Inhibitory Activities of Novel Pyrimidine Derivatives on the Contact Hypersensitivity Reaction

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In order to obtain novel topically applied anti-inflammatory compounds containing an inexpensive anti-oxidative moiety without chirality, we synthesized compound 2c derivatives having a di-*tert*-butylphenol moiety, and evaluated by topical administration their anti-inflammatory potentials on picryl chloride-(PC) induced contact hypersensitivity reaction (CHR) in mice. In the course of our structure–activity relationship (SAR) studies on the pyrimidine or the anti-oxidative moiety and the linker between them, the most potent compounds (10, 11) were obtained by the insertion of a C2 unit in compound 2c. The potencies of these compounds were 2-fold greater than that of 1. Compounds 10 and 11 were considered to be useful lead compounds having inexpensive anti-oxidative moieties without chirality.

Key words pyrimidine derivative; CX-659S; contact hypersensitivity reaction (CHR); anti-oxidative activity

We recently found that a novel pyrimidine derivative, CX-659S (**1**) [(*S*)-6-amino-5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxamido)-3-methyl-1-phenyl-2,4(1*H*,3*H*)-pyrimidinedione] (Fig. 1), had inhibitory activities against a variety of acute and chronic inflammation models when topically applied.[2—4)] This compound, having a tocopherol-related moiety with a non-steroidal structure, exerted a potent radical scavenging activity and inhibitory effects on lipid peroxidation both *in vitro* and *in vivo*.[5)]

Generally, a chiral compound such as CX-659S has many difficulties for the development of it as a new drug candidate. For example, we must establish an efficient, low cost, synthetic method to prepare it, and conduct a comparative study on its pharmacology and toxicology and so on by using each isomer (*R* or *S*) and racemate. Moreover, from the viewpoint of pharmacokinetics, we must confirm whether one isomer converts into the other one in the body or not.

In the present study, to avoid the problems mentioned above, we sought to find a new type of compound with a simple structure without chirality. Typical antioxidants without chirality are the polyphenols such as pyrogallol[6)] and methoxyphenol.[7)] So, we synthesized compounds **2a**[2)] and **2b**, having partial antioxidant structures, and evaluated their inhibitory activities toward picryl chloride (PC)-induced contact hypersensitivity reaction (CHR) in mice by the topical administration. The anti-inflammatory activities of **2a** and **2b** were almost equipotent with that of **1** (Table 1). Compounds **2a** and **2b** were evaluated as their acetate form, because their free acids were poorly soluble in application solvents such as acetone, ethyl acetate, and EtOH, and could not be used for the evaluation of anti-inflammatory activity in this study. Moreover, as both compounds were found to be unstable due to mainly their deacetylation under the moisture condition (data not shown), we judged that compounds **2a** and **2b** were not suitable as lead compounds. On the other hand, **2c**,[2)] having a di-*tert*-butylphenol moiety, showed a moderate inhibitory activity (ED_{50}=0.81 mg/ear), which was 3-fold weaker than that of **1**. The di-*tert*-butylphenol moiety is also well-known to have an anti-oxidative activity,[8)] and is widely used as an inexpensive anti-oxidative moiety without chirality. In the present study, we report and discuss our preliminary structure–activity relationship (SAR) study on **2c** derivatives as a new type anti-inflammatory drug.

Chemistry

The compounds described in this study are shown in Table 1, and their synthetic methods are outlined in Charts 1, 2. The syntheses of analogues were accomplished by the following pathway using the reported methods. Nitrosation of **14** with sodium nitrite followed by catalytic hydrogenation of the nitroso group furnished **15**.[9,10)] Compounds (**2**—**5**, **12**, **13**) were obtained by the coupling of **15** with the corresponding carboxylic acids by using diphenylphosphoryl chloride (DPP-Cl) as a condensation reagent. Compound **8** was afforded by the reaction of **14** with 2,6-di-*tert*-butyl-4-hydroxybenzyl chloride in the presence of Et_3N. The reduction of **2c** with $BH_3 \cdot Me_2S$ gave **9**. Compounds **10** was prepared by the condensation of **15** (R^4=Ph) with 2,6-di-*tert*-butyl-4-hydroxycinnamic acid, and the hydrogenation of **10** gave **11**.

