Design and Synthesis of Potent Antitumor 5,4′-Diaminoflavone Derivatives Based on Metabolic Considerations

Tsutomu Akama,*,† Hiroyuki Ishida, Yasushi Shida, Uichiro Kimura, Katsushige Gomi,‡ Hiromitsu Saito, Eiichi Fuse, Satoshi Kobayashi, Nobuyuki Yoda, and Masaji Kasai*,§

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Company, Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken 411, Japan

Received January 15, 1997⊗

Recently, we reported that 5,4′-diaminoflavone (**1**) exhibits potent and specific growth-inhibitory activity against the estrogen receptor (ER)-positive human breast cancer cell line MCF-7. However, when compound **1** was incubated with S-9 mix, its metabolites were observed. Moreover, addition of S-9 mix to the medium caused the drastic decrease in activity of compound **1**. Since the 6-, 8-, and 3′-positions were considered to be metabolized oxidatively *in vivo* from MO calculations, a series of 5,4′-diaminoflavone derivatives substituted at such putative metabolic positions with various functional groups were synthesized aiming at the metabolically stable derivatives. Among them, 5,4′-diamino-6,8,3′-trifluoroflavone (**14d**) exhibited strong growth-inhibitory activity against MCF-7 cells even in the presence of S-9 mix. Moreover, orally administered compound **14d** completely suppressed the growth of MCF-7 inoculated into nude mice, and the effect was more potent than that of compound **1**. In addition to ER-positive breast cancer cells, compound **14d** exhibited growth-inhibitory activity against a panel of human cancer cell lines including a part of ER-negative breast, endometrial, ovarian, and liver cancers. From these results, fluorine introduction to the putative metabolic positions of compound **1** was elucidated to be effective in the enhancement of the *in vivo* antitumor activity, probably due to the block of the metabolic deactivation.

Recently, we reported that 5,4′-diaminoflavone (**1**) and some of its congeners exhibit potent and specific growth-inhibitory activity against the estrogen receptor (ER)-positive human breast cancer cell line MCF-7.[1] Since they are expected to be a new type of chemotherapeutic agent, we were then interested in their *in vivo* antitumor activity. However, flavonoids are liable to be metabolized oxidatively *in vivo*.[2,3] In particular, the two benzene rings of compound **1** seemed to be suitable targets of electrophilic oxidation because of the electron-donating two amino groups. S-9 mix, the 9000 G supernatant of the enzyme-induced rat liver homogenate, is often used for the model system of metabolic activation, e.g., the Ames test.[4] When compound **1** was incubated with S-9 mix, formation of metabolites with a 16 mass increase was observed by LC-MS analysis.[5] Moreover, when S-9 mix was added to the medium, the drastic decrease in activity of compound **1** against MCF-7 cells was observed. Thus, it was suggested that the compound might be metabolically deactivated when administered *in vivo*. In order to estimate the position(s) that is (are) most susceptible to being metabolized, the superdelocalizability (*Sr*)[6] values of compound **1** were calculated. From the point of view of drug design, we hypothesized that introduction of substituents would be effective in blocking the metabolic deactivation. Especially, the fluorine atom was considered to be one of the most suitable substituents for the block of metabolism.[7,8] Therefore, we introduced a fluorine atom or some other substituent to compound **1** in order to block the presumed metabolism, retaining potent antitumor activity.

As a result, we found that 5,4′-diamino-6,8,3′-trifluoroflavone (**14d**) exhibited superior antitumor activity against MCF-7 *in vivo* as well as *in vitro*. To confirm the specificity of the growth-inhibitory activity of compound **14d**, the *in vitro* antitumor spectrum of **14d** was examined. Compound **14d** exhibited growth-inhibitory activity against not only ER-positive breast cancer cells but also certain ER-negative breast, endometrial, ovarian, and liver cancer cell lines. Herein we describe the synthesis of 6- or 8-substituted, 6,8-disubstituted, and polyfluoro-substituted 5,4′-diaminoflavone derivatives and their antitumor activity both *in vitro* and *in vivo*.

Chemistry

The AM1[9] calculation of the *Sr* values of compound **1** concerning electrophilic reaction was performed using MOPAC version 6.01.[10] The *Sr* value is known as a parameter of the reactivity of each position in the compound.[6] From the results, we expected that the metabolism would occur mainly at the 6- or 8-position and that the 3′-position would be the next candidate.[11]

To confirm a suitable substituent for the block of metabolism, various 6- or 8-substituted derivatives of compound **1** were synthesized. 6-Chloro (**2a**) and 8-chloro (**2b**) derivatives were obtained by direct chlorination of compound **1** with *N*-chlorosuccinimide (Scheme 1). The chlorinated position was determined by NMR analysis. At first, the detection of the NOE between 5-NH_2 and 6-H had failed. We successfully assigned all carbons and hydrogens by HMBC, HSQC, and, finally, deuterium shift effect, i.e., the detection of the changes in peak shapes of 4a-, 5-, and 6-C in the complete

† Present address: Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 6-6 Asahi-machi 3-chome, Machida-shi, Tokyo 194, Japan.

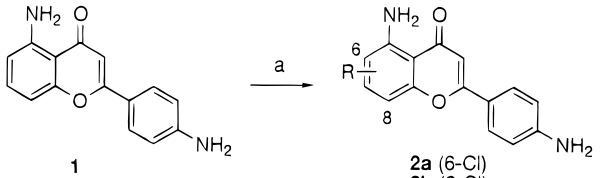
‡ Present address: Kyowa Hakko Kogyo Co., Ltd., 6-1 Ohtemachi 1-chome, Chiyoda-ku, Tokyo 100, Japan.

§ Present address: Sakai Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1-53 Takasu-cho 1-chome, Sakai-shi, Osaka 590, Japan.

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

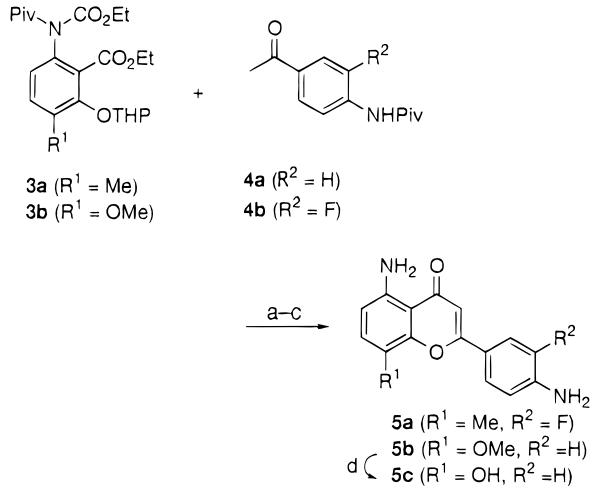
S0022-2623(97)00032-0 CCC: $14.00

Scheme 1[a]



[a] (a) *N*-Chlorosuccinimide, 1,4-dioxane, reflux (for **2a**,**b**) or Cl_2, AcOH, rt (for **2c**) or Br_2, AcOH, rt (for **2d**).

