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-5H-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 carboxbenzox-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-5H-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-naphthyl)-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-5H-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5-trimethoxybenzoyl)piperazine (I) was one of the most potent and orally active derivatives. Weber and Heuer demonstrated that the methyl group at the 9 position of the thienotiazolo-1,4-diazepin skeleton of WEB-2086 was correlated with the acetoxime moiety at the C3 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 I 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 I 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-5H-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.

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) (Chart 2). Reaction of 4 with 1 eq of 3,4,5-trimethoxybenzoyl chloride in the presence of triethylamine in CH₂Cl₂ at 0 °C gave the monoacetyl piperazine (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-trimethoxybenzoyl chloride in the presence of triethylamine in CH₂CN 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 CH₂Cl₂. Maximum absorption at 1750 cm⁻¹ in the infrared (IR) spectrum of 7, assignable to the carbonyl in 5-membered carbamates, supported the...
presumed structure. Consequently, the structures of 5a and 5b were determined as ethyl 4-(3,4,5-trimethoxy- benzoyl)piperazine-2-carboxylate and ethyl 4-(3,4,5-tri- methoxybenzyl)piperazine-2-carboxylate, respectively. It proved feasible, therefore, to obtain the monoaryl and monoalkyl derivatives 5a and 5b in a regioselective manner; these compounds were then acylated with 2,3-dimethoxy-6,7-dihydro-5H-benzocyclohepten-8-carboxylic chloride (8) to give the key intermediates (9a, 9b). The ethoxy-carbonyl 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) and triethylamine in DMF gave the amides (2c, 2d, respectively). Curtius rearrangement of 10a with diphenylphosphorylazide (DPPA), 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) 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 methylcarboxamoyloxymethyl derivatives (2n, 2o), respectively. Reaction of 11b with tributyl phosphine and dimethylsulfide gave the methythiomethyl derivative (2p), but only in low yield (7%). 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 carbethoxymethylene- triphenylphosphorane, 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 monoaryl derivative (15). Reaction of 15 with 3,4,5-trimethoxybenzyl 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 tri- substituted 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 carbobenzyoxyl-β-phenyl-L-serine (S-16) was reduced as reported by Yajima et al. to give (R)-3-benzyloxy-2-benzyloxyazetidin-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.
The aziridine ring of R-18 was opened by reaction with 2,2-diethoxyethylamine, and the resulting secondary amine was acetylated 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 tetraphyropazin derivative (R-20), 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 νmax: 1750 cm⁻¹) with triphogenase 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 carbobenzoxo-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, Chiralcel 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-
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-5H-benzocyclohepten-8-ylcarbonyl)-2-methoxypropyl 4-(3,4,5-trimethoxysalicyl)benzoylepiderazines [(R) (+)-, (S)(-) and (RS)-2f] Compound No. | Inhibitory activities against PAF-induced platelet aggregation (% inhibition) | Inhibitory activities against [3H]PAF binding (IC50 μM)
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(R)(+)-2f | 3 x 10^{-7} | 1.9
(S)(-)-2f | 3 x 10^{-6} | 0.052
(RS)-2f | 3 x 10^{-5} | 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. Methoxybenzyl (2a, 2b), acetoxyethyl (1m), and methylcarbamoyloxymethyl (2o) derivatives were equipotent with the unsubstituted compounds (1a, 1b), whereas 2-dimethylcarbamoyl (2c, 2d) and methoxy carbamylamino (2e) derivatives were less potent than 1a and 1b. Introduction of the methoxymethyl group at the 3-position of the pipеразине 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 10 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 pipеразине 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.1) 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 pipеразине 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 characterization 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 JEOI JRI-800 spectrophotometer. The proton nuclear magnetic resonance (1H-NMR) spectra were recorded in the indicated solvents on a 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-Trimethoxybenzoxy)pipеразине-2-carboxylate (5a) A solution of 3,4,5-trimethoxybenzoyl chloride (2.0 g) in CH2Cl2 (20 mL) was added dropwise to a mixture of 4 (2.0 g) and Et3N (1.0 g) in CH2Cl2 (40 mL) at 0°C with stirring. After additional stirring for 1 h, the reaction mixture was washed with H2O, dried over anhydrous MgSO4, and concentrated in vacuo. The residual product was subjected to column chromatography on silica gel (hexane:AcOEt = acetone = 2:1:2) and recrystallized from AcOEt to hexane to give 5a (2.5g, 82%) as a white powder, mp 114–115°C. 1H-NMR (CDCl3) δ: 1.29 (3H, t, J = 7 Hz, CH3), 2.05 (1H, m, NH), 2.64–4.00 (7H, m), 3.86 (9H, s, OCH3), 4.22 (2H, q, J = 7 Hz, COCH2–), 6.55 (2H, s, Ar-H). Anal. Calculated for C22H21NO3: C 75.94; H 6.86; N 10.15. Found: C 75.83; H, 6.91; N, 7.79.

