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Reactivity of kiteplatin with S-donor biomolecules and nucleotides†

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Kiteplatin, (*cis*-1,4-DACH)dichloridoplatinum(II), contains an isomeric form of the carrier ligand present in the successful antitumor drug oxaliplatin and has been recently found to be very active against oxaliplatin-resistant colon cancers, confirming that, by changing the nature of the amine ligand, it is possible to obtain platinum drugs that are not cross-resistant to those already in clinical use. Apart from interaction with DNA, another factor that can affect the activity of platinum drugs is their metabolic fate in the cellular environment. Therefore, kiteplatin has been reacted with S-donor biomolecules, such as glutathione, cysteine, and methionine. The investigation has further confirmed the different reactivity of methionine as compared to cysteine-containing peptides and has unraveled the possibility of *cis*-1,4-DACH to become mono-coordinated with one free end (a situation never seen for isomeric 1,2-DACH ligands) and to labilize *cis* ligands as a consequence of its large steric hindrance. The reaction of kiteplatin–GSH adducts with 5′-GMP has also shown how the reaction products can be different depending upon the aerobic or anaerobic reaction conditions used.

Introduction

Cisplatin (*cis*-diamminedichloridoplatinum(II)) (Fig. 1) is one of the most potent anticancer drugs known and is widely used in the treatment of solid tumors, such as testicular, ovarian, lung and bladder cancers.[1–4] Despite its efficacy, cisplatin has several limitations due to its toxicity, acquired resistance and low activity against a variety of cancers.[5–7] In order to overcome these limitations, second and third generation Pt(II) compounds have been developed. Unlike carboplatin, a second generation drug that is cross-resistant to cisplatin, oxaliplatin, (1*R*,2*R*-DACH)(oxalato-*O*,*O*′)platinum(II) (DACH = diaminocyclohexane),[8] a third generation compound, is active against colorectal cancer (Fig. 1).[9] However, resistance limitations have been observed for oxaliplatin.[10] Kiteplatin ((*cis*-1,4-DACH) dichloridoplatinum(II)),[11] a compound containing an isomeric form of the carrier ligand of oxaliplatin, has been recently investigated as a promising drug candidate against oxaliplatin-resistant colon cancers.[12–16]

It has been extensively demonstrated[17] that the nature of the amine carrier ligand(s) has a strong influence on the activity of platinum complexes. Therefore, by changing the nature of the amine ligand, it is possible to obtain complexes that are active toward cisplatin- and oxaliplatin-resistant cell lines. Moreover, since nuclear DNA is the putative target for this class of drugs, several mechanistic and structural studies of adducts of different platinum complexes with DNA have been carried out.[18,19] Another factor that can affect the activity of platinum complexes in different cell lines, is the metabolic fate of the drugs, that, once in the cellular environment, can interact with several biological substrates, particularly S-donor biomolecules[20] such as glutathione and proteins containing methionines and cysteines (Fig. 1).[21] The reaction with these platinophiles reduces the percentage of platinum reaching DNA,[22] the total of which is estimated to be only about 1%.[23]

Glutathione, GSH, is a tripeptide containing a central L-cysteine attached by peptidic bonds to the γ carboxylic group of L-glutamate and to the amino group of L-glycine. GSH acts as a biological anti-oxidant[24] and is present in concentrations of 0.5–10 mM in most cells.[25] High levels of intracellular glutathione have been correlated with low cisplatin activity[26] and high intracellular levels of glutathione have been found in cisplatin-resistant cells.[27,28] Nevertheless, co-administration of cisplatin and glutathione (as a chemoprotectant) does not appear to decrease the antitumor activity of cisplatin.[29] It has been hypothesized that Pt-GS adducts could act as a drug reservoir from which platinum could bind to DNA in a subsequent step in exerting its antitumor activity.[30] Studies of the interaction of platinum complexes with DNA in the presence of S-containing molecules indicate that, at physiological pH,

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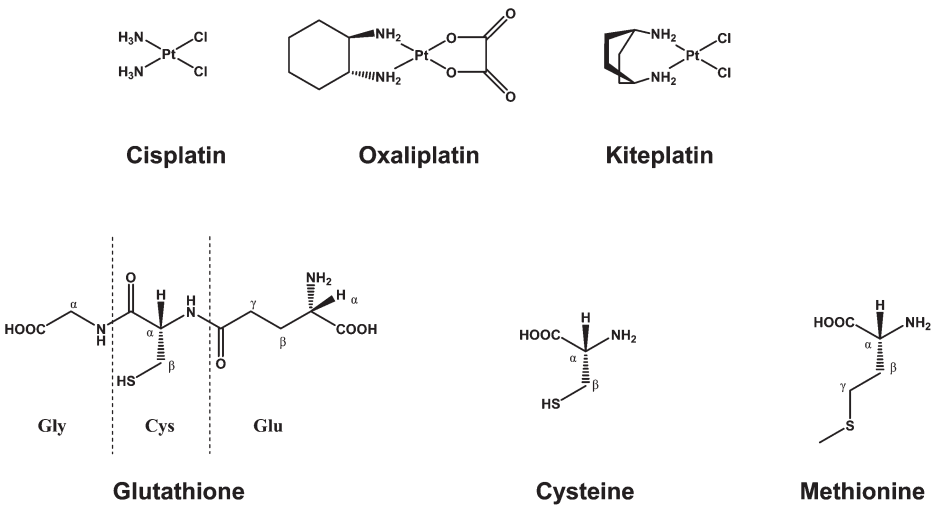


Fig. 1 Structures of antitumor Pt complexes (cisplatin, oxaliplatin, kiteplatin) and labelled structures of S-donor biomolecules (glutathione, cysteine, methionine).

the affinity of platinum for S-donors is greater than that for N-donors (such as a nucleobase).[31,32]

The reaction between GSH and diamineplatinum(II) complexes, such as $[PtCl_2(en)]$ (en = ethylenediamine), oxaliplatin or its dichlorido analogue ((1*R*,2*R*-DACH)dichloridoplatinum(II)), led to the formation of two different types of adducts.[25,33] The first adduct was a dimer formed by two glutathione residues bridging two diamineplatinum units (A in Fig. 2). The second adduct was a macrochelate formed by two diamineplatinum units bridged by a single glutathione residue (B in Fig. 2). Sadler hypothesized that the latter macrochelate adduct could be a reactive species.

