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Graphical Abstract

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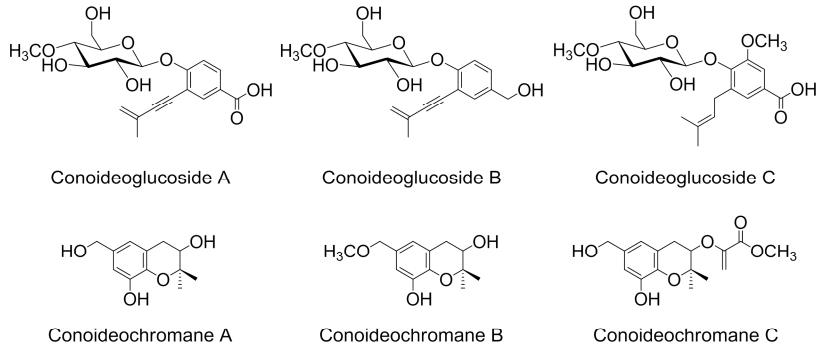

Phenolic glucosides and chromane analogs from the insect fungus *Conoideocrella krungchingensis* BCC53666

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Abstract

Six new compounds, named conoideoglucosides A – C and conoideochromanes A – C, together with eight known compounds, including eutypinic acid, 2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid, (–)-luteoskyrin, (–)-4a-oxyluteoskyrin, chrysophanol, islandicin, catenarin, and (22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol were isolated from the insect fungus *Conoideocrella krungchingensis* BCC53666. (–)-Luteoskyrin exhibited a broad range of antimicrobial activity such as antimalarial (IC_{50} 0.51 µg/mL), antitubercular (MIC 6.25 µg/mL), antibacterial (both Gram positive; MIC 0.39 – 1.56 µg/mL and Gram negative; MIC 3.13 – 12.50 µg/mL), and antifungal (against various plant pathogens; MIC 3.13 – 50.00 µg/mL) activities, while (–)-4a-oxyluteoskyrin and catenarin showed weaker antibacterial activity. Moreover, eutypinic acid, (–)-luteoskyrin, (–)-4a-oxyluteoskyrin, and catenarin showed cytotoxicity against NCI-H187 cells with IC_{50} in a range of 0.16 – 17.99 µg/mL, while eutypinic acid and catenarin had no cytotoxicity against non-cancerous (Vero) cells at maximum tested concentration (50 µg/mL). The complete NMR spectral data and biological activity of the known (–)-4a-oxyluteoskyrin was also reported for the first time.

Keywords. *Conoideocrella*; Conoideoglucosides; Conoideochromanes; (–)-4a-Oxyluteoskyrin; Biological activity

1. Introduction

The genus *Conoideocrella* is a member in the Clavicipitaceae family and has received considerable attention on the secondary metabolites due to the chemical diversity with a broad array of biological activities.[1-7] By far, only two species were identified, *Conoideocrella luteorostrata* and *C. tenuis*, which were formerly described as *Torrubiella luteorostrata* and *T. tenuis*, respectively.[8] Many compounds from *Conoideocrella* spp. were described along with their biological activities such as paecilodepsipeptide A and a naphthopyrone glycoside from *T. luteorostrata* BCC9617,[2] torrubiellutins A – C from *T. luteorostrata* BCC12904,[3] isocoumarin glucosides from *T. tenuis* BCC12732,[4] conoideocrellides A – D, hopane-type triterpenes and bioxanthracenes from *C. tenuis* BCC18627,[5] oxanthracenes and bioxanthracene analogs from *C. luteorostrata* Zimm. BCC31648,[6] and conoideocrellones A and B, conoideocin A, bioxanthracenes, and isocoumarin derivatives from *C. tenuis* BCC44534.[7] Interestingly, these compounds showed a broad range of biological activities such as antimalarial against *Plasmodium falciparum* (K1, multidrug-resistant strain),[5-7] antitubercular against *Mycobacterium tuberculosis* H37Ra,[5] antiviral against herpes simplex virus type-1 (HSV-1),[5] and antibacterial against *Bacillus cereus*[7] activities as well as cytotoxicity against cancerous (KB, MCF-7, and NCI-H187) and non-cancerous (Vero) cells.[3,5-7]

Based on the results from the chemical and biological screenings, the crude extracts from broth and mycelia of the fungus *Conoideocrella krungchingensis* BCC53666 contained several secondary metabolites detected by HPLC analyses and displayed antimalarial activity against *P. falciparum* (K1, multidrug-resistant strain) (IC_{50} 3.36 – >10 µg/mL), anti-plant pathogenic fungal activity against *Alternaria brassicicola* (MIC 12.50 – 25.00 µg/mL) and antibacterial activity against *B. cereus* (MIC 25.00 – >50 µg/mL) as well as cytotoxicity against cancerous

(MCF-7 and NCI-H187) and non-cancerous (Vero) cells (IC_{50} 3.34 – >50 µg/mL). The chemical investigation of constituents of extracts from cultures of the fungus BCC53666 were thus conducted and led to the isolation of six new compounds, including conoideoglucosides A – C (**1** – **3**) and conoideochromanes A – C (**5** – **7**), along with eight known compounds such as eutypinic acid (**4**), 2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**8**), (–)-luteoskyrin (**9**), (–)-4a-oxyluteoskyrin (**10**), chrysophanol (**11**), islandicin (**12**), catenarin (**13**), and (22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**14**). Moreover, the isolated compounds were tested for antimicrobial activity, e.g., antimalarial against *P. falciparum* (K1, multidrug-resistant strain), antitubercular against *M. tuberculosis* H37Ra, antifungal against *Candida albicans*, antibacterial against Gram-positive (*B. cereus*, *Enterococcus faecium*, *Staphylococcus aureus*) and Gram-negative (*Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria, and anti-plant pathogenic fungal against *A. brassicicola*, *Colletotrichum acutatum*, *Curvularia lunata*, *Magnaporthe grisea* activities, and for cytotoxicity against cancerous (MCF-7 and NCI-H187) and non-cancerous (Vero) cells.

2. Results and discussion

The fungus *Conoideocrella krungchingensis* BCC53666 was collected from an armored scale insect (Hemiptera: Diaspididae) at Khao Luang National Park, Nakhon Si Thammarat province, Thailand. It was cultivated in potato dextrose broth (PDB) under shaking condition at 200 rpm, 25 °C for 5 days. After separation of mycelia from broth by simple filtration, the broth was extracted with EtOAc, while the mycelia were macerated with organic solvents (MeOH and CH_2Cl_2), followed by evaporation and extraction with *n*-hexane and EtOAc, respectively. All

crude extracts were then chromatographed to provide fourteen compounds, including six new and eight known compounds.

