Supporting Information

Prodrug Activation by a Viral Protease: Evaluating Combretastatin Peptide-Hybrids to Selectively Target Infected Cells

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Chemistry. All chemicals and solvents used in synthesis were purchased from Merck (Germany), Honeywell (Germany), Carbolution (Germany), TCI (Belgium) and Carl Roth (Germany). Chemicals and solvents were of analytical grade and used without further purification, unless otherwise stated. Dry solvents were kept over molecular sieves. NMR spectra were recorded at 300 MHz and 298 K in $CDCl_3$, CD_3OD or DMSO-d_6 using a Varian NMR instrument. The chemical shift (δ) is given in parts per million (ppm) relative to tetramethylsilane (TMS). Solvent residual peaks are used as internal standard. Coupling constants (J) are given in Hertz (Hz). Multiplicity of signals is depicted as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Mass spectra were recorded on a Bruker micrOTOF-Q II (HR-ESI) instrument. Flash chromatography was run on an Isolera One purification system by Biotage (Sweden) using silica gel (0.060-0.200 mm). The chromatography was monitored with UV absorption at 254 nm and 280 nm. Preparative HPLC was performed on a GE Healthcare (Germany) ÄKTA Purifier, using RP-18 pre and main columns (Reprospher, Dr. Maisch GmbH, Germany, C18-DE, 5 µM, 30 mm x 16 mm for the pre column and 120 mm x 16 mm for the main column). Analytical HPLC was performed on an Agilent 1200 HPLC system with UV detector at 254 nm on an RP-18 column (ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany, 3 µm, 50 mm x 2 mm).

Synthesis of triphenyl(3,4,5-trimethoxybenzyl)-phosphonium bromide (1).[1] To a solution of 3 g (15.3 mmol, 1 eq) 3,4,5-trimethoxybenzaldehyde in 50 mL methanol, 1.17 g (30.6 mmol, 2 eq) $NaBH_4$ were added in small portions. The solution was stirred at room temperature for 30 minutes. When TLC showed the completion of the reaction, the solvent was removed under reduced pressure and the residue dissolved in dichloromethane, washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting (3,4,5-trimethoxyphenyl)methanol could be used without further purification. Yield 2.44 g (12.32 mmol, 81%). ^{1}H NMR ($CDCl_3$): δ = 6.61 (s, 2 H), 4.64 (d, *J* = 4.4 Hz, 2 H), 3.87 (s, 6 H), 3.84 (s, 3 H), 1.64 (t, *J* = 4.4 Hz, 1 H).

2.4 g (12.2 mmol, 1 eq) of the previously synthesized (3,4,5-trimethoxyphenyl)methanol were dissolved in 100 mL dichloromethane and cooled to 0°C in a water-ice-bath. 1.25 mL (13.3 mmol, 1.1 eq) PBr_3 were added dropwise over 10 minutes. The solution was stirred for another 30 minutes at 0°C. Then, the reaction was quenched with ice and the product was extracted with dichloromethane. It should be noted that TLC is not suitable for reaction control in this case and that the product decomposes when stirred for more than an hour. The organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting 3,4,5-trimethoxybenzylbromide could be used without further purification. Yield 2.7 g (10.4 mmol, 86%). ^{1}H NMR ($CDCl_3$): δ = 6.62 (s, 2 H), 4.46 (s, 2 H), 3.87 (s, 6 H), 3.84 (s, 3 H).

2.7 g (10.4 mmol, 1 eq) of the previously synthesized 3,4,5-trimethoxybenzylbromide and 2.7 g (10.4 mmol, 1 eq) triphenylphosphane were dissolved in chloroform. The solution was refluxed for 24 h at 65°C. Afterwards, the solvent was removed under reduced pressure to give an oily foam. This residue was collected in cyclohexane and evaporated again, yielding a white solid. The crude product was purified by recrystallization in ethanol. Yield 5.3 g (10.2 mmol, 98%). ^{1}H NMR ($CDCl_3$): δ = 7.80-7.73

(m, 9 H), 7.66-7.62 (m, 6 H), 6.46 (d, *J* = 2.7 Hz, 2 H), 5.40 (d, *J* = 13.9 Hz, 2 H), 3.76 (s, 3 H), 3.51 (s, 6 H).

Synthesis of (Z)-2-methoxy-5-(3,4,5-trimethoxystyryl)phenol (Combretastatin A-4, CA4).[1] 500 mg (0.955 mmol, 2 eq) **1** were suspended in 5 mL dry THF in a dry and nitrogen-purged flask. The suspension was cooled to 0°C in an ice bath. 955 µL (2.38 mmol, 5 eq) of a 2.5 M *n*-butyllithium (BuLi) solution in hexane were added dropwise. If the suspension does not maintain an orange color for at least 30 minutes, the addition of more *n*-BuLi is necessary. Then 73 mg (0.477 mmol, 1 eq) isovanilline were added in one portion. The reaction mixture was warmed to 65°C and stirred under reflux overnight. Upon completion, the reaction was quenched with ice-cold water. The aqueous phase was neutralized with 1 M HCl and the product was extracted three times with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and evaporated to give the crude product which was purified by flash chromatography (10%–60% ethyl acetate in cyclohexane). Yield 56 mg (0.177 mmol, 37%). ^{1}H NMR ($CDCl_3$): δ = 6.92 (d, *J* = 1.8 Hz, 1 H), 6.79 (dd, *J* = 8.3 Hz, *J* = 1.8 Hz, 1 H), 6.73 (d, *J* = 8.3 Hz, 1 H), 6.52 (s, 2 H), 6.47 (d, *J* = 12.1 Hz, 1 H), 6.41 (d, *J* = 12.1 Hz, 1 H), 5.52 (bs, 1 H), 3.86 (s, 3 H), 3.84 (s, 3 H), 3.70 (s, 6 H). ^{13}C NMR ($CDCl_3$): δ = 152.95, 145.88, 145.34, 132.80, 130.72, 129.97, 129.58, 129.12, 121.20, 115.15, 110.43, 106.17, 61.00, 56.03, 56.02. HRMS-ESI (m/z): $[M + Na]^+$ calcd for $C_{18}H_{20}O_5Na$: 339.1203, found: 339.1210.

