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Effects of 4,4-Dimethyl-5,8-dihydroxynaphtalene-1-one and 4,4-Dimethyl-5,8-dihydroxytetralone Derivatives on Tumor Cell Respiration

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Abstract—A set of structurally related compounds incorporating a carbonyl group in the *ortho* position with regard to a phenol function were tested against the TA3 mouse carcinoma cell line and its multidrug-resistant variant TA3-MTX-R. The series consists of 2′-hydroxyacetophenone, 4′-hydroxyacetophenone 2′,5′-dihydroxyacetophenone, 4-acetyl-3,3-dimethyl-5-hydroxy-2-morpholino-2,3-dihydrobenzo*b*furan, five 4,4-dimethyl-5,8-dioxygenated naphtalene-1-ones and three 4,4-dimethyl-5,8-dioxygenated tetralones. A tentative structure–activity relationship was found for this family of substances, suggesting that a coplanar *ortho*-carbonyl-1,4-hydroquinone motif is able to cause inhibition of cellular respiration.

Introduction

It was suggested long ago that semiquinone free radicals arising from the one-electron reduction of quinones by flavoproteins might be responsible for a large variety of biological activities.[1] The reduction of quinones and the oxidation of hydroquinones to afford semiquinones has been related to biological properties such as quinone cytotoxicity[2,3] and antitumor activity,[3,4] the functioning of the antioxidant defense system,[5] and the inhibition of 5-lipoxygenase.[6] In addition, oxidation of catechol or phenol moieties of a series of hydroxylated flavones, chromones and acetophenones to yield quinoid structures is a possibility that has been argued in relation with their mutagenic activity, although an aryl ketone function appears to be critical in this regard.[7]

On the other hand, it is well known that cancer cells have a lower rate of respiration than normal cells,[8–12] apparently due to mitochondrial aberrations and/or a decrease in the number of mitochondria.[8–11] Inhibition of the already low mitochondrial activity of tumor cells may be expected to cause a profound deficit in intracellular ATP needed for a broad range of cellular functions, thus triggering a complex chain of events leading eventually to cell death, while normal cells should be able to recover from such a treatment. Several classes of chemical compounds inhibiting cellular respiration have been studied with the goal of developing antineoplastic agents.[13–18] Among them, some simple or complex phenols are known for their anticarcinogenic or antitumorigenic activities.[14,15] Many of these compounds appear to act by inhibiting mitochondrial electron transport,[19–21] and/or decoupling oxidative phosphorylation,[18] although it may be hypothesized that at least in some cases these activities are due to catecholic or 1,4-hydroquinone metabolites or the corresponding semiquinone free radicals.

Two plausible mechanisms could explain the generation of free radicals arising of these phenolic compounds. (1) The abstraction of the hydrogen atom from the

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hydroxyl group as in phenolic antioxidants.[22] (2) Monoelectronic oxidation followed by the loss of the hydroxyl group's proton.[23] In both cases, the radical formed is the same.

It seems noteworthy that a phenolic aryl ketone grouping is a common feature of many of these biologically active compounds. We have now screened a set of bicyclic *ortho*-carbonyl-substituted hydroquinone derivatives, and the simple model compounds 2′-hydroxyacetophenone, 4′-hydroxyacetophenone and 2′,5′-dihydroxyacetophenone for the inhibition of oxygen consumption by TA3 murine carcinoma cells and the methotrexate-resistant subline TA3-MTX-R. This preliminary study has assessed the effects of carbonyl substitution next to the phenolic hydroxyls, which had been suggested as a critical feature for mutagenic activity.[7] Additionally the effect on cellular oxygen consumption of selective blockage of one or the other of the hydroxyl groups, or the ability of the one next to the carbonyl to form an intramolecular hydrogen bond, has been evaluated.

