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Pulcherrins D–R, potential anti-inflammatory diterpenoids from the roots of *Caesalpinia pulcherrima*

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ABSTRACT

Bioactivity-guided isolation and purification of the dichloromethane extract from the roots of *Caesalpinia pulcherrima* yielded 15 new cassane-type diterpenes, named pulcherrins D–R (**1**–**15**) together with eight known compounds. The structures of the new metabolites were determined on the basis of spectroscopic analyses including 1D- and 2D-NMR and mass spectroscopy. The anti-inflammatory activity of isolated compounds was investigated with the lipopolysaccharide (LPS)–induced murine macrophage RAW 264.7 cell lines. Compounds **8**, **9**, **11**–**15**, and **17**–**23** showed potent NO inhibitory activity.

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1. Introduction

Caesalpinia pulcherrima (L.) Swartz, locally known as 'Hang Nok Yung Thai',[1] is a large perennial shrub or small tree, that is, widely distributed in tropical areas and has been used as an ornamental plant.[1] As regards its biological activities, *C. pulcherrima* exhibits cytotoxic,[2,3] antitubercular,[3] antibacterial,[4] and antifungal activities[4] and is also active against DNA repair-deficient yeast mutant.[5] A preliminary screening of the bioactivity of the crude extract from the roots of *C. pulcherrima* has shown strong inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cell lines. Nitric oxide (NO) is one of the inflammatory mediators causing inflammation in many organs. In the preceding paper, our group have isolated pulcherrins A–C and neocaesalpins P–R from the stems of *C. pulcherrima*.[6] As a continuation of our study, we now report the isolation and structure elucidation of 15 new cassane-type diterpenes, and their anti-inflammatory activity observed with LPS-induced RAW 264.7 cell lines as well. This is the first report of the NO inhibitory activity of the isolated diterpenes from *C. pulcherrima*.

2. Results and discussion

The CH_2Cl_2 extract from the roots of *C. pulcherrima* was subjected to vacuum liquid chromatography (VLC)[7] and column chromatography over silica gel to afford 15 new diterpenes (**1**–**15**) together with eight known compounds (**16**–**23**). The known compounds were identified as vouacapen-5α-ol (**16**),[2] 6β-cinnamoyl-7β-hydroxyvouacapen-5α-ol (**17**),[2] isovouacapenol C (**18**),[4] pulcherrimin C (**19**),[5] pulcherrimin A (**20**),[5] pulcherrimin E (**21**),[8] pulcherrimin B (**22**),[5] and 8,9,11,14-didehydrovouacapen-5α-ol (**23**)[2] by comparison of their spectroscopic data with those reported in the literatures and comparison with the authentic samples. Compounds **1**–**14** showed characteristic of the 2,3-disubstituted furan by the Ehrlich reagent[9] and the UV absorptions.[10] The IR spectrum of all new compounds showed the presence of as ester carbonyl (1700–1777 cm^{-1}) and hydroxyl (3549–3425 cm^{-1}) functionalities.

Pulcherrin D (**1**) had the molecular formula $C_{22}H_{32}O_4$ ($[M]^+$ *m*/*z* 360.2301) based on HREIMS. The presence of a 2,3-furanocassane framework was inferred from the ^1H and ^{13}C NMR spectral data (Table 1). The ^1H NMR spectrum showed four singlet signals of three aliphatic methyl groups at δ 0.89 (Me-18), 1.00 (Me-19), and 1.04 (Me-20) and an acetoxy methyl group at δ 2.00 ($OCOCH_3$) and a doublet signal of a secondary methyl group at δ 0.94 (*J*=6.9 Hz, Me-17). The signal of a 2,3-disubstituted furan ring was evident from resonances at δ 6.12 and 7.16 (each d, *J*=1.8 Hz, H-15 and H-16, respectively). The ^{13}C NMR spectroscopic data displayed 22 carbons including those of an ester carbonyl carbon at δ 170.7 ($OCOCH_3$). An oxymethine proton was displayed at δ 5.22 (td, *J*=11.1, 6.0 Hz, H-7; δ_C 72.3) whose coupling constants suggested its axial orientation. This proton also showed HMBC correlations to

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Table 1
^{1}H and ^{13}C NMR (300 and 75 MHz, $CDCl_3$) spectroscopic data of compounds **1**–**3** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)[a]

Position	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	32.3	1.30–1.35 m 1.37–1.42 m	35.2	1.26–1.30 m 1.42–1.46 m	35.0	1.41–1.45 m 1.48–1.51 m
2	18.1	1.49–1.53 m 1.55–1.59 m	18.1	1.34–1.40 m 1.61–1.65 m	18.0	1.45–1.48 m 1.67–1.71 m
3α	35.8	1.11 br d (8.4)	37.5	1.05–1.10 m	37.8	1.13–1.18 m
β		1.55–1.60 m		1.54–1.58 m		1.63–1.67 m
4	38.5	—	39.3	—	39.1	—
5	77.9	—	77.7	—	77.2	—
6α	31.5	2.01 dd (12.9, 6.0)	71.3	4.15 d (3.9)	73.4	5.48 d (4.2)
β		1.64 dd (12.9, 11.1)				
7α	72.3	5.22 td (11.1, 6.0)	74.8	5.38 dd (11.4, 3.9)	69.1	4.31 dd (10.8, 4.2)
8	39.8	1.87 td (11.1, 4.8)	35.0	2.09–2.13 m	37.7	1.93 ddd (12.0, 10.8, 5.1)
9	36.8	2.42–2.48 m	37.2	2.41–2.44 m	37.1	2.36 br dd (12.0, 8.7)
10	40.9	—	40.6	—	41.2	—
11	22.4	2.30–2.34 m 2.44–2.49 m	21.7	2.39–2.42 m 2.44–2.47 m	21.6	2.44–2.49 m 2.50–2.53 m
12	149.3	—	149.4	—	149.2	—
13	121.8	—	121.6	—	121.9	—
14	27.6	2.75 qd (6.9, 4.8)	27.8	2.72 qd (6.9, 5.1)	27.3	3.02 qd (6.9, 5.1)
15	109.6	6.12 d (1.8)	109.5	6.12 d (2.1)	109.7	6.21 d (1.8)
16	140.5	7.16 d (1.8)	140.5	7.16 d (2.1)	140.5	7.23 d (1.8)
17	17.1	0.94 d (6.9)	17.3	0.92 d (6.9)	17.1	1.07 d (6.9)
18	28.0	0.89 s	27.6	0.95 s	27.7	1.04 s
19	24.7	1.00 s	25.5	1.38 s	25.3	1.21 s
20	17.4	1.04 s	17.2	1.29 s	17.0	1.34 s
$OCOCH_3$	170.7	—	170.1	—	171.4	—
$OCOCH_3$	21.3	2.00 s	21.2	2.08 s	21.7	2.12 s

[a] Assignments were based on HMQC, HMBC, and COSY experiments.

the carbons at δ 27.6 (C-14), 31.5 (C-6), 39.8 (C-8), and 170.7 ($OCOCH_3$), which suggested the location of the OAc group at C-7. In the NOESY spectrum, the correlations between the oxymethine proton at δ 5.22 (H-7) and the protons at δ 0.94 (Me-17), 2.01 (H-6α), and 2.42–2.48 (H-9) placed them on the same side of the molecule. An OH group was placed at C-5 (δ 77.9) and assumed to be α-oriented by biogenetic pathway and comparison with the previously isolated furanoditerpenoids from this plant.[2–6,8,11,12] From these data, **1** was deduced to be 7β-acetoxyvouacapen-5α-ol and named as pulcherrin D.