Synthesis of (Z)-1,2,3-trimethoxy-5-(4-methoxy-3-nitrostyryl)benzene (2).[2] 1 g (1.91 mmol, 2 eq) **1** were suspended in 10 mL dry THF in a dry and nitrogen-purged flask. The suspension was cooled to -10°C in an ice-NaCl-bath. 1.146 mL (4.77 mmol, 5 eq) of a 2.5 M *n*-butyllithium solution in hexane were added dropwise. If the suspension does not maintain an orange color for at least 30 minutes, the addition of more *n*-BuLi is necessary. Then 173 mg (0.956 mmol, 1 eq) 4-methoxy-3-nitrobenzaldehyde were added in one portion. The reaction mixture was warmed to 60°C and stirred overnight. Upon completion, the reaction was quenched with ice-cold water. The product was extracted three times with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and evaporated to give the crude product which was purified by flash chromatography (20%–40% ethyl acetate in cyclohexane). Yield 199 mg (0.577 mmol, 60%). ^{1}H NMR ($CDCl_3$): δ = 7.78 (d, *J* = 2.2 Hz, 1 H), 7.43 (dd, *J* = 2.2 Hz / 8.7 Hz, 1 H), 6.94 (d, *J* = 8.7 Hz, 1 H), 6.57 (d, *J* = 12.1 Hz, 1 H), 6.46 (s, 2 H), 6.44 (d, *J* = 12.1 Hz, 1 H), 3.93 (s, 3 H), 3.85 (s, 3 H), 3.71 (s, 6 H) (in accordance with [3]). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{20}NO_6$: 346.1285, found: 346.1285.

Synthesis of (Z)-2-methoxy-5-(3,4,5-trimethoxystyryl)aniline (Combretamin, 3). A 50 mL round bottom flask was purged with nitrogen and covered in aluminum foil. 70 mg (0.203 mmol, 1 eq) **2** were dissolved in 20 mL methanol and 258 mg (1.01 mmol, 5 eq) $SnCl_2 \cdot H_2O$ and 38 mg (1.01 mmol, 5 eq) $NaBH_4$ were added subsequently. The solution was kept under nitrogen in the dark for 24 hours. Upon

completion, the solvent was removed *in vacuo* and the residue was collected in ethyl acetate. The organic phase was washed several times with diluted aqueous ammonia and then dried over $MgSO_4$ and evaporated. The crude product was purified by reversed phase flash chromatography on an RP-18 silica column (40%–100% methanol in water). Yield 60.7 mg (0.193 mmol, 95%). ^{1}H NMR ($CDCl_3$): δ = 6.69–6.67 (m, 3 H), 6.54 (s, 2 H), 6.44 (d, *J* = 12.0 Hz, 1 H), 6.35 (d, *J* = 12.0 Hz, 1 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.69 (s, 6 H) (in accordance with [3]). ^{13}C NMR ($CDCl_3$): δ = 152.90, 146.79, 137.07, 135.62, 133.08, 130.15, 130.09, 128.49, 119.77, 115.49, 110.16, 106.11, 61.01, 56.02, 55.61. HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{22}NO_4$: 316.1543, found: 316.1544.

General Procedure for the Solid Phase Peptide Synthesis (5a, 5b, 5c, 5d). The peptide sequences were synthesized in Merrifield's solid phase synthesis[4] using Wang resin according to the Fmoc strategy: First, Wang resin was swollen overnight in dimethylformamide (DMF) under shaking in a syringe equipped with a frit. To load the resin with the first amino acid, 5 eq of the Fmoc-protected amino acid, 5 eq O-(7-azabenzo-triazole-1-yl)-N,N,N',N'-tetramethyluronium hexafluoro-phosphate (HATU) and 5 eq diisopropylethylamine (DIPEA) were dissolved in the minimal amount of DMF and added to the swollen resin in the syringe. The mixture was shaken for 3 h, then the supernatant was removed through the frit and the resin washed three times each with DMF, CH_2Cl_2 and again DMF. To mask the free binding sites on the resin, a mixture of 10% acetic anhydride and 5% DIPEA in DMF was added and incubated for 30 minutes. After removal, the resin was washed again three times with DMF. The N-terminal Fmoc group was removed by a 25% piperidine solution in DMF for 10 minutes. The deprotection was repeated for another 5 minutes, and then the resin was washed very thoroughly with DMF, CH_2Cl_2 and DMF. The next amino acid (5 eq) and HATU (5 eq) were dissolved in the minimal amount of DMF and 5 eq DIPEA were added. The faint yellow solution was added to the resin and shaken for 3 hours. The described steps of deprotection, washing and coupling were repeated for all the amino acids in the peptide. To add the benzoyl cap, 10 eq benzoyl chloride and 10 eq DIPEA were dissolved in DMF and added to the syringe. After shaking for 3 hours, the solution was removed and the resin was washed with DMF, CH_2Cl_2, DMF and eventually diethyl ether. The resin was dried under vacuum overnight and the peptide was cleaved and deprotected with a solution of 92.5% trifluoroacetic acid (TFA), 5% triisopropylsilane (TIPS) and 2.5% water for 3 hours. The cleaved and deprotected peptide was precipitated in cold diethyl ether, centrifuged, washed with diethyl ether and dried. Purification was performed by preparative HPLC (10%-80% methanol in water).

