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Inhibition of Steroid $C_{17(20)}$ Lyase with C-17-Heteroaryl Steroids

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Abstract—Steroids bearing a heteroaromatic substituent at C-17 were designed as inhibitors of $C_{17(20)}$ lyase. The thiazoles, furans, and thiophenes appended to the steroid nucleus were positioned on the α-face and the β-face of the steroid, and conjugated with a 16,17-olefin, to test their ability to coordinate the heme iron of the P450 enzyme complex. The position of the heterocycle with respect to the steroid skeleton was determined to be important for optimum affinity and, in general, compounds with the heterocycle attached to a trigonal center at C-17, had the best affinity for $C_{17(20)}$ lyase. Simple molecular models were used to compare the three types of heterocyclic-substituted steroids. Copyright © 1996 Elsevier Science Ltd

Introduction

Steroid $C_{17(20)}$ lyase,[1] a microsomal P450 enzyme found in the testes, ovaries, adrenals and placenta,[2] is responsible for the conversion of pregnenolone to dehydroepiandrosterone and progesterone to androstenedione. This conversion, which requires NADPH and oxygen, proceeds in two distinct steps and is comprised of an initial 17-α-hydroxylation followed by cleavage of the $C_{17(20)}$ bond to give the ketone at C-17. These intriguing enzymatic processes have been investigated and mechanisms for this transformation have been postulated.[2–7]

Inhibition of $C_{17(20)}$ lyase is a potential therapeutic approach for the treatment of both breast and prostate cancers.[8,9] As one approach to the inhibition of $C_{17(20)}$ lyase, we considered designing a substrate-like molecule which would form a heme complex. The use of suitably positioned heteroatoms to coordinate to the heme iron has been successfully exploited by a number of groups as inhibitors of aromatase.[10] Since certain heterocycles are known to bind to heme[11–13] and inhibit metabolic oxidation by liver P450 enzyme complexes, we reasoned that attachment of an appropriate heterocycle to the 17-position of a steroid framework may produce an inhibitor of $C_{17(20)}$ lyase. This report describes the synthesis of 17-heteroaryl steroids and their inhibition of $C_{17(20)}$ lyase at given concentrations. It should be noted that while this work was in progress, a report in the literature by Barrie and co-workers[14] appeared describing an analogous approach.

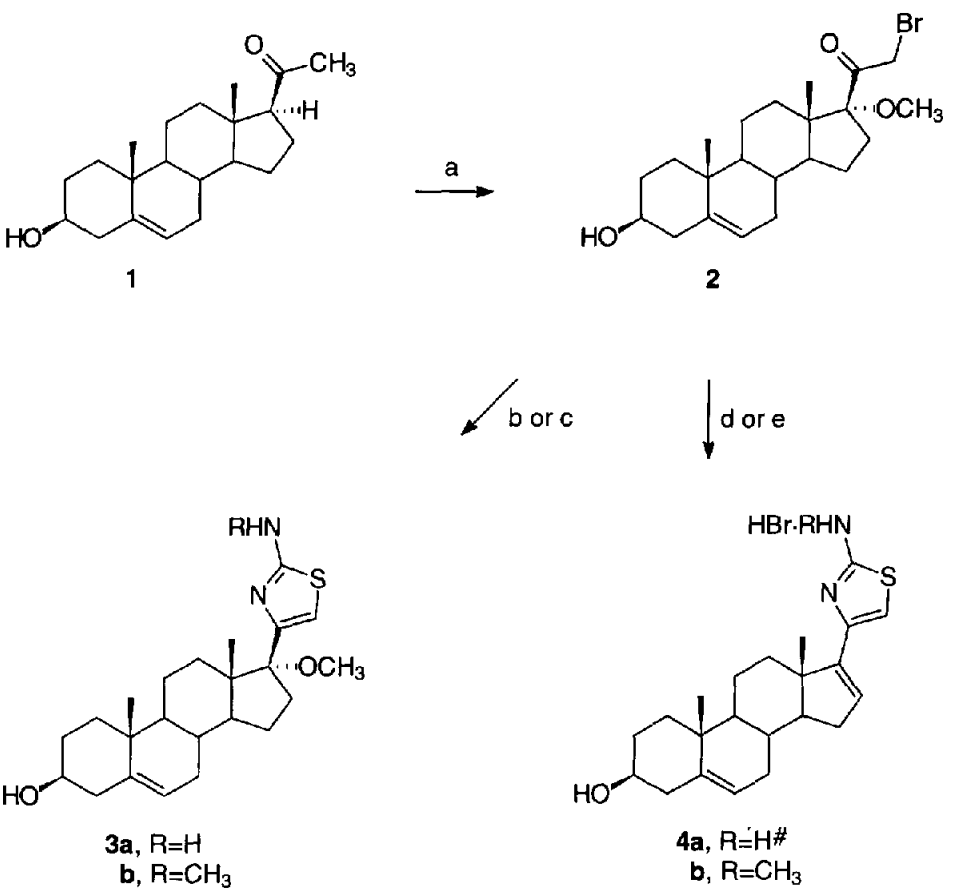
Steroids with a varicty of appended heterocycles at C-17 have been reported previously. Thus, steroids with furanyl,[15–20] thienyl,[21–23] pyrrolyl,[24] pyrazolyl,[25] isoxazolyl,[25] oxazolyl,[26] thiazolyl,[27–32] indolyl,[33,34] and pyridyl[14] rings at C-17 have been described. To our knowledge, only the recently reported C-17 pyridyl compounds[14] have been tested against $C_{17(20)}$ lyase and all compounds targeted for synthesis, with the exception of compound **16**,[35] represent novel entities.

Chemistry

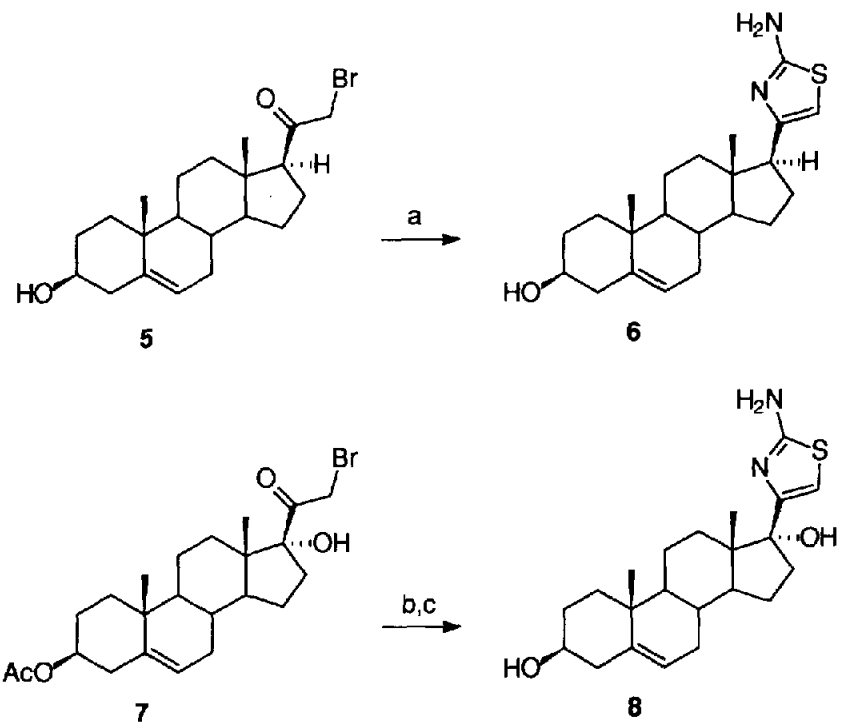
Syntheses of our first target molecules, thiazoles **3** and **4**, are shown in Scheme 1. Treatment of commercially available pregnenolone (**1**) with copper(II) bromide in methanol afforded the 17β-bromoacetyl-17α-methoxy steroid **2**.[36] Compound **2**, when treated with thiourea, triethylamine and alcohol, gave thiazole **3a**. Likewise, *N*-methylthiourea in a similar reaction gave **3b**. Interestingly, when triethylamine was omitted from these reactions, elimination of methanol occurred to give the 16,17-enes **4a** and **b**. These products undoubtedly arise from protonation of the ether oxygen by the liberated hydrobromic acid and elimination of methanol to give a stabilized carbonium ion at C-17. Subsequent loss of a proton from C-16 would then afford the unsaturated compounds **4a** and **b**.

