New Antitumor Titanocene Derivatives Containing Hydrophilic Ligands

PETRA KÖPF-MAIER*

Abteilung Anatomie der Universität Ulm, Oberer Eselsberg, D-7900 Ulm an der Donau, F.R.G.

SIEGFRIED GRABOWSKI, JOHANNES LIEGENER and HARTMUT KÖPF

Institut für Anorganische und Analytische Chemie der Technischen Universität Berlin, Strasse des 17 Juni 135, D-1000 Berlin 12, F.R.G.

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Abstract

Four titanocene derivatives containing hydrophilic ligands were tested for antiproliferative activity against Ehrlich ascites tumor in mice. The new compounds $(C_5H_5)_2TiCl(p\text{-}SC_6H_4NH_3^+Cl^-)$ **(I)** and $(C_5H_5)_2Ti(p\text{-}SC_6H_4NH_3^+Cl^-)_2$ **(II)**, containing hydrochlorinated *p*-aminothiophenolate ligands, and the known compounds $(C_5H_5)_2Ti(cis\text{-}OOC{-}CH{=}CH{-}COOH)_2$ **(III)** and $(C_5H_5)_2Ti(OOC{-}CCl_3)_2$ **(IV)** containing the carboxylic acid anions hydrogenmaleinate and trichloroacetate as acido ligands, induced maximum cure rates of 100%. The T.I. values amounted to 4.4–4.6 **(I)**, 3.5–4.1 **(II)**, 3.7–3.8 **(III)** and 5.5 **(IV)**, and were slightly increased in comparison to $(C_5H_5)_2TiCl_2$ (T.I. = 3.3). The complexes **I–III** were rather soluble in water and equally active in a DMSO/saline (1/9, v/v) mixture, in pure saline and in buffered solutions. In the case of **IV**, the toxicity was considerably low (LD_{50}, 440 mg/kg; LD_{100}, 500 mg/kg) in relation to $(C_5H_5)_2TiCl_2$ (LD_{50}, 100 mg/kg; LD_{100}, 140 mg/kg).

Introduction**

Studies into the dependence of the antitumor activity of titanocene derivatives upon structural modifications within the $(\eta^5\text{-}C_5H_5)_2TiX_2$ molecules have demonstrated the following relations [1]: pronounced antitumor activity against Ehrlich ascites tumor occurs when two non-modified cyclopentadienyl ring ligands are present within the compounds [2, 3]. On the other hand, the chloride ligands of $(C_5H_5)_2TiCl_2$ can be exchanged by other halide or pseudohalide ligands without loss of antitumor activity [4]. Thus, the preferable site for molecular modification not reducing the antitumor properties of titanocenes is obviously the position of the acido ligands X within $(C_5H_5)_2TiX_2$.

In continuation of these investigations, various titanocene derivatives were synthesized by the introduction of hydrophilic ligands X containing carboxylic functions [5, 6] or hydrochlorinated amino groups. In the present study, we report on the antiproliferative activity of these compounds against Ehrlich ascites tumor in mice.

Experimental

Preparation of Compounds

*Titanocene chloride p-aminothiophenolate hydrochloride (***I***, cf. Fig. 1)*

Prepared by dropwise addition of *p*-aminothiophenol (1.25 g, 10 mmol), dissolved in 40 ml CCl_4, within 30 min to a stirred suspension of titanocene dichloride (2.5 g, 10 mmol) in 30 ml CCl_4 under an argon atmosphere. After stirring the mixture at room temperature for 60 h, **I** was collected on a frit by suction, washed several times with CCl_4, and dried *in vacuo* to yield 3.4 g (91%) of a pale

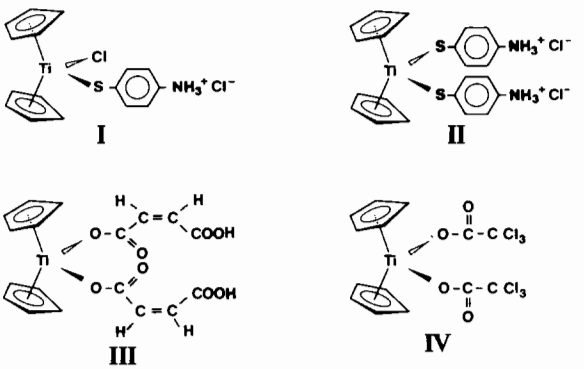

Fig. 1. Structures of compounds **I–IV**.

