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*Article*

# Synthesis of 2,3-Diyne-1,4-naphthoquinone Derivatives and Evaluation of Cytotoxic Activity against Tumor Cell Lines

***Mauro G. Silva,[a] Celso A. Camara,*,[a] Tania M. S. Silva,[a] Anderson C. S. Feitosa,[b]***
***Assuero S. Meira[b] and Cláudia Pessoa[b]***

[a]*Laboratório de Síntese de Compostos Bioativos (LSCB), Departamento de Ciências Moleculares,*
*Universidade Federal Rural de Pernambuco, 52171-900 Recife-PE, Brazil*

[b]*Laboratório de Oncologia Experimental (LOE), Departamento de Fisiologia e Farmacologia,*
*Universidade Federal do Ceará, 60431-970 Fortaleza-CE, Brazil*

Uma série de derivados 2,3-diino-1,4-naftoquinona foi sintetizada a partir do 2,3-dibromo-1,4-naftoquinona e diversos alquinos terminais funcionalizados usando a reação de acoplamento de Sonogashira catalisada por paládio. Os compostos foram submetidos à avaliação do potencial citotóxico em três linhagens de células tumorais, OVCAR-8 (ovário), PC-3M (próstata) e NCI-H358M (pulmão), apresentando, no geral, resultados satisfatórios para inibição do crescimento celular.

A series of 2,3-diyne-1,4-naphthoquinone derivatives was synthesized from 2,3-dibromo-1,4-naphthoquinone and various functionalized terminal alkynes using palladium-catalyzed Sonogashira cross-coupling reaction. The diynes were evaluated as potential cytotoxic agents against three tumor cell lines: human ovarian adenocarcinoma (OVCAR-8), human metastatic prostate cancer (PC-3M) and human bronchoalveolar lung carcinoma (NCI-H358M), presenting, in general, satisfactory results for inhibition of cell growth.

**Keywords:** 1,4-naphthoquinone, diyne, palladium, Sonogashira, cytotoxicity

## Introduction

Naphthoquinones are substances of great pharmacological interest as they exhibit a wide variety of biological activities,[1] such as antitumor,[2,3] antibacterial,[4] antifungal,[5] molluscicides,[6-8] antileishmanial[9] and anti-inflammatory.[10] The mechanism of action of naphthoquinones involves oxidants and electrophile properties influenced by their chemical structure.[11] There are two important mechanisms of quinone cytotoxicity: stimulation of oxidative stress and alkylation of cellular nucleophiles, which encompass a large range of biomolecules. Reactive oxygen species (ROS) may react directly with DNA, lipids and proteins, leading to cell damage[12,13] and shunting electrons toward oxygen, a futile pathway for reduction equivalents otherwise used for cytochrome P450 reductase-dependent reactions. Cellular damage can also occur through the alkylation of crucial proteins and nucleic acids.[14-16]

Naphthoquinones containing acetylenic substituents on the quinone ring are promising intermediates in the synthesis of heterocyclic quinones and highly reactive unsaturated compounds.[17-19] 2,3-Diyne-1,4-naphthoquinones and related compounds exhibit an enediyne system that is capable of causing damage to cellular DNA through cycloaromatization, with the formation of diradical benzenoid intermediates that remove hydrogen atoms from the sugar skeleton-phosphate of the DNA double helix.[20] Enediyne compounds are among the most effective known chemotherapy agents, highly potent for cleavage of DNA. In recent years, considerable efforts have been invested in the development of drugs such as enediyne showing a maximum activity against tumor cells and minimal toxicity in normal cells.[21]

In this work, we report the synthesis of 2,3-diyne-1,4-naphthoquinones employing Sonogashira cross-coupling reaction[22] between 2,3-dibromo-1,4-naphthoquinone and various functionalized terminal alkynes. We also report the cytotoxic activity of these derivatives in three tumor cell lines: human ovarian adenocarcinoma (OVCAR-8),

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*e-mail: ccelso@dcm.ufrpe.br

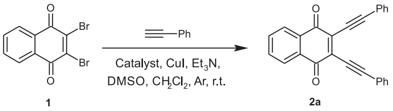
human metastatic prostate cancer (PC-3M) and human bronchoalveolar lung carcinoma (NCI-H358M), presenting, in general, satisfactory results for cell growth inhibition.

