Four New Podocarpane-Type Trinorditerpenes from *Aleurites moluccana*

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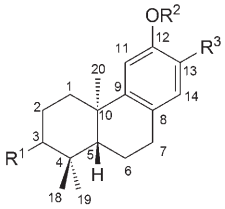
Four new podocarpane-type trinorditerpenenes, (5β,10α)-12,13-dihydroxypodocarpa-8,11,13-trien-3-one (**1**), (5β,10α)-12-hydroxy-13-methoxypodocarpa-8,11,13-trien-3-one (**2**), (5β,10α)-13-hydroxy-12-methoxypodocarpa-8,11,13-trien-3-one (**3**), and (3α,5β,10α)-13-methoxypodocarpa-8,11,13-triene-3,12-diol (**4**), together with four known diterpenes, 12-hydroxy-13-methylpodocarpa-8,11,13-trien-3-one (**5**), spruceanol (**6**), *ent*-3α-hydroxypimara-8(14),15-dien-12-one (**7**), and *ent*-3β,14α-hydroxypimara-7,9(11),15-triene-12-one (**8**), were isolated from the twigs and leaves of *Aleurites moluccana*. Their structures were elucidated by means of comprehensive spectroscopic analyses, including NMR and MS. Except **8**, all compounds were evaluated for their cytotoxicity; compound **4** exhibited moderate inhibitory activity against *Raji* cells with an IC_{50} value of 4.24 μg/ml.

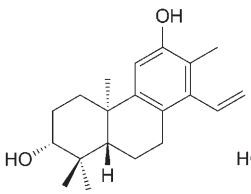
1. Introduction. – The common ornamental tree *Aleurites moluccana* (L.) WILLD (Euphorbiaceae) is found mainly in the tropic regions of Asia and Australia. The seed kernels of this plant, which contain up to 70% oil (with a fatty acid, that can be converted into biological diesel fuel), are of interest from a potential commercial vantage point [1]. However, the oil and meal produced from the fruits of *Aleurites* species are known to cause diarrhea, and irritation of the skin and internal organs. Moluccanin, a comarinolignoia, *C*-glucosyl flavonoids, acetylalueritolic acid, various fatty acids, δ-tocopherol, and 3-*O*-myristyl-20-*O*-acetyl-12-deoxyphorbol have already been isolated from various parts of *A. moluccana* [2–7].

2. Results and Discussion. – In the course of our attempts to find potential uses for this plant, four new podocarpane-type trinorditerpenes, **1**–**4**, and four known diterpenes, 12-hydroxy-13-methylpodocarpa-8,11,13-trien-3-one (**5**) [8], spruceanol (**6**) [9], *ent*-3α-hydroxypimara-8(14),15-dien-12-one (**7**) [10], and *ent*-3β,14α-hydroxypimara-7,9(11),15-triene-12-one (**8**) [11], were isolated from an acetone extract of the twigs and leaves of *A. moluccana* collected in Xishaungbannan, Yunnan Province, People's Republic of China. Except **8**, all other compounds were evaluated for their cytotoxic activity against *Raji* and HepG2 (hepatoma) cells. This report deals with the isolation and structure elucidation of these new podocarpane-type trinorditerpenes and

the results of bioactivity testing towards these two tumor cell lines. The 70% acetone extract of the twigs and leaves of *A. moluccana* was chromatographed over silica gel, RP_{18} gel, and *Sephadex LH-20* to give eight diterpenes **1**–**8**.



	R^1	R^2	R^3
1	=O	H	OH
2	=O	H	OMe
3	=O	Me	OH
4	α-OH	H	OMe
5	=O	H	Me



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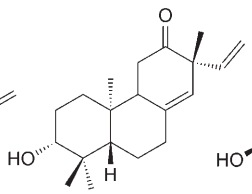

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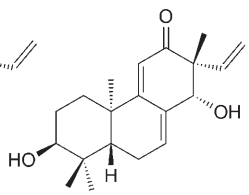

