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# Novel $P450_{17\alpha}$ inhibitors: 17-(2′-oxazolyl)- and 17-(2′-thiazolyl)-androstene derivatives

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**Abstract**

Twelve 17-(2′-oxazolyl)- and 17-(2′-thiazolyl)-androsta-5,16-diene derivatives were designed and synthesized from 3β-acetoxy-pregna-5,16-dien-20-one (**1b**) as inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$). Potent inhibitors of this enzyme could be of value as treatment of prostate cancer. Two substituents (methyl and phenyl) were introduced either at their 4′- or 5′-position in order to investigate their structure–activity relationship. Due to the 16,17-double bond, 17-thiazoles were generally obtained in low yield. The pharmacological results showed that the compounds containing 17-(2′-oxazolyl) (**14c**) and 17-(2′-thiazolyl) (**8c**) (41.5%) demonstrated reasonable inhibition against $P450_{17\alpha}$. Their 3-acetate (**13c** and **7c**) were less potent than their 3-OH counterparts. The introduction of a phenyl or methyl group generally decreased inhibitory activity. Surprisingly, 17-(5′-methyl-2′-thiazolyl) (**12a**) was the most potent compound in this series and was almost as potent as **L-39**, which has good antitumor activity.


*Keywords:* $P450_{17\alpha}$ inhibitors; 17-(2′-Oxazolyl)-androstenes; 17-(2′-Thiazolyl)-androstenes; Prostatic cancer

## 1. Introduction

17α-Hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) is a key regulatory enzyme of the androgen biosynthetic pathway that catalyzes both the 17α-hydroxylation and the cleavage of the $C_{17}$–$C_{20}$ side chain of 21-carbon steroids in both testes and adrenals. It is known that androgens play an important role in the development and progression of several prostatic diseases, most notably, benign prostatic hypertrophy (BPH) and prostatic cancer. Inhibitors of this enzyme can block androgen synthesis in its early step, and thereby may be useful in the treatment of prostatic cancer [1]. To date, only ketoconazole [2,3], an imidazole antifungal agent, has been used for this purpose to treat patients with advanced prostatic cancer. However, this agent is neither selective nor very potent and has a number of significant side effects. This highlights the need to design potent and specific inhibitors of $P450_{17\alpha}$.

Recently, we have described a number of inhibitors of $P450_{17\alpha}$, of which 17-imidazolyl, pyrazolyl, and isoxazolyl androstene are very potent [4,5] and 17-(5′-isoxazolyl)-pregna-4,16-diene-3,20-dione (**L-39**) will soon enter Phase I clinical trial [1]. Jarman et al. also reported that 17-(3′-pyridyl)-androsta-5,16-dien-3-ol is a potent inhibitor [6]. These compounds were much more active than ketoconazole. Although the detailed mechanisms of these inhibitors are not very clear, the coordination of 17-heterocycles of steroids to the heme-iron at the active site of the enzyme is thought to be an important factor related to inhibition [1,4,5]. In addition, the 16,17-double bond is also important for potent inhibitory activity against $P450_{17\alpha}$ [6]. We reasoned that 17-thiazole and -oxazole, which are other azoles and the bioisosterism of imidazole, pyrazole, and isoxazole, would also create potent inhibitors. Here we report the synthesis of twelve 17-(2′-thiazolyl)- and 17-(2′-oxazolyl)-androsta-5,16-dien-3-ol derivatives, with either a methyl or phenyl group at their 4′- or 5′-position. Their synthetic routes are shown in the Schemes 1 and 2 and their inhibition activities for $P450_{17\alpha}$ are also reported here.

## 2. Experimental

### 2.1. Synthetic methods

Melting points were determined on a $XT_4$ melting point microscope and are uncorrected. IR spectra were deter-

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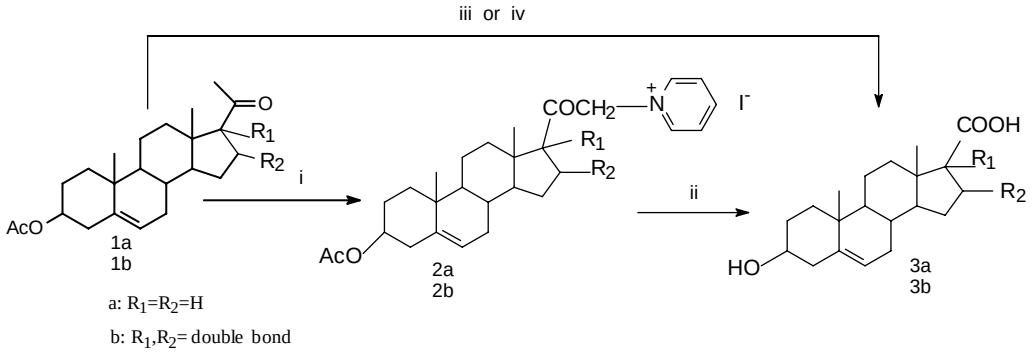
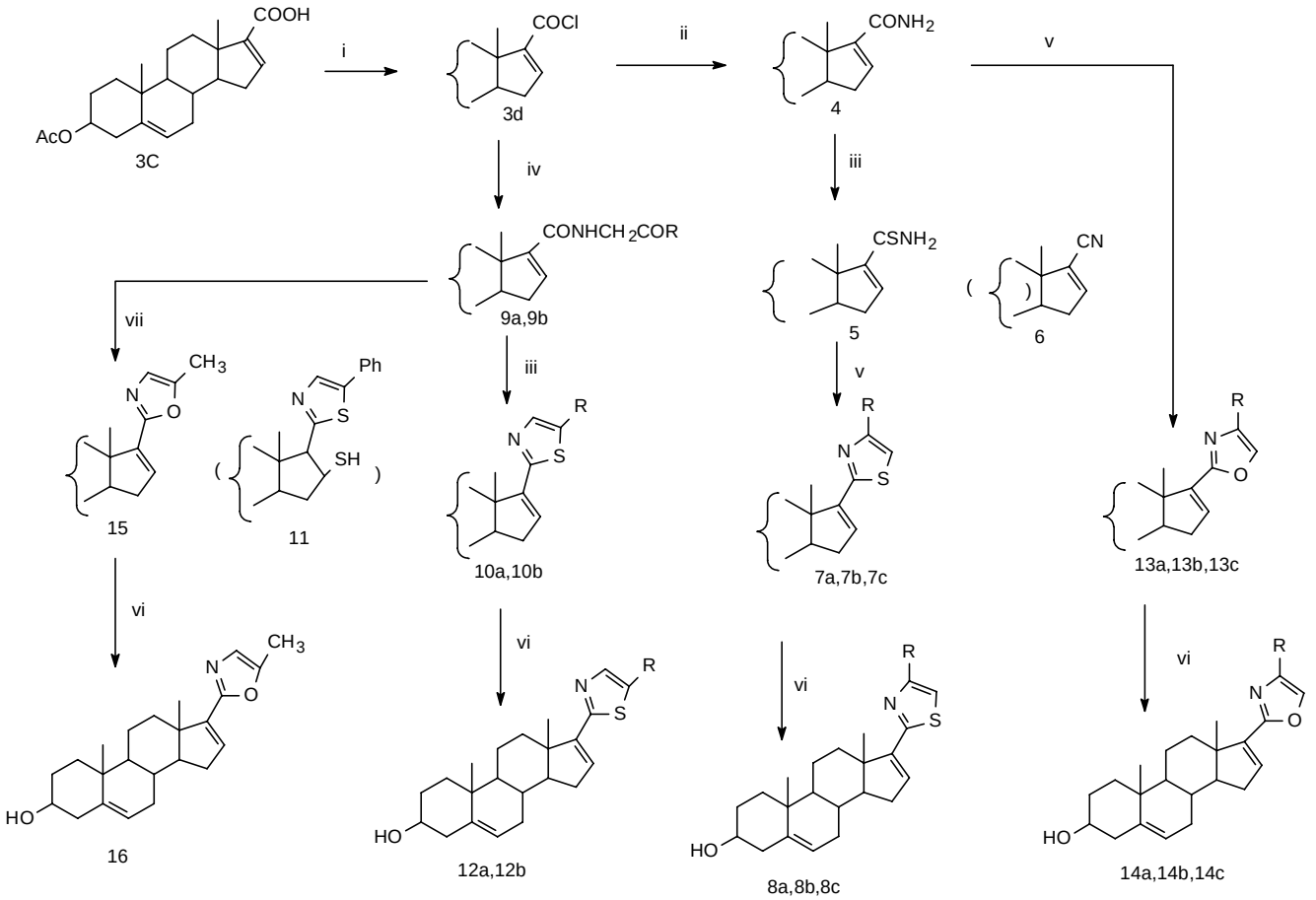



