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Synthesis of novel 4′-acylamino modified 21*E*-benzylidene steroidal derivatives and their cytotoxic activities

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ABSTRACT

A series of 4′-acylamino modified $\Delta^{1,4}$-pregnadien-21*E*-benzylidene-3,20-dione derivatives (**6a–v**) was synthesized from the commercially available progesterone (**1**). These title compounds were evaluated for their toxicity against brine shrimp (*Artemia salina*) and cytotoxic activities against two human cancer cell lines (HeLa and MCF-7). The results revealed that compound **6f** exhibited promising *in vitro* cytotoxic activity to the two cancer cell lines and the nature of acylamino functional group in the benzylidene moiety had a significant influence on cytotoxicity.

1. Introduction

Extensive research has been carried out to synthesize and characterize substituted steroids [1]. A variety of steroidal compounds with unusual and interesting structures have been synthesized and evaluated for their biological activities [2–5]. Their preparation remains a stimulating challenge to the organic chemistry community, often requiring the development of new synthetic approaches and associated chemical reactions. Moreover, the biological properties of modified steroids have proved to be of interest. For example, progesterone as a precursor for some pharmaceutical steroids, has been widely explored in synthesis of some new derivatives with biological activities [6–9].

Benzylidene derivatives are well known for their diverse array of pharmacological activities [10–17]. Their structure consists of two aromatic rings and an α,β-unsaturated carbonyl system joining them. In some specific study it was found that variations leading to conformational changes like epoxidation or substitution on the double bond would result in a decreased bioactivity [18]. Steroidal benzylidene derivatives, which were synthetized by the aldol condensation of certain steroids with various benzaldehyde derivatives, have been reported to act as dehydrogenase inhibitors [19], antimicrobial [20,21] and antineoplastic agents [22–25]. Those studies have proved an important role of the benzylidene steroidal scaffold in diverse biological activities, and it is therefore worth an effort to further investigate this class of compounds.

Amides are one of important functional groups prevalent in organic biomolecules. Recently, a series of acylamino-substituted derivative has exhibited strong inhibitory activities on several human cancer cell lines [26]. Though there are some reports for other such analogs, very few efforts have been done relating to the synthesis of acylamino modified benzylidene steroidal derivatives as well as their biological screening. Taking inspiration from the number of reported biological activities associated with structurally related analogs, and as a part of our commitment to search for novel potential anticancer agents related to steroidal derivatives [27,28], we efficiently synthesized a series of 4′-acylamino modified benzylidene compounds (**6a–v**), the steroidal derivatives from the commercially available progesterone. Brine shrimp lethality assay has been considered as a screening system for various extracts and synthetic compounds with antitumor activities [29]. In order to evaluate toxicity of these synthetic compounds, their median lethal concentration (LC_{50}) values were determined against *Artemia salina* as described previously [28], as well as their cytotoxic activities against two human cancer cell lines *in vitro* have been investigated in order to find potent and selective chemotherapy agents.

2. Experimental

2.1. General methods

The melting points of the products were determined on an X-4 apparatus (Beijing Tech Instrument Co., Beijing, P.R. China) and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance spectrometer (Unity plus 400 MHz) (Bruker Bios pin, Rheinstetten, Germany) with tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) were given in parts per million (ppm). Thin-layer chromatography (TLC) was performed on silica gel

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GF_{254} (Qingdao Marine Chemical Ltd., P. R. China). Column chromatography (CC) was performed over silica gel (200–300 mesh, Qingdao Marine Chemical Ltd.), High resolution electrospray ionization mass spectrometry (HRESIMS) data were recorded on LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Commercial solvents and reagents were of reagent grade.

2.2. Chemical synthesis

2.2.1. 21E-(4-nitrobenzylidene)pregna-1,4-diene-3,20-dione (3)

To a solution of **2** (1.56 g, 5 mmol) in ethanol (20 mL) was added 1.2 mL solution of 50% KOH. Then 4-nitrobenzaldehyde (6 mmol) was charged into the reaction mixture with stirring and the solution was stirred at room temperature for 12 h. The reaction mixture was neutralized with 1 mol/L HCl solution, and then ethyl acetate (150 mL) was added. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the corresponding compound **3** was obtained as solid powder by crystallization from ethyl acetate/petroleum ether. Yellow solid (1.82 g, 82%), mp 151–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.71 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 6.08 (1H, s, 4-H), 6.24 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.84 (1H, d, J = 16.0 Hz, 21-H), 7.03 (1H, d, J = 8.0 Hz, 1-H), 7.56 (1H, d, J = 16.0 Hz, 22-H), 7.68 (2H, d, J = 12.0 Hz, Ar-H), 8.24 (2H, d, J = 8.0 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.74, 18.69, 22.70, 22.83, 24.71, 32.76, 33.56, 35.62, 38.86, 43.47, 45.24, 52.25, 55.88, 62.27, 124.00, 124.21, 127.66, 128.83, 129.79, 138.71, 140.91, 148.51, 155.52, 168.79, 186.30, 199.44. HR-MS (ESI): m/z 446.2321 $[M + H]^+$ (calcd. for $C_{28}H_{32}NO_4$, 446.2326).

