Articles

Synthesis, Antitubulin and Antimitotic Activity, and Cytotoxicity of Analogs of 2-Methoxyestradiol, an Endogenous Mammalian Metabolite of Estradiol That Inhibits Tubulin Polymerization by Binding to the Colchicine Binding Site

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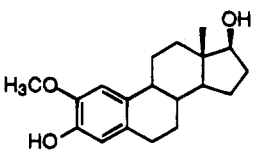
Received March 13, 1995®

In order to define the structural parameters associated with the antitubulin activity and cytotoxicity of 2-methoxyestradiol, a mammalian metabolite of estradiol, an array of analogs was synthesized and evaluated. The potencies of the new congeners as inhibitors of tubulin polymerization and colchicine binding were determined using tubulin purified from bovine brain, and the cytotoxicities of the new compounds were studied in a variety of cancer cell cultures. Maximum antitubulin activity was observed in estradiols having unbranched chain substituents at the 2-position with three non-hydrogen atoms. 2-Ethoxyestradiol and 2-((*E*)-1-propenyl)-estradiol were substantially more potent than 2-methoxyestradiol itself. The tubulin polymerization inhibitors in this series displayed significantly higher cytotoxicities in the MDA-MB-435 breast cancer cell line than in the other cell lines studied. The potencies of the analogs as cytotoxic and antimitotic agents in cancer cell cultures correlated with their potencies as inhibitors of tubulin polymerization, supporting the hypothesis that inhibition of tubulin polymerization is the mechanism of the cytotoxic action of 2-methoxyestradiol and its congeners. Several of the more potent analogs were tested in an estrogen receptor binding assay, and their affinities relative to estradiol were found to be very low.

2-Methoxyestradiol (**1**) is a naturally occurring mammalian metabolite formed by hepatic hydroxylation of estradiol followed by *O*-methylation.[1,2] Widespread interest in 2-methoxyestradiol (**1**) has resulted from its cytotoxicity in cancer cell cultures, which is associated with uneven chromosome distribution, faulty spindle formation, inhibition of DNA synthesis and mitosis, and an increase in the number of abnormal metaphases.[3,4] Recently it has been discovered that 2-methoxyestradiol (**1**) binds to the colchicine binding site of tubulin, resulting in either inhibition of tubulin polymerization or formation of polymer with altered stability properties and morphology, depending on the reaction conditions.[5] These observations suggest that 2-methoxyestradiol (**1**) could be functioning as a natural regulator of mammalian microtubule assembly and function. In addition to these antitubulin and antimitotic properties of 2-methoxyestradiol (**1**), recent *in vitro* results have shown that it also inhibits angiogenesis (the creation of new blood vessels), which is required for the growth of solid tumors.[6–8] When investigated *in vivo* by oral administration in mice, 2-methoxyestradiol was found to be a potent inhibitor of neovascularization of solid tumors and to inhibit their growth.[6] These *in vivo* antitumor effects were accompanied by no apparent signs of toxicity.[6]



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In view of these exciting results, we devised a versatile synthesis of 2-methoxyestradiol (**1**) designed to facilitate preparation of an array of congeners that would be of value in the investigation of its antitumor and antitubulin activities.[9] The exploitation of 2-methoxyestradiol (**1**) as a lead compound might also lead to novel therapeutic agents with superior biological properties for use in cancer treatment. The present report details the synthesis and the biological evaluation of a series of 2-methoxyestradiol analogs. The new compounds were evaluated for *in vitro* cytotoxicity in a variety of cancer cell cultures, for inhibition of tubulin polymerization and colchicine binding, and for antimitotic activity. The estrogen receptor binding affinities of several of the more potent compounds were also investigated.

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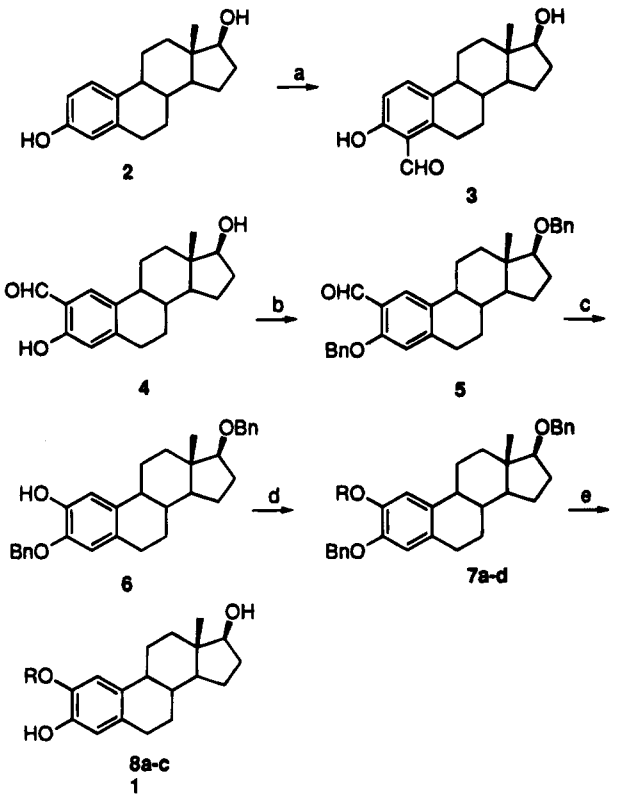
® Abstract published in *Advance ACS Abstracts,* June 1, 1995.

0022-2623/95/1838-2041$09.00/0

Scheme 1[a]



a, R = Et
b, R = *n*-Pr
c, R = *i*-Pr
1, R = Me

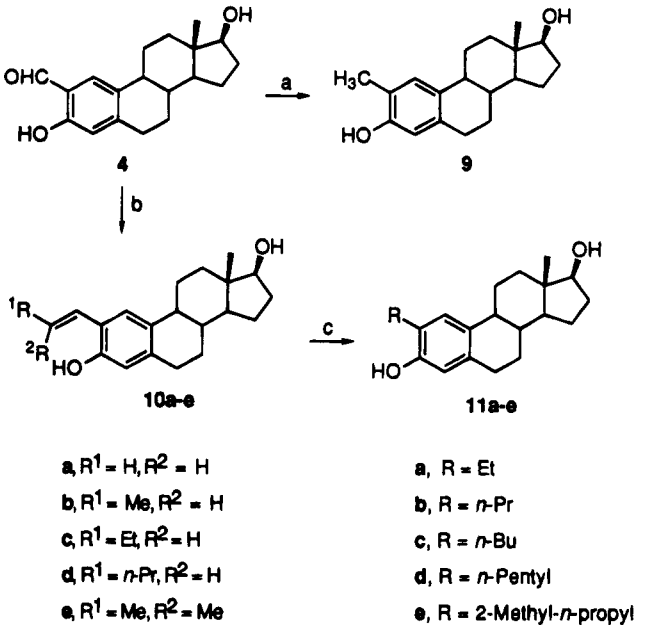
[a] Reagents and conditions: (a) hexamethylenetetramine, CF_3COOH, reflux (6 h); (b) NaH, $PhCH_2Br$, $Bu_4N^+I^-$, DMF, 23 °C (3 h); (c) MCPBA, *p*-TsOH·H_2O, CH_2Cl_2, 23 °C (3 h); (d) (1) K_2CO_3, DMF, 23 °C (10 min), (2) RBr or RI, *n*-$Bu_4N^+I^-$, 23 °C (24 h); (e) H_2, Pd–C, THF, 23 °C.

Chemistry

2-Ethoxy-, 2-propoxy-, 2-isopropoxy-, and 2-methoxy-β-estradiol were prepared as shown in Scheme 1. Formylation of estradiol (**2**) with 2 equiv of hexamethylenetetramine in refluxing trifluoroacetic acid gave a mixture of 4-formyl-β-estradiol (**3**) as the minor product and 2-formyl-β-estradiol (**4**) as the major product. Treatment of **4** with excess sodium hydride and a catalytic amount of tetra-*n*-butylammonium iodide in DMF yielded a dianion that was treated with benzyl bromide to afford the dibenzylated intermediate **5**. Baeyer–Villiger oxidation of **5** with *m*-chloroperbenzoic acid and a catalytic amount of *p*-toluenesulfonic acid in methylene chloride gave the phenol **6**. Deprotonation of the phenolic hydroxyl group of **6** with potassium carbonate in dimethylformamide afforded the corresponding phenoxide anion, which was alkylated with ethyl, propyl, and isopropyl bromide, as well as methyl iodide, to afford intermediates **7a–d**. Cleavage of the two benzyl protecting groups of the intermediates yielded the corresponding 2-alkoxy-β-estradiols **8a–c** and **1**.

