The cisplatin-based Pt(IV)-diclorofibrato multi-action anticancer prodrug exhibits excellent performances also in hypoxic conditions

Elisabetta Gabano,a* Mauro Ravera,a Francesca Trivero,a Stefano Tinello,a Andrea Gallina,a Ilaria Zanellato,a Marzia B. Gariboldi,b Elena Monti,b Domenico Osella,a

a Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Viale Michel 11, 15121 Alessandria, Italy. E-mail: elisabetta.gabano@uniupo.it

b Dipartimento di Biotecnologie e Scienze della Vita, Università dell’Insubria, Via Manara 7, 21052 Busto Arsizio (VA).

ELECTRONIC SUPPORTING INFORMATION

Content:

Figure S0: Sketch of the complexes under investigation

Figures S1-S2: NMR characterization of the clofibroyl chloride

Figures S3-S9: NMR and ESI-MS characterization of complex 1 and its 15N-labeled analogue

Figures S10-S15: NMR and ESI-MS characterization of complex 2 and its 15N-labeled analogue

Figures S16-S19: [1H, 15N] HSQC spectra for the reduction of complexes 1 and 2 with cell extracts.

Figure S20: Cell cycle distribution of A2780 cells (top plots) and HCT 116 (bottom plots) after 48 h CT with equitoxic concentrations of cisplatin and 2 in normoxic (left plots) and hypoxic conditions (right plots). The abscissa of each plot represents the percentage of cells among the cell cycle phases. Data are means ± standard error means of at least three independent samples.

Table S1: Genes analyzed by means of Quantitative Reverse Transcription PCR (RT-qPCR). The NCBI accession number is reported along with the 5’-3’ sequence of the forward and reverse primer and the expected product length.
Figure S0. Sketch of the complexes under investigation
Figure S1. $^1$H NMR spectrum of clofibroyl chloride in CDCl$_3$

Figure S2. $^{13}$C{$^1$H} NMR spectrum of clofibroyl chloride in CDCl$_3$
**Figure S3:** $^1$H NMR spectrum of complex 1 in DMSO-d$_6$ (residual acetone is observed at ca. 2.1 ppm).

**Figure S4:** $^{13}$C\{$^1$H\} NMR spectrum of complex 1 in DMSO-d$_6$. 
Figure S5: $^{195}$Pt NMR spectrum of complex 1 in DMSO-d$_6$.

Figure S6: [$^1$H, $^{13}$C] HSQC NMR spectrum of complex 1 in DMSO-d$_6$. 
**Figure S7:** ESI-MS NMR spectrum of complex 1 showing both the [M+H]$^+$ and [M+Na]$^+$ species and simulation for the [M+H]$^+$ species
Figure S8: $^{15}$N-$^1$H DEPT-45 NMR spectrum of $^{15}$N-1

Figure S9: ESI-MS NMR spectrum of complex $^{15}$N-1 showing both the [M+H]$^+$ and [M+Na]$^+$ species and simulation for the [M+H]$^+$ species
Figure S10: $^1$H NMR spectrum of complex 2 in DMSO-$d_6$.

Figure S11: $^{13}$C{$^1$H} NMR spectrum of complex 2 in DMSO-$d_6$. 
**Figure S12**: $^{195}$Pt NMR spectrum of complex 2 in DMSO-d$_6$.

