Identification of Platinum(II) Sulfide Complexes Suitable as Intramuscular Cyanide Countermeasures

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Methods

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**Table S1.** Efficacy of hexachloroplatinate when mixed with stoichiometric amounts of indicated ligand. Effectiveness for each of these samples was determined from 3-4 replicates. No rescue (NR) was observed using 1:10 Pt:ligand ratio of L-Cysteine, L-Glutathione, L-Methionine sulfoxide, and L-Alliin.

| Molar Ratio (PtCl₆²⁻ : Ligand) | S-Ligand containing ligand used | 10:1 | 1:1 | 1:10 |
|--------------------------------|---------------------------------|------|-----|------|
| S-Methyl-L-Cysteine            | 125                             | 31   | 16  |
| L-Methionine                   | 125                             | 31   | 16  |
| L-Cysteine                     | 125                             | 62   | NR  |
| L-Glutathione (reduced)        | 250                             | 62   | NR  |
| L-Methionine sulfoxide         | NR                              | NR   | NR  |
| (±)-L-Alliin                   | NR                              | NR   | NR  |

**Controls**

- **PtCl₆²⁻ + DMSO** EC100 = 62µM
- **Hydroxocobalamin** EC100 = 125µM

**Table S2.** The decarboxylated form of methionine (3-(methylthio)propylamine) was effective at enhancing efficacy of hexachloroplatinate. Additionally, less appears to be required to achieve the same antidote efficacy as the carboxylated form L-Methionine. Four biological replicates were performed. PtCl₆²⁻:DMSO exhibited an EC100 of 62 µM.

| Molar Ratio PtCl₆²⁻ : X | X = Methionine EC100 | X = 3-(methylthio)propylamine EC100 |
|-------------------------|----------------------|-------------------------------------|
| 1:1                     | 62                   | 62                                  |
| 1:2                     | 31                   | 31                                  |
| 1:5                     | 31                   | 16                                  |
| 1:10                    | 31                   | 16                                  |
| 1:25                    | 16                   | 16                                  |
| 1:50                    | 16                   | 16                                  |

**Table S3.** Platinum agents with the respective m/z determined by an LTQ Orbitrap in either negative or positive ion mode. Solvent used was 1:1 acetonitrile and water.
Table S4. Percent weight (%w/w) composition of each platinum complex measured by x-ray fluorescence. Manganese chloride served as an internal standard for quantification.

| Complex # | Empirical Formula | Predicted Monoisotopic MW (g/mol) | Predicted m/z | Measured m/z | Measured m/z (g/mol) |
|-----------|------------------|-----------------------------------|---------------|--------------|----------------------|
| 3         | [(H3N)2PtCl(DMSO)]+ | 342.0001                          | 342.000673    | 342.00084    |                       |
| 4         | Na+[PtCl6(DMSO)]- | 447.8235                          | 447.822986    | 447.82334    |                       |
| 5         | Na+[PtCl5(DMSO)]- | 377.8858                          | 377.885281    | 377.88677    |                       |
| 6         | [(H-Met-OH)2Pt]+ | 493.0669                          | 246.533443/5  | 246.53341    |                       |
| 7         | [(H-(S-Me)Cys)-OH]2Pt]2+ 2Cl- | 465.0536                           | 232.517793/5  | 232.51769    |                       |
| 8         | [(Ac-Met-OH)2PtCl2] | 647.0257                          | Not identified | Not identified |                       |
| 9         | [(H-Met-NH3)2Pt]2+ 2Cl- | 491.0988                           | 490.09103     | 490.09107    |                       |
| 10        | [(CH3SCH2CH2CH2NH2)2Pt]2+ 2Cl- | 405.0872                           | 404.079402    | 404.07977    |                       |
| 11        | [(CH3SCH2CH2NH2)2Pt]2+ 2Cl- | 377.0559                           | 188.527935    | 188.52774    |                       |

Table S5. ISE data collected of complex #4 showing that the reaction between Pt and CN- may not have been completed after 10 minutes for all complexes.

| Complex | Assay time (min) | Free cyanide removed by platinum |
|---------|------------------|---------------------------------|
| #4.2    | 10               | 1.7 ± 0.2                        |
| #4.2    | 90               | 2.9 ± 0.2                        |
Figure S1. XRF spectra of Pt (IV) and Pt(II) excitation core absorbances. Peaks associated with platinum were integrated against the manganese chloride internal standard at 6keV.

Figure S2. HPLC calibrations of analytes were performed in aqueous diluent using an Agilent 1100 equipped with a DAD for UV analysis at 260 nm. Separation conditions made use of a 2.1x50mm Restek Ultra IBD in HILIC mode and reverse-phase modes using acetonitrile:water gradients. A) Calibration curve of Pt(CN)$_2^-$ each point contains n=3 injections of standard material (error bars are small and not visible). B) Calibration curve of 6 with each point has n=3 injections of standard (error bars are small and not visible).
Figure S3. Competition reaction of 1 mM (3) and 1 mM (6) with 0.4 mM KCN in 180 mM NaPi pH 7.6 10% D$_2$O buffer at 298K, monitored by $^1$H NMR. Methyl signals at 2.08 ppm (*) and 2.67 ppm (**) indicate the release of methionine and DMSO, respectively. The apparent rate of methionine release is estimated to be about ten times that of DMSO.

Figure S4. $^1$H NMR (500 MHz) for a fresh solution of 15.6 mg complex 6 in 500 uL of 90 mM NaPi pH8 (final pH ~5.7) 10% D$_2$O at 292 K. Absence of clear signals between 3.33 pm and 3.5 ppm suggests that complex 6 is dominated by cis isomers, without any appreciable amount of trans.
Figure S5. $^1$H NMR spectra of 1 mM 3 (Cisplatin-DMSO) without cyanide (red) and with 5 mM cyanide (blue).

