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Original article

Anti-tumoral activities of dioncoquinones B and C and related naphthoquinones gained from total synthesis or isolation from plants

Gerhard Bringmann[a,*], Guoliang Zhang[a], Anastasia Hager[a,1], Michael Moos[a], Andreas Irmer[a], Ralf Bargou[b], Manik Chatterjee[b]

[a] Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany
[b] Department of Internal Medicine II, Comprehensive Cancer Center Mainfranken, Hospital of Würzburg, Versbacher Straße 5, D-97078 Würzburg, Germany

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Dedicated to Prof. Gerhard Spiteller on the occasion of his 80th birthday.

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ABSTRACT

Dioncoquinones belong to a family of natural naphthoquinone products of interest due to their promising anti-tumoral and anti-infective activities. In particular, dioncoquinones A (**5**) and B (**6**) have been shown to be highly active against *Leishmania major* and multiple myeloma cells without any significant toxicity toward normal blood cells. Their effective concentrations against multiple myeloma cell lines were similar to those of melphalan, a well known DNA-alkylating agent used in a standard therapy against B cell lymphoma and multiple myeloma. We report on the first total synthesis of the highly oxygenated anti-tumoral agent dioncoquinone B (**6**) and the isolation of its new, even higher-oxygenated analogs, dioncoquinones C (**7**), D (**8**), and E (**9**), from cell cultures of *Triphyophyllum peltatum*. In addition, several derivatives of these compounds were synthesized, including dioncoquinone C (**7**), and a small library of naphthoquinones was created. Furthermore, the first structure-activity relationship (SAR) study on this class of compounds was conducted, showing that each of the three hydroxy groups, at C-**3**, C-**5**, and C-**6**, is required for improved anti-tumoral activities and decreased cytotoxicities.

1. Introduction

Tropical lianas of the small palaeotropic families Dioncophyllaceae and Ancistrocladaceae, including *Triphyophyllum peltatum* Airy Shaw and *Ancistrocladus abbreviatus* Airy Shaw from West Africa, are the only plants known to produce naphthyl isoquinoline alkaloids [1], like, e.g., dioncophylline A (**4**) [2] (see Scheme 1). These natural products feature unique structures, usually with rotationally hindered and thus stereogenic biaryl axes, and are characterized by their promising antiprotozoal activities against *Plasmodium*, *Leishmania*, and *Trypanosoma* parasites [3], and by their unprecedented biosynthetic origin [4,5]. Feeding experiments showed that both parts of the molecule, the isoquinoline and the naphthalene portions, are biosynthetically formed from six acetate units each (see Scheme 1), *via* a mode-F type polyketide folding [6]. The phylogenetically 'young' transamination step of this unique biosynthetic pathway, which is required for the incorporation of nitrogen into the isoquinoline moiety [5], is highly susceptible to chemical, physical, or biotic stress, then leading to the formation of naphthoquinones like plumbagin (**1**) and droserone (**2**), instead [7]. These naphthoquinones, which are produced as phytoalexins [8] by several other related plant families [8,9], have been shown to possess anti-tumoral [10] and anti-malarial [11] effects.

Our studies have revealed that a systematic change of the procedure for the cultivation of *T. peltatum* cell cultures [12] can lead to an enhanced solidification of the medium, thus preventing the calli from sinking down, thus exposing the cells to more oxidative conditions, which, in turn, favor the formation of novel, even more highly oxygenated naphthoquinones [5]. We have recently reported on the isolation and structural elucidation of nine naphthoquinones, among them dioncoquinones A (**5**) and B (**6**) [13]. Dioncoquinone B (**6**) showed a high and specific activity against *Leishmania major*, while no or weak activities against other protozoic parasites were observed. Furthermore, compound **6** strongly induced apoptosis in human tumor cells

* Corresponding author.
E-mail address: bringman@chemie.uni-wuerzburg.de (G. Bringmann).
[1] Present address: Department of Chemistry, Ludwig-Maximilians-Universität, Munich, Butenandtstr. 5-13, Haus F, D-81377 Munich, Germany

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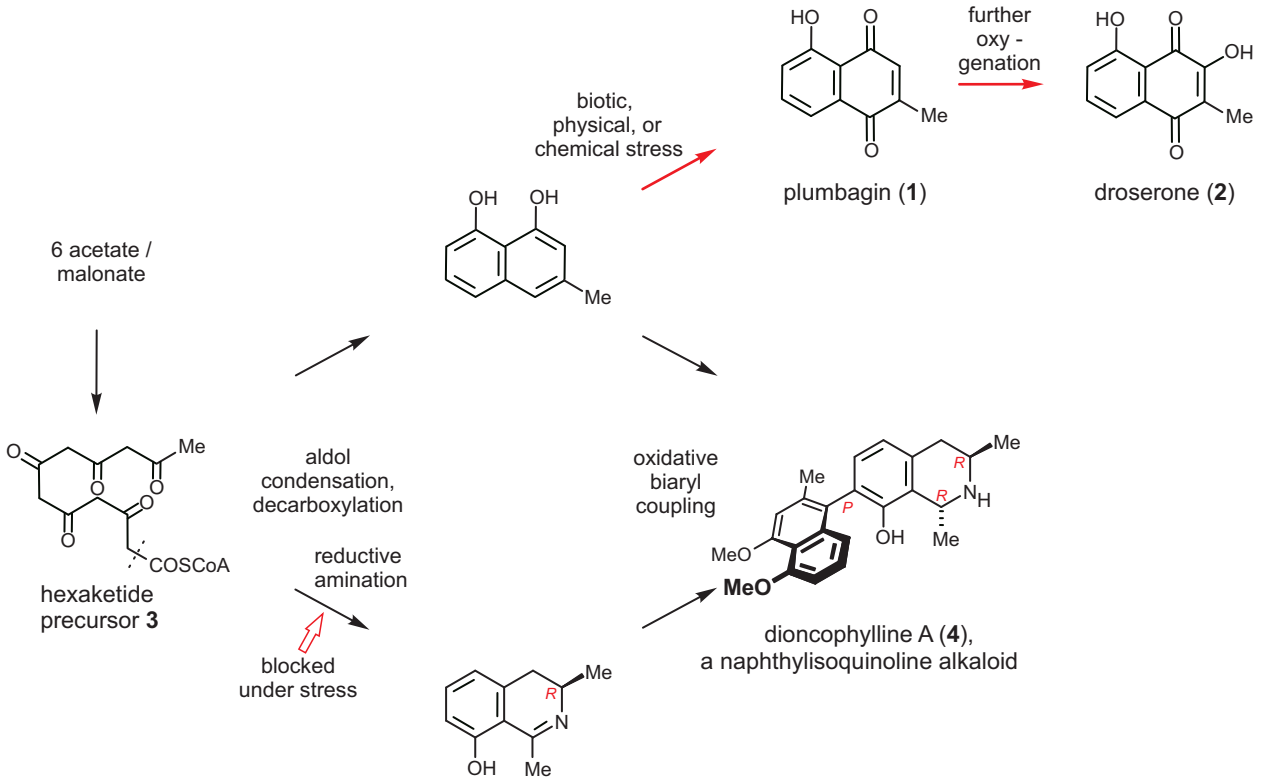


Scheme 1. Biosynthesis of the naphthyl isoquinoline alkaloid dioncophylline A (**4**), and stress-induced formation of naphthoquinones like plumbagin (**1**) and droserone (**2**), all from six acetate/malonate units *via* the postulated joint hexaketide **3**.

derived from two different B cell malignancies, namely B cell lymphoma and multiple myeloma, without any significant toxicity toward normal peripheral mononuclear blood cells [13]. In this paper, we report on the first total synthesis of dioncoquinone B (**6**) using three different synthetic approaches, and on the preparation of a range of congeners of **6**, which were then investigated in a first systematic structure-activity relationship (SAR) study for these naphthoquinone compounds. In addition, using oxidative cultivation conditions, we were able to isolate three further new dioncoquinone-related metabolites: dioncoquinones C (**7**), D (**8**), and E (**9**), and a known [14] one, 8-hydroxydroserone (**10**) (see Fig. 1). Moreover, our synthetic strategy enabled us to complete the first total synthesis of the anti-tumoral naphthoquinone **7**.

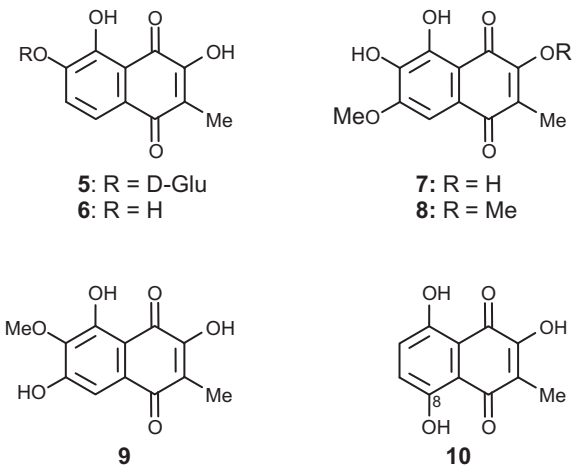


Fig. 1. Higher-oxygenated naphthoquinones isolated from *T. peltatum* cell cultures, dioncoquinones A (**5**), B (**6**), C (**7**), D (**8**), and E (**9**), and 8-hydroxydroserone (**10**).

