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β-Diketonate Titanium Compounds Exhibiting High In Vitro Activity and Specific DNA Base Binding

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This publication is dedicated in the memory of Dr. Julie Fisher.

Abstract: Herein, we report 31 new β-diketonate titanium compounds of the type $[Ti(O,O)_2X_2]$, whereby O,O = asymmetric or symmetric β-diketonate ligand and X = Cl, Br, OEt or OiPr. Thirteen new crystal structures are discussed and show that these octahedral species all adopt cis geometries in the solid state. These compounds have been tested for their cytotoxicity using SRB and MTT assays, showing several of the compounds are as potent as cisplatin against a range of tumour cell lines. Results also show the $[Ti(O,O)_2Br_2]$ complexes are more potent than $[Ti(O,O)_2Cl_2]$, $[Ti(O,O)_2(OEt)_2]$ and $[Ti(O,O)_2(OiPr)_2]$. Using a simple symmetrical heptane-3,5-dione (O,O) ligand bound to titanium, we observed more than a 50-fold increase in potency with the $[Ti(O,O)_2Br_2]$ (**28**) when compared to $[Ti(O,O)_2Cl_2]$ (**27**). One of the more potent compounds (**6**) has been added to three different sixmers of DNA, in order to analyse the potential DNA binding of the compound. NMR studies have been carried out on the compounds, in order to understand the structural properties and the species form in solution during the in vitro cell assays.

Introduction

Titanium is widely used in many applications, including pigments and coatings, aerospace, nuclear waste storage, catalysts and medical treatment.[1–5] Titanium itself is non-toxic and not rejected by the body, and due to its biocompatibility, the medical industry has embraced its use as implants in hip and joint replacements.[1] Even though there are many advantages for the uses of titanium as pro-drugs, very little work has been undertaken to optimize their potential in cancer therapy. Recent studies by Zhou et al. have shown the effects of titanium nanoparticles (n-TiO_2) on the bioavailability, metabolism and toxicity in zebra fish.[6] Treatment with n-TiO_2 did not induce lipid peroxidation, DNA damage or the generation of reactive oxygen species (ROS). The low toxicities observed in vivo show the promising effects of titanium for further research into potential titanium based pro-drugs.

The discovery of the therapeutic effects of titanocene dichloride (**Figure 1**) by Köpf and Köpf-Maier et al. in 1979 led to further research into titanium compounds as potential anti-cancer drugs.[7,8] Köpf and Köpf-Maier et al. synthesised functionalised metallocenes with differing ancillary ligands, and showed replacement of the chloride ligand with other groups had little effect on the activity of the compounds against Ehrlich ascites tumors in mice.[8,9] The activity of these compounds is thought to be due to the lability of the Ti-X bond, and the less labile the bond, the slower the rate of hydrolysis and this subsequently leads to the inability to form the active species in solution.[10] Tacke et al. reported the synthesis of the benzyl-substituted titanocene dichloride 'Titanocene Y' (**Figure 1**) with in vitro studies showing moderate IC_{50} values. Against xenograft A431 tumors in mice, Titanocene Y saw a 40% inhibition of tumor growth in comparison to control mice.[11] The oxalate derivative, 'Oxali-Titanocene Y' was more potent and caused a 38% inhibition in tumor growth in the xenograft A431 mouse model, and was also found to have an anti-angiogenic effect on tumors.[12]

Tacke et al. have recently shown drug uptake and DNA assays of Titanocene Y against HCT-8 cells (ileocecal colorectal adenocarcinoma). High DNA-adduct levels were obtained at IC_{50} concentrations, indicating DNA is a target for these metallocene drugs.[13] Computational studies of Titanocene Y with double-stranded DNA have since shown, that after the loss of the two chloride ligands, the dicationic Titanocene Y coordinates strongly to a phosphate group.[14] In addition, hydrolysis and DNA studies of Cp_2TiCl_2 and Titanocene Y, with bis(4-nitrophenyl) phosphate (BNPP) have been studied (**Figure 1**).[15] They show that Cp_2TiCl_2 solutions promoted the hydrolysis of the activated phosphate di- and mono-esters, BNPP and NPP. However, no phosphate di-ester hydrolysis was observed in solutions containing the Titanocene Y species. Their results suggest that Cp_2TiCl_2 is not able to cleave the phosphate di-ester linkages of DNA, but that coordination to DNA leads to titanocene-induced apoptosis.

