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by Tjitjik Srie Tjahjandarie

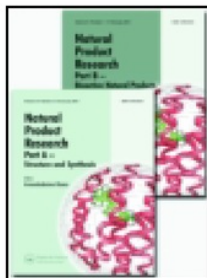
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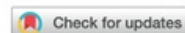
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Two novel coumarins bearing an acetophenone derivative from the leaves of *Melicope Quercifolia*

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ABSTRACT

Meliquercifolins A (1), and B (2), two new coumarins are bearing an acetophenone derivative were isolated from the leaves of *Melicope quercifolia* along with three known compounds, melicodenines E (3), F (4) and I (5). Structures of two new compounds were identified based on spectroscopic analyses (UV, HR-ESI-MS, 1D and 2D NMR). Cytotoxic activities of compounds (1–5) towards three human cancer cells (HeLa, MCF-7, P-388), compounds 1, 4 and 5 showed very potent activity against HeLa cells with IC_{50} values 2.6; 0.8; 1.1 μ M, respectively.

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Cytotoxicity; *Melicope quercifolia*; Meliquercifolins A and B



1. Introduction

Melicope quercifolia (Rutaceae) is a small tree and found as an endemic plant in West Java, Indonesia. The leaves of *M. quercifolia* have been used to treat skin diseases (Appelhans et al. 2018). Acetophenones (Nguyen et al. 2016), alkaloids (George et al. 2017), coumarins (Xu et al. 2016), flavonoids (Saputri et al. 2018), and phenylpropenoids (Nakashima et al. 2012) are phenolic compounds from the *Melicope* plants. Some of the phenolic compounds showed various Diels-Alder adduct and [2 + 2]

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cycloaddition (Nakashima et al. 2012). Melicodenines E (3), F (4) and I (5) formed a [2 + 2] cycloaddition type, and a Diels-Alder adduct type. In the ongoing phytochemical investigation of *M. quercifolia*, we report two new compounds, meliquercifolins A (1), and B (2) from the leaves of *M. quercifolia*. These structures of two new compounds are coumarins bearing an acetophenone moiety. Heterodimer compounds (1 and 2) are the first time found skeleton structure from *Melicope*. The cytotoxic properties against three human cancer cells (P-388, MCF-7, and HeLa) of isolated compounds will also report.