Compound **1** was obtained as a yellow oil and gave the molecular formula $C_{19}H_{22}O_8$, deduced from the sodium-adduct mass ion peak at *m*/*z* 401.1208 $[M+Na]^+$ in the HRESIMS spectrum. Compound **1** displayed a broad hydroxyl absorption at ν 3000 – 3600 cm^{-1} and a strong absorption at ν_{max} 1703 cm^{-1}, suggesting a conjugated carboxylic acid carbonyl in the IR spectrum, which also corresponded to a carbon resonating at δ_C 166.3 in the ^{13}C NMR spectrum. The ^1H and ^{13}C NMR spectra (Table 1) of compound **1** were similar to those of the known co-metabolite, eutypinic acid (**4**),[9] except the presence of seven additional signals of a sugar unit, including one methoxy (at δ_H 3.46), one oxymethylene (at δ_H 3.50 / 3.60), and five oxymethines (at δ_H 3.08, 3.33, 3.40 – 3.47, and 5.15). The connectivity, provided by COSY and HMBC spectral information (Fig. 2), showed correlations from H-1′ to H-2′; from H-2′ to H-3′; from H-3′ to H-4′; from H-4′ to H-5′; and from H-5′ to H_2-6′ in the COSY spectrum and in the HMBC spectrum from H-2′ to C-1′ and C-3′; from H-3′ to C-2′; from H-4′ to C-3′, C-5′, and 4′-OCH_3; and from 4′-OCH_3 to C-4′. Moreover, the HMBC spectrum showed a correlation from an anomeric proton resonating at δ_H 5.15 (H-1′) to the aromatic carbon at δ_C 160.2 (C-4), suggesting the replacement of a phenolic hydroxyl group at C-4 in compound **4** with the sugar unit. Furthermore, after an addition of D_2O, the large vicinal coupling constants of $J_{1'2'}$ = 7.7 Hz, $J_{2'3'}$ = 9.0 Hz, and $J_{3'4'}$ and $J_{4'5'}$ = 9.3 Hz were observed and in the NOESY spectrum the cross-peak correlations from H-1′ to H-3′ and H-5′ and from H-2′ to H-4′ were seen, confirming the axial position of all extra oxymethine protons (H-1′ – H-5′). Thus, the sugar unit was 4-*O*-methyl-*β*-glucopyranose and compound **1** could be depicted as shown in Fig. 1. Its trivial name is conoideoglucoside A.

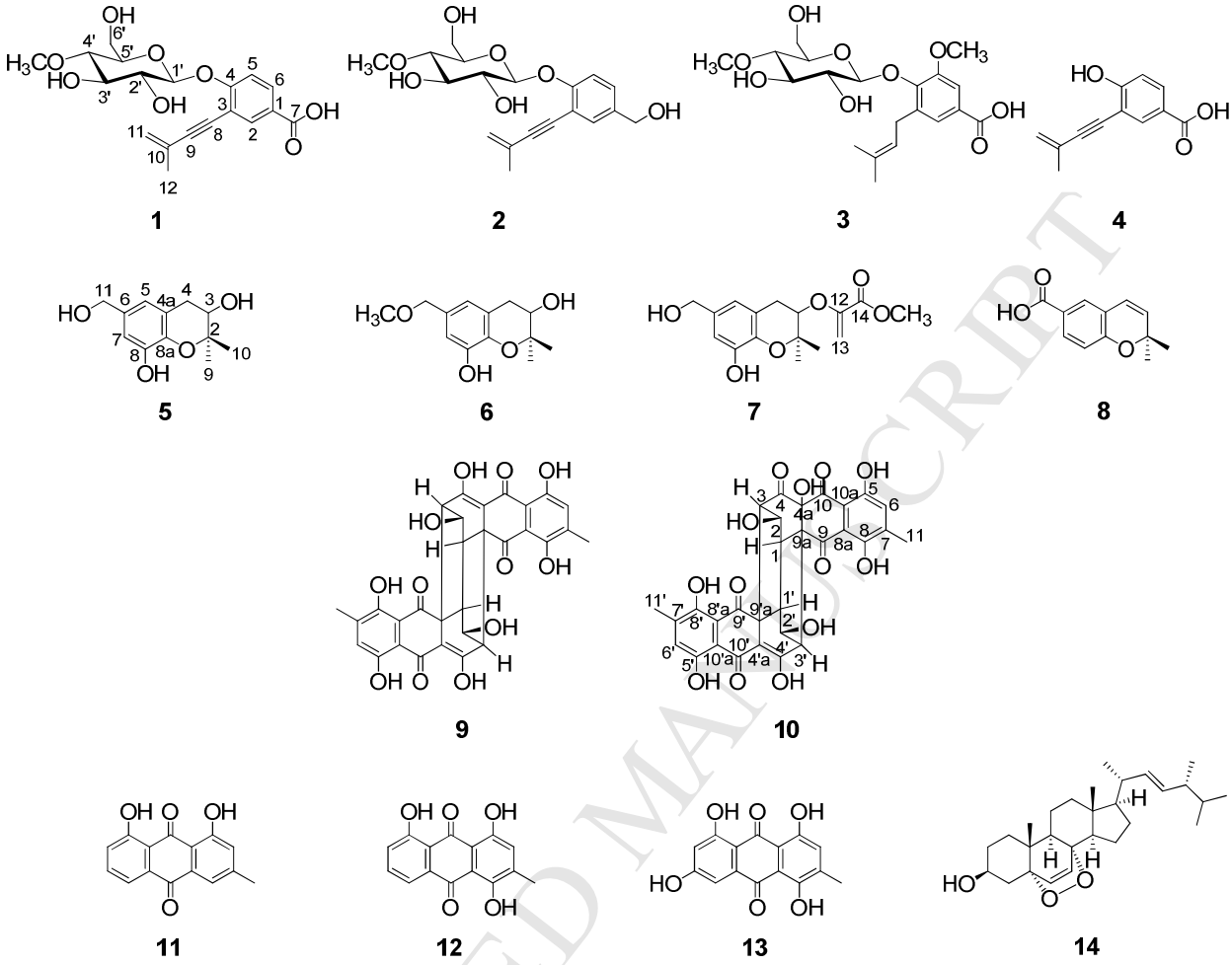

Fig. 1. Chemical structures of compounds **1** – **14**.

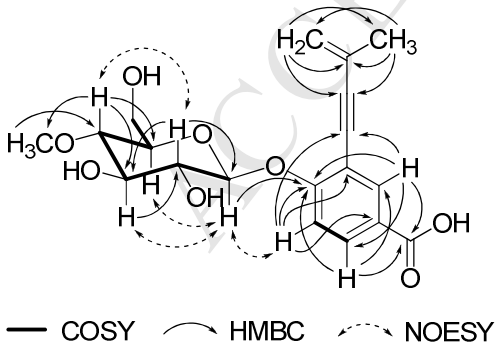

Fig. 2. COSY, key HMBC and selected NOESY correlations of compounds **1**.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignments for compounds **1** – **3** in DMSO-d_6.

Position	Compound **1**		Compound **2**		Compound **3**	
	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)
1	125.0[a], qC	–	135.7, qC	–	122.6, qC	–
2	133.9, CH	7.88 d (2.1)	130.7, CH	7.30 d (1.6)	122.8, CH	7.34 s
3	112.0, qC	–	111.8, qC	–	135.9, qC	–
4	160.2, qC	–	156.0, qC	–	146.7, qC	–
5	114.0, CH	7.22 d (8.8)	114.6, CH	7.09 d (8.7)	151.3, qC	–
6	131.9, CH	7.86 dd (8.8, 2.1)	127.9, CH	7.23 dd (8.7, 1.6)	111.6, CH	7.38 s
7	166.3, qC	–	61.9, CH_2	4.40 d (5.2)	167.0, qC	–
8	84.1, qC	–	85.2, qC	–	28.3, CH_2	3.35 – 3.45 m
9	95.0, qC	–	94.1, qC	–	122.6, CH	5.28 t (7.0)
10	126.4, qC	–	126.5, qC	–	131.7, qC	–
11	122.6, CH_2	5.40 s / 5.42 s	121.7, CH_2	5.36 s / 5.38 s	17.7, CH_3	1.68 s
12	23.0, CH_3	1.96 s	22.9, CH_3	1.95 s	25.5, CH_3	1.70 s
1′	98.9, CH	5.15 d (7.7)	99.4, CH	5.02 d (7.7)	102.5, CH	4.91 d (7.7)
2′	73.4, CH	3.33 dd (9.0, 7.7)[b]	73.5, CH	3.33 dd (8.6, 7.7)[b]	74.5, CH	3.26 dd (9.0, 7.7)[b]
3′	75.6, CH	3.40 – 3.47 m	75.5, CH	3.38 – 3.41 m	76.2, CH	3.35 – 3.40 m
4′	78.7, CH	3.08 dd (9.3, 9.3)	78.9, CH	3.05 dd (9.3, 9.3)	79.1, CH	3.00 dd (9.2, 9.2)
5′	76.5, CH	3.40 – 3.47 m	76.5, CH	3.42 – 3.45 m	75.8, CH	3.08 ddd (11.0, 4.2, 1.5)
6′	60.0, CH_2	3.50 dd (11.7, 5.1)[b]	60.2, CH_2	3.49 dd (11.2, 5.4)[b]	60.4, CH_2	3.45 dd (11.0, 4.2)[b]
		3.60 d (11.7)[b]		3.59 d (11.2)[b]		3.52 dd (11.0, 1.5)[b]
5-OCH_3		–		–	56.1, CH_3	3.81 s
7-OH		–		5.16 t (5.2)		–
2′-OH		5.31 br s		5.28 br t (4.0)		5.20 br s
4′-OCH_3	59.6, CH_3	3.46 s	59.3, CH_3	3.45 s	59.5, CH_3	3.44 s
6′-OH		4.70 t (5.1)		4.68 t (5.4)		4.47 t (4.2)

