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Design, synthesis and cytotoxic activity of a novel series of steroidal phenylpyrazoles

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ABSTRACT

Thirty novel steroidal pyrazole derivatives were designed and synthesized via a highly efficient route from pregnenolone (**1**) as starting material. The key intermediates **3a–c** were obtained under Vilsmeier conditions, and the subsequent hydrolysis, acetylation or Borch reduction afforded thirty target compounds. These compounds were mainly characterized by ^{1}H NMR, ^{13}C NMR, DEPT135°. The structure of compound **3a** was also confirmed by X-ray single crystal diffraction. The cytotoxicity of these compounds was evaluated by the SRB method against four cancer cell lines, including A549, Hela, MCF-7 and HepG2, and the results indicated that compounds **5a**, **6a**, **7a** and **8a** exhibited significant cytotoxicity with IC_{50} values ranging from 0.91 to 5.44 μM. Most importantly, compound **5a** exhibited excellent cytotoxicity against A549 with an IC_{50} value of 0.91 μM. On the basis of our research the structure–activity relationships (SAR) of these compounds were discussed. This work provides some important hints for further structural modification of steroids towards developing novel and highly effective anticancer drugs.

1. Introduction

Cancer poses a severe challenge for medical research because of its equivocal pathogenetic locations and difficulty in detection at the early stage. Though the methods of prevention, diagnosis and treatment of cancer have been progressing in recent years, it has still caused millions of people to die. Moreover, traditional anticancer drugs could cannot meet the demands of the therapy of cancer patients due to drug resistance and drug tolerance problems [1]. Therefore, in order to break the limitation of traditional cancer therapy, researchers are compelled to develop novel anticancer medicines.

Since steroid-based chemotherapeutic drugs possess higher bioavailability, less toxicity and multi-drug resistance [2], researchers are increasingly interested in the structural modification of steroidal molecules, particularly five-membered heterocycle substituted steroids and their derivatives with significant pharmacological and biological activities such as anti-bacterial, anti-inflammatory, anti-cancer and diuretic activities [3,4]. Pyrazole has always been considered as an important pharmacophore and plays a significant role in bioactivities. Therefore, it is an interesting template for combination as well as drug design [5]. Among many different D-ring substituted steroidal heterocyles, C-17 substituted steroidal pyrazole and pyrazoline derivatives show broad-spectrum bioactivities, including antimicrobial [6], anti-inflammatory, anti-nociceptive [7] and anti-tumor activities [4]. In addition, they can also be used as 17α-Hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) [8], aromatase and quinonereductase-2 [9], and 5α-reductase inhibitors [10].

To further study steroidal pyrazole analogs towards exploring excellent steroid-based lead molecules, and develop novel and highly effective anticancer drugs, thirty structurally modified 17β-pyrazolyl steroid derivatives were obtained efficiently through a concise way from pregnenolone (**1**) as the starting material. The cytotoxic activities of these compounds were tested against four cancer cell lines, including A549 (human alveolar adenocarcinoma cell line), Hela (human cervical cancer cell line), MCF-7 (human breast adenocarcinoma cell line), and HepG2 (human liver hepatocellular carcinoma cell line).

2. Experiment

2.1. General methods

Melting points were determined using a X-4 micromelting point apparatus; specific rotations were determined on a

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Perkin-Elmer 241 MC automatic polarimeter; elemental analyses were performed with a Elementar CHN analyzer model VARIO EL cubeZX21; all ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance spectrometer at 500 MHz and 125 MHz in $CDCl_3$ or CD_3OD with TMS as the reference; the Distortionless Enhancement by Polarization Transfer (DEPT) spectra (flip angle of 135°) were obtained to determine the assignments of ^{13}C chemical shifts; chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. All solvents and reagents used were obtained from commercial sources without further purification, unless otherwise noted. Column chromatography was performed on silca gel (200–300 mesh). The progresses of all reactions were monitored by thin layer chromatography (TLC) with pre-coated GF_{254} silica gel plates and visualisations were achieved with ultraviolet irradiation (254 nm) and iodine. The optical density values (OD) were recorded on a Bio-Tek ELX800 at 540 nm.

2.2. Chemical synthesis

2.2.1. General procedure for pregn-5-en-20-one-3β-ol-(p-substituted phenyl) hydrazone **2a–c**

To a solution of pregnenolone (**1**) (3.16 g, 10 mmol, 1 eq.) in acetic acid (75 ml) at room temperature, phenylhydrazine derivatives (1.1 eq.) were added and the mixture was stirred at room temperature (RT) for 6 h, and full conversion was achieved by TLC. The precipitate was filtered and dried to give corresponding product as light yellow powder, which was used directly in the next step without further purification.

