

Synthesis and Antiallergic Activity of Novel Azaazulene Derivatives

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Various azaazulene derivatives were synthesized and their antiallergic activity was examined. The structure–activity relationship among various derivatives modified by introducing substituents at the 1-, 2-, or 3-position of the azaazulene ring was investigated.

The inhibitory activities on allergic histamine release of the compounds bearing a 5-tetrazolyl group at the 3-position were more potent than those of the corresponding compounds with other groups (CN, COOH, and CHO). The compounds substituted with amino, azide and carboxymethylamino groups at the 2-position showed strong inhibitory activity. The compounds with various phenylalkyl groups at the 1-position showed a greater activity than those with other substituents. Among the compounds with substituents at the 1-, 2-, or 3-position of the azaazulene ring, 1-benzyl-7-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (18f) and 1-(4-fluorobenzyl)-7-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (19c) had the most potent inhibitory activities on histamine release from mast cells and on passive cutaneous anaphylaxis (PCA) in rats after oral administration ($ED_{50} = 0.56$ and 0.58 mg/kg, respectively).

Keywords azaazulene derivative; antiallergic agent; histamine release; passive cutaneous anaphylaxis; structure–activity relationship

Azulene derivatives have been used in the treatment of peptic ulcers, but little is known about the pharmacological action of azaazulene derivatives. We have synthesized various azaazulene derivatives and evaluated their antiallergic activity. Among these compounds, only 1-benzyl-7-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (18f) and 1-(4-fluorobenzyl)-7-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (19c) exhibited potent *in vivo* antiallergic activity as well as *in vitro* inhibitory activity on histamine release.

Compounds 1–15 were synthesized as shown in Charts 1 and 2. Compound 1 was prepared by the treatment of tropolone with anhydrous potassium carbonate and methyl iodide in dry acetonitrile in the presence of dicyclohexyl-18-crown-6.¹⁾ Compound 2 was obtained through cyclocondensation of compound 1 with sodium and α -cyanoacetamide in EtOH.²⁾ Compound 3 was produced by the treatment of 2-aminotropolone with sodium and diethyl malonate in EtOH.³⁾ Compound 4 was obtained after treatment of compound 3 with sodium hydroxide.²⁾ It was converted into the acid chloride by treatment with thionyl chloride, followed by reaction with heterocyclic amines to give 5. Treatment of 2 or 3 with concentrated hydrobromic acid afforded 6.^{2,4)} Compound 7 was obtained by the Mannich reaction of 6 and converted into the quaternary salt by treatment with methyl iodide followed by potassium cyanide.⁵⁾ Compound 8 was produced by the treatment of 7 with sodium azide and anhydrous aluminum chloride in tetrahydrofuran (THF). Compound 6 was transformed to 9 by means of the Vilsmeier reaction, while preparation of 10 was carried out by use of the Friedel–Crafts reaction.⁴⁾ The synthesis of 11 proceeded through an analogous route as shown in Chart 2, using hinokitiol instead of tropolone. Cyclocondensation of the regioisomer 11 with sodium and α -cyanoacetamide gave the azaazulene derivatives 12 and 13, resulting in a pair of regioisomers, which were separated by silica gel chromatography. Compound

15 was produced by the treatment of the isolated 12, 13, or 2 with sodium azide and anhydrous aluminum chloride in THF. The physicochemical properties and histamine release-inhibitory activities of 2–15 are shown in Table I.

Compounds 17–19 were synthesized as shown in Chart 3. Compound 2, 12, or 13 was treated with anhydrous potassium carbonate or sodium hydride and a corresponding alkyl halide to give compounds 16. Phenylalkyl derivatives 16 were treated with sodium azide and anhydrous aluminum chloride in THF to give 17, 18, and 19. The physicochemical properties and histamine release-inhibitory activities of 17, 18, and 19 are shown in Tables II, III, and IV, respectively. Compounds 21 were synthesized as shown in Chart 4. After treatment of 2, 12, or 13 with phosphorus oxychloride, compound 20 was obtained,²⁾ and treatment of 20 with a suitable alkylamine, arylamine or amino acid and NaN_3 in dimethylformamide (DMF) gave 21. The physicochemical properties and histamine release-inhibitory activities of compounds 21 are shown in Table V.

The position of the isopropyl group in 12 and 13 was determined by preparing 12 by another route that required hinokitiol methyl ether 24 as a key intermediate; it was synthesized by utilizing a blocking substituent on the ring (Chart 5).

Compound 22 was produced by the treatment of hinokitiol with iodine and anhydrous potassium carbonate. Compound 22 was alkylated with anhydrous potassium carbonate and methyl iodide in the presence of dicyclohexyl-18-crown-6 to afford 23. The iodide 23 was dissolved in MeOH containing sodium acetate and 5% Pd–C, and the resulting solution was stirred under an H_2 stream to give 24.⁶⁾ Compound 12 was produced by the treatment of 24 with sodium and α -cyanoacetamide in EtOH.

Results and Discussion

To evaluate the relationship between the structure and

TABLE I. Physicochemical and Pharmacological Data for 3-Substituted Azaazulene Derivatives

Compound No.	Z	R ¹	mp (°C)	MS (<i>m/z</i>)	Yield (%)	Inhibition (%) ^{a)} (10 µg/ml)
2	H	CN	> 290	170 (M ⁺)	75.0	62
3	H	COOC ₂ H ₅	183.5—185.0	217 (M ⁺), 171, 145	53.3	—5
4	H	COOH	200.0—202.0	189 (M ⁺), 172, 145	92.0	—9
5a	H	CONH-	203.0—205.5 (dec.)	265 (M ⁺), 247, 172, 145	38.7	16
5b	H	CONH-	204.5—206.5 (dec.)	256 (M ⁺), 215	43.0	—5
6	H	H	163.0—164.0	145 (M ⁺), 117	74.5	0
7	H	CH ₂ CN	174.0—176.5 (dec.)	187 (M ⁺), 155, 145	50.0	—4
8	H	CH ₂ -	225.0—228.0 (dec.)	227 (M ⁺), 185	50.0	26
9	H	CHO	273.0—275.0	173 (M ⁺), 145	59.3	35
10a	H	COCH ₃	293.5—294.5 (dec.)	187 (M ⁺), 172, 145	51.0	2
10b	H	COC ₆ H ₄ F(<i>p</i>)	299.5—300.5 (dec.)	267 (M ⁺), 214, 199, 172	67.6	—9
12	5-iso-Pr	CN	238.0—240.0	212 (M ⁺)	40.0	50
13	7-iso-Pr	CN	187.0—189.0	212 (M ⁺)	26.4	45
14	7-iso-Pr	H	146.0—149.0	187 (M ⁺), 172	79.5	—17
15a	H		> 290	213 (M ⁺), 170, 157	87.6	90
15b	5-iso-Pr		284.0—286.0 (dec.)	255 (M ⁺), 212, 199, 171	90.0	79
15c	7-iso-Pr		> 290	255 (M ⁺), 212, 199, 171	90.0	81

a) Effect on allergic histamine release from rat peritoneal exudate cells (PEC).

