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Further antineoplastic terpenylquinones and terpenylhydroquinones

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Abstract

Influences of the quinone/hydroquinone fragment and other structural features are considered in relation with the antineoplastic activity and selectivity of terpenylquinones/hydroquinones. Several compounds have shown IC_{50} values under the μM level. © 1998 Elsevier Science Ltd. All rights reserved.

Key words: Avarol, terpenyl-naphthoquinones/naphthohydroquinones, antineoplastic, cytotoxicity.

1. Introduction

Many terpene-hydroquinone and -quinone marine natural products have been described in the literature [1]. Among them avarol and avarone, isolated from the sponge *Dysidea avara* [2], have attracted much attention due to their cytotoxicity against several types of tumoral cells [3]. As far as the authors know, there is nothing published about the influence of the size of the terpenyl rest on the activity of these type of compounds. In a previous communication [4], we reported the synthesis and evaluation as antineoplastics of a number of terpenyl-naphthoquinones/naphthohydroquinones, which can be classified into the structural types I–VI depicted in Fig. 1.

Following this line of research and with the aim, not only of improving the activity of avarol and avarone, but also with the aim of ascertaining the influence of the functionality of the chain and the partially saturated ring of the quinone/hydroquinone fragment, we have prepared and tested a number of oxidized/degraded derivatives from those cytotoxic naphthoquinones/naphthohydroquinones previously obtained [4].

In the first instance, we planned to obtain compounds structurally similar to those possible metabolites of those terpenylquinones [5], so we started by introducing an epoxy function in the side chain. This fact will not only serve to introduce one or more hydroxyl groups in this part of the molecules but also to obtain several degradation products, and then establish some structure–activity relationship for these types of compounds.

Representative compounds of every types were evaluated for their cytotoxicity against cultured cells of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma, and Mel-28 human malignant melanoma. The IC_{50} values found ranged between 0.4–20 μM for naphthoquinone derivatives and between 8–>34 μM for anthraquinone derivatives, which were also prepared and tested. The results for several compounds were compared with IC_{50} values previously found for avarol and avarone (3–6 μM) [3], which were taken as standards in our work.

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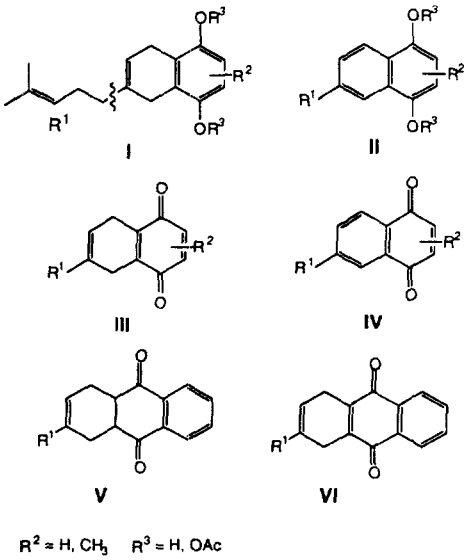


Fig. 1. Structural types of terpenylnaphthoquinone/anthraquinone derivatives.

2. Results and discussion

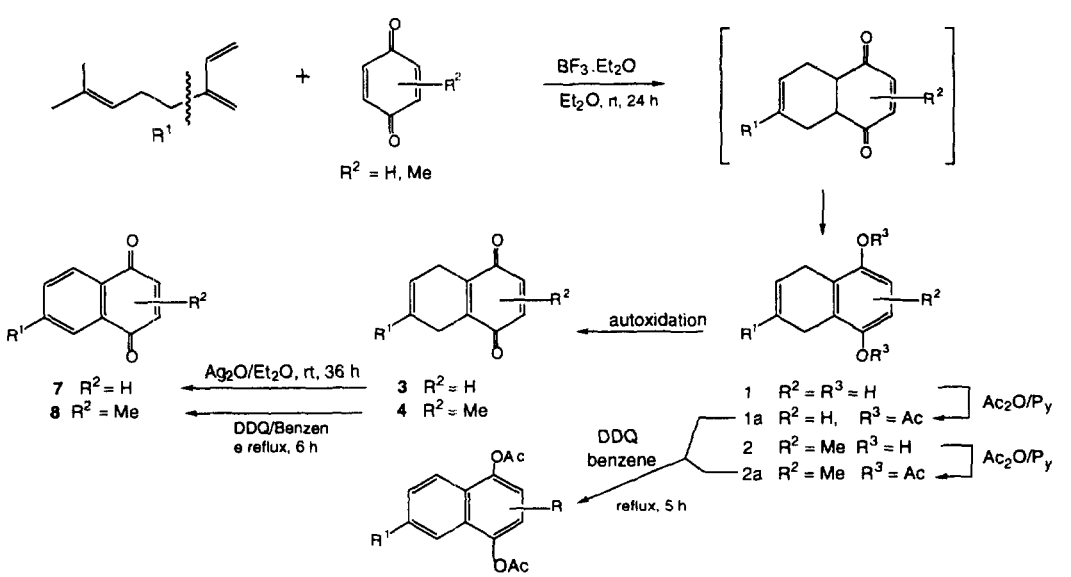
2.1 Chemistry

The monoterpenyl derivatives **1–8**, **33**, and **34** were prepared through the Diels–Alder condensation between the commercially available α-myrcene and *p*-benzoquinone, 2-methyl-*p*-benzoquinone or 1,4-naphthoquinone, in the presence of $BF_3.Et_2O$, followed in some cases by aromatization with DDQ in benzene or Ag_2O in ether as the oxidizing–dehydrogenating agents as in a previous communication [4]. (Scheme 1).

