The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Antiproliferative activity and inhibition of cancer cell migration by a cisplatin-based Pt(IV) bifunctional prodrug containing perillic acid

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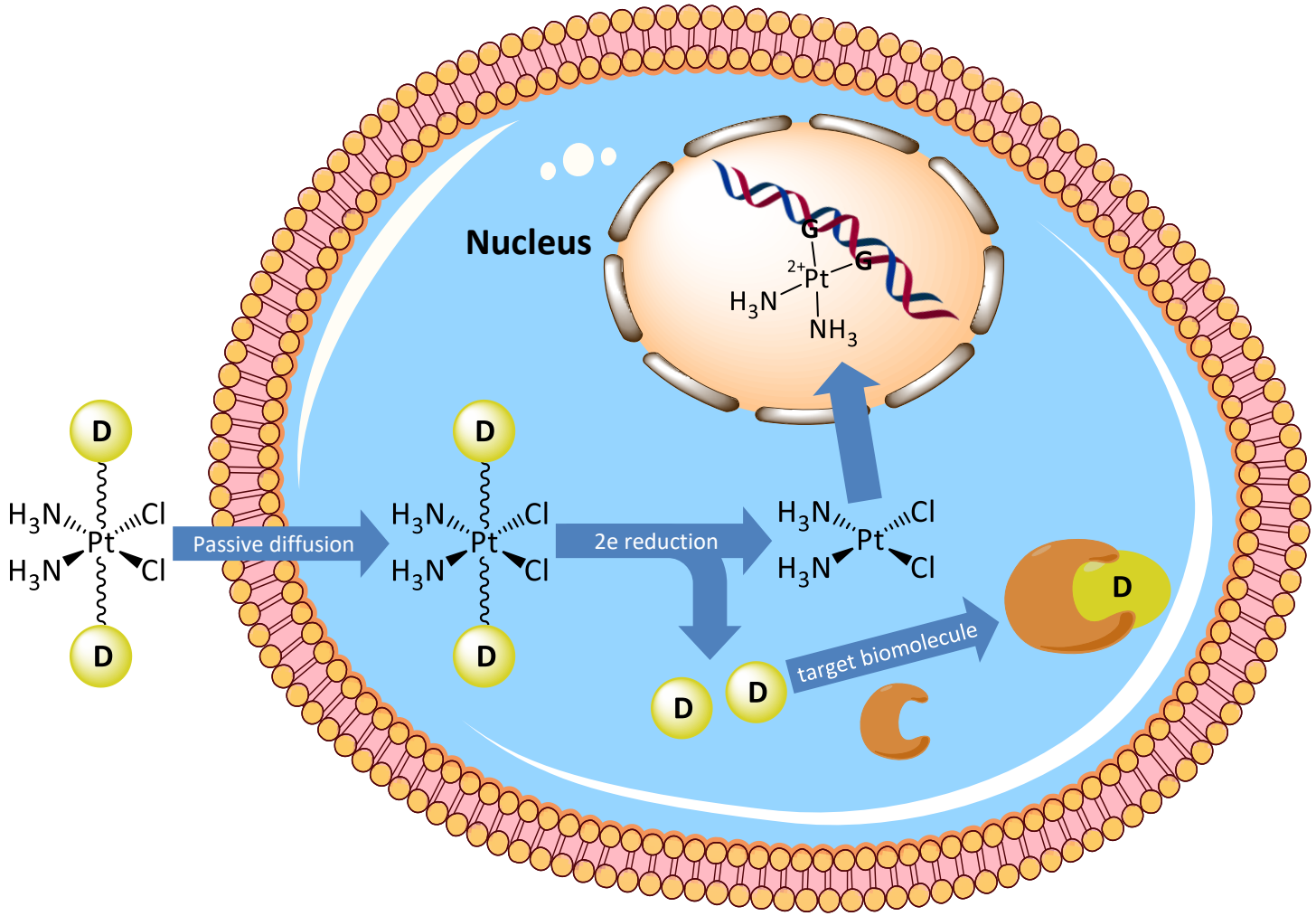
Antiproliferative activity and inhibition of cancer cell migration by a cisplatin-based Pt(IV) bifunctional prodrug containing perillic acid



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Abstract: The discovery of the anticancer properties of cisplatin in the late ’60s originated an enormous interest in the medicinal chemistry of Pt compounds. Unfortunately, like many other single-target drugs, cisplatin fails or underperforms because of redundant pathways in cancer cells, so that the inhibition of one of them has no significant effect on the global outcome. One approach to overcome this problem is the conjugation of two distinct pharmacophores together to create single molecule, dual-targeting therapeutics.

