JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp
Article

Tetrahydroxanthene-1,3(2*H*)-diones and Oxidized Hexadiene Derivatives from *Uvaria leptopoda* and Their Biological Activities

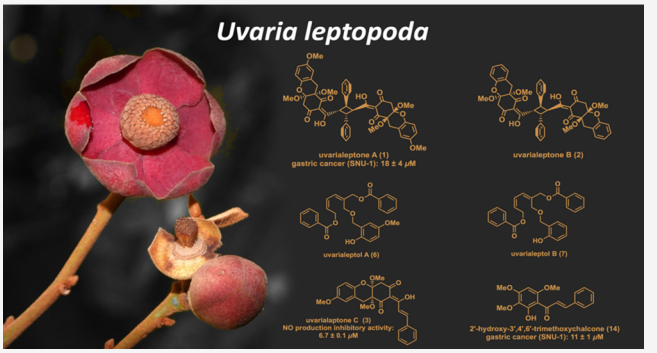
Passakorn Teerapongpisan, Thanakorn Monkantha, Saranphong Yimklan, Siau Hui Mah, Natalie Vivien Gunter, Phunrawie Promnart, Suwanna Deachathai, Tharakorn Maneerat, Thidarat Duangyod, Rawiwan Charoensup, Abdulromea Baka, Raymond J. Andersen, and Surat Laphookhieo*

Cite This: *J. Nat. Prod.* 2024, 87, 1611−1617

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The first phytochemical investigation of the twig extract of *Uvaria leptopoda* resulted in the isolation and identification of three new tetrahydroxanthene-1,3(2*H*)-diones, uvarialeptones A−C, two new oxidized hexadiene derivatives, uvarialeptols A and B, together with ten known compounds. Their structures were elucidated by spectroscopic techniques and mass spectrometry. Uvarialeptones A and B were unprecedented tetrahydroxanthene-1,3(2*H*)-dione dimers which exhibited a cyclobutane ring via [2 + 2] cycloaddition from uvarialeptone C and 9a-*O*-methyloxymitrone, respectively. The structure of uvarialeptone A was confirmed by X-ray diffraction analysis using Mo Kα radiation. Compound **3** inhibited NO production at an IC_{50} value of 6.7 ± 0.1 μM.



Uvaria (Annonaceae) genus, a shrub or climbing plant, is widely distributed throughout Asian countries, including Thailand, the Philippines, and Malaysia.[1] Some species of this genus have been used in traditional medicines. For example, the roots of *U. cherrevensis* have been used for treating urinary disorders,[2,3] while the stem of *U. dulcis*[4] and the root of *U. rufa*[5] have been used for fever[4,5] and kidney failure treatments.[5] *Uvaria* species produce diverse chemical compounds, including highly oxygenated cyclohexenes,[2,6,7] flavonoids,[3,6,8−11] chalcones,[3,8−10] alkaloids,[3,11] oxepinones,[7] naphthalenes,[8,10] and tetrahydroxanthene-1,3(2*H*)-dione derivatives.[12] Among them, highly oxygenated cyclohexenes were the major compounds found in this genus. Compounds isolated from *Uvaria* species displayed a wide range of biological activities. For example, valderramenol A, isolated from the leaves of *U. valderramensis*, showed antitubercular activity with a MIC value of 10 μg/mL.[12] Oxoanolobine, and ergosta-4,6,8(14),22-tetraen-3-one, isolated from the aerial parts of *U. rufa*, displayed cytotoxic activity against human lung adenocarcinoma cell line (LU-1) with IC_{50} values of 9.22 μg/mL and 10.21 μg/mL, respectively.[13] Uvarin B, a dimeric chalcone, isolated from the roots of *U. siamensis*, showed antiplasmodial activity with an IC_{50} value of 3.02 μg/mL,[10] while 2-hydroxy-3-methoxy-6-(4′-hydroxyphenyl)naphthalene isolated from the stem and root extracts of *U. cherrevensis*, exhibited cytotoxicity against the human mouth epidermal carcinoma cells (KB) with an IC_{50} value of 5.05 μM.[8]

Uvaria leptopoda (King) J. Sinclair is a woody climber grown as an ornamental plant in the lowland rainforests of Thailand.[1] No phytochemical investigations or biological evaluations of this plant have been reported in the SciFinder Scholar database (Chemical Abstracts Service). This information led us to investigate their phytochemicals and biological activities. Five new (**1**−**3**, **6**, and **7**) and ten known compounds (**4**, **5**, and **8**−**15**) were isolated, and some isolated compounds were evaluated for their cytotoxic effects toward SNU-1 human gastric cancer cells and anti-inflammatory activities through nitric oxide (NO) production inhibitory effect using LPS-induced RAW264.7 cells.

