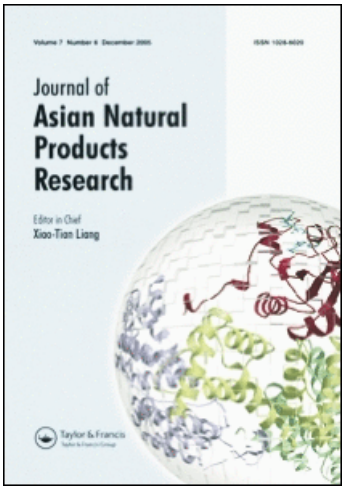

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMIDE DERIVATIVES OF GINKGOLIDE A

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Amide derivatives of ginkgolide A were prepared and evaluated for their *in vitro* ability to inhibit the PAF-induced aggregation of rabbit platelets. They showed less activities than their parent compound ginkgolide A.

Keywords: Amide derivatives of ginkgolide A; Structure and activity relationship

INTRODUCTION

Most alkaloids have strong bioactivities, and they are usually active substances of many medicinal plants. It is therefore of interest to synthesize and study biological activities of ginkgolide analogues containing nitrogen atoms. In our previous studies [1, 2], we found that nor-C-ring-ginkgolide analogues were less active and some etheric ginkgolide analogues of 1-hydroxyl or 10-hydroxyl were more active in anti-PAF potency than their parent compounds, ginkgolide A and B. As part of our continuing efforts to investigate the relationships between structure and anti-PAF activity, we describe in this paper preparation and structure-activity relationships of amide analogues of ginkgolide A, which showed weak inhibitory activities on rabbit platelet aggregation induced by PAF.

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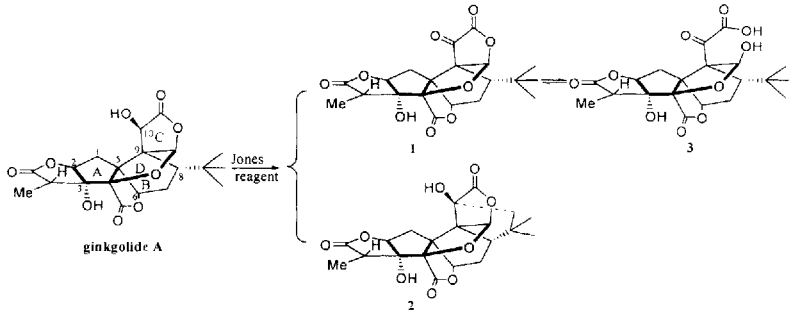
Ginkgolides are unique twenty-carbon cage molecules incorporating a *tert*-butyl group and six five-membered rings A - F: a spiro[4,4]nonane system, three lactonic rings, and a tetrahydrofuran cycle. Ginkgolide A has two secondary hydroxyl groups on C_1 and C_{10}. There are usually two ways for preparation of amine from alcohol, one is a hydroxyl group directly replaced by an amino group, and the other is a secondary hydroxyl group firstly oxidized to a ketone and successively aminated and reduced. We plan to introduce amino group into ginkgolide skeleton by the latter route.

RESULTS AND DISCUSSION

Oxidation of Ginkgolides A

The starting materials for our synthetic approach to amide derivatives of ginkgolide A were isolated from the alcoholic extract of *Ginkgo biloba* leaves. Treatment of ginkgolide A with Jones reagent yielded 10-oxoginkgolide (**1**), which was in equilibrium with its ring-C-open analogue, and a new heptacyclic ginkgolide analogue (**2**) (Scheme 1).

Compound **2** was analyzed for $C_{20}H_{22}O_9$ by its HREIMS (*m/z* $[M]^+$ 406.12698). Comparison with ginkgolide A ($C_{20}H_{24}O_9$), it had 2 H less, but no new ketone carbon signal appeared in the ^{13}C NMR spectrum of **2**. Careful examination of the NMR data, we found that the C_{10} second hydroxyl group in ginkgolide A was replaced by a C_{10} tertiary hydroxyl in compound **2**. The obvious changes were that **2** lost the typical *t*-butyl group of ginkgolides, which was replaced by two geminal methyls and a methylene group. So we deduced that one of the *t*-butyl methyls formed the seventh



SCHEME 1 Oxidation of ginkgolides A.

ring with C_{10}. Its proton and carbon signals were unambiguously assigned on the basis of its ^1H-^1H COSY and HMQC spectra.

