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Isopimarane diterpenoids from *Kaempferia pulchra* rhizomes collected in Myanmar and their Vpr inhibitory activity

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ABSTRACT

Viral protein R (Vpr), an accessory gene of HIV-1, plays important roles in viral pathogenesis. Screening of Myanmar medicinal plants that are popular as primary treatments for HIV/AIDS and for HIV-related problems revealed the potent anti-Vpr activity of the $CHCl_3$-soluble extract of *Kaempferia pulchra* rhizomes, in comparison with that of the positive control, damnacanthal. Fractionation of the active $CHCl_3$-soluble extract led to the identification of 30 isopimarane diterpenoids, including kaempulchraols A–W (**1**–**23**). All isolates were assayed for anti-Vpr activity against TREx-HeLa-Vpr cells, in which Vpr expression is tightly regulated by tetracycline. Kaempulchraols B (**2**), D (**4**), G (**7**), Q (**17**), T (**20**), U (**21**), and W (**23**) exhibited potent anti-Vpr activity, at concentrations ranging from 1.56 to 6.25 μM. The structure–activity relationships of the active kaempulchraols suggested that the presence of a hydroxy group at C-14 in an isopimara-8(9),15-diene skeleton and the presence of an acetoxy group at C-1 or C-7 in an isopimara-8(14),15-diene skeleton are the critical factors for the inhibitory effects against TREx-HeLa-Vpr cells.

Viral protein R (Vpr) is a small, basic protein (14 kDa) that is well conserved in human immune deficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus (SIV).[1,2] This protein performs pleiotropic pathobiological functions, such as nuclear import of preintegration complex,[3] transactivation of several viral promoters, including long terminal repeats,[4,5] and induction of cell cycle arrest at G2/M phase (G2 arrest) and apoptosis.[6] Many reports have suggested that Vpr is a promising drug target for comprehensive AIDS therapy.[7,8] To date, only four natural small molecule Vpr inhibitors, fumagillin, damnacanthal, vipirinin, and quercetin, have been reported.[9–12] The structures of these Vpr inhibitors are quite different from each other, at the level of their scaffolds. Thus, other structurally quite different Vpr inhibitor candidates are expected to be found in natural medicines. In the present study, we developed a screening system using the TREx-HeLa-Vpr cell line to investigate Vpr inhibitors.[13–15] In this assay system, the addition of tetracycline leads to the expression of Vpr, which then causes cell death. In contrast, Vpr-induced cell death does not occur in the presence of a Vpr inhibitor. We screened the crude extracts of Myanmar medicinal plants against the TREx-HeLa-Vpr cell line, and found that the $CHCl_3$-soluble extract of the rhizomes of *Kaempferia pulchra* Ridl. (Zingiberaceae) exhibited anti-Vpr activity at 25 μg/mL. *K. pulchra* is cultivated in various tropical countries, including Myanmar, Indonesia, Malaysia, and Thailand. It is commonly known as 'Shan-pan-oot' in Myanmar, and has been extensively used as a treatment for coughs, blood stimulation, quenching heat, urinary tract infection, and diabetes mellitus, and as a deodorant, diuretic, and carminative.[16] It reportedly possesses anti-inflammatory[17] and anti-tumor activities.[18] In Myanmar, the rhizomes are used locally for self-medication by cancer and AIDS patients. A previous chemical study of a rhizome extract reported the presence of sandaracopimaradiene diterpenoids and ethyl 4-methoxy *trans*-cinnamate.[17,19] In the present study, the isolation of the active $CHCl_3$ extract led to the identification of 31 compounds, consisting of isopimarane diterpenoids, kaempulchraols A–W (**1**–**23**), 9α-hydroxyisopimara-8(14),15-dien-7-one (**24**),[20] 7β,9α-dihydroxypimara-8(14),15-diene (**25**),[21] (1*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,11-dihydroxypimara-8(14),15-diene (**26**),[22] sandaracopimaradien-1α,2α-diol (**27**),[17] (2*R*)-*ent*-2-hydroxy isopimara-8(14),15-diene (**28**),[23] (1*R*,2*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,2,11-trihydroxypimara-8(14),15-diene (**29**),[22] 7α-hydroxyisopimara-8(14),15-diene (**30**)[24], and ethyl 4-methoxy-*trans*-cinnamate (**31**)[17] (Fig. 1). We previously reported