Shortly after the discovery of titanocene dichloride, Keppler et al. discovered the therapeutic effects of budotitane.[16–18] The in vivo results highlighted this compound as an attractive therapeutic drug due to its high activity against a range of transplantable tumors, with no known evidence of mutagenicity. However, the clinical trials were terminated at Phase I due to severe adverse side effects.[19] These compounds can exist as five different isomers (**Figure 2**), and even though budotitane has been

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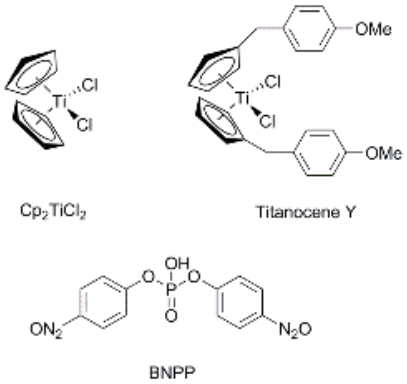


Figure 1 Structures of Titanocene dichloride (Cp_2TiCl_2), Titanocene Y and BNPP, by Tacke et al.

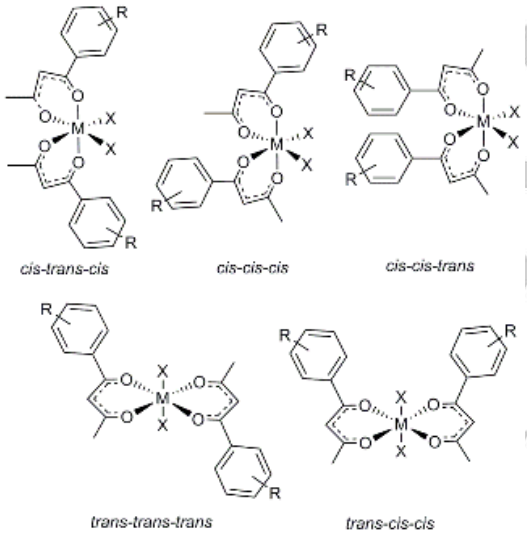


Figure 2 Five possible isomers of compounds of the type $[M(O,O)_2X_2]$

crystallised by Dubler et al., the solid state structure is only present in 19% concentration when considering the solution studies. It is postulated that overcoming this isomer issue in solution could prevent the side effects observed with this compound.[20]

Work has since continued in this area, with promising results from Huhn et al. and Tshuva et al., using salan type ligands (**Figure 3**).[21] Huhn et al. have synthesised sulfonamide functionalised Ti^{IV}-salan dipic bis-chelates and the preliminary in vitro evalutions reveal they are cytotoxic in the sub micromolar range, and 7 times more cytotoxic than cisplatin.[22] Tschuva et al. have carried out the in vitro assays with $Ti(O^iPr)_4$ and $TiCl_4(THF)_2$, two labile Ti^{IV} compounds, and reported both as being inactive against colon and ovarian cells. It is thought this inactivity is due to the rate of hydrolysis towards unreactive aggregates being too fast for any DNA binding to occur, and thus the need for inert ligands for anti-cancer activity is confirmed.[23] Consequently a new class of Ti^{IV} anti-cancer drug was reported containing amine-phenolato (salan) ligands, designed to provide relatively high hydrolytic stability.[23–25] In vitro testing against HT-29 and OVCAR-1 cell lines were very promising, and values are much lower than titanocene dichloride and are significantly lower than cisplatin.[24]

