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α-Glucosidase Inhibitory Isomeric Corniculatolides from the Stems of the Indian Mangrove Plant, *Xylocarpus granatum*

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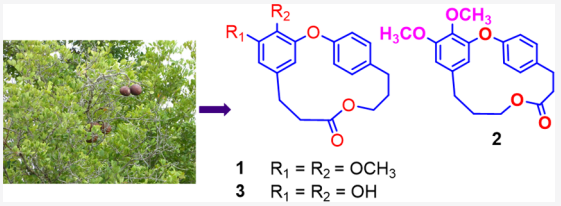
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Supporting Information

ABSTRACT: Three new isomeric corniculatolides (**1**, **2**, and **3**) with an unusual caffrane and isocorniculane framework, and five known metabolites were isolated from the chloroform extract of the stems of *Xylocarpus granatum*. The structures of the new metabolites were deduced as corniculatolide B (**1**), isocorniculatolide B (**2**), and corniculatolide C (**3**) by spectroscopic data analysis and a combination of chemical transformations and supported by single-crystal X-ray crystallographic data of **1** and **3**. The isolated compounds were evaluated for their in vitro cytotoxicity and α-glucosidase (*Saccharomyces cerevisiae*) inhibitory potential. Compound **3** possessed α-glucosidase inhibitory activity with an IC_{50} value of 24.8 μM, whereas these rare macrolides showed no effect on the mammalian cancer cell lines MIAPaCa-2, DU145, MCF-7, and HTC-116.



Macrocyclic lactones with a rare caffrane or isocorniculane (17-membered exterior or 15-atom interior) framework constitute a remarkable subclass of cyclic diaryl ether heptanoids (DAEHs), which possess an oxa[1.7]-metaparacyclophane skeleton. These macrolides differ in the position of the lactone moiety as part of the seven-atom tether. The first two representatives of potent cytotoxic caffrane macrolides were obtained from the South African bush willow tree *Combretum caffrum*[1,2] followed by two more congeners from *Getonia floribunda*[3] of the Combretaceae family. Subsequently, we reported four members of isomeric caffrane and isocorniculane macrolides from the Indian mangrove plant, *Aegiceras corniculatum*[4] (Aegiceratacеae). In our search for structurally and biologically significant secondary metabolites from the Indian mangrove flora, we found three more isomeric corniculatolides, named corniculatolide B (**1**), isocorniculatolide B (**2**), and corniculatolide C (**3**), as rare metabolites along with the known macrolides **4**−**7**[4] and friedelin (**8**)[5] from the stems of the *Xylocarpus granatum* (Figure 1).

The cannonball mangrove, *Xylocarpus granatum* Koening (Meliaceae), is widely distributed in Southeast Asia along the coastlines of the Indian Ocean.[6] This plant is used as a folk medicine in Southeast Asia to treat diarrhea, cholera, malaria, and viral diseases such as influenza.[7] The fruits of this plant are edible, and the seed paste is used in curing breast tumors.[8] The crude extracts and fractions of the leaves and the bark of this plant were reported to exhibit significant in vitro α-glucosidase inhibitory and antioxidant activities. Further, these extracts of the bark showed excellent in vivo antioxidant and antidiabetic activity in streptozotocin-induced mice.[9,10] This plant has been a rich source of bioactive and structurally intriguing cytotoxic limonoids.[11−14] Hitherto, more than 50 limonoids of the phragmalin, mexicanolide, and andirobin types have been reported from *X. granatum* collected from China, India, and Thailand.[15] The Indian species from the Krishna estuary and the Sunbardans region have been found to be prolific in elaborating an equally large number of limonoids,[16−18] which have not been found in the present investigation of the chloroform extract of the stems of *X. granatum* collected from the Nizampatnam coastline of Andhra Pradesh, India. This region is specific in the production of extremely rare isomeric macrolides, which carry a highly shielded aromatic proton in the proton NMR data. The known compounds were identified as corniculatolide A (**4**), isocorniculatolide A (**5**), 11-*O*-methylcornicultolide A (**6**), 12-hydroxy-11-*O*-methylcorniculatolide A (**7**), previously reported macrolides from *Aegiceras corniculatum*,[4] and friedelin (**8**)[5] by comparison of observed and reported spectroscopic data. Herein, we report the isolation and structural elucidation of the metabolites **1**, **2**,

