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DNA-binding property and antitumor activity of bismuth(III) complex with 1,4,7,10-tetrakis(2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane †

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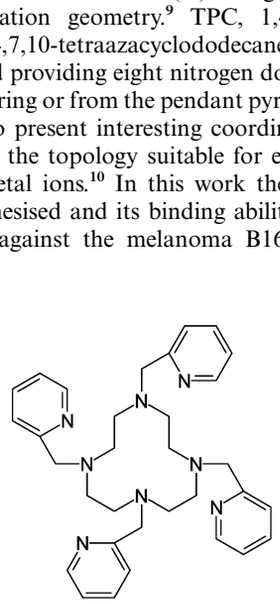
The title Bi(III) complex is highly cytotoxic against melanoma B16-BL6 cells and able to bind to calf thymus DNA noncovalently.

There is an enormous potential for the application of metals in medicine,[1] and selection of metal ions offers the possibility for the discovery of metallodrugs with novel mechanism of action.[2] Bismuth has long been associated with medicine, and its compounds have been used for the treatment of gastrointestinal diseases for nearly two centuries.[3] Today bismuth compounds are primarily used as antiulcer drugs because of their bactericidal actions against *Helicobacter pylori* (*H. pylori*). The applications of bismuth compounds in medicine have been thoroughly reviewed recently.[4]

The exploration of antitumor potential of bismuth compounds has been a subject of interest for the last few decades.[5] For example, a Bi(III)-mercaptopurine complex was found active against Dunning ascitic leukemia;[6] organobismuth complexes with thiolate or hydroxyquinoline have been reported to be cytotoxic.[7] However, the studies on bismuth antitumor compounds have been largely limited compared to other metal ions such as Pt(II) and Ru(II)/Ru(III). This fact may be partly due to the lack of evidence for DNA-binding of bismuth, although it is known that proteins such as transferrin or metallothionein appear to be the biological target for Bi(III).[8]

The coordination number of Bi(III) is highly variable with irregular coordination geometry.[9] TPC, 1,4,7,10-tetrakis(2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane, is a potential octadentate ligand providing eight nitrogen donors either from the flexible cyclen ring or from the pendant pyridyls. This ligand has been found to present interesting coordinating behaviors, especially to offer the topology suitable for effective encapsulation of large metal ions.[10] In this work the Bi(III) complex of TPC was synthesised and its binding ability towards DNA and cytotoxicity against the melanoma B16-BL6 cells were investigated.



TPC

† Electronic supplementary information (ESI) available: ^{1}H-NMR, ES-MS and CD spectra. See http://www.rsc.org/suppdata/dt/b3/b305290g/

TPC was synthesized according to the method reported in the literature.[11] Its Bi(III) complex BiTPC was obtained by the reaction of TPC with $Bi(NO_3)_3 \cdot 5H_2O$ (molar ratio 1:1) in ethanol solution at ambient temperature. The complex BiTPC was fully characterised by ^{1}H NMR, ES-MS, IR and elemental analysis. It is highly water soluble, which provides a superior property for biological testing. The ^{1}H NMR spectroscopy showed that upon binding to Bi(III) the pyridyl proton signals shifted upfield and those of $-CH_2-$Py and cyclen methylene signals shifted downfield which suggested coordination of both cyclen and pyridyl nitrogen donor atoms to Bi(III). It is notable that all methylene signals in BiTPC are very well resolved in contrast to those of free TPC which were broad and overlapped. This may be caused by the limited conformational variation due to the coordination of TPC to Bi(III). In the ES-MS spectrum of BiTPC in solution there were two peaks observed at 992.8 and 966.0 which could be assigned to one negatively charged $[BiTPC(NO_3)_4]^-$ and $[BiTPC(H_2O)(OH)(NO_3)_3]^-$, respectively. The data suggest that the coordination number of Bi(III) in solution may be higher than eight and labile coordination sites may exist.

BiTPC exhibited a very high cytotoxic activity against melanoma B16-BL6 cells with an inhibition rate of 81.8% at a concentration of 2.5×10^{-7} M (48 h). The IC_{50} of BiTPC is 4.1×10^{-8} M which is 100 times more potent than the currently used antitumor drug cisplatin. ‡

It is well known that DNA is the primary intracellular target for antitumor drugs. Small molecule drugs interact with DNA and induce DNA damage, which leads to the blockage of the cell division and eventually to the cell death.[12] A model compound of DNA, guanosine-5′-monophosphate (5′-GMP), was initially used to study the DNA-binding property of BiTPC *via* ES-MS spectroscopy (see ESI †). Two adduct peaks were observed in the ES-MS spectrum: one at 1106.2 attributable to one positively charged species $[BiTPC + 5'\text{-GMP}]^+$ ($C_{42}H_{52}N_{13}O_8PBi$), and the other at 553.6 to two positively charged species $[BiTPC + (5'\text{-GMP})]^{2+}$. However, the ^{1}H NMR and VT ^{1}H NMR spectra did not show significant shift for all the ^{1}H resonances of the complex. A slight shift of the ^{31}P signal was observed and can be attributed to the pH difference of the reaction solution from that of free 5′-GMP. Thus, the adducts detected in ES-MS are likely to be the ion pairs of BiTPC and 5′-GMP stabilized by electrostatic interactions.