Scheme 1. Regent: (i) $I_2$/pyridine; (ii) NaOH, 50% aqueous EtOH; (iii) $I_2$, NaOH; (iv) $Br_2$, NaOH. a: $R_1 = R_2 = H$; b: $R_1$, $R_2$ = double bond.

mined in a Perkin-Elmer 983 spectrometer (wave numbers in $cm^{-1}$). $^1H$ NMR data (300 MHz) (internal standard $Me_4Si$, $\delta = 0$) were recorded in a VARIAN VAN-300, with samples dissolved in $CDCl_3$ unless otherwise stated. Reactions were monitored by TLC on silica gel plates (GF254) and visualized by dipping in 4% sulfuric acid in ethanol followed by heating at ca. 120–150 °C. Flash column chromatography was carried out on silica gel (230–400 mesh) in the solvent systems indicated. PE refers to light petroleum ether, bp 60–90 °C.




R: a = $CH_3$, b = Ph, c = H

Scheme 2. Regent: (i) $SOCl_2$; (ii) $NH_4OH$; (iii) $P_2S_5$; (iv) $RCOCH_2NH_2 \cdot HCl/Et_3N$; (v) $RCOCH_2Br$ or $ClCH_2CHClOEt$; (vi) $KOH/CH_3OH$; (vii) $PPh_3/I_2/NEt_3$. R: a = $CH_3$; b = Ph; c = H.

### 2.2. 3β-Hydroxy-androsta-5,16-dien-17-carboxylic acid (**1a**)

#### 2.2.1. Method 1: the hydrolysis of 21-pyridinium iodide salt (**2b**)

3β-Acetoxy-pregna-5,16-dien-20-one (DPA, **1b**) (0.213 g, 0.6 mmol) was dissolved in 0.5 ml of pyridine and heated under reflux, iodine (0.163 g, 0.64 mmol) was then added within 0.5 h and the solution was heated under reflux for 2 h. After cooling naturally, water was added and the precipitate was filtered, washed with water, and dried. Crude product was recrystallized from $CH_2Cl_2$/ethyl acetate to give pure pyridinium iodide **2b** (0.31 g, 91.7%), mp 200–202 °C. $^1$H NMR: $\delta$ 8.92 (d, $J = 5.4$, 2H, 2′,6′-H of Py), 8.69 (t, 1H, 4′-H of Py), 8.23 (t, 2H, 3′,5′-H of Py), 7.26 (ws, 1H, 16-H), 6.10 (q, 2H, 17-$CH_2$), 5.39 (d, $J = 4.8$, 1H, 6-H), 4.46 (m, 1H, 3α-H), 1.99 (s, 3H, AcO), 1.02 (s, 3H, 19-$CH_3$), 0.90 (s, 3H, 18-$CH_3$). Anal. $C_{28}H_{36}O_3NI{\cdot}1/2(H_2O)$, C 58.94%, H 6.53%, N 2.45%; found C 59.23%, H 6.54%, N 1.83%.

Sodium hydroxide (175 mg, 4.375 mmol) was added to a suspension of the above pyridinium iodide **2b** (190 mg, 0.34 mmol) in 50% aqueous ethanol. The mixture was heated under reflux for 2 h and acidified with 3N HCl to pH 3. The acidic fraction was collected by filtration. The crude product (60 mg, 26%) was crystallized from methanol to give a dark yellow product **3b** (48 mg, 18%) with mp 249–257 °C (lit. [8]: 255–257 °C).

#### 2.2.2. Method 2: bromoform reaction

A solution of sodium hydroxide (1.46 g, 36.5 mmol) in 12.5 ml water was cooled to −5 °C in an ice-salt bath at a rate that maintained the reaction temperature below 0 °C. While stirring, bromine (1.5 g, 9 mmol, 0.48 ml) was added from a separatory funnel at a rate that maintained the reaction temperature below 0 °C. The ice-cold solution was diluted with 8.3 ml cold dioxane. This solution was kept at 0 °C until required.

A solution of DPA **1b** (1 g, 2.8 mmol) in dioxane (38.2 ml) and water (11 ml) was cooled in an ice bath. When the internal temperature had fallen to 8 °C, the above cold hypobromite solution was added. The temperature of the mixture was maintained at 8–10 °C throughout the reaction. After the solution became colorless, the mixture was stirred for an additional 2 h. The excess sodium hypobromite was destroyed by the addition of 10% aqueous sodium sulfite. The mixture was refluxed for 15 min, and the solution, while still hot (90 °C), was acidified to pH 6. The solution was kept in water for 24 h. The precipitate was collected by suction filtration, washed with water, and dried. The white product was crystallized from ethanol to give pure **3b**, mp 255–257 °C (0.91 g, 81%, lit. [8]: 255–257 °C). IR ($cm^{-1}$): 3173, 1710 (COOH, OH). $^1$H NMR: $\delta$ 12.03 (s, 1H, COOH), 6.66 (ws, 1H, 16-H), 5.28 (d, $J = 4.2$, 1H, 6-H), 4.62 (s, 1H, OH), 3.26 (m, 1H, 3α-H), 0.98 (s, 3H, 19-$CH_3$), 0.87 (s, 3H, 18-$CH_3$).

