Novel and Neuroprotective Tetranortriterpenoids from Chinese Mangrove *Xylocarpus granatum* Koenig

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Eight new tetranortriterpenoids (1–8) were isolated from the twigs and leaves of the Chinese mangrove plant *Xylocarpus granatum*, together with four related known ones (9–12). The structures of new compounds were elucidated by detailed spectroscopic analysis. The absolute configuration of 9-epi-xylogranatin A (1) was determined by time-dependent density functional theory-electronic circular dichroism (TDDFT-ECD) calculations of the solution formers. Xylogranatumin A (2) represents the first example of the 9, 10-seco-limonoid with an unprecedented oxygen-bridged B ring (2,7-dioxabicyclo[2.2.1]-heptane). All the isolates were evaluated for the *in vitro* neuroprotective activity, both compounds 11 and 12 displayed moderate effects against H$_2$O$_2$-induced neurotoxicity in PC12 cells at the concentration of 10 μM, with an increase in cell viability of 12.0% and 11.6%, respectively.

The mangrove plants of the genus *Xylocarpus* (family Meliaceae) are widely distributed in the coastal areas of Southeast Asia, Australia, East Africa, and Indian Ocean. *X. granatum*, one of the three *Xylocarpus* species, has been used as a folk medicine in Southeast Asia and India for the treatment of diarrhea, cholera and fever diseases. *X. granatum* was reported as a rich source of limonoids, featuring by their highly diverse and complex polycyclic skeletons. These structurally interesting and challenging molecules have attracted great attention for their total synthesis, bioactivity evaluation, and biosynthetic studies.

In the course of our ongoing search for bioactive substances from Chinese mangrove plants, the twigs and leaves of *X. granatum* were recently collected from Hainan Province, China. A preliminary chemical investigation of them has led to the isolation and characterization of the rearranged pyridine-containing limonoids, phragmalin orthoesters, and apotirucallane protolimonoids. To further explore the bioactive compounds in *X. granatum*, we continued the phytochemical analysis on the minor components in its twigs and leaves, resulting in the isolation and structure elucidation of eight new tetranortriterpenoids, named 9-epi-xylogranatin A (1), xylogranatumin A (2), 6-O-acetyl xylocarpin D (3), 14-hydroxy-14,15-dihydrogranatumin C (4), 30-O-tigloylhainangranatumin J (5), 9-O-methyl xylogranatin R (6), 30-O-acetylhainangranatumin E (7), and 1,2-dihydro-3α-hydroxy-turranolide (8), along with four related known compounds (9–12) (Fig. 1). Among the newly discovered natural products, xylogranatumin A (2) comprises an unprecedented B ring bearing an oxygen bridge between C-1 and C-8. Herein, we report the isolation, structure elucidation, and bioassay results of these compounds, as well as the plausible biosynthetic pathway of compound 2.

**Results and Discussion**

**Isolation and structure elucidation.** The air-dried powdered twigs and leaves (2.0 kg) of *X. granatum* were percolated thoroughly with MeOH at room temperature. The concentrated MeOH extract was partitioned between EtOAc and H$_2$O. The EtOAc-soluble portion was repeatedly chromatographed to afford twelve

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compounds. Four known of them were readily identified by comparison of their spectroscopic data with those reported in the literature to be xylogranatin A (9)⁴, xylocarpin D (10)¹⁷, xylocarpin B (11)¹⁷, and xylocarpin G (12) (Fig. 1).¹⁷ On the basis of careful analysis of NMR data, and by comparison with the known compounds, the eight new natural products were determined to be tetranortriterpenoids. Among them, 1–6 all showed characteristic signals of a α-furyl ring at C-17 position, whereas compound 7 possessed a γ-hydroxybutenolide group instead. At the same position, while differed from 1–7, compound 8 exhibited a γ-butyrolactone moiety. Accordingly, the detailed structure elucidation of these new molecules is described as follows.

