Structure−Activity Relationships of the 7-Substituents of 5,4′-Diamino-6,8,3′-trifluoroflavone, a Potent Antitumor Agent

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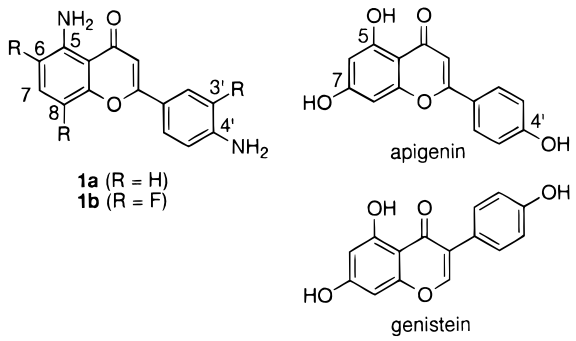
Recently, we reported that 5,4′-diamino-6,8,3′-trifluoroflavone (**1b**) exhibits potent antitumor activity against certain types of human cancer cell lines both in vitro and in vivo. Since the antiproliferative activity of 5,4′-diaminoflavone (**1a**), the lead compound of **1b**, was modulated by the addition of apigenin, we hypothesized that the 7-position is important for the interaction with a putative target molecule. On the basis of this hypothesis, the structure−activity relationships of the substituents at the 7-position of **1b** were explored. As a result, 7-methyl (**7a**), 7-hydroxymethyl (**7l**), 7-(acyloxy)methyl (**9a**,**c**,**e**,**g**,**j**), and 7-aminomethyl (**12f**) derivatives were found to exhibit comparable or superior antitumor activity to compound **1b** against MCF-7 cells both in vitro and in vivo (po administration). In particular, compounds **9e**,**g**,**j**, and **12f** were sufficiently water-soluble as compared with **1b** which hardly solubilizes in water. A lipophilic 7-(hexanoyloxy)methyl derivative (**9c**) was also found to exhibit strong antitumor activity especially in vivo. Since the modes of action and the target molecule(s) are unknown, a mechanistic study will be important in the future.

Flavonoids, either natural or synthetic, are known to exhibit various biological activities.[1] In particular, there are many compounds exhibiting antitumor[2] or related activities, such as antimitotic activity[3] or inhibition of aromatase,[4] topoisomerase,[5] protein kinase C,[6] several protein-tyrosine kinases,[7] or cyclin-dependent kinase.[8] Previously, we reported that 5,4′-diaminoflavone (**1a**) (Chart 1) and some of its congeners exhibit potent cytotoxicity against human breast cancer cell line MCF-7.[9]

In the course of the search for the pharmaceutical mechanism of compound **1a**, we found that the antiproliferative activity of compound **1a** against MCF-7 cells was modulated by the addition of apigenin which itself exhibits both growth-inhibiting and -stimulating activity to certain types of cancer cell lines.[10] The isomeric isoflavone derivative genistein exhibited no effect on the activity of **1a**. Both the mechanism of action and the target molecule of compound **1a** are still unclear. However, we hypothesized that the 7-hydroxy group of apigenin might participate in the interaction with a putative target molecule and that the introduction of a functional group to the 7-position of compound **1a** might affect the biological activity, because apigenin possesses three polar substituents at the 5-, 7-, and 4′-positions in contrast to compound **1a** which has two at the 5- and 4′-positions. In addition, substituents at the 7-position might improve the physical properties (e.g., water solubility) of compound **1a** which exhibits poor solubility both in water and in organic solvents. The hypothesis prompted us to explore the structure−activity relationships (SAR) of the substituents at the 7-position of 5,4′-diaminoflavone derivatives.

Chart 1



In direct contrast, compound **1a** was suggested to be metabolically deactivated in vivo.[11] However, the in vivo potency was enhanced in compound **1b**[11] in which fluorine atoms were introduced into potential metabolic positions, i.e., 6-, 8-, and 3′-positions, probably due to blocking metabolic deactivation. Therefore, we chose compound **1b** as a parent skeleton and synthesized various 7-substituted-5,4′-diamino-6,8,3′-trifluoroflavone derivatives. Herein, we describe the synthesis of these compounds and their antitumor activity against MCF-7 cells both in vitro and in vivo and agaist HeLa S_3 in vitro.

Chemistry

We have already developed a practical synthetic pathway to compound **2**, a key intermediate of compound **1b**, using sequential directed ortho metalations as the key step.[12] In the process, we found that the two fluorine atoms corresponding to the 6- and 8-positions can be regarded as a good directing group for the directed ortho metalation.[13] Therefore, we planned to introduce various functional groups into the 7-position or the corresponding position of an intermediate by directed ortho metalation.

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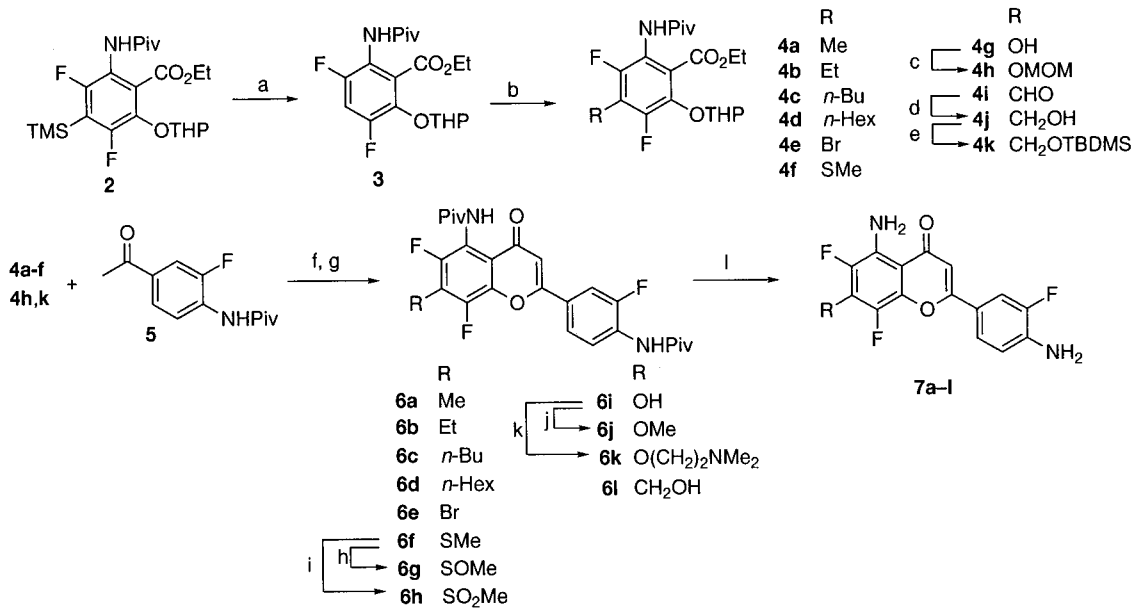
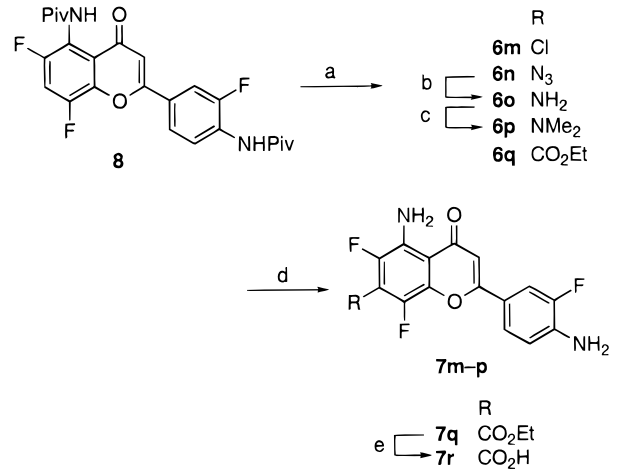
Scheme 1[a]



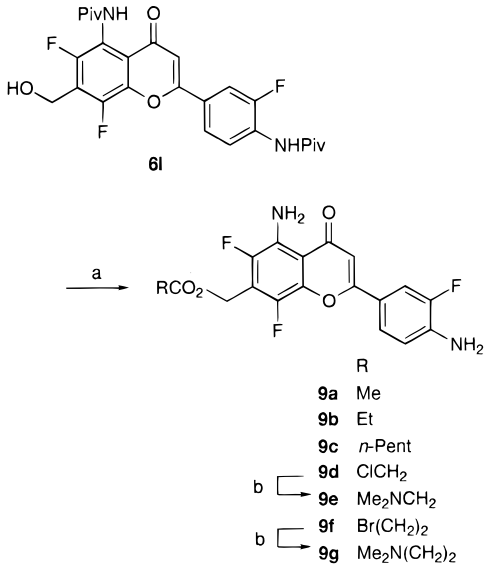
[a] (a) TBAF, THF, 0 °C; (b) LDA, electrophile, THF, −78 °C; (c) chloromethyl methyl ether, *i*-Pr_2NEt, CH_2Cl_2, rt; (d) $NaBH_4$, MeOH, 0 °C; (e) TBDMSCl, imidazole, DMF, rt; (f) NaH, 1,4-dioxane (toluene), reflux; (g) HCl, EtOH, rt; (h) *m*-CPBA (1 eq), CH_2Cl_2, 0 °C; (i) *m*-CPBA (excess), CH_2Cl_2, rt; (j) K_2CO_3, MeI, acetone, reflux; (k) NaH, $Cl(CH_2)_2NMe_2$·HCl, DMF, 80 °C; (l) HCl, 1,4-dioxane, reflux or H_2SO_4, 50 °C.

Scheme 2[a]



[a] (a) LDA, electrophile, THF, HMPA, −78 °C; (b) PPh_3, THF, 1 N HCl, rt; (c) NaH, MeI, DMF, 0 °C; (d) H_2SO_4, 50−100 °C; (e) aq NaOH, EtOH, rt.

Scheme 3[a]



[a] (a) RCO_2H, H_2SO_4, 100 °C; (b) Me_2NH·HCl, *i*-Pr_2NEt, DMF, 50 °C.

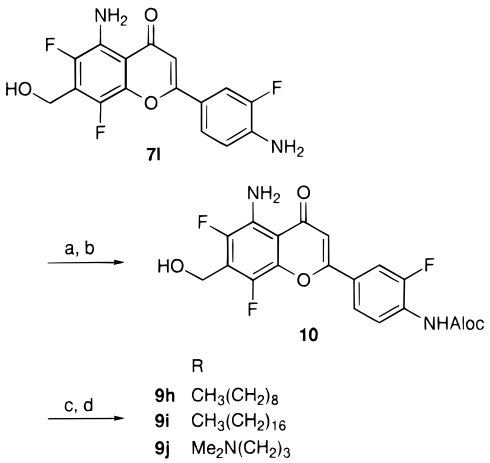
Compounds **7** were synthesized as shown in Scheme 1. The trimethylsilyl group of compound **2**, which was prepared in eight steps from 2,4-difluorophenol,[12] was removed with tetrabutylammonium fluoride to afford **3**. The 4-position and the amide group of compound **3** were lithiated by LDA, and the dianion generated was trapped with various electrophiles to afford **4**. Compounds **4** and **5**[11,12] were condensed with sodium hydride followed by cyclization with HCl to afford **6**. Target compounds **7** were obtained after removal of the two pivaloyl groups by heating with HCl or H_2SO_4.