## Results and Discussion

### Synthesis of the compounds

The diyne derivatives (**2a**-**i**) were synthesized following a procedure described in the literature,[17] via cross-coupling between 2,3-dibromo-1,4-naphthoquinone (**1**) and several terminal alkynes catalyzed by palladium complexes and co-catalyzed by copper(I) iodide.[22] Initially, the synthesis of compound **2a** from the phenylacetylene alkyne and (**1**) was performed in a mixture of solvents, DMSO (dimethylsulfoxide) and DCM (dichloromethane) (1:1), using triethylamine, CuI and a palladium complex ($Pd(PPh_3)_2Cl_2$) as catalyst. The product (**2a**) was obtained in 45% yield after isolation on silica gel column chromatography. In virtue of the importance of the catalyst to the formation of the coupling product, other palladium complexes were also tested under the same reaction conditions. Using $Pd(PPh_3)_4$, compound **2a** was obtained in only 27% yield (after consumption of **1** by TLC inspection). Other catalysts tested were unable to provide the product of interest (**2a**) (Table 1).

**Table 1.** Catalytic conditions tested to obtain **2a**

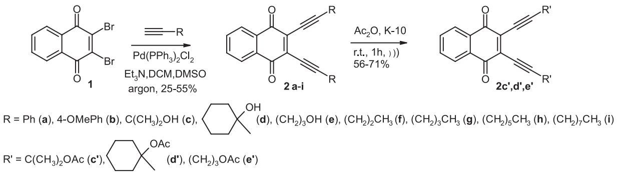


| entry | Catalyst | time / min | Yield / % |
|---|---|---|---|
| 1 | $PdCl_2 + PPh_3$[a] | not formed | – |
| 2 | $Pd(PPh_3)_2Cl_2$ | 120 | 45 |
| 3 | $Pd(PPh_3)_4$ | 50 | 27 |
| 4 | $Pd(OAc)_2$ | – | – |
| 5 | $Pd(CH_3CN)_2Cl_2$ | – | – |

[a]Included in the reaction mixture.

The preformed complex $Pd(PPh_3)_2Cl_2$ (Table 1, entry 2) was chosen as catalyst in the coupling reaction of (**1**) with several terminal alkynes due to its higher stability and solubility and better performance for **2a** formation. As a result, compounds **2a**-**i** were obtained with 25-55% yields, as shown in Table 2. Compounds **2c**-**e** were acetylated using acetic anhydride and montmorillonite clay K-10 under ultrasound treatment,[23] producing three new derivatives (**2c'**-**e'**) in yields ranging from 56 to 71%. All compounds were characterized by $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS) and Fourier transform infrared spectroscopy (FTIR). The characterization of **2a** by $^1H$ and $^{13}C$ NMR (300 and 75 MHz, $CDCl_3$) analyses showed a simplified spectrum due to a planar symmetry. A signal centered at $\delta$ 8.16 ppm (dd, 2H, *J* 3.3 and 5.7 Hz) and at $\delta$ 7.77 ppm (dd, 2H, 3.3 and 5.7 Hz) were attributed to *orto*- (5/8) and *meta*-benzenoid hydrogens. A multiplet centered at $\delta$ 7.68 ppm (4H) and another at $\delta$ 7.41 ppm (6H) were attributed to phenyl hydrogens. The $^{13}C$ NMR spectra presents a single carbonyl peak at $\delta$ 181.1 ppm, and the sp carbons of triple bond at $\delta$ 85.4 and 109.7 ppm, among the expected eight sp$^2$ signals.