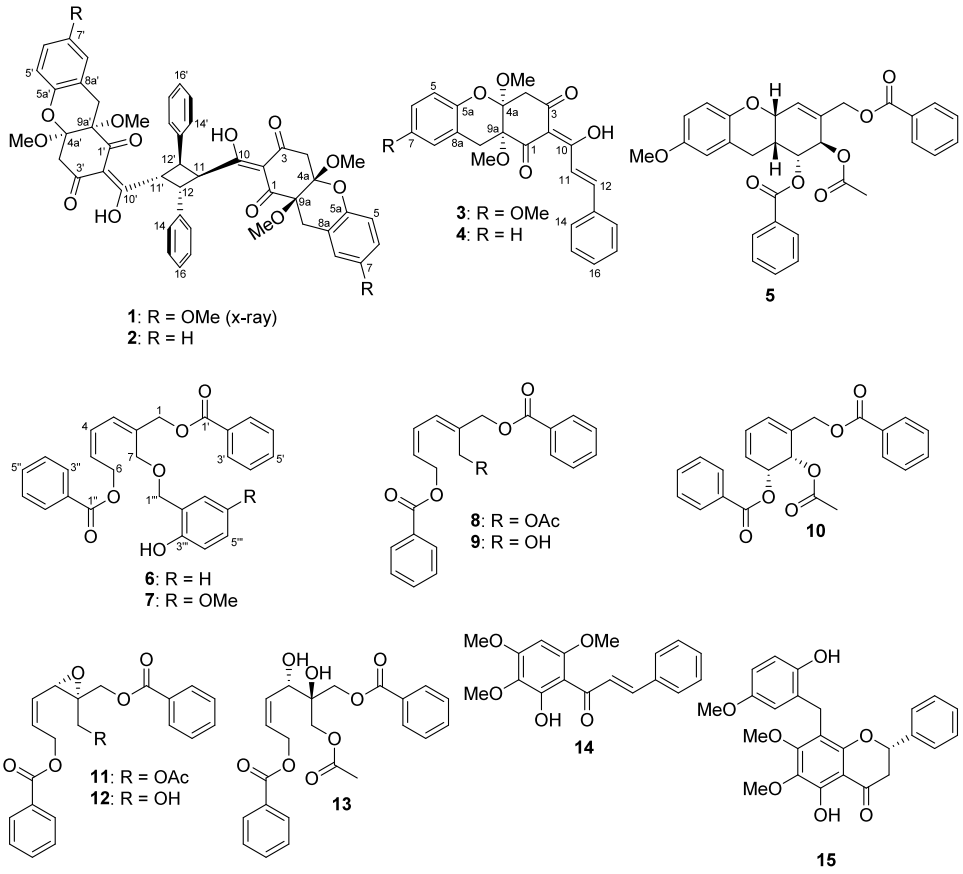
RESULTS AND DISCUSSION

The twig extract of *U. leptopoda* was subjected to various column chromatographic separations to yield 15 compounds. Among them, three tetrahydroxanthene-1,3(2*H*)-diones (**1**−**3**) and two oxidized hexadiene derivatives (**6** and **7**) were identified as new compounds. The known compounds found

Received: February 29, 2024
Revised: May 13, 2024
Accepted: May 16, 2024
Published: May 28, 2024

https://doi.org/10.1021/acs.jnatprod.4c00248
J. Nat. Prod. 2024, 87, 1611−1617

Chart 1



are 9a-*O*-methyloxymitrone (**4**),[14] cyathostemmine (**5**),[15] grandiuvarin C (**8**),[16] uvamalol G (**9**),[17] (−)-1,6-desoxytinganoxide (**10**),[18] grandiuvarin A (**11**),[16] valderepoxide (**12**),[19] grandiuvarin B (**13**),[16] 2′-hydroxy-3′,4′,6′-trimethoxychalcone (**14**),[20] and macrophyllin (**15**).[21]

Uvarialeptone A (**1**), HRESIMS *m*/*z* 871.2972 $[M - H]^-$ (calcd for $C_{50}H_{47}O_{14}$, 871.2971), was obtained as a colorless needle crystal. The X-ray diffraction data (CCDC 2326335) of compound **1** (Figure 1) confirmed that its structure is a head-to-tail symmetric tetrahydroxanthene-1,3(2*H*)-dione dimer. The dimeric compound **1** displayed only one set of NMR resonances (Table 1) as follow; a monosubstituted aromatic ring [δ_H 7.24 (4H, d, *J* = 7.1 Hz)/δ_C 128.5, C-14/C-14′ and C-18/C-18′, δ_H 7.17 (4H, dd, *J* = 7.1, 7.1 Hz)/δ_C 128.4, C-15/C-15′ and C-17/C-17′, and δ_H 7.12 (2H, d, *J* = 7.1 Hz)/δ_C 127.1, C-16/C-16′], an ABX aromatic ring [δ_H 6.64 (2H, m)/δ_C 113.7, C-6/C-6′, δ_H 6.61 (2H, m)/δ_C 117.6, C-5/C-5′, and δ_H 6.61 (2H, m)/δ_C 113.5, C-8/C-8′], two methylene groups [δ_H 3.12 (2H, d, *J* = 17.8 Hz) and 3.02 (2H, d, *J* = 17.8 Hz)/δ_C 37.2, C-4/C-4′, δ_H 3.55 (2H, t, *J* = 15.0 Hz) and 2.80 (2H, d, *J* = 15.0 Hz)/δ_C 24.5, C-9/C-9′], three methoxy groups [δ_H 3.73 (6H, s)/δ_C 55.5, OMe-7/OMe-7′, δ_H 3.24 (6H, s)/δ_C 49.2, OMe-4a/OMe-4a′, and δ_H 2.65 (6H, s)/δ_C 51.5, OMe-9a/OMe-9a′], one hydrogen-bonded hydroxy proton [δ_H 18.36 (2H, s), OH-10/OH-10a′], and a symmetric cyclobutane [δ_H 5.03 (2H, dd, *J* = 11.4, 7.6 Hz)/δ_C 41.7, C-12/C-12′ and δ_H 4.79 (2H, dd, *J* = 11.4, 7.6 Hz)/δ_C 53.0, C-11/C-11′]. The HMBC correlations supported the assignments, as shown in Figure 2. The relative configuration of compound **1**, (4a*R**,9a*R**,11*R**,12*S**,4a′*S**,9a′*S**,11′*S**,12′*R**), was determined by the analyses of X-ray diffraction data and NOESY correlations. The specific rotation was zero, and its ECD spectrum displayed no Cotton effect, suggesting compound **1** was obtained as a racemic mixture. Several attempts to isolate pure enantiomers using Chiral HPLC were not successful (Supporting Information, Figures S36−39). This is the first example of a tetrahydroxanthene-1,3(2*H*)-dione dimer isolated from a natural source.

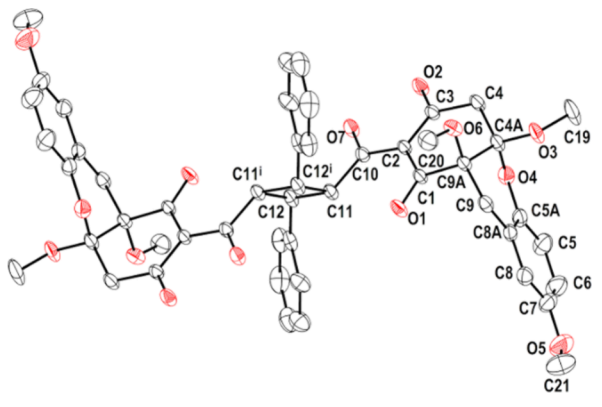


Figure 1. ORTEP diagram of compound **1**.

