Brazilian Journal of Medical and Biological Research (2007) 40: 1399-1402
ISSN 0100-879X Short Communication

Cytotoxic and DNA-topoisomerase effects of lapachol amine derivatives and interactions with DNA

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Abstract

The cytotoxic activity of amino (3a-e), aza-1-antraquinone (4a-e) lapachol derivatives against Ehrlich carcinoma and human K562 leukemia cells was investigated. Cell viability was determined using MTT assay, after 48 (Ehrlich) or 96 h (K562) of culture, and vincristine (for K562 leukemia) and quercetin (for Ehrlich carcinoma) were used as positive controls. The results showed dose-dependent growth-inhibiting activities and that the amino derivatives were active against the assayed cells, whereas the 4a-e derivatives were not. The allylamine derivative 3a was the most active against Ehrlich carcinoma, with IC_{50} = 16.94 ± 1.25 μM, and against K562 leukemia, with IC_{50} = 14.11 ± 1.39 μM. The analogous lawsone derivative, 5a, was also active against Ehrlich carcinoma (IC_{50} = 23.89 ± 2.3 μM), although the 5d and 5e derivatives showed lower activity. The interaction between 3a-d and calf thymus DNA was investigated by fluorimetric titration and the results showed a hyperchromic effect indicating binding to DNA as presented of ethidium bromide, used as positive control. The inhibitory action on DNA-topoisomerase II-α was also evaluated by a relaxation assay of supercoiled DNA plasmid, and the etoposide (200 μM) was used as positive control. Significant inhibitory activities were observed for 3a-d at 200 μM and a partial inhibitory action was observed for lapachol and methoxylapachol.

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Research supported by CNPq, CAPES, and FAPERJ-PRONEX.

Received January 25, 2007
Accepted June 28, 2007

Key words
- Lapachol
- Amino-lapachol derivatives
- Cytotoxicity
- DNA-topoisomerase
- DNA-interactions

The quinone structure is common to many natural products and is associated with anticancer, antibacterial, antimalarial, antifungal, and trypanocidal activities (1). Some of these quinones act as vital links in the electron transport chain playing important roles in the biochemistry of energy production in their natural hosts, while many others show pronounced cytotoxic and allergenic actions that might enable the hosts to defend themselves against invading pathogens (2). The presence of the nitrogen atom in simple alkylamino derivatives or in fused heterocycles is related to a wide range of biological properties in quinone compounds (3).

Lapachol 1, a prenyl naphthoquinone, is

isolated from plants of the Bignoniaceae family, such as several species of the *Tabebuia* genus, native to Central and South America (4). A number of synthetic derivatives of lapachol, such as mono-(arylimines)-*o*-quinones derived from ß-lapachone, have show cytotoxicity against human cancer cells (3), and tricyclic furano and pyrano-1,4-naphthoquinone derivatives have shown potent cytotoxicity against cell lines derived from human tumors (A-549, MCF-7, HT-29) (5,6). Furthermore, naphthoquinones related to lapachol 1 have been shown to exhibit a notable cancer preventive potential (7).

DNA supercoiling is a precisely regulated process that influences DNA replication, transcription and packaging. DNA topoisomerases are enzymes that modulate the topological state of DNA. The interest in these enzymes has increased in the last few years because they are a target for many effective drugs in cancer treatment (8).

In the present study, we report the *in vitro* cytotoxic activity of lapachol and lawsone amino derivatives toward Ehrlich carcinoma, kindly provided by Dr. Noema F. Grynberg, Instituto Nacional do Câncer (Figure 1). We also investigated the cytotoxic activity of the most active lapachol amines toward human leukemia K562 cells (kindly provided by Dr. Vivian M. Rumjanek, Departamento de Bioquímica Médica, UFRJ). The interactions of these derivatives with calf thymus DNA and their inhibitory effect on human DNA-topoisomerase II-α were also studied.

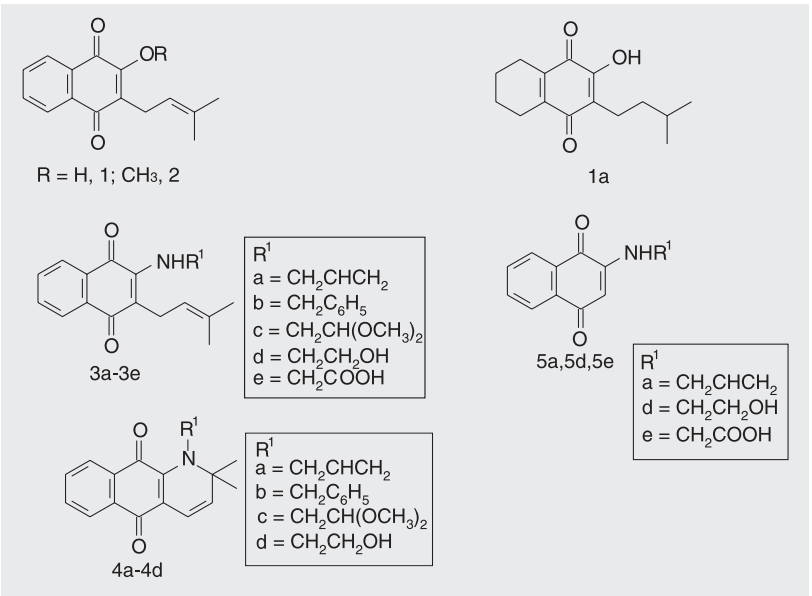

Figure 1. Structures of lapachol and lawsone derivatives.

