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### Short communication

# Cytotoxic-antineoplastic activity of hydroquinone derivatives

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#### Abstract

Several myrcenylhydroquinone derivatives have been evaluated for their cytotoxic activity against three neoplastic cell line cultures and compared with the activity previously observed on other neoplastic systems. Also a new series of this type of compounds has been prepared and the compounds synthesised have been evaluated by their  $GI_{50}$  values.  $\bigcirc$  2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Prenylhydroquinones; Naphthohydroquinones; Cytotoxicity; Antineoplastic

#### 1. Introduction

It has been reported that terpenylquinones/hydroquinones and related compounds are active against several types of neoplastic cells [1,2]. Some compounds of this type were prepared through Diels–Alder condensation between  $\alpha$ -myrcene and quinones and showed fair cytotoxic activities against cultured cells of P-388 murine leukaemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and Mel-28 human



Fig. 1. Structure of the starting hydroquinonic diacetate 1.

\* Correspondence and reprints. E-mail address: amolinar@ucv.cl (A. Molinari). malignant melanoma [3,4]. As a continuation of these studies, more recently we described the synthesis of new series of prenylhydroquinones together with the evaluation of their antineoplastic activity against the same neoplastic cells. These new series were prepared through chemical modifications, starting from the hydroquinonic diacetate 1 (Fig. 1), which was obtained by Diels–Alder condensation between the monoterpene  $\alpha$ -myrcene and *p*-benzoquinone [5].

The IC<sub>50</sub> ( $\mu$ M) values of selected previously reported compounds 2–14 (Fig. 2) with high, medium and low potency are shown in Table 3 and, as it can be observed, the most potent derivatives resulted to be compounds 2 and 7, which contain a saturated alkyl side chain, and compounds 13 and 14, containing a totally aromatized benzene ring [5]. The mentioned compounds showed the strongest activity against P-388 cultured cells (IC<sub>50</sub> = 0.3  $\mu$ M), while those compounds containing an additional lactone grouping, a spiroether or a fused epoxide ring and a functionalised open side chain, are less active. From these results, it was deduced that a higher saturation degree of the terpenic part is important for the activity. It was also confirmed that

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Fig. 2. Selected prenylhydroquinone derivatives 2-14.



(a) :  $H_5IO_6$ , THF,  $H_2O$ , r.t, 24h; (b) : LAH,  $Et_2O$ , r.t, 24 h; (c) :  $Ac_2O$ , Pyridine, r.t, 24h; (d) :  $NaCIO_2$ ,  $NaH_2PO_4$ ,  $H_2O$ , t-BuOH, 2-methyl-2-butene, r.t, 27 h; (e) :  $CH_2N_2$ ,  $Et_2O$ , r.t, 30 min.

Fig. 3. The synthesis of new hydroquinone derivatives 17-22

the aromatisation of the ring fused to the quinone moiety improved the cytotoxic potency, as was previously reported [3,4].

In order to get more information about the cytotoxic-antineoplastic properties of this type of derivative, we have prepared and evaluated the activity of a new compound 15 (Fig. 2), designed taking into account the structure of compounds with better  $IC_{50}$  values (2, 7, 13 and 14). Also, from epoxides 9 and 16 used as starting materials we have prepared and tested

the bioactivity behaviour of a new family of derivatives 17-22 (Fig. 3). Complementarily, compounds 2-14 have been submitted to further evaluation assays on the neoplastic cell lines: MCF-7, mammary gland carcinoma, H-460, human lung carcinoma and SF-268 central nervous system carcinoma.