Scheme 2[a]



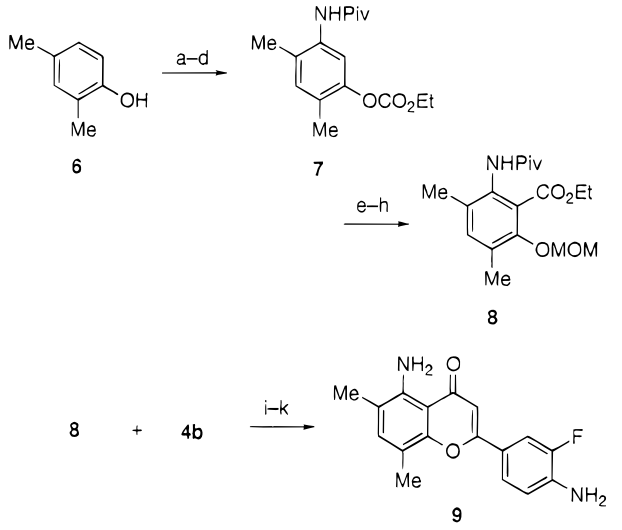
[a] (a) NaH, 1,4-dioxane, reflux; (b) HCl, EtOH, rt; (c) HCl, EtOH, reflux; (d) BBr_3, CH_2Cl_2, −78 °C to rt.

decoupled ^{13}C-NMR spectrum by the addition of CD_3OH and CD_3OD in DMSO-d_6. When chlorine or bromine was used in acetic acid, 6,8-dichloro derivative **2c** or 6,8-dibromo derivative **2d** was obtained as a major product, respectively.

8-Methyl (**5a**), 8-methoxy (**5b**), and 8-hydroxy (**5c**) derivatives were synthesized as shown in Scheme 2 in a similar manner as the synthesis of compound **1**.[1] The starting materials **3a**,**b** were prepared according to the synthesis of compound **10**[12] from 5-amino-2-methylphenol and 5-amino-2-methoxyphenol, respectively. The condensation of compounds **3a**,**b** and acetophenones **4a**,**b**[13] afforded 1,3-diketones. The *N*-ethoxycarbonyl group of compound **3** was removed during the reaction because of the strongly basic conditions. The 1,3-diketones thus obtained were treated with HCl in EtOH at room temperature to afford cyclized products. Compounds **5a**,**b** were obtained after the removal of the *N*-pivaloyl groups under more strongly acidic conditions. Compound **5c** was obtained by the demethylation of **5b** using boron tribromide in dichloromethane.

6,8-Dimethyl derivative **9** was synthesized as shown in Scheme 3. 2,4-Dimethylphenol (**6**) was converted to ethyl carbonate followed by nitration in mixed acid to give the 5-nitro product. Catalytic hydrogenation of the nitro group and amidation of the amino group formed with pivaloyl chloride afforded compound **7**. The ethoxycarbonyl group of **7** was removed by hydrolysis, and the phenol obtained was ortho brominated. Then the phenolic hydroxy group was reprotected as a methoxymethyl (MOM) ether, and the ethoxycarbonyl group was introduced to the position between the amide and the ether via a halogen−metal exchange by *n*-butyllithium to afford **8**. Compound **9** was obtained from **8** and **4b** in a similar manner as shown in Scheme 2.

Scheme 3[a]



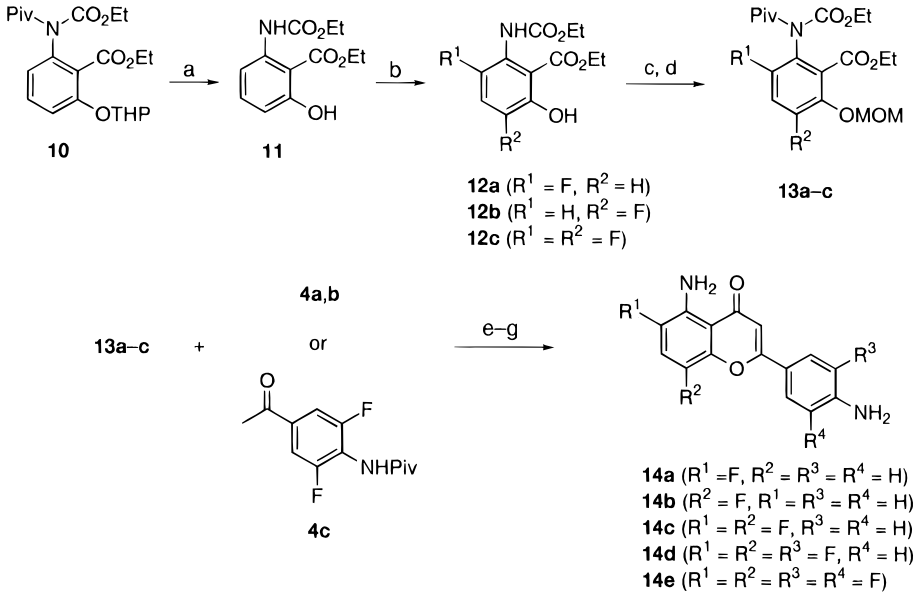
[a] (a) $ClCO_2Et$, Et_3N, CH_2Cl_2, rt; (b) fuming HNO_3, H_2SO_4, rt; (c) H_2, Pd/C, EtOAc, rt; (d) pivaloyl chloride, pyridine, rt; (e) aq NaOH, EtOH, rt; (f) Br_2, $(CH_2Cl)_2$, 0 °C; (g) chloromethyl methyl ether, *i*-Pr_2NEt, CH_2Cl_2, 0 °C; (h) *n*-BuLi, $ClCO_2Et$, THF, −78 °C; (i) NaH, toluene, 1,4-dioxane, reflux; (j) HCl, EtOH, rt; (k) HCl, EtOH, reflux.

Fluorinated derivatives were synthesized as shown in Scheme 4. Compound **10**[12] was hydrolyzed with HCl to afford compound **11**. Fluorination of the compound **11** with *N*-fluoro-3,5-dichloropyridinium triflate[14] in 1,2-dichloroethane afforded monofluoro- and difluorophenol derivatives **12a**−**c**. Each exchangeable OH and NH proton of compounds **12a**−**c** was protected as a MOM ether and a pivaloyl amide, respectively. Compounds **13a**−**c** thus obtained led to target compounds **14a**−**e** via the condensation with acetophenones **4a**−**c**[15,16] in the same way as shown in Scheme 3.