2. Result and discussion

Meliquercifolin A (1) obtained a colorless oil in which showed an optical rotation was inactive. Based on the HRESIMS measurement of 1 gave a positive ion peak $[M + H]^+$ at m/z 479.1729 correspondings for a molecular formula $C_{27}H_{27}O_8$. The IR absorptions of 1 displayed vibrations the presence of conjugated $C=O$ (1636 cm^{-1}), aromatic (1608 and 1579 cm^{-1}) and ether (1119 cm^{-1}) groups. Two maximum absorptions at λ_{max} 280 (3.86) and 332 (3.88) nm very like with benzoyl and cinnamoyl chromophore. Four signals of a 1,2,3,4-tetrasubstituted cyclobutane ring showed at δ_H 3.05 (1H, dd, $J = 9.8$; 5.8 Hz, H-3), δ_H 3.96 (1H, t, $J = 2.2$ Hz, H-4), δ_H 4.71 (1H, t, $J = 6.9$ Hz, H-6) and δ_H 5.42 (1H, ddd, $J = 8.2$; 6.2; 2.2 Hz, H-7). The signal at δ_H 5.42 implied an oxymethine attached in the cyclobutane ring. Compound 1 also demonstrated the presence of two proton signals of two aromatics at δ_H 5.98 (1H, s, H-8), δ_H 6.21 (1H, s, H-9) and a pair of *cis* vinylic of chromen-2-one ring at δ_H 6.01 (1H, d, $J = 9.8$ Hz, H-3), δ_H 7.82 (1H, d, $J = 9.8$ Hz, H-4). Based on the ^1H NMR spectrum, compound 1 likewise showed the presence of three methyl signals at δ_H 2.17 (3H, s, H-10), δ_H 1.19 (3H, s, H-11), δ_H 1.61 (3H, s, H-12) as well as three methoxyl signals at δ_H 4.04 (3H, s, 5-OCH₃), δ_H 3.67 (3H, s, 5'-OCH₃), and δ_H 3.59 (3H, s, 7-OCH₃). The ^{13}C NMR analysis (APT experiment), compound 1 showed 27 carbon signals that are completely separated, consisting of six methyl carbons, eight methine carbons, and 13 quaternary carbons. Three of them (δ_C 161.9; δ_C 139.5 and δ_C 109.7) are characteristic of C-2, C-3, and C-4 of the chromen-2-one ring (coumarin). Furthermore, two signals of the acetyl group (δ_C 32.3; δ_C 202.5) are characteristic of the acetophenone structure (Nakashima et al. 2012). The signal of a *cis* vinylic of chromen-2-one at δ_H 7.82 (H-4) in the HMBC spectrum exhibited correlation with two oxyaryl carbons, C-5 (δ_C 152.7), C-9a (δ_C 156.7) and a lactone carbonyl at C-2 (δ_C 161.9) as well as the methoxyl signal at δ_H 4.04 correlated to C-5 (δ_C 152.7) unambiguously located the methoxyl group at C-5. Another proton signal of a *cis* vinylic at δ_H 6.01 (H-3) correlated to C-2 (δ_C 161.9) and a quaternary carbon at C-4a (δ_C 108.1). Furthermore, a signal of aromatic at δ_H 6.21 correlated to two oxyaryl carbons at C-9a (δ_C 156.7); C-8a (δ_C 168.5) and two quaternary carbons at C-4a (δ_C 108.1); C-5a (δ_C 104.7) showed that the signal of δ_H 6.21 at H-9. A signal of an oxymethine at δ_H 5.42 correlated to C-8a (δ_C 168.5), and C-4' (δ_C 34.7) revealed that a part of the heterodimer is bergapten (coumarin) (Nakashima et al. 2012). The HMBC spectrum, a signal of methine at δ_H 4.71 (H-6) correlated to C-3' (δ_C 45.5), C-4' (δ_C 34.7) and C-8a (δ_C 168.5) reinforced the structure of the bergapten experiencing a [2 + 2] cycloaddition reaction on furano ring (Nakashima et al. 2012). A methine signal (cyclobutane ring) at

