Synthesis of Pyrimidine Derivatives Possessing an Antioxidative Property and Their Inhibitory Effects on Picryl Chloride-Induced Contact Hypersensitivity Reaction

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We conducted a preliminary structure–activity relationship (SAR) study of some barbituric acid and uracil derivatives against the picryl chloride-induced contact hypersensitivity reaction. The introduction of an antioxidative moiety to the side chain of the C(6)-position of uracil was effective against this model. The introduction of dimethoxyphenol (8b) or dimethylphenol (8c) instead of di-*t*-butylphenol (8a) as an antioxidative moiety gave diminished activities, so, the reactive oxygen would contribute to the inflammation of this model, and an antioxidative activity was required for exhibiting the inhibitory activity. The inhibitory activity was significantly affected by the substituent at the N(1)-phenyl moiety.

Key words uracil; barbituric acid; delayed type hypersensitivity (DTH); di-*t*-butylphenol; antioxidative activity

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 diseases.[1)] Many antiallergic drugs are clinically used for treatment of these dermatitis, but they are not very efficacious because 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. However, long-time use of glucocorticoids is limited because of its many side effects, such as atrophia cutis and infections. Therefore, development of non-steroidal agents having inhibitory activity against DTH reactions and low toxicity has been ardently desired.

In the pathogenesis of many inflammatory skin diseases, there are both direct and indirect evidences implicating a reactive oxygen species (ROS) such as superoxide and hydrogen peroxide.[2)] Overproduction of ROS has also been postulated for immune-mediated skin diseases such as atopic dermatitis, contact dermatitis, and psoriasis.[3)] Monocytes or neutrophils of these patients show an increased capacity to release ROS.[4,5)] Therefore, compounds having inhibitory activity on the production and/or scavenging of ROS may be effective against DTH reactions. For example, tocopherol was reported to show a suppressive effect on chemical hapten-induced contact hypersensitivity reaction (CHR), one of the DTH models,[6)] and showed efficacy against atopic dermatitis in clinic.[7,8)]

In a previous paper, we described antiallergic activities against DTH reaction of various 5-acylamido-6-aminouracil derivatives, which originated from the lead compound (**1**) with an antioxidative activity.[9)] We describe herein the synthesis and suppressive effects against picryl chloride (PC)-induced CHR of novel pyrimidine derivatives, such as barbituric acids and uracils, having a 2,6-di-*t*-butylphenol moiety at the side chain of the C(5) or C(6)-position of the pyrimidine ring.

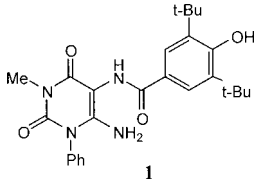

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Fig. 1. **1**

Results and Discussion

The synthetic methods of pyrimidine derivatives (**3**, **6**) possessing a 4-hydroxyphenyl group at the 5-position are described in Chart 1. 6-Amino-5-(3,5-di-*t*-butyl-4-hydroxybenzyl)uracil (**3**) was synthesized by the reaction of 6-aminouracil (**2**) with 3,5-di-*t*-butyl-4-hydroxybenzyl chloride as previously reported.[10)] For the synthesis of 5-(3,5-di-*t*-butyl-4-hydroxybenzyl)barbituric acids (**6a**—**d**), first, several *N*-substituted barbituric acids (**4a**—**d**), which were easily prepared[10)] by acid-hydrolysis of the corresponding 6-aminouracils,[11)] were condensed[12)] with 3,5-di-*t*-butyl-4-hydroxybenzaldehyde to give the 5-benzylidenebarbituric acids (**5a**—**d**) in moderate yields. The NMR analysis of an unsymmetrical product (**5a**) possessing different substituents at the 1,3-N-positions indicates them to be a mixture of *E*- and *Z*-isomers. The reduction of **5a**—**d** with $NaBH_4$ afforded the desired 3,5-di-*t*-butyl-4-hydroxybenzylbarbituric acids (**6a**—**d**).

