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 (III_3), 2-ethyl-*N*-methyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III_{14}), 2-isopropyl-*N*-methyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III_{27}), 5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2,3,4-trimethylfuro[2,3-*c*]pyrazole (IV_1) and 5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-isopropyl-3,4-dimethylfuro[2,3-*c*]pyrazole (IV_3) 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 III_{27} and IV_3 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 III_{27} and IV_3 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

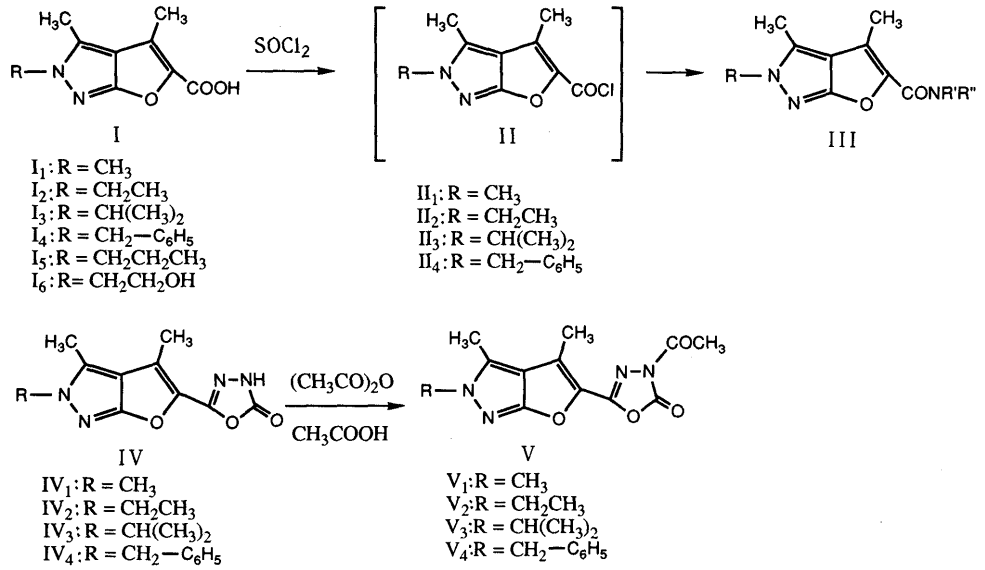
Chemistry The preparation of several 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxylic acids (I_{1-4}) and 2-substituted 5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazole (IV_{1-4}) was described in a previous paper.[2] As shown in Chart 1, compounds I were allowed to react with $SOCl_2$ to afford 2-substituted 3,4-dimethylfuro[2,3-*c*]pyrazole-5-carbonylchlorides (II_{1-4}), which were treated with a variety of amines to give the corresponding amides (III) (Table I). On the other hand, compounds IV[2] 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 (I_1) was heated with quinoline in the presence of active copper, 2,3,4-trimethylfuro[2,3-*c*]pyrazole (IV_1) was obtained.

The Vielsmeier formylation of VI_1 with $POCl_3$ in dimethylformamide (DMF) afforded 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carbaldehyde (VII_1). Finally, compound VII_1 was condensed with malonic acid to afford the corresponding acrylic acid ($VIII_1$). The ^{1}H-NMR spectrum of $VIII_1$ showed signals at δ 2.28 (3H, s, 4-CH_3), 2.43 (3H, s, 3-CH_3), 3.70 (3H, s, 2-CH_3), 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 $VIII_2$ 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 Tabe II, only compound $VIII_2$ 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 III_3 with an ethyl group on amide nitrogen



