	724.4941	724.4960	2.6	362.7507	362.7503	-1.1	722.4795	722.4824	4.0
15	$C_{28}H_{56}N_{14}O_6$	685.4580	685.4594	2.0	343.2326	343.2317	-2.6	683.4434	683.4444	1.5
16	$C_{33}H_{57}N_{13}O_6$	732.4628	732.4628	3.4	366.7350	366.7350	0	730.4482	730.4484	0.3
17	$C_{27}H_{52}N_{12}O_7$	657.4155	657.4173	2.7	329.2114	329.2120	1.8	655.4009	655.4016	1.1
18	$C_{26}H_{50}N_{12}O_6$	627.4049	627.4078	4.6	314.2061	314.2066	1.6	625.3904	625.3924	3.2
19	$C_{27}H_{50}N_{12}O_6$	639.4049	639.4078	4.5	320.2061	320.2060	-0.3	637.3904	637.3886	-2.8
20	$C_{31}H_{58}N_{12}O_6$	695.4675	695.4706	4.5	348.2374	347.2371	-0.9	693.4530	693.4547	2.4
21	$C_{27}H_{53}N_{13}O_6$	656.4315	656.4336	3.2	328.1794	328.7200	1.8	654.4169	654.4164	-0.7
22	$C_{32}H_{54}N_{12}O_6$	703.4362	703.4396	4.8	352.2217	352.2207	-2.8	701.4217	701.4237	2.9