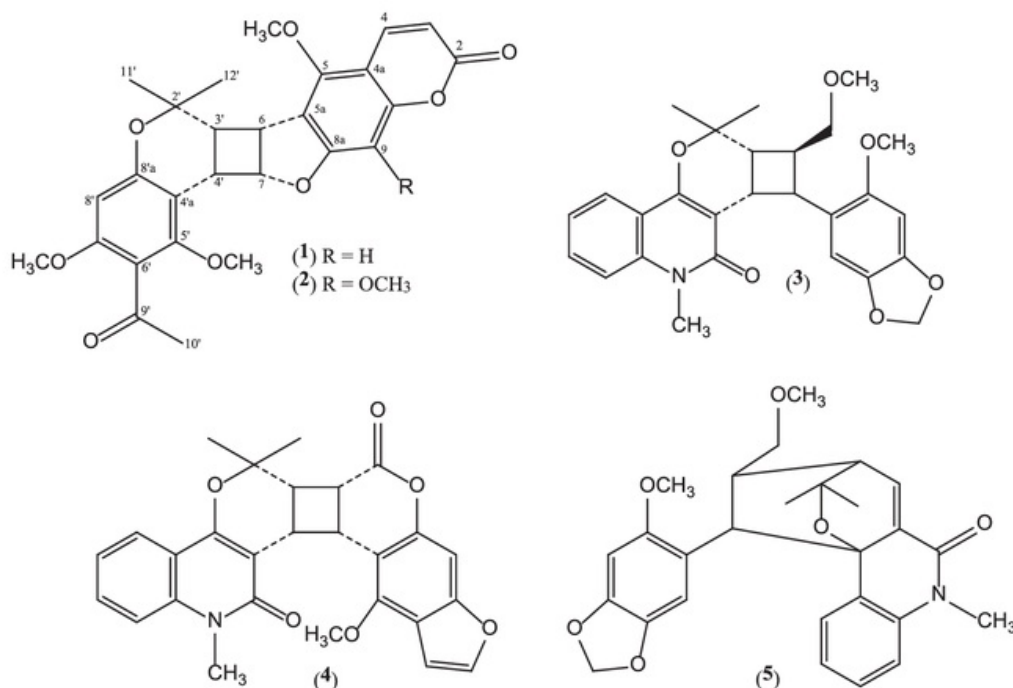


Figure 1. Chemical structures of isolated compounds from *M. quercifolia*.

43 δ_{H} 3.96 (H-4) correlated to δ_{C} 44.1 (C-6), δ_{C} 84.9 (C-7), δ_{C} 156.4 (C-8a) and δ_{C} 106.6 (C-4a). Two methyl signals of pyrano ring at δ_{H} 1.19 (H-11) and δ_{H} 1.61 (H-12) correlated to a methine carbon at C-3' (δ_{C} 45.5) and an ether cyclic carbon at C-2' (δ_{C} 74.0). Besides, a signal of aromatic isolated at δ_{H} 5.98 (H-8) correlated to an oxyaryl carbon at δ_{C} 156.8 (C-7), and two quaternary carbons at δ_{C} 106.6 (C-4a); 117.8 (C-6). A methoxyl signal at δ_{H} 3.67 (7-OCH₃) correlated to δ_{C} 156.8 (C-7). Another a methoxyl signal at δ_{H} 3.59 (5'-OCH₃) correlated to δ_{C} 155.7 (C-5). A methyl signal of acetyl at δ_{H} 2.17 (H-10) correlated to δ_{C} 202.5 (C-9). These correlations showed that a part of evodionol (Nakashima et al. 2012). This result implied that compound **1** is a reaction of [2 + 2] cycloaddition between bergapten-evodionol. Therefore, the structure of meliquercifolin A established as **1**.

Meliquercifolin B (**2**) also acquired as a colorless oil. The UV absorption bands (λ_{max} 269, 335), and IR (1620, 1601, 1571 and 1110 cm⁻¹) absorptions very semblable with **1**. The molecular formula of compound **2** was assigned to be C₂₈H₂₉O₉ exhibited positive ion peak [M + H]⁺ at m/z 509.1802 (calcd for 509.1812) by HRESIMS measurement. The ¹H and ¹³C NMR spectrum of **2** had very semblable with **1**. The main difference, the ¹H and ¹³C NMR of **2** showed a methoxyl group at C-9 (δ_{H} 3.98; δ_{C} 58.6). The HMBC and HMQC experiments confirmed the location of a methoxyl group at C-9. With the addition, a methoxyl group attached in the aromatic ring of coumarin skeleton indicated is [2 + 2] cycloaddition between isopimpinellin-evodionol. Based on UV, IR, HRESIMS, 1D, and 2D NMR measurements, the structure of meliquercifolin B assigned as **2**. The chemical structures of isolated compounds shown in (Figure 1).

Three known compounds, melicodenine E (**3**), melicodenine F (**4**) and melicodenine I (**5**) from HRESIMS, 1D and 2D NMR data showed chemical formula, and the chemical

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Table 1. Cytotoxic activities of isolated compounds (1–5).

Compound	IC ₅₀ (μM)		
	HeLa	MCF-7	P-388
1	2.6 ± 0.10	>100	78.9 ± 1.20
2	>100	>100	>100
3	>100	>100	11.9 ± 0.42
4	0.8 ± 0.01	>100	38.3 ± 0.87
5	1.1 ± 0.05	>100	>100
Artonin E	–	–	1.33 ± 0.07
Doxorubicin	0.9 ± 0.04	0.8 ± 0.02	–

shift very resemblant with published previously (Nakashima et al. 2012; George et al. 2017). Compound **3** is a form of a [2 + 2] cycloaddition reaction of *N*-methylflindersin-melicodin A, and compound **4** is *N*-methylflindersin-bergapten. Compound **5** is a [4 + 2] cycloaddition (a Diels-Alder adduct type) two *N*-methylflindersins (George et al. 2017; Nakashima et al. 2012).

