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Ionic titanocene complexes: a new type of antitumor agent

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Summary. Five ionic cyclopentadienyltitanium(IV) derivatives were investigated for their activity against fluid Ehrlich ascites tumor. Four compounds were built up by the intact bis(cyclopentadienyl)titanium(IV) ("titanocene") unit, forming the cationic moiety together with two covalently bound ligands, with certain anions being bonded via electrostatic forces: the acetonitrile complex $[(C_5H_5)_2TiCl(NCCH_3)]^+[FeCl_4]^-$ (I), the 2,2′-bipyridyl derivative $[(C_5H_5)_2Ti(bipy)]^{2+}[CF_3SO_3]_2$ (II), the *o*-phenanthroline complex $[(C_5H_5)_2Ti(phen)]^{2+}[CF_3SO_3]_2$ (III), and the *N*-methyl-*o*-aminothiophenolate derivative $\{(C_5H_5)_2Ti[o\text{-}S(NHCH_3)C_6H_4]\}^+I^-$ (IV). Another ionic cyclopentadienyltitanium derivative investigated was the five-coordinate bis(dithiolene) chelate $[(C_5H_5)Ti(1,2,4\text{-}S_2C_6H_3CH_3)_2]^-[N(C_2H_5)_4]^+$ (V), the cyclopentadienyltitanium moiety representing the anionic part of the complex salt. All complexes were ionic, salt-like compounds, distinguished by good water solubility. Whereas complexes I, III, and V, given at optimal dose levels, effected maximal cure rates of only 70%–80%, all animals were cured after receiving complexes II and IV at dose ranges of 200–220 and 240–300 mg/kg, respectively. The antitumor activity of complex I was confirmed against solid experimental tumor systems B16 melanoma, colon 38 carcinoma, and Lewis lung carcinosarcoma. Because of their improved solubility in water and pronounced antitumor activity (especially that of II and IV against fluid Ehrlich ascites tumor), ionic cyclopentadienyl titanium complexes are considered to be an interesting new type of antitumor agent.

Introduction

Numerous platinum complexes as well as diverse non-platinum-group metal compounds have been described as antitumor agents during the past decade [5, 16, 26]. Most of them are monomeric complexes containing a central metal atom surrounded by inorganic or organic ligands. Another general feature of most inorganic and organometallic antitumor complexes is electric neutrality. In platinum compounds, the neutrality of the molecules is believed to be an important prerequisite for antitumor activity [21, 24], enabling them to pass through the hydrophobic layer of cell membranes into the interior of cells [21, 25]. In antitumor non-platinum-group metal compounds [16], the complexes containing the main-group IV elements germanium and tin [6, 20, 23] as well as the early transition metal compounds titanocene dichloride [10] and vanadocene dichloride [14] or certain copper and gold complexes [7, 27, 28] are again neutral, uncharged compounds, whereas only few inorganic and organometallic antitumor compounds are actually distinguished by their ionic character.

Most titanocene complexes known to be antitumor agents are neutral, uncharged compounds containing two acido ligands covalently bound to the central metal atom [16, 17, 19]. In the present study, we investigated the antitumor properties of some new, ionic titanocene complexes, where the bis(η^5-cyclopentadienyl)titanium(IV) ("titanocene") unit represented either the cationic or anionic moiety, neutralized by appropriate counterions.

