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TIME-DEPENDENT INACTIVATION OF STEROID $C_{17(20)}$ LYASE BY 17β-CYCLOPROPYL ETHER-SUBSTITUTED STEROIDS

Michael R. Angelastro,* Angela L. Marquart, Philip M. Weintraub, Cynthia A. Gates, Marie E. Laughlin, Thomas R. Blohm, and Norton P. Peet

Marion Merrell Dow Research Institute, 2110 East Galbraith Rd, Cincinnati, OH 45215

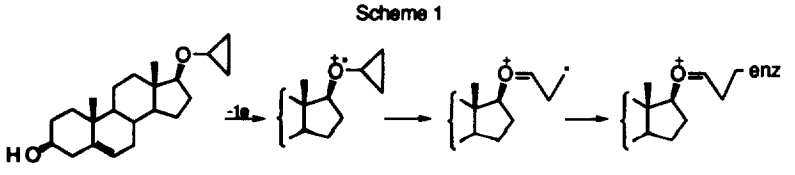
Abstract: Androstenes bearing a cyclopropyl group attached to the C-17β position with a heteroatom linker, designed as mechanism-based inhibitors of steroid $C_{17(20)}$ lyase, were found to be potent, time-dependent inhibitors of this enzyme.

Androgen deprivation is a potential therapeutic approach for the treatment of prostatic carcinoma since the majority of these tumors (80%) are androgen dependent. Microsomal cytochrome P450 dependent 17α-hydroxylase/17,20 lyase (EC1.14.99.9 /EC 4.1.2.30; P450c17), which is present in the testes, adrenal glands, the ovaries and placental tissue, is a potential target for inhibition because it converts pregnenolone to dehydroepiandrosterone and progesterone to androstenedione. These are the initial transformations in androgen biosynthesis following cholesterol side chain cleavage.[1] The oxidative deacetylations are accomplished in a two-step process, presumably involving C-17α hydroxylation followed by C-17(20) bond cleavage, to give the C-17 ketone. The enzyme activites require NADPH and oxygen, are co-purified from microsomes[2] and are expressed from a single mRNA.[3]

The mechanism of the C-17α hydroxylation has been studied extensively[4,5] and it is generally accepted that an iron-activated oxygen species abstracts a hydrogen atom from C-17, and the resulting iron-hydroxyl radical combines with the C-17 radical of the steroid to produce the C-17α hydroxylated derivative. The subsequent lyase reaction is not so well understood, but may involve the addition of an enzyme-peroxy radical to the C-20 ketone.

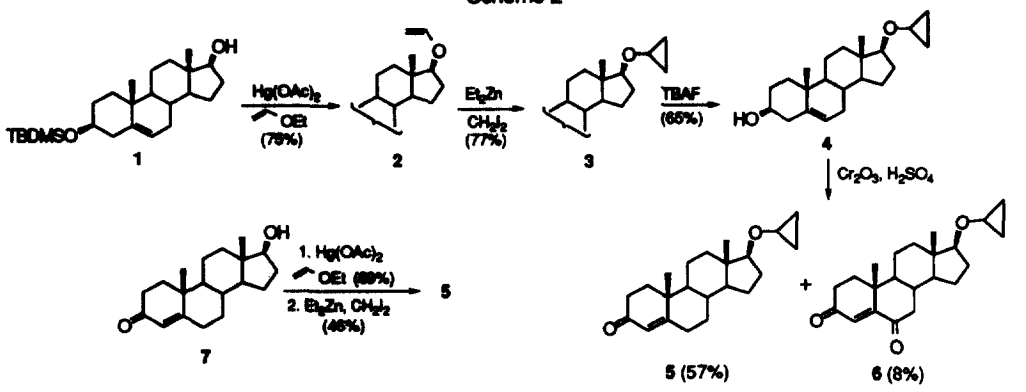
Previously, we reported the time-dependent irreversible inhibition of $C_{17(20)}$ lyase by 17β-(cyclopropylamino)androst-5-en-3β-ol.[6,7] Cyclopropylamines undergo rapid ring-opening to produce primary radicals as a result of one-electron oxidation.[8] Cyclopropylamines[9-12] and cyclopropyl ethers[11] were shown to be time-dependent inactivators of cytochrome P_{450} enzymes. Herein, we wish to report the syntheses of 17β-cyclopropyl ether-substituted steroids and their time-dependent inhibition of $C_{17(20)}$ lyase. A proposed mechanism-based inactivation is shown in Scheme 1.

