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ORIGINAL RESEARCH



# Synthesis of dehydro-α-lapachones, α- and β-lapachones, and screening against cancer cell lines

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## Abstract

14 new naphthoquinones were prepared and tested against human cancer cell lines PC-3 (prostate), HCT-116 (colon carcinoma), SNB-19 (glioblastoma), HL-60 (leukemia) and MCF-7 (breast), and a nontumor cell line L929 (murine fibroblasts) to determine cytotoxicity with the MTT assay. 8-OH-β-lapachones (**14a**, **14c**, **14d**) presented best results, showing low $IC_{50}$ values and high selectivity for HCT-116 and HL-60 tumor cells.

**Keywords** Naphthoquinone · Cancer · Dehydro-α-lapachone · Lapachone · Xyloidone

## Introduction

Naphthoquinones are important natural products that have multiple biological activities because their structural characteristics allow them to receive and transfer electrons and affect the functions of topoisomerases enzymes, and therefore, they can interfere in several biochemical mechanisms of cells (Morton 1965), bacteria, viruses, fungi, and insects (Kumagai et al. 2012; De Castro et al. 2013). For example, the family of vitamin K (e.g., vitamins K1, K2, and K3) contains several commercially available naphthoquinones used for various therapeutic purposes (Dowd et al. 1995).

Several naphthoquinones have become drugs and are available in the pharmaceutical market. Notably, doxorubicin (**1**), lapachol (**2**), atovaquone (**3**), buparvaquone (**4**), and parvaquone (**5**) may be highlighted. The natural drug **1** is used in the treatment of melanoma and prostate cancer. In the past, compound **2** aroused a great deal of pharmacological interest due to its wide spectrum of action, presenting antimicrobial action (**3**), activity against the penetration of cercariae of *Schistosoma mansoni* and use as a drug against some cancer strains in clinical treatment (Linardi et al. 1975; Epifano et al. 2014), although it is no longer a first-line option for the treatment of cancer. However, its structure served as an inspiration for the synthesis of new drugs for other diseases, such as drug **3** that is used for prevention of mild cases of infection by *Plasmodium vivax* (in combination with proguanil) (Weaver et al. 1993; Canfield et al. 1993; Olliaro and Wirth 1997), as well as for pneumocystosis, toxoplasmosis, and babesiosis (usually in combination with azithromycin). The naphthoquinones buparvaquone (**4**) and parvaquone (**5**) have their structure based on **1** and have become drugs for veterinary treatment of bovine theileriosis and leishmaniasis (Fig. 1) (Hashemi-Fesharki 1991).

Other naturally occurring naphthoquinones such as α- (**6**) and β-lapachone (**7**) and dehydro-α-lapachone or xyloidone (**8**) (De Lima et al. 1972) exhibit various biological activities. It is noteworthy that naphthoquinone **7**, which is a pro-drug currently in a Phase II clinical trial under the name ARQ 761, has demonstrated activity against a wide range of solid tumors with high levels of NQO1.

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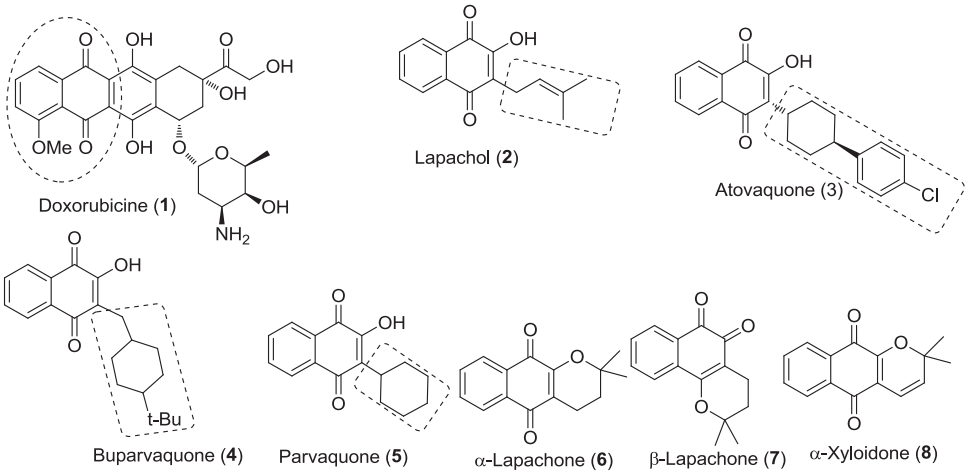



**Fig. 1** Some natural and synthetic naphthoquinones that have become drugs

Cytotoxicity against several neoplastic cell lines induced by pyran naphthoquinones is mainly related to the generation of reactive oxygen species that cause damage to cell membranes and various biomolecules. The pro-oxidant capacity of these substances is strongly affected by substituent groups on the aromatic ring (Da Rocha et al. 2011) or by modifications of the tetrahydropyran ring (Ferreira et al. 2010; De Witte et al. 2004; Shaabani et al. 2009). Therefore, several synthetic routes have been studied for the purpose of finding new, more active, and selective substances.

Dehydro-α-lapachones are found as a minor product of photochemical origin during the isolation of lapachol and can also be isolated from fungi and insects (De Oliveira et al. 1990), and more recently, their microbiological synthesis has been discovered by probiotics from lapachol (**2**) (Silva et al. 2014). These compounds present different types of biological activities such as fungicidal (Cho et al. 2006), antibacterial (De Lima et al. 1966; Machado et al. 2003), immunomodulatory (Maji et al. 2014), and antipsoriasis properties (Müller et al. 1999). Recently, it has been shown that **8** is able to inhibit vessel regeneration as it interferes with vascular anastomosis, preventing the nutrition of cancerous cells (Garkavtsev et al. 2011).

As a part of our studies aimed at the synthesis of biologically active naphthoquinones and, respectively, their antitumor evaluation, a route was elaborated to obtain dehydro-α-lapachones and subsequent transformation into α- and β-lapachones modified in the aromatic ring and pyran ring.

