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NEW DIHYDROAGAROFURANOID SESQUITERPENES FROM *CELASTRUS ANGULATUS*

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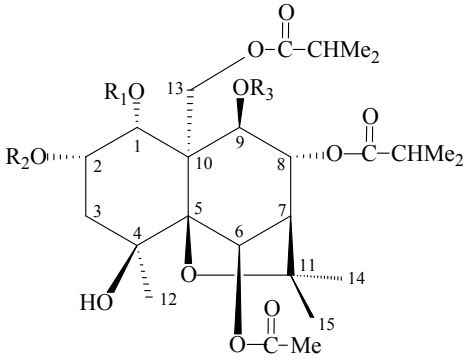
Abstract − Three new highly esterified *β*-dihydroagarofuran sesquiterpenes (**1**-**3**) were isolated from the root barks of *Celastrus angulatus* Max. together with eight (**4**–**11**) known ones. The new compounds were identified as 1*β*-picolinoyloxy-2*β*,6*α*-diacetoxy-8*β*,13-diisobutanoyloxy-9*α*-benzoyloxy-4*α*-hydroxy-*β*-dihydro-agarofuran (**1**), 1*β*-picolinoyloxy-2*β*,6*α*-diacetoxy-8*β*,13-diisobutanoyloxy-9*α*-furanoyloxy-4*α*-hydroxy-*β*-dihydroagarofuran (**2**), and 1*β*,6*α*-diacetoxy-2*β*-nicotinoyloxy-8*β*,13-diisobutanoyloxy-9*α*-benzoyloxy-4*α*-hydroxy-*β*-dihydro-agarofuran (**3**) on the basis of spectroscopic methods. Compounds **1-11** were all possessed cytotoxic activities against three human tumor cell lines by MTT assay.

There are about 50 species in the genus *Celastus*, family Celastraceae, and some of them have been applied as important folk medicines to treat fever, chill, joint pain, edema, rheumatoid arthritis, and bacterial infection or as natural insecticides in China and South America for a long history.[1] The family Celastraceae is well-known to provide various highly oxygenated dihydroagarofuran derivatives. As for the same skeleton of *Celastrus angulatus*, these compounds usually posses six different polyester substitutes in carbon positions C-1, 2, 6, 8, 9 and 13, such as acetoxy (Ac), isobutanoyloxy (iBu), (*α*-methyl)-butanoyloxy (iPet), benzoyloxy (Bz), furanoyloxy (Fu), nicotinoyloxy (Nic), picolinoyloxy (Pic) and cinnamoyloxy esters etc. Different species of genus *Celastus* may take different oxygenated level in C-2, 4, 6, 8 or 13 of the skeleton, such as *Celastrus Hindsii, Celastrus orbiculatus, Celastrus paniculatus, Celastrus Stephanotiifolius* and *Celastrus Wulcanicola,* etc. Considering the amounts of polyester substitutes and connection positions variations, these compounds have abundant molecular diversities, with more than 100 of them reported to date.[2] These ingredients were responsible in major for traditional functions of *Celastus* plants such as insecticidal, antitumor, multidrug-resistance reversing,

antituberculosis, and immunosuppressive activities. In the present study, three new highly esterified sesquiterpenes (**1**–**3**, Scheme 1) were isolated from the root barks of *Celastrus angulatus* Max., along with eight known ones (**4**–**11**), 1*α*,2*α*,6*β*-triacetoxy-8*α*,13-di(*α*-methyl)-butanoyloxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**4**),[3] 1*α*,2*α*,6*β*-triacetoxy-8*α*-isobutanoyloxy-9*β*-benzoyloxy-13-(*α*-methyl)-butanoyloxy-4*β*-hydroxy-*β*-dihydro-agarofuran (**5**),[4] 1*α*,2*α*,6*β*-triacetoxy-8*α*,13-diisobutanoyloxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**6**),[4] 1*α*,6*β*,8*α*-triacetoxy-2*α*,13-diisobutanoyloxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**7**),[5] 1*α*,2*α*,6*β*-triacetoxy-8*α*-isobutanoyloxy-9*β*-furanoyloxy-13-(*α*-methyl)-butanoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**8**),[6] 1*α*,6*β*,8*β*-triacetoxy-2*α*,13-diisobutanoyloxy-9*β*-furanoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**9**),[7] 1*α*,2*α*,6*β*-triacetoxy-8*α*,13-diisobutanoyloxy-9*β*-furanoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran (**10**),[8] and 1*α*,2*α*-diacetoxy-8*α*,13-diisobutanoyloxy-9*β*-benzoyloxy-4*β*,6*β*-dihydroxy-*β*-dihydroagarofuran (**11**).[9] In this paper we describe the isolation and structural elucidation of the three new compounds, as well as the evaluation of their cytotoxic activities against human HeLa, SMMC-7721, and HL-60 tumor cell lines.