Some of the compounds **7** were obtained by the direct lithiation of compound **8**[11,12] followed by the deprotection shown in Scheme 2. In this case, HMPA was needed as a cosolvent to dissolve the starting material **8** in THF.

Lower fatty acid ester derivatives **9** were synthesized from 7-hydroxymethyl derivative **6l** as shown in Scheme 3. When compound **6l** was heated in the corresponding carboxylic acid in the presence of H_2SO_4, simultaneous esterification of the 7-hydroxymethyl group and hydrolysis of the pivaloyl groups afforded **9a**−**d**,**f**. *N*,*N*-Dimethylglycine ester (**9e**) and *N*,*N*-dimethyl-*β*-alanine ester (**9g**) were obtained by the reactions of **9d**,**f** with dimethylamine hydrochloride, respectively.

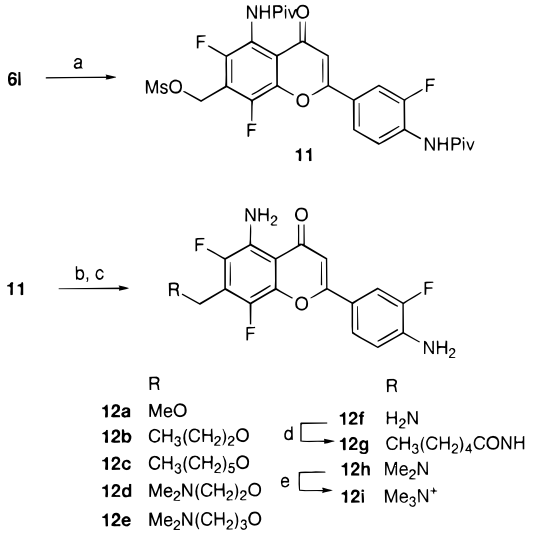
Long-chain fatty acid ester derivatives **9h**,**i** and 4-(dimethylamino)butyric acid ester derivative **9j** were synthesized as shown in Scheme 4. Since the reaction of compound **7l** and allyloxycarbonyl (Aloc) chloride in pyridine afforded a mixture of **10** (major) and 7-(allyloxycarbonyl)oxymethyl derivative of **10** (minor), the crude mixture was treated with aqueous NaOH to remove the *O*-Aloc group to give **10** as a sole product.

Scheme 4[a]



[a] (a) Ally chloroformate, pyridine, rt; (b) 1 N NaOH, THF, 0 °C; (c) RCOCl, Et_3N, CH_2Cl_2 or RCO_2H, CDI, DMF, rt; (d) $Pd(PPh_3)_4$, HCO_2H, Et_3N, THF, rt.

Scheme 5[a]



[a] (a) MsCl, Et_3N, DMF, 0 °C; (b) RH (base), DMF; (c) H_2SO_4, 50−100 °C; (d) $CH_3(CH_2)_4COCl$, Et_3N, DMF; rt; (e) MeI, THF, rt.

The 7-hydroxymethyl group of **10** was acylated followed by deprotection of the Aloc group to afford **9h**−**j**.

Various 7-alkoxymethyl and 7-aminomethyl derivatives **12** were synthesized as shown in Scheme 5. The hydroxymethyl group of compound **6l** was converted into mesylate (**11**) by treating with methanesulfonyl chloride in pyridine. Compounds **12** were obtained by a substitution reaction at the 7-methyl position of **11** with various nucleophiles, such as alcohols, alkoxides, and amines, followed by the removal of the pivaloyl groups.

All target compounds synthesized are listed in Table 1.

Biological Results

In Vitro Activity. The growth of MCF-7 cells in the absence of estradiol was completely inhibited by 0.05 μM compound **1a**.[9] When various concentrations of apigenin were added to the medium in the presence of compound **1a** (0.05 μM), the cells grew in a concentration-dependent manner up to 6.3 μM apigenin (Figure 1A). In contrast, the addition of genistein had no effect (Figure 1B). Although both compounds are known to bind the estrogen receptor (ER) and possess growth-stimulating activity to MCF-7 cells,[9,10b,14] apigenin and genistein behaved quite differently against the cytotoxicity of compound **1a**, suggesting that the effect of apigenin was mediated by an alternative pathway to the ER. Taken together with the fact that the target molecule of compound **1a** is not the ER,[9] compound **1a** and apigenin might be anticipated to interact with the same unknown molecule.

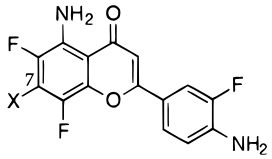
Because compounds **1a**,**b** have unique antiproliferative profiles against various human cancer cell lines,[9,11] e.g., cytotoxic against breast, ovarian, endometrial, and liver cancer cell lines but not to uterus, colon, and stomach cell lines, two cell lines considered to be sensitive and nonsensitive to **1a**,**b** were selected for in vitro evaluation of the compounds synthesized. The cytotoxicity of compounds **7a**−**r**, **9a**−**j** (except **9d**,**f**), and **12a**−**l** against HeLa S_3 as a nonsensitive cancer cell line and MCF-7 as a sensitive one was tested. The results are presented in Table 2.

With regard to the activity against MCF-7 cells, the 7-methyl derivative (**7a**) showed potent cytotoxicity comparable to that of compound **1b** among lower alkyl derivatives, and the activity was decreased when the alkyl chains became longer (**7b**−**d**). 7-Chloro (**7m**), 7-bromo (**7e**), 7-methoxy (**7j**), and 7-methylthio (**7f**) derivatives were less active than **1b**. In contrast to the 7-hydroxy derivative (**7i**) which lost activity, the 7-amino derivative (**7o**) exhibited the most potent activity against MCF-7. Although the introduction of ethoxycarbonyl (**7q**) and carboxy (**7r**) groups resulted in a large decrease in activity, the 7-hydroxymethyl derivative (**7l**), its acetate (**9a**), and hydrophilic esters possessing a dimethylamino group (**9e**,**g**,**j**) retained the activity. With increasing the length of the alkyl chain, decreasing activity occurred in fatty acid ester derivatives (**9b**,**c**,**h**,**i**). 7-Methoxymethyl (**12a**), 7-aminomethyl (**12f**), and 7-(dimethylamino)methyl (**12h**) derivatives retained activity against MCF-7 cells; however, the activity tended to decrease in derivatives possessing bulkier substituents with more than three carbons (**12b**−**e**,**g**). It is interesting that the quaternary amine derivative (**12i**) lost its potency in contrast with the tertiary amine derivative (**12h**) which showed strong cytotoxicity. 7-(Hexanoylamino)methyl derivative (**12g**) showed comparable activity to the 7-(hexanoyloxy)methyl derivative (**9c**).

With regard to the activity against HeLa S_3 cells, half of the compounds did not show cytotoxicity (IC_{50} > 100 μM). Among the compounds showing IC_{50} values below 0.1 μM against MCF-7, 7-methyl (**7a**), 7-(dimethylamino)methyl (**7p**), and 7-methoxymethyl (**12a**) showed no cytotoxicity against HeLa S_3 cells. These compounds were considered to have the same profiles as compound **1b**. Against HeLa S_3 cells compounds **7l**,**o**, **9a**,**e**,**g**,**j**, and **12f**,**h** showed cytotoxicity with IC_{50} values of 4.5−24 μM, which is more than 100 times greater than the IC_{50} values against MCF-7 cells.

In Vivo Activity. The in vivo antitumor activity of the derivatives showing strong cytotoxicity in vitro was tested. MCF-7 cells were inoculated into female nude mice and were growth-stimulated by estradiol. Compounds were orally administered on 5 consecutive days/week for 2 weeks (days 0−4 and 7−11). The results are

Table 1. Synthesized 7-Substituted-5,4′-diamino-6,8,3′-trifluoroflavone Derivatives



