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Graphical Abstract

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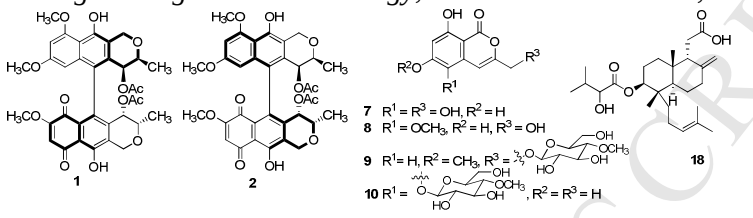

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ABSTRACT

Eleven new compounds, including two quinone derivatives of bioxanthracene, conoideocrellones A (**1**) and B (**2**), two bioxanthracenes **3** and **4**, four isocoumarins and isocoumarin glycosides **7**–**10**, two phenolic compounds **16** and **17**, and a diterpenoid compound, conoideocin A (**18**), were isolated from culture of the scale-insect pathogenic fungus *Conoideocrella tenuis* BCC 44534. Seventeen known compounds, compounds **5** and **6**, ES-242-2 and its atropisomer, isocoumarins and isocoumarin glycosides **11**–**15**, 3,4,6-trihydroxymellein, *cis*-4,6-dihydroxymellein, metarhizins A (**19**) and B (**20**), BR-050 (**21**), 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol, zeorin, and conoideocrellide A, were also isolated from this fungus. Structures of these compounds were elucidated by NMR and MS data analyses. Compound **4** was active against *Plasmodium falciparum* K1 (IC_{50} 6.6 μg/mL), while it did not show cytotoxicity. Conoideocrellone A (**1**) and compounds **3** and **7** exhibited cytotoxic activity, while conoideocin A (**18**) showed broad range of biological activities including antimalarial, antibacterial, and cytotoxic activities.

1. Introduction

Insect pathogenic fungi are known to be a good source of various biologically active compounds.[1,2] *Conoideocrella* is a small genus belonging to the Clavicipitaceae family, which specifically attacks scale-insects. There are only two currently described species, *Conoideocrella luteorostrata* and *C. tenuis*, formerly named as *Torrubiella luteorostrata* and *T. tenuis*, respectively.[3] Most of reported chemical studies on this genus are those from our group, employing many strains isolated at various locations in Thailand, and deposited and preserved in the BIOTEC Culture Collection. Our studies have revealed that this genus is a rich source of bioactive secondary metabolites with diverse structures.[4-7] Examples of the isolated compounds are isocoumarin glycosides from *T. tenuis* BCC 12732,[6] paecilodepsipeptide A and naphthopyrone glycoside from *T. luteorostrata* BCC 9617,[4] hopane triterpenes and bioxanthracenes from *C. tenuis* BCC 18627,[5] and oxanthracenes from *C. luteorostrata* BCC 31648.[7] In continuation of our study, we found an extract from *C. tenuis* BCC 44534 that showed a prolific chemical profiles from HPLC and ^{1}H NMR spectra and exhibited antimalarial activity against *Plasmodium falciparum* K1 (IC_{50} 9.53 μg/mL) and cytotoxic activity against human small-cell lung cancer (NCI-H187, IC_{50} 4.60 μg/mL) cell lines. ~~The chem~~ical investigation led to the isolation of two new quinone derivatives of bioxanthracene, conoideocrellones A (**1**) and B (**2**), two new bioxanthracenes **3** and **4**, four new isocoumarins and isocoumarin glycosides **7**–**10**, two new phenolic compounds **16** and **17**, and a new diterpenoid compound, conoideocin A (**18**) (Fig. 1), together with seventeen known compounds, including four bioxanthracenes, compounds **5** and **6**,[5,7] ES-242-2[8,9] and its atropisomer,[5] five isocoumarins and isocoumarin glycosides **11**–**15**,[10,6,11] two mellein derivatives, 3,4,6-trihydroxymellein[12] and *cis*-4,6-dihydroxymellein,[13] three pyrone-diterpene hybrids, metarhizins A (**19**) and B (**20**),[14] and BR-050 (**21**),[15] two triterpenes, 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol[16] and zeorin,[17] and conoideocrellide A.[5] Details of the isolation, structure elucidation, and biological activities of these compounds are presented herein.

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2. Results and discussion

The molecular formula of conoideocrellone A (**1**), $C_{35}H_{34}O_{13}$, was determined by HRESIMS in combination with ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum showed similarity to those of known co-metabolite ES-242-2.[8,9] Notable differences were the down field shift of one phenolic proton (δ_H 13.43) and the absence of one methoxy group and one of the *meta*-coupled aromatic proton. The ^{13}C and 2D NMR spectroscopic data (in

acetone-d_6) revealed that conoideocrellone A was a non-symmetrical C-10−C-10' dimer, wherein one of the monomeric unit was determined to be the same as that of ES-242-2. The presence of two carbonyl groups in the ^{13}C NMR spectrum and the HMBC correlations from H-7' to C-5'/C-6'/C8'/C-8a' established ring A of another monomeric unit as a quinone (Fig. 2). The H-bonding between one of the quinone carbonyl and 9'-OH resulting in the downfield chemical shifts of this quinone carbonyl carbon (C-8', δ_C 192.7) and the phenolic hydroxyl proton (9'-OH, δ_H 13.43). The *cis*-relationship between CH_3-11 and the acetoxy group, the same as known ES-242s, was deduced on the basis of NOESY analysis and the coupling constants. Thus, the correlation between H-3 and methylene proton H-1 in the NOESY spectrum suggested the pseudoaxial orientation of H-3, and the presence of broad singlet (narrow peak width) of H-4 demonstrated the pseudoequatorial orientation of this proton. With the same logic, the relative configuration at C-3' and C-4' was determined in the same manner. The previous works[7-9] showed that the known bioxanthracenes (ES-242 derivatives) isolated from invertebrate pathogenic fungi with a 4-acetoxy or 4-hydroxy group in a monomeric oxanthracene unit, including ES-242-2 and its atropisomer (co-metabolites in the present study), possess 3*S*,4*S* absolute configuration. Accordingly, it is possible that **1** should also share the same absolute configuration, 3*S*,4*S*,3'*S*,4'*S*. The intense cross peak between H-4 and H-4' in the NOESY spectrum suggested the same helicity of the chiral axis as that of ES-242-2 (Fig. 2).

