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Antiplatelet Activities of Newly Synthesized Derivatives of Piperlongumine

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Piperlongumine, a pyridone alkaloid isolated from *Piper longum* L., exhibited a potential inhibitory effect on washed rabbit platelet aggregation induced by collagen, arachidonic acid (AA) and platelet activating factor (PAF), without any inhibitory effect on that induced by thrombin. Piperlongumine was used as a lead compound for the synthesis of new antiplatelet agents. Seven synthetic compounds were newly synthesized from 3,4,5-trimethoxycinnamic acid (TMCA). They were 1-piperidin-1-yl-3-(3,4,5-trimethoxy-phenyl)prop-2-en-1-one (1′), 1-morpholin-4-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (2′), 1-(3,5-dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3′), 1-(2-methylpiperidin-1-yl)-3-(3,4,5-tri-methoxyphenyl)prop-2-en-1-one (4′), 1-(3-hydroxypiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)- prop-2-en-1-one (5′), 1-[3-(3,4,5-tri-methoxyphenyl) acryloyl]-piperidin-2-one (6′) and ethyl 1-[3-(3,4,5-trimethoxyphenyl)-acryloyl]piperidine-4-carboxylate (7′). Among those seven synthetic derivatives, 1-(3,5-dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3′) had the most inhibitory effect on platelet aggregation induced by collagen, AA and PAF.

Keywords: piperlongumine; platelet aggregation; collagen; synthesis.

INTRODUCTION

Cerebral or cardiovascular diseases are mainly caused by the increase of blood cholesterol level, change of lipid composition and excessive mental stress, which intimately correspond to blood composition. Normally, the blood is not aggregated in the blood vessels, but on an occasion of bleeding, blood aggregation is generated as a physiological defense reaction. Platelet aggregation is caused by physiological substances such as thrombin and prostaglandin endoperoxide and can lead an arterial thrombosis (Fuster *et al.*, 1992). Therefore, inhibitors of platelet aggregation can provide protection against the acute coronary syndromes.

The long pepper, *Piper longum* L., has been widely used in the tropical and subtropical regions of the world. It has multiple applications in different folk medicines such as Indian Ayurveda (Kirtikar and Basu, 1993) and exhibits numerous biological activities (Rege *et al.*, 1999; Tripathi *et al.*, 1999; Stöhr *et al.*, 2001). As part of our studies on finding pharmacologically active principles in oriental medicinal plants, the dried fruits of *P. longum* were chosen to determine its biological activities as well as the activities of its individual constituents. Four alkaloids were isolated from the fruits, piperine, piperlongumine, pipernonaline and piperoctadecalidine. Pipernonaline displayed both potent mosquito larvicidal activity and antifungal activities against phytopathogenic fungi (Lee, 2000; Lee *et al.*, 2001). Piperoctadecalidine also exhibited broad-spectrum insecticidal activities against five agricultural insect pests (Park *et al.*, 2002). Piperlongumine showed potent antiaflatoxigenic activity against *Aspergillus flavus* WRRC 3-90-42-12 (Lee *et al.*, 2002). Recently, a consecutive study on the development of antiplatelet medicine from *P. longum* has showed that piperlongumine has potential pharmacological activity on platelet aggregation (Park *et al.*, 2007). This study determined the inhibitory effect of piperlongumine (Fig. 1) on washed rabbit platelet aggregation *in vitro* in comparison with a positive control, aspirin. The antiplatelet activities of seven newly synthesized derivatives of piperlongumine were investigated.

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MATERIALS AND METHODS

Chemicals. Piperlongumine was isolated from *P. longum* dried fruits as reported previously (*6*,*7*). 3,4-Dimethylpiperidine, ethyl isonipecotate, 3-hydroxypiperidine hydrochloride, 2-methylpiperidine, morpholine, piperidine, 3,4,5-trimethoxycinnamic acid and δ-valerolactam were obtained from Aldrich Chemical Co. (St Louis, MO, USA). Collagen, AA and thrombin were from Chrono-Log Co. (Havertown, PA, USA). PAF was from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals were of analytical grade.

