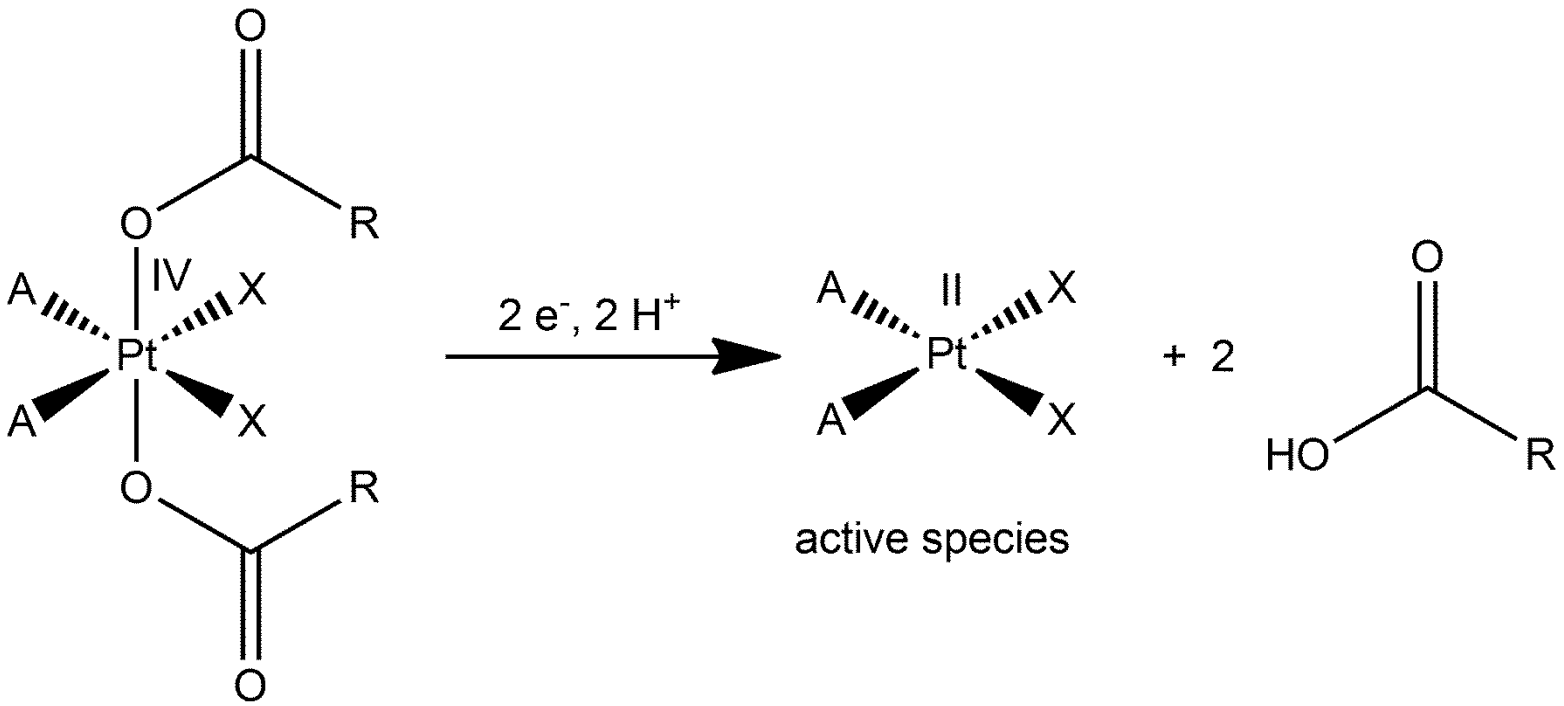
# Pt(IV) complexes in the treatment of Pt(II)-refractory tumors: an update.

