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Preparation of Analogues of Territrem B, a Potent AChE Inhibitor

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Abstract—The synthesis of analogues of the potent acetylcholinesterase inhibitor, Territrem B, was carried out starting from the naturally occurring jujubogenin glycosides. Dihydrojujubogenin-2-en-1-one (**1**), a dammarane derivative that possesses a skeleton and pharmacophore partially similar to those of Territrem B, was synthesized via three different paths. The derivative **21**, which contains two potential pharmacophores, was also synthesized. The *anti*-AChE activity of the analogues was measured. The aromatic ring moiety seemed to be less important when compared with the 2-en-1-one pharmacophore.

Introduction

The past two decades have been marked by dramatically increased public and scientific attention to late-life cognitive disorders, especially Alzheimer's Disease (AD). One of the strategies of overcoming this disorder is focused on cholinergic compounds based on the cholinergic hypothesis by Summers and his co-workers.[1] The low level of acetylcholine (ACh) in the brain is considered to be one of main reasons for memory loss. Thereby, methods for elevating the ACh level in the brain and enhance the cognition have become major issues.[2,3] One of the approaches is to investigate acetylcholinesterase (AChE) inhibitors, such as THA, physostigmine, oxotremorine, as well as the newly developed Huperzine A,[4] which can prevent the hydrolysis of ACh in the brain. Territrem B is a newly found potent AChE inhibitor (IC_{50} 47 nM, *H. Zea* AChE)[5] and, most interestingly, its inhibitory mechanism is totally different to the known AChE inhibitors.[6] A previous investigation found that Territrem B block the entrance of acetylcholine into AChE by its hydrophobic interaction with the lipophilic amino acid, which entered the binding site channel of AChE.[6] This makes Territrem B a new lead compound and this prompted us to study its analogues, since the source of Territrem B is heavily restricted from the fermentation liquid of *Aspergillus terrus*[5] and thus restricts further investigation into this type of AChE inhibitor. Furthermore, the preliminary SAR investigation of Terretrim B derivatives suggested that the 2-en-1-one moiety was essential for its inhibitory activity on AChE.[7] So we utilized a naturally occuring compound which has a similar skeleton and stereochemistry at the A and B rings to Territrem B as starting material and tried to build up the 2-en-1-one pharmacophore and assayed their *anti*-AChE activity. Based on this design, the method of preparation of a jujubogenin-2-en-1-one analog **1** from the triterpenoid jujubogenin glycosides, present in the subtropical plant *Colubrina asiatica*, was investigated.[8,9] Furthermore, to search and generalize new SAR, we have designed another analogue **21**, which contains an aromatic ring moiety attached to the terpenoid skeleton as well as the existence of a 2-en-1-one pharmacophore in the molecule. The syntheses and pharmaceutical activities of these two compounds are reported below.

Results and Discussion

The *n*-BuOH extract from the aerial part of *Colubrina asiatica* which contained jujubogenin glycosides was oxidatively cleaved under strongly alkaline conditions (O_2, NaOBu, BuOH, 90°C, overnight)[10] to afford the common aglycone–jujubogenin **2**[8] in 0.1% overall yield. Catalytic hydrogenation of **2** yielded dihydrojujubogenin **3**. This procedure was aimed to avoid any interference caused by Δ^{24} in the following reactions. Selective *O*-mesylation at the 3-OH, using a large excess of mesyl chloride due to the steric hindrance caused by 4,4-dimethyl groups, afforded **4**.[11] Treatment of **4** with DBU afforded the 2-ene product **5** in a 65% yield.[11] Allylic oxidation on C-1 of **5**, however, suffered a lot of problems, attributable to the steric hindrance of the 10-methyl group (see Table 1). Finally, we found that reaction of **5** with chromic trioxide in acetic acid

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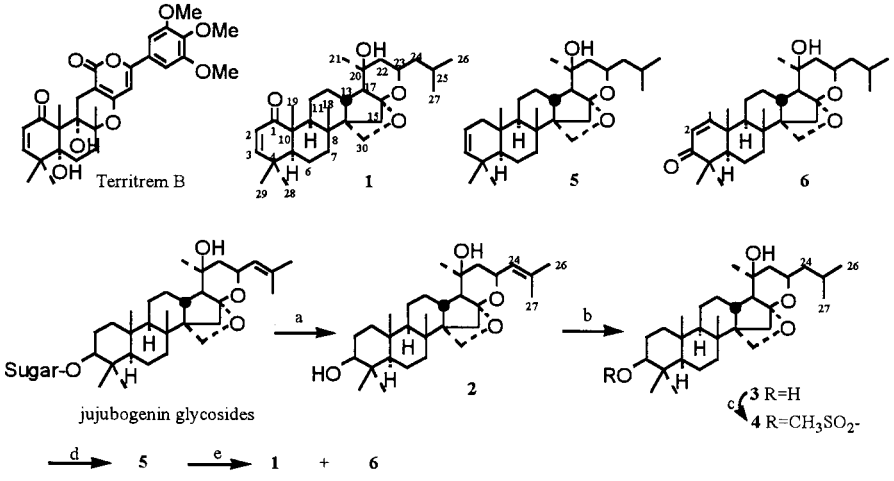
Table 1. Comparison of the yields of **1** and **6** by different allylic oxidative conditions (+: could not be detected)

Condition	Yield of **1** (%)	Yield of **6** (%)
PDC, Celite, *t*-BuOOH, toluene, reflux	8	7
PDC, Celite, *t*-BuOOH, benzene, reflux	4	4
PDC, CH_2Cl_2, 80°C, 20 h	7	4
PDC, CH_2Cl_2, sealed tube, 8 h	5	4
SeO_2/MnO_2 (1:1), CH_2Cl_2, rt, 10 days	8	+
SeO_2/MnO_2 (1:1), *t*-BuOOH, CH_2Cl_2, rt, 10 days	10	+
$HgBr_2$, hν, *t*-BuOH/ Cyclohexane (1:1), 2 days	10	+
CrO_3, HOAc, 80°C, 24 h	24	22

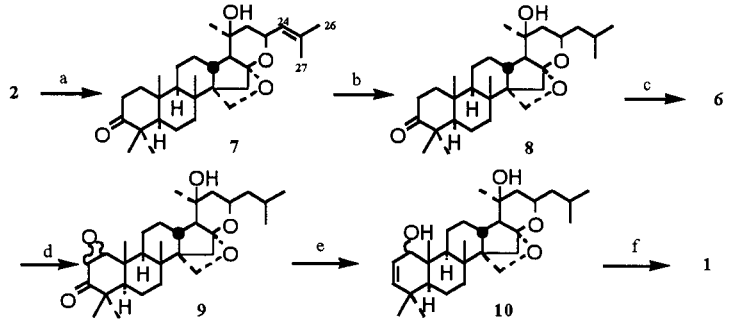
afforded the highest yield of 24% of **1** and 22% of **6**.[12] The ESI–MS of **1** showed $[M+H]^+$ at *m/z* 471 and its ^1H NMR spectrum ($CDCl_3$) showed a characteristic AX system for H-2 (δ 5.62, d) and H-3 (δ 6.26, d), J_{ax}=10.1 Hz. The EIMS spectrum of **6** showed $[M]^+$ at *m/z*470 and its ^1H NMR spectrum ($CDCl_3$) showed a characteristic AX system for H-1 (δ 7.07, d) and H-2 (δ 5.79, d), J_{ax}=10.2 Hz. The structures for **1** and **6** were distinguished by NOE difference experiments. Irradiation at the doublet of the downfield olefinic proton (H-3, δ 6.26) in **1** enhanced two methyl singlets at δ 1.07 (4β-Me) and 1.03 (4α-Me), supporting the structure for **1**. These two methyl signals were distinguished by another NOE difference experiment, which enhanced the signals of 10-Me singlet (δ1.17) and H-3 upon irradiation at the methyl singlet at δ 1.07 (Scheme 1).

Another synthetic route as depicted in Scheme 2 was explored to improve the low yield obtained with the previous route. Jujubogenin **2** was first oxidized by PDC in DMF to a 3-one product **7**, followed by hydrogenation of the 23,24-double bond to afford **8**.[13] Treatment of the 3-one **8** with LDA and phenylselenium bromide, and then $NaIO_4$ oxidation, afford the Δ^1,3-one product **6**.[14,15] Epoxidation of the 1,2-double bond in **6** by hydrogen peroxide in an ice bath afforded **9**.[16] Wharton rearrangement[16,17] of the epoxide **9** led to the production of the allylic alcohol **10**. PDC oxidation of **10** led to the expected product **1** in a high yield (82%).

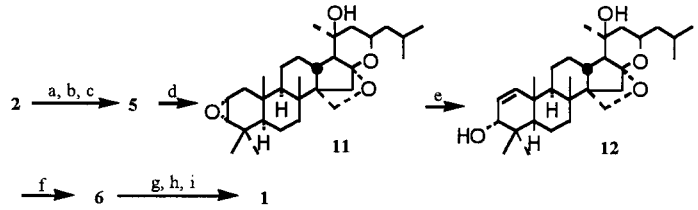
In another procedure, the 2-ene product **5** was first epoxidized by MPCBA (*meta*-perchlorobenzoic acid) to form an epoxide **11**, which was further cleaved by the phenylselenide anion and followed by an oxidation by 30% hydrogen peroxide to allow the consequent elimination of a PhSeOH. This produced an allylic alcohol **12**,[18] which could easily be subsequently oxidized to enone **6** (Scheme 3). NOE difference experiments disclosed that the epoxide of **11** adopted an α-orientation. Furthermore, this base-induced rearrangement proceeds via a cyclic *syn* elimination mechanism,[19] leading to the C-1 *syn* product of



Scheme 1. (a) $NaOBu^n$, O_2, *n*-NuOH, 90°C, 24 h; (b) Pd-C, H_2, rt, 48 h (91%); (c) MsCl, py, 0°C, 1 h (82%); (d) DBU, toluene, reflux, 34 h (65%); (e) CrO_3, HOAc, 80°C, 24 h (**1**, 24%; **6**, 22%).



Scheme 2. (a) PDC, DMF, 0°C, 10 h (93%); (b) Pd-C, H_2, rt, 48 h (90%); (c) (i) LDA, PhSeBr, THF, −78°C, 0.5 h (ii) $NaIO_4$, MeOH-H_2O (1:1), 12 h (50% in two steps); (d) 30% H_2O_2, 10% NaOH, MeOH, 0°C, 4 h (78%); (e) $N_2H_4H_2O$, MeOH, AcOH, 0°C to reflux (0.5 h+13 h) (40%); (f) PDC, DMF, reflux 60 h (82%).



Scheme 3. (a) Pd-C, H_2, rt, 48 h (91%); (b) MsCl, py, 0°C, 1 h (82%); (c) DBU, toluene, reflux, 34 h (65%); (d) MCPBA, CH_2Cl_2, 0°C-rt, 8 h (77%); (e) (i) PhSeSePh, $NaBH_4$, EtOH, rt, 2.5 h (ii) exces H_2O_2, 0°C-rt, 8 h, (77% in two steps); (f) MnO_2, Na_2CO_3, CH_2Cl_2, 0°C, 3 h (90%); (g) 30% H_2O_2, 10% NaOH, MeOH, 0°C, 4 h (78%); (h) $N_2H_4H_2O$, MeOH, AcOH, 0°C to reflux (0.5 h+13 h) (40%); (i) PDC, DMF, reflux 60 h (82%).

the 2α, 3α-epoxy. Therefore the 3-hydroxy group of the allylic alcohol **12** should also be an axial one (3α-OH).

The enone products, 2-en-1-one **1**, **6**, and **5** were assayed against eel AChE.[20] The preliminary results showed that the IC_{50} of compounds **1** and **5** was about 50 μM, at such concentration **6** inhibited AChE only about 10%. The inhibitory activities should be higher if the problem of the poor solubility of the samples get solved. This study demonstrated at least the 2-en or 2-en-1-one moiety was essential for *anti*-AChE activity.

