Antiproliferative Effect from Sesquiterpene Lactones of *Carpesium rosulatum* MlQ Consumed in South Korea on the Five Human Cancer Cell Lines

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Abstract: In search for antiproliferative compound against human cancer cells (A549, SK-OV-3, SK-MEL-2, XF498, HCT15), a chloroform soluble extract obtained by re-extraction of the methanol extract of whole plant of *Carpesium rosulatum* MlQ. (Compositae) exhibited cytotoxic activity. Four germacrane sesquiterpene lactones 2α,5-epoxy-5,10-dihydroxy-6-angeloyl-oxy-9β-isobutyloxy-germacran-8α,12-olide, 2α,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide, 2α,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-(3-methyl-butanoyloxy)-germacran-8α,12-olide, and 2β,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide were isolated from the chloroform extract of *C. rosulatum,* and 2α,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide showed the most potent cytotoxicity with IC_{50} value of 6.01 μM against SK-MEL-2.

Keywords: *Carpesium rosulatum;* Cytotoxic activity; human tumor cell lines; SRB.

1. Introduction

A huge reservoir of bioactive compounds exists in the over 400 000 species of plants on Earth, only a small percentage of which has been examined in research studies. Plants have been and continue to be an important source of anticancer agents [1]. Worldwide efforts are ongoing to identify new anticancer compounds from plants. The approaches for selecting plants to be tested for new bioactive compounds vary from random selection to more guided selection strategies such as the ethnopharmacological approach [2]. The latter strategy utilizes knowledge gained from the folk medicinal uses of a plant to guide the selection process. This approach generally increases the chance of finding active compounds compared with the random selection strategy. *Carpesium rosulatum* (Inuleae, Compositae), uncommon in Korea distributed in South Korea, has been used in

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Korean traditional medicine for its antipyretic, analgesic, vermifugic and anti-inflammatory properties [3]. Seeds of *Carpesium* species are explored as a traditional expellent of seat worms in Japan [4,5]. Previous works on *Carpesium* species report the occurrence of several germacranolides and triterpenoids in *C. divaricatum* as well as the determination of biological activities including cytotoxicity assays [6]. Maruyama et al. reported the isolation of several sesquiterpene lactones from the genus *Carpesium*; granilin [7], carpesiolin [7], carabrone [8], carabrol [9] and ivaxillin [9]. Recently, thymol compounds from aerial parts of *Carpesium* species have been evaluated for cancer treatment in South Korea [10]. The isolation, structure elucidation and *in vitro* antimalarial activity of a sesquiterpene lactone from *C. rosulatum* have been reported [11]. To the best of our knowledge, extracts prepared from *Carpesium* spp. have not been evaluated for anticancer properties. Continuing the investigations and our research on the pharmacologically active compounds, we report here the isolation and structure identification of sesquiterpene lactones as well as some source plants where potential anticancer activity could be characterized. In this paper, isolation of the chloroform fractions from whole plants of *C. rosulatum* yielded four sesquiterpene lactones. The structure of the compound was determined by chemical analyses as well as nuclear magnetic resonance spectroscopy. Among the isolates, 2α,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide showed the most potent cytotoxicity with the IC$_{50}$ value of 6.01 μM against SK-MEL-2.

