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Syntheses and antiproliferative activities of novel phosphatidylcholines containing dehydroepiandrosterone moieties

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ABSTRACT

Dehydroepiandrosterone (DHEA) is a natural hormone with many beneficial properties including an anticancer activity. Unfortunately, DHEA is unstable in the body and exhibits cytotoxicity against healthy cells. In this study, a series of new phosphocholines containing DHEA at *sn*-1 and/or *sn*-2 positions were prepared. Succinic acid was used as a linker between the active drug and *sn*-glycero-3-phosphocholine. All the compounds were evaluated in vitro for their antiproliferative activities against four cell lines: Balb/3T3, HL-60, B16, and LNCaP. The results showed that phosphocholines with DHEA at *sn*-1 and/or *sn*-2 positions did not have cytotoxic effects on the normal cell line (Balb/3T3). Mixed-chain phospholipids with DHEA and fatty acid residues showed the highest activity against tumor cell lines. The most active compound, **11c**, showed a moderate cytotoxic effect against the HL-60 and B16 cell lines.

1. Introduction

Dehydroepiandrosterone (DHEA) is a natural hormone produced mainly in the zona reticularis of human adrenal glands [1]. In peripheral tissues, DHEA is largely converted to its sulfate derivative (DHEA-S). There are very high concentrations of the two hormones in the hippocampus and the other structures of the limbic system. DHEA is a very important precursor of sex hormones such as testosterone and estradiol. DHEA levels vary among individuals. In serum, DHEA achieves its highest concentration during adolescence. It is therefore considered as one of 'the hormones of youth'. DHEA levels drop dramatically as the body ages [2,3]. The wide spectrum of DHEA's activities makes the compound very valuable, especially for use in the pharmaceutical and cosmetic industries. DHEA has been shown to exhibit anticancer activity against several cancer cell lines [4,5]. Moreover, it has been proved that DHEA is useful in the management of obesity [5] and protects against heart diseases [5,6] and diabetes [7]. It also enhances the immune system [5,8], reduces cholesterol [9], and has antiviral properties [10]. There are also reports on the antioxidant and antiinflammatory properties of DHEA [11].

Unfortunately, there are reports indicating that oral DHEA has limited clinical effects [12,13]. The low oral bioavailability of DHEA is caused by its quick conversion to DHEA-S and sex hormones [14]. Many derivatives of DHEA, especially its esters, exhibit greater biological activity than the parent compound does [15–18]. As a result, phospholipids can be effective carriers of DHEA.

Glycerophospholipids are fundamental to many life processes as they have various biological functions. Together with cholesterol and membrane proteins, phospholipids constitute an important component of biological membranes and impart a semipermeable character to the membranes. Phospholipids mediate the transport of compounds into cells. They also increase the lipophilicity and bioavailability of drugs and prevent the degradation of linked drugs [19–23].

In our previous report, we presented the synthesis of diacylphosphatidyl-DHEA [24]. In the present report, the synthesis of 9 new phosphatidylcholines (PC) containing DHEA or DHEA and fatty acids such as palmitic acid, oleic acid, conjugated linoleic acid (CLA), and natural fatty acids from soybean and egg yolk are described. Succinic acid, which is a non-toxic compound, was used as a linker between DHEA and glycerol.

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2. Results and discussion

2.1. Chemistry

The first phospholipid with a DHEA moiety at the *sn*-1 and *sn*-2 positions (**4**) was synthesized from a cadmium chloride complex of *sn*-glycero-3-phosphocholine (**3**) and DHEA hemisuccinate (**2**) (Scheme 1). DHEA (**1**) does not have any carboxyl group in its structure; thus, succinic acid was used as a linker in the study. Succinic acid is commonly found in nature as a non-toxic metabolite. For example, it is an intermediate in the Krebs cycle. Succinic acid has been used as a linker in several reactions, especially in the synthesis of prodrugs [25,26].

