Electronic supplementary information for:

Reduction of ormaplatin by an extended series of thiols unravels a remarkable correlation

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1. Experimental section

1.1. Materials

L-Cysteine (Cys), DL-Homocysteine (Hcy), D-Penicillamine (Pen), DL-Thiomalic acid (TMA), L-Cysteine ethyl ester hydrochloride (Cys-Et), Cysteamine (CA), DL-thiolactic acid (TLA), and 2-Mercaptoethanol (2-ME), potassium tetrachloroplatinate(II) ($K_2[PtCl_4]$), (±)-*trans*-1,2-diaminocyclohexane (dach) in their purest forms were obtained from Sigma-Aldrich (St. Louis, MO). Acetic acid, sodium acetate, sodium chloride, hydrochloric acid, sodium dihydrogen-phosphate, disodium hydrogen phosphate, sodium bicarbonate, sodium carbonate, trisodium phosphate, and sodium perchlorate were obtained as the purest forms available either from Alfa Aesar (Shanghai, China) or from Fisher Scientific (Beijing, China) and were used without further purification. For pH meter calibrations, standard buffers of pH 4.00, 7.00 and 10.00 were purchased from Fisher Scientific. Doubly distilled water was used in all syntheses and for all solutions used for the kinetic measurements.

1.2. Synthesis and characterization of ormaplatin ($[Pt(dach)Cl_4]$)

1.2.1. Synthesis of $[Pt(dach)Cl_2]$

1.875 g $K_2[PtCl_4]$ (4.5 mmol) was dissolved in 50 mL water (reddish-orange color), 0.56 g dach (4.9 mmol) was added to the Pt(II) solution. The reaction mixture was stirred at room temperature under nitrogen gas while the precipitate appeared. The stir continued until the color of the mixture changed from the original reddish-orange, via orange to yellow. The precipitate was collected by centrifugation, and washed with 1 M HCl to remove the excess dach. The precipitate was then washed three times with ethanol to remove the excess HCl and water, and finally washed once more with ether. The solid product was air dried in a hood for 4 h. It was then dissolved in a minimum amount of DMF and the solution was filtered. 100 ml of methanol

was added to the filtrate and the precipitate re-appeared. It was collected by centrifugation and washed once more with methanol, and then lyophilized for 12 h, yielding 1.068 g of [Pt(dach)Cl_2] (65%). Calcd for $C_6H_{14}Cl_2N_2Pt$ (MW = 380.18): C, 18.96%; H, 3.71%; N, 7.37%. Found: C, 19.01%; H, 3.55%; N, 7.35%. ^{13}C NMR (150 MHz, DMF-d_7), δ 63.97, 32.64, 25.12. *m/z* = 402.01 (highest isotopic peak for M·Na^+); *m/z* = 378.01 (highest isotopic peak for [M-H^+]$^-$).

1.2.2. Synthesis of [Pt(dach)Cl_4]·⅓H_2O

0.70 g [Pt(dach)Cl_2] (1.84 mmol) was added to 25 mL 0.50 M HCl saturated with chlorine gas; the color of the suspension formed rapidly changed to orange red. The mixture was stirred at room temperature under slow chlorine gas bubbling until the color became yellow. The yellow solid was separated by rotary evaporation. It was re-dissolved in 40 mL warm acetone and the solution was quickly filtered. Rotary evaporation of the filtrate gave a solid precipitate, which was re-dissolved in 40 mL warm acetone. 50 mL ethyl ether was added, and the mixture was kept in a fridge for 12 h. The precipitate formed was filtered off, washed three times with ethyl ether and then dried, yielding 0.47 g [Pt(dach)Cl_4]·⅓H_2O (56.7%). Calculated for [Pt(dach)Cl_4]·⅓H_2O (MW = 457.08): C 15.78%; H 3.20%; N 6.12%. Found: C 15.63%; H 3.21%; N 5.90%. ^{13}C NMR (150 MHz, DMSO-d_6), δ 63.82, 31.52, 24.64. *m/z* = 473.94 (highest isotopic peak for M·Na^+); *m/z* = 449.95 (highest isotopic peak for [M-H^+]$^-$).

1.3. Instrumentation

UV-Vis spectra were recorded by use of a TU-1900 spectrophotometer (Beijing Puxi, Inc., Beijing, China) and 1.00 cm quartz cells. Rapid scan spectra and kinetic measurements were

performed using an Applied Photophysics SX-20 stopped-flow spectrometer (Applied Photophysics Ltd., Leatherhead, U.K.). ^{13}C NMR spectra were recorded on a Bruker AVANCE III 600 MHz digital NMR spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). ESI high-resolution mass spectra were recorded using an LC/Q-Orbitrap mass spectrometer with a heated electrospray ionization source (ESI) in positive mode (Thermo Scientific, Bremen, Germany). An Accumet Basic AB15 Plus pH meter, equipped with an Accumet AccutupH® combination pH electrode (Fisher Scientific, Pittsburgh, PA), was used to measure the pH values of buffer solutions. Immediately before each pH measurement, the electrode was calibrated using standard buffers of pH 4.00, 7.00 and 10.00.

1.4. Reaction media

Combinations of HAc/NaAc, NaH_2PO_4/Na_2HPO_4, $NaHCO_3/Na_2CO_3$, and Na_2HPO_4/Na_3PO_4 (concentrations 0.15 - 0.25 M) were used to prepare buffer solutions, covering a wide pH range between ca. 2 and 11. All buffers contained 2 mM EDTA and 0.10 M NaCl and their ionic strength (μ) was adjusted to 1.0 M by use of sodium perchlorate. The presence of EDTA minimized possible catalytic effects by traces of metal ions such as Cu(II) and Fe(III) during thiol autooxidation,[1-3] and that of NaCl suppressed the hydrolysis of ormaplatin.

