Studies on Antiallergic Agents. I. Synthesis and Antiallergic Activity of Novel Pyrazine Derivatives

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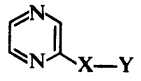
Various pyrazine derivatives were synthesized and their antiallergic activity was examined. The inhibitory activity on allergic histamine release of the compounds bearing a 5-tetrazolyl group was more potent than that of the corresponding carboxyl derivatives. The introduction of –CONH– or –NHCO– between the pyrazine ring and the 5-tetrazolyl group as a spacer greatly enhanced the activity. *N*-(1*H*-Tetrazol-5-yl)-2-pyrazinecarboxamide (I-3) was estimated to exhibit nearly the same potency as disodium cromoglycate (DSCG). The structure–activity relationship among various derivatives modified by introducing some substituents onto the 3-, 5- or 6-position of the pyrazine ring of I-3 was investigated. The activity remained unchanged or was reduced when such substituents as methyl, chloro, methoxy, methylamino and dimethylamino were introduced at the 3- or 5-position. In contrast, 6-substitution with various alkylamino groups more or less increased the activity. Among them, the 6-dimethylamino (I-17c) and 6-(1-pyrrolidinyl) (I-34) derivative were proved to be most potent. The IC_{50} values (concentration which produces 50% inhibition of the allergic histamine release) of I-17c and I-34 were determined to be 4.7×10^{-10} and 4.6×10^{-10} M, respectively. These two compounds produced a potent inhibitory activity on passive cutaneous anaphylaxis (PCA) in rat, not only by the intravenous route ($ED_{50} = 0.0096$ mg/kg for both compounds) but also by the oral route ($ED_{50} = 0.19$ and 0.18 mg/kg, respectively). On the other hand, when the pyrazine ring of some representative compounds was replaced with a pyridine ring, the inhibitory activity on histamine release was significantly reduced.

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

Several studies on the relationships between chemical structure and antiallergic activity have revealed that the combination of a flat or coplanar ring nucleus with an acidic group plays an important role in generating antiallergic activity.[1] Among acidic groups, only carboxylic acid and 5-substituted tetrazole are effective and are regarded as having biologically equivalent function.[1a] On the other hand, antiallergic agents which have recently been developed possess not only polycyclic hetero rings such as chromone and quinazoline but also monocyclic hetero rings as an aromatic moiety.[1,2] The present study was designed to investigate the relationships between the antiallergic activity and structure of various pyrazine derivatives bearing a carboxyl or 5-tetrazolyl group. We report here that the 6-dimethylamino (I-17c) and 6-(1-pyrrolidinyl) derivatives (I-34) among the compounds tested produced the most potent antiallergic activity, not only *in vitro* but also *in vivo*.

Synthesis The compounds (I-3, 8, 9, 10) bearing a carboxyl or 5-tetrazolyl group were synthesized as follows. 2-Pyrazinecarboxylic acid or 3-pyrazinyl-2-propenoic acid was treated with pivaloyl chloride in tetrahydrofuran (THF) in the presence of triethylamine. The active intermediate (mixed anhydride) obtained was condensed with

TABLE I. Physicochemical and Pharmacological Data for Pyrazine Derivatives



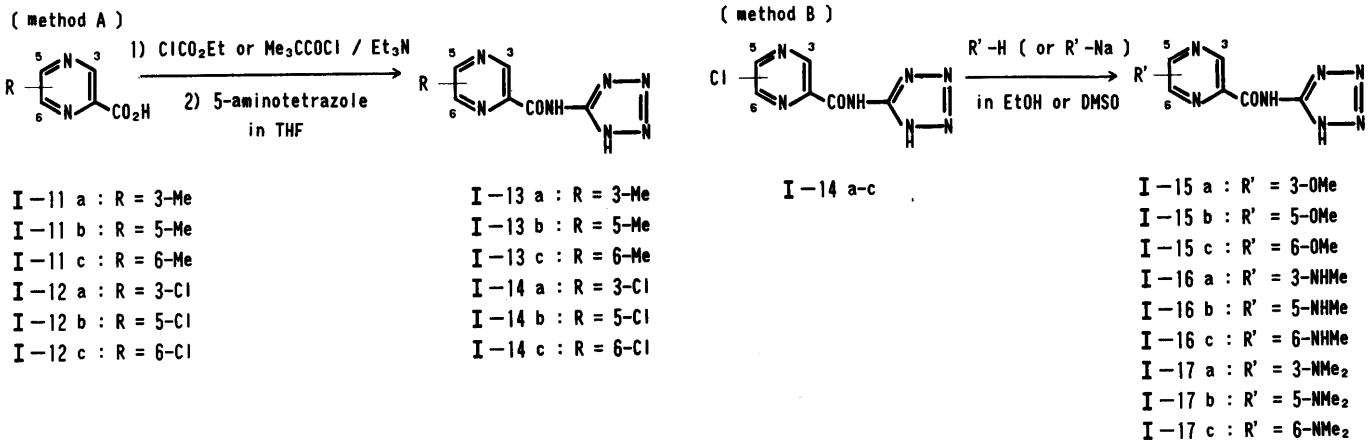
Compd. No.	X	Y	mp (°C) Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	AHR[a] IC_{50} (M)
I-1[b]	COOH	—						$>10^{-4}$
I-2[c]	5-Te[d]	—						2.6×10^{-5}
I-3	CONH	5-Te	295—298 (dec.) (DMSO)	$C_6H_5N_7O$	37.70 (37.87	2.64 2.36	51.29 51.32)	4.2×10^{-7}
I-4	NHCO	COOH	249—251 (dec.) (DMF–EtOH)	$C_6H_5N_3O_3$	43.12 (43.07	3.02 3.28	25.14 25.14)	6.4×10^{-5}
I-5[e]	NHCO	5-Te						2.0×10^{-7}
I-6[f]	CH=CH	COOH						$>10^{-4}$
I-7[g]	$CONHCH_2$	COOH						$>10^{-4}$
I-8	$CONHCH_2$	5-Te	218.5—221 (MeOH)	$C_7H_7N_7O$	40.98 (40.72	3.44 3.75	47.79 47.66)	1.0×10^{-4}
I-9	CH=CHCONH	5-Te	262—265 (dec.) (DMSO–MeOH)	$C_8H_7N_7O$	44.24 (44.56	3.25 3.58	45.14 45.28)	3.1×10^{-6}
I-10	CONH	5-Tr[h]	>300 (10% NaOH–10% HCl)	$C_7H_6N_6O$	44.21 (44.18	3.18 3.33	44.19 44.19)	$>10^{-4}$
	DSCG							3.2×10^{-7}