[a] Observed in the HMBC spectrum.

[b] Coupling constants (*J*) appeared in the presence of D_2O.

Compound **2** was obtained as a yellow oil. Its molecular formula was determined to be $C_{19}H_{24}O_7$, showing the sodium-adduct mass ion peak at *m/z* 387.1413 $[M+Na]^+$ in the HRESIMS spectrum. The ^{1}H and ^{13}C NMR spectral data (Table 1) of compound **2** showed similarity to those of compound **1**, except for the upfield shift of two aromatic protons at H-2 ($\Delta\delta$ –0.58) and H-6 ($\Delta\delta$ –0.63) in the ^{1}H NMR spectrum and the presence of an additional oxymethylene signal in both ^{1}H and ^{13}C NMR spectra resonating at δ_H 4.40 (H_2-7) and δ_C 61.9 (C-7), respectively. Moreover, in the ^{13}C NMR spectrum, the carbon belonging to carboxylic acid observed in

compound **1** was absent in compound **2**. The HMBC spectrum showed correlations from H_2-7 to C-1, C-2, and C-6, indicating the replacement of the carboxylic group with a hydroxymethyl group. In addition, after an addition of D_2O, the large vicinal coupling constants of $J_{1'2'}$ (7.7 Hz), $J_{2'3'}$ (8.6 Hz), and $J_{3'4'}$ and $J_{4'5'}$ (9.3 Hz) together with the NOESY correlations from H-1′ to H-3′ and H-5′ and from H-2′ to H-4′ suggested the axial position of all oxymethine protons (H-1′ to H-5′) in the sugar unit. Therefore, compound **2** was a reduced form of compound **1** (as shown in Fig. 1) and is named conoideoglucoside B.

Compound **3** was obtained as a yellow oil. The IR spectrum indicated the presence of a carboxylic group (ν 3000 – 3600 cm^{-1} and ν_{max} 1703 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) of compound **3** were in good agreement with those of trollioside,[10] except an extra methoxy signal at δ_H 3.44 (4′-OCH_3) in the ^1H NMR spectrum, which attributed to the carbon at δ_C 59.5 by the HSQC correlation. In the HMBC spectrum, the additional methoxy protons at δ_H 3.44 (4′-OCH_3) correlated to an oxymethine carbon at δ_C 79.1 (C-4′), suggesting that the methoxy group was attached to C-4′ of the sugar unit. Thus, compound **3** also had 4-*O*-methyl-*β*-glucopyranose moiety. The HRESIMS spectrum confirmed the molecular formula $C_{20}H_{28}O_9$ by showing the sodium-adduct mass ion peak at *m/z* 435.1621 $[M+Na]^+$. Thus, compound **3** had the chemical structure as shown in Fig. 1 and is given the trivial name conoideoglucoside C.

Moreover, compound **3** was subjected to acid hydrolysis to give the aglycone unit and glucosidic part (detail given in Experimental section 4.4). The sugar residue of compound **3** showed the positive optical rotation value ($[\alpha]_D^{20}$ +61.4), which was consistent with the reported data of 4-*O*-methyl-D-glucopyranose ($[\alpha]_D^{25}$ +80).[11] The absolute configuration of its sugar unit could thus be assigned as the D configuration. In addition, the similar spectral data of the sugar unit (Table 1) and the negative optical rotations of compounds **1** ($[\alpha]_D^{24}$ –24.0) and **2** ($[\alpha]_D^{25}$

−16.9) to those of the co-metabolite **3** ($[\alpha]_D^{24}$ −8.9) suggested the same configuration of the sugar moiety as that of compound **3**.

Compound **5** was obtained as a yellow oil. HRESIMS data showing the sodium-adduct mass ion peak at *m/z* 247.0940 $[M+Na]^+$ revealed the molecular formula of $C_{12}H_{16}O_4$, indicating five degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Table 2) of compound **5**, which incorporated with HSQC correlations, disclosed signals of two methyls, one methylene, one oxymethylene, one oxymethine, two sp^2 methines, and three hydroxyl protons. Moreover, the ^{13}C NMR spectrum, differentiated by the DEPT-135 spectrum, gave additional signals of one sp^3 quaternary and four quaternary carbons. The COSY correlations from H-3 to H_2-4 and 3-OH together with the HMBC correlations from H_2-4 to C-2, C-3, C-4a, C-5, and C-8a; from H-5 to C-4, C-7, and C-8a; from H-7 to C-5, C-8, and C-8a; from H_3-9 to C-2, C-3, and C-10; and from H_3-10 to C-2, C-3, and C-9 gave 3-hydroxy-2,2-dimethylchromane ring similar to that of acremine R (Fig. 3).[12] In addition, the cross-peaks correlations between H_2-11 and 11-OH in the COSY spectrum and the HMBC correlations from H-5 and H-7 to C-11; from H_2-11 to C-5, C-6, and C-7; and from 8-OH to C-7 and C-8a suggested that the hydroxymethyl and hydroxyl groups should be placed at C-6 and C-8, respectively. Moreover, compound **5** showed the positive optical rotation value ($[\alpha]_D^{24}$ +52.1) similar to those of 3-hydroxy-2,2-dimethylchromane analogs bearing *S* configuration,[13] which was opposite to that of acremine R having *R* configuration ($[\alpha]_D^{24}$ −8.0).[12] In order to confirm the absolute configuration at C-3, compound **5** was treated with the Mosher's reagents, (*R*)- and (*S*)-MTPACl (*α*-methoxy-*α*-trifluoromethylphenylacetyl chloride). The reaction resulted in diastereomeric mixtures of corresponding MTPA esters, indicating an enantiomeric mixture of compound **5**. Thus, it could then be summarized that compound **5** contained a non-equivalent mixture of enantiomers by having a larger amount of

(*S*)-stereoisomer. Unfortunately, the attempt to separate the enantiomers by using the chiral HPLC analysis was unsuccessful. At present, the chemical structure of compound **5** was therefore depicted as shown in Fig. 1 and conoideochromane A is named for compound **5**.

Table 2

^{1}H and ^{13}C NMR assignments for compounds **5** – **7** in DMSO-d_6.

