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Cytotoxic principles and α-pyrone ring-opening derivatives of bufadienolides from *Kalanchoe hybrida*

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Abstract

Purification of the cytotoxic fractions of the methanol extracts of *Kalanchoe hybrida* leads to three new compounds with the basic skeleton of α-pyrone ring-opening products of bufadienolides, namely, kalanhybrin A–C (**1**–**3**). Moreover, four bufadienolides (**4**–**7**) and eight other compounds were also characterized from the title plant. The isolated compounds (**1**–**7**) were evaluated for their cytotoxicity toward MCF-7, NCI-H460, and SF-268 tumor cell lines. Among them, compounds **4** and **6** displayed significant cytotoxicity toward MCF-7 and NCI-H460 tumor cell lines at the tested concentration. In addition, the biosynthetic pathway of these α-pyrone ring-opening derivatives was also proposed.

Keywords: Crassulaceae; Bufadienolides; Biosynthetic pathway; Cytotoxicity

1. Introduction

Various species of *Kalanchoe* are used medicinally in Southeast Asia, i.e., Indo-China and Philippines Islands, whereas *Kalanchoe hybrida* (family Crassulaceae) is naturalized throughout the island of Taiwan. The leaves and barks of *Kalanchoe* genus are bitter tonic, astringent to the bowels, analgesic, carminative, useful in diarrhea and vomiting.[1] The plants of this genus were known in folklore and traditional medicine for the treatment of fever, abscesses, bruises, contused wounds, coughs, and skin diseases.[2,3] In the previous investigations, antiulcer,[4] antiinflammatory,[5,6] and antimicrobial activities[7] of leaf extracts for this species were reported. The aqueous leaf extract was found to cause significant inhibition of cell-mediated and humoral responses in BALB/c mice, and was shown to protect them when infected with *Leishmania amazonensis*.[8–10] The genus *Kalanchoe* is reported to contain potent cytotoxic bufadienolides, such as bersaldegenin-1,3,5-orthoacetate and bryophyllin B.[11–15] Other chemical constituents from this plant like fatty acids,[16] triterpenoids,[17] and flavonoids[18–20] were also reported. This species is also included in the plants' species, which are used by the tribals of Kerala for treating cancer symptoms.[21] In our preliminary studies, the crude methanol extract of *K. hybrida* displayed significant cytotoxicity against MCF-7, NCI-H460, and SF-268 tumor cell lines. The bioassay directed fractionation of the crude extract of *K. hybrida* exhibited that the chloroform fraction possessed potent cytotoxicity against the tumor cell lines. At the tested concentration of 50 μg/mL, the $CHCl_3$ fraction showed 93%, 99%, and 81% inhibition against MCF-7, NCI-H460, and SF-268 tumor cell lines, respectively. On the basis of the above studies, *K. hybrida* was selected as a target due to its biological activities and herein three new compounds of α-pyrone ring-opening derivatives of bufadienolides, four cytotoxic bufadienolides, and eight other compounds were reported.

2. Results and discussion

Air-dried and powdered whole plants of *K. hybrida* were extracted with methanol under reflux and concentrated. The

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methanolic extract was suspended in water and partitioned with $CHCl_3$ and *n*-butanol, successively, and afforded $CHCl_3$-, *n*-butanol- and H_2O-solubles, respectively. Cytotoxicity-guided purification of the $CHCl_3$ fraction yielded three new (**1**–**3**) and twelve known compounds, including bersaldegenin 3-acetate (**4**),[14] bersaldegenin 1-acetate (**5**),[14] daigredorigenin 3-acetate (**6**),[14] evogenin 3-*O*-acetate (**7**),[22] *p*-methoxy benzoic acid (**8**),[23] *p*-hydroxybenzaldehyde (**9**),[24] vanillic acid (**10**),[24] *p*-hydroxybenzoic acid (**11**),[25] blumenol A (**12**),[26] cinnamic acid (**13**),[27] 4-(2,3-dihydroxy-butylidene)-3,5,5-trimethyl-cyclohex-2-enone (**14**),[28] and nicotinic acid (**15**).[29]