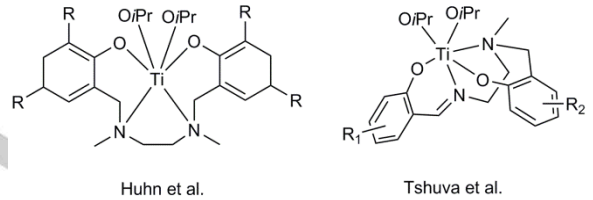


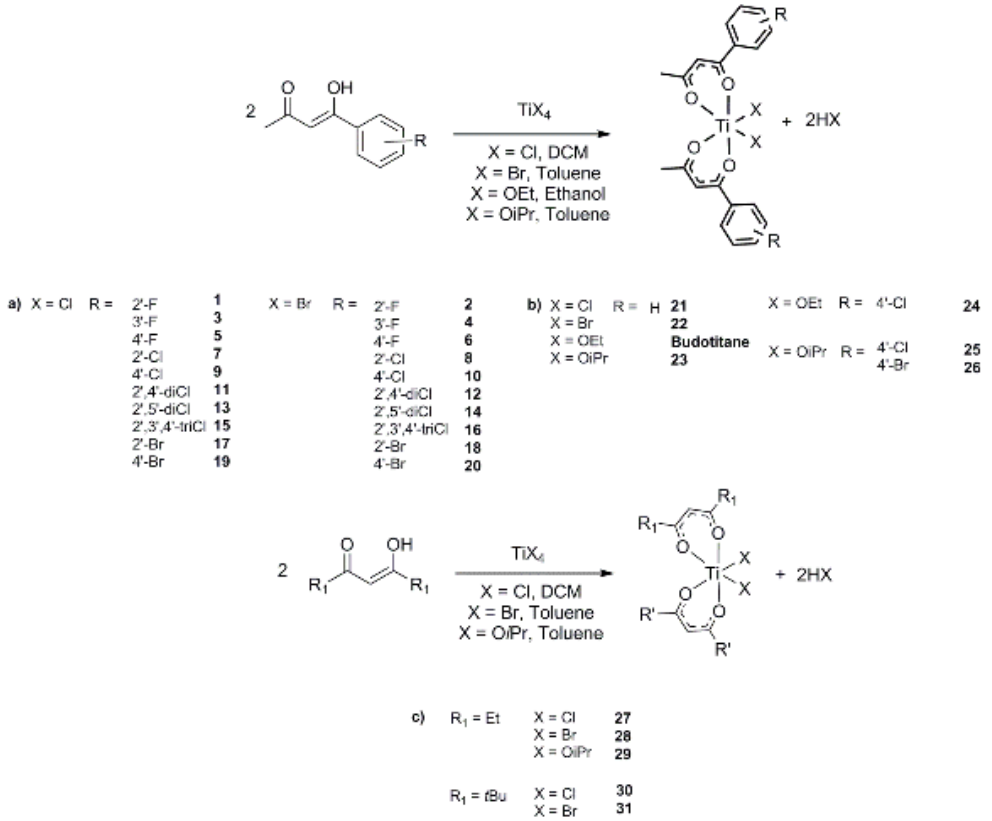
Figure 3 Examples of Ti^{IV} salan compounds by Huhn et al.[21] and Tshuva et al.[25]

We have been interested in the synthesis of group IV compounds, including both metallocenes and coordination compounds. We reported metallocenes containing functionalised Cp (cyclopentadiene) substituents, which increased solubilities and in vitro cytotoxicities.[26] These compounds have good activity against a range of cancer cell lines, in particular against the cisplatin-resistant cell line A2780cis, where one of the compounds is 10 times more active than its non-functionalised equivalent.[27] Recently, we reported a series of functionalised budotitane analogues, showing a general increase in cytotoxicity from Ti < Zr < Hf. We showed the first cytotoxic seven coordinate hafnium acac complexes, in which the complexes with symmetrical acac ligands are > 8-fold more potent than the asymmetric β-diketonate ligands.[28] Herein, we report a series of asymmetric and symmetric titanium compounds incorporating functionalised β-diketonate ligands, with thirteen new crystal structures discussed. Their cytotoxicity values using the SRB assay has been evaluated in order to gain structure-activity-relationships (SARs). We have further enhanced the SARs by the synthesis of titanium compounds with varying ancillary ligands (Cl, Br, OEt and iOPr). As a possible target, DNA binding studies were undertaken for one of the more potent compounds, to determine if DNA binding occurs and contributes to the drugs mode of action. In order to assess the consistency of the IC_{50} values, a 5 day MTT assay was carried out on selected compound and mechanistic studies are discussed in order to determine the lability of these compounds during in vitro solution.

Results and Discussion

Synthesis and Characterization

Compounds **1**-**31** were all synthesised according to **Scheme 1a), b)** and **c)** and isolated as analytically pure samples. **Scheme 1a)** shows the synthesis of functionalised asymmetric ligands with titanium chloride and titanium bromide. **Scheme 1b)** shows the synthesis of titanium compounds with different ancillary ligands; chloride, bromide, ethoxide and isoproxide. **Scheme 1c** shows symmetric ligands with different titanium starting precursors. Compound **5** was previously reported,[28] and all other compounds have been fully characterised by ^1H NMR



Scheme 1 Synthetic pathway **a)**, **b)** and **c)** for bis(*β*-diketonate)titanium compounds **1**-**31** and **Budotitane**.[16]

spectroscopy, $^{13}C\{^{1}H\}$ NMR spectroscopy and microanalysis. X-ray crystallographic data has been obtained for compounds **1**, **3**, **4**, **9**-**12**, **14**, **16**, **17**, **19**, **20** and **3.**