The synthesis of uracil derivatives (**8a**—**g**, **10**) possessing an antioxidant moiety at the terminal of the side-chain of the 6-position is shown in Chart 2. Thus, 6-hydrazinouracils (**7a**, **d**—**g**, **9**) were obtained by chlorination[13)] of barbituric acids (**4a**, **d**—**g**) with phosphorus oxychloride followed by treatment with hydrazine or methylhydrazine as previously reported.[14—16)] Chlorination of unsymmetrical 3-methyl-1-phenylbarbituric acids (**4a**, **f**, **g**) proceeded regioselectively to afford the corresponding 6-chloro-3-methyl-1-phenyluracils as previously reported.[17)] The desired hydrazones (**8a**—**g**, **10**) were prepared by the reaction of 6-hydrazinouracils (**7a**, **d**—**g**, **9**) with 3,5-disubstituted 4-hydroxybenzaldehydes in good yields.

The antiallergic activities of the test compounds were evaluated by PC-induced CHR in mice at a dose of 10 mg/kg.

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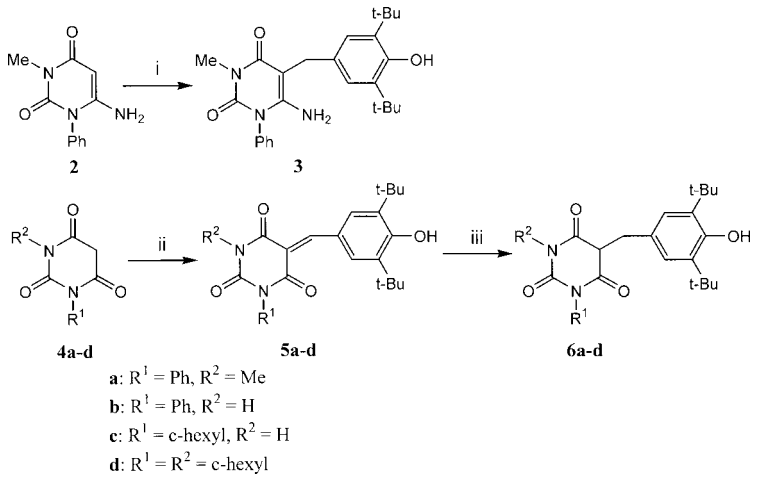
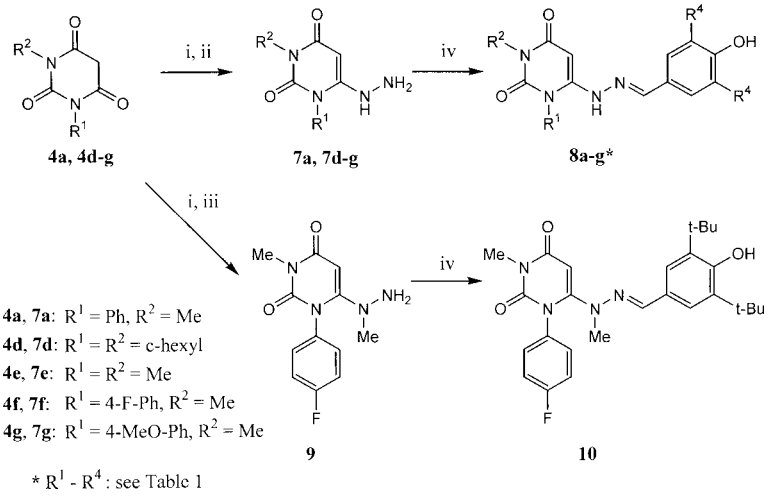


Reagents and conditions: i) 3,5-Di-*t*-butyl-4-hydroxybenzyl chloride, Et_3N, *i*-PrOH, reflux; ii) 3,5-di-*t*-butyl-4-hydroxybenzaldehyde, EtOH, reflux; iii) $NaBH_4$, EtOH, rt.

Chart 1



Reagents and conditions: i) $POCl_3$, reflux; ii) NH_2NH_2, *i*-PrOH, reflux; iii) $MeNHNH_2$, *i*-PrOH; iv) 3,5-disubstituted-4-hydroxybenzaldehyde, EtOH, 50 °C.