Compound **1** was isolated as colorless optically active prisms from chloroform/methanol with $[\alpha]_D^{25}$ +36.2. The molecular formula of **1** was established to be $C_{27}H_{38}O_9$ by HRFABMS data (*m*/*z* 507.2598 for $[M+H]^+$) together with ^1H and ^{13}C NMR spectral data, thus requiring nine degrees of unsaturation. The UV spectrum showed absorption maxima at 237 nm and indicated the presence of a conjugated carbonyl group.[30] The IR spectrum exhibited absorption bands, which were attributed to hydroxyls (3450 and 3437 cm^{-1}), an aldehyde (1715 cm^{-1}), and a conjugated carbonyl group (1676 cm^{-1}). The ^{13}C NMR together with the DEPT and HMQC spectra revealed 27 carbon signals including characteristic signals due to an hemiacetal (*δ* 95.5), two aldehydes (*δ* 190.2 and 207.9), one acetyl group (*δ* 21.5 and 170.5), one set of conjugated double bond (*δ* 126.2 and 161.2), two tertiary oxygenated carbons (*δ* 73.8 and 86.4), two secondary oxygenated carbons (*δ* 67.7 and 67.9), one methoxyl (*δ* 55.2), one methyl (*δ* 16.4), eight methylenes, four methines, and two quaternary carbons. These data indicated **1** to be a pentacyclic structure with a hemiacetal moiety. The characteristic signals in the ^1H NMR spectrum of **1** were quite similar to that of bersaldegenin 3-acetate,[14] particularly the signals in the A, B, and C rings. The essential differences between the ^1H NMR spectra of **1** and bersaldegenin 3-acetate consisted of the absence of α-pyrone ring and appearance of a hemiacetal proton [*δ* 5.71 (1H, s)], a methoxy group [*δ* 3.45 (3H, s)], and one set of conjugated aldehyde functionality [*δ* 9.82 (1H, d, *J*=8.1 Hz) and 5.73 (1H, d, *J*=8.1 Hz)]. It revealed that **1** was an α-pyrone ring-opening product of bufadienolide. The COSY spectrum of **1** showed correlations in C1–C2–C3–C4, C6–C7–C8–C9–C11–C12, and C15–C16–C17, indicating the presence of steroidal-like structure in **1** and remained the cholane basic skeleton. The long-range coupling HMBC experiment was carried out to determine the partial structure due to an α-pyrone ring cleavage. The methoxy signal at *δ* 3.45 was correlated to the hemiacetal carbon (*δ* 95.5), and the signals of olefinic proton at *δ* 5.73 were correlated to the aldehyde (*δ* 190.2) and hydoxylated olefinic carbons (*δ* 161.2), indicating that a conjugated aldehyde was built up at C-22, C-23, and C-24. It also supported the occurrence of a tetrahydropyran ring made up of C-13, C-14, C-21, C-20, and C-17. The signal of H-21 (*δ* 5.71) displayed ^3J-correlation with C-14 (*δ* 86.4), attributable to an ether bridge formed between C-21 and C-14. Other correlations between H-21/C-17 (*δ* 54.5), C-14 (*δ* 86.4), and C-22 (*δ* 161.2); and H-17/C-15 (*δ* 28.2), C-13 (*δ* 45.1), C-14 (*δ* 86.4), C-21 (*δ* 95.5), and C-22 (*δ* 161.2), confirmed the connectivity of the tetrahydropyran ring and conjugated aldehyde group between C-20 and C-22. The relative configurations were determined to be the same as those of bersaldegenin 3-acetate on the basis of NOESY correlations (Fig. 2). In the differential NOE experiment, irradiation of the CH_3-19 at *δ* 0.82 enhanced the signal intensity of four methine protons (H-8, H-11, H-12, and H-17), indicating that CH_3-19, H-8, and H-11 have a 1,3-diaxial relationship to each other. In addition, the NOE correlations of H-21/H-23 and H-24 inferred that the conjugated aldehyde group adapted trans-configuration. Consequently, the structure of this new bufadienolide derivative **1** is shown in Figure 1 and named as kalanhybrin A, in which this ring-opening basic skeleton has been reported in the literature and identified as a natural metabolite.[31]

