

Synthesis and structure-activity relationships of cassiarin A as potential antimalarials with vasorelaxant activity

by Wiwied Ekasari

Submission date: 17-May-2021 12:25PM (UTC+0800)

Submission ID: 1587639592

File name: C-19 - Bioorg Med Chem_naskah.pdf (475.31K)

Word count: 7178

Character count: 32222



Synthesis and structure–activity relationships of cassiarin A as potential antimalarials with vasorelaxant activity

Hiroshi Morita^{a,*}, Yuichiro Tomizawa^a, Jun Deguchi^a, Tokio Ishikawa^a, Hiroko Arai^a, Kazumasa Zaima^a, Takahiro Hosoya^a, Yusuke Hirasawa^a, Takayuki Matsumoto^a, Katsuo Kamata^a, Wiwied Ekasari^b, Aty Widawaruyanti^b, Tutik Sri Wahyuni^b, Noor Cholies Zaini^b, Toshio Honda^a

^a Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan

^b Faculty of Pharmacy, Airlangga University, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

ARTICLE INFO

Article history:

Received 21 September 2009

Revised 6 October 2009

Accepted 7 October 2009

Available online 13 October 2009

Keywords:

Cassiarin A

Cassia siamea

Plasmodium falciparum

Antimalarial activity

Vasorelaxant activity

Nitric oxide

SAR

ABSTRACT

Cassiarin A **1**, a tricyclic alkaloid, isolated from the leaves of *Cassia siamea* (Leguminosae), shows powerful antimalarial activity against *Plasmodium falciparum* in vitro as well as *P. berghei* in vivo, which may be valuable leads for novel antimalarials. Interactions of parasitized red blood cells (pRBCs) with endothelium in aorta are especially important in the processes contribute to the pathogenesis of severe malaria. Nitric oxide (NO) reduces endothelial expression of receptors/adhesion molecules used by pRBC to adhere to vascular endothelium, and reduces cytoadherence of pRBC to vascular endothelium. Cassiarin A **1** showed vasorelaxation activity against rat aortic ring, which may be related with NO production. A series of a hydroxyl and a nitrogen-substituted derivatives and a dehydroxy derivative of **1** have been synthesized as having potent antimalarials against *P. falciparum* with vasodilator activity, which may reduce cytoadherence of pRBC to vascular endothelium. Cassiarin A **1** exhibited a potent antimalarial activity and a high selectivity index in vitro, suggesting that the presence of a hydroxyl and a nitrogen atom without any substituents may be important to show antimalarial activity. Relative to cassiarin A, a methoxy derivative showed more potent vasorelaxant activity, although it did not show improvement for inhibition of *P. falciparum* in vitro. These cassiarin derivatives may be promising candidates as antimalarials with different mode of actions.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Malaria caused by *Plasmodium falciparum* is a major parasitic infection disease in the world and continues to cause morbidity and mortality on a large scale in tropical countries.¹ According to WHO, it is a threat to over 2 billion people living in areas of high incidence.² A major contributor to malarial morbidity and mortality is almost certainly the increasing resistance of malaria parasites to available drugs.³ Such a situation has heralded the need for alternative antiplasmodial therapy. Antimalarial potential of drugs derived from plants has been proven by examples such as quinine from *Cinchona* species and artemisinin from *Artemisia annua*.^{4,5}

Nitric oxide (NO), a highly diffusible cellular mediator involved in a wide range of biological effects, has been implicated as one of the agents to counteract malaria infection.⁶ NO and L-Arg (the substrate for NO synthase) reducing cytoadherence of pRBC to vascular endothelium are low in clinical severe malaria.⁷ Agents that can improve endothelial NO production and endothelial function may

have potential as adjunctive therapy early during the course of severe malaria.^{8,9}

Recently we have isolated two new chromone alkaloids, cassiarins A **1** and B **2** from the leaves of *Cassia siamea* (Leguminosae),¹⁰ which has been used in traditional Indonesian medicine for the treatment of fevers caused by malaria (Chart 1).¹¹ Cassiarin A **1** is a promising antimalarial drug, although mode of action of cassiarin A on *P. falciparum* has not been known. Since **1** shows powerful in vitro antimalarial activity against *P. falciparum* and in vivo activity against mouse malaria, *P. berghei*,¹² the potent antimalarial activity of **1** has stimulated medicinal chemists to pursue deriva-

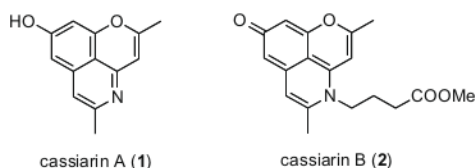


Chart 1. Structures of cassiarins A (**1**) and B (**2**).

* Corresponding author. Tel./fax: +81 3 5498 5778.

E-mail address: moritah@hoshi.ac.jp (H. Morita).

tives of **1**, which may provide valuable leads for novel drugs. In addition, we evaluated vasorelaxant activity against rat aortic rings whether **1** possessed potential to improve endothelial NO production. In this article, we report the syntheses of the cassiarin A derivatives and evaluate their in vitro antimalarial activity as well as vasorelaxant activity against rat aortic rings.

2. Results and discussion

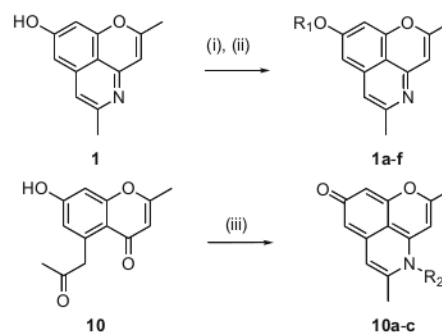
2.1. Chemistry

As outlined in Scheme 1, cassiarin A **1** was prepared in eight steps through 5-acetyl-7-hydroxy-2-methylchromone **10** from **3** by modified biomimetic methods as described previously.¹³ Under the literature conditions,¹⁴ 5,7-dihydroxy-2-methyl-4H-chromen-4-one **5** was prepared in a large scale from the commercially available material, 2,4,6-trihydroxyacetophenone **3**. With assistance of the intramolecular H-bonding between C-5 hydroxyl and C-4 carbonyl, selective protection of C-7 hydroxyl group of chromone **5** was achieved using MOMCl and DIPEA in CH₂Cl₂ to give MOM ether **6** in 83% yield. Then, treatment of chromone **6** with PhN(Tf)₂ in the presence of NaH at 0 °C gave the corresponding triflate **7** in 97% yield. Sonogashira coupling¹⁵ of triflate **7** with in situ generated propyne gave alkyne **8** in 85% yield.^{10c} Conversion from **8** into 5-acetyl-7-hydroxy-2-methylchromone **10**, which is a potential biogenetic precursor for cassiarins, was carried out by treatment with a catalytic amount of AgNO₃ and 3 equiv of TFA in 61% yield, and then acidic deprotection of MOM ether **9** in 96% yield. Cassiarin A **1** was easily prepared in good yield (91%) by treating the chromone **10** with AcONH₄ in AcOH.^{10b}

We prepared ester derivatives **1a–c** and ether derivatives **1d–f** from **1** in good yields by use of appropriate acid chloride and alkyl iodide or bromide, respectively (Scheme 2). The nitrogen-substituted derivatives **10a–c** were synthesized from 5-acetyl-7-hydroxy-2-methylchromone **10** by appropriate amines in AcOH. Dehydroxy derivative **11** was synthesized by way of trifluoromethanesulfonate **1g** (Scheme 3), followed by reductive deoxygenation with TES in the presence of Pd(PPh₃)₂Cl₂.

