

Novel 2-(R-phenyl)amino-3-(2-methylpropenyl)-[1,4]-naphthoquinones: Synthesis, Characterization, Electrochemical Behavior and Antitumor Activity

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Novas 2-(R-fenil)amino-3-(2-metilpropenil)-[1,4]-naftoquinonas (R = H, 4-OMe, 4-Ferrocenil, 4-Me, 3-Me, 4-I, 3-I, 4-CN, 3-CN, 4-NO₂ e 3-NO₂) derivadas do *nor*-lapachol [2-hidroxi-3-(2-metilpropenil)-1,4-naftoquinona] foram obtidas em bons rendimentos. A estrutura dos compostos foi proposta com base em estudos de difração de raios-X (R = OMe, **2b**), dados de RMN de ¹H e ¹³C e cálculos teóricos utilizando o funcional B3LYP e a base 6-311+G(2d,p). Os potenciais de meia-onda das aminonaftoquinonas e o deslocamento químico do hidrogênio da cadeia 3-propenil dos compostos **2a-k** mostraram boa correlação com as constantes de Hammett dos substituintes presentes no anel fenílico. A avaliação da citotoxicidade evidenciou atividade antitumoral promissora para o substrato metóxi-*nor*-lapachol **1** e o derivado 4-ferrocenil **2c**.

Novel 2-(R-phenyl)amino-3-(2-methyl-propenyl)-[1,4]-naphthoquinones (R = H, 4-OMe, 4-Ferrocenyl, 4-Me, 3-Me, 4-I, 3-I, 4-CN, 3-CN, 4-NO₂ and 3-NO₂) derived from *nor*-lapachol [2-hydroxy-3-(2-methylpropenyl)-1,4-naphthoquinone] were obtained in good yields. Their structures were proposed on the basis of a single crystal X-ray diffraction study (R = OMe, **2b**), ¹H and ¹³C NMR studies and calculations using the B3LYP functional and the 6-311+G(2d,p) basis set. The half-wave potentials of the aminonaphthoquinones and ¹H NMR chemical shifts of the 3-propenyl hydrogen in **2a-k** show good correlation with the substituent Hammett constants on the phenylamino ring. The antitumor assays showed promising activity for substrate methoxy-*nor*-lapachol **1** and the 4-ferrocenyl derivative **2c**.

Keywords: *Nor*-lapachol, arylamine, aminonaphthoquinone, electrochemistry, B3LYP, antitumor activity

Introduction

Naphthoquinones are widely distributed in nature and some of these molecules have an important role in the biochemistry of microbial energy production, by means of photosynthesis and respiratory chain.¹ Compounds containing the quinone group are known for exhibiting antitumor,² trypanocide,³ moluscicide,⁴ fungicide⁵ and antimalarial⁶ activities. The presence of an amino group

in quinones has led to interesting biologically active compounds.⁷⁻¹²

Biological activity of quinones is often related to their electrochemical behavior.¹³ The ability to accept one or two electrons to form the corresponding radical anion (Q^{•-}) or dianion (Q²⁻) species is believed to induce formation of reactive oxygen species, responsible for the oxidative stress in cells.¹⁴ The electron-accepting capacity of naphthoquinones may be tuned by carbonyl position changes (1,2- x 1,4-naphthoquinones)¹⁵ or different substituents or functions attached to the naphthoquinone moiety, and the use of electrochemical methods to study

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this type of molecules has proven to be useful.^{16,17} We reported recently¹⁸ the synthesis of a series of 2-arylamino-1,4-naphthoquinones from 2-methoxy-1,4-naphthoquinone and arylamines in the presence of $MgCl_2 \cdot 6H_2O$ and *p*-toluenesulfonic acid as catalysts. The reactions of both electron-donor and electron-attracting substituted anilines having given good yields of the respective products, we decided to investigate the analogous reactions of the methoxy-derivatives of *nor*-lapachol [2-hydroxy-3-(2-methyl-propenyl)-1,4-naphthoquinone]⁴ and lapachol [2-hydroxy-3-(3-methyl-2-but-enyl)-1,4-naphthoquinone].¹⁹ *Nor*-lapachol is obtained from lapachol by the Hooker oxidation²⁰ and has been used as a substrate for the synthesis of several active compounds.^{9,12} The incorporation of polyamines to this quinone, for example, has led to significant increase in the DNA topoisomerase II- α inhibition, compared to the original naphthoquinone.^{13,14} Furthermore, arylamino derivatives of *nor*- α and *nor*- β lapachones present potent antitumor¹¹ and trypanocide activities.⁹

Herein is the first report on the synthesis and characterization of the novel 2-arylamino derivatives of *nor*-lapachol **2a-k**, including 2-(4-ferrocenyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone **2c** (Figure 1) and cytotoxic screening against the several cancer cell lines (SF-295, HCT-8, MDAMB-435 and HL-60). Because correlations between electrochemical potentials and the inhibitory activity of naphthoquinones on Epstein-Barr virus early antigen activation²¹ and their cytotoxicity²² has been reported, we also investigated the redox properties of these compounds by cyclic voltammetry.

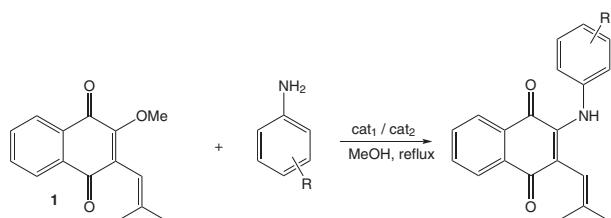


Figure 1. Synthesis of the novel arylamines derived from methoxy-*nor*-lapachol **1**.

