Synthesis and Antiallergic Activity of *N*-[4-(4-Diphenylmethyl-1-piperazinyl)butyl]-1,4-dihydro-4-oxopyridine-3-carboxamides[1)]

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A new series of oxopyridinecarboxamide derivatives **3a—g** and **5a** were synthesized and evaluated for their antiallergic activity. 1,4-Dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamides **3a** and **5a** exhibited potent antiallergic activity (inhibitory rates of 80.7 and 88.3%, respectively, at 20 mg/kg, *p.o.*) in the rat passive cutaneous anaphylaxis (PCA) test and also exhibited much more potent *in vitro* inhibitory activity than caffeic acid against the enzyme 5-lipoxygenase (5-LO). Their *in vitro* antihistamine activity, however, was weaker than that of ketotifen. Compounds **3a** and **5a** are viewed as promising candidates for antiallergic agents.

Keywords   *N*-[4-(4-diphenylmethyl-1-piperazinyl)butyl]-1,4-dihydro-4-oxopyridine-3-carboxamide derivative; antiallergic agent; anti-passive cutaneous anaphylaxis activity; 5-lipoxygenase inhibitory activity; antihistamine activity

We have recently studied[2−4)] a series of *N*-[4-(4-di-phenylmethyl-1-piperazinyl)butyl]-3-heteroarylacryl-amides **1** (Chart 1), which exhibited antiallergic activity when orally administered. Among them, *N*-[4-(4-diphenyl-methyl-1-piperazinyl)butyl]-3-(6-methyl-3-pyridyl)acryl-amide (**2**, AL-3264)[3)] (Chart 2) was five times more potent than ketotifen[5)] in the rat passive cutaneous anaphylaxis (PCA) test and was characterized as an antagonist of histamine, as well as an inhibitor of the enzyme 5-lipoxy-genase (5-LO), which catalyzes the generation of leuko-trienes (LTA₄, LTB₄, LTC₄, LTD₄ and LTE₄) from ara-chidonic acid, and an inhibitor of histamine release from healthy human basophils induced by anti-human immuno-globulin E (IgE) antibody. The leukotrienes generated from arachidonic acid have extremely potent biological activities: the peptido leukotrienes LTC₄, LTD₄ and LTE₄ are potent bronchoconstrictors and lead to an increase in vascular permeability.[6)] Recently, the peptido leukotrienes have been proposed to be important mediators in a variety of disease states such as asthma, arthritis, psoriasis and allergy.[7)] 5-LO inhibitors may be useful candidate drugs for treating allergic diseases.

The present study was undertaken to find compounds having an enhanced 5-LO inhibitory activity with retention of the other biological properties of **2**. A structure–activity study of the acrylamides **1** revealed that the 3-(6-methyl-3-pyridyl)acrylamide moiety of compound **2** is important for inhibitory activity against the rat PCA reaction and 5-LO,
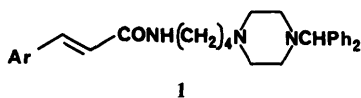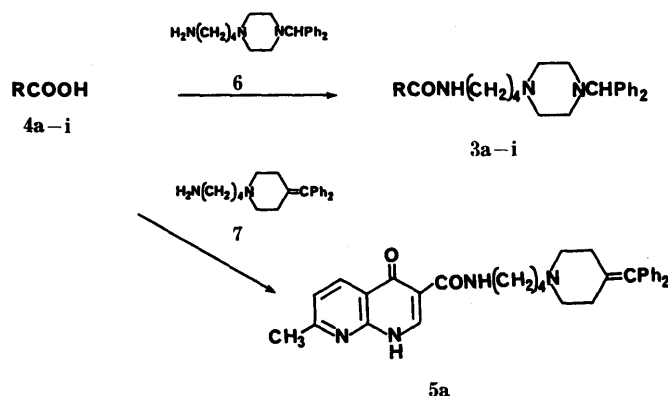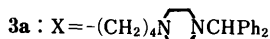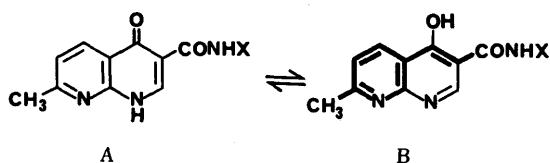
Chart 1

