Kinetico-mechanistic study on the reduction/complexation sequence of PtIV/PtII organometallic complexes by thiol-containing biological molecules

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ABSTRACT:

The kinetics of the reaction of [PtIV(4X-Cph,N,N')Cl(Y)2] complexes (2-X-Y) (X=Cl or F and Y=OH or Cl) with biological thiols (glutathione, cysteine, thiolactic acid) and methionine, has been monitored by UV–Vis spectrophotometry. The reactions have been followed at varying pHs and chloride concentrations (within the physiological range) and different temperatures and pressures. The bis-chlorido derivatives, 2-X-Cl, have been found to react with cysteine, glutathione and thiolactic acid, while the bis-hydroxido 2-X-OH derivatives are not reduced due to the high potential of the PtIV/PtII pair, as measured in aqueous solution. The lack of reactivity of methionine is related with its tioether nature preventing deprotonation of the S donor. In all remaining cases, two consecutive reaction steps have been found to occur. For cysteine the two steps can be kinetically resolved, the first step being neatly related to a PtIV to PtII reduction and the second step corresponding to the substitution of the remaining Cl− ligand by cysteine. The nature of the second step has been also confirmed by ESI-MS, as well as by the associative character of the activation parameters determined (low $\Delta H^{\ddagger}$ and very negative $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$). For glutathione and thiolactic acid, the rate and thermal and pressure activation parameters for the reduction step has been found similar to that obtained for the reaction with cysteine, but the substitution step could not be resolved kinetically. The substitution step, as measured from the reduced [PtII(4X-Cph,N,N')Cl] complex, is faster than the reduction process, and also much faster than that observed for the reaction with cysteine. In both cases the final product resulting for the reduction reactions corresponds thus to the final substituted complex as found for the reaction with cysteine.

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1. INTRODUCTION

Since the discovery of the anticancer effects of cisplatin (cis- [PtCl2(NH3)2]) by Rosenberg, the antitumor activity of certain PtIV compounds such as cis-[PtCl4(NH3)2] was also noticed [1–3]. In recent years, platinum complexes with non-classical structures such as PtIV compounds or with different mechanisms of action than cisplatin have been thoroughly investigated [4–5]. Nevertheless, despite the worldwide application of PtII complexes such as cisplatin, carboplatin and oxaliplatin, there is no PtIV complex approved for clinical use so far [6]. Given the inertness of PtIV compounds, it is generally accepted that the PtIV compounds may be reduced in vivo by molecules present in the cell, such as glutathione, L-cysteine, L-methionine or ascorbic acid, to PtII compounds which, in turn, could exert their cytotoxic activity. Properly designed PtIV complexes display several advantages since their inertness diminishes side effects and prevents deactivation before entering the cancer cell, and their higher lipophilicity renders them more suitable for oral administration. The prototype of PtIV anticancer agents involves, in addition to two non-leaving groups and two substitutionally active groups as for cisplatin analogues, the presence of two axial ligands which can dissociate after biological reduction. These ligands can be used to modulate the reduction parameters, kinetic stability, lipophilicity and pharmacological properties of the prodrug. Therefore, a versatile strategy is to combine clinically relevant PtII drugs with adequate axial ligands [2,6–12]. A common route to introduce these two additional axial ligands consists of a two-electron oxidation of a PtII precursor and the most widely used oxidizing agents are hydrogen peroxide and chlorine, which give trans addition products [3].

Since PtIV compounds act as prodrugs which are activated by reduction, the reduction potential is a key pharmacological parameter to predict the activity of these compounds. On one hand, very high redox potentials might lead to straightforward fast reduction and severe side effects, as those found for cisplatin, thus representing a serious drawback despite its original more benign design. On the other hand, a lack of anticancer activity might be related with too low reduction potentials which would keep the complex with rather inert characteristics, thus not allowing its interaction with the DNA molecule [7]. The reduction potentials are dependent on the nature of both axial and equatorial ligands, but in general the effect of the leaving axial ligands is more relevant. In particular, for a series of PtIV compounds with axial chlorido, acetato and hydroxido ligands, the ease of reduction follows the trend Cl > OAc > OH [13,14]. Hydroxido ligands possess strong electron donating properties and the resulting complexes are difficult to be reduced [15].

It was originally assumed that a correlation exists between the biological activity of the PtIV prodrugs, the reduction potentials and the rates of reduction. However, the reduction potentials have been found to not necessarily correlate with the rates of reduction of PtIV complexes [16,17]. In fact, the rates of reduction depend on the ability of the ligands in the coordination sphere of the PtIV species to associate with the reducing agent which facilitates the electron transfer [18]. Moreover, although the reduction of PtIV complexes produces the loss of axial ligands to form the corresponding square-planar PtII

complexes [19], several studies indicate that reductants such as glutathione might coordinate to the resulting PtII species, thus resulting in an already substituted cisplatinum analogue that might, or might not, interact with the DNA molecules [20–22]. Therefore, in spite of the great deal of attention recently devoted to PtIV antitumor drugs, more studies aimed at disclosing the nature and substitutional reactivity of the produced PtII compounds are needed. By doing so, it should be possible to fully understand the precise mechanisms of the reduction process and thus develop a rational design of new compounds with better pharmacokinetic tuning properties which have been found to be the keystone for its activity.

On the other hand, recent studies indicate that organometallic compounds are promising anticancer agents in spite of the initial idea that these compounds would be unstable under physiological conditions. The presence of some strong M−C bonds improves the stability of these compounds and greatly influences the lability of the other bonds present. In addition, organometallic compounds are easily modified and thus suitable for the establishment of structure–activity relationships [23–25]. In particular, cyclometallated compounds [26] are appealing since they are easily obtained for a wide range of different ligands and metals; furthermore, they can be rather stable versus hydrolysis in aqueous media and present a good tuning of the polarity needed for going across membranes. In this respect the careful choice of complexes with just one Pt-C bond is extremely relevant for the maintenance of the substitutional associative activation of the final PtII complex formed, thus enabling its reactive discrimination among the wealth of potential substituting ligands in the reaction medium.

Following our interest in cyclometallated platinum compounds, we have recently shown that PtIV metallacycles present cytotoxic properties [27], and, on the other hand, they are adequate platforms for kinetico-mechanistic studies of oxidative addition and reductive elimination processes [28]. Therefore, cyclometallated PtIV compounds are excellent candidates to study their reactivity towards reductants such as glutathione and other biologically relevant thiols. Furthermore, the resulting PtII species can be independently prepared and its substitutional activity also monitored under the same conditions. Although several kinetico-mechanistic studies of reactions of PtIV compounds with this type of reductants have been reported [19,29–33], to the best of our knowledge this is the first one which involves cyclometallated compounds which can be easily tuned, both in the reduction potential and in the substitutionally reactivity aspects of the reduced PtII species obtained. The aim of this work is to use [C,N,N′] cyclometallated compounds as models to establish the reactivity sequence of PtIV compounds with biologically relevant reductants in order to provide a deeper understanding of these processes so that more efficient PtIV prodrugs can be designed.

