

Synthesis and Antiallergic Activities of 2-Alkyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides and Related Compounds

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A series of 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides and related compounds have been synthesized and their antiallergic activities were evaluated. Most derivatives with a lower alkyl group at position 2 were orally active. Among them, *N*-ethyl-2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III₃), 2-ethyl-*N*-methyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III₁₄), 2-isopropyl-*N*-methyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III₂₇), 5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2,3,4-trimethylfuro[2,3-*c*]pyrazole (IV₁) and 5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-isopropyl-3,4-dimethylfuro[2,3-*c*]pyrazole (IV₃) showed promising antiallergic effects. The structure–activity relation of these 3,4-dimethylfuro[2,3-*c*]pyrazole derivatives was examined. An amide or 5-oxo-1,3,4-oxadiazole substituent at position 5 was favorable, while introduction of a carboxylic acid or acrylic acid moiety was unfavorable. However, none of these compounds exerted a significant inhibitory effect on mast cell degranulation. Compound III₂₇ and IV₃ showed potent anti-allergic activity. We found that they also suppressed histamine-, serotonin-, bradykinin- and substance P-induced ear edema in mice. In compound 48/80-pretreated mice, the preformed mediators in mast cells in the ear were greatly reduced. Under this condition, the bradykinin- and substance P-induced ear edema was suppressed by compound III₂₇ and IV₃ to a significantly greater extent than by diphenhydramine combined with methylsergide. These results indicated that the antiallergic effect of 3,4-dimethylfuro[2,3-*c*]pyrazole derivatives probably involves protection of the vasculature against the effects of challenge by several mediators.

Keywords furopyrazole; antiallergic activity; structure–activity relationship; furo[2,3-*c*]pyrazole-5-carboxamide; mast cell degranulation; vascular permeability

In the course of our investigations of the synthesis and biological activities of furo[2,3-*c*]pyrazole compounds, we have synthesized 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxylic acids (I) and examined their antiplatelet activities.^{1,2)} In a continuation of our research program, some 2-alkyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides and related compounds were synthesized and their antiallergic activities were investigated. We also report herein their inhibitory effect on the cutaneous vascular permeability of these furopyrazole compounds and some preliminary findings on the structure–activity relationship.

Results and Discussion

Chemistry The preparation of several 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxylic acids (I_{1–4}) and 2-substituted 5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazole (IV_{1–4}) was described in a previous paper.²⁾ As shown in Chart 1, compounds I were allowed to react with SOCl₂ to afford 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxylchlorides (II_{1–4}), which were treated with a variety of amines to give the corresponding amides (III) (Table I). On the other hand, compounds IV²⁾ were treated with acetic anhydride in acetic acid to afford the corresponding 2-substituted 5-(4-acetyl-4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazoles (V_{5–8}).

The synthetic pathway to 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-acrylic acids (VIII_{1–2}) is shown

in Chart 2. When 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carboxylic acid (I₁) was heated with quinoline in the presence of active copper, 2,3,4-trimethylfuro[2,3-*c*]pyrazole (IV₁) was obtained.

The Vielsmeier formylation of VI₁ with POCl₃ in dimethylformamide (DMF) afforded 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carbaldehyde (VII₁). Finally, compound VII₁ was condensed with malonic acid to afford the corresponding acrylic acid (VIII₁). The ¹H-NMR spectrum of VIII₁ showed signals at δ 2.28 (3H, s, 4-CH₃), 2.43 (3H, s, 3-CH₃), 3.70 (3H, s, 2-CH₃), 5.97 (1H, d, *J* = 16.0 Hz, –CH = CH–COOH), 7.37 (1H, d, *J* = 16.0 Hz, –CH = CH–COOH), suggesting that the structure was the *trans* form of 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-acrylic acid. In a like manner, compound VIII₂ was prepared.

Biological Activity. **a. Evaluation of Antiallergic Effect of 3,4-Dimethylfuro[2,3-*c*]pyrazole** The 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxylic acids (I_{1–6}) and 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-acrylic acids (VIII_{1–2}) were assayed *in vivo* for antiallergic effect on the passive cutaneous anaphylactic (PCA) reaction. As shown in Table II, only compound VIII₂ exerted a significant inhibitory effect on PCA.

Next, the antiallergic effect of 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides was examined. As shown in Table III, greater activity was observed with the introduction of lower alkyl groups on amide nitrogen among 2-methyl derivatives (III_{1–12}). Compound III₃ with an ethyl group on amide nitrogen

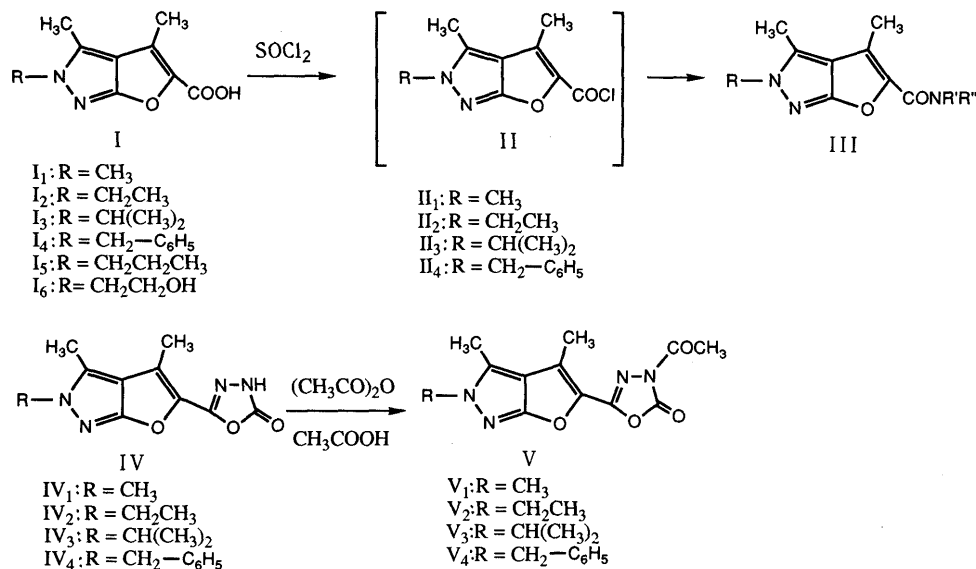


Chart 1

TABLE I. Physical Constants and Spectral Data for 2-Alkyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III₁₋₃₂)