2 (AL-3264)

3a : X=-(CH₂)₄N⌒NCHPh₂
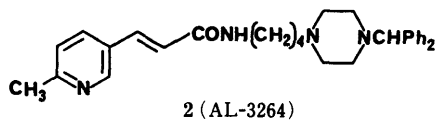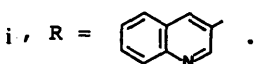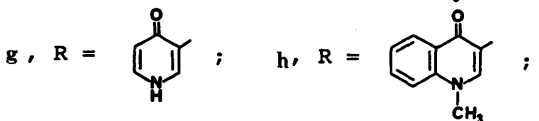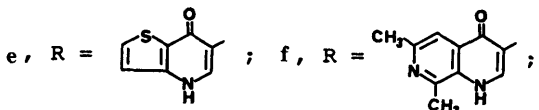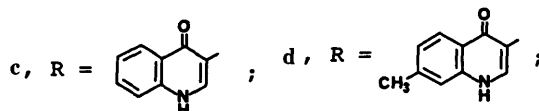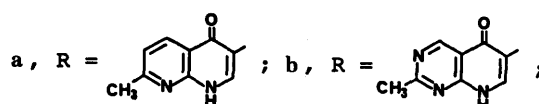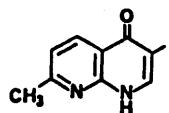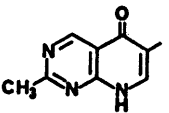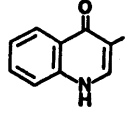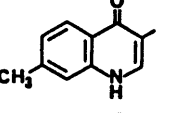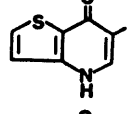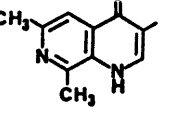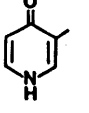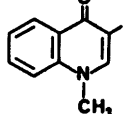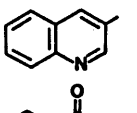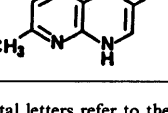
Chart 2

Chart 3

TABLE I. 4-Oxopyridine-3-carboxamides

$$RCONH(CH_2)_4N\bigcirc Y$$
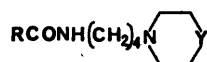
Compd.	R	Y	Procedure[a]	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
3a		NCHPh₂	A	202—205 (MeCN)	33	$C_{31}H_{35}N_5O_2 \cdot 1/2 H_2O$	71.79 (71.69	7.00 6.94	13.50 13.69)
3b		NCHPh₂	A	125—129 (Toluene)	16	$C_{30}H_{34}N_6O_2 \cdot 3/4 H_2O$	68.75 (69.02	6.83 6.83	16.03 16.23)
3c		NCHPh₂	A	205—208 (MeCN)	71	$C_{31}H_{34}N_4O_2$	75.28 (74.98	6.93 6.92	11.33 11.14)
3d		NCHPh₂	A	217—219 (Toluene)	64	$C_{32}H_{36}N_4O_2$	75.56 (75.28	7.13 6.99	11.01 10.75)
3e		NCHPh₂	A	156—158 (Toluene)	70	$C_{29}H_{32}N_4O_2S^{b)}$	68.34 (68.31	6.53 6.42	10.99 10.99)
3f		NCHPh₂	B	202—204 (Toluene)	50	$C_{32}H_{37}N_5O_2$	73.39 (73.20	7.12 7.09	13.37 13.32)
3g		NCHPh₂	C	93—95 (MeCN)	63	$C_{27}H_{32}N_4O_2$	72.94 (72.68	7.26 7.45	12.60 12.38)
3h		NCHPh₂	D	150—152 (MeCN)	46	$C_{32}H_{36}N_4O_2$	75.56 (75.86	7.13 7.02	11.01 10.86)
3i		NCHPh₂	A[c]	134—136 (iso-PrOH)	50	$C_{31}H_{34}N_4O$	77.79 (77.69	7.16 7.26	11.71 11.61)
5a		C=CPh₂	A	168—170 (MeCN)	32	$C_{32}H_{34}N_4O_2 \cdot 1/4 H_2O$	75.19 (75.36	6.80 7.05	10.96 10.90)

a) Capital letters refer to the procedures in Experimental.   b) Analysis for S: Calcd 6.29; Found 6.23.   c) Tetrahydrofuran was used as a solvent instead of CHCl₃.

