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Thanika Promchai
Chiang Mai University

Atchara Jaidee
Chiang Mai University

Sarot Cheenpracha
University of Phayao

Kongkiat Trisuwan
Chiang Mai University

Roonglawan Rattanajak
National Science and Technology Development Agency, Thailand

See next page for additional authors

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Abstract

Five new oxoprotoberberine alkaloids, miliusacunines A-E (1-5), along with nine known compounds, 6-14, were isolated from an acetone extract of the leaves and twigs of Miliusa cuneata. Their structures were elucidated by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicities against the KB and Vero cell lines and for antimalarial activities against the Plasmodium falciparum strains TM4 and K1 (a sensitive and a multi-drug-resistant strain, respectively). Compound 1 showed in vitro antimalarial activity against the TM4 strain, with an IC50 value of 19.3 ± 3.4 μM, and compound 2 demonstrated significant activity against the K1 strain, with an IC50 value of 10.8 ± 4.1 μM. Both compounds showed no discernible cytotoxicity to the Vero cell line at the concentration levels evaluated.

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Authors

Thanika Promchai, Atchara Jaidee, Sarot Cheenpracha, Kongkiat Trisuwan, Roonglawan Rattanajak, Sumalee Kamchonwongpaisan, Surat Laphookhieo, Stephen G. Pyne, and Thunwadee Ritthiwigrom

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Antimalarial Oxoprotoberberine Alkaloids from the Leaves of *Miliusa cuneata*

Thanika Promchai,[†] Atchara Jaidee,[†] Sarot Cheenpracha,[‡] Kongkiat Trisuwan,[†] Roonglawan Rattanajak,[§] Sumalee Kamchonwongpaisan,[§] Surat Laphookhieo,[⊥] Stephen G. Pyne,[∥] and Thunwadee Ritthiwigrom*[,†]

[†]Department of Chemistry, Faculty of Science, Chiang Mai University, Sutep, Muang, Chiang Mai 50200, Thailand

[‡]School of Science, University of Phayao, Maeka, Muang, Phayao 56000, Thailand

[§]Medical Molecular Biology Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumthani 12120, Thailand

[⊥]Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand

[∥]School of Chemistry, University of Wollongong, Wollongong, New South Wales 2522, Australia

Five new oxoprotoberberine alkaloids, miliusacunines A-E (**1-5**), along with nine known compounds, **6-14**, were isolated from an acetone extract of the leaves and twigs of *Miliusa cuneata*. Their structures were elucidated by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicities against the KB and Vero cell lines and for antimalarial activities against the *P. falciparum* strains TM4 and K1 (a sensitive and a multidrug-resistant strain, respectively). Compound **1** showed in vitro antimalarial activity against the TM4 strain, with an IC_{50} value of 19.3 ± 3.4 μM, and compound **2** demonstrated significant activity against the K1 strain, with an IC_{50} value of 10.8 ± 4.1 μM. Both compounds showed no discernible cytotoxicity to the Vero cell line at the concentration levels evaluated.

The plant genus *Miliusa* (Annonaceae) comprises about 50 species and is distributed from the Indian subcontinent to Indochina, Peninsula Malaysia, the Southeast Asian islands, to New Guinea and northern Australia.[1] Some species have been utilized in traditional medicine, and, for example, *M. velutina* has been used as a tonic and aphrodisiac[2] and *M. balansae* for gastropathy and glomerulonephropathy.[3] The plants from this genus are reported to contain secondary metabolites inclusive of alkaloids,[4-6] acetogenins,[7] homogentisic acid derivatives,[8-10] flavonoids,[5,8-13] dihydrochalcones,[5,8] neolignans and lignans,[14,15] and terpenoids.[5] Several of these chemical constituents exhibited antibacterial,[7] cytotoxic[5,7,12-15] and antiherpetic[14,15] activities.

Miliusa cuneata Craib is known locally in Thailand as "Ra-Khang-Khieo". Recently, two new isoquinoline alkaloids and thirteen known alkaloids were isolated from the stems and leaves of *M. cuneata*.[4] Herein are reported the isolation and structure elucidation of five new oxoprotoberberines, miliusacunines A-E (**1-5**), together with nine known compounds (**6-14**), from an acetone extract of the leaves and twigs of *M. cuneata* as well as an evaluation of their in vitro biological activities against the *P. falciparum* strains TM4 and K1 and the human oral epidermal carcinoma (KB) cell line.

