Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 15, pp. 1859-1864, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00326-5

SYNTHESIS AND BIOACTIVITY OF NEW ANTINEOPLASTIC TERPENYLQUINONES

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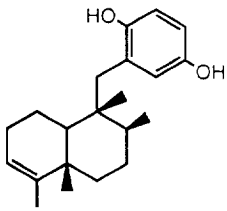
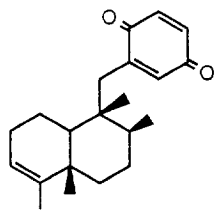
Abstract: Several monoterpenylquinones have been prepared and evaluated for their cytotoxic activity against four cell lines cultures. The size of the quinone fragment is important for the activity, being the naphtalene derivatives the most potent compounds tested. The presence of substituents on the quinone ring decreases the potency but increases the selectivity. Copyright © 1996 Elsevier Science Ltd

Avarol (**1**) and its related quinone, avarone (**2**) are two merosesquiterpenoids isolated from the sponge *Dysidea avara* [1]. They have attracted much attention due to their bioactivity and, particularly, to their cytoxicity against several types of tumoral cells[2]. A number of derivatives of these compounds have been prepared[3] and many other structurally related substances have been isolated from other natural sources[4].



1 Avarol



2 Avarone

Following the interest originated by **1** and **2**, most of their analogs have been tested to evaluate their cytotoxic/antineoplastic activity. Their published IC_{50} values ranged around the μM or higher levels for cultures of P-388 murine leukemia cells and several types of human tumoral cells[5]. It is well know that quinone compounds are cytotoxics but at the sight of the IC_{50} for benzoquinone and naphtoquinone, which have been included in our assays for comparison, the cytotoxic activity of these products seems depend not only on the quinone chromophore but on the structure of the terpenyl moiety. As far as the authors know, SAR studies to

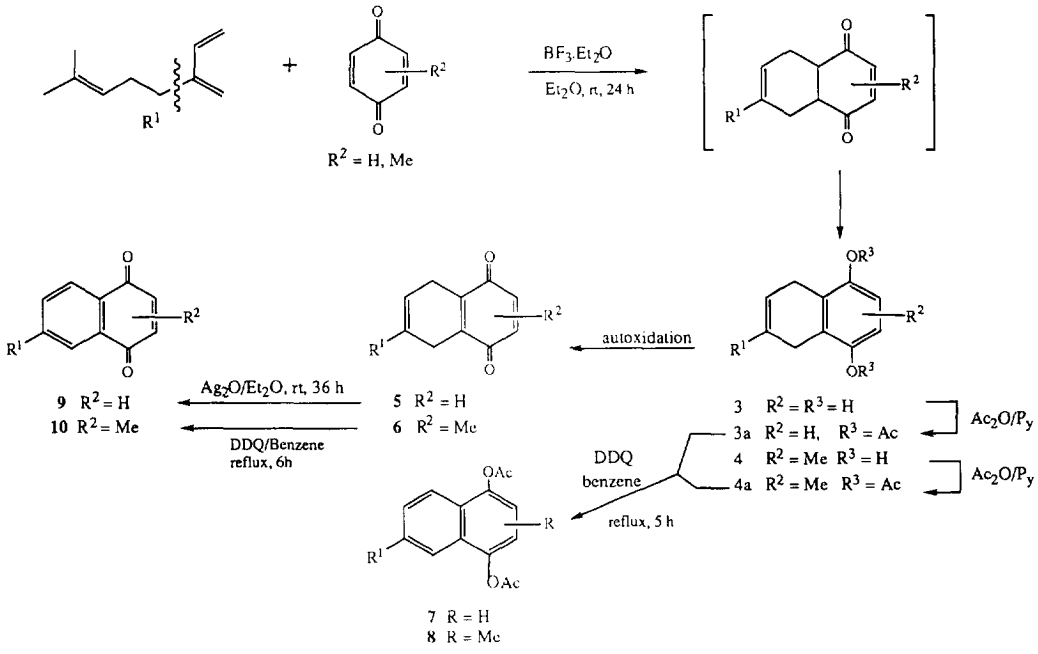
analyze the importance of sizes and types of the terpene and quinone fragments of the molecule, on the activity of this type of antineoplastics, have not yet been published. At this respect we have planned and partially performed the preparation of representative monoterpenyl and diterpenyl naphtho and anthraquinones, in order to evaluate their cytotoxicities, to compare their activity levels with those of the natural or synthetic sesquiterpenoid derivatives cited above and to establish the influence of changing the structure of the two main fragments of the molecule on the antineoplastic activity.