Animals. Male white rabbits were purchased from Samtako Bio Korea Inc. (Osan, Korea) and acclimated for 1 week at a temperature of 24 ± 1 °C and a humidity of 55 ± 5%. The animals had free access to a commercial

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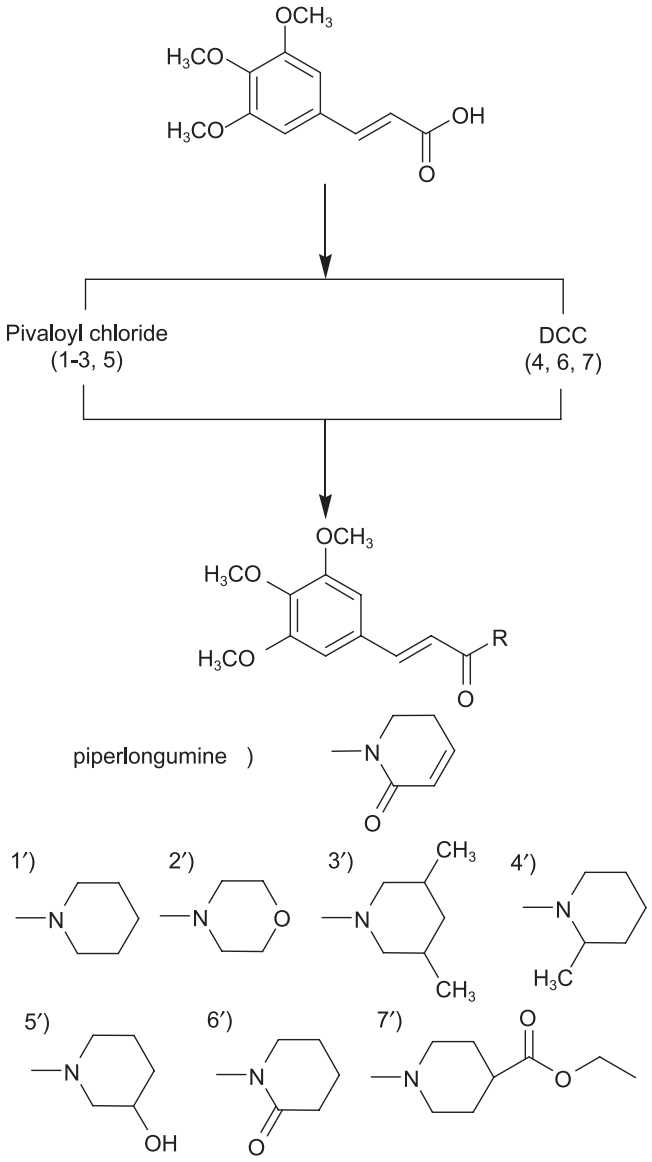

Figure 1. Synthetic scheme of piperlongumine derivatives.

pellet diet (Samyang Co., Wonju, Korea) and drinking water before experiments. All experimental animals were treated according to U.S. Environmental Protection Agency guidelines.

***In vitro* antiplatelet aggregation activity.** Washed platelets were prepared and platelet aggregation was measured as described previously (Lee *et al.*, 2006; Born and Cross, 1963). Platelet rich plasma (PRP) was obtained from male white rabbit blood anticoagulated with a one-tenth volume of 1% EDTA by centrifugation at 230 × **g** for 10 min. Platelets were sedimented by centrifugation of the PRP at 800 × **g** for 15 min, then washed twice with HEPES buffer (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose and 3.8 mM HEPES, pH 6.5) containing 0.35% bovine serum albumin and 0.4 mM EGTA. The washed platelets were resuspended in HEPES buffer (pH 7.4). The platelets were counted by a Coulter counter (Coulter Electronics, Hialeah, FL, USA) and adjusted to a concentration of 3×10^8 platelets/mL. Platelet aggregation was measured using an aggregometer (470-vs, Chrono-log Co., Havertown, PA, USA). Washed rabbit platelets (3×10^8 platelets/mL) were incubated at 37 °C for 3 min in the aggregometer with various concentrations of samples for 3 min in the presence of 1 mM $CaCl_2$, then platelet aggregation was induced by the addition of collagen (2 μg/mL), AA (100 μM), PAF (10 nM) and thrombin (100 μM). The resulting aggregation, measured as the change in light transmission, was recorded for 10 min. Each inhibition rate was obtained from the maximal aggregation induced by the respective agonist at the concentration using the equation: inhibition rate = (maximal aggregation rate (MAR) of vehicle-treated PRP – MAR of sample-treated PRP/MAR of vehicle-treated PRP) × 100. Aspirin (acetylsalicylic acid) was used as a positive control (Kim *et al.*, 1999). Student's *t*-test was used to test the significance of differences between the tested compounds and control.