**Mauro RAVERA**

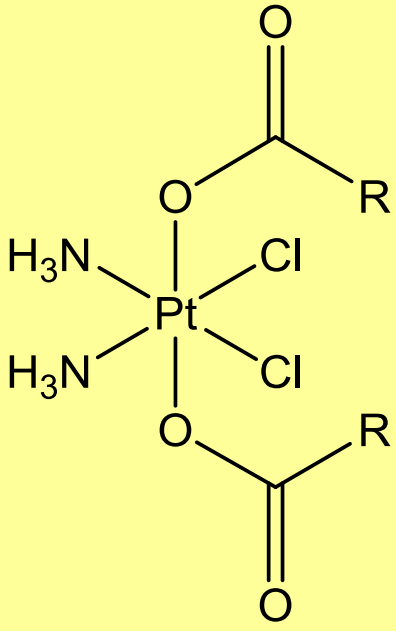
*Dipartimento di Scienze e Innovazione Tecnologica,*
*Università del Piemonte Orientale,*
*Viale Teresa Michel 11,*
*15121 Alessandria (Italy)*

Pt(IV) antitumor drug candidates are generally considered *prodrugs* since they can be reduced in the hypoxic (and then reducing) intracellular *milieu* of tumour cells to the corresponding cytotoxic Pt(II) metabolite with the (*usual*) loss of their two axial ligands (*activation by reduction*).



# *Madrid's final considerations*

- High lipophilicity (high log $P_{o/w}$) = high cell accumulation and DNA platination = high cytotoxicity;
- Compounds with low log $P_{o/w}$ are not able to "charge" the cells of adequate amounts of Pt to have an important biological effect;
- Compounds with high log $P_{o/w}$ fill the cells by a double effect: high uptake and reduced efflux.

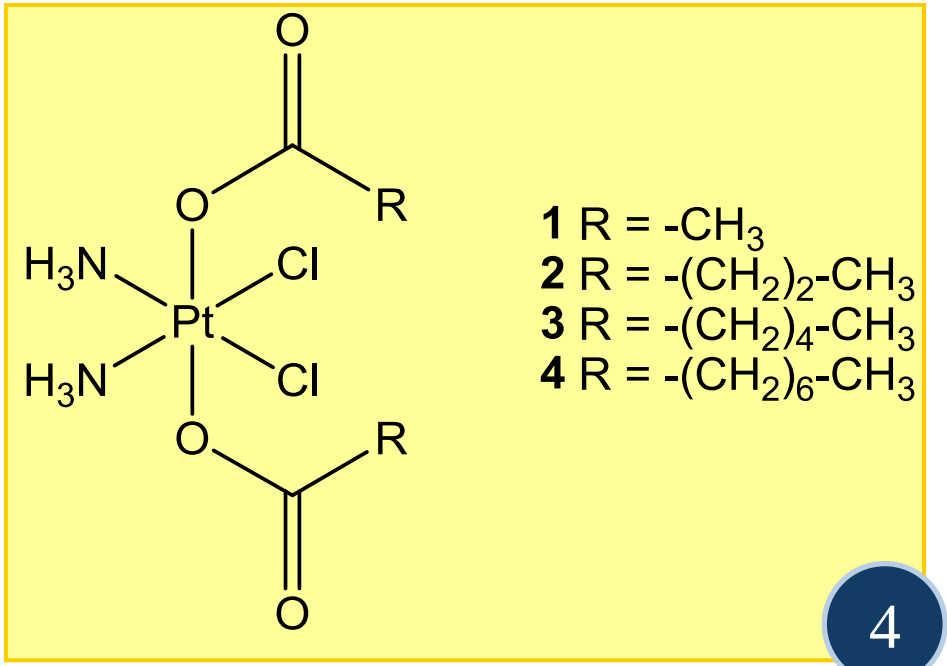



**1** R = -$CH_3$
**2** R = -$(CH_2)_2$-$CH_3$
**3** R = -$(CH_2)_4$-$CH_3$
**4** R = -$(CH_2)_6$-$CH_3$

M. Ravera et al., *unpublished results*

# *Madrid's final considerations*

- Multicellular tumor spheroids (MCTS) were used to study the effect on cell proliferation after prolonged treatment (up to 14 d).
- Complexes **1**-**4** gave a concentration-dependent response on MCTS with a potency similar to that observed in 2D experiments
- Differently from cisplatin, **1**-**4** exerted a prolonged antiproliferative action even when they were removed from the cell culture.