Biological Activity and Discussion

The growth-inhibitory activities of various 6- or 8-substituted and polyfluoro-substituted 5,4′-diaminoflavone derivatives in both the absence and the presence of S-9 mix are shown in Table 1. In the absence of S-9 mix, introduction of methoxy (**5b**) and hydroxy (**5c**) groups resulted in a drastic decrease in activity compared to compound **1**. It was suggested that if these compounds were formed *in vivo* as metabolites of compound **1**, compound **1** would not exhibit expected antitumor activity enough *in vivo*. It seems probable because metabolites considered to be hydroxylated products were formed *in vitro* by the addition of S-9 mix.[5] Methylation (**5a**) and 6,8-dimethylation (**9**) resulted in a somewhat decreased activity. Chlorination (**2a**,**b**) and fluorination (**14a**,**b**) seemed to retain the activity. However, 6,8-dichlorination (**2c**) resulted in a decrease in activity, despite that 6,8-difluorination (**14c**) retained the activity. 6,8-Dibromination (**2d**) resulted in a further decrease in activity. Thus the most suitable substituent was considered to be fluorine. Fluorine is the only element which can replace hydrogen without notable steric consequences.[7] Bulkiness of the substituents seemed to be important for decreasing the growth-inhibitory activity; however, the electron-withdrawing property did not seem to be important. Then fluorine

Scheme 4[a]



[a] (a) HCl, EtOH, reflux; (b) *N*-fluoro-3,5-dichloropyridinium triflate, $(CH_2Cl)_2$, reflux; (c) chloromethyl methyl ether, *i*-Pr_2NEt, CH_2Cl_2, rt; (d) pivaloyl chloride, NaH, THF, 0 °C; (e) NaH, 1,4-dioxane, toluene, reflux; (f) HCl, EtOH, rt; (g) HCl, EtOH, reflux.

Table 1. Growth-Inhibitory Activity of 5,4′-Diaminoflavone Derivatives against MCF-7 Cells in the Presence or Absence of S-9 Mix

		IC_{50} (μM)[a]	
compd	substituents	S-9 mix (−)	S-9 mix (+)
1	H	0.0073	0.21
2a	6-Cl	0.0039	>10
2b	8-Cl	0.0098	0.25
2c	6,8-diCl	0.053	0.61
2d	6,8-diBr	6.3	NT[b]
5b	8-OMe	21	19
5c	8-OH	5.3	16
5a	8-Me, 3′-F	0.015	0.18
9	6,8-diMe, 3′-F	0.036	0.11
14a	6-F	0.0044	0.13
14b	8-F	0.0069	0.17
14c	6,8-diF	0.0060	0.046
14d	6,8,3′-triF	0.0012	0.025
14e	6,8,3′,5′-tetraF	0.0040	0.089

[a] MCF-7 cells were treated with each compound for 6 days and counted by a microcell counter. [b] Not tested.

was introduced to the 3′- or 3′,5′-positions, which are the next candidates for metabolism. 6,8,3′-Trifluoro derivative **14d** and 6,8,3′,5′-tetrafluoro derivative **14e** exhibited comparable or somewhat enhanced activity to compound **1**.

On the other hand, the addition of S-9 mix decreased the growth-inhibitory activity in all cases except for compound **5b** which have already lost the potency in the absence of the S-9 mix. The growth-inhibitory activity of compound **1** was decreased about 30 times by S-9 mix addition. Hence it was suggested that these compounds might be metabolically deactivated in the liver, and the deactivation would not be blocked by the introduction of a substituent to at least one position. While the degree of the deactivation (the ratio of S-9 mix (+) and (−)) was not improved, 6,8-difluoro derivatives **14c**−**e** exhibited more potent growth-inhibitory activity than other compounds in the presence of S-9 mix. Compound **14d** exhibited the most potent activity (IC_{50} 0.025 μM).

To confirm the effect of fluorine introduction against metabolic deactivation, the *in vivo* antitumor activity of compounds **1** and **14d** was tested. MCF-7 cells were inoculated into female nude mice and were growth-stimulated by estradiol. Compounds were administered 25 mg/kg po on 5 consecutive days in 1 week for 2 weeks (days 0−4 and 7−11). As shown in Figure 1 A, while compound **1** suppressed the tumor growth from day 7 to day 18, compound **14d**, as expected, completely suppressed the tumor growth during the experiment. It is noteworthy that the growth inhibition by compound **14d** was maintained for 2 weeks after the drug administration. In addition, no significant body weight change was observed in each group compared to the control group during the experiment (Figure 1B).

Then the *in vitro* antitumor spectrum of compound **14d** was examined to confirm the effects against other cell lines, and the results are shown in Table 2. In addition to ER-positive breast cancer cell lines, compound **14d** exhibited growth-inhibitory activity against one of two ER-negative breast cancer cell lines (SK-BR-3) and endometrial, ovarian, and liver cancer cell lines with IC_{50} values in the range of 10^{-2}−10^{-3} μM order. However, **14d** was inactive up to 1 μM against another ER-negative cell line (MDA-MB-453) and stomach, colon, kidney, pancreas, and vulva cell lines.

The pharmacological mechanism which explains the selectivity of antitumor activity of **14d** has not, as yet, been clear. Compound **14d** did not compete with estradiol for the ER up to 100 μM, and it did not cause topoisomerase (I and II) dependent DNA cleavage (data not shown). Recently, a series of 2-(4-aminophenyl)-benzothiazoles were reported to exhibit selective growth-inhibitory activity to breast cancer cells.[17] The antitumor profile of **14d** seems to resemble that of these compounds; however, **14d** did not show biphasic dose−response against MCF-7 (data not shown) compared to these compounds which showed biphasic dose−response. Moreover, the antitumor spectrum of **14d** seems to be somewhat different as compared to the spectra of these compounds. 6,4′-Diaminoflavone and 6-hydroxy-5,7,3′-triaminoflavone were reported as inhibitors of protein-tyrosine kinases including the EGF

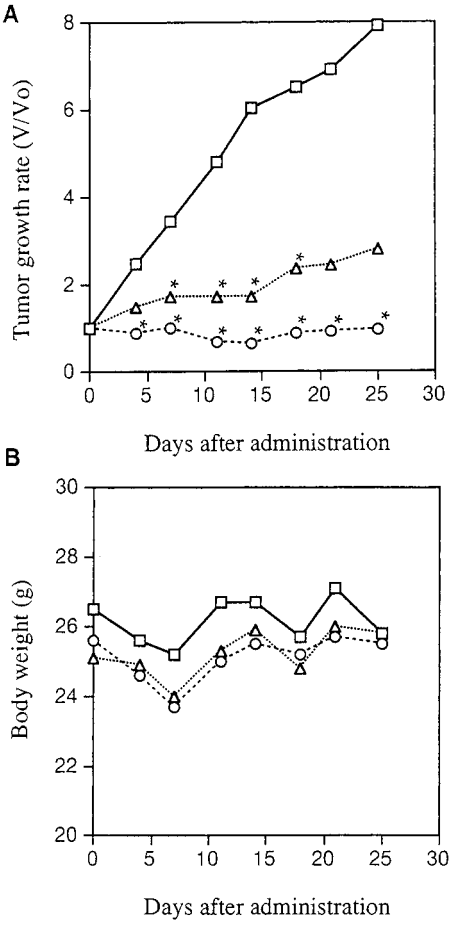


Figure 1. Antitumor activity of compounds **1** and **14d** against MCF-7. Tumor fragments were implanted sc into BALB/c-nu/nu female mice on day −20. Compounds were administered po 5 consecutive days in 1 week for 2 weeks: (A) tumor growth rate of the control group (□), compound **1**-treated group (△), and compound **14d**-treated group (○); (B) body weight change of each group (symbols are the same as in A). *P < 0.05 vs control group.