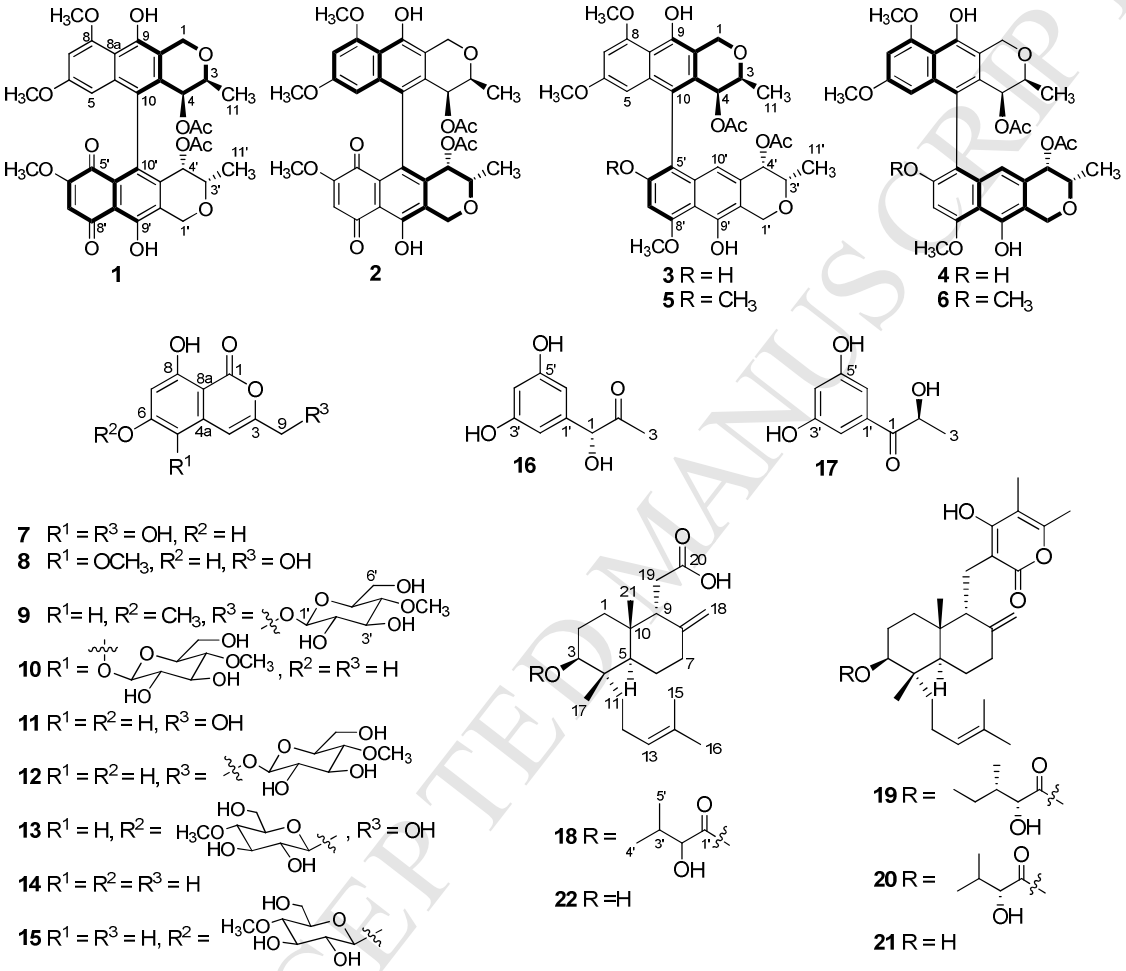

Fig. 1. Chemical structures of compounds **1** – **21**

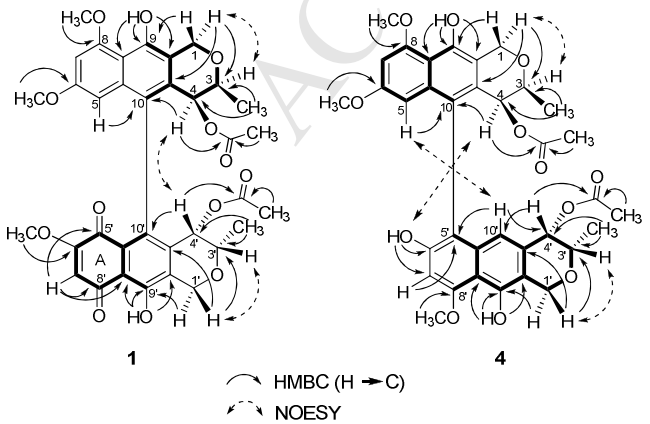

Fig. 2. Selected HMBC and NOESY correlations of compounds **1** and **4**

Conoideocrellone B (**2**) possessed the same molecular formula as conoideocrellone A (**1**). The UV, IR as well as ^{1}H and ^{13}C NMR spectra of both compounds were almost identical (Table 1). Detailed analysis of 2D spectroscopic data, in particular HMBC correlation, established the same structural feature to that of conoideocrellone A. The relative configurations at C-3/C-4 and C-3'/C-4' were also deduced to be identical to conoideocrellone A (**1**) and other ES-242 derivatives (*cis* relation between CH_3-11 and 4-OAc) on the same basis as previously described for **1**. Conoideocrellide B (**2**) should share the same absolute configuration with **1** and other known ES-242 derivatives, 3*S*,4*S*,3'*S*,4'*S*. Therefore it should be the atropisomer of **1,** which differ in the configuration at C-10–C-10' junction. This configurational assignment was confirmed by the intense NOESY correlation between H-5 and H-4' (Fig. 1).

HRESIMS data of compounds **3** and **4** deduced the same molecular formula as $C_{35}H_{36}O_{12}$. Their ^{1}H NMR spectra showed

good similarity to those of known C-10−C-5' dimers **5**[5] and **6**,[7] respectively, except for the presence of an additional phenolic hydroxyl group replacing a methoxy group. By analysis of 2D NMR spectroscopic data, the planar structures of compounds **3** and **4** were established to be the same. The HMBC correlations from an additional hydroxyl proton to C-6' in both compounds indicated the hydroxyl group at C-6'. The resonance as a broad singlet of H-4/H-4' suggested the same relative configurations at C-3/C-4 and C-3'/C-4' of both compounds to those of other ES-242s. The difference between compounds **3** and **4** was determined by the analysis of NOESY correlations. The cross peak between H-4 and H-10' in NOESY spectrum of compound **3** indicated the same helicity of the chiral axis as that of **5**, while the intense correlations from H-5 to H-10' and H-4 to OH-6' in NOESY spectrum of compound **4** determined the opposite axial stereochemistry to that of compound **3**. The selected HMBC and NOESY correlations of compound **4** are presented in Fig. 2.