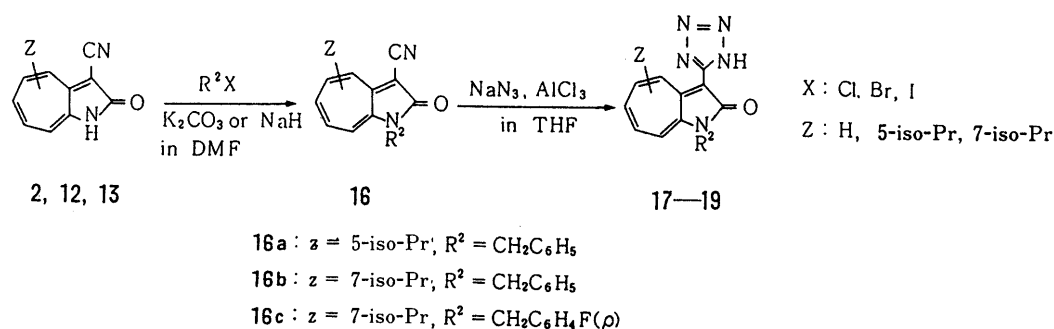
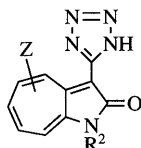


Chart 3

antiallergic activity of the newly synthesized azaazulene derivatives, their inhibitory activity on histamine release from peritoneal exudate cells (PEC) was examined. The results are summarized in Tables I—V. Table I shows the effects of substituents at the 3-position of the azaazulene ring. Among 3-substituted compounds (**2**—**15**), those with a 5-tetrazolyl (**15**), CN (**2**, **12**, **13**) or CHO (**9**) group showed activity. In particular, the compound with a 5-tetrazolyl group showed stronger activity. However, the 5-tetrazolyl aminocarbonyl derivative (**5b**) or 5-tetrazolyl methyl derivative (**8**) showed weaker activity.

In order to assess the effect of 1-alkyl substitution on the histamine release-inhibitory activity, various 1-alkyl substituted derivatives (**17a**—**f**) with a 5-tetrazolyl group were prepared and evaluated (Table II). The longer alkyl chain derivatives (**17a**—**c**) showed weaker activity, and the optimal activity was observed with the butyl group (**17b**). The activity of compounds with an alkyl chain containing a heteroatom (**17d**, **17e**) or double bond (**17f**) was slightly decreased. Next, we examined the activity of compounds with an alkyl group substituted with a phenyl group, which is more lipophilic. The results are shown in Table III.

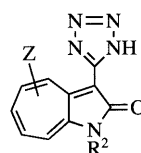
TABLE II. Physicochemical and Pharmacological Data for 1-Substituted Azaazulene Derivatives



Compd. No.	Z	R ²	mp (°C)	MS (m/z)	IR (KBr) cm ⁻¹	Yield (%)	Inhibition ^{a)} (%) (1 µg/ml)
17a	H	CH ₃	> 290	227 (M ⁺), 184, 171	3150—2850, 1670—1650	95.0	62.0
17b	H	(CH ₂) ₃ CH ₃	228—230 (dec.)	269 (M ⁺), 226, 213, 170	3150—2850, 1650	71.4	75.8
17c	H	(CH ₂) ₅ CH ₃	202—204 (dec.)	297 (M ⁺), 254, 241, 212	3160—2850, 1660—1640	87.4	60.5
17d	H	(CH ₂) ₂ OC ₂ H ₅	215—217	285 (M ⁺), 229, 213, 197	3160—2875, 1680—1670	90.0	55.2
17e	H	(CH ₂) ₃ CN	276—278 (dec.)	280 (M ⁺), 237, 224, 170	3150—2850, 2230, 1665	75.0	50.3
17f	H	CH ₂ CH=CH ₂	278—280 (dec.)	253 (M ⁺), 210, 197	3140—2860, 1660—1650	80.1	34.7

a) Effect on allergic histamine release from rat peritoneal exudate cells (PEC).

TABLE III. Physicochemical and Pharmacological Data for 1-Substituted Azaazulene Derivatives



Compd. No.	Z	R ²	mp (°C)	MS (m/z)	IR (KBr) cm ⁻¹	Yield (%)	Inhibition ^{a)} (%) (1 µg/ml)
18a	H	CH ₂ C ₆ H ₅	273—275 (dec.)	303 (M ⁺), 260, 247	3150—2850, 1660—1650	89.0	63.1
18b	H	(CH ₂) ₂ C ₆ H ₅	264—266 (dec.)	317 (M ⁺), 274, 261	3120—2850, 1660—1650	100.0	79.2
18c	H	(CH ₂) ₃ C ₆ H ₅	269—271 (dec.)	331 (M ⁺), 288, 275	3175—2850, 1650—1640	100.0	79.2
18d	H	(CH ₂) ₄ C ₆ H ₅	230—232 (dec.)	345 (M ⁺), 317, 302, 289	3150—2850, 1660—1640	100.0	34.6
18e	H	(CH ₂) ₅ C ₆ H ₅	188—190 (dec.)	359 (M ⁺), 331, 316, 303	3160—2850, 1650—1640	100.0	21.9
18f	7-iso-Pr	CH ₂ C ₆ H ₅	268—270 (dec.)	345 (M ⁺), 317, 302, 289	3180—2850, 1680—1670	98.0	53.8
18g	7-iso-Pr	(CH ₂) ₂ C ₆ H ₅	265—267 (dec.)	359 (M ⁺), 316, 303	3160—2850, 1670	100.0	44.8
18h	7-iso-Pr	(CH ₂) ₃ C ₆ H ₅	211—213 (dec.)	373 (M ⁺), 330, 317	3140—2850, 1680—1660	100.0	51.5
18i	7-iso-Pr	(CH ₂) ₄ C ₆ H ₅	212—214 (dec.)	387 (M ⁺), 359, 344, 331	3130—2850, 1670	98.9	31.9
18j	7-iso-Pr	(CH ₂) ₅ C ₆ H ₅	184—186 (dec.)	401 (M ⁺), 373, 358, 345	3130—2850, 1680—1670	91.9	5.3

a) Effect on allergic histamine release from rat peritoneal exudate cells (PEC).

