A new class of unsymmetric Pt(IV) antitumor prodrugs

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Introduction

The platinum(II) complexes are the most important drugs in anticancer chemotherapy. In recent years more attention has been paid to Pt(IV) complexes as anticancer prodrugs. These complexes can be reduced in vivo, through a two electron reduction, in the hypoxic, reducing environment of the tumor tissue so that the octahedral Pt(IV) complexes are transformed into their active square-planar Pt(II) metabolites by loss of the axial ligands. The Pt(IV) complexes exhibit greater chemical inertness than their Pt(II) counterparts and undergo fewer side reactions with biomolecules. The choice of the ligands is essential to modulate their lipophilicity (and related cellular uptake) and their redox properties. The axial ligands may also be biologically active molecules themselves or simply chemical linker between the Pt core and an active molecule. If the complex contains two axial functionalities, it will probably link two biomolecules or macromolecules leading to cross-links and possibly to the precipitation of the resulting diadducts. Therefore, it is useful to have two different axial ligands: the former can be used for the coupling with drug delivery vectors (biomolecules, nanoparticles, nanotubes, etc..), whereas the latter may be used to modulate the reduction potential (e.g. chloride can facilitate the reduction).

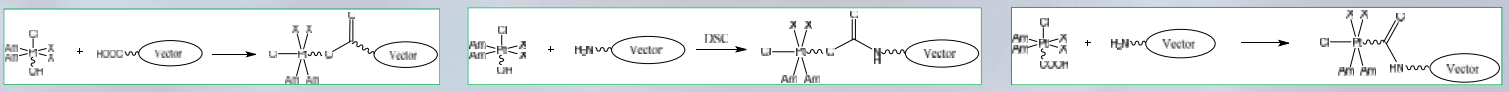


Figure 1 : General coupling reaction of a Pt(IV) complex to an appropriate vector

Synthesis

Pt(IV) complexes are usually prepared by oxidation of the corresponding Pt(II) counterparts, typically using hydrogen peroxide or chlorine. A different way to oxidize the Pt(II) compounds is represented by the use of N-chlorosuccinimide (NCS). The reaction between Pt(II) complexes and NCS in different coordinating solvents was set up to get the final asymmetric complex [PtA_2ClX_2(Solv)]. When the reaction are carried out in a non coordinative solvent in presence of another nucleophile the complex [PtA_2ClX_2(Nucl)] (Figure 2) can be obtained.

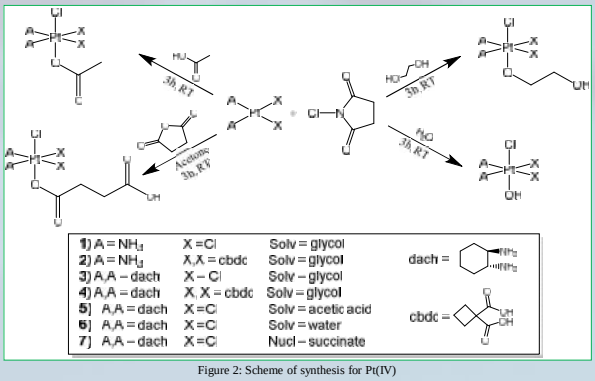
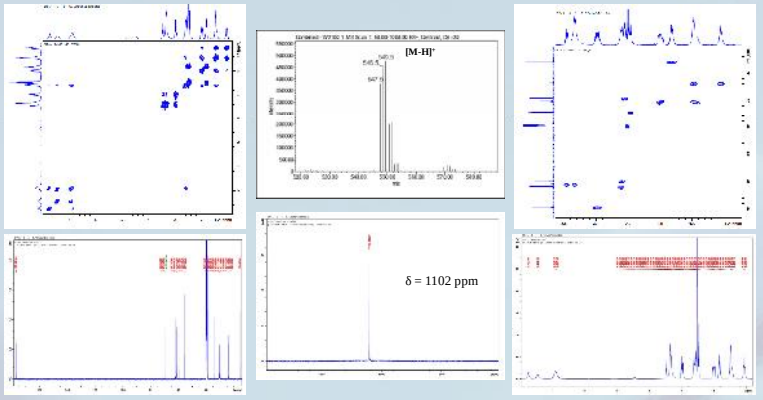


Figure 2: Scheme of synthesis for Pt(IV)

Characterization

All of these complexes have been characterized by HPLC-MS, mono- and bi-dimensional NMR spectroscopy (^{1}H, ^{13}C, ^{195}Pt, COSY, HSQC) and, only for [Pt(dach)Cl(glyc)(cbdc)] **(4),** by single crystal X-rays diffraction, to confirm the formation of Pt(IV) complexes with solvent moiety as axial ligand (Figure 3).



