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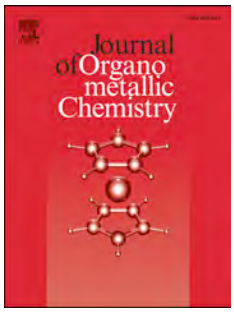

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Study of the Anticancer Properties of Optically Active Titanocene Oximato Compounds

Isabel de la Cueva-Alique,[‡] *Sara Sierra,*[‡] *Adrián Pérez-Redondo,*[‡] *Isabel Marzo,*[⊥] *Lourdes Gude,*[‡] *Tomás Cuenca,*[‡] *Eva Royo*[‡*]

Dedicated to the memory of Prof. Dr. Pascual Royo, who loved aquo titanium chemistry

[‡] Departamento de Química Orgánica y Química Inorgánica, Instituto de Investigación en Química Andrés del Río (IQAR), Universidad de Alcalá, 28805 Alcalá de Henares, Madrid, Spain

[⊥] Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, 50009 Zaragoza, Spain

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Corresponding Author

*E-mail: eva.royo@uah.es. Phone Nr. (+34) 918854629; Fax Nr. (+34) 918854683

ABSTRACT

New water soluble and optically active cyclopentadienyl titanium derivatives [(η^5-C_5H_5)$_2$Ti{(1*R*,4*S*)-κON,(R)NH}Cl] (R = Bn (Benzyl) **1a'**, 2-pic (2-picolylamine) **1b'**) have been synthesized. The novel compounds along with those previously described [(η^5-C_5H_5)$_2$Ti{(1*S*,4*R*)-κON,(R)NH}Cl] (R = Bn **1a**, 2-pic **1b**) were evaluated by polarimetry, ultra-violet and circular dichroism spectroscopy. The structure of **1b** was determined by single crystal X-ray crystallography and showed a unique terminal monohapto Ti-O disposition of the oximato ligand. All enantiomers have been tested against several cancer cell lines *in vitro*: prostate PC-3 and DU-145, lung A-549, pancreas MiaPaca-2, colorectal HCT-116, leukemia Jurkat and cervical HeLa. In addition, **1a**, **1b** and **1b'** were tested against non-tumorigenic prostate RWPE-1 cell line. After 24 h of incubation, **1b** and **1b'** were moderately active against Jurkat and A-549 cells. The anti-proliferative effect of titanium compounds on prostate PC-3, DU-145 and RWPE-1 cell lines was also assessed after 72 h of drug exposure. The cytotoxic profile of the enantiomers was similar, exception made for the PC-3 cells, with *S,R*-isomers exhibiting cytotoxicities 2 to 3 times higher than *R,S*-compounds. Under these conditions, derivative **1b** showed calculated IC_{50} values better than those of Tacke´s Titanocene-Y (bis-[(*p*-methoxybenzyl)cyclopentadienyl]titanium(IV) dichloride) on both the prostate PC-3 and DU-145 cells. **1a** and **1b** cytotoxic behaviour shows certain selectiveness, with activities 2-4 times lower on normal prostate RWPE-1 than on cancer PC-3 cells. Furthermore, **1b** produces higher cytotoxicity on prostate PC-3, DU-145 and RWPE-1 cells than the additive dose of titanocene dichloride and pro-ligand **b·HCl**. Additionally, compound-DNA interactions have been investigated by equilibrium dialysis, Fluorescence Resonance Energy Transfer (FRET) melting assays and viscometric titrations, which suggest that these metal complexes and/or their hydrolysis products bind DNA either in the minor groove or externally.

1. Introduction

Since the successful introduction of cisplatin (*cis*-[$PtCl_2(NH_3)_2$]) as an anticancer drug, much effort has been devoted to investigation of the anticancer activity of other coordination and/or organometallic transition metal compounds [1-7]. The titanium derivatives titanocene dichloride ([$(\eta^5\text{-}C_5H_5)_2TiCl_2$], TDC) [8,9] and budotitane ([*cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV)]) [10,11] were the first metal compounds to enter clinical trials after platinum complexes. Although these derivatives showed promising properties in preliminary studies, they failed advanced clinical trials due to low antitumor efficacy *in vivo*, rapid hydrolysis and limited solubility in biological media [12-19]. Since then, a plethora of modified titanium based compounds have been synthesized and studied as potential antitumor agents [17-29].

The effect of stereochemistry on biological activity is of great importance in medicinal chemistry, as many of the biological targets are chiral [30,31]. The anticancer properties of chiral metal derivatives have been largely studied [32-46], but the role of the stereochemistry in the biological activity of non-platinum based compounds has been less investigated [22,47-61]. Effect of the absolute configuration on the anticancer efficiency of titanium compounds was firstly explored by Tshuva in 2010 [50]. The enantiomers of C_2-symmetrical Ti(IV) compounds with chiral diamine bis(phenolato) ligands showed different antitumor activities by factors of 2-4 on human colorectal (HT-29) and ovarian (OVCAR-1) carcinoma cells [50,51,56,60]. According to these results, the authors proposed that stereochemistry should be considered in the design, modification, and improvement of active compounds [60]. The same year, Baird published a family of enantiomerically pure titanocene derivatives bearing chiral alkylammonium groups, but a relationship between the anticancer activity and chirality could not be established due to the low cytotoxicity showed on the cancer cell lines

evaluated [62]. Enantiomer-dependent activity was found in chiral substituted titanocene compounds by Cini et al [22,58], with the (*S*,*S*) enantiomer of $Cp^R{}_2TiCl_2$ (Cp^R = η^5-$C_5H_4CH(CH_2CH_3)C_6H_5OMe$) being twice as active as the (*R*,*R*) isomer towards pancreatic, breast and colon cancer cell lines, after 24 h of treatment. Interestingly, lack of enantiomer recognition was observed at 72 h when screening the compounds in MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays.

Within this context, enantiomerically pure, naturally occurring terpenes are useful building blocks for asymmetric synthesis [63,64]. They are inexpensive and commercially available reagents in optically pure form, and easily tailored by stereoselective functionalization [65]. On the other hand, oxime groups are presented as excellent chemical modifiers, with a wide versatility of coordination modes going from mono κNO, κON, to dihapto κ^2N,O; either with *side on* or bridging coordination, which could offer an increased stability of the final compounds when bonded to Ti(IV) acid centres [66-68].

We have recently reported a new family of enantiopure cyclopentadienyl titanium(IV) compounds with amino-oximato ligands derived from *R*-limonene, of formula [(η^5-$C_5H_5)_2$Ti{(1*S*,*4R*)-κON,(R)NH}Cl] (R = Bn **1a**, 2-pic **1b**) (Fig. 1), with relevant antitumor properties. Our compounds show significant effects on cytotoxicity, cell adhesion to collagen and migration of androgen-independent prostate cancer cells while they do not seem to exhibit strong interactions with plasmid DNA by electrophoretic mobility shift assays. Compounds **1a** or **1b** suffered hydrolysis in water or phosphate buffered saline (PBS) solutions. However, the additive doses of TDC and **a·HCl** or **b·HCl** produced lower antiproliferative effects on prostate cancer PC3 cells than those observed after treatment with oximato titanocenes **1a** or **1b**, respectively. This fact led

us to the conclusion that the active operating titanium species was positively influenced by the presence of the oximato ligand [69].

