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Synthesis and Biological Evaluation of CX-659S and its Related Compounds for their Inhibitory Effects on the Delayed-Type Hypersensitivity Reaction

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Abstract—In order to find novel nonsteroidal compounds possessing an inhibitory activity against delayed-type hypersensitivity (DTH) reactions, we conducted random screening using a picryl chloride (PC)-induced contact hypersensitivity reaction (CHR) in mice, and found compound **1** as a lead compound. Then we synthesized and evaluated an extensive series of 5-carboxamidouracil derivatives focused on both the uracil and the antioxidative moieties. Among them, we found that the hindered phenol moiety was necessary to exhibit the activities; especially, compounds **28a**–**28c** having the partial structure of vitamin E were found to exert potent activities against the DTH reaction by both oral and topical administration. And compound **28c** showed antioxidative activity against lipid peroxidation with an IC_{50} of 5.9 μM. Compound **28c** (CX-659S) was chosen as a candidate drug for the treatment of cutaneous disorders such as atopic dermatitis and allergic contact dermatitis.

Introduction

Recently, patients suffering from allergic cutaneous disorders such as atopic dermatitis and allergic contact dermatitis have been increasing in number. T cell-mediated delayed-type hypersensitivity (DTH) reactions are thought to be involved in these deseases.[1] Many antiallergic drugs such as anti-histamine agents and mast cell stabilizers are clinically used for the treatment of these types of dermatitis, but are not very efficacious against them, for these drugs are not effective against DTH reactions. Glucocorticoids have a suppressive effect against DTH reactions, and are widely used for the treatment of these disorders.[2,3] However, long-time use of glucocorticoids is limited because of its many side effects, such as atrophia cutis and infection. Therefore, nonsteroidal agents having an inhibitory activity against DTH reactions and a less toxicity are awaited.

On the other hand, there are both direct and indirect evidences implicating reactive oxygen species (ROS) such as superoxide and hydrogen peroxide in the pathogenesis of many inflammatory skin diseases.[4] ROS may be produced in the skin, for example, by ionizing or UV radiation under aerobic conditions,[5] or by infiltration of leukocytes.[6] Overproduction of ROS has also been postulated for immune-mediated skin diseases such as atopic dermatitis, contact dermatitis, and psoriasis. Monocytes or neutrophils of these patients show an increased capacity to release ROS.[7,8] Therefore, compounds having inhibitory activities on the production and/or scavenging of ROS may be effective against DTH reactions. For example, AD0261 (Fig. 1), a compound having antioxidative activities, was reported to show a suppressive effect on picryl chloride (PC)-induced contact hypersensitivity reaction (CHR) by topical administration.[9]

In a search for new compounds that have an inhibitory activity against DTH reactions, we found that compound **1**, containing the 3-methyl-1-phenyluracil moiety and the antioxidative di-*tert*-butyl phenol moiety in its structure (Fig. 1), inhibited PC-induced CHR in mice (54% inhibition at a dose of 10 mg/kg, po). However, the diarrhea was observed in mice administered **1**. Therefore, we performed studies to find more potent and lower toxic compounds than **1** by the modification and the optimization of both the uracil moiety and the antioxidative moiety.

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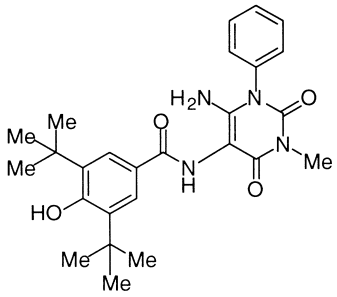

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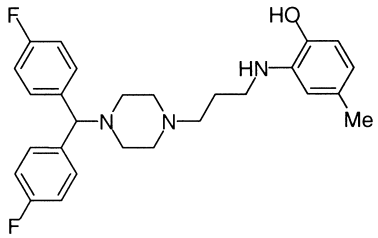


AD0261

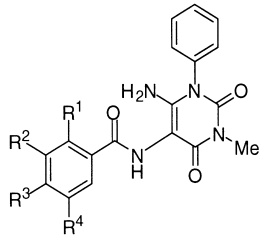
Figure 1. Structures of compound **1** and AD0261.

In this paper, we describe the synthesis and antiallergic activities against DTH reactions in mice of various novel uracil derivatives. In addition, we report the antioxidative activities of several of these compounds.

Chemistry

The compounds described in this study are shown in Tables 1–4 and their synthetic methods are outlined in Schemes 1–6. Synthesis of 1,3-disubstituted-5,6-diaminouracil derivatives was accomplished in the following way using the reported method (Scheme 1).[10,11] Reaction of appropriate amines with suitable isocyanates gave **2a**–**2d** in good yield. Compounds **3a**–**3d** were prepared by acylation of the respective **2a**–**2d** with cyanoacetic acid. Alkaline catalyzed cyclization of **3a**–**3d** afforded **4a**–**4d**, respectively. Nitrosation of **4a**–**4d** with sodium nitrite followed by catalytic hydrogenation of the nitroso group furnished **5a**–**5d**, respectively.

Table 1. Effects of compounds **1** and **7a**–**7i** on PC-induced CHR in mice by oral administration



Compound	R^1	R^2	R^3	R^4	% Inhibition[a] (10 mg/kg)
1	H	*t*-Bu	HO	*t*-Bu	54
7a	H	Me	AcO	Me	24
7b	H	Me	AcO	H	−1
7c	Me	AcO	H	H	24
7d	H	AcO	Me	H	4
7e	AcO	Me	H	H	−2
7f	AcO	*i*-Pr	H	H	−10
7g	H	AcO	AcO	H	34
7h	H	AcO	H	AcO	65**
7i	H	AcO	AcO	AcO	48
Prednisolone					85***

[a]Percent inhibition was calculated from the values of percent responses of drug-treated and control groups ($n = 6$). The results are expressed as the mean values of 6 animals

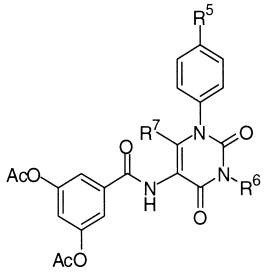
**$P < 0.01$

***$P < 0.001$ versus control (Dunnet's test).

Schemes 2 and 3 show the synthetic routes for the preparation of arylcarboxamide-type compounds. Since most of this type of compounds having a free phenolic hydroxyl group were unstable in air and were poorly soluble in organic solvent, the phenolic hydroxyl groups of these compounds were protected by the acetyl group. Acetylation of **6a**–**6i** with acetic anhydride in pyridine followed by condensation with **5a** by using diphenylphosphoryl chloride (DPP-Cl) as a condensation reagent provided **7a**–**7i** (Scheme 2). Coupling of 3,5-diacetoxybenzoic acid with the corresponding **5b**–**5d** in the presence of DPP-Cl gave **8a**, **8b** and **8d**, respectively (Scheme 3).

Modification of the amino group at the 6-position of the uracil ring is shown in Scheme 4. Compounds **9a** and **9b** were obtained from **4a** and **4d**, respectively, by heating the latter with concentrated HCl in acetic acid. Compounds **10a** and **10b** were prepared by chlorination of **9a** and **9b**, respectively, with phosphorus oxychloride as reported previously.[12] The substitution of **10a** and **10b** with methylamine or dimethylamine, respectively, yielded **11a** and **11b**. Compound **13** was obtained by the same

Table 2. Effects of compounds **8a**–**8e** on PC-induced CHR in mice by oral administration



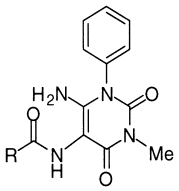
Compound	R^5	R^6	R^7	% Inhibition[a] (10 mg/kg)
7h	H	Me	NH_2	65**
8a	H	*n*-Pr	NH_2	11
8b	H	CH_2Ph	NH_2	21
8c	H	Me	NHMe	9
8d	F	Me	NH_2	46
8e	F	Me	NMe_2	18
Prednisolone				85***