9-epixylogranatin A (1) was isolated as a colorless gum. The molecular formula of 1 was determined to be C₃₄H₄₂O₁₂ by HRESIMS (m/z 665.2554 [M + Na]⁺, calcd. 665.2574), corresponding to the molecular formula C₃₄H₄₂O₁₂, which is the same as that of the co-occurring tetranortriterpenoid, xylogranatin A (9), previously isolated from the seeds of the same species.⁴ The NMR data of 1 showed diagnostic signals of a furan ring [δC 109.8, 119.7, 141.2, and 142.9; δH 6.34 (1H, d, J = 0.9 Hz), 7.41 (1H, s), and 7.41 (1H, t, J = 0.9 Hz)], an α,β-unsaturated lactone group (δC 117.7, 163.7, and 165.5; δH 6.12 s), and a tetrasubstituted double bond (δC 119.7, C-10 and 136.6, C-1). Detailed analysis of 1D (Tables 1 and 2) and 2D NMR (Fig. 2) spectra revealed that the planar structure of 1 was identical to that of 9.⁴ In fact, the 1³C NMR data from C-3 to C-7 and C-16 to C-23 of 1 are almost the same as those of 9, and the main differences at C-1, C-2, C-3, C-5, C-13, C-17, and C-30 were elucidated to be the same as those of 9 by the comparison of their ROESY spectral results, as well as by analysis of the coupling constants and splitting patterns of H-2 (δH 3.30, dd, J = 11.1, 3.8 Hz), H-3 (δH 5.02, d, J = 3.8 Hz), and H-30 (δH 5.10, d, J = 11.1 Hz). NOE correlations observed of H-2/H-3, H-2/CH₃-28, H-3/CH₃-28, and H-3/Ac-30 (Fig. 2) suggested that both H-2 and H-3 were axial orientation, whereas H-3 was equatorial orientation, which further supported the relative configurations of H-2, H-3, and H-30. The diagnostic NOE correlations of H-30/H₂-11 and H-30/H₂-12 revealed that rings B and C were cis-fused, and both 8-OH and 9-OH were α-oriented. Thus, compound 1 was established as 9-epimer of xylogranatin A (9).

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Figure 1. Structures of compounds 1–17.
With the aim to determine the absolute configuration of 1, time-dependent density functional theory electronic circular dichroism calculations (TDDFT-ECD) of its solution conformers were carried out. The ECD spectrum of 1 was recorded in acetonitrile (MeCN), which showed positive, negative and positive Cotton effects (CEs) at 270, 243 and 225 nm, respectively. The initial Merck Molecular Force Field (MMFF) conformational analysis of the arbitrarily chosen (2R,3R,5S,8S,9R,13R,17R,30R) absolute configuration of 1 resulted in 151 conformers, which were re-optimized at B3LYP/6-31G(d) level of theory in vacuo, as well as at B3LYP/TZVP level with Polarizable Continuum Model (PCM) solvent model for MeCN. The gas-phase re-optimization afforded 9 conformers with Boltzmann population above 2%, while the PCM one yielded 14 geometries above 2% (see S46 in supporting information). These structures were used as input for ECD calculations. The C-3 tigloyl group is more suitable for an axial orientation in the low-energy conformers (conformers A-H), which is in accordance with the

Table 1. 1H NMR spectroscopic data for compounds 1–8a. aSpectra measured at 400 MHz in CDCl3.

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coupling constant between H-2 and H-3 (\(J_{2,3} = 3.8\) Hz). Ring B possesses a twist-boat conformation with equatorial 30-OAc group in most of the conformers, which is in agree with the coupling constants between H-3 and H-30 (\(J_{3,30} = 11.1\) Hz). As to prevent the side effects of the solvent, the ECD spectra were also calculated for the (2R, 3R, 5S, 7S, 8R, 9R, 13R, 17R, 30R) enantiomers with the same functions (B3LYP, BH&HLYP, PB08) and TZVP basis set for both the in vacuo and the solvent model re-optimized structures. The computed ECD curves of the individual conformers were quite similar to each other, and as well as to the experimental ECD spectrum, since their rings D and E share the same orientation. Moreover, all the Boltzmann-weighted ECD spectra reproduced well the experimental ECD curve with BH&HLYP/TZVP PCM/McCN giving the most agreement (Fig. 3). Thus, the absolute configuration of 1 was unambiguously determined as (2R, 3R, 5S, 7S, 8R, 9R, 13R, 17R, 30R). To our best knowledge, this is the first report on the configurational assignment of limonoids with 9, 10-seco skeleton by TDDFT ECD calculations.

The related xylogranatin A (9) was only reported for its relative configuration by the X-ray diffraction analyses, while as it shared the similar planar structure as that of 9-epi-xylogranatin A (1), the absolute configuration of 9 could then be determined by comparing their ECD spectra, which rely upon the unsaturated \(\delta\)-lactone and furan chromophores of ring D governed by the C-13 and C-17 chiralities. The similar ECD pattern of 9 [225 nm (\(\Delta e + 23.66\)), 269 nm (\(\Delta e + 1.57\)) in MeCN] and 1 indicated the same (13R, 17R) absolute configuration for both compounds. Thus the absolute configuration of xylogranatin A (9) can be deduced as (2R, 3R, 5S, 7S, 8R, 13R, 17R, 30R), which could further allow the absolute configurational assignment of the related xylogranatins B–D.