In Scheme 2 are shown preparations of two additional analogues of **3a** and **4a**. 21-Bromopregnenolone (**5**)[36] was treated with thiourea in ethanol to afford aminothiazole **6**. Likewise, 3-*O*-acetyl-17-α-hydroxyl-21-bromopregnenolone (**7**)[36] was converted to the 17 α-hydroxy derivative of **6** (**8**) in a two-step procedure. Treatment of **7** with thiourea and triethylamine in ethanol to produce the heterocycle followed by hydrolysis of the acetyl group with lithium hydroxide gave **8**.

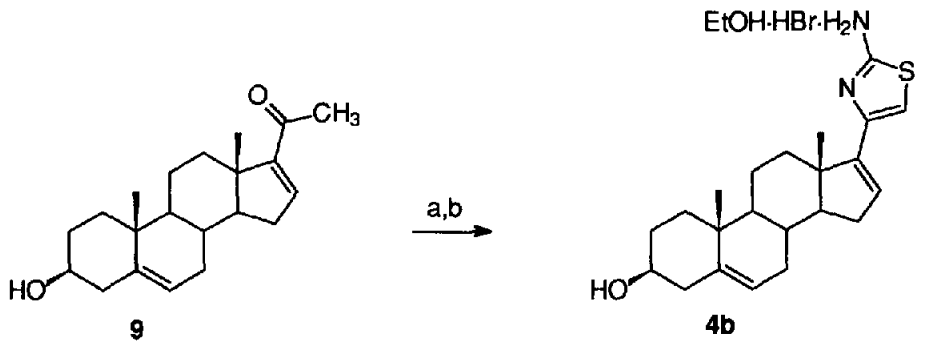
Since thiazole **4a** was the most potent compound in the set of compounds shown in Table 1 (*vide infra*), we investigated alternate synthetic routes for preparing a larger sample. In Scheme 3 is shown our optimum procedure. Treatment of commercially available 16-dehydropregnenolone (**9**) with 5,5-dibromobarbituric acid and aqueous hydrobromic acid in tetrahydrofuran gave 21-bromo-3β-hydroxypregna-5,16-dien-20-



Scheme 1. Reagents: (a) $CuBr_2$, MeOH; (b) thiourea, Et_3N, EtOH; (c) *N*-methylthiourea, Et_3N, EtOH; (d) thiourea, EtOH; (e) *N*-methylthiourea, EtOH. #One equivalent of EtOH was complexed with this molecule.



Scheme 2. Reagents: (a) thiourea, Et_3N, EtOH; (b) thiourea, Et_3N, EtOH; (c) LiOH, MeOH, THF, H_2O.



Scheme 3. Reagents: (a) 5,5-dibromobarbituric acid, THF, concd HBr; (b) thiourea, EtOH.

one, which was then cyclized by treatment with thiourea in ethanol to give **4a** (MDL 101,119B) in 74% overall yield.

In Scheme 4 are shown preparations of 17-furanyl- and 17-thienyl-substituted steroids. Since **4a** and related compounds supplied proof of concept for inhibiting $C_{17(20)}$ lyase with steroids bearing heterocyclic substituents at C-17, these additional compounds were prepared to optimize this inhibition. Treatment of dehydroepiandrosterone (**10**) with *t*-butyldimethylsilyl chloride gave the 3-*O*-protected steroid **11**, to which was added 2-lithiofuran and 2-lithiothiopene to give adducts **12a** and **b**, respectively. None of the epimeric products, arising from addition of the lithiated heterocycle to the β-face of the C-17 ketone, were observed. Dehydration with hydrochloric acid in dioxane cleanly gave the 16-dehydro steroids **13a** and **b**, respectively. In similar fashion were prepared the 3-furanyl (**15a**) and 3-thienyl (**15b**) isomers.

Scheme 5 shows the synthesis of diol **16**. Intermediate **14a**, from Scheme 4, is deprotected using tetrabutylammonium fluoride to provide compound **16**.[35]

Enzymology

Percent inhibition values for compounds evaluated against cynomolgus monkey testicular $C_{17(20)}$ lyase at given concentrations are presented in Tables 1 and 2. In Figure 1 is shown a plot of percent enzyme inhibition versus inhibitor concentration for compound **4a**, which was used for determination of the IC_{50} value for **4a**. Methods used for the determination of inhibitory values reported in Tables 1 and 2 and Figure 1 are detailed in the Experimental section.

Table 1. Inhibition of cynomolgus monkey testicular $C_{17(20)}$ lyase with aminothiazoles

Compd	Concn (μM)	Inhibition %
8	10	13 ± 5
	1	0
4b	10	40 ± 3
	1	28 ± 3
6	10	50 ± 3
	1	39 ± 10
3a	10	57 ± 1
	1	49 ± 2
4a	1	71 ± 2
	0.1	58 ± 1

Results and Discussion

The aminothiazoles synthesized in Schemes 1 and 2 provided a concise series of compounds for evaluating biological activity related to structural variation at the point of attachment of the heterocycle to the steroid. Each analogue possessed the 5-ene-3-ol so that A-ring configuration would be a constant for comparison of compounds.[37] In Table 1 are shown percent inhibition values at two concentrations for these compounds when evaluated in an in vitro enzyme assay[38] using cynomolgus monkey testicular $C_{17(20)}$ lyase. The compounds are arranged in order of increasing inhibitor potency. The 16,17-dehydro compound **4a** displayed the best affinity. A plot of percent enzyme inhibition versus inhibitor concentration for **4a** is presented in Figure 1.

To understand the relationship between the potential positions which the heterocycles could assume with respect to the steroid skeletons with the compounds in Table 1, we compared compounds **8** and **4a**. Figure 2 shows an overlay of these energy-minimized structures.[39] Surprisingly, this comparison showed that the thiazole rings of **8** and **4a** can assume similar orientations, even though the heterocycle stems from an sp^3 center in **8** and an sp^2 center in **4a**.[40]

The compound sets in Tables 1 and 2 are too limited to adequately address the components which are important for heme binding. However, a common feature of all of the compounds with affinity is at least one heteroatom (sulfur or oxygen) in the heterocyclic ring attached to the C-17 position. Thus, we speculate that this heteroatom is involved in heme binding.