Results and Discussion

Syntheses

The compounds **2a-k** (Figure 2) were synthesized from 2-methoxy-3-(2-methylpropenyl)-[1,4]-naphthoquinone with various aromatic amines, in the presence of the catalysts 4-toluenesulfonic acid and $MgCl_2 \cdot 6H_2O$ in methanol under reflux.¹⁸ The products are stable in the solid state and in solution. Compounds **2a**, **2b** were obtained

in a pure state, whereas **2c-k** were purified by column chromatography using a mixture of ethyl acetate / hexane (1:5) as eluent. They were obtained in yields ranging from 84 to 73% and formulated on the basis of analytical and spectroscopic data (see Experimental).

The 1H NMR and infrared spectra of compounds **2a-k** are consistent with their composition and structure. The 1H NMR spectra exhibit signals in the δ 7.5-8.2 ppm region as double doublets and triple doublets, attributed to the four naphthoquinone aromatic hydrogens H5-H8. Attributions were made on the basis of $^1H \times ^1H$ -COSY experiments, *J* values and multiplicity. All expected resonances were observed in the ^{13}C NMR spectra of compounds **2a-k**. The carbonyl peaks appear around δ 183 and 180, and those attributed to C2 bound to the nitrogen at about δ 145. The other chemical shifts are compatible with the structures proposed for these compounds. We observed that the chemical shift of H18 (Figure 2) in the 1H NMR spectra of **2a-k** is directly influenced by the nature of the R substituent group in the phenylamino ring [5.80 (4-OMe) < $\delta_{H(18)}$ < 6.08 (4-NO₂)].

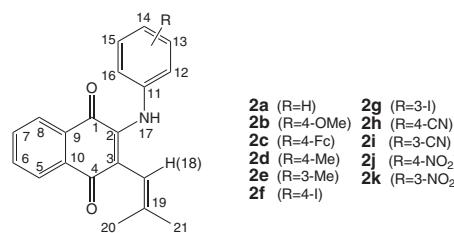


Figure 2. Compounds **2a-k**.

X-ray structure / Theoretical calculations

The structure of 2-(4-methoxy-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone **2b** was determined by a single crystal X-ray diffraction study (Figure 3). The average C-C, C-O, C=O and C-N bond lengths are in good agreement with the literature.³⁶ The naphthoquinone ring system of **2b** is approximately planar, the dihedral angle between the naphthoquinone plane and the arylamine phenyl ring being $47.3^\circ(1)$. The torsion angles around the fragments involved in the H-bond are: C(2)-N(1)-C(11)-C(12), $-158.94(17)^\circ$, C(1)-C(2)-N(1)-C(11) $-154.47(16)^\circ$ and N(1)-C(2)-C(1)-O(2) $-3.2(2)^\circ$. The planar unsaturated side chain is twisted about 54° with respect to the ring system. This is the first reported structure of an amine derivative of *nor*-lapachol.

The packing of **2b** involves molecules that interact through classical and non-classical hydrogen bonds, forming a 1D infinite network along the crystallographic [100] direction (Figures 4 and 5). The carbonyl O1ⁱ atom

makes a classical and a non-classical hydrogen bonds with H1 to the N1 atom (amino group) and with H12 to the C12 atom (C11-C16 phenyl ring) of a neighboring molecule, forming a six-membered ring [symmetry code: $i=x-1, y, z$]. In addition the other C=O group interacts *via* O2ⁱⁱ with H5 to the C5 forming now a ten-membered ring. For more details of the crystal structure, see Supplementary material.

Starting from the experimental structure of **2b** (Figure 3), the geometries of **2a-2e** and **2h-2i** were fully optimized with the B3LYP/6-31G(d) method.³⁷ Energies and molecular properties were obtained from a single-point calculation on the optimized geometries using the 6-311+G(2d,p) basis set³⁸ and the B3LYP functional.³⁹ To confirm that the most stable conformation in the gas-phase is similar to that found in the solid state the geometry of an alternative conformation for **2b**, with the 2-methylpropenyl group bonded to position 3 of the naphthoquinone ring rotated by 180° was also optimized. This alternative

conformation is 0.4 kcal mol⁻¹ less stable than the solid state conformation. The barrier for conversion between the two conformers calculated at the 6-31G(d) level is 5.5 kcal mol⁻¹.

Calculations of the absolute ¹H NMR chemical shifts using the GIAO approach⁴⁰ confirmed that electron-attracting groups yield higher $\delta_{\text{H}(18)}$ values than electron-donor groups. Calculations including solvent (chloroform) show essentially the same behavior. Interestingly, the $\delta_{\text{H}(18)}$ value for the alternative conformation with the 2-methylpropenyl side chain rotated by 180° is shifted highfield by 1.67 ppm, compared to the same hydrogen in the most stable conformation. The fact that the experimental values are intermediate between the calculated values for the two conformations suggests that these conformations are in equilibrium in solution, although the variable temperature ¹H NMR spectra of **2a** do not show broadening of H18 down to -90 °C in CD₂Cl₂.

