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Photo-DHEA—A functional photoreactive dehydroepiandrosterone (DHEA) analog

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ABSTRACT

The steroid hormone dehydroepiandrosterone (DHEA) has beneficial effects on vascular function, survival of neurons, and fatty acid metabolism. However, a specific receptor for DHEA has not been identified to date. Here, we describe the synthesis of a photoreactive DHEA derivative (Photo-DHEA). In Photo-DHEA, typical characteristics of DHEA are conserved: (i) a "planar" tetracyclic ring system with a Δ^5 double bond, (ii) a 3β-hydroxyl group, and (iii) a keto group at C17. In cell-based assays, Photo-DHEA showed the same properties as DHEA. We conclude that Photo-DHEA is suitable for radioiodination to yield a tool for the identification of the elusive DHEA receptor.

1. Introduction

The steroid dehydroepiandrosterone (Fig. 1(1)) is produced by the adrenals and the central nervous system [1]. Besides its role as a precursor of androgens and estrogens, DHEA has been reported to be neuroprotective [2] and to have beneficial effects on diabetes, atherosclerosis, and obesity [3–5]. Declining DHEA and DHEA sulfate (DHEAS) levels during aging is associated with pathophysiological effects such as neuronal degeneration [6]. Although several DHEA activated intracellular pathways have been characterized, a specific DHEA binding receptor has not been identified to date. Photoreactive steroid analogs are appropriate tools for analysis or identification of steroid binding proteins. The formation of covalent bonds between the photoreactive steroid and the protein upon irradiation allows the application of denaturating preparative and analytical methods such as SDS polyacrylamide gelelectrophoresis and mass spectrometry.

Here, we report the synthesis of the photoreactive DHEA-analog Photo-DHEA (Fig. 1(2)). As starting compound we chose 5-androsten-3β-ol-7,17-dione-7-O-carboxymethyloxime (DHEA-CMO (8), Scheme 1) in order to retain an unaltered DHEA core structure with a hydroxyl group at C3 in β-configuration, a Δ^5 double bound and a keto group at C17. DHEA-CMO was used successfully for the synthesis of the fluorescent DHEA analog DHEA-Bodipy (Fig. 1(3)), which we introduced previously as a tool to study intracellular trafficking and localization of DHEA [7]. As photoreactive group, we employed the aryl azide 4-azidosalicylic acid that forms a nitrene upon irradiation which can react with appropriate nucleophiles such as primary amines, and cysteinyl or histidyl residues of proteins. In Photo-DHEA, DHEA-CMO is connected with 4-azidosalicoyl-ethylenediamine by an amide bond. The long spacer between DHEA and the aryl azide provides a flexible link between the steroid hormone and the photoreactive group. Thereby, potentially interfering effects of the photoreactive aryl azide on the functions of DHEA should be minimized. Moreover, a certain flexibility between the photoreactive group and the steroid is beneficial because this might enable the labeling of several amino acids at the ligand binding site [8]. Aryl azides are the most commonly used photoactivatable reagents because they are easy to synthesize, relatively stable, have high extinction coefficients, and a relatively short half-life upon irradiation [9].

The biological functionality of Photo-DHEA was confirmed by two cell-based assays: displacement of DHEA-Bodipy from specific DHEA binding-sites on neuronal cells and induction of cAMP synthesis by Photo-DHEA in liver cells. Our results indicate that radioiodinated Photo-DHEA should be a useful tool for photoaffinity labeling and identification of DHEA receptor(s).