For preparation of the dimethylamino derivative of **2c** (**7**), nitration of **14** was needed instead of nitrosation. But the nitration of **14** gave a di-nitro compound (nitration proceeded at not only the C(5) position of pyrimidine, but also at the *para* position of the phenyl group). To avoid this, we used 4-fluorophenyl compound. The conversion of **16** with phosphorus oxychloride, followed by the treatment with methylamine or dimethylamine, led to **17** or **20**, respectively. Treatment of **20** with sodium nitrate in sulfuric acid followed by catalytic hydrogenation provided **22**. The condensation of **19** or **22** with 2,6-di-*tert*-butyl-4-hydroxybenzoic acid afforded **6** or **7**,

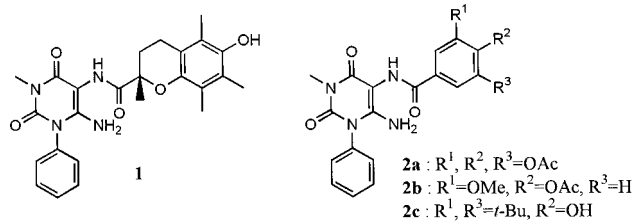

Fig. 1. The Structures of **1** and **2a**—**c**

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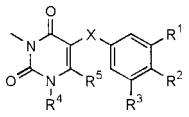
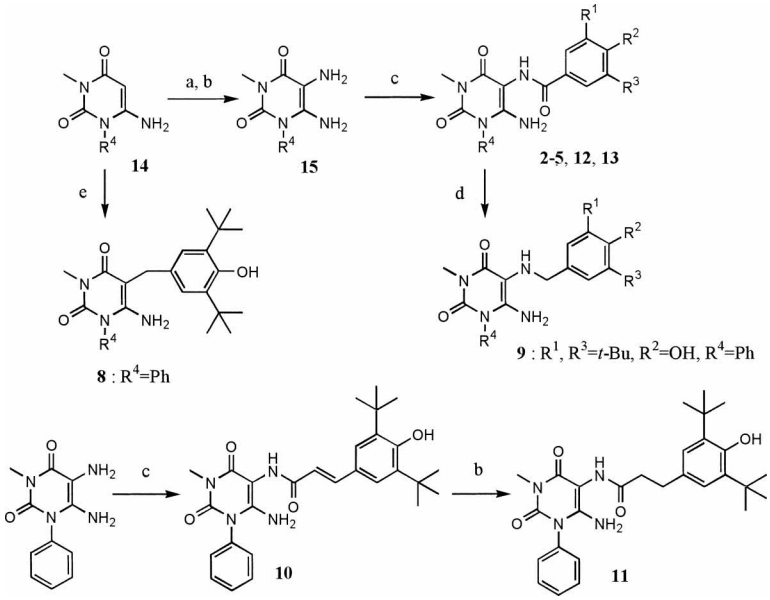
Table 1. Inhibitory Effects of the Test Compounds on PC-Induced CHR in Mice