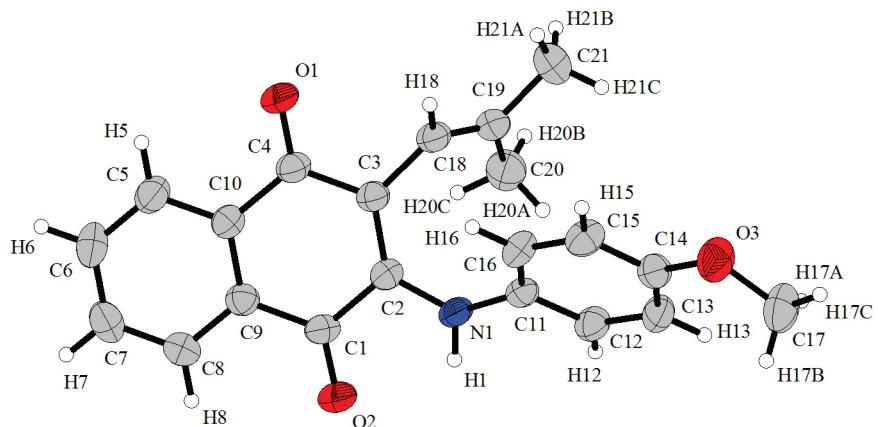


Figure 3. View of the ORTEP plot for **2b** with labeled atoms and 50% probability ellipsoids.

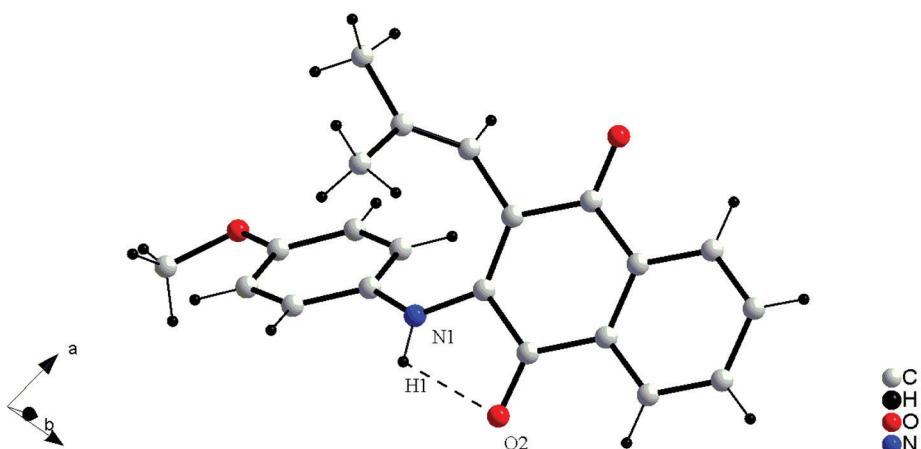


Figure 4. View of the intramolecular interaction.

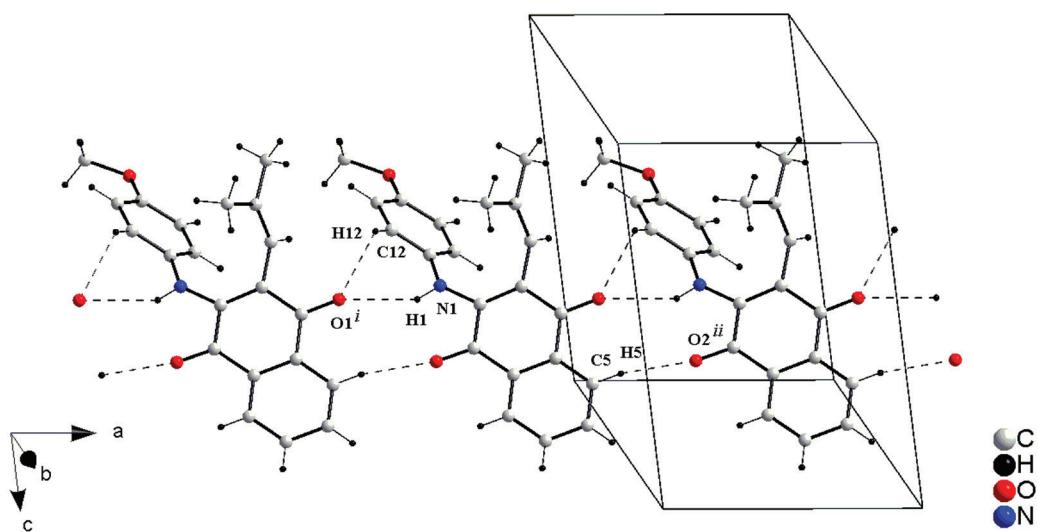


Figure 5. View of the self-assembly 1D by classical and non-classical hydrogen bonds along the [100] crystallography direction. [Symmetry codes: (i) $x-1, y, z$; (ii) $x+1, y, z$].

UV-Vis spectra

The UV-Vis spectra of the compounds obtained in CHCl_3 show two absorption bands. PBE1PBE/6-311+G(2d,p) calculations indicate that the band in the 275–290 nm region can be attributed to the aromatic and quinone $\pi-\pi^*$ transitions and the low-energy band in the visible region between 456 and 512 nm is attributed predominantly to π phenyl- π^* naphthoquinone transitions. Electron-donor substituents blue shift the latter band, whereas electron-attracting groups red shift it.

Cyclic voltammetry

The redox behavior of compounds **2a-k** was evaluated by cyclic voltammetry (CV) at room temperature in acetonitrile/ Bu_4NPF_6 (0.1 mol L⁻¹). The CVs were obtained in the potential range from +1.3 to -2.1 V vs FcH/FcH⁺ as internal standard (Table 1). Two quasi-reversible pairs of waves were observed for compounds **2a-i** in the negative region of the CV, which are attributed to the one-electron transfer to the naphthoquinone moiety. The redox potentials of the naphthoquinone unit are directly influenced by the substituents in the phenylamino ring: electron-donor groups present lower $E_{1/2}$ when compared to electron-releasing groups. The complexity of the CV observed for **2j** indicated that the nitro group is also electroactive in the cathodic region studied⁴¹ and because the reduction potentials for the nitro and the quinone moieties are similar, we were unable to assess the voltammetric parameters for this derivative. The data indicate that derivative **2c**, R= ferrocenyl (Fc, $E_{1/2} = -1.23$ V) exhibit electronic properties similar to

compound **2b**. This was also observed for the Fc-arylamine derivative of lawsone.¹⁸

