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Strategies for the Development of Novel Pt Anticancer Compounds: Shifting the Paradigms from Pt(II) to Pt(IV) Complexes.

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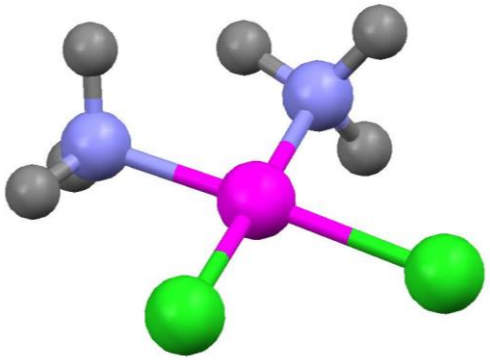
Cisplatin: a leading anticancer drug



***cis*-diamminedichloridoplatinum(II)**

$[PtCl_2(NH_3)_2]$

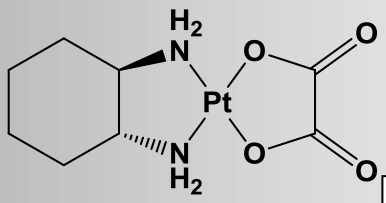
- cisplatin was included (1999) into the WHO list of 17 essential anticancer drugs;
- cisplatin was approved (1978) to be used alone or with other drugs to treat: bladder, cervical, ovarian, testicular, non-small cell lung cancer, malignant mesothelioma, and squamous cell carcinoma;
- DNA is the main final target.

Main problems associated with the use of cisplatin:

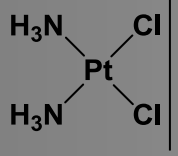
- severe side effects (*e.g.*, kidney damage, vomiting, neurotoxicity, etc.);
- development of resistance (acquired during the treatment).

"New" kids on the block

Timeline | **Milestones in the development of platinum drugs for cancer therapy**



1965 — Discovery of the biological properties of cisplatin in bacteria

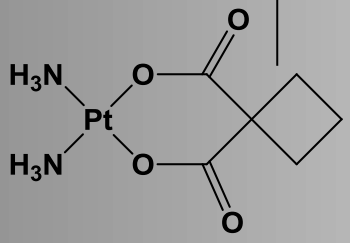


1968 — Cisplatin shown to be active against a mouse model of cancer

1971 — First patient treated with cisplatin

1978 — Regulatory approval of cisplatin (testicular and bladder cancer)

1982 — First patient treated with carboplatin



1985 — Description of the various cisplatin-induced adducts formed on DNA

1989 — Regulatory approval of carboplatin (ovarian cancer)

1993 — First patient treated with satraplatin

1997 — Announced positive results for satraplatin in phase III trial in hormone refractory prostate cancer

2003 — Worldwide approval of oxaliplatin (colorectal cancer)

2006 — Announcement that satraplatin trial did not achieve the endpoint of overall survival

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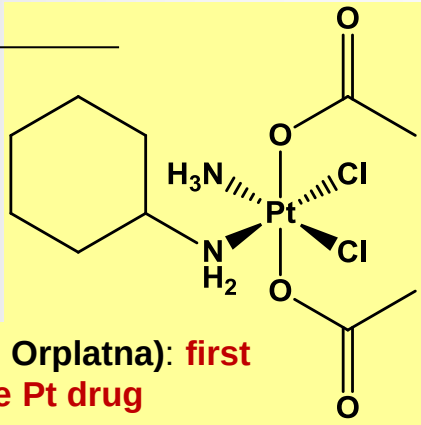
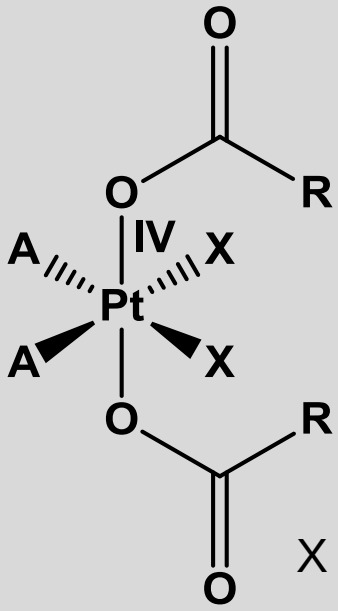