I
I_1: R = CH_3
I_2: R = CH_2CH_3
I_3: R = $CH(CH_3)_2$
I_4: R = CH_2—C_6H_5
I_5: R = $CH_2CH_2CH_3$
I_6: R = CH_2CH_2OH

II
II_1: R = CH_3
II_2: R = CH_2CH_3
II_3: R = $CH(CH_3)_2$
II_4: R = CH_2—C_6H_5

III

IV
IV_1: R = CH_3
IV_2: R = CH_2CH_3
IV_3: R = $CH(CH_3)_2$
IV_4: R = CH_2—C_6H_5

V
V_1: R = CH_3
V_2: R = CH_2CH_3
V_3: R = $CH(CH_3)_2$
V_4: R = CH_2—C_6H_5

Chart 1

TABLE I. Physical Constants and Spectral Data for 2-Alkyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III_{1-32})

Compd. No.	Recrystallization method[a]	Yield (%)	mp (°C)	Formula	Analysis (%)[b] Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	IR (KBr) $\nu_{C=O}$ cm^{-1}	UV λ_{max} (Solvent), nm
III_1	A	61	148—150	$C_9H_{11}N_3O_2$	55.95	5.74	21.75	55.83	5.70	21.71	1670	($CHCl_3$) 288
III_2	A	82	186—188	$C_{10}H_{13}N_3O_2$	57.96	6.32	20.28	57.79	6.30	20.28	1630	($CHCl_3$) 279
III_3	A	95	172—174	$C_{11}H_{15}N_3O_2$	59.71	6.83	18.99	59.79	6.69	18.87	1630	($CHCl_3$) 285
III_4	A	98	168—170	$C_{12}H_{17}N_3O_2$	61.26	7.28	17.86	61.30	7.35	17.69	1630	($CHCl_3$) 280
III_5	A	97	146—148	$C_{14}H_{21}N_3O_2$	63.85	8.04	15.96	63.77	8.14	15.87	1630	($CHCl_3$) 285
III_6	A	62	138—140	$C_{15}H_{21}N_3O_2$	65.43	7.69	15.26	65.49	7.61	15.32	1630	($CHCl_3$) 280
III_7	A	50	235—238	$C_{14}H_{22}N_4O_2$	60.41	7.97	20.13	60.40	7.89	20.23	1630	($CHCl_3$) 286
III_8	A	66	213—215	$C_{19}H_{21}N_3O_4$	64.21	5.96	11.82	64.30	5.84	11.70	1660	($CHCl_3$) 305
III_9	A	90	116—118	$C_{11}H_{15}N_3O_2$	59.71	6.83	18.99	59.60	6.71	18.75	1610	($CHCl_3$) 285
III_{10}	A	81	152—154	$C_{13}H_{19}N_3O_2$	62.62	7.68	16.85	62.71	7.60	16.81	1605	($CHCl_3$) 285
III_{11}	A	85	156—158	$C_{14}H_{19}N_3O_2$	64.35	7.33	16.08	64.22	7.30	16.12	1610	($CHCl_3$) 280
III_{12}	A	90	183—185	$C_{13}H_{17}N_3O_3$	59.30	6.51	15.96	59.20	6.39	15.81	1610	($CHCl_3$) 280
III_{13}	A	93	145—147	$C_{14}H_{20}N_4O_2$	60.85	7.29	20.27	60.74	7.32	20.35	1615	($CHCl_3$) 288
III_{14}	A	63	113—115	$C_{12}H_{19}N_3O_2$	61.26	7.28	17.86	61.35	7.31	17.69	1630	($CHCl_3$) 275
III_{15}	A	85	109—110	$C_{13}H_{19}N_3O_2$	62.63	7.68	16.85	62.60	7.64	16.83	1630	($CHCl_3$) 284
III_{16}	A	73	106—107	$C_{14}H_{21}N_3O_2$	63.85	8.04	15.96	63.91	8.12	15.84	1630	($CHCl_3$) 285
III_{17}	A	71	265—267	$C_{10}H_{13}N_3O_2$	57.96	6.32	20.28	57.79	6.38	20.34	1680	(C_2H_5OH) 283
III_{18}	A	87	171—172	$C_{11}H_{15}N_3O_2$	59.71	6.83	18.99	59.65	6.80	18.95	1640	($CHCl_3$) 277
III_{19}	A	58	129—131	$C_{12}H_{19}N_3O_2$	61.26	7.28	17.86	61.32	7.32	17.75	1640	($CHCl_3$) 281
III_{20}	A	60	113—115	$C_{13}H_{19}N_3O_2$	62.63	7.68	16.85	62.70	7.72	16.85	1640	($CHCl_3$) 275
III_{21}	A	56	79—80	$C_{14}H_{21}N_3O_2$	63.85	8.04	15.96	63.80	8.16	15.92	1640	($CHCl_3$) 286
III_{22}	A	68	110—113	$C_{13}H_{19}N_3O_2$	62.63	7.68	16.85	62.59	7.60	16.74	1630	($CHCl_3$) 286
III_{23}	A	48	63—65	$C_{14}H_{21}N_3O_2$	63.85	8.04	15.96	63.88	8.15	15.92	1610	($CHCl_3$) 275
III_{24}	A	46	133—135	$C_{17}H_{19}N_3O_2$	68.67	6.44	14.13	68.74	6.49	14.00	1640	($CHCl_3$) 280
III_{25}	A	77	56—55	$C_{14}H_{22}N_4O_2$	60.41	7.97	20.13	60.74	7.49	20.00	1635	($CHCl_3$) 276
III_{26}	A	76	134—135	$C_{25}H_{27}N_3O_2$	74.79	6.78	10.47	74.84	6.69	10.52	1630	($CHCl_3$) 286
III_{27}	A	55	128—130	$C_{20}H_{24}N_4O_2$	68.16	6.86	15.90	68.20	6.91	15.86	1625	($CHCl_3$) 277
III_{28}	A	61	129—131	$C_{19}H_{23}N_5O_2$	64.57	6.56	19.82	64.49	6.46	19.75	1618	($CHCl_3$) 280
III_{29}	A	50	225—226	$C_{13}H_{18}N_4O_4$	53.05	6.16	19.04	53.11	6.10	19.12	1660, 1740	($CHCl_3$) 280
III_{30}	A	76	176—177	$C_{11}H_{15}ClN_3O_2$	59.71	6.83	18.99	59.68	6.91	18.90	1680	($CHCl_3$) 302
III_{31}	A	85	148—150	$C_{12}H_{17}ClN_3O_2$	61.26	7.28	17.86	61.31	7.32	17.65	1650	($CHCl_3$) 286
III_{32}	A	60	128—130	$C_{13}H_{19}N_3O_2$	62.63	7.68	16.85	62.70	7.72	16.74	1650	($CHCl_3$) 287