#### 2. Chemistry

Compounds 2-14 and 16 were prepared from the hydroquinonic diacetate 1 as starting material, by means of reactions such as epoxidation with *m*-chloroperbenzoic acid (MCPBA), catalytic hydrogenation by bubbling  $H_2$  in the presence of Pd/CaCO<sub>3</sub> or Pd/C, reduction of aldehydes or epoxides with lithium aluminium hydride (LAH), oxidation of the aldehydes with pyridinium dichromate (PDC), methylation of carboxylic acid by diazomethane, acid hydrolysis of epoxides with HCl/H<sub>2</sub>O/t-BuOH and acetylation of alcohols with acetic anhydride/pyridine, according to previously reported procedures [3-6]. Compound 15 was prepared by aromatisation of compound 7 with 2,3 dichloro-5,6dicyano-1,4-benzoquinone (DDQ). The new polyfunctional derivatives of the naphthohydroquinonic diacetate 1, were prepared by oxidative cleavage of epoxides 9 and 16, using periodic acid to afford the ketoaldehydes 17 and 18. Compounds 19, 20, 21 and 22 were obtained by reduction/acetylation or oxidation/ methylation of 17 or 18 (Fig. 3).

Physical and analytical data of compounds 15 and 17-22 are given in Section 4, while the most significant data of <sup>1</sup>H- and <sup>13</sup>C-NMR spectra are listed in Tables 1 and 2, according to carbon numbering (Fig. 4).

Table 1

<sup>1</sup> H-NMR	data	of	compounds	15,	17-22	synthesised
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#### 3. Biological results and discussion

For comparison purposes, these selected derivatives 2-14 previously evaluated (through their IC<sub>50</sub> values) on P-388, A-549, HT-29 and Mel-28 neoplastic cells, have now been evaluated (GI<sub>50</sub>) on cultures of MCF-7, H-460 and SF-268 cells. The GI<sub>50</sub> results of cytotoxicity evaluation of these compounds and the reported  $IC_{50}$ values are included in Table 3. In general, the  $GI_{50}$ values observed with the new cell systems, are higher than those IC<sub>50</sub> values found in previous assays [5]. In spite of the fact that the cell lines and the experimental methods to evaluate the bioactivity are different, it can be seen in Table 3 that those compounds with better  $IC_{50}$  values (2, 7, 13 and 14) also display better  $GI_{50}$ values against the new group of cells. Furthermore, the new GI<sub>50</sub> values confirm the importance of the saturated side chain and of the complete aromatisation of the ring fused to the guinone moiety, for the antineoplastic cytotoxicity of these kind of compounds.

It is interesting to note that according to these results, it could be predicted that a hydroquinonic derivative containing a fused aromatic ring and a saturated side chain should lead to a compound with higher potency than 2, 7, 13 and 14. In fact, this was confirmed by preparing and evaluating compound 15, which simultaneously contains both mentioned structural characteristics. The GI<sub>50</sub> values found for this new compound were < 0.3, 0.4 and < 0.3  $\mu$ M, representing a cytotoxicity enhancement by, at least, a factor of 5–17 with respect to 2, 7, 13 and 14 on the new cell lines.

The new compounds 17 and 19–22 were similarly evaluated for their cytotoxicity and the  $GI_{50}$  values found for each compound are shown in Table 4.

H <sup>a</sup>	15	17	18	19	20	21	22
1	0.85 d (6.6)	0.85 d (6.5)	_	0.85 d (6.5)	_	0.85 d (6.6)	_
3	_	_	4.10 t (6.6)	_	4.12 m	_	4.03 t (6.4)
4	_	-	-	-	_	-	1.87 m
5	_	2.39 t (7.4)	2.41 t (7.3)	_	_	2.39 t (7.2)	2.49 t (7.1)
6	_	_	-	5.03 m	5.07 m	_	_
7	7.37–7.77m	9.50 t (1.9)	9.50 t (2.0)	4.14 t (7.6)	4.12 m	3.59 s	_
8	7.37–7.77m	3.54 d (2.0)	3.52 d (2.0)	-	_	_	3.59 s
9	7.37–7.77m	3.63 s	3.65 s	_	_	3.73 s	3.61 s
10	0.85 d (6.6)	0.85 d (6.5)	_	0.85 d (6.5)	_	0.85 d (6.6)	_
3′	7.10 s	7.10 s	7.10 s	6.99 s	6.98 s	7.08 s	7.08 s
4′	7.70 s	7.10 s	7.10 s	6.99 s	6.98 s	7.08 s	7.08 s
Ac	2.40 s	2.26 s	2.26 s	2.35 s	2.34 s	2.27 s	2.30 s
Ac	2.43 s	2.27 s	2.27 s	2.35 s	2.34 s	2.30 s	2.27 s
Ac	_	_	2.00 s	2.04 s	2.03 s	_	2.01 s
Ac	_	_	_	1.93 s	2.01 s	_	_
Ac	_	_	_	_	1.94 s	_	_
MeO	_	_	_	_	_	3.62 s	3.73 s

<sup>a</sup> According to carbon numbering given in Fig. 4.