Table 1. NMR spectroscopic data for compounds **1**–**4**

Position	**1** (in acetone-d_6)[a]		**2** (in acetone-d_6)[a]		**3** (in acetone-d_6)[b]		**4** (in acetone-d_6)[a]	
	δ_C	δ_H mult(*J* in Hz)	δ_C	δ_H mult(*J* in Hz)	δ_C	δ_H mult(*J* in Hz)	δ_C	δ_H mult(*J* in Hz)
1	65.3	4.70 d (15.5) 5.08 d (15.5)	66.0	4.72 d (15.6) 4.99 d (15.6)	64.5	4.72 d (15.6) 5.07 d (15.6)	65.5	4.64 d (15.5) 5.09 d (15.5)
3	73.5	3.81-3.90 m	73.7	3.60-3.64 m	72.9	3.72 dq (1.6, 6.5)	73.9	3.80 dq (0.7, 6.3)
4	67.7	5.30 brs	66.4	5.31 brs	66.9	5.19 d (1.6)	67.2	5.56 d (0.7)
4a	129.1	-	131.1	-	133.9	-	134.5	-
5	98.7	6.11 s	96.8	6.07 s	98.0	6.13 s	99.2	6.10 s
6	158.4	-	158.9	-	157.9	-	158.5	-
7	98.5	6.61 s	98.6	6.57 s	98.0	6.64 s	98.5	6.64 s
8	158.5	-	158.5	-	157.6	-	158.5	-
8a	111.5	-	111.6	-	110.8	-	111.3	-
9	150.3	-	150.7	-	149.9	-	150.3	-
9a	116.0	-	116.9	-	115.5	-	116.6	-
10	125.6	-	125.2	-	121.9	-	123.6	-
10a	128.9	-	128.2	-	136.3	-	136.8	-
11	17.0	0.99 d (6.4)	16.7	0.99 d (6.4)	16.4	0.96 d (6.5)	17.2	1.01 d (6.3)
1'	64.8	4.76 d (17.5) 5.10 d (17.5)	65.1	4.77 d (17.6) 5.01 d (17.6)	63.8	4.62 d (15.4) 5.01 d (15.4)	64.9	4.60 d (15.4) 5.02 d (15.4)
3'	73.8	3.81-3.90 m	73.6	3.71-3.75 m	71.9	3.86 dq (2.0, 6.0)	73.0	3.80 dq (0.7, 6.3)
4'	65.5	5.35 brs	64.8	5.17 brs	68.4	5.52 d (2.0)	69.1	5.52 d (0.7)
4a'	140.4	-	141.0	-	133.4	-	133.2	-
5'	177.8	-	179.4	-	109.7	-	110.6	-
6'	162.9	-	162.8	-	153.4	-	153.3	-
7'	108.9	6.28 s	109.1	6.27 s	98.5	6.73 s	99.2	6.77 s
8'	192.7	-	192.7	-	157.8	-	158.2	-
8a'	114.9	-	114.9	-	110.3	-	111.0	-
9'	158.2	-	158.7	-	149.6	-	150.4	-
9a'	135.2	-	135.3	-	114.0	-	114.6	-
10'	132.9	-	133.0	-	115.4	6.37 s	117.9	6.34 s
10a'	136.1	-	134.5	-	135.6	-	136.7	-
11'	17.2	0.96 d (6.5)	16.8	0.88 d (6.4)	15.8	1.10 d (6.0)	17.0	1.10 d (6.3)
4-O*CO*CH_3	169.7	-	169.9	-	168.7	-	169.4	-
4-OCO*CH_3*	20.3	1.63 s	20.3	1.84 s	19.7	1.96 s	19.3	1.04 s
6-OCH_3	55.5	3.56 s	55.5	3.52 s	54.5	3.45 s	55.3	3.42 s
8-OCH_3	57.0	4.16 s	56.9	4.15 s	56.2	4.17 s	56.8	4.18 s
9-OH	-	9.55 s	-	9.55 s	-	9.67 s	-	9.63 s
4'-O*CO*CH_3	168.7	-	169.1	-	169.3	-	170.6	-
4'-OCO*CH_3*	19.1	1.06 s	20.3	1.84 s	19.4	1.62 s	20.7	1.92 s
6'- OCH_3/OH	57.3	3.86 s	57.3	3.86 s	-	7.43 s	-	7.84 s
8'-OCH_3	-	-	-	-	56.2	4.18 s	57.0	4.18 s
9'-OH	-	13.43 s	-	13.45 s	-	9.59 s	-	9.58 s

[a] 500 MHz for ^1H and 125 MHz for ^{13}C
[b] 400 MHz for ^1H and 100 MHz for ^{13}C

Table 2. NMR spectroscopic data for compounds **7**–**10** (500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position	**7** (in acetone-d_6)		**8** (in acetone-d_6)		**9** (in DMSO-d_6)		**10** (in DMSO-d_6)	
	δ_C	δ_H mult(J in Hz)	δ_C	δ_H mult(J in Hz)	δ_C	δ_H mult(J in Hz)	δ_C	δ_H mult(J in Hz)
1	165.9	-	166.6	-	165.5	-	165.2	-
3	155.6	-	158.0	-	153.8	-	154.0	-
4	97.8	6.89 s	98.3	6.83 s	106.1	6.84 s	99.5	6.98 s
4a	124.5	-	135.4	-	139.5	-	131.8	-
5	131.7	-	135.8	-	102.7	6.62 d (2.2)	131.4	-
6	153.6	-	159.2	-	167.2	-	158.0	-
7	101.5	6.48 s	103.3	6.49 s	101.6	6.57 d (2.2)	101.9	6.41 s
8	156.8	-	160.6	-	163.3	-	159.3	-
8a	97.8	-	98.3	-	100.3	-	97.1	-
9	60.3	4.41 d (0.6)	61.2	4.43 s	66.5	4.42 d (13.9) 4.58 d (13.9)	19.0	2.23 s
1'	-	-	-	-	102.9	4.29 d (7.8)	106.0	4.48 d (7.8)
2'	-	-	-	-	74.3	3.01-3.07 m	74.0	3.31-3.35 m
3'	-	-	-	-	77.1	3.26-3.32 m	75.5	3.38-3.42 m
4'	-	-	-	-	80.0	2.95 t (9.3)	78.9	3.07 t (9.3)
5'	-	-	-	-	76.4	3.14-3.17 m	75.8	3.24 ddd (1.9, 4.8, 9.3)
6'	-	-	-	-	61.2	3.46-3.51 m 3.60-3.64 m	60.3	3.49-3.53 m 3.59-3.62 m
5-OH/OCH_3	-	-	61.9	3.79 s	-	-	-	-
6- OH/OCH_3	-	-	-	-	56.6	3.86 s	-	-
8-OH	-	10.75 s	-	10.92 s	-	10.95 s	-	10.87 s
9-OH	-	-	-	4.74 brs	-	-	-	-
2'-OH	-	-	-	-	-	5.29 d (4.9)	-	-
3'-OH	-	-	-	-	-	5.19 d (5.2)	-	5.43 d (5.8)
4'- OCH_3	-	-	-	-	60.3	3.42 s	59.6	3.44 s
6'-OH	-	-	-	-	-	4.71 brt (5.7)	-	4.75 brt (5.7)