In the first step, DHEA (**1**) was esterified with succinic anhydride in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP). This resulted in the production of DHEA hemisuccinate (**2**), which was lyophilized and reacted with a cadmium complex of *sn*-glycero-3-phosphocholine (**3**) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling agent and DMAP as a catalyst.

The structure of compound **4** was confirmed by ^{1}H, ^{13}C, and ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Correlation spectroscopy (heteronuclear multiple-quantum correlation spectroscopy and heteronuclear multiple-bond correlation spectroscopy) was also applied. The phosphatidylcholine **4** contains two identical groups at the *sn*-1 and *sn*-2 positions; however, identification of the groups by NMR was difficult and inconclusive. In determining the structure of phosphatidylcholine **4**, the use of mass spectroscopy was very important. In the electrospray ionization (ESI)-high-resolution mass spectra (HRMS), an intensive signal at *m*/*z* 998.5414 was detected. This confirmed the presence of DHEA hemisuccinate at both *sn* positions. In the ^{1}H NMR spectrum, the signals of the DHEA moiety, glycerol and choline, were visible. Signals of CH_2-1′ from the glycerol skeleton at δ = 4.20 as a doublet of doublets (J = 12.0, 6.7 Hz) and at δ = 4.37 as a multiplet were observed. The multiplet of H-2′ from the *sn*-2 position at 5.20 ppm was observed. This chemical shift is characteristic of *sn*-2 substituted phosphocholines (Table 1). At δ = 3.21, the signals of three *N*-methyl groups from the choline were visible as a singlet. Most of the signals from DHEA in the ^{1}H NMR and ^{13}C NMR spectra overlapped, whereas the others were well separated. Characteristic signals of two groups in DHEA hemisuccinate were found at 0.87 and 1.03 ppm as two singlets of CH_3-18 and CH_3-19, respectively. A characteristic multiplet at 4.55 ppm from the H-3 proton in DHEA was also detected. The olefinic H-6 proton was identified at δ = 5.38 as a multiplet. The methylene protons in the linker were observed as two multiplets at 2.55–2.64 ppm. In the ^{13}C NMR spectrum of **4**, signals of the ester carbon atoms at δ = 171.94, 171.97, 172.04, and 172.35 were detected. These chemical shifts confirmed linker ester connections between DHEA and glycerol. Signals of C5 and C6 carbon atoms were observed at δ = 121.93 and 139.73, respectively. Interestingly, only the C4 and C17 carbon atoms in the DHEA moiety were differentiated. These atoms showed double signals at 37.92 and 37.93 ppm for C4 and at 222.57 and 222.60 ppm for C17. The ^{31}P NMR data confirmed the presence of a phosphocholine **4** as a singlet at −1.07 ppm.

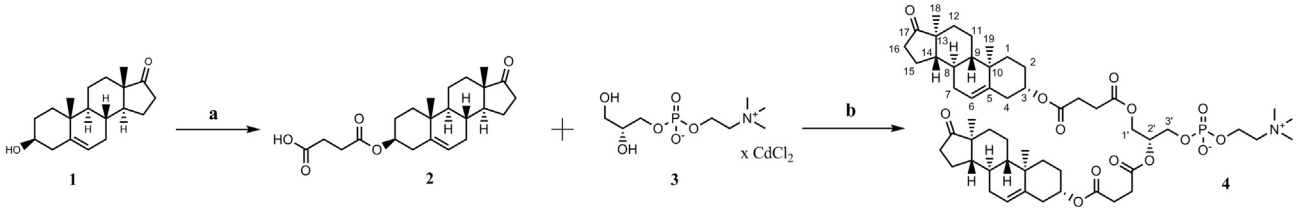
Two phosphocholines containing DHEA hemisuccinate at the *sn*-1 position (**9a** and **b**) were obtained according to the reaction in Scheme 2. Selective acylation at the *sn*-1 position of the stannylene acetal (**7**) was carried out with the chloride (**5**) of DHEA hemisuccinate. This last compound was obtained from a dried monoester (**2**) in the reaction with oxalyl chloride in a high yield (98%). In the ^{1}H NMR spectrum of **5**, the signals of linker methylene protons appeared as triplets (J = 6.5 Hz) at δ = 2.63 and 3.18. The same signals in the hemisuccinate **2** were observed at δ = 2.59–2.69 as two multiplets.