To further mimic Territrem B by constructing an aromatic moiety in the molecule, which is possibly another pharmacophore, jujubogenin glycosides were utilized. After an acidic hydrolysis, the glycosides were not only hydrolyzed but also cleaved on the acidic-sensitive ketal group and afforded *trans*-Ebelin lactone **13** and *cis*-Ebelin lactone **14**.[21,22] The mixture of **13** and **14** was mesylated and subjected to ozonolysis, which formed three aldehydes including the totally ozone-oxidized product, **15**, as well as a pair of partial ozone-oxidized aldehydes, **16** and **17**.[23] The formation of the unexpected aldehydes **16** and **17** is possibly due to the steric hinderince of C-20 as well as the nonuniformity of the electric density of the conjugated triene. DBU treatment of **15** afforded 2-en-Ebehyde **18**,[11] which was subsequently reacted with phenyl magnesium bromide, under kinetic control. The expected alcohol **19** was obtained in 28% yield as well as a rearranged alcohol **20** isolated in 23% yield, 16% of unreacted aldehyde **18**.

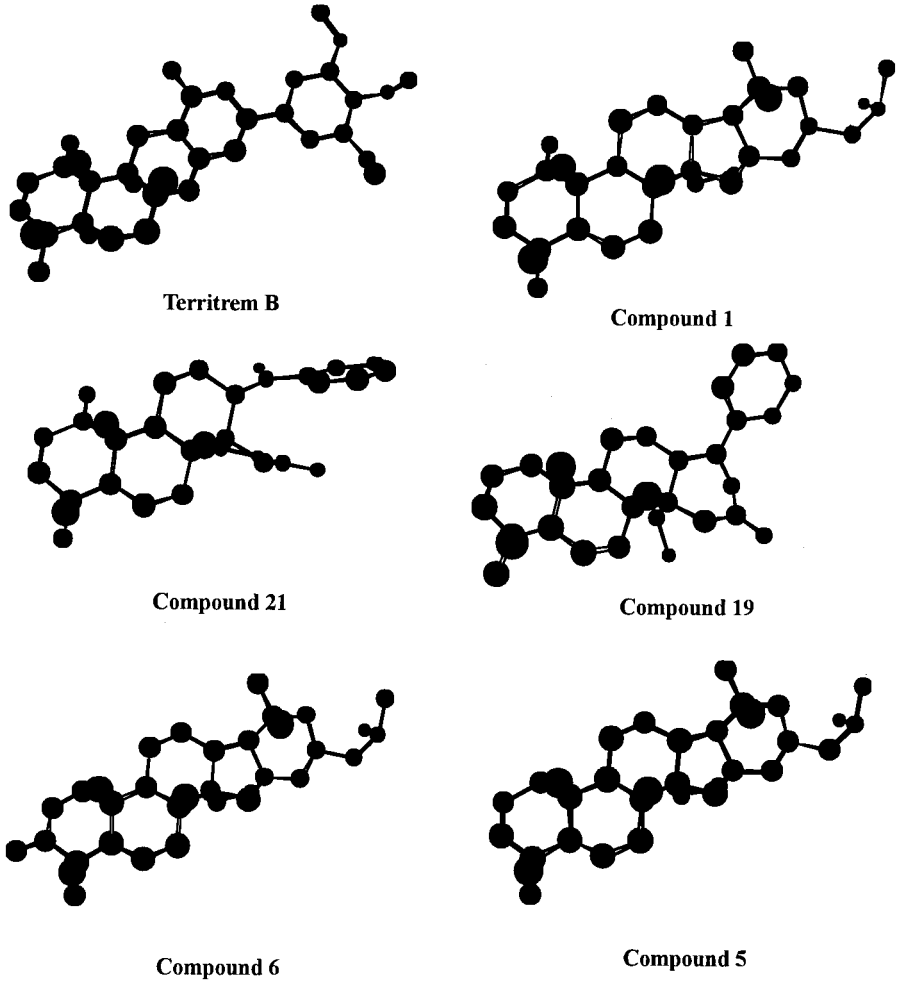
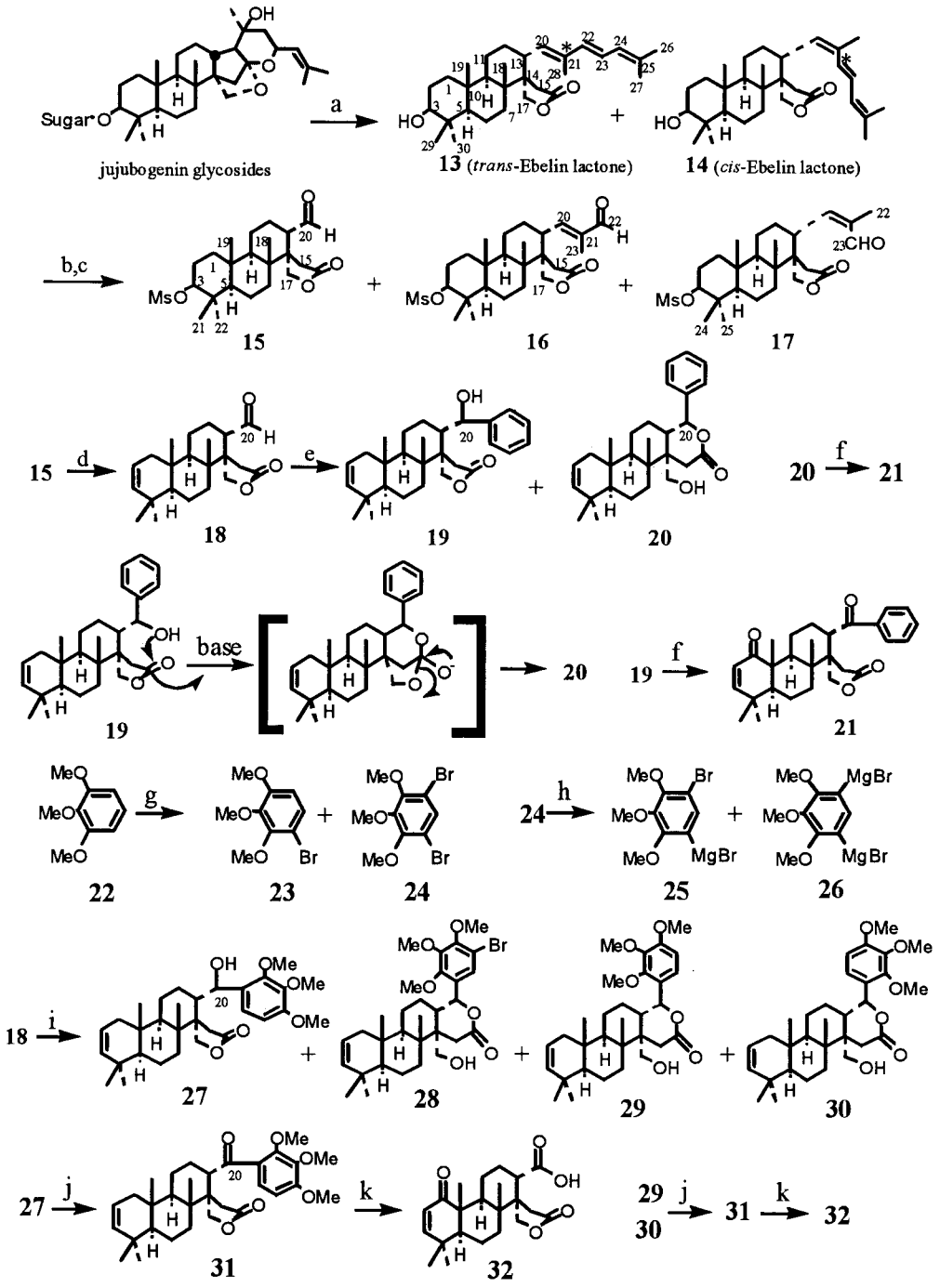


Figure 1. Energy-minimized configuration analysis of the Territrem B analogues



Scheme 4. (a) HCl:EtOH:H_2O (1:3:3, v/v/v), 80°C, 5 h, **13:14** in a 45:55 ratio for total yield of 4.5% of the crude extract; (b) MsCl, Py,)°C, 10 H, 83%; (c) 1. O_3, MeOH, −78°C, <1 h; 2. thiourea, 0°C–rt, 6 h; (d) DBU, toluene, reflux, 10 h, 54%; (e) 0.8 equiv. of C_6H_5MgBr, CH_2Cl_2, −78°C, 20 min, 28% of **19**, 23% of **20**, 19% of unreacted **18**; (f) CrO_3, HOAc, 80°C, 2 h, 69%; (g) Br_2, pyridine, 0–80°C, 1.5 h, **23:24** in ca 1:1 ratio for total yield of 86%; (h) 1 equiv. of Mg, THF, rt, 15 min; (I) 0.8 equiv. of **25** and **26**, CH**2**Cl**2**, −78°C, 20 min, 12% of **27**, 16% of **28**, 12% of **27**, 16% of **28**, 12% of **29** and 8% of **30**, 17% of unreacted **18**; (j) CrO_3, HOAc, 80°C, 2 h, 84%; (k) excess CrO_3 in HOAc, reflux 12 h, 56%.

The side reaction producing **20** consumed the expected product **19** but it can be seen that the structure of **20** possesses more structural similarity to Territrem B (Fig. 1). Unfortunately, further oxidation of **20** by chromic trioxide or pyridinium dichromate could only afford **21**, which is the oxidative product of the secondary alcohol **19** under the same conditions[12] (Scheme 4).

The combination of 2-en-Ebehyde **18** with a trimethoxyphenyl Grignard reagent was also investigated. During preparation of the trimethoxy phenyl Grignard reagent from 1,2,3-trimethoxybenzene **22**, the monobromide **23** and dibromide **24** were formed in a 1:1 ratio. The dibromide **24** was further converted into Grignard reagents **25** and **26**, which were directly reacted with 2-en-Ebehyde **18**. Four products were isolated after work-up. The expected alcohol **27** was obtained in a 12% yield and three other rearranged alcohols were obtained: **28** in a 16% yield, **29** in a 12% yield

as well as **30** in a 8% yield. Furthermore, 18% of the unreacted **18** was recovered.

In the final step chromic trioxide was applied to oxidize the C-1 of **27**–**30** to form a 2-en-1-one moiety. Alcohol **27** and the rearranged alcohols **29** and **30** were easily oxidized to the mono-ketone product **31**.[12] However, treatment of **31** with much excess of chromic trioxide only gave compound **32**, which possesses the 2-en-1-one moiety but lost the aromatic ring, since the electron-rich trimethoxy phenyl part is fragile under these oxidative conditions.

The *anti*-AChE activity of **21**, which contains the 2-en-1-one pharmacophore and a aromatic ring moiety, was also tested against eel AChE.[20] The IC_{50} was ca. 10 μM, a little bit stronger than that of dihydrojujubogenin-2-en-1-one **1**. This suggested that the aromatic ring present in Territrem B is perhaps not the major factor of the *anti*-AChE activity. Therefore further investigation into the preparation Territrem B analogues possessing both 5α-OH and 9α-OH together with the 2-en-1-one moiety will be designed and synthesised.

Experimental

General

Diethyl ether and toluene were dried over sodium; tetrahydrofuran was distilled from sodium benzophenone ketyl. Organic solutions were dried over anhydrous magnesium sulfate. Column chromatographies were performed using Merck silica gel 60 (70–230 Mesh) and TLC were carried out using Merck Kiesel gel 60 F254 plates. Melting points were determined on a Fisher–Johns melting point apparatus (uncorrected). IR spectra were recorded on a Jasco IR Report-100 infrared spectrometer. NMR spectra were recorded on a Bruker AMX 400 (400/100 MHz) and/or a Bruker DPX 200 (200/50 MHz). Selected ^{1}H NMR data is reported. ESIMS and EIMS data were measured on a Finnigan Mat TSQ-7000 mass spectrometer while HRMS was recorded on JEOL-JMS-HX 110 mass spectrometer.

