Synthesis of Analogs of 2-Methoxyestradiol with Enhanced Inhibitory Effects on Tubulin Polymerization and Cancer Cell Growth

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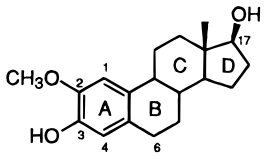
A new series of estradiol analogs was synthesized in an attempt to improve on the anticancer activity of 2-methoxyestradiol, a naturally occurring mammalian tubulin polymerization inhibitor. The compounds were evaluated as inhibitors of tubulin polymerization and the binding of [^{3}H]colchicine to tubulin, as well as for *in vitro* cytotoxicity in human cancer cell cultures. Overall, the most potent of the new compounds were 2-(2′,2′,2′-trifluoroethoxy)-6-oximinoestradiol, 2-ethoxy-6-oximinoestradiol, and 2-ethoxy-6-methoximinoestradiol. These agents lacked significant affinity for the estrogen receptor. The cytotoxicities of the compounds correlated in general with their abilities to inhibit tubulin polymerization, thus supporting inhibition of tubulin polymerization as the primary mechanism causing inhibition of cell growth.

In mammals, hepatic hydroxylation of estradiol followed by *O*-methylation results in the formation of 2-methoxyestradiol **(1)**, which has very low affinity for the estrogen receptor.[1,2] General interest in 2-methoxyestradiol **(1)** has been stimulated by its cytotoxicity in cancer cell cultures, which is associated with inhibition of mitosis, uneven chromosome distribution, and an increase in the number of abnormal metaphases.[3,4] The binding of 2-methoxyestradiol **(1)** to the colchicine binding site of tubulin results in either inhibition of tubulin polymerization or formation of polymer with altered morphology and stability properties, depending on reaction conditions.[5,6] 2-Methoxyestradiol **(1)** or an unknown related compound might therefore be functioning as a natural regulator of mammalian microtubule assembly and function. Recent *in vitro* and *in vivo* results have demonstrated that 2-methoxyestradiol **(1)** also inhibits angiogenesis (the formation of new blood vessels),[7,8] required for the growth of solid tumors.[9,10] After oral administration to mice, 2-methoxyestradiol **(1)** acts as a potent inhibitor of neovascularization of solid tumors and inhibits their growth at doses which produce no apparent signs of toxicity.[7,8]



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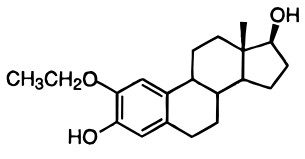

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In an effort to investigate the structural parameters associated with the biological activities of 2-methoxyestradiol **(1)** and to exploit it as a lead compound for the design of more potent anticancer agents, we recently synthesized **1** and a series of structurally related compounds.[4,11] The most potent of these new analogs proved to be 2-ethoxyestradiol **(2)**, which was found to be more potent than **1** as a cytotoxic agent in cancer cell cultures as well as a tubulin polymerization inhibitor.[4] The present investigation was undertaken in order to further maximize the anticancer and antitubulin activities of compounds related structurally to 2-methoxyestradiol and to obtain active analogs with enhanced metabolic stabilities. As described below, this effort resulted in additional potent inhibitors of cancer cell growth, and these active analogs, like **1**, interact negligibly with the estrogen receptor.

Chemistry

Treatment of the protected 2-iodoestradiol derivative **3**[12] with triethylamine and $Pd(PPh_3)_4$ in THF at room temperature, followed by propyne and CuI, gave the desired intermediate **4** (Scheme 1).[13,14] Deprotection of intermediate **4** with tetra-*n*-butylammonium fluoride in THF at room temperature afforded the diol **5** as a minor product in 18% yield along with the partially deprotected compound **6** as the major product in 38% yield. Further treatment of **6** with tetra-*n*-butylammonium fluoride in refluxing THF resulted in the formation of compound **7**, having a fused furan ring, as indicated by the disappearance of the acetylene absorption at 2361 cm^{-1} in the IR spectrum of **6** and the appearance of an additional aromatic broad singlet assigned to the furan ring at δ 6.27 in the ^{1}H NMR spectrum of **7**.[15,16] The conversion of **6** to **7** most likely involves deprotonation of the phenolic hydroxyl group to generate the corresponding phenoxide anion, followed by 5-*endo-dig* ring closure.[17] 2-Methylbenzofuran itself has been prepared in a related process involving the reaction of 1-(*o*-methoxyphenyl)-1-propyne with lithium iodide in refluxing 2,4,6-trimethylpyridine.[18] In addition, the con-

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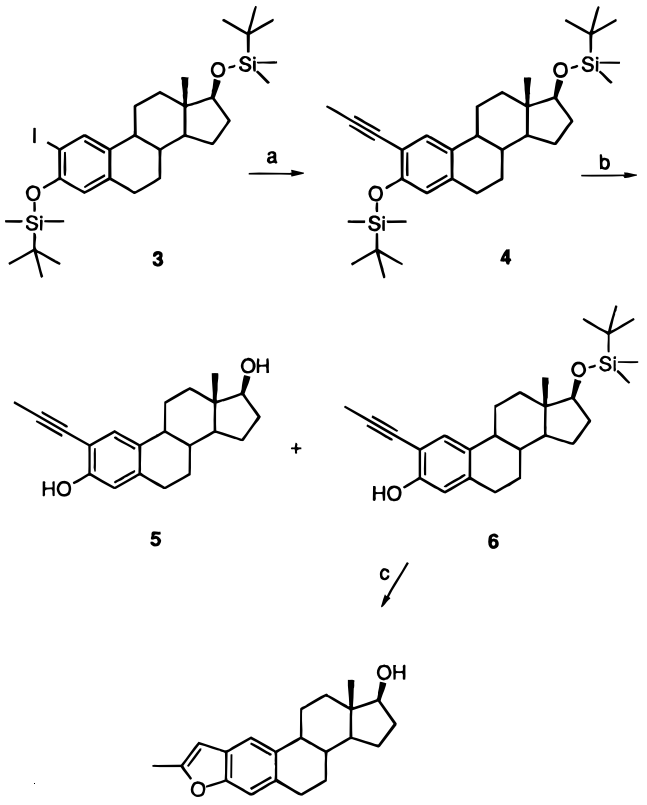
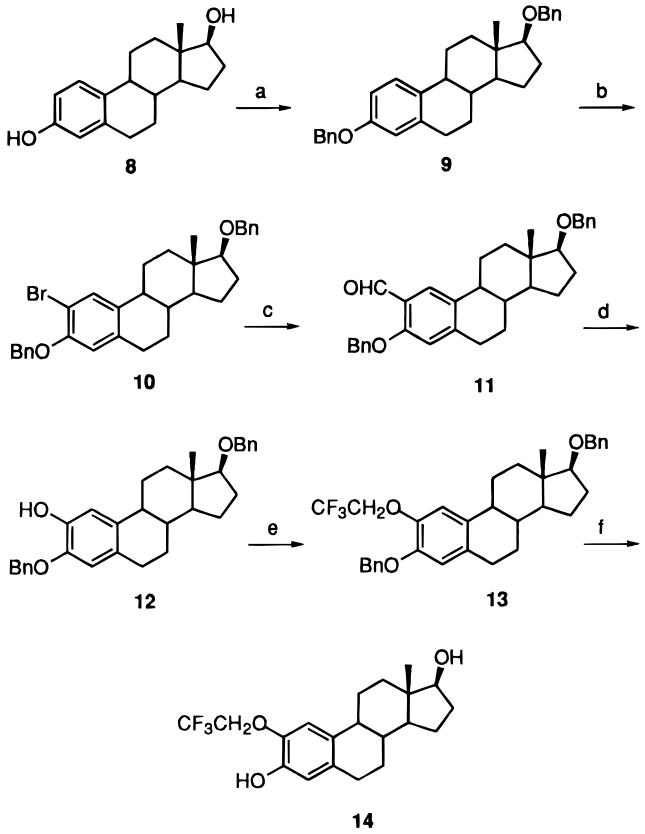
Scheme 1[a]



[a] Reagents and conditions: (a) $CH_3C{\equiv}CH$, $Pd(PPh_3)_4$, CuI, Et_3N, THF, 23 °C (41 h); (b) *n*-$Bu_4N^+F^-$, THF, 23 °C (24 h); (c) *n*-$Bu_4N^+F^-$, THF, reflux (6.5 h).

Scheme 2[a]



[a] Reagents and conditions: (a) (1) NaH, DMF, 0 °C (15 min), (2) $C_8H_5CH_2Br$, *n*-Bu_4NI, 2 days, 23 °C; (b) Br_2, AcOH, THF, 0 to 23 °C (1.5 h); (c) (1) *n*-BuLi, THF, −78 °C (2 h), (2) DMF, −78 to 0 °C (1 h); (d) (1) MCPBA, TsOH, CH_2Cl_2, 23 °C (15 h), (2) MeOH, H_2SO_4, reflux (1 h); (e) K_2CO_3, DMF, CF_3CH_2I, 110 °C (3 h), 130 °C (2 h); (f) H_2, $Pd(OH)_2$−C, THF, 23 °C (24 h).

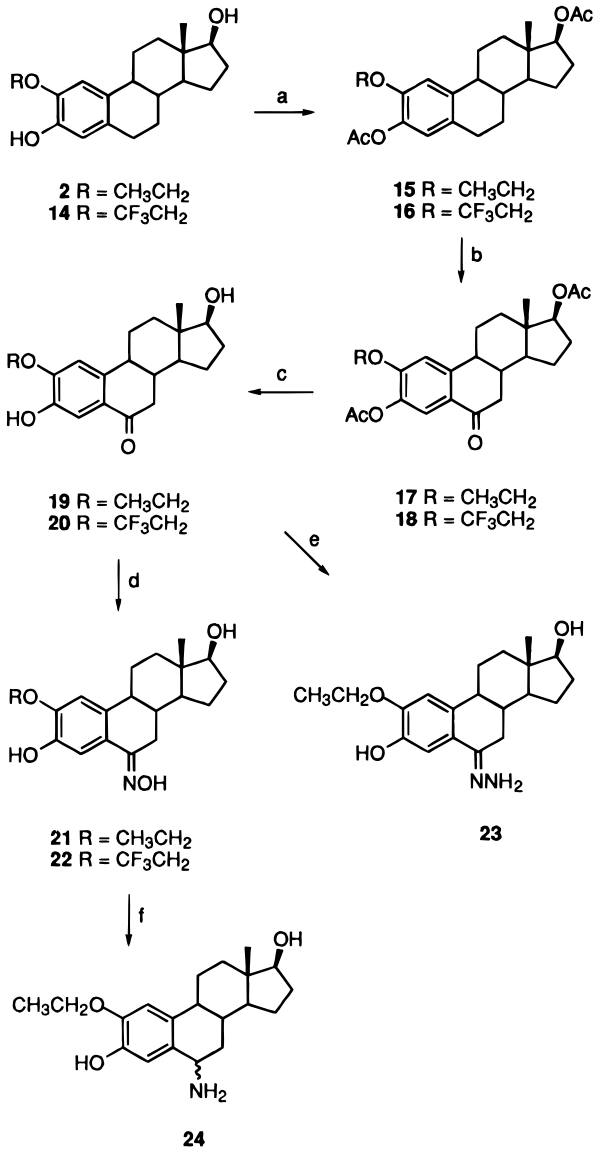
version of **6** to **7** is in agreement with several reported cyclizations of 2-alkynylphenols to benzofurans under acidic[19] and basic[12,20−22] conditions, although the temperatures utilized for these reactions (100−171 °C) are somewhat higher than the temperature of refluxing THF (66 °C) used here.

