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Dual- and multi-action Pt(IV) antitumor prodrugs or *how to kill two birds with one stone*

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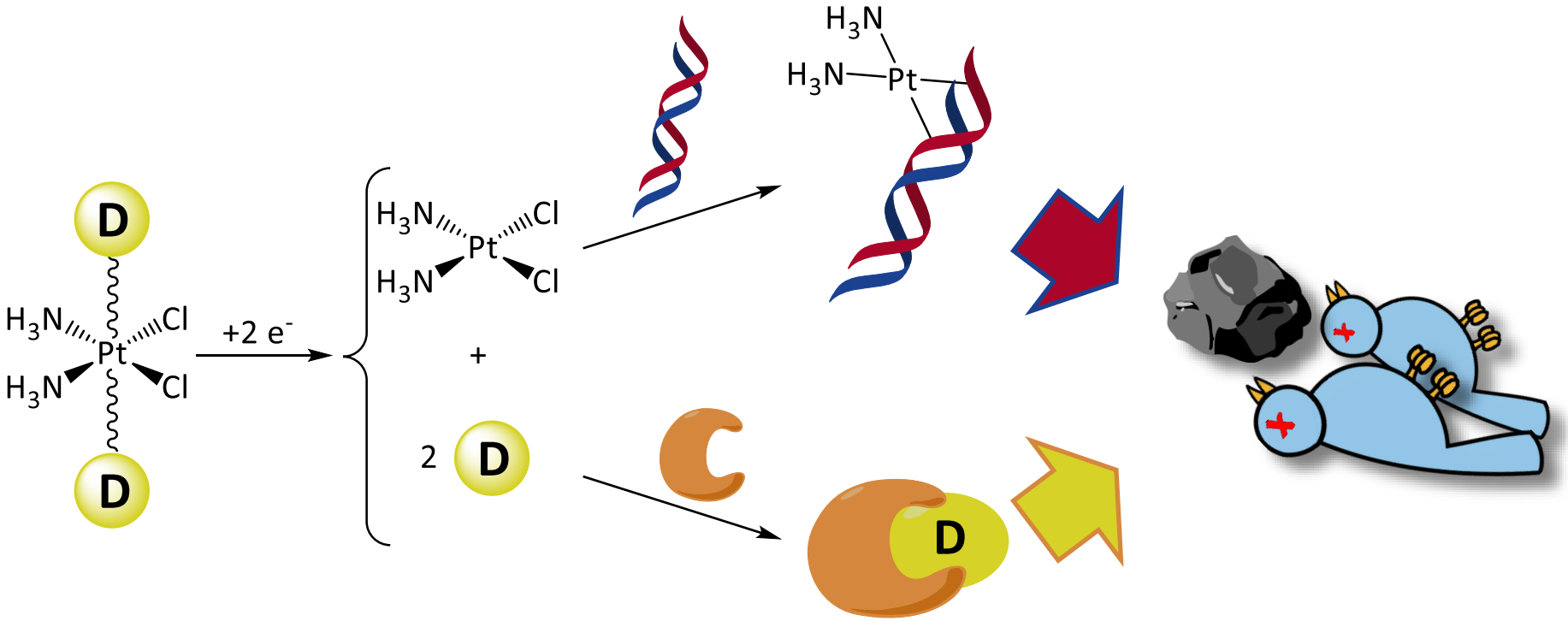
UNIVERSITÀ DEL PIEMONTE ORIENTALE

Dual- and multi-action Pt(IV) antitumor prodrugs or *how to kill two birds with one stone*

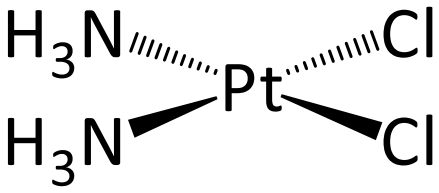


Abstract:
Although targeted- and immuno- therapies are nowadays considered among the most promising tools in fighting cancer, combination protocols including new biologically-smart agents and old cytotoxic drugs (the Pt(II)-based ones, in particular) result to be useful, especially against very aggressive tumors.
Octahedral Pt(IV) derivatives are intensively studied as *prodrugs*, being deprived of most of the heavy side effects of cisplatin and suitable to be administered orally. These complexes reach tumor cells in their intact form and then are reduced (ideally only) in the hypoxic intracellular *milieu* to cytotoxic cisplatin, with the simultaneous loss of the two remaining ligands from the axial position (*activation by reduction*). The well-established Pt(IV) chemistry permits to design dual-action drug candidates that can act as *single-molecule combination-therapy*, often called *combo*. Indeed, two adjuvant/synergistic drugs (generally in form of carboxylates) are conjugated to the Pt(IV) core (frequently obtained by cisplatin oxidation) in axial positions. These Pt(IV) derivatives are much more lipophilic than their progenitors (*i.e.*, the hydrophilic cisplatin and the amphiphilic carboxylate anions). Their assembly permits a more efficient cellular uptake (via passive diffusion) than that of the separate components (*synergistic cellular accumulation*), increasing the intracellular concentration of the two drugs.
Nowadays, dozens of dual- or even multi-action antitumor Pt(IV) prodrugs (mitochondria-targeted complexes, glutathione-S-transferase-targeted complexes, cyclooxygenases inhibitors, histone deacetylase inhibitors, etc.) have been designed, synthesized and tested *in vitro* and *in vivo*, and a number of them moved into clinical trials. In this presentation some prototypal Pt(IV) prodrugs will be shown as case studies.

Keywords: anticancer activity; cisplatin; metal-based drugs; prodrugs; Pt complexes.

