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New depsidones and xanthone from the roots of *Garcinia schomburgkiana*

Edwin Risky Sukandar [a], Pongpun Siripong [b], Suttira Khumkratok [c], Santi Tip-pyang [a,*]

[a] Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[b] Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand
[c] Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44000, Thailand

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ABSTRACT

Two new depsidones, schomburgdepsidones A and B (**1** and **2**), and one new xanthone, schomburgxanthone A (**3**), together with eight known compounds (**4**–**11**) were isolated from the roots of *Garcinia schomburgkiana*. Their chemical structures were established on the basis of spectroscopic analysis. The *in vitro* cytotoxicity of all 11 compounds was evaluated against the KB, HeLa S-3, HT-29, MCF-7 and Hep G2 human cancer cell lines. Compound **7** performed a good cytotoxicity against the KB, Hela S-3 and MCF-7 cell lines with IC_{50} values in the range of 3.17–6.07 μM. Compound **3** exhibited a good cytotoxicity against the KB cell line only, with an IC_{50} value of 8.14 μM.

1. Introduction

The genus *Garcinia* (family Clusiaceae) is comprised of about 29 species in Thailand [1]. The species *Garcinia schomburgkiana* Pierre, locally named "Ma dan" in Thai, is a small evergreen plant and can be found in Thailand, Laos and Vietnam as an indigenous plant [2,3]. This plant has been used by Thai people in folk medicine as an expectorant, laxative and menstrual treatment [4]. Previous phytochemical studies on *G. schomburgkiana* have revealed the presence of xanthones [3–6], biflavonoids [3,7], biphenyl derivatives [3–5], benzophenones [3,8] and steroids [3], and some of these exhibited an antimalarial activity [8] or cytotoxicity against cancer cell lines [3–6]. As a part of our ongoing search for bioactive constituents [9–11], we report herein the isolation and structure elucidation of two new depsidones, schomburgdepsidones A and B (**1** and **2**), and one new xanthone, schomburgxanthone A (**3**), along with eight known compounds (**4**–**11**) from the roots of *G. schomburgkiana*. Furthermore, all these 11 compounds were evaluated for their *in vitro* inhibitory activities against five human cancer cell lines (KB, HeLa S-3, HT-29, MCF-7 and Hep G2).

2. Experimental

2.1. General experiment procedures

The UV spectra were analyzed using a UV-2550 UV–vis spectrometer (Shimadzu, Kyoto, Japan), while IR data were obtained on Nicolet 6700 FT-IR spectrometer using the KBr disc method. The 1D- and 2D-NMR spectra were measured on a Bruker 400 AVANCE spectrometer in $CDCl_3$. The HR-ESI-MS were analyzed using a Bruker MICROTOF model mass spectrometer. Column chromatography (CC) was performed using silica gel 60 (Merck) and Sephadex LH-20. For TLC analysis, precoated silica gel plates (Merck silica gel 60 GF_{254}, 0.25 mm) were used. Spots were visualized under UV light and sprayed with anisaldehyde solution followed by heating.

2.2. Plant material

The roots of *G. schomburgkiana* were collected from a riparian zone along the Chi River, Mahasarakham Province, Thailand. The plant was identified and deposited with a voucher specimen (Khumkratok no. 92-08) by Dr. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Thailand.

2.3. Extraction and isolation

The air-dried roots of *G. schomburgkiana* (13.0 kg) were ground into powder and extracted twice with 30 L each time of dichloromethane (CH_2Cl_2) at room temperature for six days. The solvent was removed from the extract *in vacuo* to yield 104.0 g of crude extract. The crude CH_2Cl_2 extract was then fractionated by CC on silica gel (1.5 kg) with a step gradient elution of hexane:CH_2Cl_2 (80:20, 60:40, 40:60, 20:80 and 0:100, v/v, each 2.0 L), CH_2Cl_2:EtOAc (80:20, 60:40, 40:60, 20:80 and 0:100, v/v, each 2.0 L) and MeOH, respectively, to obtain 13 fractions (A–M). Fraction F (10.1 g) was further subjected to Sephadex LH-20 CC (300.0 g) eluted with CH_2Cl_2:MeOH (1:1, v/v) to yield three subfractions (F1–F3). Subfraction F3 (5.3 g) was separated by using

* Corresponding author.
E-mail address: Santi.Ti@chula.ac.th (S. Tip-pyang).

