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Synthesis, Characterization, Cytotoxicity and Anticomplementary Activity of a Novel Dimethyltin(IV) Cyclobutyl Dicarboxylate Complex of 8-Hydroxyquinaldine

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The reaction of equimolar quantities of dimethyltin(IV)-cyclobutyl dicarboxylate (prepared from dimethyltin(IV) dichloride and silver nitrate followed by addition of dipotassium cyclobutyl dicarboxylate) and 8-hydroxyquinaldine (L) in ethanol under reflux, afforded a novel complex of the formula

$$[Me_2Sn\{O_2(CO)_2\overline{CCH_2CH_2CH_2}\}L].$$

The complex was characterized by its elemental analysis and IR, NMR and mass spectral data. The complex was subjected to cytotoxic bioassay against one fluid suspension cell line, *i.e.*, P338 and four solid cell lines, *i.e.*, Hep-2, HeLa, L_{20B} and RD using the MTT assay technique. It is also tested for its anticomplementary activity (using a test that detects complement proteins inhibition). These activities were compared with those of the reference standards, cisplatin, carboplatin and oxaliplatin as well as with those of the starting organotin compound against the same cell lines. The significance of these results is given and discussed.

INTRODUCTION

In our recent articles about the synthesis and biological evaluations of organotin(IV) compounds and their complexes, we have given a brief survey about the antitumour, antipathogenic bacteria and anticomplementary activities of these compounds.[1–3] Several review articles have been reported during the last decade about organotin compounds, their complexes and their biological activities.[4–7] In the present article, we are presenting the synthesis of a novel dimethyl-tin(IV) cyclobutyl dicarboxylate complex with the ligand 8-hydroxyquinaldine (Fig. 1) and its biological activity against some tumour cell lines as well as its anticomplementary activity.

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EXPERIMENTAL

The ^1H NMR spectrum was recorded at Yarmook University, Irbid, Jordan, on a Burker-WH80DS spectrometer, using $CDCl_3$ as a solvent with TMS as internal standard. IR spectrum was recorded on a Burker FT-IR spectrometer in the range 4000–400 cm^{-1} using KBr discs. Analysis of the complex was performed by Atlantic Microlab., Inc., Norcross, Georgia 30091 (USA). Mass spectrum (EI) was determined *via* an SSQ 7000 at Ulm University, Germany.

The free ligand 8-hydroxyquinaldine was purchased from Fulka and the tin compound $Me_2Sn\{O_2(CO_2)\overline{CCH_2CH_2CH_2}\}$ was prepared according to our previous method[1].

Preparation of the complex $[Me_2Sn\{O_2(CO_2)\overline{CCH_2CH_2CH_2}\}L]$

The compound $Me_2Sn\{O_2(CO)_2\overline{CCH_2CH_2CH_2}\}$ (0.3 g, 1.03 mmol) was suspended in ethanol (50 mL) and the ligand 8-hydroxyquinaldine (0.16 g, 1.01 mmol) was added to the suspension. The reaction mixture was refluxed for *ca.* 30 min, then taken to dryness by evaporation of all ethanol. The resulting yellow oil was treated with *n*-hexane with stirring until complete solidification. The pale yellow solid thus formed was filtered off, washed several times with *n*-hexane and dried under vacuum at 80°C for several hours.

Biological methods: The complex was dissolved in 10% DMSO. Three serial dilutions of 0.1, 1.0 and 10.0 μg mL^{-1} were used and millipore (0.2 nm) filtered under laminar flow conditions. Reference standards (cisplatin and carboplatin) were purchased from Bristol Myers (USA) and oxaliplatin was prepared, characterized and purified (HPLC) in our laboratories.