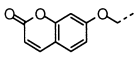
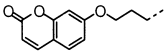
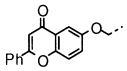
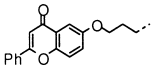
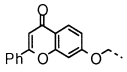
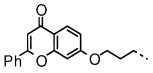
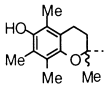
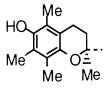
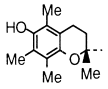
[a]Percent inhibition was calculated from the values of percent responses of drug-treated and control groups ($n = 6$). The results are expressed as the mean values of 6 animals

**$P < 0.01$

***$P < 0.001$ versus control (Dunnet's test).

Table 3. Effects of compounds **19a–19c, 24a–24d** and **28a–28c** on PC-induced CHR in mice by oral administration



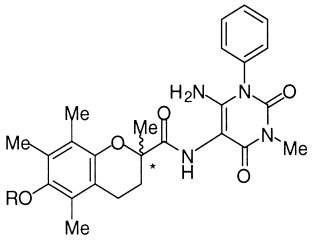
Compound	R	% Inhibition[a] (10 mg/kg)
19a		−36
19b		−32
19c		4
24a		8
24b		2
24c		−13
24d		13
28a		55*
28b		46*
28c (CX-659S)		48*
Prednisolone		85***

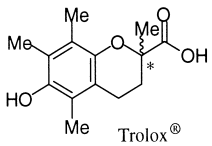
[a]Percent inhibition was calculated from the values of percent responses of drug-treated and control groups ($n=6$). The results are expressed as the mean values of six animals.
*$P<0.05$.
***$P<0.001$ versus control (Dunnet's test).

procedure used to prepare **5a–5d**. Treatment of **11b** with fuming nitric acid in concentrated sulfuric acid followed by catalytic hydrogenation provided **15**. Similar condensation of **13** and **15** with 3,5-diacetoxybenzoic acid described above afforded **8c** and **8e**, respectively.

Preparation of compounds containing coumarin or flavone structure is shown in Scheme 5. Alkylation of **16**, **21** and **25** was performed by the reaction with haloesters in the presence of sodium hydride. Compounds **17b**, **18b**, **22b**, **23b**, **26b** and **27b** were obtained by the hydrolysis of the corresponding esters. Coupling of **5a** and the carboxylic acids in the presence of *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) and

Table 4. Effects of compounds **28a–28d** on PC-induced CHR in mice by topical administration





Compound	R	*	Mg/site	% Inhibition[a]
28a	H	*RS*	0.03	6
			0.1	15
			0.3	52**
28b	H	*R*	0.03	21
			0.1	28
			0.3	65*
28c (CX-659S)	H	*S*	0.03	21
			0.1	35
			0.3	69*
28d	Me	*RS*	0.3	7
			1	33
Trolox®	—	*RS*	0.3	0
			1	18
(±)-α-tocopherol	—	—	0.3	−1
			1	−18
Prednisolone	—	—	0.1	77***

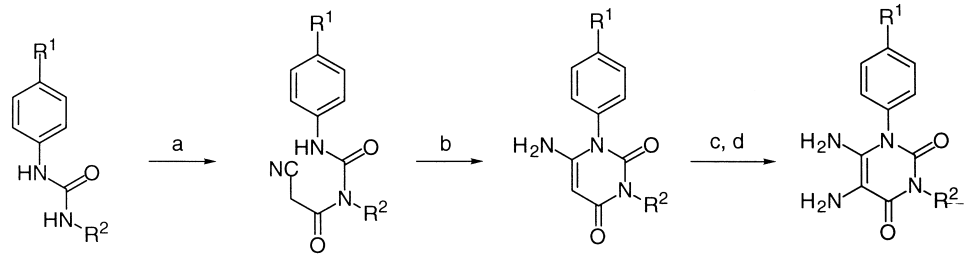
[a]Percent inhibition was calculated from the values of percent responses of drug-treated and control groups ($n=6$ or 8). The results are expressed as the mean values of 6–8 animals.
*$P<0.05$.
**$P<0.01$.
***$P<0.001$ versus control (Dunnet's test).

1-hydroxybenzotriazole (HOBt) provided compounds **19a–19c** and **24a–24d**.

Synthesis of compounds having chroman structure is shown in Scheme 6. Compounds **28a–28c** were prepared by the same procedure used for preparation of **19a–19c**. (*R*)-, (*S*)-, and (*RS*)-Trolox® were commercially available. Alkylation and esterification of Trolox® with methyl iodide using potassium carbonate as a base were conducted to afford **29**. Compound **29** was hydrolyzed to give **30** which was then coupled with **5a** to give **28d**.

Results and Discussion

We used the PC-induced CHR in mice as a typical DTH reaction model to evaluate the effect of the compounds.[13] In the initial step of our screening, we used oral administration of compounds at a dose of 10 mg/kg. During the course of the randomized screening test using this model, 6-amino-5-(3,5-di-*tert*-butyl-4-hydroxyphenyl) carboxamido-3-methyl-1-phenyluracil (**1**) was identified as a potent inhibitor, which gave a 54% inhibition of PC-induced CHR at a dose of 10 mg/kg. As the structure of **1** containing the di-*tert*-butylphenol moiety is well-known to have antioxidative activity,[14] we firstly conducted a structure–activity relationship study focused on the introduction of a substituent such as an alkyl or/and acetoxy group at the phenyl ring. The



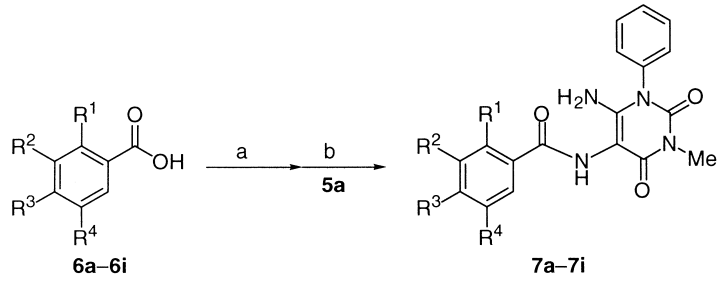
2a: R^1 = H, R^2 = Me
2b: R^1 = H, R^2 = *n*-Pr
2c: R^1 = H, R^2 = CH_2Ph
2d: R^1 = F, R^2 = Me

3a: R^1 = H, R^2 = Me
3b: R^1 = H, R^2 = *n*-Pr
3c: R^1 = H, R^2 = CH_2Ph
3d: R^1 = F, R^2 = Me

4a: R^1 = H, R^2 = Me
4b: R^1 = H, R^2 = *n*-Pr
4c: R^1 = H, R^2 = CH_2Ph
4d: R^1 = F, R^2 = Me

5a: R^1 = H, R^2 = Me
5b: R^1 = H, R^2 = *n*-Pr
5c: R^1 = H, R^2 = CH_2Ph
5d: R^1 = F, R^2 = Me

Scheme 1. Synthesis of compounds **5a**–**5d**. Reagents and conditions: (a) $NCCH_2COOH$, Ac_2O, AcOH, 80 °C; (b) 2 N NaOH; (c) $NaNO_2$, 5 N HCl; (d) H_2, Pd-C, EtOH, reflux.

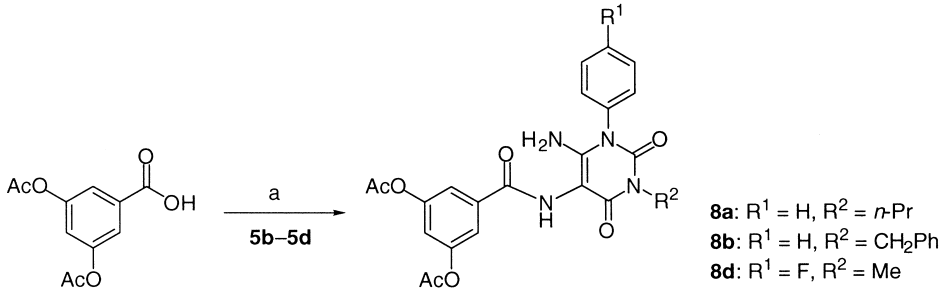


6a–6i **7a–7i**

6	R^1	R^2	R^3	R^4
a	H	Me	HO	Me
b	H	Me	HO	H
c	Me	HO	H	H
d	H	HO	Me	H
e	HO	Me	H	H
f	HO	*i*-Pr	H	H
g	H	HO	HO	H
h	H	HO	H	HO
i	H	HO	HO	HO