1 R_1 = Pic R_2 = Ac R_3 = Bz
2 R_1 = Pic R_2 = Ac R_3 = Fu
3 R_1 = Ac R_2 = Nic R_3 = Bz

Figure 1. Chemical structure of compounds **1-3**

Compound **1** was obtained as a white amorphous powder ($CHCl_3$) and gave positive Vanillin reaction. Its moleucular formula was analyzed for $C_{40}H_{49}O_{14}N$ at *m/z* 768.3222 ($[M+H]^+$, calcd 768.3226) by HR-ESIMS. The ^{13}C-NMR spectrum of **1** revealed nine methyls, two methylenes, nine oxygenated carbon signals (between δ_C 65.9–91.5), and six ester C=O groups (δ_C 163.9-177.2). Two signals at δ_C 91.5 (5-C) and 54.4 (10-C) are common characteristics of *β*-dihydroagarofuran skeleton. The ^{1}H-NMR spectrum of **1** indicated the proton signals of a *β*-dihydroagarofuran skeleton, such as three tertiary methyls and seven oxygenated methylenes (between δ_H 4.92–6.33), and the proton signals of six ester groups to be two methyls of two acetate esters, four secondary methyls of two isobutanoate esters, five aromatic proton signals of one benzoate ester, and four aromatic proton signals of one picolinoylate ester,

by comparisons with the corresponding signals of angulatusine A (1*α*,2*α*,8*β*-triacetoxy-9*α*-benzoyloxy-13-picolinoyloxy-*β*-dihydroagarofuran)[10] and other known compounds. Assignments of the H- and C-atom signals of **1** were obtained by extensive 2D NMR (HSQC, ^{1}H-^{1}H COSY, HMBC and NOESY) analyses as shown in Table 1 and Figure 1. The linkage of the six esters to the skeleton locations were confirmed by the HMBC correlations, as between δ_H 5.30 (H-8) and δ_C 175.8 (8-C=O of iBu), δ_H 4.92/5.02 (H-13) and δ_C 177.2 (13-C=O of iBu), δ_H 5.88 (H-1) and δ_C 163.9 (1-C=O of Pic), δ_H 5.72 (H-9) and δ_C 164.3 (9-C=O of Bz), δ_H 6.33 (H-6) and δ_C 169.5 (6-C=O of Ac). The HMBC correlation of remaining one acetyl group could not be observed, and it should be connected to the remaining position at C-2. Assignments of the relative configurations of parent skeleton of compound **1** were based on the splitting patterns and the coupling constants of proton signals, and by comparisons with the corresponding signals of known compounds.[10,11] The singlet peak of H-6 could be observed as the dihedral angels of H-6 and H-7 were near 90°. Similarly, the singlet peak of H-9 suggested H-8 and H-9 both take equatorial orientations. The coupling constant between H-1 (J = 3.6 Hz) and H-2 suggested they take same orientations. Usually, H-1 and H-6 should have same axial orientations by comparing with other compounds of this class. In the NOESY spectrum of compound **1**, cross-peaks between H-1 (δ_H 5.88)/H-2 (δ_H 5.71) /H-3β (δ_H 2.34), Me-12 (δ_H 1.52)/H-6 (δ_H 6.33)/ H-7 (δ_H 2.40), H-6 (δ_H 6.33)/CH_2-13 (δ_H 4.92 and 5.02) /H-1 (δ_H 5.88) further confirmed their stereochemistry configurations. Accordingly, structure of **1** was elucidated as 1*α*-picolinoyloxy-2*α,*6*β*-diacetoxy-8*α*,13-diisobutanoyl-oxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran.