Material and methods

Substances

Five ionic, salt-like titanocene complexes were tested for their antitumor properties. Most of them correspond to the general formula $[(C_5H_5)_2TiXL]^+Y^-$ or $[(C_5H_5)_2TiL_2]^{2+}(Y^-)_2$, where X is an anion or a ligand anionic donor site and L is a donor molecule or ligand neutral donor site. They contain the intact titanocene unit, forming the cationic complex moiety in tetrahedral configuration together with the covalently bound X and L or two L ligands. Various anions Y^- are bonded via electrostatic forces. Four compounds of this type were investigated in the present study:

1. $[(C_5H_5)_2TiCl(NCCH_3)]^+[FeCl_4]^-$ (I), bis(η^5-cyclopentadienyl)acetonitrile(chloro)titanium(IV) tetrachloroferrate (III)
2. $[(C_5H_5)_2Ti(bipy)]^{2+}[CF_3SO_3]_2$ (II), bis(η^5-cyclopentadienyl)-2,2′-bipyridyltitanium(IV) bis(trifluoromethanesulfonate)
3. $[(C_5H_5)_2Ti(phen)]^{2+}[CF_3SO_3]_2$ (III), bis(η^5-cyclopentadienyl)-*o*-phenanthrolinetitanium(IV) bis(trifluoromethanesulfonate)
4. $\{(C_5H_5)_2Ti[o\text{-}S(NHCH_3)C_6H_4]\}^+I^-$ (IV), bis(η^5-cyclopentadienyl)-*N*-methyl-*o*-aminothiophenolato titanium(IV) iodide

Another ionic cyclopentadienyl titanium derivative tested for its antitumor properties was the five-coordinate mono(cyclopentadienyl)titanium(IV) bis(dithiolene) chelate, $[(C_5H_5)Ti(1,2,4\text{-}S_2C_6H_3CH_3)_2]^-[N(C_2H_5)_4]^+$ (V), tetraethylammonium η^5-cyclopentadienylbis(toluene-3,4-dithiolato)titanate(IV). It belongs to type $M^+[(C_5H_5)TiX_4]^-$,

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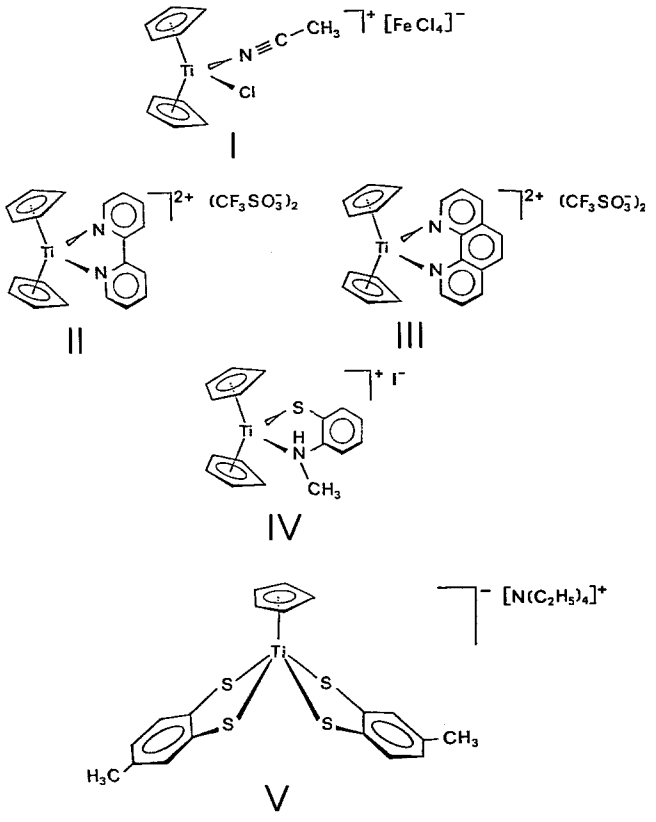

Fig. 1. Structures of titanocene complexes I–V

in which the cyclopentadienyl titanium moiety represents the anionic part of the complex salt.

The structures of compounds I–V, which were prepared by recently described methods [11, 12, 22, 29], are illustrated in Fig. 1. All compounds were characterized by elemental analysis (C, H, N), infrared (IR), ^{1}H-NMR (nuclear magnetic resonance) and mass spectrometry; complexes I–III were additionally analyzed by X-ray structural analysis [29, 30]. No impurities were detectable by these methods.