Octahedral Pt(IV) complexes are an attractive platform to develop such a class of molecules. The rationale behind their development was that the nontoxic Pt(IV) prodrugs, upon entry into the reducing environment of the tumor tissue, would be activated by a two-electron reduction, to form a Pt(II) active metabolite with concomitant loss of the two axial ligands. If such positions are occupied by a second anticancer agent/pharmacophore, the complex will release two kinds of molecules: the Pt(II) moiety will enter the nucleus where it will react with DNA inducing apoptosis, whereas the axial ligands will interact with their own target. This approach allows the bifunctional molecule to aim at multiple targets simultaneously and often synergistically, achieving a greater therapeutic advantage over single-targeting agents.

In this framework, two Pt(IV) complexes containing perillic acid were synthesized and tested in vitro on several human tumor cell lines. In particular, the complex $[PtCl_2(NH_3)_2(perillato)_2]$ exhibited excellent antiproliferative and anti-migration (antimetastatic) activity on A-549 lung tumor cells at nanomolar concentrations.

Keywords: Antimetastatic activity; Antiproliferative activity; Bifunctional drugs; Pt(IV) prodrugs

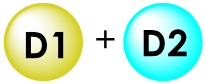
Introduction

*"One ring to rule them all"** 

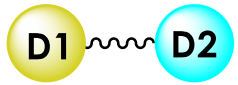
Most of the current drug discovery is centered on **single-agent / single-target therapeutics**.

For multifactorial diseases (such as cancer), **single target drugs alone are often unable to provide effective treatment** and can be more susceptible to drug resistance.

Alternative #1: the simultaneous administration of two or more drugs (**combination therapy**).



Alternative #2: the conjugation of two distinct pharmacophores together to create **single-molecule / dual-targeting therapeutics** = **bifunctional drugs**.



(More) Predictable pharmacokinetic profile, reduced patient compliance difficulties, reduced drug-drug interactions (if any), etc.

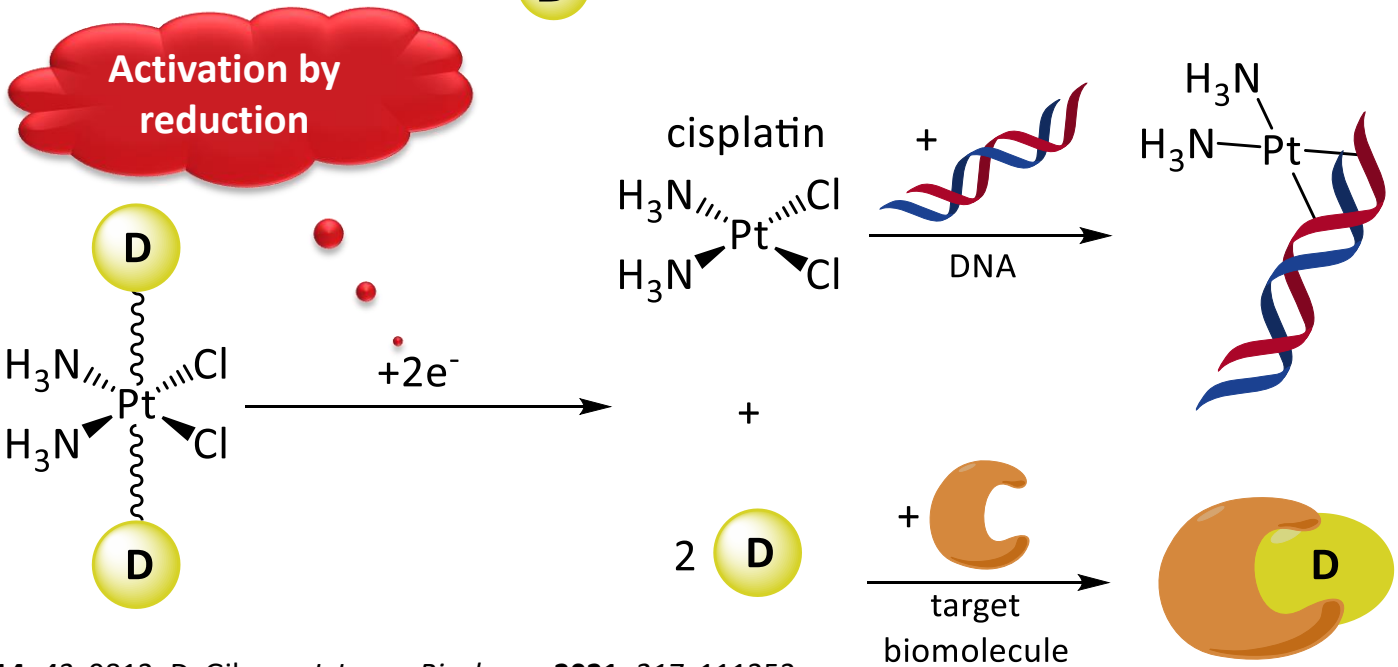
The drug must retain effective binding to the two targets while also maintaining desirable physiochemical and pharmacokinetic properties.

