UNIVERSITÀ DEL PIEMONTE ORIENTALE

DIPARTIMENTO DI SCIENZE E INNOVAZIONE TECNOLOGICA

Biological activity of bis(carboxylato) cisplatin-based Pt(IV) prodrug candidates: how long the axial ligands should be?

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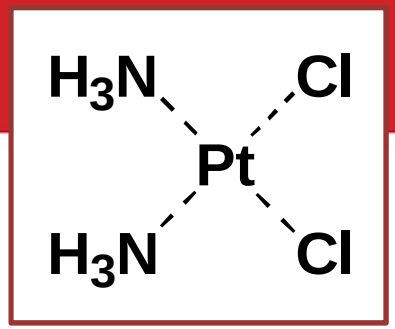
Some milestones in the development of Pt drugs



1965 — Discovery of the biological properties of **cisplatin**

1978 — Approval of cisplatin (testicular and bladder cancer)

1985 — Description of Pt-DNA adducts

1989 — Approval of **carboplatin** (ovarian cancer)

1991 1992 — The role of GSH in the resistance to cisplatin; The role of NER in the low response to cisplatin

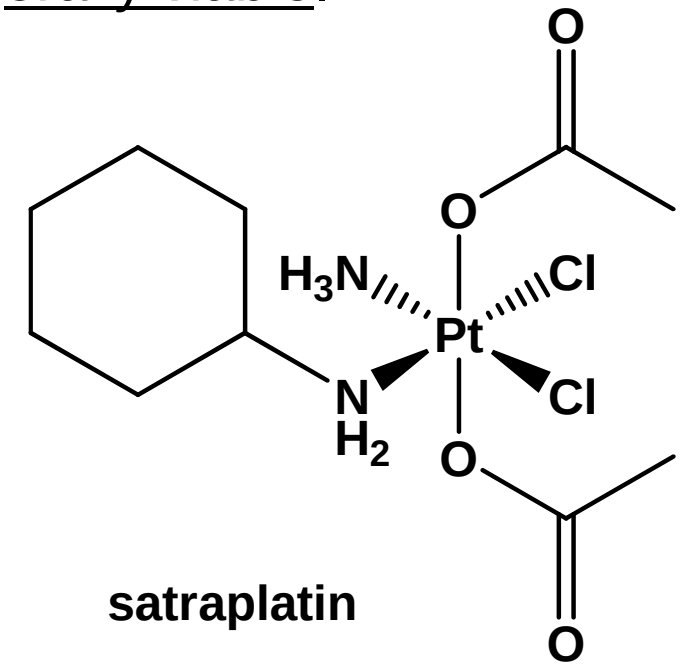
2002 — Approval of **oxaliplatin** (colorectal cancer)

2007 — Satraplatin Pt(IV) under approval (hormone-refractory prostate cancer)

Pt(IV)-based anticancer prodrugs

Pt(IV) complexes (d^6 low-spin electronic configuration) are quite inert towards ligand substitution:

- they give fewer side-reactions;
- they are not deactivated by gastric juices and therefore are orally viable.



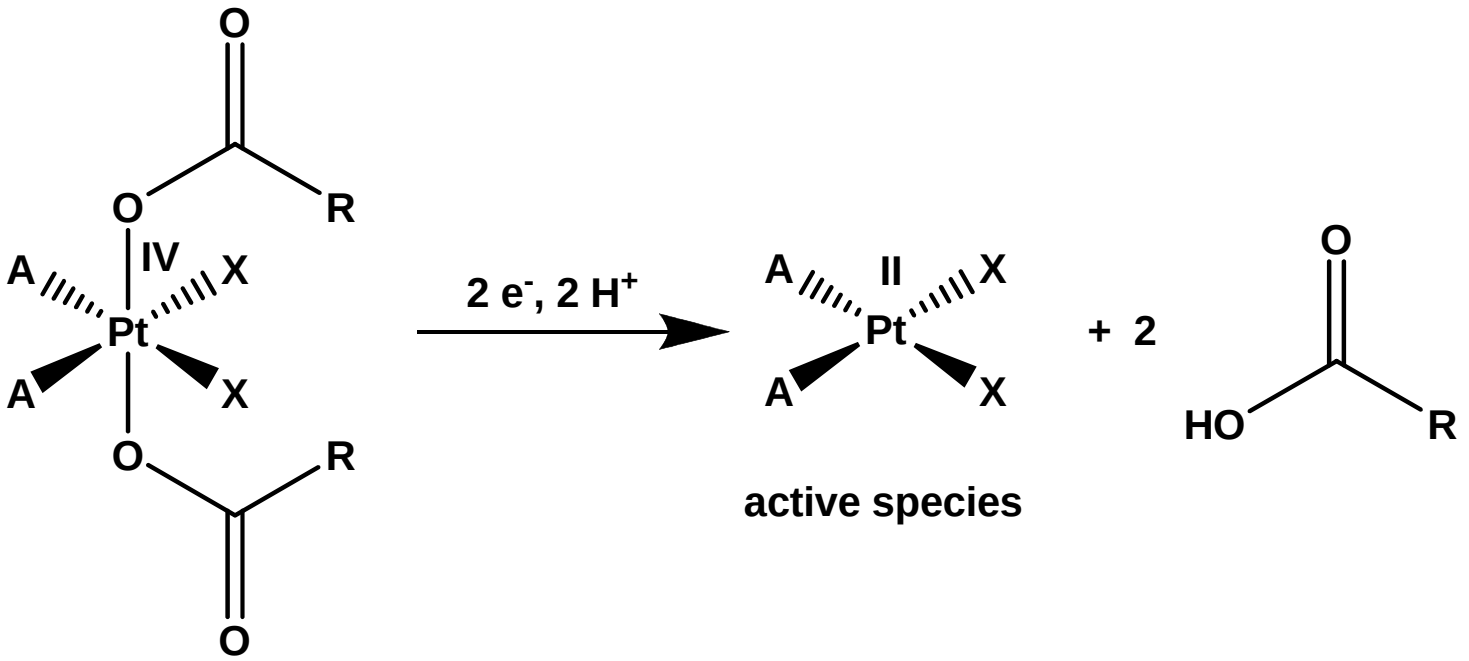
satraplatin

JM216 (satraplatin): after phase III, under approval for the cure of hormone-refractory prostate cancer.

