Structure–Activity Studies on Triazolothienodiazepine Derivatives as Platelet-Activating Factor Antagonists

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A series of triazolodiazepines was synthesized and evaluated for anti-platelet activating factor (PAF) activities. Structure–activity relationship (SAR) studies on this series revealed that the introduction of a methyl group into the 8-position of the thienodiazepine nucleus can lead to a lengthening of the duration of action. Introduction of a methyl group produced an asymmetric center and the enantiomers so formed were separated with an optical resolving column. In the *in vitro* assay system, the (+)-isomers displayed 50—200 times more potent anti-PAF activity than the (−)-isomers. After comparison of toxicology and pharmacokinetics, (+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (35(+)-isomer, E6123) was selected from among the compounds synthesized as a candidate for clinical study.

Keywords platelet activating factor (PAF); PAF antagonist; triazolothienodiazepine; E6123

Platelet activating factor (PAF) is a phospholipid that appears to be involved in pathophysiological processes such as asthma, allergic inflammation, DIC (disseminated intravascular coagulation) and shock.[1] In particular, the following facts suggest that PAF plays an important role in asthma; 1) Inhaled PAF induced bronchoconstriction and hyperresponsiveness in man.[2] 2) An increase in the bronchoalveolar lavage fluid PAF level was found in asthmatic patients.[3] 3) PAF antagonist BN-52021 has effects on PAF and antigen-induced bronchial hyperreactivity and eosinophil accumulation in guinea-pigs.[4] In view of these facts, we have developed PAF receptor antagonists for the treatment of asthma. In 1984, E. Kornecki *et al.*, found that triazolobenzodiazepines such as alprazolam, triazolam showed weak anti-PAF activities,[5] and based on their finding, WEB2086, WEB2170, STY2108, WEB2347[6] and Y-24180[7] were developed as thieno-triazolo-1,4-diazepine (hetrazepines) PAF antagonists (Fig. 1).

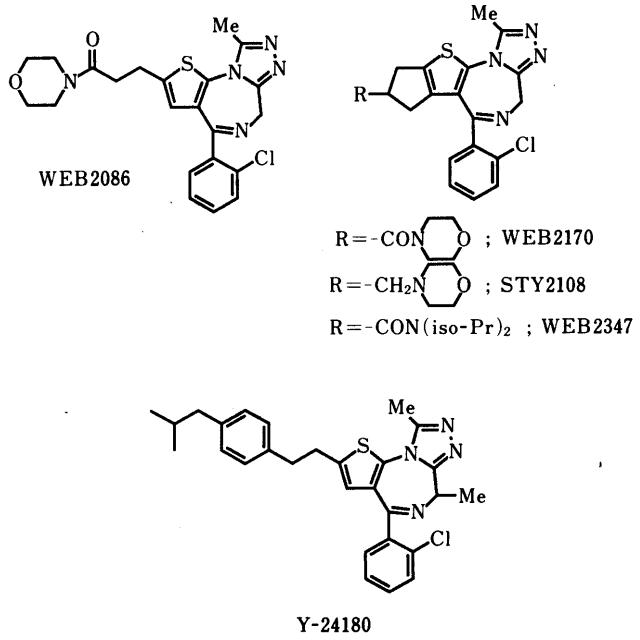


Fig. 1. Structural Formulas of WEB2086, WEB2170, STY2108, WEB2347 and Y-24180

We, too, have been interested in this class of compounds and have carried out structural modification in attempts to obtain compounds with a long duration of action and with good pharmacokinetics. During our study, we discovered that the introduction of a methyl group into the 8-position of the thienodiazepine nucleus can lead to a lengthening of the duration of action. Introduction of a methyl group produced an asymmetric center and the enantiomers so formed were separated with an optical resolving column. In the *in vitro* assay system, the (+)-isomers displayed 50—200 times more potent anti-PAF activities than the (−)-isomers. After comparison of toxicology and pharmacokinetics, (+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**35**(+)-isomer, E6123)[8] was selected from the compounds synthesized as a candidate for clinical study. We herein report the synthesis and structure–activity studies on triazolothienodiazepines as novel and potent PAF receptor antagonists.

Chemistry The mother skeleton, 6-(2-chlorophenyl)-11-methyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**1**) was prepared from 2-chlorocyanoacetophenone, *N*-acetylpiperidone and sulfur based on previous literature.[9] The synthetic method for introducing the side chain was chosen according to the type of junction (Chart 1). To introduce an alkyl side chain, compound **1** was deprotonated with a base such as sodium hydride and then reacted with the corresponding alkylating agent (method A). To form ureas, an amine was converted into its phenylcarbamate with phenyl chloroformate, and this was then heated with amine **1** to give urea type compounds **3** (method B). Carbamate type compounds **4** were prepared in a similar fashion (method C). Namely, an alcohol was activated in the form of its phenyl carbonate and this was coupled with amine **1**. Two different methods were used to prepare the amides **5** depending on the availability of the carboxylic acid com-

