Antitumor Titanium Compounds

Francesco Caruso*[a] and Miriam Rossi[b]

[a]*Instituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Piazzale Aldo Moro, 5 00185, Rome, Italy*

[b]*Vassar College, Department of Chemistry, Poughkeepsie, NY, 12604-0484, USA*

Abstract: Most anticancer titanium compounds act against tumors in the gastrointestinal tract. Activity towards breast, lung and skin (melanoma) cancers is shown by some as well. Among their appealing properties is that they do not show common side effects of widely used cytostatic agents such as emesis, alopecia or bone marrow impairment. These features make titanium compounds interesting for combined therapy and further study. This review focuses on two drugs that reached clinical trials, namely, titanocene dichloride and budotitane. We try to integrate the biological fate of the related Ti–cyclopentadienyl and Ti–β-diketonato families of drugs, delineating the structure-activity relationship. We also discuss novel related species with increased solubility for improved drug delivery and some potentially useful polynuclear compounds.

Keywords: Titanium, cancer, chemotherapy, cytotoxicity, tumor.

INTRODUCTION

Several metal containing compounds (Ti, Ru, Au, Cu, Ga, Ge, Rh, Sn) have been investigated for antitumor properties [1, 2], after it became clear that the platinum agent, *cis*-diaminodichloroplatinum(II) (**1**), (cisplatin) (Figure (**1**)), was successful in the treatment of some tumors [3, 4]. Designed by following cisplatin models, the octahedral titanium species *cis*-diethoxy-bis(1-phenylbutane-1,3-dionato)titanium(IV), $[(bzac)_2Ti(OEt)_2]$ (budotitane **2**) (see Figure (**2**)), was the first non-Pt metal compound that reached clinical trials [5]. The ligand 1-phenylbutane-1,3-dionato = bzac = benzoylacetonato is an asymmetric β-diketone chelator. It should be noted that *cis*-octahedral Pt(IV) compounds such as iproplatin (**3**) shown in Figure (**3**) also display antitumor activity [6]. Another Ti antitumor agent is titanocene dichloride (**4**), $(Cp)_2TiCl_2$, where Cp = cyclopentadienyl (see Figure (**4**)).

A clue to budotitane's activity comes from examining its structure and noting that cisplatin and budotitane both have 2 labile *cis* groups: chloro and ethoxy, respectively. However, marked differences are also seen between the cytotoxic Pt and Ti mechanisms. For instance, cisplatin hydrolysis of the Pt–Cl bond occurs within the tumor cell [4]. In contrast, Ti–O(ethoxy) cleavage in budotitane is fast and so the hydrolysis happens outside the tumor cell [5]. This agrees with results from phase I clinical studies of titanocene dichloride showing 70-80% Ti found bound to proteins in blood [7, 8].

ANTITUMOR ACTIVITY

Budotitane

The principal targets are gastrointestinal tumors, as shown in pre-clinical studies [5]. Tumors sensitive to budotitane include sarcoma 180 on ascitic tumor-bearing mice (T/C values around 300%; T/C higher than 100% implies increase of survival time) and carcinosarcoma Walker 256 on Sprague Dawley mice (T/C about 200%) [9-11]. In contrast to cisplatin, activity against P338 and L1210 leukemia by budotitane was marginal [12]. Budotitane activity was greater than those of cisplatin and 5-fluorouracil in colon rectal tumors [13]. Liver toxicity was observed. Budotitane clinical phase I was performed on 18 patients refractory to all other known treatments [14]. Minimal acute toxicity was observed and there was cardiac arrhythmia at the highest dose (230 mg/m^2). From these studies the

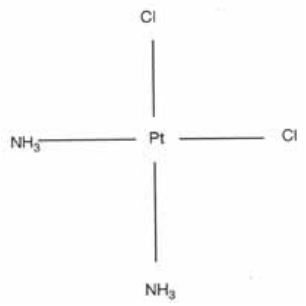

Fig. (1). cisplatin **1**.

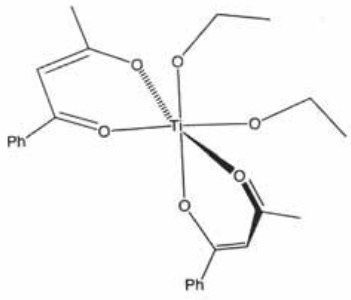

Fig. (2). A budotitane stereoisomer **2**.

*Address correspondence to this author at the Instituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Piazzale Aldo Moro, 5, 00185, Rome, Italy; Fax: (39) 06 49913628; E-mail: francesco.caruso@icb.cnr.it

1389-5575/04 $45.00+.00

recommended dosage was determined to be 180 mg/m^2. Subsequent clinical evaluation has not yet begun because its formulation did not meet modern standards and is being revised [2].

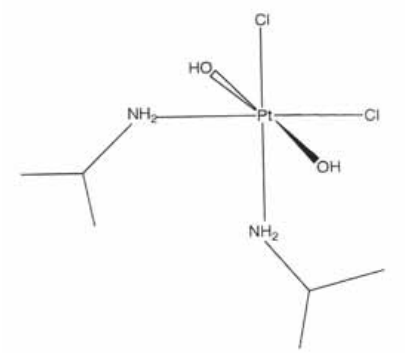

Fig. (3). Iproplatin, a *cis* octahedral antitumor platinum compound **3**.

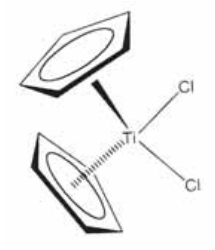

Fig. (4). Titanocene dichloride **4**.

Titanocene Dichloride

Excellent reviews on this compound are available [15, 16]. Initial studies showed an Ehrlich ascites cure of 100% and Colon 38 adenocarcinoma inhibition was better than that of cisplatin [17]. Carcinomas in the gastrointestinal tract, lung and breast tumors were receptive and titanocene dichloride was chosen for development among other metallocenes. Titanocene dichloride pre-clinical tests showed liver and intestinal toxicity but no nephrotoxicity or bone marrow depletion. Transaminases showed up as Ti dose dependent but were reversible after stopping treatment [18].

In one clinical phase I study the maximum dose administered was 560 mg/Kg and subsequently recommended for phase II. Nephrotoxicity was dose limiting. Some patients showed decreased glucose levels, suspected due to involvement of an insulin receptor, and a reversible metallic taste was also felt. Stomach and intestine mucosa damage were observed [7]. Of the 40 patients treated there were 2 minor responses for bladder carcinoma and non-small cell lung cancer.

In another clinical phase I the doses were escalated. The treatment was performed on 20 patients having 83 (range, 2 to 12) cycles on a weekly schedule. The main problems were hepatic toxicity (bilirubinemia) and nephrotoxicity that was partly reversible as the increasing creatinine levels returned to normal in 1 to 10 weeks after cessation of treatment, whereas albuminuria and glucosuria did not return to pretreatment levels. Fatigue was reversible and a metallic taste was observed a few hours after drug infusion. From this study it was concluded that creatinine and bilirubin are dose limiting and the recommended dose for phase II was 140 mg/m^2/wk [8].

Pre-clinical tests on mice did not indicate nephrotoxicity, and a clinical investigation of the drug against renal metastasis (a difficult cancer to treat) was performed [19]. The results were unsuccessful. However, from phase I published trials [7, 8] it was realized that nephrotoxicity was dose limiting. That is, the results obtained from animal studies were misleading because the effect of the drug on humans was clearly different.

Other Potential Drugs

Variations on $(Cp)_2TiCl_2$ by substituting the Cl anions with neutral ligands (CH_3CN, bipyridine or o-phenanthroline) is useful. This generates cationic complexes where the neutral ligands are bound to the metal. Of these, the CH_3CN derivative was the most active species having similar activity to titanocene dichloride for human gastrointestinal and breast carcinomas [20]. Extensive modification of Cp ring and Cl leaving group was performed, see ref [15, 16].

FORMULATION

Budotitane undergoes hydrolysis and evolves towards polynuclear forms, where O(oxo) atoms bridge Ti units; further hydrolysis of Ti–bzac ultimately forms titanium dioxide [5]. These properties have made budotitane development difficult and ethanol is added to the formulation to counterbalance the Ti–OEt bond cleavage [5]. There is poor solubility of budotitane in water, though organic solutions of budotitane hydrolyze easily. Budotitane decomposes in moist acetonitrile in a few seconds due to fast hydrolysis with the formation of polynuclear compounds. Besides ethanol, the formulation also contains Cremophore propylene glycol [21]. This formulation was iv applied in clinical phase I. Formulation is still a limiting feature for budotitane development and new delivery methods are underway [2].

Titanocene dichloride was dispensed in a saline-DMSO (10%) mixture. The drug is very acidic with a pH around 1 due to Cl hydrolysis that liberates H^+ and to prevent tissue damage created by the acid, a malate buffer is used to stabilize the pH around 3. Higher pH induces Ti–Cp hydrolysis, which is detrimental to antitumor activity (variation of these conditions has been recently published for **4** and related titanocenes [22]). After phase I studies no change of formulation was recommended for phase II [7-8].