Compound **2**, obtained as optically active colorless needles with mp 235–237 °C and $[\alpha]_D^{25}$ +45.8, was also shown to have the same molecular formula as **1** from pseudomolecular ion peak at *m*/*z* 507.2597 in HRFABMS analysis. The UV absorption maxima at 238 nm indicated the presence of a conjugated carbonyl group[30] and the IR absorption bands at 3447, 1715, and 1676 cm^{-1} were in agreement with the occurrence of hydroxyl, aldehyde, and conjugated carbonyl functionalities, respectively. The typical signals in the ^1H NMR spectrum inferred that **2** possessed an aldehydic proton at *δ* 9.97 (1H, s), a hemiacetal proton at *δ* 5.70 (1H, s), two oxygenated methines at *δ* 5.70 (1H, s) and 4.19 (1H, m), a methoxy group at *δ* 3.45 (3H, s), and one set of conjugated aldehyde functionality at *δ* 9.82 (1H, d, *J*=7.8 Hz) and 5.76 (1H, br t), respectively. The ^{13}C NMR spectrum also exhibited characteristic signals for an hemiacetal (*δ* 95.6), two aldehydes (*δ* 190.2 and 205.7), one acetyl group (*δ* 21.3 and 170.3), and one set of conjugated double bond (*δ* 126.2 and 161.2). The ^1H and ^{13}C NMR spectra of **2** were almost same as **1**, only differed in the positions of oxygenated methines. According to the long-range HMBC spectral analysis, correlation peaks through

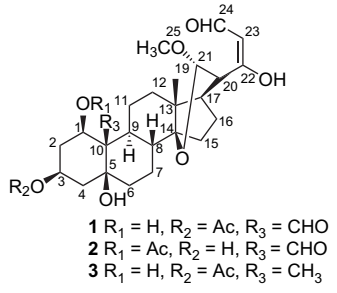

Figure 1. Structures of α-pyrone ring-opening bufadienolides **1**–**3**.

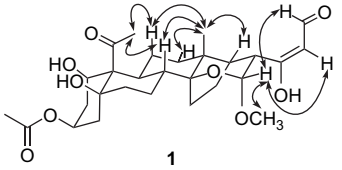

Figure 2. Relative stereochemistry and significant NOESY correlations of kalanhybrin A (**1**).

H-1 (δ 5.76) to C-3 (δ 65.8), C-5 (δ 74.3), and C-26 (δ 170.3) indicated the location of acetoxy group at C-1. Similar HMBC correlations as in **1**, like H-21/C-17, and C-14; and H-17/C-15, C-13, C-14, C-21, and C-22, further established the α-pyrone ring cleavage partial structure in **2**. The relative stereochemistry of structure **2** was determined to be the same as **1** based on the NOESY spectral analysis. The NOE correlations between CH_3-19 and H-8, H-11, H-12, and H-17, suggested that CH_3-19, H-8, and H-11 were arranged in 1,3-diaxial configurations as in **1**. In addition, similar NOE crosspeaks between H-21 and H-24 also inferred that the conjugated aldehyde group adapted trans-configuration. Thus the structure of compound **2** was elucidated as displayed in Figure 1 and trivially named as kalanhybrin B.

