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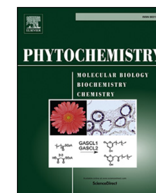

Antiplasmodial dimeric chalcone derivatives from the roots of *Uvaria siamensis*

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ABSTRACT

Four dimeric chalcone derivatives, 8″,9″-dihydrowelwitschin H, uvarins A-C, a naphthalene derivative, 2-hydroxy-3-methoxy-6-(4′- hydroxyphenyl)naphthalene, and the known dimeric chalcones, dependensin and welwitschin E, flavonoids, a cyclohexane oxide derivative, an aromatic aldehyde were isolated from the roots of *Uvaria siamensis* (Annonaceae). The structures of the compounds were elucidated by spectroscopic analysis, as well as by comparison with literature data. The isolated compounds with a sufficient amount for biological assays were evaluated for their antimalarial, antimycobacterial, and cytotoxic activities. The dimeric chalcones 8″,9″-dihydrowelwitschin H, uvarins B and C, dependensin and welwitschin E showed strong antiplasmodial activity with IC_{50} values of 3.10, 3.02, 3.09, 4.21 and 3.99 μg/mL, respectively. A possible biosynthesis pathway of the dimeric chalcones is discussed.

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1. Introduction

The genus *Uvaria* (Annonaceae) is widely distributed over the tropical zones of Africa, Asia, and Australia and there are about 18 species occurring in Thailand (Chalermglin, 2001). Several secondary metabolites were isolated from the plants of this genus, including acetogenins (Hisham et al., 1991; Chen et al., 1996; Raynaud et al., 1997; Zhou et al., 2000; Fall et al., 2004, 2006; Dai et al., 2012), alkaloids (Panichpol and Waterman, 1978; Akendengue et al., 2002), flavonoids (Nkunya et al., 1993; Achenbach et al., 1997; Okorie, 1977; Fleischer et al., 1998; Moriyasu et al., 2011), polyoxygenated cyclohexenes (Awale et al., 2012a, b), pyrenes (Moriyasu et al., 2012), and xanthenes (Macabeo et al., 2014), some of which showed biological activities such as antimycobacterial (Macabeo et al., 2014) and cytotoxic (Fall et al., 2004, 2006; Awale et al., 2012a, b; Moriyasu et al., 2012; Macabeo et al., 2014) effects. The isolation, characterization, and bioactivities of two *C*-benzylated dihydrochalcones, along with seven flavonoids and four aromatic compounds from the leaves of *Melodorum siamensis* (Prawat et al., 2013a) (synonym: *Uvaria siamensis* (Scheff.) L.L. Zhou, Y.C.F. Su & R.M.K. Saunders (Zhou et al., 2009)) were recently reported. As part of a continuing search for bioactive compounds from Thai medicinal plants (Prawat et al., 2012a, b, 2013a, b; Tuntiwachwuttikul et al., 2008), an EtOAc extract of the roots of *U. siamensis* was shown to be active against *Mycobacterium tuberculosis* (MIC = 100 μg/mL). Herein, the isolation of four new dimeric chalcones, 8″,9″-dihydrowelwitschin H (**1**), uvarins A-C (**2**–**4**) and a new naphthalene derivative (**5**), and 10 known compounds including dependensin (**6**) (Nkunya et al., 1993), welwitschin E (**7**) (Moriyasu et al., 2011), 2′-hydroxy-3′,4′,6′-trimethoxychalcone (**8**) (Panichpol and Waterman, 1978), 2′-hydroxy-3′,4′,6′-trimethoxydihydrochalcone (**9**) (Lien et al., 2000), 2-hydroxy-3,4,6-trimethoxychalcone (**10**) (Nkunya et al., 1993; Colegate et al., 1992), 2-hydroxy-3,4,6-trimethoxydihydrochalcone (**11**) (Lien et al., 2000), welwitschin A (**12**) (Moriyasu et al., 2011), 5,3′-dihydroxy-7-methoxyflavone (**13**) (Prawat et al., 2012b), (2*R*)-*trans*-2,3-diacetoxy-1-[(benzoy1oxy)

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methyl]cyclohexa-4,6-diene (**14**) (Kodpinid et al., 1983), and 2-hydroxy-3,4,6-trimethoxybenzaldehyde (**15**) (Paul and Wang, 1979) from the roots of *U. siamensis* (Fig. 1) are reported. The antimalarial, antimycobacterial and cytotoxic activities of some of the isolated compounds were investigated.

2. Results and discussion

Compound **1** was obtained as a dark green solid with $[\alpha]_D^{25}$ + 3.4 (*c* 0.11, $CHCl_3$), and had the molecular formula $C_{36}H_{36}O_8$ by HRTOFESIMS and ^{13}C NMR, indicating that **1** had two protons more than the known welwitschin H (Moriyasu et al., 2011). The UV spectrum of **1** exhibited absorptions at 265 and 281 nm and the IR spectrum showed an absorption band for aromatic (1610-1460 cm^{-1}) functional groups. The ^1H and ^{13}C NMR data of **1** (Table 1) were closely related to those of welwitschin H (Moriyasu et al., 2011). The major difference was the replacement of the $\Delta^{8'',9''}$double bond of welwischin H, with the −CH−CH_2− moiety at δ_C 36.7/δ_H 2.88, td (J = 6.0, 5.6 Hz) for C-8″ and δ_C 20.5/δ_H 2.59, dd (J = 16.8, 6.0 Hz) and 2.72, dd (J = 16.8, 5.6 Hz) for C-9″ supporting **1** as 8″,9″-dihydrowelwitschin H. The small coupling constant between H-9 and H-8″ (J = 6.0 Hz) indicated a *cis quasi*-axial- *quasi*-equatorial arrangement. NOESY correlations (Fig. 3) were observed between H-9 (δ 3.72) and H-8″ (δ 2.88); H-8″ (2.88) and H_α-9″ (δ 2.59), and the aromatic protons, H-2″ and H-6″ (δ 7.51). The NOESY spectrum also revealed correlations between: H-7 (δ 6.31) and H-2, H-6 (δ 7.02); H-8 (δ 6.08) and H-2, H-6 (δ 7.02). The relative configuration of compound **1** was derived from the above data. Thus, *quasi*-axial H-9, *quasi*-equatorial H-8″, the *quasi*-axial C-7″ aromatic ring, and Hα-9″ have α-orientations, while the *quasi*-

