Synthesis and Platelet-Activating Factor (PAF)-Antagonistic Activities of Trisubstituted Piperazine Derivatives

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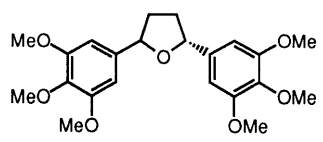
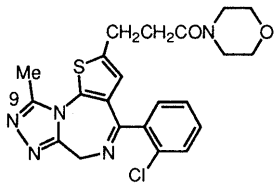
2- or 3-Substituted 1-(2,3-dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)- and 4-(3,4,5-trimethoxybenzyl)piperazines (2a—s, 3a, b) were prepared and evaluated for antagonistic activities against platelet-activating factor (PAF)-induced platelet aggregation and blood pressure reduction. The 2-methoxymethyl derivative (2f) showed the most potent activities in this series. The enantiomers (*R*)-(+)-2f and (*S*)-(−)-2f were synthesized from carbobenzoxy-*O*-benzyl-L- and D-serine in several steps. In the binding experiment, (*S*)-(−)-2f showed thirty times greater affinity than the *R* isomer for the PAF receptor.

Keywords PAF antagonist; structure–activity relationship; trisubstituted piperazine; 1-(2,3-dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxymethyl-4-(3,4,5-trimethoxybenzoyl)piperazine

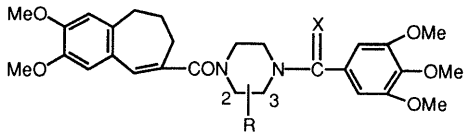
In the course of our search for orally active antagonists against platelet-activating factor (PAF), we discovered 1-(6-methoxy-3,4-dihydro-2-naphthoyl)-4-(3,4,5-trimethoxybenzyl)piperazine and its 3,4,5-trimethoxybenzoyl derivatives as lead compounds and reported the structure–activity relationships (SARs) with regard to the PAF-antagonistic activities of the 1,4-disubstituted piperazine derivatives.[1] In those series, 1-(2,3-dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)piperazine (**1**) was one of the most potent and orally active derivatives. Weber and Heuer demonstrated that the methyl group at the 9 position of the thienotriazolo-1,4-diazepin skeleton of WEB-2086 was correlated with the acetoxy moiety at the C_2 position of PAF, from studies of the quantitative SARs between the K_i values and the bulkiness of the substituents at the 9 position.[2] Based on calculation of three-dimensional electrostatic maps for six PAF antagonists, gingolides, kadsurenone, a furanoid lignan L-652731, and WEB-2086, Dive *et al.* proposed that PAF receptors recognized two wells of negative potential at both ends within 10—12 Å and a small hydrophobic binding site in the middle of the molecular structure of PAF antagonists.[3] The polymethoxy moieties in the structure of L-652731 were defined as the ones providing the negative electrostatic potential.[3] In our study of SARs, terminal polyalkoxy groups in the structure **1** were found to be critical for manifestation of PAF-antagonistic activities. Therefore, we planned to introduce various kinds of small hydrophobic moieties into the piperazine ring of compound **1** as the third binding site. In this paper, we describe the synthesis and biological activities of 2- and 3-substituted 1-(2,3-dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)piperazine derivatives (**2a—s, 3a, b**) and each of enantiomers of the 2-methoxymethylpiperazine analogue (**2f**), which showed the most potent activity in this series.



L-652731



WEB-2086



1 : R = H ; X = O

2a–s : R = 2 - substituted series ; X = O or H_2

3a : R = 3 - CH_3OCH_2- ; X = O

3b : R = 3 - CH_3OCH_2- ; X = H_2

Chart 1

Synthesis

Trisubstituted piperazine derivatives were prepared as described below. First, we studied the regioselective acylation and alkylation at the 4-position of ethyl piperazine-2-carboxylate (**4**)[4] (Chart 2). Reaction of **4** with 1 eq of 3,4,5-trimethoxybenzoyl chloride in the presence of triethylamine in CH_2Cl_2 at 0 °C gave the monoacylpiperazine (**5a**) as a single isomer in 82% yield. Similarly, the monoalkylpiperazine (**5b**) was obtained in 61% yield by the reaction of **4** with 1 eq of 3,4,5-trimethoxybenzyl chloride in the presence of triethylamine in CH_3CN at 70 °C. To clarify the regiochemistry of the reactions, **5a** and **5b** were reduced with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) to give the corresponding amino alcohol (**6**), which was converted into the oxazolidinone (**7**) by treatment with triphosgene and triethylamine in CH_2Cl_2. Maximum absorption at 1750 cm^{-1} in the infrared (IR) spectrum of **7**, assignable to the carbonyl in 5-membered carbamates, supported the

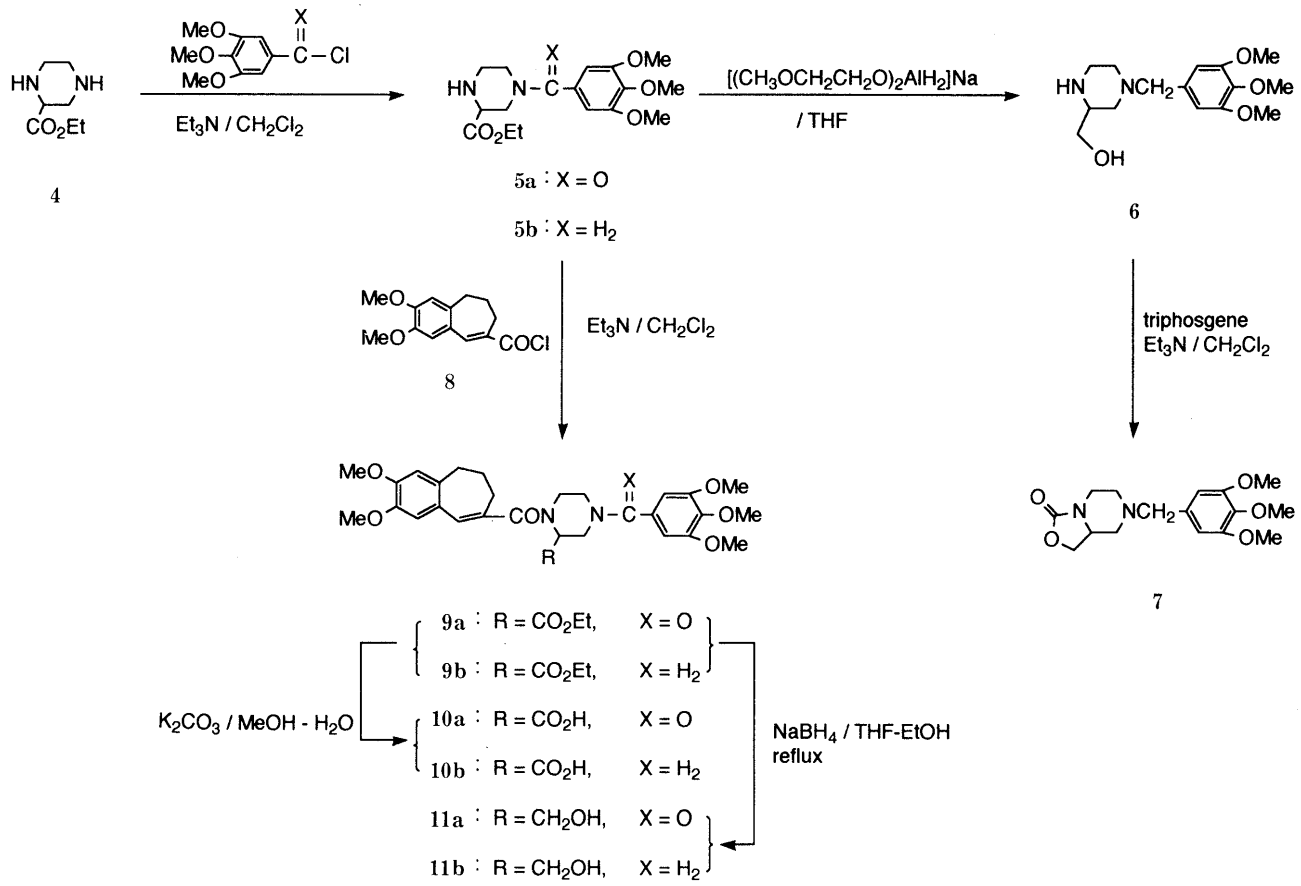


Chart 2

starting material	conditions	products	R	X
10a	MeI, $NaHCO_3$ / DMF	2a	CO_2Me	O
10b	MeI, $NaHCO_3$ / DMF	2b	CO_2Me	H_2
10a	Me_2NH, DEPC, Et_3N / DMF	2c	$CON(Me)_2$	O
10b	Me_2NH, DEPC, Et_3N / DMF	2d	$CON(Me)_2$	H_2
10a	DPPA / toluene, then MeOH	2e	$NHCO_2Me$	O
11a	MeI, NaH / DMF	2f	CH_2OMe	O
11b	MeI, NaH / DMF	2g	CH_2OMe	H_2
11a	EtI, NaH / DMF	2h	CH_2OEt	O
11b	EtI, NaH / DMF	2i	CH_2OEt	H_2
11b	Propyl bromide, NaH / DMF	2j	CH_2OPr	H_2
11b	allyl bromide, NaH / DMF	2k	$CH_2OCH_2CH{=}CH_3$	H_2
11b	propargyl bromide, NaH / DMF	2l	$CH_2OCH_2C{\equiv}CH_2$	H_2
11b	cyclopropylmethyl bromide, NaH / DMF	2m	CH_2-cyclopropyl	H_2
11b	Ac_2O, DMAP / CH_2Cl_2	2n	CH_2OAc	H_2
11b	MeNCO, Et_3N / CH_2Cl_2	2o	$CH_2OCONHMe$	H_2
11b	$(MeS)_2$, $(Bu)_3P$ / DMF	2p	CH_2SCH_3	H_2
11b	Swern oxidation, then Me_2NH $NaBH_3CN$ / MeOH	2q	$CH_2N(Me)_2$	H_2
11b	Swern oxidation, then Et_2NH $NaBH_3CN$ / MeOH	2r	$CH_2N(Et)_2$	H_2

