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Semisynthesis and antitumoral activity of 2-acetylfuranonaphthoquinone and other naphthoquinone derivatives from lapachol

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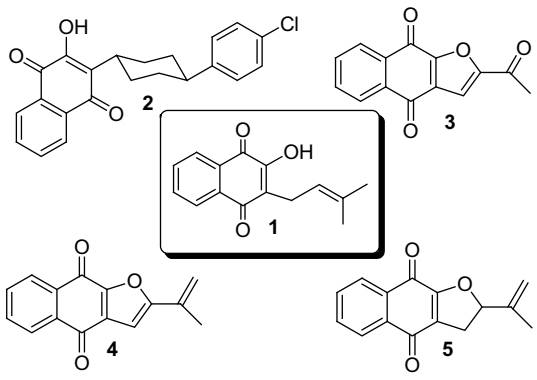
ABSTRACT

Ozonolysis of lapachol (**1**), resulting in an unusual formation of a potent antitumor agent 2-acetylfuranonaphthoquinone (**3**) along with the expected aldehyde **6**, is described. The reaction of lapachol (**1**) with CAN in dry acetonitrile leading to biologically active furanonaphthoquinones is also reported. The antitumoral activity of the tested compounds on human DU-145 prostate carcinoma cells was evaluated following XTT assay. The results revealed that 2-(1-methylethenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione (**5**), β-lapachone (**10**) and dehydro-β-lapachone diacetate (**11**) showed 100% inhibition at 25 μg/ml. All the tested samples showed dose-dependent activity.

Lapachol (**1**), a principal constituent of *Newbouldia leavis* (Bignoniaceae family)[1,2] exhibits wide spectrum of biological activities such as antibacterial, antiplasmodial, antioxidant and trypanocidal activities.[2] Lapachol has also been used as anti-microbial and anti-protozoal agents and many of its derivatives are proved to be potent antimalarial and anti-HIV agents. Atovaquone® (**2**), a derivative of lapachol, has already been approved for the treatment of *Pneumocystis* pneumonia, toxoplasmosis and malaria.[3]



In addition, furanonaphthoquinones (**3**–**5**), also phytoconstituents of Bignoniaceae family, are known to exhibit significant biological activities. Among those, 2-acetylfuranonaphthoquinone (**3**) in particular shows superior antitumoral activity[4] and the influence of furan/pyran-ring in the antitumoral activity has recently been demonstrated.[5] As a consequence of its biological significances, 2-acetylfuranonaphthoquinone (**3**) has become an important synthetic target and several studies have been carried out towards the synthesis of this potent antitumor agent.[6]

Since 2-acetylfuranonaphthoquinone (**3**) is available in minute amounts from nature, it is essential to synthesize this biologically active molecule in large quantities for conducting structure–activity investigation as well as to make various analogs of this compound for biological assay. Lapachol is abundant in nature and hence it is of our interest to develop methods for the semisynthesis of biologically active and pharmaceutically important furanonaphthoquinone molecules through efficient manipulation of lapachol. In this communication, we report simple approaches for the synthesis of 2-acetylfuranonaphthoquinone (**3**) and other furanonaphthoquinones from lapachol.

Intriguingly, exposure of lapachol under ozonolysis conditions resulted in the formation of expected aldehyde **6** in 70% yield along with the unusual compound, 2-acetylfuranonaphthoquinone, **3** in 30% yield.

The structure of compound **3** is established by spectroscopic data[6g] and further unambiguously confirmed by single crystal X-ray analysis (Fig. 1).[7]

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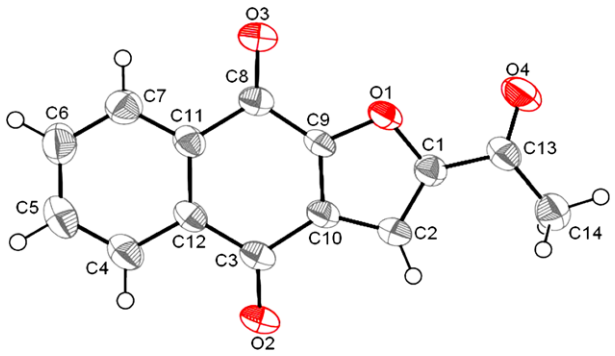


Figure 1. ORTEP diagram of compound **3.**

Further reaction of compound **6** with acetic anhydride in pyridine at room temperature resulted in (*E*)-2-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalene-2-yl)vinyl acetate (**7**) in good yield (80%) (Scheme 1).

Encouraged by the interesting reaction of lapachol with ozone leading to the biologically important compound **3**, we were motivated to gain deeper insight into the mechanism and also to improve the yield of this unusual transformation. We believed that lapachol under ozonolysis reaction conditions forms an intermediate through radical cyclization, which on further ozonolysis results in the formation of compound **3**. In order to understand this novel transformation leading to **3**, a systematic investigation on the mode of cyclization of lapachol under radical conditions was initiated.