## Materials and methods

### Chemistry

The reagents were purchased from Sigma-Aldrich Brazil and were used without further purification. Column chromatography was performed with silica gel 60 (Merck 70-230 mesh). Analytical thin layer chromatography was performed with silica gel plates (Merck, TLC silica gel 60 F254), and the plots were visualized using UV light or aqueous solutions of ammonium sulfate. The indicated yields refer to chromatographically and spectroscopically homogeneous materials. Melting points were obtained on a Fischer-Johns apparatus and were uncorrected. Infrared spectra were measured with KBr pellets on a PerkinElmer model 1420 FT-IR Spectrophotometer, and the spectra were calibrated relative to the 1601.8 $cm^{-1}$ absorbance of polystyrene. NMR spectra were recorded on a Varian Unity Plus VXR (300 and 500 MHz) instrument in DMSO-$d_6$ or $CDCl_3$ solutions. The chemical shift data were reported in units of $\delta$ (ppm) downfield from solvent and was used as an internal standard; coupling constants ($J$) are reported in hertz and refer to apparent peak multiplicities. High-resolution mass spectra (HRMS) were recorded on a Mass spectrometer MICROMASS Q-TOF (Waters).

### Synthesis of 2,8-dihydroxynaphthalene-1,4-dione (11)

To a solution of 0.200 g of **10** (0.96 mmol) and 10 mL of methanol was added a solution of $K_2CO_3$ (1.38 g in 30 mL of MeOH and 10 mL of $H_2O$). The reaction was kept at room temperature for 15 min and additionally for 40 min under reflux. After the total consumption of the starting material **10**, 20 mL of water was added and the reaction medium was neutralized with 2 M $H_2SO_4$ solution, maintaining the reaction under stirring at room temperature for a further 10 min. The methanol was then evaporated under reduced pressure and the residue was extracted with ethyl acetate. The organic phase was washed with saturated NaCl solution, dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography employing silica gel using hexane/ethyl acetate as the eluent. The product **11** was obtained in 90% yield, and its

spectroscopy data were identical to the one in a previous report (MacLeod and Thomson 1960; Wurm et al. 1986).

## Synthesis of dehydro-α-lapachones or α-xyloidones (12a–d)

Into a microwave tube were added 0.190 g of compound **11** (1 mmol), 10 mL of a 1:1 (v/v) ethanol/water mixture, the appropriate aldehyde (1 mmol) and 1 mL of formic acid. The reaction was heated under microwave irradiation at 120 °C for a period of 2–3 h. After the total consumption of the starting material, observed by thin layer chromatography, the ethanol was evaporated under reduced pressure. To the residue was added water (10 mL) that was then extracted with ethyl acetate. The combined organic phase was washed with saturated NaCl solution and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography employing silica gel, using a mixture of hexane/ethyl acetate as the eluent to give the substituted compounds (**12a–d**) (Da Rocha et al. 2014).

### 9-hydroxy-2,2-dimethyl-2*H*-benzo[*g*]chromene-5,10-dione (12a)

The α-xyloidone (**12a**) was obtained in 80% yield, after 2 h of reaction, as a red solid of mp consistent with the literature (Matsumoto et al. 1985). IR (KBr, $cm^{-1}$): $\nu$ 1621, 1306, 1283, 1199, 1155, 1130, 730. $^{1}$H NMR ($CDCl_3$, 500 MHz): 11.87 (s, 1H, OH), 7.62 (dd, 1H, $J = 7.4$ and 1.2 Hz, H-6), 7.58 (dd, 1H, $J = 8.2$ and 7.4 Hz, H-7), 7.20 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-8), 6.63 (d, 1H, $J = 10.0$ Hz, H-4), 5.73 (d, 1H, $J = 10.0$ Hz, H-3), 1.56 (s, 6H, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 126 MHz): 184.6 (C-10), 181.0 (C-5), 161.5 (C-9), 152.1 (C-10a), 136.6 (C-7), 131.5 (C-5a), 131.3 (C-3), 123.9 (C-8), 119.0 (C-6), 118.5 (C-4a), 115.3 (C-4), 114.5 (C-9a), 80.7 (C-2), 28.4 (C-1′) ppm. HRMS: Calcd for $C_{15}H_{13}O_4^+$: 257.0808. Found: 257.0821 $(M + H)^+$.

### 9-hydroxy-3-methyl-2*H*-benzo[*g*]chromene-5,10-dione (12b)

The α-xyloidone (**12b**) was obtained in 52% yield, after 3 h of reaction, as a red solid; mp 160–163 °C. IR (KBr, $cm^{-1}$): $\nu$ 3422, 1633, 1595, 1447, 1239, 1210. $^{1}$H NMR ($CDCl_3$, 500 MHz): 11.84 (s, 1H, OH), 7.60 (dd, 1H, $J = 7.5$ and 1.4 Hz, H-6), 7.57 (dd, 1H, $J = 8.1$ and 7.5 Hz, H-7), 7.20 (dd, 1H, $J = 8.1$ and 1.4 Hz, H-8), 6.44–6.43 (m, 1H, H-4), 4.93–4.92 (m, 2H, H-2), 1,86–1,85 (m, 3H, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz): 184.1 (C-10), 181.1 (C-5), 161.4 (C-9), 151.0 (C-10a), 136.5 (C-7), 133.9 (C-3), 131.4 (C-5a), 124.0 (C-8), 120.4 (C-4a), 119.0 (C-6), 114.2 (C-9a), 112.2 (C-4), 70.6 (C-2), 19.3 (C-1′) ppm. HRMS: Calcd for $C_{14}H_9O_4^-$: 241.0506. Found: 241.0508 $(M - H)^-$.