Pulcherrin E (**2**) had the molecular formula $C_{22}H_{32}O_5$ ($[M]^+$ m/z 376.2250) inferred from HREIMS. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **2** were closely related to those of **1**. The only difference was found as replacement of the methylene protons at δ 1.64 and 2.01 (2H-6) in **1** with an oxymethine proton at δ 4.15 (d, J=3.9 Hz; δ_C 71.3) in **2**. The HMBC correlations of the latter proton with the carbons at δ 35.0 (C-8), 39.3 (C-4), 40.6 (C-10), 74.8 (C-7), and 77.7 (C-5) suggested its location at C-6 whose α-orientation was suggested by its NOESY cross-peaks with Me-18 (δ 0.95) and H-7 (δ 5.38) and the small vicinal coupling constants ($J_{7ax,6eq}$=3.9 Hz). Therefore, **2** was 6β-hydroxy-7β-acetoxyvouacapen-5α-ol and was named as pulcherrin E.

Pulcherrin F (**3**) had the same molecular formula $C_{22}H_{32}O_5$ as **2**. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **3** were closely related to those of **2**, which differed only in the chemical shifts of positions 6 and 7. The oxymethine proton H-6 of **3** appeared at δ_H 5.48 (δ_C 73.4) more downfield than that of **2** (δ_H 4.15; δ_C 71.3) as a result of the deshielding effect of the OAc group while H-7 of **3** resonanced at δ_H 4.31 (δ_C 69.1), higher field than that of **2** (δ_H 5.38; δ_C 74.8). The HMBC correlations of an oxymethine proton at δ 5.48 (H-6) with the carbons at δ 37.7 (C-8), 39.1 (C-4), 41.2 (C-10), 69.1 (C-7), 77.2 (C-5), and 171.4 ($OCOCH_3$) and of an oxymethine proton at δ 4.31 (H-7) with the carbons at δ 27.3 (C-14), 37.7 (C-8), and 73.4 (C-6) confirmed the locations of the OAc group at C-6 and OH at C-7, respectively. The NOESY cross-peaks of H-6/H-7/H-9 and H-7/H-6/H-17 confirmed the α-orientations of H-6 and H-7. Thus, **3** was assigned to be 6β-acetoxy-7β-hydroxyvouacapen-5α-ol and was named as pulcherrin F.

The molecular weight of pulcherrin G (**4**), $C_{27}H_{34}O_5$, was assigned at m/z 438.2410 $[M]^+$ by HREIMS. The NMR spectra (Table 2) of **4** displayed characteristic similar to those of **2** except for the replacement of an acetoxy group at δ 2.08 in **2** with a benzoyloxy group at δ 7.40 (br t, J=7.5 Hz; H-4′, H-6′), 7.53 (tt, J=7.5, 1.2 Hz; H-5′), and 8.02 (br d, J=7.5 Hz; H-3′, H-7′) in **4**. This evidence was confirmed by HMBC correlations of an oxymethine proton at δ 5.62 (dd, J=10.8, 3.9 Hz; H-7) to the carbons at δ 27.7 (C-14), 35.2 (C-8), and 165.6 (C-1′), and of H-6 (δ 4.31) with the carbons at δ 35.2 (C-8), 39.3 (C-4), 40.7 (C-10), 75.6 (C-7), and 77.8 (C-5). An oxymethine proton H-6 was deduced to be equatorially oriented by a small vicinal coupling constant ($J_{6eq,7ax}$=3.9 Hz), whereas H-7 was an axial proton by the large vicinal coupling constant ($J_{7ax,8ax}$=10.8 Hz). It was further supported by NOESY cross-peaks of H-7 with Me-17, H-9, and H-6. Thus, **4** was assigned to be 6β-hydroxy-7β-benzoyloxyvouacapen-5α-ol and was named as pulcherrin G. This compound was first isolated from natural product, however it was previously obtained from the partial synthesis.[8]

Pulcherrin H (**5**) had the molecular formula $C_{27}H_{34}O_4$ by HREIMS. The ^{1}H and ^{13}C NMR spectra (Table 2) were comparable to those of **1** except that the signals of an acetoxy group in **1** was replaced by those of a benzoyloxy group in **5** shown as the resonances at δ 7.37 (t, J=7.2 Hz; H-4′, H-6′), 7.48 (tt, J=7.2, 1.5 Hz; H-5′), and 7.98 (dt, J=7.2, 1.5 Hz; H-3′, H-7′). The correlations of an oxymethine proton at δ 5.29 (H-3) with the carbons at δ 19.6 (C-19), 23.1 (C-18), 23.8 (C-2), 43.5 (C-4), and 166.2 (C-1′) in the HMBC spectrum placed the benzoyloxy group at C-3. The relative stereochemistry of H-3 was assigned to be axially oriented by the large and small vicinal coupling constants ($J_{3ax,2ax}$=11.4 Hz, $J_{3ax,2eq}$=4.8 Hz). In the NOESY spectrum, the benzoyloxy protons at δ 7.98 (H-3′, H-7′) displayed a cross-peak with the methyl protons at δ 1.17 (Me-19), confirming the β-orientation of the benzoyloxy

Table 2
^{1}H and ^{13}C NMR (300 and 75 MHz, $CDCl_3$) spectroscopic data of compounds **4**–**7** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)[a]

Position	**4**		**5**		**6**		**7**		**4** (from synthesis)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	35.2	1.31–1.36 m 1.47–1.52 m	31.2	1.40 td (8.4, 2.7) 1.70–1.77 m	34.9	1.34–1.40 m 1.50–1.56 m	34.8	1.31–1.40 m 1.45–1.54 m	35.2	1.38 m 1.54 m
2	18.2	1.40–1.44 m 1.60–1.66 m	23.8	1.73–1.77 m 1.80–1.87 m	18.3	1.38–1.44 m 1.60–1.68 m	18.2	1.35–1.43 m 1.60–1.70 m	18.2	1.50 m 1.74 m
3α	37.5	1.08–1.12 m	77.8	5.29 dd (11.4, 4.8)	38.1	1.02 br d (8.4)	38.1	1.05 br d (9.3)	37.5	1.16 m
β		1.60–1.65 m				1.60–1.68 m		1.61–1.68 m		1.65 m
4	39.3	—	43.5	—	39.0	—	39.0	—	39.3	—
5	77.8	—	78.6	—	76.4	—	76.3	—	77.8	—
6α	71.4	4.31 d (3.9)	26.1	1.55 br d (12.0)	72.8	5.47 t (2.7)	72.3	5.31 dd (3.0, 2.4)	71.5	4.38 d (3.8)
β				1.80–1.90 m						
7α	75.6	5.62 dd (10.8, 3.9)	24.1	1.42–1.50 m	31.6	1.53 ddd (14.4, 3.9, 2.7)	31.5	1.50 dt (13.8, 2.4)	75.5	5.71 dd (11.4, 3.8)
β				1.67–1.73 m		2.23 td (14.4, 2.7)		2.18 td (13.8, 3.0)		
8	35.2	2.33 ddd (12.0, 10.8, 4.8)	34.3	1.70–1.76 m	30.7	1.91–2.05 m	30.6	1.91–2.04 m	35.3	2.42 td (11.4, 5.0)
9	37.3	2.47–2.50 m	37.6	2.27–2.31 m	38.0	2.30–2.40 m	38.0	2.26–2.44 m	37.3	2.51 m
10	40.7	—	41.0	—	41.3	—	41.4	—	40.7	—
11	21.8	2.46–2.50 m	22.4	2.29–2.35 m 2.41–2.45 m	21.9	2.31–2.38 m 2.39–2.47 m	21.8	2.31–2.39 m 2.39–2.49 m	21.8	2.56 m
12	149.5	—	149.4	—	149.5	—	149.5	—	149.5	—
13	121.6	—	122.6	—	122.4	—	122.4	—	121.6	—
14	27.7	2.82 qd (6.9, 4.8)	31.3	2.55 qd (6.9, 3.9)	31.2	2.40–2.49 m	31.1	2.44–2.54 m	27.7	2.89 dq (7.0, 5.0)
15	109.5	6.10 d (1.8)	109.5	6.11 d (1.5)	109.5	6.06 d (1.8)	109.5	6.09 d (1.8)	109.5	6.17 d (1.9)
16	140.6	7.16 d (1.8)	140.4	7.15 d (1.5)	140.4	7.11 d (1.8)	140.4	7.14 d (1.8)	140.6	7.23 d (1.9)
17	17.4	0.94 d (6.9)	17.5	0.95 d (6.9)	17.6	0.90 d (7.2)	17.6	0.92 d (6.6)	17.4	1.02 d (7.0)
18	27.8	0.96 s	23.1	0.97 s	27.8	0.93 s	27.7	0.94 s	17.3	1.41 s
19	25.5	1.39 s	19.6	1.17 s	26.0	1.13 s	25.9	1.17 s	27.8	1.04 s
20	17.3	1.34 s	17.2	1.05 s	17.2	1.44 s	16.9	1.37 s	25.5	1.47 s
1′	165.6	—	166.2	—	165.8	—	166.0	—	165.6	—
2′	130.0	—	131.0	—	130.6	—	118.6	6.33 d (15.9)	130.0	—
3′[b]	129.7	8.02 br d (7.5)	129.5	7.98 dt (7.2, 1.5)	129.7	7.95 br d (7.2)	145.2	7.60 d (15.9)	129.7	8.01 dd (8.4, 1.3)
4′[b]	128.6	7.40 br t (7.5)	128.3	7.37 t (7.2)	128.6	7.33 br t (7.2)	134.3	—	128.6	7.48 dd (8.4, 8.4)
5′[c]	133.3	7.53 tt (7.5, 1.2)	132.7	7.48 tt (7.2, 1.5)	133.1	7.45 br t (7.2)	128.6	7.42–7.46 m	133.3	7.60 tm (8.4)
6′[c]							129.7	7.28–7.30 m		
7′							130.4	7.28–7.30 m		

