J. Braz. Chem. Soc., Vol. 21, No. 1, 169-178, 2010.
Printed in Brazil - ©2010 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

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

Acácio I. Francisco,[a] Annelise Casellato,[a,#] Amanda P. Neves,[a] J. Walkimar de M. Carneiro,[a] Maria D. Vargas,*,[a] Lorenzo do C. Visentin,[b] Alviclér Magalhães,[c] Celso A. Câmara,[d] Claudia Pessoa,[e] Letícia V. Costa-Lotufo,[e] José D. B. Marinho Filho[e] and Manoel O. de Moraes[e]

[a]*Instituto de Química, Universidade Federal Fluminense, 24020-141 Niterói-RJ, Brazil*

[b]*Instituto de Química, Universidade Federal do Rio de Janeiro, 22640-000 Rio de Janeiro-RJ, Brazil*

[c]*Instituto de Química, Universidade Estadual de Campinas, 13083-970 Campinas-SP, Brazil*

[d]*Instituto de Química, Universidade Federal Rural de Pernambuco, 52171-900 Recife-PE, Brazil*

[e]*Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, 60430-270 Fortaleza-CE, Brazil*

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-NO_2 e 3-NO_2) 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 ^{1}H e ^{13}C 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 fenileno. 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-NO_2 and 3-NO_2) 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**), ^{1}H and ^{13}C 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 ^{1}H 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] fungicide[5] and antimalarial[6] 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 ($Q^{\bullet-}$) or dianion (Q^{2-}) 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

*e-mail: mdvargas@vm.uff.br
#Present address: Instituto de Química, Universidade Federal do Rio de Janeiro, 22640-000 Rio de Janeiro-RJ, Brazil

this type of molecules has proven to be useful.[16,17] We reported recently[18] 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-butenyl)-1,4-naphthoquinone].[19] *Nor*-lapachol is obtained from lapachol by the Hooker oxidation[20] 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 antitumor[11] and trypanocide activities.[9]

Herein is the first report on the synthesis and characterization of the novel 2-arylamine 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 activation[21] and their cytotoxicity[22] has been reported, we also investigated the redox properties of these compounds by cyclic voltammetry.

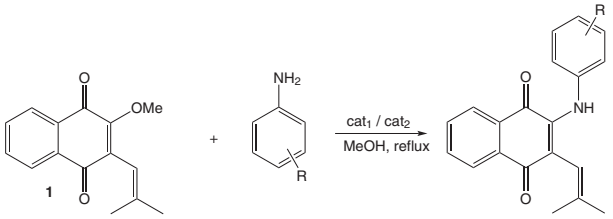


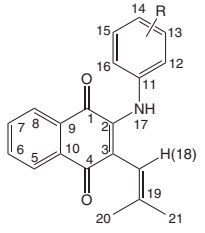
Figure 1. Synthesis of the novel arylamines derived from methoxy-*nor*-lapachol **1**.

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 ^{1}H NMR and infrared spectra of compounds **2a**-**k** are consistent with their composition and structure. The ^{1}H NMR spectra exhibit signals in the δ 7.5-8.2 ppm region as double dublets and triple dublets, attributed to the four naphthoquinone aromatic hydrogens H5-H8. Attributions were made on the basis of ^{1}H x ^{1}H-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 ^{1}H 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-NO_2)].



2a (R=H) **2b** (R=4-OMe) **2c** (R=4-Fc) **2d** (R=4-Me) **2e** (R=3-Me) **2f** (R=4-I) **2g** (R=3-I) **2h** (R=4-CN) **2i** (R=3-CN) **2j** (R=4-NO_2) **2k** (R=3-NO_2)

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°(1). The torsion angles around the fragments involved in the H-bond are: C(2)-N(1)-C(11)-C(12), −158.94(17)°, C(1)-C(2)-N(1)-C(11) −154.47(16)° and N(1)-C(2)-C(1)-O(2) −3.2(2)°. 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 O1^i 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* $O2^{ii}$ 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 set[38] 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 ^{1}H NMR chemical shifts using the GIAO approach[40] confirmed that electron-attracting groups yield higher $\delta_{H(18)}$ values than electron-donor groups. Calculations including solvent (chloroform) show essentially the same behavior. Interestingly, the $\delta_{H(18)}$ 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 ^{1}H NMR spectra of **2a** do not show broadening of H18 down to –90 °C in CD_2Cl_2.

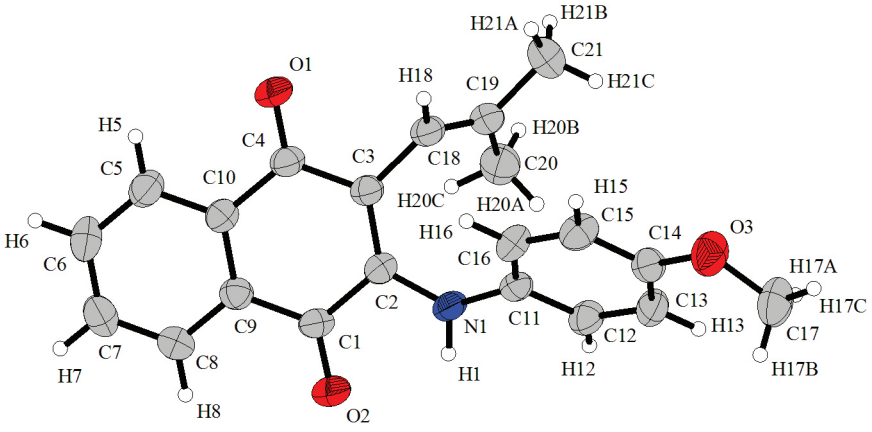


Figure 3. View of the ORTEP plot for **2b** with labeled atoms and 50% probability ellipsoids.

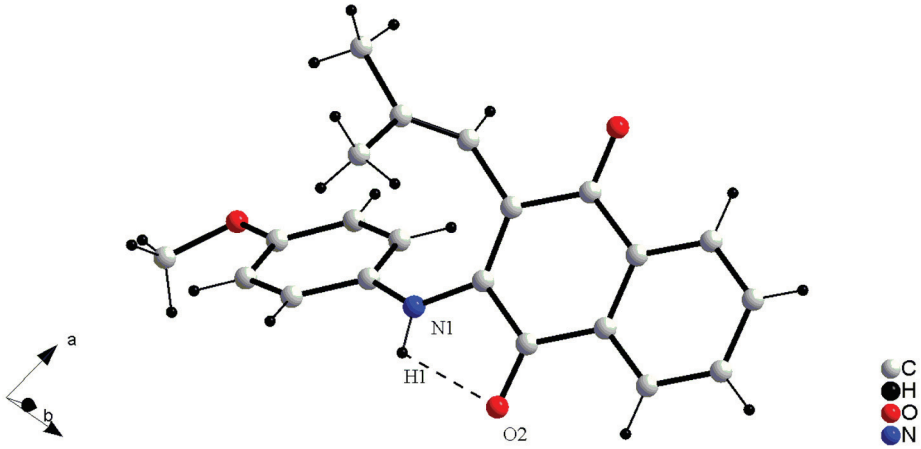


Figure 4. View of the intramolecular interaction.

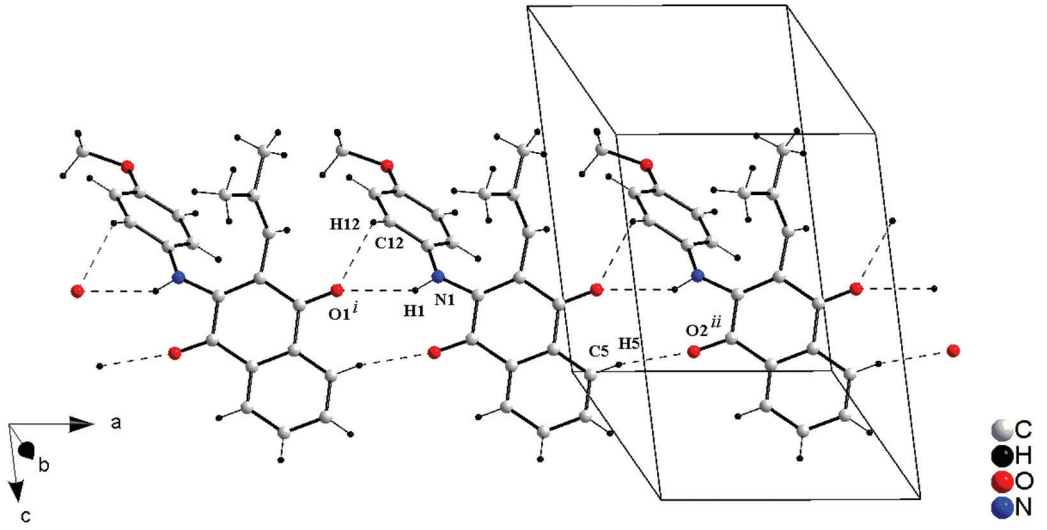


Figure 5. View of the self-assembly 1D by classical and non-classical hydrogen bonds along the [100] crystallography direction. [Symmetry codes: (*i*) *x*–1, *y*, *z*; (*ii*) *x*+1, *y*, *z*].

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 π-π^* 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 –2.1V *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 studied[41] 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 constants[42] 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 assay[35] and the results, summarized in the

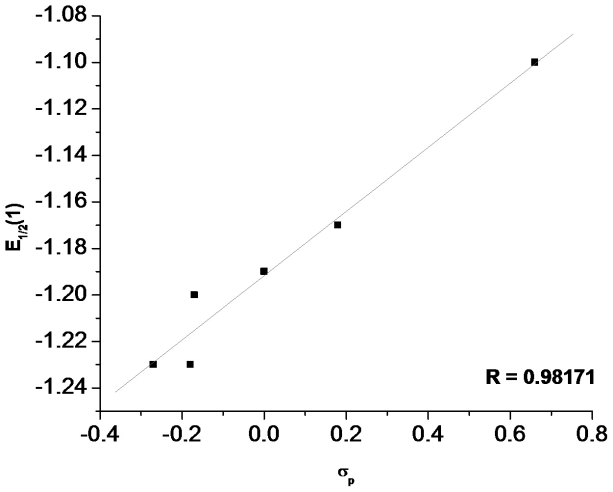


Figure 6. Correlation between $E_{1/2}(1)$ and σ_p.

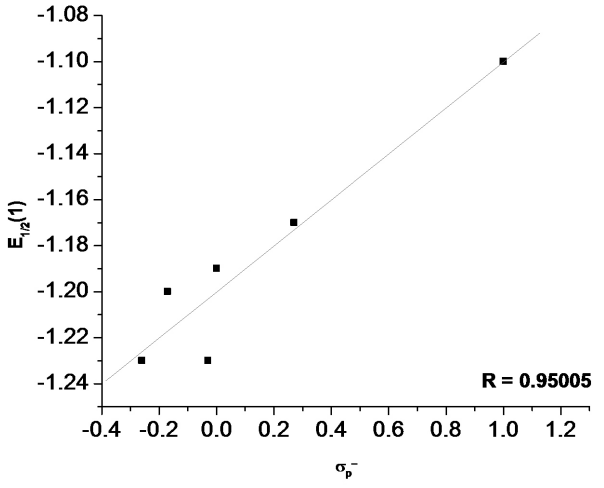


Figure 7. Correlation between $E_{1/2}(1)$ and σ_p^-.

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

Table 2. Cytotoxic activity expressed by IC_{50} in μg 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

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 $^1H \times {}^1H$ - 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 functional[24] with the standard 6-31G(d) basis set[25] 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 model[26] (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-arylamine-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 $MgCl_2 \cdot 6H_2O$ (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-anthraquinones 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 $C_{20}H_{17}NO_2$: C 79.14; H 5.47; N 4.67%; found: C 79.19; H 5.65; N 4.62%. ^{1}H NMR (300 MHz, $CDCl_3$): 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* 6.51, *J* 1.51 Hz), 7.65 (td, 1H, *J* 6.51, *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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 $C_{21}H_{19}NO_3$: C 75.66; H 5.74; N 4.20%; found: C 75.56; H 5.69; N 4.22%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 $C_{30}H_{25}FeNO_2$: C 73.93; H 5.17; N 2.87%; found: C 73.84; H 5.11; N 2.81%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 $C_{21}H_{19}NO_2$: C 79.47; H 6.03; N 4.41%; found: C 79.43; H 6.01; N 4.38%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 $C_{21}H_{19}NO_2$: C 79.47; H 6.03; N 4.41%; found: C 80.01; H 6.03; N 4.53%. ^{1}H NMR (300 MHz, $CDCl_3$): 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. ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 $C_{20}H_{16}INO_2$: C 55.96; H 3.76; N 3.26%; found: C 55.91; H 3.71; N 3.24%.