**The One Ring, is a central plot element in J. R. R. Tolkien's The Lord of the Rings (1954–55).*

Introduction

Bifunctional Pt(IV) complexes

- ✓ Pt(IV) complexes are **prodrugs** that can be selectively reduced *in vivo* under the hypoxic conditions of the tumor tissue.
- ✓ After **activation by reduction**, a bifunctional Pt(IV) complex may release simultaneously **the DNA damaging Pt(II) metabolite AND the second drug** D that should operate in a synergistic or (at least) an additive mode:

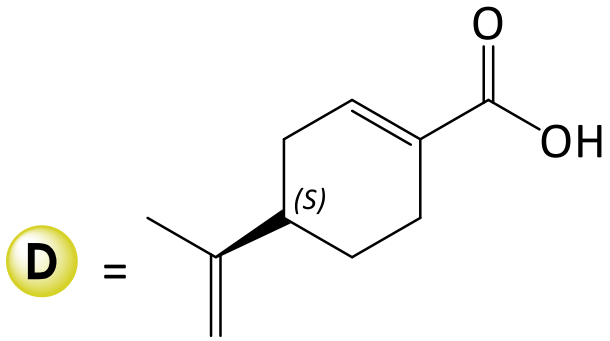


E. Gabano *et al.*, *Dalton Trans.*, **2014**, *43*, 9813; D. Gibson, *J. Inorg. Biochem.*, **2021**, *217*, 111353.

Introduction

PA-containing Pt(IV) complexes

- ✓ Limonene is quickly metabolized in humans to produce more soluble metabolites like perillic acid (4-isopropenylcyclohexene-1-carboxylic acid, **PA**).
- ✓ **PA** increases apoptosis in A-549 and H520 lung cancer cells, induce the cleavage of PARP [poly(ADP-ribose)polymerase], etc.
- ✓ Combination studies revealed that exposure of the cells to **PA** sensitizes them to cisplatin (**CDDP**), increasing sensibly the potency of the Pt drug.
- ✓ *In vivo* models showed that **PA** exhibits antimetastatic activity, modulates the immune system, etc.

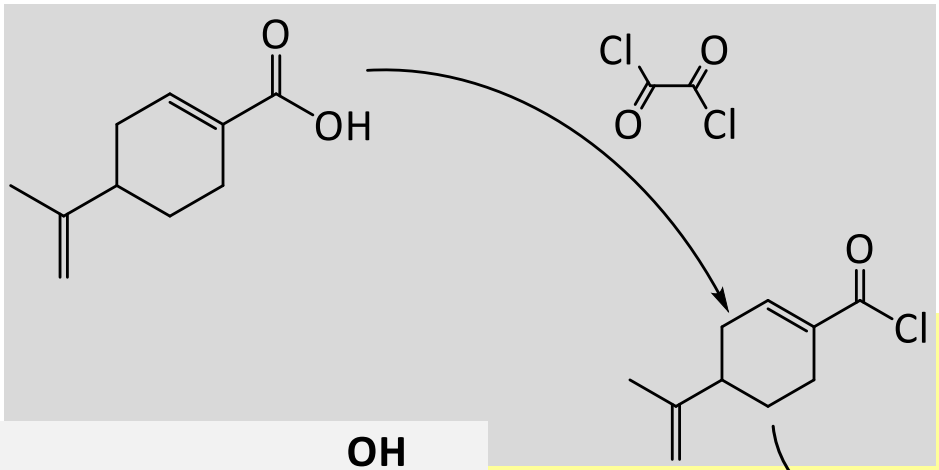


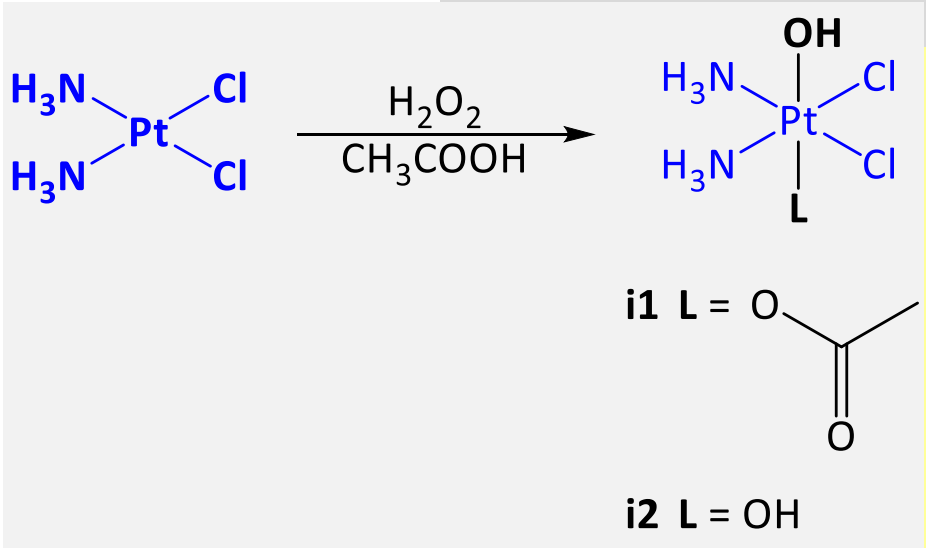
Perillic acid (**PA**)
(4-isopropenylcyclohexene-1-carboxylic acid)