[a] Assignments were based on HMQC, HMBC, and COSY experiments.
[b] Compounds **4**–**6**: 3′=7′ and 4′=6′.
[c] Compound **7**: 5′=9′ and 6′=8′.

group. Thus, **5** was assigned to be 3β-benzoyloxyvouacapen-5α-ol and was named as pulcherrin H.

Pulcherrin I (**6**) showed the molecular ion peak at m/z 422.2459 $[M]^+$ by HREIMS corresponding to a molecular formula of $C_{27}H_{34}O_4$. The ^{1}H and ^{13}C NMR spectroscopic data (Table 2) were closely related to those of **5** except for the arrangement of a benzoyloxy group whose location in **6** was at C-6, whereas that of **5** at C-3. The observed HMBC correlations of a proton at δ 5.47 (H-6) with the carbons at δ 30.7 (C-8), 31.6 (C-7), 39.0 (C-4), 41.3 (C-10), 76.4 (C-5), and the carbonyl carbon of a benzoyloxy group at δ 165.8 (C-1′) supported the assignment. The small vicinal coupling constants ($J_{6eq,7ax,}$=2.7 Hz and $J_{6eq,7eq}$=2.7 Hz) suggested the relative stereochemistry of H-6 to be equatorially oriented. In the NOESY spectrum, an oxymethine proton at δ 5.47 (H-6) showed cross-peaks with the methyl protons at δ 0.93 (Me-18) and the aromatic protons at δ 7.95 (H-3′, H-7′) correlated with the methyl protons at δ 1.44 (Me-20), confirming a β-orientation of a benzoyloxy group. Thus, **6** was assigned to be 6β-benzoyloxyvouacapen-5α-ol and was named as pulcherrin I.

Pulcherrin J (**7**) showed the molecular formula $C_{29}H_{36}O_4$ ($[M]^+$ m/z 448.2617) by HREIMS. The ^{1}H and ^{13}C NMR spectroscopic data (Table 2) were closely related to those of **6** except for the replacement of a benzoyloxy group at δ 7.33 (br t, J=7.2 Hz; H-4′, H-6′), 7.45 (br t, J=7.2 Hz; H-5′), and 7.95 (br d, J=7.2 Hz; H-3′, H-7′) in **6** with a *trans*-cinnamoyloxy moiety in **7** at δ 6.33 and 7.60 (each d, J=15.9 Hz, H-2′ and H-3′, respectively) and 7.28–7.46 (m, H-5′ to H-9′). The HMBC correlation of H-6 (δ 5.31) to the carbonyl carbon of the cinnamoyloxy group at δ 166.0 (C-1′) suggested the location of the *trans*-cinnamoyloxy side chain at C-6. Thus, **7** was assigned to be 6β-cinnamoyloxyvouacapen-5α-ol and was named as pulcherrin J.

Pulcherrin K (**8**) had the molecular formula $C_{27}H_{32}O_6$ ($[M]^+$ m/z 452.2198), based on HREIMS. The ^{1}H and ^{13}C NMR spectral data (Table 3) were related to those of **6**. The major differences were the replacement of the ^{1}H NMR signals of Me-19 at δ 1.13 and the methylene protons at δ 1.53 (ddd, J=14.4, 3.9, 2.7 Hz; H_{eq}-7) and 2.23 (td, J=14.4, 2.7 Hz; H_{ax}-7) of **6** with an aldehydic proton at δ 9.65 (d, J=1.2 Hz; H-19) and an oxymethine proton at δ 4.33 (dd, J=11.1, 4.2 Hz; H-7), respectively in **7**. The HMBC correlation of an oxymethine proton at δ 4.33 (H-7) with the carbons at δ 27.2 (C-14), 37.7 (C-8), and 73.8 (C-6), of an aldehydic proton at δ 9.65 (H-19) with the carbons at δ 29.1 (C-3), 55.8 (C-4), and 78.6 (C-5) and of the methyl protons at δ 1.10 (Me-18) with the carbons at δ 29.1 (C-3), 55.8 (C-4), 78.6 (C-5), and 202.3 (C-19) confirmed the attachments of an OH and an aldehyde groups at C-7 and C-4, respectively. In the NOESY spectrum, the aldehydic proton at δ 9.65 (H-19) displayed a cross-peak with the methyl protons at δ 1.18 (Me-20) indicating a β-orientation. The large and small coupling constants ($J_{7ax,8ax}$=11.1 Hz, $J_{7ax,6eq}$=4.2 Hz) of H-7 and its NOESY cross-peaks with H-6, H-9, and Me-17 confirmed an α-axial orientation. Thus, **8** was deduced to be 6β-benzoyloxy-7β-hydroxy-19-formylvouacapen-5α-ol and was named as pulcherrin K.