Figure S6. $^1$H NMR spectra of 1 mM 5 without cyanide (red) and with 5 mM cyanide (blue).
Figure S7. $^1$H NMR spectra of 1 mM 7 without cyanide (red), with 1 mM cyanide (green) and 5 mM cyanide (blue).

Figure S8. $^1$H NMR spectra of 1 mM 8 without cyanide (red), with 1 mM cyanide (green) and 5 mM cyanide (blue).
Figure S9: $^1$H NMR spectra of 1 mM 9 (NH$_2$-Met$_2$Pt) without cyanide (red), with 1 mM cyanide (green) and 5 mM cyanide (blue).
Figure S10: $^1$H MMR spectra of 1 mM 10 without cyanide (red), with 1 mM cyanide (green) and 5 mM cyanide (blue).

Figure S11: $^1$H NMR spectra of 1 mM 11 without cyanide (red), with 1mM cyanide (green) and 5 mM cyanide (blue).
Figure S12 Proton NMR of 9 reaction with cyanide under alkaline ISE assay conditions. 1H NMR spectra here show no observable change between the sample (50µM of 9) with cyanide and without cyanide, suggesting that the reaction occurred within an hour of preparation.
Figure S13 Blood plasma concentrations of total platinum content were collected by ICP-MS of rabbits dosed with 3 and 6 in a the non-lethal cyanide model. Blood samples were collected for up to 180 min, data presented as µg/mL vs. time as total platinum concentration. ICP-MS sample infusion was performed using an Arridus peristaltic pump and analysis of each sample using a collection time of 90 sec each. Platinum 195 peak intensities were converted to micrograms per milliliter using values obtained from a hexachloroplatinate standard curve in rabbit serum.
Figure S14 Monitoring the stability of 4 by $^{195}$Pt NMR and $^1$H NMR. A sample of 100 mM 4 in 20 mM pH 6.0 phosphate buffer and was monitored for up to 18 d. The sample was shielded from light during storage to prevent accelerated decomposition due to light. An equilibrium was established after 4 d with approximately 70% of bound DMSO on platinum released.
Methods

Safety Disclosure: no unexpected or unusually high safety hazards were encountered in this work.

Zebrafish efficacy: Zebrafish embryos 6 days post fertilization were grown in baths containing lethal amounts of cyanide and varying amounts of platinum and ligand ratios. Efficacy reported is the minimum concentration of platinum required to achieve 100% survival of zebrafish embryos.

NMR: The dried powder samples were dissolved and analyzed by $^{195}$Pt NMR to monitor the deshielding effect on the platinum core and observed as shifts in the ≤ -2800ppm with hexachloroplatinate reference. At low pH (pH < 7.0), we observed rapid conversion between bicyclic and hemicyclic isomers in the bidentate ligands. Assignments for the $^1$H spectra were not made for these complexes due to the complexity. For example, complex 0 shows a mixture of isomers (Supplemental Figure S1) in $^{195}$Pt NMR with 4 signals from -3550 to -3700 ppm which we observed as pH dependant. Norman et al. proposed the dominate peaks under acidic conditions (e.g. -3625ppm and -3675ppm) as the bidentate ring closed structure.\(^{54}\) $^{195}$Pt spectra simplified between pH 7 and 8 and are consistent with Norman’s observations suggesting a ring-closed dominate form.

Kinetics: Platinum was reacted with cyanide under first order conditions in platinum. Pt(CN)$_4$$^{2-}$, a product for these for Pt(II) complexes. Signal at 255nm increased over time is transcribed as Pt(CN)$_4$$^{2-}$ formation. Results were plotted as production of Pt(CN)$_4$ over time and observed rate constants are obtained by fitting the curve to a first order process. Conditions for the reaction were performed in 12.5mM phosphate buffer pH 7.6 with 0.8mM KCN and 0.02mM platinum.

Mass spectrometry (high resolution): Platinum agents were dissolved in 50:50 acetonitrile and water solvent. The sample was infused into a LTQ Orbitrap mass spectrometer using electrospray ionization in either positive or negative mode.

Mass spectrometry (low resolution): Platinum agent 8 was dissolved in 50:50 acetonitrile and water solvent. The sample was infused into an Advion Expression spectrometer using electrospray ionization in either positive or negative mode.
Additional Supporting Data:

Mass spectrum of 3 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 342.00084 was obtained with an error of -0.48ppm.

Mass spectrum of 4 using the LTQ Orbitrap in electrospray ionization negative ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 447.82334 was obtained with an error of -0.79ppm.
Mass spectrum of 5 using the LTQ Orbitrap in electrospray ionization negative ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 377.88677 was obtained with an error of 3.94ppm.

Mass spectrum of 6 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 246.53341 was obtained with an error of -0.13ppm.
Mass spectrum of 7 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 232.51769 was obtained with an error of -0.44ppm.

Mass spectrum of 8 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of ____ was obtained with an error of ____ppm. 626 m/z is identified as [Ac-Met₂Pt(HO)(H₂O) Na]+.
Mass spectrum of 8 using an Advion Expression spectrometer in electrospray ionization positive ion (top) and negative ion (bottom) mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z spectra of 8 was obtained. 646 m/z is identified as [Ac-Met₂PtCl₂ Na]⁻.
Mass spectrum of 9 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 490.09107 was obtained with an error of -0.08ppm.

Mass spectrum of 10 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 404.07977 was obtained with an error of 0.91ppm.
Mass spectrum of 11 using the LTQ Orbitrap in electrospray ionization positive ion mode. The solvent used was a 1:1 mixture of acetonitrile and water. M/z of 188.52774 was obtained with an error of -0.08ppm.