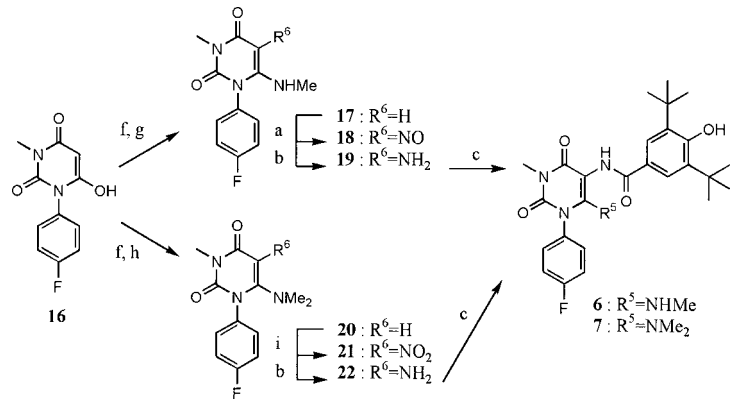
No	R^4	R^5	X	R^1	R^2	R^3	ED_{50} [a)]	AlogP
1							0.29	
2a	Ph	NH_2	NHCO	OAc	OAc	OAc	0.21	
2b	Ph	NH_2	NHCO	OMe	OAc	H	0.27	
2c	Ph	NH_2	NHCO	*tert*-Bu	OH	*tert*-Bu	0.81	4.86
3	Me	NH_2	NHCO	*tert*-Bu	OH	*tert*-Bu	4%	3.28
4	1-Napth	NH_2	NHCO	*tert*-Bu	OH	*tert*-Bu	0.31	5.77
5	4-F-Ph	NH_2	NHCO	*tert*-Bu	OH	*tert*-Bu	0.58	5.07
6	4-F-Ph	NHMe	NHCO	*tert*-Bu	OH	*tert*-Bu	0.25	5.27
7	4-F-Ph	NMe_2	NHCO	*tert*-Bu	OH	*tert*-Bu	0.26	5.48
8	Ph	NH_2	CH_2	*tert*-Bu	OH	*tert*-Bu	nd	6.57
9	Ph	NH_2	$NHCH_2$	*tert*-Bu	OH	*tert*-Bu	20%	5.52
10	Ph	NH_2	NHCOCH=CH	*tert*-Bu	OH	*tert*-Bu	0.14	5.22
11	Ph	NH_2	$NHCO(CH_2)_2$	*tert*-Bu	OH	*tert*-Bu	0.13	5.35
12[2)]	Ph	NH_2	NHCO	Me	OAc	Me	0%	3.07
13	Ph	NH_2	NHCO	H	OAc	H	17%	2.09
Prednisolone							0.03	

a) ED_{50} (mg/ear) or % inhibition at 1 mg/ear. nd; not determined owing to its poor solubility in application solvents.



Reagents and conditions: (a) $NaNO_2$, 12 N HCl, H_2O, (b) 5% Pd/C, H_2, MeOH, (c) R–COOH, DPP–Cl, Et_3N, CH_2Cl_2, then **15**, Et_3N, (d) $BH_3 \cdot Me_2S$, THF, reflux, (e) 3,5-di-*tert*-butyl-4-hydroxybenzyl chloride, Et_3N, 2-PrOH, reflux.

Chart 1



Reagents and conditions : (f) $POCl_3$, reflux, (g) $MeNH_2$, EtOH, reflux, (h) Me_2NH, EtOH, reflux, (i) $NaNO_3/H_2SO_4$.

Chart 2

respectively.

Results and Discussions

We examined the inhibitory effects of topically applied test compounds on PC-induced CHR in mice,[11] and investigated the relationship between the anti-inflammatory activity and the lipophilicity (AlogP) with regard to the skin tissue permeability. AlogP values were computed by using a Cerius 4.6 AlogP98 descriptor.[12]