a) A, silica-gel–$CHCl_3$. *b*) Analyzed for C, H, N; analytical results were within ±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 $-NH_2 < -NHCH_3 < -NHCH_2CH_3 > -NH(CH_2)_2CH_3 \geq NH(CH_2)_3CH_3$.

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

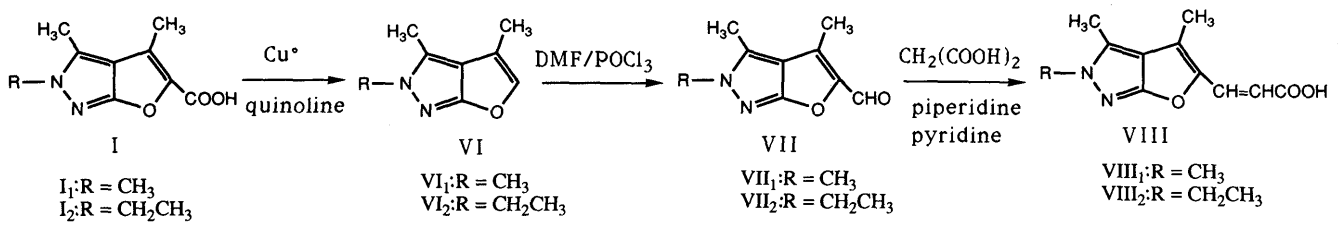
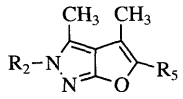