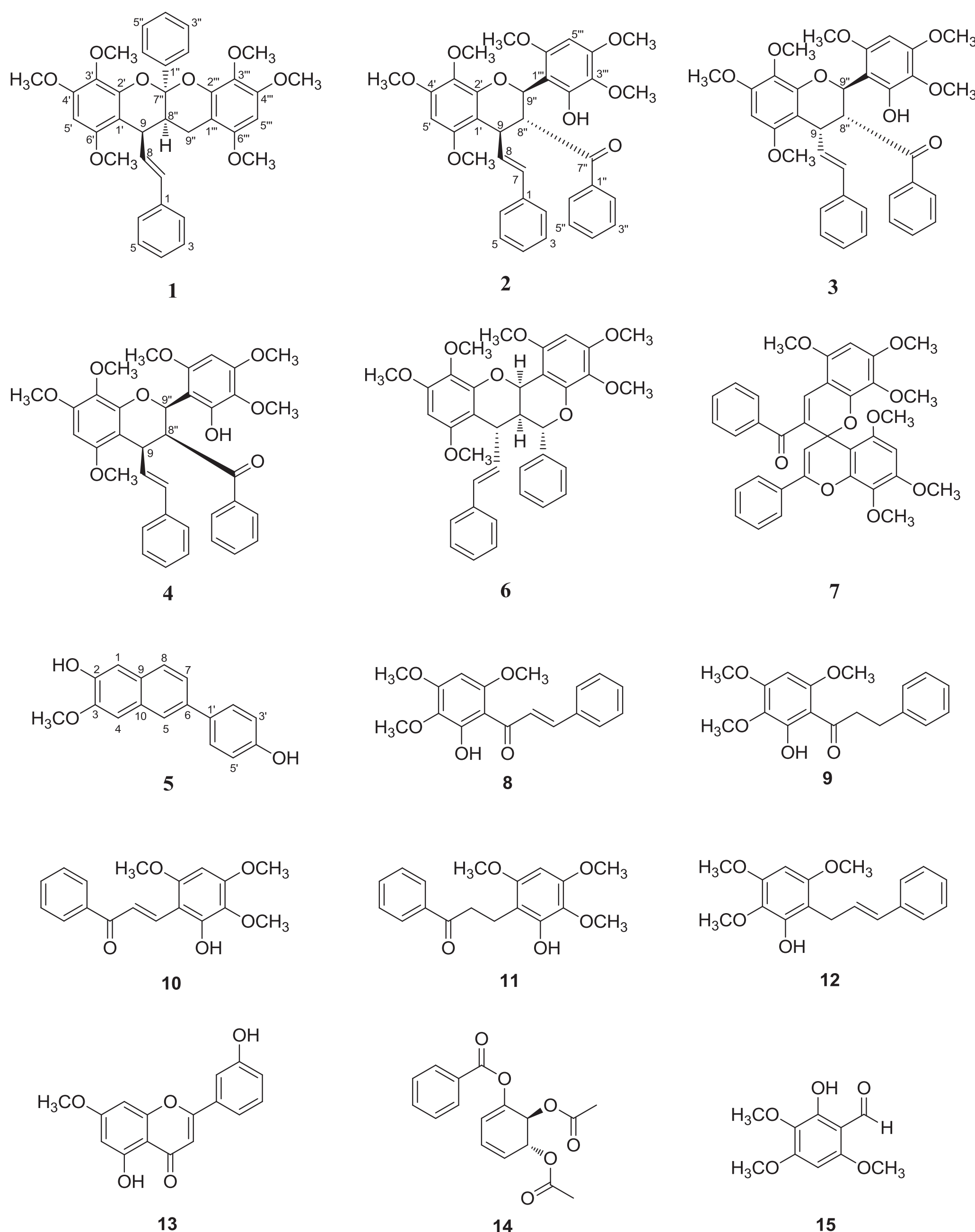

Fig. 1. Structures of compounds **1**–**15**.

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Table 1
NMR (400 MHz, $CDCl_3$) spectroscopic data for 8″,9″-dihydrowelwitschin H (**1**).

Position	δ_C, type	δ_H (J in Hz)
1	138.0, C	
2,6	126.0, CH	7.02, d (7.2)
3,5	128.1, CH	7.17, t (7.2)
4	126.6, CH	7.11, t (7.2)
7	129.5, CH	6.31, d (15.6)
8	131.3, CH	6.08, dd (15.6, 9.2)
9	38.4, CH	3.72, dd (9.2, 6.0)
1′	105.2, C	
2′	146.5, C	
3′	131.6, C	
4′	152.3, C	
5′	90.8, CH	6.16, s
6′	154.0, C	
1″	140.5, C	
2″, 6″	125.9, CH	7.51, m
3″, 5″	128.4, CH	7.31, m
4″	128.6, CH	7.31, m
7″	101.2, C	
8″	36.7, CH	2.88, td (6.0, 5.6)
9″	20.5, CH_2	2.59, dd (16.8, 6.0) 2.72, dd (16.8, 5.6)
1‴	104.2, C	
2‴	147.5, C	
3‴	131.3, C	
4‴	151.6, C	
5‴	89.9, CH	5.95, s
6‴	152.6, C	
3′-OCH_3	61.1, CH_3	3.76, s
4′-OCH_3	56.5, CH_3	3.87, s
6′-OCH_3	56.0, CH_3	3.68, s
3‴-OCH_3	56.4, CH_3	3.78, s
4‴-OCH_3	61.0, CH_3	3.79, s
6‴-OCH_3	55.5, CH_3	3.59, s

equatorial C-9 styryl group and H_β-9″ are β-oriented. The interpretation of the complete set of NMR data (DEPT, COSY, HMQC, HMBC and NOESY) permitted assignment of the structure of **1**, with the relative configuration shown, as 8″,9″-dihydrowelwitschin H.