2. Materials and Methods

2.1. Plant materials and isolation of active compounds

Carpesium rosulatum (Inuleae, Compositae), collected at Mt. O-de, Kangwondo, Korea in August 2000, was identified by Prof. Seung-Jo Yoo. A voucher specimen (No. 2000-0815-2101) has been deposited in the Pharmacy Herbarium of the Sung Kyun Kwan University (Suwon, Korea). For anticancer bioassay from methanol extract, two sets (50 g each) of roughly ground air-dried plant materials of every selected species were extracted twice separately with methanol (200 mL for each extraction) by refluxing for 4 h on a sonication bath at 35°C. For the bioassay, the fractions were dissolved with 3 mg/mL in dimethyl sulfoxide and further diluted with incubation buffer. These were again pooled by methanol extracts, of which n-hexane, chloroform, ethylacetate and butanol fractions of C. rosulatum was the most active (Table 1). Attempts were then made to isolate the active components from the chloroform-soluble fraction. The air-dried plant material (1.0 kg) was finely grounded and extracted at room temperature with MeOH (2 L×3) for 2 weeks. The resulting MeOH extract (103 g) was suspended in H$_2$O (500 mL) and partitioned with n-hexane (1L×2), chloroform (1L×2), ethyl acetate (1 L×2) and n-butanol (1L×2), successively, to give n-hexane (52 g)-, chloroform (10 g)-, ethyl acetate (3 g)-, butanol (8 g)- and H$_2$O (32 g)-soluble fractions. The most active chloroform fraction was applied to a silica gel column and eluted with hexane–EtOAc mixtures of increasing polarity (5:1～1:5) to give five subfractions whose main subfraction (subfraction 2; 2.3 g) was chromatographed with silica gel eluted with chloroform/EtOAc (15:1) followed by CH$_2$Cl$_2$/EtOAc (9:1) to give five fractions (CR1- CR5). CR1.3 (1.7 g) was purified by Lobar A (Merck; CH$_2$Cl$_2$/EtOAc, 20:1 ~ 9:1) to yield four main compounds **1** (12 mg), **2** (8 mg), **3** (10 mg) and **4** (13 mg), (Figure 1).

2α,5-epoxy-5,10-dihydroxy-6-angeloyl-oxy-9β-isobutyloxy-germacran-8α,12-olide *(1):* White needle crystals; mp: 210-215°C; [α]$_D^{25}$ -14.5°(MeOH, c 1.00); UV λ$_{max}$ (MeOH) nm : 224.2; IR ν$_{max}$ cm^{-1}(KBr): 3442(OH); 1768(α-methyl-γ-lactone moiety), 1720(C=O), 1650(C=C); EI-MS(70eV) [m/z] (rel. int. %): 466[M]$^+$(3.6), 448(3.3), 383(8.4), 365(10.1), 83(100), ^{1}H-NMR(600 MHz, CDCl$_3$, δ): 1.16(3H, d, *J*=6.48, H-15), 1.21(3H, d, *J*=7.0, H-4`), 1.25(3H, d, *J*=7.0, H-3`), 1.26(3H, s, H-14), 1.76(1H, m, H-3), 1.91(3H, s, H-5``), 1.97(3H, d, *J*=7.26, H-4``), 1.97(1H, m, H-3), 2.56(1H, m, H-4), 2.68(1H, seq, H-2`), 3.09(1H, m, H-7), 4.62(1H, d, *J*=10.0, H-9), 4.71(1H, m, H-

2), 5.25(1H, d, *J*=9.6, H-6), 5.26(1H, m, H-8), 5.67(1H, d, *J*=1.6, H-13β), 6.14(1H, q, H-3``), 6.34(1H, d, *J*=1.6, H-13α); $^{13}$C-NMR(150MHz, CDCl$_3$, δppm): 14.5(C-15), 15.9(C-4``), 18.9(C-4`), 19.0(C-3`), 20.3(C-5``), 30.8(C-14), 34.0(C-2`), 36.6(C-4), 37.4(C-3), 43.9(C-1), 45.0(C-7), 72.1(C-10), 73.9(C-2), 75.6(C-6), 77.4(C-8), 77.9(C-9), 106.1(C-5), 126.2(C-2``), 127.2(C-13), 133.1(C-11), 141.6(C-3``), 166.5(C-1``), 168.3(C-12), 176.4(C-1`) [14].