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the structure elucidations of kaempulchraols A–T (**1**–**20**) and their antiproliferative activities.[25–27] Most of the compounds were found to be non-cytotoxic, and thus we were encouraged to study their inhibitory activities against the expression of Vpr. Herein, we describe the isolation[28] and structure elucidation of the remaining three new compounds, kaempulchraols U–W (**21**–**23**), the anti-Vpr activities of the isolated compounds, and their structure–activity relationships.

Kaempulchraol U (**21**)[29] was obtained as colorless oil, and its molecular formula was determined as $C_{20}H_{32}O$ from the HREIMS and ^{13}C NMR data. The IR spectrum of **1** showed absorption bands of hydroxy and olefinic groups at 3480 and 1635 cm^{-1}, respectively. The ^{1}H NMR spectroscopic data (Table 1) displayed signals due to terminal vinyl protons [δ_H 5.74, dd, (J = 17.6, 11.2 Hz, H-15), 4.97, dd (J = 17.6, 1.3 Hz, H-16a), 4.98, dd (J = 11.3, 1.3 Hz, H-16b)], two methine signals, including an oxygenated one [δ_H 3.39, br s (H-14β), 1.11, dd (J = 12.8, 2.0 Hz, H-5α)], seven methylene protons, and four singlets of tertiary methyls [δ_H 1.05 (H_3-17), 0.85 (H_3-18), 0.90 (H_3-19), 0.98 (H_3-20)]. The ^{13}C NMR data (Table 2) revealed 20 signals, including those for four olefins (δ_C 144.0, 141.6, 127.4, 112.4), one oxygenated methine (δ_C 74.9), one methine (δ_C 51.2), three quaternary carbons (δ_C 39.5, 37.6, 33.3), seven methylenes, including two overlapping signals (δ_C 41.6, 36.3, 29.9, 29.2, 21.4, 18.9), and four methyls (δ_C 33.1, 23.0, 21.7, 19.8). These data suggested that **1** is a monohydroxy derivative of a $\Delta^{8(9),15}$-isopimaradiene. The key HMBC correlations from an oxygenated methine signal (δ_H 3.39, br s) to C-8/C-9/C-12/C-15 placed the hydroxy group at C-14 (Fig. 2). The relative configuration of **21** was assigned on the basis of a 2D NOESY experiment. The NOESY correlations between H-14 and H_3-17 suggested the α orientation of the C-14 hydroxy group. Therefore, the structure of **21** (kaempulchraol U) was assigned as 14α-hydroxy-isopimara-8(9),15-diene.

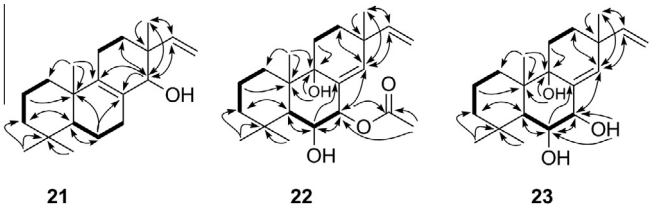
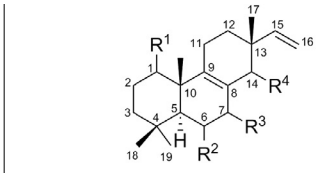
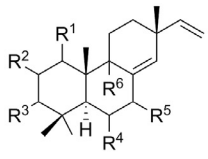


Figure 2. COSY (bold lines) and key HMBC ($^{1}H \rightarrow ^{13}C$) (arrows) correlations in compounds **21**–**23**.