TI BIOLOGICAL TRANSPORT

The presence of titanium in humans is ubiquitous. Ti content in black pepper, cloves, thyme, lettuce, pork, chicken and margarine is high and only 3% of titanium contained in food is absorbed and excreted in the urine, implying an intestinal absorption barrier due to insolubility of Ti compounds [23]. Ti has replaced lead in paints and inhalation of TiO_2 containing particles contributes to Ti

accumulation in skin, lung, spleen, liver and heart. Pharmacokinetics of titanocene dichloride phase I study showed that Ti elimination from plasma was between 8.6 and 88.3 hours [24].

Titanocene dichloride shows interesting properties regarding the molecular antitumor Ti mechanism. Its interaction with transferrin, a protein associated with iron transport, suggests a possible entry into the tumor cell. A Ti atom occupies one of the two metal binding sites. That is, the protein, with a Ti atom bound to one of its 2 domains [25, 26], would cross the tumor cell wall, which is characterized by greater concentration of transferrin receptors than present in normal cells. Once in the cell Ti can disrupt normal activity by interacting with some yet unidentified target, ATP being suggested. It is interesting that Ti atoms were seen bound to transferrin after being soaked with titanium citrate too [25].

INTERACTION WITH MOLECULES IN THE BODY

1) DNA

Unlike the very well studied mechanism for cisplatin whereby it crosslinks the DNA disturbing replication, interaction of Ti derivatives with DNA is poorly understood. When budotitane was studied for growth inhibition in a cell proliferation assay no metal–DNA cross-link was seen, although dose dependent inhibition was observed; the authors suggested DNA–budotitane intercalation [27]. Supporting this, planar aromatic groups in the β-diketone moiety of budotitane derivatives correlate to antitumor activity [5]. Stable nucleosides adducts were obtained due to budotitane binding to ribose hydroxy groups. Unlike cisplatin no Ti–N interaction was detected. Reaction with DNA was faster for budotitane than for cisplatin [28].

Ti–DNA interaction in metallocenes has been recently described [16]. Titanium was first detected in the nuclear chromatin of sensitive human carcinoma cells 12 hours after application of $(Cp)_2TiCl_2$ and its location was near rich phosphorus areas, suggesting Ti–DNA interaction [29]. Sadler and co-workers, however, propose ATP as a potential target for Ti [25]. A unique mechanism of action for metallocenes *in vivo* describes a non-specific electrostatic interaction between the metal and DNA after Ti enters the cell [16]. A related study shows that $(Cp)_2TiCl_2$ overcomes Pt resistance in CH1cisR and 2780CP cell lines. In the latter, Ti–DNA adducts were detected and, in agreement with this finding, p53 accumulated rapidly in these cells [30]. The existence and pH dependence of DNA-$(Cp)_2TiCl_2$ adducts was determined with inductively coupled plasma spectroscopy [31].

2) Proteins

We already mentioned that transferrin seems to play an important role in titanocene dichloride antitumor activity. The fact that about 70% of titanocene dichloride is protein-bound after injecting the drug clearly shows a Ti-protein affinity. Ti interaction with proteins during tumor suppression has been suggested [32] after identification of carboxyl groups from two amino acids (Asp) that bind metal ions at the active site of telomerases [33]. The chloro atoms of titanocene dichloride can be replaced by amino acids but these derivatives are not stable as the amino acids hydrolyze easily in water [34]. This suggests that specific conditions are needed for establishing a stable Ti-protein species. A lipophilic environment created by Cp favoring interaction of titanocene dichloride with hydrophobic areas of molecules has been suggested [35]. Recent studies describe the interaction of titanocene dichloride with topoisomerase II resulting in inhibition of its unwinding. This implies that inhibition of topoisomerase II is possible if a $Ti(Cp)_2$ moiety enters the cell [36].

DEVELOPMENT OF NEW DRUGS

Assuming that budotitane and titanocene dichloride both use transferrin as the carrier into the cell, we should expect some similarities in behavior. Already mentioned was a fast hydrolysis rate for their leaving groups, Cl in titanocene dichloride [37] and ethoxy in budotitane [5], and a much slower cleavage of the Ti–Cp [37] and Ti–bzac [5] bonds, respectively. These features should prove useful in developing novel Ti drugs.

Since β-diketone asymmetry is an essential feature for high activity in budotitane and related compounds [5], we are studying Ti derivatives closely related to budotitane but having 4-acyl-5-pyrazolones (**5**), see Figure (**5**), as ligands. These are asymmetric β-diketones having 3 possible substitution options. That is, any substituent (R^1, R^3 and R^4) provides asymmetry because the fused pyrazole ring is intrinsically asymmetric. In contrast, classical β-diketones used in budotitane studies need 2 different substituents at unique positions for this purpose, specifically those of Me and Ph groups in budotitane.

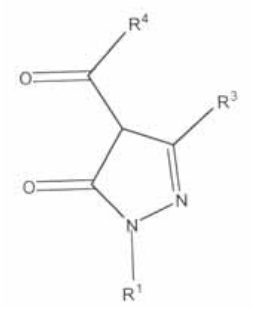

Fig. (5). 4-acyl-5-pyrazolone diketo form, HQ **5**.

A molecule showing *in vivo* antitumor activity (T/C about 300%) against TA-3 (mouse mammary adenocarcinoma) in AJ mice of the same order found for budotitane activity against other tumors [5] was synthesized by our group. It is a bis(4-acyl-5-pyrazolonato)titanium(IV) tetranuclear cyclic species, $[(Q^B)Ti(\mu\text{-}O)]_4$ (**6**); Q^B has R^1 = R^4 = Ph, R^3 = Me, see Figure (**6**) [38]. This tumor model was chosen for drug development after *in vitro* studies on TA-3, HEP-2 (human epithelial larynx carcinoma) and VERO (African green monkey kidney); the latter is a benign tumor that was insensitive to **6**. Activity in HEP-2 tumor (T/C about 200%) was less than in TA-3. Compound **6** is a stable intermediate in the Ti-monomer hydrolysis/polymerization pathway that interfered with budotitane studies.

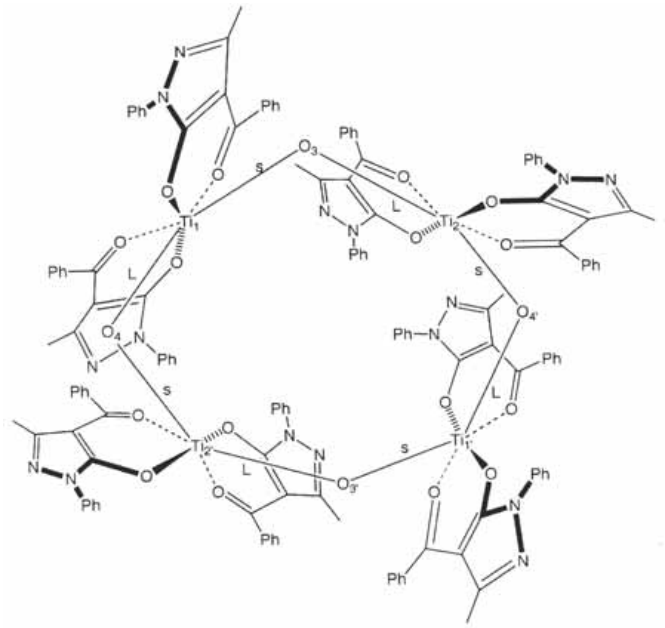

Fig. (6). Tetranuclear 4-acyl-5-pyrazolonato-Ti species $[(Q^B{}_2)Ti(\mu\text{-}O)]_4$, ($Q^B$: $R^1 = R^4$ = Ph, R^3 = Me), **6**. Dashed bonds indicate weak Ti-O bonds; L and (s) indicate long (short) bonds in the 8-membered ring.

Budotitane has low water solubility due to fast hydrolysis of Ti–O(ethoxy) bonds and subsequent polymerization also leading to Ti–bzac cleavage. Although compound **6** is stable to further polymerization, it is less water-soluble than budotitane, as expected for a larger molecule. To solve this problem, compound **6** was encapsulated as a liposome (dipalmitoylphosphatidylcholine-DPPC) adduct [38]. Besides the effective antitumor activity shown by **6**-DPPC, there was an excellent response of healthy mice treated with the same adduct and IC_{50} could not be obtained in the range 10^{-4}—10^{-2} M (in Ti-complex) because all mice survived the treatment; the behavior of these mice was absolutely normal. Another promising feature shown by compound **6** is tumor weight decrease, observed for CF-1 treated mice [38]. In the body, compound **6** generates a Ti distribution [39] as that observed for titanocene dichloride, mainly in the liver followed by intestinal tissue [40].

Since ligand asymmetry is an unexplained relevant parameter in budotitane studies the X-ray crystal structure of **6** in the solid is of interest [38]. The molecular structure of a related tetranuclear compound, cyclo-tetrakis[μ-oxo-bis(2,2,6,6-tetramethylheptane-3,5-dionato)titanium(IV)], $[(tmhd)_2Ti(\mu\text{-}O)]_4$ (**7**), containing the symmetric β-diketonato ligand tmhd, Figure (**7**), shows 3 sets of Ti-O bond distances [41]. Set (a) describes the shortest bonds that are located in the 8-membered ring (Ti–μ-O bonds), with values about 1.81 Å. Set (b) includes the longest bonds (about 2.12 Å) that are *trans* to the Ti–O bonds forming set (a). Set (c) shows the intermediate Ti bonds that are *trans* to each other (about 1.97 Å); sets (b) and (c) are formed by Ti-O(tmhd) bonds. The driving force responsible for these relevant geometrical features is described by the *trans* influence, a feature some metals bestow upon their coordination complexes.