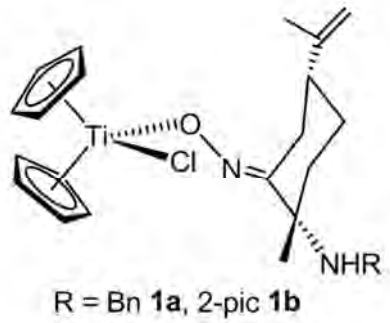

Fig. 1 Optically active titanocene compounds containing ligands derived from *R*-limonene

Encouraged by these previous results, we decided to explore the reactions of TDC with the already described amino-oxime chiral compounds (1*R*,4*S*)-{NH(R),NOH} (R = Bn **a'**, 2-pic **b'**, see Fig. 2) [65,70,71], derived from *S*-limonene.

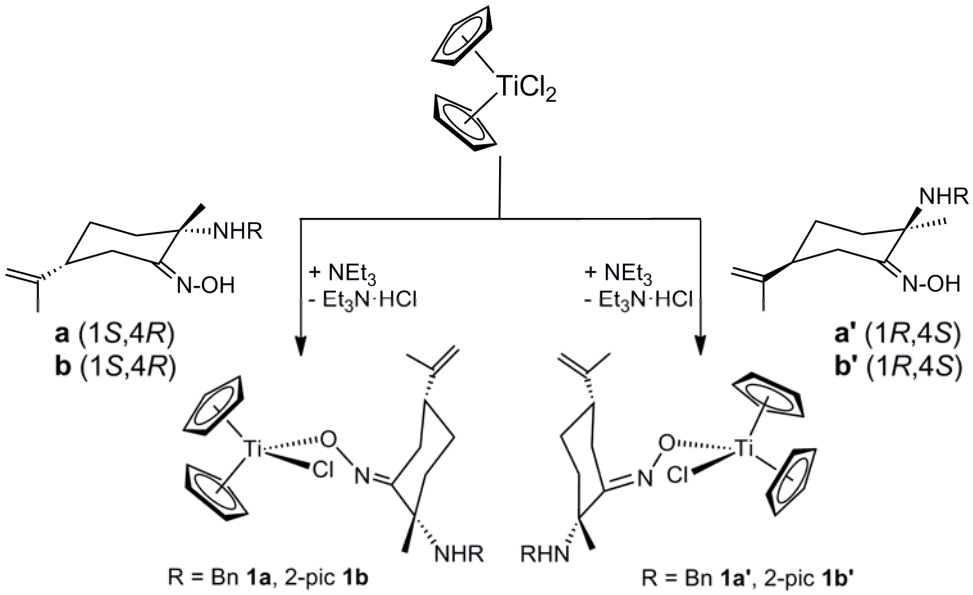

Fig. 2 Synthesis of optically active titanocene oximato compounds

We report here on the synthesis and characterization of corresponding cyclopentadienyl Ti(IV) enantiomers [(η^5-C_5H_5)$_2$Ti{(1*R*,*4S*)-κON,(R)NH}Cl] (R = Bn

1a', 2-pic **1b'**). Their hydrolytic behaviour has been studied by ^{1}H NMR, Ultraviolet-visible (UV-Vis) spectroscopy and circular dichroism (DC). These novel compounds along with those previously described have been evaluated against several cancer cell lines *in vitro*: prostate PC-3 and DU-145, lung A-549, pancreas MIA PaCa-2, colorectal HCT-116, leukemia Jurkat and cervical HeLa. In addition, the compounds were tested against the non-tumorigenic human prostate RWPE-1 cell line. DNA interactions of the metal derivatives and/or their hydrolysis products have been further investigated by FRET melting assays, equilibrium dialysis and viscometric titrations experiments.

2. Experimental Section

2.1. Chemicals and synthesis

Manipulations involving the synthesis of titanium compounds **1a**, **1a'**, **1b** and **1b'** and Titanocene-Y (bis-[(*p*-methoxybenzyl)cyclopentadienyl]titanium(IV) dichloride) were performed at an argon/vacuum manifold using standard Schlenk techniques or in a MBraun MOD System glove-box. Solvents were dried by known procedures and used freshly distilled. Titanocene-Y [72], (1*S*,4*R*)-{NH(R),NOH}, (R = Bn **a** [70], 2-pic **b**); (1*R*,4*S*)-{NH(R),NOH} (R = Bn **a'**; 2-pic **b'**); corresponding adducts (1*S*,4*R*)-{NH(R)·HCl,NOH}, (R = Bn **a·HCl**, 2-pic **b·HCl**); (1*R*,4*S*)-{NH(R)·HCl,NOH} (R = Bn **a'·HCl**, 2-pic **b'·HCl**) [63,73] and metal compounds [(η^5-C_5H_5)$_2$Ti{(1*S*,*4R*)-κON,(R)NH}Cl] (R = Bn **1a**, 2-pic **1b**) [69] were prepared according to previous reports. *R*- or *S*-limonene and isopentyl nitrite were reacted following the standard method described by Carman et al in 1977 [73]. *R*-limonene, *S*-limonene, TDC and cisplatin were purchased from Sigma-Aldrich. Commercially available reagents were used without further purification. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker 400 Ultrashield. ^{1}H and ^{13}C chemical shifts are reported relative to

tetramethylsilane. ^{15}N chemical shifts are reported relative to liquid ammonia (25 ºC). Coupling constants *J* are given in Hertz. Elemental analysis was performed on a LECO CHNS 932 Analyzer at the Universidad de Alcalá or, alternatively, at the Universidad Autónoma de Madrid. Fourier Transform Infrared (FT IR) spectra were recorded on IR FT Perkin Elmer (Spectrum 2000) spectrophotometer on KBr pellets. The pH was measured in a HANNA HI208 pHmeter in distilled water solutions. Circular Dichroism (CD) spectra were recorded on a J-715 CD spectropolarimeter (Jasco, UK) at ambient temperature (297 K). The spectra were determined at a concentration of 0.5 mM in water using a quartz cuvette of 0.5 cm path length, scan speed of 20 $nm \cdot min^{-1}$, 0.1 nm band width, 0.5 nm data pitch and 0.5 s of response time. Optical rotations of all the compounds solutions were recorded on a Perkin Elmer 341 polarimeter, using the sodium D line (589 nm) at ambient temperature (297 K) in a quartz cell of 1 dm path length. Specific optical rotation values were calculated according to the equation $[\alpha]^{24}_D = 100 \cdot \alpha_{obs}/l \cdot c$ [74]. Analytical balance and volumetric pipettes (2.0 mL) were used to prepare $CHCl_3$ solutions of the compounds at concentrations within a range of 7.50-7.80 $g \cdot dL^{-1}$. UV-Vis spectra were measured at room temperature on water solutions of the compounds with a Perkin Elmer Lambda 35 spectrophotometer.