Chart 3

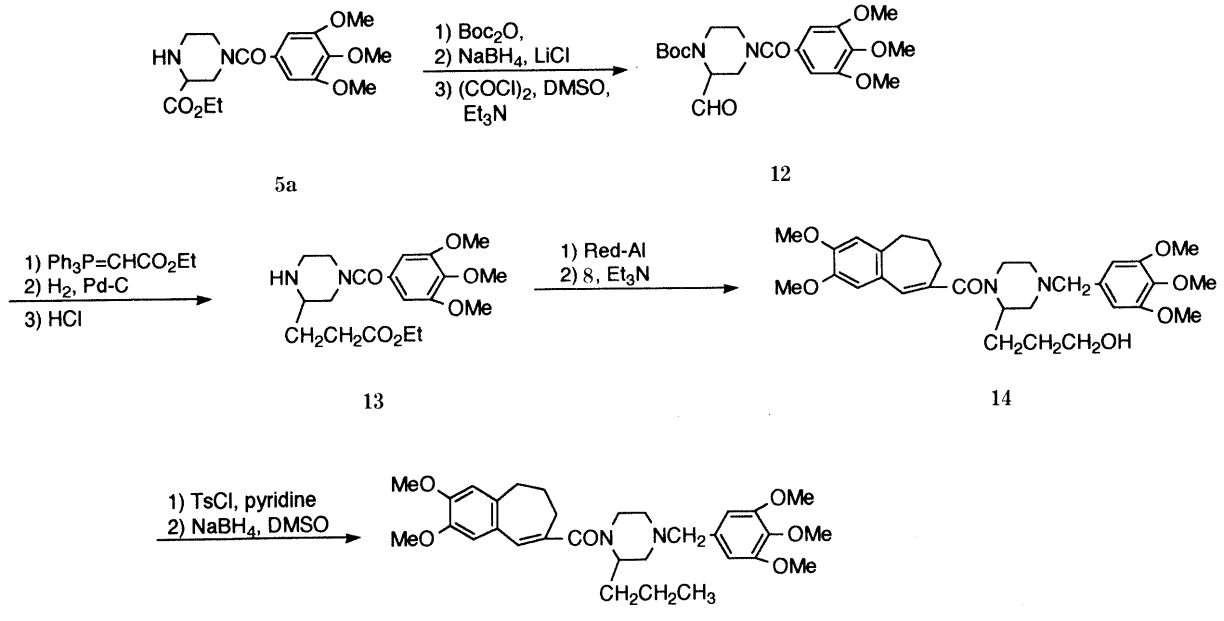
presumed structure. Consequently, the structures of **5a** and **5b** were determined as ethyl 4-(3,4,5-trimethoxybenzoyl)piperazine-2-carboxylate and ethyl 4-(3,4,5-trimethoxybenzyl)piperazine-2-carboxylate, respectively. It proved feasible, therefore, to obtain the monoacyl and monoalkyl derivatives **5a** and **5b** in a regioselective manner; these compounds were then acylated with 2,3-dimethoxy-6,7-dihydro-5*H*-benzocycloheptene-8-carbonyl chloride (**8**) to give the key intermediates (**9a**, **9b**). The ethoxycarbonyl groups of **9a** and **9b** were converted into various groups as summarized in Table I (Chart 3).

Carboxylic acids (**10a**, **10b**), obtained by the alkaline hydrolysis of **9a** and **9b**, were esterified with methyl iodide in the presence of sodium hydrogen carbonate in dimethylformamide (DMF) to give the esters (**2a**, **2b**). Condensation of **10a** and **10b** with dimethylamine using diethyl cyanophosphonate (DEPC)[5] and triethylamine in DMF gave the amides (**2c**, **2d**, respectively). Curtius rearrangement of **10a** with diphenylphosphorylazide (DPPA),[6] and subsequent trapping of the resulting isocyanate with MeOH gave the methylcarbamate (**2e**). Hydroxymethyl derivatives (**11a**, **11b**), obtained on reduction of **9a** and **9b** with sodium borohydride in tetrahydrofuran (THF)–EtOH,[7] were alkylated with various kinds of alkyl halides in the presence of sodium hydride in DMF to give the alkoxymethyl derivatives (**2f**—**m**). Acylation of **11b** with acetic anhydride and methyl isocyanate gave the acetoxymethyl and methylcarbamoyloxymethyl derivatives (**2n**, **2o**), respectively. Reaction of **11b** with tributyl phosphine and dimethyldisulfide gave the methylthiomethyl derivative (**2p**), but only in low yield (7%).[8] Swern oxidation of **11b** and subsequent reductive amination of the resulting carboxaldehyde gave the alkylaminomethyl derivatives (**2q**, **2r**).

As a representative of the alkyl-substituted compounds, the 2-propyl derivative (**2s**) was prepared from **5a** as outlined in Chart 4. After protection of the amino group, the ethoxycarbonyl moiety was reduced with sodium borohydride in the presence of lithium chloride in THF–EtOH to give the corresponding alcohol, which was converted into the carboxaldehyde (**12**) by Swern oxidation. Treatment of **12** with carbethoxymethylenetriphenylphosphorane, hydrogenation on Pd–C and final deprotection under acidic conditions gave the propionate (**13**). Reduction of both the ester and amide groups of **13** with Red-Al, and subsequent acylation with the acid chloride **8** gave the 2-piperazinepropanol derivative (**14**). Conversion of **14** into **2s** was accomplished in a two-step process involving tosylation of the hydroxy group and reduction of the resulting tosylate with sodium borohydride in dimethyl sulfoxide (DMSO).

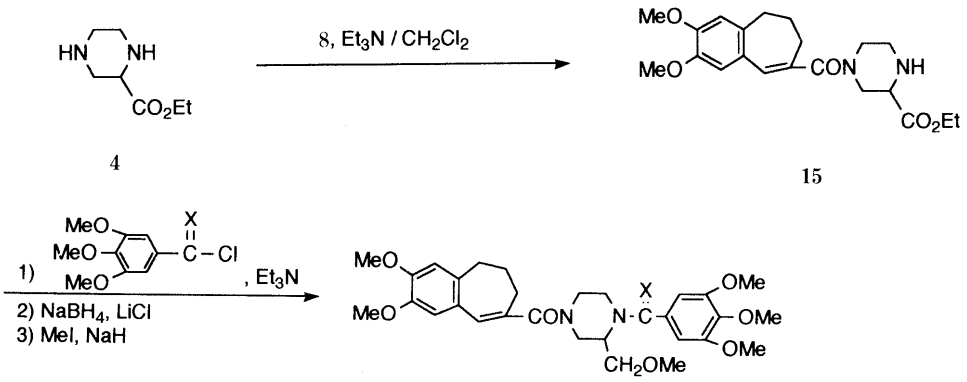
To investigate the effect of the position of substitution, 3-methoxymethyl derivatives (**3a**, **3b**) were synthesized as outlined in Chart 5. Regioselective acylation of **4** at the 4-position with the acid chloride **8** gave the monoacyl derivative (**15**). Reaction of **15** with 3,4,5-trimethoxybenzoyl chloride or 3,4,5-trimethoxybenzyl chloride followed by reduction of the ester groups gave the hydroxymethyl derivatives; these were converted into **3a** and **3b** by methylation with methyl iodide. The physicochemical properties of the compounds synthesized are summarized in Table I.