Chart 2

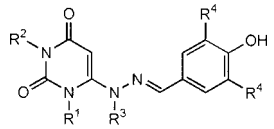
The antioxidative activities were measured by the inhibition toward auto-oxidation using rat brain homogenate.

Compounds **3**, **5**, **6** did not show efficacy against this *in vivo* model by oral administration (Inhibition of these compounds was less than 10%); however, compounds **1** and **5a** showed moderate inhibitory activities against this model by topical administration. The percent of inhibition of compounds **1** and **5a** at a dose of 1 mg/ear was 55% and 46%, respectively. Moreover, antioxidative activities of **1** and **5a** indicated IC_{50} of 33 and 34 μM, respectively, suggesting that compound **5a** did not exhibit the activities against PC-induced CHR owing to its poor oral bioavailability. Therefore, an amino group at the C(6) position and/or carboxamide as the linker between the uracil ring and the di-*t*-butylphenol moiety as shown in the lead compound (**1**) would be needed to exhibit the inhibitory activity by oral administration.

The inhibitory activities of 6-substituted uracil derivatives (**8a**—**g**, **10**) are summarized in Table 1. Compound **8a** showed inhibitory activity against this model by oral administration, but the inhibitory potency of **8a** was somewhat weaker than that of **1** at a dose of 10 mg/kg. The uracils (**8b**, **c**) possessing the dimethoxyphenol or the dimethylphenol instead of the di-*t*-butylphenol at the 6-position were synthesized in order to evaluate the contribution of the reactive oxygen on this *in vivo* model. The inhibitory activities of **8b** and **8c** were diminished, corresponding well with their results of weak antioxidative activities. Accordingly, we thought that the reactive oxygen contributed to the process of inflammation of this *in vivo* model. The 1-(4-fluorophenyl)uracil (**8f**) showed 27% inhibition at this dose, which was nearly equivalent with that of **8a**. On the other hand, compounds **8d**, **8e**, and **10** did not exhibit inhibitory activity at this dose. From these results, it was suggested that the phenyl group for R^1 and the hydrogen atom for R^3 were needed for exhibiting the inhibitory activities.

Next, we evaluated the dose-dependency of **8a** and **8f** on this model. Compound **8a** did not exhibit dose–dependent inhibition; the most potent inhibition was observed at a dose of 3 mg/kg (Fig. 2). The reason why the dose–response inhibition indicated a bell-shaped curve is not clear. Different from the result of **8a**, compound **8f** showed inhibition in a dose dependent manner against this model, suggesting modification

Table 1. Inhibitory Effects of 6-Benzylidenehydrazinouracils (**8a**—**g**, **10**) on PC-Induced CHR and Lipid Peroxidation



Compound	R^1	R^2	R^3	R^4	PC-induced CHR % of inhibition (10 mg/kg)	Lipid peroxidation IC_{50} (μM)
1					54	33
8a	Ph	Me	H	*t*-Bu	32	27
8b	Ph	Me	H	OMe	−8	>100
8c	Ph	Me	H	Me	−9	>100
8d	*c*-Hexyl	*c*-Hexyl	H	*t*-Bu	4	22
8e	Me	Me	H	*t*-Bu	16	32
8f	4-F-Ph	Me	H	*t*-Bu	27	29
8g	4-MeO-Ph	Me	H	*t*-Bu	−21	nd
10	Ph	Me	Me	*t*-Bu	10	20
Tranilast					−10[a)]	nd
Prednisolone					67	nd

nd: not determined. *a*) 200 mg/kg.

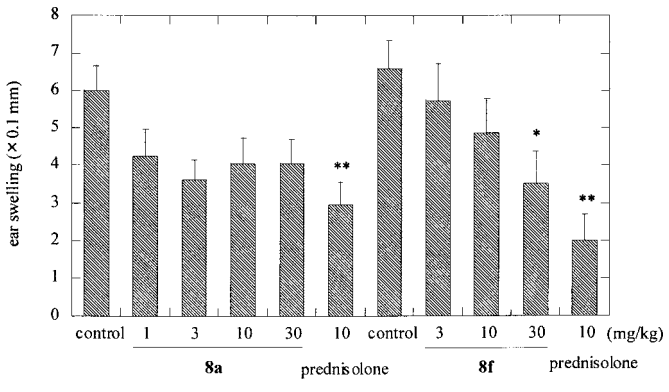


Fig. 2. Inhibitory Activities of **8a** and **8f** against PC-Induced CHR

of the N(1)-substituent at uracil would be possible to enhance the inhibitory potency. In addition, diarrhea, which was observed in mouse treated with **1**, was not monitored in **8a** or **8f** administered animals.