7	R^1	R^2	R^3	R^4
a	H	Me	AcO	Me
b	H	Me	AcO	H
c	Me	AcO	H	H
d	H	AcO	Me	H
e	AcO	Me	H	H
f	AcO	*i*-Pr	H	H
g	H	AcO	AcO	H
h	H	AcO	H	AcO
i	H	AcO	AcO	AcO

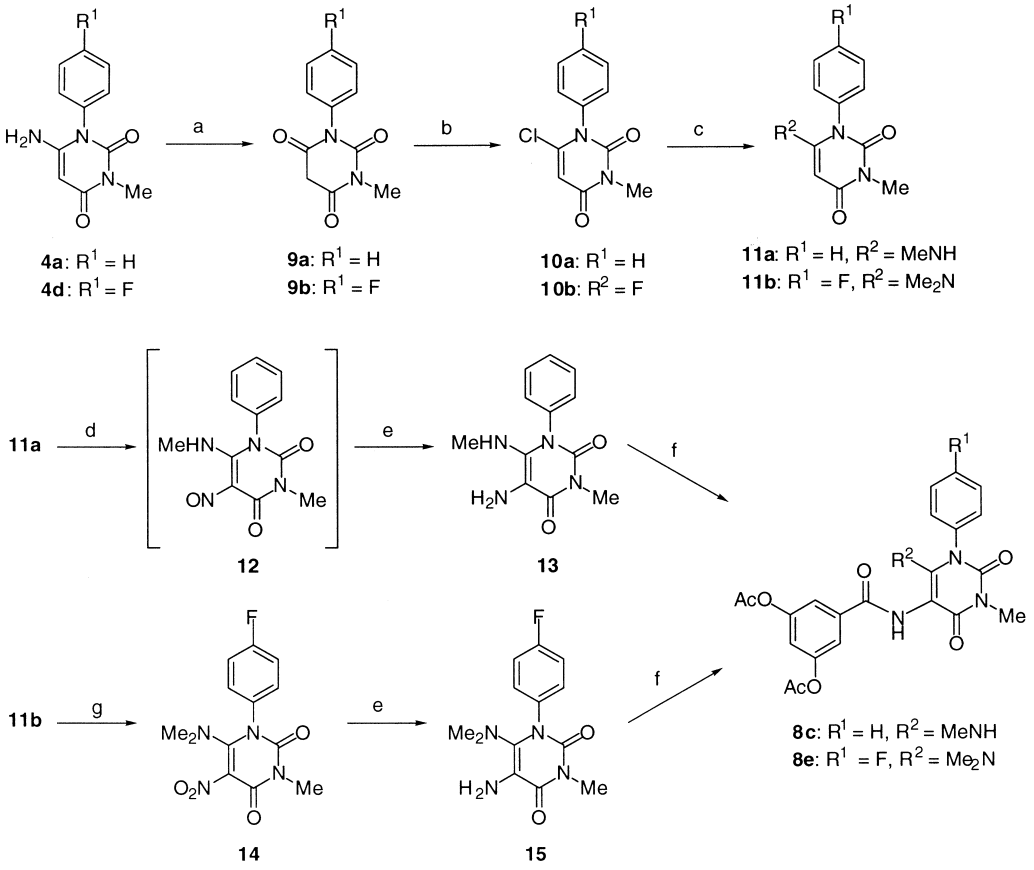
Scheme 2. Synthesis of compounds **7a**–**7i.** Reagents and conditions: (a) Ac_2O, pyridine, AcOEt; (b) DPP-Cl, triethylamine, CH_2Cl_2.



8a: R^1 = H, R^2 = *n*-Pr
8b: R^1 = H, R^2 = CH_2Ph
8d: R^1 = F, R^2 = Me

Scheme 3. Synthesis of compounds **8a**, **8b** and **8d.** Reagents and conditions: (a) DPP-Cl, triethylamine, CH_2Cl_2.

results are shown in Table 1. The inhibitory activities of compound **7a** and **7b**, having the 4-acetoxy-3,5-dimethylphenyl and the 4-acetoxy-3-methylphenyl moiety, respectively, in place of the 3,5-di-*tert*-butyl-4-hydroxyphenyl moiety of **1**, showed reduced activity. Comparing the activity of **1**, **7a**, and **7b**, we found that the deletion of bulky *tert*-butyl group was less effective. Both the *ortho* and the *meta* substituted derivatives **7c**–**7f** of the phenyl ring showed lowered activity. The role of the di-*tert*-butylphenol moiety as an antioxidant is well-known; i.e.

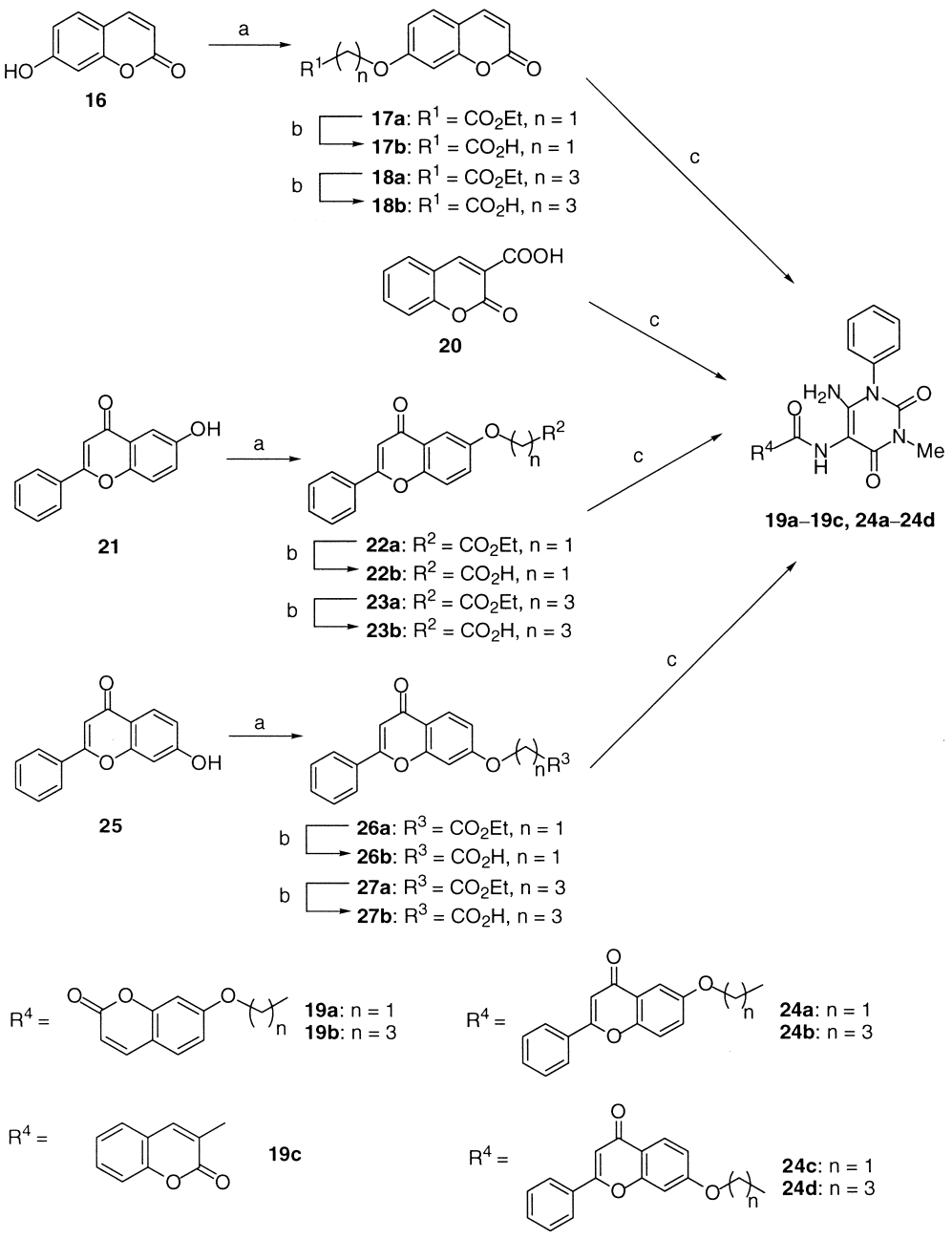


Scheme 4. Synthesis of compounds **8c** and **8e**. Reagents and conditions: (a) c-HCl, AcOH, 80 °C; (b) $POCl_3$, 80 °C; (c) 40% $MeNH_2$-H_2O or 40% Me_2NH-H_2O, *i*-PrOH, 70 °C; (d) $NaNO_2$, 5 N HCl; (e) H_2, Pd-C, EtOH, reflux; (f) 3,5-diacetoxybenzoic acid, DPP-Cl triethylamine, CH_2Cl_2; (g) concentrated H_2SO_4, fuming HNO_3.