Compound **7** was obtained as a light brown solid, possessing the molecular formula $C_{10}H_8O_6$ as determined by HRESIMS. The ^{1}H NMR spectrum showed one D_2O exchangeable phenolic proton (when a few drop of D_2O was added), two aromatic protons, and two oxygenated methylene protons (Table 2). The presence of a conjugated lactone functional group was indicated by the signal at δ_C 165.9 in the ^{13}C NMR spectrum, corresponding to the absorption band at 1681 cm^{-1} in IR spectrum.[5,6] The HMBC correlations from H-4 to C-3/C-4a/C-5/C-8a/C-9, H-7 to C-5/C-6/C-8/C8a, and H-9 to C-3/C-4 revealed the 3-methylisocoumarin unit. The hydroxyl group at C-5, C-6, C-8 and C-9 were established on the basis of the molecular formula and their chemical shifts. Thus, compound **7** was determined as 3-hydroxymethyl-5,6,8-trihydroxy isocoumarin.

The ^{1}H NMR spectrum of compound **8** showed a resemblance to compound **7** except for the presence of an additional methoxy signal at δ_H 3.79. The molecular formula $C_{11}H_{10}O_6$ as deduced by HRESIMS also indicated an atomic mass 14 units higher than that of **7**. The correlation form the methoxy protons to C-5 in HMBC spectrum suggested the 5-*O*-methyl derivative of **7**. Compound **8** was, therefore, determined to be 6,8-dihydroxy-3-hydroxymethyl-5-methoxyisocoumarin.

The ^{1}H and ^{13}C NMR spectra of compound **9** were similar to those of the known co-metabolite **12**.[6] The molecular formula $C_{18}H_{22}O_{10}$ as established by HRESIMS suggested that **9** has an additional methyl group as compared to **12**. The additional methoxy signal at δ_H 3.86 in ^{1}H NMR spectrum was indicated to present at C-6 instead of the hydroxyl group in **12** on the basis of HMBC correlation analysis. The identical sugar moiety to that of **12** was assigned as 4'-*O*-methyl-*β*-glucopyranose. It was suggested to attached at C-9 by the HMBC correlation from an anomeric proton (H-1', δ_H 4.29) to C-9. The large coupling constants of $J_{1',2'}$ = 7.8 Hz, $J_{3',4'}$ = $J_{4',5'}$ = 9.3 Hz confirmed the axial orientation of all methine protons (H-1' to H-5') in the sugar unit. The correlation from methoxy protons (4'- OCH_3, δ_H 3.42) to C-4' indicated the methoxy group at C-4'. The D configuration of 4'-*O*-methyl-*β*-glucopyranose was established by comparison of the specific rotations of the aqueous layer of its acid hydrolysate ($[\alpha]^{25}_D$ +55.4, *c* 0.08, MeOH) with that of 4-*O*-methyl-D-glucopyranose ($[\alpha]^{25}_D$ +80, *c* 1.30, MeOH).[18] The ^{1}H NMR spectrum of the aglycone unit, obtained by acid hydrolysis of **9**, was identical to that of cytogenin.[19] Therefore compound **9** was determined as 9-*O*-(4'-*O*-methyl-*β*- D-glucopyranosyl)cytogenin.

The molecular formula of compound **10** was deduced to be $C_{17}H_{20}O_{10}$ by HRESIMS in combination with ^{13}C NMR spectroscopy. The ^{1}H and ^{13}C NMR spectra revealed the closely related structure of **10** and the known co-metabolite **14** except for the presence of the sugar unit and the missing of one of the *meta*-coupled aromatic proton. The HMBC correlation from an anomeric proton (H-1') to C-5 suggested the linkage of the sugar

moiety to C-5. The 2D NMR spectroscopic data of the sugar unit were in good agreement with those of the sugar moiety in **9**. Comparison of the specific rotation of the aqueous layer of hydrolysate ($[\alpha]^{23}_D$ +58.7, *c* 0.08, MeOH) with that of 4-*O*-methyl-D-glucopyranose ($[\alpha]^{25}_D$ +80, *c* 1.30, MeOH)[18] indicated its D configuration.

Table 3. NMR spectroscopic data for compounds **16** and **17** (500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position	**16** (in DMSO-d_6)		**17** (in DMSO-d_6)	
	δ_C	δ_H mult(*J* in Hz)	δ_C	δ_H mult(*J* in Hz)
1	79.2	4.81 d (3.6)	201.5	-
2	208.4	-	68.7	4.90, dq (6.7)
3	24.8	2.00 s	20.8	1.24 d (6.7)
1'	141.2	-	136.6	-
2', 6'	104.6	6.23 d (1.6)	106.4	6.81 d (2.1)
3', 5'	158.4	-	158.5	-
4'	101.9	6.11 d (1.6)	107.2	6.44 d (2.1)
1-OH	-	5.83 d (3.6)	-	-
2-OH	-	-	-	5.19 d (6.7)
3', 5'-OH	-	9.26 s	-	9.61 s

The HRMS data of compounds **16** and **17** indicated the same molecular formula as $C_9H_{10}O_4$. The ^{1}H and ^{13}C NMR spectra were also similar (Table 3), which showed the presence of three hydroxyl groups, 1,3,5-trisubstitued benzene ring, one oxygenated methine proton, one methyl and one carbonyl group. Detailed analysis of 2D spectroscopic data established 1-(3,5-dihydroxyphenyl)-1-hydroxypropane-2-one and 1-(3,5-dihydroxyphenyl)-2-hydroxypropane-1-one for **16** and **17**, respectively. The assignment of these two compounds were assured by the HMBC correlations, depicted as shown in Fig. 3. The absolute configuration at C-1 of **16** was determined as *R* by comparing the optical rotation ($[\alpha]^{23}_D$ −230.0, *c* 0.002, MeOH) to those of phenylhydroxypropanone analogues, e.g. −181 for (*R*)-(–)-1-hydroxy-1-phenylpropane-2-one and −225 for (*R*)-(–)-1-(4-hydroxyphenyl)-1-hydroxypropane -2-one.[20] On the same basis, comparison of the optical rotation of **17** ($[\alpha]^{25}_D$ −81.1, *c* 0.01, MeOH) to those of similar derivatives, e.g. −83 for (*S*)-(–)-2-hydroxy-1-phenylpropane-1-one and −79 for (*S*)-(–)-2-hydroxy-1-(4-methoxyphenyl)propane -1-one,[21] deduced *S* configuration at C-2.