RESULTS AND DISCUSSION

Column chromatographic separation of an acetone extract of the leaves of *M. cuneata* afforded five new alkaloids, miliusacunines A-E (**1-5**), along with five known compounds, namely, 5-hydroxy-3,7-dimethoxy-3′,4′-methylenedioxyflavone (**6**),[16] pachypodol (**7**),[12,17] 4'-hydroxy-3,5,7,3′-tetramethoxyflavone (**8**),[18] (+)-miliusol (**9**),[10] and (+)-syringaresinol (**10**).[19] Also, an acetone extract of the twigs of *M. cuneata* was subjected to column chromatography over silica gel and Sephadex LH-20 to provide six known substances, compounds **6** and **7**, and chrysoplenetin (**11**),[17] *N-trans*-feruloyltyramine (**12**),[20] *N-trans*-caffeoyltyramine (**13**),[21] and *N-trans*-coumaroyltyramine (**14**).[20,22]

Compound **1** was isolated as a bright pink solid with mp 218-220 ºC and demonstrated a molecular formula of $C_{19}H_{18}NO_6$, as assigned from the HRESIMS ion peak at *m/z* 356.1136 $[M+H]^+$ (calcd 356.1134). The UV spectrum displayed maximal absorption bands at λ_{max} 224, 338, 371 and 389 nm and the IR spectrum revealed the presence of one or more hydroxy groups (3419 cm^{-1}), a conjugated amide group (1649 cm^{-1}), and an aromatic (1584 and 1504 cm^{-1}) ring system, indicating the presence of an oxoprotoberberine nucleus.[23,24] The ^1H NMR spectrum (Table 1) displayed resonances for a chelated hydroxy group (δ_H 13.17, 1H, br s), two *ortho*-coupled aromatic protons [δ_H 7.25 (1H, d, J = 8.4 Hz) and 7.02 (1H, d, J = 8.4 Hz)], a singlet aromatic proton (δ_H 7.12), a singlet olefinic proton (δ_H 7.24), a set of coupled methylene protons [δ_H 4.24 (2H, t, J = 6.2 Hz) and 2.96 (2H, t, J = 6.2 Hz)], and two methoxy groups at δ_H 3.95 and 3.84 (each, 3H, s). Compound **1** showed resonances for an amide carbonyl (δ_C 165.8), ten quaternary carbons (δ_C 152.9, 148.1, 147.5, 143.2, 137.7, 135.0, 130.6, 126.1, 116.2 and 111.6), four methines (δ_C 123.1, 116.7, 104.9 and 100.7), two methylenes (δ_C 39.4 and 21.4), and two methoxy groups (δ_C 60.8 and 56.2) in the ^{13}C NMR and DEPT spectra (Table 1). The chelated hydroxy proton at δ_H 13.17 was located at C-9 (δ_C 148.1) due to being hydrogen bonded to the amide carbonyl carbon at C-8 (δ_C 165.8). Two *ortho*-coupled aromatic protons at δ_H 7.25 and 7.02 were assigned to H-11/δ_C 123.1 and H-12/δ_C 116.7, respectively, on the basis of the correlations of H-11 with C-9, C-10 (δ_C 143.2) and C-12a (δ_C 130.6) and H-12 with C-8a (δ_C 111.6), C-10 and C-13 (δ_C 104.9) in the HMBC spectrum. The olefinic proton resonating at δ_H 7.24 was assigned as H-13 based on HMQC and HMBC correlations. These data together with the carbon chemical shifts of C-9 and C-10 confirmed the presence of the hydroxy groups at C-9 and C-10. The low field methylene protons at δ_H 4.24 were assigned to H-6 due to the inductive effect of the *N* atom of the amide group and HMBC correlations with C-4a (δ_C 116.2), C-5 (δ_C 21.4), C-8 (δ_C 165.8), and C-13a (δ_C 135.0). The remaining coupled methylene protons at δ_H 2.96 were

attributed to H-5. The substituent at C-4 was identified as a hydroxy group from its ^{13}C NMR chemical shift (δ_C 147.5) and the HMBC correlation between H-5 with C-4. The two methoxy groups (δ_H 3.95 and 3.84) were located at C-2 (δ_C 152.9) and C-3 (δ_C 137.7), respectively, on the basis of HMBC correlations and their ^{13}C NMR chemical shifts. The resonance for C-3 appeared at a high field (δ_C 137.7) due to the occurrence of two *ortho* oxygen substituents. The singlet aromatic proton resonance at δ_H 7.12 was assigned to H-1, on the basis of the HMBC correlations with C-2, C-3, and C-13a. Consequently, compound **1** (miliusacunine A) was elucidated as 8-oxo-4,9,10-trihydroxy-2,3-dimethoxy-5,6-dihydroberberine.

