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Growth inhibitory activity for cancer cell lines of lapachol and its natural and semi-synthetic derivatives

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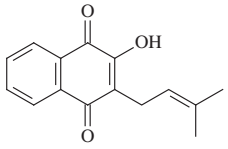
ABSTRACT

A series of 17 selected natural and semisynthetic 1,4-naphthoquinones were synthesized, and their growth inhibitory activity was evaluated in vitro. The compounds were tested on six human cancer cell lines using the MTT colorimetric assay. The data revealed that of the chemicals under study only lapachol, its acetate and 3-geranyllawsone displayed the highest activity, recording mean IC_{50} values ranging from 15 to 22 μM.

Every year approximately seven million people suffer from cancer, making this disease responsible for at least 12% of deaths worldwide.[1] A number of important new commercialized anticancer drugs have been obtained from natural sources,[2] including vinblastine, vincristine, vinorelbine, etoposide, teniposide, taxol®, taxotere®, topotecan and irinotecan from plants.[3] Trabectedin (Yondelis®) became the first marketed marine anticancer drug in 2007.[4] In fact, as emphasized by Tan et al.,[5] natural products have been the most significant source of drugs, accounting for approximately 74% of anticancer drugs. Thus, as claimed both by Coseri[1] and Gordaliza,[2] natural products represent the most valuable potential source of novel anticancer agents. We have accumulated experience in this domain over the last two decades and have developed an original screening approach based on the combined use of the conventional MTT colorimetric assay[6–9] and computer-assisted phase-contrast microscopy, that is, quantitative videomicroscopy,[7–9] to identify anticancer drugs with potentially novel mechanisms of action. As detailed below, we adopted a similar strategy of research in the current work to investigate the potential of natural and semi-synthetic 1,4-napthtoquinones structurally related to lapachol **1** as anticancer agents.

1,4-Naphthoquinones represent a huge class of natural products and are found in a wide range of plant families as well as in fungi and bacteria. Examples of naturally occurring



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1,4-naphthoquinones worthy to be mentioned include the K vitamins, juglone (isolated from the black walnut, *Juglans nigra* L. [Juglandaceae]), and plumbagin (obtained from Plumbago, *Drosera*, and *Nepenthes* spp.). Naphthoquinone derivatives have been found to exert valuable pharmacological effects, acting as cytotoxic, antibacterial, antifungal, antiviral, antiprotozoal, insecticidal, anti-inflammatory, and antipyretic agents (Grolig and Wagner, 2005).[10] Atovaquone®, a derivative of lapachol **1**, has been approved for the treatment of *Pneumocystis pneumonia*, toxoplasmosis, and malaria.[11] This latter was also tested as an anti-tumor agent by the National Cancer Institute (Bethesda, MD, USA) in the 60-cell-line panel under the NSC-759582 NCI code, but displaying a poor activity as compared to lapachol with a maximal growth inhibition percentage of 24.6% in UO-31 renal cancer cells at 10 μM. The mechanisms of action underlying the observed effects of naphthoquinone derivatives are mainly due to their capacity to interact with topoisomerases and to generate semiquinone radicals and reactive oxygen species (ROS) inside the cell.[12] Plants

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containing naphthoquinones are widely used in Southeast Asia and South America to treat malignant and parasitic diseases. In this context, lapachol **1** (2-hydroxy-3-isopentenyl-1,4-naphthoquinone) represents one of the best described examples of a research topic in natural product chemistry. Its phytochemical and pharmacological properties has been recently exhaustively reviewed.[13]

In the current work, we characterized the in vitro growth inhibitory activity of 17 selected 1,4-naphthoquinones, including **1** and its structurally related natural and semi-synthetic derivatives. The in vitro IC_{50} growth inhibitory concentration was determined for each compound in a panel of six human cancer cell lines exhibiting different levels of resistance to pro-apoptotic stimuli using the MTT colorimetric assay. The lack of a reference drug is justified upon the fact that practically all, except PC3 cells, cancer cell lines have been selected to be resistant to the most common therapeutically used chemotherapeutics.

Compounds were synthesized following three criteria: (a) esterification of the hydroxyl function of lapachol **1** with acids having different lengths of the carbon skeleton (C2, C12, C14, C16, C18 either saturated or monounsaturated); it is thought that such a chemical modification may allow these highly lipophilic derivatives to permeate the cell membrane and release the active portion inside the cell upon cleavage by esterases; (b) decrease in polarity of **1** by etherification of the OH function with 1–5 carbons chains or its transformation into –Cl; (c) replacement of the isopentenyl chain in position 3 of lapachol with other allylic or benzylic moieties. The effects of lawsone **17** was also studied to highlight the role of the *C*-side chain of lapachol on the biological activity. The chemical structures of the compounds under study are illustrated in Figure 1.