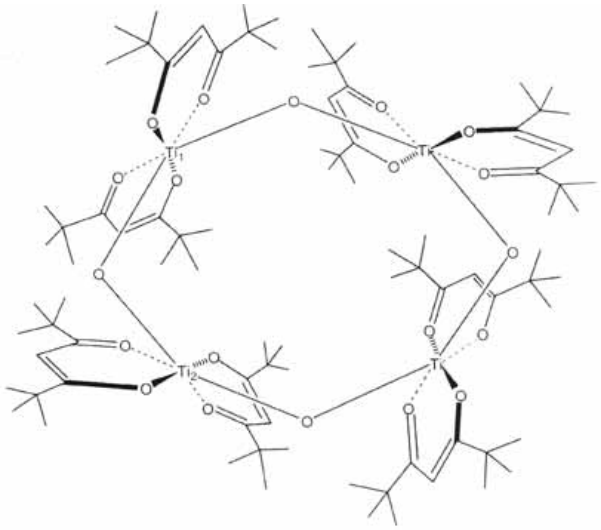

Fig. (7). Tetranuclear diketonato Ti compound **7** containing a symmetric ligand and equivalent Ti-O bond lengths in the 8-membered ring.

Compound **6** shows further splitting of sets (a) and (b) and there is an alternate sequence of short-long lengths in the Ti–μ-O bonds (Ti_1–O_3 = 1.767(6) Å, O_3–Ti_2 = 1.859(6) Å, Ti_2–$O_{4'}$ = 1.758(5) Å, $O_{4'}$–$Ti_{1'}$ = 1.868(4) Å), indicated as s (for short) or L (for long) in Figure (**6**). *Trans* to each Ti–μ-O bond a Ti–O(acyl) bond is found; these are also split. Since the O(pyrazolonato) is *trans* to another O(pyrazolonato) no *trans* influence is present and these Ti–O(pyrazolonato) bonds do not split; their lengths are similar to those of set (b) above. Marked ligand asymmetry is seen in structures of Q organotin derivatives [42] (ligands Q are defined in Figure **5**) as Sn–O(acyl) bond distances are longer than Sn-O(pyrazolonato) ones, in agreement with sets (b) and (c) of compound **6**.

The 8-membered ring common to **6** and **7** is further affected by ligand asymmetry, as the latter is planar whereas the former is not. In addition, **7** has a strained ring conformation with Ti–O–Ti bond angles about 170°, very far from a normal bond angle centered on oxygen. Instead, the asymmetric ligand in **6** induces a Ti–O–Ti bond angle of about 150°, creating a more relaxed ring. It appears that an asymmetric chelating agent defines a more stable polynuclear conformation.

It could not be ascertained whether there is asymmetry in the Ti–bzac binding because no useful crystals were obtained for budotitane. However, from the structures of other metal–bzac compounds, examples of different M–O(Ph) and M–O(Me) bond lengths are known, although this difference is not as marked as for 4-acyl-5-pyrazolonato metal species [43]. Based on the fact that asymmetric ligands provide higher activity, the geometrical features observed above should be considered in designing novel antitumor octahedral Ti drugs.

The tendency of Ti to polymerize, frequently through O(oxo) bridges, raises the question as to whether a polynuclear form is an entity needed to induce antitumor activity. So far, studies on the dinuclear species $[(Cp)_2TiCl]_2(\mu\text{-}O)$ (**8**), derived from hydrolysis of titanocene

dichloride [44], show activity although less than for titanocene dichloride [45], see Figure (**8**). Also, the dinuclear compound $[(bzac)_2TiCl]_2(\mu\text{-}O)$ (**9**), closely related to budotitane, has antitumor activity, Figure (**9**) [5].

Fig. (8). $[(Cp)_2TiCl]_2(\mu\text{-}O)$ **8**, Ti-O-Ti bond angle = 174º.

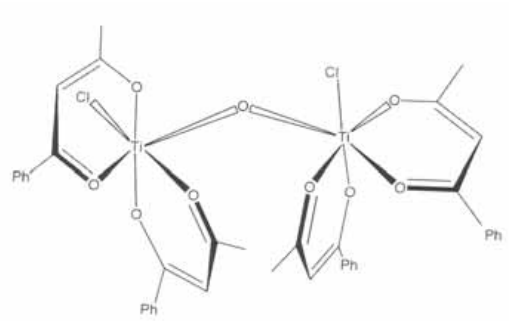

Fig. (9). $[(bzac)_2TiCl]_2(\mu\text{-}O)$ **9**.

However, there are many examples of polynuclear Ti-Cp compounds not tested, probably because their poor water solubility impedes their entry into cells. We mention some of them, outline their large structural variety and indicate the parent antitumor compound they stem from, if applicable. Dinuclear compounds include, $\{[(Cp)_2Ti(H_2O)]_2(\mu\text{-}O)\}^{2+}$ (**10**), obtained from titanocene dichloride [46] and $[(Cp)TiCl_2]_2(\mu\text{-}O)$ (**11**) [47] obtained from $(Cp)TiCl_3$.

Some trinuclear complexes include $[(Cp)_3Ti_3(\mu_3\text{-}O)(\mu\text{-}OH)_3(\mu\text{-}HCOO)_3]^+$ (**12**), a cationic cyclic species obtained after hydrolysis of titanocene dichloride and addition of formic acid [48]; the cationic cyclic species $[(Cp)_3Ti_3(\mu_3\text{-}O)(\mu\text{-}OMe)_3(OMe)_3]^+$ (**13**), [49]; $[(Cp)_2TiCl(\mu\text{-}O)]_2(Cp)TiCl]^{3+}$ (**14**), a non-cyclic species obtained from titanocene dichloride hydrolysis [50] and the neutral species $[(Me_5\text{-}Cp)_2TiCl(\mu\text{-}O)]_3$ (**15**) [51]. They are obtained after Ti-Cp cleavage, an important property in titanocene dichloride mechanism of action.

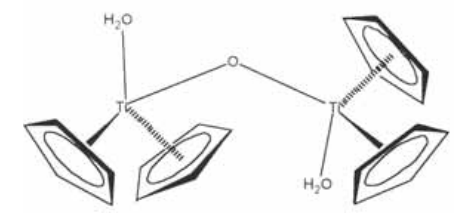

Fig. (10). $\{[(Cp)_2Ti(H_2O)]_2(\mu\text{-}O)\}^{2+}$ **10**, Ti-O-Ti bond angle = 177º.

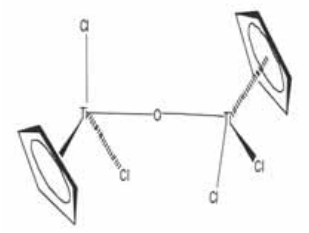

Fig. (11). $[(Cp)TiCl_2]_2(\mu\text{-}O)$ **11**, Ti-O-Ti bond = 180º.

Some tetranuclear compounds are $[Br(CH_2)_2\text{-}Cp)TiBr(\mu\text{-}O)]_4$ (**16**) [52] and $[(Me_5\text{-}Cp)Ti(\mu\text{-}O)Br]_4$ (**17**) [53]. A tetranuclear species with additional O(oxo) bridges is $[(Me_5\text{-}Cp)_4Ti_4(\mu\text{-}O)_5Cl_2]$ (**18**) [54], whereas $[(MePh_4\text{-}Cp)_4Ti_4(\mu\text{-}O)_6]$ (**19**) shows an additional stage of Cl hydrolysis [55]. Even more complex structures are cluster compounds, such as $[(Cp)_6Ti_6(\mu_3\text{-}O)_6(\mu_3\text{-}Cl)_2]$ (**20**), shown in Figure (**20**) [56]. A cage example is $[(Cp)_8Ti_8(\mu\text{-}O)_{12}]$ (**21**) [57]. Some could aid in clarifying the mechanism of Ti antitumor action if appropriate formulations become available.

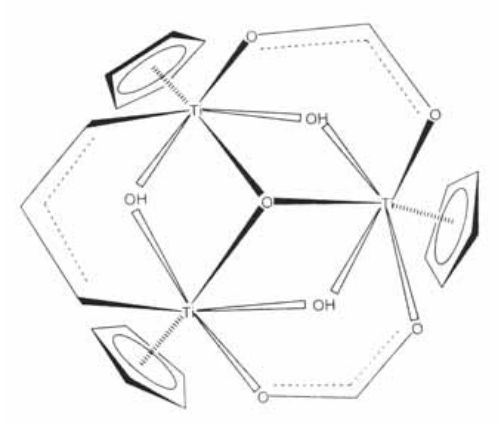

Fig. (12). $[(Cp)_3Ti_3(\mu_3\text{-}O)(\mu\text{-}OH)_3(\mu\text{-}HCOO)_3]^+$ **12**.