Results and Discussion

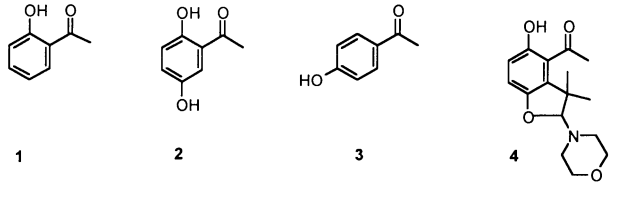
Compounds **1**–**3** are simple models meeting the primary requirement of a carbonyl group *ortho* to a phenol function. It may be seen in Table 1 that **1** and **2** show moderate activity against both cell lines, and that the introduction of a second hydroxyl group in **2** *para* to the first appears to improve the activity somewhat in the non-resistant line. On the other hand, benzo[*b*]furan **4** is inactive, possibly due to the fact that the acetyl carbonyl group would be forced out of the ring plane by the neighboring methyl groups with the attending loss of conjugation. The results for compound **3** suggest that the aryl ketone moiety may be sufficient for the inhibition of the tumor cell respiration, although the presence of the *para* hydroxyl group may be critical.

Table 2 shows biological results for naphthalenones **5**–**9**, in which incorporation of the second unsaturated ring keeps the carbonyl group practically coplanar with the aromatic ring. For compounds **5** and **6**, their inhibitory activity against TA3 cells rises approximately two to threefold with regard to the monocyclic prototypes. Compound **7**, however, in which the phenol group neighboring the carbonyl oxygen is blocked, is inactive. Along the lines suggested above, it might be hypothesized that the electron-withdrawing character of the carbonyl group, whether *ortho* or *para*, should weaken the O–H bond and stabilize a hypothetical free radical which might be involved in the inhibition of oxygen uptake. This effect should be maximal with the carbonyl group in a strictly coplanar relationship with the hydroxylated aromatic ring, and the inactivity of **3**, in which the acetyl group is forcibly twisted out of the ring plane, is in agreement with this proposal. Furthermore, as an additional factor, the intramolecular hydrogen bond to carbonyl in compounds **5**, **6**, and **8**–**12** also weakens the O–H bond like hydrogen bond-acceptor solvents do.[24] In contrast, however, conjugated carbonyl groups would be expected to increase the oxidation potential, making interpretation less straightforward.

The fact that compound **6** is more active than **5** against both cell lines might be correlated with the stability of the corresponding radical intermediates or with a decrease of the oxidation potential. The antioxidant activity of closely related phenols that have an oxy substituent *para* to the hydroxyl group has been correlated with the degree of stabilization of the phenoxyl radical.[22] The presence of the *gem*-dimethyl groups on C-4 forces the methoxyl carbon atom to remain close to the ring plane, a conformation in which orbital overlap between the methoxyl oxygen lone pair and the aromatic π system is optimal, lowering the oxidation potential and stabilizing the radical formed.

A comparison between the naphthalenone and tetralone systems indicates that the loss of the C-2/C-3 double bond, which should lead to greater flexibility of the partially unsaturated ring and may be expected to modify the charge distribution due to decreased conjugation of the aromatic system with the carbonyl group, only has a small effect upon the activities of the analogues on both cell lines. Thus, comparing **5** and **10** (Table 3), the difference in activities in TA3 cells may well not be significant, although in the methotrexate-resistant cells the tetralone seems to be more potent. A

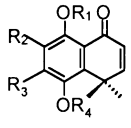
Table 1. Inhibitory activities of acetophenones **1**–**4**



1 2 3 4

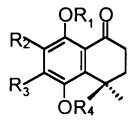
Compd	$I_{50}TA_3$ (mM)	$I_{50}TA_{3RMTX}$ (mM)
1	3.73±0.015	3.73±0.01
2	2.25±0.01	3.64±0.01
3	3.75±0.01	4.1±0.01
4	Inactive	Inactive

Table 2. Inhibitory activities of naphtalenones **5**–**9**



Compd	R_1	R_2	R_3	R_4	I_{50} TA3* (mM)	I_{50} TA3-MTX-R* (mM)
5	H	H	H	H	1.25±0.014	1.83±0.07
6	H	H	H	Me	1.09±0.014	1.5±0.1
7	Me	H	H	H	Inactive	Inactive
8	H	Br	H	H	Inactive	Inactive
9	H	H	Br	H	2.83	3.15

Table 3. Inhibitory activities of tetralones **10–12**



Compd	R_1	R_2	R_3	R_4	I_{50} TA3 (mM)	I_{50} TA3-MTX-R (mM)
10	H	H	H	H	1.50±0.07	1.12±0.02
11	H	H	H	Me	0.48±0.032	0.75±0.011
12	H	Br	H	Me	0.23±0.014	1.24±0.01

similar comparison of **6** and **11** indicates a twofold increase in potency in both cell lines. In the tetralone series, methylation of the C-5 hydroxyl group appears to be a more favorable structural change versus both cell lines, that in the naphthalenones. It should be pointed out that the loss of planarity of the carbonyl-containing ring might also lead to both a lowered oxidation potential, and a somewhat decreased stability of the free radical.

