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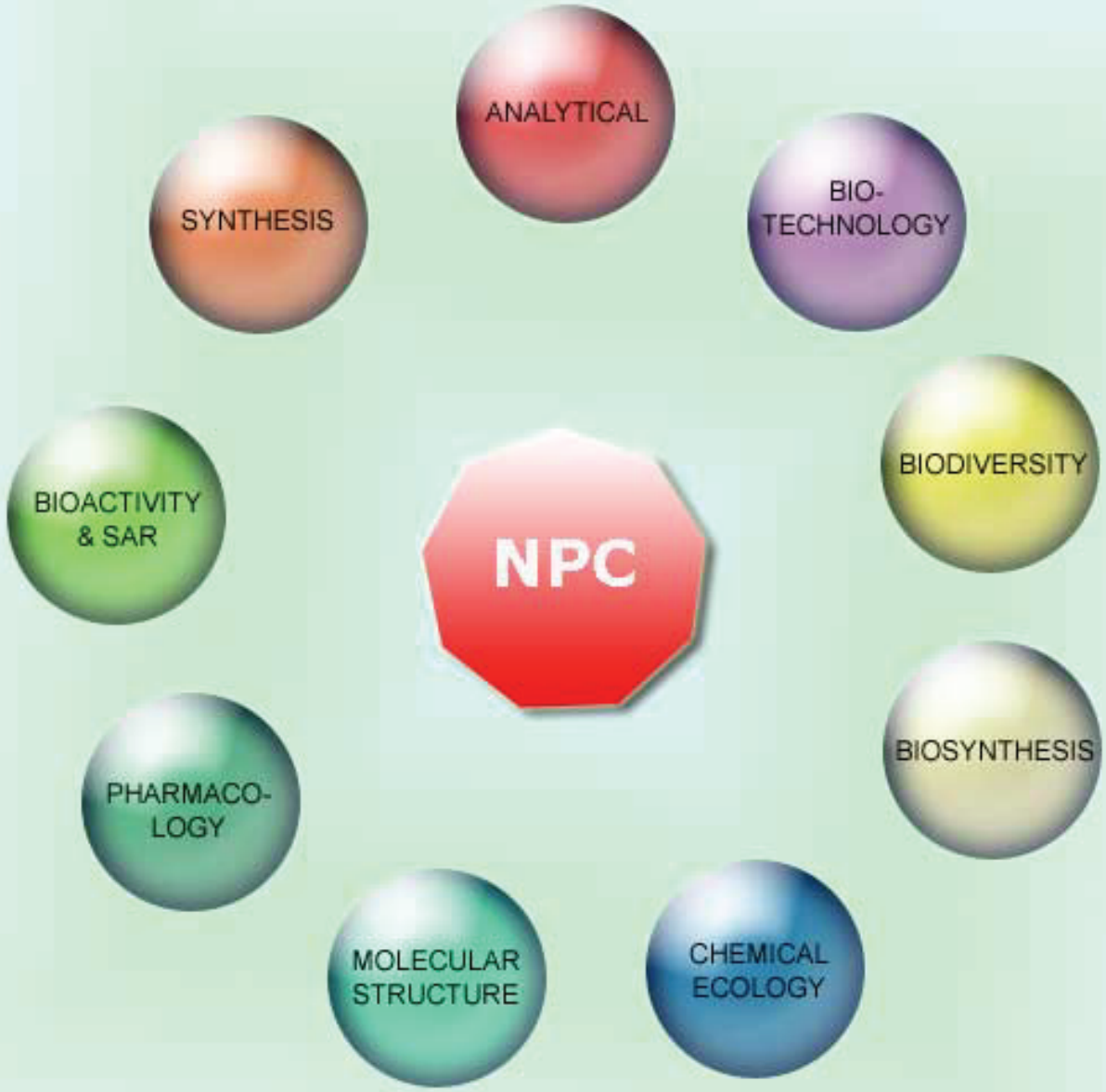

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A New Cytotoxic Tetrahydroxanthene-1,3(2H)-dione Derivative from *Uvaria cordata* and Structure Revision of Valderramenol A

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A new tetrahydroxanthene-1,3(2H)-dione derivative (**1**) was isolated from the leaves of *Uvaria cordata* collected in Viet Nam. Its structure was elucidated to be a mixture of four tautomers (**1a**–**1d**) by a combination of extensive spectroscopic analyses and the theoretical calculation of Gibbs free energies. Compound **1** exhibited moderate cytotoxicity against KB, LNCaP, Hep-G2, MKN-7, SW-480, HL-60, and SK-Mel-2 cancer cell lines with IC_{50} values ranging from 25.92 ± 2.33 to 44.29 ± 4.36 μg/mL. In addition, the previously reported structure of valderramenol A has been revised to **1a**/**1b**.