2.2. Antimalarial activity in vitro

All compounds synthesized were tested for their antiparasitic activity on the chloroquine (CQ)-sensitive *P. falciparum* strain 3D7. In parallel, cytotoxicity of the compounds was determined on human MCF7 cell line. The results are expressed as IC₅₀ values representing the drug concentration required to inhibit the growth of parasites and human cells with selectivity index, SI (IC₅₀ for MCF7)/(IC₅₀ for 3D7) (Table 1). Among the ester and ether



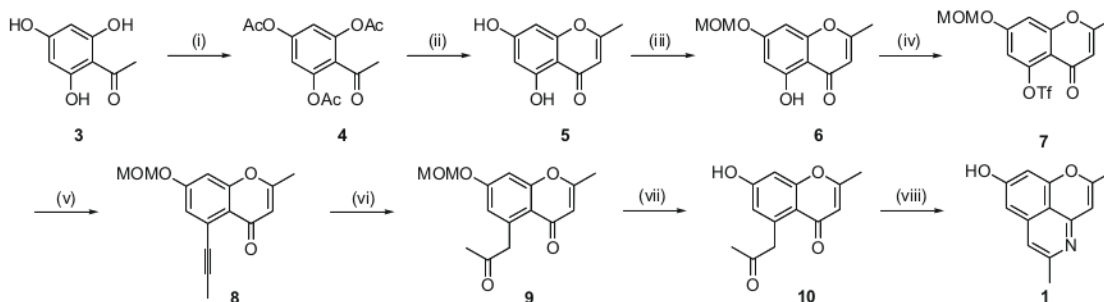
Scheme 2. Synthesis of **1a–f**. Reagents and conditions: (i) appropriate acid chloride (or Ac₂O), pyridine, CH₂Cl₂; (ii) appropriate alkyl iodide (or bromide), K₂CO₃, acetone, reflux; (iii) appropriate amine, AcOH, reflux.

derivatives at C-7 (Table 1), the acetyl and methyl ether derivatives **1a** and **1d** exhibited relatively potent antimalarial effect with high selectivity index. There was a tendency that the ester derivatives showed more potent than the ether ones. Derivatives with bulky substituents such as benzoyl and benzyl showed less effective against *P. falciparum* 3D7. However, the excellent activity was not consistent with the activity of a dehydroxy derivative **11**. The design of nitrogen-substituted derivatives **10a–c** built in this study and cassiarin B **2**, did not afford improved antimalarial effects with respect to the parent cassiarin A (Table 1). Interestingly the N-Ph derivative **10c** still showed potent antimalarial activity and good selectivity index. Significant antimalarial effect for the parent cassiarin A **1**, diminished depending on size of the substitution both of the O-alkyl (R₁), O-acyl (R₁), and N-alkyl (R₂) side chain.

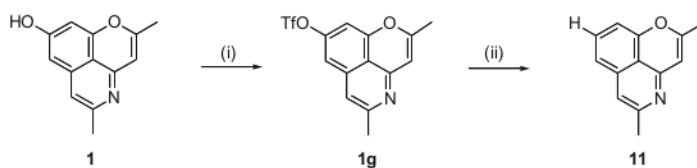
2.3. Vasorelaxant activity in ex vivo

It has been shown that the endothelium plays an important role in controlling vascular tone by releasing NO,¹⁶ which is widely known to inhibit platelet and leukocyte adhesion to endothelium through its regulatory effect on adhesion molecule expression.⁷ Recently, NO has been reported to be protective against *P. falciparum* infection by inhibiting cytoadherence, and emphasize the therapeutic potential of NO in the treatment of severe malaria.^{7–9} The present widely used artemisin derivatives have vascular effects.¹⁷ Artemisin causes relaxation of precontracted rat aortic rings which is partly mediated by NO.¹⁷

When phenylephrine (PE, 3 × 10⁻⁷ M) was applied to thoracic aortic rings with endothelium after achieving a maximal response, cassiarin A **1** showed vasorelaxant actions at 3 × 10⁻⁵ M and



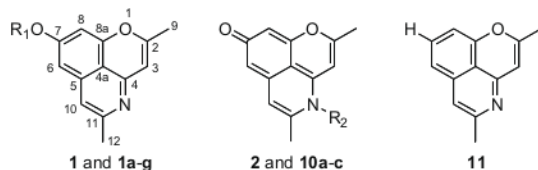
Scheme 1. Synthesis of **1** and **10**. Reagents and conditions: (i) acetic anhydride, pyridine; (ii) LiH, THF, reflux, then aq HCl 0 °C, and aq Na₂CO₃, reflux; (iii) chloromethyl methyl ether, DIPEA; (iv) PhN(Tf)₂, NaH; (v) 1-bromo-1-propyne, *n*-BuLi, then Pd(PPh₃)₂Cl₂, CuI, THF-*i*-Pr₂NH; (vi) AgNO₃, TFA, ClCH₂CH₂Cl, –24 °C, then aq NaOAc, reflux; (vii) 2 N aq HCl, MeOH, reflux; (viii) AcONH₄, AcOH, reflux.



Scheme 3. Synthesis of **1g** and **11**. Reagents and conditions: (i) Tf₂O, pyridine, CH₂Cl₂, rt; (ii) TES, Pd(PPh₃)₂Cl₂, DMF, reflux.

Table 1

Antimalarial activity of cassiarin derivatives **1**, **2**, **1a–g**, **10a–c**, and **11**



Compd	R ₁ or R ₂	IC ₅₀ 3D7 ^a (μM)	IC ₅₀ MCF7 ^b (μM)	SI ^c
CQ		0.011	36.1	3281
1	H	0.023	>100	>4348
1a	COMe	1.2	>100	>83
1b	COPr	4.1	>100	>24
1c	COPh	1.2	>100	>83
1d	Me	3.6	87.8	>24
1e	Bu	3.0	76.7	>26
1f	Bn	14.9	72.2	4.8
1g	Tf	0.41	>100	>244
2	(CH ₂) ₃ -COOMe	22.0	>100	>4.5
10a	Me	23.3	>100	>4.2
10b	Bu	4.2	>100	>23
10c	Ph	0.090	>100	>1112
11		5.4	>100	>18

^a In this assay, 3D7 is CQ-sensitive *Plasmodium falciparum* strain. The standard drug chloroquine (CQ) served as positive control for CQ-sensitive *P. falciparum* 3D7 strain.