Dimeric A-type adducts were shown to be formed also by Pt(II) complexes with monoamine ligands, such as cisplatin,[34,35] together with the $[Pt(GS)_2]$ adduct, resulting from the displacement of both labile and inert ligands.[21,36] Also, proteins containing L-methionine, and L-cysteine can act as platinophiles.[35]

Oxaliplatin biotransformation products containing monocysteine and mono-methionine have been found in rat kidney,[37] confirming the role of these amino acids in the metabolism of Pt drugs.

The reaction with L-cysteine, cys, does not lead to displacement of the carrier ligand(s) even in the case of monodentate amines, such as cisplatin, where the dimeric $[\{Pt(NH_3)_2(L\text{-cys-}\mu\text{-}S)\}_2]^{2+}$ adduct was the major product, and $[Pt(NH_3)_2(L\text{-cys-}S)_2]$ and $[Pt(NH_3)_2(L\text{-cys-}N,S)]^+$ were the less abundant products.[34,35]

In contrast, reaction with L-methionine, met, can lead to displacement of the carrier ligand(s) forming the $[Pt(L\text{-met-}S,N)_2]^{2+}$ adduct.[35] In the case of complexes with diamine ligands, as in oxaliplatin, it was found that methionine displaces only the labile ligands without displacement of the diamine (forming $[Pt(1R,2R\text{-DACH})(Met\text{-}S,N)]^+$ in the case of oxaliplatin).[36,39]

It is clear, from the above considerations, that a detailed investigation of the interaction between a potentially new Pt antitumor drug, such as kiteplatin, and S-donor biomolecules is a necessary step to understand the metabolic fate of this drug candidate and to learn to what extent it is able to overcome the deactivating action of platinophiles.

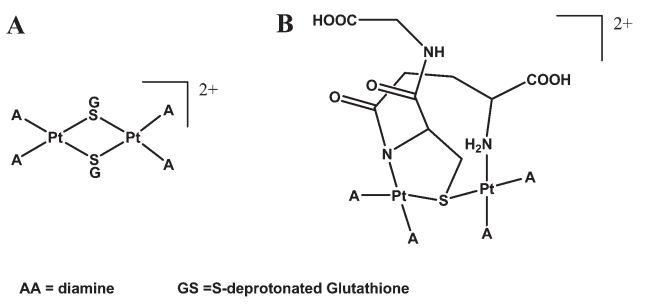


Fig. 2 Structure of dimeric (A) and macrochelate (B) adducts obtained by reaction of diamineplatinum(II) complexes with glutathione.

Experimental section

Materials and methods

Commercial reagent grade chemicals and solvents were used as received without further purification.

NMR spectra were recorded using Jeol ECX 400 MHz and Bruker Avance DPX 300 MHz instruments at 25 °C. NMR spectra were recorded on samples of concentration of *ca.* 5 mM, 32 scans were performed for ^{1}H 1D NMR experiments and 256 iterations were performed for ^{1}H 2D COSY NMR experiments. ^{1}H NMR experiments were referenced to the residual HOD peak (^{1}H), positioned at 4.78 ppm.[40] NMR data were processed with Mestre-C software.

ESI-MS analyses were performed using a Waters LCT Premier mass spectrometer. Samples were prepared by dissolving the solid compounds in water with 0.1% formic acid, obtaining a final concentration of *ca.* 10 μM, and then injected with a flow rate of 0.2 mL min^{-1} of 50/50 water–acetonitrile mixture into the mass spectrometer ion source.

Reaction mixtures were fractionated using a Thermo Scientific Dionex ICS-5000 station, equipped with a reverse-phase column (Hypersil GOLD 250 × 4.6 mm, 5 μm, 175 Å) and a UV detector at 220 nm, and eluting with water for 5 min followed by a 0–30% acetonitrile gradient over 25 min with 0.1% trifluoroacetic acid (TFA) as an ion-pairing agent.

A Thermo Scientific Orion 4 star pH meter, equipped with a Thermo Scientific Orion 8192BNUWP combination electrode standardized with buffers at pH 4.01, 7.00 and 10.01, was used for pH measurements.

Kiteplatin was prepared using a method derived from that of Khokhar,[41] and developed in our laboratories.[42]

Reaction between kiteplatin and S–platinophiles. Kiteplatin (5.7 mg, 15.0 μmol) was dissolved in water (14.3 mL) and then treated with the stoichiometric amount (1 : 1 ratio) of the selected platinophile (GSH, cysteine, or methionine) dissolved in water (0.70 mL) and adjusted to pH 7 with NaOH. The final solution (1.0 mM in both Pt complex and platinophile) was kept at 37 °C for 24 h.

Reaction between kiteplatin, glutathione and 5′-guanosine monophosphate (GMP). Kiteplatin (5.7 mg, 15.0 μmol) was dissolved in water (13.0 mL H_2O and 1.5 mL D_2O), and treated with a stoichiometric amount (1 : 1 ratio) of GSH dissolved in water (0.50 mL) and adjusted to pH 7 with NaOH. The final solution (1.0 mM in both Pt complex and GSH) was kept at 37 °C for 24 h under magnetic stirring and then treated with 2 equivalents of GMP (12.2 mg, 30.0 μmol). The reaction course was monitored by ^{1}H NMR spectroscopy.

Reaction between kiteplatin, glutathione and 5′-guanosine monophosphate (GMP) under N_2 atmosphere. This experiment was performed under the same conditions used for the previous one, except for bubbling N_2 gas into solvents and solutions for 2 minutes in order to remove dissolved oxygen.

Results and discussion

Kiteplatin and glutathione

The reaction between kiteplatin and glutathione (GSH) has previously been investigated by means of biophysical techniques.[14] The results showed that, compared to cisplatin, kiteplatin reacts with GSH at a similar or slightly higher rate. Despite this high reactivity, the efficacy of kiteplatin toward A2780cisR tumor cells, whose resistance to cisplatin appears also to be dependent upon an elevated levels of GSH, remains good.

