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Xanthones and biphenyls from the stems of *Garcinia cylindrocarpa* and their cytotoxicity

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ABSTRACT

Six new compounds including four new xanthones, cylindroxanthones D–G (**1**–**4**), and two new biphenyls, cylindrobiphenyls A and B (**5** and **6**), were isolated from the stems of *Garcinia cylindrocarpa* together with 28 known compounds (**7**–**34**). The structures of the new compounds were established on the basis of extensive 1D and 2D NMR and HRESIMS spectroscopic analysis. Their cytotoxicity was evaluated against five human cancer cell lines including KB, HeLa S-3, MCF-7, Hep G2, and HT-29. Compound **23** showed strong cytotoxicity against KB, HeLa S-3, MCF-7, and Hep G2 cells with IC_{50} values in the range of 2.20–6.00 μM. Furthermore, compound **25** selectively exhibited good cytotoxicity against MCF-7 cells with IC_{50} value of 8.77 μM, while **31** showed good cytotoxicity against HT-29 cells with IC_{50} value of 9.18 μM.

1. Introduction

The genus *Garcinia* contains over 300 species, that are distributed in the lowland tropical rainforest such as Southeast Asia, West and Central Africa, and South America [1]. This genus is a prolific source of structurally diverse secondary metabolites such as xanthones [2], biphenyls [3,4], benzophenones [3,5], depsidones [6], biflavonoids [7], and triterpenoids [8]. Many of these compounds showed a wide range of biological and pharmacological properties including α-glucosidase inhibition [2], antioxidant [4], anti-HIV [3], anti-inflammatory [5], cytotoxic [6], antibacterial [7], and cholinesterase inhibition activities [9].

Garcinia cylindrocarpa Kosterm is a woody plant distributed mainly in Maluku Island, Indonesia. This plant has been used in Indonesian folk medicine as fever remedy. In a previous phytochemical investigation, we reported the isolation of three pyranoxanthones, cylindroxanthones A–C, from the stem bark of *G. cylindrocarpa* with their cytotoxic activities [10]. As a continuation of our research interest to explore bioactive compounds from *G. cylindrocarpa*, we describe herein the isolation and structure elucidation of four new xanthones, cylindroxanthones D–G (**1**–**4**), and two new biphenyls, cylindrobiphenyls A and B (**5** and **6**), along with 28 known compounds from the stems of this plant. The cytotoxic evaluation of the isolated compounds against five human cancer cell lines were also reported.

2. Experimental

2.1. General experiment procedures

Melting points were determined by Fischer-Johns melting point apparatus. Optical rotations were measured on a Jasco (Oklahoma City, OK, USA) P-1010 polarimeter. IR data were obtained using a Nicolet (Thermo Scientific, Waltham, MA, USA) 6700 FT-IR spectrometer using KBr discs. UV–visible absorption spectra were obtained on a Shimadzu (Kyoto, Japan) UV-2550 UV–vis spectrometer. NMR spectra were recorded on a Bruker 400 AVANCE spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in $CDCl_3$ and acetone-d_6. HRESIMS spectra were recorded using a Bruker MICROTOF model mass spectrometer. Vacum liquid chromatography (VLC), column chromatography (CC), and radial chromatography (chromatotron model 7924 T, Harrison Research) were carried out on silica gel 60 GF_{254} (Merck) and silica gel 60 (63–200 μm; Merck). Size-exclusion chromatography was performed with Sephadex LH-20 (25–100 μm; GE Healthcare). For TLC analysis, precoated silica gel 60 GF_{254} (0.25 mm; Merck) was used.

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2.2. Plant material

The stems of *Garcinia cylindrocarpa* were collected from Saumlaki Forest, Southeast West Maluku Islands, Indonesia. The plant was identified by Mrs. Rismita Sari (a botanist at Bogor Botanical Garden, Indonesia). A voucher specimen (No. 630) was deposited at the Herbarium Bogoriense, Bogor Botanical Garden, Indonesia.