Compounds (18a—c, f—h) showed high inhibitory activity, but the compounds with a longer alkyl chain (18d, 18e, 18i and 18j) showed relatively weak activity.

Since the phenylalkyl compounds showed activity, the effect of substituents in the phenyl ring was evaluated. As shown in Table IV, compounds substituted with a halogen group (19b—f, i), methoxy group (19n) or ester group (19h) showed activity. Among the halogen-substituted compounds, fluorine derivatives showed high activity. The compounds with *meta* substitution showed lower activity. Compounds substituted with polar groups such as a hydroxyl group (19m) or carboxyl group (19g) showed low activity. A compound with a partial structure of FPL55712 an antiallergic drug (19p), did not show high activity.

Table V shows the physicochemical properties of 2-substituted compounds and the histamine release-inhibitory activities. Activity was observed in the compounds with simple amino acids (21e—h), while the compounds with aromatic amino acids, such as phenylalanine (21j, 21k), were inactive. The activity among the amino acid-substituted derivatives decreased in the

following order: Gly (21e—g) > Ala (21h) > Pro (21i, 21m) > Ser (21i) > Phe (21j, 21k). The anthranilic acid derivatives (21n, 21o), in which methylene of glycine was replaced with benzene, showed lower activity than the glycine derivatives (21e—g). Compounds with an amino group (21r, 21s) also showed activity. Concerning the activity of carboxyl derivatives, those with an ester (21m, 21o) showed lower activity than those with a free acid (21i, 21n).

The skin passive cutaneous anaphylaxis (PCA) test, a typical *in vivo* antiallergic test, was performed in rats using representative compounds. As shown in Table VI, the compounds substituted with *n*-butyl in the 1-position (17b) and with glycine in the 2-position (21e) had no inhibitory effect on *in vivo* PCA. The compounds substituted with benzyl at the 1-position (18a, 18f, 19a, 19c and 19d) showed higher inhibitory activity than repirinast and WP-833, the positive control compounds. The compounds substituted with an isopropyl group had no effect. The compounds with azide groups (21b) and amino groups (21r, 21s) were ineffective.

One of the actions of theophylline as an antiasthmatic

TABLE IV. Physicochemical and Pharmacological Data for 1-Substituted Azaazulene Derivatives

Compd. No.	Z	R ²	mp (°C)	MS (<i>m/z</i>)	IR (KBr) cm ⁻¹	Yield (%)	Inhibition ^{a)} (%) (10 µg/ml)
19a	5-iso-Pr	CH ₂ C ₆ H ₅	224—226 (dec.)	345 (M ⁺), 317, 302, 289	3190—2850, 1660—1650	81.8	84.0
19b	H	CH ₂ C ₆ H ₄ F (<i>p</i>)	282—284 (dec.)	321 (M ⁺), 278, 265	3200—2850, 1670—1660	95.0	90.0
19c	7-iso-Pr	CH ₂ C ₆ H ₄ F (<i>p</i>)	283—285 (dec.)	363 (M ⁺), 335, 320, 307	3160—2850, 1670—1660	100.0	86.0
19d	5-iso-Pr	CH ₂ C ₆ H ₄ F (<i>p</i>)	266—268 (dec.)	363 (M ⁺), 335, 320, 307	3120—2850, 1660—1645	90.9	94.0
19e	H	CH ₂ C ₆ H ₄ F (<i>o</i>)	289—291 (dec.)	321 (M ⁺), 278, 265	3160—2850, 1660	92.0	87.0
19f	H	CH ₂ C ₆ H ₄ F (<i>m</i>)	271—273 (dec.)	321 (M ⁺), 293, 278, 265	3150—2850, 1665—1650	99.0	67.0
19g	H	CH ₂ C ₆ H ₄ COOH (<i>p</i>)	175—177	347 (M ⁺), 330, 304, 291	3200—2850, 1710, 1650—1640	90.0	12.0
19h	H	CH ₂ C ₆ H ₄ CO ₂ C ₂ H ₅ (<i>p</i>)	279—281 (dec.)	375 (M ⁺), 332, 330, 319	3150—2850, 1715, 1670—1650	95.0	65.0
19i	H	CH ₂ C ₆ H ₄ Cl (<i>p</i>)	255—257 (dec.)	339 (M ⁺ + 2), 337 (M ⁺), 294, 281	3160—2850, 1650—1640	95.7	86.0
19j	H	CH ₂ C ₆ H ₄ CF ₃ (<i>p</i>)	286—288 (dec.)	371 (M ⁺), 343, 328, 315	3175—2850, 1670	94.1	54.0
19k	H	CH ₂ C ₆ H ₄ CN (<i>p</i>)	251—253 (dec.)	328 (M ⁺), 300, 285, 272	3180—2850, 2220, 1660—1650	94.9	43.0
19l	H	CH ₂ COC ₆ H ₄ Cl (<i>p</i>)	> 290	367 (M ⁺ + 2), 365 (M ⁺), 322, 297	3150—2850, 1685, 1660—1640	83.6	43.0
19m	H	CH ₂ C ₆ H ₄ OH (<i>p</i>)	230—232 (dec.)	319 (M ⁺), 276, 263	3600, 3050—2850, 1680—1670	80.0	0
19n	H	CH ₂ C ₆ H ₄ OCH ₃ (<i>p</i>)	223—225 (dec.)	333 (M ⁺), 290, 277	3150—2850, 1660—1650	95.0	83.0
19o	H	CH ₂ C ₆ H ₃ Cl ₂ (2, 4)	285—287	373 (M ⁺ + 2), 371 (M ⁺), 328, 315	3150—2850, 1660	71.9	19.0
19p	H		222—224 (dec.)	447 (M ⁺), 419, 404, 319	3400, 3200—2850, 1670—1640	64.0	63.0
19q	H		288—290 (dec.)	459 (M ⁺), 431, 416, 403	3590, 3140—2850, 1680, 1660	99.1	10.0
19r	H	CH ₂ C ₆ H ₂ (CH ₃) ₃ (2, 4, 6)	279—281 (dec.)	345 (M ⁺), 317, 302, 289	3200—2850, 1650	82.4	13.0

a) Effect on allergic histamine release from rat peritoneal exudate cells (PEC).

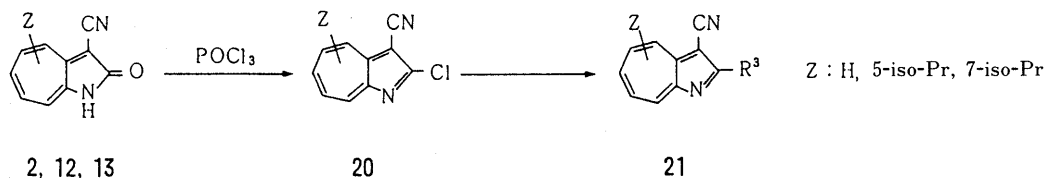


Chart 4

drug is known to depend on phosphodiesterase (PDE) inhibition. Therefore, representative compounds that showed activity in the rat PCA test were examined for PDE-inhibitory activity. As shown in Table VII, each compound showed similar activity to that of papaverine.