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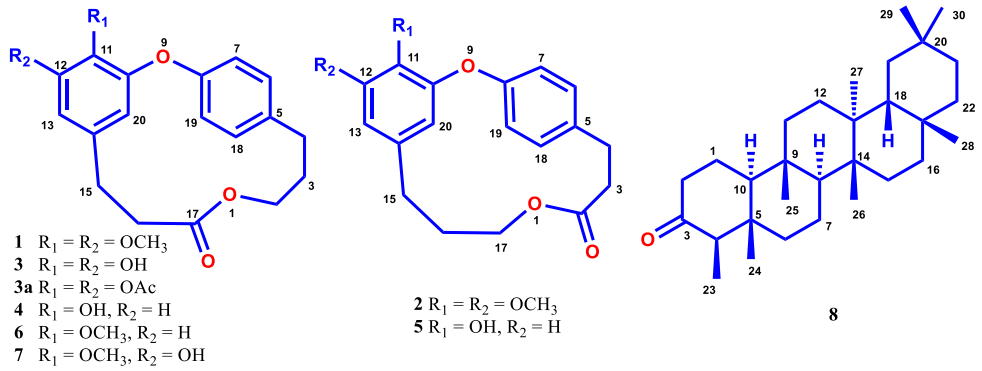


Figure 1. Compounds isolated from the stems of *Xylocarpus granatum*.

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) Chemical Shifts of Compounds 1, 2, 3, and 3a

	1 (400 MHz)	**2** (400 MHz)	**3** (400 MHz)	**3a** (500 MHz)		**1** (125 MHz)	**2** (125 MHz)	**3** (100 MHz)
position	δ_H (*J*, Hz)	δ_H (*J*, Hz)	δ_H (*J*, Hz)	δ_H (J, Hz)	position	δ_C	δ_C	δ_C
2	4.05−4.07, m		4.03−4.06, m	4.05−4.07, m	2	64.0	173.2	64.0
3	2.06−2.11, m	2.47−2.53, m	2.06−2.11, m	2.00−2.13, m	3	28.7	38.6	28.7
4	2.78−2.86, m	3.00−3.03, m	2.76−2.82, m	2.80, t (6.5)	4	34.0	32.4	34.0
6	7.28, d (8.4)	7.22, d (8.4)	7.28, d (8.4)	7.28, d (8.4)	5	137.4	137.4	137.9
7	7.02, d (8.4)	7.03, d (8.4)	7.00, d (8.4)	7.01, d (8.4)	6	130.9	130.4	131.0
12					7	123.6	123.8	123.5
13	6.30, d (2.0)	6.29, d (1.8)	6.33, d	6.53, d (2.0)	8	154.9	156.5	154.4
15	2.78−2.86, m	2.47−2.53, m	2.76−2.82, m	2.85−2.88, m	10	155.1	155.3	149.4
16	2.25−2.28, m	1.72−1.78, m	2.22−2.25, m	2.00−2.13, m	11	153.3	153.5	129.9
17		3.60, t (7.0)			12	135.7	136.1	144.3
18	7.28, d (8.4)	7.22, d (8.4)	7.28, d (8.4)	7.28, d (8.4)	13	105.2	105.8	108.4
19	7.02, d (8.4)	7.03, d (8.4)	7.00, d (8.4)	7.01, d (8.4)	14	135.7	134.7	132.4
20	5.00, d (2.0)	5.10, d (2.0)	4.86, d (2.0)	5.32, d (2.0)	15	27.7	28.8	27.3
OH			5.42, br s		16	32.3	25.9	32.6
OH			5.39, br s		17	173.6	62.3	174.0
OCH_3	3.99, s	4.01, s			18	130.9	130.4	131.0
OCH_3	3.84, s	3.84, s			19	123.6	123.8	123.5
OAc				2.37, s	20	106.6	109.6	104.9
OAc				2.29, s	OMe	56.2	56.2	
					OMe	61.2	61.4	