that the hindered phenol plays the role of a scavenger for active oxygen[14] and the antioxidative activity is dependent on this hindrance.[15] Therefore, the reason for the behavior of **7a**–**7f** in this model may be thought to be reduced hindrance compared with the hindrance in compound **1**. Based on the above information and the present results, the antiallergic activity of the tested compounds may be related to the antioxidative activity due to the hindered phenol. Not only a monophenol like di-*tert*-butylphenol, but also polyphenols such as catechol,[16] resorcinol,[17,18] and pyrogallol[16,19,20] have been reported to be typical antioxidants. Then, several compounds, **7g**–**7i**, having these partial structures were further designed and synthesized. The inhibitory activities of compounds **7g**, **7h** and **7i**, corresponding to the respective catechol, resorcinol, and pyrogallol structures were 34, 65, and 48%, respectively. These results taken together suggest that the structures of di-*tert*-butylphenol and resorcinol were favorable to show the inhibitory activity. However, since the former moiety is reported to induce disorder of liver function,[21,22] we next conducted a structural optimization study on the uracil ring of **7h**.

As shown in Table 2, the substitution by *n*-propyl (**8a**) or benzyl (**8b**) group at the 3-position of the uracil ring resulted in decreased activity compared with the activity of **7h**. The change of the amino group to the methylamino (**8c**) or to the dimethylamino (**8e**) one at the 6-position of the uracil ring also resulted in a reduced antiallergic effect. These results suggest that the free amino group also had an important role in remaining activity. Moreover, the substitution of hydrogen to fluorine (**8d**) at the para position of the phenyl group of the uracil ring had a tendency to inhibit PC-induced CHR more weakly than **7h**. In the course of study, we confirmed that **7h** suppressed the DTH reaction very strongly; however, this compound was found to be unstable under the moisture condition (the deacetylation was observed).

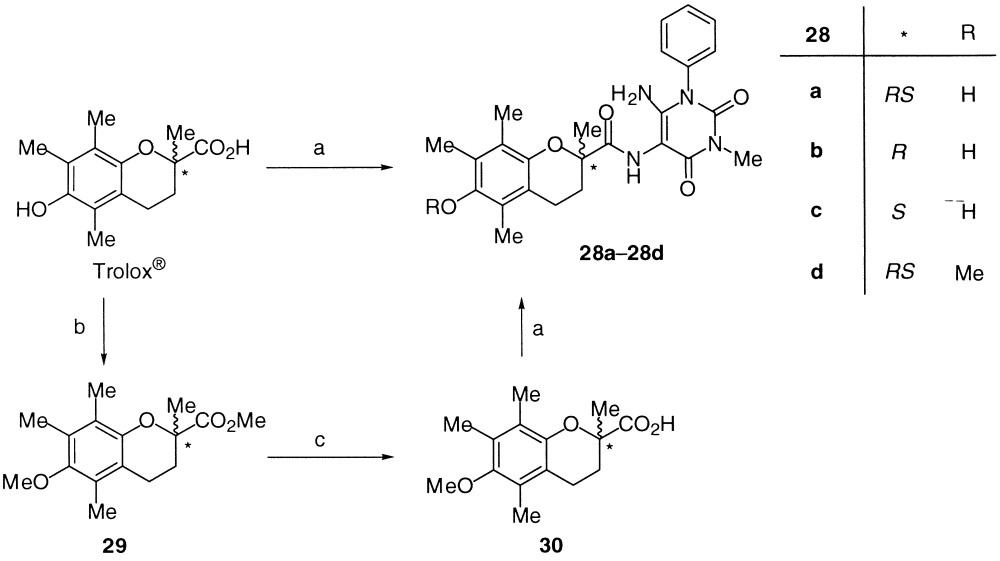
To further clarify the role of the antioxidative moiety in antiallergic activities and to find a stable structure, we designed and synthesized a series of compounds having coumarin,[23–25] flavone,[26–28] and chroman[29,30] structures. As shown in Table 3, the inhibitory activities of **19a**–**19c**, having the coumarin structure, were −36, −32, and 4%, respectively. Compounds **24a**–**24d**, having the flavone structure, showed drastically reduced activity. On the other hand, compounds **28a–28c,** chroman analogues, exerted inhibitory activities ranging from 46 to 55% inhibition and no significant difference in efficacy was observed between **28b** and **28c**. In regard to the physicochemical stability, compounds **28a**–**28c** were confirmed to be more stable than **7h** (data not shown). Then we next investigated the activity of the two stereoisomers, **28b** and



Scheme 5. Synthesis of compounds **19a**–**19c** and **24a**–**24d**. Reagents and conditions: (a) $Br(CH_2)_nCO_2Et$, NaH, DMF; (b) 3 N NaOH, EtOH; (c) **5a**, EDC·HCl, HOBt, DMF.

28c, applied topically to mice. As shown in Table 4, both **28b** and **28c** showed almost the same inhibitory activity against DTH reactions at the dose range from 0.03–0.3 mg/site. Whereas compound **28d**, in which the hydroxyl group of the chroman ring was blocked by a methoxy group, showed reduced activity, and Trolox® and (±)-α-tocopherol (vitamin E) also had drastically lowered activity. These findings suggest that the structure of uracil and chroman units might be an essential structure to exhibit the activities.

To further confirm the role of the antioxidative moiety against DTH reactions, we analyzed the inhibitory activity of **28c**, **28d**, and Trolox® on lipid peroxidation in rat brain homogenates in vitro (Fig. 2).[31,32] Compound **28c** was found to possess potent dose-dependent antioxidative activity, with an IC_{50} of 5.9 μM. The IC_{50} value of Trolox® was 17.4 μM, and **28d** had no effect. In the case of the Trolox® study, the failure of Trolox® to show strong inhibition of the DTH reaction in spite of the fact that the drug had a moderate antioxidative activity could considered to be the result of poor skin permeability, but the detail was not examined in the present study. From all these results, compound **28c** (CX-659S) has emerged as a novel nonsteroidal compound with potent antiallergic activities for both oral and topical administration. Further pharmacological studies are now under way.



28	*	R
a	RS	H
b	R	H
c	S	H
d	RS	Me

Scheme 6. Synthesis of compounds **28a**–**28d**. Reagents and conditions: (a) **5a**, EDC·HCl, HOBt, DMF; (b) MeI, K_2CO_3, DMF; (c) 3 N NaOH, MeOH.

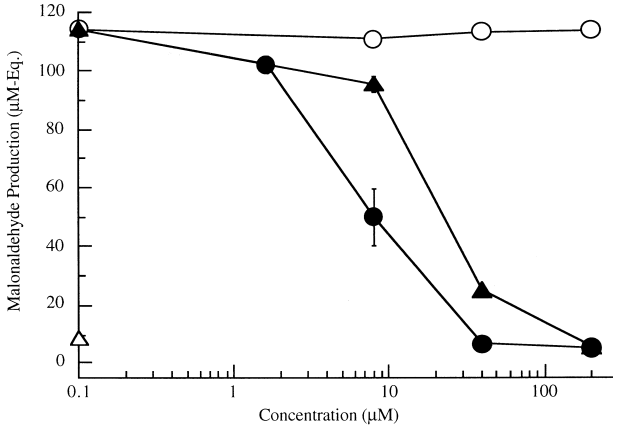


Figure 2. Inhibitory activities of **28c** (CX-659S) and its related compounds on lipid peroxidation in rat brain homogenate. The lipid peroxidation was measured as described in the text. The control level of lipid peroxidation was 114.4±1.5 mM equivalents of malondialdehyde. Each point represents the mean of triplicate experiments. ●, **28c** (CX-659S); ○, **28d**; ▲, Trolox®; △, endogenous.