Pulcherrin L (**9**) was deduced as $C_{27}H_{34}O_5$ from an exact mass measurement ($[M]^+$ m/z 438.2405) by HREIMS. The ^{1}H and ^{13}C NMR spectral data (Table 3) of **9** were comparable to those of **6**. The difference was shown as the replacement of a singlet methyl at δ 1.13 (Me-19) in **6** with an oxymethylene proton signals at δ 4.88

Table 3
^{1}H and ^{13}C NMR (300 and 75 MHz, $CDCl_3$) spectroscopic data of compounds **8**–**11** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)[a]

Position	**8**		**9**		**10**		**11**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	34.2	1.45–1.49 m 1.51–1.55 m	34.6	1.30–1.36 m 1.40–1.44 m	34.7	1.45–1.50 m 1.70–1.75 m	33.1	1.52–1.60 m 1.86–1.92 m
2	17.8	1.40–1.50 m 1.60–1.70 m	17.9	1.43–1.47 m 1.65–1.70 m	18.7	1.40–1.44 m 1.62–1.66 m	24.3	1.80–1.86 m 2.50–2.58 m
3	29.1	1.35–1.45 m 1.85–1.95 m	31.8	1.44–1.52 m 1.58–1.70 m	34.1	1.35–1.40 m 1.75 br d (13.8)	77.7	5.28 dd (12.0, 4.5)
4	55.8	—	44.0	—	48.4	—	53.3	—
5	78.6	—	76.7	—	76.5	—	78.5	—
6	73.8	5.92 d (4.2)	71.0	4.17 t (3.6)	70.7	5.45 t (2.7)	70.9	5.57 br s
7	69.0	4.33 dd (11.1, 4.2)	35.4	1.38–1.44 m 2.19 dt (13.5, 3.6)	30.8	1.58 dt (14.1, 2.7) 2.11–2.18 m	30.4	1.60–1.74 m 2.15–2.23 m
8	37.7	1.99 td (11.1, 5.1)	29.8	2.03–2.12 m	30.7	1.92–2.04 m	30.5	2.04 br t (11.4)
9	36.7	2.20–2.33 m	38.6	2.20–2.34 m	38.0	2.11–2.25 m	37.8	2.35 td (11.4, 8.7)
10	41.2	—	41.1	—	41.7	—	41.8	—
11	22.2	2.42–2.61 m	21.9	2.36–2.46 m	22.2	2.34–2.56 m	22.2	2.40–2.60 m
12	148.8	—	149.4	—	149.3	—	149.1	—
13	121.8	—	122.5	—	122.2	—	122.2	—
14	27.2	2.96 qd (6.9, 5.1)	31.2	2. 54 qd (7.2, 5.4)	31.0	2.41–2.53 m	30.9	2.44–2.55 m
15	109.6	6.12 d (1.8)	109.5	6.12 d (1.8)	109.5	6.08 d (1.5)	109.5	6.10 d (1.8)
16	140.7	7.17 d (1.8)	140.4	7.16 d (1.8)	140.5	7.13 d (1.5)	140.6	7.16 d (1.8)
17	17.0	0.97 d (6.9)	17.7	0.94 d (7.2)	17.5	0.92 d (6.9)	17.6	0.94 d (6.9)
18	19.1	1.10 s	20.8	1.11 s	24.2	0.97 s	19.9	1.22 s
19	202.3	9.65 d (1.2)	68.2	4.88 d (11.4) 5.01 d (11.4)	181.9	—	177.4	—
20	17.0	1.18 s	16.2	1.31 s	17.6	1.32 s	16.7	1.55 s
1′	167.3	—	166.6	—	165.7	—	166.1	—
2′	129.2	—	130.5	—	130.6	—	130.1	—
3′/7′	129.9	7.92 d (7.2)	129.5	7.97 br d (7.5)	129.5	7.84 br d (7.2)	129.6	7.85 d (7.5)
4′/6′	128.8	7.38 t (7.2)	128.5	7.38 t (7.5)	128.4	7.32 t (7.2)	128.3	7.27 t (7.5)
5′	133.8	7.52 br t (7.2)	132.9	7.55 br t (7.5)	132.8	7.43 tt (7.2, 1.2)	133.2	7.36 br t (7.5)
1"							165.8	—
2"							130.2	—
3"/7"							129.4	7.85 d (7.5)
4"/6"							128.5	7.18 t (7.5)
5"							133.1	7.43 br t (7.5)

[a] Assignments were based on HMQC, HMBC, and COSY experiments.

and 5.01 (each d, J=11.4 Hz; 2H-19) in **9**. In addition the oxymethine proton H-6 in **9** appeared at δ 4.17 (t, J=3.6 Hz), more highfield than that of **6** (δ 5.47, t, J=2.7 Hz) indicating the OH group at C-6 instead of a benzoyloxy group as in **6**. The HMBC correlations of the oxymethylene proton signals at δ 4.88 and 5.01 (2H-19) with the carbons at δ 20.8 (C-18), 31.8 (C-3), 44.0 (C-4), 76.7 (C-5), and 166.6 (C-1′) suggested the attachment of a benzoyloxy group at C-19. In the NOESY spectrum, the cross-peaks of the oxymethylene protons at δ 4.88 and 5.01 (2H-19) with the methyl protons at δ 1.31 (Me-20), and of an oxymethine proton at δ 4.17 (H-6) with the methyl protons at δ 1.11 (Me-18) indicated an oxymethylene protons to be β-oriented and H-6 as α-oriented, respectively. Therefore, **9** was assigned as 6β-hydroxy-19-benzoyloxyvouacapen-5α-ol and was named as pulcherrin L.

Pulcherrin M (**10**) showed the molecular ion $[M]^+$ at m/z 452.2196 by HREIMS spectrum in agreement with the formula $C_{27}H_{32}O_6$. The ^{1}H and ^{13}C NMR spectral data (Table 3) of **10** showed characteristics similar to those of **6** except for the disappearance of a methyl singlet at δ_H 1.13 (Me-19; δ_C 26.0) and the appearance of a carboxyl carbon at δ_C 181.9 in **10**. This finding was supported by HMBC spectrum in which the methyl protons at δ 0.97 (Me-18) were correlated with the carbons at δ 34.1 (C-3), 48.4 (C-4), 76.5 (C-5), and 181.9 (C-19). The relative stereochemistry of **10** was assigned by NOESY experiment, in which Me-18 (δ 0.97) showed a cross-peak with δ 5.45 (H-6), whereas the benzoyloxy protons H-3′/H-7′ (δ 7.84) with δ 1.32 (Me-20). Therefore, **10** was assigned as 6β-benzoyloxy-19-carboxyvouacapen-5α-ol and was named as pulcherrin M.

The molecular weight of pulcherrin N (**11**), $C_{34}H_{36}O_8$ ($[M]^+$ was assigned at m/z 572.2411) by HREIMS. The NMR spectroscopic data (Table 3) of **11** displayed similarities with pulcherrin M (**10**) except for the presence of an additional monosubstituted benzene ring in the range δ 7.18–7.85 and an oxymethine proton at δ 5.28 (dd, J=12.0, 4.5 Hz; H-3) in **11**. The latter proton was attached to the oxymethine carbon at δ 77.7 in the HMQC spectrum and showed HMBC correlations to the carbons at δ 19.9 (C-18), 24.3 (C-2), 53.3 (C-4) 166.1 (C-1′), and 177.4 (C-19), confirming the location of a benzoyloxy group at C-3. The stereochemistry of H-3 as α-axial oriented was determined from the results of the large and small coupling constants ($J_{3ax,2ax}$=12.0 Hz, $J_{3ax,2eq}$=4.5 Hz) and by the observed cross-peak with Me-18 (δ 1.22) in the NOESY experiment. Thus, **11** was 3β,6β-dibenzoyloxy-19-carboxyvouacapen-5α-ol and was named as pulcherrin N.

