Studies on Antiulcer Agents. II.[1] Antiulcer Properties of *N*-(1*H*-Tetrazol-5-yl)-2-anilino-5-pyrimidinecarboxamides Inhibiting Release of Histamine from Passively Sensitized Rat Peritoneal Mast Cells

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With the aim of applying mast cell-stabilizing agents as antiulcer agents, *N*-(1*H*-tetrazol-5-yl)-2-anilino-5-pyrimidinecarboxamides were synthesized, and initially evaluated pharmacologically for activity in the rat passive cutaneous anaphylaxis test by oral administration. The most active compound 6 was proved to inhibit potently the release of histamine from passively sensitized rat peritoneal mast cells *in vitro*. When compared with other mast cell-stabilizing agents and an antiulcer agent, compound 6 was found to show excellent gastric mucosal protection and gastric antisecretion activities. Furthermore, compound 6 revealed good activity against acidified aspirin ulcer in rats and water-immersion stress ulcer in rats.

Key words antiulcer agent; pyrimidine derivative; passive cutaneous anaphylaxis test; gastric mucosal protection; gastric antisecretion

Histamine exists in the gastrointestinal tract, and the highest concentration is observed in the stomach.[2] This chemical mediator is well known to play an important role in the physiological regulation of gastric acid secretion. It has also been suggested by Oates and Hakkinen that histamine is closely correlated with gastric hyperemia and disturbance of the microvasculature in gastric lesions.[3] Therefore, we decided to search for a new type of antiulcer agent with an ability to regulate physiological histamine. From the same point of view, there have been several reports on antiulcer action of mast cell-stabilizing agents, such as disodium cromoglicate (DSCG) and 5-(2-hydroxypropyl)-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylic acid sodium salt (FPL-52694) (Fig. 1).[2,4]

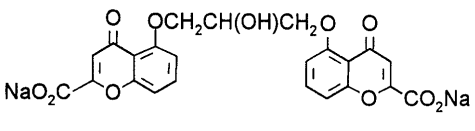
We have previously reported the synthesis and antiallergic activity of a series of 2-anilino-1,6-dihydro-6-oxo-5-pyrimidinecarboxylic acids,[5] and selected the 2-(2-methylpropoxy)anilino derivative (**1**) as a candidate mast cell-stabilizing agent.[6] In accord with our expectation, compound **1** was later proved not only to have significant gastric mucosal protection and gastric antisecretion activities but also to be effective in various experimental ulcer models in rats.[7] These attractive pharmacological properties of **1** prompted us to search for new compounds which had further improved antiulcer properties, but the same mode of action. The basic chemical modification was a transformation of **1** which led to a series of *N*-(1*H*-tetrazol-5-yl)carboxamides as illustrated by the general formula **2**. This modification was intended to provide compounds with a more potent mast cell-stabilizing action after oral administration.

In the present paper, we describe the antiulcer properties of a new mast cell-stabilizing agent selected on the basis of performance in the rat passive cutaneous anaphylaxis (PCA) test.

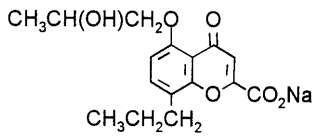
Synthesis All of the desired *N*-(1*H*-tetrazol-5-yl)-2-anilino-5-pyrimidinecarboxamides (**3**—**11**) listed in Table 1 were prepared by coupling reaction between the corresponding 2-anilino-5-pyrimidinecarboxylic acids[5] and 5-amino-1*H*-tetrazole using *N*,*N*′-carbonyldiimidazole (CDI) in *N*,*N*-dimethylformamide (DMF).

Results and Discussion

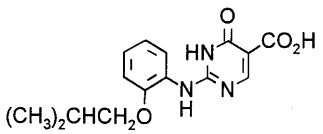
We employed the rat PCA test for primary screening,



DSCG



FPL-52694



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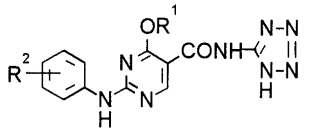

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Fig. 1

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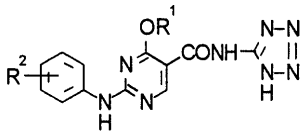
Table 1. *N*-(1*H*-Tetrazol-5-yl)-2-anilino-5-pyrimidinecarboxamides



