Bioinorganic Drugs

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The Biological Activity of Zeise's Salt and its Derivatives**

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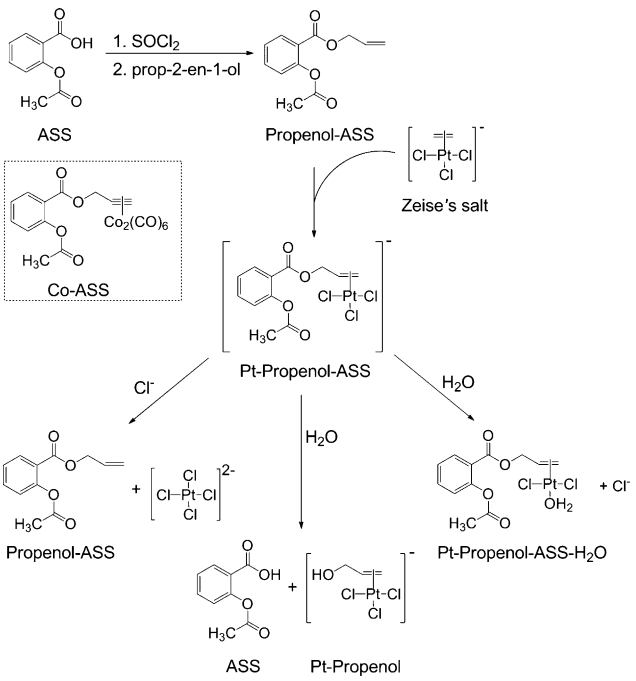
Dedicated to Professor Helmut Schönenberger on the occasion of his 90th birthday

Abstract: *With the aim to design new biologically active bioinorganic drugs of aspirin, whose mode of action is based on the inhibition of the cyclooxygenase(COX) enzymes, derivatives of Zeise's salt were synthesized in this structure–activity relationship study. Surprisingly, not only these Zeise–aspirin compounds but also Zeise's salt itself showed high inhibitory potency against COX enzymes in in vitro assays. In contrast, potassium tetrachloroplatinate and cisplatin did not influence the enzyme activity at equimolar concentrations. It was demonstrated by LC-ESI tandem-mass spectrometry that Zeise's salt platinates the essential amino acids Tyr385 (active site of the enzyme) and Ser516 (will be acetylated by aspirin) of COX-1, thereby strongly impairing the function of the enzyme. This finding demonstrates for the first time that Zeise's salt is pharmacologically active and is a potent enzyme inhibitor.*

The discovery of the antitumor activity of cisplatin and the subsequent use of platinum complexes in medicinal chemistry was the consequence of a fortunate coincidence. In 1965 Barnett Rosenberg investigated the influence of an electric field on the growth of *Escherichia coli* bacteria and observed a changed growth of the bacteria.[1] His great academic performance was the finding that not the electric field caused this phenomenon but platinum complexes which were formed in the medium from the chemically inert platinum electrode. From this time on, cisplatin, known since 1844 as Peyron's salt, has been a permanent feature in chemotherapy and in clinical trials.

Continuous development with the aim of increasing its efficiency, reducing the side effects, and overcoming intrinsic and acquired resistance led to the approval of further platinum complexes for medical use.[2] All these complexes cause cytotoxicity mainly by interaction with DNA.

A possibility to circumvent resistance is the design of organometallic compounds which have another mode of action. A very interesting representative of this class of compounds is the [prop-2-inyl-2-acetoxybenzoat]dicobalt hexacarbonyl complex (Co-ASS, see Scheme 1).[3,4] In cell-culture experiments this derivative of acetylsalicylic acid (ASS, aspirin) showed cytotoxicity comparable to cisplatin and inhibited the cyclooxygenase enzymes COX-1 und COX-2 distinctly more than aspirin.



Scheme 1. Design and reactivity of potassium [η²-(prop-2-enol)-2-acetoxybenzoat]trichloroplatinate(II) (Pt-Propenol-ASS).