Orange-red single crystals were obtained and the compounds crystallized in a triclinic (**1**), orthorhombic (**3**, **9**, **19** and **20**) or monoclinic (**4**, **10**, **11**, **12**, **14**, **16**, **17**, and **30**) cell. The molecular structures are shown in **Figure 4** and the structures adopt a mix of geometries either cis-trans-cis or cis-cis-trans with half or one molecule in the asymmetric unit cell. The crystallographic data is presented in **Table S1-S2** (see SI). Selected bond lengths are presented in **Table S3**, and the cis bond angles around the titanium center are all in the range 83.17(19)-98.02(16)° (**Table S4**, SI). The single crystal structure of budotitane has previously been reported by Dubler et al. and showed that in solid state this analogous compound also adopts a cis-cis-trans geometry, showing a similarity to the structures presented here.[20]

SRB Chemosensitivity Studies

In the first instance compounds were chosen to be tested using the SRB assay, these were selected according to previous cytotoxicity results we have obtained.[16] Compounds **5, 6, 9-14**, **19**-**23, 25** and **26**, and cisplatin were incubated with A2780 (human ovarian carcinoma), A2780cis (cisplatin-resistant human ovarian carcinoma), CaSki (human cervical carcinoma), HT-29 (human colorectal adenocarcinoma), LoVo (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma) and PC3 (human prostate cancer) cell lines, and results are presented in **Table 1**. The results show a general trend that the *β*-diketonate titanium bromide compounds are more cytotoxic than their corresponding *β*-diketonate titanium chloride compounds. The most promising result was observed for compound **6**, which is as active as cisplatin against the HT-29 cell line. The 4-fluoro-*β*-diketonate ligand was tested and showed no cytotoxicity, with IC_{50} values > 100 μM, meaning the activity seen for compound **6** is due to the titanium complex. Compound **6** appears to be selective in its activity against HT-29, as when tested against other cell lines this compound is only moderately active. Compounds **13** and **14** show the highest cytotoxicity against all cell lines tested, and increasing the number of electron withdrawing substituents increases the potency. This can be seen when comparing IC_{50} values against A2780, the mono-substituted 4-chloro compounds **9** (X = Cl) and **10** (X = Br) have IC_{50} values of 15.84 μM and 11.77 μM, whereas the 2',4'-dichloro compounds **13** (X = Cl) and **14** (X = Br) have IC_{50} values of 2.3 μM and 2.6 μM respectively. Up to a 6.8-fold increase in potency was observed upon addition of another electron withdrawing substituent.

The ancillary ligand is thought to be significant for the cytotoxicity of a compound, and this ligand is usually hydrolysed in vivo and replaced by -OH_2/-OH. Therefore size and lability of the ligands can affect the rates of hydrolysis.[5] It is essential to choose the correct ligand to make sure hydrolysis occurs only once the drug has entered the cell. We synthesised a library of compounds to compare the IC_{50} values when ancillary ligand

