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Novel achiral indole-substituted titanocenes: Synthesis and preliminary cytotoxicity studies

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ABSTRACT

A series of eight new titanocene dichloride derivatives has been synthesised and characterized. Four compounds from the series are lypophilic indole-functionalised titanocenes and four are hydrochloride salts of their dimethylaminomethyl-functionalised counterparts, which are water soluble. The compounds were tested for their *in vitro* cytotoxicities against the human kidney cancer cell line CAKI-1 and their results are compared with previously synthesised structural analogues. Surprisingly, two of the compounds showed no activity against the CAKI-1 cell line; however six compounds exhibited medium to high potency with IC50 values as low as 7.0 μM. These six complexes were tested further on this cell line using the co-solvent Soluphor P, which has been shown to improve both solubility and cytotoxicity of similar complexes. One of the compounds carrying a N-methylindole-substituent was obtained in the form of single crystals and allowed for the characterisation by X-ray crystallography; the compound crystallised in the space group $P2_1/n$ (#14) with four molecules present in the monoclinic unit cell.

1. Introduction

Ever since the discovery of cisplatin for the treatment of a variety of tumours, there has been an increased effort devoted to the identification and clinical development of novel organometallic compounds which overcome cross resistance in patients and have more favourable toxicity profiles [1,2]. Despite this effort, movement of other transition-metal antitumour agents toward the clinic has been extremely slow. This is perhaps due to the assumption that organometallic chemistry and biology are mutually incompatible with many organometallic compounds being sensitive to water and oxidation. Research over the last decade by Alberto [3] and Jaouen [4] has shown that organometallic pharmaceuticals can infact be formulated.

Köpf first identified the antitumour activity of metallocenes [5], most notably of which is titanocene (bis-cyclopentadienyl titanium) dichloride or Cp_2TiCl_2 and has been investigated for use against various animal and xenografted human tumours including fluid and solid sarcoma 180 [6], colon 38 and B16 melanoma [7] and fluid and solid Ehrlich ascites tumour (EAT) [8]. Based on these encouraging results titanocene dichloride was the first non-platinum metal complex to enter phase I clinical trials in 1993 [9]. The compound exhibited hydrolytic instability under a pH of 5, which held back the identification of the active species responsible for antitumour activity. A formulation of titanocene dichloride in malic acid was required for administration of the compound in clinical trials [10]. The maximum tolerated dose was an IV injection of 560 mg/kg, contrary to preclinical trials where liver toxicity was the dose-limiting effect, renal toxicity was identified as the dose-limiting side effect. Two minor responses out of the 40 patients were observed, those with bladder carcinoma and non-small cell lung cancer [10]. Despite this low rate of success the compound proceeded to phase II clinical trials in 1998 for patients with metastatic renal cell Carcinoma [11]. Previous to this study Kurbacher et al. showed that the *in vitro* activity of titanocene dichloride in renal cell carcinoma (RCC) specimens was more favourable over conventional antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine [12]. The results from phase II trials indicated that using titanocene dichloride as a single agent in chemotherapy was not sufficiently promising to warrant further studies and titanocene dichloride has been discontinued from further clinical trials.

As previously stated, a limiting property of titanocene dichloride is its very low aqueous solubility, generally requiring formulation for administration as a drug. Derivatives of titanocene dichloride can be

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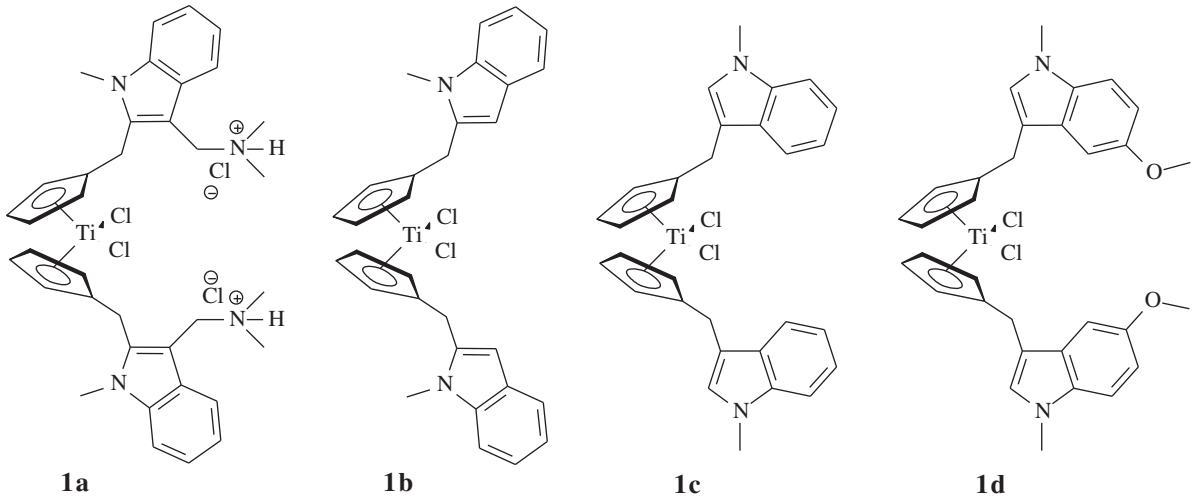


