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Anti-allergic and Anti-inflammatory actions of 2'-(tetrazole-5-yl)-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxanilide 1,1-dioxide

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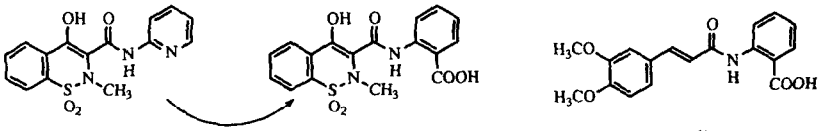
Abstract: 2'-(Tetrazole-5-yl) 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxanilide 1,1-dioxide which was designed by the structural hybridization of tranilast and piroxicam, markedly exhibited the inhibitory effects on the antigen-induced histamine release from rat PEC, 48 h homologous PCA in rats and the carrageenin-induced paw edema in mice.

3 Carboxamides of 4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide have been reported to possess excellent anti-inflammatory activities,[1,2] and N-(2-pyridinyl)-4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide (piroxicam) is currently applied for the clinical treatment of various inflammatory diseases as a non-steroidal acidic anti-inflammatory drug. On the other hand, N-(3',4'-dimethoxycinnamoyl) anthranilic acid (tranilast) has been clinically used as an orally applicable anti-allergic drug which specifically inhibits the IgE-mediated reaction.[3-9] In the experimental models, tranilast has been shown to inhibit the release of chemical mediators induced by antigen-antibody reactions and the homologous passive cutaneous anaphylaxis (PCA) as its characteristic pharmacological actions. In the present study, we investigated the anti-allergic and anti-inflammatory properties of 2'-carboxyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (I) bearing anthranilic moiety like the structure of tranilast.



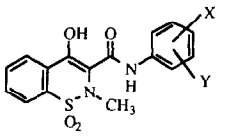
Piroxicam　　I　　Tranilast

3-Carboxanilides of 4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide prepared in this work are listed in Table 1. The synthesis of I is given as an example. A suspension of 1.0 g (0.0037 mol) of 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide which was synthesized according to Lombardino et al.[1], and 0.6 g (0.0044 mol) of 2-aminobenzoic acid in 100 ml of dry xylene was placed under N_2 and refluxed for 48 h in an oil bath. The refluxing was followed by slow distillation to a final volume of about 40 ml. After cooling the reaction mixture the resulting yellow solid wa sfiltrated and recrystallized from H_2O-MeOH to give 1.08 g (yield 78%) of I,

mp 259-262℃ (see Table I).

The inhibitory effects of the compounds synthesized, piroxicam and tranilast on the histamine release from rat peritoneal exudate cells induced by antigen-antibody reaction were examined and the results are shown in Table I. The preparation of sensitized rat peritoneal exudate cells (PEC) and the inhibition assay were performed according to the methods previously described.[10] The histamine release from sensitized rat PEC by 100 μg/ml of egg albumin (antigen) was 54.7 ± 5.2 %. The spontaneous histamine release was 7.8 ± 4.0 %. All test drugs at 2×10^{-4} M significantly ($p<0.01$, Student's t-test) suppressed the histamine release. In this case, I and tranilast were approximately equal in inhibitory activity, and also V, VI and piroxicam were equally effective as inhibitors.

Table I. Inhibitory effects of 3-carboxamides of 4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide on antigen-induced histamine release from PEC of rats.



	X	Y
I:	2-COOH	H
II:	3-COOH	H
III:	4-COOH	H
IV:	2-COOH	5-Cl
V:	2-COOH	4-OH
VI:	2-Tetrazole-5-yl	H

Compd no.	Mp, ℃	Yield, %	Formula	Inhibition, %
I	259-262	78	$C_{17}H_{14}N_2O_6S$	47.7 ± 4.6
II	289-291	93	$C_{17}H_{14}N_2O_6S$	25.2 ± 4.2
III	>290	86	$C_{17}H_{14}N_2O_6S$	21.7 ± 3.8
IV	264-267	65	$C_{17}H_{13}N_2O_6ClS$	72.3 ± 4.7
V	289-291	31	$C_{17}H_{14}N_2O_7S$	94.2 ± 4.1
VI	256 258	84	$C_{17}H_{14}N_6O_4S$	102.7 ± 3.3
Tranilast				42.2 ± 4.4
Piroxicam				92.5 ± 5.0

Each value indicates the mean ± S.E.M. of 4 observations.
Concentration of drug was 2×10^{-4} M.

With respect to the position of carboxyl group of the benzene ring, the

ortho-substituent (I) was more effective than corresponding meta- and para-substituent (II and III, respectively). Among the ortho-carboxyl compounds tested, 5'-Cl- and 4'-OH-substituents (IV and V, respectively) showed more potent activities than I. Among the compounds tested, VI in which structure was replaced carboxyl group at the ortho-position of the benzene ring of I with tetrazole-5-yl group, gave the strongest inhibitory activity, suggesting that tetrazole-5-yl group seems to be more important than carboxyl group for increasing the inhibitory potency.