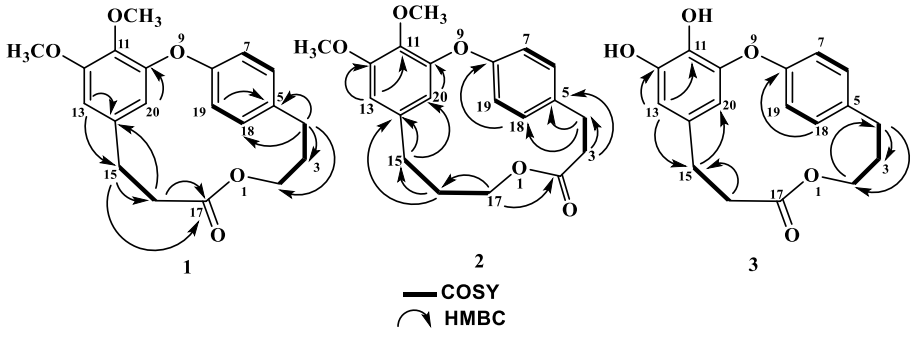


Figure 2. Key COSY and HMBC correlations of compounds **1**, **2**, and **3**.

and **3** from the stems of *X. granatum* and evaluation of the AGH inhibitory and cytotoxicity of the isolated compounds.

RESULTS AND DISCUSSION

Compound **1** was obtained as colorless needles from [*n*-hexane/acetone (8.5:1.5)], mp 194−195 °C. Its HRESIMS data exhibited a protonated molecular ion $[M^+ + H]$ at *m/z* 343.1563 (calcd for 343.1545), corresponding to the molecular formula $C_{20}H_{22}O_5$, requiring 10 indices of hydrogen deficiency. Its IR spectrum displayed absorption bands for a lactone or ester carbonyl (1726 cm^{-1}), ether (1100 cm^{-1}), and aromatic ring (1591, 1505 cm^{-1}) functionalities. The ^{1}H NMR data (Table 1) of **1** revealed the presence of two methoxy groups at δ_H 3.84 (s, 3H) and 3.99 (s, 3H), an oxymethylene group at δ 4.05−4.07 (m, 2H), a *para*-disubstituted aromatic ring at δ_H 7.02 (d, 2H, *J* = 8.4 Hz) and 7.28 (d, 2H, *J* = 8.4 Hz), a 1,2,3,5-tetra-substituted aromatic ring at δ_H 6.30 (d, 1H, *J* = 2.0 Hz), and a highly shielded aromatic proton at δ_H 5.00 (d, 1H, *J* =

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Table 2. Key HMBC, ^{1}H−^{1}H COSY, and ^{1}H−^{1}H NOESY Correlations of Compounds 1, 2, and 3

	1			2			3		
position	HMBC	COSY	NOESY	HMBC	COSY	NOESY	HMBC	COSY	NOESY
2	C-4	H-3					C-3, C-4	H-3	H-18
3		H-2, H-4	H-18	C-2, C-4, C- 5	H-4		C-5	H-2, H-4	H-18
4	C-2, C-3, C-5, C-18	H-3		C-2, C-3, C-5, C-18	H-3	H-18	C-2, C-3, C-5, C-18	H-3	H-18
11							C-11, C-13		
12							C-12, C-13		
13	C-14, C-15, C-20		H-15, H-22	C11, C12, C-15, C-20		H-22	C-11, C-12 C-15, C-20		
15	C-13, C-14, C-16, C-17, C-20	H-16	H-13	C-13, C-14, C-16, C-17, C-20	H-16		C-13, C-14 C-16, C-20	H-16	
16	C-14, C-15, C-17	H-15	H-20	C-14, C-15, C-17	H-15, H-17	H-20	C-14, C-15	H-15	H-20
17				C-2, C-15, C-16	H-16				
18	C-4, C-6, C-8	H-19	H-3	C-4, C-6, C-8, C-19	H-19		C-4, C-8, C-18	H-19	H-2, H-3, H-4
19	C-5, C-7, C-8	H-18	H-20	C-5, C-7, C-8, C-18	H-18	H-20	C-5, C-7, C-8	H-18	H-20
20	C-11, C-13, C-14, C-15		H-16, H-19	C-10, C-12 C-13, C-15		H-16, H-19	C-10, C-11 C-13, C-15		H-16, H-19
OMe	C-11			C-11					
OMe	C-12		H-13	C-12		H-13			

