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Alkenyl-substituted titanocene dichloride complexes: Stability studies, binding and cytotoxicity

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ABSTRACT

Four alkenyl-substituted titanocene dichloride complexes [Ti(η^5-C_5H_5){η^5-C_5H_4(CMeR(CH_2CH_2CH=CH_2))}Cl_2] (R = Me (**8**), Ph (**9**)) and [Ti(η^5-C_5H_5){η^5-C_5H_3(CMeR(CH_2CH_2CH=CH_2))($SiMe_3$)}] (R = Me (**10**), Ph (**11**)) have been synthesized and characterized.

The cytotoxic activity of **8**–**11** has been tested against human tumour cell lines from four different tissue origins [8505C (anaplastic thyroid cancer), DLD-1 (colon cancer), FaDu (head and neck cancer), A2780 (ovarian cancer) and A549 (lung carcinoma)] and compared with those of the reference complexes [Ti(η^5-C_5H_5)$_2Cl_2$] and cisplatin. The majority of the studied titanocene compounds are more active than the reference complex [Ti(η^5-C_5H_5)$_2Cl_2$] indicating that the presence of alkenyl substituents leads to an increase in the cytotoxic activity. In addition, the presence of a trimethylsilyl group on the cyclopentadienyl ring also leads to an increase in the cytotoxic activity of **10** with respect to **8**. The contrary is observed for **9** and **11** (except on the DLD-1 cell line) with **9** (without –$SiMe_3$) being more active than **11** (with –$SiMe_3$). However, all synthesized complexes, exhibited lower cytotoxic activity than cisplatin.

Stability and binding studies based on cyclic voltammetry and UV–visible spectroscopy have been carried out in order to explore possible interactions between titanocene derivatives and various intracellular molecules, such as the nitrogenous bases cytosine and thymine, the nucleotides adenosine and guanosine, and single-strand fish sperm DNA (FS-DNA). These experiments have allowed us to construct models to examine the interactions and action mechanisms of titanocene complexes inside the cells. In addition, this is one of the first studies on the interactions of titanocene derivatives with DNA fragments using cyclic voltammetry.

Introduction

A large number of metal compounds have shown antiproliferative activity against tumour cells in animals or *in vitro* cell cultures. However, in spite of their severe side effects, only platinum-based compounds have had a clinical significance in chemotherapy. Thus, many groups have focused their research on finding alternatives to platinum-based drugs, in order to minimize these negative side effects, by studying metal complexes of Au, Co, Ga, Ge, Pd, Ru, Sn and Ti [1–12].

In recent years, titanocenes have been tested in the treatment of certain types of cancer [13–15]. Cytotoxicity of titanocene dichloride was evaluated against animal and human tumours [16], and in spite of the stability problems in aqueous solutions of titanocene derivatives the high cytotoxicity of these compounds led to titanocene dichloride being tested in phase II clinical trials [17]. Unfortunately, titanocene dichloride is not effective against advanced renal and breast metastatic carcinomas [18]. Despite

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these drawbacks, many research groups, including our own, have focused their efforts on the synthesis of titanocene complexes incorporating different substituents and polar functional groups on the cyclopentadienyl rings [19–22], with the purpose of improving the cytotoxic activity of the corresponding compounds.

Further studies have revealed that titanium is able to bind to nucleic acids of tumour cells [23], hampering DNA replication and cancer propagation [24]. This fact suggests that DNA is probably one of the molecular targets of this type of drugs inside cells. Sadler and co-workers [25], found that titanium could enter into the cells assisted by the major iron transport protein, "transferrin", although transport into cancer cells *via* albumin interactions is also possible [26].

In this paper, we present the synthesis and characterization of alkenyl-substituted titanocene dichloride complexes. The cytotoxic activity has been tested against the human tumour cell lines, 8505C, DLD-1, FaDu, A2780 and A549. Hydrolysis and binding studies by UV–visible spectroscopy and cyclic voltammetry methods have been carried out in order to shed light on the possible action mechanism of the cytotoxic active titanocene complexes. The work reported here constitutes one of the first studies on the interactions of titanocene derivatives with DNA fragments using cyclic voltammetry.

Experimental

General manipulations

All reactions were performed using standard Schlenk tube techniques in an atmosphere of dry nitrogen. Solvents were distilled from the appropriate drying agents and degassed before use. Cyclopentadiene dimer, pyrrolidine, Li^{n}Bu (1.6 M in hexane), LiMe (1.6 M in Et_2O), LiPh (1.8 M in dibutylether), $SiMe_3Cl$ and $CH_3COCH_2CH_2CH{=}CMe_2$ were purchased from Aldrich and used directly. (C_5H_4)=CMe($CH_2CH_2CH{=}CH_2$) (**1**) and Li{C_5H_4(CMe_2($CH_2CH_2CH{=}CH_2$))} (**2**) and [Ti(η^5-C_5H_5){η^5-C_5H_4(CMe_2($CH_2CH_2CH{=}CH_2$))}Cl_2] (**8**) were synthesized as previously described by us [27]. [Ti(η^5-C_5H_5)Cl_3] was prepared by the reaction of $TiCl_4$ with $C_5H_5SiMe_3$ [28]. IR spectra were recorded on a Thermo Nicolet Avatar 330 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C{^{1}H} NMR spectra were recorded on a Varian Mercury FT-400 spectrometer. Microanalyses were carried out with a Perkin–Elmer 2400 microanalyzer. EI-MS spectroscopic analyses were performed on a MASPEC II system [II32/A302] (*m*/*z* 50–1000).