^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 ºC. Anal Calc. for $C_{20}H_{16}INO_2$: C 55.96; H 3.76; N 3.26%; found: C 55.92; H 3.74; N 3.23%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 ºC. Anal. Calc. for $C_{21}H_{16}N_2O_2$: C 76.81; H 4.91; N 8.53%; found: C 76.79; H 4.87; N 8.50%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 ºC. Anal Calc. for $C_{21}H_{16}N_2O_2$: C 76.81; H 4.91; N 8.53%; found: C 76.77; H 5.01; N 8.55%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 ºC. Anal. Calc. for $C_{20}H_{16}N_2O_4$: C 68.96; H 4.63; N 8.04%; found: C 68.86; H 4.55; N 8.03%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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 ºC. Anal. Calc. for $C_{20}H_{16}N_2O_4$: C 68.96; H 4.63; N 8.04%; found: C 68.49; H 4.66; N 8.01%. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 ($CHCl_3$) 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-CCD[27] diffractometer with graphite monochromatized Mo K_α radiation. The cell parameters were obtained and refined using PHICHI[28] and EvalCCD[29] programs. Intensities for (**1**) were corrected by Lorentz polarization and absorption with the SADABS[30] 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 0.93Å for C*sp*^{2}, and refined as riding on

their respective C atoms, with $U_{iso}(H) = 1.2Ueq(Csp^2)$. H atoms bonded to C atoms in the methyl group were located geometrically and with the C-H distances fixed at 0.96Å for Csp^3 and with $U_{iso}(H) = 1.5Ueq(Csp^3)$. 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-3[34] for Windows was used to draw the Figures.

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

Formula	$C_{21}H_{19}O_3N$
Formula weight	333.37
Crystal system, space group	Triclinic, P-1
Crystal size / mm	0.30 × 0.20 × 0.15
Unit cell dimensions a, b ,c /Å α, β, γ /°	a =7.8709(16) b = 9.3748(19) c = 12.117(2) α = 86.11(3)° β = 81.60(3)° γ = 78.26(3)°
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% CO_2. 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.sbq.org.br, as a PDF file and contain the results of the theoretical calculations, crystallographic data, NMR spectra (^{1}H 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).

Acknowledgments

The authors thank Prof. A. V. Pinto and Dr. M. C. F. R. Pinto for providing *nor*-lapachol and the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for financial support. Pronex-FAPERJ (grant number E-26/171.512/2006) is acknowledged. J. W. D. Carneiro, M. D. Vargas, A. P. Neves and C. A. Camara are recipients of CNPq research fellowships. A. I. Francisco and A. Casellato were benefited with CAPES fellowships. We also thank the X-ray diffraction laboratory (LDRX) of Universidade Federal Fluminense for data collection.

References

1. Goodwin, T. W.; Mercer, E. I.; *Introduction to Plant Biochemistry*; Pergamon Press: New York, 1972.

2. Fry, F. H.; Jacob, C.; *Curr. Pharm. Design* **2006**, *12*, 4479; Asche, C.; *Mini-Rev. Med. Chem.* **2005**, *5*, 449; Hassani, M.; Cai, W.; Holley, D. C.; Lineswala, J. P.; Maharjan, B. R.; Ebrahimian, G. R.; Seradj, H.; Stocksdale, M. G.; Mohammadi, F.; Marvin, C. C.; Gerdes, J. M.; Beall, H. D.; Behforouz, M.; *J. Med. Chem.* **2005**, *48*, 7733; Lee, J. H.; Cheong, J. H.; Park, Y. M.; Choi, Y. H.; *Pharmacol. Res.* **2005**, *51*, 553; Liu, K. K. C.; Li, J.; Sakya, S.; *Mini-Rev. Med. Chem.* **2004**, *4*, 1105; Kongkathip, N; Siripong, P.; Sangma, C.; Luangkamin, S.; Niyomdecha, M.; Pattanapa, S.; Piyaviriyalgul, S.; Kongsaeree, P.; *Bioorg. Med. Chem.* **2003**, *11*, 3179; da Silva, A. J. M.; Netto, C. D.; Pacienza-Lima, W.; Torres-Santos, E. C.; Rossi-Bergmann, B.; Maurel, S.; Valentin, A.; Costa, P. R. R.; *J. Braz. Chem. Soc.* **2009**, *20*, 176.
3. Pinto, C. N.; Dantas, A. P.; De Moura, K. C. G.; Emery, F. S.; Polequevitch, P. F.; Pinto, M. D.; De Castro, S. L.; Pinto, A. V.; *Arzneim. Forsch./Drug Res*. **2000**, *50*, 1120; Goulart, M. O. F.; Zani, C. L.; Tonholo, J.; Freitas, L. R.; de Abreu, F. C.; Oliveira, A. B.; Raslan, D. S.; Starling, S.; Chiari, E.; *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2043.
4. Barbosa, T. P.; Camara, C. A.; Silva, T. M. S.; Martins, R. M.; Pinto, A. C.; Vargas, M. D.; *Bioorg. Med. Chem.* **2005**, *13*, 6464; dos Santos, A. F.; Ferraz, P. A. L.; de Abreu, F. C.; Chiari, E.; Goulart, M. O. F.; Sant'Ana, A. E. G.; *Planta Med.* **2001**, *67*, 92; Dos Santos, A. F.; Ferraz, P. A. L.; Pinto, A. V.; Pinto, M.C. F. R.; Goulart, M. O. F.; Sant'Ana, A. E. G.; *Int. J. Parasitol*. **2000**, *30*, 1199.
5. Gafner, S.; Wolfender, J.-L.; Nianga, M.; Stoeckli- Evans, H.; Hostettmann, K.; *Phytochemistry* **1996**, *42*, 1315.
6. Dos Santos, E. V. M.; Carneiro, J. W. de M.; Ferreira, V. F.; *Bioorg. Med. Chem.* **2004**, *12*, 87.
7. Stefanska, J. B.; Dzieduszycka, M.; Morteli, S.; Antonini, I.; Borowski, E.; *J. Org. Chem.* **1993**, *58*, 1568; Lin, T. –S.; Xu, S.-P.; Zhu, L.-Y.; Cosby, L.; Sartonelli, A.; *J. Med. Chem.* **1989**, *32*, 1467; Konoshima, T.; Kozuka, M.; Koyami, J.; Okatani, J.; Tagahara, K.; Tokuda, H.; *J. Nat. Prod.* **1989**, *52*, 987; Ossowski, T.; Goulart, M. O. F.; de Abreu, F. C.; Sant'Ana, A. E. G.; Miranda, P. R. B.; Costa, C. O.; Liwo, A.; Falkowski, P.; Zarzeczanska, D.; *J. Braz. Chem. Soc*. **2008**, *19*, 175; Neves, A. P.; Barbosa, C. C.; Greco, S. J.; Vargas, M. D.; Visentin, L. C.; Pinheiro, C. B.; Mangrich, A. S.; Barbosa, J. P.; da Costa, G. L.; *J. Braz. Chem. Soc*. **2009**, *20*, 712.
8. Lin, T.-S; Xu, S.-P; Zhu, L.-Y; Divo, A.; Sartonelli, A.; *J. Med. Chem.* **1991**, *34*, 1634.
9. da Silva Junior, E. N.; de Souza, M. C. B. V.; Fernandes, M. C.; Menna-Barreto, R. F. S.; Pinto, M. do C. F. R.; Lopes, F. de A.; de Simone, C. A.; Andrade, C. K. Z.; Pinto, A. V.; Ferreira, V. F.; de Castro, S. L.; *Bioorg. Med. Chem.* **2008**, *16*, 5030; da Silva Junior, E. N.; de Moura, M. A. B. F.; Pinto, A. V.; Pinto, M. C. F. R.; de Souza, M. C. B. V.; Araujo, A. J.; Pessoa, C.; Costa-Lotufo, L. V.; Montenegro, R. C.; de Moraes, M. O.; Ferreira, V. F.; Goulart, M. O. F.; *J. Braz. Chem. Soc.* **2009**, *20*, 635; Tapia, R. A.; Cantuarias, L.; Cuellar, M.; Villena, J.; *J. Braz. Chem. Soc.* **2009**, *20*, 999.
10. da Silva Junior, E. N.; Menna-Barreto, R. F. S.; Pinto, M. do C. F. R.; Silva, R. S. F.; Teixeira, D. V.; de Souza, M. C. B. V.; de Simone, C. A.; de Castro, S. L.; Ferreira, V. F.; Pinto, A. V.; *Eur. J. Med. Chem.* **2008**, *43*, 1774; da Silva Junior, E. N.; de Souza, M. C. B. V.; Pinto, A. V.; Pinto, M. do C. F. R.; Goulart, M. O. F.; Barros, F. W. A.; Pessoa, C.; Costa-Lotufo, L. V.; Montenegro, R. C.; de Moraes, M. O.; Ferreira, V. F.; *Bioorg. Med. Chem.* **2007**, *15*, 7035.
11. Cunha, A. S.; Lima, E. L. S.; Pinto, A. C.; Esteves-Souza, A.; Echevarria, A.; Camara, C. A.; Vargas, M. D.; Torres, J. C.; *J. Braz. Chem. Soc*. **2006**, *17*, 439; Esteves-Souza, A.; Figueiredo, D. V.; Esteves, A.; Câmara, C. A.; Vargas, M. D.; Pinto, A. C.; Echevarria, A.; *Braz. J. Med. Biol. Res.* **2007**, *30*, 1399; Cunha, A. S.; Vargas, M. D.; Gattass, C. R.; Pinto, A. C.; Camara, C. A.; Esteves, A. S.; Lima, E. L. S.; *Oncol. Rep.* **2008**, *20*, 225.
12. Silva, R. S. F.; Costa, E. M.; Trindade, U. L. T.; Teixeira, D. V.; Pinto, M. de C. F. R.; Santos, G. L.; Malta, V. R. S.; de Simone, C. A.; Pinto, A. V.; de Castro, S. L.; *Eur. J. Med. Chem.* **2006**, *41*, 526.
13. Kovacic, P.; Becvar, L. E.; *Curr. Pharm. Des.* **2000**, *6*, 143; Monks, T. J.; Hanzlik; P.; Cohen, G. M.; Ross, D.; Graham, D. G.; *Toxicol. Appl. Pharmacol.* **1992**, *112,* 2.
14. Ferraz, P. A. L.; de Abreu, F. C.; Pinto, A. V.; Glezer, V.; Tonholo, J. Goulart, M. O. F.; *J. Electroanal. Chem.* **2001**, *507*, 275; Kumagai, Y.; Tsurutani, Y.; Shinyashiki, M.; Takeda, S. H.; Nakai, Y.; Yoskikawa, T.; Shimojo, N.; *Environ. Toxicol. Pharmacol.* **1997**, *3*, 245.
15. Fragoso, T. P.; Carneiro J. W. D.; Vargas, M. D.; *J. Mol. Model.*, submitted.
16. González, F. J.; Aceves, J. M.; Miranda, R.; González, I.; *J. Electroanal. Chem.* **1991**, *310*, 293.
17. Ferraz, P. A. L.; Abreu, F. C.; Pinto, A. V.; Glezer, V.; Tonholo, J.; Goulart, M. O. F.; *J. Electroanal. Chem.* **2001**, *507*, 275.
18. Francisco, A. I.; Vargas, M. D.; Carneiro, J. W. de M.; Lanznaster, M.; Torres, J. C.; Camara, C. A.; Pinto, A. C.; *J. Mol. Struct.* **2008**, *891*, 228.
19. Camara, C. A.; Pinto, A. C.; Rosa; M. A.; Vargas, M. D.; *Tetrahedron* **2001**, *57*, 9569.
20. Fieser, L. F.; Fieser, M.; *J. Am. Chem. Soc.* **1948**, *70*, 3215.
21. Koyama, J.; Morita, I.; Kobayashi, N.; Osakai, T.; *Cancer Lett.* **2004**, *212*, 1.
22. Pan, A-S.; Gonzalez, H.; *Mol. Pharmacol*. **1990**, *37*, 966.
23. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreen Jr., T.; Kudin, K. N.; Burat, J. C.; Millam, J. M.; Iyengar, S. S.; Tomsi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota,

K.; Fukua, R.; Hasegawa, J.; Ishida, M.; Nakajim, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochteski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Faras, O.; Malick, D. K.; Rabu, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C.; Nanayakkra, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; *Gaussian 03, Revision B.02,* Gaussian, Inc.: Pittsburg P. A., 2003.

24. Beck, A. D.; *J. Chem. Phys.* **1993**, *98*, 5648.
25. Hariharan, P. C.; Pople, J. A.; *Theor. Chim. Acta* **1973**, *28*, 213.
26. Cossi, M.; Rega, N.; Scalmani, G.; Barone, V.; *J. Comput. Chem.* **2003**, *24*, 669.
27. Nonius; *COLLECT*, Nonius BV: Delft, The Netherlands, 1998.
28. Duisenberg, A. J. M.; Hooft, R. W. W.; Schreurs, A. M. M.; Kroon, J.; *J. Appl. Crystallogr.* **2000**, *33*, 893.
29. Duisenberg, A. J. M.; Kroon-Batenburg, L. M. J.; Schreurs, A. M. M.; *J. Appl. Crystallogr.* **2003**, *36*, 220.
30. Sheldrick, G. M.; *SADABS; Program for Empirical Absorption Correction of Area Detector Data*; University of Göttingen, Germany, 1996.
31. Sheldrick, G. M.; *SHELXS97; Program for Crystal Structure Solution*; University of Göttingen, Germany, 1997.
32. Sheldrick, G. M.; *SHELXL97; Program for Crystal Structure Refinement*; University of Göttingen, Germany, 1997.
33. Farrugia, L. J.; *J. Appl. Crystallogr.* **1999**, *32*, 837.
34. Farrugia, L.J.; *J. Appl. Crystallogr.* **1997**, *30*, 565.
35. Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.
36. Larsen, I. K.; Andersen, L. A.; *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1992**, *48*, 2009.
37. Rassalov, V. A.; Pople, J. A.; Ratner, M. A. Windus, T. L.; *J. Chem. Phys.* **1998**, *109*, 1223.
38. McLean, A. D.; Chandler, G. S.; *J. Chem. Phys.* **1980**, *72*, 5639.
39. Becke, A. D.; *J. Chem. Phys.* **1992**, *96*, 2155.
40. Wolinski, K.; Hilton, J. F.; Pulay, P.; *J. Am. Chem. Soc.* **1990**, *112*, 8251.
41. Aguilar-Martínez, M.; Cuevas, C.; Jiménez-Estrada, M.; González, I.; Lotina-Hennsen, B.; Macías-Ruvalcaba, N.; *J. Org. Chem.* **1999**, *64*, 3684.
42. Hansch, C.; Leo, A.; Taft, R. W.; *Chem. Rev.* **1991**, *91*, 165.
43. Nesmeyanov, A. N.; Perevalova, E. G.; Gubin, S. P.; Grandberg, K. I.; Kozlovsky, A. G.; *Tetrahedron Lett.* **1966**, *7*, 2381.