2.1.1. (*1R*,4*S*)-{NH(2-pic),NOH} (**b'**). An analogous procedure to that described before for the synthesis of **b** [63] was used, starting from *S*-limonene [70,71,73]. $[\alpha]^{23}_D$ ($deg \cdot dm^{-1} \cdot cm^3 \cdot g^{-1}$) -126 ± 1.3 (**b'** at c = 0.7839 $g \cdot dL^{-1}$, α_{obs} = -0.957 deg); +127 ± 1.3 (**b** at c = 0.7604 $g \cdot dL^{-1}$, α_{obs} = +0.954 deg). All analytical and spectroscopic data are identical to those observed for **b**. Anal. Calcd. for $C_{16}H_{23}N_3O$: C, 70.30; H, 8.48; N, 15.37; Found: C, 70.13; H, 8.07; N, 15.20. FT IR (KBr, λmax/cm^{-1}): 3086-3314 (br, νOH/NH), 1650, 1598 (νC=N). UV-Vis (0.1 mM in H_2O:DMSO 99:1): λmax (ε): 261 (316), 340 (10). ^1H NMR (plus two dimensional correlation spectroscopy (COSY),

400.1 MHz, 293 K, chloroform-d_1): δ 9.80 (=NOH), 8.49, 7.60, 7.28, 7.11 (m, each 1H, NC_5H_4), 4.75 (br, 2H, $=CH_2$), 3.87, 3.61 (both d, each 1H, $^3J_{HH}$ = 6, -CH_2-C_5H_4N), 3.28 (d, 1H, $^2J_{HH}$ = 12, -CH_2^3), 2.60 (br, 1H, NH), 2.09 (m, 1H, -CH^4), 2.03 (dd, 1H, $^2J_{HH}$ = 12, $^3J_{HH}$= 3, -CH_2^3), 2,00, 1.69 (m, each 1H, -CH_2^6 + -CH_2^5), 1.85 (m, 1H, CH_2^6), 1.75 (s, 3H, CH_3-C=), 1.65 (m, 1H, CH_2^5), 1.32 (s, 3H, - CH_3-Cq-N). ^{13}C NMR (plus Attached Proton Test (APT), plus gradient Heteronuclear Single Quantum Coherence (gHSQC), plus Heteronuclear Multiple Bond Correlation (HMBC), 100.6 MHz, 293 K, chloroform-d_1): δ 162.4 (Cq=NOH, Cq is quaternary carbon), 161.1 (C_{ipso}-C_5H_4N), 148.9 (*C*=CH_2), 149.2, 136.8, 122.7, 122.1 (C_5H_4N), 109.6 ($=CH_2$), 56.9 (Cq-NH), 48.1 (CH_2-C_5H_4N), 45.0 (CH^4), 40.5 (-CH_2^6), 26.4 (-CH_2^5), 25.6 (-CH_2^3), 23.5 (-*C*H_3-CNH), 21.0 (*C*H_3-C=). ^{15}N NMR (gHMBC, 40.5 MHz, 293 K, chloroform-d_1): δ 346.7 (C=N-), 305.3 (C_5H_4N), 51.8 (-NHpic).

2.1.2. [(η^5-C_5H_5)$_2$Ti{(1*R*,*4S*)-*κ*ON,(Bn)NH}Cl] (**1a'**). An analogous procedure to that described for [(η^5-C_5H_5)$_2$Ti{(1*S*,*4R*)*κ*ON,(Bn)NH}Cl] [69] was followed, starting from TDC (0.20 g, 0.80 mmol), (1*R*,*4S*)-{NH(Bn),NOH} (0.22 g, 0.80 mmol) and NEt_3 (0.11 mL, 0.80 mmol). Compound **1a'** was obtained as a yellow-orange solid. Yield: 0.32 g (88%). $[\alpha]^{23}_D$ (deg·dm^{-1}·cm^3·g^{-1}) -88.9 ± 1.2 (**1a'** at c = 0.7602 g·dL^{-1}, α_{obs} = -0.676 deg), +89.2 ± 1.2 (**1a** at c = 0.7497 g·dL^{-1}, α_{obs} = +0.681 deg). Analytical and spectroscopic data of the compound are identical to those already reported [69]. Solubility in H_2O at 24 ºC (mM): 6.6 ± 0.2. Value of pH ([2.0 mM]) in H_2O at 24 ºC: 5.54. Anal. Calcd for $C_{27}H_{33}ClN_2OTi$: C, 66.88; H, 6.86; N, 5.78; Found: C, 66.80; H, 6.90; N, 5.76. FT IR (KBr, λmax/cm^{-1}): 3370 (m, NH), 1646, 1601 (both m, C=N). ^{1}H NMR (plus HSQC, plus HMBC, plus COSY, 400.1 MHz, 293 K, chloroform-d_1): δ 7.32 (m, 5H, -C_6H_5), 6.39, 6.39 (both s, each 5H, C_5H_5), 4.76, 4.74 (both s, each 1H,

=CH_2), 3.76, 3.55 (both m, each 1H, -CH_2Ph), 2.92 (m, 1H, -CH_2^3), 2.05 (m, 1H, -CH-C=), 1.90 (m, 1H, -CH_2^6), 1.72 (m, 1H, -CH_2^3), 1.68 (m, 1H, -CH_2^5), 1.59 (m, 1H, -CH_2^6), 1.56 (m, 1H, -CH_2^5), 1.25 (br, 1H, NH), 1.47, 1.25 (both s, each 3H, NC-CH_3 + CH_3C=). ^{13}C NMR (plus APT, plus gHSQC, plus HMBC, 100.6 MHz, 293 K, chloroform-d_1): δ 159.2 (Cq=N), 149.3 (=Cq-Me), 141.6 (C_{ipso}Ph), 128.7, 128.7, 127.2 (C_6H_5), 117.1, 117.1 (C_5H_5), 109.4 (=CH_2), 57.1 (Cq-NH), 47.3 (-CH_2Ph), 45.6 (-CH^4), 41.2 (-CH_2^6), 27.8 (-CH_2^3), 26.2 (-CH_2^5), 23.9, 21.3 (CH_3-CNH + CH_3-C=). ^{15}N NMR (gHMBC, 40.5 MHz, 293 K, chloroform-d_1): δ 398.9 (C=N), 60.0 (NHBn).

2.1.3. [(η^5-C_5H_5)$_2$Ti{(1*R*,4*S*)-κON,(2-pic)NH}Cl] **(1b').** An analogous procedure to that described for [(η^5-C_5H_5)$_2$Ti{(1*S*,4*R*)-κON,(2-pic)NH}Cl] [69] was followed, starting from TDC (0.30 g, 1.20 mmol), (1*R*,4*S*)-{NH(2-pic),NOH} (0.33 g, 1.20 mmol) and NEt_3 (0.11 mL, 1.20 mmol). Compound **1b'** was obtained as a yellow-orange solid. Yield: 0.35 g (60%). $[\alpha]^{23}_D$ (deg·dm^{-1}·cm^3·g^{-1}) -75.7 ± 1.2 (**1b'** at c = 0.7534 g·dL^{-1}, α_{obs} = -0.570 deg), +74.2 ± 1.2 (**1b** at c = 0.7772 g·dL^{-1}, α_{obs} = +0.570 deg). Solubility in H_2O at 24 ºC (mM): 15.7 ± 1.7. Value of pH ([2.0 mM]) in H_2O at 24 ºC: 5.22. Anal. Calcd for $C_{26}H_{32}ClN_3OTi$: C, 64.27; H, 6.64; N, 8.65; Found: C, 64.62; H, 7.25; N, 8.54. FT IR (KBr, λmax/cm^{-1}): ῡ 3304 (m, NH), 1640, 1591, 1569 (all s, C=N). ^{1}H NMR (plus HSQC, plus HMBC, plus COSY, 400.1 MHz, 293 K, chloroform-d_1): δ 8.50, 7.60, 7.30, 7.12 (all m, each 1H, -NC_5H_4), 6.38, 6.38 (both s, each 5H, C_5H_5), 4.77, 4.74 (both s, each 1H, =CH_2), 3.91, 3.70 (both m, each 1H, CH_2-C_5H_4N), 2.84 (m, 1H, -CH_2^3), 2.07 (m, 1H, -CH-C=), 1.98 (m, 2H, overlapped -CH_2^{6+3}), 1.78 (s, 3H, CH_3C=), 1.64 (m, 1H, -CH_2^6), 1.62 (m, 1H, -CH_2^5), 1.60 (m, 1H, -CH_2^5), 1.48 (br, 4H, NC-CH_3 + NH). ^{13}C NMR (plus APT, plus gHSQC, plus HMBC, 100.6 MHz, 293 K, chloroform-d_1): δ 157.6 (Cq=N), 148.1 (=Cq-Me), 160.2 ($C_{ipso}C_5H_4$N), 149.3, 136.7,

122.9, 122.9 (C_5H_4N), 117.1, 117.1 (C_5H_5), 109.6 (=CH_2), 48.5 (-***C***H_2-C_5H_4N), 45.3 (-CH^4), 41.1 (-CH_2^6), 27.7 (-CH_2^3), 26.2 (-CH_2^5), 23.9, 21.3 (*C*H_3-CNH + *C*H_3-C=). ^{15}N NMR (gHMBC, 40.5 MHz, 293 K, chloroform-d_1): δ 402.1 (C=N), 312.5 (C_5H_4N), 52.6 (NHpic).