Keywords: *Uvaria cordata*, Annonaceae, Tetrahydroxanthene-1,3(2H)-dione derivative, Tautomerism, Cytotoxicity.

Uvaria cordata (Dun.) Wall. ex Alston (Annonaceae), also known as *Uvaria macrophylla* or *Guatteria cordata*, is a long woody climber widely distributed in India, Myanmar, Thailand, Malaysia, Sri Lanka, Indonesia and Viet Nam [1]. Previous phyto-chemical studies of this species have led to the isolation of flavonoids [2a, 2b], polyoxygenated cyclohexenes and polyoxygenated *seco*-cyclohexenes [2c-2e], and triterpenoids [2e].

As part of our ongoing research for novel and anticancer compounds from *Uvaria* genus [3a, 3b], the *U. cordata* species was selected for bioassay-guided fractionation. In our previous work, we reported the isolation and structural elucidation of one new aromatic compound, cordauvarin A, together with one tetrahydroxanthene-1,3(2H)-dione derivative (**1**) from the leaves of *U. cordata* [3c]. At that time, compound **1** has been misassigned to be cyathoviridine [4] because we did not place much importance on the signal of hydroxyl proton and tautomeric behavior.

Recently, Macabeo and co-workers [5] described the isolation and structural elucidation of valderramenol A from the Philippines endemic Annonaceous species *Uvaria valderramensis*. The structure **1c** (Figure 1) was proposed for valderramenol A on the basis of its MS and NMR spectral analysis. Surprisingly, the NMR data of valderramenol A were found to be essentially identical to those of compound **1** as reported in our previous paper [3c]. However, careful examination of the reported data for valderramenol A revealed a number of inconsistencies. In particular, the ^{13}C chemical shift of C-1 (δ_C 190.9), the carbon of a hydrogen bond acceptor carbonyl group, has been assigned lower than that of C-3 (δ_C 199.0) (Table 1). Moreover, in the process of structural determination, the long-range HMBC correlation ($^4J_{C\text{-}H}$) from 1′-OH to C-3 seemed to be ignored as well as the *Z* configuration of the enol double bond Δ-2 was chosen arbitrarily for theoretical calculation without explanation. These intriguing findings inspired us to reinvestigate the structure of **1** more thoroughly.

Herein, we report the structural elucidation of compound **1** as well as its cytotoxicity against seven human cancer cell lines (KB, epidermoid carcinoma; LNCaP, prostate carcinoma; Hep-G2, hepatoma cancer; MKN-7, stomach cancer; SW-480, colon adenocarcinoma; HL-60, acute leukemia; SK-Mel-2, malignant melanoma). In addition, the structural revision of valderramenol A [5] is also described.

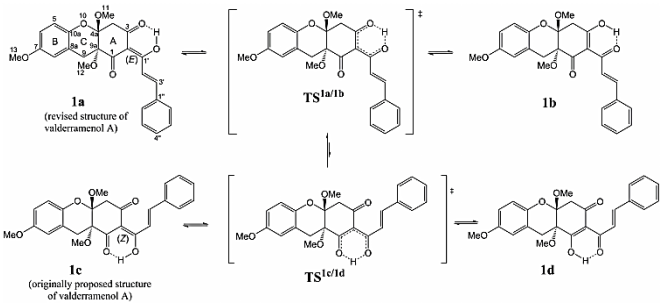

Figure 1: Structure of **1** depicted as a tautomeric equilibrium.

Compound **1** was obtained as an optically inactive pale yellow powder from $CHCl_3$-soluble portion. The HRESIMS of **1** showed only one pseudo-molecular ion peak at *m/z* 459.1400 $[M+Na]^+$. Its molecular formula was thus determined to be $C_{25}H_{24}O_7$ by HRESIMS in conjunction with NMR data analysis, which contains fourteen degrees of unsaturation.

The ^1H NMR spectrum of **1** in $CDCl_3$ at room temperature displayed two similar sets of signals with a ratio of approximately 85:15 (derived from the signal intensities). This fact suggested that compound **1** existed as an inseparable mixture of tautomers in solution. The NMR analysis was thus carried out on the mixture, with a focus on the major component.

The ^1H NMR spectrum of the major form showed typical signals of two *trans*-olefinic protons at δ_H 8.18 (d, *J* = 15.5 Hz, H-2′) and 7.98 (d, *J* = 15.5 Hz, H-3′), five aromatic protons of a phenyl group at δ_H 7.61 (2H, dd, *J* = 7.5, 1.5 Hz, H-2″ and H-6″) and 7.38 (3H, overlapped). In addition, the signals of three aromatic protons of an ABX spin system [δ_H 6.74 (2H, overlapped) and 6.67 (dd, *J* = 9.0, 2.5 Hz, H-6)], three methoxy groups [δ_H 3.32 (H_3-12), 3.35 (H_3-11),

Table 1: ^{1}H and ^{13}C NMR data for **1a**–**1d** and valderramenol A in $CDCl_3$ [δ (ppm), J (Hz)].