Received: August 11, 2009
Web Release Date: November 12, 2009

FAPESP helped in meeting the publication costs of this article.

J. Braz. Chem. Soc., Vol. 21, No. 1, S1-S18, 2010.
Printed in Brazil - ©2010 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

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

Acácio I. Francisco,[a] Annelise Casellato,[a#] Amanda P. Neves,[a] J. Walkimar de M. Carneiro,[a] Maria D. Vargas,*,[a] Lorenzo do C. Visentin,[b] Alviclér Magalhães,[c] Celso A. Câmara,[d] Claudia Pessoa,[e] Letícia V. Costa-Lotufo,[e] José D. B. Marinho Filho[e] and Manoel O. de Moraes[e]

[a]*Instituto de Química, Universidade Federal Fluminense, 24020-141 Niterói-RJ, Brazil*

[b]*Instituto de Química, Universidade Federal do Rio de Janeiro, 22640-000 Rio de Janeiro-RJ, Brazil*

[c]*Instituto de Química, Universidade Estadual de Campinas, 13083-970 Campinas-SP, Brazil*

[d]*Instituto de Química, Universidade Federal Rural de Pernambuco, 52171-900 Recife-PE, Brazil*

[e]*Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, 60430-270 Fortaleza-CE, Brazil*

1. Theoretical Calculations

Starting from the experimental structure for **2b** (4-Me), the geometries of **2a**-**2e** and **2h**-**2i** were fully optimized with the B3LYP/6-31G(d) method. Energies and molecular properties were obtained from a single-point calculation using the 6-311+G(2d,p) basis set and the B3LYP functional. 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-methyl-propenyl group bonded to the position 3 of the naphthoquinone ring rotated by 180º was also optimized. This alternative conformation was found 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}.

The orientation of the 2-phenylene ring is stabilized by an intramolecular hydrogen bond with one carbonyl group of the naphthoquinone ring and was, therefore, not further investigated. 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. Absolute energies and GIAO nuclear magnetic shielding tensors for H(18) are given in Table S1. Interestingly, the δ_{H18} value for the alternative conformation (with the 2-methyl-propenyl group rotated by 180º, entry **2b**-4 in Table S1) is 1.67 ppm shifted highfield as compared to the same hydrogen in the most stable conformation. Calculation for **2b** on a geometry with the methoxy group rotated by 90º (entry **2b**-2 in Table S1) yielded essentially the same δ_{H18} value as that obtained for the most stable conformation. To verify the effect of the solvent on the chemical shifts some calculations were repeated with the solvent chloroform, using the CPCM approach. These calculations revealed that the solvent has only marginal influence on the relative chemical shifts.

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. All calculations were carried out with the G03W package of molecular orbital calculation.

2. Crystallographic Data

2.1. Computing details

Data collection: *COLLECT* (Nonius, 1998); cell refinement: *PHICHI* (Duisenberg, 2000); data reduction: *EVALCCD* (Duisenberg, 2003); program(s) used to solve structure: *SHELXS97* (Sheldrick, 1997); program(s) used to refine structure: *SHELXL97* (Sheldrick, 1997); molecular graphics: *ORTEP-3 for Windows* (Farrugia, 1997); software used to prepare material for publication: *WinGX* publication routines (Farrugia, 1999).

2.2. Experiment X-ray diffraction

The X-ray diffraction data for (**2b**) were collected at 295 K from a *Enraf-Nonius Kappa*-CCD diffractometer with graphite-monochromatized Mo K_α radiation. The cell parameters were obtained and refined using *PHICHI* and

*e-mail: *e-mail: mdvargas@vm.uff.br
#Present address: Instituto de Química, Universidade Federal do Rio de Janeiro, 22640-000 Rio de Janeiro-RJ, Brazil

Table S1. Absolute energies (Hartree) and GIAO nuclear magnetic shielding tensors (ppm) for compounds **2a**-**2e** and **2h**-**2i**. All calculations were carried out on a optimized B3LYP/6-31G(d) geometry

	Absolute energies / hartree		GIAO nuclear magnetic shielding tensors / ppm	
Derivative	B3LYP/6-31+G(d,p)	B3LYP/6-311+G(2d,p)	B3LYP/6-31+G(d,p)[a]	B3LYP/6-311+G(2d,p)[b]
2a (H)	-977.62466	-	24.64	-
2b-1 (4-OMe)	-1092.15228	-1092.40283	24.64	24.97
2b-2 (4-OMe)[c]	-1092.14589	-	24.67	-
2b-3 (4-OMe)[d]	-1092.16808	-	24.64	-
2b-4 (4-OMe)[e]	-1092.16687	-	26.31	-
2l (3-OMe)	-1092.15346	-1092.40399	24.62	24.89
2c (4-Fc)	-2627.17389	-2627.55599	24.66	24.79
2m (3-Fc)	-	-2627.55562	-	24.85
2d (4-Me)	-1016.94536	-1017.17288	24.60	24.86
2e (3-Me)	-	-1017.17322	-	24.81
2h-1 (4-CN)	-1069.87032	-1070.11410	24.50	24.79
2h-2 (4-CN)[d]	-1069.89020	-	24.46	-
2i-1 (3-CN)	-1069.86851	-1070.11232	24.54	24.75
2i-2 (3-CN)[d]	-1069.88800	-	24.49	-

[a]At this level, the absolute GIAO nuclear magnetic shielding of TMS is 32.09 ppm. [b]At this level, the absolute GIAO nuclear magnetic shielding of TMS is 31.82 ppm. [c] Conformation with the methoxy group rotated by 90º. [d]Calculation with inclusion of solvent (CPCM, chloroform). The absolute GIAO nuclear magnetic shielding of TMS at this level is 31.63 ppm [e]Alternative conformation, with the 2-methyl-propenyl group rotated by 180º, including solvent effect (chloroform).

EvalCCD programs. Intensities for (**2b**) were corrected by Lorentz polarization and absorption with the *SADABS* program. The structure was solved by *SHELXS*-97 Direct Methods, and refined with *SHELXL*-97, contained within the *WinGX*-32 crystallography program. 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 0.93Å for Csp^2, and refined as riding on their respective C atoms, with U_{iso}(H) = 1.2Ueq(Csp^2). H atoms bonded to C atoms in the methyl group were located geometrically and with the C-H distances fixed at 0.96Å for Csp^3 and with U_{iso}(H) = 1.5Ueq(Csp^3). The positional parameters of H(1) bonded to N(1) was obtained from a Fourier difference map and refined freely with an isotropic displacement parameter; the distance for N(1)-H(1) is 0.87(2). X-ray data are listed in Table S2.

Crystallographic data for the structural analysis of compound **2b** 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).

The packing of **2b** involves molecules that interact intra- and intermolecularly through classical and non-classic hydrogen bonds, forming a 1D infinite network along the [100] crystallographic direction (Figures S2 and S3). The naphthoquinone carbonyl O1^{i} interacts *via* classical and non-classic hydrogen bonds with H(1) to N(1) (imine group) and with H(12) to C(12) [C(11)-C(16) phenyl ring] of a neighboring molecule forming a six membered ring [symmetry code: $i = x-1, y, z$]. In addition the other carbonyl group interacts *via* O2^{ii} with H(5) to C(5) forming a ten membered ring in association with N(1)-H(1)···O(1). The H-bond geometric parameters are listed in Table S4. Table S5 gathers the atomic coordinates and equivalent isotropic displacement parameters.

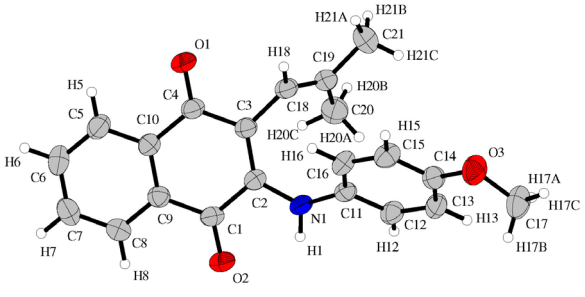


Figure S1. View of the ORTEP plot for **2b** with labeled atoms and 50% probability ellipsoids.

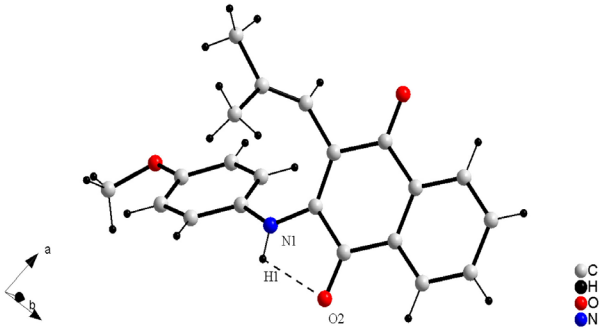


Figure S2. View of the intramolecular interaction.

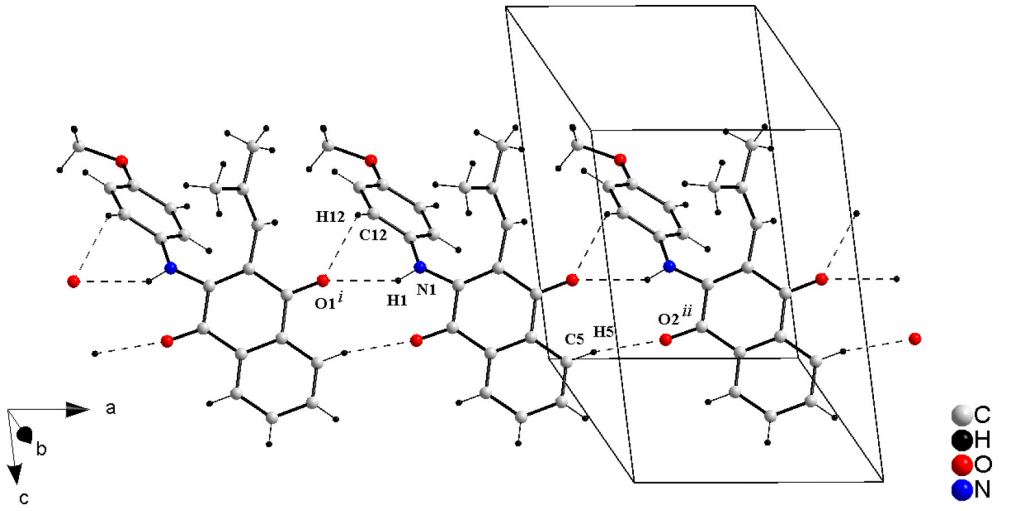


Figure S3. View of the self-assembly 1D by classical and non classical hydrogen bonds along the [100] crystallography direction. [Symmetry codes: (*i*) *x*–1, *y*, *z*; (*ii*) *x*+1, *y*, *z*].

Table S2. Crystal data and structure refinement for **2b**.

