

Cassiarins C–E, Antiplasmodial Alkaloids from the Flowers of *Cassia siamea*

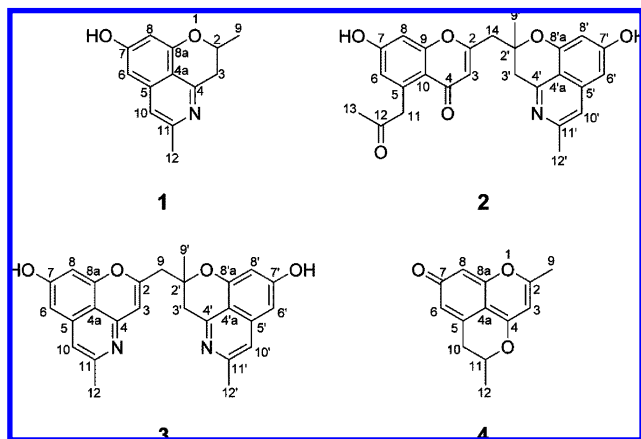
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Three new alkaloids, cassiarins C–E (**1–3**), and a new chromone, 10,11-dihydroanhydrobarakol (**4**), which showed moderate antiplasmodial activity against *Plasmodium falciparum* 3D7, were isolated from flowers of *Cassia siamea*, and the structures of **1–4** were elucidated by 2D NMR analysis and chemical transformation. Cassiarin D (**2**) was a dimeric compound consisting of 5-acetyl-7-hydroxy-2-methylchromone and cassiarin C (**1**), and cassiarin E (**3**) was a dimer of cassiarins A and C (**1**).

Cassia siamea Lam. (Leguminosae) has been used widely in traditional medicine, particularly for treatment of periodic fever and malaria in Indonesia.¹ Earlier we isolated cassiarin A,² an alkaloid with an unprecedented tricyclic skeleton and potent antiplasmodial activity, and chrobisiamone A,³ a bischromone, from leaves of *C. siamea*. We then reported an efficient total synthesis of cassiarin A,⁴ which also showed in vivo antimalarial activity.⁵ Our continuing search for bioactive constituents of *C. siamea* has resulted in the isolation of three additional new alkaloids, cassiarins C–E (**1–3**), and a new chromone, 10,11-dihydroanhydrobarakol (**4**), from the flowers of *C. siamea*. Also isolated were cassiarin A, anhydrobarakol (**5**),⁶ and 5-acetyl-7-hydroxy-2-methylchromone (**6**).⁷ This paper describes the isolation and structure elucidation of **1–4** and their antiplasmodial activity against *Plasmodium falciparum* 3D7.



Cassiarin C (**1**), $[\alpha]_D^{20} -70$ (c 0.3, MeOH), had the molecular formula $C_{13}H_{13}NO_2$, which was determined by HRESITOFMS [m/z 216.1025, $(M + H)^+$, $\Delta +1.7$ mmu]. IR absorptions implied the presence of hydroxy and/or amino (3401 cm^{-1}) functionalities. ^1H and ^{13}C NMR data are presented in Table 1. The ^{13}C NMR spectrum revealed 13 signals due to six sp^2 quaternary carbons, three sp^2 methines, one sp^3 methine, one sp^3 methylene, and two methyl groups. Among them, four quaternary carbons (δ_C 149.9, 153.6, 156.9, and 159.1) and one sp^3 methine (δ_C 73.7) were ascribed to those bearing nitrogen or oxygen atoms. Two partial structures,

C-10 to C-12 and C-9 to C-3, were deduced from analysis of the $^1\text{H}-^1\text{H}$ COSY spectrum, which included a long-range coupling from H_3-12 to H-10 of **1**. The HMBC correlations of H-10 to C-4a (δ_C 110.6) and C-5 (δ_C 139.1) and of H_2-3 to C-4 (δ_C 153.6) and C-4a indicated connectivity of two partial structures through a nitrogen and C-4 atoms. Connection between the partial structure and a benzene ring was determined by a HMBC correlation between H-10 and C-6 (δ_C 100.0). The presence of an ether linkage between C-2 (δ_C 73.7) and C-8a (δ_C 156.9) to form a dihydropyran ring was also evident. Thus, cassiarin C (**1**) was concluded to be a 2,3-dihydrocassiarin A.