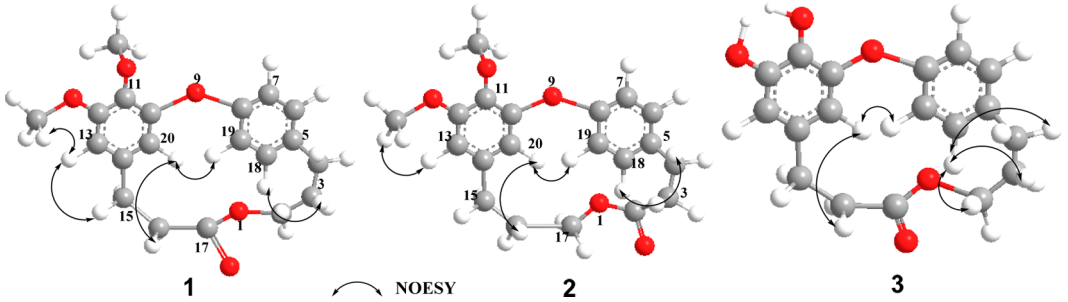


Figure 3. Key NOESY correlations of compounds 1, 2, and 3.

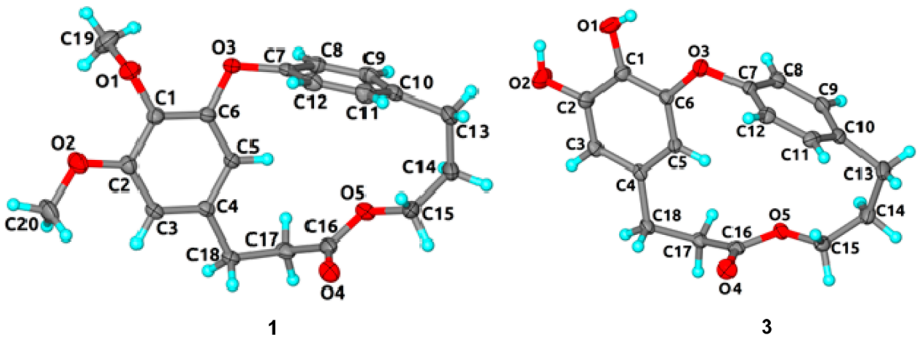


Figure 4. ORTEP drawing of compounds 1 and 3.

2.0 Hz) analogous to that of corniculatolide A (4)[4] and isocorniculatolide A (5).[4]

Its ^{13}C NMR data (Table 1) showed signals for 20 carbon resonances, which were resolved into two methyls, five methylenes, six methines, two quaternary carbons, a carbonyl, and four oxygenated tertiary carbons by DEPT and HSQC data. Its 1D NMR data indicated that 1 was composed of a carbonyl group and two aromatic rings, which accounted for nine indices of hydrogen deficiency. The remaining index of hydrogen deficiency could be ascribed to a third ring system in 1. The aforementioned data suggested that compound 1 featured a corniculane rather than an isocorniculane scaffold. Fragments H_2-2-H_2-3-H_2-4/H_2-15-H_2-16/H-6-H-7/H-18-H-19 were established by the ^{1}H−^{1}H COSY correlations (Figure 2 and Table 2).

