# Potential Antipsoriatic Agents: Lapacho Compounds as Potent Inhibitors of HaCaT Cell Growth

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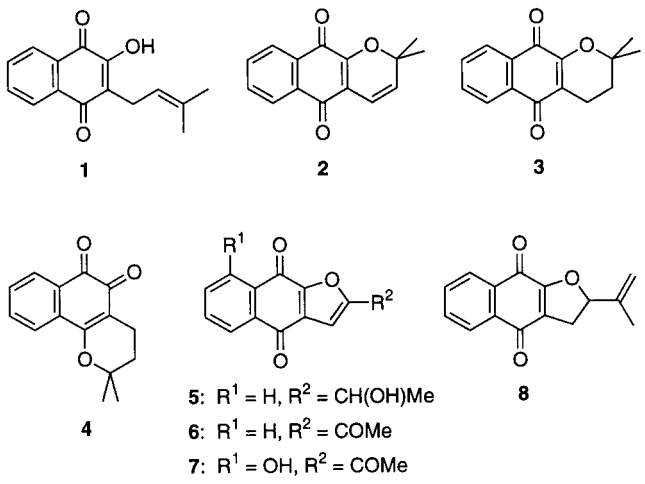
A number of lapacho compounds, representing the most common constituents of the inner bark of *Tabebuia impetiginosa*, together with some synthetic analogues, were evaluated in vitro against the growth of the human keratinocyte cell line HaCaT. With an $IC_{50}$ value of 0.7 $\mu$M, $\beta$-lapachone (**4**) displayed activity comparable to that of the antipsoriatic drug anthralin. 2-Acetyl-8-hydroxynaphtho[2,3-*b*]furan-4,9-dione (**7**), which was prepared in a four-step synthesis from 2,8-dihydroxy-1,4-naphthoquinone, was the most potent inhibitor among the known lapacho-derived compounds and inhibited cell growth with an $IC_{50}$ value of 0.35 $\mu$M. Furthermore, other active constituents of lapacho inhibited keratinocyte growth, with $IC_{50}$ values in the range of 0.5−3.0 $\mu$M. However, as already observed with anthralin, treatment of HaCaT cells with these potent lapacho compounds also caused remarkable damage to the plasma membrane. This was documented by leakage of lactate dehydrogenase into the culture medium, which significantly exceeded that of the vehicle control. Because of their potent activity against the growth of human keratinocytes, some lapacho-derived compounds appear to be promising as effective antipsoriatic agents.

Lapacho ("pau d'arco", "ipê-roxo", "taheebo") is a commercial natural product obtained from the bark of *Tabebuia* trees, which are found in the rainforests throughout Central and South America.[1] The particular species used more than any other is *T. impetiginosa* (Martius ex DC.) Standley (Bignoniaceae).[1] Lapacho has been used as a folk medicine for many years by the Maka and Callawaya Indians for the treatment of a variety of diseases,[2,3] in particular cancer.[4] Traditionally used to treat a wide range of ailments, lapacho is today used against disorders of the immune system, for example, psoriasis.[1]

The occurrence of naphthoquinones in various members of the genus *Tabebuia* is well-known.[5−8] Although localized in the heartwood of *T. impetiginosa*,[5,9] lapachol (**1**) and dehydro-α-lapachone (**2**) are also minor constituents of the inner bark[10] but not detectable in aqueous extracts.[11] Other known constituents, α-lapachone (**3**) and $\beta$-lapachone (**4**), appear to be restricted to the heartwood of *T. impetiginosa*.[5,9] A further interesting feature includes the occurrence of another group of naphthoquinones, the naphtho[2,3-*b*]furan-4,9-diones, typified by lapacho compounds **5**−**7**, and dehydro-*iso*-α-lapachone (**8**), which predominate in the lapacho extracts.[10,11] By contrast, anthracenediones occur exclusively in the heartwood.[12] In addition, a number of benzoic acid derivatives and some benzaldehydes have been isolated from the inner bark.[10]

Lapachol was shown to be active against different types of tumors[13,14] and shows antiinflammatory activity.[15] Naphtho[2,3-*b*]furan-4,9-diones **5** and **6** were active against KB cells,[6] and these compounds were also found to have immunomodulating effects on human granulocytes and lymphocytes.[16] Of the constituents isolated from the heartwood, $\beta$-lapachone has been the most extensively studied naphthoquinone. It is active in tumor models,[17,18] and its mechanism of action has been linked to reactive oxygen species generated by redox cycling of the compound.[19,20] $\beta$-Lapachone also induces apoptosis in tumor cells[21−23] and topoisomerase II-mediated DNA cleavage.[24]