Those cytotoxic activities of isolated compounds, compounds **1**, **4** and **5** showed high activity against HeLa cells while compounds **2** and **3** were inactive. However, all of the isolated compounds were inactive against MCF-7 and P-388 cells (Table 1). For coumarin bearing an acetophenone derivative, compound **1** more active than compound **2** against HeLa cells. The influence of a methoxyl group at C-9 suggested as a critical element to decrease the cytotoxic effect towards HeLa cells. The melicodenine E (**4**) more active than meliquercifolins A (**1**). However, compound **4** slightly active than compound **5**. The *N*-methylflindersin factor is a crucial element to enhance the cytotoxic effect of HeLa cells. In addition to the *N*-methylflindersin factor tend to increase cytotoxic activity against human colon cancer cells (DLD-1) (Nakashima et al. 2012).

28 3. Experimental

3.1. Plant material

The fresh leaves of *M. quercifolia* obtained from Cianten Farm, Cigudek District, Bogor, West Java, Indonesia on Nov. 2017. The plant identified by Mr. Ismail Rachman, a botanist senior from the Herbarium Bogoriense, Bogor, Indonesia. A specimen (MQ 20171104) deposited as a reference.

5 3.2. Extraction and isolation

The air-dried leaves of *M. quercifolia* (2.0 kg) were extracted with MeOH three times (6 L, each for two days) at room temperature. Evaporation of the solvent with evaporator gave a MeOH extract (450 g) and then partitioned with *n*-hexane three times. Furthermore, the MeOH extract was added with H₂O (4:1 v/v) and partitioned with EtOAc three times gave the crude extract (5 g). The EtOAc extract (4.8 g), fractionated by CC on silica gel, eluted with *n*-hexane-EtOAc (from 9:1 to 3:7) providing two fractions, A and B. Fraction A (1.3 g) was fractionated by CC chromatography on polyamide, eluted with *n*-hexane-CHCl₃ (from 9:1 to 1:4) gave two subfractions, A₁ and A₂. Compounds **3** (13 mg) and **4** (15 mg) were isolated from subfraction A₂ (655 mg) using radial planar chromatography with the same eluent. Fraction B (800 g) was

separated by CC chromatography on polyamide, and eluted *n*-hexane-EtOAc (from 4:1 to 1:1) gave subfractions B₁-B₂. Compounds **1** (5 mg) and **2** (7 mg) was obtained from subfraction B₁ (265 mg) by radial planar chromatography, eluted with *n*-hexane-diisopropylether (from 3:9 to 7:3), and diisopropylether as a solvent system. By the same methods, compound **5** (16 mg) isolated from subfraction B₂ (148 mg).

3.3. Spectral data

Meliquercifolin A (**1**): yellow solid, UV/Vis (MeOH) λ_{\max} (nm) (log ϵ): 217 (3.03), 269 (3.08), 289 (3.20) and 325 (2.81) nm. IR (KBr) ν_{\max} (cm⁻¹): 3411, 2972, 2925, 2852, 1649, 1604, 1579 and 1159. ¹H-NMR (400 MHz, CDCl₃) δ_{H} ppm: 6.01 (1H, *d*, *J* = 9.8 Hz, H-3), 7.81 (1H, *d*, *J* = 9.8 Hz, H-4), 4.04 (3H, *s*, 5-OCH₃), 4.71 (1H, *t*, *J* = 6.9 Hz, H-6), 5.42 (1H, *ddd*, *J* = 8.2; 6.2; 2.2 Hz, H-7), 6.21 (1H, *s*, H-9), 3.05 (1H, *dd*, *J* = 9.8; 5.8 Hz, H-3), 3.96 (1H, *t*, *J* = 2.2 Hz, H-4), 3.67 (3H, *s*, 5'-OCH₃), 3.59 (3H, *s*, 7'-OCH₃), 5.98 (1H, *s*, H-8), 2.17 (3H, *s*, H-10), 1.19 (3H, *s*, H-11) and 1.61 (3H, *s*, H-12). ¹³C-NMR (100 MHz, CDCl₃) δ_{C} ppm: 161.1 (C-2), 109.7 (C-3), 139.5 (C-4), 108.1 (C-4a), 152.7 (C-5), 104.7 (C-5a), 44.1 (C-6), 84.9 (C-7), 168.5 (C-8a), 91.7 (C-9), 156.7 (C-9a), 74.0 (C-2), 45.5 (C-3), 34.7 (C-4), 106.6 (C-4a), 155.7 (C-5), 62.6 (5'-OCH₃), 117.8 (C-6), 156.8 (C-7), 55.8 (7'-OCH₃), 96.8 (C-8), 156.4 (C-8a), 202.5 (C-9), 32.3 (C-10), 25.3 (C-11) and 25.7 (C-12). HRESIMS: *m/z* [M + H]⁺ calcd. for C₂₇H₂₇O₈ 479.1706, found 479.1729.