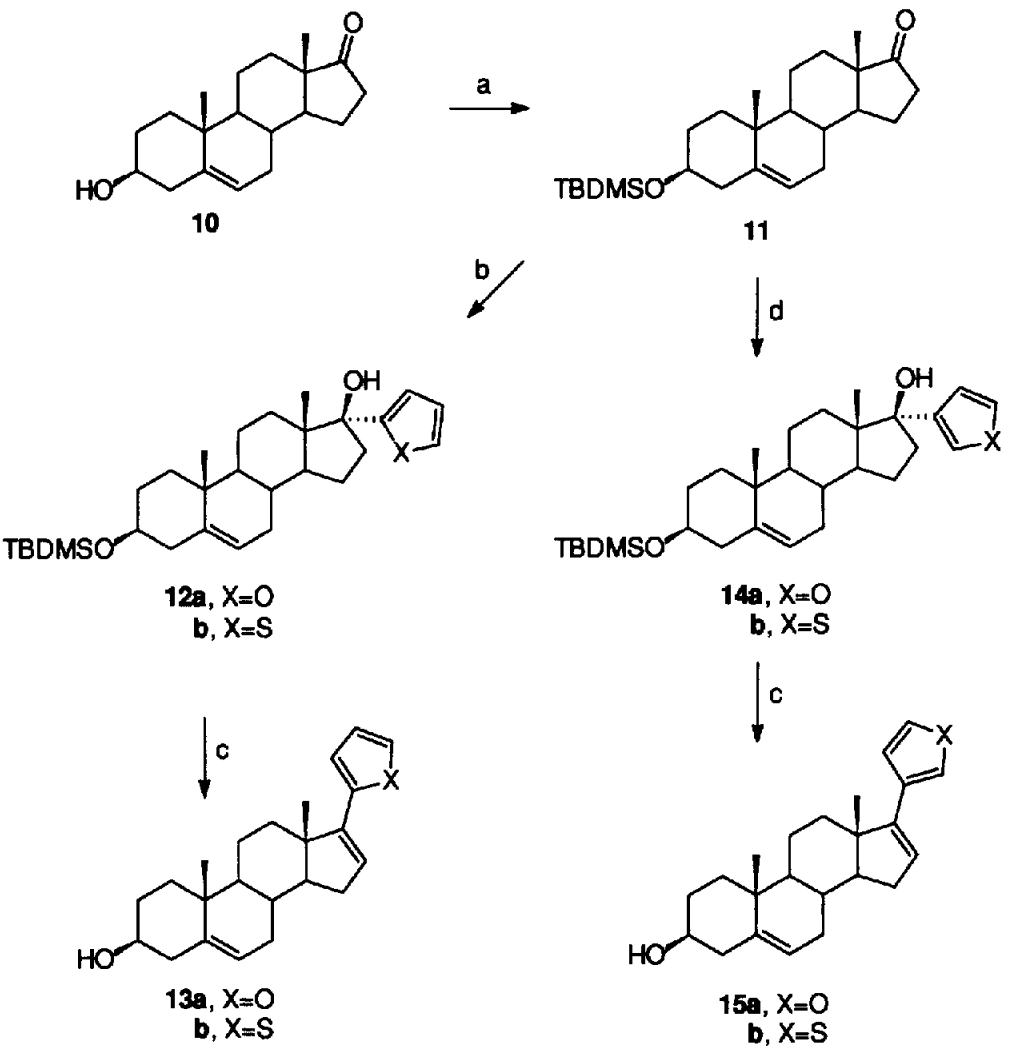
In Table 2 are shown percent inhibition values of cynomolgus monkey testicular $C_{17(20)}$ lyase for two 17-furanyl compounds. Compound **15a** was the most potent from the set of compounds prepared in Scheme 4. Compound **16** was also evaluated. Compound **16** was

chosen for comparison with **15a** since the furan ring in **16** is positioned on the α-face of the steroid. Affinity of **16** for the enzyme was poor, as shown in Table 2.

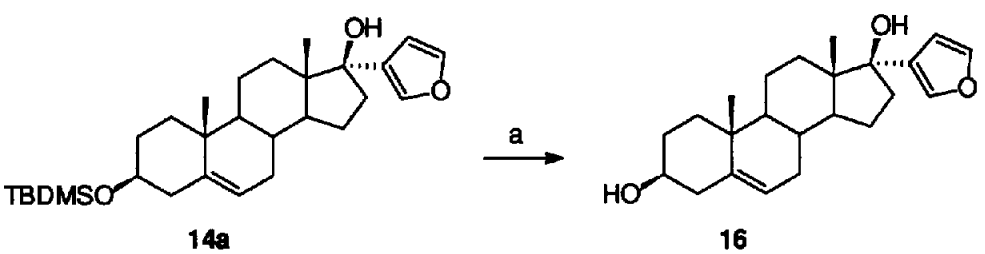
In Figure 3 is shown an overlay of energy-minimized versions of compounds **15a** and **16**.[39] It is clear that with respect to the steroid skeletons, the furan ring of **16** occupies a very different region of space than does the furan ring of **15a**. Thus, the heterocycles when attached to the β-face of the steroid or through a trigonal C-17 position are better positioned to interact with the heme. In addition, the compounds bearing a C-17 trigonal center are more potent inhibitors, in general, than those with the heterocycle attached to the β-face of the steroid through a C-17 tetrahedral center.

Conclusion

In summary, we have designed effective inhibitors of cynomolgus monkey testicular $C_{17(20)}$ lyase by attaching



Scheme 4. Reagents: (a) *t*-butyldimethylsilyl chloride, DMAP, Et_3N, DMF; (b) furan or thiophene, BunLi, THF; (c) 4 N HCl in dioxane; (d) 3-bromofuran or 3-bromothiophene, BunLi, THF.



Scheme 5. Reagents: (a) tetrabutylammonium fluoride, THF.

heterocycles as C-17 steroidal substituents, which apparently inhibit the enzyme by binding to the heme of the P450 enzyme complex. Molecular modeling studies suggest preferred positions for the heterocycles, with respect to the steroid, for heme-binding.

Experimental

General methods and materials

Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. TLC analyses were performed with Merck DC F_{254} or Analtech GHLF silica gel plates, with visualization by I_2, alkaline permanganate, or UV irradiation. Flash chromatography was performed with Merck silica gel 60 (0.040–0.063 mm). NMR spectra were recorded on Varian VSR-300, Unity 300, or Gemini-300 spectrometers in $CDCl_3$, unless otherwise stated. ^1H and ^{13}C NMR signals are reported in ppm from tetramethylsilane, and coupling constants are reported in Hz. IR spectra were recorded on a Perkin–Elmer model 1800 or Mattson Galaxy 5020 FTIR spectrophotometer. MS data were collected at 70 eV on a Finnigann MAT 4600, MAT TSQ-700, or VG Analytical Limited ZAB2-SE mass spectrophotometer and computerized peak matching with perfluorokerosense as the reference was utilized for HRMS. Combustion analysis performed using a Perkin–Elmer model 2400 elemental analyzer fell within $\pm 0.4\%$ of the calculated values.

Table 2. Inhibition of cynomolgus monkey testicular $C_{17(20)}$ lyase with furans

Compd	Concn (μM)	Inhibition %
15a	1	91 ± 2
	0.1	53 ± 3
16	10	25 ± 15
	1	0

Dehydroepiandrosterone (**1**), anhyd THF, anhyd DMF, 5,5-dibromobarbituric acid, furan, thiopene, 3-bromothiophene and 3-bromofuran were all purchased from Aldrich Chemical Company. Pregnenolone (**10**) was purchased from Searle and 16-dehydropregnenolone (**9**) was purchased from Sigma Chemical Company. $CuBr_2$ was obtained from Allied Chemical Company, thiourea from Fisher Scientific Company and 1-methyl-2-thiourea from Pfaltz and Bauer.