*Author to whom correspondence should be addressed.

**Abbreviations: DMSO dimethylsufoxide; I.L.S. increase in life span; i.p. intraperitoneal(ly); p.t.t. post transplantationem tumoris; T.I. therapeutic index.

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violet powder, decomposing above 115 °C. *Anal.* Calcd. for $C_{16}H_{17}Cl_2NSTi$ (374.19): C, 51.36; H, 4.58; N, 3.74. Found: C, 50.97; H, 4.52; N, 3.68%. IR spectrum (KBr disc, cm^{-1}): 3300w; 3240w; 3080w (C_5H_5, νCH); 2820m,br; 2560m; 1780m; 1540w; 1480s; 1430s (C_5H_5, ωCC); 1400w; 1360w; 1300vw; 1270vw; 1250s; 1200m; 1170m; 1125w; 1070vw; 1010s (C_5H_5, δCH); 940sh; 920s; 870s; 820vs (C_5H_5, γCH); 700w; 630m; 590vw; 520sh; 500m; 490sh; 410sh; 395s; 355s; 295s.

*Titanocene bis(p-aminothiphenolate) bis(hydrochloride) (***II***, cf. Fig. 1)*

Obtained analogously to **I** from 1.25 g (5 mmol) titanocene dichloride in 10 ml CCl_4. The deep red powder of **II** (2.3 g, 91%) decomposed above 135 °C. *Anal.* Calcd. for $C_{22}H_{24}Cl_2N_2S_2Ti$ (499.38): C, 52.92; H, 4.84; Cl, 14.20; N, 5.61; S, 12.84. Found: C, 52.77; H, 4.85; Cl, 14.56; N, 5.55; S, 12.68%. IR spectrum (KBr disc, cm^{-1}): 3400m,br; 3100m (C_5H_5, νCH); 2820s,br; 2550m,br; 1580m; 1480vs; 1435m (C_5H_5, ωCC); 1405m; 1350w; 1200w; 1170w; 1110w; 1070w; 1010s (C_5H_5, δCH); 865m; 820vs (C_5H_5, γCH); 625vw; 490m; 410w; 395w; 355w; 295w.

*Titanocene bis(hydrogenmaleinate) (***III***, cf. Fig. 1)*

Synthesized by the method described by Döppert and Thewalt [5, 6]. *Anal.* Calcd. for $C_{18}H_{16}O_8Ti$ (408.22): C, 52.96; H, 3.95. Found: C, 52.98; H, 3.99%. IR spectrum (KBr disc, cm^{-1}): 3100m (C_5H_5, νCH); 1920w,br; 1880w,br; 1705vs; 1620s; 1525-vs,br; 1400s (C_5H_5, ωCC); 1335sh; 1310vs; 1210m; 1015m (C_5H_5, δCH); 955w; 860s (C_5H_5, γCH); 840sh; 800sh; 640m; 600w; 480m; 430w; 350w; 305m. Mass spectrum (70 eV, 130 °C; Cp = C_5H_5): $Cp_2TiC_4H_2O_4^+$ (m/e 292, relative intensity 48%); $Cp_2TiC_3H_2O_2^+$ (248, 17); $CpTiC_4H_2O_4^+$ (227, 34); $Cp_2TiC_2H_2^+$ (204, 8); $Cp_2TiC_2^+$ (202, 4); Cp_2TiO^+ (194, 25); $CpTiC_3H_2O_2^+$ (183, 3); Cp_2Ti^+ (178, 28); $CpTiC_2H_2^+$ (139, 79); $CpTiO^+$ (129, 100); $CpTi^+$ (113, 30).