Chart 2

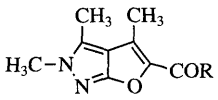
TABLE II. Antiallergic Effect of ^2N-Substituted 3,4-Dimethylfuro[2,3-*c*]pyrazole-5-carboxylic Acids (I_{1-6}) and ^2N-Substituted 3,4-Dimethylfuro[2,3-*c*]pyrazole-5-acrylic Acids ($VIII_{1-2}$)



Compd.	Dose (mg/kg) *p.o.*	R_2	R_5	Inhibition (%)	*n*
I_1	80	$-CH_3$	–COOH	5.2	(4)
I_2	80	$-CH_2CH_3$	–COOH	2.9	(3)
I_3	80	$-CH(CH_3)_2$	–COOH	−2.9	(3)
I_4	80	$-CH_2-C_6H_5$	–COOH	2.1	(4)
I_5	80	$-CH_2CH_2CH_3$	–COOH	−5.7	(3)
I_6	80	$-CH_2CH_2OH$	–COOH	9.6	(3)
$VIII_1$	80	$-CH_3$	–CH=CH–COOH	−2.2	(4)
$VIII_2$	80	$-CH_2CH_3$	–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 (III_{1-12})

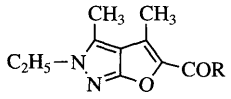


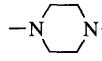
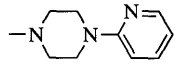
Compd.	Dose (mg/kg) *p.o.*	R	Inhibition (%)	*n*
III_1	80	$-NH_2$	5.2	(3)
III_2	80	$-NHCH_3$	39.9[a]	(3)
III_3	80	$-NHCH_2CH_3$	49.0[a]	(3)
III_4	80	$-NH(CH_2)_2CH_3$	31.0[a]	(4)
III_5	80	$-NHCH(CH_3)_2$	13.2	(3)
III_6	80	$-NH(CH_2)_3CH_3$	31.0[a]	(4)
III_7	80	$-NH(CH_2)_2CH(CH_3)_2$	29.6[a]	(3)
III_8	80	$-NH(CH_2)_4CH_3$	1.5	(3)
III_9	80	$-N(CH_3)_2$	20.2[a]	(3)
III_{10}	80		1.9	(4)
III_{11}	80		9.3	(3)
III_{12}	80		12.6	(4)

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

than others among 2-ethyl deri atives (III_{13-25}) and 2-isopropyl derivatives (III_{26-28}), as shown in Tables IV and V, respectively. With a methyl substituent on amide nitrogen (III_{14}, III_{27}), the greatest activity was observed. The relative antiallergic activities of compounds with

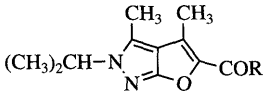
TABLE IV. Antiallergic Effect of 2-Methyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III_{13-25})



Compd.	Dose (mg/kg) *p.o.*	R	Inhibition (%)	*n*
III_{13}	80	$-NH_2$	15.7	(3)
III_{14}	80	$-NHCH_3$	51.6[a]	(4)
III_{15}	80	$-NHCH_2CH_3$	19.9[a]	(3)
III_{16}	80	$-NH(CH_2)_2CH_3$	23.1[a]	(3)
III_{17}	80	$-NH(CH_2)_3CH_3$	32.4[a]	(4)
III_{18}	80	$-NHCH(CH_3)_2$	26.2[a]	(3)
III_{19}	80	$-N(CH_2CH_3)_2$	30.1[a]	(4)
III_{20}	80	$-N-CH_2-C_6H_5$	6.5	(3)
III_{21}	80	$-NH(CH_2)_2N(CH_3)_2$	9.0	(3)
III_{22}	80	$-NH(CH_2)_2CH(C_6H_5)_2$	21.2[a]	(4)
III_{23}	80	$-NHNHCOOC_2H_5$	13.1	(3)
III_{24}	80		4.4	(3)
III_{25}	80		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 (III_{26-28})