### 2.3. 3β-Acetoxy-androsta-5,16-dien-17-carboxylic acid (**3c**)

17-Acid **3b** (0.75 g, 2.37 mmol) was dissolved by warming in 3 ml of pyridine. After the solution had cooled to room temperature, 1 ml of acetic anhydride was added and the mixture was allowed to stand overnight. It was then treated with 4 ml of water and heated to a boil until the precipitate had dissolved. Another 10 ml of water was added and the mixture was cooled. The crystalline product was collected and recrystalized from glacial acetic acid to give 3β-acetate **3c** 0.78 g (91%) as white crystals, mp 240–244 °C (lit. [9]: 255 °C). IR ($cm^{-1}$): 1726 (AcO).

#### 2.3.1. 3β-Acetoxy-androsta-5,16-dien-17-carboxylic acid chloride (**3d**) and 17-carboxamide (**4**)

Compound **3c** (2.5 g, 6.98 mmol) was mixed with $SOCl_2$ (10 ml) at 0 °C, and then stirred for 6 h at room temperature. The solvent was evaporated under reduced pressure after addition of $C_6H_6$ to the mixture in order to get rid of any remaining $SOCl_2$. This gave the acid chloride **3d**. This compound was stirred vigorously with concentrated aqueous $NH_4OH$ (10 ml), the precipitate was collected and dried, and yielded the amide **4** (2.345 g, 94.1%), mp 217–220 °C (from ethanol). IR ($cm^{-1}$): 3465, 3326 ($NH_2$), 1668 ($CONH_2$), 1726 (AcO). $^1$H NMR: $\delta$ 6.49 (ws, 1H, 16-H), 5.70 (ws, 2H, $NH_2$), 5.39 (d, $J = 5.1$, 1H, 6-H), 4.60 (m, 1H, 3α-H), 2.04 (s, 3H, AcO), 1.07 (s, 3H, 19-$CH_3$), 1.02 (s, 3H, 18-$CH_3$). Anal. $C_{22}H_{37}O_3N$, C 73.92%, H 8.74%, N 3.92%; found C 74.20%, H 8.39%, N 3.64%.

### 2.4. 3β-Acetoxy-androsta-5,16-dien-17-thiocarboxamide (**5**)

Phosphorus pentasulfide (62 mg, 0.28 mmol) was stirred vigorously in anhydrous dioxane (4 ml) to become a powdered solid, to which the amide **4** (100 mg, 0.28 mmol) was added. The mixture was stirred at 14 °C for 2 h and then filtered. The solution was adjusted to neutral by adding 50% aqueous KOH. Then the solvent was evaporated under reduced pressure and the residue purified by flash chromatography. Elution with acetone/PE (1:4) yielded thiocarboxamide **5** (50 mg, 48.3%), mp 173–175 °C (from acetone/PE). IR ($cm^{-1}$): 3314, 3191 ($NH_2$), 1382 ($CSNH_2$), 1707 (AcO). $^1$H NMR: $\delta$ 7.46 and 6.59 (s, each 1H, –$CSNH_2$↔–HS–C=NH–, exchangeable with $D_2O$), 5.38 (d, $J = 5.1$, 1H, 6-H), 4.62 (m, 1H, 3α-H), 4.15 (m, 1H, 16-H), 2.04 (s, 3H, AcO), 1.02 (s, 3H, 19-$CH_3$), 0.79 (s, 3H, 18-$CH_3$). Anal. $C_{22}H_{31}ONS{\cdot}2(H_2O)$, C 64.51%, H 8.61%, N 3.42%; found C 64.56%, H 8.24%, N 3.10%.

### 2.5. 3β-Acetoxy-17-cyano- (**6**) and -17-(4′-methyl-2′-thiazolyl)-androsta-5,16-diene (**7a**)

A suspension of powdered $P_2S_5$ (23.2 mg, 0.10 mmol) and the amide **4** (200 mg, 0.5 mmol) in anhydrous diox-

ane (10 ml) was stirred at 14 °C for 2 h. The bromoacetone (43.2 mg, 0.32 mmol, bp 68–75 °C, prepared from the bromination of acetone in acetic acid [10]) was added and the mixture was refluxed for 2 h, 50% KOH was added to neutralize the solution. Solvent was removed under reduced pressure and the residue was flash chromatographed. Elution with PE/ethyl acetate (20:1) yielded a white product (**7a**) (20 mg, 8.8%), mp 199–200 °C (from acetone). IR ($cm^{-1}$): 1717, 1243, 1034 (AcO), 1406 (thiazolyl). $^1H$ NMR: $\delta$ 6.75 (s, 1H, 5′-H), 6.50 (ws, 1H, 16-H), 5.40 (d, $J = 5.1$, 1H, 6-H), 4.63 (m, 1H, 3α-H), 2.45 (s, 3H, 4′-$CH_3$), 2.04 (s, 3H, AcO), 1.08 (s, 3H, 19-$CH_3$), 1.07 (s, 3H, 18-$CH_3$). Anal. $C_{25}H_{33}O_2NS$, C 72.95%, H 8.08%, N 3.40%; found C 72.74%, H 8.32%, N 3.11%.

Further elution with PE/ethyl acetate (9:1) yielded 17-cyanide **6** (20 mg, 14%), mp 200–202 °C (from acetone). IR ($cm^{-1}$): 2209 (CN), 1720 (AcO). $^1H$ NMR: $\delta$ 6.64 (m, 1H, 16-H), 5.38 (d, $J = 4.8$, 1H, 6-H), 4.60 (m, 3H, 3α-H), 2.04 (s, 3H, AcO), 1.06 (s, 3H, 19-$CH_3$), 0.95 (s, 3H, 18-$CH_3$). Anal. $C_{22}H_{29}O_2N$, C 77.55%, H 8.85%, N 4.14%; found C 77.84%, H 8.61%, N 4.13%.