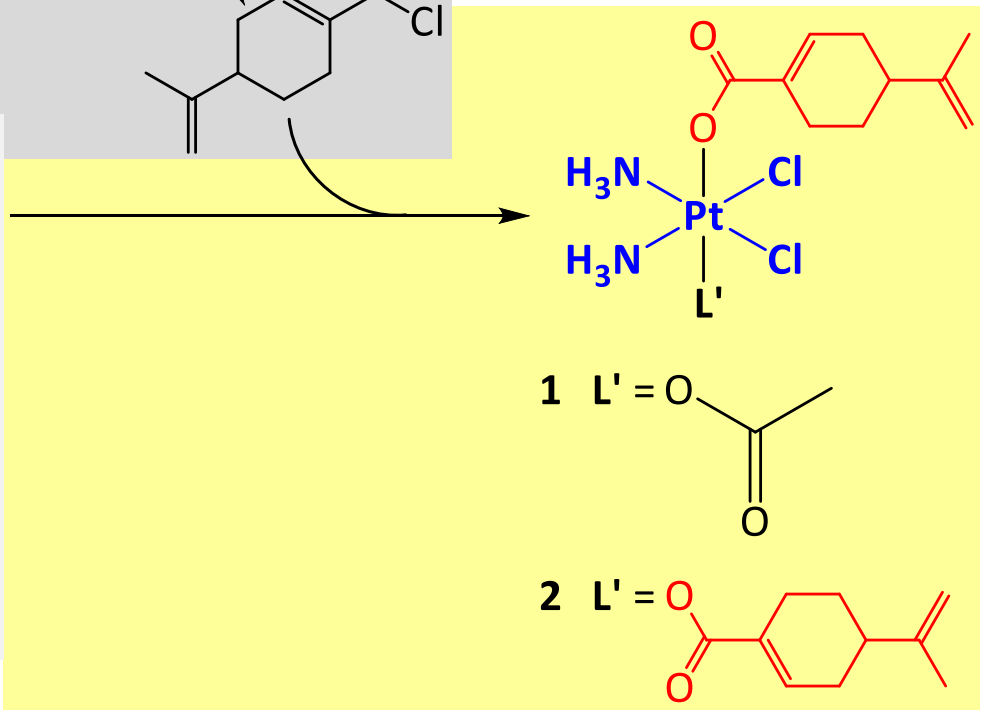
See, for instance: G. Kuttan *et al.*, *Pharm. Biol.*, **2011**, *49*, 995; L. Yeruva *et al.*, *Cancer Letters*, **2007**, *257*, 216.

Results and discussion

The synthesis







M. Ravera *et al.*, *Dalton Trans*. **2021**, *50*, 3161

Results and discussion

Cytotoxicity (Half-maximal inhibitory concentrations (IC_{50}, μM)

	A2780 ovarian	**NT2/D1** embryonal	**A-549** Lung	**HCT 116** colon	**HT-29** colon	**MCF-7** breast	**BR95** MPM	**MG06** MPM	**MM98** MPM
CDDP	0.460 ± 0.110	0.106 ± 0.039	3.60 ± 0.90	2.30 ± 0.30	2.83 ± 0.33	6.50 ± 0.90	6.20 ± 0.90	4.10 ± 1.50	3.20 ± 1.20
PA	639 ± 140	1265 ± 318	1043 ± 496	1346 ± 469	1770 ± 398	2660 ± 523	974 ± 412	919 ± 355	3210 ± 879
1	0.056 ± 0.014	0.063 ± 0.034	0.575 ± 0.147	0.304 ± 0.129	1.06 ± 0.36	0.810 ± 0.301	1.73 ± 0.52	2.34 ± 0.91	0.501 ± 0.103
2	0.0022 ± 0.0004	0.0037 ± 0.0011	0.0081 ± 0.0036	0.011 ± 0.003	0.010 ± 0.002	0.041 ± 0.004	0.150 ± 0.051	0.094 ± 0.018	0.028 ± 0.005

The data were obtained after 72 h of treatment. MPM = malignant pleural mesothelioma. Data are mean ± standard deviation (sd) of at least three independent replicates, performed in triplicate.

