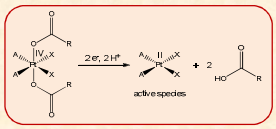
Relationship between cellular accumulation, DNA platination and antiproliferative activity for a series of Pt(IV) complexes: the effect of the length of the axial ligand

E. Perin,[1] M. Alessio,[1] I. Zanellato,[1] I. Bonarrigo,[1] E. Gabano,[1] M. Ravera,[1] D. Osella[1]

[1]*Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "Amedeo Avogadro", Viale Teresa Michel 11, 15121 Alessandria (Italy); elena.perin@mfn.unipmn.it*

Introduction

The antitumour activity of Pt(II) drugs, after their cell entrance, is related to their ability in making adducts with nucleic acids, which in turn correlates with their cytotoxicity. It is generally accepted the hypothesis that the reduction to their parental Pt(II) complexes is the basis of the antitumour activity of the Pt(IV) compound (*Figure 1*). This is the reason for which Pt(IV) complexes are considered Pt(II) prodrugs and they can be selectively activated in the hypoxic and reducing *milieu* of tumour cells (*Figure 2*).



The reduction of the Pt(IV) complexes to their Pt(II) metabolites takes place mainly within the cells due to species such as ascorbate or glutathione (GSH), present in higher concentration than in the extracellular fluid, and implies the release of the axial ligands .

Figure 1. Generally accepted Pt(IV) complexes mechanism of action: the so-called "activation by reduction"

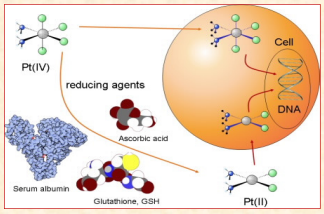


Figure 2. Fate of a Pt(IV) drug, once inside the cell

The resulting Pt(II) active species binds to their main biological target, DNA. The greater kinetic inertness of Pt(IV) complexes, compared to Pt(II) compounds, results in a greater resistance to side reactions with biomolecules or gastric juices: this feature allows the drug to be orally administered. Furthermore, the choice of the coordinated ligands Pt(IV) complexes is fundamental to tune the chemico-physical properties, such as lipophilicity ($logP_{o/w}$) and reduction potential, of these complexes.

Synthesis

In the presence of hydrogen peroxide we can assist to the oxidation of cisplatin (*Figure 3*). The resulting diidroxido-complex reacts with an anhydride in DMF to synthesize a series of homologous dicarboxilato-compounds characterized by an increasing chain length of the axial ligands (**1**, **2** and **3**). Compound **4**, instead, derives from the reaction between the diidroxido-complex and octanoyl chloride in acetone, in presence of pyridine.

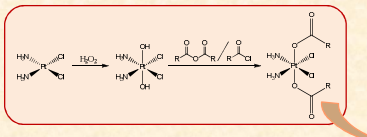


Figure 3. Synthesis scheme: cisplatin oxidation and subsequent reaction to form dicarboxilato-complexes

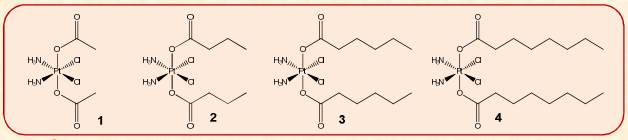


Figure 4. Scheme of the Pt(IV) complexes under investigation

Relationship between lipophilicity and solubility

$$logP_{o/w} = log\left(\frac{[C]_o}{[C]_w} \times \frac{V_o}{V_w}\right) = log\left(\frac{[C]_{in\ octanol} - [C]_{in\ final}}{[C]_{in\ final}} \times \frac{V_{oct}}{V_w}\right)$$

$$k' = \frac{t_R - t_0}{t_0}$$ [b]

Compound	Water volume (mL)	Octanol volume (mL)	$logP_{o/w}$ [a]	t_R (min)	$logk'_{90}$	Solubility (mM)
1	1.0	19.0	-1.92	2.60	-0.4	0.60 ± 0.01
2	5.0	5.0	-0.39	2.73	-0.33	0.13 ± 0.05
3	9.0	1.0	1.14	3.04	-0.2	(2.0 ± 0.1) x 10^{-3}
4	-	-	-	3.74	4x10^{-3}	< 0.8 x 10^{-4}

Table 1. $logP_{o/w}$ determined by shake-flask method, $logk'_{90}$, and solubility values of complexes **1**-**4**

$logP_{o/w}$ = 4.14

[a] cisplatin $logP_{o/w}$ value is -2.27.
[b] t_R is the retention time of analyzed species and t_0 is the one of the unretained compound (KCl in this case).

The lipophilicity of a drug is usually evaluated by means of the *n*-octanol/water partition coefficient, $logP_{o/w}$ (*n*-octanol is a rough model of the cell membrane and water represents the fluid inside and outside the cells).
Since RP-HPLC retention is due to partitioning between (polar) mobile and (apolar) stationary phases, there is a correlation between $P_{o/w}$ and HPLC capacity factors k'.

$$logP_{o/w} = m\ logk'_0 + q$$

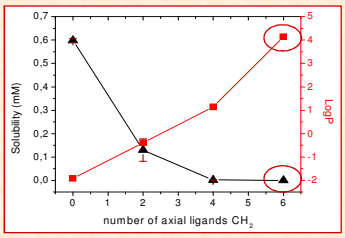
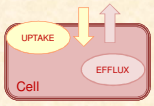


Figure 5. Correlation between lipophilicity and solubility of the studied Pt(IV) compounds

Accumulation Ratio (AR)



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

- Despite lower log $P_{o/w}$, AR of cisplatin is slightly higher than that of **1**.
- AR of cisplatin and **1**-**3** increases from 4h to 24h CT. Complex **4** needs only 4h to reach maximum accumulation.
- AR of cisplatin, **1** and **2** drops during 20h R. On the contrary, AR of **3**-**4** remains almost unchanged during R.