2. RESULTS

2.1. Compounds

The syntheses of [C,N,N'] cyclometallated PtII compounds containing a chloro (1-Cl in Scheme 1) [34] or a fluoro (1-F in Scheme 1) [35] substituent have been previously reported. As for cisplatin, these cyclometallated PtII compounds contain two substitutionally active positions: the chlorido ligand and the dimethylamino moiety of the terdentate [C,N,N'] ligand. This has been evidenced upon the reactions of 1-Cl with monodentate and bidentate phosphines which lead respectively to dissociation of the NMe2 moiety to produce a neutral compound, or to dissociation of both the NMe2 and the chlorido ligand to produce an ionic compound [34]. Oxidative addition of either Cl2 or H2O2 on these compounds has been carried out in this work following reported procedures [36,37] and gave the desired cyclometallated PtIV compounds 2-Cl-Cl, 2-Cl-OH and 2-F-Cl depicted in Scheme 1.

The structure of compound 2-Cl-Cl was determined (CCDC deposition number 1828170) by single crystal XRD and is shown in Fig. 1. As expected from NMR studies, the platinum atom displays an octahedral coordination with a meridional tridentate [C,N,N'] ligand. An equatorial and two axial chlorido ligands complete the coordination around the platinum, and the axial chlorido ligands form a Cl-Pt-Cl angle of 174.55 °. Although three distinct Pt-Cl bond distances are observed, all values are in the same range within experimental error and do not give conclusive evidence of the relative strength of these bonds. The main distortion from the ideal octahedral coordination is due to the small bite angle of the metallacycles (80.9 (2) °). The metallacycle is flat and nearly coplanar with both the coordination plane and the mean plane of the (N,N') chelate. A comparison of the bond distances with those for previously reported PtII compound 1-Cl reveals [34] that the equatorial Pt-Cl, Pt-Namine and Pt-C bond lengths are moderately longer, and the Pt-Nimine bond length decreases for the PtIV compound. As a result, the Pt-Namine distance is consistently longer than the Pt-Nimine distance, which suggests a weaker bond with the NMe2 moiety.

The 1H NMR spectrum of compound 2-Cl-Cl in CDCl3 show the features expected, in particular both NMe2 and imine protons are coupled with platinum and the J(H-Pt) values are smaller than those of the parent PtII compound. Analogous results were obtained for the 1H NMR spectrum recorded in d6-DMSO; addition of deuterated water to the NMR sample in d6-DMSO did not produce significant changes. Moreover, the addition of an excess of NaCl did not produce any changes in the NMR spectrum which, in all cases, only shows one set of resonances. Analogous results were obtained for 2-F-Cl and 2-Cl-OH. According to the NMR studies, all three compounds are stable in the DMSO-water media used for the kinetic study.

In this work, complexes 2-Cl-Cl, 2-Cl-OH and 2-F-Cl were investigated by cyclic voltammetry in DMSO-D2O solution. All compounds showed an irreversible reduction peak, a common feature for

PtIV compounds resulting in loss of the axial ligands. As previously observed for non-cyclometallated similar compounds [1], reduction occurs most readily for chlorido than for hydroxido axial ligands (see Table 1 and Fig. 2). In contrast only a small difference of the potential is obtained upon changing a chloro for a fluoro substituent in the meridional [C,N,N'] ligand with the fluoro derivative 2-F-Cl being slightly less stable towards reduction. Consecutive cycles also shown the presence of an oxidation signal around 1.25 V associated to the oxidation of some reduced platinum complex, as described in similar systems.

2.2. Reaction with cysteine and methionine

The reactivity of the PtIV complexes shown in Scheme 1 with cysteine and methionine have been studied kinetically at pH values between 6.5 and 8.0, using HEPES buffer at 0.1M ionic strength (NaClO4); different chloride concentrations were also used, as described in the Experimental section. As it may be expected from the values determined for the reduction potential of the PtIV complexes (see before, Table 1), only the bis-chlorido derivatives of the organometallic skeleton were found to be reactive under the conditions of the study; the bis-hydroxido complexes were unreactive under the same conditions. The lack of reactivity of the bis-hydroxido compound 2-Cl-OH was confirmed by 1H and mass spectra (see Experimental section) of the residue obtained after reacting for 24 h with an excess of the biomolecules in a DMSO-water solvent mixture.

Furthermore, although the reaction with cysteine was readily monitored for the chlorido 2-Cl-Cl and 2-F-Cl derivatives, all attempts to have these complexes reacted with methionine were unsuccessful, the UV–Vis spectrum of the initial bis-chlorido PtIV complexes were found invariable for hours at room temperature at pH=7.0. Fig. 3a shows the typical spectral changes observed for the chlorido reacting systems (2-Cl-Cl and 2-F-Cl). From the time-resolved spectral changes, and using the Specfit or ReactLab software, two consecutive steps could be assigned to the reactivity sequence observed. The pattern of the spectral changes agrees with an initial PtIV to PtII reduction (decrease of the charge transfer band at ca. 300 nm and increase of that at ca. 350 nm), followed by a reaction (probably substitution, see below) occurring on the PtII derivative produced.

The values of the pseudo-first order rate constants observed (kobs1=kobs red; kobs2=kobs subst) were found to depend linearly on the cysteine concentration, as well as on pH, as shown in Fig. 3b; no significant intercept has been observed. From these plots the second order rate constants (kred and kPtII subs) indicated in Table 2 were obtained, and from its dependence with temperature and pressure the thermal and pressure activation parameters, also shown, were derived.

Given the fact that the reduction with thiol-containing biomolecules on similar PtIV [PtCl4(NN)] complexes is known to take place via a reductive elimination process [18] leading to RSCl molecules,

plus chloride, and the corresponding [PtIICl2(NN)] compounds, the reactivity of the corresponding PtII reduced complexes 1-Cl and 1-F with cysteine was also pursued. In all cases the spectral changes observed for the reaction of the compounds with cysteine at pH=7.0, under the same conditions than those used for the reduction of the PtIV complexes, match exactly with the second step observed. Even the rate constants derived agree within error with those obtained for kobs2, being thus clear that the full process shown in Fig. 3a corresponds to the expected sequence of reduction reaction plus substitution indicated above Scheme 2). The associative character of the activation for the substitution process is also corroborated by the thermal and pressure activation parameters associated, shown in Table 2 (low $\Delta H^{\ddagger}$ and very negative $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$). Interestingly, the differences in the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the substitution processes can only be associated with changes at the peripheral site of the bound ligand. This fact suggests that the resonance effect of fluorine is de facto increasing the electron density of the platinum centre in the para position, thus making the substitution processes less associative (higher $\Delta H^{\ddagger}$ and less negative $\Delta S^{\ddagger}$). The product of the reaction of the PtII compound 1-Cl with cysteine in DMSO-water mixtures was too insoluble to be characterised by 1H NMR, but the final residue obtained after reacting for 24 h a 1:2 (PtIV:cysteine) mixture was analysed by ESI (+)-MS {H2O:CH3CN (1:1)}. Its mass spectra shows a peak at 539.0926 with an isotopic distribution corresponding to [Pt(CNN)(cysteine)] (calc. for C15H23ClN3O2PtS 539.043) [M−Cl+cys]+.

The dependence of the second order rate constants of the PtIV complexes reduction, as well as those for the PtII substitution, on pH was also studied for a comprehensive understanding of the reactivity observed. The pH-dependence is similar for both processes, and is associated with the deprotonation of the –SH group of cysteine at pHs close to its pKa (≈ 8.0); Fig. 4a shows the trend observed for the reduction process as an example. No further quantification of these trends has been carried out due to the fact that pKa values are much higher than the physiological pH range where the systems have been studied, Fig. 4a being the initial branch of a typical pKa titration curve. With respect to the dependence on chloride added to the reaction medium, Fig. 4b indicates clearly that for the PtIV complex reduction process no significant dependence is observed, in line with the absence of substitution reactions occurring on PtIV compounds containing a single Pt-C bond within the reduction time range [38–40].

