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Full Length Research Paper

New amides from the seeds of *Clausana lansium*

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An investigation of *Clausena lansium* seed extract led to the isolation and identification of two new amides, clausenalansamide A (3) and clausenalansamide B (4), along with three known amides (1, 2 and 5). All structures were characterized by spectroscopic methods. Some of isolated compounds (1, 2 and 4) were evaluated for their anti-cancer activity against three human cancer cell lines, KB, MCF7 and NCI-H187. Compounds 1, 2 and 4 showed cytotoxicity against KB and NCI-H187 cancer cell lines with IC_{50} value ranging from 13.73 to 28.48 µg/ml. Compound 1 was also exhibited weakly cytotoxicity against MCF7 cell line with the IC_{50} value of 48.67 µg/ml while compounds 2 and 4 were found to be inactive.

Key words: *Clausena lansium*, Rutaceae, amides, cytotoxic activity, clausenalansamide A and B.

INTRODUCTION

Clausena is a small genus belonging to the Rutaceae family in which several species have been used as folk medicines in many Asian countries. For example, the leaves of *Clausena lansium* have been used for the treatment of coughs, asthma and gastro-intestinal diseases, in China and Taiwan (Lin, 1989; Adebajo et al., 2009) whereas several parts of *Clausena excavata*, a well-known Thai and Chinese medicinal plant, have been used as a detoxification agent (Wu et al., 1999) and treat snake bites, colds, malaria (Sunthitikawinsakul et al., 2003) and HIV-1 infection (Kongkathip et al., 2005). Previous chemical investigations of *Clausena* plants led to the discovery of a number of coumarins and alkaloids and many of them showed interesting pharmacological activities (Maneerat et al., 2010; Maneerat and Laphookhieo, 2010; Bhattacharyya et al., 1984; Chakraborty et al., 1995; Wang et al., 2010; Xin et al., 2008). As part of our searching for bioactive compounds from Rutaceae plants, we report herein the isolation and structure elucidation of two new and three known amides which isolated from the seeds of *C. lansium* as well as anti-cancer activity against three cancer human cell lines, including oral human epidermal carcinoma (KB), breast cancer (MCF7) and small cell lung cancer (NCI-H187).

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MATERIALS AND METHODS

Plant material

The seeds of *C. lansium* were collected in April 2008 from Nan Province, northern part of Thailand. Botanical identification was achieved through comparison with a voucher specimen number QBG 25077 in the herbarium collection of Queen Sirikit Garden, Mae Rim District, Chiang Mai, Thailand.

Extraction and isolation

The air-dried seeds of *C. lansium* (2.40 kg) were grinded and extracted with *n*-hexane for 3 days at room temperature then evaporated under reduced pressure to give *n*-hexane extract (46.26 g). The extract was separated by quick column chromatography (QCC) over silica gel using a gradient of *n*-hexane-EtOAc (100% *n*-hexane to 100% EtOAc) to give 12 fractions (A1-A12).The yellow solid was precipitated at room temperature from fraction A6 (41.11 g) and washed with *n*- hexane to afford compound 1 (1.96 g). Fraction A9 (2.81 g) was isolated by silica gel column

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **3**- **4** in $CDCl_3$.

Position	3		4	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	139.6	-	135.0
2	7.33 (*m*)	126.4	7.54 (*m*)	127.9
3	7.37 (*m*)	128.5	7.38 (*m*)	128.8
4	7.35 (*m*)	128.3	7. 37(*m*)	129.9
5	7.37 (*m*)	128.5	7.38 (*m*)	128.8
6	7.33 (*m*)	126.4	7. 54 (*m*)	127.9
7	4.94 (*d*, 2.8)	75.9	6.84 (*d*, 15.6)	143.8
8	4.68 (*d*, 2.8)	72.1	7.75 (*d*, 15.6)	116.6
9	-	172.5	-	169.0
11	6.00 (*d*, 8.4)	127.3	3.70 (*m*)	58.3
12	6.14 (*d*, 8.4)	126.1	5.05 (*m*)	73.8
13	-	133.7	-	142.4
14	7.15 (*m*)	128.7	7.43 (*m*)	125.8
15	7.29 (*m*)	128.8	7.38 (*m*)	128.4
16	7.28 (*m*)	128.5	7.28 (*m*)	127.8
17	7.29 (*m*)	128.8	7.38 (*m*)	128.4
18	7.16 (*m*)	128.7	7.43 (*m*)	125.8
19	3.05 (*s*)	34.7	3.03 (*s*)	37.7
11-OH	-	-	4.75 (*br s*)	-

chromatography (CC) with 20% EtOAc- *n*-hexane to give compound 2 (6.0 mg). Fraction A12 (865.0 mg) was further purified by QCC with a gradient of *n*-hexane-EtOAc (100% *n*-hexane to 25% EtOAc-*n*-hexane) to give 12 subfractions (B1-B12). Subfraction B12 (90.5 mg) was repeated the purification by CC with 35% EtOAc-*n*-hexane to yield compounds 3 (2.5 mg), 4 (4.4 mg) and 5 (2.0 mg).

