Novel 5-Aminoflavone Derivatives as Specific Antitumor Agents in Breast Cancer

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In the course of our search for new antitumor agents in breast cancer, novel amino-substituted flavone derivatives were synthesized and examined for antitumor activities. Among them, 5,4′-diaminoflavone and some of its congeners showed remarkable antiproliferative activity against the estrogen receptor (ER)-positive and estrogen-responsive human breast cancer cell line MCF-7. The activity was observed irrespective of the presence or absence of estrogen. The 5-aminoflavone derivatives (5-AFs) are not classical anti-estrogens because they did not compete with [^{3}H]estradiol to bind the estrogen receptor. Moreover, 5-AFs showed antitumor activity highly selective to the ER-positive breast cancer cell line, and they showed no effects against the ER-negative human cancer cell lines HeLa S_3, WiDr, and MDA-MB-453. Although the mechanism of their selective antitumor activity to ER-positive breast cancer cells is unclear, 5-AFs are expected to be a new type of antitumor agents in breast cancer.

The blockade of estrogen action is a major approach for the treatment of hormone-dependent breast cancer.[1−4] Triarylethylene anti-estrogens, such as tamoxifen (TAM) (Chart 1), are representative of this strategy.[5] However, because these anti-estrogens generally possess partial estrogenic activity, considerable efforts by many groups have been devoted to the search for pure anti-estrogens without estrogenic activity.[6−10] Indeed, pure anti-estrogens might be more effective than partial agonists in reducing the mitogenic action of estrogen on the growth of breast cancer cells, but they would exhibit the antiproliferative effect mostly in the case when the growth is estrogen-dependent. In addition, there is another problem that many breast tumors become resistant to TAM during treatment. Tumors that lose hormone dependency usually become resistant to TAM.

Among estrogen receptor (ER)-positive breast cancer tissues, approximately 60% of them respond to anti-estrogens, whereas the remaining 40% are nonresponsive.[1,2,11−13] On the other hand, it is known that MCF-7, the estrogen receptor-positive and estrogen-responsive human breast cancer cell line, can proliferate in the absence of estrogen.[14] Recently, a new breast cancer cell line which expresses ER but grows estrogen independently was reported.[15] It seems that the growth of breast cancer cells, which are not responsive to anti-estrogens but are ER-positive, is due to estrogen-independent growth, although it is poorly understood how estrogen-independent growth in an ER-positive tumor is mediated.[14] This circumstance prompted us to search for novel anti-breast-cancer agents which are effective against both estrogen-dependent and -independent growth.

Flavonoids, either natural or synthetic, are well known to exhibit various biological activities.[16] For example, antioxidant,[17] anti-inflammatry,[18] gastroprotective,[19] antiviral,[20] antimutagenic,[21] topoisomerase II inhibitory,[22] protein kinase C inhibitory,[23] and cytotoxic[24−26] activities, etc., have been reported. Particularly, we have been interested in the relationship between flavonoids and anti-breast-cancer activities because large numbers of flavonoids are known to exhibit antiproliferative effects against breast cancer cells or binding affinities for the ER. For example, apigenin (**1**) and some of its congeners were reported to possess antiproliferative activity against the human breast cancer cell line ZR-75-1.[27] 6,4′-Dihydroxyflavone (**2**) has a binding affinity for the ER,[28] and flavanone derivative **3** (BE-14348B[29]) exhibits strong estrogenic activity. The ER binding affinity and the estrogenic

Chart 1

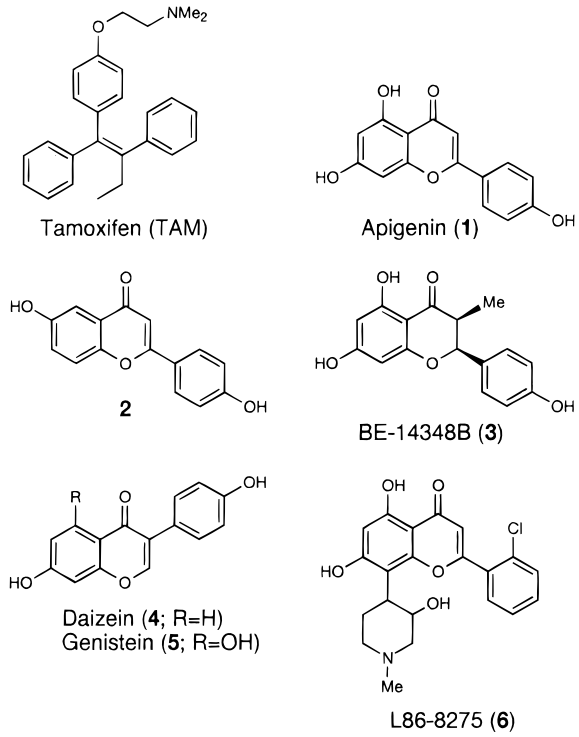

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Table 1. Antiproliferative Activity and ER Binding of Compounds **10** against MCF-7

		IC_{50} (μM)[a]		
		estradiol[b]		ER binding
compounds	X	−	+	competition
10a	H	>100	13	>100
10b	4′-OH	>100	1.5	18
10c	4′-OMe	>100	12	>100
10d	4′-Br	9.5	1.0	>100
10e	4′-CN	94	>100	>100
10f	4′-CO_2H	>10	>10	NT[c]
10g	4′-NH_2	0.0098	0.0072	>100
10h	4′-NHAc	18	13	>100
10j	4′-NHMe	0.0054	0.0023	>100
10k	4′-NHEt	0.0017	0.0013	>100
10l	4′-$NHPr^n$	0.0087	0.0067	>100
10m	4′-$NHBu^n$	0.0087	0.0051	>100
10p	4′-$NHHex^n$	0.44	0.075	NT[c]
10q	4′-$NHCH_2Ph$	4.3	4.1	>100
10n	4′-NMe_2	0.0050	0.0040	>100
10i	4′-NEt_2	0.010	0.0020	NT[c]
apigenin (**1**)		95	>100	14
genistein (**5**)		77	11	0.99
tamoxifen (TAM)		19	0.14	3.9
estradiol				0.0016

[a] IC_{50} values were measured by cell count method described in the Experimental Section. [b] 10^{-4} μM. [c] Not tested.

activity were also reported concerning the isoflavone derivatives daizein (**4**) and genistein (**5**).[30] Recently, compound **6** (L86-8275) was reported to exhibit antitumor activities against several types of human breast cancer cell lines.[31]

As mentioned above, most flavonoids exhibiting biological activities associated with breast cancer or estrogenic action possess at least one hydroxyl group in their skeletons. On the other hand, some flavonoids possessing amino groups are known, but their previously mentioned biological activities are scarcely known.[32−35] Moreover, to the best of our knowledge, 5-aminoflavones are not known except for only a few examples.[36,37] Therefore, we hypothesized that flavone derivatives substituted with amino groups, which can function as hydrogen bond donors or acceptors as hydroxyl groups, might exhibit antitumor activity in breast cancer.

In this study, we synthesized various amino-substituted flavones and evaluated their antitumor activity against MCF-7 in both the presence and absence of estrogen. In addition, to characterize their physiological properties, their binding affinity for the ER was studied by binding competition with [^{3}H]estradiol. Furthermore, we evaluated the antiproliferative activity against hormone-independent human cancer cell lines HeLa S_3 (uterus), WiDr (colon), and MDA-MB-453 (breast, without the ER). We wish to describe the synthesis of amino-substituted flavones and their attractive antitumor activity.

Chemistry

The 5-aminoflavone derivatives (5-AFs) listed in Table 1 were synthesized as shown in Scheme 1. The condensation of the benzoate **7**[38] and various acetophenone derivatives **8** with sodium hydride afforded 1,3-diketones. If compound **8** was substituted with a functional group, which is unstable or reactive under basic conditions, suitable protecting groups (THP, MOM, pivaloyl, etc.) were used. During the reaction, the *N*-ethoxycarbonyl group of compound **7** was removed because of the strongly basic conditions. The 1,3-diketones thus obtained were used for the next step without purification. They were treated with HCl in EtOH to afford cyclized products **9**. Various compounds **10** were obtained after the removal of the *N*-pivaloyl group and other protecting groups of **9** under more strongly acidic conditions. Some derivatives were prepared from the obtained compounds **10**. The 4′-carboxy derivative **10f** was obtained by hydrolysis of the corresponding cyano derivative **10e**. The 4′-acetamido derivative **10h** was obtained by selective acetylation of **10g**. The 4′-diethylamino derivative **10i** was obtained as a major product of the mixture of mono- and trialkylated products by alkylation of **10g**. Compounds **10p**,**q** were synthesized by alkylation of **9p** followed by deprotection.