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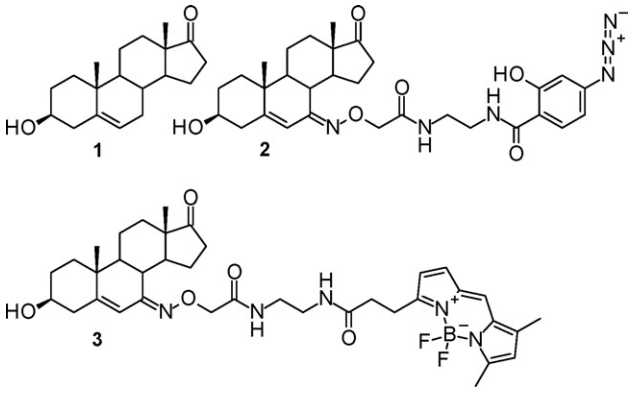
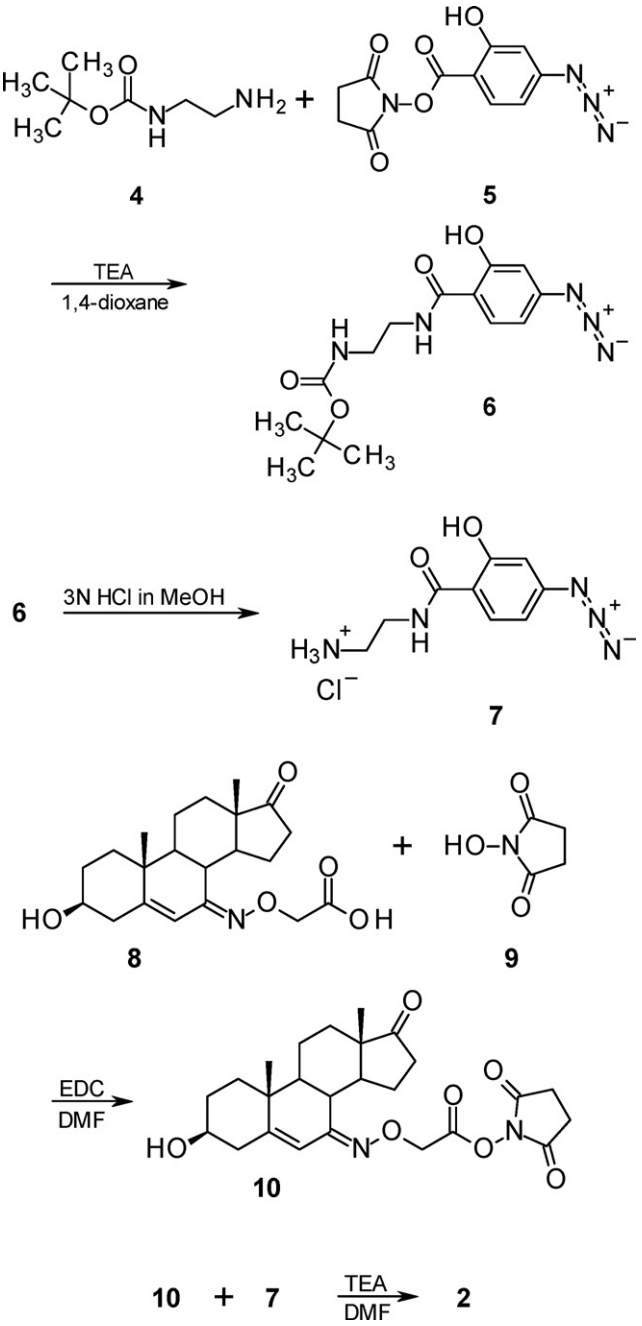


Fig. 1. DHEA (1), Photo-DHEA (2), and DHEA-Bodipy (3).



Scheme 1. Synthesis of Photo-DHEA.

2. Experimental

2.1. General methods

NMR spectra were recorded on Bruker AC-300 instrument in $CDCl_3$ at 300 MHz unless otherwise indicated. Chemical shift values are given in δ (ppm) and coupling constants are given in Hz. ESI mass spectra were recorded on Micromass Q-TOF Ultima API instrument. IR spectra were recorded on Bruker Tensor 27 instrument. Analytical thin layer chromatography (TLC) was performed on 0.5 mm thick silica gel 60 F_{254} plates. Compounds were revealed by UV light (λ = 254 nm) or by staining with ninhydrin. Commercial reagents were used without further purification unless otherwise noted.

