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A trimethoxyphenyl substituted *ansa*-titanocene: A possible anti-cancer drug

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Abstract

Starting from 6-(3′,4′,5′-trimethoxyphenyl) fulvene (**1**) [1,2-di(cyclopentadienyl)-1,2-di-(3′,4′,5′-trimethoxyphenyl)ethanediyl] titanium dichloride (**2**) was synthesised. When titanocene **2** was tested against pig kidney carcinoma cells (LLC-PK), an inhibitory concentration (IC_{50}) of 9.0×10^{-4} M was observed.

Keywords: Anti-cancer drug; *cis*-Platinum; Titanocene; Fulvene; LLC-PK; DFT calculations

1. Introduction

Despite the resounding success of *cis*-platinum and closely related platinum antitumor agents, the movement of other transition-metal anti-cancer drugs towards clinical trials has been exceptionally slow [1–3]. Metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo show remarkable antitumor activity [4,5]. However, only titanocene dichloride has reached Phase I clinical trials so far, with a maximum tolerable dose of 315 mg/m^2 per week. The dose limiting effects of titanocene dichloride include nephrotoxicity and elevation of creatinine and bilirubin levels [6,7]. Unfortunately, the efficacy of Cp_2TiCl_2 in Phase II clinical trials in patients with metastatic renal-cell carcinoma [8] or metastatic breast cancer [9] was too low to be pursued. Nevertheless, little synthetic effort has been employed to increase the cytotoxicity of any titanocene dichloride derivatives [10–12], despite the existence of a novel synthetic method starting from titanium dichloride and fulvenes [13–16], which allows direct access to highly substituted *ansa*-titanocenes [17–20]. Recently, using this method we have synthesised [1,2-di-(cyclopentadienyl)-1,2-di-(4-*N*,*N*-dimethylaminophenyl) ethanediyl] titanium dichloride, which has an IC_{50} value of 2.7×10^{-4} M when tested for cytotoxic effects on the LLC-PK cell line [21]. It was followed by reports about heteroaryl [22] and methoxyphenyl [23] substituted *ansa*-titanocenes. This paper reports the synthesis of a novel 1,2-diarylsubstituted ethanediyl-*ansa*-titanium dichloride, which combines the reactivity of the titanium dichloride moiety with a trimethoxyphenyl substituted Cp ligand leading to an improved water solubility.

2. Experimental

Titanium tetrachloride (1 mol solution in toluene), *n*BuLi (*n*-butyl lithium, 2 mol solution in pentane) and

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3,4,5-trimethoxybenzaldehyde were obtained commercially from Aldrich Chemical Co. THF and toluene were dried over and distilled from Na/benzophenone prior to use. Cyclopentadiene was collected under an atmosphere of nitrogen from freshly cracked dicyclopentadiene and pyrrolidine was distilled under argon prior to use. Manipulation of air and moisture sensitive compounds was carried out using standard Schlenk techniques under an argon atmosphere. NMR spectra were measured on a Varian 300 MHz spectrometer. Chemical shifts are reported in ppm and are referenced to TMS. IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR Spectrometer employing a KBr disk.

The GCMS spectrum for fulvene **1** was measured on a FINNIGAN TRACE GCMS 2000 Series (70 eV) and 1×10^{-5} M solutions in ethyl acetate were used.

For mass spectrometric analysis of titanocene **2**, a stock solution of the sample was prepared by dissolving the compound in 0.5 ml dichloromethane. A 10-fold dilution of these solutions was made in acetonitrile and electrospray mass spectrometry was performed on a quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Waters Corp., USA) in a negative ion mode.

With a view to elucidate the structures, spectroscopic data, bonding properties and energies of formation, the application of theoretical methods is advantageous. For this purpose, the GAUSSIAN 98 Revision A11 [24] running under Red Hat Linux was used. DFT calculations were performed at the B3LYP level using the 6-31G* basis set for the species of interest.

2.1. 6-(3′,4′,5′-Trimethoxyphenyl)fulvene (*1*)

The synthesis of fulvene **1** was carried out under argon as outlined in reference [25]. Pyrrolidine (2.5 ml, 30.0 mmol) was added to a solution of 3,4,5-trimethoxybenzaldehyde (3.9 g, 20.0 mmol) and cyclopentadiene (4.1 ml, 60.0 mmol) in 30 ml of methanol. After this addition the solution turned from colourless to deep red. When TLC analysis (silica/dichloromethane) showed only one product band after 2 h, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was partitioned between 20 ml of ether and 40 ml water and extracted with a total of 3×20 ml ether. The combined organic extracts were washed with a saturated aqueous NaCl solution. The organic solution was dried over magnesium sulfate and the solvent removed under reduced pressure. The crude product was triturated with pentane. After solvent removal under reduced pressure a deep red/orange product was obtained. 3.8 g (85% yield wrt 3,4,5-trimethoxybenzaldehyde); m.p. 41.0–43.0 °C.