Compound **2** was isolated as a pale yellow solid with $[\alpha]_D^{25}$ + 5.9 (*c* 0.07, $CHCl_3$), and had the molecular formula $C_{36}H_{36}O_9$ as determined by HRTOFESIMS (*m/z* 635.2232 $[M+Na]^+$, calcd for $C_{36}H_{36}O_9Na^+$ 635.2252) and ^{13}C NMR spectroscopic data. The IR spectrum indicated the presence of hydroxy (3406 cm^{-1}), carbonyl (1674 cm^{-1}), and aromatic (1606-1460 cm^{-1}) groups and the UV spectrum exhibited absorptions at 267, 284 and 293 nm. Close examination of the ^1H, ^{13}C and 2D NMR spectra data of **2** (Table 2) revealed the presence of two sets of monosubstituted phenyl rings, two sets of 2-hydroxy-3,4,6-trimethoxyphenyl groups, two olefinic protons, three methines, and one carbonyl carbon, indicating the dimeric chalcone. Important HMBC correlations (Fig. 2) were observed between: the C-7″ (δ 201.2) carbonyl, and H-9″ (δ 5.53), H-9 (δ 4.44), and H-2″, H-6″ (δ 7.66); C-9 (δ 40.2), and H-7 (δ 6.33), H-8 (δ 6.13), H-8″ (δ 4.78), and H-9″ (δ 5.53), and C-2′ (δ 150.0), and H-9 (δ 4.44). In addition, long-range correlations between H-9″ (δ 5.53), and C-2‴ (δ 149.3), C-6‴ (δ 154.2), C-1‴ (δ 105.0), C-9 (δ 40.2), and C-8″ (δ 50.2) were also observed. These HMBC correlations suggested that the dimeric chalcone **2** possessed a pyran ring. The positions of the connection between the two chalcone units were C-9 and C-8″ and the oxygen atom of the pyran ring located between C-2′ and C-9″. The relative configuration of **2** was derived from NOESY correlations (Fig. 3) and the coupling constants between H-9 and H-8″ and H-8″ and H-9″. The large coupling constants of H-9/H-8″ (J = 10.0 Hz) and H-8″/H-9″ (J = 10.0 Hz) indicated that the three protons (H-9, H-8″ and H-9″) were *trans quasi*-diaxially oriented. A NOESY correlation was observed between H-9 (δ 4.44) and H-9″ (δ 5.53), but not between H-8″ (δ 4.78) and H-9″ (δ 5.53). Thus, the *quasi*-axial H-9, the *quasi*-equatorial C-8″ benzoyl group and the *quasi*-axial H-9″ have α-orientations and the *quasi*-equatorial C-9 styryl, the *quasi*-axial H-8″ and the *quasi*-equatorial C-9″ 2-hydroxy-3,4,6-trimethoxyphenyl group occupy the β-face. The interpretation of the complete set of NMR data (DEPT, COSY, HSQC, HMBC, and NOESY) permitted assignment of the structure of **2**, with the relative configuration shown, as uvarin A.

Compound **3** was isolated as a pale yellow solid with $[\alpha]_D^{25}$ + 3.6 (*c* 0.11, $CHCl_3$), and had the molecular formula $C_{36}H_{36}O_9$ by ^{13}C NMR data and HRTOFESIMS (m/z 635.2238 $[M+Na]^+$, calcd for $C_{36}H_{36}O_9Na^+$ 635.2252); is indicated that **3** had same molecular formula to compound **2**. The structure of **3** was closely related to **2** based on the ^1H NMR, ^{13}C NMR, IR and UV spectroscopic data, except for the configuration at C-9. The small coupling constant (J = 4.4 Hz) between H-9 and H-8″ and the large coupling constant (J = 10.8 Hz) between H-8″ and H-9″ indicated the *cis quasi*-equatorial-*quasi*-axial and *trans quasi*-diaxial configurations, respectively. The NOESY spectrum of **3** showed correlations between H-9 (δ 4.20) and H-8″ (δ 5.13), but not between H-9″ (δ 6.12) and H-8″ (δ 5.13). Thus, the *quasi*-equatorial H-9, the *quasi*-axial H-8″, and the *quasi*-equatorial C-9″ 2-hydroxy-3,4,6-trimethoxyphenyl have β-orientations while the *quasi*-axial C-9 styryl, the *quasi*-equatorial C-8″ benzoyl group and the *quasi*-axial H-9″ occupy the α-face. The interpretation of the complete set of NMR data (DEPT, COSY, HMQC, HMBC, and NOESY) permitted assignment of the structure of **3**, with the relative configuration shown, as uvarin B.

Compound **4** was obtained as a pale green solid with $[\alpha]_D^{25}$ + 3.2 (*c* 0.11, $CHCl_3$), and had the molecular formula $C_{36}H_{36}O_9$ by HRTOFESIMS (m/z 635.2270 $[M+Na]^+$, calcd for $C_{36}H_{36}O_9Na^+$ 635.2252) and ^{13}C NMR spectroscopic data. Its structure was closely related to **2** and **3** based on ^1H NMR, ^{13}C NMR, IR and UV spectral data. The ^1H NMR spectrum of **4** indicated that the configuration of C-9 and C-8″ were different from that of **2** and **3**. The ^1H NMR spectrum of **4** showed signals at δ 4.07 (br d, J = 6.0 Hz, H-9), 4.20 (br s, H-8″) and 5.90 (br s, H-9″). The small coupling constants between H-9 and H-8″, and between H-8″ and H-9″ indicated the *cis quasi*-equatorial-*quasi*-axial orientation between H-9 and H-8″, and *cis quasi*-axial-*quasi*- equatorial between H-8″ and H-9″. The ^1H NOE spectra of **4** were observed between: H-8″ (δ 4.20) and H-9 (δ 4.07) and H-9″ (δ 5.90); H-9 (δ 4.07) and H-8″ (δ 4.20); H-9″ (δ 5.90) and H-8″ (δ 4.20), but not between H-9 (δ 4.07) and H-9″ (δ 5.90). Thus, the *quasi*-equatorial H-9, the *quasi*-axial H-8″, and the *quasi*-equatorial H-9″ have α-orientations, while the *quasi*-axial C-9 styryl, the *quasi*-equatorial C-8″ benzoyl and the *quasi*-axial C-9″ 2-hydroxy-3,4,6-trimethoxyphenyl occupy the β-face. The interpretation of the complete set of NMR data (DEPT, COSY, HSQC, HMBC, NOESY, and NOE) permitted assignment of the structure of **4**, with the relative configuration shown, as uvarin C.