a) Effect on allergic histamine release from rat PEC (see text). Each value represents the mean of 2 to 6 experiments. *b*) Commercially available. *c*) Ref. 4*a*. *d*) 5-Tetrazolyl. *e*) Ref. 4*b*. *f*) Ref. 4*c*. *g*) Ref. 4*d*. *h*) 5-Triazolyl.

5-aminotetrazole, 5-(aminomethyl)tetrazole or 5-aminotriazole. Compound I-4 was prepared by the hydrolysis of (2-pyrazinyl)oxamic acid ethyl ester synthesized according to the method of Sellstedt *et al.*[3] The other compounds (I-2, 5, 6 and 7) were prepared by the methods described previously.[4] The physicochemical properties of the compounds (I-1—10) are summarized in Table I.

N-(1*H*-Tetrazol-5-yl)-2-pyrazinecarboxamide derivatives (I-13—17) bearing various substituents at the 3-, 5- or 6-position of the pyrazine ring of I-3 were synthesized according to methods A and B (Chart 1).

Methyl (I-13) and chloro (I-14) derivatives were obtained through the mixed anhydride (method A) and methoxy (I-15), methylamino (I-16) and dimethylamino (I-17) derivatives were synthesized by the treatment of I-14 with methylate, methylamine or dimethylamine, respectively, in



I−11 a : R = 3-Me
I−11 b : R = 5-Me
I−11 c : R = 6-Me
I−12 a : R = 3-Cl
I−12 b : R = 5-Cl
I−12 c : R = 6-Cl

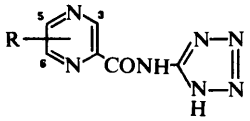
I−13 a : R = 3-Me
I−13 b : R = 5-Me
I−13 c : R = 6-Me
I−14 a : R = 3-Cl
I−14 b : R = 5-Cl
I−14 c : R = 6-Cl

I−14 a-c

I−15 a : R' = 3-OMe
I−15 b : R' = 5-OMe
I−15 c : R' = 6-OMe
I−16 a : R' = 3-NHMe
I−16 b : R' = 5-NHMe
I−16 c : R' = 6-NHMe
I−17 a : R' = 3-NMe_2
I−17 b : R' = 5-NMe_2
I−17 c : R' = 6-NMe_2

Chart 1

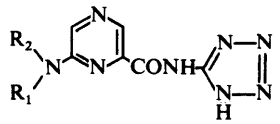
TABLE II. Physicochemical and Pharmacological Data for Pyrazinecarboxamide Derivatives