#### *2.5.1. 3β-Acetoxy-17-(4′-phenyl-2′-thiazolyl)-androsta-5,16-diene (**7b**)*

A suspension of powdered $P_2S_5$ (100 mg, 0.45 mmol) and the amide **4** (200 mg, 0.5 mmol) in anhydrous dioxane (6 ml) was stirred at 14 °C for 2 h. The $P_2O_5$ and other precipitates were removed by filtration and then 2-bromoacetophenone (100 mg, 0.53 mmol) was added into the filtrate, and then refluxed for 0.5 h. The solution was concentrated under reduced pressure and the residue was diluted with $CH_2Cl_2$, then 50% KOH was added to make the solution neutral. The whole mixture was washed with water to remove the residual dioxane. The water layer was extracted with $CH_2Cl_2$ and the combined organic layer was dried over sodium sulfate. After evaporation, the residue was purified by flash chromatography. Elution with PE/ethyl acetate yielded **7b** (30 mg, 10%), mp 173–174 °C (from acetone). $^1H$ NMR: $\delta$ 7.92 (d, $J = 7.5$, 2H, Ph–H), 7.24–7.43 (m, 4H, Ph–H and 5′-H), 6.54 (ws, 1H, 16-H), 5.39 (d, $J = 5.1$, 1H, 6-H), 4.58 (m, 1H, 3α-H), 2.03 (s, 3H, AcO), 1.08 (s, 3H, 19-$CH_3$), 1.05 (s, 3H, 18-$CH_3$).

#### *2.5.2. 3β-Acetoxy-17-(2′-thiazolyl)-androsta-5,16-diene (**7c**)*

The 17-thiamide **5** (60 mg, 0.14 mmol) was dissolved in warm anhydrous DMF, α,β-dichloroether (20 ul, 0.14 mmol) added and the mixture heated under reflux for 0.5 h. The solution was concentrated under reduced pressure and the residue was diluted with $CH_2Cl_2$ and washed with water to remove the residual DMF. The water layer was extracted with $CH_2Cl_2$, the combined organic layer was dried over sodium sulfate. The solvent was removed and the residue was purified by chromatography. Elution with PE/ethyl acetate (15:1) yielded 17-thiazole **7c** (10 mg, 15.75%), mp 160–162 °C (from acetone). $^1H$ NMR: $\delta$ 7.77 (d, $J = 3.3$, 1H, 4′-H), 7.20 (d, $J = 3.3$, 1H, 5′-H), 6.54 (ws, 1H, 16-H), 5.36 (d, $J = 5.1$, 1H, 6-H), 4.58 (m, 1H, 3α-H), 2.09 (s, 3H, AcO), 1.07 (s, 3H, 19-$CH_3$), 1.04 (s, 3H, 18-$CH_3$).

#### *2.6. 3β-Hydroxy-17-(4′-methyl-2′-thiazolyl)-(**8a**), -17-(4′-phenyl-2′-thiazolyl)- (**8b**), and -17-(2′-thiazolyl)-androsta-5,16-diene (**8c**)*

Compound **7a** (45 mg, 0.11 mmol) was dissolved by warming in methanol (10 ml), 0.86N KOH–$CH_3OH$ (2 ml) was added, and the reaction solution was heated under reflux for 0.5 h. The solvent was removed under reduced pressure and the residue was chromatographed. Elution with PE/ethyl acetate (4:1) yielded **8a** (28 mg, 70%), mp 236–237 °C (from acetone). $^1H$ NMR: $\delta$ 6.74 (s, 1H, 5′-H), 6.48 (ws, 1H, 16-H), 5.38 (d, $J = 4.8$, 1H, 6-H), 3.52 (m, 1H, 3α-H), 2.44 (s, 3H, 4′-$CH_3$), 1.07 (s, 6H, 18,19-$CH_3$). Anal. $C_{23}H_{31}ONS$, C 74.75%, H 8.45%, N 3.79%; found C 74.95%, H 8.70%, N 3.54%.

Following the same procedure described above for **8a**, compound **7b** (20 mg, 0.04 mmol) gave 3β-ol **8b** (18 mg, 99%), mp 205–207 °C (from acetone). IR ($cm^{-1}$): 3344 (OH), 1437, 1372 (thiazolyl). $^1H$ NMR: $\delta$ 7.87 (d, $J = 7.2$, 2H, Ph–H), 7.24–7.43 (m, 4H, Ph–H and 5′-H), 6.45 (ws, 1H, 16-H), 5.32 (d, $J = 5.1$, 1H, 6-H), 3.50 (m, 1H, 3α-H), 1.08 (s, 3H, 19-$CH_3$), 1.05 (s, 3H, 18-$CH_3$). Anal. $C_{28}H_{33}ONS$, C 77.91%, H 7.71%, N 3.24%; found C 77.64%, H 7.88%, N 3.18%.

Similarly, compound **7c** (14 mg, 0.03 mmol) gave 3β-ol **8c** (10 mg, 80%), mp 185–187 °C (from acetone). IR ($cm^{-1}$): 3267 (OH), 1595, 1497 (thiazolyl). $^1H$ NMR: $\delta$ 7.80 (d, $J = 3.3$, 1H, 4′-H), 7.22 (d, $J = 3.3$, 1H, 5′-H), 6.61 (ws, 1H, 16-H), 5.38 (d, $J = 5.1$, 1H, 6-H), 3.53 (m, 1H, 3α-H), 1.09 (s, 3H, 19-$CH_3$), 1.08 (s, 3H, 18-$CH_3$). Anal. $C_{22}H_{29}ONS{\cdot}1/2(H_2O)$, calculated C 72.48%, H 8.29%, N 3.84%; found C 72.26%, H 8.06%, N 3.63%; MS (EI): 355 ($M^+$).

#### *2.7. N-Acetylmethyl-3β-acetoxy-androsta-5,16-diene-17-carboxamide (**9a**)*

Aminoacetone hydrochloride (16.4 mg, 0.15 mmol, prepared from glycine and acetic anhydride [11]) and triethylamine (35 ul, 0.25 mmol) were added to a solution of 17-acid chloride **3** (50 mg, 0.12 mmol) in dry dichloromethane at 0 °C. The mixture was stirred at room temperature for 0.5 h. Then, it was washed successively with 5% hydrochloric acid, 5% sodium hydrogen carbonate, water, and then dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed. Elution with PE/acetone (4:1) gave β-methylketo amide **9a** (50 mg, 92%), bp 190–192 °C (from acetone). IR ($cm^{-1}$): 1726, 1246, 1037 (AcO), 3375, 3326, 1649 (CONH). $^1H$ NMR: $\delta$ 6.45 (ws, 2H, 16-H and NH), 5.39 (d, $J = 5.1$, 1H, 16-H), 4.60 (m, 1H, 3α-H), 4.27 (s, 2H, NH$\underline{CH_2}$CO), 2.23 (s, 3H, $CH_2CO\underline{CH_3}$), 2.04 (s, 3H, AcO), 1.06 (s,

3H, 19-$CH_3$), 1.02 (s, 3H, 18-$CH_3$). Anal. $C_{25}H_{35}O_4N$, C 72.61%, H 8.53%, N 3.39%; found C 72.51%, H 8.56%, N 3.30%.