2.2.2. 21E-(4-aminobenzylidene)pregna-1,4-diene-3,20-dione (4)

A mixture of compound **3** (1.78 g, 4 mmol), Tin(II) chloride (4.512 g, 20 mmol), concentrated hydrochloric acid (2 mL), and ethyl acetate (40 mL) was stirred under reflux for 1 h. After cooled to room temperature, the mixture was treated with 40 mL water, and neutralized with a solution of 1 mol/L NaOH to pH was 8–9. The aqueous phase was extracted with of ethyl acetate (3 × 80 mL). The combined organic phase was dried with Na_2SO_4 and filtered. The filtrate was concentrated to give **4** as yellow powder (1.23 g, 74%). mp 223–225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 1.23 (3H, s, 19-CH_3), 4.01 (2H, s, 4′-NH_2), 6.09 (1H, s, 4-H), 6.24 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.58 (1H, d, J = 16.0 Hz, 21-H), 6.66 (2H, d, J = 8.0 Hz, Ar-H), 7.05 (1H, d, J = 8.0 Hz, 1-H), 7.38 (2H, d, J = 8.0 Hz, Ar-H), 7.49 (1H, d, J = 16.0 Hz, 22-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.62, 18.71, 22.78, 22.89, 24.81, 32.85, 33.66, 35.66, 38.82, 43.59, 44.98, 52.40, 55.89, 61.49, 114.87, 122.66, 123.92, 124.78, 127.56, 130.26, 142.35, 148.97, 155.83, 169.17, 186.42, 199.89. HR-MS (ESI): m/z 416.2589 $[M + H]^+$ (calcd for $C_{28}H_{34}NO_2$, 416.2584).

2.2.3. Synthesis of 4-acylaminobenzylidene derivatives (6a–v)

A mixture of compound **4** (42 mg, 0.1 mmol) and a series of acyl chloride **5a–v** (0.2 mmol) in dry pyridine (2.0 mL) was stirred at room temperature. When the reaction was completed (monitored by TLC), the solvent was removed under reduced pressure and the residue obtained was purified by thin-layer chromatography over silica gel GF_{254} (chloroform/acetone = 8:1) to afford 4′-acylamino derivative **6a–v** in 70–88% yield. The spectral data of various compounds is given as below.

2.2.3.1. 21E-(4-acetamidobenzylidene)pregna-1,4-diene-3,20-dione (6a).

White powder (39 mg, 86%), mp 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 2.19 (3H, s, CH_3 of acetyl), 6.08 (1H, s, 4-H), 6.24 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.68 (1H, d, J = 16.0 Hz, 21-H), 7.03 (1H, d, J = 12.0 Hz, 1-H), 7.48–7.51 (3H, m), 7.58 (2H, d, J = 8.0 Hz, Ar-H), 7.97 (1H, s, 4′-NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.46, 18.48, 22.53, 22.68, 24.50, 24.57, 32.64, 33.43, 35.42, 38.60, 43.43, 44.87, 52.15, 55.66, 61.50, 119.53, 123.67, 125.16, 127.30, 129.06, 130.05, 140.03, 141.10, 155.84, 168.47, 169.22, 186.31, 199.76. HR-MS (ESI): m/z 458.2698 $[M+H]^+$ (calcd for $C_{30}H_{36}NO_3$, 458.2690).

2.2.3.2. 21E-(4-n-propionamidobenzylidene)pregna-1,4-diene-3,20-dione (6b).

White powder (38 mg, 81%), mp 142–144 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.70 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 1.25 (3H, t, J = 4.0 Hz, CH_3 of propionyl), 6.08 (1H, s, 4-H), 6.24 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.68 (1H, d, J = 16.0 Hz, 21-H), 7.05 (1H, d, J = 8.0 Hz, 1-H), 7.48–7.59 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 9.45, 13.56, 18.60, 22.67, 22.78, 24.68, 30.71, 32.73, 33.54, 35.56, 38.73, 43.48, 44.96, 52.27, 55.80, 61.63, 119.55, 123.83, 125.29, 127.46, 129.18, 130.18, 140.05, 141.15, 155.72, 169.05, 172.06, 186.31, 199.78. HR-MS (ESI): m/z 472.2852 $[M+H]^+$ (calcd for $C_{31}H_{38}NO_3$, 472.2847).