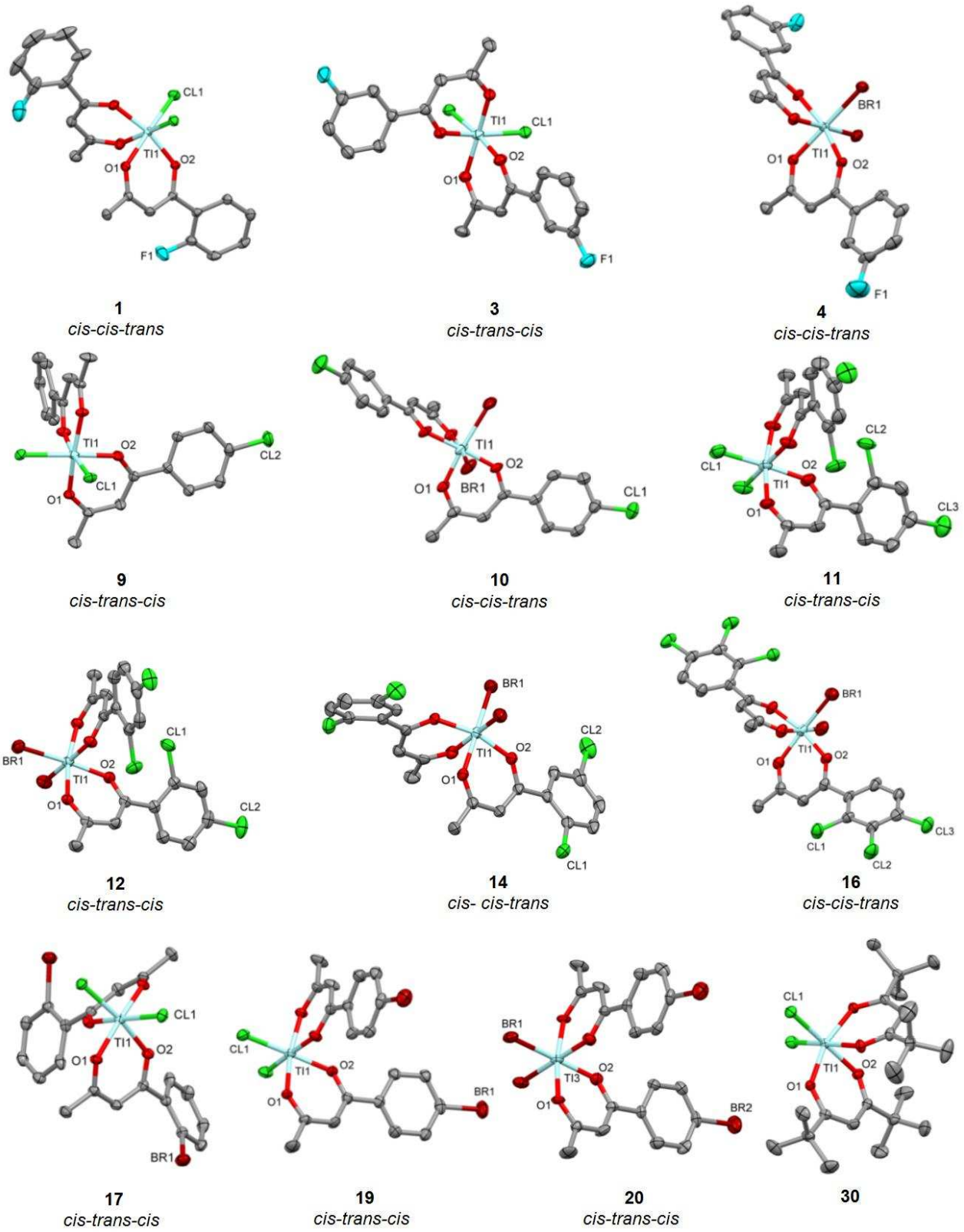


Figure 4 Molecular structures of compounds **1**, **3**, **4**, **9**-**12**, **14**, **16**, **17**, **19, 20** and **30**. Hydrogen atoms and solvent molecules are omitted for clarity and displacement ellipsoids are at the 50% probability level

Table 1 IC_{50} values (μM) for the SRB assay for cisplatin, compounds **5, 6, 9-13, 19-23, 24, 25** and **budotitane** against a range of cell lines.

Compound	A2780	A2780cis	CaSki	HT-29	LoVo	MCF-7	PC3
Cisplatin	0.38	2.74	1.66	2.29	0.63	0.62	0.3
5	42.12	40.68	44.7	>100.0	42.25	40.05	36.32
6	15.06	17.65	24.16	2.61	19.56	23.51	21.81
9	15.84	20.83	23.97	23.14	19.61	19.27	19.16
10	11.77	20.91	23.12	23.73	20.7	20.61	17.57
11	13.09	31.8	15.11	10.44	30.97	24.35	35.82
12	12.27	18.41	13.51	>25.0	12.54	17.71	16.71
13	2.3	13.0	-	-	4.2	6.2	-
14	2.6	9.5	-	-	4.7	6.8	-
19	16.51	40.95	29.47	41.17	30.62	18.49	36.12
20	11.28	12.73	12.92	>25.0	13.41	15.66	13.9
21	38.45	49.75	33.69	23.51	26.85	43.68	41.45
22	1.64	1.87	20.57	23.48	7.11	8.9	4.25
Budotitane	3.9	3.17	3.64	42.19	4.65	5.49	5.34
23	>25.0	19.43	>25.0	>25.0	>25.0	21.62	>25.0
25	>12.5	7.34	8.67	>12.5	>12.5	11.68	>12.5
26	11.81	11.29	9.08	>25.0	11.23	9.58	11.59

X = Cl (**21**), Br (**22**), OEt (**Budotitane**) and OiPr (**23**). The results show that budotitane, which is the current leading compound of this type, has high activity against all cell lines. However, compound **22** which has a bromide ancillary ligand, shows a 2-fold increase in activity against A2780 (1.64 μM) and A2780cis (1.87 μM) cell lines, when compared to budotitane (3.9 μM and 3.17 μM respectively). The unsubstituted β-diketonate ligand was also tested and shows no cytotoxicity, having IC_{50} values >100 μM.

