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Structure and Activity Relationships of Novel Uracil Derivatives as Topical Anti-Inflammatory Agents

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Abstract—In order to create novel, topical anti-inflammatory compounds exhibiting more potent activities than lead compound CX-659S (**1**), we designed and synthesized various derivatives of **1** focusing on the uracil *N*(1)- and *N*(3)-substituents, and evaluated their anti-inflammatory activities via inhibition of the picryl chloride-induced contact hypersensitivity reaction (CHR) in mice. In the course of our structure and activity relationship study, we found that compounds **6k**, **6q**, and **6r** inhibited by approximately 50% the CHR, at 0.1 mg/ear. These activities were essentially equipotent with that of Tacrolimus, a strong immunosuppressant.

Introduction

Drug therapy for the major inflammatory skin diseases, such as atopic dermatitis, contact dermatitis, and psoriasis is often inadequate due to poor efficacy and/or toxicity. The mainstays of therapy have been oral antihistamines and topical corticosteroids. Side effects associated with higher potency topical corticosteroids have limited their use in children and around the facial areas. Therefore, much of the recent research has been focused on developing new classes of therapeutic agents. Recently T cell inhibitors such as Cyclosporine A[1,2] and Tacrolimus (FK-506) have been considered to be clinically promising agents for immunotherapy. However Tacrolimus, despite its high efficacy, caused side effects of burning and irritation in 80% of the patients.[3–5] Thus new classes of topical anti-inflammatory agents which have lower toxicities than the current therapies are highly desired.

We have recently reported that a series of 5-substituted uracil derivatives, such as CX-659S (**1**), are a new class of non-steroidal anti-inflammatory agent possessing anti-oxidative activity.[6] Although **1** exerts promising anti-inflammatory activities in both an acute inflammation model and various delayed type hypersensitivity models,[7] the inhibitory potency of **1** was about 10-fold weaker than that of Tacrolimus, in inhibiting the picryl chloride (PC)-induced contact hypersensitivity reaction (CHR). Recently, modification of the anti-oxidative hydroxy chroman moiety in **1** and the linker between the uracil and the anti-oxidative moiety was investigated, but great improvements in the inhibitory activities could not be achieved.[8] The present work, described below, involved a detailed structure–activity relationship study (SAR) focusing on the uracil *N*(1)- and *N*(3)-substituents, and evaluating their anti-inflammatory activities by their ability to inhibit the CHR in mice, induced by PC. As the inhibitory potency of **1** was equivalent to that of its (*R*)-isomer,[6] we conducted the present SAR study using the racemate (**2**) (Fig. 1).

Chemistry

The synthetic methods of **6a**–**v** are outlined in Scheme 1. The syntheses of the 5,6-diaminouracil derivatives (**5a**–**v**) were achieved using previously reported methods.[9,10] A series of 6-aminouracil derivatives (**4a**–**v**) were prepared by acylation of the respective urea derivatives (**3a**–**v**) with cyanoacetic acid, followed by alkaline-catalyzed cyclization. Nitrosation with sodium nitrite followed by catalytic hydrogenation of the nitroso group furnished **5a**–**v**. The desired compounds (**6a**–**v**) were obtained by coupling the respective compounds **5a**–**v**

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with Trolox®, using diphenylphosphoryl chloride as the coupling reagent. The syntheses of **12** and **13** are described in Scheme 2. The amino group of **4a**[11] was protected using dimethylformamide-dimethylacetal, alkylated with ethyl iodoacetate and subsequently deprotected with concentrated HCl to give the desired 6-aminouracil derivative **9**. Conversion of **9** to **11** was achieved using the same procedures described above. Catalytic hydrogenation of **11** gave **12**, and alkaline hydrolysis of **11** followed by catalytic hydrogenation gave **13**. Compound **14** was obtained by catalytic hydrogenation of **6l**.

Results and Discussion

We evaluated the inhibitory effects of topically applied test compounds on the PC-induced CHR in mice, and their inhibitory potencies are shown as a percentage (%) of inhibition in Tables 1 and 2.[12]

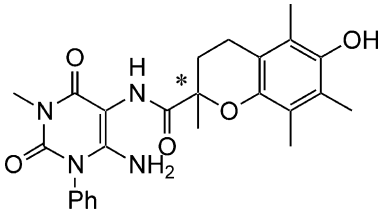
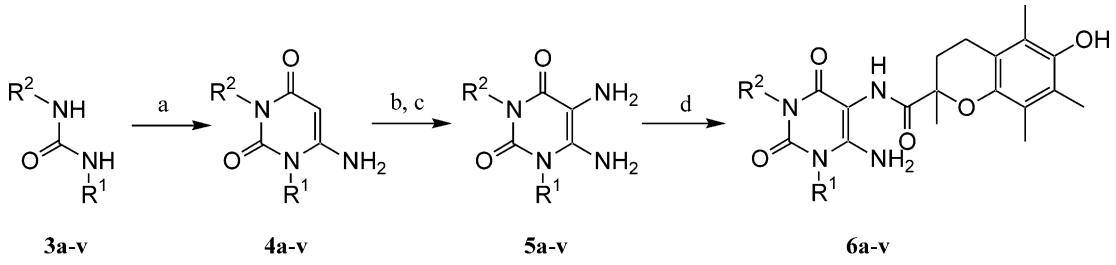


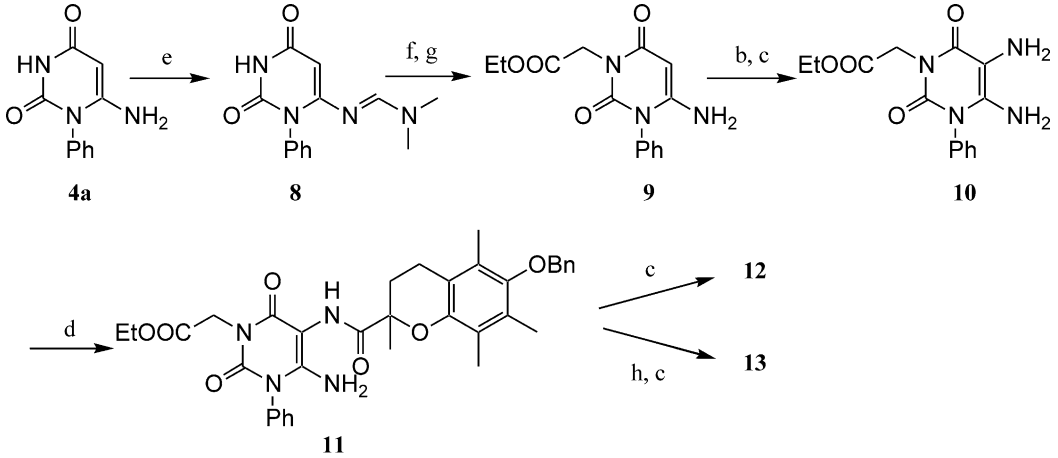
Figure. 1. Structures of compounds **1** and **2**.