In order to modify the functionality of the terpenic part of those molecules, we introduced some changes on the side chain. This was done by treating with MCPBA [6], the epoxidation of several derivatives with different degree of oxidation in the quinonic moiety: three dihydronaphthohydroquinones (**1**, **1a**, and **2a**), two naphthohydroquinones (**5** and **6**), two naphthoquinones (**7** and **8**) and two anthraquinone derivatives (**33** and **34**). In the case of **1** and **2**, equimolecular amounts of MCPBA led to the epoxidation of the double bond in the side chain, leaving unreacted the endocyclic one. The latter can be epoxidized if greater amounts of MCPBA is used. Second, we wanted to have a hydroxyl group on the side chain. By reduction of the epoxides **10** and **11** with lithium aluminum hydride (LAH), the corresponding tertiary alcohols were obtained as major products. In the case of epoxide **10**, the secondary alcohol **12** and the diol **17** were also isolated in small quantities.

Another step was to change the functionality of B ring in compounds **12** and **14**. So, by treatment with MCPBA, the naphthalene moiety was transformed into the corresponding epoxides **18** and **19**. Acid hydrolysis of the epoxide **19** led to the corresponding tetrahydropyrane **20**. The next reaction was to aromatize **13** and **15** to obtain the tertiary alcohols **21** and **22**.

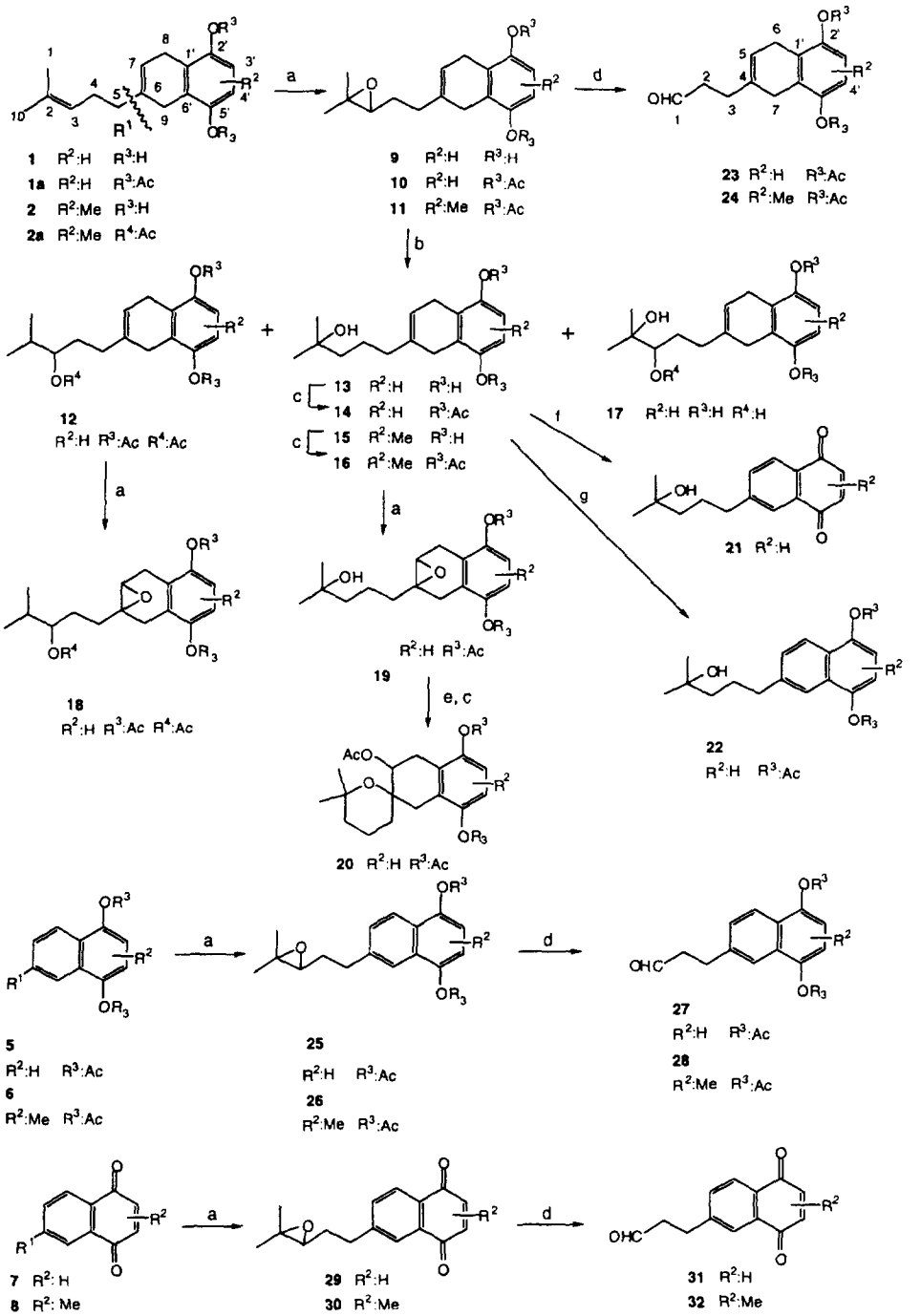
Finally, we considered degrading the side chain to obtain reactive aldehydes. To achieve this, all the derivatives with and epoxy function at the side chain (**10**, **11**, **25**, **26**, **29**, **30**) were treated with a mixture of



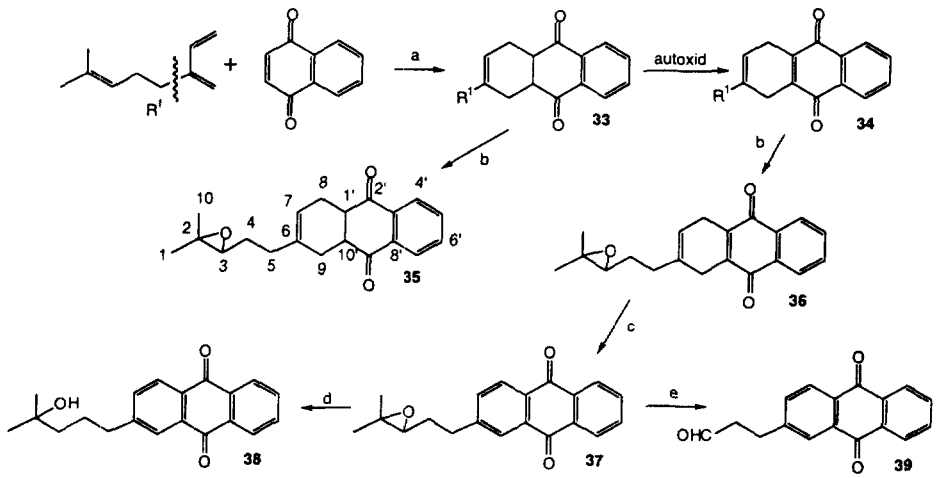
Scheme 1. Preparation of naphthoquinones from α-myrcene.