Synthesis of compounds

*Synthesis of Li{C_5H_4($CMePh$($CH_2CH_2CH{=}CH_2$))} (**3**)*

LiPh (10.75 mL, 19.35 mmol, 1.80 M in dibutylether) was added dropwise to (C_5H_4)=CMe($CH_2CH_2CH{=}CH_2$) (**1**) (2.57 g, 17.60 mmol) in hexane at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The solvent was removed *in vacuo* and the resulting white solid washed with hexane (2 × 50 mL) and dried under vacuum to yield a free-flowing white powder. Yield: 2.47 g, 78%. ^{1}H NMR (400 MHz, THF-d_8, 25 °C): δ = 1.62 (s, 3H, C*Me*Ph), 1.80–2.14 (m, 4H, $CH_2CH_2CH{=}CH_2$), 4.81 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{cis}, $^2J_{gem}$ = 2.4 Hz, $^3J_{cis}$ = 10.4 Hz), 4.97 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{trans}, $^2J_{gem}$ = 2.4 Hz, $^3J_{trans}$ = 17.2 Hz), 5.56 (m, 4H, C_5H_4), 5.80 (m, 1H, $CH_2CH_2CH{=}CH_2$), 6.98 (t, 1H, *H* in *para* position of Ph), 7.13 (m, 2H, *H* in *meta* position of Ph), 7.35 (d, 2H, *H* in *ortho* position of Ph) ppm. ^{13}C{^{1}H} NMR (100 MHz, THF-d_8, 25 °C): δ = 27.5 and 30.1 ($CH_2CH_2CH{=}CH_2$), 42.7 (C*Me*Ph), 43.3 (Cp*C*), 101.3, 101.5 and 153.4 (C_5H_4), 112.6 ($CH_2CH_2CH{=}CH_2$), 124.1, 126.7, 127.2 and 128.7 (C_6H_5), 140.4 ($CH_2CH_2CH{=}CH_2$) ppm. $C_{17}H_{19}Li$ (230.3): calcd C 88.67, H 8.32; found C 88.21, H 8.47%.

*Synthesis of C_5H_4(CMe_2($CH_2CH_2CH{=}CH_2$))($SiMe_3$) (**4**)*

$SiMe_3Cl$ (1.30 mL, 10.26 mmol) was added dropwise to a solution of Li{C_5H_4(CMe_2($CH_2CH_2CH{=}CH_2$))} (**2**) (1.40 g, 8.34 mmol) in tetrahydrofuran (THF) at 0 °C for 5 min. The reaction mixture was warmed to room temperature and stirred for 6 h. The solvent was then removed *in vacuo* to give an oily solid, which was extracted with hexane (2 × 50 mL). The mixture was filtered and hexane removed from the filtrate to give a yellow oily solid. Yield: 1.73 g, 89%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, for the predominant isomer): δ = −0.03 (s, 9H, Si*Me*$_3$), 1.18 (s, 6H, C*Me*$_2$), 1.59 (t, 2H, C*H*$_2$$CH_2CH{=}CH_2$), 1.94 (m, 2H, $CH_2CH_2CH{=}CH_2$), 3.24 (s, 1H, *H*C_5), 4.90 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{cis}, $^2J_{gem}$ = 1.2 Hz, $^3J_{cis}$ = 10.2 Hz), 4.97 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{trans}, $^2J_{gem}$ = 1.2 Hz, $^3J_{trans}$ = 17.2 Hz), 5.82 (m, 1H, $CH_2CH_2CH{=}CH_2$), 6.06, 6.45 and 6.59, (m, each 1H, C_5H_3) ppm. ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 °C, for the predominant isomer): δ = −1.7 (Si*Me*$_3$), 28.3 and 29.6 ($CH_2CH_2CH{=}CH_2$), 35.4 (C*Me*$_2$), 42.4 (Cp*C*), 50.6 (C^1–C_5H_4), 113.8 ($CH_2CH_2CH{=}CH_2$), 140.0 ($CH_2CH_2CH{=}CH_2$), 125.3, 130.3, 133.9 and 154.1 (C_5H_4) ppm. $C_{15}H_{26}Si$ (234.5): calcd C 76.84, H 11.18; found C 76.54, H 11.09%.