We first examined the substituent at the N(1)-position of the pyrimidine ring of **2c**. As shown in Table 1, the inhibitory activity was diminished when a methyl group (**3**) replaced the phenyl one (**2c**) at this position, whereas the inhibitory effect with the 1-naphthyl group (**4**) or 4-fluorophenyl one (**5**) was increased. We could not observe the relationship between the anti-oxidative activity and the inhibitory activity on PC-induced CHR, because anti-oxidative activities (Fenton reaction) of these compounds were nearly the same (data not shown). On the other hand, the order of potency of these compounds was well consistent with that of the calculated AlogP value, suggesting that the increase in lipophilicity of the compounds may be contributed to enhanced skin tissue permeability. Next, to further elevate the lipophilicity of **5**, we changed the amino group at the C(6)-position to the methylamino group (**6**) or the dimethylamino one (**7**). The activities of **6** and **7** were 2-fold more potent than that of **5** at the most. These results show the limitation of enhancing the inhibitory activity by improving the AlogP value. In addition, to improve the lipophilicity, we changed the linker moiety between the pyrimidine ring and di-*tert*-butylphenol. Especially, the hint of the distance between the hydroxy group at the chroman and the pyrimidine moiety of **1** prompted us to synthesize **10** and **11**. Both **10** and **11** showed the most potent inhibitory activities, with an ED_{50} value of 0.14 mg/ear for **10** and that of 0.13 mg/ear for **11**. As shown in Fig. 2, the inhibitory activities of **10** and **11** were about 3-fold greater than that of **1** at a dose of 0.1 mg/ear. In spite of compound **9**, having a high lipophilicity, its inhibitory activity was significantly diminished compared with **2c**. We considered that an active NH group at the C(5) of **9** might react with some proteins before reaching to inflamed tissues, therefore, causing **9** to have poor inhibitory activity. These findings suggest that the presence of the carbonyl moiety in the **2c**-type compounds may be needed to exhibit the inhibitory activity and that a favorable AlogP value (around 5.0—5.5) would potentiate the inhibitory activity.

In conclusion, we conducted a preliminary SAR study on topically applied **2c** derivatives with respect to anti-inflammatory activity, and found that the inhibitory activities were improved with an increase in the AlogP value. Among the compounds, **10** and **11** showed 2-fold more potent activity than **1**. They could be considered to be useful lead compounds, having inexpensive anti-oxidative moieties without chirality. A study for further optimization is still underway.

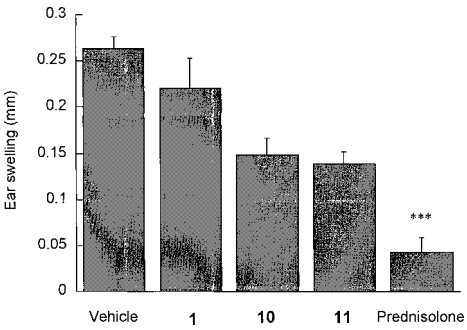


Fig. 2. The Inhibitory Activities of **1**, **10** and **11** on PC-Induced CHR at a Dose of 0.1 mg/ear

***$p<0.001$ *versus* vehicle (Dunnet's test).

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) were recorded on a Compact MALDI 3 V4.0.0 spectrometer. 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 by using Merck 60 F_{254} silica gel, glass-supported TLC plates, and visualization with UV light (254, 365 nm). The 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), and HOBt (1-hydroxybenzotriazole).

5-(4-Acetoxy-3-methoxyphenylcarboxamido)-6-amino-3-methyl-1-phenyl-2,4-(1*H*,3*H*)-pyrimidinedione (2b) To a solution of 4-acetoxy-3-methoxybenzoic acid (200 mg, 1.0 mmol) and Et_3N (0.16 ml, 1.2 mmol) in EtOAc (10 ml), DPP-Cl (0.24 ml, 1.2 mmol) was added. After 1 h of stirring at room temperature, **15** (190 mg, 0.9 mmol) was added, and the mixture was then stirred for 12 h at room temperature. After partitioning with water (10 ml), the organic layer was dried over $MgSO_4$ and concentrated. The residue was sonicated in EtOAc to give **2b** as a white solid (230 mg, 66%). mp 160—162 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.01 (1H, s), 7.72 (1H, s), 7.53—7.72 (4H, m), 7.34—7.38 (2H, m), 7.19 (1H, d, J=8.1 Hz), 6.08 (2H, s), 3.85 (3H, s), 3.16 (3H, s), 2.28 (3H, s). MS (TOF): *m/z* 425 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{20}N_4O_6\cdot H_2O$: C, 57.01; H, 5.01; N, 12.66. Found: C, 57.24; H, 4.73; N, 12.66.