Compd. No.	Position	Method[a]	R	Yield (%)	mp (°C) Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	AHR[b] IC_{50} (M)
I-13a	3	A	Me	70	259—261 (dec.) (DMF–EtOH)	$C_7H_7N_7O$	40.98 (41.00	3.44 3.79	47.79 47.45)	1.3×10^{-6}
I-14a	3	A	Cl	76	242—250 (dec.) (DMF–EtOH)	$C_6H_4ClN_7O$	31.94 (31.98	1.79 2.19	43.46 43.85)	2.3×10^{-6}
I-15a	3	B	OMe	53	215—222 (dec.) (DMF–EtOH)	$C_7H_7N_7O_2$	38.01 (38.24	3.19 3.21	44.33 44.37)	2.8×10^{-6}
I-16a	3	B	NHMe	82	263—268 (dec.) (DMF–EtOH)	$C_7H_8N_8O$	38.18 (37.94	3.66 3.98	50.89 51.23)	2.1×10^{-7}
I-17a	3	B	NMe_2	83	265—270 (dec.) (DMF–EtOH)	$C_8H_{10}N_8O$	41.02 (40.77	4.30 4.59	47.84 48.06)	5.5×10^{-5}
I-13b	5	A	Me	75	278—283 (dec.) (DMSO–MeOH)	$C_7H_7N_7O$	40.98 (41.24	3.44 3.13	47.79 47.75)	2.6×10^{-6}
I-14b	5	A	Cl	72	>300 (DMSO)	$C_6H_4ClN_7O$	31.94 (31.72	1.79 2.11	43.46 43.54)	4.6×10^{-6}
I-15b	5	B	OMe	80	270—277 (dec.) (DMSO–MeOH)	$C_7H_7N_7O_2$	38.01 (37.66	3.19 3.63	44.33 44.34)	5.4×10^{-6}
I-16b	5	B	NHMe	51	>300 (DMSO–MeOH)	$C_7H_8N_8O$	38.18 (37.99	3.66 3.93	50.89 50.88)	2.5×10^{-6}
I-17b	5	B	NMe_2	83	>300 (DMSO–MeOH)	$C_8H_{10}N_8O$	41.02 (40.64	4.30 4.35	47.84 48.24)	8.2×10^{-7}
I-13c	6	A	Me	69	268—273 (dec.) (DMF–EtOH)	$C_7H_7N_7O$	40.98 (41.02	3.44 3.75	47.79 47.54)	1.0×10^{-7}
I-14c	6	A	Cl	75	263—268.5 (dec.) (DMSO–MeOH)	$C_6H_4ClN_7O$	31.94 (32.03	1.79 1.70	43.46 43.68)	1.6×10^{-7}
I-15c	6	B	OMe	75	258—262 (dec.) (DMF–EtOH)	$C_7H_7N_7O_2$	38.01 (38.05	3.19 3.55	44.33 44.00)	1.1×10^{-7}
I-16c	6	B	NHMe	71	260 (dec.) (DMSO–MeOH)	$C_7H_8N_8O$	38.18 (38.16	3.66 3.85	50.89 51.14)	2.5×10^{-9}
I-17c	6	B	NMe_2	74	267—269 (dec.) (DMSO–MeOH)	$C_8H_{10}N_8O$	41.02 (40.95	4.30 4.63	47.84 47.83)	4.7×10^{-10}

a) See Chart 1. *b*) Effect on allergic histamine release from rat PEC. Each value represents the mean of 3 to 6 experiments.

TABLE III. Physicochemical and Pharmacological Data for 6-Alkylamino-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamides



Compd. No.	R_1	R_2	Yield (%)	mp (°C) Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	AHR[a] IC_{50} (M)
I-3									4.2×10^{-7}
I-16c	H	Me							2.5×10^{-9}
I-18	H	Et	71	272—273.5 (dec.) (DMSO–MeOH)	$C_8H_{10}N_8O$	41.02 (40.95	4.30 4.45	47.84 47.99)	4.0×10^{-9}
I-19	H	*n*-Pr	73	278—279.5 (dec.) (DMSO–MeOH)	$C_9H_{12}N_8O$	43.54 (43.42	4.87 5.23	45.14 44.91)	2.1×10^{-8}
I-20	H	iso-Pr	55	272—274 (dec.) (DMSO–MeOH)	$C_9H_{12}N_8O$	43.54 (43.51	4.87 5.00	45.14 45.49)	1.3×10^{-8}
I-21	H	*n*-Bu	76	269—272 (dec.) (DMF–EtOH)	$C_{10}H_{14}N_8O$	45.80 (45.65	5.38 5.53	42.72 42.83)	3.0×10^{-9}
I-22	H	iso-Bu	80	265—275 (dec.) (DMF–EtOH)	$C_{10}H_{14}N_8O$	45.80 (45.77	5.38 5.38	42.72 42.82)	1.7×10^{-8}
I-23	H	*sec*-Bu	49	265—269 (dec.) (DMF–EtOH)	$C_{10}H_{14}N_8O$	45.80 (45.54	5.38 5.36	42.72 42.49)	9.2×10^{-8}
I-24	H	*tert*-Bu	41	263—273 (dec.) (DMF–EtOH)	$C_{10}H_{14}N_8O$	45.80 (45.39	5.38 5.35	42.72 42.76)	1.3×10^{-7}
I-25	H	*n*-Hex	67	263—267 (dec.) (DMF–EtOH)	$C_{12}H_{18}N_8O$	49.64 (49.54	6.25 6.26	38.60 38.48)	2.5×10^{-9}
I-26	H	*n*-Oct	86	268—272 (dec.) (DMF–EtOH)	$C_{14}H_{22}N_8O$	52.82 (52.54	6.96 6.79	35.19 35.24)	3.0×10^{-9}
I-27	H	*n*-Dec	87	263—267 (dec.) (DMF–EtOH)	$C_{16}H_{26}N_8O$	55.47 (55.34	7.56 7.45	32.34 32.44)	3.4×10^{-8}
I-28	H	*n*-Dodec	58	250—259 (dec.) (DMSO–EtOH)	$C_{18}H_{30}N_8O$	57.73 (57.85	8.07 8.09	29.92 29.92)	3.3×10^{-7}
I-17c	Me	Me							4.7×10^{-10}
I-29	Me	Et	72	224—226 (DMF–EtOH)	$C_9H_{12}N_8O$	43.54 (43.42	4.87 4.81	45.14 45.49)	1.8×10^{-9}
I-30	Me	*n*-Pr	69	243—245 (DMF–EtOH)	$C_{10}H_{14}N_8O$	45.80 (45.79	5.38 5.41	42.72 43.02)	2.9×10^{-9}
I-31	Me	*n*-Bu	83	204.5—205.5 (EtOH)	$C_{11}H_{16}N_8O$	47.82 (47.76	5.84 5.98	40.55 40.31)	9.7×10^{-10}
I-32	Me	*n*-Hex	81	192.5—193.5 (EtOH)	$C_{13}H_{20}N_8O$	51.30 (51.16	6.62 6.44	36.82 36.78)	4.2×10^{-9}
I-33	Et	Et	57	217—218 (DMSO–MeOH)	$C_{10}H_{14}N_8O$	45.80 (45.57	5.38 5.67	42.72 42.84)	1.5×10^{-8}
I-34	$-(CH_2)_4-$		84	288—294 (dec.) (DMSO)	$C_{10}H_{12}N_8O$	46.15 (46.17	4.65 4.59	43.05 42.75)	4.6×10^{-10}
I-35	$-(CH_2)_5-$		72	274—250 (dec.) (DMSO–MeOH)	$C_{11}H_{14}N_8O$	48.17 (48.12	5.14 5.38	40.85 40.93)	5.6×10^{-9}
I-36	$-(CH_2)_2O(CH_2)_2-$		75	276—278 (dec.) (DMSO–MeOH)	$C_{10}H_{12}N_8O_2$	43.48 (43.47	4.38 4.56	40.56 40.70)	3.6×10^{-8}
I-37	$-(CH_2)_2N(CH_2)_2-$ H		60	>300 (10% NaOH–10% HCl)	$C_{10}H_{13}N_9O\cdot1/2H_2O$	42.25 (41.90	4.96 4.89	44.34 44.23)	1.5×10^{-7}