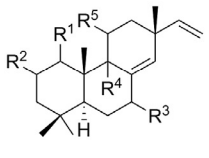
Kaempulchraol V (**22**)[30] was obtained as amorphous solid. The molecular formula was determined as $C_{22}H_{34}O_4$, from the HRCIMS and ^{13}C NMR data. The IR spectrum showed absorption bands at 3434, 1687, and 1650, due to the presence of hydroxy, ester, and olefinic groups. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **22** were similar to those of kaempulchraol T (**20**).[27] The significant differences were the absence of one methine signal and the presence of a hydroxy signal (δ_H 2.63, br d, J = 0.9 Hz) and an additional oxygenated quaternary carbon (δ_C 75.0) in **22**. The HMBC correlations (Fig. 2) from the hydroxy signal to C-8 (δ_C 130.8)/C-9 (δ_C 75.0)/C-10 (δ_C 42.3)/C-11 (δ_C 25.6) suggested that the hydroxy group was located at C-9. The NOESY correlations between H-6 (δ_H 4.17, quin, J = 2.7 Hz) and H-5 (δ_H 1.84, d, J = 2.2 Hz)/H-7 (δ_H 5.13, d, J = 2.7 Hz)/H_3-19 (δ_H 0.99, s), and between HO-9 (δ_H 2.63, br d, J = 0.9 Hz) and H-7 suggested the β



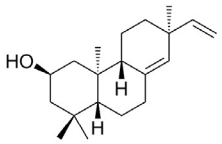
	R^1	R^2	R^3	R^4
1	H	β-OH	H	α-OMe
2	H	β-OH	H	β-OMe
3	H	β-OH	H	α-OH
4	H	β-OH	H	β-OH
7	H	β-OH	H	=O
14	α-OH	β-OH	H	α-OH
15	α-OH	β-OH	H	β-OMe
19	H	H	=O	α-OH
21	H	H	H	α-OH



	R^1	R^2	R^3	R^4	R^5	R^6
5	α-OH	H	H	β-OH	H	α-H
6	α-OH	H	α-OH	H	H	α-H
8	α-OH	H	α-OH	β-OH	H	α-H
9	α-OH	H	H	H	H	α-H
10	α-OH	H	H	H	=O	α-H
11	H	H	H	β-OAc	H	α-OH
12	H	H	H	β-OH	H	α-OMe
13	α-OH	α-OH	H	H	H	α-OH
16	H	H	H	β-OH	H	α-H
17	α-OAc	H	H	β-OH	H	α-H
18	H	H	H	H	α-OAc	α-OH
20	H	H	H	β-OH	α-OAc	α-H
22	H	H	H	β-OH	β-OAc	α-OH
23	H	H	H	β-OH	β-OH	α-OH



	R^1	R^2	R^3	R^4	R^5
24	H	H	=O	α-OH	H
25	H	H	β-OH	α-OH	H
26	α-OH	H	H	α-H	α-OH
27	α-OH	α-OH	H	α-H	H
29	α-OH	α-OH	H	α-H	α-OH
30	H	H	α-OH	α-H	H



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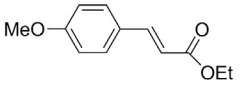

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Figure 1. Structures of compounds isolated from *K. pulchra*.

Table 1
^{1}H NMR spectroscopic data (600 MHz, $CDCl_3$) for kaempulchraols U–W (**21**–**23**), (δ in ppm and J values in (Hz) in parentheses)