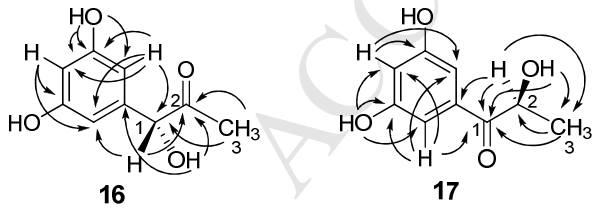

Fig. 3. Selected HMBC correlations of compounds **16** and **17**

Conoideocin A (**18**) with the molecular formula $C_{26}H_{42}O_5$ by HRESIMS was obtained as a pale yellow solid. The IR spectrum displayed major absorption bands at 3432 and 1723 cm^{-1} corresponding to a hydroxyl and a carbonyl group, respectively. The ^{1}H and ^{13}C NMR spectra showed the presence of two carbonyl, six methyl, eight methylene, and six methine groups. The COSY correlations observed for H-1–H-2–H-3 and H-5–H-6–H-7 together with the HMBC correlations from H_2-11 and H_3-17 to C-3/C-4/C-5, H_3-21 to C-1/C-5/C-9/C-10, and H_2-18 to C-7/C-8/C-9 established a substituted decalin skeleton. The existence of the prenyl unit at C-11 was deduced by the correlations between H-11, H-12, and H-13 in COSY spectrum and the correlations from H-5 and H_3-17 to C-11, H_3-15 and H_3-16 to C-13/C-14 in HMBC spectrum. The 2-hydroxy-3-methylbutanoyloxy moiety at C-3 was indicated by the HMBC correlations from H-3 and H-2' to C-1' and H_3-4' and H_3-5' to C-2'/C-3'. The correlations from H-9 to C-20 and H_2-19 to C-8/C-9/C-10/C-20 suggested an acetyl group at C-9. The observed NOESY correlations, presented in Fig. 4, showed the resemblance relative configurations of decalin system to those of the known co-metabolites **19**–**21**. The absolute configurations at C-3, C-4, C-5, C-9, and C-10 were determined by converting conoideocin A (**18**) into **22** by metholysis with NaOMe. The similar specific rotation of **22** ($[\alpha]^{24}_D$ −44.9, *c* 0.03, MeOH) to that of BR-050 (**21**, $[\alpha]^{24}_D$ −32.1, *c* 0.03, MeOH)[14] indicated the same absolute configurations of these two compounds. Many attempts to converted conoideocin A (**18**) to (*R*)- and (*S*)-MTPA esters failed, no desired products were found in the reaction. Thus, the absolute configuration at C-2' could not be determined.

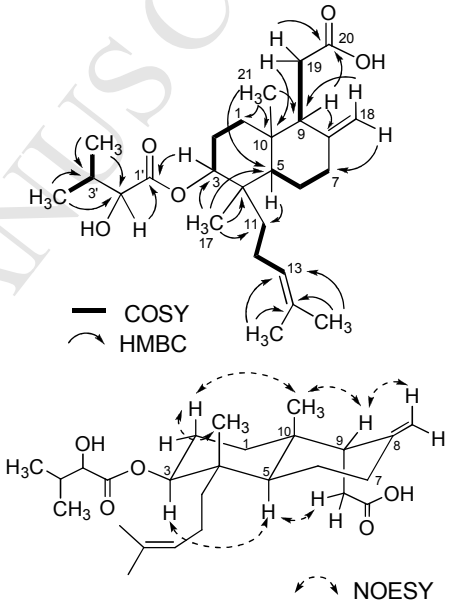

Fig. 4. Selected COSY, HMBC and NOESY correlations of compounds **18**

The structures of seventeen known compounds, compounds **5**–**6**,[5,7] ES-242-2,[8,9] atropisomer of ES-242-2,[5] isocoumarins and isocoumarin glycosides **11**–**15**,[10,6,11] 3,4,6-trihydroxymellein[12] *cis*-4,6-dihydroxymellein,[13] metarhizins A (**19**) and B (**20**),[14] BR-050 (**21**),[15] 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol,[16] zeorin,[17] and conoideocrellide A,[5] were elucidated by analysis of their spectroscopic and physical property data which were in good agreement with those appeared in the literature data.

All new compounds apart from **2** and **8**, due to the sample shortage, were tested for antibacterial (*Bacillus cereus*), antimalarial (*P. falciparum* K1), and cytotoxicity against KB, MCF-7, NCI-H187, and Vero cells (Table 4). Cyclohexadepsipeptides and most of isocoumarins and isocoumarin glycosides, including new compounds **9** and **10**, were inactive in our biological assays at the maximum tested concentrations,[5,6] however, compound **7** was moderately active to NCI-H187 and Vero cells (IC_{50} 27.7 and 9.6 µg/mL, respectively). Bioxanthracenes are known to exhibit antimalarial activity[22,23] and a new analogue **4** also showed activity against *P. falciparum* K1 (IC_{50} 6.6 µg/mL) without cytotoxicity,

while conoideocrellone A (**1**) and compound **3** had no antimalarial activity but exhibited cytotoxic activity. Conoideocin A (**18**), was active against *P. falciparum* (IC_{50} 6.6 µg/mL), *B. cereus* (MIC 25 µg/mL), and human cancer cell lines KB and NCI-H187 (IC_{50} 25.8 and 47.4 µg/mL, respectively). Pyrone diterpene compounds **19**-**21** were reported to exhibit antimalarial and antiproliferative activity on both insect and human cancer cell lines.[14,24] Compounds **16** and **17** were not active in our biological assays.

Table 4. Biological activities of compounds **1**, **3**, **4**, **7**, **9**, **10** and **16**–**18**

compound	*P. falciparum*, IC_{50} (µg/mL)	*B. cereus*, MIC (µg/mL)	cytotoxicity, IC_{50} (µg/mL)			
			KB	MCF-7	NCI-H187	Vero
Conoideocrellone A (**1**)	>10	>50	29.1	11.5	7.3	23.6
Compound **3**	>10	>50	31.4	43.0	28.1	>50
Compound **4**	6.6	>50	>50	>50	>50	>50
Compound **7**	>10	>50	>50	>50	27.7	9.6
Compound **9**	>10	>50	>50	>50	>50	>50
Compound **10**	>10	>50	>50	>50	>50	>50
Compound **16**	>10	>50	>50	>50	>50	>50
Compound **17**	>10	>50	>50	>50	>50	>50
Conoideocin A (**18**)	6.6	25.0	25.8	>50	47.4	>50
Dihydroartemisinin[a]	0.0010	-	-	-	-	-
Mefloquine hydrochloride[a]	0.020	-	-	-	-	-
Vancomycin hydrochloride[b]	-	2.0	-	-	-	-
Doxorubicin hydrochloride[c]	-	-	0.5	6.9	0.1	-
Ellipticine[c]	-	-	2.8	-	4.0	1.9

[a]Positive control for the antimalarial assay. [b]Positive control for the antibacterial assay. [c]Positive control for the cytotoxicity assay.