The synthesis of the lapachol amine derivatives 3a-3e, 1-azaanthraquinones 4a-4d (9,10) and the amine compounds 5a, 5d and 5e (3) has been reported previously.

The cytotoxic activity of these compounds (see Figure 1) was investigated against murine Ehrlich carcinoma (1 x 10^5 cells), and that of compounds 3a-3d, against human K562 leukemia (2 x 10^4 cells) in RPMI complete medium (supplemented with 10% fetal calf serum, 50 µM 2-mercaptoethanol, 100 IU/mL penicillin, and 100 µg/mL streptomycin), seeded onto 96-well microplates. The compounds were dissolved in DMSO (0.3%, v/v), diluted at concentrations of 100, 50, 25, and 12.5 µM, and added to the cells, which were incubated for 48 (Ehrlich) and 96 h (K562) at 37ºC in the presence of 5% CO_2. The same volume of DMSO was used as negative control.

Cell viability was determined by the 3,4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide (MTT) assay (11) in quadruplicate. After 45 (Ehrlich) or 93 h (K562) of cell culture, MTT was added to the samples and absorbance was measured at 570 nm after 3 h at 37ºC. The IC_{50} values (µM) are reported as means ± SD of three independent experiments. Vincristine (for K562 leukemia) and quercetin (for Ehrlich carcinoma) were used as positive controls.

The lapachol derivatives presented concentration-dependent growth-inhibiting activities on cultured Ehrlich carcinoma and K562 leukemia cells. The IC_{50} values were 16.94 ± 1.25 (3a), 23.70 ± 1.80 (3b), 25.28 ± 1.73 (3c), 17.47 ± 1.06 (3d), and 19.71 ± 0.06 (3e) µM for Ehrlich carcinoma cells, and 14.11 ± 1.39 (3a), 18.95 ± 1.80 (3b),

17.80 ± 1.77 (3c), and 23.51 ± 0.81 (3d) µM for K562 leukemia cells. Compound 3a was the most active against both types of cancer cells. Furthermore, all amine derivatives of lapachol (3a-e) showed higher antiproliferative activities against Ehrlich carcinoma than the positive control (quercetin, IC_{50} = 44.0 µM). However, despite the promising IC_{50} values of compounds 3a-d against K562 leukemia cells, the positive control (vincristine, IC_{50} = 60.0 nM) showed better antiproliferative activity.

Lapachol (1), hydrogenated lapachol (1a) and methoxylapachol (2) were also assayed against Ehrlich carcinoma cells, but presented only 30, 40, and 6% cell growth inhibition at 50 µM, respectively. Substitution of hydroxyl or methoxyl in 1 or 2, respectively, with amine groups led to increased cytotoxicity; however, the cyclic derivatives 4a-e did not exhibit cytotoxic activity against Ehrlich and K562 cells under the same conditions.

In order to evaluate the importance of the isoprenyl moiety for the activity of the 2-amino-1,4-naphthoquinone structure, the amine derivatives of lawsone 5a, 5d, and 5e were also assayed against Ehrlich carcinoma cells. These compounds showed less activity than the corresponding lapachol derivatives, indicating a contribution by the isoprenyl fragment. However, significant antiproliferative activity was observed for 5a (IC_{50} = 23.89 ± 2.3 µM), which suggests a correlation between hydrophilic effect and activity. Compounds 5d and 5e did not show activity at the concentration of 50 µM.

The effects of cytotoxic lapachol amine derivatives (3a-d) on calf thymus DNA were evaluated by fluorescence studies as described previously (12). The excitation wavelength was 276 nm, and emission was monitored at 552 nm for 3a and at 825 nm for 3b-d. The fluorescence emission spectra of 3a-d in the presence of increasing DNA concentrations (1:1, 1:3, 1:5, 1:7, and 1:10) was compared to 3a-d in the absence of DNA after 24-h incubation at 37ºC. The concentrations of bound and free 3a-d could be determined from the emission spectra assuming formation of single-association complexed species. The data were analyzed according to the Scatchard procedure (13).