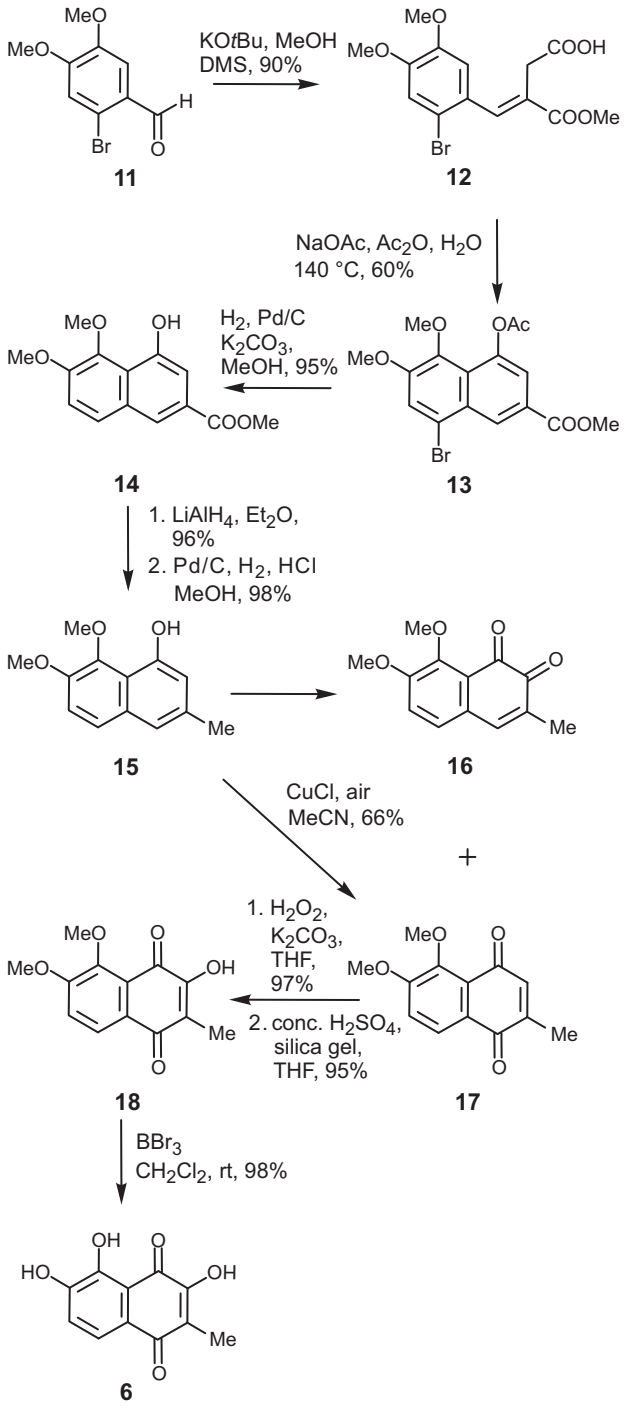
2. Results and discussion

2.1. Synthetic chemistry

2.1.1. The first route to dioncoquinone B (6): by a Stobbe approach

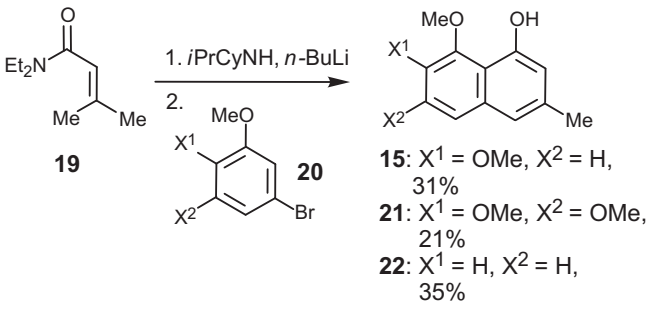
The synthesis of highly substituted naphthoquinones has been well studied during the past decades [15]. Many strategies have been developed to build up these systems, among them the Dötz reaction [16], the Hauser-Kraus annulation [17], and [4 + 2]-cycloadditions [18]. In analogy to previous work [19], our initial approach to dioncoquinone B (**6**) was based on the well-developed Stobbe-condensation route to a naphthalene, which was then oxidized and modified to provide the required naphthoquinone. This pathway, thus, started with the synthesis of the naphthalene **15** from the known [19] bromo veratrum aldehyde **11** (see Scheme 2). A Stobbe condensation [20] with dimethyl succinate resulted in the formation of the *E*-configured (NMR) acid **12**, which was cyclized in acetic anhydride in the presence of sodium acetate [21] to produce the naphthalene **13**.[2] Using palladium on charcoal in methanol in the presence of K_2CO_3 [22], the bromine and the acetate protecting

[2] Initial attempts to cyclize the corresponding non-brominated analog of **12** had resulted in an exclusive formation of the bromine-free analog of the undesired regioisomer of **13**, showing the necessity to block the *para*-cyclization reaction by a bromine substituent.



Scheme 2. First total synthesis of dioncoquinone B (**6**), *via* a Stobbe-condensation route.

groups were removed to give the free naphthol **14** in an almost quantitative yield. Reduction of the ester group using $LiAlH_4$ [22] and palladium on charcoal [22] provided the methylnaphthalene **15** [23] in a 94% yield over two steps. By oxidation of **15** with CuCl in the presence of air [24], the *para*-naphthoquinone **17** [23] was obtained in a 66% yield, accompanied by the respective *ortho*-quinone **16** (ratio 6.5:1). The required α-hydroxy functionality of the target molecule was introduced by epoxidation of the 2,3-double bond [25] of **17** followed by ring opening [26] of the resulting α, β-epoxydiketone. Under optimized conditions using sulfuric acid on silica gel, this reaction sequence provided ancistroquinone C (**18**),



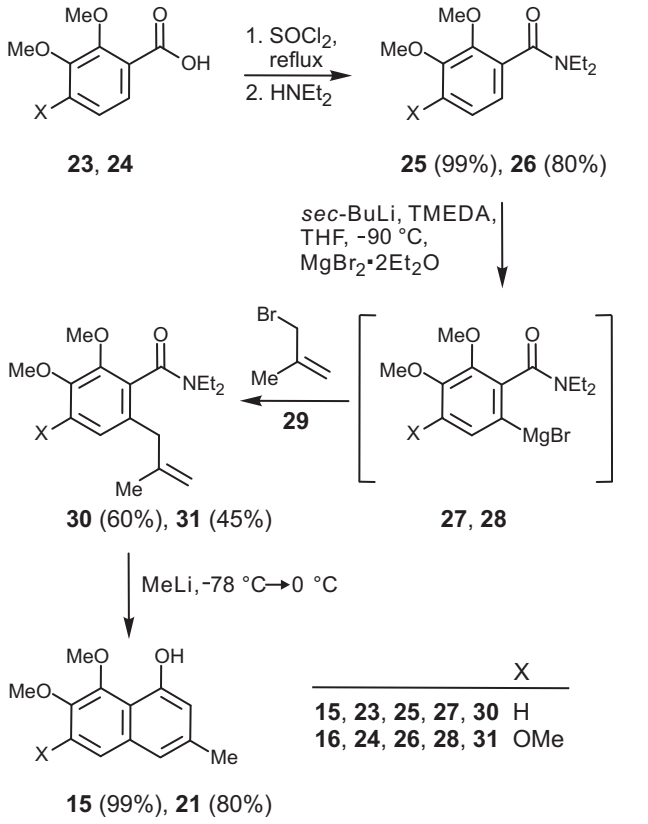
Scheme 3. Synthesis of naphthols *via* a Diels–Alder route.

a natural product that we had previously isolated from the related plant, *A. abbreviatus* [13]. *O*-demethylation of **18** with boron tribromide furnished dioncoquinone B (**6**) in a 98% yield, thus completing its first total synthesis (see Scheme 2).

This first synthetic route, although reliably providing access to dioncoquinone B (**6**) and offering the possibility to synthesize derivatives of this natural product by varying the starting materials, contains some tedious isolation and purification steps, which prompted us to explore less time-consuming alternatives. The improved second-generation synthesis of the same naphthalene intermediate **15** is discussed in the following section.

*2.1.2. Diels–Alder route to precursors of dioncoquinone B (**6**) and dioncoquinone C (**7**), and to the related naphthol **22***

For a better access to naphthols **15**, **21**, and **22**, we envisioned a strategy based on the Diels–Alder (DA) reaction between



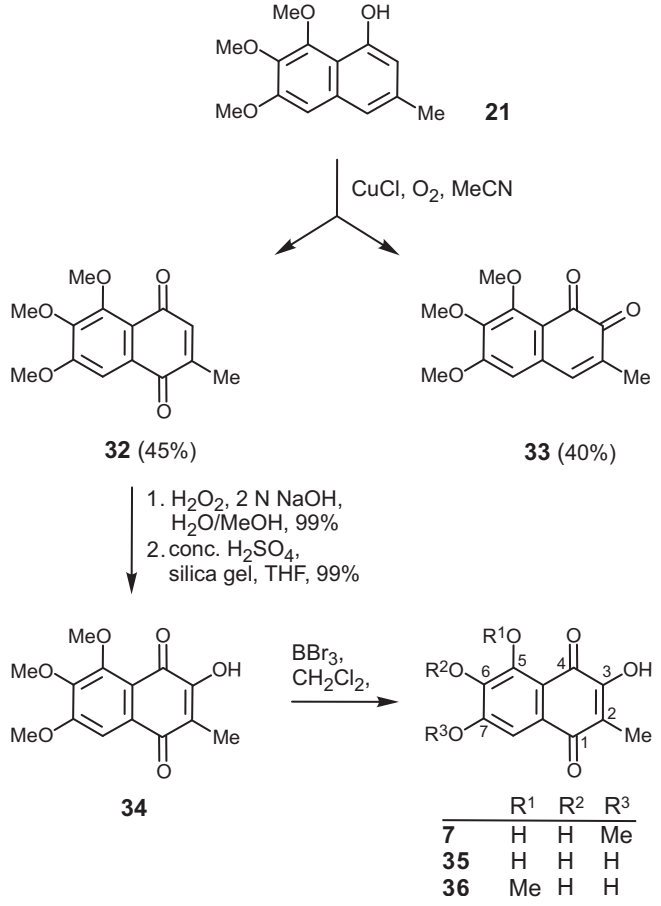
Scheme 4. Synthesis of the key intermediates **15** and **21** by Grignard reaction of the benzamides **25** and **26**, and 2-methylallyl bromide (**29**), and MeLi-induced cyclization.

benzynes as dienophiles and lithiated unsaturated amides as dienes established by Watanabe et al. [23]. This required the amide **19** and 3,4-dimethoxybenzyl bromide (**20**) as starting materials for the DA reaction, through which **15** and its congeners **21** and **22** were prepared, with different substitution patterns in the left ring part. This synthetic route permitted a concise access to several derivatives of dioncoquinone B (**6**). The yield of the naphthalene products depended on the number of electron-donating groups in the benzyne ring. The best results were achieved using mono- and di-substituted bromobenzenes (see Scheme 3).

With this Diels–Alder route to the intermediates **15**, **21**, and **22**, a significantly shorter synthetic route to dioncoquinone derivatives was established, yet in unsatisfactory yields.

2.1.3. The Grignard approach to precursors of dioncoquinone B (*6*) and dioncoquinone C (*7*)

Therefore, another pathway to naphthols **15** and **21** was established, this time based on the addition of a Grignard reagent derived from benzamides **25** and **26** to an allyl bromide [27]. This route started with the commercially available benzoic acids **23** and **24**, which were converted to the known [28,29] benzamides **25** and **26** using procedures described for other compounds [30]. After the directed *ortho*-deprotonation of **25** and **26** using *sec*-BuLi, the resulting lithium species was transmetallated with $MgBr_2 \cdot 2Et_2O$ [31], providing Grignard reagents **27** and **28**, respectively. Their reaction with 2-methylallyl bromide (**29**) resulted in the formation of the new *ortho*-allyl benzamides **30** and **31** in satisfactory yields. By treatment with methyl lithium at −78 °C [27], **30** and **31** were converted to the desired naphthalenes **15** and **21** in excellent yields (see Scheme 4). This route enabled us to synthesize these key intermediates in a very short manner and with high yields.



Scheme 5. Synthesis of derivatives of dioncoquinone B (**6**) possessing an additional oxygen function at C-7.

Table 1
Yields of **7**, **35**, and **36** using different reaction conditions in the last step.

Entry	BBr_3 (eq.)	Time (h)	T (°C)	**7** (%)[a]	**35** (%)[a]	**36** (%)[a]
1	2	2	−78	0	0	0
2	3	2	−78→0	41	10	15
3	10	2	−78→0	67	13	15
4	10	16	−78→rt	0	98	0

[a] Yields after normal-phase column chromatography and preparative HPLC on reversed-phase material.

2.1.4. Synthesis of dioncoquinone C (*7*) and its analogs

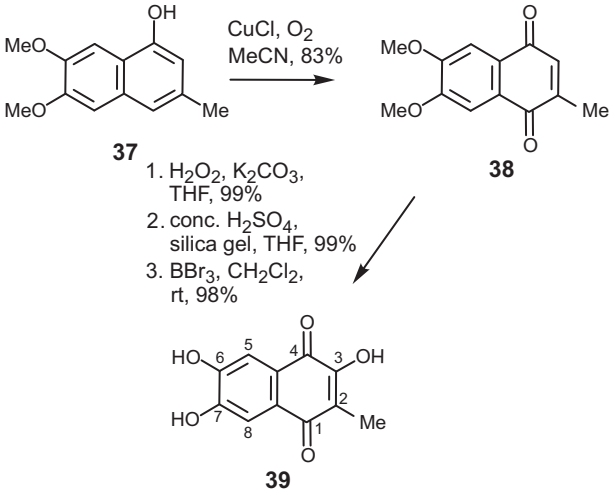
This good synthetic access to several differently substituted naphthalene compounds **15** and **21** now permitted to synthesize a small library of further dioncoquinone B analogs, among them the natural product dioncoquinone C (**7**) (see Scheme 5 and Table 1).