The synthesis of a series of 2-alkyl- and 2-alkenyl-β-estradiols is outlined in Scheme 2. 2-Methylestradiol (**9**) was prepared by catalytic reduction of 2-formylestradiol (**4**)[9] using palladium on charcoal as the catalyst.

Scheme 2[a]

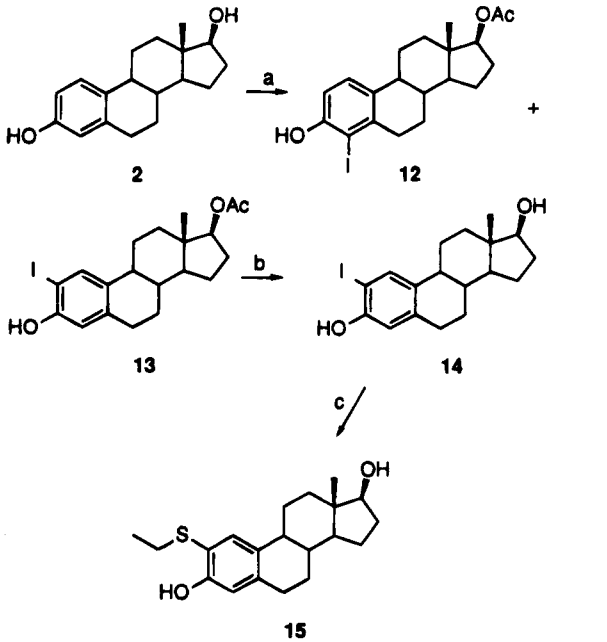


a, R^1 = H, R^2 = H
b, R^1 = Me, R^2 = H
c, R^1 = Et, R^2 = H
d, R^1 = *n*-Pr, R^2 = H
e, R^1 = Me, R^2 = Me

a, R = Et
b, R = *n*-Pr
c, R = *n*-Bu
d, R = *n*-Pentyl
e, R = 2-Methyl-*n*-propyl

[a] Reagents and conditions: (a) H_2, Pd–C, EtOH, THF, 23 °C (18 h); (b) (1) $RPh_3P^+Br^-$, $Li[N(SiMe_3)_2]$, THF, 0 °C (10 min), (2) **5**, THF, 0 °C (4 h); (c) H_2, Pd–C, THF, 23 °C.

Scheme 3[a]

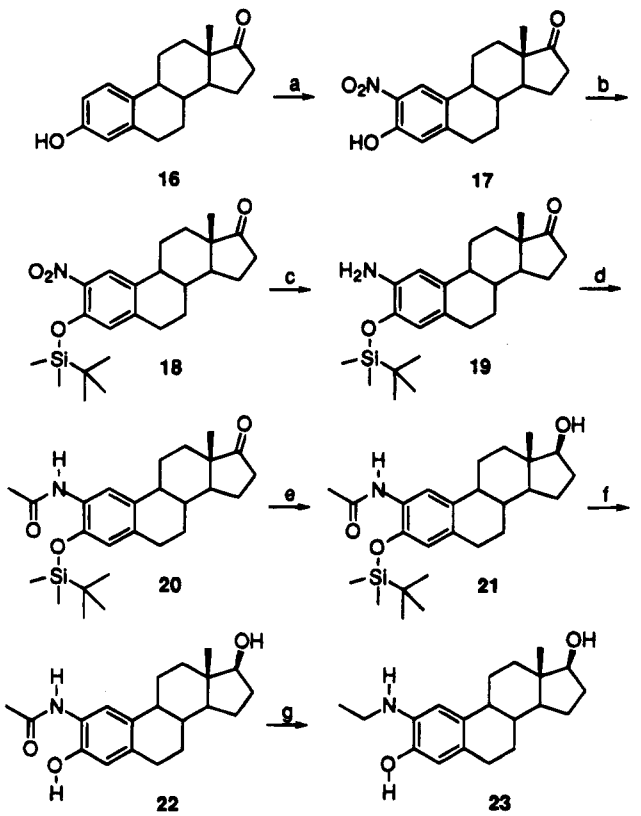


[a] Reagents and conditions: (a) I_2, $CuCl_2$, AcOH, 60 °C (35 h); (b) LiOH·H_2O, aq THF, 23 °C (48 h); (c) NaSEt, CuI, 18-crown-6, DMF, reflux (24 h).

The aldehyde **4** was also reacted with a series of Wittig reagents to afford the corresponding 2-(*E*)-alkenylestradiols **10a–e**. The expected *E* geometry of the alkenes **10a–d** was supported by the 14–18 Hz trans coupling constants observed in their ^1H NMR spectra. The alkenes **10a–e** were subjected to catalytic reduction to afford the 2-alkylestradiols **11a–e**.

As depicted in Scheme 3, iodination of estradiol (**2**) with iodine in acetic acid in the presence of cupric chloride yielded a mixture of 4-iodoestradiol-17-acetate (**12**) and 2-iodoestradiol-17-acetate (**13**) as the minor and major products, respectively. The 2-iodo compound **13** was isolated from the mixture in 63% yield by fractional

Scheme 4[a]



[a] Reagents and conditions: (a) HNO_3, AcOH, 23 °C (12 h); (b) *tert*-butyldimethylsilyl chloride, imidazole, DMF, 23 °C (24 h); (c) H_2, Pd–C, THF; (d) Ac_2O, pyridine, 0 °C (24 h); (e) $NaBH_4$, THF, 23 °C (20 h); (f) n-$Bu_4N^+F^-$, THF, 23 °C (5 h); (f) $LiAlH_4$, THF, reflux (1 h).

crystallization from methanol. The 2,3-disubstitution pattern of **13** was indicated by the appearance of the two aromatic protons as singlets in the ^1H NMR spectrum. Hydrolysis of the 17β-acetate **13** with lithium hydroxide in aqueous THF afforded 2-iodoestradiol (**14**). Treatment of **14** with sodium thiolate and cuprous iodide in refluxing DMF in the presence of 18-crown-6 afforded 2-(ethylthio)estradiol (**15**).

The synthesis of 2-(ethylamino)estradiol (**23**) is outlined in Scheme 4. Nitration of estrone (**16**) with nitric acid in acetic acid afforded 2-nitroestrone (**17**). As with compound **13**, the 2,3-disubstitution pattern of **17** was established by the appearance of the two aromatic protons as singlets in the ^1H NMR spectrum. Treatment of **17** with *tert*-butyldimethylsilyl chloride in DMF in the presence of imidazole afforded the *tert*-butyldimethylsilyl ether **18**. The 2-amino compound **19** was obtained by catalytic reduction of the nitro group of **18** using palladium on charcoal as the catalyst. The amino group of **19** was acetylated with acetic anhydride in pyridine to give the 2-acetamido intermediate **20**. Sodium borohydride reduction of the ketone **20** afforded the expected 17β-alcohol **21**.[10] Removal of the *tert*-butyldimethylsilyl protecting group from **21** with tetra-n-butylammonium fluoride in THF, followed by reduction of the acetamido group of **22** with lithium aluminum hydride, provided the desired 2-(ethylamino)estradiol (**23**).

Biological Results and Discussion

The inhibitory activities of 2-methoxyestradiol (**1**) and analogs on tubulin polymerization, [^{3}H]colchicine binding to tubulin, and cancer cell growth are listed in Table 1. Inhibition of tubulin polymerization was tested using electrophoretically homogeneous tubulin from bovine brain, and eight of the new analogs displayed significant activity ($IC_{50} \leq 5.0$ μM). In fact, three of the analogs were more potent than the natural product, 2-methoxyestradiol (IC_{50} 2.9 μM). The most potent of these proved to be 2-ethoxyestradiol (**8a**) (IC_{50} 0.91 μM) followed closely by 2-(1-(*E*)-propenyl)estradiol (**10b**) (IC_{50} 1.1 μM). In addition, **10a** showed good inhibitory activity with an IC_{50} value of 2.4 μM. An additional 10 congeners had lower tubulin polymerization inhibitory activity than 2-methoxyestradiol (**1**), with IC_{50} values ranging from 3.0 to 17 μM.