HRMS positive mode



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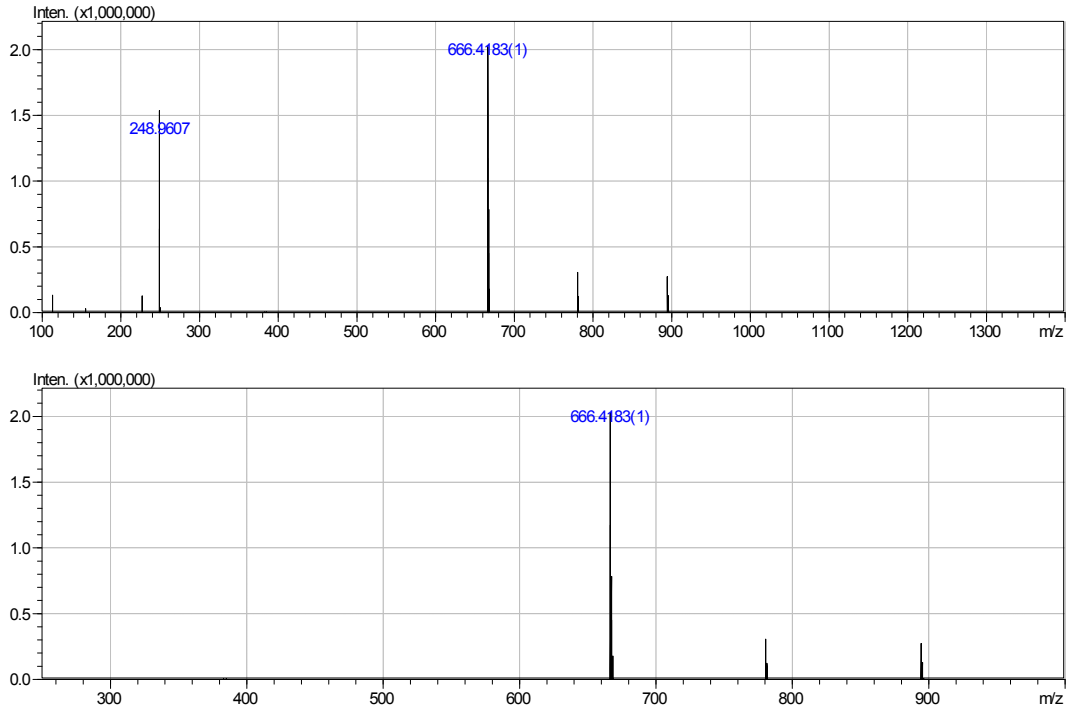
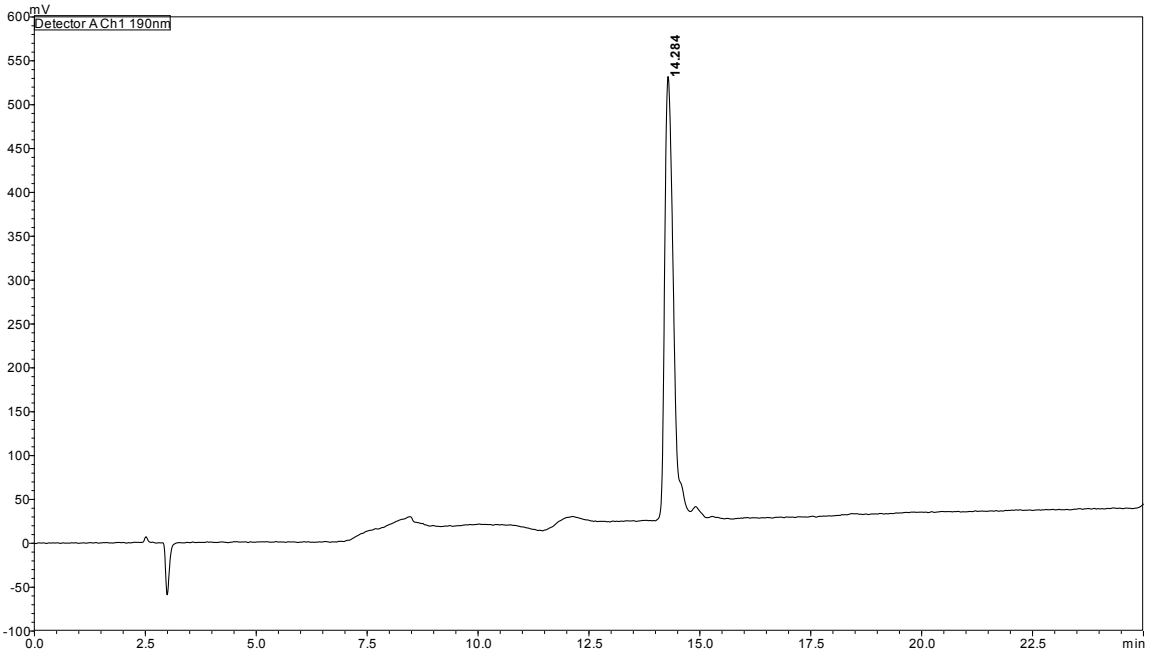


Figure S1. HPLC chromatogram (190 nm) and HRMS spectra of puriefied compound **13**.