2.1.5. Synthesis of the dioncoquinone B analog *39*

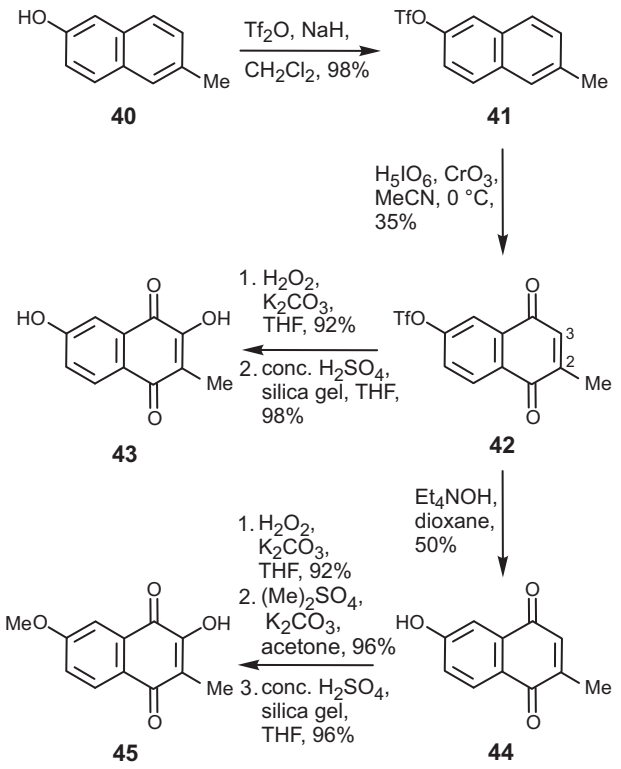
For SAR studies on dioncoquinone compounds, derivatives of dioncoquinone B (**6**) with a different OH and OMe substitution pattern were prepared. To synthesize 6,7-substituted analogs, which were difficult to access by the described methods, we started with the known naphthol **37** [32], which was oxidized to the quinone **38** [33]. Introduction of a hydroxy group at C-3 over three steps furnished the new analog **39** of **6** (see Scheme 6).

2.1.6. Synthesis of the dioncoquinone B analog *43*

To evaluate the contribution of the 5-OH function to the anti-MM activity of dioncoquinone B (**6**), compound **43**, *i.e.*, the analog without that hydroxy group, was prepared. The synthesis started with the preparation of the new triflate **41** by treatment of the known [22,34] naphthol **40** with triflic anhydride (see Scheme 7). Selective oxidation of **41** using periodic acid and chromium trioxide [35] at 0 °C afforded the new naphthoquinone **42**. Epoxidation of the 2,3-double bond in **42** with concomitant cleavage of the triflate ester, followed by epoxide opening, produced the dihydroxynaphthoquinone **43**. For the preparation of the monophenolic derivative



Scheme 6. Synthesis of the 5-deoxy-7-hydroxy derivative **39** of dioncoquinone B (**6**), i.e., with a hydroxy pattern shifted to C-6 and C-7. For reasons of comparability with the 'parent compound' dioncoquinone B (**6**), the framework numbering was adapted to that of **6** for all naphthoquinones prepared in this manuscript.



Scheme 7. Synthesis of dioncoquinone B derivatives without a hydroxy function at C-5.

45 [36], the naphthol **44** obtained from the triflate **42** [37] was epoxidized, *O*-methylated, and then converted to the target compound **45** under acidic conditions.

2.2. New natural naphthoquinones by isolation work

In addition to the above described synthetic work, the isolation of similar naphthoquinones from cell cultures of *T. peltatum* Airy Shaw [13] proved to be a rewarding source of further related analogs, too. Thus, three new natural products, dioncoquinones C (**7**), D (**8**), and E (**9**, see Fig. 2), and the known [14] naphthoquinone

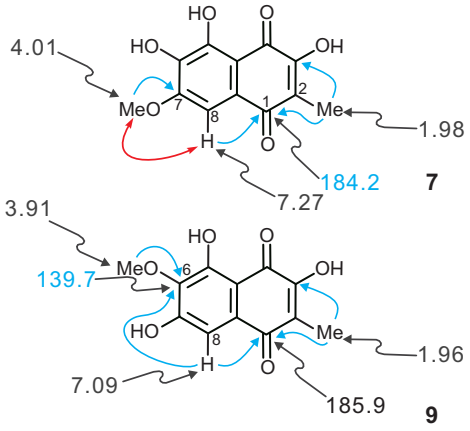


Fig. 2. Selected NMR data of 7 (in CD_3COCD_3) and 9 (in CD_3OD): ^1H and ^{13}C NMR shifts (δ in ppm), NOESY correlations (red arrow) and HMBC couplings (blue arrows) indicative for their constitutions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

8-hydroxydroserone (**10**, see Fig. 1) were isolated from this productive plant species.

The calli of the cell cultures were removed from the medium, lyophilized, ground, and extracted with CH_2Cl_2/MeOH (v/v 1:1). The crude extract was subjected to an ion-exchange column to remove naphthyl isoquinoline alkaloids, followed by normal-phase chromatography permitting isolation of three main naphthoquinones: droserone (**2**) and dioncoquinones A (**5**) and B (**6**) [13]. In the course of these investigations, three further naphthoquinones, **7**–**9**, were found in trace quantities, but easily recognized already by their UV absorption spectra. Specifically, the maximum UV absorption, around 419 nm, is characteristic of 3,5-dihydroxy substituted naphthoquinones [38]. In addition, a 3,5,8-trihydroxy substituted naphthoquinone, **10**, was found, possessing a maximum absorption at 485 nm. All these natural products were isolated and purified by preparative HPLC and characterized by ^1H-, ^{13}C- and 2D-NMR spectroscopy.

Naphthoquinones **7**–**9** all showed one aromatic proton by ^1H NMR, suggesting that their ring systems were higher-substituted as compared to those of dioncoquinones A (**5**) and B (**6**). The highly polar dioncoquinones C (**7**) and E (**9**) were found to have, both, molecular formulas of $C_{12}H_{10}O_6$, as deduced from the number of signals in the ^{13}C NMR spectrum and from HRESIMS, which is more than that of **6** by 30 units (corresponding to CH_2O). The substitution pattern of these naphthoquinones was established using 2D-NMR spectroscopy. The spectra of dioncoquinone C (**7**) showed HMBC correlations from the methyl protons at δ 1.98 (2-CH_3) and the only aromatic proton at δ 7.27 (H-8) to the carboxyl carbon at δ 184.2 (C-1), suggesting that this aromatic proton was located at C-8 (see Fig. 2). The NOESY correlation between H-8 and the methoxy group at δ 4.01 indicated that the latter was at C-7. In the naphthoquinone **9**, by contrast, no such NOESY correlation was observed between the methoxy group and the aromatic proton, while the methoxy protons at δ 3.91 (6-OCH_3) and H-8 at δ 7.09 displayed HMBC correlations to the carbon signal at δ 139.7 (C-6), indicating that the methoxy group was located at C-6 in **9**. In the structure of naphthoquinone **8**, an additional *O*-methyl group was found to be linked to the oxygen function at C-3 as compared to that of **7**. The fourth naphthoquinone isolated from the callus cultures was identified as the known 8-hydroxydroserone (**10**), previously found in Droseraceae and Nepenthaceae [14], but not yet in *T. peltatum*. Its structure has one more chelated hydroxy group as compared to compounds **7**–**9** (see Fig. 1).

From a biosynthetic point of view, the new naphthoquinones **7**–**9** and the known one, **10**, are apparently closely related to their less oxygenated analogs, plumbagin (**1**) and droserone (**2**, see Scheme 1).

3. SAR studies

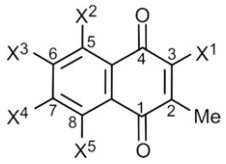
The promising results of the biological screening of dioncoquinones A (**5**) and B (**6**) [13], especially the high and selective activity of **6** against multiple myeloma (MM) cells, prompted us to

Table 2
EC_{50} values (μM) of INA-6 multiple myeloma cells and peripheral mononuclear blood cells (PBMCs) treated with compounds **1**, **2**, or **5**–**9**, or with melphalan.

	1	**2**	**5**	**6**	**7**	**8**	**9**	Melphalan
INA–6[a]	0.8	>100	29	11	14	80	100	2 [13]
PBMCs	0.8	NR	NR	NR	NR	NR	NR	3 [13]

NR: not reached.
[a] Multiple myeloma cells were treated with different concentrations of **1**, **2**, or **5**–**9**, or melphalan. The viable fractions of the treated cells were determined by annexin V-FITC/PI staining.

Table 3
EC_{50} values of INA-6 multiple myeloma cells and PBMCs treated with naphthoquinones.



Compd.	X^1	X^2	X^3	X^4	X^5	EC_{50} (μM) (INA-6)[a]	EC_{50} (μM) (PBMC)[b]
10	OH	OH	H	H	OH	>100	NR
17	H	OMe	OMe	H	H	15	13
18	OH	OMe	OMe	H	H	>100	NR
32	H	OMe	OMe	OMe	H	8	7.5
34	OH	OMe	OMe	OMe	H	>100	NR
35	OH	OH	OH	OH	H	7	70
38	H	H	OMe	OMe	H	6	6.5
39	OH	H	OH	OH	H	52	NR
43	OH	H	OH	H	H	>100	NR
44	H	H	OH	H	H	13	17
45	OH	H	OMe	H	H	>100	50
46	H	OH	OH	H	H	75	100
47	OH	OH	OMe	H	H	>100	NR
48	OMe	OH	OH	H	H	>100	NR
49	OH	H	OMe	OMe	H	>100	NR
50	H	OMe	H	H	H	4	16

NR: not reached.
[a] Activity against the multiple myeloma cell line INA-6.
[b] Cytotoxicity against normal peripheral mononuclear blood cells (PBMC).

test the biological activities of the newly synthesized naphthoquinones, of their synthetic intermediates, and of the isolated natural analogs. Dioncoquinone C (**7**) showed a similar activity against the MM cells as that of dioncoquinone B (**6**), but it was inactive against peripheral mononuclear blood cells (PBMCs). Some of the compounds induced strong apoptosis in MM cell lines in concentrations comparable to that of the melphalan, which is a well known DNA-alkylating agent [39] routinely used in a standard therapy against multiple myeloma [40]. Dioncoquinones D (**8**) and E (**9**) were less active than **6** and **7** (see Table 2).

To identify the pharmacophore of these molecules, several synthetic intermediates and isolated compounds were examined for their biological activity against multiple myeloma cells. The results are summarized in Table 3.

The first results of our biological tests enabled us to localize the preliminary structural elements of naphthoquinone derivatives that are responsible for their anti-MM activity and cytotoxicity. To examine the individual roles of the hydroxy functions at the C-3, C-5, and C-6 positions in dioncoquinone B (**6**), we synthesized or isolated derivatives that each contain only two hydroxy groups. The test results (see structure **2** in Table 2 and structures **43** and **46** in Table 3) show that removal of one OH group in any of these positions leads to an almost complete loss of bioactivity. The observation that substance **46** bearing two hydroxy groups at C-5 and C-6 exhibits lower activity and higher toxicity than **6**, combined with the activities of compounds **43** and **44**, jointly evidence that the lack of a hydroxy function at C-3 makes the compound more toxic against normal blood cells. This tendency is supported by the pairwise comparison of the properties of compounds **17** and **18**, of **32** and **34**, and of **38** and **49** with respect to the structural variation at C-3 (see Table 3). A possible reason for this is that the hydroxy group and other substituents at C-3 may block Michael addition reactions of possible nucleophiles (e.g., SH groups of proteins) in the cell to the α,β-unsaturated carbonyl fragments in naphthoquinones **17**, **32**, and **38**, thereby inhibiting a variety of cellular functions that direct the cells into apopotosis [41]. On the other hand, the possibility of a Michael addition reaction seems to be necessary for the anti-MM activities of these compounds, too. It is worth mentioning that shifting the OH groups along the aromatic ring has a significant impact on the biological activities of naphthoquinones. Hydroxy shifts from position 5, 6—6, 7 (structure **39**) or 5, 8 (structure **10**) both result in a decrease of the activities of the corresponding compounds. The introduction of an additional OH function in position 7 of **6** as in the naphthoquinone **35** does not have any significant effect on the anti-MM activity of this compound, while its toxicity is increased. No pronounced toxicity was observed, however, in the 7-*O*-methylated derivative of **35**, dioncoquinone C (**7**), while its bioactivity was still high (Table 2). Among the numerous derivatives tested, **7** was the most active candidate, with no measurable cytotoxicity against peripheral mononuclear blood cells (PBMCs). This activity is highly specific, and thus rewarding, because in our tests all other mixed OH/OMe- or completely OMe-substituted structures were entirely inactive (structures **18**, **34**, **45**, and **47**—**50**).