6-Amino-4′-(dimethylamino)flavone (**13**) was synthesized as shown in Scheme 2. Ethyl 5-nitrosalicylate (**11**) was converted to its methoxymethyl ether. The nitro group was then reduced to the amino group with hydrazine and palladium on charcoal and protected as a pivaloyl amide to afford compound **12**. Compound **13** was obtained from compounds **12** and **8n** in the same way as shown in Scheme 1.

6-Amino-3′-(dimethylamino)flavone (**16a**) and 7-amino-4′-(dimethylamino)flavone (**16b**) were synthesized as shown in Scheme 3. Compounds **14a**,[39]**b** were treated with the derivatives of 3- and 4-(dimethylamino)benzoic acid under basic condition to afford the corresponding 1,3-diketones, which were successively cyclized and deprotected under acidic conditions to afford compounds **16a**,**b**.

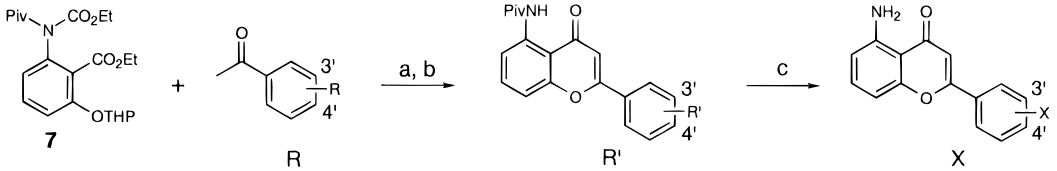
8-Amino-4′-(dimethylamino)flavone (**19**) was prepared in another way (Scheme 4). Although the coupling of **17** with **15b** or some of its derivatives was unsuccessful, the aldol condensation of compound **17** and 4-(dimethylamino)benzaldehyde (**18**) successfully afforded a chalcone derivative. Compound **19** was obtained by oxidative cyclization[40] of the chalcone followed by acidic deprotection.

Biological Activity and Discussion

5-AFs **10a**−**q** and compounds **13**, **16a**,**b**, and **19** were evaluated for their antiproliferative activity against MCF-7, the ER-positive and estrogen-responsive human breast cancer cell line, in both the presence and absence of estrogen. Although the growth rate was very slow, estrogen-independent growth of MCF-7 was observed in the absence of estrogen.[14] Under this condition, we could evaluate not only antiproliferative activity of test compounds against the estrogen-independent growth of MCF-7 but also estrogenic activity (growth-stimulating activity). In addition, the binding affinity of 5-AFs for the ER was studied by the binding competition with [^{3}H]-estradiol. These results are listed in Table 1.

TAM exhibited antiproliferative activity against MCF-7 in the presence of estrogen (IC_{50} 0.14 μM). Apigenin was almost inactive. Genistein exhibited weak antiproliferative activity in the presence of estrogen (IC_{50} 11 μM) and had an affinity to the ER 4 times stronger than TAM. 5-Aminoflavone **10a** exhibited weak estrogenic activity at 6.3 μM in the absence of estradiol (data not shown) and weak antiproliferative activity (IC_{50} 13 μM) in the presence of estradiol. In the course of the chemical modification of **10a**, 5-amino-4′-hydroxyflavone (**10b**) showed a similar property to **10a** with enhanced

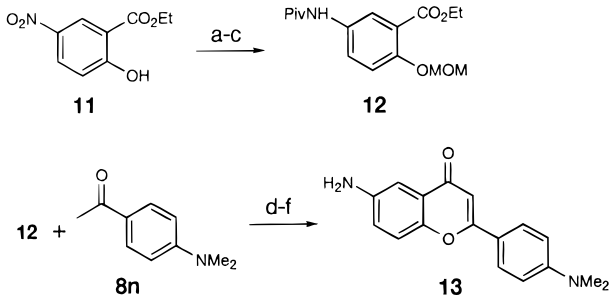
Scheme 1[a]



	R		R'		X
8a	H	**9a**	H	**10a**	H
8b	4'-OTHP	**9b**	4'-OH	**10b**	4'-OH
8c	4'-OMe	**9c**	4'-OMe	**10c**	4'-OMe
8d	4'-Br	**9d**	4'-Br	**10d**	4'-Br
8e	4'-CN	**9e**	4'-CN	**10e**	4'-CN
				10f	4'-CO_2H
8g	4'-NHPiv	**9g**	4'-NHPiv	**10g**	4'-NH_2
				10h	4'-NHAc
				10i	4'-NEt_2
8j	4'-NHMe	**9j**	4'-NHMe	**10j**	4'-NHMe
8k	4'N(Ac)Et	**9k**	4'N(Ac)Et	**10k**	4'NHEt
8l	4'-N(Ac)Pr^n	**9l**	4'-N(Ac)Pr^n	**10l**	4'-NHPr^n
8m	4'-N(Ac)Bu^n	**9m**	4'-N(Ac)Bu^n	**10m**	4'-NHBu^n
8n	4'-NMe_2	**9n**	4'-NMe_2	**10n**	4'-NMe_2
8o	3'-NHPiv	**9o**	3'-NHPiv	**10o**	3'-NH_2
8p	4'-N(Ac)MOM	**9p**	4'-NHAc		
				10p	4'-NHHex^n
				10q	4'-$NHCH_2Ph$

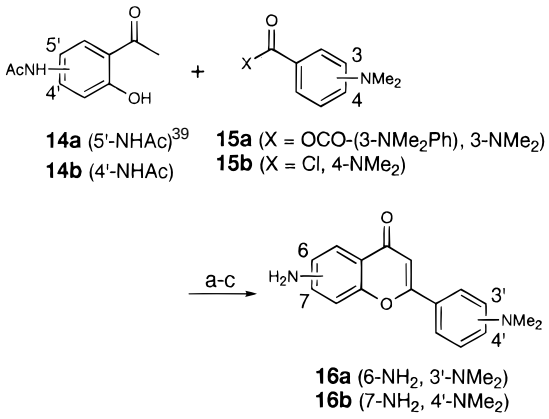
[a] (a) NaH, 1,4-dioxane, reflux; (b) HCl, EtOH, rt; (c) HCl, EtOH or 1,4-dioxane, reflux; (d) H_2SO_4, AcOH, H_2O, 100 °C; (e) Ac_2O, pyridine, 0 °C; (f) NaH, EtI, DMF, 0 °C; (g) NaH, DMF, rt, *n*-HexI (for **10p**) or $PhCH_2Br$ (for **10q**).

Scheme 2[a]



[a] (a) $MeOCH_2Cl$, NaH, THF, reflux; (b) $H_2NNH_2 \cdot H_2O$, Pd/C, EtOH, rt; (c) pivaloyl chloride, pyridine, rt; (d) NaH, 1,4-dioxane, reflux; (e) HCl, EtOH, rt; (f) HCl, EtOH, reflux.

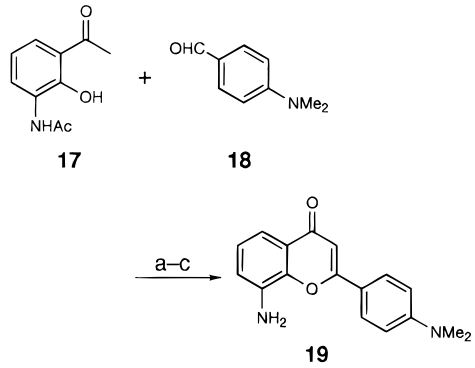
Scheme 3[a]



[a] (a) NaH, THF, rt; (b) HCl, EtOH, rt; (c) HCl, EtOH, reflux.