Compd. No.	Recrystallization method ^{a)}	Yield (%)	mp (°C)	Formula	Analysis (%) ^{b)}			Found			IR (KBr) $\nu_{\text{C=O}}$ cm ⁻¹	UV λ_{max} (Solvent), nm
					C	H	N	C	H	N		
III ₁	A	61	148—150	C ₉ H ₁₁ N ₃ O ₂	55.95	5.74	21.75	55.83	5.70	21.71	1670	(CHCl ₃) 288
III ₂	A	82	186—188	C ₁₀ H ₁₃ N ₃ O ₂	57.96	6.32	20.28	57.79	6.30	20.28	1630	(CHCl ₃) 279
III ₃	A	95	172—174	C ₁₁ H ₁₅ N ₃ O ₂	59.71	6.83	18.99	59.79	6.69	18.87	1630	(CHCl ₃) 285
III ₄	A	98	168—170	C ₁₂ H ₁₇ N ₃ O ₂	61.26	7.28	17.86	61.30	7.35	17.69	1630	(CHCl ₃) 280
III ₅	A	97	146—148	C ₁₄ H ₂₁ N ₃ O ₂	63.85	8.04	15.96	63.77	8.14	15.87	1630	(CHCl ₃) 285
III ₆	A	62	138—140	C ₁₅ H ₂₃ N ₃ O ₂	65.43	7.69	15.26	65.49	7.61	15.32	1630	(CHCl ₃) 280
III ₇	A	50	235—238	C ₁₄ H ₂₂ N ₄ O ₂	60.41	7.97	20.13	60.40	7.89	20.23	1630	(CHCl ₃) 286
III ₈	A	66	213—215	C ₁₉ H ₂₁ N ₃ O ₄	64.21	5.96	11.82	64.30	5.84	11.70	1660	(CHCl ₃) 305
III ₉	A	90	116—118	C ₁₁ H ₁₅ N ₃ O ₂	59.71	6.83	18.99	59.60	6.71	18.75	1610	(CHCl ₃) 285
III ₁₀	A	81	152—154	C ₁₃ H ₁₉ N ₃ O ₂	62.62	7.68	16.85	62.71	7.60	16.81	1605	(CHCl ₃) 285
III ₁₁	A	85	156—158	C ₁₄ H ₁₉ N ₃ O ₂	64.35	7.33	16.08	64.22	7.30	16.12	1610	(CHCl ₃) 280
III ₁₂	A	90	183—185	C ₁₃ H ₁₇ N ₃ O ₃	59.30	6.51	15.96	59.20	6.39	15.81	1610	(CHCl ₃) 280
III ₁₃	A	93	145—147	C ₁₄ H ₂₀ N ₄ O ₂	60.85	7.29	20.27	60.74	7.32	20.35	1615	(CHCl ₃) 288
III ₁₄	A	63	113—115	C ₁₂ H ₁₉ N ₃ O ₂	61.26	7.28	17.86	61.35	7.31	17.69	1630	(CHCl ₃) 275
III ₁₅	A	85	109—110	C ₁₃ H ₁₉ N ₃ O ₂	62.63	7.68	16.85	62.60	7.64	16.83	1630	(CHCl ₃) 284
III ₁₆	A	73	106—107	C ₁₄ H ₂₁ N ₃ O ₂	63.85	8.04	15.96	63.91	8.12	15.84	1630	(CHCl ₃) 285
III ₁₇	A	71	265—267	C ₁₀ H ₁₃ N ₃ O ₂	57.96	6.32	20.28	57.79	6.38	20.34	1680	(C ₂ H ₅ OH) 283
III ₁₈	A	87	171—172	C ₁₁ H ₁₅ N ₃ O ₂	59.71	6.83	18.99	59.65	6.80	18.95	1640	(CHCl ₃) 277
III ₁₉	A	58	129—131	C ₁₂ H ₁₉ N ₃ O ₂	61.26	7.28	17.86	61.32	7.32	17.75	1640	(CHCl ₃) 281
III ₂₀	A	60	113—115	C ₁₃ H ₁₉ N ₃ O ₂	62.63	7.68	16.85	62.70	7.72	16.85	1640	(CHCl ₃) 275
III ₂₁	A	56	79—80	C ₁₄ H ₂₁ N ₃ O ₂	63.85	8.04	15.96	63.80	8.16	15.92	1640	(CHCl ₃) 286
III ₂₂	A	68	110—113	C ₁₃ H ₁₉ N ₃ O ₂	62.63	7.68	16.85	62.59	7.60	16.74	1630	(CHCl ₃) 286
III ₂₃	A	48	63—65	C ₁₄ H ₂₁ N ₃ O ₂	63.85	8.04	15.96	63.88	8.15	15.92	1610	(CHCl ₃) 275
III ₂₄	A	46	133—135	C ₁₇ H ₁₉ N ₃ O ₂	68.67	6.44	14.13	68.74	6.49	14.00	1640	(CHCl ₃) 280
III ₂₅	A	77	56—55	C ₁₄ H ₂₂ N ₄ O ₂	60.41	7.97	20.13	60.74	7.49	20.00	1635	(CHCl ₃) 276
III ₂₆	A	76	134—135	C ₂₅ H ₂₇ N ₃ O ₂	74.79	6.78	10.47	74.84	6.69	10.52	1630	(CHCl ₃) 286
III ₂₇	A	55	128—130	C ₂₀ H ₂₄ N ₄ O ₂	68.16	6.86	15.90	68.20	6.91	15.86	1625	(CHCl ₃) 277
III ₂₈	A	61	129—131	C ₁₉ H ₂₃ N ₅ O ₂	64.57	6.56	19.82	64.49	6.46	19.75	1618	(CHCl ₃) 280
III ₂₉	A	50	225—226	C ₁₃ H ₁₈ N ₄ O ₄	53.05	6.16	19.04	53.11	6.10	19.12	1660, 1740	(CHCl ₃) 280
III ₃₀	A	76	176—177	C ₁₁ H ₁₅ ClN ₃ O ₂	59.71	6.83	18.99	59.68	6.91	18.90	1680	(CHCl ₃) 302
III ₃₁	A	85	148—150	C ₁₂ H ₁₇ ClN ₃ O ₂	61.26	7.28	17.86	61.31	7.32	17.65	1650	(CHCl ₃) 286
III ₃₂	A	60	128—130	C ₁₃ H ₁₉ N ₃ O ₂	62.63	7.68	16.85	62.70	7.72	16.74	1650	(CHCl ₃) 287