MTT Chemosensitivity Studies

To allow us to make comparisons with our previously published work, cisplatin compounds **1-4**, **7-10**, **15-31** and **budotitane** were tested using the MTT assay. The compounds were tested against A2780, HT-29 and MCF-7, and additionally using a one hour exposure time against MCF-7 (**Table 2**). The results are not in the same magnitude as those seen from the SRB assay (**Table 1**), however the trends are still consistent. When comparing the titanium chloride compound **9** (23 ± 2 μM) with the corresponding titanium bromide compound **10** (9 ± 2 μM), the trend shows again that the bromide compound is the most cytotoxic, with up to a 2.5-fold increase in IC_{50} against MCF-7. When comparing the unsubstituted β-diketonate ligand on titanium chloride (**21**) and titanium bromide (**22**), the same trend is seen with that of the SRB assay, whereby the bromide ancillary ligand is consistently more active against all cell lines tested.

The compounds were also tested against MCF-7 using a one hour exposure time. In order to assess how potent the compounds are upon initial exposure and determine the rate at which cytotoxicity is attained. The results (**Table 2**) show that after a one hour incubation with compound **10**, the IC_{50} value of 18 ± 5 μM is lower than that seen for cisplatin, 53 ± 8 μM. Compound **10** has a bromide ancillary ligands, which as stated previously has lower IC_{50} values than its chloride analogue, compound **9**. On comparing compounds **9/ 10** and **21/ 22**, there is an 8.1 and 9.9-fold increase in potency observed on changing the ancillary ligands from chloride to bromide. The high in vitro cytotoxicity seen after just one hour exposure highlights these compounds as attractive candidates for further assays.

When comparing the IC_{50} values of the symmetric β-diketonate compounds, the most significant result was seen for **27** and **28**. These two compounds have a simple symmetrical heptane-3,5-dione ligand bound to either titanium chloride (**27**) or titanium bromide (**28**). Compound **27** is inactive against all cell lines tested, whereas upon changing the ancillary ligand to bromide, the compound becomes active against all cell lines with up to a 50-fold increase observed against MCF-7 (**27** > 500 μM versus **28** = 10 ± 2 μM). Also when considering the 1 hour exposure for compound **28** (46 ± 6 μM), it can be seen to be as active as budotitane (64 ± 19 μM) and cisplatin (53 ± 8 μM). Against MCF-7, the isopropoxide compound **29** (22 ± 4 μM) is also over 22.7-fold more cytotoxic than the analogues chloride compound **27**

Table 2 IC_{50} values (μM) for the MTT assay for cisplatin compounds **1-4, 7-10, 15-31** and **Budotitane** against a range of cell lines.

Compound	A2780	HT-29	MCF-7	MCF-7, 1 hr exp
Cisplatin	2.2 ± 0.5	10 ± 3	3 ± 1	53 ± 8
1	13 ± 3	30 ± 5	24 ± 5	346 ± 46
2	9 ± 2	20 ± 8	19 ± 3	278 ± 77
3	9 ± 2	25 ± 9	24 ± 5	353 ± 28
4	6 ± 1	10 ± 2	11 ± 4	53 ± 29
7	5.4 ± 0.7	25 ± 4	19 ± 1	350 ± 16
8	5 ± 2	18 ± 5	12 ± 1	290 ± 48
9	13 ± 4	29 ± 6	23 ± 2	147 ± 37
10	5.8 ± 0.7	12 ± 6	9 ± 2	18 ± 5
15	9 ± 2	14 ± 6	16 ± 5	273 ± 29
16	6 ± 1	10 ± 2	11 ± 4	233 ± 25
17	12 ± 3	14 ± 4	23 ± 4	364 ± 21
18	10.3 ± 0.4	14 ± 4	21 ± 2	330 ± 44
21	19.0 ± 0.8	61 ± 16	42 ± 5	458 ± 42
22	12 ± 1	38 ± 12	33 ± 12	46 ± 18
26	-	-	18 ± 3	-
24	-	-	12 ± 1	-
Budotitane	9 ± 2	26 ± 4	22 ± 6	64 ± 19
27	93 ± 46	>500	>500	-
28	18 ± 4	17 ± 5	10 ± 2	46 ± 6
29	-	32 ± 10	22 ± 4	100 ± 21
30	169 ± 31	346 ± 30	353 ± 19	440 ± 38
31	175 ± 13	175 ± 11	45 ± 9	376 ± 41

(>500 μM), showing the ancillary ligand can affect the observed toxicity and further highlighting the compounds with ancillary bromides as attractive compounds for future studies.