The application of a cytotoxic COX inhibitor opens a new option for the therapy of tumors for which it is known that an over expression of COX results in pathological variations. During the last years it was demonstrated that various mammary carcinoma show increased expression of COX-2 and that inhibitors of this enzyme can reduce tumor growth and tumor progression. An increased COX-2 expression in gynecologic tumors is further accompanied by a bad prognosis for patients with mammary carcinoma as well as prostate carcinoma.[5,6]

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In a structure activity relationship study, the metal cluster of Co-ASS was systematically modified and the influence on cytotoxicity and COX inhibition was investigated.[7–9] In this context, the ASS moiety was connected to Zeise's salt (see Scheme 1). This complex was discovered in 1827 by the Danish pharmacist William Christopher Zeise and is believed to be the first organometallic compound. In contrast to cisplatin, nothing is known about its capability as drug in medicinal use or as pharmacophor for the design of new metal-based drugs. Therefore, analogously to Co-ASS, acetylsalicylic acid was esterified with prop-2-enol and treated with Zeise's salt to give the anionic Pt-Propenol-ASS complex (see Scheme 1; counterion K^+).

Before starting the pharmacological testing, the stability of Pt-Propenol-ASS in aqueous solution was determined by HPLC analysis. On the one hand, the strong *trans* effect of the alkene can force the exchange of a chlorido ligand by water, and on the other, a high Cl^- concentration can cause the release of the alkene from platinum.[10] Interestingly, neither of these reactions is observed when the complex is dissolved in water. With a half-life of 20.2 ± 1.4 min the ester is cleaved to give ASS and Pt-Propenol (see Scheme 1 and Figure 1). The release of the acetyl group was not observed.

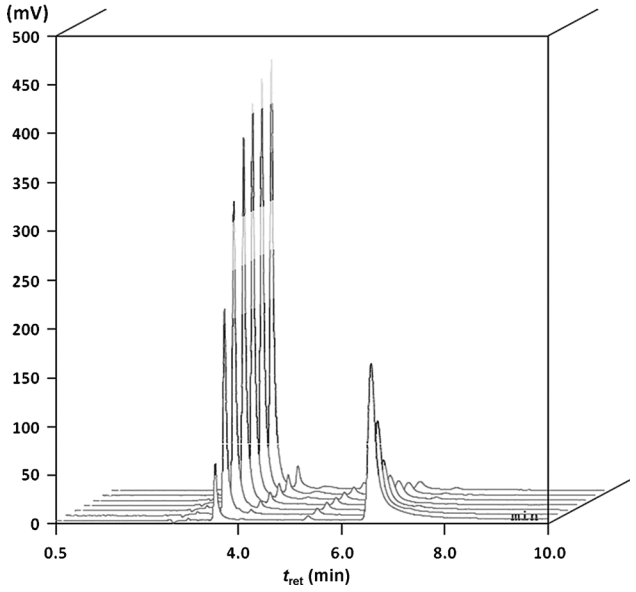

Figure 1. 3D plot of HPLC chromatograms of the degradation of Pt-Propenol-ASS in aqueous solution at 37 °C; starting at $t = 0$ min (front) to $t = 90$ min (back) in intervals of 15 min. t_{ret}(ASS) = 3.56 min; t_{ret}(Pt-Propenol-ASS) = 6.45 min.

The ligand Propenol-ASS was stable under the same conditions, thus it is very likely that the platinum catalyzes the ester cleavage. We assume that in solution a five-membered chelate ring is built from the platinum to the oxygen of the ester, resulting in a strong electron withdrawal at the α-C atom of the side chain and activation for a nucleophilic attack.

The stability of the complex can be increased if a further methylene group is inserted between the ester function and the "Zeise moiety" (→Pt-Butenol-ASS). Pt-Butenol-ASS was stable for 24 h under the conditions used.

Pt-Propenol-ASS and Pt-Butenol-ASS were investigated in the COX-1/2 assay to evaluate the influence of the "Zeise-component", in comparison with the $\{Co_2(CO)_6\}$ cluster, on the pharmacological properties. Both complexes were 10-fold stronger COX inhibitors than Co-ASS. They reduced the activity of the enzymes at a concentration of 10 μM by 100% (COX-1) and about 35% (COX-2), respectively. Even at 1 μM an inhibition of the COX-1 by about 70% was determined for both complexes (Table 1).

Table 1: COX inhibition using isolated COX-1 (ovine) and COX-2 (human recombinant).