2′,2′,2′-Trifluoroethoxyestradiol **(14)** was prepared by a modification of our previous synthesis of 2-alkoxyestradiols involving the 2-formylestradiol dibenzyl ether intermediate **11** (Scheme 2).[4,11] The synthesis of **14** was prompted by the increased metabolic stability of trifluoroethoxy ethers relative to ethyl ethers.[23−25] Whereas the prior two-step synthesis of intermediate **11** was carried out in 10% yield by formylation of estradiol and benzylation of the two hydroxyl groups,[4] the present three-step synthesis afforded the product in 37% overall yield. The low yield of **11** in the prior synthesis[4] was due to the fact that the direct formylation of estradiol gave a mixture of 2-formylestradiol and 4-formylestradiol, which were separated by chromatographic techniques in low yield. In the present modification, the two hydroxyl groups of estradiol were protected as benzyl ethers in 60% yield by deprotonation with sodium hydride in DMF followed by alkylation of the alkoxide anions with benzyl bromide.[26,27] Bromination of the resulting intermediate **9** with bromine in acetic acid gave the 2-bromo derivative **10** in 75% yield. Halogen−lithium exchange was carried out on the 2-bromo compound **10** to afford the 2-lithio derivative, which was formylated with DMF to afford **11** in 82% yield from **10**. Baeyer−Villiger oxidation of **11** with *m*-chloroperbenzoic acid and a catalytic amount of *p*-toluenesulfonic acid in methylene chloride, followed by hydrolysis of the resulting formate, yielded the phenol **12**.[4] The phenolic hydroxyl group of **12** was then alkylated with 1,1,1-trifluoro-2-iodoethane using potassium carbonate in anhydrous DMF as the base. The desired analog **14** was then obtained after hydrogenolysis of the two benzyl ether protecting groups with palladium hydroxide on charcoal as the catalyst.

The syntheses of certain 6-keto, 6-oximino, 6-hydrazono, and 6-amino derivatives of 2-ethoxyestradiol and 2-(2′,2′,2′-trifluoroethoxy)estradiol are outlined in Scheme 3. Acetylation of the two hydroxyl groups of 2-ethoxyestradiol **(2)** and 2-(2′,2′,2′-trifluoroethoxy)estradiol **(14)** with acetic anhydride in pyridine afforded the corresponding diacetates **15** and **16**, respectively. The key oxidation at the C-6 benzylic carbons of **15** and **16**, to form **17** and **18**, respectively, was accomplished with chromium trioxide in acetic acid.[28] The acetates of **17** and **18** were hydrolyzed with potassium hydroxide in methanol to afford **19** and **20**, which were converted to the corresponding oximes **21** and **22**, respectively. Treatment of 2-ethoxy-6-ketoestradiol **(19)** with hydrazine afforded the hydrazone **23**. Sequential reduction of the oxime group of 2-ethoxy-6-oximinoestradiol **(21)** with sodium borohydride in the presence of titanium tetrachloride,[29,30] followed by catalytic hydrogenolysis over palladium on charcoal, provided the 6-amino derivative **24**.

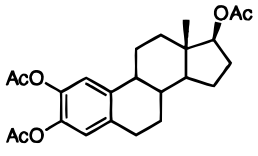
The triacetate **25** was a minor product obtained during the conversion of **14** to **16**. It resulted from acetylation of 2,3,17-trihydroxyestra-1,3,5(10)-triene,

Scheme 3[a]



[a] Reagents and conditions: (a) Ac_2O, pyridine, 110 °C (1 h); (b) CrO_3, AcOH; (c) KOH, MeOH; (d) $H_2NOH{\cdot}HCl$, AcONa, MeOH; (e) H_2NNH_2, AcOH, EtOH, 95 °C (3 h); (f) (1) $TiCl_4$, $NaBH_4$, DME, 23 °C (3.5 days), (2) H_2, Pd/C, THF, 23 °C (20 h).

present as an impurity in the starting material **14** in one experiment.



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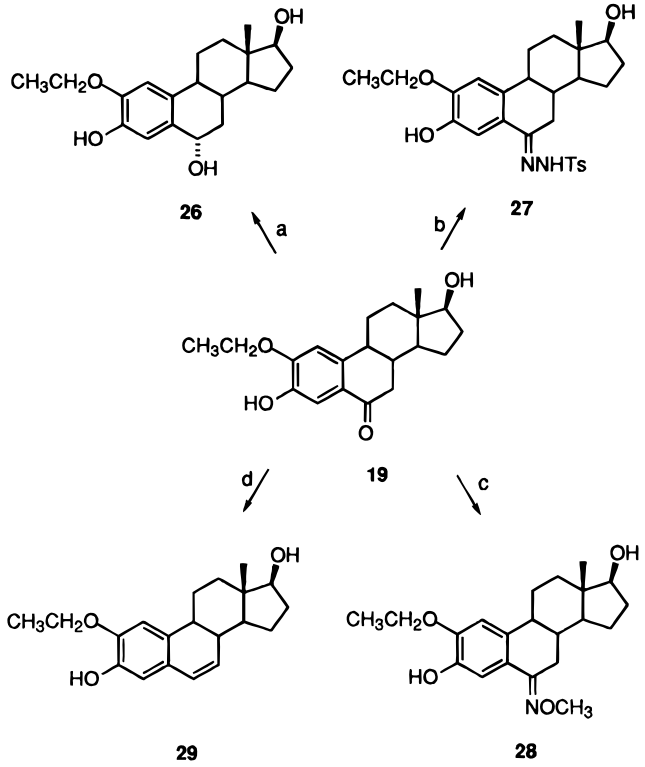
The syntheses of 6-hydroxyl, 6-tosylhydrazono, 6-methoximino, and 6,7-dehydro congeners are reported in Scheme 4. The 6α-alcohol derivative **26** was obtained by sodium borohydride reduction of the ketone **19**. The ^1H NMR spectrum of **26**, as compared with that of commercially available estra-1,3,5(10)-triene-3,6β,17β-triol (STERALOIDS, Inc.), indicated the presence of about 20% of 6β-isomer and 80% of 6α-isomer. Repeated recrystallization of **26** resulted in material of 90% isomeric purity (6α-hydroxysteroid), which was not further improved by recrystallization. In the purified sample of **26**, which contained about 90% of the 6α-

Scheme 4[a]



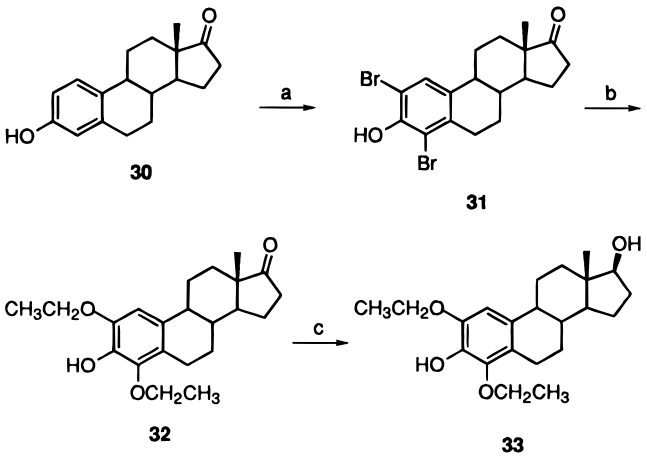
[a] Reagents and conditions: (a) $NaBH_4$, MeOH, 23 °C (6 h); (b) $TsNHNH_2$, AcOH, 90−95 °C, (4.5 h); (d) (1) $NaBH_4$, MeOH, 23 °C (3.5 h), (2) HCl, THF, 0 °C (45 min).

hydroxysteroid, the expected resonance signal due to the axial 6β-hydrogen appeared as a broad multiplet at δ 4.60 ppm, which is approximately 0.1 ppm lower field than the narrower equatorial 6α-hydrogen signal of the 6β-hydroxysteroid epimer. The sodium borohydride reduction of 6-ketoestradiol derivatives to afford the corresponding 6α-alcohols is well precedented.[31−35] Treatment of **19** with tosylhydrazine in acetic acid afforded the tosylhydrazone **27**. The oxime ether **28** was obtained by reaction of the ketone **19** with methoxylamine in methanol in the presence of sodium acetate. The 6,7-dehydro derivative **29** was obtained by sodium borohydride reduction of the ketone **19** followed by acid-catalyzed dehydration.

The synthesis of an analog of 2-ethoxyestradiol containing an additional ethoxy group in the 4-position is portrayed in Scheme 5. Bromination of estrone **(30)** in acetic acid afforded 2,4-dibromoestrone **(31)**, which was converted to 2,4-diethoxyestrone **(32)** by reacting **31** with sodium ethoxide in ethanol and DMF using CuI as the catalyst. Sodium borohydride reduction of **32** yielded the desired 2,4-diethoxyestradiol analog **33**. The 17β-configuration of the alcohol **33** is assigned based on the known sodium borohydride reduction of estrone and certain estrone derivatives to 17β-alcohols, which is dictated by the approach of the hydride from the sterically less hindered side of the carbonyl, opposite the adjacent angular 13β-methyl group.[36−39]

The synthesis of an analog of 2-ethoxyestradiol in which the 17β-hydroxyl group is replaced by a 17β-amino group is shown in Scheme 6. The 3-hydroxyl group of 2-ethoxyestradiol **(2)** was selectively benzylated with benzyl bromide in ethanol with potassium carbonate as the base to afford **34**. The 17β-hydroxyl group of **34** was then oxidized to the ketone **35** with manganese dioxide in methylene chloride. Reaction of **35** with

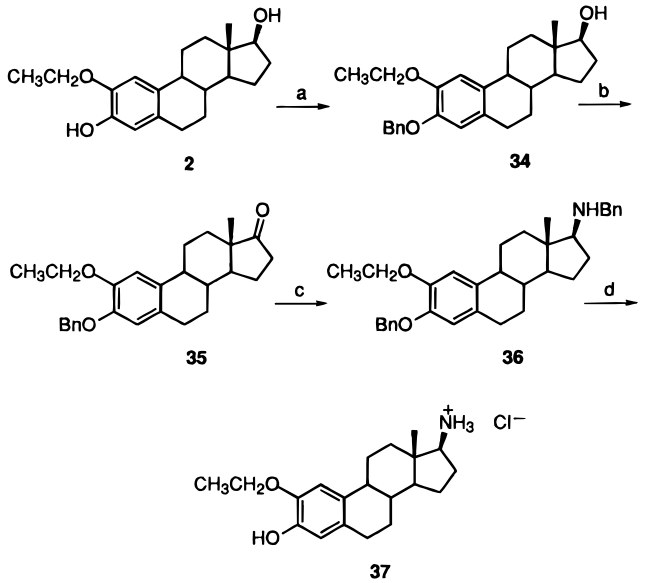
Scheme 5[a]



30 31 32 33

[a] Reagents and conditions: (a) Br_2, Fe, AcOH, 17 °C (30 min); (b) C_2H_5ONa, CuI, DMF, C_2H_5OH; (c) $NaBH_4$, MeOH, 23 °C (5.5 h).