Satraplatin (JM216, Orplatna): **first orally administrable Pt drug**

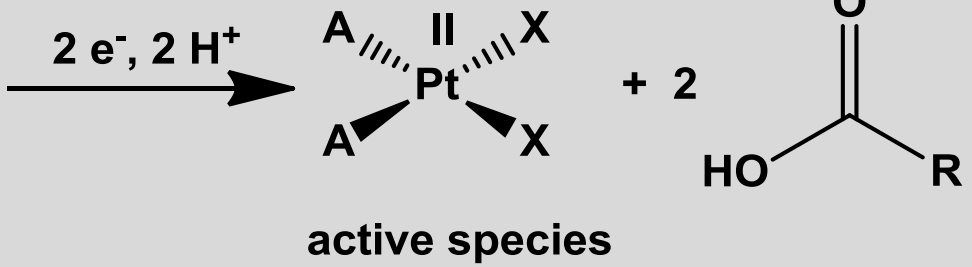
adapted from: Kelland, *Nat. Rev. Cancer*, **2007**, *7*, 573

Pt(IV)-based anticancer prodrugs

Pt(IV) complexes are supposed to act as **prodrugs** being *reduced in vivo* to their active Pt(II) metabolites in the hypoxic and reducing conditions of the tumor tissue.



activation by reduction



active species

X = carboxylates or halogens, A = NH_3 and amines

Activation **ONLY** in the tumour tissue through a rational choice of the ligands to modulate the key features (*i.e.*, reduction potential, reduction rate, lipophilicity, nature of the final Pt(II) metabolite).

Pt(IV) and cisplatin-resistant tumors

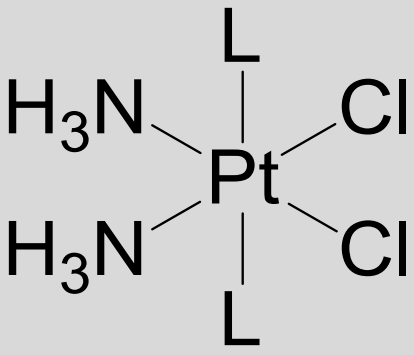
KEY QUESTION #1: may Pt(IV) complex be useful to bypass chemoresistance ?

Cisplatin resistance can occur by several ways, in particular by increased drug efflux (through “chemical” efflux pumps).

- **The rationale:** Pt(IV) complexes are not the target of efflux pumps.
- **The case study:** 4 cisplatin-based Pt(IV) complexes were tested on 8 cancer cell lines sensitive, intrinsically or made resistant to cisplatin + 1 non-tumoral as healthy control.

Antiproliferative activity is evaluated as **IC_{50}** = half maximal inhibitory concentration, *i.e.*, concentration required to reduce the viability of cells by 50% as compared with the control cells.