Compound	COX-1 inhibition [%] at 10 μM	1 μM	COX-2 inhibition [%] at 10 μM
ASS	29 ± 2.0		0
Co-ASS	68.0 ± 5.4		63.0 ± 5.0
Pt-Propenol-ASS	100	69.7 ± 1.2	39.1 ± 1.7
Pt-Butenol-ASS	100	72.1 ± 2.3	33.6 ± 2.4
Propenol-ASS	0		13.3 ± 2.4
Pt-Propenol	80.7 ± 14.2		0
$K_2[PtCl_4]$	18.5 ± 4.5		0
Zeise's salt	100	35.8 ± 2.7	13.5 ± 0.9
Cisplatin	0		29.2 ± 2.0

Because it cannot be excluded that the complexes were unstable under the in vitro conditions, Pt-Propenol, Propenol-ASS, and $[PtCl_4]^{2-}$ as possible degradation products of Pt-Propenol-ASS were investigated, too.

The free ligand Propenol-ASS and $K_2[PtCl_4]$ showed at the highest concentration of 10 μM only marginal COX inhibition (Table 1). In contrast, Pt-Propenol was very active against COX-1 (inhibition: 80.7%). Because ASS inhibited COX-1 at this concentration by only 29%, we assumed that the "Zeise component" caused this effect. Consequently, Zeise's salt itself was included in this study. And indeed, in contrast to cisplatin and $K_2[PtCl_4]$, Zeise's salt had excellent activity in this assay. At a concentration of 10 μM, COX-1 was inhibited by 100% and COX-2 by 13.5%. At 1 μM a COX-1 inhibition of 35.8% was determined.

To get insight into the mode of action, COX-1 was incubated with Zeise's salt and subsequently enzymatically digested with trypsin. The resulted peptide fragments were analyzed by LC-ESI tandem-mass spectrometry.[11–13] Interestingly, four fragments contained platinated amino acids (Table 2): Ser516 (or His513), Glu347 (or Glu346), Cys512 and His513, as well as Tyr385 (or His386), in parentheses are potential alternative coordination sites in the same sequence with $\Delta Cn \leq 0.10$ compared to the first position with highest Xcorr value are given.

Of highest relevance is the finding that the active site of COX-1, the amino acid Tyr385, was platinated with the $\{PtCl_2\}$ fragment (Figure 2). Because $K_2[PtCl_4]$ did not cause COX inhibition, it is very likely that in the first step Zeise's salt binds to the amino acid and in the second step the alkene is released from the complex during the MS fragmentation. Another observation is the binding of the (η^2-ethylene)pla-

Table 2: Platination (@) of COX-1 by Zeise's salt, determined by ESI tandem-mass spectrometry.

Peptide sequence	Amino acid	$z^{[a]}$	SEQUEST parameter Xcorr[d]	ΔCn[d]	Ions[b,c]
C.HPNS@IFGESMIEMGAPFSLK.G	S516/H513	3	4.31	0.25	28/76
V.IEE@YVQQLSGYFLQLK.F	E347/E346	3	3.76	0.52	24/60
L.EKC@H@PNSIFGESMIEMGAPFSLK.G	C512 H513	3	5.87	0.17	33/88
E.FNQLY@HWHPLMPDSFR.V	Y385/H386	3	3.67	0.31	20/60

[a] Charge. [b] Observed/possible b^+ and y^+ ions. [c] @ represents the following platinum fragments in the peptide ions, with the relevant binding site being given in parentheses: $\{(C_2H_4)Pt\}^{2+}$ (S516), $\{PtCl\}^+$ (E347), $\{PtCl_2\}$ (C512, H513, and Y385). [d] Xcorr gives the cross-correlation score and ΔCn the delta correlation value with respect to the platinated peptide sequence with the next best Xcorr value.