To confirm the anti-allergic actions in vivo of I and VI, we proceeded to examine the effects of these compounds on 48 h homologous PCA in rats. The preparation of anti-serum containing homocytotropic antibody and the analysis of the inhibitory potency of drugs were performed according to the procedures previously mentioned.[10] The test drugs were suspended in 10 % polyoxyethylene hydrogenated castor oil (Nikkol HCO-60; Nikko Chemical Co., Tokyo) and given orally 60 min prior to challenge with antigen. As shown in Fig. 1, I, VI and tranilast at doses of 100 and 200 mg/kg dose-dependently prevented PCA in rats, the effects of VI and tranilast at all doses being significant, and also piroxicam at a dose of 200 mg/kg, which obviously can produce gastric lesion in rats as an adverse effect (Table III), significantly inhibited PCA in rats. In this case, VI and tranilast were approximately equal in inhibitory activity, whereas piroxicam was less effective than VI and tranilast. On the other hand,

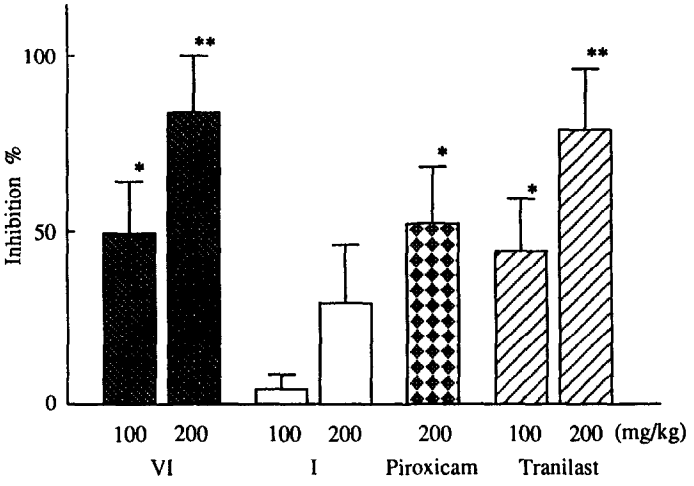


Fig. 1. Effects of I, VI and tranilast on 48h homologous passive cutaneous anaphylaxis (PCA) in rats.
Each experiment included 5-6 observations. Vertical bars indicate S.E.M. * and **: Statistical significance from the control at $p < 0.05$ and 0.01, respectively.

the inhibitory effect of I was very little. These results were not concerned with their inhibitory effects on the histamine release from rat PEC induced by antigen-antibody reaction.

Next, to find out whether VI possess anti-inflammatory activity, we evaluated their inhibitory effects on the formation of edema induced by carrageenin in mice. The initial hind paw thickness (mm) of male ICR mice was measured using a dissecting microscope (×10) with a scale. A solution of λ-carrageenin in sterile saline (0.02 ml/animal) at concentration of 1% was injected subcutaneously into the plantar of the hind paw The thickness (mm) of each hind paw was measured 3 h after carrageenin injection and the results were represented as (Et - En/En) ×100, where En = thickness of hind paw before injection of carrageenin, and Et = thickness after injection. Test drugs were administered orally 60 min bofore the injection of carrageenin. As summarized in Fig 2, VI at doses of 30 and 100 mg/kg dose-dependently reduced the formation of carrageenin-induced edema in mice, and showed statistically significant inhibition at dose of 100 mg/kg. Piroxicam at doses of 10 and 30 mg/kg exhibited marked inhibitory effect in a dose dependent manner. The inhibitory effect of VI on the carrageenin induced inflammation was more than 10 times less than that of piroxicam Azuma et al. previously reported that tranilast at a oral dose of 150 mg/kg significantly ($p< 0.05$) inhibited the carrageenin-induced

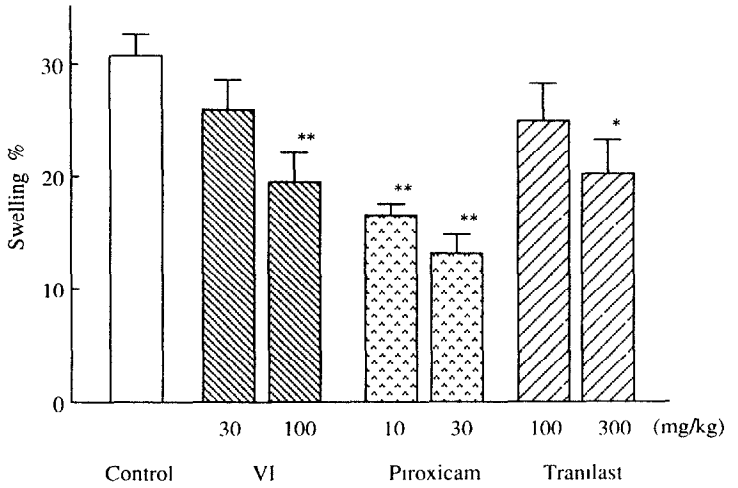


Fig. 2. Effects of VI, piroxicam and tranilast on edema of mice hind paw induced by carrageenin.
Each experiment included 5-6 observations. Vertical bars indicate S.E.M. * and **: Statistical significance from the control at $p< 0.05$ and $p<0.01$, respectively. Swelling was measured 3h after carrageenin injection .

edema in rats.[4] In agreement with this finding, tranilast at oral dose of 300 mg/kg significantly prevented the edema-formation induced by carrageenin, this effect being approximately 3 times less potent than Ⅵ.