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Compound **1** was isolated as colorless cubic crystals. Its molecular formula was established as $C_{17}H_{22}O_3$ by the HR-ESI-MS molecular-ion peak at *m/z* 297.1461 ([*M* + Na]$^+$; calc 297.1466). The IR spectrum displayed peaks indicating an OH group (3466 cm^{-1}), a benzene ring (2966, 1612, 1519, 1449 cm^{-1}), and C=O groups (1681 cm^{-1}). UV Absorptions at λ_{max} 289 nm suggested that **1** had an aromatic moiety. The ^{1}H-NMR spectrum displayed three Me *singlet*s at δ (H) 1.08, 1.11, and 1.18 (Me(19), Me(18), and Me(20)), and two aromatic H-atom *singlet*s at δ (H) 6.41 and 6.64 (H–C(14) and H–C(11)). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*, resp.) indicated that **1** is a podocarpane-type trinorditerpene analogue [12–16]. Comparison of the ^{13}C-NMR data of **1** with those of the known 12-hydroxy-13-methylpodocarpa-8,11,13-trien-3-one (**5**) indicated that **1** possessed a similar structure except the presence of an OH group at C(13) instead of a Me group (δ(C) 144.5). Among 17 ^{13}C signals observed (*Table 1*), six corresponded to aromatic C-atoms, two of which were oxygenated (δ(C) 144.3 and 144.5). Aromatic H-atom *singlet*s at δ(H) 6.41 and 6.64 indicated the presence of a 1,2,4,5-tetrasubstituted benzene moiety with two OH groups. On the basis of HMBC correlations of δ(H) 6.64 (*s*, H–C(11)) with δ(C) 127.2 (*s*, C(8)), 37.9 (*s*, C(10)), 144.5 (*s*, C(13)), and 115.9 (*d*, C(14)), and of δ(H) 6.41 (*s*, H–C(14)) with δ(C) 31.3 (*t*, C(7)), 139.9 (*s*, C(9)), 113.2 (*d*, C(11)), and 144.3 (*s*, C(12)), the OH were groups at C(12) and C(13). H–C(5) (δ (H) 1.86), Me(18) (δ(H) 1.11), and Me(19) (δ(H) 1.08) exhibited HMBC correlations with the C=O signal (δ(C) 220.5), suggesting that the C=O group was at C(3). Considering that four known diterpenenes **6**–**8** isolated from the same species were *ent*-enantiomers, and C(5) and C(10) are (*S*)- and (*R*)-configured, respectively, the configurations at C(5) and C(10) of **1** were presumed to be the same as in **6**–**8**. This can be confirmed by the negative optical rotation ($[\alpha]_D^{24} = -128.7$) of **1**, which is similar to that of **5** ($[\alpha]_D^{20} = -103.4$), while (5α,10β)-13-hydroxy-12-methoxypodocarpa-8,11,13-trien-3-one [12] and (5β,10β)-13-hydroxy-12-methoxypodocarpa-8,11,13-triene [13] exhibit positive optical rotations ($[\alpha]_D^{15} = +25.1$, and $[\alpha]_D^{18} = +17.8$, resp.). On the basis of above evidence, the

Table 1. *^{1}H-NMR-Spectroscopic Data of Compounds* **1**–**4**. δ in ppm, J in Hz.

