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Organotin(IV) derivatives with 5,7-disubstituted-1,2,4-triazolo [1,5-*a*]pyrimidine and their cytotoxic activities: The importance of being conformers

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ABSTRACT

The organotin(IV) compounds $Me_2SnCl_2(dbtp)$(**1**), $Me_2SnCl_2(dbtp)_2$ (**2**), $Et_2SnCl_2(dbtp)$ (**3**), $Et_2SnCl_2(dbtp)_2$ (**4**), $Et_2SnCl_2(dptp)$ (**5**), $^nBu_2SnCl_2(dbtp)_2$ (**6**), $^nBu_2SnCl_2(dptp)$ (**7**), $Ph_2SnCl_2(dbtp)$ (**8**), $Ph_2SnCl_2(EtOH)_2(dptp)_2$ (**9**), where **dbtp** = 5,7-di-*tert*-butyl-1,2,4-triazolo[1,5-*a*]pyrimidine and **dptp** = 5,7-diphenyl-1,2,4-triazolo [1,5-*a*]pyrimidine, have been tested by MTT for their cytotoxic activity on three tumor cell lines, HepG2 (human hepatocellular carcinoma), HeLa (human cervix adenocarcinoma) and MCF-7 (human breast cancer). Except for **1** and **2**, which were ineffective, all compounds significantly showed a dose-dependent anti-proliferative effect against the three cell lines. By calculated IC_{50} values, the cytotoxicity of the complexes followed the order ^{n}Bu > Ph > Et > Me for all the selected tumor cells. The cell death of HepG2, induced by organotin(IV) compounds **6–9**, was considered to be apoptotic by measuring the exposure of phosphatidylserine to the outer membrane and observing the typical apoptotic morphological change by acridine orange/ethidium bromide staining. Flow cytometric analysis of propidium iodide-stained cells also demonstrated that organotin(IV) complexes caused apoptosis of HepG2 cells through cell arrest at G0–G1 phase. The crystal structure of **7**, investigated by X-ray diffraction study, exhibited a distorted trigonal bipyramidal geometry with N, Cl as axial atoms and Cl and butyl groups in the equatorial plane. The triazolopyrimidine unit coordinates to the Sn atom through N(3) in a monodentate mode. Two conformational isomers (molecule **A** and **B** in the crystallographic independent unit) are co-crystallized in the solid state, a phenomenon that has been observed only occasionally. Conformational mobility of the cytotoxic complex **7** can sum up to the ligands ability to form H-bonds and $\pi\cdots\pi$ stacking, facilitating its intracellular uptake.

1. Introduction

The chemistry of organotins has attracted much attention in recent years, owing to potential biological and industrial applications. The organotin(IV) compounds have shown a wide variety of biological activities: bactericidal, fungicidal, biocidal and pesticidal [1]. In addition, many organotin(IV) compounds have been tested for their *in vitro* activity against a large variety of tumor cell lines and have been found to be as effective, or better, than traditional heavy metal anticancer drugs such as cisplatin [2]. In general, the biochemical activity of organotin(IV) complexes is influenced greatly by the structure of the molecule and the coordination number of the tin atoms [3]; it is well known that the biological activity of organotin(IV) complexes is related to the number of Sn–C bonds and the type of groups in the organotin moiety. The organotin(IV) compounds with triazolopyrimidine ligands have not been investigated to a large extent. Considering that organotin(IV) compounds are promising also in cancer therapy, we have decided to study a class of $R_2SnCl_2(L)_2$ and $R_2SnCl_2(L)$ type complexes (R = Me, Et, ^{n}Bu, Ph and L = dbtp or dptp).