compd	X	empirical formula[a]	mp (°C)	^{1}H NMR (δ, ppm)[b] of C-7 substituents
7a	Me	$C_{16}H_{11}F_3N_2O_2$	239	2.33 (t, J = 2.0 Hz, 3H)
7b	Et	$C_{17}H_{13}F_3N_2O_2$	204−205	1.21 (t, J = 7.6 Hz, 3H), 2.74 (q, J = 7.6 Hz, 2H)
7c	*n*-Bu	$C_{19}H_{17}F_3N_2O_2$	178−180	0.95 (t, J = 7.4 Hz, 3H), 1.40 (sextet, J = 7.4 Hz, 2H), 1.63 (quint, J = 7.4 Hz, 2H), 2.79 (t, J = 7.4 Hz, 2H)
7d	*n*-Hex	$C_{21}H_{21}F_3N_2O_2$	179−180	0.89 (t, J = 6.9 Hz, 3H), 1.2−1.5 (m, 6H), 1.64 (quint, J = 7.4 Hz, 2H), 2.78 (t, J = 7.4 Hz, 2H)
7m	Cl	$C_{15}H_8ClF_3N_2O_2$	243−244	
7e	Br	$C_{15}H_8BrF_3N_2O_2$[c]	256−258	
7i	OH	$C_{15}H_9F_3N_2O_3$	>300	11.3 (br s, 1H)
7j	OCH_3	$C_{16}H_{11}F_3N_2O_3$	215−217	4.09 (t, J = 1.5 Hz, 3H)
7k	$O(CH_2)_2NMe_2$	$C_{19}H_{18}F_3N_3O_3\cdot$HCl	250 dec	2.89 (s, 6H), 3.56 (t, J = 5.0 Hz, 2H), 4.67 (t, J = 5.0 Hz, 2H)
7f	SMe	$C_{16}H_{11}F_3N_2O_2S$	205−207	2.59 (s, 3H)
7g	SOMe	$C_{16}H_{11}F_3N_2O_3S$	280 dec	3.19 (s, 3H)
7h	SO_2Me	$C_{16}H_{11}F_3N_2O_4S\bullet0.6H_2O$	300 dec	3.50 (s, 3H)
7n	N_3	$C_{15}H_8F_3N_5O_2$	182 dec	
7o	NH_2	$C_{15}H_{10}F_3N_3O_2$[d]	290−291	5.99 (br s, 2H)
7p	NMe_2	$C_{17}H_{14}F_3N_3O_2$	210−212	3.00 (t, J = 2.5 Hz, 6H)
7q	CO_2Et	$C_{18}H_{13}F_3N_2O_4$	237−239	1.34 (t, J = 7.2 Hz, 3H), 4.43 (q, J = 7.2 Hz, 2H)
7r	CO_2H	$C_{16}H_9F_3N_2O_4\cdot0.3H_2O$	254−255	
7l	CH_2OH	$C_{16}H_{11}F_3N_2O_3$	277 dec	4.5−4.6 (m, 2H), 5.44 (t, J = 4.9 Hz, 1H)
9a	CH_2OCOMe	$C_{18}H_{13}F_3N_2O_4$	220 dec	2.11 (s, 3H, $COCH_3$), 5.29 (t, J = 1.5 Hz, 2H)
9b	CH_2OCOEt	$C_{19}H_{15}F_3N_2O_4$	214−215	1.04 (t, J = 7.6 Hz, 3H), 2.36 (q, J = 7.6 Hz, 2H), 5.24 (br s, 2H)
9c	$CH_2OCO(CH_2)_4CH_3$	$C_{22}H_{21}F_3N_2O_4$	166−167	0.88 (t, J = 6.4 Hz, 3H), 1.2−1.3 (m, 4H), 1.64 (quint, J = 7.4 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 5.29 (s, 2H)
9h	$CH_2OCO(CH_2)_8CH_3$	$C_{26}H_{29}F_3N_2O_4$	149−150	0.86 (t, J = 6.7 Hz, 3H), 1.1−1.4 (m, 12H), 1.64 (quint, J = 7.4 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 5.29 (s, 2H)
9i	$CH_2OCO(CH_2)_{16}CH_3$	$C_{34}H_{45}F_3N_2O_4$	139−140	0.87 (t, J = 6.7 Hz, 3H), 1.1−1.4 (m, 28H), 1.63 (quint, J = 6.9 Hz, 2H), 2.34 (t, J = 7.7 Hz, 2H), 5.29 (s, 2H)
9e	$CH_2OCOCH_2NMe_2$	$C_{20}H_{18}F_3N_3O_4\cdot$HCl$\cdot0.4H_2O$	220 dec	2.84 (s, 6H), 4.27 (s, 2H), 5.42 (s, 2H)
9g	$CH_2OCO(CH_2)_2NMe_2$	$C_{21}H_{20}F_3N_3O_4\cdot$HCl$\cdot0.3H_2O$	227−228	2.75 (d, J = 4.5 Hz, 6H), 2.93 (t, J = 7.4 Hz, 2H), 3.31 (t, J = 7.4 Hz, 2H), 5.31 (s, 2H)
9j	$CH_2OCO(CH_2)_3NMe_2$	$C_{22}H_{22}F_3N_3O_4\cdot$HCl	233 dec	1.94 (quint, J = 7.9 Hz, 2H), 2.48 (t, J = 7.9 Hz, 2H), 2.73 (s, 6H), 3.04 (br s, 2H), 5.27 (s, 2H)
12a	CH_2OMe	$C_{17}H_{13}F_3N_2O_3$	221	3.31 (s, 3H), 4.57 (br s, 2H)
12b	$CH_2O(CH_2)_2CH_3$	$C_{19}H_{17}F_3N_2O_3$	170−171	0.93 (t, J = 7.4 Hz, 3H), 1.63 (sextet, J = 6.9 Hz, 2H), 3.50 (t, J = 6.9 Hz, 2H), 4.69 (t, J = 2.0 Hz, 2H)
12c	$CH_2O(CH_2)_5CH_3$	$C_{22}H_{23}F_3N_2O_3$	162−163	0.88 (t, J = 6.9 Hz, 3H), 1.2−1.4 (m, 6H), 1.60 (q, J = 6.9 Hz, 2H), 3.52 (t, J = 6.9 Hz, 2H), 4.68 (t, J = 2.0 Hz, 2H)
12d	$CH_2O(CH_2)_2NMe_2$	$C_{20}H_{20}F_3N_3O_3\cdot$HCl$\cdot0.6H_2O$	265	2.76 (d, J = 5.0 Hz, 6H), 3.29 (q, J = 5.0 Hz, 2H), 3.83 (t, J = 5.0 Hz, 2H), 4.72 (br s, 2H)
12e	$CH_2O(CH_2)_3NMe_2$	$C_{21}H_{22}F_3N_3O_3\cdot$HCl$\cdot1.6H_2O$	155 dec	1.8−2.0 (m, 2H), 2.74 (d, J = 4.9 Hz, 6H), 3.0−3.2 (m, 2H), 3.55 (t, J = 5.9 Hz, 2H), 4.64 (br s, 2H)
12f	CH_2NH_2	$C_{16}H_{12}F_3N_3O_2\cdot2$HCl	>300	3.80 (s, 2H)
12h	CH_2NMe_2	$C_{18}H_{16}F_3N_3O_2\cdot$HCl$\cdot0.5H_2O$	269−270	2.84 (s, 6H), 4.47 (br s, 2H)
12i	$CH_2N^+Me_3I^-$	$C_{19}H_{19}F_3IN_3O_2$	244 dec	3.19 (s, 9H), 4.73 (s, 2H)
12g	$CH_2NHCO(CH_2)_4CH_3$	$C_{22}H_{22}F_3N_3O_3\cdot0.2H_2O$	239−240	0.83 (t, J = 6.9 Hz, 3H), 1.1−1.3 (m, 4H), 1.48 (quint, J = 7.3 Hz, 2H), 2.07 (t, J = 7.3 Hz, 2H), 4.39 (d, J = 5.0 Hz, 2H)

[a] All compounds were analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values unless otherwise noted. [b] The solvent was DMSO-d_6 except for **7a**,**c**,**d**, **9c**,**h**,**i**, and **12b**,**c** ($CDCl_3$). [c] N: calcd, 7.27; found, 6.74. [d] N: calcd, 13.08; found, 12.62.

listed in Table 3 by the T/C values which is the ratio of tumor growth rate of the drug-treated group relative to that of the control group. Representative results (compounds **9c**,**e** and **12f**) are graphed in Figure 2.

The 7-methyl derivative (**7a**) showed 89% reduction of the growth rate at 12.5 mg/kg with some body weight decrease (−11%), which was equal to the efficacy of **1b** at 25 mg/kg dosage. The 7-amino derivative (**7o**) which was the most potent in vitro also showed strong antitumor activity; however, the body weight decrease was more serious (−22% at 12.5 mg/kg). The efficacy was diminished when the 7-amino group of **7o** was dimethylated (**7p**). The 7-hydroxymethyl derivative (**7l**) showed activity comparable to that of compound **1b** at 25 mg/kg with slight body weight decrease, and further, the dose of 50 mg/kg was also tolerable. The acetylated derivative of **7l** (**9a**) showed strong activity, but dose dependency was not observed, probably due to poor solubility. Compound **9b**'s lack of effect seemed to be due to the same reason. Although compounds **9a**,**b** have poor solubility both in water and in organic solvents, compound **9b** is especially insoluble. Interestingly, the

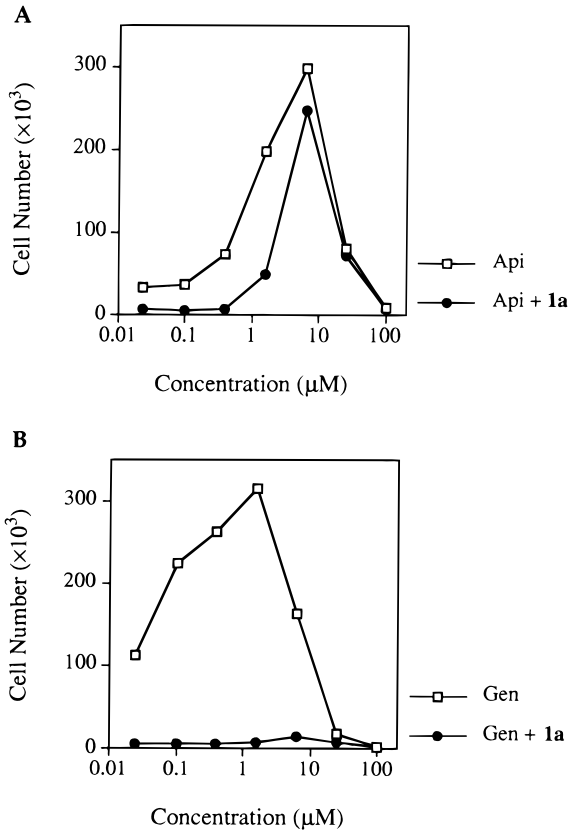


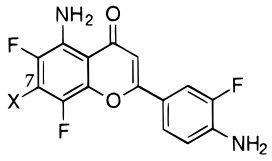
Figure 1. Interaction of apigenin with compound **1a** on the growth of MCF-7 cells. MCF-7 cells were incubated with various concentrations of apigenin (Api) (A) or genistein (Gen) (B) with or without 0.05 μM compound **1a** for 6 days and counted by a microcell counter (ref 9).

(hexanoyloxy)methyl derivative (**9c**) exhibited intensive antitumor activity from 6.3 to 25 mg/kg without serious body weight decrease (also see Figure 2A). The long-chain fatty acid ester derivatives (**9h**,**i**) exhibited 80−87% reduction of the growth rate at 25 mg/kg. These long-chain ester derivatives would have high lipid solubility which might improve the oral absorption. The ester derivatives possessing a dimethylamino group (**9e**,**g**,**j**) showed strong antitumor activity; especially, compound **9e** showed excellent activity (also see Figure 2B). 7-Aminomethyl derivative **12f** showed activity comparable to 7-hydroxymethyl derivative **7l** at 25 and 50 mg/kg (also see Figure 2C). As observed in the case of compounds **7o**,**p**, dimethylation of the amino group at the 7-methyl position of compound **12f** (compound **12h**) did not improve activity.

Discussion

The SAR of the 7-position of 5,4′-diamino-6,8,3′-trifluoroflavone (**1b**) was explored. Interesting findings were obtained from in vitro experiments: (1) although both an amino group and a hydroxy group are capable of being a hydrogen bond donor/acceptor, the 7-amino derivative (**7o**) showed excellent cytotoxicity against MCF-7 cells in contrast that the 7-hydroxy derivative (**7i**), which showed no activity; (2) although the 7-methyl (**7a**) and 7-hydroxymethyl (**7l**) derivatives showed activity comparable to that of **1b** against MCF-7 cells, the more oxygenated 7-ethoxycarbonyl (**7q**) and 7-carboxyl (**7r**) derivatives showed weak or no activity.