The results of fluorimetric titration of 3a-d with calf thymus DNA indicated a hyperchromic effect similar to that of ethidium bromide, an intercalator used as positive control (14), thus suggesting that these compounds interact with DNA. Figure 2A shows the superposed fluorescence emission spec-

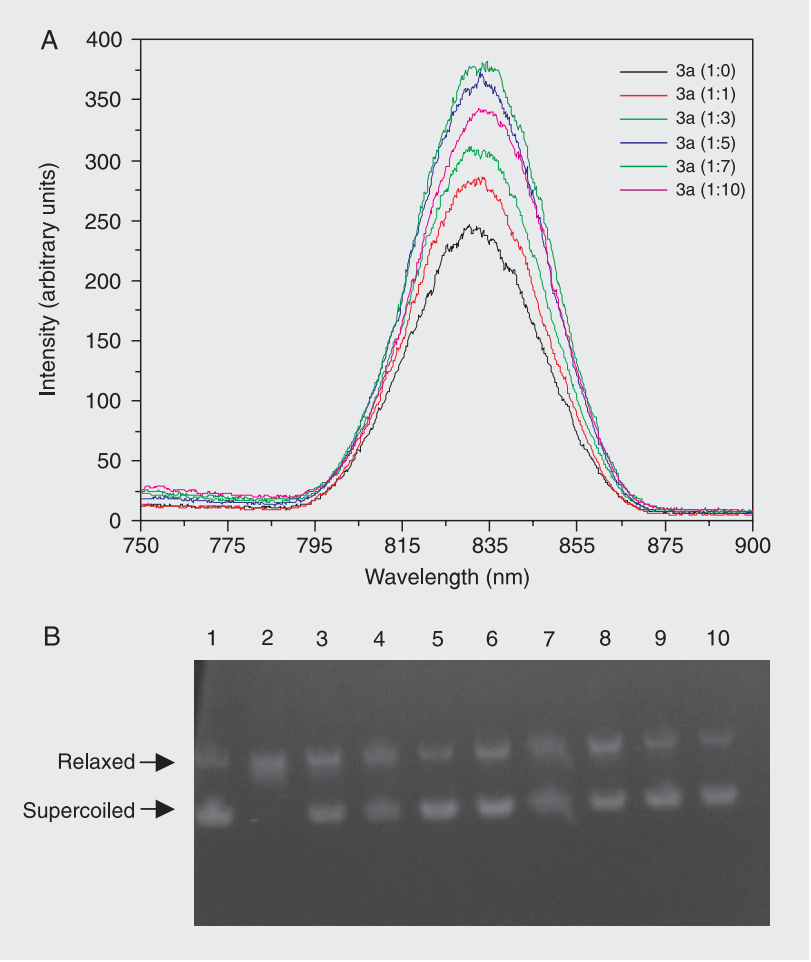

Figure 2. *A*, Fluorescence emission spectra for free 3a (0.12 µM) and 3a bound to DNA (1.5 µM). *B*, Effect of lapachol derivatives on DNA-topoisomerase II-α (Topo II-α). *Lane 1*, pBR322 (0.250 µg) only; *lane 2*, pBR322 + Topo II-α (1 U); *lane 3*, Topo II-α + 1 (200 µM); *lane 4*, Topo II-α + 2 (200 µM); *lane 5*, Topo II-α + 3a (200 µM); *lane 6*, Topo II-α + 3d (200 µM); *lane 7*: Topo II-α + 3c (200 µM); *lane 8*, Topo II-α + 3b (200 µM); *lane 9*, Topo II-α + 3e (100 µM); *lane 10*, Topo II-α + etoposide (200 µM). 1 = lapachol; 2 = methoxylapachol; 3a-e = lapachol amine derivatives.

tra of 3a in the absence and in the presence of increasing DNA concentrations. The quenching constants *K*s were obtained by Scatchard's procedure in the range of *K*s = 2 to 3 x 10^3 M^{-1}.

The inhibitory effects of lapachol and lawsone amine derivatives on human DNA-topoisomerase II-α were also studied. Topoisomerase II-α was assayed by relaxation of supercoiled plasmid DNA, as suggested by TopoGen, Columbus, OH, USA. Briefly, 0.25 µg supercoiled pBR322 was incubated with 1 unit human topoisomerase II-α and 0.1 M ATP in the presence or absence of drugs (200 µM) for 30 min at 37ºC in reaction buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.15 M NaCl, 0.1 mM spermidine, 4% glycerol, and 0.1% BSA) and the reaction was stopped with 0.1 volume of stopping solution (5% SDS, 0.025% bromophenol blue, and 40% glycerol). The relaxation products were analyzed in TAE buffer (40 mM Tris-acetate, pH 8.5, and 10 mM Na_2EDTA) by electrophoresis on 1% agarose gels at 50 V. Gels were stained with ethidium bromide and photographed under UV light.

The inhibitory effects of human DNA-topoisomerase II-α by lapachol amine derivatives are shown in Figure 2B. A significant inhibitory action of the enzyme was observed for all compounds (200 µM, 3a-d), as indicated by the marked presence of the supercoiled band. The same effect was observed for etoposide (200 µM) used as positive control (15). Interestingly, a partial inhibitory action was observed for lapachol (1) and methoxylapachol (2).

In conclusion, substitution of the hydroxyl and methoxyl groups in lapachol and lawsone by amine groups led to a moderate increase in the cytotoxic activity against Ehrlich cells. However, the azaanthraquinones resulting from cyclization of the amines were inactive. All amine derivatives of lapachol were more active than the corresponding lawsone derivatives, thus indicating that the hydrophobic isoprenyl moiety is important for activity.

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