In the present work, we have monitored the reaction of kiteplatin with glutathione by HPLC, performed at 37 °C (Fig. 3). In the course of the reaction the pH decreased from 7 to a value between 4 and 5. Nevertheless, we preferred not to use a buffer, such as phosphate, since it could interfere with platinum complexation.[25]

Pure kiteplatin showed two peaks eluting after *ca.* 10 and 11 min, assigned to the monoaqua and the dichlorido species, respectively (Fig. 3a).[43] After addition of GSH to the kiteplatin solution, a new peak, having a retention time of *ca.* 9 min, was observed (Fig. 3b); this peak was assigned to GSH by comparison with the pure reagent. After 3 h of reaction at 37 °C, a new peak with a retention time of *ca.* 15 min (**I**) and corresponding to the major reaction product was observed (Fig. 3c). Peak **I** was collected, frozen, and lyophilized for subsequent mass-spectrometry analysis. ESI-MS analysis of **I** revealed the presence of a doubly-charged cationic peak at m/z = 615.1 ($[M]^{2+}$), corresponding to the molecular formula $[C_{32}H_{60}N_{10}O_{12}S_2Pt_2]^{2+}$, as confirmed by isotopic pattern distribution (Fig. S1 in ESI†). This peak can be assigned to the dimeric adduct [{Pt(*cis*-1,4-DACH)(GS)}$_2$] (GS denotes deprotonated GSH) with two S-atoms bridging the platinum units (Fig. 4). This assignment was confirmed by a negative peak detected at m/z = 1227.1,

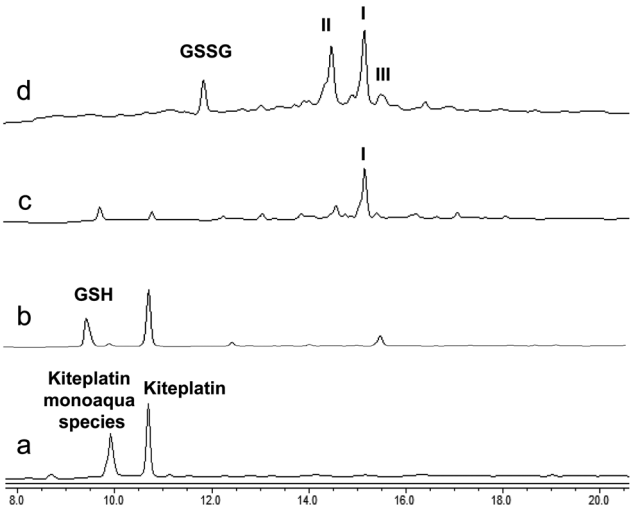


Fig. 3 HPLC chromatograms of the reaction between kiteplatin and GSH. (a) Kiteplatin solution; (b) soon after addition of GSH; (c) after 3 h at 37 °C; (d) after 24 h at 37 °C.

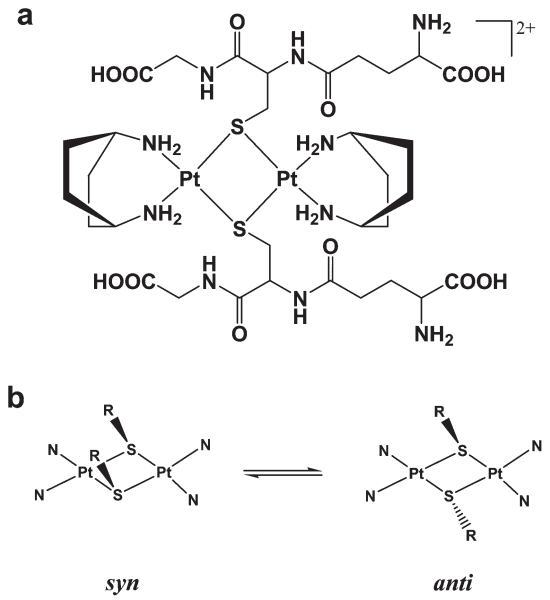


Fig. 4 Structure of kiteplatin–glutathione adduct **I** (a) and possible equilibrium between *syn* and *anti* conformers of adduct **I** (b).

Table 1 ^{1}H NMR chemical shifts (ppm) for kiteplatin–glutathione adducts I, II and III in D_2O

	Glutathione										
	Glutamate				Cysteine			Glycine		*cis*-1,4-DACH		
Adduct	N*H*	*Hα*	*Hβ*	*Hγ*	N*H*	*Hα*	*Hβ*	N*H*	*Hα*	NH_2	C*H*	CH_2
Kiteplatin[a]										4.91	3.19	1.74–1.73
GSH[b]	—	3.78	2.13	2.48	—	4.53	2.88	—	3.93			
GSSG	—	3.84	2.18	2.55	—	4.77	3.30, 3.00	—	3.99			
I	—	3.76	1.68	2.18	—	4.76	3.71	—	4.04	—	3.38	1.40–0.78
II	6.91, 5.59	2.88	1.93, 1.60	2.67, 2.11	—	5.29	2.08	8.97	3.93	6.41, 5.90, 5.10, 4.49	3.67, 3.57, 3.31, 3.06	1.45–0.80
III	—	4.09	2.34	3.30 2.52	—	5.72	2.61	7.82	3.95	5.69, 5.34, 5.11, 4.67, 4.28	3.70, 3.52, 3.17	1.89–1.48

[a] From ref. 42. [b] From ref. 36.

whose molecular formula, $[C_{32}H_{57}N_{10}O_{12}S_2Pt_2]^-$, is consistent with $[M - 3H]^-$, as confirmed by isotopic distribution pattern (Fig. S1 in ESI†).

Adduct **I** was characterized also by ^{1}H 1D and 2D COSY NMR (Fig. S2 in ESI† and Table 1). Besides the main signals, other minor signals were detected. This finding suggests the presence of an equilibrium between two conformers, *syn* and *anti*, in solution, with one conformer largely favoured over the other (Fig. 4).[33,36]

The HPLC chromatogram recorded after 24 h reaction between kiteplatin and GSH at 37 °C showed a new peak having a retention time of *ca*. 12 min which was assigned to the oxidized form of glutathione[44] (GSSG) by comparison with the chromatogram of pure GSSG recorded in the same conditions (data not shown). The chromatogram also showed the presence of a major peak with a retention time of *ca*. 14 min (peak **II** in Fig. 3d). The fraction containing peak **II** was collected and lyophilized. Once redissolved in water, this product was not stable and was partially transformed into another peak having a retention time of 15.5 min (peak **III** in Fig. 5). This latter peak was present also in the HPLC chromatogram taken after 24 h at 37 °C, (**III** in Fig. 3d). Peak **III** was collected and lyophilized. Also compound **III** was not stable in water where it was partially transformed into **II**. These data indicate that **II** and **III** are in equilibrium.