1.5. Kinetic measurements

Stock solutions of thiols were prepared by adding the needed amount of the thiol to a buffer solution of specific pH. The solutions were flushed with nitrogen for 10 min. Stock solutions of 1.0 mM ormaplatin were prepared by dissolving the desired amount of ormaplatin in a solution containing 0.90 M $NaClO_4$, 0.09 M NaCl and 0.01 M HCl; these solutions were only used for a

couple of hours. Solutions of ormaplatin and of thiol for kinetic measurements were prepared by dilution of the stock solutions mentioned with the same pH buffer. They were nitrogen-flushed for 10 min before loading onto the stopped-flow spectrometer. Kinetic traces were run by mixing equal volumes of ormaplatin and thiol solutions directly in the stopped-flow instrument and followed between 260 and 280 nm. All kinetic runs were carried out under pseudo first-order conditions with a 10-fold excess of $[\text{thiol}]_{\text{tot}}$ over [Pt(IV)], where $[\text{thiol}]_{\text{tot}}$ denotes the total concentration of thiol. The reported pseudo first-order rate constants, k_{obsd}, were obtained as the average values of 5-7 duplicate runs; standard deviations were usually less than 5%.

1.6. Mass spectra

To identify the oxidation products of Cys, Hcy and Pen by ormaplatin under reaction conditions similar to the kinetic measurements, high-resolution ESI mass spectra were recorded for fresh solutions of 8 mM Cys, Hcy, and Pen in 10 mM acetic acid, and for reaction mixtures containing 8 mM Cys/Hcy/Pen and 1 mM ormaplatin in 10 mM acetic acid after a reaction time of 10 min.

2. Calculation of rate constants

2.1. Reduction of ormaplatin by 2-Mercaptoethanol (2-ME)

The reaction mechanism is described in Figure S7. The rate expression of Eq. (i) can be readily derived:

$$k' = \frac{k_1 a_{\text{H}} + k_2 K_{\text{a1}}}{a_{\text{H}} + K_{\text{a1}}} \qquad \text{(i)}$$

The protolysis constant was reported to be pK_{a1} = 9.61 at 25.0 °C and μ = 1.0 M.[4] Eq. (i) was used to simulate the k' – pH dependence data, resulting in the good fit shown in Figure S8. Values of k_1 = 0.056 ± 0.09 $M^{-1}s^{-1}$ and k_2 = (2.2 ± 0.1) x 10^5 $M^{-1}s^{-1}$ were obtained from the simulation.

2.2. Reduction of ormaplatin by Thiolactic Acid (TLA)

The reduction mechanism of ormaplatin by TLA is similarly described in Figure S9. A corresponding rate expression is deduced as Eq. (ii):

$$k' = \frac{k_1 a_H^2 + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \qquad \text{(ii)}$$

Protolysis constants were reported as pK_{a1} = 3.38 and pK_{a2} = 9.93 at 25.0 °C and μ = 0.50 M.[5] Eq. (ii) was used to simulate the k' – pH dependence data in Figure S10 with the protolysis constants as direct inputs; the simulation showed that k_1 was too small to determine with any confident accuracy. Neglecting the k_1 -term in Eq. (ii) gives Eq. (iii), which was employed to

$$k' = \frac{k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \qquad \text{(iii)}$$

simulate the k' – pH dependence data; the results are given in Figure S10, simultaneously affording k_2 = 4.1 ± 0.2 $M^{-1}s^{-1}$ and k_3 = (4.2 ± 0.1) x 10^5 $M^{-1}s^{-1}$.

2.3. Reduction of ormaplatin by L-Cysteine Ethyl Ester (Cys-Et) and Cysteamine (CA)

Reduction of ormaplatin by Cys-Et and CA proceeds via similar reaction mechanisms which are outlined in Figures S11 and S13, respectively, The rate expressions derived are identical to Eq. (ii) above. Protolysis constants were reported to be pK_{a1} = 6.53 and pK_{a2} = 8.94 at 25.0 ^{o}C and μ = 0.16 M for Cys-Et;[6] and pK_{a1} = 8.37 and pK_{a2} = 10.44 for CA at 25.0 ^{o}C and μ = 0.50 M.[7] When Eq. (ii) was employed to simulate the k' – pH dependence data in Figures S12 and S14, respectively, excellent curve fits were obtained. The simulations gave rise to: k_1 = 0.12 ± 0.03 $M^{-1}s^{-1}$, k_2 = (5.7 ± 0.2) x 10^3 $M^{-1}s^{-1}$ and k_3 = (1.73 ± 0.06) x 10^5 $M^{-1}s^{-1}$ for Cys-Et and k_1 = 0.11 ± 0.02 $M^{-1}s^{-1}$, k_2 = (8.8 ± 0.2) x 10^4 $M^{-1}s^{-1}$ and k_3 = (1.89 ± 0.08) x 10^5 $M^{-1}s^{-1}$ for CA.

2.4. Reduction of ormaplatin by Thiomalic Acid (TMA)

Figure S15 is the proposed reaction mechanism for the reduction of ormaplatin by TMA. The rate expression derived from this mechanism is identical to Eq. (3) in the main text. Protolysis constants for TMA were reported to be pK_{a1} = 2.7, pK_{a2} = 4.42, and pK_{a3} = 9.79 at 25.0^{o}C and μ = 1.0 M.[8] Since the k_1 value was indeterminate, Eq. (4) in the main text was used to simulate the k' – pH dependence data in Figure S16. The simulation result is good, producing k_2 = 3.3 ± 0.2 $M^{-1}s^{-1}$, k_3 = 10.7 ± 0.4 $M^{-1}s^{-1}$ and k_4 = (3.0 ± 0.1) x 10^5 $M^{-1}s^{-1}$.

All pK_a values and rate constants of the rate-determining steps calculated are summarized in Table S1.

Table S1. Values of rate constants of the rate-determining steps of various thiol species towards reduction of ormaplatin at 25.0 °C and μ = 1.0 M.

