

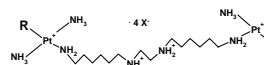
**Background**

Mononuclear platinum complexes represent a new class of platinum agents, characterized by a peculiar DNA binding and higher potency on a wider range of preclinical tumor models than cisplatin. Unlike the mononuclear complexes currently used in clinic (cisplatin, carboplatin), they give rise to 1,4-intrastrand and 1,5-interstrand cross-links with DNA.

Unconvincing efficacy results were obtained in Phase II with 1<sup>st</sup> generation binuclear platinum complex, due to extensive metabolism and irreversible protein binding in human plasma.

As part of an ongoing program aimed to reduce the high irreversible plasma protein binding and improve the chemical and metabolic stability, a series of bis(carboxylatoplatinum) compounds were prepared starting from CT-3610 ((bis(trans)(diamino)(chloro)platinum(II))μ-(1,16-diamino-7,10-diazahexadecane-N1,N16)) tetrinitrate) by replacement of the chloride ligands with alkylcarboxylates.

These new bisplatinum complexes have been evaluated for i) *in vitro* protein binding in murine and human plasma, ii) *in vitro* antiproliferative activity on human ovarian carcinoma cell lines sensitive and resistant to cisplatin and iii) *in vivo* antitumor efficacy on xenograft tumor models in comparison with clinical standard platinumes.



CT-3610 R = Cl, X<sup>-</sup> = NO<sub>3</sub><sup>-</sup>  
 CT-47613 R = CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>COO<sup>-</sup>, X<sup>-</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>11</sub>OSO<sub>3</sub><sup>-</sup>  
 CT-47609 R = CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>COO<sup>-</sup>, X<sup>-</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>11</sub>OSO<sub>3</sub><sup>-</sup>

**Materials and Methods**

**Plasma protein binding**

The *in vitro* protein binding in murine and human plasma was evaluated for selected compounds up to 8 h incubation at 37°C followed by acetonitrile protein precipitation and LC/MS/MS analysis. Binding kinetics was characterized through the estimation of the half-life for each compound and species.

**In vitro antiproliferative activity**

Antiproliferative effect was evaluated by WST assay in A2780 human ovarian carcinoma cell line and in A2780/CDDP, a cisplatin (CDDP) resistant variant.

**In vivo activity**

The antitumor efficacy of bisplatinum complexes was evaluated in comparison with standard platinumes cisplatin (CDDP), carboplatin (CBDCA) and oxaliplatin on several xenograft tumor models: A2780 ovarian and a cisplatin resistant variant A2780/CDDP, LoVo (colon) and LX-1 (lung).

Human tumors were subcutaneously (s.c.) transplanted in female athymic (nu/nu) mice. The animals were randomized in experimental groups (6-8 mice each experimental group). Treatment started when the tumor reached an average weight of about 100-150 mm<sup>3</sup>.

Compounds were administered intravenously as a solution.

RTW% = Relative Tumor weight inhibition%: 100-(mean tumor weight of treated/mean tumor weight of control or vehicle group) × 100 evaluated 7 days after last treatment.

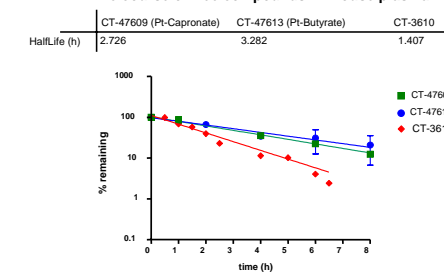
TGD tumor growth delay; TG treated – TG Vehicle/Control (TG: mean time, in days, to reach a weight of 1g or 2g).

Statistical analyses were performed using one-way ANOVA Bonferroni:

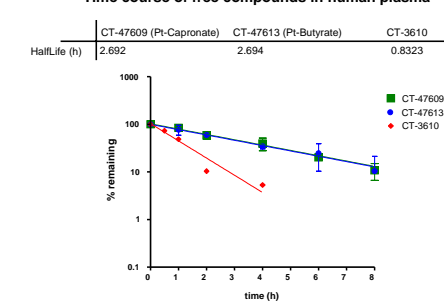
\*p<0.05; \*\*p<0.01; \*\*\*p<0.001

**In vitro protein binding in murine and human plasma**

**Time course of free compounds in mouse plasma**



**Time course of free compounds in human plasma**

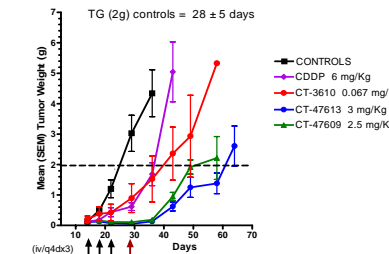


**Antiproliferative activity on human ovarian carcinoma cell lines: sensitive (A2780) and resistant (2780/CDDP)**

| Compound | A2780 IC <sub>50</sub> nM(±SE) |              |              | A2780/CDDP IC <sub>50</sub> nM(±SE) |              |               |
|----------|--------------------------------|--------------|--------------|-------------------------------------|--------------|---------------|
|          | 1h                             | 24h          | 120h         | 1h                                  | 24h          | 120h          |
| CDDP     | 5835.0 (±22.0)                 | 261.6 (±2.0) | 265.1 (±2.0) | 3527.0 (±22.0)                      | 204.5 (±2.0) | 1537.0 (±2.0) |
| CT-3610  | 0.2 (±0.1)                     | 0.1 (±0.1)   | 0.1 (±0.1)   | 6.8 (±0.1)                          | 3.5 (±0.1)   | 2.9 (±0.1)    |
| CT-47613 | 4.8 (±0.1)                     | 0.5 (±0.1)   | 0.4 (±0.1)   | 194.7 (±0.8)                        | 8.8 (±0.1)   | 7.2 (±0.1)    |
| CT-47609 | 6.6 (±0.1)                     | 4.0 (±0.1)   | 6.8 (±0.1)   | 71.6 (±0.1)                         | 29.1 (±0.1)  | 17.9 (±0.1)   |

