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New 30-ketophragmalins with anti-breast cancer activity against MDA-MB-453 cells from the Godavari mangrove, *Xylocarpus moluccensis* (Lam.) M. Roem

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ABSTRACT

30-Ketophragmalins are a small group of limonoids. To date, only 21 ones have been exclusively reported from the mangrove, *Xylocarpus moluccensis* (Lam.) M. Roem. In this paper, three new 30-ketophragmalins, named godavarins L-N (**1**-**3**), were obtained from the seeds of *X. moluccensis*, collected in the mangrove swamp of Godavari estuary, Andhra Pradesh, India, together with a mexicanolide, named godavarin O (**4**). The structures of these compounds, including absolute configurations of godavarins L-M (**1**-**2**), were determined on the basis of HRESIMS, extensive 1D and 2D NMR spectroscopic data, and electronic circular dichroism (ECD) quantum-chemical calculations. Compounds **1**, **2**, and **4** contain a $\Delta^{8,14}$ double bond, whereas **3** possesses $\Delta^{8,9}$, $\Delta^{14,15}$ conjugated double bonds. Two 30-ketophragmalins, *viz.* **1** and **3**, exhibited antitumor activities against the triple-negative breast cancer MDA-MB-453 cells with IC_{50} values of 2.1 ± 0.06 and 9.0 ± 1.0 μM, respectively. This is the first report of the anti-breast cancer activity of 30-ketophragmalins.

1. Introduction

Plants of the family Meliaceae are well known resources for the production of limonoids, being highly-oxidized tetranortriterpenoids derived from a biogenetic precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. Due to plentiful structural diversity and a wide range of bioactivities, limonoids have attracted considerable attention in natural products research during recent years (Behenna and Corey, 2008; Peng et al., 2016; Schuster et al., 2011; Shi et al., 2017; Yamashita et al., 2015). Up to now, more than 1300 limonoids with at least 35 types have been reported (Wu et al., 2008; Fang et al., 2011; Tan and Luo, 2011; Ye et al., 2016). Limonoids exhibited insect antifeedant, antibacterial, antiviral, and antitumor activities (Armelle et al., 2016; Matos et al., 2009; Sanna et al., 2015).

Xylocarpus moluccensis (Meliaceae) is a mangrove tree mainly distributed in tropical and sub-tropical tidal coastal zones and river deltas (Aritra and Amit, 2013). Phragmalins and mexicanolides, firstly obtained from the timber of the African *X. moluccensis* in 1976 and the seeds of *X. granatum* in 1970, respectively (Connolly et al., 1976; Okorie and Taylor, 1970), are two main types of limonoids reported from mangroves of the genus *Xylocarpus* (Tan and Luo, 2011; Hu and Wu, 2010; Shen et al., 2009). 30-Ketophragmalins are a small group of limonoids. To date, only 21 ones have been exclusively reported from mangroves of *X. moluccensis* (Pudhom et al., 2010; Wu et al., 2010; Ravangpai et al., 2011; Li et al., 2012a,b, Li et al., 2009; Li et al., 2017). As a part of our ongoing investigation of limonoids from mangroves (Li et al., 2012a,b; Li et al., 2017, 2016, 2015; Chen et al., 2014; Dai et al., 2017), three new 30-ketophragmalins (**1**-**3**) and a mexicanolide (**4**) (Fig. 1) were isolated from the seeds of the Godavari *X. moluccensis*. Herein, we report the isolation, structure elucidation, antitumor and anti-HIV activities of these limonoids.

2. Experimental

2.1. General experimental procedures

Optical rotations were determined on a MCP 200 polarimeter (*Anton Paar* GmbH, Germany). UV spectra were obtained on a GENESYS 10S UV–vis spectrophotometer (Thermo Fisher Scientific, Shanghai, China). NMR spectra were recorded on a Bruker AV-400 spectrometer (Bruker

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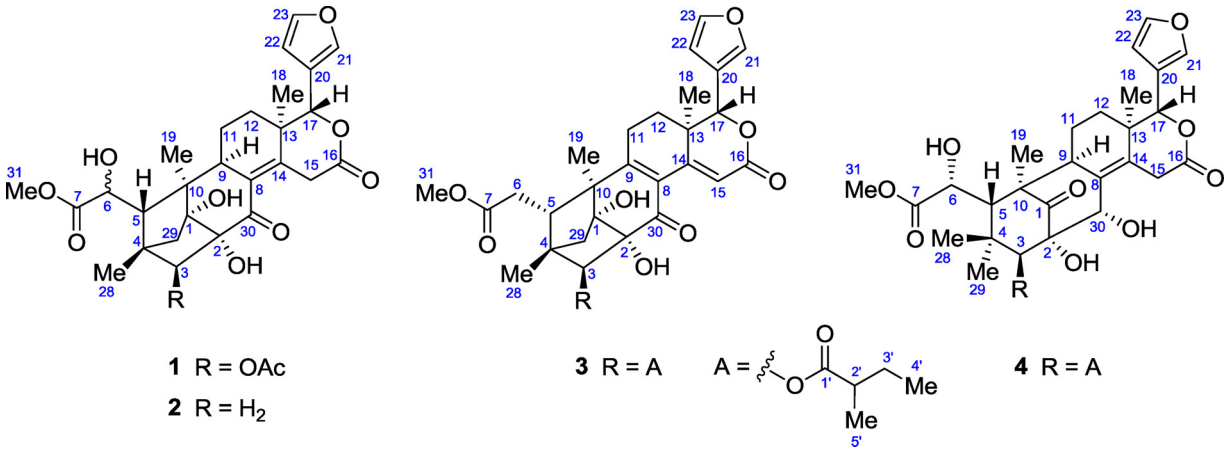

Fig. 1. Structures of compounds **1**-**4**.