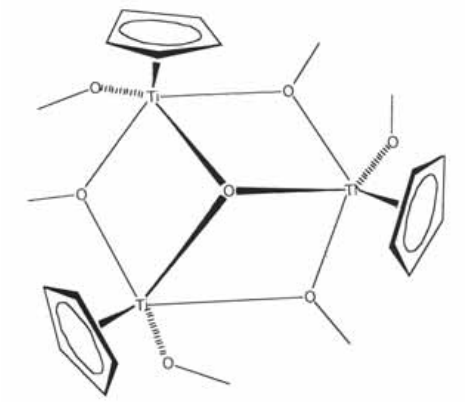

Fig. (13). $[(Cp)_3Ti_3(\mu_3\text{-}O)(\mu\text{-}OMe)_3(OMe)_3]^+$ **13**.

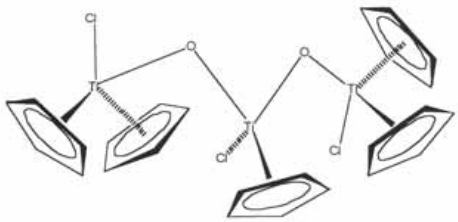

Fig. (14). $[(Cp)_2TiCl(\mu\text{-}O)]_2(Cp)TiCl]^{3+}$ **14**, Ti-O-Ti bond angles = 162º and 176º.

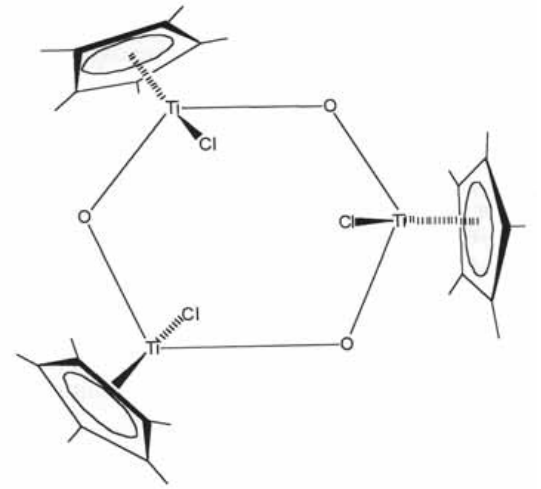

Fig. (15). $[(Me_5\text{-}Cp)_2TiCl(\mu\text{-}O)]_3$ **15**, Ti-O-Ti bond angles = 131º-135º.

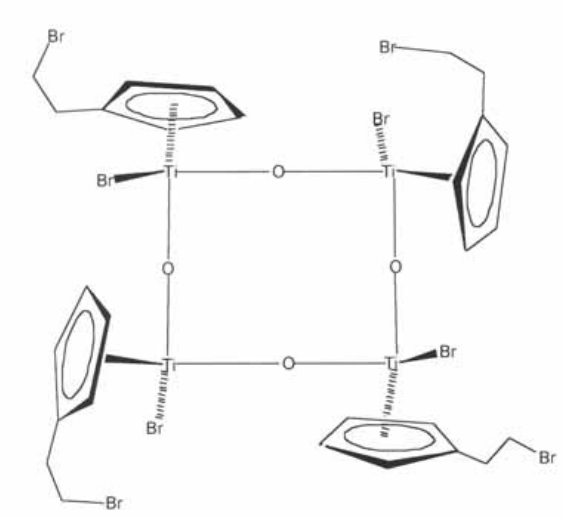

Fig. (16). $[Br(CH_2)_2\text{-}Cp)TiBr(\mu\text{-}O)]_4$ **16**, Ti-O-Ti bond angles = 155º and 162º.

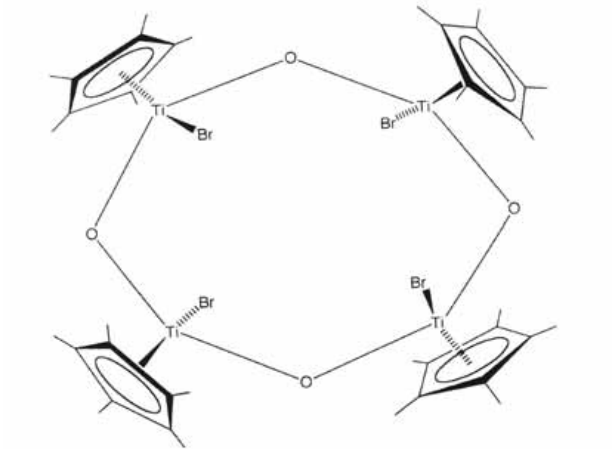

Fig. (17). $[(Me_5\text{-}Cp)Ti(\mu\text{-}O)Br]_4$ **17**, Ti-O-Ti bond angles = 161º-164º.

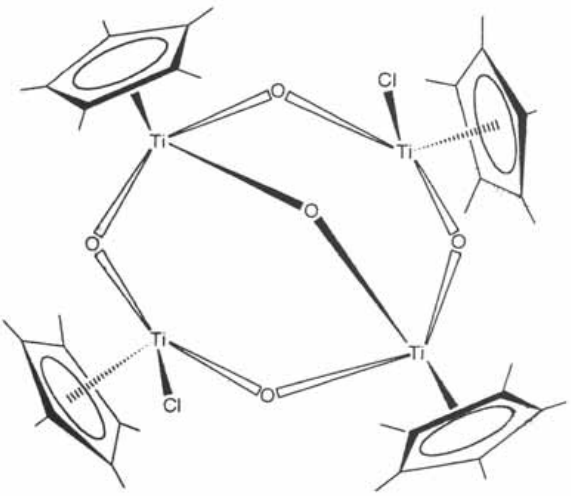

Fig. (18). $[(Me_5\text{-}Cp)_4Ti_4(\mu\text{-}O)_5Cl_2]$ **18**, Ti-O-Ti bond angles = 122º-128º.

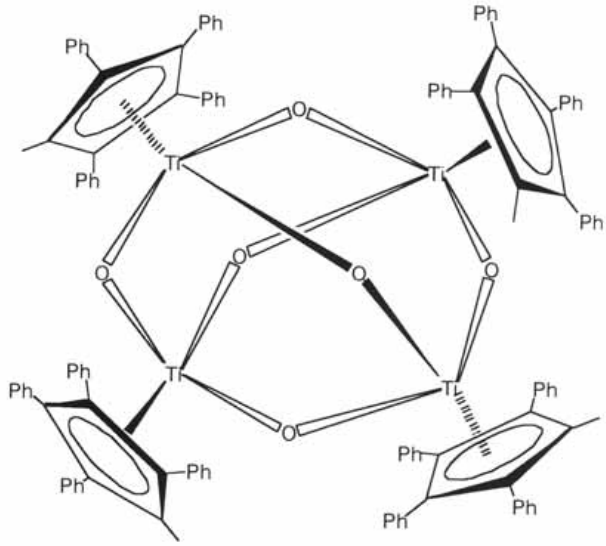

Fig. (19). $[(MePh_4\text{-}Cp)_4Ti_4(\mu\text{-}O)_6]$ **19**, Ti-O-Ti bond angles = 96º-124º.

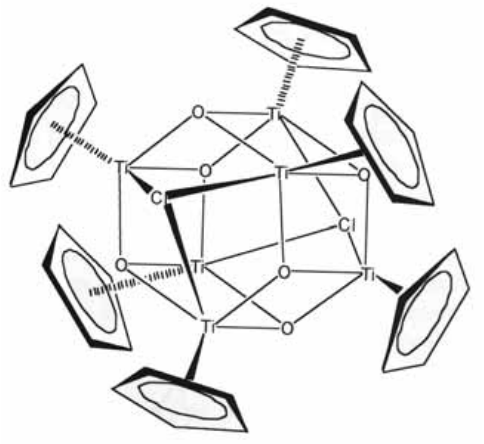

Fig. (20). $[(Cp)_6Ti_6(\mu_3\text{-}O)_6(\mu_3\text{-}Cl)_2]$, **20**.

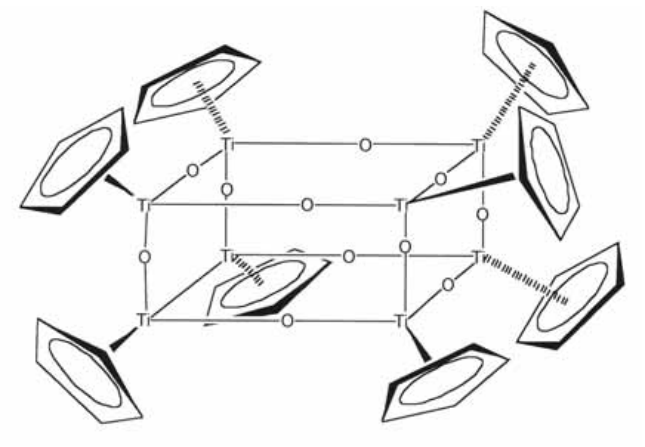

Fig. (21). $[(Cp)_8Ti_8(\mu\text{-}O)_{12}]$ **21**. Ti-O-Ti bond angles = 152º-161º.