Fig. 1. Previously synthesised indole-substituted titanocenes.

generated either by substitution of the chloride anions or through alteration of the cyclopentadienyl ring to increase water solubility. One approach has involved the substitution of the chlorides anions by thiols, this improved the hydrolytic stability of the compound and increased cell growth inhibition in both the HeLa and COLO 320DM cell lines [13]. Baird [14,15] and McGowan [16,17] have both exploited the use of aryl and alkyl ammonium salts to produce water soluble derivatives of titanocene dichloride. These compounds were shown to have significantly better activity and stability than their nonfunctionalized counterpart titanocene dichloride and gave a potent cytotoxic effect on cisplatin-resistant ovarian tumour cell lines. Another important observation made by both groups is the fact that the dicationic analogues demonstrated quite significant potency when compared to their monocationic counterparts [18]. Gansäuer et al. synthesised water soluble carbonyl-substituted titanocenes and tested their activity for apoptosis on a range of cells including A375, MCF-7 cells and Jurkat cells. These compounds showed promising *in vivo* activity on SCID mice bearing human lymphomas [19]. Melëndez et al. have showed that incorporation of various steroidal esters into titanocene dichloride have potential as vectors for anticancer compounds such as these [20].

In an attempt to exploit this alkyl ammonium substituent for the development of new water soluble drug candidates, we recently reported the use of indole-substituted titanocenes [21]. Substitution of the Cp ring with a gramine side arm was the most logical choice for this type of compound to give a dihydrochloride derivative of bis[(1-methyl-3-dimethylaminomethylindol-2-yl)cyclopentadienyl]titanium(IV) dichloride **1a** (Fig. 1). This compound was tested on the CAKI-1 cell line, to give an IC50 value of 13 μM when tested without any solubilising agent, and 8.2 μM when 0.7% DMSO was used as a co-solvent, which is five-fold increase in cytotoxicity when compared to indole-substituted titanocene **1c**. A complimentary study also carried out within our research group was to compare the cytotoxic behaviour of compound **1a** with different formulation reagents such as the non-ionic surfactants Cremophor EL and Tween 80, pegylated reagents PEG-400 and Solutol HS 15, and the co-solvent Soluphor P (Fig. 2) [22]. It was found that Soluphor P increased the cytotoxicity of compound **1a** ten-fold to give an IC50 value of 0.71 μM against CAKI-1 cells *in vitro*.

This paper is now reporting the synthesis and cytotoxicity of achiral indole-substituted titanocene dichloride derivatives, which explore structural analogues of compounds **1a** and **1b**. Where these compounds were substituted to the CP on the 5-membered ring of the indole, we wished to investigate substitution of the CP to all available positions of the indoles 6-membered ring. Structure and shape of complexes such as these is of utmost importance for biological activity. Varying the position of attachment of the CP ring would give us an insight into the difference in activity of these compounds and allow us to build on these results for future work. Both the indole-substituted titanocenes and corresponding "gramine" substituted titanocenes have been synthesised for comparison purposes.

2. Results and discussion

2.1. Synthesis

1H-Indole-4-carbaldehyde, 1H-Indole-5-carbaldehyde and 6-Bromo-1H-indole were available commercially and used as received. The methylation of indoles was achieved by N-alkylation with NaH and methyliodide [23]. Introduction of an aldehyde group into indoles **3c** and **3d** was achieved using a halogen-lithium exchange with *n*-BuLi followed by a DMF quench and subsequent work up in aq HCl [24]. The Bartoli indole synthesis, whereby 2-nitrobromobenzene and three equivalents of vinyl magnesium bromide were used to generate indole 2, Scheme 1 [25]. The Mannich reaction, whereby an indole is reacted with formaldehyde and dimethylamine in the presence of acetic acid was used to incorporate the dimethylaminomethyl functionality to the four indoles **3a**–**d** to give compounds **3e**–**h** [26] (Fig. 3).

The synthesis of fulvenes **4a**–**h** (Fig. 4) from aldehydes **3a**–**h** is based on a modified version of the Stone and Little method [27] and

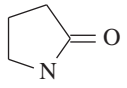
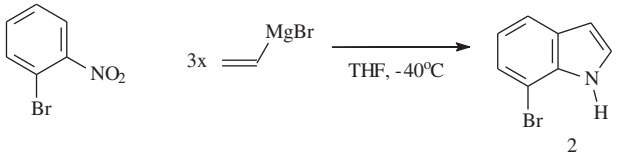


Fig. 2. Soluphor P.



Scheme 1. Bartoli synthesis of indole 2.