In summary, for a good balance of activity versus toxicity, it seems essential to have OH groups in positions 3, 5, and 6, while position 7 has little influence, which can be seen from the similar activities of dioncoquinone B (**6**), dioncoquinone C (**7**), and 7-hydroxydioncoquinone B (**35**) (Table 2 and Table 3). The role of an OH group in position 8 is not quite clear, but — at least in combination with the lack of oxygen functions at C-6 and C-7 — it seems to lower the activity as seen in structure **10**.

4. Conclusion

For a first synthetic access to the anti-tumoral product dioncoquinone B (**6**) in sufficient quantities for *in vivo* tests, three approaches are described in this paper. The third strategy, based on a directed *ortho* metalation (DOM) reaction, proved to be the best one as compared to the first two approaches, which required either too many steps or gave too low overall yields. Utilizing the third strategy, the likewise highly anti-tumoral metabolite dioncoquinone C (**7**) was synthesized, too. For the elaboration of the first preliminary SAR studies, a number of dioncoquinone B analogs were obtained by synthesis and by isolation from cell cultures of *T. peltatum*. Among them, only the new dioncoquinone C (**7**) strongly induced apoptosis in the multiple myeloma cell line INA-6 without any significant toxicity. Thus, the two natural products dioncoquinones B (**6**) and C (**7**) constitute two promising basic structures to develop anti-MM candidates. Remarkably both antitumoral compounds have three hydroxy functions at C-3, C-5, and C-6, which are required for their biological properties. Extended SAR studies to further optimize the structures and to explore the *in vivo* anti-MM activity of **6** and **7** and their analogs are currently in progress.

5. Experimental

5.1. Instrumentations and chemicals

Melting points were determined with a Kofler hot stage microscope (Reichert) and are uncorrected. IR spectra were measured with a Jasco FT/IR-410 spectrometer and are reported in wave numbers (cm^{-1}). NMR spectra were recorded either on a Bruker AC 250, a Bruker AV 400 or a Bruker DMX 600 spectrometers at ambient temperature. The chemical shifts are given in δ units (ppm) taking the signals of the deuterated solvents as internal reference for ^1H and ^{13}C NMR spectroscopy; the coupling constants *J* are given in Hertz (Hz). Mass spectra were recorded on a Finnigan MAT 8200 mass spectrometer at 70 eV for EI and on a Bruker Daltonics micrOTOF-focus for ESI. All reactions with air and/or moisture sensitive material were carried out in flame dried glasware using the Schlenk tube

technique under an inert nitrogen or argon atmosphere. Preparative HPLC was performed on a SymmetryPrep-C18 column (*Waters*; 19 × 300 mm); flow rate of 10 mL/min; mobile phase: (A) CH_3CN (0.05% trifluoroacetic acid), (B) H_2O (0.05% trifluoroacetic acid); room temperature; detection by a photodiode array.

5.2. Chemistry

5.2.1. The first route to dioncoquinone B (**6**)

*5.2.1.1. (E)-4-(2′-bromo-4′,5′-dimethoxyphenyl)-3-methoxycarbonyl-3-butenoic acid (**12**).* Dimethyl succinate (775 mg, 5.30 mmol) and 2-bromo-4,5-dimethoxybenzaldehyde [19] (**11**) (500 mg, 2.04 mmol) were added to a solution of potassium-*tert*-butoxide (916 mg, 8.16 mmol) in dry methanol (8 mL). The mixture was refluxed for 48 h, then poured into stirred aqueous 10% HCl solution at 0 °C, and extracted with EtOAc (3 × 80 mL). The extract was washed with water (100 mL) and extracted with 0.5 N aqueous NaOH (3 × 50 mL). The aqueous phase was acidified with half-concentrated HCl solution, and extracted with EtOAc (3 × 100 mL). The organic layer was washed with H_2O, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was recrystallized from EtOAc to afford **12** as a yellow solid (660 mg, 1.84 mmol, 90%). Mp 148–150 °C (EtOAc). IR (KBr, cm^{-1}) ν 2961, 2836, 2361, 2111, 1717, 1697, 1596, 1502, 1466, 1436, 1387, 1330, 1262, 1206, 1167, 1092, 1023, 920, 871, 814, 770, 745, 607. ^{1}H NMR ($CDCl_3$) δ 3.49 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 7.04 (s, 1H, C=CH), 7.09 (s, 1H, Ar–H), 7.93 (s, 1H, Ar–H), 10.72 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$) δ 34.5, 53.1, 56.5, 56.5, 113.1, 115.5, 115.8, 124.9, 126.8, 142.9, 148.7, 150.6, 168.9, 173.3. EI-MS (70 eV) *m/z* (%): 358 (3), 279 (100), 220 (11). MS (ESI) exact mass calcd for $C_{14}H_{16}BrO_6$: 359.01248 $[M + H]^+$; found: 359.01248 $[M + H]^+$.

*5.2.1.2. Methyl 4-acetoxy-8-bromo-5,6-dimethoxy-2-naphthoate (**13**).* The acid **12** (2.67 g, 7.43 g) and anhydrous sodium acetate (612 mg, 7.46 mmol) in acetic anhydride (300 mL) were refluxed under dry nitrogen for 2 h. After addition of H_2O (1300 mL), the reaction mixture was stirred for 2 h at room temperature until the acetic anhydride had been hydrolyzed. The crude product was neutralized with saturated aqueous $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was dried over Na_2SO_4, concentrated in vacuo, and chromatographed on silica gel to give product **13** (1.70 g, 4.44 mmol, 60%). Mp 110–113 °C (EtOAc). IR (KBr, cm^{-1}) ν 3097, 2956, 2849, 2117, 1767, 1715, 1593, 1467, 1439, 1335, 1281, 1255, 1227, 1195, 1100, 1046, 972, 821, 765, 729, 607. ^{1}H NMR (CD_3COCD_3) δ 2.38 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.09 (s, 3H, OCH_3), 7.67 (d, J = 1.6 Hz, 1H, Ar–H), 7.97 (s, 1H, Ar–H), 8.78 (d, J = 1.6 Hz, 1H, Ar–H). ^{13}C NMR (CD_3COCD_3) δ 20.8, 52.9, 57.5, 62.0, 119.9, 120.9, 121.8, 127.5, 128.8, 129.2, 143.3, 147.4, 147.4, 153.1, 166.4, 170.1. EI-MS (70 eV) *m/z* (%): 382 (21), 340 (100), 287 (28). Anal. calcd for $C_{16}H_{15}BrO_3$: C 50.15; H 3.95; found: C 49.70; H 3.71.

*5.2.1.3. Methyl 4-hydroxy-5,6-dimethoxy-2-naphthoate (**14**).* A solution of **13** (216 mg, 560 μmol) in methanol (20 mL) was stirred with palladized charcoal (5%, 10.0 mg) and K_2CO_3 (390.0 mg, 2.82 mmol) under a hydrogen atmosphere at room temperature. After the catalyst had been separated by filtration through Celite, the crude mixture was diluted with water, neutralized with half-concentrated HCl solution, and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried with Na_2SO_4, and the solvent evaporated to afford a crude product, which was purified by crystallization in CH_2Cl_2 to give **14** as a yellow solid (139.0 mg, 530 μmol, 95%). Mp 99–100 °C (CH_2Cl_2). IR (KBr, cm^{-1}) ν 3303, 2944, 1728, 1612, 1578, 1510, 1487, 1371, 1272, 1215, 1093, 1055, 996, 958, 887, 809, 780, 761, 644, 620, 606. ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.09 (s, 3H, OCH_3), 7.30 (d, J = 9.0 Hz, 1H, Ar–H), 7.40 (d, J = 1.6 Hz, 1H, Ar–H), 7.69 (d, J = 9.1 Hz, 1H, Ar–H), 8.04 (d, J = 1.4 Hz, 1H, Ar–H), 9.63 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ 52.4, 56.9, 63.3, 109.5, 115.6, 120.6, 122.4, 127.1, 127.2, 130.4, 142.9, 149.3, 153.7, 167.3. EI-MS (70 eV) *m/z* (%): 262 (100), 247 (48), 231 (9), 219 (15). Anal. calcd for $C_{14}H_{14}O_5$: C 64.12; H 5.38; found: C 63.76; H 4.97.

5.2.1.4. 4-Hydroxy-5,6-dimethoxy-2-hydroxymethylnaphthalene. The ester **14** (139 mg, 530 μmol) in dry Et_2O (3 mL) was added dropwise to a stirred suspension of lithium aluminum hydride (61.0 mg, 1.59 mmol) in 30 mL Et_2O under a nitrogen atmosphere at room temperature. The solution was stirred for 4 h, then water and half-concentrated aqueous HCl solution (20 mL) were added. The crude mixture was extracted with CH_2Cl_2 (3 × 20 mL), and the organic layer was dried over Na_2SO_4, concentrated in vacuo and the residue was purified by crystallization in CH_2Cl_2 to afford the 2-hydroxymethylnaphthalene as a yellow solid (119.0 mg, 510 μmol, 96%). Mp 80–83 °C (CH_2Cl_2). IR (KBr, cm^{-1}) ν 3234, 2937, 2931, 2362, 1643, 1613, 1578, 1514, 1489, 1367, 1148, 1044, 949, 833, 646. ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3H, OCH_3), 4.13 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 5.66 (s, 1H, OH), 6.82 (d, J = 1.4 Hz, 1H, Ar–H), 7.30 (s, 1H, Ar–H), 7.43 (d, J = 9.0 Hz, 1H, Ar–H), 7.64 (d, J = 9.0 Hz, 1H. Ar–H), 9.55 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ 57.1, 62.2, 65.5, 109.4, 115.7, 116.6, 117.8, 125.3, 131.6, 138.5, 143.2, 147.5, 153.9. EI-MS (70 eV) *m/z* (%): 234 (100), 219 (54), 191 (10). Anal. calcd for $C_{13}H_{14}O_4$: C 64.12; H 5.38; found: C 63.98; H 5.33.

*5.2.1.5. 5,6-Dimethoxy-2-methyl-4-naphthol (**15**).* 5,6-Dimethoxy-4-hydroxy-2-hydroxymethylnaphthalene (100.0 mg, 43.0 μmol) was stirred in a mixture of methanol, concentrated HCl (100 μL), and palladized charcoal (5%, 5.0 mg) for 40 min under a hydrogen atmosphere at room temperature. After the catalyst had been separated by filtration through Celite, the eluent was diluted with water and half-concentrated HCl solution, and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, and concentrated by evaporation. Crystallization of the residue from CH_2Cl_2 gave **15** as a pale-yellow solid (92.0 mg, 420 μmol, 98%). Mp 36–37 °C (EtOAc); Ref. [23], 35–37 °C (petroleum ether). Spectroscopic data (IR, ^{1}H NMR, MS) in agreement with those reported in the literature [23].