HRMS positive mode

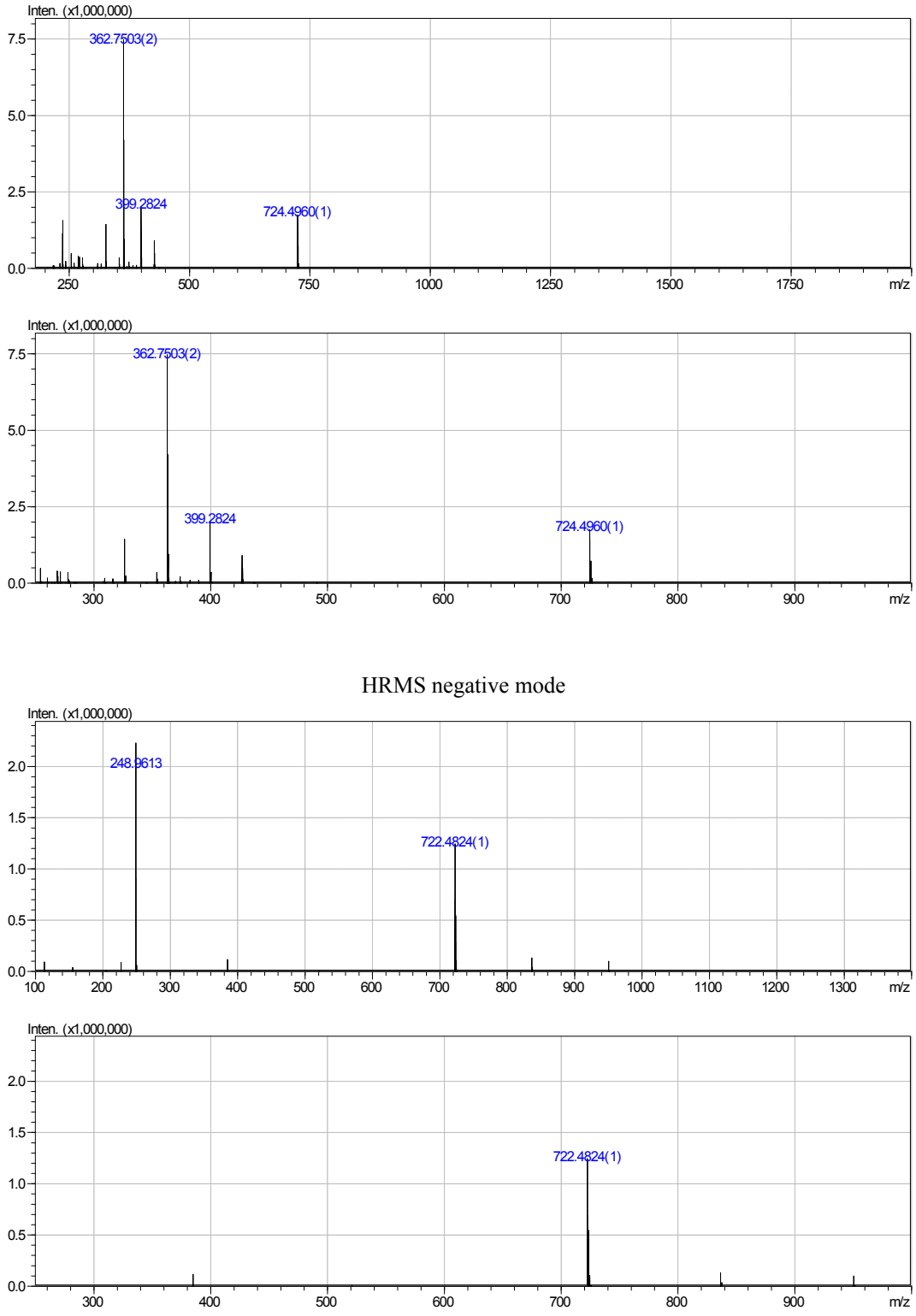


Figure S2. HPLC chromatogram (190 nm) and HRMS spectra of puriefied compound **14**.

3. Analytical data of plasma degradation. Analysis of plasma degradation products was carried out by HPLC-ESI-Q-MS with a Prominence HPLC system (binary pump system LC-20AD and autosampler SIL-20AC HT coupled to a SPD-20A UV detector and LCMS-2020

quadrupole mass detector). Chromatographic separation was achieved on Phenomenex Jupiter Proteo C12 column (250 × 4.6 mm) at 35˚C. Mobile phases consisted of H_2O:TFA (99.95:0.05 v/v, phase A) and ACN:TFA (99.95:0.05 v/v, phase B) at a flow rate of 1.2 mL/min. The eluent was split at a ratio of 1:3 after UV-detector to reduce flow for MS to 0.3 mL/min.

Elution was performed with a gradient as follows: t = 20 min., 0% - 18% B. The injection volume was 15 µl. UV spectra were recorded at 200 nm. Retention time is only given for untouched compound and identified degradation products. Rest of the signals were not identified and are supposedly from plasma and its natural degradation in time.