**Table 2.** 2,3-Diyne-1,4-naphthoquinone derivatives



| entry | Compound | Yield[a] / % | time / h / Temperature / °C |
|---|---|---|---|
| 1 | **2a** | 45 | 2 / 28 |
| 2 | **2b** | 46 | 1 / 28 |
| 3 | **2c** | 42 | 4 / 40 |
| 4 | **2d** | 30 | 2.5 / 40 |
| 5 | **2e** | 25 | 12 / 28 |
| 6 | **2f** | 38 | 1 / 28 |
| 7 | **2g** | 55 | 1 / 28 |
| 8 | **2h** | 38 | 1 / 28 |
| 9 | **2i** | 30 | 2 / 28 |

**2c'**: 56%, **2d'**: 65% and **2e'**: 71%; [a]All yields after column chromatography.

DMSO was the solvent of choice for the preparation of compounds **2a**-**i** due to the relatively good stability of **1** in this solvent, as reported by Romanov *et al.*[17] in contrast to what is observed in other solvents (pyridine, Dimethylformamide-DMF, $Et_3N$, etc.). $CH_2Cl_2$ was previously reported as a suitable co-solvent to reduce resinification produced in reaction.[17] The use of THF (tetrahydrofuran) as solvent in the synthesis of **2a** was unsuccessful, resulting in a rather complex mixture of products, not allowing isolation. Alternatively, when this reaction was performed in acetonitrile, **2a** was produced in 20 min, but with 36% yield after column chromatography purification procedure.

The low yields in Sonogashira coupling products (**2a**-**i**) are related to several factors, e.g., low reactivity of 2,3-dibromo-1,4-naphthoquinone (**1**) in performing coupling reactions. In addition, vinyl bromides are less reactive than their iodide and triflate analogs in the Sonogashira type coupling reactions.[24] Another problem is the undesirable formation of alkyne homocoupling due to reaction conditions and exposure to oxidizing agents.[24]

Several terminal alkynes with different substituents can be submitted to Sonogashira coupling, but some specific alkynes have a low reactivity or even do not react.[25] The reaction with 4-methoxyphenylacetylene afforded compound **2b** with 46% yield. It is possible to observe that the electron donor methoxy substituent increased the reactivity, and this reaction occurred two times faster than that from unsubstituted phenyl (entry 2, Table 2). Even though, the yields were not significantly different. The coupling with alkynes containing the alcohol function resulted in products **2c**, **2d** and **2e** with yields of 42, 30 and 25%, respectively. It was observed that the hydroxyl group influences the yield of the coupling, so that the less hindered the hydroxyl group, the lower the reaction yields. The coupling of **1** with different alkyne alkyl (1-pentyne, 1-hexyne, 1-octyne and 1-decyne) resulted in products **2f**, **2g**, **2h** and **2i** yields with 38, 55, 38 and 30%, respectively. No obvious relation between the size of the alkyne chain and the reaction yield could be noticed.

Using the same above reaction conditions, the coupling between 2,3-dibromo-1,4-naphthoquinone (**1**) and some terminal alkynes were not successful. The reaction with alkyne prop-2-yn-1-ol (propargyl alcohol) resulted in a complex mixture of products which could not be isolated. The reaction with ethyl propiolate also did not result in the desired coupling product, probably due to electron-withdrawing alkyne conjugation, thus lowering the reactivity of the alkyne to Sonogashira coupling reaction.[26] Attempts to react **1** with trimethylsilylacetylene were also unsuccessful, confirming previously reported results.[27]

### Cytotoxicity assay

The cytotoxicity of the 2,3-diyne-1,4-naphthoquinone derivatives (**2a**-**i**; **2c'**-**e'**) were evaluated *in vitro* against three tumor cell lines of different histotypes, PC-3M, OVCAR-8 and NCI-H358M, using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay.[28] Doxorubicin was used as a positive control. The cytotoxic activity of the samples is shown in Table 3, with their respective percentage of cell growth inhibition (GI in %). Substances which showed inhibition of tumor cell growth greater than 75% in at least two tumor cell lines tested in the preliminary test with a single dose of 25 µg mL$^{-1}$ were selected to determine the IC$_{50}$ value.