*5.2.1.6. 5,6-Dimethoxy-2-methyl-1,4-naphthoquinone (**17**).* A suspension of CuCl (25.0 mg, 250 μmol) and methylnaphthol **15** (70.0 mg, 320 μmol) in acetonitrile (12 mL) was stirred for 2 h with a strong current of air bubbling through it. The reaction mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give **17** as an orange-colored solid (49.2 mg, 210 μmol, 66%). Mp 182–185 °C (EtOAc); Ref. [23], 184–185 °C (petroleum ether). Spectroscopic data (IR, ^{1}H NMR, MS) in agreement with those reported in the literature [23].

*5.2.1.7. 5,6-Dimethoxy-2-methyl-1,2-naphthoquinone (**16**).* Furthermore, a red solid (6.0 mg, 25 μmol, 8%) was isolated from the above reaction. Mp 133 °C (EtOAc). IR (KBr, cm^{-1}) ν 3079, 2944, 2843, 1691, 1650, 1624, 1567, 1485, 1450, 1415, 1378, 1328, 1269, 1246, 1166, 1114, 1078, 1032, 991, 960, 906, 835, 813, 772, 753, 700, 641. ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.72 (s, 1H, H-3), 7.17 (d, J = 8.6 Hz, 1H, Ar–H), 7.93 (d, J = 8.6 Hz, 1H, Ar–H). ^{13}C NMR ($CDCl_3$) δ 15.4, 30.6, 56.2, 61.4, 117.1, 124.1, 125.6, 128.7, 133.6, 142.5, 153.9, 155.3, 178.9, 181.7. MS (ESI) exact mass calcd for $C_{13}H_{13}O_4$: 233.08084 $[M + H]^+$; found: 233.08080 $[M + H]^+$.

5.2.1.8. 5,6-Dimethoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide. Aqueous H_2O_2 solution (30%, 1.5 mL) was added with stirring to naphthoquinone **17** (35.0 mg, 150 μmol) in THF (4 mL) at ambient

temperature. 1 N aqueous Na_2CO_3 solution was added dropwise until the orange solution had turned colorless. Stirring was continued at ambient temperature for 1 h. The mixture was diluted with saturated aqueous NaCl solution and extracted with EtOAc (3 × 15 mL). The organic layer was dried with Na_2SO_4, and the solvent evaporated to dryness. The crude epoxide was crystallized from EtOAc to give a colorless solid (36.0 mg, 145 μmol, 97%). Mp 151–153 °C (EtOAc). IR (KBr, cm^{-1}) ν 2925, 2900, 1706, 1687, 1581, 1488, 1451, 1423, 1343, 1277, 1235, 1212, 1171, 1147, 1093, 1060, 1045, 1023, 984, 967, 869, 845, 809, 725, 674. ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 1H, CH_3), 3.81 (s, 1H, CH), 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.18 (d, J = 8.6 Hz, 1H, Ar–H), 7.78 (d, J = 8.6 Hz, 1H, Ar–H). ^{13}C NMR ($CDCl_3$) δ 14.9, 56.5, 61.9, 62.1, 62.2, 116.3, 124.9, 125.7, 126.7, 148.5, 158.9, 191.7, 191.8. MS (ESI) exact mass calcd for $C_{13}H_{12}O_5Na$: 271.05769 $[M + Na]^+$; found: 271.05769 $[M + Na]^+$. Anal. calcd for $C_{13}H_{12}O_5$: C 62.90; H 4.87; found: C 62.58; H 4.62.

*5.2.1.9. 3-Hydroxy-5,6-dimethoxy-2-methyl-1,4-naphthoquinone (anci-stroquinone C, **18**).* To a solution of the epoxide (20.0 mg, 81.0 μmol) in THF (20 mL) silica gel (116.0 mg) and concentrated H_2SO_4 (100 μL) were added. The solvent was evaporated in vaccum (200 mbar) at 70 °C for 20 min on a rotary evaporator. To the resultant red solid, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2. The organic layer was extracted with 5% aqueous K_2CO_3 solution until it had turned colorless. The water layer was acidified with half-concentrated aqueous HCl solution and extracted with CH_2Cl_2 until the color in the water layer was colorless again. Removal of the organic solvent gave the crude product **18** as a red solid, which was purified by crystallization from CH_2Cl_2 (19.0 mg, 760 μmol, 95%). Mp 218 °C (CH_2Cl_2); Ref. [13], 217 °C (H_2O/MeCN). All spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) in agreement with those of the isolated natural product [13].

*5.2.1.10. 3,5,6-Trihydroxy-2-methyl-1,4-naphthoquinone (dioncoquinone B, **6**).* A solution of BBr_3 in CH_2Cl_2 (1 M, 120 μmol, 0.12 mL) was added dropwise to a solution of naphthoquinone **18** (10.0 mg, 40.0 μmol) in dry CH_2Cl_2 (200 μL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, then diluted with water (4 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was extracted with 5% aqueous K_2CO_3 solution, and the water layer was acidified with half-concentrated aqueous HCl solution, extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over Na_2SO_4, concentrated, and subjected to crystallization in $CHCl_3$ affording **6** as a red solid (9.1 mg, 39 μmol, 98%). Mp 216–217 °C (CH_2Cl_2); Ref. [13], 218 °C. All spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) in agreement with those of the isolated natural product [13].

*5.2.2. The second route to naphthols **15**, **21**, and **22***

*5.2.2.1. 5,6-Dimethoxy-2-methyl-4-naphthol (**15**).* Diethylamine (180 mmol, 18.6 mL) was added slowly to a solution of senecioyl chloride (84.4 mmol, 9.4 mL) in 220 mL dry THF under a nitrogen atmosphere at 0 °C. The mixture was kept at room temperature for 1 h to give *N*,*N*-diethylsenecioamide (**19**), which was purified by distillation under reduced pressure. *N*-isopropylcyclohexylamide (LCI) was prepared by adding a 1.6 M solution of *n*-BuLi in *n*-hexane (67.6 mmol, 42.1 mL) to *N*-isopropylcyclohexylamine (67.6 mmol, 11.3 mL) at 0 °C with stirring and kept at room temperature for 1.5 h. The *n*-hexane was removed in vaccum and dry THF (50.0 mL) was added. To the freshly prepared solution of LCI in THF (50.0 mL), *N*,*N*-diethylsenecioamide (**19**) (3.00 g, 19.3 mmol) was added at −78 °C under nitrogen and the mixture was stirred for 1 h. The bromide **20** (38.6 mmol, 5.54 mL, X^1 = OMe, X^2 = H) was injected to the flask, which was then allowed to warm to room temperature and stirred for 18 h. Aqueous ammonium chloride solution and 10% aqueous HCl solution were added and the reaction mixture was stirred for another 30 min. THF was removed by evaporation and the residue was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on silica gel and crystallized to afford the naphthol **15**. as a colorless solid (1.33 g, 6.07 mmol, 31%). Mp (36–37 °C, EtOAc) and spectroscopic data in agreement with those described in Section 5.2.1.5 and as reported in the literature [23] (Mp 35–37 °C, petroleum ether).

*5.2.2.2. 5,6,7-Trimethoxy-2-methyl-4-naphthol (**21**).* Following the protocol described above (Section 5.2.2.1), *N*,*N*-diethylsenecioamide (**19**) (325 mg, 2.10 mmol) and bromide **20** (1.03 g, 4.18 mmol, X^1 = OMe, X^2 = OMe) were reacted affording **21** as a colorless solid (108 mg, 435 μmol, 21%). Mp 80 °C (petroleum ether/ethyl acetate); Ref. [42], 80–81 °C (ethanol). Since the spectroscopic data were not fully given in the literature [42], they are presented here: ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, OCH_3), 3.94 (s, 6H, OCH_3), 4.13 (s, 3H, OCH_3), 6.63 (s, 1H, Ar–H), 6.83 (s, 1H, Ar–H), 6.97 (s, 1H, Ar–H), 9.34 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ 21.7, 55.8, 61.2, 62.2, 102.9, 110.5, 110.9, 117.3, 132.8, 137.1, 139.0, 148.3, 152.9, 155.6. EI-MS (70 eV) *m/z* (%): 248 (100), 233 (42), 205 (19).

*5.2.2.3. 5-Methoxy-2-methyl-4-naphthol (**22**).* According to the procedure described in Section 5.2.2.1, *N*,*N*-diethylsenecioamide (**19**) (1.50 g, 9.66 mmol) and bromide **20** (2.77 mL, 21.9 mmol, X^1 = H, X^2 = H) were reacted to yield **22** as a colorless solid (684 mg, 3.38 mmol, 35%). Mp 89 °C (petroleum ether/ethyl acetate); Ref. [23], 89 °C (*n*-hexane). Spectroscopic data (IR, ^{1}H NMR, MS) in agreement with those reported in the literature [23].

*5.2.3. The third route to naphthols **15** and **21***

*5.2.3.1. N,N-diethyl-2,3-dimethoxybenzamide (**25**).* A mixture of thionyl chloride (1.40 g, 11.8 mmol, 837 μL) and benzoic acid **23** (429 mg, 2.36 mmol) in a 10-mL one-necked flask was refluxed at 80 °C overnight. The excess of thionyl chloride was evaporated under reduced pressure to give the acyl chloride. It was added carefully with stirring at 0 °C to diethylamine (517 mg, 7.1 mmol, 732 μL) in anhydrous THF (10 mL) in a 100-mL one-necked flasks. The reaction was continued at room temperature with stirring overnight. The mixture was diluted with water, acidified with 0.5 N aqueous HCl solution, and neutralized with saturated aqueous $NaHCO_3$ solution. The solvent was evaporated under reduced pressure and the residue was extracted with $CHCl_3$ (100 mL × 3), dried with anhydrous Na_2SO_4, concentrated in vacuo, and subjected to column chromatography to afford the product **25**. Yellow oil (553 mg, 2.33 mmol, 99%). Spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) in agreement with those reported in the literature [28].

*5.2.3.2. N,N-diethyl-2,3,4-trimethoxybenzamide (**26**).* In a similar way as described above, benzoic acid **24** (500 mg, 2.36 mmol) was converted to **26**. Yellow oil (504 mg, 1.89 mmol, 80%). In the literature [29], no spectroscopic data of **26** were reported, so they are presented here: IR (KBr, cm^{-1}) 2972, 2937, 1610, 1458, 1433, 1315, 1265, 1220, 1002, 837, 794, 758. ^{1}H NMR ($CDCl_3$): δ 1.01 (t, J = 7.0 Hz, 3H, CH_3), 1.21 (t, J = 7.0 Hz, 3H, CH_3), 3.15 (q, J = 7.0 Hz, 2H, CH_2), 3.20 and 3.40 (broad signal, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.64 (d, J = 8.4 Hz, 1H, Ar–H), 6.87 (d, J = 8.4 Hz, 1H, Ar–H). ^{13}C NMR ($CDCl_3$): δ 12.7 (CH_3), 13.9 (CH_3), 39.0 (CH_2), 43.1 (CH_2), 56.0 (CH_3O-4), 60.9 (CH_3O-2), 61.6 (CH_3O-3), 107.6 (C-5), 121.6 (C-6), 124.8 (C-1), 142.0 (C-2), 149.8 (C-3), 154.2 (C-4), 168.4 (CO). EI-MS (70 eV) *m/z* (%): 267 (19), 195 (100).

*5.2.3.3. N,N-diethyl-2,3-dimethoxy-6-(2-methylallyl)benzamide (**30**).* Preparation of $MgBr_2 \cdot 2Et_2O$ following a literature known [31] procedure (yet in German)

To 2.43 g magnesium (100 equiv) in dry diethylether (50 mL) in a three-neck flask under argon, 1,2-dibromoethane (0.5 mL) was added by syringe injection. After the reaction had started, the remaining 1,2-dibromoethane (7.9 mL, total 97.4 mmol) was added under reflux. After 30 min, the flask was cooled to room temperature. The lower, brown phase, containing the $MgBr_2 \cdot 2Et_2O$ solution (2.62 N) was freshly utilized for the transmetalation step.