Table 1. Voltammetric data (V) for **2a-k** vs FcH/FcH⁺ as internal standard

Compound	$E_{1/2}$ (1)	$E_{1/2}$ (2)	$E_{1/2}$ (1) – $E_{1/2}$ (2)
2a	-1.19	-1.54	0.35
2b	-1.23	-1.59	0.36
2c	-1.23	-	-
2d	-1.20	-1.58	0.38
2e	-1.23	-1.50	0.27
2f	-1.17	-1.57	0.40
2g	-1.16	-1.48	0.32
2h	-1.10	-1.47	0.37
2i	-1.15	-1.53	0.38
2j	-	-	-
2k	-1.18	-1.65	0.47

Good correlation of the $E_{1/2}$ (1) potentials with the σ_p and σ_p^- Hammett constants⁴² was obtained (Figures 6 and 7, respectively) except for the Fc group (Figure 7) for which the low σ_p^- value (-0.03)⁴³ has been correlated to low resonance contribution. The linear correlation coefficients for both plots suggest that all naphthoquinones of this series are reduced by the same mechanism. $E_{1/2}$ (1) potentials do not show linear correlation with σ_m values.

Antitumor assays

The antitumor screening of compounds **2a-k** was carried out against three cancer cell lines: SF-295 (central nervous system), HCT-8 (colon), MDAMB-435 (breast) through an MTT assay³⁵ and the results, summarized in the

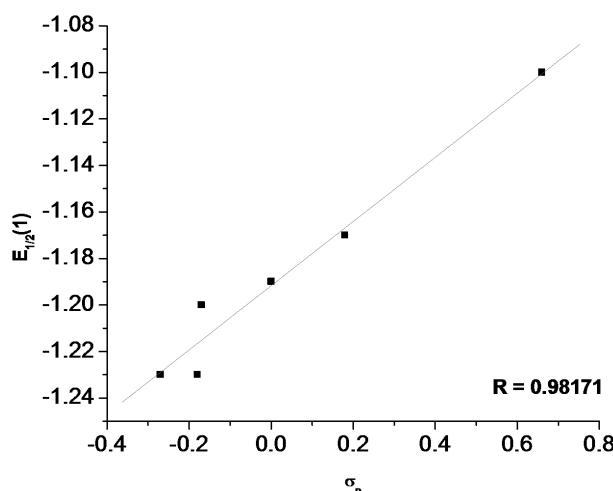


Figure 6. Correlation between $E_{1/2}(1)$ and σ_p^+ .

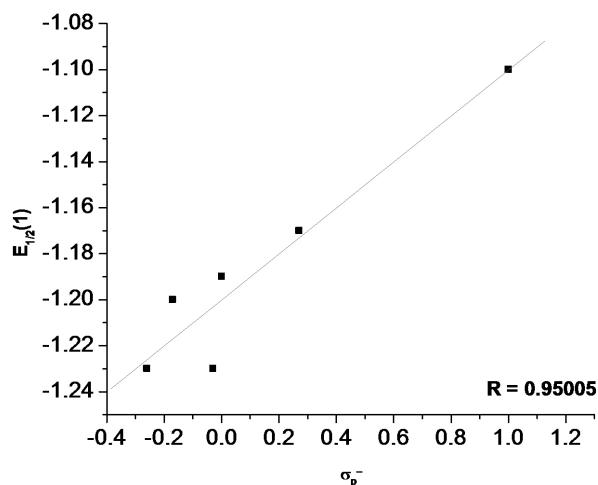


Figure 7. Correlation between $E_{1/2}(1)$ and σ_p^- .

Supplementary information, show that the Fc-derivative **2c** and the methoxy-substrate **1** presented significant proliferation inhibition against MDA-MB435, higher than the positive control doxorubicin (DOX). In a second set of experiments, four cell lines were used for IC_{50} determination of previously selected compounds (**1** and **2c**). Only methoxy-nor-lapachol **1** was highly active against MDA-MB435 and moderately active against HL-60 and HCT-8 cell lines (Table 2). The loss of activity of the ferrocenyl derivative **2c** may be due to decomposition during dilution and defreezing

Table 2. Cytotoxic activity expressed by IC_{50} in $\mu\text{g mL}^{-1}$ of compounds **1**, **2c** and doxorubicin (DOX), with the respective confidence intervals

Compound	SF295	HCT-8	MDA-MB435	HL-60
2c	> 5	> 5	> 5	> 5
1	> 5	2.53 (1.38-4.66)	0.30 (0.14-0.64)	1.17 (0.59-2.32)
DOX	0.42 0.35-0.46	0.07 0.05-0.09	0.86 0.62-1.20	0.04 0.02-0.04

of the solution, since this compound is slightly unstable in solution in the presence of oxygen.

