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Synthesis, anti-inflammatory and anti-ulcer evaluations of thiazole, thiophene, pyridine and pyran derivatives derived from androstenedione

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ABSTRACT

The reaction of androstenedione with bromine gave the 16-bromo derivative 2. The latter reacted with either cyanothioacetamide or thiourea to give the 2-cyanomethylthiazole derivative **4** and the 2-aminothiazole derivative **13**. Compound **4** and **13** were used underwent some condensation, coupling and heterocyclization reactions to give thiophene, pyridine and pyran derivatives. The anti-inflammatory and anti-ulcer evaluations of the newly synthesized products were evaluated and the results showed that **23f** showed the maximum antiulcer activity. In addition, toxicity of the most active compounds was studied against shrimp larvae and showed that compounds **2**, **23c** and **23f** showed non-toxicity against the tested organisms.

1. Introduction

Androstenedione, a naturally-occurring steroidal compound, has been commonly available for use as a dietary supplement [1–3]. Androstenedione is a frequent component in many performance-enhancing and body building products. The fact that minor modifications of the androstenedione structure can result in extensive changes in biological activity has led to the development of various androstenedione derivatives. These can be classified into two different categories. The first consists of modification of the steroid ring system itself, either through substitution of an androstenedione core carbon atom with a heteroatom [4], or modification of the ring systems through expansion, contraction, or additional cyclic features [5]. The second category involves addition of one or more functional groups to the androstenedione core structure. The most common of which tends to be the latter due to the extensive synthetic work that is necessary for the incorporation of heteroatoms into the steroid structure. A number of research groups have been able to expand upon the known biological activity of steroid compounds by combining them with important structural features from other natural products, creating new hybrid molecules for testing [6–11]. Chemical modification of the steroid D-ring provides a way to alter the functional groups, sizes and stereochemistry of the D-ring, and numerous structure–activity relationships have been established by such synthetic alterations. Steroids bearing heterocycles fused to the D-ring of the steroid nucleus have been of pharmaceutical interest [12–14]. Recently our research group reported the heterocyclization of some steroids followed by their cytotoxic evaluation towards cancer cell lines [15–17]. In the present paper, we report on the efficient synthesis of androstenedione possessing thiazole, thiophene, pyridine and pyran ring systems. This study focused on the synthesis and biochemical evaluation of the newly synthesized heterocyclic which were then subjected through anti-inflammatory and anti-ulcer evaluations.

2. Experimental

2.1. Synthetic methods, analytical and spectral data

The starting steroid, androstenedione (**1**), was purchased from Sigma Company, USA. All solvents were dried by distillation prior

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to using. Melting points were recorded on Buchi melting point apparatus D-545; ^{13}C NMR and ^{1}H NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ and DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit Ludwig-Maximilians-Universitaet-Muenchen, Germany. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The purity of all compounds was determined through used for peak purity determination in high performance liquid chromatography (HPLC). Where in this work the purity of compounds was improved through changing the eluents being used and the solvent introduced through the data was that which minimize the peak distortion and consequently indicating the best purity of the described compounds [18,19]. The nomenclature of the newly synthesized compounds were according to the ChemBioDraw Ultra12.

2.2. Chemical syntheses

2.2.1. (8R,9S,10R,13S,14S)-16-Bromo-10,13-dimethyl-7,8,9,10,11,12,13,14,15,16-decahydro-1H-cyclopenta[a]phenanthrene-3,17(2H,6H)-dione (**2**)

To a solution of androstenedione (0.286 g, 1 mmol) in acetic acid (40 mL) at 40 °C, bromine solution (0.50 mL, 0.01 mol) in acetic acid (10 mL) was added drop wise then the reaction mixture was stirred at room temperature for 3 h. The whole mixture was poured onto ice water and the formed solid product was collected by filtration.