Among the tested samples, ten (**2a**-**f**, **2h** and **2c'**-**e'**) were selected as the most active as cytotoxic agents, as they presented cell growth inhibition greater than 75% in at least two tested tumor cell lines. In addition, compound **2g**, 2,3-di(hex-1-yn-1-yl)-1,4-naphthoquinone, was also selected for IC$_{50}$ determination as it presented high specificity (95.83% inhibition) towards OVCAR-8 cell line. Only compound 2,3-di(dec-1-yn-1-yl)-naphthoquinone **2i** showed no cytotoxic potential higher than 75% in any of the three tested tumor cell lines. In general, compounds having aliphatic side chains had lower percentage of cell

**Table 3.** Percent inhibition of cell growth obtained with the samples in three tumor cell lines in a single dose of 25 µg mL$^{-1}$

| Compound | OVACAR-8 | | PC-3M | | NCI-H358M | |
|---|---|---|---|---|---|---|
| | GI / % | SD | GI / % | SD | GI / % | SD |
| **2a** | 100 | 0.08 | 72.52 | 3.05 | 85.46 | 2.55 |
| **2b** | 99.28 | 0.39 | 71.12 | 4.86 | 77.77 | 3.97 |
| **2c** | 100.50 | 0.08 | 99.63 | 0.05 | 98.21 | 0.13 |
| **2c'** | 97.67 | 1.41 | 98.55 | 2.39 | 100.84 | 3.91 |
| **2d** | 104.70 | 0.00 | 110.69 | 1.19 | 112.75 | 0.07 |
| **2d'** | 98.61 | 0.39 | 90.71 | 3.26 | 103.24 | 1.19 |
| **2e** | 100.89 | 0.79 | 100.07 | 0.00 | 98.25 | 0.19 |
| **2e'** | 101.72 | 3.36 | 96.85 | 2.17 | 99.88 | 1.87 |
| **2f** | 102.35 | 1.53 | 93.89 | 4.41 | 84.02 | 2.98 |
| **2g** | 95.83 | 1.49 | 55.51 | 2.61 | 59.39 | 3.82 |
| **2h** | 93.76 | 3.03 | 52.17 | 3.76 | 98.80 | 3.06 |
| **2i** | 50.01 | 3.63 | 50.82 | 2.41 | 32.25 | 0.70 |
| Doxorubicin | 98 | 1.1 | 97.8 | 2.4 | 99.8 | 0.89 |

GI: growth inhibition percentage; SD: standard deviation.

**Table 4.** Cytotoxic activity expressed as $IC_{50}$ of compounds **2a**-**h** against tumor cell lines

| Compound | Cell lines $IC_{50}$ / µM (confident range 95%) | | | |
|---|---|---|---|---|
| | PBMC | NCI-H358M | OVCAR-8 | PC-3M |
| **2a** | 0.388 (0.307-0.490) | 6.55 (4.93-8.70) | 4.49 (4.04-4.96) | 14.26 (13.11-15.50) |
| **2b** | 0.323 (0.232-0.449) | 4.57 (3.67-5.69) | 3.90 (2.69-5.65) | 9.03 (5.62-14.49) |
| **2c** | ND | 2.98 (2.43-3.64) | 2.28 (2.02-2.57) | 4.28 (3.44-5.33) |
| **2c’** | ND | 4.95 (4.71-5.20) | 5.63 (3.77-8.39) | 5.74 (3.91-8.41) |
| **2d** | 0.549 (0.410-0.736) | 5.07 (4.15-5.42) | 5.98 (5.32-6.71) | 6.56 (5.72-7.52) |
| **2d’** | 0.045 (0.038-0.053) | 2.74 (2.57-2.92) | 3.09 (2.08-4.58) | 5.26 (4.13-6.68) |
| **2e** | 0.213 (0.161-0.281) | 6.23 (4.72-8.21) | 6.56 (5.96-7.21) | 8.94 (7.51-10.63) |
| **2e’** | 0.310 (0.270-0.360) | 5.48 (5.10-5.75) | 7.40 (6.25-8.75) | 6.92 (5.35-8.94) |
| **2f** | 0.361 (0.289-0.451) | 8.03 (6.82-9.44) | 9.01 (7.94-10.22) | 9.17 (8.10-10.37) |
| **2g** | 0.568 (0.123-2.64) | 6.42 (4.38-9.41) | 9.70 (7.48-12.57) | 20.23 (15.48-26.42) |
| **2h** | 0.307 (0.270-0.349) | 3.99 (3.62-4.38) | 7.53 (5.31-10.66) | 17.70 (13.17-23.78) |
| Doxorubicin | 0.970 (0.520-1.80) | 0.258 (0.173-0.385) | 0.357 (0.278-0.463) | 0.476 (0.322-0.705) |