ESI-MS analysis of **II** showed a doubly-charged cation at m/z = 461.5, consistent with the molecular formula $[C_{22}H_{43}N_7O_6Pt_2S]^{2+}$ ($[M]^{2+}$), as also confirmed by the isotopic pattern distribution. Negative ESI-MS showed a peak at m/z = 966.2 corresponding to $[C_{23}H_{42}N_7O_8Pt_2S]^-$ ($[M - 2H + HCOO]^-$), as confirmed by comparison of experimental and theoretical isotopic pattern distributions (Fig. S3 in ESI†). Formic acid was added to the solution used for ESI-MS analysis for improving volatility.

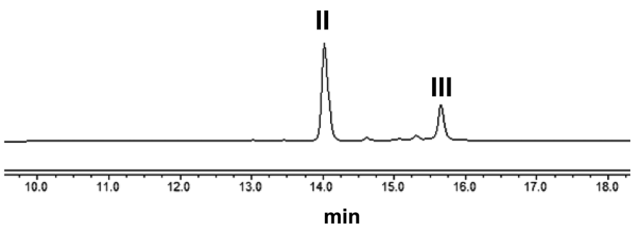


Fig. 5 HPLC chromatogram of adduct **II** redissolved in water and generating also adduct **III**.

Positive ESI-MS analysis of **III** showed the same mass peak reported for **II** as a major peak, and also a minor doubly-charged cationic peak at m/z = 493.0, consistent with an adduct of formula $[C_{22}H_{43}N_7Na_2O_7Pt_2S]^{2+}$, as confirmed by the isotopic distribution pattern (Fig. S4 in ESI†), and assigned to $[M + H_2O + 2Na - 2H]^{2+}$. These data led us to conclude that adducts **II** and **III** have a molecular composition similar to the macrochelate reported by Sadler,[25,33] *i.e.*, two Pt(*cis*-1,4-DACH) moieties and one molecule of glutathione, with the addition, in the case of **III**, of a water molecule.

Both **II** and **III** were characterized also by ^{1}H NMR spectroscopy (Fig. S5 in ESI†) and the assignments are reported in Table 1.

Fig. 6 reports the glutamate region of the ^{1}H 2D COSY spectra of **II** and **III**, where the largest differences between the two adducts are observed. Glutamate Hα shifts downfield from 2.88 to 4.09 ppm on passing from **II** to **III**. Moreover, in **II** the $C_\beta H_2$ methylene protons are magnetically non-equivalent and are observed at 1.60 and 1.93 ppm. In contrast, in **III** the Hβ methylene protons are magnetically equivalent and are found at 2.34 ppm. Finally, while in **II** glutamate aminic protons were observed at 6.91 and 5.59 ppm, no aminic protons were observed in **III**. The slow exchange with deuterium in the case of **II** could be due to coordination of the aminic group to a metal center, while the fast exchange with deuterium of the solvent in the case of **III** is indicative of uncomplexed aminic group.

All these data are in accord with compound **II** having a molecular arrangement similar to the macrochelate reported by Sadler for other diamine complexes of Pt(II) (Fig. 2).[25,33] Coordination of the aminic group of glutamate to platinum and consequent formation of a 9-membered chelate ring can fully explain not only the slow exchange rate of the aminic protons of glutamate, but also the remarkable diastereotopic splitting of the aminic and β-methylene protons of glutamate

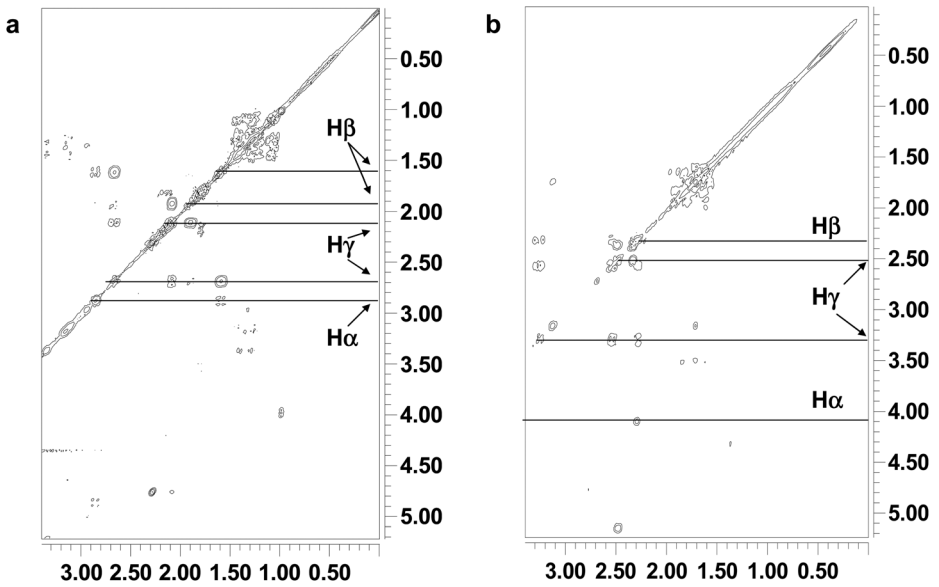


Fig. 6 Comparison of ^{1}H 2D COSY NMR spectra of **II** (a) and **III** (b) recorded in D_2O, with assignments of glutamate protons.

as a consequence of hindered rotation of the C–C and C–N bonds within the macrochelate. In contrast, in the case of **III** the glutamate aminic group would be uncomplexed and one platinum core would complete the coordination shell by binding a water molecule. Thus, in the case of **III**, the set of chemical shifts is consistent with a glutathione coordinated only by the S and N atoms of cysteine.[21]

Attempts to shift the equilibrium between **II** and **III** by changing the pH (by addition of TFA or NaOH) were unsuccessful. Sadler reported that the oxaliplatin–glutathione macrochelate was stable in the pH range 2.8–8.7.[25] In the case of kiteplatin, the corresponding macrochelate **II** is quite reactive, and the aminic group of glutamate can be easily displaced by a water molecule, leading to the formation of **III** (Fig. 7).