In conclusion, we have conducted a preliminary SAR study of some pyrimidine derivatives against the PC-induced CHR. In addition to the 5-substituted lead compound (**1**), the introduction of an antioxidative moiety to the C(6)-position of uracil was effective against this model. The inhibitory activity against PC-induced CHR was dependent on antioxidative activity and oral bioavailability.

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 or $CDCl_3$. 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 on Yanagimoto MT-3. Wakogel C-200 (Wako; 70—150 mm) was used for column chromatography. Monitoring of reactions was carried out by using Merck 60 F_{254} silica gel, glass-supported TLC plates, and visualization with UV light (254, 365 nm).

General Procedure for the Synthesis of 5-Benzylidenebarbituric Acids (5a—d) A mixture of barbituric acids (**4a**—**d**) (1.0 mmol) and 3,5-di-*t*-butyl-4-hydroxybenzaldehyde (1.0 mmol) in EtOH was refluxed for 1—3 h. After cooling, the resulting precipitate was filtered and recrystallized from an appropriate solvent to give **5a**—**d**.

5-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)-3-methyl-1-phenylbarbituric Acid (5a) Yield 66% (a mixture of *E* and *Z* isomer); mp 156—158 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.52, 8.61 (1H, s, Ar-C<u>H</u>=), 8.26, 8.32 (2H, s, ArH), 7.21—7.55 (5H, m, Ph), 6.02, 6.08 (1H, s, OH), 3.48 (3H, s, *N*-Me), 1.47 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 435 $(M+H)^+$; *Anal.* Calcd for $C_{26}H_{30}N_2O_4 \cdot 0.1H_2O$: C, 71.57; H, 6.98; N, 6.42. Found: C, 71.45; H, 7.12; N, 6.35.

5-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)-1-phenylbarbituric Acid (5b) Yield 98%; mp 295—296 °C; ^{1}H-NMR (DMSO-d_6) δ: 11.57 (1H, s, NH), 8.38 (1H, s, Ar-C<u>H</u>=), 8.33 (2H, s, ArH), 7.38—7.58 (5H, m, Ph), 1.48 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 421 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{28}N_2O_4$: C, 71.41; H, 6.71; N, 6.66. Found: C, 71.28; H, 6.84; N, 6.46.

1-Cyclohexyl-5-(3,5-di-*t*-butyl-4-hydroxybenzylidene)barbituric Acid (5c) Yield 96%; mp 223 °C; ^{1}H-NMR (DMSO-d_6) δ: 8.34 (1H, s, Ar-C<u>H</u>=), 8.31 (2H, s, ArH), 4.63 (1H, t, *J*=12.0 Hz, CH), 1.22—2.35 (10H, m, c-Hex), 1.49 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 427 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{34}N_2O_4$: C, 70.39; H, 8.03; N, 6.57. Found: C, 70.47; H, 8.13; N, 6.52.

1,3-Dicyclohexyl-5-(3,5-di-*t*-butyl-4-hydroxybenzylidene)barbituric Acid (5d) Yield 71%; mp 215—216 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.39 (1H, s, Ar-C<u>H</u>=), 8.14 (2H, s, ArH), 5.93 (1H, s, OH), 4.72 (2H, t, *J*=12.0 Hz, 2×CH), 1.24—2.42 (20H, m, 2×c-Hex), 1.47 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 509 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{44}N_2O_4$: C, 73.19; H, 8.72; N, 5.51. Found: C, 73.24; H, 9.07; N, 5.32.