For antitumor testing, compounds I–V were given at the dose ranges listed in Table 1, the doses rising by increments of 20 mg/kg and being dissolved in pure saline. At higher doses (>200–300 mg/kg), a portion of 10% dimethylsulfoxide (DMSO) was added to the injection solution. The substance concentrations were selected such that each animal received a total volume of 0.4–0.5 ml (0.02 ml/g body weight). The preparations were injected i.p. within 30 min after dissolution.

Antitumor bioassay

Ehrlich ascites tumor. On day 0 of the experiment, female CF1 mice (20–25 g) kept under standard conditions (20°–22° C, Altromin and tap water ad libitum) were inoculated i.p. with 6×10^6 Ehrlich ascites tumor cells. On day 1 (24 h later), the animals received a single injection of the substances dissolved as described above. Each dose group consisted of ten animals. Five additional groups, each consisting of ten tumor-bearing mice, served as control groups. The control animals received 0.5 ml solvent without drug on day 1 of the experiment.

The antitumor activity of complexes I–V against Ehrlich ascites tumor was estimated by determining the length of survival of the animals. Deaths occurring within 7 days after drug injection, corresponding to 8 days after tumor transplantation, were considered to be due to toxicity; those occurring later were defined as deaths due to disease. All animals that died after day 8 showed macroscopic signs of intraperitoneal tumor development. The key date for the evaluation of the experiment as well as the determination of survival was day 120 after tumor transplantation. The survival of controls ranged from 15 to 21 (mean, 17.2 ± 1.7) days. All animals that were still alive on day 120 had no signs of tumor diesease and were considered to be cured.

Solid experimental tumors. The solid tumors B16 melanoma, colon 38 adenocarcinoma, and Lewis lung carcinosarcoma were established as subcutaneously growing tumor lines in male C57BL/6J mice. For the experiments, the tumors were transplanted on day 0 into the right flank of female $B_6D_2F_1$ mice, resulting in the growth of solitary solid tumors. Complex I was given either in three injections on days 1, 3, and 5 or in five injections on days 1, 3, 5, 7, and 9. The doses used are listed in Table 2, each dose group consisting of eight animals. The number of deaths was recorded daily. Deaths occurring within 11 (threefold injections) or 15 days (fivefold injections) after tumor transplantation were defined as deaths due to substance toxicity. In the case of B16 melanoma and Lewis lung carcinoma, the tumors were removed and weighed on day 10 after threefold and on day 15 after fivefold injections, whereas colon 38 carcinomas were always evaluated on day 15 of the experiments. The mean tumor weights and the T/C ratios, the latter being calculated by relating the tumor weights of treated groups to those of the appropriate control groups (expressed as a percentage), were used as parameters for determining the antitumor activity. The values obtained are listed in Table 2.

Table 1. Pharmacologic and toxicologic data of ionic titanocene complexes

Compound	Experimental dose range (mg/kg)	Optimal dose range (mg/kg)	Maximal cure rate (%)	LD_{50} (mg/kg)	LD_{100} (mg/kg)	TI[a]
I	20–300	80–140	80	180	220	1.5
II	20–400	140–220	100	260	300	1.9
III	20–400	140–200	80	240	340	1.5
IV	20–500	200–300	100	380	500	1.9
V	20–200	40	70	60	120	–

[a] Therapeutic index, defined as LD_{50}/ED_{80}. TI values can only be given when the optimal cure rate is $\geq 80\%$

Table 2. Experimental data of the antitumor testing of complex I against various solid experimental tumors