Thiols	pK_a Values	k_m	Value/$M^{-1}s^{-1}$
Cys	pK_{a1} = 1.9	k_1	not obsd
	pK_{a2} = 8.07	k_2	0.26 ± 0.06
	pK_{a3} = 9.95	k_3	(7.1 ± 0.1) x 10^4
		k_4	(2.7 ± 0.1) x 10^5
Hcy	pK_{a1} = 2.27	k_1	not obsd
	pK_{a2} = 8.66	k_2	0.22 ± 0.05
	pK_{a3} = 10.55	k_3	(6.4 ± 0.1) x 10^4
		k_4	(1.15 ± 0.05) x 10^5
Pen	pK_{a1} = 1.95	k_1	not obsd
	pK_{a2} = 7.92	k_2	0.16 ± 0.03
	pK_{a3} = 10.50	k_3	(3.5 ± 0.1) x 10^4
		k_4	(2.04 ± 0.08) x 10^5
2-ME	pK_{a1} = 9.61	k_1	0.056 ± 0.09
		k_2	(2.2 ± 0.1) x 10^5
TLA	pK_{a1} = 3.38	k_1	not obsd
	pK_{a2} = 9.93	k_2	4.1 ± 0.2
		k_3	(4.2 ± 0.1) x 10^5
Cys-Et	pK_{a1} = 6.53	k_1	0.12 ± 0.03
	pK_{a2} = 8.94	k_2	(5.7 ± 0.2) x 10^3
		k_3	(1.73 ± 0.06) x 10^5
CA	pK_{a1} = 8.37	k_1	0.11 ± 0.02
	pK_{a2} = 10.44	k_2	(8.8 ± 0.2) x 10^4
		k_3	(1.89 ± 0.08) x 10^5
TMA	pK_{a1} = 2.7	k_1	not obsd
	pK_{a2} = 4.42	k_2	3.3 ± 0.2
	pK_{a3} = 9.79	k_3	10.7 ± 0.4
		k_4	(3.0 ± 0.1) x 10^5

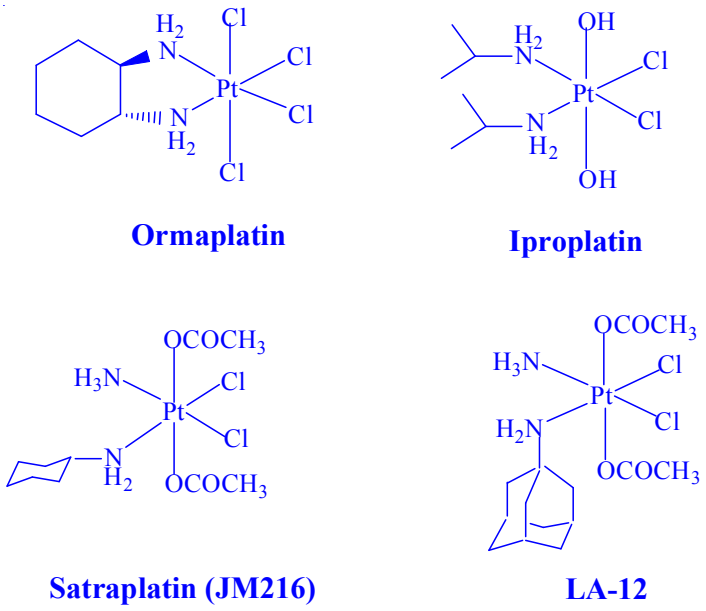

Figure S1. Pt(IV) anticancer prodrugs which have entered clinical trials.

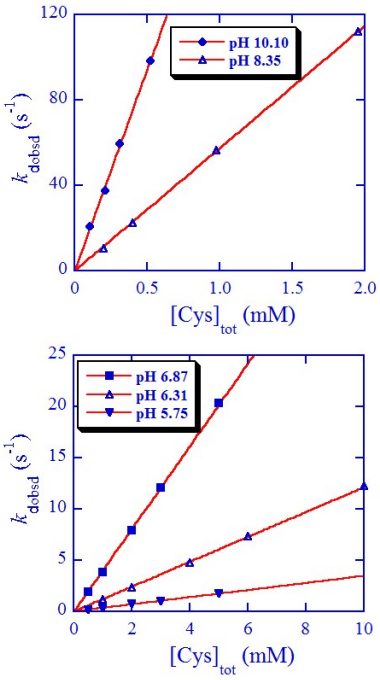

Figure S2. Plots of k_{obsd} *versus* $[Cys]_{tot}$ in different pH buffer solutions in the reduction of ormaplatin by L-cysteine at 25.0 °C and μ = 1.0 M. All plots are linear and passing through the origin, demonstrating that the reduction process is first-order with respect to Cys.

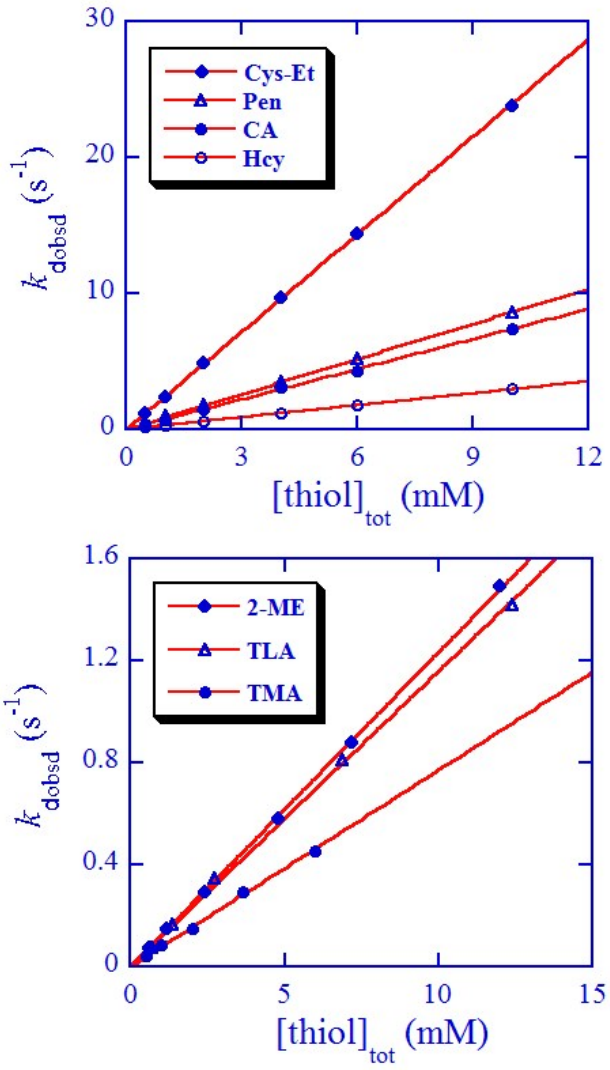

Figure S3. Plots of k_{obsd} *versus* $[thiol]_{tot}$ in a pH 6.31 buffer solution in the reduction of ormaplatin by various thiol-containing compounds at 25.0 °C and μ = 1.0 M. All plots are linear and passing through the origin, demonstrating that the reduction processes are first-order with respect to the thiols.