Scheme 6[a]



2 34 35 36 37

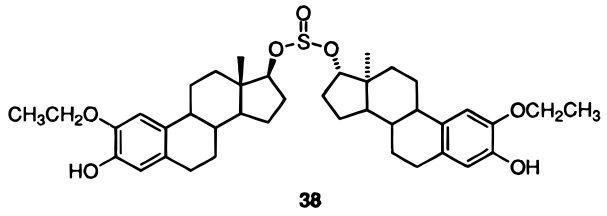
[a] Reagents and conditions: (a) (1) $C_6H_5CH_2Br$, K_2CO_3, EtOH, reflux (7.5 h); (b) MnO_2, CH_2Cl_2, 23 °C (26 h); (c) $C_6H_5CH_2NH_2$, $NaCNBH_3$, $ClCH_2CH_2Cl$, 23 °C (96 h); (d) (1) H_2, $Pd(OH)_2$/C, 23 °C (27 h), (2) HCl, Et_2O.

benzylamine afforded an intermediate Schiff base which was reduced to the secondary amine **36** with sodium cyanoborohydride. The ^1H NMR spectrum of **36** shows a triplet at about δ 3.0 without any additional shoulder, suggesting the steroid is predominantly a single diastereomer. We assume that the hydride attacks the imino carbon from the less sterically hindered α-face of the molecule, opposite the neighboring axial 13β-methyl group, to afford the 17β-amine **36**.[39−41] The benzyl groups of **36** were cleaved by hydrogenolysis with palladium hydroxide on charcoal as the catalyst to afford the corresponding primary amine, which was converted to the hydrochloride salt **37**.

The sulfite **38** was prepared by treatment of 2-ethoxyestradiol **(2)** with thionyl chloride in benzene. Bromination of estradiol in acetic acid afforded the 2,4-dibromoestradiol derivative **39**.

Biological Results and Discussion

The newly synthesized compounds were examined for antiproliferative activity against human cancer cell lines



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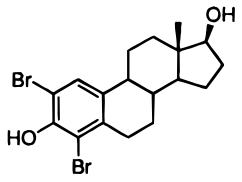

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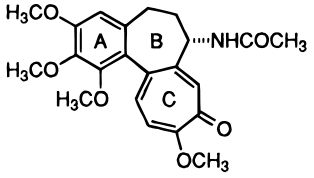

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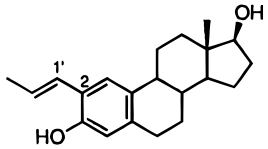

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in the NCI screen, in which the activity of each compound was evaluated with approximately 55 different cell lines of diverse tumor origins. In addition, the new agents were examined for inhibitory effects on the polymerization of purified bovine brain tubulin under reaction conditions where 2-methoxyestradiol **(1)** had shown maximal inhibitory effect.[7] Compounds that inhibited tubulin assembly were further evaluated for their ability to inhibit the binding of [^3H]colchicine to tubulin. The activities against tubulin, the mean graph midpoints (MGMs) for 50% growth inhibition of all human cancer cell lines successfully tested, and the GI_{50} values obtained with selected cell lines are summarized in Table 1. The MGM is based on a calculation of the average GI_{50} for all of the cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-4}−10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.[42] Table 1 includes published cell line data for 2-methoxyestradiol **(1)** and 2-ethoxyestradiol **(2)**, which was the most active of the 2-methoxyestradiol analogs prepared previously,[4] but the antitubulin data presented in the table for **1** and **2** were obtained in experiments performed contemporaneously with those performed with the new compounds.

Inhibition of Tubulin Assembly. Eleven of the new analogs showed significant activity (defined as IC_{50} values substoichiometric to the tubulin concentration of 12 μM) as inhibitors of tubulin polymerization. Our experience with this assay is that a difference in IC_{50}

Table 1. Cytotoxicities and Antitubulin Activities of 2-Methoxyestradiol and Analogs

	cytotoxicity (GI_{50}, μM)[a]									inhibn of tubulin polymn[c] (IC_{50}, μM ± SD)	% inhibn of colchicine binding[d] ± SD
no.	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]		
1	0.70	0.47	0.32	0.36	0.21	0.95	1.8	0.080	1.3	3.1 ± 0.5	19 ± 3
2	0.018	0.026	0.014	0.016	0.016	0.039	0.065	<0.01	0.076	1.3 ± 0.2	51 ± 6
5	1.7	0.48	0.95	0.50	0.34	6.9	3.6	0.13	1.7	4.8 ± 1	11 ± 4
7	19	0.51	14	16	18	16	17	23	14	>40	
14	12	3.2	1.4	2.3	0.46	4.5	7.0	0.28	2.6	1.5 ± 0.2	11 ± 7
18	20	21	23	18	24	20	20	18	20	>40	
19	0.03	0.02	0.71	0.01	0.63	0.18	0.17	<0.01	0.13	4.1 ± 0.8	19 ± 6
20	8.7	3.7	5.1	3.3	3.5	24	15	27	9.8	24 ± 2	1.7 ± 2
21	0.011	0.011	0.013	0.017	0.017	0.021	0.026	0.010	0.079	1.1 ± 0.02	63 ± 4
22	0.017	0.031	0.021	0.015	0.016	0.045	0.049	0.010	0.066	2.3 ± 0.06	28 ± 3
23	1.2	2.1	2.8	0.88	5.4	17	17	6.5	4.2	4.3 ± 1	9.6 ± 4
24	26	40	–	19	45	59	>100	16	40	>40	
25	4.8	5.1	3.5	2.5	6.4	5.1	3.2	0.32	3.7	>40	
26[e]	–	0.35	–	0.28	0.35	0.66	0.71	5.4	0.83	2.9 ± 0.2	12 ± 2
27	5.1	6.7	3.5	–	3.2	12	15	0.92	6.2	>40	
28	0.11	0.049	–	0.052	0.026	0.39	0.33	0.029	0.27	1.4 ± 0.1	34 ± 1
29	3.9	1.1	2.0	4.8	1.9	4.6	2.1	0.34	3.8	4.4 ± 1	8.6 ± 6
33	1.3	0.80	–	–	0.09	4.8	3.3	0.20	2.7	8.8 ± 2	1.5 ± 2
37	11	3.2	4.0	3.6	1.3	5.2	1.9	1.7	3.3	>40	
38	0.83	0.49	0.57	1.3	0.27	2.6	1.1	0.22	1.1	5.9 ± 1	24 ± 3
39	–	20	21	17	17	28	40	27	25	>40	

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines seccessfully tested. [c] Minimum of two independent determinations. Tubulin concentration was 1.2 mg/mL (12 μM). [d] Reaction mixtures contained 1.0 μM tubulin (0.1 mg/mL), 5.0 μM [^{3}H]colchicine, and 50 μM inhibitor. Assays were performed with triplicate samples in each experiment. [e] The sample tested contains 15% of the 6β-alcohol.

values greater than 20% is probably significant. Three compounds (**14**, **21**, and **28**), with IC_{50} values from 1.1 to 1.5 μM, had activities comparable to that of 2-ethoxyestradiol **(2)** (1.3 μM), and two additional compounds (**22** and **26**) had activities between those of **2** and **1** (IC_{50} value, 3.1 μM). An additional seven compounds (in order of activity **19**, **23**, **29**, **5**, **38**, **33**, and **20**) were less active than 2-methoxyestradiol **(1)** as inhibitors of tubulin polymerization (IC_{50} values ranging from 4.1 to 24 μM), and another seven were essentially inactive (**7**, **18**, **24**, **25**, **27**, **37**, and **39**) with IC_{50} values over 40 μM.

Inhibition of Colchicine Binding. In the inhibition of colchicine binding assay, in which compound **2** was significantly more active than **1** in both the previous[4] and current experiments, only compound **21** had activity greater than that of compound **2**. The other two strongest inhibitors of tubulin assembly had significantly weaker inhibitory effects on colchicine binding. Compound **28** had activity intermediate between the activities of **1** and **2**, while **14** had less inhibitory activity than 2-methoxyestradiol **(1)**. Three additional compounds (**19**, **22**, and **38**) had inhibitory effects on colchicine binding comparable to that of **1**, while the remainder of those examined had minimal or negligible inhibitory effects.

Inhibition of Cancer Cell Growth. Selected antiproliferative data obtained in the *in vitro* cancer cell screen[42] of the NCI Developmental Therapeutics Program are summarized in Table 1 [GI_{50} results with HOP-62 (non-small-cell lung), HCT-116 (colon), SF-59 (central nervous system), UACC-62 (melanoma), OVCAR-3 (ovarian), SN12C (renal), DU-145 (prostate), and MDA-MB-435 (breast), together with the MGM values for 50% inhibition of cell growth of all of the cell lines tested].

Two of the new compounds had antiproliferative activity comparable to that of 2-ethoxyestradiol **(2)**, which was the most active of the analogs prepared previously (MGM, 76 nM).[4] These were the two 6-oximino derivatives **21** and **22**. MGM values intermediate between those of **2** and 2-methoxyestradiol **(1)** (1.3 μM) were obtained with compounds **19**, **26**, **28**, and **38**. The remaining compounds were less inhibitory for cell growth than 2-methoxyestradiol. The highest MGM value was 40 μM for compound **24**.

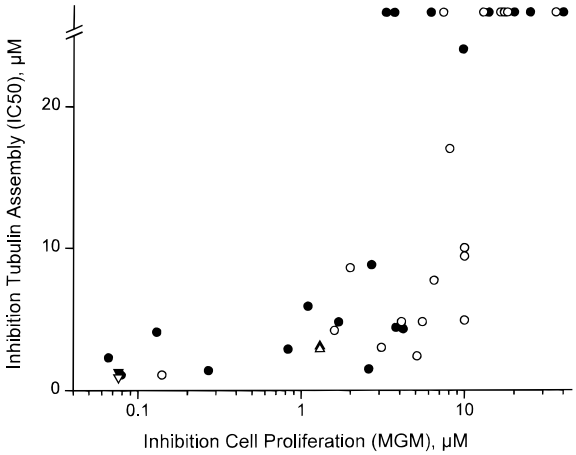

Figure 1. Activities of 2-methoxyestradiol analogs as inhibitors of tubulin assembly compared to their activities as inhibitors of cancer cell growth. The solid symbols represent compounds shown in Table 1; the open symbols, compounds described in ref 4. The upright triangles represent the data obtained with 2-methoxyestradiol (**1**); the inverted triangles, the data obtained with 2-ethoxyestradiol (**2**).

Correlation of Cell Growth Effects with Antitubulin Activity. There appears to be a rough correlation between the inhibition of tubulin polymerization and cytotoxicity in the 2-methoxyestradiol series of compounds. Figure 1 displays this graphically. All of the compounds having MGM values less than 1 μM had IC_{50} values for inhibition of tubulin polymerization of 4.1 μM or less. On the other hand, all of the compounds with IC_{50} values greater than 40 μM for inhibition of tubulin polymerization had MGM values of 3.3 μM or greater. A low IC_{50} value for inhibition of tubulin polymerization seems to be a necessary, but not sufficient condition for potent cytotoxicity. The chief problem with the assembly assay data is that it fails to

distinguish agents with nanomolar effects on cell growth (MGM values < 0.5 μM) from those that require low micromolar concentrations to inhibit cell growth.

There appears to be less correlation in the current study than previously between inhibition of colchicine binding and inhibition of either tubulin polymerization or cell growth. In the earlier study,[4] all three compounds more active than **1** as polymerization inhibitors also inhibited colchicine binding more strongly than **1**. With the preparation of five additional agents that inhibit assembly better than **1** (compounds **14**, **21**, **22**, **26** and **28**), this observation no longer holds. Only compounds **21** and **28**, and perhaps **22** as well, are more active than **1** as inhibitors of colchicine binding.

Structure−Activity Correlation. The synthetic chemistry effort summarized here has yielded two additional compounds comparable to 2-ethoxyestradiol as inhibitors of the growth of human tumor cells. These are the two 6-oximino derivatives **21** and **22**, which are also among the most potent inhibitors of tubulin assembly. On the basis of the polymerization assay and also the colchicine binding assay, **21** has a higher affinity for tubulin than does either **22** or **2**. Compounds **21** and **22** differ structurally only in the C-2 substituent, an ethoxy group in the former and a 1,1,1-trifluoroethoxy group in the latter.

