Antitumor Agents. 254. Synthesis and Biological Evaluation of Novel Neo-tanshinlactone Analogues as Potent Anti-Breast Cancer Agents

Xihong Wang,[†] Kyoko Nakagawa-Goto,[†] Kenneth F. Bastow,[†] Ming-Jaw Don,[‡] Yun-Lian Lin,[‡] Tian-Shung Wu,[§] and Kuo-Hsiung Lee[†,*]

Natural Products Research Laboratories, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, National Research Institute of Chinese Medicine, Taipei, Taiwan, and Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan

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In our previous study, neo-tanshinlactone (**1**) showed potent and selective anti-breast cancer activity. To explore the SAR of **1**, nine analogues (**15**−**18**, **24**−**28**) were designed and synthesized. Together with **1** and tamoxifen (TAM), all newly synthesized compounds and some intermediates were evaluated for in vitro anticancer activity against several human tumor cell lines. Compounds without a ring D did not show promising activity, while compounds with a methylated furan ring D showed better activity than those with unsubstituted furan or hydroxy-dihydrofuran rings. Among all newly synthesized compounds, compound **15** with an ethyl group at the 4-position showed the best activity and selectivity with ED_{50} values of 0.45 and 0.18 μg/mL against MCF-7 and ZR-75-1 (ER+) and 13.5 and 10.0 μg/mL against MDA MB-231 and HS 587-1 (ER−), respectively. Furthermore, **15** also showed potent activity against SK-BR-3 (ER−, HER2+) with an ED_{50} value of 0.10 μg/mL. Our preliminary SAR studies showed that a methylated furan ring D and the C-4 substituent in ring A are critical for anti-breast cancer activity. Further development of **1** and **15** as anti-breast cancer drug candidates is warranted.

Introduction

Breast cancer is the most frequent cancer and the second leading cause of cancer deaths in women today.[1,2] According to the World Health Organization, more than 1.2 million people will be diagnosed with breast cancer this year worldwide, even though the incidence leveled off and the death rate of breast cancer declined significantly after the 1990s. Medical experts attribute the decline in breast cancer deaths to earlier detection and more effective treatments.[3,4]

Estrogens are well recognized to play the predominant role in breast cancer development and growth, and much effort has been devoted to block estrogen formation and action.[5,6] The most widely used therapy for breast cancer is the use of an antiestrogen such as tamoxifen (TAM). However, the present breast cancer therapies achieve meaningful clinical results in only 30−40% of patients,[5] because drug resistance usually develops after one or two years of treatment. This resistance is linked to the presence of estrogen-independent pathways for breast cancer cell growth.[7,8] Recently, it was reported that expression of the human estrogen receptor-2 (HER-2) is also a significant factor associated with breast cancer morbidity.[9] It is also becoming clearer that cross-talk between estrogen and growth factor receptor pathways occurs and likely is a factor in the pathology and treatment of breast cancer.[10] Therefore, more potent anti-breast cancer agents that combine the desired, tissue-selective effects with novel structures or new mechanism(s) of action must be developed.

In our prior paper, we reported the isolation of neo-tanshinlactone (**1**) from Tanshen and its first total synthesis. Compound **1** showed significant inhibition against two ER+ human breast cancer cell lines and was 10-fold more potent and 20-fold more selective as compared to TAM. Compound **1** also potently inhibited an ER−, HER-2 overexpressing breast cancer cell line.[11] Although the mechanism of action of **1** is not yet determined, these promising results strongly encouraged us to explore novel **1**-analogues as potential anti-breast cancer agents. Accordingly, we recently designed and synthesized compounds with variously substituted rings A and B or a modified ring D (Figure 1). In this paper, we report the synthesis of these **1**-analogues and their cytotoxic activity against several human tumor cell lines.

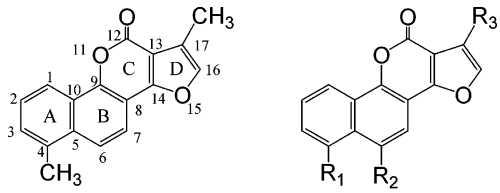

Figure 1. Structures of neo-tanshinlactone and its synthetic analogues.