periodate/formic acid [7] and the corresponding degradation aldehydes **23**, **24**, **27**, **28**, **31**, and **32** were obtained. All the transformations referred to above are shown in Scheme 2.

The side chain in the anthraquinone derivatives **33** and **34** was transformed in a similar way as described for the naphthoquinone derivatives: (a) epoxidation of the side chain double bond only; (b) hydroxylation of



Scheme 2. Oxidised/degraded derivatives of monoterpenylquinone/hydroquinones. (a) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 15–60 min, 75–88%; (b) $LiAlH_4$, Et_2O, rt, 4–20 h, 63–93%; (c) Ac_2O, Py, rt, o.n.; (d) $NaIO_4$-HCOOH, ^tBuOH-H_2O, rt, 24–48 h, 68–92%; (e) ^tBuOH, 2 N HCl, rt, 16 h, 77%; (f) Ag_2O, Et_2O; (g) DDQ, benzene.



Scheme 3. Preparation of anthraquinone derivatives. (a) BF_3-Et_2O, Et_2O, rt, 24 h; (b) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 45 min, 76–86%; (c) DDQ, benzene; (d) $LiAlH_4$, Et_2O, rt, 85%; (e) $NaIO_4$-HCOOH, ^tBuOH-H_2O, rt, 24 h, 9%.

C-2; (c) aromatization of ring B; (d) degradation of the side chain in order to form an aldehyde on it. The compounds obtained are shown in Scheme 3.

All the compounds were fully characterized by NMR and other spectroscopic methods and most of them are reported for the first time. In Table 2 are listed only the most significant data in the ^1H NMR spectra. The complete ^{13}C NMR data are reported in Table 3.

2.2 Bioactivity

Evaluation was performed as previously reported [8] against cultured cells of P-388, A-549, HT-29, and Mel-28 neoplastic systems. The results for representative compounds of the different types are shown in Table 1. Bioactivity data for avarol and avarone [3] are also included for comparison purposes.

As can be deduced from the data shown in Table 1, most changes made in the structure of the side chain of a defined type of naphthoquinone (naphthohydroquinone) do not affect substantially the potency of the compounds but some effects could be observed with respect to the structures.

All the compounds tested showed a certain degree of selectivity against P-388. In addition, derivatives **2**, **2a**, **6**, **11**, **15**, **16**, **18**, **19**, **33**, and **34**, some of them having a substituent on the naphthoquinone moiety, are also selective against A-549.

Those compounds lacking the double bond in the ring B in the naphthalene core (**19** and **20**) are among the less potent compounds of this series. This fact is in agreement with our previous observation [4], which is also observed here (**22** versus **13**), that the aromatization of the naphthalene ring improves the activity.

Table 1
Cytotoxicity of several terpenylquinones (hydroquinones) against neoplastic cultrued cells (IC_{50} values, μM)

Compound[a]	P-388	A-549	HT-29	Mel-28
Avarol	3.1	6.0	6.0	6.0
Avarone	3.1	6.0	6.0	6.0
1	2.0	4.9	4.9	4.9
1a	1.5	3.6	3.6	3.6
2	3.8	3.8	19.4	19.4
2a	2.9	2.9	14.6	14.6
3	1.0	2.1	2.1	2.1
4	3.9	3.9	19.5	9.8
6	2.9	1.5	7.3	3.5
7	0.4	1.0	1.0	0.4
8	3.9	3.9	19.6	9.8
9	2.0	4.9	4.9	4.9
10	1.4	7.3	7.3	7.3
11	2.8	2.8	14.0	14.0
12	0.3	2.5	2.5	2.5
13	1.9	9.5	9.5	9.5
14	1.4	7.2	7.2	7.2
15	3.6	3.6	9.0	9.0
16	2.8	2.8	13.9	13.9
17	3.4	4.3	4.3	4.3
18	2.9	5.9	11.9	11.9
19	6.9	6.9	13.8	13.8
20	5.9	11.8	11.8	11.8
22	0.3	2.9	2.9	2.9
29	1.9	3.8	3.8	3.8
30	3.7	3.7	4.4	4.4
31	5.0	12.5	12.5	5.0
32	4.4	4.4	5.3	5.3
33	8.5	8.5	> 34.0	> 34.0
34	17.0	17.0	> 34.0	> 34.0

[a]Bioactive data for compounds **1–4**, **6–8**, **33**, and **34** are cited from the literature previously reported [4].