### 2.7.1. *N-Benzoylmethyl-3β-acetoxy-androsta-5,16-diene-17-carboxamide (**9b**)*

Following the same procedure described above, acylation of 2-aminoacetophenone hydrochloride (25 mg, 0.14 mmol) with 17-acid chloride **3** yielded **9b** (50 mg, 78%), mp 163–165 °C (from acetone). IR ($cm^{-1}$): 3327 (NH), 1686 (CONH), 1727 (AcO). $^1H$ NMR: $\delta$ 7.5–8.0 (m, 5H, Ph–H), 6.80 (s, 1H, NH), 6.55 (ws, 1H, 16-H), 5.41 (d, $J = 5.1$, 1H, 6-H), 4.84 (s, 2H, $NHCH_2CO$), 4.61 (m, 1H, 3α-H), 2.04 (s, 3H, AcO), 1.09 (s, 3H, 19-$CH_3$), 1.08 (s, 3H, 18-$CH_3$). Anal. $C_{30}H_{37}O_4N$, C 75.76%, H 7.84%, N 2.94%; found C 75.35%, H 7.88%, N 2.88%.

### 2.8. *3β-Acetoxy-17-(5′-methyl-2′-thiazolyl)-androsta-5,16-diene (**10a**)*

Compound **9a** (50 mg, 0.11 mmol) and phosphorus pentasulfide (33.3 mg, 0.15 mmol) in dioxane (3 ml) was stirred at room temperature for 3.5 h, and then refluxed for 1 h. The solution was concentrated and washed with water. The water layer was extracted with $CH_2Cl_2$ and the combined $CH_2Cl_2$ layer was dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed. Elution with PE/ethyl acetate (15:1) yielded product **9a** (14 mg, 28%), mp 162–163 °C (from acetone). $^1H$ NMR: $\delta$ 7.38 (s, 1H, 4′-H), 6.38 (ws, 1H, 16-H), 5.38 (d, $J = 4.8$, 1H, 6-H), 4.60 (m, 1H, 3α-H), 2.42 (s, 3H, 5′-$CH_3$), 2.05 (s, 3H, AcO), 1.06 (s, 3H, 19-$CH_3$), 1.05 (s, 3H, 18-$CH_3$).

### 2.8.1. *3β-Acetoxy-17-(5′-phenyl-2′-thiazolyl)- (**10b**) and 3β-acetoxy-16α-thiol-17-(5′-phenyl-2′-thiazolyl)-androsta-5,16-diene (**11**)*

Compound **9a** (100 mg, 0.2 mmol) and phosphorus pentasulfide (67 mg, 0.3 mmol) in toluene (2 ml) were heated at 80 °C for 2 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography. Elution with PE/ethyl acetate (15:1) yielded product **10b** (20 mg, 21.1%), mp 155–158 °C (from acetone). IR ($cm^{-1}$): 1717 (AcO), 1457 (aromatic rings). $^1H$ NMR: $\delta$ 7.94 (s, 1H, 4′-H), 7.20–7.59 (m, 5H, Ph–H), 6.65 (ws, 1H, 16-H), 5.39 (d, $J = 4.8$, 1H, 6-H), 4.60 (m, 1H, 3α-H), 2.02 (s, 3H, AcO), 1.09 (s, 3H, 19-$CH_3$), 1.08 (s, 3H, 18-$CH_3$).

Following the same procedure described above except dioxane was used to replace toluene, 16α-thiol **11** (13 mg, 12.7%) was obtained, mp 173–175 °C (from acetone). $^1H$ NMR: $\delta$ 7.89 (s, 1H, 4′-H), 7.27–7.54 (m, 5H, Ph–H), 5.37 (d, $J = 5.1$, 1H, 6-H), 4.60 (m, 1H, 3-H), 4.07 (m, 1H, 16-H), 3.04 (d, 1H, 17-H), 2.02 (s, 3H, AcO), 1.08 (s, 3H, 19-$CH_3$), 0.63 (s, 3H, 18-$CH_3$). $^{13}C$ NMR: $\delta$ 170 (CO), 167 (2′-C), 139 (Ph–C linked with thiazole), 138 (5-C), 137 (4′-C), 131 (5′-C), 129 (*m*-Ph–C), 128 (*p*-Ph–C), 126 (*o*-Ph–C), 122 (6-C), 73 (3-C), 67 (16-C), 54 (17-C), 49 (9-C), 46 (13-C), 38 (14-C), 31 (8-C), 21 (AcO–C), 19 (19-C), 13 (18-C); MS (EI): 507 ($M^+$), 474 ($M^+$–SH), 414 ($M^+$–SH–AcOH) NOESY: 17-H has no NOE with both 18-$CH_3$ and 16-H. This suggests the configuration was 17α-H, 16β-H.

### 2.8.2. *3β-Hydroxy-17-(5′-methyl-2′-thiazolyl)- (**12a**) and -17-(5′-phenyl-2′-thiazolyl)-androsta-5,16-diene (**12b**)*

Following the same procedure described above for the preparation of **8a**, 3β-acetate **10a** (20 mg, 0.044 mmol) was hydrolyzed in KOH/methanol to give 3β-ol **12a** (17 mg, 94%), mp 163–167 °C (from acetone/PE). IR ($cm^{-1}$): 3370 (OH), 1458 (thiazole). $^1H$ NMR: $\delta$ 7.39 (s, 1H, 4′-H), 6.41 (ws, 1H, 16-H), 5.37 (d, $J = 5.1$, 1H, 6-H), 3.50 (m, 1H, 3α-H), 2.43 (s, 3H, 5′-$CH_3$), 1.08 (s, 3H, 19-$CH_3$), 1.05 (s, 3H, 18-$CH_3$). Anal. $C_{23}H_{31}ONS$, C 74.75%, H 8.45%, N 3.79%; found C 74.84%, H 8.32%, N 3.62%.

And from 3β-acetate **10b** (20 mg, 0.042 mmol) gave **12b** (18 mg, 99%), mp 218–218.8 °C (from acetone). IR ($cm^{-1}$): 3314 (OH), 1591, 1478 (aromatic ring). $^1H$ NMR: $\delta$ 7.90 (s, 1H, 4′-H), 7.30–7.54 (m, 5H, Ph–H), 6.48 (ws, 1H, 16-H), 5.37 (d, $J = 5.1$, 1H, 6-H), 3.56 (m, 1H, 3α-H), 1.10 (s, 3H, 19-$CH_3$), 1.07 (s, 3H, 18-$CH_3$). Anal. $C_{28}H_{33}ONS\cdot(H_2O)$, C 74.79%, H 7.85%, N 3.11%; found C 75.17%, H 7.53%, N 2.91%.