We initially investigated the uracil *N*(3)-substituent. As shown in Table 1, the compounds substituted with various alkyl groups (**6d–f**) showed almost equipotent activities with that of **2**, in which the alkyl group is methyl, except for **6c** which was less potent than **2**. Compounds substituted with ethoxycarbonylmethyl (**12**) or carboxylmethyl (**13**), however, showed decreased activities. From these results, we selected the methyl group as the preferable *N*(3)-substituent and next focused on optimizing the *N*(1)-phenyl moiety.

Introducing substituents at the *para* position of the phenyl ring, such as halogen atoms, and methyl or methoxy groups gave compounds which were poorly soluble in application solvents, such as acetone, ethyl acetate, and ethanol, so they were unable to be used in the PC-induced CHR experiments. Thus, we investigated substituents at the *ortho* and *meta* positions, the inhibitory potencies of which are summarized in Table 2. Small substituents such as methyl (**6g**) and hydroxy (**14**) did not lead to potent inhibition, whereas methoxy (**6h**), methylthio (**6i**), bromo (**6k**) and benzyloxy (**6l**) showed over 70% inhibition, at a dose of 1 mg/ear. In addition, the inhibitory activities of **6i**, **6k**, and **6l**, at a dose of 0.1 mg/ear, were about 3- to 4-fold greater than those of **2** and **6h**. The results also show that there is a difference in activities between the *ortho* and the *meta* substituted compounds. Electron withdrawing substituents give rise to higher potencies in the *ortho* series (**6q**, **6r**) than electron donating substituents (**6m–p**) whereas in the *meta* series both electron withdrawing

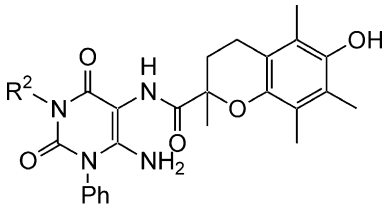


Scheme 1. Synthetic route to compounds **6a–v**. Reagents and conditions: (a) (i) $NCCH_2COOH$, Ac_2O, AcOEt, reflux; (ii) 3 N NaOH aq rt; (b) $NaNO_2$, 12 N HCl, H_2O; (c) 5% Pd/C, H_2, MeOH; (d) Trolox®, diphenylphosphoryl chloride, Et_3N, CH_2Cl_2, then **5**, Et_3N, rt.



Scheme 2. Synthetic route to compounds **12** and **13**. Reagents and conditions: (e) $Me_2NCH(OMe)_2$, MeOH, rt; (f) ICH_2COOEt, K_2CO_3, DMF, rt; (g) 12 N HCl, 40 °C; (h) 3 N NaOH aq, EtOH, rt.

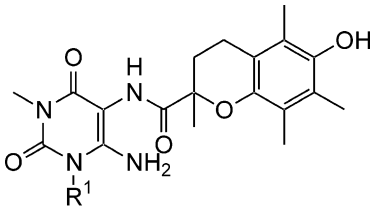
Table 1. Inhibitory effects of the test compounds on PC-induced CHR in mice



Compd	R^2	% of inhibition (1 mg/ear)
2	Me	53
6a	H	Nt[a]
6b	Et	Nt[a]
6c	Pr	29
6d	*n*-Bu	53
6e	*n*-Pen	46
6f	*c*-Hex	51
12	CH_2COOEt	17
13	CH_2COOH	5
Tacrolimus		81

Percent inhibition was calculated from the values of percent responses of drug-treated and control groups ($n=6$).
[a]Not tested due to its poor solubility for application solvents.

Table 2. Inhibitory effects of the test compounds on PC-induced CHR in mice



Compd	R^1	% of inhibition	
		0.1 mg/ear	1 mg/ear
2	Ph	15	53
6g	3-Me-Ph		40
6h	3-OMe-Ph	16	77
6i	3-SMe-Ph	41	87
6j	3-Cl-Ph		50
6k	3-Br-Ph	55	90
6l	3-OBn-Ph	44	74
14	3-OH-Ph		61
6m	2-Me-Ph		65
6n	2-OMe-Ph		64
6o	2-OEt-Ph		55
6p	2-SMe-Ph		48
6q	2-Cl-Ph	50	82
6r	2-Br-Ph	53	82
6s	2,3-OMe-Ph		39
6t	3,4-OMe-Ph		42
6u	3-*O*-*c*-Pen,4-OMe-Ph	30	89
6v	2,3-Cl-Ph		48
Tacrolimus		53	81

Percent inhibition was calculated from the values of percent responses of drug-treated and control groups ($n=6$).

and donating substituents give rise to high potencies (**6h**, **6i**, **6k**, **6l**), though **6j** is weakly activity so other effects, such as steric size, may be important in this series (cf. **6h** and **6l**). Next we examined the activities of the dimethoxy (**6s**, **6t**) and dichloro (**6v**) substituted compound. In contrast to the monosubstituted compounds, the disubstituted compounds were all weakly active. Compound **6u**, which contains a bulky substituent at *meta* position, showed greater activity than **6t**, this result being consistent with the importance of steric size at the *meta* position, vide supra. Among all of the compounds tested, compounds **6k**, **6q**, and **6r** were the most active and essentially equipotent to Tacrolimus, a potent immunosuppressant. Thus substitution at the *N*(1)-position of **2** proved to be effective for potentiating the inhibitory activity.

As lipophilicity and molecular size are generally important physicochemical properties for dermal absorption, we calculated the AlogP values of the test compounds, as an indicator of their lipophilicity. Unfortunately, there was no clear correlation between the AlogP values and the inhibitory activities. The anti-oxidative activities of these compounds were presumed to be related to their tocopherol-related structure and we in fact found that the hydroxyl radical-scavenging activities of **6k**, **6q**, and **6r** were the same as **2** (data not shown).[13] The mechanism of action of the compounds is unknown, but is presently being investigated by us, although the activity is considered to be not only anti-oxidative activity but also anti-inflammatory in nature.