Position	Compound **5**[a,c]		Compound **6**[b]		Compound **7**[a,d]	
	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)
2	76.7, qC	–	76.8, qC	–	77.4, qC	–
3	68.4, CH	3.54 – 3.62 m	68.2, CH	3.58 – 3.63 m	67.8, CH	3.60 – 3.65 m
4	31.2, CH_2	2.53 dd (16.7, 8.2) 2.82 dd (16.7, 5.4)	31.1, CH_2	2.54 dd (16.6, 8.1) 2.83 dd (16.6, 5.4)	31.0, CH_2	2.61 dd (16.8, 7.7) 2.92 dd (16.8, 5.1)
4a	120.4, qC	–	120.6, qC	–	122.3, qC	–
5	117.8, CH	6.42 d (1.6)	119.2, CH	6.43 d (1.6)	124.2, CH	6.87 d (1.5)
6	133.5, qC	–	129.2, qC	–	134.2, qC	–
7	111.9, CH	6.55 d (1.6)	112.8, CH	6.53 d (1.6)	117.0, CH	6.76 d (1.5)
8	145.4, qC	–	145.5, qC	–	142.0, qC	–
8a	139.7, qC	–	140.4, qC	–	142.7, qC	–
9	20.0, CH_3	1.12 s	20.1, CH_3	1.13 s	20.3, CH_3	1.10 s
10	25.7, CH_3	1.28 s	25.7, CH_3	1.28 s	25.4, CH_3	1.22 s
11	62.9, CH_2	4.27 s	73.7, CH_2	4.18 s	62.2, CH_2	4.35 s
12	–	–	–	–	150.0, qC	–
13	–	–	–	–	100.5, CH_2	4.57 d (2.3) 5.45 d (2.3)
14	–	–	–	–	162.4, qC	–
3-OH	–	5.09 s	–	5.09 s	–	–
8-OH	–	8.54 s	–	8.60 s	–	–
11-OH	–	4.90 s	–	–	–	5.08 br s
11-OCH_3	–	–	57.1, CH_3	3.21 s	–	–
14-OCH_3	–	–	–	–	52.3, CH_3	3.77 s

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C.

[b] 500 MHz for ^{1}H and 125 MHz for ^{13}C.

[c] Approx. 92% purity examined by HPLC analysis.

[d] Approx. 89% purity examined by HPLC analysis.

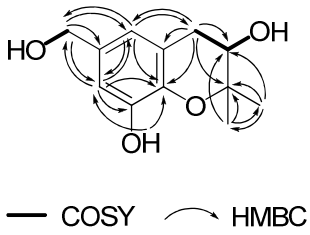

Fig. 3. COSY and key HMBC correlations of compounds **5**.

Compound **6** was obtained as a yellow oil. The ^{1}H and ^{13}C NMR spectra (Table 2) were almost identical to those of compound **5**, apart from the presence of an additional signal of a methoxy group (11-OCH_3) resonating at δ_H 3.21 and δ_C 57.1 in the ^{1}H and ^{13}C NMR spectra, respectively. The extra methoxy protons (11-OCH_3) showed HMBC correlation to an oxymethylene carbon at δ_C 73.7 (C-11), bearing the methylene protons at δ_H 4.18 (H_2-11) in the HSQC spectrum, which was in turn correlated in HMBC spectrum to C-5, C-6, C-7, and 11-OCH_3, corroborating the position of the methoxy group at C-11. The remaining structure was reassured by an extensive analysis of 2D NMR spectroscopic information (HSQC, HMBC, and COSY). HRESIMS data confirmed the structure by revealing the sodium-adduct mass ion peak at *m/z* 261.1099 $[M+Na]^+$, corresponding to the molecular formula of $C_{13}H_{18}O_4$. The reaction with Mosher's reagents also resulted in diastereomeric mixtures of corresponding MTPA esters, suggesting an enantiomeric form of compound **6**. The positive optical rotation of compound **6** ($[\alpha]_D^{25}$ +5.1) suggested a non-racemic mixture of compound **6**, which was slightly rich quantity in (*S*)-stereoisomer. The chemical structure of compound **6** was thus illustrated as shown in Fig. 1 and the name conoideochromane B is given.

Compound **7** was obtained as a yellow oil. The ^{1}H and ^{13}C NMR spectra (Table 2) of compound **7** contained those signals present in compound **5** and three extra signals in the ^{1}H NMR spectrum including one methoxy (at δ_H 3.77, s) and two olefinic protons [at δ_H 4.57 (d,

2.3) and 5.45 (d, 2.3)], which attributed in the HSQC spectrum to the carbons resonating at δ_C 52.3 and 100.5, respectively. In the HMBC spectrum, the extra methoxy at δ_H 3.77 (14-OCH_3) correlated to C-14, while two additional olefinic protons at δ_H 4.57 and 5.45 (H_2-13) correlated to C-12 (δ_C 150.0) and C-14 (δ_C 162.4). The spectroscopic information suggested the presence of an additional methyl acrylate unit in compound **7**. The carbon resonating at δ_C 150.0 (C-12) indicating an attachment to an oxygen together with the evidence from NOESY spectrum showing correlations from H_3-9 (δ_H 1.10) to H_b-13 (δ_H 4.57) suggested that the methyl acrylate moiety was linked to an oxygen at C-3. The remaining chemical structure was affirmed by extensive 2D NMR spectroscopic analyses (HSQC, HMBC, and COSY). The molecular formula of $C_{16}H_{20}O_6$ was approved by HRESIMS spectral data, establishing the sodium-adduct mass ion peak at *m/z* 331.1151 $[M+Na]^+$. In addition, compound **7** should be derived from the same biosynthesis as the co-metabolite **5**, thus it was likely to obtain as an enantiomeric mixture, although it was fail to separate by chiral HPLC analysis. Compound **7** displayed the positive optical rotation value ($[\alpha]_D^{25}$+35.4), which was the same sign as that of compound **5** ($[\alpha]_D^{24}$ +52.1), suggesting the enantiomeric mixture with a larger amount of (*S*)-stereoisomer. The chemical structure of compound **7** was therefore depicted as shown in Fig. 1 and conoideochromane C is given as its informal name.

Compound **10** was obtained as a yellowish brown solid with the molecular formula of $C_{30}H_{22}O_{13}$, deduced from the negative mass ion peak at *m/z* 589.0987 $[M–H]^-$ in the HRESIMS spectrum. The molecular formula suggested twenty degrees of unsaturation. The ^{1}H NMR spectrum (Table 3) showed signals of two methyls, four sp^3 methines, two sp^3 oxymethines, and two sp^2 methines. In the ^{13}C NMR spectrum, there were additional signals of fifteen quaternary and five carbonyl carbons, which was differentiated by the DEPT-135 spectrum. The 2D NMR

spectral information, including COSY, HSQC, HMBC spectra led to a bisanthraquinone heterodimer, whose two subunit were linking at C-1–C-1′, C-3–C-9′a, and C-9a–C-3′. Compound **10** (as illustrated in Fig. 1), known as (–)-4a-oxyluteoskyrin, was previously isolated only from pigments of the fungus *Penicillium islandicum* Sopp NRRL1036 as a minor constituent, reported only ^{1}H NMR data by Takeda and co-workers in 1973.[14] Compound **10** was also given from the reaction of (–)-luteoskyrin (**9**) with pertrifluoroacetic acid, reported by Takeda et al.[14] Moreover, the CD spectrum of compound **10** showed a similar pattern to that previously reported for compound **9** (Fig. 4),[15] demonstrating the same absolute stereochemistry of a central cage structure. However, it cannot be concluded for the absolute configuration at C-4a, which probably causing a strong positive peak at λ 356 nm in the CD spectrum. Herein we also describe the complete ^{1}H and ^{13}C NMR spectral data in the Table 3.

