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Cis,cis,trans-[Pt^{IV}Cl₂(NH₃)₂(perillato)₂], a dual-action prodrug with excellent cytotoxic and antimetastatic activity

Mauro Ravera,[§] Elisabetta Gabano,[§] Ilaria Zanellato,[§] Beatrice Rangone,[§] Elena Perin[§] Beatrice Ferrari,[#] Maria Grazia Bottone,[#] and Domenico Osella*

[§] Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Viale Michel 11,

15121 Alessandria, Italy.

[#] Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Via Ferrata 9, 27100 Pavia, Italy.

Electronic Supplementary Information

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Figure S1. ¹H NMR spectrum of perillyl chloride in CDCl₃



Figure S2. ¹³C{¹H} NMR spectrum of perillyl chloride in CDCl₃



Figure S3. ¹H NMR spectrum of complex 1 in DMSO-d₆.



Figure S4. ${}^{13}C{}^{1}H$ NMR spectrum of complex 1 in DMSO-d₆.



Figure S5. ¹⁹⁵Pt NMR spectrum of complex 1 in DMSO-d₆.



Figure S6. [¹H, ¹H] COSY NMR spectrum of complex 1 in DMSO-d₆.



Figure S7. [¹H, ¹³C] HSQC NMR spectrum of complex 1 in DMSO-d₆.



Figure S8. [¹H, ¹³C] HMBC NMR spectrum of complex 1 in DMSO-d₆.



Figure S9. (top) RP-HPLC chromatogram (C_{18} column, eluent: CH₃OH 70% v/v + aqueous 15 mM HCOOH 30% v/v), (bottom) ESI-MS spectrum of complex 1 showing both the [M+H]⁺ and [M+Na]⁺ species and simulation for the [M+H]⁺ species.



Figure S10. (top) RP-HPLC chromatograms (C₁₈ column, eluent: CH₃OH 50% v/v + aqueous 15 mM HCOOH 50% v/v) and (left) corresponding normalized peak area of complex 1 within 72 h of incubation in RPMI 1640 with 10% methanol cosolvent at 37°C; (right) ESI-MS spectrum of hydrolyzed complex 1 [M- $Cl+H_2O$]⁺ and its fragmentation [M-Cl]⁺.



Figure S11. ¹⁵N{¹H} DEPT-45 NMR spectrum of complex ¹⁵N-1 in DMSO-d₆.



Figure S12. (top) RP-HPLC chromatogram (C_{18} column, eluent: CH₃OH 70% v/v + aqueous 15 mM HCOOH 30% v/v), (bottom) ESI-MS spectrum of complex ¹⁵N-1 showing both the [M+H]⁺ and [M+Na]⁺ species and simulation for the [M+H]⁺ species.



Figure S13: ¹H NMR spectrum of complex 2 in DMSO-d₆.



Figure S14. ${}^{13}C{}^{1}H$ NMR spectrum of complex 2 in DMSO-d₆.



Figure S15.¹⁹⁵Pt NMR spectrum of complex 2 in DMSO-d₆.



Figure S16. (top) RP-HPLC chromatogram (C₁₈ column, eluent: CH₃OH 80% v/v + aqueous 15 mM HCOOH 20% v/v), (bottom) ESI-MS spectrum of complex **2** showing both the [M+H]⁺ and [M+Na]⁺ species and simulation for the [M+H]⁺ species.



Figure S18. (top) RP-HPLC chromatogram (C_{18} column, eluent: CH₃OH 80% v/v + aqueous 15 mM HCOOH 20% v/v), (bottom) ESI-MS spectrum of complex ¹⁵N-2 showing both the [M+H]⁺ and [M+Na]⁺ species and simulation for the [M+H]⁺ species.



Figure S19. [¹H, ¹⁵N] HSQC spectrum of complex ¹⁵N-1



Figure S20. [¹H, ¹⁵N] HSQC spectrum for the reduction of complex ¹⁵N-1 with cell extracts



Figure S21. [¹H, ¹⁵N] HSQC spectrum of complex ¹⁵N-2



Figure S22. [¹H, ¹⁵N] HSQC spectrum for the reduction of complex ¹⁵N-2 with cell extracts



Figure S23. Colonies of A-549 and HCT 116 after the clonogenic assay. Cells were treated with equitoxic concentrations of PA (1 mM), cisplatin (5 μ M), 1

(0.5 µM), and 2 (10 nM). After 72 h, culture medium was removed and refurbished with fresh one. Cells were cultured for a further week in the absence of

drugs; after that time cells were stained with crystal violet. NT = control ("non-treated")



Figure S24. Images of A-549 spheroids. The fresh spheroids (day 0) were treated for 72 h (day 3) with equitoxic concentrations of cisplatin (**CDDP**), **PA**, **1**, and **2** (corresponding to 1× and 10× 72 h IC₅₀, respectively). After that time culture medium was removed and refurbished with fresh one. Spheroids were cultured for a total of 14 d.



Figure S25. Multicellular Tumor Spheroids (MCTS) growth inhibition. MCTS obtained from A-549 were treated with 72 h-equitoxic concentrations ($1 \times IC_{50}$) and $10 \times IC_{50}$ of **CDDP**, **PA**, **1**, and **2** from day 0 to day 3, then followed in drug-free medium (recovery). Data are mean of volume fold increase ± standard deviation

(sd) of at least 3 independent replicates.

Table S1. Genes analyzed by means of Quantitative Reverse Transcription PCR (RT-qPCR). The NCBI accession number is reported along with the 5'-3'

Gene	Accession n.	Forward	Reverse	Product length (bp)
GAPDH	NG_007073.2	ATCCCTGAGCTGAACGGGAA	GGCAGGTTTTTCTAGACGGC	99
HPRT1	NM_000194.2	TTGCTTTCCTTGGTCAGGCA	ATCCAACACTTCGTGGGGTC	85
RNA18SN1	NR_145820.1	CGTCTGCCCTATCAACTTTCG	TGCCTTCCTTGGATGTGGTAG	124
BAD	NM_004322.3	GAGACCTGTGCGCCGTCA	AGGACCTCAGTCTCCCCTCAG	74
BAX	NM_001291428.1	GACCATCTTTGTGGCGGGAG	GAGGAAAAACACAGTCCAAGGC	94
BCL-2	NM_000633.2	CTTTGAGTTCGGTGGGGTCA	GGGCCGTACAGTTCCACAAA	162
CDKN1A	NG_009364	GCGACTGTGATGCGCTAATG	GAAGGTAGAGCTTGGGCAGG	141
TP53	NG_017013.2	GCCCCTCCTCAGCATCTTATC	CTCATAGGGCACCACCACAC	99

sequence of the forward and reverse primer and the expected product length.

Note: GAPDH, HPRT1, and RNA18SN1 are the reference genes used for normalization.