21-Bromo-3β-hydroxy-17α-methoxy-5-pregnen-20-one (2). To a stirred solution of pregnenolone (1.58 g, 5.00

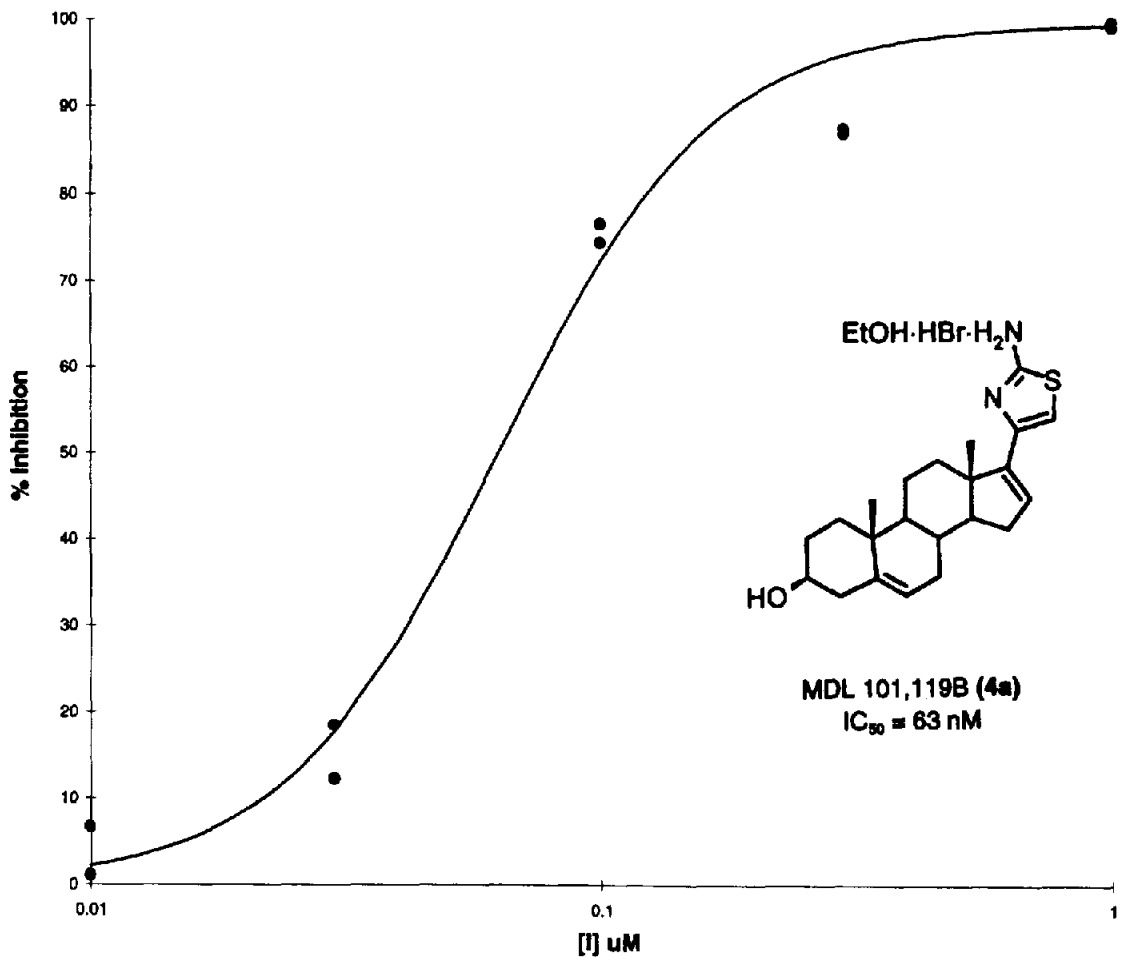


Figure 1. Determination of IC_{50} value for MDL 101,119B (**4a**).

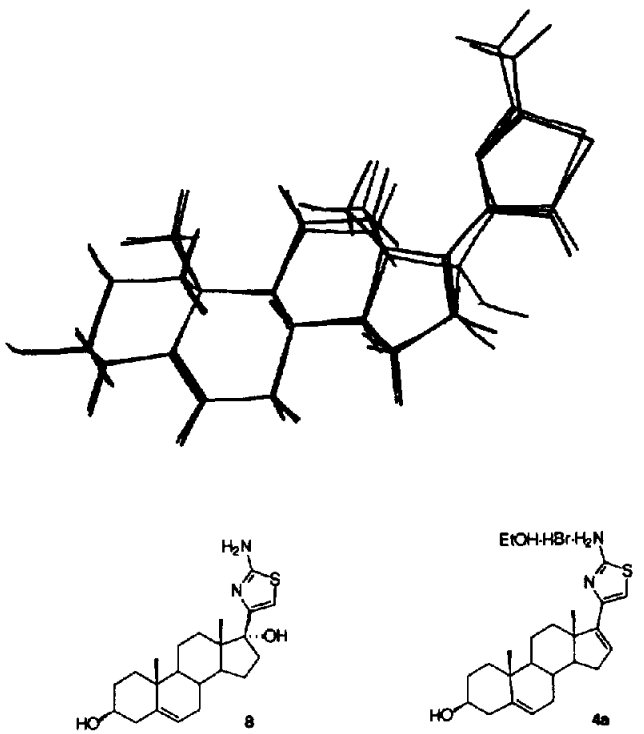


Figure 2. Overlay of compound **8** (C-17 sp^3 center; β-heterocycle) with compound **4a** (C-17 sp^2 center; α-hyterocycle).

mmol) in CH_3OH (180 mL) was added $CuBr_2$ (6.7 g, 30.0 mmol) and the reaction mixture heated to reflux. After 24 h, the reaction mixture was cooled to room temperature and suction filtered to remove CuBr. The filtrate was concentrated and the residue dissolved in CH_2Cl_2 (75 mL) and washed with H_2O (125 mL), half-saturated aq $NaHCO_3$ (2 × 100 mL) followed by brine (50 mL). Drying (Na_2SO_4) and concentration gave crude **2**. Two recrystallizations from acetone provided **2** (428 mg, 20%) as a white crystalline solid (contaminated by <5% 3β-hydroxy-17α-methoxy-5-pregnen-20-one); ^{1}H NMR: δ 5.38–5.32 (m, 1H, vinyl), 4.25 and 4.12 (pr of d, 2H, J = 15 Hz, CH_2Br), 3.60–3.47 (m, 1H, CHO), 3.18 (s, 3H, OCH_3), 1.00 (s, 3H, 19-CH_3), 0.63 (s, 3H, 18-CH_3).

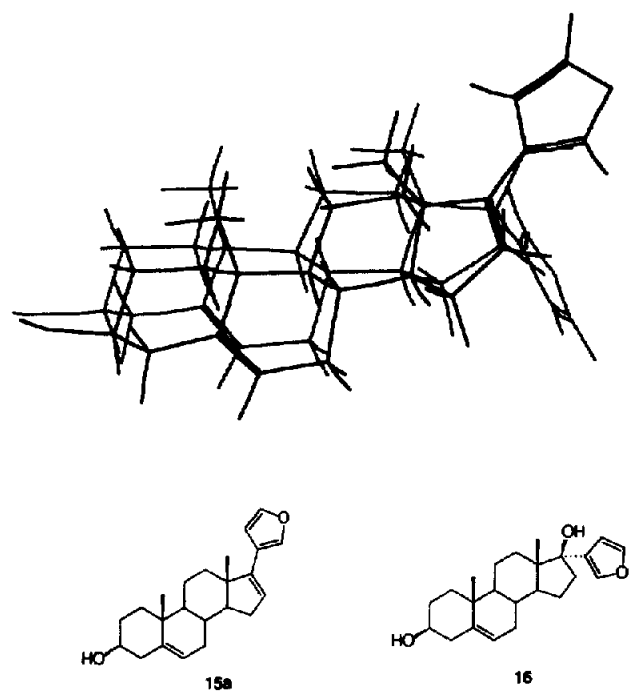


Figure 3. Overlay of compound **15a** (C-17 sp^2 center) with compound **16** (C-17 sp^3 center; α-heterocycle).