Instrumental analysis. Purity and structural determination of the synthetic derivatives was made by spectroscopic analyses. The UV/VIS spectrophotometer (model V-550) was purchased from Jasco (Easton, MD, USA). ^{1}H NMR spectra were recorded with a JNM-LA 400 F7 spectrometer (JEOL, Tokyo, Japan) and chemical shifts were given in δ (ppm) with a reference of tetramethylsilane. The HPLC system (model LC-10AT) from Shimadzu (Tokyo, Japan) consisted of a binary pump (model G1312A) and an autosampler (model G1313A). The analytical HPLC column (Supercosil LC-C18, average particle size – 5 μm, 150 × 1.5 mm i.d.) was purchased from Waters (Milford, MA). The mobile phase was a mixture of methanol/water (30:70 by volume) with a flow rate of 1.0 mL/min at room temperature. The mobile phase was filtered through a 0.45 μm Whatman nylon membrane filter (Maidstone, UK) and degassed under vacuum before use. The GC-MS system consisted of a Shimadzu GC-2010 coupled to a Shimadzu QP-2010 mass spectrometer (capillary direct interface). A 60 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column was used. Helium carrier gas was used at a column head pressure of 26.0 kPa. The oven temperature was programmed from 35 °C (4 min isothermal) to 220 °C at 2 °C/min and then to 300 °C (held for 5 min at the final temperature) at 30 °C/min.

Synthesis

3,4,5-Trimethoxyphenylpropenone derivatives 1′, 2′ and 3′, General Procedure. Triethylamine (5.0 mmol, 0.7 mL) was added to a stirred solution of 3,4,5-trimethoxycinnamic acid (4.2 mmol, 1.0 g) in dichloromethane (5 mL). The mixture was stirred for 10 min at 0 °C. Pivaloyl chloride (4.3 mmol, 0.53 mL) was slowly added to this mixture which was stirred for 2 h at –10 to –5 °C. The reaction mixture was cooled and the respective derivatives were formed by adding piperidine (6.8 mmol, 0.67 mL) (**1′**), morpholine (7.6 mmol, 0.67 mL) (**2′**) or 3,5-dimethylpiperidine (5.0 mmol, 0.67 mL) (**3′**). The resulting mixtures were stirred in an ice cooled bath for 2 h. Dilute HCl and 20 mL of water were added and the organic phase was separated and dried (magnesium sulfate). The solvent was evaporated to yield a residue, which was purified by column chromatography.

1-Piperidin-1-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (1′). Derivative **1′** was obtained as a pale yellow solid (1.7 g), yield 89.7%: R_f = 0.56 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 236; mass spectrum (*m/z*) 305,

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306 (M+1), 221 (base peak); HPLC (%) = 99.2; ^{1}H-NMR ($CDCl_3$) 7.7 (*d*, 1H, H-7′), 7.4 (*d*, 1H, H-8′), 6.8 (*d*, 2H, H-2′, H-6′), 3.9 (*s*, 9H, 3 –OC<u>H</u>$_3$), 3.1 (*m*, 4H, -N-C<u>H</u>$_2$), 1.7-1.3 (*m*, 6H).

1-Morpholin-4-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (2′). Derivative **2′** was obtained as a pale yellow solid (1.16 g), yield 90.0%: R_f = 0.47 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 228; Mass spectrum (*m/z*) 307, 221 (base peak); HPLC(%) = 97.7; ^{1}H-NMR($CDCl_3$) 7.6 (*d*, 1H, H-7′), 7.3 (*d*, 1H, H-8′), 6.7 (*d*, 2H, H-2′, H-6′), 3.8 (*s*, 9H, 3 –OC<u>H</u>$_3$), 3.6 (*t*, 4H, O-C<u>H</u>$_2$), 3.2 (*t*, 4H, -N-C<u>H</u>$_2$).