3. Conclusion

In conclusion, the present study further demonstrated that the genus *Conoideocrella* is a potent source of the diverse structures of bioactive secondary metabolites. Various types of compounds, including isocoumarins and isocoumarin glycosides, bioxanthracenes, cyclohexadepsipeptides, diterpenes, and triterpenes, were reported from the different strains of the fungi in this genus. Interestingly, all these type of compounds could be isolated from the strain in this study, BCC 44534, and the quinone derivatives of bioxanthracene, conoideocrellone A (**1**) and B (**2**), were reported here for the first time. Compound **4** was active against *P. falciparum*, conoideocrellone A (**1**) and compounds **3** and **7** exhibited cytotoxic activity while conoideocin A (**18**) showed various activities including antimalarial, antibacterial, and cytotoxic activities.

4. Experimental

4.1. General procedures

Melting points were measured using a Metler MP90 melting point apparatus. Optical rotation measurements were conducted by using a JASCO P-1030 digital polarimeter. UV and FT-IR spectra were recorded on an Analytic Jena Spekol 1200 spectrophotometer and a Bruker Alpha spectrometer. NMR spectra were recorded on Bruker AV400 and Bruker AV500D spectrometers. ESITOF MS data were obtained on a Bruker micrOTOF mass spectrometer. Column chromatography was performed on silica gel 60 (70-230 Mesh ASTM, Merck). HPLC were performed using Dionex-Ultimate 3000 series equipped with a binary pump, an autosampler, and a diode array detector.

4.2. Fungal material

The fungus was isolated from a scale-insect (Hemiptera), collected in Khao Yai National Park, Nakhon Ratchasima Province, Thailand. The living culture was deposited in the BIOTEC Culture Collection as BCC 44534 on November 12, 2010. This fungus was identified as *Conoideocrella tenuis* of the order Hypocreales, family Clavicitaceae, on the basis of morphology, by Ms. Kanoksri Tasanathai, BIOTEC, and the partial nuclear large subunits rDNA (nc28S) (GenBank accession number MG198773), the internal transcribed spacer (ITS1-5.8S-ITS2) rDNA (GenBank accession number MG230251), and the partial translation elongation factor 1-alpha region (EF-1alpha) (GenBank accession number MG230541), analysed by Dr. Nattawut Boonyuen, BIOTEC.

4.3. Fermentation and isolation

The fungus BCC 44534 was maintained on potato dextrose agar at 25°C. The agar was cut into pieces (1×1 cm) and inoculated into 8×250-mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB, potato starch 4.0 g/L, dextrose 20.0 g/L). After incubation at 25°C for 7 days on a rotary shaker (200 rpm), each primary culture was transferred into 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated under the same conditions for 5 days. Each 25 mL portion of the secondary culture was transferred into 40 × 1 L Erlenmeyer flasks containing 250 mL of M102 medium [40 × 250 mL, composition: sucrose (30.0 g/L), malt extract (20.0 g/L), bacto-peptone (2.0 g/L), yeast extract (1.0 g/L), KCl (0.5 g/L), $MgSO_4.7H_2O$ (0.5 g/L), and KH_2PO_4 (0.5 g/L)] and the fermentation was carried out under static conditions, 25 °C for 19 days.

After filtration of the mycelium, the cells were macerated in MeOH (2 L) for 3 days, followed by CH_2Cl_2 (2 L) for 3 days. The MeOH and CH_2Cl_2 extracts were combined and evaporated under reduced pressure. Water (500 mL) was added, and the mixture was extracted with hexanes (3×500 mL), followed by EtOAc (3×500 mL). The brown solid (6.46 g), obtained from EtOAc extract, was fractionated using Sephadex LH-20, eluted with 100% MeOH, to give seven fractions (A1–A7). Compound **11** (44.8 mg) was obtained from fraction A7. Further purification

of fraction A2 (815.4 mg) by preparative HPLC using reverse phase column (SunFire C18 OBD, 10 μm, 19×250 mm, step gradient elution with 40−95% MeCN/H_2O, flow rate 15.0 mL/min) afford compound **18** (8.5 mg). Fraction A3 (4.25 g) was subjected to silica gel column chromatography, using 50% EtOAc/hexanes as an eluent, to provide thirteen fractions (A3-1–A3-13). Zeorin (265.7 mg) and conoideocrellide A (2.2 g) were obtained, respectively, from fractions A3-4 and A3-13. Fraction A3-5 and A3-12 were purified by preparative HPLC, step gradient elution with 40−95% MeCN/H_2O, to furnish 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol (5.2 mg) and compound **5** (7.1 mg), respectively. Consecutive purification of fraction A3-6 by preparative HPLC, step gradient elution with 40−95% MeCN/H_2O, yielded compounds **19** (2.1 mg) and **20** (320.4 mg). Compounds **18** (17.3 mg), **20** (4.7 mg), **21** (3.3 mg), and atropisomer of ES-242-2 (6.4 mg) were obtained from fraction A3-7 after purification by preparative HPLC, step gradient elution with 60−95% MeCN/H_2O. Fraction A3-8 was subjected to preparative HPLC, step gradient elution with 60−80% MeCN/H_2O, to give compounds **18** (1.3 mg), **21** (3.5 mg), ES-242-2 (1.1 mg), and atropisomer of ES-242-2 (4.6 mg). Further purification of fraction A3-9 by preparative HPLC, step gradient elution with 50−90% MeCN/H_2O, afforded compound **3** (4.6 mg), ES-242-2 (48.6 mg), and atropisomer of ES-242-2 (20.5 mg). Fraction A3-10 was purified by preparative HPLC, step gradient elution with 50−70% MeCN/H_2O, followed by preparative thin layer chromatography, using 40% EtOAc/hexanes as an eluent, to provide compounds **3** (1.5 mg), **4** (0.9 mg), and ES-242-2 (16.3 mg). Compound **6** (4.5 mg) was obtained after trituration of fraction A3-11 with MeOH followed by filtration. Further purification of the filtrate yielded compound **5** (3.2 mg). Fraction A4 (141.5 mg) was subjected to preparative HPLC, step gradient elution with 5−100% MeCN/H_2O, to furnish compounds **1** (1.8 mg), **3** (3.5 mg), **4** (4.2 mg), **5** (3.2 mg), **12** (5.5mg), **15** (45.4 mg), conoideocrellide A (4.2 mg), ES-242-2 (4.0 mg), and atropisomer of ES-242-2 (7.1 mg). More of compound **12** (40.7 mg) was obtained after trituration of fraction A5 (213.7 mg) with MeOH followed by filtration. Consecutive purification of the filtrate with preparative HPLC, step gradient elution with 5−70% MeCN/H_2O, give compounds **10** (3.8 mg), **12** (32.4 mg), **13** (9.7 mg), **14** (5.5 mg), **15** (21.1 mg), and 3,4,6-trihydroxymellein (13.6 mg). Fraction F6 (32.9 mg) was further purified by preparative HPLC, step gradient elution with 10−70% MeCN/H_2O, to afford compounds **11** (11.5 mg) and **14** (5.7 mg). Purification of the crude hexanes extract (1.51 g) by silica gel column chromatography, using 100% CH_2Cl_2 as an eluent, followed by preparative HPLC, step gradient elution with 30−80% MeCN/H_2O, provided compounds **1** (2.6 mg), **2** (1.1 mg), **3** (7.8 mg), **5** (17.6 mg), **11** (3.3 mg), **15** (5.6 mg), **18** (17.6 mg), ES-242-2 (58.2 mg), atropisomer of ES-242-2 (50.5 mg), zeorin (24.1 mg), 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol (4.6 mg), and conoideocrellide A (24.7 mg).