### 9-hydroxy-2-methyl-2*H*-benzo[*g*]chromene-5,10-dione (12c)

The α-xyloidone (**12c**) was obtained in 61% yield, after 3 h of reaction, as a red solid; mp 160–163 °C. IR (KBr, $cm^{-1}$): $\nu$ 3412, 1628, 1589, 1456, 1265, 1199, 731. $^{1}$H NMR ($CDCl_3$, 300 MHz): 11.82 (s, 1H, OH), 7.64–7.55 (m, 2H, H-6 and H-7), 7.20 (dd, 1H, $J = 7.7$ and 1.9 Hz, H-8), 6.67 (dd, 1H, $J = 10.0$ and 1.6 Hz, H-4), 5.80 (dd, 1H, $J = 10.0$ and 3.4 Hz, H-3), 5.33–5.24 (m, 1H, H-2), 1.55 (d, 3H, $J =$ 6.6 Hz, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz): 184,5 (C-10), 180,9 (C-5), 161,5 (C-9), 152,4 (C-10a), 136,7 (C-7), 131,5 (C-5a), 127,5 (C-3), 124,0 (C-8), 119,1 (C-4a), 119,0 (C-6), 116,6 (C-4), 114,4 (C-9a), 74,43 (C-2), 21,6 (C-1′) ppm. HRMS: Calcd for $C_{14}H_{11}O_4^+$: 243.0652. Found: 243.0654 $(M + H)^+$.

### 9-hydroxy-2-propyl-2*H*-benzo[*g*]chromene-5,10-dione (12d)

The α-xyloidone (**12d**) was obtained in 67% yield, after 2 h of reaction, as a orange solid; mp 109–110 °C. IR (KBr, $cm^{-1}$): $\nu$ 3435, 1642, 1622, 1584, 1453, 1309, 1261, 1201, 1156, 726. $^{1}$H NMR ($CDCl_3$, 500 MHz): 11.84 (s, 1H, OH), 7.62 (dd, 1H, $J = 7.4$ and 1.3 Hz, H-6), 7.58 (dd, 1H, $J = 8.2$ and 7.6 Hz, H-7), 7.21 (dd, 1H, $J = 8.2$ and 1.3 Hz, H-8), 6.68 (dd, 1H, $J = 10.1$ and 1.6 Hz, H-4), 5.81 (dd, 1H, $J = 10.1$ and 3.4 Hz, H-3), 5.20–5.16 (m, 1H, H-2), 1.90–1.84 (m, 1H, H-1′), 1.80–1.73 (m, 1H, H-1′), 1.56- 1.47 (m, 2H, H-2′), 0.98 (t, 3H, $J = 7.4$ Hz, H-3′) ppm. $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$: 184.4 (C-10), 180.9 (C-5), 161.5 (C-9), 152.6 (C-10a), 136.6 (C-7), 131.5 (C-5a), 126.6 (C-3), 123.9 (C-8), 119.3 (C-4a), 119.0 (C-6), 116.9 (C-4), 114.4 (C-9a), 77.9 (C-2); 37.7 (C-1'), 17.5 (C-2'), 13.8 (C-3′) ppm. HRMS: Calcd for $C_{16}H_{15}O_4^+$: 271.0965. Found: 271.0973 $(M + H)^+$.

## Synthesis of α-lapachones (13a–d)

Into a high-pressure reactor was added the corresponding compounds **12a–d** (0.4 mmol) dissolved in ethyl acetate (100 mL) and 7 mg of 10% Pd/C as a catalyst. The reactor was loaded with 27 psi $H_2$ and stirred at 50 °C for 2 h. After total consumption of the starting material, observed by thin layer chromatography, the solution was filtered to remove the catalyst. Thereafter, the resulting filtrate was evaporated under reduced pressure to give the appropriately substituted compounds **13a–d** in moderate to high yields (Lee et al. 2005).

### 9-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (13a)

The α-lapachone (**13a**) was obtained in quantitative yield as a yellow solid of mp consistent with the literature (Da Rocha et al. 2011). IR (KBr, $cm^{-1}$): $\nu$ 3411, 1640, 1608, 1457, 1282, 1198, 1157, 1114. $^{1}$H NMR ($CDCl_3$, 500

MHz11.83 (s, 1H, OH), 7.61–7.56 (m, 2H, H-6 and H-8), 7.19–7.17 (m, 1H, H-7), 2.62–2.59 (m, 2H, H-4), 1.83–1.81 (m, 2H, H-3), 1.44 (s, 6H, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 126 MHz): 184.9 (C-10), 183.4 (C-5), 161.6 (C-10a), 154.1 (C-9), 136.5 (C-7), 132.0 (C-5a), 123.5 (C-8), 121.1 (C-9a), 118.7 (C-6), 114.1 (C-4a), 78.4 (C-2), 31.3 (C-4), 26.5 (C-1′), 16.8 (C-3) ppm. HRMS: Calcd for $C_{15}H_{15}O_4^+$: 259.0965. Found: 259.0978 $(M + H)^+$.

#### 9-hydroxy-3-methyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione (13b)

The α-lapachone (13b) was obtained in quantitative yield as a yellow solid of mp 160–163 °C. IR (KBr, $cm^{-1}$): ν 1637, 1609, 1452, 1250, 1192, 1019, 757, 740. $^1$H NMR ($CDCl_3$, 500 MHz): 11.77 (s, 1H, OH), 7.61–7.56 (m, 2H, H-6 and H-7), 7.20 (dd, 1H, $J = 8.0$ and 1.2 Hz, H-8), 4.40–4.37 (m, 1H, H-2), 3.80–3.76 (m, 1H, H-2), 2.82–2.77 (m, 1H, H-3), 2.14–2.08 (m, 2H, H-4), 1.11–1.09 (m, 3H, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 126 MHz): 184.6 (C-10), 183.4 (C-5), 161.6 (C-10a), 154.9 (C-9), 136.6 (C-7), 132.0 (C-5a), 123.5 (C-8), 122.1 (C-9a), 118.7 (C-6), 114.0 (C-4a), 74.5 (C-3), 27.2 (C-4), 20.4 (C-1′), 18.4 (C-2) ppm. HRMS: Calcd for $C_{14}H_{11}O_4^-$: 243.0663. Found: 243.0655 $(M - H)^-$.