Data are presented as $IC_{50}$ values and 95% confidence intervals obtained by nonlinear regression for all cell lines. Doxorubicin was used as positive control. Only compounds with an $IC_{50}$ value lower than 5 µg $mL^{-1}$ for at least one cell line were considered active. ND: not determined.

growth inhibition. The calculation of $IC_{50}$ was measured by MTT assay after 72 h of incubation at concentrations from 0.01 to 25 µg $mL^{-1}$. Peripheral mononuclear blood cells (PBMC) were tested to confront the data in normal cells. The results are shown in Table 4.

From the eleven selected samples for determination of $IC_{50}$, eight (**2b**-**f**, **2c’**-**e’**) showed inhibitory concentration smaller than 10 µM for the three tested tumor cell lines, characterizing them as potent cytotoxic agents. The compounds (**2c**, **2d** and **2e**) which bear hydroxyl groups have approximately the same cell growth inhibition ($IC_{50}$) of acylated compounds (**2c’**, **2d’** and **2e’**). Thus, it is possible to see that the hydroxyl group in these compounds did not significantly influence the cytotoxic action for the three analyzed tumor cell lines. Samples **2a**, **2g** and **2h** exhibited $IC_{50}$ higher than 10 µM for PC-3M, showing lower cytotoxic action for this cell line and higher selectivity for inhibitory action against OVCAR-8 and NCI-H358M lines. The increase of the aliphatic chain reduced the cytotoxic effect on PC-3M cell line. The 2,3-di(3-hydroxy-3-methylbut-1-in-yl)-1,4-naphthoquinone (**2c**) presented the lower $IC_{50}$ (2.28-4.28 µM) for all three tested cell lines, characterizing this substance as a potent cytotoxic agent. Although the excellent cytotoxicity in cancer cell lines, all the compounds presented high inhibitory effect in peripheral blood mononuclear cells, which means non-selectivity effect and high cytoxicity. Further investigation aiming at mechanisms of action and hemolytic activity are required for a better understanding of the cytotoxic effects and for determining the potential of the compounds reported in this work as future antitumor agents.

## Conclusions

In this work, new potential antitumor active diyne-naphthoquinones were synthesized (twelve derivatives), nine of them new entities. In general, they exhibited inhibitory concentrations ($IC_{50}$) smaller than 10 µM for all three tested tumor cell lines, characterizing potent cytotoxic action. These results are significant for continuing studies of cytotoxicity in this class of bioactive compounds.

## Experimental

### General remarks

$^1H$ and $^{13}C$ NMR spectra were recorded on a Varian unity plus-300 or 400 MHz spectrometer in $CDCl_3$ or DMSO-$d_6$. All IR spectra were recorded on a Bruker IFS66 spectrophotometer using KBr pellets. High resolution mass spectra were obtained by electrospray on a Shimadzu LC-MS-IT-TOF spectrometer. Sonication was performed using a Model USC-1400A ultracleaner with a frequency of 40 kHz. Melting points are uncorrected and were determined with an electrically heated block apparatus. Monitoring of the course of the reactions was performed by $GF_{254}$ thin layer chromatography (TLC) and the column chromatography was performed on silica gel $G_{60}$ (70-230 mesh, Merck). All solvents and reagents were purchased from commercial suppliers (Sigma-Aldrich, Tedia, Merck, Cinética, Fluka), and used as received or purified by standard procedures.

