Synthesis and Structure−Activity Profiles of A-Homoestranes, the Estratropones

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2-Methoxyestradiol, a mammalian metabolite of estradiol, has reported antiangiogenic activity which has been proposed to be mediated through interaction at the colchicine binding site on the tubulin monomer. Subsequent structure−activity studies of 2-methoxyestradiol have yielded highly potent steroidal inhibitors of tubulin polymerization. In an effort to probe the scope of binding at the colchicine binding site and the nature of the relationship between 2-methoxyestradiol and colchicine, a series of colchicine/2-methoxyestradiol hybrids was synthesized. These A-homoestrane hybrid systems, collectively termed estratropones, possessed an A-ring tropone system with the keto functionality at either the C-2, C-3, or C-4 position of the steroid nucleus. The estratropones were evaluated for their ability to inhibit the polymerization of tubulin using an *in vitro* purified bovine brain assay. Most of these hybrids inhibit polymerization with greater potency than either of the natural products. The most potent of these congeners possessed an approximate 5-fold enhancement of the activity of colchicine for the inhibition of tubulin polymerization. α-Substituents on the tropone ring showed varied effects on the activities for the two classes of estratropones studied in this regard, the C-3 oxo and the C-4 oxo species. The 3-substituted 4-oxoestratropones exhibited antitubulin activity according to Cl ≈ Br > OCH_3, whereas the 4-substituted 3-oxoestratropones exhibited activity according to OCH_3 > Br ≈ Cl. It is unclear if these substituent factors are purely electronic or steric effects or if the substituent operates indirectly by altering the conformation of the nonplanar troponoid ring. The estratropones represent a new class of tubulin binding agents with potential antiangiogenic utility.

Angiogenesis, the process of vascularization, has been implicated in a host of biological disorders including cancer, macular degeneration and arthritis.[1] Spawned by the therapeutic potential associated with the inhibition of pathological angiogenesis, a flurry of activity has led to the discovery of a variety of antiangiogenic compounds and to their clinical evaluation.[1] The potent antiangiogenic activity of 2-methoxyestradiol (2ME, Figure 1) was discovered in 1994 by Folkman *et al.*[2] Their studies provided evidence for antiangiogenic activity by the estrane steroid family and identified 2ME as the most potent endogenous inhibitor of mammalian tubulin polymerization yet discovered.[2] Additionally, it has been shown that 2ME exhibits *in vitro* both antimitotic properties and the reversible inhibition of cell proliferation without inducing cytotoxic responses in confluent cultures.[3] Preclinical and clinical trials have also shown 2ME to be promising in the treatment of several disorders associated with aberrant angiogenesis.[1−4]

2-Methoxyestradiol has been reported to exhibit antiangiogenic activity through the inhibition of tubulin polymerization by binding at the colchicine binding site.[2] In contrast to 2ME, colchicine (Figure 1) exhibits minimal selectivity, is highly cytotoxic, and, as a result, has limited clinical use due to its low therapeutic index.[5] Since the discovery of 2ME, SAR studies have yielded several 2-substituted estradiol derivatives that exhibit both greater affinity for the colchicine binding site and greater induction of cytotoxic responses from neoplastic cellular assays than the parent, 2ME.[6] While the full clinical potential of 2ME and these related compounds

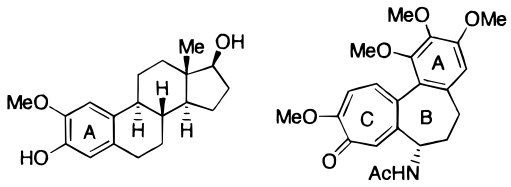

Figure 1. 2ME and colchicine.

continues to be investigated, the relationship between the observed antiangiogenic activity of 2ME and the ability of 2ME to bind to tubulin has not been defined. Motivated by the potent antiangiogenic activity of 2ME and by its ability to bind to tubulin, either of which may provide potential therapeutic benefits, our research program was directed toward the synthesis and characterization of 2ME derivatives to address critical issues of the biological activity of this estrane class. These molecules incorporate structural features of colchicine in an effort to explore the relationship between the antiangiogenic and tubulin binding activities and the scope of binding at the colchicine binding site. We disclose here the synthesis of A-homoestradiol derivatives, which we term "estratropones".

Design

As the result of preliminary structure−activity studies and spectroscopic evidence, the structural relationship between 2ME and colchicine (Figure 1) has been hypothesized independently by Folkman *et al.* and by Rava *et al.*[2,7] Their proposal suggests correspondence of 2ME's A ring and colchicine's C ring. This hypothesis provides plausible areas of molecular and functional analogy between 2ME and colchicine, thereby supplying

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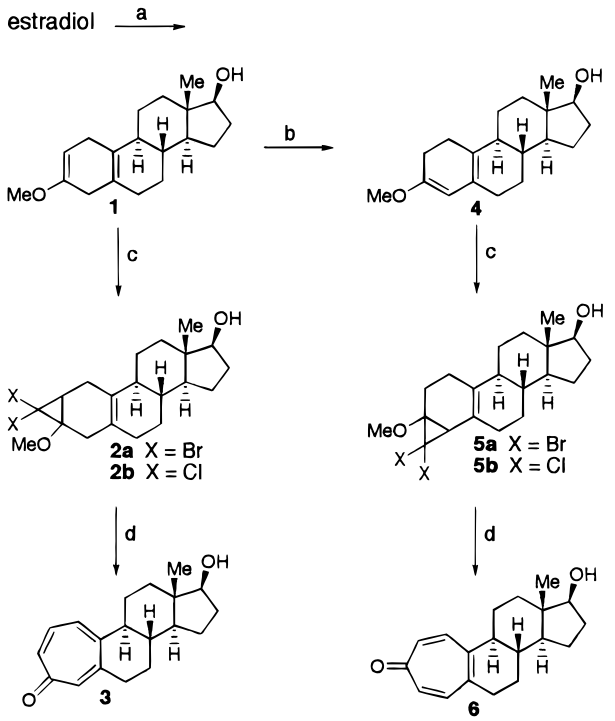
Scheme 1[a]