ponent. If carboxylic acid chloride was available, method D was applied; otherwise, the carboxylic acid and amine **1** were coupled in the presence of a condensing agent such as dicyclohexylcarbodiimide (method E). To introduce alkyl substituents at the 8-position, the unsubstituted compound was reacted with the corresponding alkyl halide in the presence of sodium hydride as a base (Table II). Among these compounds, mono methyl-substituted compound **22** showed higher biological activity than the mother compound **10**. Therefore, we synthesized 6-(2-chlorophenyl)-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]-dioazepine (**33**) as the new mother skeleton (Chart 2). 2-Amino-3-benzoyl thiophene **27** was prepared by the reaction of 2-chlorocyanoacetophenone with *N*-acetylpiperidone **26** and sulfur in the presence of triethylamine. Compound **27** was then converted into haloacetoamide intermediate **28** with 2-bromopropionyl bromide under Schotten–Baumann reaction conditions. Compound **28** was ammonoloyzed by passing ammonia gas into a solution of the compound in dichloromethane–ethylacetate, giving amine **29**. The ring closure reaction of **29** to **30** was carried out by refluxing a mixture of **29** and a molar equivalent of acetic acid in a pyridine–toluene solution with azeotropic removal of water. Conversion of **30** to triazole **32** was accomplished by thioamidation (P_2S_5–$NaHCO_3$/1,2-dimethoxyethane, reflux) and successive cyclization with acetic hydrazide. The thioacetamide of **32** was cleaved under basic conditions to give **33**. The mono methyl-substitution at the 8-position of its triazolodiazepine skeleton introduces an assymmetric center into molecule **32**. These enantiomers were separated with an optical resolving column; ChiraSpher.

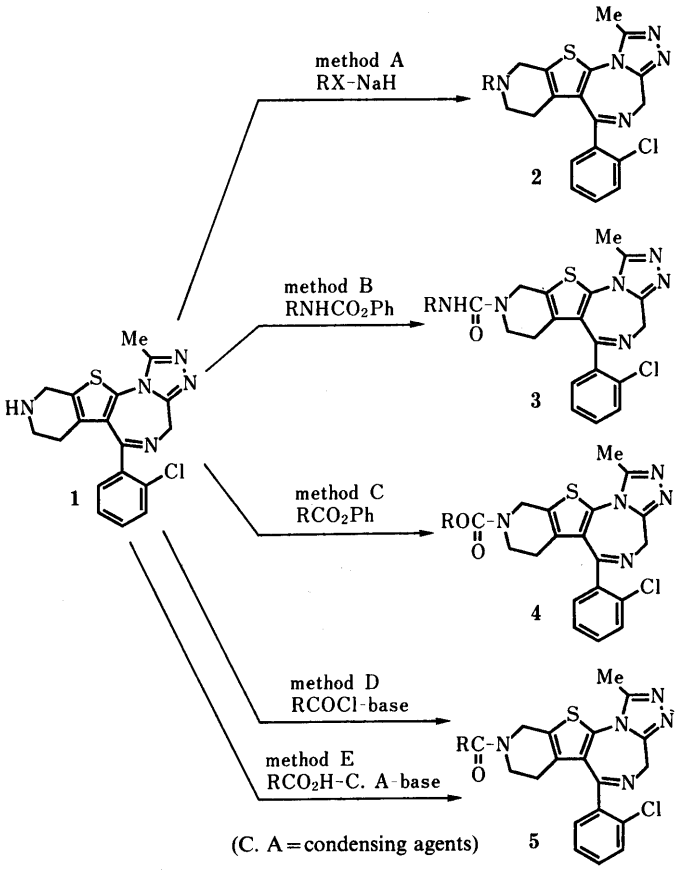
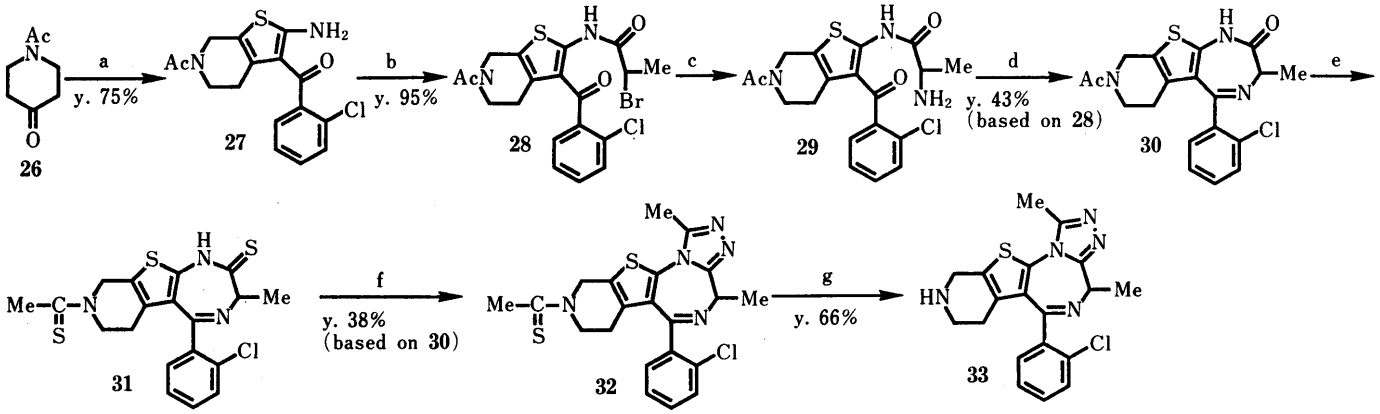