The molecular formula of xylogranatumin A (2), \(C_{29}H_{40}O_{12}\), was deduced by HRESIMS with ion peak at \(m/z\) 627.2431 [\(M + Na\)^+] (calcd 624.2417), suggesting the presence of twelve degrees of unsaturation. In the \(^1H\) NMR spectrum, resonances arising from seven methyl groups were observed, including two geminal (\(\delta 0.92\) and 0.94, each 3H, s), one terminal (\(\delta 1.08\), 3H, s), and one secondary (\(\delta 1.02\), 3H, s, d, \(J = 6.6\) Hz), one methoxyl (\(\delta 3.68\), 3H, s) and two acetyl groups (\(\delta 2.11\) and 2.15, each 3H, s). Diagnostic proton signals represent a \(\beta\)-furan ring (\(\delta 6.38, 7.26, 7.41, 7.43\)) was also obtained. The \(^{13}C\) NMR spectrum of 2 disclosed seven methyl groups, four sp\(^3\) methylenes, three sp\(^2\) methines, seven sp\(^3\) methines, and ten quaternary carbons (four carbonyl groups). With regard to the 12 degree of unsaturation, and bearing in mind the presence of a furan ring and four carbonyl groups, xylogranatumin A (2) should possess five other rings.

The aforementioned data implied that compound 2 bore a similar skeleton as that of compound 1. The comparison of the 1D and 2D NMR data of 2 with those of the co-occurring limonoid 1 indicated that they shared the similar rings C, D, and E (Fig. 4). Correspondingly, the construction of rings A and B was important for the structure determination of 2. Two proton-bearing partial structures of C-19 \(\rightarrow\) C-10 \(\rightarrow\) C-5 \(\rightarrow\) C-6 and C-3 \(\rightarrow\) C-2 \(\rightarrow\) C-30 were readily recognized from the \(^1H\) COSY spectrum (Fig. 5). These two structural segments were connected to the quaternary carbons, C-1 and C-4, respectively, by the observation of HMBC and two acetyl groups (\(\delta 2.11\) and 2.15, each 3H, s). Diagnostic proton signals represent a \(\beta\)-furan ring (\(\delta 6.38, 7.26, 7.41\)) was also obtained. The \(^{13}C\) NMR spectrum of 3 disclosed seven methyl groups, four sp\(^3\) methylenes, three sp\(^2\) methines, seven sp\(^3\) methines, and ten quaternary carbons (four carbonyl groups). With regard to the 12 degree of unsaturation, and bearing in mind the presence of a furan ring and four carbonyl groups, xylogranatumin A (2) should possess five other rings.

The relative configuration of compound 2 was elucidated by the analysis of ROESY spectrum and proton coupling constants, as well as by analogy with that of I. The same relative stereochemistry of C-2, C-3, C-5, C-13 and C-17 in 2 was deduced from the similar carbon chemical shifts, proton coupling constants, and ROESY correlations with those of I and the known compounds 10–16 (for C-13 and C-17), as well as by a biogenetic consideration of such limonoids in Nature. In addition, the NOE correlations between H-5 and CH\(_2\)-19, H-10 and CH\(_2\)-28, CH\(_2\)-28 and H-3, H-3 and H-2, H-14 and CH\(_2\)-18, H-14 and H-30, H-30 and OH-9 (Fig. 4) further confirmed the relative configuration of 2R, 3R, 5S, 7S, 8R, 9S, 10R, 13R, 14R, 17R, 30S. Although the relative configurations of C-1 and C-8 cannot be determined by distinct ROE correlations, the correlation between H-30 and OH-9 linked to C-9, which was further connected to C-8. The methoxyl group was located at C-7 on the basis of the HMBC cross-peak from MeO- to C-7, and the assignment of the two acetyl groups at C-3 and C-30 were clearly indicated by HMBC correlations of H-3 (\(\delta 5.52\), 1H, d, \(J = 3.5\) Hz) to \(\delta 169.8\) and H-30 (\(\delta 5.30\), 1H, d, \(J = 3.5\) Hz) to \(\delta 170.2\) (Fig. 4).

In terms of ring B, the presence of one acetal carbon at \(\delta 109.2\) (C-30, sp\(^3\)), one oxygenated quaternary carbon signal at \(\delta 89.6\) (C-8, sp\(^3\)), a typical hemiacetal carbon at \(\delta 99.8\) (C-9, and a tertiary oxygenated carbon at \(\delta 71.3\) (C-30) suggested that ring B was constructed via the C-8 \(\rightarrow\) C-30 \(\rightarrow\) C-2 \(\rightarrow\) C-1 \(\rightarrow\) O \(\rightarrow\) C-9 \(\rightarrow\) C-8 bond to form a tetrahedrpyran ring with an oxygen bridge between C-8 and C-1. The linkage of C-30 to C-8 was also confirmed by the distinct HMBC correlation from H-30 to C-9. A planar structure of 2 was thus proposed as depicted in Fig. 1, which was consistent with its molecular composition and degrees of unsaturation.