a) A, silica-gel-CHCl₃. b) Analyzed for C, H, N; analytical results were within $\pm 0.4\%$ of the theoretical values.

showed the greatest antiallergic activity, while compounds with a shorter or longer alkyl substituent as well as with two alkyl groups on amide nitrogen had weak or no activity. The order of relative antiallergic activities

among different structures was $-\text{NH}_2 < -\text{NHCH}_3 < -\text{NHCH}_2\text{CH}_3 > -\text{NH}(\text{CH}_2)_2\text{CH}_3 \geq \text{NH}(\text{CH}_2)_3\text{CH}_3$.

As in the 2-methyl derivatives, compounds with lower alkyl groups on amide nitrogen appeared to be more active

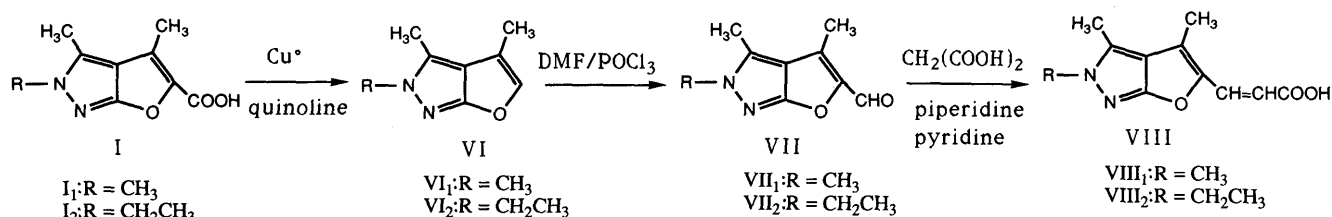
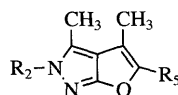
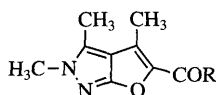


Chart 2

TABLE II. Antiallergic Effect of 2-N-Substituted 3,4-Dimethylfuro[2,3-c]pyrazole-5-carboxylic Acids (I₁₋₆) and 2-N-Substituted 3,4-Dimethylfuro[2,3-c]pyrazole-5-acrylic Acids (VIII₁₋₂)

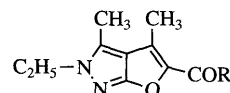
Compd.	Dose (mg/kg) <i>p.o.</i>	R ₂	R ₅	Inhibition (%)	<i>n</i>
I ₁	80	-CH ₃	-COOH	5.2	(4)
I ₂	80	-CH ₂ CH ₃	-COOH	2.9	(3)
I ₃	80	-CH(CH ₃) ₂	-COOH	-2.9	(3)
I ₄	80	-CH ₂ -C ₆ H ₅	-COOH	2.1	(4)
I ₅	80	-CH ₂ CH ₂ CH ₃	-COOH	-5.7	(3)
I ₆	80	-CH ₂ CH ₂ OH	-COOH	9.6	(3)
VIII ₁	80	-CH ₃	-CH=CH-COOH	-2.2	(4)
VIII ₂	80	-CH ₂ CH ₃	-CH=CH-COOH	31.6 ^a	(4)
Theophylline				67.8 ^a	(4)

a) Significantly different from the control group, *p* < 0.01. *n*: number of rats.TABLE III. Antiallergic Effect of 2-Methyl-3,4-dimethylfuro[2,3-c]pyrazole-5-carboxamides (III₁₋₁₂)

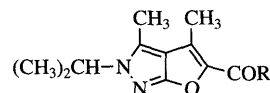
Compd.	Dose (mg/kg) <i>p.o.</i>	R	Inhibition (%)	<i>n</i>
III ₁	80	-NH ₂	5.2	(3)
III ₂	80	-NHCH ₃	39.9 ^a	(3)
III ₃	80	-NHCH ₂ CH ₃	49.0 ^a	(3)
III ₄	80	-NH(CH ₂) ₂ CH ₃	31.0 ^a	(4)
III ₅	80	-NHCH(CH ₃) ₂	13.2	(3)
III ₆	80	-NH(CH ₂) ₃ CH ₃	31.0 ^a	(4)
III ₇	80	-NH(CH ₂) ₂ CH(CH ₃) ₂	29.6 ^a	(3)
III ₈	80	-NH(CH ₂) ₄ CH ₃	1.5	(3)
III ₉	80	-N(CH ₃) ₂	20.2 ^a	(3)
III ₁₀	80	-N	1.9	(4)
III ₁₁	80	-N-CH ₃	9.3	(3)
III ₁₂	80	-N	12.6	(4)

a) Significantly different from the control group, *p* < 0.01.