and introduction of methyl and ethyl groups into the α-position of the acryloyl moiety caused no decrease in these activities. These findings prompted us to study a bicyclic compound, the 1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide derivative 3a; compound 3a can formally take two tautomeric forms A and B, the latter of which involves the 3-(6-methyl-3-pyridyl)acrylamide moiety as shown by the bold lines in Chart 2. Biological evaluation revealed it to be equivalent in anti-PCA activity and superior in 5-LO inhibitory activity to compound 2. We thought consequently that the 4-oxopyridine-3-carboxamide moiety involved in compound 3a, instead of

the acrylamide moiety of compound 2, would play an important role in inhibiting 5-LO activity and the rat PCA reaction. As an extension of this modification, various 4-oxopyridine-3-carboxamides were prepared and evaluated for antiallergic activity in the rat PCA and 5-LO assays.

Chemistry

The requisite carboxylic acids 4b—h were prepared according to the reported method; 4a and 4i were commercially available.

The carboxylic acids 4a—i were allowed to condense with 1-(4-aminobutyl)-4-diphenylmethylpiperazine (6) or 1-(4-

aminobutyl)-4-diphenylmethylenepiperidine (7) by the use of ethyl chlorocarbanate (procedure A), N,N'-carbonyldiimidazole (procedure B), p-nitrophenyl trifluoroacetate (procedure C), or thionyl chloride (procedure D) (Chart 3), giving the desired carboxamides 3a—i and 5a (Table I); we reported previously[2,3] that 4-(4-diphenylmethyl-1-piperazinyl)butylamino and 4-(4-diphenylmethylene-1-piperidyl)butylamino groups were efficient for showing anti-PCA activity. An attempt to condense 1,4-dihydro-4-oxo-1,5- and 1,4-dihydro-4-oxo-1,6-naphthyridine-3-carboxylic acids with 6 was unsuccessful.

Pharmacological Results

Compounds 3a—i and 5a were evaluated for their anti-allergic activity in the rat PCA and 5-LO assays. The results are shown in Table II. Compound 3a obtained by molecular modification of 2 was equivalent to 2 in anti-PCA activity and superior to 2 in 5-LO inhibitory activity. Compound 3b containing a fused pyrimidine ring, instead of the pyridine ring, exhibited potent anti-PCA activity, similarly to 3a but, surprisingly, it showed weak inhibitory activity against 5-LO. On the other hand, compounds 3c, 3d and 3e containing a fused benzene or thiophene ring were potent inhibitors of 5-LO although they were weak inhibitors of the rat PCA reaction. The 1,7-naphthyridine derivative 3f also exhibited potent 5-LO inhibitory activity but showed weak inhibitory activity against the PCA reaction. Compound 5a, substituted with a 4-diphenylmethylene-1-piperidyl group instead of a 4-diphenylmethyl-1-piperazinyl group, displayed potent inhibitory

activities against the rat PCA reaction and 5-LO activity, similarly to 3a.

Replacement of the bicyclic ring by 4-pyridone itself significantly influenced the activities; the 4-pyridone derivative 3g showed fairly weak anti-PCA activity and no 5-LO inhibitory activity. Compound 3c was alkylated at the 1-position of the 4-quinolone moiety to fix the keto form (the tautomeric form A in Chart 2). The N-methyl analogue 3h retained potent 5-LO inhibitory activity. The 3-quinolinecarboxamide derivative 3i had weak 5-LO inhibitory activity. In view of these facts, the 4-oxopyridine-3-carboxamide moieties containing a fused aryl or heteroaryl group, shown by the bold lines in Chart 4, seem generally to play an important role in 5-LO inhibition.

Among the bicyclic 4-oxopyridine-3-carboxamides prepared in the present study, several compounds exhibited anti-PCA activity equivalent to and 5-LO inhibitory activity superior to those of 2. In addition to potent inhibitory activities against 5-LO and the rat PCA reaction, compounds 3a and 5a possessed potent in $vitro$ antihistamine activity. Compounds 3a and 5a are hence considered to be promising candidates as antiallergic agents.