2.3. Extraction and isolation

The air-dried stems of *G. cylindrocarpa* (3.0 kg) were ground into powder and extracted by maceration at room temperature with MeOH (3 × 15 L) for three days. The solvent was evaporated under reduced pressure to obtain a residue (31.4 g). The crude extract was then suspended in distilled H_2O and partitioned with CH_2Cl_2 and EtOAc to afford a CH_2Cl_2 fraction (6.04 g), an EtOAc fraction (9.23 g), and the remaining aqueous solution. The CH_2Cl_2-soluble fraction was subjected to VLC on silica gel (125.0 g) using a gradient of n-hexane:EtOAc (90:10–0:100) to obtain nine fractions (D1–D9). Fraction D2 (488.8 mg) was chromatographed on Sephadex LH-20 column (50.0 g) eluted with CH_2Cl_2:MeOH (1:1, *v/v*) to yield subfractions D2.1–D2.3. Subfraction D2.1 (93.2 mg) was separated by repeated Sephadex LH-20 CC (50.0 g) using CH_2Cl_2:MeOH (1:1, *v/v*) to afford compounds **4** (4.3 mg) and **7** (4.2 mg). Compound **8** (16.7 mg) was yielded from subfraction D2.2 (70.4 mg) by separation using radial chromatography (chromatotron) with n-hexane:EtOAc (95:5) as eluent. Subfraction D2.3 (112.0 mg) was separated by repeated chromatotron eluted with n-hexane:EtOAc (95:5) to afford compounds **9** (3.1 mg), **10** (2.0 mg), **11** (2.6 mg), **12** (9.3 mg), **13** (1.5 mg), and **14** (4.4 mg). Fraction D3 (270.3 mg) was loaded to Sephadex LH-20 column (50.0 g) eluted with CH_2Cl_2:MeOH (1:1, *v/v*) to provide three subfractions (D3.1–D3.3). Compound **1** (2.7 mg) was obtained by purification of subfraction D3.1 (54.6 mg) using chromatotron with n-hexane:CH_2Cl_2 (25:75) as eluent. Fraction D4 (229.6 mg) was chromatographed using Sephadex LH-20 column (50.0 g) with CH_2Cl_2:MeOH (1:1, *v/v*) as eluent to give four subfractions (D4.1–D4.4). Compounds **15** (11.6 mg) and **16** (1.4 mg) were yielded from subfraction D4.3 (56.5 mg) using chromatotron eluted with n-hexane:EtOAc (85:15). Subfraction D4.4 (29.1 mg) was purified by chromatotron using n-hexane:CH_2Cl_2 (25:75) as eluent to obtain compound **3** (1.2 mg). Fractionation of fraction D5 (202.1 mg) using Sephadex LH-20 CC (50.0 g) with CH_2Cl_2:MeOH (1:1, *v/v*) as eluent was performed to obtain subfractions D5.1–D5.4. Compound **17** (6.0 mg) was yielded from subfraction D5.1 (67.4 mg) using chromatotron eluted with n-hexane:EtOAc (85:15), while compound **18** (1.7 mg) was obtained from subfraction D5.2 (42.7 mg) using the same technique with n-hexane:acetone (75:25) as eluent. Fraction D6 (581.0 mg) was subjected to Sephadex LH-20 column (50.0 g) using solvent system of CH_2Cl_2:MeOH (1:1, *v/v*) to obtain subfractions D6.1–D6.6. Compounds **5** (7.2 mg), **19** (24.7 mg), and **20** (3.6 mg) were successfully afforded from subfraction D6.2 (97.2 mg) using chromatotron with CH_2Cl_2:MeOH (97:3) as eluent. Fractionation of subfraction D6.3 (102.1 mg) was conducted using chromatotron with CH_2Cl_2:MeOH (97:3) as eluent to furnish compounds **6** (31.1 mg), **21** (1.4 mg), and **22** (1.7 mg). A separation technique using chromatotron was employed to obtain compounds **23** (7.2 mg) and **24** (11.6 mg) from subfraction D6.4 (45.3 mg) with eluent system of CH_2Cl_2:MeOH (97:3). Subfraction D6.5 (85.4 mg) was applied to chromatotron with CH_2Cl_2:MeOH (97:3) as eluent to give compounds **25** (14.4 mg) and **26** (15.5 mg). Compound **27** (14.0 mg) was afforded using chromatotron with n-hexane:EtOAc (70:30) as eluent from subfraction D6.6 (41.0 mg). Fraction D7 (204.3 mg) was separated by Sephadex LH-20 CC (50.0 g) with CH_2Cl_2:MeOH (1:1, v/v) as eluent to obtain five subfractions (D7.1–D7.5). A chromatotron technique using eluent system of CH_2Cl_2:MeOH (97:3) was performed to obtain compounds **28** (1.4 mg) and **29** (1.7 mg) from subfraction D7.1 (17.2 mg) and compound **2** (5.8 mg) from subfraction D7.4 (24.5 mg). The same separation technique was conducted to afford compounds **30** (20.4 mg) and **31** (7.6 mg) from subfraction D7.2 (80.8 mg) using n-hexane:EtOAc (60:40) as eluent. Subfraction D7.3 (56.2 mg) was applied on Sephadex LH-20 CC (25.0 g) with CH_2Cl_2:MeOH (1:1, *v/v*) as eluent to get compound **32** (7.7 mg). Finally, fraction D8 (92.1 mg) was repeatedly separated using Sephadex LH-20 CC (50.0 g) with CH_2Cl_2:MeOH (1:1, *v/v*) to obtain compounds **33** (10.5 mg) and **34** (18.9 mg).