Table 2. Growth-Inhibitory Activity of Compound **14d** against Various Human Cancer Cell Lines

cell lines	type	ER	IC_{50} (μM)[a]
MCF-7	breast	+	0.0012
T-47D	breast	+	0.031
SK-BR-3	breast	−	0.0025
MD- 453	breast	−	>1.0
Ishikawa	endometrial	+	0.013
A2780	ovarian	−	0.0013
OVCAR-3	ovarian	−	0.014
Hep-G2	liver		0.0028
MKN-28	stomach		>1.0
WiDr	colon		>1.0
ACHN	kidney		>1.0
PSN-1	pancreas		>1.0
A431	vulva		>1.0

[a] Cells were treated with compound **14d** for 6 days and counted by a microcell counter.

receptor.[18] Although the direct inhibition of such kinases concerning **14d** has not been tested, the inhibition of [^{3}H]thymidine uptake by the EGF receptor expressing NRK-49F cells stimulated by EGF (10 ng/mL) in the serum free medium was examined. The IC_{50} value of **14d** was >10 μM, suggesting that the EGF dependent pathway is not the major target of the compound.

Moreover, some compounds which exhibit growth-inhibitory activity against MCF-7 cells without ER binding were reported.[19,20] One possible mechanism of their growth-inhibitory activity was thought to be the depletion of the ER or the ER−ERE complex, which probably resulted in the inhibition of the ER responsive gene expression. In preliminary results, compound **14d** slightly suppressed the estradiol-stimulated ER transactivation in the $(ERE)_2$−luciferase reporter gene assay (data not shown). However, even if compound **14d** possesses such activity, it is not enough to explain its strong antitumor effect and the activities against ER-negative cell lines.

The pharmacological mechanism is still unclear; however, our hypothesis that metabolic deactivation of compound **1** *in vivo* would be overcome by introducing substituents such as fluorine atom to the putative metabolic positions was supported by the *in vivo* experiment. Although further investigation is required to disclose the real effect of fluorine introduction, it might be one methodology for enhancing the *in vivo* potency of a drug which is susceptible to being metabolized.

Conclusion

We prepared a series of 5,4′-diaminoflavone derivatives substituted at putative metabolic positions with various functional groups aiming at the metabolically stable derivatives. Among them, we found that 5,4′-diamino-6,8,3′-trifluoroflavone (**14d**) exhibited strong growth-inhibitory activity *in vivo* as well as *in vitro*. Since the compound was considered to be a new type of antitumor agent, further evaluation of the compound and research on structure−activity relationships of related derivatives are in progress.

Experimental Section

All melting points were determined on a Yanako micromelting point apparatus and are uncorrected. IR spectra were recorded on JASCO IR-400 and JASCO IR-810 spectrometers. ^{1}H-NMR spectra were recorded on HITACHI R-90H (90 MHz), JEOL JNM-GX-270 (270 MHz), and JEOL JNM-EX-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 (NaH) was a 60% oil dispersion. THF was freshly distilled from sodium and benzophenone.

5-Amino-2-(4-aminophenyl)-6-chloro-4*H*-1-benzopyran-4-one (2a) and 5-Amino-2-(4-aminophenyl)-8-chloro-4*H*-1-benzopyran-4-one (2b). To a solution of compound **1**[1] (2.00 g, 7.93 mmol) in 1,4-dioxane (50 mL) was added *N*-chlorosuccinimide (1.06 g, 7.93 mmol). The mixture was refluxed for 19 h, and the solvent was evaporated. The mixture was extracted with EtOAc, and the organic layer was washed with brine. The residue was chromatographed (4:1 $CHCl_3$/acetone) and recrystallized from $CHCl_3$/MeOH to afford **2a** (415 mg, 18%) and **2b** (266 mg, 12%). **2a**: mp 242−243 °C; IR (KBr) 1642 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.58 (s, 1H, 3-H), 6.67 (d, J = 8.9 Hz, 2H, 3′,5′-H), 6.75 (d, J = 8.9 Hz, 1H, 8-H), 7.54 (d, J = 8.4 Hz, 1H, 7-H), 7.73 (d, J = 8.9 Hz, 2H, 2′,6′-H); EIMS m/z 286, 288 (M^+). Anal. ($C_{15}H_{11}ClN_2O_2$) C, H, N. **2b**: mp 275−276 °C; IR (KBr) 1640 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.52 (d, J = 8.9 Hz, 1H, 6-H), 6.58 (d, J = 8.9 Hz, 2H, 3′,5′-H), 6.68 (s, 1H, 3-H), 7.42 (d, J = 8.9 Hz, 1H, 7-H), 7.75 (d, J = 8.9 Hz, 2H, 2′,6′-H); EIMS m/z 286, 288 (M^+). Anal. ($C_{15}H_{11}ClN_2O_2$) C, H, N.

5-Amino-2-(4-aminophenyl)-6,8-dichloro-4*H*-1-benzopyran-4-one (2c). To a suspension of compound **1** (940 mg, 3.73 mmol) in AcOH (50 mL) was added a solution of chlorine in AcOH (2.7 wt %, 24 g, 9.2 mmol). The mixture was stirred

for 18 h at room temperature and poured into water. The mixture was extracted with $CHCl_3$/MeOH (9:1), and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The residue was chromatographed (4:1 $CHCl_3$/acetone) and recrystallized from $CHCl_3$/MeOH to afford **2c** (218 mg, 18%): mp 255−259 °C; IR (KBr) 1624 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 6.58 (s, 1H, 3-H), 6.68 (d, J = 9.4 Hz, 2H, 3′,5′-H), 7.76 (s, 1H, 7-H), 7.76 (d, J = 8.9 Hz, 2H, 2′,6′-H); EIMS m/z 320, 322, 324 (M^+). Anal. ($C_{15}H_{10}Cl_2N_2O_2$) C, H, N.

5-Amino-2-(4-aminophenyl)-6,8-dibromo-4*H*-1-benzopyran-4-one (2d). To a suspension of compound **1** (1.46 g, 5.80 mmol) in AcOH (100 mL) was added bromine (0.45 mL, 18 mmol). The mixture was stirred for 18 h at room temperature. The precipitate was collected by filtration and triturated with MeOH to afford **2d** as a hydrobromide (1.23 g, 43%): mp 240 °C dec; IR (KBr) 1621 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 6.68 (s, 1H, 3-H), 6.69 (d, J = 8.8 Hz, 2H, 3′,5′-H), 7.79 (d, J = 8.6 Hz, 2H, 2′,6′-H), 7.97 (s, 1H, 7-H); EIMS m/z 408, 410, 412 (M^+). Anal. ($C_{15}H_{10}Br_2N_2O_2$·0.6HBr) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-8-methyl-4*H*-1-benzopyran-4-one (**5a**). To a refluxing suspension of NaH (800 mg, 20.0 mmol) in 1,4-dioxane/toluene (1:1, 10 mL) under argon atmosphere was added dropwise a solution of **3a** (2.56 g, 7.05 mmol) and **4b** (1.39 g, 5.88 mmol) in the above solvent (35 mL) over 5 min. The reaction mixture was refluxed for 2 h and cooled on an ice bath. Water was added, and the basic solution was washed with *n*-hexane. Then the solution was extracted with EtOAc, and the organic layer was washed with brine. The combined extracts were concentrated and dissolved in EtOH (40 mL). Concentrated HCl (10 mL) was added, and the reaction mixture was stirred for 16 h at room temperature. Water (80 mL) was added, and the precipitated product was collected by filtration to afford 2-[3-fluoro-4-(pivaloylamino)-phenyl]-8-methyl-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (2.03 g, 76%).