Pt(IV) and cisplatin-resistant tumors



L = $CH_3(CH_2)_nCOO^-$

1 n = 0
2 n = 2
3 n = 4
4 n = 6

Lipophilicity increases

resistancefactor $RF = \frac{IC_{50}\ (MM98R)}{IC_{50}\ (MM98)}$

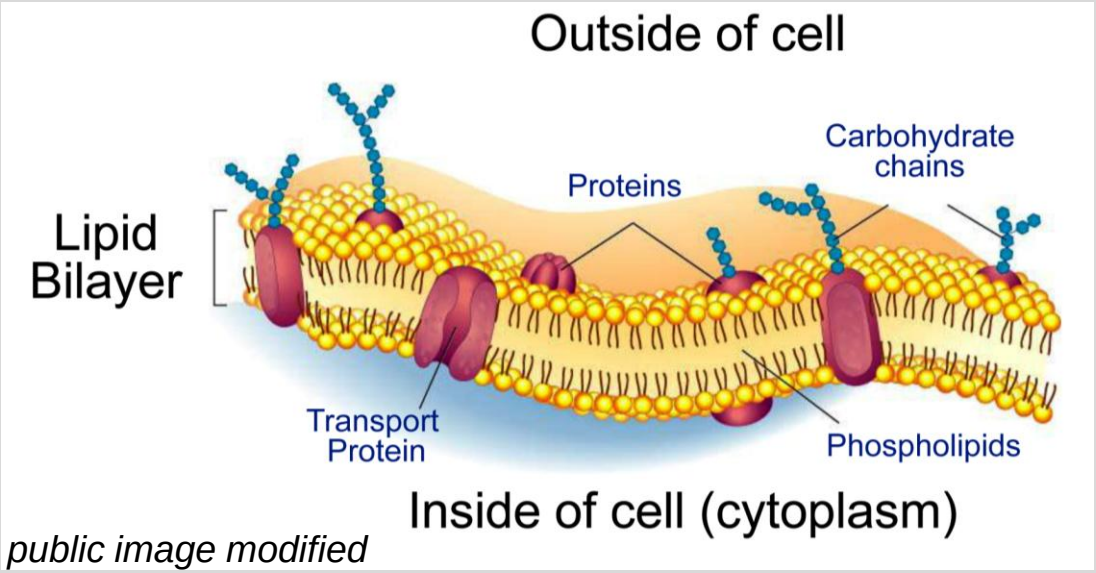
Cmpd	IC_{50} (μM)			
	BR95	**MM98**	**MM98R**	**RF**
cisplatin	6.2±0.9	3.2±1.0	19.4±2.8	6.1
3	0.9±0.1	0.3±0.1	0.8±0.1	2.7
4	64±19×10^{-3}	17±4×10^{-3}	22±3×10^{-3}	1.3

In general: high lipophilicity → high activity
(also on cisplatin-resistant sublines)

Legend Malignant pleural mesothelioma cell lines: *BR95* epithelioid and *MM98* sarcomatoid phenotypes; *MM98R* = cisplatin-resistant subline derived from MM98.

Pt(IV) and cisplatin-resistant tumors

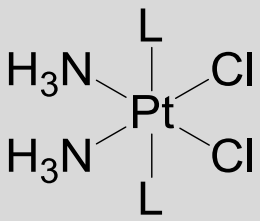
KEY QUESTION #2: is lipophilicity important only because intracellular [Pt] is high?



public image modified

- **The rationale:** there is affinity between lipophilic compounds and (lipophilic) cell membrane. Is it enough?
- **The case study:** measurement of the cell accumulation and DNA platination of the 4 cisplatin-based Pt(IV).