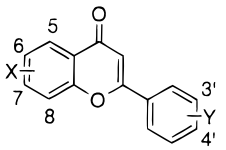
potency (IC_{50} 1.5 μM in the presence of estradiol). In addition, **10b** bound to the ER with an affinity 5 times less than TAM. Substitution of the 4′-position with electron-withdrawing groups such as cyano (compound **10e**) and carboxy (compound **10f**) groups resulted in a decrease in activity. Only the 4′-bromo derivative **10d** showed comparable activity to TAM. From a further investigation of the substituents at the 4′-position, we found that the 4′-amino derivative **10g** exhibited an intensive antiproliferative effect against MCF-7 irrespective of the presence or absence of estradiol (IC_{50} 0.0072 and 0.0098 μM, respectively). Acetylation of the 4′-amino group of **10g** resulted in a decrease in activity (compound **10h**). Activity was somewhat enhanced when the 4′-amino group was alkylated (compounds **10j**−**m**), whereas with increasing bulkiness, decreasing activity occurred with compounds **10p**,**q**. Dialkylated derivatives **10n**,**i** also seemed to enhance antitumor activity; hence, the 4′-amino hydrogen was not important as a hydrogen bond donor. Further, the basicity of the 4′-amino group is important because secondary

Scheme 4[a]



[a] (a) NaH, DMF, rt; (b) SeO_2, *i*-AmOH, 150 °C; (c) HCl, 1,4-dioxane, reflux.

Table 2. Antiproliferative Activity of Various Diaminoflavones against MCF-7



			IC_{50} (μM),[a] estradiol[b]	
compounds	X	Y	−	+
10g	5-NH_2	4′-NH_2	0.0098	0.0072
10o	5-NH_2	3′-NH_2	>100	27
13	6-NH_2	4′-NMe_2	0.035	0.013
10n	5-NH_2	4′-NMe_2	0.0050	0.0040
16a	6-NH_2	3′-NMe_2	13	13
16b	7-NH_2	4′-NMe_2	6.9	5.0
19	8-NH_2	4′-NMe_2	2.0	0.021
tamoxifen			19	0.14

[a] IC_{50} values were measured by cell count method described in the Experimental Section. [b] 10^{-4} μM.

or tertiary amines seemed to enhance the activity. These data indicated that 4′-amino groups with some basicity and without bulkiness are essential for exhibiting strong antitumor activity. However, the alkylation of the 4′-amino group is not essential for exhibiting the antiproliferative activity because compound **10g** showed sufficiently strong activity. In addition, when the compounds are used in *in vivo*, the lower alkyl side chain might be metabolically removed. So we considered compound **10g** as a prototype. Interestingly, all the compounds exhibiting strong antitumor activity did not compete with [^{3}H]estradiol in terms of receptor binding even at a concentration of 100 μM.

We then investigated the relationship between the positions of the amino groups and antitumor activities (Table 2). The relocation of the 4′-amino group to the 3′-position (compound **10o**) resulted in a drastic decrease in activity. The 4′-dimethylamino derivative **10n** also exhibited a very strong antiproliferative effect which is comparable to **10g**. The relocation of the 5-amino group of compound **10n** to the 6-position (compound **13**) resulted in a decrease in activity. A further decrease in activity was observed when the 4′-dimethylamino group of **13** relocated to the 3′-position (compound **16a**). Although the 7-amino derivative **16b** exhibited only weak effects, the 8-amino derivative **19** exhibited antiproliferative activity at an IC_{50} of 0.021 μM in the presence of estradiol. Reviewing the results, the best positions of amino groups seemed to be the 5- and 4′-positions. Because the decrease in activity was larger when the 4′-amino group was relocated to the 3′-position than when the 5-amino group was relocated to the 6-, 7-, or 8-position, the 4′-amino group evidently plays a critical role in growth inhibition of MCF-7.

The antiproliferative activity of compound **10g** against MCF-7 in both the presence and absence of estradiol is shown in Figure 1. In the absence of estradiol, the cell numbers increased from 2.5×10^3 to 42×10^3 in 6 days, and this seemed to be the estrogen-independent growth (lane 1). The growth of MCF-7 cells was stimulated 8.3-fold by the addition of 10^{-4} μM estradiol (lane 4 vs lane 1). TAM (0.5 μM) stimulated the growth of MCF-7 cells in the absence of estradiol (lane 2); hence, the partial agonistic activity of TAM was confirmed,[41,42] and it inhibited the growth of MCF-7 about 55% in the

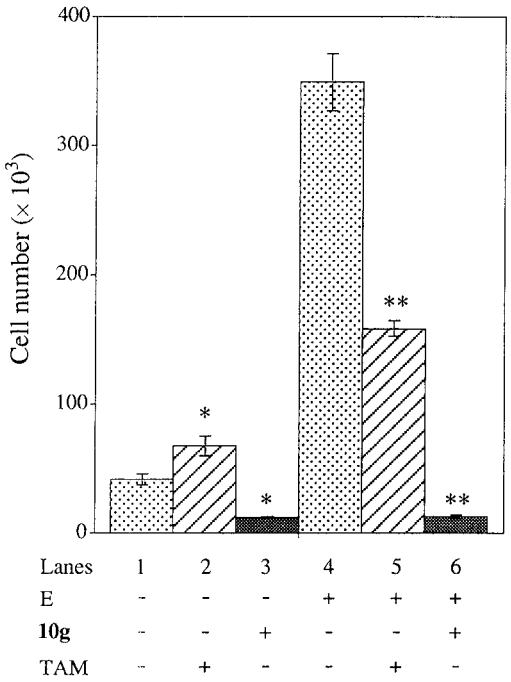


Figure 1. Response of MCF-7 cells to compound **10g** (0.5 μM) and tamoxifen (TAM; 0.5 μM) in both the presence and absence of estradiol (E; 10^{-4} μM). MCF-7 cells were preincubated in phenol red free MEM containing 5% calf serum for 5 days. The cells (2.5×10^3) were treated with each compound in both the presence and absence of estradiol and counted 6 days later. The results presented are means ± SD of three independent replications, and the vertical bars represent the standard deviations. *P < 0.05 vs lane 1. **P < 0.05 vs lane 4.

Table 3. Specificity of Antiproliferative Activity to Breast Cancer Cell Line

	IC_{50} (μM)[a]		
compounds	MCF-7[b]	HeLa S_3	WiDr
10g	0.052	>10	>10
10j	0.076	6.3	>10
10k	0.092	>10	>10
adriamycin	0.25	0.030	0.62

[a] IC_{50} values were measured by neutral red dye uptake method described in the Experimental Section. [b] In the presence of 10^{-2} μM estradiol.

presence of estradiol (lane 5). On the other hand, compound **10g** (0.5 μM) inhibited almost completely the growth of MCF-7 cells in both the absence (lane 3) and presence (lane 6) of estradiol.

The 5,4′-diaminoflavone derivatives exhibited remarkable antiproliferative activity against MCF-7 cells without ER competition; thus, we were next interested in the antiproliferative effects of these compounds against other human cancer cell lines and the ER-negative breast cancer cell line. As shown in Table 3, a representative cytotoxic agent, adriamycin, exhibited antitumor activity against not only MCF-7 but also HeLa S_3 and WiDr, while compounds **10g**,**j**,**k** exhibited selective and strong antiproliferative activity against the breast cancer cell line. However, compound **10g** exhibited no effect against the growth of MDA-MB-453, the ER-negative human breast cancer cell line, at least up to 5 μM (Table 4).