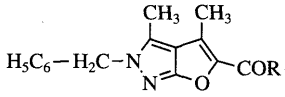
Compd.	Dose (mg/kg) *p.o.*	R	Inhibition (%)	*n*
III_{26}	80	$-NH_2$	36.5[a]	(3)
III_{27}	80	$-NHCH_3$	53.5[a]	(4)
III_{28}	80	$-NHCH_2CH_3$	30.5[a]	(4)

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

different 2-alkyl substituents were as follows: $-NH_2 < -NHCH_3 > -NHCH_2CH_3 < -NH(CH_2)_2CH_3 < NH-(CH_2)_3CH_3$ for 2-ethyl derivatives and $-NH_2 < -NHCH_3 > -NHCH_2CH_3$ 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.

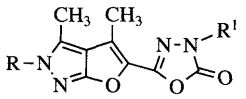
The antiallergic effects of 4,5-dihydro-5-oxo-1,3,4-

TABLE VI. Antiallergic Effect of 2-Benzyl-3,4-dimethylfuro[2,3-*c*]pyrazole-5-carboxamides (III_{29-31})



Compd.	Dose (mg/kg) *p.o.*	R	Inhibition (%)	*n*
III_{29}	80	$-NH_2$	4.4	(3)
III_{30}	80	$-NHCH_3$	−8.6	(3)
III_{31}	80	$-NHCH_2CH_3$	−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 (IV_{1-4}, V_{1-4})



Compd.	Dose (mg/kg) *p.o.*	R	R′	Inhibition (%)	*n*
IV_1	80	$-CH_3$	$-H$	53.0[a]	(3)
IV_2	80	$-CH_2CH_3$	$-H$	14.1	(4)
IV_3	80	$-CH(CH_3)_2$	$-H$	46.1[a]	(4)
IV_4	80	$-CH_2-C_6H_5$	$-H$	−27.3	(4)
V_1	80	$-CH_3$	$-COCH_3$	19.8[a]	(4)
V_2	80	$-CH_2CH_3$	$-COCH_3$	25.8[a]	(4)
V_3	80	$-CH(CH_3)_2$	$-COCH_3$	22.7[a]	(4)
V_4	80	$-CH_2-C_6H_5$	$-COCH_3$	5.3	(4)