Introduction

***cis*-diamminedichloridoplatinum(II),**
cisplatin, CDDP



- ✓ **FDA Approval**: Dec 19, 1978.
- ✓ **Awards**: *WHO Model List of Essential Medicines* since 1991.[1,2]
- ✓ **Indications**: cervical, head and neck, nasopharyngeal, non-small cell lung cancers, osteosarcoma, ovarian and testicular germ cells tumors. [2]
- ✓ **(Main) Final target**: DNA
- ✓ **Possible side effects**: nausea, vomiting, diarrhea, hair loss, hiccups, nephrotoxicity, ototoxicity, neuropathy and myelosuppression, etc.
- ✓ **Resistance**: reduced drug uptake and/or increased drug efflux; improved repair or tolerance of DNA–cisplatin adducts.
- ✓ **Alternative metal-based compounds**: different DNA-binding modes or different cellular targets, complexes accumulated or activated only in the tumor tissue, etc.

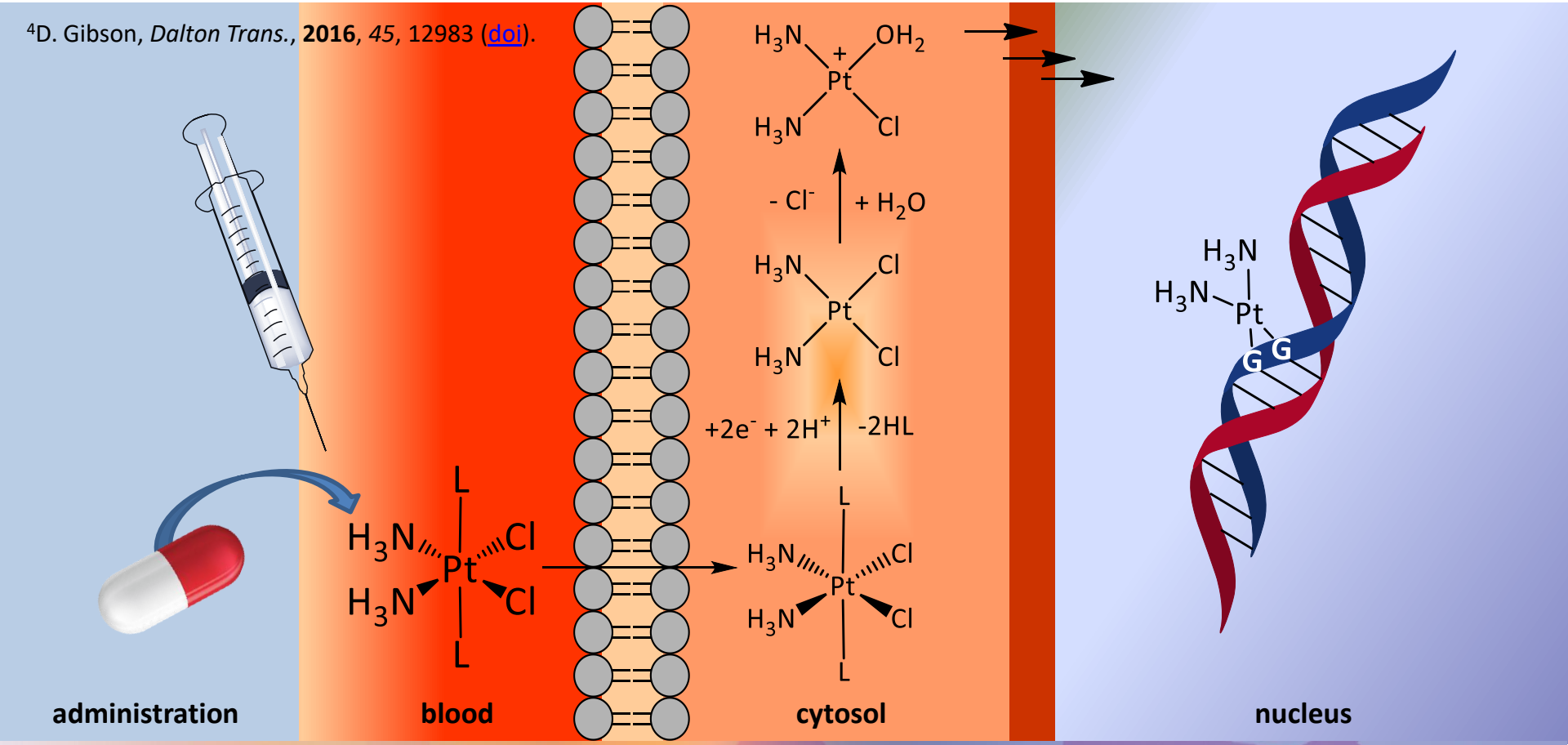
[1]K. Sikora *et al.*, *Ann. Oncol.*, **1999**, *10*, 385 (doi); [2]*WHO Model List of Essential Medicines*, 21st ed., June **2019**. http://www.who.int/medicines/publications/essentialmedicines/; [3]T.C. Johnstone *et al.*, *Chem. Rev.*, **2016**, *116*, 3436 (doi).

Introduction

Why Pt(IV) complexes?

- ✓ **kinetic inertness**: limited ligand substitution, fewer side effects, administration *per os*.
- ✓ **redox properties**: *in vivo* reduction under the hypoxic conditions of the tumor site.[4]

[4]D. Gibson, *Dalton Trans.*, **2016**, *45*, 12983 (doi).



