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Synthesis, X-ray crystal structure, DNA/protein binding and cytotoxicity studies of five α-aminophosphonate N-derivatives

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ABSTRACT

Five new α-aminophosphonates are synthesized and characterized by EA, FT-IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, ESI-MS and X-ray crystallography. The X-ray analyses reveal that the crystal structures of ***1–5*** are monoclinic or triclinic system with the space group *P* 21/c, *P* – 1, *P* – 1, *P*2(1)/c and *P* – 1, respectively. All *P* atoms of ***1–5*** have tetrahedral geometries involving two O-ethyl groups, one C_{α} atom, and a double bond O atom. The binding interaction of five new α-aminophosphonate N-derivatives (***1–5***) with calf thymus(CT)-DNA have been investigated by UV–visible and fluorescence emission spectrometry. The apparent binding constant (Kapp) values follows the order: ***1*** (3.38×10^5 M^{-1}) > ***2*** (3.04×10^5 M^{-1}) > ***4*** (2.52×10^5 M^{-1}) > ***5*** (2.32×10^5 M^{-1}) > ***3*** (2.10×10^5 M^{-1}), suggesting moderate intercalative binding mode between the compounds and DNA. In addition, fluorescence spectrometry of bovine serum albumin (BSA) with the compounds ***1–5*** showed that the quenching mechanism might be a static quenching procedure. For the compounds ***1–5***, the number of binding sites were about one for BSA and the binding constants follow the order: ***1*** (2.72×10^4 M^{-1}) > ***2*** (2.27×10^4 M^{-1}) > ***4*** (2.08×10^4 M^{-1}) > ***5*** (1.79×10^4 M^{-1}) > ***3*** (1.17×10^4 M^{-1}). Moreover, the DNA cleavage abilities of ***1*** exhibit remarkable changes and the in vitro cytotoxicity of ***1*** on tumor cells lines (MCF-7, HepG2 and HT29) have been examined by MTT and shown antitumor effect on the tested cells.

1. Introduction

Due to the structural similarity of α-aminophosphonates with α-amino acids, it is received an increasing attention [1]. A considerable number of α-aminophosphonate derivatives are known to be antiviral [2,3], antibacterial [4], anticancer [5,6], haptens of catalytic antibodies [7,8], antibiotics and pharmacological agents [9–11] and herbicides [12]. The potential of α-aminophosphonates such as a novel series of diphenyl 1-(arylamino)-1-(pyridin-3-yl)ethylphosphonates, containing difluoro-methylenephosphonate group, 6-(phosphono-difluoromethyl)-2-naphthoic acid and so on, acting as enzyme inhibitors pharmacological agents has been established [13–19]. Moreover, our early research indicated that a-aminophosphonate N-derivatives with the rigid structures can potently inhibit PTPs with some selectivity [20].

The field of anticancer metallodrugs is dominated by platinum based complex and the mechanism of metallodrugs relies on direct DNA damage. DNA is the carrier of human genetic information [21], the formation of human tumors has a close relationship with DNA [22], and most of tumor was caused by the mutation of genetic [23]. The change of DNA structure were detected in a variety of cancer cells, such as liver cancer [24], digestive system tumors [25] and renal cell carcinoma [26], so DNA is always used as one of the most important targets for drug design. While the toxic side of platinum based metallodrugs limited their for further use, so many metal complexes and organic compounds with fewer side effects were investigated in recent years for their tumor inhibiting properties. As early as 1961, Lerman discovered that molecules with an aromatic structure could interact with DNA first, and this has been confirmed by many molecules with such structures [27]. Therefore, α-aminophosphonate may also be developed to interact with DNA.

Abbreviations: MTT, 3-[4,5-dimethylthiazo-2-yl]-2,5-diphenpyltetra-zolium bromide; BSA, Bovine Serum Albumin; CT-DNA, calf thymus-DNA.

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Binding to plasma proteins is regarded as a reason for the α-aminophosphonate low general toxicity. Bovine serum albumin is the most important and the most abundant carrier protein in the plasma [28]. It plays an important role in the distribution and absorption of the drug [29,30]. Therefore, the study of the interaction mechanism between drug and bovine serum albumin can provide the basis of theory for the design and development of drugs. Serum albumin contains many kinds of ligands [31], which can interact with various endogenous and exogenous compounds [32,33], so serum albumin can play a role in the storage and transportation of some drugs or some other biological activities [34]. When smaller molecules interact with serum albumin in the form of covalent bond, the serum albumin conformation will be changed [35,36], which could provide valuable information and data to the disease prevention, diagnosis, curative effect.

Here, we intend to report the preparation of five new α-aminophosphonate N-derivatives (Scheme 1) and their DNA and BSA binding, cytotoxicity and preliminary apoptotic.

2. Results and discussion

2.1. Synthesis of α-aminophosphonate N-derivatives 1–5

A powerful and direct method for construction of P—C—N bonds is *Pudovik* reaction [37–39]. It could form an asymmetric center in α-position to the phosphorus atom if there are two different substituents in C-terminal. In this paper, five new α-aminophosphonate N-derivatives were prepared, which exhibited characteristic either the rigid structure with one α-aminoalkylphosphonate fragment.