The 2-methoxymethylpiperazine derivative **2f** was the most potent PAF antagonist among the series of trisubstituted compounds tested. To elucidate the enantiospecificity at the 2-position of the piperazine ring in PAF-antagonistic activity, both enantiomers of **2f** were prepared. Synthesis of (*R*)-(+)-**2f** is outlined in Chart 6. The starting material carbobenzoxy-*O*-benzyl-L-serine (*S*-**16**) was reduced as reported by Yajima *et al.*[9] to give (*R*)-3-benzyloxy-2-benzyloxycarbonylamino-1-propanol (*R*-**17**), which was converted into the aziridine (*R*-**18**) by mesylation followed by treatment with potassium carbonate in the presence of 18-crown-6 in DMF at 70 °C.



5a 12 13 14 2s

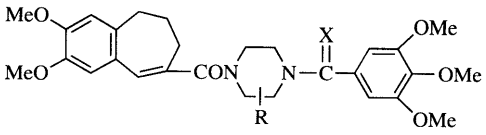
Chart 4



3a : X = O, or 3b : X = H_2

Chart 5

TABLE I. Physicochemical Data for 2- or 3-Substituted 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)- and 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazines (**2a—s, 3a, 3b**)



Compound No.	R	X	mp (°C)	Formula	Analysis (%) Calcd			Found		
					C	H	N	C	H	N
2a	2-CO_2CH_3	O	134	$C_{30}H_{36}N_2O_9 \cdot 1/2H_2O$	62.38	6.46	4.85	62.30	6.31	4.86
2b	2-CO_2CH_3	H_2	—	$C_{30}H_{38}N_2O_8 \cdot HCl$	60.96	6.65	4.74	60.82	6.92	4.53
2c	2-$CON(CH_3)_2$	O	190—191	$C_{31}H_{39}N_3O_8$	64.01	6.76	7.22	63.85	6.95	7.01
2d	2-$CON(CH_3)_2$	H_2	165—166	$C_{31}H_{41}N_3O_7$	65.59	7.28	7.40	65.31	7.29	7.23
2e	2-$NHCO_2CH_3$	O	143—145	$C_{30}H_{37}N_3O_9$	61.74	6.39	7.20	61.62	6.12	7.05
2f	2-CH_2OCH_3	O	173	$C_{30}H_{38}N_2O_8$	64.97	6.91	5.05	64.58	6.88	4.68
2g	2-CH_2OCH_3	H_2	—	$C_{30}H_{40}N_2O_7 \cdot HCl \cdot 4/5H_2O$	60.91	7.26	4.74	60.92	6.98	4.75
2h	2-$CH_2OC_2H_5$	O	—	$C_{31}H_{40}N_2O_8$	65.48	7.09	4.93	65.18	7.25	4.64
2i	2-$CH_2OC_2H_5$	H_2	169—171	$C_{31}H_{42}N_2O_7 \cdot HCl \cdot 1/2H_2O$	62.04	7.39	4.67	62.45	7.32	4.75
2j	2-$CH_2OC_3H_7$	H_2	152—156	$C_{32}H_{44}N_2O_7 \cdot HCl \cdot 1/2H_2O$	62.58	7.55	4.56	62.29	7.71	4.48
2k	2-$CH_2OCH_2CH{=}CH_2$	H_2	154—157	$C_{32}H_{42}N_2O_7 \cdot HCl$	63.72	7.19	4.64	63.70	7.31	4.63
2l	2-$CH_2OCH_2C{\equiv}CH$	H_2	—	$C_{32}H_{40}N_2O_7 \cdot HCl$	63.94	6.87	4.66	63.84	6.87	4.73
2m	2-CH_2OCH_2- 	H_2	—	$C_{33}H_{44}N_2O_7 \cdot HCl$	64.22	7.35	4.54	64.10	7.60	4.58
2n	2-CH_2OCOCH_3	H_2	135—139	$C_{31}H_{40}N_2O_8 \cdot HCl \cdot 1/2H_2O$	60.63	6.89	4.56	60.53	7.03	4.38
2o	2-$CH_2OCONHCH_3$	H_2	161—163	$C_{31}H_{41}N_3O_8 \cdot HCl$	60.04	6.83	6.78	60.01	6.98	6.49
2p	2-CH_2SCH_3	H_2	—	$C_{30}H_{40}N_2O_6 \cdot HCl \cdot 2H_2O$	57.26	7.21	4.45	57.50	7.05	4.42
2q	2-$CH_2N(CH_3)_2$	H_2	167—169	$C_{31}H_{43}N_3O_6 \cdot 2HCl$	59.42	7.24	6.71	59.18	7.33	6.68
2r	2-$CH_2N(C_2H_5)_2$	H_2	157—160	$C_{33}H_{47}N_3O_6 \cdot HCl$	60.54	7.54	6.42	60.47	7.60	6.40
2s	2-C_3H_7	H_2	172—176	$C_{31}H_{42}N_2O_6 \cdot HCl$	64.74	7.54	4.87	64.50	7.44	4.80
3a	3-CH_2OCH_3	O	—	$C_{30}H_{38}N_2O_8$	64.97	6.91	5.05	64.80	7.13	4.79
3b	3-CH_2OCH_3	H_2	136—138	$C_{30}H_{40}N_2O_7 \cdot HCl \cdot 4/5H_2O$	60.91	7.26	4.74	61.09	7.29	4.70

The aziridine ring of *R*-**18** was opened by reaction with 2,2-diethoxyethylamine, and the resulting secondary amine was acylated with 3,4,5-trimethoxybenzoyl chloride to give a single diacylethylenediamine (*R*-**19**). Cyclization of *R*-**19** with catalytic amounts of *p*-toluenesulfonic acid in toluene at 50 °C gave the labile tetrahydropyrazine derivative (*R*-**20**),[10] which was converted, without further purification, into the amino alcohol (*R*-**21**) by hydrogenation on Pd–C. Regiochemistry of the ring opening reaction of *R*-**18** was confirmed at this stage by the conversion of *R*-**21** to the oxazolidinone derivative (IR ν_{max}: 1750 cm^{-1}) with triphosgene as mentioned before. Finally, (*R*)-(+)-**2f** was obtained by the acylation of *R*-**21** with the acid chloride **8** followed by methylation with methyl iodide in the presence of sodium hydride in DMF. In an identical manner, (*S*)-(−)-**2f** was prepared from carbobenzoxy-*O*-benzyl-D-serine (*R*-**16**). Optical purity of the reaction products was evaluated by analytical HPLC on a column designed for optical resolution (Daicel, Chiracel OD), and estimated to be greater than 98% ee.

Biological Activity and Discussion

The inhibitory activities of the compounds against PAF induced rabbit platelet aggregation *in vitro* was examin-