The relative configuration of compound 2 was elucidated by the analysis of ROESY spectrum and proton coupling constants, as well as by analogy with that of I. The same relative stereochemistry of C-2, C-3, C-5, C-13 and C-17 in 2 was deduced from the similar carbon chemical shifts, proton coupling constants, and ROESY correlations with those of I and the known compounds 10–16 (for C-13 and C-17), as well as by a biogenetic consideration of such limonoids in Nature. In addition, the NOE correlations between H-5 and CH\(_2\)-19, H-10 and CH\(_2\)-28, CH\(_2\)-28 and H-3, H-3 and H-2, H-14 and CH\(_2\)-18, H-14 and H-30, H-30 and OH-9 (Fig. 4) further confirmed the relative configuration of 2R, 3R, 5S, 7S, 8R, 9S, 10R, 13R, 14R, 17R, 30S. Although the relative configurations of C-1 and C-8 cannot be determined by distinct ROE correlations, the correlation between H-30 and OH-9 linked the \(\beta\)-orientation of the oxygen bridge due to the smaller transannular strain.

Biogenetically, this interesting molecule might be derived from hainangranatumin D (Fig. 5), a limonoid previously isolated from X. granatum with absolute configuration established, by a first plausible weak acid promoted nucleophilic addition of acetoxyl group at C-3 position, which allowed the double bond migration and the epoxidation from C-1 to C-9. The resulting intermediate then underwent a C-1 hydration, followed by a second acid promoted epoxidation from C-1 to C-8 with the elimination of H\(_2\)O. Finally, a C-30 epimerization, which possibly occurred during the previous epoxidation to lower the energy of the molecule, allowed the production of compound 2. Since the relative stereochemistry has been established, the common biosynthetic origin of 2, compound 1, the known compounds 9–16 and hainangranatumin D, suggested the corresponding chiral centers, especially C-13 and C-17 adjacent to the furan core should be the same. Therefore, the absolute configuration of compound 2 was deduced as (15S, 2R, 3R, 5S, 7S, 8S, 9R, 13R, 17R, 30S). Based on the above information, xylogranatumin A (2) was determined as a novel limonoid characterized by a 9, 10-seco skeleton bearing an oxygen-bridge between C-1 and C-8 (Fig. 1), and the discovery of xylogranatumin A provided a new example to the extremely diverse and complex family of limonoids.
6-O-acetyl xylocarpin D (3) gave a HRESIMS pseudomolecular ion peak at m/z 769.2634 [M + Na]+, a plus of 42 mass units on that of the co-occurring xylocarpin D (10), which was previously isolated from the fruits of X. granatum with absolute configuration determined17, indicating the presence of an additional acetyl group in 3, which was further confirmed by the careful comparison of their 1H and 13C NMR data (Tables 1 and 2), with an observation of additional peaks of δH at 2.19 and δC at 169.6 and 21.0 on 3. The location of the acetyl group at C-6 was established by the expected downfield shifted 1H NMR resonance of H-6 (from δH 4.29 to 5.30). Therefore, compound 3 was determined as the 6-acetyl derivative of xylocarpin D (10).

The HRMS data for 14-hydroxy-14,15-dihydrogranatumin C (4) displayed a pseudomolecular ion peak at 584.2643 [M]+ (calcld 584.2621), consistent with the molecular formula C32H40O10. Detailed analysis of the 1H and 13C NMR data of 4 were reminiscent of those of 13, which was previously isolated from the seeds of a Krishna mangrove, X. granatum14. Their main differences were an oxygenated quaternary carbon at C-14 (δC 62.7) and a