Chart 1

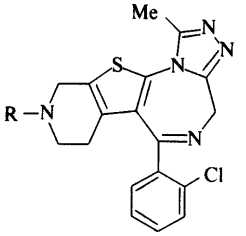
Structure–Activity Relationships Initial evaluation of the compounds was performed with two *in vitro* assay systems; 1) Inhibition of PAF-induced human platelet aggregation[10] and 2) [^{3}H]-PAF receptor binding assay.[11] Compounds with strong PAF antagonistic activity *in vitro* were further evaluated by measuring their inhibitory effect on PAF-induced bronchoconstriction in guinea pigs,[12] in which we employed two different dosages and time points to examine the potency and the duration of action of each compound. We first prepared a series of 8-unsubstituted thienotriazolodiazepines. The results are expressed in Table I. The alkyl-substituted derivative **6** showed very low activity in both of the *in vitro* assay systems, while compounds such as **8** and **9** having a urea junction group showed more potent activity than WEB2086, or WEB2170 in the receptor binding assay system. However compounds **8** and **9** were not so effective in PAF-induced bronchoconstriction at a dose of 0.05 mg/kg *p.o.* when given 90 min before challenge. Next we examined the biological activities of compounds such as **10**—**15** having a carbamate junction. All of them showed more potent activity than WEB2086, or WEB2170



y., yield. a, 2-chlorocyanoacetophenone, S, NEt_3/DMF, 60°C; b, 2-bromopropionyl bromide-$NaHCO_3$/toluene-H_2O, 60°C; c, NH_3/AcOEt-dichloroethane, 100°C; d, AcOH/toluene-pyridine, reflux; e, P_2S_5-$NaHCO_3$/DME, reflux; f, $CH_3CONHNH_2$/dioxane, reflux; g, NaOH/aq. MeOH, reflux

Chart 2

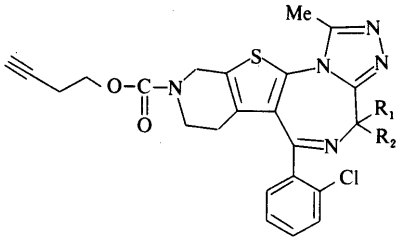
TABLE I. PAF Antagonist Property in Triazolothienodiazepine Series: Structure–Activity Relationships



No.	R	*In vitro* P.A.	*In vitro* R.B.A.	*In vivo* 0.05 mg/kg 90 min	*In vivo* 0.1 mg/kg 8 h
6	$HC\equiv C-CH_2-$	3.0	5.8	—	—
7	NC-⟨⟩-N(Me)CO-	0.3	0.019	43.8±24.3	—
8	⟨⟩(NHCO-)	0.14	0.0066	17.7	—
9	⟨⟩(NHCO-)	0.71	0.006	40.8±21.4	—
10	⟨⟩-OCO-	0.031	0.0035	93.0± 1.3	80.1± 1.0
11	$MeC\equiv C$-⟨⟩-OCO-	0.029	0.00074	100± 0	16.9±22.7
12	Me, ⟨⟩-OCO-	0.034	0.0019	97.4± 0.9	44.8±13.1
13	Ph, ⟨⟩-OCO-	0.03	0.0011	0	—
14	Me, NC, Me, OCO-	0.076	0.0033	99.0± 1.0	91.5± 2.9
15	⟨⟩(N, OCO-)	0.034	0.0088	7.3± 9.7	—
16	NC-⟨⟩-CO-	0.14	0.022	45.6±20.2	—
17	⟨⟩-C≡C-CO-	0.015	0.00056	0	—
18	⟨⟩-CO-	0.15	0.0038	100	90.3± 7.2
19	⟨⟩(N, CO-)	0.016	0.0105	0	—
20	⟨⟩(CO-, OMe)	0.066	0.0058	48.3±33.6	—
21	F-⟨⟩-CH_2CO-	0.31	0.0056	12.6±25.4	—
	WEB2086	0.03	0.046	7.9± 7.7	12.4± 5.6
	WEB2170	0.017	0.017	98.3± 1.7	72.4± 6.2

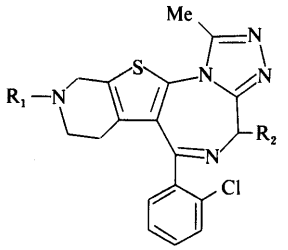
In vitro: P.A. = inhibition of human platelet aggregation induced by PAF IC_{50} (μM), R.B.A. = [^{3}H]-PAF receptor binding assay IC_{50} (μM). *In vivo*: Inhibitory effect on PAF-induced bronchoconstriction at 90 min and 8 h after oral administration of each dose. The inhibition rate was calculated by the following equation: inhibition (%) = 100 − (% peak bronchoconstriction in the test group/% peak bronchoconstriction in the vehicle group) × 100.