Position	**1a/1b** (major tautomers)		**1c/1d** (minor tautomers)		**Valderramenol A**[†]	
	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C	δ_H
1	190.9	–	198.5[#]	–	190.9	–
2	108.5	–	110.0[#]	–	108.5	–
3	199.0	–	192.5[#]	–	199.0	–
4	39.5	3.25 d (18.0) 3.37[*]	42.5[#]	3.21[#] d (18.0) 3.37[*]	39.4	3.26 d (18.0) 3.28 d (18.0)
5	117.5	6.74[*]	117.5	6.74[*]	117.5	6.73 d (8.8)
6	113.7	6.67 dd (9.0, 2.5)	113.7	6.67 dd (9.0, 2.5)	113.7	6.67 dd (8.8, 3.0)
7	154.7	–	154.7	–	154.7	–
8	113.5	6.74[*]	113.5	6.74[*]	113.4	6.73 d (3.0)
9	24.9	3.00 d (15.0) 3.70 d (15.0)	24.5[#]	3.09[#] d (15.0) 3.70 d (15.0)	24.9	3.01 d (15.0) 3.69 d (15.0)
4a	97.2	–	97.5[#]	–	97.2	–
8a	122.3	–	122.3	–	122.3	–
9a	79.2	–	78.0[#]	–	79.2	–
10a	143.8	–	143.8	–	143.8	–
11	49.2	3.35 s	49.2	3.35 s	49.2	3.35 s
12	52.2	3.32 s	52.2	3.32 s	52.2	3.33 s
13	55.5	3.76 s	55.5	3.76 s	55.5	3.76 s
1′	187.6	–	186.0[#]	–	187.6	–
2′	121.5	8.18 d (15.5)	121.5	8.28 d (16.0)[#]	121.5	8.17 d (16.0)
3′	146.7	7.98 d (15.5)	146.7	7.96 d (16.0)	146.9	7.97 d (16.0)
1″	134.7	–	134.7	–	134.7	–
2″/6″	129.1[c]	7.61 dd (7.5, 1.5)[c]	129.1	7.65[*#]	128.0	7.37 d (7.5)
3″/5″	128.9[c]	7.38[*c]	128.9	7.42[*#]	129.1	7.62 dd (8.0, 7.5)
4″	131.1	7.38[*]	131.1	7.42[*#]	131.1	7.37 d (8.0)
OH	–	18.39 s	–	17.30[#] s	–	18.40 s

Measured at [a]125 MHz, [b]500 MHz; [c]revised signals; [#]signals were clearly differentiated from those in major form; [*]overlapping signals; [†]data (600 MHz for ^{1}H, 150 MHz for ^{13}C NMR) taken from ref. [5].

3.76 (H_3-13) (each, s)], and a strong intramolecularly hydrogen-bonded proton [δ_H 18.39 (s, OH)] were observed. The ^{13}C NMR and DEPT spectra revealed twenty-five signals for three methyl, two methylene, ten methine, and ten quaternary carbons. Moreover, the ^{13}C NMR spectrum indicated characteristic signals corresponding to three carbonyl carbons [δ_C 190.9 (C-1), 199.0 (C-3) and 187.6 (C-1′)], one oxygenated methine carbon [δ_C 79.2 (C-9a)], and three methoxy groups [δ_C 49.0 (C-11), 52.0 (C-12) and 55.4 (C-13)].

The HMBC correlations of H-2″ and H-6″ (δ_H 7.61) to C-3′ (δ_C 146.7), of H-2′ (δ_H 8.18) to C-1″ (δ_C 134.7), of H-3′ (δ_H 7.98) to C-1″/C-1′ (δ_C 187.6) suggesting the presence of an (*E*)-cinnamoyl moiety in the molecule. The appearance of one 1,2,4-trisubstituted aromatic ring was confirmed by the HMBC correlations of H-5 (δ_H 6.74) to C-7 (δ_C 154.7)/C-8a (δ_C 122.3), of H-6 (δ_H 6.67)/H-8 (δ_H 6.74) to C-10a (δ_C 143.8) as well as the COSY correlation between H-5 and H-6. Similarly, the key HMBC correlations from the isolated methylene protons H-4 (δ_H 3.25 and 3.37) to C-2 (δ_C 108.5)/C-3/C-4a (δ_C 97.2)/C-9a (δ_C 79.2), from H-9 (δ_H 3.00 and 3.70) to C-1/C-8 (δ_C 113.5)/C-4a/C-8a (δ_C 122.3)/C-9a/C-10a allowed the construction of the tetrahydroxanthene-1,3(2H)-dione skeleton. The strong cross-peaks in HMBC spectrum of H_3-11 to C-4a, of H_3-12 to C-9a, of H_3-13 to C-7 confirmed the location of three methoxy groups at C-4a, C-9a and C-7, respectively. Additionally, the absence of NOESY cross-peak from H_3-11 to H_3-12 allowed us to assign the *trans* relationship between 4a-OMe and 9a-OMe groups.