2.3. Reaction with glutathione

Once the reduction of the PtIV complexes with cysteine was found relevant for the generation of more substitutionally active organometallic PtII complexes, the use of glutathione (another reducing biomolecule) was also studied with complex 2-Cl-Cl using the same methodology. Given the fact that the biomolecule reacts with DMSO (in DMSO-water mixtures as those used in this study), the concentration conditions to be used had to be carefully screened. Effectively, under the concentration conditions used for the reaction with cysteine, although the reduction process from PtIV is obvious from

the pattern of the UV–Vis spectral changes, the process is masked by a secondary slower reaction, occurring even in the absence of the platinum complex. As a consequence, the platinum complex and glutathione concentrations were diminished to 25–33% of those previous used. Under these conditions a clearly defined PtIV to PtII process is observed (kobs=kobs red, Fig. 5a), which could be resolved from the secondary glutathione- DMSO/water reaction, observed as a small drift in the final absorbance readings as described in the Experimental section (Fig. 5a, inset). Fig. 5b collects the linear dependence of the pseudo-first order rateconstants for reaction with concentration of the reductant, chloride and pH. The trends observed fully agree with those of the reaction with cysteine with no significant intercept observed. In order to ascertain any possible substitution by glutathione on the PtII reduced complex, the reaction of the PtII complex 1-Cl with glutathione was pursued under the same concentration conditions at pH=7.0 and 25 °C. Fig. 5b also shows the glutathione concentration dependence observed for this process, kobs=kobs subs. Interestingly, the substitution reaction monitored is much faster than the reduction process indicated above, which implies that the final product upon reduction of the 2-Cl-Cl PtIV is, de facto, the 1-Cl PtII substituted complex. Table 3 collects the relevant kinetic and thermal activation parameters for the two series of reactions observed.

2.4. Reaction with thiolactic acid

Finally the reaction on compound 2-Cl-Cl with a smaller and less acidic thiol with no amine groups, thiolactic acid, was also tried at pH=7.0. The purpose was to study the possible changes of activity due to its pKa, the effect of the absence of amine groups in the biomolecule, and the possible chelation on the final reduced 1-Cl PtII complex formed. As a whole, the time-resolved spectral changes proved to be much slower, and much more complex, than for the systems studied before, once the parent PtII 1-Cl complex was considered. For the simple reduction reaction of the 2Cl-Cl PtIV compound, a final decomposition of the biomolecule under the conditions of the study had to be quantified in the absence of PtIV compound (and further eliminated as explained in the Experimental section from the reactivity pattern) as already done for the glutathione molecule. Even under these conditions, though, the spectral pattern of a single PtIV to PtII reduction reaction was clearly observed (Fig. 6a), and the corresponding pseudo-first order rate constants, kobs=kobs red, were derived by the standard Specfit or ReactLab software [41,42]. From its linear variation with thiolactic concentration, the values of kred at different temperatures (Fig. 6b) and pressures were derived, as well as the associated activation parameters at pH=7.0 (Table 4).

In view of the data obtained, it is clear that either no reaction of thiolactic acid occurs on the 1-Cl PtII reduced compound or this is faster that the reduction process determined, as found for glutathione (see before). Consequently the reaction of the PtII complex with thiolactic acid under the same conditions was pursued (avoiding the final decomposition of the biomolecule). Fig. 7a shows the spectral changes

associated with a set of two consecutive reactions observed; in Fig. 7b the dependence of the derived two pseudo-first order rate constants, kobs1 and kobs2 (kobs subst1 and kobs subst2), on the thiolactic acid concentration are also shown at pH=7.0 and 25 °C. Table 4 collects also the summary of these data.

From the data is seems clear that the process associated with kobs1 is a clearly defined substitution process on the PtII 1-Cl species. Nevertheless, the reaction associated with kobs2 has to correspond to a chelation of the thiolactic molecule on the PtII species independent on the concentration of ligand; the smaller bite angle with the already deprotonated carboxylate moiety of the molecule can easily explain this fact.

3. DISCUSSION

From the data collected in the Results section (plus the available data corresponding to the acidity and redox characteristics at pH=7.0 of the free biomolecules used in this study and collected in Table 5), it is clear that the observed lack of reactivity of the methionine molecules with the PtIV complexes could not be associated with the reduction potential. Methionine is, in fact, the most readily oxidised of the thiol derivatives used [43], which indicates, as stated before, that the thermodynamic oxidation of these biomolecules does not correspond to the processes by which the reduction of PtIV complexes are reduced [16–18,44]. Neither the formation of SeS bonds nor sulfoxide formation are relevant for the reaction observed, the process being already described as a reductive elimination S-Cl reaction occurring on the PtIV starting material (Scheme 2) [17]. The fastest reaction observed corresponds, in fact, to the less thermodynamically favourable redox process, thus reinforcing the importance of the study of the proper mechanistic reaction paths for biologically relevant reactivity [45].

The only clear reactivity trend observed within the data collected corresponds to that associated with acidity changes of the biomolecules. The reaction rates for the reduction process increase on increasing the amount of thiolate present in the reaction medium at pH 7.0 [16–18]; even so, the amount of free thiolate units at the physiological pH range studied is rather small thus indicating the high reactivity involved (see Table 5). This fact is in line with the expected, provided an attack of the thiolate group on a chlorido ligand to produce the reductive elimination of RSCl is the responsible of the reduction process (see Fig. 4a and Scheme 2). Taking this fact into account the lack of reactivity observed with methionine, despite the highly favourable potential, can be easily related with its thioether nature that does not allow for a thiolate attack to the bound chlorido ligand. In this line, the values of the entropies and volumes of activation are rather negative due to the ordering (and contraction) of an external thiol-containing molecule when interacting with the chlorido ligand to produce the leaving –SCl unit; the reaction being de facto the reverse of a SN2 oxidative addition process. The effect of the intra- and extra-cellular chloride concentration gradient has been found to be irrelevant for the redox reactivity observed, thus indicating that no chlorido aquation from the PtIV complexes is occurring during the reactivity studied. Thus the fact that the original PtIV complex keeps as such before the reduction process studies is reinforced, in important contrast with what occurs with the cisplatin analogues [45].

Much more interesting are the dramatic changes observed in the thermal and pressure activation parameters when the amine groups present in cysteine and glutathione are absent, as in thiolactic acid (see Table 5). The value on the activation enthalpy decreases noticeably, while the entropy and volume of activation become much more negative. Given the fact that the difference in pKa between cysteine and glutathione is of the same magnitude than that between glutathione and thiolactic acid, and that only minimal reactivity changes are observed, this effect cannot be related solely to acidity tuning. Clearly a higher degree of ordering and contraction is occurring for the RSH group to reach the chlorido ligand

316 (producing simultaneously a less enthalpy demanding RSCl reductive elimination) on its way to the
317 final PtII complex with this biomolecule. Taking into account that the final PtII reduced complex has
318 been proved kinetically to contain a thiolactate chelate unit (see Results section), probably the bite angle
319 of this biomolecule allows for an extra Pt-O interaction with the reducing PtIV centre when losing one
320 of the axial chlorido groups on reduction [19].