Pulcherrin O (**12**) with the molecular formula $C_{30}H_{38}O_9$ by HERIMS showed comparable ^{1}H and ^{13}C NMR spectral data (Table 4) with those of **3** except for the appearance of the additional signals of an oxymethine proton at δ_H 5.24 (H-3; δ_C 76.7) and a benzoyloxy group (δ_H 7.37–7.95; δ_C 128.4, 129.5, 130.6, 133.0, 166.1) in **12** whose location of the latter at C-3 was supported by the HMBC correlations of H-3 with the carbons at δ 19.2 (C-19), 22.7 (C-18), 43.9 (C-4), and 166.1 (C-1′). In addition the methyl doublet at δ_H 1.07 (Me-17; δ_C 17.1) in **3** was replaced with a singlet signal of a methyl ester at C-17 (δ_H 3.68; δ_C 52.2) and an ester carbonyl at δ_C 175.9 in **12**. The location of a CO_2Me group was confirmed by HMBC spectrum, in which the methine proton H-14 (δ 3.38) showed the correlations with the ester carbonyl carbon at δ 175.9. The large vicinal coupling constant of H-3 ($J_{3ax,2ax}$=10.8 Hz) and H-14 ($J_{14ax,8ax}$=8.4 Hz) suggested the relative stereochemistry of H-3 and H-14 to be α-axially oriented. In the NOESY spectrum, the hydroxyl

Table 4
^{1}H and ^{13}C NMR (300 and 75 MHz, $CDCl_3$) spectroscopic data of compounds **12**–**15** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)[a]

Position	**12**		**13**		**14**		**15**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	32.6	1.42–1.49 m 1.73–1.82 m	32.3	1.37–1.45 m 1.74–1.86 m	31.7	1.25–1.30 m 1.70–1.77 m	38.1	0.74–0.84 m 1.45–1.53 m
2	23.9	1.73–1.78 m 1.82–1.89 m	23.9	1.75–1.83 m	23.7	1.71–1.79 m 1.84–1.94 m	18.6	1.33–1.40 m 1.43–1.53 m
3	76.7	5.24 dd (10.8, 5.7)	76.7	5.31 dd (10.8, 4.8)	76.9	5.23 dd (11.7, 4.5)	42.0	0.98–1.10 m 1.26–1.38 m
4	43.9	—	48.2	—	43.1	—	33.2	—
5	78.8	—	79.0		77.2	—	54.6	0.80 dd (10.8, 2.7)
6	73.4	5.42 d (4.2)	71.3	4.16 d (3.3)	55.0	3.25 d (4.2)	22.0	1.16–1.23 m 1.55–1.63 m
7	74.0	4.05 dd (10.2, 4.2)	78.2	5.19 dd (11.1, 3.3)	54.0	3.01 d (4.2)	35.2	1.15–1.23 m 1.94–2.23 m
8	37.6	3.38 ddd (10.5, 9.9, 8.1)	34.3	2.76 ddd (11.1, 9.0, 8.4)	35.6	2.21–2.26 m	36.9	1.55–1.65 m
9	41.2	2.24–2.35 m	41.5	2.32 ddd (9.0, 7.5, 4.8)	35.3	2.24–2.38 m	56.9	1.04 td (14.1, 2.1)
10	41.1	—	40.9	—	39.1	—	37.3	—
11	21.5	2.44–2.54 m	21.4	2.47–2.55 m	23.6	2.25–2.31 m 2.38–2.44 m	36.4	1.79 t (14.1) 2.13 dd (14.1, 2.1)
12	150.7	—	150.5	—	149.8	—	211.8	—
13	113.1	—	112.8	—	122.1	—	33.4	—
14	45.6	3.38 d (8.1)	45.4	3.29 d (8.4)	31.0	2.90 qd (6.9, 5.4)	38.5	0.94 dd (5.7, 1.5)
15	108.8	6.13 d (1.8)	108.3	6.07 d (1.8)	109.3	6.15 d (1.8)	37.3	1.37–1.45 m
16	141.2	7.17 d (1.8)	144.4	7.17 d (1.8)	141.0	7.17 d (1.8)	62.3	3.47 dd (11.7, 8.1) 3.73 dd (11.7, 5.7)
17	175.9	—	174.6	—	17.1	1.11 d (6.9)	14.1	1.17 s
18	22.7	1.03 s	15.2	1.06 s	23.2	1.16 s	33.4	0.78 s
19	19.2	1.26 s	64.0	4.63 d (12.0) 5.39 d (12.0)	19.6	1.34 s	21.5	0.73 s
20	16.5	1.40 s	15.7	1.35 s	16.4	1.24 s	14.1	0.71 s
OCH_3	52.2	3.68 s	52.1	3.68 s				
$OCOCH_3$	170.8		170.7					
$OCOCH_3$	21.7	2.10 s	21.0	2.00 s				
19-$OCOCH_3$			171.6					
19-$OCOCH_3$			21.0	1.98 s				
1′	166.1	—	166.1	—	166.2	—		
2′	130.6	—	130.4	—	130.8	—		
3′/7′	129.5	7.95 br d (7.2)	129.7	8.03 br d (7.8)	129.6	8.00 br d (7.5)		
4′/6′	128.4	7.37 t (7.2)	128.3	7.38 t (7.8)	128.4	7.39 t (7.5)		
5′	133.0	7.92 tt (7.2, 2.1)	133.0	7.50 br t (7.8)	132.9	7.51 tt (7.5, 1.5)		
5-OH		2.01 br s		2.21 s				

[a] Assignments were based on HMQC, HMBC, and COSY experiments.

proton at C-5 (δ 2.01) showed cross-peaks with H-3, H-6, H-7, H-9, and Me-18, whereas the methine proton H-14 (δ 3.38) displayed cross-peaks with H-7 and H-9 but not with H-8 supporting a benzoyloxy and CO_2Me group as β-oriented. Thus, **12** was deduced to be 3β-benzoyloxy-6β-acetoxy-7β-hydroxy-14β-methoxycarbonylvouacapen-5α-ol and was named as pulcherrin O.

The molecular formula of pulcherrin P (**13**) was determined to be $C_{32}H_{38}O_{11}$ ([M]$^+$ m/z 598.2423) by HREIMS. The ^{1}H and ^{13}C NMR spectral data (Table 4) of **13** were similar to those of **12**. The differences were shown as a replacement of a singlet at δ 1.26 (Me-19) in **12** with an oxymethylene protons at δ 4.63 and 5.39 (each, d, J=12.0 Hz; 2H-19) and an acetyl group (δ_H 1.98: δ_C 21.0, and δ_C 171.6) in **13**, whose position was supported by the HMBC correlations of oxymethylene protons at δ 4.63 and 5.39 (2H-19) with the carbons at δ 15.2 (C-18), 48.2 (C-4), 76.7 (C-3), 79.0 (C-5), and 171.6 ($OCOCH_3$). Furthermore the HMBC correlations of an oxymethine proton at δ 5.19 (H-7) with the carbons at δ 34.3 (C-8), 45.4 (C-14), and 170.7 ($OCOCH_3$) and of an oxymethine proton at δ 4.16 (H-6) with carbons at δ 34.3 (C-8), 40.9 (C-10), 78.2 (C-7), and 79.0 (C-5) implied the locations of an OAc group and an OH at C-7 and C-6, respectively. The relative stereochemistry of **13** was analyzed by NOESY experiment, in which the oxymethylene protons (2H-19) showed a cross-peak with the methyl protons at δ 1.35 (Me-20). Therefore, **13** was 3β-benzoyloxy-6β-hydroxy-7β,19-diacetoxy-14β-methoxycarbonylvouacapen-5α-ol and was named as pulcherrin P.