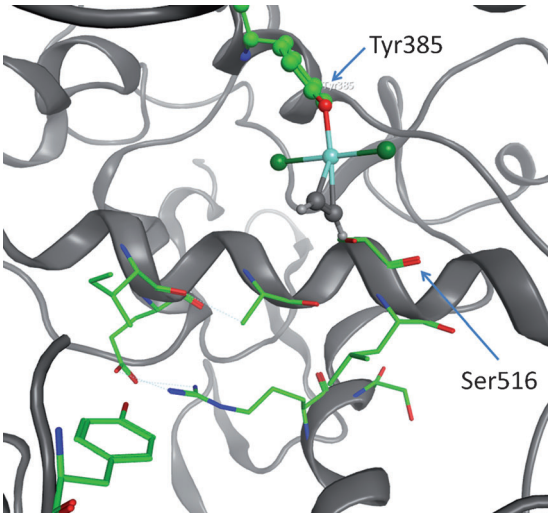

Figure 2. Platination of essential amino acids at the active site of COX-1 by Zeise's salt (Tyr385).

tinum(II) fragment to Ser516, which is the essential acetylation position of ASS in the binding pocket. It can be assumed that Zeise's salt binds to the hydroxy group of the amino acid after hydrolytic release of the Cl^- leaving group.

Interestingly, platination with cisplatin resulting in COX inhibition seems not to take place, although cisplatin can be activated by hydrolysis of Pt–Cl bonds and bind to proteins or DNA. Cisplatin was inactive in the COX assays.

Because Pt-Propenol-ASS, Pt-Butenol-ASS, and Zeise's salt can generally be activated by the same hydrolysis reaction allowing DNA binding, they were tested for growth inhibitory effects against MCF-7 and MDA-MB 231 breast cancer cell lines (Table 3). In these tests, Zeise's salt and Pt-Propenol-ASS were inactive ($IC_{50} > 50$ μM), while Pt-Butenol-ASS caused a marginal reduction of the cell growth (IC_{50} (MCF-7) = 30.1 ± 1.5 μM; IC_{50} (MDA-MB 231) = 46.7 ± 2.2 μM).

Table 3: Cytotoxicity studies against breast cancer cells lines.

Complex	MCF-7 IC_{50} [μM]	MDA-MB 231 IC_{50} [μM]
Cisplatin	2.0 ± 0.3	3.3 ± 0.5
Zeise's salt	> 50	> 50
Pt-Propenol-ASS	> 50	> 50
Pt-Butenol-ASS	30.1 ± 1.5	46.7 ± 2.2

Under cell culture conditions, the complexes are negatively charged, which poses the question as to whether the low cytotoxicity was a consequence of a reduced accumulation in the tumor cells and/or by an insufficient platination of the DNA.

The general ability for DNA binding was determined using isolated salmon sperm DNA. Zeise's salt and Pt-Butenol-ASS bound distinctly faster to DNA than cisplatin, with a lower nucleoside/compound ratio (Figure 3).

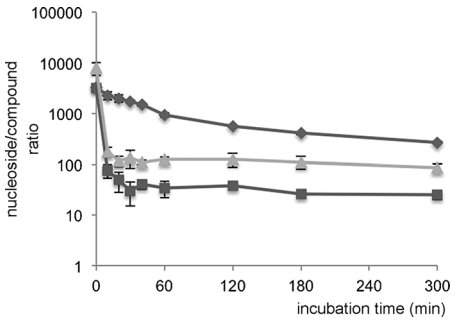

Figure 3. DNA binding to isolated salmon sperm DNA (◆ Cisplatin; ▲ Zeise's salt; ■ Pt-Butenol-ASS).

In cellular systems, the complexes have to cross the cell membrane to cause cytotoxic effects. When MCF-7 cells were incubated for 24 h with the complexes, a nearly identical uptake kinetic was found for cisplatin and Zeise's salt (Figure 4). The same is true for the degree of accumulation in nuclei and the cellular DNA binding measured after 2 h of incubation (Table 4). Pt-Butenol-ASS is taken up in the cells and the nuclei in a 4–5-fold higher amount because of its more-hydrophobic character. The lower or absent cytotoxic effects compared to cisplatin could be the consequence of

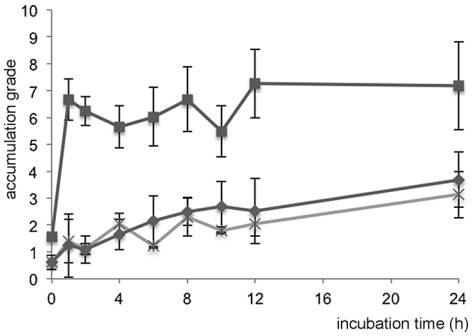

Figure 4. Accumulation in MCF-7 cells (◆ Cisplatin; *x* Zeise's salt, ■ Pt-Butenol-ASS).