Compd. No.	R^1	R^2	Yield (%)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	Rat PCA test inhibition (%) 100 mg/kg, *p.o.*
3	H	2-$OCH_2CH(CH_3)_2$	71	290—292 (DMF–H_2O)	$C_{16}H_{18}N_8O_3$	51.89 (51.90)	4.90 (4.73)	30.25 (30.16)	11
4	CH_3	2-$OCH_2CH(CH_3)_2$	53	244—246 (DMF–H_2O)	$C_{17}H_{20}N_8O_3$	53.12 (53.25)	5.24 (5.08)	29.15 (29.37)	48
5	CH_3	2-$O(CH_2)_3CH_3$	67	212—214 (DMF–H_2O)	$C_{17}H_{20}N_8O_3$	53.12 (53.01)	5.24 (5.14)	29.15 (29.13)	72
6[a]	CH_3	2-$O(CH_2)_3CH_3$	91	167—170 (EtOH–H_2O)	$C_{17}H_{19}N_8NaO_3$	50.25 (50.11)	4.71 (4.83)	27.57 (27.47)	97
7	CH_3	2-$S(CH_2)_3CH_3$	92	196—198 (DMF–MeOH)	$C_{17}H_{20}N_8O_2S$	50.99 (50.91)	5.03 (5.11)	27.98 (27.80)	55
8	$(CH_2)_2CH_3$	2-OCH_3	77	265—267 (DMF–MeOH)	$C_{16}H_{18}N_8O_3$	51.89 (51.59)	4.90 (4.80)	30.25 (30.03)	58
9	$(CH_2)_2CH_3$	3-OCH_3	76	250—251 (DMF–H_2O)	$C_{16}H_{18}N_8O_3$	51.89 (51.74)	4.90 (5.16)	30.25 (30.08)	17
10	$(CH_2)_2CH_3$	4-OCH_3	74	239—241 (DMF–MeOH)	$C_{16}H_{18}N_8O_3$	51.89 (51.74)	4.90 (4.81)	30.25 (30.34)	31
11	$(CH_2)_2CH_3$	2-$O(CH_2)_3CH_3$	62	176—178 (DMF–H_2O)	$C_{19}H_{24}N_8O_3$	55.33 (55.09)	5.87 (6.01)	27.17 (27.55)	66

a) Compound **6** is the sodium salt of **5**.

Table 2. Pharmacological Properties of **1**, **6**, DSCG, Tranilast and Cimetidine

Compound No.	Rat PCA test ED_{50} (mg/kg) *p.o.*	Inhibition of histamine release from mast cells IC_{50} (M)	Mucosal protective activity ED_{50} (mg/kg)	Antisecretory activity ED_{50} (mg/kg), i.d. Acidity	Volume	TAO[a]	Antiulcer activity ED_{50} (mg/kg), *p.o.* Acidified aspirin ulcer	Stress ulcer
1	39	2.1×10^{-6}	7.2	>100	36.8	11.6	35.6[b]	6.0[b]
6	1.2	3.8×10^{-7}	0.71	>100	2.3	1.3	9.8	5.8
DSCG	NA[c] (0.85)[d]	4.1×10^{-6}	100 (4.0)[e]	>100	>100	>100	>300	>100
Tranilast	110	5.8×10^{-5}	>100	>100	>100	>100	>100	>100
Cimetidine			>100	61.4	>100	10.2	14.7	31.4

a) TAO = Total acid output. *b*) Compound **1** was given orally 15 min before administration of HCl–aspirin or exposure of water-immersion stress. *c*) NA = not active. *d*) Intravenous administration. *e*) Intraperitoneal administration.

and the results are summarized in Table 1. Compound **6**, which is the sodium salt of **5**, was found to possess the most potent activity in this series and thus was selected for further evaluation.

Table 2 summarizes the various pharmacological properties of **1**, **6**, DSCG, tranilast and cimetidine. Compound **6** was found to be 32 times more active than the lead compound **1** in the rat PCA test. Also, this compound was proved to inhibit potently histamine release from passively sensitized rat peritoneal mast cells, as can be seen from the comparison with **1**, DSCG and tranilast. Mucosal protective activity was next evaluated. In gastric lesions induced by hydrochloric acid–ethanol in rats, compound **6** was 10 times more protective than **1**. On the other hand, DSCG and tranilast exhibited weak activity and a lack of activity, respectively, after oral administration, while DSCG exhibited significant potency after intraperitoneal administration, and so presumably has poor oral bioavailability. Subsequently, the antisecretory activity of **6** was tested in pylorus-ligated rats and the inhibitory effect on total acid output (TAO) was evaluated by measuring both acidity and volume of gastric juice secreted. Compound **6** was approximately 6 times more potent than **1** in inhibition of TAO. Although the effect of **6** on TAO was obviously due to reduction of both the acidity and volume, compound **6** suppressed volume output more significantly than acidity. The mode of action **6** on gastric antisecretion was quite different from that of the histamine H_2-receptor antagonist cimetidine. The characteristic antisecretory property of **6** was also different from that of FPL-52694, which was reported to suppress predominantly the acidity of gastric juice.[4a,e] On the other hand, DSCG and tranilast had no antisecretory effect in this model after oral administration. These results

demonstrated that **6** possesses dual actions, gastric mucosal protection and antisecretion activities, expected to be useful for the treatment of peptic ulcer disease. Compound **6** was further examined in gastric ulcer models induced by hydrochloric acid–aspirin (acidified aspirin ulcer) in rats and by water-immersion stress (stress ulcer) in rats. In the acidified aspirin ulcer model, compound **6** was nearly 4 times as active as **1** and somewhat more potent than cimetidine. The potency of **6** in the stress ulcer model, although 5 times higher than that of cimetidine, was nearly equal to that of **1**, which might be a result of the limited antiulcer effect exhibited by the mast cell-stabilizing agent itself.

