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Two new cytotoxic constituents from the *Clausena lansium* (Lour.) Skeels

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Cytotoxic activity

ABSTRACT

Two new compounds 6-methoxy-9H-carbazole-3-carboxylic acid (**1**) and 9-[3-methyl-4-(4-methyl-5-oxo-tetrapydro-furan-2-yl)-but-2-enyloxy]-furo[3,2-g]chromen-7-one (**5**) along with four known compounds clausine D (**2**), claulansines J (**3**), O-demethylmurrayanine (**4**) and pabularinone (**6**) were isolated from the *Clausena lansium*. The chemical structures of the new compound were elucidated by 1D and 2D NMR as well as HR-ESIMS spectral analysis. All the compounds isolated were evaluated for cytotoxic effects against human breast cancer (MCF-7), non-small lung carcinoma (H1299) and liver cancer (SMMC-7721). Compounds **2** and **4** exhibited strong cytotoxicity against MCF-7 and SMMC-7721 with IC_{50}values in the range 2.63–7.59 μg/ml, and Compounds **1** and **5** showed moderate cytotoxicity against MCF-7, H1299 and SMMC-7721 tumor cell lines.

1. Introduction

Clausena lansium (Lour.) Skeels (Rutaceae) is widely distributed in the south part of China. In Traditional Chinese Medicine, the leaves of *C. lansium* are used for cough, asthma, viral hepatitis, dermatological and gastrointestinal diseases. The leaves are also used as folk medicines for the treatment of acute and chronic viral hepatitis in China (Yang et al., 1988; Liu et al., 1996; Flecher, 2001). It was reported that carbazoles and coumarins from *C. lansium* exhibited a variety of bioactivities such as antimicrobial (Kuvatanasuchati et al., 2011; Liu and Wan, 2008; Tada et al., 2002; Chakraborty et al., 1995a,b; Wu et al., 1996), anti-inflammatory (Bandgar et al., 2012; Shen et al., 2012; Menghini et al., 2010; Kang et al., 2009), cytotoxicity (Itoigawa et al., 2000; Maneerat et al., 2012; Chakraborty et al., 1995a,b; Zhang et al., 2010) and anti-HIV (Sancho et al., 2004; Olomola et al., 2013).

Here, this work was concentrated on searching for more biologically active constituents from *C. lansium*. A new carbazole alkaloid and a new coumarin along with four known compounds were isolated and identified. The structures of these compounds were determined by extensive NMR and mass spectroscopy. All the compounds were tested against MCF-7, H1299 and SMMC-7721 tumor cell lines *in vitro*. In this paper, the processes of the isolation were presented, the elucidation of the molecular structure was discussed and the results of the cytotoxic activity tests were given for the above six compounds.

2. Results and discussion

With a silica gel column, the chromatography was carried out on the ethanol extract and petroleum ether–ethyl acetate extract obtained from the stems of *C. lansium*. From the former portion, four compounds, which were labeled as compounds **1**–**4** were obtained. While compounds **5** and **6** were isolated from the later

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portion. Compound **1** and compound **5** were new and identified as 6-methoxy-9H-carbazole-3-carboxylic acid and 9-[3-methyl-4-(4-methyl-5-oxo-tetrapydro-furan-2-yl)-but-2-enyloxy]-furo[3,2-g]chromen-7-one, respectively. The other four belonged to known compounds, which were clausine D (**2**) (Wu and Huang, 1992), claulansines J (**3**) (Liu et al., 2012), O-demethylmurrayanine (**4**) (Maneerat et al., 2012) and pabularinone (**6**) (Chakraborty et al., 1972).