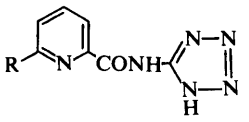
a) Effect on allergic histamine release from rat PEC. Each value represents the mean of 3 to 6 experiments.

an appropriate organic solvent such as ethanol (EtOH) or dimethyl sulfoxide (DMSO) (method B). In method A, ethyl chloroformate or pivaloyl chloride was used to activate carboxyl groups for the synthesis of 3- and 5-substituted pyrazine derivatives, respectively. Reactions with pivaloyl chloride for the synthesis of 3-substituted derivatives failed, and almost all the starting materials were recovered. This may be due to the steric hindrance of the bulky *tert*-butyl group in the formation of the mixed anhydride. Preparation of the compounds (I-15—17) bearing a methoxy, methylamino or dimethylamino group by method A gave low yields due to the difficulty in the isolation of intermediates, methoxy-, methylamino- or dimethylamino-2-pyrazinecarboxylic acid, because of their high water-solubility. The physicochemical properties of the obtained pyrazinecarboxamide derivatives (I-13—17) are summarized in Table II.

In addition, the physicochemical properties of several 6-alkylamino-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamides (I-18—37) prepared by method B are summarized in Table III.

Novel 6-alkylamino-*N*-(1*H*-tetrazol-5-yl)-2-pridinecarboxamides (II-5—10) were also prepared by method B described above. However, a long time and high temperature were required for the reaction due to the low reactivity of the 6-position on the pyridine ring as compared to that of

TABLE IV. Physicochemical and Pharmacological Data for 6-Substituted *N*-(1*H*-Tetrazol-5-yl)-2-pyridinecarboxamides



Compd. No.	R	Yield (%)	mp (°C) (Recrystn. solvent)	Formula	Analysis (%) Calcd			Analysis (%) Found			AHR[a] IC_{50} (M)
					C	H	N	C	H	N	
II-1[b]	H										2.3×10^{-7}
II-2[b]	Me										4.6×10^{-8}
II-3[b]	Cl										1.7×10^{-7}
II-4[b]	OMe										2.6×10^{-7}
II-5	NHMe	28	254—260 (dec.) (DMF–EtOH)	$C_8H_9N_7O$	48.83	4.14	44.73	43.78	4.39	45.02	1.9×10^{-8}
II-6	NHEt	51	255—270 (dec.) (DMSO–MeOH)	$C_9H_{11}N_7O$	46.35	4.75	42.04	46.28	4.70	42.13	2.0×10^{-8}
II-7	NH-*n*-Pr	63	267—273 (dec.) (DMSO–MeOH)	$C_{10}H_{13}N_7O$	48.58	5.30	39.65	48.50	5.19	39.65	2.1×10^{-7}
II-8	NMe_2	62	271—274 (dec.) (DMSO–MeOH)	$C_9H_{11}N_7O$	46.35	4.75	42.04	46.06	4.87	42.02	4.6×10^{-9}
II-9	1-Pyrrolidinyl	71	283—285 (dec.) (DMSO–MeOH)	$C_{11}H_{13}N_7O$	50.96	5.05	37.82	50.87	5.23	37.69	6.0×10^{-9}
II-10	1-Piperidinyl	59	266—267 (dec.) (DMSO–MeOH)	$C_{12}H_{15}N_7O$	52.74	5.53	35.88	52.58	5.61	35.73	3.6×10^{-8}

a) Effect on allergic histamine release from rat PEC. Each value represents the mean of 3 to 6 experiments. *b*) Ref. 2*c*.

pyrazine derivatives. The physicochemical properties are summarized in Table IV.

