Protolimonoids and Limonoids from the Chinese Mangrove Plant *Xylocarpus granatum*

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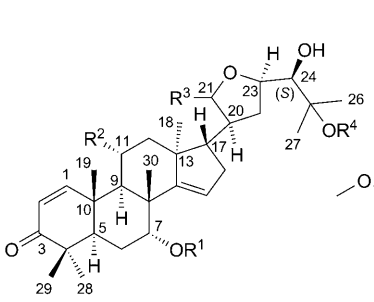
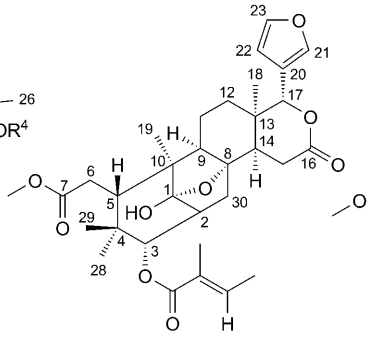
The phytochemical examination of the fruits of a Chinese mangrove plant *Xylocarpus granatum* led to the isolation of five new protolimonoids, protoxylocarpins A – E (**1** – **5**, resp.), and two new limonoids xylocarpins J and K (**6** and **7**, resp.), together with xyloccensins M and Y (**9** and **10**, resp.). Their structures were determined on the basis of extensive spectroscopic data analysis. The cytotoxic activity of the isolated compounds against tumor cell-lines HCT-8, Bel-7402, BGC-823, and A2780 were evaluated.

Introduction. – The mangrove plant *Xylocarpus granatum* KOENIG (Meliaceae) is used as a folk medicine in South-East Asia for the treatment of diarrhea, cholera, fever diseases, such as malaria and also as an antifeedant [1][2]. *X. granatum* is a rich source of structurally unique limonoids with various structural patterns, which could be classified into phragmalin, mexicanolide, and andirobin-types based on their parent ring rearrangements [3–23]. Naturally occurring limonoids normally contain a furan ring linked to C(17) of the *D*-ring, while the positions at C(3), C(4), C(7), C(16), and C(17) are frequently oxygenated. Limonoids have been found in all *Xylocarpus* plants investigated so far. They are stereochemically homogenous compounds with a prototypical structure either containing or derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton [2]. Previous chemical investigation of *X. granatum* growing in different mangrove regions resulted in the isolation and characterization of more than 40 limonoid derivatives. In continuation of our previous chemical examination [7][16], nine additional compounds were isolated as minor components, of which protoxylocarpins A – E (**1** – **5**, resp.) are determined as a group of new apotirucallane triterpenes, while xylocarpins J and K (**6** and **7**, resp.) are new mexicanolide and polyhydroxyphragmalin derivatives, respectively.

Results and Discussion. – Protoxylocarpin A (**1**) was isolated as a white powder, its molecular formula was determined as $C_{32}H_{50}O_6$ on the basis of HR-FAB-MS (*m*/*z* 531.3670, $[M+H]^+$; calc. 531.3679) and NMR data. The IR absorptions at 3508, 1734, and 1699 cm^{-1} suggested the presence of OH and C=O groups. The ^{1}H-NMR data of **1** exhibited seven Me *singlet*s at δ(H) 1.32 (*s*, Me(27)), 1.29 (*s*, Me(26)), 1.19 (*s*, Me(19)),



1 $R^1 = R^2 = R^4 = H$, $R^3 = \alpha$-EtO
2 $R^1 = R^2 = R^4 = H$, $R^3 = \beta$-EtO
3 $R^1 = R^2 = H$, $R^3 = \alpha$-EtO, $R^4 = Et$
4 $R^1 = R^2 = R^4 = H$, $R^3 = \beta$-MeO
5 $R^1 = Ac$, $R^2 = AcO$, $R^3 = \alpha$-MeO, $R^4 = H$



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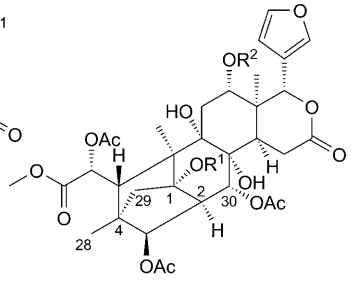
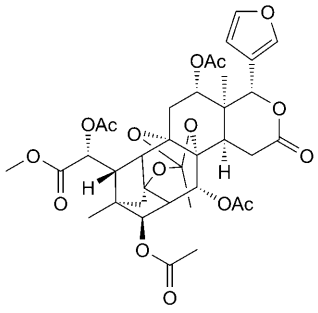


10 $R^1 = R^2 = H$
7 $R^1 = R^2 = Ac$



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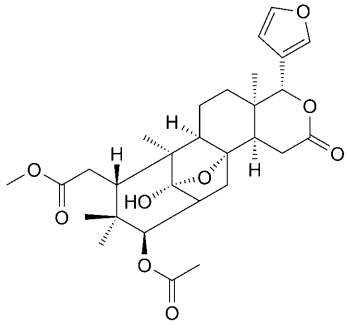

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Me(29)), 1.15 (*s*, Me(30)), 1.09 (*s*, Me(18)), and 1.11 (*s*, Me(28)), two vicinal coupled vinyl H-atoms at δ(H) 7.18 (*d*, $J = 10.0$, H–C(1)) and 5.86 (*d*, $J = 10.0$, H–C(2)), and an olefinic *singlet* at δ(H) 5.51 (br. *s*, H–C(15)). The ^{1}H- and ^{13}C-NMR spectral data (*Table 1*) were characteristic of an apotirucallane triterpene, closely related to those for protolimonoids holstinones A–C [24]. The ^{13}C-NMR resonances at δ(C) 158.2 (*d*, C(1)), 125.5 (*d*, C(2)), 205.1 (*s*, C(3)), 161.6 (*s*, C(14)), and 119.7 (*d*, C(15)) were consistent with an apotirucalla-1,14-dien-3-one nucleus [24][25]. The OH group at C(7) found in holstinones A–C was also present in **1**, which was evidenced by the HMBC correlations from Me(30) to C(7) (δ(C) 71.5, *d*), C(8) (δ(C) 44.8, *s*), C(9) (δ(C) 36.7, *d*), and C(14), and from H–C(7) (δ(H) 4.01, br. *s*) to C(30) (δ(C) 27.6, *q*). Compound **1** and holstinone C could be distinguished by the substitution at C(21), where an EtO group in **1** replaced the MeO group in the latter. This was clarified by the ^{1}H-NMR resonances at δ(H) 1.26 (*t*, $J = 7.2$, Me(2′)), 3.75 (*dq*, H_a–C(1′)), and 3.46 (*dq*, H_b–C(1′)) and their corresponding C-atoms at δ(C) 15.4 (*q*, C(2′)) and 64.1 (*t*, C(1′)). The attachment of the EtO group at C(21) was confirmed by the HMBC correlation between H–C(1′) and C(21) (δ(C) 108.3, *d*), and in turn between H–C(21) (δ(H) 4.93, *d*, $J = 3.7$) and C(1′).