Compound **5** was isolated as a pale red solid and had the molecular formula $C_{17}H_{14}O_3$ by HRTOFESIMS (*m/z* 265.0872 $[M-H]^-$, calcd for $C_{17}H_{13}O_3$ 265.0870) and ^{13}C NMR spectroscopic data. The UV spectrum showed absorption bands at λ_{max} 273, 295 and 334 nm and the IR spectrum indicated the hydroxy (3411 cm^{-1}) and aromatic (1615–1540 cm^{-1}) groups. The ^1H NMR spectrum of **5** (Table 3) showed signals for five aromatic protons at δ 7.27 (s, H-1), 7.16 (s, H-4), 7.83 (br s, H-5), 7.55 (dd, J = 8.4, 1.6 Hz, H-7), 7.70 (d, J = 8.4 Hz, H-8), suggesting a 2,3,6-trisubstituted naphthalene skeleton. Its ^1H NMR spectrum also showed two doublets at δ 6.94 (2H, J = 8.4 Hz, H-3′, H-5′) and δ 7.57 (2H, J = 8.4 Hz, H-2′, H-6′) of a *para*-substituted aromatic ring, a three protons singlet of a methoxy group at δ 4.04 and a phenolic hydroxy group at δ 5.94 (br s). This was consistent with the ^{13}C NMR data of **5** (Table 3) which exhibited signals of a 2,3,6-trisubstituted naphthalene at δ 109.2 (C-

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Table 2
NMR (400 MHz, $CDCl_3$) spectroscopic data for uvarins A-C (**2**–**4**).

Position	uvarin A (2)		uvarin B (3)		uvarin C (4)	
	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	137.8, C		137.5, C		137.5, C	
2,6	126.0, CH	7.18, m	126.6, CH	7.28, t (7.6)	126.3, CH	7.42, m
3,5	128.3, CH	7.19, m	128.3, CH	7.25, t (7.6)	128.2, CH	7.31, m
4	126.7, CH	7.13, m	127.0, CH	7.19, t (7.6)	127.3, CH	7.23, m
7	129.9, CH	6.33, d (16.0)	132.3, CH	6.09, d (15.2)	131.1, CH	6.41, d (16.0)
8	132.5, CH	6.13, dd (16.0, 8.8)	128.0, CH	6.23, dd (15.2, 7.6)	131.8, CH	6.57, dd (16.0, 6.0)
9	40.2, CH	4.44, dd (10.0, 8.8)	37.4, CH	4.20, dd (7.6, 4.4)	36.6, CH	4.07, br d (6.0)
1′	108.1, C		105.9, C		104.4, C	
2′	150.0, C		149.2, C		148.0, C	
3′	132.3, C		131.6, C		131.6, C	
4′	154.7, C		152.1, C		152.1, C	
5′	90.9, CH	6.19, s	88.8, CH	6.14, s	90.2, CH	6.21, s
6′	151.8, C		153.3, C		153.7, C	
1″	138.4, C		137.3, C		136.6, C	
2″, 6″	127.9, CH	7.66, d (7.2)	128.3, CH	7.92, d (7.6)	128.4, CH	7.72, d (7.6)
3″, 5″	128.0, CH	7.20, t (7.2)	128.7, CH	7.45, t (7.6)	128.6, CH	7.30, t (7.6)
4″	132.3, CH	7.33, t (7.2)	132.9, CH	7.56, t (7.6)	133.0, CH	7.34, t (7.6)
7″	201.2, C		199.6, C		199.6, C	
8″	50.2, CH	4.78, t (10.0)	46.7, CH	5.13, dd (10.8, 4.4)	45.7, CH	4.20, br s
9″	73.4, CH	5.53, d (10.0)	68.6, CH	6.12, d (10.8)	72.2, CH	5.90, br s
1‴	105.0, C		106.7, C		104.8, C	
2‴	149.3, C		149.2, C		150.4, C	
3‴	129.9, C		130.2, C		132.1, C	
4‴	152.9, C		152.3, C		152.2, C	
5‴	88.8, CH	5.77, s	89.4, CH	6.04, s	88.3, CH	6.00, s
6‴	154.2, C		155.5, C		153.2, C	
3′-OCH_3	61.2, CH_3	3.79, s	60.9, CH_3	3.80, s	61.5, CH_3	3.88, s
4′-OCH_3	55.9, CH_3	3.70, s	56.3, CH_3	3.90, s	56.3, CH_3	3.91, s
6′-OCH_3	56.4, CH_3	3.89, s	55.8, CH_3	3.73, s	56.7, CH_3	3.75, s
2‴-OH		6.49, s		6.23, s		8.21, s
3‴-OCH_3	61.0, CH_3	3.65, s	61.1, CH_3	3.77, s	60.7, CH_3	3.54, s
4‴-OCH_3	55.9, CH_3	3.72, s	55.8, CH_3	3.82, s	56.1, CH_3	3.72, s
6‴-OCH_3	56.0, CH_3	3.64, s	56.3, CH_3	3.74, s	56.2, CH_3	3.80, s

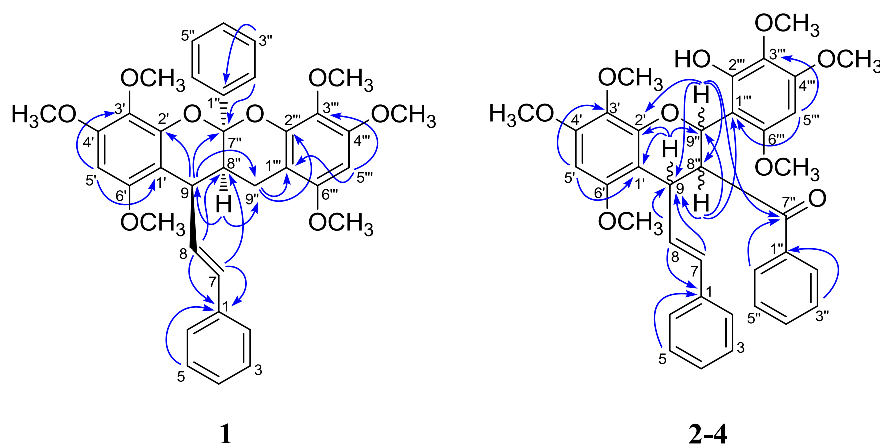

Fig. 2. Selected HMBC correlations of compounds **1**–**4**.