I. Zanellato et al., *J. Inorg. Biochem.*, in press, doi: 10.1016/j.jinorgbio.2014.07.018

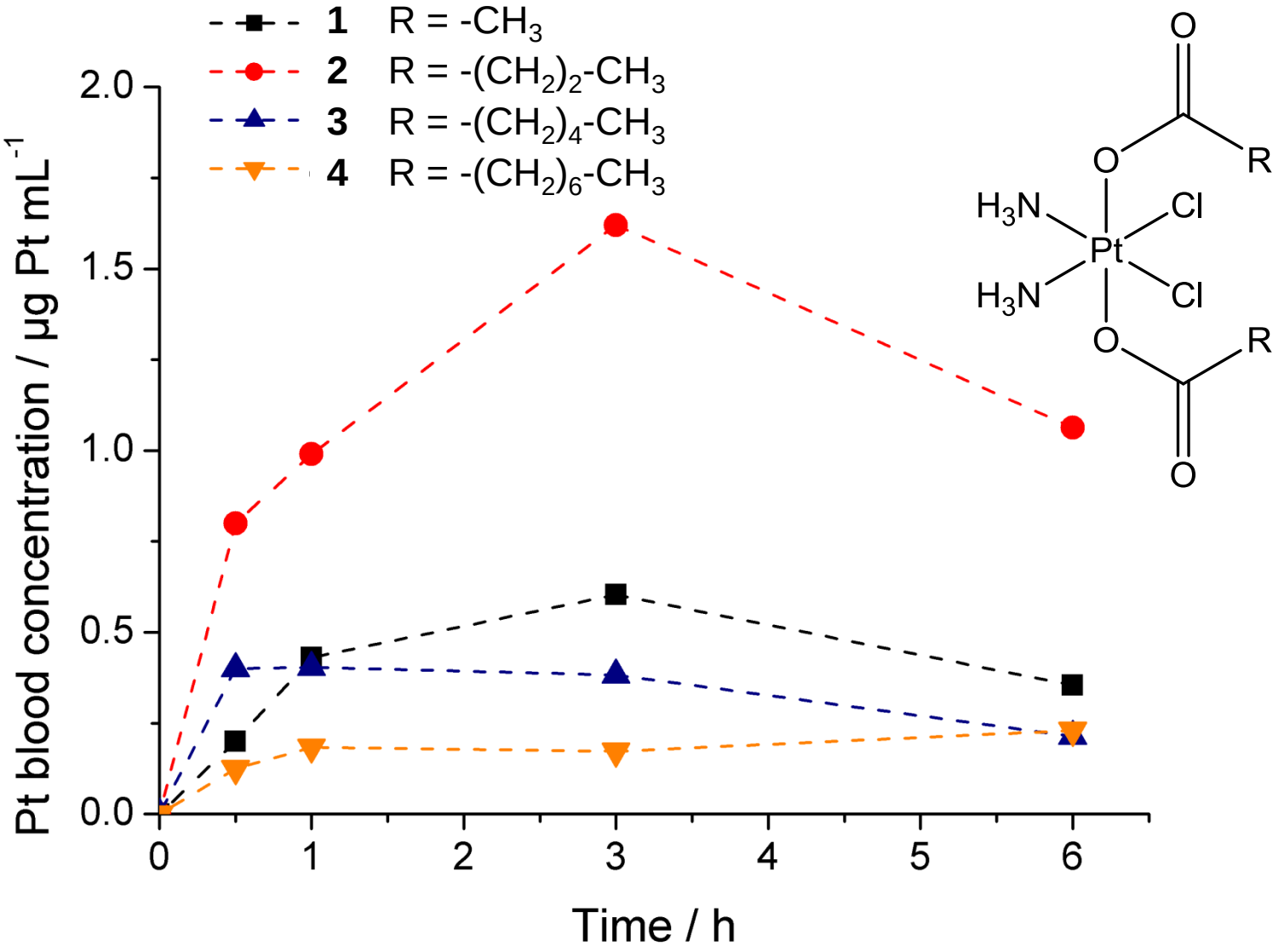
## *One step beyond…*

- Complexes **1**-**4** were challenged *ex vivo* in different pH conditions (from 1.0 to 9.0, in some cases up to 24 h) to simulate the hypothetical conditions for an oral route of administration, showing always a high stability (>90%).
- Pt(IV) compounds were administered in saline by oral gavage to a cohort of syngeneic FVB female mice. Whole blood was mineralized and analyzed for Pt content by ICP-MS.

I. Zanellato et al., *J. Inorg. Biochem.*, in press, doi: 10.1016/j.jinorgbio.2014.07.018

*One step beyond...*

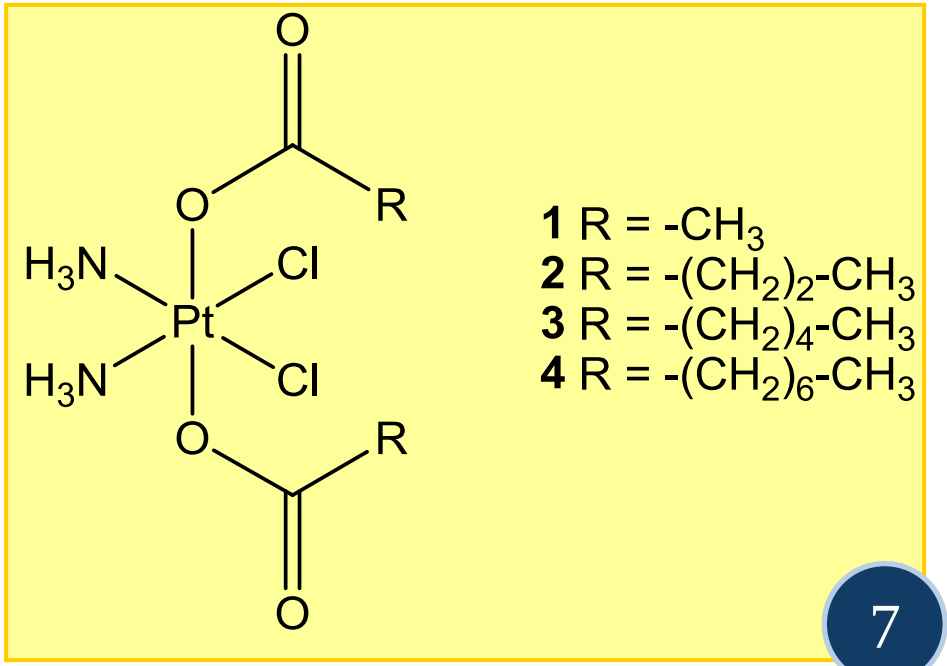
Total [Pt] in blood after the oral administration of a single dose (20 mg $kg^{-1}$) of compounds **1**-**4**. Data are means of experiments on 3 animals (syngeneic FVB female mice) for each time point. SD are omitted for sake of clarity (ca. ± 22%).




I. Zanellato et al., *J. Inorg. Biochem.*, in press, doi: 10.1016/j.jinorgbio.2014.07.018

# Zurich's final considerations

- The very high lipophilicity that makes compound **4** so active *in vitro* is detrimental of its oral absorption.
- Compound **2** gave the best results in terms of Pt concentration in blood. It seems to represents the best compromise between lipophilicity and water solubility for oral administration.