Experimental

Materials and methods

Reagents and solvents were used without further purification. Microanalyses were performed using a Perkin-Elmer CHN 2400 micro analyser at the Central Analítica, Instituto de Química, USP-São Paulo, Brazil. Melting points were obtained with a Mel-Temp II, Laboratory Devices-USA apparatus and are uncorrected. IR spectra (KBr pellets) were recorded on a FT-IR Spectrum One (Perkin Elmer) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Varian Unit Plus 300 MHz spectrometer in CDCl_3 ; coupling constants are reported in Hertz (Hz) and chemical shifts in parts per million (ppm) relative to internal standard Me_4Si . The hydrogen signals were attributed through coupling constant values and $^1\text{H} \times ^1\text{H}$ - COSY experiments. Electronic spectra were taken on a Diode Array 8452A (Hewlett Packard-HP) spectrophotometer using spectroscopic grade solvents (Tedia Brazil) in 10^{-3} and 10^{-4} mol L^{-1} solutions. Cyclic voltammograms were obtained on an Epsilon-BAS potentiostat-galvanostat from 1×10^{-3} mol L^{-1} solutions in chloroform containing 0.1 mol L^{-1} of TBABF_4 as supporting electrolyte, at room temperature and under argon atmosphere. A standard three component system was used: a carbon-glassy working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl reference electrode for organic media. Ferrocene was used as an internal standard ($E_{1/2}$ 0.40 V *vs* NHE). Density functional calculations were carried out using the Gaussian03W molecular orbital package.²³ Geometries were fully optimized using the B3LYP functional²⁴ with the standard 6-31G(d) basis set²⁵ Solvent effects (chloroform) were estimated by single-point calculations on the gas-phase optimized geometries by mean of the continuum solvation model using the conductor-like polarisable continuum model²⁶ (CPCM) at the same level. NMR absolute chemical shifts were calculated using the GIAO (Gauge Independent Atomic Orbital) method with the B3LYP/6-311+G(2d,p) approach on the B3LYP/6-31G(d) optimized geometry. The electronic spectra were calculated using the TD (Time Dependent) methodology available in Gaussian. The PBE1PBE functional together with the 6-311+G(2d,p) basis set was employed.

Synthesis of the compounds **2a-k**

Compounds **2a-k** were synthesized by the same procedure we reported recently for the synthesis of

2-arylamino-1,4-naphthoquinones derived from 2-hydroxy-1,4-naphthoquinone and were obtained in yields that varied from 71% (**2c**) to 84% (**2h**).¹⁸ [2-methoxy-3-(2-methylpropenyl)-[1,4]-naphthoquinone] (155 mg, 0.64 mmol) **1** in MeOH (6.00 mL) was heated under reflux in the presence of 4-toluenesulfonic acid (17.2 mg, 0.1 mmol) and MgCl₂·6H₂O (20.3 mg, 0.1 mmol) as catalysts for 10 min. to dissolve most of **1**. After addition of the respective arylamine (0.96 mmol), the reactions were monitored by TLC (1:9 ethyl acetate/hexane) and were stopped when **1** was no more observed. The resulting solids were filtered, washed with water and cold MeOH and dried under vacuum. The melting points and elemental analysis data are indicative of pure compounds. In contrast, the analogous reactions of methoxylapachol under the same conditions yielded the corresponding 1-aza-1,2-dihydro-5,10-anthaquinones in low yields described previously.¹⁹

2-(Phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2a)

Yield: 78%. mp 131-133 °C. Anal. Calc. for C₂₀H₁₇NO₂: C 79.14; H 5.47; N 4.67%; found: C 79.19; H 5.65; N 4.62%. ¹H NMR (300 MHz, CDCl₃): δ 8.13 (ddd, 1H, J 6.51, J 1.51, J 0.49 Hz), 8.10 (ddd, 1H, J 6.51, J 1.51, J 0.49 Hz), 7.73 (td, 1H, J 6.51, J 1.51 Hz), 7.65 (td, 1H, J 6.51, J 1.51 Hz), 7.23 (br t, 1H, J 7.52 Hz), 7.07 (tt, 1H, J 7.52, J 2.05, J 1.13 Hz), 7.91 (m, 1H), 6.89 (dt, 1H, J 7.93, J 2.05, J 1.34 Hz), 5.87 (m, 1H), 1.38 (d, 3H, J 1.54 Hz), 1.24 (d, 3H, J 1.32 Hz). ¹³C NMR (75 MHz, CDCl₃): δ 188.2, 184.3, 140.2, 139.5, 137.9, 134.8, 133.6, 132.6, 130.8, 127.9, 126.7, 124.2, 120.1, 118.9, 117.0, 100.2, 25.6, 20.6. IR (KBr) ν_{max}/cm⁻¹: 3278, 3102, 3051, 2902, 1670, 1590, 1567. UV-Vis (CHCl₃) λ_{max}/nm: 290 (log ε 4.15), 463 (3.37).

2-(4-Methoxy-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2b)

Yield: 80%. mp 167-169 °C. Anal. Calc. for C₂₁H₁₉NO₃: C 75.66; H 5.74; N 4.20%; found: C 75.56; H 5.69; N 4.22%. ¹H NMR (300 MHz, CDCl₃): δ 8.10 (m, 2H), 7.72 (td, 1H, J 7.49, J 7.49, J 1.54 Hz), 7.63 (td, 1H, J 7.70, J 1.54 Hz), 5.80 (m, 1H), 6.85 (br d, 2H, J 9.25 Hz), 6.77 (br d, 2H, J 9.25 Hz), 3.80 (s, 3H), 1.40 (d, 3H, J 1.54 Hz), 1.25 (d, 3H, J 1.32 Hz). ¹³C NMR (75 MHz, CDCl₃): 183.7, 182.9, 156.5, 140.4, 138.8, 134.4, 133.0, 132.1, 130.8, 130.4, 126.0, 124.5, 118.4, 115.4, 114.7, 112.9, 55.4, 25.3, 20.2. IR (KBr) ν_{max}/cm⁻¹: 3288, 3100, 3031, 2889, 1668, 1600, 1577. UV-Vis (CHCl₃) λ_{max}/nm: 289 (log ε 4.20), 511 (3.45).