Table 2. In Vitro Cytotoxicity of 7-Substituted-5,4′-diamino-6,8,3′-trifluoroflavone Derivatives against HeLa S_3 and MCF-7 Cell Lines

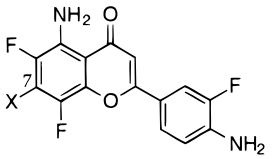


compd	X	IC$_{50}$ (μM)[a] HeLa S_3	IC$_{50}$ (μM)[a] MCF-7
1b	H	>100	0.040
7a	Me	>100	0.013
7b	Et	>100	0.13
7c	*n*-Bu	>100	8.8
7d	*n*-Hex	21	>10
7m	Cl	>100	0.21
7e	Br	>100	0.16
7i	OH	>100	>10
7j	OCH_3	34	0.10
7k	$O(CH_2)_2NMe_2$	1.5	1.4
7f	SMe	>100	0.19
7g	SOMe	>100	2.5
7h	SO_2Me	>100	>10
7n	N_3	1.2	0.46
7o	NH_2	19	0.0077
7p	NMe_2	>100	0.039
7q	CO_2Et	>100	1.4
7r	CO_2H	>100	>10
7l	CH_2OH	24	0.062
9a	CH_2OCOMe	4.9	0.026
9b	CH_2OCOEt	69	0.13
9c	$CH_2OCO(CH_2)_4CH_3$	34	0.16
9h	$CH_2OCO(CH_2)_8CH_3$	>100	0.10
9i	$CH_2OCO(CH_2)_{16}CH_3$	>100	>10
9e	$CH_2OCOCH_2NMe_2$	9.2	0.026
9g	$CH_2OCO(CH_2)_2NMe_2$	11	0.019
9j	$CH_2OCO(CH_2)_3NMe_2$	4.5	0.029
12a	CH_2OMe	>100	0.046
12b	$CH_2O(CH_2)_2CH_3$	78	1.2
12c	$CH_2O(CH_2)_5CH_3$	>100	7.1
12d	$CH_2O(CH_2)_2NMe_2$	4.6	>10
12e	$CH_2O(CH_2)_3NMe_2$	12	>10
12f	CH_2NH_2	8.8	0.026
12h	CH_2NMe_2	8.9	0.072
12i	$CH_2N^+Me_3I^-$	32	3.1
12g	$CH_2NHCO(CH_2)_4CH_3$	>100	0.34

[a] Cells were treated with each compound for 3 days. The uptake of neutral red dye was measured (ref 9).

Various 7-(acyloxy)methyl derivatives were synthesized, and their antitumor activity was tested to find derivatives with improved solubility because the 7-hydroxymethyl derivative (**7l**) had a potency comparable to that of compound **1b** despite its poor physical properties. Long-chain fatty acid ester derivatives (**9c**,**h**,**i**) were inactive or less active than the corresponding alcohol (**7l**) or its acetate (**9a**) in vitro; nevertheless all the ester derivatives except compound **9b** were active in vivo. Therefore the ester derivatives were considered to act as prodrugs and to be hydrolyzed to generate compound **7l** in vivo. With respect to the acetoxymethyl derivative **9a**, it is not evident whether the compound is hydrolyzed in vivo because this compound showed cytotoxicity as potent as that of compound **7l** in vitro. Compound **9b** and the methoxymethyl derivative **12a** also showed potent activity in vitro but no effect in vivo. A potential cause of these results can be traced to the poor solubility of the compounds in both water and organic solvents. Hydrophilic ester derivatives (**9e**,**g**,**j**), showed potent antitumor activity both in vitro and in

Table 3. In Vivo Antitumor Activity of 7-Substituted-5,4′-diamino-6,8,3′-trifluoroflavone Derivatives against MCF-7 Cells Implanted into Nude Mice[a]



compd	X	dose (mg/kg)	T/C min (%)	body weight change (%)
1b	H	25	11	−7.4
7a	Me	6.3	55	−8.7
		12.5	11	−11
7o	NH_2	3.1	55	−5.7
		6.3	26	−13
		12.5	18	−22
7p	NMe_2	12.5	39	−19
7l	CH_2OH	25	17	−4
		50	1.6	−6.7
9a	CH_2OCOMe	25	6.1	−5.9
		50	11	−4.5
9b	CH_2OCOEt	25	79	−2.4
9c	$CH_2OCO(CH_2)_4CH_3$	6.3	27	−5.9
		12.5	7.9	−8.4
		25	0.8	−7.9
9h	$CH_2OCO(CH_2)_8CH_3$	25	13	−5.0
9i	$CH_2OCO(CH_2)_{16}CH_3$	25	20	−4.8
9e	$CH_2OCOCH_2NMe_2$	6.3	68	−4.8
		25	4.0	−9.0
		50	1.0	−11
9g	$CH_2OCO(CH_2)_2NMe_2$	25	4.1	−12
9j	$CH_2OCO(CH_2)_3NMe_2$	25	15	−8.4
12a	CH_2OMe	50	89	0
12d	$CH_2O(CH_2)_2NMe_2$	50	57	−7.9
12f	CH_2NH_2	6.3	60	−5.8
		25	11	−8.5
		50	1.1	−11
12h	CH_2NMe_2	25	16	−17
12g	$CH_2NHCO(CH_2)_4CH_3$	50	76	0

[a] Tumor fragments were implanted sc into BALB/c-nu/nu female mice on day −20. Compounds were administered po 5 consecutive days/week for 2 weeks.

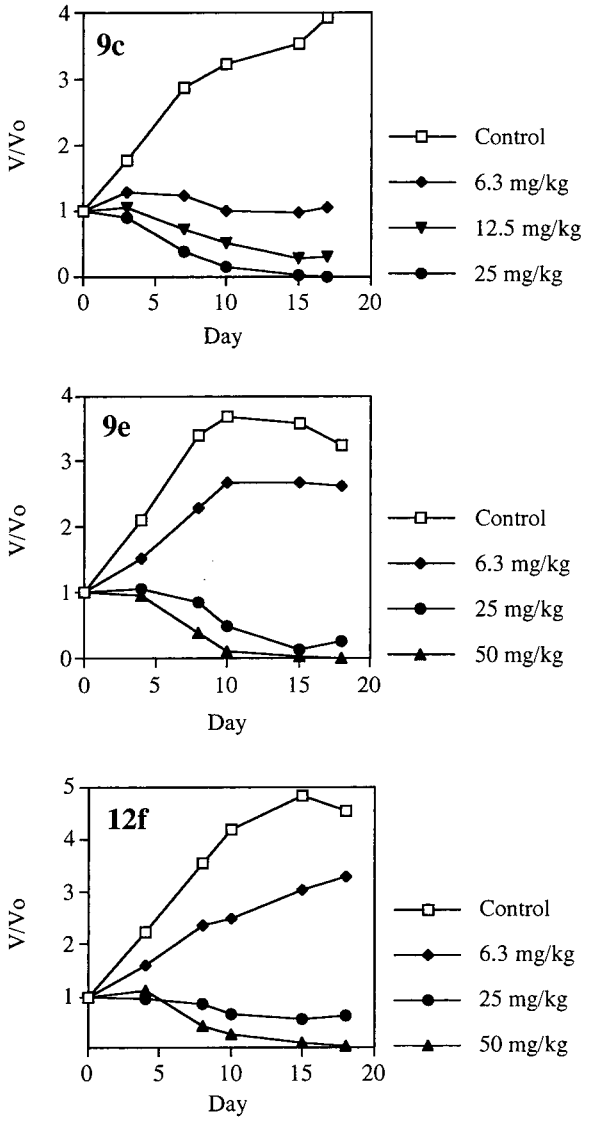


Figure 2. In vivo antitumor activity of compounds **9c**,**e**, and **12f**.

vivo. However, the ether derivatives (**12d**,**e**), which correspond to decarbonylated derivatives of compounds **9e**,**g** and cannot be hydrolyzed, were inactive both in vitro and in vivo. Thus, it was suggested that compounds **9e**,**g**,**j** also act as prodrugs of compound **7l**. On the other hand, the amide type derivative **12g** which exhibited activity comparable to that of the ester derivative **9c** in vitro showed no effect in vivo despite the fact that the aminomethyl derivative **12f** exhibited strong antitumor activity in vivo, suggesting that the amide bond was not cleaved in vivo.

Since the lead compound **1b** possesses poor solubility in both water and organic solvents,[15] the improvement of a physical property such as solubility may be crucial. In addition to the potent antitumor activity, compounds **9e**,**g**,**j** and **12f** which are hydrochlorides exhibited improved water solubility. Preliminary data of water solubility are >10 mg/mL for compounds **9e**,**j** and >5 mg/mL for compounds **9g** and **12f**. Water solubility is an advantage for a drug with respect to the formulation and the oral absorption. The prodrug formation is one potential approach for improving the physical properties and pharmacokinetics.[16] In this study, it is suggested from the biological data that 7-(acyloxy)methyl derivatives act as prodrugs of the 7-hydroxymethyl derivative **7l**. On the other hand, compound **9c**, which also exhibits remarkable antitumor activity in vivo, does not exhibit water solubility, but it is more soluble in organic solvents such as methanol (ca. 2 mg/mL) than compound **1b** (ca. 1 mg/mL). The increased lipophilicity might enhance the membrane permeability, which could improve the oral absorption compared to that of compound **1b**. We cannot conclude that the prodrugs are preferable to the intact drugs from the present data because the intact compounds, such as **7a**,**l**, are sufficiently potent in vivo despite the poor water solubility. In fact, the formulation and administration route of the intact compounds are restricted; however, other problems such as species-specific activation make the development of the prodrugs difficult.

The real role of the 7-substituents is still unclear. To clarify it, the target molecule of aminoflavone derivatives and how they interact with the molecule must be identified. Neither the simple alkylation nor the cleavage of the DNA is considered to be the mode of action, because the sensitivities of the two cell lines are quite different. Therefore, the potential candidates of the target molecule include various protein kinases, nuclear receptors, transcriptional factors, and other key en-

zymes or cofactors in certain types of tumor cell growth. It will be important to determine the mechanism of the antitumor activity of the aminoflavone derivatives in the future. At least, we found that a physical property of the parent compound **1b** can be improved by modification at the 7-position, while retaining the antitumor activity.

In conclusion, the structure−activity relationships of the substituents at the 7-position of 5,4′-diamino-6,8,3′-trifluoroflavone (**1b**) were explored based on the hypothesis that the 7-substituents would affect the biological activity of 5,4′-diaminoflavone derivatives. As a result, some derivatives, such as compounds **7a**,**l**, **9a**,**c**,**e**,**g**,**j** and **12f**, were found to exhibit comparable or superior antitumor activity to the parent compound **1b** against MCF-7 cells both in vitro and in vivo. In addition, compounds **9e**,**g**,**j** and **12f** were sufficiently water-soluble compared to **1b** which barely solubilizes in water. A lipophilic derivative (**9c**) was also found to exhibit strong antitumor activity in vivo.

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. EIMS and FABMS 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.

Ethyl 3,5-Difluoro-2-(2-tetrahydropyranyloxy)-6-(pivaloylamino)benzoate (3). To a solution of compound **2** (44.0 g, 96.3 mmol) in THF (400 mL) was added tetrabutylammonium fluoride (1.0 M in THF, 28 mL) at 0 °C, and the mixture was stirred for 30 min. Brine was added, and the mixture was extracted with Et_2O. The organic layer was washed with brine. The residue was triturated with hexane to afford **3** (37.1 g, 100%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.28 (s, 9H, $C(CH_3)_3$), 1.38 (t, J = 7.0 Hz, CH_2CH_3), 1.4−2.0 (m, 6H, 3′,4′,5′-CH_2), 3.3−4.1 (m, 2H, 6′-CH_2), 4.36 (q, J = 7.0 Hz, CH_2CH_3), 5.32 (br s, 1H, 2′-H), 7.00 (dd, J = 10.7, 9.6 Hz, 1H, 4-H), 7.57 (br s, 1H, NH); FABMS m/z 386 (M + H$^+$).