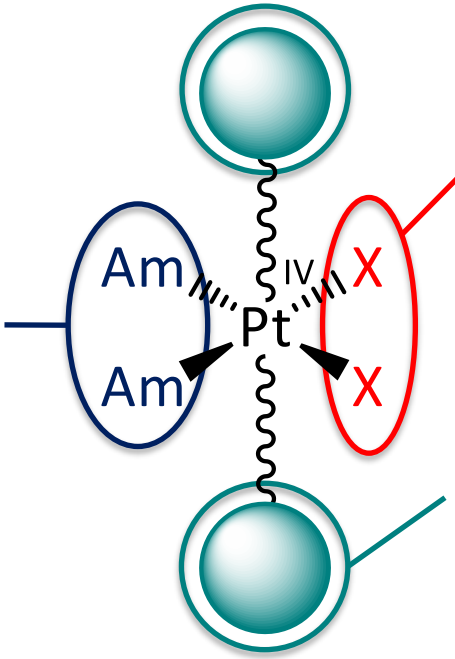
Introduction

Why Pt(IV) complexes?

- ✓ **kinetic inertness**: limited ligand substitution, fewer side effects, administration *per os*.
- ✓ **redox properties**: *in vivo* reduction under the hypoxic conditions of the tumor site.[4]
- ✓ **modular platform**:

Carrier ligands:

- Nature of Pt-DNA adduct
- Selectivity
- Resistance profile



Leaving ligands:

- Activation kinetics
- Systemic toxicity (off-target reactions)

Axial ligands:

- Lipophilicity / solubility
- Reduction (thermodynamic / kinetics)
- Vectors for drug targeting and delivery
- **Second drug**

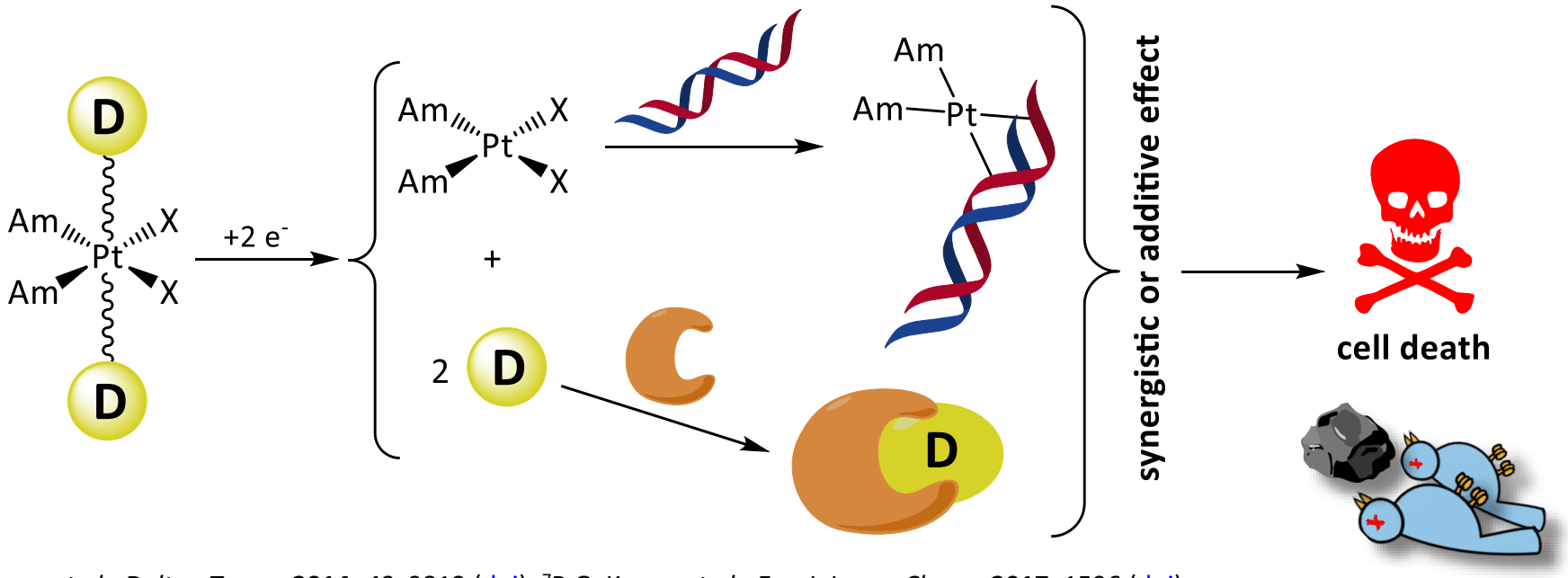
Am = am(m)ine/s; X = carboxylatos, halidos, etc.[5]

[4]D. Gibson, *Dalton Trans.*, **2016**, *45*, 12983 (doi); [5]Figure adapted from: J.J. Wilson, S.J. Lippard, *Chem. Rev.*, **2014**, *114*, 4470 (doi).

Introduction

Why bifunctional or dual-action compound?