Clausenalansamide A (3)

A white solid; mp 81.2-82.3°C; $[\alpha]^{25}_{D}$ -49.4° (*c* = 0.10, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 265 (4.17) and 204 (4.32); IR ν_{max} (neat) cm^{-1}: 3407 and 1651; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HR-TOF-MS *m/z* 298.1437 $[M+H]^+$ (calcd for $C_{18}H_{20}NO_3$, 298.1443).

Clausenalansamide B (4)

A white solid; mp 84.3-85.2 °C; $[\alpha]^{25}_{D}$ -33.25° (*c* = 0.12, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 280 (3.96), 224 (3.72), 2.17 (3.91) and 206 (3.95); IR ν_{max} (neat) cm^{-1}: 3364 and 1647; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), (Table 1); HR-TOF-MS *m/z* 282.1478 $[M+H]^+$ (calcd for $C_{18}H_{19}NO_2$, 282.1494).

Cytotoxic assay

The procedures for cytotoxic assay were performed by Resazurin microplate assay (REMA) as described by Brien et al. (2000). Doxorubicin and ellipticine were the reference substance in this study.

General experimental procedure

Melting points were determined using a Fisher-John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 digital polarimeter. UV spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The HR-TOF-MS was obtained from a MicroTOF, Bruker Daltonics mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5-40 µm) and silica gel 100 (Merck, 63-200 µm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

RESULTS AND DISCUSSION

The *n*-hexane of air-dried seeds of *C. lansium* was separated by chromatographic technique to yield two new amides, clausenalansamide A (3) and B (4), together with three known compounds (1, 2 and 5) (Figure 1). All structures were elucidated using spectroscopic data and compared with those reported in literatures.

Clausenalansamide A (3) was isolated as a white solid (mp 81.2-82.3°C), $[\alpha]^{25}_{D}$ -49.4° (*c* 0.10, $CHCl_3$). The molecular formula was established as $C_{18}H_{20}NO_3$ by HR-TOF-MS data, according to the $[M+H]^+$ ion peak at *m/z* 298.1437 (calcd. for 298.1443). The UV spectrum displayed two absorption bands at λ_{max} 265 and 204 nm

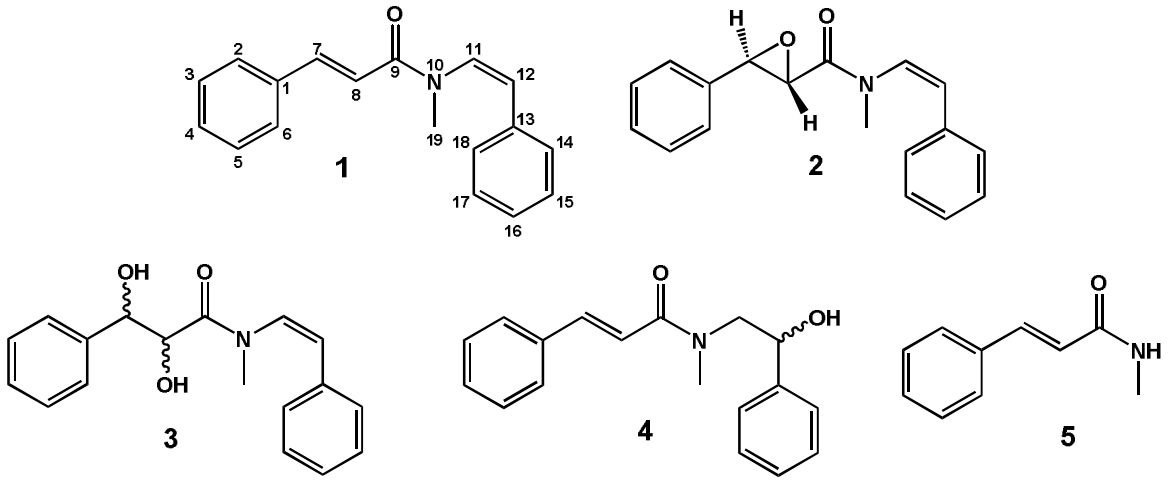

Figure 1. Structure of compounds 1-5.

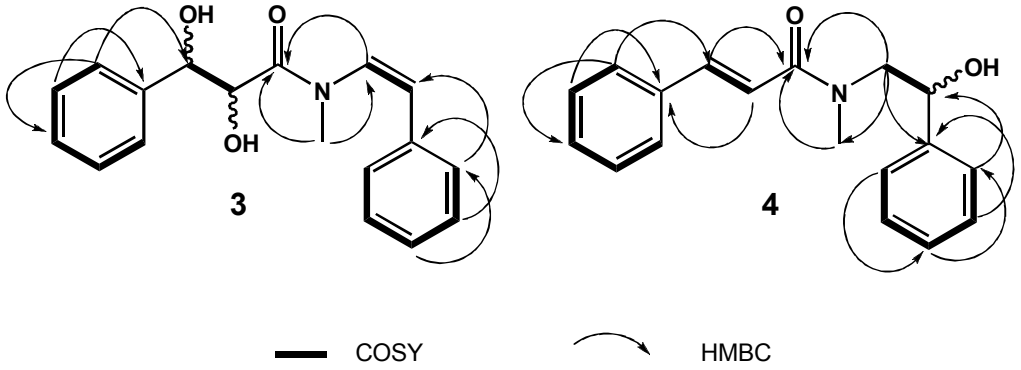

Figure 2. COSY and selective HMBC correlations of 3 and 4.