Thus, lapachol on treatment with DDQ in dry benzene under reflux conditions underwent 6-*endo-trig* cyclization to give the corresponding dehydro-α-lapachone (**8**) in low yield (10%).[8] On the other hand, lapachol on treatment with CAN in dry acetonitrile underwent 5-*exo-trig* cyclization to give 1-(2,3,4,9-tetrahydro-4,9-dioxonaphtho[2,3-b]furany-2-yl)-1-methylethyl nitrate **9** (55%) and 2-(1-methylethenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione **5** (35%) in good yields along with 2-(1′-methylethenyl)-naphtho[2,3-b]furan-4,9-dione (**4**) in low yield (5%) (Scheme 2).[9] The structure of the major compound **9** was unambiguously confirmed by single crystal X-ray analysis (Fig. 2).[7]

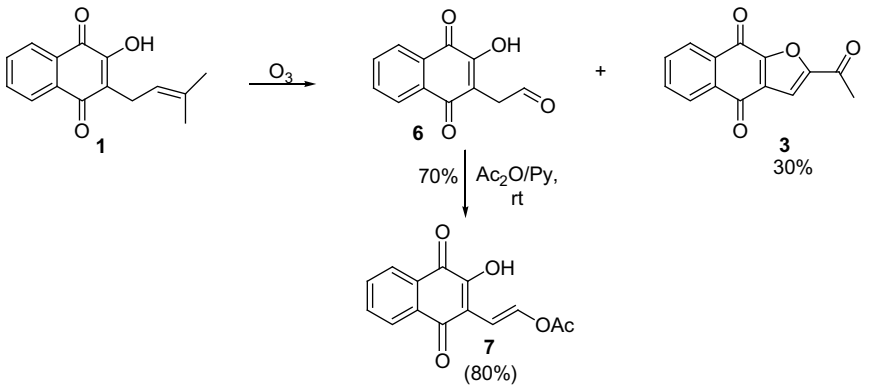
The CAN mediated radical cyclization reaction suggests that the antitumor agent **3** is likely to be formed *via* intermediate **4** during the ozonolysis of lapachol.

A plausible mechanism for the formation of compounds **4**, **5** and **9** from lapachol (**1**) under radical condition is shown in Scheme 3.

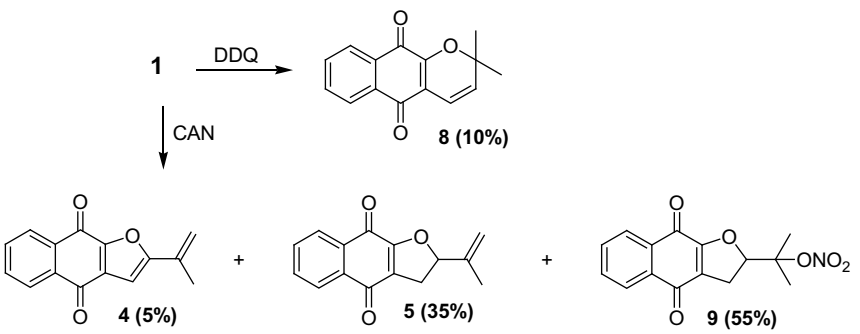
After achieving the synthesis, the anticancer activity of the synthetic compounds on human DU-145 prostate carcinoma cells was evaluated following XTT assay as described[10,11] and the results are summarized in both Figure 3 and Table 1. The results in Figure 3 indicate that compounds **5**, β-lapachone (**10**) and dehydro-β-lapachone diacetate (**11**) showed 100% inhibition at 25 μg/ml.[12] All the tested samples showed dose-dependent activity.

However, the anticancer efficacy of the tested samples is mostly highlighted by their IC_{50} values (Table 1).

From the activity obtained with a reference antineoplastic compound (doxorubicin), some of the tested compounds such as **5**, **6**, **9**, β-lapachone (**10**) and dehydro-β-lapachone diacetate (**11**) can be considered as promising antitumor candidates. Compounds **5** and **9** are known to exhibit significant cytotoxic activities.[5a]



Scheme 1. Ozonolysis reaction of lapachol.



Scheme 2. Reaction of lapachol with CAN and DDQ.