First orally administrable Pt(IV) drug

Activation by reduction

The 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.

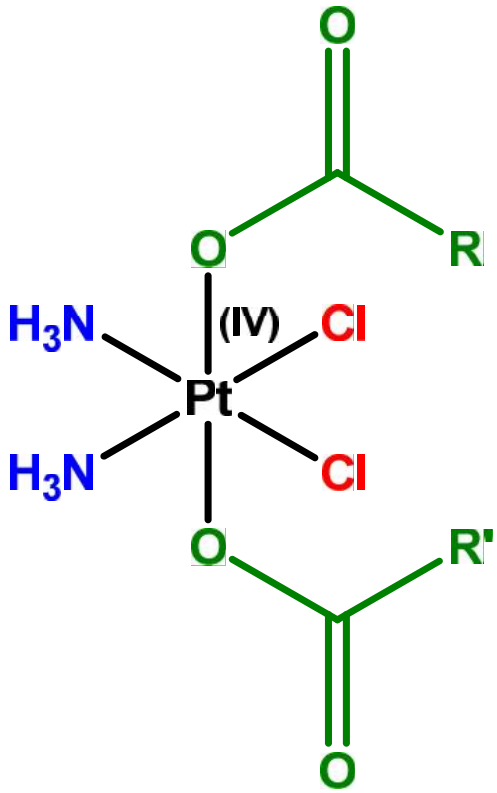


In the case of carboxylato ligands the Pt(IV)→Pt(II) reduction is further favored by low pH.

Key features of Pt(IV) prodrugs

A rational choice of the ligands is fundamental to modulate the key features of these drugs

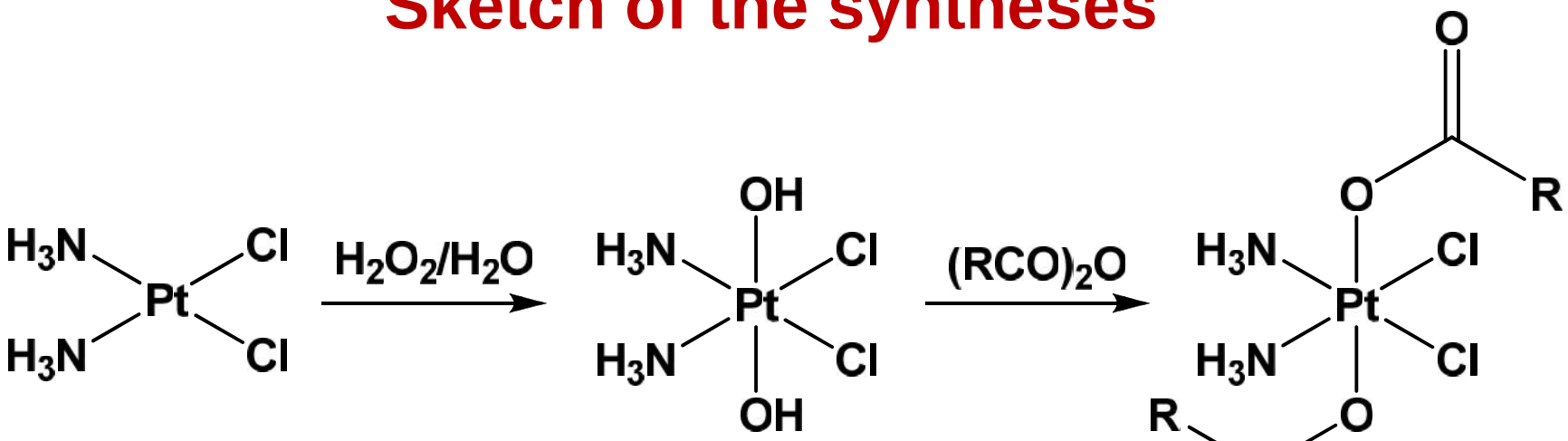
carrier ligands
(activity of the Pt(II) metabolite)



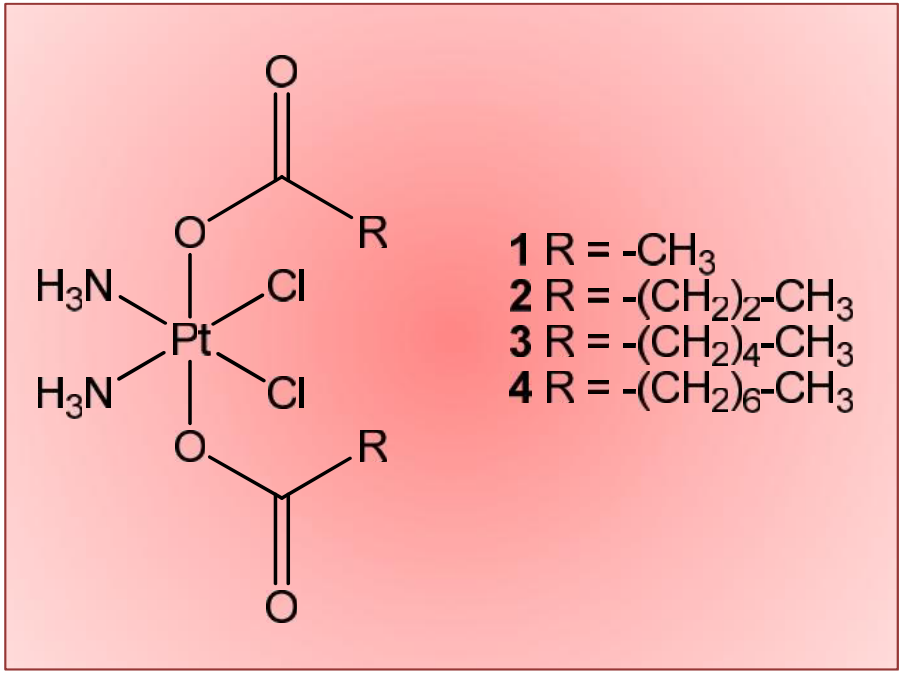
leaving ligands
(hydrolysis of the Pt(II) metabolite)

axial ligands
(lipophilicity, redox properties, functionalisation)