1-(3,5-Dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3′). Derivative **3′** was obtained as a pale yellow solid (1.05 g), yield 75.0%: R_f = 0.74 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 234; Mass spectrum (*m/z*) 333, 221(base peak); HPLC(%) = 97.6; ^{1}H-NMR($CDCl_3$) 7.6 (*d*, 1H, H-7′), 7.3 (*d*, 1H, H-8′), 6.7 (*d*, 2H, H-2′, H-6′), 3.8 (*s*, 9H, 3 –OC<u>H</u>$_3$), 3.1 (*t*, 4H, -N-C<u>H</u>$_2$), 1.7-1.4 (*m*, 4H), 0.9 (*d*, 6H, -CH-C<u>H</u>$_3$).

1-(2-Methylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (4′). 2-Methylpiperidine (5.1 mmol, 0.60 mL) and dicyclohexylcarboiimide (4.6 mmol, 0.95 g) were added to a stirred solution of 3,4,5-trimethoxycinnamic acid (4.2 mmol, 1.0 g) in dichloromethane (20 mL) maintained at –15 to –10 °C. The mixture was stirred for 10 min at this temperature and then stirred at room temperature for 7 h. The mixture was adjusted to pH 8 with 5% $NaHCO_3$, and the organic phase was separated and dried (magnesium sulfate). The solvent was evaporated to yield a residue, which was purified by column chromatography. Derivative **4′** was obtained as a pale yellow solid (1.03 g), yield 84.0%: R_f = 0.50 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 224; mass spectrum (*m/z*) 321, 221 (base peak); HPLC(%) = 94.1; ^{1}H-NMR($CDCl_3$) 7.8 (*d*, 1H, H-7′), 7.3 (*d*, 1H, H-8′), 6.7 (*d*, 2H, H-2′, H-6′), 3.8 (*m*, 9H, 3 –OC<u>H</u>$_3$), 3.2 (*m*, 1H, N-C<u>H</u>-CH$_3$). 3.1 (*m*, 2H, -N-C<u>H</u>$_2$), 1.6 (*m*, 6H), 1.3 (*d*, 3H, N-CH-C<u>H</u>$_3$).

1-(3-Hydroxypiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (5′). Triethylamine (4.6 mmol, 0.64 mL) was added to a stirred solution of 3,4,5-trimethoxycinnamic acid (4.2 mmol, 1.0 g) in dichloromethane (15 mL) and stirring was maintained for 10 min at 0 °C. Pivaloyl chloride (4.3 mmol, 0.53 mL) was slowly added to the mixture which was stirred for 2 h at –10 to –5 °C. The reaction mixture was cooled to below –20 °C. The mixture was added to 3-hydroxypiperdine hydrochloride solution [prepared by adding triethylamine (5.1 mmol, 0.71 mL) to a stirred solution of 3-hydroxypiperidine hydrochloride (3.3 mmol, 0.46 g) in isopropyl alcohol (5 mL) and then stirring for 30 min at room temperature]. The resulting mixture was stirred at room temperature for 48 h and then adjusted to pH 8 with 5% $NaHCO_3$. The organic phase was separated and dried (magnesium sulfate). The solvent was evaporated to yield a residue, which was purified by column chromatography. Derivative **5′** was obtained as a pale yellow solid (0.46 g), yield 34.0%: R_f = 0.23 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 236; mass spectrum (*m/z*) 319, 320(M+1), 221 (base peak); HPLC (%) = 94.1; ^{1}H-NMR($CDCl_3$) 7.7 (*d*, 1H, H-7′), 7.4 (*d*, 1H, H-8′), 6.6 (*d*, 2H, H-2′, H-6′), 3.9 (*m*, 9H, 3 –OCH_3), 3.4 (*m*, 1H, HO-C<u>H</u>-CH$_2$), 3.2 (*m*, 2H, N-C<u>H</u>$_2$-CH-OH), 3.1 (*t*, 2H, -N-C<u>H</u>$_2$), 1.7-1.5 (*m*, 4H).