H-Atom	**1**[a])[b])	**2**[a])[c])	**3**[a])[c])	**4**[c])[d])
CH_2(1)	1.83–1.85 (*m*), 2.35 (*ddd*, J = 13.8, 8.6, 3.2)	1.85–1.87 (*m*), 2.37 (*ddd*, J = 13.6, 10.0, 3.2)	1.82–1.84 (*m*), 2.45 (*ddd*, J = 14.0, 10.4, 3.2)	1.50 (*ddd*, J = 13.2, 8.8, 4.0), 2.22 (*ddd*, J = 12.8, 7.0, 3.2)
CH_2(2)	2.51–2.56 (*m*), 2.60–2.63 (*m*)	2.55–2.58 (*m*), 2.62–2.64 (*m*)	2.53–2.55 (*m*), 2.57–2.59 (*m*)	1.74–1.76 (*m*), 1.82–1.84 (*m*)
H–C(3)	–	–	–	3.31 (*dd*, J = 3.7, 12.9)
H–C(5)	1.86 (br. *s*)	1.86–1.88 (*m*)	1.85–1.87 (*m*)	1.30 (*dd*, J = 1.6, 10.0)
CH_2(6)	1.66–1.70 (*m*), 1.74–1.78 (*m*)	1.67–1.70 (*m*), 1.76–1.78 (*m*)	1.65–1.67 (*m*), 1.72–1.75 (*m*)	1.75 (*dd*, J = 6.6, 12.6), 1.84–1.86 (*m*)
CH_2(7)	2.66 (*ddd*, J = 16.4, 7.6, 4.8), 2.71 (*ddd*, J = 16.4, 5.2, 3.2)	2.74–2.76 (*m*), 2.80 (*ddd*, J = 16.0, 7.0, 5.2)	2.76–2.78 (*m*), 2.83 (*ddd*, J = 16.5, 7.5, 5.0)	2.60 (*ddd*, J = 17.0, 7.2, 5.2), 2.78 (*ddd*, J = 16.8, 6.8, 4.0)
H–C(11)	6.64 (*s*)	6.68 (*s*)	6.75 (*s*)	6.80 (s)
H–C(14)	6.41 (*s*)	6.53 (*s*)	6.45 (*s*)	6.51 (*s*)
Me(18)	1.11 (*s*)	1.12 (*s*)	1.12 (*s*)	1.07 (*s*)
Me(19)	1.08 (*s*)	1.09 (*s*)	1.09 (*s*)	0.88 (*s*)
Me(20)	1.18 (*s*)	1.20 (*s*)	1.22 (*s*)	1.17 (*s*)
MeO		3.77 (*s*)	3.78 (*s*)	3.83 (*s*)

[a]) Recorded in CD_3OD. [b]) Recorded at 400 MHz. [c]) Recorded at 500 MHz. [d]) Recorded in $CDCl_3$.

Table 2. *^{13}C-NMR-Spectroscopic Data of Compounds* **1**–**4**. δ in ppm.

C-Atom	**1**[a])[b])	**2**[a])[c])	**3**[a])[c])	**4**[c])[d])
C(1)	38.9 (*t*)	38.8 (*t*)	38.8 (*t*)	37.0 (*t*)
C(2)	35.6 (*t*)	35.5 (*t*)	35.5 (*t*)	28.0 (*t*)
C(3)	220.5 (*s*)	220.2 (*s*)	220.2 (*s*)	78.7 (*d*)
C(4)	48.4 (*s*)	48.4 (*s*)	48.5 (*s*)	38.9 (*s*)
C(5)	52.1 (*d*)	52.1 (*d*)	52.2 (*d*)	49.9 (*d*)
C(6)	21.6 (*t*)	21.5 (*t*)	21.5 (*t*)	18.9 (*t*)
C(7)	31.3 (*t*)	31.5 (*t*)	31.3 (*t*)	30.5 (*t*)
C(8)	127.2 (*s*)	127.9 (*s*)	128.7 (*s*)	126.4 (*s*)
C(9)	139.9 (*s*)	141.0 (*s*)	139.8 (*s*)	142.2 (*s*)
C(10)	37.9 (*s*)	38.0 (*s*)	38.3 (*s*)	37.1 (*s*)
C(11)	113.2 (*d*)	113.1 (*d*)	110.1 (*d*)	110.5 (*d*)
C(12)	144.3 (*s*)	147.2 (*s*)	145.5 (*s*)	143.4 (*s*)
C(13)	144.5 (*s*)	145.7 (*s*)	147.5 (*s*)	144.4 (*s*)
C(14)	115.9 (*d*)	112.6 (*d*)	115.9 (*d*)	110.6 (*d*)
C(18)	27.4 (*q*)	27.4 (*q*)	27.4 (*q*)	28.1 (*q*)
C(19)	21.4 (*q*)	21.4 (*q*)	21.4 (*q*)	15.3 (*q*)
C(20)	25.1 (*q*)	24.9 (*q*)	24.9 (*q*)	24.8 (*q*)
MeO		56.3 (*q*)	56.5 (*q*)	55.8 (*q*)

[a]) Recorded in CD_3OD. [b]) Recorded at 400 MHz. [c]) Recorded at 500 MHz. [d]) Recorded in $CDCl_3$.

structure of **1** was established as (5β,10α)-12,13-dihydroxypodocarpa-8,11,13-trien-3-one.