Although the mechanism of selective antitumor activity of 5-AFs to ER-positive MCF-7 breast cancer cells is unclear, it is noteworthy that they are not competitive inhibitors of estrogen like TAM and pure anti-estrogens

Table 4. Antiproliferative Activity of Compound **10g** against the ER-Negative Breast Cancer Cell Line MDA-MB-453

	IC_{50} (μM)[a]	
compounds	MCF-7[b]	MDA-MB-453
10g	0.0072	>5.0
tamoxifen	0.14	>5.0

[a] IC_{50} values were measured by cell count method described in the Experimental Section. [b] In the presence of 10^{-4} μM estradiol.

because they did not compete with [^{3}H]estradiol even at a concentration of 100 μM. Recently, some amino-substituted flavones were reported as inhibitors of protein-tyrosine kinases.[27] Thus, the possibility cannot be excluded that 5-AFs may affect the signal transduction downstream of the ER, including the phosphorylation of the ER.[43−46] However, although some 5-AFs reported here weakly inhibit a certain type of protein-tyrosine kinase (data not shown), it is probably not enough to explain their strong and selective antitumor activity. On the other hand, compound **6** (L86-8275) was reported to exhibit antiproliferative activities against some cancer cell lines, including breast cancer cells, by inhibiting Cdc2 kinase activity.[47] But the antiproliferative activity of compound **6** is not selective to breast cancer cells.[48] Therefore, it is unlikely that 5-AFs exhibited antitumor activity by the same mechanism as compound **6**.

These results suggest the presence of an alternative receptor or a signal transduction pathway to the ER in the ER-positive breast cancer cells because the 5-AFs only inhibited the growth of MCF-7 cells among the human cancer cell lines tested, including ER-negative breast cancer cell line. Further, it would appear that there is a new therapeutic target for the treatment of breast cancer.

Conclusions

We prepared a series of amino-substituted flavones. Among them, 5,4′-diaminoflavone (**10g**) and some of its congeners exhibited a remarkable antiproliferative effect against the human breast cancer cell line MCF-7 irrespective of the presence or absence of estrogen. Although the mechanism of selective antitumor activity of 5-AFs to the ER-positive MCF-7 breast cancer cells is unclear, they are expected to be a new type of chemotherapeutic agent in breast cancer. Further evaluation of the antitumor activities against other cell lines and research on the structure−activity relationships of related derivatives are in progress.

Experimental Section

All melting points were determined on Yanako micromelting point apparatus and are uncorrected. IR spectra were recorded on a JASCO IR-400 spectrometer. ^{1}H-NMR spectra were recorded on HITACHI R-90H (90 MHz) and JEOL JNM-GX-270 (270 MHz) spectrometers. Electron impact mass spectra (EIMS) were recorded on a JEOL JMS-D-300 spectrometer. Elemental analyses were performed by a Perkin-Elmer 2400 C, H, N analyzer. Organic extracts were dried over anhydrous Na_2SO_4, and the solvents were evaporated under reduced pressure. Merck Kieselgel 60 was used for column chromatography. Sodium hydride used was a 60% oil dispersion.

Typical Procedure for Preparation of 2-Aryl-4*H*-1-benzopyran-4-ones 9a−e,g,j−p: 5-(Pivaloylamino)-2-[4-(pivaloylamino)phenyl]-4*H*-1-benzopyran-4-one (9g). To a refluxing suspension of sodium hydride (4.60 g, 115 mmol) in 1,4-dioxane (50 mL) under argon atmosphere was added dropwise a solution of **7** (20.0 g, 47.5 mmol) and **8g** (12.6 g, 57.3 mmol) in 1,4-dioxane (110 mL) over 10 min. The reaction mixture was refluxed for 3 h and cooled on an ice bath. Water was added, and the basic solution was washed with *n*-hexane. Then the solution was twice extracted with EtOAc, and the organic layer was washed with brine. The combined extracts were concentrated and dissolved in EtOH (250 mL). Concentrated HCl (30 mL) was added, and the reaction mixture was stirred for 2 h at room temperature. The precipitated product was collected by filtration, washed with EtOH, and dried to afford **9g** (15.3 g, 77%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.33 (s, 9H, $C(CH_3)_3$), 1.35 (s, 9H, $C(CH_3)_3$), 6.73 (s, 1H, 3-H), 7.25 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.66 (t, J = 8.4 Hz, 1H, 7-H), 7.77 (d, J = 9.0 Hz, 2H, 2′,6′-H), 7.91 (d, J = 9.0 Hz, 2H, 3′,5′-H), 8.68 (dd, J = 8.3, 1.0 Hz, 1H, 6-H), 12.8 (br s, 1H, 5-NH).

2-Phenyl-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9a). This compound was obtained from **7** and **8a** (19%) and was used for the next step without purification.

2-(4-Hydroxyphenyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9b). This compound was obtained from **7** and **8b** (29%): ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.30 (s, 9H, $C(CH_3)_3$), 6.82 (s, 1H, 3-H), 6.95 (d, J = 9.0 Hz, 2H, 3′,5′-H), 7.31 (dd, J = 8.3, 1.1 Hz, 1H, 8-H), 7.69 (t, J = 8.3 Hz, 1H, 7-H), 7.92 (d, J = 8.8 Hz, 2H, 2′,6′-H), 8.57 (dd, J = 8.3, 1.1 Hz, 1H, 6-H).

2-(4-Methoxyphenyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9c). This compound was obtained from **7** and **8c** (39%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 3.89 (s, 3H, OCH_3), 6.65 (s, 1H, 3-H), 7.03 (d, J = 8.8 Hz, 2H, 3′,5′-H), 7.17 (d, J = 8.6 Hz, 1H, 8-H), 7.61 (t, J = 8.4 Hz, 1H, 7-H), 7.96 (d, J = 9.0 Hz, 2H, 2′,6′-H), 8.70 (d, J = 8.1 Hz, 1H, 6-H), 12.9 (br, 1H, NH).

2-(4-Bromophenyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9d). This compound was obtained from **7** and **8d** (67%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 6.71 (s, 1H, 3-H), 7.21 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.65 (t, J = 8.6 Hz, 1H, 7-H), 7.68 (d, J = 8.8 Hz, 2H, 3′,5′-H), 7.77 (d, J = 8.9 Hz, 2H, 2′,6′-H), 8.74 (dd, J = 8.3, 1.0 Hz, 1H, 6-H), 12.8 (br, 1H, NH).

2-(4-Cyanophenyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9e). This compound was obtained from **7** and **8e** (58%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 6.79 (s, 1H, 3-H), 7.22 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.68 (t, J = 8.6 Hz, 1H, 7-H), 7.84 (d, J = 8.8 Hz, 2H, 2′,6′-H), 8.03 (d, J = 8.9 Hz, 2H, 3′,5′-H), 8.76 (dd, J = 8.3, 1.0 Hz, 1H, 6-H), 12.7 (br, 1H, NH).

2-[4-(Methylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9j). This compound was obtained from **7** and **8j** (26%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 2.93 (d, J = 5.1 Hz, 3H, CH_3), 4.28 (br, 1H, 4′-NH), 6.60 (s, 1H, 3-H), 6.67 (d, J = 7.0 Hz, 2H, 3′,5′-H), 7.17 (dd, J = 8.6, 1.0 Hz, 1H, 8-H), 7.59 (t, J = 8.4 Hz, 1H, 7-H), 7.77 (d, J = 7.1 Hz, 2H, 2′,6′-H), 8.69 (dd, J = 8.3, 1.0 Hz, 1H, 6-H), 13.0 (br, 1H, NH).

2-[4-(*N*-Ethylacetamido)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9k). This compound was obtained from **7** and **8k** (78%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.15 (t, J = 7.1 Hz, 3H, CH_3), 1.39 (s, 9H, $C(CH_3)_3$), 1.93 (s, 3H, $COCH_3$), 3.81 (q, J = 7.2 Hz, 2H, CH_2), 6.76 (s, 1H, 3-H), 7.22 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.30 (d, J = 8.5 Hz, 2H, 3′,5′-H), 7.67 (t, J = 8.4 Hz, 1H, 7-H), 7.97 (d, J = 8.8 Hz, 2H, 2′,6′-H), 8.75 (dd, J = 8.3, 1.0 Hz, 1H, 6-H), 12.8 (br, 1H, NH).

5-(Pivaloylamino)-2-[4-(*N*-propylacetamido)phenyl]-4*H*-1-benzopyran-4-one (9l). This compound was obtained from **7** and **8l** and used for the next step without isolation.