The relative configuration of **1** was determined through the NOESY data and coupling constants, along with its NMR data compared to those reported for structurally related holstinones A–C [24][25]. The NOESY correlations between Me(19)/Me(30), Me(19)/Me(29), H–C(5) (δ(H) 2.42, *dd*)/H–C(9), and Me(18)/H–C(9) designated the tetracyclic nucleus as being in *trans*-junction, as the case of known apotirucallane triterpenes [24]. The broad *singlet* of H–C(7) was indicative of its equatorial orientation, while the NOE correlation between H–C(7) and Me(30) allowed the assignment of H–C(7) in β-position. With respect to the side chain, the NOE interactions between H–C(20) (δ(H) 2.34–2.37, *m*)/H–C(23) (δ(H) 4.31, *dd*) and H–C(20)/CH_2(1′) supposed the same face of H–C(20) and H–C(23), while H–C(21) (δ(H) 4.93, *d*) was on the opposite face. Since H–C(17) was exclusively assigned to the β-form as observed in all apotirucallane triterpenes, thus, the NOE correlations of H–C(20) (δ(H) 2.34–2.37, *m*)/Me(18) (δ(H) 1.09, *s*), and H–C(21)/H–C(17) (δ(H) 1.65–1.77, *m*) (*Fig. 1*) allowed the assignment of α-orientations for H–C(20), H–C(23), and Me(18), while H–C(17) and H–C(21) were β-oriented. The broad *singlet* of H–C(24) required approximately 90° of the dihedral angle between H–C(24)/H–C(23), implying the C(23)–C(24) bond to be fixed. This fact suggested a H-bond formed between HO–C(25) and the acetal oxygen, as the cases in gentinones C and D [25]. A favorable conformation of the H-bond toward the furan ring was

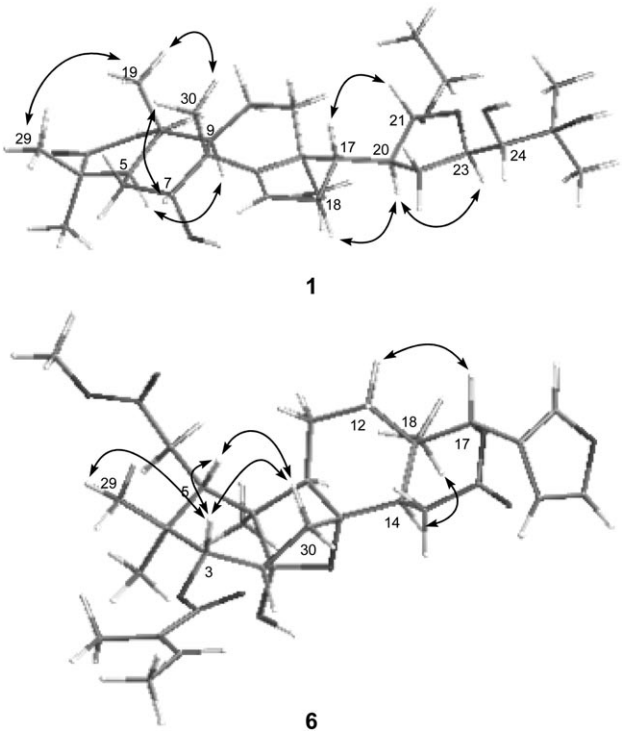

Fig. 1. *Main NOE correlations of compounds* **1** *and* **6**

Table 1. *^{1}H- and ^{13}C-NMR Data of Protoxylocarpins A–E* (**1**–**5**) *in $CDCl_3$*