In addition, an acute toxicity test of representative compounds was performed. The results are shown in Table VIII. The 1-benzyl and 1-(4-fluorobenzyl)-7-isopropyl-3-(5-tetrazolyl)-1-azaazulene-2-one (**18f**, **19c**), having low toxicity with marked inhibitory effects on rat skin PCA and histamine release, and strong PDE inhibitory activity, appear to be promising candidates as antiasthmatic drugs.

Experimental

Melting points were determined by means of a Yanagimoto micro melting point apparatus, without correction. Infrared (IR) spectra

were recorded using a Shimadzu IR-430 spectrophotometer. Mass spectra (MS) were obtained with a Hitachi M-52 mass spectrometer. Proton nuclear magnetic resonance (¹H-NMR) spectra were measured with a JEOL JNM-EX270 spectrometer. Chemical shifts are given in δ values with tetramethylsilane (TMS) as an internal standard and the following abbreviations are used: s, singlet; d, doublet; dd, double doublet; br s, broad singlet.

2-O-Methyltropolone (1) and 2-O-Methylhinokitiol (11) A mixture of tropolone (17.0 g, 0.14 mol) or hinokitiol (23.0 g, 0.14 mol), K₂CO₃ (58.0 g, 0.42 mol), dicyclohexyl-18-crown-6 (5.2 g, 0.014 mol), acetonitrile (750 ml) and methyl iodide (99.4 g, 0.7 mol) was refluxed for 10 h. The mixture was filtered and the filtrate was evaporated. The residue was dissolved in CH₂Cl₂ and the organic layer was washed with water, dried and evaporated. The residue was purified by column chromatography (AcOEt: *n*-hexane = 1:1—3:1) to give **1** (17.1 g, 90.0%), MS *m/z*: 136 (M⁺), 105, or **11** (24.1 g, 96.4%), MS *m/z*: 178 (M⁺), 147, 135.

3-Cyano-1-azaazulene-2-one (2) α-Cyanoacetamide (7.9 g, 0.094 mol) was added dropwise to a solution of sodium (2.2 g, 0.094 mol) in anhydrous EtOH (340 ml) at room temperature. Then **1** (12.8 g, 0.094 mol) dissolved in anhydrous EtOH (24 ml) was added, and the

TABLE V. Physicochemical and Pharmacological Data for 2-Substituted Azaazulene Derivatives

Compd. No.	Z	R ³	mp (°C)	MS (<i>m/z</i>)	IR (KBr) cm ⁻¹	Yield (%)	Inhibition ^{a)} (%) (10 μg/ml)
21a	H	NHCH ₂ CH(C ₆ H ₅) ₂	158—160	349 (M ⁺), 245, 153	3250, 3050—2800, 2200	78.6	18.0
21b	H	N ₃	170 (dec.)	195 (M ⁺), 167, 140	3030—2950, 2200, 2150—2100	94.0	65.0
21c	5-iso-Pr	N ₃	145—147 (dec.)	237 (M ⁺), 209, 194	3050—2850, 2200, 2150	86.7	71.0
21d	7-iso-Pr	N ₃	150—152	237 (M ⁺), 209, 194	3050—2850, 2200, 2150—2140	95.2	4.0
21e	H	NHCH ₂ COOH	260—262 (dec.)	227 (M ⁺), 209, 181, 169, 154	3350, 3100—2850, 2200, 1640—1620	65.0	83.0
21f	5-iso-Pr	NHCH ₂ COONa	267—270 (dec.)	n.d.	3500—3350, 3050—2850, 2200, 1610	70.0	85.0
21g	7-iso-Pr	NHCH ₂ COONa	>280	n.d.	3500—3350, 3050—2850, 2200, 1610	78.8	86.0
21h	H	NHCH(CH ₃)COOH	211—213 (dec.)	241 (M ⁺), 223, 197	3200, 3050—2850, 2200, 1660—1640	73.0	61.0
21i	H	NHCH(CH ₂ OH)COONa	210—213 (dec.)	n.d.	3500—3260, 3050—2850, 2200	70.0	23.0
21j	H	NHCH(COOH)CH ₂ C ₆ H ₅	129—131	317 (M ⁺), 299, 273, 208	3250, 3100—2850, 2200, 1650—1630	63.2	8.0
21k	H	NHCH(COOC ₂ H ₅)CH ₂ C ₆ H ₅	82—84	345 (M ⁺), 272, 254	3300, 3050—2850, 2200, 1740	67.7	4.0
21l	H		>280	n.d.	3050—2850, 2200, 1600	95.0	51.0
21m	H		120—122	281 (M ⁺), 250, 222	3050—2850, 2200, 1740—1730	80.0	36.0
21n	H	NHC ₆ H ₄ COONa (<i>p</i>)	>280	n.d.	3300, 3050—2850, 2200	80.0	57.0
21o	H	NHC ₆ H ₄ COOC ₂ H ₅ (<i>p</i>)	220—222	317 (M ⁺), 288, 272, 244	3300, 3050—2850, 2200, 1620	78.5	21.0
21p	H	NHC ₆ H ₄ COOCH ₃ (<i>o</i>)	249—251	303 (M ⁺), 288, 272, 244	3300, 3150—2850, 2200, 1710	97.2	9.0
21q	H		228—230	404 (M ⁺), 251, 237, 222, 208	3100—2750, 2200	90.0	33.0
21r	H	NH ₂ ·HCl	240—242 (dec.)	169 (M ⁺), 143	3400, 3300, 2200	78.0	65.0
21s	5-iso-Pr	NH ₂ ·HCl	217—219 (dec.)	211 (M ⁺), 185	3400, 3300, 3050—2850, 2200	73.5	68.0

a) Effect on allergic histamine release from rat PEC. n.d.: not detected.