To a solution of the compound obtained (1.50 g, 3.32 mmol) in 1,4-dioxane (40 mL) was added concentrated HCl (20 mL), and the reaction mixture was refluxed for 4 h and cooled on an ice bath. The mixture was neutralized with 10 N NaOH, and the precipitated product was collected by filtration. Chromatography (40:1 $CHCl_3$/MeOH) followed by trituration with *i*-Pr_2O/EtOAc afforded **5a** (528 mg, 58%): mp 249−250 °C; IR (KBr) 1637 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.98 (br, 2H, 4′-NH_2), 6.44 (d, J = 8.3 Hz, 1H, 6-H), 6.60 (s, 1H, 3-H), 6.87 (t, J = 8.6 Hz, 1H, 5′-H), 7.19 (br, 2H, 5-NH_2), 7.21 (d, J = 8.3 Hz, 1H, 7-H), 7.5−7.7 (m, 2H, 2′,6′-H); EIMS m/z 284 (M^+). Anal. ($C_{16}H_{13}FN_2O_2$) C, H, N.

5-Amino-2-(4-aminophenyl)-8-methoxy-4*H*-1-benzopyran-4-one (**5b**)**.** This compound was obtained from **3b** and **4a** in a similar manner as described for **5a** (overall 22%): mp 216−217 °C; IR (KBr) 1641 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 5.92 (br, 2H, 4′-NH_2), 6.43 (d, J = 8.8 Hz, 1H, 6-H), 6.50 (s, 1H, 3-H), 6.68 (d, J = 8.8 Hz, 2H, 3′,5′-H), 6.89 (br, 2H, 5-NH_2), 7.18 (d, J = 8.8 Hz, 1H, 7-H), 7.71 (d, J = 8.8 Hz, 2H, 2′,6′-H); EIMS m/z 282 (M^+). Anal. ($C_{16}H_{14}N_2O_3$) C, H, N.

5-Amino-2-(4-aminophenyl)-8-hydroxy-4*H*-1-benzopyran-4-one (**5c**)**.** To a suspension of **5b** (286 mg, 1.01 mmol) in CH_2Cl_2 (25 mL) was added BBr_3 (1.0 M in CH_2Cl_2, 5.6 mL, 5.6 mmol) at −78 °C under argon atmosphere. The cold bath was removed, and the mixture was stirred for 9.5 h. Water was added, and the aqueous layer was neutralized with 2 N NaOH. Insoluble precipitate was collected by filtration and recrystallized from EtOH/*n*-hexane to afford **5c** (166 mg, 61%): mp 250 °C dec; IR (KBr) 3330, 1641 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 5.88 (br, 2H, 4′-NH_2), 6.35 (d, J = 8.6 Hz, 1H, 6-H), 6.45 (s, 1H, 3-H), 6.66 (d, J = 8.8 Hz, 2H, 3′,5′-H), 6.71 (br, 2H, 5-NH_2), 6.99 (d, J = 8.8 Hz, 1H, 7-H), 7.78 (d, J = 8.8 Hz, 2H, 2′,6′-H), 8.72 (br, 1H, OH); EIMS m/z 268 (M^+). Anal. ($C_{15}H_{12}N_2O_3$·0.3H_2O) C, H; N: calcd, 10.24; found, 9.81.

2,4-Dimethyl-*O*-(ethoxycarbonyl)-5-(pivaloylamino)-phenol (**7**)**.** To a solution of 2,4-dimethylphenol (17.8 mL, 150 mmol) were added Et_3N (25.0 mL, 180 mmol) and a solution of ethyl chloroformate (17.2 mL, 180 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred for 15 min at room temperature. Water was added, and the mixture was extracted with $CHCl_3$. The organic layer was washed twice with brine and concentrated. The crude product was dissolved in concentrated H_2SO_4 (50 mL) on an ice bath, and fuming HNO_3 (4.0 mL) was added. The mixture was stirred for 40 min and was poured into 500 mL of ice−water. The mixture was extracted with EtOAc, and the organic layer was washed with water and brine. Chromatography (9:1 *n*-hexane/EtOAc) gave 2,4-dimethyl-*O*-(ethoxycarbonyl)-5-nitrophenol (12.8 g, two steps, 36%).

The obtained nitrophenol (12.2 g, 51.0 mmol) and 10% palladium on charcoal (1.22 g) in EtOAc (150 mL) were stirred under hydrogen atmosphere for 1 day at room temperature. Hydrogen was replaced with nitrogen, and the mixture was filtered through a Celite pad. The solvent was evaporated, and the residue was dissolved in pyridine (50 mL) on an ice bath. Pivaloyl chloride (5.2 mL, 42 mmol) was added, and the mixture was stirred for 1 h and poured into water. The mixture was extracted with EtOAc, and the organic layer was washed with 2 N HCl, H_2O, and brine. Recrystallization from *n*-hexane gave **7** (10.0 g, two steps, 67%): mp 82−83 °C; IR (KBr) 1760, 1650 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.32 (s, 9H, $C(CH_3)_3$), 1.37 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.16 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 4.29 (q, J = 7.0 Hz, 2H, CH_2), 7.01 (s, 1H, 3-H), 7.20 (br, 1H, NH), 7.79 (s, 1H, 6-H); EIMS m/z 293 (M^+). Anal. ($C_{16}H_{23}NO_4$) C, H, N.

Ethyl 3,5-Dimethyl-2-(methoxymethoxy)-6-(pivaloylamino)benzoate (**8**). To a solution of **7** (6.28 g, 21.4 mmol) in EtOH (30 mL) was added 10 N NaOH (6 mL, 60 mmol) at 0 °C. The mixture was stirred for 30 min and acidified with 2 N HCl. The precipitated product was collected by filtration. To the solution of the crude product in dichloroethane (100 mL) was added dropwise bromine (1.0 mL, 18 mmol) at 0 °C. The mixture was stirred for 30 min and poured into water. The mixture was extracted with $CHCl_3$, and the organic layer was washed with brine. Chromatography (3:1 *n*-hexane/EtOAc) gave 2-bromo-4,6-dimethyl-3-(pivaloylamino)phenol (4.09 g, two steps, 64%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.36 (s, 9H, $C(CH_3)_3$), 2.13 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 5.48 (br, 1H, OH), 6.93 (s, 1H, 5-H), 6.98 (br, 1H, NH); EIMS m/z 299, 301 (M^+).