2.2.2. General procedure for 17β-(1-p-substituted-phenyl-4-formyl-3-pyrazolyl) androst-5-ene-3β-ylformate **3a–c**

Phosphorus oxychloride (0.92 mL, 5 eq) was added dropwise to anhydrous *N*,*N*-dimethylformamide (20 mL), and the mixture was stirred at RT for 20 min. Then the solution of **2a–c** (2 mmol, 1 eq) in anhydrous *N*,*N*-dimethylformamide (30 mL) was added dropwise to the above mixture and stirred at 0 °C for 24 h. After full conversion by TLC, the reaction mixture was quenched with cooled saturated $NaHCO_3$ solution and the mixture was stirred until bubbling ceased. The precipitate was filtered, dried and purified by column chromatography (CH_2Cl_2:CH_3OH = 10:1, v/v) to give the corresponding pyrazole derivatives (76–93%) as white solid.

*2.2.2.1. 17β-(1-phenyl-4-formyl-3-pyrazolyl)androst-5-ene-3β-yl formate (**3a**).* **3a**, white solid, Yield: 92%, mp 272–274 °C; $[\alpha]_D^{25}$ +40.6 (*c* 1 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.96 (s, 1H, CHO), 8.39 (s, 1H, pyrazole 5′-H), 8.04 (s, 1H), 7.72 (d, 2H, *J* = 7.5 Hz), 7.48 (t, 2H, *J* = 7.5 Hz), 7.34 (t, 1H, *J* = 7.5 Hz), 5.43–5.42 (m, 1H, 6-H), 4.78–4.71 (m, 1H, 3-H), 3.28 (t, 1H, *J* = 10.0 Hz, 17-H), 1.03 (s, 3H), 0.64 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.90 (CH), 160.64 (CH), 155.53 (C), 139.42 (C), 139.33 (C), 130.65 (CH), 129.59 (CH), 129.59 (CH), 127.49 (CH), 123.93 (C), 122.82 (CH), 119.58 (CH), 119.58 (CH), 73.92 (CH), 56.52 (CH), 50.14 (CH), 48.38 (CH), 44.65 (C), 38.07 (CH_2), 37.99 (CH_2), 36.95 (CH_2), 36.69 (C), 32.38 (CH), 31.90 (CH_2), 27.76 (CH_2), 25.96 (CH_2), 24.62 (CH_2), 20.89 (CH_2), 19.34 (CH_3), 13.46 (CH_3).

*2.2.2.2. 17β-(1-(4-nitrophenyl)-4-formyl-3-pyrazolyl)androst-5-ene-3β-yl formate (**3b**).* **3b**, light yellow solid, Yield: 82%, mp 268–270 °C; $[\alpha]_D^{25}$ +93.6 (*c* 1 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 10.01 (s, 1H, CHO), 8.52 (s, 1H, pyrazole 5′-H), 8.38 (d, 2H, *J* = 9.0 Hz), 8.05(s, 1H), 7.94 (d, 2H, *J* = 9.0 Hz), 5.45–5.44 (m, 1H, 6-H), 4.79–4.73 (m, 1H, 3-H), 3.31 (t, 1H, *J* = 10.0 Hz, 17-H), 1.04 (s, 3H), 0.66 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.61 (CH), 160.62 (CH), 156.74 (C), 146.20 (C), 143.51 (C), 139.43(C), 131.06 (CH), 125.43 (CH), 125.43 (CH), 125.05 (C), 122.75 (CH), 119.25 (CH), 119.25 (CH), 73.85 (CH), 56.55 (CH), 50.09 (CH), 48.39 (CH), 44.80 (C), 38.06 (CH_2), 38.01 (CH_2), 36.96 (CH_2), 36.68 (C), 32.37 (CH), 31.87 (CH_2), 27.75 (CH_2), 26.02 (CH_2), 24.60 (CH_2), 20.88 (CH_2), 19.34 (CH_3), 13.51 (CH_3).

*2.2.2.3. 17β-(1-(4-chlorophenyl)-4-formyl-3-pyrazolyl)androst-5-ene-3β-yl formate (**3c**).* **3c**, white solid, Yield: 93%, mp 254–256 °C; $[\alpha]_D^{25}$ +101.6 (*c* 1 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.95 (s, 1H, CHO), 8.36 (s, 1H, pyrazole 5′-H), 8.04 (s, 1H), 7.67 (d, 2H, *J* = 9.0 Hz), 7.45 (d, 2H, *J* = 9.0 Hz), 5.43–5.42 (m, 1H, 6-H), 4.78–4.71 (m, 1H, 3-H), 3.27 (t, 1H, *J* = 10.0 Hz, 17-H), 1.02 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.75 (CH), 160.62 (CH), 155.77 (C), 139.42 (C), 137.85 (C), 133.10 (C), 130.51 (CH), 129.70 (CH), 129.70 (CH), 124.17 (C), 122.79 (CH), 120.67 (CH), 120.67 (CH), 73.89 (CH), 56.52 (CH), 50.12 (CH), 48.35 (CH), 44.66 (C), 38.07 (CH_2), 37.99 (CH_2), 36.95 (CH_2), 36.69 (C), 32.37 (CH), 31.89 (CH_2), 27.76 (CH_2), 25.98 (CH_2), 24.60 (CH_2), 20.88 (CH_2), 19.34 (CH_3), 13.46 (CH_3).