Two methoxy groups, whose protons resonated at δ_H 3.84 and 3.99, were distinguished by HSQC and were located at C-11 and C-12 by the corresponding HMBC correlations of the methoxy group protons to C-11 and C-12. The oxymethylene group at C-2 was assigned based on the HMBC correlations from H_2-4 to C-2, C-3, C-4, C-5, and C-18, while the C-17 carbonyl group was confirmed by the HMBC cross-peaks of H_2-15 to C-16, C-17 and H_2-16 to C-14 (Figure 2 and Table 2). The structure of corniculatolide B (1) was confirmed by comparison of its ^{13}C NMR data with those of corniculatolide A,[4] which possessed the identical corniculane core. The diaryl

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ether moiety was confirmed by the ^{1}H−^{1}H NOESY correlations between H-20 and H-19 (Figure 3 and Table 2). The macrolide structure of compound **1** was confirmed by single-crystal X-ray crystallography data (Figure 4 and Table S1, Supporting Information).

Compound **2** was isolated as an amorphous solid that showed a protonated molecular ion $[M^+ + H]$ at m/z 343.1547 (calcd for 343.1545) in its HRESIMS data to deduce a molecular formula of $C_{20}H_{22}O_5$. The mass spectrum was identical to that of corniculatolide B (**1**), indicating isomeric structures. The ^{1}H NMR data of these compounds were similar except for the upfield shift of an oxymethylene group [δ_H 3.60 (t, 2H, J = 7.0 Hz)]. The ^{1}H−^{1}H COSY spectrum of compound **2** showed the same correlations as those observed for compound **1**, suggesting that both compounds have the same gross structure but differ in the position of the lactone carbonyl moiety, probably at C-2 or C-17, analogous to that of isocorniculatolide A.[4] Unlike corniculatolide B (**1**), its HMBC data confirmed the position of a lactone carbonyl at C-2 based on the correlation between C-2 and H-17. A 2D NMR analysis (^{1}H−^{1}H COSY, NOESY, HSQC, and HMBC) (Table 2, Figures 2 and 3) confirmed the structure of compound **2** as isocorniculatolide B (**2**).

Compound **3** was obtained as colorless needles from [*n*-hexane/EtOAc (8:2)], mp 173−175 °C. It exhibited a protonated molecular ion $[M + H^+]$ m/z at 315.1228 (calcd 315.1232) to establish its molecular formula as $C_{18}H_{18}O_5$, indicating 10 indices of hydrogen deficiency. Its IR spectrum revealed the presence of hydroxy (3440 cm^{-1}) and lactone carbonyl (1696 and 1238 cm^{-1}) functional groups. It formed a diacetate (**3a**) on acetylation with pyridine/Ac_2O, which confirmed the presence of two free hydroxy groups in **3**. The ^{1}H NMR spectroscopic data of corniculatolide C (**3**) were similar to those of corniculatolide B (**1**) (Table 1), except for the signals of two methoxy groups in **1** and the presence of two phenolic protons in **3**. Diagnostic ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY (Table 2, Figure 2, and Figure 3) correlations confirmed that **3** was the dide-*O*-methyl analogue of corniculatolide B (**1**). Finally, the structure of corniculatolide C (**3**) was confirmed by single-crystal X-ray data analysis.

The first series of caffrane macrolides was confined to the Combretaceae family of Myrtales and hardly found anywhere else. Later, the presence of caffrane and isocorniculane macrolides was extended to the Aegiceratacaeae (Ericales). The absence of limonoids, the taxonomic character of *Xylocarpus granatum* (Sapindales), and the presence of isomeric macrolides indicated the close relationship with Myrtales in a Malvidae clade.[19] The overview of these macrolides as distinct taxonomic characters is reported for the first time in the *Xylocarpus* genus. These macrolides could be of taxonomic significance in the Meliaceae family.

