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Homoisoflavanones from *Disporopsis aspera*

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Abstract

From cytotoxic extracts of the roots of *Disporopsis aspera* Engl. (Liliaceae) a homoisoflavanone, disporopsin (3-(2′,4′-dihydroxy-benzyl)-5,7-dihydroxy-chroman-4-one) (**1**) and three rare methyl-homoisoflavanones, 3-(4′-hydroxy-benzyl)-5,7-dihydroxy-6-methyl-chroman-4-one (**2**), 3-(4′-hydroxy-benzyl)-5,7-dihydroxy-6,8-dimethyl-chroman-4-one (**3**) and 3-(4′-hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4- one (**4**) along with five other known compounds, *N-trans*-feruloyl tyramine (**5**), adenine (**6**), 5-(hydroxy-methyl)-2-furfural (**7**), β-sitosterol (**8**) and β-sitosteryl glucopyranoside (**9**) were isolated. The structures of compounds **1**–**2** were elucidated by spectral data (1, 2-D NMR and EIMS). The four homoisoflavanones (**1**–**4**) were found to be cytotoxic against a series of human cancer cell lines (HCT15, T24S, MCF7, Bowes, A549 and K562) with IC_{50} ranging from 15 to 200 μM. Possible biosynthesis routes for homoisoflavonoids (**1**–**4**) are discussed.

Keywords: *Disporopsis asapera*; Liliaceae; Homoisoflavanones; Disporopsin; Cytotoxicity

1. Introduction

Disporopsis aspera Engl. (Liliaceae) is a perennial herb, 20–50 cm high, well known in Vietnam with the name of "Ngoc Truc Hoang Linh". Its rhizomes in decoction are prescribed as tonic in asthenia, persistent fever, night sweats, spermatorrhea, dry cough, polyuria and for the treatment of cancer (Nguyen et al., 2005; WHO and IMM, 1990). Homoisoflavanones, which are widely distributed in the Liliaceae family, harbor antibacterial and anti-angiogenic activities, and inhibit *in vitro* the growth and sporogenesis of several microorganisms (Heller and Tamm, 1981; Sup et al., 2004). In a preliminary communication, we reported the cytotoxicity of different extracts *i.e.* CH_2Cl_2, EtOAc, MeOH, MeOH–H_2O (1:1) and H_2O from *Disporopsis aspera* rhizomes (Nguyen et al., 2005). In continuing our research on bio-active compounds from Vietnamese medicinal plants, we present herein the isolation and the structure elucidation of four homoisoflavanones (**1**–**4**) (Fig. 1) along with five known compounds (**5**–**9**) from the most bio-active EtOAc extract of the rhizomes of *Disporopsis aspera*. The four isolated homoisoflavanones (**1**–**4**) were also evaluated for their cytotoxic activity against six human cancer cell lines *e.g.* HCT15, T24S, MCF7, Bowes, A549 and K562 and the possible biosynthesis pathways of these homoisoflavanones are proposed.

2. Results and discussion

The EtOAc extract of *Disporopsis aspera* powdered rhizomes was fractionated and purified by silica gel CC and *prep.* TLC to yield a new homoisoflavanone that was named disporopsin (**1**), three homoisoflavanones (**2**–**4**), along with other known compounds: *N-trans*-feruloyl

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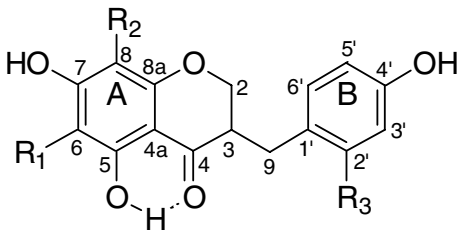

	R_1	R_2	R_3
1	H	H	OH
2	CH_3	H	H
3	CH_3	CH_3	H
4	CH_3	OCH_3	H

Fig. 1. Structure of homoisoflavanones (**1**–**4**) isolated from *Disporopsis aspera*.

tyramine (**5**), adenine (**6**), 5-(hydroxymethyl)-2-furfural (**7**), β-sitosterol (**8**) ($[\alpha]_D^{25}$ – 37° (*c* 0.5, $CHCl_3$)) and β-sitosteryl glucopyranoside (**9**). The structures of known compounds (**3**–**9**) were identified by 1, 2-D NMR, EIMS and by comparison with published data (Camarda et al., 1983; Vastano et al., 2002; Huang et al., 1997; Ma et al., 2004; Budavari et al., 1996; Wright et al., 1978; Tsai et al., 1999).