Compound **2** was obtained as a yellow solid (mp 217-218 °C), which showed a $[M+Na]^+$ ion at *m/z* 392.1112 in the HRESIMS, consistent with a molecular formula of $C_{20}H_{19}NO_6Na$ (calcd 392.1110). The spectroscopic data of compound **2** was similar to those of **1**, except that compound **2** displayed an additional methoxy group resonance at δ_H 3.89. This methoxy group was located at C-4 (δ_C 151.1) based on the HMBC correlation with C-4. This assignment was further supported from the HMBC correlation between the methylene protons H-5 [δ_H 2.98 (t, J = 6.2 Hz)] with C-4. Therefore, compound **2** (miliusacunine B) was characterized as the 4-*O*-methyl ether of compound **1**.

Compound **3** was obtained as a reddish brown gum, and its UV and IR data were similar to those of compound **1**. Its ^{1}H NMR and ^{13}C NMR data indicated one of the methoxy groups in **3** to be located to a different position as that found in **1**. The methoxy group resonance at δ_H 3.91 showed a HMBC correlation to C-4 (δ_C 143.8), while a HMBC correlation between the methylene protons H-5 [δ_H 2.98 (t, J = 5.6 Hz)] and C-4 was also observed. This indicated that the methoxy group is located at C-4. The other methoxy group (δ_H 4.00) was located at C-2 (δ_C 147.2), according to its HMBC correlation with C-2. The

substituent at C-3 was assigned as a hydroxy group by the relative high field ^{13}C NMR chemical shift of C-3 at δ_C 139.8 due to its two *ortho* oxygen substituents. The locations of the methoxy and hydroxy groups attached at C-2 and C-3, respectively, were also confirmed by the HMBC correlations between the aromatic proton H-1 (δ_H 7.04/δ_C 103.0) and C-2 and C-3. Thus, the structure of **3** (miliusacunine C) was assigned as 8-oxo-3,9,10-trihydroxy-2,4-dimethoxy-5,6-dihydroberberine.

Compound **4** was obtained as a brownish yellow gum and gave a specific rotation of $[\alpha]^{28}_D$ –175 (*c* 0.07, MeOH). The ^{1}H NMR spectroscopic data of **4** were similar to those of **3**. The main differences found were the additional methine ^{1}H NMR signal [δ_H 4.83 (1H, br d, J = 13.2 Hz)], which was coupled to two diastereotopic methylene protons [δ_H 3.14 (1H, br d, J = 15.3 Hz) and 2.89 (1H, br t, J = 15.3 Hz)] for compound **4**. This methylene group was situated at C-13 (δ_C 37.1) by the observation of its HMQC cross peak and the HMBC correlations of H-13 to C-1a (δ_C 126.7), C-8a (δ_C 111.2), C-12 (δ_C 116.8), C-12a (δ_C 128.2) and C-13a (δ_C 55.7) and between the aromatic proton H-12 [δ_H 6.61 (d, J = 8.0 Hz)] and C-13. The methine proton (δ_H 4.83) was located at C-13a (δ_C 55.7) from the correlation between the singlet aromatic proton at δ_H 6.52 (H-1) and C-13a in the HMBC spectrum. Therefore, the structure of **4** (miliusacunine D) was analyzed as (–)-(*S*)-8-oxo-3,9,10-trihydroxy-2,4-dimethoxy-5,6,13,13a-tetrahydroberberine. Its configuration was assigned based on the sign of its specific rotation when compared to that of the related compound, (*S*)-(–)-2,3-dimethoxy-8-oxoberbine, $[\alpha]_D$ –413.8 (*c* 0.36, $CHCl_3$),[25] for which the structure was assigned using X-ray crystallography.

Compound **5** was obtained as a yellow gum with a specific rotation of $[\alpha]^{28}_D$ –93 (*c* 0.02, MeOH). Comparison of its NMR data with those of **4** showed the presence of an additional resonance for a methoxy group (δ_H 3.89) in **5**, which was located at C-3 on the basis of its HMBC correlation with C-3 (δ_C 140.9). The aromatic proton H-1 (δ_H 6.53)

correlated to C-3 in the HMBC spectrum, further supporting the location of the additional methoxy group. Thus, the structure of **5** (miliusacunine E) was assigned as the (–)-(*S*)-3-*O*-methyl ether of compound **4**.