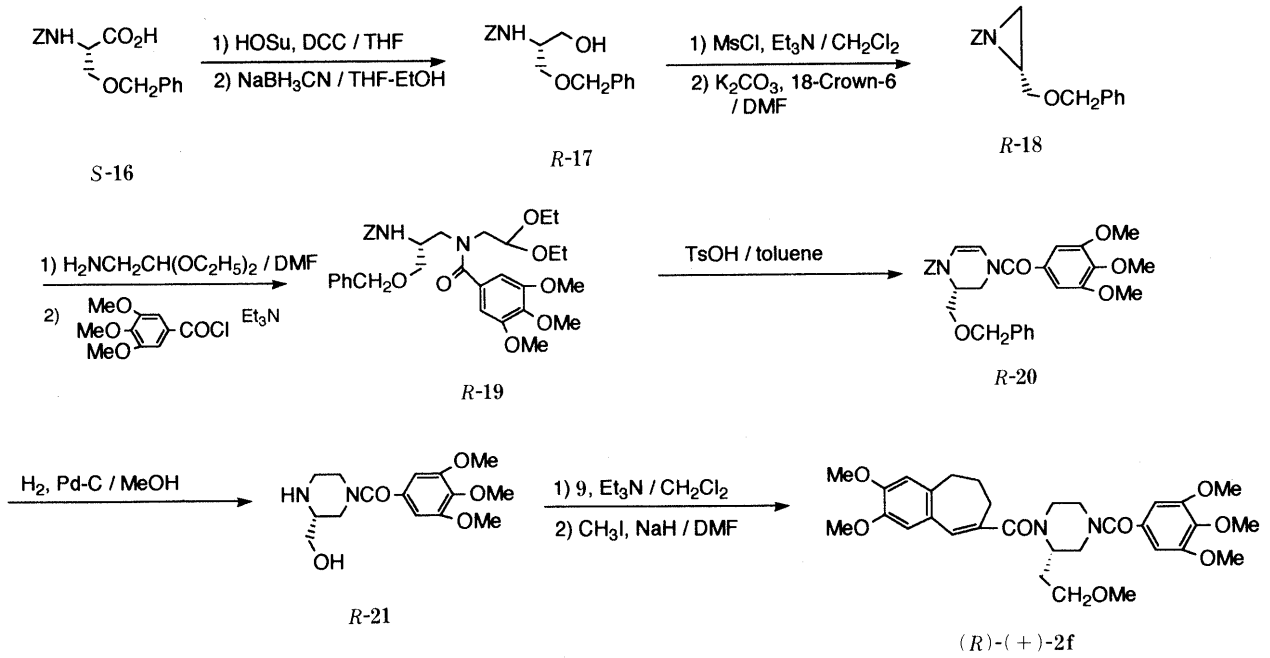


Chart 6

TABLE II. PAF-Antagonistic Activities of Trisubstituted Piperazine Derivatives

Compound No.	Inhibitory activities against PAF-induced platelet aggregation (% inhibition)			Inhibitory activities against PAF-induced hypotension in rat (% inhibition)					
	3×10^{-7}	3×10^{-6}	3×10^{-5} M	Dose (*p.o.*) (mg/kg)	1 h	2 h	4 h	6 h	8 h
1a		64	100	30	100	100	100	100	87
				3	78	72	53	24	18
1b		28	100	30	94	97	88	72	41
				3	78	66	33	17	3
2a		61	100	30	51	87	95	95	95
2b		84	100	30	94	94	90	39	32
2c		0	89	30	12	25	12		
2d		20	100	30	93	100	100	83	60
2e		26	100		ND				
2f	45	100	100	30	100	100	100	100	97
				3	69	55	39	11	
2g	14	100	100	30	100	100	100	100	83
				3	78	58	38	17	
2h	15	100	100	30	100	100	100	100	100
2i	31	100	100	30	100	100	79	24	
2j		88	100	30	100	100	100	85	56
2k	10	100	100	30	100	100	57	6	
2l	6	100	ND		ND				
2m	0	75	ND		ND				
2n		67	100	30	100	100	24		
2o		54	100	30	100	50.	16		
2p		0	100		ND				
2q	17	88	100	30	100	100	91	89	66
2r	0	100	ND		ND				
2s		47	100		ND				
3a		80	100	30	100	100	100	92	92
3b		43	100	30	100	100	100	100	88
L-652731	12	88	99	30	34	63	49	31	—
WEB-2086	81	100	—	30	85	81	59	45	17

ND: Not determined.

ed as a primary bioassay (Table II). Variation of the substituents at the 2 position of the piperazine ring caused marked changes in the inhibitory activities. Introduction of the methoxymethyl and the ethoxymethyl groups resulted in an increase of potency by about one order of magnitude (**2f—i**). Some alkoxymethyl derivatives (**2h—m**), with bulkier alkoxy groups, showed a decrease of antiaggregatory activity. Dialkylaminomethyl derivatives

TABLE III. PAF-Antagonistic Activities of (*R*)-(+)-, (*S*)-(−)-, and Racemic 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxymethyl-4-(3,4,5-trimethoxybenzoyl)piperazines [(*R*)-(+)-, (*S*)-(−)- and (*RS*)-**2f**]

Compound No.	Inhibitory activities against PAF-induced platelet aggregation (% inhibition)			Inhibitory activities against [^{3}H]PAF binding (IC_{50}: μM)
	3×10^{-7}	3×10^{-6}	3×10^{-5} M	
(*R*)-(+)-**2f**		17	100	1.9
(*S*)-(−)-**2f**	27	100	100	0.052
(*RS*)-**2f**	0	100	100	0.1

(**2q**, **2r**) showed double the activity. Interestingly, introduction of a propyl (**2s**) or methylthiomethyl (**2p**) group, providing length and bulkiness comparable to those obtained with the methoxymethyl group, resulted in only a slight increase of the activity or caused great loss of potency. These results may suggest that a lone pair of electrons on the oxygen atom of the methoxymethyl group plays a significant role in the interaction with the PAF receptor. Methoxycarbonyl (**2a**, **2b**), acetoxymethyl (**1n**), and methylcarbamoyloxymethyl (**2o**) derivatives were equipotent with the unsubstituted compounds (**1a**, **1b**), whereas 2-dimethylcarbamoyl (**2c**, **2d**) and methoxycarbonylamino (**2e**) derivatives were less potent than **1a** and **1b**. Introduction of the methoxymethyl group at the 3-position of the piperazine ring (**3a**, **3b**) had little effect upon PAF-antagonistic activity.

The compounds were further evaluated for inhibitory activity against PAF-induced hypotension on oral administration in rats, but no pronounced effect was observed on the activity.

Significant differences were observed in the inhibitory activity against PAF-induced platelet aggregation between (*R*)-(+)-**2f** and (*S*)-(−)-**2f**. The latter was ten times more potent than the former. This observation was confirmed by the PAF receptor binding assay, in which the *S* isomer showed over thirty times greater affinity than the *R* isomer (Table III).

The above results suggest that the substituents at the 2-position of the piperazine skeleton play a significant role at the third binding site in the interaction of the compounds with the PAF receptor. The binding model proposed by Dive *et al.*[3] seems to be applicable to the compounds tested in the present study, because the two wells of negative potential generated by the methoxy groups at both ends of the molecule are essential for manifesting PAF-antagonist activity. Recently, Honda *et al.*[11] reported the expression cloning of PAF receptor from guinea-pig lung, and demonstrated that the receptor belonged to the superfamily of G protein-coupled receptors. Although PAF contains a positively charged choline moiety, the aspartic acid in the third helix of the seven cell-spanning domains, a putative counter ion for cationic ligands (catecholamines and acetylcholine), is not present. Trisubstituted piperazine derivatives, as well as kadsurenone and L-652731, lack a cationic center in their molecular structure. The SARs obtained in the present study will provide useful information on the interaction between PAF receptor and ligands. Further pharmacological characterizations of the compounds are in progress.

Experimental

Melting points were determined on a Yanagimoto micro melting apparatus and are uncorrected. The IR spectra were recorded with a JEOL IR-800 spectrophotometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded in the indicated solvent on Varian EM-390 or Varian XRD-200 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane (TMS) as an internal standard. Melting points and analytical data for **2a**—**s**, **3a** and **3b** are listed in Table I.

Ethyl 4-(3,4,5-Trimethoxybenzoyl)piperazine-2-carboxylate (5a) A solution of 3,4,5-trimethoxybenzoyl chloride (2.0 g) in CH_2Cl_2 (20 ml) was added dropwise to a mixture of **4** (2.0 g) and Et_3N (1.0 g) in CH_2Cl_2 (40 ml) at 0 °C with stirring. After additional stirring for 1 h, the reaction mixture was washed with H_2O, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual product was subjected to column chromatography on silica gel (hexane : AcOEt : acetone = 1 : 2 : 2) and recrystallized from AcOEt–hexane to give **5a** (2.5 g, 82%) as a white powder, mp 114—115 °C. IR ν_{max}^{KBr} cm^{-1}: 3300, 2920, 1740, 1630, 1580, 1440, 1240. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J = 7 Hz, CH_3), 2.05 (1H, m, NH), 2.64—4.00 (7H, m), 3.86 (9H, s, OCH_3), 4.22 (2H, q, J = 7 Hz, $-CO_2CH_2-$), 6.65 (2H, s, Ar-H). *Anal.* Calcd for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.86; N, 7.95. Found: C, 57.83; H, 6.91; N, 7.79.

Ethyl 4-(3,4,5-Trimethoxybenzyl)piperazine-2-carboxylate (5b) A solution of **4** (1.0 g), 3,4,5-trimethoxybenzyl chloride (0.94 g) and Et_3N (2.4 ml) in CH_3CN (15 ml) was stirred for 15 min at 70 °C. The reaction mixture was concentrated to dryness, and then the residue was dissolved in CH_2Cl_2 and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was subjected to column chromatography on silica gel (hexane : acetone : CH_2Cl_2 : EtOH = 4 : 4 : 4 : 1), and then the residual oil was treated with HCl–AcOEt and recrystallized from EtOH–Et_2O to give **5b**·2HCl (0.9 g, 61%) as colorless prisms, mp 150—153 °C. IR ν_{max}^{KBr} cm^{-1}: 3359, 1740, 1595, 1450, 1240, 1015. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J = 7 Hz, CH_3), 2.00—3.75 (10H, m), 3.85 (3H, s, OCH_3), 3.87 (6H, s, OCH_3), 4.17 (2H, q, J = 7 Hz, $-CO_2CH_2-$), 6.53 (2H, s, Ar-H). *Anal.* Calcd for $C_{17}H_{26}N_2O_5\cdot2HCl\cdot H_2O$: C, 47.56; H, 7.04; N, 6.52. Found: C, 47.57; H, 7.07; N, 6.31.

