Supplementary Material

Development of potent CPP6-gemcitabine conjugates against human prostate cancer cell line (PC-3)

Cristiana Correia\textsuperscript{a,b,c}, Cristina P. R. Xavier\textsuperscript{b,c}, Diana Duarte\textsuperscript{a,b,c}, Abigail Ferreira\textsuperscript{a,d}, Sara Moreira\textsuperscript{a,b,c}, M. Helena Vasconcelos\textsuperscript{b,c,e}, Nuno Vale\textsuperscript{a,b,c,f,*}

\textsuperscript{a}Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
\textsuperscript{b}Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Rua Júlio Amaral de Carvalho, 45, 4200-135 Porto, Portugal
\textsuperscript{c}Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal
\textsuperscript{d}LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
\textsuperscript{e}Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
\textsuperscript{f}Department of Molecular Pathology and Immunology, Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

* nuno.vale@ff.up.pt
Figure S1- Chemical structure of CPP6-1.

Figure S2- Chromatogram of the CPP6-1 after purification, acquired with a HPLC-DAD system, with a C18 column, using ACN in water with 0.05% TFA as eluent, in gradient mode (0 - 100%), for 30 minutes, at a flow of 1 mL/min and detection at $\lambda = 220$ nm.

Figure S3- Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown) of CPP6-1.
Figure S5 - Chromatogram of the CPP6-2 after purification, acquired with a HPLC-DAD system, with a C18 column, using ACN in water with 0.05% TFA as eluent, in gradient mode (0 - 100%), for 30 minutes, at a flow of 1 mL/min and detection at $\lambda = 220$ nm.

Figure S6 - Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown) of CPP6-2.
Figure S8 - Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown) of CPP6-3.

Figure S7 - Chemical structure of CPP6-3.

Figure S8 - Chromatogram of the CPP6-3 after purification, acquired with a HPLC-DAD system, with a C18 column, using ACN in water with 0.05% TFA as eluent, in gradient mode (0 - 100%), for 30 minutes, at a flow of 1 mL/min and detection at $\lambda = 220$ nm.

Figure S9 - Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown) of CPP6-3.
Figure S10 - Chemical structure of Gem-C2-CPP6-1 conjugate.

Figure S11 - Chromatogram of the Gem-C2-CPP6-1 conjugate after purification, acquired with a HPLC-DAD system, with a C18 column, using ACN in water with 0.05% TFA as eluent, in gradient mode (0 - 100%), for 30 minutes, at a flow of 1 mL/min and detection at $\lambda = 220$ nm.

Figure S12 - Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown) of Gem-C2-CPP6-1.
**Figure S13** - Chemical structure of Gem-C2-CPP6-2 conjugate.

**Figure S14** - Chromatogram of the Gem-C2-CPP6-2 conjugate after purification, acquired with a HPLC-DAD system, with a C18 column, using ACN in water with 0.05% TFA as eluent, in gradient mode (0 - 100%), for 30 minutes, at a flow of 1 mL/min and detection at \( \lambda = 220 \) nm.

**Figure S15** - Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown), of Gem-C2-CPP6-2.
**Figure S16** - Chemical structure of Gem-C2-CPP6-2 conjugate.

**Figure S17** - Chromatogram of the Gem-C2-CPP6-3 conjugate after purification, acquired with a HPLC-DAD system, with a C18 column, using ACN in water with 0.05% TFA as eluent, in gradient mode (0 - 100%), for 30 minutes, at a flow of 1 mL/min and detection at λ = 220 nm.

**Figure S18** - Mass spectrum (LC-ESI/MS Orbitrap, positive mode) of the major peak of the LC-MS chromatogram (data not shown) of Gem-C2-CPP6-3.
**Figure S19.** IC$_{50}$ of dFdC and conjugates with or without the presence of the inhibitor NBMPR in BxPC-3 cells: percentage of variation between the two values for each compound. +I: with inhibitor; -I: without inhibitor.

**Figure S20.** Chemical structures of drugs tamoxifen (7) and metformin (8)
**Table S1.** Molecular properties such as the molecular weight (MW), hydrophobicity parameter logP, lipophilicity parameter logD, and the total polar surface area (T_PSA) for peptides 4 and 6. For all CPP6 and dFdC-CPP6: Number of residue, 6; Extension coefficient (M^{-1}cm^{-1}), 5690; isoeletric-point, 14 and net charge at pH 7 is +2 for all peptides 4 and 6. (Calculated using GastroPlusTM Software)

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<tr>
<th>#</th>
<th>Compound</th>
<th>Sequence</th>
<th>MW/gmol^{-1}</th>
<th>logP</th>
<th>logD</th>
<th>T_PSA</th>
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<td>4.1</td>
<td>CPP6-1</td>
<td>KLPVMW</td>
<td>772.02</td>
<td>1.94</td>
<td>0.65</td>
<td>247.63</td>
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<tr>
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<td>2.01</td>
<td>0.59</td>
<td>247.63</td>
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<tr>
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<td>2.51</td>
<td>1.51</td>
<td>364.39</td>
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