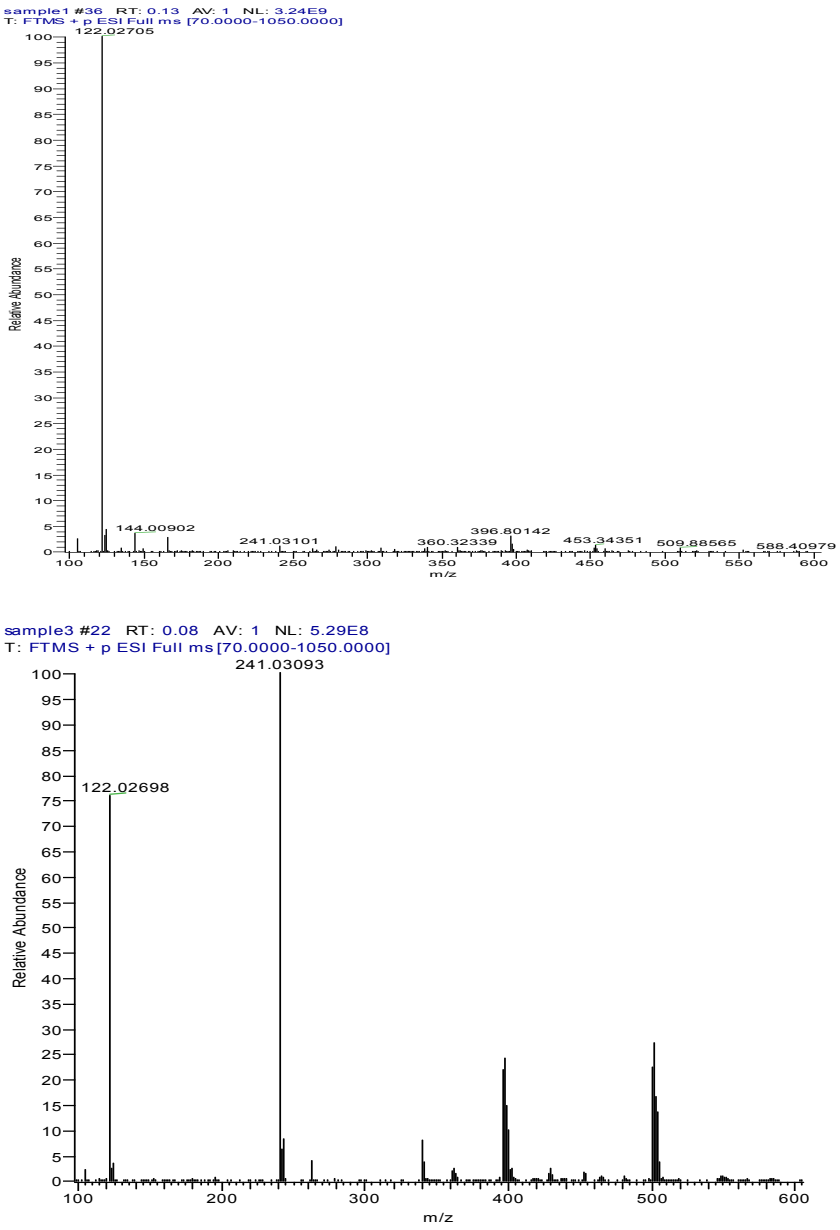

Figure S4. High-resolution mass spectra: (top): 8 mM L-Cysteine in 10 mM HAc. (bottom): A reaction mixture of 8 mM L-Cysteine with 1 mM ormaplatin in 10 mM HAc after a reaction time of 20 min.

Peak assignments: *m/z* = 122.027 for Cys·H^+; *m/z* = 244.03 for CysS-SCys·H^+; *m/z* around 400 and 500 are likely due to derivatives of Pt(II) complexes which are formed after reduction of ormaplatin.