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

oxadiazole derivatives (IV_{1-4}, VI_{1-4}) 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 (IV_4, V_4) 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 (IV_1, IV_3) 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 Mass 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 (I_3, III_{27}, III_{30}, IV_3, IV_4, V_3) 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.	(μM)	Inhibition (%)	*n*
I_3	30	8.8± 4.2	(4)
	100	15.2±13.5	(4)
III_{27}	30	2.3± 4.8	(3)
	100	24.4±12.0	(4)
III_{30}	30	9.8± 9.6	(4)
	100	10.3± 5.3	(4)
IV_3	30	18.7±14.6	(3)
	100	16.7± 8.2	(4)
IV_4	30	25.8± 2.7	(3)
	100	22.1± 9.9	(3)
V_3	30	20.3±15.4	(3)
	100	26.8±10.2	(3)
Mepacrine	10	40.2± 3.7[a]	(3)
	30	75.2± 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 (III_{27}, IV_3) were chosen to investigate the protective effect against phlogist-induced vascular plasma extravasation. As shown in Table IX, compounds III_{27} and IV_3 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 III_{27} and IV_3 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 III_{27} and IV_3 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 /methysergide	10		
	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 methylsergide, 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 III_{27} and IV_3 inhibited edema formation to a greater extent than did diphenhydramine in combination with methylsergide (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-oxadiazo-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 $CDCl_3$ 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 I_1[3] (7.8 g, 0.04 mol) in dried benzene (50 ml) was treated dropwise with $SOCl_2$ (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 (II_1). The crude intermediate (II_1) 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 $CHCl_3$–EtOH to give 3.9 g of 2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III_1). 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 (IV_1) One gram (0.036 mol) of *N*-(ethoxycarbonylamino)-2,3,4-trimethylfuro[2,3-*c*]pyrazole-5-carboxamide (III_{32}) 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 IV_1 0.8 g (93%). mp 236—239 °C. IR ν_{max}^{KBr}: 3050 (–NH), 1780 (C=O) cm^{-1}. UV $\lambda_{max}^{C_2H_5OH}$: 287 nm ($\varepsilon=3.0\times10^4$). ^{1}H-NMR δ: 2.45 (6H, s, 3-CH_3, 4-CH_3), 3.83 (3H, s, N–CH_3). MS *m/z*: 234 (M^+). *Anal.* Calcd for $C_{10}H_{12}N_4O_3$: 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 (V_1) One gram (0.0043 mol) of compound IV_1 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 $CHCl_3$–acetonitrile gave 1.0 g (85%) of V_1. mp 236—239 °C. IR ν_{max}^{KBr}: 3050 (–NH), 1720 (C=O) cm^{-1}. UV $\lambda_{max}^{C_2H_5OH}$: 314 nm ($\varepsilon=3.5\times10^3$). ^{1}H-NMR δ: 2.45 (9H, s, 3-CH_3, 4-CH_3, –$COCH_3$), 3.83 (3H, s, N–CH_3). MS *m/z*: 276 (M^+). *Anal.* Calcd for $C_{12}H_{12}N_4O_4$: 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 (V_2) 5-(4,5-Dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-ethyl-3,4-dimethylfuro[2,3-*c*]pyrazole (IV_2) (1 g, 0.004 mol) was treated as described for the preparation of V_1 to afford V_2 0.9 g (72%). mp 208—210 °C. IR ν_{max}^{KBr}: 3300 (–NH), 1740 (C=O), 1640 (C=O) cm^{-1}. UV $\lambda_{max}^{C_2H_5OH}$: 311 nm ($\varepsilon=5.5\times10^3$). ^{1}H-NMR δ: 1.33 (3H, t, N–CH_2CH_3), 2.46 (9H, m, 3-CH_3, 4-CH_3, –$COCH_3$), 4.17 (2H, q, N–CH_2–CH_3). MS *m/z*: 306 (M^+). *Anal.* Calcd for $C_{13}H_{14}N_6O_4$: 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 (V_3) 5-(4,5-Dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-2-isoporpyl-3,4-dimethylfuro[2,3-*c*]pyrazole (IV_3) (1.0 g, 0.0038 mol) was treated as described for the preparation of V_1 to afford V_3 0.9 g (77%). mp 208—210 °C. IR ν_{max}^{KBr}: 3000 (–NH), 1730 (C=O), 1640 (C=O) cm^{-1}. UV $\lambda_{max}^{CHCl_3}$: 314 nm ($\varepsilon=3.5\times10^4$). ^{1}H-NMR δ: 1.33—1.50 (6H, d, N–CH(CH_3)$_2$, 2.48 (9H, m, 3-CH_4, 4-CH_3, –$COCH_3$). 