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New cytotoxic diterpenylnaphthohydroquinone derivatives obtained from a natural diterpenoid

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Abstract—Diterpenylquinone/hydroquinone derivatives were prepared through Diels–Alder cycloaddition between natural myrcecommunic acid or its methyl ester and *p*-benzoquinone (*p*-BQ), using $BF_3{\cdot}Et_2O$ as catalyst or under microwave (Mw) irradiation. Acetyl, methyl and benzyl derivatives of several diterpenylnaphthohydroquinone were prepared from cycloadducts following two basic synthetic strategies, either protection before aromatisation or viceversa. Some of them were further functionalised at the B-ring of the decaline core. Most of the new compounds were evaluated and some of them resulted cytotoxic against several tumour cell lines with IC_{50} values under the μM level.

1. Introduction

Terpenylquinones constitute an interesting group of marine natural products,[1] for which a wide variety of biological activities have been described, including anti-inflammatory, antifungal, anti-HIV and most often antineoplastic activities. Their cytotoxicity has been justified on their ability for undergoing redox cycling and on the generation of reactive oxygen species, which would damage tumour cells.[2] Most of these meroterpenoids are characterised by possessing a benzoquinone (BQ) fragment attached to a terpenoid, which usually includes a decaline core, mostly without other functions, as in the case of avarol and avarone (Fig. 1), which present cytotoxic and antiviral activities.[3] A few reported examples present oxygenated functions at the decaline moiety, as it is the case of the cytotoxic drimanoid yahazunol[4] (Fig. 1). The 1,4-naphthoquinone system (1,4-NQ) is ubiquitously distributed in nature[5] and it is also present in numerous biologically active compounds, such as menadione (vitamin K_3) and drugs like the antiparasitic atovaquone (Fig. 1).[6] Both facts

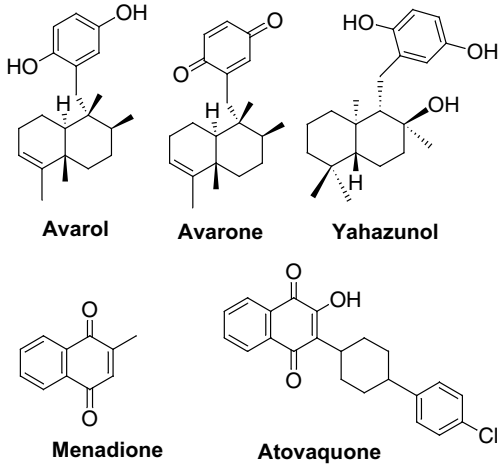

Figure 1. Structures of biologically active quinones.

prompted us, a few years ago, to study the influence of the terpenyl and quinone sizes and functionalities on the bioactivity of this type of meroterpenoids. Thus, several monoterpenylbenzo-,[7] naphtho-[8] and anthraquinones[8d,9] (AQ) with different functions in the side chain and in the quinone ring were prepared and their antineoplastic cytotoxicity assayed. Additionally, several diterpenyl-NQ and AQs were also synthesised.[10] In those studies it became clear that all the compounds

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prepared were more cytotoxic than the 1,4-NQ itself, being the NQ system more interesting than BQ and AQ, with cytotoxic potencies in the μM range. The most active compounds had the naphthalene ring either as 1,4-NQ or as diacetyl naphtho-1,4-hydroquinone (1,4-NHQ). Also, few of those diterpenyl-NQ derivatives had oxygenated the C-17 position of the terpenoid and showed a very interesting cytotoxicity and selectivity.[10]

These facts prompted us to continue our research and to prepare several new series of NHQ/NQ prodrugs, in order to extend the evaluation to in vivo assays. With this aim, we planned to block the redox-cycling ability of the hydroquinone hydroxyls through their protection with groups displaying different chemical and metabolic stability. The groups selected to protect the hydroquinone moiety were the acetyl, benzyl and methyl groups. Acetates are hydrolysable and removable, either in vitro or in vivo and, consequently, they could easily liberate the active hydroquinone. Indeed, saponification would occur progressively at neutral pH, along the 3 days needed for culminating the in vitro assays and also, according to common metabolic pathways,[11–14] they would be removed in vivo under the action of lipases and esterases. On the other hand, methyl ethers of NHQ are chemically stable under the in vitro evaluation conditions, and the methyl group could be removed as a formaldehyde unit, through in vivo metabolic cytochrome oxidation and acetal hydrolysis.[12] At an intermediate position, the benzyl group can be more easily oxidised and degraded than the methyl by chemical reagents, but the benzylic protons would be less accessible to the in vivo oxidative action of macromolecular enzymes than those of the methyl group.[13]

Complementarily to those hydroxyls' protections, several transformations of the B-ring in the decaline moiety were also planned in order to prepare yahazunol analogues, for ascertaining the influence of oxygen functions located around the position C-8 on the cytotoxicity. In addition, the Diels–Alder reaction conditions have been analysed, in order to optimise the yields of diterpenyl-NHQs, as well as those of their subsequent transformations.

2. Results and discussion

2.1. Chemistry

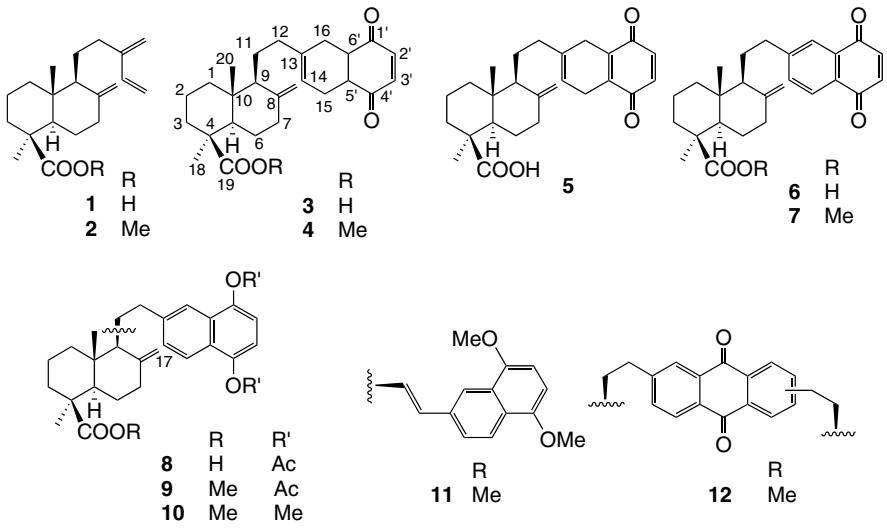
2.1.1. Diels–Alder cycloaddition. Diterpenylquinone/hydroquinone derivatives have been prepared through a Diels–Alder cycloaddition between myrcecommunic acid **1** or methyl myrcecommunate **2** and *p*-benzoquinone (*p*-BQ). The natural labdane acid used as starting material was isolated from berries of *Juniperus oxycedrus*.[15] It was transformed into its methyl ester by treatment with an ethereal solution of diazomethane. The condensation of **1** and **2** with *p*-BQ gave the cycloadducts, **3** and **4**, respectively. The presence and proportion of the original diketone cycloadducts in the crude reaction product had to be estimated by ^{1}H NMR, before purification, because if the cycloaddition product is subjected to chromatography on silica, it enolises to the corresponding hydroquinones, which can undergo further autoxidation to dihydronaphthoquinones.[10] In order to optimise the synthesis of cycloadducts **3** and **4** and looking for shortening the reaction time, two Diels–Alder procedures were considered, one in ethereal solution using $BF_3{\cdot}Et_2O$ as catalyst and other, under Mw irradiation in absence of solvent. Some variations were tested in both procedures, such as the *p*-BQ/diene ratio, the type of solid support and the irradiation power. The proportion of components in the crude reaction products was established by ^{1}H NMR analysis of the mixtures and the results found are summarised in Table 1.

Considering cycloadditions under Mw irradiation,[16] in all cases the reaction of **1** (or **2**) with *p*-BQ (entries 1–8 or 9, Table 1) led to the corresponding cycloadduct **3** (or **4**) as the main reaction product. However, when silica or alumina was used as solid supports, compound **3** was accompanied by its autoxidation products **5** and **6**. In some cases, when SiO_2 was used (entries 2 and 5 of Table 1), the quinone **5** was the major component. It should also be mentioned that with Al_2O_3 as support, the cycloaddition was not completed, remaining a part of the starting diene unreacted, even with doubled amount of *p*-BQ (entries 1, 3 and 4 of Table 1). On the other hand, the cycloadduct **3** (or **4**) was obtained as the unique product (entries 6–9 of Table 1) when using an excess of *p*-BQ ($\geqslant$1.5 equiv) and montmorillonite K-10 as solid support. With this support, only when the *p*-BQ/diene ratio was 1:1 (entry 7 of Table 1), some unreacted diene was detected in the cycloaddition crude. The good results obtained with K-10 should be due to the acidity of this support, comparable to that of mineral acids.[16]

Similarly, the reaction between **1** (or **2**) and *p*-BQ in ether, using $BF_3{\cdot}Et_2O$ as catalyst, led to the cycloadduct **3** (or **4**) as the unique detected product in the reaction crude in all of the cases (entries 10–13 of Table 1), making this conditions even better than those of the Mw/K-10 procedure. Although the Mw irradiation has the advantage of shortening the reaction time, this procedure needs an excess of the *p*-BQ that difficults the purification of the final products while the aromatised products also appear with variable proportions in the reaction crude.

Acetylated and methylated derivatives of hydroquinones **8–10** were prepared from cycloadducts **3** and **4**, following two basic synthetic strategies (see Table 1). In the first one, the acetylation or the methylation was accomplished prior to completion of the aromatisation (methods A and D). In the second, the oxidation to the NQ was followed by the reduction to the NHQ and the final acetylation or methylation (methods B and C). Method A applied to cycloadducts **3** and **4**, obtained through Mw irradiation (Table 1, entries 8 and 9), afforded the corresponding acetylated derivatives **8** and **9** in good yield, though they were accompanied by the corresponding autoxidation quinones NQ **6** and **7**, respectively.