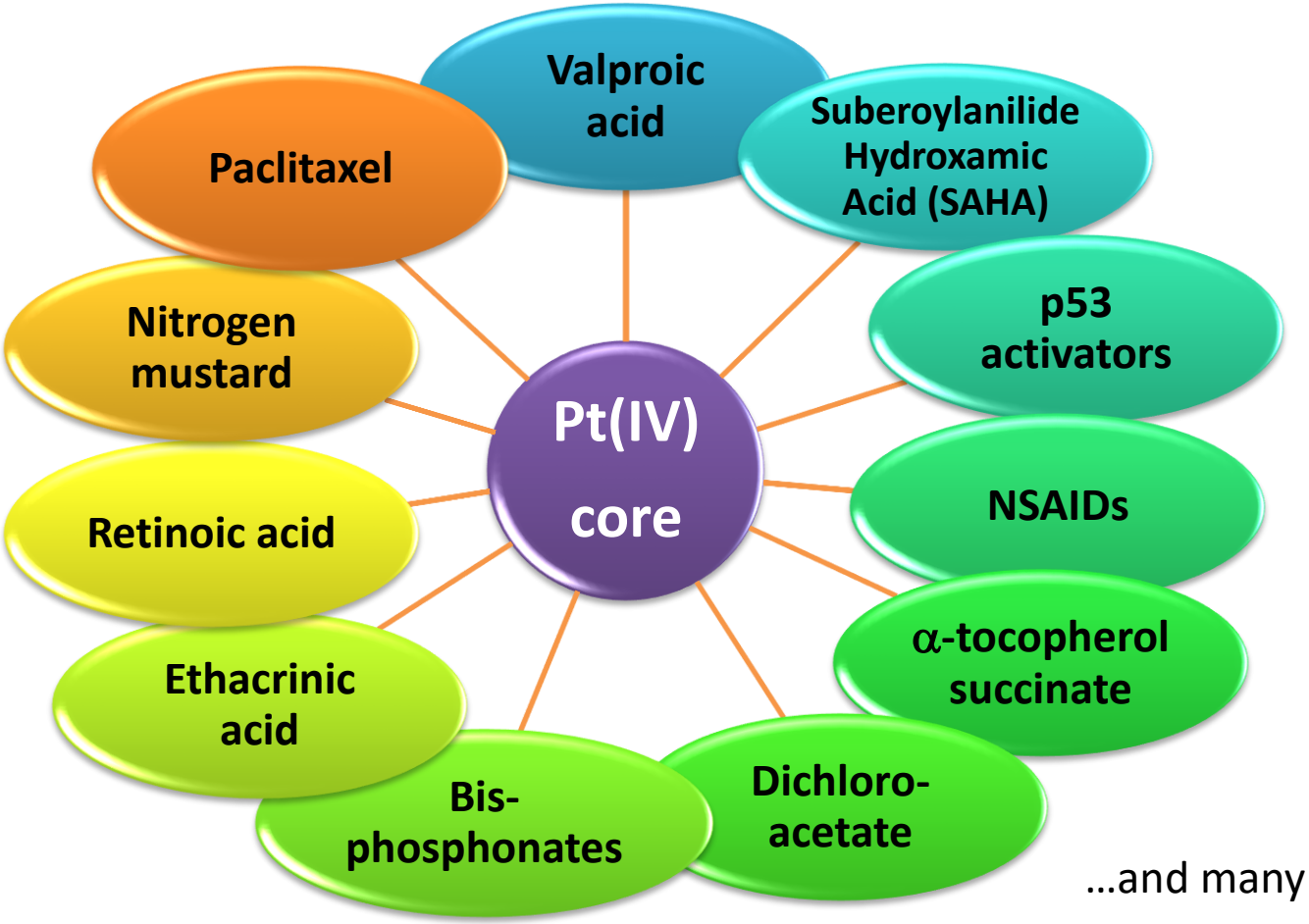
- ✓ Treatment of cancer by attacking one pathway at a time and disregarding its complexity and redundancy is destined to fail (**combination therapy**).
- ✓ A drug (D) with a complementary mode of action can be coordinated to the Pt(IV) core, so that the final conjugate can operate on multiple targets with greater potency than a single-target drug (a sort of **intramolecular combination therapy**).[6,7]



[6]E. Gabano *et al.*, *Dalton Trans.*, **2014**, *43*, 9813 (doi); [7]R.G. Kenny *et al.*, *Eur. J. Inorg. Chem.*, **2017**, 1596 (doi)

Introduction

Which bifunctional or dual-action compound?



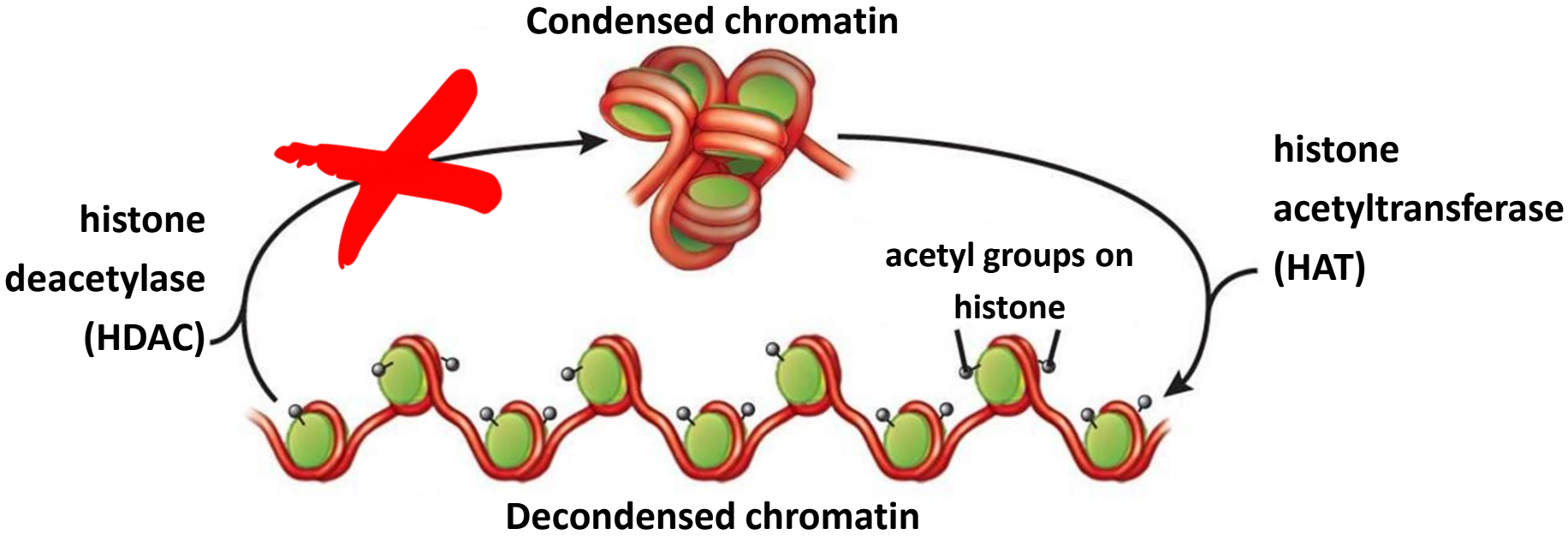
…and many others…

[6]E. Gabano *et al.*, *Dalton Trans.*, **2014**, *43*, 9813 (doi); [7]R.G. Kenny *et al.*, *Eur. J. Inorg. Chem.*, **2017**, 1596 (doi)