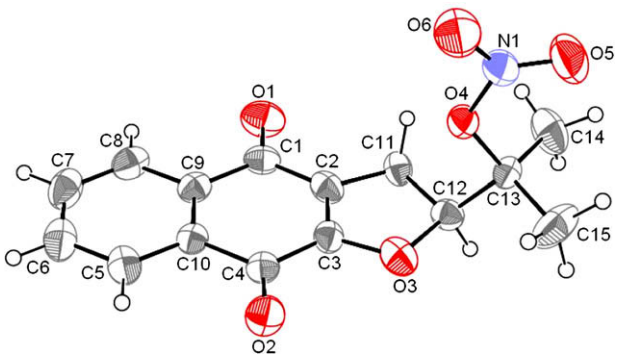
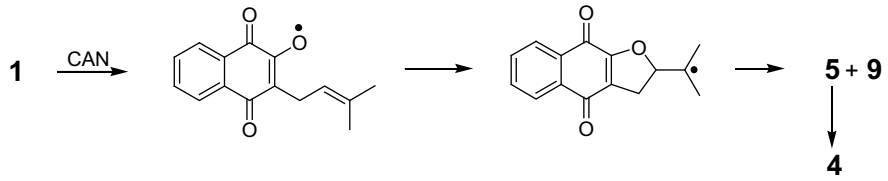


Figure 2. ORTEP diagram of compound **9**.

The activity of β-lapachone (IC_{50}: 7.4 nM) is only 1.9-fold lower that of doxorubicin (IC_{50}: 3.90 nM) while those of **6** (8.80 nM), dehydro-β-lapachone diacetate (11.14 nM) and **5** (11.67 nM) are less than 3-fold. Upon comparing the structure–activity relationship of the tested compounds, it could generally be noted that acetylation reduces the anticancer activity (compare **6** and **7**; β-lapachone and dehydro-β-lapachone diacetate). However, the formation of the pyran ring (conversion of **1** to β-lapachone) increases antitumoral activity. It has previously been shown that the pyran and the furan rings do influence the antitumoral activity of naphthoquinones.[5a] In this study, it is also confirmed that a modification in the furan ring (**3** and **5**) as well as on the furan ring substituents (**4** and **9**) induce changes in the antitumoral activity.

In conclusion, a one step protocol was developed for the conversion of lapachol into the potent antitumor agent, 2-acetylfuranonaphthoquinone (**3**), through ozonolysis,[13] while treatment of the ozonolysis co-product **6** with acetic anhydride in pyridine led to the novel vinyl acetate **7** in good yield. Since compound **4** is also a phytoconstituent of *N. leavis*, the reaction of lapachol with CAN has thrown some light on the biosynthesis of different furanonaphthoquinones isolated from *N. leavis*. The antitumoral activity of some of the synthesized naphthoquinone derivatives gives a promising basis for their possible use in the treatment of prostate cancer.



Scheme 3. Proposed mechanism for the formation of compounds **4**, **5** and **9** from lapachol.

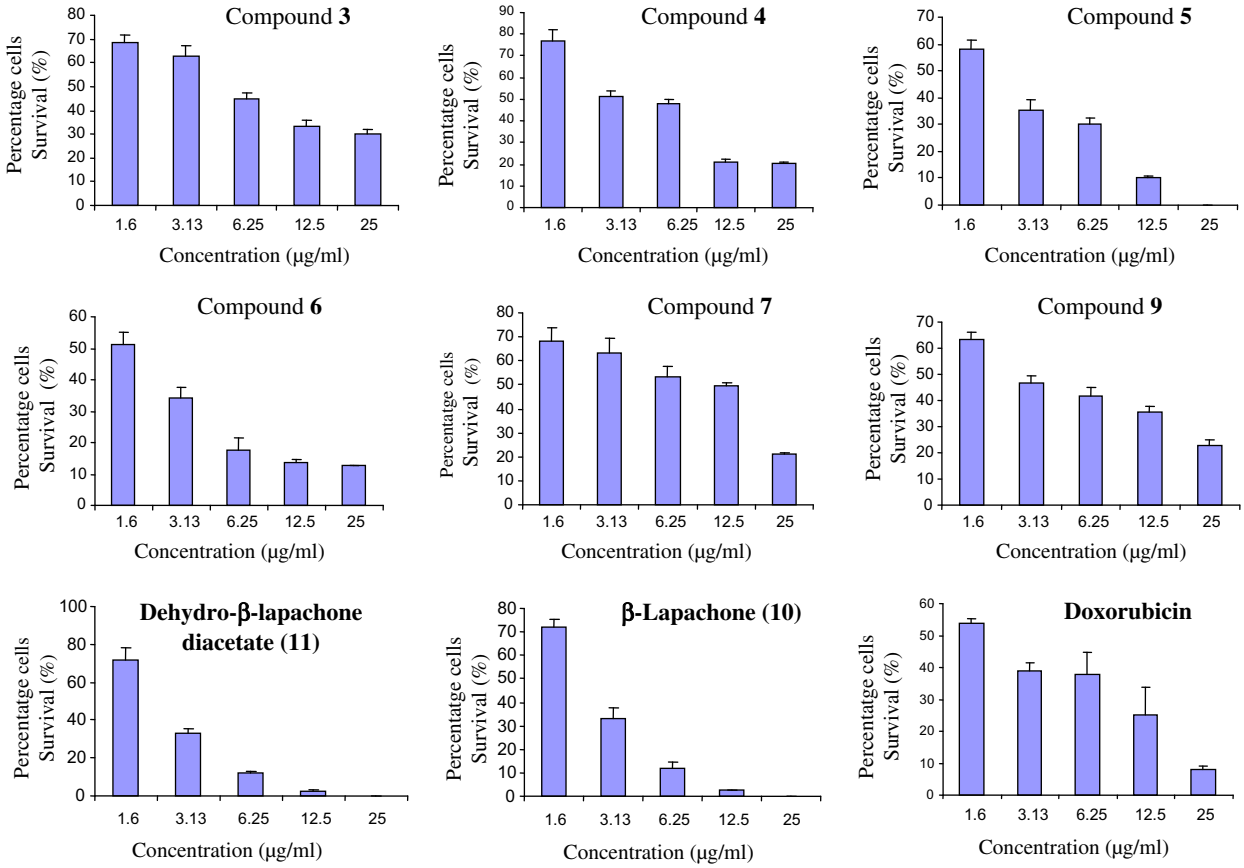
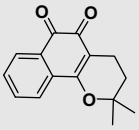
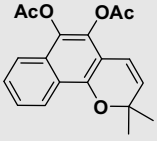