The formation of **2** can be explained as follows. The *t*-butyl methyls of 10-oxoginkgolide A formed radicals under sunlight irradiation, and it subsequently attacked the 10-ketone to form the seventh ring. Owing to the α-*t*-butyl configuration, we determined the 10-hydroxyl as β-configuration in order to form the new ring.

Synthesis of Amide Derivatives of Ginkgolide A

Our original plan was to prepare amine derivatives of ginkgolide A from 10-oxoginkgolide A by reductive-amination. However, when benzylamine was added to the THF solution of 10-oxoginkgolide A and refluxed for 2 hours, an unexpected product **4**, an amide, was formed. Compound **4** was obtained as white powder. A molecular formula of $C_{26}H_{31}NO_8$ was deduced by its HREI mass spectrum (*m/z*: 471.20254). Its IR spectrum showed strong esters (1785.8 cm^{-1}) and an amide (1677.8 cm^{-1}) absorption. Comparison of its ^{13}C NMR data with those of **1** showed the presence of the ketone carbon signal (201.7 ppm) indicating that the ketone did not react with amine RNH_2. The major changes in its NMR data (Tab. I) were losses of C-12 and one quaternary carbon (C_5 or C_9), which was replaced by a new tertiary carbon. From the crosspeak between the proton of new tertiary carbon (4.34 ppm) and H-8 (1.80 ppm) in its ^1H-^1H COSY spectrum, we

TABLE I Summary of two-dimensional NMR correlations of **4**

Proton	*^1H NMR*[a]	*COSY*	*HMQC*	*HMBC*
H-1	2.06 *dd* (13.6, 5.9 Hz)	H-1′, H-2	C_1	C_2, C_3, C_4, C_5
H-1′	1.49 *dd* (13.4, 8.6 Hz)	H-1, H-2	C_2	C_2, C_5,
H-2	3.98 *dd* (8.5, 6.1 Hz)	H-1, H-1′	C_3	C_3
H-6	4.99 *d* (5.8 Hz)	H-7	C_4	C_{13}, C_8
H-7	2.33 *m*	H-6, H-7′, H-8	C_5	
H-7′	1.80 *m*	H-7, H-8	C_6	
H-8	1.80 *m*	H-7, H-7′, H-9	C_7	C_7, C_9
H-9	4.34 *d* (6.1 Hz)	H-8	C_7	C_4, C_7, C_8, C_{10}
H-14	2.87 *q* (7.3 Hz)	H-16	C_8	C_2, C_3, C_{15}, C_{16}
H-16	1.15 *d* (7.4 Hz)	H-14	C_9	C_3, C_{14}, C_{15}
t-Bu	0.80 *s*		C_{14}	C_8, C_{17}
NH	9.39 *t* (6.2 Hz)	CH_2	C_{16}	C_{11}
Other	4.4 *dd* (15.1, 6.5 Hz)		$C_{t\text{-Bu}}$	
Signals	4.27 *dd* (15.0, 6.0 Hz)			
	7.31 *t* (7.3 Hz, 2 H)			
	7.23 *t* (7.2 Hz, 2 H)			

[a] Recorded in DMSO-d_6 at 400 MHz.

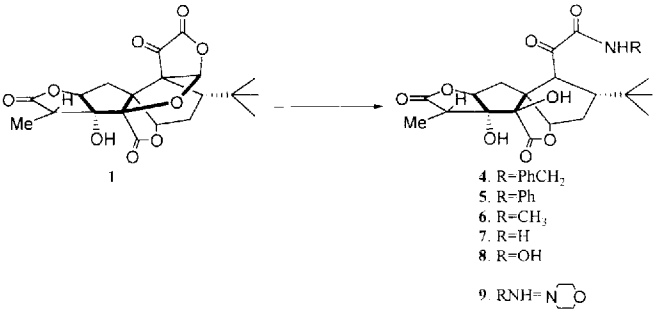
determined that the new tertiary carbon was C_9, which was also confirmed by presence between H-9 and C_{10}, C_4, C_8, C_7 in its HMBC spectrum. The crosspeak between NH (9.39 ppm, *t*) and C_{11} (161.9 ppm) established that the $PhCH_2NH$- group was connected to C_{11}. Its proton and carbon signals were assigned on the basis of ^1H-^1H COSY, HMQC, and HMBC spectra.