Scheme 1



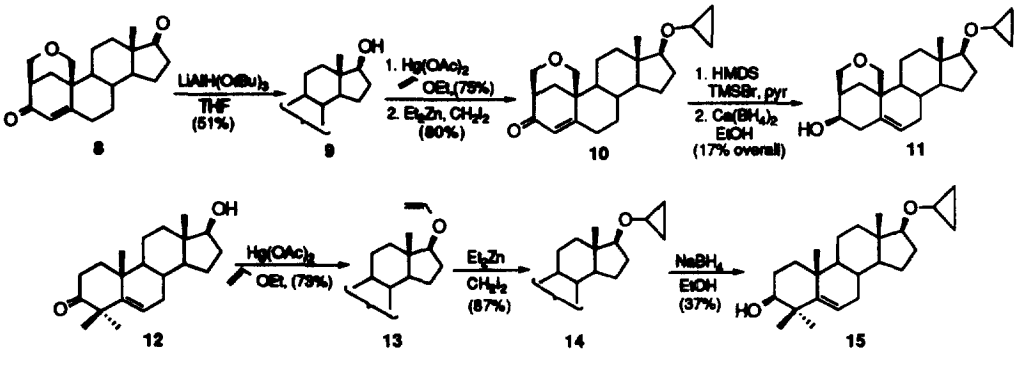
The steroidal cyclopropyl ethers were prepared by vinylation and subsequent cyclopropanation of 17β-hydroxy-substituted steroids. Treatment of 3β-{[(1,1-dimethyl)ethyl]dimethyl}silyloxyandrost-5-en-17β-ol (**1**) with ethyl vinyl ether in the presence of mercuric acetate afforded vinyl ether **2**, which was cyclopropanated using a Simmons-Smith procedure to produce cyclopropyl ether **3**. Deprotection of **3** with tetrabutylammonium fluoride gave **4**,[13] which was oxidized (Cr_2O_3, H_2SO_4) to give the enone **5**[14] and the 3,6-dione **6**[15] as a minor by-product (Scheme 2). The enone **5** was also prepared with an increase in effeciency by the vinylation and cyclopropanation of testosterone (**7**).

Scheme 2



The syntheses of A-ring modified steroidal cyclopropyl ethers containing a 2,19-methyleneoxy bridge (**11**) and 4,4-dimethyl substitution (**15**) are shown in Scheme 3. Compounds **11** and **15** were designed to preclude a potential for metabolic conversion to androgenic steroids.[16] The reduction of 2,19-(methyleneoxy)androst-4-ene-3,17-dione (**8**)[17] with lithium tri-*tert*-butoxyaluminohydride in THF at 0 °C gave the alcohol **9**, which was vinylated and cyclopropanated to produce the cyclopropyl ether **10**. Treatment of **10** with hexamethyldisilylazide and trimethylsilyl bromide in pyridine gave the expected trimethylsilyldienol which was immediately reduced with calcium borohydride in ethanol to give the cyclopropyl ether **11**.[18] Similarly, the vinylation and cyclopropanation of ketone **12**[19] gave a 63% yield of the cyclopropyl ether **14**, which was reduced with sodium borohydride in ethanol to produce 17β-cyclopropyloxy-4,4-dimethylandrost-5-en-3β-ol (**15**).[20]