Cassiarin D (**2**), $[\alpha]_D^{20} -10$ (c 0.1, MeOH), had the molecular formula $C_{26}H_{23}NO_6$ (by HRESITOFMS). IR absorptions implied the presence of hydroxy and/or amino (3673 cm^{-1}) and carbonyl (1713 and 1632 cm^{-1}) functionalities. ^1H and ^{13}C NMR data are presented in Table 2. The ^{13}C NMR spectrum revealed 26 signals due to two carbonyl carbons, 11 sp^2 quaternary carbons, one sp^3 quaternary carbon, three sp^3 methylenes, six sp^2 methines, and three methyl groups. Among them, 10 quaternary carbons (δ_C 78.9, 152.4, 152.5, 154.9, 160.1, 162.5, 162.9, 163.1, 178.4, and 204.6) were ascribed to those bearing nitrogen or oxygen atoms. The gross structure of **2** consisted of two units, a chromone, and cassiarin C, as deduced from extensive analyses of the two-dimensional NMR data, including the $^1\text{H}-^1\text{H}$ COSY and HMBC spectra in pyridine- d_5 . The presence of an acetyl group at C-5 was supported by HMBC correlations of H_3-13 (δ_H 2.37) to C-11 (δ_C 49.8) and C-12 (δ_C 204.6) and H_2-11 (δ_H 4.37) to C-5 (δ_C 139.2), C-6 (δ_C 119.4), and C-10 (δ_C 115.1). HMBC correlations of H_2-14 (δ_H 2.83 and 2.96) to C-2 (δ_C 163.1), C-2' (δ_C 78.9), C-3 (δ_C 114.0), and C-3' (δ_C 42.3) and those of H_3-9' (δ_H 1.42) to C-14 (δ_C 42.9), C-2', and C-3' indicated connectivity of two units through C-14 and C-2'. Thus, cassiarin D (**2**) was concluded to be a unique dimeric compound consisting of 5-acetyl-7-hydroxy-2-methylchromone² and cassiarin C (**1**).

Cassiarin E (**3**), $[\alpha]_D^{20} -20$ (c 0.3, MeOH), had the molecular formula $C_{26}H_{22}N_2O_4$ (by HRESITOFMS). IR absorptions implied the presence of hydroxy and/or amino (3743 cm^{-1}) and carbonyl (1622 cm^{-1}) functionalities. The gross structure of **3** was elucidated by analyses of 2D NMR data including $^1\text{H}-^1\text{H}$ COSY, HOHAHA, HMQC, and HMBC spectra in pyridine- d_5 (Figure S3). Each pair of observed ^1H and ^{13}C NMR signals seemed to be due to each half moiety of a dimeric compound of cassiarin A.² Methylene signals (δ_H 2.79 and 2.90; δ_C 43.1) were observed in place of a methyl signal (C-12) in cassiarin A. Methylene signals (δ_H 3.38 and 3.52; δ_C 42.3) were observed as in the case of chrobisiamone A.³ The connection between the two units, C-9 to C-2', was provided by HMBC correlations of H_2-9 to C-2' (δ_C 79.1) and C-3'

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Table 1. ^1H NMR Data [δ_{H} (J , Hz)] and ^{13}C NMR Data [δ_{C}] for Cassiarin C (**1**) and 10,11-Dihydroanhydrobarakol (**4**) in CD_3OD at 300 K a

position	1		4	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2	4.49 (1H, m)	73.7		165.3
3a	3.06 (1H, dd, 16.8, 10.9)	37.8	6.04 (1H, d, 0.6)	110.0
3b	3.13 (1H, dd, 16.8, 3.2)			
4		153.6		160.2 b
4a		110.6		111.8
5		139.1		143.0
6	6.57 (1H, d, 2.0)	100.0	6.70 (1H, d, 2.5)	118.1
7		159.1		180.5
8	6.48 (1H, d, 2.0)	103.1	6.71 (1H, d, 2.5)	110.9
8a		156.9		161.6 b
9	1.56 (3H, d, 6.0)	19.9	2.34 (3H, d, 0.6)	18.4
10a	7.20 (1H, s)	115.6	3.05 (1H, dd, 12.4, 7.6)	43.9
10b			3.51 (1H, dd, 12.4, 4.4)	
11		149.9	4.00 (1H, m)	68.2
12	2.53 (3H, s)	22.1	1.22 (3H, d, 6.4)	22.2