Table 2
^{1}H NMR data of compounds **1–39**

H	**1a**	**2**	**2a**	**4**	**9**	**10**	**11**	**12**
1	1.64 s	1.61 s	1.63 s	1.57 s	1.25 s	1.26 s	1.25 s	0.90 d (8.0)
3	5.13 bs	5.14 t (7.0)	5.13 bs	5.06 bs	3.14 m	3.15 m	3.10 m	4.70 m
7	5.57 bs	5.55 bs	5.55 bs	5.45 bs	5.54 sa	5.60 sa	5.59 sa	5.05 bs
10	1.71 s	1.67 s	1.70 s	1.64 s	1.30 s	1.30 s	1.30 s	0.90 d (8.0)
3′, 4′	6.94 s	6.80 s	6.83 s	6.52 s	6.48 s	6.92 s	6.80 s	6.92 s
Me-Ar	—	2.11 s	2.14 s	2.01 s	—	—	2.12 s	
Ac	2.33 s	—	2.33 s	—	—	2.32 s	2.28 s	2.04 s
Ac	2.30 s	—	2.35 s	—	—	2.29 s	2.34 s	2,29s
Ac	—	—	—	—	—	—	—	2.33 s

H	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	1.18 s	1.21 s	1.17 s	1.21 s	1.18 s	0.89 d (8.0)	1.22 s	1.22 s
3	—	—	—	—	3.25 m	4.75 m	—	
7	5.60 bs	5.50 bs	5.58 bs	5.54 bs	5.68 bs	3.25 m	3.27 m	5.25 t (4.4)
10	1.18 s	1.21 bs	1.17 s	1.21 s	1.21 s	0.89 d (8.0)	1.22 s	1.22 s
3′,4′	6.47	6.92 s	6.38 s	6.80 s	6.48 s	6.91 s	6.92 s	6.92 s
Me-Ar	—	—	2.12 s	2.14 s	—	—	—	—
Ac	—	2.33 s	—	2,30s	—	2.05 s	2.31 s	2.00 s
Ac	—	2.30 s	—	2.35 s	—	2.29 s	2.32 s	2.30 s
Ac	—	—	—	—	—	2.31 s	—	2.33 s

H	**5**	**6**	**8**	**21**	**22**	**25**	**26**	**29**	**30**
1	1.62 s	1.60 s	1.50 s	1.20 s	1.18 s	1.15 s	1.15 s	1.18 s	1.22 s
3	5.24 t	5.23 t	5.05 t (7.1)	—	—	2.95 m	2.95 m	2.93 m	2.85 m
7	7.46 dd (1.7, 6.7)	7.38 dd (8.6, 1.6)	7.50 dd (7.9, 1.9)	7.56 dd (7.9, 1.9)	7.40 dd (8.7, 1.8)	7.41 dd (1.6, 8.7)	7.39 dd (8.6, 1.8)	7.60 dd (7.9, 1.9)	7.52 dd (7.9, 1.8)
8	7.86 d (8.7)	7.77 d (8.6)	7.95 d (7.9)	7.99 d (7.9)	7.79 d (8.7)	7.81 d (8.7)	7.76 d (8.6)	8.02 d (7.9)	7.97 d (7.9)
9	7.69 d (1.7)	7.54 d (1.6)	7.85 d (1.9)	7.87 d (1.9)	7.61 d (1.8)	7.67 d (1.6)	7.54 d (1.8)	7.92 d (1.9)	7.85 d (1.8)
10	1.76 s	1.73 s	1.64 s	1.20 s	1.18 s	1.27 s	1.28 s	1.29 s	1.23 s
3′	7.26 s	7.12 s	6.76 s	6.93 s	7.19 s	7.21 s	7.09 s	6.96 s	6.76 s
4′	7.25 s	—	—	6.93 s	7.18 s	7.20 s	—	6.96 s	—
Me-Ar	—	2.34 s	2.14 s	—	—	—	2.31 s	—	2.13 s
Ac	2.45 s	2.45 s	—	—	2.44 s	2.42 s	2.44 s	—	—
Ac	2.47 s	2.51 s	—	—	2.46 s	2.45 s	2.49 s	—	—

H	**23**	**24**	**27**	**28**	**31**	**32**
1	9.35 s	9.31 s	9.77 s	9.81 s	9.28 s	9.50 s
5	5.56 sa	5.54 sa	7.36 dd	7.31 dd	7.55 dd (7.9;1.9)	7.59 dd (7.9;1.9)
6	—	—	7.80 d	7.69 d	7.98 d (7.9)	7.98 d (7.9)
7	—	—	7.64 d	7.53 d	7.86 d (1.9)	7.89 d (1.9)

(continued)

Table 2—*contd*

H	23	24	27	28	31	32
3′, 4′	6.93 s	6.81 s	7.21 s	7.12 s	6.92 s	6.79 s
Me-Ar	—	2.12 s	—	2.31 s	—	2.17 s
Ac	2.32 s	2.35 s	2.45 s	2.47 s	—	—
Ac	2.32 s	2.29 s	2.43 s	2.44 s	—	—

H	34	35	36	37	38	39
1	1.58 s	1.28 s	1.17 s	1.16 s	1.23 s	9.84 s
3	5.05 t	3.30 m	3.20 m	2.95 m	—	—
5	—	—	—	—	—	7.67 s
6		—	—	—	—	8.16 d
7	5.38 bs	5.58 bs	5.63 bs	7.61 dd (7.8, 1.9)	7.62 dd (8.0, 1.9)	8.06 d
8	—	—	—	8.21 d (7.9)	8.23 d (8.0)	—
9	—	—	—	8.11 d (1.9)	8.12 d (1.9)	—
10	1.56 s	1.30 s	1.29 s	1.28 s	1.23 s	—
4′	7.60 m	8.03 m	8.23 m	8.27 m	8.29 m	8.21 m
5′, 6′	7.98 m	7.75 m	7.74 m	7.77 m	7.79 m	7.76 m
7′	7.90 m	8.03 m	8.23 m	8.27 m	8.29 m	8.21 m

The oxidation of the side chain did not modify significantly the activity, although in the case of **22** an improvement in the selectivity against P-388 can be observed. On the contrary the degradation of the same side chain produced a decrease in the activity (**31** and **32** versus **29** and **30**). Also the anthracene derivatives were much less potent than the naphthoquinone derivatives.

In conclusion, it can be said that most of the compounds tested show IC_{50} in the same order of magnitude as avarol and avarone, even some of them, such as **7**, **12**, and **22**, are several times more potent. It is also worth noting that the majority of the derivatives tested shown a certain degree of selectivity against P-388 and A-549.