2.2. Characterization of α-aminophosphonate N-derivatives **1–5**

The elemental analysis data for ***1–5*** were consistent with the compositions of desired products. All the compounds showed absorption bands of infrared in the region 3148–3244, 2983–2988, 1221–1228 and 970–975 cm^{-1} for stretch vibrations of N—H, C_α—H, P═O and P—C_α, respectively [20].

The ^{31}P NMR spectroscopy is the most precise method and a very convenient tool for determining the structure of the organophosphorus compounds. Chemical shifts of ^{31}P NMR spectra in the α-aminophosphonic acids are in range of 15–25 ppm referenced to 85% H_3PO_4 [37]. The ^{31}P NMR spectra of all the compounds show one single peak. The ^{31}P NMR spectra of ***1–5*** have signals with the chemical shifts of 20.447–22.573 ppm. The values agree with other diester aminophosphonates, such as diethyl (1*R*)-1-{[(carbobenzyloxy]amino}ethylphosphonate (^{31}P NMR, DMSO-d_6 25.89 ppm) [40], *p*-[*N*-Methyl(dietoxy-phosphonyl)(2-furyl)]tolui dine (^{31}P NMR at 101 MHz, $CDCl_3$ 21.13 ppm) [41], it is also agree with our early results [20].

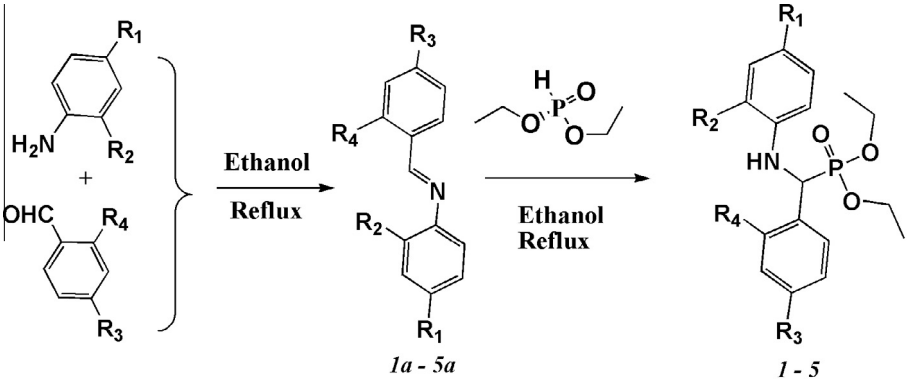
2.3. Crystal structures of 1–5

In order to comprehensively understand the structural features of ***1–5***, the crystals in several solvents or mix-solvents were attempted to grow. Suitable crystals for X-ray single crystal diffraction were obtained from ethanol (***1–4***) and ethanol/H_2O (***5***). Crystallographic data and hydrogen bonds are listed in Tables 1 and S1 (see supporting information). Also the X-ray structures of the α-aminophosphonates N-derivatives ***1–5*** are shown in Figs. 1–5.

The crystal structures of ***1–5*** are monoclinic or triclinic system with the space group *P* 21/c, *P* − 1, *P* − 1, *P*2(1)/c and *P* − 1, respectively. Seen from Figs. 1–5, all *P* atoms have tetrahedral geometries involving two O-ethyl groups, one C_α atom, and a double bond O atom. In the structures, C_α atoms are chiral centres, which are responsible for the existence of optically activity. The C—P and P═O bond lengths are almost comparable with the similar structures [20]. There are a lot of weak interactions, such as O—H···O, *N*—H···O, C—H···O, C—H···π and *N*—O···π, resulting in stabilization in the structures.

2.4. DNA-binding studies

It is reported that one of an effective method in examining the binding mode of DNA with the metal complex was electronic absorption spectroscopy [42]. The intercalative binding usually results in a large hypochromism or hyperchromic and red shift due to the interaction between the base pairs of DNA and aromatic chromophores. Absorption spectral changes of ***1*** in the absence (dash line) and presence (solid line) of CT-DNA are shown in Fig. 6. The addition of CT-DNA to ***1*** solution results in hyperchromic and no bathochromic shift. It is suggested the association interaction between ***1*** and DNA is intercalation [43]. The binding constant between ***1*** and CT-DNA was calculated according to the Eqs. (1) and (2), is $1.69 \times 10^4\ M^{-1}$. The binding constants K_b for compounds ***2–5*** were also calculated and were shown in Table 2. From Table 2, we can see that the similar binding constants K_b for compounds ***1–5***.



1, R_1 = -Cl; R_2 = -H; R_3 = -H; R_4 = $-OCH_3$; ***2***, R_1 = -Cl; R_2 = -H; R_3 = $-OCH_3$; R_4 = -H
3, R_1 = -Cl; R_2 = -H; R_3 = -H; R_4 = $-NO_2$; ***4***, R_1 = -Cl; R_2 = -H; R_3 = $-NO_2$; R_4 = -H
5, R_1 = $-NO_2$; R_2 = H; R_3 = -Cl; R_4 = -H

Scheme 1. The synthesis of the rigid α-aminophosphonate N-derivatives ***1–5*** in two-step by *Pudovik* reaction.