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New 5α-reductase inhibitors: In vitro and in vivo effects

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Abstract

The enzyme 5α-reductase is responsible for the conversion of testosterone (T) to its more potent androgen dihydrotestosterone (DHT). This steroid had been implicated in androgen-dependent diseases such as: benign prostatic hyperplasia, prostate cancer, acne and androgenic alopecia. The inhibition of 5α-reductase enzyme offers a potentially useful treatment for these diseases.

In this study, we report the synthesis and pharmacological evaluation of several new 3-substituted pregna-4, 16-diene-6, 20-dione derivatives. These compounds were prepared from the commercially available 16-dehydropregnenolone acetate. The biological activity of the new steroidal derivatives was determined in vivo as well as in vitro experiments.

In vivo experiments, the anti-androgenic effect of the steroids was demonstrated by the decrease of the weight of the prostate gland of gonadectomized hamster treated with T plus finasteride or the new steroids. The IC_{50} value of these steroids was determined by measuring the conversion of radio labeled T to DHT.

The results of this study carried out with 5α-reductase enzyme from hamster and human prostate showed that four of the six steroidal derivatives (**5**, **7**, **9**, **10**) exhibited much higher 5α-reductase inhibitory activity, as indicated by the IC_{50} values than the presently used Proscar **3** (finasteride).

The comparison of the weight of the hamster's prostate gland indicated that compound **5** had a comparable weight decrease as finasteride. The overall data of this study showed very clearly those compounds **5**, **7**, **9**, **10** are good inhibitors for the 5α-reductase enzyme.

Keywords: 5α-Reductase; 5α-Reductase inhibitors; Prostate; Pregnadiene derivatives; In vivo effect of 5α-reductase inhibitors; Epoxide toxicity

1. Introduction

The normal activity of the NADPH-dependent 5α-reductase enzyme (EC 1.3.99.5) maintains testosterone's **1** (T) biological functions: anabolic actions and spermatogenesis of humans as well as the dihydrotestosterone **2** mediated effects such as, increased facial and body hair, acne, scalp hair recession, and prostate enlargement [1]. Abnormally high 5α-reductase activity in humans results in excessively high DHT levels in peripheral tissues, which is implicated in the pathogenesis of prostate cancer, benign prostatic hyperplasia (BPH), acne, and male patter baldness [2,3], thus suggesting that both the enzyme 5α-reductase and DHT play important physiological and pathological roles in humans. Therefore, the suppression of androgen action by 5α-reductase inhibitors is a logical treatment for 5α-reductase activity disorders. Furthermore, since the beginning of the last decade, two types of 5α-reductase enzyme had been identified **1** and **2** [4,5]; the identification of these two isozymes opened the door for specific and selective inhibition.

The most extensively studied class of 5α-reductase inhibitors are the 4-azasteroids [6,7], which include the drug finasteride **3**. This compound is the first 5α-reductase inhibitor approved in the USA for the treatment of BPH. This drug has approximately a 100-fold greater affinity for type

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2–5α-reductase, than for the type 1 enzyme. In humans, finasteride decreases prostatic DHT levels by 70–90% and reduces prostate size, while T tissue levels remain constant [8]. The use of finasteride demonstrated a sustained improvement in the treatment of androgen-dependent diseases and it also reduces the prostate specific antigen (PSA) levels [8].

Recently, our group synthesized several new progesterone derivatives that considerably decreased the prostate growth produced by T [9–12]. Since these compounds showed a high biological activity [9–12], in this paper, we describe the synthesis and pharmacological evaluation of six similar compounds (**5**–**11**) based on the progesterone skeleton as inhibitors of hamster and human 5α-reductase enzyme.

2. Experimental

2.1. Chemical and radioactive material

Solvents were laboratory grade or better. (1, 2, 6, 7–^{3}H) Testosterone [^{3}H] T specific activity: 95 Ci/mmol was provided by New England Nuclear Corp. (Boston, MA). Radioinert T and 5α-dihydrotestosterone were supplied by Steraloids (Wilton, NH, USA). Sigma Chemical Co. (St. Louis, MO) supplied NADPH. Finasteride was obtained by extraction from Proscar (Merck, Sharp & Dohme). The tablets were crushed, extracted with chloroform and the solvent was eliminated in vacuum; the crude product was purified by silica gel column chromatography.

2.2. Synthesis of the steroidal derivatives

*2.2.1. Synthesis of steroidal derivatives **5**–**11***

*2.2.1.1. 3β-Acetoxy-5α, 6α-epoxypregn-16-ene-20-one **5**.* A solution of steroid **4** (1 g, 2.82 mmol) and *m*-chloroperbenzoic acid (1.62 g) in chloroform (50 ml) was stirred at room temperature for 30 min. Upon termination of the epoxidation, a saturated aqueous solution of sodium bicarbonate (84 ml) containing sodium bisulfite (1 g) was added. The reaction mixture was extracted three times with chloroform; the organic phase was washed with water, dried with sodium sulfate and the solvent was removed in vacuum. The crude product was recrystallized from methanol. Yield 1.01 g, 2.7 mmol (97%) of pure product **5**, mp 170–172 °C. UV (nm): 238 (ε = 10100). IR (KBr) cm^{-1}: 2943, 1734, 1665 and 1588. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3Hs, H-18), 1.1 (3Hs, H-19), 2.0 (3Hs, H-21), 2.2 (3Hs, acetoxy), 2.9 (1Hd, J = 2 Hz, epoxy at C-6), 4.7 (1Hd, J = 2 Hz, H-3). ^{13}C-NMR ($CDCl_3$) δ: 15.8 (C-18), 16.9 (C-19), 21.3 (C-21), 27.2 (acetoxy), 63.2 (C-6), 65.3 (C-5), 144.1 (C-16), 155.2 (C-17), 170.5 (acetoxy carbonyl), 196.7 (C-20). MS *m*/*z* 372 (M+).