From these results and those previously reported [4], it can be concluded that the size of the quinone fragment is important for the activity, being the benzoquinone and the naphthoquinone the better size. On the other hand, the size of the terpenic part is also important since its degradation led to less potent compounds. Thus, it is foreseeable that an increase in the terpenic size towards sesquiterpene, diterpene, etc., will improve the biological properties of this type of compounds. Studies in this sense, are currently in progress.

3. Experimental

UV spectra were recorded on a Hitachi 100-60 spectrophotometer in ethanol solution. IR spectra were obtained on a Beckmann (Acculab VIII) spectrophotometer in chloroform solution. NMR spectra were recorded at 200 MHz for ^1H and 50.3 MHz for ^{13}C in deuterochloroform using TMS as internal reference, on a Bruker WP 200 SY. Chemical shift values are expressed in ppm followed by multiplicity and coupling constants (J) in Hz. Column chromatography (CC) was performed on silica-gel (Merck no. 9385). Melting points were determined by heating in an external silicone bath and were uncorrected. All the described compounds were of oil appearance, unless otherwise stated.

3.1 Chemistry

3.1.1 Diels–Alder cyclocondensation

To a solution of *p*-benzoquinone (1.58 g, 14.7 mmol) in dry ether, were added α-myrcene (2.0 g, 14.7 mmol) and $BF_3.Et_2O$ cat. The mixture was stirred at room temperature under argon atmosphere for 24 h, then it was diluted with ether, washed with water, dried over Na_2SO_4 and the solvent evaporated off. The reaction product was chromatographed to yield: (a) 390 mg (19%) of α-myrcene; (b) 360 mg (10%) of quinone **3** [4]; (c) 2.4 g (67%) of hydroquinone **1** [4].

Acetylation of **1** with acetic anhydride and pyridine yielded the diacetate **1a**: UV λ_{max} (ε) 260(530); IR cm^{-1} 3090, 1600, 1740, 1370, 1230, 1200, 1130, 1050, 1025, 840, 790; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

From the reaction product between 2-methyl-*p*-benzoquinone and α-myrcene, according to the procedure

Table 3
^{13}C NMR data of compounds **1–39**

C	**1a**	**2**	**2a**	**4**	**5**	**6**	**8**	**9**	**10**
1	17.7	17.8	17.8	17.6	17.7	17.7	17.6	18.7	18.7
2	131.7	132.3	131.9	133.0	132.5	132.4	133.0	59.6	58.3
3	124.1	125.4	124.1	123.7	123.6	123.8	122.8	64.9	63.9
4	26.2	26.1	26.3	26.0	29.7	29.7	29.3	26.9	27.1
5	37.1	38.4	37.1	38.9	36.4	36.5	36.2	33.9	33.8
6	134.0	135.5	134.0	134.0	141.4	141.4	149.0	133.4	133.3
7	117.2	119.0	117.1	116.7	128.6	128.9	133.7	118.2	117.7
8	25.3	27.4	25.6	25.1	120.2	120.7	126.2	25.2	25.2
9	27.7	28.9	27.5	27.1	121.7	121.5	126.6	27.6	27.7
10	25.7	25.8	25.6	25.5	25.7	25.6	25.5	24.8	24.8
1′	128.9	123.5	128.4	140.0	128.0	127.8	132.0	122.8	128.5
2′	146.2	148.5	146.1	187.1	144.5	144.4	185.7	147.0	146.2
3′	119.9	114.9	121.4	133.0	117.7	119.8	135.7	112.7	119.9
4′	119.9	119.0	128.4	131.9	116.8	124.4	140.1	112.7	119.9
5′	146.2	145.9	145.1	187.1	144.2	144.4	184.7	147.0	146.2
6′	128.5	120.5	126.1	139.5	126.4	126.5	130.1	122.5	128.3
7′		—	—	15.5	—	16.5	16.2	—	—
Ac	169.0	—	168.9	—	169.1	169.1	—	—	168.9
Ac	20.7	—	20.8	—	20.9	20.9	—	—	20.7
Ac		—	168.4	—	—	168.8	—	—	—
Ac		—	20.3	—	—	20.4	—	—	—

C	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
1	18.7	17.5	29.2	29.3	29.2	29.3	25.1	17.5	29.3
2	58.5	31.6	71.5	70.8	71.2	70.9	73.8	31.5	70.8
3	63.7	78.2	44.4	43.5	44.3	43.5	79.2	78.0	43.7
4	27.1	29.1	23.3	22.1	23.4	22.1	26.2	26.0	19.5
5	33.8	33.1	38.9	37.4	38.3	37.5	35.5	32.2	36.7
6	133.3	133.5	135.7	134.0	136.4	133.9	135.8	58.4	58.8
7	117.7	117.3	119.0	117.2	117.8	117.3	113.1	56.1	56.4
8	25.4	25.2	26.2	25.2	26.6	25.1	28.6	24.8	24.8
9	27.5	27.7	28.4	27.7	28.7	27.7	30.5	27.3	27.2
10	25.1	18.5	29.2	29.3	29.2	29.3	25.6	18.5	29.4
1′	121.8	128.6	124.0	128.8	127.6	128.8	127.5	126.5	126.7
2′	146.0	146.2	148.3	146.2	149.5	146.1	148.5	146.7	146.6
3′	119.9	119.9	113.0	119.9	119.1	121.4	119.1	120.3	120.3
4′	128.5	119.9	113.0	119.9	126.5	125.8	119.1	120.3	120.3
5′	144.3	146.2	148.3	146.2	148.6	145.1	148.5	146.6	146.6
6′	121.5	128.4	123.6	128.3	126.5	128.5	127.0	125.7	125.5
7′	24.7	—	—	—	16.2	16.0	—	—	—
Ac	169.1	168.8	—	169.0	—	169.1	—	168.6	168.7
Ac	20.7	20.7	—	20.7	—	20.7	—	20.7	20.7
Ac	168.7	170.4	—	—	—	168.6	—	170.7	—
Ac	20.4	21.2	—	—	—	20.4	—	20.9	—