TABLE II. Inhibitory Activities against the Rat PCA Reaction and 5-LO, and Antihistamine Activity

Compd.	Rat PCA test Inhibition (%) 20 mg/kg, $p.o.$	5-LO Inhibition (%) at 10 μM	Anti-hist.[a] Inhibition (%) at 1 μM
2	81.9[b]	45.7[b]	48.0[b]
3a	80.7[b]	79.6[b]	53.0[b]
3b	79.1[b]	17.1[b]	—
3c	36.7[b]	83.7[b]	—
3d	36.9[b]	93.0[b]	—
3e	25.8[c]	60.9[b]	—
3f	34.9[c]	52.8[b]	—
3g	16.8[b]	3.5[c]	—
3h	25.7[c]	89.5[b]	—
3i	26.7[b]	35.2[b]	—
5a	88.3[b]	92.4[b]	38.4[b]
Caffeic acid[d]	—	22.7[b,e]	—
Ketotifen	54.6[b]	11.5[c,f]	77.8[b,g]
Oxatomide[h]	42.2[b]	6.2[c]	68.6[b,i]

a) Anti-hist.: antihistamine activity. b) $p<0.05$, significantly different from the matched vehicle control. c) Not statistically significant. d) This compound was reported as an inhibitor of 5-LO.[23] e) Inhibition (%) at 30 μM. f) Inhibition (%) at 100 μM. g) Inhibition (%) at 0.01 μM. h) This compound was reported as an antiallergic agent.[24] i) Inhibition (%) at 0.3 μM. —: not tested.

Y = NCHPh$_2$ or C=CPh$_2$
Z = CH, N or S

Chart 4

Experimental

Carboxylic Acids 4a—i The following compounds were prepared according to the cited literature: 5,8-dihydro-2-methyl-5-oxopyrido[2,3-d]pyrimidine-6-carboxylic acid (4b)[8]; 4,7-dihydro-7-oxothieno[3,2-b]-pyridine-6-carboxylic acid (4e)[9]; 1,4 dihydro-4-oxo- (4c),[10] 1,4-dihydro-7-methyl-4-oxo- (4d)[11] and 1,4-dihydro-1-methyl-4-oxoquinoline-3-carboxylic acid (4h)[12]; 1,4-dihydro-6,8-dimethyl-4-oxo-1,7-naphthyridine-3-carboxylic acid (4f)[13]; 1,4-dihydro-4-oxopyridine-3-carboxylic acid (4g).[14] Commercially available 4a and 4i were used.

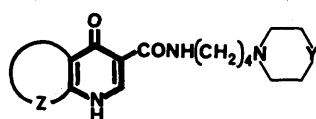Carboxamides 3a—i and 5a (Table I). Procedure A. N-[4-(4-Diphenylmethyl-1-piperazinyl)butyl]-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide (3a) Triethylamine (49.5 g, 0.49 mol) was added at room temperature to a suspension of 4a (50 g, 0.24 mol) in dry CHCl$_3$ (1.7 l). The resulting mixture was cooled to −10——5 °C, and ethyl chlorocarbonate (53 g, 0.49 mol) was added slowly. The mixture was stirred at the same temperature for 2 h, then a solution of 160 g of 1-(4-aminobutyl)-4-diphenylmethylpiperazine (6)[2] in dry CHCl$_3$ (100 ml) was added. The reaction mixture was stirred for 1 h at −10——5 °C and then at room temperature overnight, washed successively with 600 ml of 10% K$_2$CO$_3$ and two 300-ml portions of water, and dried. The solvent was removed by distillation in $vacuo$. The residue was chromatographed on silica gel and eluted with CHCl$_3$-MeOH (40 : 1) to give an oily product, which was crystallized from EtOH to give 3a (40.6 g, 33%). EIMS m/z: 509 (M$^+$). IR: 1655 (C=O) cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 2.64 (3H, s, -CH$_3$), 4.18 (1H, s, -CHPh$_2$), 8.56 (1H, d, $J=8$ Hz, C$_5$-H), 8.87 (1H, s, C$_2$-H).

