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Oxali-Titanocene Y: A Potent Anticancer Drug

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Outside of platinum and ruthenium anticancer drugs, there is significant unexplored space for further metal-based drugs that target cancer, such as titanocene dichloride.[1,2] One of these promising drug candidates is bis-[(*p*-methoxybenzyl)cyclopentadienyl]titanium(IV) dichloride (titanocene Y, **1**), which is accessible via the hydridolithiation reaction of 6-anisylfulvene and subsequent transmetallation with titanium tetrachloride.[3] Compound **1** exhibits an IC_{50} value of 21 μM toward the LLC-PK cell line, which has proven to be a good mimic of a kidney carcinoma cell line and a reliable tool for the optimisation of titanocenes against this type of cancer. Additionally, the antiproliferative activity of **1** and other titanocenes has been studied in 36 human tumour cell lines[4] and against explanted human tumours.[5,6] These in vitro and ex vivo experiments showed that renal cell cancer is the prime target for this novel class of titanocenes, but there is significant activity against ovarian, prostate, cervical, lung, colon, and breast cancers as well. Furthermore, titanocene derivatives give a positive immune response by up-regulating the number of natural killer (NK) cells in mice.[7] These results were underscored by initial mechanistic studies of the effects of these titanocenes on apoptosis and the apoptotic pathway in prostate[8] and cervical cancer cells.[9] Recently, animal studies demonstrated the successful treatment of mice bearing xenografted A431, Caki-1, and MCF-7 tumours with **1**.[9–11]

Herein the synthesis and initial cytotoxicity studies of titanocene oxalate and oxali-titanocene Y (**2**) are presented. The structures and syntheses of **1** and **2** are shown in Scheme 1.

A simple anion-exchange reaction in THF employing silver oxalate eliminates insoluble silver chloride and produces **2** or titanocene oxalate. An X-ray crystallographic study[12] established the molecular structure of **2** (Figure 1). Despite the addition of the oxalate bidentate ligand in replacement of the two chlorides on the titanium centre, there is almost no apparent variance in the molecular structures of **1** and **2**. The length of the bond between the titanium centre and the carbon atoms of the cyclopentadienide rings are very similar for both **1** and **2**. They vary from 2.34 to 2.41 Å for **1** and from 2.33 to 2.40 Å for **2**. Very slight differences can be observed in comparing the titanium centroid distances in **1** (2.06 Å) and **2** (2.04 Å).

The centroid–titanium–centroid angle is 130.7° for **1**, whereas the corresponding angle in **2** is 134.8°. This widening goes nicely with the more blade-like shape of the oxalate anion, compared with the spherical chloride anions.

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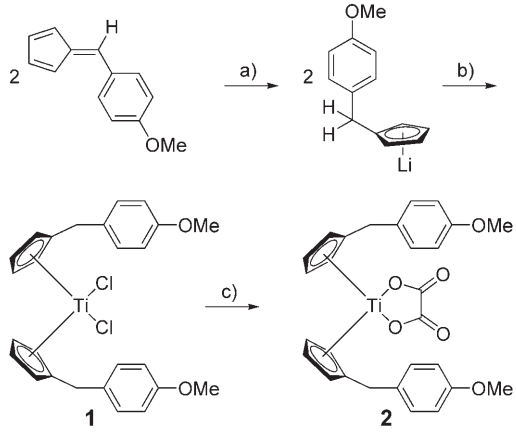

Scheme 1. Structures and syntheses of titanocene Y (**1**) and oxali-titanocene Y (**2**): a) 2 $LiBEt_3H$, Et_2O, −2 BEt_3; b) $TiCl_4$, THF, −2 LiCl(s); c) $Ag_2C_2O_4$, THF, −2 AgCl(s).

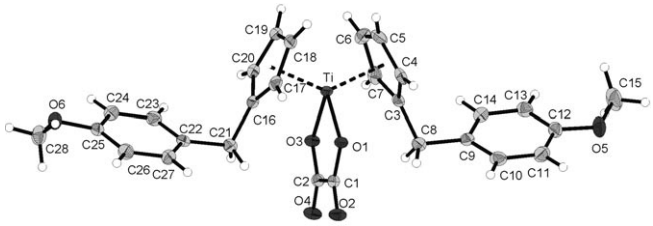

Figure 1. Molecular structure of **2**; (thermal ellipsoids are drawn on the 50% probability level).

As expected, the average titanium–chloride bond in **1** is considerably longer (2.37 Å) than the average titanium–oxygen bond in **2** (1.99 Å). The average carbon–oxygen single and double bonds in **2** are, with 1.30 Å and 1.21 Å, quite close to those found in free carboxylic acids, showing that there is very little, if any, negative charge located on the oxygen atoms that are not bonded to titanium.

