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Combining anti-cancer drugs with artificial sweeteners: Synthesis and anti-cancer activity of saccharinate (sac) and thiosaccharinate (tsac) complexes *cis*-[Pt(sac)₂(NH₃)₂] and *cis*-[Pt(tsac)₂(NH₃)₂]

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ABSTRACT: The new platinum(II) complexes *cis*-[Pt(sac)₂(NH₃)₂] (sac = saccharinate) and *cis*-[Pt(tsac)₂(NH₃)₂] (tsac = thiosaccharinate) have been prepared, the X-ray crystal structure of *cis*-[Pt(sac)₂(NH₃)₂].H₂O reveals that both saccharinate anions are N-bound in a *cis*-arrangement being inequivalent in both the solid-state and in solution at room temperature. Preliminary anti-cancer activity has been assessed against A549 human alveolar type-II like cell lines with the thiosaccharinate complex showing good activity.

Keywords: cisplatin, ammine, saccharinate, thiosaccharinate, X-ray structure, anti-cancer

A key feature of cisplatin activity [1-7] is the slow displacement of one chloride by water, which in turn is readily displaced by a range of nucleic bases, most commonly guanine [8-12] and then crosslink DNA *via* displacement of the second chloride [13-18]. Due primarily to its widespread use of saccharin (sacH) as an artificial sweetener, the coordination chemistry of the saccharinate anion (sac) has been extensively studied [19]. While it is capable of binding to transition metals in a number of different ways, by far the most common is the simple N-

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bound coordination [19]. This mode of binding is observed exclusively at four-coordinate platinum(II) and palladium(II) centres [20-27]. Most pertinent to the work described herein are pyridine (py) and substituted pyridine adducts, *cis*-[Pt(sac)₂(py)₂], prepared upon addition of sodium saccharinate to the relevant dichlorides [20]. This behavior is different to that noted for [PtCl₂(κ²-dppf)] [dppf = bis(diphenylphosphino)ferrocene], which even under forcing conditions adds only a single equivalent of saccharinate to afford [PtCl(sac)(κ²-dppf)] [27]. Given the importance of both cisplatin and saccharin we were surprised to find that the interaction of the two had not to be noted in the literature. Herein we report that saccharinate readily displaces both chlorides of cisplatin to afford *cis*-[Pt(sac)₂(NH₃)₂] (**1**) which has been crystallographically characterized and give preliminary details of its anti-cancer activity together with that of the related thiosaccharinate (tsac) complex, *cis*-[Pt(tsac)₂(NH₃)₂] (**2**), which we postulate contains a PtN₂S₂ core.

Cis-[Pt(sac)₂(NH₃)₂] (**1**) was obtained as colorless crystals upon treatment of cisplatin with two equivalents of sodium saccharinate in boiling water¹. It is soluble in dimethylformamide (DMF) and DMSO, but insoluble in other common organic solvents. For comparison the related thiosaccharinate (tsac) complex *cis*-[Pt(tsac)₂(NH₃)₂] (**2**) was prepared² (Scheme 1). It shows far greater solubility in common organic solvents, believed to be a result of the binding of this ligand through sulfur [19,27]. The facile formation of **2** is somewhat surprising as

¹ Synthesis and characterization of *cis*-[Pt(sac)₂(NH₃)₂].H₂O (**1**.H₂O) - A solution of sodium saccharinate (0.27 g, 1.33 mmol) in hot water (10 cm³) was added to a solution of *cis*-[PtCl₂(NH₃)₂] (0.20 g, 0.667 mmol) in hot water (10 cm³). The mixture was refluxed for 1 h. The colorless-white crystals formed were filtered off and dried in *vacuo*. Yield: 85% (0.33 g). Anal. Calcd. for C₁₄H₁₆N₄O₇PtS₂: C, 27.50; H, 2.64; N, 9.16. Found: C, 27.60; H, 2.60; N, 9.22%. IR (KBr) (ν, cm⁻¹): 3524s, 3298s, 3277s, 3134s, 1693s, 1666s, 1585s, 1462m, 1285s, 1246s, 1169s, 978s, 795m, 565m. ¹H NMR (d⁷-DMF): 8.09-8.05 (m, 1H), 7.99-7.93 (m, 2H), 7.91-7.87 (m, 1H), 4.78 (brs, 1 H), 4.56 and 4.52 (brs, 5H). ¹³C{¹H} NMR (d⁷-DMF): 165.6, 164.9, 142.0, 141.8, 133.1, 132.8, 132.8, 132.6, 131.9, 131.6, 123.2, 123.1, 119.8, 119.5 ppm. Positive mode ESI-FTICR *m/z* 594.007284 [M+H], calcd for [C₁₄H₁₅N₄O₆PtS₂]⁺ 594.007284.