Ethyl 4-(3,4,5-Trimethoxybenzoxy)pipеразине-2-carboxylate (5b) A solution of 4 (1.0 g), 3,4,5-trimethoxybenzoyl chloride (0.94 g) and Et3N (2.4 mL) in CH2CN (15 mL) was stirred for 15 min at 70°C. The reaction mixture was concentrated to dryness, and then the residue was dissolved in CH2Cl2, and washed with H2O. The organic layer was dried over anhydrous MgSO4 and concentrated in vacuo. The crude product was subjected to column chromatography on silica gel (hexane:AcOEt = acetone = 2:1:2) and recrystallized from EtO–EtO to give 5b (2HCl) (0.9 g, 61%) as colorless prisms, mp 150–153°C. 1H-NMR (CDCl3) δ: 1.27 (3H, t, J = 7 Hz, CH3), 2.00–3.75 (10H, m), 3.85 (9H, s, OCH3), 3.87 (6H, s, OCH3), 4.17 (2H, q, J = 7 Hz, -COCH2–), 6.53 (2H, s, Ar-H). Anal. Calculated for C23H21NO4: 2HCl · H2O: C 47.56; H 7.04; N, 6.52. Found: C 47.57; H, 7.07; N, 6.31.

Ethyl 4-(3,4,5-Trimethoxybenzoxy)pipеразине-2-carboxylate (6) A solution of 5 (36.0 g) in toluene (100 mL) was added over 1 h to a mixture of sodium bis(2-methoxyethoxy)aluminum hydride (50g) 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. 1H-NMR (CDCl3) δ: 2.05–2.17, 2.67–2.72 (2H, m), 2.84–3.11 (5H, m), 3.42 (2H, o), 3.56 (1H, dd, J = 10 Hz, 7.4 Hz), 3.59 (1H, dd, J = 10 Hz, 4.4 Hz), 3.84 (3H, s, OCH3), 3.86 (6H, s, OCH3), 6.56 (2H, s, Ar-H). Anal. Calculated for C21H21NO4: C 76.79; H, 7.86; N 9.43. Found: C 76.77; H, 7.86; N, 9.18.

4-(3,4,5-Trimethoxybenzoyl)pipеразине-9-oxo-1,4-diazaoxa-8-oxacyclo(4.3.0)-nonane (7) A mixture of triphenogly (755 mg) and CH2Cl2 (5 mL) was added over 5 min to a solution of 6 (1.5 g) and Et3N (1.0 g) in CH2Cl2 (20 mL) at 0°C. After additional stirring for 1 h, the reaction mixture was washed with 10% aqueous NaHCO3, dried over anhydrous MgSO4 and concentrated in vacuo. The residual product was subjected to column chromatography on silica gel (AcOEt:hexane = acetone = 1:4:1) and recrystallized from AcOEt to give 7 (1.3 g, 80%) as colorless needles, mp 107°C. 1H-NMR (CDCl3) 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, OCH3), 3.86 (6H, s, OCH3), 6.54 (2H, s, Ar-H). Anal. Calculated for C24H20NO4: C 59.62; H, 6.88; N, 8.66. Found: C 59.62; H, 7.02; N, 8.69.