1), 145.6 (C-2), 147.5 (C-3), 105.9 (C-4), 123.9 (C-5), 136.3 (C-6), 123.9 (C-7), 126.8 (C-8), 129.3 (C-9), and 133.6 (C-10), one *para*-substituted aromatic ring at δ 134.2 (C-1′), 128.4 (C-2′ and C-6′), 115.7 (C-3′ and C-5′) and 155.0 (C-4′), and one methoxy carbon at δ 55.9. The 2D HMBC data of **5** revealed correlations between: the proton signal of 3-OCH_3 (δ 4.04) and C-3 (δ 147.5); H-1 (δ 7.27), and C-2 (δ 145.6), C-3 (δ 147.5) and C-8 (δ 126.8); H-4 (δ 7.16), and C-3 (δ 147.5), C-2 (δ 145.6), C-9 (δ 129.3), and C-5 (δ 123.9); H-2′, H-6′ (δ 7.57), and C-6 (δ 136.3), and C-4′ (δ 155.0), and H-3′, H-5′ (δ 6.94), and C-1′ (δ 134.2), and C-4′ (δ 155.0). These results indicated that the methoxy group, the two hydroxy groups and the *para*-substituted aromatic ring were attached to C-3, C-2, C-4′, and C-6, respectively. Compound **5** was, therefore, assigned as 2-hydroxy-3-methoxy-6-(4′-hydroxyphenyl)naphthalene.

The skeletons of 8″,9″-dihydrowelwitschin H (**1**), uvarins A-C (**2**–**4**) and dependensin (**6**) could be biosynthetically formed from chalcone **8** (Fig. 4). Reduction and subsequent loss of water of chalcone **8** would generate the *o*-quinone methide **A**, which would condense with another molecule of **8** to give the addition adduct **B**. Reduction of **B** would give rise to the dihydro derivative **C**, which would undergo ketal formation to give the novel skeleton **1**. Alternatively, [4 + 2] cycloaddition between *o*-quinone methide **A** and chalcone **8** would give uvarins A-C (**2**–**4**) which would be transformed to dependensin (**6**) via hemiketal formation followed

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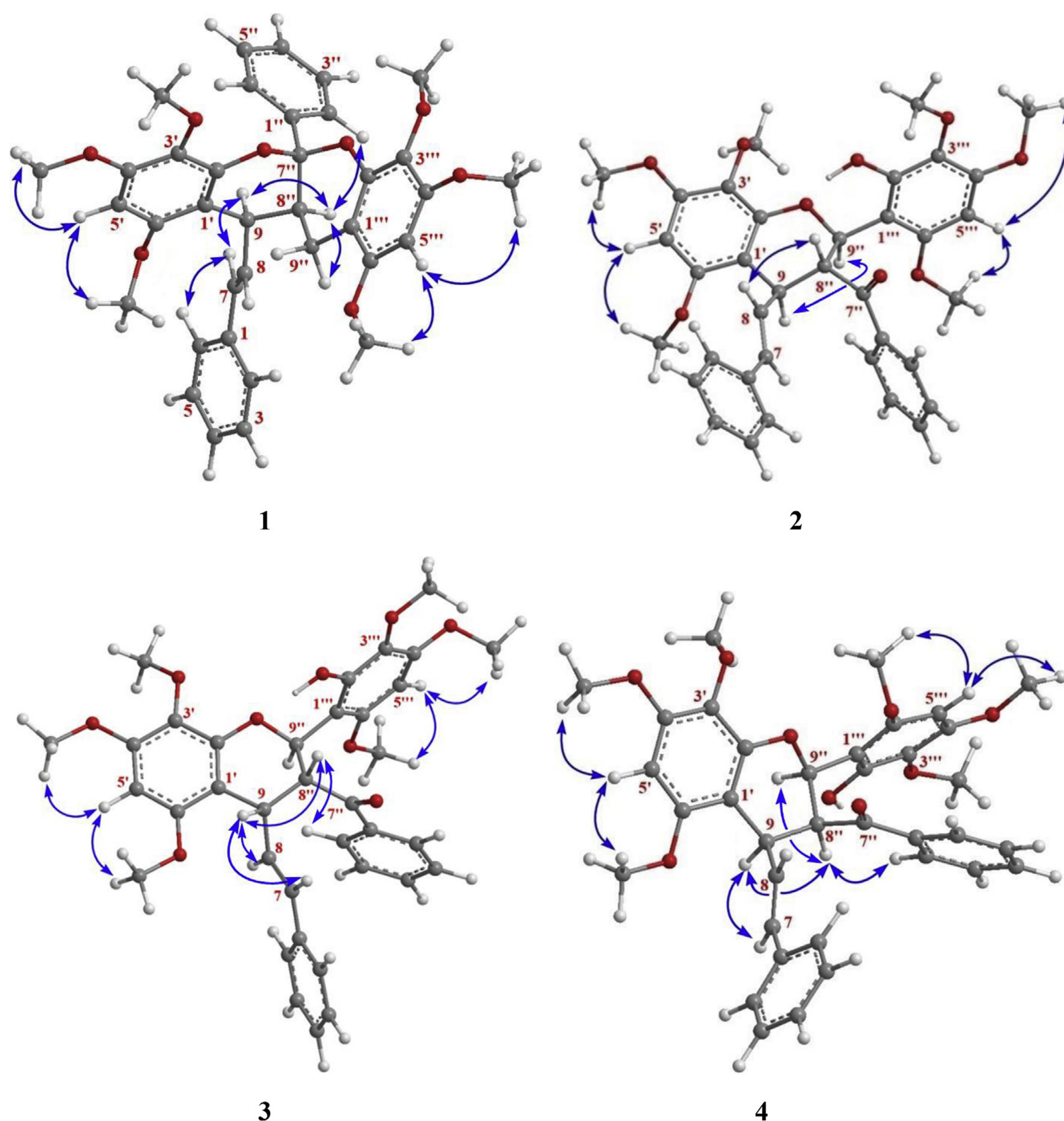

Fig. 3. Selected NOESY and NOE correlations of compounds **1**–**4**.

Table 3
NMR (400 MHz, $CDCl_3$) spectroscopic data for compound **5**.