Uvarialeptone B (**2**), HRESIMS *m*/*z* 813.2922 $[M + H]^+$ (calcd for $C_{48}H_{45}O_{12}$, 813.2906), was isolated as a yellow amorphous powder. The ^1H and ^{13}C NMR spectroscopic data (Table 1) of compound **2** were similar to that of compound **1**. The only difference observed was the missing methoxy resonance in compound **2**. In addition, the ABX aromatic protons of compound **1** changed to a 1,2-disubstituted

https://doi.org/10.1021/acs.jnatprod.4c00248
J. Nat. Prod. 2024, 87, 1611−1617

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data for Compounds 1 and 2 in $CDCl_3$

	1			2		
Position	δ_C	type	δ_H, mult (J in Hz)	δ_C	type	δ_H, mult (J in Hz)
1/1′	188.8,	C		188.8,	C	
2/2′	109.7,	C		109.8,	C	
3/3′	194.7,	C		194.6,	C	
4/4′	37.2,	CH_2	3.02 d (17.8); 3.12 d (17.8)	37.3,	CH_2	3.04 d (17.7); 3.13 d (17.7)
4a/4a′	97.2,	C		97.4,	C	
5/5′	117.6,	CH	6.61 m	117.1,	CH	6.70 d (7.2)
5a/5a′	143.7,	C		150.0,	C	
6/6′	113.7,	CH	6.64 m	127.7,	CH	7.04 t (7.2)
7/7′	154.6,	C		122.4,	CH	6.92 m
8/8′	113.5,	CH	6.61 m	129.5,	CH	7.11 m
8a/8a′	121.9,	C		121.4,	C	
9/9′	24.5,	CH_2	2.80 d (15.0); 3.55 d (15.0)	24.3,	CH_2	2.83 d (14.9); 3.58 d (14.9)
9a/9a′	78.0,	C		78.1,	C	
10/10′	203.1,	C		203.2,	C	
11/11′	53.0,	CH	4.79 dd (11.4, 7.6)	53.1,	CH	4.78 dd (11.4, 7.7)
12/12′	41.7,	CH	5.03 dd (11.4, 7.6)	41.8,	CH	5.02 dd (11.4, 7.7)
13/13′	139.7,	C		139.8,	C	
14/14′, 18/18′	128.5,	CH	7.24 d (7.1)	128.6,	CH	7.23 d (7.2)
15/15′, 17/17′	128.4,	CH	7.17 dd (7.1, 7.1)	128.5,	CH	7.16 dd (7.2, 7.2)
16/16′	127.1,	CH	7.12 d (7.1)	127.2,	CH	7.11 m
OMe-4a/OMe-4a′	49.2,	CH_3	3.24 s	128.5,	CH_3	3.25 s
OMe-7/OMe-7′	55.5,	CH_3	3.73 s			
OMe-9a/OMe-9a′	51.5,	CH_3	2.65 s	51.6,	CH_3	2.68 s
OH-10/OH-10′			18.36 s			18.33 s

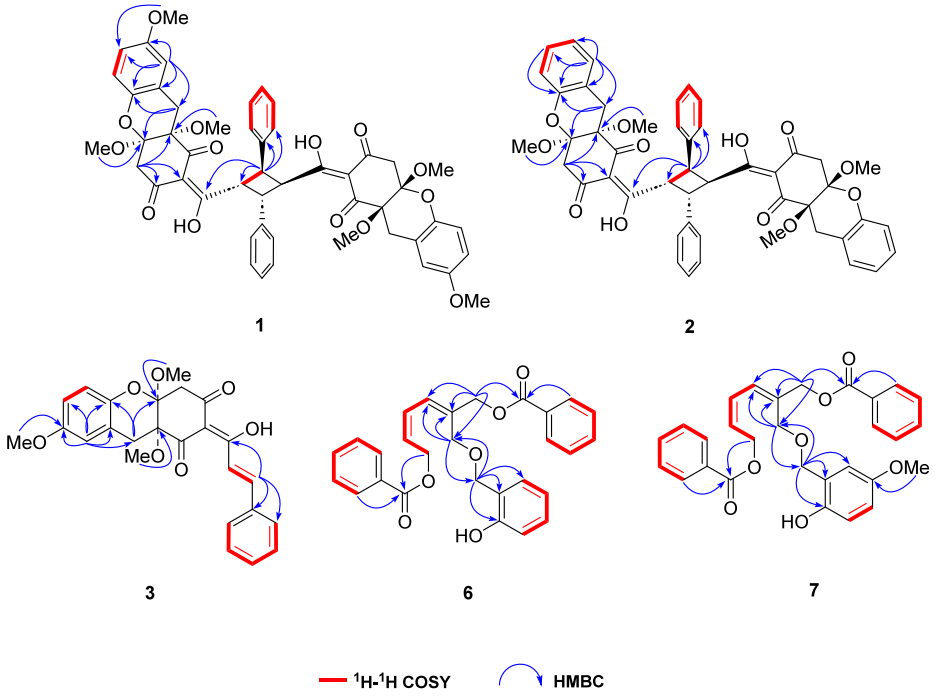


Figure 2. Key $^{1}H-^{1}H$ COSY and HMBC correlations of compounds 1−3 and 6−7.

benzene proton in compound 2 [δ_H 7.11 (2H, m)/δ_C 129.5, C-8 and C-8′, δ_H 7.04 (2H, t, J = 7.2 Hz)/δ_C 127.7, C-6 and C-6′, δ_H 6.92 (2H, m)/δ_C 122.4, C-7 and C-7′, and δ_H 6.70 (2H, d, J = 7.2 Hz)/δ_C 117.1, C-5 and C-5′], which were confirmed by COSY and HMBC as shown in Figure 2. Thus, compound 2 was named as uvarialeptone B.

