Inorganica Chimica Acta, 153 (1988) 119–122

Antitumor Properties of Some Titanocene Chalcogenates

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(Received March 2, 1988)

Abstract

Five chalcogen-coordinated bis(η^5-cyclopentadienyl)titanium(IV) chalcogenolates were tested against fluid Ehrlich ascites tumor for antitumor properties: the titanocene phenolates $(C_5H_5)_2TiCl(2,4,6\text{-}OC_6H_2Cl_3)$ (I) and $(C_5H_5)_2Ti(OC_6F_5)_2$ (II); the titanocene thiophenolate $(C_5H_5)_2Ti(SC_6F_5)_2$ (III); the titanocene dithiolene chelate $(C_5H_5)_2Ti[cis\text{-}1,2\text{-}S_2C_2(CN)_2]$ (IV); and the titanocene selenophenolate $(C_5H_5)_2TiCl(SeC_6H_5)$ (V). The best antitumor activity and an optimum cure rate of 100% were observed in the case of the pentafluorophenyl derivatives **II** and **III**, followed by **IV** and **V** which induced cure rates of 90 and 80%, respectively. These results confirm that bis(η^5-cyclopentadienyl)titanium(IV) diacido complexes can be widely varied at the position of the acido ligands without loss of antitumor potency. The titanocene derivatives described in the present study are the first neutral mercapto and seleno titanocene derivatives for which strong antiproliferative properties have been shown.

Introduction

Bis(cyclopentadienyl)metal(IV) ('metallocene') diacido complexes $(C_5H_5)_2MX_2$, containing as the central atom early transition metals such as titanium or vanadium, have shown antitumor activity against numerous experimental and human tumors [1–3]. Studies into the structure–activity relationship of metallocene complexes revealed a strong dependence of the antiproliferative properties upon the central metal atom M within the metallocene dichloro complexes [1] and pointed to the importance of the presence of two unsubstituted cyclopentadienyl ring ligands [1, 4]. On the other hand, the acido ligands X within the metallocene molecules can be obviously varied widely without loss of the antineoplastic properties. Besides titanocene dihalides and dipseudohalides [5], carboxylato derivatives are also characterized by antitumor properties [6]. Titanocene complexes modified at the acido ligands X which did not exhibit antiproliferative properties against fluid Ehrlich ascites tumor are the polychalcogenide chelates $(C_5H_5)_2TiS_5$ and $(C_5H_5)_2TiSe_5$. It is supposed that, within these metallacycles, the pentasulfido and pentaselenido ligands are bound too strongly to the titanium atom, thus preventing dissociation of the acido ligands in aqueous systems [1].

In the present study, we further modified titanocene complexes at the position of the acido ligands X by incorporating phenolate, thiophenolate or selenophenolate ligands into the complex molecules. The antitumor properties of these complexes and of a dithiolene chelate were investigated against fluid Ehrlich ascites tumor.

Experimental

Substances

Five bis(η^5-cyclopentadienyl)titanium(IV) ('titanocene') chalcogenolates with symmetrically or unsymmetrically coordinated ligands, $(C_5H_5)_2TiX_2$ or $(C_5H_5)_2TiXY$, were investigated in the present study for antiproliferative properties: the titanocene phenolates bis(η^5-cyclopentadienyl)-2,4,6-trichlorophenolato(chloro)titanium(IV) $(C_5H_5)_2TiCl(2,4,6\text{-}OC_6H_2Cl_3)$ (I) and bis(η^5-cyclopentadienyl)bis(pentafluorophenolato)titanium(IV) $(C_5H_5)_2Ti(OC_6F_5)_2$ (II); the titanocene thiophenolate bis(η^5-cyclopentadienyl)bis(pentafluorothiophenolato)titanium(IV) $(C_5H_5)_2Ti(SC_6F_5)_2$ (III); the titanocene dithiolene chelate bis(η^5-cyclopentadienyl)maleonitriledithiolatotitanium(IV) $(C_5H_5)_2Ti[cis\text{-}1,2\text{-}S_2C_2(CN)_2]$ (IV), containing a five-membered TiS_2C_2 chelate ring; and the titanocene selenophenolate bis(η^5-cyclopentadienyl)selenophenolato(chloro)titanium(IV) $(C_5H_5)_2TiCl(SeC_6H_5)$ (V).