^b The cytotoxicity is evaluated against MCF7 cells. Vincristine exhibited an IC₅₀ value of 2.4 μM against the human MCF7 cell line.

^c SI is selectivity index (IC₅₀ MCF7/IC₅₀ 3D7).

1×10^{-5} M (Fig. 1), whereas cassiarin B **2** was found to have no vasorelaxant effect. All compounds synthesized were tested for vasorelaxant activity against rat aorta (Fig. 2). Derivatives with bulky substituents such as benzoyl **1c**, butyl **1e**, and benzyl **1f** were found to be less potent than **1**, although those with smaller substituents, such as acetyl **1a** and propionyl **1b**, still showed potent vasodilator effect. Methyl ether derivative **1d** showed more potent vasorelaxant effect than **1**. However, the excellent activity could not be observed for a dehydroxy derivative **11**. The nitrogen-substituted derivatives **10a–c** including **2** did not show vasorelaxant activity (Fig. 2). There has been shown to be slightly different tendency as antimalarial activity.

The activity of **1d**-induced vasorelaxation was observed in a concentration-dependent manner (Fig. 3). The vasorelaxant effects of **1** and **1d** were attenuated by endothelium removal or pretreatment with a NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA, 10⁻⁴ M).¹⁸ Our results suggested that the vasorelaxant effect was attributed to its actions on the endothelial cells to release NO.

In conclusion, we synthesized and evaluated a new series of cassiarin derivatives. Among them, natural cassiarin A **1** exhibited potent antimalarial activity and a high selectivity index against cytotoxicity of human cells in vitro. In addition, we found **1** and methyl ether derivative **1d** showed vasorelaxant activity against rat aorta. Further studies on **1**, such as mode of action mechanisms

about antimalarial and vasorelaxant activities which may be mediated by NO production from endothelium, are necessary to develop a novel antimalarial drug with controlling vascular tone by releasing NO, which may inhibit cytoadherence of *P. falciparum* infection.

3. Experimental section

3.1. General experimental procedures

IR spectra on a JASCO FTIR-230 spectrometer. Mass spectra were obtained with a Micromass LCT spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AV 400 spectrometer and chemical shifts were referenced to the residual solvent peaks (δ_{H} 3.31 and δ_{C} 49.0 for methanol-*d*₄). Analytical HPLC was carried out on with a Shimadzu CLASS-M10A Series HPLC with a photodiode array detector. Separations were done with a 5 μm, 4.6 mm × 250 mm, column (Waters, SunFire), (method A): flow rate 0.5 mL/min, 50% MeOH with 0.1% TFA; (method B): 60% MeOH with 0.1% TFA. The purity of derivatives by HPLC (methods A or B) was higher than 95%.

3.2. Synthesis

3.2.1. 5,7-Dihydroxy-2-methyl-4H-chromen-4-one (5)

To a stirred solution of **3** (3.0 g, 17.9 mmol) in pyridine (6 mL) at room temperature was added Ac₂O (7.6 mL, 68.8 mmol) and the reaction mixture was stirred for 4 h. The reaction was quenched by addition of satd aq NaHCO₃ and then extracted with ethyl acetate. The combined organic phases were successively washed with 1 M HCl and dried over anhydrous Na₂SO₄, and concentrated in vacuo to give a crude product of **4**.

After working up, a solution of crude product of **4** (6.3 g) in THF (150 mL) was treated with LiH (650 mg, 81.3 mmol) and the mixture was stirred at 60 °C for 4 h. To this, aq HCl was added dropwise at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was adjusted to pH 10 with satd aq Na₂CO₃ and stirred for a further 2 h at reflux. The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. Purification with column chromatography (toluene/ethyl acetate = 1/1) gave a pale yellow solid (**5**, 2.3 g, 67% from **3**). Spectroscopic data were corresponding to those of the article.¹³

3.2.2. 5-Hydroxy-7-(methoxymethoxy)-2-methyl-4H-chromen-4-one (6)

To a stirred solution of 5,7-dihydroxy-2-methyl-chromen-4-one (**5**, 920 mg, 4.79 mmol) in CH₂Cl₂ (15 mL) was added DIPEA (1.0 mL, 5.74 mmol) at 0 °C under Ar. After the solution was stirred for 10 min, MOMCl (0.47 mL, 4.95 mmol) was added at the same temperature. The reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. The reaction was quenched with satd aq NaHCO₃ and then extracted with ethyl acetate. The combined organic phases were washed with 1 M HCl, water, and

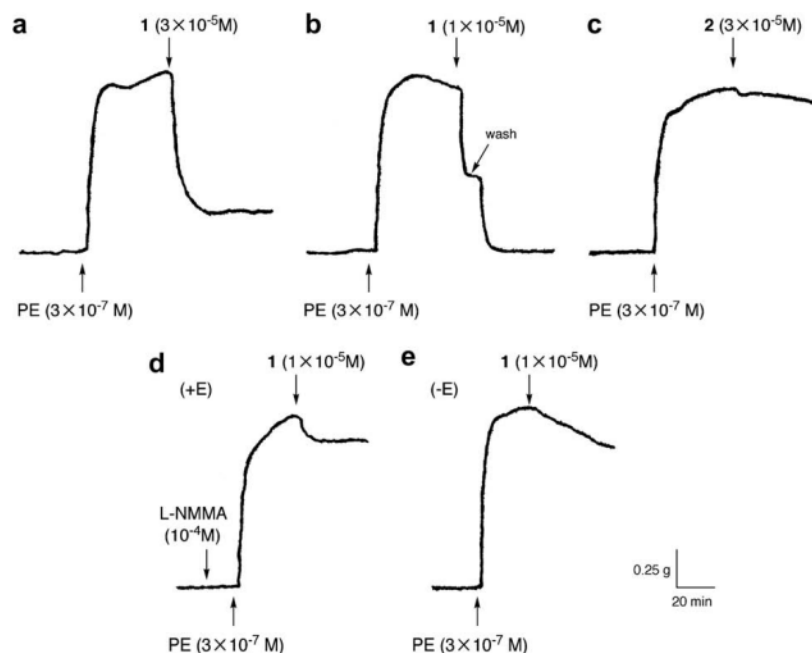


Figure 1. Typical recording of the vasorelaxation effects of **1** and **2** on the rat aortic rings precontracted with 3×10^{-7} M PE: (a) and (b) for cassiarin A **1** (3×10^{-5} M and 1×10^{-5} M, respectively). (c) For cassiarin B **2** (3×10^{-5} M). (d) Pretreatment with L-NMMA with endothelium (+E). (e) Without endothelium (-E).