[a] (a) NaOH, Me_2SO_4 then NH_3, Li^0, EtOH; (b) ^{t}BuOK; (c) ^{t}BuOK, CHX_3; (d) $AgNO_3$.

the most reasonable regions for structure−activity exploration in the synthesis of 2ME/colchicine hybrid molecules. The positional and functional importance of the A, B, and C rings of colchicine has been extensively investigated and is well established. The influence of colchicine B-ring substituents has been reduced to steric accommodation in the colchicine binding site and is otherwise considered independent of ligand binding.[8]

As a starting point we have chosen to focus on modifications to the A ring of 2ME in an effort to explore the effect of colchicine's dominating methoxytropone functionality on tubulin binding and antiangiogenic activity in hybrid molecules. Placement of the α-substituted tropone functionality at a variety of positions about the steroidal A ring provides an array of diverse spatial probes for the preliminary investigation of the structural requirements for binding to the colchicine binding site.

Chemistry

The synthesis of estrone derived tropones has been previously reported.[9] Two classes of estratropones could be accessed through modification of this protocol (Scheme 1). Thus, selective methylation of estradiol followed by Birch reduction provided **1**. The conversion of **1** to **4** was realized by base-catalyzed isomerization. Subsequent exposure of the requisite dienes to dihalocarbene conditions afforded **2** and **5**. Treatment of the dibromocarbene monoadducts **2a** and **5a** with silver nitrate in aqueous acetone at reflux cleanly afforded **3** and **6**, respectively, in good overall yield.

A survey of the literature provided several general routes to the required α-substituted tropones; however, limiting our endeavors to the modification of readily available steroid nuclei, few synthetic alternatives proved viable.[10] The methods of Banwell et al.[10a] in the synthesis of α-methoxytropone derivatives of **13** (Scheme 2) failed to yield ring expanded products under a variety of conditions (Figure 2). The method of Macdonald[10b] proved efficient in the synthesis of α-chlorotropone **8b** (Scheme 2), but this method did not provide a general entry into the wide variety of α-substituted tropones required for the present study. Suitable chemodivergent routes were realized by the utilization of base-catalyzed ring expansion and oxidation of **2** and **5**. Accordingly, a wide variety of α-substituted tropones could be accessed from trienes **7** and **9** (Scheme 2).

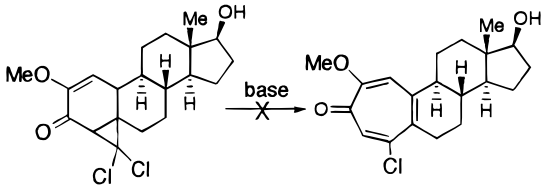


Figure 2. Chloro derivative of **13** (Scheme 2).

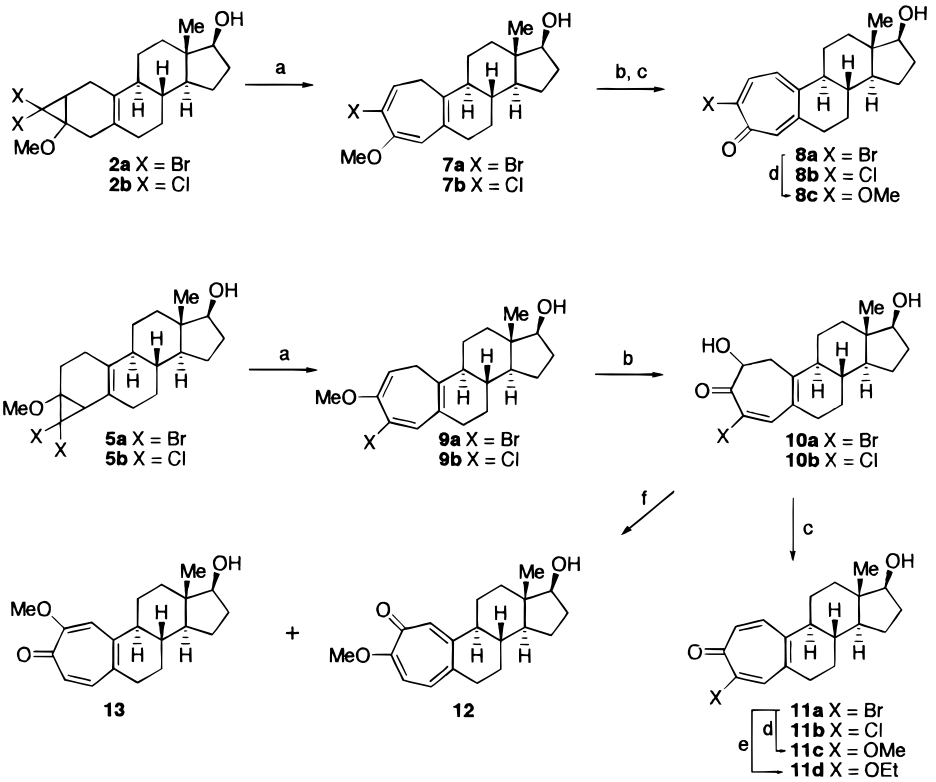
Thus, treatment of **2** and **5** with pyridine at reflux afforded the corresponding highly sensitive A-homodienes **7** and **9**. The failure of **7** and **9** to afford monooxidized products by treatment with mCPBA or MMPP[11] was surmounted by the use of dimethyldioxirane,[12] which afforded intermediate α-hydroxy dienones that underwent aromatization to **8** and **11**, respectively, on exposure to acid impregnated SiO_2 or prolonged standing at ambient temperature. The treatment of intermediate α-hydroxy dienone **10a** with silver nitrate in water at reflux, followed by exposure to (trimethylsilyl)diazomethane,[13] afforded **12** and **13** as an equimolar mixture of isomers separable by flash chromatography.