The electrospray ionization (ESI) was operated in positive and negative mode. Nitrogen was used as nebulizing gas, set at 1.5 ml/min and as a drying gas set at 17 ml/min. Desolvation line and heat block temperature were set at 250°C and 300°C respectively. Needle voltage was set at +4.5 kV (positive mode) and -4.5kV (negative mode). Detector voltage was set to -1.25kV. Mass spectrometer was used in scan mode in range 150-1000 m/z.

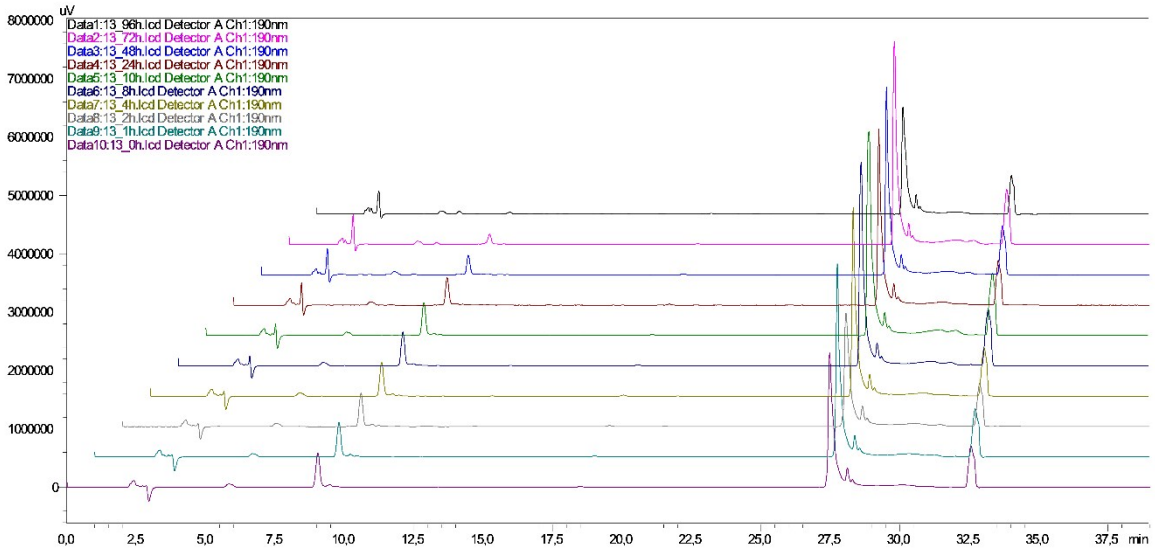


Figure S3. Full chromatogram of **13** after degradation in different time intervals