Previously 2-methoxyestradiol (**1**) was shown to be a relatively weak competitive inhibitor of the binding of [^{3}H]colchicine to tubulin[5] (apparent K_i value of 22 μM, as compared with apparent K_i values of 0.5 and 0.1 μM

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

		cytotoxicity (GI_{50}, μM)[a]										
no.	C-2 R[b]	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12-C	prostate DU-145	breast MDA-MB-435	MGM[c]	inhibn of tubulin polymn IC_{50} (μM)	% inhibn of colchicine binding[d]
1	CH_3O	0.70	0.47	0.32	0.36	0.21	0.95	1.8	0.080	1.3	2.9 ± 0.6	38
8a	C_2H_5O	0.018	0.026	0.014	0.016	0.016	0.039	0.065	<0.01	0.076	0.91 ± 0.5	71
8b	n-C_3H_7O	1.7	1.4	1.4	1.0	1.0	4.8	3.8	0.30	1.6	4.2 ± 0.8	33
8c	i-C_3H_7O	3.5	3.3	2.4	2.2	2.4	5.5	7.2	0.76	4.1	4.8 ± 0.5	24
9	CH_3	11	3.6	7.4	5.4	9.1	17	16	1.8	8.1	17 ± 3	21
10a	$H_2CH{=}CH$	5.0	2.6	2.1	2.5	2.6	5.1	4.4	1.1	5.1	2.4 ± 0.04	47
10b	$CH_3CH{=}CH$	0.083	0.033	0.037	0.041	0.028	0.080	0.19	<0.01	0.14	1.1 ± 0.06	71
10c	$C_2H_5CH{=}CH$	2.7	1.0	0.64	0.48	0.26	5.1	2.8	0.25	2.0	8.6 ± 3	19
10d	n-$C_3H_7CH{=}CH$	22	17	20	17	17	17	19	16	17	>40	0
10e	$(CH_3)_2CH{=}CH$	15	10	7.6	11	5.3	15	14	3.0	10	9.4 ± 0.8	22
11a	C_2H_5	6.1	3.5	3.3	–	2.9	7.3	11	1.7	6.5	7.7 ± 2	23
11b	n-C_3H_7	16	6.5	11	6.0	12	12	19	2.2	10	4.9 ± 0.7	19
11c	n-C_4H_9	28	14	26	12	14	19	18	17	17	>40	6
11d	n-C_5H_{11}	14	17	24	19	14	16	16	16	18	>40	4
11e	$(CH_3)_2CHCH_2$	16	7.3	20	9.3	6.4	9.5	10	12	7.4	>40	7
13	I (17-OAc)	11	16	15	12	17	18	15	13	13	>40	–
14	I	4.2	5.0	3.0	3.6	3.5	6.6	6.9	2.1	5.5	4.8 ± 1	32
15	C_2H_5S	16	8.5	16	11	14	18	24	2.0	10	10 ± 3	18
22	CH_3CONH	72	40	35	31	9.1	49	>100	20	36	>40	12
23	C_2H_5N	0.58	3.1	0.74	0.31	0.81	16	3.8	0.051	3.1	3.0 ± 0.9	33

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. The values for compounds **8a–c**, **10b,d**, **11e**, **14**, and **23** are the averages of at least two determinations. The remaining values are the results of a single determination. [b] Substituent in the 2-position of 2-substituted estradiols (except for **13**, which is the 17-acetate). [c] Mean graph midpoint for all human cancer cell lines (approximately 55) tested. [d] Reaction mixtures contained 1.0 μM tubulin (0.1 mg/mL), 5.0 μM [^{3}H]colchicine, and 50 μM inhibitor.

Table 2. Cyclotoxicities of 2-((*E*)-1-Propenyl)estradiol (**10b**)

panel/cell line	$\log_{10}$ TGI
leukemia	
CCRF-CEM	−4.2
MOLT-4	−4.9
RPMI-8226	>−4.0
SR	>−4.0
non-small-cell lung cancer	
A549/ATCC	−4.8
EKCX	−4.7
HOP-62	−4.7
HOP-92	−4.6
NCI-H226	−4.8
NCI-H23	−5.0
NCI-H322M	−4.8
NCI-H460	−4.9
NCI-H522	<−8.0
colon cancer	
COLO 205	−4.6
HCC-299B	−6.1
HCT-116	−5.0
HT29	−4.7
KM12	−4.8
SW-620	−4.7
CNS cancer	
SF-268	−4.7
SF-295	−4.7
SF-539	−6.9
SNB-19	−4.9
U251	−4.9
melanoma	
LOX IMVI	−4.8
MALME-3M	−4.7
M14	−4.3
SK-MEL-2	−4.9
SK-MEL-28	−4.7
SK-MEL-5	−4.9
UACC-257	−4.7
UACC-62	−4.8
ovarian cancer	
IGR-OV1	−4.8
OVCAR-3	−4.9
OVCAR-4	−4.7
OVCAR-5	−4.7
OVCAR-8	−4.8
SK-OV-3	−4.9
renal cancer	
786-0	−4.8
A498	−5.4
ACHN	−4.7
CAKI-1	−4.7
SN12C	−4.8
TK-10	−4.7
UO-31	−4.8
prostate cancer	
PC-3	−4.8
DU-145	−6.2
breast cancer	
MCF7	−7.1
MCF7/ADR-RES	−6.9
MDA-MB-231/ATCC	−4.9
MDA-MB-435	<−8.0
MDA-N	<−8.0
SK-BR-3	−6.4
MDA-MB-468	−4.8
MAXF401	−7.4
average (all cell lines)	−5.2

for podophyllotoxin and combretastatin A-4, respectively). We therefore examined the activity of the current series of analogs as inhibitors of colchicine binding, with tubulin at 1.0 μM, [^{3}H]colchicine at 5.0 μM, and the potential inhibitors at 5.0 and 50 μM. Results at the higher inhibitor concentration are summarized in Table 1, and these results are in accord with the polymerization data. The only analogs more effective than 2-methoxyestradiol (**1**) were **8a** and **10a,b**. These analogs, too, are relatively weak inhibitors of colchicine binding. At 5.0 μM the greatest effect, 33% inhibition, was observed with compound **10b**, as compared with 89% inhibition obtained with combretastatin A-4.

The antitubulin results presented in Table 1 strongly suggest that there is a critical size factor of the 2-substituent which modulates the interactions with tubulin of this series of compounds. The two most active compounds, **8a** and **10b**, have an unbranched chain in the 2-position containing three atoms from the second row of the periodic table. This is also true of compound **23**, which has activity comparable to that of 2-methoxyestradiol. The lower activity displayed by 2-(ethylthio)-β-estradiol (**15**) may reflect the large size of the sulfur, being present in the third row of the periodic table. The van der Waals radius of sulfur is 1.85 Å, as opposed to 1.40 Å for oxygen.[11] Comparison of the structures of **8a**, **10b**, and **23** with that of the less active 2-(*n*-propyl) analog **11b** also indicates that the increased electron density next to the aromatic ring contributed by the double bond in **10b**, the nonbonded electrons of the oxygen in **8a**, and the nonbonded electrons of the nitrogen in **23** may enhance the inhibitory activity. This electronic effect also seems to be operating in the series **1**, **10a**, and **11a**, which contain two-atom side chains (excluding hydrogens), as well as in the series **8b**, **10c**, and **11c**, which contain four-atom side chains (excluding hydrogens). The optimal type of substituent in the 2-position for inhibition of tubulin polymerization appears to be an unbranched chain containing three atoms from the second row of the periodic table, with biological activity dropping off as the chain is either lengthened or shortened.

We also wished to confirm the findings[3] that 2-methoxyestradiol (**1**) caused mitotic disruption in cultured cells and extend the observation to the new analogs. We chose to examine human Burkitt lymphoma CA46 cells, which we have found to reliably yield a high percentage of mitotic figures when treated with antimitotic drugs. With these cells we obtained a GI_{50} value of 2 μM for 2-methoxyestradiol (**1**), and compounds **8a** and **10b** were 10-fold more active (GI_{50} values of 0.2 μM). All other agents examined had GI_{50} values greater than 5 μM. Cells were examined for mitotic arrest at equivalent drug concentrations (4 times the GI_{50} value). The mitotic index values obtained were 42%, 37%, and 31% for compounds **1**, **8a**, and **10b**, respectively (control value 5%).

The effects of 2-methoxyestradiol (**1**) and analogs on cancer cell growth were examined in the National Cancer Institute (NCI) Developmental Therapeutics Program's *in vitro* panel of approximately 55 human cancer cell lines. Representative results are listed in Table 1 for HOP-62 non-small-cell lung cancer cells, HCT-116 colon cancer cells, SF-539 central nervous system (CNS) cancer cells, UACC-62 melanoma cells, OVCAR-3 ovarian cancer cells, SN12-C renal cancer cells, DU-145 prostate cancer cells, and MDA-MB-435 breast cancer cells. Several conclusions can be drawn on the basis of the cytoxicity data given in Table 1. The close correlation between the potencies of these compounds as cytotoxic agents in multiple cell lines and their potencies as tubulin polymerization inhibitors supports the hypothesis that inhibition of tubulin polymerization is the mechanism of the cytotoxicity of the 2-methoxyestradiol analogs. Further, the analogs that

Table 3. Relative Estrogen Receptor Binding Affinities of Selected 2-Substituted Estradiol Analog Tubulin Polymerization Inhibitors

compd	RBA[a] 0 °C	RBA[a] 25 °C
1	0.245 (±0.007)	0.0225 (±0.007)
8a	0.011 (±0.003)	0.020 (±0.001)
10b	0.0095 (±0.005)	0.021
15	0.285 (±0.163)	0.235 (±0.050)
23	0.35 (±0.106)	0.014 (±0.001)

[a] Relative binding affinity determined in a competitive radiometric assay, estradiol = 100%. Values are the average of two experiments. Details are given in the Experimental Section and ref 19.

inhibit tubulin polymerization, along with 2-methoxyestradiol (**1**) itself, have significantly greater cytotoxicity in MDA-MB-435 breast cancer cells than in the other lines. This selective cytotoxic effect is not present with the analogs which do not inhibit tubulin polymerization (IC_{50} > 40 μM).