Ethyl 1-(2,3-Dimethoxy-6,7-dihydro-5H-benzocyclohepten-8-ylcarbonyl)-4-(3,4,5- trimethoxybenzoyl)piperazine-2-carboxylate (9a) A mixture of 8 (550 mg) and CH2Cl2 (5 mL) was added dropwise to a solution of 5a (700 mg) and Et3N (300 mg) in CH2Cl2 (10 mL) at 0°C with stirring. After additional stirring for 1 h at room temperature, the reaction mixture was washed with 10% aqueous NaHCO3. The organic layer was dried over anhydrous MgSO4 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 (KBr) ν cm⁻¹: 3421, 2960, 1630, 1590, 1240, 1130. 1H-NMR (CDCl₃): δ: 1.25 (3H, t, J = 7 Hz, CH₃), 1.85—5.30 (13H, m), 3.84, 3.86 (each 3H, s, OCH₃), 3.90 (9H, s, OCH), 4.22 (2H, q, J = 7 Hz, -CO₂CH₂-), 6.48 (1H, br, vinyl), 6.67 (4H, s, Ar-H). Anal. Calc'd for C₁₇H₁₄O₅N₌: C, 63.90; H, 6.57; N, 4.87. Found: C, 63.81; H, 6.63; N, 4.87.

Ethyl 1-(2,3-Dimethoxy-6,7-dihydropyrido[3,4-d]pyrimidin-6-yl)cyclohexene-1-carboxylate (9b) This was prepared from 9b in 80% yield by the method similar to that described for 9a. The free base was converted into the hydrochloride (amorphous powder). IR (KBr) ν cm⁻¹: 2936, 1736, 1650, 1591, 1124. 1H-NMR (DMSO-d₆): δ: 1.25 (3H, t, J = 7 Hz, CH₃), 2.05—2.84 (101, m), 3.33—3.59 (5H, m), 3.84, 3.87 (each 3H, s, OCH₃), 3.86 (6H, s, OCH₃), 3.89 (3H, s, OCH), 4.21 (2H, q, J = 7.2 Hz, -CO₂CH₂-), 6.46 (1H, s, 54, 2H, s, Ar-H). Found: C, 63.82; H, 7.08; N, 4.31.

1-(2,3-Dimethoxy-6,7-dihydropyrido[3,4-d]pyrimidin-6-yl)cyclohexene-1-carboxylate (10b) The mixture of 2c and 9b was subjected to column chromatography on silica gel (hexane:AcOEt:acetone = 1:1:1) and recrystallized from AcOEt-hexane to give 10b (101 mg, 99% yield) as colorless prisms. IR (KBr) ν cm⁻¹: 2950, 1760, 1590, 1590, 1120. 1H-NMR (CDCl₃): δ: 1.85—2.54, 3.22—3.65, 3.17—3.79 (17H, m), 3.80, 3.86 (7H, s, OCH₃), 3.89 (7H, s, OCH), 4.21 (2H, q, J = 7.2 Hz, -CO₂CH₂-), 6.46 (1H, s, 54, 2H, s, Ar-H). Anal. Calc'd for C₁₇H₁₄O₅N₃: C, 63.76; H, 6.89; N, 4.56. Found: C, 63.82; H, 7.08; N, 4.31.