Tumor	Applied doses (mg/kg)	Toxic deaths/ animals treated	Tumor weight[a] (g)	T/C (%)
B 16 melanoma	3×40	–/5	1.63±0.39[c]	92
	3×50	–/5	1.19±0.28[c]	67
	3×60[b]	1/5	0.99±0.30[c]	56
	5×30	–/5	1.18±0.50[d]	52
	5×40[b]	1/5	0.97±0.45[d]	43
Colon 38 carcinoma	3×40	–/5	0.94±0.35[e]	34
	3×50	–/5	0.83±0.29[e]	30
	3×60[b]	–/5	0.88±0.27[e]	32
	5×30	–/5	0.99±0.39[f]	38
	5×40[b]	1/5	0.63±0.21[f]	24
Lewis lung carcinoma	3×40	–/5	0.72±0.32[g]	54
	3×50	–/5	0.46±0.21[g]	34
	3×60[b]	1/5	0.40±0.29[g]	30
	5×30	–/5	1.06±0.39[h]	58
	5×40[b]	–/5	0.43±0.21[h]	24

[a] Data given represent the mean values ± SD for tumor weights on the day of evaluation (cf. *Materials and methods*)
[b] Doses correspond to LD_{10} doses in BDF_1 mice
[c–h] Corresponding values of control groups consisting of 10 animals each:
[c] 1.77±0.30 g; [d] 2.26±0.57 g; [e] 2.30±0.55 g;
[f] 2.61±0.81 g; [g] 1.34±0.50 g; [h] 1.82±0.51 g

Results

All five substances tested were ionic, salt-like compounds, distinguished by good water solubility. They all effected antitumor activity against fluid Ehrlich ascites tumor, the maximal cure rates ranging between 70% and 100%. The data obtained after treatment with complexes I–V are summarized in Table 1 and illustrated in Figs. 2–6. Whereas the chelate complex V, containing a mono(cyclopentadienyl)titanium bis(dithiolene) unit as the anionic complex moiety, induced a maximal cure rate of only 70% at 40 mg/kg and toxic values of 60 (LD_{50}) and 120 (LD_{100}) mg/kg, resulting in a rather narrow therapeutic range (Fig. 6), the acetonitrile complex I and the phenanthroline derivative III caused maximal cure rates of 70%–80% over dose ranges of 80–140 and 140–200 mg/kg (Figs. 2 and 4), respectively, with respective toxic threshold values (LD_{20}) of 160 and 220 mg/kg. For the bipyridyl compound II and the aminothiophenolate derivative IV, the therapeutic ranges were much broader (Figs. 3 and 5) than those of the other three compounds. A maximal cure rate of 100% was attained by complexes II and IV over dose ranges of 200–220 and 240–300 mg/kg, respectively. This means that following the delivery of these doses, all animals survived until the key date without any signs of developing tumor disease and were therefore considered to be cured. The LD_{20} values of complexes II and IV amounted to 240 and 360 mg/kg, respectively, with the corresponding therapeutic index (TI) value, which numerically expresses the width of the therapeutic range, running to 1.9 in both cases.

Pilot experiments with solid experimental tumors, which grew at a site different from the route of administration of the compounds, revealed growth-inhibiting properties for complex I against solid tumors (Table 2) and, thus, confirmed systemic antitumor activity for ionic titanocene complexes. In the case of melanoma B16, multiple injections suppressed tumor growth by 33%–44% and 48%–57% to T/C values ranging between 56%–67% and 43%–52%, respectively, for triple and fivefold doses (Table 2). On the treatment of animals bearing colon 38 carcinoma with complex I according to the regimens listed in Table 2, depressions of tumor growth were observable in clear depen-

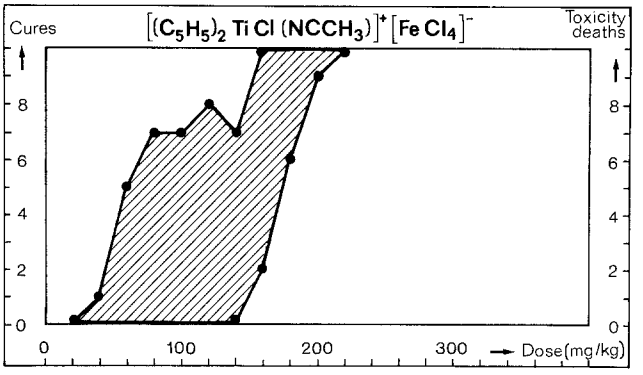

Fig. 2. Dose-activity (*left graph*) and dose-lethality (*right graph*) relationships of complex I against fluid Ehrlich ascites tumor. The *shaded area* indicates the range of surviving, cured animals