In conclusion, we conducted a SAR study on **2**, in order to find the optimal substituents to enhance the inhibition of the PC-induced CHR in mice. A methyl group was found to be the most favorable substituent at the uracil *N*(3) position and the *N*(1) substituent was found to have a great influence on the inhibitory activities. The most potent compounds were **6k**, **6q**, and **6r**, which showed a 10-fold improvement in inhibitory activities compared to **2**, and were almost equipotent to Tacrolimus. We expect these new compounds to be useful anti-inflammatory agents for treatment of skin deseases, such as atopic dermatitis and contact dermatitis.

Experimental

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) was recorded on a Compact MALDI 3 V4.0.0 spectrometer. High-resolution mass spectra were obtained on a JEOL JMS-700 mass spectrometer. Elemental analyses were performed at the Toray Research Center. Wakogel C-200 (Wako; 70–150 mm) was used for column chroma-

tography. Monitoring of reaction was carried out by using Merck 60 F_{254} silica gel, glass-supported TLC plates, and visualization with UV light (254 and 365 nm).

Chemistry

6-Amino-3-ethyl-1-phenyluracil (4b). A suspension of *N*-ethyl-*N*-phenylurea (820 mg, 5 mmol), cyanoacetic acid (638 mg, 7.5 mmol) and acetic anhydride (1 mL) in ethyl acetate (20 mL) was refluxed for 4 h, and was concentrated. Water (20 mL) was added to the residue, and adjusted to pH 10 with 3 N NaOH solution. After 6 h of stirring, neutralized to pH 7 with 1N HCl solution, and the resulting precipitate was collected to give **4b** (950 mg, 78%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 7.49–7.57 (3H, m), 7.31–7.35 (2H, m), 6.09 (2H, brs), 4.80 (1H, s), 3.75 (2H, q, J = 6.8 Hz), 1.06 (3H, t, J = 6.8 Hz); MS (TOF) m/z 232 $(M+H)^+$.

Compounds **4c**–**v** were also prepared by using the same procedure as for **4b**. ^{1}H NMR data (DMSO-d_6: δ) of these compounds are shown below.

(**4c**) 7.46–7.56 (3H, m), 7.31–7.34 (2H, m), 6.10 (2H, brs), 4.80 (1H, s), 3.67 (2H, t, J = 7.3 Hz), 1.49 (2H, dq, J = 7.3, 7.6 Hz), 0.82 (3H, t, J = 7.3 Hz), (**4d**) 7.46–7.57 (3H, m), 7.30–7.34 (2H, m), 6.09 (2H, brs), 4.79 (1H, s), 3.71 (2H, t, J = 7.0 Hz), 1.41–1.52 (2H, m), 1.24 (2H, tt, J = 7.0, 7.4 Hz), 0.87 (3H, t, J = 7.0 Hz). (**4e**) 7.48–7.56 (3H, m), 7.29–7.33 (2H, m), 6.10 (2H, brs), 4.70 (1H, s), 3.77 (2H, t, J = 6.8 Hz), 1.45–1.56 (2H, m), 1.20–1.33 (4H, m), 1.19–1.26 (2H, m), 0.85 (3H, t, J = 6.8 Hz). (**4f**) 7.48–7.56 (3H, m), 7.29–7.32 (2H, m), 6.05 (2H, brs), 4.64 (1H, tt, J = 8.6, 5.9 Hz), 2.18–2.31 (2H, m), 1.60–1.76 (2H, m), 1.46–1.60 (3H, m), 0.96–1.26 (3H, m). (**4g**) 7.38–7.44 (1H, m), 7.29–732 (1H, m), 7.09–7.14 (2H, m), 6.09 (2H, brs), 4.80 (1H, s), 3.08 (3H, s), 2.35 (3H, s). (**4h**) 7.42 (1H, t, J = 8.0 Hz), 7.04–7.08 (1H, m), 6.87–6.95 (2H, m), 6.13 (2H, brs), 4.80 (1H, s), 3.78 (3H, s), 3.08 (3H, s). (**4i**) 7.35–7.47 (2H, m), 7.22–7.24 (1H, m), 7.07–7.11 (1H, m), 6.17 (2H, brs), 4.80 (1H, s), 3.08 (3H, s), 2.49 (3H, s). (**4j**) 7.51–7.59 (3H, m), 7.31–7.35 (1H, m), 6.25 (2H, brs), 4.80 (1H, s), 3.08 (3H, s). (**4k**) 7.64–7.72 (2H, m), 7.45–7.51 (1H, m), 7.37 (1H, d, J = 8.4 Hz), 6.24 (2H, brs), 4.80 (1H, s), 3.08 (3H, s). (**4l**) 7.31–7.49 (6H, m), 7.11–7.15 (1H, m), 6.91–6.94 (2H, m), 5.08 (2H, s), 5.03 (1H, s), 4.40 (2H, brs), 3.32 (3H, s). (**4m**) 7.23–7.35 (3H, m), 7.15 (1H, d, J = 3.8 Hz), 6.04 (2H, brs), 4.73 (1H, s), 3.01 (3H, s), 1.97 (3H, s). (**4n**) 7.45–7.52 (1H, m), 7.18–7.27 (2H, m), 7.03–7.09 (1H, m), 6.10 (2H, brs), 4.78 (1H, s), 3.76 (3H, s), 3.07 (3H, s). (**4o**) 7.46–7.48 (1H, m), 7.21–7.29 (2H, m), 7.07–7.09 (1H, m), 6.13 (2H, brs), 4.80 (1H, s), 4.09 (2H, q, J = 6.8 Hz), 3.10 (3H, s), 1.23 (3H, t, J = 6.8 Hz). (**4p**) 7.45–7.54 (3H, m), 7.30–7.36 (1H, m), 6.14 (2H, brs), 4.80 (1H, s), 3.09 (3H, s), 2.42 (3H, s). (**4q**) 7.65–7.69 (1H, m), 7.47–7.58 (3H, m), 6.30 (2H, brs), 4.82 (1H, s), 3.09 (3H, s). (**4r**) 7.81–7.83 (1H, m), 7.45–7.55 (3H, m), 6.30 (2H, brs), 4.82 (1H, s), 3.10 (3H, s). (**4s**) 7.14–7.24 (2H, m), 6.84–6.88 (1H, m), 6.19 (2H, brs), 4.78 (1H, s), 3.87 (3H, s), 3.70 (3H, s), 3.08 (3H, s). (**4t**) 7.05 (1H, d, J = 8.1 Hz), 6.94 (1H, s), 6.82–6.86 (1H, m), 6.14 (2H, brs), 4.80 (1H, s), 3.81 (3H, s), 3.74 (3H, s), 3.08 (3H, s). (**4u**) 6.78–6.98 (3H, m), 5.05 (1H, s), 4.76 (1H, tt, J = 5.0, 5.0 Hz), 3.89 (3H, s), 3.33 (3H, s), 1.76–1.95 (6H, m), 1.60–1.67 (2H, m). (**4v**) 7.79–7.82 (1H, m), 7.48–7.56 (2H, m), 6.43 (2H, s), 4.81 (1H, s), 3.09 (3H, s).