Position	21	22	23
1α	1.02, m	1.86, td (13.1, 3.9)	1.78, td (13.0, 3.8)
1β	1.73, m	1.35, m	1.36, m
2α	1.48,[a] m	1.51, m	1.54, m
2β	1.60, m	1.60,[a] m	1.63, m
3α	1.17, m	1.28, m	1.31, m
3β	1.40, m	1.38, m	1.41, m
5α	1.11, dd (12.8, 2.0)	1.84, d (2.2)	1.97, d (2.1)
6α	1.48,[a] m	4.17, quin (2.7)	4.26, quin (2.8)
6β	1.74, m		
7α	1.93, m	5.13, d (2.7)	3.95, q (2.8)
7β	2.49, m		
9α			
11α	1.94,[a] m	1.60,[a] m	1.51, m
11β	1.94,[a] m	1.94, td (13.0, 3.7)	2.02, td (16.0, 3.4)
12α	1.54, m	1.73, td (13.0, 3.5)	1.74, m
12β	1.42, m	1.46, dt (13.0, 3.7)	1.49, m
14		5.93, br d (1.4)	5.80, br d (1.1)
14β	3.39, br s		
15	5.74, dd (17.6, 11.2)	5.82, dd (17.5, 10.6)	5.86, dd (17.4, 10.6)
16a	4.97, dd (17.6, 1.3)	5.01, dd (17.5, 1.0)	5.06, dd (17.4, 1.0)
16b	4.98, dd (11.3, 1.3)	4.97, dd (10.6, 1.0)	5.01, dd (10.6, 1.0)
17	1.05, s	1.08, s	1.08, s
18	0.85, s	1.26, s	1.29, s
19	0.90, s	0.99, s	1.06, s
20	0.98, s	1.08, s	1.21, s
HO-7			3.17, d (5.7)
HO-9		2.63, br d (0.9)	2.98, s
*Me*OCO-7		2.07, s	

[a] Overlapping resonances within the same column.

orientations of the C-6 hydroxy and C-7 acetoxy groups and the α orientation of the C-9 hydroxy group, respectively. Hence, the structure of **22** (kaempulchraol V) was established as 7β-acetoxy-6β,9α-dihydroxy-isopimara-8(14),15-diene.

Kaempulchraol W (**23**)[31] was obtained as amorphous solid, and its molecular formula was determined to be $C_{20}H_{32}O_3$ from the HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data of **23** (Tables 1 and 2) were similar to those of **22**, except for the absence of signals corresponding to an acetoxy group in the ^{1}H and ^{13}C NMR spectra. In contrast, the resonances of an oxygenated methine (δ_H 3.95, q, J = 2.8 Hz) and a hydroxy group (δ_H 3.17, d, J = 5.7 Hz) appeared in the spectra of **23**. The HMBC correlations between an oxygenated methine (δ_H 3.95, q, J = 2.8 Hz) and C-5/C-6/C-9/C-14, and between the hydroxy group (δ_H 3.17, d, J = 5.7 Hz) and C-6/C-7/C-8 (Fig. 2) confirmed that the hydroxy group was attached to C-7. On the basis of the NOESY correlations between H-6 and H-5/H-7/H_3-19 and between H-7 and OH-9, the orientations of OH-6 and OH-7 were determined as β, and that of OH-9 was assigned as α, respectively. Accordingly, **23** (kaempulchraol W) was identified as 6β,7β,9α-trihydroxy-isopimara-8(14),15-diene.

Table 2
^{13}C NMR spectroscopic data (150 MHz, $CDCl_3$) for kaempulchraols U–W (**21**–**23**), (δ in ppm)

Position	21	22	23
1	36.3	33.9	34.1
2	18.9[a]	18.6	18.7
3	41.6	43.5	43.6
4	33.3	33.8	33.9
5	51.2	43.8	42.9
6	18.9[a]	69.4	72.6
7	29.9	80.4	79.5
8	127.4	130.8	134.3
9	141.6	75.0	76.3
10	37.6	42.3	42.6
11	21.4	25.6	26.6
12	29.2	31.0	31.3
13	39.5	38.4	38.1
14	74.9	144.4	140.4
15	144.0	147.1	147.3
16	112.4	111.3	111.4
17	23.0	23.4	23.6
18	21.7	24.7	24.8
19	33.1	33.6	33.8
20	19.8	21.6	21.5
*Me*OCO-7		21.5	
Me*O*CO-7		169.0	

[a] Overlapping resonances within the same column.