Formula	$C_{21}H_{19}O_3N$
formula weight	333.37
crystal system, space group	Triclinic, P-1
crystal size / mm	0.30 x 0.20 x 0.15
a /Å	7.8709(16)
b/Å	9.3748(19)
c/Å	12.117(2)
α / (°)	86.11(3)
β / (°)	81.60(3)
γ / (°)	78.26(3)
V / Å^3	865.3(3)
Z	2
T / K	295
$\rho_{calc.}$ / (g cm^{-3})	1.279
μ / mm^{-1}	0.086
2θ range	3.99 to 25.00
F(000)	352
Reflections collected	10324
Reflections unique	3023
R_{int}	0.0358
Max. and min. transmission	0.9873 and 0.9748
data[b]/restraints/parameters	3023 / 0 / 232
S[b,c]	1.027
R_1[a,d]	0.0430
wR_2[b,e]	0.1031
largest difference peak and hole / (e Å^{-3})	0.153 and -0.180

[a] $F_o^2 \geq 2\sigma(F_o^2)$. [b] $F_o^2 \geq 3\sigma(F_o^2)$. [c] $S = [\Sigma w(F_o^2 - F_c^2)^2/(n-p)]^{1/2}$. [d] $R_1 = \Sigma||F_o| - |F_c||\Sigma|F_o|$. [e] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^4)]^{1/2}$

Table S3. Geometric parameters for molecule **2b** (Å, °)

Bonds distances	
N(1)-C(11)	1.424(2)
N(1)-C(2)	1.367(2)
O(1)-C(4)	1.234(2)
O(2)-C(1)	1.223(2)
O(3)-C(14)	1.378(2)
O(3)-C(17)	1.423(2)
C(3)-C(18)	1.480(2)
C(19)-C(18)	1.328(3)
C(19)-C(20)	1.502(3)
C(19)-C(21)	1.510(3)
Bond angles	
C(2)-N(1)-C(11)	129.43(15)
C(14)-O(3)-C(17)	116.93(16)
O(1)-C(4)-C(3)	120.81(16)
O(1)-C(4)-C(10)	119.19(16)
O(2)-C(1)-C(9)	121.52(16)
O(2)-C(1)-C(2)	119.54(16)
C(19)-C(18)-C(3)	126.38(17)
C(20)-C(19)-C(21)	115.62(18)

Table S4. Geometric parameters for non-classical H bonds for molecule (**2b**) (*Å*, °)

D-H···A	*D*-H	H···A	*D*···A	∠*D*-H···A
N(1)—H(1) ···O(2)	0.87 (2)	2.18 (2)	2.620 (2)	111.2 (18)
N(1)—H(1) ···O(1)^i	0.87 (2)	2.46 (2)	3.241 (2)	150.2 (18)
C(12)—H(12)···O(1)^i	0.93	2.58	3.246 (2)	129
C(5)—H(5) ···O(2)^{ii}	0.93	2.39	3.304 (2)	169

[Symmetry codes: (*i*) *x*-1, *y*, *z*; (*ii*) *x*+1, *y*, *z*]

Table S5. Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (A^2 × 10^3) for (**2b**). U(eq) is defined as one third of the trace of the orthogonalized *Uij* tensor

	x	y	z	U(eq)
C(19)	–974(2)	–1627(2)	5732(2)	43(1)
C(20)	–2387(3)	–494(2)	5299(2)	52(1)
C(21)	–31(3)	–2776(3)	4922(2)	70(1)
O(1)	1355(2)	–120(2)	7804(1)	54(1)
O(2)	–5563(2)	1227(2)	9030(1)	54(1)
C(2)	–3178(2)	–323(2)	8018(1)	33(1)
N(1)	–4432(2)	–1001(2)	7743(1)	38(1)
C(1)	–3973(2)	916(2)	8784(1)	36(1)
C(9)	–2800(2)	1709(2)	9233(1)	35(1)
O(3)	–4254(2)	–6772(1)	6747(1)	57(1)
C(10)	–984(2)	1312(2)	8931(1)	35(1)
C(4)	–239(2)	152(2)	8106(2)	36(1)
C(3)	–1409(2)	–604(2)	7634(1)	34(1)
C(11)	–4275(2)	–2456(2)	7413(1)	34(1)
C(12)	–5544(2)	–2751(2)	6832(2)	40(1)
C(18)	–559(2)	–1641(2)	6757(2)	39(1)
C(16)	–2999(2)	–3610(2)	7728(2)	40(1)
C(8)	–3498(2)	2840(2)	9955(2)	44(1)
C(14)	–4310(2)	–5325(2)	6920(2)	39(1)
C(15)	–3008(2)	–5025(2)	7473(2)	43(1)
C(5)	112(2)	2050(2)	9372(2)	44(1)
C(13)	–5574(2)	–4176(2)	6589(2)	43(1)
C(6)	–601(3)	3166(2)	10106(2)	51(1)
C(7)	–2398(3)	3565(2)	10390(2)	51(1)
C(17)	–5788(3)	–7133(2)	6440(2)	61(1)

3. ^{1}H NMR and APT Spectra ($CDCl_3$) of Compounds 2a-k

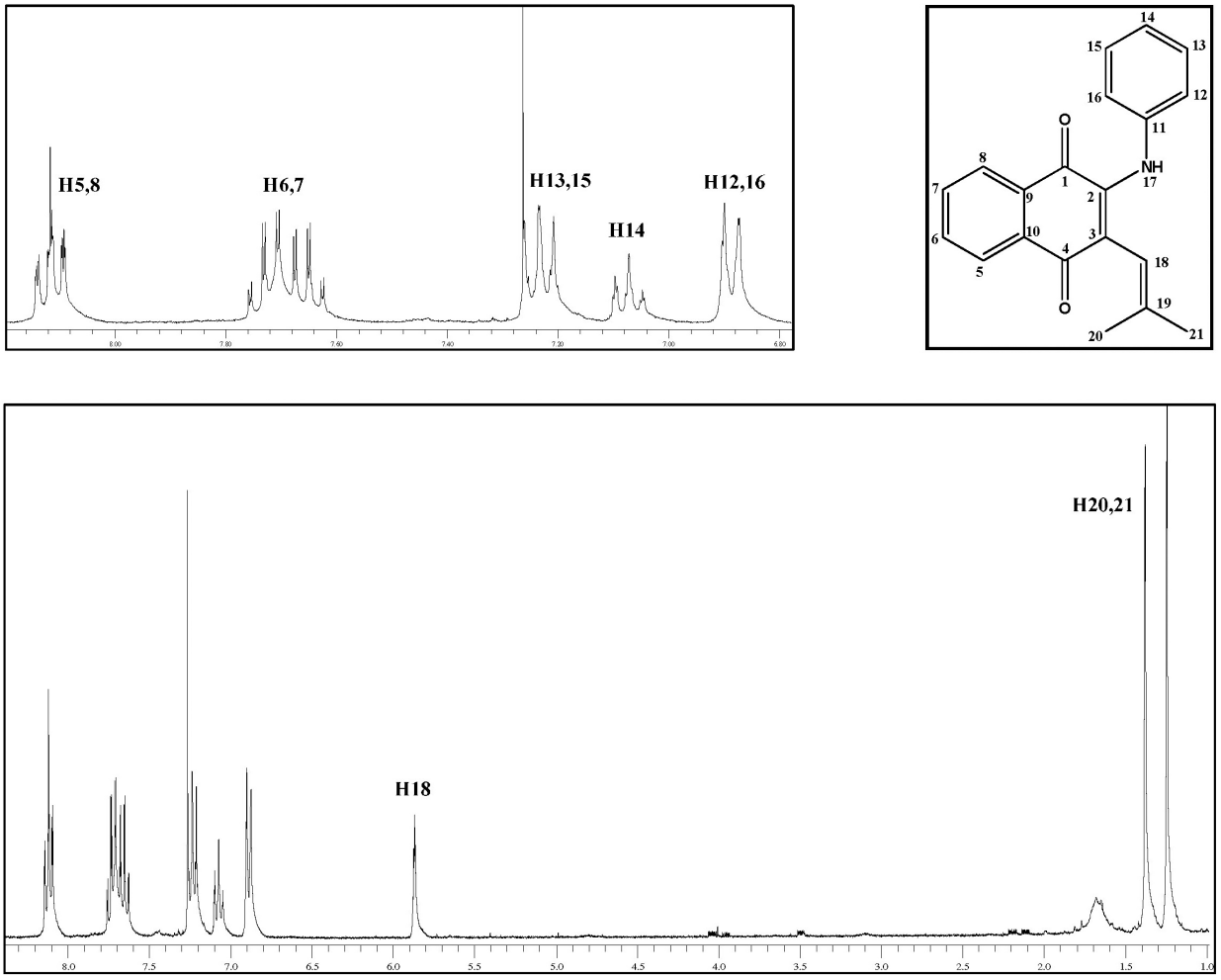


Figure S4. ^{1}H NMR spectrum of 2-(phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2a**).

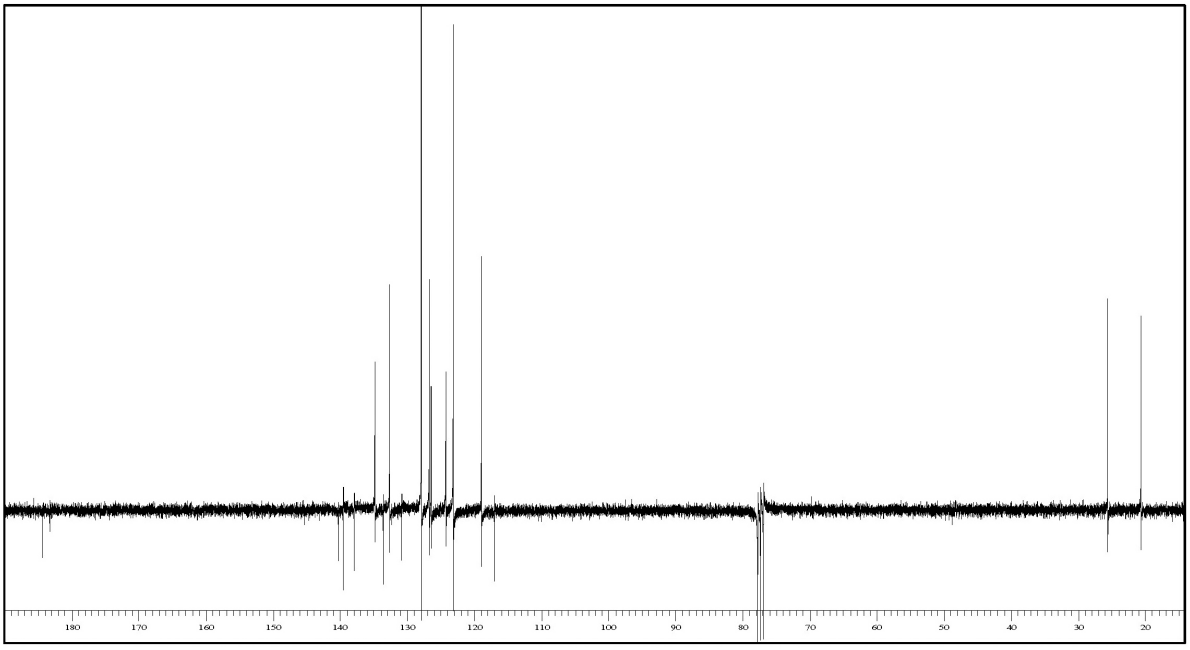


Figure S5. APT spectrum of 2-(phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2a**).

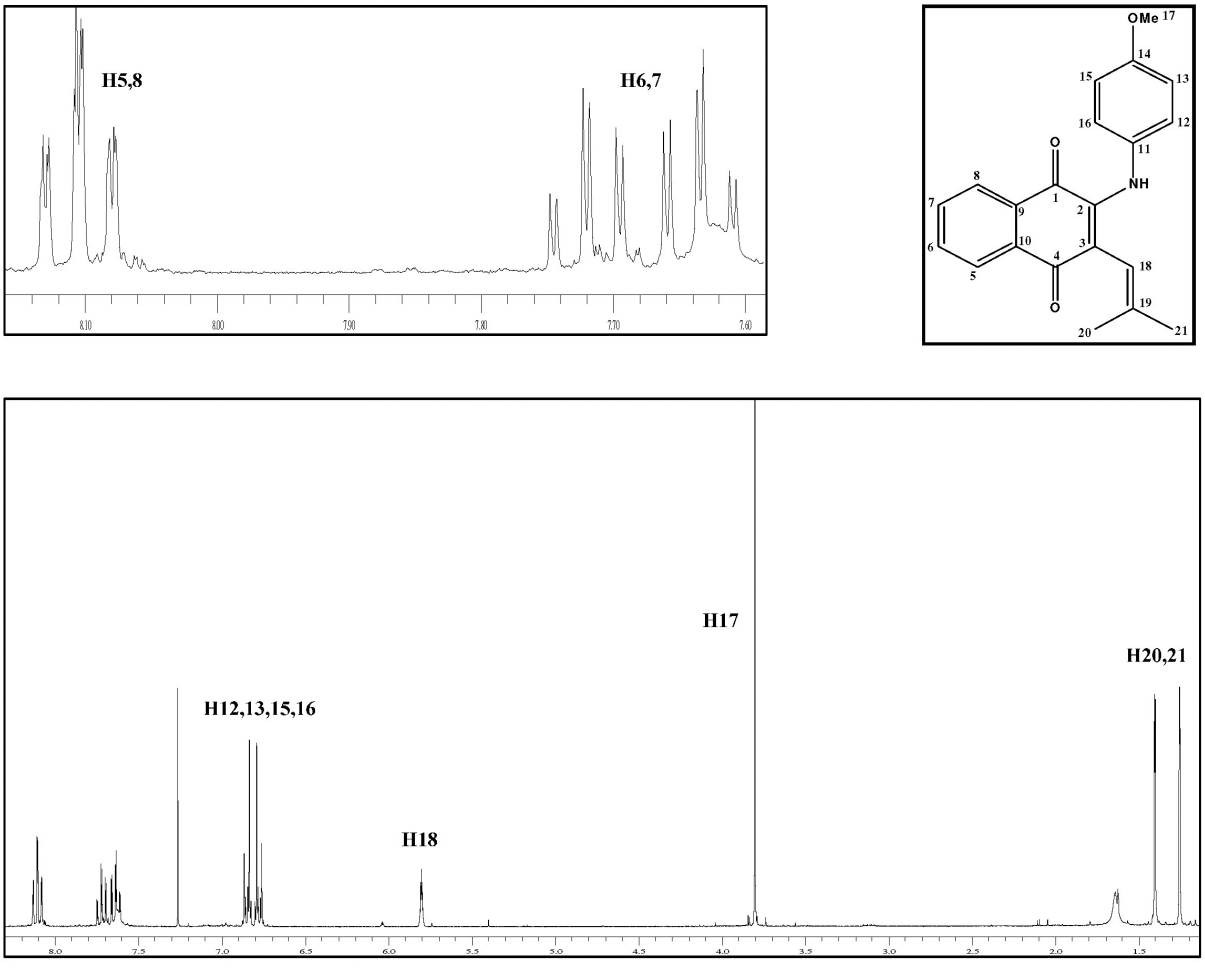


Figure S6. ^{1}**H NMR** spectrum of 2-(4-methoxy-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2b**).