Uvarialeptone C (3), HRESIMS m/z 435.1442 $[M - H]^-$ (calcd for $C_{25}H_{23}O_7$, 435.1449) was isolated as a yellow amorphous powder. Intensive analysis of the NMR spectroscopic data of 3 (Table 2) suggested that the structure of compound 3 is similar to that of compound 1. The major difference between these compounds is that compound 3 displayed resonances for a Δ^{11} double bond, which contributed to the NMR resonances at δ_H 8.17 (1H, d, J = 15.9 Hz)/δ_C 121.6 (C-11) and 7.97 (1H, d, J = 15.9 Hz)/δ_C 146.9 (C-12). In contrast, compound 1 carries a cyclobutene moiety. The

https://doi.org/10.1021/acs.jnatprod.4c00248
J. Nat. Prod. 2024, 87, 1611−1617

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data for Compound 3 in $CDCl_3$

	3		
Position	δ_C,	type	δ_H, mult (J in Hz)
1	191.1,	C	
2	108.6,	C	
3	199.2,	C	
4	39.6,	CH_2	3.25 d (17.1); 3.38 d (17.1)
4a	97.3,	C	
5	117.7,	CH	6.73 d (8.9)
5a	143.9,	C	
6	113.8,	CH	6.66 dd (8.9, 3.1)
7	154.8,	C	
8	113.6,	CH	6.73 d (3.1)
8a	122.5,	C	
9	25.1,	CH_2	3.00 d (15.0); 3.69 d (15.0)
9a	79.4,	C	
10	187.8,	C	
11	121.6,	CH	8.17 d (15.9)
12	146.9,	CH	7.97 d (15.9)
13	134.8,	C	
14/18	129.1,	CH	7.37 d (7.6)
15/17	129.2,	CH	7.62 dd (6.7, 2.9)
16	131.3,	CH	7.37 d (7.6)
OMe-4a	49.4,	CH_3	3.35 s
OMe-7	55.7,	CH_3	3.76 s
OMe-9a	52.3,	CH_3	3.32 s
OH-10			18.42 s

large J value (15.9 Hz) of H-11/H-12 identified the E geometry of the Δ^{11} double bond. The HMBC correlation (Figure 2) between H-12 to C-10 (δ_C 187.8), C-13 (δ_C 134.8), and C-14/C-18 (δ_C 129.1) supported the assignment above. Comparing structures **3** and **1**, it was suggested that structure **3** might be a monomer of structure **1** via the [2 + 2] cycloaddition at the Δ^{11} double bond. Since the structure of compound **1** was confirmed by X-ray diffraction data, the *cis* dimethoxy at C4a and C-9a of compound **3** should be identified as the same as compound **1**. The specific rotation of compound **3** was zero, and its ECD spectrum displayed no Cotton effect, suggesting compound **3** is a racemic mixture.

Uvarialeptol A (**6**), HRESIMS m/z 457.1654 $[M - H]^-$ (calcd for $C_{28}H_{25}O_6$, 457.1657), was obtained as a colorless viscous oil. The ^{1}H and ^{13}C NMR spectra of **6** (Table 3) displayed characteristics of an oxidized hexadiene derivative, similar to those of grandiuvarin C (**8**),[16] including two benzoyl units [(δ_H 8.05 (2H, dd, J = 8.4, 1.3 Hz)/δ_C 130.4, C-3′ and C-7′, δ_H 7.50 (4H, ddd, J = 8.4, 8.4, 1.6 Hz)/δ_C 129.5, C-4′, C-6′, C-4″, and C-6″, and δ_H 7.63 (2H, m)/δ_C 134.0, C-5′ and C-5″)], three olefinic protons [δ_H 6.92 (1H, dd, J = 11.4, 1.1 Hz)/δ_C 126.3, C-3, δ_H 6.72 (1H, t, J = 11.4 Hz)/δ_C 127.9, C-4, and δ_H 5.87(1H, dt, J = 11.4, 7.0 Hz)/δ_C 128.4, C-5)], and three oxymethylene groups [δ_H 5.07 (2H, dd, J = 7.0, 1.3 Hz)/δ_C 61.2, C-6, δ_H 5.01 (2H, s, H-1)/δ_C 67.5, C-1, and δ_H 4.43 (2H, s, H-1)/δ_C 66.3, C-7)]. However, a 2-hydroxy benzyloxy moiety ([δ_H 7.28 (1H, dd, J = 7.5, 1.5 Hz)/δ_C 130.0, C-7‴, δ_H 7.11 (1H, ddd, J = 7.5, 7.5, 1.5 Hz)/δ_C 129.5, C-5‴, δ_H 6.83 (1H, dd, J = 7.5, 1.5 Hz)/δ_C 116.1, C-4‴, and δ_H 6.79 (1H,

Table 3. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data for Compounds 6 and 7 in acetone-d_6

	6			7		
Position	δ_C,	type	δ_H, mult (J in Hz)	δ_C,	type	δ_H, mult (J in Hz)
1	67.5,	CH_2	5.01 s	67.4,	CH_2	5.01 s
2	136.7,	C		136.6,	C	
3	126.3,	CH	6.92 dd (11.4, 1.1)	126.2,	CH	6.92 dd (11.0, 1.0)
4	127.9,	CH	6.72 t (11.4)	127.8,	CH	6.71 m
5	128.4,	CH	5.87 dt (11.4, 7.0)	128.3,	CH	5.87 dt (11.0, 7.0)
6	61.2,	CH	5.07 dd (7.0, 1.3)	61.1,	CH	5.07 dd (7.0, 1.3)
7	66.3,	CH_2	4.43 s	66.2,	CH_2	4.43 s
OBz-1′						
1′	166.6,	C		166.5,	C	
2′	131.2,	C		131.1,	C	
3′/7′	130.4,	CH	8.05 dd (8.4, 1.3)	130.3,	CH	8.04 dd (8.4, 1.3)
4′/6′	129.5,	CH	7.50 ddd (8.4, 8.4, 1.6)	129.4,	CH	7.50 ddd (8.4, 8.4, 2.4)
5′	134.0,	CH	7.63 m	134.0,	CH	7.63 m
OBz-6						
1″	166.7,	C		166.6,	C	
2″	131.3,	C		131.1,	C	
3″/7″	130.3,	CH	8.02 dd (8.4, 1.3)	130.2,	CH	8.01 dd (8.4, 1.3)
4″/6″	129.5,	CH	7.50 ddd (8.4, 8.4, 1.6)	129.4,	CH	7.50 ddd (8.4, 8.4 2.4)
5″	134.0,	CH	7.63 m	133.9,	CH	7.63 m
Benzyloxy-7						
1‴	68.7,	CH_2	4.64 s	68.4,	CH_2	4.59 s
2‴	125.5,	C		126.2,	C	
3‴	156.2,	C		149.7,	C	
4‴	116.1,	CH	6.83 dd (7.5, 1.5)	116.6,	CH	6.75 d (8.7)
5‴	129.5,	CH	7.11 ddd (7.5, 7.5, 1.5)	114.5,	CH	6.67 dd (8.7, 3.1)
6‴	120.3,	CH	6.79 ddd (7.5, 7.5, 1.5)	153.9,	C	
7‴	130.0,	CH	7.28 dd (7.5, 1.5)	114.9,	CH	6.89 d (3.1)
OMe-6‴				55.8,	CH_3	3.68 s