Numerous attempts to introduce alkoxy substituents via substitution of the α-halotropones **8a** and **11a** by the direct conjugate addition of the corresponding metalloalkoxides proved unfruitful. Under a variety of conditions these reactions afforded the corresponding ring contracted adducts as the sole isolable products.[10b,14] Conversion of α-halotropones **8a** and **11a** to the corresponding α-alkoxy derivatives, **8c**, **11c**, and **11d**, was eventually realized via a modification of the methods recently described by Fukuyama.[15]

Results and Discussion

The evaluation of the estratropones for their abilities to inhibit tubulin polymerization is summarized in Table 1. The least potent of the compounds studied, 3-methoxy-2-oxo-A-homo-1(10),3,4a-estratrien-17β-ol (**12**)- and 2-methoxy-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (**13**), had IC_{50} values greater than 25 μM, exhibiting 41% and 38% of the inhibition activity of colchicine, respectively. One congener (**11a**) was equipotent with colchicine, and the remaining eight displayed greater inhibitory activity than colchicine. Several of these colchicine−2ME hybrids displayed significantly greater activities in the inhibition of tubulin polymerization than either of the parent compounds. 4-Methoxy-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (**11c**) was the most potent (IC_{50} = 2.1 μM), possessing approximately 5-fold greater activity than colchicine for the inhibition of polymerization. Compounds **8a** and **8b** were also found to be highly potent (IC_{50} = 2.9 and 2.8 μM, respectively) for the inhibition of tubulin polymerization. The remaining compounds, **3**, **6**, **8c**, **11b**, and **11d**, displayed IC_{50} values ranging from 9.8 to 4.4 μM.

Scheme 2[a]



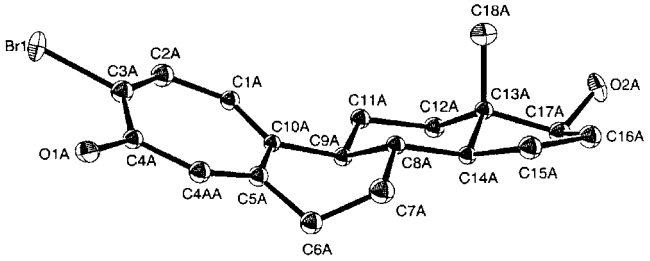
[a] (a) Pyridine, reflux; (b) dimethyldioxirane; (c) acid impregnated silica gel; (d) $Pd(PPh_3)_4$, THF, MeOH, K_2CO_3; (e) $Pd(PPh_3)_4$, THF, EtOH, K_2CO_3; (f) $AgNO_3$, H_2O, reflux then $TMSCHN_2$, MeOH, CH_2Cl_2.

Table 1. Effects of Estratropones on Tubulin Polymerization

compound	inhibn of tubulin polym IC_{50} (μM)	activity (IC_{50} colchicine/ IC_{50} drug)	R[a] C−2	C−3	C−4
colchicine	11.2 ± 5.0	1			
2ME	14.2 ± 1.2	0.79			
3	7.2 ± 1.7	1.5	H	H	O
6	4.4 ± 1.7	2.5	H	O	H
8a	2.9 ± 0.7	3.9	H	Br	O
8b	2.8 ± 0.9	4	H	Cl	O
8c	8.1 ± 4.1	1.4	H	OMe	O
11a	11.2 ± 5.5	1	H	O	Br
11b	9.8 ± 5.6	1.1	H	O	Cl
11c	2.1 ± 0.5	5.3	H	O	OMe
11d	5.9 ± 1.1	1.9	H	O	OEt
12	27.5 ± 5.2	0.41	O	OMe	H
13	29.8 ± 2.9	0.38	OMe	O	H

[a] Substituent at the stated position on the estratropone nucleus.

Interestingly, α-tropone substituent effects on activity show a reversal in the 3-oxo- and 4-oxoestratropones. For the series comprised of compounds **3** and **8**, α-substituents at C-3 attenuate activity with greater electron releasing potential and for the series comprised of **6** and **11,** α-substituents at C-4 attenuate activity with greater electron-withdrawing potential. It is unclear if these substituent factors are purely electronic or if the substituent operates "indirectly" by altering the conformation of the nonplanar troponoid ring. The extent of tropone ring pucker, illustrated in the X-ray crystal structure of **8a** (Figure 3), has been shown to correlate with the electronic contribution of α-substituents. Enhanced π-electron donation promotes a more planar tropone framework.[10] The results of previous investigations[6] and the minimal steric differences among the α-substituents of the estratropone systems suggest that the **3,8/6,11** activity differences are not due to the varying steric requirements of the α-substituent *per se*,



1 - 2 - 3 - 4	angle	1 - 2 - 3 - 4	angle
C1 - C2A - C3A - C4A	-4	C1A - C10A - C5A - C4AA	+2
C2A - C3A - C4A - C4AA	+10	C2A - C1A - C10A - C5A	-1
C3A - C4A - C4AA - C5A	-10	Br1 - C3A - C4A - O1A	+5
C4A - C4AA - C5A - C10A	+3		

Figure 3. Structure of **8a** as determined by single-crystal X-ray diffraction. Hydrogen atoms have been omitted for clarity. Torsional angles (deg) have been provided to display the extent of tropone ring derivation from planarity.

but rather is due to the structural requirements of the tropone ring as a whole.

The three most potent inhibitors of tubulin polymerization, **8a**, **8b**, and **11c**, were also assayed for the competitive inhibition of colchicine binding to tubulin. Compound **8a** displayed only 22.7% (±1%) inhibition of colchicine binding to tubulin; however, **11c** provided 87.8% (±6%) inhibition. Interestingly, **8b** provided an exceptional 377% (±12%) inhibition of colchicine binding to tubulin. In this assay 2ME displayed 84.6% (±1%) inhibition of colchicine binding, which is in accord with previous studies.[2] These data are consistent with the proposal that the estratropones, like 2ME, bind to the colchicine binding site on tubulin.