Typical Procedure for the Preparation of Compounds 4: Ethyl 3,5-Difluoro-4-methyl-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4a). To a solution of diisopropylamine (70 mL, 500 mmol) in THF (140 mL) was added *n*-butyllithium (1.6 M in hexane, 288 mL) at −10 to 0 °C under argon atmosphere. The reaction mixture was cooled to below −60 °C, and a solution of compound **3** (77.0 g, 200 mmol) in THF (600 mL) was added dropwise. After 2 h of stirring, iodomethane (19.0 mL, 300 mmol) was added, and the reaction mixture was stirred for 30 min. Water was added, and the mixture was warmed to room temperature. The mixture was extracted with EtOAc, and the organic layer was washed with brine. The residue was triturated with hexane to afford **4a** (75.9 g, 95%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.19 (s, 9H, $C(CH_3)_3$), 1.37 (t, J = 7.0 Hz, CH_2CH_3), 1.4−2.0 (m, 6H, 3′,4′,5′-CH_2), 2.23 (t, J = 2.2 Hz, 3H, 4-CH_3), 3.4−4.2 (m, 2H, 6′-CH_2), 4.34 (q, J = 7.0 Hz, CH_2CH_3), 5.28 (br s, 1H, 2′-H), 7.69 (dd, J = 10.7, 9.6 Hz, 1H, 4-H), 7.57 (br s, 1H, NH); FABMS m/z 400 (M + H$^+$). NMR data of compounds **4** are listed in Table 4.

Ethyl 3,5-Difluoro-4-ethyl-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4b). Iodoethane was used as an electrophile, and chromatography (3:1 hexane/EtOAc) of the crude product gave **4b** (67%): FABMS m/z 414 (M + H$^+$).

Table 4. ^{1}H NMR of the C-4 (C-7 of Target Compounds) Substituents of Compounds **4**

compd	^{1}H NMR (δ, ppm) in $CDCl_3$
4b	1.20 (t, J = 7.7 Hz, 3H), 2.72 (q, J = 7.7 Hz, 2H)
4c	0.92 (t, J = 5.9 Hz, 3H), 1.4−2.0 (m, 4H), 2.69 (br t, 2H)
4d	0.7−1.0 (m, 3H), 1.4−2.0 (m, 8H), 2.5−2.9 (m, 2H)
4f	2.50 (br s, 3H)
4h	3.57 (s, 3H), 5.21 (s, 2H)
4i	10.3 (s, 1H)
4j	4.7−4.8 (m, 2H)
4k	0.03 (s, 6H), 0.83 (s, 9H), 4.70 (t, J = 1.7 Hz, 2H)

Ethyl 4-(1-Butyl)-3,5-difluoro-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4c). 1-Iodobutane was used as an electrophile, and chromatography (4:1 hexane/EtOAc) of the crude product gave **4c** (29%): FABMS (negative) m/z 440 (M − H$^-$).

Ethyl 3,5-Difluoro-4-(1-hexyl)-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4d). 1-Iodohexane was used as an electrophile, and chromatography (4:1 hexane/EtOAc) of the crude product gave **4d** (71%): FABMS (negative) m/z 468 (M − H$^-$).

Ethyl 4-Bromo-3,5-difluoro-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4e). 1,2-Dibromoethane was used as an electrophile, and chromatography (4:1 hexane/EtOAc) of the crude product gave **4e** (49%): FABMS (negative) m/z 464, 462 (M − H$^-$).

Ethyl 3,5-Difluoro-4-(methylthio)-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4f). Dimethyl disulfide was used as an electrophile (89%): FABMS (negative) m/z 430 (M − H$^-$).

Ethyl 3,5-Difluoro-4-formyl-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4i). *N*,*N*-Dimethylformamide was used as an electrophile, and chromatography (4:1 hexane/EtOAc) of the crude product gave **4i** (54%): FABMS m/z 414 (M + H$^+$).

Ethyl 3,5-Difluoro-4-(hydroxymethyl)-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4j). To a solution of **4i** (22.3 g, 54.0 mmol) in MeOH (260 mL) was added $NaBH_4$ (1.02 g, 27.0 mmol) at 0 °C. The reaction mixture was stirred for 1 h, and water was added. The mixture was extracted with EtOAc twice, and the organic layer was washed with brine to afford **4j** (22.0 g, 98%): FABMS m/z 416 (M + H$^+$).

Ethyl 4-[(*tert*-Butyldimethylsilyloxy)methyl]-3,5-difluoro-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4k). To a solution of **4j** (213 mg, 0.500 mmol) in CH_2Cl_2 (5 mL) were added imidazole (68 mg, 1.0 mmol) and *tert*-butyldimethylsilyl chloride (302 mg, 2.0 mml). The reaction mixture was refluxed for 30 min. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, and the residue was purified by chromatography (4:1 hexane/EtOAc) to afford **4k** (256 mg, 98%): FABMS m/z 528 (M + H$^+$).

Ethyl 3,5-Difluoro-4-hydroxy-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4g). To a solution of diisopropylamine (7.00 mL, 50.0 mmol) in THF (14 mL) was added *n*-butyllithium (1.6 M in hexane, 30 mL) at −10 to 0 °C under argon atmosphere. The reaction mixture was cooled to below −60 °C, and a solution of compound **3** (7.70 g, 20.0 mmol) in THF (60 mL) was added dropwise. After 2 h of stirring, trimethylborate (2.7 mL, 24 mmol) was added, and the reaction mixture was warmed to 0 °C during 20 min. Acetic acid (4.0 mL) and 30% H_2O_2 (8.0 mL) were added, and the mixture was stirred for 20 h at room temperature; 10% $NaHSO_3$ was added, and the mixture was washed with EtOAc. The mixture was acidified with 2 N HCl and extracted with EtOAc. The organic layer was washed with brine, and the residue was triturated with hexane to afford **4g** (4.33 g, 54%): FABMS (negative) m/z 400 (M − H$^-$).

Ethyl 3,5-Difluoro-4-(methoxymethoxy)-6-(pivaloylamino)-2-(2-tetrahydropyranyloxy)benzoate (4h). To a solution of **4g** (4.23 g, 10.5 mmol) in CH_2Cl_2 (50 mL) were added diisopropylethylamine (2.4 mL, 13.7 mmol) and chlo-

Table 5. ^{1}H NMR of the C-7 Substituents of Compounds **6**

compd	^{1}H NMR (δ, ppm) in $CDCl_3$
6b	1.35 (t, J = 7.5 Hz, 3H), 2.7−3.1 (m, 2H)
6c	0.96 (t, J = 6.3 Hz, 3H), 1.2−1.9 (m, 4H), 2.7−3.0 (m, 2H)
6d	0.8−1.0 (m, 3H), 1.1−1.8 (m, 8H), 2.7−3.0 (m, 2H)
6f	2.66 (t, J = 1.3 Hz, 3H)
6g	3.23 (s, 3H)
6h	3.43 (s, 3H)
6i	10.9 (br s, 1H)
6j	4.22 (t, J = 1.8 Hz, 3H)
6k	2.36 (s, 6H), 2.79 (t, J = 5.5 Hz, 2H), 4.48 (t, J = 5.5 Hz, 2H)
6l	4.6−4.7 (m, 2H)[a]
6o	6.58 (br s, 2H)[a]
6p	3.12 (t, J = 2.6 Hz, 6H)
6q	1.43 (t, J = 7.0 Hz, 3H), 4.49 (q, J = 7.0 Hz, 2H)

[a] The solvent was DMSO-d_6.

romethyl methyl ether (0.96 mL, 12.6 mmol) at 0 °C, and the reaction mixture was stirred for 30 min. Water was added, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, and the residue was triturated with hexane to afford **4h** (4.33 g, 93%): FABMS (negative) m/z 444 ($M - H^-$).

Typical Procedure for the Preparation of Compounds 6a−f,i,l: 6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-methyl-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6a). To a refluxing suspension of NaH (27.7 g, 693 mmol) which was washed with hexane three times in 1,4-dioxane/toluene (1:1, 300 mL) under argon atmosphere was added dropwise a solution of **4a** (99.8 g, 250 mmol) and **5** (50.0 g, 210 mmol) in the above solvent (800 mL). The reaction mixture was refluxed for 2 h and was cooled on an ice bath. Water was added, the mixture was extracted twice with EtOAc, and the organic layer was washed with brine. The combined extracts were concentrated and dissolved in EtOH (800 mL). Concentrated HCl (200 mL) was added, and the reaction mixture was stirred for 14 h at room temperature. Water was added, and the precipitated product was collected by filtration. Recrystallization from $CHCl_3$ (1250 mL)/hexane (500 mL) afforded **6a** (50.3 g, 49%). The mother liquid was concentrated and recrystallized from $CHCl_3$ (300 mL)/hexane (200 mL) to afford additional **6a** (12.0 g, 21%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.36 (s, 9H, $C(CH_3)_3$), 1.38 (s, 9H, $C(CH_3)_3$), 2.41 (t, J = 2.2 Hz, 3H, 7-CH_3), 6.64 (s, 1H, 3-H), 7.5−7.9 (m, 3H, 2′,6′-H and 4′-NH), 8.59 (t, J = 8.4 Hz, 1H, 5′-H), 10.5 (br s, 1H, 5-NH); EIMS m/z 488 (M^+). NMR data of compounds **6** are listed in Table 5.

6,8-Difluoro-7-ethyl-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6b). This compound was obtained from **4b** and **5** in a similar manner as described for **6a** (41%): EIMS m/z 502 (M^+).

7-(1-Butyl)-6,8-difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6c). This compound was obtained from **4c** and **5** in a similar manner as described for **6a** (36%): EIMS m/z 530 (M^+).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-(1-hexyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6d). This compound was obtained from **4d** and **5** in a similar manner as described for **6a** (43%): EIMS m/z 558 (M^+).