The compounds, the molecular structures of which are illustrated in Fig. 1, were synthesized

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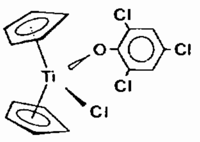
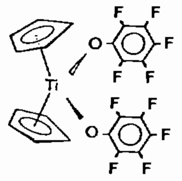


I



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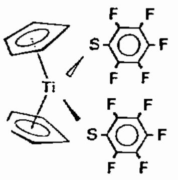

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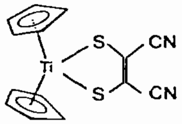
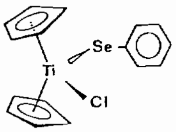


IV



V

Fig. 1. Structures of complexes I–V.

as described before ([7] for **I**, [8] for **II** and **III**, [9–11] for **IV**, [12] for **V**) and characterized by IR, NMR and mass spectroscopy. No impurities were detectable by these methods. Elemental analyses (C, H, N) revealed deviations ⩽0.5% of the calculated values.

Animals

Female CF1 mice weighing 20–25 g were kept under standard conditions (20–22 °C, Altromin® and tap water *ad libitum*).

Testing of Antitumor Activity

The antitumor activity of compounds **I**–**V** was tested against Ehrlich ascites tumor growing intraperitoneally as a fluid tumor. On day 0 of the experiment, 6×10^6 Ehrlich ascites tumor cells were inoculated into the peritoneal cavity of the mice. On day 1 (*i.e.* 24 h later) the animals were given a single intraperitoneal injection of the substances, which had been dissolved in saline containing a 10% admixture of dimethyl sulfoxide (DMSO). The doses given are listed in Table I, the substance concentrations being so selected that each mouse received a total volume of 0.4–0.5 ml (0.02 ml/g body weight). Every dose group consisted of 10 animals; five additional groups of 10 animals served as untreated, tumor-bearing controls. They received 0.5 ml of the DMSO–saline (1/9, *v*/*v*) mixture without drug addition on day 1.

The parameter evaluated was the survival time of the animals. Deaths within 8 days after tumor transplantation were defined as toxic deaths due to substance toxicity and those occurring later as tumor deaths. All animals dying after day 8 showed macroscopic signs of intraperitoneal tumor development. The key-date for determining the survival rate was day 120 after tumor transplantation. All animals that were still alive on the key-date showed no recognizable signs of tumor disease and were considered to be cured. The optimum dose ranges, the maximum cure rates obtained after treatment with **I**–**V**, and the values of the therapeutic index (*TI*) calculated by the relation LD_{50}/ED_{80} are summarized in Table I.

Results

All untreated control animals died from tumor disease between day 14 and 20 (mean value 16.1 ± 2.1 d). The influence of treatment with **II**–**V** on the occurrence of tumor deaths, toxic deaths and cures is illustrated in Figs. 2–5.

Following application of the titanocene monochloro complex **I**, containing as a second acido ligand an oxygen-coordinated trichlorophenolato group, half of the animals which were treated with optimum dose survived (Table I). No increase in cure rate was effected by higher doses of **I**, the LD_{20} and LD_{50} values amounting to 160 and 200 mg/kg. On the other hand, administration of the symmetrically ligated bis(pentafluorophenolato)

TABLE I. Pharmacological and Toxicological Data of Titanocene Chalcogenolates

Compounds	Experimental dose range (mg/kg)	Optimum dose range[a] (mg/kg)	Maximum cure rate (%)	LD_{50} (mg/kg)	LD_{100} (mg/kg)	TI[b]
I	20, 40 ... 400	60–140	50	200	300	
II	20, 40 ... 600	140–360	100	480	540	1.5
III	20, 40 ... 300	60–180	100	260	320	4.3
IV	20, 40 ... 300	60–140	90	240	260	4.0
V	20, 40 ... 300	40–80	80	150	200	1.9

[a]Defined as dose range with cure rates ⩾50%. [b]Defined as LD_{50}/ED_{80}. TI values can only be given when optimum cure rate ⩾80%.

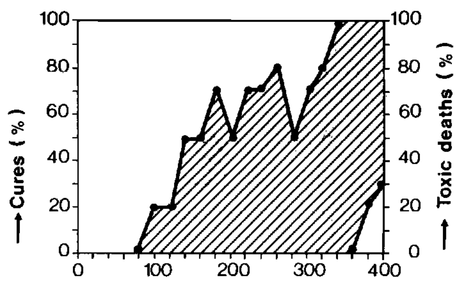


Fig. 2. Dose–activity (left graph) and dose–lethality (right graph) relationships of **II** against fluid Ehrlich ascites tumor. The shaded area indicates the range of surviving, cured animals.

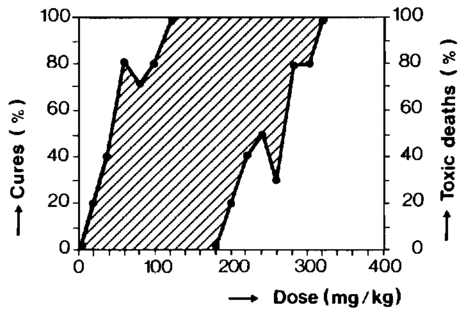


Fig. 3. Dose–activity and dose–lethality relationships of **III**. For further explanations, *cf.* legend to Fig. 2.