https://doi.org/10.1021/acs.jnatprod.4c00248
J. Nat. Prod. 2024, 87, 1611−1617

ddd, J = 7.5, 7.5, 1.5 Hz)/δ_C 120.3, C-6‴] and one oxymethylene group [δ_H 4.64 (2H, s)/δ_C 68.7, C-1‴]) were detected in **6** instead of the acetoxy group in **8**. The HMBC correlations (Figure 2) between H-1‴ with C-7 and H-7 with C-1‴ confirmed that the 2-hydroxy benzoyloxy moiety was located at C-7. Two benzoyl groups were placed on C-1 and C-6 according to the HMBC correlations of H-1 with C-1′ and H-6 with C-6″, respectively. The geometries of the double bonds at C-2/C-3 (Δ^2) and C-4/C-5 (Δ^4) were designated as *E* and *Z*, respectively, based on the NOESY correlation between H_2-1/H-3 and H-4/H-5, as well as the observed J coupling constants (11.4 Hz) between H-3/H-4 and H-4/H-5.

Uvarialeptol B (**7**), HRESIMS m/z 487.1750 $[M - H]^-$ (calcd for $C_{29}H_{27}O_7$, 487.1762), was isolated as a colorless viscous oil. The ^1H and ^{13}C NMR spectroscopic data of **7** (Table 3) were similar to those of **6**, except that the resonances for the 2-hydroxy benzoyloxy moiety of **6** were replaced with a 2-hydroxy-5-methoxybenzoyloxy moiety in **7**. The ^1H NMR data of **7** contained resonances for an aromatic ring ABX spin system [δ_H 6.89 (1H, d, J = 3.1 Hz)/δ_C 114.9, C-7‴, δ_H 6.75 (1H, d, J = 8.7 Hz)/δ_C 116.6, C-4‴, and δ_H 6.67 (1H, dd, J = 8.7, 3.1 Hz)/δ_C 114.5, C-5‴] and methoxy group [δ_H 3.68 (3H, s)/δ_C 55.8, OMe-6‴]. The key HMBC correlations used to confirm the structure of **7** are shown in Figure 2.

Compounds **1**, **3**, **5**, **11**, **13**, and **14** were evaluated for their cytotoxicity against SNU-1 human gastric cancer cells, and the results were presented in Table 4. Compounds **1**, **3**, **11**, and **14**

Table 4. IC_{50} Values of Compounds against SNU-1 Gastric Cancer Cells

Compounds	IC_{50} (μM)
1	18 ± 4
3	34 ± 5
5	130 ± 10[a]
11	37 ± 2
13	82 ± 7[a]
14	11 ± 1
Cisplatin	25 ± 1

[a]$p < 0.0001$ compared to cisplatin. Data are presented as mean ± SD from three independent experiments.

had IC_{50} values comparable with cisplatin, which has been used as a clinical drug against gastric cancer.[22] No significant differences were found between cisplatin and these compounds ($p > 0.05$). Due to cisplatin resistance in gastric cancer treatment, compounds with comparable activity to the clinical drug may have chemotherapeutic potential. Compounds **1** and **14** displayed IC_{50} values of 18 and 11 μM, respectively, lower than that of cisplatin (IC_{50} = 25 μM).

The anti-inflammatory potential of compounds **1**, **3**, **5**, **11**, **13**, and **14** was also evaluated based on their inhibitory effects on LPS-induced nitric oxide (NO) production by RAW264.7 macrophages (Table 5). All the compounds tested are nontoxic toward RAW264.7 cells at 15 μM with cell viabilities of >90%. Thus, the bioassay was performed at this concentration. Among the tested compounds, **3** was the most potent NO production inhibitor, showing the highest percentage of inhibition at 73%. In comparison, other compounds showed minimal NO inhibition effect at the same concentration with a 6.5 to 15 μM percentage range. Thus, only compound **3** was further assessed at a range of concentrations, and its IC_{50} value was observed at 6.7 μM,

Table 5. Inhibitory Effects of Compounds on LPS-Induced NO Production in RAW264.7 Macrophages

Compounds	NO inhibition (%)[a]	IC_{50} (μM)
1	6.5 ± 1	–
3	73 ± 2	6.7 ± 0.1[b]
5	15 ± 1	–
11	12 ± 3	–
13	14 ± 2	–
14	−11 ± 3	–
Diclofenac sodium	–	331 ± 2

[a]Test concentration = 15 μM. [b]$p < 0.0001$ compared to diclofenac sodium. –, not tested. Data are presented as mean ± SD from three independent experiments.

which is almost 50 times lower than the standard drug, diclofenac sodium (IC_{50} = 331 μM). The results showed that **3** has a high potential as a primary anti-inflammatory agent. On the other hand, **14** did not inhibit NO production and instead upregulated the NO production without affecting the viability of the RAW264.7 cells, indicating a pro-inflammatory effect. Thus, the activity of **14** against SNU-1 gastric cancer cells may occur via pro-inflammatory mechanisms. Further studies are recommended to confirm this hypothesis.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting point was recorded on a Buchi M-560 apparatus. The specific rotations were measured on a Jasco P-2000 polarimeter. The UV−vis spectra were recorded with a Varian Cary 5000 spectrometer. The electronic circular dichroism (ECD) spectra were measured on a JASCO J-1500 spectropolarimeter. The IR spectra were determined on a PerkinElmer FTS FT-IR spectrometer. The NMR spectra were recorded on a 500 MHz Bruker FT-NMR Ultra Shield in $CDCl_3$ and acetone-d_6 with TMS as an internal standard; J values are reported in Hz. The HRESIMS were obtained on an Agilent 1290 infinity II/G6545B QTOF mass spectrometer. High-performance liquid chromatography (HPLC) analysis was performed on an Agilent Technology HPLC 1260 Infinity II system, coupled to a 1260 Infinity II Diode Array Detector HS. A CHIRALCEL OD-H @ 5 μm (4.6 mmφ × 250 mmL) column was used for chiral HPLC. Silica gel C60 (0−20 μm, SiliCycle Inc.) and silica gel G60 (60−200 μm, SiliCycle Inc.) were used to perform quick column chromatography (QCC) and column chromatography (CC), respectively. Precoated TLC plates of silica gel $60F_{254}$ were used for analytical purposes. The X-ray crystallography was performed on a Rigaku SuperNova, Mo Kα, Single source at offset/far, HyPix3000.