All of the isolated compounds were evaluated for their cytotoxic activities against the KB (human oral epidermoid cancer cell line) and a non-cancerous cell line (African green monkey kidney cell line) as well as for antimalarial activity against the *Plasmodium falciparum* strains, TM4 and K1 (multidrug resistant strain) (Table 3). Compound **9** exhibited cytotoxic activity against the KB cell line with an IC_{50} value of 10.2 ± 0.1 μM and showed antimalarial activity against both strains with IC_{50} values of 11.1 ± 2.0 and 9.1 ± 1.0 μM, respectively. However this compound was relatively cytotoxic with an IC_{50} value of 13.5 ± 0.5 μM against the Vero cell line. In earlier work, compound **9** was isolated from the leaves, twigs and flowers of *Miliusa sinensis*, and was demonstrated as being cytotoxic to the human oral epidermoid carcinoma (KB), human prostate carcinoma (LNCaP), human lung carcinoma (Lu-1), human colon carcinoma (Col-2), and human umbilical vein endothelial (HUVEC) cell lines.[26] Compounds **1-5**, **8**, and **11-13** displayed weaker antimalarial activity than compound **9**, with the IC_{50} values ranging from 19.3-41.4 and 10.8-54.9 μM against the TM4 and K1 strains, respectively. None of these was cytotoxic for the Vero cell line. Among these, compound **1** showed antimalarial activity against the TM4 strain with an IC_{50} value of 19.3 ± 3.4 μM and compound **2** demonstrated significant activity against the K1 strain with an IC_{50} value of 10.8 ± 4.1 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured with a Sanyo Gallenkamp melting point apparatus and are uncorrected. Optical rotation values were determined on a Bellingham and Stanley APD440 polarimeter. The UV spectra were

recorded with a Perkin-Elmer UV-vis spectrophotometer, whereas the IR spectra were obtained using a Bruker Tensor FT-IR spectrophotometer. NMR spectroscopic data were obtained on a 400 MHz Bruker Ultra Shield FT-NMR and a 500 MHz Varian Unity INOVA NMR spectrometer. Chemical shifts are recorded in parts per million (δ) in $CDCl_3$ (δ_H 7.26 and δ_C 77.2) and/or acetone-d_6 (δ_H 2.05 and δ_C (CO) 206.2 and (CH_3) 29.8, with TMS as an internal reference. Mass spectrometric data were obtained on a Micro TOF, Bruker Daltonics mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was performed on Sephadex LH-20, and silica gel (Merck) type 100 (63-200 μm) and type 60 (5-40 μm for quick column chromatography; QCC). All solvents for extraction and chromatography were routinely distilled prior to use.

Plant Material. The leaves and twigs of *M. cuneata* were collected at Doi Tung, Chiang Rai Province, Thailand in September 2013. The plant was identified by Mr. James F. Maxwell from the CMUB Herbarium, Chiang Mai University, where a voucher specimen has been deposited (specimen no. Martin van de Bult 1328).

Extraction and Isolation. The air-dried leaves (0.60 kg) of *M. cuneata* were extracted with acetone (15 L) over a period of three days for three times at room temperature. Removal of the solvent under reduced pressure provided an acetone extract (88.41 g) as a dark green gum. The crude extract was separated by QCC over silica gel, eluting with a gradient of hexanes and acetone (1:0 to 2:3), to give seven fractions (L1-L7). Fraction L2 (603.1 mg) was separated over Sephadex LH-20 with CH_2Cl_2-MeOH (1:4) to afford two subfractions (L2A and L2B), and the latter subfraction (31.6 mg) was fractionated further over Sephadex LH-20 with MeOH to provide three subfractions (L2B1-L2B3). The second subfraction (7.0 mg) was purified by CC over silica gel using EtOAc-hexanes (1:9) as mobile phase to afford compound **6** (2.5 mg) as a brownish yellow solid, mp 181-183 °C, lit. 182-183 °C.[16] Fraction L4 (1.30 g) was washed with MeOH and then hexanes, and the remaining solid was dried