To a stirred THF solution of *sec*-BuLi (34.0 mL, 47.7 mmol) and TMEDA (7.1 mL, 47.7 mmol) the benzamide **25** (10.3 g, 43.4 mmol) in THF (100 mL) was added at −90 °C under argon by syringe injection and stirred for 1.5 h. The solution of the lithiated species was then warmed to −78 °C over 30 min and freshly prepared $MgBr_2 \cdot 2Et_2O$ (33.0 mL, 86.8 mmol) was added. The mixture was stirred for 30 min, allowed to warm to room temperature, again cooled to −78 °C, and stirred for 1 h. The freshly distilled 3-bromo-2-methylpropene (**29**) (8.7 mL, 86.8 mmol) was added and the solution was allowed to warm to room temperature, and stirred overnight. The reaction mixture was treated with saturated aqueous NH_4Cl. Removal of THF by rotary evaporation, extraction with CH_2Cl_2, drying with Na_2SO_4, removal of the solvent, and purification by column chromatography gave **30** as a slightly yellow liquid. Pale-yellow oil (7.60 g, 26.0 mmol, 60%). IR (KBr, cm^{-1}) 2977, 2937, 1627, 1430, 1376, 1272, 1058, 890, 804. ^{1}H NMR ($CDCl_3$): δ 1.08 (t, J = 7.0 Hz, 3H, CH_3), 1.27 (t, J = 7.0 Hz, 3H, CH_3), 1.72 (s, 3H, CH_3), 3.18 (qd, J = 7.0, 1.6 Hz, 2H, CH_2), 3.25 (d, J = 6.4 Hz, 2H, CH_2), 3.61 (qd, J = 7.0, 6.4 Hz, 1H, CH_2), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.62 (s, 1H), 4.85 (s, 1H), 6.88 (s, J = 8.0 Hz, 1H, Ar−H), 6.92 (s, J = 8.0 Hz, 1H, Ar−H). ^{13}C NMR ($CDCl_3$): δ 12.5, 13.5, 22.1, 38.2, 40.0, 42.7, 55.5, 61.1, 112.0, 112.4, 125.1, 128.3, 132.4, 143.8, 144.5, 150.6, 167.2. EI-MS (70 eV) *m/z* (%): 291 (8), 219 (100), 218 (58). MS (ESI) exact mass calcd for $C_{17}H_{25}NO_3Na$: 314.17321 $[M + Na]^+$; found: 314.17350 $[M + Na]^+$.

*5.2.3.4. N,N-diethyl-2,3,4-trimethoxy-6-(2-methylallyl)benzamide (**31**).* In a similar way as described above, the benzamide **26** (2.49 g, 9.33 mmol) was converted to the 2-methylallyl product **31**. Pale-yellow oil (1.35 g, 4.2 mmol, 45%). IR (KBr, cm^{-1}) 2977, 2937, 1625, 1456, 1425, 1400, 1319, 1284, 1141, 1105, 1033, 890. ^{1}H NMR ($CDCl_3$): δ 1.03 (t, J = 7.0 Hz, 3H, CH_3), 1.22 (t, J = 7.0 Hz, 3H, CH_3), 1.68 (s, 3H, CH_3), 3.09 (qd, J = 7.0, 1.6 Hz, 2H, CH_2), 3.15 (s, 2H, CH_2), 3.54 (qd, J = 7.0, 6.4 Hz, 1H, CH_2), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.70 (s, 1H), 4.84 (s, 1H), 6.52 (s, 1H, Ar−H). ^{13}C NMR ($CDCl_3$): δ 12.8, 13.9, 22.5, 38.6, 41.0, 43.1, 56.2, 61.1, 61.8, 108.7, 113.1, 125.1, 131.7, 140.4, 144.0, 149.6, 153.5, 167.7. EI-MS (70 eV) *m/z* (%): 321 (10), 249 (100). MS (ESI) exact mass calcd for $C_{18}H_{27}NO_4Na$: 344.18378 $[M + Na]^+$; found: 344.18330 $[M + Na]^+$.

*5.2.3.5. 5,6-Dimethoxy-2-methyl-4-naphthol (**15**).* To the *ortho*-allyl benzamide **30** (2.41 g, 8.2 mmol) in 50 mL THF, 2.2 equiv of MeLi were added with stirring at −78 °C. The reaction mixture was slowly warmed up to 0 °C over 5 h, followed by addition of saturated aqueous NH_4Cl solution, evaporation of the solvent, and extraction with EtOAc. The organic layer was dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography to afford the naphthol **15** as a colorless solid (1.76 g, 8.1 mmol, 99%). Mp (36−37 °C, EtOAc) and spectroscopic data in agreement with those described in Section 5.2.1.5 and reported in the literature [23] (Mp 35−37 °C, petroleum ether).

*5.2.3.6. 5,6,7-Trimethoxy-2-methyl-4-naphthol (**21**).* In a similar way as described above (Section 5.2.3.5), the amide **31** (3.48 g, 10.7 mmol) was converted to **21** (2.16 g, 8.6 mmol, 80%). Mp 80 °C (petroleum ether/ethyl acetate); Ref. [42], 80−81 °C (ethanol). All spectroscopic data in agreement with those described in Section 5.2.2.2.

*5.2.4. The route to 3,5,6,7-tetrahydroxy-2-methyl-1,4-naphtho quinone (dioncoquinone C, **7**)*

*5.2.4.1. 5,6,7-Trimethoxy-2-methyl-1,4-naphthoquinone (**32**).* Following the protocol described in Section 5.2.1.6, 5,6,7-trimethoxy-2-methyl-4-naphthol (**21**) (760 mg, 3.10 mmol) was converted to the quinone **32**. Yellow powder (365.0 mg, 1.40 mmol, 45%). Mp 142 °C (petroleum ether/ethyl acetate); Ref. [42], 142−143 °C. Since the spectroscopic data were not fully given in the literature [42], they are presented in the following: IR (KBr, cm^{-1}) ν 2945, 2837, 1654, 1629, 1572, 1484, 1457, 1433, 1408, 1375, 1355, 1315, 1279, 1224, 1198, 1181, 1159, 1116, 1077, 1019, 1005, 987, 934, 911, 900, 865, 820, 811, 781, 722, 701, 650, 631. ^{1}H NMR ($CDCl_3$): δ 2.11 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.99 (s, 1H, OCH_3), 6.66 (s, 1H, Ar−H), 7.46 (s, 1H, Ar−H). ^{13}C NMR ($CDCl_3$) δ 15.8, 56.4, 61.3, 61.6, 106.3, 119.7, 129.5, 132.5, 137.6, 145.5, 147.9, 154.0, 157.2, 183.6, 185.1. EI-MS (70 eV) *m/z* (%): 262 (100), 247 (69).

*5.2.4.2. 5,6,7-Trimethoxy-2-methyl-1,2-naphthoquinone (**33**).* Furthermore, a red solid (384.0 mg, 1.46 mmol, 47%) was isolated from the above reaction. Mp 184 °C (petroleum ether/ethyl acetate). IR (KBr, cm^{-1}) ν 3092, 2945, 2920, 2847, 2359, 1661, 1648, 1632, 1575, 1491, 1455, 1406, 1368, 1329, 1267, 1200, 1147, 1084, 1030, 1000, 976, 964, 934, 919, 865, 809, 780, 738, 686, 668, 647. ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.72 (s, 1H, H-3), 7.17 (d, J = 8.6 Hz, 1H, Ar−H), 7.93 (d, J = 8.6 Hz, 1H, Ar−H). ^{13}C NMR ($CDCl_3$) δ 15.5, 56.4, 61.4, 61.5, 109.0, 118.1, 133.2, 135.6, 141.6, 143.7, 158.4, 159.1, 176.8, 181.8. MS (ESI) exact mass calcd for $C_{14}H_{14}O_5Na$: 285.07334 $[M + Na]^+$; found: 285.07330 $[M + Na]^+$.

5.2.4.3. 5,6,7-Trimethoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide. According to the protocol described in Section 5.2.1.8, the naphthoquinone **32** (68.1 mg, 260 μmol) was converted to the corresponding epoxide. Pale-yellow powder (72.0 mg, 257 μmol, 99%). Mp 110 °C (MeOH/H_2O). IR (KBr, cm^{-1}) ν 2979, 2946, 1681, 1654, 1631, 1571, 1485, 1461, 1409, 1346, 1315, 1279, 1193, 1161, 1116, 1099, 1072, 999, 987, 928, 902, 871, 820, 806, 785, 762, 731. ^{1}H NMR ($CDCl_3$): δ 1.70 (s, 3H, CH_3), 3.79 (s, 1H, H-3), 3.94 (s, 6H, OCH_3), 3.97 (s, 3H, OCH_3), 7.29 (s, 1 H, Ar−H). ^{13}C NMR ($CDCl_3$) δ 14.6, 56.4, 61.2, 61.6, 62.1, 106.3, 120.1, 129.0, 137.6, 148.2, 153.5, 157.7, 183.6, 185.1. MS (ESI) exact mass calcd for $C_{14}H_{14}NaO_6$: 301.06826 $[M + Na]^+$; found: 301.06821 $[M + Na]^+$.

*5.2.4.4. 3-Hydroxy-5,6,7-trimethoxy-2-methyl-1,4-naphthoquinone (**34**).* Following the protocol described in Section 5.2.1.9, 5,6,7-trimethoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide (53.9 mg, 194 μmol) was converted to the naphthoquinone **34**. Yellow powder (53.0 mg, 192 μmol, 99%). Mp 177 °C ($CHCl_3$). IR (KBr, cm^{-1}) ν 3329, 2948, 2841, 1655, 1641, 1573, 1486, 1460, 1412, 1382, 1346, 1258, 1210, 1181, 1144, 1105, 1080, 1021, 998, 965, 910, 869, 816, 787, 761, 746, 720, 704. ^{1}H NMR ($CDCl_3$): δ 2.05 (s, 3H, CH_3), 3.94 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 7.54 (s, 1H, OH), 7.66 (s, 1H, Ar−H). ^{13}C NMR ($CDCl_3$) δ 8.5, 56.5, 61.3, 61.6, 106.8, 116.3, 117.7, 130.7, 146.6, 153.7, 154.7, 158.5, 178.9, 184.3. MS (ESI) exact mass calcd for $C_{14}H_{14}NaO_6$: 301.06826 $[M + Na]^+$; found: 301.06821 $[M + Na]^+$.

*5.2.4.5. 3,5,6-Trihydroxy-7-methoxy-2-methyl-1,4-naphthoquinone (dioncoquinone C, **7**).* *O*-Demethylation to dioncoquinone C (**7**) and its two analogs **35** and **36** from the quinone **34** was achieved following the procedure described for the preparation of dioncoquinone B (**6**) from **18** in 5.2.1.9, with modifications of the reaction time, the temperature, and the equivalents of BBr_3 used (see Table 1). Under different reaction conditions, with increasing temperature from −78 °C to 0 °C within 2 h and 10 equiv of BBr_3, **34** was converted to its 5,6-*O*-didemethyl derivative, dioncoquinone C

(**7**), with a satisfactory yield. Red powder (14.0 mg, 56 μmol, 67%). Mp > 340 °C (ethyl acetate). IR (KBr, cm^{-1}) ν 3384, 3921, 1600, 1571, 1463, 1319, 1238, 1151, 1072, 997, 804, 740. ^{1}H NMR (CD_3COCD_3): δ 1.98 (s, 3H, 2-CH_3), 4.01 (s, 1H, 7-OCH_3), 7.27 (s, 1H, 8-H). ^{13}C NMR (CD_3COCD_3) δ 8.6 (CH_3-2), 56.8 (CH_3O-7), 104.8 (C-8), 109.8 (C-10), 121.0 (C-2), 125.3 (C-9), 139.4 (C-6), 150.5 (C-5), 153.6 (C-7), 154.8 (C-3), 184.2 (C-1), 185.1 (C-4). ^{1}H NMR (CD_3OD): δ 1.97 (s, 3H, 2-CH_3), 3.99 (s, 1H, 7-OCH_3), 7.29 (s, 1H, 8-H). ^{13}C NMR (CD_3OD) δ 8.6 (CH_3-2), 56.9 (CH_3O-7), 105.1 (C-8), 110.3 (C-10), 121.5 (C-2), 125.5 (C-9), 140.0 (C-6), 151.2 (C-5), 154.0 (C-7), 156.4 (C-3), 185.7 (C-4 or C-1), 185.8 (C-1 or C-4). EI-MS (70 eV) *m/z* (%): 250 (16), 249 (100). (ESI) exact mass calcd for $C_{12}H_9O_6$ $[M - H]^+$: 249.03990; found: 249.04040 $[M - H]^+$.