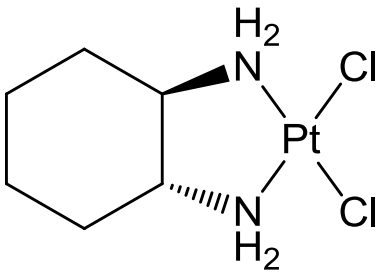

I. Zanellato et al., *J. Inorg. Biochem.*, in press, doi: 10.1016/j.jinorgbio.2014.07.018

# *Resistance to oxaliplatin*

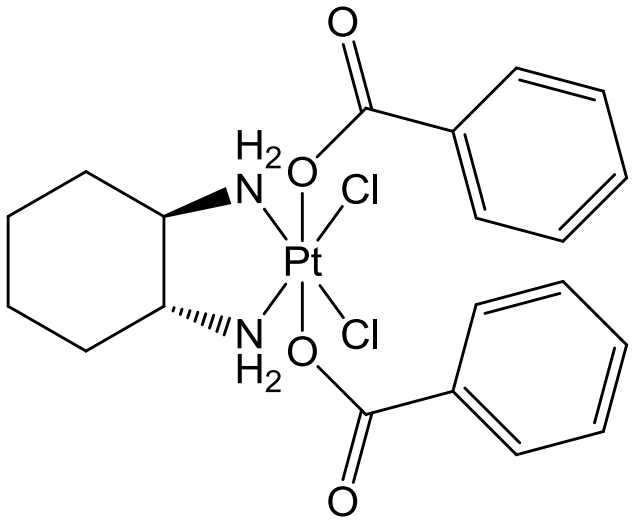
Colorectal cancer is the third most common type of cancer. The gold standard for its treatment consists of folonic acid, 5-fluorouracil and oxaliplatin.

However, treated patients may experience a relapse of the disease because of the eventual emergence of drug-resistant tumor clones.

May Pt(IV) complexes help?




**5**




**6**

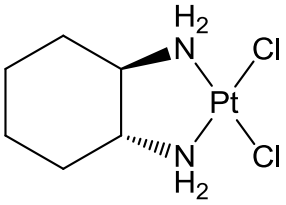
# Cytotoxicity

| Cmpds | $IC_{50}$ [μM] (mean ± SD , 72 h CT) | | | | |
|---|---|---|---|---|---|
| | **HCT-15** colon | **CCD18-Co** colon fibroblasts | **2008** ovarian | **C13*** CDDP resistant | **A549** lung |
| **Cisplatin CDDP** | 11.32±1.51 | 28.30±1.53 [2.5] | 2.22±1.14 | 29.27±3.17 (13.2) | 9.24±1.08 |
| **Oxaliplatin OXP** | 1.15±0.96 | 27.14±2.17 [23.6] | 1.53±1.15 | 2.93±1.31 (1.9) | 1.46±0.54 |
| **5** | 13.26±2.73 | 33.89±3.38 [2.6] | 14.35±3.00 | 27.52±3.63 (1.9) | 17.36±3.19 |
| **6** | 0.051±0.01 | 0.57±0.02 [11.2] | 0.10±0.05 | 0.058±0.010 (0.6) | 0.11±0.02 |

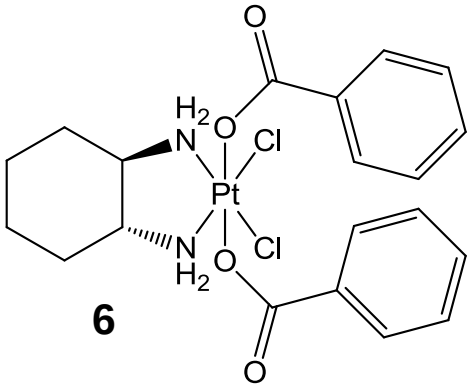
parentheses: **resistance factor** **RF** = $IC_{50}$ (resistant subline) / $IC_{50}$ (wild-type cells)
brackets: **selectivity index** **SI** = $IC_{50}$ (CCD18-Co) / $IC_{50}$ (HCT-15).