Interestingly, the hydroxyl proton at δ_H 18.39 (OH) correlated to five carbons (C-2, C-3, C-4, C-1′, C-2′) but not to C-1 and C-9a in the HMBC spectrum. This observation suggested that the major form appeared as the fast equilibrium of two internal tautomers (**1a**/**1b**) via a transition state (**TS**$^{\mathbf{1a/1b}}$) containing pseudo-six-membered heterocyclic ring (Figure 2). As a result, these two tautomeric structures were not differentiated on the NMR time scale and the obtained NMR data are mean values for **1a** and **1b** [6a-6e].

The formation of the intramolecular hydrogen bond as shown in **1a**/**1b** explained a strong downfield shift of C-3 (δ_C 199.0) comparing to the other carbonyl carbon [C-1 (δ_C 190.9)] within the

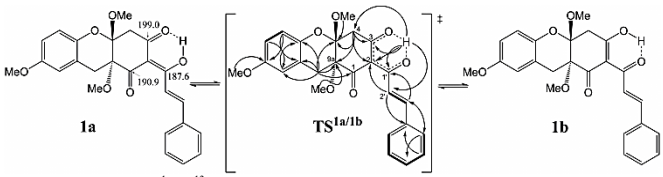

Figure 2: Key HMBC (^{1}H→^{13}C, arrows) and COSY (bold lines) correlations of preferred tautomers **1a**/**1b**.

A ring. This phenomenon was observed popularly in many phloroglucinol compounds such as flavesone, isoleptospermone, leptospermone, grandiflorone, papuanone [7a], ialibinones A–E [7b], enaimeones A–C [7c], hyperatomarin [7d], champanone A [7e], norflavesone, norisoleptospermone, norleptospermone, myrigalone A, and nortriketone [7f]. It was also found in 2-[1-hydroxy-3-phenyl-(*Z*,2*E*)-2-propenylidene]-4-methyl-4-cyclopentene-1,3-dione [8a], 7-*epi*-clusianone [8b], and watsonianone C [6b].

From the keto-enol tautomerism point of view, the remaining set of NMR signals was assigned for the minor indistinguishable form (**1c**/**1d**) [9]. Detailed examination of 2D-NMR spectra also allowed the complete assignment of NMR data for **1c**/**1d** (Table 1) [6a, 7e]. Notably, the interconversion between **1a**/**1b** and **1c**/**1d** structures was confirmed by chemical exchange cross-peak from the enolic hydroxy proton of **1a**/**1b** (δ_H 18.39) to that of **1c**/**1d** (δ_H 17.30) in the NOESY spectrum [7d, 10].

In order to support the experimental results, the Gibbs free energy G values (at 298 K) were calculated for four tautomers (**1a**–**1d**) and two transition states (**TS**$^{\mathbf{1a/1b}}$, **TS**$^{\mathbf{1c/1d}}$) using density functional theory (Table 2). The B3LYP/6-31G(d) method was selected for the geometric optimization [5, 6a]. As expected, the theoretical calculation indicated that the Gibbs free energies of **1a**/**1b** (major) were lower than those of **1c**/**1d** (minor). The existence of the fast keto-enol equilibrium between **1a** and **1b**, between **1c** and **1d** was deduced from the low-energy transition states (**TS**$^{\mathbf{1a/1b}}$, **TS**$^{\mathbf{1c/1d}}$), which can be easily obtained at room temperature.

Table 2: Density functional theory calculations for **1**.

Structures	G_{rel} (298.15 K) [kcal/mol]
(**1a**)	0.00
TS$^{\mathbf{1a/1b}}$	-0.56[1]
(**1b**)	0.68
(**1c**)	1.14
TS$^{\mathbf{1c/1d}}$	1.37
(**1d**)	2.69

G_{rel}: relative Gibbs free energies respect to **1a**. [1]Relative energy at 0 K is 1.50 kcal/mol.