4-(3,4,5-Trimethoxybenzoyl)piperazine-2-methanol (6) A solution of **5a** (36.6 g) in toluene (100 ml) was added over 1 h to a mixture of sodium bis(2-methoxyethoxy)aluminum hydride (50 g) and toluene (250 ml) with stirring at room temperature. After additional stirring for 1 h, the excess reductant was quenched with 40% aqueous NaOH. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residual product was recrystallized from MeOH to yield **6** (10.5 g, 34%) as a white powder, mp 93 °C. IR ν_{max}^{KBr} cm^{-1}: 2818, 1591, 1421, 1237, 1126. ^{1}H-NMR ($CDCl_3$) δ: 2.05—2.17, 2.67—2.74 (2H, m), 2.84—3.11 (5H, m), 3.42 (2H, s), 3.56 (1H, dd, J = 10.0 Hz, 7.4 Hz), 3.59 (1H, dd, J = 10.0 Hz, 4.4 Hz), 3.84 (3H, s, OCH_3), 3.86 (6H, s, OCH_3), 6.56 (2H, s, Ar-H). *Anal.* Calcd for $C_{15}H_{24}N_2O_4$: C, 60.79; H, 8.16; N, 9.45. Found: C, 60.77; H, 8.06; N, 9.18.

4-(3,4,5-Trimethoxybenzyl)-9-oxo-1,4-diaza-8-oxa-bicyclo[4.3.0]-nonane (7) A mixture of triphosgene (755 mg) and CH_2Cl_2 (5 ml) was added over 5 min a solution of **6** (1.5 g) and Et_3N (1.0 g) in CH_2Cl_2 (20 ml) at 0 °C. After additional stirring for 1 h, the reaction mixture was washed with 10% aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual product was subjected to column chromatography on silica gel (AcOEt : hexane = 4 : 1) and recrystallized from AcOEt to give **7** (1.3 g, 80%) as colorless needles, mp 107 °C. IR ν_{max}^{KBr} cm^{-1}: 2934, 1751, 1591, 1458, 1239, 1125. ^{1}H-NMR ($CDCl_3$) δ: 1.93—2.10, 2.78—2.92 (2H, m), 3.13—3.50 (5H, m), 3.40, 3.54 (1H, d, J = 13.2 Hz), 3.84 (3H, s, OCH_3), 3.86 (6H, s, OCH_3), 6.54 (2H, s, Ar-H). *Anal.* Calcd for $C_{16}H_{22}N_2O_5$: C, 59.62; H, 6.88; N, 8.69. Found: C, 59.62; H, 7.02; N, 8.69.

Ethyl 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)piperazine-2-carboxylate (9a) A mixture of **8** (550 mg) and CH_2Cl_2 (5 ml) was added dropwise to a solution of **5a** (700 mg) and Et_3N (300 mg) in CH_2Cl_2 (10 ml), at 0 °C with stirring. After additional stirring for 1 h at room temperature, the reaction mixture was washed with 10% aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The

residual product was subjected to column chromatography on silica gel (hexane : AcOEt : acetone = 1 : 1 : 1) and recrystallized from AcOEt–hexane to give **9a** (1.1 g, 93%) as colorless prisms, mp 108—110 °C. IR ν_{max}^{KBr} cm^{-1}: 2950, 1740, 1630, 1580, 1240, 1130. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7 Hz, CH_3), 1.85—5.30 (13H, m), 3.84, 3.86 (each 3H, s, OCH_3), 3.90 (9H, s, OCH_3), 4.22 (2H, q, J = 7 Hz, $-CO_2CH_2-$), 6.48 (1H, br s, vinyl), 6.67 (4H, s, Ar-H). *Anal.* Calcd for $C_{31}H_{38}N_2O_9$: C, 63.90; H, 6.57; N, 4.81. Found: C, 63.68; H, 6.63; N, 4.87.

Ethyl 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine-2-carboxylate (9b) 9b was prepared from **5b** in 80% yield by the method similar to that described for **9a**. The free base was converted into the hydrochloride (amorphous powder). IR ν_{max}^{KBr} cm^{-1}: 2936, 1736, 1605, 1591, 1124. ^{1}H-NMR (DMSO-d_6) δ: 1.25 (3H, t, J = 7.2 Hz, CH_3), 2.05—2.84 (10H, m), 3.33—3.59 (5H, m), 3.84, 3.87 (each 3H, s, OCH_3), 3.86 (6H, s, OCH_3), 3.89 (3H, s, OCH_3), 4.21 (2H, q, J = 7.2 Hz, $-CO_2CH_2-$), 6.46 (1H, s), 6.54 (2H, s, Ar-H), 6.66, 6.69 (1H, s, Ar-H). *Anal.* Calcd for $C_{31}H_{40}N_2O_8 \cdot HCl \cdot 1/2H_2O$: C, 60.63; H, 6.89; H, 4.56. Found: C, 60.82; H, 7.08; N, 4.31.

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)piperazine-2-carboxylic Acid (10a) A mixture of **9a** (1.5 g), K_2CO_3 (1.1 g), EtOH (5 ml) and H_2O (5 ml) was stirred for 2 h at 70 °C. After removal of EtOH by evaporation, the solution was acidified with concentrated HCl and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give **10a** (1.0 g, 70%) as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 1738, 1640, 1583, 1122. ^{1}H-NMR ($CDCl_3$) δ: 1.95—2.15, 2.43—2.51, 2.72—2.88 (2H, m), 2.95—3.38 (7H, m), 3.85, 3.86 (each 6H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.38 (1H, s), 6.83, 6.92 (2H, s, Ar-H). *Anal.* Calcd for $C_{29}H_{34}N_2O_9 \cdot 1/2H_2O$: C, 61.80; H, 6.26; N, 4.97. Found: C, 61.79; H, 6.52; N, 4.70.

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine-2-carboxylic Acid (10b) 10b was synthesized from **9b** in 73% yield by the procedure similar to that described for **10a**. Recrystallization from AcOEt gave colorless prisms, mp 143—146 °C. IR ν_{max}^{KBr} cm^{-1}: 3446, 2938, 1734, 1637, 1595, 1254, 1124. ^{1}H-NMR ($CDCl_3$) δ: 1.85—2.03, 2.35—2.57, 2.71—2.85 (2H, m), 2.66—3.67 (9H, m), 3.69, 3.74, 3.77 (each 3H, s, OCH_3), 3.82 (6H, s, OCH_3), 6.45 (1H, br s), 6.80, 6.89 (2H, s, Ar-H). *Anal.* Calcd for $C_{29}H_{36}N_2O_8 \cdot 3/2H_2O$: C, 61.36; H, 6.93; N, 4.94. Found: C, 61.33; H, 6.72; N, 4.98.

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine-2-methanol (11a) EtOH (5 ml) was added dropwise over 1 h to a solution of **9a** (1.16 g) and $NaBH_4$ (0.76 g) in THF (20 ml) at reflux. The reaction mixture was concentrated to dryness, and then the residue was dissolved in CH_2Cl_2 and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was subjected to column chromatography on silica gel (AcOEt) and recrystallized from AcOEt–Et_2O to give **11a** (950 mg, 88%) as colorless prisms, mp 169 °C. IR ν_{max}^{KBr} cm^{-1}: 3344, 2922, 1640, 1600, 1585, 1123. ^{1}H-NMR ($CDCl_3$) δ: 2.05—2.10, 2.51—2.57, 2.80—2.85 (2H, m), 3.07—3.38 (4H, m), 3.78 (2H, d, J = 7.6 Hz, $-OCH_2-$), 3.85, 3.88 (each 3H, s, OCH_3), 3.89 (9H, s, OCH_3), 4.01—4.25, 4.41—4.78 (2H, m), 6.44 (1H, s), 6.65, 6.68 (2H, s, Ar-H). *Anal.* Calcd for $C_{29}H_{36}N_2O_8$: C, 64.63; H, 6.71; N, 5.18. Found: C, 64.18; H, 6.66; N, 5.09.

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine-2-methanol (11b) 11b was synthesized from **9b** in 70% yield by the procedure similar to that described for **11a**. Recrystallization from AcOEt–Et_2O gave colorless prisms, mp 172 °C. IR ν_{max}^{KBr} cm^{-1}: 2932, 1603, 1590, 1419, 1232, 1128. ^{1}H-NMR ($CDCl_3$) δ: 2.04—2.13, 2.18—2.31 (2H, m), 2.35—3.07 (11H, m), 3.39, 3.52 (1H, d, J = 12 Hz), 3.85, 3.86, 3.89 (each 3H, s, OCH_3), 3.87 (6H, s, OCH_3), 4.07—4.15 (1H, m), 6.43 (1H, s), 6.53 (2H, s, Ar-H), 6.65, 6.67 (each 1H, s, Ar-H). *Anal.* Calcd for $C_{29}H_{38}N_2O_7$: C, 66.14; H, 7.27; N, 5.32. Found: C, 66.02; H, 7.36; H, 5.27.

Methyl 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)piperazine-2-carboxylate (2a) A solution of **10a** (550 mg), methyl iodide (284 mg) and $NaHCO_3$ (170 mg) in DMF (5 ml) was stirred for 12 h at room temperature. The reaction mixture was dissolved in AcOEt and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (3% MeOH in AcOEt) and recrystallized from EtOH to give **2a** (450 mg, 74%) as colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 2950, 1750, 1630, 1590, 1240, 1130. ^{1}H-NMR ($CHCl_3$) δ: 1.81—2.03 (2H, m), 2.29—4.86 (11H, m), 3.70 (3H, s, OCH_3), 3.87 (12H, s, OCH_3), 4.01 (3H, s, $-CO_2CH_3$), 6.43 (1H, s), 6.63 (4H, br s, Ar-H).