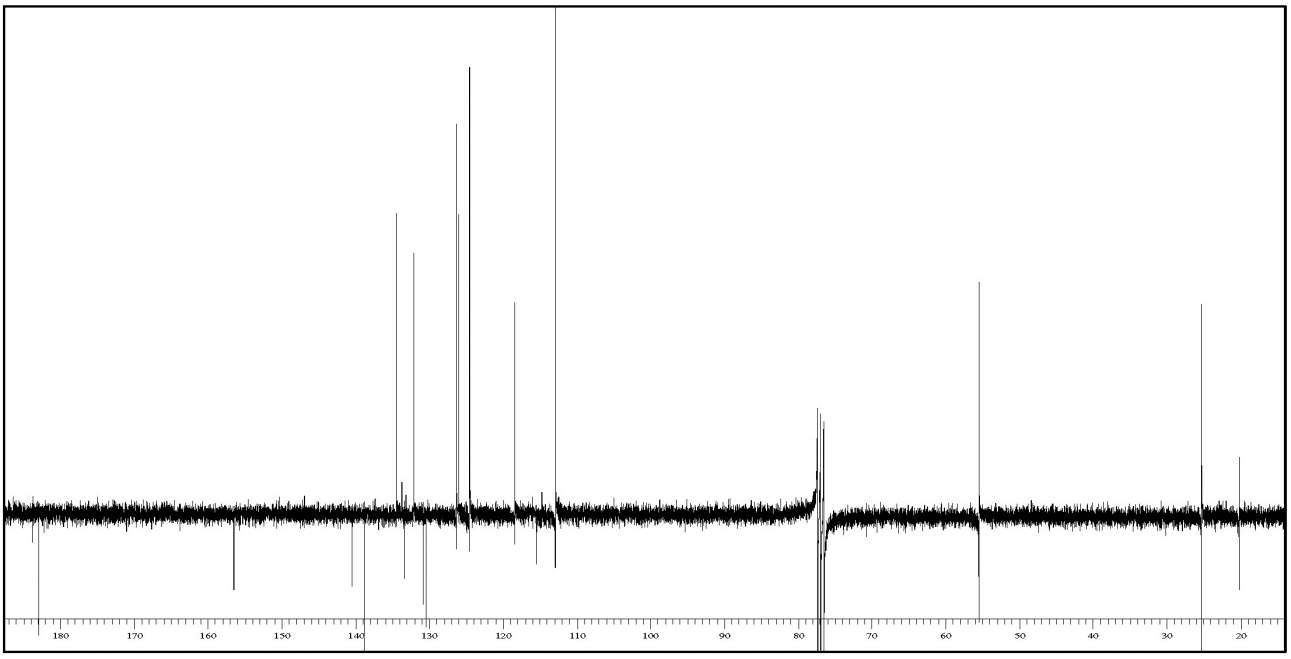


Figure S7. APT spectrum of 2-(4-methoxy-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2b**).

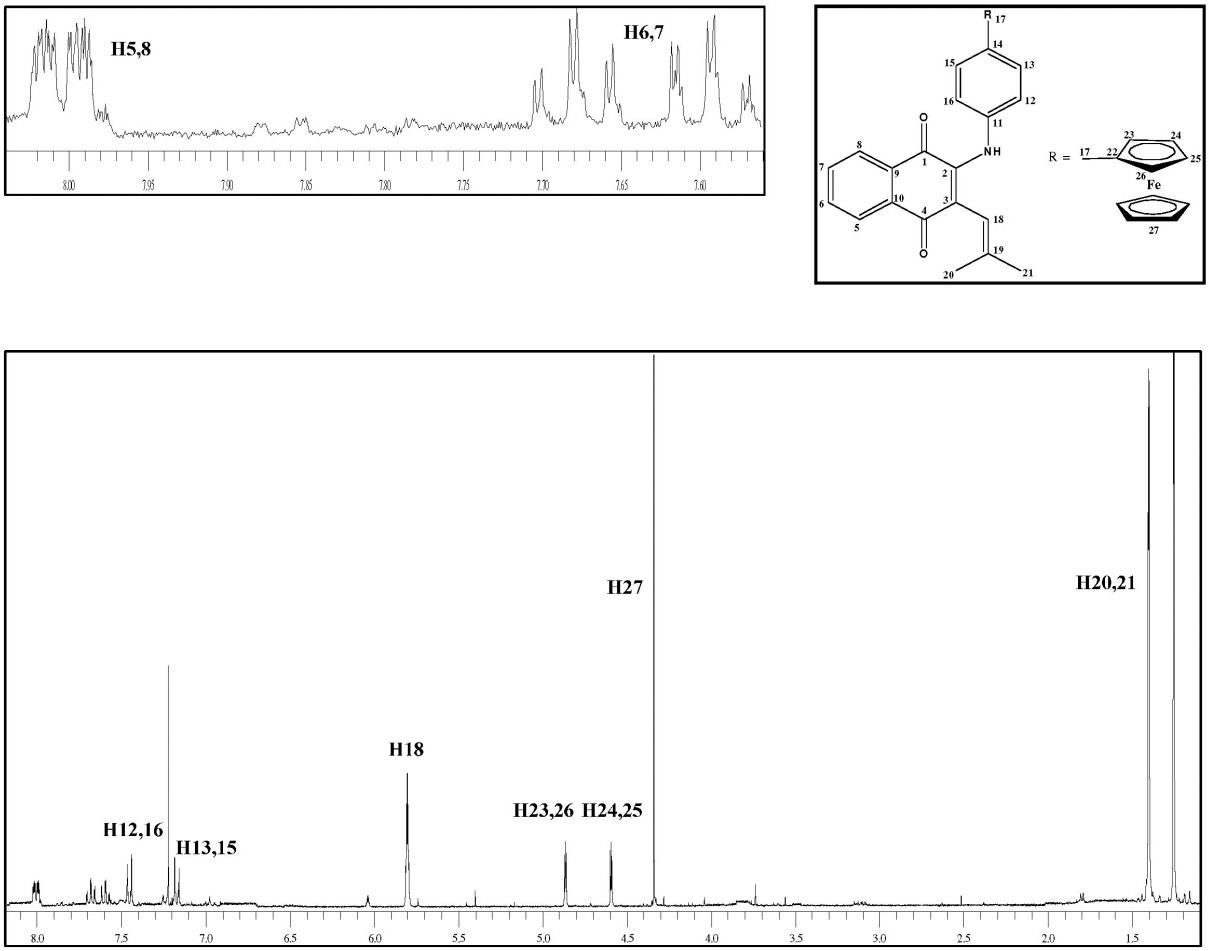


Figure S8. ^{1}H NMR spectrum of 2-(4-ferrocenyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2c**).

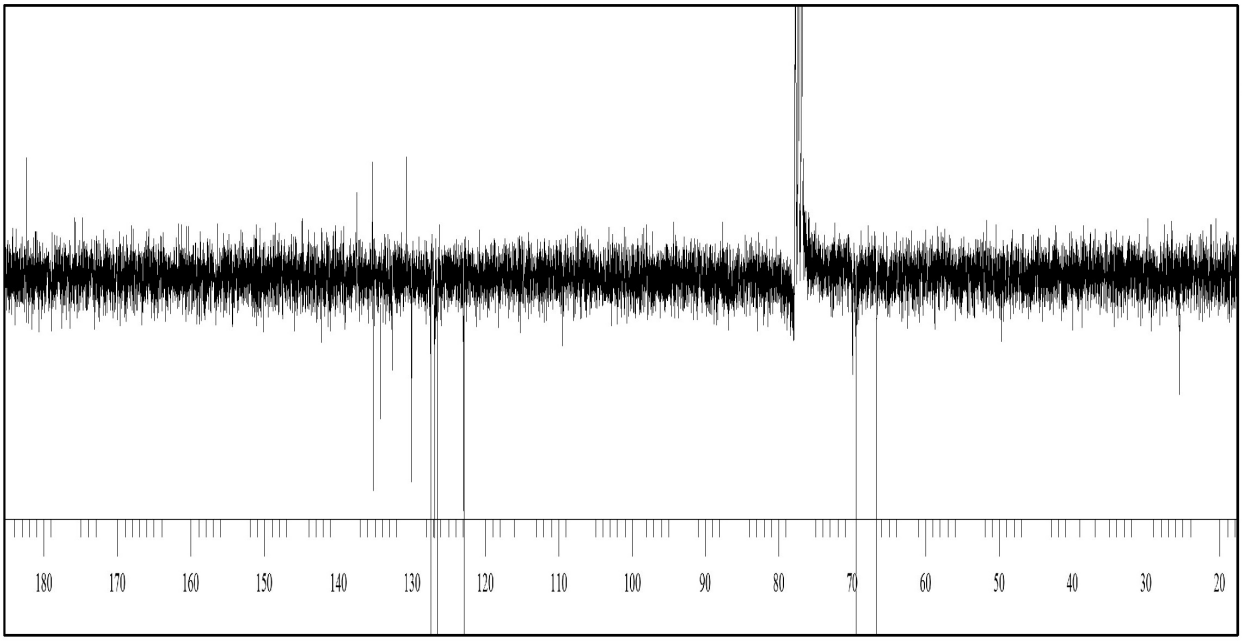


Figure S9. APT spectrum of 2-(4-ferrocenyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2c**).

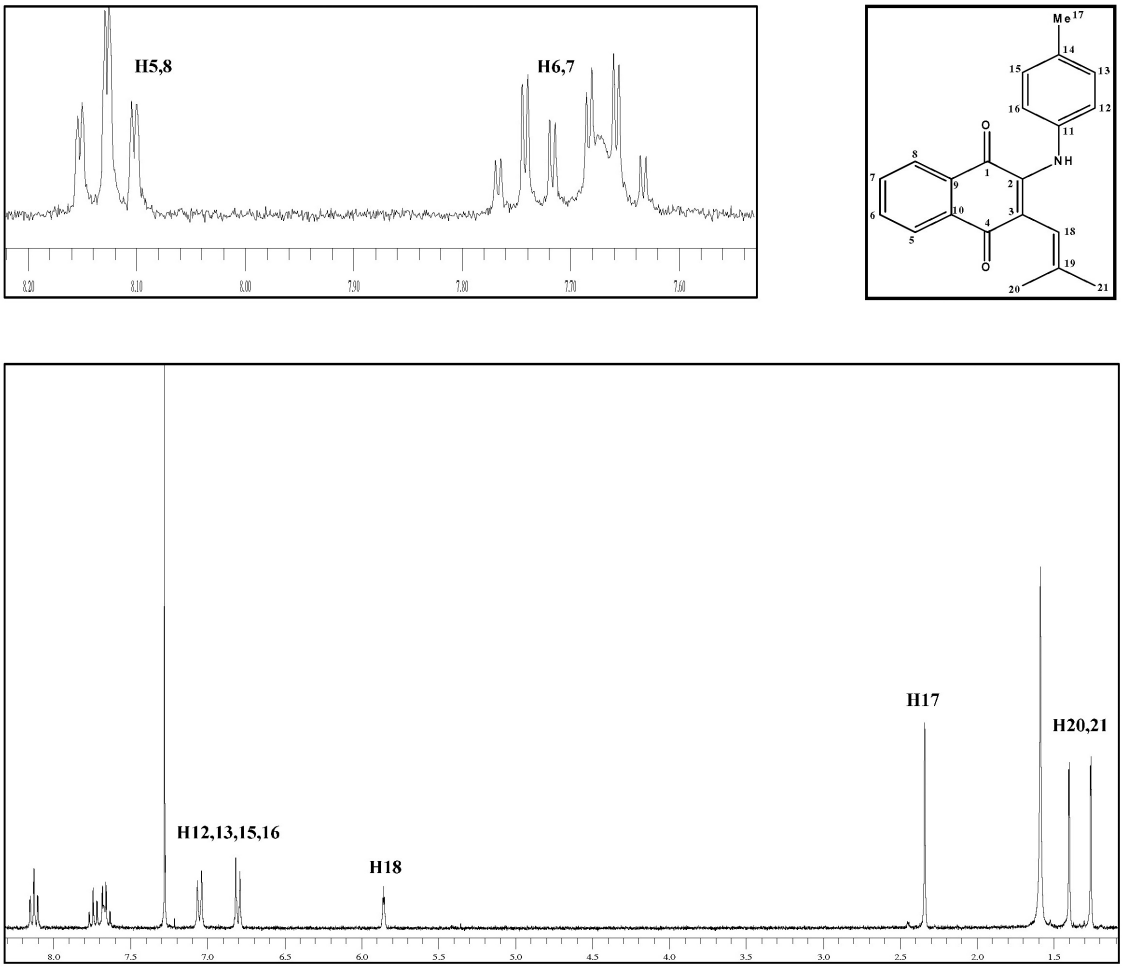


Figure S10. ^{1}H NMR spectrum of 2-(4-methyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2d**).