Results and discussion

Inhibition of histone deacetylase

Histones are a family of positively charged proteins that are bound to the negatively charged DNA. The *N*-ε-Lys acetylation and deacetylation of histone are controlled by two groups of enzymes: **HAT** and **HDAC**.



Inhibitors of HDAC (HDACi) decrease expression of chromatin maintenance proteins. The chromatin's morphology transforms into a more chemo-sensitive conformation.

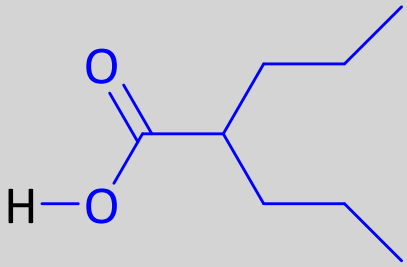
Results and discussion

The target

HDAC

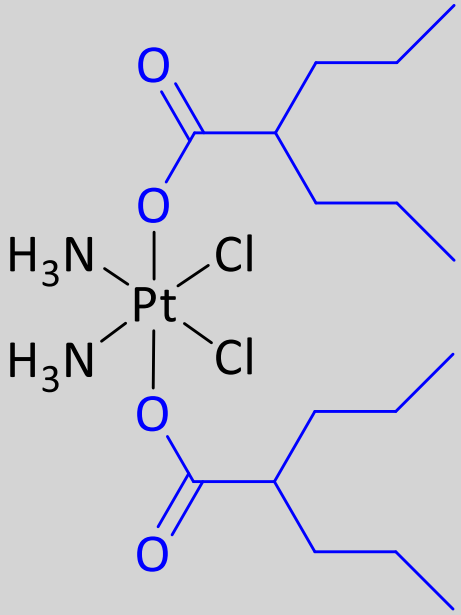
The "*bullet*"

Medium chain fatty acids are a class of HDAC inhibitors.



Valproic acid,
VPA

The conjugate



Pt-(VPA)$_2$

J. Yang *et al.*, *Mol. Pharm.*, **2012**, *9*, 2793; M. Ravera *et al.*, *J. Inorg. Biochem.*, **2013**, *129*, 52; V. Novohradsky *et al.*, *J. Inorg. Biochem.*, **2014**, *140*, 72 and *Biochem. Pharmacol.*, **2015**, *95*, 133; R. Raveendran *et al.*, *Chem. Sci.*, **2016**, *7*, 2381; M. Ravera *et al.*, *Dalton Trans.*, **2017**, *46*, 1559; V. Novohradsky *et al.*, *Sci. Rep.*, **2017**, *7*, 3751.

Results and discussion

The results

Pt-$(VPA)_2$ shows a much higher antiproliferative activity than CDDP and VPA in a variety of cancer cell lines.

Cell lines	Pt-$(VPA)_2$ IC_{50} [nM]	CDDP IC_{50} [μM]	VPA IC_{50} [mM]
A2780	11.1±0.3	0.5±0.1	1.8±0.7
HCT116	77.1±1.3	2.3±0.3	1.3±0.2
A549	158±35	3.8±0.7	0.8±0.5
MCF-7	155±49	6.5±0.9	4.8±1.1
MM98	273±42	3.2±1.0	3.9±0.9
MM98R	245±53	19.4±2.8	3.5±0.7

Comparison of IC_{50} values. *Key: ovarian A2780, colon HCT116, lung A549, breast MCF7 carcinomas, malignant pleural mesothelioma MM98 and CDDP-resistant subline MM98R.*

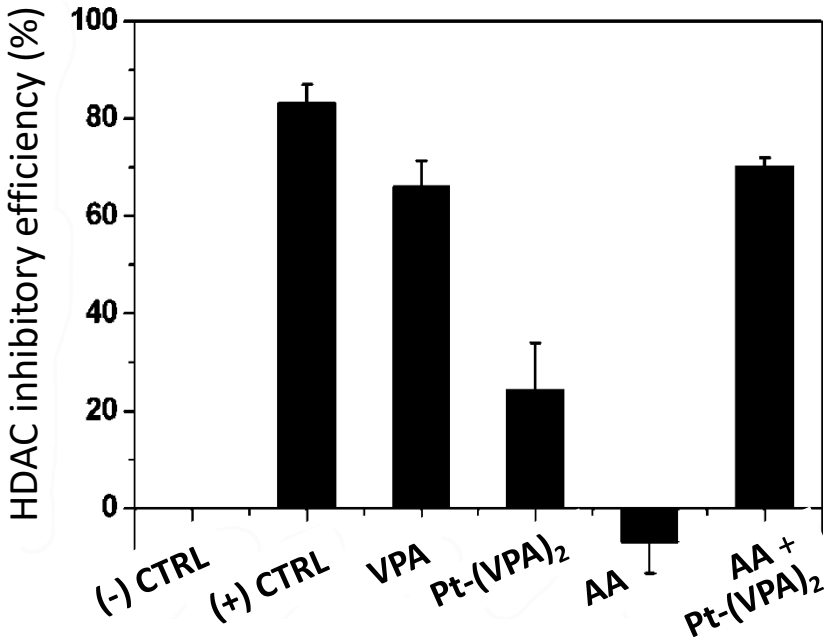
M. Ravera *et al.*, *J. Inorg. Biochem.*, **2013**, *129*, 52 (doi)