### 2.9. *3β-Acetoxy-17-(4′-methyl-2′-oxazolyl)-androsta-5,16-diene (**13a**)*

The 17-carboxamide **4** (100 mg, 0.25 mmol) and bromoacetone (34 mg, 0.25 mmol, prepared from the bromination of acetone [10]) in DMF (2 ml) was refluxed for 0.5 h, the solution was concentrated under reduced pressure. Water (10 ml) was added and extracted with $CH_2Cl_2$ (3 × 10 ml), the combined $CH_2Cl_2$ was evaporated, and the residue was purified by chromatography. Elution with PE/ethyl acetate (9:1) gave 4′-methyloxazole **13a** (44 mg, 40.2%), mp 196–198 °C (from acetone). IR ($cm^{-1}$): 1728, 1242, 1035 (AcO), 1615, 1507, 1370 (oxazolyl). $^1H$ NMR: $\delta$ 7.28 (s, 1H, 5′-H), 6.56 (ws, 1H, 16-H), 5.40 (d, $J = 4.8$, 1H, 6-H), 4.60 (m, 1H, 3α-H), 2.19 (s, 3H, 4′-$CH_3$), 2.04 (s, 3H, AcO), 1.08 (s, 3H, 19-$CH_3$), 1.02 (s, 3H, 18-$CH_3$).

### 2.9.1. *3β-Acetoxy-17-(4′-phenyl-2′-oxazolyl)-androsta-5,16-diene (**13b**)*

The same procedure described above for **13a**, was followed except 2-bromoacetophenone (50 mg, 0.27 mmol) replaced bromoacetone, to obtain 4′-phenyloxazole **13b** (68 mg, 67.9%), mp 196–197.8 °C (from acetone). IR ($cm^{-1}$): 1720, 1253 (AcO), 1618, 1564, 1482, 1372 (oxazolyl). $^1H$ NMR: $\delta$ 7.83 (s, 1H, 5′-H), 7.26–7.78 (m, 5H, Ph–H), 6.61 (m, 1H, 16-H), 5.41 (d, $J = 5.4$, 1H, 6-H), 4.62 (m, 1H, 3α-H), 2.04 (s, 3H, AcO), 1.10 (s, 3H, 19-$CH_3$), 1.07 (s, 3H, 18-$CH_3$). Anal. $C_{30}H_{35}O_3N$, C 78.74%, H 7.71%, N 3.06%; found C 78.80%, H 7.70%, N 2.97%.

*2.9.2. 3β-Acetoxyl-17-(2′-oxazolyl)-androsta-5,16-diene (**13c**)*

The same procedure described above for **13a** was followed but using α,β-dichloroether (30 ul, 0.21 mmol) instead of bromoacetone. The mixture was refluxed for 1 h and gave oxazole **13c** (16 mg, 15.1%), mp 178–179.5 °C (from acetone/PE). IR ($cm^{-1}$): 1726, 1238, 1031 (AcO), 1615, 1529, 1370 (oxazolyl). $^1$H NMR: $\delta$ 7.60 (d, 1H, 5′-H), 7.10 (d, 1H, 4′-H), 6.60 (ws, 1H, 16-H), 5.40 (d, $J = 4.8$, 1H, 6-H), 4.60 (m, 1H, 3α-H), 2.04 (s, 3H, AcO), 1.09 (s, 3H, 19-$CH_3$), 1.03 (s, 3H, 18-$CH_3$). Anal. $C_{24}H_{31}O_3N$, C 75.56%, H 8.19%, N 3.67%; found C 75.37%, H 8.22%, N 3.43%.

*2.9.3. 3β-Hydroxy-17-(4′-methyl-2′-oxazolyl)-(**14a**), -17-(4′-phenyl-2′-oxazolyl)- (**14b**), and -17-(2′-oxazolyl)-androsta-5,16-diene (**14c**)*

Following the same procedure described above for **8a**, 3β-acetate **13a** (40 mg) gave product **14a** (10 mg, 56%), mp 249–250 °C (from acetone). IR ($cm^{-1}$): 3280 (OH), 1601, 1529 (oxazolyl). $^1$H NMR: $\delta$ 7.24 (s, 1H, 5′-H), 6.52 (ws, 1H, 16-H), 5.33 (d, $J = 5.4$, 1H, 6-H), 3.50 (m, 1H, 3α-H), 2.14 (s, 3H, 4′-$CH_3$), 1.10 (s, 3H, 19-$CH_3$), 1.07 (s, 3H, 18-$CH_3$). Anal. $C_{23}H_{31}O_2N$, C 78.15%, H 8.84%, N 3.96%; found C 78.40%, H 8.87%, N 3.77%.

Acetate **13b** (30 mg, 0.065 mmol) yielded **14b** (20 mg, 73.5%), mp 142–144 °C (from acetone). $^1$H NMR: $\delta$ 7.75–7.83 (m, 2H, 5′-H, Ph–H), 7.30–7.48 (m, 3H, Ph–H), 6.65 (ws, 1H, 16-H), 5.38 (d, $J = 5.1$, 1H, 6-H), 3.55 (m, 1H, 3α-H), 1.09 (s, 3H, 19-$CH_3$), 1.08 (s, 3H, 18-$CH_3$). Anal. $C_{28}H_{33}O_2N$, calculated C 80.93%, H 8%, N 3.37%; found C 80.90%, H 8.20%, N 3.09%; MS (EI): 415 ($M^+$).

The acetate **13c** (30 mg, 0.078 mmol) gave the product **14c** (20 mg, 77%), mp 193.8–195 °C (from acetone). IR ($cm^{-1}$): 3422 (OH), 1616, 1530 (oxazole). $^1$H NMR: $\delta$ 7.56 (ws, 1H, 5′-H), 7.13 (ws, 1H, 4′-H), 6.58 (ws, 1H, 16-H), 5.37 (d, $J = 4.8$, 1H, 6-H), 3.52 (m, 1H, 3α-H), 1.08 (s, 3H, 19-$CH_3$), 1.04 (s, 3H, 18-$CH_3$). Anal. $C_{24}H_{31}O_3N$, calculated C 77.84%, H 8.61%, N 4.12%; found C 77.45%, H 8.61%, N 3.90%; MS (EI): 355 ($M^+$).

*2.9.4. 3β-Acetoxy-17-(5′-methyl-2′-oxazolyl)-androsta-5,16-diene (**15**)*

The β-keto amide **9a** (100 mg, 0.23 mmol) was added into a flask containing a freshly prepared solution of triphenylphosphine (118 mg, 0.46 mmol), iodine (117 mg, 0.46 mmol) and triethylamine (130 ul) in $CH_2Cl_2$ (5 ml). The mixture was stirred at room temperature for 2 days. The solution was diluted with $CH_2Cl_2$ (10 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the residue was chromatographed. Elution with PE/ethyl acetate (9:1) yielded 5′-methyloxazole **15** (20 mg, 21%), mp 183–184 °C (from ethyl acetate/PE). $^1$H NMR: $\delta$ 6.70 (s, 1H, 4′-H), 6.50 (t, 1H, 16-H), 5.40 (d, $J = 4.8$, 1H, 6-H), 4.60 (m, 1H, 3α-H), 2.31 (s, 3H, 5′-$CH_3$), 2.04 (s, 3H, AcO), 1.08 (s, 3H, 19-$CH_3$), 1.02 (s, 3H, 18-$CH_3$).