5a: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{34}N_9O_5$: 492.2677, found: 492.2668.

5b: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{36}N_9O_5$: 506.2839, found: 506.2827.

5c: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{36}N_9O_5$: 506.2839, found: 506.2827.

5d: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{23}H_{37}N_{10}O_6$: 549.2892, found: 549.2890.

General Procedure for the Solid Phase Peptide Synthesis while Preserving the Protective Groups in the Amino Acid Side Chains (5e, 5f, 5g, 5h). This procedure equals the general procedure for the solid phase peptide synthesis described above except for the cleavage step from the resin. For this purpose, a mild cleavage solution of 4% TFA in CH_2Cl_2 was prepared. 2 mL of this solution were aspirated into the syringe with the bound peptide and incubated while shaking for 30 minutes. Then, the solution was injected into 30 mL cold water. This step was repeated three more times. The mixture of water, CH_2Cl_2 and TFA was evaporated under reduced pressure to yield the crude protected peptide. This was purified by preparative HPLC (50%-100% methanol in water).

5e: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{52}H_{76}N_9O_{12}S_2$: 1082.5055, found: 1082.5007.

5f: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{39}H_{58}N_7O_{10}S$: 816.3960, found: 816.3938.

5g: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{44}H_{68}N_7O_{11}S$: 902.4692, found: 902.4659.

5h: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{60}N_7O_{10}S$: 830.4117, found: 830.4106.

General Procedure for the Peptide Coupling in Solution (4a, 4b, 6a, 6b, 6c, 6d). In a dry round bottom flask, 1 eq carboxylic acid, 1.05 eq HATU and 1.1 eq 1-hydroxy-7-azabenzotriazole (HOAt) were dissolved in 3 mL dry DMF. The solution was cooled to 0°C and 1.3 eq (DIPEA) were added. The resulting yellow solution was stirred for 3 minutes, then 1 eq amine was added and the reaction was stirred overnight at room temperature. After completion, the reaction was quenched with ice and the solvent was evaporated *in vacuo*. The residue was purified by preparative HPLC (10%-80% methanol in water).

4a: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{25}N_2O_5$: 373.1758, found: 373.1654.

4b: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{27}N_2O_5$: 387.1914, found: 387.1919.

6a: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{39}H_{53}N_{10}O_8$: 789.4048, found: 789.4016.

6b: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{55}N_{10}O_8$: 803.4204, found: 803.4172.

6c: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{55}N_{10}O_8$: 803.4204, found: 803.4211.

6d: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{41}H_{56}N_{11}O_9$: 846.4262, found: 846.4232.

General Procedure for the Peptide Coupling in Solution Followed by Deprotection of the Amino Acid Side Chains (6e, 6f, 6g, 6h). This procedure equals the general procedure for the peptide coupling in solution described above. In addition, the crude product was dissolved in a mixture of 95% TFA and 5% water and stirred for 2 hours at room temperature. After evaporation of the solvents, the residue was purified by preparative HPLC (10%-80% methanol in water).

6e: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{55}N_{10}O_9$: 819.4148, found: 819.4131.

6f: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{39}H_{53}N_8O_8$: 761.3981, found: 761.3990.

6g: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{55}N_8O_9$: 791.4087, found: 791.4084.

6h: HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{40}H_{55}N_8O_8$: 775.4137, found: 775.4132.

Synthesis of 2-Abz-Nle-Lys-Arg-Arg-Ser-(3-NO_2)-Tyr-NH_2 (FRET substrate). The DENV protease FRET substrate (K_m = 105 µM) was synthesized by solid phase supported peptide synthesis using the Fmoc-protocol as described by Nitsche *et al*.[5]

Expression and Purification of DENV Protease. Serotype 2 DENV NS2B-NS3 protease construct was used with a glycine-serine GGGGSGGGG linker that connects the NS3 protease domain with the NS2B cofactor.[6,7] The protease was expressed and purified as reported by Steuer *et al.*[8,9]

Virus Titer Reduction Assay (Plaque Assay). Huh-7 and Vero E6 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 units penicillin G per mL and 0.1 mg/mL streptomycin (DMEMcplt) at 37°C, 5% CO_2 and 95% relative humidity. During infection of Huh-7 cells, DMEM was supplemented with 10 mM HEPES. 1 x 10^4 Huh-7 cells per well were seeded into 96-well plates in 50 µL DMEMcplt and incubated overnight at 37°C. On the next day, the cells were infected with wild type DENV serotype 2 or ZIKV for 2 h with a multiplicity of infection (MOI) of 1 before medium change and compound addition (usually 25 µM). After incubation for 24 h at 37°C the supernatant was harvested, the triplicates were pooled and stored at -80°C until further usage. 50 µl of fresh DMEMcplt was added to the cells and cell viability was determined using Cell-Titer Glo Luminescent Viability Assay (Promega). For measurement of virus titers by plaque assay, Vero E6 cells were seeded into 24-well plates with a density

of 2.5 x 10^5 cells per well. After overnight incubation at 37°C, the cells were infected with the harvested virus supernatant. The virus (and compound) containing medium was diluted with DMEMcplt ranging from 10^{-1} to 10^{-6} prior to infection. After incubation of the cells with 100 µl of the virus dilution at 37°C with agitation for 1 h, the medium was removed and 1 mL of plaque medium was added. After further incubation for 7 days at 37°C the cells were fixed with 5% (v/v) formaldehyde for 2 h, stained with crystal violet and plaques were counted at a suitable dilution. Titer reduction was calculated compared to a control treated with DMSO alone.