Results and discussion

The results

Two explanations have been proposed

1. **synergism between CDDP and VPA metabolites**



HDAC inhibitory activities *of the nuclear extract of HeLa cells treated with* ***5 mM*** *VPA-equivalent dose of* ***VPA***, ***Pt-(VPA)$_2$***, *or* ***Pt-(VPA)$_2$*** *pretreated with 5 mM ascorbic acid,* ***AA***, *at 37 °C for 10 h. In the case of* ***Pt-(VPA)$_2$***, *5 mM is ~25,000×IC$_{50}$, for* ***VPA*** *is ~IC$_{50}$.*

J. Yang *et al.*, *Mol. Pharm.*, **2012**, *9*, 2793 (doi)

Results and discussion

The results

Two explanations have been proposed

2. high lipophilicity

Cell lines	VPA IC_{50} [mM]	CDDP IC_{50} [μM]	Pt-$(VPA)_2$ IC_{50} [nM]	CDDP AR	Pt-$(VPA)_2$ AR
A2780	1.8±0.7	0.5±0.1	11.1±0.3	1.5±0.5	18.6±5.4
HCT116	1.3±0.2	2.3±0.3	77.1±1.3	2.0±1.2	15.4±2.3

***Accumulation Ratio, AR**, is the ratio between intra- and extra-cellular [Pt] after 4 h treatment with 10 μM Pt complexes.*

M. Ravera *et al.*, *J. Inorg. Biochem.*, **2013**, *129*, 52 (doi)

Results and discussion

The results

It has been demonstrated that free **VPA** hardly enter cells, but, as a result of the high lipophilicity of **Pt-$(VPA)_2$**, there was an enhanced uptake of Pt that obviously parallels the enhanced uptake of **VPA** (***synergistic accumulation***).

Compounds	moles Pt per 10^6 cells	moles VPA per 10^6 cells
Pt-$(VPA)_2$	2.2×10^{-11}	4.4×10^{-11}
VPA	-	6.2×10^{-15}

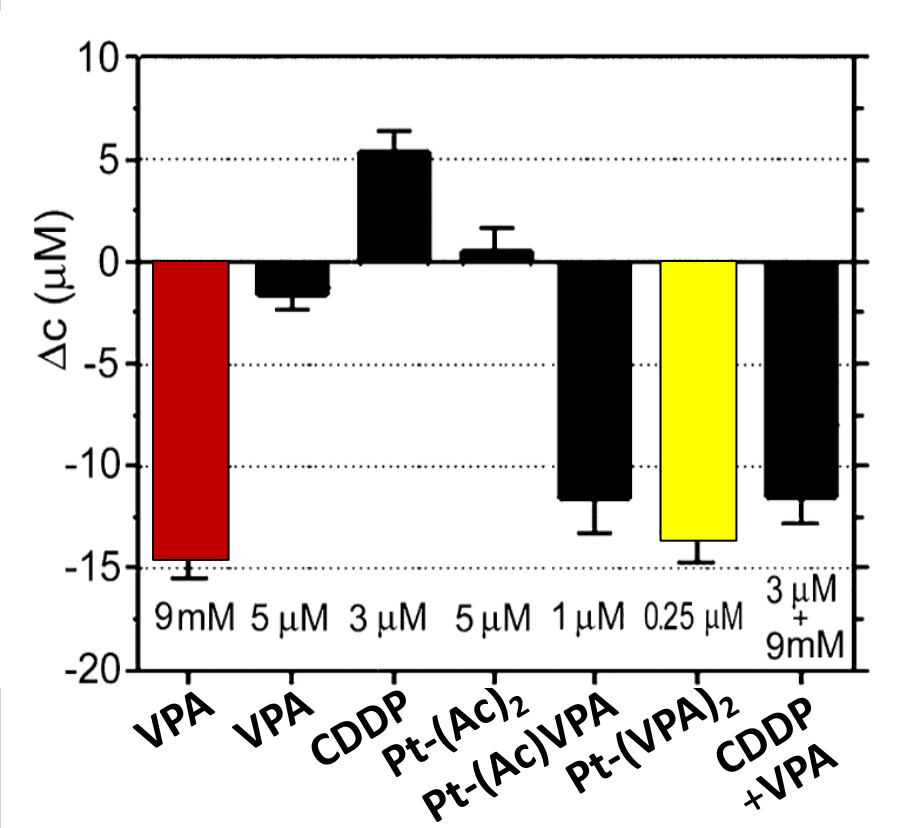
Uptake of the compounds *(0.35 μM) into ovarian A2780 cells after 24 h of exposure.*

V. Novohradsky *et al.*, *Biochem. Pharmacol.*, **2015**, *95*, 133 (doi)

Results and discussion

The results

What about HDAC inhibition?