brine, dried over anhydrous Na_2SO_4 , and concentrated in vacuo. Purification with column chromatography (hexane/ethyl acetate = 3/1) gave a pale yellow solid (**6**, 1.0 g, 83%). Spectroscopic data were corresponding to those of the article.¹³

3.2.3. 7-(Methoxymethoxy)-2-methyl-4-oxo-4H-chromen-5-yl trifluoromethanesulfonate (**7**)

To a stirred solution of **6** (400 mg, 1.70 mmol) in THF (10 mL) was added NaH (60%, 120 mg, 2.54 mmol) at 0 °C under Ar. The suspended yellow solution was stirring for 10 min, and then a solution of PhNTf_2 (910 mg, 2.54 mmol) in THF (2 mL) was added. The resulting mixture was allowed to warm to room temperature after

it became clear. After being stirred for an additional 1 h, the reaction was quenched with satd aq NH_4Cl . THF was evaporated, and the residue was diluted with water and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated. Purification with column chromatography (hexane/ethyl acetate = 3/1) gave a pale yellow solid (**7**, 610 mg, 97%). Spectroscopic data and corresponding to those of the article.¹³

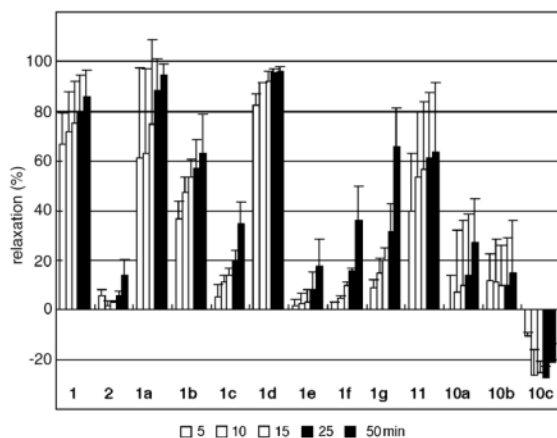


Figure 2. Vasorelaxation effects of **1**, **2**, **1a-g**, **11**, and **10a-c** (3×10^{-5} M) on the rat aortic rings precontracted with 3×10^{-7} M PE. Values are the mean \pm SE ($n = 3$).

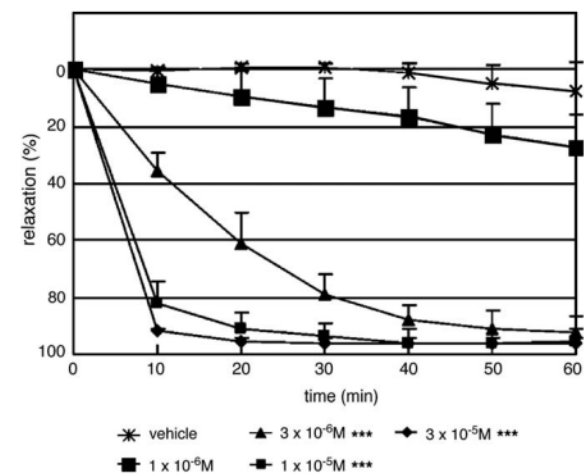


Figure 3. Time course and dose dependency of vasorelaxation effects of **1d** on the rat aortic rings precontracted with 3×10^{-7} M PE. Values are the mean \pm SE ($n = 3$). Each relaxation response is expressed as a percentage of the contraction induced by PE. *** $P < 0.001$ versus vehicle.

3.2.4. 7-(Methoxymethoxy)-2-methyl-5-(prop-1-ynyl)-4H-chromen-4-one (8)

1-Bromo-1-propene (1.05 mL, 12.2 mmol) was dissolved in THF (18 mL). After cooling to -78°C , to the solution was added *n*-BuLi (1.5 M in hexane, 10.9 mL, 16.3 mmol). The resulting mixture was stirred at -78°C for 1 h. Water (0.3 mL, 16.3 mmol) was added, and the temperature was allowed to rise to 0°C where the mixture was further stirred for 30 min. To the mixture was added a solution of triflate **7** (1.5 g, 4.08 mmol) in THF (12 mL), Pd(PPh₃)₂Cl₂ (143 mg, 0.20 mmol), CuI (77.5 mg, 0.408 mmol), and *i*-Pr₂NH (12 mL). The resulting mixture was stirred at room temperature for 2.5 h. The reaction was quenched by addition of satd aq NH₄Cl. After separation, the water layer was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄, and concentrated. Purification with column chromatography (hexane/ethyl acetate = 3/1) gave a pale yellow solid (**8**, 900 mg, 85%). Spectroscopic data were corresponding to those of the article.¹³

3.2.5. 7-(Methoxymethoxy)-2-methyl-5-(2-oxopropyl)-4H-chromen-4-one (9)

To a solution of **8** (50 mg, 0.19 mmol) and AgNO₃ (1.5 mg, 5 mol %) in (CH₂Cl)₂ (7.5 mL) was added dropwise trifluoroacetic acid (TFA) (45 μL , 0.56 mmol) at -24°C under Ar. The orange solution was stirred for 1 h at this temperature, and then allowed to warm up. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated. Purification with column chromatography (hexane/ethyl acetate = 3/2) gave a pale yellow solid (**9**, 33 mg, 61%). Spectroscopic data were corresponding to those of the article.¹³

3.2.6. 7-Hydroxy-2-methyl-5-(2-oxopropyl)-4H-chromen-4-one (10)

A mixture of **9** (110 mg, 0.24 mmol) and 2 N aq HCl (0.5 mL) in MeOH (10 mL) was refluxed for 4 h. MeOH was removed and water was added. The mixture was extracted with ethyl acetate. The organic layer were washed with brine, dried over Na₂SO₄, and concentrated. Purification with flash chromatography (CHCl₃/MeOH = 19/1) gave a pale yellow solid (**10**, 89 mg, 96%). Spectroscopic data were corresponding to those of the article.¹³

3.2.7. Cassiarin A (1)

To a stirred solution of **10** (10 mg, 0.036 mmol) in AcOH (2 mL) at room temperature was added AcONH₄ (30 mg) and the reaction mixture was stirred for 48 h at reflux. The solvent was removed and satd aq NaHCO₃ was added. The mixture was extracted with CHCl₃. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. Purification with flash chromatography (CHCl₃/MeOH = 9/1) gave a pale yellow solid (**1**, 8.4 mg, 91%). Spectroscopic data were corresponding to those of the article.^{10a}

3.2.8. Acetylcassiariin A (2,5-dimethylpyrano[2,3,4-*ij*]isoquinolin-8-yl acetate) (1a)

To a stirred solution of **1** (2.0 mg, 9.39 μmol) in pyridine (100 μL) at room temperature was added Ac₂O (100 μL) and the reaction mixture was stirred for 4 h. The reaction was quenched by addition of satd aq NaHCO₃ and then extracted with CHCl₃. The combined organic phases were dried over anhydrous Na₂SO₄, and concentrated in vacuo. Purification with column chromatography (CHCl₃/MeOH = 9/1) gave a pale yellow solid (**1a**, 2.1 mg, 87%). IR (neat) 1572, 1621, 1659, 1762, 2871, 2936, 2970 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 2.27 (3H, d, *J* = 0.7 Hz), 2.32 (3H, s), 2.45 (3H, d, *J* = 0.5 Hz), 6.22 (1H, d, *J* = 0.7 Hz), 6.84 (1H, d, *J* = 1.9 Hz), 6.70

(1H, d, *J* = 1.9 Hz), 7.05 (1H, br s); ¹³C NMR (400 MHz, CD₃OD) δ 19.9, 21.0, 23.4, 105.2, 105.5, 110.8, 115.3, 115.9, 139.2, 152.4, 153.0, 155.4, 156.3, 163.0, 170.4; HRESIMS *m/z* 214.0865 [calcd for C₁₃H₁₂O₂N (M–Ac+H)⁺, 214.0863].