Table 2 <sup>13</sup>C-NMR data of compounds **15**, **17–22** synthesised

C <sup>a</sup>	15	17	18	19	20	21	22
1	22.6	22.4	_	22.5	_	22.5	_
2	29.2	27.8	_	27.9	_	27.9	_
3	27.9	38.3	64.2	26.7	63.0	33.1	63.4
4	36.6	21.6	21.2	21.1	26.7	21.6	23.7
5	38.6	41.5	41.2	31.7	30.4	36.3	33.1
6	142.0	206.4	205.9	73.7	72.9	206.7	205.6
7	128.6	196.0	197.0	63.0	63.9	170.3	171.1
8	121.6	42.7	42.5	34.4	38.7	42.0	42.1
9	121.6	42.6	47.2	38.6	38.7	42.1	37.6
10	22.6	22.4	_	22.6	_	22.5	_
1′	127.8	126.0	126.0	130.0	130.0	127.5	127.5
2′	144.0	147.0	147.0	147.3	147.3	146.9	146.9
3′	117.7	122.1	122.5	121.6	128.8	122.2	122.3
4′	116.7	122.5	122.5	121.7	129.8	122.2	122.3
5′	144.4	147.4	147.2	147.3	147.3	147.0	147.1
6′	126.2	128.2	126.8	130.3	130.7	128.1	127.9
Ac	169.5	169.0	168.9	169.5	169.2	168.8	168.8
Ac	21.1	20.8	20.8	21.0	20.9	20.8	20.8
Ac	169.5	168.8	168.9	169.3	169.4	168.9	168.9
Ac	21.1	20.8	20.9	21.0	21.1	20.9	20.8
Ac	_	_	171.0	171.0	170.5	_	170.3
Ac	_	_	21.2	23.0	23.0	_	20.9
Ac	_	_	_	170.5	170.9	_	_
Ac	_	_	_	23.4	23.7	_	_
Ac	_	_	_	_	171.1	_	_
Ac	_	_	_	_	24.8	_	_
MeO	-	_	_	_	_	52.3	52.3

<sup>a</sup> Carbon numbering given in Fig. 4.

In general, the  $GI_{50}$  values found for the hydroquinone derivatives 17 and 19–22 are higher than those found for the naphthohydroquinones and show that these polyfunctional monocyclic structures are less cytotoxic. These results are in agreement with those observed for the previously reported series [3–5]. The presence of oxygenated functions in the non-quinonic part of the structure decreased the cytotoxic potency. The slightly enhanced bioactivity of ketoaldehyde 17 could probably be justified on the interaction of this electrophilic function with free amino or other nucleophilic groups of the receptor biomolecules. This behaviour has also been observed for the neoplastic activity of cyclolignan aldehydes [7].

#### 4. Experimental protocols

#### 4.1. Chemistry

All the compounds evaluated were prepared by previously reported procedures [3–6] and characterised by IR on a Perkin–Elmer FT IR 1600 spectrophotometer, as film over sodium chloride discs. NMR spectra were recorded at 200 MHz for <sup>1</sup>H and 50 MHz for <sup>13</sup>C in deuterochloroform using TMS as internal reference, on a Bruker AC 200 spectrophotometer. Chemical shifts are expressed in ppm followed by multiplicity and coupling constants (J) in hertz. Elemental analysis of C and H were obtained with a Perkin–Elmer 2400 Series II CHN Elemental Analyzer within  $\pm 0.4\%$  of the theoretical values. Column chromatographic separations were performed on Silicagel 60, 230-400 mesh ASTM.