Results and discussion

Cytotoxicity (Half-maximal inhibitory concentrations (IC_{50}, μM)

	MM98R cisplatin-resistant subline	Resistance Factor, RF	**HMC** human mesothelial cell	Selectivity Index, SI
CDDP	19.4 ± 1.2	6.1	6.7 ± 1.3	1.5
PA	2520 ± 654	0.8	2850 ± 380	1.7
1	0.304 ± 0.101	0.6	4.42 ± 0.73	2.9
2	0.031 ± 0.013	1.1	0.290 ± 0.092	3.2

1 and **2** were:

- more active than **CDDP** and **PA**
- able to bypass **CDDP** resistance (*cf.* RF)
- but poorly selective (*cf.* SI)

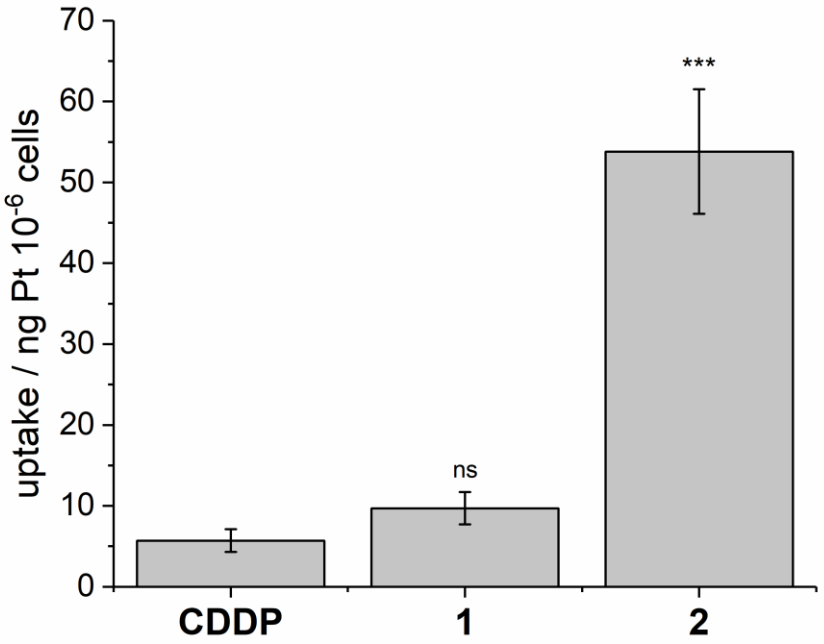
The data were obtained after 72 h of treatment. MPM = malignant pleural mesothelioma. Data are mean ± standard deviation (sd) of at least three independent replicates, performed in triplicate.
Resistance Factor RF = IC_{50} (MM98R) / IC_{50} (MM98); Selectivity Index SI = IC_{50} (HMC) / mean IC_{50} (BR95, MG06, MM98).

Results and discussion

Other biological features of 2 (A-549 lung cancer cells)

1

2 enters cells via passive diffusion more efficiently than **CDDP** and **PA** (***synergistic accumulation***) (*cf.* intracellular Pt quantification by ICP-MS).



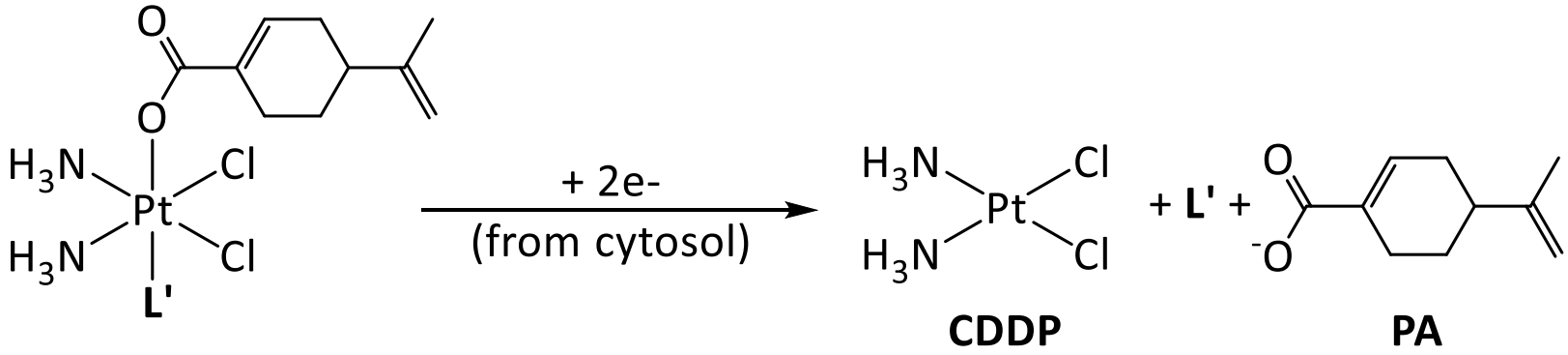
Cell uptake *of* ***CDDP***, ***1***, *and* ***2*** *in A-549 cells treated for 4 h CT at 10 μM concentrations. Statistical analysis:* ***CDDP*** *vs.* ***1*** *or* ***2*** *ns = not significant, (***) p < 0.001.*

Results and discussion

Other biological features of 2 (A-549 lung cancer cells)

2

Once in the cells, **2** is reduced 100% in 2 h supporting the ***activation by reduction*** mechanism of action (*cf.* [^{1}H, ^{15}N] HSQC experiments on ^{15}N-**2** challenged with cell cytosol)