1-[3-(3,4,5-Trimethoxyphenyl)acryloyl]piperidin-2-one (6′). *δ*-Valerolactam solution [prepared by adding triethylamine (5.1 mmol, 0.71 mL) to a stirred solution of *δ*-valerolactam (4.6 mmol, 0.46 g) in dichloromethane (2 mL) and maintaining stirring for 10 min at –10 to –5 °C] and dicyclohexylcarboiimide (4.3 mmol, 0.88 g) were added to a stirred solution of 3,4,5-trimethoxycinnamic acid (4.2 mmol, 1.0 g) in dichloromethane (20 mL) controlled at –10 to –5 °C and stirred for 10 min. The mixture was then stirred at room temperature for 3 h. Twenty-five mL of water was added and the organic phase was separated and dried (magnesium sulfate). The solvent was evaporated to yield a residue, which was purified by column chromatography. Derivative **6′** was obtained as a pale yellow solid (1.02 g), yield 76.0%: R_f = 0.39 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 232; mass spectrum (*m/z*) 319, 320(M+1), 221(base peak); HPLC(%) = 94.1; ^{1}H-NMR($CDCl_3$) 7.6 (*d*, 1H, H-7′), 7.2 (*d*, 1H, H-8′), 6.6 (*d*, 2H, H-2′, H-6′), 3.8 (*m*, 9H, 3 –OC<u>H</u>$_3$), 3.1 (*t*, 2H, -N-C<u>H</u>$_2$), 2.1 (*t*, 2H, O=C-C<u>H</u>$_2$), 1.5-1.3 (*m*, 4H).

Ethyl 1-[3-(3,4,5-trimethoxyphenyl)acryloyl]piperidine-4-carboxylate (7′). Ethyl isonipecotate (4.6 mmol, 0.71 mL) and dicyclohexylcarboiimide (4.6 mmol, 0.95 g) were added to a stirred solution of 3,4,5-trimethoxycinnamic acid (4.2 mmol, 1.0 g) in dichloromethane (20 mL) maintained between –10 and –5 °C. The mixture was stirred for 10 min at this temperature and then stirred at room temperature for 48 h. Water (25 mL) was added and the organic phase was separated and dried (magnesium sulfate). The solvent was evaporated to yield a residue which was subsequently purified by column chromatography. Derivative **7′** was obtained as a pale yellow solid (1.14 g), yield 72.0%; R_f = 0.68 (ethyl acetate/hexane 2:1 v/v); UV λ_{max} (nm) = 238; Mass spectrum (*m/z*) 377, 221 (base peak); HPLC(%) = 99.9; ^{1}H-NMR($CDCl_3$) 7.6 (*d*, 1H, H-7′), 7.3 (*d*, 1H, H-8′), 6.6 (*d*, 2H, H-2′, H-6′), 4.3 (*m*, 2H, O=C-OC<u>H</u>$_2$), 3.9 (*s*, 9H, 3 –OCH_3), 3.1 (*m*, 4H, -N-C<u>H</u>$_2$),? 2.0 (*m*, 1H, O=C-C<u>H</u>-CH$_2$), 1.7-1.3 (*m*, 7H).

RESULTS

Synthesis of piperlongumine derivatives

Figure 1 showed the synthetic scheme of seven synthetic derivatives of piperlongumine. The synthetic procedure was indicated changing carboxylic acid group of 3,4,5-trimethoxycinnamic acid to the following seven esters **(1′–7′)**. Among those derivatives, **1′–3′**, and **5′** were synthesized from formation of mixed anhydride from TMCA reaction with pivaloyl chloride in the presence of triethylamine using piperidine (**1′**), morpholine (**2′**), 3,5-dimethylpiperidine (**3′**) and 3-hydroxypiperdine hydrochloride (**5′**), respectively. However, compounds **4′**, **6′** and **7′** were obtained from 3,4,5-trimethoxycinnamic acid reaction with dicyclohexylcarboiimide using 2-methylpiperidine (**4′**), *δ*-valerolactam (**6′**), ethyl isonipecotate (**7′**), respectively. Structural confirmation and purity of these synthetic compounds were determined by HPLC, GC/MS and ^{1}H-NMR. The derivatives were