Accumulation of Pt(IV) complexes



L = $CH_3(CH_2)_nCOO^-$

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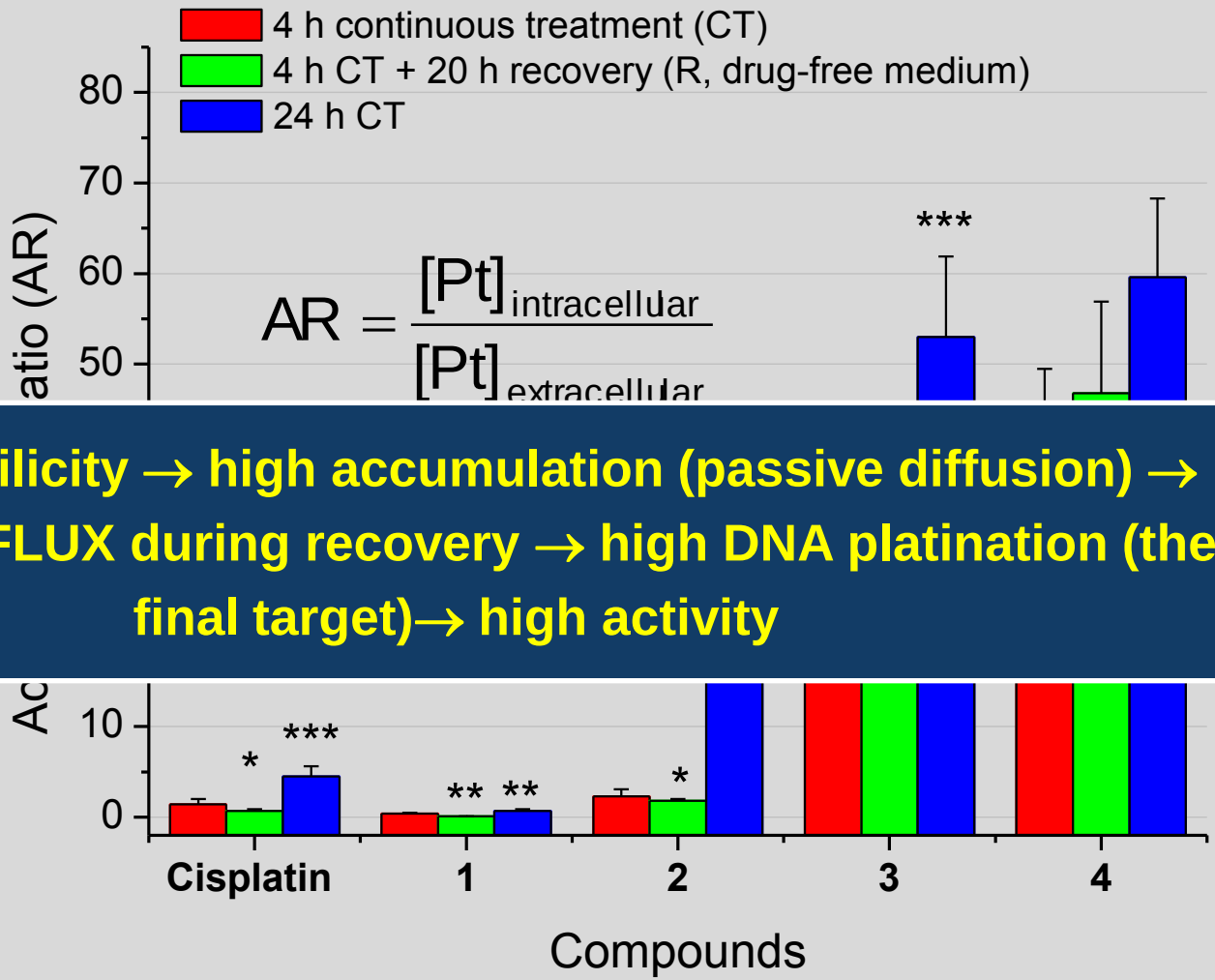


$$AR = \frac{[Pt]_{intracellular}}{[Pt]_{extracellular}}$$

High lipophilicity → high accumulation (passive diffusion) → LIMITED EFFLUX during recovery → high DNA platination (the final target)→ high activity

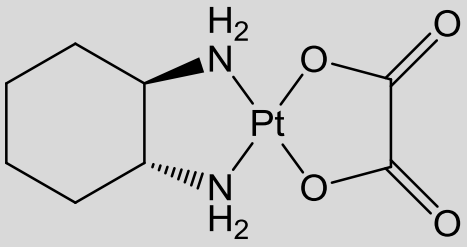
Notes: AR of A2780 ovarian cancer cells treated with 10 μM of the Pt complexes. Data are means ± SD of at least 3 independent replicates and were compared to those obtained for each drug after 4 h CT by means of the two sample t-test (*p<0.05;** p<0.01; ***p<0.001).

Pt(IV) and oxaliplatin-resistant tumors

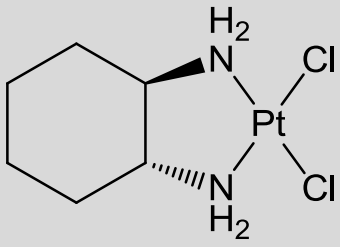
KEY QUESTION #3: may Pt(IV) complex be useful to bypass chemoresistance to other Pt drugs ?