Toxicity Assay. Determination of cell viability was performed using the Cell Titer Blue assay with HeLa or Huh-7 cells. All tested compounds were diluted in a dilution series from 50 µM down to 3.2 nM in a sterile flat-bottom 96-well plate with a zero compound control. HeLa and Huh-7cells are cultivated in Dulbecco's modified Eagle Medium (DMEM). After splitting, the cells were diluted with DMEM and 5000 HeLa cells or 7000 Huh-7 cells were placed into each well of the initial plate and incubated for 72 h at 37°C. After the incubation, the medium was removed with a vacuum pump and 100 µL of a mixture of 20% Resazurin and 80% DMEM was aliquoted into the wells. The dye was incubated for 2 h at 37°C. Afterwards, the percentage of cell viability was calculated by the difference of absorption at 570 nm and 600 nm. CC_{50} values were derived from fitting the data to a four-parameter logistic model (variable-slope, nonlinear regression model) to generate a dose-response curve using the GraphPad Prism software package (GraphPad Software, San Diego, CA).

Dengue protease reporter cell line. HeLa wildtype cells were kept in DMEM (supplemented with 10% FCS and 100U/ml Pen and 100µg/ml Strep) at 37°C if not stated otherwise. All transfections were done using Lipofectamine 3000 transfection reagent (Life technologies), according to the manufacturer's instruction. In brief, plasmid DNA, encoding for DENV serotype 2 full length NS2B and NS3 proteins, was diluted in Opti-MEM with 0.2 µl P3000 reagent per 100 ng DNA and mixed with 0.3 µl Lipofectamine 3000 transfection reagent per 100 ng DNA, which was also diluted in Opti-MEM. Ten minutes after mixing, the cell suspension was added and seeded into the respective plates or flasks. In order to generate a stably transfected cell line, selection antibiotics were added 24 h post transfection and monoclonal cell lines generated from surviving single cell clones. Successful transfection and expression of the protease construct was confirmed via a luciferase reporter system as described in detail elsewhere (Kühl *et al.*, in preparation).

The cell-based proteolytic assay was performed analogous to the **Toxicity Assay** by seeding 5000 cells/well into a 96-well plate and treatment of cells with the respective compound dilutions for 72 h before readout of cell viability with the CellTiter-Blue reagent, according to the manufacturer's instructions.

Prodrug Hydrolysis by DENV Protease. The biochemical assay was performed in a 96-well U-bottom plate. In assay buffer (50 mM TRIS pH 9, 10% ethylene glycol, 0.0016% Brij 58), a 10 mM prodrug stock solution in DMSO was diluted to 500 µM. DENV protease solution was added to a final concentration of 1 µM. The plate was incubated at 37°C for 2 h. Subsequently, the assay was quenched by addition of 30 µL 4% TFA per well. The relative concentration of the prodrug was then determined by integration of HPLC signals and compared to a control without enzyme.

DENV Protease Inhibition. The biochemical DENV protease inhibition assay was performed as described by Nitsche *et al.*[5,10] and Behnam *et al.*[11] in black 96 well V-bottom plates on a BMG Labtech Fluostar OPTIMA Microtiter fluorescence plate reader using 320 nm as excitation wavelength and 405 nm as emission wavelength. The inhibitors (final concentration 50 µM, from 10 mM stock solutions in DMSO) were preincubated for 15 min with the DENV protease (100 nM) in the assay buffer (50 mM Tris-HCl pH 9, ethylene glycol (10% v/v), and 0.0016% Brij 58). Subsequently, the reaction was initiated by the addition of the FRET substrate (K_m = 105 µM, final concentration 50 µM) to obtain a final assay volume 100 µL per well. The enzymatic activity was determined as slope per second (relative fluorescence units per second) and monitored for 15 min. Percentage inhibition was calculated relative to a positive control (without the inhibitor). All experiments were performed in triplicate and averaged.

Tubulin Polymerization Assay. The assay was performed in a transparent 96-well flat-bottom plate using a porcine tubulin polymerization assay kit by tebu-bio GmbH (Germany). To prepare the 5 mg/mL stock solution of tubulin 4 mg of lyophilized tubulin was dissolved in 800 µL of cold general tubulin buffer (GTB: 80 mM PIPES, 0.5 mM EGTA, 2 mM $MgCl_2$, pH 6.9). 100 µM stock solutions of the compounds were prepared by diluting 5 µL of the 10mM DMSO based solution of the compound in 495 µL GTB. In each analytical well 80 µL of tubulin solution in GTB, 10 µL 10-fold concentrated compound solution in GTB and 10 µL GTP solution in GTB were added reaching the respective final concentrations of 4 mg/mL tubulin, 10 µM compound, and 1 mM GTP. In each control well 80 µL of tubulin solution in GTB, 10 µL of DMSO in GTB (0.1 mL in 9.9 mL), and 10 µL GTP in GTB were added to yield final concentrations of 4 mg/mL tubulin and 1 mM GTP. The plate was pre-incubated at 37°C for 30 min with added solutions of tubulin and the test compound to allow slow binding molecules to bind to tubulin, followed by chilling to 0°C to depolymerize formed microtubules. Subsequently, GTP solution was added. Immediately after the addition of GTP, the read-out began. For read-out, the absorption at 340 nm and 37°C was measured by a microtiter plate reader (BMG labtech Omega).

Table S1. Kinetic parameters of dodecapeptides that resemble the polyprotein cleavage sites.

Natural cleavage site in dengue polyprotein	**Oligopeptide**[12]	**Kinetic parameters**[12]
NS2A/NS2B	RTSKKR/SWPLNE	K_m = 184 µM k_{cat} = 0.0012 s^{-1}
NS2B/NS3	EVKKQR/AGVLWD	K_m = 3890 µM k_{cat} = 0.0062 s^{-1}
NS3/NS4A	FAAGRK/SLTLNL	K_m = 432 µM k_{cat} = 0.0052 s^{-1}
NS4B/NS5	TTSTRR/GTGNIG	K_m = 416 µM k_{cat} = 0.0045 s^{-1}

Table S2. Hydrolysis of combretastatin peptide hybrids by other serine proteases.