In conclusion, derivatization to the *N*-(1*H*-tetrazol-5-yl)carboxamide led to an increase of intrinsic inhibitory activity on the release of histamine from mast cells, which should represent an improvement of antiulcer properties, though not all compounds possessing mast cell-stabilizing action exhibit antiulcer effect, as observed in tranilast.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded in a Nujol mull on a Hitachi 270-30 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken in dimethyl sulfoxide-d_6 with a Bruker AC250 instrument using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a JEOL JMS-300 mass spectrometer using a direct inlet system.

***N*-(1*H*-Tetrazol-5-yl)-4-methoxy-2-(2-butoxyanilino)-5-pyrimidinecarboxamide Sodium Salt (6)** CDI (4.4 g, 27.2 mmol) was added portionwise to a stirred solution of 4-methoxy-2-(2-butoxyanilino)-5-pyrimidinecarboxylic acid (8.0 g, 25.2 mmol) in dry DMF (60 ml) and the mixture was heated at 50 °C for 1 h with stirring. After addition of 5-amino-1*H*-tetrazole (2.5 g, 29.4 mmol), the solution was further heated at the same temperature for 3 h. The solvent was removed under reduced pressure and the resulting residue was recrystallized from DMF–H_2O to give **5** (6.5 g) in 67% yield as white needles. A suspension of compound **5** (3.8 g, 10 mmol) in EtOH (30 ml) was added to 2% aqueous $NaHCO_3$ solution (12 mmol) and the mixture was heated until the white precipitates disappeared. The solution was then allowed to stand at room temperature to give **6** (3.7 g) in 91% yield. IR: 3410 and 3350 (NH), 1662 (C=O) cm^{-1}. ^{1}H-NMR δ: 0.91 (3H, t, J=7.4 Hz, CH_3), 1.38 (2H, m, CH_2), 1.71 (2H, m, CH_2), 4.04 (2H, t, J=7.4 Hz, OCH_2), 4.04 (3H, s, OCH_3), 6.97 (1H, m, aromatic H), 7.07 (2H, m, aromatic H), 7.97 (1H, d, J=7.6 Hz, aromatic H), 8.63 (1H, s, NH), 8.69 (1H, s, pyrimidine H), 9.83 (1H, s, NH). Compounds **3**, **4** and **7**—**10** were obtained in a manner similar to that described for the preparation of **5**.

Rat PCA Test The preparation of anti-dinitrophenylated Ascaris (anti-DNP-As) rat serum (titer 1 : 128) was performed according to the method of Tada and Okumura.[8] The anti-DNP-As serum (0.1 ml), which was diluted 50-fold with physiological saline, was injected intradermally at 4 sites on the shaved back of rats. After 48 h, physiological saline (1 ml) containing dinitrophenylated Ascaris (DNP-AS) (7.1 mg) and Evans blue (5 mg) was injected intravenously to induce an antigen–antibody reaction. The animals were killed 30 min after challenge and the skins were removed for the colorimetric measurement of the bluing spots. Test compounds suspended in 0.5% aqueous carboxymethyl cellulose (CMC) solution were administered orally 30 min before antigen challenge.

Inhibition of Histamine Release from Rat Peritoneal Mast Cells Rat peritoneal mast cells, which were sensitized with anti-DNP-AS, were obtained according to the methods described by Sullivan *et al.*[9] and Saijo *et al.*[10] The cell suspension was preincubated for 20 min at 37 °C in the presence of phosphatidyl-serine (10 μg/ml) and then incubated with DNP-As. The reaction was stopped by cooling in ice bath. After centrifugation (100 × *g*) for 10 min, histamine content of the suspension was measured by the method of Watanabe *et al.*[11] A test compound was given just before the antigen challenge.

Basal Secretion in Pylorus-Ligated Rats The animals were anesthetized and the pylorus was ligated according to the method of Shay *et al.*[12] A suspension of a test compound in 0.5% aqueous CMC solution was administered into the duodenum immediately after pylorus ligation and the abdomen was closed by suturing. Four hours later, the animals were killed. The stomachs were removed and the gastric juice was collected by centrifugation (900 × *g*) for 10 min. The volume of each sample was measured and the acidity was determined using an autoburette. The total acid output was calculated.

Stress Ulcer[13] A suspension of test compound in 0.5% aqueous CMC solution was given orally 30 min before the immersion of rats into water. Each rat was placed in a stress cage and immersed to the level of the xiphoid process for 7 h in a water bath maintained at 23 °C. The animals were killed and the stomachs were examined for ulcers.

Acidified Aspirin Ulcer A suspension of a test compound in 0.5% aqueous CMC solution was given orally 30 min before the oral administration of a solution of aspirin (150 mg/kg) in 150 mM HCl. One hour later, the rats were killed and the stomachs were removed. The isolated stomachs were examined for ulcers.

Gastric Lesions Induced by Acidified Ethanol in Rats The experiment was carried out according to the method reported by Mizui and Doteuchi.[14] The acidified ethanol solution (60% ethanol in 150 mM HCl) was given orally in a volum of 5 ml/kg 30 min after oral administration of test compounds. One hour later, the animals were killed and the stomachs were examined.

References and Notes

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