Table 1. Quinones and acetylated and methylated hydroquinones obtained from myrcecommunic acid

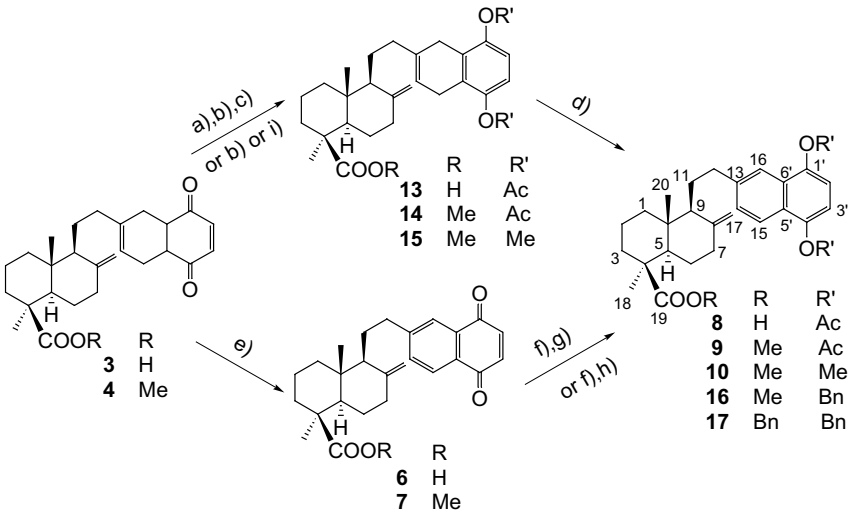


Entry	Diene	*p*-BQ/**1**(**2**) Ratio	Solid support	Conditions	Time	Products (ratio)	Method	Products (yields)
1	**1**	2:1	Al_2O_3	Mw[b]	8 min	**1/3/5** (1:2:1)	—	—
2	**1**	2:1	SiO_2	Mw[c]	8 min	**3/5** (1:2)	—	—
3[a]	**1**	2:1	Al_2O_3	Mw[c]	4 min	**1/3/5/6** (1:2:2:1)	—	—
4[a]	**1**	2:1	Al_2O_3	Mw[b]	4 min	**1/3/5** (2:2:3)	—	—
5[a]	**1**	2:1	SiO_2	Mw[c]	8 min	**3/5** (1:5)	—	—
6	**1**	2:1	K-10	Mw[c]	4 min	**3**	—	—
7	**1**	1:1	K-10	Mw[c]	4 min	**1/3** (1:2)	—	—
8	**1**	1.5:1	K-10	Mw[c]	8 min	**3**	A	**6** (9%), **8** (68%)
9	**2**	1.5:1	K-10	Mw[c]	8 min	**4**	A	**7** (10%), **9** (69%)
10	**1**	1:1		BF_3/Et_2O	24 h	**3**	B	**8** (60%)
11	**2**	1:1		BF_3/Et_2O	24 h	**4**	B	**9** (55%)
12	**2**	0.9:1		BF_3/Et_2O	24 h	**4**	C	**10** (58%), **12** (7%)
13	**2**	1:1		BF_3/Et_2O	24 h	**4**	D	**10** (25%), **11** (8%)

Method A: (i) Ac_2O, py, rt, 12 h; (ii) DDQ, benzene, rt, 0.5–1 h.
Method B: (i) MnO_2, benzene, reflux, 16 h. (ii) $Na_2S_2O_4$, dioxane, H_2O, rt, 2 h; (iii) Ac_2O, py, rt, 12 h.
Method C: (i) MnO_2, benzene, reflux, 16 h; (ii) $Na_2S_2O_4$, dioxane, H_2O, rt, 2 h; (iii) MeI, K_2CO_3, DMF, 110 °C, 1 h.
Method D: (i) Me_2SO_4, NaOH, EtOH, reflux, 1.5 h; (ii) DDQ, benzene, rt, 0.5–1 h.
[a] Suspended in Hex overnight before the extraction with EtOAc.
[b] Power, 750 W.
[c] Power, 500 W.

When method B, consisting of MnO_2 oxidation, followed by reduction with sodium ditionite and acetylation, was applied to the cycloadducts **3** and **4** (Table 1, entries 10 and 11), only the desired acetylated naphthohydroquinones **8** and **9** were, respectively, isolated, although the yield was somewhat lower than with method A. When the crude cycloadduct **4** was submitted to method C, MnO_2 oxidation, reduction and methylation (Table 1, entry 12), unexpectedly, the methylated derivative **10** was accompanied by a small amount of the symmetrical diterpenylanthraquinone **12**. The formation of this AQ should be consequence of a second Diels–Alder reaction between **4** and the residual unreacted diene–ester **2,** due to the relative starting excess of the latter (Table 1, entry 12, 0.9:1 molar ratio). As an alternative, method D, consisting in subjecting the crude reaction product to methylation before the oxidation, was applied to compound **4**; however, the yield was lower and the side-chain suffered dehydrogenation leading to compound **11,** due to an extended DDQ oxidation.

2.1.2. Optimisation of diterpenylnaphthohydroquinone synthesis. Methods A–D, leading directly to the final terpenylhydroquinone derivatives **8**–**10,** were conducted without purification of the intermediates; so we decided to isolate those intermediate diketones **3** and **4** and to optimise the remaining steps for the preparation of the acetylated, methylated and benzylated NHQ derivatives (Scheme 1). Thus, we followed the two strategies mentioned above, either protection before aromatisation or vice versa. The dihydronaphthalene diacetates **13** and **14** were obtained in good yields by direct acetylation of cycloadducts **3** and **4**, respectively (Scheme 1, entries 1 and 2). The direct methylation of cycloadduct **4** (Scheme 1, entry 4) afforded **15** in moderate yield only. Due to that, compound **3** was previously enolised by keeping it with silica gel and quickly treating with dimethyl sulfate. However, under these conditions the partial methylation of the carboxylic group at C-19 was observed. This prompted us to bring methylation to completion by treating the mixture with diazomethane,



Entry	Substrate	Reaction conditions	Product (Yield)	Further reaction conditions	Final products (Yields)
1	**3**	i)	**13** (78 %)	d)	**8** (91 %)
2	**4**	i)	**14** (77 %)	d)	**9** (46 %)
3	**3**	a), b), c)	**15** (54 %)	d)	**10** (51 %)
4	**4**	b)	**15** (39 %)		
5	**3**	e)	**6** (84 %)	f), h)	**16** (46 %), **17** (22 %)
6	**4**	e)	**7** (86 %)	f), g)	**10** (56 %)
				f), h)	**16** (67 %)

Scheme 1. Preparation of acetylated, methylated and benzylated hydroquinones from cycloadducts **3** and **4**. Reagents and conditions: (a) SiO_2, benzene, rt, 7 h; (b) Me_2SO_4, NaOH, EtOH, reflux, 1.5 h; (c) CH_2N_2, ether, 12 h; (d) DDQ, benzene, rt, 0.5–1 h; (e) MnO_2, benzene, reflux, 16 h; (f) $Na_2S_2O_4$, dioxane, H_2O, rt, 2 h; (g) MeI, K_2CO_3, DMF, 110 °C, 1 h; (h) NaH, DMF, 0 °C, 15 min then BnBr, rt, 1 h; (i) Ac_2O, py, rt, 12 h.

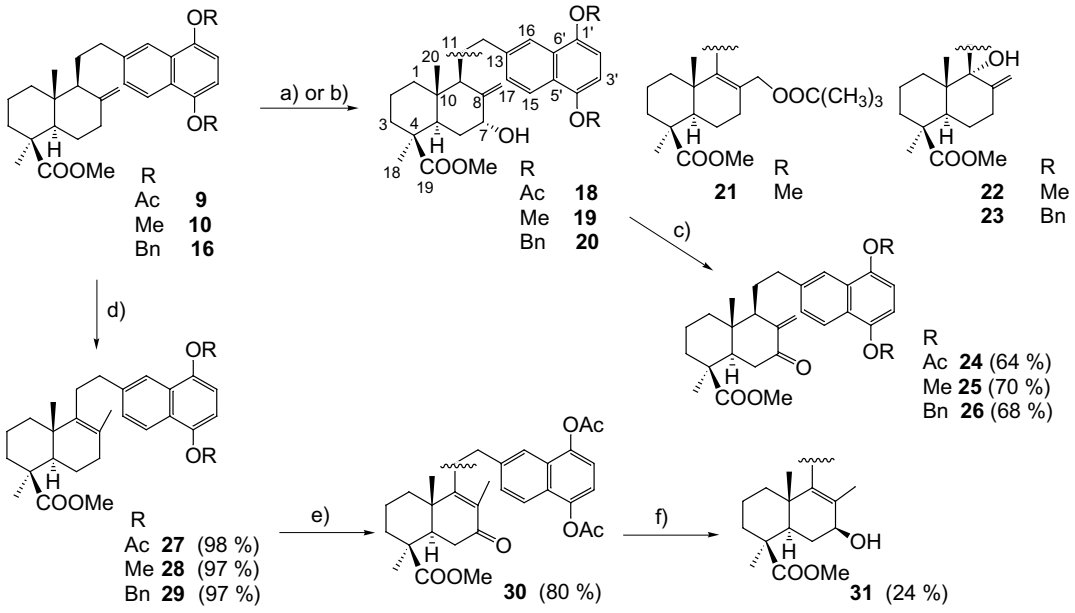
thus improving the yield in the trimethyl derivative **15** up to 54% (Scheme 1, entry 3). Further treatment of compounds **13**–**15** with DDQ afforded the NHQ derivatives **8**–**10** in moderate to good yields.