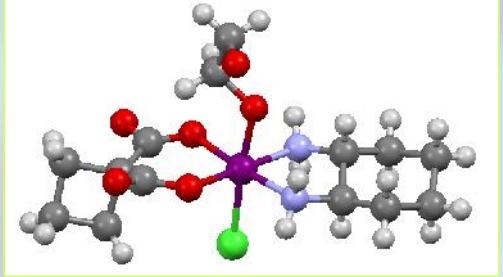


Figure 3: Example of characterization and molecolar structure of complex **4** (NMR in DMSO-d_6)

Stability and reductions

The stability of complexes **1**-**4** was studied both in carbonated water (pH = 6.4) and in HEPES buffer (pH 7.5) by monitoring the peak's area of the Pt(IV) over the time. Complexes **1** and **3**, with equatorial chloride, undergo fast hydrolysis, while complexes **2** and **4**, with cbdc ligand, are 100% recovered after 3 days. Moreover, **1** and **3** are more stable at neutral pH (1 t½ = 50 h, 3 t½ = 70 h) than at slightly acidic pH (t½ ca. 21 h for both complexes) (Figure 4).
Through ESI-MS spectra analysis (positive ion mode), aged solutions show that the first hydrolysis product contains water instead of glycol. This intermediate further loses chlorides that are replaced by water/hydroxyl groups (Figure 5).
The stable complexes **2** and **4** were challenged with the two most common biological reductants: ascorbic acid (AsA) and glutathione. HPLC data show that corresponding $t_{½}$ for complex **2** and **4** are 1.6 h and 2.4 h with respectively GSH and 13.1 h and 46.3 h with AsA.

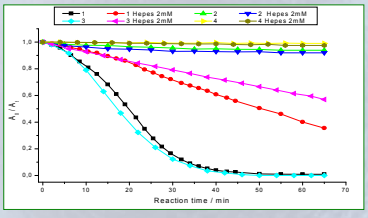


Figure 4: Normalized area (A_i/A_0) of the HPLC chromatographic peaks *vs.* aging time in water (pH 6.4) and HEPES buffer (pH 7.5).

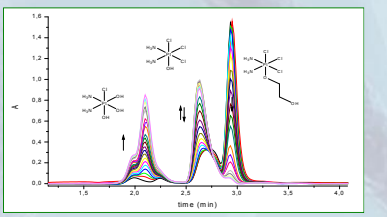


Figure 5: HPLC chromatograms of complex **1** vs. aging time (0 - 45 h) in water

Coupling Reactions

Complex **4** was used in coupling reactions with carboxylic acids and amines as models for useful in DTD strategies (Figure 6). Esterification of **4** was performed with uronium salts, in particular HBTU, as coupling agents in dry DMF (complexes **8** and **9**). Another type of coupling was performed with a modification agent, *N*,*N*'-disuccinimidylcarbonate (DSC), which is able to temporarily activate the hydroxyl group as a succinimidyl carbonate group, that can further react with an amine to form a stable carbamate. In both cases simple models protected amino acid (alanine) were used to test the reactivity of **4** with –COOH and -NH_2 groups (complexes **10** and **11**).

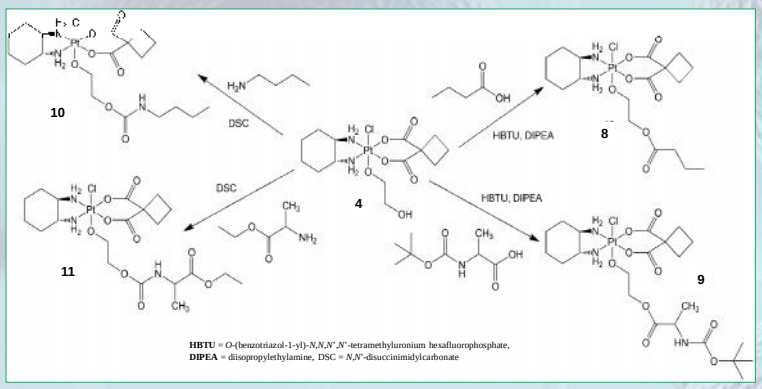


Figure 6: Scheme of the reaction between complex **4** and acids, amines or aminoacids.

Perspectives

In this work we have reported the synthesis of several new asymmetric platinum (IV) complexes with a chlorido and a solvent molecule as axial ligands. In particular, stable complexes **4** contains a functional group that can be used for the linking to a wide variety of vectors (e.g. . nanomaterial or bioactive species). The future of this work is the efficient coupling of this prodrug to molecules suitable in the context of the drug delivery strategy.

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