Using reactions according to previously reported procedures, the following new compounds were prepared:

#### 4.1.1. Fully aromatized diacetate 15

Obtained by aromatisation of compound 7 with DDQ in refluxing benzene [3,4] (86%); m.p. 52-53 °C; IR cm<sup>-1</sup> 3067 (aromatic CH), 2954–2866 (aliphatic C–H), 1763 (C=O ester); <sup>1</sup>H-NMR (Table 1);



Fig. 4. Numbering of basic carbon skeletal of compounds 15 and 17-22.

Table 3 Cytotoxicity of several hydroquinonic derivatives of 1 against neoplastic cultured cells

Compound	P-388 a	A-549 <sup>a</sup>	HT-29 <sup>a</sup>	Mel-28 <sup>a</sup>	MCF-7 <sup>b</sup>	H-460 <sup>b</sup>	SF-268 <sup>b</sup>
Avarol <sup>c</sup>	3.1	6.0	6.0	6.0	nt	nt	nt
2	0.3	1.5	1.5.	1.5	1.5	3.5	1.2
3	2.6	6.4	6.4	6.4	6.1	19.8	11.4
4	2.9	14.5	14.5	14.5	>25.6	>25.6	>25.6
5	3.0	3.7	14.9	3.7	>28.7	16.8	>28.7
6	3.1	6.2	6.2	5.2	10.0	15.8	11.1
7	0.3	1.5	1.5	1.5	1.6	5.6	2.2
8	1.4	2.9	2.9	2.9	2.9	8.7	2.9
9	6.9	6.9	6.9	13.8	18.5	>27.0	15.5
10	15.1	15.1	15.1	15.1	> 30.0	> 30.0	> 30.0
11	13.8	13.8	13.8	13.8	>27.0	>27.0	>27.0
12	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0
13	0.3	3.6	3.6	3.6	3.2	3.3	3.9
14	0.3	2.9	2.9	2.9	2.2	5.9	2.4
15	nt	nt	nt	nt	< 0.3	0.4	< 0.3

nt: not tested.

<sup>a</sup> IC<sub>50</sub> values, µM.

<sup>b</sup> GI<sub>50</sub> values, μM.

<sup>c</sup> IC<sub>50</sub> values included for comparison purposes [1,2].

<sup>13</sup>C-NMR (Table 2). Anal. Found: C, 72.90; H, 7.09. Calc. for  $C_{20}H_{24}O_4$ : C, 73.17; H, 7.32%.

#### 4.1.2. Ketoaldehyde diacetate 17

Obtained by degradative  $H_5IO_6$  oxidation of the epoxide **9** in aqueous tetrahydrofuran [3,4] (72%); oil; IR cm<sup>-1</sup> 3084 (aromatic CH), 2954–2869 (aliphatic CH), 2725 (aldehyde CH), 1765 (C=O ester), 1721 (C=O aldehyde); <sup>1</sup>H-NMR (Table 1); <sup>13</sup>C-NMR (Table 2). Anal. Found: C, 66.23; H, 7.10. Calc. for  $C_{20}H_{26}O_6$ : C, 66.30; H, 7.18%.

#### 4.1.3. Ketoaldehyde triacetate 18

Obtained by degradative  $H_5IO_6$  oxidation of the epoxide **16** in aqueous tetrahydrofuran (66%); oil; IR cm<sup>-1</sup> 3060 (aromatic CH), 2960–2870 (aliphatic CH) 2731 (aldehyde CH), 1765 (C=O ester), 1720 (C=O aldehyde); <sup>1</sup>H-NMR (Table 1); <sup>13</sup>C-NMR (Table 2). Anal. Found: C, 60.26; H, 5.90. Calc. for  $C_{19}H_{22}O_8$ : C, 60.32; H, 5.87%.

#### 4.1.4. Tetraacetate 19

Obtained by LAH reduction of **17** in anhydrous ethyl ether, followed by pyridine/acetic anhydride acetylation [3–6] (53%); m.p. 98–99 °C; IR cm<sup>-1</sup> 3061 (aromatic CH), 2952–2863 (aliphatic CH), 1769 (C=O ester, 1739 (C=O ester); <sup>1</sup>H-NMR (Table 1); <sup>13</sup>C-NMR (Table 2). Anal. Found: C, 63.85; H, 7.50. Calc. for  $C_{24}H_{34}O_8$ : C, 64.00; H, 7.56%.