More extensive cytotoxicity testing results are listed in Table 2 for 2-(1-(*E*)-propenyl)estradiol (**10b**) in 55 cancer cell lines in the NCI screen. The values in Table 2 are $\log_{10}$ molar concentrations for total growth inhibition (TGI). These results suggest a more general, selective cytotoxicity for breast cancer cell lines. The average $\log_{10}$ molar cytotoxic concentration for TGI by **10b** in all cell lines was −5.2. The $\log_{10}$ TGI molar concentrations in six of the eight breast cancer cell lines were significantly lower, indicating increased sensitivity of these cells to the cytotoxic effect of **10b** by factors in the 100−1000 range. The selective cytotoxicity of **10b** for human breast cancer cells is obviously of interest in relation to the potential therapeutic application of **10b** and related compounds for the treatment of breast cancer.

One concern about the potential therapeutic application of the present series of tubulin polymerization inhibitors is that they might bind to estrogen receptors and display either agonist or antagonist effects. In order to investigate this question, several analogs were analyzed for their binding affinity to the estrogen receptor. More specifically, 2-methoxyestradiol (**1**), 2-ethoxyestradiol (**8a**), 2-(1-(*E*)-propenyl)estradiol (**10b**), 2-(ethylthio)estradiol (**15**), and 2-(ethylamino)estradiol (**23**) were tested, and the results are displayed in Table 3. Competitive radiometric binding assays were used to determine the relative binding affinities (RBA) as compared with estradiol (RBA = 100, K_d = 0.3 nM). Assays were performed at both 0 and 25 °C (Table 3). All analogs tested bound weakly to estrogen receptors relative to estradiol, but the affinities were not negligible. The two compounds most potent in the tubulin polymerization and cytotoxicity assays, **8a** and **10b**, displayed very low affinities for estrogen receptors. This, together with the fact that of the breast cancer cell lines tested only the two MCF7 lines are positive for estrogen receptors, makes it unlikely that the high cytotoxicity of these compounds in breast cancer cells is mediated to any significant degree by their binding to the estrogen receptor. The cytotoxicity of the compounds in the MCF7 cell lines was not significantly different from that observed in the other cell lines.

In conclusion, a new class of tubulin polymerization inhibitors has been synthesized using 2-methoxyestradiol (**1**) as a lead compound. Several of the compounds are more potent as cytotoxic agents in cancer cell cultures and as tubulin polymerization inhibitors than the natural metabolite **1** itself, and these analogs are being investigated *in vivo* as anticancer agents. Future work will concentrate on the design and the synthesis of additional 2-substituted estradiols in which the side chain approximates that of **8a**, **10b**, and **23** in size and electron density. Hopefully, some fine tuning of these steric and electronic parameters will result in additional compounds with therapeutic potential 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 Varian VXR-500S and Brüker ARX-300 spectrometers, and IR spectra on a Beckman IR-33 spectrometer or a Perkin Elmer 1600 series FTIR. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values are within ±0.4% of the calculated compositions.

2-Formylestradiol (3) and 4-Formylestradiol (4). A solution of 17β-estradiol (**2**) (5.0 g, 98%, 18 mmol) and hexamethylenetetramine (7.6 g, 99%, 54 mmol) in trifluoroacetic acid (30 mL) was heated at reflux under argon for 6 h. An orange solution developed. The reaction mixture was poured into ice-cooled water (100 mL), and the products were extracted with ether (100, 50 mL). The combined ether phase was washed twice with 30 mL of saturated aqueous NaCl solution and dried over Na_2SO_4. Evaporation of the filtered ether solution at 40 °C under vacuum gave the crude product as an oil. The oil was subjected to flash chromatography (silica gel, 230-400 mesh; ether:hexane, 1:6 by volume) to afford compound **3** as a yellow solid (0.71 g, 13.2%): mp 173−176 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.01 (s, 1 H), 10.39 (s, 1 H), 7.49 (d, J = 8.7 Hz, 1 H), 6.81 (d, J = 8.7 Hz, 1 H), 4.91 (t, J = 7.8 Hz, 1 H), 3.35 (dd, J = 5.2, 5.9 Hz, 1 H), 3.15 (m, 1 H), 2.34 (m, 3 H), 1.95 (m, 2 H), 1.77 (m, 3 H), 1.47 (m, 6 H), 0.91 (s, 3 H); CIMS (isobutane) *m/z* 301 (MH^+, 14), 283 ($MH - H_2O$, 100).

The above chromatography followed by recrystallization from ethanol also gave compound **4** as a white solid (1.34 g, 24.8%): mp 230−232 °C (lit.[12] mp 231−233 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.79 (s, 1 H), 9.82 (s, 1 H), 7.41 (s, 1 H), 6.71 (s, 1 H), 4.90 (t, J = 7.8 Hz, 1 H), 2.91 (m, 2 H), 2.34 (m, 3 H), 1.95 (m, 2 H), 1.78 (m, 3 H), 1.52 (m, 6 H), 0.90 (s, 3 H); CIMS (isobutane) *m/z* 301 (MH^+, 5.71), 283 ($MH - H_2O$, 100).

3,17β-Bis(benzyloxy)estra-1,3,5(10)-triene-2-carbaldehyde (5). A solution of compound **4** (2.87 g, 9.56 mmol) in toluene (40 mL) was azeotropically evaporated to dryness under reduced pressure, and the residue was dissolved in anhydrous DMF (50 mL) under argon. The solution was cooled to 0−5 °C, and NaH (60%, 3.82 g, 95.6 mmol, 10.0 equiv) was added in portions with stirring. The resulting yellow suspension was stirred at ca. 0 °C under argon for 10 min. Benzyl bromide (11.6 g, 8.07 mL, 67.8 mmol, 7.1 equiv) and tetra-*n*-butylammonium iodide (100 mg) were added sequentially to the suspension, and the mixture was stirred at room temperature under argon for 3 h. Excess NaH was quenched by adding 50% ethanol dropwise at ca. 0 °C with stirring. The reaction mixture was acidified to pH 5, and the product was extracted twice with ether (50, 30 mL) in the presence of saturated aqueous NaCl solution (20 mL). The combined ether layer was washed with saturated NaCl solution (10 mL) and dried over $MgSO_4$. Evaporation of the filtrate gave the crude product as an oil that was subjected to flash chromatography (silica gel, 230−400 mesh; ether:hexane, 1:5−1:4 by volume) to give compound **5** as a white solid (1840 mg, 40.2%). The analytical sample was recrystallized from chloroform and hexane: mp 124−125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, 1 H), 7.79 (s, 1 H), 7.38 (m, 10 H), 6.75 (s, 1 H), 5.15 (s, 2 H), 4.58 (s, 2 H), 3.51 (t, J = 8.4 Hz, 1 H), 2.88 (m, 2 H), 2.36

(m, 1 H), 2.09 (m, 4 H), 1.87 (m, 1 H), 1.41 (m, 7 H), 0.87 (s, 3 H). Anal. ($C_{33}H_{36}O_3$) C, H.

3,17β-Bis(benzyloxy)-2-hydroxyestra-1,3,5(10)-triene (6). A solution of the aldehyde **5** (480 mg, 1.0 mmol), *m*-chloroperbenzoic acid (80–90%, 259 mg, 1.2 mmol), and *p*-TsOH·H_2O (5 mg) in CH_2Cl_2 (10 mL) was stirred at room temperature under argon for 3 h. A pale yellow solution developed. Excess *m*-chloroperbenzoic acid was destroyed by adding Na_2SO_3 solution (20 mL) to the reaction mixture and stirring for 0.5 h. The product was extracted with CH_2Cl_2 (30, 20 mL). The combined CH_2Cl_2 layer was washed with saturated NaCl solution (10 mL) and dried over anhydrous $MgSO_4$. Evaporation of the filtrate gave the crude product as a pale brown oil, which was subjected to flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:4 by volume) to give compound **6** as a colorless oil (270 mg, 57.7%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 10 H), 6.90 (s, 1 H), 6.64 (s, 1 H), 5.45 (s, 1 H, exchangeable with D_2O), 5.06 (s, 2 H), 4.57 (s, 2 H), 3.50 (t, *J* = 8.0 Hz, 1 H), 2.76 (m, 2 H), 2.11 (m, 3 H), 1.43 (m, 10 H), 0.87 (s, 3 H); CIMS (isobutane) *m/z* 469 (MH^+, 100).