The molecular formula $C_{16}H_{14}O_6$ for **1** was determined by HREI-MS, *m/z* $[M+H]^+$ 302.075218 (calcd. 302.079038), ^{1}H-, ^{13}C-, DEPT 90 and DEPT 135 NMR. Its ^{1}H NMR spectrum exhibited the characteristic spin system of a 3-benzyl-4-chromanone structure with the signals –(2)CH_2–(3)CH–(9)CH_2– grouping at δ_H 4.35 (*dd*) and 4.16 (*dd*) (CH_2 – 2), 3.05 (*m*) (CH – 3), 3.20 (*dd*) and 2.60 (*dd*) (CH_2 – 9) (Adinolfi et al., 1989), together with signals of five aromatic protons, three exchangeable protons at δ_H 8.20, 8.50 and 9.88 (each 1H, *s*), and the expected chelated hydroxyl at δ_H 12.25 (1H, *s*). The presence of two *meta*-related aromatic protons at δ_H 5.90 and 5.95 (*d*, $J = 1.8$) suggested ring A had two oxygenated functions. Other three protons appeared at δ_H 6.95 (*d*, $J = 8.1$), 6.32 (*dd*, $J = 8.1$, 2.1) and 6.45 (*d*, $J = 2.1$) characteristic for a spin system of 1′,2′,4′-trisubstituted benzene ring B. The HMBC spectrum (Fig. 2) showed correlations of C-6′ to C-9 and to two aromatic carbons bearing oxygen (C-2′and C-4′); of the chelated OH proton to two quaternary carbons (C-4a and C-5), and to an aromatic carbon bearing one of the *meta*-protons at δ_H 5.95 (C-6) confirming the locations of hydroxy groups at C-2′, C-4′, C-5 and C-7. Detailed analysis of the 1, 2-D NMR spectra allowed to complete ^{1}H and ^{13}C NMR assignments and the unequivocal establishment of the new structure of **1** as 3-(2′,4′-dihydroxy-benzyl)-5,7-dihydroxy-chroman-4-one which we have named disporopsin.

The molecular formula of **2**, $C_{17}H_{16}O_5$ was determined by HREI-MS, *m/z* $[M+H]^+$ 300.097977 (calcd. 300.099774), ^{1}H, ^{13}C, DEPT 90 and DEPT 135 NMR. Its ^{1}H NMR spectrum was similar to that of **1** except for the signals of five aromatic protons and an additional methyl signal at δ_H 1.95 (3H, *s*). The two *ortho*-related aromatic protons at δ_H 7.10 and 6.73 (*d*, $J = 8.5$, each 2H) suggested a symmetrical substituted aromatic ring B. Detailed analysis of the HMBC spectrum (Fig. 2) allowed to complete ^{1}H and ^{13}C NMR assignments and the unequivocal establishment of **2** as 3-(4′-hydroxy-benzyl)-5,7-dihydroxy-6-methyl-chroman-4-one (Camarda et al., 1983). This is the first report of ^{13}C NMR assignments for **2**. Some of ^{1}H NMR assignments by Carmada et al. had also to be revised. In fact, the two methylenic protons at C-2 are diastereotopic, therefore, the peaks must be double doublets. The chemical shift of the methyl group can not reach 2.95 ppm as reported (Camarda et al., 1983); we recorded ^{1}H NMR of the molecule in different solvents and all gave methyl chemical shift ranging from 1.95 (in MeOD) to 2.00 ppm (in CD_3COCD_3). This can only be explained by a typing mistake in Carmada's paper (1.95 instead of 2.95). Compounds **3** and **4** were identified as 3-(4′-hydroxy-benzyl)-5,7-dihydroxy-6,8-dimethyl-chroman-4-one (Vastano et al., 2002) and 3-(4′-hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one (Huang et al., 1997; Vastano et al., 2002), respectively, by detailed 1, 2-D NMR and MS spectra analysis. These compounds (**2**–**4**) with an unusual *C*-methylated homoisoflavanone skeleton are quite rare. They were found only in *Polygonatum* species and in *Dracaena draco* (Camarda et al., 1983; Huang et al., 1997; Vastano et al., 2002).