Colorectal cancer is the third most common type of cancer. Standard treatment consists of oxaliplatin, but a relapse of the disease may occur because of the emergence of drug-resistant clones.

- **The case study:** oxaliplatin and two cyclohexane-1*R*,2*R*-diamine complexes were tested on oxaliplatin-resistant cell lines.



oxaliplatin



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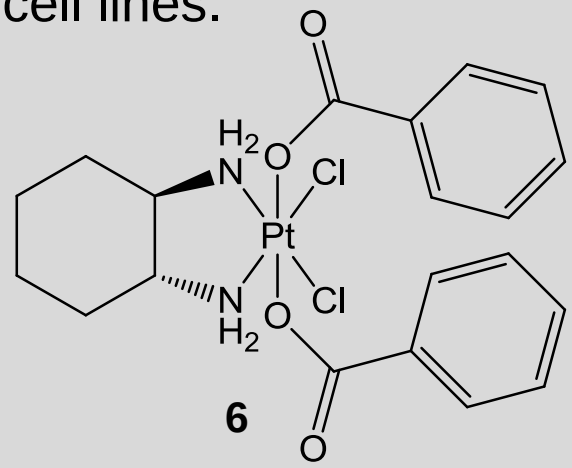

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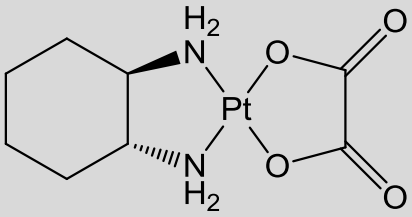
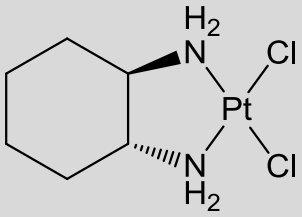
Pt(IV) and oxaliplatin-resistant tumors



oxaliplatin



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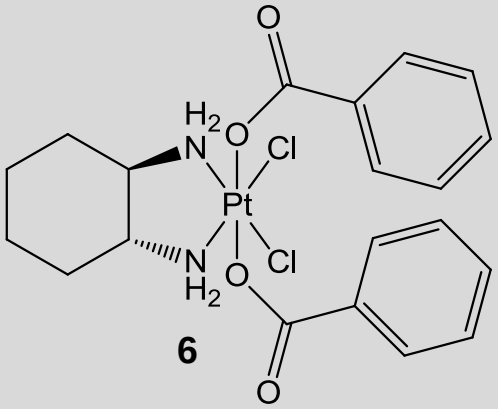