Chemistry[6]

In the first instance, starting from commercially available compounds and through simple chemical routes, we have prepared a number of monoterpenyl derivatives of naphtho and anthraquinone displaying different functionalization patterns on the terpene fragment.

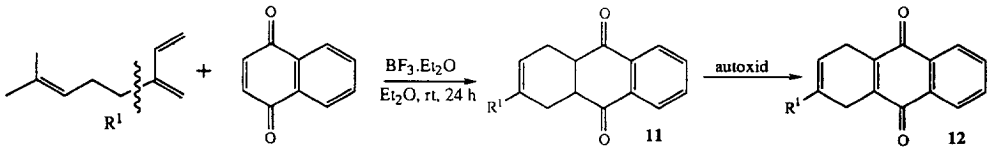
Diels-Alder condensation between α-myrcene and *p*-benzoquinone or 2-methyl-*p*-benzoquinone, in the presence of $BF_3{\cdot}Et_2O$, yielded reaction mixtures from which, after chromatography, the expected hydroquinone derivatives **3** (67 %) and **4** (43 %), along with the autoxidation quinones **5** (10 %) and **6** (9 %) were isolated[7]. **3** and **4** were stabilized respectively as their diacetates **3a** and **4a** (Scheme 1).

Aromatization of **3a**, **4a** and **6** with DDQ in benzene[8] yielded the naphthalene diacetates **7** (83 %) and **8** (72 %) and the quinone **10** (74 %), whereas the aromatization of quinone **5** to **9** (81 %) was achieved by using Ag_2O in ether as the oxidizing-dehydrogenating agent[9,10] (Scheme 1).



Scheme 1. Preparation of prenylnaphthoquinones from α-myrcene

The reaction between α-myrcene and naphthoquinone, worked up similarly and after chromatography, yielded the direct product of cyclocondensation, the diketone **11** (35 %), instead of the expected hydroquinone tautomer, along with the autoxidation quinone **12** (17 %)[11] (Scheme 2).



Scheme 2. The reaction of α-myrcene and naphthoquinone

Both the double bond in the prenyl chain and the one resulting from the Diels-Alder condensation could be easily functionalized. This will help to stablish the influence of different substituents on the activity of this type of compounds. This part of the work is currently in progress.

Bioactivity

Most of the compounds prepared, were evaluated for determining their antineoplastic activities against cultured cells of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and Mel-28 malign human melanoma.[12] The results obtained are shown in Table 1. Avarol (**1**), avarone (**2**) and the simple quinones 1,4-benzoquinone and 1,4-naphtoquinone were also included in the tests for comparison.

From these results the following observations and deduction can be made:

- The simple quinones are much less cytotoxic that the considered terpenylquinones. This points out the importance of the terpenyl moiety on the cytotoxicity of these compounds.

- The prenylnaphthoquinone **9**, the most potent compound of the new series, is 8 to 6 times more potent than avarone in the neoplastic systems assayed.

- The size of the quinone (hydroquinone) fragment seems to be important for the activity. Naphthalene derivatives being equally or more potent than benzene derivatives and both clearly more potent than those evaluated anthracenes. Exceptions are the hydroquinone **4**, its diacetate **4a** and the methylquinones **6** and **10** which are less potent than avarol in the HT-29 and MEL-28 assays.

Within the series of naphthalene derivatives it can be observed:

- The substitution of the ring supporting the quinone (hydroquinone) by methyl group at positions 2 or 3, decreases the antineoplastic potency in all the cases and cell systems (**3**, **3a**, **5** and **9** *versus* **4**, **4a**, **6** and **10** respectively).

- Acetylation, initially performed to avoid autoxidation of hydroquinones before evaluation, seems to improve their cytotoxicity slightly.

- Quinone derivatives are, in general, more potent than the corresponding hydroquinones (**5** *vs* **3**).

-The 5,8-dihydrogenated quinone **5** is less potent than the corresponding aromatic analog **9**, which is also the most potent member of the series.

If comparison of results takes into account the different cell systems in relation with every compound tested, it can be observed that the most potent derivatives are less selective, the leukemia cells being always most sensible than the other cells by a factor of two or more. On the other hand, the presence of one methyl group on the quinone (hydroquinone) ring, while decreasing the potency, increases the selectivity by a factor of around five times.