Figures **16-19** show tetranuclear Ti–Cp derivatives closely related to those of β-diketone ligands in Figures (**6**) and (**7**). The evolution of Ti–Cp towards TiO_2 can be seen following the sequence of figures 4, 8, 10, 14, 15, 17, 18, 19, that is, hydrolysis of Cl, formation of Ti–O–Ti bridges and hydrolysis of Cp. The same trend is seen for Ti-β-diketone compounds in the order 2, 9, 6, 7. These parallel trends suggest that titanocene dichloride and budotitane share similar biological pathways.

To gather more information on the Ti-Q system we synthesized other polymeric Ti-Q_2 derivatives using Q ligands with modified substituents, R^1, R^3 and R^4; the ligands used are shown in Scheme 1 [58].

4-acyl-5-pyrazolone proligand	R^1	R^3	R^4
Q^BH	Ph	Me	Ph
Q^TH	Ph	Me	CH_2Bu^t
Q^AH	Ph	Me	Ph-4-OMe
Q^NH	Ph	Me	Ph-4-NO
Q^CH	Ph	Me	Cy
Q^LH	Ph	Me	CH_2Ph
Q^EH	Ph	Me	$(CH_2)_5Me$
Q^PH	Ph	Ph	Ph

Scheme 1. 4-Acyl-5-pyrazolone proligands reacted

The formation of polynuclear compounds could be indicated as:

$$n\ TiCl_4 + 2n\ HQ + n\ H_2O \rightarrow [(Q_2)Ti\text{-}\mu\text{-}O]_n + 4n\ HCl \quad (\mathbf{R1})$$

or

$$n\ Ti(OR)_4 + 2n\ HQ + n\ H_2O \rightarrow [(Q_2)Ti\text{-}\mu\text{-}O]_n + 4n\ ROH \quad (\mathbf{R2})$$

Generally, however, we obtain compounds including OH and/or H_2O as well; $[(Q^T)_2Ti(O)(H_2O)]_n$ (**22**), $[(Q^C)_2Ti(O)(H_2O)_2]_n$ (**23**), $(Q^B)_2Ti(OPr^n)_2$ (**24**), $(Q^T)_2Ti(OMe)_2$ (**25**), $(Q^T)_2Ti(OPr^n)_2$ (**26**) and $(Q^T)_2Ti(OBu^n)_2(H_2O)$ (**27**). Compound $[(Q^P)_2Ti(O)(H_2O)]_n$ (**28**), containing a Ph substituent in position R^3, is equivalent to **22** (R^3 = Me). Some alternative structural schemes are also possible, for instance **22** may also be indicated as $[(Q^T)_2Ti(OH)_2]_n$ (see Figure (**22**)).

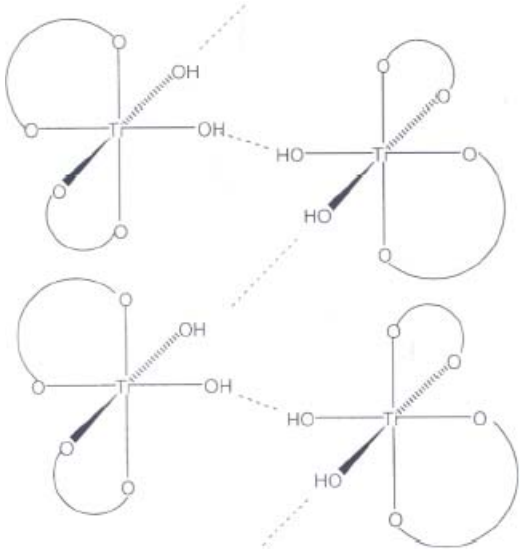

Fig. (22). A possible structural scheme for an open form of $[(Q_2)Ti(OH)_2]_n$ **22**. Oligomers may form if tail and head of the chain interact.

Molecular weight determinations carried out in Cl_3CH show some polynuclear compounds with n as fractional numbers. For instance, compound $[(Q^N)_2Ti(O)(H_2O)]_n$, **(29)** has n = 2.8, suggesting a mixture containing di- and other poly-nuclear species. In a related study of budotitane after dissolution in CH_3CN/H_2O, there is formation of [(bzac)$_2$Ti-

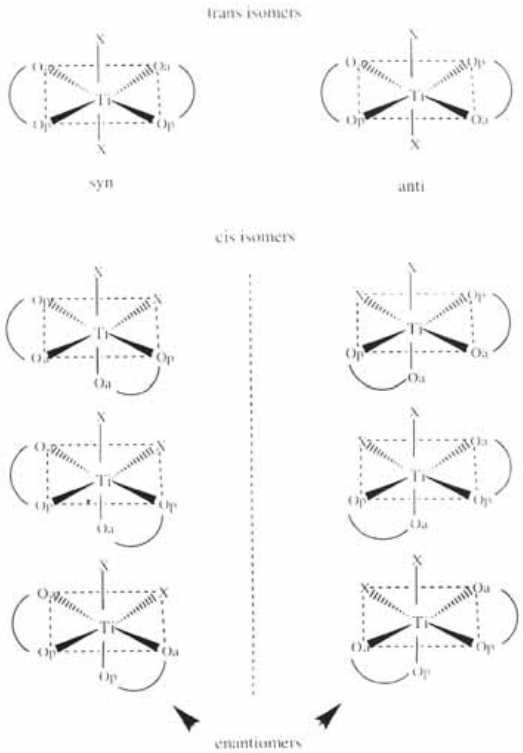

Fig. (23). Q_2TiX_2 possible stereoisomers.

μ-O]$_n$ with n = 2.4, that was interpreted as a mixture of di- and tri-nuclear compounds [5].

Octahedral 6-coordinate titanium derivatives can theoretically exist in solution as a mixture of several *cis* and *trans* isomers, see Figure (**23**). However, for related dialkoxy- and dihalo- bis(acetylacetonato)titanium(IV) derivatives, it was shown that steric interactions influence the relative stability of the isomers and *cis* forms are strongly preferred [59, 60]. From budotitane studies, only iodo derivatives form *trans* conformers [5].

The low temperature ^{1}H NMR spectrum of budotitane exhibits four signals assigned to the different methyl groups due to the three *cis* isomers. We have performed a ^{1}H NMR variable temperature experiment on compounds **24** and **26** and observed, also at 273 K, only one signal for each equivalent proton of the propoxy groups and two signals having the same area for the methyl group of Q^B and Q^T. These can be assigned to the equatorial and axial methyl groups of the *cis-cis-cis* isomer.

It is worth noting that 20 min after dissolution of **24** and **26** in Cl_3CH formation of Pr^nOH was observed, that is hydrolysis of the Ti-OPr^n bond occurred. Hydrolysis is fast at high temperature and is complete 48 hours after dissolution. The hydrolysis process is reversible as addition of Pr^iOH or Pr^nOH to the NMR solutions showed signals due to Ti(OPr^i)- and Ti(OPr^n)- containing species. Hydrolysis was effected 10 min after dissolution for derivative **25**.

The reverse hydrolysis is related to that obtained when EtOH is added to polynuclear species stemming from previous budotitane hydrolysis [5]. We describe a stage of hydrolysis of bis-β-diketonato titanium species as studied by theoretical methods. To simplify calculations we selected $(acac)_2Ti(OH)(OCH_3)$ as the reagent, acac = acetylacetonato.

$(acac)_2Ti(OH)(OCH_3) + H_2O$ ———> $(acac)_2Ti(OH)_2 + CH_3OH$ (**R3**)

The geometry of the molecules involved was calculated with Density Functional Theory methods. Interestingly, the energy associated with the products is slightly higher than that of the reagents. Nonetheless, this feature is in agreement with the reverse hydrolysis of Ti-OEt and Ti-OPr bonds mentioned above. Therefore, the reversible hydrolysis, confirmed theoretically, is likely determined by environmental conditions such as acidity and reagent/product concentration. This may be related to the fast reaction of titanocene dichloride with proteins in the body [61].

Accurate experimental control of the hydrolysis reaction allowed us also to isolate a mononuclear species Q_2TiCl_2 for Q^B and Q^T ligands.

$TiCl_4.2THF + 2HQ + 2NaOMe$ ———> $Q_2TiCl_2 + 2NaCl + 2MeOH + 2THF$ (**R4**)

Thus, reaction in basic (KOH) alcohol solution yields polynuclear products whereas replacing KOH with NaOMe, under anhydrous conditions, induces formation of the corresponding mononuclear compounds.

We also synthesized derivatives with Ti/Q ratio 1:1, namely, $[(Q^A)Ti(OH)_3(H_2O)]_n$ (**30**), $(Q^C)Ti(OH)_3$ (**31**), $[(Q^B)Ti(OH)_2(OPr^n)](H_2O)$ (**32**), $[(Q^B)Ti(OH)_2(OBu^n)](H_2O)$ (**33**) and $[(Q^L)Ti(O)(OH)]_n$ (**34**) (see Figures (**24**) and (**25**)) [58].