Scheme 3



Compounds were tested for inhibition of human or cynomolgus monkey testicular microsomal $C_{17(20)}$ lyase activity. Microsomes were prepared, and $C_{17(20)}$ lyase activity from human tissue was determined as described previously.[12] Radiolabeled substrate used for both species was [17-^{3}H]-17α-hydroxypregnenolone (10-11 Ci/mmol; 0.2 μCi per assay; Amersham).[21] Compounds **4**, **10**, **11** and **15** were evaluated with human testicular $C_{17(20)}$ lyase (Table 1) and cynomolgus testicular $C_{17(20)}$ lyase was used for compounds **5** and **6** (Table 2). All of the compounds which displayed activity (**4**, **5**, **6** and **10**) were clearly time-dependent, which supports the proposed mechanism-based inhibition shown in Scheme 1. Compound **4** was the most potent of the compounds tested with the human enzyme, whereas compounds **5** and **6** were essentially equal in potency against the monkey enzyme. Interestingly, compounds with the modified (with respect to normal substrate) A-rings (**10**, **11** and **15**) showed little affinity for $C_{17(20)}$ lyase, which suggests that these modifications prevent binding at the catalytic site of the enzyme.

Table 1

Inhibition of Human Testicular $C_{17(20)}$ Lyase

Compound	Inhibitor Conc. (μM)	Preincubation (minutes)	Percent Inhibition
4	0.8	0	11
		30	64
10	0.8	0	1
		30	17
11	0.8	0	0
		30	0
15	0.8	0	0
		30	0

Table 2

Inhibition of Cynomolgus Testicular $C_{17(20)}$ Lyase

Compound	Inhibitor Conc. (μM)	Preincubation (minutes)	Percent Inhibition
5	1	0	73
		40	90
	0.1	0	26
		40	55
6	1	0	52
		40	79
	0.1	0	17
		40	51

In summary, we described the preparation of several 17β–cyclopropyl ether-substituted steroids and evaluated these compounds with $C_{17(20)}$ lyase enzymes. Compounds which inhibited the enzyme were time-dependent, which is consistent with our suggested mechanism for enzyme inactivation.