C	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
1	30.1	29.3	29.3	201.6	201.5	18.7	18.7	200.8	200.9
2	71.3	70.7	70.8	29.1	29.1	58.5	58.5	28.4	28.5
3	36.5	43.3	43.4	41.7	41.7	63.3	63.7	44.9	45.0
4	26.8	25.6	36.6	132.4	132.4	30.5	30.6	139.5	139.5
5	33.4	36.5	25.8	118.0	118.1	33.1	33.2	128.0	128.8
6	72.2	149.5	141.4	25.2	25.1	140.0	141.9	120.3	122.0
7	71.6	133.9	128.4	27.8	28.0	128.3	128.4	122.1	127.0

(*continued*)

Table 3—*contd*

C	20	21	22	23	24	25	26	27	28
8c	30.1	126.1	120.1	—	—	120.3	120.0	—	—
9	31.2	126.7	121.7	—	—	121.9	121.8	—	—
10	30.1	29.3	29.3	—	—	24.7	24.7	—	—
1′	128.9	132.0	127.9	128.3	128.6	127.9	126.7	126.5	126.0
2′	146.5	184.7	144.5	146.2	146.0	144.5	144.3	144.1	144.3
3′	119.9	138.7	117.7	120.1	121.5	117.8	120.9	118.0	119.0
4′	119.9	138.5	116.7	120.1	125.3	116.9	139.4	117.2	138.4
5′	146.5	184.7	144.1	146.2	145.8	144.1	144.0	144.1	144.0
6′	127.5	130.1	126.3	128.1	128.6	126.4	125.0	126.5	125.0
7′	—	—	—	—	16.0	—	16.5	—	16.5
Ac	168.7	—	169.2	169.0	169.0	169.0	169.4	169.1	169.2
Ac	20.7	—	20.9	20.7	20.6	20.8	20.9	20.8	20.9
Ac	170.4	—	—	—	169.0	—	168.8	—	168.7
Ac	21.2	—	—	—	20.6	—	20.5	—	20.5

C	29	30	31	32	33	34	35	36	37	38	39
1	18.9	18.2	199.7	199.9	17.6	17.7	22.5	18.7	18.7	29.4	200.1
2	58.3	58.4	28.2	28.2	131.7	131.8	54.3	58.4	58.4	70.7	28.2
3	63.3	63.3	44.2	44.4	124.0	124.0	63.4	63.4	63.4	43.4	44.2
4	30.2	30.5	147.4	148.0	26.3	26.3	29.8	30.2	30.1	25.6	147.6
5	33.1	33.0	133.9	135.8	37.5	37.5	34.6	33.1	33.1	36.6	133.6
6	148.5	148.4	125.9	126.6	134.0	134.0	133.9	134.2	148.6	149.7	126.7
7	134.0	133.7	126.7	127.1	118.3	118.3	121.3	117.3	134.3	134.3	127.8
8	126.1	126.3	—	—	24.8	24.8	25.8	25.6	126.8	126.8	—
9	126.8	126.5	—	—	27.7	27.7	27.4	27.6	127.6	127.6	—
10	24.7	18.7	—	—	25.5	25.5	22.5	24.8	24.7	29.4	—
1′	132.0	132.4	132.0	132.5	47.2	47.2	44.6	141.9	133.8	133.8	132.1
2′	184.7	185.0	184.9	184.6	197.8	197.8	184.0	184.2	183.2	186.5	184.2
3′	138.8	138.0	138.7	138.7	135.7	135.7	133.8	133.9	131.9	131.8	132.0
4′	138.8	135.7	138.5	138.5	126.8	126.8	127.0	127.2	127.2	127.2	127.2
5′	184.7	185.0	184.5	184.5	134.1	134.1	134.4	134.0	134.0	134.0	134.1
6′	130.1	132.4	130.1	130.9	134.1	134.1	134.4	134.0	134.0	134.0	134.1
7′	—	24.7	—	18.2	126.8	126.8	126.2	126.2	127.2	127.2	127.2
8′	—	—	—	—	135.7	135.7	133.5	133.4	131.9	131.8	132.0
9′	—	—	—	—	197.8	197.8	184.0	184.1	182.8	186.5	184.2
10′	—	—	—	—	46.6	46.6	44.4	141.9	133.8	133.8	132.1

described above, the following compounds were obtained: (a) 210 mg (10%) of α-myrcene; (b) 340 mg (9%) of **4**, UV λ_{max} (ε) 253(4300), IR cm^{-1} 3000, 1675, 1660, 1625, 1600, 1450, 1380, 1180, 1000, 840, 800, 700, ^1H NMR (Table 2), ^{13}C NMR (Table 3); (c) 197 mg (11%) of 2-methyl-*p*-benzoquinone. (d) 1.64 g (43%) of **2**, UV λ_{max} (ε) 290 (4100), IR cm^{-1} 3350, 1660, 1625, 1600, 1470, 1440, 1385, 1300, 1240, 1120, 1100, 840, 820, 760, ^1H NMR (Table 2), ^{13}C NMR (Table 3).

Acetylation of **2** yielded **2a**; UV λ_{max} (ε) 252(510); IR cm^{-1} 3000, 1760, 1625, 1600, 1480, 1440, 1370, 1210, 1200, 1100, 1050, 100, 900, 850, 820, 790; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

From the reaction product between α-myrcene and 1,4-naphthoquinone, the following compounds were isolated after CC: (a) 290 mg (29%) of α-myrcene; (b) 355 mg (17%) of 34, UV λ_{max} (ε) 240(23500), 276 (19400), 325(2700), IR cm^{-1} 1675, 1660, 1640, 1600, 1450, 1430, 1330, 1290, 1170, 930, 880, 800, 750, 700, ^1H NMR (Table 2), ^{13}C NMR (Table 3); (c) 747 mg (35%) of **33** [4]; (d) 110 mg (9%) of 1,4-naphthoquinone.