HPLC purity = 89% (C-18 NovaPak column; MeOH:EtOAc/87:13), t_r = 22 min; pale yellow crystals from EtOAc: hexane (82%), m.p. 180–183 °C; IR (KBr) cm^{-1}: 3059, 2930, 1680, 1561; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.03 (2s, 6H), 1.30–2.87 (m, 10H), 2.85–2.96 (m, 4H), 5.01, 5.20 (2s, 2H), 5.38 (s, 1H), 6.10 (s,1H), 6.40 (d, 1H, *J* = 2.50 Hz); ^{13}C-NMR ($CDCl_3$): δ 17.2,17.6, 19.9, 23.6, 25.8, 29.0, 30.2, 33.9, 34.6, 34.8, 42.0, 43.9, 44.0, 53.2, 55.4, 124.2, 158.8, 190.8, 192.3. MS: *m*/*e* = 365 (M^+, 22%); *Analysis Calcd* for $C_{19}H_{25}BrO_2$: C, 62.47; H, 6.90; Br, 21.87%. Found: C, 62.61; H, 7.21; Br, 21.69%.

2.2.2. 2-((6aR,6bS,8aS,12aS,12bR)-6a,8a-Dimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-10-yl)acetonitrile (**4**)

To a solution of compound **2** (0.365 g, 1 mmol) in absolute ethanol (40 mL) cyanothioacetamide (0.100 g, 1 mmol) was added. The reaction mixture was heated under reflux for 2 h and the reaction was monitored under TLC control then poured onto ice/water containing few drops of sodium hydroxide (1 mL, 10%) and the formed solid product was collected by filtration.

Compound **4**: HPLC purity = 90% (C-18 NovaPak column; MeOH:EtOAc/88:12), t_r = 18 min; yellow crystals from EtOAc: hexane (80%), m.p. 210–212 °C; IR (KBr) cm^{-1}: 3054, 2930, 2220, 1680, 1633, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.87, 1.04 (2s, 6H), 1.32–2.85 (m, 10H), 2.88–2.96 (m, 4H), 4.80 (s, 2H), 5.21 (1s, 1H), 5.37 (s, 1H), 6.29 (s, 1H), 6.43 (d, 1H, *J* = 3.02 Hz); ^{13}C-NMR ($CDCl_3$): δ 17.3,17.6, 19.8, 21.8, 23.6, 25.4, 29.4, 30.0, 33.7, 34.6, 34.8, 41.5, 43.9, 44.4, 53.2, 117.2, 128.2, 133.6, 144.8, 146.3, 158.2, 190.3. MS: *m*/*e* = 366 (M^+, 38%); *Analysis Calcd* for $C_{22}H_{26}N_2OS$: C, 72.09; H, 7.15; N, 7.64; S, 8.75%. Found: C, 72.24; H, 6.89; N, 7.43; S, 8.64%.

2.2.3. 2-((6aR,6bS,8aS,12aS,12bR)-6a,8a-Dimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-10-yl)-3-phenylacrylonitrile (**6a**), 3-(4-chlorophenyl)-2-((6aR,6bS,8aS,12aS,12bR)-6a,8a-dimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-10-yl)acrylonitrile (**6b**) and 3-(4-methoxyphenyl)-2-((6aR,6bS,8aS,12aS,12bR)-6a,8a-dimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d]thiazol-10-yl)acrylonitrile (**6c**)

General procedure: To a solution of compound **4** (0.366 g, 1 mmol) in ethanol (30 mL) containing piperidine (0.50 mL), either benzaldehyde (0.108 g, 1 mmol), 4-chlorobenzaldehyde (0.140 g, 1 mmol) or 4-methylbenzaldehyde (0.137 g, 1 mmol) was added with stirring. The reaction mixture was heated under reflux for 2 h till thin layer chromatography revealed just a single spot which proved the presence of a single product. The formed solid product upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration.

Compound **6a**: HPLC purity = 88% (C-18 NovaPak column; MeOH:EtOAc/85:15), t_r = 21 min; yellow crystals from EtOAc: hexane (80%), m.p. 166–168 °C; IR (KBr) cm^{-1}: 3056, 2933, 2222, 1687, 1664, 1636, 1568; ^{1}H-NMR ($CDCl_3$): δ 0.87, 1.04 (2s, 6H), 1.32–2.87 (m, 10H), 2.86–2.99 (m, 4H), 5.06, 5.16 (2s, 2H), 6.10 (s, 1H), 6.48 (d, 1H, *J* = 3.80 Hz), 7.01 (s, 1H, CH = C), 7.31–7.38 (m, 5H); ^{13}C-NMR ($CDCl_3$): δ 17.20,17.9, 19.5, 20.3, 23.6, 25.8, 29.4, 30.0, 33.9, 34.4, 34.8, 41.7, 43.9, 44.2, 106.9, 119.0, 117.2, 120.8, 122.7, 128.0, 128. 3, 130.8, 149.0, 149.6, 152.8, 154.3, 190.8. MS: *m*/*e* = 454 (M^+, 22%); *Analysis Calcd* for $C_{29}H_{30}N_2OS$: C, 76.61; H, 6.65; N, 6.16; S, 7.05%. Found: C, 76.41; H, 6.47; N, 6.08; S, 6.88%.