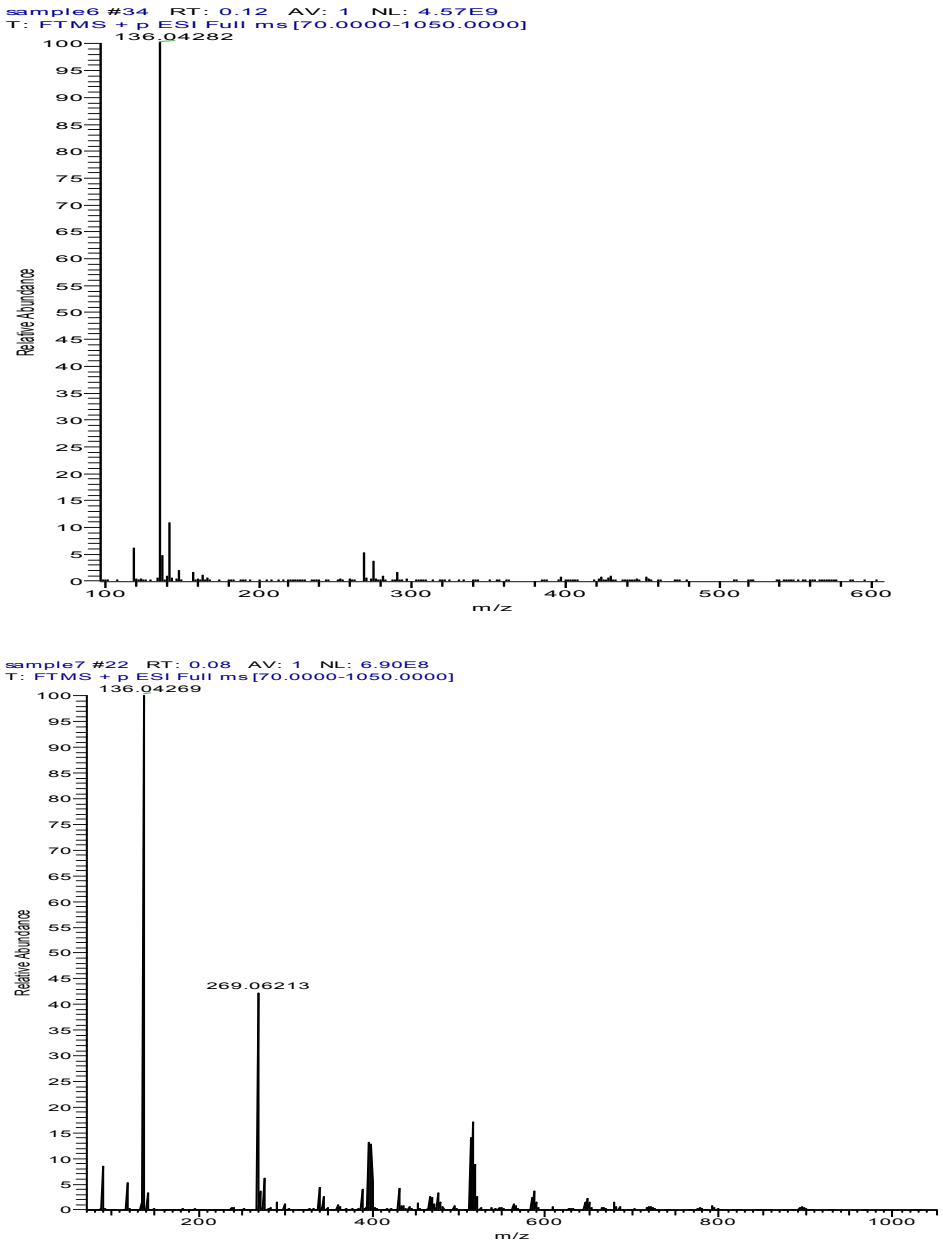

Figure S5. High-resolution mass spectra: (top): 8 mM DL-homocysteine in 10 mM HAc. (bottom): A reaction mixture of 8 mM Dl-homocysteine with 1 mM ormaplatin in 10 mM HAc after a reaction time of 20 min.

Peak assignments: *m/z* = 136.04, Hcy·H^+; *m/z* = 269.06, HcyS-SHcy·H^+; *m/z* around 400 and 510-520 are likely due to derivatives of Pt(II) complexes which are formed after reduction of ormaplatin.

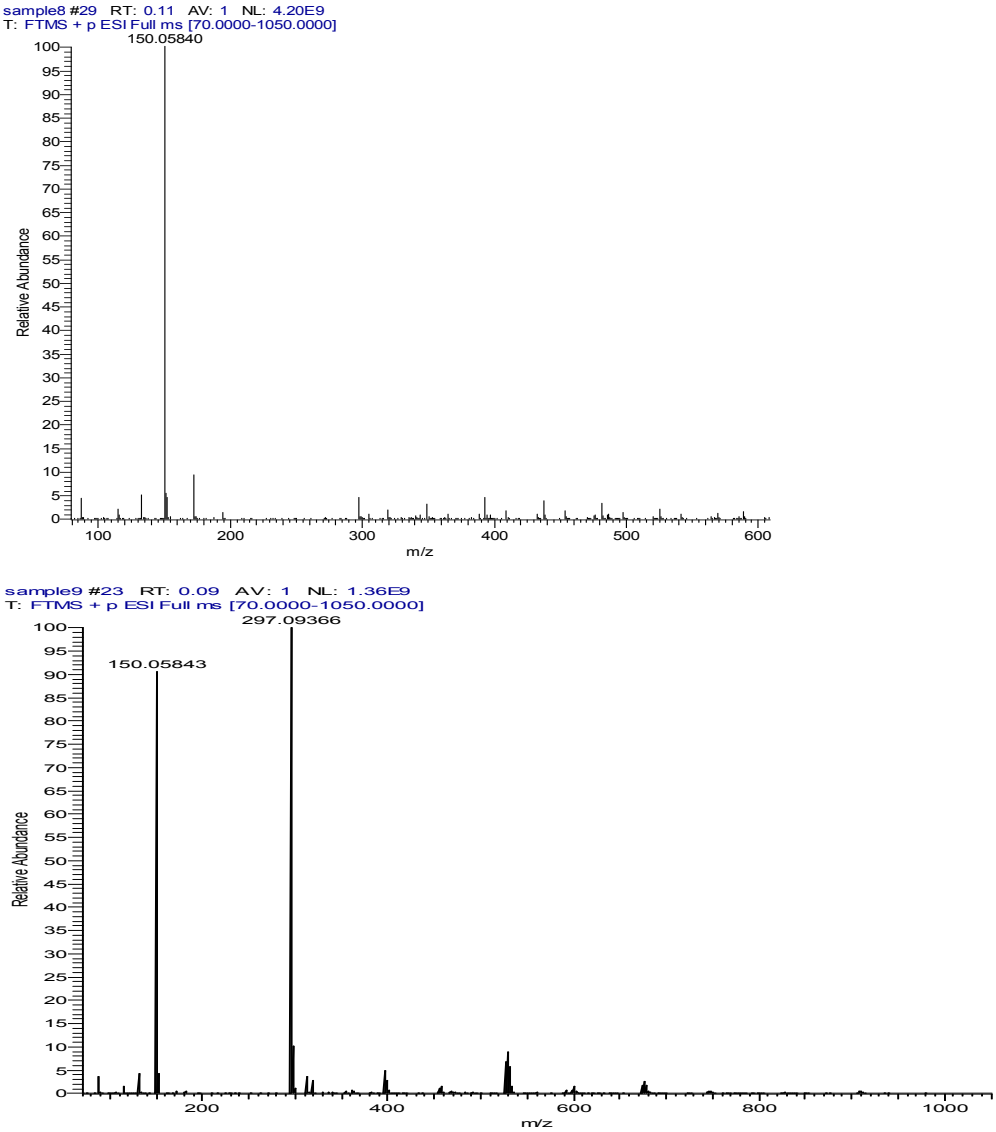

Figure S6. High-resolution mass spectra: (top): 8 mM D-penicillamine in 10 mM HAc. (bottom): A reaction mixture of 8 mM D-penicillamine with 1 mM ormaplatin in 10 mM HAc after a reaction time of 20 min.

Peak assignments: *m/z* = 150.058 for Pen·H^+; *m/z* = 297.09 for PenS-SPen·H^+; *m/z* around 400 and 530 are likely due to derivatives of Pt(II) complexes which are formed after reduction of ormaplatin.