Pulcherrin Q (**14**) showed the molecular ion [M]$^+$ at m/z 436.2250 by HREIMS spectrum in agreement with the formula $C_{27}H_{32}O_5$. The ^{1}H and ^{13}C NMR spectral data (Table 4) of **14** showed characteristics similar to those of **5** except for the presence of a 1,2-disubstituted epoxide ring resonanced as two oxymethine protons at δ_H 3.25 and 3.01 (each d, J=4.2 Hz; δ_C 55.0, 54.0, respectively) instead of 2 sets of methylene protons as in **5**. The signal at δ_H 3.25 was deduced to be an oxymethine proton H-6 from its HMBC correlations with the carbons at δ 39.1 (C-10), 43.1 (C-4), 54.0 (C-7), and 77.2 (C-5), and the other proton as H-7 (δ_H 3.01) from its HMBC correlations with the carbons at δ 31.0 (C-14), 35.3 (C-9), 35.6 (C-8), and 55.0 (C-6), whose data suggested an epoxide ring between C-6 and C-7. The relative stereochemistry of **14** was determined on the basis of coupling constants and the results of NOESY experiments. The large J values for H-6 and H-7 (J=4.2 Hz) suggested a *cis* epoxide ring. From the NOESY correlations, an oxymethine proton at δ 3.25 (H-6) showed cross-peaks with the protons at δ 1.16 (Me-18) and 3.01 (H-7), and an oxymethine proton at δ 3.01 (H-7) with the methyl protons at δ 1.11 (Me-17) indicating that this *cis* epoxide ring should be β-oriented. Thus, **14** was assigned as 3β-benzoyloxy-6β,7β-epoxyvouacapen-5α-ol and was named as pulcherrin Q.

Pulcherrin R (**15**) had the molecular formula $C_{20}H_{32}O_2$ ([M]$^+$ m/z 304.2405) based on HREIMS. The ^{13}C NMR (Table 4) and DEPT spectral data exhibited 20 carbons including a carbonyl at δ 211.8 (C-12) and an oxymethylene carbon at δ 62.3 (C-16). The ^{1}H NMR spectral

data (Table 4) showed four aliphatic methyl groups at δ 0.71 (Me-20), 0.73 (Me-19), 0.78 (Me-18), and 1.17 (Me-17), and the oxymethylene protons at δ 3.47 (dd, J=11.7, 8.1 Hz; H-16) and 3.73 (dd, J=11.7, 5.7 Hz; H-16). The presence of a cyclopropane ring was deduced from the ^{1}H NMR, COSY, and HMQC spectra that exhibited two signals at δ_H 0.94 (dd, J=5.7, 1.5 Hz, H-14: δ_C 38.5) and 1.37–1.45 (m, H-15: δ_C 37.3). The observed HMBC correlations of a singlet methyl group at δ 1.17 (Me-17) with the carbons at δ 33.4 (C-13), 37.3 (C-15), 38.5 (C-14), and 211.8 (C-12), and of the oxymethylene protons at δ 3.47 and 3.73 (2H-16) with the carbons at δ 33.4 (C-13), 37.3 (C-15), and 38.5 (C-14) supported the assignments. These data suggested a carbonyl group at C-12 and an OH group at C-16, whereas C-13, C-14, and C-15 formed a cyclopropane ring. The NOESY cross-peaks of the proton signal at δ 1.79 (t, J=14.1 Hz; H_{ax}-11) with the protons at δ 0.71 (Me-20), 1.37–1.45 (H-15), and 1.55–1.65 (H-8), of a methine proton at δ 0.94 (H-14) with the methyl protons at δ 1.17 (Me-17), 1.04 (H-9) and oxymethylene protons at δ 3.47 and 3.73 (2H-16) but no correlation with H-15 supported the α-orientation of H-14, Me-17, and 2H-16 hence suggesting a *cis* cyclopropyl ring with an α-hydroxy methyl side chain. The stereochemistry of compound **15** was implied by biogenetic pathway from the pimarane skeleton.[13] Therefore, **15** was assigned as 13,14,15-cyclopropa-12-oxo-16-hydroxypimarane and was named as pulcherrin R.

All the furanoditerpenes isolated have similar stereochemistry. The three six-membered rings A, B, and C are fused as a *trans*/*anti*/*trans* system. The configurations of H-8, Me-20, and Me-19 are β-oriented, whereas those of H-9, Me-17, Me-18, and OH-5 are α-oriented. With substituent groups, the configurations of H-3, H-6, and H-7 are α-oriented.

The CH_2Cl_2 extract from the roots of *C. pulcherrima* showed an inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cell lines with an IC_{50} value of 6.1 μg/ml. Further separation and purification led to the isolation of 23 diterpenes (**1**–**23**) as shown in Fig. 1. The results indicated that compound **14** was the most potent inhibitor of NO production (Table 5) with an IC_{50} value of 2.9 μM and compounds **8**, **9**, **11**–**15**, and **17**–**23** significantly reduced LPS-stimulated NO production with the IC_{50} values in the range of 3.4–10.2 μM better than that of the positive control, indomethacin (IC_{50}=14.5 μM), whereas other compounds exhibited weak activity. Compounds **17** (IC_{50}=5.3 μM) and **18** (IC_{50}=8.2 μM) showed much better activity than **3** (IC_{50}=59.7 μM) suggesting that the cinnamoyloxy and benzoyloxy groups at C-6 may increase the activity more than the acetoxy group. The substitution of a benzoyloxy group at C-3 (**11**, IC_{50}=4.2 μM) and C-7 (**19**, IC_{50}=6.0 μM) demonstrated significantly increase in NO inhibitory activity compared to that of **10** (IC_{50}=26.7 μM). The oxidation at C-19 of **10** (IC_{50}=26.7 μM) resulted in 2-fold increase in activity against NO production compared to that of **6** (IC_{50}=47.5 μM).

3. Experimental

3.1. General experimental procedures

Melting points were determined on the Fisher–John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. The IR spectra were measured with a Perkin–Elmer FTS FT-IR spectrophotometer. The UV spectra were measured with an UV-160A spectrophotometer (Shimadzu) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. The ^{1}H and ^{13}C NMR spectra were recorded using 300 MHz Bruker FTNMR Ultra Shield™ spectrometers in $CDCl_3$ solution. Chemical shifts are recorded in parts per million (δ) with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively.

3.2. Reagents

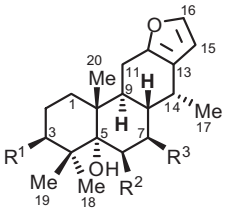
Lipopolysaccharide (LPS, from *Escherichia coli*), RPMI-1640 medium, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), indomethacin and phosphate buffer saline (PBS) were purchased from Sigma Aldrich (Sigma Aldrich, Missouri, USA). Fetal calf serum (FCS) was bought from Gibco (Invitrogen, California, USA). Penicillin–streptomycin was purchased from Invitrogen (Invitrogen, California, USA). 96-Well microplates were obtained from Nunc (Nunc, Birkrød, Denmark). ELISA test kits of PGE_2 and TNF-α were from R&D systems (R&D systems, Minnesota, USA). Other chemicals were from Sigma Aldrich (Sigma Aldrich, Missouri, USA).

3.3. Plant material

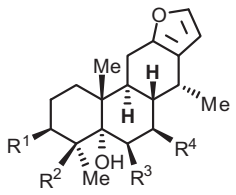
C. pulcherrima (L.) Swartz. was collected from Songkhla province, Thailand in October 2007. Identification was made by Assoc. Prof. Dr. Kitichate Sridith, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. SC51) was deposited at the Prince of Songkla University Herbarium.