	1		**2**		**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	7.18 (*d*, *J* = 10.0)	158.2 (*d*)	7.11 (*d*, *J* = 10.2)	158.2 (*d*)	7.11 (*d*, *J* = 10.2)	158.2 (*d*)	7.15 (*d*, *J* = 10.2)	158.1 (*d*)	7.06 (*d*, *J* = 10.5)	157.9 (*d*)
H–C(2)	5.86 (*d*, *J* = 10.0)	125.5 (*d*)	5.81 (*d*, *J* = 10.2)	125.5 (*d*)	5.81 (*d*, *J* = 10.2)	126.5 (*d*)	5.85 (*d*, *J* = 10.2)	125.5 (*d*)	5.81 (*d*, *J* = 10.5)	124.1 (*d*)
C(3)		205.1 (*s*)		205.2 (*s*)		205.0 (*s*)		205.5 (*s*)		203.8 (*s*)
C(4)		44.2 (*s*)		44.2 (*s*)		44.2 (*s*)		44.2 (*s*)		45.8 (*s*)
H–C(5)	2.42 (*dd*, *J* = 3.0, 12.0)	44.5 (*d*)	2.41 (*dd*, *J* = 12.0, 3.0)	44.5 (*d*)	2.40 (*dd*, *J* = 11.0, 4.0)	44.5 (*d*)	2.42 (*dd*, *J* = 11.6, 3.0)	44.5 (*d*)	2.58 (*d*, *J* = 9.0)	45.7 (*d*)
CH_2(6)	1.82–1.89 (*m*)	24.3 (*t*)	1.87–1.91 (*m*)	24.2 (*t*)	1.86–1.92 (*m*)	24.3 (*t*)	1.89–1.92 (*m*)	24.2 (*t*)	1.84–1.89 (*m*)	23.5 (*t*)
H–C(7)	4.01 (br. *s*)	71.5 (*d*)	4.00 (br. *s*)	71.5 (*d*)	3.96 (br. *s*)	71.5 (*d*)	4.01 (br. *s*)	71.5 (*d*)	5.29 (br.)	73.6 (*d*)
C(8)		44.8 (*s*)		44.8 (*s*)		44.8 (*s*)		44.8 (*s*)		44.2 (*s*)
H–C(9)	2.25 (*dd*, *J* = 5.0, 10.0)	36.7 (*d*)	2.21–2.26 (*m*)	36.6 (*d*)	2.20–2.25 (*m*)	36.7 (*d*)	2.19–2.24 (*m*)	36.7 (*d*)	2.59 (*d*, *J* = 10.0)	44.5 (*d*)
C(10)		40.2 (*s*)		40.2 (*s*)		40.2 (*s*)		40.2 (*s*)		40.6 (*s*)
CH_2(11) or H–C(11)	1.73–1.79 (*m*), 1.95–2.03 (*m*)	16.4 (*t*)	1.72–1.77 (*m*), 1.96–2.02 (*m*)	16.4 (*t*)	1.70–1.76 (*m*), 1.94–1.99 (*m*)	16.3 (*t*)	1.71–1.75 (*m*), 1.97–2.02 (*m*)	16.4 (*t*)	5.45 (*ddd*, *J* = 2.0, 8.5, 10.0)	73.7 (*d*)
CH_2(12)	1.80–1.86 (*m*), 1.56 (*ddd*, *J* = 2.0, 10.0, 12.0)	32.7 (*t*)	1.73–1.80 (*m*), 1.53–1.59 (*m*)	32.7 (*t*)	1.81–1.89 (*m*), 1.54–1.58 (*m*)	32.6 (*t*)	1.72–1.79 (*m*), 1.55–1.60 (*m*)	32.7 (*t*)	1.94–1.99 (*m*), 1.86–1.91 (*m*)	41.5 (*t*)
C(13)		46.9 (*s*)		46.5 (*s*)		46.9 (*s*)		46.5 (*s*)		45.7 (*s*)
C(14)		161.6 (*s*)		161.2 (*s*)		161.7 (*s*)		161.3 (*s*)		158.6 (*s*)
H–C(15)	5.51 (br. *s*)	119.7 (*d*)	5.53 (br. *s*)	120.1 (*d*)	5.46 (br. *s*)	119.6 (*d*)	5.53 (br. *s*)	120.1 (*d*)	5.32 (br. *s*)	118.4 (*d*)
CH_2(16)	2.08–2.11 (*m*), 2.05–2.28 (*m*)	34.8 (*t*)	2.15–2.21 (*m*), 2.08–2.14 (*m*)	35.0 (*t*)	2.06–2.11 (*m*), 2.12–2.18 (*m*)	34.8 (*t*)	2.10–2.20 (*m*)	35.0 (*t*)	2.15–2.21 (*m*), 2.15–2.21 (*m*)	34.5 (*t*)
H–C(17)	1.65–1.77 (*m*)	57.7 (*d*)	2.04–2.08 (*m*)	52.4 (*d*)	1.72–1.80 (*m*)	57.9 (*d*)	2.01–2.06 (*m*)	52.5 (*d*)	2.03–2.07 (*m*)	57.0 (*d*)
Me(18)	1.09 (*s*)	19.6 (*q*)	1.04 (*s*)	20.2 (*q*)	1.06 (*s*)	19.6 (*q*)	1.04 (*s*)	20.2 (*q*)	1.23 (*s*)	29.7 (*q*)
Me(19)	1.19 (*s*)	18.9 (*q*)	1.19 (*s*)	18.9 (*q*)	1.14 (*s*)	18.9 (*q*)	1.19 (*s*)	18.9 (*q*)	1.30 (*s*)	20.3 (*q*)
H–C(20)	2.34–2.37 (*m*)	46.0 (*d*)	2.21–2.25 (*m*)	44.5 (*d*)	2.16–2.21 (*m*)	46.3 (*d*)	2.15–2.21 (*m*)	44.6 (*d*)	2.19–2.26 (*m*)	44.5 (*d*)
H–C(21)	4.93 (*d*, *J* = 3.7)	108.3 (*d*)	4.89 (*d*, *J* = 5.0)	103.3 (*d*)	4.88 (*d*, *J* = 3.9)	108.0 (*d*)	4.80 (*d*, *J* = 3.7)	104.6 (*d*)	4.72 (*d*, *J* = 4.5)	110.0 (*d*)
CH_2(22)	1.91–1.94 (*m*), 1.75–1.79 (*m*)	33.8 (*t*)	1.91–1.96 (*m*), 1.98–2.02 (*m*)	31.5 (*t*)	1.90–1.96 (*m*), 1.59–1.65 (*m*)	35.6 (*t*)	1.92–1.97 (*m*), 1.96–2.02 (*m*)	31.4 (*t*)	1.93–1.97 (*m*), 1.82–1.89 (*m*)	33.8 (*t*)
H–C(23)	4.31 (*dd*, *J* = 10.5, 4.6)	76.9 (*d*)	4.48 (*dd*, *J* = 3.0, 10.0)	78.6 (*d*)	4.22 (*dd*, *J* = 5.5, 10.5)	76.6 (*d*)	4.47 (*ddd*, *J* = 2.0, 8.0, 8.0)	78.9 (*d*)	4.29 (*dd*, *J* = 10.0, 4.5)	77.3 (*d*)
H–C(24)	3.27 (br. *s*)	75.5 (*d*)	3.20 (br. *s*)	76.7 (*d*)	3.35 (br. *s*)	76.8 (*d*)	3.20 (br. *s*)	76.6 (*d*)	3.25 (br. *s*)	75.4 (*d*)

Table 1 (cont.)

	1		**2**		**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(25)		73.1 (*s*)		73.0 (*s*)		76.7 (*s*)		72.9 (*s*)		73.1 (*s*)
Me(26)	1.29 (*s*)	26.4 (*q*)	1.28 (*s*)	26.3 (*q*)	1.12 (*s*)	20.7 (*q*)	1.28 (*s*)	26.3 (*q*)	1.32 (*s*)	26.5 (*q*)
Me(27)	1.32 (*s*)	26.5 (*q*)	1.29 (*s*)	26.3 (*q*)	1.22 (*s*)	22.4 (*q*)	1.29 (*s*)	26.4 (*q*)	1.28 (*s*)	26.3 (*q*)
Me(28)	1.11 (*s*)	21.5 (*q*)	1.10 (*s*)	21.5 (*q*)	1.07 (*s*)	21.5 (*q*)	1.11 (*s*)	21.5 (*q*)	1.08 (*s*)	21.1 (*q*)
Me(29)	1.19 (*s*)	27.1 (*q*)	1.18 (*s*)	27.1 (*q*)	1.14 (*s*)	27.1 (*q*)	1.19 (*s*)	27.1 (*q*)	1.11 (*s*)	26.4 (*q*)
Me(30)	1.15 (*s*)	27.6 (*q*)	1.15 (*s*)	27.6 (*q*)	1.10 (*s*)	27.5 (*q*)	1.15 (*s*)	27.6 (*q*)	1.23 (*s*)	29.7 (*q*)
EtO–C(21) or MeO–C(21)	3.75 (*dq*, J = 7.2, 12.0), 3.46 (*dq*, J = 7.2, 12.0), 1.26 (*t*, J = 7.2)	64.1 (*t*), 15.4 (*q*)	3.80 (*dq*, J = 7.0,12.0), 3.43 (*dq*, J = 7.0,12.0), 1.24 (*t*, J = 7.0)	63.8 (*t*), 15.2 (*q*)	3.74 (*dq*, J = 7.0,12.0)), 3.43 (*dq*, J = 7.0,12.0), 1.20 (*t*, J = 7.0)	63.8 (*t*), 15.4 (*q*)	3.40 (*s*)	55.1 (*q*)	3.35 (*s*)	55.8 (*q*)
EtO–C(25)					3.41 (*q*, J = 7.0), 1.12 (*t*, J = 7.0)	56.5 (*t*), 16.1 (*q*)				
AcO–C(7)									2.11 (*s*)	21.8 (*q*), 170.3 (*s*)
AcO–C(11)									2.00 (*s*)	21.3 (*q*), 170.0 (*s*)