3.1.2 Aromatization

To a solution of the corresponding quinone in dry benzene was added DDQ. The mixture was kept at room temperature for 1 h. Then it was filtered, the organic solvent was evaporated and the product was purified by CC. Aromatization of **1a** gave **5** (83%); UV λ_{max} (ε) 220(200), 298(430), 324(120); IR cm^{-1} 3050, 1775, 1690, 1625, 1470, 1440, 1370, 1220, 1050, 840, 720; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Aromatization of **2a** yielded **6** (72%); UV λ_{max} (ε) 254(200), 279(525); IR cm^{-1} 3010, 1770, 1640, 1450, 1370, 1240, 1180, 1160, 1050, 900, 820, 750; ^1H NMR Table 2); ^{13}C NMR (Table 3).

Aromatization of **4** afforded **8** (74%); UV λ_{max} (ε) 254(18300), 335(3200); IR cm^{-1} 3000, 1670, 1635, 1600, 1450, 1370, 1350, 840, 800, 700, 690; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Aromatization of **14** afforded **22** (84%); UV λ_{max} (ε) 280(790), 320(119); IR cm^{-1} 3600, 3000, 1790, 1650, 1625, 1470, 1440, 1370, 1240, 1050, 900, 830, 750; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Aromatization of **36** gave **37** (85%); UV λ_{max} (ε) 242(19800), 275(18000), 325(1400); IR cm^{-1} 1680, 1600, 1450, 1380, 1230, 1200, 940, 870, 850, 800, 700; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Aromatization of **3** was performed by treating a solution of **3** (316 mg, 1.3 mmol) in dry ether with Ag_2O (475 mg) at room temperature for 36 h. The excess of Ag_2O was removed by filtration and the reaction product was purified by CC to yield 256 mg (81%) of **7** [4].

Treatment of **13** in the same way as that described for **3**, yielded **21** (76%); UV λ_{max} (ε) 256(14300), 249(17800), 339(3200); IR cm^{-1} 3500, 3000, 1675, 1625, 1475, 1310, 1150, 1050, 840, 820; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

3.1.3 Epoxidation reaction

The corresponding quinone was dissolved in dichloromethane and MCPBA was added in the presence of $NaHCO_3$. The mixture was kept at room temperature for 15–60 min. Then, CH_2Cl_2 was added and 10% aq. $Na_2S_2O_3$ until the oxidant was decomposed. The organic layer was washed with water, dried over Na_2SO_4 and the solvent was evaporated. The reaction products were purified by CC using hexene/EtOAc as eluant.

Epoxidation of **1**, for 25 min, yielded **9** (86%); UV λ_{max} (ε) 290(3250); IR cm^{-1} 3350, 3040, 1710, 1670, 1600, 1490, 1390, 1250, 1150, 1050, 810, 750; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **1a**, for 15 min, yielded **10** (83%); UV λ_{max} (ε) 255(425); IR cm^{-1} 3030, 1770, 1690, 1625, 1470, 1440, 1380, 1240, 1200, 840; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **2a**, for 50 min, yielded **11** (88%); UV λ_{max} (ε) 269(560); IR cm^{-1} 1775, 1625, 1600, 1475, 1440, 1375, 1240, 1210, 1050, 1020, 900, 840, 820; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **5**, for 45 min, yielded **25** (81%); UV λ_{max} (ε) 297(6350), 320(3000); IR cm^{-1} 1760, 1640, 1610, 1460, 1430, 1360, 1250, 1220, 1050, 890, 830, 750; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **6**, for 45 min, yielded **26** (75%); UV λ_{max} (ε) 290(400), 324(130); IR cm^{-1} 3090, 1765, 1650, 1625, 1450, 1380, 1230, 1200, 1100, 1070, 1050, 900, 830, 770; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **7**, for 60 min, yielded **29** (75%); UV λ_{max} (ε) 245(21000), 335(1950); IR cm^{-1} 3030, 1675, 1625, 1600, 1470, 1260, 760; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **8**, for 45 min, yielded **30** (77%); UV λ_{max} (ε) 256(20300), 335(4000); IR cm^{-1} 1670, 1625, 1600, 1470, 1450, 1390, 1300, 1270, 900, 850, 800, 760, 690; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **12**, for 1 h and 45 min, yielded **18** (70%); UV λ_{max} (ε) 268(5300); IR cm^{-1} 3600, 1770, 1740, 1620, 1474, 1380, 1250, 1000, 850; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **14**, for 1 h and 45 min, yielded **19** (78%); UV λ_{max} (ε) 268(620); IR cm^{-1} 3400, 3030, 1770, 1740, 1475, 1375, 1375, 1240, 1210, 1050, 840; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **33**, for 45 min, yielded **35** (76%); UV λ_{max} (ε) 222(21300), 253(18800); IR cm^{-1} 3020, 1700, 1675, 1600, 1570, 1450, 1300, 1260, 1230, 1150, 910, 800, 700; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Epoxidation of **34**, for 45 min, yielded **36** (86%); UV λ_{max} (ε) 240(24500), 263(23000), 270(9700), 324(4300), 405(115); IR cm^{-1} 3010, 1700, 1675, 1600, 1570, 1450, 1300, 1260, 1230, 1150, 1150, 910, 800, 700; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