3β-17-(2-Amino-4-thiazolyl)-17α-methoxy-androst-5-ene-3-ol (3a). To a stirred suspension of **2** (638 mg, 1.50 mmol) in abs EtOH (30 mL) was added Et_3N (0.42 mL, 3.00 mmol) and thiourea (126 mg, 1.65 mmol). The reaction mixture was heated at reflux for 90 min allowing solvent to slowly distil off until 15 mL remained. The reaction mixture was allowed to cool to room temperature and the precipitated **3a** (401 mg, 66%) collected, as a white solid, by suction filtration; mp 209–211 °C (dec); ^{1}H NMR (DMSO-d_6): δ 6.80 (br s, 2H, NH_2), 6.34 (s 1H, SCH), 5.27 (br d, 1H, J = 3.8 Hz, Δ^5 vinyl), 4.60 (d, 1H, J = 4.4 Hz, OH), 3.32–3.20 (m, 1H, CHO), 2.84 (s, 3H, OCH_3), 0.93 (s, 3H, 19-CH_3), 0.43 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6): δ 167.1, 152.1, 142.1, 120.4, 103.8, 89.0, 70.0, 50.1, 49.8, 49.7, 48.1, 42.2, 37.0, 36.2, 32.1, 31.6, 31.4, 29.7, 26.8, 23.8, 20.2, 19.2, 15.4; MS (CI, CH_4): m/z (relative intensity) 403 (MH^+, 34), 385 (72), 371 (100), 369 (42), 353 (81), 329 (25), 315 (21), 297 (19); anal. ($C_{23}H_{34}N_2O_2S$), C, H, N, S.

3β-17α-Methoxy-17-[2-(methylamino)-4-thiazolyl]-androst-5-ene-3-ol (3b). To a stirred suspension of **2** (638 mg, 1.50 mmol) in abs EtOH (25 mL) was added Et_3N (0.42 mL, 3.0 mmol) and 1-methyl-2-thiourea (149 mg, 1.65 mmol). The reaction mixture was heated at reflux for 90 min allowing solvent to slowly distil off until 10 mL remained. The reaction mixture was allowed to cool to room temperature and concentrated. Flash chromatography, eluting with EtOAc:hexane (1:1) gave an oil which was triturated with Et_2O:hexane to give **3b** (216 mg, 35%) as a white solid, mp 180–182 °C; TLC: R_f 0.27 (EtOAc:hexane 1:1); ^{1}H NMR (DMSO-d_6): δ 7.34 (br q, 1H, J = 4.8 Hz, NH), 6.41 (s, 1H, SCH), 5.27 (br d, 1H, J = 4.3 Hz, Δ^5 vinyl), 4.59 (d, 1H, J = 4.5 Hz, OH), 3.32–3.19 (m, 1H, CHO), 2.84 (s, 3H, OCH_3), 2.76 (d, 3H, J = 4.7 Hz, NCH_3), 0.93 (s, 3H, 19-CH_3), 0.43 (s, 3H, 18 CH_3); ^{13}C NMR (DMSO-d_6): δ 168.6, 152.6, 141.3, 120.4, 103.2, 89.0, 70.0, 50.1, 49.84, 49.79, 48.1, 42.2, 37.0, 36.2, 32.1, 31.6, 31.4, 31.1, 29.8, 26.8, 23.8, 20.2, 19.2, 15.4; MS (CI, CH_4): m/z (rel intensity) 417 (18), 399 (69), 385 (100), 383 (54), 367 (90); anal. ($C_{24}H_{36}N_2O_2S$) C, H, N, S.

17-(2-Amino-4-thiazolyl)-androsta-5,16-diene-3β-ol, hydrobromide salt, ethanol solvate (4a). To a stirred suspension of **2** (638 mg, 1.50 mmol) in abs EtOH (25 mL) was added thiourea (126 mg, 1.65 mmol). The reaction mixture was heated at reflux for 90 min allowing solvent to slowly distil off until 10 mL remained. The reaction mixture was allowed to cool to room temperature and the resultant precipitate was

suction filtered, washed with EtOH (2 × 1 mL), and dried under vacuum to provide **4a** (360 mg, 49%) as a white solid, mp 256–260 °C (dec); ^{1}H NMR (DMSO-d_6): δ 8.85 (br s, NH_2), 6.81 (s, 1H, SCH), 6.28 (dd, 1H, J = 1.9, 3.3 Hz, Δ^{16} vinyl), 5.29 (br d, 1H, J = 4.7 Hz, Δ^5 vinyl), 3.43 (q, 2H, J = 7.0 Hz, CH_2O of EtOH), 3.32–3.18 (m, 1H, CHO), 1.05 (t, 3H, J = 7.0 Hz, CH_3 of EtOH), 0.99 (s, 3H, 19-CH_3), 0.94 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6): δ 169.4, 142.5, 141.6, 135.6, 131.0, 120.1, 102.0, 69.9, 56.4, 55.9, 49.7, 46.1, 42.2, 36.8, 36.2, 34.4, 31.3, 31.2, 30.8, 29.7, 20.4, 19.0, 18.5, 15.8; MS (CI, CH_4): m/z (relative intensity) 371 (MH^+, 76), 370 (33), 369 (34), 353 (100), 83 (26), 81 (27); anal. ($C_{22}H_{30}N_2OS \cdot HBr \cdot 1EtOH$) C, H, N.

Alternative preparation of 17-(2-amino-4-thiazolyl)-androsta-5,16-diene-3β-ol, hydrobromide salt, ethanol solvate (4a). To a stirred solution of **9** (5.00 g, 15.90 mmol) and 5,5-dibromobarbituric acid (4.55 g, 15.90 mmol) in THF (60 mL) was added concd aq HBr (100 μL of a 48% solution) and the reaction mixture heated at reflux for 1 h. The reaction mixture was allowed to cool to room temperature and concentrated. The residue was dissolved in CH_2Cl_2 (350 mL) and washed with half-satd aq $NaHCO_3$ (2 × 200 mL) and brine (125 mL). Drying ($MgSO_4$) and concentration provided 6.53 g of crude 21-bromo-3β-hydroxypregna-5,16-diene-20-one as an orange–brown solid which was used without further purification. The crude 21-bromo-3β-hydroxypregna-5,16-diene-20-one was suspended in EtOH (260 mL) and thiourea (1.33 g, 17.5 mmol) added. The reaction mixture was heated at reflux for 90 min allowing solvent to slowly distil off until 130 mL remained. The reaction mixture was allowed to cool to room temperature and crude **4a** (3.62 g) precipitated. Concentration furnished further **4a** (0.63 g). The two crops were combined and recrystallized from EtOH to give **4a** (2.77 g, 35%) as a white crystalline solid, mp 258–260 °C.