4.33—4.67 (1H, m, N–CH). MS *m/z*: 307 (M^+). *Anal.* Calcd for $C_{14}H_{16}N_4O_4$: 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 (V_4) 2-Benzyl-5-(4,5-dihydro-5-oxo-1,3,4-oxadiazol-2-yl)-3,4-dimethylfuro[2,3-*c*]pyrazole (IV_4) (1.0 g, 0.0032 mol) was treated as described for the preparation of V_1 to afford V_4 0.8 g (70%). mp 168—170 °C. IR ν_{max}^{KBr}: 3200 (–NH), 1740 (C=O), 1640 (C=O) cm^{-1}. UV $\lambda_{max}^{C_2H_5OH}$: 297 nm ($\varepsilon=2.0\times10^4$). ^{1}H-NMR δ: 2.47 (9H, m, 3-CH_3, 4-CH_3, –$COCH_3$), 5.37 (2H, s, –CH_2–), 7.25 (5H, m, –C_6H_5). MS *m/z*: 325 (M^+). *Anal.* Calcd for $C_{18}H_{16}N_4O_4$: 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 (VI_1) 2,3,4-Trimethylfuro[2,3-*c*]-pyrazole-5-carboxylic acid (1_1) (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 $CHCl_3$, and the $CHCl_3$ layer was washed with water, dried with $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography (silica gel–$CHCl_3$) to give compound VI_1 2.5 g (70%). mp 68—71 °C. UV $\lambda_{max}^{CHCl_3}$: 249 n, ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s, 4-CH_3), 2.33 (3H, s, 3-CH_3), 3.37 (3H, s, N–CH_3), 6.86 (1H, s, 5-H). MS *m/z*: 150 (M^+). *Anal.* Calcd for $C_8H_{10}N_2O$: 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 (VI_2) 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 $\lambda_{max}^{CHCl_3}$: 259 nm. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, $-CH_2CH_3$), 2.11 (3H, s, 4-CH_3), 2.40 (3H, s, 3-CH_3), 4.03 (2H, q, $-CH_2CH_3$), 6.89 (1H, s, 5-H). MS *m/z*: 164 (M^+). *Anal.* Calcd for $C_9H_{12}N_2O$: 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 (VII_1) DMF (30 g, 0.41 mol) was added dropwise to $POCl_3$ (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 Na_2CO_3 then allowed to settle at room temperature for 8 h. The solution was extracted with $CHCl_3$, dried with $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography (silica gel–$CHCl_3$) to give compound VII_1 0.95 g (80%). mp 122—124 °C. IR ν_{max}^{KBr}: 1645 (C=O) cm^{-1}. UV $\lambda_{max}^{CHCl_3}$: 320 nm. ^{1}H-NMR ($CDCl_3$) δ: 2.50 (3H, s, 3-CH_3), 2.53 (3H, s, 4-CH_3), 3.86 (3H, s, N–CH_3), 9.69 (1H, s, CHO). MS *m/z*: 178 (M^+). *Anal.* Calcd for $C_9H_{10}N_2O_2$: 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 (VII_2) 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 ν_{max}^{KBr}: 2720 (–CHO), 1680 (C=O) cm^{-1}. UV $\lambda_{max}^{CHCl_3}$: 320 nm. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, t, $-CH_2CH_3$, 2.53 (3H, s, 4-CH_3), 2.57 (3H, s, 3-CH_3), 4.13 (2H, q, $-CH_2CH_3$), 9.65 (1H, s, 5-H). MS *m/z*: 192 (M^+). *Anal.* Calcd for $C_{10}H_{12}N_2O_2$: 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 ($VIII_1$) 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–$CHCl_3$+EtOH) to give compound $VIII_1$ 0.6 g (78%). mp 254—256 °C. IR ν_{max}^{KBr}: 2700—3000 (–COOH), 1685 (CH=CH–COOH) cm^{-1}. UC $\lambda_{max}^{CHCl_3}$: 350 nm. ^{1}H-NMR δ: 2.22 (3H, s, 4-CH_3), 2.43 (3H, s, 3-CH_3), 3.70 (3H, s, N–CH_3), 5.97 (1H, d, J=16 Hz, –CH=CH–COOH), 7.37 (1H, d, J=16 Hz, –CH=COOH). MS *m/z*: 220 (M^+). *Anal.* Calcd for $C_{11}H_{12}N_2O_3$: 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 ($VIII_2$) 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 ν_{max}^{KBr}: 2500—3100 (–OH), 1600 (CH=CH–COOH) cm^{-1}. UV $\lambda_{max}^{C_2H_5OH}$: 355 nm. ^{1}H-NMR δ: 1.30 (3H, t, N–CH_2CH_3), 2.25 (3H, s, 4-CH_3), 2.42 (3H, s, 3-CH_3), 4.06 (3H, 1, N–CH_2CH_3), 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 $C_{12}H_{14}N_2O_3$: 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 mm^2. 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 × 10^6 cells/ml. Cell viability was assessed by use of the typan 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 μg for the first three injections and 3 μg for the last three injections.

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