indicating the conjugated benzene chromophore in the molecule. The IR spectrum showed the absorption bands of hydroxy and amide carbonyl functionalities at 3407 and 1651 cm^{-1}, respectively. All ^{1}H and ^{13}C NMR spectral data of 3 (Table 1) were similar to that of 2 (Milner et al., 1996), except that the *trans*-oxirane ring with J = 1.6 Hz of H-7 (δ_H 3.79/δ_C 57.9) and H-8 (δ_H 3.77/δ_C 56.8) of 2 was opened to a vicinal dihydroxy group in 3 which appeared as two sets of doublet at δ_H 4.94/δ_C 75.9 and δ_H 4.68/δ_C 72.1 with the same J value 2.8 Hz. This result was also confirmed by HMBC experiments (Figure 2), in which the ^{2}J and ^{3}J HMBC correlations of oxymethine protons H-7 (δ_H 4.94) and H-8 (δ_H 4.68) with C-1 (δ_C 139.6), C-2/C-6 (δ_C 126.4) and C-9 (δ_C 172.5) were observed. Thus, the structure of 3 was identified to be clausenalansamide A.

Clausenalansamide B (4) was isolated as a white solid (mp 84.3-85.2°C), $[\alpha]^{25}_{D}$ -33.25° (*c* 0.12, $CHCl_3$). The molecular formula was established as $C_{18}H_{19}NO_2$ from an exact mass measurement which showed the $[M+H^+$ ion peak at *m/z* 282.1478 (calcd. for 282.1494) in HR-TOF-MS spectrum.The presence of hydroxy (3364 cm^{-1}) and amide carboxyl (1647 cm^{-1}) functionalities was evident from IR absorptions. Its ^{1}H and ^{13}C NMR spectral data (Table 1) of 4 were closely related to those of 1 (Dhan et al., 1980; Lin, 1989). The major difference was the replacement of the *cis*-configuration of double bond of *N*-styryl moiety signals at δ_H 6.51 (*d*, J = 8.4)/δ_H 128.7 and δ_H 6.24 (*d*, J = 8.4)/δ_C 125.0 with the -CH_2CH(OH)- unit at δ_H 3.70 (*m*) and 5.05 (*m*) which connected to methylene and methine carbons at δ_C 58.3 and 73.8, respectively, from the HMQC experiment. This finding was supported by ^{2}J and ^{3}J HMBC correlations of H-11 (δ_H 3.70) to carbons at C-9 (δ_C 169.0) and C-13 (δ_C 142.4) (Figure 2).

Therefore, the structure of 4 was identified to be clausenalansamide B which has been synthesized as an intermediate for proving the structure of lansamide-I by Dhan et al. (1980). The absolute configurations of clausenalansamide A (C-7 and C-8) and clausenalansamide B (C-12) were not determined due to the inadequacy of samples.

Table 2. Anti-cancer activity of compounds 1, 2, and 4 isolated from the seeds of *C. lansium*.

Compound	Anti-cancer activity (IC_{50}, µg/ml)		
	KB[a]	MCF7[b]	NCI-H187[c]
1	18.20	47.67	28.46
2	19.08	Inactive	13.73
4	26.75	Inactive	28.48
Elliticine	0.311	not tested	0.526
Doxorubicin	0.180	1.25	0.077

[a] KB = oral cavity cancer, [b] MCF7 = breast cancer, [c] NCI-H187 = small cell lung cancer.

The remaining three known amides, lansiumamide B (1) (Gooßen et al., 2010; Lin, 1989), SB-204900 (2) (Milner et al., 1996), and *N*-methyl-3-phenyl-2-propanamide (5) (Robert et al., 2003) were identified by 1D and 2D NMR spectral data and also compared with their reported physical and spectroscopic data.

Compounds 1, 2, and 4 were evaluated for their anti-cancer activity against three human cancer cell lines including oral human epidermal carcinoma cancer (KB), breast cancer (MCF7) and small cell lung cancer (NCI-H187). Unfortunately, compounds 1, 2 and 4 showed weakly cytotoxicity against KB and NCI-H187 cancer cell lines with IC_{50} value ranging from 13.73-28.48 µg/ml (Table 2). Compound 1 was also exhibited weakly cytotoxicity against MCF-7 cell line with the IC_{50} value of 48.67 µg/ml while compounds 2 and 4 were found to be inactive.

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