Plant Material. The twigs of *U. leptopoda* were collected in March 2022 from Narathiwat Province (N: 6.156754°, E: 101.664661°). The plant was identified by Mr. Abdulromae Baka (Independent Research Group on Plant Diversity in Thailand, Sichon, Nakhon Si Thammarat, 80120, Thailand). A voucher specimen (MFU-NPR0216) was deposited at the Natural Products Research Laboratory of Mae Fah Luang University.

Extraction and Isolation. The air-dried twigs of *U. leptopoda* (2.0 kg) were extracted with EtOAc (3 × 4 L, for 3 days) at room temperature and concentrated under reduced pressure to provide the EtOAc extract (299.68 g), which was subjected to QCC (100% hexanes to 100% EtOAc) to give five fractions (ULA−ULE). Fraction ULC (257.3 mg) was further separated by CC (1:4 v/v, EtOAc−hexanes) to obtain three fractions (ULC1−ULC3). Further purification of fraction ULC1 (13.7 mg) by Sephadex LH20 (100% MeOH) gave compounds **2** (1.0 mg) and **4** (2.1 mg). Fraction ULC2 (65.2 mg) was subjected to Sephadex LH20 (100% MeOH) to afford compounds **1** (22.1 mg) and **3** (31.3 mg). Fraction ULD (385.7 mg) was purified by CC (1:9 v/v, EtOAc−hexanes), yielding eight fractions (ULD1−ULD8) and compound **11** (5.8 mg). Fraction

https://doi.org/10.1021/acs.jnatprod.4c00248
J. Nat. Prod. 2024, 87, 1611−1617

ULD2 (10.9 mg) was further purified by Sephadex LH20 (1:4 v/v, MeOH−CH_2Cl_2) to obtain compound **8** (1.4 mg). Fraction ULD4 (37.2 mg) was separated by CC (1:4 v/v, acetone−hexanes) to yield compound **5** (8.6 mg). Fraction ULD5 (25.8 mg) was separated by CC over silica gel (1:4 v/v, acetone−hexanes) to afford three counds **6** (0.9 mg), **14** (7.1 mg), and **15** (1.2 mg). Compounds **7** (0.8 mg), **10** (1.0 mg), and **13** (6.5 mg) were isolated from Fraction ULD7 (30.2 mg) by CC (1:4 v/v, acetone−hexanes). Fraction ULD8 (31.2 mg) was separated by CC over silica gel (2:3 v/v, acetone−hexanes) to obtain **9** (1.1 mg) and **12** (0.9 mg).

*Uvarialeptone A (**1**).* Colorless needles: mp 174−175 °C; $[\alpha]_D^{25}$ 0 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.4), 230 (4.7), and 285 (3.8) nm; IR (neat) ν_{max} 2925, 1728, 1598, 1456, 1263, 1092, 804 cm^{-1}; ^1H and ^{13}C NMR, see Table1; HRESIMS *m*/*z* 871.2972 $[M - H]^-$ (calcd for $C_{50}H_{47}O_{14}$, 871.2971).

*Uvarialeptone B (**2**).* Yellow amorphous powder; $[\alpha]_D^{25}$ 0 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.3), 236 (3.1), and 287 (3.6) nm; IR (neat) ν_{max} 2927, 1728, 1671, 1549, 1495, 1451, 1261, 1095, 805 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 813.2922 $[M + H]^+$ (calcd for $C_{48}H_{45}O_{12}$, 813.2906).

*Uvarialeptone C (**3**).* Yellow amorphous powder; $[\alpha]_D^{25}$ 0 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.3), 236 (3.1), and 287 (3.6) nm; IR (neat) ν_{max} 2937, 1666, 1575, 1490, 1417, 1201, 1049 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 435.1442 $[M - H]^-$ (calcd for $C_{25}H_{23}O_7$, 435.1449).

*Uvarialeptol A (**6**).* Colorless oil: UV (MeOH) λ_{max} (log ε) 232 (3.62) and 277 (2.69) nm; IR (neat) ν_{max} 3392, 2960, 1720, 1600, 1453, 1263, and 1098 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRESIMS *m*/*z* 457.1654 $[M - H]^-$ (calcd for $C_{28}H_{25}O_6$, 457.1657).

*Uvarialeptol B (**7**).* Colorless oil: UV (MeOH) λ_{max} (log ε) 232 (3.47) and 272 (2.81) nm; IR (neat) ν_{max} 3423, 2959, 1719, 1601, 1453, 1264, and 1098 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRESIMS *m*/*z* 487.1750 $[M - H]^-$ (calcd for $C_{29}H_{27}O_7$, 487.1762).