3.2.9. Butyroylcassiariin A (2,5-dimethylpyrano[2,3,4-*ij*]isoquinolin-8-yl butyrate) (1b)

To a stirred solution of **1** (5.0 mg, 23 μmol) in DCM (200 μL) and pyridine (100 μL) at 0°C was added butyryl chloride (12 μL) and the solution was allowed to warm to room temperature. The reaction mixture was stirred for 1.5 h. The reaction was quenched by addition of satd aq NaHCO₃ and then extracted with CHCl₃. The combined organic phases were dried over anhydrous Na₂SO₄, and concentrated in vacuo. Purification with column chromatography (CHCl₃/MeOH = 49/1) gave a pale yellow solid (**1b**, 6.1 mg, 97%). IR (neat) 1573, 1622, 1659, 1762, 2871, 2936, 2970 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 1.06 (3H, dd, *J* = 7.3, 7.3 Hz), 1.78 (3H, m), 2.25 (3H, d, *J* = 0.7 Hz), 2.43 (3H, d, *J* = 0.4 Hz), 2.60 (2H, dd, *J* = 7.3, 7.3 Hz), 6.17 (1H, d, *J* = 0.7 Hz), 6.77 (1H, d, *J* = 1.9 Hz), 6.94 (1H, d, *J* = 1.9 Hz), 7.01 (1H, s); ¹³C NMR (400 MHz, CD₃OD) δ 13.9, 19.3, 19.9, 23.7, 36.9, 104.9, 105.9, 110.6, 115.2, 116.0, 139.2, 152.6, 153.8, 155.2, 156.3, 162.3, 173.1; ESIMS 284 (M+H)⁺, HRESIMS *m/z* 284.1281 [calcd for C₁₇H₁₈O₃N (M+H)⁺, 284.1281].

3.2.10. Benzoylcassiariin A (2,5-dimethylpyrano[2,3,4-*ij*]isoquinolin-8-yl benzoate) (1c)

To a stirred solution of **1** (10.0 mg, 46 μmol) in pyridine (500 μL) at 0°C was added benzoyl chloride (27 μL) and the solution was allowed to warm to room temperature. The reaction mixture was stirred for 1 h. The reaction was quenched by addition of satd aq NaHCO₃ and then extracted with CHCl₃. The combined organic phases were dried over anhydrous Na₂SO₄, and concentrated in vacuo. Purification with column chromatography (hexane/ethyl acetate = 1/2) gave a pale yellow solid (**1c**, 14.0 mg, 94%). IR (neat) 1573, 1620, 1657, 2921, 2953, 3063 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 2.22 (3H, d, *J* = 0.8 Hz), 2.42 (3H, d, *J* = 0.4 Hz), 6.14 (1H, d, *J* = 0.8 Hz), 6.86 (1H, d, *J* = 1.9 Hz), 6.97 (1H, s), 7.04 (1H, d, *J* = 1.9 Hz), 7.54 (1H, dd, *J* = 8.0, 8.0 Hz), 7.69 (1H, m), 8.16 (2H, dd, *J* = 8.0, 8.0 Hz); ¹³C NMR (400 MHz, CD₃OD) δ 19.9, 23.8, 104.9, 106.0, 110.6, 115.2, 116.0, 129.9, 129.9, 130.4, 131.2, 131.2, 135.1, 139.2, 152.5, 153.8, 155.2, 156.3, 162.2, 166.0; ESIMS 318 (M+H)⁺, HRESIMS *m/z* 318.1125 [calcd for C₂₀H₁₆O₃N (M+H)⁺, 318.1125].

3.2.11. Cassiariin A methyl ether (8-methoxy-2,5-dimethylpyrano[2,3,4-*ij*]isoquinoline) (1d)

To a stirred solution of **1** (4.5 mg, 21 μmol) in acetone (2 mL) at room temperature were added K₂CO₃ (30 mg) and MeI (10 μL) and the reaction mixture was refluxed for 5 h. The solvent was removed and the crude products was purified by flash chromatography (CHCl₃/MeOH = 9/1) gave a pale yellow solid (**1d**, 2.7 mg, 56%). IR (neat) 1575, 1624, 1663, 2849, 2925 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 2.21 (3H, d, *J* = 0.8 Hz), 2.41 (3H, s), 3.88 (3H, s), 6.09 (1H, d, *J* = 0.8 Hz), 6.57 (1H, d, *J* = 2.1 Hz), 6.67 (1H, d, *J* = 2.1 Hz), 6.94 (1H, s); ¹³C NMR (400 MHz, CD₃OD) δ 18.5, 22.1, 54.8, 98.2, 98.2, 103.8, 112.0, 113.5, 138.4, 150.5, 155.0, 160.3, 163.1; HRESIMS *m/z* 228.1023 [calcd for C₁₄H₁₄O₂N (M+H)⁺, 228.1019].

3.2.12. Cassiariin A butyl ether (8-butoxy-2,5-dimethylpyrano[2,3,4-*ij*]isoquinoline) (1e)

To a stirred solution of **1** (10.0 mg, 46 μmol) in acetone (1.5 mL) at room temperature were added K₂CO₃ (32 mg) and 1-iodobutane (27 μL) and the reaction mixture was refluxed for 24 h. The solvent was removed and the crude product was purified by flash chromatography (CHCl₃/MeOH = 9/1) to give a pale yellow solid (**1e**, 9.3 mg, 67%). IR (neat) 1575, 1623, 1659, 2871, 2936, 2958 cm⁻¹;

^1H NMR (400 MHz, CD_3OD) δ 1.01 (3H, dd, $J = 7.3, 7.3$ Hz), 1.50 (2H, m), 1.78 (2H, m), 2.19 (3H, d, $J = 0.8$ Hz), 2.38 (3H, s), 4.02 (2H, dd, $J = 6.4, 6.4$ Hz), 6.03 (1H, d, $J = 0.8$ Hz), 6.50 (1H, d, $J = 2.1$ Hz), 6.58 (1H, d, $J = 2.1$ Hz), 6.85 (1H, s); ^{13}C NMR (400 MHz, CD_3OD) δ 14.2, 19.9, 20.3, 23.8, 32.4, 69.2, 100.1, 100.1, 105.5, 113.5, 114.8, 140.0, 152.1, 153.3, 156.5, 161.4, 163.9; ESIMS 270 (M+H) $^+$. HRESIMS m/z 270.1490 [calcd for $\text{C}_{17}\text{H}_{20}\text{O}_2\text{N}$ (M+H) $^+$, 270.1489].