The alternative procedure started with the oxidation[9] of the cycloadducts **3** and **4** with MnO_2 to give NQs **6** and **7**, respectively, in good yields (Scheme 1, entries 5 and 6). Reduction of NQ **7** followed by methylation with MeI or benzylation with BnBr gave, respectively, **10** and **16** in acceptable yields (Scheme 1, entry 6). Attempts to obtain the dibenzylated hydroquinone from NQ **6**, in presence of the free carboxylic acid at the terpenic core, were not too successful because a mixture of methyl (**16**) and benzyl (**17**) esters was obtained (Scheme 1, entry 5). The formation of both esters was due to the partial benzylation of the carboxylic group, similarly to that occurring in the methylation of cycloadduct **3**, while methylation of the remaining free carboxylic acid occurred during hydride excess destruction with methanol, in the workup after benzylation.

2.1.3. Preparation of NHQs oxidised in the terpene fragment. As mentioned above, another objective of this research was focused on the introduction of oxygen functions at C-7 of the terpene core in order to compare the activity of the resulting compounds with that of yahazunol. We chosed diterpenylhydroquinones **9**, **10** and **16** as the starting materials for those transformations. They possess the exocyclic $\Delta^{8(17)}$ double bond that facilitates the introduction of the desired functionalities.

The first transformation performed was the allylic oxidation[17] of the diterpenyl-NHQ derivatives **9**, **10** and **16** with SeO_2 under different reaction conditions (Scheme 2). When the diacetate **9** was treated with SeO_2 excess (entry 1, Scheme 2), the 7α-hydroxyl derivative **18** was obtained in good yield. When the same conditions were applied to the dimethyl ether **10** (entry 2, conditions a) and to the dibenzyl ether **16** (entry 3), the corresponding alcohols **19** and **20** were obtained, respectively, but in these cases, the other possible allylic oxidation products, the tertiary 9α-hydroxyl derivatives **22** and **23**, were also obtained. Trying to improve the yields on the latter cases, new conditions were applied to compound **10** using SeO_2 and *t*-BuOOH (entry 2, conditions b), however, the yield of the oxidation was somewhat lower and compound **19** was accompanied by the allylic peroxide **21**.

The absolute configuration at C-7 in compounds **18**–**20** was deduced from the analysis of the pattern of the H-7 signal in their ^1H NMR spectra, that derived from small J_{vec} coupling constants of 2.6–3.1 Hz, characteristic for equatorial protons.[17c,18] This was in accordance with the mechanism of the SeO_2 oxidation,[19] that also served to deduce the C-9 configuration proposed for compounds **22** and **23**.



Entry	Substrate	Reaction conditions	Products (Yield)
1	**9**	a)	**18** (80 %)
2	**10**	a)	**19** (67 %), **22** (25 %)
		b)	**19** (62 %), **21** (18 %)
3	**16**	a)	**20** (60 %), **23** (24 %)

Scheme 2. Preparation of oxidised derivatives of diterpenylnaphthohydroquinones. Reagents and conditions: (a) SeO_2 (3.5 equiv), EtOH, 60 °C, 16 h; (b) *t*-BuOOH, SeO_2 (0.7 equiv), CH_2Cl_2, rt, 5 h; (c) DMSO, $(COCl)_2$, CH_2Cl_2, −55 °C, 30 min; then Et_3N, 0 °C, 30 min; (d) C_6H_6, aq 57% HI, 80 °C, 10 min; (e) Na_2CrO_4, AcONa, AcOH, Ac_2O, C_6H_6, 70 °C, 8 h; (f) $NaBH_4$, $CeCl_3$, MeOH/THF, 1:1, 0 °C, 45 min; then at rt, 3 h.

The allylic C-7 alcohols **18**–**20** were further oxidised to the corresponding ketones **24**–**26**. Attempts to oxidise the alcohol **19** with PCC and PDC were unsuccessful, leading to complex mixtures. However, on treating the allylic alcohols **18**–**20** with DMSO/oxalyl chloride at −55 °C in presence of Et_3N (Swern procedure), the corresponding α,β-unsaturated ketones **24**–**26** were obtained as the unique products, with yields between 64% and 70%.

In order to obtain other functionality configurations we planned the previous isomerisation of the $\Delta^{8(17)}$ double bond, to the more stable endocyclic tetrasubstituted position, that also would allow the oxidation at positions C-7 and C-17. So the treatment of **9**, **10** and **16** with aq 57% HI at 80 °C led to the corresponding Δ^8 derivatives **27**–**29**. Nevertheless, when the previous allylic oxidation conditions were applied to the Δ^8 olefins, no oxidation products were detected and the starting material was recovered unreacted. Consequently, the introduction of the hydroxyl group at C-7 was indirectly achieved through reduction of the corresponding carbonyl group, which was easily formed by direct oxidation with sodium dichromate. So, compound **27** treated with Na_2CrO_4 in benzene[20] gave the desired ketone **30**. Several attempts to reduce the ketone function by treating **30** with $NaBH_4$ in different solvents and temperatures were unsuccessful, and only when **30** was treated with $NaBH_4$ in the presence of $CeCl_3$[21] in MeOH/THF at 0 °C, the reaction led to the C-7 alcohol **31** in 24% yield. As it would be expected, and unlike compound **18**, **31** possessed an equatorial 7β-hydroxyl group, as it was confirmed by the coupling pattern of the corresponding axial H-7 signal (δ 4.09 ppm, dd, J_1 = 9.8 Hz, J_2 = 6.2 Hz) in its ^{1}H NMR spectrum.

2.2. Cytotoxicity

Most of the synthesised diterpenyl-NQ/NHQ derivatives were evaluated in vitro for determining their antineoplastic cytotoxicity[22] against the human cell lines: A-549 lung carcinoma, HT-29 colon carcinoma and MEL-28 malignant melanoma. The cytotoxicity results are shown in Table 2 as GI_{50} values expressed in μM.

From these results, it can be stated that the compounds assayed were cytotoxic and that the protection of the hydroquinone hydroxyls, while blocking their redox properties, displays an important influence on the activity. As it was expected for in vitro assays, the hydrolysable acetyl derivatives were the most potent, having GI_{50} values under the μM level, whereas the methylated and benzylated hydroquinones were much less or almost non-cytotoxic at the maximum concentrations tested. It could be noted as exceptions the μM cytotoxicity levels displayed by the methyl and benzyl ethers **25** and **26**, comparable to those of the diacetate **24**, but this fact would be justified on the basis of the intrinsic cytotoxicity of the electrophilic α,β-unsaturated carbonyl, present in the terpene core of these molecules.

Table 2. Antineoplastic cytotoxicity for terpenylnaphthohydroquinone derivatives

Compound	A-549[a]	HT-29[a]	MEL-28[a]
6	>7.4	5.7	5.7
7	0.20	0.20	0.20
8	0.24	0.24	0.24
9	0.12	0.50	0.12
10	>6.7	>6.7	>6.7
11	>22.3	>22.3	n.t.
12	>4.1	>4.1	>4.1
13	2.5	10.1	5.0
14	0.51	1.38	0.68
16	>5.0	>5.0	>5.0
17	6.5	4.4	n.t.
18	1.6	1.0	n.t.
19	>6.4	>6.4	>6.4
20	>4.9	>4.9	>4.9
21	>18.6	>18.6	>18.6
22	>21.5	>21.5	n.t.
23	12.4	12.4	9.0
24	2.3	4.4	n.t.
25	3.7	3.2	n.t.
26	14.8	8.1	n.t.
27	0.24	0.24	n.t.
28	>6.7	>6.7	>6.7
29	15.2	>16.6	n.t.
30	0.19	0.19	n.t.
31	4.8	4.8	n.t.

n.t., not tested.
[a] GI_{50} values expressed in μM.

Most of the SAR observations will mainly refer to structure variations in the decaline fragment of the diacetyl NHQ derivatives. It can be seen that transformation of the free carboxylic acid into the methyl ester enhances by more than one order the cytotoxicity of diterpenyl-NQ derivatives (**6** vs **7**), while this transformation seems to have not a significant effect in the case of the diacylated NHQs (**8** vs **9**). The introduction of the 7α-hydroxyl group slightly decreased the potency (**9** vs **18**). The same happened with the carbonyl group at that position (**9** vs **24**), but the decrease attained more than one order of magnitude for the 7β-hydroxyl group (**9** and **17** vs **31**). The double bond isomerisation from the exocyclic to the endocyclic position did not modify significantly the activity (**9** vs **27**), while a slight increase was induced by the presence of the 7-oxo function (**24** vs **30**).

The NQ **7** and the NHQs, **8**, **9**, **27** and **30** (Fig. 2) were the most cytotoxic compounds with small differences in potency. All of them displayed GI_{50} values under the μM level and the NHQ derivatives share in common the diacetate functionality. The practical equipotency of NQ **7** and NHQ diacetate **9** could be explained assuming that along the 3 days of the in vitro assay in the atmospheric ambient, the acetate groups of NHQ **9** should be hydrolytically removed and the resulting unprotected and less stable NHQ **8a** would be oxidised by air leading to NQ **7**. This could support the design hypothesis of this research, related to the prodrug nature of the protected NHQs, although for the methyl and benzyl ethers it remains to be proven through in vivo assays; for which, NHQ diacetate **8** and the dimethyl and dibenzyl ethers **16** and **29** have been selected.

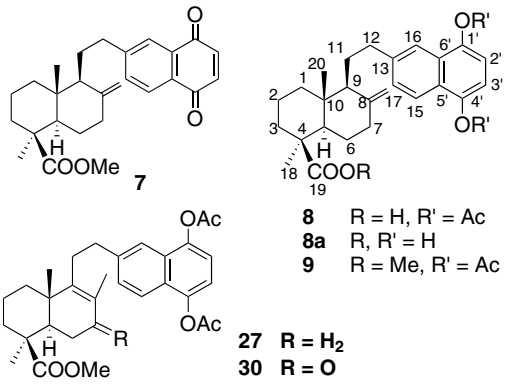


Figure 2. The most potent NQ and NHQ derivatives.