Compound **3** was isolated as optically active colorless syrup with $[\alpha]_D^{25}$ −40.4. The UV absorption maxima at 240 nm suggested that **3** had a conjugated carbonyl group.[30] The IR spectrum displayed typical absorption bands at 3418, 1727, 1676 cm^{-1}, representative to hydroxyl, aldehyde, and conjugated carbonyl groups, respectively. The ^1H NMR spectrum of compound **3** displayed the characteristic signals for the α-pyrone ring-opening products as in **1** at δ 9.84 (1H, d, J=7.6 Hz), 5.75 (1H, d, J=7.6 Hz), 5.73 (1H, s), and 3.46 (3H, s). There were also two oxygenated methines at δ 5.27 (1H, br s) and 3.93 (1H, br s), assignable to be the 1,3-oxygenated A-ring of bufadienolide basic skeleton. However, one more methyl group at δ 1.28 (3H, br s) appeared in the ^1H NMR spectrum of **3** and the downfield singlet typically for aldehydic proton disappeared. It suggested the difference between **3** and **1** was only that the 18-formyl group in **1** was reduced to methyl group. Analysis of the ^{13}C NMR, DEPT, and HMQC spectra revealed typical signals for an hemiacetal (δ 95.6), an aldehyde (δ 190.3), one acetyl group (δ 21.6 and 170.3), one set of conjugated double bond (δ 126.1 and 161.8), two tertiary oxygenated carbons (δ 74.2 and 87.2), two secondary oxygenated carbons (δ 69.1and 72.8), one methoxyl (δ 55.2), and two methyls (δ 12.5 and 16.7). These data also suggested that **3** should be a derivative of kalanhybrin A, which possessed 18,19-dimethyl functionalities. The HMBC proton/carbon correlations through H-21 to C-17 and C-14; H-17 to C-14, C-15, C-21, and C-22; CH_3-18 to C-1, C-5, C-9, and C-10; and CH_3-19 to C-12, C-13, C-14, and C-17, established the basic skeleton of **3** as kalanhybrin A (Fig. 1). The relative stereochemistry of **3** was also determined by NOESY proton/proton correlations of CH_3-18/H-6, H-8, and H-11; and CH_3-19/H-8, H-11, H-12, and H-17. Consequently, following the trivial nomenclature convention, the structure of **3** was named as kalanhybrin C.

Compounds **1**–**7** were screened for in vitro cytotoxicity against MCF-7, NCI-H460, and SF-268 tumor cell lines as described previously[32] and the data are given in Table 1. Compounds **1**–**3** did not exhibit any significant cytotoxicity at the tested concentration (data not shown). Compounds **4** and **6** displayed almost complete inhibition towards MCF-7 and NCI-H460 tumor cell lines at the concentration of 4 μg/mL. Compounds **5** and **7** displayed weaker cytotoxicity towards MCF-7 cell lines but were also effective to inhibit the growth of NCI-H460 tumor cell lines. Compounds **4**–**7** all displayed relatively weaker cytotoxicity towards SF-268 tumor cell lines compared with another two cell lines.

In the previous report,[31] the α-pyrone ring-opening product of bersaldegenin 3-acetate was identified as a natural metabolite from the title plant while the metabolic pathway was not discussed yet. Here we proposed the possible steps in metabolism as shown in Figure 3. First, the α-pyrone ring of bersaldegenin 3-acetate was protonated and addition of water into the β-position of α,β-unsaturated carbonyl group afforded Michael addition product. Further protonation on 20-position induced 14-hydroxy group to attack the 21-position, thus the dihydropyran ring was formed. Successive methoxylation at the hemiacetal carbon resulted in the cleavage of the α-pyrone ring. Consequently, reduction of the carboxylic acid to formyl group resulted in α-pyrone ring-opening derivative **1**. Although the bufadienolide may be converted into its α-pyrone ring-opening derivatives under the condition of base-methanol/acid in common chemical reactions, these α-pyrone ring-opening derivatives (**1**–**3**) from this species should be identified as natural products followed the possible metabolic pathway as shown.