Position	δ_C, type	δ_H (J in Hz)	HMBC
1	109.2, CH	7.27, s	2,3,8
2	145.6, C		
3	147.5, C		
4	105.9, CH	7.16, s	2,3,5,9
5	123.9, CH	7.83, br s	4,7,1′
6	136.3, C		
7	123.9, CH	7.55, dd (8.4, 1.6)	5
8	126.8, CH	7.70, d (8.4)	1,6,10
9	129.3, C		
10	133.6, C		
1′	134.2, C		
2′,6′	128.4, CH	7.57, d (8.4)	6,4′
3′,5′	115.7, CH	6.94, d (8.4)	1′,4′
4′	155.0, C		
3-OCH_3	55.9, CH_3	4.04, s	3
4′-OH		5.94, br s	

by reduction. Another possibility is that of intramolecular cycloaddition of *o*-quinone methide to generate the intermediate **D**, which underwent cycloaddition with the second molecule of *o*-quinone methide to give dependensin (**6**).

The isolated compounds **1**, **3**, **4**, **6**–**8**, **10**, **11** and **15** were individually evaluated for antiplasmodial activity against *Plasmodium falciparum*, antimycobacterial activity against *Mycobacterium tuberculosis* and cytotoxicity against three cancer cell lines (Table 4). The dimeric chalcones, 8″,9″-dihydrowelwitschin H (**1**), uvarin B (**3**), and C (**4**), dependensin (**6**) and welwitschin E (**7**) showed strong antiplasmodial activity with IC_{50} values of 3.10, 3.02, 3.09, 4.21 and 3.99 μg/mL, respectively. The dimeric chalcones were found to be inactive for antimycobacterial and cytotoxic activities. Chalcone **8** and dihydrochalcone **11** showed moderate cytotoxic activity against KB and NCI-H187 cell lines with IC_{50} values in the range of 10.47–33.75 μg/mL and chalcone **10** was weakly active against NCI-H187 cell line with IC_{50} value of 48.61 μg/mL. The three chalcones **8**, **10** and **11** did not showed cytotoxic activity against

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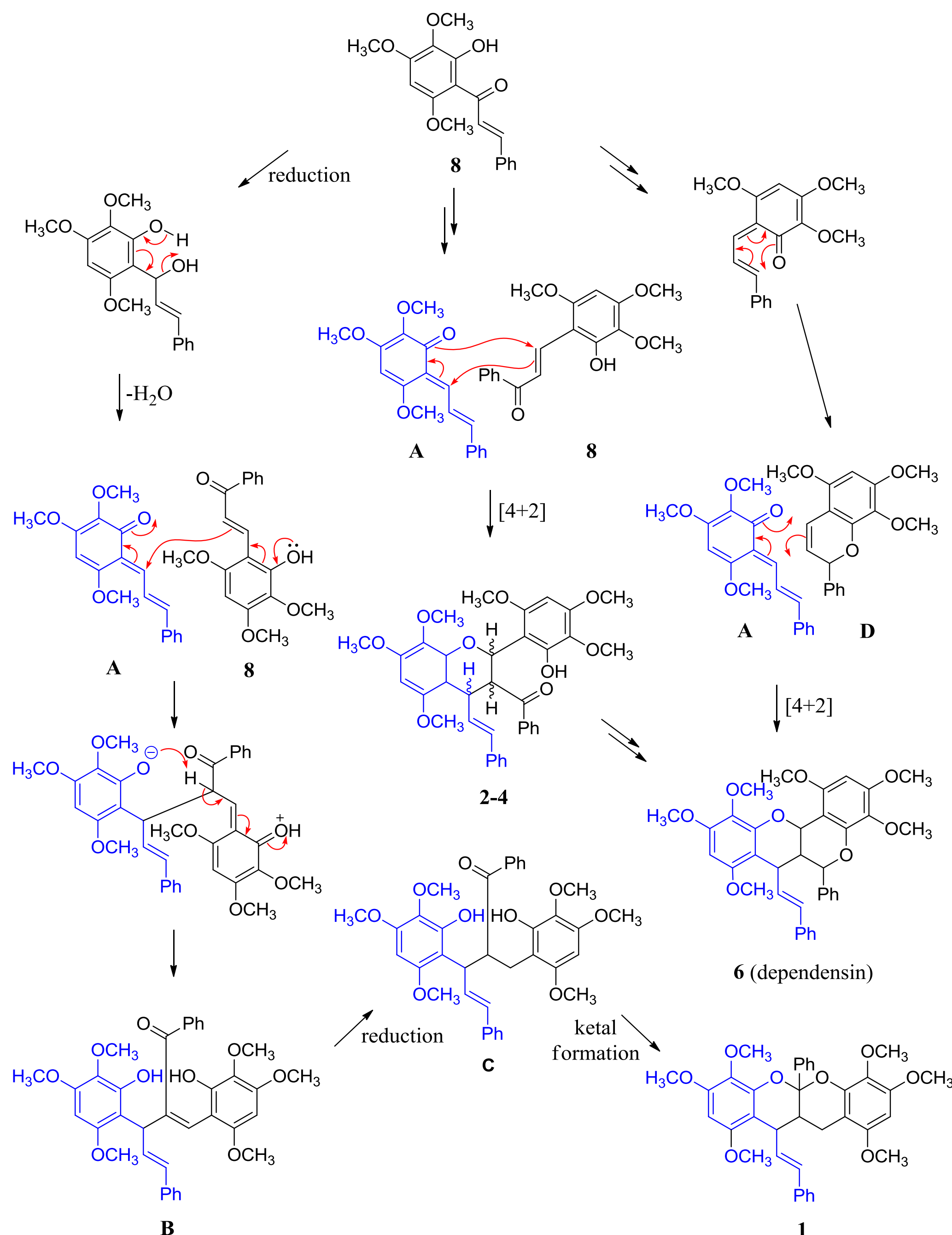

Fig. 4. Possible biosynthesis pathway of 8″,9″-dihydrowelwitschin H (**1**), uvarins A-C (**2**–**4**) and dependensin (**6**).

MCF-7 cell line and were also inactive for antiplasmodial and antimycobacterial activities. The benzaldehyde derivative **15** was moderately active against NCI-H187 cell line with IC_{50} value of 23.08 μg/mL and was found to be inactive against KB and MCF-7 cell lines and also showed no activity for antiplasmodial and antimycobacterial activities.