In conclusion, the synthesis of colchicine−2ME hybrids, termed the estratropones, has been disclosed. Many of these hybrids exhibit greater potency for the *in vitro* inhibition of bovine brain tubulin polymerization

than either of the parent compounds, colchicine and 2ME. Several of the most active leads are under preclinical investigation *in vitro* and *in vivo* as agents for the treatment of disorders associated with abnormal angiogenesis. Continuing investigations of these and other α-substituted estratropones are underway. Progress directed toward a greater understanding of these novel substrates, their antiangiogenic activity, and the scope of binding for this new class of tubulin binders will be reported in due course.

Experimental Section

All reactions were carried out under argon with magnetic stirring unless otherwise noted. All solvents were distilled from appropriate desiccant under nitrogen immediately prior to use in reactions unless otherwise noted. All nuclear magnetic resonance spectra were obtained with a General Electric QE300 spectrometer at 300 MHz, and chemical shifts are reported in ppm. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Melting points were determined using a Thomas-Hoover Uni-Melt melting point apparatus and are uncorrected. Analytical thin-layer chromatography was performed on Merck silica gel 60 F-254 precoated plates (aluminum), and visualizations were affected with phosphomolybdic acid in ethanol. Radial chromatography was executed on a Chromatotron 7924 Harrison Research system. Column chromatography was performed on E. Merck silica 60 (230−400 mesh).

3-Methoxyestra-2,5(10)-dien-17*β*-ol (1). To a solution of estradiol (10.0 g, 36.7 mmol) in ethanol (100 mL) at reflux was added NaOH (100 mmol) in water (10 mL) followed by the dropwise addition of Me_2SO_4 (100 mmol). Concentration of the mixture followed by filtration through silica gel with ethyl ether afforded 3-methoxy-1,3,5(10)-estratrien-17*β*-ol as white needles. Dissolving metal reduction of 3-methoxy-1,3,5(10)-estratrien-17*β*-ol was carried out via the methods described by Wilds and Nelson, affording the known dihydroestradiene derivative as a white solid (86% overall yield).[16]

Dibromocarbene Monoadducts of 3-Methoxyestra-2,5-(10)-dien-17*β*-ol (2a). 3-Methoxyestra-2,5(10)-dien-17*β*-ol (**1**) (1.0 g, 3.5 mmol) and ^{t}BuOK (1.17 g, 10.4 mmol) were suspended in anhydrous ethyl ether (30 mL) at −30 °C followed by the dropwise addition of bromoform (1.3 mL, 14.9 mmol) in ethyl ether (30 mL) over 1 h. After an additional 2 h of stirring, the mixture was filtered through silica gel with ethyl ether and the filtrate concentrated. Flash chromatography of the residue eluting with 50% ethyl ether/petroleum ether yielded monoadducts **2a** as an equimolar mixture of diastereomers (white foam, R_f =0.26, 0.82 g, 51%): ^{1}H NMR ($CDCl_3$) δ 3.61 (t, J = 8.1 Hz, 1H), 3.464 (s, 1.5H), 3.460 (s, 1.5H), 2.7−2.15 (m, 4H), 2.1−0.95 (complex, 17H), 0.704 (s, 1.5H), 0.688 (s, 1.5H); ^{13}C NMR ($CDCl_3$) δ 126.68, 126.28, 124.72, 124.58, 82.18, 81.89, 64.57, 64.11, 54.73, 54.67, 51.65, 51.09, 50.36, 50.26, 46.18, 45.77, 43.78, 43.71, 39.69, 39.47, 37.55, 37.45, 34.78, 34.36, 32.10, 31.84, 31.58, 31.32, 30.99, 30.91, 27.10, 27.04, 26.31, 25.87, 25.73, 25.61, 23.49, 23.49, 11.79, 11.79.

Dichlorocarbene Monoadducts of 3-Methoxyestra-2,5-(10)-dien-17*β*-ol (2b). 3-Methoxyestra-2,5(10)-dien-17*β*-ol (**1**) (1.2 g, 4.2 mmol) and ^{t}BuOK (95%, 1.49 g, 12.6 mmol) were suspended in anhydrous ethyl ether (36 mL) at −40 °C followed by the dropwise addition of chloroform (1.0 mL, 12.5 mmol) in ethyl ether (36 mL) over 1 h. After an additional 3 h of stirring, the mixture was filtered through silica gel with ethyl ether and the filtrate concentrated. Flash chromatography of the residue eluting with 50% ethyl ether/petroleum ether yielded monoadducts **2b** as an equimolar mixture of diastereomers (white powder, R_f = 0.24, mp 142−146 °C, 0.70 g, 45%): ^{1}H NMR ($CDCl_3$) δ 3.67 (t, J = 8.09 Hz, 1H), 3.488 (s, 1.5H), 3.485 (s, 1.5H), 2.65−2.21 (m, 4H), 2.05−0.95 (complex, 17H), 0.677 (s, 1.5H), 0.665 (s, 1.5H); ^{13}C NMR ($CDCl_3$) δ 136.38, 136.38, 124.92, 124.92, 82.28, 81.98, 55.14, 55.10, 51.22, 50.39, 50.31, 50.13, 47.42, 46.93, 39.87, 39.69, 39.64, 39.53, 39.42, 39.07, 37.51, 37.45, 36.47, 36.44, 31.11, 31.07, 30.86, 30.25, 27.07, 26.61, 25.43, 24.60, 24.21, 23.49, 23.13, 22.95, 11.81, 11.75.