Conclusion

We examined the structure activity relationship of 5-carboxamidouracil derivatives on the DTH reaction. Among them, compounds **28a**–**28c** had an inhibitory activity against PC-induced CHR in mice by both oral and topical administration. Compound **28c**, containing the partial structure of vitamin E, exhibited antioxidative activity on lipid peroxidation in the rat brain homogenates in vitro. Furthermore, no toxicity was observed by the single or repeated administration of **28c**.

6-Amino-5-[(*S*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido]-3-methyl-1-phenyluracil (**28c**), named CX-659S, is now being prepared for use in clinical studies in Japan as a drug for cutaneous disorders such as atopic dermatitis and allergic contact dermatitis.

Experimental

Chemistry

General. All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were measured with a BÜCHI 535 melting point apparatus and were uncorrected. Proton NMR spectra were recorded on a JEOL GSX270 FT NMR spectrometer. Chemical shifts were given in parts per million (ppm) using tetramethylsilane as the internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. TOF MS (time-of-flight mass spectrometry) were recorded on a Kompact MALDI 3 V4.0.0 spectrometer. Optical rotations were determined by using a JASCO DIP-370 digital polarimeter. Elemental analyses were performed at the Toray Research Center. Wakogel C-200 (Wako; 70–150 mm) was used for column chromatography. Monitoring of reactions was carried out using Merck 60 F_{254} silica gel, glass-supported TLC plates, and visualization with UV light (254 and 365 nM). Following abbreviations are used for reagents and solvents: DMF (*N,N*-dimethylformamide), DPP-Cl (diphenylphosphoryl chloride), EDC·HCl (*N*-ethyl-*N*′-[3-(dimethylamino)propyl] carbodiimide hydrochloride), EtOAc (ethyl acetate), HOBt (1-hydroxybenzotriazole).

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)carboxamido-3-methyl-1-phenyluracil (1). To a solution of 5,6-diamino-3-methyl-1-phenyluracil **5a** (200 mg, 0.86 mmol), 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (259 mg, 1.03 mmol), and HOBt (128 mg, 0.95 mmol) in DMF (4.3 mL) was added EDC·HCl (182 mg, 0.95 mmol) portionwise at ambient temperature under a nitrogen atmosphere. After 6 h of stirring, the reaction mixture was evaporated in vacuo. The residue was partitioned with CH_2Cl_2 and saturated aqueous Na_2CO_3 solution. The organic layer was washed with water three times, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH 50:1 elution) and recrystallized from

i-PrOH to give **1** (246 mg, 61.5%) as white crystals, mp 178 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 1.42 (18H, s), 3.15 (3H, s), 5.90 (2H, s), 7.33–7.55 (8H, m), 8.79 (1H, brs); MS (TOF) *m*/*z* 465 $(M+H)^+$. Anal. calcd for $C_{26}H_{32}N_4O_4 \cdot 0.5H_2O$: C, 65.94; H, 7.02; N, 11.83. Found: C, 65.85; H, 7.07; N, 11.79.

6-Amino-5-(3,5-diacetoxyphenyl)carboxamido-3-methyl-1-phenyluracil (7h). To a solution of 3,5-dihydroxybenzoic acid (1.85 g, 12.0 mmol) in EtOAc (27.8 mL) were added acetic anhydride (2.94 mL, 31.2 mmol) and pyridine (1.94 mL, 24.0 mmol) in an ice-water bath under a nitrogen atmosphere. The mixture was stirred on ice for 30 min and then stirred at ambient temperature for 3 h. To the reaction mixture was added 98% formic acid (680 μL, 14.4 mmol), and the mixture was stirred at ambient temperature for 1 h. Next, it was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was recrystallized from *n*-heptane-EtOAc to give 3,5-diacetoxybenzoic acid (2.04 g, 71.4%) as white crystals. To a suspension of 3,5-diacetoxybenzoic acid (1.05 g, 4.41 mmol) in CH_2Cl_2 (11 mL) were added triethylamine (737 μL, 5.29 mmol) and DPP-Cl (1.10 mL, 5.29 mmol) in an ice-water bath under a nitrogen atmosphere. After 1 h of stirring, to the mixture were added **5a** (931 mg, 4.01 mmol) and triethylamine (737 μL, 5.29 mmol) at the same temperature. The reaction mixture was stirred for another 4 h and was poured into water (15 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was crystallized from *i*-PrOH to give **7h** (1.15 g, 63.4%) as a white solid, mp 144 °C (Dec.); ^{1}H NMR ($CDCl_3$) δ 2.31 (6H, s), 3.38 (3H, s), 5.29 (2H, s), 7.13 (1H, s), 7.36–7.39 (2H, m), 7.53–7.60 (5H, m), 8.01 (1H, s); MS (TOF) *m*/*z* 453 $(M+H)^+$. Anal. calcd for $C_{22}H_{20}N_4O_7 \cdot 0.8H_2O$: C, 56.60; H, 4.66; N, 12.00. Found: C, 56.82; H, 4.64; N, 12.05.

5-(4-Acetoxy-3,5-dimethylphenyl)carboxamido-6-amino-3-methyl-1-phenyluracil (7a). The title compound was prepared from **5a** (430 mg, 1.85 mmol) and 4-acetoxy-3,5-dimethylbenzoic acid (424 mg, 2.04 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (458 mg, 58.6%), mp 176 °C (Dec.); ^{1}H NMR ($CDCl_3$) δ 2.21 (6H, s), 2.36 (3H, s), 3.39 (3H, s), 5.31 (2H, brs), 7.35–7.38 (2H, m), 7.54–7.62 (5H, m), 8.04 (1H, s); MS (TOF) *m*/*z* 423 $(M+H)^+$. Anal. calcd for $C_{22}H_{22}N_4O_5 \cdot 1.0H_2O$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.87; H, 5.49; N, 13.02.

5-(4-Acetoxy-3-methylphenyl)carboxamido-6-amino-3-methyl-1-phenyl-uracil (7b). The title compound was prepared from **5a** (375 mg, 1.61 mmol) and 4-acetoxy-3-methylbenzoic acid (345 mg, 1.78 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (214 mg, 32.4%), mp 142 °C (Dec.); ^{1}H NMR ($CDCl_3$) δ 2.25 (3H, s), 2.35 (3H, s), 3.40 (3H, s), 5.33 (2H, brs), 7.13 (1H, d, J = 8.4 Hz), 7.37–7.41 (2H, m), 7.56–7.64 (3H, m), 7.73–7.79 (2H, m), 8.03 (1H, s); MS (TOF) *m*/*z* 409 $(M+H)^+$. Anal. calcd for $C_{21}H_{20}N_4O_5 \cdot 0.7H_2O$: C, 59.91; H, 5.12; N, 13.31. Found: C, 59.78; H, 5.10; N, 13.53.

5-(3-Acetoxy-2-methylphenyl)carboxamido-6-amino-3-methyl-1-phenyl-uracil (7c). The title compound was prepared from **5a** (278 mg, 1.20 mmol) and 3-acetoxy-2-methylbenzoic acid (256 mg, 1.32 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (374 mg, 76.6%), mp 235–236 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 2.33 (3H, s), 3.27 (3H, s), 5.63 (2H, brs), 7.08–7.61 (8H, m), 8.78 (1H, s); MS (TOF) *m*/*z* 409 $(M+H)^+$. Anal. calcd for $C_{21}H_{20}N_4O_5 \cdot 0.1H_2O$: C, 61.49; H, 4.96; N, 13.66. Found: C, 61.33; H, 4.96; N, 13.92.

5-(3-Acetoxy-4-methylphenyl)carboxamido-6-amino-3-methyl-1-phenyl-uracil (7d). The title compound was prepared from **5a** (305 mg, 1.31 mmol) and 3-acetoxy-4-methylbenzoic acid (281 mg, 1.44 mmol) by a similar to that for **7h** and was crystallized from EtOH to give white solid (332 mg, 61.9%), mp 142 °C (Dec.); ^{1}H NMR ($CDCl_3$) δ 2.25 (3H, s), 2.34 (3H, s), 3.38 (3H, s), 5.31 (2H, brs), 7.34–7.67 (8H, m), 8.04 (1H, s); MS (TOF) *m*/*z* 409 $(M+H)^+$. Anal. calcd for $C_{21}H_{20}N_4O_5 \cdot 0.8H_2O$: C, 59.65; H, 5.15; N, 13.25. Found: C, 59.38; H, 5.13; N, 13.52.