TABLE II. PAF Antagonist Property in Triazolothienodiazepine Series: Structure–Activity Relationships



No.	R_1	R_2	*In vitro* P.A.	*In vitro* R.B.A.	*In vivo* 0.05 mg/kg 90 min	*In vivo* 0.1 mg/kg 8 h
10	H	H	0.031	0.0035	93.0±1.3	80.1±1.0
22	Me	H	0.013	0.0015	97.9±0.6	95.9±0.4
23	Me	Me	0.68	0.16	—	—
24	Et	H	0.031	0.022	—	—
25	Et	Et	0.12	0.029	42.0±5.2	—
WEB2170			0.017	0.017	98.3±1.7	72.4±6.2

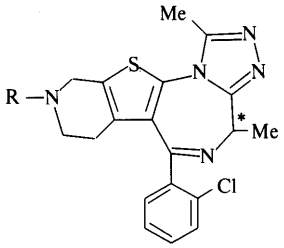
TABLE III. PAF Antagonist Property in Triazolothienodiazepine Series: Structure–Activity Relationships

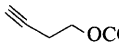
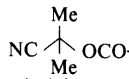


No.	R_1	R_2	*In vitro* P.A.	*In vitro* R.B.A	*In vivo* 0.01 mg/kg 90 min	*In vivo* 0.05 mg/kg 8 h
22	⟨⟩-OCO-	Me	0.013	0.0015	65.0± 6.1	93.9± 2.4
10	⟨⟩-OCO-	H	0.031	0.0035	59.9±16.5	—
34	Me, NC, Me, OCO-	Me	0.03	0.011	90.2± 6.9	92.6± 1.1
14	Me, NC, Me, OCO-	H	0.076	0.0033	95.8± 2.4	83.7± 7.0
35	⟨⟩-CO-	Me	0.0072	0.03	98.8± 1.2	100± 0
18	⟨⟩-CO-	H	0.15	0.0038	70.0±15.5	53.6±11.9
WEB2170			0.017	0.017	59.8±17.9	58.3± 6.8

in the receptor binding assay system. Compounds **10**—**12** and **14** showed the same potency as WEB2170 in the *in vivo* assay system I (0.05 mg/kg, *p.o.* given 90 min before challenge), but only compounds **10** and **14** showed approximately equivalent potency to WEB2170 in the *in vivo* assay system II (0.1 mg/kg, *p.o.* given 8 h before challenge). Compounds **16**—**21** bearing an amido junction had varied biological activity in the inhibition of PAF-induced platelet aggregation and, excepting compound **16**, most of them showed significantly higher activity than WEB2170 in the receptor binding assay system. But in the *in vivo* assay system I (0.05 mg/kg, *p.o.* given 90 min before challenge), only compound **18** showed the same potency as WEB2170. Next we introduced an alkyl substituent into the 8-position in order to induce the higher activity, and especially to prolong the duration of action. From the metabolic study of triazolodiazepines, psychotropic agents such as brotizolam, hydroxylation of the 8-position is known to be one of the main metabolic pathways in dogs and man.[13] Also, the introduction of an alkyl substituent into the 8-position seems to lead to a decrease in the effect on the central nervous system according to previous

TABLE IV. PAF-Antagonist Property in Triazolothienodiazepine Series: Structure–Activity Relationships



No.	R	*In vitro*		*In vivo*	
		R.A.	R.B.A.	0.01 mg/kg 90 min	0.05 mg/kg 8 h
22					
	(−)-isomer	0.69	0.082	—	—
	(+)-isomer	0.0034	0.00034	97.2	92.9±2.9
34					
	(−)-isomer	0.34	0.5	—	—
	(+)-isomer	0.007	0.0031	97.7± 0.4	99.5±0.5
35					
	(−)-isomer	1.4	0.42	—	—
	(+)-isomer	0.013	0.0028	100± 0	99.8±0.2
	WEB2170	0.017	0.017	59.8±17.9	58.3±6.8

structure activity relationship studies of the benzodiazepines.[14] Considering these facts, we prepared alkyl-substituted derivatives such as **22**—**25** based on compound **10** (Table II). Among them, the mono-methylated compound **22** showed more potent PAF antagonistic activity than the mother compound **10** in both of the *in vitro* assay systems, and it showed a greater effect against PAF-induced bronchoconstriction in guinea-pigs at a preadministered dose of 0.1 mg/kg. Next, we investigated the effect of a monomethyl substituent in the 8-position of the triazolodiazepine skeleton when introduced into other active compounds (**14**, **18**). These results are expressed in Table III. As we expected, each compound showed more potent PAF antagonistic activity than its mother compound, especially in the *in vivo* assay system. Mono methyl-substitution at the 8-position results in a new asymmetric center in the skeleton, so we investigated separation of the enantiomers by an optical resolving column, ChiraSpher, and we examined their biological activities with *in vitro* and *in vivo* assay systems. These results are expressed in Table IV. In each case the (+)-isomer showed more potent activity than the (−)-isomer. From the results of the biological evaluation, and comparison of the toxicity and pharmacokinetics of these three compounds, (+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*]-[1,4]diazepine (E6123) was selected as a candidate for clinical study.