Since the $CHCl_3$-soluble extract of the rhizomes of *K. pulchra* exhibited anti-Vpr activity at a 25 μg/mL concentration (Fig. 3A), the anti-Vpr activities of all of the isolates, including kaempulchraols A–W (**1**–**23**) and eight known compounds (**24**–**31**) (Fig. 1), were evaluated against TREx-HeLa-Vpr cells. Damnacanthal, a natural product that is a potent inhibitor of Vpr-induced cell death,[10] was used as a positive control in the present study. Among the tested compounds, only kaempulchraols B (**2**), D (**4**), G (**7**), Q (**17**), T (**20**), U (**21**), and W (**23**) inhibited the expression of Vpr, with concentrations ranging from 1.56 to 6.25 μM (Fig. 3B), and their inhibition potencies are comparable to that of 5 μM of the positive control, damnacanthal. Kaempulchraols B (**2**), D (**4**), G (**7**), and U (**21**) are the isopimara-8(9),15-dienes. In contrast, kaempulchraols Q (**17**), T (**20**), and W (**23**) are the isopimara-8(14),15-dienes. The structure–activity relationship study suggested that the presence of the β-OH groups at C-6 and C-14 on the isopimara-8(9),15-diene skeleton increased the inhibition potency; that is, more viable cells were found as compared to the treatments with **2** and **7**. Thus, the presence of either a methoxy or carbonyl group led to a decrease in the inhibition potency. Moreover, the lack of a β-OH group at C-6 in **7** resulted in a decrease in the inhibition effect. Since **17**, **20**, and **23** exhibited similar inhibitory effects at 1.56 μM, we concluded that the presence of an α-OAc group at either C-1 or C-7 on the 6β-hydroxy-isopimara-8(14),15-diene skeleton is important for the inhibition of Vpr