Methyl 1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine-2-carboxylate (2b) 2b was prepared from **10b** in 77% yield by the method similar to that described for **2a**. The free base was treated with HCl–AcOEt to give the hydrochloride as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 2940, 1745, 1640, 1590, 1420, 1330, 1130. ^{1}H-NMR (DMSO-d_6) δ: 1.87—2.74 (12H, m), 3.76 (3H, s, OCH_3), 3.88 (12H, s, OCH_3), 3.90—5.30 (3H, m), 3.93 (3H, s, $-CO_2CH_3$), 6.43 (1H, s), 6.52, 6.63 (2H, s, Ar-H).

1-(2,3-Dimethyl-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-dimethylaminocarbonyl-4-(3,4,5-trimethoxybenzoyl)piperazine (2c) A mixture of diethyl cyanophosphonate (330 mg) and DMF (5 ml) was added dropwise to a solution of **10a** (550 mg), dimethylamine hydrochloride (600 mg) and Et_3N (500 mg) in DMF (5 ml) at 0 °C with stirring. After additional stirring for 1 h at room temperature, the reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (AcOEt) and recrystallized from EtOH to give **2c** (406 mg, 70%) as colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 2950, 1650, 1620, 1600, 1590, 1120. ^{1}H-NMR ($CDCl_3$) δ: 1.75—2.10, 2.33—2.65, 2.86—2.91 (2H, m), 2.88, 3.10 (3H, s, $-NCH_3$), 3.20—4.68 (6H, m), 3.80 (15H, s, OCH_3), 5.20—5.46 (1H, m), 6.45 (1H, s), 6.69 (4H, s, Ar-H).

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-dimethylaminocarbonyl-4-(3,4,5-trimethoxybenzyl)piperazine (2d) 2d was prepared from **10b** in 88% yield by a method similar to that described for **2c**. Recrystallization from AcOEt gave colorless needles. IR ν_{max}^{KBr} cm^{-1}: 2940, 1650, 1520, 1420, 1230, 1120. ^{1}H-NMR ($CDCl_3$) δ: 1.95—2.88 (10H, m), 3.05 (6H, br s, $-NCH_3$), 3.85 (3H, s, OCH_3), 3.89 (9H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.92—4.79 (5H, m), 6.46 (1H, s), 6.65, 6.67 (2H, s, Ar-H).

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxycarbonylamino-4-(3,4,5-trimethoxybenzoyl)piperazine (2e) A mixture of diphenylphosphorylazide (370 mg) and DMF (1 ml) was added to a solution of **10a** (750 mg) and Et_3N (140 mg) in DMF (4 ml) at 0 °C with stirring. After additional stirring for 2 h, the reaction mixture was poured into ice-water and extracted with benzene. The organic layer was dried over anhydrous $MgSO_4$, heated with MeOH (5 ml) at refluxing point for 1 h and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (hexane : AcOEt : acetone = 1 : 1 : 1) and recrystallized from AcOEt to give **2e** (600 mg, 80%). IR ν_{max}^{KBr} cm^{-1}: 3280, 2934, 1688, 1632, 1412, 1242, 1124. ^{1}H-NMR ($CDCl_3$) δ: 1.98—2.26, 2.59—2.64, 2.82—2.89 (2H, m), 3.58, 3.58, 3.89 (each 3H, s, OCH_3), 3.87 (9H, s, OCH_3), 3.45—4.51 (7H, m), 6.08 (1H, br s, –NH), 6.50 (1H, s), 6.67, 6.70 (2H, s, Ar-H).

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxymethyl-4-(3,4,5-trimethoxybenzoyl)piperazine (2f) A solution of **11a** (1.08 g) and methyl iodide (250 mg) in DMF (10 ml), was treated portionwise with 60% NaH (120 mg) at 0 °C with stirring. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (AcOEt : hexane = 4 : 1) and recrystallized from AcOEt to give **2f** (860 mg, 79%) as colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 2950, 1640, 1590, 1240, 1130. ^{1}H-NMR ($CDCl_3$) δ: 2.05—2.11, 2.52—2.58, 2.80—2.85 (2H, m), 3.33 (3H, s, OCH_3), 3.56 (2H, d, J = 7.2 Hz, $-CH_2O-$), 3.86, 3.87 (each 3H, s, OCH_3), 3.88 (6H, s, OCH_3), 3.89 (3H, s, OCH_3), 4.00—4.85 (7H, m), 6.44 (1H, s), 6.66 (4H, s, Ar-H).

The alkoxymethyl derivatives **2g**—**m** were similarly synthesized from the alcohol **11a** or **11b** by reaction with the appropriate alkyl halide in the presence of sodium hydride in DMF.

2-Acetoxymethyl-1-(2,3-dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine (2n) A solution of **11b** (500 mg), acetic anhydride (153 mg) and 4-dimethylaminopyridine (10 mg) in CH_2Cl_2 (5 ml) was stirred for 20 h at room temperature. The reaction mixture was washed with 1 N HCl and then 10% aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (AcOEt) to give **2n** (420 mg, 75%). The free base was treated with HCl–AcOEt to give the hydrochloride as colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 2938, 1742, 1627, 1505, 1424, 1125.

^{1}H-NMR (DMSO-d_6) δ: 1.75—2.13 (2H, m), 1.95 (3H, s, $COCH_3$), 2.30—2.60, 2.68—2.90 (2H, m), 3.68 (3H, s, OCH_3), 3.73 (9H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.89—4.53 (11H, m), 6.47 (1H, s), 6.77, 6.85 (1H, s, Ar-H), 7.10 (2H, s, Ar-H).

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-(*N*-methylaminocarbonyloxymethyl)-4-(3,4,5-trimethoxybenzyl)piperazine (2o) A solution of **11b** (500 mg), methyl isocyanate (100 mg) and Et_3N (200 mg) in CH_2Cl_2 (5 ml) was stirred at room temperature for 5 h. The reaction mixture was washed with 10% aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual oil was subjected to column chromatography on silica gel (AcOEt : hexane : MeOH = 5 : 4 : 1) to give **2o** (440 mg, 78%) as a colorless oil. The free base was treated with HCl–AcOEt and recrystallized from EtOH to give the hydrochloride as colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 2934, 1732, 1596, 1252, 1124. ^{1}H-NMR (DMSO-d_6) δ: 1.70—2.14, 2.34—2.63 (2H, m), 2.50 (3H, s, $-NCH_3$), 2.63—2.94 (2H, m), 3.04—4.89 (11H, m), 3.70, 3.73, 3.75 (each 3H, s, OCH_3), 3.80 (6H, s, OCH_3), 5.97 (1H, br s, NH), 6.47 (1H, s), 6.73, 6.87 (1H, s, Ar-H), 7.12 (2H, s, Ar-H).

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methylthiomethyl-4-(3,4,5-trimethoxybenzyl)piperazine (2p) A mixture of **11b** (500 mg), dimethyl disulfide (940 mg), tributylphosphine (2.0 g) and DMF (10 ml) was stirred at room temperature for 120 h. The reaction mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (AcOEt) to give **2p** (40 mg, 7%) as a colorless oil. The free base was treated with HCl–AcOEt to give the hydrochloride as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 2934, 1654, 1593, 1510, 1242, 1123. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.26 (8H, m), 2.20 (3H, s, SCH_3), 3.67 (3H, s, OCH_3), 3.70 (9H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.30—4.87 (9H, m), 6.52 (3H, s), 6.56, 6.68 (1H, s, Ar-H).

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-(*N*,*N*-dimethylaminomethyl)-4-(3,4,5-trimethoxybenzyl)piperazine (2q) DMSO (220 mg) was added to a mixture of oxalyl chloride (180 mg) and CH_2Cl_2 (5 ml) at -78°C, followed by a solution of **11b** (500 mg) in CH_2Cl_2 (1 ml). After additional stirring for 5 min, Et_3N (650 mg) was added and the resulting mixture was allowed to warm to room temperature. The reaction mixture was diluted with AcOEt and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the corresponding aldehyde. A solution of the aldehyde, dimethylamine hydrochloride (200 mg) and $NaBH_3CN$ (130 mg) in MeOH (5 ml) was stirred at room temperature for 48 h. After removal of MeOH by evaporation, the residue was dissolved in CH_2Cl_2 and washed with 1 N aqueous NaOH. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was subjected to column chromatography on silica gel (AcOEt : MeOH = 85 : 15) to give **2q** (200 mg) as a colorless oil. The free base was treated with HCl–AcOEt and recrystallized from EtOH to give the hydrochloride as colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 3414, 2936, 1625, 1596, 1255, 1124. ^{1}H-NMR (DMSO-d_6) δ: 1.75—2.18, 2.33—2.65, 2.69—2.95 (2H, m), 2.80 (6H, s, NCH_3), 3.00—4.43 (11H, m), 3.83 (6H, s, OCH_3), 3.89 (9H, s, OCH_3), 6.72 (1H, s), 7.00, 7.07 (2H, s, Ar-H).