From a biosynthesis perspective, it is very interesting to find in the same plant, *Disporopsis aspera*, both *C*-methylated homoisoflavanones (**2**–**4**) and an ordinary homoisoflavanone, disporopsin (**1**). Dewick (1975) has shown in *Eucomis bicolor* that 2′-methoxychalcone (II), obtained from phenylalanine (tyrosine), acetate and methionine (*S*-adenosylmethionine), was the precursor of 3-benzylchroman-4-one (III), likely involved in homoisoflavanone ring closure (Dewick, 1975; Dewick et al., 1970) (Fig. 3). The ordinary homoisoflavanone, disporopsin (**1**), could be easily obtained from intermediary (III) by aromatic hydroxylation, a common reaction

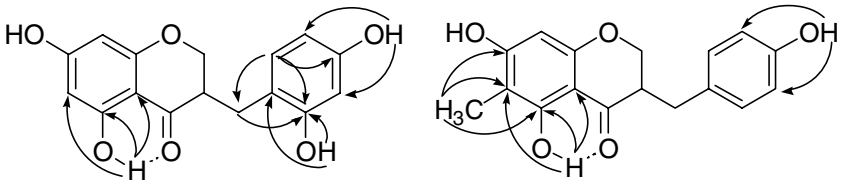

Fig. 2. Key ^{1}H–^{13}C long range correlations observed in HMBC spectra for **1** and **2**.