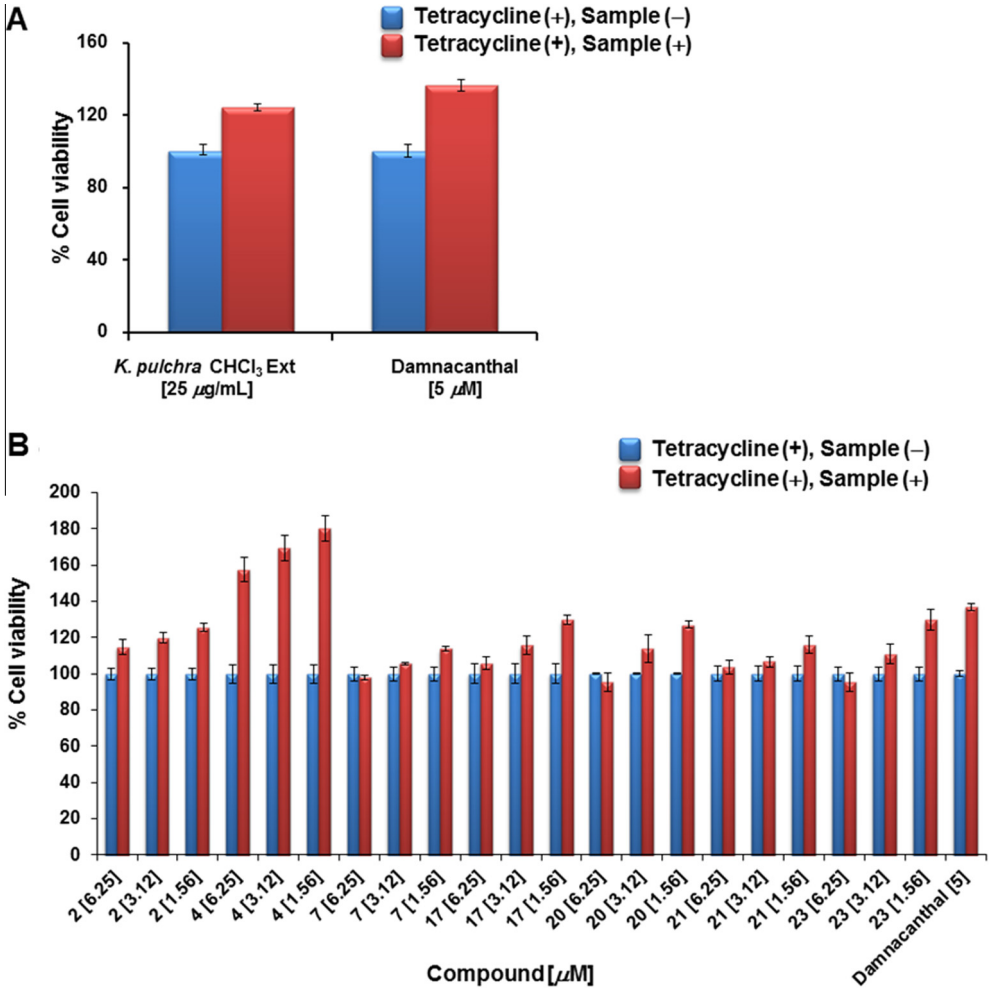

Figure 3. The inhibitory effects of (A) the $CHCl_3$-soluble extract of *K. pulchra* and (B) the isolated compounds and the positive control damnacanthal, against the expression of Vpr in TREx-HeLa-Vpr cells.

expression. These studies illustrate that the naturally occurring and chemically quite different isopimara-8(14),15-dienes and isopimara-8(9),15-dienes are potent Vpr inhibitors, as compared to the previously reported inhibitors.[9–12]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.036.

References and notes

1. Tristem, M.; Marshall, C.; Karpas, A.; Hill, F. *EMBO J.* **1992**, *11*, 3405.
2. Richter, S. N.; Frasson, I.; Palù, G. *Curr. Med. Chem.* **2009**, *16*, 267.
3. Popov, S.; Rexach, M.; Zybarth, G.; Reiling, N.; Lee, M. A.; Ratner, L.; Lane, C. M.; Moore, M. S.; Blobel, G.; Bukrinsky, M. *EMBO J.* **1998**, *17*, 909.
4. Agostini, I.; Navarro, J. M.; Rey, F.; Bouhamdan, M.; Spire, B.; Vigne, R.; Sire, J. *J. Mol. Biol.* **1996**, *261*, 599.
5. Forget, J.; Yao, X. J.; Mercier, J.; Cohen, E. A. *J. Mol. Biol.* **1998**, *284*, 915.
6. Jowett, J. B.; Planelles, V.; Poon, B.; Shah, N. P.; Chen, M. L.; Chen, I. S. *J. Virol.* **1995**, *69*, 6304.
7. Stewart, S. A.; Poon, B.; Jowett, J. B.; Chen, I. S. *J. Virol.* **1997**, *71*, 5579.
8. Stewart, S. A.; Poon, B.; Song, J. Y.; Chen, I. S. *J. Virol.* **2000**, *74*, 3105.
9. Watanabe, N.; Nishihara, Y.; Yamaguchi, T.; Koito, A.; Miyoshi, H.; Kakeya, H.; Osada, H. *FEBS Lett.* **2006**, *580*, 2598.
10. Kamata, M.; Wu, R. P.; An, D. S.; Saxe, J. P.; Damoiseaux, R.; Phelps, M. E.; Huang, J.; Chen, I. S. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 1101.
11. Ong, E. B.; Watanabe, N.; Saito, A.; Futamura, Y.; Abd El Galil, K. H.; Koito, A.; Najimudin, N.; Osada, H. *J. Biol. Chem.* **2011**, *286*, 14049.
12. Shimura, M.; Zhou, Y.; Asada, Y.; Yoshikawa, T.; Hatake, K.; Takaku, F.; Ishizaka, Y. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 308.
13. *Construction of Vpr expression plasmids:* The gene encoding the full-length Vpr from HIV-1_{NL4-3} (GenBank accession No.: AAB60574.1) was purchased from Eurofins Genomics. The DNA, encoding a c-myc tag at the C-terminus of the gene, was amplified using the following pair of primers: 5′-GCGGGATCCATGGAACAAGCCCC-3′ (*BamH* I site is underlined), and 5′-CGCACCGGTATGCATATT*CAGATCCTCTTCTGAGATGAGTTTTTGTTC*GGCACCGGATCTACTGGCTCCATTTCTTGC-3′ (*Age*I site is underlined and c-myc site is italicized). The amplified sequence was inserted into the *BamH*I/*Age*I sites of the modified pcDNA4/TO vector (QIAGEN), with a His_6 tag sequence between the *Age*I/*Xho*I sites, using a DNA ligation kit (TaKaRa) for expression as a fusion protein with c-myc and His_6 tags at the C terminus. The resultant plasmid (Vpr-c-myc-His_6) was transformed into the *E. coli* DH5α strain and purified with an Endo® Free Plasmid Maxi Kit (10) (QIAGEN).
14. *Establishment of TREx-HeLa-Vpr cell line:* TREx™-HeLa (Invitrogen) (1×10^5) cells were incubated in α-minimal essential medium (α-MEM, Wako), supplemented with 10% fetal bovine serum (FBS, Nichirei Bioscience), 1% antibiotic antimycotic solution (Sigma–Aldrich), and 5 μg/mL of blasticidin (Invitrogen), at 37 °C under a 5% CO_2 and 95% air atmosphere. When the cells