Triazolopyrimidines [4] represent an interesting class of heterocycles due to the number and the arrangement of the nitrogen atoms in the aromatic cycle as well as the nature of the ring substituents. Some transition metal complexes (e.g. Ag(I), Cu(II), Pt(II), and Pd(II)) with triazolopyrimidines significantly inhibit the *in vitro* cell

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growth of Gram(+) and Gram(−) bacteria. The antitumor activity studies (*in vitro*) of *cis*-dichloro platinum(II) compounds with triazolopyrimidine ligands dbtp and dptp [5] indicate a moderate antiproliferative activity against the cells of rectal, breast and bladder cancer. Cytotoxicity parameters pointed out that the antitumor activity of the investigated platinum(II) compounds containing 1,2,4-triazolo[1,5-*a*]pyrimidines depends directly on the geometry around platinum(II) and the kinds of ligands involved. When the antitumor activity of the triazolopirymidine complexes is related to substitutions in the pyrimidine ring, their electronic and stereochemical impact is unequivocal. Previous studies on *cis*-dichloro platinum(II) compounds with 5,7-disubstituted-1,2,4-triazolo [1,5-*a*]pyrimidines suggested that the presence of a bulky ligand (*tert*-butyl or a phenyl group in the triazolopyrimidine ring) might be a major factor in the antitumor activity of the platinum(II) compounds [6]. Reported X-ray structures confirmed the presence of monodentate triazolopyrimidines in many coordination compounds; in each case the heterocyclic ligand binds *via* N(3), in mononuclear Pt(II) [7], Pd(II) [8] and Ru(III) [9] coordination compounds. A special feature of these complexes is that they were modeled on the active square-planar Pt(II) complexes which have *cis* halogen groups and yet the mode of action for the formation of metal-based cross-links is reported to follow a different route for organotin adducts [10]. Among the diorganotin(IV) compounds, di-*n*-butyltin(IV) derivatives have received more attention due to their antitumor activity stronger than that of dimethyltin(IV) or diethyltin(IV) analogs. However, their antitumor mechanisms of action are still not elucidated [11]. Investigations on 'induction of apoptosis' by metal-based drugs have become popular with cisplatin and ruthenium compounds, suggesting that apoptosis is a possible key event in mediating the *in vitro* antitumor activity of these compounds. Inspired by the apoptosis mechanism of these metal-based antitumor complexes, we decided to check if the *in vitro* antitumor activity of organotin(IV) complexes could be related to apoptosis by cellular biochemical studies.

Recently, we described complexes of diorganotin(IV) dichlorides with the heterocyclic ligands [1,2,4]triazolo-[1,5-*a*]pyrimidine (**tp**), 5,7-dimethyl-[1,2,4]triazolo-[1,5-*a*]pyrimidine (**dmtp**) [12], 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine (**dbtp**) and 5,7-diphenyl-1,2,4-triazolo[1,5-*a*]pyrimidine (**dptp**) [13] that exhibit antibacterial activity against a group of reference pathogen micro-organisms, thus showing antibacterial activities against a group of reference pathogen micro-organisms, which shows their inhibitory effect. *In vitro* antimicrobial tests showed that $^{n}Bu_2SnCl_2$(dmtp) [12] has interesting properties as anti Gram-positive and antibiofilm agent.

The present study was aimed to widen the knowledge about bio-activity of organotin(IV) compounds. We here investigate inhibitory effects of complexes with triazolopyrimidine on the growth of a number of human malignant cell lines and assess the influence of the substituents (*tert*-butyl or phenyl) attached to the heterocyclic ring and the type of alkyl or phenyl groups attached to the tin atom, on their biological activity. The formerly characterized organotin(IV) compounds [13] assayed in this study were: Me_2SnCl_2(dbtp)(**1**), $Me_2SnCl_2(dbtp)_2$ (**2**), Et_2SnCl_2(dbtp) (**3**), $Et_2SnCl_2(dbtp)_2$ (**4**), Et_2SnCl_2(dptp) (**5**), $^{n}Bu_2SnCl_2(dbtp)_2$ (**6**), $^{n}Bu_2SnCl_2$(dptp) (**7**), Ph_2SnCl_2(dbtp) (**8**), $Ph_2SnCl_2(EtOH)_2(dptp)_2$ (**9**). All complexes were evaluated for their *in vitro* anti-proliferative activity against three human cancer cell lines: HepG2 (human hepatocellular carcinoma), HeLa (human cervix adenocarcinoma) and MCF-7 (human breast cancer). The apoptosis of HepG2 cells induced by the most active complexes was also investigated using flow cytometry as well as fluorescence microscopy. Moreover, crystal structure of $^{n}Bu_2SnCl_2$(dptp), **7**, whose spectroscopical parameters have been previously reported [13], is also discussed.