Effect of several compounds on HDAC activity in A2780 cells. *The cells were treated for 24 h with the equitoxic concentrations of the agents corresponding to their IC_{50} values (with **VPA** also at the 5 μM concentration). The Δc values were computed by subtracting the concentration of deacetylated product in the control (untreated) cells, from the concentration of deacetylated product in the treated cells.*

V. Novohradsky *et al.*, *Biochem. Pharmacol.*, **2015**, *95*, 133 (doi)

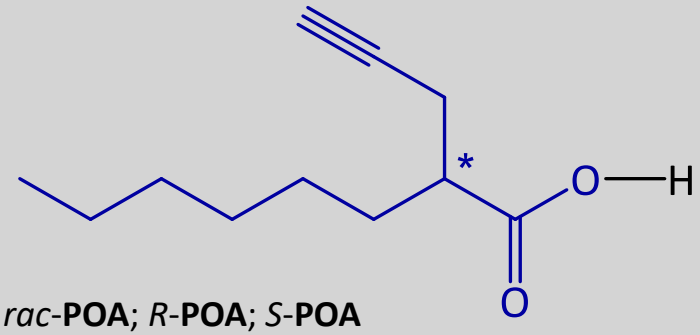
Results and discussion

The target

HDAC

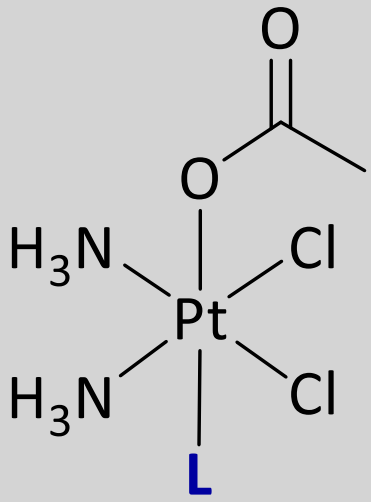
The "*bullet*"

2-(2-propynyl)octanoic acid, **POA**.



rac-**POA**; *R*-**POA**; *S*-**POA**

The conjugate



Pt-POA
L = *rac*-POA$^-$

Pt-*R*-POA
L = *R*-POA$^-$

Pt-*S*-POA
L = *S*-POA$^-$

E. Gabano *et al.*, *Dalton Trans.*, **2017**, *46*, 14174 (doi)

Results and discussion

E. Gabano *et al.*, *Dalton Trans.*, **2017**, *46*, 14174 (doi)

The results (*in vitro*)

Also in this case the conjugates are better than the "fragments" **CDDP** and **POA**.

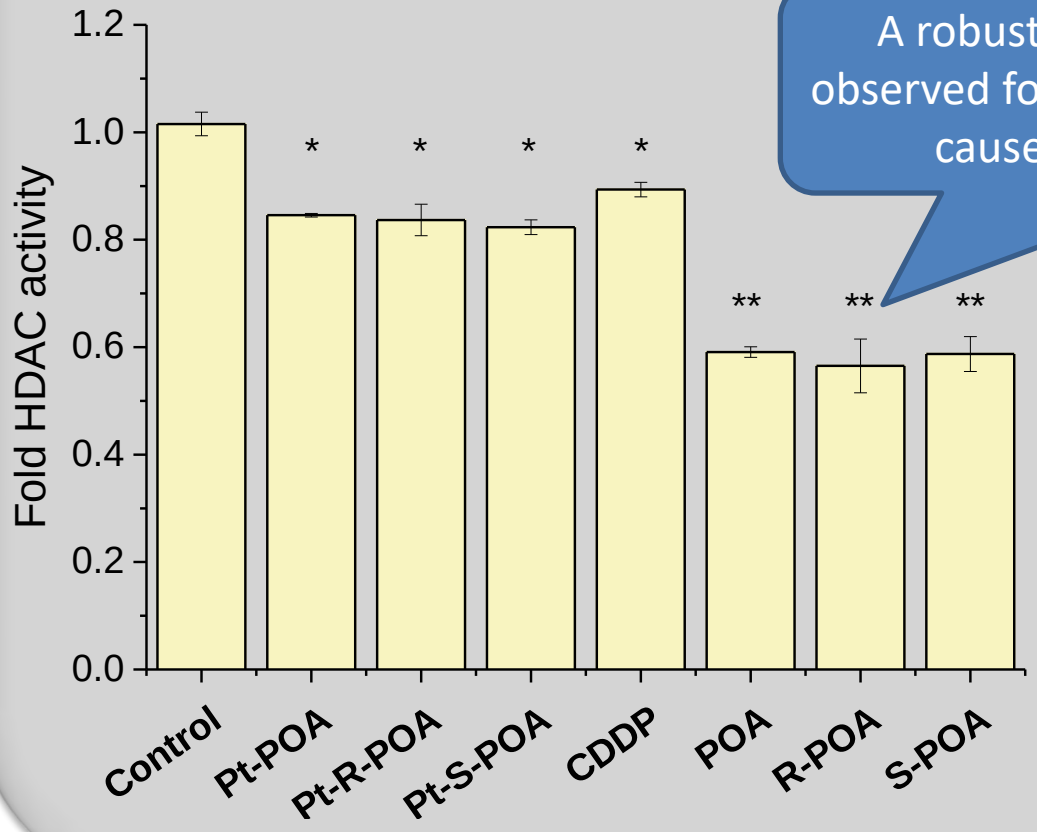
Cmpd	Unit	IC_{50} (mean±standard deviation, sd)				
		A2780	NT2/D1	A549	HCT 116	MCF-7
POA	μM	237±65	94±18	521±288	439±279	319±119
CDDP	μM	0.5±0.1	0.10±0.04	3.6±0.9	2.3±0.3	3.3±0.2
Pt-POA	nM	8±1	4±1	129±44	29±11	474±56
Pt-*R*-POA	nM	13±4	5±2	115±27	50±24	215±119
Pt-*S*-POA	nM	14±5	4±2	84±40	37±2	301±68

Antiproliferative activity (IC_{50}) *obtained after 72 h of treatment of ovarian carcinoma A2780, testicular cancer NT2-D1, colorectal cancer HCT 116, lung carcinoma A549, and breast adenocarcinoma MCF-7 cell lines.*

Results and discussion

E. Gabano *et al.*, *Dalton Trans.*, **2017**, *46*, 14174 (doi)

The results (*in vitro*)



A robust inhibition of HDAC activity was observed for **POA**s, whereas the **Pt-POA** series caused a less important decrease.