General Procedure for the Synthesis of 5-Benzylbarbituric Acids (6a—d) To a suspension of 5-benzylidenebarbituric acids (**5a**—**d**) (1.0 mmol) in EtOH (10 ml) was added $NaBH_4$ (1.0 mmol). The reaction mixture was stirred at room temperature for 2 h, and was concentrated. Water (10 ml) was added to the residue and neutralized to pH 7 with 1 N HCl. The resulting precipitate was collected and washed with water to give **6a**—**d**.

5-(3,5-Di-*t*-butyl-4-hydroxybenzyl)-3-methyl-1-phenylbarbituric Acid (6a) Yield 92%; mp 147—149 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.39 (3H, m, Ph), 6.90 (2H, s, ArH), 6.76 (2H, m, Ph), 5.23 (1H, s, OH), 3.88—3.92 (2H, m, C<u>H</u>$_2$Ar), 3.50 (1H, Abq, *J*=23.2, 4.9 Hz, H-5), 3.29 (3H, s, *N*-Me), 1.40 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 437 $(M+H)^+$; *Anal.* Calcd for $C_{26}H_{32}N_2O_4$: C, 71.53; H, 7.39; N, 6.42. Found: C, 71.48; H, 7.43; N, 6.40.

5-(3,5-Di-*t*-butyl-4-hydroxybenzyl)-1-phenylbarbituric Acid (6b) Yield 65%; mp 162—164 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.27 (1H, s, NH), 7.36—7.39 (3H, m, Ph), 6.95 (2H, s, ArH), 6.74 (2H, m, Ph), 5.23 (1H, s, OH), 3.87 (2H, m, C<u>H</u>$_2$Ar), 3.46—3.55 (1H, m, H-5), 1.41 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 423 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{30}N_2O_4$: C, 71.07; H, 7.16; N, 6.63. Found: C, 71.01; H, 7.22; N, 6.56.

1-Cyclohexyl-5-(3,5-di-*t*-butyl-4-hydroxybenzyl)barbituric Acid (6c) Yield 75%; mp 159—160 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.25 (1H, s, NH), 6.87

(2H, s, ArH), 5.13 (1H, s, OH), 4.33 (1H, m, CH), 3.64—3.67 (2H, m, C$\underline{H}_2$Ar), 3.41 (1H, Abq, J=23.2, 4.9 Hz, H-5), 1.98—2.09 (2H, m, c-Hex), 1.62—1.77 (2H, m, c-Hex), 1.11—1.60 (6H, m, c-Hex), 1.41 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 429 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{36}N_2O_4$: C, 70.06; H, 8.47; N, 6.54. Found: C, 69.84; H, 8.69; N, 6.31.

1,3-Dicyclohexyl-5-(3,5-di-*t*-butyl-4-hydroxybenzyl)barbituric Acid (6d) Yield 85%; mp 148 °C; ^{1}H-NMR ($CDCl_3$) δ: 6.85 (2H, s, ArH), 5.10 (1H, s, OH), 4.46 (2H, m, 2×CH), 3.60 (1H, m, H-5), 3.37—3.39 (2H, m, C$\underline{H}_2$Ar), 2.10—2.20 (4H, m, c-Hex), 1.76—1.78 (4H, m, c-Hex), 1.18—1.65 (12H, m, c-Hex), 1.41 (18H, s, 2×*t*-Bu); MS (TOF): *m/z* 511 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{46}N_2O_4$: C, 72.91; H, 9.08; N, 5.49. Found: C, 73.00; H, 9.38; N, 5.29.

General Procedure for the Synthesis of 6-Benzylidenehydrazinouracils (8a—g) A mixture of barbituric acids (**4a**, **d**—**g**) in $POCl_3$ was refluxed for 2 h and concentrated. The residue was poured into crashed ice and the resulting precipitate was collected to give 6-chlorouracil derivatives, which were used to next step. A solution of 6-chlorouracil derivatives and hydrazine hydrate in 2-PrOH was refluxed for 30 min. The reaction mixture was concentrated to half volume and stood at room temperature overnight. The resulting precipitate was filtered and washed with water to give 6-hydrazinouracils (**7a**, **d**—**g**).[14—16] A mixture of the 6-hydrazinouracils and 4-hydroxybenzaldehyde derivatives in EtOH was heated 50 °C for 15 min. After cooling, the resulting precipitate was filtered and recrystallized from an appropriate solvent to give the hydrazones (**8a**—**g**).