The effect of the oximino substituent at C-6 can be evaluated by comparison of **2** vs **21** and **14** vs **22**. The effect on tubulin binding and polymerization is small, but the increase in cytotoxicity resulting from oxime incorporation at C-6 is dramatic in the case of **14** vs **22**. The reason for the increase in cytotoxicity in this case is not known, but it does not appear to result from a direct effect on tubulin polymerization.

Considering only C-6 modifications with a C-2 ethoxy group, replacing the oximino group of **21** with a methoximino group in **28** or a keto group in **19** only modestly reduced inhibitory effects in both the cell growth and tubulin assays. Comparing a C-6 α-hydroxy group in **26** with the C-6 keto group in **19**, the former was slightly more potent as an inhibitor of assembly and the latter as an inhibitor of cell growth. Replacement of the oximino group of **21** with either a hydrazone group in **23** or a C-6/C-7 double bond with no C-6 substituent in **29** yielded compounds with inhibitory effects on tubulin similar to that of the 6-keto derivative **19**, but with over a 30-fold reduction in antiproliferative activity relative to **19**. Compounds with a bulky C-6 substituent (the tosylhydrazone **27**) or a C-6 amino group (the amine **24**) had little activity as inhibitors either of cell growth or tubulin polymerization. This variability of the C-6 effects may support the hypothesis[5] that the A ring of methoxyestradiol **(1)** interacts with the same portion of the tubulin molecule as the C ring of colchicine **(40)**. The C-6 substituents in 2-methoxyestradiol analogs are probably analogous to C-7 side chains in colchicinoids.

The effect of the 2′,2′,2′-trifluoroethoxy group replacing the ethoxy group was somewhat unpredictable. Analogs with this substituent were therefore prepared in order to investigate whether they would retain activity against cancer cells and tubulin. Comparing the 6-oximino derivatives **21** (ethoxy) and **22** (trifluoroethoxy), the ethoxy compound **21** had a moderately higher affinity for tubulin (2-fold lower IC_{50} value in assembly), while both compounds had similar cytotoxicity. Comparing 2-ethoxyestradiol **(2)** with the trifluoroethoxy analog **(14)**, the latter differed little in its effect on tubulin assembly, was significantly less potent as an inhibitor of colchicine binding, and was substantially (over 30-fold) less cytotoxic than **2**. Finally, comparing the 6-keto derivatives **19** (2-ethoxy) and **20** [2-(2′,2′,2′-trifluoroethoxy)], the 2-ethoxy compound was substantially more active than **20** in both tubulin and cell-based assays.

Table 2. Relative Estrogen Receptor Binding Affinities of Selected 2-Substituted Estradiol Analog Tubulin Polymerization Inhibitors

compd	RBA[a] (0 °C)
1	0.245[b]
2	0.011[b]
21	<0.001
22	0.007
28	<0.001

[a] Relative binding affinity determined at 0 °C in a competitive radiometric assay, estradiol = 100; details are given in the Experimental Section and in reference 29. [b] Data published previously in ref 4.

Compound **5** represents an additional extension of the earlier study,[4] which primarily examined the effects of C-2 substituents. At that time, we found that the most active agents were 2-ethoxyestradiol **(2)** and 2-[(*E*)-1′-propenyl]estradiol **(41)**, both of which have three-atom side chains. In the prior investigation, the 2-[(*E*)-1′-propenyl] analog **41** displayed an IC_{50} value of 1.1 μM for inhibition of tubulin polymerization.[4] The 1′-propynyl substituent at C-2 in **5** resulted in decreased interaction with tubulin and decreased cytotoxicity relative to the 2-ethoxy and 2-[(*E*)-1′-propenyl] compounds. Compound **5** and the corresponding 2-*n*-propyl analog[4] had nearly identical, diminished effects on tubulin assembly and cell growth relative to the more active 2-[(*E*)-1′-propenyl] analog **41**.[4] It is interesting to note that the conformationally restricted analog **7** of the 2-[(*E*)-1′-propenyl] analog **41** was inactive. The inactivity of **7** could be due to the effect of restricted rotation of the conformationally free C-2−C-1′ single bond present in **41**. This would indicate that the biological activity of the 2-[(*E*)-1′-propenyl] analog **41** is due to a specific conformation about the C-2 to C-1′ single bond. On the other hand, the inactivity of **7** might simply indicate a requirement for a free hydroxyl group at C-3 for activity.

Finally, compound **38** deserves mention. Although modification at C-17 has generally resulted in substantially reduced activity,[4,5] compound **38**, a dimer of 2-ethoxyestradiol **(2)**, was comparable to 2-methoxyestradiol **(1)** as an inhibitor of cancer cell growth and of colchicine binding to tubulin and half as active as an inhibitor of assembly. These activities may result from hydrolysis of **38** to **2**, but the compound or a related dimer may be worth considering as a possible strategy for cross-linking tubulin molecules.

Affinities for Estrogen Receptor. One potential concern about the present series of tubulin polymerization inhibitors is that they might have some affinity for estrogen receptors and therefore possess either estrogenic or antiestrogenic activity or be estrogen antagonists, thus compromising their use as anticancer drugs with selective actions. We had previously shown[4] that **1** and **2** had limited affinity for the estrogen receptor, and the data shown in Table 2 extends this observation to the most potent compounds in the present series, namely analogs **21**, **22**, and **28**. The previously

obtained relative estrogen receptor binding affinities of 2-methoxyestradiol **(1)** and 2-ethoxyestradiol **(2)** are also presented in Table 2. To obtain these data, comparative radiometric binding assays were employed using rat uterine cytosol at 0 °C to determine the relative binding affinities (RBA) as compared to estradiol (RBA = 100, K_d = 0.3 nM). Both 2-ethoxy-6-oximinoestradiol **(21)** and 2-ethoxy-6-methoximinoestradiol **(28)** had no affinity for the estrogen receptor within the limits of the assay (RBA < 0.001), while 2-(2′,2′,2′-trifluoroethoxy)-6-oximinoestradiol **(22)** displayed very weak affinity for the receptor (RBA = 0.007). These new analogs had even lower affinity for the estrogen receptor than either of the very weak binders 2-methoxyestradiol **(1)** (RBA = 0.245) or 2-ethoxyestradiol **(2)** (RBA = 0.011). These results make it clear that effects due to estrogen receptor binding should not be a factor in the therapeutic application of compounds **21**, **22**, and **28**.

Conclusion. An array of new estradiol analogs has been synthesized in an effort to improve on the anticancer and antitubulin activities of 2-methoxyestradiol **(1)** and 2-ethoxyestradiol **(2)** and to further decrease their affinity for the estrogen receptor. These objectives have resulted in compounds **21**, **22**, and **28**, which have negligible estrogen receptor affinities. These compounds, along with 2-ethoxyestradiol **(2)**, are being investigated *in vivo* as anticancer agents.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: CI mass spectra on a Finnegan 4000 spectrometer; FAB mass spectra and EI mass spectra on a Kratos MS50 spectrometer; ^{1}H NMR spectra on a Varian VXR-500S or XL-200A spectrometer or on a Bruker AC 300 spectrometer; IR spectra on a Beckman IR-33 spectrophotometer, a Perkin-Elmer 1600 series FTIR spectrophotometer, or a Nicolet FT-IR Impact 410 spectrophotometer. Microanalyses were performed at the Purdue Microanalysis Laboratory or by Micro-Atlantic Laboratories, Atlanta, and all values were within ±0.4% of the calculated compositions.

3,17β-Bis[(*tert*-butyldimethylsilyl)oxy]-2-(1-propynyl)-1,3,5(10)-estratriene (4). To a solution of 2-iodoestradiol derivative **3** (3.04 g, 4.85 mmol) in anhydrous THF (80 mL) were added Et_3N (0.85 mL, 6.06 mmol, 1.25 equiv) and $Pd(PPh_3)_4$ (566 mg, 99%, 0.49 mmol, 0.1 equiv). The reaction mixture was stirred at room temperature under Ar for 0.5 h. A dark yellow solution developed. Propyne gas (25 g, 620 mmol, 128 equiv) was bubbled into the reaction mixture at room temperature. CuI (745.4 mg, 3.9 mmol, 0.8 equiv) was added, and the reaction mixture was stirred at room temperature under Ar for 41 h. The reaction mixture was diluted with saturated aqueous NaCl solution (50 mL) and extracted twice with ether (100, 50 mL). The combined ether layer was washed with aqueous saturated NaCl solution (2 × 30 mL) and dried over anhydrous Na_2SO_4. Evaporation of the filtrate gave the crude product as a black solid. Flash chromatography (silica gel; ether−hexane, 1:60 to 1:30 by volume) gave compound **4** (0.31 g, 12%) as a pale yellow solid: mp 56 °C; IR (neat) 3419, 2363, 1654, 999 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (s, 1 H), 6.48 (s, 1 H), 3.62 (t, J = 8.6 Hz, 1 H), 2.76 (m, 2 H), 2.25 (m, 1 H), 2.10 (m, 1 H), 2.04 (s, 3 H), 1.86 (m, 3 H), 1.70−1.10 (m, 8 H), 1.02 (s, 9 H), 0.89 (s, 9 H), 0.73 (s, 3 H), 0.20 (s, 6 H), 0.03 (s, 3 H), 0.02 (s, 3 H); LRCIMS (isobutane) m/z (rel intensity) 539 (MH^+, 56).

2-(1-Propynyl)-1,3,5(10)-estratriene-3,17β-diol (5) and 17β-[(*tert*-butyldimethylsilyl)oxy]-2-(1-propynyl)-1,3,5-(10)-estratrien-3-ol (6). A solution of the silyl ether **4** (200 mg, 0.37 mmol) in THF (20 mL) containing TBAF (1.0 M in THF, 1.5 mL, 2 equiv) was stirred at room temperature for 24 h. The reaction mixture was acidified to pH 5 with 2 N HCl, saturated NaCl solution (20 mL) was added, and the products were extracted with ether (2 × 20 mL). The combined ether layer was washed with aqueous saturated NaCl solution (20 mL) and dried over anhydrous Na_2SO_4. Evaporation of the filtrate gave the crude product as an oil, which was subjected to preparative TLC purification (silica gel uniplate 1000 microns, ether−hexane, 1:3, by volume) to give compound **5** (20 mg, 17%) as a yellow solid: mp 96−98 °C. Analytical HPLC (C18 reverse phase; solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile; gradient, 0−90% B in 30 min at a flow rate of 1 mL/min) indicated that compound **5** was pure: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (s, 1 H), 6.62 (s, 1 H), 5.59 (s, 1 H, exchangeable), 3.72 (t, J = 8.6 Hz, 1 H), 2.81 (m, 2 H), 2.27 (m, 1 H), 2.11 (s, 3 H), 2.10 (m, 2 H), 1.90 (m, 2 H), 1.68 (m, 1 H), 1.60−1.10 (m, 7 H), 0.90 (m, 1 H), 0.77 (s, 3 H); LRCIMS (isobutane) m/z (rel intensity) 311 (MH^+, 84); HRCIMS (isobutane) calcd $C_{21}H_{27}O_2$ MH^+ m/z 311.2011, found 311.1998.

The above preparative TLC also gave compound **6** (33.4 mg, 38%) as a pale yellow solid: mp 168−170 °C; IR (neat) 2927, 2361, 1725, 1676, 1604, 1492, 1460, 1256, 1094, 888, 836, 776 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (s, 1 H), 6.63 (s, 1 H), 5.58 (s, 1 H), 3.62 (t, J = 8.6 Hz, 1 H), 2.79 (m, 2 H), 2.15 (m, 2 H), 2.12 (s, 3 H), 1.85 (m, 4 H), 1.65−1.05 (m, 7 H), 0.89 (s, 9 H), 0.72 (s, 3 H), 0.05 (s, 3 H), 0.04 (s, 3 H); LRCIMS (isobutane) m/z 424 (M^+, minute), 367 ($M^+ - Me_3C$, minute), 291 ($M^+ - {}^tBuMe_2SiOH$, 2).