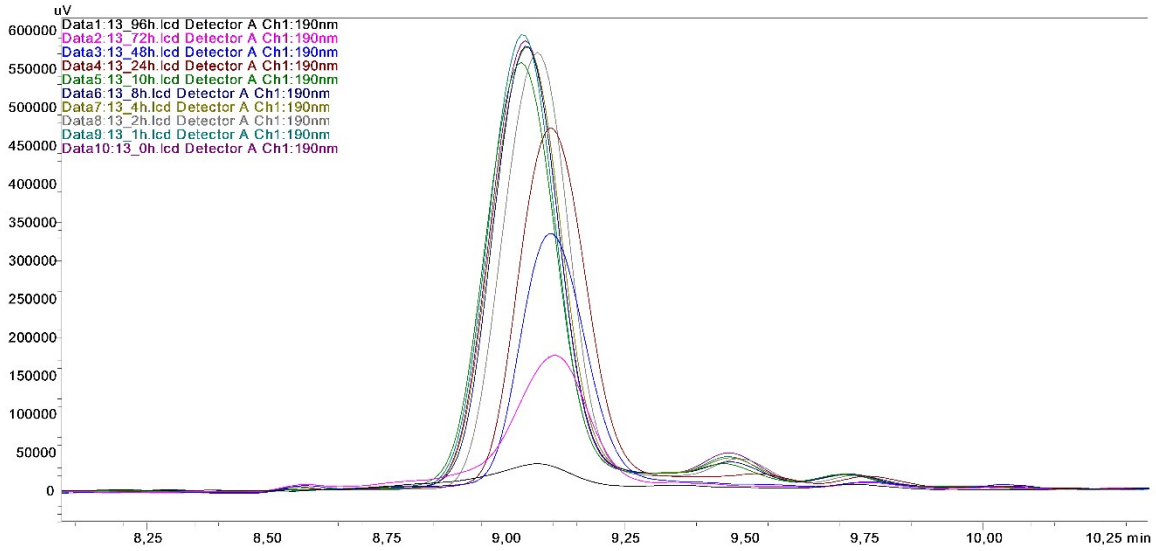


Figure S4. Zoom on **13** signal after degradation in different time intervals.

Time [h]	Area	Area [%]
0	5678319	100
24	4467576	79
48	2994031	53
72	1640084	29
96	285416	5

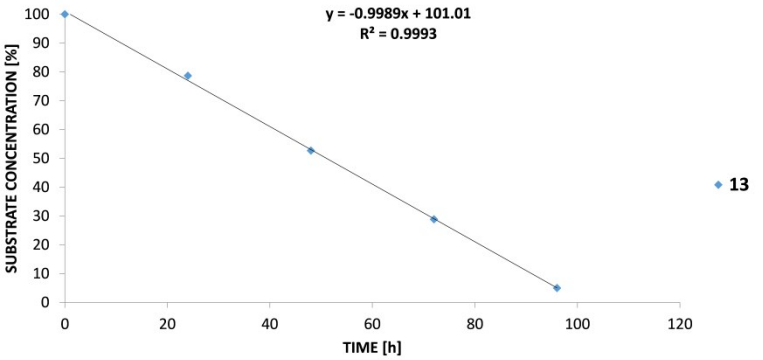


Figure S5. Data used for $t_{1/2}$ estimation of compound **13**.

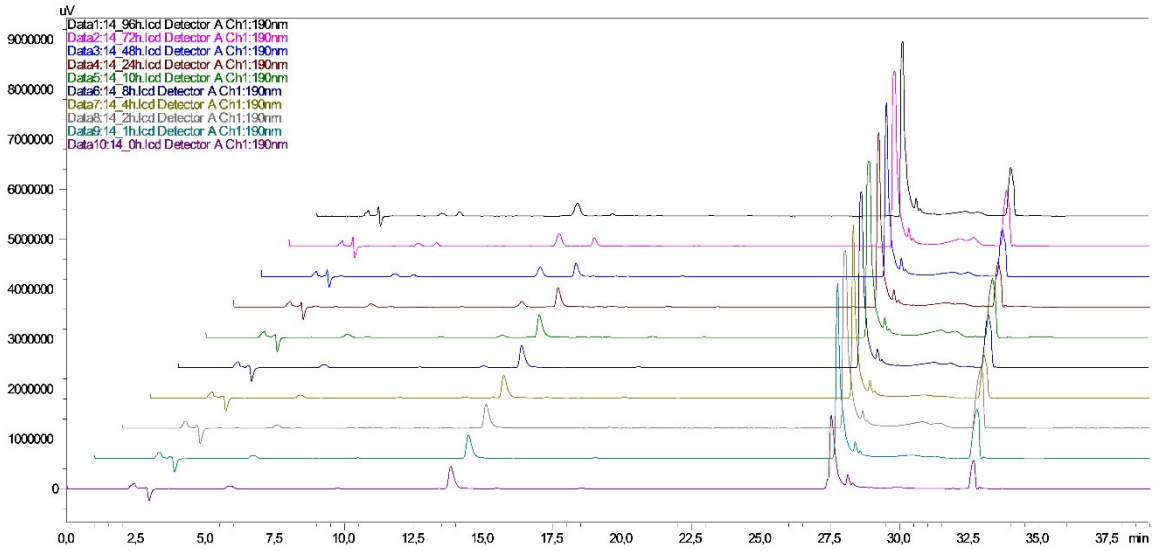


Figure S6. Full chromatogram of **14** after degradation in different time intervals.