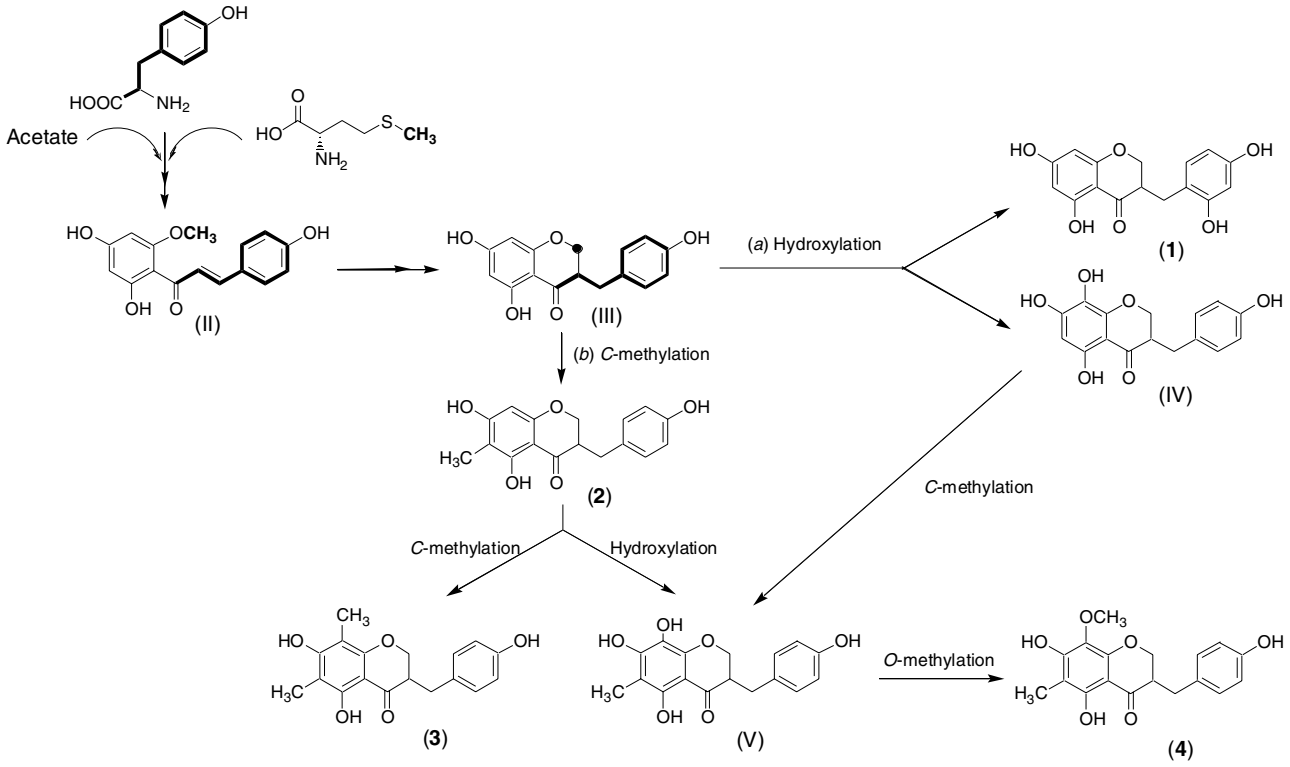

Fig. 3. Possible routes for the synthesis of an ordinary homoisoflavanone (**1**) and three *C*-methylated homoisoflavanones (**2-4**) in *Disporopsis aspera*.

catalyzed by cytochrome P-450-dependent mono-oxygenases (O_2 and NADPH). By this pathway (*a*), compound (IV) could also be formed, a possible precursor of (V) and (**4**). The pathway (*b*) to compounds **2** and **3** could be by *C*-methylation of (III) using *S*-adenosylmethionine (Dewick, 2003), whereas the intermediary (V) could be obtained by hydroxylation of **2**. Finally, *O*-methylation using SAM might involve establishing compound **4** (Dewick, 2003). As *ortho* positions to phenol groups activated easily lead to the generation of C-methyl linkage; compounds **3** and **4** therefore are more likely coming from compound **2** following route (*b*). These putative pathways involve aromatic *C*-and *O*-methylations, which may support the highly probable key methylation step involved in 3-benzylchroman-4-one biosynthesis.

The four isolated homoisoflavanones (**1**–**4**) were tested for their cytotoxicity against six human cancer cell lines (HCT15, T24S, MCF7, Bowes, A549 and K562) at concentrations ranging from 0.003 to 300 μM using MTT assay. The results are summarized in Table 3. The *C*-methylated homoisoflavanones (**2**–**4**) are more active on the cells tested than the ordinary homoisoflavanone, disporopsin (**1**), compound **3** being the most active with IC_{50} around 20 μM on the three cell lines (HCT15, T24S, MCF7 and Bowes) and on the two cell lines, K562 and A549 ($IC_{50} = 32$ and 48 μM, respectively). Compound **2** is also cytotoxic on HCT15, T24S and Bowes cells with IC_{50} about 30 μM. This is the first report of the cytotoxicity of homoisoflavanones. As the *C*-methylated homoisoflavanones (**2**–**4**) are more active than the ordinary homoisoflavanone, disporopsin (**1**), the methyl group(s) at C-6 and C-8 thus may play an important role for the cytotoxicity. The presence of a methyl group at C-8 enhanced the cytotoxic activity of **3** whereas a methoxyl group at this carbon reduced the cytotoxic activity of **4**. Without substitution at C-8, **2** showed a stronger cytotoxic activity than **4** but less than the one observed with **3**. Bowes cells were the most sensitive cells among the six tested cell lines. Reducing the polarity of these homoisoflavanones by methylation or by alkylation at C-6 and/or C-8 might therefore increase the cytotoxic activity of the compounds and possibly lead to new potential drugs.

3. Experimental

3.1. General

TLC was carried out on precoated silica gel 60 F254 plates (Merck). Spots were detected under UV (254 and 366 nm) before and after spraying with an anisaldehyde sulphuric acid solution followed by heating the plate at 150 °C for 10 min. *Prep*. TLC was performed on precoated silica gel plates, layer thickness 0.5 mm (Merck). Column chromatography was carried out on silica gel 60 (230–400 mesh, *i.d.* 2 × 30 cm, Merck). ^1H and ^{13}C (BBD, DEPT 135, DEPT 90) NMR spectra were measured on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively, with TMS as an internal standard; 2-D NMR spectra including COSY, HMQC and HMBC were recorded in $CDCl_3$ and MeOD and CD_3COCD_3 on a Varian Unity 600 at 25 °C.

HREI mass spectra were recorded on an Autospec M instrument (Micromass, Manchester, UK) at an ion source temperature of 200 °C, an electron energy of 70 eV and a mass resolution of approximately 500. The optical rotations were recorded on a Perkin–Elmer 141 polarimeter at 25 °C. UV spectra were measured on a Shimadzu UV–Vis spectrometer UV-265FS.