3. Experimental

3.1. General

Melting points were determined using Yanagimoto MP-S3 micro melting point apparatus without correction. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi UV-3210 spectrophotometer and IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. ^1H NMR (300 and 400 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker AMX-400 and AVANCE-300 spectrometers using

Table 1
Cytotoxic activity of compounds **4**–**7** against three human cancer cell lines

Cancer cell lines	Percentages of cell growth inhibition MCF-7		NCI-H460		SF-268	
Concentrations	20 μg/mL	4 μg/mL	20 μg/mL	4 μg/mL	20 μg/mL	4 μg/mL
Compounds						
4	100	102	101	102	96	93
5	97	91	101	101	87	69
6	102	99	101	101	92	85
7	92	50	101	93	77	55

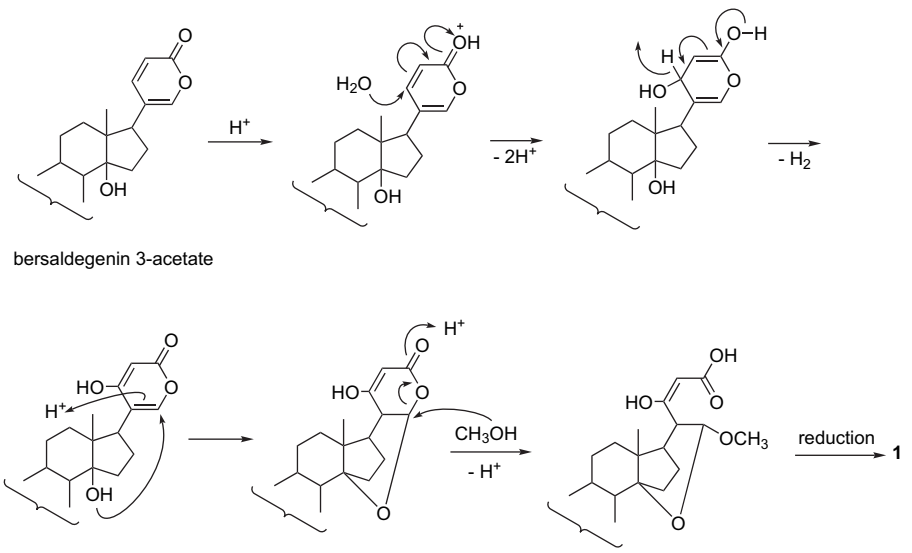

Figure 3. Possible metabolic pathway of **1**.

$CDCl_3$ as the solvent. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal standard. Low and high FAB (positive-ion mode) mass spectra were measured on a JEOL JMS-700 spectrometer with samples being dispersed in glycerol and bombarded with a beam of Xe atoms with an acceleration of 8 kV. Reversed-phase column chromatography was accomplished with Diaion HP-20 and Sephadex LH-20 columns. Silica gel column chromatography was carried out using Kieselgel 60 (70–230 and 230–400 mesh, Merck). Thin layer chromatography (TLC) was executed on precoated Kieselgel 60 F_{254} plates (Merck), with compounds visualized by UV light or spraying with 10% (v/v) H_2SO_4 followed by charring at 110 °C for 10 min.

3.2. Plant material

The whole plants of *K. hybrida* were collected in the campus of National Cheng Kung University, Tainan, Taiwan, Republic of China, in August 2003 and was authenticated by Prof. C.S. Kuoh, Department of Life Science, National Cheng Kung University. A voucher specimen (Wu 2003010001) has been deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