2-[4-(*N*-Butylacetamido)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9m). This compound was obtained from **7** and **8m** (59%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.90 (t, J = 6.8 Hz, 3H, CH_3), 1.1−1.6 (m, 4H, $NCH_2CH_2CH_2$), 1.39 (s, 9H, $C(CH_3)_3$), 1.93 (s, 3H, $COCH_3$), 3.76 (t, J = 6.8 Hz, 2H, NCH_2), 6.76 (s, 1H, 3-H), 7.1−7.4 (m, 3H, 8,3′,5′-H), 7.66 (t, J = 8.4 Hz, 1H, 7-H), 7.99 (d, J = 8.1 Hz, 2H, 2′,6′-H), 8.75 (d, J = 8.4 Hz, 1H, 6-H), 12.8 (br, 1H, NH).

2-[4-(Dimethylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9n). This compound was obtained from **7** and **8n** (51%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.38 (s, 9H,

$C(CH_3)_3$), 3.08 (s, 6H, $N(CH_3)_2$), 6.60 (s, 1H, 3-H), 6.74 (d, J = 9.0 Hz, 2H, 3′,5′-H), 7.17 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.59 (t, J = 8.3 Hz, 1H, 7-H), 7.80 (d, J = 9.0 Hz, 2H, 2′,6′-H), 8.69 (dd, J = 8.6, 1.0 Hz, 1H, 6-H), 13.0 (br, 1H, NH).

5-(Pivaloylamino)-2-[3-(pivaloylamino)phenyl]-4*H*-1-benzopyran-4-one (9o). This compound was obtained from **7** and **8o** (49%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.37 (s, 9H, $C(CH_3)_3$), 1.39 (s, 9H, $C(CH_3)_3$), 6.75 (s, 1H, 3-H), 7.25 (dd, J = 8.1, 1.0 Hz, 1H, 8-H), 7.48 (t, J = 7.9 Hz, 1H, 5′-H), 7.50 (br, 1H, 3′-NH), 7.63 (dd, J = 8.0, 1.2 Hz, 1H, 6′-H), 7.64 (t, J = 8.3 Hz, 1H, 7-H), 7.70 (ddd, J = 8.0, 2.2, 1.0 Hz, 1H, 4′-H), 8.19 (t, J = 2.0 Hz, 1H, 2′-H), 8.73 (dd, J = 8.3, 0.7 Hz, 1H, 6-H), 12.8 (br, 1H, 5-NH).

2-(4-Acetamidophenyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (9p). This compound was obtained from **7** and **8p** (52%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.29 (s, 9H, $C(CH_3)_3$), 2.10 (s, 3H, $COCH_3$), 6.96 (s, 1H, 3-H), 7.39 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.45 (t, J = 8.3 Hz, 1H, 7-H), 7.79 (d, J = 9.3 Hz, 2H, 2′,6′-H), 8.06 (d, J = 8.8 Hz, 2H, 3′,5′-H), 8.57 (dd, J = 8.3, 0.7 Hz, 1H, 6-H), 10.3 (br, 1H, 4′-NH), 12.9 (br, 1H, 5-NH).

Typical Procedure for Preparation of 2-Aryl-4*H*-1-benzopyran-4-ones 10a−e,g,j−o: 5-Amino-2-(4-aminophenyl)-4*H*-1-benzopyran-4-one (10g). To a suspension of **9g** (14.5 g, 34.5 mmol) in EtOH (200 mL) was added concentrated HCl (200 mL). The mixture was refluxed for 5 h. During the reaction, starting material gradually dissolved and the product precipitated. After cooling on an ice bath, the precipitate was collected by filtration, washed with 2-propanol, and dried to afford **10g** (9.44 g, 84%) as a hydrochloride: mp 186−188 °C; IR (KBr) 1624 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.52 (dd, J = 8.3, 0.9 Hz, 1H, 6-H), 6.59 (s, 1H, 3-H), 6.64 (dd, J = 8.1, 0.9 Hz, 1H, 8-H), 6.91 (d, J = 8.6 Hz, 2H, 3′,5′-H), 7.33 (t, J = 8.2 Hz, 1H, 7-H), 7.36 (d, J = 8.8 Hz, 2H, 2′,6′-H); EIMS m/z 252 (M^+). Anal. ($C_{15}H_{12}N_2O_2$·1.9HCl) C, H, N.

5-Amino-2-phenyl-4*H*-1-benzopyran-4-one (10a). This compound was obtained in a similar manner as described for **10g** (70%): mp 191−193 °C; IR (KBr) 1644 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.24 (dd, J = 8.1, 0.9 Hz, 1H, 6-H), 6.62 (s, 1H, 3-H), 6.69 (dd, J = 8.1, 0.9 Hz, 1H, 8-H), 7.24 (t, J = 8.1 Hz, 1H, 7-H), 7.4−7.6 (m, 3H, 3′,4′,5′-H), 7.8−8.0 (m, 2H, 2′,6′-H); EIMS m/z 237 (M^+). Anal. ($C_{15}H_{11}NO_2$·HCl) C, H, N.

5-Amino-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (10b). This compound was obtained in a similar manner as described for **10g** (77%): mp 244−245 °C; IR (KBr) 1634 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.59 (dd, J = 8.1, 1.1 Hz, 1H, 6-H), 6.62 (s, 1H, 3-H), 6.71 (dd, J = 8.1, 0.9 Hz, 1H, 8-H), 6.95 (d, J = 8.9 Hz, 2H, 3′,5′-H), 7.46 (dd, J = 8.1, 8.3 Hz, 1H, 7-H), 7.86 (d, J = 8.2 Hz, 2H, 2′,6′-H); EIMS m/z 253 (M^+). Anal. ($C_{15}H_{11}NO_3$·HCl) C, H, N.

5-Amino-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one (10c). The reaction mixture was concentrated, poured into water, and extracted with $CHCl_3$. The organic layer was washed with brine. Chromatogaphy (50:1 $CHCl_3$/MeOH) and recrystallization from EtOAc/*n*-hexane afforded **10c** as a free base (48%): mp 144−145 °C; IR (KBr) 1647 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.88 (s, 3H, OCH_3), 6.43 (dd, J = 8.1, 0.9 Hz, 1H, 6-H), 6.51 (br, 2H, NH_2), 6.55 (s, 1H, 3-H), 6.69 (dd, J = 8.3, 0.9 Hz, 1H, 8-H), 7.00 (d, J = 9.0, 2H, 3′,5′-H), 7.31 (t, J = 8.2 Hz, 1H, 7-H), 7.84 (d, J = 9.0 Hz, 2H, 2′,6′-H); EIMS m/z 267 (M^+). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

5-Amino-2-(4-bromophenyl)-4*H*-1-benzopyran-4-one (10d). This compound was obtained in a similar manner as described for **10g** (57%): mp 197−199 °C; IR (KBr) 1653 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.55 (dd, J = 8.2, 0.9 Hz, 1H, 6-H), 6.68 (dd, J = 8.4, 1.0 Hz, 1H, 8-H), 6.85 (s, 1H, 3-H), 7.37 (t, J = 8.3 Hz, 1H, 7-H), 7.77 (d, J = 8.6 Hz, 2H, 3′,5′-H), 7.99 (d, J = 9.0 Hz, 2H, 2′,6′-H); EIMS m/z 315, 317 (M^+). Anal. ($C_{15}H_{10}BrNO_2$·0.5HCl) C, H, N.

5-Amino-2-(4-cyanophenyl)-4*H*-1-benzopyran-4-one (10e). This compound was obtained as a free base in a similar manner as described for **10c** (82%): mp 270−273 °C; IR (KBr) 2234, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.48 (dd, J = 8.2, 0.8 Hz, 1H, 6-H), 6.67 (s, 1H, 3-H), 6.70 (dd, J = 8.1, 0.9 Hz, 1H, 8-H), 7.36 (t, J = 8.3 Hz, 1H, 7-H), 7.80 (d, J = 8.6 Hz, 2H, 3′,5′-H), 7.99 (d, J = 8.6 Hz, 2H, 2′,6′-H); EIMS m/z 262 (M^+). Anal. ($C_{16}H_{10}N_2O_2$) C, H, N.