3.2. Plant material

The *Disporopsis aspera* rhizomes were collected in Cao bang, Vietnam in September 2002. The voucher specimen (No. 508) has been deposited in the Herbarium of Hanoi University of Pharmacy, Vietnam.

3.3. Extraction and isolation

Air-dried rhizomes of *Disporopsis aspera* (500 g) were ground into fine powder, macerated (24 h) and exhaustively percolated with 5 l ethyl acetate. The extract was concentrated under reduced pressure at 40 °C and then completely dried in a Speedvac yielding 9.3 g EtOAc extract. A part of the EtOAc extract (6 g) was chromatographed on a silica gel column (*i.d.* 2.5 × 30 cm) eluted with a gradient $CHCl_3$–MeOH [99:1 (100 ml), 95:5 (100 ml), 90:10 (700 ml), 85:5 (200 ml), 80:20 (200 ml)] followed by $CHCl_3$–MeOH–H_2O (70:30:1, 60:40:1, each 100 ml), yielding 15 100-ml fractions (1–15). The biological testing of these fractions on MCF7 cells permitted to select the active fractions for further investigation. Fractions 4 (0.4 g) and 6 (95 mg) were found to be the most active on MCF7 cells. Separation of fraction 4 through silica gel column (*i.d.* 2.5 × 30 cm) with a gradient *n*-hexane–EtOAc (1:0, 9:1, 8:2, 7:3, 6:4, 1:1, each 100 ml), afforded six 100-ml subfractions. Purification of subfraction 4.4 on *prep.* TLC using mobile phase *n*-hexane–EtOAc (7:3) (3 developments) yielded **2** (20 mg, $R_f = 0.45$) and **3** (27.1 mg, $R_f = 0.5$). From subfraction 4.5, **4** (43.8 mg, $R_f = 0.6$) was isolated after purification on *prep.* TLC (2 developments) using mobile phase *n*-hexane–EtOAc (6:4). Fraction 6 was purified by *prep.* TLC using mobile phase *n*-hexane–EtOAc (4:6) (2 developments) yielding **1** (3.4 mg, $R_f = 0.6$). Similar purifications of fractions 8–15 yielded **5** (3.8 mg), **6** (1.1 mg), **7** (2.3 mg), **8** (7.8 mg) and **9** (4.3 mg).

Compound **1**: white crystals, $[\alpha]_D^{25}$ – 22.7° (*c* 0.11, MeOH); UV (MeOH): λ_{max} (log ε) 206 (4.90), 288 (4.60); EIMS *m/z* (%): 302 $[M + H]^+$ (58), 193 (45), 153 (32), 123 (100), 108 (31). ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **2**: white powder, $[\alpha]_D^{25}$ – 67.1° (*c* 0.49, MeOH); UV (MeOH): λ_{max} (log ε) 212 (4.38), 225 (4.34), 293 (4.33), 340 (3.52); CIMS *m/z* (%): 318 $[M + 18]^+$ (29), 301 $[M + 1]^+$ (100), 285 (5), 195 (33), 124 (16). ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **3**: pale yellow oil, $[\alpha]_D^{25}$ – 58.2° (*c* 0.28, MeOH); UV (MeOH): λ_{max} (log ε) 210 (4.54), 225 (4.43), 296 (4.35), 342 (3.61); CIMS *m/z* (%): 315 $[M + 1]^+$ (100), 209 (33), 124 (13), 107 (7).

Table 1
^{13}C NMR assignments [δ (ppm)] of compounds **1**–**2**

Position	Compound **1**	Compound **2**	
	In CD_3COCD_3	In MeOD	In CD_3COCD_3
2	70.4	70.4	70.0
3	45.9	48.6	48.2
4	199.5	199.9	199.0
4a	103.0	102.9	102.2
5	165.6	163.2	162.5
6	96.8	105.6	104.7
7	167.1	166.3	165.0
8	95.5	95.1	94.8
8a	164.4	162.5	161.3
9	27.4	33.5	33.2
1′	116.2	130.6	130.0
2′	157.0	131.4	131.0
3′	103.4	116.7	116.8
4′	158.0	157.5	157.6
5′	107.5	116.7	116.8
6′	132.5	131.4	131.0
6-CH_3	–	7.3	7.3