Compound **2** had the molecular formula $C_{18}H_{24}O_3$ on the basis of HR-MS data. The ^{1}H-NMR spectrum showed three Me *singlet*s at δ(H) 1.09, 1.12, 1.20, one MeO *singlet* at δ(H) 3.77, and two aromatic H-atom *singlet*s at δ(H) 6.53 and 6.68, suggesting that **2** has a podocarpane-type trinorditerpene skeleton similar to that of **1**. The NMR and MS data indicated that **2** had an additional MeO group at the benzene moiety. The NOE correlations of H–C(14) (δ(H) 6.53) with the MeO group (δ(H) 3.77) and CH_2(7) (δ(H) 2.74–2.76, 2.80) revealed that the MeO group is attached to C(13). Furthermore, compound **2** had the same negative optical rotation (–104.2) as **1**. Therefore, the structure of **2** was elucidated as (5β,10α)-12-hydroxy-13-methoxypodocarpa-8,11,13-trien-3-one.

Compound **3** had the same molecular formula as **2** according to HR-ESI-MS. Its IR spectrum and UV absorption were similar to those of **2**. Four Me *singlet*s at δ(H) 1.09, 1.12, 1.22, and 3.78, and two aromatic H-atom *singlet*s at δ(H) 6.45 and 6.75 in its ^{1}H-NMR spectrum indicated that **3** is also a podocarpane-type trinorditerpenoid with substituents at both C(12) and C(13). These evidences implied that **3** is an isomer of **2**. The NMR-spectral data revealed that the difference between **2** and **3** lies in the position of the OH and MeO group. The position of the aromatic MeO group at C(12) and of the OH group at C(13) were supported by the observation that H–C(11) (δ(H) 6.75) was related to H_α–C(1) (δ(H) 2.45) and the MeO H-atom (δ(H) 3.78) in the ROESY experiment. Its optical rotation was negative (–63.7). Thus, the structure of **3** was thus determined to be (5β,10α)-13-hydroxy-12-methoxypodocarpa-8,11,13-trien-3-one.

Compound **4** showed a molecular ion at *m/z* 290 (M^+), and the molecular formula $C_{18}H_{26}O_3$ was established by HR-ESI-MS (313.1783 ([M + Na]$^+$; calc. 313.1779)). The NMR data of **4** were similar to those of **2**, except for signals indicating the disappearance of a C=O group and the presence of a OH group at C(3) (δ(C) 78.7). Therefore, we assumed that the C(3)=O group was reduced to a OH group in compound **4**. This assumption was confirmed by HMBC correlations between H–C(3) (δ(H) 3.31) and C(1) (δ(C) 37.0), C(5) (δ(C) 49.9), Me(18) (δ(C) 28.1), and Me(19) (δ(C) 15.3), and the upfield shift of C(2) and C(4) (δ(C) –7.5 and 9.5 ppm, resp.). The relative configuration of the OH group at C(3) was established by a ROESY experiment (*Fig.*). The ROESY correlations of H–C(3) (δ(H) 3.31) with H–C(5) (δ(H) 1.30) and Me(18) (δ(H) 1.07), and of the MeO group (δ(H) 3.83) with H–C(14) (δ(H) 6.51) revealed that the OH group at C(3) was α-oriented, and the MeO group was at C(13). Accordingly, the structure of **4** was assigned as (3α,5β,10α)-13-methoxypodocarpa-8,11,13-triene-3,12-diol.