321 In this respect the study of the substitution processes of the resulting 1-Cl PtII complex with the same
322 biomolecules, and under the same pH and concentration conditions, has been extremely revealing. Only
323 for cysteine the substitution process has been found slower than that of reduction (see Table 2), thus
324 allowing for the reduced 1-Cl complex to associatively discriminate between the ligands present in the
325 reaction medium even in the presence of the soft thiol containing biomolecule. The process is clearly
326 associatively activated [40], as expected for a square planar complex containing a single Pt-C bond, and
327 as also indicated by the activation parameters collected [54]. For glutathione and thiolactic acid the
328 substitution reaction on complex 1-Cl is ca. one order of magnitude faster than the reduction reaction
329 (see Tables 3 and 4) which implies that the PtII species resulting from the reduction of the PtIV
330 precursor may very well have the biomolecule already coordinated. In this case the final compound,
331 although being discriminating due to the associative substitution mechanism expected to be operating
332 for these organometallic single Pt-C containing complexes [40], has a definitive clear kinetic preference
333 for these two biomolecules, thus affecting an effective selective coordination with the rest of bioligands
334 that could be present in the reaction medium.

335

4. **CONCLUSIONS**

Only the tris-chlorido derivatives from the cyclometallated skeleton indicated in Scheme 1 can be reduced by cysteine, glutathione and thiolactic acid. The mono-chlorido, bis-hydroxido derivatives from the same cyclometallated unit are not reduced due to the high potential of the PtIV/PtII pair, as measured in aqueous solution. The reduction reaction is found to be clearly dependent on the amount of deprotonated thiolate present in the medium, which agrees with a reductive elimination process involving an attack of thiolate to one of the chloride ligands to produce RSCl. No intervening chlorido substitution reactions are involved during this process, thus ensuring the maintenance of the PtIV complex structure before its reduction. The thermal and pressure activation parameters agree with this assumption, as well as the fact that methionine is found unreactive under the same conditions, despite the more favourable redox potential. Interestingly for the reduction reaction with thiolactic acid an unexpected increase in ordering and contraction is observed from the data collected; the fact that the ligand can actuate as a perfect chelating unit to one of the other PtIV coordination positions losing a chlorido ligand can be held responsible for this fact.

The study of the substitution reactions occurring at the [PtII(CNN')Cl] reduced species has also provided support for the reactivity observed. While for the reduction with cysteine, the PtII complex can be detected in solution under the conditions of the study, for the reduction with glutathione and thiolactic acid the consecutive substitution process is very fast. For these reactions the final PtII complex after reduction already has the reducing agent present in solution coordinated to the metal centre (even in a chelate fashion for thiolactic acid reduction). Despite the associativeness character of the substitution reactions occurring on these single Pt-C containing organometallic complexes, these reactions become less capable of distinguishing between ligands. The thermal and pressure activation parameters of the substitution reaction have been determined for the reaction with cysteine provides further corroboration of the latter assumption.

The results obtained confirm the expected reactivity sequence of the PtIV compounds with thiols consisting of reduction and substitution, and, in addition, the present study reveals that the relative rates of these reactions are relevant for the design of more efficient PtIV prodrugs.

5. EXPERIMENTAL

5.1. General

Microanalyses were performed at the Centres Científics i Tecnològics (Universitat de Barcelona). Mass spectra were performed at the Unitat d'Espectrometria de Masses (Universitat de Barcelona) in a LC/MSD-TOF spectrometer using H2O-CH3CN 1:1 to introduce the sample. NMR spectra were performed at the Unitat de RMN d'Alt Camp de la Universitat de Barcelona using a Mercury-400 (1H, 400 MHz; 13C, 100.6 MHz; 19F, 376.5 MHz) or a Bruker 400 MZ AvanceIII Avance (1H, 400 MHz) and referenced to SiMe4 (1H and 13C) or CFCl3 (19F). δ values are given in ppm and J values in Hz. Abbreviations used: s=singlet; d=doublet; t=triplet; m=multiplet; sh=shoulder.

5.2. Compounds

[PtCl3{(CH3)2N(CH2)3NCH(4-ClC6H3)}] (2-Cl-Cl) was obtained from 0.050 g (0.090 mmol) of the cyclometallated [PtCl{(CH3)2N (CH2)3NCH(4-ClC6H3)}] and the equimolar amount of PhICl2 in acetone (15 mL) following the method described in the literature for similar compounds [37]. Yield: 35 mg (60%). 1H NMR (400 MHz, CDCl3): δ=8.22 [s, 3J(Pt-H)=8.0, 1H, He], 8.05 [d, 4J(HeH)=2.0, 3J(Pt-H)=24.0, 1H, Hh], 7.43 [d, 3J(HeH)=8.0, 1H, Hf], 7.23 [dd, 3J (HeH)=8.0, 4J(HeH)=2.0, 1H, Hg], 4.00 [m, 2H, Hd], 2.97 [s, 3J(Pt- H)=11.2, 6H, Ha], 2.95 [m, 2H, Hb], 2.26 [q, 3J(HeH)=4.8.0, 2H, Hc]. 1H NMR (400 MHz, d6-DMSO): δ=8.79 [s, 3J(Pt-H)=96.0, 1H, He], 7.69 [d, 4J(HeH)=2.0, 1H, Hh], 7.69 [d, 3J(HeH)=8.0, 1H, Hf], 7.37 [dd, 3J(HeH)=8.0, 4J(HeH)=2.0, 1H, Hg], 3.95 [m, 2H, Hd], 2.8.05 [m, 2H, Hb], 2.8.00 [s, 3J(Pt-H) ca. 8.0 (sh), 6H, Ha], 2.06 [m, 2H, Hc]. 13C NMR (100.6 MHz, d6-DMSO): δ=178.03 [Ce], 141.63, 140.42, 136.61, 132.10 [Cf], 131.41 [Ch], 126.76 [Cg], 62.11 [Cb], 57.29 [Cd], 49.79 [Ca], 25.21 [Cc]. Anal. Calc. for C12H16Cl4N2Pt (%): C, 27.45; H, 3.07; N, 5.33. Found (%): C, 27.27; H, 3.02; N, 5.15.