The possibility that the 9-membered macrochelate ring of **II** can easily open up, making a platinum coordination site available for coordination of other nucleophiles, such as water or a guanine base of DNA, could render compound **II** not an end product, but a drug reservoir able to interact, in a subsequent step, with biomolecules and exert an anticancer effect.

We also investigated the reaction between kiteplatin and oxidized glutathione. It has been reported that GSSG can react with chloridoamine–Pt(II) compounds leading to the formation of the same products formed in the case of GSH. This was explained with the possibility of chloridoamine–Pt(II) complexes to catalyze the disproportionation of GSSG into GSH and GSO_2H (2 GSSG + 2 H_2O → 3 GSH + GSO_2H).[45] Reaction of kiteplatin with GSSG (molar ratio 1 : 0.67 so to allow for formation of one GSH per platinum after disproportionation) led to formation of the same adducts obtained in the direct reaction between kiteplatin and GSH (Fig. S6 in ESI†).

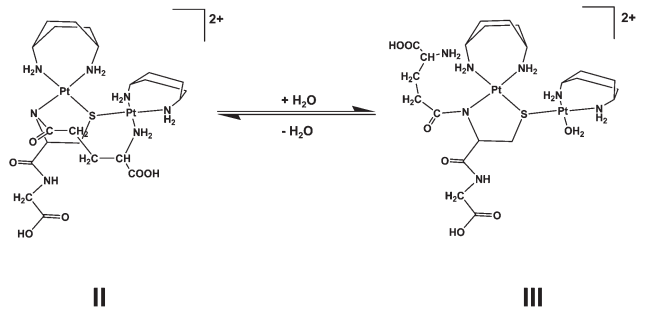


Fig. 7 Equilibrium between adducts **II** (left) and **III** (right).

Reaction of kiteplatin–glutathione adducts with 5′-GMP. As already reported, reaction of Pt(II) complexes with S-donor biomolecules is thought to cause deactivation of Pt(II)-based drugs, although the co-administration of cisplatin and glutathione (the latter as chemoprotectant) does not appear to influence the efficacy of the drug in terms of therapeutic outcome.[29]

It has also been hypothesized that platinum–glutathione adducts could act as a drug reservoir in tumor cells.

In order to assess if one of the adducts formed by kiteplatin and a S-containing biomolecule can react with nucleic acids, we have carried out a preliminary NMR investigation using 5′-GMP as a DNA model.

Therefore, in one experiment kiteplatin was allowed to react with glutathione for 24 h at 37 °C (a time sufficient for the reaction to be completed) and then treated with 5′-GMP (2 equivalents). The reaction was monitored by ^{1}H NMR focusing our attention on H8 and H1′ signals of 5′-GMP (Fig. 8).[30]

The spectrum recorded immediately after addition of 5′-GMP shows the signals of the free nucleotide: a sharp singlet at 8.16 ppm assigned to H8, a broad signal at 6.35 ppm assigned to the NH_2 group and a doublet at 5.91 ppm assigned to H1′ of the ribose (Fig. 8a). The pH of the solution, initially acidic, shifted to neutral values after addition of GMP.

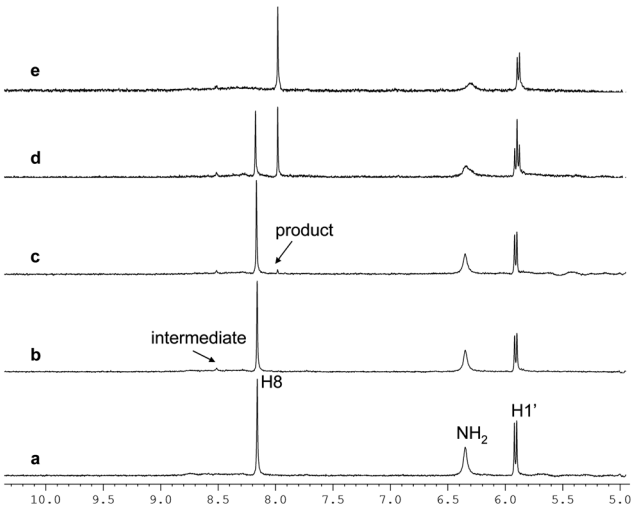


Fig. 8 ^{1}H NMR spectra of the reaction between kiteplatin–GSH adducts and GMP: (a) soon after addition of GMP to a solution of kiteplatin and glutathione kept for 1 d at 37 °C, (b) after 1 day at 37 °C, (c) after 6 days, (d) after 10 days, (e) after 13 days.

After 1 day at 37 °C, only a very weak signal was detected at 8.51 ppm. Most likely this signal belongs to H8 of a mono-adduct between Pt and GMP (Fig. 8b).[38] After 6 days at 37 °C, the signal at 8.51 ppm remained very weak and a new weak, more shielded, signal was detected at 7.98 ppm (Fig. 8c). The latter signal grew with time and, after 10 days reaction, its intensity was comparable to that of unreacted GMP (Fig. 8d). After 13 days, no free GMP remained. The singlet detected at 7.98 ppm belongs to the H8 protons of GMP in the bis-adduct $[Pt(cis\text{-}1,4\text{-DACH})(5'\text{-GMP})_2]^{2+}$, as shown in a previous work.[42] The latter compound is also characterized by NH_2 (6.31 ppm) and H1′ GMP protons (5.68 ppm) slightly shifted to higher field with respect to free GMP (Fig. 8e).

This experiment proves that kiteplatin already bound to a S-containing biomolecule is still able to react with 5′-GMP at non-acidic pH values. We were surprised by the extremely low reactivity observed in the first 6 days (spectra a–c in Fig. 8) as opposed to the quite high reactivity observed in the following 4 days and hypothesized that handling and shaking of the NMR sample could have favoured GSH oxidation by air. Therefore we repeated the experiment under anaerobic conditions (flushing with N_2). In the latter conditions, no reaction was observed even after a prolonged reaction time. Therefore we can argue that the displacement of glutathione by GMP is triggered by oxygen which, by oxidizing GSH, shifts the equilibrium in favour of the formation of the kiteplatin-$(GMP)_2$ adduct. When the reaction is carried out under a N_2 atmosphere, the oxidation process cannot take place, and thus the glutathione remains in its reduced form that has higher affinity for the Pt(II) center than for GMP.