2. Experimental

2.1. Diorganotin(IV) compounds, $R_2SnCl_2(L)_2$ and $R_2SnCl_2(L)$

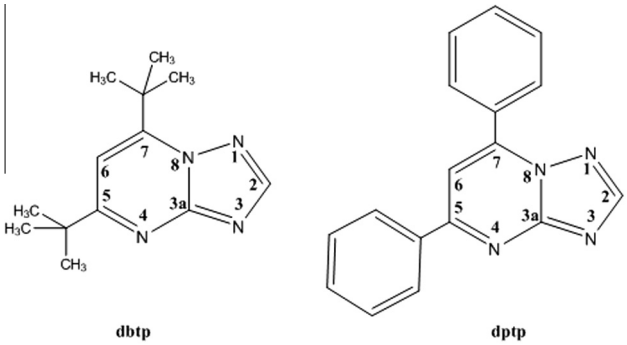
The synthesis and characterization of the compounds $R_2SnCl_2(L)_2$ and $R_2SnCl_2(L)$ (**1–9**) where R = Me, Et, ^{n}Bu and Ph, while L = 5,7-di-*tert*-butyl-1,2,4-triazolo[1,5-*a*]pyrimidine (**dbtp**) and/or 5,7-diphenyl-1,2,4-triazolo[1,5-*a*]pyrimidine (**dptp**) (Scheme 1) were reported earlier [13] and will not be discussed here. Scheme 2 shows the proposed structures for R_2SnL_2 and R_2SnL complexes.

2.2. X-ray crystallography of $^{n}Bu_2SnCl_2$(dptp) (**7**)

Crystals of **7**, suitable for the X-ray diffraction studies, were grown on slow evaporation of a methanol solution of the complex (Fig. 1). Preliminary examination and data collection were carried out at ambient temperature on a Sapphire CCD detector (Oxford Diffraction Ltd., Agilent Technologies, USA) with Mo Kα radiation, λ = 0.71073 Å, monochromator graphite.

Data reduction was automatically performed by CrysAlisPRO (Oxford Diffraction Ltd., UK). The cell parameters were obtained and refined using the PHICHI [14] and DIRAX [15] programs, respectively, catching reflections with random orientation in *hkl* planes. Intensities were corrected by Lorentz polarization and absorption with the SADABS [16] program. The XPREP [17] program was used for analysis of the data reduction and revealed an orthorhombic P group. The structure was solved by direct methods using the SHELXS-97 [18] program. SHELX-97 was used for structure solution and refinement based on F^2. The asymmetric unit contains two crystallographically independent molecules, **A** and **B**, two conformers differing in both the butyl groups and the phenyl ring orientations Fig. 2. The butyl moieties, particularly those of molecule **B**, are characterized by large thermal vibrations; however, no satisfactory alternative model for the disordered atoms could be refined despite repeated attempts. The two independent molecules were separately refined by blocked-matrix least squares methods, giving to each correspondent atom the same crystallographic numbering with **A** or **B** labels. Non-hydrogen atoms were refined isotropically, apart from Sn and Cl atoms. Hydrogen atoms bound to carbon atoms were added in calculated positions. Aromatic carbon atoms were refined with U_{iso}(H) = 1.2 U_{eq} C sp^2 and methyl carbon s with U_{iso}(H) = 1.5 U_{eq} C sp^3. Final R indexes for 249 parameters refined R_1 = 0.0582 [$(I) > 2\sigma(I)$] and 0.1807 for all 9256 data, wR_2 = 0.11146 [$(I) > 2\sigma(I)$], GooF S = 0.910 for all data. Flack x parameter = −0.04 with e.s.d = 0.05.

Crystal data and details of measurements are reported in Table 1. Molecular graphics were prepared using ORTEP-3 for Windows [19].



Scheme 1. The structure of ligands **dbtp** and **dptp**.