2.1.5. ^{1}H NMR experiments at physiological pH. Phosphate buffered saline solution (PBS) was prepared according to Cold Spring Harbor Protocols (http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8247) using NaCl, KCl, Na_2HPO_4 and K_2HPO_4 in D_2O. Adjustment of pD (pD = pH* + 0.4, where pH* = pHmeter reading in D_2O) was carried out using a solution of DCl (0.01M) or NaOD (0.01M) in D_2O, with the help of a HANNA HI208 pHmeter. Titanium compounds were then dissolved in 2000 μL of the freshly prepared PBS, final pD measured (7.30-7.38) and time-dependent ^{1}H NMR spectra of 500 μL aliquots of final solutions were carried out at 25 ºC.

2.2. Single-crystal X-ray structure determination

Yellow crystals of pure enantiomer **1b** were grown from a hexane-toluene solution. The crystals were removed from the vial and covered with a layer of a viscous perfluoropolyether. A suitable crystal was selected with the aid of a microscope, mounted on a cryo-loop, and placed in the low-temperature nitrogen stream of the diffractometer. The intensity data sets were collected at 200 K on a Bruker-Nonius Kappa CCD diffractometer equipped with an Oxford Cryostream 700 unit. The molybdenum radiation (λ = 0.71073) was used in both cases, graphite monochromated, and enhanced with an MIRACOL collimator.

The structure was solved, using WINGX package [75], by intrinsic phasing methods (SHELXT) [76], and refined by least-squares against F^2 (SHELXL-2014/7) [77].

Crystals of **1b** were refined as a two-component inversion twin, and also had two independent molecules in the asymmetric unit with no significant differences. All non-hydrogen atoms were anisotropically refined. Positions of the amine hydrogen atoms, H(2) and H(21), were located in the difference Fourier map. H(2) was refined isotropically, while U_{iso} for H(21) was fixed with a value of 0.05. The rest of the hydrogen atoms were positioned and refined by using a riding model. *Crystal data for* **1b**: ($C_{26}H_{32}ClN_3OTi$), FW = 485.89, Monoclinic, space group $P2_1$, crystal dimensions (mm^3) 0.30 x 0.27 x 0.27, a = 10.470(1), b = 11.631(1), β = 91.53(1), c = 19.856(3) Å, V = 2417.2(5) $Å^3$, Z = 4, ρ_{calcd} = 1.335 g cm^{-3}, μ = 0.488 mm^{-1}, F(000) = 1024, θ range = 3.08 to 27.50 deg, no. of rflns collected = 42638, no. of indep rflns / R_{int} = 10939 / 0.074, no. of data / restraints / params = 10939 / 1 / 589, $R1$ / $wR2$ ($I>2\sigma(I)$) = 0.068 / 0.141, $R1$ / $wR2$ (all data) = 0.089 / 0.151, GOF (on F^2) = 1.167, Absolute structure parameter = 0.04(5). Final difference Fourier maps did not show peaks higher than 0.695 nor deeper than –0.329 $eÅ^{-3}$. CCDC-1572920 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

2.3. Cell culture, cytotoxicity assays and cell death analysis

2.3.1. Cell culture

The prostate androgen-unresponsive cancer cell line PC-3 was obtained from the American Type Culture Collection (Manassas, VA) and may be related to recurrent prostate cancers that have achieved androgen independence. All culture media were supplemented with 1% penicillin/streptomycin/amphoterycin B (Life Technologies, Barcelona, Spain). The culture was performed in a humidified 5% CO_2 environment at 37 °C. After the cells reached 70–80% confluence, they were washed with PBS, detached with 0.25% trypsin/0.2% ethylenediaminetetraacetic acid (EDTA) and seeded

at 30,000–40,000 cells·cm^{-2}. The culture medium was changed every 3 days. A549 (lung carcinoma) cells were maintained in high glucose DMEM (Dulbecco´s Modified Eagle´s Medium) and RWPE-1 (non-tumorigenic prostate) cells in DMEM/F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12), supplemented with 5% fetal bovine serum (FBS), 200 U·mL^{-1} penicillin, 100 µg·mL-1 streptomycin and 2 mM L-glutamine. DU-145 (prostate carcinoma), MIA PaCa-2 (pancreas carcinoma), HCT-116 (colorectal carcinoma), HeLa (cervical cancer) and Jurkat (leukemic cancer) cells were maintained in RPMI (Roswell Park Memorial Institute) 1640 medium supplemented with 5% FBS, 200 U·mL^{-1} penicillin, 100 µg·mL^{-1} streptomycin and 2 mM L-glutamine. Cultures were maintained in a humidified atmosphere of 95% air:5% CO_2 at 37 °C. Adherent cells were allowed to attach for 24 h prior to addition of compounds.

2.3.2. MTT Toxicity Assays

For toxicity assays, cells (5×10^4 for Jurkat cells and 10^4 for adherent cell lines) were seeded in flat-bottom 96-well plates (100 µL/well) in complete medium. Adherent cells were allowed to attach for 24 h prior to addition of cisplatin or tested compounds. Stock solutions of Titanocene-Y, TDC and ammonium-oxime pro-ligands were freshly prepared in 1% of dimethyl sulfoxide (DMSO) in water, while cisplatin and oximato titanium compounds were dissolved in culture medium. The stock solutions were then diluted in complete medium and used for sequential dilutions to desired concentrations. The final concentration of DMSO in the cell culture medium did not exceed 0.1%. Control groups with and without DMSO (0.1%) were included in the assays. Compounds were then added at different concentrations in quadruplicate. Cells were incubated with compounds for 24 h or 72 h, and then cell proliferation was determined by a modification of the MTT-reduction method. Briefly, 10 µL/well of [3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) (5 $mg \cdot mL^{-1}$ in PBS) was added, and plates were incubated for 1−3 h at 37 °C. Finally, formazan crystals were dissolved by adding 100 µL/well *i*PrOH (0.05 M HCl) and gently shaking. The optical density was measured at 550 nm using a 96-well multi-scanner auto-reader Enzyme-Linked Inmuno Sorbent Assay (ELISA).