### Synthesis of the 2,3-diyne-1,4-naphthoquinone derivatives (**2**)

A mixture of terminal alkynes (1.0 mmol), CuI (0.13 g, 0.68 mmol) and triethylamine (0.07 g, 0.8 mmol) in anhydrous DMSO (4 mL) and $CH_2Cl_2$ (3 mL) was stirred for 2 min under argon atmosphere, then 2,3-dibromo-1,4-naphthoquinone (0.100 g, 0.32 mmol) and $Pd(PPh_3)_2Cl_2$ (0.005 g, 0.007 mmol) were added to the reaction mixture. The stirring was continued until total consumption of the starting material (**1**), after that, $CH_2Cl_2$ was removed by reduced pressure and water was added to the reaction mixture. The precipitate formed was separated by vacuum filtration and the compounds were isolated by silica gel column chromatography.

### 2,3-di(Phenylethynyl)-1,4-naphthoquinone (**2a**)

Using dichloromethane/hexane 30:70 as eluent, **2a** was obtained as a red solid (mp 151-152)[17] in 45% (51 mg, 0.143 mmol) yield.

### 2,3-di(4-Methoxylphenylethynyl)-1,4-naphthoquinone (**2b**)

Using dichloromethane/hexane 50:50 as eluent, **2b** was obtained as a red-winesh solid (mp 162-163 °C) in 46% (61 mg, 0.146 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2175, 1664, 1605, 1531, 1376, 1330, 1302, 1257, 1156, 1026, 904, 826, 711, 535; $^{1}$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.15 (dd, 2H, 5.7/3.3 Hz), 7.76 (dd, 2H, 5.7/3.3 Hz), 7.63 (m, 4H), 6.92 (m, 4H), 3.86 (s, 6H); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 180.9, 161.1, 134.2, 133.9, 133.1, 132.1, 126.8, 114.3, 109.9, 84.8, 55.4. LC-MS calcd. for $[C_{28}H_{19}O_4]^+$, 419.1278; found: 419.1261.

### 2,3-di(3-Hydroxy-3-methylbutynyl)-1,4-naphthoquinone (**2c**)

Using ethyl acetate/hexane 30:70 as eluent, **2c** was obtained as a dark yellow solid (mp 138-139 °C)[17] in 42% (43 mg, 0.134 mmol) yield.

### 2,3-di[(1-Hydroxycyclohexyl)ethynyl]-1,4-naphthoquinone (**2d**)

Using ethyl acetate/hexane 25:75 as eluent, **2d** was obtained as a yellow solid (mp 109-110 °C) in 30% (38 mg, 0.095 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 3227, 2934, 2856, 2208, 1666, 1594, 1550, 1449, 1345, 1277, 1185, 1073, 986, 966, 788, 712; $^{1}$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.07 (d, 2H, 3.2 Hz), 7.73 (d, 2H, 3.6 Hz), 2.07 (s, 4H), 1.71 (m, 16H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 180.7, 134.1, 133.9, 131.8, 126.8, 113.6, 79.1, 69.5, 39.5, 25.2, 23.2. LC-MS calcd. for $[C_{26}H_{26}O_4Na]^+$, 425.1729; found: 425.1705.

### 2,3-di(5-Hydroxypent-1-yn-1-yl)-1,4-naphthoquinone (**2e**)

Using ethyl acetate/dichloromethane 80:20 as eluent, **2e** was obtained as a pale yellow solid (mp 130-131 °C) in 25% (25 mg, 0.078 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 3328, 3237, 2949, 2213, 1667, 1597, 1358, 1322, 1292, 1194, 1031, 954, 796, 713, 642; $^{1}$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 7.96 (dd, 2H, 6.0/3.3 Hz), 7.85 (dd, 2H, 6.0/3.3 Hz), 4.60 (t, 2H, 5.2 Hz), 3.57 (dd, 4H, 6.3/5.1 Hz), 2.65 (t, 4H, 7.0 Hz), 1.73 (quin, 4H, 6.6 Hz); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 180.6, 134.3, 133.9, 131.5, 126.3, 110.6, 76.3, 59.2, 31.5, 16.3. LC-MS calcd. for $[C_{20}H_{19}O_4]^+$, 323.1284; found: 323.1295.