*2.2.1.2. 3β-Acetoxy-5-hydroxypregn-16-ene-6,20-dione **6**.* To a solution of steroid **5** (1 g, 2.7 mmol) in acetone (50 ml) was added dropwise a solution of chromium trioxide (1.05 g, 10.5 mmol) in water (5 ml) at 0 °C during 10 min. The resulting mixture was allowed to warm up to room temperature and again the same amount of chromium trioxide was added in the same manner. The mixture was diluted with cold water (150 ml) and the precipitated product was filtered and dried. It was recrystallized from methanol; yield 852 mg, 2.19 mmol (81%) of pure product **6**, mp 244–245 °C. UV (nm) 238 (ε = 10200). IR (KBr) cm^{-1}: 3409, 2940, 1730, 1700, 1685 and 1600. ^{1}H-NMR ($CDCl_3$) δ: 0.8 (3Hs, H-18), 1.1 (3Hs, H19), 2.0 (3Hs, H-21), 2.2 (3Hs, acetoxy), 4.8 (1Hm, H-3), 6.6 (1Hq, J = 2 Hz, H-16). ^{13}C-NMR ($CDCl_3$) δ: 15.8 (C-18), 17.0 (C-19), 22.0 (C-21), 28.0 (acetoxy), 80.0 (C5), 143.8 (C-16), 155.0 (C-17), 173.0 (acetoxy carbonyl), 197.2 (C-20), 212.8 (C-6). MS *m*/*z* 388 (M+).

*2.2.1.3. 3β-Acetoxypregna-4,16-diene-6,20-dione **7**.* To a solution of steroid **6** (1 g, 2.57 mmol) in pyridine (10 ml) was added dropwise under a nitrogen atmosphere at 0 °C thionyl chloride (1 ml). The resulting solution was stirred at room temperature for 45 min. Iced water (100 ml) was added and it was extracted three times with ethyl acetate. The organic phase was washed with 10% aqueous hydrochloric acid, 5% aqueous sodium bicarbonate and water. It was dried with sodium sulfate and the solvent was removed in vacuum. The crude product was recrystallized from ethyl acetate-hexane. Yield 623 mg, 1.69 mmol (66%) of pure product **7**, mp 193–195 °C. UV (nm): 239 (ε = 10300), 243 (ε = 6200). IR (KBr) cm^{-1}: 2942, 1735, 1691 and 1635. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (3Hs, H-18), 1.0 (3Hs, H-19), 2.1 (3Hs, H-21), 2.3 (3Hs, acetoxy), 5.1 (1Hm, H-3), 6.0 (1Hq, J = 2 Hz, H-4), 6.7 (1Hq, J = 2 Hz, H-16). ^{13}C-NMR ($CDCl_3$) δ: 15.7 (C-18), 19.6 (C-19), 22.0 (C-21), 29.5 (acetoxy), 70.1 (C-3), 129.0 (C-4), 143.7 (C-16), 147.2 (C-5), 154.6 (C-17), 170.6 (acetoxy carbonyl), 195.0 (C-20), 201.7 (C-6). MS *m*/*z* 370 (M+).

*2.2.1.4. 3β-Hydroxypregna-4,16-diene-6,20-dione **8**.* A solution of steroid **7** (1 g, 2.7 mmol), 2% aqueous sodium hydroxide solution (10 ml) in methanol (150 ml) was stirred at room temperature for 20 min. Upon termination of the hydrolysis, water (200 ml) was added and the precipitated product was filtered and dried. It was recrystallized from methanol. Yield 531 mg, 1.62 mmol (60%) of pure product **8**, mp 168–170 °C. UV (nm) 238 (ε = 10100), 242 (ε = 6100). IR (KBr) cm^{-1}: 3430, 2941, 1688, 1663 and 1630. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (3Hs, H-18), 1.0 (3Hs, H-19), 2.2 (3Hs, H-21), 4.3 (1Ht, J = 2 Hz, H-3), 6.2 (1Hq, J = 2, H-4), 6.7 (1Hq, J = 2, H-16). ^{13}C-NMR ($CDCl_3$) δ: 15.7 (C-18), 19.8 (C-19), 27.1 (C-21), 67.1 (C-3), 133.2 (C-4), 143.9 (C-16), 146.5 (C-5), 154.8 (C-17), 196.6 (C-20), 202.4 (C-6). MS *m*/*z* 328 (M+).

*2.2.1.5. Pregna-4,6-diene-3,6,20-trione **9**.* To a solution of steroid **8** (1 g, 2.57 mmol) in acetone (50 ml) was added dropwise a solution of chromium trioxide (1.05 g, 10.5 mmol) in water (5 ml) at 0 °C during 10 min. The resulting mixture was allowed to warm to room temperature and again the same amount of chromium trioxide was added in the same manner. The reaction product was diluted with cold water (150 ml)