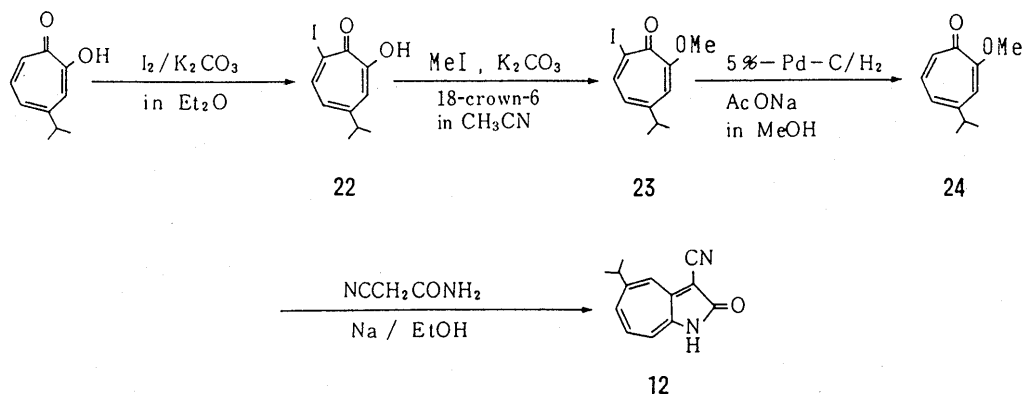


Chart 5

mixture was stirred at room temperature for 2 d. The mixture was filtered and the filtrate was evaporated. The residue was dissolved in water and acidified with 1N HCl. The precipitate was collected by filtration and dried to give **2** (10.7 g, 75.0%). *Anal.* Calcd for C₁₀H₆N₂O: C, 70.61; H, 3.55; N, 16.46. Found: C, 70.45; H, 3.57; N, 16.21.

3-Ethoxycarbonyl- and 3-Carboxy-1-azaazulene-2-one (3, 4) A mixture of 2-aminotropolone (5.6 g, 0.046 mol), anhydrous EtOH (30 ml) and diethyl malonate (11.4 g, 0.07 mol) was added dropwise to a solution of sodium (1.8 g, 0.078 mol) in anhydrous EtOH (18 ml), and the mixture was reacted in a sealed tube for 6 h at 100 °C. The mixture was evaporated.

TABLE VI. Inhibitory Effects on Allergic Histamine Release and 48 h PCA in Rats

Compound No.	Inhibition ^{a)} (%)	Compound No.	Inhibition ^{a)} (%)
17b	-2 ± 9	21b	0 ± 2
18a	95 ± 5	21e	14 ± 4
18f	99 ± 4	21r	-2 ± 4
18h	11 ± 1	21s	5 ± 2
19a	78 ± 4	Repirinast ^{b)}	36 ± 6
19c	98 ± 2	WP-833 ^{c)}	43 ± 5
19d	85 ± 2		

a) Test compounds were given orally 1 h before the antigen challenge ($N=5$) at a dose of 5 mg/kg. b) Isopentyl 5,6-dihydro-7,8-dimethyl-4,5-dioxo-4H-pyrano-[3,2-c]quinoline-2-carboxylate. c) 5-(3-Butyloxyalylaminoisophenyl)-1H-tetrazole.

TABLE VII. PDE-Inhibitory Activity

Compound No.	Inhibition (%) (10^{-5} M)
15b	45.3
18a	33.3
18f	52.6
19c	55.2
Papaverine	49.8

TABLE VIII. Acute Toxicity

Compound No.	Dose (mg/kg <i>p.o.</i>)	Mortality
18f	500	0/5
	1000	0/5
	2000	0/5
19c	500	0/5
	1000	0/5
	2000	0/5

The residue was dissolved in water and the solution was adjusted to pH 4.5 and extracted with CHCl_3 . The organic layer was washed with water, dried, evaporated and recrystallized from a mixture of EtOH and AcOEt to give **3** (5.3 g, 53.3%). Compound **3** (1.0 g, 0.0046 mol) was suspended in 2N NaOH solution and stirred at 90–100 °C for 1 h for complete dissolution. The reaction solution was acidified with 1N HCl. The precipitate was collected by filtration, dried and recrystallized from MeOH to give **4** (0.8 g, 92.0%). *Anal.* Calcd for $\text{C}_{10}\text{H}_7\text{NO}_3$: C, 63.49; H, 3.73; N, 7.40. Found: C, 63.72; H, 3.58; N, 7.64.

3-(2-Pyridyl or 5-Tetrazolyl)carbamoyl-1-azaazulen-2-one (5a, b) Compound **4** (0.0053 mol) was dissolved in thionyl chloride (20 ml) and refluxed for 1.5 h. When excess thionyl chloride was removed, a precipitate was obtained. This precipitate was dissolved in CH_2Cl_2 (50 ml) and an amine (2-aminopyridine or 2-aminotetrazole, 0.0053 mol) and pyridine (0.8 g, 0.011 mol) were added. The mixture was stirred at room temperature for 1 d. The mixture was evaporated, and the residue was poured into ice water and acidified with 1N HCl. The precipitates were collected by filtration, and purified on silica gel plates (CHCl_3 : C_6H_6 : MeOH = 15:3:2) to give **5a** (38.7%) or **5b** (43.0%).

5a *Anal.* Calcd for $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_2$: C, 67.92; H, 4.18; N, 15.84. Found: C, 67.82; H, 4.27; N, 15.88. **5b** *Anal.* Calcd for $\text{C}_{11}\text{H}_8\text{N}_6\text{O}_2$: C, 51.57; H, 3.15; N, 32.80. Found: C, 51.78; H, 3.10; N, 32.63.

1-Azaazulen-2-one (6) and 7-Isopropyl-1-azaazulen-2-one (14) Compound **2**, **3**, or **13** (0.047 mol) was mixed with hydrobromic acid (50 ml) and the mixture was refluxed for 7.5 h. It was then poured into ice-water and extracted with CHCl_3 . The organic layer was washed with water, dried and evaporated. The residue was recrystallized from a mixture of EtOH, AcOEt and *n*-hexane to give **6** (74.5%) or **14** (79.5%). **6** *Anal.* Calcd for $\text{C}_9\text{H}_7\text{NO}$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.29; H,

4.64; N, 9.80. **14** *Anal.* Calcd for $\text{C}_{12}\text{H}_{13}\text{NO}$: C, 76.98; H, 7.00; N, 7.48. Found: C, 76.83; H, 7.23; N, 7.33.

3-Cyanomethyl-1-azaazulen-2-one (7) A mixture of **6** (0.7 g, 0.0048 mol), paraformaldehyde (0.14 g), acetic acid (10 ml) and 50% dimethylamine (1.5 g) was allowed to react at 40–50 °C for 12 h. The mixture was poured into ice-water and neutralized. Unreacted substances were removed by extraction with AcOEt. Then the aqueous layer was adjusted to pH 10 and extracted with AcOEt. The organic layer was washed with water, dried and evaporated to give 3-dimethylaminomethyl-1-azaazulen-2-one (0.8 g, 66.7%). A mixture of this compound (0.65 g, 0.0032 mol), EtOH (10 ml) and methyl iodide (1.0 g) was stirred at room temperature for 2–2.5 h. The precipitates were collected by filtration and dried. A mixture of this precipitate, KCN (0.6 g, 0.0092 mol) and 60% EtOH solution (24 ml) was stirred at room temperature for 8 h and evaporated. The residue was extracted with CHCl_3 . The organic layer was evaporated and the residue was recrystallized from a mixture of AcOEt and petroleum ether to give **7** (0.32 g, 50.0%). *Anal.* Calcd for $\text{C}_{11}\text{H}_8\text{N}_2\text{O}$: C, 71.73; H, 4.38; N, 15.21. Found: C, 71.91; H, 4.38; N, 15.29.