To a solution of the bromophenol (3.98 g, 13.3 mmol) in CH_2Cl_2 (100 mL) were added diisopropylethylamine (2.7 mL, 16 mmol) and chloromethyl methyl ether (1.2 mL, 16 mmol) at 0 °C. The mixture was stirred for 3 h, and 1 N NaOH was added. The mixture was extracted with $CHCl_3$, and the organic layer was washed with water and brine. Trituration with *n*-hexane gave a MOM ether (4.09 g, 89%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.36 (s, 9H, $C(CH_3)_3$), 2.17 (s, 3H, $ArCH_3$), 2.32 (s, 3H, $ArCH_3$), 3.62 (s, 3H, OCH_3), 5.03 (s, 2H, OCH_2O), 7.00 (s, 1H, 5-H), 7.05 (br, 1H, NH); EIMS m/z 343, 345 (M^+).

To a solution of *n*-butyllithium (1.6 M in *n*-hexane, 16 mL, 25 mmol) in THF (20 mL) were added a solution of the MOM ether (3.78 g, 11.0 mmol) in THF (50 mL) and ethyl chloroformate (2.1 mL, 22 mmol) under argon atmosphere below −60 °C. The mixture was stirred for 10 min, and water was added. The mixture was extracted with EtOAc, and the organic layer was washed with brine. Chromatography (4:1 *n*-hexane/acetone) gave **8** (2.32 g, 63%): mp 85−86 °C; IR (KBr) 1715, 1650 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.28 (s, 9H, $C(CH_3)_3$), 1.37 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.14 (s, 3H, $ArCH_3$), 2.28 (s, 3H, $ArCH_3$), 3.53 (s, 3H, OCH_3), 4.34 (q, J = 7.0 Hz, 2H, CH_2-CH_3), 4.96 (s, 2H, OCH_2O), 7.12 (s, 1H, 4-H), 7.73 (br, 1H, NH); EIMS m/z 337 (M^+). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-dimethyl-4*H*-1-benzopyran-4-one (**9**)**.** This compound was obtained from **8** and **4b** in a similar manner as described for **5a** (overall 54%): mp 222−223 °C; IR (KBr) 1647 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 2.05 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 5.98 (br, 2H, 4′-NH_2), 6.60 (s, 1H, 3-H), 6.88 (t, J = 8.6 Hz, 1H, 3′-H), 7.07 (br, 2H, 5-NH_2), 7.17 (s, 1H, 7-H), 7.5−7.7 (m, 2H, 2′,6′-H); EIMS m/z 298 (M^+). Anal. ($C_{17}H_{15}N_2O_2$·0.2H_2O) C, H, N.

Ethyl 2-[*N*-(Ethoxycarbonyl)amino]-6-hydroxybenzoate (**11**)**.** To a solution of **10**[10] (200 g, 475 mmol) in EtOH (900 mL) was added concentrated HCl (300 mL). The mixture

was refluxed for 3.5 h and cooled on an ice bath. Water (500 mL) was added, and the precipitated product was collected by filtration to afford **11** (89.1 g, 74%): mp 76−77 °C; IR (KBr) 3380, 1735, 1655 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.31 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.50 (t, J = 7.0 Hz, 3H, CH_2CH_3), 4.22 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.53 (q, J = 7.0 Hz, 2H, CH_2CH_3), 6.65 (dd, J = 8.2, 1.2 Hz, 1H, Ar-H), 7.37 (t, J = 8.4 Hz, 1H, 4-H), 7.86 (dd, J = 8.4, 1.1 Hz, 1H, Ar-H), 9.48 (br, 1H, NH), 10.7 (s, 1H, OH); EIMS m/z 253 (M^+).

Ethyl 2-[*N*-(Ethoxycarbonyl)amino]-3-fluoro-6-hydroxybenzoate (12a), Ethyl 6-[*N*-(Ethoxycarbonyl)amino]-3-fluoro-2-hydroxybenzoate (12b), and Ethyl 2-[*N*-(Ethoxycarbonyl)amino]-3,5-difluoro-6-hydroxybenzoate (12c). A mixture of **11** (12.7 g, 50.0 mmol) and *N*-fluoro-3,5-dichloropyridinium triflate (31.6 g, 100 mmol) in CH_2Cl_2 (100 mL) was refluxed for 22 h. Et_2O (200 mL) was added to remove the pyridinium salt as the precipitate, and the mixture was filtered. The filtrate was washed with 1 N HCl, water, and brine. Chromatography (6:1−5:1 *n*-hexane/EtOAc) gave **12a** (5.70 g, 42%), **12b** (1.29 g, 9.5%), and **12c** (1.76 g, 12%). **12a**: mp 110−112 °C; IR (KBr) 3280, 1745, 1710 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.30 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.42 (t, J = 7.0 Hz, 3H, CH_2CH_3), 4.21 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.44 (q, J = 7.0 Hz, 2H, CH_2CH_3), 6.80 (dd, J = 9.2, 4.4 Hz, 1H, 5-H), 7.07 (br, 1H, NH), 7.22 (t, J = 9.2 Hz, 1H, 4-H), 10.4 (s, 1H, OH); EIMS m/z 271 (M^+). **12b**: mp 97−99 °C; IR (KBr) 3400, 1730, 1660 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.31 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.51 (t, J = 7.0 Hz, 3H, CH_2CH_3), 4.22 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.56 (q, J = 7.0 Hz, 2H, CH_2CH_3), 7.22 (t, J = 9.2 Hz, 1H, 4-H), 7.79 (dd, J = 9.2, 4.4 Hz, 1H, 5-H), 9.22 (br, 1H, NH), 10.4 (s, 1H, OH); EIMS m/z 271 (M^+). **12c**: mp 146−148 °C; IR (KBr) 3270, 1705, 1690 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.29 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.43 (t, J = 7.1 Hz, 3H, CH_2CH_3), 4.21 (q, J = 7.2 Hz, 2H, CH_2CH_3), 4.47 (q, J = 7.1 Hz, 2H, CH_2CH_3), 6.83 (br, 1H, NH), 7.13 (t, J = 9.9 Hz, 1H, 4-H), 10.5 (s, 1H, OH); EIMS m/z 289 (M^+).

Typical Procedure for the Preparation of Compound 13: Ethyl 3,5-Difluoro-2-[*N*-(ethoxycarbonyl)-*N*-pivaloylamino]-6-(methoxymethoxy)benzoate (13c). To a solution of **12c** (5.72 g, 19.8 mmol) in CH_2Cl_2 (70 mL) were added diisopropylethylamine (4.1 mL, 24 mmol) and chloromethyl methyl ether (1.8 mL, 24 mmol) at 0 °C. The mixture was stirred for 20 min, and 1 N NaOH was added. The mixture was extracted with Et_2O, and the organic layer was washed with water and brine. To a solution of the crude product in THF (35 mL) were added NaH (792 mg, 19.8 mmol) and pivaloyl chloride (1.7 mL, 20 mmol) at 0 °C. The mixture was stirred for 1.5 h, and saturated aqueous NH_4Cl was added. The mixture was extracted with ether, and the organic layer was washed with brine. Chromatography (4:1 *n*-hexane/EtOAc) gave **13c** (6.63 g, two steps, 80%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.33 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.39 (s, 9H, $C(CH_3)_3$), 3.55 (s, 3H, OCH_3), 4.18 (q, J = 7.2 Hz, 2H, CH_2CH_3), 4.33 (q, J = 7.1 Hz, 2H, CH_2CH_3), 5.12 (s, 2H, OCH_2O), 7.00 (dd, J = 10.2, 9.1 Hz, 1H, 4-H); EIMS m/z 417 (M^+).