5,6-Diamino-3-ethyl-1-phenyluracil (5b). To a suspension of **4b** (696 mg, 3 mmol) and sodium nitrite (280 mg, 4 mmol) in water (20 mL) was added and 12 N HCl (0.3 mL). After 2 h of stirring, the reaction mixture was neutralized to pH 7 with 10% NaOH solution, and the resulting red-violet solid was collected (700 mg). To a suspension of this solid (700 mg) in MeOH (30 mL) was added 5% Pd/C (50 mg). After 1 h of stirring under a H_2 atmosphere, the catalyst was removed by filtration, and the filtrate was concentrated. The residue was triturated with ethyl acetate, and the resulting solid was filtered to give **5b** (652 mg, 88%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.50–7.56 (4H, m), 7.31–7.35 (3H, m), 5.34 (2H, s), 3.82 (2H, q, J = 6.8 Hz), 1.08 (3H, t, J = 6.8 Hz); MS (TOF) m/z 247 $(M+H)^+$.

Compounds **5a**–**v** and **10** were also prepared by using the same procedure as for **5b**. ^{1}H NMR data (DMSO-d_6: δ) of these compounds are shown below.

(**5a**) 7.25–7.55 (7H, m), 5.34 (2H, s), (**5c**) 7.49–7.56 (3H, m), 7.31–7.34 (2H, m), 5.35 (2H, brs), 3.74 (2H, t, J = 7.3 Hz), 1.53 (2H, m), 0.84 (3H, t, J = 7.3 Hz). (**5d**) 7.46–7.56 (2H, m), 7.30–7.38 (3H, m), 5.34 (2H, brs), 3.77 (2H, t, J = 7.3 Hz), 1.37–1.58 (2H, m), 1.19–1.27 (2H, m), 0.88 (3H, t, J = 7.3 Hz). (**5e**) 7.49-7.57 (3H, m), 7.30–7.34 (2H, m), 5.34 (2H, brs), 3.77 (2H, t, J = 6.8 Hz), 1.46–1.56 (2H, m), 1.21–1.33 (4H, m), 1.19–1.27 (2H, m), 0.85 (3H, t, J = 6.8 Hz). (**5f**) 7.24–7.77 (7H, m), 6.56 (2H, brs), 4.65 (1H, m), 2.25 (2H, m), 1.51–1.80 (5H, m), 1.15–1.28 (3H, m). (**5g**) 7.32–7.42 (4H, m), 7.14 (2H, m), 5.34 (2H, brs), 3.19 (3H, s), 2.36 (3H, s). (**5h**) 9.51 (2H, brs), 7.46 (1H, t, J = 8.4 Hz), 7.08–7.12 (1H, m), 6.92–7.01 (4H, m), 3.78 (3H, s), 3.17 (3H, s). (**5i**) 9.35 (2H, brs), 7.39–7.51 (2H, m), 7.29–7.30 (1H, m), 7.12–7.18 (1H, m), 6.89 (2H, brs), 3.14 (3H, s), 2.50 (3H, s). (**5j**) 9.46 (2H, brs), 7.55–7.63 (3H, m), 7.36–7.39 (1H, m), 7.05 (2H, s), 3.17 (3H, s). (**5k**) 7.69–7.71 (2H, m), 7.47–7.51 (1H, m), 7.37–7.39 (1H, m), 5.54 (2H, s), 3.17 (3H, s). (**5l**) 7.36–7.49 (6H, m), 6.90–7.06 (3H, m), 5.42 (2H, s), 5.11 (2H, s), 3.16 (3H, s). (**5m**) 9.52 (2H, s), 7.29–7.47 (4H, m), 6.99 (2H, s), 3.19 (3H, s), 2.08 (3H, s). (**5n**) 9.50 (2H, s), 7.50–7.53 (1H, m), 7.23–7.33 (2H, m), 7.08–7.12 (1H, m), 7.03 (2H, s), 3.77 (3H, s), 3.17 (3H, s). (**5o**) 9.49 (2H, s), 7.47–7.50 (1H, m), 7.22–7.32 (2H, m), 7.08–7.10 (1H, m), 7.00 (2H, s), 4.09 (2H, q, J = 7.0 Hz), 3.18 (3H, s), 1.22 (3H, t, J = 7.0 Hz). (**5p**) 9.58 (2H, s), 7.48–7.57 (2H, m), 7.32–7.38 (2H, m), 7.10 (2H, s), 3.18 (3H, s), 2.44 (3H, s). (**5q**) 9.58 (2H, s), 7.85–7.89 (1H, m), 7.46–7.58 (2H, m), 7.13 (2H, s), 3.17 (3H, s). (**5r**) 9.59 (2H, s), 7.84–7.87 (1H, m), 7.49–7.62 (3H, m), 7.27 (2H, s), 3.20 (3H, s). (**5s**) 7.11–7.23 (3H, m), 6.79–6.87 (2H, m), 5.44 (2H, brs), 3.87 (3H, s), 3.66 (3H, s), 3.15 (3H, s). (**5t**) 6.81–6.98 (3H, m), 3.80 (3H, s), 3.72 (3H, s), 3.32 (3H, s). (**5u**) 9.46 (2H, s), 7.09 (2H, s), 6.78–6.98 (3H, m), 5.05 (1H, s), 4.76 (1H, tt, J = 5.0, 5.0 Hz), 3.89 (3H, s), 3.33 (3H, s), 1.76–1.95

(6H, m), 1.60–1.67 (2H, m). (**5v**) 9.51 (2H, brs), 7.83–7.87 (1H, m), 7.53–7.63 (2H, m), 7.32 (2H, s), 3.19 (3H, s). (**10**) 7.52–7.60 (2H, m), 7.34–7.37 (3H, m), 4.71 (2H, brs), 4.68 (2H, brs), 4.21 (2H, q, $J=7.4$ Hz), 1.28 (3H, t, $J=7.3$ Hz).