Results and Discussion

In order to examine the structure–activity relationship of the newly synthesized pyrazine derivatives, the inhibitory activity on allergic histamine release from mast cells was tested. The results are summarized in Tables I to III. In this model, the IC_{50} value (concentration which produces 50% inhibition) of the reference compound, disodium cromoglycate (DSCG), was determined as 3.2×10^{-7} M.

As can be seen in Table I, the inhibitory activity of the compounds bearing a 5-tetrazolyl group (I-2, 5 and 8) was more potent than that of the corresponding carboxyl derivatives (I-1, 4 and 7).

The activity of I-1 and I-2, in which a carboxyl and a 5-tetrazolyl group directly bind to the pyrazine ring, respectively, was relatively low ($IC_{50}=>10^{-4}$ M and 2.6×10^{-5} M). Thus, we decided to join the 5-tetrazolyl group to the pyrazine ring through an appropriate spacer. When –CONH– (compound I-3) or –NHCO– (compound I-5) was employed as a spacer, the activity was greatly enhanced and became equipotent to that of DSCG. On the other hand, the introduction of other spacers such as –CH=CH– (compound I-6) and $-CONHCH_2-$ (compound I-7, 8) did not enhance the activity. Even in the case of –CH=CHCONH– (compound I-9), enhancement of the activity was no more than approximately 10 times compared to I-2 without any spacer. In addition, compound I-10 in which the 5-tetrazolyl group of I-3 was replaced with a less acidic 5-triazolyl group exhibited a marked reduction of the activity. Consequently, compound I-3 was selected as a mother structure for the investigation of the structure–activity relationship, because various derivatives of this compound could be easily synthesized compared with those of I-5. It is known that in several compounds both the *N*-(1*H*-tetrazol-5-yl)carboxamide moiety and the reversal *N*-(1*H*-tetrazol-5-yl)carbamoyl fragment of the amide linkage contribute to produce effective antiallergic activity.[1,2c-e,5]

Table II shows the influence of substitution at the 3-, 5- or 6-position of the pyrazine ring of I-3. Methyl, chloro, methoxy, methylamino and dimethylamino groups were used as substituents. The activity of 3-substituted compounds (I-13a—17a) was less potent than that of I-3, except for the 3-methylamino derivative (I-16a), which produced nearly the same activity as I-3. The reduction of the activity was most prominent when a dimethylamino (I-17a) group was introduced at the 3-position, suggesting that the dimethylamino group interferes sterically to maintain the best conformation of the pyrazine ring and the 5-tetrazolyl group. All 5-substituted derivatives (I-13b—17b) exhibited lower activity than I-3. In contrast, 6-substitution resulted in an increase in the activity. It is noteworthy that the inhibitory activity was greatly enhanced by the introduction of a methylamino (I-16c) or dimethylamino (I-17c) group. The 6-dimethylamino derivative (I-17c) was estimated to be 900 times more potent than the mother compound (I-3). The above results indicate that the introduction of alkylamino groups at the 6-position of the pyrazine ring is favorable to enhance the activity.

Thus, the activity of various 6-alkylamino derivatives was further investigated. As shown in Table III, the activity was more or less increased by the introduction of any monoalkylamino group as compared to the unsubstituted compound (I-3). The most potent activity was observed when a methylamino (I-16c), ethylamino (I-18), *n*-butylamino (I-21), *n*-hexylamino (I-25) or *n*-octylamino (I-26) group was introduced at the 6-position of the pyrazine ring of I-3. It seems likely that the increase of activity produced by introducing the monoalkylamino groups is brought about independently of the length of the alkyl chain attached, except for *n*-decyl (I-27) and *n*-dodecyl (I-28) groups, which may be too long to increase the activity. With regard to compounds I-21, 22, 23 and 24 bearing *n*-, iso-, *sec*- and *tert*-butylamino groups, respectively, the reduction in the rank order of potency coincided well with the rank order of bulk of the butyl group. It is, therefore, suggested that the bulk of the alkyl chain may influence the overall activity of 6-monoalkylamino derivatives.

As described previously, the introduction of a dimethyl-

amino (I-17c) group at the 6-position of I-3 led to a marked increment of inhibitory activity on allergic histamine release. However, when the methyl group at R_2 (Table III) was replaced with such longer alkyl chains as ethyl, *n*-propyl, *n*-butyl and *n*-hexyl group, the activity was rather reduced. In addition, the introduction of a diethylamino (I-33) group instead of a dimethylamino group failed to increase the activity (Table III). These results indicate that the steric conformation of the 6-dimethylamino group may be most favorable for the inhibition of allergic histamine release. This speculation might be at least partly supported by the finding that compound I-34 bearing a 1-pyrrolidinyl group, which possesses a similar steric conformation to a dimethylamino group exhibited nearly the same activity as I-17c. In addition, when a 1-piperidinyl (I-35), 4-morpholinyl (I-36) or 1-piperazinyl (I-37) moiety instead of the 1-pyrrolidinyl moiety was introduced at the 6-position of I-3, the inhibitory activity of these compounds did not exceed that of I-34.