The formation of amide derivatives of ginkgolide A could be explained by the plausible mechanism proposed in Scheme 3. Owing to the presence of two vicinal carbonyl groups in the five-membered ring C, it was easy to open ring C to give an intermediate **10** when RNH_2 attacked the less hindered carbonyl function. The aldehyde **10** was subsequently oxidized to acid **11** by air. Under refluxed condition the β-carbonyl acid underwent rapid decarboxylation to yield the amide.

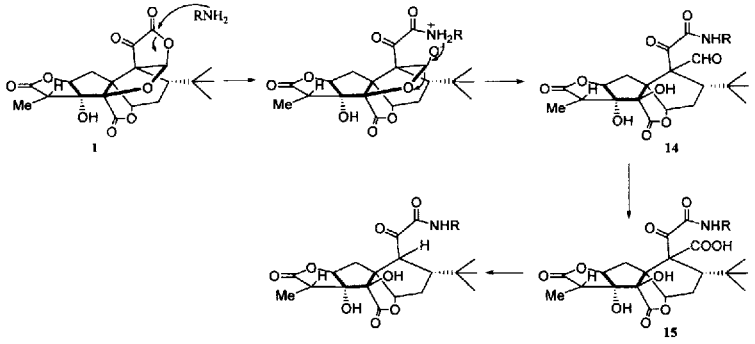
Other amide derivatives of ginkgolide A (**4**–**9**) were prepared from 10-oxoginkgolide A and appropriate amines using the same method.

Biological Evaluation of Oxidation Products and Amide Derivatives of Ginkgolide A

Compounds **1**, **2** and **4**$\sim$**9** were evaluated as PAF antagonist *in vitro* using an assay involving rabbit platelets. Jones oxidation products **1** and **2** gave only slightly less active than ginkgolide A. Its amide derivatives (**4**$\sim$**9**) lacking rings C and D definitely reduced their anti-PAF potency comparable to ginkgolide A (Tab. II). But it is of interest that the N-substituted groups largely affected their anti-PAF activity. Replacement of the phenyl group with morpholino, benzyl, hydroxyl, methyl, hydrogen groups showed that the inhibitory activities were 5$\sim$79 times decrease.



SCHEME 2 Preparation of amide derivatives of ginkgolide A.



SCHEME 3 Plausible mechanism of formation of amide derivatives of ginkgolide A.

TABLE II *In Vitro* biological evaluation of oxidant and amide derivatives of ginkgolide A

Compound	*PAF-induced platelet aggregation IC_{50}[a] (uM)*	*Compounds*	*PAF-induced platelet aggregation IC_{50}[a] (uM)*
ginkgolide A	0.410 (0310 ~ 0.557)	**6**	80.8 (75.6 ~ 85.1)
1	0.547 (0.493 ~ 0.618)	**7**	123 (116 ~ 132)
2	0.536 (0.471 ~ 0.587)	**8**	68.2 (59.8 ~ 73.9)
4	65.9 (63.4 ~ 69.7)	**9**	7.97 (6.23 ~ 8.91)
5	1.56 (1.26 ~ 1.82)		

[a] Concentration required to inhibit PAF-induced maximum aggregation by 50%. Parentheses contain 95% confidence limits.

EXPERIMENTAL SECTION

General Experimental Procedures

IR spectra were measured with a Perkin-Elmer 559B apparatus. ^{1}H-NMR spectra were obtained on Bruker AMX-400 spectrometer, using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were measured on a MAT-711 mass spectrometer. C-18-PAF acether was purchased from Sigma Co. Ltd.

Plant Material

Ginkgolide A, isolated from Chinese medicinal herb *Ginkgo biloba* L., was used as starting material.