In **1** the chloride–titanium–chloride angle is 95.9°, which is in the range of other titanocene dichlorides. In **1**, however, the oxygen atoms are part of a chelate ring which forces the oxygen–titanium–oxygen angle to 79.0°. Despite the addition of the bidentate oxalate ligand. minimal structural change incurred.

In tests against LLC-PK cells,[13] which have proven to be a valuable in vitro model for kidney cancer, titanocene oxalate shows an IC_{50} value of 140 μM, which is a significant improvement (14-fold) relative to titanocene dichloride, with an IC_{50} value of 2000 μM. Similar behaviour is observed for the *p*-dimethoxybenzyl-substituted species; **2** is 13-fold more cytotoxic against LLC-PK than **1** and exhibits an IC_{50} value of 1.6 μM (see Figure 2), which is twice as cytotoxic as cisplatin (3.3 μM[3]). The

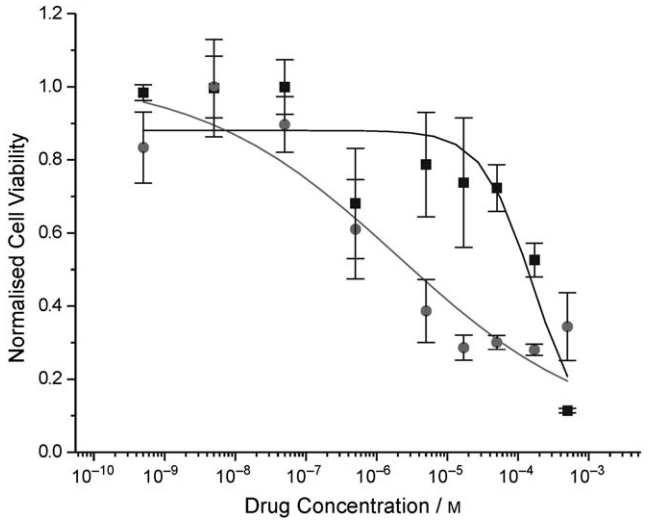

Figure 2. Cytotoxicity curves from typical MTT assays showing the effect of titanocene oxalate (■, IC_{50}: 140±48 μM) and **2** (●, IC_{50}: 1.6±3.7 μM) on the viability of LLC-PK cells.

cytotoxicity of **2** against LLC-PK is now >1000-fold greater than titanocene dichloride, and to the best of our knowledge, **2** is the most cytotoxic titanocene known so far.

After optimising the substitution pattern on the Cp ring of titanocene dichloride derivatives, we are ready to start to exchange the chlorides for more suitable anions, and oxalate is a clear choice. Oxali-titanocene Y will be tested for in vivo potential against renal-cell cancer in a xenograft mouse model in the near future.

Experimental Section

All reactions were carried out under a protective nitrogen atmosphere. The synthesis of silver oxalate is a variation of a published procedure.[14]

Silver oxalate, $Ag_2C_2O_4$: Oxalic acid dihydrate (0.80 g, 6.33 mmol) was dissolved in ethanol (30 mL) to give a colourless solution. To this solution, freshly distilled triethylamine (0.90 mL, 12.64 mmol) was added, which remained colourless. Silver nitrate (1.16 g, 6.80 mmol) was dissolved in acetonitrile (5 mL) and ethanol (30 mL) to give a colourless solution. The two solutions were added and shielded from light exposure with stirring for two hours. When the stirring was stopped, the white precipitate was allowed to stand for one more hour. The solution was filtered, and the white solid was dried in vacuo whilst being shielded from the light (0.77 g, 40.3% yield, 2.55 mmol). IR (KBr): $\tilde{\nu}$=1593, 1305 cm^{-1}; elemental analysis calcd (%) for $Ag_2C_2O_4$: C 7.9%, found: C 8.1%.

Titanocene oxalate, $Ti_{12}O_4H_{10}$: Silver oxalate (0.20 g, 0.62 mmol) and titanocene dichloride (0.12 g, 0.48 mmol) were added to a round-bottom flask, shielded from the light. THF (100 mL) was then added, and the solution was left to stir for 24 h. The solution was gravity filtered to give a red–orange coloured filtrate. The solvent was removed in vacuo to yield a red–orange solid (0.080 g, 59.4%, 0.29 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C, TMS): δ= 6.67 ppm (s, 10H, C_5H_5); ^{13}C NMR ($CDCl_3$, 100 MHz, proton decoupled): δ=122.7 (C_5H_5), 162.0 ppm (C_2O_4); IR (KBr): $\tilde{\nu}$=3453, 3081, 1697, 1331, 1020, 820, 767, 532, 349 cm^{-1}; elemental analysis calcd (%) for $TiC_{12}O_4H_{10}$: C 54.2, H 3.8, Cl 0, found: C 53.6, H 3.9, Cl 0.1.