Plant material

Colubrina asiatica (L.). Brongn belongs to the genus *Colubrina* and the aerial parts were collected in June 1996 in Tong Sha islands, China. A voucher specimen was deposited at the School of Pharmacy, College of Medicine, National Taiwan University.

Preparation of aglycone-jujubogenin (2)

(a) *Crude extraction by solvent partition.* The stems of *Colubrina asiatica* were air-dried (8.48 kg) and chopped to pieces, which was followed by extraction with 95% EtOH (30 L×6). The EtOH extract was further extracted by a mixed solvent composed of MeOH and hexane (6L:4L, four times) to afford hexane extract (75 g) and methanol layer (ca. 650 mL), which is difficult to be dried thoroughly. This methanol layer was subjected to partition between water (1L) and $CHCl_3$ (1 L×2). The $CHCl_3$ extract (278 g) was subjected to the next step.

(b) *Oxidative cleavage of saponins to jujubogenin.* To a stirred solution of the above-mentioned extract (140 g) in dry BuOH (500 mL) at room temperature was added carefully, fine sodium bar (58 g) in one hour. Temperature was controlled by periodically emerging the reaction flask into an ice bath so as not to exceed 100°C. After another 20 min stirring, oxygen gas was carefully introduced, the vigorous reaction must be controlled by an ice bath and by adjusting the bubble speed of oxygen gas. After 40 min stirring, all the sodium bar was dissolved and the reaction was calmed down and oxygen was bubbled in for another 24 h. The reaction was quenched by water (950 mL) and was washed out by water (800 mL) to a separating funnel. The mixture was washed by dist. H_2O (500 mL×3). The BuOH layer was evaporated under vacuum to afford an extract of 85 g, while the water was combined and extracted with $CHCl_3$ (500 mL×3). The $CHCl_3$ solution was combined and evaporated to give a residue (24 g). The BuOH extract and $CHCl_3$ extract was combined and subjected to a 1500 g silica gel column, eluting with a gradient of 3–100% $Me_2CO/CHCl_3$. The fractions containing jujubogenin were combined, evaporated and recrystalized from EtOAc to give finally jujubogenin (**2**) (8.07 g). [R_f (10% $Me_2CO/CHCl_3$) 0.38, its IR, UV, MS, ^{1}H- and ^{13}C NMR spectra were compared with the literature,[20] and was directly compared with the authentic sample.]

23,24-Dihydrojujubogenin (3). To a mixture of jujubogenin **2** (2.0 g, 4.24 mmol) and 10% Pd–C (300 mg) was added EtOAc (35 mL). The suspension was slightly pressured by hydrogen atmosphere and stirred for 48 h. The suspension was filtered through a celite funnel and washed by $CHCl_3$ (30 mL×3). The filtrate was combined and evaporated to provide crude product (1.89 g). Recrystalization from EtOAc afforded finally 23,24-dihydrojujubogenin **3** (1.81 g, 91%) as a white powder, mp 270–271°C; [Found: C, 75.81; H, 10.50. $C_{30}H_{50}O_4$ requires C, 75.90; H, 10.62%]; R_f (10% Acetone /$CHCl_3$) 0.42; ν_{max} (KBr) 3530 (OH), 2951, 2477, 2455, 1386, 1289, 1029, 1010 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 3.97 (2H, brs, H-30 and H-30′), 3.91 (1H, m, H-23), 3.13 (1H, dd, J=10.3, 5.9 Hz, H-3), 2.29 (1H, m, H-13), 1.13 (3H, s, Me-21), 2.0–1.0 (4H, m), 1.06 (3H, s, Me-18), 0.93 (3H, s, Me-28), 0.85 (3H, d, J=6.5 Hz, Me-26), 0.83 (3H, d, J=6.6 Hz, Me-27), 0.79 (3H, s, Me-19), 0.73 (3H, s, Me-29); δ_C (50 MHz, $CDCl_3$) 110.23 (C-16), 79.26 (C-3), 69.88 (C-20), 69.87 (C-23), 66.28 (C-30), 56.10 (C-5), 54.05 (C-14), 53.37 (C-17), 53.20 (C-9), 45.37 (C-24), 45.14 (C-22), 39.44 (C-4), 39.00 (C-1), 37.87 (C-8), 37.74 (C-10), 37.49 (C-13), 36.44 (C-15), 36.11 (C-7), 30.64 (C-21), 28.48 (C-25), 28.45 (C-12), 27.75 (C-2), 24.56 (C-26), 23.77 (C-27), 22.51 (C-18), 21.88 (C-11), 19.13 (C-28), 18.63 (C-6), 16.63 (C-29), 15.88 (C-19); *m/z* (ESI–MS) 475 (4, MH^+), 457 (4), 439 (12), 421 (3), 396 (10), 383 (14), 371 (14), 257 (14), 229 (13), 217 (20), 203 (33), 189 (44), 163 (50), 149 (51), 135 (100), 109 (72); HRMS (EI): M^+, found 474.3703. $C_{30}H_{50}O_4$ requires: 474.3711.

3-Methanesulfonyl-23,24-dihydrojujubogenin (4). To a stirred solution of **3** (1.79 g, 3.81 mmol) in pyridine (10 mL) at 0°C was added, 890 μL of methanesulfonyl-chloride (11.43 mmol, 3 equiv.) under a nitrogen

atmosphere. After 30 min stirring, the reaction was quenched by a Py:H_2O (2:1) mixture (4 mL of pyridine and 2 mL of water) and the solution was stirred for another 15 min at 0°C. The solution was then evaporated under vacuum to a residue, the residue was dissolved in H_2O (50 mL) and was subjected to a partition by dichloromethane and water (50 mL×3). The combined organic extract (1.83 g) was purified by flash chromatography (7–10% EtOAc/$CHCl_3$) to afford **4** (1.57 g, 82%) as a white powder, mp 147–148°C (EtOAc); [Found: C, 67.26; H, 9.49; S, 5.91. $C_{31}H_{52}O_6S$ requires C, 67.35; H, 9.48; S, 5.80%]; R_f (10% Acetone /$CHCl_3$) 0.61; ν_{max} (KBr) 3540 (OH), 2950, 1486, 1475, 1406, 1308, 1240, 1201, 1044, 1023, 1002 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.27 (1H, dd, *J*=11.5, 5.2 Hz, H-3), 3.98 (1H, d, *J*=7.8 Hz, H-30), 3.95 (1H, d, *J*=8.0 Hz, H-30′), 3.91 (1H, m, H-23), 2.98 (3H, s, SO_2*Me*), 2.32 (1H, ddd, *J*=6.4, 5.8, 5.1 Hz, H-13), 1.14 (3H, s, Me-21), 1.07 (3H, s, Me-18), 0.98 (3H, s, Me-28), 0.86 (3H, d, *J*=6.6 Hz, Me-26), 0.85 (3H, d, *J*=6.6 Hz, Me-27), 0.84 (3H, s, Me-19), 0.83 (3H, s, Me-29); δ_C (100.6 MHz, $CDCl_3$) 109.6 (C-16), 90.1 (C-3), 69.2 (C-20), 68.4 (C-23), 65.5 (C-30), 55.7 (C-5), 53.3 (C-17), 52.3 (C-9), 44.7 (C-24), 44.4 (C-22), 38.6 (SO_2*Me*), 38.5 (C-4), 38.1 (C-1), 37.1 (C-10), 37.0 (C-8), 36.7 (C-13), 35.8 (C-15), 35.3 (C-7), 32.7 (C-14), 28.0 (C-25), 27.7 (C-29), 27.0 (C-21), 25.0 (C-12), 23.9 (C-2), 23.1 (C-26), 21.8 (C-27), 21.3 (C-11), 18.4 (C-18), 18.0 (C-6), 16.1 (C-28), 15.9 (C-19); *m/z* (ESI–MS) 553 (37, MH^+), 507 (3), 451 (6), 439 (24), 421 (9), 383 (16), 371 (27), 355 (11), 311 (14), 295 (8), 269 (14), 247 (16), 217 (25), 203 (50), 191 (100), 163 (49), 162 (48), 149 (45), 109 (59), 95 (27), 81 (15); HRMS (EI): M^+, found 552.3475 $C_{31}H_{52}O_6S$ requires 552.3486.

Elimination reaction of 4 by DBU. To a stirred yellow solution of **4** (0.90 g, 1.79 mmol) in dry toluene (10 mL) was added DBU (3 mL, 20.10 mmol). After 10 min stirring at room temperature, the temperature of oil bath was elevated and the solution was refluxed 34 h. The products mixture was dried and subjected to a silica gel column chromatography (hexane:EtOAc 20:1–1:1) to afford **5** (0.526 g, 65%).

Elimination reaction of 4 by NaI/HMPA. To a stirred solution of **4** (0.15 g, 0.30 mmol) in hexamethylphosphoramide (5 mL) was added sodium iodide (0.138 g, 0.87 mmol). The temperature of the oil bath was elevated to 130°C and keep reflux for 8 h. The solvent was evaporated under vacuum (0.01 mmHg) at 120°C. The residue was washed out by sodium thiosulfate (8 g in 15 mL of water), and was extracted by diethyl ether (25 mL×4). The organic layer was combined, dried ($MgSO_4$), evaporated to a residue (0.158 g), which was purified by flash chromatography to afford **5** (0.082 g, 66%).

2-Ene-23,24-dihydrojujubogenin (5). White powder (EtOAc), mp 238–239°C; [Found: C, 78.79; H, 10.49; $C_{30}H_{48}O_3$ requires C, 78.90; H, 10.59%]; R_f (8% Me_2CO/$CHCl_3$) 0.64; ν_{max} (KBr) 3522 (OH), 2953, 2920, 1462, 1454, 1383, 1286, 1222, 1116, 1039, 998 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.38 (1H, ddd, *J*=10.1, 5.1, 1.0 Hz, H-2), 5.33 (1H, d, *J*=10.1, 2.1 Hz, H-3), 4.00 (2H, brs, H-30 and H-30′), 3.93 (1H, m, H-23), 2.33 (1H, ddd, *J*=6.2, 5.8, 5.2 Hz, H-13), 1.85 (1H, m, H-1), 1.69 (1H, m, H-1′), 1.16 (3H, s, Me-21), 1.10 (3H, s, Me-18), 0.92 (3H, s, Me-28), 0.87 (3H, d, *J*=6.6 Hz, Me-26), 0.86 (6H, s, Me-29), 0.85 (3H, d, *J*=6.7 Hz, Me-27); δ_C (100.6 MHz, $CDCl_3$) 137.9 (C-3), 121.2 (C-2), 109.6 (C-16), 69.4 (C-23), 68.6 (C-20), 65.8 (C-30), 53.5 (C-14), 52.9 (C-5), 52.4 (C-17), 51.4 (C-9), 44.9 (C-24), 44.6 (C-22), 40.9 (C-1), 40.9 (C-4), 37.4 (C-13), 37.1 (C-8), 37.1 (C-10), 36.5 (C-15), 35.9 (C-7), 31.7 (C-21), 30.1 (C-25), 24.0 (C-26), 23.3 (C-29), 22.6 (C-18), 22.0 (C-27), 21.7 (C-11), 19.2 (C-6), 17.9 (C-28), 16.3 (C-19); *m/z* (ESI–MS) 457 (29, MH^+), 439 (7), 421 (4), 403 (1), 393 (4), 383 (10), 371 (12), 364 (4), 355 (4), 343 (3), 323 (6), 267 (18), 229 (12), 217 (23), 203 (22), 191 (30), 177 (33), 161 (45), 133 (77), 123 (100), 121 (85), 109 (67), 95 (45); HRMS (EI): M^+, found 456.3607. $C_{30}H_{48}O_3$ requires 456.3605.