5-Amino-2-[4-(methylamino)phenyl]-4*H*-1-benzopyran-4-one (10j). This compound was obtained as a free base in a similar manner as described for **10c** (42%): mp 184−185 °C; IR (KBr) 1646 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.91 (d, J = 2.0 Hz, 3H, CH_3), 6.41 (dd, J = 8.2, 1.0 Hz, 1H, 6-H), 6.49 (s, 1H, 3-H), 6.65 (d, J = 8.8 Hz, 2H, 3′,5′-H), 6.66 (dd, J = 8.4, 1.0 Hz, 1H, 8-H), 7.29 (t, J = 8.1 Hz, 1H, 7-H), 7.74 (d, J = 8.8 Hz, 2H, 2′,6′-H); EIMS m/z 266 (M^+). Anal. ($C_{16}H_{14}N_2O_2$) C, H; N: calcd, 10.52; found, 9.81.

5-Amino-2-[4-(ethylamino)phenyl]-4*H*-1-benzopyran-4-one (10k). This compound was obtained as a free base in a similar manner as described for **10c** (72%): mp 194−195 °C; IR (KBr) 1640 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.29 (t, J = 7.2 Hz, 3H, CH_3), 3.23 (m, 2H, CH_2), 4.05 (br, 1H, NH), 6.41 (dd, J = 8.2, 0.9 Hz, 1H, 6-H), 6.48 (s, 1H, 3-H), 6.50 (br, 2H, NH_2), 6.64 (d, J = 7.0 Hz, 2H, 3′,5′-H), 6.67 (dd, J = 8.3, 1.0 Hz, 1H, 8-H), 7.29 (t, J = 8.2 Hz, 1H, 7-H), 7.72 (d, J = 7.0 Hz, 2H, 2′,6′-H); EIMS m/z 280 (M^+). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

5-Amino-2-[4-(propylamino)phenyl]-4*H*-1-benzopyran-4-one (10l). This compound was obtained in a similar manner as described for **10g** (29%): mp 159−162 °C; IR (KBr) 1641 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.95 (t, J = 7.3 Hz, 3H, CH_3), 2.50 (m, 2H, NCH_2CH_2), 3.07 (t, J = 7.0 Hz, 2H, NCH_2), 6.50 (s, 1H, 3-H), 6.51 (d, J = 8.2 Hz, 1H, 6-H), 6.64 (d, J = 8.2 Hz, 1H, 8-H), 6.72 (d, J = 8.8 Hz, 2H, 3′,5′-H), 7.31 (t, J = 8.2 Hz, 1H, 7-H), 7.76 (d, J = 8.9 Hz, 2H, 2′,6′-H); EIMS m/z 294 (M^+). Anal. ($C_{18}H_{18}N_2O_2$·1.8HCl) C, H, N.

5-Amino-2-[4-(butylamino)phenyl]-4*H*-1-benzopyran-4-one (10m). This compound was obtained as a free base in a similar manner as described for **10c** (44%): mp 138−139 °C; IR (KBr) 1639 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.97 (t, J = 7.2 Hz, 3H, CH_3), 1.4−1.7 (m, 4H, $NCH_2CH_2CH_2$), 3.18 (q, J = 6.4 Hz, 2H, NCH_2), 4.10 (br, 1H, NH), 6.41 (dd, J = 8.3, 0.9 Hz, 1H, 6-H), 6.48 (s, 1H, 3-H), 6.51 (br, 2H, NH_2), 6.64 (d, J = 9.0 Hz, 2H, 3′,5′-H), 6.67 (dd, J = 8.2, 0.9 Hz, 1H, 8-H), 7.28 (t, J = 8.2 Hz, 1H, 7-H), 7.72 (d, J = 8.8 Hz, 2H, 2′,6′-H); EIMS m/z 308 (M^+). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

5-Amino-2-[4-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one (10n). This compound was obtained as a free base in a similar manner as described for **10c** (77%): mp 229−230 °C; IR (KBr) 1626 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.06 (s, 6H, $N(CH_3)_2$), 6.50 (dd, J = 8.0, 0.9 Hz, 1H, 6-H), 6.50 (br, 2H, NH_2), 6.51 (s, 1H, 3-H), 6.68 (dd, J = 8.2, 1.1 Hz, 1H, 8-H), 6.75 (d, J = 7.0 Hz, 2H, 3′,5′-H), 7.29 (t, J = 8.0 Hz, 1H, 7-H), 7.77 (d, J = 7.0 Hz, 2H, 2′,6′-H); EIMS m/z 280 (M^+). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

5-Amino-2-(3-aminophenyl)-4*H*-1-benzopyran-4-one (10o). This compound was obtained in a similar manner as described for **10g** (88%): mp 238−239 °C; IR (KBr) 1637 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.56 (dd, J = 8.3, 0.8 Hz, 1H, 6-H), 6.64 (dd, J = 8.2, 0.8 Hz, 1H, 8-H), 6.77 (s, 1H, 3-H), 7.42 (t, J = 8.4 Hz, 1H, 5′-H), 7.44 (dd, J = 8.6, 1.3 Hz, 1H, 4′-H), 7.59 (t, J = 8.0 Hz, 1H, 7-H), 7.85 (s, 1H, 2′-H), 7.91 (d, J = 8.1 Hz, 1H, 6′-H); EIMS m/z 252 (M^+). Anal. ($C_{15}H_{12}N_2O_2$·2HCl) C, H, N.

5-Amino-2-(4-carboxyphenyl)-4*H*-1-benzopyran-4-one (10f). A mixture of **10e** (100 mg, 0.375 mmol), AcOH (1 mL), H_2SO_4 (1 mL), and water (1 mL) was heated at 100 °C for 16 h. After cooling, the precipitated solid was collected by filtration, washed with water, and chromatographed (90:10:1 $CHCl_3$/MeOH/Et_3N) to afford **10f** (92 mg, 86%): mp >300 °C; IR (KBr) 1711, 1642 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.56 (dd, J = 8.3, 0.7 Hz, 1H, 6-H), 6.69 (d, J = 8.1 Hz, 1H, 8-H), 6.89 (s, 1H, 3-H), 7.38 (t, J = 8.2 Hz, 1H, 7-H), 7.46 (br s, 2H, NH_2), 8.08 (d, J = 8.6 Hz, 2H, 2′,6′-H), 8.15 (d, J = 8.6 Hz, 2H, 3′,5′-H); EIMS m/z 281 (M^+). Anal. ($C_{16}H_{11}NO_4$) C, H, N.

2-(4-Acetamidophenyl)-5-amino-4*H*-1-benzopyran-4-one (10h). To a solution of **10g** (100 mg, 0.397 mmol) in pyridine (2 mL) was added acetic anhydride (41 μL, 0.40 mmol), and the mixture was stirred at 0 °C for 20 min. The reaction mixture was dissolved in $CHCl_3$ and washed with 10% aqueous citric acid, 5% aqueous copper sulfate, and brine.

Chromatography (20:1 $CHCl_3$/MeOH) and recrystallization from $CHCl_3$/MeOH/*n*-hexane afforded **10h** (44 mg, 37%): mp 272−274 °C; IR (KBr) 1689, 1633 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 2.09 (s, 3H, CH_3), 6.52 (dd, J = 8.3, 0.9 Hz, 1H, 6-H), 6.65 (dd, J = 8.1, 0.9 Hz, 1H, 8-H), 6.71 (s, 1H, 3-H), 7.35 (t, J = 8.2 Hz, 1H, 7-H), 7.44 (br, 2H, NH_2), 7.76 (d, J = 8.8 Hz, 2H, 2′,6′-H), 7.98 (d, J = 8.8 Hz, 2H, 3′,5′-H), 10.3 (br, 1H, NH); EIMS *m*/*z* 294 (M^+). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