**Figure S13**: ESI-MS NMR spectrum of complex 2 showing both the [M+H]$^+$ and [M+Na]$^+$ species and simulation for the [M+H]$^+$ species
**Figure S14**: $^{15}\text{N}\{^1\text{H}\}$ DEPT-45 NMR spectrum of complex $^{15}\text{N}-2$

**Figure S15**: ESI-MS NMR spectrum of complex $^{15}\text{N}-2$ showing both the $[\text{M+H}]^+$ and $[\text{M+Na}]^+$ species and simulation for the $[\text{M+H}]^+$ species
Figure S16: $[^{1}H, ^{15}N]$ HSQC spectrum of complex $^{15}$N-1

Figure S17: $[^{1}H, ^{15}N]$ HSQC spectrum for the reduction of complex $^{15}$N-1 with cell extracts
Figure S18: $[^1\text{H}, ^{15}\text{N}]$ HSQC spectrum of complex $^{15}\text{N}$-2

Figure S19: $[^1\text{H}, ^{15}\text{N}]$ HSQC spectrum for the reduction of complex $^{15}\text{N}$-2 with cell extracts
**Figure S20:** Cell cycle distribution of A2780 cells (top plots) and HCT 116 (bottom plots) after 48 h CT with equitoxic concentrations of cisplatin and 2 in normoxic (left plots) and hypoxic conditions (right plots). The *abscissa* of each plot represents the percentage of cells among the cell cycle phases. Data are means ± standard error means of at least three independent samples.
Table S1. Genes analyzed by means of Quantitative Reverse Transcription PCR (RT-qPCR). The NCBI accession number is reported along with the 5’-3’ sequence of the forward and reverse primer and the expected product length.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession n°</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACOX1</td>
<td>NM_004035.6</td>
<td>GCTGGAGCTGCGGATTTAGA</td>
<td>TGTTCTCGATCTCTCGGCGG</td>
<td>187</td>
</tr>
<tr>
<td>ACOX1</td>
<td>NM_001876.3</td>
<td>CACTGAGCAGCGCAAGATGAG</td>
<td>AGGCGAGGCAGCGATGTC</td>
<td>111</td>
</tr>
<tr>
<td>Cyclin D1 (CCND1)</td>
<td>NM_053056.2</td>
<td>TGAGGGACGCTTTGTCTGTC</td>
<td>GCCTTTGGCCTCTCGATACA</td>
<td>75</td>
</tr>
<tr>
<td>p21 (CDKN1A)</td>
<td>NG_009364</td>
<td>GCGACTGTGTGATCGCCTAATG</td>
<td>GAAGGTAGAGCTTGGGCAGG</td>
<td>141</td>
</tr>
<tr>
<td>Cyclin A2 (CCNA2)</td>
<td>NM_001237.4</td>
<td>TGGTGCTCTGTTTCTGTGA</td>
<td>TGCCAGTCTTACTCAGCTGA</td>
<td>136</td>
</tr>
<tr>
<td>Cyclin E (CCNE)</td>
<td>NM_001238</td>
<td>GCAGGATCCAGATGAAGAATG</td>
<td>TAATCCGAGGCTTGCAGTT</td>
<td>173</td>
</tr>
<tr>
<td>HMOX1</td>
<td>NM_002133.2</td>
<td>TCTTTGGCTGCTTTGCCTTACC</td>
<td>GGATGTGCTTTTCGTGGGG</td>
<td>123</td>
</tr>
<tr>
<td>TP53 (p53)</td>
<td>NG_017013.2</td>
<td>GCCCGCTCCTTGAGCATTTATC</td>
<td>CTCATAGGGCACACACAC</td>
<td>99</td>
</tr>
<tr>
<td>GAPDH</td>
<td>NG_007073.2</td>
<td>ATCCCTGAGCTGAACGGGAA</td>
<td>GGCAGGTTTTCTAGACGAC</td>
<td>99</td>
</tr>
<tr>
<td>HPRT1</td>
<td>NM_000194.2</td>
<td>TGTCTTTCTGTGTCAGGGCA</td>
<td>ATCCAACACTTCGTGGGGTC</td>
<td>85</td>
</tr>
<tr>
<td>RNA18SN1</td>
<td>NR_145820.1</td>
<td>CGTCTGGCCCTATCAACTTTTC</td>
<td>TGCCCTCTTGGATGTTGTA</td>
<td>124</td>
</tr>
</tbody>
</table>