3.3. Extraction and isolation

Dried and powdered whole plants of *K. hybrida* (16.4 kg) was refluxed with methanol (20 L×7) and filtered. The filtrate was concentrated to afford the crude extracts (1.0 kg). The crude extracts were suspended in water and partitioned with chloroform and *n*-butanol, successively, and then resulted in chloroform extracts (70 g), *n*-butanol-solubles (250 g), and H_2O-fraction (125 g), respectively. The chloroform extracts showed inhibition percentages of 93%, 99%, and 81% at 50 μg/mL against MCF-7, NCI-H460, and SF-268 tumor cell lines, respectively. Accordingly, the $CHCl_3$-solubles were subjected to column chromatography over silica gel, eluted using a step gradient of *n*-hexane/ethyl acetate (50:1 to 1:1), to obtain nine fractions (F1–F9) based on TLC profile. Each fraction was concentrated in vacuo and monitored by an in vitro cytotoxicity assay. Active fractions F7, F8, and F9 showed inhibitory percentage values of 94%, 99% and 81%; 88%, 99%, and 76%; 87%, 93%, and 68%, respectively, against MCF-7, NCI-H460, and SF-268 cell lines at a concentration of 50 μg/mL. Further purification of active fraction F7 on column chromatography over silica gel with the mixing eluents of benzene and acetone (9:1) and repeated column chromatography and thin layer chromatography gave compounds **7** (3.0 mg), **8** (2.0 mg), **9** (5.0 mg), **10** (10.0 mg), **11** (12.0 mg), **12** (3.5 mg), and **13** (1.0 mg). Active fraction F8 was further subjected to column chromatography over silica gel using a stepwise gradient of *n*-hexane/acetone (20:1 to 1:1) and preparative thin layer chromatography purification to afford **1** (5.0 mg), **2** (6.5 mg), **3** (2.0 mg), **4** (10.0 mg), **5** (4.5 mg), **6** (7.0 mg), **7** (6.0 mg), **10** (5.3 mg), **12** (2.0 mg), and **14** (2.0 mg). F9 was further chromatographed over silica gel eluted with $CHCl_3$/MeOH (from 50:1 to 1:1), and then purified by preparative TLC with *n*-hexane/acetone (3:1) to yield **4** (1.5 mg), **6** (1.0 mg), and **15** (25.0 mg).

3.3.1. Kalanhybrin A (*1*)

Colorless needles (MeOH); mp 250–252 °C; $[\alpha]_D^{25}$ +36.2 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 237 (3.20) nm; IR (KBr) ν_{max} 3450, 3437, 2949, 1715, 1676, 1448, 1378 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.82 (3H, s, CH_3-19), 2.04 (3H, s, CH_3-27), 3.45 (3H, s, OCH_3-25), 3.72 (1H, br s, OH, D_2O exchangeable), 4.12 (1H, br s, OH, D_2O exchangeable), 4.65 (1H, br t, H-1), 5.21 (1H, m, H-3), 5.71 (1H, s, H-21), 5.73 (1H, d, *J*=7.8 Hz, H-23), 9.82 (1H, d, *J*=7.8 Hz, H-24), 10.15 (1H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.4 (C-19), 20.3 (C-11), 21.5 (C-27), 23.9 (C-16), 25.1 (C-7), 28.2 (C-15), 30.2 (C-6), 31.3 (C-12), 36.0 (C-2 and C-4), 39.1 (C-8), 40.9 (C-9), 45.1 (C-13), 54.5 (C-17), 55.2 (C-25 and

C-20), 57.7 (C-10), 67.7 (C-1), 67.9 (C-3), 73.8 (C-5), 86.4 (C-14), 95.5 (C-21), 126.2 (C-23), 161.2 (C-22), 170.5 (C-26), 190.2 (C-24), 207.9 (C-18); FABMS *m/z* 507 ($[M+H]^+$, 17); HRFABMS *m/z* 507.2598 ($[M+H]^+$) (calcd for $C_{27}H_{39}O_9$ 507.2594).

3.3.2. Kalanhybrin B (*2*)

Colorless needles (MeOH); mp 235–237 °C; $[\alpha]_D^{25}$ +45.8 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (3.50) nm; IR (KBr) ν_{max} 3447, 2939, 1715, 1676, 1447, 1378 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.83 (3H, s, CH_3-19), 2.04 (3H, s, CH_3-27), 3.45 (3H, s, OCH_3-25), 4.19 (1H, m, H-3), 5.70 (1H, s, H-21), 5.73 (1H, d, *J*=7.8 Hz, H-23), 5.76 (1H, br t, H-1), 9.82 (1H, d, *J*=7.8 Hz, H-24), 9.97 (1H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.3 (C-19), 20.1 (C-11), 21.3 (C-27), 23.9 (C-16), 25.2 (C-7), 28.5 (C-15), 31.3 (C-6), 31.5 (C-12), 36.8 (C-4), 37.5 (C-2), 39.4 (C-8), 41.1 (C-9), 45.0 (C-13), 54.5 (C-17), 55.3 (C-25 and C-20), 56.9 (C-10), 65.8 (C-3), 69.6 (C-1), 74.3 (C-5), 86.4 (C-14), 95.6 (C-21), 126.2 (C-23), 161.2 (C-22), 170.3 (C-26), 190.2 (C-24), 205.7 (C-18); FABMS *m/z* 507 ($[M+H]^+$, 20); HRFABMS *m/z* 507.2597 ($[M+H]^+$) (calcd for $C_{27}H_{39}O_9$ 507.2594).