It has recently been reported[2c] that pyridinecarboxamide derivatives inhibit passive cutaneous anaphylaxis (PCA) in rats on oral administration. Therefore, the inhibitory activity of several 6-substituted pyrazinecarboxamides on allergic histamine release was compared with that of the corresponding pyridine derivatives. As shown in Table IV, although the unsubstituted compound (II-1) and methyl derivative (II-2) were slightly more potent than I-3 and I-13c, respectively, some alkylamino derivatives tested (II-5—10) were approximately 5 to 10 times less potent than the corresponding pyrazine derivatives (I-16c, 17c, 18, 19, 34 and 35). Chloro (II-3) and methoxy (II-4) derivatives exhibited nearly the same potency as the corresponding pyrazine derivatives (I-14c and I-15c). These results may be based on the greater electron-withdrawing character of pyrazinecarboxamide. In the case of pyridinecarboxamides, alkylamino groups were more favorable as substituents than methyl, chloro and methoxy group to increase the activity.

Finally, the inhibitory activity of some compounds on PCA in rats was examined. The results are shown in Table V. The inhibitory activity of the compounds other than I-17c and I-34 on PCA was relatively low on oral administration, that is, in this series of compounds, there was no relationship between the *in vitro* potency as a release inhibitor and the inhibitory activity on PCA by oral administration. This may indicate that the compounds other than I-17c and I-34 are poorly absorbed from the gastrointestinal tract. As can be seen in Table VI, I-19 as well as DSCG markedly inhibited PCA in rat by the intravenous route, but not by the oral route (Table VI). It is well established that DSCG is not absorbed through the gastrointestinal tract.[6]

Among the compounds tested, I-17c and I-34, which markedly inhibited allergic histamine release *in vitro*, produced an extremely potent inhibitory activity on PCA not only by the intravenous route ($ED_{50}=0.0096$ mg/kg for both compounds) but also by the oral route ($ED_{50}=0.19$ and 0.18 mg/kg, respectively). In addition, these two compounds exhibited low toxicity ($LD_{50}=>2000$ mg/kg, *p.o.*, in mice). The pharmacological and toxicological properties of these compounds will be reported in detail elsewhere.

The effects of various substituents at the 6-position of the pyrazine ring of the lead compound (I-3) on allergic histamine release were analyzed quantitatively by using substituent parameters and regression analysis. The results will be reported separately.

TABLE V. Comparison of Inhibitory Effect on Allergic Histamine Release and 48-h PCA in Rats

Compd. No.	IC_{50}[a] (M)	Inhibition[b] (%)	Compd. No.	IC_{50}[a] (M)	Inhibition[b] (%)
I-16c	2.5×10^{-9}	40[c]	I-26	3.0×10^{-9}	19
I-17c	4.7×10^{-10}	84[d]	I-29	1.8×10^{-9}	50[c]
I-18	4.0×10^{-9}	40[c]	I-30	2.9×10^{-9}	65[d]
I-19	2.1×10^{-8}	0	I-31	9.7×10^{-10}	31
I-20	1.3×10^{-8}	4	I-32	4.2×10^{-9}	13
I-21	3.0×10^{-9}	19	I-33	1.5×10^{-8}	8
I-22	1.7×10^{-8}	0	I-34	4.6×10^{-10}	81[d]
I-25	2.5×10^{-9}	35[c]	I-35	5.6×10^{-9}	32

a) IC_{50} values are the same as described in Tables II and III. *b*) Test compounds were given orally 30 min before the antigen challenge ($N=6$—9) at a dose of 3 mg/kg (see text). *c, d*) Statistically significant difference from the control group at $p<0.05$ and $p<0.01$, respectively.

TABLE VI. ED_{50} Values on 48-h PCA with Intravenous or Oral Administration

Compound	ED_{50} value[a] (mg/kg) (95% confidence limits)	
	i.v.	*p.o.*
I-19	0.047 (0.0071—0.33)	>10
I-17c	0.0096 (0.0032—0.030)	0.19 (0.063—0.62)
I-34	0.0096 (0.0042—0.021)	0.18 (0.064—0.52)
DSCG	0.39 (0.093—1.12)	>100

a) ED_{50} values (doses which produce 50% inhibition of the PCA with 95% confidence limits) were calculated according to the probit method ($N=6$—9). Test compounds were given i.v. just before or *p.o.* 5 min before the antigen challenge.

Experimental

Melting points were determined by means of a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a JASCO A-202 or Hitachi 270-30 spectrophotometer. Mass spectra (MS) were obtained with the aid of a Hitachi RMU-6M or JEOL DX-300 mass spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured with a JEOL FX-90Q spectrometer using tetramethylsilane as an internal standard.

***N*-(1*H*-Tetrazol-5-yl)-2-pyrazinecarboxamide (I-3): Method A** Ethyl chloroformate (1.27 ml, 13.3 mmol) was added dropwise to an ice-cooled solution of 2-pyrazinecarboxylic acid (I-1) (1.50 g, 12.1 mmol) and triethylamine (1.85 ml, 13.3 mmol) in THF (50 ml). The mixture was stirred at 5 °C for 30 min, then 5-aminotetrazole monohydrate (1.37 g, 13.1 mmol) was added and the whole was stirred at room temperature for 28.5 h. The precipitate was collected by filtration, washed with dilute HCl, dried at 80 °C, and recrystallized from DMSO to give I-3 (2.05 g, 89%) as colorless crystals: mp 295—298 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1680 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 8.84 (1H, dd, $J=1.5$, 2.5 Hz), 8.97 (1H, d, $J=2.5$ Hz), 9.32 (1H, d, $J=1.5$ Hz), 12.48 (1H, br s). MS *m/z*: 192 ($M^+ +1$). *Anal.* Calcd for $C_6H_5N_7O$: C, 37.70; H, 2.64; N, 51.29. Found: C, 37.87; H, 2.36; N, 51.32.