Compound **6b**: HPLC purity = 92% (C-18 NovaPak column; MeOH:EtOAc/80:20), t_r = 19 min; yellow crystals from EtOAc: hexane (68%), m.p. 222–224 °C; IR (KBr) cm^{-1}: 3058, 2930, 2220, 1689, 1662, 1638, 1564; ^{1}H-NMR ($CDCl_3$): δ 0.88, 1.03 (2s, 6H), 1.30–2.89 (m, 10H), 2.86–2.98 (m, 4H), 5.06, 5.16 (2s, 2H), 6.10 (s, 1H), 6.48 (d, 1H, *J* = 3.80 Hz), 7.01 (s, 1H, CH═C), 7.26, 7.36 (2d, 4H); ^{13}C-NMR ($CDCl_3$): δ 17.22, 17.8, 19.5, 20.4, 23.6, 25.9, 29.6, 30.2, 33.9, 34.4, 34.7, 41.9, 42.3, 43.6, 44.0, 106.6, 117.0, 119.2, 120.9, 123.8, 125.3, 126.3, 127.1, 129.8, 149.2, 149.7, 153.0, 170.1, 190.4. MS: *m*/*e* = 489 (M^+, 20%); *Analysis Calcd* for $C_{29}H_{29}ClN_2OS$: C, 71.22; H, 5.98; N, 5.73; S, 6.56%. Found: C, 71.37; H, 6.08; N, 5.80; S, 6.73%.

Compound **6c**: HPLC purity = 88% (C-18 NovaPak column; MeOH:EtOAc/90:10), t_r = 19 min; yellow crystals from EtOAc: hexane (68%), m.p. 222–224 °C; IR (KBr) cm^{-1}: 3058, 2930, 2220, 1689, 1662, 1638, 1564; ^{1}H-NMR ($CDCl_3$): δ 0.87, 1.04 (2s, 6H), 1.31–2.89 (m, 10H), 2.88–2.95 (m, 4H), 3.11 (s, 3H), 5.06, 5.16 (2s, 2H), 6.20 6.48 (d, 1H, *J* = 3.80 Hz), 7.01 (s, 1H, CH═C), 7.26, 7.36 (2d, 4H); ^{13}C-NMR ($CDCl_3$): δ 17.22, 17.8, 19.5, 20.4, 23.6, 25.8, 29.6, 30.2, 33.9, 34.4, 34.7, 41.9, 43.6, 44.0, 55.4, 106.8, 117.0, 119.9, 120.4, 121.5, 125.6, 127.0, 128.2, 148.5, 149.2, 153.3, 170.1, 190.6. MS: *m*/*e* = 484 (M^+, 16%); *Analysis Calcd* for $C_{30}H_{32}N_2O_2S$: C, 74.35; H, 6.66; N, 5.78; S, 6.60%. Found: C, 74.57; H, 6.49; N, 5.92; S, 6.53%.

2.2.4. (6aR,6bS,8aS,12aS,12bR,E)-6a,8a-Dimethyl-4-oxo-N′-phenyl-2,4,5,6,6a,6b,7,8,8a,-12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazole-10-carbohydrazonoyl cyanide (**8a**) (6aR,6bS,8aS,12aS,12bR,E)-N′-(4-chlorophenyl)-6a,8a-dimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazole-10-carbohydrazonoyl cyanide (**8b**) and (6aR,6bS,8aS,12aS,12bR,E)-N'-(4-chlorophenyl)-6a,8a-dimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]-indeno[1,2-d]thiazole-10-carbohydrazonoyl cyanide (**8c**)

General procedure: To a solution of compound **4** (0.366 g, 1 mmol) in ethanol (30 mL) containing sodium acetate (1.50 g), either benzenediazonium chloride (1 mmol), 4-chlorobenzenediazonium chloride (1 mmol) or 4-methybenzenediazonium chloride (1 mmol) [prepared by adding sodium nitrite solution