PDC oxidation of 5 with celite and *t*-BuOOH

(1) **Preparation of 3 M *t*-BuOOH.** To a 70% TBHP (*tert*-butylhydroperoxide) solution (43 mL) was added dichloromethane (70 mL) at 4°C in a cool room, the milk sap was vortex for 10 min, then transferred to a separating funnel. After 1 h, the clear organic layer was collected, transferred to a septum-equipped bottle using cannulation technique, and stored in a 4°C refrigerator until use.

(2) **Oxidation of 5.** To a stirred solution of **5** (53 mg, 0.12 mmol) was added celite (100 mg) and PDC (150 mg, 0.4 mmol) at 0°C, then the 3 M TBHP in CH_2Cl_2 solution was added (140 μL, 0.42 mmol). The suspension was stirred at 0°C for 45 min, then reflux for 24 h. The mixture was cooled to room temperature and diethyl ether (20 mL) was added, followed by a partition with 1 M potassium carbonate solution (25 mL). The aqueous layer was extracted by diethyl ether (20 mL). The organic layer was combined and was evaporated to a residue (45 mg), which was subjected to column chromatography (10–50% EtOAc/hexane) to afford **1** (4.4 mg, 8%) and **6** (3.8 mg, 7%). **5** (36 mg, 9%) was recovered.

23,24-Dihydrojujubogenin-2-en-1-one (1). White powder, mp 262–263°C (EtOAc); [Found: C, 76.68; H, 9.96. $C_{30}H_{46}O_4$ requires C, 76.55; H, 9.85%]; R_f (33% EtOAc/hexane) 0.36; ν_{max} (KBr) 3480 (OH), 2955, 1669, 1471, 1458, 1383, 1368, 1285, 1259, 1233, 1220, 1015, 819 cm^{-1}; UV 228 nm (ϵ 18200); δ_H (400 MHz, $CDCl_3$) 6.25 (1H, d, *J*=10.1 Hz, H-3), 5.62 (1H, d, *J*=10.1 Hz, H-2), 4.08 (1H, d, *J*=7.5 Hz, H-30), 3.94 (1H, dd, *J*=7.6, 1.8 Hz, H-30′), 3.91 (1H, dddd, *J*=8.7, 8.7, 4.3, 4.3 Hz, H-23), 2.37 (1H, ddd, *J*=6.2, 5.8, 5.2 Hz, H-13), 1.16 (6H, s, Me-19 and Me-21), 1.15 (3H, s, Me-28), 1.06 (3H, s, Me-18), 1.03 (3H, s, Me-29), 0.86 (3H, d, *J*=6.6 Hz, Me-26), 0.85 (3H, d, *J*=6.6 Hz, Me-27); δ_C (100.6 MHz, $CDCl_3$) 206.9 (C-1), 154.4 (C-3), 124.2 (C-2), 109.8 (C-16), 69.2 (C-20), 68.6 (C-23), 66.3 (C-30), 53.6 (C-17), 52.7 (C-14), 51.4 (C-5), 48.0 (C-10), 44.8 (C-24), 44.6 (C-22), 43.2 (C-9), 37.5 (C-13), 36.4 (C-15), 36.0 (C-8), 34.8 (C-7), 31.0 (C-21), 30.9 (C-25), 30.2 (C-26), 28.0 (C-12), 24.8 (C-11), 24.1 (C-18), 23.3 (C-29), 22.0 (C-27), 18.6 (C-28), 15.8 (C-19); *m/z* (ESI–MS) 471 (29, MH^+), 453 (6), 435 (28), 417 (8), 385 (18), 367 (14), 337 (10), 325 (17), 283 (18), 231 (38), 217 (100), 187 (48), 173 (95),

149 (75), 109 (45); HRMS (EI): M^+, 470.3393. $C_{30}H_{46}O_4$ requires 470.3398.

23,24-Dihydrojujubogenin-1-en-3-one (6). Colourless gum, [Found: C, 76.73; H, 10.03. $C_{30}H_{46}O_4$ requires C, 76.55; H, 9.85]; R_f (33% EtOAc/hexane) 0.22; ν_{max} (KBr) 3446 (OH), 2950, 1668, 1477, 1382, 1290, 1261, 1239, 1220, 1214, 1160, 994 cm^{-1}; UV 229 nm (ϵ 16500); δ_H (400 MHz, $CDCl_3$) 7.07 (1H, d, J=10.2 Hz, H-1), 5.79 (1H, d, J=10.2 Hz, H-2), 3.98 (2H, brs, H-30 and H-30′), 3.94 (1H, dddd, J=8.7, 8.7, 4.4, 4.4 Hz, H-23), 2.42 (1H, m, H-13), 1.16 (3H, s, Me-28), 1.11 (3H, s, Me-19), 1.07 (3H, s, Me-18), 1.04 (3H, s, Me-29), 0.88 (3H, d, J=6.6 Hz, Me-26), 0.86 (1H, d, J=6.6 Hz, Me-27); δ_C (100.6 MHz, $CDCl_3$) 206.9 (C-3), 154.4 (C-1), 124.2 (C-2), 109.8 (C-16), 69.2 (C-20), 68.6 (C-23), 65.9 (C-30), 53.6 (C-5), 52.7 (C-17), 51.4 (C-14), 48.0 (C-9), 44.9 (C-24), 44.6 (C-22), 43.2 (C-4), 37.5 (C-13), 37.5 (C-8), 36.4 (C-10), 36.0 (C-15), 34.9 (C-7), 30.2 (C-21), 28.0 (C-12), 28.0 (C-25), 24.8 (C-26), 24.1 (C-29), 23.3 (C-18), 22.0 (C-27), 21.9 (C-11), 19.2 (C-6), 18.9 (C-28), 15.8 (C-19); m/z (EIMS) 470 (100 M^+), 452 (46), 422 (41), 395 (80), 377 (42), 343 (13), 285 (8), 237 (18), 203 (23), 159 (18), 150 (25), 109 (48), 91 (36), 69 (66); HRMS (EI): M^+, 470.3392. $C_{30}H_{46}O_4$ requires 470.3398.

PDC oxidation of 5 in CH_2Cl_2. To a stirred suspension of pyridinium dichromate (500 mg, 1.33 mmol) in dichloromethane (5 mL) was added a solution of **5** (100 mg, 0.22 mmol) in dichloromethane (5 mL) in 5 min. The suspension was then refluxed under a N_2 atmosphere for 20 h. The solution was evaporated to dryness, then dissolved in water (30 mL) and was extracted by diethyl ether (30 mL×4). The combined ether layer was dried, the residue (56 mg) was separated by column chromatography (10–50% EtOAc/hexane) to afford **1** (7.3 mg, 7%) and **6** (4.2 mg, 4%). **5** (42 mg, 42%) was recovered.

Allylic oxidation of 5 to 1 by SeO_2/MnO_2 mixture. To a stirred solution of SeO_2 (20 mg, 0.32 mmol) and MnO_2 (35 mg, 0.4 mmol) in dichloromethane (3 mL) was added, a solution of **5** (23 mg, 0.05 mmol) in dichloromethane (2 mL). The suspension was stirred at room temperature for 10 days. The solvent was filtrate and evaporated, while the residue was further purified by flash chromatography (7–25% EtOAc/$CHCl_3$) to afford **1** (2.4 mg, 8%). **5** (11 mg, 48%) was recovered.

Photocatalic oxidation of 5 to 1. To a stirred solution of **5** (20 mg, 0.044 mmol) in cyclohexane (5 mL) and *tert*-butanol (5 mL) was added of mercury (II) bromide (40 mg, 0.1 mmol, 2.3 equiv. of **5**). The suspension was stirred under an UV lamp at 254 nm for 2 days. The solution was filtrate, evaporated and the residue was subjected to flash chromatography (10% EtOAc/$CHCl_3$) to afford **1** (1.9 mg, 10%). 5 (6.6 mg, 33%) was recovered.

Allylic oxidation of 5 by CrO_3/HOAc. To a stirred solution of **5** (23 mg, 0.05 mmol) in acetic acid (5 mL) at 0°C was added, 3 equiv. of chromium trioxide (16 mg, 0.15 mmol) in acetic acid (3 mL). After 30 min, the solution was allowed to be warmed up to room temperature, then stirred for another 6 h. The acidic mixture was neutralized by a saturated $NaHCO_3$ solution, followed by an extraction of diethyl ether (20 mL×3). The ether layer was combined and dried ($MgSO_4$), evaporated, and the residue (16 mg) subjected to a flash column (10% EtOAc/$CHCl_3$) to afford **1** (2.3 mg, 10%). Repeat this experiment and elevate the reaction temperature to 80°C for 24 h gave finally **1** (5.7 mg, 24%) and **6** (5.2 mg, 22%).

PDC oxidation of 3. To a stirred solution of PDC (1.316 g, 3.5 mmol) in dry DMF (9 mL) was added, a solution of **3** (260 mg, 0.55 mmol) in dry DMF (5 mL) in an ice bath under N_2 atmosphere. The suspension was stirred for another 4 h and warmed up to room temperature in 7 h. The mixture was evaporated to a small volume (4 mL) and water (50 mL) was added while the mixture was extracted by diethyl ether (50 mL×4). The combined organic layer was evaporated to a dryness (233 mg) and was subjected to flash chromatography (10% EtOAc/CH_2Cl_2) to afford **8** (199 mg, 77%) and of **7** (23 mg, 9%).

3-Oxo-jujubogenin (7). White powder (EtOAc), mp 243–244°C, [Found: C, 76.66; H, 9.99. $C_{30}H_{46}O_4$: C, 76.55; H, 9.85%]; R_f (10% $Me_2CO/CHCl_3$) 0.67; ν_{max} (KBr) 3524, 2955, 1705, 1458, 1384, 1288, 1216, 1184, 1018, 1008, 998, 838 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.16 (1H, dq, J=8.2, 1.4 Hz, H-24), 4.62 (1H, ddd, J=10.8, 10.7, 2.9 Hz, H-23), 3.98 (2H, brs, H-30 and H-30′), 2.40 (1H, m, H-13), 1.85 (1H, m, H-2′), 1.66 (3H, d, J=1.1 Hz, Me-26), 1.64 (3H, d, J=1.2 Hz, Me-27), 1.16 (3H, s, Me-21), 1.12 (3H, s, Me-28), 1.05 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.90 (3H, s, Me-19); δ_C (100.6 MHz, $CDCl_3$) 217.8 (C-3), 135.4 (C-25), 125.1 (C-24), 109.5 (C-16), 69.3 (C-20), 68.0 (C-23), 65.7 (C-30), 55.0 (C-5), 53.3 (C-14), 52.6 (C-17), 52.0 (C-9), 47.3 (C-4), 44.5 (C-22), 39.2 (C-1), 37.2 (C-8), 37.0 (C-10), 37.0 (C-13), 36.1 (C-15), 34.9 (C-7), 33.9 (C-2), 29.9 (C-21), 28.0 (C-12), 26.8 (C-29), 25.6 (C-26), 21.9 (C-11), 21.0 (C-18), 19.5 (C-6), 18.4 (C-27), 18.2 (C-28), 16.0 (C-19); m/z (ESI–MS) 471 (2 MH^+), 453 (100), 435 (10), 407 (3), 393 (14), 385 (22), 367 (35), 339 (29), 325 (6), 321 (5), 247 (8), 219 (10), 205 (20), 191 (14), 188 (16), 133 (8), 109 (9); HRMS (EI): M^+, found 470.3407. $C_{30}H_{46}O_4$ requires 470.3398.