than others among 2-ethyl derivatives (III₁₃₋₂₅) and 2-isopropyl derivatives (III₂₆₋₂₈), as shown in Tables IV and V, respectively. With a methyl substituent on amide nitrogen (III₁₄, III₂₇), the greatest activity was observed. The relative antiallergic activities of compounds with

TABLE IV. Antiallergic Effect of 2-Methyl-3,4-dimethylfuro[2,3-c]pyrazole-5-carboxamides (III₁₃₋₂₅)

Compd.	Dose (mg/kg) <i>p.o.</i>	R	Inhibition (%)	<i>n</i>
III ₁₃	80	-NH ₂	15.7	(3)
III ₁₄	80	-NHCH ₃	51.6 ^a	(4)
III ₁₅	80	-NHCH ₂ CH ₃	19.9 ^a	(3)
III ₁₆	80	-NH(CH ₂) ₂ CH ₃	23.1 ^a	(3)
III ₁₇	80	-NH(CH ₂) ₃ CH ₃	32.4 ^a	(4)
III ₁₈	80	-NHCH(CH ₃) ₂	26.2 ^a	(3)
III ₁₉	80	-N(CH ₂ CH ₃) ₂	30.1 ^a	(4)
III ₂₀	80	-N-CH ₂ -C ₆ H ₅	6.5	(3)
III ₂₁	80	-NH(CH ₂) ₂ N(CH ₃) ₂	9.0	(3)
III ₂₂	80	-NH(CH ₂) ₂ CH(C ₆ H ₅) ₂	21.2 ^a	(4)
III ₂₃	80	-NHNHCOOC ₂ H ₅	13.1	(3)
III ₂₄	80	-N-C ₆ H ₅	4.4	(3)
III ₂₅	80	-N	7.0	(3)

a) Significantly different from the control group, *p* < 0.01.TABLE V. Antiallergic Effect of 2-Isopropyl-3,4-dimethylfuro[2,3-c]pyrazole-5-carboxamides (III₂₆₋₂₈)

Compd.	Dose (mg/kg) <i>p.o.</i>	R	Inhibition (%)	<i>n</i>
III ₂₆	80	-NH ₂	36.5 ^a	(3)
III ₂₇	80	-NHCH ₃	53.5 ^a	(4)
III ₂₈	80	-NHCH ₂ CH ₃	30.5 ^a	(4)

a) Significantly different from the control group, *p* < 0.01.

different 2-alkyl substituents were as follows: -NH₂ < -NHCH₃ > -NHCH₂CH₃ < -NH(CH₂)₂CH₃ < -NH(CH₂)₃CH₃ for 2-ethyl derivatives and -NH₂ < -NHCH₃ > -NHCH₂CH₃ for 2-isopropyl derivatives. However, loss of activity occurred with a benzyl substituent at position 2 (Table VI). These results suggested that 3,4-dimethylfuro[2,3-c]pyrazole-5-carboxamide with a lower alkyl group at position 2 and one or two lower alkyl groups on amide nitrogen would show potent antiallergic activity.

The antiallergic effects of 4,5-dihydro-5-oxo-1,3,4-

TABLE VI. Antiallergic Effect of 2-Benzyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III₂₉₋₃₁)

Compd.	Dose (mg/kg) <i>p.o.</i>	R	Inhibition (%)	<i>n</i>
III ₂₉	80	-NH ₂	4.4	(3)
III ₃₀	80	-NHCH ₃	-8.6	(3)
III ₃₁	80	-NHCH ₂ CH ₃	-18.7	(3)

TABLE VII. Antiallergic Effect of 2-Alkyl-5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazoles (IV₁₋₄, V₁₋₄)

Compd.	Dose (mg/kg) <i>p.o.</i>	R	R'	Inhibition (%)	<i>n</i>
IV ₁	80	-CH ₃	-H	53.0 ^{a)}	(3)
IV ₂	80	-CH ₂ CH ₃	-H	14.1	(4)
IV ₃	80	-CH(CH ₃) ₂	-H	46.1 ^{a)}	(4)
IV ₄	80	-CH ₂ -C ₆ H ₅	-H	-27.3	(4)
V ₁	80	-CH ₃	-COCH ₃	19.8 ^{a)}	(4)
V ₂	80	-CH ₂ CH ₃	-COCH ₃	25.8 ^{a)}	(4)
V ₃	80	-CH(CH ₃) ₂	-COCH ₃	22.7 ^{a)}	(4)
V ₄	80	-CH ₂ -C ₆ H ₅	-COCH ₃	5.3	(4)

a) Significantly different from the control group, $p < 0.01$.

oxadiazole derivatives (IV₁₋₄, VI₁₋₄) were also evaluated as shown in Table VII. Compounds with lower alkyl groups at position 2 demonstrated greater activity, while compounds with a 2-benzyl substituent (IV₄, V₄) appeared inactive. It has been reported that the antiallergic activities of *N*-acetyl derivatives of 4,5-dihydro-5-oxo-1,3,4-oxadiazole derivatives were greater than those of the parent compounds.³⁾ Nevertheless, we found that *N*-acetylation of 2-alkyl-5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazoles (IV₁, IV₃) reduced the antiallergic activity.