7-Bromo-6,8-difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6e). This compound was obtained from **4e** and **5** in a similar manner as described for **6a** (57%): FABMS m/z 555, 553 ($M + H^+$).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-(methylthio)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6f). This compound was obtained from **4f** and **5** in a similar manner as described for **6a** (58%): EIMS m/z 520 (M^+).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-(methylsulfinyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6g). To a solution of **6f** (204 mg, 0.392 mmol) in CH_2Cl_2 (5 mL) was added *m*-chloroperbenzoic acid (86 mg, 0.39 mmol) at 0 °C, and the reaction mixture was stirred for 2 h; 10% aqueous $NaHSO_3$ was added, and the mixture was extracted with $CHCl_3$. The organic layer was washed with aqueous $NaHCO_3$, H_2O, and brine. Chromatography (100:1 $CHCl_3$/MeOH) gave **6g** (200 mg, 95%): EIMS m/z 536 (M^+).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-(methylsulfonyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6h). To a solution of **6f** (203 mg, 0.391 mmol) in CH_2Cl_2 (5 mL) was added *m*-chloroperbenzoic acid (850 mg, 3.91 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h; 10% aqueous $NaHSO_3$ was added, and the mixture was extracted with $CHCl_3$. The organic layer was washed with aqueous $NaHCO_3$, H_2O, and brine. Chromatography (100:1 $CHCl_3$/MeOH) gave **6h** (204 mg, 94%): EIMS m/z 552 (M^+).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-hydroxy-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6i). This compound was obtained from **4h** and **5** in a similar manner as described for **6a** (26%): EIMS m/z 490 (M^+).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-methoxy-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6j). To a solution of **6i** (348 mg, 0.710 mmol) in acetone (35 mL) were added K_2CO_3 (166 mg, 1.20 mmol) and iodomethane (0.45 mL, 7.1 mmol), and the reaction mixture was refluxed for 40 min. The mixture was filtered, and to the filtrate were added water and EtOAc. The organic layer was washed with 1 N NaOH, H_2O, and brine. Chromatography ($CHCl_3$) gave **6j** (280 mg, 78%): EIMS m/z 504 (M^+).

6,8-Difluoro-7-[2-(dimethylamino)ethoxy]-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6k). To a solution of **6i** (490 mg, 1.00 mmol) in DMF (40 mL) were added K_2CO_3 (3.60 g, 26.0 mmol) and 2-(dimethylamino)ethyl chloride hydrochloride (2.88 g, 20.0 mmol), and the reaction mixture was stirred at 50 °C for 12 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. To the residue were added water and EtOAc. The organic layer was washed with H_2O and brine. Chromatography (30:1 $CHCl_3$/MeOH) gave **6k** (230 mg, 41%): EIMS m/z 561 (M^+).

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-(hydroxymethyl)-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6l). This compound was obtained from **4k** and **5** in a similar manner as described for **6a** (40%): EIMS m/z 504 (M^+).

Typical Procedure for the Preparation of Compounds 6m,n,q: 7-Chloro-6,8-difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6m). To a solution of diisopropylamine (1.54 mL, 11.0 mmol) was added *n*-butyllithium (1.6 M in hexane, 6.3 mL) at −10 to 0 °C under argon atmosphere. The reaction mixture was cooled to below −60 °C, and a solution of compound **8** (1.19 g, 2.50 mmol) in THF (50 mL) and HMPA (10 mL) was added dropwise. After 2 h of stirring, *N*-chlorosuccinimide (802 mg, 6.00 mmol) was added, and the reaction mixture was stirred for 10 min. Water was added, and the mixture was warmed to room temperature. The mixture was extracted with EtOAc, and the organic layer was washed with brine. Chromatography (60:1 $CHCl_3$/acetone) gave **6m** (384 mg, 30%): EIMS m/z 511, 509 (M^+).

7-Azido-6,8-difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6n). *p*-Toluenesulfonylazide[17] was used as an electrophile, and the precipitated product after adding water to the reaction mixture was collected by filtration to afford **6n** (81%): IR (KBr) 2140 cm^{-1}; FABMS (negative) m/z 514 ($M - H^-$).

6,8-Difluoro-7-(ethoxycarbonyl)-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6q). Ethyl chloroformate was used as an electrophile, and chromatography (40:1 $CHCl_3$/EtOAc) gave **6q** (66%): EIMS m/z 546 (M^+).

7-Amino-6,8-difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6o). To a solution of **6n** (3.50 g, 6.80 mmol) in THF (120 mL) was added PPh_3 (1.96 g, 7.48 mmol), and the reaction mixture was stirred for 2.5 h at room temperature; 1 N HCl (50 mL) was added, and the mixture was further stirred for 10 h. The pH was adjusted to 9 with 10 N NaOH, and the precipitated

product was collected by filtration. Chromatography (40:1 $CHCl_3$/MeOH) gave **6o** (2.83 g, 85%): EIMS *m/z* 489 (M^+).

6,8-Difluoro-7-(dimethylamino)-2-[3-fluoro-4-(pivaloylamino)phenyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (6p). To a solution of **6o** (510 mg, 1.04 mmol) in DMF (15 mL) were added NaH (212 mg, 5.30 mmol) and iodomethane (0.17 mL, 2.6 mmol) at 0 °C, and the reaction mixture was stirred for 2.5 h. Water was added, and the mixture was extracted with $CHCl_3$. The organic layer was washed with water and brine. The residue was purified by chromatography (100:1 $CHCl_3$/acetone) to afford **6p** (290 mg, 54%): EIMS *m/z* 517 (M^+).

Typical Procedure for the Preparation of Compounds 7a−e: 5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-methyl-4*H*-1-benzopyran-4-one (7a). A mixture of **6a** (800 mg, 1.64 mmol), 1,4-dioxane (50 mL), and HCl (25 mL) was refluxed for 2 h. The mixture was poured into ice−water and was basified with 10 N NaOH. The mixture was extracted with $CHCl_3$, and the organic layer was washed with brine. Chromatography (40:1 $CHCl_3$/MeOH) followed by recrystallization from EtOAc/hexane gave **7a** (183 mg, 35%): IR (KBr) 1654 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 2.33 (t, J = 2.0 Hz, 3H, 7-CH_3), 4.17 (br s, 2H, 4′-NH_2), 6.12 (br s, 2H, 5-NH_2), 6.45 (s, 1H, 3-H), 6.84 (t, J = 8.4 Hz, 1H, 5′H), 7.5−7.6 (m, 2H, 2′,6′H); EIMS *m/z* 320 (M^+). Anal. ($C_{16}H_{11}F_3N_2O_2$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-ethyl-4*H*-1-benzopyran-4-one (7b). This compound was obtained from **6b** in a similar manner as described for **7a** (44%): IR (KBr) 1658 cm^{-1}; EIMS *m/z* 334 (M^+). Anal. ($C_{17}H_{13}F_3N_2O_2$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-7-(1-butyl)-6,8-difluoro-4*H*-1-benzopyran-4-one (7c). This compound was obtained from **6c** in a similar manner as described for **7a** (47%): IR (KBr) 1653 cm^{-1}; EIMS *m/z* 362 (M^+). Anal. ($C_{19}H_{17}F_3N_2O_2$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(1-hexyl)-4*H*-1-benzopyran-4-one (7d). This compound was obtained from **6d** in a similar manner as described for **7a** (53%): IR (KBr) 1655 cm^{-1}; EIMS *m/z* 390 (M^+). Anal. ($C_{21}H_{21}F_3N_2O_2$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-7-bromo-6,8-difluoro-4*H*-1-benzopyran-4-one (7e). This compound was obtained from **6e** in a similar manner as described for **7a** (38%): IR (KBr) 1649 cm^{-1}; EIMS *m/z* 386, 384 (M^+). Anal. ($C_{15}H_8BrF_3N_2O_2$) C, H; N: calcd, 7.27; found, 6.74.

Typical Procedure for the Preparation of Compounds 7f−q: 5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(methylthio)-4*H*-1-benzopyran-4-one (7f). 6f (511 mg, 0.983 mmol) in H_2SO_4 (20 mL) was stirred at 50 °C for 10 min. The reaction mixture was poured into ice−water and neutralized with 10 N NaOH. The precipitated product was collected by filtration. Chromatography ($CHCl_3$) followed by recrystallization from EtOAc/hexane gave **7f** (309 mg, 89%): IR (KBr) 1641 cm^{-1}; EIMS *m/z* 352 (M^+). Anal. ($C_{16}H_{11}F_3N_2O_2S$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(methylsulfinyl)-4*H*-1-benzopyran-4-one (7g). This compound was obtained from **6g** in a similar manner as described for **7f** (89%): IR (KBr) 1635 cm^{-1}; FABMS *m/z* 369 (M + H^+). Anal. ($C_{16}H_{11}F_3N_2O_3S$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(methylsulfonyl)-4*H*-1-benzopyran-4-one (7h). This compound was obtained from **6h** in a similar manner as described for **7f** (64%): IR (KBr) 1637 cm^{-1}; FABMS *m/z* 385 (M + H^+). Anal. ($C_{16}H_{11}F_3N_2O_4S\cdot 0.6H_2O$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-hydroxy-4*H*-1-benzopyran-4-one (7i). This compound was obtained from **6i** in a similar manner as described for **7f** (52%): IR (KBr) 1651 cm^{-1}; EIMS *m/z* 322 (M^+). Anal. ($C_{15}H_9F_3N_2O_3$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-methoxy-4*H*-1-benzopyran-4-one (7j). This compound was obtained from **6j** in a similar manner as described for **7f** (78%): IR (KBr) 1647 cm^{-1}; EIMS *m/z* 336 (M^+). Anal. ($C_{16}H_{11}F_3N_2O_3$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[2-(dimethylamino)ethoxy]-4*H*-1-benzopyran-4-one Hydrochloride (7k). This compound was obtained from **6k** in a similar manner as described for **7f** (97%). To a solution of the free base of **7k** (170 mg) in 2-PrOH (10 mL) were added HCl (1 N in 2-PrOH, 0.5 mL) and 2-Pr_2O (5 mL). The precipitated hydrochloride was collected: IR (KBr) 1651 cm^{-1}; EIMS *m/z* 393 (M^+). Anal. ($C_{19}H_{18}F_3N_3O_3\cdot$HCl) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-hydroxy-4*H*-1-benzopyran-4-one (7l). This compound was obtained from **6l** in a similar manner as described for **7f** (60%): IR (KBr) 3500, 3350, 1657 cm^{-1}; EIMS *m/z* 336 (M^+). Anal. ($C_{16}H_{11}F_3N_2O_3$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-7-chloro-6,8-difluoro-4*H*-1-benzopyran-4-one (7m). This compound was obtained from **6m** in a similar manner as described for **7f** (73%): IR (KBr) 1653 cm^{-1}; EIMS *m/z* 342, 340 (M^+). Anal. ($C_{15}H_8ClF_3N_2O_2$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-7-azido-6,8-difluoro-4*H*-1-benzopyran-4-one (7n). This compound was obtained from **6n** in a similar manner as described for **7f** (84%): IR (KBr) 2135, 1653 cm^{-1}; EIMS *m/z* 347 (M^+). Anal. ($C_{15}H_8F_3N_5O_2$) C, H, N.