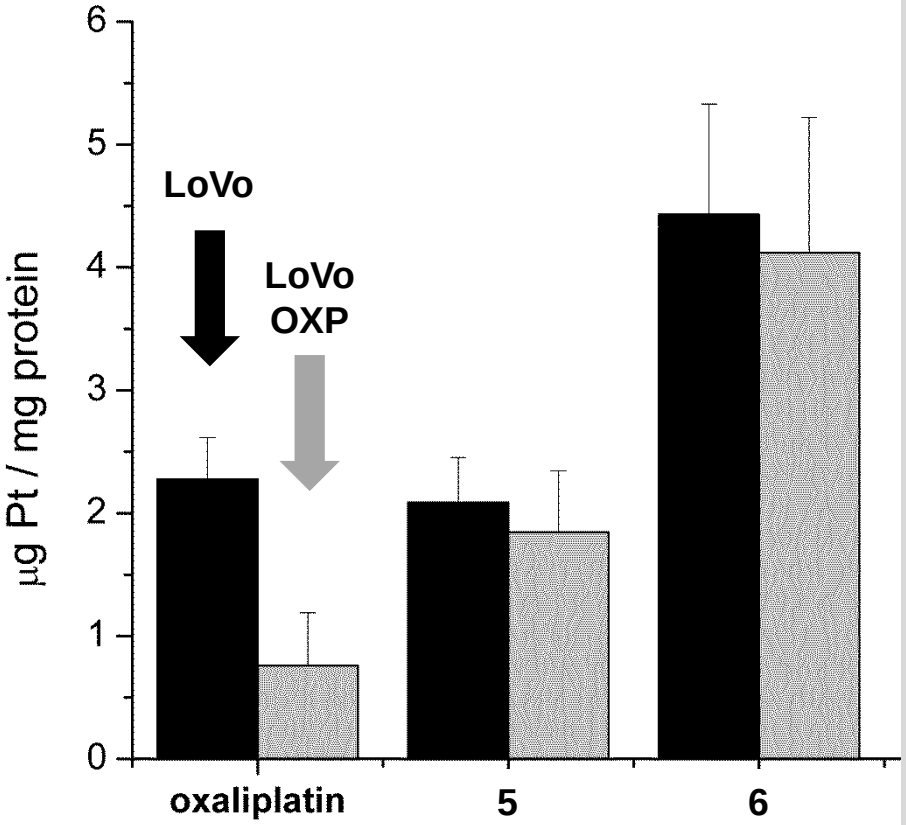
6

Cmpd	IC_{50} [μM]		$RF = \frac{IC_{50}\ (\text{LoVo - OXP})}{IC_{50}\ (\text{LoVo})}$
	LoVo	LoVo-OXP	
cisplatin	9.12 ± 1.35	16.16 ± 3.36	1.8
oxaliplatin	0.89 ± 0.46	15.25 ± 2.24	17.1
5	6.23 ± 2.34	12.86 ± 1.32	2.0
6	0.093 ± 0.030	0.068 ± 0.02	0.7

Data represent the mean ± SD of at least three independent experiments performed in triplicate (72 h CT). LoVo = colon carcinoma cell line, LoVo-OXP = oxaliplatin-resistant subline derived from LoVo.

Gandin *et al.*, *ChemMedChem*, 9 (2014) 1299

Pt(IV) and oxaliplatin-resistant tumors



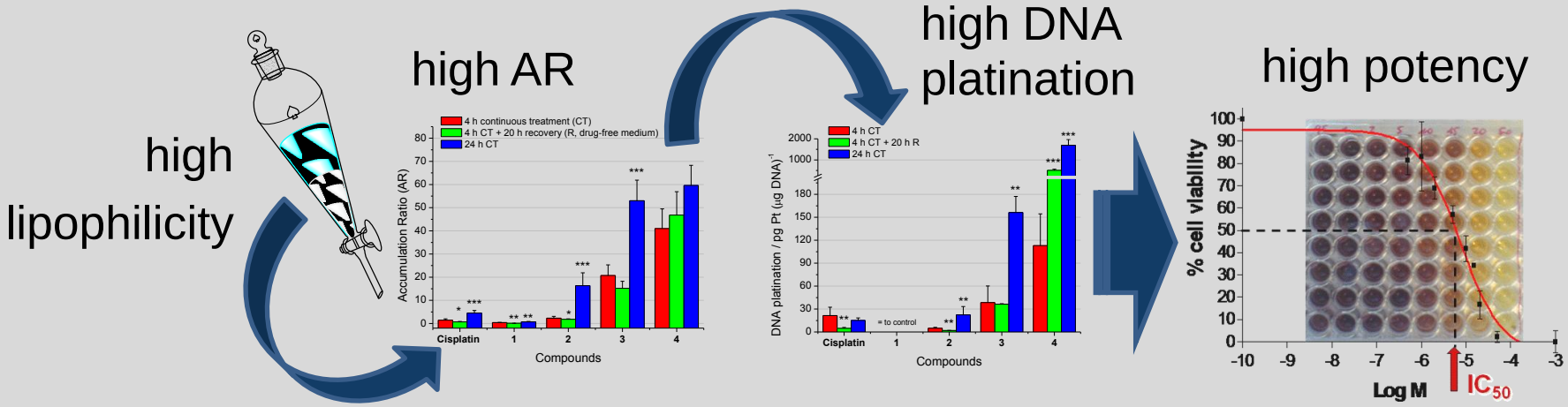
Intracellular accumulation of Pt complexes detected by GF-AAS analysis. LoVo (black bars) and LoVo-OXP (grey bars) cells were incubated for 24 h with 5 µM of complexes **5**, **6**, and oxaliplatin.