In the key step of the synthesis, *sn*-glycerophosphocholine was treated with dibutyltin oxide (DBTO), and the resulting acetal (**7**) was subjected to reaction with triethylamine (TEA) and DHEA hemisuccinate chloride (**5**). In the next step, the lysophosphocholine (LPC) (**8**) formed was esterified using a reported method [27,28] with palmitic or oleic acid to give the phosphatidylcholines **9a** and **9b**, respectively.

The structure of 2-LPC **8** was confirmed from the spectroscopic data. In the ^{31}P NMR, a characteristic signal for LPCs as a singlet at δ = −0.06 was detected (Table 1). The ^{1}H NMR spectrum of **8** showed a multiplet of proton H-2′ at δ = 3.95. In comparison to disubstituted phosphocholines, the chemical shift of this signal proved that the *sn*-2 position was non-esterified (Table 1). In the ^{13}C NMR, two ester carbon atom signals at 172.14 and 172.64 ppm were observed. The signals of carbon atoms and protons in DHEA moiety were identified. The carbon-phosphorus coupling gave characteristic doublets in the ^{13}C NMR spectrum as follows: C-2′ at δ = 68.53 (J = 7.0 Hz), C-3′ at δ = 66.75 (J = 5.6 Hz), and C-α at δ = 59.05 (J = 5.1 Hz). After esterification at the *sn*-2 position of compound **8**, triplets from terminal methyl groups were observed in the ^{1}H NMR spectrum at δ = 0.81 (J = 7.1 Hz) from palmitic acid (**9a**) and δ = 0.84 (J = 7.0 Hz) from oleic acid (**9b**). The multiplets from olefinic protons H-9 and H-10 in the oleic acid (**9b**) residue were detected at 5.27–5.32 ppm. Furthermore, the H-2′ multiplet was shifted from 3.95 ppm in **8** to 5.15 ppm in **9a** and **9b** (Table 1) upon esterification. In the ^{13}C NMR spectra of **9a** and **9b**, three carbon atom signals from ester groups were identified at δ = 171.78, 171.90, and 173.78 for compound **9a** and at δ = 171.89, 172.28, and 173.50 for compound **9b**. The ^{31}P NMR spectra of **9a** and **9b** showed singlets at δ = −1.12 and −0.68, respectively.

The third group of phospholipids, phosphocholines with DHEA hemisuccinate at the *sn*-2 position (**11a**–**e**), was synthesized with good yields (61–82%) by reacting DHEA hemisuccinate (**2**) with 1-acyl-2-hydroxy-*sn*-glycero-3-phosphocholines (**10a**–**e**) (Scheme 3).

The LPCs (**10a**–**c**) containing fatty acids such as palmitic acid, oleic acid, or conjugated linoleic acid (CLA) at the *sn*-1 position were synthesized by the reaction described above for LPC **8**. The natural 2-LPCs (**10d** and **e**) were obtained by enzymatic hydrolysis of natural PCs isolated from soybean and egg yolk. The hydrolysis was catalyzed with phospholipase A_2 (PLA_2) from porcine pancreas. The physical and spectral data of the LPCs (**10a**–**e**) were confirmed by NMR spectroscopy and were in accordance with the data reported in literature [28–31]. The compositions of fatty acids at



Scheme 1. Reagents and conditions. (a) Succinic anhydride, DMAP, and pyridine at 60 °C. (b) DMAP, DCC, $CHCl_3$, and N_2 at 25 °C.