Cell lines: Hep-2 (human carcinoma of larynx), Hela (human cervical carcinoma), RD (human embryonal rhabdomyosarcoma), L_{20B} (mouse L-cells containing human polio-virus receptors)[8], P388 (myelogenous leukaemia) were kindly supplied by Dr. M. Abdul-Majeed, Al-Basheer Hospital, Amman, Jordan. All cells except L_{20B} were maintained in Minimum Essential Medium (MEM) and supplemented with 5% fetal calf serum (ICN-Flow Laboratories, UK), L-glutamine and antibiotics (100 units of penicillin and 100 μg mL^{-1} of streptomycin). L_{20B} cells were maintained in Dulbecco's MEM (DMEM) (Sigma Chemical Co., USA) and supplemented with 10% fetal calf serum and antibiotics.

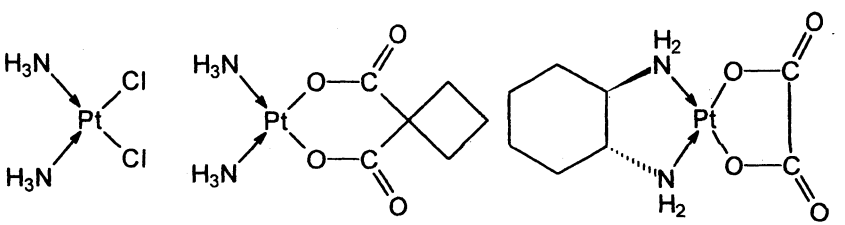
Cytotoxicity tests: MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) colorimetric assay was performed in a 96-well plate[9,10]. The above cell lines (1×10^6 cells mL^{-1}) were seeded in each well with 10 μL of growth medium and 10% fetal calf serum and antibiotics. After overnight incubation (37°C, 5% CO_2), 10 μL of the sample solution was added to each well and incubated for 72 h. Then 10 μL of MTT (5 mg mL^{-1}) was added to each well and the plates were incubated for a further 4 h. Later, 25 μL of 10% SDS-0.01M HCl solution was added to each well. The optical density was recorded using a microplate reader at 540 nm. Three separate sets of controls containing the solvents (10% DMSO) were used in each plate. The IC_{50} (μg mL^{-1}) was calculated using the probit test.

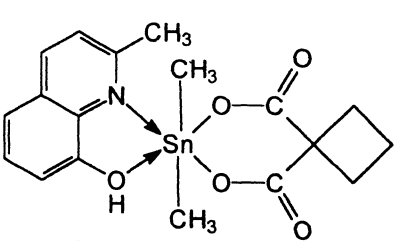
Anticomplementary: The inhibition of complement activity was determined

as described by Shahat *et al.*[11] The test complex and the standard reference compounds were dissolved in 10% dimethylsulphoxide (DMSO) and serial concentrations (10, 1.0, 0.1, 0.01, 0.001, 0.0001 μg mL^{-1}) were prepared. The assay was performed in v-well microtitre plates. Rabbit complement (C901 Virion/Serion Immunodiagnostic GmbH) and hemolysing anti-sheep erythrocyte serum (C902 Virion/Serion Immunodiagnostic GmbH) were used as recommended by the manufacturer. 50 μL of the complement solution (diluted 1 : 50) was added to 50 mL of each sample concentration. After an incubation at 37°C for 30 min, 50 μL of a suspension of sensitized sheep erythrocytes were added to each well. Hemolysis was observed optically after an incubation at 37°C for 1 h. Controls consisted of sensitized sheep erythrocytes incubated in buffer + DMSO (no hemolysis), with working solution complement (100% hemolysis), with 1 : 2 and 1 : 3 diluted working solution complement (partial hemolysis). Data were obtained as the results for duplicated samples. The IC_{50} values were calculated using the probit test.