2.2. Chemical synthesis

All synthesis steps were performed under dim light. The synthetic pathways are depicted in Scheme 1.

2.2.1. 4-Azidosalicoyl-N-Boc-ethylenediamine (**6**)

Reaction and purification of **6** were performed according to Bidasee et al. [10]. 500 mg (1.81 mmol) of **5** was dissolved in dioxane (4 ml). 320 mg (2.0 mmol) of N-Boc-ethylenediamine (4) and 2.5 ml triethylamine were added. The reaction mixture was stirred under N_2 at room temperature for 3.5 h. The mixture was evaporated, the solid was dissolved in 10 ml of chloroform and chromatographed on a silica gel column (33 cm, Ø 2.5 cm) with chloroform/methanol (99/1) to afford 408.05 mg (1.3 mmol, 70%) of **6** as colorless crystals.

^{1}H NMR ($CDCl_3$, 300 MHz), δ [ppm]: 1.41 (s, 9H, tert-Butyl), 3.41–3.42. (t, 2H, CH_2–N), 3.46–3.48. (t, 2H, CH_2–N), 5.06 (t, 1H, –OH), 6.46 (dd, 1H, ar), 6.58 (d, 1H, ar), 7.44 (d, 1H, ar), 7.84 (s, –NH–) IR (nujol) $\tilde{\nu}$ [cm^{-1}] = 2120 (azide) (compare: IR (KBr) $\tilde{\nu}$ [cm^{-1}] = 2130 (N_3, **7**) [11], IR (nujol) $\tilde{\nu}$ [cm^{-1}] 2102 (azide, N-(4-azido-salicyloyl-β)-alanine ethyl ester)) [10].

TLC: detection UV and ninhydrin (yellow spot). R_f: 0.2 ($CHCl_3$/MeOH (99:1, v/v)), 1.0 (ethylacetate).

2.2.2. 4-Azidosalicoyl-ethylenediamine hydrochloride (**7**)

400 mg (1.24 mmol) of **6** were dissolved in 8 ml of HCl/methanol (1/4), stirred under N_2 at room temperature for 20 h, and allowed to stand at 4 °C [12]. The resulting crystals were filtered, washed with diethylether and dried to afford 237.1 mg (0.9 mmol, 74%) of **7** as colorless crystals.

^{1}H NMR (CD_3OD, 300 MHz), δ [ppm]: 2.97–3.01 (t, 2H, CH_2–N), 3.51–3.57 (q, 2H, CH_2–NH), 6.63 (d, 1H, ar), 6.65–6.69 (dd, 1H, ar), 7.97 (d, 1H, ar), 9.04–9.07 (m, –NH–). *Note*: lack of the tert-butyl singlet (1.41 ppm) indicates successful N-Boc deprotection.

TLC: detection UV and ninhydrin (red-purple spot). R_f: 0.3 (ethylacetate).

2.2.3. Photo-DHEA (**2**) (4-azido-2-hydroxy-N-(2-(2-((E)-((3S,8R,9S,10R,13S,14S)-3-hydroxy-10,13-dimethyl-17-oxo-3,4,9,11-tetrahydro-1H-cyclopenta[a]phenanthren-7(2H,8H,10H,12H,13H,14H,15H,16H,17H)-ylidene)aminooxy)acetamido)ethyl)benzamide)

To a solution of 200 mg (0.53 mmol) of DHEA-CMO (**8**) in 1.5 ml of DMF, 124 mg (1.08 mmol) of **9** and 104 mg (0.54 mmol) of EDC were added similar to [13]. The reaction mixture was stirred under N_2 at room temperature for 22 h. 136 mg (0.53 mmol) of **7** in 0.5 ml of DMF and 80 μl of triethylamine were added. The reaction mixture was stirred at room temperature for 24 h, filtrated and diluted with H_2O. The mixture was extracted with ethylacetate. The combined organic layers were washed with saturated NaCl solution, dried with Na_2SO_4, filtered and evaporated. The resulting yellow oil was chromatographed on a silica gel column (33 cm,