2: Compound **1** (0.10 g, 0.20 mmol) and silver oxalate (0.08 g, 0.25 mmol) were added to a Schlenk flask, which was shielded from the light. Dry THF (40 mL) was added, and the reaction mixture was left to stir for six days at room temperature. The solution was allowed to stand for one hour and was filtered to leave a grey residue on the filter paper and a brown filtrate. The solvent was removed in vacuo to give a brown solid (0.050 g, 49.0% yield, 0.10 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.68 (s, 4H, C_5H_4-CH_2), 3.78 (s, 6H, C_6H_4-(OCH_3)), 6.19 (m, 4H, C_5H_4), 6.53 (m, 4H, C_5H_4), 6.83 (d, 4H, J=8.4 Hz, C_6H_4-(OCH_3)), 7.03 ppm (d, 4H, J=8.4 Hz, C_6H_4-(OCH_3)); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C, TMS): δ=33.9 (C_5H_4-CH_2), 54.3 (C_6H_4-(OCH_3)), 113.0, 113.1, 115.1, 118.9, 120.6, 121.2, 129.0, 129.1, 136.7, 142.6, 157.6, 161.9 ppm; IR (KBr): $\tilde{\nu}$=3437, 2918, 1691, 1610, 1512, 1327, 1249, 1177, 1095, 1034, 804, 538 cm^{-1}; UV/Vis (CH_2Cl_2): $\lambda_{max}(\varepsilon)$=248 (7500), 275 (6100), 307 (3600), 328 (3100), 350 (2400), 395 (1400), 422 nm (960); elemental analysis calcd (%) for $TiC_{28}O_6H_{26}$: C 66.4, H 5.2, Cl 0.0, found: C 65.9, H 5.7, Cl 0.2.

Acknowledgements

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Keywords: antitumor agents · fulvenes · hydridolithiation · oxalates · titanocenes

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[12] Crystal data for **2** ($C_{28}H_{26}O_6Ti$) at 100(2) K: 0.5×0.5×0.01 mm^3; orthorhombic; *Pbca*; a=19.813(2), b=8.1788(7), c=29.243(3) Å, U=4738.7(7) $Å^3$; ρ_{calcd}=1.420 mg m^{-3}; $2\theta_{max}$=52.00°; $Mo_{K\alpha}$ (λ=0.71073 Å); instrument: Bruker SMART APEX CCD diffractometer; ϕ-ω-scans; 26995 reflections collected, 4644 unique, R_{int}=0.036; μ=0.751 mm^{-1}, max and min transmission 0.8644 and 0.7227; solution: direct methods, refinement: full-matrix least-squares against $|F^2|$, 895 parameters; hydrogen atoms were located in the difference Fourier map and allowed to refine freely; R_1=0.050, wR_2=0.101 (all data); max/min residual electron density: 0.474/−0.182 e^- $Å^{-3}$. CCDC 664290 contains the supplementary crystallographic data for this paper. These data can be ob-

tained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

[13] The LLC-PK cell line was obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's medium (DMEM) containing foetal calf serum (FCS, 10% *v/v*), penicillin–streptomycin (1% *v/v*) and L-glutamine (1% *v/v*). Cells were seeded in 96-well plates containing 200-μL microtitre wells at a density of 5000 cells per 200 μL medium and were incubated at 37 °C for 24 h to allow exponential growth. Compounds for testing were then dissolved in the minimal amount of dimethyl sulfoxide (DMSO) possible and diluted with medium to obtain stock solutions at concentrations of 5×10^{-4} M, containing <0.7% DMSO. The cells were then treated with varying concentrations of the compounds and incubated for 48 h at 37 °C. The solutions were then removed from the wells, and the cells were washed with phosphate-buffered saline (PBS), and fresh medium was added to the wells. Following a recovery period of 24 h incubation at 37 °C, individual wells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 200 μL); the solution consisted of 30 mg MTT in 30 mL medium. The cells were incubated for 3 h at 37 °C. The medium was then removed, and the purple formazan crystals were dissolved in 200 μL DMSO per well, and the absorbance was read at $\lambda=$ 540 nm. Cell viability was expressed as a percentage of the absorbance recorded for control wells. The values used for the dose–response curves represent the values obtained from four consistent MTT-based assays for each compound tested.

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