#### 9-hydroxy-2-methyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (13c)

The α-lapachone (**13c**) was obtained in quantitative yield as a yellow solid of mp 105–109 °C. IR (KBr, $cm^{-1}$): ν 1636, 1600, 1454, 1267, 1188, 1110, 739. $^1$H NMR ($CDCl_3$, 500 MHz): 11.81 (s, 1H, OH), 7.61–7.59 (m, 1H, H-6), 7.59–7.56 (m, 1H, H-7), 7.20–7.18 (m, 1H, H-8), 4.34–4.28 (m, 1H, H-2), 2.72 (ddd, 1H, $J = 18.9$, 5.6 and 3.6 Hz, H-4), 2.51 (ddd, 1H, $J = 18.9$, 10.1 and 6.4 Hz, H-4), 2.11–2.06 (m, 1H, H-3), 1.73–1.65 (m, 1H, H-3), 1.51 (d, 3H, $J =$ 6.4 Hz, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz): 184.6 (C-10), 183.4 (C-5), 161.6 (C-10a), 154.9 (C-9), 136.6 (C-7), 132.0 (C-5a), 123.5 (C-8), 122.1 (C-9a), 118.7 (C-6), 114.0 (C-4a), 74.5 (C-2), 27.2 (C-4), 20.4 (C-1′), 18.4 (C-3) ppm. HRMS: Calcd for $C_{14}H_{12}NaO_4^+$: 267.0628. Found: 267.0629 $(M + Na)^+$.

#### 9-hydroxy-2-propyl-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (13d)

The α-lapachone (**13d**) was obtained in quantitative yield as a yellow solid of mp 123–125 °C. IR (KBr, $cm^{-1}$): ν 1636, 1605, 1262, 1200, 1156, 1119, 1094, 1072, 963, 834, 739, 707. $^1$H NMR ($CDCl_3$, 500 MHz): 11.82 (s, 1H, OH), 7.62–7.54 (m, 2H, H-7 and H-8), 7.18 (dd, 1H, $J = 7.5$ and 2.0 Hz, H-6), 4.20–4.12 (m, 1H, H-2), 2.71 (ddd, 1H, $J =$ 18.9, 5.7 and 3,9 Hz, H-4), 2.49 (ddd, 1H, $J = 18.9$, 9.8 and 6.4, H-4), 2.12–2.03 (m, 1H, H-3), 1.92–1.79 (m, 2H, H-1′), 1.76–1.47 (m, 3H, H-3 and H-2′), 0.99 (t, 3H, $J = 7.2$, H-3′) ppm. $^{13}$C NMR ($CDCl_3$, 126 MHz): 184.6 (C-10), 183.5 (C-5), 161.5 (C-10a), 155.0 (C-9), 136.5 (C-7), 132.0 (C-5a), 123.5 (C-8), 122.2 (C-9a), 118.7 (C-6), 114.0 (C-4a), 78.0 (C-2), 36.4 (C-1′), 25.3 (C-4), 18.5 (C-2′), 18.4 (C-3), 13.9 (C-3′) ppm. HRMS: Calcd for $C_{16}H_{16}NaO_4^+$: 295.0941. Found: 295.0935 $(M + Na)^+$.

### Synthesis of β-lapachones (14a, 14c, and 14d)

Into a round bottom flask was added the corresponding α-lapachones (0.2 mmol) of compounds **14a, 14c**, and **14d** and 3 mL of $H_2SO_4$. The mixture was left under stirring at room temperature or with heating in some cases. After the total consumption of the starting material, observed by thin layer chromatography, the reaction mixture was poured into crushed ice and the product was filtered under vacuum and washed with a small amount of ice water. Subsequently, the formed solid was transferred to a round bottom flask and 3 mL of dichloromethane was added. Finally, the solvent was evaporated under reduced pressure to give the substituted β-lapachones (**14a**, **c**, **d**) (Watson et al. 2016). All attempts to obtain compound **14b** failed.

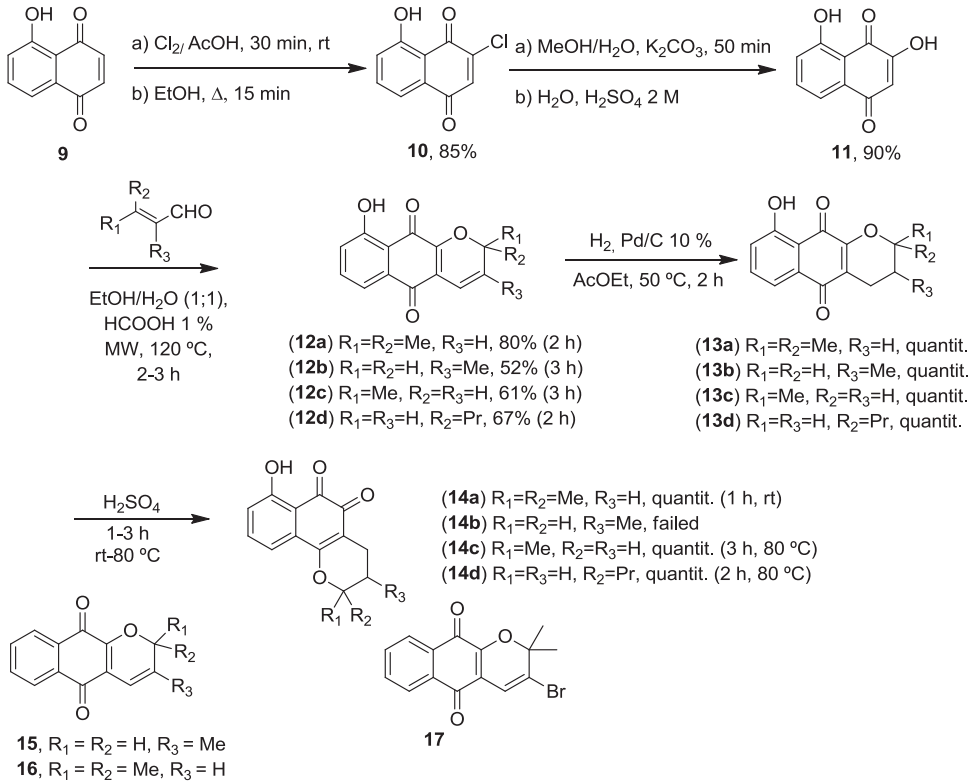
#### 7-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (14a)

