Steroids 112 (2016) 103–108

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis and antiproliferative activity of D-ring substituted steroidal benzamidothiazoles

Ning-Juan Fan [a], Qiu-Rui He [b], Min Duan [a], Yu-Bin Bai [b], Jiang-Jiang Tang [b,*]

[a] Biochemistry and Molecular Biology Research Platform, College of Life Science, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Shaanxi Engineering Center of Bio-resource Chemistry & Sustainable Utilization, College of Science, Northwest A&F University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 30 March 2016
Accepted 21 April 2016
Available online 29 April 2016

Keywords:
Progesterone
Thiazole
Steroidal derivatives
Antiproliferative activity

ABSTRACT

Using progesterone as the starting material, we synthesized a series of steroidal derivatives possessing a D-ring substituted benzamidothiazole. All of the final structures were reported and identified by NMR and HRMS spectrometry for the first time. The antiproliferative activity of the synthesized compounds against PC-3 (human prostate cancer cell line) and SKOV-3 (ovarian cancer cells) were investigated. The preliminary results showed that compounds **8b**, **8d** and **8g** possessed moderate antiproliferative activities.

1. Introduction

Steroidal derivatives in which the D-ring is modified with *exo*-heterocycles exhibit numerous forms of biological activity and are attractive for medicine [1]. Recently, a large number of steroidal derivatives containing five- or six-membered 17-*exo*-heterocycles (preferably nitrogen containing), such as pyrazolinyl [2,3], triazolyl [4,5] and pyrazolyl [6], were found to exert potential cytotoxic activity, and abiraterone (17-(3′-Pyridyl)androsta-5,16-dien-3β-ol) has been successfully applied in the treatment of prostatic carcinoma [7]. Thiazole moieties have various pharmacological activities, such as antimalarial [8], antimicrobial [9], anti-inflammatory [10] and anti-hypolipidemic [11] agents. Additionally, the antitumor activity [12–14] of thiazole derivatives such as benzamidothiazoles has been reported. In drug design, molecular hybridization is a prevailing concept that aims primarily at combating drug resistance and enriching the existing arsenals of anti-infective agents [15,16]. Molecular hybridization usually occurs in two or more pharmacophores or chemical entities that are either linked or fused together to create a new molecule. These pharmacophores are always chosen based on their known bio-properties to achieve synergistic or additive pharmacological activities [17–19].

Progesterone has been explored in our research group as a precursor for some pharmaceutical steroids. Our previous results have shown that some steroidal derivatives of progesterone that introduced an unsaturated double bond at C1-C2 and then introduced a chlorine atom into the C-4 position could significantly improve cytotoxicity against certain cancer cell lines [20,21]. As a continuation of our research program and based on the hybridization concept, we herein report on the introduction of new structural elements into progesterone: a C1-C2 double bond (Δ^1) or a chlorine atom at C-4 along with the double bond as well as a benzamidothiazole (benzamido group attached to a thiazole unit) incorporated with a D-ring. Alternative substituents on the benzamido skeleton were also investigated. Furthermore, we evaluated the antiproliferative effects of the newly synthetized steroidal derivatives and studied the influence of the benzamido group on the activity. Although some steroidal thiazole derivatives are reported in the literature [22,23], this is the first report on the antiproliferative activities of this group of steroidal derivatives bearing a benzamidothiazole scaffold.

2. Experimental

2.1. General methods

The melting points of the products were determined on an X-4 apparatus (Beijing Tech Instrument Co., Beijing, PR China) and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance spectrometer (Unity plus 500 MHz)

* Corresponding author.
E-mail addresses: fannj1980@nwsuaf.edu.cn (N.-J. Fan), tangjiang11@nwsuaf.edu.cn (J.-J. Tang).

http://dx.doi.org/10.1016/j.steroids.2016.04.009
0039-128X/

(Bruker Bios pin, Rheinstetten, Germany) with tetramethylsilane (TMS) as the internal standard. Chemical shift values (δ) were given in parts per million (ppm). Thin-layer chromatography (TLC) was performed on silica gel 60 F254 (Qingdao Marine Chemical Ltd., PR China). Column chromatography (CC) was performed over silica gel (200–300 mesh, Qingdao Marine Chemical Ltd.), and 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. Synthesis of 21-bromo-pregn-1,4-diene-3,20-dione (3) and 21-bromo-4-chloro-pregn-1,4-diene-3,20-dione (**4**)

Compound **1** (or **2**) (8 mmol) and cupric bromide (5.36 g, 24 mmol) in methanol (100 mL) with pyridine (1.90 g, 24 mmol) were refluxed for 24 h. After completion, the reaction mixture was cooled to room temperature and suction filtered to remove CuBr. The filtrate was concentrated and the residue dissolved in dichloromethane (150 mL) and washed with water (100 mL) and saturated sodium bicarbonate solution (100 mL) followed by brine (100 mL). The organic layer was dried with anhydrous Na_2SO_4 and evaporated in vacuo, and the corresponding compound **3** (or **4**) was obtained as a solid powder by crystallization from acetone.