2-(*N*,*N*-Diethylaminomethyl)-1-(2,3-dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine (2r) 2r was synthesized from **11b** in 40% yield by the method similar to that described for **2q**. Recrystallization from EtOH gave colorless prisms. IR ν_{max}^{KBr} cm^{-1}: 3434, 2938, 1620, 1595, 1252. ^{1}H-NMR (DMSO-d_6) δ: 1.16—1.46 (6H, m, CH_3), 1.83—2.14, 2.33—2.65, and 2.71—2.94 (2H, m), 2.99—3.46 (8H, m), 3.67, 3.70, 3.75 (each 3H, s, OCH_3), 3.83 (6H, s, OCH_3), 3.88—4.41 (7H, m), 6.73 (1H, s), 6.80, 7.00 (1H, s, Ar-H), 7.11 (2H, s, Ar-H).

1-*tert*-Butoxycarbonyl-4-(3,4,5-trimethoxybenzoyl)piperazine-2-carboxaldehyde (12) A solution of di-*tert*-butyl dicarbonate (3.3 g) in CH_2Cl_2 (5 ml) was added dropwise to a mixture of **5a** (4.8 g), Et_3N (1.5 g) and CH_2Cl_2 (20 ml) at 0 °C with stirring. After additional stirring for 1 h, the reaction mixture was treated with *N*,*N*-dimethylethylenediamine and washed with 5% aqueous citric acid. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in THF (20 ml), and to this solution LiCl (567 mg), $NaBH_4$ (514 mg) and EtOH (20 ml) were added successively at room temperature with stirring. After additional stirring for 12 h, the reaction mixture was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the corresponding alcohol. This alcohol was converted into **12** (colorless prisms, mp 160—161 °C, AcOEt), in 82% yield from **5a**, by a method similar to that described for the synthesis of **2q**. IR ν_{max}^{KBr} cm^{-1}: 2924, 2666, 1724, 1647, 1151. ^{1}H-NMR ($CDCl_3$) δ: 1.53 (9H, s, CH_3), 3.90 (9H, s, OCH_3), 3.00—4.91 (7H, m), 6.62 (2H, s, Ar-H), 9.65 (1H, s, CHO). *Anal.* Calcd for $C_{20}H_{28}N_2O_7$: C, 58.81; H, 6.91; N, 6.86. Found: C, 58.42; H, 7.01; N, 6.75.

Ethyl 4-(3,4,5-Trimethoxybenzoyl)piperazine-2-propionate (13) A mixture of **12** (3.8 g), carbethoxymethylenetriphenylphosphorane (3.9 g) and toluene (30 ml) was stirred for 3 h at 50 °C. The reaction mixture was concentrated *in vacuo*, and the residue was chromatographed on silica gel (AcOEt) to give the corresponding enoate. This enoate was hydrogenated at atmospheric pressure of H_2 in MeOH (40 ml) in the presence of 10% Pd–C (0.4 g) for 3 h at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The residual oil was dissolved in 4 N HCl–AcOEt and the solution was allowed to stand at room temperature for 1 h. The precipitates were collected by filtration and recrystallized from EtOH to give **13** (1.5 g, 39%) as colorless prisms, mp 182—184 °C. IR ν_{max}^{KBr} cm^{-1}: 2928, 1732, 1638, 1620, 1125. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, J = 6 Hz, CH_3), 1.82—2.67 (4H, m), 3.00—3.56 (7H, m), 3.70 (3H, s, OCH_3), 3.83 (6H, s, OCH_3), 4.05 (2H, q, J = 6 Hz, $-CO_2CH_2-$), 6.71 (2H, s, Ar-H). *Anal.* Calcd for $C_{19}H_{28}N_2O_6 \cdot HCl$: C, 58.81; H, 6.91; N, 6.86. Found: C, 58.42; H, 7.01; N, 6.75.

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzyl)piperazine-2-propanol (14) A solution of sodium bis(2-methoxyethoxy)aluminum hydride (70%) in toluene (9 g) was added to a mixture of **13** (1.3 g) and toluene (10 ml) at room temperature for 30 min with stirring. After quenching of the excess reductant with H_2O, insoluble material was removed by filtration. The filtrate was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in CH_2Cl_2 (20 ml), and to this solution, Et_3N (1.5 g) and a mixture of **8** (713 mg) and CH_2Cl_2 (5 ml) were added at 0 °C with stirring. The reaction mixture was washed with H_2O, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography (AcOEt : hexane : MeOH = 5 : 4 : 1) and recrystallized from AcOEt to give **14** (1.05 g, 76%) as colorless prisms, mp 144—145 °C. IR ν_{max}^{KBr} cm^{-1}: 2934, 1620, 1599, 1127. ^{1}H-NMR ($CDCl_3$) δ: 1.40—2.11 (9H, m), 2.45—2.58 (2H, m), 2.70—2.96 (4H, m), 3.23—3.79 (7H, m), 3.84 (6H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (6H, s, OCH_3), 6.37 (1H, s), 6.58 (2H, s, Ar-H), 6.65, 6.66 (1H, s, Ar-H). *Anal.* Calcd for $C_{31}H_{42}N_2O_7$: C, 67.13; H, 7.63; N, 5.05. Found: C, 66.76; H, 7.74; N, 4.98.

1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-propyl-4-(3,4,5-trimethoxybenzyl)piperazine (2s) A mixture of **14** (500 mg) and pyridine (2 ml) was treated portionwise with *p*-TsCl (190 mg) at -20 °C with stirring. After additional stirring for 1 h, the reaction mixture was washed with 10% aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. A mixture of the resulting tosylate, $NaBH_4$ (340 mg) and DMSO (5 ml) was stirred at 80 °C for 1 h. The reaction mixture was poured into H_2O and extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt : hexane = 5 : 4) to give **2s** (220 mg, 42%) as a colorless oil, which was treated with HCl–AcOEt and recrystallized from EtOH to give the hydrochloride. IR ν_{max}^{KBr} cm^{-1}: 2934, 1634, 1595, 1307, 1125. ^{1}H-NMR (DMSO-d_6) δ: 1.76—2.92 (13H, m), 3.00—4.73 (9H, m), 3.62, 3.73, 3.77 (each 3H, s, OCH_3), 3.87 (6H, s, OCH_3), 6.40 (1H, s), 6.74, 6.83 (1H, s, Ar-H), 7.10 (2H, s, Ar-H).

Ethyl 4-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-piperazine-2-carboxylate (15) A solution of **8** (1.1 g) in CH_2Cl_2 (12 ml) was added dropwise to a mixture of **4** (1.0 g), Et_3N (0.6 ml) and CH_2Cl_2 (20 ml) at 0 °C with stirring. After additional stirring for 1 h, the reaction mixture was washed with H_2O and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (hexane : AcOEt : acetone = 2 : 2 : 1) and recrystallized from AcOEt–hexane to give **15** (1.2 g) as colorless prisms, mp 105—106 °C. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1730, 1620, 1500, 1120. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7 Hz, CH_3), 1.8—4.1 (14H, m), 3.86, 3.92 (each 3H, s, OCH_3), 4.22 (2H, q, J = 7 Hz, CH_2), 6.40 (1H, s), 6.67 (2H, s, Ar-H). *Anal.* Calcd for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21. Found: C, 64.89; H, 7.32; N, 7.13.

4-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxymethyl-1-(3,4,5-trimethoxybenzoyl)piperazine (3a) A solution of 3,4,5-trimethoxybenzoyl chloride (2.4 g) in CH_2Cl_2 (10 ml) was added

to a mixture of **15** (3.9 g), Et_3N (1.2 g) and CH_2Cl_2 (30 ml) at 0 °C with stirring. After additional stirring for 1 h, the reaction mixture was washed with H_2O, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual product was dissolved in THF (20 ml), and to this solution $NaBH_4$ (380 mg), LiCl (430 mg) and EtOH (20 ml) were added at room temperature. This mixture was stirred for 12 h, and then concentrated to dryness. The residue was dissolved in CH_2Cl_2 and the solution was washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the corresponding alcohol. This alcohol was converted into **3a** (amorphous powder), in 49% yield from **15**, by a method similar to that described for **2f**. IR ν_{max}^{KBr} cm^{-1}: 2940, 1630, 1590, 1420, 1240, 1130. ^{1}H-NMR ($CDCl_3$) δ: 1.9—4.8 (15H, m), 3.35, 3.83 (each 3H, s, OCH_3), 3.88 (12H, s, OCH_3), 6.38 (1H, s), 6.62, 6.66 (2H, s, Ar-H).