Experimental

General Methods Reagents and solvents were purchased from usual commercial sources. Silicagel (Kiesel gel 60, Merck) was used for column chromatography and silicagel (Kiesel gel 60 F_{254}, Merck) for analytical thin layer chromatography (TLC). Melting points were measured on a Yanagimoto micro melting apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-100 (100 MHz) or Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. Infrared (IR) spectra were obtained on a Hitachi 260-30 IR spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer. Optical rotations were measured with a JASCO DIP-140 digital polarimeter.

6-(2-Chlorophenyl)-11-methyl-3-(2-propynyl)-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (6) Sodium hydride (60% content) (30 mg, 0.8 mmol) was added to a dimethylformamide (DMF, 20 ml) solution of 6-(2-chlorophenyl)-11-methyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]-triazolo[4,3-*a*][1,4]diazepine (**1**, 0.12 g, 0.3 mmol), and this solution was heated at 60 °C for 1 h. The reaction mixture was cooled to room temperature and propargyl bromide (60 mg, 0.5 mmol) was added. The reaction mixture was heated at 60 °C for 1 h again. The reaction mixture was poured into water and extracted with AcOEt. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (1.5%MeOH/$CHCl_3$) to give **6** (20 mg, 7%) as a yellow-colored amorphous solid. **6**: MS (Pos, Fab) *m/z*: 407 (M^+). IR (Nujol): 1640, 1595, 1540 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.52—2.12 (m, 2H), 2.25 (t, J=2 Hz, 1H), 2.16—2.84 (m, 2H), 2.66 (s, 3H), 3.45 (d, J=2 Hz, 2H), 3.74 (m, 2H), 3.90—4.40 and 5.20—5.76 (m, 2H), 7.27 (m, 4H).

6-(2-Chlorophenyl)-3-(1-cyano-1-methylethoxycarbonyl)-11-methyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (14) a) 1-Cyano-1-methylethylphenylcarbonate. To a solution of acetone cyanohydrin (0.85 g, 10 mmol) in pyridine (20 ml) was added phenyl chloroformate (1.40 g, 8.9 mmol) at 0 °C. The reaction mixture was stirred for 30 min. The reaction mixture was concentrated in a vacuum and the residue was dissolved in $CHCl_3$ and then washed successively with 1 N-HCl and sat. $NaHCO_3$. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (1% AcOEt/*n*-hexane) to give 1-cyano-1-methylethyl-phenylcarbonate (1.83 g, quant.) as a white, waxy solid.

b) A mixture of 1-cyano-1-methylethylphenylcarbonate (0.15 g, 0.7 mmol) and **1** (0.15 g, 0.4 mmol) in $CHCl_3$ was heated at 120 °C for 1 h, boiling off the solvent. The residue was purified by column chromatography (1% MeOH/$CHCl_3$) to give **14** (0.18 g, 92%) as a pale yellow-colored amorphous solid. **14**: MS (Pos, Fab) *m/z*: 481 $(M+H)^+$. IR (Nujol): 1700, 1595, 1450 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.77 (s, 6H), 1.80—2.20 (m, 2H), 2.68 (s, 3H), 3.10—3.60 (m, 2H), 4.22 (m, 1H), 4.50—4.88 (m, 2H), 5.60 (m, 1H), 7.35 (m, 4H).

6-(2-Chlorophenyl)-3-cyclopropanecarbonyl-11-methyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (18) Cyclopropanecarbonyl chloride (90 mg, 0.9 mmol) was added to a solution of **1** (150 mg, 0.4 mmol) and triethylamine (210 mg, 2.1 mmol) in DMF (6 ml) at −60 °C and the reaction mixture was stirred for 30 min at this temperature. The reaction mixture was poured into sat. $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (1% MeOH/$CHCl_3$) to give **18** (140 mg, 79%) as a yellow-colored amorphous solid. **18**: MS (Pos, Fab) *m/z*: 438 $(M+H)^+$. IR (Nujol): 1635, 1590, 1540 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.4—1.3 (m, 4H), 1.4—2.7 (m, 3H), 2.67 (s, 3H), 2.8—5.8 (m, 6H), 7.1—7.6 (m, 4H).