17-[2-(Methylamino)-4-thiazolyl]-androsta-5,16-diene-3β-ol, hydrobromide salt (4b). Compound **2** (250 mg, 0.59 mmol) and 1-methyl-2-thiourea (64 mg, 0.71 mmol) were reacted as for the preparation of **4a** to give **4b** (152 mg, 55%) as a white solid, mp 256–259 °C (dec); ^{1}H NMR (DMSO-d_6): δ 6.86 (br s, 1H, NH), 6.38 (s, 1H, SCH), 6.38 (dd, 1H, J = 1.9, 3.1 Hz, Δ^{16} vinyl), 5.30 (br d, 1H, J = 4.9 Hz, Δ^5 vinyl), 3.33–3.20 (m, 1H, CHO), 3.02 (s, 3H, NCH_3), 1.00 (s, 3H, 19-CH_3), 0.95 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6): δ 169.3, 142.7, 141.6, 135.6, 131.5, 120.1, 101.7, 69.9, 56.5, 49.8, 46.3, 42.2, 36.8, 36.2, 34.4, 32.5, 31.4, 31.2, 30.9, 29.8, 20.5, 19.0, 15.9; MS (CI/CH_4): m/z (relative intensity) 385 (MH^+, 80), 367 (100), 83 (58), 81 (60); HRMS $C_{23}H_{33}N_2O_6$ (MH^-) calcd 385.2314, obsd 385.2296.

(3β,17β)-17-(2-Amino-4-thiazolyl)-androst-5-ene-3-ol (6). To a stirred solution of **5**[36] (190 mg, 0.48 mmol) in abs EtOH (15 mL) was added Et_3N (0.17 mL, 1.25 mmol) and thiourea (76 mg, 1.00 mmol). The reaction mixture was heated at reflux for 1 h allowing solvent to slowly distil off until 5 mL remained. The suspension was allowed to cool to room temperature and the white solid collected by suction filtration. Recrystallization from EtOH gave **6** (61 mg, 34%) as a white crystalline solid; mp 280–285 °C (dec); TLC: R_f 0.24 (EtOAc:hexane 1:1); ^{1}H NMR (DMSO-d_6): δ 6.71 (br s, 2H, NH_2), 6.10 (s, 1H, SCH), 5.27 (br d, 1H, J = 3.9 Hz, Δ^5 vinyl), 4.59 (d, 1H, J = 4.5 Hz, OH), 3.32–3.20 (m, 1H, CHO), 0.94 (s, 3H, 19-CH_3), 0.46 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6): δ 167.0, 153.0, 141.3, 120.4, 100.3, 70.0, 55.5, 52.4, 49.9, 43.2, 42.2, 37.9, 37.0, 36.2, 31.9, 31.4, 25.5, 24.2, 20.4, 19.2, 12.9; MS (CI/CH_4): m/z (relative intensity) 373 (71), 355 (100); anal. ($C_{22}H_{32}N_2OS$) C, H, N.

(3β,17α)-17-(2-Amino-4-thiazolyl)-androst-5-ene-3,17-diol (8). To a stirred suspension of **7**[36] (0.20 g, 0.44 mmol) in abs EtOH (25 mL) was added Et_3N (0.12 mL, 0.88 mmol) and thiourea (50 mg, 0.66 mmol). The reaction mixture was heated at reflux for 90 min allowing solvent to slowly distill off until 10 mL remained. The suspension was allowed to cool to room temperature and loaded directly onto a column for chromatography. Flash chromatography, eluting with EtOAc:hexane (1:1), gave the 3-acetoxy derivative of **8** (0.15g, 79%) as a white solid. Treatment of a solution of the 3-acetoxy derivative in a mixture of THF (15 mL), CH_3OH (10 mL) and H_2O (2 mL) with 1.0 N aq LiOH (0.66 mL, 0.66 mmol) for 2 h followed by concentration gave crude **8**. Recrystallization from EtOH gave **8** (42 mg, 32%) as a tan solid; mp 273–277 °C (dec); TLC: R_f 0.17 (EtOAc:hexane 1:1); ^{1}H NMR (DMSO-d_6): δ 6.75 (br s, 2H, NH_2), 6.30 (s, 1H, SCH), 5.31–5.24 (m, 1H, Δ^5 vinyl), 4.60 (d, 1H, OH), 4.20 (s, 1H, OH), 3.32–3.20 (m, 1H, CHO), 0.94 (s, 3H, 19-CH_3), 0.47 (s, 3H, 18-CH_3); MS (CI/CH_4): m/z (relative intensity) 389 (MH^+, 38), 388 (21), 387 (23), 371 (100), 353 (45); anal. ($C_{22}H_{32}N_2O_2S$), C, H, N.

3β-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-androst-5-ene-17-one (11). To a stirred solution of **10** (10.00 g, 34.67 mmol) in anhyd DMF (150 mL) under N_2 was added t-butyl dimethylsilyl chloride (5.23 g, 34.67 mmol), 4-dimethylaminopyridine (0.21 g, 1.73 mmol) and Et_3N (5.32 mL, 38.14 mmol). The resultant light suspension was stirred at room temperature for 2 days and then poured into rapidly stirred H_2O (1.5 L). The suspension was filtered and the collected white solid recrystallized from aq acetone to give **11** (12.32 g, 88%) as a white crystalline solid, mp 146–148 °C; TLC: R_f 0.78 (EtOAc:hexane 1:1); ^{1}H NMR: δ: 5.38–5.32 (m, 1H, Δ^5 vinyl), 3.55–3.43 (m, 1H, CHO), 1.03 (s, 3H, CH_3), 0.89 [s, 9H, $C(CH_3)_3$], 0.88 (s, 3H, CH_3), 0.06 (s, 6H, 2 × $SiCH_3$); MS (CI/CH_4): m/z (relative intensity) 403 (MH^+, 3), 401 (5), 387 (9), 345 (18), 271 (100); anal. ($C_{25}H_{42}O_2Si$) C, H.

(3β,17β)-3-[[1,1-Dimethylethyl)dimethylsilyl]oxy]-17-(2-furanyl)-androst-5-ene-17-ol (12a). To a stirred solution of furan (0.60 mL, 8.25 mmol) in anhyd THF (15 mL) under N_2 and cooled in an ice H_2O bath was added BunLi (4.69 mL of a 1.6 M solution in hexane,

7.50 mmol). After 5 min, the reaction mixture was allowed to warm to room temperature and 15 min later, a solution of **11** (604 mg, 1.50 mmol) in anhyd THF (6 mL) was added. After 2 h, the reaction mixture was diluted with Et_2O (100 mL) and washed with 0.5 N aq HCl (45 mL), satd aq $NaHCO_3$ (2 × 45 mL) and brine (45 mL). Drying ($MgSO_4$) and concentration gave crude **12a**. Flash chromatography, eluting with EtOAc:hexane (1:9), followed by recrystallization from aq methanol gave **12a** (302 mg, 43%) as a white crystalline solid, mp 129–133 °C; TLC: R_f 0.25 (EtOAc:hexane 1:9); ^{1}H NMR: δ 7.36 (dd, 1H, J = 0.8, 1.8 Hz, H-5′), 6.33 (dd, 1H, J = 1.8, 3.2 Hz, H-4′), 6.14 (dd, 1H, J = 0.8, 3.2 Hz, H-3′), 5.35–5.26 (m, 1H, Δ^5 vinyl), 3.52–3.35 (m, 1H, CHO), 0.99 (s, 6H, 2 × CH_3), 0.88 [s, 9H, $C(CH_3)_3$], 0.04 (s, 6H, 2 × $SiCH_3$); MS (CI/CH_4): *m/z* (relative intensity) 471 (MH^+, 10), 470 (13), 469 (20), 455 (30), 413 (21), 339 (40), 321 (100); anal. ($C_{29}H_{46}O_3Si$) C, H.