2-(4-Ferrocenyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2c)

Yield: 71%. mp 125-126 °C. Anal. Calc. for C₃₀H₂₅FeNO₂: C 73.93; H 5.17; N 2.87%; found: C 73.84; H 5.11; N 2.81%. ¹H NMR (300 MHz, CDCl₃): δ 8.16 (ddd, 1H, J 7.40, J 1.57, J 0.51 Hz), 8.13 (ddd, 1H, J 7.40, J 1.57, J 0.51 Hz), 7.79 (td, 1H, J 7.40, J 7.40, J 1.57 Hz), 7.69 (td, 1H, J 7.40, J 7.40, J 0.51 Hz), 7.55 (dd, 2H, J 6.12, J 2.09 Hz), 7.17 (dd, 2H, J 6.12, J 2.09 Hz), 5.82 (m, 1H), 4.61 (dd, 2H, J 2.37, J 1.98 Hz), 4.31 (dd, 2H, J 2.37, J 1.98 Hz), 4.04 (s, 5H), 1.35 (d, 3H, J 1.61 Hz), 1.24 (d, 3H, J 1.47 Hz). ¹³C NMR (75 MHz, CDCl₃): 184.1, 181.8, 155.8, 139.4, 138.1, 131.7, 133.5, 131.3, 130.2, 129.6, 125.8, 124.3, 118.2, 114.9, 114.5, 112.3, 69.2, 68.7, 66.3, 24.9, 19.8. IR (KBr) ν_{max}/cm⁻¹: 3281, 3098, 2880, 1670, 1599, 1571, 1108, 994. UV-Vis (CHCl₃) λ_{max}/nm: 294 (log ε 4.29), 510 (3.64).

2-(4-Methyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2d)

Yield: 81%. mp 141-142 °C. Anal. Calc. for C₂₁H₁₉NO₂: C 79.47; H 6.03; N 4.41%; found: C 79.43; H 6.01; N 4.38%. ¹H NMR (300 MHz, CDCl₃): δ 8.13 (m, 2H), 7.74 (td, 1H, J 7.57, J 1.47 Hz), 7.66 (td, 1H, J 7.57, J 1.47 Hz), 7.05 (br d, 2H, J 8.06 Hz), 6.80 (br d, 2H, J 8.06 Hz), 5.85 (m, 1H), 2.34 (s, 3H), 1.40 (d, 3H, J 1.47 Hz), 1.26 (d, 3H, J 1.22 Hz). ¹³C NMR (75 MHz, CDCl₃): 182.1, 181.3, 157.4, 141.4, 137.5, 133.9, 132.9, 132.0, 131.0, 130.9, 125.6, 123.9, 119.1, 114.3, 114.1, 112.5, 49.3, 25.1, 20.8. IR (KBr) ν_{max}/cm⁻¹: 3272, 3101, 3045, 2900, 1668, 1587, 1565. UV-Vis (CHCl₃) λ_{max}/nm: 286 (log ε 4.00), 505 (3.25).

2-(3-Methyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2e)

Yield: 84%. mp 151-152 °C. Anal. Calc. for C₂₁H₁₉NO₂: C 79.47; H 6.03; N 4.41%; found: C 80.01; H 6.03; N 4.53%. ¹H NMR (300 MHz, CDCl₃): δ 8.12 (m, 2H); 7.74 (td, 1H, J 7.48, J 7.48, J 1.51 Hz), 7.66 (td, 1H, J 7.48, J 7.48, J 1.51 Hz), 7.70 (m, 2H); 7.06 (br d, 2H, J 8.30 Hz); 5.84 (m, 1H); 6.80 (br d, 2H, J 8.30 Hz); 2.34 (s, 3H); 1.40 (d, 1H, J 1.22 Hz); 1.26 (d, 1H, J 1.33 Hz). ¹³C NMR (75 MHz, CDCl₃): 181.9, 181.2, 157.3, 141.5, 137.2, 133.8, 133.5, 132.4, 131.9, 131.0, 130.5, 129.5, 125.3, 123.4, 118.9, 114.0, 113.8, 112.1, 50.3, 24.5, 20.3. IR (KBr) ν_{max}/cm⁻¹: 3277, 3106, 3049, 2906, 1669, 1588, 1570. UV-Vis (CHCl₃) λ_{max}/nm: 287 (log ε 4.15), 507 (3.31).

2-(4-Iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2f)

Yield: 73%. mp 111-112 °C. Anal. Calc. for C₂₀H₁₆INO₂: C 55.96; H 3.76; N 3.26%; found: C 55.91; H 3.71; N 3.24%.

¹H NMR (300 MHz, CDCl₃): δ 8.13 (dd, 1H, J 7.52, J 1.58 Hz), 8.10 (dd, 1H, J 7.52, J 1.39 Hz), 7.74 (td, 1H, J 7.52, J 7.52, J 1.39 Hz), 7.66 (td, 1H, J 7.52, J 7.52, J 1.58 Hz), 7.54 (br d, 2H, J 8.71 Hz), 6.65 (br d, 2H, J 8.71 Hz), 5.90 (m, 1H), 1.47 (d, 3H, J 1.53 Hz), 1.24 (d, 3H, J 1.33 Hz). ¹³C NMR (75 MHz, CDCl₃): 184.3, 183.0, 140.3, 139.8, 137.7, 136.8, 134.9, 133.4, 132.8, 130.7, 126.5, 124.6, 118.9, 117.7, 100.3, 87.2, 25.7, 20.8. IR (KBr) ν_{max}/cm⁻¹: 3327, 3065, 2994, 1664, 1594, 1566. UV-Vis (CHCl₃) λ_{max}/nm: 290 (log ε 4.01), 460 (3.47).