2.2.3. General procedure for 17β-(1-p-substituted-phenyl-4-formyl-3-pyrazolyl) androst-5-ene-3β-ol **4a–c**

To a solution of **3a–c** (1 mmol, 1 eq) in MeOH/THF (10 mL/10 mL), K_2CO_3 (151 mg, 1.1 mmol, 1.1 eq) was added at RT then the mixture was stirred at RT for 1 h. After completion of reaction, the solvent was evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 8:1, v/v) to give the corresponding products(94–96%) as white solid.

*2.2.3.1. 17β-(1-phenyl-4-formyl-3-pyrazolyl)androst-5-ene-3β-ol (**4a**).* **4a**, white solid, Yield: 96%, mp 246–248 °C; $[\alpha]_D^{25}$ +36.5 (*c* 1 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.96 (s, 1H, CHO), 8.39 (s, 1H, pyrazole 5′-H), 7.73 (d, 2H, *J* = 7.5 Hz), 7.48 (t, 2H, *J* = 7.5 Hz), 7.35 (t, 1H, *J* = 7.5 Hz), 5.38–5.37 (m, 1H, 6-H), 3.57–3.51 (m, 1H, 3-H), 3.27 (t, 1H, *J* = 10.0 Hz, 17-H), 1.01 (s, 3H), 0.64 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.92 (CH), 155.57 (C), 140.89 (C), 139.34 (C), 130.65 (CH), 129.59 (CH), 129.59 (CH), 127.49 (CH), 123.92 (C), 121.52 (CH), 119.58 (CH), 119.58 (CH), 71.75 (CH), 56.61 (CH), 50.26 (CH), 48.40 (CH), 44.67 (C), 42.29 (CH_2), 38.06 (CH_2), 37.29 (CH_2), 36.63 (C), 32.45 (CH), 31.93 (CH_2), 31.66 (CH_2), 25.97 (CH_2), 24.64 (CH_2), 20.94 (CH_2), 19.44 (CH_3), 13.46 (CH_3).

*2.2.3.2. 17β-(1-(4-nitrophenyl)-4-formyl-3-pyrazolyl)androst-5-ene-3β-ol (**4b**).* **4b**, light yellow solid, Yield: 94%, mp 222–224 °C; $[\alpha]_D^{25}$ +39.3 (*c* 1 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 10.01 (s, 1H, CHO), 8.47 (s, 1H, pyrazole 5′-H), 8.37 (d, 2H, *J* = 9.0 Hz), 7.91 (d, 2H, *J* = 9.0 Hz), 5.36–5.35 (m, 1H, 6-H), 3.48–3.42 (m, 1H, 3-H), 3.32 (t, 1H, *J* = 10.0 Hz, 17-H), 1.05 (s, 3H), 0.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.44 (CH), 159.84 (C), 146.07 (C), 143.46 (C), 140.71 (C), 131.48 (CH), 125.44 (CH), 125.44 (CH), 124.96 (C), 121.58 (CH), 119.11 (CH), 119.11 (CH), 71.74 (CH), 50.65 (CH), 49.69 (CH), 46.68 (CH), 45.33 (C), 42.22 (CH_2), 37.17 (CH_2), 36.48 (CH_2), 34.65 (C), 32.28 (CH), 32.15 (CH_2), 31.56 (CH_2), 27.12 (CH_2), 26.04 (CH_2), 20.84 (CH_2), 20.22 (CH_3), 19.38 (CH_3).

*2.2.3.3. 17β-(1-(4-chlorophenyl)-4-formyl-3-pyrazolyl)androst-5-ene-3β-ol (**4c**).* **4c**, white solid, Yield: 96%, mp 234–236 °C; $[\alpha]_D^{25}$ +53.7 (*c* 1 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.92 (s, 1H, CHO), 8.50 (s, 1H, pyrazole 5′-H), 7.72 (d, 2H, *J* = 9.0 Hz), 7.46 (d, 2H, *J* = 9.0 Hz), 5.38–5.37 (m, 1H, 6-H), 3.52–3.46 (m, 1H, 3-H), 3.29 (t, 1H, *J* = 10.0 Hz, 17-H), 1.01 (s, 3H), 0.64 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 185.84 (CH), 156.25 (C), 141.23 (C), 138.04 (C), 133.42 (C), 130.58 (CH), 129.89 (CH), 129.89 (CH), 124.21 (C), 121.58 (CH), 121.09 (CH), 121.09 (CH), 71.74 (CH),