3-(5-Tetrazolylmethyl)-1-azaazulen-2-one (8) A mixture of **7** (1.0 g, 0.0054 mol), anhydrous aluminum chloride (2.0 g, 0.0162 mol), sodium azide (3.2 g, 0.0486 mol) and THF (50 ml) was stirred at room temperature for 1 h and then heated for 6 h. The mixture was poured into ice-water, acidified with 1N HCl and extracted with EtOAc. The organic layer was washed with water, dried and evaporated. The residue was purified on silica gel plates (CHCl_3 : C_6H_6 : MeOH = 15:3:2) to give **8** (0.6 g, 50.0%). *Anal.* Calcd for $\text{C}_{11}\text{H}_9\text{N}_5\text{O}$: C, 58.15; H, 3.99; N, 30.82. Found: C, 58.01; H, 3.88; N, 30.52.

3-Formyl-1-azaazulen-2-one (9) Compound **6** (0.5 g, 0.0034 mol) was dissolved in DMF (3 ml) and a mixture of DMF (4 ml) and POCl_3 (1.0 g) was added. The whole was stirred at room temperature for 1 d, then sodium acetate (0.5 g) was added, and the reaction was allowed to proceed at 50 °C for 1 h. The mixture was poured into ice water, and adjusted to pH 5. The precipitate was collected by filtration, dried and recrystallized from a mixture solvent of CHCl_3 and *n*-hexane to give **9** (0.35 g, 59.3%). *Anal.* Calcd for $\text{C}_{10}\text{H}_8\text{NO}_2$: C, 68.96; H, 4.63; N, 8.04. Found: C, 68.80; H, 4.80; N, 8.04.

3-(Acetyl or 4-fluorobenzoyl)-1-azaazulen-2-one (10a, b) Compound **6** (1.0 g, 0.007 mol) was dissolved in toluene (35 ml). The solution was cooled to 0–5 °C, and acetyl chloride or 4-fluorobenzoyl chloride (0.007 mol) and then anhydrous aluminum chloride (0.9 g, 0.007 mol) were added. The reaction was allowed to proceed at 80–90 °C for 1 h, then the mixture was poured into ice-water and acidified to give **10a** (51.0%) or **10b** (67.6%). **10a** *Anal.* Calcd for $\text{C}_{11}\text{H}_9\text{NO}_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.71; H, 5.05; N, 7.43. **10b** *Anal.* Calcd for $\text{C}_{16}\text{H}_{10}\text{FNO}_2$: C, 71.91; H, 3.77; N, 5.24. Found: C, 71.77; H, 3.78; N, 5.39.

5- or 7-Isopropyl-3-cyano-1-azaazulen-2-one (12, 13) Sodium (13.8 g, 0.6 mol) was dissolved in anhydrous EtOH (2,200 ml) and α -cyanoacetamide (50.4 g, 0.6 mol) was added, followed by a solution of **11** (103.0 g, 0.58 mol) in anhydrous EtOH (150 ml). The mixture was stirred at room temperature for 2 d, then filtered and the filtrate was evaporated. The residue was dissolved in water and acidified. The precipitate was collected by filtration, dried and purified by silica gel column chromatography (AcOEt). The first eluate afforded the 7-isopropyl compound **13** (16.1 g), and the later eluate yielded the 5-isopropyl compound **12** (25.4 g). The position of the isopropyl group was confirmed by an alternative synthesis.

12: $^1\text{H-NMR}$ (in $(\text{CD}_3)_2\text{SO}$) δ : 1.30 (6H, d, $J=6.9$ Hz), 3.14 (1H, m), 7.42–7.71 (4H, m), 12.13 (1H, brs). *Anal.* Calcd for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}$: C, 73.57; H, 5.70; N, 13.20. Found: C, 73.34; H, 5.63; N, 13.20.

13: $^1\text{H-NMR}$ (in $(\text{CD}_3)_2\text{SO}$) δ : 1.28 (6H, d, $J=6.6$ Hz), 3.08 (1H, m), 7.40–7.77 (4H, m), 12.07 (1H, brs). *Anal.* Calcd for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}$: C, 73.57; H, 5.70; N, 13.20. Found: C, 73.67; H, 5.63; N, 13.35.

Alkylation Reaction (Compounds 16) In general, these compounds were prepared using NaH or anhydrous potassium carbonate in DMF at room temperature to 80 °C for 2 h to 1 d. Some cases are shown.

1-Benzyl-3-cyano-5-isopropyl-1-azaazulen-2-one (16a) A mixture of **12** (2.1 g, 0.01 mol), DMF (20 ml) and K_2CO_3 (1.4 g, 0.01 mol) was stirred at room temperature for 15 min and then benzyl bromide (1.7 g, 0.01 mol) was added. The mixture was stirred at room temperature for 3 h, then poured into ice-water. The precipitates were collected by filtration and washed with petroleum ether to give **16a** (2.75 g, 91.7%) mp 128–130 °C. MS m/z : 302 (M^+), 259. $^1\text{H-NMR}$ (in $(\text{CD}_3)_2\text{SO}$) δ : 1.30 (6H, d,

$J=6.9$ Hz), 3.15 (1H, m), 5.32 (2H, s), 7.24–7.80 (9H, m). *Anal.* Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.26. Found: C, 79.23; H, 6.00; N, 9.12.

1-Benzyl-3-cyano-7-isopropyl-1-azaazulen-2-one (16b) A mixture of **13** (2.1 g, 0.01 mol), DMF (20 ml) and K_2CO_3 (1.4 g, 0.01 mol) was stirred at room temperature for 15 min and then benzyl bromide (1.7 g, 0.01 mol) was added. The mixture was stirred at room temperature for 3 h and poured into ice-water. The precipitates were collected by filtration and washed with petroleum ether to give **16b** (2.80 g, 93.5%) mp 142–144°C. *MS* m/z : 302 (M^+), 259. 1H -NMR (in $(CD_3)_2SO$) δ : 1.16 (6H, d, $J=6.9$ Hz), 3.06 (1H, m), 5.41 (2H, s), 7.25–7.87 (9H, m). *Anal.* Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.26. Found: C, 79.33; H, 5.99; N, 9.26.