Figure 3. The effect of test samples on DU-145 prostate cancer cells. The values are expressed as means ± SEM (n = 3).

Table 1
Anticancer activity of the tested compounds on DU-145 prostate cancer cells

Tested compounds	IC_{50} in μg/ml (nM)	Inhibition percentage (%) at 25 μg/ml
1	15.63 (64.59)	78.12 ± 6.12
3	5.53 (23.04)	69.79 ± 4.18
4	4.73 (19.87)	79.79 ± 5.34
5	2.80 (11.67)	100.00 ± 0.00
6	1.90 (8.80)	87.50 ± 4.72
7	12.48 (48.37)	79.17 ± 6.08
9	4.41 (15.37)	77.08 ± 4.78
β-lapachone (**10**) 	1.80 (7.4)	100.00 ± 0.00
Dehydro-β-lapachone diacetate (**11**) 	3.63 (11.14)	100.00 ± 0.00
Doxorubicin	2.12 (3.90)	92.13 ± 1.09

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.053.

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7. Crystallographic details have been deposited at the Cambridge Crystallographic Data Centre (deposition number for compound **3**: CCDC 669086 and for compound **9**: CCDC 669085). Please see Supplementary data for more information.
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9. Under aqueous conditions, the reaction of lapachol with CAN is known to give compounds **5** (15%) and **9** (19%) in low yields Perez-Sacau, E.; Estevez-Braun, A.; Ravelo, A. G.; Yapu, D. G.; Turba, A. G. *Chem. Biodiversity* **2005**, *2*, 264.
10. *Cytotoxicity assay:* Cells were cultured in Dulbecco's minimum essential medium (DMEM) supplemented with 5% fetal calf serum (FCS), gentamycin sulfate (0.004%), glucose (0.57%) and $NaHCO_3$ (0.12%). Cells were seeded into 96-well flat-bottomed plates at a concentration of 3.0×10^5 cells per ml. After 24 h, cells were treated with compounds, which were diluted with culture medium to a final concentration of 25 μg/ml. XTT labeling reagent (50:1) was added and the absorbance (560 nm) read after 72 h.[11] Experiments were carried out three times in triplicate. Active samples (with less than 50% survival) after an exposure time of 72 h were serially diluted in a concentration range of 1.6–25 μg/ml and tested. The concentration of the sample that inhibited 50% cell proliferation (IC_{50}) was determined graphically. Doxorubicin, a known anti-tumour agent, was used as positive control.
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12. β-Lapachone (**10**)[5a] and dehydro-β-lapachone diacetate (**11**) were prepared as described in the literature Manners, G. D.; Jurd, L.; Wong, R.; Palmer, K. *Tetrahedron* **1975**, *31*, 3019.
13. *Ozonolysis reaction of lapachol:* A solution of lapachol **1** (242 mg, 1 mmol) in dry DCM (50 ml) was cooled to −78 °C and ozonised oxygen was passed till the completion of the reaction. Then the ozonide was quenched with dimethylsuphide (2 ml) at −78 °C and stirred for 1 h. The reaction mixture was washed with water and extracted with DCM (3 × 20 ml). Organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography over silica gel using 30% EtOAc in hexane as an eluent to give the corresponding aldehyde **6** (70%) and 2-acetylnaphtho[2,3-b]furan-4,9-dione **3** (30%) in good yields.