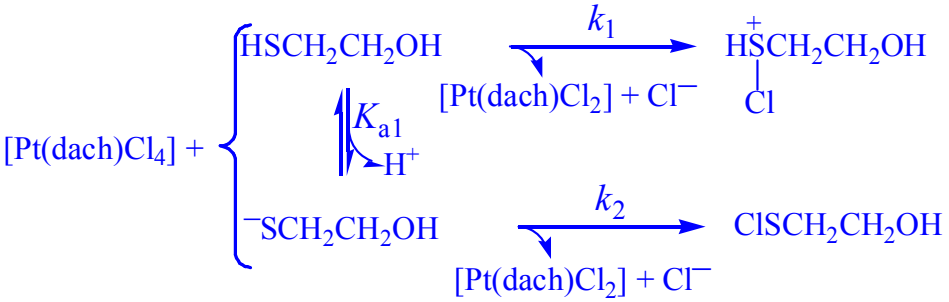

Figure S7. Reaction mechanism proposed for reduction of ormaplatin by 2-ME.

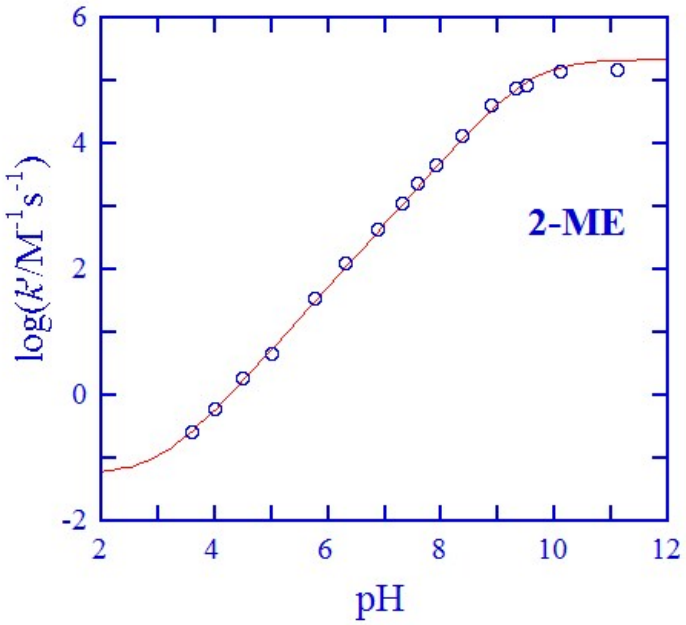

Figure S8. The k' – pH dependence data for reduction of ormaplatin by 2-ME. The solid line was obtained by simulation of Eq. (i) to the experimental data using a weighted nonlinear least-squares method.

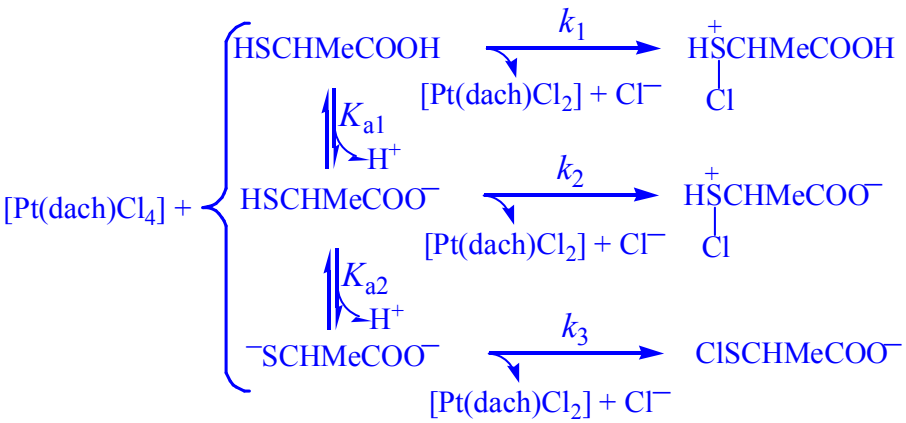

Figure S9. Reaction mechanism proposed for reduction of ormaplatin by thiolactic acid (TLA).

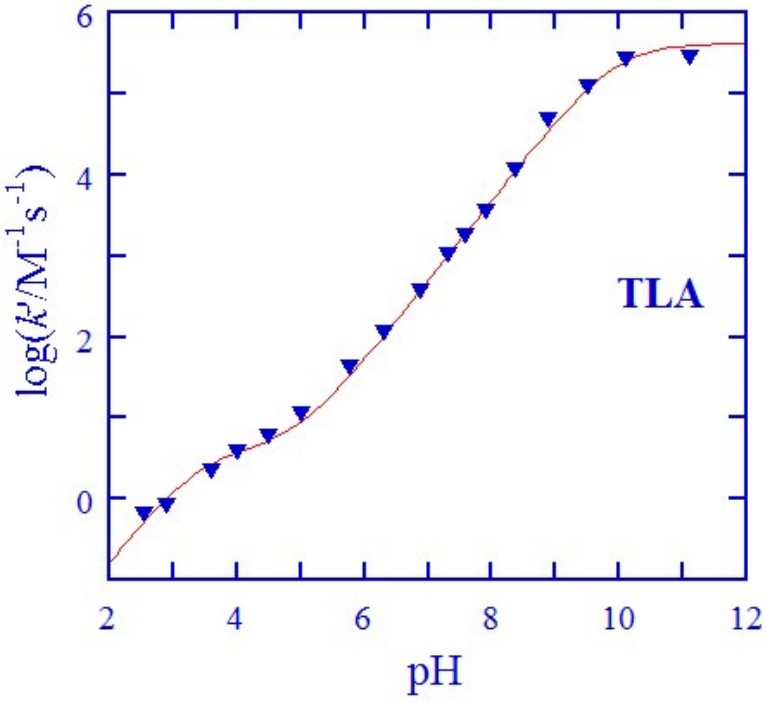

Figure S10. The k' – pH dependence data for reduction of ormaplatin by TLA. The solid line was obtained by simulation of Eq. (iii) to the experimental data using a weighted nonlinear least-squares method.