Chemistry

Target compounds **15**−**18** and **24**−**28** were synthesized from various substituted 1-naphthols (**2**−**6**), as shown in Scheme 1. 5-Methyl- (**2**) and 5-ethyl-1-naphthol (**3**) were prepared with the previously reported methodology.[11] Treating naphthols **2**−**6** with malonic acid in the presence of polyphosphoric acid (PPA) provided benzochromenones **7**−**11**,[11,12] which were converted into analogues **15**−**18** by two different methods. In the first method, O-alkylation of **8**, **9**, and **11** provided **12**−**14**,[13,14,15] which were cyclized using PPA to give target compounds **15**, **16**, and **18**.[16] Second, benzochromene **10** was directly converted to benzofurochromenone **17** according to previous methodology.[11,17] Furthermore, compounds **7**−**11** were reacted with chloroacetaldehyde in the presence of potassium carbonate and provided compounds **19**−**23** with a hydroxyl dihydrofuran ring

* To whom correspondence should be addressed. Phone: (919)-962-0066, Fax: (919)-966-3893, E-mail: khlee@unc.edu.
† University of North Carolina.
‡ National Research Institute of Chinese Medicine.
§ National Cheng Kung University.

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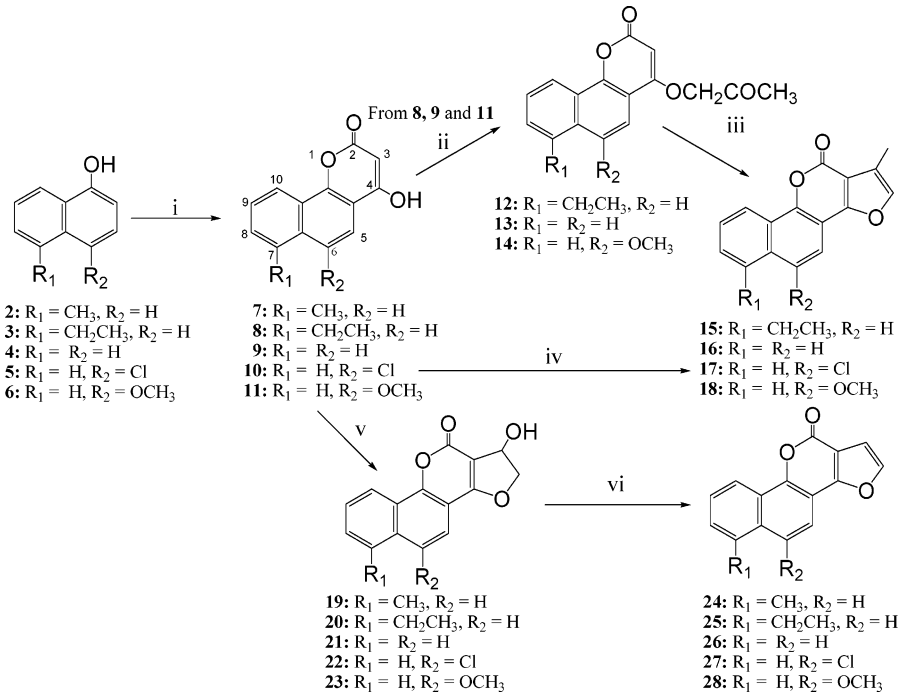
Scheme 1. Synthetic Routes to Target Compounds[a]



[a] (i) Malonic acid, PPA, 75 °C; (ii) $ClCH_2COCH_3$, K_2CO_3, DMF; (iii) PPA, 110 °C; (iv) $ClCH_2COCH_3$, HOAc/NH_4OAc, toluene/EtOH, reflux, 24 h; (v) $ClCH_2CHO$, K_2CO_3, H_2O; (vi) 2 N HCl, 50 °C.

Table 1. Cytotoxicity of Compounds against Tumor Cell Lines

	mean ED_{50} (μg/mL),[a] cell line							
compd	MCF-7 (ER+)	ZR-75-1 (ER+)	MDA MB-231 (ER−)	HS587-1 (ER−)	SK-BR-3 (ER−,HER2+)	A431	SW620	KB
1	0.60	0.25	10.0	16.0	0.18	>10	>10	>10
15	0.45	0.18	13.5	10.0	0.10	>10	>10	>10
16	4.0	4.0	10.3	7.5				
17	5.0	8.6			4.5	8.0	>10	>10
18	5.0	>10			8.6	9.5	9.0	>10
24	5.5	>10			8.5	8.3	>10	>10
25	>10		>10					
26	>20		10.5					
27	7.0	>10			>10	>10	>10	>10
28	3.4	8.5			4.0	9.5	4.0	9.5
TAM	5.0	3.6	8.5	7.0	5.0	7.0	4.0	9.0

[a] Standard error of independent determinations was less than 5%.