In our continuing efforts aimed at the discovery of plant-derived potent antiproliferative agents and the development of potential antipsoriatic drug candidates, we have chosen lapacho compounds **1**−**8**, representing the most common constituents of the inner bark of *T. impetiginosa*, for biological evaluation. Evaluation of the usefulness of a potential antipsoriatic drug requires a demonstration that the compound is able to arrest the excessive growth of keratinocytes characteristic of psoriasis.[25] As a model for a highly proliferative epidermis, we used the nontransformed human keratinocyte line HaCaT.[26] In this study, we identified the naphtho[2,3-*b*]furan-4,9-diones **6** and **7** as highly potent inhibitors of the growth of HaCaT cells. Although **7** is a relatively simple compound, its structure, which was determined originally by spectroscopic analysis,[10] has not yet been accessible by total synthesis. Herein, we also report a preparative route to this lapacho compound.

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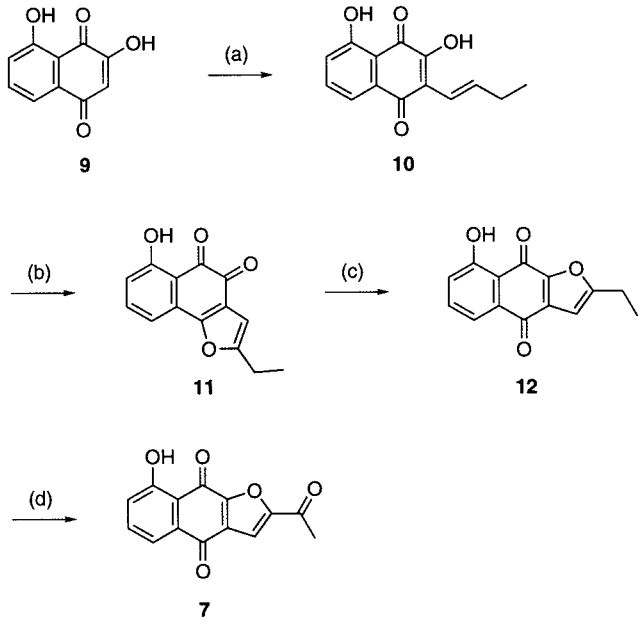
**Scheme 1.** Reagents: (a) *n*-butyraldehyde, HOAc, concentrated HCl; (b) $Hg(OAc)_2$, HOAc; (c) EtOH, HCl; (d) $Ac_2O$, HOAc, $CrO_3$




## Results and Discussion

Scheme 1 illustrates the synthesis of compound **7**, which was prepared from 2,8-dihydroxy-1,4-naphthoquinone[27] (**9**) by condensation[28] with *n*-butyraldehyde and oxidative cyclization[29] of the resulting **10** with mercuric acetate to afford the *ortho*-quinone **11**. The desired *para*-quinone **12** was obtained from **11** by rearrangement[29] under strong acid conditions. The structure of the *para*-quinone was specifically assigned[30] based on its IR and UV spectra. In the IR spectrum, the nonchelated carbonyl showed an absorption at 1672 $cm^{-1}$, whereas in **11**, the interaction by the two carbonyl groups shifts the absorption to a higher wavenumber. In the UV spectrum, the red **11** showed absorption bands at a longer wavelength than the orange-yellow **12**. Final oxidation of the ethyl side chain of **12** using chromium(VI) oxide gave 2-acetyl-8-hydroxynaphtho[2,3-*b*]-furan-4,9-dione (**7**).

All of the lapacho compounds and the synthetic intermediates **10**−**12** were evaluated for antiproliferative activity against the HaCaT keratinocyte cell line as demonstrated by reduction in cell number over time as compared to control plates. Activity was measured directly by counting the dispersed cells under a phase-contrast microscope. To have a direct antipsoriatic activity comparison model, the antipsoriatic drug anthralin was used as a standard. The concentrations required to inhibit 50% of cell growth are shown in Table 1. With the exception of lapachol, **2**, **9**, and **10**, all compounds inhibited 50% keratinocyte growth, with $IC_{50}$ values in the range of 0.35−10 $\mu$M. $\beta$-Lapachone displayed activity comparable to that of the antipsoriatic anthralin. The 2-acetylated naphtho[2,3-*b*]furan-4,9-diones **6** and **7** were the most potent inhibitors. Reducing the acetyl moiety of these compounds to hydroxyethyl or ethyl groups as in **5** and **12**, respectively, decreased antiproliferative activity by about one order of magnitude. However, rearranging the linear *para*-quinone **12** to the angular *ortho*-quinone **11** once more increased activity against keratinocyte growth. Furthermore, compounds preserving the O atom of the anellated furan of the most potent inhibitors **6** and **7**, while eliminating the furan ring (**1**, **9**, **10**), were inactive. Finally, antiproliferative activity of the linear analogue **3** of $\beta$-lapachone and its dehydrogenated congener **2** was dramatically decreased or not apparent at 10-$\mu$M concentration, respectively.