3-Chloro-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamide (I-14a): Method A Ethyl chloroformate (0.34 ml, 3.61 mmol) was added dropwise to an ice-cooled solution of 3-chloro-2-pyrazinecarboxylic acid (I-12a) (520 mg, 3.28 mmol) and triethylamine (0.50 ml, 3.61 mmol) in THF (10 ml). After stirring of the mixture at 5 °C for 1 h, 5-aminotetrazole monohydrate (370 mg, 3.61 mmol) was added and stirring was continued at room temperature for 17 h. The precipitate was collected by filtration, washed

with water, dried at 80 °C, and recrystallized from dimethylformamide (DMF)–EtOH to give I-14a (0.56 g, 76%) as colorless crystals: mp 242—250 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1698 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 8.74 (1H, d, J=2.5 Hz), 8.79 (1H, d, J=2.5 Hz), 12.56 (1H, br s). MS m/z: 225, 227 (M^+). *Anal.* Calcd for $C_6H_4ClN_7O$: C, 31.94; H, 1.79; N, 43.46. Found: C, 31.98; H, 2.19; N, 43.85.

6-Chloro-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamide (I-14c): Method A Pivaloyl chloride (4.06 ml, 33 mmol) was added dropwise to an ice-cooled solution of 6-chloro-2-pyrazinecarboxylic acid (I-12c) (4.76 g, 30 mmol) and triethylamine (4.60 ml, 33 mmol) in THF (90 ml). The mixture was stirred at 5 °C for 1 h, then 5-aminotetrazole monohydrate (3.40 g, 33 mmol) was added and the whole was stirred at room temperature for 1 h and then refluxed for 6 h. The reaction mixture was cooled to 5 °C and the precipitate was collected by filtration, washed with water, dried at 80 °C, and recrystallized from DMSO–MeOH to give I-14c (5.07 g, 75%) as colorless crystals: mp 263—268.5 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1710 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 9.10 (1H, s), 9.26 (1H, s), 12.67 (1H, br s). MS m/z: 225, 227 (M^+). *Anal.* Calcd for $C_6H_4ClN_7O$: C, 31.94; H, 1.79; N, 43.46. Found: C, 32.03; H, 1.70; N, 43.68.

6-Methoxy-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamide (I-15c): Method B A solution (NaOMe/MeOH) of Na (0.70 g, 30 mmol) in MeOH (60 ml) was prepared, and I-14c (2.26 g, 10 mmol) was added at room temperature. The mixture was refluxed for 3 h and then evaporated in a vacuum. Water was added to the residue and the solution was acidified with concentrated HCl to pH 2. The precipitate was collected by filtration, washed with water, dried at 80 °C, and recrystallized from DMF–EtOH to give I-15c (1.66 g, 75%) as colorless needles; mp 258—262 °C (dec.) IR ν_{max}^{KBr} cm^{-1}: 1690 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 4.14 (3H, s), 8.60 (1H, s), 8.87 (1H, s), 12.56 (1H, br s). MS m/z: 222 (M^++1). *Anal.* Calcd for $C_7H_7N_7O_2$: C, 38.01; H, 3.19; N, 44.33. Found: C, 38.05; H, 3.55; N, 44.00.

6-Methylamino-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamide (I-16c): Method B A mixture of I-14c (2.26 g, 10 mmol) and CH_3NH_2/EtOH solution (30%, 6.50 ml) in EtOH (30 ml) was heated at 80—90 °C for 24 h in a sealed tube. The reaction mixture was cooled to room temperature and acidified with ethanolic HCl to pH 3. The precipitate was collected by filtration, washed with EtOH, dried at 80 °C, and recrystallized from DMSO–MeOH to give I-16c (1.57 g, 71%) as pale yellow needles: mp 260 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1690 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 2.94 (3H, d, J=4.0 Hz), 7.40 (1H, br s), 7.61 (1H, s), 8.32 (1H, s), 11.69 (1H, br s). MS m/z: 220 (M^+). *Anal.* Calcd for $C_7H_8N_8O$: C, 38.18; H, 3.66; N, 50.89. Found: C, 38.16; H, 3.85; N, 51.14.

6-(1,1-Dimethyl ethyl)amino-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamide (I-24): Method B A mixture of I-14c (2.26 g, 10 mmol) and *tert*-butylamine (5.25 ml, 50 mmol) in DMSO (15 ml) was heated at 80—90 °C for 41 h in a sealed tube. After cooling, the reaction mixture was made alkaline with aqueous NaOH and then washed with $CHCl_3$. The aqueous layer was filtered and the filtrate was adjusted with 10% HCl to pH 3. The precipitate was collected by filtration and recrystallized from DMF–EtOH to give I-24 (1.07 g, 41%) as colorless crystals, mp 263—273 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1750 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 1.47 (9H), 7.19 (1H, br), 8.20 (1H, s), 8.31 (1H, s), 11.01 (1H, s). MS m/z: 262 (M^+). *Anal.* Calcd for $C_{11}H_{14}N_8O$: C, 45.80; H, 5.38; N, 47.72. Found: C, 45.40; H, 5.32; N, 43.12.