Compounds **3**—**5**, **10**, **13** were also prepared by using the same procedure as for **2b**.

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxyphenylcarboxamido)-1,3-dimethyl-2,4-(1*H*,3*H*)-pyrimidinedione (3) Yield 77%. mp 234—236 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.74 (1H, s), 7.74 (2H, s), 7.38 (1H, s), 6.62 (2H, s), 3.31 (3H, s), 3.13 (3H, s), 1.42 (18H, s). MS (TOF): *m/z* 403 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{30}N_4O_4\cdot 1.5H_2O$: C, 58.72; H, 7.74; N, 13.04. Found: C, 58.59; H, 7.34; N, 12.81.

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxyphenylcarboxamido)-3-methyl-1-(1-naphthyl)-2,4-(1*H*,3*H*)-pyrimidinedione (4) Yield 53%. mp 172—174 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.85 (1H, s), 8.08—8.14 (2H, m), 7.77 (2H, s), 7.57—7.71 (5H, m), 7.39 (1H, s), 6.03 (2H, s), 3.19 (3H, s), 1.42 (18H, s). MS (TOF): *m/z* 515 $(M+H)^+$. *Anal.* Calcd for $C_{30}H_{34}N_4O_4\cdot 0.7H_2O$: C, 68.53; H, 6.65; N, 10.66. Found: C, 68.20; H, 6.77; N, 10.66.

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxyphenylcarboxamido)-1-(4-fluorophenyl)-3-methyl-2,4-(1*H*,3*H*)-pyrimidinedione (5) Yield 71%. mp 231—233 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.75 (1H, s), 7.73 (2H, s), 7.37 (2H, m), 7.41 (2H, m), 6.03 (2H, s), 3.15 (3H, s), 1.41 (18H, s). MS (TOF): *m/z* 483 $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{31}FN_4O_3\cdot 0.6H_2O$: C, 63.30; H, 6.58; N, 11.36. Found: C, 63.09; H, 6.40; N, 11.31.

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxycinnamoylamido)-3-methyl-1-phenyl-2,4-(1*H*,3*H*)-pyrimidinedione (10) Yield 36%. mp 178—180 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.57—7.62 (4H, m), 7.49 (1H, br s), 7.37—7.40 (4H, m), 6.50 (1H, d, J=15.7 Hz), 5.53 (2H, br s), 5.50 (1H, s), 3.40 (3H, s), 1.46 (18H, s). MS (TOF): *m/z* 491 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{38}N_4O_4\cdot H_2O$: C, 66.12; H, 7.13; N, 11.02. Found: C, 66.33; H, 6.84; N, 11.01.

5-(4-Acetoxyphenylcarboxamido)-6-amino-3-methyl-1-phenyl-2,4-(1*H*,3*H*)-pyrimidinedione (13) Yield 63%. mp 156—157 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.99 (1H, s), 8.02 (2H, dd, J=7.2, 1.8 Hz), 7.53—7.58 (3H, m), 7.36 (2H, m), 7.23 (2H, d, J=7.2 Hz), 6.09 (2H, s), 3.16 (3H, s), 2.30 (3H, s). MS (TOF): *m/z* 395 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{18}N_4O_5\cdot H_2O$: C, 58.25; H, 4.89; N, 13.59. Found: C, 58.15; H, 4.76; N, 13.52.

1-(4-Fluorophenyl)-3-methyl-6-methylamino-2,4-(1*H*,3*H*)-pyrimidinedione (17) A solution of **16** (3.1 g, 13.1 mmol) in $POCl_3$ (30 ml) was re-

fluxed for 1 h. The solvent was concentrated and then partitioned with CH_2Cl_2 (100 ml) and water (100 ml). And the organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give 6-chloro compound as a pale yellow solid (3.37 g, 100%). A suspension of this solid (0.95 g, 3.7 mmol) and 40% methylamine (40% in H_2O, 4 ml) in 2-PrOH (40 ml) was refluxed for 3 h. After cooling, the resulting precipitate was collected and washed with EtOH to give **17** as a white solid (0.82 g, 88%).