Scientific Technology Co. Ltd., Karlsruhe, Germany) with TMS as the internal standard in $CDCl_3$ or acetone-d_6. HRESIMS data were detected on a 6210 ESI/TOF mass spectrometer (Agilent, CA, USA). Semi-preparative HPLC was performed on a Waters 2535 pump equipped with a 2489 UV detector (Waters Corporation, Milford, MA, USA) and YMC ODS columns (250 × 10 mm *i.d.,* 5 μm, or 250 × 4.6 mm i.d., 5 μm). Silica gel (100–200 mesh) (Qingdao Marine Chemical Industrial Co. Ltd., Qingdao, PR China) and C_{18} reversed-phase silica gel (YMC*GEL ODS-A-HG 12 nm, 50 μm, YMC, Japan) were used for column chromatography.

2.2. Plant material

The seeds of *Xylocarpus moluccensis* were collected during October 2009 in the mangrove swamp of Godavari estuary, Andhra Pradesh, India. The identification of the mangrove was done by Mr. Tirumani Satyanandamurty. A voucher sample (No. IXM200901) was deposited in Marine Drugs Research Center, College of Pharmacy, Jinan University, Guangzhou, PR China.

2.3. Extraction and isolation

The air-dried and powdered seeds (15.0 kg) of *X. moluccensis* were extracted with EtOH (95%, 5 × 15 L) at room temperature. After removal of the solvent under vacuum, the residue was partitioned between water and EtOAc (1: 2, v/v) to afford the EtOAc portion (320.1 g). The EtOAc portion was subjected to silica gel column chromatography (940 × 150 mm i.d.), eluted with a gradient mixture of $CHCl_3$/MeOH (from 100:0 to 5:1), to produce 251 fractions. The combination of fractions 90–112 (29.2 g, except the fraction 95, $CHCl_3$/MeOH, 75:1, v/v for elution) and further separation on a glass column packed with C_{18} reversed-phase silica gel (750 × 70 mm i.d., acetone/H_2O, from 2:3 to 1:0) gave 92 subfractions. Subfractions 11 and 12 (158.5 mg) were combined and purified by semi-preparative HPLC (MeCN/H_2O, 40:60, v/v, 3 mL min^{-1}) to afford compounds **1** (t_R = 7.5 min, 43.1 mg) and **2** (t_R = 8.1 min, 42.3 mg). The combination of fractions 81–85 (22.0 g) and further separation on an RP-18 column (640 × 70 mm i.d., acetone/H_2O, from 2:3 to 1:0) gave 80 subfractions. The subfraction 30 (102.8 mg) was purified by semi-preparative HPLC (MeOH/H_2O, 52:48, v/v, 3 mL min^{-1}) to yield compound **3** (t_R = 40.2 min, 1.8 mg). The fraction 95 (200.0 mg) was purified by semi-preparative HPLC (MeCN/H_2O, 40:60, v/v, 3 mL min^{-1}) to afford compound **4** (t_R = 25.2 min, 56.4 mg).

2.3.1. Godavarin L (1)

White amorphous powder; $[a]_D^{25}$ − 16° (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ε): 192 (4.28), 259 (3.93) nm; HRESIMS *m/z*: 559.2189 [M + H]$^+$ (calcd. for $C_{29}H_{35}O_{11}$, 559.2174); ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2.

2.3.2. Godavarin M (2)

White amorphous powder; $[a]_D^{25}$ − 14° (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ε): 193 (4.75), 251 (4.35) nm; HRESIMS *m/z*: 501.2129 [M + H]$^+$ (calcd. for $C_{27}H_{33}O_9$, 501.2119); ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2.

2.3.3. Godavarin N (3)

White amorphous powder; $[a]_D^{25}$ + 138° (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ε): 193 (3.89), 272 (3.81) nm; HRESIMS *m/z*: 583.2545 [M + H]$^+$ (calcd. for $C_{32}H_{39}O_{10}$, 583.2538); ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2.

2.3.4. Godavarin O (4)

White amorphous powder; $[a]_D^{25}$ − 100° (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ε): 195 (4.60) nm; HRESIMS *m/z*: 603.2810 [M + H]$^+$ (calcd. for $C_{32}H_{43}O_{11}$, 603.2800); ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2.

2.4. Computational details

Conformational analyses were carried out for godavarin L (**1**) using both BALLOON (Vainio and Johnson, 2007) and confab programs (O'Boyle et al., 2011). The BALLOON program searched conformational space with multi-objective genetic algorithm, whereas the confab program systematically generated diverse low energy conformations. The conformations generated by both programs were grouped together by removing duplicated conformations whose root mean square (RMS) distance was less than 0.5 Å. Semi-empirical PM3 quantum mechanical geometry optimizations were performed on conformations using the Gaussian 09 program (Frisch et al., 2009). Duplicated conformations after geometry optimization were then identified and removed. Remaining conformations were further optimized at B3LYP/6-31G* level of theory in acetonitrile solvent with IEFPCM solvation model using Gaussian 09 program (Tomasi et al., 2005), and duplicated conformations emerging after these calculations were removed according to the same RMS criteria above. Harmonic vibrational frequencies were performed to confirm the stability of the finally obtained conformers. ECD spectra were calculated for each conformer using the TDDFT methodology at the B3LYP/6-311 + + G**//B3LYP/6-31G* level of theory with acetonitrile as the solvent by the IEFPCM solvation model implemented in Gaussian 09 program. The calculated spectra for each conformer were combined after Boltzmann weighting according to their population contribution in which the Gibbs free energy of each conformer were obtained at B3LYP/6-311 + + G**//B3LYP/6-31G* level of