In summary, we have prepared cytotoxic terpenyl-NHQs protected as acetyl, methyl or benzyl derivatives from the cycloadducts **3** and **4**, obtained by Diels–Alder addition between natural diterpenoids and *p*-BQ. The final protected compounds were attained by two alternative procedures, involving hydroquinone protection and aromatisation or vice versa. In general, the best results were obtained through the second option, that is, via NQ reduction and final hydroquinone protection. Additionally, several oxidised derivatives at the decaline core were obtained through allylic oxidation procedures. The bioevaluation revealed the interesting antineoplastic cytotoxicity of the diacetyl NHQ derivatives, which, while being enough stable for most chemical synthetic work and ready to be hydrolysed by aqueous in vitro or enzymatic in vivo media, constitute a group of good candidates for further developments, in the search for more potent and selective antineoplastic agents. In addition to the SAR mentioned above, the results found and the global comparisons made contribute to support the redox-based mechanism of action proposed for quinone/hydroquinone antineoplastics.[2]

3. Experimental

3.1. Chemistry

Optical rotations were determined on a Perkin-Elmer 241 polarimeter in $CHCl_3$ and UV spectra on a Hitachi 100-60 spectrophotometer in $CHCl_3$ solution. IR spectra were obtained on a Niconet Impact 410 spectrophotometer in NaCl film. All NMR spectra were recorded on Bruker WP 200 SY (200 MHz for ^1H and 50.3 MHz for ^{13}C) or Bruker Avance 400 DRX (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometers in deuterochloroform using TMS as internal reference. Chemical shift values are expressed in ppm and coupling constants are reported in Hz. GSMS spectra were measured on a Hewlett–Packard 5890 series II gas chromatograph (5971 series mass selective detector) and electron impact (EIMS) were run in a VG-MICROMASS ZAB-2F. High-resolution mass spectra (HRMS) and the corresponding low-resolution spectra (EIMS and FABMS) were obtained on a VG TS-250 spectrometer by EI and fast atom bombardment (FAB) working at 70 eV and using nitrobenzyl alcohol as the matrix with xenon

Table 3. ^{1}H NMR ($CDCl_3$-TMS, δ ppm, J Hz) data for compounds **3**–**31**

H	**3**	**4**	**8**	**9**	**10**	**11**	
5	1.33 m	—	—	—	1.25 m	1.37 m	
9	1.56 m	—	—	—	1.69 m	2.50 m	
11	1.51 m	—	—	—	2.02 m; 1.69 m	6.45 dd (15.6, 9.7)	
12	—	—	2.95 m; 2.61 m	2.97 m; 2.63 m	2.97 m; 2.60 m	6.59 d (15.6)	
14	5.36 m	5.35 m	7.38 dd (8.8, 1.5)	7.39 dd (8.8, 1.6)	7.34 dd (8.6, 1.5)	7.59 dd (8.8, 1.8)	
15	2.14 m[a]	—	7.78 d (8.8)	7.78 d (8.8)	8.11 d (8.6)	8.12 d (8.8)	
16	2.40 m[a]; 2.08 m[a]	—	7.55 s	7.56 d (1.6)	7.95 d (1.5)	8.07 d (1.8)	
17	4.83 br s; 4.48 br s	4.82 br s; 4.46 br s	4.97 br s; 4.68 br s	4.97 br s; 4.68 br s	4.97 br s; 4.72 br s	4.80 br s; 4.62 br s	
18	1.23 s	1.17 s	1.20 s	1.16 s	1.16 s	1.23 s	
20	0.58 s	0.48 s	0.61 s	0.51 s	0.51 s	0.74 s	
2′	6.66 s	6.65 s	7.22 d (8.0)	7.21 d (8.4)	6.69 d (8.4)	6.69 d (8.2)	
3′			7.17 (8.0)	7.16 d (8.4)	6.63 d (8.4)	6.64 d (8.2)	
5′, 6′	3.24–3.16 m	3.24–3.12 m	—	—	—	—	
OAc	—	—	2.45 s; 2.46 s	2.45 s; 2.46 s	—	—	
OMe	—	—	—	—	3.96 s; 3.95 s	3.96 s; 3.95 s	
COOMe	—	3.60 s	—	3.60 s	3.61 s	3.65 s	
	12	**13**	**14**	**15**	**16**	**17**	
5	—	—	—	—	1.28 m	—	
9	—	—	—	—	1.72 m	—	
11	—			—	2.02 m; 1.72 m	—	
12	2.96 m			—	2.97 m; 2.64 m	2.99 m; 2.62 m	
14	7.56 d (8.0)	5.54 m	5.51 m	5.56 m	7.53–7.20 m	7.55–7.35 m	
15	8.21 d (8.0)	3.25–3.01 m	3.24–2.95 m	3.32–3.12 m	8.22 d (8.6)	8.24 d (8.8)	
16	8.07 d (1.1)	3.25–3.01 m	3.24–2.95 m	3.32–3.12 m	8.05 br s	8.06 br s	
17	4.96 br s; 4.66 br s	4.88 br s; 4.54 br s	4.86 br s; 4.52 br s	4.87 br s; 4.58 br s	4.96 br s; 4.72 br s	4.96 br s; 4.72 br s	
18	1.16 s	1.23 s	1.17 s	1.18 s	1.16 s	1.20 s	
20	0.51 s	0.62 s	0.51 s	0.51 s	0.52 s	0.50 s	
2′	—	6.93 s	6.92 s	6.63 s	6.74 d (8.3)	6.75 d (8.4)	
3′	—				6.69 d (8.3)	6.69 d (8.4)	
COOMe	3.60 s	—	3.61 s	3.61 s	3.61 s	—	
OAc	—	2.31 s; 2.32 s	2.30 s; 2.32 s	—	—	—	
OMe	—	—	—	3.79 s; 3.77 s	—	—	
OBn	—	—	—	—	7.53–7.20 m (10H), 5.21 s (2H), 5.19 s (2H)	7.55–7.35 m(15H), 5.21 s (2H), 5.20 s (2H), 5.16 d (12.8, 1H), 4.99 d (12.8, 1H)	
	18	**19**	**20**	**21**	**22**	**23**	**24**
7	4.42 t (2.9)	4.44 t (3.1)	4.38 t (2.6)	—	—	—	—
12	2.89 m	2.92 m	2.94 m	2.85 m	2.87 m	—	—
14	7.38 dd (8.4, 1.5)	7.33dd (8.3, 1.3)	(7.51–7.38) m	7.38 dd (8.6, 1.6)	7.37 dd (8.6, 1.6)	(7.50–7.37) m	7.37 dd (8.8, 1.7)
15	7.79 d (8.4)	8.12 d (8.3)	8.24 d (8.8)	8.12 d (8.6)	8.12 d (8.6)	8.23 d (8.8)	7.80 d (8.8)
16	7.52 br s	7.92 d (1.3)	8.04 d (1.1)	7.99 br s	7.99 br s	8.10 br s	7.53 br s
17	5.16 br s; 4.78 br s	5.18 br s; 4.82 br s	5.16 br s; 4.83 br s	4.64 d (10.0); 4.47 d (10.0)	5.09 br s; 4.89 br s	5.09 br s; 4.88 br s	5.86 br s; 5.23 br s
18	1.15 s	1.16 s	1.16 s	1.23 s	1.22 s	1.22 s	1.15 s
20	0.49 s	0.49 s	0.50 s	0.84 s	0.64 s	0.63 s	0.66 s
2′	7.21 d (8.0)	6.68 d (8.3)	6.74 d (8.4)	6.68 d (8.4)	6.69 d (8.2)	6.74 d (8.4)	7.22 d (8.3)
3′	7.16 d (8.0)	6.63 d (8.3)	6.68 d (8.4)	6.64 d (8.4)	6.63 d (8.2)	6.69 d (8.4)	7.17 d (8.3)
OAc	2.45 s; 2.47 s	—	—	—	—	—	2.47 s; 2.45 s
OMe	—	3.96 s; 3.95 s	—	3.96 s; 3.95 s	3.96 s; 3.95 s	—	—

OBn	—	—	(7.51–7.38) m (10H); 5.21 s (2H); 5.19 s (2H)	—	—	(7.50-7.37) m (10H); 5.21 s (2H); 5.20 s (2H)	—		
COOMe	3.61 s	3.61 s	3.61 s	3.64 s	3.62 s	3.62 s	3.64 s		
$C(CH_3)_3$	—	—	—	1.27 s	—	—	—		
	25	**26**	**27**	**28**	**29**	**30**	**31**		
7	—	—	—	—	—	—	4.09 dd (9.8, 6.2)		
12	—	—	2.79 m	2.75 m	2.81 m	2.88 m	2.82 t (8.8)		
14	7.32 dd (8.8, 1.8)	(7.53–7.35) m	7.42 dd (8.6, 1.6)	7.38 dd (8.8, 1.8)	(7.54–7.34) m	7.43 dd (8.6, 1.6)	7.42 dd (8.4; 1.5)		
15	8.12 d (8.8)	8.23 d (8.4)	7.78 d (8.6)	8.13 d (8.8)	8.24 d (8.4)	7.82 d (8.6)	7.79 d (8.4)		
16	7.94 d (1.8)	8.04 d (1.1)	7.60 d (1.6)	7.99 d (1.8)	8.09 br s	7.62 d (0.8)	7.59 sa		
17	5.88 br s; 5.28 br s	5.87 br s; 5.28 br s	1.70 s	1.74 s	1.74 s	1.91 s	1.82 s		
18	1.15 s	1.16 s	1.21 s	1.23 s	1.23 s	1.23 s	1.23 s		
20	0.66 s	0.67 s	0.80 s	0.81 s	0.81 s	0.96 s	0.86 s		
2′	6.69 d (8.4)	6.74 d (8.4)	7.21 d (8.2)	3.69 d (8.4)	6.74 d (8.4)	7.24 d (8.2)	7.22 d (8.0)		
3′	6.64 d (8.4)	6.69 d (8.4)	7.17 d (8.2)	3.63 d (8.4)	6.68 d (8.4)	7.20 d (8.2)	7.17 d (8.0)		
OAc	—	—	2.45 s; 2.47 s	—	—	2.45 s; 2.47 s	2.45 s; 2.47 s		
OMe	3.96 s; 3.95 s	—	—	3.97 s; 3.95 s	—	—	—		
OBn	—	(7.53–7.35) m (10H); 5.21 s(2H); 5.19 s (2H)	—	—	(7.54–7.34) m (10H); 5.22 s (2H); 5.19 s (2H)	—	—		
COOMe	3.64 s	3.65 s	3.64 s	3.64 s	3.64 s	3.68 s	3.65 s		

[a] Interchangeable assignments.

as the fast atom. Column chromatography (CC) was performed on silica gel (Merck No. 9385). TLC were carried aut on silica gel 60 F_{254} (Merck, 0.25 mm thick) and were detected by UV and by spraying with 10% (v/v) sulfuric acid in ethanol and subsequent heating. HPLC analyses were carried out on a Waters Delta 600 high-pressure liquid chromatograph with a Waters 2996 spectrophotometric detector (200–600 nm) and equipped with a Xterra® Prep MS C_{18} column, 150×10 mm, flow rate 5 mL/min. Elemental analyses were obtained with a Perkin-Elmer 2400 CHN. Solvents and reagents were purified by standard procedures as necessary.