Compound	**WNV Protease**	**Thrombin**	**Trypsin**
6b	5.8% ± 14.2%	n.h.	13.2% ± 4.9%
6c	n.h.	n.h.	9.8% ± 1.8%
6d	15.9% ± 4.3%	n.h.	32.6% ± 6.7%
6e	n.h.	n.h.	100%

[Enzyme] = 0,2 µM, [compound] = 100 µM in TRIS buffer pH 9, incubated for 2 h at 37°C

n.h. = no hydrolysis

Table S3. Determination of DENV titer in the "plaque" assay: treated and non-treated samples.

sample name	**n° plaques**	**n° plaques**	**average**	**error (s)**	**dilution**	**titer**	**log titer**	**% inhib.**
0 µM	48	37	42,5	7,78	1,E-04	2,E+06	6,33	
0 µM	47	37	42	7,07	1,E-04	2,E+06	6,32	
0 µM	39	41	40	1,41	1,E-04	2,E+06	6,30	
non-treated*				0,01		2,E+06	6,32	0,00
3	13	14	13,5	0,71	1,E-04	7,E+05	5,83	67,47
4a	12	14	13	1,41	1,E-04	7,E+05	5,81	68,67
4b	14	16	15	1,41	1,E-04	8,E+05	5,88	63,86
6a	6	7	6,5	0,71	1,E-04	3,E+05	5,51	84,34
6b	10	27	18,5	12,02	1,E-04	9,E+05	5,97	55,42
6c	13	24	18,5	7,78	1,E-04	9,E+05	5,97	55,42
6d	19	19	19	0,00	1,E-04	1,E+06	5,98	54,22
6e	21	20	20,5	0,71	1,E-04	1,E+06	6,01	50,60
6f	20	20	20	0,00	1,E-04	1,E+06	6,00	51,81
6g	12	21	16,5	6,36	1,E-04	8,E+05	5,92	60,24
6h	15	9,5	12,25	3,89	1,E-04	6,E+05	5,79	70,48

*mean value from three biological replicates

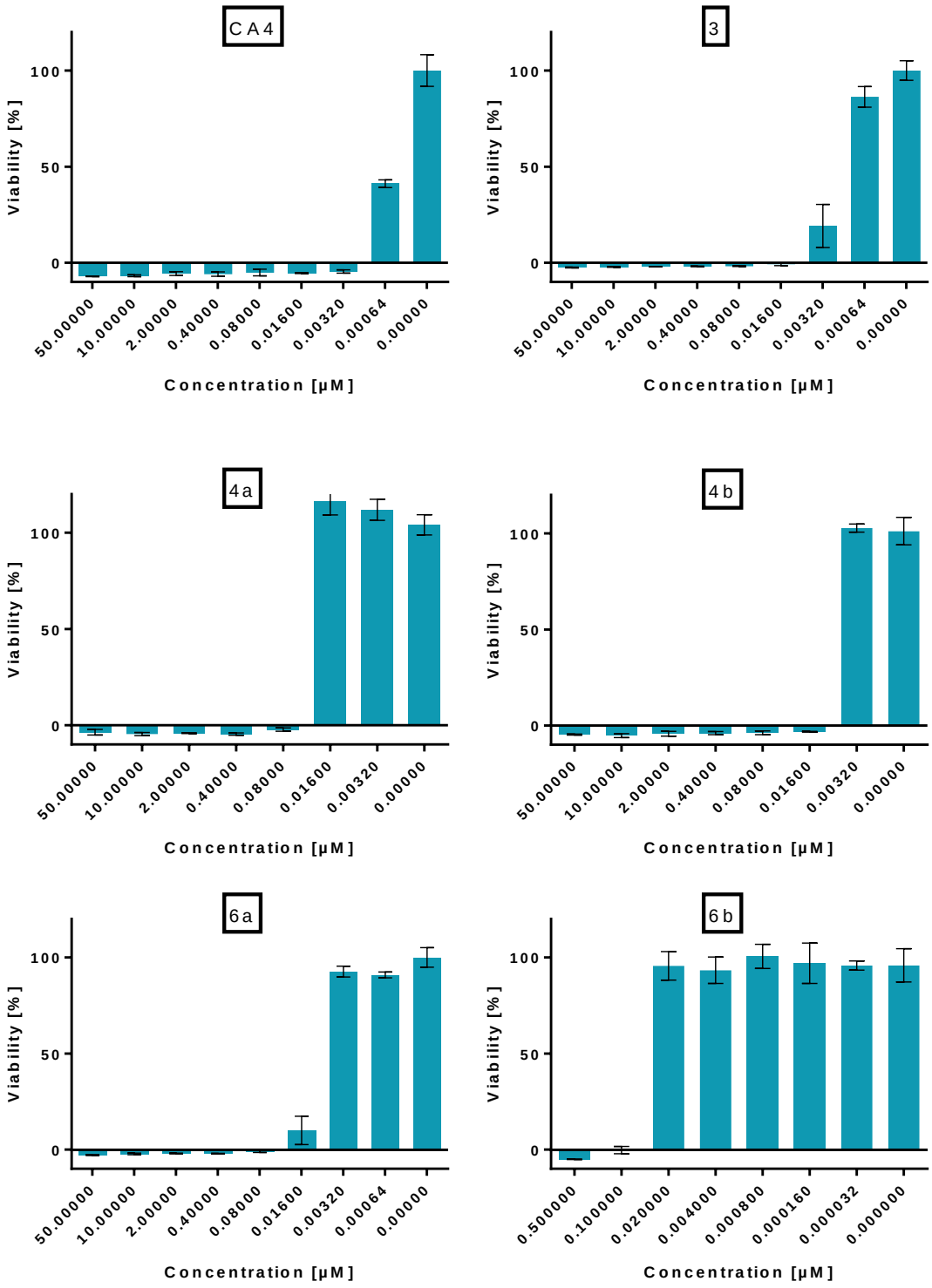
Table S4. Cytotoxicity of hydrolyzable and non-hydrolyzable peptide hybrids in wildtype HeLa and DENV2proHeLa after 72 h of incubation.