DNA Binding Studies

To gain further understanding regarding the mode of action of these compounds, DNA binding experiments were carried out. Using a cGMP machine, three sixmers were synthesised (**Figure 5**), incorporating adenosine/thymine (Strand 1), a mixture of all four bases (Strand 2) or cytosine/guanine (Strand 3). Compound **6** was incubated with the individual strands and the HPLC data analysed of the strand alone and then further after a period of 1 and 2 weeks incubation with compound 6 (Figure S1-S3, SI).

Compound **6** was incubated with Strand 1 and after one week no significant changes were observed and the major starting material peak was still present. However, after a period of two weeks this major peak disappears and a new peak at 16.69 minutes can be identified as a cleaved section of Strand 1.Compound **6** was incubated with Strand 2 and after a period of one week there was a significant decrease in the amount of starting strand present and a second peak was observed. After a further week, the peak corresponding to the parent strand was essentially non-existent, with a new major peak now occurring at 7.39 minutes and a secondary peak occurring at 16.29 minutes. This again suggests the DNA strand is cleaved into smaller portions; however in this case it suggests that there are two portions of differing sizes produced with the larger of these being converted to the smaller by further cleavage. Lastly, compound **6** was incubated with Strand 3, and the chromatograms show how after a period of 1 week incubation there is a decrease in the amount of starting strand present in solution, corresponding to the peak at 20.47 minutes. There is the appearance of one cleavage product at 9.60 minutes, and after two weeks this

Strand 1: 5'-ATATAT-3'
Strand 2: 5'-ATGCAT-3'
Strand 3: 5'-GCGCGC-3'

Figure 5 Three different sixmers of DNA synthesised using cGMP.

cleavage product appears to be the major product, corresponding to the peak at 7.76 minutes. These DNA binding studies suggest that the mode of action of this class of compound is different to that of cisplatin in that they appear to cleave the DNA sequence as opposed to performing cross-linking.[29–31] Also the presence of the guanine base in the DNA chain appears to help facilitate the compounds action but is not a necessity.

NMR Studies

We have previously shown preliminary results on the displacement of the ancillary ligands when compounds are incubated with dimethylsulfoxide (DMSO), this was to mimic the MTT assay, and showed a new DMSO-titanium compound.[16] Further mechanistic studies have been carried out to understand the solvolysis of these titanium compounds with DMSO and water, in particular compound **10** which contains a bromide ancillary ligand. It is thought that determining the mechanism of action of these drugs in the MTT assay should help to gain an understanding of the behavior of titanium drugs in the body. NMR studies were conducted using compound **6** and addition of two equivalents of DMSO, then the ^{1}H NMR spectra recorded after 20 minutes, 1 day, 2 days and then 2 weeks. Changes were observed in the aromatic region, a decrease in the diketonate resonances and new signals corresponding to free ligand and free DMSO area now visible (**Figure S4**, SI). It is postulated that the labile ancillary chloride ligands are substituted for DMSO solvent. Equimolar solutions of both compounds **9** and **10** in d_6-DMSO were prepared and ^{1}H NMR spectra were recorded after 5 minutes, 1 hour, 5 hours and 1-5 days, to investigate changes on the same time scale as the MTT assay. After 5 minutes, the majority of compound **10** appears to have dissociated, with the major resonances all corresponding to free diketonate ligand [(O,O)] and additional resonances for the complex $[Ti(O,O)_2(DMSO)_2][2Br]$ (**10-DMSO**) (**Figure S5**, SI). Integration of the signals in the diketonate region shows after 5 minutes a ratio of 0.6:1:4.2 is observed for $[Ti(O,O)_2Br_2]$: $[Ti(O,O)_2(DMSO)_2][2Br]$: [(O,O)]. The NMR spectra for compounds **9** and **10** in d_6-DMSO show clear differences in rates of reaction (**Figure S6-S7**, SI). For compound **9**, after five minutes there is $[Ti(O,O)_2Cl_2]$ remaining, indicating that reaction with DMSO or ligand dissociation has given a solution containing only $[Ti(O,O)_2(DMSO)_2][2Cl]$ (**9-DMSO**) and free diketonate ligand. For compound **10**, the $[Ti(O,O)_2Br_2]$ is observed until one day after dissolution, indicating the rate of reaction with DMSO or the rate of ligand dissociation is much slower than for compound **9**. Once there is no original compounds left, there is more **10-DMSO** present in solution than **9-DMSO**. If the DMSO compounds are the active species, as hypothesized, this observation offers a possible explanation for the increased activity of the titanium bromide compounds over the corresponding chlorides.