[PtCl(OH)2{(CH3)2N(CH2)3NCH(4-ClC6H3)}] (2-Cl-OH) was obtained from 0.050 g (0.090 mmol) of the cyclometallated [PtCl{(CH3)2N (CH2)3NCH(4-ClC6H3)}] and the equimolar amount of H2O2 in dichloromethane (15 mL) following the method described in the literature for similar compounds [36]. Yield: 27 mg (50%). 1H NMR (400 MHz, CDCl3): δ=8.30 [s, 3J(Pt-H)=108.0, 1H, He], 8.00 [d, 4J (HeH)=2.0, 3J(Pt-H)=28.0, 1H, Hh], 7.41 [d, 3J(HeH)=8.0, 1H, Hf], 7.26 [dd, 3J(HeH)=8.0, 4J(HeH)=2.0, 1H, Hg], 3.98.0 [t, 3J (HeH)=5.2, 2H, Hd], 2.91 [m, 2H, Hb], 2.78.0 [s, 6H, Ha], 2.17 [m, 2H, Hc]. 1H NMR (400 MHz, d6-DMSO): δ=8.46 [s, 3J(Pt-H)=104.0, 1H, He], 7.75 [d, 4J(HeH)=2.0, 1H, Hh], 7.52 [d, 3J(HeH)=8.0, 1H, Hf], 7.28.0 [dd, 3J(HeH)=8.0, 4J(HeH)=2.0, 1H, Hg], 3.8.02 [m, 2H, Hd], 2.76 [m, 2H, Hb], 2.56 [s, 6H, Ha], 1.98.0 [m, 2H, Hc]. 13C NMR (100.6 MHz, d6-DMSO): δ=178.33 [Ce], 143.32, 142.77, 135.35, 131.64 [Cf], 131.13 [Ch], 125.94 [Cg], 62.05 [Cb], 57.12 [Cd],

47.44 [Ca], 25.02 [Cc]. ESI(+)-MS {H2O:CH3CN (1:1)}, m/z: 471.0343 (calc. for C12H17Cl2N2OPt 471.0444) [M−OH]+, 48.09.0448.0 (calc. for C12H19Cl2N2O2Pt 48.09.0449) [M+H]+. Anal. Calc. for C12H18.0Cl2N2O2Pt·CH2Cl2 (%): C, 27.24; H, 3.52; N, 4.8.09. Found (%): C, 27.04; H, 3.30; N, 5.27.

[PtCl3{(CH3)2N(CH2)3NCH(4-FC6H3)}] (2-F-Cl) was obtained from 0.050 g (0.090 mmol) of the cyclometallated [PtCl{(CH3)2N (CH2)3NCH(4-FC6H3)}] and the equimolar amount of PhICl2 in acetone (15 mL) following the method described in the literature for similar compounds [37]. Yield: 33 mg (57%). 1H NMR (400 MHz, d6-DMSO): δ=8.74 [s, 3J(Pt-H)=96.0, 1H, He], 7.77 [dd, 3J(HeH)=8.4, 4J(HF)= 6.0, 1H, Hf], 7.43 [dd, 3J(H-F)=9.6, 4J(HeH)=2.0, 1H, Hh], 7.14 [td, 3J(HeH)=3J(H-F)=8.4, 4J(HeH)=2.0, 1H, Hg], 3.94 [m, 2H, Hd], 2.8.06 [m, 2H, Hb], 2.8.00 [s, 6H, 3J(Pt-H)=8.0, Ha], 2.06 [m, 2H, Hc]. 19F NMR (376.5 MHz, d6-DMSO): δ =−100.73 [td, 3J(FH)= 9.0, 4J(F-H)=5.9]. Anal. Calc. for C12H16Cl3FN2Pt (%): C, 28.33; H, 3.17; N, 5.51. Found (%): C, 28.32; H, 3.24; N, 5.32.

5.3. Instruments and methods

Buffer solutions were prepared using the standard procedures using HEPES; in all cases the concentration of the buffer was at least 10-fold (0.01 M) that of the reactants and the ionic strength was set at 0.1 with NaClO4.

Electrochemistry experiments were carried out with a BioLogic SP- 150 instrument using a glassy carbon working electrode, a Ag/AgCl (3M KCl) reference electrode, and platinum wire counter electrode; potential values are given versus SHE. The samples were dissolved in 1:1 a water-DMSO mixture at the 1×10−3M level concentration and using 0.1M (Bu4N)ClO4 as supporting electrolyte.

5.4. X-ray diffraction

Suitable crystals of compound 2-Cl-Cl were grown at room temperature in dichloromethane-methanol. X-ray diffraction data were collected for a yellow prism-like specimen on a D8.0 VENTURE system equipped with a multilayer monochromator and a Mo microfocus source (λ=0.71073 Å) at 100 K. The structures were solved and refined using the Bruker SHELXTL Software package [55]; crystallographic details are given in CCDC 1828170.

5.5. Kinetics

The time-resolved kinetic profiles for the reactions at ambient pressure with were followed by UV–Vis spectroscopy in the full 700–275 nm range on HP8.0453 or Cary50 instruments equipped with thermostated multicell transports. For runs carried out at elevated pressures the evolutions of the systems

were followed with an already described pressurizing cell system setup was used connected to a TIDAS J&M instrument. The general technique used for these experiments where a linear dependence on the concentration is observed has already been described [56]. The kinetic experiments were conducted under pseudo-first order conditions by mixing the appropriate amounts of aqueous buffer stock solution, 0.1M NaClO4 (or 0.1M NaCl) aqueous solutions, aqueous solution of the appropriate reductant, water, DMSO, and a stock solution of the PtIV complex in neat DMSO. In all cases the final DMSO volume percentage of the solutions was 20% to allow the full solution of the PtIV complexes used. Observed rate constants were derived from the absorbance versus time traces at the wavelengths where a maximum increase and/or decrease of absorbance were observed. Calculation of the observed rate constants from the absorbance versus time monitoring of reactions were carried out using the SPECFIT or ReactLab software packages [40,41]. In all cases the systems were set to a single: A→B or a two: A→B→C step scheme. For systems showing a final drift of the absorbance due to undesired decomposition processes of the reductant in the reaction medium, a final linear drift step was also included for final refinement. All post-run fittings were carried out by the standard available commercial programs.

ACKNOWLEDGEMENTS

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Legends to figures

Scheme 1 Synthesis of the PtIV compounds involved in this study including the numbering scheme for NMR assignments (see Experimental section).

Figure. 1. Molecular structure of compound 2-Cl-Cl showing 50% probability ellipsoids; hydrogen atoms were omitted for the sake of clarity. Selected bond lengths (Å) and angles (°) with estimated standard deviations: Pt(1)-C(1): 2.002(6); Pt(1)-Cl(2): 2.3116(15); Pt(1)-N(1): 2.032(5); Pt(1)-N(2): 2.259(6); Pt (1)-Cl(3): 2.3192(15); Pt(1)-Cl(4): 2.3184(15); C(1)-Pt(1)-N(1): 80.9(2);C(1)-Pt (1)-N(2): 177.0(2); N(1)-Pt(1)-N(2): 96.5(2); C(1)-Pt(1)-Cl(2): 88.22(18); N(1)-Pt(1)-Cl(2): 88.67(15); N(2)-Pt(1)-Cl(2): 90.26(15); C(1)-Pt(1)-Cl(4):93.73(19); N(1)-Pt(1)-Cl(4): 174.43(15); N(2)-Pt(1)-Cl(4): 88.95(15); Cl(2)-Pt (1)-Cl(4): 92.50(6); C(1)-Pt(1)-Cl(3): 86.80(17); N(1)-Pt(1)-Cl(3): 88.35(15); N (2)-Pt(1)-Cl(3): 94.60(15); Cl(2)-Pt(1)-Cl(3): 174.55(6); Cl(4)-Pt(1)-Cl(3): 90.05(5).

Figure. 2. 1st cycle of the cyclic voltammograms of compounds 2-Cl-Cl and 2-Cl-OH in DMSO-water solution at a scan rate of 100 mV/s and the electrochemical setup described in the Experimental section.

Figure. 3. a) UV–Vis spectral changes obtained on the reaction of PtIV complex 2-F-Cl, 4×10−5 M, with cysteine, 4×10−4 M, at pH 7.0 and 25 °C. b) Plot of the rate constants derived for the two steps observed (kobs1 and kobs2) for the 2-Cl-Cl complex, 1×10−5 M, as a function on the cysteine concentration at different pH values and 25 °C.