^{1}H NMR (δppm $CDCl_3$): 6.75, 6.65, 6.40 (C_5H_4, 4H m); 6.95 (C_6H_2, 2H s); 3.95 (*p*-OCH_3 and *o*-OCH_3, 9H s); 7.20 (Ph-C*H*-Cp, 1H s).

^{13}C NMR (δppm $CDCl_3$): 153.3, 144.7, 139.4, 138.3, 135.5, 132.3, 130.6, 127.3, 120.0, 108.0 (C_5H_4 and C_6H_2); 61.0 (*o*-OCH_3); 56.2 (*m*-OCH_3).

IR absorptions (cm^{-1} KBr): 3001 (m), 2937 (m), 1576 (s), 1503 (m), 1417 (m), 1329 (s), 1242 (m).

GCMS: 244.2 (M^+ 70%), 229.1 ($M^+ - CH_3$ 50%), 213.1 ($M^+ - OCH_3$ 40%), 201.2 ($M^+ - COCH_3$ 13%), 155.1($M^+ - CCH(C_5H_4)$ 17%), 115.1 ($M^+ - COCH_3)_3$ 100%).

Anal. Calc. for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60; Found: C, 72.31; H, 6.50%.

2.2. [1,2-Di(cyclopentadienyl)-1,2-di(3′-4′-5′-trimethoxyphenyl)-ethanediyl] titanium dichloride [1,2-(3′,4′,5′-(MeO)$_3$-C_6H_2)$_2$$C_2H_2${$\eta^5$-$C_5H_4$}$_2$]$TiCl_2$ (*2*)

$TiCl_4$ (6.25 mmol, 1 M in toluene) was added to 90 ml of dry toluene and 10 ml dry THF. The solution turned immediately from colourless to pale yellow. The solution was stirred and cooled down to −78 °C, and then was treated dropwise with *n*BuLi (7.8 ml, 12.5 mmol). The solution turned from yellow to brown during the addition. After this addition, the mixture was allowed to warm up slowly to r.t. and the solution finally turned black. After 20 h stirring, a solution of **1** (3.05 g, 12.5 mmol) in dry toluene was added to the solution of $TiCl_2 \cdot 2THF$ at r.t. under argon. It was then stirred under reflux for another 16 h. The solvent was removed under vacuum. The resulting black solid was extracted with 3×20 ml of chloroform and filtered on celite. The solvent was removed under vacuum and the residue dissolved in 8 ml of chloroform and filtered twice through Whatman No. 1 filter paper. The solvent was removed again under vacuum and the residue triturated with a total of 40 ml pentane to give 2.5 g (66% yield) black solid. The ratio of *trans* and *cis* isomers was 58–42%. The mixture cannot be purified or separated by column chromatography or crystallisation; therefore the elemental analysis shows some discrepancy between measured and calculated values and an X-ray crystal structure is not available.

^{1}H NMR (δppm $CDCl_3$): 6.36 (*cis*-C_6H_2, 4H s); 6.33 (*trans*-C_6H_2, 4H s); 7.23–6.00 (C_5H_4, 8H m); 5.34 (*trans*-PhC*H* Cp, 2H s); 4.63 (*cis*-PhC HCp, 2H); 3.80 (*cis-m*-CH_3, 6H s); 3.78 (*cis-m*-CH_3, 12H s); 3.74 (*trans-p*-CH_3, 6H s); 3.67 (*trans-m*-CH_3, 12H s).

^{13}C NMR (δppm $CDCl_3$): 153.3, 153.1, 152.8, 137.5, 137.0, 136.5, 135.8, 133.9, 129.0, 126.6, 120.3, 117.4, 116.5, 115.4, 109.7, 106.5, 105.9, 105.2 (*cis* and *trans*-C_6H_3 and C_5H_4); 60.9, 60.8 (*cis* and *trans-o*-O(CH_3)$_2$), 56.3, 56.2 (*cis* and *trans-m*-O(CH_3)$_2$); 54.4, 52.2 (*cis* and *trans*-PhCHCp).

IR absorptions (cm^{-1} KBr): 3104 (m), 2991 (s), 2976 (m), 2965 (s), 1583 (m), 1461 (m), 1412 (m), 1120 (m), 1000 (m), 821 (m).