2-(4-Amino-3-fluorophenyl)-5,7-diamino-6,8-difluoro-4*H*-1-benzopyran-4-one (7o). This compound was obtained from **6o** in a similar manner as described for **7f** (69%): IR (KBr) 1662 cm^{-1}; FABMS *m/z* 322 (M + H^+). Anal. ($C_{15}H_{10}F_3N_3O_2$) C, H; N: calcd, 13.08; found, 12.62.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(dimethylamino)-4*H*-1-benzopyran-4-one (7p). This compound was obtained from **6p** in a similar manner as described for **7f** (97%): IR (KBr) 1653 cm^{-1}; EIMS *m/z* 349 (M^+). Anal. ($C_{17}H_{14}F_3N_3O_2$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(ethoxycarbonyl)-4*H*-1-benzopyran-4-one (7q). This compound was obtained from **6q** in a similar manner as described for **7f** (50%): IR (KBr) 1726, 1637 cm^{-1}; EIMS *m/z* 378 (M^+). Anal. ($C_{18}H_{13}F_3N_2O_4$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-7-carboxy-6,8-difluoro-4*H*-1-benzopyran-4-one (7r). To a suspension of **7q** (121 mg, 0.320 mmol) in EtOH (10 mL) and MeOH (5 mL) was added 5 N NaOH (0.4 mL), and the reaction mixture was stirred for 2.5 h at 50 °C. The mixture was cooled on an ice bath, and the pH was adjusted to 4 with HCl. The precipitated product was collected by filtration to afford **7r** (101 mg, 90%): IR (KBr) 1734, 1624 cm^{-1}; FABMS *m/z* 351 (M + H^+). Anal. ($C_{16}H_9F_3N_2O_4\cdot 0.3H_2O$) C, H, N.

Typical Procedure for the Preparation of Compounds 9a−d,f: [5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl Acetate (9a). A mixture of **6l** (6.02 g, 11.9 mmol), AcOH (160 mL), and H_2SO_4 (40 mL) was stirred at 100 °C for 35 min. The reaction mixture was cooled on an ice bath and poured into ice−water. The mixture was extracted with EtOAc, and the organic layer was washed with 1 N NaOH twice, water, and brine. The residue was purified by chromatography (100:1 $CHCl_3$/MeOH) followed by recrystallization from $CHCl_3$/MeOH/hexane to afford **9a** (2.43 g, 54%): IR (KBr) 1738, 1622 cm^{-1}; FABMS *m/z* 379 (M + H^+). Anal. ($C_{18}H_{13}F_3N_2O_4$) C, H, N.

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl Propionate (9b). This compound was obtained in a similar manner as described for **9a** except that propionic acid was used instead of AcOH and the product was purified by chromatography (20:1 $CHCl_3$/MeCN) (32%): IR (KBr) 1728, 1651 cm^{-1}; EIMS *m/z* 392 (M^+). Anal. ($C_{19}H_{15}F_3N_2O_4$) C, H, N.

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl Hexanoate (9c). This compound was obtained in a similar manner as described for **9a** except that hexanoic acid was used instead of AcOH and the product was purified by chromatography (20:1 $CHCl_3$/MeCN) followed by recrystallization from EtOAc/hexane

(55%): IR (KBr) 1728, 1651 cm^{-1}; EIMS *m/z* 392 (M^+). Anal. ($C_{19}H_{15}F_3N_2O_4$) C, H, N.

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl Chloroacetate (9d). This compound was obtained in a similar manner as described for **9a** except that chloroacetic acid was used instead of AcOH and the crude product was triturated with isopropyl ether (97%): ^1H NMR (90 MHz, DMSO-d_3) δ 4.40 (s, 2H, $ClCH_2$), 5.36 (s, 2H, 7-CH_2), 6.00 (br s, 2H, 4′-NH_2), 6.66 (s, 1H, 3-H), 6.87 (t, J = 8.9 Hz, 1H, 5′-H), 7.06 (br s, 2H, 5-NH_2), 7.5−7.7 (m, 2H, 2′,6′-H); FABMS *m/z* 415, 413 ($M + H^+$).

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl 3-Bromopropionate (9f). This compound was obtained in a similar manner as described for **9a** except that 3-bromopropionic acid was used instead of AcOH and the crude product was purified by chromatography (20:1 $CHCl_3$/MeCN) (35%): ^1H NMR (90 MHz, $CDCl_3$) δ 2.97 (t, J = 6.8 Hz, 2H, $COCH_2$), 3.58 (t, J = 6.8 Hz, 2H, $BrCH_2$), 5.36 (t, J = 1.5 Hz, 2H, 7-CH_2), 6.49 (s, 1H, 3-H), 6.83 (t, J = 8.7 Hz, 1H, 5′−H), 7.4−7.7 (m, 2H, 2′,6′-H); FABMS *m/z* 473, 471 ($M + H^+$).

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl (Dimethylamino)acetate (9e). To a solution of **9d** (1.50 g, 3.63 mmol) in DMF (30 mL) were added dimethylamine hydrochloride (1.48 g, 18.2 mmol) and K_2CO_3 (2.50 g, 18.2 mmol), and the reaction mixture was stirred at 50 °C for 30 min. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine. The residue was recrystallized from EtOAc/hexane to afford the free base of **9e** (1.18 g, 77%). To a solution of the free base in EtOAc was added 1 N HCl in 2-PrOH, and the precipitated hydrochloride was collected by filtration: IR (KBr) 1753, 1653 cm^{-1}; FABMS *m/z* 422 ($M+H^+$). Anal. ($C_{20}H_{18}F_3N_3O_4 \cdot HCl \cdot 0.4H_2O$) C, H, N.

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl 3-(Dimethylamino)propionate (9g). This compound was obtained in a similar manner as described for **9e** (86%) and converted into the hydrochloride: IR (KBr) 1738, 1622 cm^{-1}; FABMS *m/z* 436 ($M + H^+$). Anal. ($C_{21}H_{20}F_3N_3O_4 \cdot HCl \cdot 0.3H_2O$) C, H, N.

2-[4-[(Allyloxycarbonyl)amino]-3-fluorophenyl]-5-amino-6,8-difluoro-7-(hydroxymethyl)-4*H*-1-benzopyran-4-one (10). To a solution of **7l** (6.72 g, 20.0 mmol) in pyridine (200 mL) was added allyl chloroformate (10.6 mL, 100 mmol), and the reaction mixture was stirred at room temperature for 1.5 h. Water was added, and the precipitated product was collected by filtration. This was dissolved in EtOH (500 mL), and 2 N NaOH (18 mL) was added. The mixture was stirred at room temperature for 1.5 h. Water was added, and the precipitated product was collected by filtration to afford **10** (6.92 g, 82%): ^1H NMR (90 MHz, DMSO-d_6) δ 4.5−4.7 (m, 4H, 7-CH_2 and OCH_2), 5.1−5.5 (m, 2H, CH=CH_2), 5.7−6.2 (m, 1H, CH=CH_2), 6.90 (s, 1H, 3-H), 7.01 (br s, 2H, 5-NH_2), 7.7−8.0 (m, 3H, 2′,5′,6′-H), 9.79 (br s, 1H, 4′-NH); FABMS *m/z* 421 ($M + H^+$).

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl Decanoate (9h). To a solution of **10** (420 mg, 1.00 mmol) in DMF (20 mL) were added Et_3N (1.4 mL, 10 mmol) and decanoyl chloride (1.3 mL, 6.0 mmol), and the reaction mixture was stirred at room temperature for 7 h. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, and the residue was purified by chromatography (4:1 $CHCl_3$/hexane) to afford a decanoate of **10** (258 mg, 45%): ^1H NMR (90 MHz, $CDCl_3$) δ 0.7−1.0 (m, 3H, CH_3), 1.0−1.8 (m, 14H, $(CH_2)_7CH_3$), 2.35 (t, J = 6.4 Hz, 2H, $COCH_2$), 4.72 (d, J = 5.5 Hz, 2H, OCH_2), 5.2−5.5 (m, 2H, CH=CH_2), 5.30 (s, 2H, 7-CH_2), 5.7−6.2 (m, 1H, CH=CH_2), 6.58 (s, 1H, 3-H), 7.09 (br d, J = 2.6 Hz, 1H, 4′-NH), 7.5−7.7 (m, 2H, 2′,6′-H), 8.31 (t, J = 8.6 Hz, 1H, 5′-H); FABMS *m/z* 575 ($M + H^+$). To a solution of the decanoate (249 mg, 0.434 mmol) in THF (20 mL) were added HCO_2H-Et_3N (0.285 mL, 2.17 mmol) and $Pd(PPh_3)_4$ (51 mg, 0.044 mmol), and the mixture was stirred under argon atmosphere at room temperature for 50 min. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, and the residue was purified by preparative TLC (20:1 $CHCl_3$/MeOH) followed by recrystallization from EtOAc/hexane to afford **9h** (125 mg, 59%): IR (KBr) 1743, 1659 cm^{-1}; FABMS *m/z* 491 ($M + H^+$). Anal. ($C_{26}H_{29}F_3N_2O_4$) C, H, N.

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl Stearoate (9i). This compound was obtained in a similar manner as described for **9h** except that stearoyl chloride was used instead of decanoyl chloride (two steps, 29%): IR (KBr) 1738, 1657 cm^{-1}; FABMS *m/z* 603 ($M + H^+$). Anal. ($C_{34}H_{45}F_3N_2O_4$) C, H, N.

[5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-4*H*-4-oxo-1-benzopyran-7-yl]methyl 4-(Dimethyamino)butyrate (9j). To a solution of 4-(dimethylamino)butyric acid hydrochloride (3.99 g, 23.8 mmol) in DMF (50 mL) was added 1,1′-carbonyldiimidazole (3.86 g, 23.8 mmol), and the mixture was stirred at 80 °C for 2.5 h. Compound **10** (1.00 g, 2.38 mmol) was added, and the mixture was further stirred for 2 h. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, and the residue was purified by chromatography (9:1 $CHCl_3$/MeOH) to afford the 4-(dimethylamino)butyrate of **10** (1.27 g, 100%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.6−2.0 (m, 2H, $COCH_2CH_2$), 2.1−2.5 (m, 4H, $COCH_2CH_2CH_2$), 2.23 (s, 6H, $N(CH_3)_2$), 4.72 (d, J = 5.7 Hz, 2H, OCH_2), 5.2−5.5 (m, 2H, CH=CH_2), 5.30 (s, 2H, 7-CH_2), 5.7−6.2 (m, 1H, CH=CH_2), 6.23 (br s, 2H, 5-NH_2), 6.56 (s, 1H, 3-H), 7.10 (br s, 1H, 4′-NH), 7.5−7.7 (m, 2H, 2′,6′-H), 8.31 (t, J = 7.9 Hz, 5′-H); FABMS *m/z* 534 ($M + H^+$). To a solution of the ester (1.27 g, 2.38 mmol) in THF (20 mL) were added HCO_2H-Et_3N (1.6 mL, 12 mmol) and $Pd(PPh_3)_4$ (275 mg, 0.24 mmol), and the mixture was stirred under argon atmosphere at room temperature for 19 h. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, and the residue was purified by chromatography (9:1 $CHCl_3$/MeOH) followed by recrystallization from EtOAc/hexane to afford the free base of **9h** (567 mg, 53%). This was converted into the hydrochloride in a similar manner as described for **9e**: IR (KBr) 1732, 1624 cm^{-1}; FABMS *m/z* 450 ($M + H^+$). Anal. ($C_{22}H_{22}F_3N_3O_4 \cdot HCl$) C, H, N.