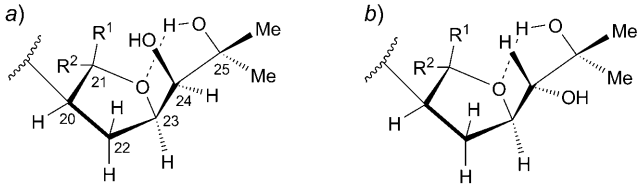

Fig. 2. *The H-bond formation between HO–C(25) and the acetal O-atom. a*) C(24*S*) caused an axial-equatorial coupling of H–C(23)/H–C(24) ($J(23,24_{eq}) = ca.\ 0$). *b*) C(24*R*) caused an axial-axial coupling of H–C(23)/H–C(24) ($J(23,24_{ax}) = ca.\ 10$).

established (*Fig. 2*) according to the structure modeling calculated by MOPAC minimizing energy. Thereby, the broad *singlet* of H–C(24) was attributed to an axial-equatorial coupling in contrast to $J(23,24) = 10.0$ Hz in holstinone C [24] for axial-axial coupling (*Fig. 2*). Accordingly, C(24) configuration of **1** was assumed to be (*S*), which was the same as gentinones C and D.

The molecular formula of protoxylocarpin B (**2**) was the same as that of **1** as determined from HR-FAB-MS and NMR data. Elucidation of 2D-NMR and comparison of NMR data (*Table 1*) revealed that **2** had the same structure as **1**, and the *trans*-geometry of the tetracyclic nucleus was determined on the basis of the similar NOESY interactions as indicated in **1**. The sole significant difference was the chemical shifts of C(17) and C(21) which were shifted upfield to δ(C) 52.4 (– 5.3 ppm) and 103.3 (– 5.0 ppm) compared to **1** due to the γ-gauche effect. This finding suggested that the EtO group at C(21) of **2** was β-oriented, while the NOE correlation between H–C(20) (δ(H) 2.21–2.25, *m*)/H–C(21) (δ(H) 4.89, *d*) further confirmed the assignment. Thus, **2** was identified as the C(21) epimer of **1**. The broad *singlet* of H–C(24) implied the (*S*)-form of C(24) as indicated in **1**.

Protoxylocarpin C (**3**) had a molecular formula of $C_{34}H_{54}O_6$ as determined by HR-FAB-MS (*m*/*z* 581.3831, $[M + Na]^+$; calc. 581.3812) and NMR data, being 28 amu higher than that of **1**. The comparable NMR and IR data indicated that **3** shared mostly the structure of **1**, with the exception of an additional Et group which showed the ^{13}C-NMR resonances at δ(C) 56.5 (*t*) and 16.1 (*q*), and the corresponding H-atoms at δ(H) 3.41 (*q*, $J = 7.0$, CH_2(1″)) and 1.12 (*t*, $J = 7.0$, Me(2″)). This Et group was deduced to form an ether with HO–C(25) based on the C(25) data appearing more downfield shifted at δ(C) 76.7 (*s*) compared to δ(C) 73.1 (*s*) in **1**. This assignment was also evident from the HMBC correlation from Me(27), Me(26), and CH_2(2″) to C(25). The chemical shifts of C(17) (δ(C) 57.9, *d*) and C(21) (δ(C) 108.0, *d*) were comparable to those of **1**, indicating H–C(21) to be β-oriented. The *singlet* H-atom H–C(24) (δ(H) 3.35, br. *s*) was assignable to (24*S*). The configuration of the remaining centers was identical to **1** on the basis of close similarity of NMR and NOE data.

The 1D- and 2D-NMR, and ESI-MS spectroscopic data analysis revealed that the gross structure of protoxylocarpin D (**4**) was identical to holstinone B [24], as apparent from ^{13}C-NMR signals at δ(C) 205.5 (*s*, C(3)), 125.5 (*d*, C(2)), 158.1 (*d*, C(1)), and their corresponding H-atoms at δ(H) 7.15 (*d*, $J = 10.2$, H–C(1)) and 5.85 (*d*, $J = 10.2$, H–C(2)) for α,β-unsaturated ketone of ring *A*, an vinyl group at δ(C) 161.3 (*s*, C(14)) and 120.1 (*d*, C(15)), and a HO–CH group at δ(H) 4.01 (br. *s*, H–C(7)), along with

the presence of seven Me *singlet*s (*Table 1*). The difference was recognized by the chemical shifts of C(17) (δ(C) 52.5) and C(21) (δ(C) 104.6) instead of δ(C) 57.7 (C(17)) and δ(C) 109.5 (C(21)) of holstinone B. Those findings suggested β-orientation of the MeO group at C(21) of **3**. The NOESY correlations between H–C(20) (δ(H) 2.15–2.21, *m*)/H–C(23) (δ(H) 4.47, *ddd*), H–C(20)/Me(18) (δ(H) 1.04, *s*), H–C(23)/H–C(24) (δ(H) 3.20, br. *s*), and H–C(21) (δ(H) 4.80, *d*)/H–C(20) further confirmed the relative configuration of **4** to be in agreement with **2**.

Protoxylocarpin E (**5**) had a molecular formula of $C_{35}H_{52}O_9$, as determined by HR-FAB-MS (*m*/*z* 639.3508, $[M+Na]^+$; calc. 639.3503) and NMR data. Interpretation of the NMR data (*Table 1*) led to the proposal that **5** is an apotirucalla-1,14-dien-3-one based analogue, closely related to **4**. The ^{13}C-NMR spectrum exhibited totally 35 C-atoms, of which 30 were attributed to the apotirucallane nucleus, while the remaining five C-atoms were assigned to two AcO groups at δ(H) 2.00 (*s*, 3 H) and 2.11 (*s*, 3 H) and one MeO group at δ(H) 3.35 (*s*, 3 H). C(7)–O was assigned by the HMBC between C(7) (δ(C) 73.6, *d*) and Me(30) (δ(H) 1.23, *s*), and its corresponding H-atom H–C(7) appeared at δ(H) 5.29 (br.) rather than δ(H) *ca.* 4.00 ppm in **1**–**4** with a free OH group at C(7), suggesting the location of an AcO group at C(7), which was confirmed through the HMBC between H–C(7) and an AcO CO group at δ(C) 170.3 (*s*). In addition, the COSY correlation between H–C(9) (δ(H) 2.59, *d*, $J=10.0$) and H–C(11) (δ(H) 5.45, *ddd*, $J=2.0$, 8.5, 10.0), and the fact that the latter H-atom correlated to the second AcO CO group at δ(C) 170.0 (*s*) in the HMBC spectrum, led to the assignment of the second AcO group linked to C(11). The MeO group (δ(H) 3.35, *s*) was located at C(21) based on the HMBC correlation between the Me H-atoms and C(21) (δ(C) 110.0, *d*). The downfield ^{13}C-NMR values of C(17) (δ(C) 57.0, *d*) and C(21) were in agreement with MeO in α-position at C(21), while the *singlet* H-atom H–C(24) (δ(H) 3.25, br. *s*) was consistent with (24*S*). The *J* value of H–C(9) (10.0 Hz) implied axial/axial coupling to H–C(11), while the NOESY correlation between H–C(11)/Me(30) and H–C(7)/Me(30) revealed the β-orientation of H–C(11) and H–C(7).