D.[15] Subsequently, dehydration of **19**−**23** at 50 °C in the presence of 2 N hydrochloric acid gave analogues **24**−**28** without a methyl group on the furan ring D.[15]

Results and Discussion

Together with **1** and TAM, the newly synthesized **1**-analogues (**15**−**18**; **24**−**28**) were tested for in vitro anticancer activity against several human tumor cell lines (Table 1). In limited testing of the reaction intermediates (data not shown), the benzochromenones **9**−**11** generally did not show promising activity, and the hydroxyl-dihydrofurans **19**−**23** did not show comparable activity with the most potent compounds **1** and **15**.

Like the lead compound (**1**), target analogues **15**−**18** contain a methylated furan ring D but have different substituents on ring A or B. Although **16** showed similar activity to TAM with ED_{50} values of 4.0 μg/mL against MCF-7 and ZR-75-1 (ER+) and 10.3 and 7.5 μg/mL against MDA MB-231 and HS 587-1 (ER−), respectively, it was significantly less active than **1**. However, compound **15** did show comparable activity to **1** with ED_{50} values of 0.45 and 0.18 μg/mL against MCF-7 and ZR-75-1 (ER+) and 13.5 and 10.0 μg/mL against MDA MB-231 and HS 587-1 (ER−), respectively. Indeed, **1** and **15** showed 10 times better activity and more than 20 times higher selectivity against ER+ cell lines than **16** and TAM, and as seen in Table 1, were the most potent among all tested compounds. Compounds **1**, **15**, and **16** differ structurally only at the 4-position in ring A, and thus, this position is important for the anticancer activity. The rank order of potency was ethyl (**15**) ∼ methyl (**1**) > no substituent (**16**). Compounds **17** and **18** with chlorine and methoxy groups, respectively, at the 6-position in ring B did not show strong activity.

Unlike **1** and **15**−**18**, compounds **24**−**28** have an unsubstituted furan ring D. By comparing the results of **16**, **21**, and **26** against MCF-7 cells, we concluded that the methyl group on the furan ring D is critical for the anti-breast cancer activity. Although **16**, with a methylated furan ring D, had an ED_{50} of 4.0 μg/mL, its hydroxyl-dihydrofuran precursor **21** and corresponding unmethylated analogue **26** had ED_{50} values of 12.5 and >20 μg/mL, respectively. The same conclusion was obtained from the compounds with methyl (**1** vs **19** vs **24**) and ethyl (**15** vs **20** vs **25**) substituents on ring A.

Overall, these studies showed that the furan ring D is important to the cytotoxic activity and the methyl furan ring D resulted in better activity than an unsubstituted furan ring D.

Methyl and ethyl groups at the 4-position of ring A can increase the activity dramatically, while chlorine and methoxy groups at the 6-position of ring B do not affect the activity significantly. The activity of these compounds does not seem to correlate with ER− status or EGFR/HER2 status; however, the mechanism of action should be further investigated. Further analogue synthesis is ongoing to place other substituents on rings A and B. Compounds with bulkier groups at the 4-position of ring A are particularly interesting, as new compound **15** contained a larger ring A substituent than lead compound neo-tanshinlactone (**1**), yet had comparable activity and selectivity.

Experimental Section

Materials and Methods. Melting points were measured with a Fisher Johns melting apparatus without correction. The ^{1}H NMR spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Mass spectra were measured on a PE-SCIEX API 3000 instrument with turbo ion spray source or Agilent-1100, LC/MSD-Trap. Thin-layer chromatography (TLC) and preparative TLC were performed on precoated silica gel GF plates purchased from Merck, Inc. Biotage Flash+ or Isco Companion systems were used for flash chromatography. Silica gel (200−400 mesh) from Aldrich, Inc., was used for column chromatography. All other chemicals were obtained from Aldrich, Inc.

Preparation of Intermediates for Target Compounds 15−18. Compounds **7**−**11** were prepared from naphthols **2**−**6**, respectively, by the methods described in our previous paper. The benzochromenones **12**−**14** were prepared from compounds **8**, **9**, and **11**, respectively, by the following procedure.