Sketch of the syntheses



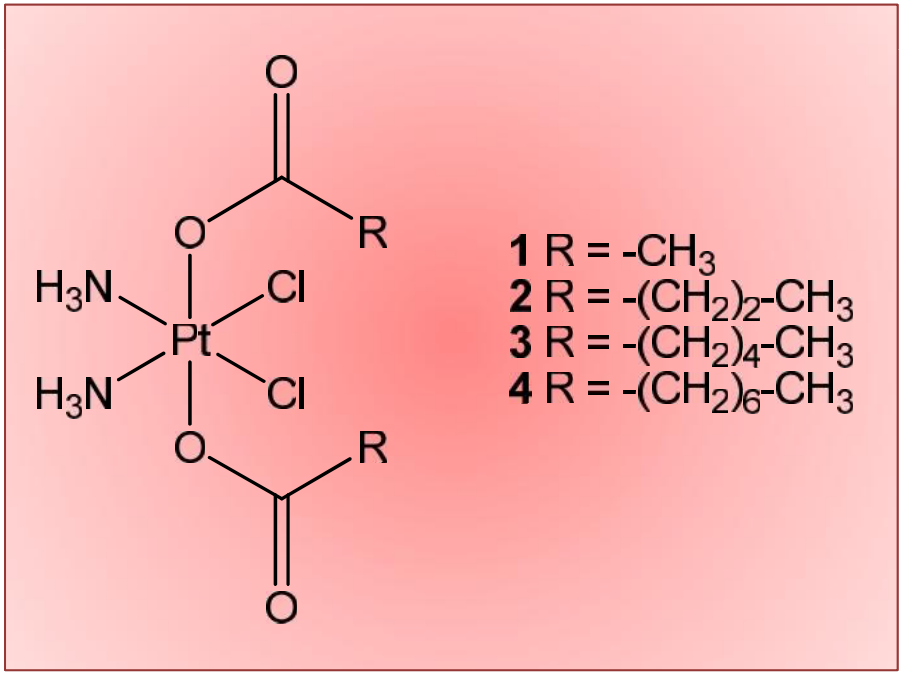
bis(carboxylato)

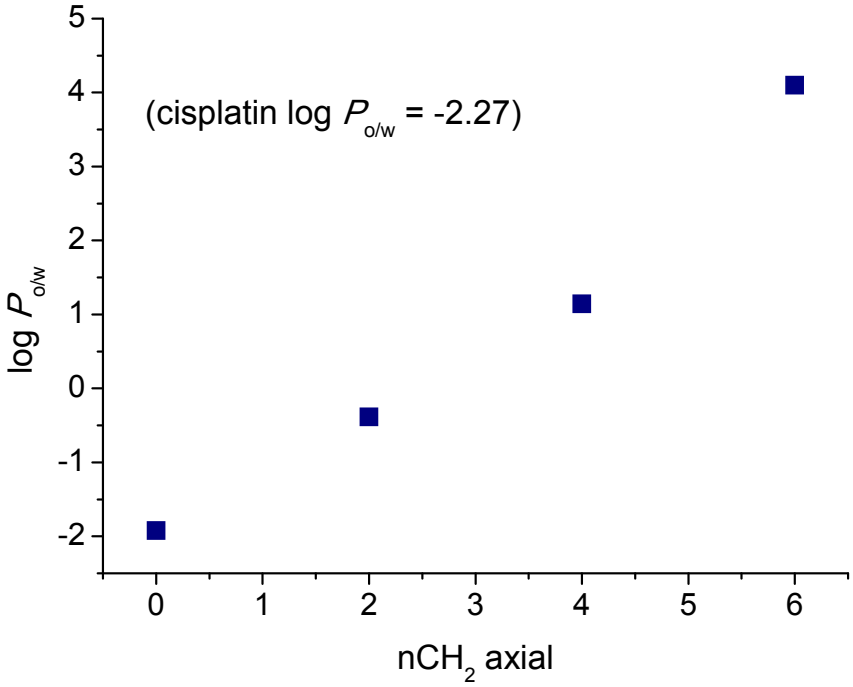


1 R = -CH_3
2 R = -$(CH_2)_2$-CH_3
3 R = -$(CH_2)_4$-CH_3
4 R = -$(CH_2)_6$-CH_3

Aim of the work

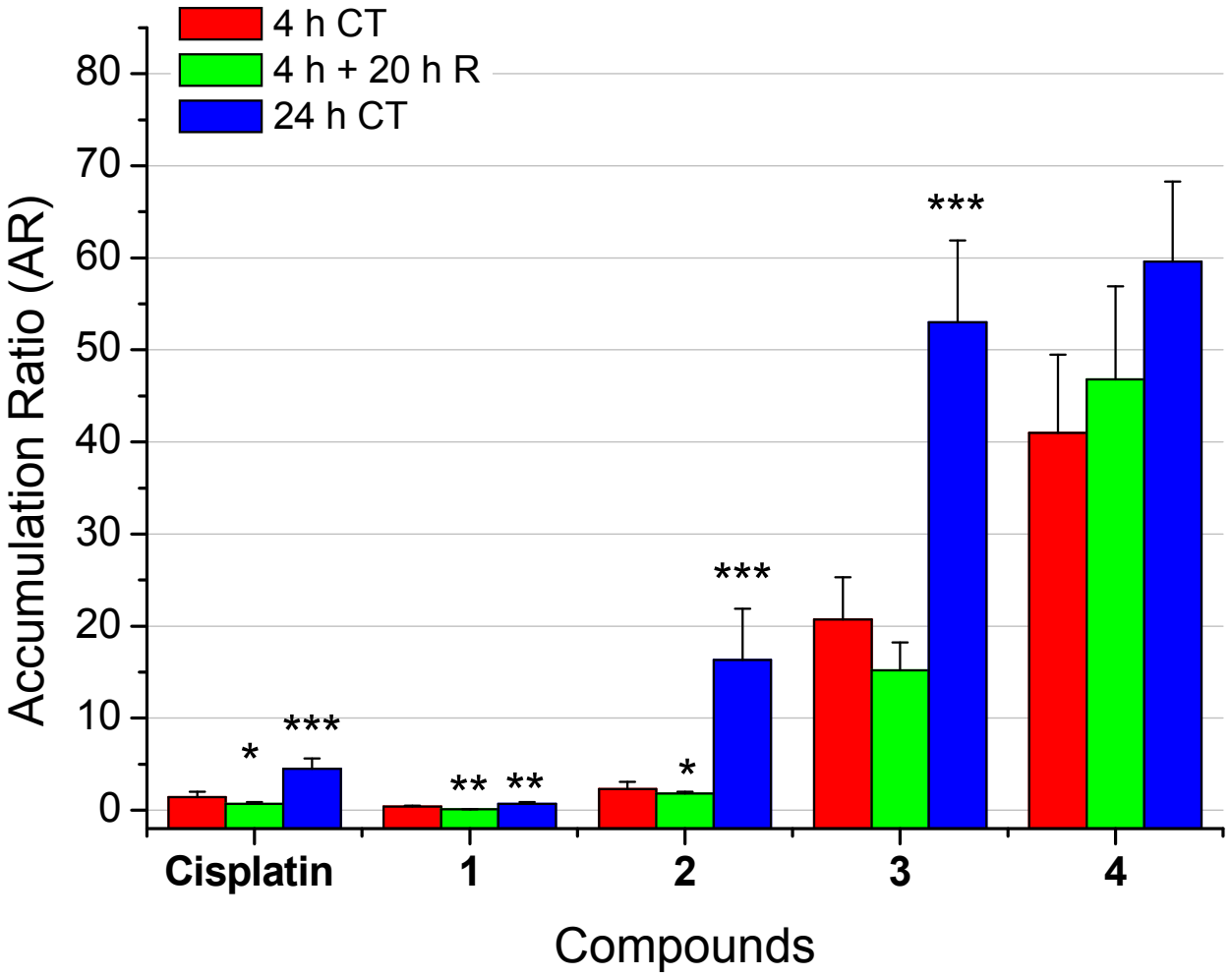
The relationship between lipophilicity, cell accumulation, DNA platination and antiproliferative activity (ovarian A2780) of a small series of homologous complexes has been studied and compared with that of cisplatin.