3-Oxo-23,24-dihydrojujubogenin (8). White powder (EtOAc), mp 240–241°C; [Found: C, 76.36; H, 10.19. $C_{30}H_{48}O_4$ requires C, 76.23; H, 10.24%]; R_f (10% $Me_2CO/CHCl_3$) 0.71; ν_{max} (KBr) 3522, 2952, 1710, 1457, 1386, 1376, 1288, 1221, 1082, 1042, 1022, 998, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.98 (2H, brs, H-30 and H-30′), 3.94 (1H, m, H-23), 2.40 (1H, m, H-2), 2.37 (1H, m, H-13), 1.84 (1H, m, H-2′), 1.14 (3H, s, Me-21), 1.10 (3H, s, Me-28), 1.04 (3H, s, Me-18), 1.00 (3H, s, Me-29), 0.88 (3H, s, Me-19), 0.86 (3H, d, J=6.6 Hz, Me-26), 0.84 (3H, d, J=6.5 Hz, Me-27); δ_C (100.6 MHz, $CDCl_3$) 217.7 (C-3), 109.5 (C-16), 69.3 (C-20), 68.6 (C-23), 65.6 (C-30), 55.0 (C-5), 53.4 (C-14), 52.9 (C-17), 52.0 (C-9), 47.3 (C-4), 44.8 (C-24), 44.6 (C-22), 39.2 (C-1), 37.2 (C-8), 37.0 (C-10), 37.0 (C-13), 35.9 (C-15), 34.8 (C-7), 33.9 (C-2), 30.1 (C-21), 28.0 (C-12), 26.8 (C-25), 24.0 (C-26), 23.3 (C-29), 22.0 (C-18), 21.9 (C-11), 21.0 (C-27), 19.5 (C-6), 18.1 (C-28), 16.0 (C-19). m/z (ESI–MS) 473 (18 MH^+), 455 (7), 437 (8), 419 (2), 409 (3), 399 (13), 387 (9), 377

(6), 369 (11), 351 (8), 343 (10), 324 (15), 313 (12), 257 (14); HRMS (EI): M^+, found 472.3550. $C_{30}H_{48}O_4$ requires 472.3554.

Catalytic hydrogenation of 7 to 8. The mixture of **7** (20 mg, 0.043 mmol) and Pd–C (10%) (5 mg) was dissolved in EtOAc (6 mL), then pressured by H_2 gas for overnight. Filtration through Celite and removal of solvent afforded **8** (18.2 mg, 91%).

Preparation of 8 from jujubogenin 2. To a stirred suspension of PDC (5.414 g, 14.4 mmol) in dry DMF (9 mL) in an ice bath was added, a solution of **2** (0.98 g, 2.1 mmol) in DMF (8 mL) under a nitrogen atmosphere. The solution was stirred at 0°C for 1 h, then another 7 h in room temperature. Evaporation of part of the solvent to a 7 mL volume, water (50 mL) was added and the mixture was extracted by ether (60 mL×4). The combined organic layer was evaporated and the dryness was subjected to recrystallization and afford **7** (903 mg, 93%). The pure **7** was hydrogenated by H_2 gas under Pd–C catalization as above and afforded finally **8** (814 mg, 90%).

α-Dehydrogenation of 8. To a stirred solution of diisopropylamine (17 μL, 0.12 mmol) in dry THF (2 mL) at −78°C was added, 1.6 M *n*-Buli in hexane (75 μL, 0.12 mmol) under a nitrogen atmosphere. After 10 min, **8** (47 mg, 0.1 mmol) in THF (2 mL) was added dropwise. After another 10 min, phenylselenylbromide (28.2 mg, 0.12 mmol) in THF (1 mL) was added quickly. 1.6 M *n*-BuLi in hexane (150 μL, 0.24 mmol) was added and the solution was stirred for 20 min. Then a solution of $NaIO_4$ (36 mg, 0.72 mmol) in a mixture of MeOH (1.4 mL) and H_2O (0.7 mL) was added carefully and the mixture was allowed to warm up to room temperature in 2 h then refluxed for 10 h. The cooled solution was partitioned by water (10 mL) and ether (25 mL×2). The organic layer was washed and dried, and evaporated to dryness. Purification by column chromatography (5–20% EtOAc/$CHCl_3$) provided **6** (23 mg, 50%).

1α,2α-Epoxy-3-oxo-23,24-dihydrojujubogenin (9). To a stirred solution of **6** (160 mg, 0.4 mmol) in MeOH (4 mL) at 0°C was added 30% aqueous hydrogen peroxide (300 μL, 2.4 mmol). After stirring for 4 h, the solution was evaporated to a 2 mL volume and was partitioned between water (10 mL) and dichloromethane (20 mL×2). The organic layer was dried and evaporated to afford a residue (155 mg), which was subjected to flash chromatography (33% EtOAc/hexane) to provide **9** (129 mg, 78%) as colorless gum; [Found: C, 73.96; H, 9.46. $C_{30}H_{46}O_5$ requires C, 74.03; H, 9.53%]; R_f (12.5% EtOAc/$CHCl_3$) 0.61; ν_{max} (KBr) 3524, 2952, 1712, 1458, 1385, 1371, 1288, 1080, 1025, 998, 836 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.06 (1H, d, *J*=8.0 Hz, H-30), 4.00 (1H, dd, *J*=7.8, 1.5 Hz, H-30′), 3.94 (1H, dddd, *J*=8.8, 8.8, 4.3, 4.3 Hz, H-23), 3.54 (1H, d, *J*=4.7 Hz, H-2), 3.33 (1H, d, *J*=4.7 Hz, H-1), 2.41 (1H, m, H-13), 1.17 (3H, s, Me-21), 1.13 (3H, s, Me-28), 1.07 (3H, s, Me-28), 1.01 (3H, s, Me-21), 0.88 (3H, d, *J*=6.6 Hz, Me-26), 0.86 (3H, s, Me-19), 0.85 (3H, d, *J*=6.6 Hz, Me-27); δ_C (100.6 MHz, $CDCl_3$) 212.4 (C-3), 109.5 (C-16), 69.4 (C-20), 68.6 (C-23), 65.6 (C-30), 63.4 (C-2), 56.8 (C-1), 53.4 (C-14), 52.8 (C-17), 46.1 (C-5), 45.6 (C-9), 44.9 (C-24), 44.8 (C-4), 44.6 (C-22), 38.6 (C-10), 37.6 (C-8), 37.0 (C-13), 36.0 (C-15), 34.6 (C-7), 30.9 (C-21), 27.7 (C-25), 27.0 (C-12), 24.1 (C-26), 23.3 (C-29), 22.3 (C-11), 22.0 (C-18), 20.1 (C-27), 18.9 (C-6), 18,4 (C-28), 15.2 (C-19); *m/z*(ESI–MS) 487 (60 M^+), 469 (10), 440 (8), 412 (11), 403 (10), 304 (10), 355 (18), 343 (16), 311 (32), 268 (23), 233 (62), 199 (34), 189 (57), 175 (73), 147 (100), 123 (46), 69 (51); HRMS (EI): M^+, found 486.3348. $C_{30}H_{46}O_5$ requires 486.3347.

1α-Hydroxy-2-ene-23,24-dihydrojujubogenin (10). To a stirred solution of **9** (96 mg, 0.2 mmol) in dry MeOH (15 mL) at 0°C was added, hydrazine hydrate (150 μL, 0.3 mmol) dropwise. After 0.5 h, the solution was allowed to warm up to room temperature. Acetic acid (20 μL) was added and the solution was stirred for another 1 h, then refluxed for12 h. The cooled solution was evaporated to a dryness and the residue was partitioned by water (30 mL) and diethyl ether (30 mL×3). The organic layer was combined, dried and evaporated. Purification by flash column chromatography (10–50% EtOAc/$CHCl_3$) afford of **10** (38 mg, 40%) as white powder (EtOAc), mp 228–229°C, [Found: C, 76.32; H, 10.12. $C_{30}H_{48}O_4$ requires C, 76.23; H, 10.24%]; R_f (12.5% EtOAc/$CHCl_3$) 0.28; ν_{max} (KBr) 3481 (OH), 2951, 1462, 1453, 1385, 1289, 1218, 1033, 1018, 992 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.68 (1H, dd, *J*=9.9, 5.8 Hz, H-2), 5.51 (1H, d, *J*=9.9 Hz, H-3), 4.09 (1H, d, *J*=7.6 Hz, H-30), 4.03 (1H, dd, *J*=7.6, 1.7 Hz, H-30′), 3.96 (1H, ddddd, *J*=8.7, 8.7, 4.4, 4.4, 1.4 Hz, H-23), 3.59 (1H, d, *J*=5.8 Hz, H-1), 2.34 (1H, m, H-13), 1.16 (3H, s, Me-21), 1.12 (3H, s, Me-18), 0.96 (3H, s, Me-28), 0.87 (3H, d, *J*=6.7 Hz, Me-26), 0.86 (3H, d, *J*=6.6 Hz, Me-27), 0.85 (3H, s, Me-19), 0.79 (3H, s, Me-29); δ_C (100.6 MHz, $CDCl_3$) 142.1 (C-3), 124.1 (C-2), 110.2 (C-16), 69.9 (C-23), 69,0 (C-1), 68.7 (C-20), 66.4 (C-30), 53.4 (C-14), 53.3 (C-17), 45.7 (C-5), 45.3 (C-24), 45.1 (C-22), 42.4 (C-9), 40.8 (C-10), 37.6 (C-4), 37.5 (C-13), 36.6 (C-15), 35.6 (C-8), 34.9 (C-7), 32.0 (C-21), 30.6 (C-25), 28.3 (C-12), 24.5 (C-26), 23.7 (C-29), 22.9 (C-18), 22.9 (C-27), 22.2 (C-11), 19.0 (C-6), 18.5 (C-28), 16.6 (C-19); *m/z*(ESI–MS) 473 (19 MH^+), 455 (2), 437 (11), 419 (5), 391 (2), 377 (8), 313 (14), 253 (14), 215 (29), 201 (51), 175 (42), 147 (45), 133 (100), 109 (60); HRMS (EI): M^+, found 472.3549. $C_{30}H_{48}O_4$ requires 472.3554.

PDC oxidation of 10

(1) To a mixture of **10** (10 mg, 0.021 mmol) and PDC (80.4 mg, 0.21 mmol) was added dichloromethane (5 mL). The solution was stirred at room temperature for 8 h and reflux for 60 h. After evaporation, the residue was purified by flash chromatography (10–33% EtOAc/$CHCl_3$) to afford **1** (4.5 mg, 45%).

(2) Similar experiment was carried out using DMF as solvent. Utilizing **10** (10 mg, 0.021 mmol) provided finally **1** (8.2 mg, 82%).

2α,3α-Epoxy-23,24-dihydrojujubogenin (11). To a stirred solution of **5** (57 mg, 0.13 mmol) in dichloromethane (5 mL) at 0°C was added dropwise, MCPBA (35 mg, 0.2 mmol) in ice-cooled dichloromethane (2 mL). After

2 h, the solution was allowed to warm up to room temperature and stirred for another 8 h. Saturated $NaHCO_3$ (5 mL) solution was added, the dichloromethane layer was separated while the water layer was extracted by dichloromethane (10 mL×2). The organic layer was combined and dried ($MgSO_4$). Evaporation followed by flash chromatography (12.5% EtOAc/hexane) provided **11** (55 mg, 80%) as white powder (EtOAc), mp 236–237°C, [Found: C, 76.05; H, 10.12. $C_{30}H_{48}O_4$ requires C, 76.23; H, 10.24%]; R_f (50% EtOAc/hexane) 0.55; ν_{max} (KBr) 3519 (OH), 2949, 1458, 1386, 1286, 1219, 999, 834 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.94 (2H, brs, H-30 and H-30′), 3.91 (1H, ddddd, J=8.7, 8.7, 4.4, 4.4, 1.4 Hz, H-23), 3.14 (1H, dd, J=12.0, 7.8 Hz, H-2), 2.75 (1H, d, J=7.6 Hz, H-3), 2.32 (1H, m, H-13), 1.13 (3H, s, Me-21), 1.06 (3H, s, Me-18), 1.04 (3H, s, Me-19), 0.98 (3H, s, Me-28), 0.85 (3H, d, J=6.5 Hz, Me-26), 0.84 (3H, d, J=6.6 Hz, Me-27), 0.82 (3H, s, Me-29); δ_C (100.6 MHz, $CDCl_3$) 109.7 (C-16), 69.4 (C-20), 68.6 (C-23), 65.7 (C-30), 61.6 (C-2), 61.6 (C-3), 53.3 (C-14), 52.4 (C-17), 51.3 (C-9), 47.1 (C-5), 44.9 (C-24), 44.6 (C-22), 40.2 (C-1), 37.2 (C-4), 37.0 (C-13), 36.2 (C-15), 35.9 (C-8), 34.7 (C-7), 32.8 (C-10), 29.7 (C-21), 28.1 (C-25), 28.0 (C-12), 24.1 (C-26), 23.3 (C-29), 22.0 (C-18), 22.0 (C-27), 21.7 (C-11), 18.6 (C-28), 18.4 (C-6), 17.6 (C-19); *m/z* (ESI–MS) 473 (17 MH^+), 455 (1), 437 (4), 419 (2), 409 (1), 392 (1), 371 (3), 325 (3), 277 (2), 213 (14), 200 (15), 187 (31), 159 (34), 133 (100), 119 (44); HRMS (EI): M^+, found 472.3553. $C_{30}H_{48}O_4$ requires 472.3554.