To a solution of **8** (260 mg, 1.1 mmol) in anhydrous DMF was added potassium carbonate (480 mg, 3.3 mmol). After stirring at 75 °C for 10 min, chloroacetone (277 μL, 3.3 mmol) in DMF (1 mL) was added and stirring maintained at 75 °C for 3 h. After the reaction was complete, water was added and the reaction was extracted three times with EtOAc. After drying over Na_2SO_4 and evaporation of the organic solvent in vacuo, purification with flash chromatography eluting with hexane:EtOAc = 3:1 provided compound **12**.

7-Ethyl-4-(2-oxopropoxy)benzo[*h*]chromen-2-one (12). 62% yield; MS-ESI+ (*m*/*z*, %): 297 (M^+ + 1, 100); ^{1}H NMR δ 1.39 (3H, t, J = 7.5 Hz, *CH*$_3$CH$_2$-), 2.40 (3H, s, CO-*CH*$_3$), 3.14 (2H, q, J = 7.5 Hz, CH$_3$*CH*$_2$-), 4.78 (2H, s, O*CH*$_2$COCH$_3$), 5.63 (1H, s, H-3), 7.56 (2H, m, H-8, 9), 7.90 (1H, d, J = 9.3 Hz, H-5), 7.95 (1H, d, J = 9.3 Hz, H-6), 7.45 (1H, d, J = 8.4 Hz, H-10).

4-(2-Oxopropoxy)benzo[*h*]chromen-2-one (13). 51% yield; ^{1}H NMR δ 2.40 (3H, s, CO-*CH*$_3$), 4.78 (2H, s, O*CH*$_2$COCH$_3$), 5.63 (1H, s, H-3), 7.65 (2H, m, H-8, 9), 7.70 (1H, d, J = 8.1 Hz, H-5), 7.87 (1H, d, J = 8.1 Hz, H-6), 7.90 (1H, m, H-7), 8.53 (1H, m, H-10).

6-Methoxy-4-(2-oxopropoxy)benzo[*h*]chromen-2-one (14). 35% yield; ^{1}H NMR (DMSO) δ 2.37 (3H, s, OCH$_2$CO*CH*$_3$), 4.06 (3H, s, OCH$_3$-6), 4.82 (2H, s, O*CH*$_2$COCH$_3$), 5.61 (1H, s, H-3), 7.11 (1H, s, H-5), 7.67 (2H, m, H-8, 9), 8.30 (1H, m, H-7), 8.52 (1H, m, H-10).

Preparation of Target Compounds 15−18. Compounds **15**, **16**, and **18** were prepared from **12**−**14**, respectively, using method 1, and compound **17** was prepared from **10** using method 2.

Method 1. Compound **12** (100 mg) and PPA (2 g) was mixed and stirred at 110 °C for 5 h. The reaction mixture was cooled and cold water was added to decompose excess PPA. The solid was filtered and purified by flash chromatography eluting with hexane: EtOAc = 30:1 to obtain the product.

4-Ethyl-17-methyl-11,15-dioxacyclopenta[*a*]phenanthren-12-one (15). 49% yield; mp 145−147 °C; MS-ESI+ (*m*/*z*, %): 279 (M^+ + 1, 100); ^{1}H NMR δ 1.39 (3H, t, J = 7.5 Hz, *CH*$_3$CH$_2$-), 2.41 (3H, s, CH$_3$-17), 3.15 (2H, q, J = 7.5 Hz, CH$_3$*CH*$_2$-), 7.47 (1H, d, J = 1.2 Hz, H-16), 7.48 (1H, d, J = 6.9 Hz, H-3), 7.58 (1H, dd, J = 6.9 Hz, 8.4 Hz, H-2), 7.87 (1H, d, J = 9.0 Hz, H-7), 7.98 (1H, d, J = 9.0 Hz, H-6), 8.50 (1H, d, J = 8.4 Hz, H-1). Anal. ($C_{15}H_{14}O_3 \cdot {}^1/_8H_2O$) C, H.