10-oxoginkgolide A (1) and Compound 2

2.0 g of ginkgolide A was dissolved in 20 mL of acetone, then 1 mL of Jone reagent was added to the solution with stirring at 0°C. After 10 min the

solution was warmed to room temperature for overnight. 5 mL of isopropanol was added, and the solvent was removed under reduced pressure. The residue was taken up in 5 mL of water and 5 mL of ethyl acetate, and the aqueous layers were evaporated to give a solid, which was chromatographied on silica gel, elution with cyclohexane-actone (3 : 1) gave **1** (1.68 g) as colorless needles in 84.4% yield and **2** (58 mg) as white powder in 2.9% yield. **1**: IR (KBr) ν_{max} 3620, 3442, 3386, 1786, 1711 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.53 (1H, *dd*, $J = 15.5$, 5.9 Hz, H-1), 2.04 (1H, *dd*, $J = 15.3$, 5.2 Hz, H-1′), 4.84 (1H, *dd*, $J = 5.6$, 5.3 Hz, H-2), 5.04 (1H, *d*, $J = 3.0$ Hz, H-6), 2.29 (1H, *m*, H-7), 2.50 (1H, *m*, H-7′), 2.29 (1H, *m*, H-8), 6.62 (1H, *s*, H-12), 3.04 (1H, *q*, $J = 7.4$ Hz, H-14), 1.10 (3H, *d*, $J = 7.5$ Hz, H-16), 0.87 (9H, *s*, *t*-Bu). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 38.2 (C-1), 84.8 (C-2), 87.7 (C-3), 96.3 (C-4), 73.8 (C-5), 87.2 (c-6), 36.9 (C-7), 56.5 (C-8), 71.3 (C-9), 198.5 (C-10), 164.7 (C-11), 104.2 (C-12), 171.8 (C-13), 40.4 (C-14), 178.0 (C-15), 8.0 (C-16), 33.2 (C-17), 29.2 (C-*t*-Bu). HREIMS: *m/z* 406.12634, $C_{20}H_{22}O_9$ requires 406.12639; EIMS *m/z* 406 $[M]^+$, 391, 362, 316, 278, 221, 205, 149, 91, 57. **2**: IR (KBr) ν_{max} 3556, 3403, 1774 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.74 (1H, *dd*, $J = 15.8$, 6.5 = Hz, H-1), 2.11 (1H, *m*, H-1′), 4.85 (1H, *dd*, $J = 5.1$, 6.2 Hz, H-2), 4.98 (1H, *d*, $J = 2.5$ Hz, H-6), 2.11 (1H, *m*, H-7), 2.11 (1H, *m*, H-7′), 2.45 (1H, *m*, H-8), 6.14 (1H, *s*, H-12), 2.81 (1H, *q*, $J = 7.3$ Hz, H-14), 1.12 (3H, *d*, $J = 7.4$ Hz, H-16), 2.11 (1H, *m*, H-18), 1.96 (1H, *d*, $J = 19.6$ Hz, H-18′), 1.16 (3H, *s*, H-19), 1.13 (3H, *s*, H-20). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 36.6 (C-1), 89.1(C-2), 86.3 (C-3), 102.7 (C-4), 79.4 (C-5), 90.0 (C-6), 35.8 (C-7), 55.7 (C-8), 66.8 (C-9), 85.0 (C-10), 174.7 (C-11), 110.1 (C-12), 171.4 (C-13), 40.8 (C-14), 176.9 (C-15), 9.5 (C-16), 32.9 (C-17), 55.4 (C-18), 27.9 (C-19, 20). HREIMS: *m/z* 406.12698, $C_{20}H_{21}O_9$ requires 406.12639; EIMS *m/z* 406 $[M]^+$, 404, 362, 101, 57.

General Procedure of Amidation of 10-oxoginkgolide A (4 ~ 9)

The appropriate amine (1.5 mmole) was added to a solution of 200 mg of 10-oxoginkgolide A in 5 mL of THF. The mixture was refluxed for 2 h, then the solvent was removed under reduced pressure and the residue was purified by flash chromatography.