2α,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide (2): White needle crystal; mp: 217-220 °C; [α]$_D^{25}$ +25.3° (CHCl$_3$, c 1.00); UV λ$_{max}$ (MeOH) nm : 225.4; IR ν$_{max}$ cm^{-1}(KBr): 3450(-OH); 1760(α-methyl-γ-lactone moiety), 1720(C=O), 1648(C=C); FAB-MS (rel. int. %): 479[M$^+$+H](38.6), 461(54.6), 379(17), 83(100); ^{1}H-NMR(600 MHz, CDCl$_3$, δ): 1.16(3H, d, *J*=7.0, H-15), 1.23(3H, s, H-14), 1.67(1H, dd, *J*=4.1, 15.6, H-1), 1.82(1H, m, H-3), 1.92(3H, s, H-5``), 1.93(1H, s, H-5`), 1.94(3H, d, *J*=7.26, H-4``), 1.94(1H, m, H-3), 1.97(3H, d, *J*=7.26, H-4`), 2.11(1H, dd, *J*=12.1, 15.6, H-1), 2.77(1H, m, H-4), 3.39(1H, dd, *J*=1.1, 10.8), 4.60(1H, m, H-2), 4.73(1H, d, *J*=10.0), 5.21(1H, d, *J*=10.8), 5.32(1H, dd, *J*=1.1, 9.6, H-8), 5.72(1H, d, *J*=1.5, H-13β), 6.15(1H, q, H-3``), 6.16(1H, d, *J*=1.5, H-13α); $^{13}$C-NMR(150MHz, CDCl$_3$, δppm): 15.1(C-15), 16.0(C-4`), 16.1(C-4``), 20.7(C-5` & C-5``),30.8(C-14), 37.3(C-4), 38.6(C-3), 45.4(C-1), 46.5(C-7), 72.2(C-10), 75.3(C-2), 77.2(C-6), 79.4(C-8), 79.8(C-9), 107.2(C-5), 127.1(C-13), 128.5(C-2``), 129.2(C-2`), 136.0(C-11), 139.1(C-3`), 140.6(C-3``), 167.8(C-1``), 169.3(C-1`), 171.4(C-12) [6].

2α,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-(3-methyl-butanoyloxy)-germacran-8α,12-olide

(3): White needle crystal; mp: 190 ∼ 193.6°C; [α]$_D^{25}$ -4.3° (MeOH, c 1.00); UVλ$_{max}$ (MeOH) nm : 217.2; IR ν$_{max}$ cm^{-1}(KBr): 3450(-OH), 1760(α-methyl-γ-lactone moiety), 1723(C=O), 1648(C=C); HREI-MS(70eV) [m/z] (rel. int. %): 480[M$^+$](8.6), 462(11.6), 83(100); ^{1}H-NMR(600 MHz, CDCl$_3$, δ): 0.97(3H, d, *J*=1.6, H-5`), 0.99(3H, d, *J*=1.84, H-4`), 1.67(3H, d, *J*=6.5, H-15), 1.24(3H, s, H-14), 1.73(1H, m, H-1), 1.76(1H, m, H-3), 1.83(1H, m, H-1), 1.91(3H, s, H-5``), 1.96(3H, d, *J*=7.2, H-4``), 2.02(1H, m, H-3), 2.14(1H, m, H-3`), 2.27(1H, dd, *J*=7.2, 15.2, H-2`), 2.37(1H, dd, *J*=7.2, 15.2, H-2`) 2.56(1H, m, H-4), 3.09(1H, d, *J*=10.6, H-7), 4.62 (1H, d, *J*=10.1, H-9), 4.71(1H, m, H-2), 5.23(1H, d, *J*=10.1, H-8), 5.25(1H, d, *J*=10.6, H-6), 5.66(1H, d, *J*=1.6, H-13β), 6.14(1H, q, H-3``), 6.32(1H, d, *J*=1.6, H-13α); $^{13}$C-NMR(150MHz, CDCl$_3$, δppm): 14.4(C-15), 15.8(C-4``), 20.3(C-5``), 22.3(C-4` & C-5``),25.3(C-3`), 30.8(C-14), 36.6(C-4), 37.4(C-3), 43.0(C-2`), 43.8(C-1), 45.0(C-7), 72.0(C-10), 73.9(C-2), 75.6(C-6), 77.4(C-8), 77.9(C-9), 106.1(C-5), 126.2(C-2``), 127.2(C-13), 133.1(C-11), 141.6(C-3``), 166.4(C-1``), 168.4(C-12), 172.5(C-1`) [6].