4-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxymethyl-1-(3,4,5-trimethoxybenzyl)piperazine (3b) **3b** was synthesized from **15** in 56% yield by a method similar to that described for **3a** by using 3,4,5-trimethoxybenzyl chloride. The free base was treated with HCl–AcOEt to give the hydrochloride as a white powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 2925, 1640, 1590, 1460, 1420, 1240, 1120. ^{1}H-NMR (DMSO-d_6) δ: 1.80—4.85 (17H, m), 3.70, 3.85 (each 3H, s, OCH_3), 3.87 (9H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.37 (1H, s), 6.54 (2H, s, Ar-H), 6.63, 6.67 (1H, s, Ar-H).

(*R*)-3-Benzyloxy-2-benzyloxycarbonylamino-1-propanol (*R*-17) A mixture of dicyclohexylcarbodiimide (9.4 g) and THF (20 ml) was added dropwise to a solution of *S*-**16** (10.0 g), *N*-hydroxysuccinimide (5.3 g) in THF (100 ml) at 0 °C with stirring. After additional stirring for 1 h at room temperature, the mixture was added to a solution of $NaBH_4$ (12 g) in MeOH (160 ml) and H_2O (40 ml) for 10 min at −10 °C. After additional stirring for 2 h, MeOH and THF were removed by evaporation, and the resulting aqueous solution was extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane : AcOEt = 3 : 2) to give *R*-**17** (7.2 g, 75%) as a colorless oil. IR ν_{max}^{KBr} cm^{-1}: 3416, 1703, 1516, 1238. ^{1}H-NMR ($CDCl_3$) δ: 3.63—3.83 (6H, m), 4.49, 5.08 (2H, s, benzyl), 5.43 (1H, br s, NH), 7.30, 7.33 (5H, s, Ar-H). $[\alpha]_D^{22}$ +16.1° (c = 1.1 $CHCl_3$). *Anal.* Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found C, 68.29; H, 6.90; N, 4.25.

(*R*)-1-Benzyloxycarbonyl-2-benzyloxymethylazirizine (*R*-18) A solution of methanesulfonyl chloride (4.35 g) in CH_2Cl_2 (10 ml) was added dropwise to a mixture of *R*-**17** (10.0 g), Et_2N (4.7 g) and CH_2Cl_2 (30 ml) at −10 °C with stirring. After additional stirring for 1 h, the reaction mixture was washed with 10% aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The mixture of the crude mesylate, K_2CO_3 (10.5 g), 18-crown-6 (5.0 g) and DMF (20 ml) was stirred at 70 °C for 3 h. The reaction mixture was diluted with AcOEt and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (hexane : AcOEt = 7 : 3) to give *R*-**18** (9.2 g, 82%) as a colorless oil. IR ν_{max}^{neat} cm^{-1}: 2860, 1722, 1297, 1200. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (1H, d, J = 3.6 Hz), 2.35 (1H, d, J = 6 Hz), 2.69—2.78 (1H, m, methine), 3.61 (2H, d, J = 4.6 Hz, $-CH_2O$), 4.50, 4.59 (1H, d, J = 12 Hz), 5.11 (2H, s), 7.30, 7.34 (5H, s, Ar-H). $[\alpha]_D^{22}$ +54.7° (c = 0.57, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.56; H, 6.71; N, 4.42.

(*R*)-2-Benzyloxycarbonylamino-1-benzyloxymethyl-4-[*N*-(2,2-diethoxyethyl)-*N*-(3,4,5-trimethoxybenzoyl)]aminopropane (*R*-19) A mixture of *R*-**18** (4.5 g), 2,2-diethoxyethylamine (6.0 g) and DMF (9 ml) was stirred at 70 °C for 3 h. After cooling of the mixture, Et_3N (2.5 g) and a solution of 3,4,5-trimethoxybenzoyl chloride (3.0 g) in DMF (10 ml) were added to it at 0 °C. The reaction mixture was diluted with AcOEt and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (AcOEt : hexane = 1 : 1) to give *R*-**19** (2.1 g, 56%) as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3320, 2970, 1700, 1633, 1584, 1414, 1123. ^{1}H-NMR ($CDCl_3$) δ: 1.61—1.24 (6H, m), 3.43—4.55 (11H, m), 3.83 (3H, s, OCH_3), 3.86 (6H, s, OCH_3), 4.45, 5.10 (each 2H, s), 5.50—5.60 (2H, m), 6.68 (2H, s, Ar-H), 7.31 (10H, s, Ar-H). $[\alpha]_D^{22}$ +16.8° (c = 0.53, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{44}N_2O_9$: C, 65.37; H, 7.10; N, 4.48. Found: C, 65.09; H, 7.16; N, 4.41.

(*R*)-4-(3,4,5-Trimethoxybenzoyl)piperazine-2-methanol (*R*-21) A mixture of *R*-**19** (2.1 g), *p*-TsOH (200 mg) and toluene (50 ml) was stirred at 50 °C for 30 min. After cooling, the mixture was washed with 10% aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give *R*-**20** as a colorless oil. The crude *R*-**20** was dissolved in MeOH (50 ml) containing 10% Pd–C (100 mg), and the resulting solution was stirred under an H_2 stream for 24 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give *R*-**21** (506 mg, 63%) as a colorless oil. The free base was treated with 4 N HCl–AcOEt and recrystallized from EtOH to give the hydrochloride as white crystals, mp 220—223 °C. IR ν_{max}^{KBr} cm^{-1}: 3320, 1631, 1584, 1417. ^{1}H-NMR (DMSO-d_6) δ: 2.72—2.95 (3H, m), 3.05 (2H, d, J = 8 Hz, $-CH_2O-$), 3.79—4.02 (4H, m), 3.88 (3H, s, OCH_3), 3.89 (6H, s, OCH_3), 4.46 (1H, t, J = 8 Hz), 6.64 (2H, s, Ar-H). $[\alpha]_D^{22}$ +33.0° (c = 1.3 MeOH). *Anal.* Calcd for $C_{15}H_{22}N_2O_5 \cdot HCl$: C, 51.95; H, 6.68; N, 8.08. Found: C, 52.00; H, 6.71; N, 8.18.

(*R*)-1-(2,3-Dimethoxy-6,7-dihydro-5*H*-benzocyclohepten-8-ylcarbonyl)-2-methoxymethyl-4-(3,4,5-trimethoxybenzoyl)piperazine [(*R*)-2f] (*R*)-**2f** was synthesized from *R*-**21** in 67% yield by a method similar to that described for **3a**. The oily product was treated with petroleum ether to give an amorphous powder. *Anal.* Calcd for $C_{30}H_{38}N_2O_8 \cdot 1/2H_2O$: C, 63.93; H, 6.97; N, 4.97. Found: C, 64.15; H, 6.91; N, 5.04. $[\alpha]_D^{22}$ +22.5° (c = 1.0 $CHCl_3$). In the chiral synthesis of (*R*)-(+)-**2f** and (*S*)-(−)-**2f**, the IR and ^{1}H-NMR ($CDCl_3$) spectra of (*R*)-**2f** and (*S*)-**2f** were superimposable on those of racemic **2f**.

Similarly, (*S*)-**2f** was synthesized from *R*-**16** in 15% yield. $[\alpha]_D^{22}$ −22.3° (c = 1.0 $CHCl_3$). *Anal.* Calcd for $C_{30}H_{38}N_2O_8 \cdot 1/2H_2O$: C, 63.93; H, 6.97; N, 4.97. Found: C, 63.98; H, 6.88; N, 4.84. The optical purity of these products were more than 99% on HPLC: column, Chiralcel OD (4.6 × 250 mm, Daicel Chemical Industries, Ltd.); temperature, room temperature; eluant, hexane–EtOH (4 : 1), flow rate, 1.0 ml/min; detector, 254 nm; retention time, 62.2 min for (*R*)-(+)-**2f** and 48.2 min for (*S*)-(−)-**2f**.

Evaluation of Inhibitory Activity Against PAF-Induced Platelet Aggregation and Effect on PAF-Induced Hypotension in Conscious Rat Experiments were done according to the method reproted in the preceding paper.[1)]

Binding of [^{3}H]PAF to Rabbit Platelet Membranes The effect of (*R*)-(+)-, (*S*)-(−)-, and (*R*,*S*)-**2f** on the binding of [^{3}H]PAF to rabbit platelet membranes was studied according to the method reported by Hwang *et al.*[12)] In brief, rabbit platelet membranes, 100 μg of protein, were added to 1 ml (final volume) of 10 nM Tris–HCl buffer (pH 7.0) containing 1 pmol of [^{3}H]PAF, a known concentration of test compound; 10 mM $MgCl_2$, and 0.25% bovine serum albumin (BSA). After incubation of membranes with [^{3}H]PAF and vehicle or the test compound for 2 h, bound and unbound [^{3}H]PAF were separated using a Whatman GF/C filter under a vacuum. Nonspecific binding was defined as the amount of binding of [^{3}H]PAF in the presence of 1 μM unlabeled PAF.

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