² Synthesis and characterization of *cis*-[Pt(tsac)₂(NH₃)₂] (**2**) - A solution of thiosaccharinate (0.26g, 1.33 mmol) in hot water (30 cm³) was added to a solution of *cis*-[PtCl₂(NH₃)₂] (0.20 g, 0.667 mmol) in hot water (15 cm³). The mixture was refluxed for 2 h. The light-brown solid formed was filtered off washed several times with distilled water and dried in *vacuo*. Yield: 89% (0.39 g). Anal. Calcd. for C₁₄H₁₄N₄O₄PtS₄: C, 26.88; H, 2.26; N, 8.96. Found: C, 26.33; H, 2.09; N, 8.86%. IR (KBr) (ν, cm⁻¹): 3471m, 3413m, 3286m, 1627s, 1440w, 1373s, 1315s, 1236m, 1155m, 1126s, 1008w, 817s, 515m. ¹H NMR (d⁶-DMSO): 7.96-7.90 (m, 4H), 7.82-7.75 (m, 4H), 4.54 (s, 6 H). ¹³C{¹H} NMR (d⁶-DMSO): 182.8, 137.6, 133.7, 133.4, 133.1, 124.3, 120.7 ppm.

reactions of cisplatin with thiols generally result in loss of ammonia rather [28-31]. Formation of **1-2** probably results from direct attack of the saccharinate or thiosaccharinate anions on cisplatin [32,33] since it is known that aquation of cisplatin is slow [13-18].

A crystallographic study of **1** (which crystallizes with a molecule of water) was carried out³ (Figure 1). The Pt-N1 and Pt-N2 bond distances 2.042(4) and 2.018(4) Å are similar to other Pt(II)-saccharinate complexes [19-27]. The Pt-N3 and Pt-N4 distances of 2.044(4) and 2.065(4) Å compare well with those of 2.05(4) and 1.95(3) Å in cisplatin [34] and also related Pt(II)-ammine complexes [35-38]. The saccharinate ligands are inequivalent in the solid-state and adopt a relative *anti*-arrangement and each is twisted with respect to the PtN₄ plane, by 52.1° for the ligand containing N(1) and 87.6° for N(2). A similar arrangement is seen in related species of the type *cis*-[Pt(NH₃)₂(nucleobase)₂]²⁺ as probed by crystallographic [35] and NMR [40] studies. For example in *cis*-[Pt(NH₃)₂(9-methyladenine)(9-ethylguanine)][NO₃]₂·2H₂O [37], the nucleobases adopt a relative *syn*-arrangement being rotated by 66.07 and 88.26° respectively with respect to the PtN₄ plane. The asymmetric unit contains a molecule of water which sits in the cleft made by the two saccharinate ligands. One of the protons on the water has a short contact with O(3) from an SO₂ group of the N(1) saccharinate ligand [O(3)...H(15) 1.970 Å]. The same oxygen atom also has a relatively short intramolecular contact with the nitrogen atom of the second saccharinate group [O(3)...N(2) 2.928 Å; sum of van der Waals radii: 3.05 Å].

NMR spectra for **1** are more complex than expected for a simple *cis*-MX₂L₂ system, with broad signals at δ 4.78, 4.56, 4.52 (in *d*⁷-DMF) and δ 4.79, 4.69 (in *d*⁶-DMSO) seen in the ¹H NMR spectrum for the NH₃ protons. In comparison, the NH₃ resonances in cisplatin appear as a singlet at δ 4.06 in H₂O/D₂O [40], although a more complex spectrum is observed in DMSO [41]. At 80 °C in DMSO the broad ammonia resonances collapse into the baseline and by 120 °C no amine resonances could be detected. Upon cooling the sample back to room temperature amine resonances were still absent and we conclude that amine substitution (by DMSO) has occurred. Complex **2** shows good solubility in a range of organic solvents, attributed to the S-bound coordination of the thiosaccharinate ligand [19]. Spectroscopic data

³ Crystallographic Data: white block, size 0.50 × 0.30 × 0.12 mm, monoclinic, space group P2₁/c, a = 12.642(1) Å, b = 9.7435(8) Å, c = 14.786(1) Å, β = 90.59(1)°, V = 1821.2(3) Å³, Z = 4, d_{calc} = 2.230 g/cm³, μ = 7.982 mm⁻¹, F(000) = 1176, unique refns.(R_{int}) 3559 (0.0536), R (I ≥ 2σ(I), R₁ = 0.025, wR₂ = 0.045, R (all data) R₁ = 0.042, wR₂ = 0.049.

are in accord with a symmetrical MX_2L_2 species: in the ^1H NMR spectrum, the six NH_3 protons appear as a singlet at δ 4.54. In the absence of a crystallographic study we cannot unequivocally deduce the regioselectivity but strongly favour a relative *cis* arrangement of ligands.