Kiteplatin and cysteine

Reaction between kiteplatin and cysteine led to the formation of a major product that was isolated by HPLC and characterized by ESI-MS and NMR spectroscopy.

ESI-MS analysis showed a doubly-charged positive peak at m/z = 386.57, assignable to the molecular formula $[C_{15}H_{34}ClN_5O_2Pt_2S]^{2+}$ (M^{2+}). This is consistent with a molecular arrangement formed by two [Pt(*cis*-1,4-DACH)] moieties, a chloride and a deprotonated cysteine, as confirmed by the isotopic distribution pattern. A second peak was detected at m/z = 772.14, corresponding to the molecular formula $[C_{15}H_{33}ClN_5O_2Pt_2S]^{+}$ and consistent with $[M - H]^+$, as also confirmed by the isotopic distribution pattern (Fig. S7 in ESI†).

The isolated adduct was characterized by NMR (Fig. S8 in ESI†) and the proton chemical shifts are reported in Table 2.

Two signals (at 3.77 and 3.51 ppm) were assigned to the methynic protons of coordinated DACH. These two signals correlate with the multiplet centered at 1.87 ppm, that was assigned to the methylenic protons of DACH.

A signal detected at 3.71 ppm was assigned to the α protons of cysteine. It correlates with a multiplet centered at 3.12 ppm, that was assigned to the β protons of cysteine.

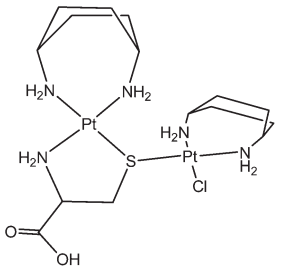
The presence of two different signals for the methynic protons of DACH suggests that the two aminic groups have different ligands in the *trans* position.

All the reported data are consistent with a molecular arrangement having two (*cis*-1,4-DACH)Pt(II) units bridged by a single S-atom of cysteine. One Pt(II) unit could complete its coordination shell by coordinating the aminic group of cysteine, while the second Pt(II) center could complete its coordination shell by coordinating a chlorido ligand. The overall formula is sketched in Fig. 9 (compound **IV**). For the first platinum, the overall arrangement would be similar to that reported for the cysteine–cisplatin adduct $[Pt(NH_3)_2(L\text{-cys-}N,S)]^+$,[34] while for the second platinum the arrangement corresponds to the substitution of the first chloride by the incoming cysteine nucleophile.

Compound **IV** has strong relationship with the glutathione-adducts **II** and **III** previously described. In all cases, one cysteine–sulfur bridges two (*cis*-1,4-DACH)Pt(II) units, and the

Table 2 ^{1}H NMR chemical shifts (ppm) for adducts of kiteplatin with cysteine and methionine in D_2O

	Amino acid				*cis*-1,4-DACH		
Adduct	*Hα*	*Hβ*	*Hγ*	CH_3	NH_2	*CH*	CH_2
Cysteine	4.00	3.15–3.02					
Methionine	3.87	2.24–2.09	2.65	2.14			
IV	3.71	3.12			—	3.77, 3.51	1.87
V	3.61, 3.33	1.91, 1.60	2.73, 2.54, 2.24	2.10, 2.09, 2.00, 1.97	5.54–4.99	3.41, 3.12, 2.69, 2.51	1.46–0.76



IV

Fig. 9 Sketch of kiteplatin–cysteine adduct (**IV**).

N atom of cysteine coordinates to one platinum unit forming a 5-membered ring. Unlike compound **II**, in which the aminic group of glutamate coordinates to the second Pt(II) unit, in the case of **III** and **IV** the coordination shell of the second platinum is completed by an extra ligand (a water molecule and a chlorido ligand, respectively).

Both Pt centers retain their *cis*-1,4-DACH ligand. This fact could allow kiteplatin to retain its antitumor activity after interacting with cysteine or cysteine containing peptides, since the intact (*cis*-1,4-DACH)Pt(II) moiety could still be able to coordinate to DNA and exert a pharmacological effect.

Kiteplatin and methionine

The interaction between cisplatin and methionine has been considered a possible cause of nephrotoxicity observed in patients treated with cisplatin.[46] Therefore, it was of interest to investigate the reaction of kiteplatin with methionine.

Reaction between kiteplatin and methionine led to the formation of a major adduct that was isolated by HPLC and characterized by ESI-MS and ^{1}H NMR spectroscopy.

ESI-MS showed a cationic peak at m/z = 493.95, corresponding to the molecular formula, $[C_{11}H_{25}ClN_3O_2PtS]^+$ (M^+), as also confirmed by the isotopic distribution pattern. An anionic peak was also detected at m/z = 527.94, corresponding to the molecular formula $[C_{11}H_{24}Cl_2N_3O_2PtS]^-$ ($[M + Cl - H]^-$), as also confirmed by the isotopic distribution pattern (Fig. S9 in ESI†). These data are consistent with a reaction product having the formula [PtCl(*cis*-1,4-DACH)(met)]$^+$ (compound **V**).