3.1.4 Treatment of the epoxides with $LiAlH_4$

A solution of **10** (395 mg, 1.15 mmol) in dry ether was added to a suspension of $LiAlH_4$ (LAH) (174 mg, 4.58 mmol) in the same solvent. This was stirred at room temperature under argon for 4.5 h. The excess of hydride was decomposed with wet ether and then acidified with 2 N HCl, extracted with EtOAc, washed with sat. aq. $NaHCO_3$ and water, dried, and evaporated. The reaction product was chromatographed on column to yield: (a) 70 mg (16%) of **12**, which was purified as its diacetate: UV λ_{max} (ε) 280(360), IR cm^{-1} 3600, 3010, 1770, 1730, 1625, 1600, 1445, 1370, 1240, 1210, 900, 820, 760, ^1H NMR (Table 2), ^{13}C NMR (Table 3); (b) 280 mg (70%) of **13**, eluted with hexene:EtOAc (1:1); UV λ_{max} (ε) 290(3200), IR cm^{-1} 3400, 1670, 1660, 1600, 1490, 1470, 1380, 1150, 1050, 1820, 750, ^1H NMR (Table 2), ^{13}C NMR (Table 3). Acetylation of **13** yielded **14**: UV λ_{max} (e) 268(207); IR cm^{-1} 3400, 1770, 1650, 1625, 1450, 1380, 1240, 1050, 820, 760; ^1H NMR (Table 2); ^{13}C NMR (Table 3); (c) 23 mg (5%) of **17** eluted with hexene:EtOAc (4:6); Mp 137–140°C (hexane:AcOEt), UV λ_{max} (ε) 295(3100), IR cm^{-1} 3500, 3350, 1660, 1625, 1490, 1470, 1380, 1260, 1050, 820, ^1H NMR (Table 2), ^{13}C NMR (Table 3).

In the same way as described for **10** and after CC of the reaction product, the following compounds were obtained using the corresponding starting materials.

Compound **15**. From 360 mg (1 mmol) of **11** and 153 mg (4.02 mmol) of LAH were obtained 250 mg (90%) of 15; Mp 130–132°C (hexane:AcOEt); UV λ_{max} (ε) 293(3500); IR cm^{-1} 3400, 3300, 3010, 1660, 1625, 1475, 1440, 1390, 1250, 1150, 860, 840; ^1H NMR (Table 2); ^{13}C NMR (Table 3). Acetylation gave the diacetate **16**: UV λ_{max} (ε) 264(460); IR cm^{-1} 3450, 1760, 1660, 1625, 1480, 1460, 1360, 1240, 1210, 1040, 1010, 900, 820, 800; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Compound **38**. From 350 mg (1.14 mmol) of **37** and 165 mg of LAH were obtained 297 mg (85%) of **38**: UV λ_{max} (ε) 243(24700), 275(21600), 324(2350); IR cm^{-1} 3400, 1680, 1660, 1600, 1470, 1330, 1300, 1200, 1170, 940, 850; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

3.1.5 Degradation of the epoxides with $NaIO_4$

To a solution of compound **10** (160 mg, 0.46 mmol) in 10 ml of ^{t}BuOH, was added a mixture of $NaIO_4$ (265 mg, 1.22 mmol) and 0.5 ml of formic acid in 3 ml of water. The reaction mixture was kept stirring at room temperature under argon for 24 h. It was diluted with EtOAc, basified with sat. aq. Na_2CO_3, extracted with EtOAc and washed with water. The reaction product was purified by CC and 123 mg (88%) of **23** were eluted with hexene:EtOAc (6:4); UV λ_{max} (ε) 220(630), 275(327); IR cm^{-1} 3000, 1790, 1765, 1650, 1625, 1470, 1440, 1370, 1210, 1050, 825; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

In the same way, the following aldehydes were obtained:

Compound **24**. From 200 mg (0.56 mmol) of **11**, 130 mg (74%) of **24** were obtained: UV λ_{max} (ε) 222(445), 271(300); IR cm^{-1} 2720, 1770, 1720, 1640, 1615, 1475, 1440, 1370, 1240, 1200, 1050, 1000, 900, 820; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Compound **27**. From 200 mg (0.58 mmol) of **25**, 162 mg (92%) of **27** were obtained: UV λ_{max} (ε) 285(360), 320(130); IR cm^{-1} 3010, 2720, 1760, 1650, 1625, 1460, 1440, 1375, 1240, 1050, 1010, 840, 760; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Compound **28**. From 66 mg (0.18 mmol) of **26**, 40 mg (69%) of **28** were obtained: UV λ_{max} (ε) 297(830), 324(275); IR cm^{-1} 3010, 2720, 1760, 1720, 1650, 1610, 1450, 1365, 1220, 1200, 1100, 1070, 1020, 900, 820; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Compound **31**. From 167 mg (0.65 mmol) of **29**, 95 mg (68%) of **31** were obtained: UV λ_{max} (ε) 241(23200), 339(2980); IR cm^{-1} 3090, 2720, 1679, 1625, 1475, 1150, 1050, 900, 840, 820; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Compound **32**. From 164 mg (0.60 mmol) of **30**, 105 mg (76%) of **32** were obtained: UV λ_{max} (ε) 249 (21000), 335(2500); IR cm^{-1} 3010, 2720, 1720, 1670, 1625, 1600, 1440, 1300, 1150, 840, 800, 750, 700; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

Compound **39**. From 230 mg (0.75 mmol) of **37**, 180 mg (90%) of **39** were obtained: UV λ_{max} (ε) 243(17700), 275(18500), 263(19100), 270(19000), 325(7600); IR cm^{-1} 3020, 2720, 1725, 1670, 1600, 1375, 1330, 1300, 1250, 1050, 930, 850, 700; ^1H NMR (Table 2); ^{13}C NMR (Table 3).

3.2 Bioactivity

A screening procedure [8] was used to assess the cytotoxic activity against the following cell lines: P-388 (lymphoid neoplasma from DBA/2 mouse), A-549 (human lung carcinoma), HT-29 (human colon carcinoma), and MEL-28 (human melanoma).

Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of 1×10^4 (P-388), 2×10^4 (A-549, HT-29 and MEL-28) cells/well, respectively, in 1 ml aliquots of MEM 10FCS medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After four days at 37°C, under a 10% CO_2, 98% humid atmosphere, P-388 cells were observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls, whereas A-549, HT-29 and MEL-28 were stained with crystal violet before examination.

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