17-Methyl-11,15-dioxacyclopenta[*a*]phenanthren-12-one (16). 38% yield; mp 205−207 °C; MS-ESI+ (*m*/*z*, %): 251 (M^+ + 1, 100), 273 (M^+ + Na, 65); IR (KBr) 1731, 762 cm^{-1}; ^{1}H NMR δ 2.41 (3H, d, J = 1.2 Hz, CH$_3$-17), 7.45 (1H, d, J = 1.2 Hz, H-16), 7.64 (2H, m, H-2, 3), 7.75 (1H, d, J = 8.7 Hz, H-7), 7.86 (1H, d, J = 8.7 Hz, H-6), 7.90 (1H, m, H-4), 8.62 (1H, dd, J = 2.4 Hz, 8.4 Hz, H-1). Anal. ($C_{16}H_{10}O_3$) C, H.

6-Methoxy-17-methyl-11,15-dioxacyclopenta[*a*]phenanthren-12-one (18). 45% yield; mp 203−205 °C; LC/MSD-Trap-positive (*m*/*z*, %): 303 (M^+ + Na, 100), 281 (M^+ + 1, 10); ^{1}H NMR δ 2.41 (3H, d, J = 1.2 Hz, CH$_3$-17), 4.08 (3H, s, OCH$_3$-6), 7.08 (1H, s, H-7), 7.42 (1H, d, J = 1.2 Hz, H-16), 7.65 (2H, m, H-2, 3), 8.29 (1H, d, J = 8.4 Hz, H-4), 8.55 (1H, d, J = 7.5 Hz, H-1). Anal. ($C_{17}H_{12}O_4 \cdot {}^1/_8H_2O$) C, H.

Method 2. To a solution of **10** (260 mg, 1 mmol) in toluene (25 mL) was added a mixture of HOAc (300 μL, 5 mmol) and ammonium acetate (384 mg, 5 mmol) in EtOH (8 mL) and chloroacetone (420 μL, 5 mmol). The reaction mixture was heated to reflux for 24 h. The mixture was then diluted and extracted with EtOAc. After drying over Na_2SO_4 and evaporation of the organic solvent in vacuo, the residue was purified with flash chromatography eluting with hexane: EtOAc = 100:1 to give **17**.

6-Chloro-17-methyl-11,15-dioxacyclopenta[*a*]phenanthren-12-one (17). 45% yield; mp 220−221 °C; MS-ESI+ (*m*/*z*, %): 285 (M^+ + 1, 100), 287 (30); ^{1}H NMR δ 2.40 (3H, d, J = 1.2 Hz, CH$_3$-17), 7.44 (1H, q, J = 1.2 Hz, H-16), 7.71 (2H, m, H-2, 3), 7.91 (1H, s, H-7), 8.27 (1H, m, H-4), 8.60 (1H, m, H-1). Anal. ($C_{16}H_9ClO_3$) C, H.

Preparation of Intermediates 19−23. Compounds **19**−**23** were prepared from **7**−**11**, respectively, using the following procedure. To a suspension of **7** (100 mg, 0.50 mmol) in water (5 mL) was added potassium carbonate (100 mg, 0.55 mmol). The resulting mixture was stirred at room temperature for 5 min, and then chloroacetaldehyde (130 μL, 1.0 mmol) was added to the reaction mixture. Stirring was maintained at room temperature for 5 h. After extraction three times with EtOAc, drying over Na_2SO_4, and evaporation of the organic solvent in vacuo, purification with flash chromatography eluting with $CHCl_3$:MeOH = 100:1 provided compound **19**.

4-Methyl-17-hydroxy-16,17-dihydro-11,15-dioxacyclopenta-[*a*]phenanthren-12-one (19). 36% yield; amorphous solid; MS-ESI+ (*m*/*z*, %): 292 (M^+ + H + Na, 100); ^{1}H NMR δ 2.74 (1H, s, CH$_3$-4), 4.82 (1H, dd, J = 2.7 Hz, 11.1 Hz, H-16α), 4.92 (1H, dd, J = 7.2 Hz, 11.1 Hz, H-16*β*), 5.68 (1H, dd, J = 2.7 Hz, 7.2 Hz, H-17), 7.55 (2H, m, H-2, 3), 7.70 (1H, d, J = 8.7 Hz, H-7), 7.88 (1H, d, J = 8.7 Hz, H-6), 8.48 (1H, m, H-1).