Table 1. Cytotoxicity of several terpenyl quinones (hydroquinones) against neoplastic cultured cells (IC_{50} values, μM)

Compound	P-388	A-549	HT-29	MEL-28
Benzene derivatives				
1,4-benzoquinone	11.1	46.2	46.2	46.2
1	3.1	6.0	6.0	6.0
2	3.1	6.0	6.0	6.0
Naphthalene derivatives				
1,4-naphtoquinone	>63.2	>63.2	>63.2	>63.2
3	2.1	4.9	4.9	4.9
3a	1.5	3.6	3.6	3.6
4	3.8	3.8	19.4	19.4
4a	2.9	2.9	14.6	14.6
5	1.0	2.1	2.1	2.1
6	3.9	3.9	19.5	9.8
8	2.9	1.5	7.3	3.5
9	0.4	1.0	1.0	0.4
10	3.9	3.9	19.6	9.8
Anthracene derivatives				
11	8.5	8.5	>34	>34
12	17	17	>34	>34

In an attempt of determining the possible mechanism of cytotoxicity for these compounds, assays of enzyme inhibition (DFHR, TS, TOPO-I and TOPO-II, DNA and RNA polymerases) were performed as well as interference of biosynthesis of proteins, DNA and RNA. The results were not very significants. The most relevant observations related the synthesis of biomolecules. RNA synthesis was inhibited by compounds **3**, **5** and **8** at rates of IC_{50} = 4.1, 6.2 and 8.3 μM respectively and protein synthesis was similarly interfered at rates IC_{50} = 8.2, 4.0 and 4.2 μM respectively.

Although the number of compounds prepared and tested is low, it can be concluded that the preferred size of the quinone fragment of terpenylquinones for their antineoplastic activity is that of naphthoquinone. Furthermore, that the inclusion of substituents on the quinonic part of the naphthalene ring can serve to induce a certain degree of selectivity against different kinds of neoplasms.

Clearly, more research in this field must be done to ascertain the mechanism of cytotoxicity as well as, the effect of other structural changes on the activity and selectivity of this type of compounds. Particularly, the effect of functionalization of the side chain and of increasing the size of the prenyl chain are being the subject of our current work in this field.

Acknowledgments:
Financial support for this work came from Spanish DGICYT (PB 93/616) and Junta de Castilla y León (Consejería de Educación y Cultura, SA-35/94).

References and Notes:

1. (a) Minale, L. ; Riccio, R.; Sodano, G. *Tetrahedron Lett.* **1974**, 3401. (b) De Rosa, S.; Minale, L.; Riccio, R.; Sodano, G. *J. Chem. Soc., Perkin Trans. I*, **1976**, 1408.
2. (a)Müller, W.E.G.; Maidhof, A.; Zahn, R.K.; Schröeder, H.C.M.; Gasic, M.J.; Heidemann, D.; Bernd, A.; Kurelec, B.; Eich, E.; Seibert, G. *Cancer Res.* **1985**, *45*, 4822. (b)Sarin, P.S.; Sun, D.; Thornton, A.; Müller, W.E.G. *J. Nat. Cancer Inst.*, **1978**, *78*, 663. (c) García-Gravalos, M.D.; Gordaliza, M.; Miguel del Corral, J.M.; Mahiques, M.M.; San Feliciano, A. Patent WO 96/04230.
3. (a) De Giulio, A; De Rosa, S.; Strazzullo, G.; Diliberto, L.; Obino, P.; Marongiu, M.E.; Pani, A.; La Colle, P. *Antiviral Chem. Chemother.* **1991**, *4*, 223. (b) De Rosa, S.; De Giulio, A.; Strazzullo, G. *Trends in Org. Chem.* **1991**, *2*, 1991.
4. (a) Gil, B.; Sanz, M.J.; Terencio, M.C.; De Giulio, A.; De Rosa, S.; Alcaraz, M.J.; Payá, M. *Eur. J. Pharmacol.* **1995**, *285*, 281. (b) Kobayashi, J.; Murayama, T.; Ohizumi, Y.; Ohta, T.; Nozoe, S.; Sasaki, T. *J. Nat. Prod.* **1989**, *52*, 1173. (c) De Pasquale, R.; Circosta, C.; Occhiuto, F.; De Rosa, S.; De Stefano, S. *Phytotherapy Res.* **1991**, *5*, 49. (d) Hirsch, S.; Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1991**, *54*, 92.
5. (a)Belisario, M.A.; Pece, R.; Arene, A.R.; De Giulio, A.; Strazzullo, G.; De Rosa, S. *Toxicol. Lett.* **1991**, *57*, 183.(b) Crispino, A.; De Giulio, A.; De Rosa, S.; Strazzullo, G. *J. Nat. Prod.* **1989**, *52*, 646. (c) Schroder, H.C.; Wenger, R.; Gerner, H.; Renter, P.; Kuchino, Y.; Sladic, D.; Muller W.E. *Cancer Res.* **1989**, *49*, 2069. (d) Muller, W.E.; Sobel, C.; Diehl-Seifer, B.; Maidhof, A.; Schroder, H.C. *Biochem. Pharmacol.* **1987**, *36*, 1489.
6. All the compounds were completely characterized by spectroscopic and analytical methods and are reported for the first time.
7. Compound **3**. Mp 120-123 °C (Hexane-EtOAc). UV $\lambda_{max}(\varepsilon)$ (EtOH): 290(4400). IR: 3400, 1670, 1600, 1475, 1300, 1150, 840, 800 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ in ppm: 1.50 (3H,s), 1.58 (3H,s), 2.04 (4H, m), 3.14 (4H, m), 5.04 (1H,t), 5.50 (1H,sa), 6.44 (2H,s). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ in ppm: 17.7, 25.6(2C), 26.6, 27.9, 37.7, 112.5(2C), 118.0, 123.1, 123.5, 124.6, 131.7, 134.7, 147.4. Compound **5**. UV $\lambda_{max}(\varepsilon)$ (EtOH): 245(25300), 285(300), 430(23). IR: 3030, 1675, 1600, 1450, 1300, 1050, 840, 740 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ in ppm: 1.59 (3H,s), 1.66 (3H,s), 2.09 (4H, m), 3.00 (4H, m), 5.07 (1H,t), 5.50 (1H,sa), 6.71 (2H,s). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ in ppm: 17.7, 24.9, 25.9, 26.0, 27.0, 36.9, 116.5, 123.7, 132.0, 134.0, 136.3(2C), 138.5, 140.0, 186.9, 187.0.
8. Yamaguchi, H.; Arimoto, M.; Tanoguchi, M.; Ishida, T.; Inove, M. *Chem. Pharm. Bull* **1982**, *30*, 3212.

9. (a) Liebeskind, L.S.; Baysdon, S,L.; South, M.S. Iyer, S.; Leeds, J.P. *Tetrahedron* **1985**, *41*, 5839. (b) Watanabe, M.; Morimoto, H.; Nogami, K.; Ijichi, S.; Furukawa, S. *Chem. Pharm. Bull.* **1993**, *41*, 968.
10. Compound **9**. UV $\lambda_{max}(\varepsilon)$ (EtOH): 246(19100), 340(1900). IR: 3030, 1675, 1600, 1475, 1300, 1150, 840, 800 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ in ppm: 1.49 (3H,s), 1.63 (3H,s), 2.30 (2H, c), 2.73 (2H, t), 5.09 (1H,t), 6.90 (2H,s), 7.51 (1H,dd), 7.83 (1H,d), 7.93 (1H,d). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ in ppm: 17.6, 25.5, 29.2, 36.2, 122.7, 126.1, 126.5, 130.1, 132.0, 133.0, 134.0, 138.5, 138.7, 149.4, 184.7, 185.2.
11. Compound **11**. UV $\lambda_{max}(\varepsilon)$ (EtOH): 221(21600), 250(15000). IR: 3030, 1675, 1660, 1600, 1440, 1370, 1290, 1200, 1150, 1070, 840, 800, 750 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ in ppm: 1.52 (3H,s), 1.62 (3H,s), 2.00-2.50 (6H, m), 3.40 (4H, m), 5.05 (1H,t), 5.39 (1H,sa), 7.73 (2H,m), 8.03 (2H,m) . ^{13}C NMR (200 MHz, $CDCl_3$) δ in ppm: 17.6, 24.8, 25.5, 26.3, 27.7, 37.5, 46.6, 47.2, 118.3, 124.0, 126.8(2C), 131.7, 134.0, 134.1(2C), 135.7(2C), 197.8(2C).
12. Antineoplastic assays: Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of $1x10^4$ (P-388), $2x10^4$ (A-549, HT-29 and MEL-28) cell/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 three 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.

(*Received in Belgium* 19 *February* 1996; *accepted* 4 *July* 1996)