5-(3,5-Di-*tert*-butyl-4-hydroxyphenylcarboxamido)-1-(4-fluorophenyl)-3-methyl-6-methylamino-2,4-(1*H*,3*H*)-pyrimidinedione (6) To a suspension of **17** (0.72 g, 2.9 mmol) in H_2O (20 ml), sodium nitrate (0.24 g, 3.5 mmol) and 12 N HCl (0.7 ml) was added, and the mixture was stirred for 2 h at room temperature. The resulting solid was collected and washed with H_2O to give **18** as a violet solid (0.54 g, 68%). A suspension of **18** (0.5 g, 1.8 mmol) and 5% Pd/C (25 mg) in MeOH (30 ml) was stirred under a H_2 atmosphere for 12 h. The catalyst was removed by the filtration, and the solution was concentrated to give crude **19**. Compound **19** was used for the next step without further purification. A solution of 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (60 mg, 0.24 mmol) and HOBt (36 mg, 0.26 mmol) in DMF (5 ml) was stirred at room temperature for 1 h. To this solution, **19** (100 mg, 0.37 mmol) and EDC·HCl (51 mg, 0.26 mmol) were added, and this mixture was stirred for 12 h. The solvent was concentrated and partitioned with CH_2Cl_2 and 1 N HCl, then the organic layer was washed with 5% $NaHCO_3$ solution and water, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH=100 : 1), and recrystallized from EtOAc/hexane to give **6** (36 mg, 19%) as a white solid. mp 154—157 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.75 (2H, s), 7.38—7.43 (3H, m), 7.22 (2H, d, J=7.3 Hz), 5.63 (1H, s), 4.49 (1H, br s), 3.36 (3H, s), 2.67 (3H, d, J=5.4 Hz), 1.47 (18H, s). MS (TOF): m/z 497 $(M+H)^+$. High resolution (HR)-MS. Calcd for $C_{27}H_{33}FN_4O_4$: 496.2486; Found: 496.2489.

6-Dimethylamino-1-(4-fluorophenyl)-3-methyl-2,4-(1*H*,3*H*)-pyrimidinedione (20) A suspension of 6-chloro compound prepared from **16** (0.95 g, 3.7 mmol) and dimethylamine (50% in H_2O, 1.64 ml) in 2-PrOH (30 ml) was refluxed for 8 h. After concentration, water (20 ml) was added, and the resulting solid was collected to give **20** as a white solid (0.87 g, 89%).

5-(3,5-Di-*tert*-butyl-4-hydroxyphenylcarboxamido)-6-dimethylamino-1-(4-fluorophenyl)-3-methyl-2,4-(1*H*,3*H*)-pyrimidinedione (7) To a solution of **20** (0.77 g, 2.9 mmol) in sulfuric acid (17 ml), sodium nitrite (0.26 g, 3.1 mmol) was added and the mixture was stirred on ice for 1 h. The resulting solid was collected and washed with water to give **21** as a yellow solid (0.78 g, 87%). A suspension of **21** (0.7 g, 2.3 mmol) and 5% Pd/C (35 mg) in MeOH (30 ml) was stirred under a H_2 atmosphere for 12 h. The catalyst was removed by the filtration, and the solution was concentrated to give crude **22**. Compound **22** was used for the next step without further purification. A solution of 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (58 mg, 0.23 mmol) and HOBt (34 mg, 0.25 mmol) in DMF (10 ml) was stirred at room temperature for 1 h. To this solution, **22** (100 mg, 0.35 mmol) and EDC·HCl (49 mg, 0.25 mmol) were added, and this mixture was stirred for 12 h. The solvent was concentrated and partitioned with CH_2Cl_2 and 1 N HCl, then the organic layer was washed with 5% $NaHCO_3$ solution and water, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH=100 : 1) to give **7** as a white solid (38 mg, 21%). mp 164—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.70 (2H, s), 7.31 (2H, d, J=8.1 Hz), 7.18 (2H, d, J=8.1 Hz), 5.61 (1H, s), 3.36 (3H, s), 2.50 (6H, s), 1.47 (18H, s). MS (TOF): m/z 511 $(M+H)^+$. HR-MS. Calcd for $C_{28}H_{35}FN_4O_4$: 510.2642; Found: 510.2649.