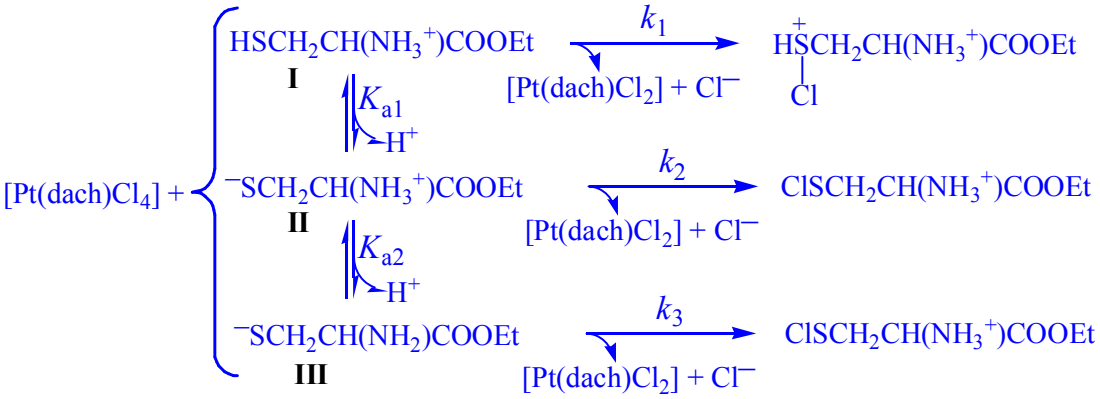

Figure S11. Reaction mechanism proposed for reduction of ormaplatin by Cys-Et.

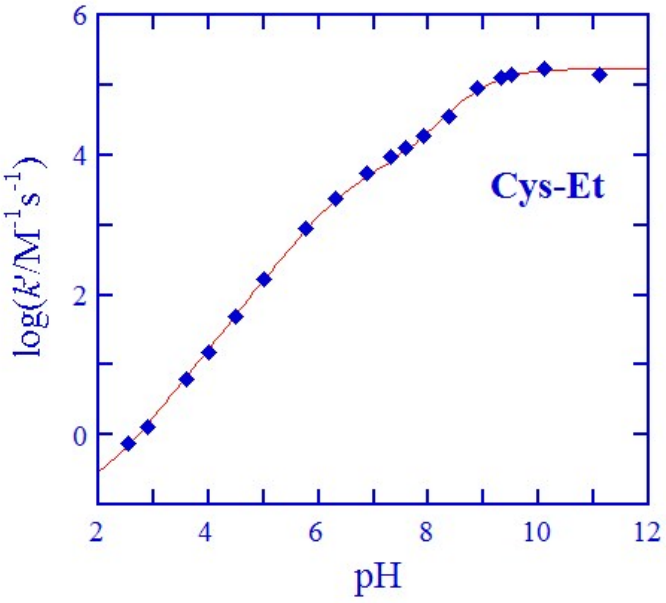

Figure S12. The k' – pH dependence data for reduction of ormaplatin by Cys-Et. The solid line was obtained by simulation of Eq. (ii) to the experimental data using a weighted nonlinear least-squares method.

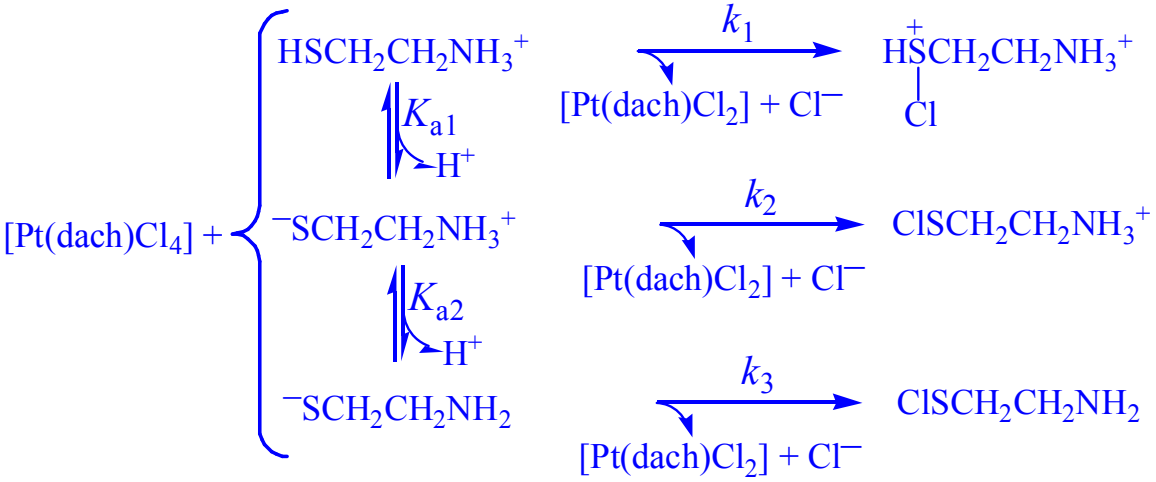

Figure S13. Reaction mechanism proposed for reduction of ormaplatin by cysteamine (CA).

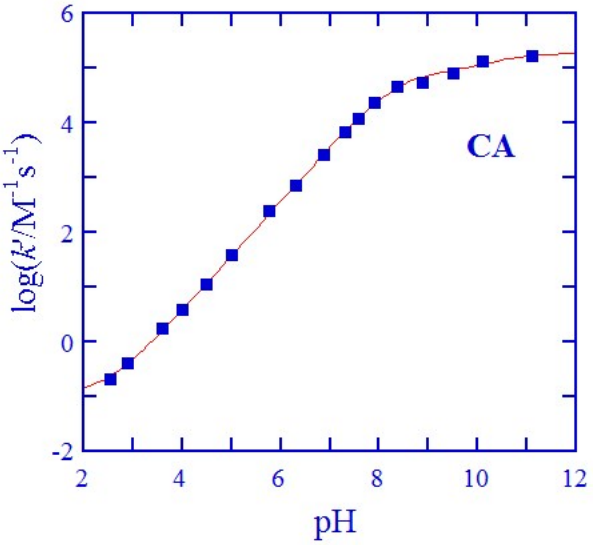

Figure S14. The k' – pH dependence data for reduction of ormaplatin by CA. The solid line was obtained by simulation of Eq. (ii) to the experimental data using a weighted nonlinear least-squares method.