Scheme 2. Reduction plus substitution reactivity sequence expected for the processes studied.

Figure. 4. a) pH-dependence of the second order rate constants for the reduction of the PtIV complexes 2-Cl-Cl and 2-F-Cl at 25 °C. b) Plot of the rate constants obtained for the reduction reaction of the same complex with cysteine as a function of cysteine and chloride concentration at pH 7.0 and 25 °C.

Figure. 5 a) UV–Vis spectral changes obtained on the reaction of PtIV complex 2-Cl-Cl, 1×10−5 M, with glutathione, 1×10−4M at pH 7.0 and 25 °C. b) Plot of the pseudo-first order rate constants derived for the reduction observed on complex 2-Cl-Cl (kobs red) at different pHs (empty square points correspond to 0.1M NaCl added), and those of the substitution (kobs subs) occurring on the 1-Cl PtII complex at pH=7.0, with the same ligand and as a function on glutathione concentration at 25 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Figure. 6 a) UV–Vis spectral changes obtained on the reaction of the PtIV complex 2-Cl-Cl, 1×10−6 M, with thiolactic acid, 1×10−4M at pH 7.0 and 25 °C. b) Plot of the rate constants derived for the reduction observed on the same complex, kobs=kobs red, at pH=7.0 (empty points correspond to 0.1M NaCl added) at different temperatures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Figure. 7 a) UV–Vis spectral changes obtained on the reaction of the PtII complex 1-Cl, 3×10−6 M, with thiolactic acid, 1×10−4M at pH 7.0 and 25 °C. b) Plot of the rate constants derived for the two sequential processes observed at pH=7.0 and 25 °C.

SCHEME 1

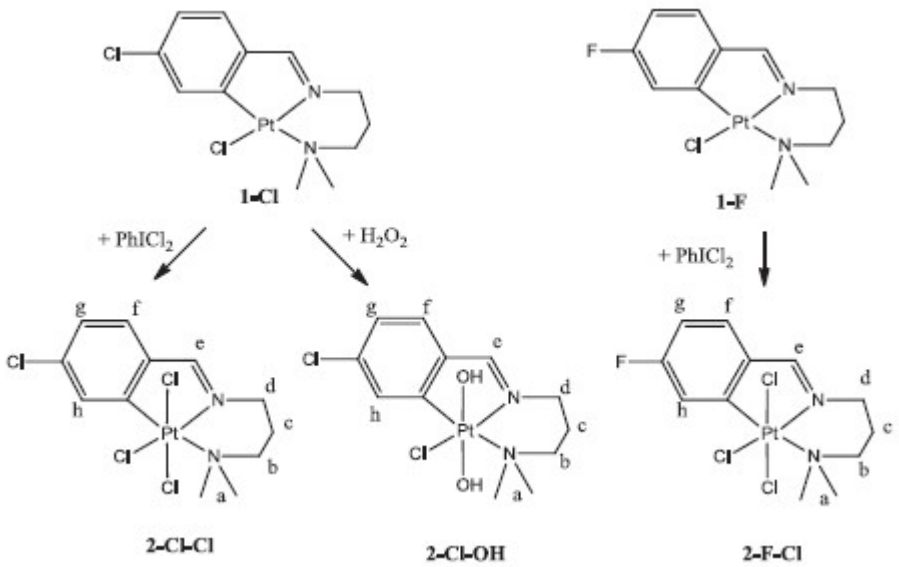
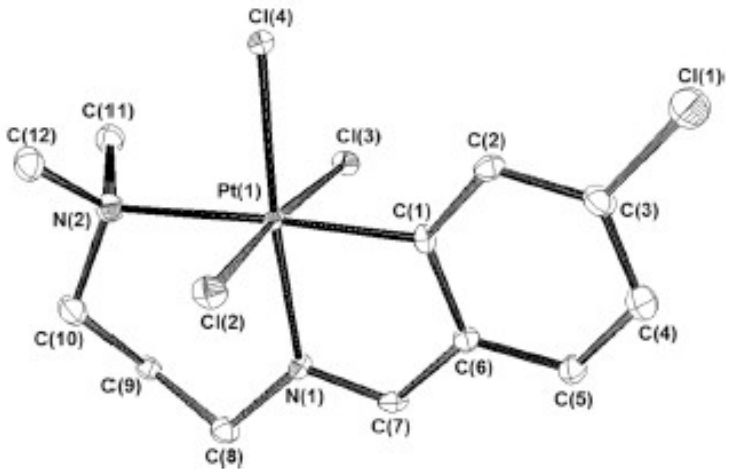


FIGURE 1



582 **FIGURE 2**

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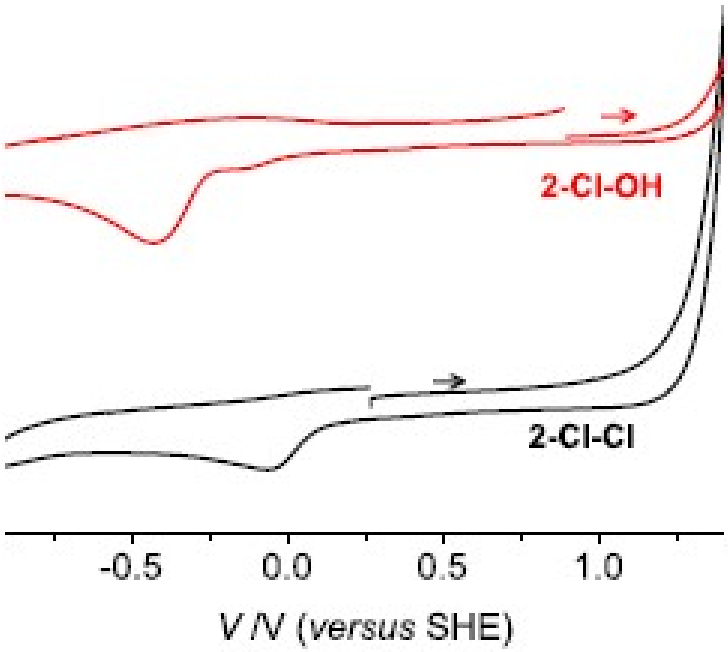
584



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586

587 **FIGURE** 3

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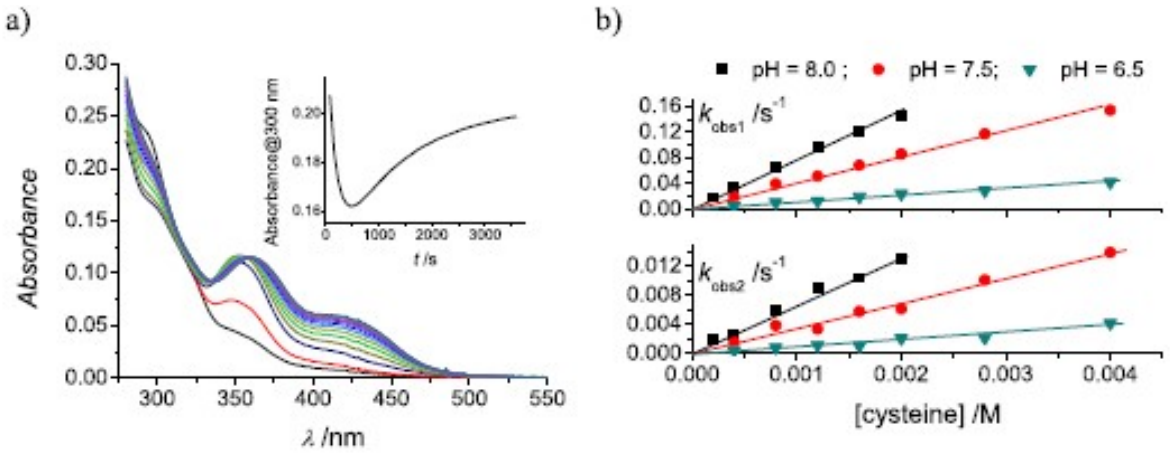