Based on the above evidences, the chemical structure of **1** was elucidated unambiguously as a mixture of four tautomeric forms (**1a**–**1d**) (Figure 1). Since the MS and NMR data of valderramenol A are exactly identical to those of the preferred tautomers **1a**/**1b**, the correct structure of valderramenol A was confirmed to be **1a**/**1b** instead of **1c** as shown in previous paper. Also, valderramenol B [5] was described as a 9a-*O*-demethyl derivative of valderramenol A, and consequently the proposed structure for valderramenol B should be revised.

To further compare the reported structure of cyathoviridine [4] with **1a**/**1b**, the ^{1}H, ^{13}C NMR, and HMBC spectra of **1** were re-measured by changing solvent from $CDCl_3$ to C_6D_6:$CDCl_3$ (7:3). The structural difference of **1a**/**1b** and cyathoviridine was deduced from their ^{1}H and ^{13}C NMR data (Table 3). This result led us to re-examine the structural elucidation of cyathoviridine which was described in the previous work [4]. We notice that the signal of hydroxyl proton of cyathoviridine at 6.42 ppm is consistent with "free" enolic [7e], but very different from chelated enolic proton (δ_H 17-19 ppm) [11a, 11b]. In fact, Mahmood and co-workers [4] also

proposed without assertiveness the presence of intramolecularly hydrogen bonding only based on the NOESY correlation of H_3-12 to H-2′. Thus, we now assume that cyathoviridine might be existed as a conformational isomer of **1b**, in which the formation of intramolecularly hydrogen bonding is unfavorable. Further investigation regarding the above hypothesis is required.

Table 3: ^{1}H and ^{13}C NMR data for **1a**/**1b** and cyathoviridine in C_6D_6:$CDCl_3$ (7:3) [δ (ppm), J (Hz)].

Position	1a/1b		Cyathoviridine[†]	
	δ_C[a]	δ_H[b]	δ_C	δ_H
1	191.7	–	195.0	–
2	109.9	–	–	–
3	200.5	–	199.6	–
4	40.7	3.19[*]	39.8	3.26 d (17.0)
		3.30[*]		3.40 d (17.0)
5	118.8	6.73[*]	117.9	6.82 d (8.7)
6	115.2	6.59[*]	114.1	6.67 dd (8.7, 2.5)
7	156.3	–	155.2	–
8	115.0	6.73[*]	114.2	6.77 d (2.5)
9	26.3	3.05[*]	25.4	3.14 d (15.0)
		3.74[*]		3.81 d (15.0)
4a	98.8	–	97.9	–
8a	124.0	–	123.1	–
9a	80.6	–	79.7	–
10a	145.5	–	144.3	–
11	49.9	3.04 s	49.0	3.12 s
12	52.9	3.15 s	52.0	3.23 s
13	56.3	3.39 s	55.4	3.49 s
1′	189.2	–	188.3	–
2′	123.2	8.40 d (15.5)	122.3	7.98 d (15.5)
3′	147.7	7.89 d (15.5)	146.8	8.45 d (15.5)
1″	136.0	–	135.4	–
2″/6″	130.3[c]	7.33[*]	129.1	7.41 d (6.2)
3″/5″	130.0[c]	7.02[*]	128.1	7.05-7.15 m
4″	132.0	7.02[*]	131.2	7.05-7.15 m
OH	–	**18.86 brs**	–	**6.42**

Measured at [a]125 MHz, [b]500 MHz; [*]overlapping signals; [†]data (400 MHz for ^{1}H, 62.5 MHz for ^{13}C NMR) taken from ref. [4].

The cytotoxicity of compound **1** against the growth of seven human cancer cell lines (KB, LNCaP, Hep-G2, MKN-7, SW-480, HL-60, SK-Mel-2) was evaluated by a sulforhodamine B assay [3a], and the results are displayed in Table 4. These data revealed that compound **1** exhibited moderate inhibitory effect against tested cancer cell lines with IC_{50} values ranging from 25.92 ± 2.33 to 44.29 ± 4.36 μg/mL. Remarkably, the cytotoxicity of **1** on the normal 3T3 cell line was significantly lower than that of the positive control, ellipticine.

Table 4: Cytotoxicity of **1** against human cancer cell lines.

Cell lines	IC_{50}[a] (μg/mL)	
	1	Ellipticine[b]
KB	25.92 ± 2.33	0.47 ± 0.08
LNCaP	33.44 ± 1.31	0.45 ± 0.07
Hep-G2	30.66 ± 1.73	0.45 ± 0.03
MKN-7	44.29 ± 4.36	0.50 ± 0.04
SW-480	32.85 ± 3.33	0.46 ± 0.08
HL-60	42.90 ± 2.03	0.61 ± 0.08
SK-Mel-2	39.17 ± 4.64	0.56 ± 0.08
3T3 (normal cell)	28.85 ± 1.80	0.38 ± 0.05

[a]IC_{50} (concentration that inhibits of 50% of cell growth). [b]Positive control.