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silica gel CC (200.0 g) with a gradient of hexane:EtOAc (10:1, 8:1, 6:1, 4:1 and 2:1, v/v, each 300.0 mL) to give ten subfractions (F3.1–F.3.10).

Subfraction F3.1 (40.2 mg) was subjected to Sephadex LH-20 CC (30.0 g) eluted by CH_2Cl_2:MeOH (1:1, v/v) to afford **11** (4.1 mg). Compound **2** (10.4 mg) was obtained by separation of subfraction F3.2 (150.0 mg) on Sephadex LH-20 CC (50.0 g) with CH_2Cl_2:MeOH (1:1, v/v). Subfraction F3.7 (1.0 g) was applied to CC over silica gel (50.0 g) with a hexane:CH_2Cl_2 gradient solvent system (50:50, 40:60 and 30:70, v/v, each 200.0 mL) to obtain **1** (11.1 mg), **3** (5.9 mg) and **4** (5.8 mg). Compound **6** (4.3 mg) was afforded by purification of subfraction F3.10 (67.0 mg) on Sephadex LH-20 CC (30.0 g) eluted with CH_2Cl_2:MeOH (1:1, v/v). Subfraction F3.9 (1.4 g) was chromatographed into Sephadex LH-20 CC (100.0 g) with CH_2Cl_2:MeOH (1:1, v/v) to give three subfractions (F3.9.1–F3.9.3). Compound **8** (1.6 mg) and **10** (1.8 mg) were achieved from subfraction F3.9.2 (106.8 mg) by repeated Sephadex LH-20 CC (50.0 g) with a CH_2Cl_2:MeOH (1:1, v/v) solvent system. Subfraction F3.9.3 (570.0 mg) was treated by silica gel CC (50.0 g) eluted with a step gradient of hexane:CH_2Cl_2 (50:50, 40:60 and 30:70, v/v, each 100.0 mL) to yield **5** (7.1 mg), **7** (4.2 mg) and **9** (14.5 mg).

2.3.1. Schomburgdepsidone A (*1*)

Brownish gum; UV (MeOH) λ_{max}: 320, 272 and 217 nm; IR ν_{max} (KBr): 3415, 2974, 1656, 1613, 1465 and 1177 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 1; and HR-ESI-MS *m/z* 503.2051 $[M + Na]^+$ (calcd. for $C_{28}H_{32}O_7Na$, 503.2046).

2.3.2. Schomburgdepsidone B (*2*)

Brownish gum; UV (MeOH) λ_{max}: 316, 279 and 212 nm; IR ν_{max} (KBr): 3408, 2970, 1660, 1621, 1467 and 1201 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 1; and HR-ESI-MS *m/z* 495.2390 $[M + H]^+$ (calcd. for $C_{29}H_{35}O_7$, 495.2383).

2.3.3. Schomburgxanthone A (*3*)

Yellow powder; UV (MeOH) λ_{max}: 325, 281 and 224 nm; IR ν_{max} (KBr): 3337, 2965, 1636, 1609, 1433 and 1214 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 1; and HR-ESI-MS *m/z* 411.1818 $[M + H]^+$ (calcd. for $C_{24}H_{27}O_6$, 411.1808).