X-ray Crystal Structure Analysis of Compound 1. Single crystals of compound **1** ($C_{50}H_{48}O_{14}$) were crystallized in MeOH/CH_2Cl_2 upon slow evaporation under ambient conditions. A suitable single crystal was selected and mounted on a SuperNova, Single source at offset/far, HyPix3000 diffractometer. The crystal was kept at 293(2) K during data collection. The structure was solved with the SHELXT structure solution program using Intrinsic Phasing and refined with the SHELXL refinement package using Least Squares minimization via Olex2 program.[23−25] All non-hydrogen atoms were refined anisotropically. The crystallographic data for **1** (CCDC 2326335) can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

*Crystal Data for **1**.* colorless needle-shaped crystals, $C_{50}H_{48}O_{14}$, monoclinic, space group $P2_1/c$ (no. 14), a = 14.4012(11) Å, b = 12.2540(7) Å, c = 13.9106(9) Å, β = 115.338(9)°, V = 2218.7(3) $Å^3$, Z = 2, Z' = 0.5, T = 293(2) K, μ(MoKα) = 0.096 mm^{-1}, D_{calc} = 1.307 g/cm^3, 17620 reflections measured (4.566° ≤ 2θ ≤ 55.014°), 4713 unique (R_{int} = 0.0395, R_{sigma} = 0.0454) which were used in all calculations. The final R_1 was 0.0512 (I > 2σ(I)), and *w*R2 was 0.1490 (all data).

Cell Culture. Human gastric epithelial carcinoma cells, SNU-1 (CRL-5971) and murine macrophages RAW264.7 (TIB-71) were obtained from American Type Culture Collection (ATCC) and cultured in RPMI 1640 (Gibco, Thermo-Fisher Scientific) and Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo-Fisher Scientific), respectively. Both culture media were supplemented with 10% fetal bovine serum (Sigma-Aldrich). The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

MTT Bioassay. Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Merck) assay according to the experimental literature procedure with some modifications.[26] For the adherent RAW264.7 macrophages, cells were seeded in a 96-well plate at a concentration of 1 × 10^6 cells/mL and incubated for 24 h to allow cell attachment. After that, the cells were treated with compounds (using DMSO as a vehicle at the maximum concentration of 0.1%) and further incubated for 24 h. The culture media was carefully removed and replaced with fresh media, followed by adding 5 mg/mL of MTT reagent in phosphate-buffered saline (PBS; Sigma-Aldrich) and a 4-h incubation. The culture media was carefully removed, and DMSO was added to dissolve the formazan crystals. The plates were shaken for 30 s before measuring the absorbance at 570 nm.

For the SNU-1 cells, the suspension cells were evaluated for cytotoxicity according to a previously reported method.[27] Briefly, the cells were seeded in a 96-well plate and treated with the compounds. After incubating for 24 h, the plates were centrifuged at 1500 rpm for 5 min. Culture media was replaced with fresh media, and MTT reagent was added. After 4 h of incubation, the plates were centrifuged once again. The supernatant was carefully removed, and the formazan crystals were dissolved in DMSO. The plates were shaken before measuring the absorbance. Cisplatin was used as a standard drug, while untreated cells were used as a negative control in the MTT assay for both cell lines.

Nitric Oxide Production Inhibition Bioassay. The evaluation of NO production inhibitory effect of the compounds was carried out using Griess assay according to Mah et al. (2017) with some modifications.[28] Briefly, RAW264.7 cells were seeded in a 96-well plate at a concentration of 1 × 10^6 cells/mL. After 24 h, the cells were induced with lipopolysaccharide (LPS; Merck) in a final concentration of 10 μg/mL and treated with the compounds at a nontoxic concentration toward RAW264.7 cells (cell viability >90%) as determined using the MTT assay. The plates were incubated for 24 h, and 100 μL of the cell supernatant was aliquoted and replaced with Griess reagent. The plates were incubated in the dark at room temperature before measuring the absorbance at 550 nm. Untreated LPS-induced cells were used as a negative control, while diclofenac sodium was used as a positive control. Percentage NO production inhibition was calculated as $[(A_0\text{-}A_1)/A_0 \times 100\%]$, where A_1 is the absorbance of treated cells and A_0 is the absorbance of the negative control.

Statistical Analysis. All biological experiments were performed in three independent biological and technical replicates. The data are presented as mean ± standard deviation. Statistical analyses were performed using one-way ANOVA in GraphPad Prism (version 7.04) at a 5% significance level ($p < 0.001$). The comparison of means was evaluated using Tukey's posthoc test.

■ ASSOCIATED CONTENT

Data Availability Statement

The NMR data for uvarialeptones A−C and uvarialeptols A and B have been deposited in the Natural Products Magnetic Resonance Database (NP-MRD; www.np-mrd.org) and can be found at NP033240 (uvarialeptone A), NP033241 (uvarialeptone B), NP033242 (uvarialeptone C), NP033243 (uvarialeptol A), and NP033244 (uvarialeptol B).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.4c00248.

HRESIMS, 1D and 2D NMR spectra of new compounds **1**−**3**, **6**, and **7** (PDF)

X-ray single-crystal structure of **1** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

Surat Laphookhieo − *Center of Chemical Innovation for Sustainability (CIS) and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand;* orcid.org/0000-0002-4757-2781; Email: surat.lap@mfu.ac.th

https://doi.org/10.1021/acs.jnatprod.4c00248
J. Nat. Prod. 2024, 87, 1611−1617

Authors

Passakorn Teerapongpisan − *Center of Chemical Innovation for Sustainability (CIS) and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Thanakorn Monkantha − *Center of Chemical Innovation for Sustainability (CIS) and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Saranphong Yimklan − *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand;* orcid.org/0000-0003-4008-0845

Siau Hui Mah − *School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, 47500 Subang Jaya, Selangor, Malaysia;* orcid.org/0000-0003-0370-4866

Natalie Vivien Gunter − *School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, 47500 Subang Jaya, Selangor, Malaysia*

Phunrawie Promnart − *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Suwanna Deachathai − *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Tharakorn Maneerat − *Center of Chemical Innovation for Sustainability (CIS) and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Thidarat Duangyod − *Medicinal Plant Innovation Center of Mae Fah Luang University and School of Integrative Medicine, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Rawiwan Charoensup − *Medicinal Plant Innovation Center of Mae Fah Luang University and School of Integrative Medicine, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Abdulromea Baka − *Chanae, Narathiwat 96220, Thailand*

Raymond J. Andersen − *Departments of Chemistry and Earth, Ocean & Atmospheric Sciences, University of British Columbia, Vancouver, BC V6T 1Z1, Canada;* orcid.org/0000-0002-7607-8213

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.4c00248

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Thailand Science Research and Innovation, the National Science, Research, and Innovation Fund, and Mae Fah Luang University through the Fundamental Fund (672A01030). The financial support from the National Research Council of Thailand and Mae Fah Luang University (N42A650373) was also acknowledged.