**Table 1.** Antiproliferative Activity and Cytotoxicity against HaCaT Cells by Lapacho Compounds

| compound | AA[a] $IC_{50}$ ($\mu$M) | LDH[b] (mU) |
|---|---|---|
| lapachol (**1**) | >10 | ND |
| dehydro-α-lapachone (**2**) | >10 | ND |
| α-lapachone (**3**) | 10[c] | ND |
| $\beta$-lapachone (**4**) | 0.7[c] | 329 |
| **5** | 3.7[c] | ND |
| **6** | 0.5[c] | 331 |
| **7** | 0.3[c] | 346 |
| dehydro-*iso*-α-lapachone (**8**) | 4.3[c] | ND |
| **9** | >10 | ND |
| **10** | >10 | ND |
| **11** | 0.9[c] | 235 |
| **12** | 3.0[c] | ND |
| anthralin[d] | 0.7[c] | 294 |

[a] Antiproliferative activity against HaCaT cells. [b] Activity of LDH (mU) release in HaCaT cells after treatment with 2-$\mu$M test compound (vehicle control: 135 mU/mL), $n = 3$, SD < 10%, $p < 0.05$. [c] Inhibition of cell growth was significantly different with respect to that of the control, $n = 3$, $p < 0.05$. [d] Standard antipsoriatic agent. ND = not determined.

Because of the well-known in vitro cytotoxicity associated with naphthoquinones, which results from reactive oxygen species generated during redox cycling between the quinone and reduction products,[31] keratinocytes were also tested for their susceptibility to cytotoxic action of the most potent lapacho compounds on plasma membrane integrity. To confirm that inhibition of keratinocyte growth by the most potent inhibitors is not a result of membrane damage, cell viability was assessed on the basis of leakage of lactate dehydrogenase (LDH) from cells. Treatment of HaCaT cells with standard anthralin caused remarkable damage, documented by LDH release, which significantly exceeded that of the vehicle control. Also, treatment of the cells with the highly potent inhibitors $\beta$-lapachone, **6**, and **7** at a 2-$\mu$M concentration resulted in substantially increased LDH release as compared to controls (Table 1). The relatively high amounts of LDH leakage document elevated membrane damage by these lapacho compounds. The results obtained with $\beta$-lapachone are consistent with observations that this compound is a stimulator of lipid peroxidation.[19,32] Oxygen-radical-induced peroxidative damage to membrane lipids has long been regarded as a critical event leading to cell injury.[33]

In conclusion, a number of lapacho compounds are potent inhibitors of human keratinocyte growth at sufficiently low concentrations to warrant investigation as antipsoriatic agents in more sophisticated systems. In particular, naphtho[2,3-*b*]furan-4,9-diones are considered to be the therapeutic active ingredients of lapacho for the treatment of psoriasis. These findings encourage the design and synthesis of new lapacho compounds and their evaluation for antipsoriatic activity in a variety of biological systems. Because naphthoquinones are capable of undergoing redox cycling with the generation of oxygen radicals that relate to their cytotoxic action, which could preclude a possible use in dermatology, an optimal balance of antiproliferative activity and redox properties of the compounds has to be achieved. We are now pursuing structural modifications in these series of compounds in order to more closely define structure−activity relationships.

## Experimental Section

**General Experimental Procedure.** For analytical instruments and methods, see Müller et al.[25] α-Lapachone (**3**) and

$\beta$-lapachone (**4**) were prepared according to known methods.[34] Compounds **1**, **3**, and **8** and compounds **2**, **5**, and **6** were kind gifts from Prof. Dr. Dr. H. Wagner, University of Munich, and Dr. J. Steinert, Medizinische Hochschule of Hannover, respectively.