Ethyl 3-Fluoro-2-[*N*-(ethoxycarbonyl)-*N*-pivaloylamino]-6-(methoxymethoxy)benzoate (13a). This compound was obtained from **12a** in a similar manner as described for **13c** (two steps, 82%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.32 (t, J = 7.2 Hz, 3H, CH_2CH_3), 1.39 (s, 9H, $C(CH_3)_3$), 3.48 (s, 3H, OCH_3), 4.19 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.33 (q, J = 7.2 Hz, 2H, CH_2CH_3), 5.14 (s, 2H, OCH_2O), 7.0−7.3 (m, 2H, Ar-H); EIMS m/z 399 (M^+).

Ethyl 3-Fluoro-6-[*N*-(ethoxycarbonyl)-*N*-pivaloylamino]-2-(methoxymethoxy)benzoate (13b). This compound was obtained from **12b** in a similar manner as described for **13c** (two steps, 95%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.19 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.35 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.35 (s, 9H, $C(CH_3)_3$), 3.55 (s, 3H, OCH_3), 4.17 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.32 (q, J = 7.0 Hz, 2H, CH_2CH_3), 5.17 (s, 2H, OCH_2O), 6.85 (dd, J = 8.8, 4.4 Hz, 1H, 5-H), 7.16 (dd, J = 10.3, 9.0 Hz, 1H, 4-H); EIMS m/z 399 (M^+).

5-Amino-2-(4-aminophenyl)-6-fluoro-4*H*-1-benzopyran-4-one (14a). This compound was obtained from **12a** and **4a** in a similar manner as described for **5a** (overall 50%): mp 247−249 °C; IR (KBr) 1641 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 5.97 (br, 2H, 4′-NH_2), 6.51 (s, 1H, 3-H), 6.65 (d, J = 8.9 Hz, 2H, 3′,5′-H), 6.67 (dd, J = 9.2, 3.7 Hz, 1H, 8-H), 7.25 (br, 2H, 5-NH_2), 7.37 (dd, J = 11.4, 9.4 Hz, 1H, 7-H), 7.72 (d, J = 8.4 Hz, 2H, 2′,6′-H); EIMS m/z 270 (M^+). Anal. ($C_{15}H_{11}FN_2O_2$) C, N; H: calcd, 4.10; found, 3.64.

5-Amino-2-(4-aminophenyl)-8-fluoro-4*H*-1-benzopyran-4-one (14b). This compound was obtained from **12b** and **4a** in a similar manner as described for **5a** (overall 66%): mp 248−250 °C; IR (KBr) 1619 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 6.00 (br, 2H, 4′-NH_2), 6.43 (d, J = 8.9 Hz, 2H, 3′,5′-H), 6.54 (s, 1H, 3-H), 6.67 (dd, J = 8.9, 3.5 Hz, 1H, 6-H), 7.19 (br, 2H, 5-NH_2), 7.34 (t, J = 9.4 Hz, 1H, 7-H), 7.69 (d, J = 8.4 Hz, 2H, 2′,6′-H); EIMS m/z 270 (M^+). Anal. ($C_{15}H_{11}FN_2O_2$) C, H, N.

5-Amino-2-(4-aminophenyl)-6,8-difluoro-4*H*-1-benzopyran-4-one (14c). This compound was obtained from **12c** and **4a** in a similar manner as described for **5a** except that the final product was isolated as a hydrochloride by filtration of the precipitate from the cooled reaction mixture (overall 17%): mp 242−244 °C; IR (KBr) 1653 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 6.58 (s, 1H, 3-H), 6.68 (d, J = 8.9 Hz, 2H, 3′,5′-H), 7.67 (t, J = 10.9 Hz, 1H, 7-H), 7.71 (d, J = 8.9 Hz, 2H, 2′,6′-H); EIMS m/z 288 (M^+). Anal. ($C_{15}H_{10}F_2N_2O_2 \cdot HCl \cdot 0.7H_2O$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-1-benzopyran-4-one (14d). This compound was obtained from **12c** and **4b** in a similar manner as described for **5a** (overall 35%): mp 228−229 °C; IR (KBr) 1653 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 6.10 (br, 2H, 4′-NH_2), 6.69 (s, 1H, 3-H), 6.68 (t, J = 8.7 Hz, 1H, 5′-H), 7.05 (br, 2H, 5-NH_2), 7.59 (dd, J = 8.4, 2.0 Hz, 1H, 6′-H), 7.65 (dd, J = 12.9, 2.0 Hz, 1H, 2′-H), 7.69 (t, J = 11.1 Hz, 1H, 7-H); EIMS m/z 306 (M^+). Anal. ($C_{15}H_9F_3N_2O_2$) C, H, N.

5-Amino-2-(4-amino-3,5-difluorophenyl)-6,8-difluoro-4*H*-1-benzopyran-4-one (14e). This compound was obtained from **12c** and **4c** in a similar manner as described for **5a** (overall 14%): mp 230 °C dec; IR (KBr) 1637 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 6.17 (br, 2H, 4′-NH_2), 6.80 (s, 1H, 3-H), 7.06 (br, 2H, 5-NH_2), 7.60 (dd, J = 7.7, 2.7 Hz, 2H, 2′,6′-H), 7.71 (t, J = 11.1 Hz, 1H, 7-H); EIMS m/z 324 (M^+). Anal. ($C_{15}H_8F_4N_2O_2$) C, H, N.

Cell Growth-Inhibitory Activity. Assays were conducted according to the published method.[1] T-47D (3×10^3 cells/well), OVCAR-3 (3×10^3 cells/well), MKN-28 (1.5×10^3 cells/well), and PSN-1 (1.5×10^3 cells/well) cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; GIBCO, NY); SK-BR-3 (1×10^4 cells/well) cells were cultured in McCoy's 5a medium supplemented with 10% FBS; Ishikawa (3×10^3 cells/well), Hep-G2 (5×10^3 cells/well), WiDr (2.5×10^3 cells/well), and ACHN (1.5×10^3 cells/well) cells were cultured in modified Eagle's medium supplemented with 10% FBS; A2780 (1×10^3 cells/well) cells were cultured in RPMI 1640 medium supplemented with 5% FBS; and A431 (1.5×10^3 cells/well) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS.

S-9 Mix Treatment. A 30% S-9 mix solution was prepared from S-9 (0.3 mL, Oriental yeast; Tokyo, Japan), glucose 6-phosphate (1.3 mg), β-NADPH (3.6 mg), 20 mM HEPES buffer (0.2 mL), 50 mM $MgCl_2$ (0.1 mL), 330 mM KCl (0.1 mL), and distilled water (0.3 mL). The solution was added to the medium at a concentration of 0.125%. No influence against the cell growth was observed under this condition.