The β-lapachone (**14a**) was obtained in quantitative yield, after 2 h of reaction at room temperature, as a red solid of mp consistent with the literature (Da Rocha et al. 2011). IR (KBr, $cm^{-1}$): ν 3435, 1637, 1586, 1573, 1451, 1400, 1193, 1118, 714. $^1$H NMR ($CDCl_3$, 300 MHz): 11.97 (s, 1H, OH), 7.53 (dd, 1H, $J = 8.5$ and 7.6 Hz, H-9), 7.34 (dd, 1H, $J =$ 7.6 and 0.9 Hz, H-10), 7.05 (dd, 1H, $J = 8.5$ and 0.9 Hz, H-8), 2.55 (t, 2H, $J = 6.6$ Hz, H-4), 1.84 (t, 2H, $J = 6.6$ Hz, H-3), 1.45 (s, 6H, H-1′) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz): 183.2 (C-6), 178.1 (C-5), 164.3 (C-10b), 161.6 (C-7), 137.9 (C-9), 132.4 (C-10a), 121.5 (C-8), 116.7 (C-10), 113.6 (C-6a), 112.7 (C-4a), 79.2 (C-2), 31.5 (C-4), 26.7 (C-1′), 16.1 (C-3) ppm. HRMS: Calcd for $C_{15}H_{14}NaO_4^+$: 281.0784. Found: 281.0788 $(M + Na)^+$.

#### 7-hydroxy-2-methyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (14c)

The β-lapachone (**14c**) was obtained in quantitative yield, after 2 h of reaction at room temperature, as a red solid of mp 155–160 °C. IR (KBr, $cm^{-1}$): ν 1630, 1580, 1445, 1401, 1386, 1323, 1186, 1165, 1147, 1115, 1057, 1038, 932, 907, 740, 714, 704. $^1$H NMR ($CDCl_3$, 500 MHz): 11.96 (s, 1H), 7.54–7.51 (m, 1H), 7.33 (dd, 1H, $J = 7.5$, 0.8 Hz), 7.04 (dd,

**Scheme 1** Compounds synthesized and screened against cancer cells




1H, $J = 8.6$, 0.8 Hz), 4.39–4.33 (m, 1H), 2.68 (ddd, 1H, $J = 17.5$, 5.5, 3.3 Hz), 2.43 (ddd, 1H, $J = 17.5$, 10.8, 6.3 Hz), 2.11–2.06 (m, 1H), 1.73–1.63 (m, 1H), (d, 3H, $J =$ 6.4 Hz) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz): 183.7 (C-6), 178.2 (C-5), 164.4 (C-10b), 137.9 (C-9), 132.0 (C-10a), 121.5 (C-8), 116.7 (C-10), 113.7 (C-6a), 113.4 (C-4a), 75.3 (C-2), 27.6 (C-4), 20.6 (C-1′), 18.0 (C-3) ppm. HRMS: Calcd for $C_{14}H_{12}NaO_4^+$: 267.0628. Found: 267.0622 $(M + Na)^+$.

### 7-hydroxy-2-propyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (14d)

The β-lapachone (**14d**) was obtained in quantitative yield, after 2 h of reaction at room temperature, as a red solid of mp 115–117 °C. IR (KBr, $cm^{-1}$): $\nu$ 3434, 1637, 1584, 1453, 1401, 1199. $^1$H NMR ($CDCl_3$, 500 MHz): 11.97–11.96 (m, 1H, OH), 7.54–7.51 (m, 1H, H-9), 7.34–7.31 (m, 1H, H-10), 7.05–7.04 (m, 1H, H-8), 4.25–4.20 (m, 1H, H-2), 2.67 (ddd, 1H, $J = 17.7$, 5.5 and 3.4 Hz, H-4), 2.59–2.39 (m, 1H, H-4), 2.10–2.04 (m, 1H, H-3), 1.89–1.77 (m, 2H, H-1′), 1.75–1.60 (m, 3H, H-3 and H-2′), 1.02 (t, 3H, $J =$ 7.3 Hz, H-3′) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz): 183.1 (C-6), 178.2 (C-5), 164.4 (C-10b), 137.9 (C-9), 132.2 (C-10a), 121.5 (C-8), 116.6 (C-10), 114.0 (C-6a), 113.4 (C-4a), 78.7 (C-2), 36.8 (C-1′), 25.9 (C-4), 18.6 (C-2′), 18.0 (C-3), 13.9 (C-3′) ppm. HRMS: Calcd for $C_{16}H_{16}NaO_4^+$: 295.0941. Found: 295.0933 $(M + Na)^+$.

## Biology

### Cell lines and culture

The human tumor cell lines used in this study were SNB19 (human glioblastoma), HCT-116 (human colon carcinoma), PC3 (prostate), MCF-7 (human breast adenocarcinoma) and HL-60 (leukemia), which were kindly provided by the National Cancer Institute (Bethesda, MD, USA). The selectivity of the compounds towards a nontumor cell line was investigated using the murine cell line L929 (murine fibroblasts), obtained from ATCC and deposited in the Rio de Janeiro Cell bank (BCRJ). All tumor cell lines and L929 were, respectively, maintained in RPMI 1640 and DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U $mL^{-1}$ penicillin, and 100 µg $mL^{-1}$ streptomycin, at 37 °C with 5% $CO_2$. For the survival assays, cells were plated in 96-well plates ($0.1 \times 10^6$ cells/well for PC3, SNB19 and MCF-7 cells; $0.3 \times 10^6$ cells/well for HL-60 cells; and $0.7 \times 10^5$ cells/well for HCT-116 cells). All tested compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich®) and tested at increasing concentrations (highest concentration of 10 µg/mL). The final concentration of DMSO in the culture medium was always kept lower than 0.1%, v/v. Doxorubicin (DOX, Sigma-Aldrich®) was used as a positive control in all experiments.