2′-Methylfurano[2,3-*b*]-1,3,5(10)-estratrien-17β-ol (7). A solution of **6** (60.0 mg, 0.14 mmol) in THF (10 mL) containing TBAF (1.0 M in THF, 0.3 mL, 2.1 equiv) was heated at reflux under Ar for 6.5 h. The reaction mixture was diluted with aqueous saturated NaCl solution (10 mL) and extracted twice with ether (30, 20 mL). The combined ether layer was washed with aqueous saturated NaCl solution (2 × 20 mL) and dried over anhydrous Na_2SO_4. Evaporation of the filtrate gave the crude product as an oil. Preparative TLC purification (silica gel uniplate 1000 μm, ether−hexane, 1:2 by volume) gave compound **7** (40 mg, 92%) as a yellow solid after recrystallization from $CHCl_3$−ether−hexane: mp 126−128 °C; IR (neat) 2924, 2867, 1605, 1467, 1433, 1264, 1054, 873, 732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1 H), 7.10 (s, 1 H), 6.27 (s, 1 H), 3.73 (t, J = 8.2 Hz, 1 H), 2.95 (m, 2 H), 2.41 (s, 3 H), 2.30 (m, 2 H), 2.11 (m, 1 H), 1.95 (m, 2 H), 1.75−1.15 (m, 9 H), 0.78 (s, 3 H); LRCIMS (isobutane) m/z 311 (MH^+, 20), 293 ($MH - H_2O$, 100). Anal. ($C_{21}H_{26}O_2$) C, H.

***β*-Estradiol Dibenzyl Ether (9).** Sodium hydride (2.6 g, 65 mmol) was added in four portions to a solution of β-estradiol (**8**, 5.0 g, 18 mmol) in anhydrous DMF (150 mL) under Ar at 0 °C, and the resulting mixture was stirred at 0 °C for 15 min. Benzyl bromide (12.5 mL, 105 mmol) was added, followed by tetrabutylammonium iodide (0.50 g, 1.35 mmol). Stirring was continued for 2 days at room temperature. The reaction mixture was cooled to 0 °C, and 50% aqueous ethanol (20 mL) was added slowly, followed by dropwise addition of 3 N HCl (18 mL) at 0−5 °C. The compound was extracted into ether (3 × 200 mL). The ether layer was washed with water (100 mL) and brine (100 mL) and dried over anhydrous sodium sulfate. Evaporation of the ether layer under reduced pressure gave a viscous oil. Trituration with hexane afforded a white solid (5.0 g, 60%), which was collected by filtration and dried under vacuum: mp 74−78 °C [lit.[26] mp 81−82 °C (ethanol, acetone); lit.[27] mp 62 °C (aqueous methanol)]; R_f 0.334 [hexane−ethyl acetate (95:5), silica gel]; IR (KBr) 2910, 2850, 1600, 745, 685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 3 H), 1.05−2.45 (m, 14 H), 2.83 (m, 1 H), 3.5 (t, J = 8.5 Hz, 1 H), 4.57 (s, 2 H), 5.05 (s, 2 H), 6.67−6.82 (m, 2 H), 7.12−7.57 (m, 11 H). Anal. ($C_{32}H_{36}O_2$) C, H.

2-Bromo-β-estradiol Dibenzyl Ether (10). Bromine (0.58 mL, 11.3 mmol) was added by syringe to an ice-cold, stirred solution of **8** (4.5 g, 10 mmol) in acetic acid−THF mixture (3:2, 45 mL). The resulting reaction mixture was allowed to warm to room temperature with stirring. After 1.5 h, the mixture was poured onto an ice−water mixture (200 mL), and compound was extracted with dichloromethane (3 × 200 mL). The combined organic layer was washed with water (200 mL), saturated aqueous sodium bicarbonate (200 mL), 10% aqueous sodium thiosulfate (150 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic

layer, on evaporation under vacuum, followed by trituration with hexane−ethyl acetate mixture, gave the product **10** (3.9 g, 75%). An analytical sample was prepared by crystallization from ethyl acetate: mp 156−158 °C; IR (KBr) 2960−2840, 1600, 1590, 730−720, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (s, 3 H), 1.10−2.40 (m, 13 H), 2.75−2.85 (m, 2 H), 3.5 (t, J = 8.5 Hz, 1 H), 4.6 (s, 2 H), 5.63 (s, 2 H), 6.67 (s, 1 H), 7.25−7.60 (m, 11 H). Anal. ($C_{32}H_{35}BrO_2$) C, H.

2-Formyl-3,17β-bis(benzyloxy)estra-1,3,5(10)-triene (11). A 1.6 M solution of *n*-BuLi (17.5 mL) in hexanes was added dropwise by syringe under Ar at −78 °C to a solution of compound **10** (5.88 g, 11.2 mmol) in anhydrous THF (150 mL), and the resulting reaction mixture was stirred at −78 °C for 2 h. Anhydrous DMF (5.5 mL) was added, and stirring was continued for 1 h at −78 °C. The mixture was warmed to 0 °C, stirred for 1 h, and poured onto an ice-cold solution of 3 N HCl (100 mL). The aqueous layer was extracted with ether (3 × 300 mL). The combined organic layer was washed with 50% brine−water (100 mL) and brine (150 mL) and dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to afford impure **11**, which was purified over a silica gel column using hexane−ethyl acetate as eluant. Appropriate fractions were combined and evaporated under reduced pressure to afford compound **11** (4.36 g, 82%): mp 145−147 °C; IR (KBr) 2920, 2820, 1675, 1600,728, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (s, 3 H), 1.1−2.5 (m, 13 H) , 2.80−2.97 (m, 2 H), 3.50 (t, J = 8.5 Hz, 1 H), 4.56 (s, 2 H), 5.65 (s, 2 H), 6.75 (s, 1 H), 7.20−7.50 (m, 10 H), 7.78 (s, 1 H), 10.50 (s, 1 H). Anal. ($C_{33}H_{36}O_3$) C, H.

2-Hydroxy-3,17β-bis(benzyloxy)estra-1,3,5(10)-triene (12). MCPBA (3.3 g, 19.1 mmol) and *p*-toluenesulfonic acid monohydrate (0.16 g, 0.84 mmol) were added successively to a solution of compound **11** (5.8 g, 12.1 mmol) in anhydrous dichloromethane (150 mL). The resulting mixture was stirred at room temperature, and the reaction was followed by TLC (dichloromethane, silica gel). After 10 h, additional amounts of MCPBA (1.0 g) and *p*-toluenesulfonic acid (0.040 g) were added, and stirring was continued for 5 h. The reaction mixture was diluted with dichloromethane (300 mL), and the organic layer was washed with 10% sodium sulfite solution (100 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer on evaporation under reduced pressure afforded crude compound **12** (4.85 g). This was suspended in anhydrous methanol (250 mL), four drops of concentrated H_2SO_4 were added, and the resulting mixture was stirred at reflux for 1 h. The methanol was removed under reduced pressure at 50 °C, and the resulting residue was dissolved in dichloromethane (400 mL). The organic layer was washed with water (100 mL), saturated sodium bicarbonate solution (100 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The dichloromethane layer on evaporation under reduced pressure gave crude compound **12** (4.15 g) as a gummy solid, which on purification over a silica gel column using hexane−dichloromethane mixture as an eluant provided pure product **12** (3.8 g, 67%) as a white solid: mp 117−118 °C; R_f 0.364 (dichloromethane, silica gel); IR (KBr) 3540, 2960−2840, 1510, 1450, 735−720, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (s, 3 H), 1.05−2.40 (m, 14 H), 2.80 (m, 1 H), 3.50 (t, J = 8.5 Hz, 1 H), 4.60 (s, 2 H), 5.08 (s, 2 H), 5.5 (s, 1 H), 6.65 (s, 1 H), 6.82 (s, 1 H), 7.20−7.50 (m, 10 H). Anal. ($C_{32}H_{36}O_3$) C, H.

2-(2′,2′,2′-Trifluoroethoxy)-3,17β-bis(benzyloxy)estra-1,3,5(10)-triene (13). Powdered K_2CO_3 (1.5 g, 11 mmol) was added to a solution of compound **12** (4.0 g, 8.5 mmol) in anhydrous DMF (50 mL), followed by dropwise addition of CF_3CH_2I (5.0 mL, 51 mmol) at room temperature. The resulting reaction mixture was heated at 110 °C for 3 h. Additional K_2CO_3 (2.5 g) and CF_3CH_2I (6 mL) were added, and the mixture was heated again at 130 °C for 2 h. The mixture was cooled in an ice bath and poured onto ice cold 3 N HCl (125 mL). The aqueous layer was extracted with ether (2 × 200 mL). The combined ether layer was washed with 3 N HCl (100 mL), water (100 mL), and brine (100 mL) and dried over anhydrous $MgSO_4$. The ether layer was evaporated under reduced pressure, and the resulting crude product on purification on a silica gel column gave product **13** (4.5 g, 95%) as a colorless oil: IR (neat) 2910, 2850, 1600, 1500, 730, 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (s, 3 H), 1.1−2.4 (m, 13 H), 2.65−2.85 (m, 2 H), 3.50 (t, J = 8.5 Hz, 1 H), 4.40 (q, J = 8.5 Hz, 2 H), 4.56 (s, 2 H), 5.05 (s, 2 H), 6.70 (s, 1 H), 6.98 (s, 1 H), 7.2−7.55 (m, 10 H). Anal. ($C_{34}H_{37}F_3O_3$) C, H.

2-(2′,2′,2′-Trifluoroethoxy)estra-1,3,5(10)-triene-3,17β-diol (14). $Pd(OH)_2$−C (20%, 1.0 g) was added *carefully under Ar* to a solution of **13** (1.0 g, 1.8 mmol) in anhydrous THF (50 mL). The resulting mixture was hydrogenated at 45−50 psi on a Parr apparatus for 24 h. The catalyst was removed by filtration using a Celite pad under Ar, and the pad was washed with dichloromethane (200 mL). Evaporation of the filtrate under reduced pressure, followed by purification on a silica gel column, gave compound **14** (0.5 g, 75%): mp 167−168 °C; IR (KBr) 3550, 2960−2840, 1590, 1510, 870 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (s, 3 H), 1.10−2.40 (m, 13 H), 2.70−2.85 (m, 2 H), 3.75 (m, 1 H), 4.40 (q, J = 8.5 Hz, 2 H), 5.35 (s, 1 H, OH), 5.5 (s, 1 H), 6.70 (s, 1 H), 6.80 (s, 1 H); MS m/z (relative intensity) 370 (100), 311 (13), 270 (8), 244 (8), 205 (6). Anal. ($C_{20}H_{25}O_3 \cdot 0.7H_2O$) C, H.

2-Ethoxy-3,17β-bis(acetyloxy)estra-1,3,5(10)-triene (15). Acetic anhydride (1.7 mL, 18 mmol) was added dropwise under Ar at 0 °C to a solution of 2-ethoxyestra-1,3,5(10)-triene-3,17β-diol **(2)** (0.44 g, 1.4 mmol) in anhydrous pyridine (6 mL). The resulting mixture was stirred at 110 °C for 1 h. The reaction mixture was cooled to room temperature and poured onto ice cold 3 N HCl (50 mL). The compound was extracted with ethyl acetate (150 mL × 2). The combined organic layer was washed with water (80 mL), sodium bicarbonate (100 mL), water (80 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The ethyl acetate layer, on evaporation under reduced pressure, gave compound **15** (0.50 g, 90%): mp 135−136 °C; R_f 0.66 [hexane−ethyl acetate (7:3), silica gel]; IR (KBr) 2970−2860, 1765, 1725, 1505, 920, 885 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (s, 3 H), 1.15−2.00 (m, 13 H), 2.10 (s, 3 H), 2.12−2.38 (m, 3 H), 2.35 (s, 3 H), 2.70−2.90 (m, 2 H), 4.05 (q, J = 6.4 Hz, 2 H), 4.71 (t, J = 8.5 Hz, 1 H), 6.75 (s, 1 H), 6.9 (s, 1 H). Anal. ($C_{24}H_{32}O_5$) C, H.