3.1.1. Starting materials. Myrcecommunic acid **1** was isolated from berries of *J. oxycedrus* and transformed into methyl myrcecommunate **2** by a reported procedure.[10]

3.1.2. Cycloadducts 3 and 4: General procedures for the Diels–Alder cycloadditions. *In the presence of $BF_3{\cdot}Et_2O$.* To a solution of *p*-BQ (178 g, 1.65 mmol) in dry ether, **1** (499 mg, 1.65 mmol) or **2** (522 mg, 1.65 mmol) and $BF_3{\cdot}Et_2O$ cat. were added. The mixture was stirred at room temperature under argon atmosphere for 24 h, then, it was diluted with ether, washed with brine, dried over anhydrous Na_2SO_4 and the solvent evaporated off to yield cycloadduct **3** or **4**.

Compound **3**. Six hundred and thirty-seven milligrams (94%). IR cm^{-1} (film): 3500–2500, 1680, 1470, 1380, 1180, 1030, 910, 850, 730. ^1H NMR (Table 3). ^{13}C NMR (Table 4). HRMS (FAB, M+1): calcd $C_{26}H_{34}O_4$ 411.2535; found 411.2495.

Compound **4**. Six hundred and eighty-six milligrams (98%). IR cm^{-1} (film): 1722, 1689, 1656, 1448, 1153, 1030, 887, 730. ^1H NMR (Table 3). ^{13}C NMR (Table 4). HRMS (FAB, M+1): calcd $C_{27}H_{37}O_4$ 425.2692; found 425.2703.

Under microwave irradiation. Diterpenoids **1** or **2** and *p*-BQ were dissolved in dichloromethane and the corresponding solid support (five times the reactant weights) was added and well mixed. Then, the organic solvent was evaporated and dried material was subjected to microwave irradiation for a specified time (2 min intervals, see Table 1). The solid mixture was treated according to the description in Table 1, and the reaction product was extracted from the support with EtOAc. After evaporation of the organic solvent the samples were analysed by NMR to determine the product ratios (Table 1).

Spectroscopic data of **5** and **6** were in agreement with those previously described by us.[10]

3.1.3. Diterpenylnaphthohydroquinone diacetate (8). *Method A*. The cycloaddition product under microwave irradiation between **1** (293 mg, 0.97 mmol) and *p*-BQ (157 mg, 1.45 mmol) was treated with acetic anhydride and pyridine. The mixture was kept at room temperature and darkness for 12 h. After this period, the reaction mixture was quenched with ice, diluted in water

Table 4. ^{13}C NMR ($CDCl_3$–TMS, δ ppm) data for compounds **3**–**31**

C	3	4	8	9	10	11	12	13	14	15	16	17	18
1	39.0	39.1	38.8	39.2	39.0	40.9	39.1	39.1	39.2	39.1	39.0	39.0	38.7
2	19.8	19.8	19.8	20.0	19.9	19.7	19.8	20.0	20.1	19.9	19.9	19.9	19.9
3	37.9	38.1	37.7	38.2	38.1	38.4	38.1	37.9	38.3	38.2	38.1	38.1	37.9
4	44.1	44.2	44.1	44.4	44.3	44.3	44.2	44.3	44.4	44.3	44.3	44.4	43.9
5	56.2	56.1	55.9	56.4	56.2	55.8	56.2	56.3	56.3	56.3	56.2	56.3	48.6
6	26.0	26.1	26.0	26.4	26.3	25.1	26.2	26.2	26.4	26.3	26.3	26.3	32.4
7	38.6	38.6	38.6	38.6	38.8	37.3	38.7	38.8	38.6	38.8	38.7	38.7	73.9
8	147.8	147.8	147.8	148.2	148.0	149.5	147.6	148.1	148.2	148.1	148.0	147.9	149.1
9	55.1	55.0	54.8	55.3	55.3	60.9	55.3	55.3	55.3	55.6	55.2	55.1	48.4
10	40.4	40.1	40.3	40.4	40.2	39.8	40.2	40.5	40.3	40.2	40.2	40.3	40.2
11	21.6	21.5	25.2	25.3	25.9	128.7	25.4	21.5	21.5	21.8	25.7	25.7	24.7
12	36.2	36.0	34.6	34.7	35.0	133.0	34.8	35.7	35.7	36.2	34.8	34.8	34.4
13	135.7	136.4	141.9	141.9	140.8	135.1	150.3	134.3	134.4	134.8	140.8	140.9	141.6
14	118.0	117.9	128.4	128.5	127.2	123.6	133.5	116.7	116.7	117.4	127.9	128.1[a]	128.3
15	24.6[a]	24.5[a]	121.6	121.7	121.8	122.0	127.4	27.7	27.7	27.5	122.0	122.0	121.8
16	27.3[a]	27.3[a]	119.8	120.0	120.2	119.4	126.7	25.2	25.2	25.3	120.4	120.5	120.0
17	106.4	106.3	106.6	106.5	106.6	108.2	106.7	106.5	106.4	106.5	106.5	106.7	109.4
18	28.9	28.7	28.8	28.8	28.8	28.7	28.7	29.0	28.8	28.8	28.7	28.9	28.5
19	183.9	177.8	183.7	177.6	177.8	177.7	177.5	184.0	177.8	177.8	177.7	177.0	177.8
20	12.7	12.5	12.7	12.7	12.6	13.5	12.5	12.9	12.7	12.6	12.6	12.8	11.6
1′	200.0[a]	200.1[a]	143.9	144.1	149.2	149.5[a]	183.9	146.2	146.2	151.0	148.5	148.5	143.9
2′	139.2	139.2	116.6	116.7	102.3	103.1	—	119.9	119.9	106.8	104.0	104.0	116.7
3′	139.2	139.2	117.6	117.6	103.2	103.6	—	119.9	119.9	106.8	105.2	105.2	117.7
4′	200.2[a]	200.4[a]	144.2	144.5	149.6	149.7[a]	183.7	146.2	146.2	151.0	148.9	148.9	144.4
5′	46.2	46.2	126.1	126.3	124.7	125.3	131.5	128.6	128.6	124.6	126.8	125.1	126.2
6′	46.8	46.7	127.7	128.0	126.5	126.5	134.1	128.9	128.7	124.9	127.2	126.8	127.7
COO*Me*	—	51.1	—	51.0	51.1	51.1	51.0	—	51.1	51.1	51.0	—	51.2
OAc	—	—	169.4 20.9	169.1 20.9	—	—	—	169.3 20.8	169.2 20.8	—	—	—	169.5 169.4 21.0
OMe	—	—	—	—	55.7	55.7	—	—	—	55.6	—	—	—
OBn	—	—	—	—	—	—	—	—	—	—	70.4, 70.5, 137.5, 137.6, 127.9[a], 128.5(5C)[a], 127.4(4C)[a]	70.5,70.4, 65.9,137.6, 137.5,136.1, 128.1(5C)[a], 128.0(2C)[a], 127.9(2C)[a], 127.8(2C)[a], 127.3(4C)[a]	—