6-(2-Chlorophenyl)-3-(4-fluorophenyl)acethyl-11-methyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]-diazepine (21) *N*,*N*′-Dicyclohexylcarbodiimide (80 mg, 0.4 mmol) was added to a solution of **1** (120 mg, 0.3 mmol), 4-fluorophenylacetic acid (60 mg, 0.4 mmol) and 1-hydroxybenzotriazole hydrate (60 mg, 0.4 mmol) in DMF (8 ml). The reaction mixture was stirred at 4 °C fof 12 h and then stirred at room temperature for 1 h. The reaction mixture was poured into sat. $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified with column chromatography (1% MeOH/CH_2Cl_2) to give **21** (80 mg, 49%) as a yellow colored amorphous solid. **21**: MS (Pos, Fab) *m/z*: 506 $(M+H)^+$. IR: 1610, 1590 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.0—2.4 (m, 2H), 2.66 (s, 3H), 2.8—5.9 (m, 6H), 3.65 (br s, 2H), 6.65—7.6 (m, 8H).

3-(3-Butynyloxycarbonyl)-6-(2-chlorophenyl)-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]-diazepine (22) To a solution of 3-(3-butynyloxycarbonyl)-6-(2-chlorophenyl)-11-methyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*]-

[1,2,4]triazolo[4,3-*a*][1,4]diazepine (**10**, 57 mg, 0.12 mmol) in DMF (2 ml) were added successively sodium hydride (55% content) (28 mg, 0.64 mmol) and iodomethane (0.2 ml, 3.2 mmol), and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with AcOH and concentrated. The residue was extracted with CH_2Cl_2 and the extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (1% MeOH/CH_2Cl_2) to give **22** (24 mg, 32%) as a yellow-colored amorphous solid. **22**: MS (Pos, Fab) *m/z*: 480 (M^+). IR (Nujol): 1690, 1590, 1520 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 2.0—3.0 (m, 5H), 2.1 (d, J=7 Hz, 3H), 2.5 (dt, J=1, 7 Hz, 2H), 2.7 (s, 3H), 4.1 (t, J=8 Hz, 2H), 4.2 (m, 1H), 4.5 (d, J=18 Hz, 1H), 4.9 (d, J=18 Hz, 1H), 7.4 (s, 5H).

3-(3-Butynyloxycarbonyl)-6-(2-chlorophenyl)-8,8,11-trimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4',3':4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]-diazepine (23) Compound **10** (0.68 g, 1.5 mmol) was dissolved in DMF (15 ml) and the solution was cooled to 0 °C. Sodium hydride (60% content) (0.153 g, 3.8 mmol) and iodomethane (0.45 ml, 7.2 mmol) were added. The reaction was quenched with water, then neutralized with sodium dihydrogen phosphate. The solvent was evaporated off, and the residue was portioned between CH_2Cl_2 (50 ml) and H_2O (50 ml). The aqueous layer was extracted with CH_2Cl_2 (50 ml), and combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silicagel, using CH_2Cl_2–1%MeOH/CH_2Cl_2 as an eluent while the fractions were analyzed by high performance liquid chromatography (HPLC). HPLC conditions: column Nucleosil $_5C_{18}$ 4.6 i.d. × 250 mm, mobile phase CH_3CN–H_2O–H-NEt_2 (40:60:0.01), flow rate 1.0 ml/min, detector UV 240 nm. The mono-methylated compound **22** (0.173 g, 25%) and the dimethylated compound **23** (0.050 g, 7%) were obtained, along with fractions containing a mixture of the two. **23**: MS (Pos, Fab) *m/z*: 494 (M^+). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 2.0—3.1 (m, 5H), 2.5 (dt, J=1, 8 Hz, 2H), 2.7 (s, 3H), 3.1 (s, 3H), 3.7 (s, 3H), 4.2 (t, J=8 Hz, 2H), 4.5 (d, J=17 Hz, 1H), 4.9 (d, J=17 Hz, 1H), 7.4 (s, 4H).

6-Acetyl-2-amino-3-(2-chlorobenzoyl)-4,5,6,7-tetrahydro-thieno[2,3-*c*]-pyridine (27) To a mixture of *N*-acetylpiperidone **26** (440 g, 3.11 mol), sulfur (100 g, 3.11 mol) and 2-chlorocyanoacetophenone (559 g, 3.11 mol) in DMF (1 l) was added triethylamine (290 ml, 2.08 mol), and the mixture was heated at 60 °C for 1 h. The mixture was concentrated by vacuum pump and MeOH was added. The yellow powder that formed was filtered and washed with a small amount of MeOH to give **27** (712.4 g, 75%). **27**: mp 205 °C. MS (Pos, Fab) *m/z*: 335 ($M+H)^+$. IR (Nujol): 2900, 1620, 1585 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.7—1.9 (m, 2H), 2.08, 2.12 (each s, total 3H), 3.4, 3.58 (each t like, total 2H), 4.37, 4.50 (br s, total 2H), 7.2—7.42 (m, 4H).