A comparison of ^{13}C-NMR data of compounds **1**–**5** in association with the reported data in literature revealed that the configuration of the RO group at C(21) significantly affected the chemical shifts of C(17) and C(21). The chemical shifts of C(17) and C(21) appeared shifted upfield at δ(C) *ca.* 52 ppm for C(17), and δ(C) *ca.* 104 ppm when RO is β-oriented due to the γ-gauche effect, whereas the α-form of RO induced significant downfield shifted signals for C(17) and C(21) which appeared at δ(C) *ca.* 57 ppm and *ca.* 108 ppm, respectively. The resonances of C(17) and C(21) can be used to distinct the C(21) epimers.

Xylocarpin J (**6**) had a molecular formula of $C_{32}H_{42}O_9$ as determined by HR-FAB-MS (*m*/*z* 593.2738, $[M+Na]^+$; calc. 593.2721), implying 12 degrees of unsaturation. The 1D- and 2D-NMR data analyses (*Table 2*) and the comparison of the NMR data reported for xylocarpin A [4] revealed that both shared the same gross structure. The sole difference was ascribed to the configuration at C(3). The H–C(3) in **6** was determined as β-orientated based on the NOE interactions from H–C(3) (δ(H) 5.02, *d*) to H–C(5) (δ(H) 2.87, *d*), Me(29) (δ(H) 0.78, *s*), CH_2(30) (δ(H) 1.71–1.79, 2.24–2.30, 2*m*). The NOESY cross peaks from H–C(5) to H–C(3), and to one H-atom of CH_2(30) assumed the β-orientation of H–C(5). The remaining NOE correlations were

Table 2. *^{1}H- and ^{13}C-NMR Data of Xylocarpins J and K* (**6** and **7**) *and Xyloccensin Y* (**10**) ($CDCl_3$)

	6		**7**		**10**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
HO–C(1)		108.0 (*s*)		87.0 (*s*)		81.9 (*s*)
H–C(2)	2.14–2.19 (*m*)	45.1 (*d*)	2.23 (*dd*, *J* = 4.2, 11.6)	51.2 (*s*)	2.32 (*dd*, *J* = 4.4, 11.4)	51.1 (*s*)
H–C(3)	5.02 (*d*, *J* = 8.7)	75.3 (*d*)	5.39 (*d*, *J* = 11.6)	76.6 (*d*)	5.26 (*d*, *J* = 11.4)	76.6 (*d*)
C(4)		35.7 (*s*)		45.3 (*s*)		45.3 (*s*)
H–C(5)	2.87 (*d*, *J* = 10.0)	40.2 (*d*)	3.45 (*s*)	45.0 (*d*)	3.33 (*s*)	45.4 (*d*)
CH_2(6) or H–C(6)	2.28 (*d*, *J* = 14.0), 2.42 (*dd*, *J* = 10.0, 14.0)	32.4 (*t*)	5.48 (*s*)	72.2 (*d*)	5.49 (*s*)	72.1 (*d*)
C(7)		174.3 (*s*)		170.8 (*s*)		170.8 (*s*)
C(8)		80.1 (*s*)		73.4 (*s*)		77.3 (*s*)
H–C(9) or C(9)	1.53–1.57 (*m*)	63.2 (*d*)		83.1 (*s*)		78.1 (*s*)
C(10)		44.0 (*s*)		54.3 (*s*)		53.5 (*s*)
CH_2(11)	1.14–1.19 (*m*), 1.67–1.72 (*m*)	19.1 (*t*)	2.17–2.22 (*m*), 2.30–2.35 (*m*)	34.1 (*t*)	2.38–2.44 (*m*), 2.29–2.34 (*m*)	34.1 (*t*)
CH_2(12) or H–C(12)	1.32–1.38 (*m*), 1.71–1.77 (*m*)	35.5 (*t*)	5.43 (*dd*, *J* = 2.3, 11.0)	79.1 (*d*)	3.77–3.82 (*m*)	70.4 (*d*)
C(13)		38.1 (*s*)		38.9 (*s*)		40.9 (*s*)
H–C(14)	2.83 (*dd*, *J* = 5.2, 11.0)	45.3 (*d*)	2.72 (*dd*, *J* = 9.0, 3.9)	44.6 (*d*)	2.55 (*d*, *J* = 8.3)	43.1 (*d*)
CH_2(15)	2.82 (*dd*, *J* = 5.2, 12.0), 2.45 (*dd*, *J* = 11.0, 12.0)	27.4 (*t*)	2.58 (*dd*, *J* = 16.2, 3.9), 2.69 (*dd*, *J* = 16.2, 9.0)	27.0 (*t*)	3.38 (*d*, *J* = 19.4), 2.75 (*dd*, *J* = 8.3, 19.5)	28.0 (*t*)
C(16)		170.0 (*s*)		169.7 (*s*)		169.7 (*s*)
H–C(17)	5.40 (*s*)	77.8 (*d*)	5.75 (*s*)	77.1 (*d*)	5.80 (*s*)	77.1 (*d*)
Me(18)	1.23 (*s*)	22.1 (*q*)	0.93 (*s*)	19.7 (*q*)	1.15 (*s*)	19.2 (*q*)
Me(19)	1.07 (*s*)	20.8 (*q*)	1.18 (*s*)	17.1 (*q*)	1.18 (*s*)	14.8 (*q*)
C(20)		121.2 (*s*)		121.1 (*s*)		121.1 (*s*)
H–C(21)	7.48 (br. *s*)	140.8 (*d*)	7.50 (br. *s*)	141.4 (*d*)	7.46 (br. *s*)	140.4 (*d*)
H–C(22)	6.41 (br. *s*)	110.0 (*d*)	6.44 (br. *s*)	109.3 (*d*)	6.37 (br. *s*)	109.5 (*d*)
H–C(23)	7.44 (br. *s*)	143.0 (*d*)	7.46 (br. *s*)	143.7 (*d*)	7.47 (br. *s*)	143.7 (*d*)
Me(28)	1.02 (*s*)	22.3 (*q*)	0.99 (*s*)	15.7 (*q*)	1.01 (*s*)	15.7 (*q*)
Me(29) or CH_2(29)	0.78 (*s*)	24.5 (*q*)	2.29 (*d*, *J* = 10.8), 1.45 (*d*, *J* = 10.8)	45.3 (*t*)	2.26 (*d*, *J* = 10.5), 2.55 (*d*, *J* = 10.5)	45.2 (*t*)
CH_2(30) or H–C(30)	1.71–1.79 (*m*), 2.24–2.30 (*m*)	29.1 (*t*)	5.44 (*d*, *J* = 4.2)	69.7 (*d*)	5.48 (*d*, *J* = 4.4)	70.5 (*d*)
MeO	3.70 (*s*)	51.8 (*q*)	3.77 (*s*)	53.0 (*q*)	3.78 (*s*)	53.0 (*q*)
AcO–C(3)			2.03 (*s*)	23.0 (*q*), 170.2 (*s*)	2.13 (*s*)	21.2 (*q*), 170.2 (*s*)
AcO–C(6)			2.10 (*s*)	21.3 (*q*), 170.5 (*s*)	2.11 (*s*)	21.5 (*q*), 170.5 (*s*)
AcO–C(30)			2.18 (*s*)	21.3 (*q*), 170.5 (*s*)	2.22 (*s*)	21.0 (*q*), 169.5 (*s*)
AcO–C(12)			2.19 (*s*)	21.2 (*q*), 169.5 (*s*)		
AcO–C(1)			2.20 (*s*)	20.7 (*q*), 169.5 (*s*)		
Tigloyl:						
C=O		167.4 (*s*)				
C–C=O		128.4 (*s*)				
H–C	6.87 (*qq*, *J* = 1.40, 7.0)	138.1 (*d*)				
Me	1.80 (*d*, *J* = 1.40)	14.5 (*q*)				
Me	1.84 (*d*, *J* = 7.0)	12.1 (*q*)				