3.2.13. Cassiarin A benzyl ether (8-(benzyloxy)-2,5-dimethylpyrano[2,3,4-ij]isoquinoline) (1f)

To a stirred solution of **1** (10.0 mg, 46 μmol) in acetone (2.0 mL) at room temperature were added K_2CO_3 (40 mg) and BnBr (34 μL) and the reaction mixture was refluxed for 24 h. The solvent was removed and the crude products was purified by flash chromatography ($\text{CHCl}_3/\text{MeOH} = 18/1$) to give a pale yellow solid (**1f**, 9.1 mg, 64%). IR (neat) 1573, 1622, 1659, 2925, 3037, 3067 cm^{-1} ; ^1H NMR (400 MHz, CD_3OD) δ 2.19 (3H, s), 2.39 (3H, s), 5.13 (2H, s), 6.06 (1H, s), 6.61 (1H, d, $J = 2.1$ Hz), 6.71 (1H, d, $J = 2.1$ Hz), 6.88 (1H, s), 7.38 (5H, m); ^{13}C NMR (400 MHz, CD_3OD) δ 19.9, 23.8, 71.4, 100.4, 100.8, 105.6, 113.7, 114.9, 128.7, 128.7, 129.1, 129.6, 129.6, 138.0, 140.0, 152.2, 153.6, 156.7, 161.5, 163.5; HRESIMS m/z 304.1336 [calcd for $\text{C}_{20}\text{H}_{18}\text{O}_2\text{N}$ (M+H) $^+$, 304.1332].

3.2.14. 2,4,5-Trimethylpyrano[2,3,4-ij]isoquinolin-8-(4H)-one (10a)

To a stirred solution of **10** (5.0 mg, 22 μmol) in AcOH (1.0 mL) at room temperature was added aq 40% $\text{MeNH}_2\text{-MeOH}$ (100 μL) and the resulting mixture was heated at reflux for 1.5 h. The reaction was quenched by addition of satd aq NaHCO_3 and then extracted with CHCl_3 . The combined organic phases were dried over anhydrous Na_2SO_4 , and concentrated in vacuo. Purification with column chromatography ($\text{CHCl}_3/\text{MeOH} = 8/2$) gave a pale yellow solid (**10a**, 3.5 mg, 72%). IR (neat) 1443, 1593, 1658 cm^{-1} ; ^1H NMR (400 MHz, CD_3OD) δ 2.38 (3H, s), 2.43 (3H, d, $J = 0.5$ Hz), 3.61 (3H, s), 6.31 (1H, d, $J = 2.0$ Hz), 6.44 (1H, d, $J = 2.0$ Hz), 6.47 (1H, br s), 6.74 (1H, br s); ^{13}C NMR (400 MHz, CD_3OD) δ 20.6, 20.7, 35.9, 97.4, 105.7, 108.1, 108.5, 116.0, 136.8, 142.4, 148.6, 156.9, 167.4, 177.9; HRESIMS m/z 228.1022 [calcd for $\text{C}_{14}\text{H}_{14}\text{O}_2\text{N}$ (M+H) $^+$, 228.1019].

3.2.15. 4-Butyl-2,5-dimethylpyrano[2,3,4-ij]isoquinolin-8-(4H)-one (10b)

To a stirred solution of **10** (5.0 mg, 22 μmol) in AcOH (1.0 mL) at room temperature was added *n*-butylamine (100 μL) and the resulting mixture was heated at reflux for 24 h. The reaction was quenched by addition of satd aq NaHCO_3 and then extracted with CHCl_3 . The combined organic phases were dried over anhydrous Na_2SO_4 , and concentrated in vacuo. Purification with column chromatography ($\text{CHCl}_3/\text{MeOH} = 5/1$) gave a pale yellow solid (**10b**, 3.5 mg, 60%). IR (neat) 1354, 1467, 1595, 1653, 2872, 2932, 2958 cm^{-1} ; ^1H NMR (400 MHz, CD_3OD) δ 1.06 (3H, dd, $J = 7.3, 7.3$ Hz), 1.51 (2H, m), 1.72 (2H, m), 2.41 (3H, s), 2.50 (3H, s), 4.09 (2H, dd, $J = 8.3, 8.3$ Hz), 6.42 (1H, d, $J = 2.0$ Hz), 6.53 (1H, s), 6.54 (1H, d, $J = 2.0$ Hz), 6.84 (1H, s); ^{13}C NMR (400 MHz, CD_3OD) δ 12.6, 18.7, 19.2, 19.3, 30.1, 48.6, 96.0, 104.3, 107.1, 107.9, 115.8, 135.7, 140.7, 147.4, 155.9, 166.7, 175.2; ESIMS 270 (M+H) $^+$. HRESIMS m/z 270.1488 [calcd for $\text{C}_{17}\text{H}_{20}\text{O}_2\text{N}$ (M+H) $^+$, 270.1489].

3.2.16. 2,5-Dimethyl-4-phenylpyrano[2,3,4-ij]isoquinolin-8-(4H)-one (10c)

To a stirred solution of **10** (5.0 mg, 22 μmol) in AcOH (1.0 mL) at room temperature was added PhNH_2 (100 μL) and the resulting mixture was heated at reflux for 5 h. The reaction was quenched by addition of satd aq NaHCO_3 and then extracted with CHCl_3 . The combined organic phases were dried over anhydrous Na_2SO_4 , and concentrated in vacuo. Purification with column chromatography ($\text{CHCl}_3/\text{MeOH} = 5/1$) gave a pale yellow solid (**10c**, 3.5 mg, 56%). IR (neat) 1478, 1545, 1597, 1656, 2924, 3059 cm^{-1} ; ^1H

NMR (400 MHz, CD_3OD) δ 2.07 (3H, d, $J = 0.5$ Hz), 2.27 (3H, s), 5.59 (1H, s), 6.64 (1H, d, $J = 1.9$ Hz), 6.69 (1H, d, $J = 1.9$ Hz), 7.04 (1H, d, $J = 0.5$ Hz), 7.48 (2H, m), 7.73 (3H, m); ^{13}C NMR (400 MHz, CD_3OD) δ 20.6, 21.0, 98.5, 105.5, 109.0, 116.0, 129.0, 129.0, 132.0, 132.2, 137.5, 138.6, 142.4, 150.0, 157.9, 168.4, 176.8; HRESIMS m/z 290.1179 [calcd for $\text{C}_{19}\text{H}_{16}\text{O}_2\text{N}$ (M+H) $^+$, 290.1176].