Podocarpane diterpenes do not occur widely in nature but are present in several genera, such as *Azadirachta* [14–18], *Humianther* [19], *Micrandropsis* [20], *Taiwania* [12][13][21][22], *Podocarpus* [23], and *Pinus* [24]. Some of these podocapane phenols displayed bioactivity, and semi-synthesis and total synthesis of podocarpane diterpenes have been reported [25–27]. Therefore, we evaluated the cytotoxicity of these compounds except for **8**, against human lymphoma *Raji* cells and human hepatoma HepG2 cells, using cisplatin as positive control. The results showed that compound **4** was the most active against human lymphoma *Raji* cells with an IC_{50} value of 4.24 μg/ml, and compounds **1**, **3**, **6**, and **7** had weak activities against *Raji* cells. Compounds **2**

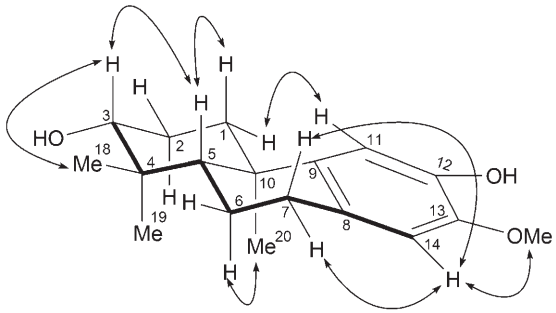

Figure. *Key ROESY correlations of compound* **4**

and **4** showed weak activity against HepG2 cells, while compounds **1**, **3**, **5**, **6**, and **7** were completely inactive ($IC_{50} > 100$ μg/ml) (*Table 3*).

Table 3. *Cytotoxicity Data of Compounds* **1**–**7** *with* IC_{50} *Values* [μg/ml]

Compound	*Raji*[a])	HepG2[b])
1	7.60	> 100
2	> 100	27.53
3	56.67	> 100
4	4.24	27.39
5	> 100	> 100
6	13.46	> 100
7	22.55	> 100
Cisplatin	0.70	0.20

[a]) Human lymphoma cell line. [b]) Human hepatoma cell line.

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Experimental Part

General. MPLC Instrument includes a *Büchi Pump Module C-605*, a *Büchi Pump Manager C-615*, and *Büchi Fraction Collector C-660*. Column chromatography (CC): Silica gel (200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Ltd. Co.*), *Lichroprep RP-18* (43–63 μm; *Merck*), and *Sephadex LH-20* (*Pharmacia*). TLC: On silica-gel plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. M.p.: *XRC-1* Apparatus; uncorrected. Optical rotations: *JASCO DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-2401 PC* spectrophotometer; λ_{max} in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* instrument (400/100 MHz) and *Bruker DRX-500* instrument (500/125 MHz); δ in ppm rel. to TMS as internal standard, J in Hz. EI-MS: *VG Auto Spec-3000* mass spectrometer; in *m/z*. HR-ESI-MS: *API Qstar Pulsar LC/TOF* instrument.

Plant Material. The twigs and leaves of *A. moluccana* were collected from Xishaungbannan, Yunnan Province, P. R. China, in August 2005. The material was identified by Prof. *Guo-Da Tao* at Xishuangbannan Tropical Botanical Garden, the Chinese Academy of Sciences (CAS). A specimen (CLS-004) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

Extraction and Isolation. The air-dried twigs and leaves of *A. moluccana* (6.0 kg) were extracted with 70% Me_2CO (3×15 l) at r.t. for three weeks and filtered. The filtrate was then concentrated, and the gummy residue (318 g) was subjected to CC (silica gel (3 kg, 200–300 mesh); $CHCl_3/Me_2CO$ 1:0 to 0:1) to afford *Fractions A–E*, of which *Fr. B* was separated by CC (*RP-18*; MeOH/H_2O 5:5 to 7:3) to afford *Fr. 1–12*. *Fr. 1–12* were combined and chromatographed (silica gel; petroleum/AcOEt 3:1 to 2:1; and *Sephadex LH-20*; MeOH) to give **2** (10 mg), **3** (9 mg), **5** (120 mg), and **6** (15 mg). Similarly, *Fr. C* was separated by CC (silica gel; petroleum/Me_2CO 4:1 to 2:1; *RP-18* column and *Sephadex LH-20*; MeOH/H_2O) to yield **1** (100 mg), **4** (47 mg), **6** (12 mg), **7** (25 mg), and **8** (7 mg).