Accumulation Ratio (AR)

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



AR of A2780 treated with 10 μM of all the Pt-based 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).

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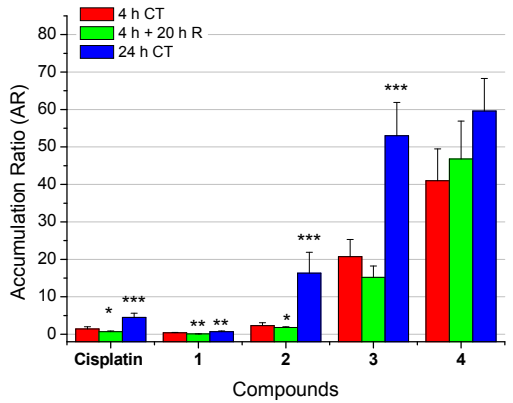
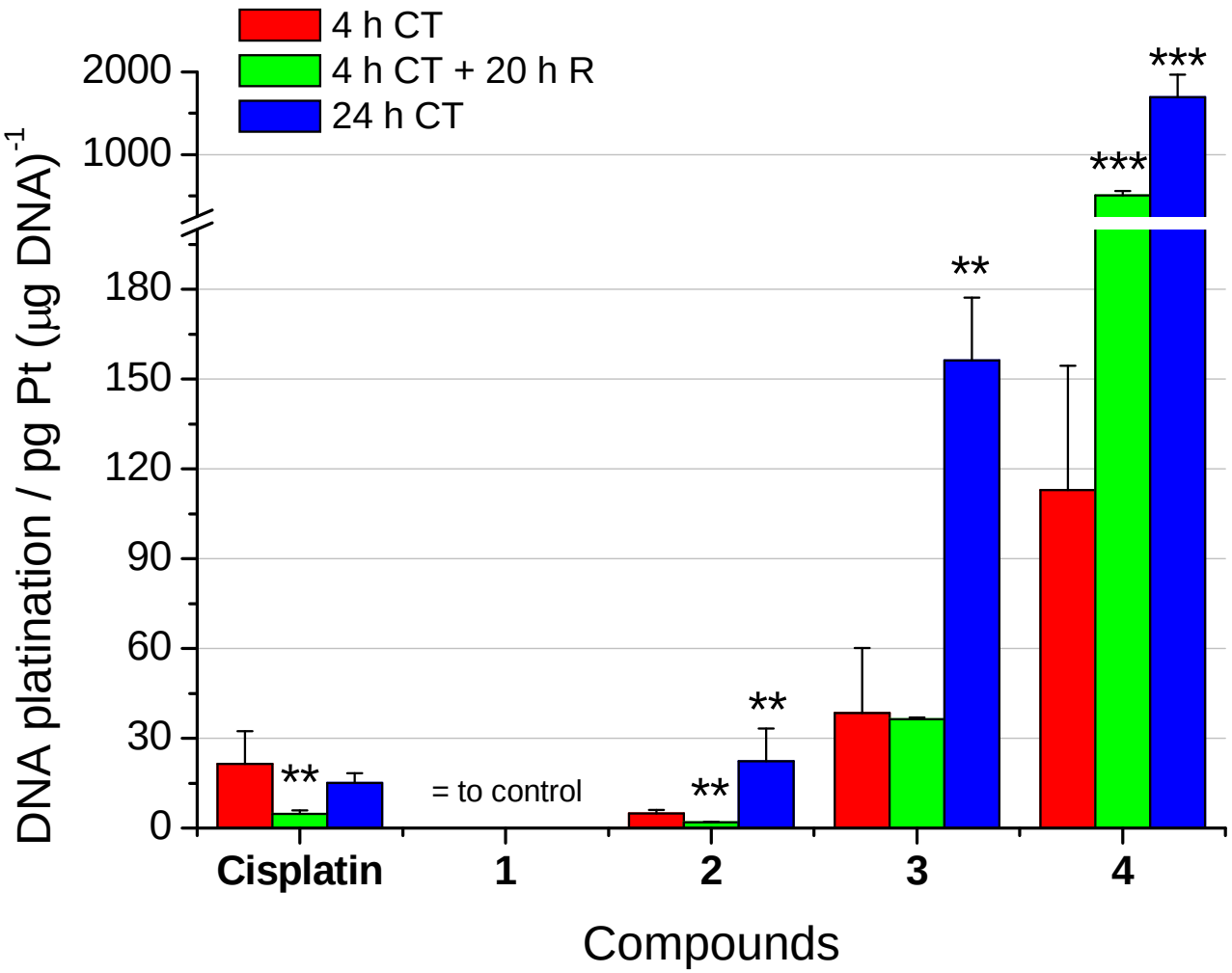
Accumulation Ratio (AR)