*2.9.5. 3β-Hydroxy-17-(5′-methyl-2′-oxazolyl)-androsta-5,16-diene (**16**)*

Following the same procedure described above for **8a**, acetate **15** (20 mg, 0.046 mmol) gave the 3β-ol **16** (10 mg, 50%), mp 200–202 °C (from acetone). $^1$H NMR: $\delta$ 6.72 (s, 1H, 4′-H), 6.47 (ws, 1H, 16-H), 5.37 (d, $J = 4.8$, 1H, 6-H), 3.54 (m, 1H, 3α-H), 2.31 (s, 3H, 5′-$CH_3$), 1.07 (s, 3H, 19-$CH_3$), 1.02 (s, 3H, 18-$CH_3$); MS (EI): 353 ($M^+$).

## 3. $P450_{17\alpha}$ (lyase) assay [17]

Abbreviations used: IPTG, isopropyl β-D-thiogalactopyranoside; ALA, δ-aminolevulenic acid; LBAG, LB medium with ampicillin and glucose; TBA, terrific broth containing ampicillin; DTT, dithiothretiol; MOPS, 3-(*N*-morpholino)propanesulfonic acid; ACN, acetonitrile.

### 3.1. Materials

The vector pCWH17modHis was a generous gift of Dr. M.R. Waterman (University of Texas, Southern Medical Center, Dallas, TX). JM109 bacterial strain was from Gibco-BRL (Grand Island, NY). Terrific Broth, LB Agar, IPTG, Ampicillin were from Gibco-BRL. ALA and EDTA were from Sigma (St. Louis, MO). Protease Inhibitor Cocktail was from Boehringer-Mannheim (Mannheim, Germany). [21-$^3$H]Hydroxypregnenolone (specific activity 13.61 mCi/mmol) was prepared in our laboratory as described previously [5]. HPLC grade acetonitrile was purchased from Sigma-Aldrich. The reference standards for the inhibitors of androgen synthesis were ketoconazole, and **L-39** (**1**).

### 3.2. Bacterial stock preparation

JM109/H mod17His was incubated overnight in LBAG medium (containing 20 mM glucose and 1 mg/ml of ampicillin) and grown overnight at 37 °C with agitation at 250 rpm. Overnight cultures were diluted 1:100 in terrific broth containing 100 mg/ml ampicillin (TBA) and allowed to grow to $OD_{600} = 0.8$–0.9 at 37 °C for 4–5 h. Cytochrome $P450_{17\alpha}$ synthesis was stimulated by adding 1 mM of IPTG, and heme precursor ALA. After incubation for 16–18 h at 27 °C with agitation at 150 rpm, bacteria were collected by centrifugation at 2000 × *g* for 20 min at 4 °C. The pellet was resuspended and washed with 10 volumes of buffer A (20 mM glucose, 2 mM DTT, 1 mM EDTA, and 100 mM MOPS). After centrifugation at 2000 × *g* for 20 min at 4 °C, the pellet was resuspended in buffer B (buffer A and protease inhibitor cocktail) to $2 \times 10^9$ cells in 1 ml ($OD_{600} = 0.6$).

### 3.3. Measuring $P450_{17\alpha}$ activity in cultured bacteria

Substrate ([21-$^3$H]hydroxypregnenolone (specific activity 13.61 mCi/mmol)) and different concentrations of in-

hibitors were mixed in the sample tube (13 mm × 100 mm polystyrene tubes, Becton Dickinson, Lincoln Park, NJ) and the solvent (ethanol) was evaporated under airflow. The control used in this experiment was the substrate and bacteria without the presence of inhibitor.

The reaction was started by adding 1 ml of prepared bacterial culture to each tube. Tubes were sealed and mixed in a Dynal Sample Mixer (Dynal, Inc., Long Island, NY) at 40 rpm for 5–6 h at room temperature. The reaction was stopped by adding 1 ml of ACN, which precipitated bacterial and medium proteins. After centrifugation at 2000 rpm for 15 min at 4 °C, the supernatant was collected (∼1.9 ml), transferred into borosilicate glass tubes, and the steroids were extracted with 2 ml of chloroform at 4 °C for 30 min. After 30 min, the tubes were centrifuged at 2000 rpm for 15 min at 4 °C. The aqueous phase which contains the [$^3$H]acetic acid, was collected (∼0.9 ml), and this was mixed with 300 μl of 8.5% charcoal suspension for 30 min at 4 °C. After centrifugation at 2000 rpm for 15 min at 4 °C, the supernatant, which contained [$^3$H]acetic acid, was removed and radioactivity measured in a Liquid Scintillation Analyzer (Packard Instrument Co., Meriden, CT).

## 4. Results and discussion

### 4.1. Chemistry

The key starting material $\Delta^{16}$-17-carboxylic acid **3b** could be obtained by three synthetic routes shown in the Scheme 1, as reported in the literature. The first route was the nucleophilic addition of cyano anion to the 17-keto group of epiandrostene, followed by dehydration and hydrolysis [9,12]. This was not tried due to the difficulty of handling toxic cyanide in the lab. The second route was the hydrolysis of 21-pyridinium iodide (**2a**) [13]. Practically, 17-acid **3a**, which was the starting material for finasteride, was prepared in kg quantities by this route. We tried this procedure and $\Delta^{16}$-21-pyridinium **2b** was obtained in 90% yield, but the hydrolysis of **2b** to **3b** only gave $\Delta^{16}$-17-acid **3b** in poor (20%) yield. The third method was the iodoform reaction: the DPA **1b** was treated with $I_2$ in strong base and gave 17-acid **3b** directly, but the yield was less than 10% [8]. When we used bromine to replace iodine to prepare hypobromide instead of hypoiodide, the yield was increased to 80%. This important improvement enabled us to obtain a sufficient supply of **1a** for the following steps. After 3-OH was protected with the acetate group to give **3c**, this was further converted to key intermediates 17-acid chloride **3** and 17-amide **4**.

Although the syntheses of 17β-(2′-thiazolyl) derivatives have been reported by Urbansky and Drasar [7], the synthesis of their $\Delta^{16}$ counterpart was achieved with difficulty.