We have undertaken preliminary anti-cancer activity studies [42] on both complexes against A549 human alveolar type-II cell lines⁴ and using methotrexate as a positive control and an MTT assay was used to determine their dose-dependent cytotoxicity⁵ after 24 and 48 h (Figure 2). These show that **2** has higher activity than **1** and the activity of the former at higher concentrations (100 $\mu\text{g}/\text{ml}$ and 250 $\mu\text{g}/\text{ml}$) is significantly greater than the control at these concentrations. Reasons for this are not clear, but it is well-known that a significant amount of cisplatin is hydrolysed outside of the cell and renally excreted upon binding to plasma proteins, and thus only *ca.* 10% binds to DNA. It may be that the strong Pt-S bond results in more of the complex penetrating into the cell and becoming DNA bound. An alternative explanation is that the *trans* labilization of thiolates leads to a quite different mechanism of action in which ammine loss may be significant [28-31]. It is somewhat surprising that *cis*- $[\text{Pt}(\text{SR})_2(\text{NH}_3)_2]$ complexes remain elusive given the strength of the Pt-S interaction. Indeed sulfur-coordinating ligands are potential “rescue agents” or “protecting agents” providing competition with methionine and cysteine residues in proteins to reduce adverse binding thereby diminishing toxic side effects [29]. With this in mind we are

⁴ Human alveolar type-II (ATII)-like cell lines A549 were cultured in Dulbecco’s Modified Eagle Medium (DMEM) with L-Glutamine (Lonza, Switzerland) supplemented with 10% (v/v) FCS (Lonza, Switzerland), Penicillin (10000 U/mL) and Streptomycin (10000 U/mL) (Lonza, Switzerland). Cells were cultivated in 75 cm^2 tissue culture flasks and maintained under standard cell culture conditions (5.0% CO_2 , 95% humidity and 37°C in incubators). Cells were passaged twice per week.

⁵ A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine dose-dependent cytotoxicity with Methotrexate as a positive control. Cells were seeded out in 96-well-tissue plates (Sarstedt, USA) with a volume of 200 μL . Cultivation took 3 days at 37 °C, 5.0% CO_2 and 100% humidity. After cultivation wells were grown until reaching confluence. A-549 cells were washed once in PBS and treated with the samples (**1** and **2**) and Methotrexate from 12.5 to 240 $\mu\text{g}/\text{mL}$ concentrations for 24 and 48 h. Samples were then removed completely and cells were incubated in 110 $\mu\text{L}/\text{well}$, 10% MTT solution (5.0 mg/mL PBS) in supplement-free medium for 4 h. With this incubation time the formazan complex was produced inside the cells. To release the purple salt, 100 μL SDS solution (1.0 g SDS in 10 mL 0.01 M HCl) were added per well and after 24 h of incubation, the UV-vis absorption was measured at 570 nm with 630 nm as reference wavelength using a micro-plate reader.

currently further exploring the cytotoxicity of **2** together with that of related Pt(II)-thiosaccharinate complexes.

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Captions for figures

Figure 1. The molecular structure of *cis*-[Pt(sac)₂(NH₃)₂].H₂O (**1**).H₂O with selected bond lengths (Å) and angles (°): Pt-N(1) 2.042(4), Pt-N(2) 2.018(4), Pt-N(3) 2.044(4), Pt-N(4) 2.065(4), O(3)..N(2) 2.928, O(3)...H(15) 1.970, N(1)-Pt-N(2) 92.5(1), N(3)-Pt-N(4) 93.0(2), N(1)-Pt-N(3) 178.0(2), N(2)-Pt-N(4) 178.0(2), O(2)...H(13) 2.370, O(2)...H(12) 2.947, O(1)...H(19) 2.653, O(1)...H(10) 2.644, O(5)...H(16) 1.947

Figure 2. Dose dependent cytotoxicity of *cis*-[Pt(sac)₂(NH₃)₂] (**1**) (denoted A) and *cis*-[Pt(tsac)₂(NH₃)₂] (**2**) (denoted B) on a A549 cell line after 24 h (red-dark) and 48 h (green-light) incubation periods.