 a δ in ppm. b Interchangeable.**Table 2.** ^1H NMR Data [δ_{H} (J , Hz)] and ^{13}C NMR Data [δ_{C}] for Cassiarins D and E (**2** and **3**) in Pyridine- d_5 at 300 K a

position	2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		163.1		158.0
3	6.24 (1H, s)	114.0	6.44 (1H, s)	110.0
4		178.4		150.7
4a				112.9
5		139.2		139.2
6	7.02 (1H, d, 1.5)	119.4	6.91 (1H, s)	101.6
7		162.9		162.2
8	7.04 (1H, d, 1.5)	102.7	6.91 (1H, s)	100.0
8a				155.9
9a		160.1	2.79 (1H, d, 14.2)	43.1
9b			2.90 (1H, d, 14.2)	
10		115.1	6.84 (1H, s)	113.3
11	4.37 (2H, s)	49.8		153.9
12		204.6	2.50 (3H, s)	24.6
13	2.37 (3H, s)	30.1		
14a	2.83 (1H, d, 14.2)	42.9		
14b	2.96 (1H, d, 14.2)			
2'		78.9		79.1
3'a	3.32 (1H, d, 16.6)	42.3	3.38 (1H, d, 16.6)	42.3
3'b	3.42 (1H, d, 16.6)		3.52 (1H, d, 16.6)	
4'		152.5		152.7
4'a		110.3		110.4
5'		139.0		139.1
6'	7.02 (1H, d, 1.0)	101.0	7.02 (1H, d, 2.0)	100.9
7'		162.5		162.6
8'	6.98 (1H, d, 1.0)	103.8	7.04 (1H, d, 2.0)	103.8
8'a		154.9		155.2
9'	1.42 (3H, s)	25.1	1.54 (3H, s)	25.2
10'	7.17 (1H, brs)	115.8	7.19 (1H, s)	115.7
11'		152.4		152.5
12'	2.61 (3H, s)	24.4	2.62 (3H, s)	24.5

 a δ in ppm.

(δ_{C} 42.3), and $\text{H}_3\text{-9}'$ (δ_{H} 1.54) to C-9 and C-3'. Thus, the gross structure of cassiarin E (**3**) was assigned to be a unique dimeric compound consisting of cassiarin A and cassiarin C (**1**).

Compound **4**, 10,11-dihydroanhydrobarakol, [α_{D}^{20} +47 (c 1.0, MeOH), had the molecular formula $\text{C}_{13}\text{H}_{12}\text{O}_3$, which was determined by HRESITOFMS. The IR spectrum implied the presence of a carbonyl group (1652 cm^{-1}). ^1H and ^{13}C NMR data are presented in Table 1. The ^{13}C NMR spectrum revealed 13 carbon signals due to one carbonyl carbon, five sp^2 quaternary carbons, three sp^2 methines, one sp^3 methine, one sp^3 methylene, and two methyl groups. Among them, four quaternary carbons (δ_{C} 160.2, 161.6, 165.3, and 180.5) and one sp^3 methine (δ_{C} 68.2) were

ascribed to those bearing an oxygen atom. Two partial structures, C-10 to C-12, and C-9 to C-2 and C-3, were deduced from ^1H - ^1H COSY analysis of **4** in CD_3OD . Connectivity of partial structures through an oxygen and C-4 atoms in the tricyclic skeleton was supported by HMBC correlations of H-3, H-6, H-8, and H-10 to C-4a (δ_{C} 111.8). Connection between partial structure and the dienone ring could be assigned by a HMBC correlation of H-10 to C-6 (δ_{C} 118.1). In addition, the presence of an ether linkage between C-2 (δ_{C} 165.3) and C-8a to form a pyran ring was also indicated.