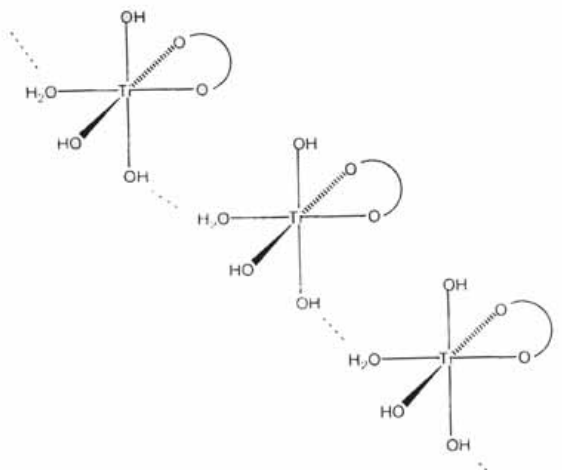

Fig. (24). A possible structural scheme of $[QTi(OH)_3(H_2O)]_n$ **30**.

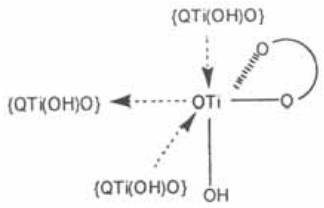

Fig. (25). A possible structural scheme for the monomer unit of **34**, $[QTiO(OH)]_n$. The Ti atom posses a small number of ligands and binding contributions from other Ti units determine a non-linear polymer.

These are water soluble, useful for drug formulation and do not disproportionate, as do equivalent compounds containing symmetrical classical β-diketones as acetylacetonato.

2 $(acac)Ti(OR)_3$ → $(acac)_2Ti(OR)_2 + Ti(OR)_4$ (**R5**)

Further rearrangement stabilizes $[(acac)Ti(OR)_3]_2$ (**35**), which contains alkoxy RO bridges (Figure (**26**)) [62]. Such features are not shown by Ti-Q (1:1) compounds, which are instead stable.

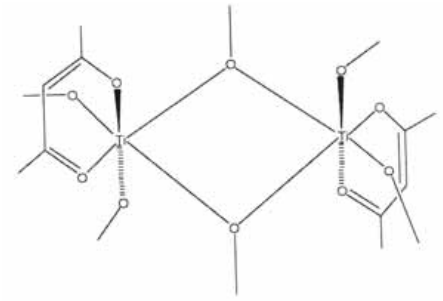

Fig. (26). $[(acac)Ti(OR)_3]_2$ obtained from an unstable 1:1 Ti/acac species.

As already mentioned, a first step of hydrolysis is seen in budotitane and titanocene dichloride, with cleavage of Ti–(OEt) ($T_{1/2}$ = 20 sec in moist acetonitrile [5]) and Ti–Cl,

(rapid reversible 1st Cl^- and $T_{1/2}$ = 50 min for 2nd Cl^- [37]), respectively. The slow hydrolysis of benzoylacetonato (2.5 h) [5] and Cp ($T_{1/2}$ = 57 h [37]) provides sufficient stability to allow both Ti species to react with proteins before formation of titanium dioxide.

In contrast with budotitane and titanocene dichloride, our studies do not show any Ti–Q cleavage. This specific feature is a novel contribution to this area because our Q ligands, by avoiding promotion to TiO_2 and enforcing the effective asymmetry structure-activity relationship mentioned earlier, provide more stability to Ti species than classical β-diketones. A related property is that as Ti–Cp bond strength increases (using Me–Cp rings) the antitumor activity decreases suggesting that lability of Ti-Cp may be critical [63]. Furthermore, the antitumor activity of the Ti–Cp (1:1) species, $(Cp)TiCl_3$, was also observed, though it was lower than for $(Cp)_2TiCl_2$ [45]. Therefore our scheduled biological tests will clarify whether our Ti–Q (1:1) derivatives having marked stability will improve antitumor action.

Table 1. Selected Structural Parameters of Bis(4-acyl-5-Pyrazolon-5-Ato)Titanium Conformers, Q_2TiY_2, Obtained with DFT Methods, and Related Polynuclear Ti-β-Diketonato Compounds, Obtained with Diffraction Methods

Conformer	Ti-Y3 [b]	Ti-Y4 [b]	Ti-O1	Ti-O2 [c]	Ti-O51	Ti-O52 [c]	Y-Ti-Y [b]	Ti ligands
energy [a]			1st Chelate		2nd Chelate			
CIS1	2.235 Y = Cl	2.233 Y = Cl	1.952	2.038	1.966	2.027	102.6 Y = Cl	Q^T Cl
–3512.92248								
CIS2	2.233 Y = Cl	2.236 Y = Cl	2.021	1.968	1.955	2.048	102.3 Y = Cl	Q^T Cl
–3512,91900								
CIS3	2.234 Y = Cl	2.237 Y = Cl	2.041	1.950	2.047	1.957	98.8 Y = Cl	Q^T Cl
–3512.91935								
CIS3	1.797 Y = O	1.801 Y = O	2.085	2.012	2.042	2.010	95.0 Y = O	Q^B CH_3O
–2892.38076								
CIS3	1.801 Y = O	1.816 Y = O	2.084	1.994	2.046	1.992	95.1 Y = O	Q^T CH_3O
–2823.92115								
Polynuclear Comps.	Ti-O3	Ti-O4	Ti-O1	Ti-O2	Ti-O51	Ti-O52	O-Ti-O[d]	Ti-O-Ti[e]
			1st Chelate		2nd Chelate			
$[(Q^B)_2Ti(\mu\text{-}O)]_4$ [f]	1.767	1.868	1.979	2.146	1.980	2.056	99.2	150.5 153.8
2nd Ti unit	1.758	1.859	1.983	2.163	1.978	2.076	100.2	150.5 153.8
$[L_2Ti(\mu\text{-}O)]_4$ [g]	1.804	1.818	1.969	2.116	1.969	2.117	100.5	170.3 169.3
2nd Ti unit	1.810	1.810	1.968	2.120	1.968	2.120	100.3	170.3 169.3
3rd unit	1.793	1.793	1.969	2.132	1.969	2.132	99.4	170.3 169.3
$[(L')_2Ti(\mu\text{-}O)]_2$ [h]	1.831	1.824	1.968	2.059	1.974	2.059	83.4	97.1 97.1

[a] Energy units (Hartree) for geometrically converged conformers; 1 Hartree = 627.50959 Kcal/mol.

[b] Y3 and Y4 are: O associated to methoxy groups in $(Q^T)_2Ti(OCH_3)_2$ and $(Q^B)_2Ti(OCH_3)_2$; Cl in $(Q^T)_2TiCl_2$; Q^B = 1-phenyl-3-methyl-4-benzoyl-pyrazolon-5-ato, Q^T = 1-phenyl-3-methyl-4-neo-pentyl-carbonyl-pyrazolon-5-ato. In the corresponding columns for the polynuclear compounds Ti-O(oxo) bonds are shown.

[c] O1 and O2 belong to the 1st β-diketonato chelate; O51 and O52 belong to the 2nd β-diketonato chelate.

[d] This parameter is O3-Ti-O4.

[e] These parameters are Ti-O3-Ti and Ti-O4-Ti.

[f] $[(Q^B)_2Ti(\mu\text{-}O)]_4$ has an inversion center that makes two Ti units equivalent to the other two [38]. Its conformation is similar to CIS1.

[g] $[L_2Ti(\mu\text{-}O)]_4$ has a 2-fold axis passing on two Ti atoms; L = 2,2,6,6-tetramethylheptane-3,5-dionato = tmhd [41].

[h] The 2nd Ti unit is crystallographically related by an inversion center; L' = acetylacetonato [60].

As we were not able to obtain crystals of the monomers useful for X-ray diffraction studies, we calculated minimum energy geometry using theoretical methods (Density Functional Theory, DFT). We name the three stereoisomer conformers CIS1, CIS2 and CIS3. For $(Q^T)_2TiCl_2$ the three possible *cis* conformers have equivalent calculated energies, see Table 1 and Figures (**27**) – (**29**). This agrees with budotitane isomer interconversion as shown by NMR [64].

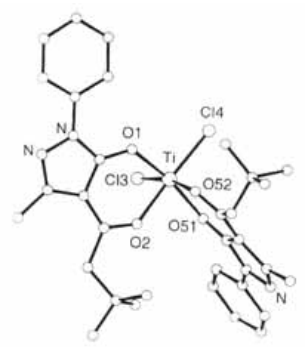

Fig. (27). DFT structure of the $(Q^T)_2TiCl_2$ conformer CIS1.

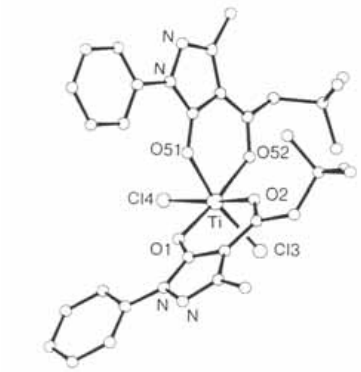

Fig. (28). DFT structure of the $(Q^T)_2TiCl_2$ conformer CIS2.

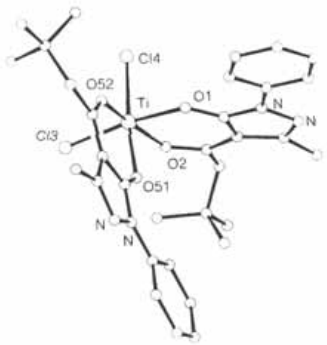

Fig. (29). DFT structure of the $(Q^T)_2TiCl_2$ conformer CIS3.