5-Amino-2-[4-(diethylamino)phenyl]-4*H*-1-benzopyran-4-one (10i). To a suspension of sodium hydride (1.44 g, 36.0 mmol) in DMF (5 mL) was added a solution of **10g** (3.02 g, 12.0 mmol) in DMF (30 mL) at 0 °C. Then EtI (2.1 mL, 26 mmol) was added and the mixture stirred at 0 °C for 50 min. The reaction mixture was poured into aqueous NH_4Cl and extracted with $CHCl_3$. The organic layer was washed with water and brine. Chromatography (4:1−1:0 $CHCl_3$/*n*-hexane) and recrystallization from EtOAc/*n*-hexane afforded **10i** (0.99 g, 21%): mp 219−220 °C; IR (KBr) 1626 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.21 (t, J = 7.4 Hz, 6H, $N(CH_2CH_3)_2$), 3.43 (q, J = 7.4 Hz, 4H, $N(CH_2CH_3)_2$), 6.41 (dd, J = 7.9, 1.0 Hz, 1H, 6-H), 6.48 (s, 1H, 3-H), 6.50 (br, 2H, NH_2), 6.67 (dd, J = 7.4, 1.5 Hz, 1H, 8-H), 6.71 (d, J = 9.4 Hz, 2H, 3′,5′-H), 7.28 (t, J = 8.2 Hz, 1H, 7-H), 7.75 (d, J = 8.9 Hz, 2H, 2′,6′-H); EIMS *m*/*z* 308 (M^+). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

5-Amino-2-[4-(hexylamino)phenyl]-4*H*-1-benzopyran-4-one (10p). To a solution of **9p** (3.00 g, 7.93 mmol) in DMF (30 mL) were added sodium hydride (634 mg, 15.9 mmol) and 1-iodohexane (1.40 mL, 9.52 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h and poured into ice and water. The mixture was extracted with $CHCl_3$; the extracts were washed with water and brine and chromatographed (1:1 EtOAc/*n*-hexane) to afford 2-[4-(*N*-hexylacetamido)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (2.66 g, 70%). The compound (2.16 g, 4.67 mmol) was then treated in a similar manner as described for compound **9g**. Recrystallization from $CHCl_3$/MeOH gave **10p** (1.40 g, 73%): mp 162−165 °C; IR (KBr) 1645 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 0.88 (t, J = 6.7 Hz, 3H, CH_3), 1.3−1.4 (m, 6H, $(CH_2)_3CH_3$), 1.5−1.6 (m, 2H, NCH_2CH_2), 3.11 (t, J = 7.1 Hz, 2H, NCH_2), 6.52 (s, 1H, 3-H), 6.53 (d, J = 8.2 Hz, 2H, 6-H), 6.58 (br, 2H, NH_2), 6.66 (dd, J = 8.3, 0.9 Hz, 1H, 8-H), 6.71 (d, J = 9.0 Hz, 2H, 3′,5′-H), 7.32 (t, J = 8.2 Hz, 1H, 7-H), 7.78 (d, J = 8.8 Hz, 2H, 2′,6′-H); EIMS *m*/*z* 336 (M^+). Anal. ($C_{21}H_{24}N_2O_2 \cdot 2HCl$) C, H, N.

5-Amino-2-[4-(benzylamino)phenyl]-4*H*-1-benzopyran-4-one (10q). This compound was obtained by the same way as described for compound **10p** except that benzyl bromide was used instead of 1-iodohexane (overall 26%): mp 157−159 °C; IR (KBr) 1638 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 4.37 (s, 2H, CH_2), 6.48 (s, 1H, 3-H), 6.50 (dd, J = 8.3, 0.9 Hz, 1H, 6-H), 6.61 (dd, J = 8.1, 0.9 Hz, 1H, 8-H), 6.71 (d, J = 9.0 Hz, 2H, 3′,5′-H), 7.30 (t, J = 8.4 Hz, 2H, 2′,6′-H), 7.3−7.4 (m, 5H, Ph); EIMS *m*/*z* 342 (M^+). Anal. ($C_{22}H_{18}N_2O_2 \cdot 1.9HCl$) C, H, N.

Ethyl 2-(Methoxymethoxy)-5-(pivaloylamino)benzoate (12). To a solution of ethyl 5-nitrosalicylate (5.00 g, 23.4 mmol) in THF (50 mL) were added sodium hydride (1.23 g, 30.8 mmol) and chloromethyl methyl ether (2.3 mL, 31 mmol) at 0 °C. The reaction mixture was refluxed for 30 min. The mixture was then poured into ice and water and extracted with $CHCl_3$. The organic layer was washed with brine and concentrated. The residue was dissolved in EtOH (30 mL), and 10% palladium on charcoal (610 mg) and a solution of hydrazine monohydrate (2.38 g, 47.5 mmol) in EtOH (3 mL) were added. The reaction mixture was stirred at room temperature for 30 min. The mixture was then filtered, concentrated, and extracted with $CHCl_3$. The organic layer was washed with brine and chromatographed (3:2 *n*-hexane/EtOAc) to afford ethyl 5-amino-2-(methoxymethoxy)benzoate (3.75 g, 71%). To a solution of this amino MOM ether (1.91 g, 8.48 mmol) in pyridine (20 mL) was added pivaloyl chloride (1.2 mL, 9.3 mmol), and the reaction mixture was stirred at room temperature for 30 min. The mixture was then filtered, concentrated, and extracted with $CHCl_3$. The organic layer was washed with 5% aqueous copper sulfate and brine and chromatographed (7:3 *n*-hexane/EtOAc) to afford **12** (2.01 g, 77%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.31 (s, 9H, $C(CH_3)_3$), 1.38 (t, J = 7.0 Hz, 3H, CH_2CH_3), 3.51 (s, 3H, OCH_3), 4.35 (q, J = 7.0 Hz, 2H, CH_2-CH_3), 5.20 (s, 2H, OCH_2O), 7.14 (d, J = 9.2 Hz, 1H, 3-H), 7.30 (br, 1H, NH), 7.6−7.8 (m, 2H, 4,6-H).

6-Amino-2-[4-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one (13). This compound was obtained from **12** and **8n** in a similar manner as described for compounds **9g** and **10g** and isolated as a free base in a similar manner as described for **10c** (overall 5.5%): mp 248 °C; IR (KBr) 1681 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 3.02 (s, 6H, $N(CH_3)_2$), 5.44 (br s, 2H, NH_2), 6.64 (s, 1H, 3-H), 6.81 (d, J = 9.2 Hz, 2H, 3′,5′-H), 7.02 (dd, J = 8.8, 2.9 Hz, 1H, 7-H), 7.09 (d, J = 2.8 Hz, 1H, 5-H), 7.43 (d, J = 8.8 Hz, 1H, 8-H), 7.86 (d, J = 9.0 Hz, 2H, 2′,6′-H); EIMS *m*/*z* 280 (M^+). Anal. ($C_{17}H_{16}N_2O_2 \cdot 0.3H_2O$) C, H, N.

6-Amino-2-[3-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one (16a). To a solution of **15a** (1.61 g, 5.16 mmol) in THF (30 mL) were added **14a** (710 mg, 3.68 mmol) and sodium hydride (475 mg, 11.9 mmol). The reaction mixture was stirred at room temperature for 5 h. Then 1 N KOH was added, and the mixture was washed with $CHCl_3$. The aqueous layer was acidified with 6 N HCl and extracted with $CHCl_3$. The organic layer was washed with brine and concentrated to afford 1-(5-acetamido-2-hydroxyphenyl)-3-[3-(dimethylamino)-phenyl]propane-1,3-dione (400 mg, 44%). The propanedione obtained (400 mg, 1.18 mmol) was dissolved in EtOH (10 mL), and concentrated HCl (2 mL) was added. The reaction mixture was stirred at room temperature for 4 h. The precipitated product was filtered to afford 6-acetamido-2-[3-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one hydrochloride (327 mg, 77%). The compound (200 mg, 0.620 mmol) was dissolved in a mixture of EtOH (10 mL) and concentrated HCl (5 mL) followed by refluxing for 2.5 h. After cooling, the precipitated product was collected by filtration and triturated with $CHCl_3$/MeOH to afford **16a** (100 mg, 57%): mp 175 °C; IR (KBr) 1634 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 3.08 (s, 6H, $N(CH_3)_2$), 7.11 (s, 1H, 3-H), 7.29 (br d, J = 7.9 Hz, 1H, 4′-H), 7.50 (t, J = 7.9 Hz, 1H, 5′-H), 7.62 (br d, J = 7.3 Hz, 1H, 6′-H), 7.69 (br, 1H, 2′-H), 7.74 (dd, J = 8.9, 2.6 Hz, 1H, 7-H), 7.89 (d, J = 8.9 Hz, 1H, 8-H), 7.93 (br d, J = 2.6 Hz, 1H, 5-H); EIMS *m*/*z* 280 (M^+). Anal. ($C_{17}H_{16}N_2O_2 \cdot 2HCl \cdot 0.1H_2O$) C, H, N.