4-Oxo-A-homo-1(10),2,4a-estratrien-17*β*-ol (3). Monoadduct **2a** (240 mg, 0.52 mmol) was dissolved in acetone (2 mL), and water (20 mL) was added, followed by the addition of silver nitrate (0.50 g, 3.0 mmol). Reflux was initiated and after 1 h, the reaction was cooled, and the mixture was concentrated, diluted with ethyl acetate, chromatographically filtered, and concentrated. Radial chromatography eluting with 15% acetone in chloroform and recrystallization from ethyl acetate of the residue yielded **3** (white needles, R_f = 0.27, mp 178−180 °C, 140 mg, 91%): ^{1}H NMR ($CDCl_3$) δ 7.114 (d, J = 9.24 Hz, 1H), 7.075 (d, J = 8.86 Hz, 1H), 7.004 (s, 1H), 6.896 (t, J = 8.84 Hz, 1H), 3.723 (t, J = 8.09 Hz, 1H), 2.808−2.695 (m, 1H), 2.510 (ddd, J = 15.02, 3.08, 3.08, 1H), 2.23−1.17 (complex, 14H), 0.761 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 187.89, 153.00, 151.25, 140.26, 139.58, 136.39, 128.00, 81.83, 52.40, 44.88, 43.74, 36.57, 36.00, 33.63, 30.93, 25.57, 25.37, 23.82, 11.50. Anal. ($C_{19}H_{24}O_2$) C, H.

3-Methoxyestra-3,5(10)-dien-17*β*-ol (4). 3-Methoxyestra-2,5(10)-dien-17*β*-ol (**1**) (2.0 g, 6.9 mmol) was added to a solution consisting of tetrahydofuran (15 mL), DMSO (5 mL), and ^{t}-BuOK (2.3 g, 20.7 mmol). The resultant solution was stirred at ambient temperature for 10 h and then concentrated. Flash chromatography eluting with 50% ethyl ether/petroleum ether afforded **4** (white powder, R_f = 0.33, mp 97−100 °C, 1.30 g, 65% conversion, 91% based on recovered **1**). Alternatively, crystallization by selective seeding (ether/methanol) was utilized, thereby circumventing chromatographic purification: ^{1}H NMR (acetone-d_6) δ 4.629 (s, 1H), 3.505 (t, J = 8.09 Hz, 1H), 3.431 (s, 3H), 2.15−0.98 (complex, 20H), 0.644 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 156.71, 126.56, 123.88, 96.55, 81.31, 54.19, 50.18, 46.03, 39.91, 37.54, 30.64, 30.58, 30.21, 27.71, 27.17, 25.67, 25.24, 23.22, 11.37.

Dibromocarbene Monoadducts of 3-Methoxyestra-3,5-(10)-dien-17*β*-ol (5a). 3-Methoxyestra-3,5(10)-dien-17*β*-ol (**4**) (2.6 g, 9.0 mmol) and ^{t}BuOK (3.0 g, 27 mmol) were suspended in anhydrous ethyl ether (30 mL) at −40 °C followed by the dropwise addition of bromoform (5.2 mL, 59.5 mmol) via syringe over 1 h. After an additional 3 h of stirring, the mixture was filtered through silica gel with ethyl ether and the filtrate concentrated. Flash chromatography of the residue eluting with 50% ethyl ether/petroleum ether yielded monoadducts **5a** as an equimolar mixture of diastereomers (white foam, R_f = 0.24, 2.65 g, 64%): ^{1}H NMR ($CDCl_3$) δ 3.61 (t, J = 8.08 Hz, 1H), 3.488 (s, 1.5H), 3.482 (s, 1.5H), 2.8−1.05 (complex, 21H), 0.761 (s, 1.5H), 0.748 (s, 1.5H).

Dichlorocarbene Monoadducts of 3-Methoxyestra-3,5-(10)-dien-17*β*-ol (5b). 3-Methoxyestra-3,5(10)-dien-17*β*-ol (**4**) (2.0 g, 6.9 mmol) and ^{t}BuOK (2.3 g, 20.8 mmol) were suspended in anhydrous ethyl ether (30 mL) at −30 °C followed by the dropwise addition of chloroform (1.66 mL, 20.8 mmol) via syringe over 1 h. After an additional 3 h of stirring, the mixture was filtered through silica gel with ethyl ether and the filtrate concentrated. Flash chromatography of the residue eluting with 50% ethyl ether/petroleum ether yielded monoadducts **5b** as an equimolar mixture of diastereomers (white foam, R_f = 0.26, 2.65 g, 64%): ^{1}H NMR ($CDCl_3$) δ 3.63 (t, J = 7.70 Hz, 1H), 3.467 (s, 1.5H), 3.463 (s, 1.5H), 2.8−1.00 (complex, 21H), 0.752 (s, 1.5H), 0.740 (s, 1.5H).

3-Oxo-A-homo-1,4,5(10)-estratrien-17*β*-ol (6). The monoadduct **5a** (100 mg, 0.22 mmol) was dissolved in acetone (1 mL), and water (10 mL) was added, followed by the addition of silver nitrate (170 mg, 1.0 mmol). Reflux was initiated and after 0.5 h, the reaction was cooled, the mixture was concentrated, diluted with ethyl acetate, and filtered through silica gel, and the filtrate was concentrated. Flash chromatography of the resultant residue eluting with 15% acetone in chloroform followed by recrystalization from ethyl acetate yielded **6** (white needles, R_f = 0.28, mp 201−202 °C, 51 mg, 82%): ^{1}H NMR ($CDCl_3$) δ 7.268 (d, J = 13.09 Hz, 1H), 6.921 (d, J = 12.70 Hz, 1H), 6.882 (d, J = 13.09 Hz, 1H), 6.874 (d, J = 12.71 Hz, 1H), 3.728 (t, J = 8.08 Hz, 1H), 2.90−2.53 (m, 2H), 2.28−1.18 (complex, 14H), 0.785 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 187.68, 146.44, 143.87, 142.33, 138.66, 138.33, 138.12, 82.01, 50.52,

48.00, 43.74, 38.36, 37.53, 34.94, 31.13, 27.66, 26.32, 23.49, 11.71. Anal. ($C_{19}H_{24}O_2$) C, H.

General Procedure A: Pyridine-Induced Ring Expansion. The requisite dihalocarbene monoadduct (1 g) was dissolved in pyridine (10 mL), and reflux was initiated and held for 5 h. After cooling, the mixture was filtered through silica gel with ethyl ether and the filtrate concentrated under reduced pressure. The resultant gum was taken up in dichloromethane and flash chromatographed eluting with 30% ethyl ether/petroleum ether to afford the corresponding triene.