under a vacuum to give compound **7** (140 mg) as a yellow solid, mp 173-174 °C, lit. 171-172 °C.[27] Separation of fraction L5 (10.73 g) by QCC over silica gel, with gradient elution using CH_2Cl_2-MeOH (1:0 to 9:1), gave five subfractions (L5A-L5E). Purification of subfraction L5B (20.0 mg) over Sephadex LH-20 with MeOH gave compound **5** (1.3 mg) as a yellow viscous oil and **2** (9.7 mg) as a yellow solid, respectively. Subfraction L5D (402.5 mg) was separated over Sephadex LH-20 with MeOH to produce three subfractions (L5D1-L5D3). Subfraction L5D2 (286.5 mg) was further purified by CC with acetone-hexanes (3:7) to yield compound **9** (149.4 mg) as a yellow viscous oil. Fraction L6 (7.82 g) was separated over silica gel by QCC with a gradient of CH_2Cl_2-MeOH (1:0 to 9:1) to afford three subfractions (L6A-L6C). Subfraction L6B (408.2 mg) was further separated over Sephadex LH-20 with MeOH to provide four additional subfractions (L6B1-L6B4). The second subfraction, L6B2 (18.6 mg) was further purified over Sephadex LH-20 with MeOH to afford compound **4** (4.4 mg) as a brownish yellow gum. Subfraction L6B4 (28.0 mg) was further separated by CC over silica gel with EtOAc-hexanes (3:7) to provide compound **1** (8.1 mg) as a bright pink solid. Fraction L7 (5.96 g) was separated over silica gel by QCC with a gradient of CH_2Cl_2-MeOH (1:0 to 9:1) to afford three subfractions (L7A-L7C). Subfraction L7B (111.4 mg) was separated over Sephadex LH-20 with MeOH to obtain compounds **8** (10.1 mg) as a yellow gum and **3** (3.7 mg) as a brownish red gum. Subfraction L7B2 (20.3 mg) was further purified by CC over silica gel using EtOAc-hexanes (3:2) as mobile phase to afford compound **10** (2.8 mg) as a yellow viscous oil.

The air-dried twigs (2.21 kg) of *M. cuneata* were macerated with acetone (15 L) over a period of three days (three times) at room temperature. The combined extracts were evaporated under reduced pressure to afford an acetone extract (35.0 g) as a dark brown gum. The crude extract was separated by QCC over silica gel and eluted with a gradient of hexanes-CH_2Cl_2-MeOH (1:0:0 to 0:4:1) to give nine fractions (T1-T9). Fraction T2 (860.0

mg) was separated by CC over silica gel eluting with CH_2Cl_2 to give three subfractions (T2A-T2C). Subfraction T2B (46.0 mg) was purified over Sephadex LH-20 with MeOH to provide compound **6** (1.1 mg). Fraction T4 (2.79 g) was fractionated by QCC using EtOAc-hexanes (3:7 to 1:1) for elution to give three subfractions (T4A-T4C). Subfraction T4B (149.2 mg) was further purified by CC with CH_2Cl_2 to provide compound **7** (40.0 mg). Fraction T6 (556.3 mg) was purified over Sephadex LH-20 with MeOH to afford compound **11** as a brownish red gum (1.2 mg). Fraction T8 (1.25 g) was separated over Sephadex LH-20 with MeOH to provide three subfractions (T8A-T8C). Subfraction T8B (425.2 mg) was purified by CC with EtOAc-CH_2Cl_2 (9:1) to give compound **12** as an orange gum (17.7 mg). Fraction T9 (1.40 g) was separated over Sephadex LH-20 with MeOH to give three fractions (T9A-T9C). Subfraction T9B (194.6 mg) was further fractionated by CC with acetone-hexanes (3:7) to yield three fractions (T9B1-T9B3). Separation of subfraction T9B2 (24.0 mg) over Sephadex LH-20 with MeOH provided compound **13** as an orange-yellow gum (2.7 mg). Purification of subfraction T9B3 (19.0 mg) over Sephadex LH-20 with MeOH furnished compound **14** (9.7 mg) as a white solid, with mp 247-249 °C, lit. 248 °C.[22]

*Miliusacunine A (**1**)*: bright pink solid; mp 218-220 °C; UV (MeOH) λ_{max} (log ε) 224 (4.5), 338 (4.2), 371 (4.1), 389 (4.1) nm; IR (neat) ν_{max} 3419, 1649, 1617, 1584, 1504, 1463, 1278, 1126 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 356.1136 $[M+H]^+$, calcd for $C_{19}H_{18}NO_6$, 356.1134.

*Miliusacunine B (**2**)*: yellow solid; mp 217-218 °C; UV (MeOH) λ_{max} (log ε) 224 (4.6), 341 (4.2), 371 (4.2), 389 (4.1) nm; IR (neat) ν_{max} 3388, 1648, 1586, 1494, 1461, 1280, 1032 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 392.1112 $[M+Na]^+$, calcd for $C_{20}H_{19}NO_6Na$, 392.1110.

*Miliusacunine C (**3**)*: brownish red gum; UV (MeOH) λ_{max} (log ε) 225 (4.3), 340 (4.0), 372 (3.9), 389 (3.9) nm; IR (neat) ν_{max} 3421, 1648, 1611, 1583, 1502, 1463, 1278,

1126, 1093 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 378.0958 $[M+Na]^+$, calcd for $C_{19}H_{17}NO_6Na$, 378.0954.