2β,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide (4): White needle crystal; mp: 206°C; [α]$_D^{25}$ -36.73° (MeOH, c 1.2); UVλ$_{max}$ (MeOH) nm : 218.3; IR ν$_{max}$ cm^{-1}(KBr): 3513(-OH), 1760(α-methyl-γ-lactone moiety), 1720(C=O), 1650(C=C); EI-MS(70eV) [m/z] (rel. int. %): 478[M$^+$,11); ^{1}H-NMR(600 MHz, CDCl$_3$, δ): 1.05(3H, d, *J*=7.5, H-15), 1.42(3H, s, H-14), 1.95(3H, s, H-5``) ,1.96(3H, s, H-5`), 2.00(3H, d, *J*=7.5, H-4``), 2.01(3H, d, *J*= 7.5, H-4`), 2.35(1H, m, H-4), 2.59(1H, m, H-3α), 3.91(1H, m, H-7), 4.40(1H, m, H-2), 4.78(1H, dd, *J*=6.5, H-8), 5.13(1H, d, *J*=7.5, H-6), 5.24(1H, d, *J*=5.1, H-9), 5.59(1H, d, *J*=3.1, H-13β), 6.11(1H, q, *J*=7.5, H-3``), 6.22(1H, q, *J*= 7.5, H-3`), 6.23(1H, d, *J*=3.1, H-13α).; $^{13}$C-NMR(150MHz, CDCl$_3$, δppm): 13.2(C-15), 15.8(C-4``), 15.9(C-4`), 20.4(C-5``), 20.6(C-5`), 25.6(C-14), 40.8(C-3α), 44.9(C-4), 45.7(C-7), 47.9(C-1α), 72.0(C-2), 73.1(C-10), 74.0(C-6), 77.2(C-8), 80.5(C-9), 106.0(C-5), 125.5(C-13α), 126.7(C-2``), 127.1(C-2`), 134.2(C-11), 139.0(C-3``), 141.3(C-3`), 165.8(C-1`), 167.5(C-1``), 169.4(C-12) [14].

2.2. Cell lines and culture

The following cancer cell lines were used in this study: A549 (non small cell lungcarcinoma), SK-OV-3 (adenocarcinoma, ovary malignant ascites), SK-MEL-2 (malignant melanoma, metastasis to skin of thigh), XF498 (central nervous system tumor) and HCT15 (colon adenocarcinoma). All lines were maintained in 90% DMEM supplemented with 2 mm l-glutamine, penicillin (100 IU/mL), streptomycin (100 µg/mL) and 10% heat-inactivated fetal bovine serum. Cells at 70–80% confluence were used for plating for growth inhibition assays [12].
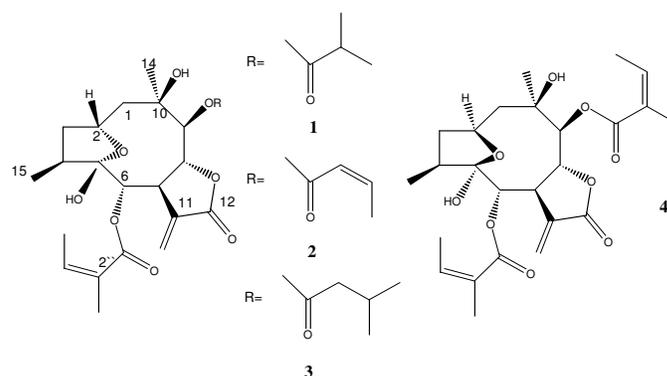
Figure 1. Sesquiterpene lactones isolated from *Carpesium rosulatum* MIQ

2.3. Antiproliferative assay

Cells were plated in 96-well flat bottom plates at 5000–10000 cell/well. The difference in cell numbers plated adjusts for differences in the growth rates of the various cell lines. Cells were allowed to adhere to the wells overnight, then the herbal extracts were added to triplicate wells in serial 3-fold dilutions. Water was added to the control wells at a 1:10 dilution in medium. These plates were incubated at 37 °C, 5% CO_2 for 3 days, then assayed from growth inhibition using a sulforhodamine B (SRB) assay [13].