2.2.3.3. 21E-(4-n-butyrylamidobenzylidene)pregna-1,4-diene-3,20-dione (6c).

White powder (41 mg, 85%), mp 129–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 0.99 (3H, t, J = 8.0 Hz, CH_3 of butyryl), 1.22 (3H, s, 19-CH_3), 6.08 (1H, s, 4-H), 6.24 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.67 (1H, d, J = 16.0 Hz, 21-H), 7.05 (1H, d, J = 8.0 Hz, 1-H), 7.47–7.51 (3H, m), 7.59 (2H, d, J = 8.0 Hz, Ar-H), 7.87 (1H, br, 4′-NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.45, 13.56, 18.47, 18.78, 22.53, 22.66, 24.55, 32.63, 33.41, 35.41, 38.58, 39.44, 43.42, 44.85, 52.13, 55.65, 61.48, 119.48, 123.64, 125.09, 127.27, 129.05, 129.95, 140.05, 141.14, 155.87, 169.27, 171.47, 186.33, 199.78. MS (ESI): m/z: 486$[M+H]^+$; HR-MS (ESI): m/z 486.3009 $[M+H]^+$ (calcd for $C_{32}H_{40}NO_3$, 486.3003).

2.2.3.4. 21E-(4-n-Pentanamidobenzylidene)pregna-1,4-diene-3,20-dione (6d).

White powder (43 mg, 86%), mp 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 0.93 (3H, t, J = 8.0 Hz, CH_3 of valeryl), 1.22 (3H, s, 19-CH_3), 6.08 (1H, s, 4-H), 6.24 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.67 (1H, d, J = 16.0 Hz, 21-H), 7.05 (1H, d, J = 8.0 Hz, 1-H), 7.47–7.51 (3H, m), 7.59 (2H, d, J = 8.0 Hz, Ar-H), 7.85 (1H, br, 4′-NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.46, 13.64, 18.48, 22.19, 22.54, 22.68, 24.57, 27.40, 32.64, 33.43, 35.42, 37.34, 38.60, 43.43, 44.87, 52.15, 55.66, 61.50, 119.48, 123.66, 125.09, 127.30, 129.07, 129.94, 140.11, 141.15, 155.85, 169.23, 171.62, 186.32, 199.78. HR-MS (ESI): m/z 500.3166 $[M + H]^+$ (calcd for $C_{33}H_{42}NO_3$, 500.3160).

2.2.3.5. 21E-(4-benzamidobenzylidene)pregna-1,4-diene-3,20-dione (6e).

White powder (41 mg, 79%), mp 234–236 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.70 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 6.07 (1H, s, 4-H), 6.23 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.71 (1H, d, J = 16.0 Hz, 21-H), 7.04 (1H, d, J = 8.0 Hz, 1-H), 7.47–7.51 (3H, m), 7.54–7.56 (3H, m), 7.72 (2H, d, J = 8.0 Hz, Ar-H), 7.88 (2H, d, J = 8.0 Hz, Ar-H), 8.18 (1H, s, 4′-NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.57, 18.60, 22.68, 22.78, 24.68, 32.73, 33.53, 35.56, 38.74, 43.47, 44.97, 52.26, 55.80, 61.67, 120.08, 123.83, 125.48, 127.04, 127.47, 128.77, 129.23, 130.60, 132.04, 134.53, 140.01, 141.09, 155.68, 165.68, 169.00, 186.30, 199.80. HR-MS (ESI): m/z 520.2857 $[M+H]^+$ (calcd for $C_{35}H_{38}NO_3$, 520.2846).

2.2.3.6. 21E-(4-(2-fluorobenzamido)benzylidene)pregna-1,4-diene-3,20-dione (6f).

White powder (44 mg, 82%), mp 223–225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.70 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 6.08 (1H, s, 4-H), 6.23 (1H, dd, J = 4.0, 12.0 Hz, 2-H), 6.71 (1H, d, J = 16.0 Hz, 21-H), 7.04 (1H, d, J = 12.0 Hz, 1-H), 7.17–7.22 (1H, m), 7.31–7.35 (1H, m), 7.51–7.57 (4H, m), 7.72 (2H, d, J = 8.0 Hz, Ar-H), 8.14–8.19 (1H, m), 8.60 (1H, d, J = 16.0 Hz, 4′-NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 13.68, 18.72, 22.76, 22.88, 24.79, 32.83, 33.63, 35.65, 38.83, 43.56, 45.08, 52.34, 55.89, 61.76, 116.16, 120.46, 123.89, 125.23, 125.64, 127.54, 129.30, 130.93, 132.26, 134.09, 139.66, 141.07, 152.80, 155.73, 160.30 (d, J = 246 Hz, C-F), 161.30,

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