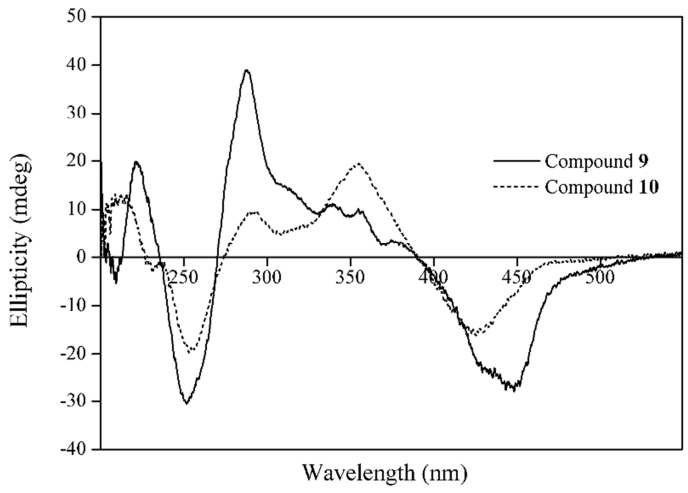

Fig. 4. CD spectra of compounds **9** and **10**.

Table 3

^{1}H (400 MHz) and ^{13}C (100 MHz) NMR assignments for compound **10** in acetone-d_6.

Position	Compound **10**[a]	
	δ_C, type	δ_H, mult (*J* in Hz)
1	45.0, CH	3.99 dd (3.7, 1.7)
2	71.1, CH	5.02 dd (4.9, 3.7)
3	66.8, CH	3.22 br d (4.9)
4	198.4, qC	–
4a	75.5, qC	–
5	157.1 / 157.2, qC	–
6	128.6(5) / 128.7(3), CH	7.25 s
7	140.7 / 140.8, qC	–
8	155.0 / 155.3, qC	–
8a	113.4, qC	–
9	201.3 / 201.4, qC	–
9a	65.4, qC	–
10	193.7 / 193.8, qC	–
10a	112.3, qC	–
11	16.4, CH_3	2.34 s
1′	50.9, CH	3.62 dd (3.5, 1.8)
2′	71.5, CH	4.62 dd (4.1, 3.5)
3′	58.0, CH	3.16 br d (4.1)
4′	177.5, qC	–
4′a	108.3, qC	–
5′	156.6(7) / 156.7(4), qC	–
6′	129.9, CH	7.30 s
7′	140.4 / 140.5, qC	–
8′	156.9 / 157.0, qC	–
8′a	112.0, qC	–
9′	201.0(7) / 201.1(3), qC	–
9′a	55.2, qC	–
10′	187.3, qC	–
10′a	113.0, qC	–
11′	16.4, CH_3	2.34 s
5-OH	–	11.27 s
8-OH	–	11.56 s
2′-OH	–	4.77 br s
4′-OH	–	14.15 s
5′-OH	–	11.89 br s
8′-OH	–	12.42 s

[a] The ^{1}H NMR spectral information of compound **10** was previously reported in DMSO-d_6.[14] In our structural determination, the 1D and 2D NMR spectral data of this compound **10** were recorded in both DMSO-d_6 and acetone-d_6. It was found that acetone-d_6

provided better resolution NMR spectra. Therefore, the NMR characterization of compound **10** was performed in acetone-d_6, which should be the preferred solvent for future studies.

The chemical structures of other known compounds (**4**, **8**, **9**, **11** – **14**) were established by comparison of the ^{1}H and ^{13}C NMR spectroscopic information with those formerly reported data for eutypinic acid (**4**),[9] 2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**8**),[16] (–)-luteoskyrin (**9**),[14,17] chrysophanol (**11**),[18] islandicin (**12**),[19] catenarin (**13**),[20] and (22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**14**)[21,22] along with their physical properties.

The isolated compounds were assessed for antimicrobial activity, including antimalarial against *P. falciparum* (K1, multidrug-resistant strain), antitubercular against *M. tuberculosis* H37Ra, antifungal against *C. albicans*, *A. brassicicola*, *C. acutatum*, *C. lunata*, and *M. grisea*, antibacterial activities against Gram-positive (*B. cereus*, *E. faecium*, *S. aureus*) and Gram-negative (*A. baumannii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*) bacteria, and for cytotoxicity against cancerous (MCF-7 and NCI-H187) and non-cancerous (Vero) cells. Since compounds **1**, **3**, and **8** were obtained in poor amounts, their biological activity was then not evaluated. Compound **14** was not subjected for biological evaluation due to its biological activity has previously been done by our group.[23]

Only compounds **9** and **12** exhibited antimalarial activity against *P. falciparum* with IC_{50} values of 0.51 and 8.81 µg/mL, respectively (Table 4). (–)-Luteoskyrin (**9**) possessed a broad range of antimicrobial activity (MIC 0.39 – 50.00 µg/mL), while (–)-4a-oxyluteoskyrin (**10**) had anti-*M. tuberculosis* H37Ra (MIC 50.00 µg/mL) and antibacterial (against *B. cereus*, *E. faecium* and *S. aureus*) activities (MIC12.50 – 25.00 µg/mL) (Table 4 and 5). Both compounds were inactive to *E. coli* and *K. pneumoniae* at the maximum tested concentration of 50 µg/mL, but in combination with agent that increases permeability of outer bacterial membrane such as

phenylalanine-arginine *β*-naphthylamide (PA*β*N), these compounds became active against *E. coli* with respective MIC values of 12.50 and 25.00 µg/mL. The results (Table 5) suggested that anti-Gram negative bacterial activity of compound **9** against *K. pneumoniae* and *A. baumannii* (MIC of 3.13 and 6.25 µg/mL) was improved in the presence of PA*β*N. Furthermore, catenarin (**13**) displayed anti-Gram positive bacterial activity against *B. cereus*, *E. faecium*, and *S. aureus* with MIC values in a range of 6.25 – 25.00 µg/mL. In combination with PA*β*N, compound **13** was also active to Gram-negative bacterial such as *E. coli* and *K. pneumoniae* with MIC values of 12.50 and 50.00 µg/mL, respectively. Moreover, compound **13** exhibited anti-plant pathogen activity against *M. grisea* at the maximum tested concentration (MIC of 50 µg/mL) (Table 4). For cytotoxicity (Table 6), compounds **4**, **9**, **10**, and **13** were toxic to NCI-H187 cells with IC_{50} values ranging from 0.16 to 17.99 µg/mL. Only compounds **10** and **13** had weak cytotoxicity against MCF-7 cells with respective IC_{50} values of 12.49 and 34.49 µg/mL. Compounds **9**, **10**, and **12** showed cytotoxicity against non-cancerous (Vero) cells with IC_{50} values of 0.48, 17.38, and 17.03 µg/mL, respectively. All tested compounds were inactive for anti-*C. albicans* and anti-*P. aeruginosa* at the maximum tested concentration (50 µg/mL).

Table 4

Antimalarial, antitubercular, and anti-plant pathogen activities of compounds **2**, **4** – **7**, and **9** – **13**.

Compounds	Anti-*P. falciparum*[a] (IC_{50}, µg/mL)	Anti-*M. tuberculosis* H37Ra[b] (MIC, µg/mL)	Anti-plant pathogen activity[b] (MIC, µg/mL)		
			A. brassicicola	*C. acutatum*	*M. grisea*
2	>10	>50	>50	>50	>50
4	>10	>50	>50	>50	>50
5	>10	>50	>50	>50	>50
6	>10	>50	>50	>50	>50
7	>10	>50	>50	>50	>50
9	0.51	6.25	3.13	50.00	12.50
10	>10	50.00	>50	>50	>50
11	>10	>50	>50	>50	>50
12	8.81	>50	>50	>50	>50
13	>10	>50	>50	>50	50.00
Dihydroartemisinin	4.35×10^{-4}	–	–	–	–
Chloroquine	0.10	–	–	–	–
Rifampicin	–	0.01 – 0.05	–	–	–
Ofloxacin	–	0.39 – 0.78	–	–	–
Streptomycin	–	0.63	–	–	–
Isoniazid	–	0.05	–	–	–
Ethambutol	–	0.94	–	–	–
Amphotericin B	–	–	1.56	0.78 – 3.13	0.78 – 1.56

[a] Maximum tested concentration was done at 10 µg/mL.