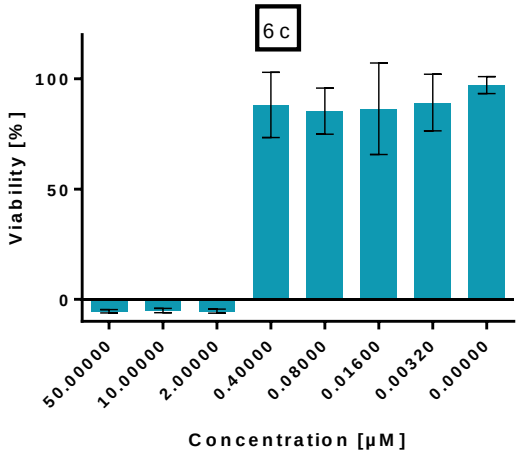
Compound	Hydrolyzable?	CC_{50} in DENV2proHeLa [nM]	CC_{50} in wildtype HeLa [nM]	Toxicity change by factor*	Relative fold increase/ decrease**
CA4	no	0.45	0.43	0.96	-0.04
3	no	1.69	1.47	0.87	-0.13
6a	yes	0.85	7.2	8.45	+7.45
6b	yes	1.58	65.7	41.58	+40.58
6c	no	657	952	1.45	+0.45
6d	yes	11.9	19.5	1.64	+0.63
6e	no	54.6	29.2	0.53	-0.47
6f	yes	34.7	78.2	2.25	+1.25
6g	no	55.8	61.8	1.11	+0.11

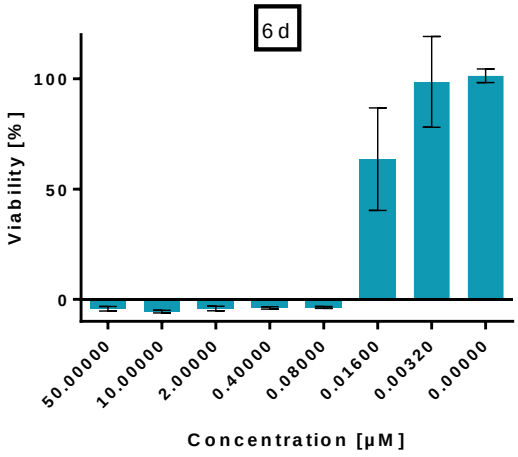
*calculated as CC_{50} in wildtype HeLa /CC_{50} in DENV2proHeLa

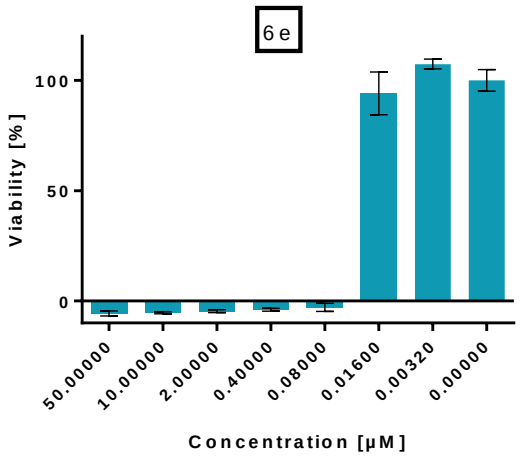
** calculated as (CC_{50} in wildtype HeLa/CC_{50} in DENV2proHeLa)-1 for better distinctiveness

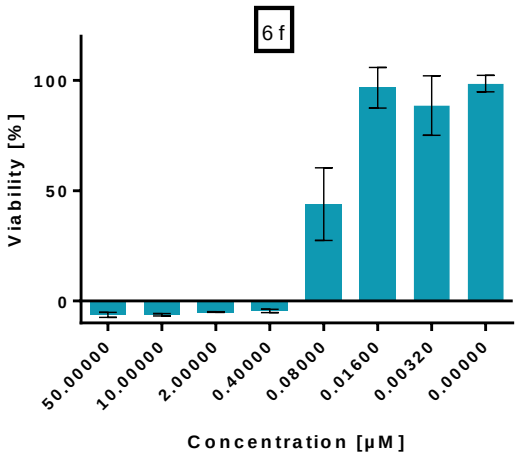
Figure S2. Dose-response diagram to find cytotoxic concentration in wildtype HeLa cells.

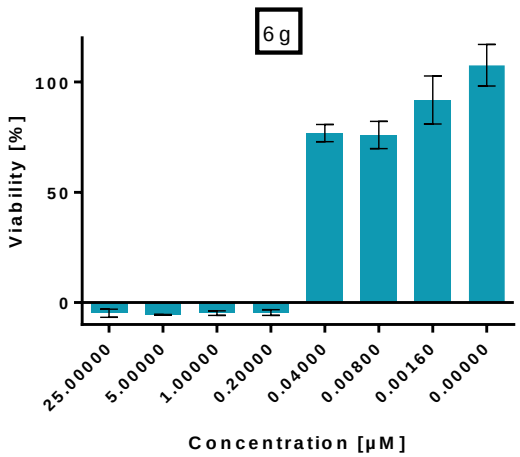



6 c
Viability [%]
100
50
0
50.00000
10.00000
2.00000
0.40000
0.08000
0.01600
0.00320
0.00000
Concentration [µM]