Benzyl 11-ginkgamide (4)

Compound **4** was prepared from 200 mg of 10-oxoginkgolide A and 160 mg of benzyl amine as described by the preceding general procedure to obtain white solid **4** (175 mg, 73.2%) after flash chromatography (cyclohexane:

acetone 2 : 1). **4**: IR (KBr) ν_{max} 3388, 2690, 1786, 1678 cm^{-1}; ^{1}H NMR data see in Table I. ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 40.0 (C-1), 75.3 (C-2), 88.8 (C-3), 92.2 (C-4), 57.0 (C-5), 89.2 (C-6), 34.2 (C-7), 54.8 (C-8), 47.8 (C-9), 201.7 (C-10), 161.9 (C-11), 171.3 (C-13), 38.5 (C-14), 177.9 (C-15), 10.3 (C-16), 31.8 (C-17), 29.4 (C-*t*-Bu), 42.9 (**C**H$_2$), 138.9, 128.8 × 2, 127.6 × 3 (Ph-). HREIMS: *m/z* 485.20871, $C_{26}H_{31}NO_8$ requires 485.20497; EIMS *m/z* 485 [M]$^+$, 470, 452, 428, 351, 324, 259, 241, 135, 91.

Phenyl 11-ginkgamide (5)

Compound **5** was prepared from 200 mg of 10-oxoginkgolide A and 140 mg of aniline as described by the preceding general procedure to obtain white solid **5** (202 mg, 86.9%) after flash chromatography (cyclohexane : acetone 2 : 1). **5**: IR (KBr) ν_{max} 3440, 1783, 1684 cm^{-1}. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.67 (1H, *dd*, *J* = 13.6, 7.5 Hz, H-1), 1.87 (1H, *m*, H-1′), 4.76 (1H, *t*, *J* = 8.5 Hz, H-2), 6.04 (1H, *m*, H-6), 2.29 (1H, *m*, H-7), 1.98 (1H, *dd*, *J* = 13.7, 4.4 Hz, H-7′), 1.87 (1H, *m*, H-8), 5.01 (1H, *d*, *J* = 4.4, Hz, H-9), 3.72 (1H, *q*, *J* = 7.4 Hz, H-14), 1.04 (3H, *d*, *J* = 7.3 Hz, H-16), 1.01 (9H, *s*, *t*-Bu), 10.5 (1H, brs, NH), 7.70 (2H, *d*, *J* = 6.7 Hz), 7.34 (2H, *t*, *J* = 7.4 Hz), 7.12 (1H, *t*, *J* = 7.3 Hz) (Ph-). HREIMS: *m/z* 471.18529, $C_{25}H_{29}NO_8$ requires 471.18932; EIMS *m/z* 471 [M]$^+$, 456, 414, 362, 351, 334, 278, 205, 93.

Methyl 11-ginkgamide (6)

Compound **6** was prepared from 200 mg of 10-oxoginkgolide A and 120 mg of methylamine solution in water (40 wt.%) as described by the preceding general procedure to obtain white solid **6** (162 mg, 80.4%) after flash chromatography (cyclohexane : acetone 2 : 1). **6**: IR (KBr) ν_{max} 3377, 1786, 1676 cm^{-1}. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.02 (1H, *dd*, *J* = 13.5, 5.8 Hz, H-1), 1.48 (1H, *dd*, *J* = 13.4, 8.7 Hz, H-1′), 3.97 (1H, *m*, H-2), 4.96 (1H, *d*, *J* = 5.7 Hz, H-6), 2.35 (1H, *m*, H-7), 1.79 (1H, *dd*, *J* = 13.7, 4.4 Hz, H-7′), 1.79 (1H, *m*, H-8), 4.53 (1H, *d*, *J* = 5.4, Hz, H-9), 2.87 (1H, *q*, *J* = 7.2 Hz, H-14), 1.15 (3H, *d*, *J* = 7.3 Hz, H-16), 0.81 (9H, *s*, *t*-Bu), 8.72 (1H, brs, N**H**), 2.67 (3H, *d*, *J* = 4.4 Hz, Me). ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 39.2 (C-1), 75.3 (C-2), 88.8 (C-3), 92.2 (C-4), 56.9 (C-5), 89.2 (C-6), 34.2 (C-7), 54.8 (C-8), 47.4 (C-9), 201.6 (C-10), 162.1 (C-11), 171.3 (C-13), 38.5 (C-14), 178.0 (C-15), 10.3 (C-16), 31.9 (C-17), 29.4 (C-*t*-Bu), 26.3 (**C**H$_3$). HREIMS: *m/z* 409.17034, $C_{20}H_{27}NO_8$ requires 409.17367; EIMS *m/z* 409 [M]$^+$, 376, 351, 323, 259, 241, 203.