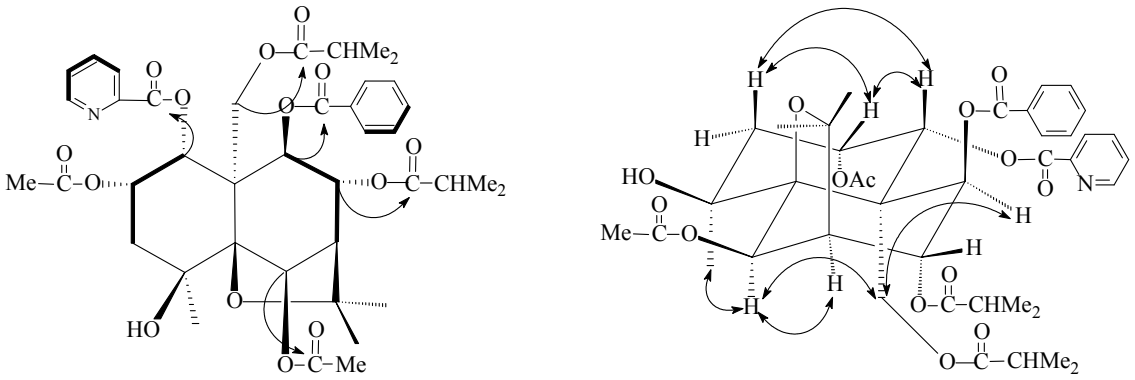


Figure 2. Key COSY (H ━ H), HMBC (H→C) and NOESY (H↔H) correlations of compound **1**

Compound **2** was obtained as a white amorphous powder ($CHCl_3$). Its moleucular formula was determined to be $C_{38}H_{47}O_{15}N$ at *m/z* 758.3016 ([M+H]$^+$, calcd 758.3018) by HR-ESIMS. By comparisons with 1*α*-nicolinoyloxy-2*α,*6*β,*8*α*-triacetoxy-13-isobutanoyloxy-9*β*-furanoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran[11] and **1**, the ^{1}H and ^{13}C-NMR data of **2** were similar to those of **1**, with difference of a furanoyloxy ester (δ_C 109.4, 117.6, 143.7, 148.7, 160.7) substituted at C-9 instead of a benzoyloxy group

Table 1.^{1}H (400 Hz) and ^{13}C (100 MHz) NMR data of compounds **1-3** ($CDCl_3$ δ ppm)

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	72.1	5.88 d (3.6)*	71.9	5.84 d (3.6)	70.8	5.75 d (3.6)
2	68.2	5.71 m	68.1	5.69 m	69.3	5.89 m
3	42.1	2.34 m, 2.07 m	42.0	2.25 m, 2.09 m	42.2	2.37 m, 2.07 m
4	69.9	-	69.9	-	69.7	-
5	91.5	-	91.4	-	91.4	-
6	75.6	6.33 s	75.5	6.29 s	75.5	6.34 s
7	53.0	2.40 d (2.8)	52.9	2.38 d (2.8)	53.1	2.39 d (2.8)
8	76.3	5.30 d (2.8)	76.2	5.25 d (2.8)	76.3	5.31 d (2.8)
9	72.5	5.72 s	71.8	5.61 s	72.4	5.66 s
10	54.4	-	54.2	-	54.0	-
11	83.5	-	83.5	-	83.6	-
12	24.6	1.52 s	24.6	1.51 s	25.0	1.58 s
13	65.9	4.92 d, 5.02 d (12.8)	65.8	4.98 d, 4.92 d (12.8)	65.8	4.73 d, 5.12 d (12.8)
14	25.7	1.68 s	25.5	1.63 s	25.7	1.68 s
15	29.5	1.60 s	29.6	1.58 s	29.6	1.60 s
4-OH		2.79 br s		2.77 br s		2.82 br s
2xCH_3CO						
CH_3	21.0, 21.5	2.12 s, 2.02 s	21.0 s, 21.5 s	2.11 s, 2.03 s	20.4,2 1.5	2.11s, 1.45 s
C=O	169.5 x2	-	169.5, 169.6	-	169.5x 2	-
2x$(CH3)_2CHCO$						
1′	33.9	2.86 m	33.9	2.84 m	33.9	2.68 m
1″	34.0	2.66 m	34.0	2.65 m	34.0	2.36 m
2′	19.0	1.27 d (7.2)	19.0	1.27 d (7.2)	19.0	1.17 d (7.2)
2″	19.2	1.31 d (7.2)	19.2	1.31 d (7.2)	19.0	1.14 d (7.2)
3′	18.8	1.24 d (7.2)	18.8	1.24 d (7.2)	18.8	1.25 d (6.8)
3″	18.9	1.25 d (7.2)	18.9	1.25 d (7.2)	18.9	1.25 d (6.8)
8-C=O	175.8	-	175.7	-	175.9	-
13-C=O	177.2	-	177.1	-	176.9	-
1-Pic-2′	125.1	-	125.3	-		
3′	150.2	8.56 br s	150.4	8.78 d (1.2)		
4′	136.2	7.61 ddd (8.0, 1.2,1.2)	136.4	7.83 ddd (8.0, 1.2,1.2)		
5′	122.8	7.11 dd (8.0,4.8)	123.0	7.23 dd(8.0,4.8)		
6′	153.2	8.60 br d (4.8)	153.4	8.66 dd(4.8,1.2)		
C=O	163.9		164.2			
9-Fu-2′			148.7	7.75 dd (1.6)		
3′			117.6	-		
4′			143.7	7.28 dd (1.6)		
5′			109.4	6.36 dd (1.6)		
C=O			160.7	-		
9-Bz′-1′	130.1	-			130.2	-
2, 6′	129.8	7.65 d (7.6)			128.5	8.01 d (7.6)
3′, 5′	128.2	7.27 dd (both 7.6)			128.2	7.46 dd (7.2,7.6)
4′	133.6	7.49 dd (both 7.6)			133.9	7.60 dd (both 7.2)
C=O	164.3	-			164.4	-
2-Nic-2′					151.1	9.24 d (1.6)
3′					125.3	-
4′					137.0	8.31 ddd (8.0, 1.6, 1.6)
5′					123.6	7.61 dd (8.0, 4.8)
6′					153.8	8.81 dd (4.8, 1.6)
C=O					164.4	-