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SCHEME 2

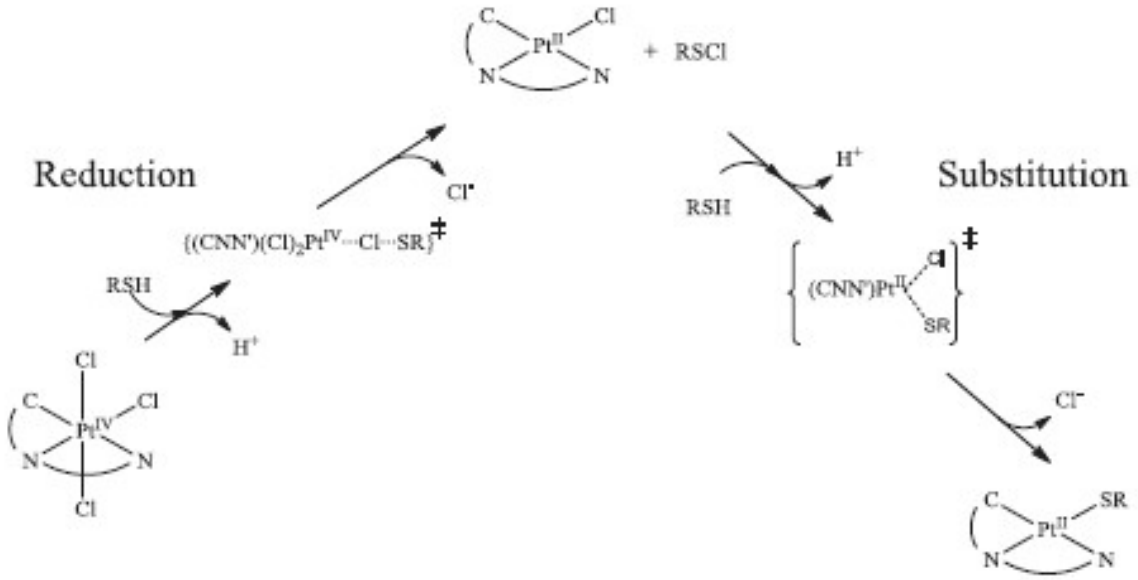
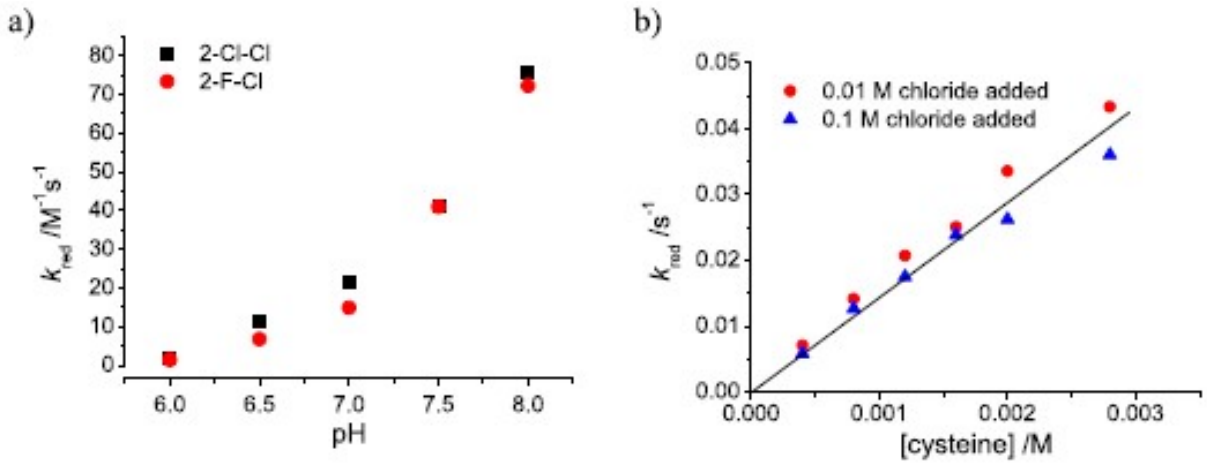


FIGURE 4

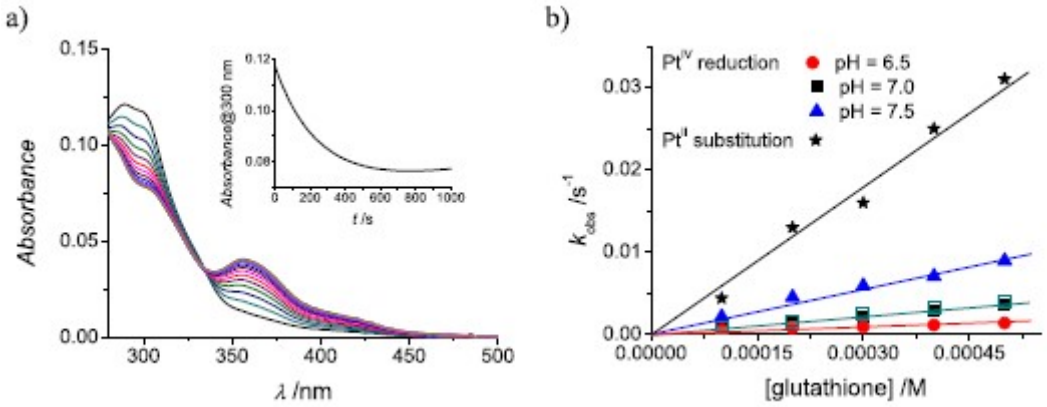

a)
2-Cl-Cl
2-F-Cl
k_{red} /M^{-1}s^{-1}
pH
b)
0.01 M chloride added
0.1 M chloride added
k_{red} /s^{-1}
[cysteine] /M

602 **FIGURE 5**

603

604

605



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608 **FIGURE 6**

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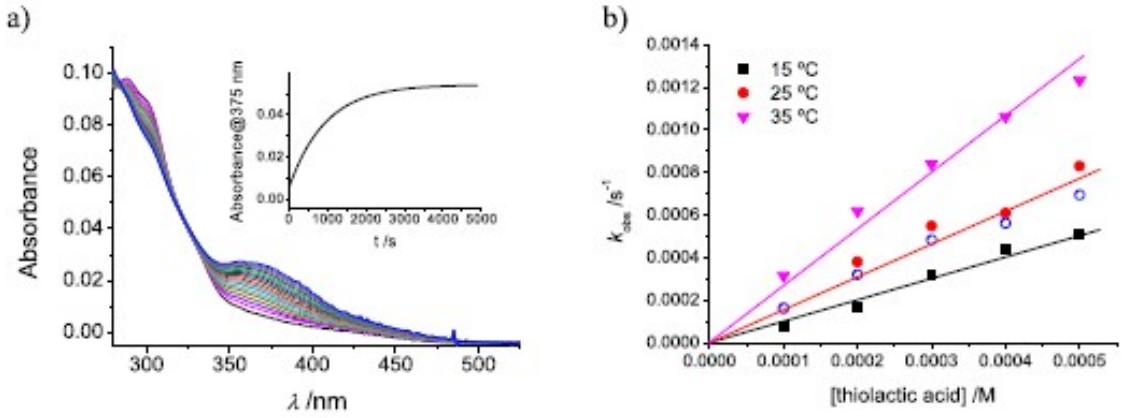