Meliquercifolin B (**2**): yellow solid, UV/Vis (MeOH) λ_{\max} (nm) (log ϵ): 217 (3.33), 234 (3.12), 255 (3.02), 268 (2.98) and 299 (2.69) nm. IR (KBr) ν (cm⁻¹): 3448, 2966, 2923, 2854, 1651, 1627, 1460 and 1186. ¹H-NMR (400 MHz, CDCl₃) δ_{H} ppm: 6.02 (1H, *d*, *J* = 9.6 Hz, H-3), 7.79 (1H, *d*, *J* = 9.6 Hz, H-4), 3.68 (3H, *s*, 5-OCH₃), 4.75 (1H, *t*, *J* = 6.8 Hz, H-6), 5.50 (1H, *dd*, *J* = 6.8; 3.9 Hz, H-7), 3.98 (3H, *s*, 9-OCH₃), 3.09 (1H, *dd*, *J* = 9.9; 5.8 Hz, H-3), 3.99 (1H, *dd*, *J* = 5.8; 1.8 Hz, H-4), 3.68 (3H, *s*, 5'-OCH₃), 3.57 (3H, *s*, 7'-OCH₃), 5.92 (1H, *s*, H-8), 2.21 (3H, *s*, H-10), 1.12 (3H, *s*, H-11), and 1.66 (3H, *s*, H-12). ¹³C-NMR (100 MHz, CDCl₃) δ_{C} ppm: 160.9 (C-2), 109.9 (C-3), 139.6 (C-4), 104.4 (C-4a), 126.0 (C-5), 106.9 (C-5a), 44.4 (C-6), 85.3 (C-7), 159.8 (C-8a), 148.1 (C-9), 149.1 (C-9a), 74.1 (C-2), 45.7 (C-3), 34.5 (C-4), 106.2 (C-4a), 156.9 (C-5), 62.7 (5'-OCH₃), 117.3 (C-6), 156.8 (C-7), 55.8 (7'-OCH₃), 96.8 (C-8), 163.8 (C-8a), 209.2 (C-9), 32.3 (C-10), 25.4 (C-11), and 25.7 (C-12). HRESIMS: *m/z* [M + H]⁺ calcd. for C₂₈H₂₉O₉ 509.1812, found 509.1802.

3.4. Cytotoxic assay

Effect cytotoxic of compounds (**1-5**) against HeLa (human cervical cancer cells), MCF-7 (human breast cancer cells), P-388 (human murine leukemia cells) were evaluated using the MTT colorimetric methods (Mah et al. 2015; Tanjung et al. 2017, 2018; Segun et al. 2019). Doxorubicin used as a control positive for HeLa, and MCF-7 as well as artonin for P-388 cells.

4. Conclusions

Meliquercifolins A (**1**) and B (**2**), two new coumarins bearing an acetophenone derivative were isolated from the leaves of *M. quercifolia* together with three known

compounds, melicodenines E (3), F (4) and I (5). Compounds (1 and 2) are the first time coumarin bearing an acetophenone derivative found on *Melicope*.

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Disclosure statement

No potential conflict of interest reported by the authors.

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