*Miliusacunine D (**4**)*: brownish yellow gum; $[\alpha]^{28}_{D}$ –175 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.5), 262 (4.0), 341 (3.8) nm; IR (neat) ν_{max} 3421, 1636, 1615, 1584, 1459, 1267, 1120, 1091 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 380.1104 $[M+Na]^+$, calcd for $C_{19}H_{19}NO_6Na$, 380.1110.

*Miliusacunine E (**5**)*: yellow viscous oil; $[\alpha]^{28}_{D}$ –93 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.4), 265 (3.8), 325 (3.5) nm; IR (neat) ν_{max} 3421, 1635, 1590, 1459, 1271, 1121, 1097, 1029 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 372.1454 $[M+H]^+$, calcd for $C_{20}H_{22}NO_6$, 372.1447.

Cytotoxicity Assays. KB cells (human oral epidermal carcinoma cells) and normal Vero cells (kidney epithelial cells of the African green monkey, *Cercopithecus aethiops*)[28] were obtained from the Bioassay Laboratory, BIOTEC, NSTDA, Pathumthani, Thailand. Vero cells were maintained and cultured in MEM/EBSS supplemented with heated fetal bovine serum (10%), $NaHCO_3$ (2.2 g/L), and sodium pyruvate (1%). KB cells were cultured in DMEM/low glucose supplemented with heated fetal bovine serum (10%), $NaHCO_3$ (3.7 g/L), and NEAA (1%). Cytotoxicity was evaluated using the sulforhodamine B (SRB) assay.[29] Ellipticine and doxorubicin were used as the standard compounds.

Antimalarial Assay. The in vitro antimalarial activity against two strains of *Plasmodium falciparum* (TM4, a wild-type chloroquine and antifolate-sensitive strain and K1, a multidrug-resistant strain) were obtained from Prof. S. Thaithong, Department of Biology, Faculty of Science, Chulalongkorn, Bangkok, Thailand. The parasites were maintained in human red blood cells in RPMI 1640 medium supplemented with HEPES (25 mM), sodium bicarbonate (0.2%), and human serum (8%) in a carbon dioxide (3%) incubator maintained at 37°C. The test samples were made up in DMSO solution (25 µL, diluted in the culture

medium) and placed in triplicate in a 96-well plate where parasitized erythrocytes (200 μL) with a cell suspension (1.5%) of parasitemia (0.5-1%) were then added to the wells. The culture was incubated (18-20 h). The antiplasmodial activity testing was carried out using the Microdilution Radioisotope Technique as described by Yuthavong et al.[30] Chloroquine, cycloguanil, and pyrimethamine were used as reference substances.[31] Human erythrocytes and serum were obtained from donors after providing informed consent, following a protocol approved by the NSTDA Ethics Committee for Human Research, document no. 0007/2557.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS publications website at DOI: 10.1021/acs.jnatprod.

1D and 2D NMR data of compounds **1**−**5** (PDF)

AUTHOR INFORMATION

Corresponding Author
*Tel: +66 5394 3341 ext 222. Fax: +66 5389 2277. E-mail address: thunwadee.r@cmu.ac.th, othunwadee@gmail.com.

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Table 1. NMR ((^{1}H 400 MHz) and ^{13}C (100 MHz)) Data of Miliusacunines A and B (1 and 2) in Acetone-d_6 and Miliusacunine C (3) in $CDCl_3$