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

Gandin *et al.*, *ChemMedChem*, 9 (2014) 1299

Final (for the moment!) considerations

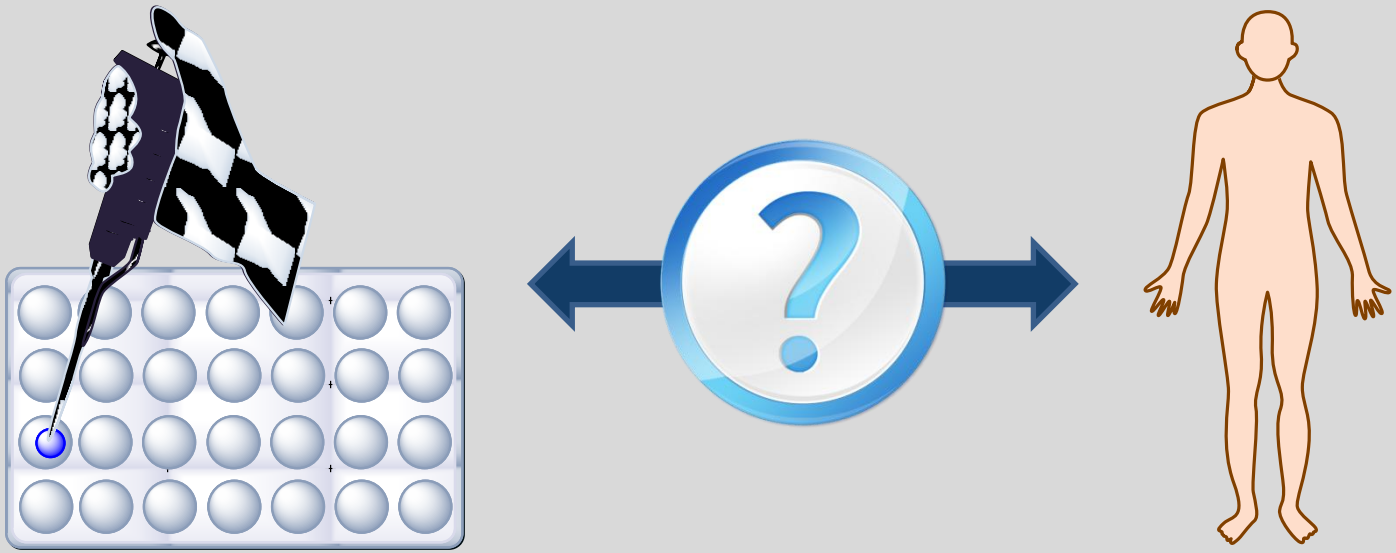
- with axial aliphatic carboxylato ligands, the lipophilicity increases as the number of carbon atoms in the chain increases, resulting in a beneficial effect on the cellular accumulation (*passive diffusion*), DNA platination, as well as on the *in vitro* antiproliferative activity also *vs.* Pt(II)-resistant cell lines.
- Highly lipophilic compounds “fill” the cells by a double effect: high uptake and limited efflux.



Final (for the moment!) considerations

BUT…

- there is a limit to lipophilicity when the corresponding drop in water solubility makes almost impossible to test these compounds without the extensive use of organic co-solvents.
- from a clinical point of view, the oral absorption may decrease since excessive lipophilicity decreases the transport across intestinal cells.



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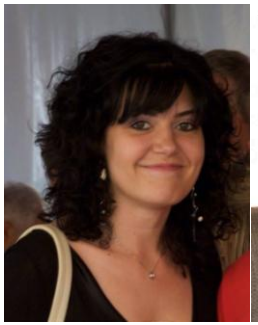

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