*Synthesis of C_5H_4($CMePh$($CH_2CH_2CH{=}CH_2$))($SiMe_3$) (**5**)*

The preparation of **5** was carried out in an identical manner to **4**. $SiMe_3Cl$ (0.98 mL, 7.73 mmol) and Li{C_5H_4($CMePh$($CH_2CH_2CH{=}CH_2$))} (**3**) (1.48 g, 6.44 mmol). Yield: 1.78 g, 93%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, two principal isomers): δ = 0.00 and 0.01 (s, each 9H, Si*Me*$_3$), 1.54 and 1.55 (s, each 3H, C*Me*Ph), 1.84–2.07 (m, 8H, $CH_2CH_2CH{=}CH_2$), 3.26 and 3.28 (s, each 1H, *H*C_5), 4.90–5.00 (m, 4H, $CH_2CH_2CH{=}CH_2$), 5.80 (m, 2H, $CH_2CH_2CH{=}CH_2$), 6.19–7.29 (m, 16H, C_5H_3 and C_6H_5) ppm. ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 °C, two principal isomers): δ = −1.6 and −1.5 (Si*Me*$_3$), 26.6, 26.9, 29.4 and 29.9 ($CH_2CH_2CH{=}CH_2$), 40.8 and 40.9 (C*Me*Ph), 43.5 and 43.5 (Cp*C*), 50.4 and 50.5 (C^1–C_5H_4), 114.1 and 114.2 ($CH_2CH_2CH{=}CH_2$), 139.6 and 139.6 ($CH_2CH_2CH{=}CH_2$), 125.7, 125.7, 131.5, 131.6, 133.8, 133.9, 153.5 and 153.6 (C_5H_4), 126.2, 126.3, 126.6, 126.8, 127.0, 127.2, 128.0 and 128.2 (C_6H_5) ppm. $C_{20}H_{28}Si$ (296.5): calcd C 81.01, H 9.52; found C 80.88, H 9.49%.

*Synthesis of Li{C_5H_3(CMe_2($CH_2CH_2CH{=}CH_2$))($SiMe_3$)} (**6**)*

Li^{n}Bu (5.10 mL, 8.16 mmol, 1.60 in hexane) was added dropwise to a solution of C_5H_4(CMe_2($CH_2CH_2CH{=}CH_2$))($SiMe_3$) (**4**) (1.73 g, 7.42 mmol) in hexane at −78 °C. The mixture was warmed to 25 °C and stirred for 16 h. Solvent was then removed *in vacuo* giving a white solid, which was washed with hexane (2 × 50 mL) and dried under vacuum to yield the title compound. Yield: 1.59 g, 89%. ^{1}H NMR (400 MHz, THF-d_8, 25 °C): δ = 0.10 (s, 9H, Si*Me*$_3$), 1.21 (s, 6H, C*Me*$_2$), 1.58 (m, 2H, C*H*$_2$$CH_2CH{=}CH_2$), 1.92 (m, 2H, CH_2C*H*$_2$$CH{=}CH_2$), 4.77 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{cis}, $^2J_{gem}$ = 2.4 Hz, $^3J_{cis}$ = 10.0 Hz), 4.88 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{trans}, $^2J_{gem}$ = 2.4 Hz, $^3J_{trans}$ = 19.2 Hz), 5.77–5.84 (m, 4H, $CH_2CH_2CH{=}CH_2$ and C_5H_3) ppm. ^{13}C{^{1}H} NMR (100 MHz, THF-d_8, 25 °C): δ = 0.8 (Si*Me*$_3$), 30.1 and 30.3 ($CH_2CH_2CH{=}CH_2$), 34.60 (C*Me*$_2$), 45.5 (Cp*C*), 104.0, 106.6, 107.4, 110.1 and 132.0 (C_5H_3), 112.3 ($CH_2CH_2CH{=}CH_2$), 140.8 ($CH_2CH_2CH{=}CH_2$) ppm. $C_{15}H_{25}LiSi$ (240.4): calcd C 74.95, H 10.48; found C 74.51, H 10.22%.

*Synthesis of Li{C_5H_3($CMePh$($CH_2CH_2CH{=}CH_2$))($SiMe_3$)} (**7**)*

The preparation of **7** was carried out in an identical manner to **6**. Li^{n}Bu (4.13 mL, 6.61 mmol, 1.60 M in hexane) and C_5H_4($CMePh$($CH_2CH_2CH{=}CH_2$))($SiMe_3$) (**5**) (1.78 g, 6.01 mmol). Yield: 1.14 g, 63%. ^{1}H NMR (400 MHz, THF-d_8, 25 °C): δ = 0.09 (s, 9H, Si*Me*$_3$), 1.62 (s, 3H, C*Me*Ph), 1.90 (m, 2H, C*H*$_2$$CH_2CH{=}CH_2$), 2.11 (m, 2H, CH_2C*H*$_2$$CH{=}CH_2$), 4.81 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{cis}, $^2J_{gem}$ = 2.4 Hz, $^3J_{cis}$ = 10.0 Hz), 4.91 (dd, 1H, $CH_2CH_2CH{=}CH_2$, H_{trans}, $^2J_{gem}$ = 2.4 Hz, $^3J_{trans}$ = 17.0 Hz), 5.70–5.83 (m, 4H, $CH_2CH_2CH{=}CH_2$ and C_5H_3), 6.98 (t, 1H, *H* in *para* position of Ph), 7.15 (t, 2H, *H* in *meta*