4-Ethyl-17-hydroxy-16,17-dihydro-11,15-dioxacyclopenta[*a*]-phenanthren-12-one (20). 42% yield; mp 163−165 °C; MS-ESI+ (*m*/*z*, %): 283 (M^+ + 1, 40), 265 (M^+ + 1 − H_2O, 100); ^{1}H NMR δ 1.38 (3H, t, J = 7.5 Hz, *CH*$_3$CH$_2$-), 3.13 (2H, q, J = 7.5 Hz, CH$_3$*CH*$_2$-), 4.80 (1H, dd, J = 3.0 Hz, 9.9 Hz, H-16α), 4.92 (1H, dd, J = 6.9 Hz, 9.9 Hz, H-16*β*), 5.68 (1H, dd, J = 3.0 Hz, 6.9 Hz, H-17), 7.58 (2H, m, H-2, 3), 7.68 (1H, d, J = 9.0 Hz, H-7), 7.92 (1H, d, J = 9.0 Hz, H-6), 8.40 (1H, d, J = 7.8 Hz, H-1).

17-Hydroxy-16,17-dihydro-11,15-dioxacyclopenta[*a*]phenanthren-12-one (21). 85% yield; mp 203−205 °C; MS-ESI+ (*m*/*z*, %): 277 (M^+ + Na, 100), 255 (M^+ + 1, 30), 237 (M^+ + 1-H_2O, 45); ^{1}H NMR δ 4.80 (1H, dd, J = 3.0 Hz, 11.1 Hz, H-16α), 4.92 (1H, dd, J = 6.9 Hz, 11.1 Hz, H-16*β*), 5.68 (1H, dd, J = 3.0 Hz, 6.9 Hz, H-17), 7.65−7.70 (4H, m, H-2, 3, 6, 7), 7.90 (1H, m, H-4), 8.60 (1H, m, H-1).

6-Chloro-17-hydroxy-16,17-dihydro-11,15-dioxacyclopenta-[*a*]phenanthren-12-one (22). 15% yield; mp 150 °C (sublime); LC/MSD-Trap-positive(*m*/*z*, %): 311 (M^+ + Na, 100); ^{1}H NMR δ 4.82 (1H, dd, J = 3.0 Hz, 10.8 Hz, H-16α), 4.92 (1H, dd, J = 6.9 Hz, 10.8 Hz, H-16*β*), 5.66 (1H, dd, J = 3.0 Hz, 6.9 Hz, H-17), 7.76 (2H, m, H-2, 3), 7.78 (1H, s, H-7), 8.32 (1H, d, J = 8.1 Hz, H-4), 8.62 (1H, d, J = 8.4 Hz, H-1).

6-Methoxy-17-hydroxy-16,17-dihydro-11,15-dioxacyclopenta-[*a*]phenanthren-12-one (23). 30% yield; mp 210−212 °C; LC/MSD-Trap-positive(*m*/*z*, %): 307 ($M^+ + Na$, 100); ^{1}H NMR δ 4.04 (3H, s, OCH_3-6), 4.85 (1H, dd, $J = 2.7$ Hz, 10.8 Hz, H-16α), 4.92 (1H, dd, $J = 6.9$ Hz, 10.8 Hz, H-16β), 5.68 (1H, dd, $J = 2.7$ Hz, 6.9 Hz, H-17), 6.86 (1H, s, H-7), 7.68 (2H, m, H-2, 3), 8.31 (1H, m, H-4), 8.53 (1H, m, H-1).

Preparation of Target Compounds 24−28. Compounds **24**−**28** were prepared from **19**−**23**, respectively, using the following procedure. To a suspension of compound **19** (12 mg) in water (1 mL) was added 2 N HCl and stirring continued at 50 °C until the reaction was complete as monitored by TLC. After extracting with $CHCl_3$ three times and drying over Na_2SO_4, the organic layer was evaporated and the residue was purified with flash chromatography to provide compound **24**.

4-Methyl-11,15-dioxacyclopenta[*a*]phenanthren-12-one (24). 86% yield; mp 220−222 °C; LC/MSD-Trap-positive (*m*/*z*, %): 273 ($M^+ + Na$, 100), 251($M^+ + 1$, 45); ^{1}H NMR δ 2.75 (1H, s, CH_3-4), 7.07 (1H, d, $J = 2.7$ Hz, H-17), 7.53 (2H, m, H-2, 3), 7.69 (1H, d, $J = 2.7$ Hz, H-16), 7.92 (1 H, d, $J = 8.7$ Hz, H-7), 7.96 (1 H, d, $J = 8.7$ Hz, H-6), 8.50 (1H, d, $J = 8.1$ Hz, H-1). Anal. ($C_{16}H_{10}O_3$) C, H.