3.3.3. Kalanhybrin C (*3*)

Colorless syrup; $[\alpha]_D^{25}$ −40.4 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 240 (3.93) nm; IR (KBr) ν_{max} 3418, 2951, 1727, 1676, 1447, 1379 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.90 (3H, s, CH_3-19), 1.28 (3H, s, CH_3-18), 2.06 (3H, s, CH_3-27), 3.46 (3H, s, OCH_3-25), 3.93 (1H, m, H-1), 5.27 (1H, m, H-3), 5.73 (1H, s, H-21), 5.75 (1H, d, *J*=7.6 Hz, H-23), 9.84 (1H, d, *J*=7.6 Hz, H-24); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.5 (C-18), 16.7 (C-19), 20.1 (C-11), 21.6 (C-27), 23.4 (C-16), 25.4 (C-7), 28.9 (C-15), 31.1 (C-6), 31.7 (C-12), 35.4 (C-4), 35.7 (C-2), 37.6 (C-8 and C-20), 40.5 (C-9), 43.3 (C-10), 45.4 (C-13), 54.8 (C-17), 55.2 (C-25), 69.1 (C-3), 72.8 (C-1), 74.2 (C-5), 87.2 (C-14), 95.6 (C-21), 126.1 (C-23), 161.8 (C-22), 170.3 (C-26), 190.3 (C-24).

3.4. Cytotoxicity assay

Three human cancer cell lines, MCF-7, NCI-H460, and SF-268, were seeded in 96-well microliter plates at a density of 6000/well in 10 μL culture medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid) and maintained at 37 °C in a humidified incubator with 5% CO_2. After an overnight adaptation period, test compounds at concentrations of 20 and 4 μg/mL, respectively, in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)-tetrazolium salt (MTS) reduction assay.[32] Actinomycin D (10 μM, final concentration) and DMSO (0.3%, final concentration) were used as positive and vehicle controls. Results were expressed as percent of DMSO control.