(*RS*)-6-Amino-3-ethyl-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-1-phenyluracil (6b). To a solution of (*RS*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (310 mg, 1.2 mmol) and triethylamine (0.2 mL) in ethyl acetate (12 mL) was added diphenylphospholyl chloride (0.31 mL, 1.5 mmol) in an ice-water bath under a N_2 atmosphere. After 1 h of stirring, **5b** (272 mg, 1.1 mmol) and triethylamine (0.2 mL) were added to the mixture at same temperature. The reaction mixture was stirred for another 3 h, and was partitioned with water (25 mL). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was triturated with diethyl ether, and the resulting white solid was filtered to give **6b** (315 mg, 58%). Mp 229–230 °C; ^{1}H NMR (DMSO-d_6) δ 8.01 (1H, s), 7.50–7.58 (4H, m), 7.32–7.35 (2H, m), 5.69 (2H, s), 3.78 (2H, q, $J=7.0$ Hz), 2.50–2.59 (2H, m), 2.23–2.27 (1H, m), 2.13 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.78–1.83 (1H, m), 1.46 (3H, s), 1.07 (3H, t, $J=7.0$ Hz); MS (TOF) m/z 479 $(M+H)^+$. Anal. calcd for $C_{26}H_{30}N_4O_5\cdot0.9H_2O$: C, 63.12; H, 6.30; N, 11.32. Found: C, 63.20; H, 6.21; N, 11.03.

Compounds **6a**–**v** were also prepared by using the same procedure as for **6b**.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-1-phenyluracil (6a). Yield 95%. Mp 280–281 °C; ^{1}H NMR (DMSO-d_6) δ 10.85 (1H, s), 7.93 (1H, s), 7.50–7.57 (3H, m), 7.30–7.33 (3H, m), 5.69 (2H, s), 2.50–2.56 (2H, m), 2.22–2.28 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.00 (3H, s), 1.77–1.82 (1H, m), 1.46 (3H, s); MS (TOF) m/z 451 $(M+H)^+$. Anal. calcd for $C_{24}H_{26}N_4O_5\cdot H_2O$: C, 61.53; H, 5.81; N, 11.96. Found: C, 61.39; H, 6.00; N, 11.97.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-1-phenyl-3-propyluracil (6c). Yield 63%. Mp 200–202 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (1H, s), 7.54–7.58 (3H, m), 7.28–7.37 (2H, m), 5.12 (2H, s), 3.85–92 (2H, m), 2.57–2.65 (2H, m), 2.32–2.39 (1H, m), 2.29 (3H, s), 2.19 (3H, s), 2.08 (3H, s), 1.93–1.98 (1H, m), 1.66 (2H, m), 1.61 (3H, s), 0.92 (3H, t, $J=7.0$ Hz); MS (TOF) m/z 493 $(M+H)^+$. Anal. calcd for $C_{27}H_{32}N_4O_5\cdot0.3H_2O$: C, 65.12; H, 6.54; N, 11.25. Found: C, 65.12; H, 6.55; N, 11.39.

(*RS*)-6-Amino-3-butyl-5-(6-hydroxy-2,5,7,8-tetramethylchroman - 2 - carboxamide) - 1 - phenyluracil (6d). Yield 43%. Mp 129–131 °C; ^{1}H NMR (DMSO-d_6) δ 8.01 (1H, s), 7.51–7.56 (3H, m), 7.31–7.34 (2H, m), 5.68 (2H, s), 3.73 (2H, m), 2.54 (2H, m), 2.21–2.24 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.84 (1H, m), 1.46 (5H, m), 1.22–1.30 (2H, m), 0.87 (3H, t, $J=7.0$ Hz); MS (TOF) m/z 507 $(M+H)^+$. Anal. calcd for $C_{28}H_{34}N_4O_5$: C, 66.38; H, 6.76; N, 11.06. Found: C, 66.31; H, 6.85; N, 11.02.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-pentyl-1-phenyluracil (6e). Yield 61%. Mp 125-127 °C; ^{1}H NMR (DMSO-d_6) δ 8.01 (1H, s), 7.51–7.58 (3H, m), 7.31–7.33 (2H, m), 5.68 (2H, s), 3.69–3.74 (2H, m), 2.54 (2H, m), 2.22–2.24 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.84 (1H, m), 1.46 (5H, m), 1.25–1.30 (4H, m), 0.85 (3H, t, $J=7.0$ Hz); MS (TOF) m/z 521 $(M+H)^+$. Anal. calcd for $C_{29}H_{36}N_4O_5\cdot0.2H_2O$: C, 66.49; H, 7.26; N, 10.32. Found: C, 66.44; H, 6.96; N, 10.69.

(*RS*)-6-Amino-3-cyclohexyl-5-(6-hydroxy-2,5,7,8-tetramethylchroman - 2 - carboxamide) - 1 - phenyluracil (6f). Yield 69%. Mp 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 7.99 (1H, s), 7.51–7.57 (3H, m), 7.30–7.33 (2H, m), 5.66 (2H, s), 4.58–4.67 (1H, m), 2.51 (2H, m), 2.22–2.24 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.72–1.76 (3H, m), 1.52 (3H, m), 1.46 (3H, s), 1.21–1.25 (3H, m), 1.07–1.09 (2H, m); MS (TOF) m/z 533 $(M+H)^+$. Anal. calcd for $C_{30}H_{36}N_4O_5$: C, 67.65; H, 6.81; N, 10.52. Found: C, 67.63; H, 6.94; N, 10.51.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyl-1-(3-methylphenyl)uracil (6g). Yield 61%. Mp 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 8.41 (1H, s), 7.42–7.46 (1H, m), 7.32–7.35 (1H, m), 7.07–7.15 (2H, m), 5.19 (2H, s), 3.35 (3H, s), 2.58–2.67 (2H, m), 2.40 (1H, m), 2.33 (3H, s), 2.19 (3H, s), 2.08 (3H, s), 1.93–2.01 (1H, m), 1.61 (3H, s); MS (TOF) m/z 479 $(M+H)^+$. Anal. calcd for $C_{26}H_{30}N_4O_5\cdot0.5H_2O$: C, 64.05; H, 6.31; N, 11.49. Found: C, 64.01; H, 6.39; N, 11.28.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2 - carboxamide) - 1 - (3 - methoxyphenyl) - 3 - methyluracil (6h). Yield 85%. Mp 150–152 °C; ^{1}H NMR (DMSO-d_6) δ 7.96 (1H, s), 7.52 (1H, s), 7.45 (1H, t, $J=8.1$ Hz), 7.07–7.11 (1H, m), 6.88–6.94 (2H, m), 5.78 (2H, s), 3.78 (3H, s), 3.11 (3H, s), 2.57 (2H, m), 2.20–2.30 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.78-1.85 (1H, m), 1.46 (3H, s); MS (TOF) m/z 495 $(M+H)^+$. Anal. calcd for $C_{26}H_{30}N_4O_6\cdot0.4H_2O$: C, 62.24; H, 6.11; N, 11.17. Found: C, 62.27; H, 6.28; N, 11.03.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyl-1-(3-methylthiophenyl)uracil (6i). Yield 31%. Mp 148–150 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (1H, s), 7.37–7.51 (3H, m), 7.22 (1H, s), 7.08 (2H, d, $J=7.8$ Hz), 5.82 (2H, s), 3.30 (3H, s), 3.11 (3H, s), 2.57 (2H, m), 2.23–2.27 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.78–1.83 (1H, m), 1.46 (3H, s); MS (TOF) m/z 513 $(M+H)^+$. Anal. calcd for $C_{26}H_{32}N_4O_5S\cdot0.4H_2O$: C, 60.07; H, 6.28; N, 10.78. Found: C, 59.91; H, 5.95; N, 11.16.