3. Conclusions

Four dimeric chalcone derivatives, 8″,9″-dihydrowelwitschin H (**1**), uvarins A-C (**2**–**4**), one naphthalene derivative, 2-hydroxy-3-methoxy-6-(4′-hydroxyphenyl)naphthalene (**5**), two known dimeric chalcones, dependensin (**6**) and welwitschin E (**7**), six known flavonoids (**8**–**13**), one cyclohexane oxide derivative (**14**) and one aromatic aldehyde (**15**) were isolated from the roots of *Uvaria siamensis* (Annonaceae). The structures of the compounds were elucidated by spectroscopic analysis, as well as by comparison with literature data. The dimeric chalcones **1**, **3**, **4**, **6** and **7** showed strong antiplasmodial activity with IC_{50} values of 3.10, 3.02, 3.09, 4.21 and 3.99 μg/mL, respectively.

4. Experimental

4.1. General experimental procedures

Melting points were determined with a Buchi melting point B-540 apparatus and are reported without correction. Optical rotations $[\alpha]_D$ were measured in $CHCl_3$ solution at the sodium D line

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Table 4
Biological Activities of compounds **1**, **3**, **4**, **6**–**8**, **10**, **11** and **15**.

Compounds	Antiplasmodial (IC_{50}, μg/mL)	Antimycobacterial (MIC, μg/mL)	Cytotoxicity (IC_{50}, μg/mL)		
			KB[a]	MCF-7[b]	NCI-H187[c]
1	3.10 ± 0.151	NA	NA	NA	NA
3	3.02 ± 0.245	NA	NA	NA	NA
4	3.09 ± 0.193	NA	NA	NA	NA
6	4.21 ± 0.328	NA	NA	NA	NA
7	3.99 ± 0.142	NA	NA	NA	NA
8	NA	NA	11.04 ± 0.86	NA	10.47 ± 1.79
10	NA	NA	NA	NA	48.61
11	NA	NA	33.75 ± 5.48	NA	18.72
15	NA	NA	NA	NA	23.08 ± 3.33
Ellipticin			0.667	0.128	0.522
Doxorubicin			0.096	0.065	0.031
Dihydroartemisinin	0.0044				
Rifampicin		0.019			
Kanamycin		1.250			
Isoniazid		0.050			

NA= not active (IC50 > 50 μg/mL or MIC >200 μg/mL.
[a] Human epidermoid carcinoma in the mouth.
[b] Human breast.
[c] Human lung.

(590 nm) on a JASCO P1010 digital polarimeter. UV and IR spectra were recorded on SPECORD S210 plus (Analytik jena) and Shimadzu FTIR-8900 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker AVANCE 400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. Coupling constants (*J*) are given in Hz. The signals in the ^{1}H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC, HSQC, HMBC, NOE and NOESY. EIMS were recorded on a MS Finnigan Polaris spectrometer. HRMS were recorded on a Bruker microTOF mass spectrometer. Column chromatography (CC) was carried out on silica gel 60 (Scharlau, 230–400 mesh) and RP-18 (Merck, 40–63 μm). Vacuum liquid chromatography (VLC) was carried out on silica gel 60H (Merck, 5–40 μm) and RP-18 (Merck, 15–25 μm). TLC analyses were performed on precoated silica gel 60 F_{254} and RP-18 F_{254s} plates (Merck); spots were detected by UV or spraying with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4, following by heating. Preparative TLC was carried out on silica gel 60 F_{254} (Merck). All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

4.2. Plant material

Roots of *Uvaria siamensis* (Scheff.) L.L.Zhou, Y.C.F.Su & R.M.K.Saunders were collected from Amphoe Khuan Niang, Songkhla Province, Thailand, December 2007. The plant was identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2007001) is deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

4.3. Extraction and isolation

Fresh roots of *U. siamensis* (2.8 kg) were exhaustively extracted with EtOAc (3 × 7 L) at room temperature, filtered and concentrated to give a dark brown crude extract (112.6 g). The EtOAc extract (111.6 g) was adsorbed onto silica gel 60H (223 g) and fractionated by vacuum liquid chromatography (VLC) over a sintered glass filter column of silica gel 60H (1.13 kg, diameter × height: 19.0 × 8.0 cm) using an increasing amount of EtOAc in hexane (1% EtOAc to 100% EtOAc) and increasing amounts of MeOH in EtOAc (1% MeOH to 100% MeOH) to yield 7 fractions (1–7).

Fraction 3 (7.4 g) was separated by CC using silica gel 60 (832.0 g, 5.5 × 70.0 cm), eluted with hexane/CH_2Cl_2/EtOAc (5:5:1) to give 15 subfractions (3.1–3.15). Subfraction 3.5 (554.6 mg) was further purified by CC over silica gel 60 (63.0 g, 2.0 × 40.0 cm), eluted with CH_2Cl_2 to give 8 subfractions (3.5.1–3.5.8). Subfraction 3.5.3 was identified as **12** (2.0 mg). Subfraction 3.5.4 (50.0 mg) was subjected to CC on silica gel 60 (7.0 g, 0.7 × 35.0 cm), eluted with CH_2Cl_2 to give **10** (4.9 mg). Subfraction 3.7 (578.4 mg) was applied to a silica gel 60 column (35.0 g, 2.1 × 20.0 cm), eluted with hexane/CH_2Cl_2/EtOAc (10:5:1) to yield 10 subfractions (3.7.1–3.7.10). Subfraction 3.7.4 was identified as **14** (19.2 mg). Subfraction 3.7.6 (271.4 mg) was separated by CC on silica gel 60 (22.0 g, 1.7 × 20.0 cm), eluted with EtOAc/hexane (4:6), to give 8 subfractions (3.7.6.1–3.7.6.8). Subfraction 3.7.6.6 (38.4 mg) was further purified by CC on silica gel 60 (3.0 g, 0.6 × 20.0 cm), eluted with CH_2Cl_2 to give **9** (6.5 mg). Subfraction 3.7.6.7 was identified as **8** (5.8 mg). Subfraction 3.10 (1.0 g) was applied to a silica gel 60 column (76.0 g, 2.2 × 40.0 cm), eluted with hexane/CH_2Cl_2/EtOAc (15:25:1) to give 5 subfractions (3.10.1–3.10.5). Subfraction 3.10.3 (241.6 mg) was separated by CC on silica gel 60 (25.5 g, 1.2 × 45.0 cm), eluted with hexane/ EtOAc/ MeOH (153:17:30) to obtain **13** (2.0 mg). Subfraction 3.10.4 (202.5 mg) was further purified by CC on silica gel 60 (19.0 g, 1.1 × 40.0 cm), eluted with hexane/ EtOAc/MeOH (153:17:30) to provide **11** (121.0 mg).