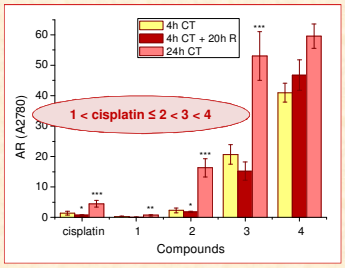


Figure 6. AR values for the ovarian cell line A2780 treated with 10 μM of all Pt-based complexes for 4h CT (continuous treatment), 4h + 20h R (recovery) and 24h CT. Data are means ± SD of at least 3 independent replicates and are compared to those obtained for each drug after 4 h CT by means of the two sample t-test (*p<0.5;** p<0.01; ***p<0.001)

DNA Platination

$$DNA\ Platination = \frac{\mu g\ Pt}{\mu g\ DNA}$$

- DNA platination reflects AR data for **2**-**4**;
- For cisplatin, R decreases platination, whereas CT maintains it;
- DNA platination increases for **2**-**4** from 4h to 24h CT, but the R has different effects: **2** is significantly reduced, **3** is unchanged, while **4** is increased.

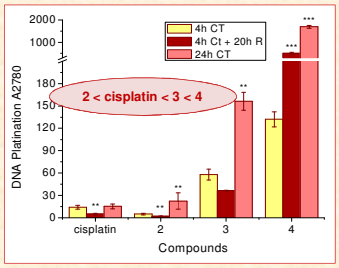


Figure 7. DNA platination for the ovarian cell line A2780 treated with 10 μM of all Pt-based complexes for 4h CT, 4h + 20h R and 24h CT. Data are means ± SD of at least 3 independent replicates and are compared to those obtained for each drug after 4 h CT by means of the two sample t-test (*p<0.5;** p<0.01; ***p<0.001)

IC_{50} (μM)

Compound	A2780	HCT-116
Cisplatin	0.5 (± 0.1)	2.3 (± 0.3)
1	12.1 (± 5.2)	55.3 (± 1.7)
2	0.5 (± 0.1)	1.8 (± 0.3)
3	0.015 (± 0.007)	0.042 (± 0.001)
4	0.0023 (± 0.0006)	0.0090 (± 0.0005)

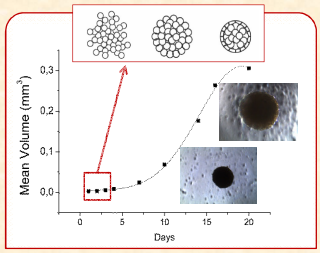
Table 2. IC_{50} values of all Pt(IV) complexes synthesized and their comparison with cisplatin, for a 72h CT on two cell lines, A2780 (ovarian carcinoma) and HCT-116 (colon carcinoma)

IC_{50} (*half inhibiting concentration*), or potency, is defined as the drug concentration capable of reducing by 50% cell vitality

2D vs. 3D cell cultures: the advantages of MCTS

2D methods are limited by cellular confluence to few days of treatment. On the contrary, multicellular tumor spheroids (MCTS) were used to perform drug screening for prolonged periods. The 3D architecture better reproduces a "true" tumor: thus, MCTS may simulate the drug penetration into the tumor tissue.

Only a few cell lines are able to give proliferating spheroids (*e. g.* HCT-116).



Treatment protocol

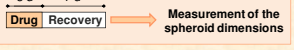
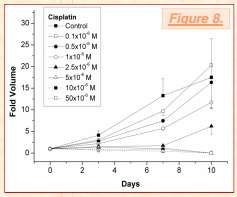


Figure 8.



- Cisplatin and **1**-**4** complexes (*Figure 8*, *9*, *10*, *11*, *12*) 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;

Figure 9.

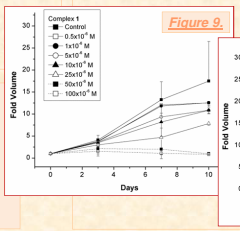
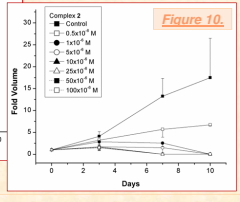


Figure 10.



- The activity of **4** is less dependent on the length of recovery than **1**-**3**, for which spheroid re-growth occurs at sub-effective concentrations

Figure 11.

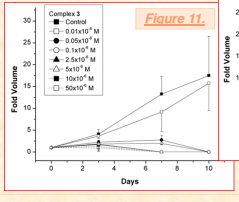
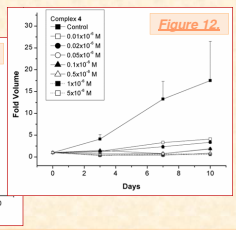


Figure 12.



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