[b] Maximum tested concentration was done at 50 µg/mL.

Table 5

Antibacterial activity of compounds **2**, **4** – **7**, and **9** – **13**.

Compounds	Antibacterial activity (MIC, µg/mL)[a]						
	B. cereus	*E. faecium*	*S. aureus*	*A. baumannii*	*A. baumannii* +PAβN[b]	*E. coli* +PAβN[b]	*K. pneumoniae* +PAβN[b]
2	>50	>50	>50	>50	>50	>50	>50
4	>50	>50	>50	>50	>50	>50	>50
5	>50	>50	>50	>50	>50	>50	>50
6	>50	>50	>50	>50	>50	>50	>50
7	>50	>50	>50	>50	>50	>50	>50
9	1.56	0.39	1.56	12.50	6.25	12.50	3.13
10	25.00	25.00	12.50	>50	>50	25.00	>50
11	>50	>50	>50	>50	>50	>50	>50
12	>50	>50	>50	>50	>50	>50	>50
13	25.00	12.50	6.25	>50	>50	12.50	50.00
Rifampicin	0.31 – 0.63	3.13	0.08 – 0.16	1.56 – 3.13	0.10	0.02 – 0.05	0.39
Vancomycin	4.00	–	1.00	–	–	–	–
Tetracycline HCl	–	0.10 – 0.20	–	–	–	–	–
Erythromycin	–	–	–	25.00	1.56 – 3.13	0.78 – 1.56	12.50

[a] Maximum tested concentration was done at 50 µg/mL.

[b] PAβN = Phenylalanine-arginine β-naphthylamide.

Table 6
Cytotoxicity of all tested compounds.

Compounds	Cytotoxicity[a] (IC_{50}, µg/mL)		
	MCF-7	NCI-H187	Vero
2	>50	>50	>50
4	>50	17.99	>50
5	>50	>50	>50
6	>50	>50	>50
7	>50	>50	>50
9	>50	0.16	0.48
10	12.49	2.27	17.38
11	>50	>50	>50
12	>50	>50	17.03
13	34.49	8.21	>50
Doxorubicin	7.01 – 9.24	0.08 – 0.12	–
Tamoxifen	6.52 – 7.36	–	–
Ellipticine	–	2.23 – 3.43	0.98 – 1.94

[a] Maximum tested concentration was done at 50 µg/mL.

3. Conclusion

Six new compounds, which included conoideoglucosides A – C (**1** – **3**) and conoideochromanes A – C (**5** – **7**), were isolated from the insect fungus *Conoideocrella krungchingensis* BCC53666 along with eight known compounds [eutypinic acid (**4**), 2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**8**), (–)-luteoskyrin (**9**), (–)-4a-oxyluteoskyrin (**10**), chrysophanol (**11**), islandicin (**12**), catenarin (**13**), and (22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**14**)]. Compound **9** had a wide range of antimicrobial activity against *P. falciparum*, *M. tuberculosis*, *B. cereus*, *E. faecium*, *S. aureus*, *A. baumannii*, *E. coli*, *K. pneumoniae*, *A. brassicicola*, *C. acutatum*, and *M. grisea* (Table 4 and 5). Compounds **10** and **13** showed antibacterial activity against Gram-positive bacteria with MIC values of 6.25 – 25.00 µg/mL and had anti-Gram negative bacterial activity such as *E. coli* in the presence of PAβN agent with MIC values of 25.00 and 12.50 µg/mL, respectively. Compound **12** was active against *P. falciparum* with an IC_{50} value of 8.81 µg/mL. In addition, compound **13** exhibited anti-*K. pneumoniae* activity in the presence of PAβN and anti-*M. grisea* activity at the maximum tested concentration (50 µg/mL). Compounds **4**, **9**, **10**, and **13** displayed cytotoxicity against NCI-H187

cells with IC_{50} values ranging from 0.16 to 17.99 µg/mL, while only compounds **10** and **13** exhibited cytotoxicity against MCF-7 cells with IC_{50} values of 12.49 and 34.49 µg/mL, respectively. Compounds **9**, **10**, and **12** had cytotoxicity against Vero cells with respective IC_{50} values of 0.48, 17.38, 17.03 µg/mL.

4. Experimental

4.1. General experimental procedures

A melting point was detected using a melting point MP90 apparatus from Mettler Toledo. Optical rotations were performed on a JASCO P-1030 digital polarimeter. UV spectra were taken in MeOH on a Spekol 1200 from Analytik Jena UV–Vis spectrophotometer. FTIR spectra were detected on a Bruker ALPHA spectrometer. CD spectra were done in MeOH on a JASCO J-810 spectropolarimeter. NMR spectra were carried out on either Bruker Avance-III 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) or Bruker Avance 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) NMR spectrometers. HRESIMS data were recorded on a Bruker MicrOTOF mass spectrometer. Semi-preparative HPLC were performed by a reverse phase column (SunFire C_{18} OBD, particle size 5 µm, diam. 19 mm × 150 mm) at the flow rate of 8 mL/min, while preparative HPLC were taken by a reverse phase column (SunFire C_{18} OBD, particle size 10 µm, diam. 19 mm × 250 mm) at the flow rate of 15 mL/min. Chiral HPLC analysis was performed using a chiral CD-Ph column (Shiseido chiral CD-Ph, particle size 5 µm, diam. 4.6 mm × 250 mm) at the flow rate of 1 mL/min. All HPLC were carried out on a Dionex–Ultimate 3000 series equipped with a binary pump, an autosampler, and a diode array detector. Purity of isolated compounds was detected using analytical reversed phase HPLC (Merck LiChroCART C18, particle size 3 µm, diam. 2

mm × 55 mm), eluted with a linear gradient system (0 – 100% MeCN in water containing 0.05% formic acid) at the flow rate 0.5 mL/min over 15 min.

4.2. Fungal material

The fungus *Conoideocrella krungchingensis* was isolated from an insect in the Hemiptera order, collected at Khao Luang National Park, Nakhon Si Thammarat province, Thailand and deposited at BIOTEC Culture Collection (BCC), the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand with a registration number BCC53666. The fungus was previously reported as new species from Thailand.[24] The fungus was identified by based on morphology and molecular phylogenetic relationships of the partial nuclear large subunit rRNA (LSU, accession number KJ435,070) and the elongation factor (EF-1α, accession number KJ435,099) genes.

4.3. Fermentation, extraction, and isolation

The fungus *Conoideocrella krungchingensis* BCC53666 was maintained on potato dextrose agar (PDA) plates at 25 °C, which was then cut into small pieces (1 cm × 1 cm each) and transferred into 8 × 250 mL Erlenmeyer flasks, which each contained 25 mL of potato dextrose broth (PDB, containing potato starch 4.0 g/L, dextrose 20.0 g/L in distilled water). After incubation at 25 °C for 5 days on a rotary shaker at 200 rpm, each flask was equally transferred into 4 × 1 L Erlenmeyer flasks, containing 250 mL of PDB medium. The production culture (20 L) was cultivated at 25 °C for 5 days under shake condition (200 rpm). The broth and mycelia were separated by simple filtration. The culture broth was extracted three times with an equal volume of EtOAc. The combined organic layer was dried over Na_2SO_4 and then concentrated under reduced pressure to provide a brown gum (3.3 g). The mycelia were macerated in MeOH

(1 L) for 3 days and consecutively in CH_2Cl_2 (1 L) for 3 days. The combined organic solvent was then evaporated to dryness in *vacuo*. Water (500 mL) was added and the mixture was extracted three times with an equal volume of *n*-hexane, followed by three times with an equal volume of EtOAc. Later, *n*-hexane and EtOAc extracts were separately dried over Na_2SO_4 and concentrated under reduced pressure to yield crude extracts as brown gum from *n*-hexane (6.5 g) and from EtOAc (0.6 g), respectively.