3,17β-Bis(benzyloxy)-2-ethoxyestra-1,3,5(10)-triene (7a). Anhydrous K_2CO_3 (383 mg, 2.7 mmol) was added to a solution of the phenol **6** (130 mg, 0.27 mmol) in anhydrous DMF (10 mL). The resulting yellow suspension was stirred under argon for 10 min. Ethyl bromide (441 mg, 4.05 mmol) was added dropwise followed by tetra-*n*-butylammonium iodide (10 mg). The resulting colorless mixture was stirred at room temperature under argon for 24 h and extracted twice with ether (20, 10 mL) in the presence of saturated NaCl solution (10 mL). The combined ether phase was washed with saturated NaCl solution (10 mL) and dried over $MgSO_4$. Evaporation of the ether gave the crude product **7a** as a pale yellow oil. Flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:4 by volume) gave product **7a** as an oil (110 mg, 82.2%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 10 H), 6.86 (s, 1 H), 6.64 (s, 1 H), 5.09 (s, 2 H), 4.59 (s, 2 H), 4.09 (q, *J* = 6.9 Hz, 2 H), 3.50 (t, *J* = 8.3 Hz, 1 H), 2.73 (m, 2 H), 2.10 (m, 4 H), 1.83 (m, 1 H), 1.40 (m, 11 H), 0.87 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 497 (MH^+, 100).

3,17β-Bis(benzyloxy)-2-*n*-propoxyestra-1,3,5(10)-triene (7b). From compound **6** (1200 mg, 2.35 mmol), a similar synthesis as described in the preparation of **7a** yielded compound **7b** as a pale brown oil (1.16 g, 96.8%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 10 H), 6.86 (s, 1 H), 6.65 (s, 1 H), 5.08 (s, 2 H), 4.57 (s, 2 H), 3.97 (t, *J* = 6.7 Hz, 2 H), 3.50 (t, *J* = 8.4 Hz, 1 H), 2.74 (m, 2 H), 2.18 (m, 4 H), 1.58 (m, 11 H), 1.06 (t, *J* = 7.3 Hz, 3 H), 0.88 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 511 (MH^+, 100).

3,17β-Bis(benzyloxy)-2-isopropoxyestra-1,3,5(10)-triene (7c). From compound **6** (530.0 mg, 1.1 mmol), a similar synthesis as described in the preparation of **7a** afforded compound **7c** as a colorless oil (230.0 mg, 50.1%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 10 H), 6.89 (s, 1 H), 6.65 (s, 1 H), 5.07 (s, 2 H), 4.57 (s, 2 H), 3.50 (t, *J* = 8.2 Hz, 1 H), 2.74 (m, 2 H), 2.10 (m, 4 H), 1.86 (m, 1 H), 1.45 (m, 9 H), 1.34 (s, 3 H), 1.32 (s, 3 H), 0.87 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 511 (MH^+, 100).

3,17β-Bis(benzyloxy)-2-methoxyestra-1,3,5(10)-triene (7d). From compound **6** (130 mg, 0.27 mmol), a similar synthesis as described in the preparation of **7a** afforded compound **7d** as a colorless oil (104 mg, 80.0%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 10 H), 6.85 (s, 1 H), 6.63 (s, 1 H), 5.11 (s, 2 H), 4.58 (s, 2 H), 3.87 (s, 3 H), 3.51 (t, *J* = 8.0 Hz, 1 H), 2.74 (m, 2 H), 2.12 (m, 4 H), 1.83 (m, 1 H), 1.44 (m, 8 H), 0.88 (s, 3 H); CIMS (isobutane) *m/z* 483 (MH^+, 100).

2-Ethoxyestradiol (8a). A solution of compound **7a** (630 mg, 1.27 mmol) in anhydrous THF (50 mL) containing 10% Pd–C (60 mg) was subjected to hydrogenolysis under 54 psi (initial hydrogen pressure) until hydrogen uptake ceased. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residual oil was subjected to flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:1 by volume) to give **8a** as white crystals (207 mg, 51.6%). The analytical sample was recrystallized from a mixture of ether and hexane: mp 154–155 °C; $[\alpha]^{25}_D$ = +90° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1 H), 6.65 (s, 1 H), 5.48 (brs, 1 H), 4.08 (qd, *J* = 6.8, 1.5 Hz, 2 H), 3.73 (t, *J* = 8.4 Hz, 1 H), 2.77 (m, 2 H), 2.18 (m, 4 H), 1.94 (m, 2 H), 1.69 (m, 1 H), 1.39 (m, 9 H), 0.79 (s, 3 H), the proton producing the peak at δ 5.48 was exchangeable with D_2O; CIMS (isobutane) *m/z* (rel intensity) 317 (MH^+, 100), 299 (MH − H_2O, 65). Anal. ($C_{20}H_{28}O_3$) C, H.

2-*n*-Propoxyestradiol (8b). From compound **7b** (1120 mg) and 10% Pd–C (110 mg), using a similar procedure as described for the synthesis of **8a**, compound **8b** was obtained as a white solid (90.0 mg, 12.4%). This solid was recrystallized from ether and hexane to give crystalline 2-*n*-propoxy-β-estradiol (**8b**) as white crystals (42.0 mg): mp 82–83 °C; $[\alpha]^{21}_D$ +102° (*c* = 0.5); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.80 (s, 1 H), 6.66 (s, 1 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 3.74 (t, *J* = 8.4 Hz, 1 H), 2.75 (m, 2 H), 2.34 (m, 3 H), 1.85 (m, 7 H), 1.35 (m, 5 H), 1.05 (t, *J* = 7.4 Hz, 3 H), 0.80 (s, 3 H); CIMS *m/z* (rel intensity) 331 (MH^+, 100). Anal. ($C_{21}H_{30}O_3$) C, H.

2-Isopropoxyestradiol (8c). From compound **7c** (160.0 mg) and 10% Pd–C (20 mg), using a similar procedure as described in the synthesis of **8a**, product **8c** was obtained as a pale yellow solid (70.0 mg, 76.6%). This solid was recrystallized from ether and hexane to give crystalline 2-isopropoxy-β-estradiol (**8c**) as pale yellow crystals (29.8 mg): mp 105–106 °C; $[\alpha]^{21}_D$ +91° (*c* = 0.5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (s, 1 H), 6.66 (s, 1 H), 5.53 (s, 1 H), 4.52 (p, *J* = 6.1 Hz, 1 H), 3.74 (t, *J* = 8.3 Hz, 1 H), 2.79 (m, 2 H), 2.18 (m, 3 H), 1.94 (m, 2 H), 1.72 (m, 1 H), 1.36 (m, 13 H), 0.80 (s, 3 H); CIMS *m/z* (rel intensity) 331 (MH^+, 100). Anal. ($C_{21}H_{30}O_3$) C, H.

2-Methoxyestradiol (1). From compound **7d** (60 mg, 0.12 mmol) and 10% Pd–C (30 mg), using a similar procedure as described for the synthesis of **8a**, product **1** was obtained as a pale yellow solid (70.0 mg, 76.6%). This solid was recrystallized from a minimum amount of acetone under argon to give pure 2-methoxyestradiol (**1**) as colorless blades (11.5 mg): mp 188–190 °C (lit.[13] mp 188–190 °C); $[\alpha]^{25}_D$ +99° (*c* = 0.5) [lit.[13] $[\alpha]^{21}_D$ +100° (*c* = 0.5)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 (s, 1 H), 6.65 (s, 1 H), 5.43 (brs, 1 H), 3.87 (s, 3 H), 3.75 (t, *J* = 8.5 Hz, 1 H), 2.77 (m, 2 H), 2.20 (m, 3 H), 1.99 (m, 1 H), 1.87 (m, 1 H), 1.69 (m, 1 H), 1.35 (m, 8 H), 0.80 (s, 3 H); CIMS (isobutane) *m/z* 303 (MH^+, 7), 302 (M^+, 100).

2-Methylestradiol (9). A solution of compound **4** (300 mg, 1 mmol) in anhydrous ethanol (20 mL) and anhydrous THF (10 mL) containing 10% Pd–C (30 mg) was subjected to hydrogenation under 42 psi (initial hydrogen pressure) at room temperature for 18 h. Evaporation of the filtered reaction mixture gave the crude product as a white solid, which was subjected to flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:3 by volume) to give compound **9** as a white solid. Pure **9** (95 mg, 33%) was obtained as silky needles after recrystallization from 80% ethanol: mp 182–184 °C (lit.[14] mp 185–186 °C from ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (s, 1 H), 6.51 (s, 1 H), 4.88 (t, *J* = 8.3 Hz, 1 H), 4.51 (s, 1 H), 2.78 (m, 2 H), 2.30 (m, 2 H), 2.21 (s, 3 H), 1.89 (m, 4 H), 1.49 (m, 8 H), 0.88 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 287 (MH^+, 6), 269 (MH − H_2O, 100).