	19	20	21	22	23	24	25	26	27	28	29	30	31
1	38.7	38.7	36.8	31.6	36.4	40.2	40.1	40.2	37.7	37.7	37.7	36.2	37.5
2	19.9	19.9	19.5[a]	19.7	20.0	19.7	19.7	19.7	19.6	19.6	19.6	19.1	19.3
3	38.0	38.0	37.6	37.8	39.1	38.7	38.7	38.7	37.0	37.0	37.0	37.3	37.3
4	43.9	43.9	43.9	44.4	44.3	44.1	44.2	44.2	43.9	43.9	43.9	43.6	43.4
5	48.8	48.6	53.1	48.3	48.3	53.2	53.8	53.8	53.5	53.5	53.5	50.8	50.5
6	32.3	32.3	30.7	25.8	26.4	37.7	37.8	37.8	20.8	20.8	20.8	36.7	31.4
7	74.0	74.0	30.1	32.3	38.2	203.9	203.7	203.9	37.3	37.3	37.3	199.6	72.9
8	149.1	149.2	126.9	148.5	148.1	148.1	148.1	148.1	127.5	127.3	128.5[a]	130.9	141.6
9	48.4	48.4	145.6	79.6	79.3	52.6	52.7	52.7	138.6	138.9	138.9	164.8	130.5
10	40.2	40.3	40.1	43.9	43.3	38.7	38.7	38.7	39.7	39.7	39.6	41.4	40.5
11	25.2	25.1	20.5[a]	33.3	38.8	25.8	26.5	26.4	30.4	30.8	30.8	31.8	30.4
12	34.5	34.4	38.4	30.7	34.9	34.4	34.9	34.8	34.3	34.4	34.3	35.2	36.3
13	140.4	140.4	140.2	140.5	140.9	140.8	139.6	139.8	142.0	140.7	140.9	140.4	142.3
14	127.0	128.5[a]	126.9	127.0	127.8[a]	128.1	126.9	127.8[a]	128.3	126.9	127.7[a]	127.8	128.2
15	121.9	122.2	121.9	122.0	122.1	121.9	122.0	122.3	121.7	121.9	122.2	122.1	121.8
16	120.2	120.5	119.8	120.1	120.5	120.2	120.4	120.5	119.4	119.7	120.0	119.7	119.5
17	109.7	109.7	75.8	110.4	106.7	117.1	117.4	117.4	19.8	19.9	19.9	11.6	17.7
18	28.5	28.5	28.4	29.0	28.9	27.9	20.8	28.0	28.4	28.4	28.4	27.7	28.2
19	177.8	177.8	178.0	178.1	177.8	176.9	176.8	176.9	178.0	178.1	178.1	177.0	177.7
20	11.6	11.7	17.9	14.6	14.7	12.4	12.4	12.4	17.8	17.7	17.7	15.8	14.7
1′	149.1	148.4	149.1	149.1	148.5	144.0	149.2	148.4	143.9	149.1	148.9	143.9	143.9
2′	102.3	104.0	102.4	102.5	104.0	116.9	102.6	104.2	116.7	102.4	104.1	117.1	117.8
3′	103.3	105.2	103.2	103.3	105.2	117.8	103.5	105.3	117.7	103.2	105.3	117.9	116.8
4′	149.6	148.9	149.5	149.6	148.9	144.2	149.6	148.8	144.3	149.6	148.5	144.3	144.3
5′	124.7	125.1	124.8	124.8	125.1	126.2	125.0	125.2	126.1	124.7	125.1	126.3	126.2
6′	126.3	126.7	126.4	126.4	126.9	1247.7	126.5	126.8	127.8	126.5	127.1[a]	127.7	127.8
COO*Me*	51.1	51.2	51.1	51.1	51.2	51.4	51.3	51.5	51.2	51.0	51.0	51.4	51.2
OAc	—	—	—	—	—	169.4 21.0	—	—	169.4 21.0	—	—	169.2 20.9 21.0	169.4 20.9
OMe	55.6	—	55.7; 55.6	55.6; 55.7	—	—	55.7	—	—	55.6	—	—	—
OBn	—	70.4; 70.5; 137.5(2C); 127.8[a], 127.3[a](8C)	—	—	70.4(2C); 137.7(2C); 128.6[a]; 127.4[a](8C)	—	—	70.6; 70.4; 137.5(2C); 128[a]; 127.3[a](8C)	—	—	70.5; 70.3; 137.6; 137.5; 127.2[a], 127.1[a](8C)	—	—
C(CH_3)$_3$	—	—	26.5	—	—	—	—	—	—	—	—	—	—
C(CH_3)$_3$	—	—	80.1	—	—	—	—	—	—	—	—	—	—

[a] Interchangeable assignments.

and extracted with EtOAc. Then the organic layer was successively washed with 2 N HCl, aq satd $NaHCO_3$ and brine and evaporated off to afford a residue that was dissolved in benzene and DDQ (330 mg, 1.45 mmol) was added. The mixture was stirred at room temperature for 0.5–1 h. Then it was filtered off, the organic solvent was evaporated and the reaction product was purified by CC, using mixtures of Hex/CH_2Cl_2, 2:8 as eluent, to yield **6**[10] (37 mg, 9%) and **8** (325 mg, 68%).

Compound **8**. $[\alpha]_D^{22}$ +37.4° (589), +38.2° (578), +40.2° (546), (*c* 0.84, $CHCl_3$). UV λ_{max} (ε): 226(11800), 257(2200). IR cm^{-1} (film): 3500–2500, 1766, 1693, 1460, 1366, 1053, 893. ^1H NMR (Table 3). ^{13}C NMR (Table 4). Anal. ($C_{30}H_{36}O_6$) C, H. HRMS (FAB, M+1): calcd $C_{30}H_{37}O_6$, 493.2590; found, 493.2576.

Method B. The cycloaddition product between **1** (293 mg, 0.97 mmol) and *p*-BQ (105 mg, 0.97 mmol) in presence of $BF_3{\cdot}Et_2O$ was treated with MnO_2 (379 mg, 4.3 mmol) under refluxed benzene (20 mL) for 16 h. Then the mixture was filtered through Celite, the solid washed with CH_2Cl_2, and the organic solvent evaporated off. The crude product was dissolved in dioxane (13 mL) and a solution of $Na_2S_2O_4$ (3.5 g) in H_2O (15 mL) was added. The mixture was kept stirring at room temperature under argon atmosphere for 2 h, then it was diluted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and the organic solvent evaporated off to yield a reduction product that was acetylated with acetic anhydride and pyridine and purified by CC using mixtures Hex/EtOAc, 8:2 as eluent, yielding **8** (287 mg, 60%).

From **13***: General procedure for aromatisation with DDQ*. To a solution of **13** (194 mg, 0.39 mmol) in benzene (10 mL) was added DDQ (134 mg, 0.59 mmol). The mixture was kept at room temperature for 0.5–1 h. Then it was filtered, the organic solvent was evaporated and the reaction product was purified by CC, using mixtures of Hex/EtOAc, 8:2 as eluent, to yield **8** (175 mg, 91%).

3.1.4. Diterpenylnaphthohydroquinone diacetate (9). *Method A*. The above method A was applied to the cycloaddition product obtained from microwave irradiation between **2** (330 mg, 1.04 mmol) and *p*-BQ (168 mg, 1.56 mmol). CC of the crude product using mixtures of Hex/CH_2Cl_2, 1:9 as eluent afforded **7**[10] (44 mg, 10%) and **9** (363 mg, 69%).

Compound **9**. $[\alpha]_D^{22}$ +42.3° (589), +43.0° (578), +44.1° (546), +50.3° (436), (*c* 0,89, $CHCl_3$). UV λ_{max} (ε): 224 (1200), 254 (1800). IR cm^{-1} (film): 1760, 1720, 1460, 1370, 1210, 1050, 920, 890. ^1H NMR (Table 3). ^{13}C NMR (Table 4). HRMS (FAB, M+1): calcd $C_{31}H_{38}O_6$ 507.2747; found, 507.2701. Anal. ($C_{31}H_{37}O_6$) C, H.

Method B. Using the method B, compound **9** was obtained in 55% overall yield from the cycloadduct **4**.

From **14**. Treatment of **14** (472 mg, 0.93 mmol) with DDQ (315 mg, 1.39 mmol) as described above following the purification by CC using mixtures of CH_2Cl_2/Hex, 9:1 as eluent yielded **9** (218 mg, 46%).

3.1.5. Methylated diterpenylnaphthohydroquinone (10). *Method C*. The cycloaddition product between **2** (511 mg, 1.61 mmol) and *p*-BQ (157 mg, 1.45 mmol) in presence of $BF_3{\cdot}Et_2O$ was oxidised with MnO_2 (665 mg, 7.65 mmol) and reduced with $Na_2S_2O_4$ (5.6 g), as described above in method B, to give a reduction product that was redissolved in DMF (15 mL) and MeI (545 μL, 8.75 mmol) and K_2CO_3 (966 mg, 7.9 mmol) were added. The mixture was stirred at 110 °C for 1 h. Then the reaction was allowed to cool down to room temperature and quenched by addition of a 2-N solution of HCl while being vigorously stirred. The resulting solution was extracted with EtOAc and the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave a reaction product, which was purified by CC using mixtures of Hex/EtOAc, 95:5 as eluent, affording **10** (425 mg, 58%) and **12** (81 mg, 7%).

Compound **10**. $[\alpha]_D^{22}$ +30.5° (589), +30.1° (578), +35.4° (546), (*c* 0.94, $CHCl_3$). UV λ_{max} (ε): 250 (9000), 319 (1800), 333 (1700). IR cm^{-1} (film): 2845, 1723, 1603, 1463, 1363, 1271, 1152, 1097, 888. ^1H NMR (Table 3). ^{13}C NMR (Table 4). EIMS *m*/*z*: 450 (M^+). HRMS (EI): calcd. $C_{29}H_{38}O_4$ 450.2770; found, 450.2824.

Compound **12**. $[\alpha]_D^{22}$ +54.6° (589), (*c* 0.78, $CHCl_3$). UV λ_{max} (ε): 267 (53800). IR cm^{-1} (film): 1724, 1673, 1446, 1227, 1153, 931, 887. ^1H NMR (Table 3). ^{13}C NMR (Table 4). HRMS (FAB, M+1): calcd. $C_{48}H_{61}O_6$ 733.4468; found 733.4387.

Method D. The cycloaddition product between **2** (1.33 g, 4.20 mmol) and *p*-BQ (454 mg, 4.20 mmol) in presence of $BF_3{\cdot}Et_2O$ was dissolved in EtOH (45 mL) and a 10-N solution of NaOH (38 mL) was added dropwise while the mixture was heated under reflux. After 10 min, Me_2SO_4 (11.5 mL, 12.1 mmol) was added dropwise and the stirring and heating was continued over 0.5 h. Then the reaction was allowed to reach rt and was quenched by the addition of a 2-N solution of HCl while being vigorously stirred. The resulting solution was extracted with EtOAc and the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave 1.35 g of reaction product. This residue was dissolved in benzene (50 mL) and treated with DDQ (1.43 g, 6.30 mmol) following the general procedure described above for **8**. CC of the reaction product, using mixtures of Hex/EtOAc, 95:5 as eluent, afforded **11** (156 mg, 8%) and **10** (468 mg, 25%).

Compound **11**. UV λ_{max} (ε): 240 (12,000), 268 (19,200). IR cm^{-1} (film): 2849, 1724, 1600, 1463, 1465, 1272, 1154, 1096. ^1H NMR (Table 3). ^{13}C NMR (Table 4). HRMS (FAB; M+1): calcd $C_{29}H_{37}O_4$ 449.2692, found, 449.2692.

From **15**. Treatment of **15** (150 mg, 0.33 mmol) with DDQ (56 mg, 0.50 mmol) following the general procedure described above for **8** afforded a reaction product that was purified by CC, using mixtures of Hex/EtOAc, 95:5 as eluent, to yield **10** (76 mg, 51%).