(5β,10α)-12,13-Dihydroxypodocarpa-8,11,13-trien-3-one (**1**). Colorless cubic crystals from MeOH. M.p. 180–182°. $[\alpha]_D^{24} = -128.7$ ($c = 0.54$, MeOH). UV (MeOH): 289 (log $\varepsilon = 3.34$). IR (KBr): 3466, 2966, 1681, 1623, 1612, 1519, 1449, 1279, 1174, 1036, 873. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. FAB-MS (pos.): 275 ($[M+H]^+$). HR-ESI-MS: 297.1461 ($[M+Na]^+$, $C_{17}H_{22}NaO_3^+$; calc. 297.1466).

(5β,10α)-12-Hydroxy-13-methoxypodocarpa-8,11,13-trien-3-one (**2**). Colorless amorphous solid. $[\alpha]_D^{13} = -104.2$ ($c = 0.22$, MeOH). UV (MeOH): 285 (log $\varepsilon = 3.30$). IR (KBr): 3430, 2964, 1702, 1592, 1511, 1463, 1273, 1192, 1061, 870. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 288 (90, M^+), 273 (100), 231 (81), 189 (24), 187 (27). HR-ESI-MS: 311.1622 ($[M+Na]^+$, $C_{18}H_{24}NaO_3^+$, calc. 311.1623).

(5β,10α)-13-Hydroxy-12-methoxypodocarpa-8,11,13-trien-3-one (**3**). Colorless amorphous solid. $[\alpha]_D^{13} = -63.7$ ($c = 0.10$, MeOH). UV (MeOH): 282 (log $\varepsilon = 3.42$). IR (KBr): 3422, 2966, 1704, 1611, 1511, 1461, 1277, 1197, 1059, 874. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 288 (95, M^+), 273 (87), 231 (77), 201 (100), 187 (61), 175 (19). HR-ESI-MS: 289.1797 ($[M+H]^+$, $C_{18}H_{25}O_3^+$; calc. 289.1803).

(3α,5β,10α)-13-Methoxypodocarpa-8,11,13-triene (**4**). Colorless crystals from $CHCl_3$. M.p. 160–162°. $[\alpha]_D^{13} = -46.5$ ($c = 0.22$, $CHCl_3$). UV ($CHCl_3$): 212, 286 (log $\varepsilon = 3.24$). IR (KBr): 3454, 2968, 1645, 1509, 1445, 1266, 1186, 1032, 869. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 290 (66, M^+), 275 (35), 257 (100), 203 (22), 189 (35), 175 (16). HR-ESI-MS: 313.1783 ($[M+Na]^+$, $C_{18}H_{26}NaO_3^+$, calc. 313.1779).

Cell-Growth Inhibition Assay. Growth inhibition of compounds on tumor cells was determined by microculture 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide (MTT) assay with minor modification [28–30]. Briefly, adherent cells (HepG2) were seeded into 96-well microculture plates and allowed to adhere for 24 h before drug addition, while suspended cells (*Raji*) were seeded just before drug addition. The cell densities were selected based on the results of preliminary tests to maintain the control cells in an exponential phase of growth during the period of the experiment, and to obtain a linear relationship between the optical density and the number of viable cells. Each tumor cell line was exposed to compounds at 0.01, 0.1, 1.0, 10, and 100 μg/ml concentrations for different periods (adherent cells 72 h, suspended cells 48 h), and each concentration was tested in triplicate. At the end of exposure, 20 μl of 5 g/l MTT (*Sigma Chemical Co.*) was added to each well, and the plates were incubated for 4 h at 37°, then 'triplex soln. (10% SDS; 5% i-BuOH; 0.012M HCl)' was added, and the plates were incubated for 12–20 h at 37°. The optical density (OD) was read on a plate reader at a wavelength of 570 nm. Media (DMSO) control wells, in which compounds were absent, were included in all the experiments in order to eliminate the influence. The inhibitory rate of cell proliferation was calculated by the following formula: Growth inhibition [%] = $OD_{control} - OD_{treated}/OD_{control} \times 100$. The cytotoxicity of compounds on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) that was calculated by the LOGIT method.

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