2-Ethenylestradiol (10a). A mixture of $MePh_3P^+Br^-$ (8.93 g, 25 mmol, 5 equiv) and 1.0 M Li[$N(SiMe_3)_2$] THF solution (37.5 mL, 37.5 mmol) was stirred in anhydrous THF (50 mL) at 0 °C for 10 min under argon. A clear yellow solution developed. Compound **4** (1.5 g, 5 mmol) in anhydrous THF (10 mL) was added dropwise under argon, yielding a turbid mixture, which was stirred under argon for 4 h and allowed to warm to room temperature. The reaction mixture was acidified to pH 5 at 0 °C with 2 N HCl and extracted twice with ether (100, 30 mL) in the presence of saturated NaCl solution (30 mL). Evaporation of the filtrate gave a brown oil that was subjected to flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:2 by volume) to give compound **10a** as a white solid (1.05 g, 70.5%). The analytical sample of **10a** was recrystallized twice from ether and hexane to give colorless needles: mp 159–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 1 H), 6.88 (dd, *J* = 17.7, 11.4 Hz, 1 H), 6.52 (s, 1 H), 5.68 (d, *J* = 17.7 Hz, 1 H), 5.29 (d, *J* = 11.4 Hz, 1 H), 4.89 (s, 1 H), 3.71 (t, *J* = 8.4 Hz, 1 H), 2.79 (m, 2 H), 2.35 (m, 1 H), 2.15 (m, 2 H), 1.98 (m, 2 H), 1.33 (m, 9 H), 0.78 (s, 3 H); CIMS

(isobutane) *m/z* 299 (MH^+, 100), 281 (MH − H_2O, 82). Anal. ($C_{20}H_{26}O_2$) C, H.

2-((*E*)-1-Propenyl)estradiol (10b). Compound **10b** was synthesized from ethyltriphenylphosphonium bromide (2.75 g, 99%, 20 mmol), 1.0 M Li[$N(SiMe_3)_2$] (7.4 mL, 7.4 mmol), and compound **5** (440 mg, 1.46 mmol) using the method detailed above for **10a**. Compound **10b** was obtained as white crystals (380 mg, 83.2%). The analytical sample was obtained as white crystals after recrystallization from ether and hexane: mp 174−175 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (s, 1 H), 6.54 (dd, J = 14.1, 1.5 Hz, 1 H), 6.51 (s, 1 H), 6.46 (qq, J = 14.1, 6.6 Hz, 1 H), 5.04 (s, 1 H), 3.71 (t, J = 8.3 Hz, 1 H), 2.77 (m, 2 H), 2.33 (m, 1 H), 2.13 (m, 2 H), 1.90 (m, 6 H), 1.40 (m, 8 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 313 (MH^+, 79), 312 (M^+, 100), 295 (MH − H_2O, 56). Anal. ($C_{21}H_{28}O_2$) C, H.

2-((*E*)-1-Butenyl)estradiol (10c). Compound **10c** was synthesized from *n*-propyltriphenylphosphonium bromide (7.86 g, 99%, 20 mmol), 1.0 M Li[$N(SiMe_3)_2$] (30 mL, 30 mmol), and compound **4** (1.29 g, 4 mmol, 1.0 equiv) using the method detailed above for **10a**. Compound **10c** was obtained as a white solid (790 mg, 58.8%). The analytical sample was recrystallized twice from ether and hexane to give compound **10c** as white needles: mp 151−152 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (s, 1 H), 6.52 (s, 1 H), 6.51 (d, J = 15.8 Hz, 1 H), 6.17 (td, J = 15.8, 6.5 Hz, 1 H), 4.92 (brs, 1 H), 3.74 (t, J = 8.4 Hz, 1 H), 2.78 (m, 2 H), 2.45 (m, 5 H), 1.95 (m, 1 H), 1.86 (m, 1 H), 1.41 (m, 9 H), 1.10 (t, J = 7.5 Hz, 3 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 327 (MH^+, 100). Anal. ($C_{22}H_{30}O_2$) C, H.

2-((*E*)-1-Pentenyl)estradiol (10d). Compound **10d** was synthesized from *n*-butyltriphenylphosphonium bromide (8.0 g, 99%, 20 mmol), 1.0 M Li[$N(SiMe_3)_2$] (30 mL, 30 mmol), and compound **4** (1.2 g, 4 mmol) using the method detailed above for **10a**. Compound **10d** was obtained as a white solid (630 mg, 46.3%). The analytical sample was recrystallized from ether and hexane to afford white needles: mp 155−156 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (s, 1 H), 6.52 (s, 1 H), 6.50 (d, J = 15.8 Hz, 1 H), 6.13 (td, J = 6.8, 15.8 Hz, 1 H), 4.79 (brs, 1 H), 3.73 (t, J = 8.3 Hz, 1 H), 2.78 (m, 2 H), 2.37 (m, 1 H), 2.19 (m, 3 H), 1.96 (m, 1 H), 1.86 (m, 1 H), 1.41 (m, 10 H), 0.95 (t, J = 7.3 Hz, 3 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 341 (MH^+, 100), 323 (MH − H_2O, 77). Anal. ($C_{22}H_{30}O_2$) C, H.

2-(2-Methyl-1-propenyl)estradiol (10e). Compound **10e** was synthesized from isopropyltriphenylphosphonium bromide (5.0 g, 99%, 11.6 mmol), 1.0 M Li[$N(SiMe_3)_2$] (17.4 mL, 17.4 mmol), and compound **4** (867 mg, 2.89 mmol) using the method detailed above for **10a**. Compound **10e** was obtained as a white solid (630 mg, 64.9%) after recrystallization from ether and hexane: mp 98−99 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 1 H), 6.62 (s, 1 H), 6.10 (brs, 1 H), 4.84 (brs, 1 H), 3.73 (t, J = 8.5 Hz, 1 H), 2.82 (m, 2 H), 2.27 (m, 1 H), 2.15 (m, 2 H), 1.94 (m, 1 H), 1.93 (d, J = 1.0 Hz, 3 H), 1.86 (m, 1 H), 1.70 (d, J = 0.5 Hz, 3 H), 1.69 (m, 1 H), 1.37 (m, 8 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* 327 (MH^+, 100), 309 (MH − H_2O, 39). Anal. ($C_{22}H_{30}O_2$) C, H.

2-Ethylestradiol (11a). A solution of compound **10a** (650 mg, 2.18 mmol) in anhydrous THF (30 mL) containing 10% Pd−C (60 mg) was hydrogenated under 30 psi (initial hydrogen pressure) at room temperature until the hydrogen uptake ceased. Evaporation of the filtered reaction mixture under vacuum gave the crude product as an oil which was subjected to flash chromatography (silica gel, 230−400 mesh; ether:hexane, 1:2 by volume) to afford product **11a** as a white solid (465 mg, 71.1%). The analytical sample was recrystallized twice from ether and hexane to give **11a** as white crystals: mp 165−166 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.07 (s, 1 H), 6.51 (s, 1 H), 4.72 (s, 1 H), 3.85 (t, J = 7.2 Hz, 1 H), 2.83 (m, 2 H), 2.61 (q, J = 7.5 Hz, 2 H), 2.34 (m, 1 H), 2.18 (m, 2 H), 1.92 (m, 2 H), 1.72 (m, 1 H), 1.36 (m, 11 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* 301 (MH^+, 199), 283 (MH − H_2O, 100). Anal. ($C_{20}H_{28}O_2$) C, H.

2-*n*-Propylestradiol (11b). Compound **11b** was synthesized from compound **10b** (180 mg, 0.576 mmol) and 10% Pd−C (30 mg) using the procedure described above for **11a**. Compound **11b** was obtained as a white solid (150 mg, 83%). The analytical sample was recrystallized twice from ether and hexane: mp 135−136 °C; ^1H NMR (acetone-d_6, 300 Hz) δ 7.69 (s, 1 H), 6.98 (s, 1 H), 6.48 (s, 1 H), 3.65 (m, 1 H), 3.55 (dd, J = 1.0, 5.0 Hz, 1 H), 2.80 (m, 3 H), 2.70 (m, 2 H), 2.52 (t, J = 7.7 Hz, 2 H), 1.59 (m, 9 H), 0.91 (m, 6 H), 0.76 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 315 (MH^+, 100), 297 (MH − H_2O, 95). Anal. ($C_{21}H_{30}O_2$) C, H.