Compound **1** was obtained as brown amorphous solid with the molecular formula $C_{14}H_{11}NO_3$ established on the basis of its HR-ESIMS *m/z* 264.0631 $[M+Na]^+$ (calculated for $C_{14}H_{11}NNaO_3$, 264.0637). The presence of the one functionality was supported by signals at δ_H 3.86 (1H, s, 6-OCH_3) in the ^1H NMR spectrum. The remainder of the spectrum consisted of two well separated ABX systems. One set of signals appeared at δ_H 7.51 (d, *J* = 8.5, 2.0 Hz), 7.98 (d, *J* = 8.5 Hz), 8.8 (d, *J* = 2.0 Hz) and the other at δ_H 7.08 (dd, *J* = 8.5, 2.0 Hz), 7.45 (d, *J* = 8.5 Hz), 7.85 (d, *J* = 2.0 Hz). The ^{13}C spectrum showed the presence of 14 carbons including a carboxyl proton (δ_C 168.6) and a methoxyl proton (δ_C 56.0). The H–H COSY spectrum exhibited the correlations between H-1 (δ_H 7.51) and H-2 (δ_H 7.98), and H-7 (δ_H 7.08) and H-8 (δ_H 7.45). The HMBC spectrum showed correlations arising from H-1 (δ_H 7.51) to C-3 (δ_C 120.8), H-2 (δ_H 7.98) to C-4 (δ_C 123.2), C-9a (δ_C 143.4) and COOH (δ_C 168.8), H-4 (δ_H 8.80) to C-2 (δ_C 127.2), C-9a (δ_C 143.4) and COOH (δ_C 168.8), H-5 (δ_H 7.85) to C-7 (δ_C 116.0) and C-8a (δ_C 135.4), H-7 (δ_H 7.08) to C-5 (δ_C 103.7) and C-8a (δ_C 135.4) and H-8 (δ_H 7.45) to C-5a (δ_C 123.5) and C-6 (δ_C 154.1) (Fig. 1). The NOESY correlations of N-H (δ_H 11.48) with H-1 (δ_H 7.51) and H-8 (δ_H 7.45), found in the NOESY spectrum, showed the position of the carboxyl proton and the methoxyl proton. On the basis of the results, the structure of compound **1** was identified as 6-methoxy-9H-carbazole-3-carboxylic acid.

Compound **5** obtained as a yellow gum. The molecular formula was established as $C_{21}H_{20}O_6$ by HR-ESI-MS that displayed a quasi-molecular ion peak at *m/z* 391.1152 $[M+Na]^+$ (calculated for $C_{21}H_{20}NaO_6$, 391.1158). The ^1H NMR spectrum showed the characteristic of furanocoumarin framework (Girennavar et al., 2006) at δ_H 8.16 (1H, d, *J* = 10.0 Hz, H-4), δ 8.14 (1H, d, *J* = 2.0 Hz, H-2′), 7.70 (1H, s, H-5), 7.10 (1H, d, *J* = 2.0 Hz, H-3′) and 6.45 (1H, d, *J* = 10.0 Hz, H-3) indicative of a substituent at C-8. This finding was supported by the HMBC correlations of H-5 to C-4a (δ_C 116.8), C-6 (δ_C 126.2), C-8a (δ_C 143.8), C-8 (δ_C 130.7) and C-7 (δ_C 116.8) (Fig. 1). Addition, the units of –OCH_2CH=C(Me)–CH_2– [δ_H 5.62 (1H, m, H-2″), 5.01 (2H, m, H-1″), 2.35 (1H, m, H-4″a), 2.22 (1H, m, H-4″b), 1.63 (3H, s, 9″-CH_3)] and α-methyl-γ-lactone ring [δ_H 4.55 (1H, m, H-5″), 2.66 (1H, m, H-7″), 1.85 (1H, m, H-6″a), 1.71 (1H, m, H-6″b), 1.09 (3H, d, 10″-CH_3)] were also observed in the ^1H NMR spectrum. On the COSY and HMBC spectra, both units are linked to each other at C-4″ and C-5″. The relative configuration of structure was determined by an analysis of the NOESY spectrum. The full assignments of ^1H NMR signals were further supported by NOESY spectra which showed NOESY correlations with H-5 (δ_H 7.70) of H-4 (δ_H 8.16) and H-2′ (δ_H 8.14). Therefore, the structure of **5** was identified to be 9-[3-methyl-4-(4-methyl-5-oxo-tetrapydro-furan-2-yl)-but-2-enyloxy]-furo[3,2-g]chromen-7-one.