*2.2.1.1. 21-Bromo-pregn-1,4-diene-3,20-dione (**3**).* White solid (2.215 g, 71%). mp 171–173 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.73 (3H, s, 18-CH_3), 1.24 (3H, s, 19-CH_3), 3.90 (2H, s, 21-CH_2), 6.08 (1H, s, 4-H), 6.25 (1H, d, J = 10.0 Hz, 2-H), 7.05 (1H, d, J = 10.0 Hz, 1-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.72, 18.73, 22.79, 23.77, 24.67, 32.76, 33.50 35.53, 35.76, 38.37, 43.46, 44.90, 52.10, 55.55, 60.10, 124.06, 127.72, 155.51, 168.72, 186.32, 201.85. HR-MS(ESI): m/z 391.1267 $[M+H]^+$ (calcd for $C_{21}H_{28}BrO_2$, 391.1270).

*2.2.1.2. 21-Bromo-4-chloro-pregn-1,4-diene-3,20-dione (**4**).* White solid (2.31 g, 68%). mp 170–172 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.73 (3H, s, 18-CH_3), 1.29 (3H, s, 19-CH_3), 3.90 (2H, s, 21-CH_2), 6.35 (1H, d, J = 10.0 Hz, 2-H), 7.07 (1H, d, J = 10.0 Hz, 1-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 13.68, 19.16, 23.11, 23.80, 24.59, 28.84, 32.36, 35.43, 35.67, 38.27, 44.80, 46.12, 52.76, 55.37, 59.99, 126.40, 128.38, 155.06, 162.15, 178.22, 201.73. HR-MS (ESI): m/z 425.0875 $[M+H]^+$ (calcd. for $C_{21}H_{27}BrClO_2$, 425.0877).

2.2.2. Synthesis of 17-(2-amino-4-thiazolyl)-androst-1,4-dien-3-one (**5**) and 17-(2-amino-4-thiazolyl)-4-chloro-androst-1,4-dien-3-one (**6**)

To a stirred solution of **3** (or **4**) (4 mmol) in ethanol (50 mL), thiourea (609 mg, 8 mmol) and triethylamine (1.02 g, 10 mmol) were added. The reaction mixture was heated under reflux for 2 h. After completion of the reaction, the mixture was evaporated slowly until approximately 15 mL solvent remained and was then moved to a refrigerator overnight to force crystallization. The white solid was filtered and washed with cooled ethanol, and then product **5** (or **6**) was produced.

*2.2.2.1. 17-(2-Amino-4-thiazolyl)-androst-1,4-dien-3-one (**5**).* White solid (1.015 g, 69%). mp 148–150 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 0.52 (3H, s, 18-CH_3), 1.19 (3H, s, 19-CH_3), 5.98 (1H, s, 4-H), 6.13 (1H, s, SCH), 6.12 (1H, d, J = 10.0 Hz, 2-H), 6.75 (2H, s, NH_2), 7.20 (1H, d, J = 10.0 Hz, 1-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 13.10, 18.48, 22.19, 24.19, 25.37, 32.02, 33.36, 35.20, 37.72, 43.30, 43.54, 52.21, 52.31, 54.19, 100.39, 123.01, 126.70, 152.71, 156.57, 167.15, 169.61, 184.96. HR-MS(ESI): m/z 369.1999 $[M+H]^+$ (calcd for $C_{22}H_{29}N_2OS$, 369.1995).

*2.2.2.2. 17-(2-Amino-4-thiazolyl)-4-chloro-androst-1,4-dien-3-one (**6**).* White solid (0.997 g, 62%). mp 241–244 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 0.57 (3H, s, 18-CH_3), 1.31 (3H, s, 19-CH_3), 6.13 (1H, s, SCH), 6.33 (1H, d, J = 10.0 Hz, 2-H), 6.75 (2H, s, NH_2), 7.33 (1H, d, J = 10.0 Hz, 1-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 13.03, 18.81, 22.53, 24.07, 25.36, 28.50, 32.23, 35.02, 37.63, 43.49, 46.33, 52.13, 52.90, 53.94, 100.37, 125.19, 126.82, 152.72, 156.72, 163.52, 167.08, 177.08. HR-MS(ESI): m/z 403.1605 $[M+H]^+$ (calcd for $C_{22}H_{28}ClN_2OS$, 403.1605).