### 2,3-di(Pent-1-yn-1-yl)-1,4-naphthoquinone (**2f**)

Using dichloromethane/hexane 40:60 as eluent, **2f** was obtained as a yellow solid (mp 92-93 °C) in 38% (35.4 mg, 0.122 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2962, 2932, 2870, 2212, 1665, 1595, 1547, 1456, 1356, 1321, 1292, 1188, 991, 794, 713, 642; $^{1}$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.09 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.59 (t, 4H, 7.2 Hz), 1.69 (sex, 4H, 7.2 Hz), 1.10 (t, 6H, 7.5 Hz); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 181.3, 134.6, 133.9, 131.9, 126.5, 111.4, 76.6, 22.5, 21.79, 13.5. LC-MS calcd. for $[C_{20}H_{19}O_2]^+$, 291.1385; found: 291.1419.

### 2,3-di(Hex-1-yn-1-yl)-1,4-naphthoquinone (**2g**)

Using dichloromethane/hexane 25:75 as eluent, **2g** was obtained as a pale yellow solid (mp 68-69 °C) in 55% (55 mg, 0.173 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 3314, 3370, 2954, 2928, 2867, 2212, 1670, 1595, 1548, 1455, 1420, 1357, 1320, 1292, 796, 711, 641; $^{1}$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.09 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.62 (t, 4H, 7.0 Hz), 1.60 (m, 8H), 0.96 (t, 6H, 7.2 Hz); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 181.3, 134.5, 133.8, 131.8, 126.8, 111.5, 76.1, 30.3, 21.9, 20.2, 13.8. LC-MS calcd. for $[C_{22}H_{23}O_2]^+$, 319.1698; found: 319.1731.

### 2,3-di(Oct-1-yn-1-yl)-1,4-naphthoquinone (**2h**)

Using dichloromethane/hexane 30:70 as eluent, **2h** was obtained as a red solid (mp 54-55 °C) in 38% (45 mg, 0.120 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2924, 2853, 2213, 1673, 1595, 1538, 1463, 1359, 1320, 1294, 1193, 1072, 989, 795, 711, 644; $^{1}$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.09 (2H), 7.72 (2H), 2.60 (t, 4H, 7.0 Hz), 1.68 (quin, 4H, 7.2 Hz), 1.50 (m, 4H), 1.33 (bs, 8H), 0.90 (t, 6H, 6.0 Hz); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 181.3, 134.6, 133.9, 131.9, 126.8, 111.6, 76.2, 31.3, 28.6, 28.3, 22.5, 20.6, 14.0. LC-MS calcd. for $[C_{26}H_{31}O_2]^+$, 375.2325; found: 375.2363.

### 2,3-di(Dec-1-yn-1-yl)-1,4-naphthoquinone (**2i**)

Using dichloromethane/hexane 30:70 as eluent, **2i** was obtained as a red solid (mp 52-53 °C) in 30% (40 mg, 0.093 mmol) yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2954, 2917,2848, 2213, 1670, 1594, 1544, 1362, 1320, 1295, 1196, 712, 642;

$^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.09 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.59 (t, 4H, 7.0 Hz), 1.66 (m, 4H), 1.29 (m, 16H), 1.49 (m, 4H), 0.87 (t, 6H, 6.7 Hz); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 181.3, 134, 133.9, 131.8, 126.8, 111.6, 76.1, 31.8, 29.2, 29.1, 28.9, 28.4, 22.6, 20.6, 14.1. LC-MS calcd. for [$C_{30}H_{39}O_2$]$^+$, 431.2951; found: 431.2909.

### Acetylation of diyne derivatives[23]

Diol (**2c**, **2d** or **2e**) (0.125 mmol) was mixed with montmorillonite K-10 (200% by weight of substrate) and 1 mL of acetic anhydride. The reaction was performed under ultrasound irradiation at room temperature for 60 min. After this time, 15 mL of dichloromethane were added and montmorillonite K-10 was removed by simple filtration. The filtrate was neutralized with a concentrated solution of NaHCO$_3$ (3 × 20 mL) in a separatory funnel and the organic phase dried with anhydrous sodium sulfate. The product was purified by column chromatography on silica gel using a mixture of ethyl acetate/hexane.