### MIT assay for determination of the cytotoxicity

The cell viability was determined by the MTT assay, which consists of the reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product by metabolically active cells (Mosmann 1983). At the end of the incubation time (72 h at 37 °C/5% $CO_2$), the plates were centrifuged, and the supernatant was replaced with fresh medium (150 µL) containing 0.5 mg $mL^{-1}$ MTT. Three hours later, the plates were centrifuged, the MTT formazan product was dissolved in DMSO (150 µL) and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc. Fullerton, California, EUA) at 525 nm. The compounds' growth inhibition effect was estimated by a reduction in absorbance when compared with the DMSO alone control. The absorbances obtained were used to calculate the $IC_{50}$ values (concentration capable of inhibiting 50% of cell growth) of each sample by nonlinear regression using the GraphPad Prism® 7.0 program. All treatments were performed in triplicate in at least three independent experiments.

## Results and discussion

### Chemistry

All of the compounds synthesized and screened against cancer cells are described in Scheme 1. We initially prepared compound **9** by a method previously described by our group (Pinto et al. 1985); **9** was easily transformed into the chlorinated derivative **10** in good yield (Wheeler and Scott 1919). The latter compound was hydrolyzed to the 2,8-dihydroxynaphthalene-1,4-dione **11** in high yield (MacLeod and Thomson 1960; Wurm et al. 1986). Compounds **12-14a, 14c, 14d** were prepared from **11** by the methods described below (Da Rocha et al. 2014; Lee et al. 2005; Watson et al. 2016). All attempts to obtain compound **14b** failed as it was necessary to use the temperature of 180 °C to observe the consumption of starting material **13b** by thin layer chromatography. This high temperature promoted the formation of many degradation products, which prevented the purification and quantification of the formed products, including **14b**. Compounds **15** and **16** were prepared by the methods described in the literature from lawsone (Da Rocha et al. 2014) and **17** from α-lapachone (Gupta and Khanna 1979).

### Evaluation of the cytotoxic activity

The fourteen naphthoquinones were tested, in increasing concentrations, against five human tumor cell lines, PC-3 (prostate), HCT-116 (colon carcinoma), SNB-19 (glioblastoma), HL-60 (leukemia), and MCF-7 (breast) and a nontumor cell line L929 (murine fibroblasts) to determine their cytotoxic activity by the MTT assay. Table 1 shows the $IC_{50}$ values for all 14 naphthoquinones, with their respective 95% confidence intervals. Figure 2 allows for a better comparative analysis.

Analysis of Table 1 and Fig. 1 shows that all compounds were active against several cell lines with different profiles. The compounds **14a**, **c**, and **d** showed lower $IC_{50}$ values (ranging from 0.42 to 3.36 µM), standing out as the most active compounds.

HL60 was the most sensitive cell line, with several values of $IC_{50}$ lower than 6 µM, reaching the lowest value of 0.42 µM for **14c**. SNB-19 and PC3 were the most resistant cell lines, allowing us to identify **12c** as the least active compound, showing $IC_{50}$ values higher than 20 µM in four of the five studied cell lines.

Table 2 shows the selectivity index (SI) (SI = $IC_{50}$ L929/$IC_{50}$ tumor cell line) for all naphthoquinones in each tumor cell line. SI indicates the selectivity of each compound towards tumor cells and can be used to determine their potential for further preclinical studies.

Figure 3 enables a better comparison of the selectivity results, highlighting the threshold of two as the limit to be considered significant in terms of selectivity index. SI values lower than 2.0 indicate low selectivity (Suffness and Pezzuto 1990).

Analysis of the SI results showed that compounds **14a**, **c**, and **d** also showed a better selectivity index, with SI values higher than 2.0 in most of the cell lines. HL-60 had significant SI values (higher than 2.0) for almost all naphthoquinones except for **13b** and **16**. The most significant selectivity was for compound **14d** in HL-60 tumor cells.

Considering the cell line SNB-19, the best results were found for compounds **12b** (3.87 µM), **14a** (3.36 µM), **12c** (2.43 µM), and **12d** (2.73 µM). For this cell line, α-lapachones and dehydro-α-lapachone presented a discrete activity, where the best results were for compounds containing a C-3 alkyl group, especially for dehydro-α-lapachone **12b** whose activity was comparable to that presented by the compound **14a**. In addition, it was shown that the presence of a hydroxyl group on the aromatic ring of **14c** led to a compound 2-fold more active than its correlate without this substituent, **15** (7.32 µM). Among the β-lapachones **14a**, **c**, and **d** it is observed that the presence of only one methyl substituent at C-2 led to a compound **14c** with higher activity than the disubstituted **14a**. At the same time, the increase of the alkyl group at C-2 (**14c**) provided a decrease in β-lapachone activity but still remained superior to the disubstituted example. All β-lapachones were shown to be much more active than the corresponding α-lapachones, demonstrating the importance of this structural pattern for their activity.