This product was characterized also by NMR (Fig. S10 in ESI†) and the proton chemical shifts are reported in Table 2. The ^{1}H NMR spectrum showed four signals assignable to CH_3 groups of coordinated methionine (Fig. 10). Since a monocoordinated, S-bonded methionine would give only one methyl signal, we deduce that, most likely, methionine coordinates to the Pt center through the S and N atoms, forming a 6-member ring and, possibly, two different isomers are present. If the methionine is chelated, we must deduce that one end of the diamine has been displaced leaving room for one chloride to coordinate. Moreover, being the *cis*-1,4-DACH monocoordinated with a chloride in *cis* position, there is the possibility to have two isomers with the methionine sulfur *trans* to nitrogen or to chloride (Fig. 11). Each isomer would have two

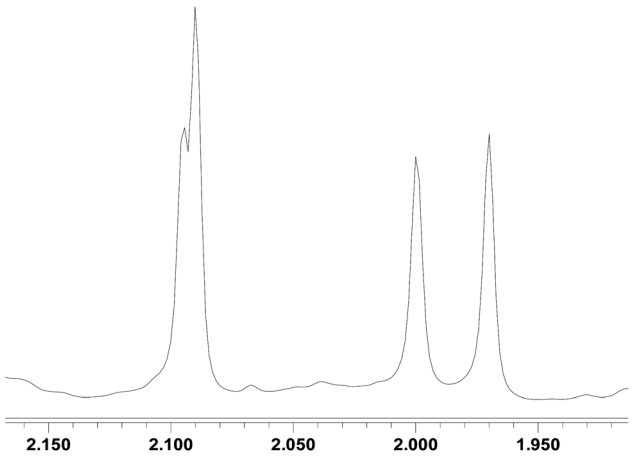
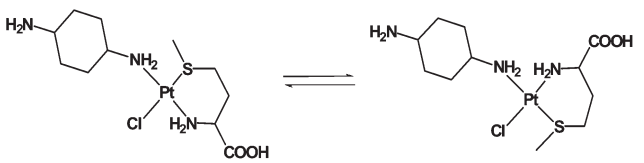


Fig. 10 Portion of ^{1}H NMR spectrum recorded on a sample of kiteplatin–methionine adduct in D_2O.



trans ***cis***

Fig. 11 Equilibrium between *trans* and *cis* isomers of the kiteplatin–methionine adduct (**V**).

possible configurations at the S atom (*R* or *S*) and the two configurations are made inequivalent by the asymmetry of the α carbon center. This would explain the observation of four methyl signals. A similar multiplicity has been observed for the [Pt(L-Methionine-*S*,*N*)$_2$]$^{2+}$ adduct.[35]

Notably, the formation of a similar adduct, with only one coordinated ammine and a chelated methionine was hypothesized in the reaction of the aqua species of cisplatin with methionine.[47]

The 7-member chelate ring of *cis*-1,4-DACH is strained, as evidenced by the NH_2–Pt–NH_2 angle of *ca.* 97°;[11,42] hence, it can easily open up with the diamine becoming monocoordinated. It is rather surprising that the diamine is not completely displaced by a second methionine molecule as observed in the case of cisplatin[46] and carboplatin.[35] It is possible that an additional interaction between the free end of the diamine and the metal core stabilizes this molecular arrangement (anchimeric assistance).

Conclusion

The investigation has shown that kiteplatin, like other platinum compounds with diamines, reacts with glutathione

forming a dimeric sulfur-bridged adduct containing two platinum units and a GSH moiety. Specific for kiteplatin is the equilibrium between two forms of the macrochelate, one with the glutamate aminic group coordinated to platinum (**II**) and the other with uncoordinated glutamate aminic group and a water molecule completing the coordination shell of one platinum (**III**). Such a dissociation equilibrium has not been observed in the case of the analogous species formed by other platinum substrates,[25,33] and could be a consequence of the steric hindrance of *cis*-1,4-DACH which can destabilize *cis* ligands.

The reaction of kiteplatin with cysteine led to the formation of an adduct having again two Pt(II)(*cis*-1,4-DACH) cores bridged by a S atom. The overall coordination of the amino acid is similar to that of GSH in compound **III** with the only difference that a chloride has replaced the coordinated water molecule.

Completely different is the adduct formed in the reaction of kiteplatin with methionine. The final product contains one methionine per platinum unit; however, unexpectedly, the DACH ligand is mono-coordinated while the amino acid is *N*,*S*-chelated. This product is different from that obtained in the reaction of methionine with cisplatin (in which two methionine molecules were *N*,*S*-coordinated to platinum and both ammines had been displaced) and with oxaliplatin (in which one methionine is *N*,*S*-coordinated to platinum and both ends of the diamine remain coordinated to platinum). The latter reaction highlights a peculiar behaviour of *cis*-1,4-DACH, *i.e.*, its ability to be mono-coordinated to platinum and leave a coordination site available for coordination to another ligand.

The reaction of kiteplatin with glutathione does not prevent a further reaction with 5′-GMP. However under anaerobic conditions the equilibrium is completely shifted in favour of the GSH derivative. In contrast, under aerobic conditions the typical Pt-$(GMP)_2$ adduct is formed. This result highlights the necessity to carefully control the experimental conditions in investigating the reaction between platinum substrates and biomolecules. We plan to extend the investigation to di- and poly-nucleotides to fully exploit the potential of Pt-GSH adducts to act as a drug reservoir.

The different reactivity of kiteplatin, with respect to cisplatin and oxaliplatin, toward the biomolecules so far investigated could explain, at least in part, the different pharmacological activity of kiteplatin.