- Relationship between log $P_{o/w}$ and AR for **1-4**. → *passive uptake*
- Despite lower log $P_{o/w}$, AR of cisplatin is slightly higher than that of **1.** → *involvement of active uptake*
- AR of cisplatin and **1-3** increased from 4 h to 24 h CT. Complex **4** reached its max accumulation within 4 h. → *saturation*
- AR of cisplatin, **1** and **2** dropped during 20 h recovery (R). On the contrary, the AR of **3-4** remained almost unchanged during R. → *efflux seems to have almost no effects on the most lipophilic complexes*

DNA platination

DNA platination [pg Pt (μg DNA)$^{-1}$] of A2780 treated with 10 μM of the Pt-based complexes for 4 h CT, 4 h+ 20 R and 24 h CT.



Data are means ± SD of at least 3 independent replicates and were compared to those obtained for each drug after 4 h of treatment by means of the two sample t-test (*p<0.05;** p<0.01; ***p<0.001).

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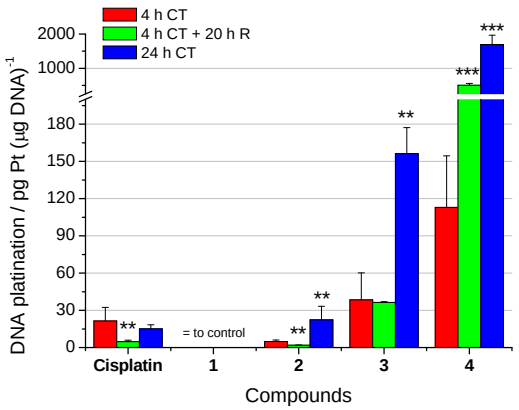
DNA platination



- DNA platination reflects AR for **1-4** (Pt = to control for **1**);

  ⇒ *DNA platination is ≈ to AR*

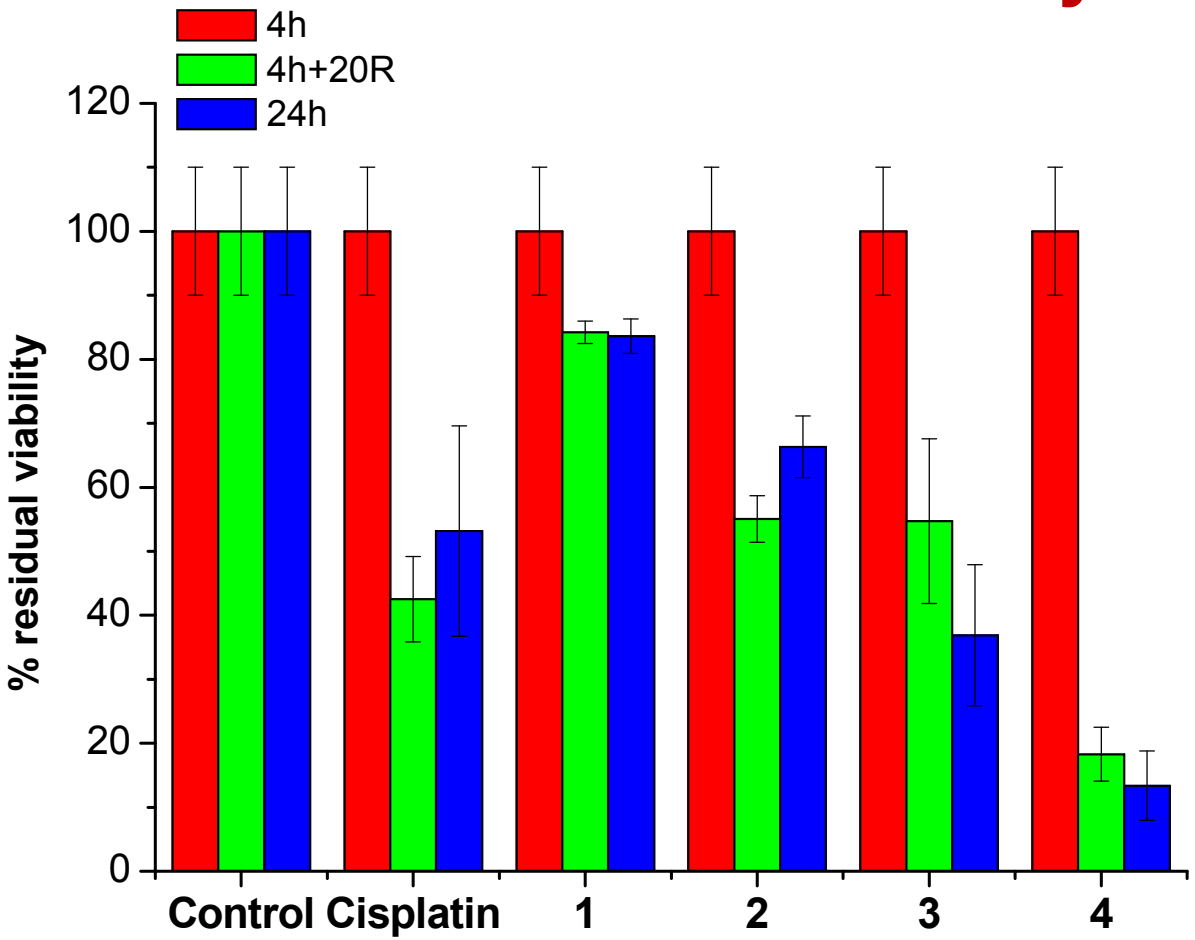
- For cisplatin, R decreased platination; CT maintained it;

  ⇒ *efflux and/or repair reduce platination?*

- DNA platination increased for **2-4** from 4 h to 24 h CT, but the R had different effects: **2** was significantly reduced, **3** was unchanged, while **4** was increased.