2.4. DNA interaction studies

2.4.1. Equilibrium Dialysis

Duplex DNA from calf thymus (CT DNA), (Deoxyribonucleic acid, Activated, Type XV) was directly purchased from Sigma Aldrich and used as provided. Duplex-forming oligonucleotides ds17-1 (5'-CCA GTT CGT AGT AAC CC-3') and ds17-2 (5'-GGG TTA CTA CGA ACT GG-3') were acquired High Performance Liquid Chromatography (HPLC) -purified and desalted from Integrated DNA Technologies (IDT). Dialysis membranes (Spectra/Por® molecular porous membrane tubing MWCO: 3.5–5.0 kDa; 6.4 mm diameter) were purchased from Spectrum Laboratories Inc. Aqueous solutions of surfactant sodium dodecyl sulphate (SDS) (10%) were purchased from Sigma Aldrich. The buffer employed in this experiment was 10 mM phosphate buffer NaH_2PO_4/Na_2HPO_4, pH = 7.2, with either 10 mM or 100 mM NaCl. The solutions of DNA were prepared in the working phosphate buffer at 75 µM monomeric unit (mum.) concentrations, in base pairs. For the preparation of the short oligonucleotide solution, an annealing step was needed, with heating at 90 °C for 10 min and then gradually cooling to 25 °C during 3 h. The solutions were left at 4 °C overnight.

Dialysis bags, previously washed with milli-Q water, were filled with 75 µM (m.u.) of DNA duplex (200 µL each bag) and placed in a beaker containing 225 mL of ca. 2 µM solution of the tested compound. The beaker was covered with parafilm and aluminium foil and allowed to equilibrate during 24 h at room temperature. Experiments

were run, at least, in triplicate. Once the dialysis process had been completed, the solutions from each dialysis bag were transferred to Eppendorf tubes. The content of each bag was then mixed with an aqueous detergent solution (10%) to reach a 1% concentration (v/v) of SDS. The concentrations of free compound in the dialysate solution and compound in the dialysis bags were determined by absorbance measurements using the extinction coefficients of the metal complexes (determined in the presence and absence of the detergent) and apparent association constants were calculated [78].

2.4.2. DNA FRET melting assay

The DNA melting assay was performed on a quantitative PCR kit ABI PRISM® 7000 Sequence Detection System (Applied Biosystems) in a 96-well plate format (96-Well Optical MicroAmp® Reaction Plate, Applied Biosystems, Life Technologies Corporation). The oligonucleotide sequence employed in this experiment, F10T (5'-FAM-AGC TAT TA /sp18/ TA TA GCT ATA-TAMRA-3') was produced, HPLC-purified and desalted by IDT. FAM is 6-carboxyfluorescein and TAMRA is carboxytetramethylrhodamine. The buffer system used in this experiment was: 10 mM sodium cacodylate, 100 mM LiCl, (pH = 7.3). First, the duplex-forming oligonucleotide was dissolved in water (grade BPC) and a 50 μM stock solution was prepared, which was then diluted to 0.5 μM. Then, the diluted DNA solution was mixed with the working buffer (2x) and water Biotechnology Performance Certified (BPC) grade. The DNA solution was heated at 90 °C for 10 min, cooled down slowly for 3 h and left at 4 ºC overnight. Compounds to be tested were dissolved in water and approximately 1 mM stock solutions were prepared. The exact concentrations were checked by UV-Vis. Stock solutions were then diluted with buffer to obtain 50 μM solutions of each compound. In a 96-well microplate, DNA solutions were mixed with solutions of tested

compound and buffer to reach a total volume of 50 μL with a F10T concentration of 0.2 μM and a compound concentration ranging between 1 and 10 μM.

The experimental protocol consisted of an incubation for 5 min at 24 °C, followed by a temperature ramp with heating rate 1 °C/min. Fluorescence values corresponding to the fluorophore FAM at wavelength of 516 nm (after excitation at 492 nm) were collected at each degree of temperature. Afterwards, the fluorescence data were normalized, plotted against temperature (ºC) at each compound concentration, and T_m values were determined.

2.4.3. Viscometric titrations

Duplex DNA from CT (Deoxyribonucleic acid, Activated, Type XV) was purchased from Sigma Aldrich and used as provided. The buffer employed in this experiment was 10 mM phosphate buffer NaH_2PO_4/Na_2HPO_4, pH = 7.2. The viscosity measurements were performed in a Visco System AVS 470 at 25.00 ± 0.01 ºC, using a microUbbelohde (K = 0.01) capillary viscometer. 6 mL of DNA solution (0.4 mM in nucleotides) in phosphate buffer were equilibrated for 20 min at 25.00 ºC and then 20 flow times were registered. Small aliquots (30–50 μL) of solutions of metal complexes (1.6–2.3 mM) were added to the same DNA solution. Before each flow time registration, the solutions were equilibrated for 20 min to 25.00 ºC and then 20 flow times were measured. With the averaged time of the different flow time measurements and the viscometer constant, the viscosities (μ) for each point were calculated. The viscosity results were plotted as $(\mu/\mu_0)^{1/3}$, where μ_0 represents the DNA solution viscosity in the absence of the ligand, versus (r), representing the ratio [ligand]/[DNA].

2.5. Data analysis

Results were subjected to computer-assisted statistical analysis using One-Way Analysis of Variance ANOVA, Bonferroni´s post-test, and Student´s t-test. Data are

shown as the means of individual experiments and presented as the mean ± SD (Standard deviation). Differences of $P < 0.05$ were considered to be significantly different from the controls.

3. Results and Discussion

3.1. Synthesis and characterization of metal compounds

Synthesis of the novel Ti(IV) compounds was carried out analogously to that of previously described enantiomers **1a** and **1b** [69]. Treatment of TDC and amino-oxime derivatives **a'** or **b'** in the presence of NEt_3 allows isolation of novel chiral-at-ligand titanium compounds **1a'** or **1b'**, respectively (Fig. 2), which are formed together with $Et_3N \cdot HCl$.

Analytical and spectroscopic data of the novel compounds **1a'** and **1b'** are identical to those reported before for **1a** and **1b**, respectively (see ref [69], Experimental Section and Online Resource, Fig. S3-S9).

Calculated data of specific optical rotation in chloroform solution for the ligands and novel metal derivatives ($[\alpha]^{23}_D$ ($deg \cdot dm^{-1} \cdot dL \cdot g^{-1}$) = -127 ± 1.3 **a'**, +130 ± 1.3 **a**, -126 ± 1.3 **b'**, +127 ± 1.3 **b**, -88.9 ± 1.2 **1a'**, +89.2 ± 1.2 **1a**, -75.7 ± 1.2 **1b'**, +74.2 ± 1.2 **1b**) evidence the enantiomeric relationship of the stereoisomers. Furthermore, absolute configuration of the compound **1b** has been confirmed through X-ray structure determination (Fig. 3, and Online Resource Table S1, S2 and Fig. S16).