**Table 1** Activity of the 13 naphthoquinones assayed against tumor and nontumor cells after 72 h of treatment[a]

| | Cancer cell line | | | | | Nontumor cell line |
|---|---|---|---|---|---|---|
| | SNB-19 (Glioblastoma) | HCT-116 (Colon) | PC-3 (Prostate) | MCF-7 (Breast) | HL-60 (Leukemia) | L929 (Fibroblast) |
| **12a** | 2.24 (8.74) | 3.35 (13.08) | 3.31 (12.94) | 4.14 (16.17) | 1.32 (5.17) | 3.33 (13.01) |
| | 2.02–2.48 | 2.32–3.01 | 3.17–3.46 | 3.75–4.57 | 1.18–1.48 | 2.91–3.81 |
| **12b** | 0.93 (3.87) | 1.26 (5.21) | 1.77 (7.32) | 1.48 (6.14) | 0.49 (2.05) | 1.14 (4.71) |
| | 0.80–1.09 | 1.10–1.44 | 1.58–1.98 | 1.35–1.63 | 0.46–0.53 | 1.00–1.3 |
| **12c** | 7.3 (30.15) | 5.14 (21.25) | 8.04 (33.21) | 11.85 (48.92) | 1.94 (8.03) | 9.96 (41.15) |
| | 7.01–7.60 | 5.59–7.09 | 6.92–9.35 | 7.26–19.32 | 1.83–2.06 | 9.01–11.02 |
| **12d** | 6.352 (23.50) | 2.31 (8.56) | 3.6 (13.35) | 5.3 (19.61) | 1.01 (3.71) | 3.53 (13.06) |
| | 5.90–6.83 | 1.98–2.70 | 3.07–4.24 | 4.55–6.17 | 0.87–1.14 | 2.17–5.73 |
| **13a** | 4.2 (16.28) | 3.22 (12.49) | 4.79 (18.55) | 3.2 (12.40) | 0.97 (3.77) | 2.02 (7.82) |
| | 3.49–5.05 | 1.96–2.80 | 4.24–5.40 | 2.88–3.55 | 0.87–1.08 | 1.88–2.16 |
| **13b** | 2.57 (10.53) | 1.66 (6.80) | 2.95 (12.08) | 1.79 (7.36) | 1.13 (4.65) | 1.68 (6.91) |
| | 2.22–2.96 | 1.25–2.20 | 1.92–4.53 | 1.58–2.03 | 0.97–1.32 | 1.52–1.86 |
| **13c** | 3.13 (12.83) | 1.8 (7.39) | 4.35 (17.82) | 3.13 (12.83) | 1.63 (6.69) | 4.61 (18.87) |
| | 2.57–3.80 | 1.69–1.92 | 3.73–5.07 | 2.70–3.63 | 1.40–1.9 | 3.67–5.78 |
| **13d** | 6.99 (25.67) | 2.88 (10.59) | 6.54 (24.03) | 4.9 (18.00) | 1.97 (7.23) | 5.76 (21.18) |
| | 5.75–8.49 | 2.43–3.42 | 4.45–9.60 | 4.22–5.68 | 1.82–2.12 | 4.66–7.13 |
| **14a** | 0.86 (3.36) | 0.26 (1.03) | 0.64 (2.48) | 0.68 (2.66) | 0.19 (0.77) | 1.41 (5.46) |
| | 0.67–1.11 | 0.23–0.30 | 0.55–0.74 | 0.61–0.77 | 0.16–0.24 | 1.20–1.65 |
| **14c** | 0.59 (2.43) | 0.2 (0.85) | 0.74 (3.06) | 0.43 (1.77) | 0.13 (0.56) | 0.73 (3.00) |
| | 0.56–0.62 | 0.14–0.29 | 0.56–0.99 | 0.39–0.46 | 0.10–0.17 | 0.62–0.85 |
| **14d** | 0.74 (2.73) | 0.43 (1.59) | 0.82 (3.01) | 0.57 (2.11) | 0.11 (0.42) | 1.5 (5.54) |
| | 0.66–0.83 | 0.38–0.48 | 0.73–0.91 | 0.54–0.60 | 0.08–0.15 | 1.33–1.70 |
| **15** | 1.65 (7.32) | 0.78 (3.46) | 2.26 (10.02) | 1.97 (8.72) | 0.72 (3.19) | 1.51 (6.68) |
| | 1.46–1.87 | 0.62–0.98 | 1.85–2.77 | 1.82–2.13 | 0.66–0.78 | 1.12–2.03 |
| **16** | 7.98 (33.23) | 4.01 (16.70) | 12.8 (53.28) | 4.67 (19.46) | 1.43 (5.95) | 6.49 (27.02) |
| | 5.78–11.02 | 3.52–4.56 | 9.66–16.94 | 4.3–5.08 | 1.16–1.75 | 5.57–7.56 |
| **17** | 4.28 (13.42) | 1.97 (6.18) | 2.59 (8.12) | 3.41 (10.68) | 0.98 (3.08) | 1.56 (4.91) |
| | 3.72–4.92 | 1.58–2.45 | 1.97–3.39 | 3.13–3.70 | 0.89–1.08 | 1.28–1.90 |
| **DOX**[b] | (2.07) | (0.21) | (0.76) | (0.15) | (0.02) | (1.72) |
| | 1.78–2.41 | 0.16–0.29 | 0.59–0.93 | 0.12–0.19 | 0.01–0.02 | 1.58–1.87 |

[a]Data are presented as $IC_{50}$ values in μg/mL and inside parenthesis (μM) with 95% confidence intervals. Data were obtained by nonlinear regression using the GraphPad Prism® 7 program from three independent experiments performed in triplicate

[b]Doxorubicin (DOX) was used as a positive control

The dehydro-α-xyloidones **12a–d** family were in almost all cases from twofold to threefold more active than the analogous α-lapachones, with dehydro-α-xyloidones substituted at C-2 with a propyl group the only exception. Comparing the compounds **16** and **17** it was observed that the introduction of Br in C-3 led to a great increase in activity but was still lower than the other outstanding examples.