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613 **FIGURE 7**

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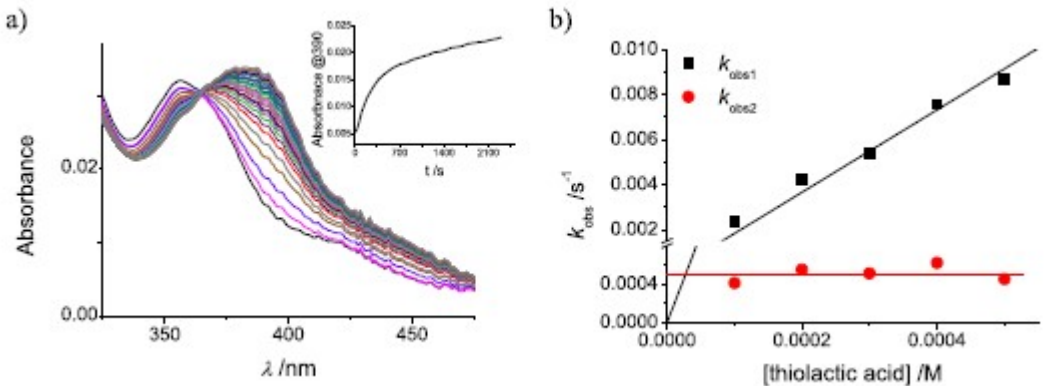

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617

618 **Table** 1 Reduction potentials versus SHE for the PtIV compounds studied in this work. Relevant
619 literature data are is also included.
620

Compound	$E°$/mV
2-Cl-Cl	−50
2-F-Cl	−8
2-Cl-OH	−400
cis,trans-$[PtCl_2X_2(en)]$ (X = Cl)[a]	−4
cis,trans-$[PtCl_2X_2(en)]$ (X = OH)[a]	−664

[a] *Versus* SHE, from Ref. [1].

621
622

Table 2 Summary of the kinetic (298.0 K) and thermal and pressure activation parameters for the processes occurring on complexes 2-Cl-Cl and 2-F-Cl with cysteine at pH 7.0.

Compound	k_{red} /M^{-1} s^{-1}	$k_{PtII\ subs}$ /M^{-1} s^{-1}	ΔH_{red}^{+} /kJ mol^{-1}	ΔS_{red}^{+} /J $K^{-1}mol^{-1}$	ΔV_{red}^{+} /cm^3mol^{-1}	$\Delta H_{PtII\ subs}^{+}$ / kJ mol^{-1}	$\Delta S_{PtII\ subs}^{+}$ /J $K^{-1}mol^{-1}$	$\Delta V_{PtII\ subs}^{+}$ /cm^3mol^{-1}
2-Cl-Cl	21 ± 1	2.0 ± 0.1	41 ± 3	−84 ± 11	−15 ± 2	27 ± 1	−150 ± 1	−25 ± 2
2-F-Cl	15 ± 1	1.3 ± 0.1	44 ± 2	−77 ± 6	n.d.	44 ± 3	−97 ± 10	n.d.

n.d.: Not determined.

628 **Table 3** Summary of the kinetic (298.0 K) and thermal and pressure activation parameters for the
629 processes occurring on complexes 2-Cl-Cl and 1-Cl with glutathione at pH 7.0.
630

Compound	k_{red} /M^{-1} s^{-1}	$k_{HH\ subst}$ /M^{-1} s^{-1}	$\Delta H_{red}^{\dagger}$ /kJ mol^{-1}	$\Delta S_{red}^{\dagger}$ /J $K^{-1}mol^{-1}$	$\Delta V_{red}^{\dagger}$ /cm^3mol^{-1}
2-Cl-Cl	7.2 ± 0.1	fast	47 ± 6	−72 ± 21	−16 ± 2
1-Cl	–	60 ± 2	n.d.	n.d.	n.d.

n.d.: Not determined.

631
632

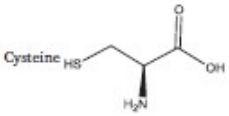
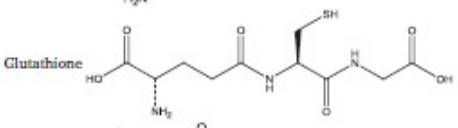
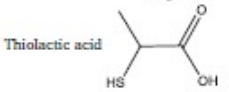
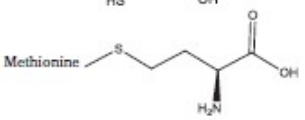
633 **Table** 4 Summary of the kinetic (298.0 K) and thermal and pressure activation parameters for the
634 processes occurring on complexes 2Cl-Cl and 1-Cl with thiolactic acid at pH 7.0.
635

Compound	k_{red} /M^{-1} s^{-1}	$k_{PH1\ rubr}$ /M^{-1} s^{-1}	$k_{PH1\ chel}$ /s^{-1}	ΔH_{red}^{+} /kJ mol^{-1}	ΔS_{red}^{+} /J $K^{-1}mol^{-1}$	ΔV_{red}^{+} /$cm^{3}mol^{-1}$
2-Cl-Cl	1.7 ± 0.1	fast	fast	32 ± 1	−134 ± 1	−22 ± 2
1-Cl	–	18.0 ± 1	5 ± 1	n.d.	n.d.	n.d.

n.d.: Not determined.

636
637

638 **Table 5** Summary of redox, acidity, kinetic and thermal and pressure activation data for the processes
639 occurring on the redox reaction between PtIV 2-Cl-Cl complex and the different thiol-containing
640 biomolecules utilised in this work.
641

Reducing agent on **2-Cl-Cl**	$E^0_{(pH\ 7.0)}$ /mV	pK_a	k_{red} /$M^{-1}\ s^{-1}$	$k_{PtII\ subs}$ /$M^{-1}\ s^{-1}$	ΔH_{red}^{+} /kJ mol^{-1}	ΔS_{red}^{+} /J $K^{-1}mol^{-1}$	ΔV_{red}^{+} /cm^3mol^{-1}
 Cysteine	280 [46]	$8.22_{(SH)}$ [47] $10.7_{(NH2)}$ [48]	21(15)[a]	slower	41(44)[a]	−84(−77) [a]	−15
 Glutathione	310 [46]	$9.48_{(SH)}$ [49,50] $10.7_{(NH2)}$ [48]	7.2	faster	47	−72	−16
 Thiolactic acid	320 [51]	$10.8_{(SH)}$ [52]	1.7	faster [b]	32	−134	–22
 Methionine	> 1 [43]	$9.21_{(NH2)}$ [53]			No reaction observed		

[a] Values in parentheses for the **2-F-Cl** complex.
[b] Chelating reaction follows.
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