*5.2.4.6. 3,5,6,7-Tetrahydroxy-2-methyl-1,4-naphthoquinone (**35**).* Following the above protocol (5.2.4.5), conducting the reaction at room temperature, overnight, and using 10 equiv of BBr_3, **34** was quantitatively converted to its 5,6,7-*O*-tridemethylated derivative, **35**. Red powder (24.0 mg, 102 μmol, 98%). Mp 245–250 °C (ethyl acetate). IR (KBr, cm^{-1}) ν 3488, 3302, 2921, 2850, 2582, 2463, 2078, 1744, 1609, 1583, 1555, 1506, 1448, 1404, 1388, 1349, 1314, 1275, 1212, 1168, 1103, 1049, 967, 934, 883, 795, 745, 633. ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H, 2-CH_3), 7.12 (s, 1H, 8-H), 9.24 (s, 1H, OH), 11.4 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ 8.7 (CH_3-2), 108.8 (C), 109.7 (CH), 121.1 (C), 126.1 (C), 137.6 (C), 151.6 (C), 152.5 (C), 155.0 (C), 184.3 (CO), 184.7 (CO). MS (ESI) exact mass calcd for $C_{11}H_7O_6$: 235.02481 $[M - H]^+$; found: 235.02485 $[M - H]^+$.

*5.2.4.7. 3,6,7-Trihydroxy-5-methoxy-2-methyl-1,4-naphthoquinone (**36**).* In the above reaction (5.2.4.5) the 6,7-*O*-didemethylated derivative of **34**, compound **36**, was isolated as a side product, too. Red powder (4.0 mg, 56 μmol, 15%). Mp > 340 °C (ethyl acetate). IR (KBr, cm^{-1}) ν 3489, 3325, 1647, 1564, 1385, 1331, 1196, 1147, 1095, 1052, 802, 746. ^{1}H NMR (CD_3COCD_3): δ 1.93 (s, 3H, 2-CH_3), 3.86 (s, 1H, 5-OCH_3), 7.39 (s, 1H, 8-H). ^{13}C NMR (CD_3COCD_3) δ 8.5, 61.6, 111.3, 116.6, 117.7, 127.8, 143.8, 149.5, 152.0, 155.3, 179.4, 184.8. EI-MS (70 eV) *m/z* (%): 250 (100). (ESI) exact mass calcd for $C_{12}H_9O_6$ $[M - H]^+$: 249.03990; found: 249.04040 $[M - H]^+$.

*5.2.5. The route to 3,6,7-trihydroxy-2-methyl-1,4-naphthoquinone (**39**)*

*5.2.5.1. 6,7-Dimethoxy-2-methyl-1,4-naphthoquinone (**38**).* Following the protocol described in Section 5.2.1.6, 6,7-dimethoxy-2-methyl-4-naphthol [32] (29.0 mg, 130 μmol) was converted to the naphthoquinone **38**. Orange-colored powder (25.0 mg, 110 μmol, 83%). Mp 210–212 °C (EtOAc); Ref. [33], 212–213 °C (Ac_2O). In the literature [33], no spectroscopic data were reported. IR (KBr, cm^{-1}) ν 2924, 2850, 1649, 1622, 1577, 1512, 1454, 1370, 1342, 1308, 1209, 1134, 1078, 1015, 985, 919, 871, 791, 742, 692, 668. ^{1}H NMR ($CDCl_3$) δ 2.17 (d, J = 1.5 Hz, 3H, CH_3), 4.02 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.74 (q, J = 1.6 Hz, 1H, C=CH), 7.48 (s, 1H, Ar–H), 7.52 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$) δ 16.6, 56.7, 56.7, 107.8, 108.2, 127.2, 127.3, 135.4, 147.9, 153.5, 153.6, 184.8, 185.2. EI-MS (70 eV) *m/z* (%): 232 (100), 217 (8).

5.2.5.2. 6,7-Dimethoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide. According to the protocol described in Section 5.2.1.8, 6,7-dimethoxy-2-methyl-1,4-naphthoquinone (**38**) (100.0 mg, 430 μmol) was converted to the corresponding epoxide. Pale-yellow powder (93.0 mg, 357 μmol, 87%). Mp 145–148 °C (EtOAc). IR (KBr, cm^{-1}) ν 3057, 2946, 1678, 1573, 1514, 1459, 1444, 1328, 1290, 1254, 1227, 1148, 1063, 1016, 984, 904, 855, 795, 767, 737, 615. ^{1}H NMR ($CDCl_3$) δ 1.72 (s, 1H, CH_3), 3.80 (s, 2H, CH_3) 4.00 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.39 (s, 1H, Ar–H), 7.45 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$) δ 15.1, 56.7, 56.8, 61.1, 61.3, 108.2, 108.9, 126.8, 126.9, 154.3, 154.5, 191.0, 191.1. EI-MS (70 eV) *m/z* (%): 248 (100), 233 (17), 220 (12), 206 (24), 191 (16). Anal. calcd for $C_{13}H_{12}O_5$: C 62.90; H 4.87; found: C 61.91; H 5.12.

5.2.5.3. 3-Hydroxy-6,7-dimethoxy-2-methyl-1,4-naphthoquinone. According to the protocol described in Section 5.2.1.9, 6,7-dimethoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide (50.0 mg, 200 μmol) was converted to the corresponding naphthoquinone. Yellow powder (28.0 mg, 110 μmol, 56%). Mp 230 °C (*n*-hexane/ethyl acetate); Ref. [43], 233 °C (ethyl acetate). Spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) in agreement with those reported in the literature [43].

*5.2.5.4. 3,6,7-Trihydroxy-2-methyl-1,4-naphthoquinone (**39**).* According to the protocol described in Section 5.2.1.10, 3-hydroxy-6,7-dimethoxy-2-methyl-1,4-naphthoquinone (10.0 mg, 40 μmol) was converted to the *O*-demethylated product **39**. Yellow powder (4.0 mg, 17.1 μmol, 46%). Mp 190–193 °C. IR (KBr, cm^{-1}) ν 3756, 3630, 2360, 2341, 2160, 2043, 1646, 1576, 1325, 1187, 1154, 668. ^{1}H NMR (CD_3COCD_3) δ 1.96 (s, 3H, CH_3), 7.44 (s, 1H, Ar–H), 7.46 (s, 1H, Ar–H), 9.00 (s, 1H, OH), 9.09 (s, 1H, OH), 9.31 (s, 1H, OH). EI-MS (70 eV) *m/z* (%): 220 (100), 192 (50), 163 (23), 137 (49).

*5.2.6. The route to 3,6-dihydroxy-2-methyl-1,4-naphthoquinone (**43**)*

*5.2.6.1. 2-Methyl-6-[(trifluoromethanesulfonyl)oxyl]-naphthalene (**41**).* To a solution of 6-hydroxy-2-methylnaphthalene (**40**) (0.90 g, 5.4 mmol) in CH_2Cl_2 (20 mL), NaH (0.65 g of a 60% powder in oil, 16.2 mmol) was added under nitrogen and the solution was stirred for 30 min at 0 °C. A solution of Tf_2O (0.82 g, 5.8 mmol) in CH_2Cl_2 (15 mL) was added dropwise over a period of 15 min and after stirring for 5 min, the reaction mixture was directly filtered through a short pad of silica gel and then washed with CH_2Cl_2 giving a white solid after removal of the solvent. (1.50 g, 5.3 mmol, 98%). Mp 45 °C (petroleum ether/ethyl acetate). IR (KBr, cm^{-1}) ν 1600, 1425, 1402, 1210, 1132, 1105, 920, 884, 822, 798, 742. ^{1}H NMR ($CDCl_3$): δ 2.53 (s, 3H, CH_3-2), 7.32 (dd, J = 8.4, 2.5 Hz, 1H, Ar–H), 7.41 (dd, J = 8.4, 2.5 Hz, 1H, Ar–H), 7.66 (d, J = 2.5 Hz, 1H, Ar–H), 7.70 (d, J = 2.5 Hz, 1H, Ar–H), 7.76 (d, J = 8.4 Hz, 1H, Ar–H), 7.82 (d, J = 8.4 Hz, 1H, Ar–H). ^{13}C NMR ($CDCl_3$) δ 21.6, 119.0, 119.1 (q, J = 320.9 Hz, CF_3), 119.5, 127.0, 127.8, 130.0, 130.0, 131.6, 132.8, 137.3, 146.7. EI-MS (70 eV) *m/z* (%): 290 (68), 157 (100), 129 (77). MS (ESI) exact mass calcd for $C_{12}H_9F_3NaO_3S$ $[M + Na]^+$: 313.01167; found: 313.01168 $[M + Na]^+$.

*5.2.6.2. 2-Methyl-6-[(trifluoromethanesulfonyl)oxyl]-1,4-naphthoquinone (**42**).* H_5IO_6 (2.44 g, 10.7 mmol) and CrO_3 (17.8 mg, 178 μmol) were dissolved in acetonitrile (30 mL) with vigorous stirring at 0 °C. The ester **41** (517 mg, 1.78 mmol) in acetonitrile (5 mL) was added dropwise to the above solution. After stirring at 0 °C overnight, the reaction mixture was filtered over a short normal-phase silica gel column, and washed quickly with CH_2Cl_2. After evaporation of the solvent in vacuo, the desired product was purified by column chromatography (petroleum ether/ethyl acetate = 97: 3). Yellow solid (199 mg, 0.62 mmol, 35%). Mp 82 °C (petroleum ether/ethyl acetate). IR (KBr, cm^{-1}) ν 1661, 1595, 1583, 1424, 1352, 1295, 1201, 1132, 918, 866, 826, 744. ^{1}H NMR ($CDCl_3$): δ 2.22 (d, J = 1.6 Hz, 3H, CH_3-2), 6.93 (d, J = 1.6 Hz, 1H, H-3), 7.61 (dd, J = 8.4, 2.5 Hz, 1H, H-7), 7.94 (d, J = 2.5 Hz, 1H, H-5), 8.25 (d, J = 8.4 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 16.7, 117.3, 118.8 (q, J = 320.9 Hz, CF_3), 126.5, 129.7, 131.8, 134.6, 136.0, 149.0, 153.1, 183.0, 184.0. EI-MS (70 eV) *m/z* (%): 320 (100), 188 (35). MS (ESI) exact mass calcd for $C_{12}H_7F_3O_5S$ $[M + Na]^+$: 342.98585; found: 342.98585 $[M + Na]^+$.