According to the structure-activity relationship among compounds I, III, IV, V and VIII, an amide or 5-oxo-1,3,4-oxadiazole substituent at position 5 is favorable for antiallergic activity, while -COOH or -CH=CHCOOH was unfavorable. This profile of 3,4-dimethylfuro[2,3-*c*]pyrazole derivatives is different from that of other antiallergic drugs. In addition, introduction of lower alkyl groups at position 2 resulted in potent activity. These results provided important information for further investigation.

b. Effect of 3,4-Dimethylfuro[2,3-*c*]pyrazoles on Mast Cell Degranulation Mast cells, which release various inflammatory mediators during immunological challenge, participate as a prominently in the PCA reaction.⁴⁾ The effect of six derivatives of 3,4-dimethylfuro[2,3-*c*]pyrazole (I₃, III₂₇, III₃₀, IV₃, IV₄, V₃) on mast cell degranulation was examined. As shown in Table VIII, none of the tested

TABLE VIII. Effect of 3,4-Dimethylfuro[2,3-*c*]pyrazole Derivatives on the Release of β -Glucuronidase from Mast Cells Stimulated with Compound 48/80

Compd.	(μ M)	Inhibition (%)	<i>n</i>
I ₃	30	8.8 $\pm$ 4.2	(4)
	100	15.2 $\pm$ 13.5	(4)
III ₂₇	30	2.3 $\pm$ 4.8	(3)
	100	24.4 $\pm$ 12.0	(4)
III ₃₀	30	9.8 $\pm$ 9.6	(4)
	100	10.3 $\pm$ 5.3	(4)
IV ₃	30	18.7 $\pm$ 14.6	(3)
	100	16.7 $\pm$ 8.2	(4)
IV ₄	30	25.8 $\pm$ 2.7	(3)
	100	22.1 $\pm$ 9.9	(3)
V ₃	30	20.3 $\pm$ 15.4	(3)
	100	26.8 $\pm$ 10.2	(3)
Mepacrine	10	40.2 $\pm$ 3.7 ^{a)}	(3)
	30	75.2 $\pm$ 2.4 ^{a)}	(3)

a) Significantly different from the control group, $p < 0.01$.

compounds, except the positive control, mepacrine, had a significantly inhibitory effect on the release reaction of mast cells induced by compound 48/80. Mepacrine, a phospholipase inhibitor, was reported to inhibit the mast cell degranulation.⁵⁾ This result was not in accordance with the antiallergic activity evaluated in the PCA reaction, indicating that the antiallergic action of 3,4-dimethylfuro[2,3-*c*]pyrazole derivatives probably does not involve the suppression of mast cell degranulation. Drugs which inhibit the generation and release of mediator from the inflammatory cells or which protect the vasculature against mediator challenge were proposed to suppress the edematous response in the PCA reaction.⁶⁻⁸⁾ Therefore, the protective effect of 3,4-dimethylfuro[2,3-*c*]pyrazole derivatives on the vascular plasma extravasation caused by some direct-acting mediators was examined.

c. Effect of 3,4-Dimethylfuro[2,3-*c*]pyrazoles on Phlogist-Induced Vascular Permeability Changes Histamine, serotonin, bradykinin and substance P could produce vascular plasma leakage by acting directly through specific receptors on the endothelial cells of postcapillary venules.⁹⁻¹³⁾ Several lines of evidence indicate that after receptor activation there is transient elevation of cytosolic calcium and contraction of actomyosin-like filaments leading to change in the shape of endothelial cells and opening of inter-endothelial cell gaps, which permit plasma extravasation.¹⁴⁻¹⁶⁾ In this study, only two derivatives of 3,4-dimethylfuro[2,3-*c*]pyrazole (III₂₇, IV₃) were chosen to investigate the protective effect against phlogist-induced vascular plasma extravasation. As shown in Table IX, compounds III₂₇ and IV₃ significantly reduced the histamine-, serotonin-, bradykinin- and substance P-induced ear edema in mice. Plasma leakage appears to be due to bradykinin and substance P acting directly on postcapillary venules as well as due to release of mast cell-derived mediator in rodent skin.¹⁷⁻¹⁹⁾ In order to investigate the vascular plasma leakage caused by the direct action of bradykinin and substance P on the receptors of the vasculature, mice were pretreated with several doses of compound 48/80 to deplete the preformed mediator in the mast cells. After this treatment, the

TABLE IX. Effect of III₂₇ and IV₃ on Histamine, Serotonin, Bradykinin and Substance P-Induced Plasma Exudation in Mice

Compd.	(mg/kg)	Plasma exudation (μl)			
		Histamine	Serotonin	Bradykinin	Substance P
Control		2.1 ± 0.2	11.9 ± 0.5	6.6 ± 0.5	4.9 ± 0.3
III ₂₇	8	0.3 ± 0.1 ^{a)}	7.8 ± 0.3 ^{a)}	3.8 ± 0.2 ^{a)}	3.4 ± 0.3 ^{a)}
IV ₃	8	0.9 ± 0.2 ^{a)}	11.3 ± 0.5	—	—
	30	0.3 ± 0.1 ^{a)}	6.3 ± 0.4 ^{a)}	4.6 ± 0.3 ^{a)}	2.5 ± 0.3 ^{a)}

Values are expressed as means ± S.E.M. of 5–6 animals. a) Significantly different from the control group, $p < 0.01$. —: Not detected.