6-(1-Piperazinyl)-*N*-(1*H*-tetrazol-5-yl)-2-pyrazinecarboxamide (I-35): Method B A mixture of I-14c (2.26 g, 10 mmol) and piperazine (8.61 g, 100 mmol) in EtOH (50 ml) was heated under reflux for 22 h. After cooling, the precipitate was collected by filtration and dissolved in 10% aqueous NaOH solution. The alkaline solution was adjusted with 10% HCl to pH 4. The precipitate was purified by dissolving it in 10% NaOH solution and the pH of the solution was adjusted to 8 with 10% HCl. The precipitate was collected by filtration to give I-35 (1.68 g, 60%) as pale yellow crystals, mp >300 °C. IR ν_{max}^{KBr} cm^{-1}: 1690 (CONH). ^{1}H-NMR (in CF_3COOD) δ: 2.70—2.95 (4H, m), 3.35—3.54 (4H, m), 8.87 (1H, s), 8.89 (1H, s). *Anal.* Calcd for $C_{10}H_{13}N_9O \cdot 1/2\,H_2O$: C, 42.25; H, 4.96; N, 44.34. Found: C, 41.90; H, 4.89; N, 44.23.

Other pyrazine derivatives were also prepared in the same manner as described above. The physicochemical properties of the products are summarized in Tables I to III.

6-Methylamino-*N*-(1*H*-tetrazol-5-yl)-2-pyridinecarboxamide (II-5): Method B A mixture of II-3 (1.13 g, 5 mmol) and methylamine aqueous solution (30%, 5.75 ml) in DMSO was heated at 100—110 °C for 24 h in a sealed tube. After cooling, the mixture was made alkaline with aqueous NaOH solution and washed with $CHCl_3$. The aqueous layer was filtered and the filtrate was adjusted with 10% HCl to pH 4—5. A precipitate was collected by filtration and recrystallized from DMF–EtOH to give II-5 (0.31 g, 28%) as pale yellow columns. mp 254—260 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1680 (CONH). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 2.94 (3H, s), 6.76 (1H, dd, J=8.5, 0.9 Hz), 6.85 (1H, br s), 7.31 (1H, dd, J=7.0, 0.9 Hz), 7.61 (1H, dd, J=8.5, 7.0 Hz), 11.36 (1H, br s). *Anal.* Calcd for $C_8H_9N_7O$: C, 48.83; H, 4.14; N, 44.73. Found: C, 43.78; H, 4.39; N, 45.02.

Other 6-alkylamino pyridine derivatives were also prepared in the same manner as described above. The physicochemical properties of the products are summarized in Table IV.

Preparation of Anti-dinitrophenylated Ascaris Extract (DNP-As) Rat Serum Anti-DNP-As rat serum was prepared by the method of Tada and Okumura.[7] The titer of the antiserum was determined as 1 : 128 or 1 : 256 according to 48-h PCA in rats.

Measurement of Allergic Histamine Release from Rat Peritoneal Exudate Cells (PEC) PEC containing mast cells were collected from the peritoneal cavity of male Wistar rats (350—450 g) and suspended in *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (HEPES) buffer (HEPES; 10 mM, NaCl; 137 mM, KCl; 2.7 mM, $CaCl_2$; 1.0 mM, Na_2HPO_4; 1.0 mM, glucose; 5.6 mM, heparin; 10 units/ml and bovine serum albumin; 3 mg/ml, pH 7.2). Mast cells were passively sensitized with anti-DNP-As rat serum under gentle agitation for 2 h at 37 °C according to the method described previously.[8] The sensitized cells (about 1×10^5 mast cells/ml) which had been rinsed and suspended in HEPES buffer were prewarmed at 37 °C for 10 min and then incubated with 10 μg of DNP-As for the following 20 min. The total volume of the reaction medium was 1 ml. Test compounds were added 1 min prior to the addition of antigen (DNP-As; final concentration: 10 μg/ml). The amount of histamine released was measured by the fluorometric method of Shore *et al.*[9] IC_{50} value is the concentration which produces 50% inhibition of the histamine release. Test compounds were dissolved in 0.1 N NaOH or distilled water and diluted with HEPES buffer to desired concentrations.

48-h PCA in Rats Male Wistar rats (180—220 g) were passively sensitized by intracutaneous injection on the back at a volume of 0.1 ml of 20- or 40-fold-diluted anti-DNP-As rat serum. Forty-eight hours later, the animals were challenged by an intravenous injection of 0.5 ml saline solution containing 0.5 mg of DNP-As and 5 mg of Evans blue. The animals were sacrificed 30 min after the challenge and the extravasated dye was extracted by the method of Katayama *et al.*[10] Test compounds were dissolved in 0.1 N NaOH, neutralized with 0.1 N HCl and administered intravenously just before antigen challenge. In the case of oral administration, the compounds suspended in 0.5% aqueous solution of sodium carboxymethyl cellulose (CMC) were administered 30 min or 5 min before antigen challenge. The inhibitory activity of a test compound was expressed as percent inhibition of PCA as compared with the control (saline or 0.5% CMC group). ED_{50} values (doses which produce 50% inhibition of the PCA with 95% confidence limits) were calculated according to the probit method.

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