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References and notes

1. Kirtikar, K. R.; Basu, B. D. *Indian Medicinal Plants*, 2nd ed.; M/s Periodical Experts: Delhi, 1975; Vol. II, p 999.
2. Heyne, K. *The Useful Indonesian Plants*; Research and Development Agency, Ministry of Forestry: Jakarta, 1987; pp 845–846.
3. Sjamsu Hidayat, S. S.; Hutapea, J. R. *Indonesian and Medicinal Plants (I)*; Research and Development Agency, Ministry of Health: Jakarta, 1991; pp 220–221.
4. Pal, S.; Nag, A. K. *J. Ethnopharmacol.* **1991**, *33*, 97.
5. Pal, S.; Nag, A. K. *Fitoterapia* **1990**, *61*, 527.
6. Pal, S.; Nag, A. K. *Fitoterapia* **1992**, *63*, 451.
7. Akinpelu, D. A. *Fitoterapia* **2000**, *71*, 193.
8. Rossi-Bergmann, B.; Costa, S. S.; Borges, M. B. S.; Da Silva, S. A.; Noleto, G. R.; Souza, M. L. M.; Moraes, V. L. G. *Phytother. Res.* **1994**, *8*, 399.
9. Da Silva, S. A. G.; Costa, S. S.; Mendonca, S. C. F.; Silva, E. M.; Moraes, V. L. G.; Rossi-Bergmann, B. *Acta Trop.* **1995**, *60*, 201.
10. Da Silva, S. A. G.; Pinheiro, R. O.; Costa, S. S.; Rossi-Bergmann, B. *Simpo'sio de Plantas Medicinais do Brasil*; Livro de Resumos: Floriano'polis, 1996; p 53.
11. Anderson, L. A. P.; Steyn, P. S.; van Heerden, F. R. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1573.
12. Yamagishi, T.; Yan, X. Z.; Wu, R. Y.; McPhail, D. R.; McPhail, A. T.; Lee, K. H. *Chem. Pharm. Bull.* **1988**, *36*, 1615.
13. Supratman, U.; Fujita, T.; Akiyama, K.; Hayashi, H.; Murakami, A.; Sakai, H.; Koshimizu, K.; Ohigashi, H. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 947.
14. Wagner, H.; Lotter, H.; Fischer, M. *Helv. Chim. Acta* **1986**, *69*, 359.
15. Wu, P. L.; Hsu, Y. L.; Wu, T. S.; Bastow, K. F.; Lee, K. H. *Org. Lett.* **2006**, *8*, 5207.
16. Almeida, A. P.; Da Silva, S. A. G.; Souza, M. L. M.; Lima, L. M. T. R.; Rossi-Bergmann, B.; Goncalves de Moraes, V. L.; Costa, S. S. *Planta Med.* **2000**, *66*, 134.
17. Gaind, K. N.; Singla, A. K.; Boar, R. B.; Copsey, D. B. *Phytochemistry* **1976**, *15*, 1999.
18. Costa, S. S.; Jossang, A.; Boda, B. *J. Nat. Prod.* **1996**, *59*, 327.
19. Liu, K. C. S.; Yang, S. L.; Roberts, M. F.; Phillipson, J. D. *Phytochemistry* **1989**, *28*, 2813.
20. Liu, K. C. S.; Yang, S. L.; Roberts, M. F.; Phillipson, J. D. *J. Nat. Prod.* **1989**, *52*, 970.
21. Mathew, P. J.; Unithan, M. C. *Indian Aryavaidyan* **1992**, *6*, 54.
22. Kislichenko, M. H. *Khim. Prir. Soedin.* **1969**, *5*, 386.
23. Yang, M. L.; Kuo, P. C.; Damu, A. G.; Chang, R. J.; Chiou, W. F.; Wu, T. S. *Tetrahedron* **2006**, *62*, 10900.
24. Lee, C. K.; Lu, C. K.; Kuo, Y. H.; Chen, J. Z.; Sun, G. Z. *J. Chin. Chem. Soc.* **2004**, *51*, 437.
25. Lo, W. L.; Chang, F. R.; Hsieh, T. J.; Wu, Y. C. *J. Chin. Chem. Soc.* **2002**, *49*, 421.
26. Chen, K. S.; Chang, F. R.; Chia, Y. C.; Wu, T. S.; Wu, Y. C. *J. Chin. Chem. Soc.* **1998**, *45*, 103.
27. Lee, H. H.; Senda, H.; Kuwae, A.; Hanai, K. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1475.
28. Takagi, Y.; Fujimori, T.; Kaneko, H.; Kato, K. *Agric. Biol. Chem.* **1981**, *45*, 787.
29. Chen, C. Y.; Chang, F. R.; Teng, C. M.; Wu, Y. C. *J. Chin. Chem. Soc.* **1999**, *46*, 77.
30. Giang, P. M.; Jin, H. Z.; Son, P. T.; Lee, J. H.; Hong, Y. S.; Lee, J. J. *J. Nat. Prod.* **2003**, *66*, 1217.
31. Supratman, U.; Fujita, T.; Akiyama, K.; Hayashi, H. *Phytochemistry* **2001**, *58*, 311.
32. Cheng, M. J.; Lee, S. J.; Chang, Y. Y.; Wu, S. H.; Tsai, I. L.; Jayaprakasam, B.; Chen, I. S. *Phytochemistry* **2003**, *63*, 603.