A plausible biogenetic pathway for cassiarins C–E (**1**–**3**) and 10,11-dihydroanhydrobarakol (**4**) was proposed as shown in Scheme 1, Supporting Information. Cassiarin C (**1**) could be derived through an imine intermediate of 2,3-dihydro-5-acetyl-7-hydroxy-2-methylchromone followed by cyclization with the acetyl group as shown in Scheme 1, whereas 10,11-dihydroanhydrobarakol (**4**) might be produced by way of anhydrobarakol (**5**) from 5-acetyl-7-hydroxy-2-methylchromone (**6**). On the other hand, cassiarins D and E (**2** and **3**) could be derived by way of chrobisiamone A from 5-acetyl-7-hydroxy-2-methylchromone (**6**), as shown in Scheme 1. Treatment of cassiarin D (**2**) with ammonium acetate as the nitrogen source caused ring cyclization, giving cassiarin E (**3**) in 63% yield. This biomimetic transformation supports the biogenetic pathway proposed for cassiarins D and E.

Cassiarins C–E (**1**–**3**) and 10,11-dihydroanhydrobarakol (**4**) showed moderate in vitro antiplasmodial activity against *Plasmodium falciparum* 3D7 (IC $_{50}$ **1**: 24.2 μM ; **2**: 3.6 μM ; **3**: 7.3 μM ; **4**: 2.3 μM) and no cytotoxicity against HL-60 (human blood premyelocytic leukemia) cells (IC $_{50}$ >100 μM for **1**–**4**). Anhydrobarakol (**5**) 6 and 5-acetyl-7-hydroxy-2-methylchromone (**6**) 7 also showed moderate antiplasmodial activity (IC $_{50}$ **5**: 4.7 μM ; **6**: 8.6 μM) and no cytotoxicity against HL-60 cells (IC $_{50}$ >100 μM for **5** and **6**). Dimeric-type compounds such as cassiarins D (**2**) and E (**3**) were less active than cassiarin A (IC $_{50}$ 0.023 μM), although they contained the cassiarin A-type skeleton. 2 10,11-Dihydroanhydrobarakol (**4**) showed activity similar to that of anhydrobarakol (**5**).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. UV spectra were obtained on an Ultrospec 2100 pro spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. High-resolution ESIMS were obtained on a LTQ Orbitrap XL (Thermo Scientific). ^1H and 2D NMR spectra were recorded on Bruker AV 400 and Inova 500 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_{H} 3.31 and δ_{C} 49.0 for methanol- d_4 and δ_{H} 7.71 and δ_{C} 135.5 for pyridine- d_5). Standard pulse sequences were employed for the 2D NMR experiments. ^1H - ^1H COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY spectra in the phase-sensitive mode were measured with a mixing time of 800 ms. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long-range C–H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

Material. Flowers of *C. siamea* were collected at Purwodadi Botanical Garden in 2007. The botanical identification was made by Ms. Sri Wuryanti, Purwodadi Botanical Garden, Indonesia. A voucher specimen (no. PU080306) has been deposited at Purwodadi Botanical Garden, Pasuruan, Indonesia.

Extraction and Isolation. Dried flowers of *C. siamea* (1.0 kg) were extracted with MeOH, and the extract was partitioned between CHCl_3 and H_2O . CHCl_3 -soluble materials were subjected to a silica gel column ($\text{CHCl}_3/\text{MeOH}$, 1:0 \rightarrow 0:1) to afford cassiarin A (0.04%), 2 anhydrobarakol (0.006%), 6 and 5-acetyl-7-hydroxy-2-methylchromone (0.007%). 7 A fraction eluted by $\text{CHCl}_3/\text{MeOH}$ (30:1) was further separated on an ODS column ($\text{MeOH}/\text{H}_2\text{O}$, 0:1 \rightarrow 1:0) and on a silica

gel column (CHCl₃/MeOH, 1:0 → 0:1) to afford cassiarin C (**1**, 0.00007%). A fraction eluted by CHCl₃/MeOH (25:1) was further purified on a silica gel column (CHCl₃ saturated with NH₃/MeOH, 1:0 → 0:1) to afford 10,11-dihydroanhydrobarakol (**4**, 0.00050%), and a fraction eluted by CHCl₃/MeOH (20:1) was dissolved in MeOH and then centrifuged to afford cassiarin D (**2**, 0.0013%). The CHCl₃/MeOH (9:1)-soluble fraction was separated on a silica gel column (CHCl₃ saturated with NH₃/MeOH, 1:0 → 0:1) to afford cassiarin E (**3**, 0.00078%).