6,8-Difluoro-2-[3-fluoro-4-(pivaloylamino)phenyl]-7-[(methylsulfonyloxy)methyl]-5-(pivaloylamino)-4*H*-1-benzopyran-4-one (11). To a solution of **6l** (3.30 g, 6.55 mmol) in DMF (70 mL) were added Et_3N (4.6 mL) and methanesulfonyl chloride (1.0 mL, 13 mmol), and the reaction mixture was stirred at room temperature for 10 min. Water was added, and the mixture was extracted with EtOAc twice. The organic layer was washed with water and brine. Evaporation of the solvent gave **11** (3.57 g, 94%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.36 (s, 9H, $C(CH_3)_3$), 1.38 (s, 9H, $C(CH_3)_3$), 3.10 (s, 3H, CH_3S), 5.48 (br s, 2H, 7-CH_2), 6.69 (s, 1H, 3-H), 7.5−7.9 (m, 3H, 2′,6′-H and 4′-NH), 8.61 (t, J = 8.4 Hz, 1H, 5′-H), 10.4 (br s, 1H, 5-NH); FABMS *m/z* 583 ($M + H^+$).

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(methoxymethyl)-4*H*-1-benzopyran-4-one (12a). A mixture of **11** (800 mg, 1.37 mmol) and MeOH (200 mL) was refluxed for 24 h, and the solvent was removed under reduced pressure. To the residue was added H_2SO_4 (15 mL), and the mixture was stirred at 50 °C for 15 min. The reaction mixture was poured into ice−water, and the pH was adjusted to 9 with 10 N NaOH. The mixture was extracted with EtOAc, and the organic layer was washed with water and brine. The residue was purified by chromatography (9:1 $CHCl_3$/MeCN) and recrystallized from EtOAc to afford **12a** (267 mg, two steps, 56%): IR (KBr) 1655 cm^{-1}; EIMS *m/z* 350 (M^+). Anal. ($C_{17}H_{13}F_3N_2O_3$) C, H, N

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[(propyloxy)methyl]-4*H*-1-benzopyran-4-one (12b). A mixture of **11** (582 mg, 1.00 mmol) and 1-propanol (100 mL) was stirred at 100 °C for 2 h, and the solvent was removed under reduced pressure. To the residue was added H_2SO_4 (30 mL), and the mixture was stirred at 100 °C for 15 min. The reaction mixture was poured into ice−water, and the pH was adjusted

to 7 with 10 N NaOH. The precipitated product was collected by filtration, purified by chromatography (100:1 $CHCl_3$/MeOH), and recrystallized from EtOAc/hexane to afford **12b** (286 mg, two steps, 76%): IR (KBr) 1626 cm^{-1}; FABMS *m/z* 379 ($M + H^+$). Anal. ($C_{19}H_{17}F_3N_2O_3$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[(hexyloxy)methyl-4*H*-1-benzopyran-4-one (12c). This compound was obtained in a similar manner as described for **12b** except that 1-hexanol was used instead of 1-propanol (two steps, 79%): IR (KBr) 1655 cm^{-1}; FABMS *m/z* 421 ($M + H^+$). Anal. ($C_{22}H_{23}F_3N_2O_3$) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[[2-(dimethylamino)ethoxy]methyl]-4*H*-1-benzopyran-4-one (12d). To a solution of **11** (800 mg, 1.38 mmol) in DMF (20 mL) were added 2-(dimethylamino)ethanol (0.28 mL, 2.8 mmol) and NaH (220 mg, 5.50 mmol) at 0 °C under argon atmosphere. The reaction mixture was stirred for 2.5 h at room temperature. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine. The residue was purified by chromatography (9:1 $CHCl_3$/MeOH) to afford a (dimethylamino)ethyl ether of **11** (477 mg, 60%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.36 (s, 9H, $C(CH_3)_3$), 1.38 (s, 9H, $C(CH_3)_3$), 2.35 (s, 6H, $N(CH_3)_2$), 2.63 (t, J = 5.7 Hz, 2H, CH_2N), 3.71 (t, J = 5.7 Hz, 2H, CH_2O), 4.79 (br s, 2H, 7-CH_2), 6.67 (s, 1H, 3-H), 7.6−7.9 (m, 3H, 2′,6′-H and 4′-NH), 8.62 (t, J = 8.4 Hz, 1H, 5′-H), 10.5 (s, 1H, 5-NH); FABMS *m/z* 576 ($M + H^+$). A mixture of the above ether (454 mg, 0.790 mmol) and H_2SO_4 (10 mL) was stirred at 50 °C for 15 min. The reaction mixture was poured into ice−water, and the pH was adjusted to 7 with 10 N NaOH. The precipitated product was collected by filtration and purified by chromatography (20:1:1 $CHCl_3$/MeOH/NH_3)[18] to afford the free base of **12d** (266 mg, 83%). This was converted into the hydrochloride in a similar manner as described for **9e**: IR (KBr) 1655 cm^{-1}; FABMS *m/z* 408 ($M + H^+$). Anal. ($C_{20}H_{20}F_3N_3O_3$·HCl·0.6H_2O) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[[3-(dimethylamino)propyloxy]methyl]-4*H*-1-benzopyran-4-one (12e). This compound was obtained in a similar manner as described for **12d** except that 3-(dimethylamino)-1-propanol was used instead of 2-(dimethylamino)ethanol (two steps, 4.6%): IR (KBr) 1653 cm^{-1}; FABMS *m/z* 422 ($M + H^+$). Anal. ($C_{21}H_{22}F_3N_3O_3$·HCl·1.6H_2O) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl]-7-(aminomethyl)-6,8-difluoro-4*H*-1-benzopyran-4-one (12f). To a solution of **11** (1.12 g, 1.91 mmol) in DMF (20 mL) was added sodium diformylamide (219 mg, 2.31 mmol),[19] and the reaction mixture was stirred at room temperature for 5 h. Water was added, and the precipitated product was collected to afford the 7-(diformylamino)methyl derivative (1.06 g, 99%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.37 (s, 18H, $C(CH_3)_3$ × 2), 5.08 (br s, 2H, 7-CH_2), 6.67 (s, 1H, 3-H), 7.6−7.8 (m, 2H, 2′,6′-H), 7.83 (d, J = 4.0 Hz, 1H, 4′-NH), 8.62 (t, J = 8.4 Hz, 1H, 5′-H), 8.94 (s, 2H, CHO × 2), 10.5 (s, 1H, 5-NH); FABMS *m/z* 560 ($M + H^+$). A mixture of the above compound (1.01 g, 1.81 mmol), EtOH (20 mL), and HCl (20 mL) was refluxed for 2.5 h and poured into ice−water. The precipitated product was collected by filtration and recrystallized from DMF/H_2O (9:1) to afford the free base of **12f** (404 mg, 67%). The free base was suspended in MeOH (15 mL). To the suspension were added 4 N HCl in EtOAc (0.67 mL) and isopropyl ether. The precipitated hydrochloride was collected by filtration: IR (KBr) 1660 cm^{-1}; FABMS *m/z* 336 ($M + H^+$). Anal. ($C_{16}H_{12}F_3N_3O_2$·2HCl) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[(hexanoylamino)methyl]-4*H*-1-benzopyran-4-one (12g). To a solution of **12f** (285 mg, 0.851 mmol) in DMF (20 mL) were added Et_3N (0.24 mL, 1.7 mmol) and hexanoyl chloride (0.20 mL, 1.4 mmol) at −50 °C. The reaction mixture was gradually warmed to 0 °C. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine. The residue was purified by chromatography (100:1 $CHCl_3$/MeOH) and trituration with isopropyl ether to afford **12g** (106 mg, 29%): IR (KBr) 1657, 1647 cm^{-1}; FABMS *m/z* 434 ($M + H^+$). Anal. ($C_{22}H_{22}F_3N_3O_3$·0.2 H_2O) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-[(dimethylamino)methyl]-4*H*-1-benzopyran-4-one (12h). To a solution of **11** (800 mg, 1.38 mmol) in DMF (10 mL) were added dimethylamine hydrochloride (558 mg, 6.85 mmol) and K_2CO_3 (945 mg, 6.85 mmol), and the reaction mixture was stirred at room temperature for 45 min. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine. The solvent was removed under reduced pressure to afford the 7-(dimethylamino)methyl derivative (727 mg, 100%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.36 (s, 9H, $C(CH_3)_3$), 1.38 (s, 9H, $C(CH_3)_3$), 2.36 (s, 6H, $N(CH_3)_2$), 3.81 (br s, 2H, 7-CH_2), 6.67 (s, 1H, 3-H), 7.5−7.8 (m, 3H, 2′,6′-H, and 4′-NH), 8.59 (t, J = 8.4 Hz, 1H, 5′-H), 10.4 (br s, 1H, 5-NH); FABMS *m/z* 532 ($M + H^+$). This compound (715 mg, 1.35 mmol) was treated with H_2SO_4 as in the case of **7f** and purified by chromatography (9:1 $CHCl_3$/MeOH) followed by recrystallization from MeOH/EtOH to afford the free base of **12h** (360 mg, 73%), which was converted into the hydrochloride in the similar manner as described for **12f**: IR (KBr) 1654 cm^{-1}; FABMS *m/z* 364 ($M + H^+$). Anal. ($C_{18}H_{16}F_3N_3O_2$·HCl·0.5H_2O) C, H, N.

5-Amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-(trimethylammoniumyl)-4*H*-1-benzopyran-4-one (12i). To a solution of **12h** (349 mg, 0.961 mmol) in THF (20 mL) was added iodomethane (0.30 mL, 4.8 mmol), and the reaction mixture was stirred at 40 °C for 30 min. The mixture was cooled on an ice bath, and the precipitated product was collected by filtration to afford **12i** (468 mg, 96%): IR (KBr) 1657 cm^{-1}; FABMS *m/z* 378 ($M + H^+$). Anal. ($C_{19}H_{19}F_3IN_3O_2$) C, H, N.

Cell Growth-Inhibitory Activity. Assays were conducted according to published method.[9]

In Vivo Antitumor Activity. Tumor fragments (8 mm^3) were transplanted subcutaneously in the flank of 7−9-week old 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/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, and 18, and the tumor volume were calculated according to the following equation:

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

The tumor volumes at initial administration (V_0) and on the day of judgment (V) were calculated, and the tumor growth rate (V/V_0) was calculated. The T/C values were obtained from the following equation:

$$\text{T/C (\%)} = (V/V_0 \text{ of treated group})/(V/V_0 \text{ of control group}) \times 100$$

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Supporting Information Available: Completely assigned ^{1}H NMR data of compounds **4**, **6**, **7**, **9**, and **12** and ^{1}H NMR spectra of compounds **7h**,**r**, **9e**,**g**, and **12d**,**e**,**g**,**h** (18 pages). Ordering information is given on any current masthead page.

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