*Data in parentheses are *J* values (in Hz).

of **1**. Assignments of the H- and C-atom signals of **2** were obtained by 2D-NMR analyses as shown in Table 1. The linkage of the five esters to the skeleton locations were confirmed by the HMBC corelations, as between δ_H 5.25 (H-8) and δ_C 175.7 (8-C=O of iBu), δ_H 4.92/4.98 (H-13) and δ_C 177.1 (13-C=O of iBu), δ_H 5.84 (H-1) and δ_C 164.2 (1-C=O of Pic), δ_H 5.61 (H-9) and δ_C 160.7 (9-C=O of Fu), δ_H 6.29 (H-6) and δ_C 169.5 (6-C=O of Ac). The left one acetyl group was then assigned to the remaining position at C-2. Compound **2** was comfirmed as 1*α*-picolinoyloxy-2*α,*6*β*-diacetoxy-8*α*,13-diisobutanoyloxy-9*β*-furanoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran.

Compound **3** was obtained as a white amorphous powder ($CHCl_3$). Its moleucular formula was analyzed for $C_{40}H_{49}O_{14}N$ at *m/z* 768.3233 ($[M+H]^+$, calcd 768.3226) by HR-ESIMS, same as that of **1**. By comparison with 1*α*-nicolinoyloxy-2*α,*6*β,*8*α*-triacetoxy-13-isobutanoyloxy-9*β*-furanoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran[11] and **1**, the ^1H and ^{13}C-NMR data of **3** were similar to those of **1**, with a different substitute ester of a nicotinoyloxy ester in stead of a picotiloxy group of **1**. Assignments of the H- and C-atom signals of **3** were obtained by comparing with the corresponding signals of **1** and by 2D-NMR analyses as shown in Table 1. In HMBC spectrum of compound **3**, correlations between δ_H 5.89 (H-2), 5.66 (H-9) and δ_C 164.4×2 (both C=O of Nic and 9-Bz) confirmed the linkage of the nicotinoyloxy ester at C-2. Other HMBC correlations could be observed between δ_H 5.31 (H-8) and δ_C 175.9 (8-C=O of iBu), δ_H 4.73/5.12 (H-13) and δ_C 176.9 (13-C=O of iBu), δ_H 5.75 (H-1), 6.34 (H-6) and δ_C 169.5 (1- and 6-C=O of Ac). Compound **3** was thus elucidated as 1*α*,6*β*-diacetoxy-2*α*-nicolinoyloxy-8*α*,13-diisobutanoyloxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydroagarofuran.