The cells were fixed by the addition of cold 50% trichloroacetic acid to a final concentration of 10%. After a 1 h incubation at 4 °C, the cells were washed five times with deionized water. The cells were then stained with 0.4% SRB (Sigma) dissolved in 1% acetic acid for 15–30 min and subsequently washed five times with 1% acetic acid to remove unbound stain. After the plates had air dried at room temperature, the bound dye was solubilized with 10 mm Tris base and the plates were analysed on a microplate reader (Bio Red, U.S.A.) at 595 nm. The percent growth inhibition was calculated as: (ave. OD control wells – ave. OD herbal extract and compound wells)/(ave OD control wells).

Table 1. The cytotoxic activity of chloroform extract fractions from *Carpesium rosulatum* MlQ on the five human cancer cell lines

Fractions	IC$_{50}$ values[*]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
CHCl$_3$ extract	22.6	34.6	20.7	24.0	22.9
CR1	13.4	12.3	14.5	14.4	12.6
CR2	> 20.0	> 20.0	14.0	> 20.0	16.2
CR3	13.8	13.3	15.9	15.5	12.5
CR4	> 20.0	17.2	> 20.0	> 20.0	> 20.0
CR5	> 20.0	> 20.0	15.3	> 20.0	> 20.0
CR1.1	> 20.0	> 20.0	> 20.0	> 20.0	> 20.0
CR1.2	11.4	> 20.0	19.5	15.9	12.3
CR1.3	7.6	6.8	7.4	10.2	11.9
CR1.4	17.6	> 20.0	18.6	> 20.0	> 20.0
CR1.5	> 20.0	> 20.0	> 20.0	> 20.0	> 20.0

[*] IC$_{50}$ value of compound against each cancer cell line, which was defined as a concentration
(uM) that caused 50% inhibition of cell growth *in vitro*
A549 : non small cell lungcarcinoma,
SK-OV-3 : adenocarcinoma, ovary malignant ascites
SK-MEL-2 : malignant melanoma, metastasis to skin of thigh
XF498 : central nerve system tumor
HCT15 : colon adenocarcinoma

3. Results and Discussion

The molecular formula of compound (**1**) was determined as 2α,5-epoxy-5,10-dihydroxy-6-angeloyl-oxy-9β-isobutyloxy-germacran-8α,12-olide by ESIMS and NMR data. Its spectral data are in good agreement with those reported data in lit. [14]. All the signal assignments in ^{1}H and ^{13}C NMR spectra are herein provided. Compound (**2**) was identified as 2α,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide by NMR and ESIMS. Its spectral data are in good agreement with those reported data in lit. [6]. Compound (**3**) was identified as 2α,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-(3-methyl-butanoyloxy)-gemacran-8α,12-olide by NMR and ESIMS. Its spectral data are in good agreement with those reported one [6]. Compound (**4**) was identified as 2β,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide by NMR and ESIMS. Its spectral data are in good agreement with those reported one [14]. Compounds (**1**), (**2**), (**3**) and (**4**) with the positive control were tested *in vitro* for their cytotoxicity activities against five cancer cell lines A549 (non small cell lungcarcinoma), SK-OV-3 (adenocarcinoma, ovary malignant ascites), SK-MEL-2 (malignant melanoma, metastasis to skin of thigh), XF498 (central nerve system tumor) and HCT15 (colon adenocarcinoma). Among the compounds tested, compounds (**1**), (**2**), (**3**) showed the strongest cytotoxic activity with IC$_{50}$ values of 8.10, 6.01, 7.00 μM against SK-MEL-2, respectively. The compound (**2**) (2α,5-epoxy-5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide) showed the most potent cytotoxicity with IC$_{50}$ value of 6.01 μM against SK-MEL-2. The positive control (adriamycin) gave IC$_{50}$ values of 1.61 μM, 2.53 μM, 1.53 μM, 1.23 μM and 4.43 μM to the corresponding cell lines of for A549, SK-OV-3, SK-MEL-2, XF498 and HCT15, respectively.