Compounds **1**−**7** were evaluated against α-glucosidase from *Saccharomyces cerevisiae* (Table 3) based on the reported antidiabetic activity of the crude extracts of this plant.[9,10] These results showed significant variations in the α-glucosidase inhibitory activity due to their structural differences. Among these compounds, corniculatolide C (**3**) exhibited the most potent AGH inhibitory activity (IC_{50} 24.8 μM), while compounds **7** and **5** showed no inhibitory activity (IC_{50} > 200 μM). The effect, being in the descending order of AGH inhibition of **3** > **6** > **1** > **2** > **4**, suggested that hydroxy as well as methoxy substituents at C-11 and C-12 played a significant role in the α-glucosidase inhibition. In addition, corniculane and isomeric corniculane scaffolds also exert a similar prime role towards AGH inhibition.

Table 3. Inhibitory Effects of Compounds and Acarbose against α-Glucosidase

compound	IC_{50} (μM)
1	72.3 ± 2.6
2	134.0 ± 2.5
3	24.8 ± 1.5
4	187.2 ± 0.1
5	>200
6	65.4 ± 2.4
7	>200
acarbose	12.2 ± 2.2

In view of the structure and activity of the reported macrolides,[1] the cytotoxicity of compounds **1**−**7** were evaluated using the sulforhodamine B (SRB) assay against a panel of four human cancer cell lines, namely, MIAPaCa-2, DU145, MCF-7, and HTC-116 (Figures S1−S4, Supporting Information). Figures S1−S4 show the concentration effectiveness of macrolides on the viability of MIAPaCa-2, DU145, MCF-7, and HTC-116 cells. Five different concentrations of each compound (1, 10, 25, 50, 100 μM) were tested. These compounds showed no cytotoxic activity in vitro.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an SMP10 Stuart melting point apparatus and are uncorrected. The IR spectra were recorded on a Thermo Nicolet Nexus 670 FT-IR spectrophotometer. The UV−vis spectra were obtained using a JASCO V-750 spectrophotometer. The HRESIMS data were acquired on a Waters Xevo G2-XS QTOF mass spectrometer. The NMR spectra were measured on a Bruker Avance (400 and 500 MHz) for ^{1}H NMR and (100 and 125 MHz) for ^{13}C NMR in $CDCl_3$ with tetramethylsilane as an internal standard. Coupling constants are given in Hz. The X-ray data for compounds **1** and **3** were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å) with the ω-scan method.

Plant Material. The stems of *X. granatum* were collected from the Nizampatnam (latitude: 15°53′ N, longitude: 80°38′ E) coastline of India in March 2017 and were identified by Prof. B. Kondala Rao, Department of Marine Living Resources, Andhra University, Visakhapatnam. A voucher specimen (#IIC-MG-120) was deposited in the herbarium of the Centre for Natural Products and Traditional Knowledge, CSIR-IICT, Hyderabad, India.

Extraction and Isolation. The air-dried stems of *X. granatum* (5 kg) were powdered and extracted successively with *n*-hexane, $CHCl_3$, and acetone in a Soxhlet apparatus. The $CHCl_3$ extract (21 g) was subjected to silica gel (230−400 mesh) vacuum liquid chromatography (VLC) eluted with *n*-hexanes and gradient *n*-hexanes/acetone/MeOH to 20% MeOH. Identical fractions were pooled based on the TLC profile to furnish a total of seven fractions (A−G).

Fraction B (5.1 g) was chromatographed over a silica gel column using *n*-hexane/acetone (70:30 to 20:80) as eluent to afford six subfractions (B1−B6). The first two subfractions on repeated column chromatography (CC) afforded a mixture of compound **1** and corniculatolide A (^{1}H NMR, S5, Supporting Information). This mixture was further chromatographed on silica gel CC to give compounds **1** (3 mg) and **4** (105 mg). Fraction D (2.7 g) was subjected to silica gel (100−200 mesh) CC using *n*-hexane/acetone as eluent to give four subfractions (D1−D4). Repeated column chromatography of D1 yielded a mixture of compounds **2**, **4**, and **5** (^{1}H NMR, S15, Supporting Information). The silica gel CC of this mixture yielded compounds **2** (2 mg) and **5** (3 mg). Fraction E (2.4 g) was subjected to silica gel CC gradient elution with *n*-hexane/