6 d
Viability [%]
100
50
0
50.00000
10.00000
2.00000
0.40000
0.08000
0.01600
0.00320
0.00000
Concentration [µM]


6 e
Viability [%]
100
50
0
50.00000
10.00000
2.00000
0.40000
0.08000
0.01600
0.00320
0.00000
Concentration [µM]


6 f
Viability [%]
100
50
0
50.00000
10.00000
2.00000
0.40000
0.08000
0.01600
0.00320
0.00000
Concentration [µM]


6 g
Viability [%]
100
50
0
25.00000
5.00000
1.00000
0.20000
0.04000
0.00800
0.00160
0.00000
Concentration [µM]

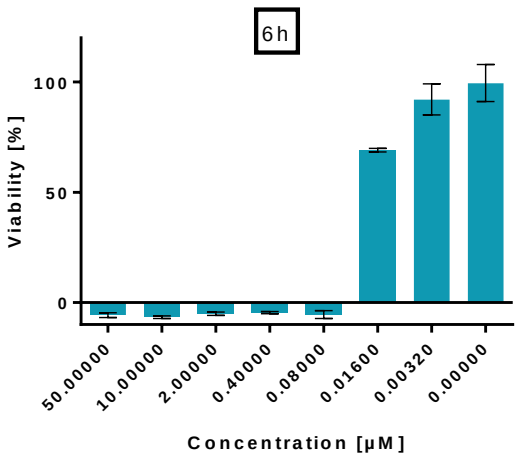
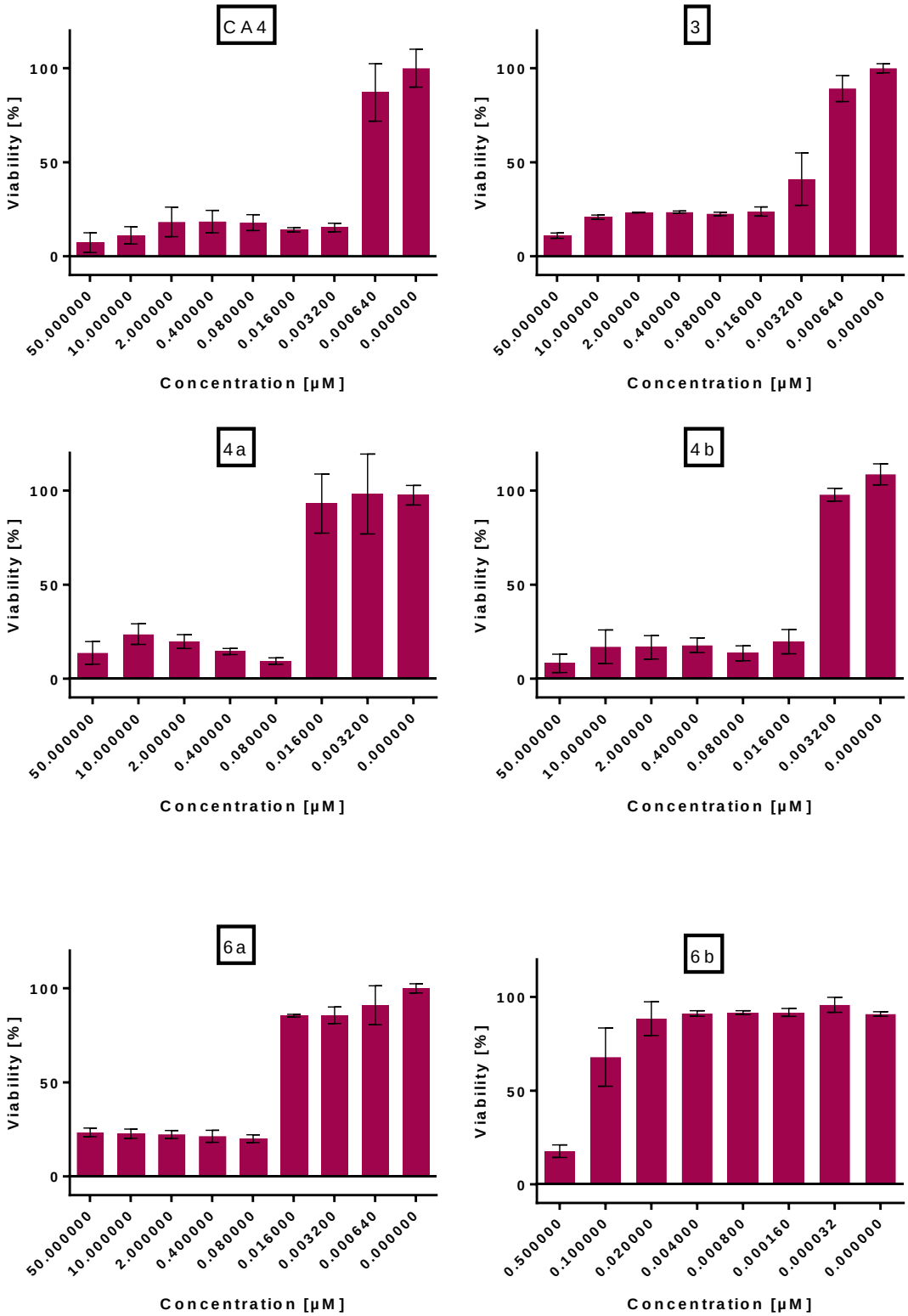
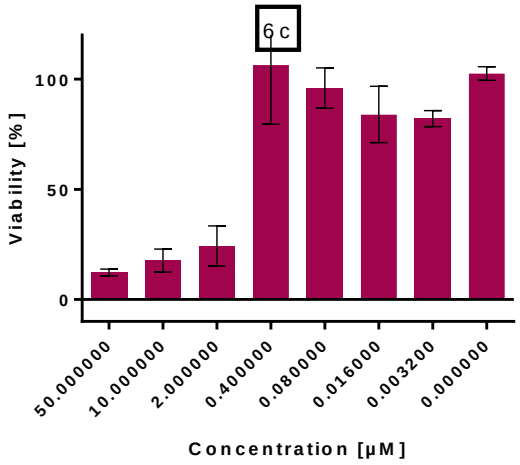
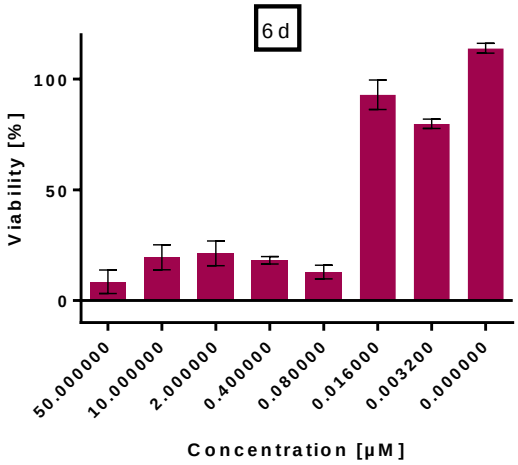
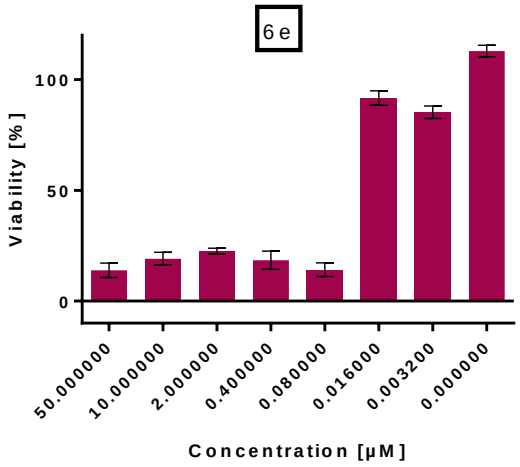