11-ginkgamide (7)

Compound **7** was prepared from 200 mg of 10-oxoginkgolide A and 90 mg of ammonium hydroxide (28% NH_3 in water) as described by the preceding general procedure to obtain white solid **7** (136 mg, 69.8%) after flash chromatography (cyclohexane : acetone 2 : 1). **7**: IR (KBr) ν_{max} 3445, 1780, 1693 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ 2.04 (1H, *dd*, $J = 13.6$, 5.9 Hz, H-1), 1.48 (1H, *dd*, $J = 13.5$, 8.5 Hz, H-1′), 3.96 (1H, *dd*, $J = 8.6$, 5.9 Hz, H-2), 4.96 (1H, *d*, $J = 5.7$ Hz, H-6), 2.33 (1H, *m*, H-7), 1.78 (1H, *dd*, $J = 13.6$, 4.3 Hz, H-7′), 1.78 (1H, *m*, H-8), 4.34 (1H, *d*, $J = 5.8$, Hz, H-9), 2.89 (1H, *q*, $J = 7.3$ Hz, H-14), 1.15 (3H, *d*, $J = 7.5$ Hz, H-16), 0.81 (9H, *s*, *t*-Bu). HREIMS: *m/z* 395.15620, $C_{19}H_{25}NO_8$ requires 395.15802; EIMS *m/z* 395 $[M]^+$, 380, 351, 287, 259, 203, 149, 95.

Hydroxyl 11-ginkgamide (8)

Compound **8** was prepared from 200 mg of 10-oxoginkgolide A and 105 mg of hydroxylamine hydrochloride as described by the preceding general procedure to obtain white solid **8** (140 mg, 69.3%) after flash chromatography (cyclohexane : acetone 2 : 1). **8**: IR (KBr) ν_{max} 3371, 1782, 1684 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ 2.35 (1H, *dd*, $J = 13.2$, 5.9 Hz, H-1), 1.48 (1H, *dd*, $J = 13.5$, 8.3 = Hz, H-1′), 3.25 (1H, *m*, H-2), 4.94 (1H, *d*, $J = 5.2$ Hz, H-6), 2.06 (1H, *m*, H-7), 1.76 (1H, *m*, H-7′), 1.76 (1H, *m*, H-8), 4.26 (1H, *d*, $J = 5.0$, Hz, H-9), 2.85 (1H, *q*, $J = 7.1$ Hz, H-14), 1.13 (3H, *d*, $J = 7.1$ Hz, H-16), 0.82 (9H, *s*, *t*-Bu). HREIMS: *m/z* 411.15726, $C_{19}H_{25}NO_9$ requires 411.15356; EIMS *m/z* 411 $[M]^+$, 378, 367, 310, 293, 203, 171, 141, 57.

Morpholino-11-ginkgamide (9)

Compound **9** was prepared from 200 mg of 10-oxoginkgolide A and 130 mg of morpholine as described by the preceding general procedure to obtain white solid **9** (196 mg, 85.7%) after flash chromatography (cyclohexane : acetone 2 : 1). **9**: IR (KBr) ν_{max} 3550, 2480, 3440, 1774, 1673 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ 2.01 (1H, *dd*, $J = 13.3$, 5.6 Hz, H-1), 1.47 (1H, *dd*, $J = 13.3$, 8.9 Hz, H-1′), 3.96 (1H, *dd*, $J = 8.9$, 5.6 = Hz, H-2), 4.97 (1H, *d*, $J = 5.7$ Hz, H-6), 2.34 (1H, *m*, H-7), 1.79 (1H, *m*, H-7′), 1.79 (1H, *m*, H-8), 4.34 (1H, *d*, $J = 4.8$, Hz, H-9), 2.87 (1H, *q*, $J = 7.3$ Hz, H-14), 1.14 (3H, *d*, $J = 7.2$ Hz, H-16), 0.80 (9H, *s*, *t*-Bu), 3.35 (4H, *t*, $J = 4.5$ Hz, OCH_2), 2.67

(4H, *t*, $J = 4.5$ Hz, NCH_2) HREIMS: m/z 465.19970, $C_{23}H_{31}NO_9$ requires 465.19988; EIMS m/z 465 $[M]^+$, 432, 351, 305, 115, 59.

Biological Method: Inhibition of Platelet Aggregation in Vitro

The procedures used were exactly as those reported previously [1].

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