Table 2. The cytotoxic activities of the sesquiterpene lactones from *Carpesium rosulatum* MlQ. on five human cancer cell lines

Compounds	IC$_{50}$ values[*]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	15.3	11.5	8.10	13.5	10.4
2	10.8	10.1	6.01	10.5	8.93
3	9.19	10.1	7.00	9.06	7.54
4	24.6	26.7	23.2	19.6	21.9
adriamycin	1.61	2.53	1.53	1.23	4.43

[*] IC$_{50}$ value of compound against each cancer cell line, which was defined as a concentration (μmol)
that caused 50% inhibition of cell growth *in vitro*
A549 : non small cell lungcarcinoma,
SK-OV-3 : adenocarcinoma, ovary malignant ascites
SK-MEL-2 : malignant melanoma, metastasis to skin of thigh
XF498 : central nerve system tumor
HCT15 : colon adenocarcinoma

However, compound (**2**) was common inhibitor of cell growth compared with adriamycin, showing only about 50% inhibition of cell growth for 19-27 μM. The antiproliferative bioactivity of the (**2**) may arise due to the double bond. Sesquiterpene lactones with such a functional group were reported as having a wide range of antifeedant, antifungal, bacteriocidal activities [6]. To our knowledge, this is the first report on the antitumor activity of the sesquiterepne lactones isolated from *C. rosulatum*. The results indicated that compound (**2**), a potent anticancer agent, may be a candidate for further study. However, the molecular mechanism of how **2** inhibit cell growth is not known, but it is being investigated by our research group.

References

[1] D.J. Newman, G.M. Cragg, S. Holbeck and E.A. Sausville (2002). Natural products and derivatives as leads to cell cycle pathway targets in cancer chemotherapy. *Curr. Cancer Drug Targets* **2**, 279-308.

[2] P.A. Cox (1994). Ethnobotanical approach to drug discovery: strengths and limitations. *Ciba Found. Symp.* **185**, 25-41.

[3] C.S. Yook (1981). Medicinal plants of Korea. Jinmyeong Publishing Co, Seoul, p 392

[4] M.R. Kim, C.S. Kim, K.H. Hwang, I.Y. Park, S.S. Hong, J.K. Son and D.C. Moon (2002). Isolation and structures of guaianolides from *Carpesium macrocephalum. J. Nat. Prod.* **65(4)**, 583-584.

[5] M. Maruyama, K. Watanabe, T. Kawakami, M. Maeda, M. Kato, S. Nozoe and T. Ohta (1995). Ineupatorolides from *Carpesium glossophyllum. Planta Med.* **61**, 388-389

[6] D.K. Kim, N.I. Baek, S.U. Choi, C.O. Lee, K.R. Lee and O.P. Zee (1996). Four new cytotoxic germacranolides from *Carpesium divaricatum. J. Nat. Prod.* **60**, 1199-1202

[7] M. Maruyama and F. Shibata (1975). Stereochemistry of granilin isolated from *Carpesium abrotanoides. Phytochemistry* **14**, 2247-2248

[8] M. Maruyama and S. Omura (1977). Carpesiolin from *Carpesium abrotanoides. Phytochemistry* **16**, 782–783

[9] M. Maruyama, A. Karube and K. Sato (1983). Sesquiterpene lactones from *Carpesium abrotanoides. Phytochemistry* **22,** 2773-2774

[10] O.P. Zee, D.K. Kim and K.R. Lee (1998). Thymol derivatives from *Carpesium divaricatum. Arch. Pharm. Res.* **21**, 618-620

[11] H.I. Moon (2007). Antiplasmodial activity of ineupatorolides A from *Carpesium rosulatum. Parasitol. Res.* **100**, 1147-1149.

[12] Z. Li, Z. Meng, A. Sayeed, R. Shalaby, B.M. Ljung and S.H. Dairkee (2002). Genome wide alleotyping of a new in vitro model system reveals early events in breast cancer progression. *Cancer Res.* **62**, 5980–5987.

[13] P. Skehan, R. Storeng and D. Scudiero (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer. Inst.* **82**, 1107-1112.

[14] M. Masao (1990). Sesquterpene lactones from *Carpesium divaricatum. Phytochemistry* **29(2)**, 547-550

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