Fraction 4 (13.4 g) was separated by CC on silica gel 60 (0.99 kg, 6.5 × 60.0 cm) using hexane/CH_2Cl_2/EtOAc (10:5:1) as the eluent to give 10 subfractions (4.1–4.10). Subfraction 4.5 (1.4 g) was subjected to VLC on silica gel 60H (50.0 g, 5.0 × 5.0 cm) using an increasing amount of EtOAc in hexane (1% EtOAc to 20% EtOAc) to yield 7 subfractions (4.5.1–4.5.7). Subfraction 4.5.3 (135.6 mg) was purified by VLC on silica gel 60H (3.9 g, 1.5 × 4.5 cm) using an increasing amount of acetone in hexane (1% acetone to 20% acetone) to obtain **15** (12.7 mg) and **1** (22.6 mg). Subfraction 4.5.5 (96.0 mg) was separated by VLC on silica gel 60H (1.7 g, 1.0 × 4.5 cm) using an increasing amount of EtOAc in hexane (1% EtOAc to 30% EtOAc) to yield **6** (33.2 mg). Subfraction 4.5.6 (114.0 mg) was subjected to VLC on silica gel 60H (1.7 g, 1.0 × 4.5 cm) using an increasing amount of EtOAc in CH_2Cl_2 (1% EtOAc to 20% EtOAc) to give **7** (52.6 mg). Subfraction 4.6 was

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identified as **8** (126.4 mg). Subfraction 4.7 (75.0 mg) was subjected to VLC on silica gel RP-18 (1.7 g, 1.0 × 4.5 cm) using H_2O/MeOH (3:7) as the eluent to provide **4** (11.5 mg). Subfraction 4.8 (520.8 mg) was separated by VLC on silica gel 60H (11.0 g, 2.5 × 4.5 cm) using an increasing amount of EtOAc in hexane (1% EtOAc to 30% EtOAc) to give 6 subfractions (4.8.1–4.8.6). Subfraction 4.8.5 (156.4 mg) was applied to a silica gel RP-18 column (3.9 g, 1.5 × 4.5 cm) using H_2O/MeOH (1:9) as the eluent to provide **5** (3.8 mg) and **3** (65.5 mg). Subfraction 4.9 (0.66 g) was separated by VLC on silica gel 60H (9.8 g, 2.5 × 4.0 cm) using an increasing amount of EtOAc in hexane (1% EtOAc to 20% EtOAc) to give 11 subfractions (4.9.1–4.9.11). Subfraction 4.9.8 (124.7 mg) was separated by VLC on silica gel 60H (1.7 g, 1.0 × 4.5 cm) using an increasing amount of acetone in hexane (1% acetone to 100% acetone) to yield 4 subfractions (4.9.8.1–4.9.8.4). Subfraction 4.9.8.3 (17.0 mg) was purified by preparative TLC on silica gel 60 F_{254} using 10% EtOAc in CH_2Cl_2 as developing solvent to afford **2** (4.0 mg).

*4.3.1. 8″,9″-Dihydrowelwitschin H (**1**)*

Dark green solid; $[\alpha]_D^{25}$ + 3.4 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 265 (3.59), 281 (3.51) nm; IR (thin film) ν_{max}: 3058, 2929, 2850, 1610, 1502, 1460, 1346, 1242, 1205, 1103, 1060, 970, 698 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRTOFESIMS: *m/z* 619.2322 $[M+Na]^+$ (calcd for $C_{36}H_{36}O_8Na^+$, 619.2302).

*4.3.2. Uvarin A (**2**)*

Pale yellow solid; $[\alpha]_D^{25}$ + 5.9 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 267 (3.66), 284 (3.57), 293 (3.40) nm; IR (KBr) ν_{max}: 3406, 2935, 2839, 1674, 1606, 1502, 1460, 1346, 1244, 1132, 1114, 964, 692 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRTOFESIMS: *m/z* 635.2232 $[M+Na]^+$ (calcd for $C_{36}H_{36}O_9Na^+$, 635.2252).

*4.3.3. Uvarin B (**3**)*

Pale yellow solid; $[\alpha]_D^{25}$ + 3.6 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 271 (3.50), 293 (3.22) nm; IR (thin film) ν_{max}: 3456, 2935, 2839, 1679, 1604, 1502, 1461, 1346, 1205, 1116, 692 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRTOFESIMS: *m/z* 635.2238 $[M+Na]^+$ (calcd for $C_{36}H_{36}O_9Na^+$, 635.2252).

*4.3.4. Uvarin C (**4**)*

Pale green solid; $[\alpha]_D^{25}$ + 3.2 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 267 (3.82), 284 (3.75), 292 (3.60) nm; IR (thin film) ν_{max}: 3409, 2935, 2839, 1677, 1612, 1504, 1460, 1344, 1207, 1134, 1109, 974, 696 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRTOFESIMS: *m/z* 635.2270 $[M+Na]^+$ (calcd for $C_{36}H_{36}O_9Na^+$, 635.2252).

*4.3.5. 2-Hydroxy-3-methoxy-6-(4′-hydroxyphenyl)naphthalene (**5**)*

Pale red solid; UV (MeOH) λ_{max} (log ε): 273 (3.22), 295 (3.30), 334 (2.99) nm; IR (KBr) ν_{max}: 3411, 2931, 2850, 1615, 1540, 1334, 1250, 1129, 763, 650 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3; HRTOFESIMS *m/z*: 265.0872 $[M-H]^-$ (calcd for $C_{17}H_{13}O_3$ 265.0870).

4.4. Antimycobacterial assay

Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997) Standard drugs, isoniazid and rifampicin, were used as the reference compounds.

4.5. Antiplasmodial assay

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen (1976). Quantitative assessments of malarial activity *in vitro* were determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. (1979). The inhibition concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of $[^3H]$-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (Table 4).

4.6. Cytotoxicity assay

Cytotoxic assays against human epidermoid carcinoma (KB), human breast cancer (MCF-7) and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method described by Skehan et al. (1990). The reference substances were ellipticine and doxorubicin.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2016.12.009

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