in agreement with the reported data for xylocarpin A. Thus, the structure of **6** was determined as the C(3) epimer of xylocarpin A.

The molecular formula ($C_{37}H_{46}O_{17}$) of xylocarpin K (**7**) was determined on the basis of HR-ESI-MS data (*m/z* 763.2817, $[M+H]^+$; calc. 763.2813). An interpretation of the NMR data (*Table 2*) revealed that **7** is a phragmalin limonoid, closely related to xyloccensin Y (**10**) [13][14]. The ^{1}H-NMR data of **7** exhibited the resonances for five AcO groups at δ(H) 2.03 (*s*), 2.10 (*s*), 2.18 (*s*), 2.19 (*s*), and 2.20 (*s*) instead of three in xyloccensin Y, and they showed HMBC correlations with CO groups at δ(C) 170.2 (*s*), 170.5 (*s*), 170.5 (*s*), 169.5 (*s*), and 169.5 (*s*), respectively. In addition to the AcO groups being attached to C(3), C(6), and C(30) like in xyloccensin Y, additional AcO groups were linked to C(1) and C(12) based on the HMBC correlation between the downfield shifted H–C(12) (δ(H) 5.43, *dd*) and an AcO CO group at δ(C) 169.5 (*s*), and the typical downfield resonance of C(1) (δ(C) 87.0, *s*) in contrast to C(1) in xyloccensin Y (δ(C) 81.9, *s*) bearing a free OH group at this position. The relative configuration of **7** was established on the basis of NOESY data. The significant NOE interaction observed from H–C(3) to H_a–C(29) (δ(H) 2.29, *d*) and from H–C(6) to H_b–C(29) (δ(H) 1.45, *d*), but not from H–C(3) to H–C(5), helped to assign α-face of H–C(3) and β-orientation of H–C(5). In addition, the NOE interactions between H–C(30)/H–C(17), H–C(30)/H–C(5), H–C(30)/H_β–C(11), H–C(30)/H–C(15), H–C(12)/H–C(17), and H–C(14)/H–C(18), indicated the *cis*-junction of rings *B*/*C* and *C*/*D*, and the β-orientation of H–C(12) and H–C(30), as observed in xyloccensin Y [14]. Thus, the structure of **7** was determined as 1,12-di-*O*-acetylxyloccensin Y.

When xylocarpin K (**7**) was left standing for two weeks in $CDCl_3$, it was completely converted to the phragmalin orthoester xylocarpin I (**8**) [7]. The putative auto-transformation is depicted in the *Scheme*. This finding led to be assumption that the orthoester derivatives may be degradation products of polyhydroxyphragmalins containing an AcO group at C(1) or C(30).

The co-existence of protolimonoids (**1**–**5**) with limonoids in this plant supports the hypothesis that limonoids derive biogenetically from protolimonoids as precursors [2]. Protolimonoid appear to be rare metabolites in the genus Xylocarpus. Protoxylogranatin A, recently isolated from the seeds of *X. granatum* [26] was the only example reported so far. The ethylated derivatives **1**–**3** may be artefacts formed during the extraction procedure.

Compounds **1**–**6**, **9**, and **10** were tested against tumor cell lines. They exhibited moderate to weak activity against HCT-8, Bel-7402, BGC-823, and A2780 cell lines (*Table 3*).

Experimental Part

General. Column chromatography (CC): Silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China); C_{18} SiO_2 (10 μm, *YMC*, Japan). TLC: *Merck* precoated plates (SiO_2 *60* F_{254}). *Sephadex LH-20* gel (18 × 110 μm, *Pharmacia*). *Kromasil RP-18* (5 μm, 20 × 250 mm; *Eka Chemicals*, Bohus, Sweden). HPLC: *Alltech* instrument (*426-HPLC PUMP*, *Alltech UV-VIS-200* Detector). Optical rotations: *Perkin-Elmer 341 LC* polarimeter. UV Spectra: *Shimadzu UV-210A* spectrophotometer; in nm. IR Spectra: *Thermo Nicolet Nexus 470 FT-IR* spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance DRX 500* spectrometer. ESI-MS: *Perkin-Elmer Q-STAR ESI-TOF-MS/MS* spectrometer. HR-FAB-MS: *Bruker FT-ICR-APEII* mass spectrometer; with glycerol as matrix.