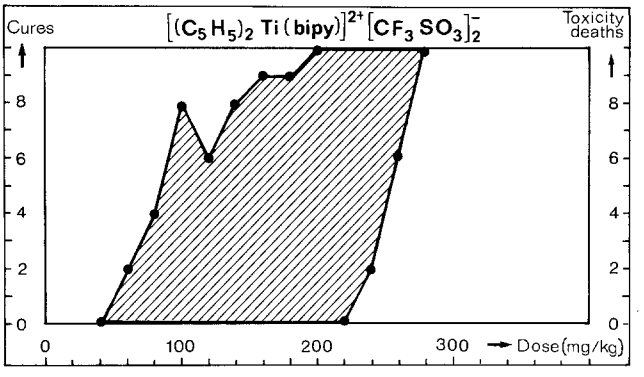

Fig. 3. Dose-activity and dose-lethality relationships of complex II. For further explanations, cf. legend to Fig. 2

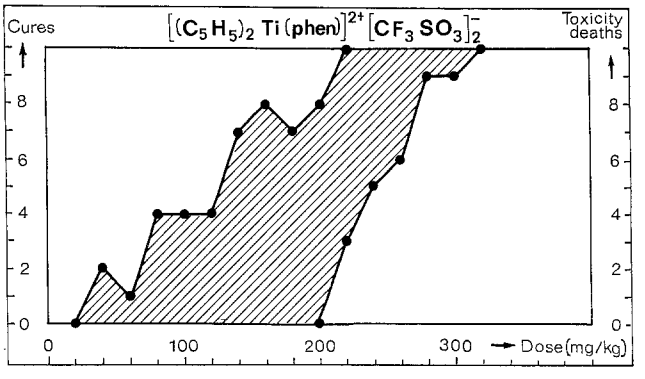

Fig. 4. Dose-activity and dose-lethality relationships of complex III. For further explanations, cf. legend to Fig. 2

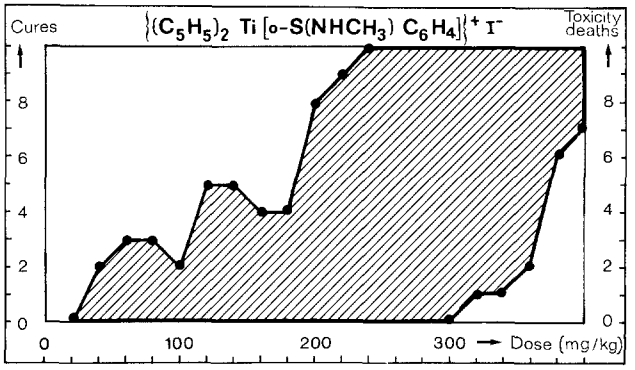

Fig. 5. Dose-activity and dose-lethality relationships of complex IV. For further explanations, cf. legend to Fig. 2