6-[2-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)hydrazino]-3-methyl-1-phenyluracil (8a) Yield 96%; mp 258—260 °C (recrystallized from MeOH); ^{1}H-NMR (DMSO-d_6) δ: 9.13 (1H, s, NH), 8.20 (1H, s, N=CH), 7.46—7.63 (5H, m, Ph), 7.44 (2H, s, ArH), 5.57 (1H, s, H-5), 3.22 (3H, s, *N*-Me), 1.45 (18H, s, 2×*t*-Bu); MS (TOF) *m/z* 449 $(M+H)^+$; *Anal.* Calcd for $C_{26}H_{32}N_4O_3$: C, 69.62; H, 7.19; N, 12.49. Found: C, 69.35; H, 7.25; N, 12.41.

6-[2-(4-Hydroxy-3,5-dimethoxybenzylidene)hydrazine]-3-methyl-1-phenyluracil (8b) Yield 92%; mp 266—268 °C (recrystallized from MeOH); ^{1}H-NMR (DMSO-d_6) δ: 9.20 (1H, s, NH), 8.13 (1H, s, N=CH), 7.47—7.65 (5H, m, Ph), 6.95 (2H, s, ArH), 5.68 (1H, s, H-5), 3.86 (6H, s, 2×OMe), 3.23 (3H, s, *N*-Me); MS (TOF) *m/z* 429 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{20}N_4O_5$: C, 60.60; H, 5.09; N, 14.14. Found: C, 60.70; H, 5.17; N, 14.14.

6-[2-(4-Hydroxy-3,5-dimethylbenzylidene)hydrazino]-3-methyl-1-phenyluracil (8c) Yield 91%; mp 287—289 °C (recrystallized from DMF-EtOH); ^{1}H-NMR (DMSO-d_6) δ: 9.14 (1H, s, NH), 8.04 (1H, s, N=CH), 7.41—7.60 (5H, m, Ph), 7.23 (2H, s, ArH), 5.61 (1H, s, H-5), 3.18 (3H, s, *N*-Me), 2.20 (6H, s, 2×Me); MS (TOF) *m/z* 397 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{20}N_4O_3$: C, 65.92; H, 5.53; N, 15.38. Found: C, 65.87; H, 5.72; N, 15.45.

1,3-Dicyclohexyl-6-[2-(3,5-di-*t*-butyl-4-hydroxybenzylidene)hydrazino]uracil (8d) Yield 90%; mp 228—230 °C; ^{1}H-NMR (DMSO-d_6) δ: 10.23 (1H, s, NH), 8.35 (1H, s, N=CH), 7.53 (2H, s, ArH), 5.45 (1H, s, H-5), 4.71 (1H, m, CH), 4.08 (1H, m, CH), 2.38—2.59 (8H, m, c-Hex), 1.49 (18H, s, 2×*t*-Bu), 1.12—1.83 (12H, m, c-Hex); MS (TOF) *m/z* 523 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{46}N_4O_3$: C, 71.23; H, 8.87; N, 10.72. Found: C, 71.01; H, 8.95; N, 10.55.

6-[2-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)hydrazino]-1,3-dimethyluracil (8e) Yield 93%; mp 262—264 °C (recrystallized from MeOH); ^{1}H-NMR (DMSO-d_6) δ: 8.37 (1H, s, N=CH), 7.53 (2H, s, ArH), 5.48 (1H, s, H-5), 3.44 (3H, s, *N*(1)-Me), 3.22 (3H, s, *N*(3)-Me), 1.49 (18H, s, 2×*t*-Bu); MS (TOF) *m/z* 387 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{30}N_4O_3$: C, 65.26; H, 7.82; N, 14.50. Found: C, 65.48; H, 7.89; N, 14.58.