3.2.17. 2,5-Dimethylpyrano[2,3,4-ij]isoquinolin-8-yl trifluoromethanesulfonate (1g)

To a stirred solution of **1** (7.8 mg, 37 μmol) in pyridine (120 μL) at 0 $^\circ\text{C}$ was added TF_2O (25 μL) and the reaction mixture was stirred for 3 h. The reaction was quenched by addition of satd aq NaHCO_3 and then extracted with CHCl_3 . The combined organic phases were dried over anhydrous Na_2SO_4 , and concentrated in vacuo. Purification with column chromatography (hexane/ethyl acetate = 1/1) gave a pale yellow solid (**1g**, 9.2 mg, 73%). IR (neat) 1571, 1617, 1659, 2921, 2958, 3093 cm^{-1} ; ^1H NMR (400 MHz, CD_3OD) δ 2.28 (3H, s), 2.47 (3H, s), 6.23 (1H, s), 6.94 (1H, d, $J = 2.1$ Hz), 7.11 (1H, s), 7.18 (1H, d, $J = 2.1$ Hz); ^{13}C NMR (400 MHz, CD_3OD) δ 19.8, 24.0, 103.4, 105.6, 110.3, 115.2, 116.8, 118.5, 139.1, 152.3, 152.5, 155.6, 162.3; HRESIMS m/z 346.0358 [calcd for $\text{C}_{14}\text{H}_{11}\text{O}_4\text{NF}_3\text{S}$ (M+H) $^+$, 346.0355].

3.2.18. 7-Dehydroxycassiariin A (2,5-dimethylpyrano[2,3,4-ij]-isoquinoline) (11)

To a solution of **1g** (21 mg, 61 μmol) and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (4.2 mg) in DMF (1.0 mL) at room temperature was added TES (140 mL) and the resulting mixture was stirred at reflux under Ar for 30 min. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a crude product, which was purified by flash chromatography (hexane/toluene = 2/1) to give a pale yellow solid **11** (12 mg, 99%). IR (neat) 1573, 1619, 1655, 2857, 2921, 3060 cm^{-1} ; ^1H NMR (400 MHz, CD_3OD) δ 2.24 (3H, s), 2.43 (3H, s), 6.15 (1H, s), 6.98 (1H, d, $J = 8.2$ Hz), 7.02 (1H, s), 7.20 (1H, d, $J = 8.2$ Hz), 7.54 (1H, dd, $J = 8.2, 8.2$ Hz); ^{13}C NMR (400 MHz, CD_3OD) δ 19.9, 23.8, 105.9, 109.4, 115.1, 118.0, 118.5, 133.3, 138.6, 152.8, 152.9, 155.4, 161.9; ESIMS 198 (M+H) $^+$. HRESIMS m/z 198.0913 [calcd for $\text{C}_{13}\text{H}_{12}\text{ON}$ (M+H) $^+$, 198.0913].

3.3. Antiplasmodial activity

Human malaria parasites were cultured according to the method of Trager and Jensen.¹⁹ The antimalarial activity of the isolated compounds was determined by the procedure described by Budimulja et al.²⁰ 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 NaHCO_3) until the final concentrations of samples in culture plate wells were 10; 1; 0.1; 0.01; 0.001 $\mu\text{g}/\text{mL}$. The malarial parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plates. Growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Giemsa stain. The antimalarial activity of each compound was expressed as an IC_{50} 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 following equation: $\text{Growth inhibition\%} = 100 - [(\text{test parasitemia}/\text{control parasitemia}) \times 100]$. Chloroquine: IC_{50} 0.011 μM .

3.4. Cytotoxic activity

MCF7 (human breast adenocarcinoma) cell line was seeded onto 96-well microtiter plates at 5×10^3 cells per well. Cells were preincubated for 24 h at 37 $^\circ\text{C}$ in a humidified atmosphere of 5%

CO₂. Different concentrations of each compound (10 μL) were added to the cultures, and then the cells were incubated at 37 °C for 48 h. On the third day, 15 μL MTT solution (5 mg/mL) was added into each well of the cultured medium. After further 2 h of incubation, 100 μL of 10% SDS–0.01 N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made using a micropipette reader (Benchmark Plus microplate spectrometer, BIO-RAD) equipped with a two wavelengths system (550 and 700 nm). In each experiment, three replicate of wells were prepared for each sample. The ratio of the living cells was determined based on the difference of the absorbance between those of samples and controls. These differences are expressed in percentage and cytotoxic activity was indicated as an IC₅₀ value. Vincristine: IC₅₀ 2.4 μM.

3.5. Vasodilation assay²¹

A male Wistar rat weighting 260 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs–Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM NaHCO₃, 1.8 mM CaCl₂, 1.2 mM NaH₂PO₄, 1.2 mM MgSO₄, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O₂, 5% CO₂) bath of 5 mL KHS solution at 37 °C with one end connected to a tissue holder and the other to a force–displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M PE. The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (ACh), and aortic ring in which 80% relaxation occurred, were regarded as tissues with endothelium. When the PE-induced contraction reached a plateau, each sample (1×10^{-6} M – 3×10^{-5} M) was added.

Data are expressed as mean ± SEM. Statistical comparisons between time–response curves were made using a one-way analysis of variance (ANOVA), with Bonferroni's correction for multiple comparisons being performed post-hoc ($P < 0.05$ being considered significant).

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

Acknowledgments

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and a grant from the Open Research Center Project. We acknowledge the Faculty of Pharmacy, Airlangga University for antimalarial activity and financial support.