***In Vivo* Antitumor Activity**. Tumor fragments (8 mm^3) were transplanted subcutaneously in the flank of 7−9 weeks of age female BALB/c-nu/nu mice (Nihon Crea, Tokyo, Japan). For promoting the growth of the tumor, 12.5 μg of estradiol propionate was intramuscularly administered in the femora on the date of transplantation and 2 weeks after; 20 days after transplantation, mice with a tumor volume of 25−200 mm^3 were selected, and the test compounds were orally administered on 5 consecutive days in 1 week for 2 weeks (n = 5). Estradiol propionate was administered on the day of initial administration of test compounds. Length and width of the tumor were determined on days 0, 4, 7, 11, 14, 18, 21, and 25,

and the tumor volumes were calculated according to the following equation:

$$\text{tumor volume (mm}^3\text{)} = \{\text{length (mm)} \times [\text{width (mm)}]^2\}/2$$

The tumor volumes at initial administration (V_0) and on the day of judgement (V) were calculated, and the tumor growth rate (V/V_0) was calculated.

Acknowledgment. We thank Mr. Shingo Kakita for the measurement of the NMR spectra and the structure elucidation of compounds **2a**,**b**, Mr. Hiroshi Magara for the LC−MS analysis, and Dr. Taisuke Nakada for the ERE−luciferase assay. We are also grateful to Mses Akiko Mimura, Miki Haibara, Taimi Sano, Miyoko Asano, and Miyoko Suzuki for their excellent technical assistance.

Supporting Information Available: Preparation and ^{1}H-NMR data of compounds **3a,b** and **4b**,**c** (2 pages). Ordering information is given on any current masthead page.

References

(1) Akama, T.; Shida,Y.; Sugaya, T.; Ishida, H.; Gomi, K.; Kasai, M. Novel 5-Aminoflavone Derivatives as Specific Antitumor Agents in Breast Cancer. *J. Med. Chem.* **1996**, *39*, 3461−3469.

(2) Griffiths, L. A. Mammalian Metabolism of Flavonoids. In *Flavonoids: Advanced Research;* Harborne, J. B., Mabry, T. J., Eds.; Chappmann & Hall: London, 1982; pp 681−718.

(3) Yoshida, K.; Tsukamoto, T.; Torii, H.; Doi, T.; Naeshiro, I.; Uemura, I.; Tanayama, S. Metabolism of Ipriflavone (TC-80) in Rats. *Radioisotopes* **1985**, *34*, 612−617.

(4) Ames, B. M.; McCann, J.; Yamasaki, E. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. *Mutat. Res.* **1975**, *31*, 347−364.

(5) The details of the metabolism of compound **1** will be published separately.

(6) Fukui, K.; Yonezawa, T.; Nagata, C. Theory of Substitution in Conjugated Molecules. *Bull. Chem. Soc. Jpn.* **1954**, *27*, 423−427.

(7) Schlosser, M. Introduction of Fluorine into Organic Molecules: Why and How. *Tetrahedron* **1978**, *34*, 3−17.

(8) Gershon, H.; Schulman, S. G.; Spevack, A. D. Organic Fluorine Compounds. III. Action of Perchloryl Fluoride on Substituted Ethyl Cyanoacetates and Animal Toxicities of the Fluorinated Products. *J. Med. Chem.* **1967**, *10*, 536−541.

(9) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: A New General Purpose Quantum Mechanical Molecular Mode. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

(10) (a) Stewart, J. J. P. MOPAC: A General Molecular Orbital Package (Ver. 6.0). *QCPE Bull.* **1990**, *10*, 86−87. (b) Hirano, T. MOPAC Version 6: Revised as Version 6.01 by Hirano for UNIX Machines. *JCPE Newslett.* **1991**, *3*, 28−29.

(11) Actually, the position 3 seemed to be the most reactive to the electrophilic reaction. However, in the case of flavonoids, hydroxylation of an aromatic ring is often observed *in vivo*.[2,3]

(12) Sugaya, T.; Mimura, Y.; Kato, N.; Ikuta, M.; Mimura, T.; Kasai, M.; Tomioka, S. Synthesis of 6*H*-Pyrazolo[4,5,1-*de*]acridin-6-one Derivative: A Useful Intermediate of Antitumor Agents. *Synthesis* **1994**, 73−76.

(13) Compound **4b** was prepared from 4-bromo-2-fluoroaniline as follows: (a) pivaloyl chloride, pyridine, room temperature (99%); (b) *n*-butyllithium, $MeCONMe_2$, THF, −78 °C (66%).

(14) Umemoto, T.; Fukami, S.; Tomizawa, G.; Harasawa, K.; Kawada, K.; Tomita, K. Power and Structure-Variable Fluorinating Agents. The *N*-Fluoropyridinium Salt System. *J. Am. Chem. Soc.* **1990**, *112*, 8563−8575.

(15) Compound **4c** was prepared by the amidation of 4′-amino-3′,5′-fluoroacetophenone[16] with pivaloyl chloride in pyridine (72%).

(16) Asato, G.; Baker, P. K.; Bass, R. T.; Bentley, T. J.; Chari, S.; Dalrymple, R. H.; France, D. J.; Gingher, P. E.; Lences, B. L.; Pascavage, J. J.; Pensack, J. M.; Ricks, C. A. Repartitioning Agents: 5-[1-Hydroxy-2-(isopropylamino)ethyl]anthranilonitrile and Related Phenethanolamines; Agents for Promoting Growth, Increasing Muscle Accretion and Reducing Fat Deposition in Meat-producing Animals. *Agric. Biol. Chem.* **1984**, *48*, 2883−2888.

(17) Shi, D.-F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. Antitumor Benzothiazoles. 3. Synthesis of 2-(4-Aminophenyl)benzothiazoles and Evaluation of Their Activities against Breast Cancer Cell Lines *in Vitro* and *in Vivo*. *J. Med. Chem.* **1996**, *39*, 3375−3384.

(18) Cushman, M.; Zhu, H.; Geahlen, R. L.; Kraker, A. J. Synthesis and Biochemical Evaluation of a Series of Aminoflavones as Potential Inhibitors of Protein-Tyrosine Kinases p56^{lck}, EGFr, and p60^{v-src}. *J. Med. Chem.* **1994**, *37*, 3353−3362.

(19) Copland, J. A.; Hendry, L. B.; Chu, C. K.; Wood, J. C.; Wrenn, R. W.; Pantazis, C. G.; Mahesh, V. B. Inhibition of Estrogen Stimulated Mitogenesis by 3-Phenylacetylamino-2,6-piperidinedione and Its para-Hydroxy Analog. *J. Steroid Biochem. Mol. Biol.* **1993**, *46*, 451−462.

(20) Bitonti, A. J.; Dumont, J. A.; Salituro, F. G.; McDonald, I. A.; Jarvi, E. T.; Frey, L. M.; Wright, P. S.; Baumann, R. J. Depletion of Estrogen Receptor in Human Breast Tumor Cells by a Novel Substituted Indole That Does Not Bind to the Hormone Binding Domain. *J. Steroid Biochem. Mol. Biol.* **1996**, *58*, 21−30.

JM9700326