4-Ethyl-11,15-dioxacyclopenta[*a*]phenanthren-12-one (25). 74% yield; mp 173−174 °C; MS-ESI+ (*m*/*z*, %): 265 ($M^+ + 1$, 100); ^{1}H NMR δ 1.40 (3H, t, $J = 7.5$ Hz, CH_3CH_2-), 3.15 (2H, q, $J = 7.5$ Hz, CH_3CH_2-), 7.07 (1H, d, $J = 2.4$ Hz, H-17), 7.50 (1H, d, $J = 6.3$ Hz, H-3), 7.59 (1H, dd, $J = 6.3$ Hz, 8.4 Hz, H-2), 7.69 (1H, d, $J = 2.4$ Hz, H-16), 7.92 (1H, d, $J = 9.0$ Hz, H-7), 8.02 (1H, d, $J = 9.0$ Hz, H-6), 8.52 (1H, d, $J = 8.4$ Hz, H-1). Anal. ($C_{17}H_{12}O_3 \cdot {}^1/_4H_2O$) C, H.

11,15-Dioxacyclopenta[*a*]phenanthren-12-one (26). 52% yield; mp 198−200 °C; MS-ESI+ (*m*/*z*, %): 237 ($M^+ + 1$, 100); ^{1}H NMR (Acetone-d_6) δ 7.12 (1 H, d, $J = 2.4$ Hz, H-17), 7.73 (2H, m, H-2, 3), 7.93 (1 H, d, $J = 8.7$ Hz, H-7), 7.97 (1 H, d, $J = 8.7$ Hz, H-6), 8.06 (2H, m, H-4, 16), 8.50 (1H, m, H-1). Anal. ($C_{15}H_8O_3$) C, H.

6-Chloro-11,15-dioxacyclopenta[*a*]phenanthren-12-one (27). 88% yield; mp 150 °C (sublime); MS-ESI+ (*m*/*z*, %): 271 ($M^+ + 1$, 100), 273 (30); ^{1}H NMR δ 7.07 (1H, d, $J = 1.8$ Hz, H-17), 7.70 (1H, d, $J = 1.8$ Hz, H-16), 7.76 (2H, m, H-2, 3), 8.01 (1H, s, H-7), 8.32 (1H, m, H-4), 8.64 (1H, m, H-1). Anal. ($C_{15}H_7ClO_3 \cdot {}^1/_2H_2O$) C, H.

6-Methoxy-11,15-dioxacyclopenta[*a*]phenanthren-12-one (28). 85% yield; mp 194−195 °C; LC/MSD-Trap-positive (*m*/*z*, %): 267 ($M^+ + 1$, 100), 289 ($M^+ + Na$, 30); ^{1}H NMR δ 4.11 (1H, s, OCH_3-6), 7.07 (1H, d, $J = 2.1$ Hz, H-17), 7.13 (1H, s, H-7), 7.68 (3H, m, H-2, 3, 16), 8.31 (1H, dd, $J = 1.8$ Hz, 6.9 Hz, H-4), 8.57 (1H, dd, $J = 1.8$ Hz, 7.8 Hz, H-1). Anal. ($C_{16}H_{10}O_4$) C, H.

Cell Growth Inhibition Assay.[18] All stock cultures are grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well with compounds added from DMSO-diluted stock. After 3 days in culture, attached cells were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The mean ED_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations. Variation between replicates was no more than 5% of the mean. The following human tumor cell lines were used in the assay: A549 (non small cell lung cancer), MCF-7 (estrogen receptor positive breast cancer), ZR-75-1 (estrogen receptor positive breast cancer), MDA MB-231 (estrogen receptor negative breast cancer), HS 587-T (estrogen receptor negative breast cancer), SK-BR-3 (estrogen receptor negative, HER-2 overexpressing breast cancer), A431 (EGFR overexpressing skin cancer), LN-CaP (AR-dependent prostate cancer), SW620 (colon cancer), PC-3 (prostate cancer), KB (nasopharyngeal carcinoma), KB-VIN (vincristine-resistant KB subline). All cell lines were obtained from the Lineberger Cancer Center (UNC−CH) or from ATCC (Rockville, MD) and were cultured in RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin.

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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