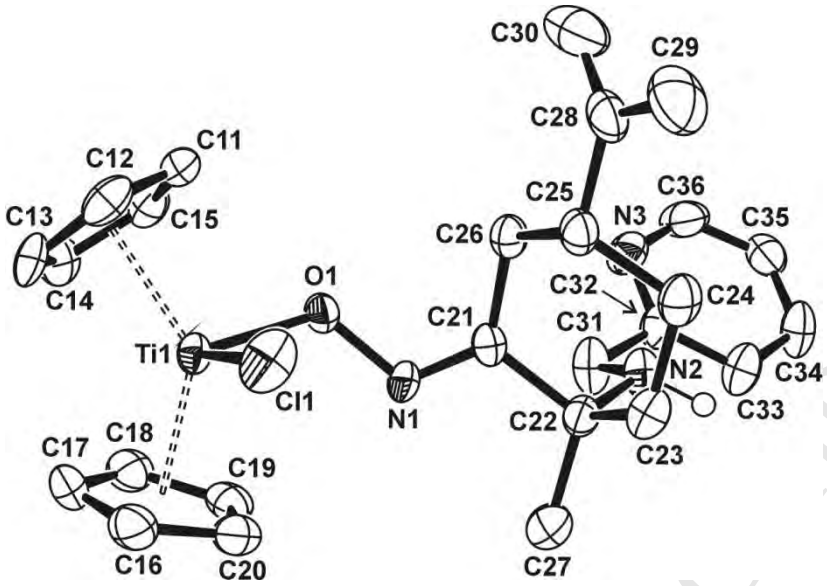

Fig. 3 ORTEP drawing of compound **1b** with 50% probability ellipsoids. Hydrogen bonded to carbon atoms have been omitted for clarity. Representative bond lengths (Å) and angles (deg): Ti(1)-Ct(1) 2.073; Ti(1)-Ct(2) 2.065; Ti(1)-Cl(1) 2.380(2); Ti(1)-O(1) 1.899(4); Ti(1)···N(1) 2.866(5); N(1)-O(1) 1.403(6); N(1)-C(21) 1.273(8); Cl(1)-Ti(1)-O(1) 92.4(2); Ti(1)-O(1)-N(1) 119.6(3); O(1)-N(1)-C(21) 114.1(5); Ct(1)-Ti(1)-Ct(2) 130.3; (Ct(1) is the centroid of the C(11)-C(15) ring, Ct(2) is the centroid of the C(16)-C(20) ring)

The X-ray crystal structure determination of **1b** shows the presence of two independent molecules in the asymmetric unit, with the same absolute configuration of the two chiral centers; an ORTEP diagram of one of them is presented in Fig. 3. The crystallographic study confirms a monohapto coordination of the oximato unit to the titanium atom. The compound shows a pseudotetrahedral environment around the metal centre, with Ti-O bond distances and O-N-C angles slightly shorter and closer (Online Resource Fig. S16), respectively, than those found in analogous biscyclopentadienyl

oximato titanium(IV) derivatives [66,68] or alcoxo oximato titanium(IV) compounds [79-82] with a dihapto κ^2NO coordination of the oximato unit to the titanium centre.

To the best of our knowledge, this is the first example found of an oximato titanium derivative with a terminal monohapto Ti-ON= coordination, where this coordination mode is probably caused by the large steric requirements of the functionalized cyclohexane residue. This terminal coordination may account for the hydrolysis suffered for the compounds in aqueous media. In contrast, dihapto titanocene oximato compounds $[(\eta^5\text{-}C_5H_5)_2Ti(H_2O)(\kappa^2O{=}NR)]^+$ (R = CMe_2; C_6H_{10}), reported by Thewalt et al [66], were described as surprisingly stable against air and water.

The reactions in water or PBS solutions of **1a** or **1b** were elucidated in a previous report and afford soluble ammonium-oxime pro-ligands (1*S*,4*R*)-{NH(R)·HCl,NOH} (R = Bn **a·HCl** or 2-pic **b·HCl**, respectively), together with aqua-oxo or –hydroxo biscyclopentadienyltitanium(IV) species [69,83,84] which are detected at least during the first three hours after dilution. The same behavior as that described before has now been observed for novel compounds **1a'** and **1b'** when their solutions in water-d_2 or PBS were studied by ^{1}H NMR spectroscopy (see Online Resource, Fig. S10).

We decided to further investigate the existence of an amino-oxime ligand containing Ti(IV) species, which could account for the observed stereoisomer-dependent cytotoxic behaviour of the compounds on the prostate cancer PC-3 cell line. Since UV-Vis spectroscopy is considered a more sensitive technique than NMR, we recorded time-dependent UV-Vis spectra for compounds **1a'** and **1b'** in PBS solution. Right after dilution, UV-Vis spectrum of **1a'** (Online Resource Fig. S13) and **1b'** (Fig. 4) shows two very broad absorption bands centered at 240 and 325, and 246 and 322 nm, respectively, ascribed to overlapping of LMCT bands due to cyclopentadienyltitanium aquo cations and the absorption bands corresponding to proligands **a'·HCl** and **b'·HCl**.

After 24 h, only the absorption bands assigned to **a'·HCl** or **b'·HCl**, at 250 and 332, and 260 and 332 nm, respectively, are detected. Similar UV-Vis spectra are obtained after 72 h. Analogous results were obtained when the compounds are diluted in pure water.

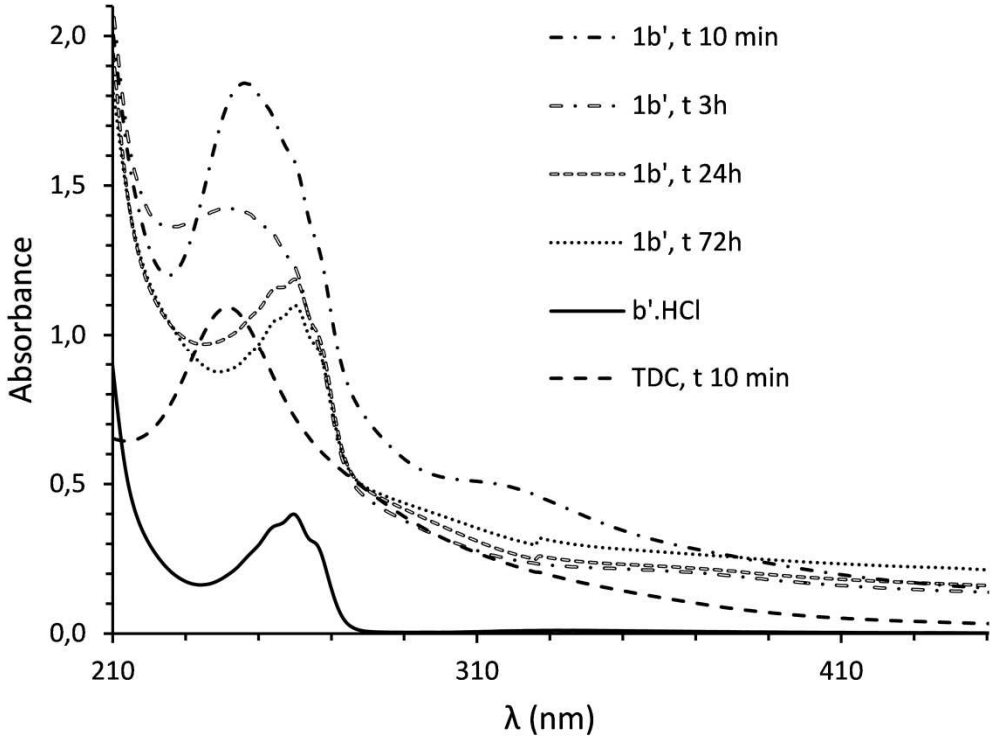

Fig. 4 Comparison of time-dependent UV-Vis spectra of **1b'** with **b'·HCl** and TDC spectra in PBS solution

CD spectra were also recorded for each pair of enantiomers. However, the spectra of derivatives **1a**, **1b** and **1a'**, **1b'** are identical to those obtained for ammonium-oxime compounds **a·HCl**, **b·HCl**, **a'·HCl**, **b'·HCl** (see Online Resource Fig. S14, S15), 15 min after dilution or after 72 h, leading to the assumption that those are the only detectable optically active, soluble in water products of the hydrolysis of titanium oximato compounds.

3.2. *In vitro* cell studies

3.2.1. Anti-proliferative studies

Chiral compounds **1a** and 1**b** have already shown their promising anticancer properties on the human prostate and renal cancer cell lines PC-3 and Caki-1. Both titanocenes, especially **1b**, are considerable less toxic to the non-tumorigenic human embryonic kidney cell line HEK-293T than to Caki-1 renal cells (7-15-fold less toxic) [69].