  ⇒ *the reduced efflux observed for more lipophilic complexes accumulate Pt inside the cell?*

Citotoxicity



% viability of A2780 cells treated with 10 μM of the Pt complexes.
Residual cell number was recorded after 4 h (red bars), 4 h + 20 h R (green bars); 24 h CT (blue bars).

Platination ì % viability

Citotoxicity

	IC_{50} [«M], A2780 cell line		
	4h CT + 68h R	24h CT + 48h R	72h CT
Cisplatin	2.4±0.2	1.5±0.9	0.43±0.07 (*)
1	48.7±19.2	12.3±2.2	8.8±1.3 (*)
2	4.3±2.2	0.7±0.3 (*)	0.42±0.08 (*)
3	0.32±0.13	0.031±0.025 (*)	0.015±0.007 (***)
4	0.034±0.022	0.0035±0.0041 (*)	0.0019±0.001 (*)

A more prolonged treatment increased the activity for all complexes, but in particular for the more lipophilic Pt(IV).

Considerations

- Higher the lipophilicity, higher the AR, higher the DNA platination, and then the potency (*passive diffusion*).
- The accumulation of compounds with low log $P_{o/w}$ is not enough to exert an important biological effect. Cisplatin may bypass this limitation through an active uptake.
- Compounds with high log $P_{o/w}$ are accumulated into the cells by both high uptake and reduced efflux.
- In the latter case, “Pt” may be stored in some intracellular trapping site and gradually released, leading to a prolonged cytotoxic effect.

…but how prolonged?

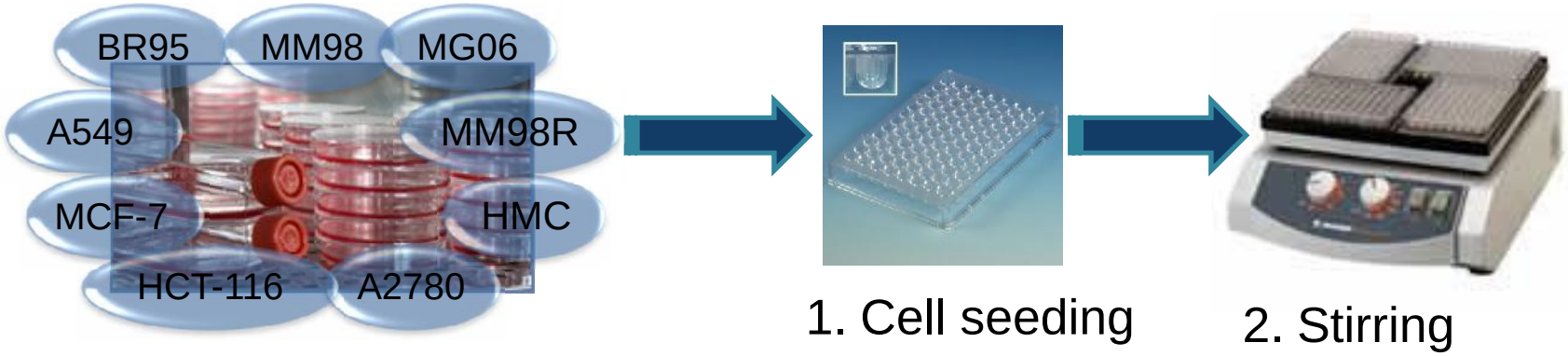
2D *vs.* 3D cell cultures

2D methods are limited by cellular confluence to few days of treatment.

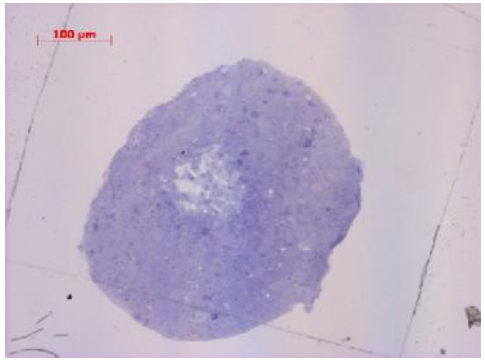
Multicellular tumor spheroids (MCTS) were used to perform drug screening for prolonged periods.

The 3D architecture better reproduces a “true” tumor (cell–cell and cell–matrix interactions, proliferating/necrotic areas, etc.).

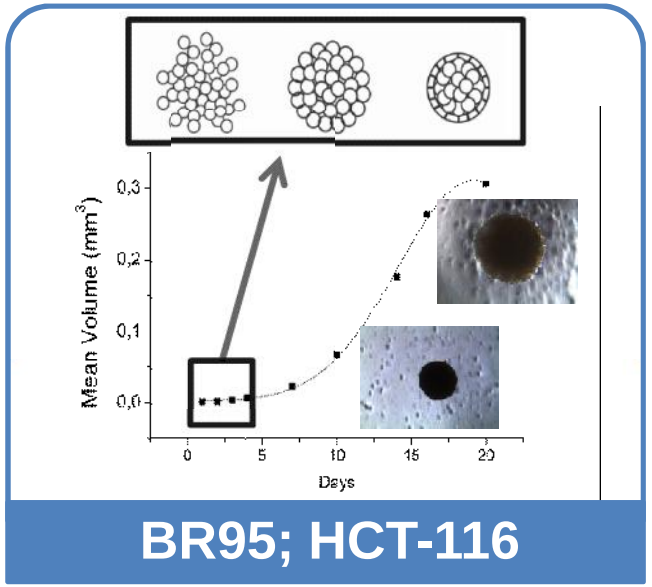
MCTS may simulate the drug penetration into the tumor tissue.



Multicellular tumor spheroids

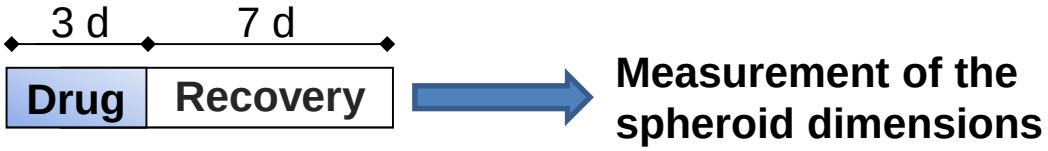


Spheroid obtained from BR95 (malignant pleural mesothelioma) cell line.