1-(2,3-Dimethoxy-6,7-dihydropyrido[3,4-d]pyrimidin-6-yl)cyclohexene-1-carboxylate (2b) 2b was prepared from 2b 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 (KBr) ν cm⁻¹: 2940, 1754, 1640, 1590, 1240, 1130. 1H-NMR (DMSO-d₆): δ: 1.85—2.74 (12H, m), 3.76 (3H, s, OCH₃), 3.88 (12H, s, OCH₃), 3.90—5.30 (20H, m), 3.93 (9H, s, -CO₂CH₂-), 6.43 (1H, s), 6.52, 6.63 (2H, s, Ar-H). 1-(2,3-Dimethoxy-6,7-dihydropyrido[3,4-d]pyrimidin-6-yl)cyclohexene-1-carboxylate (2c) 2c was prepared from 2b in 88% yield by a method similar to that described for 2c. The residue was subjected to column chromatography on silica gel (hexane:AcOEt:acetone = 1:1:1) and recrystallized from AcOEt-hexane to give 2c (100 mg, 78%) as colorless crystals. IR (KBr) ν cm⁻¹: 2950, 1750, 1630, 1590, 1240, 1130. 1H-NMR (CHCl₃): δ: 1.81—2.03 (2H, m), 2.29—4.86 (11H, m), 3.70 (3H, s, OCH₃), 3.87 (12H, s, OCH₃), 4.01 (3H, s, -CO₂CH₂-), 6.43 (1H, s), 6.52, 6.63 (3H, s, Ar-H).
1-2, (3-Dimethoxy-7,6-dihydro-5H-benza[c]cycloheptene-8-ylcarbonyl)-2-((N,N-dimethylamino)methyl)-(4,3,4,5-trimethoxybenzyl)jirimipinol (2a) A mixture of 11b (500 mg), dimethyl sulfoxide (940 mg), tributylphosphate (2.0 g) and DMF (10 ml) was stirred at room temperature for 12 h. The reaction mixture was diluted with AcOEt and washed with 10% aqueous NaHCO₃. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (AcOEt: hexane = 5:1) to give 2a (200 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 νmax cm⁻¹: 2934, 2912, 1596, 1252, 1124. 1H-NMR (DMSO-d₆): δ 1.75–2.18 (2H, m), 2.33–2.65, 2.69–2.95 (2H, m), 2.80 (6H, s, NCH₃), 3.00–4.43 (11H, m), 3.35 (6H, s, OCH₃), 3.89 (6H, s, OCH₃), 3.84–4.41 (1H, m), 6.73 (2H, s) at 7.80 (2H, s, NAr-H). 7.11 (2H, s, Ar-H).

1-tert-Butoxyacarbonyl-4-((3,4,5-trimethoxybenzyl)jirimipinol (2b) A mixture of 11b in 40% yield by the method similar to that described for 2a. Recrystallization from EtOH gave colorless prisms. IR νmax cm⁻¹: 3434, 2934, 2916, 1595, 1252. 1H-NMR (DMSO-d₆): δ 1.16–1.46 (6H, CH₃), 1.83–2.14, 2.33–2.65, and 2.71–2.94 (2H, m), 2.89–3.46 (8H, m), 3.67, 3.70, 3.75 (each 3H, s, OCH₃), 3.83 (6H, s, OCH₃), 3.84–4.16 (1H, m), 6.73 (2H, s) at 6.80 (1H, s, CH(OH)₂), 7.00 (1H, s, Ar-H), 7.11 (2H, s, Ar-H).

Ethyl 4-(3,4,5-trimethoxybenzyloxy)methyl-3,4,5-trimethoxybenzyljirimipinol-2-carboxylate (2c) A solution of 8 (1.1 g) in CH₂Cl₂ (12 ml) was added dropwise to a mixture of 4 (1.0 g), Et₃N (0.6 ml) and CH₂Cl₂ (20 ml) at 0 °C with stirring. After addition stirring for 1 h, the reaction mixture was washed with 10% aqueous NaHCO₃, dried over anhydrous MgSO₄ 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 (1:2) as colorless prisms, mp 105–106 °C. IR νmax cm⁻¹: 3450, 2920, 1730, 1620, 1510, 1450. 1H-NMR (CDCl₃): δ 1.32 (3H, s, J = 7 Hz, CH₃), 1.83–1.91 (4H, m), 3.62, 3.73, 3.77 (each 3H, s, OCH₃), 4.22 (2H, q, J = 7 Hz, CH₂), 6.40 (1H, s) at 6.67 (2H, s, Ar-H). Anal. Calcd. For C₁₅H₁₂N₂O₇C₁₅: 64.93; H, 7.27; N, 7.21. Found: C, 64.89; H, 7.32; N, 7.13.