were 80–90% confluent, the inducible expression vector (Vpr-c-myc-His_6) was cotransfected with pcDNA6/TR into TREx-HeLa cells, using the calcium phosphate transfection method (ProFection® Mammalian Transfection System—Calcium Phosphate). The medium was supplemented with 200 μg/mL of zeocin, and the culture was continued to select a single stable cell line expressing both the Tet repressor and Vpr genes.

15. *Protocol for anti-Vpr (Vpr inhibitor) screening:* The established cell line, TREx-HeLa-Vpr (6,000 cells/well, 150 μL), was seeded in 48-well plates and incubated in α-minimal essential medium (α-MEM, Wako), supplemented with 10% fetal bovine serum (FBS, Nichirei Bioscience), 1% antibiotic antimycotic solution (Sigma–Aldrich), 5 μg/mL of blasticidin (Invitrogen), and 50 μg/mL of zeocin, at 37 °C under a 5% CO_2 and 95% air atmosphere, for 24 h. Since the expression of Vpr is regulated by tetracycline, the tetracycline-treated cells were designed to express Vpr by the addition of 50 μL of tetracycline (10 μg/mL). After 24 h incubation, 50 μL portions of various samples at different concentrations (1.56, 3.13, 6.25, 12.50, 25.00, 50.00 μg/mL or μM) were added to the tetracycline-treated cells, and the wells without samples were used as controls. After 48 h incubation, 50 μL of 10% WST-8 cell counting kit (Dojindo; Kumamoto, Japan) solution was added to the wells. After 2 h incubation, the absorbance at 450 nm was measured, and the cell viability was calculated from the mean values of data from three wells, by the following equation.

$$(\%)\ \text{Cell viability} = 100 \times \left[\{\text{Abs}_{(\text{test samples})} - \text{Abs}_{(\text{blank})}\} / \{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}\}\right]$$

The inhibitory activity of the tested sample was obtained by comparing the number of viable cells treated with both tetracycline and sample to the number of viable cells treated with tetracycline without sample.