position	**1**			**2**			**3**		
	δ_C, type	δ_H, (J in Hz)	HMBC[a]	δ_C, type	δ_H, (J in Hz)	HMBC[a]	δ_C, type	δ_H, (J in Hz)	HMBC[a]
1	100.7, CH	7.12, s	1a, 2, 3, 4a, 13a	105.3, CH	7.34, s	1a, 2, 3, 4a, 13a	103.0, CH	7.04, s	2, 3, 4a, 13a
1a	126.1, C			126.3, C			121.5, C		
2	152.9, C			154.0, C			147.2, C		
3	137.7, C			144.1, C			139.8, C		
4	147.5, C			151.1, C			143.8, C		
4a	116.2, C			122.5, C			122.1, C		
5	21.4, CH_2	2.96, t (6.2)	1a, 4, 4a, 6	22.0, CH_2	2.98, t (6.2)	1a, 4, 4a, 6	21.6, CH_2	2.98, t (5.6)	1a, 4, 4a, 6
6	39.4, CH_2	4.24, t (6.2)	4a, 5, 8, 13a	39.7, CH_2	4.23, t (6.2)	4a, 5, 8, 13a	39.0, CH_2	4.25, t (5.6)	4a, 5, 8, 13a
8	165.8, C			166.1[b], C			165.0, C		
8a	111.6, C			111.9, C			109.9, C		
9	148.1, C			148.3[b], C			146.8, C		
10	143.2, C			143.4, C			141.8, C		
11	123.1, CH	7.25, d (8.4)	9, 10, 12a	122.5, CH	7.26, d (8.4)	9, 10, 12a	121.6, CH	7.28, d (8.2)	9, 10, 12a
12	116.7, CH	7.02, d (8.4)	8a, 10, 11, 12a, 13	117.0, CH	7.03, d (8.4)	8a, 10,11, 13	116.0, CH	6.97, d (8.2)	8a, 10, 13
12a	130.6, C			130.8, C			130.2, C		
13	104.9, CH	7.24, s	1a, 8a, 12, 12a, 13a	105.2, CH	7.25, s	1a, 8a, 12, 13a	103.8, CH	6.86, s	1a, 8a, 11, 13a
13a	135.0, C			134.7, C			134.7, C		
OCH_3 (2)	56.2, CH_3	3.95, s	2	56.6, CH_3	3.95, s	2	56.7, CH_3	4.00, s	2
OCH_3 (3)	60.8, CH_3	3.84, s	3	61.3, CH_3	3.88, s	3			
OCH_3 (4)				61.1, CH_3	3.89, s	4	61.0, CH_3	3.91, s	4
OH (9)		13.17, br s			13.15, br s				

[a]HMBC correlations are from proton(s) stated to the indicated carbon.
[b]Observed from the HMBC spectrum.

Table 2. NMR ((^{1}H 400 MHz) and ^{13}C (100 MHz)) Data of Miliusacunines D and E (4 and 5) in $CDCl_3$

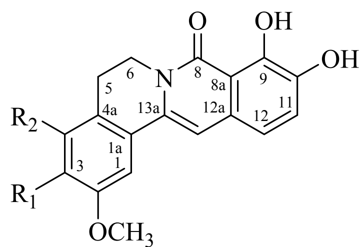
position	**4**			**5**		
	δ_C, type	δ_H, (J in Hz)	HMBC[a]	δ_C, type	δ_H, (J in Hz)	HMBC[a]
1	103.8, CH	6.52, s	1a, 2, 3, 4a, 13a	104.9, CH	6.53, s	1a, 2, 3, 4a, 13a
1a	126.7, C			131.1, C		
2	146.8, C			152.7, C		
3	137.3, C			140.9[c], C		
4	144.3, C			151.0, C		
4a	121.3, C			121.4, C		
5	23.0, CH_2	3.05, br d (15.7)	1a, 4, 4a, 6	23.0, CH_2	3.03, br d (16.2)	1a, 4a, 6
		2.70, m	1a, 4, 4a, 6		2.70, m	1a, 4, 4a, 6
6	38.0, CH_2	4.88, br d (13.2)	4a, 5, 8, 13a	38.2, CH_2	4.87, br d (13.0)	4a, 5, 13a
		2.86, br d (14.1)	4a, 5, 13a		2.85, br d (12.2)	4a, 5, 13a
8	168.5, C			168.6, C		
8a	111.2, C			111.3, C		
9	148.6, C			148.7, C		
10	143.9, C			144.1, C		
11	118.2, CH	6.99, d (8.0)	9, 10, 12a	118.4, CH	6.99, d (7.7)	9, 10, 12a
12	116.8, CH	6.61, d (8.0)	8a, 10, 11, 13	116.9, CH	6.61, d (7.7)	8a, 10, 11, 13
12a	128.2, C			128.2, C		
13	37.1, CH_2	3.14, br d (15.3)[b]	1a, 8a, 12, 12a, 13a	37.1, CH_2	3.15, br d (15.2)[b]	8a, 12, 12a, 13a
		2.89, br t (15.3)[b]	1a, 8a, 11, 12, 12a, 13a		2.91, br t (15.2)[b]	1a, 8a, 11, 12, 12a, 13a
13a	55.7, CH	4.83, br d (13.2)[b]	1, 1a, 4a, 13	55.9, CH	4.82, br d (13.5)[b]	1, 1a, 4a, 13
OCH_3 (2)	56.4, CH_3	3.92, s	2	56.4, CH_3	3.92, s	2
OCH_3 (3)				61.0, CH_3	3.89, s	3
OCH_3 (4)	60.6, CH_3	3.89, s	4	61.1, CH_3	3.89, s	4
OH (9)		12.75, br s			12.74, br s	
OH (10)		5.59, br s				

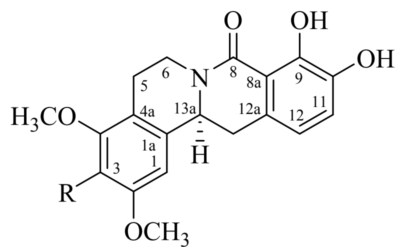
[a]HMBC correlations are from proton(s) stated to the indicated carbon.
[b]Signal partially obscured.
[c]Observed from the HMBC spectrum.