For the HCT-116 cells, some structural factors already highlighted previously were once again relevant, such as, for example, C-3 substitution by alkyl groups, leading to the best results for dehydro-α-lapachones (**12b**, 5.21 μM; **15**, 3.46 μM) and α-lapachones (**13b**, 6.80 μM). For the compounds of series **12**, it was further found that the presence of a higher C-2 alkyl group provided increased activity, although the result was lower than for the C-3 substituted compound. Similarly, for this cell line it was found that the presence of the OH group on the aromatic ring was detrimental, in view of the lower activity observed for compound **12b** when compared with its analog **15**. For the series of compounds **13**, the substitution at C-3 for a methyl group was shown to be less active than the analog dehydro-α-lapachones (**12**). Compounds with C-2 substitution were shown to be up to threefold more active than the corresponding series **12**, being substituted with a propyl group the only exception. The series

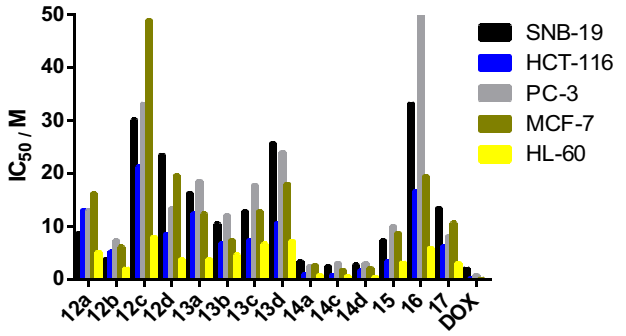



**Fig. 2** $IC_{50}$ values (μM) for the 13 naphthoquinones used against five tumor cell lines. Doxorubicin (DOX) was used as a positive control

**Table 2** Selectivity index [$IC_{50}$ (L929)/ $IC_{50}$ (tumor cell line)] towards each of the five tumor cell lines

| Quinones | Selectivity Index $IC_{50}$ (L929)/$IC_{50}$ (tumor cell line) | | | | |
|---|---|---|---|---|---|
| | SNB-19 | HCT-116 | PC-3 | MCF-7 | HL-60 |
| **12a** | 1.49 | 1.00 | 1.01 | 0.80 | 2.52 |
| **12b** | 1.22 | 0.90 | 0.64 | 0.77 | 2.29 |
| **12c** | 1.36 | 1.94 | 1.24 | 0.84 | 5.12 |
| **12d** | 0.56 | 1.53 | 0.98 | 0.67 | 3.52 |
| **13a** | 0.48 | 0.63 | 0.42 | 0.63 | 2.07 |
| **13b** | 0.66 | 1.02 | 0.57 | 0.94 | 1.49 |
| **13c** | 1.47 | 2.55 | 1.06 | 1.47 | 2.82 |
| **13d** | 0.82 | 2.00 | 0.88 | 1.18 | 2.93 |
| **14a** | 1.63 | 5.31 | 2.20 | 2.05 | 7.07 |
| **14c** | 1.23 | 3.52 | 0.98 | 1.70 | 5.32 |
| **14d** | 2.03 | 3.49 | 1.84 | 2.62 | 13.18 |
| **15** | 0.91 | 1.93 | 0.67 | 0.77 | 2.09 |
| **16** | 0.81 | 1.62 | 0.51 | 1.39 | 4.54 |
| **17** | 0.37 | 0.79 | 0.60 | 0.46 | 1.59 |
| **DOX** | 0.83 | 8.19 | 2.26 | 11.47 | 86.00 |

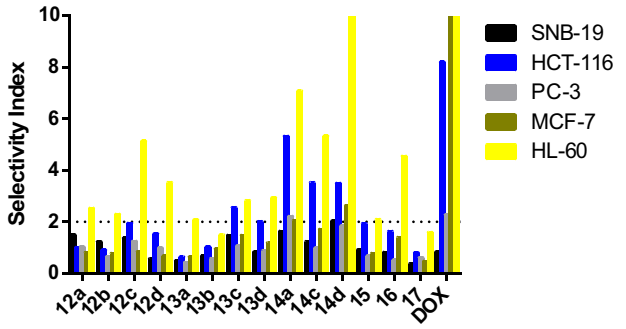



**Fig. 3** Graph showing the selectivity index for the 14 studied naphthoquinones in five tumor cell lines. The threshold of 2 is highlighted. Doxorubicin (DOX) was used as a positive control

of β-lapachones **14** were much more active than the series corresponding to compounds **13**, demonstrating the importance of this ortho-naphthoquinone structural pattern. However, the introduction of only one methyl group in C-2 significantly improved its cytotoxic activity and it increased the alkyl chain effect that was reversed and decreased. With the introduction of an electronegative atom at C-3 as in compound **17**, an increase in activity was achieved, showing the importance of this functional group.

By making the same comparison between the series for the PC-3 tumor cell line it can be seen that in this case **12b** and **15** were more active than the α- and β-lapachone series. For the β-lapachones series **14a**, **c**, and **d**, it presented similar results regardless of the substitution pattern. The same pattern was found for series **12** and **13** when evaluated against the MCF-7 cells relative to C-3 and C-2 substitution. All α-lapachones substituted at C-2 were more active than the corresponding dehydro-α-lapachones (**12**).

The most sensitive tumor cell line in the studied series was HL-60, since all of the compounds were more active in this line than the others. For the other previous cells, the compounds substituted in C-3 by an alkyl group were shown to be more active than the others and the presence of a greater lipophilic group in C-2 increased its activity. The dehydro-α-lapachones (**12**) substituted in C-2 were less active, except for those containing a propyl group, as in previous cases. Again, the series corresponding to compounds **14** were the most active, with best results for those containing only one C-2 substituent and with higher alkyl groups providing better results. The series of hydro-α-lapachones (**12**) containing the OH group on the aromatic ring were more active than their unsubstituted analogs. Considering the selectivity indices (Fig. 3) for HL-60 cells, they were always higher for the β-lapachone series of compounds (**14**).

## Conclusion

The compounds 5-OH-α-lapachones (**13a-d**) and 8-OH-β-lapachones (**14a**, **c**, **d**) were prepared in moderate to good yields. These compounds were compared with other related naphthoquinones regarding their activity against SNB-19, HCT-116, PC-3, MCF-7, and HL-60 by the MTT assay. Compounds **14a**, **14c**, and **14d** present promising activities against HCT-116 and HL-60 tumor cells with high selectivity, showing that the presence of a hydroxyl group on β-lapachones seems to be important for their antitumor activity. The results here presented are an entry point for further investigations, especially concerning the mechanism of action and in vivo antitumor activity of the best compounds.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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