5-(2-Acetoxy-3-methylphenyl)carboxamido-6-amino-3-methyl-1-phenyl-uracil (7e). The title compound was prepared from **5a** (250 mg, 1.08 mmol) and 2-acetoxy-3-methylbenzoic acid (230 mg, 1.18 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (252 mg, 57.3%), mp 235–236 °C; ^{1}H NMR ($CDCl_3$) δ 2.24 (3H, s), 2.43 (3H, s), 3.39 (3H, s), 5.18 (2H, brs), 7.27–7.60 (8H, m), 7.97 (1H, s); MS (TOF) *m*/*z* 409 $(M+H)^+$. Anal. calcd for $C_{21}H_{20}N_4O_5 \cdot 0.1H_2O$: C, 61.49; H, 4.96; N, 13.66. Found: C, 61.39; H, 5.03; N, 13.93.

5-(2-Acetoxy-3-*iso*-propylphenyl)carboxamido-6-amino-3-methyl-1-phenyluracil (7f). The title compound was prepared from **5a** (238 mg, 1.02 mmol) and 2-acetoxy-3-*iso*-propylbenzoic acid (251 mg, 1.13 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (204 mg, 45.6%), mp 145 °C (Dec.); ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, d, J = 7.3 Hz), 2.43 (3H, s), 3.07–3.12 (1H, m), 3.38 (3H, s), 5.13 (2H, brs), 7.29–7.68 (8H, m), 7.74 (1H, s); MS (TOF) *m*/*z* 437 $(M+H)^+$. Anal. calcd for $C_{23}H_{24}N_4O_5 \cdot 1.0H_2O$: C, 60.78; H, 5.77; N, 12.33. Found: C, 60.82; H, 5.71; N, 12.57.

6-Amino-5-(3,4-diacetoxyphenyl)carboxamido-3-methyl-1-phenyluracil (7g). The title compound was prepared from **5a** (140 mg, 0.60 mmol) and 3,4-diacetoxybenzoic acid (158 mg, 0.66 mmol) by a similar procedure as for **7h** and was crystallized from *i*-PrOH to give white solid (157 mg, 57.6%), mp 165 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 2.30 (3H, s), 2.32 (3H, s), 3.15 (3H, s), 6.13 (2H, s), 7.34–7.53 (3H, m), 7.55–7.58 (3H, m), 7.60–7.85 (1H, m), 7.86–7.95 (1H, m), 9.05 (1H, s); MS (TOF) *m*/*z* 453 $(M+H)^+$. Anal. calcd for $C_{22}H_{20}N_4O_7 \cdot 0.7H_2O$: C, 56.82; H, 4.64; N, 12.05. Found: C, 56.87; H, 4.67; N, 12.10.

6-Amino-3-methyl-1-phenyl-5-(3,4,5-triacetoxyphenyl)-carboxamidouracil (7i). The title compound was prepared from **5a** (222 mg, 0.96 mmol) and 3,4,5-triacetoxybenzoic acid (311 mg, 1.05 mmol) by a similar procedure as for **7h** and was crystallized from *i*-PrOH to give white

solid (242 mg, 49.6%), mp 189 °C (Dec.); ^{1}H NMR ($CDCl_3$) δ 2.30 (6H, s), 2.31 (3H, s), 3.38 (3H, s), 5.25 (2H, s), 7.36–7.39 (2H, m), 7.57–7.60 (3H, m), 7.67 (2H, s), 7.98 (1H, s); MS (TOF) m/z 511 $(M+H)^+$. Anal. calcd for $C_{24}H_{22}N_4O_9 \cdot 0.9H_2O$: C, 54.73; H, 4.55; N, 10.64. Found: C, 54.98; H, 4.43; N, 10.57.

6-Amino-5-(3,5-diacetoxyphenyl)carboxamido-1-phenyl-3-propyluracil (8a). The title compound was prepared from **5b** (300 mg, 1,15 mmol) and 3,5-diacetoxybenzoic acid (329 mg, 1.38 mmol) by a similar procedure as for **7h** and was recrystallized from EtOH to give white crystals (436 mg, 78.7%), mp 164 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, $J=7.4$ Hz), 1.49–1.55 (2H, m), 2.27 (6H, s), 3.72 (2H, t, $J=6.9$ Hz), 6.14 (2H, brs), 7.20–7.22 (1H, m), 7.34–7.37 (2H, m), 7.52–7.66 (5H, m), 9.07 (1H, s); MS (TOF) m/z 481 $(M+H)^+$. Anal. calcd for $C_{24}H_{24}N_4O_7 \cdot 0.6H_2O$: C, 58.67; H, 5.17; N, 11.40. Found: C, 58.61; H, 5.00; N, 11.15.

6-Amino-3-benzyl-5-(3,5-diacetoxyphenyl)carboxamido-1-phenyluracil (8b). The title compound was prepared from **5c** (320 mg, 1.04 mmol) and 3,5-diacetoxybenzoic acid (297 mg, 1.25 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (365 mg, 66.5%), mp 152 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 2.30 (6H, s), 4.96 (2H, s), 6.26 (2H, s), 7.24–7.66 (13H, m), 9.11 (1H, s); MS (TOF) m/z 529 $(M+H)^+$. Anal. calcd for $C_{28}H_{24}N_4O_7 \cdot 1.3H_2O$: C, 60.93; H, 4.86; N, 10.15. Found: C, 60.98; H, 4.56; N, 9.87.

5-(3,5-Diacetoxyphenyl)carboxamido-3-methyl-6-methylamino-1-phenyluracil (8c). The title compound was prepared from **13** (120 mg, 0.49 mmol) and 3,5-diacetoxybenzoic acid (139 mg, 0.59 mmol) by a similar procedure as for **7h** and was crystallized from *i*-PrOH to give white solid (126 mg, 55.4%), mp 212–213 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (6H, s), 2.62 (3H, d, $J=4.9$ Hz), 3.15 (3H, s), 5.41 (1H, q, $J=4.9$ Hz), 7.24–7.26 (1H, m), 7.33–7.37 (2H, m), 7.49–7.62 (5H, m), 9.30 (1H, s); MS (TOF) m/z 467 $(M+H)^+$. Anal. calcd for $C_{23}H_{22}N_4O_7$: C, 59.22; H, 4.75; N, 12.01. Found: C, 59.10; H, 4.78; N, 12.05.

6-Amino-5-(3,5-diacetoxyphenyl)carboxamido-1-(4-fluoropheny)-3-methyluracil (8d). The title compound was prepared from **5d** (155 mg, 0.65 mmol) and 3,5-diacetoxybenzoic acid (187 mg, 0.78 mmol) by a similar procedure as for **7h** and was crystallized from *i*-PrOH to give white solid (194 mg, 63.1%), mp 246–247 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (6H, s), 3.14 (3H, s), 6.26 (2H, brs), 7.20–7.22 (1H, m), 7.35–7.46 (4H, m), 7.65–7.66 (2H, m), 9.06 (1H, s); MS (TOF) m/z 471 $(M+H)^+$. Anal. calcd for $C_{22}H_{19}FN_4O_7 \cdot 1.3H_2O$: C, 53.51; H, 4.41; N, 11.35. Found: C, 53.52; H, 4.04; N, 11.26.

5-(3,5-Diacetoxyphenyl)carboxamido-6-dimethylamino-1-(4-fluoropheny)-3-methyluracil (8e). The title compound was prepared from **15** (100 mg, 0.36 mmol) and 3,5-diacetoxybenzoic acid (103 mg, 0.43 mmol) by a similar procedure as for **7h** and was crystallized from EtOH to give white solid (84 mg, 46.9%), mp 260–261 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (6H, s), 2.40 (6H, s), 3.19 (3H, s), 7.26–7.33 (3H, m), 7.43–7.47 (2H, m), 7.64 (2H, s), 9.52 (1H, s); MS (TOF) m/z 499 $(M+H)^+$. Anal. calcd for $C_{24}H_{23}FN_4O_7 \cdot 0.3H_2O$: C, 57.21; H, 4.72; N, 11.12. Found: C, 57.21; H, 4.65; N, 11.04.