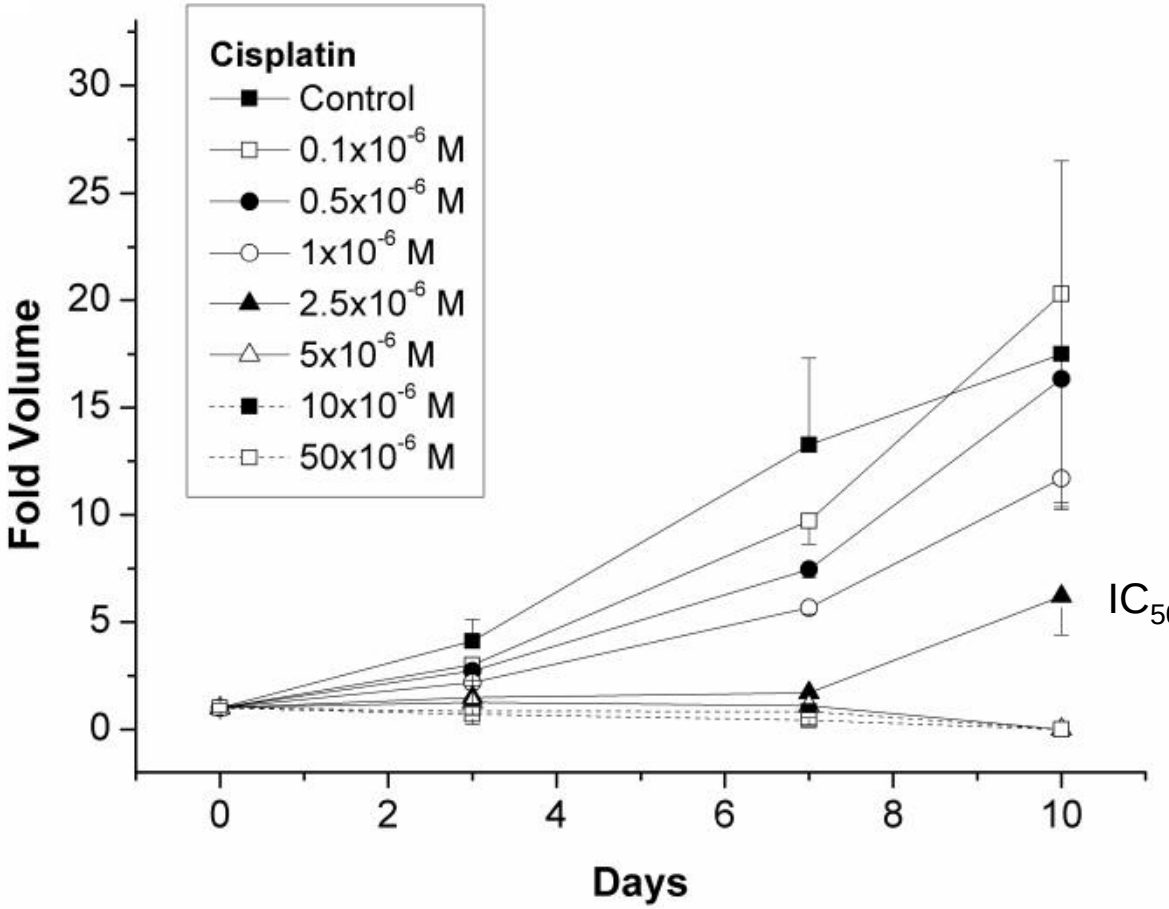
BR95 = malignant pleural mesothelioma
HCT-116 = colon carcinoma

Treatment protocol:



Treatment of HCT-116 MCTS with cisplatin

(IC_{50} monolayer HCT-116 = 2.3±0.3 μM, 72 h CT)