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) spectroscopic data of compounds **1–3** in acetone-d_6 (in ppm) and **4** in $CDCl_3$ (in ppm).

Position	**1**		**2**		**3**		**4**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		162.0		161.9		161.1		157.6
2	6.15 s	99.9	6.17 s	99.6	6.96 s	95.1		104.9
3		162.7		162.2		161.7		160.7
4		101.4		100.5		109.9	6.43 s	95.4
4a		155.6		155.8		152.4		157.2
10a		146.5		146.4		145.9		147.4
5		147.2		147.2		147.3		141.5
6	7.38 d (8.0)	121.8	7.38 dd (8.0, 1.2)	121.8	7.45 d (8.0)	122.2		150.3
7	7.28 t (8.0)	125.0	7.29 t (8.0)	125.1	7.38 t (8.0)	125.8		148.4
8	7.69 d (8.0)	116.6	7.69 dd (8.0, 1.2)	116.6	7.79 d (8.0)	116.8	7.40 s	100.5
8a		122.5		122.5		121.5		116.1
9		181.9		181.9		183.1		180.2
9a		104.2		104.4		106.3		104.2
1′	2.94 t (6.8)	17.0	2.87 dd (20.0, 11.2) 3.18 dd (20.0, 5.2)	26.2	7.87 d (1.6)	145.9	6.73 d (10.0)	115.6
2′	1.93 t (6.8)	32.4	3.92 dd (11.2, 5.2)	69.0	7.38 d (1.6)	105.3	5.61 d (10.0)	127.8
3′		77.3		80.1				78.4
4′	1.39 s	27.0	1.41 s	25.9			1.48 s	28.6
5′	1.39 s	27.0	1.35 s	21.4			1.48 s	28.6
1-OH	12.73 s		12.74 s		12.89 s		13.19 s	
2′-OH			4.48 d (5.2)					
5-OMe							4.04 s	62.1
6-OMe							3.96 s	56.4
7-OMe							4.06 s	61.7

2.3.1. Cylindroxanthone D (1)

Yellow needles; mp: 264–266 °C; UV (MeOH) λ_{max}: 328, 260, and 213 nm; IR ν_{max} (KBr): 3295, 2929, 1657, 1440, and 1255 cm^{-1}; for ^{1}H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR spectroscopic data, see Table 1; and HRESIMS *m/z* 311.0937 $[M - H]^-$ (calcd. For $C_{18}H_{15}O_5$, 311.0919).

2.3.2. Cylindroxanthone E (2)

Yellow powder; $[\alpha]_D^{20}$ + 8.3 (c 0.50, MeOH); mp: 276–278 °C; UV (MeOH) λ_{max}: 316, 252, and 206 nm; IR ν_{max} (KBr): 3256, 2900, 1630, 1454, and 1250 cm^{-1}; for ^{1}H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR spectroscopic data, see Table 1; and HRESIMS *m/z* 327.0895 $[M - H]^-$ (calcd. For $C_{18}H_{15}O_6$, 327.0869).

2.3.3. Cylindroxanthone F (3)

Yellow powder; UV (MeOH) λ_{max}: 333, 280, and 238 nm; IR ν_{max} (KBr): 3280, 2910, 1655, 1438, and 1263 cm^{-1}; for ^{1}H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR spectroscopic data, see Table 1; and HRESIMS *m/z* 267.0305 $[M - H]^-$ (calcd. For $C_{15}H_7O_5$, 267.0293).