16. *The Traditional Medicine Formulations used in Myanmar Traditional Medicine*; Department of Traditional Medicine: Ministry of Health, Myanmar, 1990.
17. Tuchinda, P.; Udchachon, J.; Reutrakul, V.; Santisuk, T.; Skelton, B. W.; White, A. H.; Taylor, W. C. *Phytochemistry* **1994**, *36*, 731.
18. Prasad, S.; Yadav, V. R.; Sundaram, C.; Reuter, S.; Hema, P. S.; Nair, M. S.; Chaturvedi, M. M.; Aggarwal, B. B. *J. Biol. Chem.* **2010**, *285*, 26987.
19. Prawat, U.; Tuntiwachwuttikul, P.; Taylor, W. C.; Engelhardt, L. M.; Skelton, B. W.; White, A. H. *Phytochemistry* **1993**, *32*, 991.
20. Chang, C. I.; Tseng, M. H.; Kuo, Y. H. *Chem. Pharm. Bull.* **2005**, *53*, 286.
21. Xia, X.; Zhang, J.; Zhang, Y.; Wei, F.; Liu, X.; Jia, A.; Liu, C.; Li, W.; She, Z.; Lin, Y. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3017.
22. Thongnest, S.; Mahidol, C.; Sutthivaiyakit, S.; Ruchirawat, S. *J. Nat. Prod.* **2005**, *68*, 1632.
23. Nagashima, F.; Murakami, M.; Takaoka, S.; Asakawa, Y. *Phytochemistry* **2003**, *64*, 1319.
24. Touchè, E. M. G.; Lopez, E. G.; Reyes, A. P.; Sánchez, H.; Honecker, F.; Achenbach, H. *Phytochemistry* **1997**, *45*, 387.
25. Win, N. N.; Ito, T.; Aimaiti, S.; Imagawa, H.; Ngwe, H.; Abe, I.; Morita, H. *J. Nat. Prod.* **2015**, *78*, 1113.
26. Win, N. N.; Ito, T.; Aimaiti, S.; Kodama, T.; Imagawa, H.; Ngwe, H.; Asakawa, Y.; Abe, I.; Morita, H. *Tetrahedron* **2015**, *71*, 4707.
27. Win, N. N.; Ito, T.; Aimaiti, S.; Kodama, T.; Tanaka, M.; Ngwe, H.; Asakawa, Y.; Abe, I.; Morita, H. *J. Nat. Prod.* **2015**, *78*, 2306.
28. *Isolation:* Fraction 1[25] (960 mg) was rechromatographed on silica gel with *n*-hexane–CH_2Cl_2 to provide kaempulchraol U (**21**, 10 mg). The purification of subfraction 6-2[25] (74 mg), using semipreparative HPLC with MeOH–H_2O (85:15) [column: Cosmosil, Nacalai Tesque, 38149-81, 10 × 250 mm, 5C 18-AR-II; flow rate: 3 mL/min], afforded kaempulchraol V (**22**, 15.5 mg; t_R 12.5 min). The purification of subfraction 7-2[26] (448 mg), using preparative HPLC with MeOH–H_2O (80:20) [column: Discovery C-18, Supelco, 19343-01, 25 cm × 21.2 mm, 5 μm; flow rate: 10 mL/min], afforded kaempulchraol W (**23**, 20 mg; t_R 25 min).
29. *Kaempulchraol U* (**21**): Colorless oil; $[\alpha]_D^{25}$ +57 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3480, 2915,1635, 1455, 1202, 1079, 910 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 288 $[M]^+$ (37); HREIMS *m*/*z* 288.2456 $[M]^+$ (calcd for $C_{20}H_{32}O$ 288.2453).
30. *Kaempulchraol V* (**22**): Amorphous solid; $[\alpha]_D^{25}$ −12 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3434, 2921, 1687, 1650, 1460, 1368, 1276, 971 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 362 $[M]^+$ (10); HRCIMS *m*/*z* 362.2453 $[M]^+$ (calcd for $C_{22}H_{34}O_4$ 362.2457).
31. *Kaempulchraol W* (**23**): Amorphous solid; $[\alpha]_D^{25}$ −21 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3300, 2919, 2362, 1703, 1636, 1462, 1406, 1226, 1037, 967 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 320 $[M]^+$ (10); HREIMS *m*/*z* 320.2358 $[M]^+$ (calcd for $C_{20}H_{32}O_3$ 320.2351).