Coplanarity between the pyrazole and its attached phenyl due to extended intraligand conjugation is seen in CIS1, CIS2 and CIS3 and this feature agrees with experimental X-ray structures of Sn–Q derivatives [42]. This may be related to Ph–bzac coplanarity found relevant for antitumor activity in budotitane studies [5] suggesting possible intercalation with DNA.

Replacing Cl by methoxy creates an energy gap between conformers of $(Q^T)_2Ti(OMe)_2$ in which the CIS1 isomer has 56 Kcal/mol more than CIS3, whereas CIS2 has 0.7 Kcal/mol more than CIS3.

A less bulky group on R^4, such as Ph instead of neo-pentyl, makes the 3 conformers energetically more similar. Thus, CIS1 has the lowest energy followed by CIS2 (0.3 Kcal/mol) and CIS3 (2.8 Kcal/mol).

In both, $(Q^B)_2Ti(OMe)_2$ and $(Q^T)_2Ti(OMe)_2$ structures, the O(methoxy) atoms form the shortest bonds while their opposite bonds are weakened. For $(Q^T)_2Ti(OMe)_2$ these data are the pair of opposite bonds: Ti–O3 = 1.801 Å, Ti–O1 = 2.084 Å, and Ti–O4 = 1.816 Å, Ti–O51 = 2.046 Å. Completing the coordination sphere are 2 O(acyl) atoms opposite to each other; here there is no dominant *trans* influence and both Ti–O(acyl) bonds are equal (Ti–O2 = 1.994 Å, Ti–O52 = 1.992 Å), see Table 1; These features are in perfect agreement with the *trans* influence obtained experimentally in related compounds [38,41] as are the corresponding data for the other 4 DFT calculated methoxy isomers (data not shown).

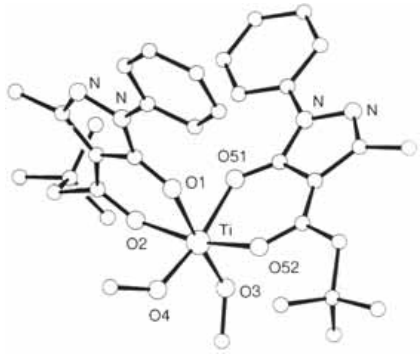

Fig. (30). DFT structure of the $(Q^T)_2Ti(OMe)_2$ conformer CIS3.

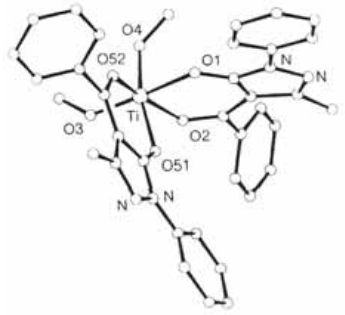

Fig. (31). DFT structure of the $(Q^B)_2Ti(OMe)_2$ conformer CIS3.

For DFT Cl and methoxy monomers, a lengthening of the Ti–Cl bond is expected due to the larger covalent radius of Cl (0.99 Å) compared with that of O (0.73 Å) [65]. However, this difference (0.26 Å) is overshadowed by calculated values of about 0.42 Å in the CIS3 arrangement of both methoxy derivatives. A partial double bond character of the Ti–O(methoxy) bonds in $Q_2Ti(OCH_3)_2$ may explain this feature and the consequent stronger Ti–chelate bonds are induced in $(Q^T)_2TiCl_2$, see Table 1. Related features for Ti–O bonds have already been described [47].

From this theoretical study we see that the *trans* influence provides the driving force for structural features in

titanium 4-acyl-5-pyrazolonates. O(oxo) in $[(Q^B)_2Ti(\mu\text{-}O)]_4$, and O(methoxy) in $Q_2Ti(CH_3O)_2$, $Q = Q^T$ and Q^B, are strongly bound to Ti, resulting in the shortest Ti–O bonds, and exert a dominant weakening of their opposite Ti-O bonds in the coordination sphere. The three *cis* Cl isomers of $(Q^T)_2TiCl_2$ are energetically equivalent, as are those of $(Q^B)_2Ti(CH_3O)_2$, whereas for the mononuclear $(Q^T)_2Ti(CH_3O)_2$ compound there is one isomer (CIS1) with marked higher energy, which is probably related to the bulkiness of the neo-pentyl substituent in R^4.

CONCLUSIONS

Cisplatin, the first metal anticancer agent, is a well-established drug in cancer therapy and still much investigated along with its derivatives (carboplatin, etc.). Ti antitumor species development occurred later than cisplatin and with titanocene dichloride in clinical phase II it is reaching important levels of protocol progress [19,66]. In addition, other interesting Ti compounds show very promising anti-cancer properties. More importantly, Ti tumor targets (slow growing tumors such as those in the gastrointestinal tract) are among the most difficult cancers to cure. Since Ti compounds with increased solubility, such as titanocenes, show activity against a larger spectrum of cancers (gastrointestinal, breast, melanoma and lung), it seems that improving water solubility (and more generally transport) of Ti drugs is an important aim in extending Ti therapy towards other tumors. Whether the antitumor effect of Ti drugs is enough to establish such drugs as useful will be known in a short time. An important feature already demonstrated by these compounds is their unusual minimal side effects, mainly the non-impairment of bone marrow and mild damage in the first part of the gastrointestinal tract. Such property is useful for combining therapy with other drugs. Note that synergistic action of $Ti(Cp)_2Cl_2$ and 5-fluoruracil was already suggested [30].

The role of asymmetry in octahedral antitumor Ti β-diketonates may be related to some of the weak bonds that such ligands induce. These bonds may play a role in the biological mechanism. For instance, the antitumor compound $[(Q^B)_2Ti(\mu\text{-}O)]_4$ has a very weak Ti–O(acyl) bond, which may favor formation of stronger Ti-electrophile bond, or induce hydrolysis, etc. Such interactions with transferrin may be critical to activity. For titanocene dichloride, the lipophilic environment created by Cp may be relevant [35] favoring interaction (including Ti-Cp cleavage) with hydrophobic regions in proteins or other biological substrates.

Further development of novel Ti drugs may come from the investigation of polynuclear species. This is concluded because polynuclear derivatives of budotitane and titanocene dichloride show antitumor activity, as does the more recent $[(Q^B)_2Ti(\mu\text{-}O)]_4$ with improved cell transport through a liposome. This approach also seems to be promising for budotitane as shown by a patent that describes a liposome formulation of lipid-soluble metal complexes for use in antitumor therapy [67]. It seems that budotitane is well suited for a liposome encapsulation. The process consists in mixing 200 mg of phosphatidylcholine, 99.2 mg of cholesterol, 9.57 mg of stearylamine and 40 mg of budotitane to obtain a lipid film that is dispersed in 15 ml of diethyl ether and 5 ml of physiological serum (calcium free). Budotitane was completely encapsulated and centrifugation (needed for cisplatin liposome) was unnecessary. The liposome is useful for parenteral administration and is stored at 4°C. This patent may be of great importance for budotitane clinical phase II, which was suspended due to problems with its formulation.