3,17β-Bis(acetyloxy)-2-(2′,2′,2′-trifluoroethoxy)estra-1,3,5(10)-triene (16). Acetic anhydride (10 mL) was added dropwise under Ar at 0 °C to a solution of 2-(trifluoroethoxy)-estra-1,3,5(10)-triene-3,17β-diol **(14)** (2.50 g, 6.7 mmol) in anhydrous pyridine (20 mL). The resulting mixture was stirred at 110 °C for 1 h. The reaction mixture was cooled to room temperature and poured onto ice cold 3 N HCl (100 mL). The compound was extracted into ether (2 × 250 mL). The combined organic layer was washed with water (60 mL), sodium bicarbonate (60 mL), water (60 mL), and brine (80 mL) and dried over anhydrous magnesium sulfate. The ether layer, on evaporation under reduced pressure, gave an almost quantitative yield (2.5 g) of compound **16**. An analytical sample was prepared by purification by column chromatography: mp 116−118 °C; R_f 0.63 [hexane−ethyl acetate (8:2), silica gel]; IR (KBr) 2960−2840, 1755, 1705, 1490, 950 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.15−2.00 (m, 10 H), 2.05 (s, 3 H), 2.15−2.40 (m, 6 H), 2.70−2.90 (m, 2 H), 4.30 (q, J = 8.5 Hz, 2 H), 4.70 (t, J = 8.5 Hz, 1 H), 6.80 (s, 1 H), 6.95 (s, 1 H). Anal. ($C_{24}H_{29}F_3O_5$) C, H.

2-Ethoxy-3,17β-diacetoxy-6-oxoestra-1,3,5(10)-triene (17). A solution of chromium trioxide (1.6 g, 16 mmol) in 90% glacial acetic acid (7.5 mL) was added dropwise at 15 °C to a solution of 2-ethoxy-3,17β-diacetyl derivative **15** (1.5 g, 3.7 mmol) in glacial acetic acid (12 mL). The resulting mixture was stirred at 15 °C for 35 min. The mixture was poured onto an ice−water mixture (150 mL), and the compound was extracted into ethyl acetate (3 × 180 mL). The combined organic layer was washed with water (100 mL), $NaHCO_3$ solution (100 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer, upon evaporation under reduced pressure, provided a crude compound, which on purification on a silica gel column using hexane−ethyl acetate mixture as eluant gave 1.20 g (55%) of compound **17:** mp 195 °C; IR (KBr) 2980−2810, 1765, 1730, 1715, 1665, 1600, 1500, 918, 880 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (s, 3 H), 1.30−1.85 (m, 9 H), 1.90−2.45 (m, 11 H), 2.47−2.85 (m, 2 H), 4.15 (q, J = 6.4 Hz, 2 H), 4.78 (t, J = 8.5 Hz, 1 H), 6.95 (s, 1 H), 7.77 (s, 1 H). Anal. ($C_{24}H_{30}O_6$) C, H.

3,17β-Bis(acetyloxy)-2-(2′,2′,2′-trifluoroethoxy)-6-estra-1,3,5(10)-triene (18). A solution of chromium trioxide (2.0 g, 20 mmol) in 90% glacial acetic acid (10 mL) was added dropwise at 9−10 °C to a solution of 2-(trifluoroethoxy)-3,17β-*O*-diacetyl derivative **16** (2.1 g, 4.6 mmol) in glacial acetic acid (14 mL). The resulting mixture was stirred at 10 °C for 60 min. The mixture was poured onto an ice−water mixture (150 mL), and the compound was extracted into ethyl acetate (3 × 200 mL). The combined organic layer was washed with water (100 mL), $NaHCO_3$ solution (100 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer, on evaporation under reduced pressure, provided a residue, which on purification over a silica gel column using hexane−ethyl acetate mixture as eluant gave the desired compound **18** (1.35 g, 61%): mp 131−133 °C; R_f 0.59 [hexane−ethyl acetate (7:3), silica gel]; IR (KBr) 2960−2840, 1770, 1730, 1675, 1610, 1500, 890, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 2.83−1.27 (m, 19 H), 4.45 (q, J = 8.5 Hz, 2 H), 4.75 (t, J = 8.5 Hz, 1 H), 6.93 (s, 1 H), 7.80 (s, 1 H). Anal. ($C_{24}H_{27}F_3O_6$) C, H.

2-Ethoxy-6-oxoestra-1,3,5(10)-triene-3,17β-diol (19). A 20% solution of KOH in methanol (5 mL) was added dropwise to a suspension of compound **17** (0.95 g, 2.3 mmol) in anhydrous methanol (12 mL). The reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure at 42−45 °C. The residue was diluted with an ice−water mixture (30 mL) and extracted with ethyl acetate (3 × 125 mL). The combined organic layer was washed with ice cold water (30 mL) and brine (30 mL) and dried over anhydrous sodium sulfate. The organic layer, on evaporation under vacuum, gave **19** (0.7 g, 92%). An analytical sample was prepared by a short column chromatography on silica gel using EtOAc, followed by EtOAc−MeOH (18:1), and finally chloroform: mp 194−196 °C; IR (KBr) 3400−3200, 2960−2842, 1650, 1590, 1500, 880, 852 cm^{-1}; ^1H NMR (DMSO-d_6 + D_2O) δ 0.85 (s, 3 H), 1.20−1.80 (m, 10 H), 1.82−2.82 (m, 8 H), 3.72 (t, J = 8.5 Hz, 1 H), 4.20 (q, J = 6.4 Hz, 2 H), 6.85 (s, 1 H), 7.58 (s, 1 H). Anal. ($C_{20}H_{26}O_4 \cdot 0.25H_2O$) C, H.

6-Oxo-2-(2′,2′,2′-trifluoroethoxy)estra-1,3,5(10)-triene-3,17β-diol (20). A 20% solution of KOH in methanol (7 mL) was added dropwise to a suspension of compound **18** (1.1 g, 2.4 mmol) in anhydrous methanol (20 mL). The resulting reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure at 42−45 °C. The residue was neutralized with ice cold 3 N HCl, and the resulting precipitate was removed by filtration. The filtrate was suspended in ethyl acetate, and residual insoluble material was removed by filtration. The organic layer, on dilution with hexane, deposited a solid which was collected by filtration and dried to afford **20**. The filtrate, on purification over a silica gel column, gave an additional amount of 2-(trifluoroethoxy)-6-oxoestra-1,3,5(10)-triene-3,17β-diol **(20)** (total yield 0.75 g, 83%): mp 186−188 °C; IR (KBr) 3400, 2940, 1660, 1600, 1505, 1440, 870 cm^{-1}; ^1H NMR (DMSO-d_6 + D_2O) δ 0.83 (s, 3 H), 1.20−2.85 (m, 13 H), 3.05 (bs, 1 H), 3.75 (t, J = 8.5 Hz, 1 H), 4.58 (q, J = 8.5 Hz, 2 H), 6.92 (s, 1 H), 7.63 (s, 1 H), 8.6 (s, 1 H). Anal. ($C_{20}H_{23}F_3O_4$) C, H.

2-Ethoxy-6-(oximino)estra-1,3,5(10)-triene-3,17β-diol (21). Sodium acetate (2.0 g, 30 mmol) was added to a stirred solution of 6-oxo derivative **19** (0.34 g, 1.03 mmol) in methanol (35 mL). Hydroxylamine hydrochloride salt (2.0 g, 29 mmol) in water (3 mL) was added at room temperature. The reaction mixture was stirred at 90 °C for 4 h. The solvent was removed under reduced pressure, the resulting residue was diluted with water (50 mL), and the product was extracted with ethyl acetate (3 × 150 mL). The combined organic layer was washed with water (100 mL) and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer, on evaporation under reduced pressure, provided the crude product, which was purified on a silica gel column using hexane−ethyl acetate mixture as eluant. Appropriate fractions were combined and evaporated under reduced pressure to afford **21** (60−68%): mp 228−230 °C; IR (KBr) 3530, 3360−3140, 2930, 1605, 1505, 870, 798 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6 + D_2O) δ 0.85 (s, 3 H), 1.10−2.45 (m, 18 H), 3.20 (dd, J = 4.3 and 12.8 Hz, 1 H), 3.75 (t, J = 8.5 Hz, 1 H), 4.18 (q, J = 8.5 Hz, 2 H), 6.80 (s, 1 H), 7.55 (s, 1 H). Anal. ($C_{20}H_{27}NO_4$) C, H.

6-(Oximino)-2-(2′,2′,2′-trifluoroethoxy)estra-1,3,5(10)-triene-3,17β-diol (22). Sodium acetate (2.86 g, 35 mmol) was added to a stirred solution of 6-oxo derivative **20** (0.45 g, 1.2 mmol) in methanol (55 mL). Hydroxylamine hydrochloride salt (2.2 g, 32 mmol) in water (3 mL) was added at room temperature. The mixture was stirred at 90 °C for 6 h. Solvent was removed under reduced pressure, the resulting residue was diluted with water (60 mL), and the product was extracted into ethyl acetate (3 × 120 mL). The combined organic layer was washed with water (70 mL) and brine (70 mL) and dried over anhydrous sodium sulfate. The organic layer on evaporation under reduced pressure provided the crude product, which on crystallization from ethyl acetate−hexane mixture gave oxime **22** (0.3 g, 65%): mp 187−191 °C; IR (KBr) 3330, 2960−2860, 1500, 880, 750 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6 + D_2O) δ 0.77 (s, 3 H), 1.10−2.38 (m, 11 H), 2.67 (s, 2 H), 3.05−3.27 (m, 2 H), 3.72 (t, J = 8.5 Hz, 1 H), 4.50 (q, J = 8.5 Hz, 2 H), 6.88 (s, 1 H), 7.57 (s, 1 H), 10.20 (s, 1 H). Anal. ($C_{20}H_{24}NF_3O_4$) C, H.

2-Ethoxy-6-oxoestra-1,3,5(10)-triene-3,17β-diol 6-Hydrazone (23). Glacial acetic acid (0.5 mL) and anhydrous hydrazine (0.2 mL) were added under Ar to a suspension of 6-oxo derivative **19** (0.4 g, 1.2 mmol) in anhydrous ethanol (25 mL). The mixture was stirred at 95 ± 5 °C. After 2 h, anhydrous hydrazine (0.1 mL) was added to the reaction mixture, and the reaction was continued for 1 h. The solvent was removed under reduced pressure, and the residue was diluted with water (25 mL). The aqueous solution was extracted with dichloromethane (3 × 100 mL). The combined organic layer was washed with water (100 mL) and dried over anhydrous sodium sulfate. The organic layer on evaporation under reduced pressure gave the desired hydrazone **23** (364 mg, 84%): mp 132−138 °C; R_f 0.24 [ethyl acetate−hexane (4:1), silica gel]; IR (KBr) 3370, 2960, 2860, 1610, 1570, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 (s, 1 H), 6.78 (s, 1 H), 5.70 (bs, 1 H, exchangeable with D_2O), 5.20 (bs, 3 H, exchangeable with D_2O), 4.15 (q, J = 6.4 Hz, 2 H), 3.77 (dd, J = 8.5 Hz, 1 H), 2.45−1.15 (m, 17 H), 0.8 (s, 3 H); MS m/z (relative intensity) 345 (MH^+, 100), 330 (43), 263 (30), 243 (100), 202 (27), 189 (25), 165 (52); exact mass calcd $(MH)^+$ 345.2178, found 345.2187. Anal. ($C_{20}H_{28}N_2O_3 \cdot 0.4CH_2Cl_2$) C, H, N.