(3β,17β)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-17-(2-thienyl)-androst-5-ene-17-ol (12b). Reaction of 2-lithothiophene [from thiophene (0.66 mL, 8.25 mmol) and BunLi (4.69 mL of a 1.6 M solution in hexane)] and **11** (604 mg, 1.50 mmol) as described for the preparation of **12a** gave crude **12b**. Flash chromatography, eluting with EtOAc:hexane (1:9), followed by recrystallization from aq acetone gave **12b** (0.51 g, 70%) as a blue solid, mp 164–168 °C; TLC: R_f 0.27 (EtOAc:hexane 1:9); ^{1}H NMR: δ 7.22 (dd, 1H, J = 1.1, 5.1 Hz, H-5′), 6.96 (dd, 1H, J = 3.5, 5.1 Hz, H-4′), 6.81 (dd, 1H, J = 1.1, 3.5 Hz, H-3′), 5.34–5.28 (m, 1H, Δ^5 vinyl), 3.47–3.34 (m, 1H, CHO), 2.19 (s, 1H, OH), 1.03 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.87 [s, 9H, $C(CH_3)_3$], 0.04 (s, 6H, 2 × $SiCH_3$); MS (CI/CH_4): *m/z* (relative intensity) 487 (MH^+, 6), 486 (5), 485 (9), 471 (16), 469 (5), 429 (20), 355 (38), 337 (100), 271 (33); anal. ($C_{29}H_{46}O_2SSi$), C, H.

17-(2-Furanyl)-androsta-5,16-diene-3β-ol (13a). Compound **12a** (1.41 g, 3.00 mmol) was dissolved in 4 N hydrogen chloride in dioxane (15 mL) under N_2 and stirred for 20 min. The reaction mixture was poured into CH_2Cl_2 (200 mL)/satd aq $NaHCO_3$ (100 mL) and the layers separated. The organic layer was washed with satd aq $NaHCO_3$ (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated to give crude **13a**. Flash chromatography, eluting with EtOAc:hexane (25:75) gave **13a** (0.75 g, 74%) as an unstable white solid, mp 106–116 °C (dec); TLC: R_f 0.24 (EtOAc:hexane 3:7); ^{1}H NMR: δ 7.35 (d, 1H, J = 1.8 Hz, H-5′), 6.36 (dd, 1H, J = 1.8, 3.3 Hz, H-4′), 6.27 (d, 1H, J = 3.3 Hz, H-3′), 6.07 (dd, 1H, J = 2.1, 3.3 Hz, Δ^{16} vinyl), 5.41–5.36 (m, 1H, Δ^5 vinyl), 3.61–3.48 (m, 1H, CHO), 1.07 (s, 3H, CH_3), 0.99 (s, 3H, CH_3); MS (CI/CH_4): *m/z* (relative intensity) 339 (MH^+, 55), 338 (42), 337 (36), 321 (100); HRMS $C_{23}H_{31}O_2$ (MH^+); calcd 339.2324, obsd 339.2328.

17-(2-Thienyl)androsta-5,16-diene-3β-ol (13b). Compound **12b** (0.22 g, 0.45 mmol) was treated with 4 N hydrogen chloride in dioxane (4 mL) as described for **13a** to give crude **13b**. Flash chromatography, eluting with EtOAc:hexane (3:7), gave **13b** (0.11 g, 69%) as a white solid, mp 190–195 °C (dec); TLC: R_f 0.39 (EtOAc:hexane 35:65); ^{1}H NMR: δ 7.14 (dd, 1H, J = 1.0, 5.1 Hz, H-5′), 7.03 (br d, 1H, J = 3.5 Hz, H-3′), 6.97 (dd, 1H, J = 3.6, 5.1 Hz, H-4′), 5.98 (dd, 1H, J = 2.0, 3.2 Hz, Δ^{16} vinyl), 5.41–5.37 (m, 1H, Δ^5 vinyl), 3.60–3.47 (m, 1H, CHO), 1.07 (s, 3H, CH_3), 1.03 (s, 3H, CH_3); MS (CI/CH_4): *m/z* (relative intensity) 355 (MH^+, 75), 337 (100); anal. ($C_{23}H_{30}OS$) C, H.

(3β,17β)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-17-(3-furanyl) androst-5-ene-17-ol (14a). To a stirred solution of BunLi (15.63 mL of a 1.6 M solution in hexanes, 25.00 mmol) in anhyd THF (30 mL) under N_2 and cooled to −78 °C was dropwise added 3-bromofuran (2.25 mL, 25.00 mmol). After 30 min, a precooled (−78 °C) solution of **11** (2.01 g, 5.00 mmol) in anhyd THF (40 mL) was added. The reaction mixture was stirred for 1.5 h and then poured into Et_2O (300 mL)/0.5 N aq HCl (125 mL). The layers were separated and the organic layer washed with 0.5 N aq HCl (125 mL), satd aq $NaHCO_3$ (3 × 75 mL) and brine (75 mL). Drying ($MgSO_4$) and concentration gave crude **14a**. Flash chromatography, eluting with EtOAc:hexane (1:9) gave **14a** (2.03 g, 86%) as a white solid. Recrystallization from aq acetone gave white platelets, mp 156–158 °C; TLC: R_f 0.19 (EtOAc:hexane 1:9); ^{1}H NMR: δ 7.36 (t, 1H, J = 1.7 Hz, H-5′), 7.27–7.24 (m, 1H, H-4′), 6.37 (dd, 1H, J = 0.8, 1.7 Hz, H-2′), 5.25–5.33 (m, 1H, Δ^5 vinyl), 3.50–3.36 (m, 1H, CHO), 1.78 (s, 1H, OH), 1.00 (s, 6H, 2 × CH_3), 0.88 [s, 9H, $C(CH_3)_3$], 0.04 (s, 6H, 2 × $SiCH_3$); MS (CI/CH_4): *m/z* (relative intensity) 471 (MH^+, 23), 470 (24), 469 (25), 455 (56), 413 (25), 339 (72), 321 (100); anal. ($C_{29}H_{46}O_3Si$) C, H.

(3β,17β)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-17-(3-thienyl)androst-5-ene-17-ol (14b). 3-Lithiothiophene [from 3-bromothiophene (1.17 mL, 12.5 mmol) and Bunli (7.81 mL of a 1.6 M solution in hexane, 12.5 mmol) was reacted with **11** (1.01 g, 2.50 mmol) as described for the preparation of **14a** to give crude **14b**. Flash chromatography, eluting with EtOAc:hexane (1:9) gave **14b** (0.88 mg, 72%) as a waxy, white solid. Recrystallization from aq acetone gave fine white needles, mp 176–178 °C; TLC: R_f 0.25 (EtOAc:hexane 1:9); ^{1}H NMR: δ 7.23 (dd, 1H, J = 2.7, 5.1 Hz, H-5′), 7.08 (dd, 1H, J = 1.2, 5.1 Hz, H-4′), 7.04 (dd, 1H, J = 1.2, 2.7 Hz, H-2′), 5.32–5.27 (m, 1H, Δ^5 vinyl), 3.47–3.35 (m, 1H, CHO), 1.03 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.88 [s, 9H, $C(CH_3)_3$], 0.05 (s, 6H, 2 × CH_3); MS (CI/CH_4): *m/z* (relative intensity) 487 (MH^+, 30), 486 (23), 485 (24), 429 (22), 355 (75), 337 (100), 271 (40); anal. ($C_{29}H_{46}O_2SSi$) C, H.