(*RS*)-6-Amino-1-(3-chlorophenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6j). Yield 60%. Mp 156–158 °C; ^{1}H NMR (DMSO-d_6) δ 7.93 (1H, s), 7.53–7.62 (4H, m), 7.31–7.35 (1H, m), 5.93 (2H, s), 3.11 (3H, s), 2.58 (2H, m), 2.21–2.30 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.76–1.85 (1H, m), 1.47 (3H, s); MS (TOF) m/z 499 $(M+H)^+$. Anal. calcd for $C_{25}H_{27}ClN_4O_5\cdot0.4H_2O$: C, 59.32; H, 5.46; N, 11.07. Found: C, 59.32; H, 5.58; N, 10.76.

(*RS*)-6-Amino-1-(3-bromophenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6k). Yield 58%. Mp 159–161 °C; ^{1}H NMR (DMSO-d_6) δ 7.93 (1H, d, J = 4.9 Hz), 7.64–7.74 (2H, m), 7.47–7.53 (2H, m), 7.35–7.38 (1H, m), 5.92 (2H, s), 3.10 (3H, s), 2.57 (2H, m), 2.23–2.28 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.78–1.83 (1H, m), 1.47 (3H, s); MS (TOF) m/z 544 $(M+H)^+$. Anal. calcd for $C_{25}H_{27}BrN_4O_5 \cdot 0.5H_2O$: C, 54.36; H, 5.02; N, 10.14. Found: C, 54.39; H, 5.24; N, 10.01.

(*RS*)-6-Amino-1-(3-benzyloxyphenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido)-3-methyluracil (6l). Yield 87%. Mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (1H, s), 7.37–7.47 (6H, m), 7.11–7.15 (2H, m), 6.87–6.96 (2H, m), 5.20 (2H, s), 5.06 (2H, s), 3.35 (3H, s), 2.61–2.65 (2H, m), 2.34 (1H, m), 2.30 (3H, s), 2.19 (3H, s), 2.09 (3H, s), 1.93–2.01 (1H, m), 1.61 (3H, s); MS (TOF) m/z 571 $(M+H)^+$. Anal. calcd for $C_{32}H_{34}N_4O_6 \cdot 0.9H_2O$: C, 65.49; H, 6.15; N, 9.55. Found: C, 65.74; H, 5.96; N, 9.16.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyl-1-(2-methyphenyl)uracil (6m). Yield 71%. Mp 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 8.32 (1H, s), 7.18–7.28 (3H, s), 7.02–7.07 (1H, m), 5.06 (2H, s), 3.19 (3H, s), 2.43–2.48 (2H, m), 2.15–2.18 (1H, m), 2.13 (3H, s), 2.04 (3H, s), 2.01 (3H, s), 1.92 (3H, s), 1.78-1.91 (1H, m), 1.42 (3H, s); MS (TOF) m/z 479 $(M+H)^+$; HRMS. calcd for $C_{26}H_{30}N_4O_5$: 478.2216; Found: 478.2223.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-1-(2-methoxyphenyl)-3-methyluracil (6n). Yield 58%. Mp 146–148 °C; ^{1}H NMR ($CDCl_3$) δ 8.49 (1H, s), 7.53 (1H, s), 7.22–7.29 (2H, m), 7.06–7.13 (2H, m), 5.14 (2H, s), 3.83 (3H, s), 3.35 (3H, s), 2.58–2.71 (2H, m), 2.35 (1H, m), 2.31 (3H, s), 2.19 (3H, s), 2.09 (3H, s), 1.92–2.02 (1H, m), 1.60 (3H, s); MS (TOF) m/z 495 $(M+H)^+$. Anal. calcd for $C_{26}H_{30}N_4O_6 \cdot 0.5H_2O$: C, 62.02; H, 6.11; N, 11.13. Found: C, 61.97; H, 6.28; N, 11.11.

(*RS*)-6-Amino-1-(2-ethoxyphenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6o). Yield 34%. Mp 124–126 °C; ^{1}H NMR ($CDCl_3$) δ 8.43 (1H, s), 7.38–7.42 (1H, m), 7.16–7.20 (1H, m), 6.98–7.01 (2H, m), 5.20 (2H, s), 4.00 (2H, q, J = 6.8 Hz), 3.29 (3H, s), 2.58 (2H, m), 2.24–2.28 (1H, m), 2.23 (3H, s), 2.12 (3H, s), 2.02 (3H, s), 1.92–2.02 (1H, m), 1.53 (3H, s), 1.28 (3H, t, J = 6.8 Hz); MS (TOF) m/z 509 $(M+H)^+$; HRMS. calcd for $C_{27}H_{32}N_4O_6$: 508.2322; Found: 508.2316.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyl-1-(2-methylthiophenyl)uracil (6p). Yield 54%. Mp 145–147 °C; ^{1}H NMR ($CDCl_3$) δ 8.46 (1H, s), 7.53 (1H, s), 7.22–7.29 (2H, m), 7.06–7.13 (2H, m), 5.14 (2H, s), 3.83 (3H, s), 3.35 (3H, s), 2.58–2.71 (2H, m), 2.35 (1H, m), 2.31 (3H, s), 2.19 (3H, s), 2.09 (3H, s), 1.92–2.02 (1H, m), 1.60 (3H, s); MS (TOF) m/z 513 $(M+H)^+$. Anal. calcd for $C_{26}H_{32}N_4O_5S \cdot 0.3H_2O$: C, 60.28; H, 6.34; N, 10.82. Found: C, 60.16; H, 6.13; N, 10.57.