The culture broth was then extracted with EtOAc (3×20 L) and evaporated to dryness, leaving a dark brown solid (5.56 g). The crude extract was pass through Sephadex LH-20 with MeOH as an eluent to give six fractions (B1–B6). Conoideocrellide A (6.4 mg) was obtained from fractions B1 after purification by preparative HPLC, step gradient elution with 40−65% MeCN/H_2O. Trituration of fraction B2 (4.3 g) with MeOH followed by filtration provided compound **12** (405.7 mg). The filtrate of fraction B2 was subjected to Sephadex LH-20, eluted with 100% MeOH, and then further purified by consecutive preparative HPLC to furnish compounds **3** (11.5 mg), **5** (33.7 mg), **8** (2.1 mg), **9** (19.6 mg), **10** (37.9 mg), **11** (16.4 mg), **12** (190.2 mg), **13** (20.9 mg), **14** (23.1 mg), **15** (217.0 mg), **16** (97.4 mg), **17** (42.1 mg), ES-242-2 (40.2 mg), atropisomer of ES-242-2 (7.3 mg), 3,4,6-trihydroxymellein (441.7 mg), *cis*-4,6-dihydroxymellein (15.3 mg), and conoideocrellide A (133.2 mg). Further purification of fraction B3 (382.2 mg) yielded compounds **7** (51.5 mg), **11** (202.5 mg), and **14** (13.3 mg).

*4.3.1 Conoideocrellone A (**1**).* Yellow solid; $[\alpha]^{23}_D$ −47.6 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.29), 293 (3.86), 339 (3.47), 355 (3.47), 423 (3.30), nm; IR (ATR) ν_{max} 3388, 2926, 2854, 1737, 1628, 1364, 1214, 1157, 1098 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 685.1890 $[M + Na]^+$ (calcd. for: $C_{35}H_{34}O_{13}Na$, 685.1892).

*4.3.2 Conoideocrellone B (**2**).* Yellow solid; $[\alpha]^{23}_D$ −129.9 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.41), 295 (4.01), 339 (3.72), 354 (3.75), 420 (3.60), nm; IR (ATR) ν_{max} 3386, 2926, 2854, 1732, 1627, 1366, 1216, 1158, 1099 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 685.1896 $[M + Na]^+$ (calcd. for: $C_{35}H_{34}O_{13}Na$, 685.1892).

*4.3.3 Compound **3**.* Light brown solid; dec. 241.6−243.2 °C; $[\alpha]^{25}_D$ +122.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.56), 296 (4.11), 310 (4.07), 341 (3.98), 355 (3.98), 425 (3.75), nm; IR (ATR) ν_{max} 3407, 2927, 2856, 1733, 1625, 1369, 1228, 1159, 1097 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 671.2090 $[M + Na]^+$ (calcd. for: $C_{35}H_{36}O_{12}Na$, 671.2099).

*4.3.4 Compound **4**.* Yellow solid; $[\alpha]^{25}_D$ −38.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.44), 295 (4.00), 307 (4.00), 340 (3.92), 355 (3.96), 426 (3.66), nm; IR (ATR) ν_{max} 3387, 2925, 2854, 1735, 1625, 1459, 1366, 1232, 1157, 1096 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 671.2097 $[M + Na]^+$ (calcd. for: $C_{35}H_{36}O_{12}Na$, 671.2099).

*4.3.5 Compound **7**.* Light brown solid; mp 222.8−224.0 °C; UV (MeOH) λ_{max} (log ε) 238 (4.35), 261 (4.10), 350 (4.04), 425 (3.55) nm; IR (ATR) ν_{max} 3430, 1681, 1638, 1630, 1479, 1295, 1176, 1080 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 247.0204 $[M + Na]^+$ (calcd. for: $C_{10}H_8O_6Na$, 247.0213).

*4.3.6 Compound **8**.* Light brown solid; UV (MeOH) λ_{max} (log ε) 240 (4.25), 258 (4.07), 335 (3.93), 422 (3.79) nm; IR (ATR) ν_{max} 3287, 2925, 2854, 1682, 1639, 1618, 1482, 1352, 1301, 1169, 1074 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 261.0364 $[M + Na]^+$ (calcd. for: $C_{11}H_{10}O_6Na$, 261.0370).

*4.3.7 Compound **9**.* Light brown solid; $[\alpha]^{25}_D$ −17.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.17), 276 (3.64), 321 (3.55) nm; IR (ATR) ν_{max} 3340, 2929, 2854, 1686, 1621, 1573, 1506, 1455, 1382, 1238, 1199, 1166, 1080 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 421.1116 $[M + Na]^+$ (calcd. for: $C_{18}H_{22}O_{10}Na$, 421.1105).

*4.3.8 Compound **10**.* . Light brown solid; mp 206.7−208.1 °C; $[\alpha]^{24}_D$ +23.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.25), 280 (3.81), 332 (3.71), 427 (3.19) nm; IR (ATR) ν_{max} 3350, 2970, 2928, 1738, 1685, 1617, 1481, 1445, 1366, 1229, 1217, 1073 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 407.0950 $[M + Na]^+$ (calcd. for: $C_{17}H_{20}O_{10}Na$, 407.0949).

*4.3.9 Compound **16**.* Light brown oil; $[\alpha]^{23}_D$ −230.0 (*c* 0.002, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.65), 285 (3.51), 423 (3.20) nm; IR (ATR) ν_{max} 3320, 2925, 2854, 1713, 1603, 1456, 1341, 1306, 1161 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 181.0505 $[M - H]^-$ (calcd. for: $C_9H_9O_4$, 181.0506).