Table 2
^1H NMR assignments [δ (ppm), *J* (Hz)] of compounds **1**–**2**

Position	Compound **1**	Compound **2**	
	In CD_3COCD_3	In MeOD	In CD_3COCD_3
2	4.16 *dd* (8.4, 11.4)	4.06 *dd* (4.1, 11.4)	4.08 *dd* (7.2, 11.4)
	4.35 *dd* (11.4, 4.6)	4.20 *dd* (11.4, 6.9)	4.27 *dd* (11.4, 4.6)
3	3.05 *m*	2.77 *m*	2.82 *m*
6	5.95 *d* (1.8)	–	–
8	5.90 *d* (1.8)	5.95 *s*	5.95 *s*
9	2.60 *dd* (9.9, 13.7)	2.64 *dd* (10.4, 13.5)	2.68 *dd* (10.2, 13.8)
	3.20 *dd* (13.7, 4.7)	3.09 *dd* (13.5, 4.2)	3.12 *dd* (13.8, 4.5)
5-OH	12.25 *s*		12.50 *s*
6-CH_3	–	1.95 *s*	2.00 *s*
7-OH	9.88 *brs*		
2′	–	7.05 *d* (8.5)	7.07 *d* (8.7)
3′	6.45 *d* (2.1)	6.73 *d* (8.5)	6.79 *d* (8.7)
5′	6.32 *dd* (8.1, 2.1)	6.73 *d* (8.5)	6.79 *d* (8.7)
6′	6.95 *d* (8.1)	7.05 *d* (8.5)	7.07 *d* (8.7)
4′-OH	8.20 *s*		
6′-OH	8.50 *s*	–	–

Compound **4**: pale yellow solid, $[\alpha]_D^{25}$ – 57.2° (*c* 0.24, MeOH); UV (MeOH): λ_{max} (log ε) 212 (4.45), 224 (4.42), 296 (4.36), 345 (3.63); CIMS *m/z* (%): 348 $[M + 18]^+$ (4), 331 $[M + 1]^+$ (100), 315 (17), 301 (8), 225.

3.4. Cytotoxicity testing

Six cell lines, HCT15 (colon cancer), T24S (bladder cancer), MCF7 (breast cancer), Bowes (skin cancer), A549 (lung cancer) and K562 (leukemia cancer) were incubated at 37 °C in minimum essential medium containing 5% inactivated foetal calf serum, 4 mM L-glutamin, penicillin–streptomycin (100 UI/ml) and gentamicin (100 μg/ml). Cells were routinely checked for mycoplasma contamination (Russell et al., 1975). They were seeded into 96-wells plate at a density of 4×10^4 cells per well in culture medium and incubated overnight before treating with fractions or

Table 3
Cytotoxicity[a] of homoisoflavanones (**1**–**4**) isolated from *Disporopsis aspera* (MTT assay: 72 h incubation, three independent experiments, each in hexaplicate)

Test product	IC_{50} (μM) Cell lines					
	HCT15	T24S	MCF7	Bowes	A549	K562
1	64.2 ± 1.5	62.5 ± 0.8	65.8 ± 0.2	61.9 ± 0.8	191.7 ± 0.6	ND
2	34.6 ± 0.4	29.6 ± 0.2	42.6 ± 0.7	23.6 ± 0.7	55.6 ± 1.0	60.3 ± 0.8
3	22.9 ± 0.6	16.5 ± 0.6	16.5 ± 1.8	14.9 ± 1.3	48.0 ± 0.6	32.4 ± 2.4
4	48.1 ± 0.5	50.3 ± 0.4	75.4 ± 1.1	41.2 ± 0.6	93.3 ± 0.5	69.0 ± 1.0
Adriamycin[b]	1.7 ± 0.1	5.8 ± 0.6	1.5 ± 0.2	0.45 ± 0.01	15.6 ± 2.7	0.07 ± 0.01

[a] The $IC_{50} \pm SD$ were determined by fitting experimental points to a parametric function by means of an original simplex algorithm: $N = N^0 \times \exp(-kC)$, where C = concentration, N = percentage of living cells at concentration C, N^0 = percentage of living cells at concentration 0 and k = parameter (Khalil et al., 1986; Dubois et al., 1989). ND, not determined.
[b] Adriamycin was used as positive control, the values are checked in every experiment.

pure compounds. The cell viability was assessed using MTT assay as reported before (Camby et al., 1996; Nguyen et al., 2004).

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