The crude broth extract (3.3 g) was fractionated by a Sephadex LH-20 column (4.2 cm × 36.5 cm), eluted with 100% MeOH to give 9 fractions. The first and seventh – ninth fractions were not further purified due to no compound of interest. The second fraction (0.9 g) was then subjected to a preparative HPLC using a linear gradient elution of 10 – 100% aqueous MeCN over 40 min to provide twenty subfractions (2.1 – 2.20), from which compounds **5** (7.8 mg), **6** (12.9 mg), and **7** (7.6 mg) were obtained in subfractions 2.2, 2.6, and 2.8, respectively. Subfraction 2.5 (14.7 mg) was further purified by a semi-preparative HPLC using a linear gradient elution of MeCN:H_2O + 0.05% formic acid (10:90 to 30:70) over 50 min to give compound **2** (4.2 mg). Compound **3** (3.2 mg) was obtained from subfraction 2.7 (10.4 mg) after separation by a preparative HPLC using a linear gradient elution of 15 – 50% aqueous MeCN over 40 min. The third fraction (0.5 g) was subjected to a preparative HPLC using a linear gradient elution of 10 – 100% aqueous MeCN over 40 min to yield twenty-two subfractions (3.1 – 3.22), from which compound **5** (4.4 mg) was obtained in subfraction 3.6. Separation of subfraction 3.9 (11.4 mg) by a preparative HPLC using a linear gradient elution of MeCN:H_2O + 0.05% formic acid (15:85 to 35:65) over 50 min afforded compounds **6** (3.7 mg) and **1** (1.3 mg), respectively, while purification of subfraction 3.17 (14.3 mg) by a preparative HPLC using a linear gradient elution of MeCN:H_2O + 0.05% formic acid (40:60 to 50:50) over 50 min

provided compound **8** (3.9 mg). After separation by a preparative HPLC using a linear gradient elution of MeCN:H_2O + 0.05% formic acid (5:95 to 15:85) over 40 min, the fourth fraction (0.3 g) gave respective compounds **4** (17.5 mg) and **11** (6.4 mg), while the fifth fraction (0.2 g) afforded compounds **11** (6.7 mg) and **12** (7.8 mg), respectively. The sixth fraction (0.2 g) was applied to a preparative HPLC using a linear gradient elution of 40 – 70% aqueous MeCN over 40 min to provide compounds **10** (14.0 mg), **9** (27.7 mg), and **13** (3.0 mg), respectively.

The *n*-hexane crude extract from mycelia (6.5 g) was passed through a Sephadex LH-20 column (4.2 cm × 36.5 cm), eluted with 100% MeOH to yield 10 fractions. The first, second, and eighth – ninth fractions were discarded since there was no compound of interest. The third fraction (1.6 g) was further fractionated by a Sephadex LH-20 column (4.2 cm × 36.5 cm), eluted with 100% MeOH to yield 4 subfractions (3.1 – 3.4). Purification of subfraction 3.2 (1.2 g) by a preparative HPLC using a linear gradient elution of 70 – 100% aqueous MeCN over 40 min provide compound **14** (62.5 mg). The fourth fraction (0.5 g) was further subjected to a preparative HPLC using a linear gradient elution of 30 – 70% aqueous MeCN over 40 min to furnish compounds **9** (2.1 mg), **11** (3.7 mg), and **12** (50.6 mg), respectively. Moreover, using the same separation method for the fourth fraction, the fifth fraction (0.2 g) gave compounds **10** (2.2 mg), **11** (2.3 mg), and **12** (2.3 mg), respectively, while the sixth fraction (0.1 g) provided respective compounds **10** (3.4 mg), **9** (12.8 mg), **11** (1.4 mg), and **12** (3.7 mg). The seventh fraction (0.3 g) was applied to a preparative HPLC using a linear gradient elution of 40 – 70% aqueous MeCN over 40 min to afford compounds **10** (22.5 mg), **9** (36.9 mg), **13** (6.1 mg), **11** (0.9 mg), and **12** (27.0 mg), respectively.

The EtOAc crude extract from mycelia (0.6 g) was triturated from MeOH to give compound **12** (131.9 mg). The filtrate was further purified by a preparative HPLC using a linear

gradient elution of 40 – 100% aqueous MeCN over 40 min to afford compounds **10** (4.7 mg) and **12** (7.2 mg), respectively.

*4.3.1 Conoideoglucoside A (**1**)*

Yellow oil; $[\alpha]_D^{24}$ –24.0 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.09), 262 (4.03), 424 (3.21) nm; FTIR (ATR): ν_{max} 3385 (br), 2957, 2924, 2853, 2195, 1703, 1641, 1599, 1535, 1456, 1387, 1250, 1107, 1084, 1059, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 401.1208 $[M+Na]^+$ (calcd for $C_{19}H_{22}NaO_8$, 401.1207).

*4.3.2 Conoideoglucoside B (**2**)*

Yellow oil; $[\alpha]_D^{25}$ –16.9 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.90), 259 (3.91), 297 (3.74) 422 (3.39) nm; FTIR (ATR): ν_{max} 3364 (br), 2953, 2922, 2855, 2193, 1659, 1605, 1497, 1452, 1416, 1377, 1240, 1099, 1078, 1053, 1018 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 387.1413 $[M+Na]^+$ (calcd for $C_{19}H_{24}NaO_7$, 387.1414).

*4.3.3 Conoideoglucoside C (**3**)*

Yellow oil; $[\alpha]_D^{24}$ –8.9 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.54), 260 (3.53), 290 (3.35) 420 (2.46) nm; FTIR (ATR): ν_{max} 3402 (br), 2956, 2924, 2854, 1703, 1650, 1591, 1458, 1419, 1377, 1299, 1206, 1072, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 435.1621 $[M+Na]^+$ (calcd for $C_{20}H_{28}NaO_9$, 435.1626).

*4.3.4 Eutypinic acid (**4**)*

Reddish brown solid; ^1H and ^{13}C NMR data in acetone-d_6, see Figs. S89 and S90; HRESIMS *m/z* 201.0559 $[M–H]^-$ (calcd for $C_{12}H_9O_3$, 201.0557).

*4.3.5 Conoideochromane A (**5**)*

Yellow oil (~92% purity); $[\alpha]_D^{24}$ +52.1 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.08), 238 (3.35), 287 (3.05) nm; FTIR (ATR): ν_{max} 3353 (br), 2928, 2874, 1599, 1497, 1449,

1380, 1371, 1319, 1272, 1252, 1235, 1218, 1188, 1135, 1062, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 247.0940 $[M+Na]^+$ (calcd for $C_{12}H_{16}NaO_4$, 247.0941).

*4.3.6 Conoideochromane B (**6**)*

Yellow oil; $[\alpha]_D^{25}$ +5.1 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.40), 236 (3.68), 286 (3.28) nm; FTIR (ATR): ν_{max} 3410 (br), 2954, 2924, 2854, 1599, 1497, 1459, 1382, 1370, 1316, 1267, 1253, 1231, 1218, 1189, 1137, 1066 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 261.1099 $[M+Na]^+$ (calcd for $C_{13}H_{18}NaO_4$, 261.1097).