The introduction of bromine atoms in the naphtalenone molecules has very different consequences depending upon the position at which the bulky, rather weakly electronegative atom is introduced and upon concomitant structural modifications. Bromination of naphthalenone **5** at C-6 gives a product (**9**) somewhat less active than its unbrominated counterpart, but bromination at C-7 leads to complete loss of activity (cf **8** versus **5**). Comparison of **11** and **12** shows that bromination at C-7 of a hydroquinone monomethyl ether can produce a more active compound. This result, which runs contrary to our observation for the non-ether naphthalenones **5** and **8**, might be a consequence either of the presence of the ether function or of the greater flexibility of the tetralone skeleton. It has been described that quinones and hydroquinones are the only products in the disproportionation of unhalogenated semiquinones and thus this reaction is completely reversible. However, when the hydroquinone is halogenated, the concentration of semiquinone decreases dramatically suggesting that the reaction is not reversible and that therefore other decay routes are available to the intermediate radical.[25] As the free radical derived from the monomethylated hydroquinone would be expected to be more stable than the semiquinone radical of the unprotected hydroquinone, the enhanced formation of free radicals from the aryl ether might determine its greater potency as an inhibitor of cellular respiration. The stability of methoxy aryloxy radicals would presumably be insensitive to bromination or might be raised depending of the position of bromine atom. The oxidation potential would be expected to be lowered by a bromine atom at the ortho position with regard to the hydroxyl group, unlike the situation observed for the semiquinone radicals, and therefore the introduction of a bulky, hydrophobic bromine atom at C-7 might be able to exert some favorable influence on the overall activity of molecules such as **12**.

In view of the opposite effects of the carbonyl group on free radical stability and on oxidation potential, a more reliable, rational structure–activity analysis would require EPR and electrochemical studies and the assessment of the activity of derivatives with different substituents, in order to determine the relative importance of the electronic properties of these groups and the free radical formation mechanisms involved.

Experimental

Chemicals

Tris–HCl was from Sigma. Compounds **1**, **2**, and **3** were purchased from Aldrich and were used without further purification. Benzofuran **4** and hydroquinones **5–7**, **10**, and **11** were synthesized by published procedures,[26,27] and the bromo derivatives **8** and **9** were obtained as previously described.[28]

The new compound **12** was obtained in 80% yield by reaction of 8-hydroxy-5-methoxytetralone with molecular bromine in $CH_2Cl_2/Et_2O/AcOH$ (1:1:0.1). Compound **12** crystallized as needles, mp 105–107 °C. ^1H NMR ($CDCl_3$) δ 1.47 (s, 6H, $2\times CH_3$), 1.93 (t, $J=6.95$ Hz, 2H, CH_2), 2.70 (t, $J=6.95$ Hz, 2H, CH_2), 3.83 (s, 3H, OCH_3), 7.36 (s, 1H), 13.08 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 26.91, 34.49, 34.85, 38.20, 56.47, 108.38, 125.05, 138.99, 150.36, 153.41, 205.29. IR (KBr) 1655; 2952; 3435 cm^{-1}. The position of the bromine atom was assessed by HMQC and HMBC NMR experiments performed with a Bruker Avance DRX 300 NMR spectrometer. Anal. Found: C: 52.17; H: 5.14; calcd for $C_{13}H_{15}BrO_3$: C: 52.19; H: 5.05.

Cell respiration

Oxygen uptake was measured polarographically at 25 °C with a Clark electrode No. 5331 (Yellow Springs Instrument) and using a YSI model 53 monitor linked to a 100 mV single channel Goerz RE 511 recorder. The 2.0-mL reaction mixture contained 150 mM NaCl, 3 mM KCl and 10 mM Tris–HCl, pH 7.4, plus 5 mM glutamine as substrate and 2.5 mg protein/mL of either TA3 ascites tumor cells derived from mouse mammary adenocarcinoma[29] or their multidrug resistant variant TA3-MTX-R[30] as described before.[14]

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