2-(3-Iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2g)

Yield: 75%. mp 117–118 °C. Anal. Calc. for C₂₀H₁₆INO₂: C 55.96; H 3.76; N 3.26%; found: C 55.92; H 3.74; N 3.23%. ¹H NMR (300 MHz, CDCl₃): δ 8.11 (dd, 1H, J 7.61, J 1.64 Hz), 8.08 (dd, 1H, J 7.61, J 1.45 Hz), 7.72 (td, 1H, J 7.61, J 1.45 Hz), 7.63 (td, 1H, J 7.61, J 1.64 Hz), 7.40 (m, 1H), 6.90 (m, 3H), 5.89 (m, 1H), 1.44 (d, 3H, J 1.49 Hz), 1.23 (d, 3H, J 1.28 Hz). ¹³C NMR (75 MHz, CDCl₃): 184.1, 182.9, 140.5, 140.1, 137.3, 136.1, 134.6, 133.1, 132.4, 130.1, 126.9, 126.3, 124.3, 123.2, 118.1, 116.9, 100.1, 87.1, 25.2, 20.5. IR (KBr) ν_{max}/cm⁻¹: 3331, 3069, 2998, 1664, 1595, 1568. UV-Vis (CHCl₃) λ_{max}/nm: 284 (log ε 4.30), 456 (3.40).

2-(4-Cyano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2h)

Yield: 79%. mp 161–163 °C. Anal. Calc. for C₂₁H₁₆N₂O₂: C 76.81; H 4.91; N 8.53%; found: C 76.79; H 4.87; N 8.50%. ¹H NMR (300 MHz, CDCl₃): δ 8.14 (dd, 1H, J 1.59, J 7.46 Hz), 8.12 (dd, 1H, J 1.49, J 7.46 Hz), 7.77 (td, 1H, J 7.46, J 7.46, J 1.49 Hz), 7.70 (td, 1H, J 7.46 Hz, J 7.46, J 1.59 Hz), 7.52 (br d, 2H, J 8.58 Hz), 6.90 (br d, 2H, J 8.58 Hz), 6.03 (m, 1H), 1.50 (d, 3H, J 1.48 Hz), 1.24 (d, 3H, J 1.27 Hz). ¹³C NMR (75 MHz, CDCl₃): 184.4, 182.8, 141.9, 141.4, 138.6, 135.1, 133.2, 132.0, 130.7, 127.0, 126.7, 121.7, 120.3, 119.2, 118.8, 106.1, 100.3, 25.9, 21.0. IR (KBr) ν_{max}/cm⁻¹: 3282, 3079, 2972, 2927, 2218, 1664, 1594, 1567. UV-Vis (CHCl₃) λ_{max}/nm: 290 (log ε 4.19), 471 (3.48).

2-(3-Cyano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2i)

Yield: 82%. mp 173–174 °C. Anal. Calc. for C₂₁H₁₆N₂O₂: C 76.81; H 4.91; N 8.53%; found: C 76.77; H 5.01; N 8.55%. ¹H NMR (300 MHz, CDCl₃): δ 8.15 (ddd, 1H, J 5.25 Hz, J 1.60 Hz, J 0.46 Hz), 8.12 (ddd, 1H, J 5.25 Hz, J 1.60 Hz, J 0.46 Hz), 7.76 (td, 1H, J 5.25, J 5.25, J 1.60 Hz), 7.69 (td, 1H, J 5.25, J 5.25, J 1.60 Hz), 7.34 (m, 2H), 7.11 (m, 2H), 5.95 (m, 1H), 1.47 (d, 3H, J 1.37 Hz), 1.25 (d, 3H, J 1.14 Hz). ¹³C NMR (75 MHz, CDCl₃): 183.7,

182.4, 140.8, 138.7, 138.2, 134.7, 132.8, 132.7, 130.2, 128.3, 126.7, 126.5, 126.2, 126.0, 124.9, 118.4, 118.1, 111.5, 25.3, 20.5). IR (KBr) ν_{max}/cm⁻¹: 3281, 3081, 2970, 2925, 2218, 1661, 1591, 1564. UV-Vis (CHCl₃) λ_{max}/nm: 284 (log ε 4.02), 475 (3.19).

2-(4-Nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2j)

Yield: 84%. mp 205–207 °C. Anal. Calc. for C₂₀H₁₆N₂O₄: C 68.96; H 4.63; N 8.04%; found: C 68.86; H 4.55; N 8.03%. ¹H NMR (300 MHz, CDCl₃): δ 8.14 (m, 4H), 7.87 (br s, 1H), 7.78 (td, 1H, J 7.59, J 7.59, J 1.47 Hz), 7.71 (td, 1H, J 7.59, J 1.57 Hz), 6.91 (br d, 2H, J 9.08 Hz), 6.08 (m, 1H), 1.27 (d, 3H, J 1.17 Hz), 1.53 (d, 3H, J 1.43 Hz). ¹³C NMR (75 MHz, CDCl₃): 184.0, 182.3, 143.4, 142.4, 141.4, 138.0, 134.7, 132.7, 132.9, 130.7, 126.6, 123.6, 120.4, 120.8, 118.3, 99.8, 25.6, 20.7. IR (KBr) ν_{max}/cm⁻¹: 3283, 3084, 2974, 2911, 1593, 1663, 1500, 1332. UV-Vis (CHCl₃) λ_{max}/nm: 281 (log ε 4.37), 469 (3.63).