2-Ethoxy-6-aminoestra-1,3,5(10)-triene-3,17β-diol (24). $TiC1_4$ (1 mL) was introduced via a syringe under Ar to an ice cold suspension of sodium borohydride (0.8 g, 21 mmol) in anhydrous DME (30 mL). A solution of the oxime **21** (0.7 g, 2.03 mmol) in anhydrous DME (15 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 1.5 h and allowed to warm to room temperature with stirring. After 2 days, an additional amount of sodium borohydride (0.24 g) was added, and stirring was continued for 1.5 days at room temperature. The mixture was cooled to 0 °C, and water (10 mL) was added *very cautiously*, followed by neutralization with saturated sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate (2 × 200 mL) and dichloromethane (2 × 200 mL). Both organic layers were washed separately with water (100 mL), saturated sodium bicarbonate (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layers on evaporation under reduced pressure gave 0.4 g of crude product. This crude compound was dissolved in anhydrous THF (25 mL), and 5% Pd−C (0.6 g) was added under Ar. The resulting reaction mixture was hydrogenated at 50−55 psi. After 5 h, an additional amount of 5% Pd−C (0.16 g) was added, and the reaction mixture was hydrogenated at 50−55 psi for an additional 15 h. The catalyst was removed by filtration through a Celite pad, and the Celite pad was washed with dichloromethane (100 mL). The organic layer on evaporation under reduced pressure provided a crude mixture, which on purification on a silica gel column using hexane−ethyl acetate and ethyl acetate−methanol mixtures as eluants provided compound **24** (160 mg, 22%): mp 208−212 °C; ^1H NMR (DMSO-d_6) δ 0.7 (s, 3 H), 1.05−2.40 (m, 14 H), 3.40 (s, 2 H), 3.55 (t, J = 8.5 Hz, 1 H), 4.05 (q, J = 8.5 Hz, 2 H), 4.40 (bs, 1 H), 4.60 (s, 1 H), 6.85 (s, 1 H), 7.05 (s, 1 H), 8.38 (bs, 2 H), 8.80 (s, 1 H). Anal. ($C_{20}H_{29}NO_3 \cdot HCl \cdot 0.5H_2O$) C, H, N.

2,3,17β-*O*-Triacetylestra-1,3,5(10)-triene (25). This minor product resulted from acylation of 2,3,17-trihydroxyestra-

1,3,5(10)-triene, which was present as an impurity in the starting material **14** used for the preparation of **16**. Compound **25**: mp 162−164 °C; R_f 0.33 [hexane−ethyl acetate (4:1)]; IR (KBr) 2910, 2820, 1765, 1715, 1490, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (s, 3 H), 1.15−2.40 (m, 22 H), 2.75−2.95 (m, 2 H), 4.72 (t, J = 8.5 Hz, 1 H), 6.87 (s, 1 H), 7.05 (s, 1 H). Anal. ($C_{24}H_{30}O_6$) C, H.

2-Ethoxy-1,3,5(10)-triene-3,6α,17β-triol (26). Sodium borohydride (0.5 g, 13.2 mmol) was added under Ar at room temperature to a solution of 6-oxo derivative **19** (0.85 g, 2.57 mmol) in anhydrous methanol (30 mL). After 6 h, the solvent was evaporated under reduced pressure. The residue was diluted with water (40 mL), and dichloromethane (170 mL) and ice cold 3 N HCl (5 mL) were added. The dichloromethane layer was removed, and the aqueous layer was extracted again with dichloromethane (150 mL). The combined organic layer was washed with water (50 mL), sodium bicarbonate (50 mL), and brine (50 mL) and dried over anhydrous sodium sulfate. The organic layer upon evaporation under reduced pressure followed by purification on a silica gel column provided a diastereomeric mixture of alcohols (0.5 g, 59%): mp 97−106 °C; R_f 0.27 [dichloromethane−methanol (95:5), silica gel]. Recrystallization from ethyl acetate gave a mixture enriched in 6α-diastereomer (>95% 6α-isomer): IR (KBr) 3380, 2910, 2850, 1500, 1270, 1105, 1050, 870, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 1 H), 6.78 (s, 1 H), 5.65 (s, 1 H), 4.60 (bm, 1 H), 4.10 (q, J = 6.4 Hz, 2 H), 3.75 (t, J = 6.4 Hz, 1 H), 2.45−1.10 (m, 18 H), 0.8 (s, 3 H). Anal. ($C_{20}H_{28}O_4 \cdot 0.7H_2O$): C, H.

2-Ethoxy-6-oxoestra-1,3,5(10)-trien-3,17β-diol 6-Tosylhydrazone (27). Acetic acid (0.5 mL) was added to a stirred solution of 6-oxo derivative **19** (0.8 g, 2.42 mmol) in ethanol (30 mL). Tosylhydrazine (0.484 g, 2.6 mmol) was added at room temperature. The resulting mixture was stirred at 90−95 °C for 5.5 h. More tosylhydrazine (0.2 g) was added, and heating continued for 3.5 h. The solvent was removed under reduced pressure, the resulting residue was diluted with water (50 mL), and the product was extracted with ethyl acetate (3 × 150 mL). The combined organic layer was washed with water (100 mL) and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer on evaporation under reduced pressure provided an impure compound **27** which, on crystallization from an ethyl acetate−hexane mixture, gave pure compound **27** (0.6 g, 50%): mp 240 °C; R_f 0.24 [dichloromethane−methanol (95:1), silica gel]; IR (KBr) 3497, 3223, 2974−2939, 1621, 1592, 1577, 1499 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3 H), 1.15−2.38 (m, 16 H), 2.45 (s, 3 H), 2.65 (dd, J = 4.3 and 17.2 Hz, 1 H), 3.72 (t, J = 8.5 Hz, 1 H), 4.15 (q, J = 6.4 Hz, 2 H), 5.60 (s, 1 H), 6.73 (s, 1 H), 7.37 (d, J = 8.5 Hz, 2 H), 7.57 (s, 1 H), 7.67 (s, 1 H), 7.95 (d, J = 8.5 Hz, 2 H).

2-Ethoxy-6-(*O*-methyloximino)estra-1,3,5(10)-triene-3,17β-diol (28). A solution of methoxylamine (1.84 g) in distilled water (3.5 mL) was added to a suspension of 6-oxo derivative **19** (0.33 g, 1 mmol) and anhydrous sodium acetate (1.94 g) in anhydrous MeOH (100 mL), and the mixture was stirred at 95 ± 5 °C for 4.5 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water (50 mL) and the solution extracted with ethyl acetate (2 × 200 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and dried over anhydrous Na_2SO_4. The organic layer on evaporation gave an amorphous powder (0.32 g). The crude mixture was chromatographed over silica gel and eluted with hexane−ethyl acetate (1:1) to afford the desired product. This was further purified by crystallization from hexane−ethyl acetate to afford **28** (0.3 g, 83%): mp 92−97 °C; R_f 0.56 (hexane−ethyl acetate (1:1), silica gel]; IR (KBr) 3460, 2960−2840, 1590, 1560, 1490, 1435, 890−872 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (s, 1 H), 6.78 (s, 1 H), 5.50 (s, 1 H), 4.15 (q, 2 H), 3.97 (s, 3 H), 3.85-3.65 (m, 1 H), 3.05 (dd, J = 8.5 and 21.4 Hz, 1 H), 2.45−1.15 (m, 15 H), 0.95 (t, J = 8.5 Hz, 1 H), 0.8 (s, 3 H). Anal. ($C_{21}H_{29}NO_4 \cdot 0.1n\text{-}C_6H_{12}$) C, H, N.

2-Ethoxy-6,7-dehydroestra-1,3,5(10)-triene-3,17β-diol (29). A solution of 2-ethoxy-6-ketoestradiol **(19)** (1.1 g, 3.3 mmol) in anhydrous methanol (30 mL) was prepared by dissolving the solid in warm methanol and then cooling to room temperature. Sodium borohydride (0.8 g, 21 mmol) was added in portions to the solution, and the mixture was stirred at room temperature for 3.5 h. The solvent was evaporated under reduced pressure. The resulting residue was suspended in THF (15 mL), and 3 N HCl (15 mL) was added to the suspension. The reaction mixture was stirred at room temperature for 45 min at 0 °C. The solvent was evaporated under reduced pressure, and the residue was diluted with dichloromethane (2 × 150 mL). The combined organic layer was washed with water (25 mL) and brine (25 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the resulting crude compound on purification on a silica gel column gave pure 6,7-dehydro compound **29** (0.63 g, 30%): R_f 0.66 [dichloromethane−methanol (95:5), silica gel]; IR (KBr) 3400, 2919, 2841, 1600, 1569, 1502, 1279 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), 6.85 (s, 1 H), 6.72 (s, 1 H), 6.40 (d, J = 8.5 and 2.1 Hz, 1 H), 5.88 (d, J = 8.5 and 2.1 Hz, 1 H), 5.50 (s, 1 H), 4.15 (q, J = 8.5 Hz, 2 H), 3.75 (t, J = 8.5 Hz, 1 H), 2.45−1.15 (m, 15 H), 0.8 (s, 3 H). Anal. ($C_{20}H_{26}O_3 \cdot 0.25H_2O$) C, H.

2,4-Dibromo-3-hydroxyestra-1,3,5(10)-trien-17-one (31). Estrone (1.5 g, 5.6 mmol) was dissolved in acetic acid (240 mL) at 75−80 °C on a steam bath and the mixture cooled to 17 °C. Some of the estrone precipitated during cooling. Iron (34 mg) was added. A solution of bromine (2 mL, 40 mmol) in glacial acetic acid (40 mL) was added dropwise to the cold stirred mixture, and stirring was continued for 30 min at 17 °C. The reaction mixture was poured onto an ice−water mixture (800−900 mL), and a yellow solid separated, which was collected by filtration and washed with water. The yellow solid was dissolved in ethyl acetate (300 mL). The organic layer was washed with water (100 mL), saturated sodium bicarbonate (2 × 100 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer, on evaporation under vacuum, gave 2.5 g of a mixture of products, which was separated by silica gel column chromatography using ethyl acetate−hexane as eluant. The desired compound **31** (1.5 g, 62%) was a major product. An analytical sample was prepared by crystallization from ethyl acetate: mp 220−226 °C (lit.[43] mp 225−226 °C); IR (KBr) 3260, 2930, 2860, 1715, 1535, 1458, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1 H), 5.87 (s, 1 H, exchangeable with D_2O), 3.10−2.90 (dd, J = 8.5 and 21.4 Hz, 1 H), 2.80−1.35 (m, 14 H), 0.91 (s, 3 H). Anal. ($C_{18}H_{20}Br_2O_2$) C, H.