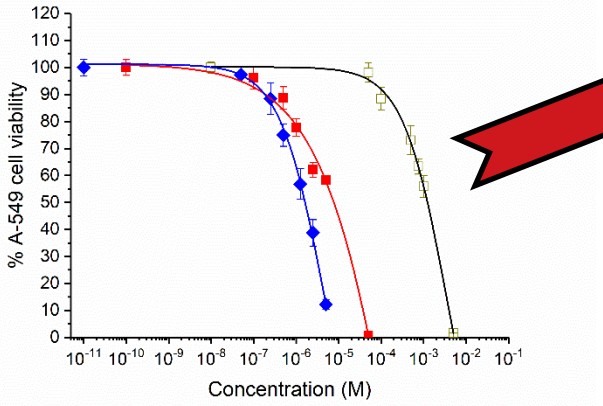


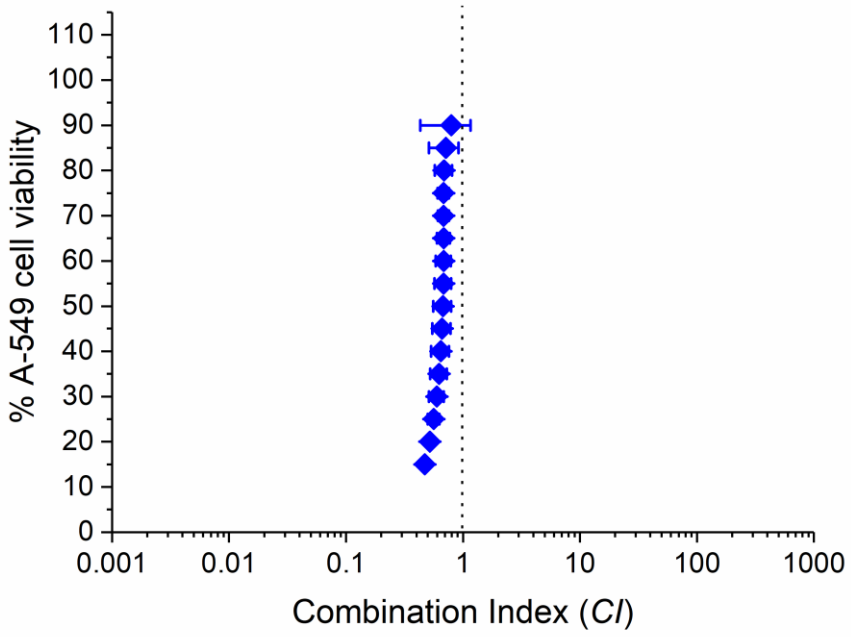
Results and discussion

Other biological features of 2 (A-549 lung cancer cells)

3

CDDP and **PA** have a **moderate synergistic action**; so, the metabolites released from **2** are expected to increase each other's effectiveness (*cf.* combination index, CI).



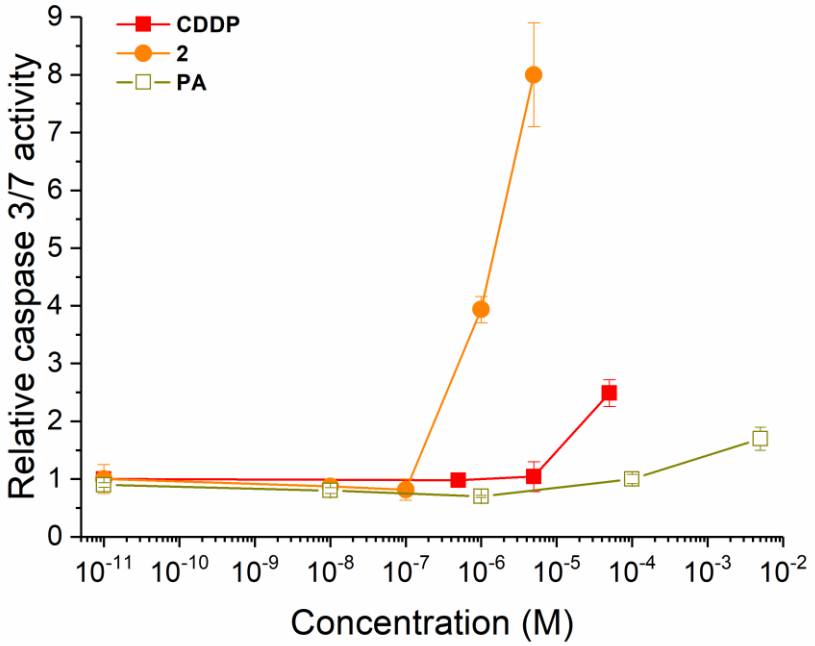


Combination index (CI) plot *of the mixture* ***CDDP-PA****. Residual viability data were compared to those obtained for* ***CDDP*** *and* ***PA*** *to obtain the CI value (CI < 1: synergism; CI ≅ 1 additive effect; CI > 1 antagonism).*

Results and discussion

Other biological features of 2 (A-549 lung cancer cells)

4 **Higher induction of apoptosis for 2 *vs.* CDDP and PA** (*cf.* activity of caspase 3/7 by fluorometric assay; loss of mitochondrial transmembrane potential by JC-1 staining method; ROS generation by DCF assay).