Ethyl 4-(3,4,5-trimethoxybenzyloxy)methyl-3,4,5-trimethoxybenzyljirimipinol-2-carboxylate (2c) A solution of 3,4,5-trimethoxybenzyl chloride (2.4 g) in CH₂Cl₂ (10 ml) was added dropwise to a mixture of 4 (1.0 g), Et₃N (0.6 ml) and CH₂Cl₂ (20 ml) at 0 °C with stirring. After addition stirring for 3 h, the reaction mixture was washed with H₂O 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 (1:2) as colorless prisms, mp 150–152 °C. IR νmax cm⁻¹: 3450, 2920, 1730, 1620, 1510, 1450. 1H-NMR (CDCl₃): δ 1.32 (3H, s, J = 7 Hz, CH₃), 1.83–1.91 (4H, m), 3.62, 3.73, 3.77 (each 3H, s, OCH₃), 4.22 (2H, q, J = 7 Hz, CH₂), 6.40 (1H, s) at 6.67 (2H, s, Ar-H). Anal. Calcd. For C₁₅H₁₂N₂O₇C₁₅: 64.93; H, 7.27; N, 7.21. Found: C, 64.89; H, 7.32; N, 7.13.
to a mixture of 15 (3.9 g), Et_N (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 (20 ml) 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 redistilled as a yellowish oil (amorphous powder), in 49% yield from 1, using a method similar to that described for 2f. IR v/cm^-1: 3290, 1630, 1590, 1420, 1410. 31H-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).

(R)-3-4-Dimethoxy-6,7-dihydro-5H-benzo[bicycloheptene-8-ylcarbonyl]-2-methoxymethyl-1-(3,4,5-trimethoxybenzoyl)piperazone (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 oil. IR v/cm^-1: 3400, 2925, 1640, 1590, 1460, 1240, 1420, 1120. 31H-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-benzoyloxycarbonyl-1-propanol (R-17) A mixture of dicyclohexylcarbodiimide (9.4 g) and THF (20 ml) was added dropwise to a solution of 36 (10.0 g), N-hydroxysuccinimide (5.3 g) in THF (20 ml), followed by stirring at room temperature. After additional stirring for 1 h, the reaction mixture was washed with H_2O (10 ml) and concentration in vacuo gave the crude product, which was recrystallized from MeOH (10 ml) at 0°C. After drying, the resulting aqueous solution was treated 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%, 5g) as a colorless oil. IR v/cm^-1: 3416, 1703, 1516, 1238. 31H-NMR (CDCl_3) δ: 3.63-3.83 (6H, m), 4.49, 5.08 (2H, s, benzyl), 5.43 (1H, brs, NH), 7.30, 7.33 (5H, s, Ar-H). [x]^22 + 22.5 (c = 1.0 CHCl_3). In the chiral synthesis of (R)-2f and (S)-2f, the IR and 31H-NMR (CDCl_3) spectra of (R)-2f and (S)-2f were superposable on those of racemic 2f.

Similarly, (S)-2f was synthesized from R-16 in 15% yield. [x]^22 + 22.3 (c = 1.0 CHCl_3). Anal. Caled for C_22H_21NO_4 (2HCl): C, 56.93; H, 6.97; N, 4.97. Found: C, 56.15; H, 6.91, N, 4.94. The optical purity of these products were more than 99% on HPLC column, Chiralcel OD (4.6 x 250 mm, Daidel Chemical Industries, Ltd.). temperature, room temperature, eluent: 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 reported in the preceding paper.

Binding of [3H]PAF to Rabbit Platelet Membranes The effect of (R)-, (S)-, and (R,S)-2f on the binding of [3H]PAF to rabbit platelet membranes by Hwang et al.22 In brief, rabbit platelet membranes, 100 μg of protein, were added to 1 ml (final volume) of 10 mM Tris-HCl buffer (pH 7.0) containing 1 pmol of [3H]PAF, a known concentration of test compound; 10 mM MgCl_2, and 0.25% bovine serum albumin (BSA). After incubation of membranes with [3H]PAF and vehicle or the test compound for 2 h, bound and unbound [3H]PAF were separated using a Whatman GF/C filter under a vacuum. Nonspecific binding was defined as the amount of binding of [3H]PAF in the presence of 1 μM unlabeled PAF.

References