| No. | 1      | 2      | 3      | 4      | 5      | 6      | 7      | 8      |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|
| 1   | 136.6 qC | 109.2 qC | 87.8 qC | 214.5 qC | 198.7 qC | 199.3 qC | 198.6 qC | 32.6 CH₂ |
| 2   | 36.2 CH  | 55.0 CH  | 46.3 CH  | 48.6 CH  | 130.4 qC | 129.2 qC | 128.4 qC | 25.0 CH₂ |
| 3   | 73.7 CH  | 76.3 CH  | 75.8 CH  | 77.1 CH  | 160.9 CH  | 158.3 CH  | 161.9 CH  | 75.9 CH  |
| 4   | 37.1 qC  | 39.4 qC  | 45.2 qC  | 39.4 qC  | 36.6 qC  | 36.8 qC  | 36.8 qC  | 36.9 qC  |
| 5   | 40.4 CH  | 41.7 CH  | 42.5 CH  | 42.4 CH  | 45.2 CH  | 43.0 CH  | 45.2 CH  | 41.8 CH  |
| 6   | 32.7 CH₃ | 33.6 CH₃ | 71.6 CH  | 33.3 CH₂ | 34.8 CH₂ | 34.7 CH₂ | 34.6 CH₂ | 23.2 CH₂ |
| 7   | 174.5 qC | 174.0 qC | 170.0 qC | 174.1 qC | 173.5 qC | 173.6 qC | 173.4 qC | 75.3 CH  |
| 8   | 75.5 qC  | 89.6 qC  | 73.3 qC  | 62.7 qC  | 201.0 CH  | 197.8 qC | 80.4 qC  | 42.2 CH  |
| 9   | 98.2 qC  | 99.8 qC  | 31.0 CH  | 54.4 CH  | 79.8 qC  | 172.8 qC | 208.2/208.0 qC | 43.0 CH  |
| 10  | 119.7 qC | 36.5 CH  | 47.2 qC  | 48.2 qC  | 43.2 CH  | 45.6 CH  | 42.8 CH  | 37.4 CH  |
| 11  | 28.2 CH₃ | 33.0 CH₃ | 28.8 CH₂ | 18.5 CH  | 29.5 CH  | 29.4 CH  | 32.9 CH  | 16.2 CH  |
| 12  | 25.5 CH₃ | 27.1 CH₃ | 71.0 CH  | 33.1 CH  | 28.6 CH  | 29.8 CH  | 25.6 CH  | 33.8 CH  |
| 13  | 38.3 qC  | 36.1 qC  | 39.3 qC  | 40.3 qC  | 41.3 qC  | 41.4 qC  | 38.6/38.5 qC | 46.6 CH  |
| 14  | 163.7 qC | 35.6 CH  | 46.4 CH  | 71.8 qC  | 156.6 qC | 158.9 qC | 162.5 qC | 159.6 qC |
| 15  | 117.7 CH | 29.0 CH  | 28.0 CH  | 40.8 CH  | 121.2 CH | 124.4 CH | 117.7 CH | 118.0 CH |
| 16  | 165.5 qC | 171.2 qC | 169.7 qC | 169.5 qC | 163.4 qC | 163.4 qC | 164.5/164.3 qC | 34.8 CH  |
| 17  | 81.1 CH  | 79.4 CH  | 76.8 CH  | 78.4 CH  | 81.4 CH  | 78.5 CH  | 78.2/77.9 CH | 58.1 CH  |
| 18  | 18.8 CH₃ | 24.4 CH₁ | 18.8 CH₁ | 20.1 CH₁ | 18.6 CH₁ | 20.4 CH₁ | 18.4/18.3 CH₁ | 19.3 CH₁ |
| 19  | 12.8 CH₃ | 10.8 CH₃ | 22.6 CH₁ | 16.4 CH₁ | 12.2 CH₁ | 11.7 CH₁ | 11.6 CH₁ | 15.4 CH₁ |
| 20  | 119.7 qC | 120.9 qC | 120.7 qC | 120.1 qC | 119.2 qC | 119.6 qC | 132.9 qC | 37.5 CH  |
| 21  | 141.2 CH | 140.8 CH | 140.4 CH | 141.2 CH | 141.4 CH | 141.6 CH | 169.1/168.8 qC | 72.6 CH₂ |
| 22  | 109.8 CH | 109.9 CH | 109.1 CH | 110.4 CH | 109.7 CH | 109.8 CH | 150.4/149.7 CH | 34.1 CH  |
| 23  | 142.9 CH | 143.3 CH | 143.9 CH | 142.9 CH | 143.3 CH | 143.4 CH | 97.4/96.7 CH | 176.8 CH |
| 24  | 24.8 CH₁ | 24.4 CH₁ | 15.6 CH₁ | 23.0 CH₁ | 20.6 CH₁ | 20.1 CH₁ | 27.8 CH₁ | 21.9 CH₁ |
| 25  | 20.1 CH₁ | 20.9 CH₁ | 41.0 CH₁ | 20.7 CH₁ | 28.0 CH₁ | 28.2 CH₁ | 20.6 CH₁ | 28.0 CH₁ |
| 30  | 68.9 CH  | 71.3 CH  | 69.7 CH  | 62.8 CH  | 66.8 CH  | 41.3 CH  | 67.8 CH  | 27.5 CH  |
| 7′ OMe| 52.0 CH₂ | 51.9 CH₂ | 53.0 CH₁ | 52.4 CH₂ | 52.0 CH₁ | 52.0 CH₂ | 52.0 CH₁ |
| 9′ OMe| 51.4 CH₁ |             |             |             |             |             |             |             |
| 7′′ OAc| 51.4 CH₁ |             |             |             |             |             |             |             |
| 1′′′ OAc| 21.0 CH₁ |             |             |             |             |             |             |             |
| 1′′′′′ OAc| 21.0 CH₁ |             |             |             |             |             |             |             |
| 1′′′′′′′ OAc| 21.0 CH₁ |             |             |             |             |             |             |             |

Table 2. 13C NMR spectroscopic data for compounds 1–8. *Spectra measured at 100 MHz in CDCl₃.
Figure 2. Key $^1$H-$^1$H COSY, HMBC, and ROESY correlations of 1.

Figure 3. Experimental solution ECD spectrum of 9-epixylogranatin A (1) and BH&HLYP/TZVP PCM/MeCN calculated ECD spectrum of (2R, 3R, 5S, 8R, 9S, 13R, 17R, 30R)-1 calculated for the low-energy solution conformers. Bars represent the calculated rotational strengths of the lowest-energy conformer.