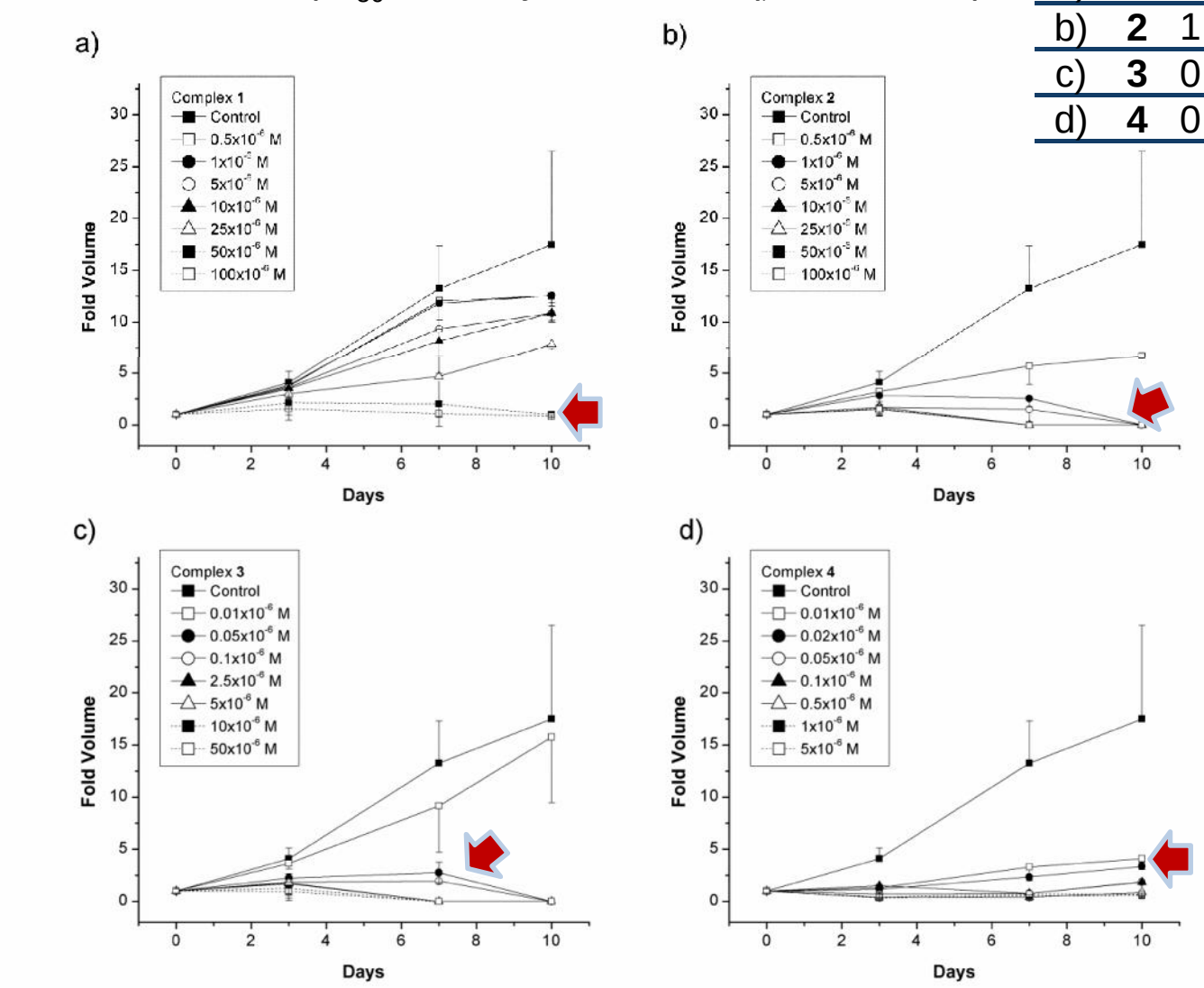


IC_{50} monolayer

Treatment of HCT-116 MCTS with 1-4

(IC_{50} monolayer HCT-116 (μM, 72 h CT) =

a)	**1**	55.3±1.7
b)	**2**	1.8±0.3
c)	**3**	0.042±0.010
d)	**4**	0.0090±0.0005



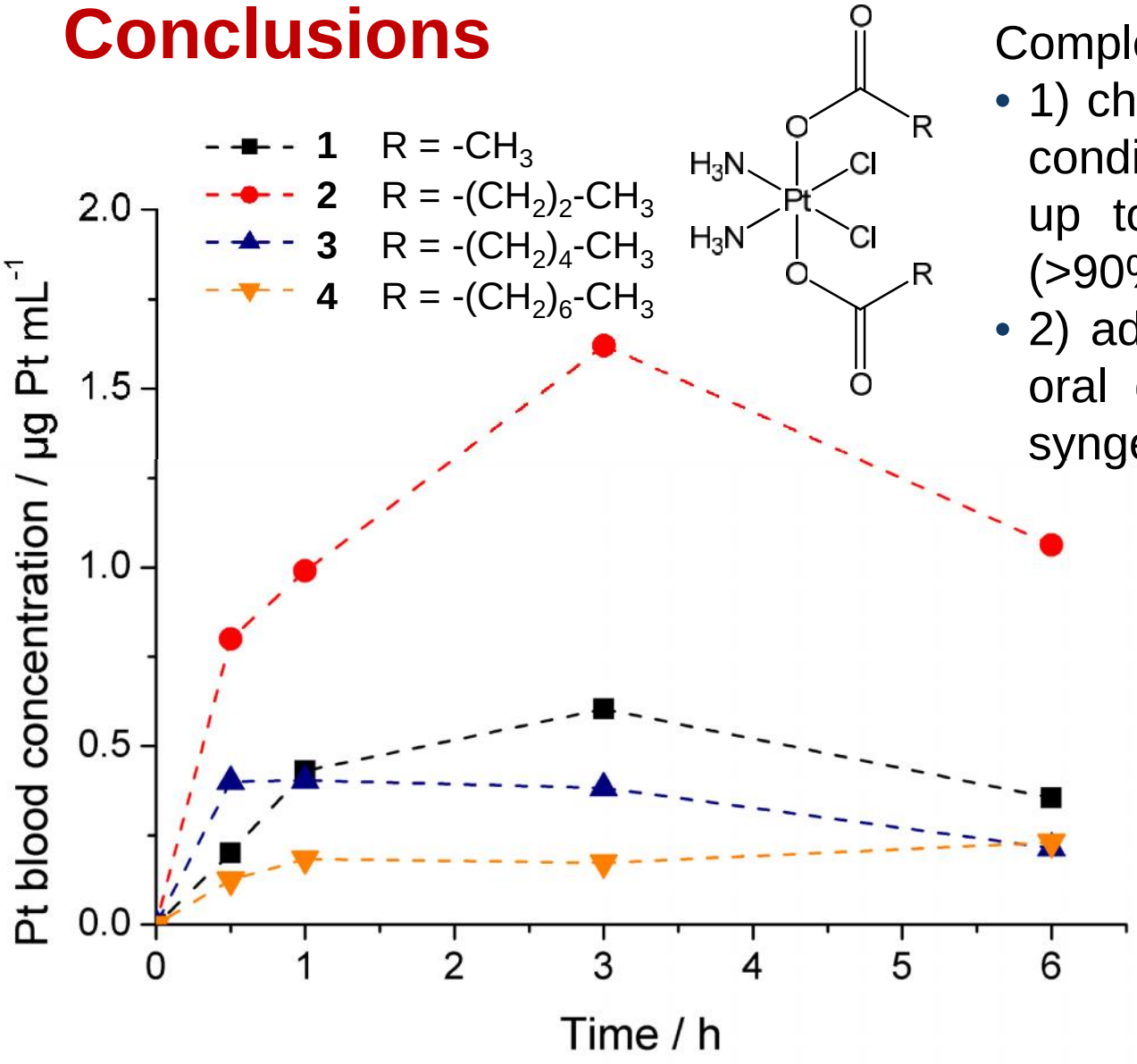
Conclusions

- Cisplatin and complexes **1-4** gave a concentration-dependent response in HCT-116 MCTS with a potency in the order **1** < cisplatin = **2** < **3** < **4** as observed for 2D experiments;
- Complexes **1-4** exert a prolonged antiproliferative action even when the drug is removed from the culture medium;

…but…

- **Preliminary *in vivo* data show that too high lipophilicity is detrimental of the oral absorption (*work in progress*).**

Conclusions



Complexes **1-4** were:

- 1) challenged in different pH conditions (from 1.0 to 9.0 up to 24 h): high stability (>90%).
- 2) administered in saline by oral gavage to a cohort of syngeneic FVB female mice.

Total [Pt] in whole 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 for each time point. SD are omitted for sake of clarity (ca. ± 22%).

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Thank you for your attention!