3-Cyano-1-(4-fluorobenzyl)-7-isopropyl-1-azaazulen-2-one (16c) A mixture of **13** (2.1 g, 0.01 mol), DMF (20 ml) and K_2CO_3 (1.4 g, 0.01 mol) was stirred at room temperature for 15 min and then 4-fluorobenzyl bromide (1.9 g, 0.01 mol) was added. The mixture was stirred at room temperature for 3 h, then poured into ice-water. The precipitate was collected by filtration and washed with petroleum ether to give **16c** (2.90 g, 90.3%) mp 152–154°C. *MS* m/z : 320 (M^+), 277. 1H -NMR (in $(CD_3)_2SO$) δ : 1.19 (6H, d, $J=6.6$ Hz), 3.08 (1H, m), 5.40 (2H, s), 7.14–7.88 (8H, m). *Anal.* Calcd for $C_{20}H_{17}FN_2O$: C, 74.98; H, 5.35; N, 8.74. Found: C, 74.78; H, 5.30; N, 8.60.

Tetrazole Reaction (Compounds 15, 17–19) In general, these compounds were prepared by reaction with NaN_3 and $AlCl_3$ in THF or with NaN_3 and NH_4Cl in DMF at room temperature to 90°C for 2 h to 1 d. Some examples are shown.

1-Benzyl-5-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (19a) A mixture of dry THF (100 ml), anhydrous aluminum chloride (3.6 g, 0.027 mol) and NaN_3 (5.3 g, 0.081 mol) was stirred under cooling for 10 min. Compound **16a** (2.75 g, 0.009 mol) was added, and the mixture was allowed to react at room temperature for 3–3.5 h, then poured into ice-water, and acidified. The precipitate was collected by filtration, washed with water and dried to give **19a** (2.5 g, 81.1%). 1H -NMR (in $(CD_3)_2SO$) δ : 1.32 (6H, d, $J=6.9$ Hz), 3.10 (1H, m), 5.41 (2H, s), 7.11–9.06 (9H, m), 16.08 (1H, brs). *Anal.* Calcd for $C_{20}H_{19}N_5O$: C, 69.55; H, 5.54; N, 20.28. Found: C, 69.35; H, 5.35; N, 20.48.

1-Benzyl-7-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (18f) A mixture of dry THF (100 ml), anhydrous aluminum chloride (3.6 g, 0.027 mol) and NaN_3 (5.3 g, 0.081 mol) was stirred under cooling for 10 min. Compound **16b** (2.75 g, 0.009 mol) was added, and the mixture was allowed to react at room temperature for 3–3.5 h, then poured into ice-water, and acidified. The precipitate was collected by filtration, washed with water and dried to give **18f** (3.0 g, 98.0%). 1H -NMR (in $(CD_3)_2SO$) δ : 1.16 (6H, d, $J=6.9$ Hz), 3.03 (1H, m), 5.51 (2H, s), 7.24–8.89 (9H, m), 16.11 (1H, brs). *Anal.* Calcd for $C_{20}H_{19}N_5O$: C, 69.55; H, 5.54; N, 20.28. Found: C, 69.49; H, 5.45; N, 20.02.

1-(4-Fluorobenzyl)-7-isopropyl-3-(5-tetrazolyl)-1-azaazulen-2-one (19c) A mixture of dry THF (100 ml), anhydrous aluminum chloride (3.6 g, 0.027 mol) and NaN_3 (5.3 g, 0.081 mol) was stirred under cooling for 10 min. Compound **16c** (2.90 g, 0.009 mol) was added, and the mixture was allowed to react at room temperature for 3–3.5 h, then poured into ice-water, and acidified. The precipitate was collected by filtration, washed with water and dried to give **19c** (3.27 g, 100.0%). 1H -NMR (in $(CD_3)_2SO$) δ : 1.19 (6H, d, $J=6.4$ Hz), 3.05 (1H, m), 5.50 (2H, s), 7.14–8.89 (8H, m), 16.11 (1H, brs). *Anal.* Calcd for $C_{20}H_{18}FN_5O$: C, 66.11; H, 4.99; N, 19.27. Found: C, 66.14; H, 4.98; N, 19.22.

Compounds **15a–c**, **17a–f**, **18a–e**, **18g–j**, **19b** and **19d–r** were synthesized by the same method as above. The physicochemical characteristics of the compounds are shown in Tables I–IV.

2-Chloro-3-cyano-1-azaazulene (20) Compound **2**, **12**, or **13** (0.01 mol) was suspended in phosphorus oxychloride (8.0 ml), refluxed for 1–1.5 h, and poured into ice-water. The precipitate was collected by filtration and purified by alumina column chromatography ($CHCl_3$). Yield, 60–70%.

Amination (Compounds 21) In general, amination was done by reaction with various amines in DMF or EtOH at room temperature by reflux for 0.5 h to 1 d. Some examples are described below.

3-Cyano-2-(2,2-diphenylethylamino)-1-azaazulene (21a) A mixture of 2-chloro-3-cyano-1-azaazulene **20a** (0.38 g, 0.002 mol) DMF (7.0 ml) and 2,2-diphenylethylamine (0.43 g, 0.0022 mol) was stirred at room temperature for 1 d, then poured into ice-water. The precipitate was collected by filtration, washed with water, dried and recrystallized from a mixed solvent of AcOEt and hexane to give **21a** (0.55 g, 78.6%).

1H -NMR (in $(CD_3)_2SO$) δ : 3.35 (2H, d, $J=7.9$ Hz), 4.19 (1H, t, $J=7.9$ Hz), 7.22–8.04 (15H, m). *Anal.* Calcd for $C_{24}H_{19}N_3$: C, 82.49; H, 5.48; N, 12.02. Found: C, 82.28; H, 5.22; N, 12.14.

2-Azido-3-cyano-1-azaazulene (21b) 2-Chloro-3-cyano-1-azaazulene **20a** (0.57 g, 0.003 mol) was dissolved in DMF (7.0 ml), then sodium azide (0.3 g, 0.0045 mol) was added. The mixture was stirred at 40–50°C for 0.5 h, and then poured into ice-water. The precipitate was collected by filtration, washed with water, and dried to give **21b** (0.55 g, 94.0%). 1H -NMR (in $CDCl_3$) δ : 7.88–8.92 (5H, m). *Anal.* Calcd for $C_{10}H_5N_5$: C, 61.54; H, 2.58; N, 35.88. Found: C, 61.33; H, 2.51; N, 35.58.