Experimental

General: Optical rotations were measured on a JASCO P-2100 polarimeter (Hachioji, Tokyo, Japan). 1D and 2D NMR were carried out using Bruker Avance 500 (Billerica, Massachusetts, USA) with TMS as an internal reference. HRESIMS data were measured on a micrOTOF-Q 10187 mass spectrometer (Bruker, Massachusetts, USA). Column chromatography was performed using silica gel (60 N, spherical, neutral, 40-50 μm, Kanto Chemical Co., Inc., Tokyo, Japan), YMC RP-18 (Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan). Analytical TLC was performed on pre-coated silica gel 60F_{254} and RP-18 F_{254} plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany). The cell lines (KB, LNCaP, Hep-G2, MKN-7, SW-480, HL-60, SK-Mel-2, and 3T3) were available and maintained in our laboratory. Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY, USA). The ELISA Plate Reader (Bio-Rad, California, USA) was used to measure the absorbance of cells in cytotoxicity assay.

Plant material: The leaves of *U. cordata* (Dun.) Wall. ex Alston. were collected from Quang Tri province, Viet Nam (N16°44'38.9" E107°14'51.1") in November, 2011 and were identified by Dr. Cuong The Nguyen, Institute of Ecology and Biological Resources, VAST, Viet Nam. A voucher specimen (AV02) was deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Viet Nam.

Extraction and isolation: The dried leaves of *U. cordata* (5.0 kg) were extracted with MeOH (10.0 L x3 times) at room temperature to yield 400 g of a dark solid extract. This was then suspended in water and successively partitioned with chloroform ($CHCl_3$) and ethyl acetate (EtOAc) (each, 5.0 L x3 times) to obtain the $CHCl_3$ (UC, 150.0 g), the EtOAc (UE, 180.0 g), and the water (UW, 50.0 g) layers after removal of the solvents *in vacuo*. The UC extract was chromatographed on a silica gel column and eluted with the gradient of *n*-hexane-acetone solvent systems (100:0→0:100, v/v) to obtain seven fractions, UC1–UC7. The fraction UC1 (30 g) was applied on a silica gel column, using *n*-hexane-EtOAc system (10:1, v/v) to obtain six sub-fractions, UC1.1–UC1.6. The sub-fraction UC1.4 (1.5 g) was chromatographed on an YMC RP-18 column eluted with MeOH-acetone-water (2:1:1, v/v) to yield compound **1** (45 mg).

Computational calculations: Geometric optimizations for the four tautomers, **1a**–**1d**, and two transition states, **TS$^{1a/1b}$**, **TS$^{1c/1d}$**, were carried out at the B3LYP level of density functional theory using the 6-31G(d) basis set. The vibrational frequencies of the transition state were calculated at the same level. All of the stationary points were confirmed to be local minima or transition states by harmonic vibrational analysis. We calculated relative Gibbs free energies G at 298 K (kcal/mol) from single point energy and thermal correction of Gibbs free energy. Single point energy calculations of all species were performed at the B3LYP/6-311G(d,p) level using the B3LYP/6-31G(d) optimized geometries. All calculations were carried out using the GAUSSIAN-09 program packages [12].

SRB assay for evaluating cytotoxic activity: Stock cultures were grown in T-75 flasks containing 50 mL of Dulbecco's Modified Eagle Medium (DMEM) with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate and 10% Fetal Bovine Serum (FBS). Media were changed at 48-hours intervals. The cells were dissociated with 0.05% Trypsin-EDTA, sub-cultured every 3-5 days with the ratio of (1:3) and incubated at 37ºC under humidified 5% carbon dioxide atmosphere. Tumor cells were cultivated in a humidified atmosphere of 5% CO_2 at 37ºC for 48 h. Cell viability was examined by sulforhodamine B (SRB) method for cell density determination, based on the measurement of cellular protein content [13]. Viable cells were seeded in the growth medium (180 μL) into 96-well microplates (4×10^4 cells per well) and allowed to attach overnight. Tested samples were added carefully into each well of 96-well plates and the cultivation was continued under the same conditions for another 72 h. Thereafter, the medium was removed and the remaining cell monolayers are fixed with the cold 20% (w/v) trichloroacetic acid for 1 h at 4ºC and stained by 1X SRB staining solution at room temperature for 30 min, after which the unbound dye was removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for optical density determination at 515 nm on an ELISA Plate Reader (Bio-Rad). DMSO 10% was used as blank sample while ellipticine was used as positive control. The cytotoxicity was

measured at doses of 100 μg/mL, 20 μg/mL, 4 μg/mL, and 0.8 μg/mL and estimated as a half maximal inhibitory concentration (IC_{50}), which was calculated by the program TableCurve Version 4.0. All experiments were prepared in triplicates. The inhibition rate (IR) of cells was calculated by the following formula IR% = {100% − [(OD_t − OD_0)/(OD_c − OD_0)] × 100}, in which: IR: Inhibition rate of cell growth, OD_t: average optical density value at day 3; OD_0: average optical density value at time-zero; OD_c: average optical density value of the blank DMSO control sample.