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Table 1. Inhibitory effects of piperlongumine derivatives on platelet aggregation induced by collagen, AA, PAF and thrombin

Compound[a]	Conc. (μM)	Inhibition (%)[b] Collagen (2 μg/mL)	Arachidonic acid (100 μM)	PAF (10 nM)	Thrombin (100 μM)
PL	300	100	100	100	23.5
	150	100	76.4	100	–
	30	49.8	12.0	29.0	–
1′	300	9.7	100	100	0
	150	0	90.3	56.9	–
	30	–	4.2	0	–
2′	300	0	0	8.6	0
	150	–	–	–	–
	30	–	–	–	–
3′	300	98.6	100	94.8	0
	150	97.2	100	56.9	–
	30	6.9	0	3.4	–
4′	300	54.2	48.6	94.8	31.0
	150	11.1	19.4	56.9	0
	30	0	0	3.4	–
5′	300	0	0	1.7	0
	150	–	–	–	–
	30	–	–	–	–
6′	300	2.8	0	62.2	5.6
	150	–	–	5.2	0
	30	–	–	–	–
7′	300	38.9	47.2	100	0
	150	12.5	9.7	89.7	–
	30	0	0	–	–
AS	300	5.8	100	0.3	0
	150	0.0	75.0	0.3	–
	30	0.0	45.9	0.3	–

[a] PL, piperlongumine; 1′, 1-piperidin-1-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one; 2′, 1-morpholin-4-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one; 3′, 1-(3.5-dimethylpiperidin-1-yl)-3-(3,4,5-tri-methoxyphenyl)prop-2-en-1-one; 4′, 1-(2-methylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one; 5′, 1-(3 hydroxypiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one; 6′, 1-[3-(3,4,5-trimethoxyphenyl)acryloyl]piperidin-2-one; 7′, ethyl 1-[3-(3,4,5-trimethoxy-phenyl)acryloyl]piperidine-4-carboxylate; AS, acetylsalicylic acid.

[b] Inhibition (%) = $[(A) - (B)/(A)] \times 100$, *A*, control aggregation %, *B*, sample aggregation %.

1-piperidin-1-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**1′**), 1-morpholin-4-yl-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (**2′**), 1-(3,5-dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3′**), 1-(2-methylpiperidin-1-yl)-3-(3,4,5-tri-methoxyphenyl)prop-2-en-1-one (**4′**), 1-(3-hydroxypiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one (**5′**), 1-[3-(3,4,5-trimethoxyphenyl)acryloyl]piperidin-2-one (**6′**) and ethyl 1-[3-(3,4,5-tri-trimethoxyphenyl)acyloyl]piperidine-4-carboxylate (**7′**).

Effects of piperlongumine and synthetic derivatives on platelet aggregation *in vitro*

The synthetic compounds were tested for their inhibitory effects on platelet aggregation in comparison with a control of aspirin and a positive control of piperlongumine. Table 1 shows the *in vitro* inhibitory effects (%) of various concentrations (30, 150 and 300 μM) of seven synthetic derivatives of piperlongumine on platelet aggregation induced by collagen (2 μg/mL), AA (100 μM), PAF (10 nM) or thrombin (100 μM) using washed rabbit platelets. Among the seven synthetic compounds tested, 1-(3,5-dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3′**) had the most potent activity, inhibiting platelet aggregation induced by AA, collagen and PAF by 100%, 98.6% and 94.8%, respectively, at a concentration of 300 μg/mL.

DISCUSSION

The dried fruits of *P. longum* have been used for the alleviation of bronchitis, stomachaches, spleen diseases and pain for several thousand years in India. *P. longum* is known to possess antiallergic effects (Chatterjee, 1999; Park *et al.*, 2007), antiinflammatory effects (Jain *et al.*, 1994) and liver protecting effects (Koul and Kapil, 1993). In the previous study, the alkaloid piperlongumine derived from *P. longum* L., inhibited *in vitro* rabbit platelet aggregation induced by collagen, AA and PAF (Park *et al.*, 2007). The alkaloids did not inhibit platelet aggregation induced by thrombin. Piperlongumine exhibited the most potent antiplatelet activity in the four isolated alkaloids from *P. longum* (Park *et al.*, 2007).