References and notes

- (a) Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5274; (b) Greenwood, B.; Mutabingwa, T. *Nature* **2002**, *415*, 670; (c) Wyley, D. J. *Clin. Infect. Dis.* **1993**, *16*, 449.
- Taylor, W. R. J.; White, N. J. *Drug Safety* **2004**, *27*, 25.
- (a) Gelb, M. H.; Hol, W. G. J. *Science* **2002**, *297*, 343; (b) Talisuna, A. O.; Bloland, P.; D'Alessandro, U. *Clin. Microbiol. Rev.* **2004**, *17*, 235; (c) Gelb, M. H. *Curr. Opin. Chem. Biol.* **2007**, *11*, 440.
- Delhaes, L.; Benoit-Vical, F.; Camus, D.; Capron, M.; Meunier, B. *Drugs* **2003**, *6*, 674.
- (a) Muhammad, I.; Samoylenko, V. *Expert Opin. Drug. Discov.* **2007**, *2*, 1065; (b) Dunbar, D. C.; Li, X.-C.; Muhammad, I. *Biomater. Aquat. Terr. Org.* **2006**, *207*.
- Ghigo, D.; Todde, R.; Ginsburg, H.; Costamagna, C.; Gautret, P.; Bussolino, F.; Ulliers, D.; Giralbaldi, G.; Deharo, E.; Gabrielli, G.; Pescarmona, G.; Bosia, A. *J. Exp. Med.* **1995**, *182*, 677.
- (a) Sobolewski, P.; Gramaglia, I.; Frangos, J.; Intaglietta, M.; van der Heyde, H. C. *Trends Parasitol.* **2005**, *21*, 415; (b) Yeo, T. W.; Lampah, D. A.; Gitawati, R.; Tjitra, E.; Kenangalem, E.; McNeil, Y. R.; Darcy, C. J.; Granger, D. L.; Weinberg, J. B.; Lopansri, B. K.; Price, R. N.; Duffull, S. B.; Celemajer, D. S.; Anstey, N. M. *J. Exp. Med.* **2007**, *204*, 2693; (c) Seriom, S.; Raharjo, W. H.; Chotivanich, K.; Loareesuwan, S.; Kubes, P.; Ho, M. *Am. J. Pathol.* **2003**, *162*, 1651.
- Weinberg, J. B.; Lopansri, B. K.; Mwaikambo, E.; Granger, D. L. *Curr. Opin. Infect. Dis.* **2008**, *21*, 468.
- Yeo, T. W.; Lampah, D. A.; Gitawati, R.; Tjitra, E.; Kenangalem, E.; McNeil, Y. R.; Darcy, C. J.; Granger, D. L.; Weinberg, J. B.; Lopansri, B. K.; Price, R. N.; Duffull, S. B.; Celemajer, D. S.; Anstey, N. M. *J. Infect. Dis.* **2008**, *198*, 602.
- (a) Morita, H.; Oshimi, S.; Hirasawa, Y.; Koyama, K.; Honda, T.; Ekasari, W.; Indrayanto, G.; Zaini, N. C. *Org. Lett.* **2007**, *9*, 3691; (b) Oshimi, S.; Tomizawa, Y.; Hirasawa, Y.; Honda, T.; Widyawaruyanti, A.; Rudyanto, M.; Ekasari, W.; Indrayanto, G.; Zaini, N. C.; Morita, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3761; (c) Rudyanto, M.; Tomizawa, Y.; Morita, H.; Honda, T. *Org. Lett.* **2008**, *10*, 1921; (d) Oshimi, S.; Deguchi, J.; Hirasawa, Y.; Ekasari, W.; Wahyuni, T. S.; Zaini, N. C.; Shirota, O.; Morita, H. *J. Nat. Prod.* **2009**, *72*, 1899–1901.
- (a) Mbatchesi, S. F.; Mbatchesi, B.; Banzouzi, J. T.; Bansimba, T.; Nsonde Ntandou, G. F.; Ouamba, J. M.; Berry, A.; Benoit-Vical, F. *J. Ethnopharmacol.* **2006**, *104*, 168; (b) Sanon, S.; Ollivier, E.; Azas, N.; Mahiou, V.; Gasquet, M.; Ouattara, C. T.; Nebie, I.; Traore, A. S.; Esposito, F.; Balansard, G.; Timon-David, P.; Fumoux, F. *J. Ethnopharmacol.* **2003**, *86*, 143.
- Ekasari, W.; Widyawaruyanti, A.; Zaini, N. C.; Syafruddin, D.; Honda, T.; Morita, H. *Heterocycles* **2009**, *78*, 1831.
- Yao, Y. S.; Yao, Z. J. *J. Org. Chem.* **2008**, *73*, 5221.
- Murti, V. V. S.; Seshadri, T. R. *Proc. Indian Acad. Sci. Sect. A* **1949**, *30A*, 107.
- Sonogashira, K.; Tohda, Y.; Hagihara, N. A. *Tetrahedron Lett.* **1975**, *16*, 4467.
- Muller, B.; Kleschyov, A. L.; Gyorgy, K.; Stoclet, J.-C. *Physiol. Res.* **2000**, *49*, 19.
- Sofola, O. A.; Raji, I.; Ladipo, C.; Coker, H. A. B. *Niger. Q. J. Hosp. Med.* **2008**, *18*, 50.
- Wang, Y. X.; Poon, C. I.; Pang, C. C. J. *Pharmacol. Exp. Ther.* **1993**, *267*, 1091.
- Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
- Budimulja, A. S.; Syafruddin, T. P.; Wilariat, P.; Marzuki, S. *Mol. Biochem. Parasitol.* **1997**, *84*, 137.
- Morita, H.; Iizuka, T.; Choo, C. Y.; Chan, K. L.; Takeya, K.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4609.

Synthesis and structure–activity relationships of cassiarin A as potential antimalarials with vasorelaxant activity

ORIGINALITY REPORT

14%

SIMILARITY INDEX

14%

INTERNET SOURCES

6%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

1	www.mnstate.edu Internet Source	2%
2	patents.google.com Internet Source	2%
3	www.jci.org Internet Source	2%
4	www.freepatentsonline.com Internet Source	1%
5	polaris.hoshi.ac.jp Internet Source	1%
6	repository.unair.ac.id Internet Source	1%
7	ajp.amjpathol.org Internet Source	1%
8	ir.lib.hiroshima-u.ac.jp Internet Source	1%
9	osdir.com Internet Source	1%

10	www.jstage.jst.go.jp Internet Source	1 %
11	Tun-Cheng Chien, David A. Berry, John C. Drach, Leroy B. Townsend. " Synthesis of 3-Aminoimidazo[4,5-]pyrazole Nucleoside via the N-N Bond Formation Strategy as a [5:5] Fused Analog of Adenosine ", Nucleosides, Nucleotides and Nucleic Acids, 2005 Publication	<1 %
12	www.yumpu.com Internet Source	<1 %
13	www.google.nl Internet Source	<1 %
14	protocolexchange.researchsquare.com Internet Source	<1 %
15	ijpt.iums.ac.ir Internet Source	<1 %
16	eprints.qut.edu.au Internet Source	<1 %
17	www.beilstein-journals.org Internet Source	<1 %
18	authors.library.caltech.edu Internet Source	<1 %
19	www.anorg.chemie.uni-frankfurt.de Internet Source	<1 %

20	ualberta.ca Internet Source	<1 %
21	www.mdpi.com Internet Source	<1 %
22	refubium.fu-berlin.de Internet Source	<1 %
23	Alberto de Juan, David Lozano, Andrew Heard, Michael Jinks, Jorge Meijide Suarez, Stephen Goldup. "A Chiral Interlocking Auxiliary Strategy for the Synthesis of Mechanically Planar Chiral Rotaxanes", American Chemical Society (ACS), 2021 Publication	<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

Synthesis and structure–activity relationships of cassiarin A as potential antimalarials with vasorelaxant activity

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7