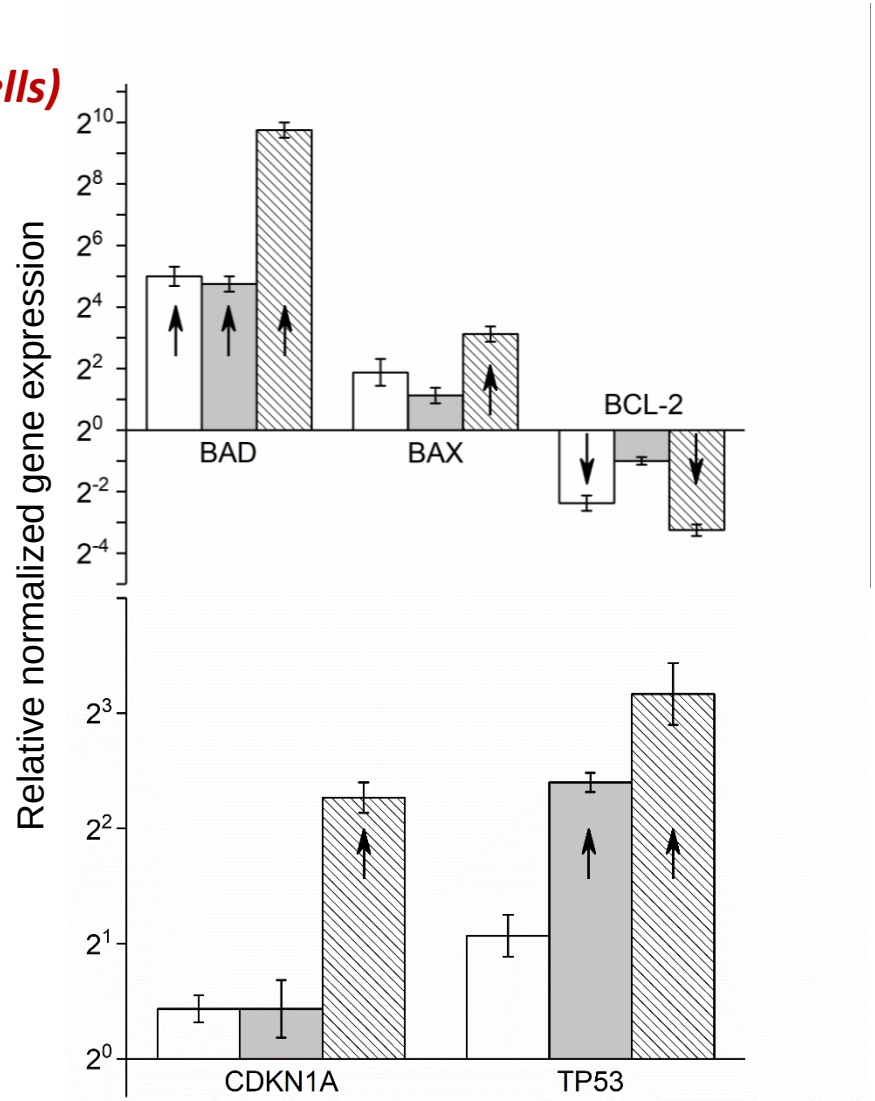


Caspase 3/7 activity assay. *A-549 cells were treated with increasing concentrations of* ***CDDP***, ***PA***, *and* ***2***. *Data were normalized over the untreated control and reported as fold activity.*

Results and discussion

Other biological features of 2 (A-549 lung cancer cells)

5 ***2 upregulates*** BAD, BAX, CDKN1A, and TP53 genes, ***whereas down-regulates*** BCL-2 better than **CDDP** and **PA** (*cf.* Quantitative Reverse Transcription PCR).