Base-induced rearrangement of epoxide 11. To a stirred solution of butyllithium (1.3 mL of the 1.6 M solution in hexane, 2.1 mmol) in dry THF (5 mL) at −78°C was added diisopropylamine (0.32 mL, 4.4 mmol). After 20 min, a solution of **11** (24 mg, 0.05 mmol) in dry THF (5 mL) was added dropwise at −78°C. After 12 h, the solution was allowed to warm up to room temperature and stirred for another 24 h. Water (10 mL) was added and the solution was extracted by ether (20 mL×3). The organic layer was combined and dried. Evaporation followed by flash column chromatography (20% EtOAc/hexane) afforded **12** (5.8 mg, 24%).

Eliminative ring opening of epoxide 11

(1) Preparation of diphenyldiselenide. The diphenyldiselenide was freshly prepared by Sharpless method.[17]

(2) To a stirred solution of diphenyldiselenide (12 mg, 0.04 mmol) in absolute ethanol (5 mL) under a nitrogen atmosphere at 0°C was added sodium borohydride (3 mg, 0.08 mmol) in three batches. After the bright yellow color changed to colorless in 10 min, the epoxide **11** (15 mg, ca. 0.035 mmol) was added in 5 min. The clear solution was stirred for another 20 min at room temperature, then was refluxed for 2 h. Absolute ethanol (5 mL) was added to the bottle and the reflux was kept overnight. The solution was cooled to 0°C and THF (10 mL) was added. 30% hydrogen peroxide (200 μL, ca. 2.4 mmol) was added in 10 min while the solution was vigorously stirred and the temperature was kept below 10°C. After 8 h the yellow color faded to colorless which indicated the reaction went thoroughly. The gray–white suspension was evaporated under vacuum to a small volume (3 mL), then water (10 mL) was added to dilute the solution, which was subjected to a extraction with diethyl ether (20 mL×2). The Organic layer was combined and washed twice with aqueous potassium carbonate, and dried by $MgSO_4$. Evaporation of the solvent afford the crude allylic alcohol **12** (13 mg). Recrystallization in EtOAc provide finally **12** (11.5 mg, 77%).

1-Ene-3α-hydroxy-23,24-dihydrojujubogenin (12). White powder (EtOAc), mp 250–251°C, R_f (50% EtOAc/hexane) 0.27; ν_{max} (KBr) 3448 (OH), 2952, 2915, 1455, 1382, 1368, 1287, 1219, 1168, 1022, 998 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.98 (1H, d, J=10.2 Hz, H-1), 5.59 (1H, dd, J=10.2, 4.8 Hz, H-2), 3.97 (2H, brs, H-30 and H-30′), 3.93 (m, 1H, H-23), 3.50 (1H, d, J=4.8 Hz, H-3), 2.34 (1H, ddd, J=6.8, 6.0, 5.9 Hz, H-13), 1.16 (3H, s, Me-21), 1.11 (3H, s, Me-18), 0.95 (3H, s, Me-28), 0.91 (3H, s, Me-19), 0.87 (3H, d, J=6.5 Hz, Me-26), 0.86 (3H, d, J=6.7 Hz, Me-27), 0.80 (3H, s, Me-29); δ_C (100.6 MHz, $CDCl_3$) 140.1 (C-2), 124.2 (C-1), 109.7 (C-16), 73.6 (C-3), 69.4 (C-20), 68.6 (C-23), 65.8 (C-30), 53.7 (C-17), 52.9 (C-14), 49.2 (C-5), 49.2 (C-9), 44.9 (C-24), 44.6 (C-22), 39.5 (C-4), 37.7 (C-10), 37.0 (C-8), 37.0 (C-13), 36.0 (C-15), 35.9 (C-7), 30.2 (C-21), 27.8 (C-12), 26.4 (C-25), 24.1 (C-26), 23.5 (C-27), 23.3 (C-29), 22.0 (C-18), 21.6 (C-11), 19.3 (C-28), 17.6 (C-6), 17.5 (C-9); *m/z*(ESIMS) 473 (12 MH^+), 455 (6), 437 (16), 419 (22), 404 (6), 378 (4), 377 (10), 313 (23), 255 (9), 253 (10), 215 (20), 201 (34), 175 (53), 147 (54), 133 (100), 109 (57); HRMS (EI): M^+, found 472.3549. $C_{30}H_{48}O_4$ requires 472.3554.

PDC oxidation of allylic alcohol 12. To a stirred solution of PDC (258 mg, 0.7 mmol) in CH_2Cl_2 (2 mL) at 0°C was added **12** (47 mg, 0.1 mmol) in CH_2Cl_2 (2 mL). After 0.5 h, the solution was stirred at room temperature for 24 h. The solution was evaporated to dry, and was partitioned by ether (20 mL×3) and water (15 mL). The ether layer was combined and evaporated to a dryness (46 mg), followed by flash chromatography (10% EtOAc/CH_2Cl_2) to afford **6** (42 mg, 89%).

MnO_2 oxidation of the allylic alcohol 12. To a stirred solution of magnesium dioxide (90%, Merck) (17.4 mg, 0.02 mmol) and sodium carbonate (10.6 mg, 0.1 mmol) was added the allylic alcohol **12** (10 mg, 0.02 mmol) at 0°C. The solution was stirred overnight while the temperature was warmed up to room temperature. Filtration through celite, the CH_2Cl_2 filtrate was evaporated and afford 9.2 mg of crude residue. Recrystallization from EtOAc afforded finally **6** (9.0 mg, 90%).

Preparation of *trans*-Ebelin lactone (13) and *cis*-Ebelin lactone (14). To a stirred solution of crude chloroform extract of jujubogenin glucosides (30 g) in ethanol (90 mL) was added, dist. Water (90 mL) and HCl (5 mL). The solution was heated at 80°C for 5 h and recovered to room temperature. Concentrated under vacuum to remove ethanol, the aqueous solution was neutralized by 1 M potassium carbonate solution, and extracted by chloroform (150 mL×4). The chloroform layer was dried ($MgSO_4$), solution evaporated off, and purified by flash column (50% EtOAc/hexane) to afford mixture of *trans*-Ebelin lactone (**13**) and *cis*-Ebelin lactone (**14**) (1.36 g, 4.5%

yield of the crude extracts). (^{1}H NMR showed the integral area of **13** and **14** in a 45:55 ratio.)

Mesylation of Ebelin lactones 13/14. To a stirred solution of Ebelin lactones (Z/E mixture, 1.39 g, 3.1 mmol) in dry pyridine (20 mL) at 0°C was added, mesyl chloride (4 mL), and the temperature was kept at 0°C for 10 h. A mixture of pyridine (5 mL) and water (5 mL) was added to the solution, which was then evaporated under vacuum. The residue was partitioned between water (20 mL) and chloroform (30 mL×3). The organic layer was combined, dried ($MgSO_4$) and solvent evaporated off. The residue (2.1 g) was purified by flash column and afforded finally mesylated ebelin lactone (MsEbelactone) (1.39 g, 2.5 mmol, 83%) as oil.

General procedure of ozonolysis of MsEbelactone

To a stirred solution of MsEbelactone (300 mg, 0.55 mmol) in anhydrous methanol (15 mL) at −78°C was introduced, ozone fluid (1 cc/s at 125 mA) for a different time (Table 2), and then nitrogen gas for another 10 min. The temperature of the solution was then recovered to 0°C (ice bath), while thiourea (150 mg) in dry MeOH (3 mL) was added portion-wise, and the solution stirred for another 6 h. The white suspension was filtered and washed by cooled dry MeOH (2 mL). The elute was concentrated under vacuum and was washed out by ether (25 mL) and 1% Na_2CO_3 (10 mL). Extracted by ether (20 mL), the ether layer were combined and washed by distilled water (10 mL), dried ($MgSO_4$), and the solvent evaporated off. Purification by flash column (33–50% EtOAc/hexane) afforded different yields of **15**, **16** and **17** (see Table 2).[22]

Table 2. The relationship of ozonolysis time and the yields of Ebehydes

T (min)	**15** (%)	**16** (%)	**17** (%)
10	5	35	34
25	26	12	10
40	22	10	0
60	18	0	0

MsEbehyde (15). Colourless gum, R_f (33% EtOAc/hexane) 0.29; ν_{max} (KBr) 2950, 1778, 1735, 1403, 1364, 1203, 1196, 977 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.61 (1H, d, J=1.1 Hz, H-20), 4.35 (1H, d, J=10.2 Hz, H-17), 4.30 (1H, dd, J=11.8, 4.6 Hz, H-3), 4.20 (1H, d, J=10.2 Hz, H-17′), 3.00 (3H, s, SO_2Me), 2.60 (1H, d, J=18.6 Hz, H-15), 2.46 (1H, d, J=18.7 Hz, H-15′), 1.01 (3H, s, Me-18), 0.97 (3H, s, Me-21), 0.88 (3H, s, Me-19), 0.85 (3H, s, Me-22); δ_C (100.6 MHz, $CDCl_3$) 203.2 (C-20), 176.4 (C-16), 89.8 (C-3), 70.5 (C-17), 55.7 (C-5), 53.0 (C-13), 52.8 (C-9), 49.6 (C-14), 40.6 (C-4), 39.2 (SO_2Me), 38.9 (C-8), 38.6 (C-1), 37.3 (C-10), 35.0 (C-7), 33.1 (C-15), 28.5 (C-22), 25.5 (C-2), 23.5 (C-12), 19.9 (C-11), 18.1 (C-18), 18.1 (C-6), 16.6(C-21), 16.4 (C-19); HRMS (EI): M^+, found 440.3175. $C_{23}H_{36}O_6S$ requires 440.3173.

MsEbehyde B (16). Colourless gum, R_f (33% EtOAc/hexane) 0.32; ν_{max} (KBr) 2953, 2677, 1776, 1699, 1418, 1405, 1365, 1194, 969 cm^{-1}; UV 239 nm (ϵ 18600); δ_H (400 MHz, $CDCl_3$) 9.36 (1H, s, H-22), 6.23 (1H, dd, J=10.4, 1.2 Hz, H-20), 5.80 (1H, d, J=10.8 Hz, H-24), 4.44 (1H, d, J=10.6 Hz, H-17), 4.36 (1H, d, J=10.6 Hz, H-17′), 4.31 (1H, dd, J=11.8 Hz, 5.0, H-3), 2.99 (3H, s, SO_2Me), 2.54 (1H, d, J=18.4 Hz, H-15), 1.92 (1H, d, J 18.4 Hz, H-15′), 1.77 (3H, d, J 1.1 Hz, Me-23), 1.06 (3H, s, Me-18), 0.89 (3H, s, Me-19), 0.85 (3H, s, Me-25); δ_C (100.6 MHz, $CDCl_3$) 195.2 (C-22), 175.8 (C-16), 151.8 (C-20), 141.6 (C-21), 90.0 (C-3), 69.6 (C-17), 55.8 (C-5), 52.9 (C-9), 51.3 (C-14), 40.6 (C-13), 39.9 (C-4), 39.3 (SO_2Me), 38.8 (C-8), 38.7 (C-1), 36.9 (C-10), 35.2 (C-15), 34.5 (C-7), 28.6 (C-25), 28.4 (C-12), 25.6 (C-2), 20.2 (C-11), 18.6 (C-18), 18.4 (C-6), 16.7 (C-24), 16.4 (C-19), 10.5 (C-23); HRMS (EI): M^+, found 480.3491. $C_{26}H_{40}O_6S$ requires 480.3486.