TABLE X. Effect of III₂₇ and IV₃ on Bradykinin- and Substance P-Induced Plasma Exudation in Compound 48/80-Pretreated Mice

Compd.	(mg/kg)	Plasma exudation (μl)	
		Bradykinin	Substance P
Control		4.7 ± 0.3	2.3 ± 0.1
Diphenhydramine	10		
/methysergide	3	3.6 ± 0.2 ^{a)}	1.5 ± 0.1 ^{b)}
III ₂₇	8	4.6 ± 0.3	2.0 ± 0.2
	30	2.6 ± 0.3 ^{b,d)}	0.8 ± 0.1 ^{b,c)}
IV ₃	8	5.2 ± 0.3	2.2 ± 0.2
	30	2.5 ± 0.2 ^{b,d)}	0.8 ± 0.1 ^{b,c)}

Values are expressed as means ± S.E.M. of 5–6 animals. Significance of differences is indicated as follows: a) $p < 0.05$, b) $p < 0.01$ (difference from control); c) $p < 0.05$, d) $p < 0.01$ (difference from diphenhydramine/methysergide-treated groups).

histamine content of the ear was reduced to less than 15% of that of control mice.^{6–8)} After treatment of compound 48/80-pretreated mice with diphenhydramine and methysergide, inhibitors of histamine and serotonin, respectively, the bradykinin- and substance P-induced plasma leakage is likely to occur predominantly as a result of their direct action on the vasculature. Under this condition, compounds III₂₇ and IV₃ inhibited edema formation to a greater extent than did diphenhydramine in combination with methysergide (Table X). These results suggest that the antiallergic effect of 2-alkyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III) and 2-alkyl-5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazoles (IV) is probably due to protection of the vasculature against mediator-induced plasma extravasation.

Experimental

Chemistry Melting points were determined in open-ended capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-440 spectrometer in KBr. NMR spectra were taken at 90 MHz on a JEOL FX-90Q spectrometer and a Varian VXR-300 FT-NMR spectrometer with tetramethylsilane (TMS) as an internal reference in CDCl₃ or in dimethyl sulfoxide (DMSO)-*d*₆ unless otherwise stated. Splitting patterns are designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Mass spectra (MS) were measured with an HP 5995 GC-MS instrument and a JEOL JMS-D-30 mass spectrometer. The UV spectra were recorded on a Hewlett Packard Diode Array UV-VIS spectrometer (HP-8452A). Elemental analyses were performed by Chung Shan Institute of Science Technology and National Cheng-Kung University, Taiwan, Republic of China.

2-Substituted 3,4-Dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III_{1–32}) A solution of compound I₃₁³⁾ (7.8 g, 0.04 mol) in dried benzene (50 ml) was treated dropwise with SOCl₂ (0.045 mol). The mixture was

refluxed for 24 h. After the reaction was completed, solvents were removed by evaporation. The residue was washed with petroleum ether to afford 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carbonylchloride (II₁). The crude intermediate (II₁) was dissolved in dried benzene (100 ml). The solution was stirred at room temperature and ammonia gas (0.1 mol) was added. After heating at reflux for 2 h, the mixture was cooled to 10 ± 2 °C. The crystals formed were collected and washed with water and recrystallized from CHCl₃-EtOH to give 3.9 g of 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III₁). In a manner similar to the above general procedure, and with appropriate starting materials and reagents compounds, III_{2–32} were prepared (Table I).

5-(4,5-Dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2,3,4-trimethylfuro[2,3-*c*]pyrazole (IV₁) One gram (0.036 mol) of *N*-(ethoxycarbonylamino)-2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III₃₂) was added with stirring to diphenyl ether (100 ml) at 230–240 °C. After heating for 1 h, the reaction mixture was diluted with a large volume of *n*-hexane to provide a dark solid that was collected, washed with hot *n*-hexane and recrystallized from EtOH to yield IV₁ 0.8 g (93%). mp 236–239 °C. IR $\nu_{\text{max}}^{\text{KBr}}$: 3050 (–NH), 1780 (C=O) cm^{–1}. UV $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$: 287 nm ($\epsilon = 3.0 \times 10^4$). ¹H-NMR δ : 2.45 (6H, s, 3-CH₃, 4-CH₃), 3.83 (3H, s, N-CH₃). MS m/z : 234 (M⁺). Anal. Calcd for C₁₀H₁₂N₄O₃: C, 51.28; H, 4.30; N, 23.92. Found: C, 51.34; H, 4.39; N, 23.90.

5-(4-Acetyl-4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2,3,4-trimethylfuro[2,3-*c*]pyrazole (V₁) One gram (0.0043 mol) of compound IV₁ in 10 ml of acetic acid containing 5 ml of acetic anhydride was heated for 1 h at 100 ± 2 °C. The mixture was poured into water, and the crystalline product was collected by filtration. Recrystallization from CHCl₃-acetonitrile gave 1.0 g (85%) of V₁. mp 236–239 °C. IR $\nu_{\text{max}}^{\text{KBr}}$: 3050 (–NH), 1720 (C=O) cm^{–1}. UV $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$: 314 nm ($\epsilon = 3.5 \times 10^3$). ¹H-NMR δ : 2.45 (9H, s, 3-CH₃, 4-CH₃, –COCH₃), 3.83 (3H, s, N-CH₃). MS m/z : 276 (M⁺). Anal. Calcd for C₁₂H₁₂N₄O₄: C, 52.17; H, 4.38; N, 20.28. Found: C, 52.20; H, 4.43; N, 20.32.

5-(4-Acetyl-4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-ethyl-3,4-dimethylfuro[2,3-*c*]pyrazole (V₂) 5-(4,5-Dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-ethyl-3,4-dimethylfuro[2,3-*c*]pyrazole (IV₂) (1 g, 0.004 mol) was treated as described for the preparation of V₁ to afford V₂ 0.9 g (72%). mp 208–210 °C. IR $\nu_{\text{max}}^{\text{KBr}}$: 3300 (–NH), 1740 (C=O), 1640 (C=O) cm^{–1}. UV $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$: 311 nm ($\epsilon = 5.5 \times 10^3$). ¹H-NMR δ : 1.33 (3H, t, N-CH₂CH₃), 2.46 (9H, m, 3-CH₃, 4-CH₃, –COCH₃), 4.17 (2H, q, N-CH₂CH₃). MS m/z : 306 (M⁺). Anal. Calcd for C₁₃H₁₄N₄O₄: C, 53.79; H, 4.86; N, 19.30. Found: C, 53.82; H, 4.74; N, 19.26.