REFERENCES

[1] Köpf-Maier, P. *Eur. J. Clin. Pharmacol.* **1994**, *47,* 1.
[2] Pieper, T.; Borsky, K.; Keppler, B. K. In *Topics in Biological Inorganic Chemistry*; M. J. Clarke, P. J. Sadler, Eds.; Springer: Berlin, **1999**; pp. 172-199.
[3] Rosenberg, B.; Van Camp, L. *Nature* **1969**, *222,* 385.
[4] Pil. P.; Lippard, S. In *Encyclopedia of Cancer*, J. R. Bertino, Ed.; Academic Press, San Diego USA **1997** 392-410.
[5] Keppler, B. K.; Friesen, C.; Vongerichten, H; Vogel, E. In *Metal Complexes in Cancer Chemotherapy*; B. K. Keppler, Ed.; VCH: Weinheim, **1993**; pp. 297-323.
[6] Kelland, L. R.; Sharp, S. Y.; O'Neill, C. F.; Raynaud, F. I.; Beale, P. J.; Judson, I. R. *J. Inorg. Biochem.* **1999**, *77*, 111.
[7] Korfel, A,; Scheulen, M. E.; Schmoll, H. J.; Grundel, O.; Harstrick, A.; Knoche, M.; Fels, L. M.; Skorzec, M.; Bach, F.; Baumgart, J.; Sass, G.; Seeber, S.; Thiel, E.; Berdel, W. E. *Clin. Cancer Res.* **1998**, *4*, 2701.
[8] Christodoulou, C. V.; Ferry, D. R.; Fyfe, D. W.; Young, A.; Doran, J.: Sheehan, T. M. T.; Eliopoulos, A.; Hale, K.; Baumgart, J.; Sass, G.; Kerr, D. J. *J. Clin. Oncol.* **1998**, *16*, 2761.
[9] Keppler, B. K.; Berger, M. R.; Heim, M. E. *Cancer Treat. Rev.* **1990**, *17*, 261.
[10] Keppler, B. K.; Diez, A.; Seifried, V. *Arzneim-Forsch. Drug Res*. **1985**, *35*, 1832.
[11] Keppler, B. K.; Friesen, C.; Moritz, H. G.; Vongerichten, H.; Vogel, E. *Struct. Bond*. **1991**, *78,* 97.
[12] Berger, M.R.; Seelig, M. H.; Galeano, A. In *Metal Complexes in Cancer Chemotherapy*; B. K. Keppler, Ed.; VCH: Weinheim, **1993**; pp. 327-349.
[13] Keppler, B. K.; Schmäl, D. *Arzneim-Forsch. Drug Res.* **1986**, *36,* 1822.
[14] Schilling, T.; B. Keppler, B. K.; Heim, M. E.; Niebch, G.; Dietzfelbinger, H.; Rastetter, J.; Hanauske, A. R. *Invest. New Drugs* **1996***, 13,* 327.
[15] Köpf-Maier, P.; Köpf, H. *Struct. Bond.* **1988**, *70,* 103.
[16] Harding, M. M.; Mokdsi, G. *Curr. Med. Chem.* **2000**, *7,* 1289.
[17] Köpf-Maier, P.; Köpf, H. *Arzneim-Forsch. Drug Res*. **1987**, *37,* 532.
[18] Köpf-Maier, P.; Gerlach, S. *J. Cancer Res. Clin. Oncol.* **1986**, *111,* 243.
[19] Lümmen, G.; Sperling, H.; Lubolt, H.; Otto, T.; Rübben, H. *Cancer Chemother. Pharmacol.* **1998**, *42*, 415.
[20] Köpf-Maier, P. In *Metal Complexes in Cancer Chemotherapy*; B. K. Keppler, Ed.; VCH: Weinheim, **1993**; pp. 259-296.
[21] Keppler, B. K.; Heim, M. E. *Drugs of the Future* **1988**, *13,* 637.
[22] Mokdsi, G.; Harding, M. M. *Metal Based Drugs* **1998**, *5,* 207.
[23] Schroeder, H. A.; Balassa, J. J.; Tripton, I. H. *J. Chron. Dis.* **1963**, *16,* 55.
[24] Berdell, W. E.; Schmoll, H. J.; Scheulen, M. E. *Onkologie* **1993**, *16,* (Suppl. 3) R172.
[25] Guo, M.; Sun, H.; McArdle, H. J.; Gambling, L.; Sadler, P. J. *Biochemistry* **2000**, *39*, 10023.
[26] Messori, L.; Orioli, P.; Banholzer, V.; Pais, I.; Zatta, P. *FEBS Letters* **1999**, *442*, 157.
[27] Fruehauf, S.; Zeller, W. J. *Cancer Res.* **1991**, *51,* 2943.
[28] Hartmann, M.; Keppler, B. K. *Comm. Inorg, Chem*. **1995**, *16*, 339.
[29] Köpf-Maier, P. *J. Struct. Biol.* **1990**, *105,* 35.
[30] Christodoulou, C. V.; Eliopoulos, A. G.; Young, L. S.; Hodgkins, L.; Ferry, D. R.; Kerr, D. J. *Br. J. Cancer* **1998**, *77,* 2088.
[31] McLaughlin, M. I.; Croman, J. M.; Schaller, T. R.; Snelling, R. D. *J. Amer. Chem. Soc*. **1990**, *112*, 8949.
[32] Schwietert, C. H.; McCue, J. P. *Coord. Chem. Rev.* **1999**, *184,* 67.
[33] Kohlstaedt, L. A.; Wang, J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. *Science* **1992**, *256,* 1783.

[34] Klapotke, M.; Köpf, H.; Tornieporth-Oetting, I. C.; White, P. S. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1518.
[35] Koepf-Maier, P.; Kahl, W.; Klouras, N.; Hermann, G.; Koepf, H. *Eur. J. Med. Chem.* **1981**, *16*, 275.
[36] Mokdsi, G.; Harding, M. M. *J. Inorg. Biochem.* **2001**, *83,* 205.
[37] Toney, J. H.; Marks, T. J. *J. Amer. Chem. Soc.* **1985**,*107*, 947.
[38] Caruso, F.; Rossi, M.; Tanski, J.; Sartori, R.; Sariego, R.; Moya, S.; Diez, S.; Navarrete, E.; Cingolani, A.; Marchetti, F.; Pettinari. C. *J. Med. Chem.* **2000**, *43*, 3665.
[39] Sartori, R.; Diez, S.; Navarrete, E.; Sariego, R.; Moya, S.; Caruso, F.; Rodriguez, S. 218th ACS National Meeting, New Orleans, Aug. 22-26 **1999**.
[40] Koepf-Maier, P.; Brauchle, U.; Henssler, A., *Toxicology* **1988**, *51*, 291.
[41] Troyanov, S. I.; Gorbenko, O. Y. *Polyhedron* **1997**, *16*, 777.
[42] Pettinari, C.; Marchetti. F.; Cingolani, A.; Lorenzotti, A.; Mundorff, E.; Rossi, M.; Caruso, F. *Inorg. Chim. Acta* **1997**, *262*, 33.
[43] Pettinari, C.; Marchetti. F.; Pettinari, R.; Gindulyte, A.; Massa, L.; Rossi, M.; Caruso, F. *Eur. J. Inorg. Chem.* **2002**, 1447.
[44] Le Page, Y.; McCowan, J. D.; Hunter, B. K.; Heyding, R. D. *J. Organomet. Chem.* **1980**, *193*, 201.
[45] Koepf-Maier, P.; Grabowski, S.; Koepf, H. *Eur. J. Med. Chem.* **1984**, *19*, 347.
[46] Thewalt, U.; Kebbel, B. *J. Organomet. Chem*. **1978**, *150*, 59.
[47] Corradini, P.; Allegra, G. *J. Amer. Chem. Soc.* **1959**, *81*, 5510.
[48] Doppert, K.; Thewalt, U. *J. Organomet. Chem.* **1986**, *301*, 41.
[49] Aslan, H.; Sielisch, T.; Fischer, R. D. *J. Organomet. Chem.* **1986**, *315*, C69.
[50] Klein, H. P.; Thewalt, U.; Doppert, K.; Sanchez-Delgado, R. *J. Organomet. Chem*. **1982**, *236*, 189.
[51] Carofiglio, T.; Floriani, C.; Sgamellotti, A.; Rosi, M.; Chiesi-Villa, A.; Rizzoli, C. *J. Chem. Soc. Dalton Trans.* **1992**, 1081.
[52] Li, Z.; Huang, J.; Qian, Y.; Chan, A. S-C.; Leung, K. S-Y.; Wong, W. T. *Inorg. Chem. Comm.* **1999**, *2*, 396.
[53] Palacios, F.; Royo, P.; Serrano, R.; Balcazar, J. L.; Fonseca, I.; Florencio, F. *J. Organomet. Chem.* **1989**, *375*, 51.
[54] Yu, P.; Pape, T.; Uson, I.; Said, Musa A.; Roesky, H. W.; Montero, M. L.; Schmidt, H. -G.; Demsar, A. *Inorg. Chem.* **1998**, *37*, 5117.
[55] Bjoergvinsson, M.; Halldorsson, S.; Arnason, I.; Magull, J.; Fenske, D. *J. Organomet. Chem.* **1997**, *544*, 207.
[56] Roth, A.; Floriani, C.; Chiesi-Villa, A.; Guastini, C. *J. Amer. Chem. Soc.* **1986**, *108*, 6823.
[57] Heshmatpour, F.; Wocadlo, S.; Massa, W.; Dehnicke, K.; Bottomley, F.; Day, R. W. *Zeit. Natur. B* **1994**, *49*, 827.
[58] Caruso, F.; Massa, L.; Gindulyte, A.; Pettinari, C.; Marchetti, F.; Pettinari, R.; Ricciutelli, M.; Costamagna, J.; Canales, J. C.; Tanski, J.; Rossi, M. *Eur. J. Inorg. Chem.* **2003,** 3221**.**
[59] Bradley, D. C.; Holloway, C. E. *J. Chem. Soc. A* **1969**, 282.
[60] Smith, G. D.; Caughlan, C. N.; Campbell, J. A. *Inorg. Chem.* **1972**, *11*, 2989.
[61] Wittrisch, H.; Schroeder, H-P.; Vogt, J.; Vogt, C. *Electrophoresis* **1998**, *19*, 3012.
[62] Errington, R. J.; Ridland, J.; Clegg, W.; Coxall, R. A.; Sherwood, J. M. *Polyhedron* **1998**, *17*, 659.
[63] Mokdsi, G.; Harding, M. M. *J. Organomet. Chem.* **1998**, *565*, 29.
[64] Comba, P.; Jakob, H.; Nuber, B.; Keppler, B. K. *Inorg. Chem.* **1994**, *33*, 3396.
[65] Pauling, L. *The Nature of the Chemical Bond* Cornell University Press, Ithaca, NY, 3rd ed., **1960**, pp. 260.
[66] Kroger, N; Kleeberg, U. R.; Mross, K.; Edler, L.; Hossfeld, D. K. *Onkologie* **2000**, *23*, 60.
[67] Max-Delbrueck Centrum. German patent DE4134158, **1993**.