2-(3-Nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (2k)

Yield: 83%. mp 210–211 °C. Anal. Calc. for C₂₀H₁₆N₂O₄: C 68.96; H 4.63; N 8.04%; found: C 68.49; H 4.66; N 8.01%. ¹H NMR (300 MHz, CDCl₃): δ 8.15 (ddd, 1H, J 7.57, J 1.46, J 0.49 Hz), 8.13 (ddd, 1H, J 7.57, J 1.46, J 0.49 Hz), 7.91 (ddd, 1H, J 8.06, J 1.93, J 0.98 Hz), 7.82 (br s, 1H), 7.77 (td, 1H, J 7.57, J 7.57, J 1.46 Hz), 7.69 (td, 1H, J 7.57, J 1.46 Hz), 7.67 (br s, 1H), 7.40 (br t, 1H, J 8.06 Hz), 7.21 (dd, 1H, J 8.06, J 1.95 Hz), 6.01 (m, 1H), 1.43 (d, 3H, J 1.46 Hz), 1.24 (d, 3H, J 0.98 Hz). ¹³C NMR (75 MHz, CDCl₃): 183.8, 182.4, 147.3, 141.1, 138.6, 134.7, 132.8, 132.7, 130.2, 128.1, 127.1, 126.5, 126.2, 118.7, 118.4, 117.9, 116.3, 25.4, 20.6). IR (KBr) ν_{max}/cm⁻¹: 3279, 3080, 2970, 2908, 1597, 1661, 1498, 1330. UV-Vis (CHCl₃) λ_{max}/nm: 275 (log ε 4.39), 470 (3.59).

X-ray crystallography

The X-ray diffraction data for **2b** were collected at 295 K from a Enraf-Nonius Kappa-CCD²⁷ diffractometer with graphite monochromatized Mo K_α radiation. The cell parameters were obtained and refined using PHICHI²⁸ and EvalCCD²⁹ programs. Intensities for (**1**) were corrected by Lorentz polarization and absorption with the SADABS³⁰ program. The structure was solved by SHELXS-97 Direct Methods,²⁹ and refined with SHELXL-97,³² contained within the WinGX-32 crystallography program.³³ The positional parameters of the H atoms bonded to C atoms in the phenyl rings were obtained geometrically, with the C-H distances fixed in 0.93 Å for Csp², and refined as riding on

their respective C atoms, with $U_{\text{iso}}(\text{H}) = 1.2 \text{ Ueq}(\text{Csp}^2)$. H atoms bonded to C atoms in the methyl group were located geometrically and with the C-H distances fixed at 0.96 Å for Csp^3 and with $U_{\text{iso}}(\text{H}) = 1.5 \text{ Ueq}(\text{Csp}^3)$. The positional parameters of atom H1 bonded to N1 was obtained from a Fourier difference map and refined freely with an isotropic displacement parameter; the distance for N1-H1 is 0.87(2). X-ray data are listed in Table 3 and ORTEP-3³⁴ for Windows was used to draw the Figures.

Table 3. Crystal data and structure refinement for compound **2b**

Formula	$\text{C}_{21}\text{H}_{19}\text{O}_3\text{N}$
Formula weight	333.37
Crystal system, space group	Triclinic, P-1
Crystal size / mm	0.30 × 0.20 × 0.15
Unit cell dimensions	$a = 7.8709(16)$ $b = 9.3748(19)$ $c = 12.117(2)$ $\alpha = 86.11(3)^\circ$ $\beta = 81.60(3)^\circ$ $\gamma = 78.26(3)^\circ$
Volume / Å ³	865.3(3)
Z, Calculated density / (g cm ⁻³)	2 / 1.279
T / K	295
Absorption coefficient / mm ⁻¹	0.086
θ range	3.99 to 25.00
F(000)	352
Reflections collected / unique	10324 / 3023
R _{int}	0.0358
Max. and min. transmission	0.9873 and 0.9748
Data / restraints / parameters	3023 / 0 / 232
S	1.027
R indexes (all data)	$R_1 = 0.0430$, $wR_2 = 0.1031$
Largest diff. peak and hole / (e ⁻ Å ⁻³)	0.153 and -0.180

Antitumor assays

The compounds (1-5 mg mL⁻¹) were tested for cytotoxic activity against four cancer cell lines: SF-295 (Central Nervous System), HCT-8 (colon), MDAMB-435 (breast) and HL-60 (human leukemia). All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mmol L⁻¹ glutamine, 100 U mL⁻¹ penicillin, and 100 µg mL⁻¹ streptomycin at 37 °C with 5% CO₂. Each compound was dissolved in DMSO and diluted with water to obtain a concentration of 1 mg mL⁻¹. They were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.5% in the highest concentration). Doxorubicin (0.1-0.58 µg mL⁻¹) was used as a positive control. The cell viability was determined by

reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-phenyl-2H-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann.³⁵

Conclusions

The eleven novel aminonaphthoquinones **2a-k**, obtained from methoxy-*nor*-lapachol and various arylamines, were synthesized in good yields and showed interesting electrochemical behavior due to the nature of the substituents in the phenylamino ring, presenting a good correlation with Hammett parameter, which confirms that the reaction with electron-donor or electron-attracting groups follow a single mechanism. Unfortunately, because the arylamine derivatives of *nor*-lapachol were not active against the tested tumor cells, correlation between structure, electrochemical data and antitumor activity could not be attempted.

Supplementary Information

Supplementary data associated with this paper are available free of charge at <http://jbcs.sqb.org.br>, as a PDF file and contain the results of the theoretical calculations, crystallographic data, NMR spectra (¹H and APT), cyclic voltammograms and antitumor assays of compounds **2a-k**. Crystallographic data for the structural analysis of the three complexes have been deposited with the Cambridge Crystallographic Data Center, CCDC 734112. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1233336 033; e-mail: deposit@ccdc.cam.ac.uk).

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