2.4. Cytotoxicity assay

All isolated compounds (**1**—**11**) were evaluated for their *in vitro* cytotoxic activities against the KB (epidermoid carcinoma), HeLa S-3 (cervix adenocarcinoma), HT-29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma) and Hep G2 (hepatocellular carcinoma) human cancer cell lines using the MTT colorimetric method [12]. Doxorubicin was used as the reference substance. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×103 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h of pre-incubation at 37 °C in a humidified atmosphere of 5% CO2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination

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

Position	1			2			Position	3		
	δ_H (*J* in Hz)	δ_C	HMBC	δ_H (*J* in Hz)	δ_C	HMBC		δ_H (*J* in Hz)	δ_C	HMBC
1		163.9			164.9		1		162.6	
2	6.28 (1H, s)	100.8	1, 3, 4, 11a	6.32 (1H, s)	96.2	1, 4, 11a	2	6.35 (1H, s)	94.6	1, 4, 9a
3		162.1			165.0		3		163.4	
4		111.2			113.7		4		106.6	
4a		158.9			158.6		4a		153.2	
5a		136.5			141.9		5		128.9	
6		133.6			117.4		6		148.2	
7		141.0			140.2		7	6.78 (1H, s)	113.4	5, 6, 8a, 16
8		123.7			140.1		8		137.8	
9		123.6			118.1		8a		111.9	
9a		136.8			136.5		9		182.8	
11		168.6			169.4		9a		103.7	
11a		99.5			98.7		10a		146.4	
12	3.64 (2H, d, 6.0)	22.5	3, 4, 4a, 13, 14	3.49 (2H, d, 6.0)	22.6	3, 4, 4a, 13, 14	11	3.48 (2H, d, 6.8)	21.9	3, 4, 4a, 12, 13
13	5.24 (1H, t, 6.0)	122.2	15, 16	5.14 (1H, t, 6.0)	122.8	15, 16	12	5.22 (1H, t, 6.8)	123.5	
14		137.1			132.3		13		131.7	
15	1.79 (3H, s)	25.9	14, 16	1.67 (3H, s)	25.8	13, 14, 16	14	1.73	25.7	12, 13, 15
16	1.86 (3H, s)	18.2	13, 14, 15	1.73 (3H, s)	18.0	13, 14, 15	15	1.85	18.0	12, 13, 14
17	3.35 (2H, d, 6.4)	25.8	7, 8, 18, 19	3.57 (2H, d, 6.4)	23.9	5a, 6, 18, 19	16	3.97 (2H, d, 7.2)	33.1	7, 8, 17, 18
18	5.08 (1H, t, 6.4)	121.9	20, 21	5.20 (1H, t, 6.4)	121.6	20, 21	17	5.35 (1H, t, 7.2)	122.7	
19		134.3			135.5		18		133.3	
20	1.71 (3H, s)	25.9	18, 19, 21	1.74 (3H, s)	25.9	19	19	1.75	26.0	20
21	1.79 (3H, s)	18.1	19, 20	1.81 (3H, s)	18.1	18, 19, 20	20	1.73	18.1	17, 18, 19
22	3.44 (2H, d, 6.4)	25.6	8, 9, 9a, 24	3.55 (2H, d, 7.2)	23.8	8, 9, 9a, 23, 24	1-OH	13.44 (1H, s)		1, 2, 9a
23	5.01 (1H, t, 6.4)	122.4	25, 26	5.27 (1H, t, 7.2)	121.2	25, 26	5-OH	5.47 (1H, brs)		
24		132.4			135.0		6-OH	5.90 (1H, brs)		
25	1.67 (3H, s)	25.9	23, 24, 26	1.73 (3H, s)	25.9	26	3-OMe	3.90 (3H, s)		3
26	1.79 (3H, s)	18.2	24, 25	1.84 (3H, s)	18.1	24				
1-OH	10.99 (1H, s)		1, 2	11.30 (1H, s)		1, 2, 11a				
3-OH	6.28 (1H, brs)									
6-OH	5.83 (1H, brs)		5a, 6, 7							
7-OH	5.42 (1H, brs)			5.54 (1H, s)		6, 7				
3-OMe				3.85 (3H, s)	56.2	3				