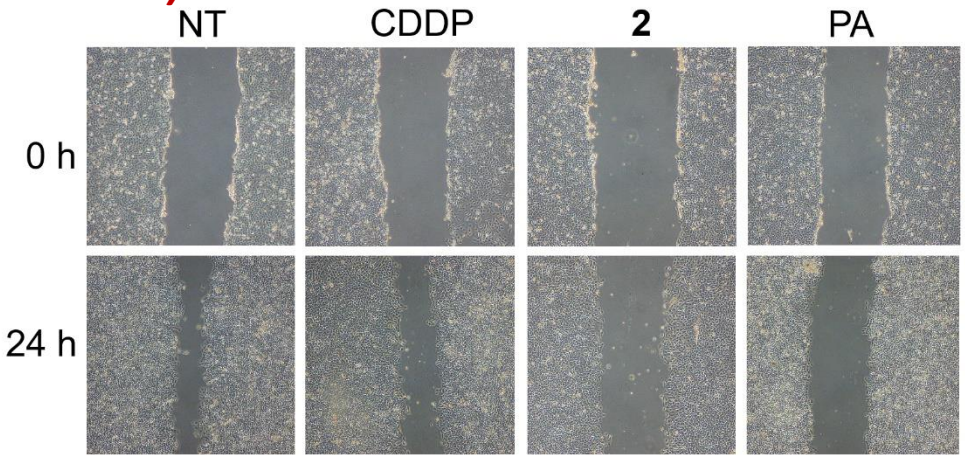


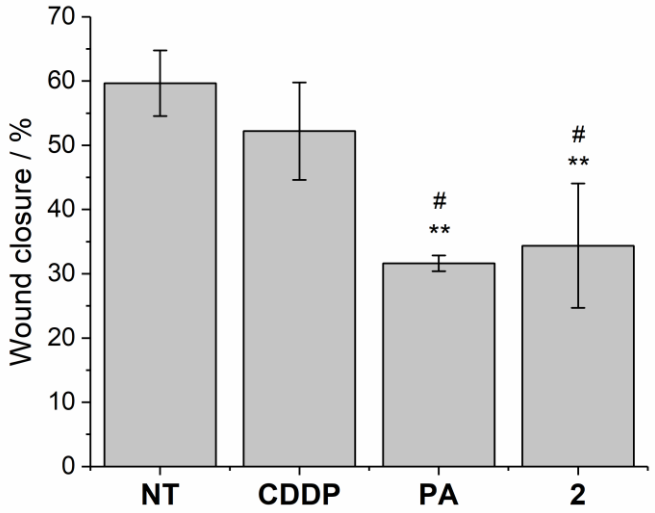
*Relative gene expression of BAD (pro-apoptotic), BAX (pro-apoptotic), BCL-2 (anti-apoptotic), CDKN1A (inhibitor of cell proliferation) and TP53 (tumor suppressing) genes in A-549 cells following 24 h treatments with equitoxic concentrations of **CDDP** (white bars), **PA** (grey bars), and **2** (patterned bars) by RT-qPCR experiments. The arrows indicate the upregulated (↑) or downregulated (↓) gene expression with respect to internal threshold value.*

Results and discussion

Other biological features of 2 (A-549 lung cancer cells)

6 The treatment with **2** induces a better ***antimigration effect*** *vs.* **CDDP**, confirming the antimetastatic propensity of the **PA** component (*cf.* wound healing assay, transwell assay and immunohistochemical tests)



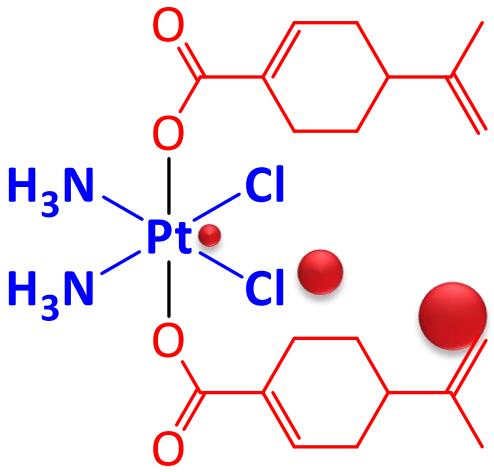


(Top) ***Images of scratches*** *obtained with A-549 cells at time point 0 h and after 24 h of treatment with* ***CDDP***, ***2***, *and* ***PA*** *at their equitoxic concentrations. (right)* ***The histogram shows the data of the average percentage of wound closure*** *obtained from three measurements inside the slide. Statistical analysis: NT vs. treated samples (**) $p < 0.01$; **CDDP** vs. **2** or **PA** (#) $p < 0.05$.*

Conclusions

1. a moderate synergistic antiproliferative activity between **CDDP** and **PA** has been demonstrated.
2. the presence of **PA** adds an additional feature on **2** at the level of cell migration.
3. after activation by reduction, the released:

CDDP exerts mainly the cytotoxic effect



PA is responsible for the antimetastatic activity

In conclusion, **2** may be considered a true bifunctional molecule

4. synergistic accumulation is behind the good performance of **2**

Acknowledgments

UNIVERSITÀ DEL PIEMONTE ORIENTALE

Elisabetta Gabano

Domenico Osella

Elena Perin

Beatrice Rangone

Ilaria Zanellato

UNIVERSITÀ DI PAVIA

Maria Grazia Bottone

Beatrice Ferrari

Funding

- **Università del Piemonte Orientale**
- **HERMES project** (*Progetto finanziato attraverso l'offerta di indennizzo ai residenti di Casale Monferrato deceduti o affetti da mesotelioma*)