6-[2-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)hydrazino]-1-(4-fluorophenyl)-3-methyluracil (8f) Yield 98%; mp 250—251 °C (recrystallized from EtOH); ^{1}H-NMR (DMSO-d_6) δ: 7.52 (1H, s, N=CH), 7.43 (2H, s, ArH), 7.26—7.40 (4H, m, 4-F-Ph), 6.81 (1H, br s, NH), 5.94 (1H, s, OH), 5.54 (1H, s, H-5), 3.37 (3H, s, *N*-Me), 1.44 (18H, s, 2×*t*-Bu); MS (TOF) *m/z* 467 $(M+H)^+$; *Anal.* Calcd for $C_{26}H_{31}FN_4O_3$: C, 66.93; H, 6.70; N, 12.01. Found: C, 66.82; H, 6.77; N, 11.92.

6-[2-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)hydrazino]-1-(4-methoxyphenyl)-3-methyluracil (8g) Yield 71%; mp 276—277 °C (recrystallized from EtOH); ^{1}H-NMR (DMSO-d_6) δ: 9.14 (1H, s, NH), 8.24 (1H, s, N=CH), 7.44 (2H, s, ArH), 7.39 (2H, d, J=8.8 Hz, Ph), 7.17 (2H, d, J=8.8 Hz, Ph), 5.55 (1H, s, H-5), 3.92 (3H, s, OMe), 3.22 (3H, s, *N*-Me), 1.46 (18H, s, 2×*t*-Bu); MS (TOF) *m/z* 479 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{34}N_4O_4$: C, 67.76; H, 7.16; N, 11.71. Found: C, 67.59; H, 7.08; N, 11.67.

1-(4-Fluorophenyl)-3-methyl-6-(1-methylhydrazino)uracil (9) A solution of 6-chloro-1-(4-fluorophenyl)-3-methyluracil (1.02 g, 4 mmol) and methylhydrazine (5 ml) in 2-propanol (10 ml) was refluxed for 5 min. The reaction mixture was concentrated to dryness and the residue was triturated with water. The resulting precipitate was filtered to give 755 mg (72%) of **9**. Recrystallization from EtOH gave a pure sample, mp 192—193 °C. *Anal.* Calcd for $C_{12}H_{13}FN_4O_4$: C, 54.54; H, 4.96; N, 21.20. Found: C, 54.76; H, 4.98; N, 21.31.

6-[2-(3,5-Di-*t*-butyl-4-hydroxybenzylidene)-1-methylhydrazino]-1-(4-fluorophenyl)-3-methyluracil (10) Yield 60%; mp 235—236 °C (recrystallized from AcOEt/benzene); ^{1}H-NMR ($CDCl_3$) δ: 7.26—7.36 (5H, s, N=CH, Ph), 7.01—7.07 (2H, m, ArH), 5.64 (1H, s, OH), 5.42 (1H, s, H-5), 3.38 (3H, s, *N*(3)-Me), 3.01 (3H, s, *N*-Me), 1.45 (18H, s, 2×*t*-Bu); MS (TOF) *m/z* 481 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{33}FN_4O_3$: C, 67.48; H, 6.92; N, 11.66. Found: C, 67.71; H, 6.93; N, 11.47.

Picryl-Chloride Induced Contact Hypersensitivity Reaction[18] 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 orally administered 1 h prior to the challenge, or dissolved in acetone and were administered 5 min after the challenge.

Lipid Peroxidation Rat brain was homogenated in 9 volumes of 0.1 M phosphate buffer (pH 7.4) at 4 °C with a Polytron homogenizer. After elimination of tissue debris by centrifugation at 120×***g*** for 5 min, the supernatant was used for the assay. The test compound in DMSO was added to the homogenate on ice, and the mixture was incubated for 1 h at 37 °C. The detection of lipid peroxidation products in the homogenate was performed by monitoring thiobarbituric acid-reactive substances according to the method of Ohkawa *et al.*[19] Sodium thiobarbituric acid 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, which was then mixed vigorously. The absorbance of the thiobarbituric acid-reactive substances extracted in the organic layer was determined at 532 nm, and the level of lipid peroxides was expressed as malondialdehyde concentration by using an external malondialdehyde standard.

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