Table 2. Cytotoxic activities of compounds **1-11** against three human tumor cell lines

Compound	IC_{50} (μM) [a]		
	HeLa	SMMC-7721	HL-60
1	17.73±2.16	21.59±2.32	16.60±2.55
2	20.80±2.81	17.73±1.68	19.16±2.40
3	15.17±1.65	21.25±2.68	21.08±2.31
4	22.38±2.83	17.40±2.49	16.95±3.07
5	17.77±2.63	16.66±1.92	18.47±2.55
6	22.51±3.02	21.09±3.58	21.42±2.82
7	20.07±2.46	24.38±3.35	20.92±2.69
8	24.07±3.20	18.45±1.86	16.92±2.74
9	14.77±2.17	22.59±2.64	23.80±2.33
10	20.06±2.58	20.78±2.15	22.33±2.81
11	23.70±2.80	17.90±1.97	20.62±2.67
Norcantharidin [b]	4.16±0.37	5.23±0.42	3.45±0.26

[a] Results are the mean of three replications; [b] reference control

Cytotoxic activities of isolated compounds **1-11** were tested by MTT assay *in vitro* and expressed as IC_{50} values. They all exhibited moderate cytotoxic activities against human HeLa, SMMC-7721, and HL-60 tumor cell lines, with IC_{50} values ranging from 14.77 to 24.38 μM (Table 2). Compounds **1-11** with a hydroxy group at C-4 showed similar cytotoxic activity results with literatures,[12] which suggested that each of these derivatives might display a unique mode of antitumor action. The present results suggested all these compounds might be, at least in part, responsible for the traditional therapeutic effect of root barks of *Celastrus angulatus*.

EXPERIMENTAL

General

Melting points were determined by the XT5 micro-melting-point apparatus (XT5, Beijing families instrument light instrument plant, China) and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. IR spectra were taken on a Perkin-Elmer 983 G spectrometer. ^{1}H-, ^{13}C-NMR and 2D NMR spectra were recorded on a Varian Inova 400 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as internal standard. EI-MS spectra were determined on a Micromass Zabspec spectrometer. HRESI-MS spectra were determined on a Micromass Q-TOF2 spectrometer. Preparative HPLC was carried out on the columns of ODS (3.5 × 400 cm, Cosmosil 75C_{18}-Prep, 250 × 20 mm i.d., 5 μm PRC-ODS column; Shimadzu Co. Ltd.), the flow rate was 2 mL/min and the wavelength for detection was 254 nm. Silica gel (200-300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory, Qingdao, China. Precoated plates of silica gel used for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. Compounds on the TLC were colored by 10% sulfuric acid alcohol solution. HeLa uterocervical carcinoma cell, SMMC-7721 hepato carcinoma cell and HL-60 leukocythemia carcinoma cell were purchased from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Plant material

The root barks of *Celastrus angulatus* Max. were collected from Liu'an city, Anhui Province, China, in March 2008, and the botanical origin of material was identified by Prof. Chun-yu Liu, College of Pharmaceutical Science, Soochow university, and the voucher specimen (No. CA080601) was deposited at department of natural medicinal chemistry, College of Pharmaceutical Science, Soochow University.

Extraction and isolation

The air-dried plants (15 kg) were extracted with 95% EtOH (60 L × 3) under reflux for 2 h. After evaporation, the residue (1.3 kg) was suspended in H_2O (15 L) and partitioned with petroleum ether (PE, 60–90 °C; 15 L × 4). The PE layer was concentracted to give a residue (169 g), which was subjected to vacuum liquid column on silica gel (30 × 25 cm, 200-300 mesh; Qingdao Haiyang Chemical Co. Ltd.)