The main natural sources of lapachol **1** and lawsone **17** have been described previously.[13,14] The compounds lapachol methyl ether **8** has been previously extracted from *Rubia tinctorum* L. (Rubiaceae),[15] 3-geranyllawsone **14** has been isolated from the roots of *Conospermum teretifolium* R. Br. and *Conospermum brownii* Meisn. (Proteaceae),[16] and finally 2-chloro-3-dimethylallyl-1,4-naphthoquinone **13** has been extracted from twigs and leaves of *Avicennia germinans* L. (Avicenniaceae).

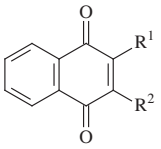
Lapachol **1** and lawsone **17** were commercially available, while the synthesis of lapachol acetate **2** and 2-chloro-3-dimethylallyl-1,4-naphthoquinone **13** were accomplished according to the procedure described previously.[18,19] All other esters (laurate, myristate, palmitate, stearate, and oleate) were obtained by reaction of lapachol **1** with acetic anhydride or the respective acyl chloride in Et_2O in the presence of Et_3N at room temperature for 30 min. The yields of the desired adducts were in the range 97–99% (Scheme 1).

O-alkylation of lapachol **1** to obtain ethers **8**–**12** was carried out in acetone at 80 °C for 1 h using methyl iodide, ethyl iodide, *n*-propyl iodide, allyl bromide, or 3,3-dimethylallyl bromide respectively in the presence of K_2CO_3 as the base, followed by acid-base work-up and crystallization from *n*-hexane. The respective adducts were obtained in 47–90% yields (Scheme 2).

Finally compounds **14**–**16**, bearing a *C*-side chain structurally different with respect to lapachol **1**, were synthesized starting from lawsone **17** using geranyl bromide, styryl bromide or the combination benzyl chloride/KI as alkylating agents and K_2CO_3 as the base in DMF at 150 °C for 2 h, followed by acid-base work-up and crystallization from *n*-hexane. The respective adducts were obtained in 37% (for the two bromides) and 38% (in the case of use of benzyl chloride) yields (Scheme 3).

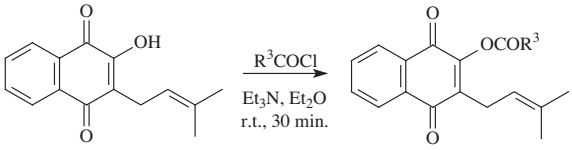
Chemical stability of esters **2**–7 was investigated by incubation of each synthesized compound in the medium used to perform pharmacological assays for 72 h. After this period in every case the percentage of recovery of each ester was >95%.

The data show that of the three chemical groups under study, esters, with the only exception of the acetate **2**, and ethers of

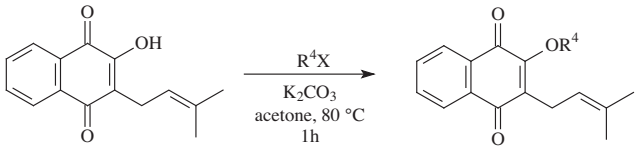


Entry	R^1	R^2
1	-OH	isopentenyl
2	-OAc	isopentenyl
3	$-OCO(CH_2)_{10}CH_3$	isopentenyl
4	$-OCO(CH_2)_{12}CH_3$	isopentenyl
5	$-OCO(CH_2)_{14}CH_3$	isopentenyl
6	$-OCO(CH_2)_{16}CH_3$	isopentenyl
7	$-OCO(CH_2)_7CH{=}CH(CH_2)_7CH_3$	isopentenyl
8	$-OCH_3$	isopentenyl
9	$-OCH_2CH_3$	isopentenyl
10	$-OCH_2CH_2CH_3$	isopentenyl
11	$-OCH_2CH{=}CH_2$	isopentenyl
12	$-OCH_2CH{=}C(CH_3)_2$	isopentenyl
13	-Cl	isopentenyl
14	-OH	geranyl
15	-OH	styryl
16	-OH	benzyl
17	-OH	H

Figure 1. Illustration of the chemical structures studied. The 17 compounds studied belong to three chemical groups, that is, esters of lapachol **1** (compounds **2**–**7** Table 1), ethers or halogen derivatives of lapachol **1** (compounds **8**–**13**; Table 1), compounds with a different *C*-side chain (compounds **14**–**16**; Table 1).



Scheme 1.



Scheme 2.

lapachol display weak or no (IC_{50} >100 μM) inhibitory activity. The same pattern has been recorded for products resulting from the substitution of the *C*-side chain of lapachol with benzyl or styryl moieties like in **15** and **16**. The retention of a terpenyl side chain in position 3 of the naphthoquinone ring like in 3-geranyllawsone **14** resulted in a decrease of activity especially against U373 and SKMEL-28 cell lines. 3-Chlorodeoxylapachol **13**, that was previously reported to exert in vitro growth inhibitory effects on Col2 (human colon cancer), KB (human oral epidermoid carcinoma), LNCaP (human hormone-dependent prostate cancer), Lu1 (human