Ethyl 4-[(4-oxo-4*H*-1-benzopyran-2-phenyl-6-yl)oxy]butyrate (23a). To a solution of 6-hydroxycoumarin (1.00 g, 4.20 mmol) in DMF (15 mL) was added 60% sodium hydride in oil (235 mg, 5.88 mmol) portionwise in an ice-water bath under a nitrogen atmosphere. The mixture was stirred at the same temperature for 30 min, and ethyl 4-bromobutyrate (721 μL, 5.04 mmol) was then added portionwise to it. After 30 min of stirring, the mixture was stirred at ambient temperature for an additional 3 h. The reaction mixture was evaporated in vacuo, and the residue was neutralized with 1N aqueous HCl and extracted with CH_2Cl_2 twice. The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2:*n*-hexane 20:1 elution) and recrystallized from EtOH–H_2O to give **23a** (920 mg, 66.2%) as white crystals, mp 102–103 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, $J=7.3$ Hz), 2.14–2.19 (2H, m), 2.54 (2H, t, $J=7.3$ Hz), 4.10–4.21 (4H, m), 6.82 (1H, s), 7.27–7.31 (1H, m), 7.50–7.59 (5H, m), 7.91–7.95 (2H, m); MS (TOF) m/z 353 $(M+H)^+$. Anal. calcd for $C_{21}H_{20}O_5$: C, 71.58; H, 5.72. Found: C, 71.51; H, 5.77.

4-[(4-Oxo-4*H*-1-benzopyran-2-phenyl-6-yl)oxy]butyric acid (23b). A solution of **23a** (800 mg, 2.27 mmol) in EtOH (7.0 mL) containing 3N aqueous NaOH solution (3.2 mL) was heated at 60 °C for 1 h. Upon cooling, the reaction mixture was adjusted to pH 4 with 1N aqueous HCl and diluted with water. The precipitate was collected by vacuum filtration, washed with EtOH and water, and dried to give **23b** (508 mg, 69.0%) as a pale yellow solid, mp 206–207 °C; ^{1}H NMR (DMSO-d_6) δ 1.99–2.04 (2H, m), 2.42 (2H, t, $J=7.3$ Hz), 4.11 (2H, t, $J=6.3$ Hz) 7.04 (1H, s), 7.43–7.46 (2H, m), 7.57–7.61 (3H, m), 7.75–7.79 (1H, m), 8.10–8.13 (2H, m), 12.25 (1H, brs); MS (TOF) m/z 325 $(M+H)^+$. Anal. calcd for $C_{19}H_{16}O_5 \cdot 0.2H_2O$: C, 69.59; H, 5.04. Found: C, 69.62; H, 5.11.

2-[(4-Oxo-4*H*-1-benzopyran-2-phenyl-6-yl)oxy]acetic acid (22b). The title compound was prepared from ethyl 2-[(4-oxo-4*H*-1-benzopyran-2-phenyl-6-yl)oxy]acetate **22a** (680 mg, 2.10 mmol) by a procedure similar to that used for **23b** and was crystallized from EtOH to give **22b** (366 mg, 58.8%) as a pale yellow solid, mp 254–255 °C; ^{1}H NMR (DMSO-d_6) δ 4.83 (2H, s), 7.05 (1H, s), 7.37 (1H, d, $J=3.5$ Hz), 7.45–7.65 (4H, m), 7.79 (1H, d, $J=8.6$ Hz), 8.10–8.13 (2H, m), 13.16 (1H, brs); MS (TOF) m/z 297 $(M+H)^+$. Anal. calcd for $C_{17}H_{12}O_5 \cdot 0.15H_2O$: C, 68.29; H, 4.15. Found: C, 68.30; H, 4.03.

4-[(4-Oxo-4*H*-1-benzopyran-2-phenyl-7-yl)oxy]butyric acid (27b). The title compound was prepared from ethyl 4-[(4-oxo-4*H*-1-benzopyran-2-phenyl-7-yl)oxy]butyrate **27a** (720 mg, 2.04 mmol) by a procedure similar to that used for **23b** and was crystallized from EtOH to give (419 mg, 63.3%) as a pale yellow solid, mp 240–241 °C; ^{1}H NMR (DMSO-d_6) δ 1.96–2.06 (2H, m), 2.43 (2H, t, $J=7.3$ Hz), 4.17 (2H, t, $J=6.3$ Hz), 6.98 (1H, s), 7.05–7.09 (1H, m), 7.35 (1H, d, $J=2.2$ Hz), 7.58–7.61 (3H, m), 7.95 (1H, d,

$J = 8.6$ Hz), 8.09–8.12 (2H, m), 12.25 (1H, brs); MS (TOF) m/z 325 $(M+H)^+$. Anal. calcd for $C_{19}H_{16}O_5 \cdot 0.15H_2O$: C, 69.78; H, 5.02. Found: C, 69.79; H, 5.09.

Compounds **19a**–**19c** and **24a**–**24d** were also prepared by using the same procedure as for **1**.

6-Amino-3-methyl-5-[2-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy] acetylamino]-phenyluracil (19a). White solid (55.9%), mp 234 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 3.14 (3H, s), 4.75 (2H, s), 6.13 (2H, br s), 6.30 (1H, d, $J = 8.6$ Hz), 7.03–7.06 (2H, m), 7.32–7.35 (2H, m), 7.54–7.66 4H, m), 7.99 (1H, d, $J = 8.6$ Hz), 8.69 (1H, s); MS (TOF) m/z 435 $(M+H)^+$. Anal. calcd for $C_{22}H_{18}N_4O_6 \cdot 0.02H_2O$: C, 60.78; H, 4.18; N, 13.19. Found: C, 60.78; H, 4.18; N, 12.89.

6-Amino-3-methyl-5-[4-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy] butanoyl-amino]-1-phenyluracil (19b). White solid (66.4%), mp 231 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 1.99–2.04 (2H, m), 2.43 (2 H, t, $J = 7.4$ Hz), 3.13 (3H, s), 4.15 (2H, t, $J = 6.3$ Hz), 5.93 (2H, s), 6.29 (1H, d, $J = 9.7$ Hz), 6.95–7.01 (2H, m), 7.32–7.35 (2H, m), 7.52–7.65 (4H, m), 8.00 (1H, d, $J = 9.7$ Hz), 8.43 (1H, s); MS (TOF) m/z 463 $(M+H)^+$. Anal. calcd for $C_{24}H_{22}N_4O_6 \cdot 0.2H_2O$: C, 61.85; H, 4.84; N, 12.02. Found: C, 61.85; H, 5.05; N, 12.11.

6-Amino-3-methyl-5-[4-(2-oxo-2*H*-1-benzopyran-3-yl)carboxamido]-1-phenyluracil (19c). Yellow solid (79.4%), mp 289–290 °C; ^{1}H NMR (DMSO-d_6) δ 3.41 (3H, s), 5.19 (2H, s), 7.36–7.45 (2H, m), 7.55–7.60 (3H, m), 7.66–7.71 (4H, m), 8.87 (1H, s), 10.56 (1H, s); MS (TOF) m/z 405 $(M+H)^+$. Anal. calcd for $C_{21}H_{16}N_4O_5$: C, 62.37; H, 3.99; N, 13.86. Found: C, 62.29; H, 3.98; N, 14.21.

6-Amino-3-methyl-5-[2-[(4-oxo-4*H*-1-benzopyran-2-phenyl-6-yl)oxy]acetylamino]-1-phenyluracil (24a). White solid (58.6%), mp 288 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 3.15 (3H, s), 4.76 (2H, s), 6.10 (2H, s), 7.04 (1H, s), 7.34–7.45 (2H, m), 7.53–7.62 (8H, m), 7.80–7.83 (1H, m), 8.10–8.13 (2H, m), 8.74 (1H, s); MS (TOF) m/z 511 $(M+H)^+$. Anal. calcd for $C_{28}H_{22}N_4O_6 \cdot 1.2H_2O$: C, 63.20; H, 4.62; N, 10.53. Found: C, 63.03; H, 4.42; N, 10.36.