From **7**. Compound **7** (823 mg, 1.96 mmol) in dioxane (31 mL) was reduced with $Na_2S_2O_4$ (6.8 g) in H_2O

(35 mL) and the reduction product was treated with MeI (590 μL, 9.45 mmol) and K_2CO_3 (1.03 g, 7.48 mmol) in DMF as described before in method C giving a residue, which was purified by CC using mixtures of Hex/EtOAc, 95:5 as eluent, to afford **10** (491 mg, 56%).

3.1.6. Diterpenylnaphthoquinone (6): General Procedure for oxidation with MnO_2. Compound **3** (850 mg, 2.07 mmol) was treated with MnO_2 (868 mg, 10.0 mmol) under refluxed benzene (40 mL) for 16 h. Then the mixture was filtered through Celite, the solid washed with CH_2Cl_2, and the organic solvent evaporated off. The residue was purified by CC using mixtures of Hex/EtOAc, 9:1 as eluent, to give **6**[10] (711 mg, 84%).

3.1.7. Diterpenylnaphthoquinone (7). Oxidation of **4** (850 mg, 2.0 mmol) with MnO_2 (868 mg, 10.0 mmol) as the general procedure described above followed by the purification by CC using mixtures of Hex/EtOAc, 95:5 as eluent, yielded **7**[10] (757 mg, 86%).

3.1.8. Dihydronaphthohydroquinone diacetate (13). Compound **3** (263 mg, 0.64 mmol) was acetylated with acetic anhydride and pyridine following the above general procedure for acetylation and purification by CC using mixtures of Hex/EtOAc, 8:2 as eluent afforded **13** (254 mg, 78%). $[\alpha]_D^{22}$ +40.7° (589), +42.5° (578), +48.5° (546), +79.1° (436), (*c* 1.01, $CHCl_3$). UV λ_{max} (ε): 260 (7200). IR cm^{-1} (film): 3500–2550, 1780, 1690, 1650, 1200, 1180, 1050, 900. ^1H NMR (Table 3). ^{13}C NMR (Table 4). Anal. ($C_{30}H_{38}O_6$) C, H. HRMS (FAB, M+1) calcd 495.2746; found 495.2673.

3.1.9. Dihydronaphthohydroquinone diacetate (14). A solution of **4** (495 mg, 1.16 mmol) in pyridine was treated with acetic anhydride following the first stage of method A described above for **8**. The obtained residue was purified by CC using mixtures of Hex/EtOAc, 9:1 as eluent, yielding **14** (472 mg, 77%). $[\alpha]_D^{22}$ +34.9° (589), +36.7° (578), +41.7° (546), +71.3° (436), (*c* 1.0, $CHCl_3$). UV λ_{max} (ε): 265 (6400). IR cm^{-1} (film): 1775, 1725, 1650, 1370, 1200, 1180, 1030, 900. ^1H NMR (Table 3). ^{13}C NMR (Table 4). GCMS (220–290 °C, 5 °C/min, HP-1 column), >99%, t_R = 23.19 min, (*m*/*z*) 508 (M^+). Anal. ($C_{31}H_{40}O_6$) C, H. HMRS (FAB, M+1) calcd 509.2903; found 509.2887.

3.1.10. Methylated dihydronaphthohydroquinone (15). *From* **4**. Treatment of **4** (414 mg, 0.97 mmol) with Me_2SO_4 (3.6 mL, 3.80 mmol), a 10-N solution of NaOH (11.8 mL) in EtOH (15 mL) as described before in the first stage of method D, gave a reaction product which was purified by CC using mixtures of Hex/EtOAc, 98:2 as eluent, to yield **15** (175 mg, 39%). IR cm^{-1} (film): 2846, 1724, 1644, 1604, 1464, 1440, 1152, 1129, 888. ^1H NMR (Table 3). ^{13}C NMR (Table 4).

From **3**.To a stirred solution of **3** (105 mg, 0.25 mmol) in benzene (20 mL) was added silica gel (1.1 g). The mixture was stirred at room temperature under argon for 7 h. The resulting mixture was filtered and washed with EtOAc. Removal of the solvent gave a residue that was dissolved in EtOH (10 mL) and treated with 10 N NaOH (2.8 mL) and Me_2SO_4 (850 μL, 0.90 mmol) as described before in the first stage of method D. After removal of the solvent the residue obtained was esterified with ethereal solution of CH_2N_2 and when the reaction was completed, the solvent was evaporated and the product was purified by CC, using mixtures of Hex/EtOAc, 98:2 as eluent, to yield **15** (61 mg, 54%).

3.1.11. Benzylated diterpenylnaphthohydroquinones (16 and 17). *From* **7**. Treatment of **7** (286 mg, 0.68 mmol) with $Na_2S_2O_4$ (2.4 g) in dioxane/H_2O (10 mL/13 mL) as described before gave 275 mg of reduction product. To a suspension of NaH (78 mg, 3,24 mmol) in DMF (9 mL) under argon was added the reduction product dissolved in DMF (3 mL) at 0 °C. After 15 min of vigorous stirring, BnBr (0.47 mL, 3.90 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of MeOH and the solvent was partially evaporated. The residue was redissolved in Et_2O and the resulting solution was washed with brine and dried over anhydrous $Na_2S_2O_4$. Removal of the solvent gave a residue, which was purified by CC using mixtures of Hex/EtOAc, 95:5 as eluent, to yield **16** (264 mg, 67%). $[\alpha]_D^{22}$ +30.7° (589), +30.5° (578), +34.8° (546), (*c* 0.91, $CHCl_3$). UV λ_{max} (ε): 248 (13,200). IR cm^{-1} (film): 2931, 2870, 2849, 1722, 1601, 1497, 1433, 1361, 1272, 1245, 1153, 1092, 1076, 734, 696. ^1H NMR (Table 3). ^{13}C NMR (Table 4). FABMS *m*/*z*: 603 $(M+1)^+$. HRMS (FAB, M+1): calcd $C_{41}H_{47}O_4$ 603.3474; found 603.3528.

From **6**. The reduction of **6** (197 mg, 0.48 mmol) with $Na_2S_2O_4$ as described before followed by benzylation according to the procedure described above gave a residue which was purified by HPLC using mixtures of MeCN/H_2O 60/40 to yield **16** (133 mg, 46%) and **17** (72 mg, 22%). $[\alpha]_D^{22}$ +22.9° (589), +23.4° (578), +25.8° (546), +23.2° (436), (*c* 0.76; $CHCl_3$). UV λ_{max} (ε): 249 (17,900). IR cm^{-1} (film): 2933, 2870, 2848, 1720, 1601, 1497, 1433, 1361, 1272, 1244, 1127, 1091, 1076, 733, 696. ^1H NMR (Table 3). ^{13}C NMR (Table 4). FABMS *m*/*z*: 679 $(M+1)^+$. HRMS (FAB, M+1): calcd $C_{47}H_{51}O_4$ 679.3787; found 679.3755.

3.1.12. Allylic alcohols (18–20): General procedure for the allylic oxidation with SeO_2. *Conditions a*. To a stirred solution of **9** (299 mg, 0.59 mmol) in EtOH (6 mL) was added SeO_2 (2.08 mmol, 3.5 equiv). The mixture was heated at 60 °C and monitored by TLC until starting material disappeared over 16 h. After evaporating the solvent, the residue was redissolved in diethyl ether and the organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated off. The reaction product was purified by CC (eluent: Hex/EtOAc, 7:3) to yield **18** (246 mg, 80%): $[\alpha]_D^{22}$ +19.1° (589), +20.6° (578), +22.1^o (546), (*c* 1.0, $CHCl_3$). UV λ_{max} (ε): 241 (7600), 277 (2900). IR cm^{-1} (film): 3515, 2930, 2870, 1770, 1725, 1610, 1450, 1365, 1200, 1095, 1050, 900, 825, 735. RMN ^1H: Table 3. RMN ^{13}C: Table 4. HMRS(ESI, M+23): calcd $C_{31}H_{38}O_7Na$ 545.2509; found 545.2524.

Following the conditions (a), CC of the product obtained from **10** (258 mg, 0.59 mmol) yielded: (a) com-

pound **22** (70 mg, 25%) using mixtures of Hex/EtOAc, 95:5 as eluent: IR cm^{-1} (film): 3529, 2944, 2873, 1723, 1632, 1603, 1463, 1455, 1428, 1368, 1271, 1243, 1195, 1154, 1097, 975, 901, 831, 800, 719. RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 467 $(M+1)^+$ (20), 281 (13), 201 (86), 136 (66). HRMS (FAB, M+1): calcd $C_{29}H_{39}O_5$ 467.2797; found, 467.2778; (b) compound **19** (184 mg, 67%) using mixtures of Hex/EtOAc, 8:2 as eluent: $[\alpha]_D^{22}$ +6.9° (589), +7.3° (578), +9.3° (546), (*c* 0.71, $CHCl_3$). UV λ_{max} (ε): 250 (14100), 319 (3100). IR cm^{-1} (film): 3427, 2934, 2872, 2852, 1723, 1603, 1464, 1450, 1368, 1331, 1271, 1243, 1195, 1152, 1097, 1040, 973, 898, 800, 737, 720. RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 466 $(M)^+$ (21), 201 (28), 136 (40). HRMS (EI, M^+): calcd $C_{29}H_{38}O_5$ 466.2719, found, 466.2754.

Starting from **16** (356 mg, 0.59 mmol) and following conditions a), CC (eluent: Hex/EtOAc, 95:5) of the reaction product yielded: (a) compound **23** (87 mg, 24%): UV λ_{max} (ε): 248 (17,400). IR cm^{-1} (film): 3520, 3064, 3031, 2942, 2871, 1721, 1601, 1497, 1453, 1433, 1361, 1273, 1242, 1201, 1154, 1093, 1075, 905, 799, 733, 696. RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 619 $(M+1)^+$ (1), 307 (13), 154 (100), 91 (71). HRMS (FAB, M+1): calcd $C_{41}H_{47}O_5$ 619.3424, found, 619.3452; (b) compound **20** (216 mg, 60%): $[\alpha]_D^{22}$ +3.8° (589), +3.8° (578), +4.2° (546), (*c* 0.73, $CHCl_3$). UV λ_{max} (ε): 250 (15600), 319 (1100). IR cm^{-1} (film): 3419, 3064, 3031, 2937, 2850, 1722, 1601, 1453, 1432, 1361, 1272, 1243, 1200, 1151, 1092, 1075, 910, 802, 734, 697. RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 619 $(M+1)^+$ (2), 307 (12), 154 (100), 91 (94). HRMS (FAB, M+1): calcd $C_{41}H_{47}O_5$ 619.3424, found, 619.3473.