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acetone (50:50 to 0:100) to afford six subfractions (E1−E6). Repeated silica gel CC of fraction E3 eluting with a mixture of *n*-hexane/EtOAc (80:20) afforded compound **3** (5 mg). Purification of fraction E1 afforded compounds **6** (5 mg) and **7** (2 mg). Friedelin (**8**) (12 mg) was obtained by crystallization of fraction A from *n*-hexane.

Acetylation of Compound 3. Compound **3** (1 mg) was dissolved in pyridine (0.1 mL), and Ac_2O (0.1 mL) was added. The solution was left overnight at room temperature. After workup, the product was purified by recrystallization from *n*-hexane/acetone (8:2) to afford compound **3a** (2 mg).

*Corniculatolide B (**1**):* $C_{20}H_{23}O_5$; colorless crystals from a mixture of *n*-hexane/acetone (8.5:1.5), R_f 0.50 [*n*-hexane/acetone (7:3)]; mp 194−195 °C; UV (CH_3OH) λ_{max} 272 nm; IR (KBr) ν_{max} 1726, 1591, 1505, 1100 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS at m/z 343.1563 $[M + H]^+$ [calcd for $C_{20}H_{23}O_5$,343.1545].

*Isocorniculatolide B (**2**):* $C_{20}H_{22}O_5$; colorless amorphous powder eluted with *n*-hexane/acetone (8:2), R_f 0.50 [*n*-hexane/acetone (7:3)]; UV (CH_3OH) λ_{max} 273 nm; IR (KBr) ν_{max} 2925, 2854, 1732, and 1212 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS at m/z 343.1547 $[M + H]^+$ [calcd for $C_{20}H_{23}O_5$,343.1545].

*Corniculatolide C (**3**):* $C_{18}H_{18}O_5$; colorless crystals from eluent *n*-hexane/EtOAc (8:2), R_f 0.5 [*n*-hexane/acetone, (6:4)]; mp 171−175 °C; UV (CH_3OH) λ_{max} 270 nm; IR (KBr) ν_{max} 3440, 1696, 1515, 1238, 1173 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS at m/z 315.1228 $[M + H]^+$ [calcd for $C_{18}H_{19}O_5$,315.1232].

*11,12-Di-O-acetylcorniculatolide C (**3a**):* $C_{22}H_{22}O_7$; IR (KBr) ν_{max} 2922, 2855, 1736, 1592, 1445, 1380, 1159; ^1H NMR (Table 1); ESIMS at m/z 421.20 $[M + Na]^+$.

X-ray Crystallographic Analysis of Compounds 1 and 3.[21] Colorless crystals of compounds **1** and **3** were obtained from *n*-hexane/acetone (8.5:1.5) and *n*-hexane/EtOAc (8:2), respectively. The X-ray crystallographic data for compounds **1** and **3** were collected at ambient temperature using a Bruker Smart Apex CCD diffractometer with a graphite-monochromated MoKα radiation (λ = 0.710 73 Å) with the ω-scan method.[21] Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined using 4538 reflections for **1** and 7992 for **3** data. Integration and scaling of intensity data were accomplished using the SAINT program.[22] The structures were solved by direct methods using SHELXS97,[23] and refinement was carried out by full-matrix least-squares technique using SHELXL97.[23] Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms, with C−H distances of 0.93−0.97 Å, and with $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}$ for methyl atoms.