**2-(But-1-enyl)-3,5-dihydroxy-1,4-naphthoquinone (10).** A mixture of 2,8-dihydroxy-1,4-naphthoquinone[27] (**9**, 8.50 g, 44.6 mmol) and *n*-butyraldehyde (16.20 g, 225 mmol) in HOAc (150 mL) and concentrated HCl (8 mL) was stirred at 80 °C for 1.5 h. Then, the hot solution was poured into ice-water (250 mL) and extracted with $Et_2O$ (4 × 50 mL), and the combined organic phase was extracted with a solution of 5% $Na_2CO_3$ (4 × 150 mL). The combined aqueous solutions were acidified with concentrated HCl. The product was filtered by suction, purified by chromatography on Si gel ($CH_2Cl_2$), and recrystallized from $CH_2Cl_2$−hexane to give red crystals: 11% yield; mp 125−126 °C; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 205 (4.41), 232 (4.24), 270 (4.35), 408 (3.68) nm; FTIR (KBr) $\nu_{max}$ 3251, 1619 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$ 11.14 (1H, s, OH-5), 7.68−7.07 (4H, m, OH-3, Ar), 7.13 (1H, td, $J$ = 16.2, 6.7 Hz, H-2′), 6.60 (1H, td, $J$ = 16.2, 1.7 Hz, H-1′), 2.33 (2H, m, $J$ = 7.5, 6.9 Hz, C$H_2$), 1.12 (3H, t, $J$ = 7.5 Hz, C$H_3$); MS (70 eV) *m*/*z* 244 (100, $M^{\bullet+}$); *anal.* C 68.62%, H 4.98%, calcd for $C_{14}H_{12}O_4$, C 68.85%, H 4.95%.

**2-Ethyl-6-hydroxynaphtho[1,2-*b*]furan-4,5-dione (11).** A solution of $Hg(OAc)_2$ (0.42 g, 1.32 mmol) in HOAc (5 mL) was treated with **10** (50 mg, 0.22 mmol) in HOAc (5 mL). After 30 min at room temperature the reaction mixture was heated to 65−70 °C for 15 min and then allowed to cool to room temperature. The precipitated $Hg_2(OAc)_2$ was removed by filtration, and the solvent was removed under vacuum. The deep red residue was dissolved in $Et_2O$ (30 mL), washed with $H_2O$ (3 × 30 mL), and dried over $Na_2SO_4$. The solvent was removed, and the product was purified by chromatography on Si gel ($CH_2Cl_2$). Several recrystallizations from $CH_2Cl_2$−hexane afforded red crystals; however, a more satisfactory elemental analysis was not obtained: 23% yield; mp 153−156 °C; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 229 (4.33), 262 (4.37), 408 (3.50), 486 (3.50) nm; FTIR (KBr) $\nu_{max}$ 3117, 1679, 1638 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$ 11.99 (1H, s, OH), 7.55−6.95 (3H, m, Ar), 6.45 (1H, s, H-3), 2.75 (2H, q, $J$ = 7.5 Hz, C$H_2$), 1.32 (3H, t, $J$ = 7.5 Hz, C$H_3$); MS (70 eV) *m*/*z* 242 (66, $M^{\bullet+}$), 199 (100, M − CO, −$Me^{\bullet}$); *anal.* C 69.42%, H 4.16%, calcd for $C_{14}H_{10}O_4$, C 68.62%, H 5.04%.

**2-Ethyl-8-hydroxynaphtho[2,3-*b*]furan-4,9-dione (12).** The crude **11** was dissolved in EtOH (15 mL) and treated with concentrated HCl (15 mL), and the mixture was heated to 75−80 °C for 2.5 h. To the hot solution was added activated charcoal, then it was filtered, cooled to room temperature, and diluted with $H_2O$ (25 mL). The crystallized product was filtered off, purified by chromatography on Si gel ($CH_2Cl_2$), and recrystallized from EtOAc to give orange-yellow crystals: 31% yield; mp 152−153 °C (subl.); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 205 (4.38), 235 (4.34), 2.49 (4.34), 294 (3.85), 424 (3.85) nm; FTIR (KBr) $\nu_{max}$ 3124, 1672, 1644 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$ 12.10 (1H, s, OH), 7.72−7.23 (3H, m, Ar), 6.62 (1H, s, H-3), 2.86 (2H, q, $J$ = 7.5 Hz, C$H_2$), 1.36 (3H, t, $J$ = 7.5 Hz, C$H_3$); MS (70 eV) *m*/*z* 242 (100, $M^{\bullet+}$); *anal.* C 68.95%, H 4.26%, calcd for $C_{14}H_{10}O_4$, C 69.42%, H 4.16%.

**2-Acetyl-8-hydroxynaphtho[2,3-*b*]furan-4,9-dione (7).** To a stirred solution of **12** (0.28 g, 1.16 mmol) in HOAc (5 mL) and $Ac_2O$ (4 mL) was added, slowly and in portions over 8 h, $CrO_3$ (0.35 g, 3.48 mmol). After 6 days (TLC control) the solution was diluted with ice-water (100 mL), the product was filtered off, purified by chromatography on Si gel ($CH_2Cl_2$), and recrystallized from $CH_2Cl_2$−hexane to give orange-yellow crystals: 7% yield; mp 215−218 °C (lit.[10] mp 212−215 °C).

**Biological Assay Methods.** HaCaT keratinocyte proliferation assay and LDH release were described previously in full detail.[25,35,36]

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