V. Gandin et al., *ChemMedChem*, **2014**, *9*, 1299

# Cytotoxicity




**5**




**6**

| Cmpds | $IC_{50}$ [μM] (mean ± SD , 72 h CT) | | | | |
|---|---|---|---|---|---|
| | **LoVo** colon | **LoVo-OXP** OXP resistant | **RF** | **LoVo-MDR** multidrug resistant | **RF** |
| **CDDP** | 9.12±1.35 | 16.16±3.36 | 1.8 | 9.06±2.03 | 1.0 |
| **OXP** | 0.89±0.46 | 15.25±2.24 | 17.1 | 1.36±0.73 | 1.5 |
| **5** | 6.23±2.34 | 12.86±1.32 | 2.1 | 8.01±0.74 | 1.3 |
| **6** | 0.093±0.030 | 0.068±0.02 | 0.7 | 0.089±0.02 | 0.9 |

**resistance factor RF** = $IC_{50}$ (resistant subline) / $IC_{50}$ (wild-type cells)

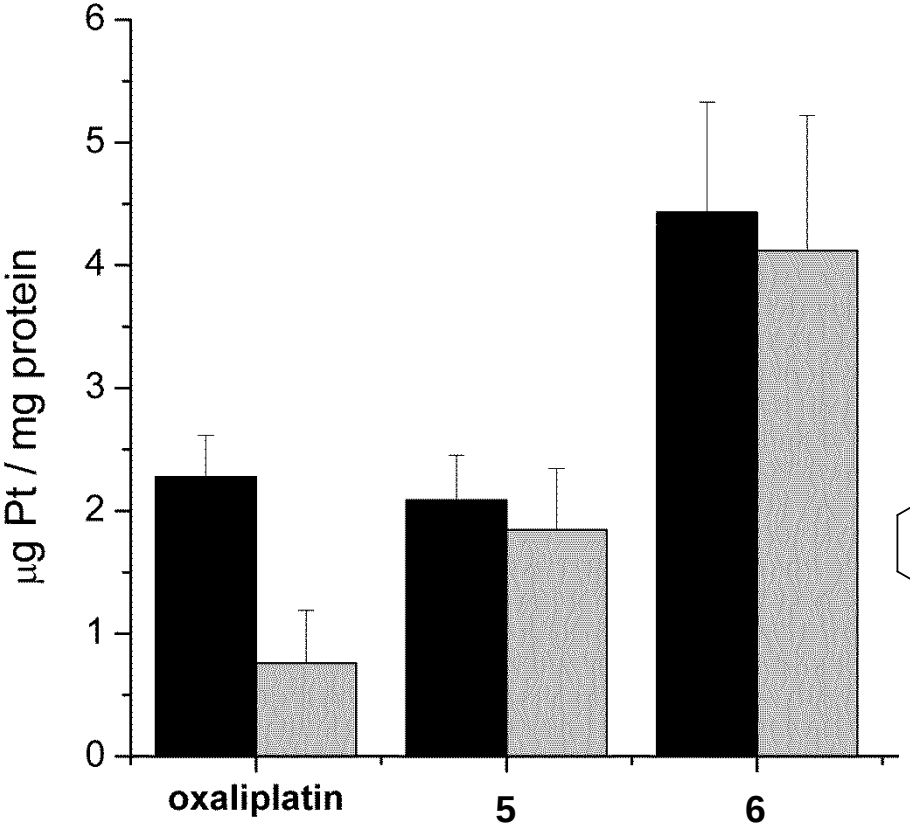
V. Gandin et al., *ChemMedChem*, **2014**, *9*, 1299

## *Taking a look at the tables…*

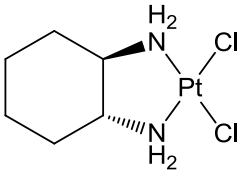
- As expected the lipophilic compound **6** was found to be the more effective on all tested cell lines.
- Compound **6** exhibits no cross-resistance with either CDDP or OXP. But **6** and OXP should produce the same active metabolite!
- Compound **6** maintains the same activity on the MDR subline suggesting it is not a P-glycoprotein (P-gp) substrate (MDR is associated with overexpression of multi-specific drug transporters, such as P-gp).
- SI is not bad, but compound **6** is really cytotoxic toward every cell line!