5-(4-Acetyl-4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-isopropyl-3,4-dimethylfuro[2,3-*c*]pyrazole (V₃) 5-(4,5-Dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-isopropyl-3,4-dimethylfuro[2,3-*c*]pyrazole (IV₃) (1.0 g, 0.0038 mol) was treated as described for the preparation of V₁ to afford V₃ 0.9 g (77%). mp 208–210 °C. IR $\nu_{\text{max}}^{\text{KBr}}$: 3000 (–NH), 1730 (C=O), 1640 (C=O) cm^{–1}. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$: 314 nm ($\epsilon = 3.5 \times 10^4$). ¹H-NMR δ : 1.33–1.50 (6H, d, N-CH(CH₃)₂), 2.48 (9H, m, 3-CH₃, 4-CH₃, –COCH₃), 4.33–4.67 (1H, m, N-CH). MS m/z : 307 (M⁺). Anal. Calcd for C₁₄H₁₆N₄O₄: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.40; H, 5.51; N, 18.46.

5-(4-Acetyl-4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-benzyl-3,4-dimethylfuro[2,3-*c*]pyrazole (V₄) 2-Benzyl-5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazole (IV₄) (1.0 g, 0.0032 mol) was treated as described for the preparation of V₁ to afford V₄ 0.8 g (70%). mp 168–170 °C. IR $\nu_{\text{max}}^{\text{KBr}}$: 3200 (–NH), 1740 (C=O), 1640 (C=O) cm^{–1}. UV $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$: 297 nm ($\epsilon = 2.0 \times 10^4$). ¹H-NMR δ : 2.47 (9H, m, 3-CH₃, 4-CH₃, –COCH₃), 5.37 (2H, s, –CH₂–), 7.25 (5H, m, –C₆H₅). MS m/z : 325 (M⁺). Anal. Calcd for C₁₈H₁₆N₄O₄: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.40; H, 4.63; N, 15.96.

2,3,4-Trimethylfuro[2,3-*c*]pyrazole (VI₁) 2,3,4-Trimethylfuro[2,3-*c*]pyrazole-5-carboxylic acid (I₁) (5.0 g, 0.026 mol) was dissolved in dry quinoline (60 ml) and active copper (1.0 g, 0.016 mol) was added, then the mixture was heated and maintained at 200 ± 5 °C for 1 h under stirring. After the reaction was completed, the reaction mixture was cooled to room temperature, acidified with 10% HCl, and then filtered. The filtrate was extracted with CHCl₃, and the CHCl₃ layer was washed with water, dried with MgSO₄ and evaporated to dryness. The residue was purified by column chromatography (silica gel-CHCl₃) to give compound VI₁ 2.5 g (70%). mp 68–71 °C. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$: 249 nm. ¹H-NMR (CDCl₃) δ : 2.06 (3H, s, 4-CH₃), 2.33 (3H, s, 3-CH₃), 3.37 (3H, s, N-CH₃), 6.86 (1H, s, 5-H). MS m/z : 150 (M⁺). Anal. Calcd for C₈H₁₀N₂O: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.79; H, 6.75; N, 18.54.

2-Ethyl-3,4-dimethylfuro[2,3-*c*]pyrazole (VI₂) 2-Ethyl-3,4-dimethyl-

furo[2,3-*c*]pyrazole-5-carboxylic acid (**I**₂) (5.0 g, 0.024 mol) was treated as described for the preparation of **VI**₁ to afford **VI**₂ 3.2 g (80%). mp 32–34°C. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$: 259 nm. ¹H-NMR (CDCl₃) δ : 1.42 (3H, t, –CH₂CH₃), 2.11 (3H, s, 4-CH₃), 2.40 (3H, s, 3-CH₃), 4.03 (2H, q, –CH₂CH₃), 6.89 (1H, s, 5-H). MS *m/z*: 164 (M⁺). Anal. Calcd for C₉H₁₂N₂O: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.80; H, 7.34; N, 17.17.

2,3,4-Trimethylfuro[2,3-*c*]pyrazole-5-carbaldehyde (VII₁) DMF (30 g, 0.41 mol) was added dropwise to POCl₃ (10 g, 0.065 mol) at 5–10°C. The solution was stirred at 10°C for an additional 20 min. To this solution, a solution of compound **VI**₁ (1.0 g, 0.0067 mol) in DMF (20 ml) was added dropwise at 0–5°C. The mixture was stirred at 30 ± 2°C for an additional 30 min and then poured into ice water, and the whole was neutralized with Na₂CO₃ then allowed to settle at room temperature for 8 h. The solution was extracted with CHCl₃, dried with MgSO₄ and evaporated to dryness. The residue was purified by column chromatography (silica gel–CHCl₃) to give compound **VII**₁ 0.95 g (80%). mp 122–124°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 1645 (C=O) cm^{−1}. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$: 320 nm. ¹H-NMR (CDCl₃) δ : 2.50 (3H, s, 3-CH₃), 2.53 (3H, s, 4-CH₃), 3.86 (3H, s, N-CH₃), 9.69 (1H, s, CHO). MS *m/z*: 178 (M⁺). Anal. Calcd for C₉H₁₀N₂O₂: C, 60.70; H, 5.66; N, 15.72. Found: C, 60.74; H, 5.53; N, 15.62.

2-Ethyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carbaldehyde (VII₂) Compound **VI**₂ (1.0 g, 0.024 mol) was treated as described for the preparation of **VII**₁ to afford **VII**₂ 0.8 g (68%). mp 93–94°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 2720 (–CHO), 1680 (C=O) cm^{−1}. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$: 320 nm. ¹H-NMR (CDCl₃) δ : 1.43 (3H, t, –CH₂CH₃), 2.53 (3H, s, 4-CH₃), 2.57 (3H, s, 3-CH₃), 4.13 (2H, q, –CH₂CH₃), 9.65 (1H, s, 5-H). MS *m/z*: 192 (M⁺). Anal. Calcd for C₁₀H₁₂N₂O₂: C, 62.49; H, 6.29; N, 14.57. Found: C, 62.35; H, 6.40; N, 15.63.