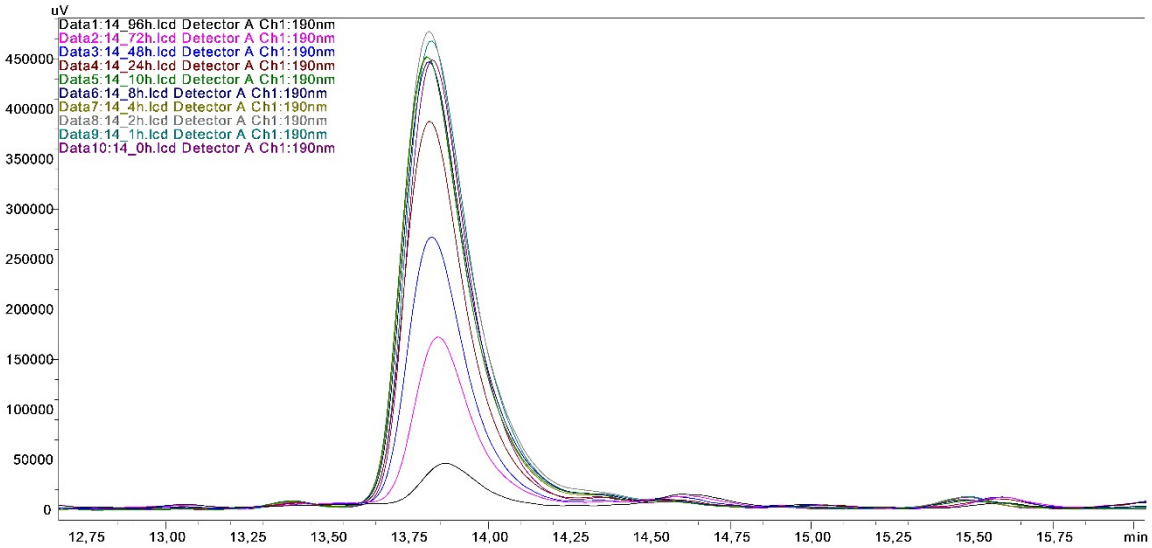


Figure S7. Zoom **14** signal after degradation in different time intervals.

Time [h]	Area	Area [%]
0	6033449	100
24	5104299	85
48	3416511	57
72	2055290	34
96	500172	8

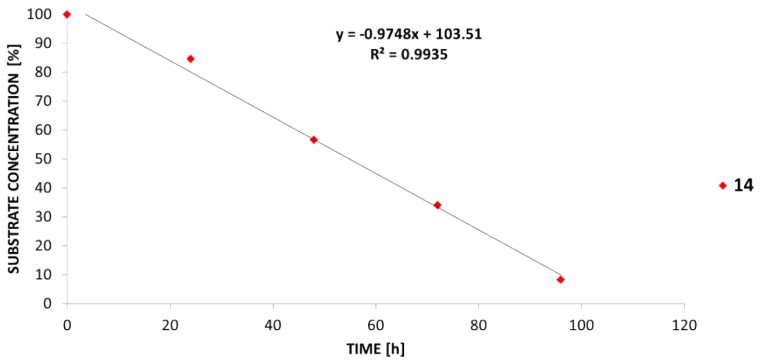


Figure S8. Data used for $t_{1/2}$ estimation of compound **14**.

The electrospray ionization (ESI) was operated in positive and negative mode. Nitrogen was used as nebulizing gas, set at 1.5 ml/min and as a drying gas set at 17 ml/min. Desolvation line and heat block temperature were set at 250°C and 300°C respectively. Needle voltage was set at +4.5 kV (positive mode) and -4.5kV (negative mode). Detector voltage was set to -1.25kV. Mass spectrometer was used in scan mode in range 150-1000 m/z.