HDAC activity *after 24 h of treatment of A2780 cells with* IC_{50} *concentrations of the complexes (1 μM) and* ***POA****s (5 mM). Data are means ± sd of three experiments performed in triplicate and were compared by means of a two-tailed t-test (* p<0.05; ** p<0.01).*

Results and discussion

E. Gabano *et al.*, *Dalton Trans.*, **2017**, *46*, 14174 (doi)

The results (*in vivo*)

Pt-POA induced an impressive reduction of the tumor mass of 94%, whereas **CDDP** induced a tumor regression of 75%.

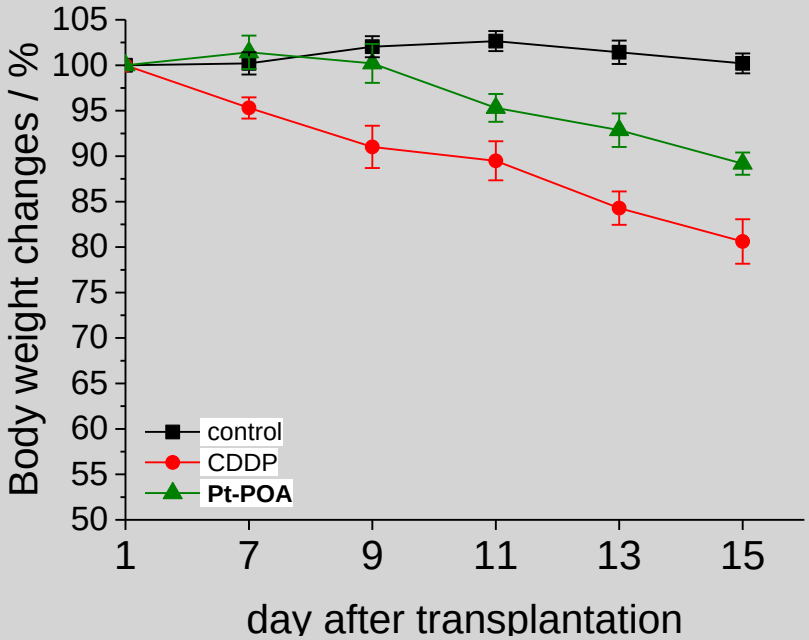
Cmpd	Daily dose [mg kg^{-1}]	Average tumor weight [g]	Inhibition of tumor growth [%]
control	0	0.542±0.16	0
CDDP	1.5	0.135±0.09	75
Pt-POA	20	0.037±0.02	94

In vivo antitumor activity of Pt-POA and CDDP. *Lewis lung carcinoma (LLC) was implanted intramuscularly in C57BL mice and from day 7 to 14 animals received daily* ***Pt-POA*** *(per os) or* ***CDDP*** *(ip). At day 15 animals were sacrificed and the inhibition of tumor growth was determined.*

Results and discussion

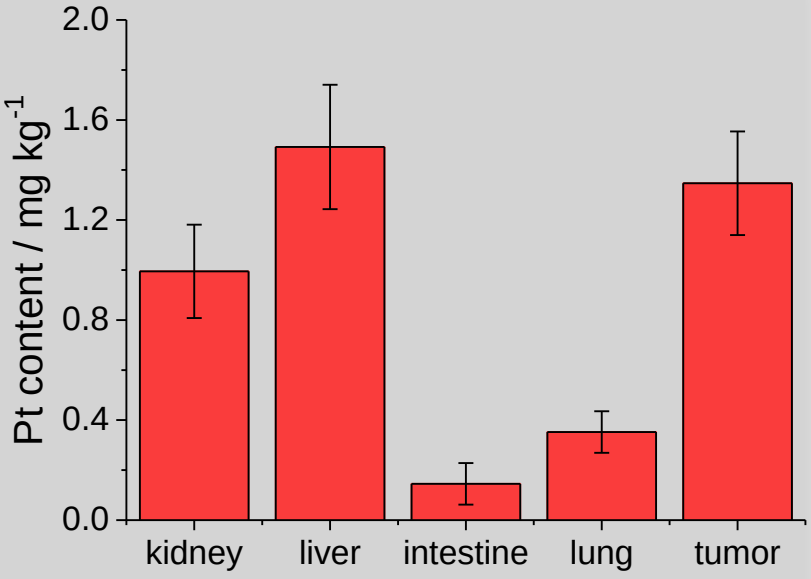
E. Gabano *et al.*, *Dalton Trans.*, **2017**, *46*, 14174 (doi)

The results (*in vivo*)



Body weight changes *of LLC-bearing C57BL mice treated with vehicle or Pt compounds.*

Pt-POA did not induce body weight loss.



Total platinum levels *determined in organs of mice treated with* ***Pt-POA*** *after a single dose oral application (20 mg kg^{-1}).*

A significant Pt content was present into the tumor mass.

Conclusions

Dual-action Pt(IV) complexes

- **Active bioligands can be coordinated to the Pt(IV) core**. The coordination should not affect its biological activity.
- **Activation by reduction** will (should) release the two metabolites (Pt(II) and bioligand).
- **A strong rationale should support the synergism**. The synergism should occur for co-treatment of the components at the same or similar concentrations. Some effect may be enhanced by **synergistic accumulation**.
- If these constrains are satisfied, **dual-action Pt(IV) antitumor prodrugs may represent an alternative worthy to be further explored in clinical practice**.

Acknowledgments

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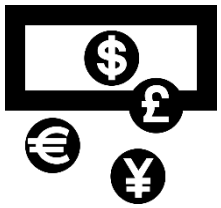

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HERMES project

Project funded by offer of compensation to the residents of Casale Monferrato (Italy) died or suffering from mesothelioma