2.2.3. General procedure for the synthesis of thiazolyl derivatives (**8a–g** and **9a–g**)

To a solution of **5** (or **6**) (0.2 mmol) in dichloromethane (2.0 mL) and triethylamine (0.6 mmol), respective acid chlorides **7a–g** (0.24 mmol) were added under cold conditions, followed by stirring at room temperature for 6–8 h. After completion of the reaction (confirmed by TLC), the solvent was removed under reduced pressure, and the residue obtained was dissolved in dichloromethane and chromatographed on silica gel with petroleum ether/ethyl acetate/dichloromethane (10:3:3, v/v) as eluent to afford **8a–g** (or **9a–g**).

*2.2.3.1. 17-(2-Benzamido-4-thiazolyl)-androst-1,4-dien-3-one (**8a**).* White solid (84 mg, 89%). mp 170–172 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.52 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 6.10 (1H, s, 4-H), 6.24 (1H, dd, J = 1.5, 10.0 Hz, 2-H), 6.62 (1H, s, SCH), 7.04 (1H, d, J = 10.0 Hz, 1-H), 7.47–7.50 (2H, m, Ar-H), 7.57–7.60 (1H, m, Ar-H), 7.95 (2H, d, J = 8.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.10, 18.80, 22.69, 24.59, 25.92, 32.88, 33.62, 35.98, 37.91, 43.63, 44.25, 52.40, 52.40, 54.87, 108.20, 124.03, 127.58, 127.61, 128.94, 132.20, 132.83, 151.89, 155.79, 157.73, 164.75, 169.02, 186.41. HR-MS(ESI): m/z 473.2258 $[M+H]^+$ (calcd for $C_{29}H_{33}N_2O_2S$, 473.2257).

*2.2.3.2. 17-(2-(3′-Flurobenzamido)-4-thiazolyl)-androst-1,4-dien-3-one (**8b**).* White solid (76 mg, 78%). mp 108–110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.54 (3H, s, 18-CH_3), 1.23 (3H, s, 19-CH_3), 6.11 (1H, s, 4-H), 6.24 (1H, dd, J = 1.5, 10.0 Hz, 2-H), 6.64 (1H, s, SCH), 7.06 (1H, d, J = 10.0 Hz, 1-H), 7.28–7.32 (1H, m, Ar-H), 7.47–7.51 (1H, m, Ar-H), 7.74 (1H, d, J = 8.0 Hz, Ar-H), 7.79 (1H, d, J = 8.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.13, 18.80, 22.70, 24.59, 25.94, 32.87, 33.65, 35.98, 37.95, 43.67, 44.32, 52.39, 52.41, 54.94, 108.43, 115.15, 119.95, 123.13, 124.12, 127.66, 130.68, 130.74, 134.35, 134.38, 155.90, 162.96 (d, J = 247 Hz, C-F), 163.51, 169.12, 186.52. HR-MS(ESI): m/z 491.2164 $[M+H]^+$ (calcd for $C_{29}H_{32}FN_2O_2S$, 491.2163).

*2.2.3.3. 17-(2-(3′-Chlorobenzamido)-4-thiazolyl)-androst-1,4-dien-3-one (**8c**).* White solid (78 mg, 77%). mp 108–110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.57 (3H, s, 18-CH_3), 1.23 (3H, s, 19-CH_3), 6.11 (1H, s, 4-H), 6.24 (1H, dd, J = 1.5, 10.0 Hz, 2-H), 6.66 (1H, s, SCH), 7.05 (1H, d, J = 10.0 Hz, 1-H), 7.46–7.49 (1H, m, Ar-H), 7.58 (1H, d, J = 8.0 Hz, Ar-H), 7.98 (1H, d, J = 8.0 Hz, Ar-H), 8.09 (1H, s, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.27, 18.82, 22.73, 24.61, 26.31, 32.90, 33.64, 36.06, 37.74, 43.67, 44.39, 52.14, 52.44, 54.92, 108.41, 124.07, 125.99, 127.65, 128.25, 129.84, 130.29, 133.00, 133.38, 133.67, 135.27, 155.85, 163.68, 169.07, 186.45. HR-MS(ESI): m/z 507.1872 $[M+H]^+$ (calcd for $C_{29}H_{32}ClN_2O_2S$, 507.1868).

*2.2.3.4. 17-(2-(4′-Chlorobenzamido)-4-thiazolyl)-androst-1,4-dien-3-one (**8d**).* White solid (83 mg, 82%). mp 110–112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.57(3H, s, 18-CH_3), 1.26 (3H, s, 19-CH_3), 6.11 (1H, s, 4-H), 6.24 (1H, dd, J = 1.5, 10.0 Hz, 2-H), 6.64 (1H, s, SCH), 7.05 (1H, d, J = 10.0 Hz, 1-H), 7.50 (2H, d, J = 8.0 Hz, Ar-H), 7.99 (1H, d, J = 8.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