General Procedure B: Epoxidation with Dimethyldioxirane. The requisite halomethoxy triene (0.25 g) was dissolved in 80% aqueous acetone (20 mL) and cooled to 0 °C in an open vessel. After portionwise addition of a freshly prepared solution of dimethyldioxirane[17] in acetone (0.1 M, 2.2 equiv), the mixture was allowed to warm to room temperature and monitored by TLC. Upon completion, solvents were removed under reduced pressure. Flash chromatography of the residue eluting with 50% ethyl ether/petroleum ether provided the requisite α-hydroxy dienone. Alternatively, flash chromatography (SiO_2 acid impregnated with acidic bromoform) of the residue eluting with 15% acetone in chloroform provided the requisite halotropone. Crystalline solids were obtained by recrystallization from ethyl acetate.

General Procedure C: Pd(PPh_3)$_4$-Mediated Alkoxylation. The requisite halotropone (10 mg) was dissolved in THF/alcohol (1:1, 5 mL), and Pd(PPh_3)$_4$ (2 equiv) was added under Ar(g). Initiation of reflux was followed by the addition of K_2CO_3 (2 equiv) in four equal portions over 1 h. After an additional hour at reflux, the mixture was diluted with 30% methanolic chloroform and filtered through silica gel and the filtrate concentrated under reduced pressure. Radial chromatography of the residue eluting with 30% acetone in chloroform afforded the corresponding α-alkoxytropone. Crystalline solids were obtained by recrystallization from ethyl acetate.

3-Bromo-4-methoxy-A-homo-2,4,5(10)-estratrien-17β-ol (7a) and 3-Chloro-4-methoxy-A-homo-2,4,5(10)-estratrien-17β-ol (7b). The title compounds **7a** (white foam, 65% yield, R_f = 0.26 with 50% ethyl ether/petroleum ether) and **7b** (oil, R_f = 0.27 with 50% ethyl ether/petroleum ether, 67% yield) were prepared from **2a** and **2b**, respectively, as described in general procedure A: ^{1}H NMR **7a** ($CDCl_3$) δ 5.84 (t, J = 8.09 Hz, 1H), 5.78 (s, 1H), 3.56 (s, 3H), 3.52 (t, J = 8.1 Hz, 1H), 2.665 (d, J = 12.7 Hz, 1H), 2.615 (d, J = 12.3 Hz, 1H), 2.2−0.95 (complex, 16H), 0.631 (s, 3H); ^{13}C NMR **7a** ($CDCl_3$) δ 153.97, 129.95, 128.75, 126.27, 114.43, 110.64, 81.21, 55.46, 49.95, 47.42, 44.04, 40.26, 37.77, 31.11, 30.78, 30.71, 28.80, 27.06, 23.27, 11.59; ^{1}H NMR **7b** ($CDCl_3$) δ 5.78 (s, 1H), 5.66 (t, J = 8.08 Hz, 1H), 3.57 (s, 3H), 3.52 (t, J = 7.3 Hz, 1H), 2.648 (d, J = 12.7 Hz, 1H), 2.620 (d, J = 13.09 Hz, 1H), 2.4−0.95 (complex, 16H), 0.634 (s, 3H).

3-Bromo-4-oxo-A-homo-1(10),2,4a-estratrien-17β-ol (8a) and 3-Chloro-4-oxo-A-homo-1(10),2,4a-estratrien-17β-ol (8b). The title compounds **8a** (crystalline needles, R_f = 0.64 with 30% acetone in chloroform, mp 132−143 °C dec, 53%) and **8b** (crystalline needles, R_f = 0.63 with 30% acetone in chloroform, mp 125−220 °C dec, 46%) were prepared from **7a** and **7b**, respectively, as described in general procedure **B**: ^{1}H NMR **8a** ($CDCl_3$) δ 7.98 (d, J = 10.01 Hz, 1H), 7.149 (s, 1H), 6.72 (d, J = 10.01 Hz, 1H), 3.69 (t, J = 8.08 Hz, 1H), 2.77 (dt, J = 15 Hz, J = 8.2 Hz, 1H), 2.55 (dd, J = 15 Hz, J = 5.0 Hz, 1H), 2.25−1.95 (m, 4H), 1.9−1.25 (complex, 10H), 0.754 (s, 3H); ^{13}C NMR **8a** ($CDCl_3$) δ 180.39, 152.90, 150.49, 139.78, 139.15, 136.36, 125.79, 81.82, 52.30, 44.92, 43.68, 36.50, 36.05, 33.54, 30.93, 25.54, 25.38, 23.79, 11.48. Anal. **8a** ($C_{19}H_{23}BrO_2$) C, H. ^{1}H NMR **8b** ($CDCl_3$) δ 7.64 (d, J = 11.24 Hz, 1H), 7.130 (s, 1H), 6.78 (d, J = 11.24 Hz, 1H), 3.678 (t, J = 9.26 Hz, 1H), 2.73 (dt, J = 14 Hz, J = 8.0 Hz, 1H), 2.51 (ddd, J = 15 Hz, J = 6.94 Hz, J = 4 Hz,1H), 2.24−1.90 (m, 4H), 1.8−1.15 (complex, 10H), 0.710 (s, 3H); ^{13}C NMR **8b** ($CDCl_3$) δ 179.32, 152.06, 150.35, 145.03, 136.89, 134.99, 120.00, 81.2, 51.78, 44.34, 43.61, 35.00, 35.57, 33.12, 30.38, 25.06, 24.80, 23.26, 10.95. Anal. **8b** ($C_{19}H_{23}ClO_2$) C, H.