■ REFERENCES

(1) Meade, C. V.; Parnell, J. A. *Aust. Syst. Bot.* **2018**, *31*, 311−356.

(2) Auranwiwat, C.; Wongsomboon, P.; Thaima, T.; Rattanajak, R.; Kamchonwongpaisan, S.; Willis, A. C.; Laphookhieo, S.; Pyne, S. G.; Limtharakul, T. *J. Nat. Prod.* **2019**, *82*, 101−110.

(3) Auranwiwat, C.; Rattanajak, R.; Kamchonwongpaisan, S.; Laphookhieo, S.; Pyne, S. G.; Limtharakul, T. *Fitoterapia* **2018**, *130*, 198−202.

(4) Promchai, T.; Thaima, T.; Rattanajak, R.; Kamchonwongpaisan, S.; Pyne, S. G.; Limtharakul, T. *Nat. Prod. Res.* **2021**, *35*, 2476−2481.

(5) Buncharoen, W.; Saenphet, K.; Saenphet, S.; Thitaram, C. *J. Ethnopharmacol.* **2016**, *194*, 483−494.

(6) Pailee, P.; Ploypradith, P.; Mahidol, C.; Ruchirawat, S.; Prachyawarakorn, V. *Phytochemistry* **2022**, *202*, 113298.

(7) Macabeo, A. P. G.; Letada, A. G.; Budde, S.; Faderl, C.; Dahse, H. M.; Franzblau, S. G.; Alejandro, G. J. D.; Pierens, G. K.; Garson, M. J. *J. Nat. Prod.* **2017**, *80*, 3319−3323.

(8) Auranwiwat, C.; Wongsomboon, P.; Thaima, T.; Rattanajak, R.; Kamchonwongpaisan, S.; Willis, A. C.; Lie, W.; Pyne, S. G.; Limtharakul, T. *Fitoterapia* **2017**, *120*, 103−107.

(9) Maeda, G.; Gilissen, P. J.; Bourgard, C.; van der Wal, J.; Munissi, J. J.; Nyandoro, S. S.; Erdelyi, M. *Fitoterapia* **2022**, *158*, 105170.

(10) Salae, A.-W.; Chairerk, O.; Sukkoet, P.; Chairat, T.; Prawat, U.; Tuntiwachwuttikul, P.; Chalermglin, P.; Ruchirawat, S. *Phytochemistry* **2017**, *135*, 135−143.

(11) Suthiphasilp, V.; Maneerat, W.; Andersen, R. J.; Patrick, B. O.; Phukhatmuen, P.; Pyne, S. G.; Laphookhieo, S. *Fitoterapia* **2019**, *138*, 104340.

(12) Macabeo, A. P. G.; Martinez, F. P. A.; Kurtán, T.; Toth, L.; Mandi, A.; Schmidt, S.; Heilmann, J.; Alejandro, G. J. D.; Knorn, M.; Dahse, H. M.; Franzblau, S. G. *J. Nat. Prod.* **2014**, *77*, 2711−2715.

(13) Nguyen, T. H.; Ho, V. D.; Do, T. T.; Bui, H. T.; Phan, V. K.; Sak, K.; Raal, A. *Nat. Prod. Res.* **2015**, *29*, 247−252.

(14) Richomme, P.; Sinbandhit, S.; David, B.; Hadi, A. H. A.; Bruneton, J. *J. Nat. Prod.* **1990**, *53*, 294−297.

(15) Mahmood, K.; Fontaine, C.; Païs, M.; Ali, H. M.; Hadi, H. A.; Guittet, E. *Tetrahedron Lett.* **1991**, *32*, 6117−6120.

(16) Ankisetty, S.; ElSohly, H. N.; Li, X. C.; Khan, S. I.; Tekwani, B. L.; Smillie, T.; Walker, L. *J. Nat. Prod.* **2006**, *69*, 692−694.

(17) Wang, S.; Chen, R. Y.; Yu, S. S.; Yu, D. Q. *J. Asian Nat. Prod. Res.* **2003**, *5* (2003), 17−23.

(18) Nguyen, N. L.; Vo, T. H.; Lin, Y. C.; Liaw, C. C.; Lin, Z. H.; Chen, M. C.; Kuo, Y. H. *Molecules* **2020**, *25*, 551.

(19) Macabeo, A. P. G.; Rubio, P. Y. M.; Higuchi, T.; Umezawa, N.; Faderl, C.; Budde, S.; Bangcaya, P. S.; Alejandro, G. J. D. *Biochem. Syst. Ecol.* **2017**, *71*, 200−204.

(20) Teixeira, A. M. R.; Santos, H. S.; Bandeira, P. N.; Julião, M. S. S.; Freire, P. T. C.; Lima, V. N.; Cruz, B. G.; da Silva, P. T.; Coutinho, H. D. M.; Sena, D. M., Jr *J. Mol. Struct.* **2019**, *1179*, 739−748.

(21) Zhang, H. L.; Wang, S.; Chen, R. Y.; Yu, D. Q. *Acta Pharm. Sin.* **2002**, *37*, 124−127.

(22) Wang, X.; Xu, Z.; Sun, J.; Lv, H.; Wang, Y.; Ni, Y.; Chen, S.; Hu, C.; Wang, L.; Chen, W.; Cheng, X. *J. Cell. Mol. Med.* **2020**, *24*, 3625−3633.

(23) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Crystallogr.* **2009**, *42*, 339−341.

(24) Sheldrick, G. M. *Acta Crystallogr.* **2015**, *A71*, 3−8.

(25) Sheldrick, G. M. *Acta Crystallogr.* **2015**, *C71*, 3−8.

(26) Wong, K. W.; Teh, S. S.; Law, K. P.; Ismail, I. S.; Sato, K.; Mase, N.; Mah, S. H. *Arch. Pharm.* **2023**, *356*, e2200418.

(27) Zamakshshari, N. H.; Ee, G. C. L.; Ismail, I. S.; Ibrahim, Z.; Mah, S. H. *Food Chem. Toxicol.* **2019**, *133*, 110800.

(28) Mah, S. H.; Teh, S. S.; Ee, G. C. *Pharm. Biol.* **2017**, *55*, 920−928.