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13. **4**: ^{1}H NMR ($CDCl_3$) 5.35 (s, 1H), 3.52 (m, 1H), 3.44 (m, 1H), 3.30 (m, 1H), 1.02 (s, 3H), 0.77 (s, 3H), 0.60-0.41 (m, 4H). ^{13}C NMR ($CDCl_3$) 140.70, 121.27, 89.10, 71.70, 11.75, 6.29, 6.03. MS (CH_4) 331 (23%, M^{+1}), 313 (100%, M^{+1}-H_2O), 273 (45%, M^{+1}-C_3H_5OH). Anal. Calcd for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found C, 79.77; H, 10.61.
14. **5**: ^{1}H NMR ($CDCl_3$) 5.73 (s, 1H), 3.44 (t, J =8.6 Hz, 1H), 3.33-3.26 (m, 1H), 1.19 (s, 3H), 0.80 (s, 3H), 0.61-0.38 (m, 4H). ^{13}C NMR ($CDCl_3$) 199.47, 171.23, 123.81, 88.88. IR (KBr) 1676, 1616 cm^{-1}. MS (CH_4) 329 (100%, M^{+1}), 271 (35%, M^{+1}-C_3H_6O). Anal. Calcd for $C_{22}H_{32}O_2$: C, 80.44; H, 9.82. Found: C, 80.73; H, 9.94.
15. **6**: ^{1}H NMR ($CDCl_3$) 6.08 (s, 1H), 3.48 (t, J = 8.3 Hz, 1H), 3.34-3.26 (m, 1H), 1.18 (s, 3H), 0.71 (s, 3H), 0.62-0.40 (m, 4H). ^{13}C NMR ($CDCl_3$) 201.80, 199.28, 160.72, 125.51, 88.50. IR ($CDCl_3$) 1687, 1604 cm^{-1}. CIMS (CH_4) 343 (100%, M^{+1}), 285 (25%, M^{+1}-C_3H_6O). Anal. Calcd for $C_{22}H_{32}O_2$: C, 77.16; H, 8.83. Found C, 77.36; H, 8.84.
16. Compound **8**, a potential metabolite of **11**, has previously been shown to lack androgenicity in an organ weight assay. See Johnston, J. O.; Wright, C. L.; Burkhart, J. P.; Peet, N. P. *J. Steroid Biochem. Molec. Biol.* **1993**, *44*, 623.
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18. **11**: ^{1}H NMR ($CDCl_3$) 5.52-5.45 (m, 1H), 4.26 (d, J = 11.6Hz, 1H), 3.96-3.84 (m, 1H), 3.77 (dd, J = 10.5, 2.4 Hz, 1H), 3.70 (d, J = 10.5 Hz, 1H), 3.51 (ddd, J = 11.6, 2.4, 1.0 Hz, 1H), 3.43 (t, J = 8.3 Hz, 1H), 3.33-3.26 (m, 1H), 3.00-2.86 (m, 1H), 2.53 (dd, J = 13.9, 6.9 Hz, 1H), 2.12 (dd, J = 12.8, 4.0 Hz, 1H), 2.12-1.90 (m, 3H), 1.84 (br s, 1H), 1.72-1.08 (series of m, 9H), 1.04-0.83 (m, 3H), 0.74 (s, 3H), 0.60-0.40 (m, 4H). ^{13}C NMR ($CDCl_3$) 139.93, 121.64, 89.02, 74.98, 71.79, 66.54, 52.33, 51.85, 47.72, 42.98, 42.13, 37.64, 36.99, 36.55, 36.09, 31.62, 31.27, 27.69, 23.08, 21.05, 11.36, 5.87, 5.64. HRMS Cacld for $C_{23}H_{36}O_3$ 359.2586, Found 359.2577.
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20. **15**: ^{1}H NMR ($CDCl_3$) 5.59-5.53 (m, 1H), 3.44 (t, J = 7.9 Hz, 1H), 3.35-3.18 (m, 2H), 2.18-1.87 (m, 3H), 1.80-1.02 (series of m, 13H), 1.15 (s, 3H), 1.10 (s, 3H), 1.07 (s, 3H), 0.98 (m, 2H), 0.76 (s, 3H), 0.61-0.40 (m, 4H). ^{13}C NMR ($CDCl_3$) 149.90, 119.87, 89.08, 77.46, 52.41, 51.08, 42.35, 41.61, 37.57, 36.79, 36.66, 32.17, 30.72, 28.00, 27.42, 27.22, 23.60, 23.30, 21.32, 20.22, 11.64, 6.16, 5.90. IR (KBr pellet) 3432, 1636 cm^{-1}. CIMS (CH_4) 359 (17%, MH^+), 341 (76%, MH-H_2O^+), 283 (base).
21. Unlabeled and radiolabeled 17α-hydroxypregnenolone dissolved in organic solvent are combined and evaporated to dryness under nitrogen gas and resuspended in DMSO and phosphate buffer. The DMSO contibuted to the assay from substrate is 2.5% (v/v). The total substrate concentration (17α-hydroxypregnenolone) was 0.8 μM for human lyase assays (= 1-2xK_m). Monkey microsomes were prepared in the same manner as human testicular tissue except that the monkey testicular tissue was previously frozen in liquid nitrogen after removal from anesthesized animals and stored at -80 °C. Assay components, procedures and analysis for monkey lyase were identical to that used for the human except that the total substrate (17α-hydroxypregnenolone) concentration was 0.05 μM (= K_m). Compounds to be screened for lyase inhibition were dissolved in DMSO and diluted in 0.05 M potassium phosphate buffer, pH 7.4, to give the desired concentrations of test compound; this solution contributes 0.1% (v/v) DMSO to the assay. Tests for time-dependent inhibition against lyase from either species were determined as follows. Microsomal protein (20-60 μg/mL), 0.05 M potassium phosphate buffer, phosphate buffer (pH 7.4), and the NADPH-regenerating system were preincubated at 34 °C for 5 minutes. The compound to be tested was next added, aliquots were removed at successive time intervals and added to 17α-hydroxypregnenolone substrate in separate tubes and assayed for remaining lyase activity for 6 minutes at 34 °C. The compound concentration used for testing against human testicular lyase was 0.8 μM. Concentrations of 1.0 and 0.1 μM were used to test for inhibition of cynomolgus lyase. Although the source of enzyme is from two different species, K_i values for other inhibitors were identical for human and cynomolgus testicular lyase.

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