(*RS*)-6-Amino-1-(2-chlorophenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6q). Yield 34%. Mp 152–154 °C; ^{1}H NMR ($CDCl_3$) δ 8.48 (1H, s), 7.43–7.46 (1H, m), 7.19–7.31 (3H, m), 5.13 (2H, s), 3.31 (3H, s), 2.55–2.59 (2H, m), 2.37 (3H, s), 2.23–2.27 (1H, m), 2.22 (3H, s), 2.13 (3H, s), 2.02 (3H, s), 1.90–1.98 (1H, m), 1.50 (3H, s); MS (TOF) m/z 499 $(M+H)^+$. Anal. calcd for $C_{25}H_{27}ClN_4O_5 \cdot 0.6H_2O$: C, 58.90; H, 5.46; N, 10.99. Found: C, 58.91; H, 5.71; N, 10.76.

(*RS*)-6-Amino-1-(2-bromophenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6r). Yield 21%. Mp 149–151 °C; ^{1}H NMR ($CDCl_3$) δ 8.46 (1H, s), 7.73–7.79 (1H, m), 7.47–7.51 (1H, m), 7.37–7.41 (2H, m), 5.13 (2H, s), 3.37 (3H, s), 2.58–2.62 (2H, m), 2.31 (1H, m), 2.29 (3H, s), 2.16 (3H, s), 2.06 (3H, s), 1.93–2.01 (1H, m), 1.56 (3H, s); MS (TOF) m/z 544 $(M+H)^+$; HRMS. calcd for $C_{25}H_{27}BrN_4O_5$: 542.1165; Found: 542.153.

(*RS*)-6-Amino-1-(2,3-dimethoxyphenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6s). Yield 77%. Mp 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 7.99 (1H, s), 7.50 (1H, s), 7.16–7.26 (2H, m), 6.84–6.87 (1H, m), 5.78 (2H, d, J = 4.9 Hz), 3.87 (3H, s), 3.70 (3H, s), 3.12 (3H, s), 2.50 (2H, m), 2.24–2.29 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.76–1.85 (1H, m), 1.46 (3H, s); MS (TOF) m/z 525 $(M+H)^+$. Anal. calcd for $C_{27}H_{32}N_4O_7 \cdot 0.5H_2O$: C, 60.78; H, 6.14; N, 10.50. Found: C, 60.67; H, 6.08; N, 10.36.

(*RS*)-6-Amino-1-(3,4-dimethoxyphenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6t). Yield 76%. Mp 157–159 °C; ^{1}H NMR (DMSO-d_6) δ 7.94 (1H, s), 7.51 (1H, s), 7.06–7.09 (1H, m), 6.92–6.94 (1H, m), 6.83–6.87 (1H, m), 5.79 (2H, s), 3.82 (3H, s), 3.74 (3H, s), 3.11 (3H, s), 2.57 (2H, m), 2.20–2.29 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.76–1.85 (1H, m), 1.46 (3H, s); MS (TOF) m/z 525 $(M+H)^+$. Anal. calcd for $C_{27}H_{32}N_4O_7 \cdot 0.6H_2O$: C, 60.57; H, 6.14; N, 10.47. Found: C, 60.78; H, 6.24; N, 10.08.

(*RS*)-6-Amino-1-(3-cyclopentyloxy-4-methoxyphenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6u). Yield 77%. Mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (1H, d, J = 11.3 Hz), 6.74–7.26 (3H, m), 5.24 (2H, d, J = 15.4 Hz), 4.72 (1H, m), 3.89 (3H, s), 3.35 (3H, s), 2.58–2.68 (2H, m), 2.30–2.39 (1H, m), 2.30 (3H, s), 2.19 (3H, s), 2.09 (3H, s), 1.84–1.99 (8H, m), 1.62 (3H, s); MS (TOF) m/z 579 $(M+H)^+$. Anal. calcd for $C_{31}H_{38}N_4O_7 \cdot 0.6H_2O$: C, 63.16; H, 6.60; N, 9.50. Found: C, 62.99; H, 6.58; N, 9.36.

(*RS*)-6-Amino-1-(2,3-dichlorophenyl)-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-3-methyluracil (6v). Yield 52%. Mp 150–152 °C; ^{1}H NMR (DMSO-d_6) δ 7.98 (1H, s), 7.81–7.84 (1H, m), 7.51–7.54 (2H, m), 6.10 (2H, brs), 3.12 (3H, s), 2.50–2.60 (2H, m), 2.23–2.28 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.78–1.83 (1H, m), 1.47 (3H, s); MS (TOF) m/z 534 $(M+H)^+$. Anal. calcd for $C_{25}H_{26}Cl_2N_4O_6 \cdot 0.3H_2O$: C,

55.73; H, 4.92; N, 10.40. Found: C, 55.75; H, 5.02; N, 10.11.

6-Dimethylaminomethylenylamino-1-phenyluracil (8). To a suspension of **4a** (7.0 g, 34.4 mmol) in MeOH (200 mL) was added *N*,*N*-dimethylformamide dimethylacetal (5.5 mL, 41.3 mmol) at room temperature. The mixture was stirred at same temperature for 10 h, and then was concentrated. The residue was triturated with EtOH, and the resulting white solid was filtered to give **8** (7.87 g, 88%). ^{1}H NMR (DMSO-d_6) δ 10.79 (1H, s), 7.96 (1H, s), 7.43–7.31 (3H, m), 7.18–7.14 (2H, m), 5.10 (1H, s), 2.99 (3H, s), 2.52 (3H, s).