*Titanocene bis(trichloroacetate) (***IV***, cf. Fig. 1)*

Precipitated from a solution of titanocene dichloride (0.5 g, 2 mmol) in 30 ml hot water by addition of trichloroacetic acid (3.3 g, 20 mmol). After cooling to room temperature, the precipitate was fritted, washed with water, and dried *in vacuo* to give 0.88 g (88%) **IV** as an orange-yellow powder, melting at 173–174 °C. *Anal.* Calcd. for $C_{14}H_{10}Cl_6O_4Ti$ (502.78): C, 33.44; H, 1.99. Found: C, 33.70; H, 2.00%. IR spectrum (KBr disc, cm^{-1}): 3100w (C_5H_5, νCH); 1680s; 1440w (C_5H_5, ωCC); 1390vw; 1330s; 1300s; 1020w (C_5H_5, δCH); 960w; 870w; 830m (C_5H_5, γCH); 730m; 680m; 600w; 550w; 520w; 430vw; 390vw; 370vw. Mass spectrum (70 eV, 135 °C; Cp = C_5H_5): $CpTiC_4Cl_6O_4^+$ (m/e 437, relative intensity 24%); $Cp_2TiC_2Cl_4O_2^+$ (376, 3); $Cp_2TiC_2Cl_3O_2^+$ (341 and 339, 15); $CpTiC_2Cl_4O_2^+$ (311, 50); $Cp_2TiCl_2^+$ (248, 8); Cp_2TiCl^+ (213, 71); $CpTiCl_2^+$ (183, 100); Cp_2Ti^+ (178, 11); $CpTiCl^+$ (148, 57); $CpTiO^+$ (129, 9). ^{1}H-NMR spectrum ($CDCl_3$): $\delta(C_5H_5)$ 6.70 ppm (singulett).

Testing of Antitumor Activity

For testing the antitumor activity of **I**–**IV**, female CF1 mice (20–25 g), kept under standard conditions, were inoculated i.p. with 6×10^6 Ehrlich ascites tumor cells on day 0 of the experiment. On day 1, *i.e.* 24 h later, the animals received a single injection of the substances. These were dissolved (i) in saline containing a 10% admixture of DMSO (**I**–**IV**), (ii) in pure saline (**I**–**III**) and (iii) in buffered DMSO/saline (1/9) solutions (**II**) adjusted to pH 5.0–6.0 by addition of 1 M $NaHCO_3$. The injection fluids were applied i.p. to the animals in volumes of 0.4–0.5 ml per mouse. The experimental details are given in Table I. Eight additional groups each consisting of 10 tumor-bearing mice served as control groups. The control animals only received 0.5 ml of the solvent without drug addition on day 1.

The antitumor activity of the substances was estimated by determination of the survival time of the animals. Deaths of animals occurring within 8 days p.t.t. were defined as toxic deaths, those occurring later as tumor deaths. The key-date for the evaluation of the experiment and for the determination of the survival rate was day 120 p.t.t. The survival times of the controls ranged from 13–20 (mean value 16.3 ± 1.5) days. The I.L.S. values were calculated by relating the mean survival time of a given dose group to that of the appertaining control groups as a percentage and by subtracting 100%, the T.I. values by relating the LD_{50} to ED_{90} values. Animals being still alive on day 120, without signs of tumor, were considered as cured.

Results

The new compounds **I** and **II** were synthesized according to eqns. (1) and (2). In these preparations, the amino group of the ligand *p*-aminothiophenol acts as an auxiliary base which adds the hydrogen chloride cleaved off under the formation of the Ti–S bonds.

$$(C_5H_5)_2TiCl_2 + HSC_6H_4NH_2 \longrightarrow (C_5H_5)_2TiCl(SC_6H_4NH_2 \cdot HCl) \quad (1)$$

I

$$(C_5H_5)_2TiCl_2 + 2HSC_6H_4NH_2 \longrightarrow (C_5H_5)_2Ti(SC_6H_4NH_2 \cdot HCl_2)_2 \quad (2)$$

II

TABLE I. Experimental Data of **I**–**IV** against Ehrlich Ascites Tumor of Mice.

Compound	Solvent[a]	Applied doses (mg/kg)	Number of animals per dose	Total number of animals
I	m	20, 40, 60 ... 240	6	72
	s	20, 40, 60 ... 200	6	60
II	m	20, 40, 60 ... 240	6	72
	s	20, 40, 60 ... 200	6	60
	b	20, 40, 60 ... 300	6	90
III	m	20, 40, 60 ... 300	6	90
	s	20, 40, 60 ... 260	6	78
IV	m	20, 40, 60 ... 540	6	162

[a]m = Mixture of DMSO and saline (1/9, v/v); s = pure saline; b = buffered DMSO/saline (1/9) solutions (pH 5.0–6.0, admixture of $NaHCO_3$).

Elemental analyses and IR spectra are consistent with the compositions and structrues of **I** and **II** (Fig. 1). In accordance with the salt-like nature of these compounds, **I** and **II** were not volatile under mass-spectrometric conditions. NMR signals of **I** and **II** could not be obtained because of poor solubility in common organic solvents.