Table 3. Cytotoxic and Antimalarial Activities of Compounds 1-14 (IC_{50}, μM)

compound	cytotoxic activity		antimalarial activity against *P. falciparum*	
	KB	Vero cells	TM4	K1
1	-[a]	-[a]	19.3 ± 3.4	26.3 ± 9.6
2	-[a]	-[a]	25.6 ± 1.2	10.8 ± 4.1
3	-[a]	-[a]	41.4 ± 1.0	32.4 ± 10.0
4	-[a]	-[a]	38.1 ± 4.6	26.1 ± 13.9
5	-[a]	-[a]	-[a]	29.1 ± 3.1
6	-[a]	-[a]	-[a]	-[a]
7	-[a]	-[a]	-[a]	-[a]
8	-[a]	-[a]	31.5 ± 18.3	27.9 ± 11.8
9	10.2 ± 0.1	13.5 ± 0.5	11.1 ± 2.0	9.1 ± 1.0
10	-[a]	-[a]	-[a]	-[a]
11	-[a]	-[a]	39.5 ± 6.5	12.4 ± 3.7
12	-[a]	-[a]	-[a]	54.9 ± 5.9
13	-[a]	-[a]	-[a]	23.8 ± 2.1
14	-[a]	-[a]	-[a]	-[a]
doxorubicin[b]	1.0			
ellipticine[b]		0.4		
chloroquine[c]			0.03	0.3 ± 0.04
cycloguanil[c]			0.04 ± 0.01	3.2 ± 0.8
pyrimethamine[c]			0.08 ± 0.01	31.0 ± 8.4

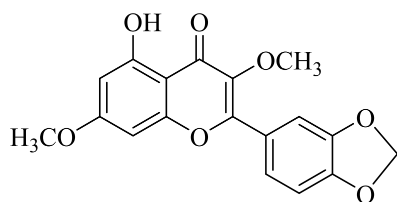
[a]Inactive at IC_{50} > 47.8 μM. [b]Positive control for cytotoxic assay.
[c]Reference drugs for antiplasmodial assays.





1 $R_1 = OCH_3$, $R_2 = OH$
2 $R_1 = OCH_3$, $R_2 = OCH_3$
3 $R_1 = OH$, $R_2 = OCH_3$

4 $R = OH$
5 $R = OCH_3$



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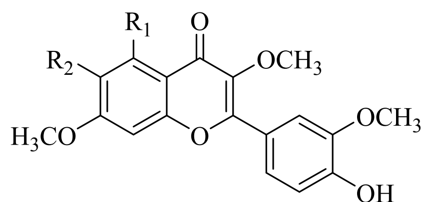
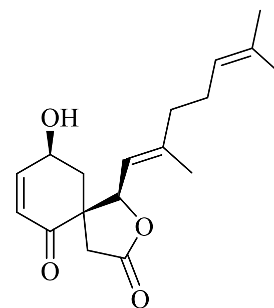


7 $R_1 = OH$, $R_2 = H$
8 $R_1 = OCH_3$, $R_2 = H$
11 $R_1 = OH$, $R_2 = OCH_3$



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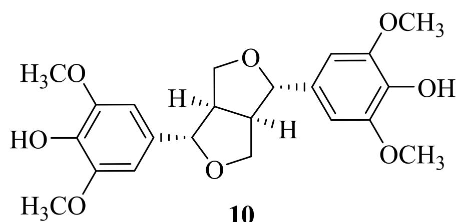

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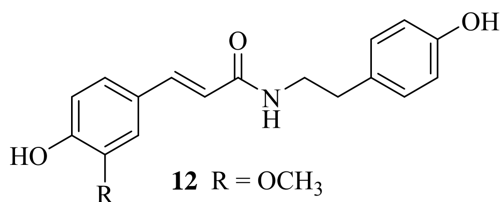

12 $R = OCH_3$
13 $R = OH$
14 $R = H$