Table 1
Cytotoxicity of compounds **1**–**6** against H1299, MCF-7, SMMC7721 cells.

Compound	IC_{50} (μg/ml)[a]		
	H1299	MCF-7	SMMC7721
1	40.20 ± 3.62	21.62 ± 1.11	75.39 ± 5.27
2	3.72 ± 0.29	2.63 ± 0.23	3.38 ± 0.87
3	23.46 ± 1.26	13.17 ± 2.07	30.56 ± 4.55
4	22.10 ± 3.26	4.42 ± 1.03	7.59 ± 1.33
5	–	49.21 ± 2.01	36.07 ± 1.92
6	–	33.74 ± 1.54	–
Doxorubicin[b]	6.79 ± 0.89	4.54 ± 0.52	1.59 ± 0.20

[a] Inhibitory activity was expressed as the mean ± SEM of 50% inhibitory concentration of triplicate determinations and was obtained by interpolation of concentration-inhibition curves.
[b] Doxorubicin used is positive control.

The cytotoxicity of six compounds was evaluated against MCF-7, H1299, SMMC-7721 cancer cell lines using the CCK-8 assay, with doxorubicin (DOX) as the positive control. The results were summarized in Table 1. Among the tested compounds, compound **2** exhibited potent cytotoxic effect against H1299, MCF-7 and SMMC7721 with IC_{50} values of 3.72, 2.63 and 3.38 μg/mL. Also, compound **4** showed strong activity toward MCF-7 and SMMC7721 with IC_{50} values of 4.42 and 7.59 μg/mL. When comparing the results obtained for these compounds, it can be suggested that the existence of a free hydroxyl or aldehyde group at C-1 and C-3 may be important for the cytotoxic activity.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 500 NMR spectrometer with TMS as the internal standard. ESI-MS and HR-ESI-MS were obtained on a Bruker Q-TOF mass spectrometer. Silica gel (160–200 mesh, 200–300 mesh, Qingdao Marine Chemical Plant, China) used for column chromatography and

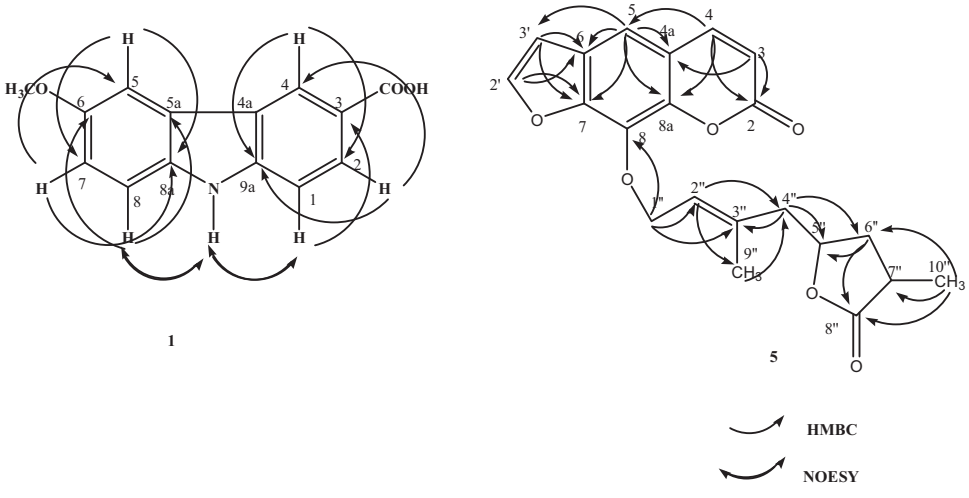

Fig. 1. HMBC correlations for **1** and **5**.