Figure 4. Key $^1$H-$^1$H COSY, HMBC, and ROESY correlations of 2.
methylene carbon at C-15 ($\delta_C$ 40.8; $\delta_H$ 3.53, 1H, $d$, $J = 16.0$ Hz; 2.75, 1H, $d$, $J = 16.0$ Hz) in 4 in place of the $\Delta^{14,15}$ double bond ($\delta_C$ 160.9 and 118.8) in 13. These data indicated that the $\Delta^{14,15}$ double bond was hydrated in 4, which is in full agreement with a plus of eighteen mass units of 4 on that of 13. The HMBC correlations from H-15 to C-14 and C-16, and CH$_3$-18 to C-14 gave further support to the structural assignment of 4.

The relative configuration of 4, except C-14 position, was determined to be the same as that of 13 due to the similar $^{13}$C NMR shifts and coupling constants in $^1$H NMR and further confirmed by ROESY experiments. Unfortunately, the absence of the proton signal of OH-14 in 4, which were usually presented in the NMR spectra of the limonoids when measuring in CDCl$_3$, such as Granaxylocarpin C 20, bearing the similar substructure as compound 4, prevented us from assigning the configuration of C-14 position via the available NMR data.

30-O-tigloylhainangranatum J (5) was isolated as an optically active white amorphous powder. The molecular formula, C$_{32}$H$_{38}$O$_{10}$, was established by HRESIMS from the ion peak at 605.2346 [M+Na]$^+$. Its $^1$H and $^{13}$C NMR data (Tables 1 and 2) were closely related to those of hainangranatum J (14)$.^9$ The only difference was the replacement of the 30-O-isobutyryl group in 14 by a tigloyl moiety ($\delta_H$ 6.78, $q$, $J = 6.8$ Hz, 1.80, $d$, $J = 6.8$ Hz, and 1.77, $s$; $\delta_C$ 166.6 $qC$, 127.6 $qC$, 139.8 CH, 14.6 CH$_3$, and 11.9 CH$_3$) in 5. Therefore, the structure of 30-O-tigloylhainangranatum J (5) was determined as shown in Fig. 1.

The molecular formula of 9-O-methyl xylogranatin R (6), C$_{28}$H$_{34}$O$_{9}$, was deduced by HRESIMS ($m/z$ 537.2100, calcd for [M+Na]$^+ 537.2101$). The $^1$H and $^{13}$C NMR spectra of 6 were almost identical to those of xylogranatin R (15), which was isolated as an antifeedant from the seeds of the same species with the absolute configuration established$^5$, except for the presence of an additional methoxy group ($\delta_H$ 3.68; $\delta_C$ 51.8), suggesting that 6 was an O-methyl derivative of 15, in agreement with an addition of 14 mass units in 6 to that of 15. The chemical shift of C-9 ($\delta_C$ 172.8) in 6 was upfield shifted $\Delta \delta 2.7$ from that of 15, indicating the carboxylic acid at C-9 was esterified to methyl ester, which was further confirmed by the HMBC correlation from 9-OCH$_3$ ($\delta_C$ 3.67) to the carbonyl carbon at C-9 ($\delta_C$ 172.8). The complete assignments of the $^1$H and $^{13}$C NMR of 6 were achieved by a comprehensive analysis of 2D NMR spectra including HSQC, COSY, HMBC, and ROESY. Compound 6 was thus determined as the methyl ether of xylogranatin R (15). In view of the presence of methyl ester moiety in a great number of limonoids previously isolated from this species, such as xylogranatins A-D$^4$, and hainangranatumins A-J$^{18}$, the authors believe that compound 6 is an original natural product rather than an artifact.

The molecular formula of 30-O-acetylhainangranatum E (7) was established as C$_{29}$H$_{34}$O$_{12}$ by HRMS ($m/z$ 597.1951, calcd for [M+Na]$^+ 597.1948$). The $\gamma$-hydroxybutenolide group was characterized by proton signals at $\delta_H$ 7.42 (H-22) and 6.24 (H-23), and by carbon signals at $\delta_C$ 132.9 (C-20), 169.1/168.8 (C-21), 150.4/149.7 (C-22), and 97.4/96.7 (C-23) in its $^1$H and $^{13}$C NMR spectra (Tables 1 and 2). The detailed NMR data analysis reminded us those of hainangranatum E (16), previously isolated from the seeds of Hainan mangrove X. granatum$^{14}$. The only differences were the presence of the acetyl group ($\delta_C$ 2.09 s; $\delta_H$ 169.9/169.8 qC, 21.0 CH$_3$) at C-30 in 7 instead of the methylbutyryl group in 16. In addition, two sets of carbon resonances at $\delta$ 208.2/208.0 (C-9), 38.6/38.5

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**Figure 5.** Plausible biosynthetic pathway of xylogranatin A from hainangranatum D.
system added a novel skeleton to the family of tetranortriterpenoids, revealing the high diversity and complexity of such beautiful molecules.

The discovery of the 9,10-

orientation of the hydroxyl at C-3 of 8 was confirmed by the ROESY correlations of H-3/CH3-29 and H-5/CH3-28. The structure of 1,2-dihydro-3α-hydroxy-turranolide (8) was thus determined as the C-3 reductive derivative of turranolide (17).