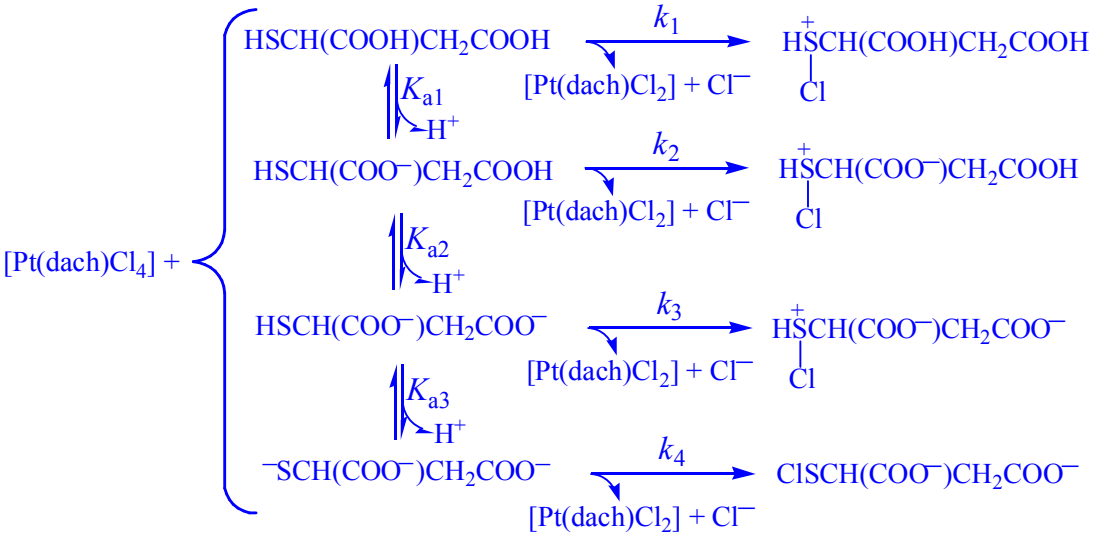

Figure S15. Reaction mechanism proposed for reduction of ormaplatin by thiomalic acid (TMA).

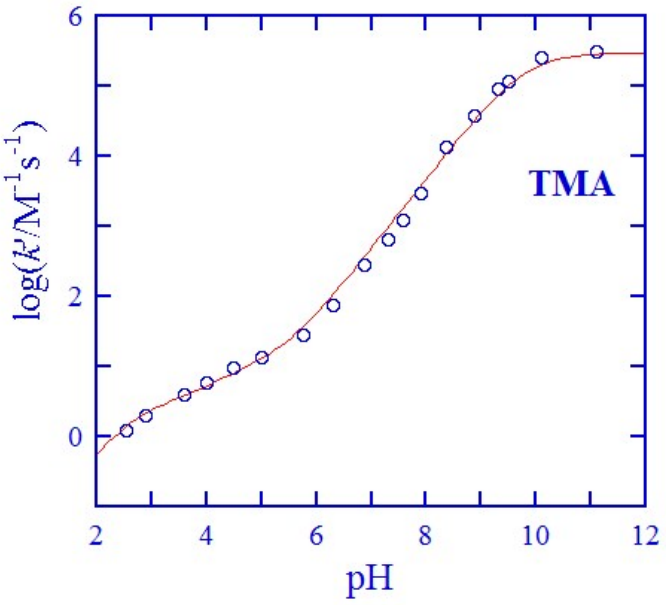

Figure S16. The k' – pH dependence data for reduction of ormaplatin by TMA. The solid line was obtained by simulation of Eq. (4) in the main text to the experimental data using a weighted nonlinear least-squares method.

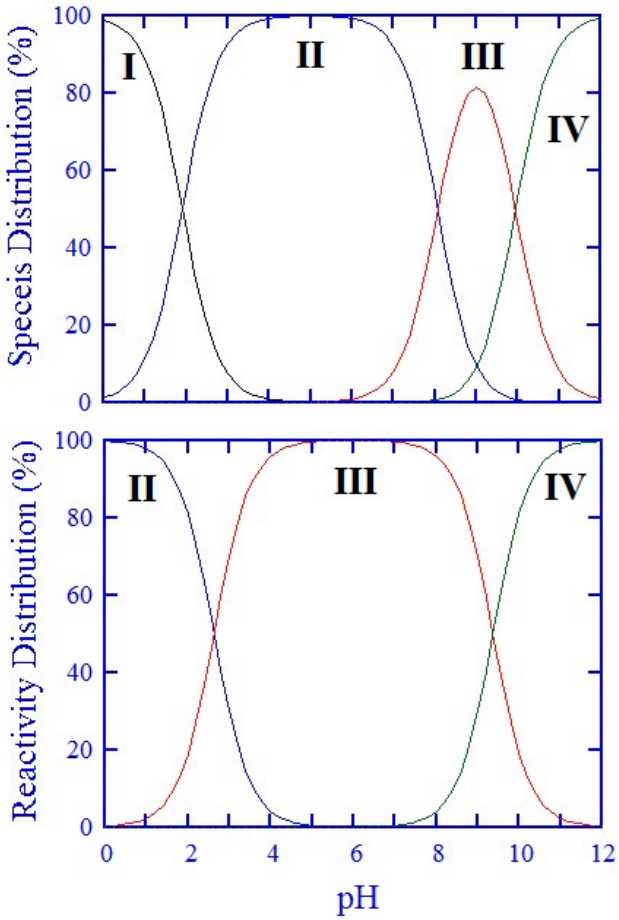

Figure S17. (Top): Cys species *versus* pH distribution diagram at 25.0 °C and μ = 1.0 M which was calculated using pK_{a1} = 1.9, pK_{a2} = 8.07 and pK_{a3} = 9.95. (Bottom): Reactivity *versus* pH distribution diagram for the Cys species in the reduction of ormaplatin; the above pK_a values and k_1 = 0, k_2 = 0.26, k_3 = 7.1 x 10^4 and k_4 = 2.7 x 10^5 $M^{-1}s^{-1}$ in Table S1 were employed in the calculation, *cf.* Figure 2 in the main text for the species **I - IV**.

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