Procedure B. N-[4-(4-Diphenylmethyl-1-piperazinyl)butyl]-1,4-dihydro-6,8-dimethyl-4-oxo-1,7-naphthyridine-3-carboxamide (3f) A mixture of 4f (1.0 g, 4.6 mmol), N,N'-carbonyldiimidazole (1.5 g, 9.3 mmol) and N,N-dimethylformamide (DMF) (50 ml) was heated at 60 °C for 6 h. A solution of 6 (3.0 g, 9.3 mmol) in DMF (5 ml) was added, the reaction mixture was heated at 60 °C for 1 h, and the solvent was removed by distillation in $vacuo$. The residue was taken up in 50 ml of CHCl$_3$. The resulting solution was washed with two 50-ml portions of water, dried, and concentrated to dryness in $vacuo$. The residue was chromatographed on silica gel and eluted with CHCl$_3$-MeOH (50 : 1) to give an oily product, which was crystallized from toluene to give 3f (1.2 g, 50%). EIMS m/z: 523 (M$^+$). IR: 1640 (C=O) cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 2.65, 2.81 (each 3H, s, 2 × -CH$_3$),

4.19 (1H, s, -CHPh$_2$), 7.81 (1H, s, C$_5$-H), 8.81 (1H, s, C$_2$-H).

Procedure C. N-[4-(4-Diphenylmethyl-1-piperazinyl)butyl]-1,4-dihydro-4-oxopyridine-3-carboxamide (3g) The 3-carboxylic acid 4g was treated with 6 and 4-nitrophenyl trifluoroacetate in a manner similar to the reported method,[4] giving 3g. EIMS m/z: 444 (M$^+$). IR: 1655 (C=O)cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 4.20 (1H, s, -CHPh$_2$), 6.50 (1H, d, J=7Hz, C$_5$-H), 8.50 (1H, d, J=1Hz, C$_2$-H).

Procedure D. N-[4-(4-Diphenylmethyl-1-piperazinyl)butyl]-1,4-dihydro-1-methyl-4-oxoquinoline-3-carboxamide (3h) Thionyl chloride (0.32 g, 2.7 mmol) was added at room temperature to a suspenion of 4h (0.5 g, 2.4 mmol) in dry CH$_2$Cl$_2$ (20 ml). The resulting mixture was heated at reflux temperature for 30 min. The mixture was concentrated to dryness in vacuo. Dry toluene (30 ml) and then a solution of 6 (0.95 g, 2.9 mmol) in dry toluene (5 ml) were added to the residue. The reaction mixture was stirred at room temperature for 2 h, then AcOEt (50 ml) and 10% K$_2$CO$_3$ (30 ml) were added. The organic layer was separated, washed with water, and dried. The solvent was removed by distillation in vacuo. The residue was chromatographed on silica gel and eluted with CHCl$_3$-MeOH (40:1) to give an oily product, which was crystallized from CH$_3$CN to give 3h (0.6 g, 46%). EIMS m/z: 508 (M$^+$). IR: 1645 (C=O)cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 3.89 (3H, s, -CH$_3$), 4.20 (1H, s, -CHPh$_2$), 8.72 (1H, s, C$_2$-H).

Reference Compounds Oxatomide[15] and ketotifen[16] were prepared according to the literature. Caffeic acid was purchased from Nacalai Tesque.

Pharmacological Methods Rat PCA Assay[17]: Male Std: Wistar rats (140—200 g) were injected with 0.1 ml of a dilute solution of mouse antiserum to egg albumin at two sites of the shaved ventral skin. Forty-eight hours later, each rat was challenged by an intravenous injection of 2 mg of the antigen together with 1 ml of a 0.5% Evan's blue saline solution. The rats were killed 30 min after the challenge. The dimensions (shortest × longest diameters) of the blueing lesions were measured on the undersurface of the skin. Test compounds were dissolved or suspended in a 0.5% gum tragacanth aqueous solution and administered orally to the rats 1 h before antigen challenge. A group of three or four rats was used for each test compound. The antiallergic activity of the compounds was expressed as percent inhibition of the dimensions compared with the control group. Mouse anti-egg albumin antiserum was produced by the method of Levine and Vaz.[18]

5-LO Assay[19]: The test was carried out according to the method of Ochi et al.,[20] and Miyamoto and Obata[21] with minor modifications. In brief, the cytosol fraction of peritoneal exudate cells of guinea pigs was used as 5-LO. The reaction mixture was incubated for 5 min at 30 °C after addition of [1-^{14}C]arachidonic acid (0.02 μCi). 5-LO activity was expressed as the conversion rate of arachidonic acid to 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) in 5 min. A group of three tubes was used for each test compound. The effect of test compounds was expressed as percent inhibition of the conversion rate compared with the control.