V. Gandin et al., *ChemMedChem*, **2014**, *9*, 1299

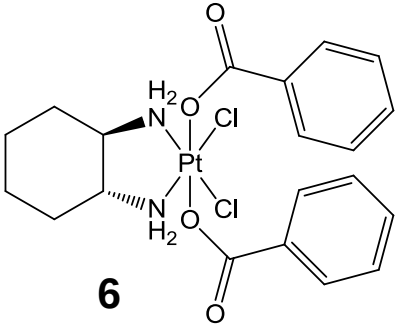
# Pt uptake




Intracellular accumulation of platinum complexes detected by GF-AAS analysis. LoVo (black bars) and LoVo-OXP (grey bars) cells were incubated for 24 h with 5 µM of complexes **5**, **6**, and OXP.




**5**




**6**

- The increased activity of **6**, showing the largest accumulation in both LoVo and LoVo-OXP cell lines, is related to its high lipophilicity that favors its cellular accumulation by passive diffusion.

V. Gandin et al., *ChemMedChem*, **2014**, *9*, 1299

## *A too simple analysis?*

- The lipophilicity seems to be the main factor to determine the *in vitro* activity of a Pt(IV) complex (uptake by passive diffusion).
- Lipophilic Pt(IV) compounds exerted a prolonged antiproliferative action in 3D models even when they were removed from the culture medium.
- Their increased activity may be related to a double effect: high uptake and reduced efflux.
- The combination of these effects fills the cell of a (high) Pt quantity that the cell is not able to eliminate and it interfere with the cell machinery.
- However, lipophilicity and oral absorption did not seem to get along.
- *In vivo* work is in progress.

# *Thanks to:*

UNIVERSITÀ DEL PIEMONTE ORIENTALE

| | |
|---|---|
| **Domenico Osella** | supervision |
| **Sabrina Bianco** | chemistry |
| **Donato Colangelo** | *in vivo* data |
| **Ilaria Bonarrigo** | *in vitro* data (spheroids) |
| **Elisabetta Gabano** | chemistry |
| **Ilaria Zanellato** | *in vitro* data (spheroids) |

***University of Padova***

| | |
|---|---|
| **Valentina Gandin** | *in vitro* data (LoVo-OXP) |
| **Cristina Marzano** | *in vitro* data (LoVo-OXP) |

# *Thanks to:*

**COST Action CM1105**

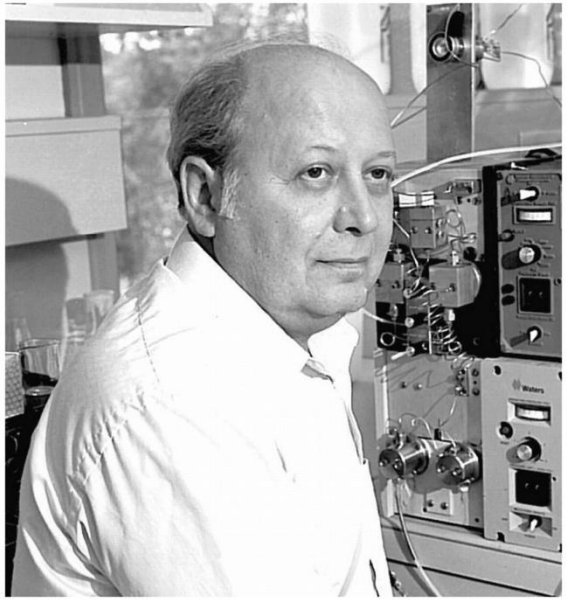
***C**onsorzio **I**nteruniversitario di **R**icerca in **C**himica dei **M**etalli nei **S**istemi **B**iologici (Bari)*

FONDAZIONE
CASSA DI RISPARMIO DI ALESSANDRIA

FONDAZIONE CRT
Cassa di Risparmio di Torino

*"If a hundred years of cancer research has taught us anything, it is that if you must get cancer, you want to be a mouse, because we can cure cancer in mice. Curing it in people is immensely harder, and most promising therapies fail at exactly the transition from mouse to man."*

G.A. Petsko, *Genome Biology*, **2001**, 3: comment1001



**Barnett Rosenberg**

(* 16 November 1926 – † 8 August 2009)