The carboxylic acid derivatives **III** and **IV** were prepared in aqueous solution following the overall reaction (3).

$$(C_5H_5)_2TiCl_2 + 2RCOOH \longrightarrow (C_5H_5)_2Ti(OOCR)_2 + 2HCl \quad (3)$$

III, IV

This method has been published in the literature [5, 6] reporting few IR-spectral and no mass-spectral data and without giving a detailed procedure for **IV**, which are therefore included in the experimental section. The preparation of **IV** under non-aqueous conditions has also been described [7].

The introduction of ligands X bearing hydrophilic groups into the titanocene species $(C_5H_5)_2TiX_2$ resulted in an increased solubility in water in the case of the compounds **I**–**III** in comparison to $(C_5H_5)_2TiCl_2$. For *in vivo* application, it was therefore possible to omit DMSO from the solvent mixture and to inject the three substances dissolved in pure saline over the whole therapeutic range. Comparable antitumor activity was observed after administration of **I**–**III** in pure saline or in a DMSO/saline mixture (Table II). The corresponding values of acute toxicity (LD_{50}, LD_{100}) were also identical or differed by only 5–20 mg/kg.

The main pharmacologic and toxicologic data, found after application of the compounds **I**–**IV** in pure saline or in DMSO/saline to mice bearing Ehrlich ascites tumor, are summarized in Fig. 2 and in Table II. For all compounds **I**–**IV**, maximum cure rates of 100% and I.L.S. values of 610–660% were attained over a dose range of 60 or 80 mg/kg (**I**, **II**, **III**) or, even of 260 mg/kg, in the case of the trichloroacetate derivative **IV**. The characteristic values of acute toxicity (LD_{50}, LD_{100}) were found to range between 165 and 240 mg/kg for **I**–**III**; in the case of **IV**, they were considerably higher and amounted to 440 (LD_{50}) and 500 (LD_{100}) mg/kg. The calculation of the therapeutic index (T.I.), which is defined as the relation of LD_{50} to ED_{90} and which indicates the width of the therapeutic range, gave values between 3.5 and 4.6 for **I**–**III**. In correspondence to the increased toxic level of the trichloroacetate derivative **IV**, the T.I. was even elevated to 5.5.

This means that the introduction of hydrophilic ligands into the titanocene molecule not only improved the water solubility in most cases but also elevated the toxic level which resulted in the widening of the therapeutic range (for comparison, $(C_5H_5)_2TiCl_2$: LD_{50}, 100 mg/kg; LD_{100}, 140 mg/kg; T.I., 3.3. [1, 4]).

The pH values of the injection fluids containing the investigated complexes in optimum doses amounted to 2.4–3.0 (**I**–**III**) or to 3.0–5.4 (**IV**). In the case of **II**, an additional experiment was performed and the injection fluids were buffered by addition of $NaHCO_3$ to adjust pH values of 5.0–6.0 in the dose range between 20 and 300 mg/kg. Again, a maximum cure rate of 100% was attained but the LD_{50} and LD_{100} values were elevated to 260 and 300 mg/kg, respectively. By this means, an improved T.I. value of 4.3 was achieved in comparison to 3.5, after the application of non-buffered solutions of **II**.

Discussion

The results of the present study confirm the concept of the structure–activity relationship of the metallocene dihalides [1, 8, 9] and indicate

TABLE II. Pharmacologic and Toxicologic Data of I–IV against Ehrlich Ascites Tumor of Mice.

Compound	Solvent[a]	Optimum dose range (mg/kg)	Optimum cure rate (%)	I.L.S.[b] at optimum dose range (%)	LD_{50} (mg/kg)	LD_{100} (mg/kg)	T.I.[c]
I	m	60–140	100	655	175	200	4.4
	s	60–140	100	642	180	200	4.6
II	m	60–140	100	636	175	200	3.5
	s	40–120	100	659	165	200	4.1
	b	100–240	100	650	260	300	4.3
III	m	60–120	100	611	170	220	3.8
	s	60–140	100	665	175	240	3.7
IV	m	100–360	100	645	440	500	5.5

[a]m = Mixture DMSO/saline (1/9, v/v); s = pure saline; b = buffered DMSO/saline (pH 5.0–6.0). [b]I.L.S. = Increase in life span. [c]T.I. = Therapeutic index, defined as the relation LD_{50}/ED_{90}.