6-Amino-3-ethoxycarbonylmethyl-1-phenyluracil (9). To a suspension of **8** (5.1 g, 19.8 mmol) and potassium carbonate (3.3 g, 23.7 mmol) in *N*,*N*-dimethylformamide (100 mL) was added ethyl iodoacetate (5.1 g, 23.7 mmol). The mixture was stirred at room temperature for 12 h, and then was concentrated. The residue was partitioned with CH_2Cl_2 and 5% aqueous citric acid solution. The organic layer was washed with water and brine, dried over Na_2SO_4. The solution was concentrated, and the residue was triturated with diethyl ether to give a white solid (5.25 g). To a solution of this solid (5.0 g) in EtOH (150 mL) was added 12 N HCl (6 mL), and was stirred at 40 °C for 12 h, and then was concentrated. The residue was partitioned with CH_2Cl_2 and 5% aqueous citric acid solution. The organic layer was washed with water and brine, dried over Na_2SO_4. The solution was concentrated, and the residue was triturated with diethyl ether, and the resulting white solid was filtered to give **9** (4.20 g, 73%). ^{1}H NMR (DMSO-d_6) δ 7.57–7.51 (3H, m), 7.33–7.29 (2H, m), 6.32 (2H, brs), 4.84 (1H, s), 4.45 (2H, s), 4.10 (2H, q, J = 7.0 Hz), 1.18 (3H, t, J = 7.0 Hz).

(*RS*)-6-Amino-5-(6-benzyloxy-2,5,7,8-tetramethylchroman-2-carboxamido)-3-ethoxycarbonylmethyl-1-phenyluracil (11). To a solution of (*RS*)-6-benzyloxy-2,5,7,8-tetramethylchroman-2-carboxyric acid (308 mg, 0.9 mmol) and triethylamine (0.14 mL, 1.0 mmol) in ethyl acetate (5 mL) was diphenylphosphoryl chloride (0.16 mL, 1.0 mmol) in an ice-water bath under a nitrogen atmosphere. After 1 h of stirring, **10** (250 mg, 0.82 mmol) and triethylamine (0.12 mL, 0.82 mmol) were added to the mixture at same temperature. The mixture was stirred for another 4 h, and then was concentrated. The residue was partitioned with CH_2Cl_2 and 5% aqueous citric acid solution. The organic layer was washed with 5% $NaHCO_3$ aqueous solution, water and brine, dried over Na_2SO_4. The solution was concentrated, and the residue was triturated with water. The resulting white solid was filtered to give **11** (433 mg, 84%). ^{1}H NMR ($CDCl_3$) δ 8.28 (1H, s), 7.60–7.31 (10H, m), 5.14 (2H, brs), 4.69 (2H, s), 4.68 (2H, s), 4.20 (2H, q, J = 7.3 Hz), 2.70–2.58 (2H, m), 2.42–2.32 (1H, m), 2.27 (3H, s), 2.24 (3H, s), 2.14 (3H, s), 2.04–1.93 (1H, m), 1.63 (3H, s), 1.26 (3H, t, J = 7.3 Hz).

(*RS*)-6-Amino-3-ethoxycarbonylmethyl-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-1-phenyluracil (12). A mixture of **11** (280 mg, 0.45 mmol) and 5% Pd/C (30 mg) in MeOH (6 mL) was stirred under a H_2 atmosphere for 12 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was triturated with diisopropyl ether, and the resulting white solid was filtered to give **12** (202 mg, 84%). Mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (1H, s), 7.54–7.60 (3H, m), 7.30–7.39 (2H, m), 5.19 (2H, s), 4.67 (2H, s), 4.19 (2H, q, J = 7.3 Hz), 2.61–2.73 (2H, m), 2.31–2.41 (1H, m), 2.27 (3H, s), 2.18 (3H, s), 2.08 (3H, s), 1.90–2.00 (1H, m), 1.60 (3H, s), 1.26 (3H, t, J = 7.3 Hz); MS (TOF) m/z 537 $(M+H)^+$. Anal. calcd for $C_{28}H_{32}N_4O_7$: C, 62.68; H, 6.01; N, 10.44. Found: C, 62.39; H, 6.10; N, 10.30.

(*RS*)-6-Amino-3-carboxymethyl-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido)-1-phenyluracil (13). A solution of **11** (400 mg, 0.64 mmol) in EtOH (7 mL) containing 3 N NaOH (2 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated. The residue was diluted with water, and then was adjusted to pH 4 with 1 N HCl. The resulting precipitate was filtered, washed with EtOH and water to give a white solid (333 mg, 87%). Next, a mixture of this solid (300 mg, 0.50 mmol) and 5% Pd/C (30 mg) in MeOH (15 mL) was stirred under a H_2 atmosphere for 10 h. The catalyst was removed by filtration, and the filtrate was concentrated. Then the residue was triturated with diethyl ether to give **13** (209 mg, 82%) as white solid. Mp 188–189 °C; ^{1}H NMR ($CDCl_3$) δ 8.18 (1H, s), 7.52–7.56 (3H, m), 7.29–7.36 (2H, m), 5.10 (2H, s), 4.58 (2H, s), 2.56–2.68 (2H, m), 2.35–2.46 (1H, m), 2.22 (3H, s), 2.13 (3H, s), 2.04 (3H, s), 1.80–1.93 (1H, m), 1.61 (3H, s); MS (TOF) m/z 509 $(M+H)^+$. Anal. calcd for $C_{26}H_{28}N_4O_7 \cdot 1.25H_2O$: C, 58.80; H, 5.55; N, 10.55. Found: C, 59.13; H, 5.94; N, 10.16.

(*RS*)-6-Amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamide)-1-(3-hydroxyphenyl)-3-methyluracil (14). A mixture of **6u** (300 mg, 0.53 mmol) and 5% Pd/C (40 mg) in MeOH (15 mL) was stirred under a H_2 atmosphere for 10 h. The catalyst was removed by filtration, and the filtrate was concentrated. Recrystallized from CH_2Cl_2/hexane gave **6u** as a white solid (245 mg, 97%). Mp 186–188 °C; ^{1}H NMR (DMSO-d_6) δ 9.86 (1H, s), 7.96 (1H, s), 7.50 (1H, s), 7.30–7.36 (1H, m), 6.88–6.92 (1H, m), 6.66–6.73 (2H, m), 5.73 (2H, s), 3.10 (3H, s), 2.55 (2H, m), 2.22–2.27 (1H, m), 2.12 (3H, s), 2.07 (3H, s), 2.01 (3H, s), 1.78–1.83 (1H, m), 1.46 (3H, s); MS (TOF) m/z 481 $(M+H)^+$. Anal. calcd for $C_{25}H_{28}N_4O_6 \cdot 1.25H_2O$: C, 59.69; H, 6.11; N, 11.14. Found: C, 59.97; H, 6.01; N, 10.75.

Biology

Picryl-chloride induced contact hypersensitivity reaction. 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 in acetone 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. Test compounds were dissolved in acetone and were administered 5 min after the challenge.

References and Notes

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