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Solid-phase synthesis of libraries of ethynylated aminosteroid derivatives as potential antileukemic agents

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ABSTRACT

Steroids possessing an ethynyl group at position 17α (tertiary alcohols) are well known to be more stable than their non-ethynyl analogs (secondary alcohols). To facilitate the development of new drugs with better metabolic stability, we developed a new diethylsilyl acetylenic linker allowing us to rapidly synthesize libraries of ethynylated steroid derivatives using a solid-phase strategy. To illustrate its usefulness, this linker was used to expand the molecular diversity of a lead compound having a hydroxy acetylenic pattern and to potentially find new compounds with interesting cytotoxic activity against leukemia cell lines. Herein, we report the chemical synthesis and the characterization of three libraries of ethynylated aminosteroid derivatives using the diethylacetylenic linker. We discuss their antiproliferative activities obtained in 2 leukemia cell lines (HL-60 and Jurkat), which results provided new structure–activity relationships. We also identified a new promising aminosteroid derivative with an azetidine moiety (compound **B1**) inhibiting 60% and 75% of HL-60 and Jurkat cell proliferation, respectively, at 1 μM. More generally, these results validate the use of a diethylsilyl acetylenic linker for researchers interested in generating libraries of alcohol derivatives with better stability and drug profile.

1. Introduction

Although there are many molecules that are active against cancer, very few with a steroid nucleus are currently used in chemotherapy. In fact, various steroid derivatives that are active against different human cancer cell lines have been isolated from natural sources or rationally synthesized [1–3], but the only compounds from steroidal families currently used in anticancer chemotherapy are glucocorticoids such as prednisone and prednisolone, and they are used in combination with other non-steroidal anticancer agents as adjuvant therapies [4,5]. 2-Methoxyestradiol has recently been proven effective to selectively induce apoptosis in a number of cancer cell lines [6], but the bioavailability of this compound seems to be problematic towards clinical use and this is why new molecular analogs are being sought [7]. In 1999, He and Jiang [8] reported that 2β-(4-methylpiperazinyl)-5α-androstane-3α,17β-diol (1) shows antiproliferative activity on human myeloid leukemia HL-60 cells (Fig. 1). This compound and its 17-keto analog **2** were also reported to induce differentiation of HL-60 cells [9]. A few years later, and using 2 different strategies (solid phase and solution phase), our team synthesized analogous compounds with the objective to improve their potency and selectivity of action. In fact, to accelerate the development and the synthesis of aminosteroid derivatives with structural similarity to compound **1**, parallel solid-phase synthesis of steroid derivatives was used to provide the first libraries of steroid derivatives of general structure **3** [10,11] and **4** [12], while a classical solution-phase approach was also used to synthesize other aminosteroid derivatives of general structure **5** [13,14].

As it is well known that 17β-hydroxy-steroids bearing an ethynyl group at position 17α are more stable drugs than corresponding secondary alcohols [15–19], we synthesized, by classic solution-phase chemistry, a first ethynylated aminosteroid to verify the impact of this modification, as well as others, on

Abbreviations: AA, amino acid; AcOH, acetic acid; 6-Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; h, hour; HOBt, 1-hydroxybenzotriazole hydrate; HPLC, high-performance liquid chromatography; IR, infrared spectroscopy; MS, mass spectrometry; NMR, nuclear magnetic resonance; Pal, pyridylalanine; Phe, phenylalanine; Pro, proline; PS-DES, polystyrene diethylsilyl resin; PyBOP, (benzotriazol-1-yloxy)tripyrrolidino phosphonium hexafluorophosphate; rt, room temperature; TBAF, tetra-n-butylammonium fluoride; TLC, thin-layer chromatography; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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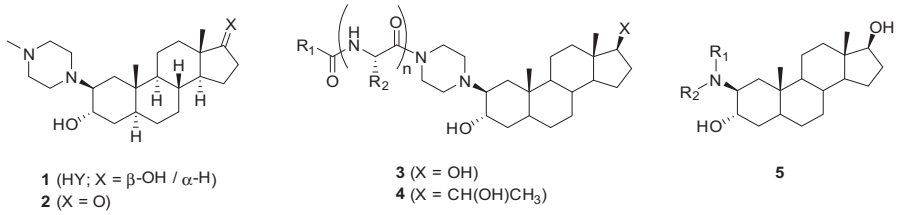

Fig. 1. Structures of compound **1**, its 17-keto analog **2**, and general structure of analog compounds represented by **3** (n = 0, 1 or 2), **4** (n = 1) and **5**.

representative biologically active aminosteroids [20]. As a result, the bioavailability expressed as the plasmatic concentration in rats of the acetylenic compound **6** was found to be more than 3 times higher than that of the corresponding unacetylenic compound **7** (Fig. 2) [20]. With this promising result obtained, and in order to accelerate the preparation of aminosteroid derivatives possessing an ethynyl group at position 17α, we have developed a new diethylsilyl acetylenic linker for the solid-phase synthesis in parallel of such analogs [21]. To expand the molecular diversity of compounds having a hydroxyacetylenic pattern, and potentially to find new steroid derivatives with interesting antiproliferative activity on cancer cells, new aminosteroids with general structure **8** (Fig. 2) were generated using this novel linker. Herein, we report the chemical synthesis and antiproliferative activity in 2 human cancer cell lines: HL-60 (myeloid leukemia) and Jurkat (lymphoid leukemia) of three libraries of ethynylated aminosteroid derivatives.