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References

1 *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, ed. B. Lippert, Verlag Helvetica Chimica Acta, Zurich, 2000, p. 563.
2 E. Reed, *Cancer Chemother. Biol. Response Modif.*, 1990, **11**, 90–96.
3 E. R. Jamieson and S. J. Lippard, *Chem. Rev.*, 1999, **99**, 2467–2498.
4 L. R. Kelland and N. Farrell, Platinum-Based Drugs in Cancer Therapy, in *Cancer Drug Discovery and Development*, ed. B. A. Teicher, Humana Press, 2000.
5 A. Eastman, *Cancer Treat. Res.*, 1991, **57**, 233–249.
6 M. J. Bloemink and J. Reedijk, *Met. Ions Biol. Syst.*, 1996, **32**, 641–685.
7 D. K. Kim, G. Kim, J. Gam, Y. B. Cho, H. T. Kim, J. H. Tai, K. H. Kim, W. S. Hong and J. G. Park, *J. Med. Chem.*, 1994, **37**, 1471–1485.
8 Y. Kidani, M. Noji and T. Tashiro, *Gann*, 1980, **71**, 637–643.
9 R. R. Barefoot, *J. Chromatogr. B: Biomed. Sci. Appl.*, 2001, **751**, 205–211.
10 S. Hector, W. Bolanowska-Higdon, J. Zdanowicz, S. Hitt and L. Pendyala, *Cancer Chemother. Pharmacol.*, 2001, **48**, 398–406.
11 J. D. Hoeschele, H. D. Hollis Showalter, A. J. Kraker, W. L. Elliott, B. J. Roberts and J. W. Kampf, *J. Med. Chem.*, 1994, **37**, 2630–2636.
12 N. Margiotta, C. Marzano, V. Gandin, D. Osella, M. Ravera, E. Gabano, J. A. Platts, E. Petruzzella, J. D. Hoeschele and G. Natile, *J. Med. Chem.*, 2012, **55**, 7182–7192.
13 V. Brabec, J. Malina, N. Margiotta, G. Natile and J. Kasparkova, *Chem. – Eur. J.*, 2012, **18**, 15439–15448.
14 J. Kasparkova, T. Suchankova, A. Halamikova, L. Zerzankova, O. Vrana, N. Margiotta, G. Natile and V. Brabec, *Biochem. Pharmacol.*, 2010, **79**, 552–564.
15 E. Petruzzella, N. Margiotta, M. Ravera and G. Natile, *Inorg. Chem.*, 2013, **52**, 2393–2403.
16 N. Margiotta, R. Ranaldo, F. P. Intini and G. Natile, *Dalton Trans.*, 2011, **40**, 12877–12885.
17 M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem.*, 1973, **2**, 187–210.
18 Y. Wu, P. Pradhan, J. Havener, G. Boyse, J. A. Swenberg, S. L. Campbell and S. G. Chaney, *J. Mol. Biol.*, 2004, **341**, 1251–1269.
19 Y. Wu, D. Bhattacharyya, C. L. King, I. Baskerville-Abraham, S. H. Huh, G. Boyse, J. A. Swenberg, B. Temple, S. L. Campbell and S. G. Chaney, *Biochemistry*, 2007, **46**, 6477–6487.
20 C. Rothenburger, M. Galanski, V. B. Arion, H. Görls, W. Weigand and B. K. Keppler, *Eur. J. Inorg. Chem.*, 2006, **18**, 3746–3752.
21 P. C. Dedon and R. F. Borch, *Biochem. Pharmacol.*, 1987, **36**(12), 1955–1964.
22 J. Reedijk, *Pure Appl. Chem.*, 1987, **59**, 181–192.
23 F. Yu, J. Megyesi and P. M. Price, *Am. J. Physiol. Renal Physiol.*, 2008, **295**, F44–F52.

24 T. Miura, S. Muraoka and T. Ogisco, *Life Sci.*, 1997, **60**, 301–309.
25 S. Fakih, V. P. Munk, M. A. Shipman, P. d. S. Murdoch, J. A. Parkinson and P. J. Sadler, *Eur. J. Inorg. Chem.*, 2003, 2106–1214.
26 L. Pendyala, R. Perez, A. Weinstein, J. Zdanowicz and P. J. Creaven, *Cancer Chemother. Pharmacol.*, 1997, **40**, 38–44.
27 T. Ishikawa and F. Ali-Osman, *J. Biol. Chem.*, 1993, **268**, 20116–20125.
28 B. A. J. Jansen, J. Brouwer and J. Reedijk, *J. Inorg. Biochem.*, 2002, **89**, 197–202.
29 F. P. T. Hamers, J. H. Brakkee, E. Cavalletti, M. Tedeschi, L. Marmonti, G. Pezzoni, J. P. Neijt and W. H. Gispen, *Cancer Res.*, 1993, **53**, 544–549.
30 S. S. G. E. van Boom, B. W. Chen, J. M. Teuben and J. Reedijk, *Inorg. Chem.*, 1999, **38**, 1450–1455.
31 E. Volckova, L. P. Dudones and R. N. Bose, *Pharm. Res.*, 2002, **19**, 124–131.
32 R. N. Bose, S. Moghaddas, E. L. Weaver and E. H. Cox, *Inorg. Chem.*, 1995, **34**, 5878–5883.
33 P. d. S. Murdoch, N. A. Kratochwil, J. A. Parkinson, M. Patriarca and P. J. Sadler, *Angew. Chem., Int. Ed.*, 1999, **38**, 2949–2951.
34 T. G. Appleton, J. W. Connor, J. R. Hall and P. D. Prenzler, *Inorg. Chem.*, 1989, **28**, 2030–2037.
35 K. J. Barnham, M. I. Djuran, P. d. S. Murdoch, J. D. Ranford and P. J. Sadler, *Inorg. Chem.*, 1996, **35**, 1065–1072.
36 P. Papadia, N. Margiotta, A. Bergamo, G. Sava and G. Natile, *J. Med. Chem.*, 2005, **48**, 3364–3371.
37 A. Bouslimani, N. Bec, M. Glueckmann, C. Hirtz and C. Larroque, *Rapid Commun. Mass Spectrom.*, 2010, **24**, 415–421.
38 O. Heudi, S. Mercier-Jobard, A. Cailleux and P. Allain, *Biopharm. Drug Dispos.*, 1999, **20**, 107–116.
39 D. B. Strickmann, A. Küng and B. K. Keppler, *Electrophoresis*, 2002, **23**, 74–80.
40 V. Maheshwari, P. A. Marzilli and L. G. Marzilli, *Inorg. Chem.*, 2011, **50**, 6626–6636.
41 S. Shamsuddin, I. Takahashi, Z. H. Siddik and A. R. Khokhar, *J. Inorg. Biochem.*, 1996, **61**, 291–301.
42 R. Ranaldo, N. Margiotta, F. P. Intini, C. Pacifico and G. Natile, *Inorg. Chem.*, 2008, **47**, 2820–2830.
43 M. El-Khateeb, T. G. Appleton, B. G. Charles and L. R. Gahan, *J. Pharm. Sci.*, 1999, **88**, 319–326.
44 A. Sionkowska, *Polym. Degrad. Stab.*, 2001, **73**, 107–112.
45 E. L. M. Lempers, K. Inagaki and J. Reedijk, *Inorg. Chim. Acta*, 1988, **152**, 201–207.
46 O. Heudi, A. Cailleux and P. Allain, *J. Inorg. Biochem.*, 1998, **71**, 61–69.
47 M. El-Khateeb, T. G. Appleton, L. R. Gahan, B. G. Charles, S. J. Berners-Price and A. M. Bolton, *J. Inorg. Biochem.*, 1999, **77**, 13–21.