3.4. Extraction and isolation

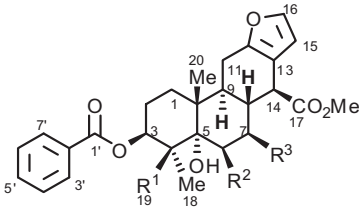
Air-dried roots (6.3 kg) of *C. pulcherrima* was extracted with CH_2Cl_2 (each 2×10 L, for 5 days) at room temperature. The crude extract was evaporated under reduced pressure to afford a brownish CH_2Cl_2 (75.3 g) extract, which was further purified by VLC using hexane as eluent and increasing polarity with EtOAc and MeOH to give sixteen fractions (P1–P16). Fraction P2 (5.9 g) was further purified by VLC with hexane–CH_2Cl_2 (1:4, v/v) to give **23** (90.5 mg), **16** (50.2 mg), **6** (275.2 mg), **7** (90.0 mg), and **5** (28.1 mg) and a mixture of two compounds (158.0 mg), which were further separated by CC with acetone–hexane (1:9, v/v) to give **4** (15.0 mg) and **1** (10.3 mg). Fraction P4 (10.0 g) was recrystallized from CH_2Cl_2 to give **18** (2.54 g), and the mother liquor (7.5 g) was further subjected to VLC with hexane as eluent and increasing polarity with CH_2Cl_2 and EtOAc to afford six subfractions (P4a–P4f). Subfraction P4c (183.4 mg) was purified by CC with acetone–hexane (1:9, v/v) to give **2** (5.8 mg). Subfraction P4d (584.9 mg) was separated by CC with CH_2Cl_2–hexane (7:3, v/v) to yield **14** (5.2 mg) and **9** (10.0 mg). Repeated recrystallization from CH_2Cl_2 of fraction P6 (624.2 mg) yielded **17** (138.0 mg). Fraction P7 (2.9 g) was separated by VLC with hexane as eluent and increasing polarity with CH_2Cl_2 and EtOAc to give nine subfractions (P7a–P7i) and **19** (355.0 mg). Each subfraction was further separated by CC with acetone–hexane (1:4, v/v) to afford **8** (18.2 mg) and **11** (25.0 mg) from subfraction P7e (401.4 mg), **3** (3.0 mg) from subfraction P7f (273.5 mg), and finally **10** (25.0 mg) from subfraction P7g (117.0 mg). Fraction P9 (5.0 g) was recrystallized from CH_2Cl_2 to give **21** (1.24 g). Fraction P10 (2.9 g) was further purified by VLC and eluted with a gradient of CH_2Cl_2–EtOAc (1:4 to 1:1, v/v) to give seven subfractions (P10a–P10g). Purification of subfraction P10c (283.2 mg) by CC with acetone–CH_2Cl_2 (2:3, v/v) afforded **15** (7.0 mg) while **22** (13.0 mg) was purified from subfraction P10f (124.7 mg) by CC with CH_2Cl_2–acetone (1:9, v/v). Fraction P13 (2.7 g) was further purified by VLC with acetone–CH_2Cl_2 (1:4, v/v) to give six subfractions (P13a–P13f). Subfraction P13c (151.7 mg) was separated by CC with EtOAc–hexane (2:3, v/v) to yield **13** (10.0 mg). Subfraction P13e (197.8 mg) was isolated by CC with EtOAc–CH_2Cl_2 (1:9, v/v) to give **12** (10.0 mg). Finally, **20** (12.5 mg) was isolated from fraction P15 (941.4 mg) by VLC (CH_2Cl_2 to MeOH–CH_2Cl_2, 3:7, v/v) and followed by CC with EtOAc–CH_2Cl_2 (1:19, v/v).



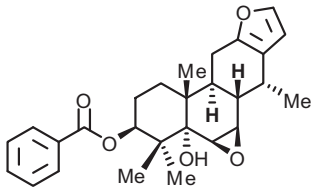
	R^1	R^2	R^3
1	H	H	OAc
2	H	OH	OAc
3	H	OAc	OH
4	H	OH	OCOPh
5	OCOPh	H	H
6	H	OCOPh	H
7	H	OCOCH=CHPh (*E*)	H
16	H	H	H
17	H	OCOCH=CHPh (*E*)	OH
18	H	OCOPh	OH



	R^1	R^2	R^3	R^4
8	H	CHO	OCOPh	OH
9	H	CH_2OCOPh	OH	H
10	H	CO_2H	OCOPh	H
11	OCOPh	CO_2H	OCOPh	H
19	H	CO_2H	OCOPh	OCOPh
20	OH	CO_2H	OCOPh	OCOPh
21	OCOPh	CO_2H	OCOPh	OAc



	R^1	R^2	R^3
12	Me	OAc	OH
13	CH_2OAc	OH	OAc



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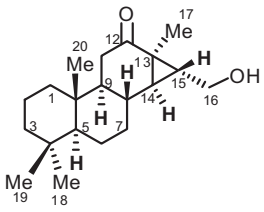
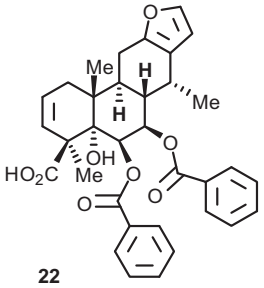


15



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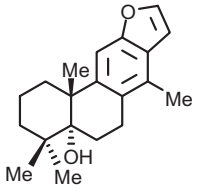

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Fig. 1. Structures of compounds **1**–**23**.

*3.4.1. Pulcherrin D (**1**).* Viscous oil; $[\alpha]_D^{25}$ +23.7 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (3.88) nm; IR (neat) ν_{max} 3453 (O–H), 2931 (C–H), 1723 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 1; HREIMS: *m*/*z* 360.2301 $[M]^+$ (calcd for $C_{22}H_{32}O_4$, 360.2301).

*3.4.2. Pulcherrin E (**2**).* Viscous oil; $[\alpha]_D^{25}$ +36.1 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (3.84) nm; IR (neat) ν_{max} 3455 (O–H), 2920 (C–H), 1723 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 1; HREIMS: *m*/*z* 376.2250 $[M]^+$ (calcd for $C_{22}H_{32}O_5$, 376.2250).

*3.4.3. Pulcherrin F (**3**).* Viscous oil; $[\alpha]_D^{25}$ +67.4 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 215 (3.78) nm; IR (neat) ν_{max} 3425 (O–H), 2929 (C–H), 1734 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 1; HREIMS: *m*/*z* 376.2252 $[M]^+$ (calcd for $C_{22}H_{32}O_5$, 376.2250).

*3.4.4. Pulcherrin G (**4**).* White solid; mp 126–128 °C; $[\alpha]_D^{25}$ +57.1 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (3.26) nm; IR (neat) ν_{max} 3494 (O–H), 2932 (C–H), 1710 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 2; HREIMS: *m*/*z* 438.2410 $[M]^+$ (calcd for $C_{27}H_{34}O_5$, 438.2406). The physical and spectral data of **4** from