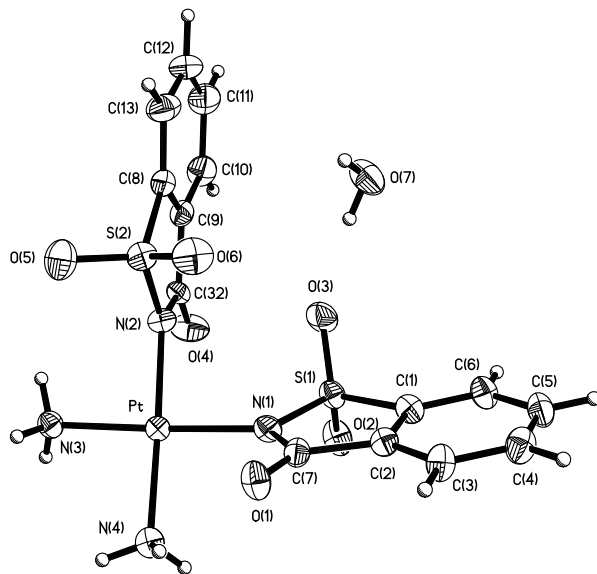


Figure 1

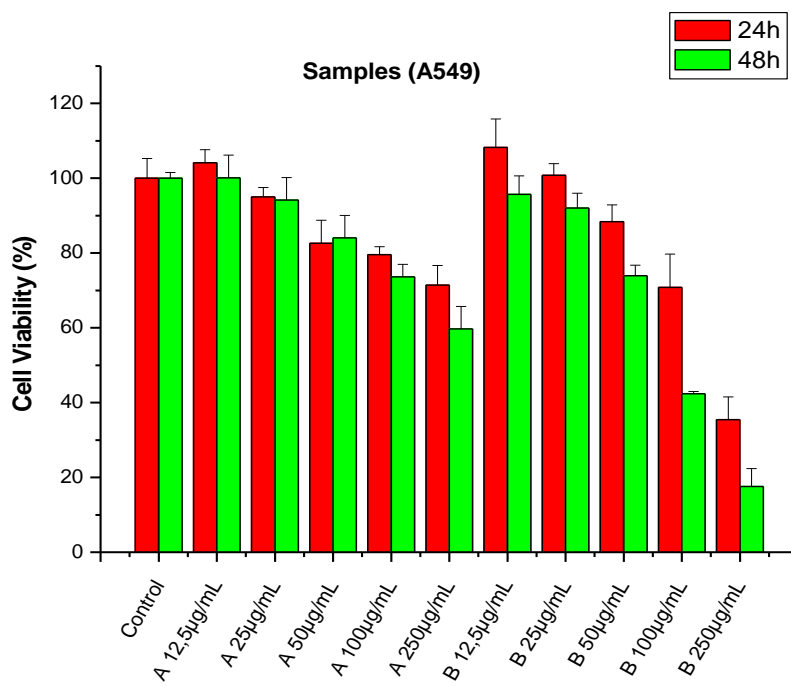
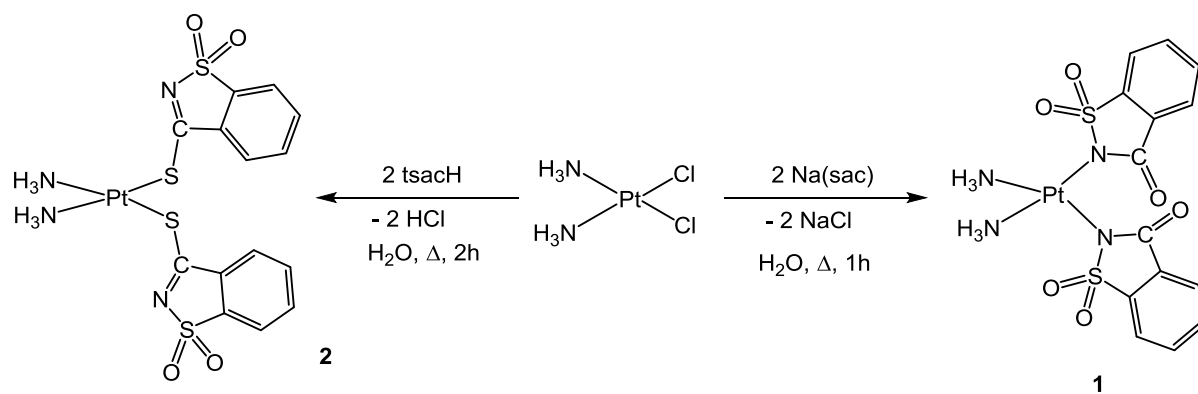


Figure 2



Scheme 1