Compound 1

Pale yellow amorphous powder.
$[\alpha]^{22}_D$: 0 (*c* 0.1, $CHCl_3$).
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) in $CDCl_3$: Table 1.
HRESIMS *m/z* 459.1400 $[M+Na]^+$ (calcd. for $C_{25}H_{24}O_7Na$, 459.1420).

Supplementary data: HRESIMS, ^{1}H and ^{13}C NMR, DEPT, HSQC, HMBC, COSY, and NOESY spectral data for compound **1**.

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References

[1] Ban NT. (**2000**) Flora of Viet Nam – Annonaceae Juss. *Science and Technology Publishing House*, *Hanoi*, *Viet Nam*, ***1***, 47.

[2] (a) Wang S, Zhang PC, Chen RY, Wang YH, He WY, Yu DQ. (**2002**) A novel dihydroflavone from the roots of *Uvaria macrophylla*. *Chinese Chemical Letters*, ***13***, 857–858; (b) Zhang HL, Chen RY. (**2001**) A new flavone from the roots of *Uvaria macrophylla*. *Chinese Chemical Letters*, ***12***, 791–792; (c) Wang S, Chen RY, Yu SS, Yu DQ. (**2003**) Uvamalols D–G: Novel polyoxygenated seco-cyclohexenes from the roots of *Uvaria macrophylla*. *Journal of Asian Natural Products Research*, ***5***, 17–23; (d) Wang S, Zhang PC, Chen RY, Dai SJ, Yu SS, Yu DQ. (**2005**) Four new compounds from the roots of *Uvaria macrophylla*. *Journal of Asian Natural Products Research*, ***7***, 687–694; (e) Mahmood K, Ali HM, Yusof R, Hadi AH, Pais M. (**1995**) Chemical components from the light petroleum soluble fraction of *Uvaria cordata* (Dunal) Alston. *Pertanika Journal* of *Science & Technology*, ***3***, 197–202.

[3] (a) Ho DV, Kodama T, Le HTB, Phan KV, Do TT, Bui TH, Le AT, Win NN, Imagawa H, Ito T, Morita H, Nguyen HT. (**2015**) A new polyoxygenated cyclohexene and a new megastigmane glycoside from *Uvaria grandiflora*. *Bioorganic & Medicinal Chemistry Letters*, ***25***, 3246–3250; (b) Nguyen TH, Ho VD, Do TT, Bui HT, Phan VK, Sak K, Raal A. (**2015**) A new lignan glycoside from the aerial parts and cytotoxic investigation of *Uvaria rufa*. *Natural Product Research*, ***29***, 247–252; (c) Nguyen TH, Ho VD, Phan VK. (**2013**) A new aromatic compound from the leaves of *Uvaria cordata*. *Vietnam Journal of Chemistry*, ***51***, 736–739 [in Vietnamese].

[4] Mahmood K, Sablé S, Païs M, Ali HM, Hadi AHA, Guittet E. (**1993**) Cyathoviridine, a cytotoxic metabolite from *Cyathostemma viridiflorum*, *Natural Product Letters*, ***3***, 245–249.

[5] Macabeo APG, Martinez FPA, Kurtán T, Tóth L, Mándi A, Schmidt S, Heilmann J, Alejandro GJ, Knorn M, Dahse HM, Franzblau SG. (**2014**) Tetrahydroxanthene-1,3(2H)-dione derivatives from *Uvaria valderramensis*. *Journal of Natural Products*, ***77***, 2711–2715.