MsEbehyde C (17). Colourless gum, R_f (33% EtOAc/hexane) 0.33; ν_{max} (KBr) 2952, 2675, 1781, 1693, 1417, 1405, 1366, 1194, 966 cm^{-1}; UV: 238 nm (ϵ 17800); δ_H (400 MHz, $CDCl_3$) 10.08 (1H, s, H-22), 6.16 (1H, dd, J=10.1, 1.2 Hz, H-20), 4.39 (1H, d, J=10.6 Hz, H-17), 4.32 (1H, d, J=10.4 Hz, H-17′), 4.31 (1H, dd, J=11.8, 4.6 Hz, H-3), 3.00 (3H, s, SO_2Me), 2.56 (1H, d, J=18.2 Hz, H-15), 2.06 (1H, d, J=18.3 Hz, H-15′), 1.80 (1H, d, J=1.2 Hz, Me-23), 1.04 (3H, s, Me-18), 1.02 (3H, s, Me-24), 0.88 (3H, s, Me-19), 0.86 (3H, s, Me-25); HRMS (EI): M^+, found 480.3489. $C_{26}H_{40}O_6S$ requires 480.3486.

2-EneEbehyde (18). To a stirred solution of **15** (50 mg, 0.11 mmol) in toluene (5 mL) was added DBU (1 mL). The brown solution was heated under reflux for 10 h. The reaction was quenched by addition of acetic acid (1 mL) in water (8 mL), stirred for 5 min. The solution was extracted by diethyl ether (20 mL×3). The aqueous layer was then basified to pH=8 and partitioned by diethyl ether (20 mL). The ether layer was combined, dried ($MgSO_4$), purified by flash column (33–50% EtOAc/hexane) to afforded finally **18** (21.1 mg, 54%) as colorless needles (acetone), mp 203–204°C; R_f (50% EtOAc/hexane) 0.40; ν_{max} (KBr) 3021, 2778, 1775, 1736, 1657, 1415, 962, 710 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.63 (1H, d, J=1.2 Hz, H-20), 5.37 (1H, m, H-2), 5.36 (1H, m, H-3), 4.19 (2H, d, J=10.3 Hz, H-17 & H-17′), 2.99 (3H, s, SO_2Me), 2.78 (1H, m, H-13), 2.60 (1H, d, J=18.3 Hz, H-15), 2.46 (1H, d, J=18.6 Hz, H-15′), 0.98 (3H, s, Me-18), 0.96 (3H, s, Me-24), 0.93 (3H, s, Me-21), 0.86 (3H, s, Me-19), 0.85 (3H, s, Me-22); δ_C (100.6 MHz, $CDCl_3$) 203.2 (C-20), 176.4 (C-16), 137.9 (C-3), 120.8 (C-2), 70.2 (C-17), 52.7 (C-5), 52.2 (C-13), 51.1 (C-9), 49.2 (C-14), 41.3 (C-5), 52.2 (C-13), 51.1 (C-9), 49.2 (C-14), 41.3 (C-1), 40.8 (C-4), 39.2 (SO_2Me), 36.3 (C-8), 34.7 (C-15), 34.5 (C-10), 31.9 (C-7), 31.6 (C-22), 23.3 (C-12), 22.6 (C-18), 19.7 (C-11), 18.7 (C-6), 16.3 (C-19); HRMS (EI): M^+, found 344.2351. $C_{22}H_{32}O_3$ requires 344.2353.

First Grignard reaction of 18

(a) **Preparation of Grignard reagent.** To a stirred solution of dry diethyl ether (2 mL) including magnesium pieces (70 mg, 2.92 mmol) and one piece of iodine was added, bromobenzene (250 μL) in dry ether (1 mL) under vigorous stirring for 20 min until the magnesium pieces disappeared.

(b) **Grignard reaction.** To a stirred solution of 2-en-Ebehyde **18** (17 mg, 0.05 mmol) in ether (1 mL) at −60°C

was added dropwise, the above-prepared Grignard reagent (45 μL) under nitrogen. The solution was stirred for 20 min and quenched by 0.5% HCl (0.5 mL) in water (3 mL) at −18°C. Stirred for another 10 h, the solution was extracted by diethyl ether (20 mL×2). The aqueous layer was basified to pH=8 and extracted by diethyl ether (10 mL×2). The ether layer was combined, washed by brine (10 mL), dried ($MgSO_4$), and the solvent evaporated off. The residue (22 mg) was purified by flash column (17% EtOAc/hexane) to afford **19** (5.8 mg, 28%) and **20** (4.8 mg, 23%), as well as unreacted **18** (3.3 mg, 19%).

2-Ene-Ebelacol A (19). Colourless gum, R_f (50% EtOAc/hexane) 0.42; IR (KBr): 3633 (OH), 3066, 3022, 1776, 1608, 1512, 1417, 1360, 1105, 1027, 712 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.31 (2H, m, H-3′ and H-5′), 7.28 (2H, m, H-2′ and H-6′), 7.27 (1H, m, H-4′), 5.36 (1H, m, H-2), 5.35 (1H, m, H-3), 4.48 (1H, dd, *J*=8.9 Hz, 1.5, H-20), 4.40 (1H, d, *J*=10.4 Hz, H-17), 4.32 (1H, d, *J*=10.3 Hz, H-17′), 3.15 (1H, d, *J*=18.3 Hz, H-15), 2.64 (1H, d, *J*=18.5 Hz, H-15′), 1.03 (3H, s, Me-18), 0.94 (3H, s, Me-21), 0.86 (3H, s, Me-22), 0.79 (3H, s, Me-19); HRMS (EI): M^+, found 422.2819. $C_{28}H_{38}O_3$ requires: 422.2823.

2-Ene-Ebelacol B (20). Colourless gum, R_f (50% EtOAc/hexane) 0.48; ν_{max} (KBr) 3599 (OH), 3060, 1752, 1611, 1515, 1346, 1334, 1031, 1023, 708 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.33 (2H, m, H-3′ and H-5′), 7.27 (2H, m, H-2′ and H-6′), 7.26 (1H, m, H-4′), 5.39 (1H, m, H-2), 5.36 (1H, m, H-3), 4.99 (1H, d, *J*=9.8 Hz, H-17), 4.96 (1H, d, *J*=3.6 Hz, H-20), 4.41 (1H, d, *J*=18.6 Hz, H-15′), 0.94 (6H, s, Me-18 and Me-21), 0.86 (3H, s, Me-22), 0.78 (3H, s, Me-19); δ_C (100.6 MHz, $CDCl_3$) 178.5 (C-16), 144.2 (C-1′), 137.9 (C-3), 128.6 (C-3′ and C-5′), 128.1 (C-4′), 126.9 (C-2′), 121.0 (C-2), 78.5 (C-20), 69.6 (C-17), 61.1 (OMe), 60.7 (OMe), 56.0 (OMe), 51.6 (C-5), 51.4 (C-9), 45.1 (C-14), 40.8 (C-1), 40.6 (C-4), 36.2 (C-8), 34.6 (C-10), 33.4 (C-7), 31.6 (C-13), 29.7 (C-22), 26.8 (C-12), 22.6 (C-18), 20.1 (C-11), 19.1 (C-6), 17.6 (C-21), 16.1 (C-19); HRMS (EI): M^+, found 422.2824. $C_{28}H_{38}O_3$ requires 422.2823.

1-Oxo-2-en-Ebelacone (21). To a stirred solution of **19** (2.2 mg, 5.2 μmol) in acetic acid (2 mL) was added, chromium trioxide (10 mg, 10 μmol) in acetic acid (1 ml). The solution was heated in an 80°C oil bath for 2 h, and the temperature was allowed to recover to room temperature and stirred for another 8 h. Solvent was evaporated under vacuum in a 50°C water bath and, residue was partitioned between CH_2Cl_2 (20 mL×3) and water (10 mL). The organic layer was combined, dried ($MgSO_4$) and purified by flash column (50% EtOAc/hexane) to afford of **21** (1.55 mg, 69%) as colorless oil, R_f (50% EtOAc/hexane) 0.31; ν_{max} (KBr) 3063, 2994, 1773, 1690, 1668, 1566, 1487, 1218, 1186 cm^{-1}; UV: 251 nm (ε 18800), 225 nm (ε 18600); δ_H (400 MHz, $CDCl_3$) 7.86 (2H, dd, *J*=8.6, 1.3 Hz, H-2′ and H-6′), 7.56 (1H, t, *J*=8.6 Hz, H-4′), 7.47 (2H, m, H-3′ and H-5′), 6.32 (1H, d, *J*=10.1 Hz, H-3), 5.67 (1H, d, *J*=10.1 Hz, H-2), 4.96 (1H, d, *J*=10.7 Hz, H-17), 4.44 (1H, d, *J*=10.2 Hz), 2.52 (1H, d, *J*=18.5 Hz, H-15), 2.15 (1H, d, *J*=18.5 Hz, H-15′), 1.19 (3H, s, Me-19), 1.18 (3H, s, Me-18), 1.10 (3H, s, Me-21), 1.07 (3H, s, Me-22); HRMS (EI): M^+, found 434.2453. $C_{28}H_{34}O_5$ requires 434.2458.

Preparation of trimethoxy bromobenzene. To a stirred solution of of trimethoxybenzene (1.076 g, 6.4 mmol) in hexane (10 mL) was added, dry pyridine (30 μL) and, after 5 min of stirring, quickly Bromine (1.8 mL). The reaction was heated at 80°C for 45 min, until the red vapor ceased and no more HBr gas created. The solution was washed by water (300 mL), and basified by 1 M NaOH solution (20 mL×3). The aqueous layer was further extracted by EtOAc (20 mL×2). The organic layer were combined and purified by flash column (10% EtOAc/hexane) to afford, 1,5-dibromo-2,3,4-trimethoxybenzene (880 mg, 42%) and 2,3,4-trimethoxybromobenzene (696 mg, 44%).

Grignard reaction of 18 with 1,5-dibromo-2,3,4-trimethoxybenzene (24)

(a) **Preparation of Grignard reagent.** To a nitrogen atmosphere bottle including of magnesium pieces (70 mg, 2.92 mmol) and one piece of iodine was added, a solution of 1,5-dibromotrimethoxybenzene **24** (720 mg, 2.2 mmol) in dry THF (3 mL) under vigorous stirring. After the reaction was initiated (in 5 min), the solution was continued stirring for another 20 min.

(b) **Grignard Reaction.** To a stirred solution of 2-en-Ebehyde **18** (35 mg, 0.1 mmol) in dry THF (3 mL) at −70°C was added slowly, the gray Grignard reagent above-mentioned (81 μL) and stirred for another 0.5 h. The reaction was quenched by 0.5% HCl (0.5 mL) in H_2O (3 mL) (*Caution!*), and the solution was basified by $NaHCO_3$ to pH=8, and then extracted by diethyl ether (10 mL×2) followed by EtOAc (10 mL). The organic layer were combined, dried ($MgSO_4$), solvent evaporated off to afford a residue (55 mg). Purified by column chromatography to afford **27** (5.8 mg, 12%), **28** (9.1 mg, 16%), **29** (6.5 mg, 12%), **30** (4.3 mg, 8%), and **18** (5.9 mg, 17%).