2-*n*-Butylestradiol (11c). Compound **11c** was synthesized from compound **10c** (600 mg, 1.81 mmol) and 10% Pd−C (50 mg) using the procedure described above for **11a**. Compound **11c** was obtained as a white solid (380 mg, 62.9%). The analytical sample was recrystallized from ether and hexane to afford the product as white needles: mp 142−143 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 7.03 (s, 1 H), 6.49 (s, 1 H), 4.77 (brs, 1 H), 3.74 (t, J = 8.5 Hz, 1 H), 2.77 (m, 2 H), 2.56 (m, 2 H), 2.32 (m, 1 H), 2.13 (m, 2 H), 1.95 (td, J = 9, 3.5 Hz, 1 H), 1.85 (m, 1 H), 1.69 (m, 1 H), 1.40 (m, 11 H), 0.9 (t, J = 7.0 Hz, 3 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 329 (MH^+, 100). Anal. ($C_{22}H_{32}O_2$) C, H.

2-*n*-Pentylestradiol (11d). Compound **11d** was synthesized from compound **10d** (320 mg, 0.94 mmol) and 10% Pd−C (50 mg) using the procedure described above for **11a**. Compound **11d** was obtained as a white solid (310 mg, 96.6%). The analytical sample was recrystallized from ether and hexane to afford the product as white needles: mp 153−154 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1 H), 6.43 (s, 1 H), 4.47 (brs, 1 H), 3.66 (t, J = 8.5 Hz, 1 H), 2.71 (m, 2 H), 2.47 (m, 2 H), 2.26 (m, 1 H), 2.07 (m, 2 H), 1.88 (td, J = 12.5, 3.0 Hz, 1 H), 1.79 (m, 1 H), 1.63 (m, 1 H), 1.52 (m, 2 H), 1.29 (m, 11 H), 0.83 (t, J = 7.0 Hz, 3 H), 0.71 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 343 (MH^+, 99), 325 (MH − H_2O, 100). Anal. ($C_{23}H_{34}O_2$) C, H.

2-(2-Methyl-*n*-propyl)estradiol (11e). Compound **11e** was synthesized from compound **10e** (230 mg, 0.68 mmol) and 10% Pd−C (40 mg) using the procedure described above for **11a**. Compound **11e** was obtained as a white solid. The analytical sample was recrystallized from ether and hexane: mp 131−132 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 6.98 (s, 1 H), 6.50 (s, 1 H), 4.48 (s, 1 H), 3.74 (brm, 1 H), 2.82 (m, 2 H), 2.43 (d, J = 7.2 Hz, 2 H), 2.36 (m, 1 H), 2.12 (m, 2 H), 1.91 (m, 3 H), 1.70 (m, 1 H), 1.39 (m, 7 H), 0.94 (d, J = 6.6 Hz, 6 H), 0.78 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 329 (MH^+, 100), 311 (MH − H_2O, 61). Anal. ($C_{22}H_{32}O_2$) C, H.

4-Iodoestradiol-17-acetate (12) and 2-Iodoestradiol-17-acetate (13). A mixture of β-estradiol (**2**) (1.0 g, 3.67 mmol) in acetic acid (100 mL) containing iodine (1.4 g, 5.51 mmol) and $CuCl_2 \cdot 2H_2O$ (940 mg, 5.5 mmol) was heated at 60 °C under argon for 35 h. TLC (silica gel, chloroform:acetone, 80:1 by volume) showed that β-estradiol had disappeared. The solvent was removed at 30 °C under vacuum, and the mixture was diluted with saturated NaCl solution (10 mL) and extracted twice with ether (2 × 20 mL). Evaporation of the ether gave the crude product as a solid, which was subjected to flash chromatography (silica gel, 230−400 mesh; chloroform to chloroform:acetone, 80:1 by volume) to give a mixture of **12** and **13**. Pure 2-iodo-β-estradiol-17-acetate (**13**) was obtained as white needles (1.02 g, 63.4%) by recrystallization of the mixture of **12** and **13** from methanol: mp 174−175 °C (lit.[15] mp 175−177 °C); ^1H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1 H), 6.74 (s, 1 H), 5.14 (s, 1 H), 4.70 (t, J = 9 Hz, 1 H), 3.51 (d, J = 5.5 Hz, 1 H), 2.81 (m, 2 H), 2.22 (m, 3 H), 2.08 (s, 3 H), 1.86 (m, 3 H), 1.39 (m, 7 H), 0.84 (s, 3 H); CIMS (isobutane) *m/z* (rel intensity) 441 (MH^+, 4), 381 (MH − HOAc, 100).

2-Iodoestradiol (14). A solution of compound **13** (800 mg, 1.82 mmol) in THF/H_2O (30 mL, 3:1) containing $LiOH \cdot 2H_2O$ (100 mg, 2.38 mmol) was stirred at room temperature under argon for 48 h. The reaction mixture was acidified to pH 2 with 2 N HCl solution and extracted twice with ether (30, 20 mL). The ether layer was washed with saturated NaCl solution (10 mL) and dried over Na_2SO_4. Evaporation of the filtered ether solution gave the crude product **14**. This was subjected to flash chromatography (ether:hexane, 2:1 by volume) to give **14** as a white solid (425 mg, 58.5%). The analytical sample was recrystallized from ether and hexane to give **14** as colorless needles: mp 154−155 °C (lit.[16] mp 146−

153 °C); $[\alpha]^{25}_D$ +113.7° (c = 0.86, $CHCl_3$) [lit.[16] $[\alpha]^{20}_D$ +114.6° (c = 0.86, $CHCl_3$)]; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1 H), 6.72 (s, 1 H), 5.15 (brs, 1 H), 3.73 (t, J = 8.3 Hz, 1 H), 2.79 (m, 2 H), 2.13 (m, 3 H), 1.93 (m, 2 H), 1.69 (m, 1 H), 1.31 (m, 8 H), 0.78 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 399 (MH^+, 57), 381 ($MH-H_2O$, 100). Anal. ($C_{18}H_{23}IO_2$) C, H, I.

2-(Ethylthio)estradiol (15). NaSEt (327 mg, 3.5 mmol), CuI (33 mg, 0.18 mmol), and 18-crown-6 (80 mg) were added to a solution of 2-iodoestradiol (**14**) (138 mg, 0.35 mmol) in anhydrous DMF (10 mL), which had been dried by azeotropic distillation with toluene (10 mL). The resulting yellow solution was heated at reflux under argon for 24 h. The products were extracted with ether (2 × 20 mL) in the presence of saturated NaCl solution (10 mL). The combined ether phase was dried over Na_2SO_4. Evaporation of the filtered ether solution gave the crude product as an oil, which was subjected to preparative TLC purification (silica gel, uniplate 1000 μm; ether:hexane, 3:1 by volume) to give the product **15** as a yellow solid (60 mg, 33%), which was recrystallized from ether and hexane: mp 166–167 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40 (s, 1 H), 6.71 (s, 1 H), 6.58 (s, 1 H, exchangeable with D_2O), 3.73 (t, J = 8.2 Hz, 1 H), 2.82 (m, 2 H), 2.67 (q, J = 7.4 Hz, 2 H), 2.30 (m, 1 H), 2.12 (m, 2 H), 1.91 (m, 2 H), 1.65–1.10 (m, 12 H), 0.78 (s, 3 H); CIMS (isobutane) m/z 333 (MH^+, 100). Anal. ($C_{20}H_{28}O_2S$) C, H, S.

2-Nitroestrone (17). Estrone (**16**) (5.0 g, 18.5 mmol) was dissolved in AcOH (150 mL) at 120 °C. The solution was cooled to 50 °C, resulting in partial precipitation. After addition of 70% HNO_3 (1.4 mL, 22.2 mmol, 1.2 equiv) in HOAc (5 mL), the solid redissolved. The reddish-brown solution was stirred at room temperature overnight and extracted with ether (2 × 100 mL) in the presence of water (100 mL). The combined ether phase was washed with saturated NaCl solution (2 × 30 mL) and dried over Na_2SO_4. Evaporation of the filtered ether solution gave the crude product as a yellowish-brown oil, which was subjected to flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:1 by volume) to give compound **17** as a yellow solid (2.62 g, 47.4%). The analytical sample was recrystallized from 95% ethanol: mp 183–184 °C (lit.[17] mp 183.5–184 °C); ^1H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1 H), 7.99 (s, 1 H), 6.87 (s, 1 H), 2.93 (m, 3 H), 2.48 (m, 3 H), 2.11 (m, 5 H), 1.53 (m, 4 H), 0.93 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 316 (MH^+, 100).