3-Methoxy-4-oxo-A-homo-1(10),2,4a-estratrien-17β-ol (8c). The title compound **8c** was prepared from **8a** as described in general procedure C (crystalline needles, R_f = 0.23, mp 219−221 °C, 77%): ^{1}H NMR **8c** ($CDCl_3$) δ 7.233 (s, 1H), 6.99 (d, J = 10.8 Hz, 1H), 6.69 (d, J = 10.4 Hz, 1H), 3.91 (s, 3H), 3.73 (t, J = 8.47 Hz, 1H), 2.83 (m, 1H), 2.61 (m, 1H), 2.3−1.2 (complex, 14H), 0.768 (s, 3H). Anal. ($C_{20}H_{26}O_3 \cdot {}^1/_2H_2O$) C, H.

4-Bromo-3-methoxy-A-homo-2,4,5(10)-estratrien-17β-ol (9a) and 4-Chloro-3-methoxy-A-homo-2,4,5(10)-estratrien-17β-ol (9b). Title compounds **9a** (foam, R_f = 0.23 with 50% ethyl ether/petroleum ether, 68%) and **9b** (foam, R_f = 0.22 with 50% ethyl ether/petroleum ether, 60% yield) were prepared from **5a** and **5b**, respectively, as described in general procedure A: ^{1}H NMR **9a** ($CDCl_3$) δ 6.89 (s, 1H), 4.70 (t, J = 8.1 Hz, 1H), 3.57 (t, J = 8.1 Hz, 1H), 3.56 (s, 3H), 2.5−1.0 (complex, 18H), 0.861 (s, 3H); ^{1}H NMR **9b** ($CDCl_3$) δ 6.58 (s, 1H), 4.79 (t, J = 8.1 Hz, 1H), 3.53 (t, J = 7.7 Hz, 1H), 3.47 (s, 3H), 2.6−0.95 (complex, 18H), 0.634 (s, 3H).

4-Bromo-3-oxo-A-homo-4,5(10)-estradien-2,17β-diol (10a) and 4-Chloro-3-oxo-A-homo-4,5(10)-estradien-2,17β-diol (10b). The title compounds **10a** (clear oil, R_f = 0.5 with ethyl ether, 62%) and **10b** (white foam, R_f = 0.57 with ethyl ether, 45%) were prepared from **9a** and **9b**, respectively, as described in general procedure B: ^{1}H NMR **10a** ($CDCl_3$) δ 7.35 (s, 1H), 4.02 (d, J = 13.9 Hz, 1H), 3.71 (t, J = 8.08 Hz, 1H), 2.65 (d, J = 15 Hz, 1H), 2.43 (t, J = 14 Hz, 1H), 2.3−1.05 (complex, 17H), 0.807 (s, 3H); ^{13}C NMR **10a** ($CDCl_3$) δ 195.0, 149.4, 147.8, 131.9, 120.7, 82.1, 77.7, 74.5, 50.2, 48.3, 43.9, 39.3, 37.6, 32.6, 31.2, 27.3, 26.8, 23.4, 11.8; ^{1}H NMR **10b** ($CDCl_3$) δ 7.08 (s, 1H), 4.60 (dd, J = 8.5 Hz, J = 2.3 Hz, 1H), 3.66 (t, J = 8.47 Hz, 1H), 2.86 (dd, J = 14.3 Hz, J = 8.9 Hz, 2H), 2.2−0.95 (complex, 17H), 0.802 (s, 3H).

4-Bromo-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (11a) and 4-Chloro-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (11b). The title compounds **11a** (oil, R_f = 0.40, 54% yield) and **11b** (needles, mp 140−240+ °C dec, R_f = 0.48, 57% yield) were prepared from **9a** and **9b**, respectively, as described in general procedure B: ^{1}H NMR **11a** ($CDCl_3$) δ 7.95 (s, 1H), 7.30 (d, J = 13 Hz, 1H), 7.01 (d, J = 13 Hz, 1H), 3.74 (t, J = 8.86 Hz, 1H), 2.85−2.70 (m, 2H), 2.25 (dq, J = 13 Hz, J = 3.5 Hz, 1H), 2.19−2.09 (m, 2H), 1.98 (dt, J = 12.3 Hz, J = 3.1 Hz, 1H), 1.9−1.8 (m, 1H), 1.7 (dq, J = 10 Hz, J = 2 Hz, 1H), 1.6−1.15 (complex, 8H), 0.794 (s, 3H). Anal. **11a** ($C_{19}H_{23}BrO_2$) C, H. ^{1}H NMR **11b** ($CDCl_3$) δ 7.64 (s, 1H), 7.34 (d, J = 13.2 Hz, 1H), 7.04 (d, J = 13 Hz, 1H), 3.74 (t, J = 8.10 Hz, 1H), 2.9−2.7 (m, 2H), 1.6−1.15 (complex, 14H), 0.789 (s, 3H). Anal. **11b** ($C_{19}H_{23}ClO_2$) C, H.

4-Methoxy-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (11c) and 4-Ethoxy-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (11d). The title compounds **11c** (crystalline needles, mp 173−175 °C, R_f = 0.23, 77% yield) and **11d** (crystalline needles, mp 177−179 °C, R_f = 0.25, 71% yield) were prepared from **11a** as described in general procedure C: ^{1}H NMR **11c** ($CDCl_3$) δ 7.233 (s, 1H), 6.99 (d, J = 10.8 Hz, 1H), 6.69 (d, J = 10.4 Hz, 1H), 3.91 (s, 3H), 3.73 (t, J = 8.47 Hz, 1H), 2.83 (m, 1H), 2.61 (m, 1H), 2.3−1.2 (complex, 14H), 0.768 (s, 3H). Anal. **11c** ($C_{20}H_{26}O_3$) C, H. ^{1}H NMR **11d** ($CDCl_3$) δ 7.223 (s, 1H), 6.92 (d, J = 10.8 Hz, 1H), 6.69 (d, J = 10.8 Hz, 1H), 4.12 (q, J = 6.93 Hz, 2H), 3.73 (t, J = 8.5 Hz, 1H), 2.9−2.5 (m, 2H), 2.3−1.2 (complex, 17H), 0.772 (s, 3H). Anal. **11d** ($C_{21}H_{28}O_3$) C, H.