6 h
Viability [%]
100
50
0
50.00000
10.00000
2.00000
0.40000
0.08000
0.01600
0.00320
0.00000
Concentration [µM]

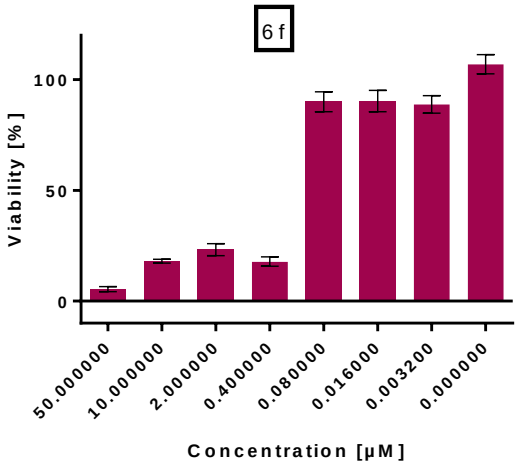
Figure S3. Dose-response diagram to find cytotoxic concentration in Huh-7 cells.

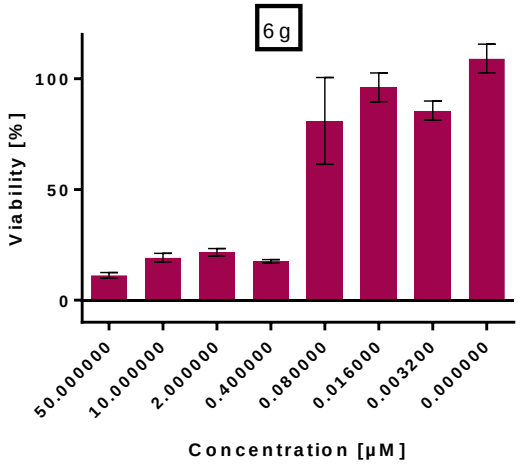


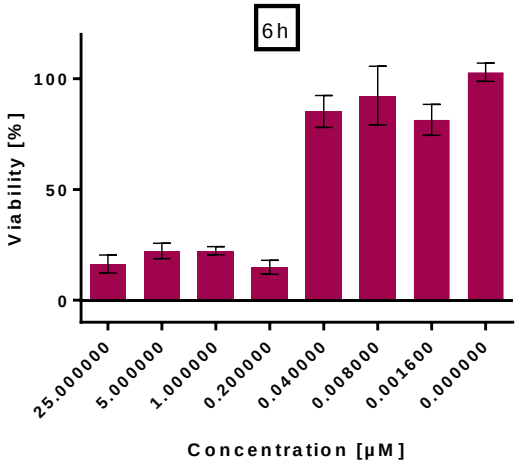

6 c
Viability [%]
100
50
0
50.000000
10.000000
2.000000
0.400000
0.080000
0.016000
0.003200
0.000000
Concentration [µM]


6 d
Viability [%]
100
50
0
50.000000
10.000000
2.000000
0.400000
0.080000
0.016000
0.003200
0.000000
Concentration [µM]


6 e
Viability [%]
100
50
0
50.000000
10.000000
2.000000
0.400000
0.080000
0.016000
0.003200
0.000000
Concentration [µM]


6 f
Viability [%]
100
50
0
50.000000
10.000000
2.000000
0.400000
0.080000
0.016000
0.003200
0.000000
Concentration [µM]


6 g
Viability [%]
100
50
0
50.000000
10.000000
2.000000
0.400000
0.080000
0.016000
0.003200
0.000000
Concentration [µM]


6 h
Viability [%]
100
50
0
25.000000
5.000000
1.000000
0.200000
0.040000
0.008000
0.001600
0.000000
Concentration [µM]

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