6-Amino-3-methyl-5-[4-[(4-oxo-4*H*-1-benzopyran-2-phenyl-6-yl)oxy]butanoyl-amino]-1-phenyluracil (24b). White solid (64.6%), mp 224–225 °C; ^{1}H NMR (DMSO-d_6) δ 2.02–2.07 (2H, m), 2.44–2.50 (2H, m), 3.13 (3H, s), 4.16 (2H, $J = 6.5$ Hz), 5.93 (2H, s), 7.03–7.04 (1H, m), 7.32–7.61 (10H, m), 7.74–7.79 (1H, m), 8.10–8.13 (2H, m), 8.44 (1H, s); MS (TOF) m/z 539 $(M+H)^+$. Anal. calcd for $C_{30}H_{26}N_4O_6 \cdot 2H_2O$: C, 62.71; H, 5.26; N, 9.75. Found: C, 62.58; H, 5.25; N, 9.59.

6-Amino-3-methyl-5-[2-[(4-oxo-4*H*-1-benzopyran-2-phenyl-7-yl)oxy]acetylamino]-1-phenyluracil (24c). White solid (61.0%), mp 214 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 3.18 (3H, s), 4.83 (2H, s), 6.17 (2H, s), 7.00 (1H, s), 7.08–7.21 (1H, m), 7.34–7.37 (2H, m), 7.46–7.61 (7H, m), 7.96–7.99 (1H, m), 8.16–8.29 (2H, m), 8.85 (1H, s); MS (TOF) m/z 511 $(M+H)^+$. Anal. calcd for $C_{28}H_{22}N_4O_6 \cdot 0.6H_2O$: C, 64.51; H, 4.49; N, 10.75. Found: C, 64.31; H, 4.60; N, 10.66.

6-Amino-3-methyl-5-[4-[(4-oxo-4*H*-1-benzopyran-2-phenyl-7-yl)oxy]butanoyl-amino]-1-phenyluracil (24d). White solid (52.7%), mp 245–246 °C; ^{1}H NMR (DMSO-d_6) δ 2.01–2.12 (2H, m), 2.44–2.50 (2H, m), 3.13 (3H, s), 4.24 (2H, $J = 6.3$ Hz), 5.95 (2H, s), 6.98 (1H, s), 7.07–7.11 (1H, m), 7.33–7.38 (3H, m), 7.50–7.62 (6H, m), 7.92–7.97 (1H, m), 8.09–8.12 (2H, m), 8.46 (1H, s); MS (TOF) m/z 539 $(M+H)^+$. Anal. calcd for $C_{30}H_{26}N_4O_6 \cdot 0.25H_2O$: C, 66.35; H, 4.92; N, 10.32. Found: C, 66.35; H, 4.90; N, 10.35.

Compounds **28a**–**28d** were also prepared by using the same procedure as for **1**.

6-Amino-5-[(*RS*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido]3-methyl-1-phenyluracil (28a). White solid (63.7%), mp 209–210 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (3H, s), 1.90–2.04 (1H, m), 2.08 (3H, s), 2.18 (3H, s), 2.29 (3H, s), 2.30–2.38 (1H, m), 2.54–2.64 (2H, m), 3.34 (3H, s), 4.32 (1H, s), 5.17 (2H, brs), 7.27–7.36 (2H, m), 7.53–7.60 (3H, m), 8.41 (1H, s); MS (TOF) m/z 465 $(M+H)^+$. Anal. calcd for $C_{25}H_{28}N_4O_5$: C, 64.64; H, 6.08; N, 12.08. Found: C, 64.75; H, 6.03; N, 12.37.

6-Amino-5-[(*R*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido]3-methyl-1-phenyluracil (28b). White solid (60.2%), mp 206–207 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (3H, s), 1.90–1.99 (1H, m), 2.06 (3H, s), 2.17 (3H, s), 2.28 (3H, s), 2.31–2.39 (1H, m), 2.53–2.65 (2H, m), 3.34 (3H, s), 4.50 (1H, s), 5.15 (2H, s), 7.27–7.34 (2H, m), 7.52–7.58 (3H, m), 8.39 (1H, s); $[\alpha]_D^{20}$ +53.9° ($c = 2.0$, $CHCl_3$); MS (TOF) m/z 465 $(M+H)^+$. Anal. calcd for $C_{25}H_{28}N_4O_5$: C, 64.64; H, 6.08; N, 12.08. Found: C, 64.58; H, 6.18; N, 12.02.

6-Amino-5-[(*S*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido]3-methyl-1-phenyluracil (28c). White solid (64.8%), mp 206–207 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (3H, s), 1.93–1.99 (1H, m), 2.07 (3H, s), 2.17 (3H, s), 2.28 (3H, s), 2.31–2.39 (1H, m), 2.57–2.65 (2H, m), 3.34 (3H, s), 4.94 (1H, s), 5.15 (2H, s), 7.27–7.34 (2H, m), 7.52–7.55 (3H, m), 8.39 (1H, s); $[\alpha]_D^{20}$ −53.9° ($c = 2.0$, $CHCl_3$); MS (TOF) m/z 465 $(M+H)^+$. Anal. calcd for $C_{25}H_{28}N_4O_5$: C, 64.64; H, 6.08; N, 12.08. Found: C, 64.75; H, 6.03; N, 12.37.

6-Amino-5-[(*RS*)-6-methoxy-2,5,7,8-tetramethylchroman-2-carboxamido]3-methyl-1-phenyluracil (28d). White crystals (57.9%), mp 210–211 °C; ^{1}H NMR ($CDCl_3$) δ 1.61 (3H, s), 1.98–2.00 (1H, m), 2.12 (3H, s), 2.21 (3H, s), 2.28 (3H, s), 2.31–2.36 (1H, m), 2.59–2.62 (2H, m), 3.35 (3H, s), 3.62 (3H, s), 5.18 (2H, s), 7.28–7.36 (2H, m), 7.52–7.58 (3H, m), 8.42 (1H, s); MS (TOF) m/z 479 $(M+H)^+$. Anal. calcd for $C_{26}H_{30}N_4O_5$: C, 65.26; H, 6.32; N, 11.71. Found: C, 65.17; H, 6.13; N, 11.72.

Biology

Picryl chloride (PC)-induced contact hypersensitivity reaction (CHR). PC-induced CHR was assessed by the method of Asherson and Ptak.[13] Male ICR mice were sensitized by applying 100 μL of 7% (w/v) PC solution in acetone to the shaved abdomen. Seven days later, the mice were challenged by applying 20 μL of 1% (w/v) PC solution, either in olive oil for po or in acetone for

topical experiment, to the left ear. The ear thickness was measured with a digital thickness gauge before and 24 h after the challenge, and the difference in thickness was calculated. In the po experiment, test compounds were suspended in 0.5% (w/v) sodium calboxymethylcellulose solution and were administered 0.5 h before and 16 h after the challenge. In the topical experiment, test compounds were dissolved in acetone and were administered immediately after the challenge.

Lipid peroxidation in vitro.[31,32] Male Sprague–Dawley rat brain was homogenized in 0.1 M phosphate buffer (pH 7.4) at 4 °C with a Polytron homogenizer. After centrifugation at $120\times g$ for 5 min, the supernatant was used as the brain homogenate. The desired test compounds in DMSO was added to the homogenate on ice, and the mixture was incubated for 1 h at 37 °C. Sodium thiobarbiturate (TBA) solution (1.2%, v/v) was added to the mixture (final 2.0 mL), and the solution was heated for 1 h at 95–97 °C. After cooling, 0.5 mL of distilled water and 2.5 mL of *n*-butanol:pyridine (15:1, v/v) were added to the solution and mixed vigorously. The absorbance of the TBA-reactive substances extracted in the organic layer was determined at 532 nm, and the level of lipid peroxides was expressed as malondialdehyde (MDA) concentration. Antioxidant activities of test compounds were calculated using the following formula:

$$\text{Inhibition}\% = [1 - (C3 - C1)/(C2 - C1)] \times 100$$

where, C1 = internal MDA concentration, C2 = MDA concentration in control sample, C3 = MDA concentration in test sample.

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