Cassiarin C (1): brown solid; $[\alpha]_D^{20}$ -70 (*c* 0.3, MeOH); IR (KBr) ν_{\max} 3401, 2924, and 1618 cm⁻¹; UV (MeOH) λ_{\max} 218 (ϵ 16 000), 243 (21 000), 289 (sh, 4300), and 330 (4000) nm (ϵ 13 000); ¹H and ¹³C NMR data (Tables 1 and 2); ESIMS *m/z* 216 (M + H)⁺; HRESITOFMS *m/z* 216.1025 [(M + H)⁺] (calcd for C₁₃H₁₄NO₂, 216.1008).

Cassiarin D (2): brown solid; $[\alpha]_D^{20}$ -10 (*c* 0.1, MeOH); IR (KBr) ν_{\max} 3673, 2992, 1713, 1632, and 1588 cm⁻¹; UV (MeOH) λ_{\max} 243 (ϵ 24 000), 298 (6000), and 330 (3000) nm (ϵ 2400); ¹H and ¹³C NMR, see Tables 1 and 2; ESIMS *m/z* 446 (M + H)⁺; HRESITOFMS *m/z* 446.1604 [(M + H)⁺] (calcd for C₂₆H₂₄NO₆, 446.1611).

Cassiarin E (3): brown solid; $[\alpha]_D^{20}$ -20 (*c* 0.3, MeOH); IR (KBr) ν_{\max} 3743, 2986, and 1622 cm⁻¹; UV (MeOH) λ_{\max} 219 (ϵ 10 000), 243 (11 600), and 338 (2400) nm; ¹H and ¹³C NMR, see Tables 1 and 2; ESIMS *m/z* 427 (M + H)⁺; HRESITOFMS *m/z* 427.1648 [(M + H)⁺] (calcd for C₂₆H₂₃N₂O₄, 427.1658).

10,11-Dihydroanhydrobarakol (4): brown solid; $[\alpha]_D^{20}$ +47 (*c* 1.0, MeOH); IR (KBr) ν_{\max} 2924, 1652, and 1589 cm⁻¹; UV (MeOH) λ_{\max} 210 (ϵ 11 200), 242 (8600), 250 (8500), and 290 (6000) nm; ¹H and ¹³C NMR, see Tables 1 and 2; ESIMS *m/z* 217 (M + H)⁺; HRESITOFMS *m/z* 217.0859 [(M + H)⁺] (calcd for C₁₃H₁₃O₃, 217.0862).

Conversion of Cassiarin D (2) to Cassiarin E (3). Ammonium acetate (1.0 mg) was added to a solution of cassiarin D (**2**, 1.0 mg) in AcOH (0.2 mL), and the mixture was kept at 60 °C for 24 h. After evaporation, the residue was applied to a silica gel column (CHCl₃/MeOH, 9:1) to give a compound (0.6 mg) whose spectroscopic data and $[\alpha]_D$ value were identical to those of natural cassiarin E (**3**).

Antiplasmodial Activity. Human malaria parasites were cultured according to the method of Trager and Jensen.⁸ The antimalarial activity of the isolated compounds was determined by the procedure described by Budimulya et al.⁹ In brief, stock solutions of the samples were prepared in DMSO (final DMSO concentrations of <0.5%) and were diluted to the required concentration with complete medium (RPMI 1640 supplemented with 10% human plasma, 25 mM HEPES, and 25 mM NaHCO₃) until the final concentrations of samples in culture plate wells were 10, 1, 0.1, 0.01, and 0.001 μg/mL. The malarial parasite *P.*

falciparum 3D7 clone was propagated in a 24-well culture plate. Growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa stain. The antimalarial activity of each compound was expressed as an IC₅₀ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

The percentage of growth inhibition was expressed according to the following equation: Growth inhibition % = 100 - [(test parasitemia/control parasitemia)] × 100. Chloroquine: IC₅₀ 0.011 μM.

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Supporting Information Available: Scheme 1: Proposed Biogenetic Pathway of Cassiarins C–E (**1–3**) and 10,11-Dihydroanhydrobarakol (**4**). ¹H and ¹³C NMR spectra and 2D NMR correlations of **1–4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

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