Scheme. *Putative Mechanism for the Spontaneous Conversion of* **7** *to* **8**

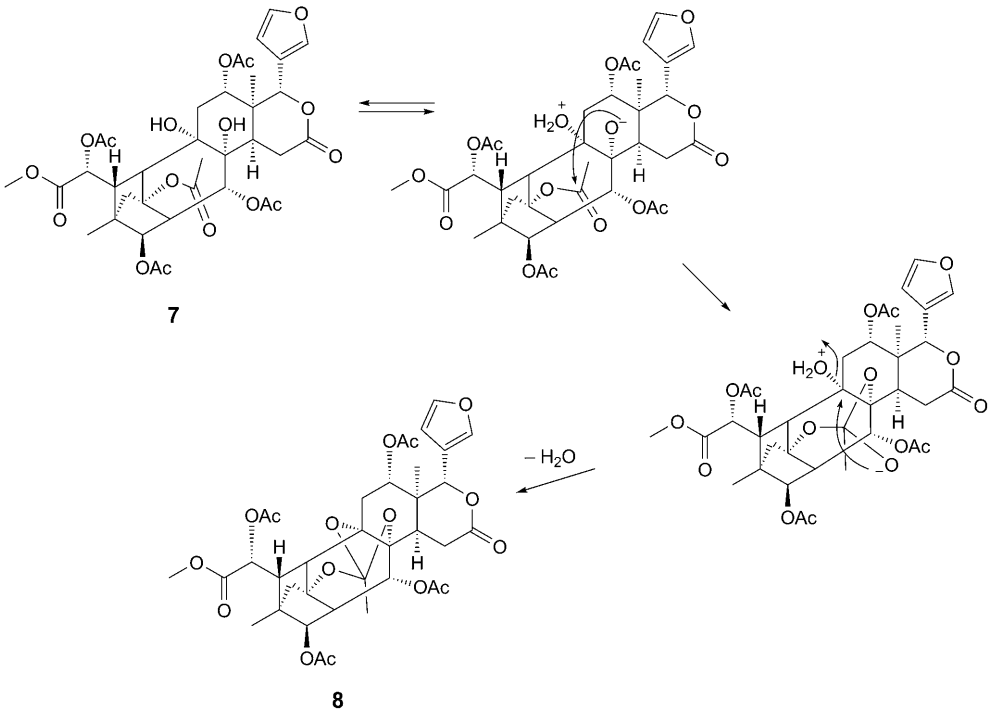

Table 3. *Cytotoxic Activities of Compounds* **1**–**6**, **9**, *and* **10**

	IC_{50} [μM]				
	HCT-8	Bel-7402	BGC-823	A549	A2780
1	2.83	2.73	3.35	19.00	0.38
2	4.52	5.52	6.76	13.52	0.97
3	4.21	3.75	5.87	16.60	4.52
4	5.38	6.21	8.82	9.62	3.35
5	3.87	4.45	4.62	7.87	2.54
6	7.75	8.22	8.38	5.35	4.77
9	14.77	12.81	8.90	18.55	16.60
10	10.43	13.55	9.87	16.23	11.64
Taxol[a])	0.21	0.02	1.37	2.76	0.03

[a]) Positive control.

Plant Material. The fresh fruits of the mangrove plant *X. granatum* were collected from the mangrove garden at Hainan Island, Southern China, in May 2003, and the species was identified by Prof. *Peng Lin* from Xiamen University. A voucher specimen (MP-HN-028) was deposited in State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

Extraction and Isolation. The air-dried fruit rind (3.5 kg) was percolated with 95% EtOH (3 × 12 l) at r.t. for 2 d each. The EtOH extract was concentrated in *vacuo* to yield a residue (706 g). The residue

was partitioned between 90% aq. MeOH (3 l) and petroleum ether (PE; 1.0 l) to remove lipids. The aq. MeOH fraction was concentrated and then re-partitioned between H_2O (1 l) and CH_2Cl_2 (0.5 l). The CH_2Cl_2 layer was collected and concentrated to afford a fraction (54.0 g), which was subjected to SiO_2 CC (200–300 mesh, 6 × 60 cm) eluting with a gradient of PE/acetone (5:1 (3 l), 2:1 (3 l), and 1:1 (3 l)) to yield six fractions (*Fr. a–g*). *Fr. b* (2.50 g) was subjected to a reverse phase SiO_2 CC (C_{18}) (10 μm, 3 × 25 cm) and eluted with MeOH/H_2O (1:1, 2.5 l) to yield xyloccensin M (**9**) (16 mg) and a portion (250 mg) which contained a mixture of limonoids as detected by ^{1}H-NMR spectroscopy. This mixture was separated on a semi-preparative HPLC (*ODS* column, 5 μm, 2 × 25 cm, MeOH/H_2O = 21:79 as mobile phase, flow rate: 3.0 ml/min, UV wavelength: 225 nm) to obtain **1** (5.8 mg, t_R 20.2 min), **2** (9.2 mg, t_R 21.3 min), **3** (5.0 mg, t_R 24.5 min), **4** (3.5 mg, t_R 22.2 min), and **5** (5.6 mg, t_R 29.4 min). *Fr. d* (890 mg) was fractionated on a *Sephadex LH-20* column (4 × 85 cm) by using MeOH as an eluent to give a limonoid-containing subfraction (50 mg), which was subsequently separated on a semi-preparative HPLC column with 75% MeOH/H_2O as a mobile phase (*ODS*, 5 μm, 2 × 25 cm, flow rate: 3.0 ml/min, UV wavelength: 210 nm) to obtain **6** (5.4 mg, t_R 26.8 min), **7** (3.7 mg, t_R 27.4 min), and xyloccensin Y (**10**) (5.8 mg, t_R 24.5 min).

When a $CDCl_3$ soln. (0.5 ml) of **7** (1.0 mg) was left standing for two weeks at r.t., it was completely converted into xylocarpin I (**8**).

Protoxylocarpin A (=*(5*R,*7*R,*8*R,*9*R,*10*R,*13*S,*17*S*)-17-{(2*R,*3*S,*5*R*)-5-[(1*R*)-1,2-Dihydroxy-2-methylpropyl]-2-ethoxytetrahydrofuran-3-yl}-4,5,6,7,8,9,10,11,12,13,16,17-dodecahydro-7-hydroxy-4,4,8,10,13-pentamethyl-3*H*-cyclopenta[a]phenanthren-3-one*; **1**). White, amorphous powder. $[\alpha]_D^{25} = -32$ (*c* = 0.9, MeOH). UV (MeOH): 205 (2.89), 236 (2.64). IR (KBr): 3508, 2972, 2823, 2872, 1734, 1699, 1460, 1382, 1248, 1160. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 553.8 ($[M+Na]^+$). ESI-MS (neg): 529.7 ($[M-H]^-$). HR-FAB-MS: 531.3670 ($[M+H]^+$, $C_{32}H_{51}O_6^+$; calc. 531.3679).