17-(3-Furanyl)-androsta-5,16-diene-3β-ol (15a). Compound **14a** (2.50 g, 5.31 mmol) was treated with 4 N hydrogren chloride in dioxane (35 mL) as described for **13a** to give crude **15a**. Flash chromatography, eluting with EtOAc:hexane (3:7) gave **15a** (1.05 g, 58%) as a white solid. Recrystallization from aq acetone gave a

white crystalline solid, mp 186–189 °C; TLC: R_f 0.43 (EtOAc:hexane, 35:65); ^{1}H NMR: δ 7.47 (br s, 1H, H-2′), 7.36 (t, 1H, J=1.7 Hz, H-5′), 6.48 (dd, 1H, J=0.8, 1.8 Hz, H-4′), 5.83 (dd, 1H, J=1.9, 3.2 Hz, Δ^{16} vinyl), 3.60–3.48 (m, 1H, CHO), 1.07 (s, 3H, CH_3), 0.96 (s, 3H, CH_3); MS (CI/CH_4): *m/z* (relative intensity) 339 (MH^+, 68), 338 (55), 337 (28), 321 (100); anal. ($C_{23}H_{30}O_2$) C, H.

17-(3-Thienyl)-androsta-5,16-diene-3β-ol (15b). Compound **14b** (0.250 g, 0.51 mmol) was treated with 4 N hydrogen chloride in dioxane (5 mL) as described for **13a** to give crude **15b**. Recrystallization from acetone gave **15b** (123 mg, 68%) as a white crystalline solid, mp 215–219 °C; TLC: R_f 0.35 (EtOAc:hexane 35:65); ^{1}H NMR: δ 7.26–7.23 (m, 1H), 7.19 (s, 1H), 7.19–7.17 (m, 1H), 5.93 (dd, 1H, J=1.9, 3.2 Hz, Δ^{16} vinyl), 5.42–5.36 (m, 1H, Δ^5 vinyl), 3.61–3.43 (m, 1H, CHO), 1.07 (s, 3H, CH_3), 1.03 (s, 3H, CH_3); MS (CI/CH_4): *m/z* (relative intensity) 355 (MH^+, 77), 354 (35), 353 (25), 337 (100); anal. ($C_{23}H_{30}OS$) C, H.

(3β,17β)-17-(3-Furanyl)androst-5-ene-3,17-diol (16). Compound **14a** (300 mg, 0.63 mmol) was dissolved in 1.0 M tetrabutylammonium fluoride (2.5 mL of a 1.0 M solution in THF) and stirred overnight. The reaction was poured into water (30 mL)/CH_2Cl_2 (50 mL), the layers separated, and the organic phase washed with saturated aq $NaHCO_3$ (30 mL) and brine (30 mL). Drying ($MgSO_4$) and concentration gave the crude product. Recrystallization from aq acetone gave **16** (137 mg, 50%) as a pale-yellow crystalline solid, mp 183–188 °C (lit.[35] stated **16** was a white solid but gave no mp); ^{1}H NMR (DMSO-d_6): δ 7.50 (dd, 1H, J=1.5, 1.7 Hz), 7.37 (dd, 1H, J=1.5, 0.7 Hz), 6.39 (dd, 1H, J=1.7, 0.7 Hz), 5.26–5.21 (m, 1H, Δ^5 vinyl H), 4.83 (s, 1H, 17-OH), 4.59 (br d, 1H, J=4.6 Hz, 3-OH), 3.30–3.10 (m, 1H, CHO), 1.06 (s, 3H, CH_3), 0.86 (s, 3H, CH_3); MS (CI, CH_4): *m/z* (relative intensity) 357 (MH^+, 25), 356 (M^+, 26), 339 ($MH^+ - H_2O$, 100), 321 ($MH^+ - 2H_2O$, 22); anal. ($C_{23}H_{32}O_3$) C, H.

Enzymes and assays

Compounds were tested for inhibition of cynomolgus monkey $C_{17(20)}$ lyase in vitro using microsomal preparations of the enzyme from testicular tissue. Testes were removed from anesthesized animals and flash frozen in liquid nitrogen. Microsomes were isolated as described previously.[38] The compound to be tested was dissolved in dimethyl sulfoxide and diluted in 0.05 M potassium phosphate buffer, pH 7.4, to give the desired concentrations of test compound; this contributes 0.1% v/v DMSO to the final mix. Assays contained 0.05 M potassium phosphate buffer, pH 7.4, an NADPH regenerating system (1 mM NADPH, 5 mM glucose-6-phosphate, 1 IU/mL glucose-6-phosphate dehydrogenase), test compound, substrate and microsomal protein in a total volume of 0.2 mL. Control assays contained all components, including dimethyl sulfoxide, but no test compound. All assays were performed in duplicate. The reaction was initiated by the addition of substrate, 7-^3H-17α-hydroxypregnenolone (11.2 mCi/mmol; 0.20 mCi per assay) plus unlabeled 17α-hydroxypregnenolone dissolved in dimethyl sulfoxide, contributing 2.5% v/v to the final assay mix, and phosphate buffer, yielding a final concentration of 0.05 mM 17α-hydroxypregnenolone (ca. equal to the K_m value), to the other assay components. The complete assay was incubated at 34 °C for 6 min. Each assay was terminated by addition of 5 mL of chloroform:methanol (2:1). Carrier steroids representing substrates and products (17α-hydroxypregnenolone, dehydroepiandrosterone, and androst-5-ene-3β,17β-diol) and 0.8 mL of distilled, deionized water were also added at this time. The steroids were extracted by the method of Moore and Wilson.[41] The organic phase containing the steroids was evaporated using nitrogen gas, the residues were dissolved in 18% tetrahydrofuran (v/v) in hexane, and the steroids were separated by HPLC on a Si60 (5 mm) column (250 × 4 mm) using a gradient of 18–22% tetrahydrofuran (v/v) in hexane. Radioactivity in the steroid peaks was measured using a Radiomatic Model HS or Model A515 Flo-One detector.

The enzyme activity for each assay was calculated from the percent conversion of substrate to products, and the results were expressed as percent inhibition of control. The IC_{50} value for compound **4a** was determined by fitting the data to the following two-parameter dose-response equation using a VAX computer:

$$f(x) = \frac{100}{1 + (X/B_3)(B_2 \times B_3/-25)}$$

In which B_2 is the slope at IC_{50} and B_3 is equal to the IC_{50}.

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39. Structures were drawn and energy minimizations and overlays were performed using ALCHEMY III software from Tripos Associates, Inc., 1699 Hanley Road, Suite #303, St Louis, MO, 63144, U.S.A.

40. We recently reported a similar finding in comparing a 2-aryltetrahydrofuran and a 2-aryloxazoline. See Peet, N. P.; Burkhart, J. P.; Brocrsma, R. J.: Heminger, E. F. *J. Heterocyc. Chem.* **1994**, *31*, 419.

41. Moore, R. J.; Wilson, J. D. In *Methods in Enzymology: Hormone Action 36*; O'Malley, B.W.; Hardman, J. G., Eds.; New York: Academic, 1975; p 466.