2,4-Diethoxy-3-hydroxyestra-1,3,5(10)-trien-17-one (32). Sodium ethoxide (2.1 g, 31 mmol) was dissolved in anhydrous ethanol (20 mL) and stirred for 15−20 min at room temperature. Anhydrous DMF (20 mL) was added to the solution under Ar, and stirring was continued for 20 min at room temperature. CuI (2.3 g, 24 mmol) and 2,4-dibromo compound **31** (0.7 g, 2.0 mmol) were added successively, and the resulting mixture was stirred at 105−110 °C for 2 h. The reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into ice cold water (200 mL) and neutralized with 3 N HCl. Insoluble material was removed by filtration through a Celite pad, and the aqueous layer was extracted with ethyl acetate (2 × 250 mL). The combined organic layer was washed with water (150 mL), saturated aqueous sodium bicarbonate (150 mL), and brine (150 mL) and dried over anhydrous sodium sulfate. The resulting compound, after removal of solvent, was purified by silica gel column chromatography using a hexane−dichloromethane system to afford the product in 37% yield: mp 57−62 °C; IR (KBr) 3540, 2980−2860, 1730, 1495 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.65 (s, 1 H), 5.45 (s, 1 H), 4.10 (q, J = 6.4 Hz, 4 H), 2.95 (dd, J = 8.5 and 21.4 Hz, 1 H), 2.80−1.10 (m, 20 H), 0.95 (s, 3 H). Anal. ($C_{22}H_{30}O_4 \cdot H_2O$) C, H.

2,4-Diethoxy-3-hydroxyestra-1,3,5(10)-trien-17β-ol (33). Sodium borohydride (0.12 g, 3.2 mmol) was added slowly under Ar to a solution of 2,4-diethoxy-17-keto derivative **32** (0.22 g, 0.61 mmol) in anhydrous methanol (8 mL). After 5.5 h, the solvent was removed under reduced pressure, and the residue was acidified with 3 N HCl. The compound was extracted with ethyl acetate (2 × 100 mL). The organic layer was washed with water (80 mL), saturated sodium bicarbonate (2 × 80 mL), water (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure, followed by purification on a silica gel column, gave **33** (55 mg, 25%): mp 67−72 °C; IR (KBr) 3400, 2960−2845, 1600, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$ + D_2O) δ 6.65 (s, 1 H), 4.82 (bs, 2 H), 4.07 (q, 4 H), 3.72 (t, J = 8.5 Hz, 1 H),

2.92 (dd, J = 8.5 and 17.1 Hz, 1 H), 2.75−2.50 (m, 1 H), 2.40−1.10 (m, 19 H), 0.82 (s, 3 H). Anal. ($C_{22}H_{32}O_4 \cdot 0.25H_2O$) C, H.

3-(Benzyloxy)-2-ethoxyestra-1,3,5(10)-trien-17β-ol (34). Benzyl bromide (4.5 g, 38 mmol) was added to a suspension of 2-ethoxy-β-estradiol **2** (4.5 g, 13 mmol) and potassium carbonate (8.6 g, 62 mmol) in anhydrous ethanol (100 mL) under Ar at 0 °C. The resulting mixture was stirred at 100 ± 5 °C for 7−8 h. The solvent was evaporated under reduced pressure, the residue was diluted with water, and the mixture was extracted with ethyl acetate (3 × 200 mL). The combined organic solution was washed with water (100 mL), sodium bicarbonate (120 mL), and brine (100 mL) and dried over sodium sulfate. The ethyl acetate layer on evaporation under reduced pressure gave a viscous oil (5.8 g), which was used in the next step without any further purification: ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 5 H), 6.75 (s, 1 H), 6.65 (s, 1 H), 5.5 (s, 1 H), 4.55 (s, 2 H), 4.04 (q, J = 6.4 Hz, 2 H), 3.5 (t, J = 8.5 Hz, 1 H), 2.75 (m, 3 H), 2.20−1.95 (m, 6 H), 1.90−1.80 (m, 2 H), 1.75−1.5 (m, 2 H), 1.45−1.1 (m, 7 H), 0.85 (s, 1 H).

2-Ethoxy-3-(benzyloxy)estra-1,3,5(10)-trien-17-one (35). Activated MnO_2 (3 g) was added under Ar to a solution of crude alcohol **34** (1.6 g, 3.9 mmol) in anhydrous dichloromethane (50 mL). After 7 h, an additional amount of MnO_2 (5 g) was added, and the reaction mixture was left overnight at room temperature. MnO_2 (4 g) was added again, and stirring was continued for 7 h. The mixture was filtered through a Celite pad, and the filtrate was evaporated under reduced pressure to give crude product, which was purified by column chromatography on silica gel using hexane, followed by hexane−CH_2Cl_2 (2:8) to afford pure **35** (0.42 g, 8%): IR (neat) 3065, 2929, 2869, 2825, 1727, 1607, 1504, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3 H), 1.30−2.60 (m, 16 H), 2.83 (m, 2 H), 4.07 (q, J = 8.5 Hz, 2 H), 5.10 (s, 2 H), 6.68 (s, 1 H), 6.88 (s, 1 H), 7.20−7.60 (m, 5 H).

2-Ethoxy-3-(benzyloxy)-17β-(benzylamino)estra-1,3,5-(10)-triene (36). Glacial acetic acid (0.8 mL), benzylamine (1.6 mL, 15 mmol), and sodium cyanoborohydride (0.9 g, 14 mmol) were added under Ar to a solution of 17-keto derivative **35** (2 g, 5 mmol) in anhydrous 1,2-dichloroethane (30 mL). The mixture was stirred at room temperature for 72 h. Additional amounts of anhydrous benzylamine (0.4 mL), acetic acid (0.2 mL), and sodium cyanoborohydride (0.25 g) were added to the mixture, and stirring was continued for 24 h. The solvent was removed under reduced pressure, and the residue was diluted with water (50 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic layer was washed with water (100 mL), sodium bicarbonate solution (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The organic layer on evaporation under reduced pressure gave crude product (3.1 g), which was subjected to column chromatography on silica gel using hexane, followed by hexane−CH_2Cl_2 (1:1), and finally CH_2Cl_2. Appropriate fractions were combined and evaporated under reduced pressure to give pure **36** (2.2 g, 89%) as a sticky solid which was used without further purification: IR (KBr) 2921, 1608, 1508, 1453, 1264, 1209, 1119, 1019, 729 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.52−7.25 (m, 11 H), 6.85 (s, 1 H), 6.65 (s, 1 H), 4.20 (q, J = 8.5 Hz, 2 H), 3.80 (s, 2 H) 2.75 (m, 1 H) 2.65 (t, J = 8.5 Hz, 1 H), 2.35−2.12 (m, 4 H), 2.10 (s, 2 H), 1.85 (m, 4 H), 1.75 −1.52 (m, 6 H) 1.40 (t, J = 8.5, 3 H), 0.78 (s, 3 H).

2-Ethoxy-3-hydroxy-17β-aminoestra-1,3,5(10)-triene Hydrochloride (37). 20% $Pd(OH)_2$−C (1.7 g) was added *carefully under Ar* to a solution of 17-benzylamino derivative **36** (1.52 g, 3.0 mmol) in anhydrous THF (20−30 mL). The resulting mixture was hydrogenated at 45−50 psi on a Parr apparatus for 27 h. After completion, the catalyst was removed via filtration using a Celite pad under Ar, and the pad was washed with THF (150 mL), followed by a 1:1 mixture of THF−CH_2Cl_2 (100 mL). The filtrate obtained on evaporation under reduced pressure gave impure compound (1.08 g), which was purified by column chromatography. The resulting material was then converted to the hydrochloride salt (0.16 g, 15%) by dissolving it in ether (100 mL) and bubbling HCl gas through the solution: mp 270 °C; IR (KBr) 3137, 2931−2261, 1612, 1499, 1396 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.60 (bs, 1 H, exchangeable with D_2O), 8.20 (bs, 2 H, exchangeable with D_2O), 6.75 (s, 1 H), 6.52 (s, 1 H), 3.95 (q, J = 8.5 Hz, 2 H), 3.60−3.20 (m, 4 H), 3.00 (t, J = 8.5 Hz, 1 H), 2.82−2.42 (m, 4 H), 2.40−1.90 (m, 4 H), 1.88−1.55 (m, 3 H) 1.27 (t, J = 8.5, 3 H), 0.78 (s, 3 H). Anal. ($C_{20}H_{29}ClNO_2 \cdot HCl \cdot 0.5C_2H_5OH$) C, H, N.

Bis[2-ethoxy-3-hydroxyestra-1,3,5(10)-trien-17β-yl] Sulfite (38). $SOCl_2$ (2 mL) was added under Ar at 0 °C to a stirred suspension of 2-ethoxy-β-estradiol **(2)** (0.5 g, 1.6 mmol) in anhydrous benzene (2 mL). The reaction mixture was allowed to warm to room temperature, and stirring was continued for 14 h. The solvent was evaporated under reduced pressure, and the residue was diluted with dichloromethane (200 mL). The organic layer was washed with water (50 mL) and dried over sodium sulfate. The organic layer, on evaporation under reduced pressure, gave a mixture of compounds, which on purification over a silica gel column using hexane−ethyl acetate mixture as eluant gave compound **38** (0.18 g, 35%): R_f = 0.46 [hexane−ethyl acetate (4:1)]; IR (KBr) 3550, 3428, 2974−2867, 1738, 1611, 1518, 1474, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.78 (s, 2 H), 6.68 (s, 2 H), 5.55 (s, 2 H), 4.55 (t, 1 H), 4.3 (t, 1 H), 4.08 (q, 4 H), 2.78 (bs, 4 H), 2.5−1.05 (m, 32 H), 0.85 (d, 3 H); MS (FAB) m/z 678 (M^+), (negative ion FAB) m/z 677 [$(M - H)^-$]. Anal. ($C_{40}H_{54}SO_7 \cdot 0.2CH_3CO_2Et$) C, H, S.

2,4-Dibromoestra-1,3,5(10)-triene-3,17β-diol (39). Estradiol (2.0 g, 7.4 mmol) was dissolved in glacial acetic acid (300 mL) at 80 °C and cooled to 15−17 °C, resulting in partial precipitation of solid. A solution of bromine (0.75 mL, 14.6 mmol) in glacial acetic acid was added dropwise, and the mixture was stirred at 15−17 °C for 32 min. The reaction mixture was poured into an ice−water mixture (1 L). A yellow solid precipitated. It was removed by filtration and dissolved in ethyl acetate (400 mL). The ethyl acetate solution was washed with water (100 mL) and brine (100 mL) and dried over sodium sulfate. The organic layer on evaporation under reduced pressure gave a crude mixture, which on crystallization from ethyl acetate gave compound **39** (1.85 g, 72%): mp 220−222°C (lit.[44] mp 214−215 °C); R_f (dichloromethane, silica gel) 0.26; ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1 H), 5.88 (s, 1 H, exchangeable with D_2O), 3.75 (t, 1 H), 3.05−2.80 (dd, 1 H), 2.78−2.45 (m, 1 H), 2.45−1.10 (m, 13 H), 0.82 (s, 3 H).

Tubulin Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[45] Determination of IC_{50} values for the polymerization of purified tubulin was performed as described in detail elsewhere.[5] In brief, tubulin was preincubated at 26 °C with varying compound concentrations, reaction mixtures were chilled on ice, GTP (required for the polymerization reaction) was added, and polymerization was followed at 26 °C by turbidimetry at 350 nm in Gilford recording spectrophotometers equipped with electronic temperature controllers. The extent of polymerization after 20 min was determined. IC_{50} values were determined graphically. All compounds were examined in at least two independent assays. Inhibition of colchicine binding to tubulin was performed as described previously.[5] Reaction mixtures contained 1.0 μM tubulin (0.1 mg/mL), 5.0 μM [^{3}H]-colchicine, and 50 μM inhibitor. Incubation was for 10 min at 37 °C.

Measurement of Estrogen Receptor Relative Binding Affinities (RBA). Estrogen receptor RBA's were determined using competitive radiometric binding assays using a tritium-labeled estrogen as the tracer and immature rat uterine cytosol as the source of receptor, according to the methods described previously.[46] By definition, estradiol is given an RBA value of 100. The assays of **21**, **22**, and **28** were conducted at 0 °C for 20 h, while those for **1** and **2** were performed at 0 °C for 18 h. The coefficient of variation in replicate experiments is typically less than 30%.

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