The 5′-methyl **10a** and the 5′-phenylthiazole **10b** were synthesized by the Gabriel method. In the presence of triethylamine, the hydrochlorides of aminoacetone, 2-aminoacetophenone were acylated, respectively, by 17-acid chloride **3c** affording the β-keto amide **9a** and **9b**. Refluxing **9a** with $P_2S_5$ in dioxane furnished the 5′-methylthiazole **10a** in 30% yield. However, the same procedure only afforded a small amount of 5′-phenylthiazole **10b**, and mainly resulted in the side-product **11**. Its $^1$H NMR showed a five-proton signal of 5′-phenyl around 7–8 ppm, the disappearance of the 16-ethylenic proton signal at 6–7 ppm as well as the carbon signal peak of $\Delta^{16}$ double bond in $^{13}$C NMR suggesting that the $\Delta^{16}$ double bond had been saturated. Mass spectrometry showed the molecular-ion peak 507 and a fragment 474 ($M^+$–SH), confirming that the structure was the 16-thiol-17-(5′-phenyl-2′-thiazolyl)-androstene derivative. NOESY spectrometry showed that there were no NOE between 17-H and 13β-$CH_3$, 16-H separately, confirming the configuration of 16α-SH **11**. The Michael addition of the $SH^-$ group at C-16 occurred preferentially at the less steric crowded α face owing to the hindrance of 13β-methyl. When toluene was used to replace dioxane, 5′-phenylthiazole **10b** was obtained albeit in lower (21%) yield. This is probably because the SH anion barely exists in the aprotic solvent toluene.

For the preparation of 4′-substituted thiazoles, the convenient one step procedure of Hantzsch [14] was employed, i.e. 17-thiamide **5** was prepared in situ without separation. Reaction of 17-amide **4** with $P_2S_5$ and then condensed with α-bromoacetophenone in dioxane gave 4′-methylthiazole **7a** (9% yield), together with the dehydrated product 17-cyanide **6**. However, the same procedure did not give 4′-phenyl **7b** and thiazole **7c**. We finally found that the solution of 17-thiamide **5** formed in situ had to be filtered first to remove $P_2O_5$ and other precipitates, and then condensed with 2-bromoacetophenone. In this way, 4′-phenylthiazole **7b** was obtained in 10% yield.

Owing to the instability of α-bromoacetaldehyde which always exists in polymer form, its more stable derivative α,β-dichloroether [14] was used as reactant for the preparation of thiazole **7c**. However, using the procedure for **7a** or **7b** gave a very poor yield. Here, pure 17-thiamide **5** was separated and then refluxed with α,β-dichlorether in DMF, to give the thiazole **7c** (16% yield). It should be pointed out that the preparation of **7a** and **7b** with pure thiamide **5** did not increase the yield.

In summary, because of the existence of 16,17-double bond, the activity of the conjugated 17-carbonyl group decreased, and the Michael addition reaction took place. This made the reaction for synthesis of 17-thiazole more complicated and the yield was generally low.

On the other hand, the synthesis of 17-oxazole derivatives were much easier. 4′-Methyloxazole **13a** was obtained in 40% yield by the direct cyclization of 17-amide **4** with bromoacetone in boiling DMF. In the same manner, 2-bromoacetophenone gave 4′-phenyloxazole **13b** in 68% yield, and oxazole **13c** was obtained with α,β-dichloroether. For the preparation of 5′-methyloxazole **15**, the classic Robinson–Gabriel cyclodehydration of β-keto amide **9a** was

Table 1
Percent inhibition of recombinant human $P450_{17\alpha}$-expressed in *E. coli* by the steroids (μM)

| Compound | Inhibition % |
|---|---|
| **7a** | 27.6 |
| **8a** | 13.2 |
| **7b** | <2 |
| **8b** | 26.7 |
| **7c** | <10 |
| **8c** | 41.5 |
| **10a** | 67.0 |
| **10b** | 0 |
| **12a** | 72.1 |
| **12b** | <10 |
| **13a** | <10 |
| **14a** | 19.4 |
| **13b** | 28.6 |
| **14b** | 26.2 |
| **13c** | 25.8 |
| **14c** | 56.0 |
| **15** | 19.9 |
| **16** | 45.0 |
| Ketoconazole | 100.0 |
| **L-39** | 116.7 |

The assay was performed in triplicate as described in Section 2 and was repeated on two occasions. Results are expressed as percent of inhibition relative to ketoconazole. Ketoconazole was used as an assay control and all values for the test compounds expressed relative to the activity of ketoconazole.

employed. However, all the dehydrating agents including concentrated sulfuric acid, $PCl_5$, $P_2O_5$, polyphosphoric acid and acetic acid anhydride [15] failed to give **9a**. A newer method [16] with mild conditions, i.e. the use of triphenylphorphine and iodine in triethylamine as dehydrating reagent was tried resulting in 21% yield of 5′-methyloxazole **15**. The freshly distilled triethylamine was crucial for the success of this reaction. However, the same method failed to give 5′-phenyloxazole.

All of the above 3β-acetate compounds were hydrolyzed in methanolic potassium hydroxide affording their 3β-ol counterparts. These were more potent inhibitors of $P450_{17\alpha}$.

### *4.2. Structure–activity relationship*

The percentage inhibition of $P450_{17\alpha}$ by the different compounds synthesized relative to ketoconazole is shown in Table 1. Since the latter is currently the only compound used clinically to inhibit this enzyme, we have compared the activities of our compounds to that of ketoconazole. The activity of the compounds as inhibitors of $P450_{17\alpha}$ was determined using recombinant human $P450_{17\alpha}$ expressed in *E. coli* [17]. The results showed that, the compounds containing 17-(2′-oxazolyl) (**14c**, 56.0% inhibition) and 17-(2′-thiazolyl) (**8c**, 23.7%) demonstrated inhibition against $P450_{17\alpha}$, although they were less potent than ketoconazole. They were also less potent than **L-39** which had greater activity than ketoconazole in the assay. The 3-acetate derivatives (e.g. **13c**, 25.8% and **7c**, <10%) were also less potent than the 3-OH counterparts, as $P450_{17\alpha}$ preferred 3-OH derivatives as substrate. The introduction of a phenyl group at either the 4′-ring position (**8b**, 26.7% and **14b**, 26.2%) or 5′-ring position (**12b**, <10%) decreased inhibition substantially. Surprisingly, although the introduction of a methyl group at the 4′-ring position decreased inhibition (**8a**, 13.2% and **14a**, 19.4%), 17-(5′-methyl-2′-oxazolyl) (**16**, 45.0%) still retained the activity to its parent compound 17-(2′-oxazolyl) (**14c**, 56.0%). The 17-(5′-methyl-2′-thiazolyl) (**12a**, 72.1%) had similar activity to ketoconazole and was the most potent inhibitor among this series. Compound **12a** was almost as potent as **L-39**, which has good antitumor activity in preclinical models and is scheduled for Phase I clinical trials.

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