### Compound **2c'**

Compound was obtained as a pale yellow solid (mp 123-124 °C) in 56% yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2992, 2937, 2211, 1739, 1673, 1587, 1547, 1351, 1254, 1131, 1016, 956, 790, 711, 608; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.06 (dd, 2H, 5.7/3.3 Hz), 7.73 (dd, 2H, 5.7/3.3 Hz), 2.06 (s, 6H), 1.80 (s, 12H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 180.4, 169.0, 134.1, 133.7, 131.7, 126.8, 109.6, 78.5, 71.9, 28.6, 21.7. LC-MS calcd. for [$C_{24}H_{22}O_6$Na]$^+$, 429.1314; found: 429.1325.

### Compound **2d'**

Compound was obtained as a pale yellow solid (mp 120-121 °C) in 65% yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2954, 2932, 2860, 2205, 1741, 1671, 1550, 1444, 1345, 1276, 1223, 1031, 994, 966, 712, 607; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.07 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.29 (m, 4H), 2.08 (s, 6H), 1.94 (m, 4H), 1.71 (m, 12H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 180.5, 168.8, 133.9, 133.3, 131.8, 126.8, 109.3, 80.9, 75.5, 36.7, 25.2, 22.6, 21.7. LC-MS calcd. for [$C_{30}H_{30}O_6$Na]$^+$, 509.1941; found: 509.1947.

### Compound **2e'**

Compound was obtained as a pale yellow solid (mp 91-92 °C) in 71% yield; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2215, 1735, 1667, 1548, 1357, 1296, 1246, 1036, 713; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.09 (dd, 2H, 6.0/3.0 Hz), 7.74 (dd, 2H, 6.0/3.0 Hz), 4.27 (t, 4H, 6.3 Hz), 2.73 (t, 4H, 7.0 Hz), 2.07 (s, 6H), 2.01 (m, 4H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 181.1, 171.0, 134.3, 134.0, 131.8, 126.9, 109.6, 62.9, 27.4, 20.9, 17.3. LC-MS calcd. for [$C_{24}H_{22}O_6$Na]$^+$, 429.1314; found: 429.1317.

### Biological

### Cytotoxicity against cancer cell lines

The cytotoxic effects of the synthesized compounds were evaluated against the following human cancer cell lines and non-tumor cells (PBMC), all transformed cell lines were obtained from the National Cancer Institute, Bethesda, MD, USA: PC-3M (prostate carcinoma), OVCAR-8 (ovarian carcinoma) and NCI-H358M (bronchoalveolar lung carcinoma). The cell lines were maintained in RPMI-1640 medium (cancer cells) supplemented with 10% fetal bovine serum, 2 mmol L$^{-1}$ glutamine, 100 µg mL$^{-1}$ penicillin and 100 µg mL$^{-1}$ streptomycin at 37 °C with 5% CO$_2$.

Cell growth was quantified by the ability of living cells to reduce a yellow dye (MTT) to a purple formazan product.[28] For all of the experiments, the cells were seeded in 96-well plates ($0.1 \times 10^5$ cells *per* well for adherent cells). After 24 h, the compounds (0.01 to 25 mg mL$^{-1}$), dissolved in DMSO, were added to each well (using an HTS (high-throughput screening) Biomek 3000, Beckman Coulter, Inc. Fullerton, California, USA) and incubated for 72 h. Doxorubicin (Sigma-Aldrich Co., St. Louis, MO, USA) was used as a positive control. At the end of the incubation, the plates were centrifuged, and the medium was replaced by fresh medium (200 µL) containing 0.5 mg mL$^{-1}$ MTT. After 3 h, the formazan product was dissolved in 150 µL DMSO, and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, California, USA). The substance effect was quantified as the percentage of the control absorbance at 595 nm.

## Supplementary Information

Supplementary information (spectral data and figures containing IR, HRMS, $^1$H and $^{13}$C NMR) are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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