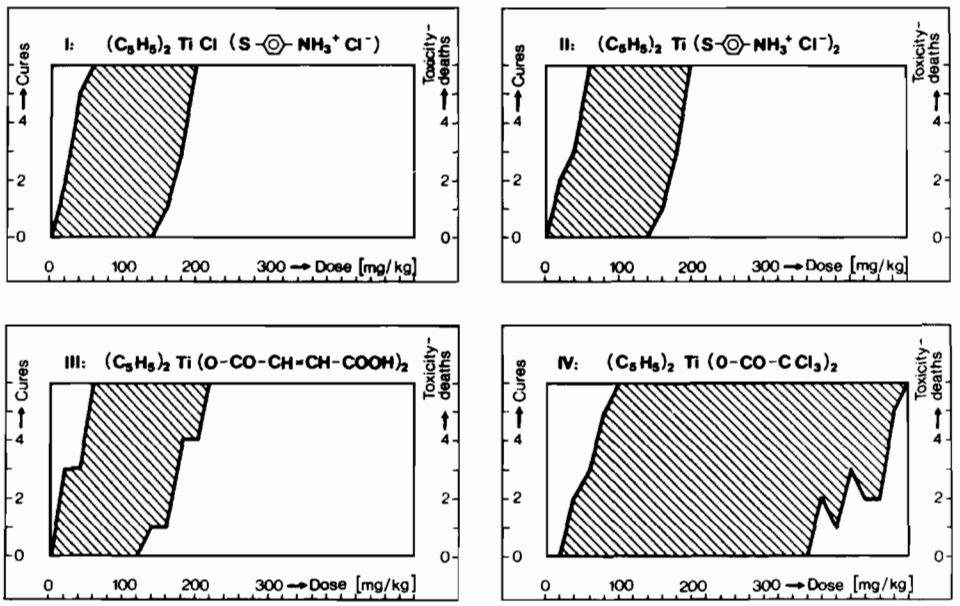

Fig. 2. Dose-activity relationships (left) and dose-lethality relationships (right) of I–IV against Ehrlich ascites tumor in mice. Surviving animals ▨. Drug administered in DMSO/saline.

the acido ligands X within $(C_5H_5)_2TiX_2$ to be that molecular site which can be modified without loss of antitumor activity. Moreover, the results show that, in comparison to titanocene dihalides, even an improvement of the biologically important properties of the titanocenes can be induced by variation at these positions X. The introduction of ligands bearing hydrophilic groups such as carboxylic functions or protonated amino substituents results, (i) in a diminution of the toxic properties, (ii) in a widening of the therapeutic range, and (iii) in an increase of the water solubility. Thus, the application in pure saline became possible for most of the investigated complexes. Moreover, the buffering experiment of the present study demonstrates that a further reduction of toxic properties and, as a result, a further improvement of the therapeutic index is attainable by pH elevation in the injected drug solutions, in a similar manner as previously shown for titanocene dihalides and dipseudohalides [4].

From the described results, the expectation may be concluded that numerous other related compounds, containing hydrophilic (ionic or polar) groups at ligands in the X positions, may also be characterized by similar improved therapeutic properties. The systematic synthesis and the *in vivo*

testing of such compounds is necessary and may perhaps achieve further amelioration of the biologic properties of titanocenes.

In this connection, it is worth mentioning that in the series of antitumor platinum compounds, the introduction of carboxylate (e.g., oxalate, malonate, substituted malonate) ligands at comparable molecular positions has also improved the biologic properties, especially the therapeutic effectivity [10–12], although these ligands are obviously bound much tighter than halide ligands [11, 12]. Thus, another intracellular mechanism must be postulated for the platinum carboxylato derivatives than for *cis*-platinum, itself. In the case of the titanocene derivatives **I**–**IV** investigated in the present paper, only a brief study is yet available related to the hydrolytic behavior of the carboxylato species **III** and **IV** and indicating the possibility of hydrolytic dissociation of the carboxylate ligands [6].

Interestingly enough, the compound titanocene chloride *o*-aminothiophenolate, $(C_5H_5)_2TiCl(o\text{-}SC_6H_4NH_2)$, which differs from **I** by the *o*-position and the neutral, non-protonated nature of the NH_2 substituent, only exhibits sporadic antitumor effectivity (sporadic cure rate: 13%) against Ehrlich ascites tumor without a strong dose-activity relationship [3]. It is not yet clear if one of the mentioned structural differences leads to the considerable difference in activity or if, perhaps, other molecular properties are responsible.

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