RESULTS AND DISCUSSION

The complex (Fig. 1) was prepared as described in the experimental part as pale yellow fine crystals, soluble in most organic solvents. The physical properties of this complex are listed in Table-1. Its elemental analysis is satisfactory for the formula $C_{18}H_{21}NO_5Sn$ solvated with two moles of C_2H_5OH, *i.e.*, the new formula being $C_{22}H_{33}NO_7Sn$. The molecular weight of this complex (541.7) is in accordance with that obtained from the mass spectrum (Fig. 2). The IR spectrum of the complex showed some characteristic bands due to ν(CO), ν(OH), ν(C=N) and the ^1H NMR spectrum recorded in $CDCl_3$ showed signals due to all the functional groups present in the complex (Table-1). All the analytical data obtained for the complex confirm the suggested structure (Fig. 1).





The Novel Complex

Fig. 1. Novel organotin(IV) complex and the known platinum anticancer agents.

TABLE-1
CHARACTERIZATION DATA OF THE COMPLEX*

Item	Description
Colour	Pale yellow
m.p. (°C)	143–145 (dec.)
Yield	75%
IR (KBr disc) cm^{-1}	3440 br, ν(OH); 1730 m, ν(CO); 1615 s, ν(C=C); 1565 s, ν(C=N)
Mass spectrum (EI)*	m/z = 542, 451, 400, 393, 369, 308, 277, 185, 159, . . .
^1H NMR ($CDCl_3$) δ (ppm) & J (Hz)	1.0 s (6H), CH_3—Sn, ^2J (^{119}Sn—CH) = 81.5; 2.8 s (3H), CH_3; 2.0 q (2H), CH_2, (J = 7.5); 7.1–7.5 m (3H) (phenolic protons), 8.15 d (2H) (pyridinic protons) (J = 8.5).

*The complex gave satisfactory elemental analysis as two moles of ethanol were solvated with it.

The novel complex was subjected to cytotoxic bioassay against five tumour cell lines. The cytotoxic activity of the complex was compared with those of the reference standards, cisplatin, carboplatin and oxaliplatin (Table-2). The complex showed significant cytotoxic activities against almost all the cell lines used. These activities are also significant when compared with those of the references against almost all the cell lines. It is worth mentioning here that the activities of the novel complex are superior when compared with those shown by the starting organotin compound, *i.e.*, $Me_2Sn\{O_2(CO)_2\overline{C—CH_2CH_2CH_2}\}$, in which the latter showed no activity against all the cell lines used ($IC_{50} > 10\ \mu g\ mL^{-1}$).[1] However, further *in vivo* tests are necessary to confirm this activity in animal models.

TABLE-2
CYTOTOXICITY AND ANTICOMPLEMENTARY ACTIVITIES OF THE COMPLEX AND THE REFERENCE STANDARDS

Cell line	Description	IC_{50} ($\mu g\ mL^{-1}$)			
		Complex	Cisplatin	Carboplatin	Oxaliplatin
P388	Lymphocytic leukaemia	1.4	0.15	> 10	NT
Hep-2	Larynx carcinoma	1.9	1.80	> 10	8.0
HeLa	Cervix carcinoma	2.4	5.30	> 10	9.0
L_{20B}	Hep-2 inactivated with polio-virus	6.5	> 10.00	> 10	> 10.0
RD	Rhabdomyosarcoma	2.1	> 10.00	> 10	> 10.0
—	Anticomplementary	No inhibition	< 0.10	< 0.1	< 0.1

NT: not tested.

On the contrary, the novel complex showed no inhibition when tested for its anticomplementary activity at all concentrations used ($IC_{50} > 10\ \mu g\ mL^{-1}$) whereas the reference standards, cisplatin, carboplatin and oxaliplatin showed

inhibition at concentrations less than 0.1 μg mL^{-1}. Similarly, the starting material $Me_2Sn\{O_2(CO)_2\overline{CCH_2CH_2CH_2}\}$ showed no inhibition at all concentrations used[3]. Thus, taking into consideration the significant cytotoxic activities of this complex against the cell lines used, it is rather difficult at the present time to correlate between the cytotoxic activity and the anticomplementary activity of this complex.

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