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-3-methyl-1-phenyl-2,4-(1*H*,3*H*)-pyrimidinedione (8) To a solution of **14** (1.1 g, 5.1 mmol) and Et_3N (10 ml) in 2-PrOH (100 ml), 2,6-di-*tert*-butyl-4-hydroxybenzyl chloride (1.9 g, 7.5 mmol) was added, and the mixture was refluxed for 30 h. The solvent was concentrated and then triturated with hexane to give a crude solid. The solid was washed with water and recrystallized from EtOH/water to give **8** (1.31 g, 59%) as a white solid. mp 280—282 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.39—7.54 (5H, m), 7.00 (2H, s), 6.63 (1H, s), 5.77 (2H, s), 3.58 (2H, s), 3.13 (3H, s), 1.32 (18H, s). MS (TOF): m/z 436 $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{33}N_3O_3$: C, 71.49; H, 7.58; N, 9.61. Found: C, 71.70; H, 7.64; N, 9.65.

6-Amino-5-(3,5-di-*tert*-butyl-4-hydroxybenzylamino)-3-methyl-1-phenyl-2,4-(1*H*,3*H*)-pyrimidinedione (9) To a solution of **2c** (700 mg, 1.5 mmol) in THF (10 ml), borane–methylsulfide complex (2 M solution in THF, 3 ml) was added, and the mixture was refluxed for 3 h under a N_2 atmosphere. The solvent was concentrated, partitioned with CH_2Cl_2 (20 ml) and water (20 ml), the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH=100 : 1) to give **9** (450 mg, 67%) as a white solid. mp 153—155 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.50—7.54 (3H, m), 7.23—7.26 (2H, m), 7.07 (2H, s), 6.78 (1H, s), 5.52 (2H, s), 6.08 (2H, s), 3.76 (2H, s), 3.15 (3H, s), 1.36 (18H, s). MS (TOF): m/z 451 $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{34}N_4O_3$: C, 69.31; H, 7.61; N, 12.43. Found: C, 69.22; H, 7.71; N, 12.45.

6-Amino-5-[2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-ethylcarboxamido]-3-methyl-1-phenyl-2,4-(1*H*,3*H*)-pyrimidinedione (11) A solution of **10** (140 mg, 0.28 mmol) and 5% Pd/C (7 mg) in MeOH (6 ml) was stirred under a H_2 atmosphere for 12 h. The catalyst was removed by the filtration, and the solution was concentrated. Recrystallized from CH_2Cl_2/hexane to give **11** as a white solid (110 mg, 79%). mp 130—132 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.55—7.69 (3H, m), 7.33—7.36 (2H, m), 7.22 (1H, br s), 7.01 (2H, s), 5.08 (1H, s), 5.02 (2H, br s), 3.36 (3H, s), 2.94 (2H, t, J=7.8 Hz), 2.70 (2H, q, J=7.8 Hz), 1.41 (18H, s). MS (TOF): m/z 493 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{36}N_4O_4 \cdot 0.4H_2O$: C, 67.28; H, 7.42; N, 11.21. Found: C, 67.46; H, 7.56; N, 10.92.

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 between the left and right ear was calculated. Test compounds were dissolved in acetone and were administered 5 min after the challenge.

References and Notes

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