using a stepwise gradient elution of PE - EtOAc (9 : 1; 8 : 2; 7 : 3; 6 : 4; 0 : 10; each 3.0 L) to afford 14 subfractions (CA-1~14). CA-3 (6.2 g) was subjected to silica gel columns (4 × 60 cm), eluted with PE - EtOAc (90:10; 80:20; 75:25) repeatedly, and purified by Sephadex LH-20 ($CHCl_3$: MeOH = 1:1) to yield **4** (14.2 mg). CA-4 (5.1 g) was chromatographed on silica gel (4 × 60 cm) with gradient elution of PE - EtOAc (90:10; 80:20; 75:25; 70:30) to afford 11 subfractions (CA-4.1 to CA-4.11); CA-4.7 (1.4 g) was then chromatographed by MPLC over ODS-C_{18} column (3.5 × 40 cm, Cosmosil 75 C_{18}-Prep), eluted with MeOH-H_2O (70:30; 75:25; 80:20; 85:15, 100:0; each 800 mL) to afford compounds **5** (18 mg), **6** (15 mg) and **7** (12 mg) from MeOH-H_2O (80:20 and 85:15) fraction. CA-5 (5.4 g) was chromatographed on silica gel (4 × 60 cm), eluted with PE - EtOAc (80:20; 75:25; 70:30; 60:40) to afford 8 subfractions (CA-5.1 to CA-5.8); CA-5.5 (1.8 g) was then chromatographed by MPLC over ODS-C_{18} column (3.5 × 40 cm, Cosmosil 75 C_{18}-Prep), eluted with MeOH-H_2O (60:40; 70:30; 75:25; 85:15, 100:0; each 800 mL) to afford compounds **8** (11 mg), **9** (10 mg) and **10** (13 mg) from MeOH-H_2O (75:25) fraction. CA-6 (4.5g) was chromatographed on silica gel (4 × 60 cm) with gradient elution of PE - EtOAc (80:20; 75:25; 70:30; 60:40; each 800 mL) to afford 6 subfractions (CA-6.1 to CA-6.6); CA-6.3 (2.3 g) was then chromatographed by MPLC over ODS-C_{18} column (3.5 × 40 cm, Cosmosil 75 C_{18}-Prep; Nacalai Tesque Inc.), eluted with MeOH-H_2O (65:35; 70:30; 75:25; 100:0; each 800 mL) to afford 11 subfractions (6.3.1 to CA-6.3.11); CA-6.3.3 (0.9 g) was further purified by HPLC (250 × 20 mm i.d., 5 μm PRC-ODS column; Shimadzu Co. Ltd.), eluted with MeOH-H_2O (70:30 – 75:25, 6 mL/min) to yield compounds **1** (11 mg) , **2** (10 mg) and **3** (13 mg). CA-7 (4.5 g) was subjected to silica gel columns (4 × 60 cm), eluted with PE - EtOAc (75:25; 70:30) repeatedly, and purified by Sephadex LH-20 ($CHCl_3$: MeOH = 1:1) to yield **11** (14 mg).

1*α*-Picolinoyloxy-2*α*,6*β*-diacetoxy-8*α*,13-diisobutanoyloxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydro-agarofuran (**1**): White amorphous powder, $[\alpha]_D^{20}$ -9.0 (*c* 0.21, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm: 226, 230 (sh), 274. IR (KBr) cm^{-1}: 3485, 2978, 1746, 1725, 1601, 1588, 1280, 1240, 745, 715. HR-ESIMS *m/z* 768.3222 $[M+H]^+$ (calcd for $C_{38}H_{47}O_{15}N$: 768.3226). ^1H- and ^{13}C- data are given in Table 1.

1*α*-Picolinoyloxy-2*α*,6*β*-diacetoxy-8*α*,13-diisobutanoyloxy-9*β*-furanoyloxy-4*β*-hydroxy-*β*-dihydro-agarofuran (**2**): White amorphous powder, $[\alpha]_D^{20}$ -17.0 (*c* 0.22, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm: 226, 245. IR (KBr) cm^{-1}: 3488, 2982, 1742, 1730, 1600, 1590, 1280, 1218, 750, 717. HR-ESIMS *m/z* 758.3016 $[M+H]^+$ (calcd for $C_{40}H_{49}O_{14}N$: 758.3018). ^1H- and ^{13}C- data are given in Table 1.

1*α*,6*β*-Diacetoxy-2*α*-nicolinoyloxy-8*α*,13-diisobutanoyloxy-9*β*-benzoyloxy-4*β*-hydroxy-*β*-dihydro-agarofuran (**3**): White amorphous powder, $[\alpha]_D^{20}$ -11.0 (*c* 0.18, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm: 216, 232 (sh), 273. IR (KBr) cm^{-1}: 3480, 2980, 1745, 1718, 1600, 1585, 1310, 1235, 741, 712. HRESI-MS *m/z* 768.3233 $[M+H]^+$ (calcd for $C_{40}H_{49}O_{14}N$: 768.3226). ^1H- and ^{13}C- data are given in Table 1.

Cytotoxic activity

MTT colorimetric assay *in vitro* was performed to evaluate the cytotoxic activities of compounds **1**-**11** (Purity higher than 90% as determined by HPLC) against human HeLa, SMMC-7721, and HL-60 tumor cell lines (Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) as reported,[13] with norcantharidin (Purity higher than 99.0% as determined by HPLC; Nanjing Zelang Medical Technology Co. Ltd.) as positive control.

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