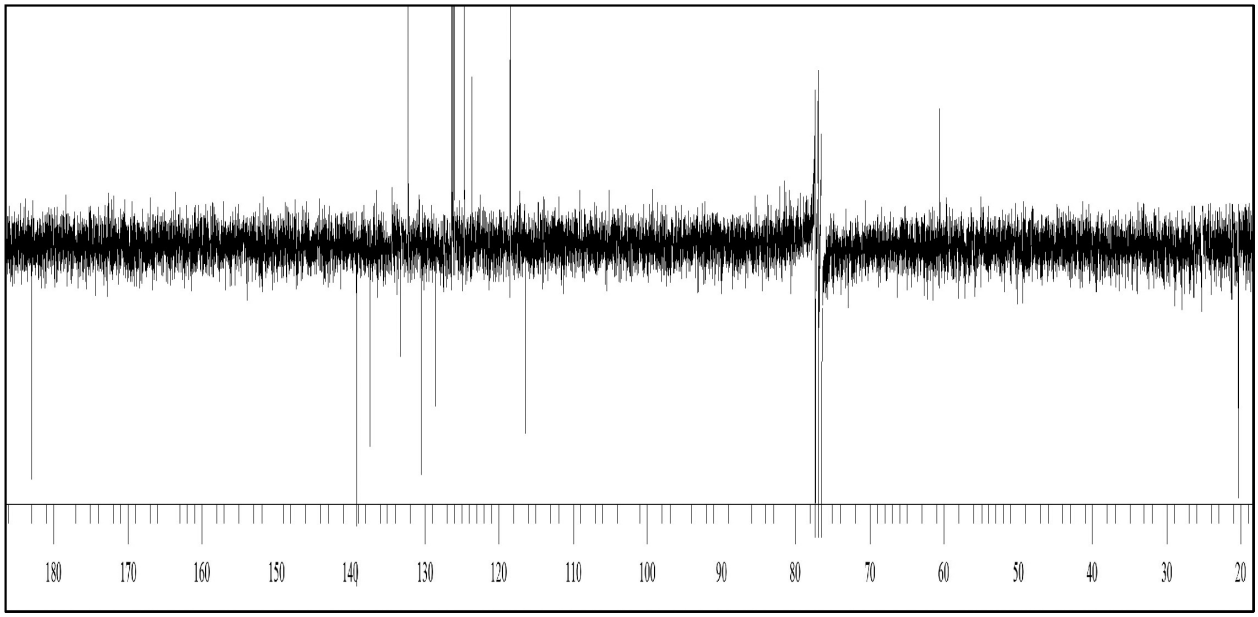
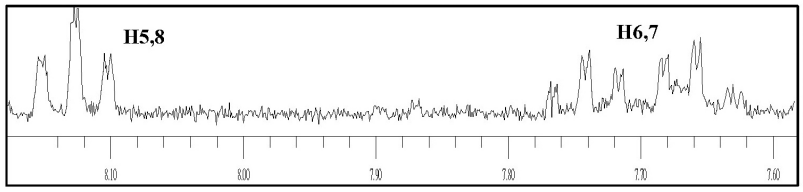
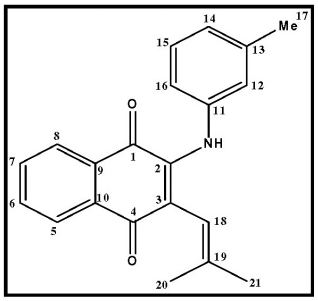


Figure S11. APT spectrum of 2-(4-methylphenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2d**).





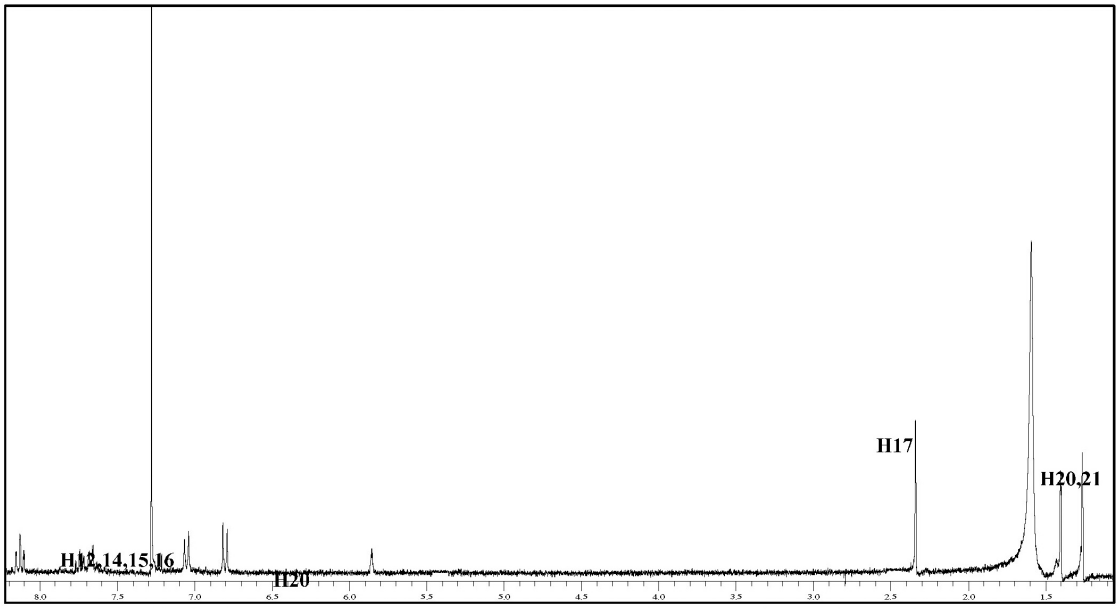


Figure S12. ^{1}H NMR spectrum of 2-(3-methylphenylene)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2e**).

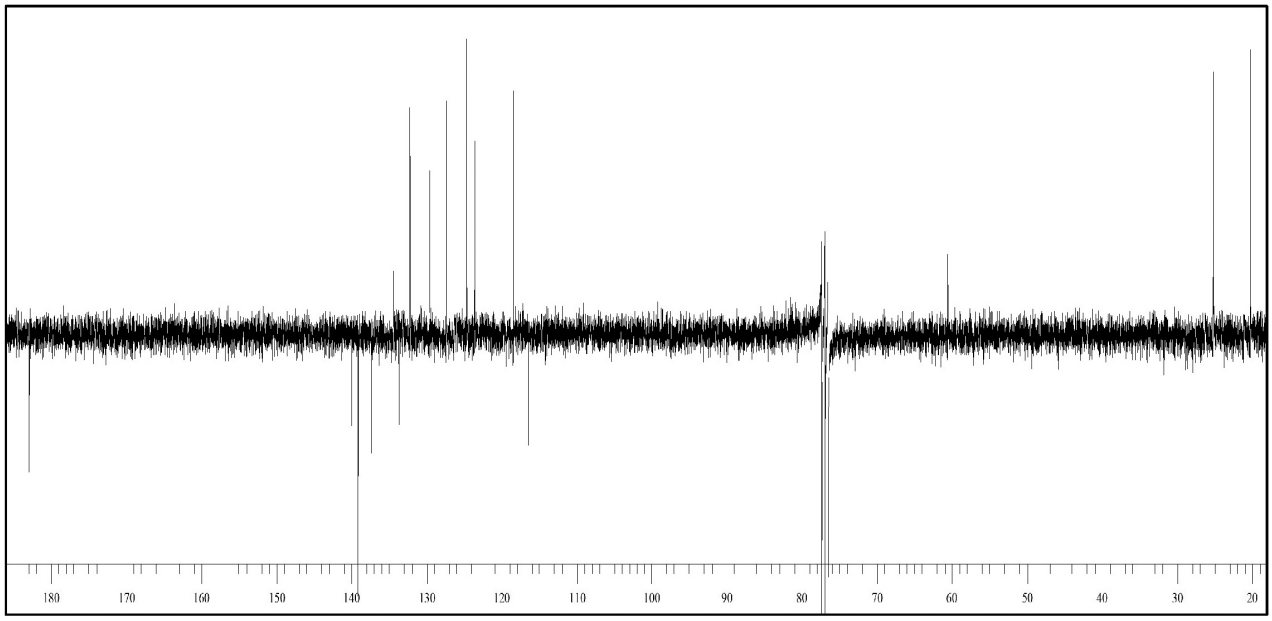


Figure S13. APT spectrum of 2-(3-methyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2e**).

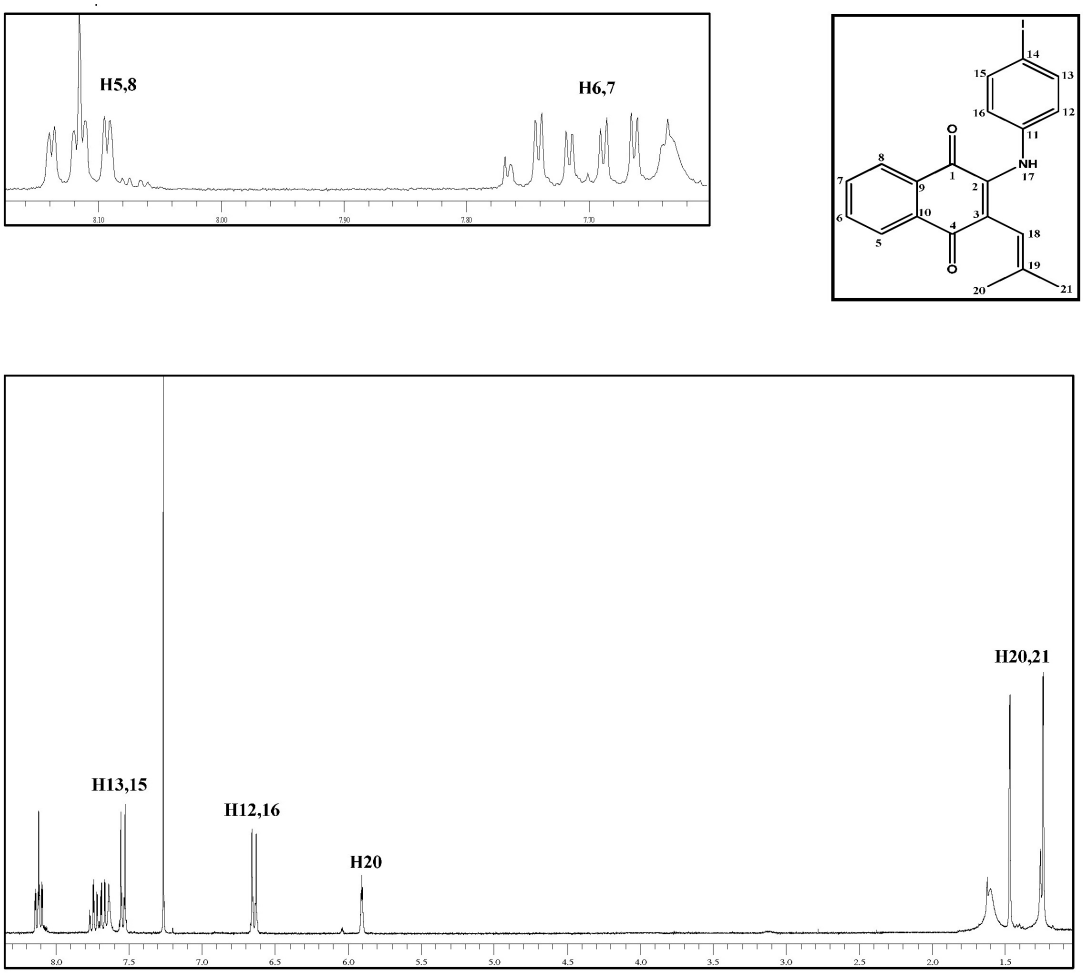


Figure S14. ^{1}H NMR spectrum of 2-(4-iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2f**).

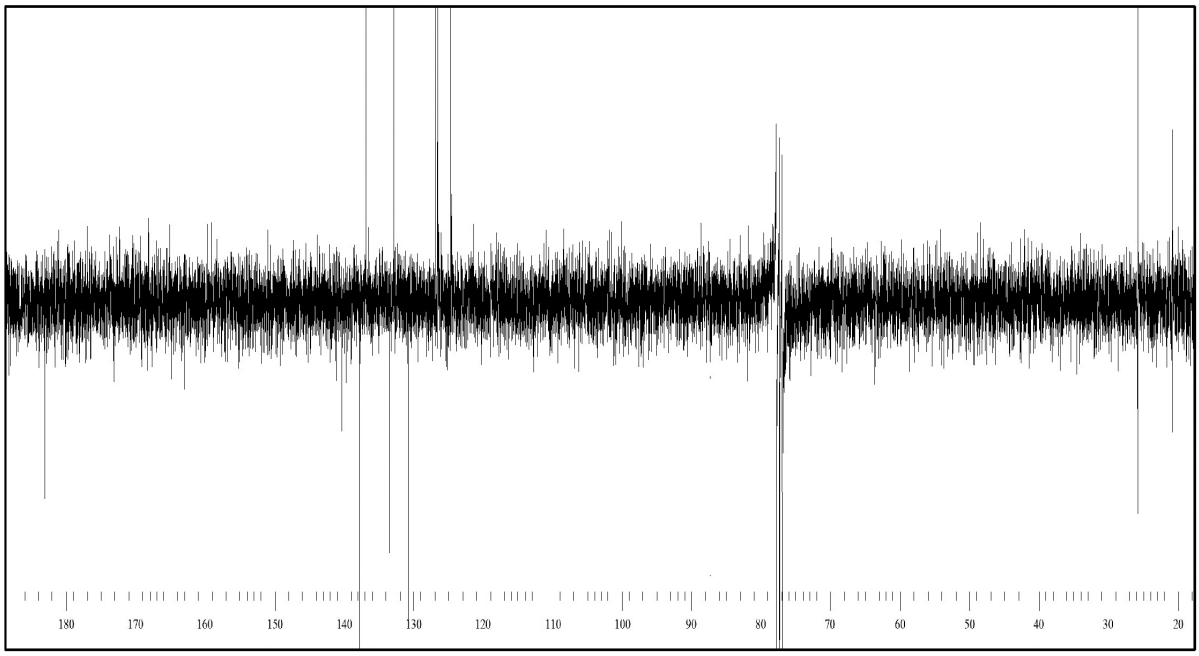


Figure S15. APT spectrum of 2-(4-iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2f**).