[6] (a) von Bargen KW, Niehaus EM, Krug I, Bergander K, Würthwein EU, Tudzynski B, Humpf HU. (**2015**) Isolation and structure elucidation of fujikurins A−D: Products of the PKS19 gene cluster in *Fusarium fujikuroi. Journal of Natural Products*, ***78***, 1809–1815; (b) Carroll AR, Avery VM, Duffy S, Forster PI, Guymer GP. (**2013**) Watsonianone A–C, anti-plasmodial β-triketones from the Australian tree, *Corymbia watsoniana*. *Organic & Biomolecular Chemistry*, ***11***, 453–458; (c) Feng ZM, He J, Jiang JS, Chen Z, Yang YN, Zhang PC. (**2013**) NMR solution structure study of the representative component hydroxysafflor yellow A and other quinochalcone *C*-glycosides from *Carthamus tinctorius*. *Journal of Natural Products,* ***76***, 270–274; (d) Socolsky C, Rates SM, Stein AC, Asakawa Y, Bardón A. (**2012**) Acylphloroglucinols from *Elaphoglossum crassipes*: Antidepressant-like activity of crassipin A. *Journal of Natural Products*, ***75***, 1007–1017; (e) Yu Q, Ravu RR, Xu QM, Ganji S, Jacob MR, Khan SI, Yu BY, Li XC. (**2015**) Antibacterial prenylated acylphloroglucinols from *Psorothamnus fremontii*. *Journal of Natural Products*, ***78***, 2748–2753.

[7] (a) van Klink JW, Brophy JJ, Perry NB, Weavers RT. (**1999**) β-Triketones from Myrtaceae: Isoleptospermone from *Leptospermum scoparium* and papuanone from *Corymbia dallachiana. Journal of Natural Products*, ***62***, 487–489; (b) Winkelmann K, Heilmann J, Zerbe O, Rali T, Sticher O. (**2000**) New phloroglucinol derivatives from *Hypericum papuanum*. *Journal of Natural Products*, ***63***, 104–108; (c) Winkelmann K, Heilmann J, Zerbe O, Rali T, Sticher O. (**2001**). Further prenylated bi- and tricyclic phloroglucinol derivatives from *Hypericum papuanum*. *Helvetica Chimica Acta*, ***84***, 3380–3392; (d) Savikin-Fodulović K, Aljancić I, Vajs V, Menković N, Macura S, Gojgić G, Milosavljević S. (**2003**) Hyperatomarin, an antibacterial prenylated phloroglucinol from *Hypericum atomarium* ssp. *degenii*. *Journal of Natural Products*, ***66***, 1236–1238; (e) Bonilla A, Duque C, Garzón C, Takaishi Y, Yamaguchi K, Hara N, Fujimoto Y. (**2005**) Champanones, yellow pigments from the seeds of champa (*Campomanesia lineatifolia*). *Phytochemistry*, ***66***, 1736–1740; (f) Killeen DP, Larsen L, Dayan FE, Gordon KC, Perry NB, van Klink JW. (**2016**) Nortriketones: Antimicrobial trimethylated acylphloroglucinols from Mānuka (*Leptospermum scoparium*). *Journal of Natural Products*, ***79***, 564–569.

[8] (a) Facundo VA, Sá AL, Silva SAF, Morais SM, Matos CRR, Braz-Filho R. (**2004**) Three new natural cyclopentenedione derivatives from *Piper carniconnectivum*. *Journal of the Brazilian Chemical Society*, ***15***, 140–145; (b) Derogis PB, Martins FT, de Souza TC, de C Moreira ME, Souza Filho JD, Doriguetto AC, de Souza KR, Veloso MP, Dos Santos MH. (**2008**) Complete assignment of the ^{1}H and ^{13}C NMR spectra of garciniaphenone and keto-enol equilibrium statements for prenylated benzophenones. *Magnetic Resonance in Chemistry*, ***46***, 278–282.

[9] Skylaris CK, Igglessi-Markopoulou O, Detsi A, Markopoulos J. (**2003**) Density functional and ab initio study of the tautomeric forms of 3-acetyl tetronic and 3-acetyl tetramic acids. *Chemical Physics*, ***293***, 355–363.

[10] Frasinyuk MS, Bondarenko SP, Khilya VP, Liu C, Watt DS, Sviripa VM. (**2015**) Synthesis and tautomerization of hydroxylated isoflavones bearing heterocyclic hemi-aminals. *Organic & Biomolecular Chemistry***,** ***13***, 1053–1067.

[11] (a) Yamaguchi T, Saito K, Tsujimoto T, Yuki H. (**1976**) NMR spectroscopic studies on the tautomerism in tenuazonic acid analogs. *Journal of Heterocyclic Chemistry*, ***13***, 533-537; (b) Agrawal PK, Thakur RS, Frahm AW, Schneider M. (**1990**) An investigation of the keto-enol tautomerism in 2-acetyl-1-tetralone by 2D NMR spectroscopy. *Magnetic Resonance in Chemistry*, ***28***, 931-933.

[12] Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery Jr. JA, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas Ö, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ. (**2010**) Gaussian 09, Revision B.01. *Gaussian, Inc., Wallingford CT*.

[13] Monks A, Scudiero D, Skehan P, Shoemake R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Campbell H, Mayo J, Boyd M. (**1991**) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *Journal of the National Cancer Institute*, ***83***, 757–766.

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