Antihistamine Assay: Zig-zag strips of guinea pig trachea were prepared by the method of Emmerson and Mackay.[22] Dose-response curves for histamine were obtained before and 5 min after the addition of test compounds. Inhibitory rate was calculated from contraction height in 3 × 10^{-5} M histamine without vs. that with a test compound.

References and Notes
1) This is part I in a series of "Oxopyridinecarboxamide Derivatives as Antiallergic Agents."
2) Y. Nishikawa, T. Shindo, K. Ishii, H. Nakamura, T. Kon and H. Uno, Chem. Pharm. Bull., 37, 100 (1989).
3) Y. Nishikawa, T. Shindo, K. Ishii, H. Nakamura, T. Kon and H. Uno, J. Med. Chem., 32, 583 (1989).
4) Y. Nishikawa, T. Shindo, K. Ishii, H. Nakamura, T. Kon and H. Uno, Chem. Pharm. Bull., 37, 684 (1989).
5) U. Martin and D. Römer, Arzneim.-Forsch., 28, 770 (1978).
6) D. M. Bailey and F. B. Casey, Annu. Rep. Med. Chem., 17, 203 (1982).
7) M. A. Bray, Agents Actions, 19, 87 (1986).
8) G. Y. Lesher, U. S. Patent 3320257 (1967) [Chem. Abstr., 68, 49643e (1968)].
9) M. A. Khan and A. E. Guarconi, J. Heterocycl. Chem., 14, 807 (1977).
10) B. Riegel, G. R. Lappin, B. H. Adelson, R. I. Jackson, C. J. Albisetti, Jr., R. M. Dodson and R. H. Baker, J. Am. Chem. Soc., 68, 1264 (1946).
11) M. Murakami, I. Isaka, A. Koda, N. Kawahara, T. Kashiwagi, M. Ageo, Y. Urawa, K. Yano, K. Nakano and I. Souzu, Ger. Offen. 2322750 (1972) [Chem. Abstr., 80, 70803q (1974)].
12) B. R. Baker and R. R. Bramhall, J. Med. Chem., 15, 230 (1972).
13) G. Y. Lesher, U. S. Patent 3429887 (1969) [Chem. Abstr., 70, 106489b (1969)].
14) W. C. J. Ross, J. Chem. Soc. (C), 1966, 1816.
15) J. Vandenberk, L. E. J. Kennis, M. J. M. C. Van der Aa and A. H. M. T. Van Heertum, Ger. Offen. 2714437 (1977) [Chem. Abstr., 88, 50920n (1978)].
16) E. Waldvogel, G. Schwarb, J. M. Bastian and J. P. Bourquin, Helv. Chim. Acta, 59, 866 (1976).
17) H. Nakamura, S. Motoyoshi, K. Ishii and T. Kadokawa, Nippon Yakurigaku Zasshi, 92, 29 (1988).
18) B. B. Levine and N. M. Vas, Int. Archs Allergy Appl. Immun., 39, 156 (1970).
19) H. Nakamura, S. Motoyoshi, K. Ishii, Y. Seto, A. Shimoda and T. Kadokawa, Nippon Yakurigaku Zasshi, 89, 15 (1987).
20) K. Ochi, T. Yoshimoto and S. Yamamoto, J. Biol. Chem., 258, 5754 (1983).
21) T. Miyamoto and T. Obata, "Perspectives in Prostaglandin Research," ed. by Y. Shiokawa, M. Katori and Y. Mizushima, Excerpta Medica, Amsterdam, Oxford and Princeton, 1983, p. 78.
22) J. Emmerson and D. Mackay, J. Pharm. Pharmacol., 31, 798 (1979).
23) Y. Koshihara, T. Neichi, S. Murota, A. Lao, Y. Fujimoto and T. Tatsuno, Biochem. Biophys. Acta, 792, 92 (1984).
24) M. B. Emanuel and G. D. W. Towse, Drugs Today, 16, 219 (1980).