Table 4: Binding to isolated DNA; uptake in MCF-7 cells and nuclei as well as DNA binding in MCF-7 cells after 2 h.

Complex	Binding to isolated DNA[a]	Uptake in MCF-7 cells[b]	Uptake in nuclei of MCF-7 cells[b]	DNA binding in MCF-7 cells[a]
Cisplatin	6.0 ± 0.3	0.08 ± 0.03	0.07 ± 0.05	0.55 ± 0.52
Zeise's salt	25.6 ± 0.5	0.10 ± 0.02	0.08 ± 0.02	0.47 ± 0.09
Pt-Butenol-ASS	31.2 ± 9.6	0.46 ± 0.09	0.50 ± 0.29	0.51 ± 0.07

[a] nmol Pt per mg DNA ± sdv. [b] nmol Pt per mg protein ± sdv.

less-effective building of intrastrand crosslinks. For this interpretation, however, further investigations are necessary.

In summary, this study showed for the first time the biological activity of Zeise's salt and related potassium (η^2-alkene)trichloroplatinate(II) complexes. They are for example, effective inhibitors of cyclooxygenase enzymes and could be further optimized. More than 40 years after the determination of the antitumor activity of cisplatin, a pharmacological effect could be demonstrated for the oldest known organometallic compound, Zeise's salt. To what extent this finding can result in the design of new tumor therapeutics remains to be seen. On the other hand, it might open the field for the development of non-toxic platinum-based COX inhibitors.

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[1] B. Rosenberg, L. Van Camp, T. Krigas, *Nature* **1965**, *205*, 698 – 699.
[2] I. Ott, R. Gust, *Arch. Pharm.* **2007**, *340*, 117 – 126.
[3] I. Ott, K. Schmidt, B. Kircher, P. Schumacher, T. Wiglenda, R. Gust, *J. Med. Chem.* **2005**, *48*, 622 – 629.
[4] I. Ott, B. Kircher, C. P. Bagowski, D. H. W. Vlecken, E. B. Ott, K. Bensdorf, W. S. Sheldrick, R. Gust, *Angew. Chem. Int. Ed.* **2009**, *48*, 1160 – 1163; *Angew. Chem.* **2009**, *121*, 1180 – 1184.
[5] G. Kristiansen, C. Denkert, K. Schluens, E. Dahl, C. Pilarsky, S. Hauptmann, *Am. J. Pathol.* **2002**, *161*, 1215 – 1221.
[6] C. Denkert, K. J. Winzer, B. M. Mueller, W. Weichert, S. Pest, M. Koebel, G. Kristiansen, A. Reles, A. Siegert, H. Guski, S. Hauptmann, *Cancer* **2003**, *97*, 2978 – 2987.
[7] G. Rubner, K. Bensdorf, A. Wellner, S. Bergemann, R. Gust, *Arch. Pharm.* **2011**, *344*, 684 – 688.
[8] G. Rubner, K. Bensdorf, A. Wellner, B. Kirchner, S. Bergemann, I. Ott, R. Gust, *J. Med. Chem.* **2010**, *53*, 6889 – 6898.
[9] G. Rubner, K. Bensdorf, A. Wellner, S. Bergemann, I. Ott, R. Gust, *Eur. J. Med. Chem.* **2010**, *45*, 5157 – 5163.
[10] J. R. Joy, M. Orchin, *Z. Anorg. Allg. Chem.* **1960**, *305*, 236 – 240.
[11] D. A. Wolters, M. P. Washburn, Y. R. Yates, *Anal. Chem.* **2001**, *73*, 5683 – 5690.
[12] J. Will, W. S. Sheldrick, D. Wolters, *J. Biol. Inorg. Chem.* **2008**, *13*, 421 – 434.
[13] D. L. Tab, J. K. Eng, J. R. Yates in *Proteome Research: Mass Spectrometry* (Ed.: P. James), Springer, Heidelberg, **2001**, pp. 125 – 142.