*Crystal Data for Compound **1**.* $C_{20}H_{23}O_5$; fw = 342.37, monoclinic space group $P2_1/n$, unit cell dimensions a = 10.2117(6) Å, b = 11.8998(7) Å, c = 14.8791(8) Å, V = 1746.70(17) $Å^3$, $\alpha = \gamma$ = 90°, β = 104.9700(10)°, Z = 4, d ρ_{calcd} = 1.318 g/cm^3, crystal dimensions 0.39 × 0.37 × 0.31 mm^3, μ = 0.093 mm^{-1}, $F(000)$ = 728. The final refinement gave R1 = 0.0509 and wR2 = 0.1388 [$I > 2\sigma(I)$].

*Crystal Data for Compound **3**.* $C_{18}H_{18}O_5$; fw = 314.32, monoclinic space group $P2_1/n$, unit cell dimensions a = 9.6693(5) Å, b = 11.1770(6) Å, c = 14.8856(9) Å, V = 1521.60(15) $Å^3$, $\alpha = \gamma$ = 90°, β = 108.9450(10)°, Z = 4, ρ_{calcd} = 1.372 g/cm^3, crystal dimensions 0.43 × 0.38 × 0.30 mm^3, μ = 0.10 mm^{-1}, $F(000)$ = 664. The final refinement gave R1 = 0.0475 and wR2 = 0.1316 [$I > 2\sigma(I)$].

α-Glucosidase Inhibitory Assay. The method for enzymatic inhibitory assay was adopted from Li et al. with a slight modification.[20] Test compounds were prepared with DMSO from a 1 mg/mL stock solution from which six different concentrations (100−3.125 μg/mL) were prepared. Both α-glucosidase enzyme (1 U/mL) and *p*NPG substrate (3 mM) were separately prepared in phosphate buffer (pH 6.9). The test compounds and 1 U/mL of α-glucosidase from *Saccharomyces cerevisiae* diluted with 1000 μL of buffer were preincubated at 37 °C for 15 min. The reaction was initiated by adding 500 μL of 3 mM *p*NPG. After 5 min, the reaction was quenched by addition of 2 M NaOH. The enzyme activity was determined by measurement of the release of *p*-nitrophenol from the *p*NPG substrate. The reaction was monitored by change in absorbance using a JASCO V-750 spectrophotometer. Each preparation was conducted in triplicate, and the results are presented as the mean ± standard error from three independent experiments. Acarbose was used as a positive control.

Cell Culture. MIAPaCa-2 (pancreatic cancer), DU145 (prostate cancer), MCF-7 (breast cancer), and HTC-116 (colon cancer) cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 10% fetal bovine serum (Invitrogen) and Penstrep (Invitrogen). The cell lines were maintained at 37 °C in a humidified atmosphere with 5% CO_2 in an incubator.

Cytotoxicity Assay for Mammalian Cells. In vitro cytotoxicity was determined against a panel of mammalian cells. Cells were placed in a 96-well culture plate at a density of 1 × 10^4/well. After overnight incubation, the cells were treated with compounds (**1**−**7**) for 24 and 48 h. Following the incubation period, cells were fixed with trichloroacetic acid and stained with SRB. For quantification, the SRB was solubilized with 10 mM Tris-HCl, and absorbance was determined at 510 nm using a spectrophotometer (Multimode Varioskan Flash, Thermo Fisher Scientific). Assays were performed in triplicates. The relative cell viability was calculated using the equation (mean OD of treated cells/mean OD of control cells) × 100. Control cells represent cells treated with vehicle DMSO only. At each time point, the treated cells were compared with the control cells. Doxorubicin and etoposide were used as a positive controls.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b00414.

HRESIMS of compounds **1**, **2**, and **3**; ^1H NMR spectrum of a mixture of compounds **1** and **4** and a mixture of compounds **2**, **4**, and **5**. ^1H and ^{13}C NMR spectra of compounds **1**, **2**, and **3**; IR spectra of compound **3**; ^1H NMR of **3a**; DEPT and 2D NMR (HSQC, HMBC $^1H-^1H$ COSY, $^1H-^1H$ NOESY) spectra of compounds **1**, **2**, and **3** (PDF)

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Notes

The authors declare no competing financial interest.

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