2-EneEbelacol C (27). Colourless gum, R_f (50% EtOAc/hexane) 0.36; ν_{max} (KBr) 3626 (OH), 3045, 3019, 1775, 1611, 1518, 1422, 1355, 1107, 1020, 708 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 6.97 (1H, d, *J*=8.6 Hz, H-5′), 6.65 (1H, d, *J*=8.6 Hz, H-6′), 5.38 (1H, m, H-2), 5.35 (1H, m, H-3), 4.80 (1H, dd, *J*=8.6, 3.8 Hz, H-20), 4.41 (1H, d, *J*=10.1 Hz, H-17), 4.37 (1H, d, *J*=10.3 Hz, H-17′), 3.90 (3H, s, O*Me*), 3.84 (s, 3, O*Me*), 3.83 (s, 3, O*Me*), 3.23 (1H, d, *J*=18.5 Hz, H-15), 2.61 (1H, d, *J*=18.5 Hz, H-15′), 1.01 (3H, s, Me-18), 0.94 (3H, s, Me-21), 0.86 (3H, s, Me-22), 0.80 (3H, s, Me-19); δ_H (100.6 MHz, $CDCl_3$) 178.6 (C-16), 153.3 (C-3′), 151.1 (C-4′), 141.7 (C-2′), 137.9 (C-3), 129.6 (C-1′), 121.0 (C-2), 107.4 (C-5′), 72.0 (C-20), 70.1 (C-17), 60.7 (O*Me*), 60.6 (O*Me*), 56.0 (O*Me*), 51.7 (C-5), 51.3 (C-9), 45.4 (C-14), 40.8 (C-1), 40.6 (C-4), 36.2 (C-8), 34.7 (C-10), 33.2 (C-7), 34.7 (C-15), 31.6 (C-13), 29.3 (C-22), 26.4 (C-12), 22.5 (C-18), 20.3 (C-11), 19.0 (C-6), 17.4 (C-21), 16.1 (C-19); HRMS (EI): M^+, found 526.2934. $C_{31}H_{42}O_6$ requires 510.9826.

2-EneEbelacol D (28). Colourless gum, R_f (50% EtOAc/hexane) 0.37; ν_{max} (KBr) 3589 (OH), 3038, 1750, 1612,

1502, 1351, 1299, 1057, 890, 656 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.35 (s, 1H, H-6′), 5.39 (1H, m, H-3), 5.06 (1H, d, *J*=3.9 Hz, H-20), 4.95 (1H, d, *J*=9.8 Hz, H-17), 4.39 (1H, d, *J*=9.8 Hz, H-17′), 3.89 (6H, s, 2×O*Me*), 3.87 (3H, s, O*Me*), 2.74 (1H, d, *J*=18.6 Hz, H-15), 2.58 (1H, d, *J*=17.8 Hz, H-15′), 0.95 (3H, s, *Me*-18), 0.93 (3H, s, *Me*-21), 0.79 (3H, s, Me-22), 0.73 (3H, s, Me-19); HRMS (EI): M^+, found 587.8845. $C_{31}H_{41}O_6Br$ requires 587.8852.

2-EneEbelacol E (29). Colourless gum, R_f (50% EtOAc/hexane) 0.38; ν_{max} (KBr) 3593 (OH), 3060, 1748, 1616, 1511, 1348, 1305, 1067, 884 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 6.94 (1H, d, *J*=8.6 Hz, H-5′), 6.63 (1H, d, *J*=8.6 Hz, H-6′), 5.38 (1H, m, H-3), 5.36 (1H, m, H-3), 5.11 (1H, d, *J*=2.4 Hz, H-20), 4.96 (1H, d, *J*=9.7 Hz, H-17), 4.40 (1H, d, *J*=9.5 Hz, H-17′), 3.84 (3H, s, O*Me*), 3.83 (3H, s, O*Me*), 3.75 (s, 3H, O*Me*), 2.73 (1H, d, *J*=18.6 Hz, H-15), 2.63 (1H, d, *J*=18.5 Hz, H-15′), 0.95 (3H, s, Me-18), 0.93 (3H, s, Me-21), 0.87 (3H, s, Me-22), 0.78 (3H, s, Me-19); δ_C (100.6 MHz, $CDCl_3$) 178.0 (C-16), 150.3 (C-3′), 148.8 (C-4′), 146.0 (C-2′), 137.8 (C-3), 134.9 (C-1′), 121.1 (C-2′), 111.2 (C-5′), 70.3 (C-17), 66.8 (C-20), 60.9 (O*Me*), 60.7 (O*Me*), 51.6 (O*Me*), 51.5 (C-9), 51.2 (C-5), 44.9 (C-14), 40.9 (C-1), 40.7 (C-4), 36.3 (C-8), 34.7 (C-15), 34.3 (C-10), 33.2 (C-7), 31.6 (C-22), 29.3 (C-13), 22.6 (C-18), 20.2 (C-11), 19.6 (C-12), 19.2 (C-6), 17.7 (C-21), 16.6 (C-19); HRMS (EI): M^+, found 510.9825. $C_{31}H_{42}O_6$ requires 510.9826.

2-EneEbelacol F (30). Colourless gum, R_f (50% EtOAc/hexane) 0.41; ν_{max} (KBr) 3615 (OH), 3047, 1749, 1618, 1510, 1344, 1301, 1053, 1019 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.08 (1H, d, *J*=8.6 Hz, H-5′), 6.64 (1H, d, *J*=8.6 Hz, H-6′), 5.39 (1H, m, H-2), 5.36 (1H, m, H-3), 5.09 (1H, brs, H-20), 4.98 (1H, d, *J*=9.8 Hz, H-17), 4.39 (1H, d, *J*=9.7 Hz, H-17′), 3.91 (s, 3H, O*Me*), 3.84 (s, 3H, O*Me*), 3.83 (s, 3H, O*Me*), 2.73 (1H, d, *J*=18.7 Hz, H-15), 2.65 (1H, d, *J*=18.6 Hz, H-15′), 0.94 (3H, s, Me-18), 0.93 (3H, s, Me-21), 0.86 (3H, s, Me-22), 0.78 (3H, s, Me-19); δ_C (100.6 MHz, $CDCl_3$) 178.1 (C-16), 149.4 (C-4′), 137.8 (C-3′), 137.8 (C-3), 130.5 (C-1′), 120.7 (C-2), 120.6 (C-2′), 106.5 (C-5′), 70.4 (C-17), 67.2 (C-20), 60.9 (O*Me*), 60.7 (O*Me*), 51.7 (C-9), 51.6 (O*Me*), 51.3 (C-5), 45.1 (C-14), 40.9 (C-1), 40.7 (C-4), 36.2 (C-8), 34.7 (C-15), 34.2 (C-10), 33.2 (C-10), 31.7 (C-22), 29.3 (C-13), 22.7 (C-18), 20.3 (C-11), 19.8 (C-12), 17.7 (C-21), 16.1 (C-19); HRMS (EI): M^+, found 510.9830. $C_{31}H_{42}O_6$ requires 510.9826.

2-EneEbelacone A (31). Colourless gum, R_f (50% EtOAc/hexane) 0.65; ν_{max} (KBr) 3015, 2942, 2918, 1776, 1689, 1644, 1621, 1505, 1465, 1382, 1182, 1100, 858, 697 cm^{-1}; UV: 282 nm (ϵ 19,200); δ_H (400 MHz, $CDCl_3$) 7.29 (1H, d, *J*=8.8 Hz, H-6′), 6.68 (1H, d, *J*=9.8 Hz, H-17), 4.41 (1H, d, *J*=9.6 Hz, H-17′), 3.96 (3H, s, O*Me*), 3.88 (3H, s, O*Me*), 3.85 (3H, s, O*Me*), 2.54 (1H, d, *J*=18.7 Hz, H-15), 2.19 (1H, d, *J*=18.7 Hz, H-15′), 1.06 (3H, s, Me-18), 0.98 (3H, s, Me-21), 0.88 (3H, s, Me-22), 0.86 (3H, s, Me-19); δ_C (100.6 MHz, $CDCl_3$) 204.1 (C-20), 177.2 (C-16), 157.3 (C-3′), 152.7 (C-4′), 141.8 (C-2′), 137.9 (C-3), 125.5 (C-1′), 120.9 (C-2), 107.5 (C-5′), 70.6 (C-17), 61.8 (O*Me*), 60.9 (O*Me*), 56.1 (O*Me*), 51.7 (C-5), 50.9 (C-9), 49.8 (C-14), 41.0 (C-1), 40.8 (C-4), 36.2 (C-8), 35.2 (C-15), 34.7 (C-10), 31.8 (C-7), 31.6 (C-13), 29.3 (C-22), 27.1 (C-12), 22.7 (C-18), 22.5 (C-11), 18.7 (C-6), 16.3 (C-21), 15.7 (C-19); HRMS (EI): M^+, found 508.2821. $C_{31}H_{40}O_6$ requires 508.2826.

1-Oxo-2-en-Ebelactonic acid (32). Colourless gum, R_f (33% EtOAc/hexane) 0.25; ν_{max} (KBr) 3507, 3056, 2987, 1774, 1718, 1668, 1221, 1187, 820 cm^{-1}; UV: 225 nm (ϵ 16900); δ_C (400 MHz, $CDCl_3$) 6.35 (1H, d, *J*=9.9 Hz, H-3), 5.68 (1H, d, *J*=9.9 Hz, H-2), 4.90 (1H, d, *J*=10.2 Hz, H-17), 4.43 (1H, d, *J*=10.2 Hz, H-17′), 2.54 (1H, d, *J*=18.6 Hz, H-15), 2.18 (1H, d, *J*=18.6 Hz, H-15′), 1.06 (3H, s, Me-19), 1.04 (3H, s, Me-18), 1.01 (3H, s, Me-21), 0.96 (s, 3H, Me-22); δ_C (100.6 MHz, $CDCl_3$) 205.6 (C-1), 180.7 (C-20), 124.1 (C-2), 153.8 (C-3), 41.2 (C-4), 51.6 (C-5), 18.7 (C-6), 32.6 (C-7), 36.1 (C-8), 45.5 (C-9), 48.2 (C-10), 24.2 (C-11), 27.8 (C-12), 35.6 (C-13), 51.1 (C-14), 35.9 (C-15), 178.0 (C-16), 70.4 (C-17), 18.0 (C-21), 24.4 (C-22); HRMS (EI): M^+, found 374.2097. $C_{22}H_{30}O_5$ requires 374.2094.

Enzyme: Electrophorus acetylcholinesterase (1000–2000/mgprotein) was purchased from Sigma.

Assay of inhibition ration of compounds 1, 5, 6 and 21

(a) The sample was dissolved in 0.1 M phosphate buffer at pH 7.0.

(b) A cellulose dialysis tube, purchased from Visking Co., was boiled for 30 min in 1% Na_2CO_3/1 mM EDTA solution and washed thoroughly with distilled water before use. To this freshly prepared cellulose tube was added 50 mL of 0.1% BSA in 0.1 M phosphate buffer at pH 7.0, 5 units AChE and 1×10^{-8} M samples. 1% MeOH was used in the controlled experiment to replace the inhibitor. The tube was made to stand at room temperature for 1 h, and subjected to a dialyzation against 10 L of 0.1 M phosphate buffer at pH 7.0 in a 4°C cool room. At predetermined time points during dialysis, 1.5 mL of the solution was taken from the tube for assay of AChE activity.

(c) The AChE activity was determined by the colorimetric method of Ellman et.al.[20] An aliquot (20–40 μL) of the working AChE assay system containing 4.8×10^{-4}M acetylthiocholine and 3.2×10-4 M DTNB in 0.1 M phosphate buffer at pH 8.0. The initiate rate of substrate hydrolysis was determined at 412 nm by a double-beam spectrometer (Hitachi model 100–50 with a model 056 recorder) at 25°C.

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