Conditions b. To a stirred solution of **10** (160 mg, 0.35 mmol) in CH_2Cl_2 (10 mL) SeO_2 (27 mg, 0.24 mmol) was added. Then a 6 M solution of *t*-BuOOH in decane (180 μL, 1.08 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise under argon atmosphere and the stirring was continued at rt for 5 h. Then it was diluted with CH_2Cl_2 and the organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated off. The reaction product was purified by CC to yield: (a) compound **21** (31 mg, 18%) using Hex/EtOAc, 95:5 as eluent: RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 538 $(M)^+$ (35), 351 (7), 307 (11), 267 (11), 201 (100), 154 (68), 91 (67). HRMS (EI, M^+): calcd $C_{33}H_{46}O_6$ 538.3532, found, 538.3562; (b) compound **19** (102 mg, 62%) using Hex/EtOAc 8/2 as eluent.

3.1.13. α,β-Unsaturated ketones (24–26): General procedure for Swern oxidation. To a precooled at −55 °C and stirred solution of 2 M oxalyl chloride (0.21 mL, 0.42 mmol) in dry CH_2Cl_2 (3 mL) a solution of DMSO (60 μL, 0.83 mmol) in dry CH_2Cl_2 (1 mL)was added dropwise. After 5 min at −55 °C, a solution of **18** (73 mg, 0.14 mmol) in dry CH_2Cl_2 (2 mL) was slowly added. The mixture was stirred at the same temperature for 30 min, then triethylamine (0.20 mL, 1.44 mmol) was added dropwise. The mixture was warmed to 0 °C over 1 h, quenched with water and extracted with CH_2Cl_2. The organic layer was washed successively with 2 N HCl, aq satd $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and evaporated off affording a crude product, which was purified by CC (eluent: Hex/EtOAc, 8:2) to yield **24** (54 mg, 64%): $[\alpha]_D^{22}$ +28.3° (589), +28.9° (578), (*c* 0.79, $CHCl_3$). UV λ_{max} (ε): 240 (12300), 287 (4500). IR cm^{-1} (film): 2938, 2873, 2852, 1765, 1722, 1694, 1608, 1463, 1432, 1366, 1268, 1202, 1155, 1053, 896, 825. RMN ^1H: Table 3. RMN ^{13}C: Table 4. HRMS (FAB, M+1): calcd $C_{31}H_{37}O_7$ 521.2539, found, 521.2532.

The Swern oxidation was applied to obtain the following derivatives:

Compound **25** (46 mg, 70%, eluent: Hex/EtOAc 95/5) from **19** (0.65 mg, 0.14 mmol): $[\alpha]_D^{22}$ +31.6° (589), +32.6° (578), +36.5° (546), +34.7° (436), (*c* 0.98, $CHCl_3$). UV λ_{max} (ε): 249 (21100), 319 (4400). IR cm^{-1} (film): 2936, 2873, 1723, 1695, 1603, 1463, 1426, 1368, 1272, 1243, 1231, 1194, 1151, 1097, 965, 803. RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 464 $(M)^+$ (14), 201 (100), 136 (56). HRMS (EI, M^+): calcd $C_{29}H_{36}O_5$ 464.2563, found, 464.2621.

Compound **26** (58 mg, 68%, eluent: Hex/EtOAc, 9:1) from **20** (84 mg, 0.14 mmol): $[\alpha]_D^{22}$ +24.2° (589), +24.3° (578), +28.5° (546), (*c* 0.80, $CHCl_3$). UV λ_{max} (ε): 249 (20100), 319 (4300). IR cm^{-1} (film): 2927, 2852, 1723, 1695, 1601, 1497, 1454, 1362, 1273, 1243, 1151, 1092, 982, 800, 734, 696. RMN ^1H: Table 3. RMN ^{13}C: Table 4. HRMS (ESI, M+Na): calcd $C_{41}H_{44}O_5Na$ 639.3081, found, 639.3066.

3.1.14. Diterpenylnaphthohydroquinones (27–29): General procedure for isomerisation. To a 10^{-1} M solution of **9** (105 mg, 0.23 mmol) in C_6H_6 (23 mL) was added HI 57% (0.53 mL, 4.01 mmol). The mixture was stirred at 80 °C for 10 min. Then, ethyl acetate was added and the organic layer was washed with aq satd $NaHCO_3$, brine, dried over Na_2SO_4, filtered and evaporated off yielding the isomerised product **27** (0.23 mmol, 98%): $[\alpha]_D^{22}$ +42.1° (589), +43.1° (578), +48.5° (546), (*c* 0.62, $CHCl_3$). IR cm^{-1} (film): 2950, 1765, 1725, 1600, 1500, 1460, 1365, 1200, 1050, 970, 910, 890, 795. RMN ^1H: Table 3. RMN ^{13}C: Table 4. HRMS (FAB, M+1): calcd $C_{31}H_{39}O_6$ 507.2747, found, 507.2702.

Starting from **10** (113 mg, 0.23 mmol) and following the above procedure for isomerisation the reaction product yielded **28** (0.22 mmol, 97%): UV λ_{max} (ε): 252 (12600), 319 (2900). IR cm^{-1} (film): 2934, 2872, 2851, 2832, 1724, 1602, 1463, 1450, 1271, 1242, 1192, 1097, 799, 719. RMN ^1H: Table 3. RMN ^{13}C: Table 4. MS *m/z* (%): 451 $(M+1)^+$ (40), 201 (85), 136 (43), 73 (100). HRMS (FAB, M+1): calcd $C_{29}H_{39}O_4$ 451,2848, found, 451,2886.

Starting from **16** (141 mg, 0.23 mmol) and following the procedure for isomerisation the reaction product yielded **29** (0.22 mmol, 97%): UV λ_{max} (ε): 249 (13,700). IR cm^{-1} (film): 3063, 3030, 2926, 2869, 2851, 1723, 1600, 1497, 1452, 1432, 1361, 1272, 1192, 1161, 1092, 1075, 1076,

1028, 984, 802, 733, 696. RMN ^{1}H: Table 3. RMN ^{13}C: Table 4. HRMS (FAB, M+1): calcd $C_{41}H_{47}O_4$ 603.3474; found, 603.3498.

3.1.15. α,β-Unsaturated ketone (30). To a stirred solution of **27** (344 mg, 0.68 mmol) in C_6H_6 (15 mL), AcOH (1.25 mL), Ac_2O (1.90 mL), NaOAc (470 mg) and Na_2CrO_4 (625 mg, 3.86 mmol) were added. The mixture was heated at 70 °C for 8 h. After evaporating the solvent, the residue was redissolved in EtOAc and the organic layer was washed with $Na_2S_2O_3$ and brine, dried over Na_2SO_4, filtered and evaporated off. The reaction product was purified by CC (eluent: Hex/EtOAc, 8:2) to yield **30** (283 mg, 80%): IR cm^{-1} (film): 2940, 1765, 1725, 1660, 1610, 1450, 1365, 1200, 1050, 1010, 895, 825, 735. RMN ^{1}H: Table 3. RMN ^{13}C: Table 4. HRMS (FAB, M+1): calcd $C_{31}H_{36}O_7$ 521.2539; found 521.2598.

3.1.16. Allylic alcohol (31). To a stirred solution of **30** (420 mg, 0.81 mmol) in MeOH/THF, 1:1 (15 mL) $CeCl_3{\cdot}7H_2O$ (605 mg, 1.63 mmol) was added. The mixture was cooled at 0 °C and $NaBH_4$ (80 mg, 2.35 mmol) was added and the stirring was continued at the same temperature for 45 min. Then the mixture was stirred at room temperature for 3 h. After this period, the solvent was evaporated off and the residue was redissolved in diethyl ether. The organic layer was washed with 2 N HCl and brine, dried over Na_2SO_4, filtered and evaporated off. The reaction product was purified by CC (eluent: $CHCl_3$/EtOAc, 8:2) to yield **31** (100 mg, 24%): $[\alpha]_D^{22}$ +22.2° (578), +24.3° (546), (*c* 0.63, $CHCl_3$). IR cm^{-1} (film): 3500, 2950, 1765, 1725, 1660, 1605, 1450, 1365, 1200, 1050, 915, 825, 735. RMN ^{1}H: Table 3. RMN ^{13}C: Table 4. HRMS (FAB, M+1): calcd $C_{31}H_{38}O_7$ 523.2696; found 523.2732.

3.2. Bioactivity: Cell growth inhibition assay

A colorimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[22] Cells were seeded in 96-well microtitre plates, at 5×10^3 cells per well in aliquots of 195 μL of RPMI medium, and they are allowed to attach to the plate surface by growing in drug-free medium for 18 h. Afterward, samples are added in aliquots of 5 μL (dissolved in DMSO/H_2O, 3:7). After 72 h exposure, the antitumour effect is measured by the SRB methodology: cells are fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates are washed with deionised water and dried; 100 μL of SRB solution (0.4% wt/vol in 1% acetic acid) is added to each microtitre well and incubated for 10 min at room temperature. Unbound SRB is removed by washing with 1% acetic acid. Plates are air-dried and bound stain is solubilised with Tris buffer. Optical densities are read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analyses are generated automatically by LIMS implementation. Using control OD values (*C*), test OD values (*T*) and time zero OD values (T_0), the drug concentration that causes 50% Growth Inhibition (GI_{50} value) was calculated from the equation: $100 \times [(T - T_0)/(C - T_0)] = 50$.

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