2,3,4-Trimethylfuro[2,3-*c*]pyrazole-5-acrylic Acid (VIII₁) Compound **VII**₁ (0.623 g, 0.0035 mol) and piperidine (1.0 ml, 0.011 mol) were added successively to a solution of dry malonic acid (1.0 g, 0.0097 mol) in pyridine (20 ml, 0.25 mol), and the mixture was refluxed for 2 h. The solvent was removed by evaporation and the residue was washed with 5% HCl and then purified by column chromatography (silica gel–CHCl₃+EtOH) to give compound **VIII**₁ 0.6 g (78%). mp 254–256°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 2700–3000 (–COOH), 1685 (CH=CH–COOH) cm^{−1}. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$: 350 nm. ¹H-NMR δ : 2.22 (3H, s, 4-CH₃), 2.43 (3H, s, 3-CH₃), 3.70 (3H, s, N-CH₃), 5.97 (1H, d, *J* = 16 Hz, –CH=CH–COOH), 7.37 (1H, d, *J* = 16 Hz, –CH=CH–COOH). MS *m/z*: 220 (M⁺). Anal. Calcd for C₁₁H₁₂N₂O₃: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.08; H, 5.54; N, 12.62.

2-Ethyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-acrylic Acid (VIII₂) Compound **VII**₂ (0.67 g, 0.0035 mol) was treated as described for the preparation of **VII**₁ to afford **VII**₂ 0.67 g (82%). mp 215–217°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 2500–3100 (–OH), 1600 (CH=CH–COOH) cm^{−1}. UV $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$: 355 nm. ¹H-NMR δ : 1.30 (3H, t, N-CH₂CH₃), 2.25 (3H, s, 4-CH₃), 2.42 (3H, s, 3-CH₃), 4.06 (3H, t, N-CH₂CH₃), 5.97 (1H, d, *J* = 16 Hz, –CH=CH–COOH), 7.37 (1H, d, *J* = 16 Hz, –CH=CH–COOH). MS *m/z*: 234 (M⁺). Anal. Calcd for C₁₂H₁₄N₂O₃: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.60; H, 6.15; N, 11.99.

Pharmacology. Materials for Bioassay Egg albumin, Evans blue, compound 48/80, histamine, serotonin, bradykinin, substance P, bovine serum albumin (BSA), DMSO, phenolphthalein- β -D-glucuronide, diphenhydramine, Triton X-100, sodium pentobarbital and trypan blue were purchased from Sigma Chem. Co. (U.S.A.). Methylsergide was supplied by Sandoz Pharmaceutical Ltd. (Switzerland).

Antiallergic Assay (PCA) Male Std. Wistar rats (140–200 g) were injected intradermally with 0.1 ml of a dilute solution of mouse antiserum (diluted 1:8 with sterile saline) to egg albumin in two sites of the shaved ventral skin.^{4,20} Forty-eight hours later, each rat was challenged by an intravenous injection of 2 mg of the antigen together with 1 ml of 0.5% Evans blue saline solution. The blueing area was measured 30 min after the challenge. The compounds (80 mg/kg) were administered orally to the rats 1 h before antigen challenge. The antiallergic activity of the compounds was expressed as percent inhibition of the blueing area compared with the vehicle control. The mean blueing area in each experiment ranged between 168 and 272 mm². Three to eight rats were used for each dose.

Mast Cell Degranulation Rat peritoneal mast cells were isolated as described.²¹ Briefly, heparinized Tyrode solution was injected into the peritoneal cavity of exsanguinated rats. After abdominal massage, the cells in the peritoneal fluid were harvested and separated in 38% BSA

in glucose-free Tyrode solution. The cell pellet was washed and suspended in tyrode solution to 1–1.5 × 10⁶ cells/ml. Cell viability was assessed by use of the trypan blue exclusion test. The mast cell suspension was preincubated at 37°C with DMSO or test compounds for 3 min, and the release reaction was triggered by the addition of compound 48/80. The reaction was stopped 15 min later by the addition of ice-cold Tyrode solution and the β -glucuronidase in the supernatant was measured, with phenolphthalein- β -D-glucuronide as substrate,²² by spectrophotometry at 550 nm. The total content was measured after treatment of the cell suspension with Triton X-100. Spontaneous release was less than 10%.

Non-immunological Phlogist-Induced Vascular Permeability Changes Permeability changes in the ear vasculature of mice (ICR, 25–30 g) were induced by a single injection of phlogist (3 nmol of histamine, 1 nmol of serotonin, 1 nmol of bradykinin or 0.3 nmol of substance P) or an equal volume of sterile saline into the right and left ears, respectively, 5 min after the intravenous injection of 0.5% Evans blue with 0.15% sodium pentobarbital in saline (4 ml/kg).^{6–8} The animals were killed 45 min after the phlogist challenge. A sample of tissue (9 mm diameter) was punched out from both the right and left ears. Extravasated Evans blue in the tissue samples was extracted as described.²³ The volume of plasma leakage of each tissue sample was calculated by interpolation on an absorbance-plasma volume standard curve which was prepared by measuring the absorbance of different volumes of plasma isolated from mice pretreated with Evans blue.

Depletion of Histamine and Serotonin Mice were injected with compound 48/80 or sterile saline into the right and left ears, respectively, twice a day for six doses.^{6–8} The doses were 1 μ g for the first three injections and 3 μ g for the last three injections.

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