*4.3.10 Compound **17***. Light brown oil; $[\alpha]^{25}_{D}$ −81.1 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.76), 259 (3.73), 417 (3.34) nm; IR (ATR) ν_{max} 3248, 2925, 2855, 1686, 1601, 1454, 1341, 1305, 1162 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 181.0507 $[M - H]^-$ (calcd. for: $C_9H_9O_4$, 181.0506).

*4.3.11 Conoideocin A (**18**)*. Pale yellow solid; $[\alpha]^{24}_{D}$ −4.1 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.18), 266 (3.11), 423 (2.34), nm; IR (ATR) ν_{max} 3432, 2938, 2876, 1723, 1454, 1387, 1257, 1216, 1177, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.01 (1H, brt, *J* = 6.9 Hz, H-13), 4.85 (1H, dd, *J* = 5.7, 10.2 Hz, H-3), 4.73 (1H, brs, Ha-18), 4.68 (1H, brs, Hb-18), 4.01 (1H, d, *J* = 3.4 Hz, H-2'), 2.63 (1H, dd, *J* = 4.1, 13.4 Hz, Ha-19), 2.37 (1H, dd, *J* = 9.7, 13.4 Hz, Hb-19), 2.30 (1H, dd, *J* = 4.1, 9.7 Hz, H-9), 2.23 (1H, m, Ha-7), 2.16 (1H, m, Hb-7), 2.04 (1H, m, H-3'), 1.93 (1H, m, Ha-12), 1.82 (1H, m, Hb-12), 1.77 (2H, m, H-2), 1.66 (3H, s, H-16), 1.58 (3H, s, H-15), 1.51 (1H, m, H-5), 1.48 (1H, m, Ha-6), 1.39 (1H, m, Hb-6), 1.29 (1H, m, Ha-11), 1.28 (2H, m, H-1), 1.15 (1H, m, Hb-11), 1.02 (3H, s, H-21), 1.01 (3H, d, *J* = 7.1 Hz, H-5'), 0.87 (3H, s, H-17), 0.86 (3H, d, *J* = 6.6 Hz, H-4'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C, C-20), 174.6 (C, C-1'), 146.8 (C, C-8), 131.7 (C, C-14), 124.0 (CH, C-13), 111.3 (CH_2, C-18), 77.6 (CH, C-3), 74.8 (CH, C-2'), 53.6 (CH, C-9), 40.0 (C, C-4), 39.5 (CH, C-5), 37.6 (CH_2, H-11), 37.1 (C, C-10), 34.2 (CH_2, C-1), 33.9 (CH_2, C-19), 32.2 (CH, C-3'), 30.6 (CH_2, C-7), 25.7 (CH_3, C-16), 23.9 (CH_2, C-2), 22.5 (CH_3, C-21), 22.5 (CH_2, C-12), 21.7 (CH_2, C-6), 18.6 (CH_3, C-5'), 18.1 (CH_3, C-17), 17.6 (CH_3, C-15), 16.1 (CH_3, C-4'); HRESIMS *m/z* 457.2933 $[M + Na]^+$ (calcd. for: $C_{26}H_{42}O_5Na$, 457.2924).

4.4. Hydrolysis of compound 9

Compound **9** (2.0 mg, 0.005 mmol) was hydrolyzed with 3M aqueous HCl (0.5 mL) at 90°C for 12 h. The reaction mixture was then diluted with H_2O (2.0 mL) and extracted with EtOAc (2.0 mL). The aqueous layer was concentrated in *vacuo* to yield 4-*O*-methyl-D-glucopyranose (0.78 mg, 80% yield, $[\alpha]^{25}_{D}$ +55.4, *c* 0.08, MeOH). The organic layer was evaporated to dryness under reduced pressure to obtain aglycone unit (0.83 mg, 75% yield) whose ^{1}H NMR spectrum was identical to those of cytogenin.[19]

4.5. Hydrolysis of compound 10

Compound **10** (2.0 mg, 0.005 mmol) was hydrolyzed by the method described for compound **9**. The aqueous layer was concentrated in *vacuo* to yield 4-*O*-methyl-D-glucopyranose (0.80 mg, 82% yield, $[\alpha]^{23}_{D}$ +58.7, *c* 0.08, MeOH). The organic layer was evaporated to dryness under reduced pressure to obtain aglycone unit (0.83 mg, 80% yield); ^{1}H NMR (400 MHz, acetone-d_6) δ 10.73 (1H, s, OH-8), 8.58 (1H, s, OH), 7.83 (1H, s, OH), 6.64 (1H, s, H-4), 6.42 (1H, s, H-7), 2.24 (3H, s, CH_3-9); HRESIMS *m/z* 207.0309 $[M - H]^-$ (calcd. for: $C_{10}H_7O_5$, 207.0299).

4.6. Methanolysis of compound 18

To a solution of **18** (6.3 mg, 0.0145 mmol) in MeOH (1.0 mL) was added NaOMe (18 mg, 0.33 mmol). The reaction mixture was left stirring at 50°C overnight then neutralized with 3M aqueous HCl, diluted with H_2O (4.0 mL), and extracted with EtOAc (4.0 mL). The organic layer was evaporated and the residue was subjected to preparative HPLC (SunFire C18 OBD, 5 µm, 4.6×150 mm, step gradient elution with 20−95% MeCN/H_2O, 35 min, flow rate 8.0 mL/min) to obtain **22** (0.33 mg, 0.001 mmol, 7% yield, $[\alpha]^{24}_{D}$ −44.9, *c* 0.03, MeOH); ^{1}H NMR (400 MHz, acetone-d_6) δ 5.12 (1H, brt, *J* = 7.2 Hz, H-13), 4.67 (1H, brs, Ha-18), 4.61 (1H, brs, Hb-18), 3.52 (1H, dd, *J* = 3.9, 10.4 Hz, H-3), 3.45 (1H, brs, OH-3), 2.63 (1H, dd, *J* = 9.5, 18.6 Hz, Ha-19), 2.30 (1H, dd, *J* = 9.5, 18.6 Hz, Hb-19), 2.20 (3H, m, H-7, H-9), 1.95 (2H, m, H-12), 1.70 (2H, m, H-2), 1.66 (3H, s, H-16), 1.62 (3H, s, H-15), 1.55 (1H, m, H-5), 1.37 (2H, m, H-6), 1.33 (2H, m, H-1), 1.22 (2H, m, H-11), 0.99 (3H, s, H-21), 0.78 (3H, s, H-17); HRESIMS *m/z* 357.2415 $[M + Na]^+$ (calcd. for: $C_{21}H_{34}O_3Na$, 357.2400).

4.7. Biological assays

Antimalarial activity against *P. falciparum* K1 was performed by using microculture radioisotope technique.[25] Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) was carried out by using the green fluorescent protein (GFP) based method.[26,27] The activities for antibacterial to *B. cereus* and anticancer against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay.[28]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:XXX. These data include NMR spectra of most important compounds described in this article.