#### 4.1.5. Pentaacetate 20

Obtained by LAH reduction of **18**, followed by pyridine/acetic anhydride acetylation (59%); m.p. 82-83 °C; IR cm<sup>-1</sup> 3068 (aromatic CH), 2919–2860 (aliphatic

CH), 1772 (C=O ester), 1731 (C=O ester); <sup>1</sup>H-NMR (Table 1); <sup>13</sup>C-NMR (Table 2). Anal. Found: C, 59.52; H, 6.52. Calc. for  $C_{23}H_{30}O_{10}$ : C, 59.23; H, 6.44%.

#### 4.1.6. Ketoester diacetate 21

Obtained by NaClO<sub>2</sub> oxidation of **17**, followed by CH<sub>2</sub>N<sub>2</sub> methylation in anhydrous ethyl ether [8] (64%); m.p. 107–109 °C; IR cm<sup>-1</sup> 3065 (aromatic CH), 2967–2880 (aliphatic CH), 1767 (C=O ester); <sup>1</sup>H-NMR (Table 1); <sup>13</sup>C-NMR (Table 2). Anal. Found: C, 64.29; H, 7.36. Calc. for C<sub>21</sub>H<sub>28</sub>O<sub>7</sub>: C, 64.29; H, 7.14%.

#### 4.1.7. Ketoester triacetate 22

Obtained by NaClO<sub>2</sub> oxidation of **18**, followed by CH<sub>2</sub>N<sub>2</sub> methylation in anhydrous ethyl ether. (71%); m.p. 114–115 °C; IR cm<sup>-1</sup> 3061 (aromatic CH), 2954–2849 (aliphatic CH), 1772 (C=O ester) 1742 (C=O ester); <sup>1</sup>H-NMR (Table 1); <sup>13</sup>C-NMR (Table 2). Anal. Found: C, 58.70; H, 6.10. Calc. for C<sub>20</sub>H<sub>24</sub>O<sub>9</sub>: C, 58.82; H 5.88%.

Table 4	
Cytotoxicity of hydroquinone derivatives 17, 19-22 against n	neoplastic
cultured cells	

Compound	MCF-7 <sup>a</sup>	H-460 <sup>a</sup>	SF-268 <sup>a</sup>
17	4.6	7.7	4.6
19	>22.0	>22.0	>22.0
20	>21.0	>21.0	>21.0
21	>25.0	>25.0	>25.0
22	>25.0	>25.0	>25.0

<sup>a</sup> GI<sub>50</sub> values, μM.

#### 4.2. Bioactivity

#### 4.2.1. Antineoplastic assays for IC<sub>50</sub>

Cells were seeded into 16 mm wells (multidishes, NUNC 42001) at concentrations of  $1 \times 10^4$  (P-388),  $2 \times 10^4$  (A-549, HT-29 and MEL-28) cells well<sup>-1</sup>, respectively, in 1 mL aliquots of MEM10FCS 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 3 days at 37 °C, under 10% CO<sub>2</sub>, 98% humid atmosphere, P388 leukaemic 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 [7].

#### 4.2.2. Antineoplastic assays for $GI_{50}$

The human tumour cell line of the cancer screening (MCF-7, H-460 and SF-268) were grown in a RPMI 1640 medium containing 5% foetal bovine serum and 1 mM L-glutamine. Cells were inoculated into 96-well microtiter, plates in 100 µL at plating densities ranging from 5000 to 40 000 cells well<sup>-1</sup>, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity for 24 h prior to addition of compound to be tested. Dilutions at twice the intended test concentration were added at time zero, in 100  $\mu$ L aliquots, to the microtiter plates wells. Usually, test compounds were evaluated at 5-10fold dilution. Incubation lasted for 48 h in 5% CO<sub>2</sub> and 100% relative humidity. The cells were assayed by using the sulforhodamine B assay. A plater reader was used to read the optical densities and a microcomputer process the optical densities [9-12].

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