In order to compare and evaluate the versatility of the different enantiomers, the cytotoxic activity of pro-ligands **a·HCl**, **a'·HCl**, **b·HCl**, **b'·HCl** and metal compounds TDC, Tacke´s Titanocene-Y [72,85,86], **1a**, **1a'**, **1b** and **1b'** was now assessed after 24 h of incubation time on a wide variety of human cancer cell lines, i.e. prostate PC-3 and DU-145, lung A-549, pancreas MIA PaCa-2, colorectal HCT-116, leukemia Jurkat and cervical HeLa. The *in vitro* effect of the compounds on cytotoxicity was firstly evaluated by monitoring their ability to inhibit cell growth using the MTT assay.

Under these conditions, pro-ligands **a·HCl**, **a'·HCl**, **b·HCl**, **b'·HCl** and metal compounds **1a** and **1a'**, TDC, and Titanocene-Y are poorly cytotoxic in all tested cell lines (IC_{50} > 150 μM under these experimental conditions). Enantiomers **1b** and **1b'** are also not effective, after 24 h of exposure, in prostate PC-3, pancreatic MIA PaCa-2 or colon HCT-116 human carcinoma cell lines, but show inhibitory activities of 40-50% and 20-25% at concentrations of 50 μM against human lung carcinoma A-549 (Online Resource Fig. S17) and leukemia Jurkat-T cell lines respectively. Cell morphology evaluation of A-549 cells indicated that titanium derivatives **1b** and **1b'** did not induced apoptotic cell death, since no apoptotic cells, characterized by condensed nuclei and membrane blebbing, were detected. Cisplatin was included in the experiment as a positive control of apoptosis.

Since compounds **1a** and **1b** had shown to be efficiently cytotoxic on the PC-3 cell line after 72 h of incubation with the cells [69], we decided to assess the anti-proliferative effect of titanium compounds on prostate PC-3 and DU-145 cell lines as the IC_{50} value after 72 h of drug exposure. The results are summarized in Table 1.

Table 1. IC_{50} values (μM) of cisplatin, Titanocene-Y and enantiomers **1a**, **1a'**, **1b** and **1b'** in prostate cancer PC-3, DU-145 and non-tumorigenic RWPE-1 cell lines,[a] (n.m. not measured)

Compound	PC-3	DU-145	RWPE-1
1a	> 150 (24 h) 48.7 ± 3.2 (72 h)	> 150 (72 h)	> 200 (72 h)
1a'	> 150 (24 h) > 150 (72 h)	> 150 (72 h)	n.m.
1b	> 150 (24 h) 14.5 ± 3.1 (72 h)	27.1 ± 1.1 (72 h)	30.8 ± 0.57 (72 h)
1b'	> 150 (24 h) 49.9 ± 7.0 (72 h)	23.9 ± 8.6 (72 h)	43.8 ± 7.2 (72 h)
1b + 1b'	37.5 ± 5.1 (72 h)	n.m.	n.m.
Titanocene-Y	> 200 (24 h) 58.1 ± 11.2 (72 h)	> 150 (72 h)	42.9 ± 0.73 (72 h)
cisplatin	104.2 ± 8.1 (24 h) 14.5 ± 2.5 (72 h)	3.7 ± 0.6 (72 h)	19.9 ± 1.1 (72 h)

[a] Each value represents the mean ± S.D. (n = 3)

Under these conditions, the enantiomer **1b** shows IC_{50} values on the prostate PC-3 and DU-145 cell lines 2-5 times lower than Tacke´s compound, Titanocene-Y. The cytotoxicity on PC-3 cells of the titanium enantiomers **1a** and **1b**, with the absolute configuration *S,R*-, is higher than that of the *R,S*-stereoisomers by a factor of ca. 2-3, while the racemic mixture of **1b** and **1b'** afford IC_{50} values average between the two enantiomers. In contrast, no enantiomer recognition is observed on the prostate DU-145 cells for derivatives **1b**, **1b'**, while **1a**, **1a'** resulted not to be efficient in this non-hormone dependent cancer cell line.

Titanocene-Y has already shown an encouraging activity in PC-3 tumour-bearing mice [85]. Other titanium compounds which have proved their *in vitro* antitumor activity in prostate cancer cell lines under similar time exposure conditions are Schiff-base titanium (IV) derivatives [87] (IC_{50} values within the range 5-18 µM, in PC-3) or heterometallic titanocene-gold compounds (IC_{50} values ranged from 27-40 µM in PC-3 [88,89], and 11.8-27.6 µM in DU-145 [24,90].

In order to analyse the cytotoxic selectiveness to healthy cells, the isomers **1a**, **1b** and **1b'** were also tested in the non-tumorigenic human prostate (RWPE-1) cells. Regarding selectivity, **1a** and **1b** are less toxic to the non-tumorigenic RWPE-1 than to the cancer PC-3 cells (from 2 to 4 times less toxic), while **1b'** shows a similar behaviour relative to the cancer DU-145 cells.

Titanium compound **1b** was selected for a further study *in vitro*. We evaluated a combination of TDC and pro-ligand **b·HCl** on the cellular viability after 72 h of exposure to the drug. As already described in the PC-3 cell line,[69] the additive dose of both starting materials also produced lower anti-proliferative effects than those observed after treatment with only **1b** (Table 2) in the prostate DU-145 and RWPE-1 cell lines. These results are consistent with the involvement of metal oxime containing species in the cytotoxicity mechanism. While water soluble hydrolysis species detected in our studies are the same as those formed from a mixture of TDC and amino-oxime proligand, the existence of polinuclear, ligand influenced species formed in a colloidal phase of hydrolysis cannot be ruled out.

Table 2. Comparison of IC_{50} values (μM) of **1b**, **b·HCl**, TDC and TDC+**b·HCl** in prostate cancer PC-3, DU-145 and non-tumorigenic RWPE-1 cell lines[a] (after 72 h of exposure to the drug).

Compound	PC-3	DU-145	RWPE-1
1b	14.5 ± 3.1	27.1 ± 1.1	30.8 ± 0.57
b·HCl	> 100	106.1 ± 10.4	140.5 ± 23.0
TDC+**b·HCl**	39.5 ± 2.1	54.9 ± 13.5	> 150
TDC	> 150	> 150	> 150

[a] Each value represents the mean ± S.D. (n = 3)

3.3. DNA binding

To date, various distinct mechanisms have been proposed for titanium-based therapeutics. DNA binding is still thought to be one important potential mode of action for titanocene compounds, although interactions with DNA have been found to be generally very weak at physiological pH conditions [16,17]. The study of DNA interactions for these particular metal complexes does often represent an experimental challenge, since the compounds can easily hydrolyse in water solutions. That said, investigation in this area may be used to shed some light about the nature of the interactions that may partially account for the biological activity observed in physiologically relevant aqueous environments, albeit the results obtained should be interpreted cautiously. Our previous results showed that titanocenes **1a** or **1b** did not exhibit strong interactions with plasmid DNA by electrophoretic mobility shift assays, but the absence of a shift in the electrophoretic bands did not allow us to rule out DNA binding. Having established the interesting antitumor properties of metal compounds **1a**, **1a'**, **1b** and **1b'**, our aim with the study presented now was to further analyse and

compare the kind of potential interactions of the enantiomers with DNA, by using other techniques to complement previous studies.

Dialysis experiments, based on the fundamental thermodynamic principle of equilibrium dialysis [78,91], were performed to determine apparent binding constants between DNA and the metal compounds, following the protocol described by Chaires [78] with some modifications. As the DNA targets, we selected CT DNA and a short oligonucleotide duplex of known sequence (ds17, 17 bp).