7-Amino-2-[4-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one (16b). This compound was obtained from **14b** and 4-(dimethylamino)benzoyl chloride (**15b**) in a similar manner as described for **16a** (overall 4%): mp 180−185 °C; IR (KBr) 1648 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 3.03 (s, 6H, $N(CH_3)_2$), 6.62 (s, 1H, 3-H), 6.67 (d, J = 2.2 Hz, 1H, 8-H), 6.71 (dd, J = 8.3, 2.1 Hz, 1H, 6-H), 6.87 (d, J = 9.2 Hz, 2H, 3′,5′-H), 7.70 (d, J = 8.6 Hz, 1H, 5-H), 7.86 (d, J = 9.0 Hz, 2H, 2′,6′-H); EIMS *m*/*z* 280 (M^+). Anal. ($C_{17}H_{16}N_2O_2 \cdot 2HCl$) C, H; N: calcd, 7.93; found, 7.40.

8-Amino-2-[4-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one (19). To a mixture of **17** (1.10 g, 5.69 mmol) and **18** (790 mg, 5.30 mmol) in DMF (30 mL) was added sodium hydride (640 mg, 16.0 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3.5 h and then neutralized with concentrated HCl and extracted with $CHCl_3$. The organic layer was washed with water and brine and chromatographed (40:1 $CHCl_3$/MeOH) to afford 3-(3-acetamido-2-hydroxyphenyl)-1-[4-(dimethylamino)phenyl]propen-3-one (1.34 g, 73%). A mixture of the propenone (500 mg, 1.54 mmol) and selenium dioxide (500 mg, 4.51 mmol) in isoamyl alcohol (25 mL) was heated at 150 °C for 2 days. The solution was filtered to remove the precipitated solid and extracted with $CHCl_3$. The organic layer was washed with water and brine. The crude product was chromatographed (20:1 $CHCl_3$/MeOH) to afford 8-acetamido-2-[4-(dimethylamino)phenyl]-4*H*-1-benzopyran-4-one (140 mg, 28%). The compound (64 mg, 0.19 mmol) was dissolved in 1,4-dioxane (4 mL), and concentrated HCl (4 mL) was added followed by refluxing for 2.5 h. After cooling, the reaction mixture was neutralized with 10% aqueous NaOH and extracted with CH_2Cl_2. The organic layer was washed with water and brine and chromatographed (20:1 $CHCl_3$/MeOH) to afford **19** (15 mg, 28%) as a free base. The compound (15 mg) was converted into the hydrochloride with hydrogen chloride in a mixture of $CHCl_3$ and 2-propanol. The precipitate was collected by filtration and dried to give the hydrochloride (15 mg): mp 230 °C dec; IR (KBr) 1605 cm^{-1};

^{1}H NMR (270 MHz, DMSO-d_6) δ 3.04 (s, 6H, N(CH_3)$_2$), 6.75 (s, 1H, 3-H), 6.81 (d, J = 9.2 Hz, 2H, 3′,5′-H), 7.17 (t, J = 7.3 Hz, 1H, 6-H), 7.27 (dd, J = 6.9, 2.7 Hz, 1H, 7-H), 7.17 (m, 1H, 5-H), 8.05 (d, J = 9.0 Hz, 2H, 2′,6′-H); EIMS m/z 280 (M^+). Anal. ($C_{17}H_{16}N_2O_2$·HCl·0.9H_2O) C, H, N.

Biological Assay. Antiproliferative Activity against MCF-7 (Cell Count Method for Tables 1 and 2). MCF-7 cells were suspended in a medium comprised of phenol red free Eagle's MEM (Nissui Pharmaceutical Co., Ltd.) and 5% calf serum (Hyclone) with or without 10^{-4} μM estradiol (Sigma Fine Chemical Inc.) (here after referred to as medium A) to a concentration of 3.3×10^3 cells/mL. The cell suspension thus prepared was put into wells of 24-well multidishes (NUNC) at 0.75 mL/well. The cells on the plate were incubated in a CO_2 incubator at 37 °C for 24 h, and 0.25 mL of a sample containing a test compound and appropriately diluted with medium A was added to each well (n = 3). The cells were further incubated in the CO_2 incubator at 37 °C for 144 h. The growth-inhibitory activity of the compounds was evaluated by counting cell numbers using a microcell counter (Toa Medical Electronics Co.).

Antiproliferative Activity against MDA-MB-453 (Cell Count Method for Table 4). MDA-MB-453 cells (3.3×10^3 cells/mL) suspended in medium A were put into wells of 24-well multidishes (NUNC) in the amount of 0.75 mL/well. The subsequent procedures were carried out in the same manner as described above.

Estrogen Receptor Binding Assay. MCF-7 cells (2.5×10^5/well) were preincubated in 24-well multidishes containing 0.75 mL of medium A in each well at 37 °C for 24 h. The cells were then treated with [^{3}H]17β-estradiol (Amersham; final concentration, 10^{-2} μM) with various concentrations of test compounds or 17β-estradiol and incubated at 37 °C for 1 h. The cells were treated with PBS containing 10% (v/v) glycerol and 0.5% (w/v) bovine serum albumin at 4 °C for 0.5 h and washed twice with the above buffer solution. The cells were lysed with 1 N NaOH at 37 °C overnight, and their radioactivity was measured with a liquid scintillation counter. Nonspecific binding was calculated using 20 μM 17β-estradiol as a competing ligand.

Antiproliferative Activity against MCF-7, HeLa S_3, and WiDr (Neutral Red Dye Uptake Method for Table 3). MCF-7 cells (5×10^4 cells/mL) prepared in a medium comprising RPMI1640 medium (Grand Island Biological Co.), 10% calf serum (Grand Island Biological Co.), 10^{-2} μM estradiol (Sigma Fine Chemical Inc.), 100 units/mL penicillin, and 100 μg/mL streptomycin (Grand Island Biological Co.) (hereinafter referred to as medium B) were put into wells of a 96-well microtiter plate in the amount of 0.1 mL/well. The cells on the plate were incubated in a CO_2 incubator at 37 °C for 20 h, and 0.05 mL of a sample containing a test compound and appropriately diluted with medium B was added to each well (n = 3). The cells were further incubated in the CO_2 incubator at 37 °C for 72 h. After the culture supernatant was removed, medium B containing 0.02% neutral red was added to the residue in an amount of 0.1 mL/well followed by incubation at 37 °C for 1 h in the CO_2 incubator, whereby the cells were stained. The culture supernatant was removed, and the residue was washed once with physiological saline solution. The pigment was extracted with 0.001 N HCl/30% EtOH, and the absorbance was determined at 550 nm with a microplate reader. The absorbance determined for intact cells was compared with that for the cells treated with the test compound at a known concentration, and the IC_{50} was calculated.

HeLa S_3 cells (3×10^4 cells/mL) prepared in a medium comprising MEM medium, 2 mM glutamine, and 10% fetal calf serum were put into wells of a 96-well microtiter plate in an amount of 0.1 mL/well. The subsequent procedures were carried out in the same manner as described above.

WiDr cells (6×10^4 cells/mL) prepared in a medium comprising MEM medium, 2 mM glutamine, 10% fetal calf serum, and 1% nonessential amino acids (Dainippon Pharmaceutical Co., Ltd.) were put into wells of a 96-well microtiter plate in the amount of 0.1 mL/well. The subsequent procedures were carried out in the same manner as described above.

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Supporting Information Available: Preparation and ^{1}H NMR data of compounds **8b**,**g**,**j**−**p**, **14b**, and **17** and ^{1}H NMR spectra of target compounds **10g**,**o**, **13**, **16a**,**b**, and **19** (9 pages). Ordering information is given on any current masthead page.

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