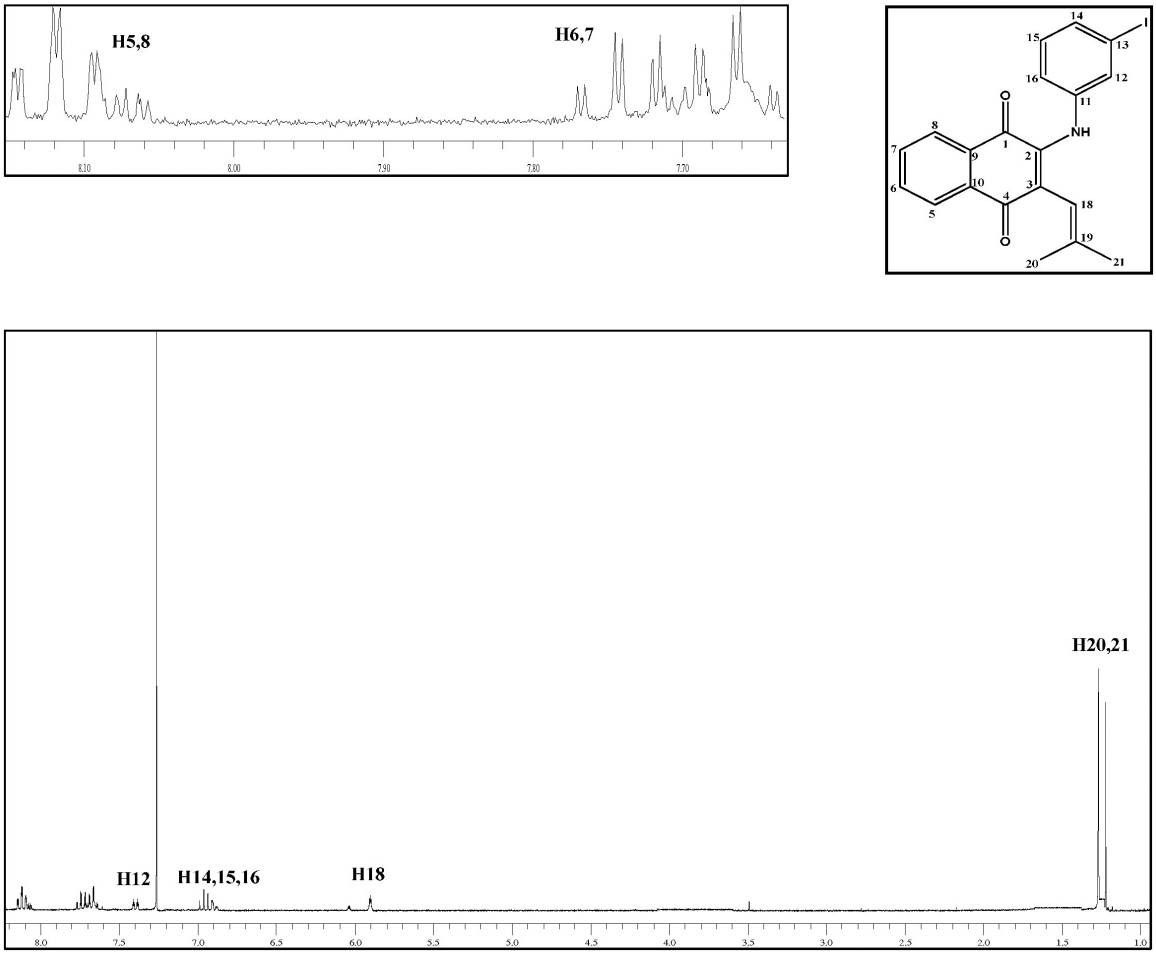


Figure S16. ^{1}H NMR spectrum of 2-(3-iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2g**).

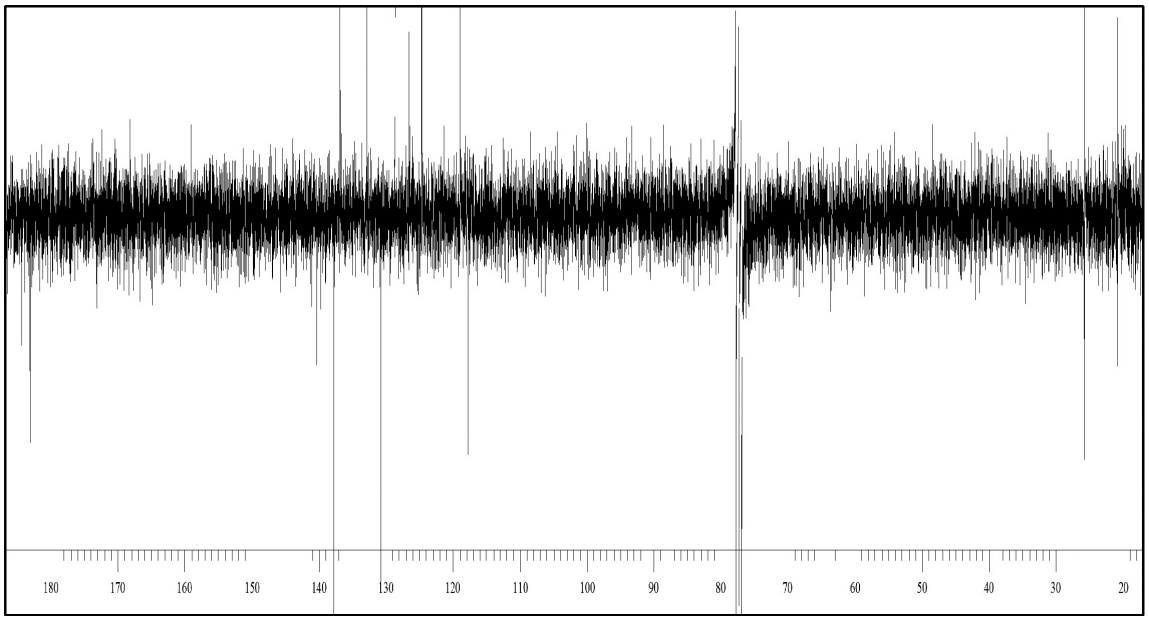


Figure S17. APT spectrum of 2-(3-iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2g**).

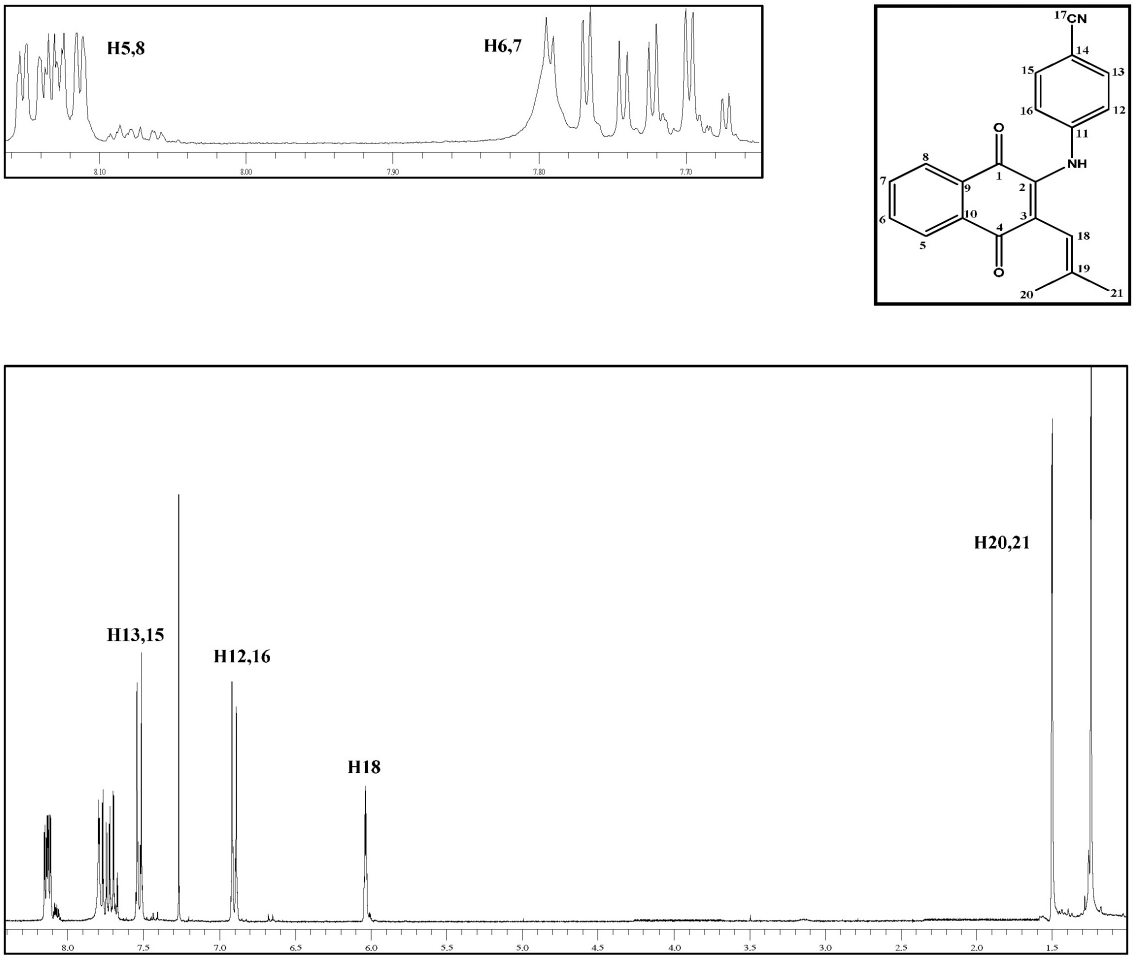


Figure S18. ^{1}**H NMR** spectrum of 2-(4-ciano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2h**).

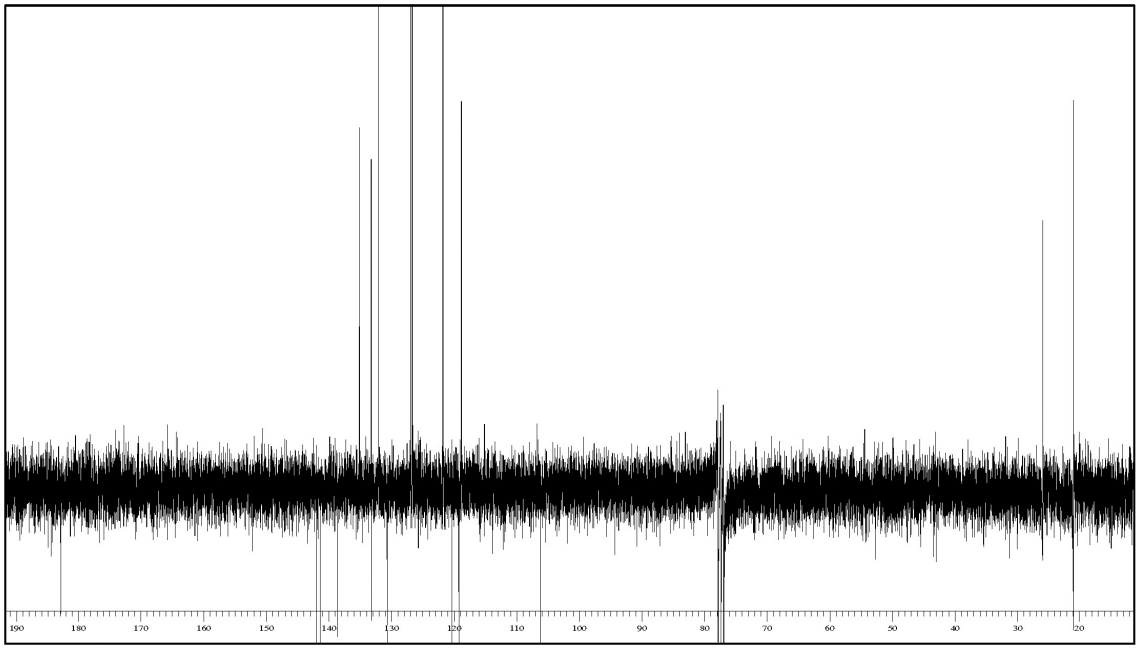


Figure S19. APT spectrum of 2-(4-ciano-phenyl)-3-(2-methylpropenyl)-1,4-naphthoquinone (**2h**).