Similar as that of compound 2, since the relative stereochemistry of the new compounds 3, 5, 6, and 8 has been established, the common biosynthetic origin of such furan limonoids2,4,17,18 suggested the corresponding chiral centers, such as C-13 and C-17 should be the same R configuration. Thus the absolute configuration of the above mentioned new compounds could be arbitrary determined as shown in Fig. 1.

In summary, eight new tetraterpenoids (1–8) together with four related known compounds (9–12) were isolated from the twigs and leaves of Chinese mangrove plant X. granatum. The structures of new compounds were elucidated by extensive spectroscopic analysis. The absolute configuration of 9-epi-xylogranatin A (1) was determined by TDDFT ECD calculations, which incidentally allowed the elucidation of the absolute configurations of xylogranatin A (9) by comparison with their ECDs, solving a puzzle of the previously reported natural products. The discovery of the 9, 10-sco limonoid 2 with a characteristic 2,7-dioxabicycle [2.2.1] heptane ring system added a novel skeleton to the family of tetraterpenoids, revealing the high diversity and complexity of such beautiful molecules.

Neuroprotective activity evaluation. In the light of a wide range of biological activities and pharmacological properties of limonoids2,8, we performed in vitro investigation of neuroprotective activity of compounds 1–12 on PC12 cells, since the isolated protolimonoids by us from Toona ciliata var. pubescens displayed significant cell protecting activity11. Both compounds 11 and 12 showed moderate neuroprotective effects against H2O2-induced neurotoxicity in PC12 cells at the concentration of 10 μM, with an increase in cell viability of 12.0% and 11.6%, respectively. N-Acetyl-L-cysteine (NAC) was used as the positive control with the increase in cell viability of 22.0% at 10 μM. In comparison with the tested structures, it is possible that the variation of rings A and B of these limonoids play an important role for the neuroprotective activity.

Methods
General experimental procedures. Optical rotations were measured on a Perkin-Elmer polarimeter 341. CD spectra were obtained on a JASCO 810 spectrometer. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. The NMR spectra were measured on Bruker DRX 400 and Varian Inova 600 spectrometers. Chemical shifts (δ) are reported with the residual CDCl3 (δH = 7.26 ppm) as the internal standards for the 1H NMR spectroscopy, and CDCl3 (δC = 77.0 ppm) for the 13C NMR spectroscopy. Chemical shifts were expressed in δ (ppm) and coupling constants (J) in Hz. 1H and 13C NMR assignments were supported by 1H–1H COSY, HSQC, HMBC and ROESY experiments. ESIMS and HRESIMS spectra were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. Reversed-phase HPLC analysis was performed on an Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semi-preparative ZORBAX ODS column (250 mm × 9.4 mm i.d., 5 mm particle size. Commercial silica gel (Qing Dao Hai Yang Chemical Group Co., 200–300 mesh) was used for column chromatography (CC), and precoated silica gel plates (Y an Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Plant materials. The twigs and leaves of X. granatum (2.0 kg) were collected in December 2009 from Dongzhai Harbor, Hainan province, China, and identified by Professor Guo-Rong Xin of Institute of Biological Science, Sun Yat-Sen University. A voucher specimen (NO. 09-P-69) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation. The air-dried powdered twigs of X. granatum were percolated with MeOH (three times, each 7 days) at room temperature. The extract was evaporated to dryness under reduced pressure to give 154.5 g of residue. The residue was partitioned with EtOAc to afford 29.7 g EtOAc extract. The EtOAc extract was separated by Sephadex LH-20, MCI and silica gel column to obtain five fractions (1–5). Fr.3 was subjected to column silica gel liquid chromatography, eluting with CHCl3/MeOH (from 100:1 to 8:2, gradient), to obtain 15 fractions (3A-3O). Fraction 3C (65.3 mg) was chromatographed on silica gel with petroleum ether/acetone (8:2 to 1:1, gradient) to yield 7 (8.6 mg), and the sub-fraction 3C1. Sub-fraction 3C1 was purified by HPLC (75:25) to afford 5 (1.8 mg). Fraction 3D was separated by silica gel column with the eluent of petroleum ether/acetone (8:2 to 7:3, gradient) and subsequently purified by semi-preparative-HPLC eluting with MeOH/H2O (70:30) to afford 4 (2.1 mg) and 6 (2.3 mg). Fraction 3E (2.17 g) was subjected to column chromatography on silica gel eluted with a gradient of petroleum ether/acetone (9:1 to 1:1) to give nine major fractions 3E0–3E9. 3E7 was separated by silica gel column with petroleum ether/acetone (8:2 to 7:3) to afford 12 (30.0 mg) and the sub-fraction 3E7a, which was purified by reversed phase HPLC (CH3CN/H2O, 67:33) to yield 8 (5.4 mg), while compounds 3 (1.0 mg) and 11 (4.8 mg) was prepared by column chromatography on silica gel eluted with petroleum ether/acetone (7:3) from fraction 3E9. Fractions 3G was first separated by column chromatography on silica gel eluted with petroleum ether/acetone (8:2 to 6:4, gradient) to give six sub-fractions (3G1–3G6). 3G3 was purified by reversed phase
Chemical structure data. All investigated compounds were ≥95% pure (HPLC, wavelength = 210 nm).