6-Acetyl-2-(2-bromopropionylamino)-3-(2-chlorobenzoyl)-4,5,6,7-tetra-hydro-thieno[2,3-*c*]pyridine (28) To a heated (60 °C) solution of **27** (600 g, 1.79 mol) and $NaHCO_3$ (301 g, 3.58 mol) in toluene (13.3 l) and H_2O (3.7 l) was added dropwise 2-bromopropionyl bromide (301 ml, 2.87 mol). A further portion of $NaHCO_3$ (170 g, 2.02 mol) and 2-bromopropionyl bromide (170 ml, 1.62 mol) was added to the above solution to drive the reaction to completion. After cooling the reactin mixture down to r.t., $NaHCO_3$ (500 g) was added to the reaction residues and the organic layer was separated. The aqueous layer was extracted twice with AcOEt and the combined organic layers were washed with H_2O, dried over $MgSO_4$, filtered and concentrated. The crystals thus formed were filtered and washed with Et_2O to give **28** (800 g, 95%) as pale yellow crystals. **28**: mp 151—153 °C. MS (Pos, Fab) *m/z*: 469 (M^+). IR (Nujol): 1680, 1630, 1610, 1510 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.7—2.4 (m, 2H), 1.99 (d, J=7.2 Hz, 3H), 2.06 and 2.12 (each s, total 3H), 3.25—3.7 (m, 2H), 4.41 (q, J=7.2 Hz, 1H), 4.4—4.8 (m, 2H), 7.0—7.5 (m, 4H).

8-Acetyl-5-(2-chlorophenyl)-3-methyl-6,7,8,9-tetrahydro-1*H*,3*H*-pyrido-[4',3':4,5]thieno[3,2-*f*][1,4]diazepine-2-one (30) To a cooled (−10 °C) solution of **28** (841 g, 1.79 mol) in 1,2-dichloroethane (0.72 l) and AcOEt (1.08 l) was added liquid NH_3, and then the above solution was heated at 100 °C for 1 h in a sealed tube. After the reaction was complete, excess NH_3 (gas) was removed and the resultant solution was poured into ice-cold 3 N-HCl and the aqueous layer was extracted with AcOEt. The aqueous layer was neutralized with $NaHCO_3$ and extracted repeatedly with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was used without further purification. A solution of **29** (636.8 g) in toluene (2.3 l), pyridine (637 ml) and AcOH (94.3 ml) was heated for 24 h with azeotropic removal of water. The mixture was concentrated and benzene was added to the residue. Filtration of the crystals formed gave **30** (300 g, 43% based on **28**) as a pale brown powder. **30**: mp 260—265 °C. MS (Pos, Fab) *m/z*: 388 ($M+H)^+$. IR (Nujol): 2900, 1690, 1630, 1590 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.3—2.6 (m, 2H), 1.76 (d, J=6.8 Hz, 3H), 2.06 and 2.12 (each s, total 3H), 2.8—4.1 (m, 2H), 3.87 (q, J=6.8 Hz, 1H), 4.1—5.1 (m, 2H), 7.1—7.5 (m, 4H), 9.0—9.5 (br s, 1H).

6-(2-Chlorophenyl)-3-thioacetyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4',3':4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (32) A mixture of compound **30** (643 g, 1.66 mol), $NaHCO_3$ (418 g, 4.89 mol) and phosphorus pentasulfide (812 g, 3.65 mol) in 1,2-dimethoxyethane (7 l) was heated at refluxing temperature for 4 h. The reaction mixture was filtered through celite and then concentrated under reduced pressure. The resulting residue was dissolved in a small amount of CH_2Cl_2–MeOH and purified by dry column chromatography (CH_2Cl_2→3% MeOH/CH_2Cl_2) to give **31** (871.2 g) as a brown cake containing inorganic salts. This product was used in the next step without further purification. The di-thioamide **31** (871.2 g) and acetic hydrazide (154 g, 2.08 mol) in 3 l of 1,4-dioxane were heated at 100 °C for 5 h. The reaction mixture was then concentrated under reduced pressure and the resulting residue was purified by column chromatography. Elution by 2% MeOH in CH_2Cl_2 gave **32** (281 g, 38% based on **30**) as a pale brown powder. **32**: MS (Pos, Fab) *m/z*: 442 ($M+H)^+$. IR (Nujol): 1680, 1585, 1520 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.7—1.9, 2.2—2.4 (m, 2H), 2.1 (d, J=7 Hz, 3H), 2.66 (s, 3H), 2.62, 2.70 (each s, total 3H), 3.5—3.6, 4.1—4.9 (m, 2H), 4.3 (t, J=7 Hz, 1H), 4.8—5.05, 5.7—5.9 (m, 2H), 7.2—7.6 (br s, 4H).

6-(2-Chlorophenyl)-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4',3':4,5]-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (33) A solution of **32** (103 g, 0.23 mol) in MeOH (800 ml) and 4 N-NaOH (500 ml) was heated at refluxing temperature for 5 h. The mixture was brine extracted with chloroform. The extract was concentrated and the residue was purified by column chromatography. Elution by 2%—5% MeOH in CH_2Cl_2 gave **33** (59.2 g, 66%) as an orange-colored amorphous solid. **33**: mp 210—213 °C. MS (Pos, Fab) *m/z*: 384 ($M+H)^+$. IR (Nujol): 3300, 1595, 1555, 1530 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.1—2.3 (m, 3H), 2.10 (d, J=6.8 Hz, 3H), 2.45—3.3 (m, 2H), 2.66 (s, 3H), 3.85—4.1 (m, 2H), 4.26 (q, J=6.8 Hz, 1H), 7.1—7.6 (m, 4H).