Table 5
Inhibitory effects on NO production[a] of compounds **1**–**23**

Compounds	% Inhibition at various concentrations (μM)						IC_{50} (μM)
	0	**1**	**3**	**10**	**30**	**100**	
1	0.0±2.0	—	—	13.7±1.6	32.1±2.0**	70.8±2.2**	48.5
2	0.0±2.0	—	—	9.9±3.4	37.3±3.1**	71.2±1.9**	46.1
3	0.0±2.0	—	—	−0.5±3.6	24.1±3.1**	67.9±4.1**	59.7
4	0.0±8.6	—	—	36.8±1.1**	39.0±1.9**	79.4±1.2**	43.2
5	0.0±2.3	—	—	25.2±1.7**	44.2±2.4**	45.1±2.2[b]**	>100
6	0.0±2.3	—	—	8.3±1.5	33.0±1.3**	72.8±1.4[b]**	47.5
7	0.0±2.3	—	—	17.5±2.4	47.6±2.9**	71.8±2.0[b]**	37.4
8	0.0±4.8	—	—	53.2±3.1**	67.0±2.1**	104.3±1.8[b]**	10.2
9	0.0±4.8	—	—	57.9±2.6**	82.4±1.9**	104.3±2.0[b]**	6.4
10	0.0±2.0	—	—	15.6±2.1	69.8±2.0**	76.4±2.0[b]**	26.7
11	0.0±4.8	27.3±2.1	34.8±2.0*	71.0±3.8**	95.0±1.7**	99.4±3.4[b]**	4.2
12	0.0±8.2	—	38.3±2.6*	78.0±4.2**	97.8±4.9[b]**	105.4±1.9[b]**	4.2
13	0.0±8.2	—	42.6±1.8**	77.4±3.3**	101.1±5.0**	104.8±4.8[b]**	3.4
14	0.0±8.2	—	49.7±2.4**	81.2±4.1**	103.8±4.7**	104.9±5.4[b]**	2.9
15	0.0±8.2	—	32.8±2.1*	67.7±4.6**	98.4±3.8**	100.5±4.6[b]**	5.4
16	0.0±2.3	—	—	9.7±2.0	35.9±2.7**	67.5±0.9[b]**	50.7
17	0.0±8.6	—	38.5±2.1*	60.1±0.4**	88.3±0.9**	104.0±0.9[b]**	5.3
18	0.0±8.6	—	29.6±1.8	55.6±1.3**	71.7±4.2**	104.5±1.6[b]**	8.2
19	0.0±9.3	−2.3±2.8	2.3±2.0	100.0±1.5[b]**	102.0±5.2[b]**	108.7±1.8[b]**	6.0
20	0.0±9.3	—	36.2±2.2*	64.7±0.5**	100.0±2.0**	106.0±5.2[b]**	5.2
21	0.0±9.3	2.3±3.2	13.0±1.3	102.2±4.2[b]**	103.8±5.0[b]**	108.7±1.5[b]**	5.6
22	0.0±8.2	—	39.5±2.2*	71.5±3.3**	105.4±2.2**	105.9±3.1[b]**	4.4
23	0.0±8.2	—	35.4±2.4*	58.1±3.7**	71.0±2.7**	100.0±3.2**	7.0
Indomethacin	0.0±4.2	—	15.5±1.7	36.4±2.3**	60.9±3.7**	104.5±1.7**	14.5

Statistical significance, *$p<0.05$, ** $p<0.01$.
[a] Each value represents mean±S.E.M. of four determinations.
[b] Cytotoxic effect was observed.

the synthesis:[8] white solid; mp 125–127 °C; $[\alpha]_D^{25}$ +23.7 (c 0.27, $CHCl_3$); ^1H NMR and ^{13}C NMR data, see Table 2.

*3.4.5. Pulcherrin H (**5**).* White solid; mp 195–196 °C; $[\alpha]_D^{25}$ +106.5 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 228 (3.96) nm; IR (neat) ν_{max} 3525 (O–H), 2929 (C–H), 1700 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 2; HREIMS: *m*/*z* 422.2454 $[M]^+$ (calcd for $C_{27}H_{34}O_4$, 422.2457).

*3.4.6. Pulcherrin I (**6**).* White solid; mp 131–133 °C; $[\alpha]_D^{25}$ +28.8 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (3.87) nm; IR (neat) ν_{max} 3549 (O–H), 2934 (C–H), 1708 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 2; HREIMS: *m*/*z* 422.2459 $[M]^+$ (calcd for $C_{27}H_{34}O_4$, 422.2457).

*3.4.7. Pulcherrin J (**7**).* White solid; mp 135–136 °C; $[\alpha]_D^{25}$ +52.8 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (3.65), 275 (3.67) nm; IR (neat) ν_{max} 3516 (O–H), 2933 (C–H), 1709 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 2; HREIMS: *m*/*z* 448.2617 $[M]^+$ (calcd for $C_{29}H_{36}O_4$, 448.2614).

*3.4.8. Pulcherrin K (**8**).* Viscous oil; $[\alpha]_D^{25}$ +20.3 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (3.94) nm; IR (neat) ν_{max} 3471 (O–H), 2935 (C–H), 1710 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 3; HREIMS: *m*/*z* 452.2198 $[M]^+$ (calcd for $C_{27}H_{32}O_6$, 452.2199).

*3.4.9. Pulcherrin L (**9**).* Viscous oil; $[\alpha]_D^{25}$ +64.1 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (3.82) nm; IR (neat) ν_{max} 3471 (O–H), 2927 (C–H), 1720 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 3; HREIMS: *m*/*z* 438.2405 $[M]^+$ (calcd for $C_{27}H_{34}O_5$, 438.2406).

*3.4.10. Pulcherrin M (**10**).* Viscous oil; $[\alpha]_D^{25}$ +19.7 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (3.90) nm; IR (neat) ν_{max} 3508 (O–H), 2931 (C–H), 1707 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 3; HREIMS: *m*/*z* 452.2196 $[M]^+$ (calcd for $C_{27}H_{32}O_6$, 452.2199).

*3.4.11. Pulcherrin N (**11**).* Viscous oil; $[\alpha]_D^{25}$ +23.6 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (3.96) nm; IR (neat) ν_{max} 3508 (O–H), 2934 (C–H), 1704 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 3; HREIMS: *m*/*z* 572.2411 $[M]^+$ (calcd for $C_{34}H_{36}O_8$, 572.2410).

*3.4.12. Pulcherrin O (**12**).* Viscous oil; $[\alpha]_D^{25}$ +26.7 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 228 (3.98) nm; IR (neat) ν_{max} 3470 (O–H), 2930 (C–H), 1720 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 4; HREIMS: *m*/*z* 540.2361 $[M]^+$ (calcd for $C_{30}H_{38}O_9$, 540.2359).

*3.4.13. Pulcherrin P (**13**).* Viscous oil; $[\alpha]_D^{25}$ +54.7 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (3.89) nm; IR (neat) ν_{max} 3468 (O–H), 2927 (C–H), 1716 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 4; HREIMS: *m*/*z* 598.2423 $[M]^+$ (calcd for $C_{32}H_{38}O_{11}$, 598.2414).

*3.4.14. Pulcherrin Q (**14**).* Viscous oil; $[\alpha]_D^{25}$ +48.8 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (3.92) nm; IR (neat) ν_{max} 3436 (O–H), 2930 (C–H), 1713 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 4; HREIMS: *m*/*z* 436.2250 $[M]^+$ (calcd for $C_{27}H_{32}O_5$, 436.2250).

*3.4.15. Pulcherrin R (**15**).* Viscous oil; $[\alpha]_D^{25}$ +83.8 (*c* 0.20, $CHCl_3$); IR (neat) ν_{max} 3372 (O–H), 2926 (C–H), 1688 (C=O) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 300 MHz), see Table 4; HREIMS: *m*/*z* 304.2405 $[M]^+$ (calcd for $C_{20}H_{32}O_2$, 304.2402).

3.5. Anti-inflammatory activity assay

Inhibitory effects of compounds on NO production from RAW 264.7 cells.

Inhibitory effects on NO production by murine macrophage-like RAW 264.7 cells were evaluated using a modified method from that previously reported.[14] Briefly, the RAW 264.7 cell line [purchased from Cell Lines Service (CLS)] was cultured in RPMI medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine,

penicillin G (100 units/mL), streptomycin (100 μg/mL) and 10% FCS. The cells were harvested with trypsin–EDTA and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere for 1 h at 37 °C in a humidified atmosphere containing 5% CO_2. After that the medium was replaced with a fresh medium containing 200 μg/mL of LPS together with the test samples at various concentrations and was then incubated for 48 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric method. Briefly, after 48 h incubation with the test samples, MTT solution (10 μL, 5 mg/mL in PBS) was added to the wells. After 4 h incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm. The test compounds were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group. Indomethacin was used as a positive control. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI (final DMSO is 1%). Inhibition (%) was calculated using the following equation and IC_{50} values were determined graphically (n=4):

$$\text{Inhibition } (\%) = \frac{A-B}{A-C}\times 100$$

A–*C*: NO_2^- concentration (μM) [*A*: LPS (+), sample (−); *B*: LPS (+), sample(+); *C*: LPS (−), sample (−)].

3.6. Statistical analysis

The results were expressed as mean±S.E.M of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

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