To estimate the mechanism of anti-inflammatory action of Ⅵ, we next evaluated the inhibitory effect of Ⅵ, piroxicam and tranilast on the biosynthesis of prostaglandins. The preparation of enzymes from bovine seminal microsomes and the analysis of the inhibitory activity of drugs was performed according the methods of Yanagi and Komatsu.[11] As a result, Ⅵ and tranilast were found to be non-inhibitors, while piroxicam concentration-dependently inhibited the prostaglandin biosynthesis at concentrations of 10^{-4} and 10^{-3} M. These facts suggest that the anti-inflammatory properties of Ⅵ and tranilast seem to differ from that of non-steroidal acidic anti-inflammatory drugs such as piroxicam, aspirin, indomethacin and mefenamic acid which mainly inhibit the biosynthesis of prostaglandins. Further investigation on the anti-inflammatory actions of Ⅵ are now in progress, relating its detailed mechanisms.

Table Ⅱ. Effects of Ⅵ, piroxicam and tranilast on the biosynthesis of prostaglandins.

	Inhibition, %		
Compd	10^{-3}	10^{-4}	10^{-5} (M)
Ⅵ	0	0	0
Piroxicam	67.0	19.0	0
Tranilast	0	0	0

Nonsteroidal anti-inflammatory drugs such as piroxicam and indomethacin are well known to induce gastric mucosal lesions through a remarkable decline in the biosynthesis of endogenous prostaglandins as gastric resistant factors in gastric mucus.[12] Therefore, we examined the ulcerogenicity of Ⅵ, piroxicam and tranilast in rats. The analysis of the ulcerogenicity of drug was performed according to the methods previously reported.[13] As shown in Table Ⅲ, piroxicam at oral doses of 100 and 300 mg/kg dose-dependently induced gastric mucosal lesions. On the other hand, Ⅵ and tranilast did not show any ulcerogenicity. These results were of concern to their inhibitory effects on the biosynthesis of prostaglandins.

In the present study, it was apparent that 2'-(tetrazole-5-yl)-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (Ⅵ) which was newly synthesized by the drug design on the basis of the structural homology

of piroxicam and tranilast, had the inhibitory effect on histamine release from rat PEC induced by antigen-antibody reaction, and also at non-ulcerate doses, markedly inhibited 48 h homologous PCA in rats and carrageenin-induced paw edema in mice, suggesting that it may appear before the foot-light as a useful leading compound for the developing of novel anti-allergic and anti-inflammatory drugs.

Table Ⅲ. Ulcerogenicities of Ⅵ, piroxicam and tranilast in rats.

Compd	Dose (mg/kg, p.o.)	Ulcer Index (mm)
Ⅵ	300	0
	500	0
Piroxicam	100	14.4 ± 8.0
	300	65.5 ± 11.7
Tranilast	300	0
	500	0

All values are means ± S.E.M., n=4-5.

References

1. Lombardino, J. G. ; Wiseman, E. H. ; McLamore, W. M. J. Med. Chem. 1971, 14, 1171-1177.
2. Lombardino, J. G. ; Wiseman, E. H. ; Chiaini, J. J. Med. Chem. 1973, 16, 493-496.
3. Nakazawa, M. ; Yoshimura, T. ; Naito, J. ; Azuma, H. Folia Pharmacol. Japon. 1978, 74, 467-472.
4. Nakazawa, M. ; Yoshimura, T. ; Naito, J. ; Azuma, H. ibid 1978, 74, 483-490.
5. Kubota, T. ; Ujiie, A. ; Naito, J. ; Nakazawa, M. ; Koda, A. Japan. J. Pharmacol. 1983, 33, 837-843.
6. Komatsu, H. ; Ujiie, A. ; Naito, J. Folia Pharmacol. Japon. 1983, 82, 47-55.
7. Komatsu, H. ; Kojima, M. ; Tsutsumi, N. ; Hamano, S. ; Kusama, H. ; Ujiie, A. ; Ikeda, S. ; Nakazawa, M. Japan. J. Pharmacol. 1988, 46, 43-51.
8. Koda, A. ; Nagai, H. ; Watanabe, S. ; Yanagihara, Y. ; Sakamoto, K. J. Allergy Clin. Immunol. 1976, 57, 396-407.
9. Azuma, H. ; Banno, K. ; Yoshimura, T. Br. J. Pharmacol. 1976, 58, 483-488.
10. Kakegawa, H. ; Matsumoto, H. ; Satoh, T. Planta Medica 1988, 54, 385-389.
11. Yanagi, Y. ; Komatsu, T. Biochem. Pharmac. 1976, 25, 937-941.
12. Robert, A. Gastroenterology 1979, 77, 761-767.
13. Yamamoto, K. ; Kakegawa, H. ; Ueda, H. ; Matsumoto, H. ; Sudo, T. ; Miki, T. ; Satoh, T. Planta Medica 1992, 58, 1091-1095.