Supporting Information

Vesicular Disruption of Lysosomal Targeting Organometallic Polyarginine Bioconjugates

Annika Grossa, d, *, Hamed Alborzinia, Stefania Piantavigna, Lisandra L. Martin, Stefan Wölfl and Nils Metzler-Noltea

a: Department of Chemistry and Biochemistry
University of Bochum, Universitätsstrasse 150, D-44801 Bochum (Germany)
Fax: +49 (0)234 - 32 14378
E-Mail: nils.metzler-nolte@ruhr-uni-bochum.de

b: Institute of Pharmacy and Molecular Biotechnology, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

c: School of Chemistry, Monash University, Clayton, Victoria, Australia

d: Current Address: Institute of Pharmacy, Technische Universität Braunschweig
Beethovenstr. 55, D-38106 Braunschweig, Germany

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I. HPLC characterization of 1, 2, 3 and 5

Figure S1. HPLC-chromatogram of the purified compounds 1 and 2 detected at 220 nm, run: 40 min (left); HPLC-chromatogram of the purified compounds 3 and 5 detected at 254 nm (right).

II. Membrane Studies

Figure S2: Δf-t plots obtained for peptide 1 (A) and peptide 3 (B) interacting with a DMPC/Cholesterol membrane. The plots are for 5 μM. The harmonic represented are: 3rd (black line), 5th (red line), 7th (green line) and 9th (blue line). The vertical dash line indicates the time when the peptide flow stopped (II.), while I. corresponds to the beginning of the peptide injection into the QCM chamber.
Figure S3: Δf-t plots obtained for peptide 2 interacting with a DMPC/Cholesterol membrane. The concentrations used are 1, 5 and 10 µM (from the lightest to the darkest trace). The harmonic examined was the 7th. The vertical dash line indicates the time when the peptide flow stopped (II.), while I. corresponds to the beginning of the peptide injection into the QCM chamber.

Figure S4: Δf-t plots obtained for peptide 1 (A) and peptide 3 (B) interacting with a DMPC/DMPG membrane. The concentrations used are 1 µM (light grey) and 10 µM (black trace). The harmonic examined was the 7th. The vertical dash line indicates the time when the peptide flow stopped (II.), while I. corresponds to the beginning of the peptide injection into the QCM chamber.
III. Biological Studies

a) Uptake Studies of 4, 5 and 6

Figure S5. Cellular uptake of 4 (10 µM) in PT45 cells after 14 h incubation. A: fluorescence image, t = 0; B: fluorescence image, t = 5 min; C: phase contrast. FITC-filter, 200x magnification. Cellular uptake of 5 (10 µM) in PT45 cells after 14 h incubation. D: fluorescence image, t = 0; E: fluorescence image, t=5 min; F: phase contrast. FITC-Filter, 200x magnification. Cellular uptake of 6 (10 µM) in PT45 cells after 14 h incubation. G: fluorescence image, t = 0; H: fluorescence image, t = 5 min; I: phase contrast. FITC-Filter, 200x magnification.

b) Cytotoxicity Assays

Cytotoxicity assays:
Resazurin Assay. 1 mL of resazurin solution per plate was diluted with 9 mL medium without phenol red. 100 µL of this solution was added per well and their absorbance was measured using a Tecan plate reader at 600 nm, using a reference wavelength of 690 nm. The plates were incubated for 2 h at 37 °C and 10% CO₂ and were measured again.

Crystal Violet Assay. To perform the crystal violet assay, the resazurin solution was removed and the cells were fixed using 0.2% glutardialdehyde for 25 min. The glutardialdehyde solution was removed and exchanged for 100 µL of 0.1% triton solution. After a short incubation, all liquids were removed and the fixed cells were stained with a 0.02 M crystal violet solution for 30 min. Afterwards the wells were washed intensely with H₂O and were filled with 100 µL of 96% ethanol, followed by shaking on a softly rocking rotary shaker for 3 h. The absorption of the ethanolic solution was measured using a micro-plate reader at 570 nm.
Table S1. IC$_{50}$ values, resazurin assay after 48 h incubation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HeLa [µM]</th>
<th>PT45 [µM]</th>
<th>HepG2 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>251 ± 19</td>
<td>290 ± 10</td>
<td>307 ± 38</td>
</tr>
<tr>
<td>2</td>
<td>62 ± 3</td>
<td>133 ± 18</td>
<td>86 ± 13</td>
</tr>
<tr>
<td>3</td>
<td>134 ± 15</td>
<td>215 ± 9</td>
<td>195 ± 17</td>
</tr>
<tr>
<td>FeC(O)OH, Rec(C(O)OH</td>
<td>&gt; 1000</td>
<td>&gt; 1000</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>cisplatin</td>
<td>1.8 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
</tbody>
</table>

c) Apoptosis Assay

Figure S6. Apoptosis induction in HeLa cells in % of cells by 1, 2 and 3 at the indicated concentrations after 48 h incubation.
d) **Cell Cycle Studies**

![Figure S7](image)

**Figure S7.** Cell cycle studies of 1, 2 and 3 in HeLa cells at indicated concentrations after 24 h incubation.

**IV. Abbreviations**

AAS, atomic absorption spectrometry; AnnV, Annexin V; ATP, adenosine triphosphate; BSA, bovine serum albumin; Cp, cyclopentadiene; CPP, cell-penetrating peptide; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMF, dimethylformamide; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; FITC, fluorescein isothiocyanate; Fmoc, 9-fluorenylmethyloxycarbonyl; HeLa, immortal cell line (cervical cancer cells); HepG2, immortal cell line (hepatocellular carcinoma); HOBt, N-hydroxybenzotriazole·H₂O; HT-29, immortal cell line (colon carcinoma); IMIM-PC2, immortal cell line (ductal pancreas carcinoma); LMP, lysosomal membrane permeabilization; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; MeCN, acetonitrile; MeOH, methanol; Mtt, 4-methyltrityl; NT, non treated cells; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; PBS, phosphate buffered saline; Pen Strep, penicillin streptomycin solution; PI, propidium iodide; PNA, peptide nucleic acids; PT45, immortal cell line (pancreatic carcinoma cells); ROS, reactive oxygen species; SPPS, solid-phase peptide synthesis; RT, room temperature; TBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIS, triisopropylsilane.