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Table S3. Identified products of **13** after 96h of incubation in human plasma.

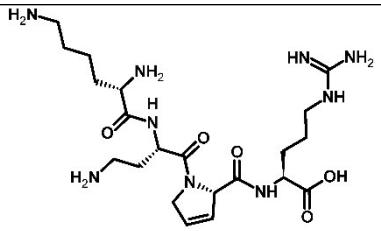
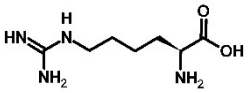
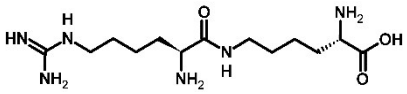
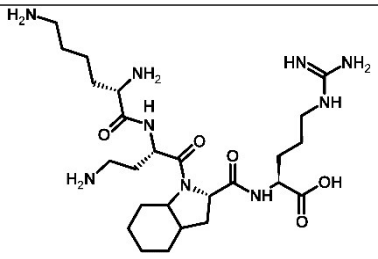
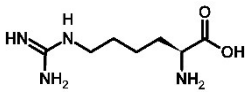
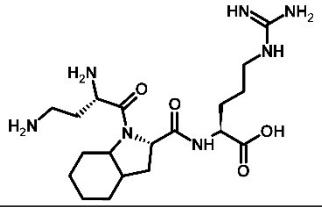
Structure	RT [min]	$[M+H]^+_{calc.}$	$[M+H]^+_{found}$
	2.79	498.3	498.5
	2.94	189.1	189.9
	2.94	317.2	317.7

Table S4. Identified products of **14** after 96h of incubation in human plasma.

Structure	RT [min]	$[M+H]^+_{calc.}$	$[M+H]^+_{found}$
	11.3	554.5	554.4
	2.96	189.1	189.9
	12.4	426.3	426..6

4. Dose-response curves of the best analogues used to calculate IC_{50} values. The concentration-dependent inhibitory dose-curve data were plotted as percentage inhibition normalized to controls with applied curve fits calculated using GraphPad Prism. Data are presented as log(inhibitor) versus normalized response-variable slope. Error bars are representing means +/- SEM for 3 independent experiments.

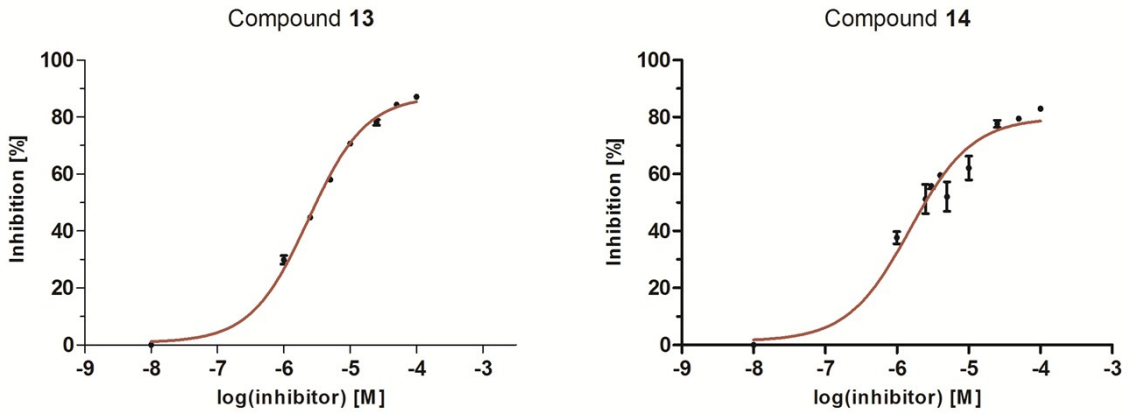


Figure S9. Dose-response curves of compounds **13** and **14**.