Protoxylocarpin B (=*(5*R,*7*R,*8*R,*9*R,*10*R,*13*S,*17*S*)-17-{(2*S,*3*S,*5*R*)-5-[(1*R*)-1,2-Dihydroxy-2-methylpropyl]-2-ethoxytetrahydrofuran-3-yl}-4,5,6,7,8,9,10,11,12,13,16,17-dodecahydro-7-hydroxy-4,4,8,10,13-pentamethyl-3*H*-cyclopenta[a]phenanthren-3-one*; **2**). White, amorphous powder. $[\alpha]_D^{25} = -22$ (*c* = 1.5, MeOH). UV (MeOH): 207 (2.87), 238 (2.67). IR (KBr): 3447, 2972, 2938, 2887, 1734, 1669, 1458, 1385, 1248, 1094. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 531.2 ($[M+H]^+$). HR-FAB-MS: 531.3699 ($[M+H]^+$, $C_{32}H_{51}O_6^+$; calc. 531.3679).

Protoxylocarpin C (=*(5*R,*7*R,*8*R,*9*R,*10*R,*13*S,*17*S*)-17-{(2*R,*3*S,*5*R*)-2-Ethoxy-5-[(1*R*)-2-ethoxy-1-hydroxy-2-methylpropyl]tetrahydrofuran-3-yl}-4,5,6,7,8,9,10,11,12,13,16,17-dodecahydro-7-hydroxy-4,4,8,10,13-pentamethyl-3*H*-cyclopenta[a]phenanthren-3-one*; **3**). White, amorphous powder. $[\alpha]_D^{25} = -28$ (*c* = 1.2, MeOH). UV (MeOH): 206 (2.91), 240 (2.70). IR (KBr): 3558, 2973, 2823, 2872, 1735, 1699, 1459, 1384, 1248, 1110. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 581.2 ($[M+Na]^+$). HR-FAB-MS 581.3831 ($[M+Na]^+$, $C_{34}H_{54}NaO_6^+$; calc. 581.3812).

Protoxylocarpin D (=*(5*R,*7*R,*8*R,*9*R,*10*R,*13*S,*17*S*)-17-{(2*S,*3*S,*5*R*)-5-[(1*R*)-1,2-Dihydroxy-2-methylpropyl]-2-methoxytetrahydrofuran-3-yl}-7-hydroxy-4,4,8,10,13-pentamethyl-4,5,6,7,8,9,10,11,12,13,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one*; **4**). White, amorphous powder. $[\alpha]_D^{25} = -24$ (*c* = 1.1, MeOH). UV (MeOH): 205 (2.77), 242 (2.55). IR (KBr): 3733, 3515, 2934, 1735, 1698, 1459, 1384, 1248, 1036. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 539.8 ($[M+Na]^+$). HR-FAB-MS: 517.3528 ($[M+H]^+$, $C_{31}H_{49}O_6^+$; calc. 517.3523).

Protoxylocarpin E (=*(5*R,*7*R,*8*R,*9*R,*10*S,*11*R,*13*S,*17*S*)-17-{(2*R,*3*S,*5*R*)-5-[(1*R*)-1,2-Dihydroxy-2-methylpropyl]-2-methoxytetrahydrofuran-3-yl}-4,5,6,7,8,9,10,11,12,13,16,17-dodecahydro-4,4,8,10,13-pentamethyl-3-oxo-3*H*-cyclopenta[a]phenanthrene-7,11-diyl Diacetate*; **5**). White, amorphous powder. $[\alpha]_D^{25} = -30$ (*c* = 0.9, MeOH). UV (MeOH): 208 (2.83), 240 (2.66). IR (KBr): 3519, 2970, 1734, 1668, 1458, 1379, 1244, 1032. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 639.0 ($[M+Na]^+$). HR-FAB-MS 639.3508 ($[M+Na]^+$, for $C_{35}H_{52}NaO_9^+$; calc. 639.3503).

Xylocarpin J (=*(4*R,*4a*R,*6a*R,*6b*S,*7*S,*9*S,*10a*S,*11a*S,*11b*R*)-4-(Furan-3-yl)-dodecahydro-10a-hydroxy-7-(2-methoxy-2-oxoethyl)-4a,6b,8,8-tetramethyl-2-oxo-2*H*,4*H*-10,11a-methano[1]benzofuro[2,3-f][2]benzopyran-9-yl (2*E*)-2-Methylbut-2-enoate*; **6**). White, amorphous powder. $[\alpha]_D^{25} = -42$ (*c* = 0.6, MeOH). UV (MeOH): 214 (3.61). IR (KBr): 3434, 2937, 2273, 1731, 1668, 1250. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS (pos.): 593.5 ($[M+Na]^+$). HR-FAB-MS: 593.2738 ($[M+Na]^+$, $C_{32}H_{42}NaO_9^+$; calc. 593.2721).

Xylocarpin K (= *Methyl (2*R*)-(Acetyloxy)[(1*R*,4a*R*,4b*R*,5*R*,6*R*,6a*S*,8*R*,9*S*,9a*R*,9b*S*,11*S*,11a*S*,12*R*)-5,12-bis(acetyloxy)-1-(furan-3-yl)-hexadecahydro-4b,6a,9b,11-tetrahydroxy-8,9a,11a-trimethyl-3-oxo-6,8-methanocyclopenta[5,6]naphtho[2,1-*c*]pyran-9-yl]ethanoate*; **7**). White, amorphous powder. $[\alpha]_D^{25} = +37$ ($c = 0.4$, MeOH). UV (MeOH): 212 (3.42). IR (KBr): 3416, 1731, 1625, 1433, 1379, 1239, 1122, 1145. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS (pos.): 763.3 ($[M+H]^+$), 780.3 ($[M+NH_4]^+$), 785.3 ($[M+Na]^+$). HR-FAB-MS: 763.2817 ($[M+H]^+$, $C_{37}H_{47}O_{17}^+$; calc. 763.2813).

Xylocarpin I (= *Methyl (2*R*)-(Acetyloxy)[(1*R*,2*R*,6*R*,7*S*,8*S*,10*S*,14*S*,16*R*,17*R*,18*R*,20*R*)-8,18,20-tris(acetyloxy)-6-(furan-3-yl)-7,12,17-trimethyl-4-oxo-5,11,13,21-tetraoxaheptacyclo[10.8.1.1^{14,17}.0^{1,10}.0^{2,7}.0^{10,15}.0^{14,19}]docos-16-yl]ethanoate*; **8**). White, amorphous powder. $[\alpha]_D^{25} = +62$ ($c = 0.8$, MeOH). UV (MeOH): 221. IR (KBr): 3424, 2954, 1744, 1702, 1619, 1384, 1225, 1183, 1134. ^{1}H- and ^{13}C-NMR: see [7]. ESI-MS: 767 ($[M+Na]^+$), 783 ($[M+K]^+$). HR-FAB-MS: 767.2513 ($[M+Na]^+$, $C_{37}H_{44}NaO_{16}^+$; calc. 767.2521).

Cytotoxicity Tests. The effects of compounds **1**–**6** and **9** and **10** on human tumor cell proliferation were determined by using a MTT-based colorimetric assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as indicated in previous work [27]. The positive control taxol was purchased from *Sigma*.

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