5.2.6.3. 6-Hydroxy-2-methyl-1,4-naphthoquinone-2,3-epoxide. According to the protocol described in Section 5.2.1.8, the naphthoquinone **42** (53.0 mg, 165 μmol) was converted to the respective

2,3-epoxide. Colorless solid (31.0 mg, 152 μmol, 92%). Mp 181 °C (MeOH/H_2O). IR (KBr, cm^{-1}) ν 3417, 1694, 1671, 1592, 1577, 1321, 1257, 1233, 1218, 1089, 1012, 785, 743. ^1H NMR ($CDCl_3$): δ 1.65 (s, 3H, CH_3-2), 3.92 and 3.54 (s, 1H, H-3), 7.27 (dd, J = 8.4, 2.5 Hz, 1H, H-7), 7.32 (d, J = 2.5 Hz, 1H, H-5), 7.92 (d, J = 8.4 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 15.0, 62.1, 62.4, 112.8, 122.4, 125.4, 130.8, 135.4, 163.8, 191.2, 192.3. EI-MS (70 eV) *m/z* (%): 204 (100), 189 (99). MS (ESI) exact mass calcd for $C_{11}H_8NaO_4$ $[M + Na]^+$: 227.03149; found: 227. 03148 $[M + Na]^+$.

*5.2.6.4. 3,6-Dihydroxy-2-methyl-1,4-naphthoquinone (**43**).* According to the protocol described in Section 5.2.1.9, the epoxide prepared in 5.2.6.3. (4.3 mg, 12.7 μmol) was converted to the corresponding naphthoquinone, **43**. Yellow solid (4.2 mg, 12.5 μmol, 98%). Mp > 340 °C (petroleum ether/ethyl acetate). IR (KBr, cm^{-1}) ν 3280, 1666, 1651, 1631, 1573, 1375, 1303, 1258, 1062, 1014, 887, 795. ^1H NMR ($CDCl_3$): δ 1.99 (s, 3H, CH_3-2), 7.07 (dd, J = 8.4, 2.5 Hz, 1H, H-7), 7.37 (d, J = 2.5 Hz, 1H, H-5), 7.89 (d, J = 8.4 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 8.5 (2–CH_3), 112.9 (C-5), 121.0 (C-2), 121.6 (C-7), 126.1 (C-10), 129.7 (C-8), 133.6 (C-9), 156.4 (C-3), 163.5 (C-6), 182.3 (C-4), 186.6 (C-1). EI-MS (70 eV) *m/z* (%): 204 (100), 176 (27). MS (ESI) exact mass calcd for $C_{11}H_8NaO_4$ $[M + Na]^+$: 227.03149; found: 227. 03148 $[M + Na]^+$.

*5.2.6.5. 6-Hydroxy-2-methyl-1,4-naphthoquinone (**44**).* To a solution of the trifluoromethanesulfonic ester **42** (150 mg, 470 μmol) in dioxane (2 mL), 10% aqueous Et_4NOH solution (689 mg, 940 μmol, 673 μL) was carefully added at ambient temperature. The mixture was stirred for 30 min, diluted with CH_2Cl_2 (10 mL) and water (10 mL), and neutralized with 1 N aqueous HCl solution. The organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by column chromatography. Yellow solid (44.0 mg, 235 μmol, 50%). Mp 175 °C (petroleum ether/ethyl acetate). IR (KBr, cm^{-1}) ν 3361, 1662, 1650, 1594, 1573, 1457, 1350, 1329, 1267, 1235, 1203, 1134, 887, 848, 692. ^1H NMR (CD_3OCD_3): δ 2.13 (d, J = 1.6 Hz, 3H, CH_3-2), 6.82 (d, J = 1.6 Hz, 1H, H-3), 7.22 (dd, J = 8.4, 2.5 Hz, 1H, H-7), 7.39 (d, J = 2.5 Hz, 1H, H-5), 7.95 (d, J = 8.4 Hz, 1H, H-8). ^{13}C NMR (CD_3OCD_3) δ 16.4 (2-CH_3), 112.4 (C-5), 121.3 (C-7), 125.9 (C-10), 130.0 (C-8), 135.6 (C-9), 135.9 (C-3), 149.4 (C-2), 163.4 (C-6), 184.5 (C-1), 185.5 (C-4). EI-MS (70 eV) *m/z* (%): 188 (100), 160 (23). MS (ESI) exact mass calcd for $C_{11}H_7O_3$ $[M - H]^+$: 187.04006; found: 187.04707 $[M - H]^+$.

5.2.6.6. 6-Methoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide. K_2CO_3 (98.0 mg, 710 μmol) was added to the solution of 6-hydroxy-2-methyl-1,4-naphthoquinone-2,3-epoxide (29.0 mg, 142 μmol) in acetone (3 mL) at 0 °C. The mixture was magnetically stirred for 30 min, then dimethylsulfate (89.5 mg, 67.4 μL, 710 μmol) was injected into the flask *via* a syringe and stirred for 3 h. The reaction mixture was quenched by addition of water (20 mL), neutralized with 1 N aqueous HCl solution, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried with Na_2SO_4, concentrated to dryness, purified by column chromatography to afford the desired epoxide as a colorless solid (30.3 mg, 139.2 μmol, 98%). Mp 89 °C (MeOH/H_2O). IR (KBr, cm^{-1}) ν 1684, 1592, 1494, 1433, 1294, 1232, 1192, 1057, 1026, 948, 856, 780, 740. ^1H NMR ($CDCl_3$): δ 1.71 (s, 3H, CH_3-2), 3.83 (s, 1H, H-3), 3.91 (s, 3H, CH_3O-6), 7.21 (dd, J = 8.4, 2.5 Hz, 1H, H-7), 7.37 (d, J = 2.5 Hz, 1H, H-5), 7.97 (d, J = 8.4 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 15.0 (2-CH_3), 56.1 (CH_3O-6), 61.4 (C-2), 61.6 (C-3), 110.0 (C-5), 121.7 (C-7), 125.5 (C-10), 130.1 (C-8), 134.3 (C-9), 164.7 (C-6), 190.8 (C-1), 192.0 (C-4). EI-MS (70 eV) *m/z* (%): 218 (100), 203 (82), 119 (51). MS (ESI) exact mass calcd for $C_{12}H_{10}NaO_4$: 241.04713 $[M + Na]^+$; found: 241.04702 $[M + Na]^+$.

*5.2.6.7. 3-Hydroxy-6-methoxy-2-methyl-1,4-naphthoquinone (**45**).* According to the protocol described in Section 5.2.1.9, 6-methoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide (14.2 mg, 65.3 μmol) was converted to **45**. Yellow solid (13.6 mg, 62.7 μmol, 96%). Mp 162–163 °C (petroleum ether/ethyl acetate); Ref. [36], 174–175 °C (benzene). In the literature [36], no spectroscopic data were reported. IR (KBr, cm^{-1}) ν 3365, 1720, 1639, 1590, 1495, 1383, 1344, 1316, 1271, 1231, 1070, 1024, 876, 863, 745. ^1H NMR ($CDCl_3$): δ 2.08 (s, 3H, CH_3-2), 3.93 (s, 3H, CH_3O-6), 7.19 (dd, J = 8.4, 2.5 Hz, 1H, H-7), 7.51 (d, J = 2.5 Hz, 1H, H-5), 8.05 (d, J = 8.4 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 8.8 (2-CH_3), 56.1 (CH_3O-6), 110.1 (C-5), 120.6 (C-2), 120.9 (C-7), 126.5 (C-10), 129.2 (C-8), 131.4 (C-9), 153.1 (C-3), 163.6 (C-6), 181.6 (C-4), 184.7 (C-1). EI-MS (70 eV) *m/z* (%): 218 (100), 190 (26). MS (ESI) exact mass calcd for $C_{12}H_{10}NaO_4$: 241.04713 $[M + Na]^+$; found: 241.04718 $[M + Na]^+$.

5.2.7. Extraction and isolation

Lyophilized cell cultures [12] of *T. peltatum* (35.2 g) were ground and extracted with CH_2Cl_2/CH_3OH (1:1). The extract was condensed under reduced pressure to give 6.6 g of a crude residue, which was subjected to ion-exchange column to remove naphthyl isoquinoline alkaloids, followed by normal-phase chromatography affording three main naphthoquinones: droserone (**2**) (50.0 mg), dioncoquinone A (**5**) (110 mg), and dioncoquinone B (**6**) (44.0 mg), and further by preparative HPLC permitting the isolation of the four further naphthoquinones, 1.0 mg of dioncoquinone C (**7**) (retention time 10.9 min), 0.8 mg of dioncoquinone D (**8**) (retention time 18.9 min), 1.6 mg of dioncoquinone E (**9**) (retention time 11.2 min), and 3.0 mg of 8-hydroxydroserone (**10**) (retention time 11.0 min).

*5.2.7.1. 3,5,6-Trihydroxy-7-methoxy-2-methyl-1,4-naphthoquinone (dioncoquinone C, **7**).* Yellow solid. Mp > 340 °C (ethyl acetate). All spectroscopic data (IR, ^1H NMR, ^{13}C NMR, MS) identical with the synthetic material in 5.2.4.5.

*5.2.7.2. 5,6-Dihydroxy-3,7-dimethoxy-2-methyl-1,4-naphthoquinone (dioncoquinone D, **8**).* Yellow solid. Mp > 340 °C ^1H NMR ($CDCl_3$): δ 2.05 (s, 3H, 2-CH_3), 4.01 (s, 3H, 7-OCH_3), 4.05 (s, 3H, 3-OCH_3), 7.26 (s, 1H, 8-H), 11.82 (s, 1H, 5-OH). ^{13}C NMR ($CDCl_3$) δ 9.6 (CH_3-2), 58.0 (CH_3O-7), 61.3 (CH_3O-3), 103.6 (C-8), 110.1 (C-10), 124.4 (C-9), 133.3 (C-2), 138.2 (C-6), 149.2 (C-5), 151.3 (C-7), 157.1 (C-3), 184.4 (C-1), 185.6 (C-4). (ESI) exact mass calcd for $C_{13}H_{11}O_6$ $[M - H]^+$: 263.05557; found: 263.05610 $[M - H]^+$.

*5.2.7.3. 3,5,7-Trihydroxy-6-methoxy-2-methyl-1,4-naphthoquinone (dioncoquinone E, **9**).* Yellow solid. Mp > 340 °C ^1H NMR (CD_3OD): δ 1.96 (s, 3H, 2-CH_3), 3.91 (s, 3H, 6-OCH_3), 7.09 (s, 1H, 8-H). ^{13}C NMR (CD_3OD) δ 8.5 (CH_3-2), 61.1 (CH_3O-6), 109.1 (C-10), 110.2 (C-8), 121.2 (C-2), 130.2 (C-9), 139.7 (C-6), 157.0 (C-3), 157.3 (C-5), 158.6 (C-7), 184.9 (C-4), 185.8 (C-1). (ESI) exact mass calcd for $C_{12}H_9O_6$ $[M - H]^+$: 249.03990; found: 249.04040 $[M - H]^+$.

*5.2.7.4. 8-hydroxydroserone (**10**).* Red solid. Mp 193 °C ($CHCl_3$); Ref. [14], 193 °C ($CHCl_3$). All spectroscopic data (IR, ^1H NMR, ^{13}C NMR, MS) were identical with those in the literature [14].

5.3. Biological tests

The effects of dioncoquinones B and C and related naphthoquinones on cell survival were analyzed either in non-malignant cells (mononuclear cells derived from the peripheral blood of healthy donors) or in malignant cells of the human multiple myeloma cell line INA-6 [44]. Apoptotic and viable cell fractions were determined by staining with annexin V-FITC and propidium iodide (PI) according to the manufactures' instructions (Bender MedSystems, Vienna, Austria). In brief, cells were washed

in PBS, incubated for 10 min in 100 mL binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) containing 2.5 mL annexin V-FITC and 1 mg/mL PI, subsequently diluted with 300 mL binding buffer and analyzed by flow cytometry (FACSCalibur/CELLQuest; Becton Dickinson, Heidelberg, Germany). In early stages of the apoptotic process phosphatidylserine translocates from the internal to the external membrane, and can be detected by annexin V-FITC. Cells in a late apoptotic stage lose their membrane integrity and the DNA-binding agent PI can be incorporated. Thus, viable cells are negative for both, annexin V-FITC and PI.

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