Ethidium bromide (EB) is a known DNA intercalator which gives a significant increase in fluorescence intensity when bound to DNA and displacement of EB from DNA results in a decrease of fluorescence.[13] To clarify the reactivity of BiTPC toward DNA and the potential binding mode, EB was employed to probe the process of binding. The experiment was carried out by titrating the solution of BiTPC to that of DNA-EB. § Fig. 1 shows the fluorescent emission spectra of the DNA-EB system in the absence and presence of BiTPC. The emission

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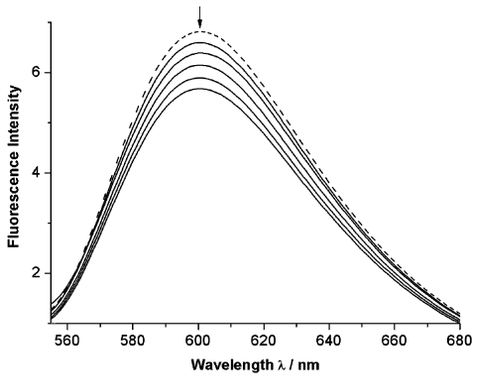

Fig. 1 Fluorescent emission spectra (excited at 526 nm) of the CT-DNA-EB system (7.33×10^{-5} mol l^{-1} EB, 4.67×10^{-5} mol l^{-1} DNA) in the absence (dashed line) and presence (solid line) of increasing amounts of 8.80×10^{-5} mol l^{-1} BiTPC (40 μl per scan).

intensity of the DNA-EB system decreased with the increase of the concentration of BiTPC, which indicated that BiTPC replaced EB from the DNA-EB system. Such a characteristic change is often observed in the intercalative DNA interaction.[14]

A UV absorption titration experiment was carried out to obtain further evidence for DNA-binding property of BiTPC. The absorption spectra of BiTPC (at a constant concentration of 1.33×10^{-6} M) in the presence of different concentrations of CT-DNA are given in Fig. 2. The absorption bands of BiTPC at about 334 nm exhibited a hypochromism of about 25.7% when DNA concentration increased from 0 to 5.13×10^{-6} M. The absorption bands at 235~265 nm showed a similar tendency. However, no obvious bathochromism was observed for both bands. It is a general observation that binding of intercalative molecules to DNA can result in hypochromism and bathochromism in the UV absorption spectra. The extent of spectral change is related to the strength of binding and the spectra for intercalators are more perturbed than those for groove binders.[15]

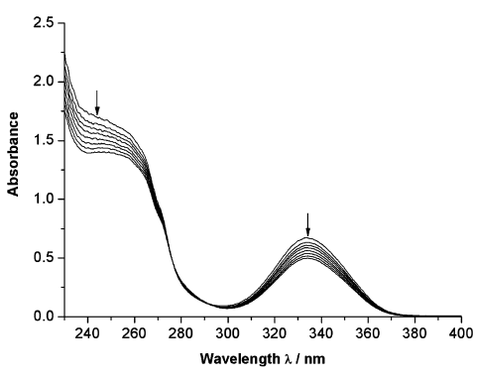

Fig. 2 UV absorption spectra of BiTPC in the buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.0) with increasing concentration of CT-DNA. [BiTPC] = 1.33×10^{-6} M, [DNA] = 0, 7.33×10^{-7}, 1.47×10^{-6}, 2.44×10^{-6}, 2.93×10^{-6}, 3.66×10^{-6}, 4.40×10^{-6}, 5.13×10^{-6} M.

The conformational changes of DNA induced by BiTPC were also assessed by circular dichroism (CD) (see ESI †). As indicated by the CD spectra, both the positive band at 276 nm and the negative band at 246 nm decreased in intensity with the increasing concnetration of BiTPC, which is an clear indication of the interations between BiTPC and DNA. As the assessment of the changes was primarily a qualitative one aimed at showing the ability of BiTPC binding to DNA, the precise amount of compound bound to DNA is not available from this experiment.

In summary, the Bi(III) complex of 1,4,7,10-tetrakis(2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane reported in this work is highly water soluble and cytotoxic. The biological target of this complex is unknown, however, we have shown here that the complex is able to bind to CT-DNA under physiological relevant conditions. Supported by the above experiments, it is likely that BiTPC interacts noncovalently with DNA. The electrostatic interactions between BiTPC and DNA may direct the bismuth compound towards DNA and facilitate the subsequent intercalative interactions. Further experiments are currently underway to extend the study to a wider range of tumour cell lines.

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Notes and references

‡ Growth inhibitory effect of BiTPC on the melanoma B16-BL6 tumour cells was measured by the microculture tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, MTT] assay.[16] The measurements of absorbance of the solutions related to the number of live cells were performed on an ELISA spectrophotometer at 540 nm. The following formula was used to evaluate the drug efficacy against the tumor cells: inhibition rate (%) = $(OD_{control} - OD_{drug})/OD_{control} \times 100$. The IC_{50} values were derived from semilog plots of percentage control *versus* drug concentration using Logit method and defined as the drug concentration that resulted in a 50% reduction in cell number compared with untreated controls.

§ The solutions of CT-DNA, EB and BiTPC were prepared with buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.0). DNA concentration was determined spectrophotometrically with an extinction coefficient of 6600 mol^{-1} l^{-1} at 260 nm. Solutions used in the following UV titration were also prepared this way. The fluorescent titration experiment was carried out in a 3 ml solution of 7.33×10^{-5} mol l^{-1} EB and 4.67×10^{-5} mol l^{-1} DNA by adding 40 μl 8.80×10^{-5} mol l^{-1} BiTPC into it per scan.

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