Unfortunately, under the conditions employed, large dispersion data sets were obtained, which prevented the precise determination of association constants between the titanium(IV) compounds and DNA. This is likely to be a consequence of the hydrolysis of these complexes in aqueous media. However, even if the results should be interpreted with caution, a significant increase in compound concentration was invariably observed in the dialysis bags of replicate experiments, suggesting effective DNA binding by metal complexes **1a**, **1a'**, **1b**, **1b'** and/or their hydrolysis products.

With the purpose of determining the effect that these compounds may exert on the DNA denaturing temperature, Tm, we used a variable-temperature (FRET-melting) assay. This experiment requires little DNA consumption, allows the assessment of a wide range of compound concentrations, can be adapted to a high-throughput fashion, and it has been extensively used to determine the degree of thermal stabilization of different DNA structures in the presence of potential ligands [92]. Thus, FRET experiments were used to establish whether metal complexes **1a**, **1a'**, **1b** and **1b'** were able to thermally stabilize duplex DNA structures.

In these experiments, a 10-bp oligonucleotide (F10T) labelled with two fluorophores, FAM at its 5' end and TAMRA at the 3' end, was selected [93]. If the metal complex binds to DNA affecting the stability of the helix, changes in the value of DNA Tm

should be expected. Stabilization of duplex DNA usually results in increased values of Tm.

Compounds **1a**, **1a'**, **1b** and **1b'** were analysed for their ability to affect duplex DNA melting within the 1-10 μM concentration range. However, under these conditions, the titanium(IV) derivatives were not able to produce a significant change in the DNA melting temperature. Furthermore, none of the enantiomers of the precursor ligand, **a·HCl**, **a'·HCl**, showed DNA stabilization (see Online Resource Fig. S18). These results suggest that the compounds may interact with DNA in an external, mainly electrostatic fashion or through partial recognition of the DNA grooves.

Finally, DNA viscometric titrations were carried out because viscosity measurements can provide a simple way to discriminate between the different binding modes of potential DNA ligands (such as intercalation *versus* groove or external binding) [94]. According to the theory of Cohen and Eisenberg [95], from gradual titration of DNA solutions with the compounds of interest, linear plots of the cubed root of the relative DNA viscosity $(\eta/\eta_o)^{1/3}$ versus the molar ratio of bound ligand to DNA nucleotide (r) can be obtained. The slope values in these plots correlate well with the DNA-ligand binding modes. Groove binding compounds normally display a slope close to 0.0, whereas classical mono-intercalants result in a slope close to 1.0 [94,95].

The tested compounds showed a linear $(\eta/\eta_o)^{1/3}$ versus r correlation in the typical r range used in these experiments (Fig. 5). Complexes **1a**, **1a'**, **1b** and **1b'**, irrespective of the amino-bound ligand and the stereochemistry of the metal complex, gave rise to slope values practically equal to zero.

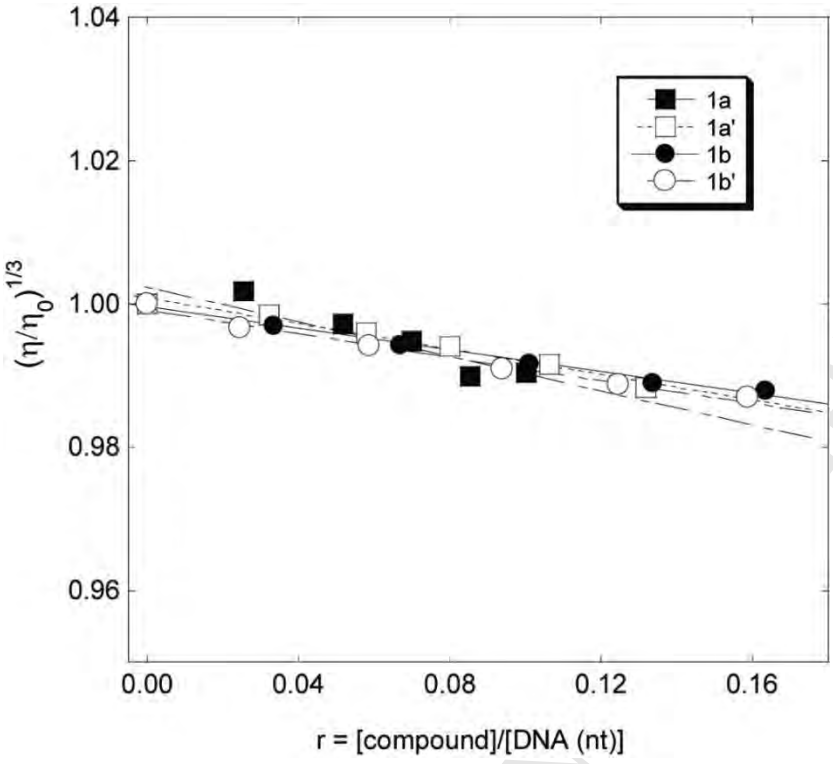

Fig. 5 Viscometric titrations of CT DNA and metal complexes **1a**, **1a'**, **1b** and **1b'** at 25 ºC (10 mM sodium phosphate buffer, pH 7.2)

These results are in good agreement with the FRET DNA melting assays and point towards an external or groove interaction of the titanium metal complexes and/or their hydrolysis products that does not result in overall changes of contour length or thermal stabilization of the DNA double helix structure.

4. Conclusions

Optically active amino-oxime ligands derived from natural products are useful and inexpensive starting materials for the design of enantiopure titanocene compounds. In contrast with the resistance to hydrolysis of other κ^2N,O oximato-Ti biscyclopentadiene compounds described before, our systems suffer hydrolysis in water at physiological conditions, most likely due to the monohapto κON coordination mode of the highly

sterically demanding limonene residue of the oximato ligand. Regarding their cytotoxic behaviour, the oxime-containing Ti(IV) compound **1b** has shown potent anticancer activities against both prostate cancer PC-3 and DU-145 cell lines after 72 h of incubation time. The cytotoxicity of the enantiomers **1a**, **1a'** and **1b'**, **1b'** towards all the cancer cell lines tested showed no significant differences, exception made for the PC-3 cells. In addition, isomers **1a** and **1b** showed certain selectivity in their toxicity against prostate cancer PC-3 versus non-tumorigenic RWPE-1 cells. Furthermore, compound **1b** shows higher activity than the additive dose of TDC and proligand **b·HCl** on the prostate PC-3, DU-145 and RWPE-1 cell lines. These results point towards the existence of an influence of the oximato-Ti unit on the hydrolysis process and/or the cytotoxicity mechanism. Compound-DNA interactions have been investigated by equilibrium dialysis, FRET melting assays and viscometric titrations. The experimental results suggest that these metal complexes and/or their hydrolysis products can bind DNA either in the minor groove or externally, irrespective of the ligand stereochemistry.

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Online Resource. Supplementary data associated with this article can be found in the online version, at http://. These data include: Representative NMR, UV-Vis and CD spectra of compounds **a**, **a'**, **b**, **b'**, **1a**, **1a'**, **1b**, **1b'**. Selected biological data. Selected crystallographic data and bond lengths and angles for X-ray molecular structures of **1b**.

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Highlights (85 characters)

- Synthesis of novel enantiopure titanocene amino-oximato compounds is reported.
- The X-ray crystal structure of one of the compounds shows a unique monohapto Ti-ON coordination.
- One enantiomer shows IC_{50} values lower than Titanocene-Y on both PC-3 and DU-145 cells.
- One enantiomer is more active than additive doses of $Ti(\eta^5\text{-}C_5H_5)Cl_2$ and oxime pro-ligand.