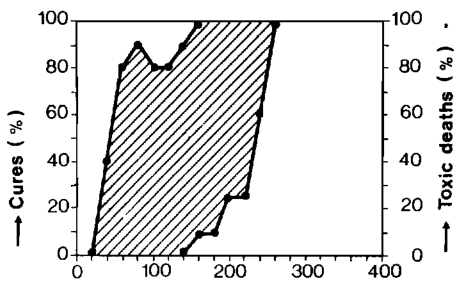


Fig. 4. Dose–activity and dose–lethality relationships of **IV**. For further explanations, *cf.* legend to Fig. 2.

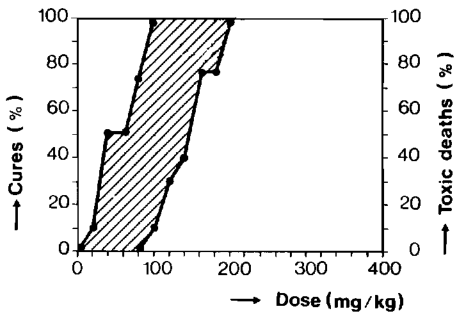


Fig. 5. Dose–activity and dose–lethality relationships of **V**. For further explanations, *cf.* legend to Fig. 2.

complex **II** caused survival of all animals treated with 340 and 360 mg/kg (Fig. 2), corresponding to a cure rate of 100% and a *TI* value of 1.5; the *TI* value expresses numerically the distance between the dose–lethality and dose–activity curves (Table I). Similar effectivity and also a maximum cure rate of 100% were attained by treatment with the analogous pentafluorothiophenolato derivative **III** over an even broader dose range of 120–180 mg/kg (Fig. 3), resulting in a *TI* value of 4.3 (Table I). In the case of the titanocene dithiolene derivative **IV**, where the sites of the acido ligands are occupied by two sulfur atoms belonging to the bifunctional maleonitriledithiolate chelate ligand, a slightly reduced maximum cure rate of 90% was provoked by application of 140 and 160 mg/kg (Fig. 4), the *TI* value amounting to 4.0. A further diminution of the maximum cure rate to 80% and the *TI* value to 1.9 was observed under the influence of the monochloroselenophenolato derivative **V** (Table I). As shown in Fig. 5 and as it becomes obvious from Table I, the therapeutic range of **V** is clearly smaller than that observed in the case of **III** and **IV**.

Discussion

The results of the present study reveal pronounced antitumor activity for some chalcogen-coordinated phenolate, thiophenolate and selenophenolate derivatives of bis(η^5-cyclopentadienyl)titanium(IV) diacido complexes and, thus, confirm the concept of a structure–activity relationship for metallocene diacido complexes, indicating the acido ligands X within $(C_5H_5)_2TiX_2$ to be those molecular sites which can be modified to some extent without diminution or loss of antitumor activity [1–3]. Other neutral thiolato and related titanocene derivatives which have been tested before include $(C_5H_5)TiCl_2(SC_6H_5)$, $(C_5H_5)_2TiCl(o\text{-}SC_6H_4CH_3)$ and $(C_5H_5)_2TiCl(o\text{-}SC_6H_4NH_2)$ [13], as well as $(C_5H_5)_2TiS_5$ and $(C_5H_5)_2TiSe_5$ [1]. Whereas the mixed chloro complexes containing the thiophenolate, *o*-thiocresolate and *o*-aminothiophenolate ligands revealed activity with cure rates below 30%, none of the pentachalcogenide complexes was able to inhibit the growth of fluid Ehrlich ascites tumor. It is not yet clear if the poor water solubility or the tight Ti–S or Ti–Se bonding of these metallacyclic complexes, preventing dissociation of the acido ligands in aqueous systems, is responsible for the observed antitumor inactivity.

The titanocene derivatives described in the present study are the first titanocene phenolates, thiophenolates and selenophenolates for which strong antitumor properties have been observed. These results enlarge the spectrum of known antitumor titanocene complexes (which at present consists of titanocene dihalides [5], dipseudohalides [5] and carboxylates [6], as well as ionic cyclopentadienyl titanium complexes [6, 14]) by an additional group of compounds and, thus, confirm the titanocene diacido complexes to be an expansible class of antitumor agents, suggesting that numerous other related compounds will also exhibit antiproliferative properties. Further investigations are necessary to show if the titanocene chalcogenolates described in the present study will retain their antitumor activity against 'stronger' animal tumor systems, e.g., B16 melanoma or colon 38 carcinoma, as well as against human tumors heterotransplanted to athymic mice.

Acknowledgements

This work was supported by grants from the Trude Goerke foundation for cancer research at the Freie Universität Berlin, and from the Fonds der Chemischen Industrie.

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