Compound **10** was dissolved in d_6-DMSO and 4-chloro-β-diketonate ligand was added, the ^{1}H NMR spectra recorded after 10 minutes, 1 hour, 1 day and 7 days. Ten minutes after the addition of free ligand, resonances for both free ligand and **10** were observed. Addition resonances were also observed at 8.06, 6.62 and 2.24 ppm, which match the signals seen in the previous study, thought to correspond to **10-DMSO.** Integrating the diketonate peaks at 6.58 and 6.62 ppm gives a ratio of 1:14.8 for compound: free ligand. After 7 days, this increased to 1:13.7, this experiment offers proof that there exists an solution equilibrium between compound and free diketonate ligand.

Two equivalents of water were added to a solution of compound **10** in anhydrous $CDCl_3$, and the ^{1}H NMR spectrum was recorded after 20 minutes, 1 day and 2 weeks (**Figure S8-S9**, SI). The spectra showed that all the diketonate ligand had dissociated from the compound and only free ligand was observed. A broad water peak is visible at 1.83 ppm, and a second broad peak is visible at 4.85 ppm, which after 1 day both they have decreased in intensity significantly and furthermore after 2 weeks. The $^{13}C\{^1H\}$ NMR spectrum recorded after 2 weeks also shows only free diketonate ligand present in solution, with no titanium compound visible.

Conclusions

A library of titanium bis(β-diketonate) compounds of the type, $[Ti(O,O)_2X_2]$, have been synthesised and characterised using both asymmetric and symmetric ligands. X-ray crystallography analysis shows that they adopt cis geometries in the solid state. Selective compounds were tested against several cell lines using the SRB assay, and when comparing the same compounds for titanium chloride and titanium bromide, the bromides always gave an increase in cytotoxicity. An increase in potency was observed upon addition of more electron withdrawing substituents to the b-diketonate ligand. Compounds were also tested against the MTT, to allow comparisons with literature results and even thought different assays were conducted and the magnitude of activity is different between the two, the general trends are similar for both the SRB and MTT assays.

Compound **6** was potent against HT-29 but had only relatively mild toxicity against other cell lines. Therefore we tested the possible binding of this compound by incubating it with three different sixmers of DNA and the results suggest that the mode of action of this class of compound differs to that of cisplatin, as that they appear to cleave the DNA sequence as opposed to cross-linking. Also the presence of the guanine base in the DNA chain appears to help facilitate the compound and therefore it is possible these compounds could interact with this base and that DNA is a potential target.

The NMR studies have shown that in the presence of a small amount of DMSO, compounds **9** and **10** react to form new titanium compounds in which the ancillary halides are substituted for dimethylsulfoxide, $[Ti(O,O)_2(DMSO)_2][2Cl]$ (**9-DMSO**) and $[Ti(O,O)_2(DMSO)_2][2Br]$ (**10-DMSO**) respectively. It is suggested that these new compounds are the cytotoxic species. In solution, there is an equilibrium between the titanium compounds (**9** and **10**) and their DMSO analogues (**9-DMSO** and **10-DMSO**). As the equilibrium of the titanium bromides lies further towards the active DMSO species, it is postulated that this is the reason the bromide compounds exhibit greater in vitro cytotoxicity than the chlorides.

Supplementary Information

The supplementary information contains the experimental details and characterization data for compounds **1**-**31**. Experimental procedures are also detailed for both the SRB and MTT assays. X-ray crystallographic data tables and tables of important bond lengths and angles are provided. HPLC chromatograms for the incubation of compounds **6** with Strands 1, 2 and 3 are discussed. The document also contains NMR time-dependent spectra are provided for compounds **9** and **10** in both d_6-DMSO and $CDCl_3$. All crystal structures have been submitted to the CCDC, with depository numbers 1495265-1495277.

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Keywords: Acac • Anti-Cancer • Coordination • Cytotoxicity • Titanium

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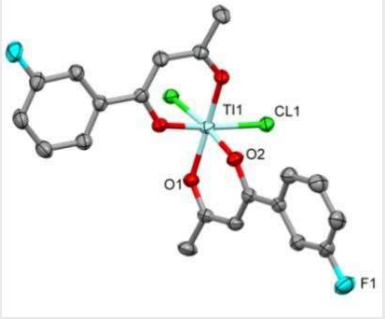
FULL PAPER

This report presents 31 new β-diketonate titanium compounds of the type $[Ti(O,O)_2X_2]$, where O,O = an asymmetric or symmetric β-diketonate ligand and X = Cl, Br, OEt or OiPr. X-ray crystallography is discussed for thirteen crystal structures, which all adopt a cis arrangement. Cytotoxic studies have been carried out against a range of cell lines, showing compounds of the type $[Ti(O,O)_2Br_2]$ are up to 50 times more potent than $[Ti(O,O)_2Cl_2]$.



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