(+)-6-(2-Chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4',3':4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]-diazepine ((+)-35) To a cooled (ice bath) solution of **33** (1.58 g, 4.2 mmol) and pyridine (0.42 ml, 5.2 mmol) in CH_2Cl_2 (10 ml) was added dropwise cyclopropionyl chloride (0.43 ml, 4.8 mmol) and the resulting solution was stirred at room temperature for 1 h. The mixture was poured into sat. aqueous $NaHCO_3$ and extracted with CH_2Cl_2 dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography. Elution first with 40% AcOEt in *n*-hexane and then successively from 1% to 5% MeOH in CH_2Cl_2 gave **35** (1.75 g, 92%) as a pale, yellow-white powder. The racemic **35** thus obtained was optically resolved on a ChiraSpher column packed by Sensyu Kagaku (Tokyo Japan) to give the two enantiomers. Preparative HPLC conditions: column ChiraSpher 25 μm 20 i.d. × 500 mm, mobile phase tetrahydrofurane–*n*-hexane (40:60), flow rate 20 ml/min, detector UV 254 nm. (+)-**35**: $[\alpha]_D$ +16.5—18.5° (c=0.1, $CHCl_3$). mp 150 °C. MS (Pos, Fab) *m/z*: 452 ($M+H)^+$. IR (Nujol): 1635, 1590, 1530 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.55—1.15 (m, 4H), 1.45—2.5 (m, 3H), 2.10 (d, J=6.8 Hz, 3H), 2.66 (s, 3H), 2.8—4.8 (m, 3H), 4.26 (q, J=6.8 Hz, m, 1H), 4.8—5.2 (m, 1H), 7.05—7.65 (m, 4H).

***In Vitro* Experiments 1) Preparation of Platelet-Rich Plasma (PRP)** Blood samples were taken from human volunteers into syringes containing one-tenth volume of 3.8% sodium citrate, then centrifuged at 100 × *g* for 10 min. The upper phase was used as PRP in the platelet aggregation study.[10]

2) [^{3}H]-PAF Binding to Platelets Human PRP containing 15% ACD-A (anticoagulant acid citrate dextrose) solution (Terumo, Tokyo, Japan) was centrifuged at 800 × *g* for 15 min and the resulting pellet washed in a standard platelet buffer composed of 4 mmol/l KH_2PO_4, 6 mmol/l Na_2HPO_4, 100 mmol/l NaCl, 0.1% glucose and 20% (w/v) citrate which was adjusted to a final pH of 6.5 with NaOH. The PAF-receptor binding assay was performed using the method of Terashita *et al.*[11] The washed platelets were resuspended at a concentration of 2.2 × 10^5/μl in 10 mmol/l phosphate buffered saline, pH 7.0, containing 0.1% (w/v) bovine serum albumin (BSA) and 0.9 mmol/l $CaCl_2$. Platelets (10^8 cells) in 460 μl of the buffer were added to a polypropylene tube (Eiken, Tokyo, Japan) and preincubated with 20 μl of unlabeled PAF or test compound for 6 min at 37 °C. [^{3}H] PAF (20 μl, 0.6 nmol/l) was then added to the tube and the whole was incubated for 6 min. The binding reaction was stopped by adding 3 ml of ice-cold 0.1% BSA in saline. Platelets were isolated by vacuum filtration on glass filters (Whatman GF/C). Each tube or filter was rapidly washed 3 times with 3 ml of ice-cold 0.1% BSA in saline. The

radioactivity on the glass filter was measured in 5 ml of ACS-II (Amersham) with a scintillation counter (Aloka LSC 900). Each experiment was done in duplicate.

3) Platelet Aggregation Study Anti-platelet aggregation effects of the test compounds were determined by Born's turbidimetric method[10] using an aggregometer (Hematracer; Nicho Bioscience Co., Ltd., Tokyo, Japan). A 200 μl aliquot of PRP and 25 μl of a test compound in saline were placed in a cuvette of the aggregometer, and incubated at 37 °C for 3 min. After incubation, platelet aggregation was induced by the addition of 25 μl of a solution of PAF at a final concentration of 50 ng/ml for humans. The inhibitory effect of the compounds or the degree of platelet aggregation was quantified by the method of Stegmeier *et al.*[10,15]

***In Vivo* Experiments PAF-Induced Bronchoconstriction in the Guinea Pig** Guinea pigs were anesthetized by intraperitoneal injection of urethane at a dose of 1.25 g/kg and prepared for the recording of bronchospasms by the method of Konzett and Rössler.[12] PAF at a dose of 100 ng/kg was intravenously injected twice with a 30 min interval in between. Since bronchospasms induced by the second PAF injection were more stable in each animal than those induced by the first PAF injection, the effects of the test compounds were evaluated on the basis of the second bronchospasm response. The bronchospasm induced by the PAF injection was 66.2±2.8% of the maximal overflow volume obtained by tracheal clamping at the end of the experiment.

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