IC<sub>50</sub> values represent an average of three experiments; single IC<sub>50</sub> was determined through a dose-response curve (6 or 7 drug applied concentrations) and each concentration assayed in quadruplicate

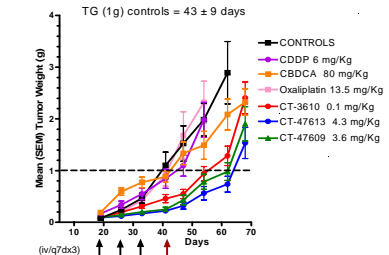
**A2780 human ovarian carcinoma**



| Compound                 | Optimal dose mg/Kg | RTW% % | CR % | PR % | TGD (days) 2g | TGD CDDP vs Bis-platinum complexes |
|--------------------------|--------------------|--------|------|------|---------------|------------------------------------|
| CDDP                     | 6                  | 89     | 0    | 0    | 14*           | -                                  |
| CT-3610                  | 0.067              | 88***  | 0    | 14   | 15**          | -                                  |
| CT-47613 (Pt-Butyrate)   | 3                  | 98***  | 0    | 75   | 29***         | ***p<0.001                         |
| CT-47609 (Pt-Caprionate) | 2.5                | 96***  | 0    | 0    | 25***         | ***p<0.001                         |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 controls (untreated) vs treated

**LoVo human colon carcinoma**



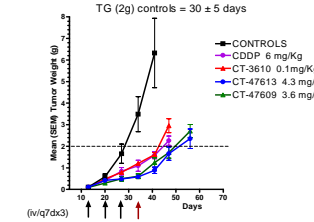
| Compound    | Optimal dose mg/Kg | RTW% %      | TGD (days) 1g | TGD Standard Platinumes vs Bis Pt-complexes |
|-------------|--------------------|-------------|---------------|---|
| CDDP        | 6                  | 55          | 9             | -   |
| CBDCA       | 80                 | 28, 48      | 5             | -   |
| Oxaliplatin | 13.5               | 27          | 1             | -   |
| CT-3610     | 0.1                | 52**, 65*** | 16            | 14**  |

CT-47613 (Pt-Butyrate) 4.3 84\*\*\* 20\*\*\* \*p<0.05 (CDDP) \*\*p<0.01 (CBDCA) \*\*\*p<0.001 (Oxaliplatin)

CT-47609 (Pt-Caprionate) 3.6 80\*\*\* 18\*\*\* \*p<0.05 (CDDP) \*\*p<0.01 (CBDCA) \*\*\*p<0.001 (Oxaliplatin)

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 controls (untreated) vs treated

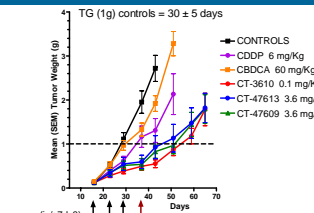
**A2780/CDDP human ovarian carcinoma**



| Compound                 | Optimal dose mg/Kg | RTW% %     | TGD (days)2g |
|--------------------------|--------------------|------------|--------------|
| CDDP                     | 6                  | 77**       | 14           |
| CT-3610                  | 0.1                | 62**, 62** | 9, 13**      |
| CT-47613 (Pt-Butyrate)   | 4.3                | 80***      | 20***        |
| CT-47609 (Pt-Caprionate) | 3.6                | 80***      | 18**         |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 controls (untreated) vs treated

**LX-1 human lung carcinoma**



| Compound                 | Optimal Dose mg/Kg | RTW% %      | TGD (days) 1g | TGD Standard Platinumes vs Bis Pt-complexes |
|--------------------------|--------------------|-------------|---------------|---|
| CDDP                     | 6                  | 46          | 13            | -   |
| CBDCA                    | 60                 | 43          | 0             | -   |
| CT-3610                  | 0.1                | 77***       | 28***         | ***p<0.001(CBDCA)                           |
| CT-47613 (Pt-Butyrate)   | 3.6                | 75**, 78*** | 21**, 20**    | **p<0.01(CBDCA)                             |
| CT-47609 (Pt-Caprionate) | 3.6                | 74**, 74*** | 23**, 19**    | ***p<0.001(CBDCA)                           |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 controls (untreated) vs treated

**Results & Conclusions**

➢ CT-47613 (Pt-Butyrate) and CT-47609 (Pt-Caprionate) have similar *in vitro* binding kinetics in human and murine plasma, but a considerably better profile when compared to the parent compound CT-3610.

➢ On human tumor xenografts A2780, A2780/CDDP, LoVo and LX-1, CT-47613 and CT-47609 showed remarkable antitumor activity as determined by tumor growth inhibition. Moreover, both complexes had Tumor Growth Delay (TGD) values significantly greater than clinical standard platinumes.

➢ CT-47613 and CT-47609 are attractive candidates for further development. Ongoing toxicology studies will determine which compound will be selected for Phase I development.