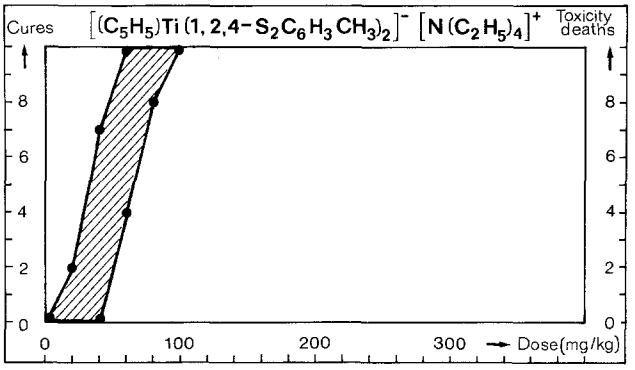

Fig. 6. Dose-activity and dose-lethality relationships of complex V. For further explanations, cf. legend to Fig. 2

dence on the frequency of injection. The growth of colon 38 tumors was inhibited below the 50% limit to T/C ratios of 30% and 24% after triple and fivefold injections, respectively (Table 2). On the administration of the titanocene tetrachloroferrate derivative I to animals bearing the Lewis lung carcinoma, the T/C values attained on the key date amounted to 54%–30% and 58%–24% for threefold and fivefold injections, respectively (Table 2).

Discussion

Electric neutrality has been postulated as an essential prerequisite for the achievement of cytostatic activity with antitumor platinum complexes, most of which are uncharged, neutral compounds [5, 15, 21, 26]. Only a few ionic platinum-metal complexes, mainly represented by the trinuclear ruthenium cation "ruthenium red" [2] and numerous monomeric cationic ruthenium(III) complexes of the type *cis*-$[(NH_3)_4RuCl_2]^+Cl^-$ or $[(en)_2RuC_2O_4][(en)Ru(C_2O_4)_2]$ (en = ethylenediamine) [4], were found to exhibit antitumor properties. Most representatives of the series of non-platinum-group metal antitumor agents [16] are also uncharged compounds, the ligands being coordinatively bound to the metal atoms. This has been reported for bis(η^5-cyclopentadienyl)titanium (IV) ("titanocene") diacido and bis(η^5-cyclopentadienyl)-vanadium(IV) ("vanadocene") diacido complexes [10, 14, 16, 17, 19] as well as for other antitumor transition or main-group metal compounds [6–9, 16, 20, 23, 27, 28].

The main examples of charged, salt-like, non-platinum-group metal compounds with antiproliferative activity are: (a) the relatively simple inorganic salt gallium tri(nitrate), which exhibits antitumor activity against various experimental tumor systems [1] and, in early clinical studies, against Hodgkin's and non-Hodgkin's lymphomas [31]; (b) the gold(I) complex bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride [3] that, in experimental studies, has inhibited the growth of P388 leukemia, M5076 reticulum cell sarcoma, and mammary adenocarcinoma 16/c; and (c) the bis(η^5-cyclopentadienyl)iron(III) ("ferricenium") compounds $[(C_5H_5)_2Fe]^+X^-$, which show cytostatic activity against diverse experimental tumor systems such as Ehrlich ascites tumor, B16 melanoma, and colon 38 and Lewis lung carcinomas [13, 18; Köpf-Maier, unpublished data] as well as some heterotransplanted human tumors (Köpf-Maier, unpublished data). Ferricenium complexes contain the electron-rich medium-transition metal iron and two cyclopentadienyl ring ligands in a parallel arrangement, the central metal atom being coordinatively saturated when bound to two η^5-cyclopentadienyl ligands.

Because of the improved water solubility observed with antitumor ferricenium complexes, some ionic titanocene complexes were synthesized for the present study such that the cationic or anionic cyclopentadienyltitanium(IV) unit, coordinatively saturated by appropriate donor ligands, and the anionic or cationic counterions were linked together by electrostatic forces, forming a salt-like crystal lattice. Similarly to the ferricenium complexes mentioned above, ionic titanocene compounds are characterized by improved water solubility in comparison with that of antitumor bis(η^5-cyclopentadienyl)titanium(IV) diacido complexes [16, 17], the strength of their antitumor activity not necessarily being reduced or lost. As water solubility is an important parameter for the biological use of chemicals, ionic cyclopentadienyl titanium complexes are interesting candidates for further experimental investigations.

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