2-Carboxymethylamino-3-cyano-1-azaazulene (21e) 2-Chloro-3-cyano-1-azaazulene **20a** (0.3 g, 0.0016 mol), EtOH (6.0 ml), DMF (1.0 ml), glycine methylester hydrochloride (0.67 g, 0.0048 mol) and tri-ethylamine (0.65 g, 0.0064 mol) was refluxed for 6 h. The mixture was evaporated, and the residue was dissolved in $CHCl_3$, washed with water, dried and purified on silica gel plates ($CHCl_3$: C_6H_6 : MeOH = 15:3:2) to give 2-methoxycarbonylmethylamino-3-cyano-1-azaazulene (0.26 g, 65.0%). *MS* m/z : 241 (M^+), 182. This compound (0.22 g, 0.0009 mol) was dissolved in 2 N NaOH solution (0.54 ml) and EtOH (12.0 ml). The mixture was stirred at 60–70°C for 30 min, then evaporated. The residue was poured into ice-water, neutralized with 1 N HCl and extracted with AcOEt several times. The organic layer was evaporated. The residue was washed with cyclohexane to give **21e** (0.17 g, 83.0%). 1H -NMR (in $(CD_3)_2SO$) δ : 4.12 (2H, d, $J=5.6$ Hz), 7.54–8.02 (5H, m), 8.23 (1H, brs). *Anal.* Calcd for $C_{12}H_9N_3O_2$: C, 63.43; H, 3.99; N, 18.49. Found: C, 63.71; H, 4.10; N, 18.60.

Compounds **21c**, **d**, **21f–s** were synthesized by the same method as above. The physicochemical characteristics of the compounds are shown in Table V.

7-Iodohinokitiol (22) A mixture of hinokitiol (1.0 g), K_2CO_3 (0.8 g), iodine (1.5 g) and ether (20.0 ml) was stirred at room temperature for 1 d. The precipitate was collected by filtration, and dissolved in water. This solution was acidified, and then extracted with ether. The ether layer was evaporated to give **22** (1.74 g, 100.0%). *MS* m/z : 290 (M^+), 247.

7-Iodo-2-O-methylhinokitiol (23) A mixture of **22** (1.74 g, 0.006 mol), K_2CO_3 (1.65 g), dicyclohexyl-18-crown-6 (0.22 g, 0.0006 mol) and methyl iodide (4.3 g, 0.03 mol) was refluxed for 10 h. The mixture was filtered, and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 , and this solution was washed with water, and evaporated. The residue was purified by silica gel column chromatography (AcOEt) to give **23** (1.4 g, 77.8%). *MS* m/z : 304 (M^+), 274, 261.

2-O-Methylhinokitiol (24) Compound **23** (1.3 g, 0.0043 mol) was dissolved in MeOH (20 ml) containing 5% Pd-C (0.2 g) and sodium acetate (0.35 g, 0.0043 mol) and the resulting solution was stirred under an H_2 stream for 2 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated. The residue was dissolved in $CHCl_3$, then this solution was washed with water, and evaporated to give **24** (0.77 g, 100.0%). *MS* m/z : 178 (M^+), 147, 135.

3-Cyano-5-isopropyl-1-azaazulen-2-one (12) Sodium (0.1 g, 0.0043 mol) was dissolved in anhydrous EtOH (20 ml), and α -cyanoacetamide (0.36 g, 0.0043 mol) was added to the mixture at room temperature, followed by **24** (0.765 g, 0.0043 mol) dissolved in anhydrous EtOH (2.0 ml). The mixture was stirred at room temperature for 1 d, then filtered and the filtrate was evaporated. The residue was dissolved in water and acidified with 1 N HCl. The precipitates were collected by filtration and dried to give **12** (0.5 g, 55.0%). 1H -NMR (in $(CD_3)_2SO$) δ : 1.30 (6H, d, $J=6.9$ Hz), 3.14 (1H, m), 7.42–7.71 (4H, m), 12.14 (1H, brs). *Anal.* Calcd for $C_{13}H_{12}N_2O$: C, 73.57; H, 5.70; N, 13.20. Found: C, 73.55; H, 5.66; N, 13.07.

Inhibition of Histamine Release One ml of rat anti-dinitrophenylated egg albumin (anti-DNP-OVA) IgE serum (48 h homologous PCA titer: 64) was intraperitoneally administered to rats for passive sensitization. After 24 h, peritoneal exudate cells were collected and incubated in test tubes ($1.5 \times 10^5/400 \mu l$) at 37°C for 10 min. Histamine release was induced with DNP-OVA (50 μl). The test compound was added 30 s before addition of the antigen. The reaction was terminated after 10 min. After centrifugation, histamine in the supernatant was measured by Shore's⁷⁾ fluorescence method. Total histamine content in the mast cells was measured after boiling at 100°C for 5 min. Inhibition (% of histamine release) was calculated as follows:

$$\text{inhibition (\%)} = [1 - (X - S)/(Y - S)] \times 100$$

X: release % with antigen and test compound

Y: release % with antigen alone

S: spontaneous release %

Histamine release (%) was calculated from total histamine content as follows:

$$\text{histamine release \%} = \frac{\text{histamine released by antigen and test compound, or antigen alone}}{\text{total histamine content}} \times 100$$

PCA Inhibition Male Slc: Wistar rats (130–150 g) were passively sensitized by intra cutaneous injection of anti-DNP-OVA rat antiserum (48 h homologous PCA titer: 256) (50 μ l/site) diluted 1/200 with physiological saline into the back (5 rats per group). After 48 h, PCA was induced by injection of 1% (w/v) Evans blue containing 1.5 mg of DNP-OVA (0.6 ml) into the caudal vein. The rats were killed by exsanguination after 30 min, and extravasated dye in the back skin was measured by Katayama's method.⁸⁾ The test compound was suspended in 0.5% (w/v) carboxymethyl cellulose (CMC) and was orally administered at 5 mg/kg 1 h before antigen challenge. Inhibition (% of PCA) was calculated as follows:

$$\text{inhibition (\%)} = (1 - Y/X) \times 100$$

X: extravasated dye amount of control rat

Y: extravasated dye amount of compound-treated rat

PDE Inhibition ³H-5-AMP released from ³H-c-AMP by swine myocardial phosphodiesterase (calmodulin-dependent) was separated by column chromatography and measured.

$$\text{inhibition (\%)} = \left(1 - \frac{{}^3\text{H-5-AMP cpm, compound}}{{}^3\text{H-5-AMP cpm, control}} \right) \times 100$$

Acute Toxicity Test Male ddy mice weighing 26–28 g (5 mice per group) orally received the test compound suspended in 0.5% CMC aqueous solution, and the occurrence or absence of deaths was observed for 2 weeks.

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