2-Nitro-3-*O*-(*tert*-butyldimethylsilyl)estrone (18). Compound **17** (900 mg, 2.86 mmol) was dissolved in toluene (20 mL), and the toluene and residual water were removed by azeotropic distillation. Anhydrous DMF (30 mL) and imidazole (820 mg, 12.0 mmol) were added, and the mixture was stirred at room temperature under argon for 0.5 h. *tert*-Butyldimethylsilyl chloride (97%, 907 mg, 6.02 mmol) was added, and the solution was stirred at room temperature under argon for 24 h. The reaction mixture was diluted with saturated NaCl solution (20 mL) and the product extracted twice with ether (30, 20 mL). The combined ether layer was washed with saturated NaCl solution (10 mL) and dried over Na_2SO_4. Evaporation of the solvent gave the crude product as a yellow solid, which was purified by flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:1 by volume) to give compound **18** as a pale yellow solid (1.07 g, 87.3%). The analytical sample was recrystallized from ethanol: mp 183–184 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1 H), 6.68 (s, 1 H), 2.91 (m, 2 H), 2.48 (m, 2 H), 2.07 (m, 5 H), 1.51 (m, 6 H), 1.01 (s, 9 H), 0.92 (s, 3 H), 0.23 (s, 6 H); CIMS (isobutane) m/z (rel intensity) 430 (MH^+, 100). Anal. ($C_{24}H_{35}NO_4Si$) C, H, N.

2-Amino-3-*O*-(*tert*-butyldimethylsilyl)estrone (19). A solution of compound **18** (400 mg, 0.93 mmol) in anhydrous THF (50 mL) containing 10% Pd–C (40 mg) was hydrogenated under 20 psi (initial hydrogen pressure) until the hydrogen uptake ceased. Evaporation of the filtrate gave the crude product, which was purified by flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:3 by volume) to give compound **19** as a pale yellow solid (316 mg, 85.2%): mp 147–149 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.67 (s, 1 H), 6.46 (s, 1 H), 2.75 (m, 2 H), 2.40 (dd, J = 17.9, 8.7 Hz, 1 H), 2.07 (m, 6 H), 1.52 (m, 6 H), 1.01 (s, 9 H), 0.92 (s, 3 H), 0.24 (s, 6 H); CIMS (isobutane) m/z (rel intensity) 400 (MH^+, 100).

2-Acetamido-3-*O*-(*tert*-butyldimethylsilyl)estrone (20). Compound **19** (790 mg, 1.98 mmol) in anhydrous pyridine (20 mL) was stirred at 0 °C for 10 min. Ac_2O (2.16 g, 1.96 mmol) was added dropwise. The resulting solution was stirred under argon for 24 h and allowed to rise to room temperature. Evaporation of the solvents gave the crude product as an oil, which was dissolved in toluene (20 mL). The solvent was evaporated, and the crude product was purified by flash chromatography (silica gel, 230–400 mesh; ether:hexane, 1:1 by volume) to give compound **20** as a white solid (460 mg, 50.5%): mp 74–76 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1 H), 7.58 (brs, 1 H), 6.53 (brs, 1 H), 2.82 (m, 2 H), 2.48 (m, 2 H), 2.16 (s, 3 H), 2.09 (m, 5 H), 1.52 (m, 6 H), 1.04 (s, 9 H), 0.92 (s, 3 H), 0.26 (s, 6 H); CIMS (isobutane) m/z (rel intensity) 442 (MH^+, 100).

2-Acetamido-3-*O*-(*tert*-butyldimethylsilyl)-β-estradiol (21). A solution of compound **20** (110 mg, 0.25 mmol) in anhydrous THF (5 mL) containing $NaBH_4$ (48.0 mg, 1.25 mmol) was stirred at room temperature under argon for 20 h and extracted twice with ether (10, 5 mL) in the presence of saturated NaCl solution (10 mL). The combined ether layer was washed with saturated NaCl solution (10 mL) and dried over anhydrous Na_2SO_4. Evaporation of the filtered ether solution gave the crude product as a pale yellow solid, which was purified by preparative TLC (silica gel, ether) to give **21** as an off-white solid (85 mg, 77%): mp 168–170 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.22 (s, 1 H), 7.58 (brs, 1 H), 6.50 (s, 1 H), 3.72 (t, J = 8.4 Hz, 1 H), 2.53 (m, 2 H), 2.33 (m, 1 H), 2.14 (s, 3 H), 1.98 (m, 3 H), 1.85 (m, 1 H), 1.66 (m, 1 H), 1.39 (m, 5 H), 1.18 (s, 9 H), 0.77 (s, 3 H), 0.24 (s, 6 H); CIMS (isobutane) m/z (rel intensity) 444 (MH^+, 100), 426 ($MH - H_2O$, 11).

2-(Acetamido)estradiol (22). A mixture of the silyl ether **21** (0.91 g, 2.05 mmol) and 1.0 M tetra-*n*-butylammonium fluoride (4.1 mL, 4.1 mmol) in THF (20 mL) was stirred at room temperature under argon for 5 h. The reaction mixture was diluted with saturated NaCl solution (10 mL), and the product was extracted with ether (2 × 20 mL). The combined ether layer was washed with NaCl solution (10 mL) and dried over Na_2SO_4. Evaporation of the filtered ether solution gave the crude product as a pale brown solid. This solid was dissolved in a minimum volume of THF and subjected to flash chromatography (silica gel, 230–400 mesh; ether to ether:THF, 5:1 by volume) to afford the product **22** as a white solid (670 mg, 99.6%). The analytical sample was recrystallized from THF–ether–hexane to give **22** as white crystals: mp 231–232 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.53 (brs, exchangeable with D_2O, 1 H), 7.52 (brs, exchangeable with D_2O, 1 H), 6.91 (s, 1 H), 6.66 (s, 1 H), 3.65 (t, J = 8.4 Hz, 1 H), 2.73 (d, J = 4.2 Hz, 1 H), 2.71 (d, J = 4.0 Hz, 1 H), 2.17 (s, 3 H), 2.07 (m, 3 H), 1.84 (m, 2 H), 1.60 (m, 3 H, 1 H exchangeable with D_2O), 1.29 (m, 6 H), 0.70 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 330 (MH^+, 100), 312 ($MH - H_2O$, 21). Anal. ($C_{20}H_{27}NO_3$) C, H, N.

2-(Ethylamino)estradiol (23). A solution of $LiAlH_4$ in THF (1.0 M, 36 mL, ca. 20 equiv) was added dropwise to a solution of the amide **22** (590 mg, 1.79 mmol) in anhydrous THF (20 mL). The resulting white suspension was stirred at gentle reflux under argon for 1 h. The reaction mixture was treated dropwise with saturated NaCl solution to decompose the excess $LiAlH_4$. The stirred mixture was adjusted to pH 7 with 2 N HCl in the presence of ether (50 mL). The water phase was extracted a second time with ether (30 mL). The combined ether phase was washed with saturated NaCl solution (50 mL) and dried over Na_2SO_4. Evaporation of the filtered ether solution under vacuum gave the crude product as a yellow solid, which was purified by flash chromatography (silica gel, 230–400 mesh; ether:hexane, 3:1 by volume) to give **23** as a yellow solid (510 mg, 90.6%). The analytical sample was recrystallized from THF–hexane–$CHCl_3$ to afford off-white crystals: mp 164–165 °C; ^1H NMR (300 MHz, acetone-d_6:TFA-*d*, ca. 1:1 by volume) δ 7.25 (s, 1 H), 6.69 (s, 1 H), 3.76 (t, J = 8.8 Hz, 1 H), 3.52 (q, J = 7.1 Hz, 2 H), 2.10–1.00 (m, 18 H), 0.69 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 316 (MH^+, 100), 298 ($MH - H_2O$, 45). Anal. ($C_{20}H_{29}NO_2$) C, H, N.

Tubulin Assays. Electrophoretically homogeneous tubulin

was purified from bovine brain as described previously.[18] 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. Four instruments were used, and two control reaction mixtures were present in each experiment. The extent of polymerization after a 20 min incubation was determined (the values for the two controls were usually within 5% of each other). IC_{50} values were determined graphically. Active compounds were examined in at least three independent assays, while inactive compounds (defined as IC_{50} value >40 μM) were examined in at least two independent experiments. 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 30 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.[19] By definition, estradiol is given an RBA value of 100. These assays were conducted in duplicate at 0 °C (18 h) and 25 °C (18 h). The coefficient of variation in replicate experiments is typically less than 30%.

Acknowledgment. This research was made possible by contract NO1-CM-17512, awarded to M.C. by the National Cancer Institute, DHHS. We also acknowledge funding from the National Institutes of Health (PHS 5R37DK15556) to J.A.K. We are grateful to Drs. Ravi K. Varma and Daniel Lednicer, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, for informative discussions during the initial stages of this research project. We are indebted to Kathryn E. Carlson and Karen Avenatti for performing estrogen receptor binding affinity measurements.

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JM950183D