As natural products have been considered as a good resource for the drugs lead, there are several natural products are known to be lead compounds of the platelet drug. For example, ajoene, isolated from green onions (*Allium fistulosum*), garlic (*Allium sativum*) and onions (*Allium cepa*), has anticoagulation properties and has long been known for cleaning the blood (Song *et al.*, 1963; Phillips and Poyser, 1978; Beretz and Cazenave, 1991). Thus, ajoene is being developed for a novel antiplatelet agent using its function of preventing the conversion of fibrinogen to fibrin. Curcumin (diferuloylmethane), a main component of the oriental spice turmeric, is known to prevent synthesis of TXA_2 and

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the transmission of Ca^{2+} signals thereby providing a potent inhibitory effect against PAF and arachidonic acid, which are platelet coagulation-inducing factors at an IC_{50} of 20–25 μM (Shah *et al.*, 1999).

Piperlongumine may be useful as a lead compound and new agent in the development of a new medicine for the prevention of thrombosis. For this purpose, several new compounds of piperlongumine were synthesized and studies on the structure-activity relationships of piperlongumine synthetic analogs were also undertaken. Reduction of the double bond in the dihydropyridone ring of piperlongumine to form 1-[3-(3,4,5-trimethoxyphenyl)acryloyl]piperidin-2-one (**6′**) caused a dramatic loss of activity, particularly toward platelet aggregation induced by collagen and AA. Removal of the ring keto group from **6′** to form 1-piperidin-1-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**1′**) enhanced inhibitory activity to platelet aggregation induced by AA and PAF but had little effect on that induced by collagen. Addition of an oxygen in the 4- position of the piperidine ring to form 1-morpholin-4-yl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**2′**) drastically reduced the inhibitory activity to platelet aggregation induced by AA and PAF. Addition of a methyl group to the C-2 position of the piperidine ring to form 1-(2-methylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**4′**) increased the inhibitory activity to platelet aggregation induced by collagen and thrombin, though **4′** displayed reduced activity to platelet aggregation induced by AA (relative to **1′**). Addition of two methyl groups at the C-3 and C-5 positions of the piperidine ring to form 1-(3,5-dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (**3′**) increased the inhibitory activity to platelet aggregation induced by collagen (relative to **1′**). Addition of a hydroxyl group to the C-3 position of the piperidine ring to form 1-(3-hydroxypiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**5′**) produced a derivative with no inhibitory activity to platelet aggregation. Addition of an ethyl carboxylate group to the C-4 position of the piperidine ring to form ethyl 1-[3-(3,4,5-trimethoxyphenyl)acyloyl]piperidine-4-carboxylate (**7′**) increased the inhibitory activity to platelet aggregation induced by collagen and PAF but decreased inhibitory activity to platelet aggregation induced by AA. The dihydropyridone ring appears to be essential for activity since reduction of the double bond in the ring caused a dramatic loss of antiplatelet activity. An oxygen atom associated with the piperidine ring had a negative effect on antiplatelet activity as the derivatives with piperidin-2-one (**6′**), morpholine (**2′**) and 3-hydroxypiperidine (**5′**) all had less activity than the derivative with piperidine (**1′**). Addition of a methyl group to the C-2 position of the piperidine ring had mixed effects, increasing inhibitory activity to platelet aggregation induced by thrombin and collagen but decreasing activity to platelet aggregation induced by AA. Addition of two methyl groups at C-3 and C-5 of the piperidine ring increased inhibitory activity to platelet aggregation induced by collagen.

Addition of the moderately polar ethyl carboxylate group at C-4 of the piperidine ring again had mixed effects, increasing inhibitory activity to platelet aggregation induced by collagen and PAF but decreasing activity to platelet aggregation induced by AA. Therefore, the results indicate that the trimethoxybenzene or the dihydropyridone moiety in piperlongumine may contribute antiplatelet effect *in vitro*.

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