*4.3.7 Conoideochromane C (**7**)*

Yellow oil (~89% purity); $[\alpha]_D^{25}$ +35.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.21), 234 (3.77), 287 (3.39) nm; FTIR (ATR): ν_{max} 3374 (br), 2955, 2925, 2856, 1730, 1632, 1592, 1488, 1441, 1415, 1371, 1323, 1249, 1203, 1168, 1133, 1068, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 331.1151 $[M+Na]^+$ (calcd for $C_{16}H_{20}NaO_6$, 331.1152).

*4.3.8 2,2-Dimethyl-2H-1-chromene-6-carboxylic acid (**8**)*

Brown solid; ^{1}H and ^{13}C NMR data in acetone-d_6, see Figs. S91 and S92; HRESIMS *m/z* 227.0680 $[M+Na]^+$ (calcd for $C_{12}H_{12}NaO_3$, 227.0679).

*4.3.9 (–)-Luteoskyrin (**9**)*

Yellow solid; $[\alpha]_D^{24}$ –728.5 (*c* 0.11, MeOH); ^{1}H and ^{13}C NMR data in DMSO-d_6, see Figs. S93 and S94; HRESIMS *m/z* 573.1035 $[M–H]^-$ (calcd for $C_{30}H_{21}O_{12}$, 573.1038).

*4.3.10 (–)-4a-Oxyluteoskyrin (**10**)*

Yellowish brown solid; mp 199.1 °C (dec); $[\alpha]_D^{24}$ –28.4 (*c* 0.92, MeOH); CD (*c* 5.08 × 10^{-5} M, MeOH): λ_{max} ($\Delta\varepsilon$) 215 (+7.1), 234 (–1.2), 255 (–11.5), 292 (+5.5), 356 (+11.4), 429 (–9.3) nm; UV (MeOH) λ_{max} (log ε) 218 (4.13), 238 (4.27), 267 (4.18), 422 (4.20) nm; FTIR

(ATR): ν_{max} 3417 (br), 2960, 2925, 2869, 1731, 1651, 1622, 1579, 1457, 1435, 1394, 1297, 1262, 1202, 1182, 1117 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 589.0987 $[M–H]^-$ (calcd for $C_{30}H_{21}O_{13}$, 589.0988).

*4.3.11 Chrysophanol (**11**)*

Orange brown solid; ^{1}H and ^{13}C NMR data in acetone-d_6, see Figs. S95 and S96; HRESIMS *m/z* 253.0509 $[M–H]^-$ (calcd for $C_{15}H_9O_4$, 253.0506).

*4.3.12 Islandicin (**12**)*

Reddish brown solid; ^{1}H and ^{13}C NMR data in $CDCl_3$, see Figs. S97 and S98; HRESIMS *m/z* 271.0603 $[M+H]^+$ (calcd for $C_{15}H_{11}O_5$, 271.0601).

*4.3.13 Catenarin (**13**)*

Reddish brown solid; ^{1}H and ^{13}C NMR data in DMSO-d_6, see Figs. S99 and S100; HRESIMS *m/z* 285.0410 $[M–H]^-$ (calcd for $C_{15}H_9O_6$, 285.0405).

*4.3.14 (22E)-5α,8α-Epidioxyergosta-6,22-dien-3β-ol (**14**)*

Colourless solid; $[\alpha]_D^{27}$ –31.9 (*c* 0.15, $CHCl_3$); ^{1}H and ^{13}C NMR data in $CDCl_3$, see Figs. S101 and S102; HRESIMS *m/z* 451.3179 $[M+Na]^+$ (calcd for $C_{28}H_{44}NaO_3$, 451.3183).

4.4. Hydrolysis of compound 3

Compound **3** (2.0 mg, 0.005 mmol) was hydrolyzed by 3 M aqueous HCl (0.5 mL) at 90 °C for 15 h. After cooling, the reaction mixture was diluted with water (2.0 mL) and then extracted with EtOAc (3 × 2 mL). The aqueous layer of hydrolysate was evaporated to dryness under reduced pressure to provide 4-*O*-methyl-D-glucopyranose (0.70 mg, 72% yield, $[\alpha]_D^{20}$ +61.4, *c* 0.07, MeOH). The combined organic layer was dried over Na_2SO_4 and then

concentrated in *vacuo* to give 4-hydroxy-3-(3-hydroxy-3-methylbutyl)-5-methoxybenzoic acid (0.97 mg, 76% yield); ^{1}H NMR (500 MHz, acetone-d_6 + D_2O) δ 1.32 (6H, s), 1.81 (2H, t, J = 6.7 Hz), 2.80 (2H, t, J = 6.7 Hz), 3.79 (3H, s), 7.33 (1H, d, J = 1.2 Hz), 7.40 (1H, d, J = 1.2 Hz); HRESIMS *m/z* 259.0946 $[M–H_2O+Na]^+$ (calcd for $C_{13}H_{16}NaO_4$, 259.0941).

4.5. Biological assays

Antimalarial activity against *P. falciparum* (K1, multidrug-resistant strain) was treated by using the microculture radioisotope technique.[25] Dihydroartemisinin and chloroquine were used as standard references. Antitubercular activity against *M. tuberculosis* H37Ra (ATCC25177) and cytotoxicity against non-cancerous Vero cells (African green monkey kidney fibroblasts, ATCC CCL-81) were carried out by using the green fluorescent protein microplate assay (GFPMA).[26] Rifampicin, ofloxacin, streptomycin, isoniazid, and ethambutol were use as standard references for anti-TB, while ellipticine was used as a standard reference for cytotoxicity against Vero cells. Antifungal activity against *C. albicans* (ATCC90028) and cytotoxicity against cancerous cells, including MCF-7 (human breast cancer, ATCC HTC-22) and NCI-H187 (human small-cell lung cancer, ATCC CRL-5804) were evaluated based on the resazurin microplate assay (REMA).[27] Amphotericin B was used as a standard reference for antifungal test. Doxorubicin and tamoxifen were used as standard references for anti-MCF-7, while doxorubicin and ellipticine were used as standard references for activity against NCI-H187 cells. The optical density microplate assay, the standard protocols published by Clinical and Laboratory Standard Institute,[28,29] was applied for an evaluation of antibacterial activity against Gram-positive bacteria [*B. cereus* (ATCC11778), *E. faecium* (ATCC51559), *S. aureus* (ATCC29213)] and Gram-negative bacteria [*A. baumannii* (ATCC19606), *E. coli* (ATCC25922), *K. pneumoniae* (ATCC700603), *P. aeruginosa* (wild type

strain PAO1, ATCC15692)]. Rifampicin and vancomycin were used as standard references for activity against *B. cereus* and *S. aureus*. Rifampicin and tetracycline HCl were used as standard references for anti-*E. faecium*. Moreover, rifampicin and erythromycin were used as standard references for assay against *A. baumannii, E. coli*, and *K. pneumonia*e, while chloramphenicol and erythromycin were used as standard references for anti-*P. aeruginosa*. Anti-plant pathogenic fungal activity against *A. brassicicola* (BCC42724), *C. acutatum* (BCC58146), *C. lunata* (BCC15558), and *M. grisea* (BCC10261) was done by employing the 5(6)-carboxyfluorescein diacetate (CFDA) fluorometric assay[30-32] and amphotericin B was used as a standard reference. Maximum tested concentration for all assays was performed at 50 µg/mL, except that antimalarial activity against *P. falciparum* was done at 10 µg/mL. All tested compounds were added in triplicate in a well plate.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/xxx.

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