2. Materials and methods

2.1. Synthesis of libraries A, B and C

2.1.1. General remarks

The butyldiethylsilane polystyrene (PS-DES resin) with a loading of 1.56 mmol/g was supplied by Biotage (Uppsala, Sweden). Chemical reagents were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada), aapptec (Louisville, KY, USA), Matrix (Sevelen, Switzerland), Oakwook Products, Inc. (West Columbia, SC, USA), Polypeptide Laboratories (San Diego, CA, USA), Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) and Calbiochem-Novabiochem Corp. (San Diego, CA, USA). The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous dichloromethane (DCM), dimethylformamide (DMF), and pyridine were obtained from Sigma–Aldrich. The loading of steroid **9** on PS-DES-Cl (resin **12**), the addition of protected amino acid giving the resins **14** and the deprotection of Fmoc giving resins **15** were performed in peptide synthesis vessels with frit equipped for vacuum filtration (ChemGlass Inc., Vineland, NJ, USA). The reaction vessels were shaken with a Burrell wrist-action shaker model 75 (Pittsburgh, PA, USA). The steps giving resins **16** and final compounds **17** were realized with an ACT LabTech manual synthesizer (Advanced ChemTech; Louisville, KY, USA) using a solid-phase reaction block (40 wells). Thin-layer chromatography (TLC) were performed on 0.20 mm silica gel 60 F^{254} plates and with 230–400 mesh ASTM silica gel 60, respectively (Silicycle, Québec, QC, Canada). Infrared spectra (IR) were recorded on an ABB model MB3000 FT-IR spectrophotometer (Québec, QC, Canada), and the significant bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA) and reported in ppm. Mass spectra (MS) were recorded on a Shimadzu Prominence apparatus equipped with an APCI (atmospheric pressure chemical ionization) (Kyoto, Japan). The HPLC purity of each library final compound released from solid support was determined with a Shimadzu apparatus using a Shimadzu SPD-M20A Photodiode array detector, a Alltima HPC18 reversed-phase column (250 mm × 4.6 mm, 5 μm) and a solvent gradient of MeOH:H_2O. The wavelength of the UV detector was selected between 190 and 205 nm.

2.1.2. Coupling of steroid **9** to PS-DES resin **11** (synthesis of resin **13**)

To a solution of steroid **9** [22] (168 mg; 0.42 mmol) in dry THF (2 mL) under an argon atmosphere at 0 °C, a methyl lithium solution (1.6 M) in diethyl ether (0.80 mL; 1.28 mmol) was added dropwise and the solution was stirred for 1.25 h at rt. To PS-DES resin **11** (134 mg; 0.21 mmol) previously dried under vacuum and swollen in dry DCM (0.8 mL), a solution of 1,3-dichloro-5,5-dimethylhydantoin (109 mg; 0.61 mmol) in dry DCM (1.5 mL) was added under an argon atmosphere. The suspension was stirred with a Burrell wrist-action shaker for 1 h at rt. The activated PS-DES-Cl resin **12** was washed with dry DCM (15 mL) then with dry THF (15 mL) and immediately used for the next step. To this resin, was added the organolithium **10** solution prepared from steroid **9** and the mixture was stirred for 19 h at rt under an argon atmosphere. Then, the resin was washed successively with DCM (65 mL), MeOH (15 mL), H_2O (15 mL), MeOH (15 mL), DCM (15 mL) and dried overnight under vacuum to give resin **13** (53% of loading by increasing weight). IR (ATR) ν: 3302 (OH and NH_2).

2.1.3. Addition of amino acids and carboxylic acids

To the resin **13** (560 mg) in DMF (2.5 mL) were added a solution of the appropriate Fmoc-protected amino acid (see Tables 1–3)

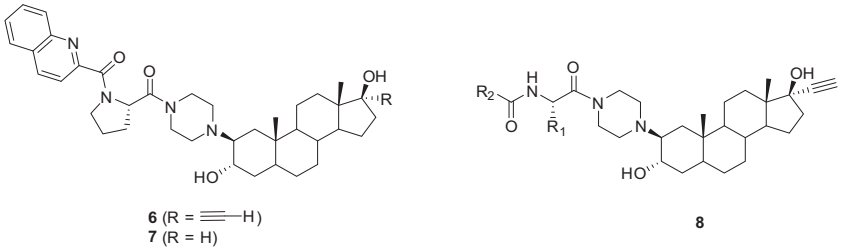

Fig. 2. Structure of acetylenic steroid **6** (tertiary alcohol), its unacetylenic analog **7** (secondary alcohol) and general structure of related ethynylated aminosteroid derivatives **8**.