The NMR spectra of the compounds are provided in the Supporting Information.

9-epixylogranatin A (1). Colorless gum, [α]D 20 +48 (c 0.12, CH3CN); ECD (CH3CN) λmax (Δε): 270 (2.23), 243 (−0.26), 225 (4.45), 206 (−1.53), 200sh (−1.43) IR (KBr) νmax 3432, 2924, 1725, 1381, 1261 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 665.2554 (calcd. for C34H42O12Na, 665.2574).

Xylogranatin A (2). Colorless gum, [α]D 20 +15.0 (c 0.08, CH3CN); ECD (CH3CN) λmax (Δε): 254 (0.12), 235 (−0.51), 212 (1.12) IR (KBr) νmax 3436, 2925, 1726, 1374, 1262, 1106 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 627.2431 (calcd. for C34H40O12Na, 627.2417).

6-O-acetyl xyloroacin D (3). White, amorphous powder, [α]D 20D +4.3 (c 0.07, CH3CN); UV (MeOH) λmax 213 nm. IR (KBr) νmax 3419, 2910, 2851, 1743, 1373, 1223, 1041 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 679.2634 (calcd. for C34H40O12Na, 679.2684).

14-Hydroxy-11,15-dihydrogranatinin C (4). White, amorphous powder, [α]D 20 +22.5 (c 0.04, MeOH); UV (MeOH) λmax 212 nm. IR (KBr) νmax 3438, 2962, 1733, 1262, 1098, 1024, 802 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 584.2643 (calcd. for C29H35O10Na, 584.2621).

30-O-tigloyl xianhiangranatinanum A (5). White, amorphous powder, [α]D 20 +22.2 (c 0.09, MeOH); UV (MeOH) λmax 210 nm. IR (KBr) νmax 3439, 2962, 1735, 1671, 1383, 1260, 1166, 1026 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 605.2346 (calcd. for C35H42O12Na, 605.2343).

9-O-methyl xylogranatin R (6). White, amorphous powder, [α]D 20 +49.1 (c 0.05, MeOH); UV (MeOH) λmax 211 nm. IR (KBr) νmax 3432, 2919, 1735, 1630, 1091, 1046 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 537.2100 (calcd. for C29H34O11Na, 537.2101).

30-O-acetyl xylogranatinanum E (7). Colorless gum, [α]D 20 +38.9 (c 0.19, MeOH); UV (MeOH) λmax 206 nm. IR (KBr) νmax 3437, 2965, 1735, 1671, 1373, 1262, 1230, 1018 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 597.1951 (calcd. for C31H37O12Na, 597.1948).

1,2-Dihydro-3o-hydroxy-turranolide (8). Colorless gum, [α]D 20 +17.6 (c 0.11, MeOH); IR (KBr) νmax 3437, 2923, 1782, 1725, 1378, 1259, 1033 cm⁻¹; for 1H NMR and 13C NMR spectroscopic data, see Tables 1 and 2; HREIMS m/z [M+Na]+ 481.2892 (calcd. for C27H36O9Na, 481.2930).

Neuroprotective bioassay. The neuroprotective activities of compounds 1–12 against hydrogen peroxide (H2O2)-induced neurotoxicity in PC12 cells were evaluated by using the MTT method25, according to the protocols described in previous literature.

Computational section. Mixed torsional/low mode conformational searches were carried out by means of the Macro model 9.7.223 software26 using Merck Molecular Force Field (MMFF) with an implicit solvent model for chloroform. Reoptimizations at B3LYP/6-31G(d) level of theory in vacuo as well as B3LYP/TZVP basis set of the Gaussian 09 package24. Boltzmann distributions were estimated from the ZPVE corrected B3LYP energies in the gas-phase calculations and from the B3LYP energies in the PCM ones. ECD spectra were generated as the sum of Gaussians25 with 2400 and 3000 cm⁻¹ half-height width (corresponding to ca. 16 and 20 nm at 260 nm, respectively), using dipole-velocity computed rotational strengths. The MOLEKEL software package27 was used for visualization of the results.

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Author Contributions
Z.-F. Zhou conducted the main experiments, data analyzes, and wrote the manuscript; T.K. and A.M. performed the ECD calculations; Y.-C. Gao assisted the biological test; L.-G. Yu assisted the sample collection and extraction; G.-R. Xie assisted the identification of the sample species; X.-W. Li and Y.-W. Gao designed the experiments, revised and polished the manuscript. All authors reviewed the manuscript.

Additional Information
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