3-Methoxy-2-oxo-A-homo-1(10),3,4a-estratrien-17β-ol (12) and 2-Methoxy-3-oxo-A-homo-1,4,5(10)-estratrien-17β-ol (13). Hydroxy dienone **10a** (20 mg, 0.05 mmol) was dissolved in acetone (1 mL), and water (10 mL) was added, followed by the addition of silver nitrate (42 mg, 0.25 mmol). Reflux was initiated and held for 5 h. After cooling, the mixture was concentrated, diluted with 15% acetone in chloroform, chromatographically filtered, and concentrated to 5 mL. The resultant mixture was treated with $TMSCHN_2$ (0.25 mmol, 2 M in hexane), and after 20 min it was concentrated. Flash chromatography of the residue eluting with 30% acetone in chloroform yielded **12** (white solid, mp 145−168 °C dec, R_f = 0.20, 42%) and **13** (white solid, mp 99−104 °C dec, R_f = 0.17, 44%): ^{1}H NMR **12** ($CDCl_3$) δ 7.05 (m, 2H), 6.95 (s, 1H), 3.92 (s, 3H), 3.76 (t, J = 8.8 Hz, 1H), 2.90−2.65 (m, 2H), 2.33−2.1 (m, 2H), 2.01 (dt, J = 12.3 Hz, J = 3.5

Hz, 1H), 1.98−1.81 (m, 1H), 1.75−1.2 (complex, 10H), 0.812 (s, 3H). Anal. **12** ($C_{20}H_{26}O_3$) C, H. ^{1}H NMR **13** ($CDCl_3$) δ 7.32 (s, 1H), 6.88 (d, J = 10.4 Hz, 1H), 6.60 (d, J = 10.4 Hz, 1H), 3.91 (s, 3H), 3.73 (t, J = 8.1 Hz, 1H), 2.90−2.5 (m, 2H), 2.3−1.95 (m, 4H), 1.8−1.2 (complex, 10H), 0.761 (s, 3H). Anal. **13** ($C_{20}H_{26}O_3$) C, H.

Materials for Tubulin Bioassay. Bovine brain tubulin was purified through two cycles of polymerization/depolymerization in a manner similar to that described by Williams and Lee.[18] [^{3}H]Colchicine was obtained from Dupont/New England Nuclear, nonradiolabeled colchicine from Sigma, and DEAE (DE81) filter paper disks from Whatman.

Tubulin Polymerization Assay. Tubulin polymerization was performed with varying drug concentrations, with at least three determinations at each concentration, in PM buffer (100 mM PIPES, 1.0 mM $MgSO_4$, 2.0 mM EGTA, pH 6.9), with a final tubulin concentration of 1.2 mg/mL (12 μM). Drug stock solutions were in DMSO, and the final DMSO concentration was 8% (v/v). GTP was added to a final concentration of 1 mM. Polymerization was monitored by the increase in solution turbidity at 351 nm for 10 min in a thermostatically controlled cuvette holder at 30 °C using a Perkin-Elmer Lambda 20 UV−vis spectrophotometer. Both initial and final A_{351} values were recorded, and the difference between the two measurements was plotted against drug concentrations to obtain IC_{50} values.

Colchicine Binding Assay. The binding of radiolabeled colchicine to tubulin was measured by a DEAE-cellulose filter technique, and the results were determined in triplicate. The 0.050 mL reaction mixtures contained tubulin (0.3 mg, 3.0 μM), PM buffer (100 mM PIPES, 1.0 mM $MgSO_4$, 2.0 mM EGTA, pH 6.9), 0.1 mM GTP, [^{3}H]colchcine in DMSO (0−16 μM), and/or drug in DMSO (0−16 μM) with a combined concentration of 16 μM and a final DMSO concentration of 8% (v/v). Each reaction was incubated at 37 °C for 15 min, after which time it was spotted onto a DEAE-cellulose paper filter disk and allowed to dry. Disks were washed in 0.05 M NaCl in PM buffer with gentle agitation for 10 min and then were allowed to dry. Each filter was allowed to soak in scintillation fluid overnight, and radioactivity was quantitated by LSC. Percent inhibition of [^{3}H]colchicine binding was determined using a Dixon type plot.

X-ray Crystallography of 8a. Crystal data: colorless needle, 0.26 × 0.24 × 0.46 mm, monoclinic, $P2_1$ (No. 4); a = 9.192(3) Å, b = 13.546(4) Å, c = 13.111(5) Å; T = −120 °C, V = 1642(2) Å^3, Z = 4; FW = 364.30, d_{calc} = 1.47 g/cm^3, μ(Mo Kα) = 24.82 cm^{-1}.

Diffraction data were collected on a Rigaku AFC6S diffractometer using Mo Kα radiation (λ = 0.710 69). All calculations were performed on a VAX station 3520 computer employing the TEXSAN 5.0 crystallographic software package (1989, Molecular Structure Corp., The Woodlands, TX 77381) and in the later stages on a Silicon Graphics Indigo Extreme computer with the teXsan 1.7 package (1995, Molecular Structure Corp., The Woodlands, TX 77381). Unit cell dimensions were determined by least-squares refinement of the setting angles of 25 high-angle reflections. Three standard reflections were monitored during the data collection showing no significant variance. The intensities were corrected for absorption by applying ψ scans of several reflections. The transmission factors ranged from 0.77 to 1.00.

The structure was solved by direct methods in SIR88. Full-matrix least-squares refinement with anisotropic displacement parameters for the Br, O, and methyl C atoms yielded the final R of 0.042 (R_W = 0.051). All hydrogen atoms were found in difference Fourier maps and included in calculations without further refinement. The final difference map was essentially featureless with the highest peak of 0.47 e/Å^3.

Supporting Information Available: X-ray crystallographic data (10 pages); structure factors (11 pages). Ordering information is given on any current masthead page.

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