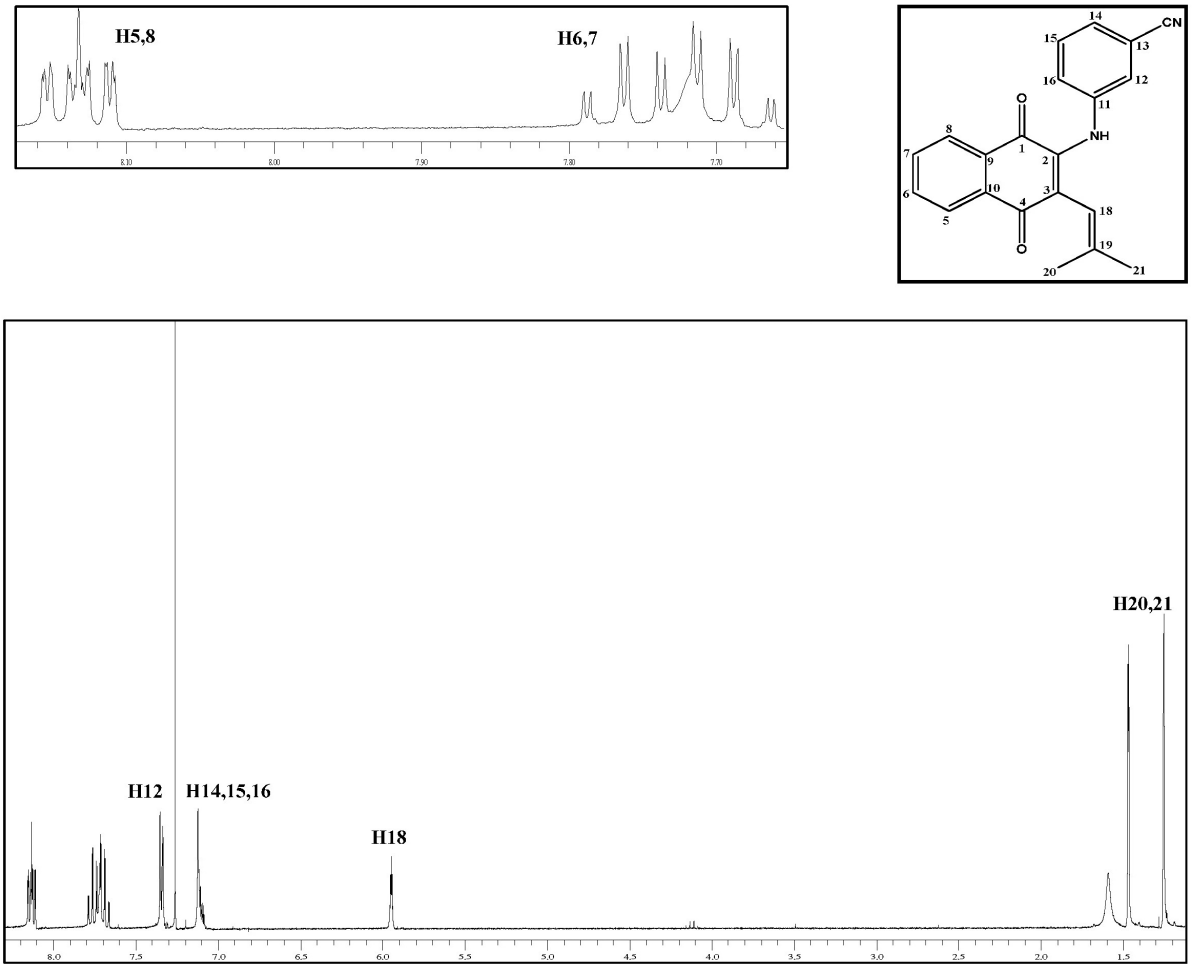


Figure S20. ^{1}H NMR spectrum of 2-(3-ciano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2i**).

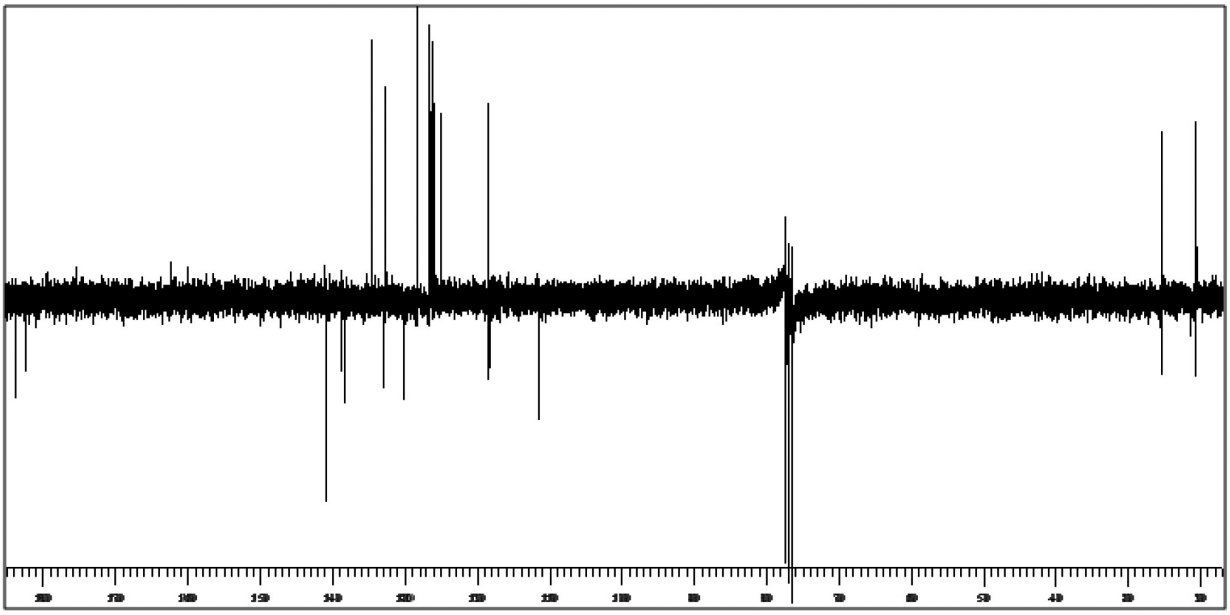


Figure S21. APT spectrum of 2-(3-ciano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2i**).

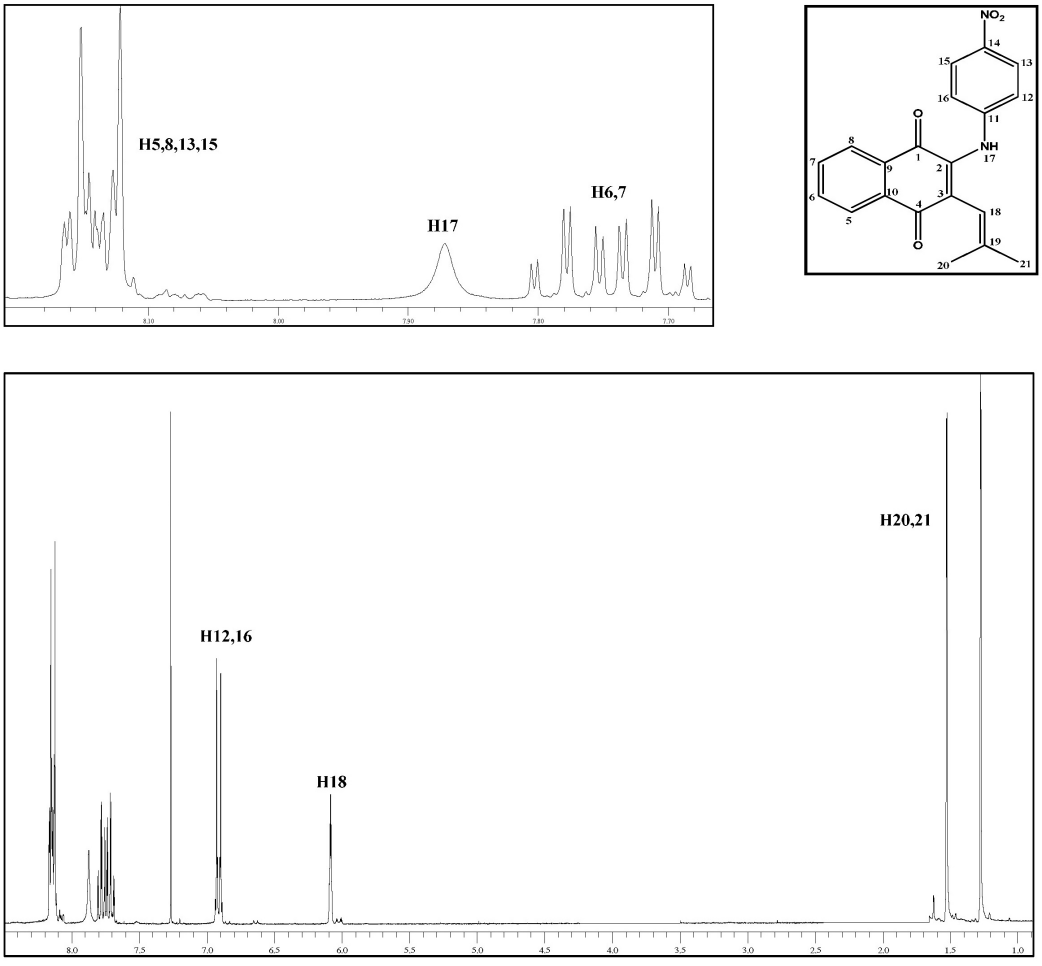


Figure S22. ^{1}**H NMR** spectrum of 2-(4-nitro-phenylene)-3-(2-methylpropenyl)-1,4-naphthoquinone (**2j**).

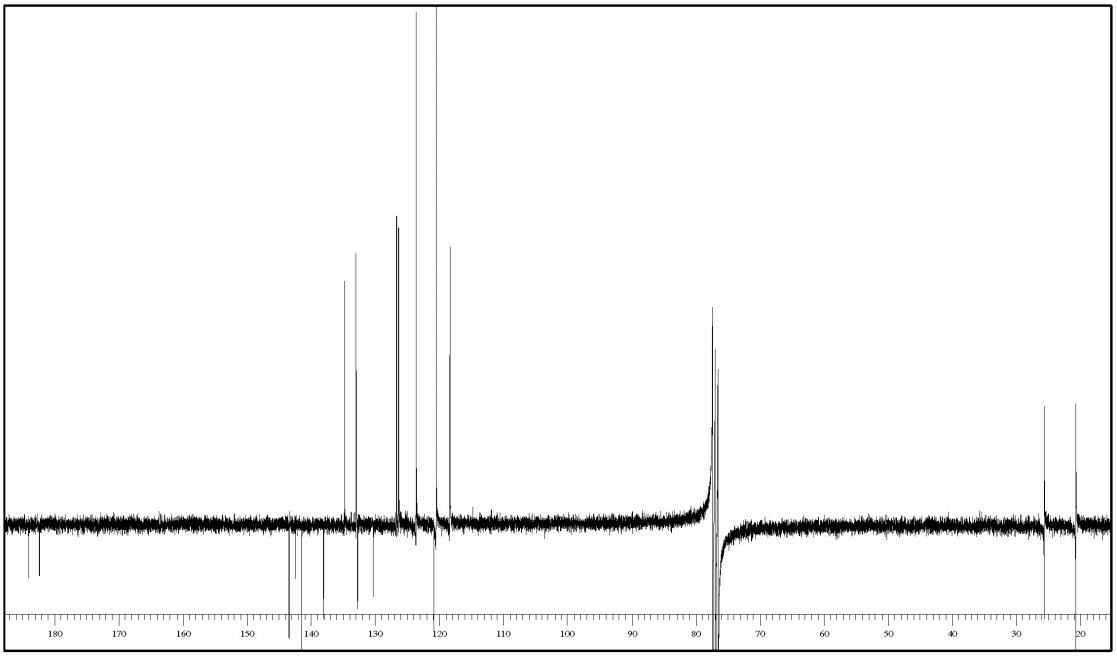
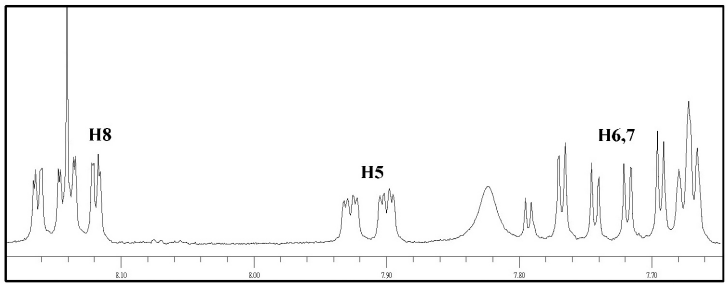
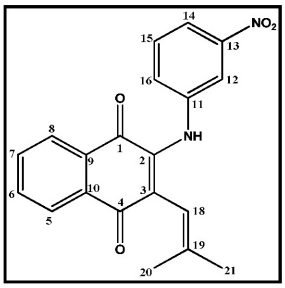


Figure S23. APT spectrum of 2-(4-nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2j**).





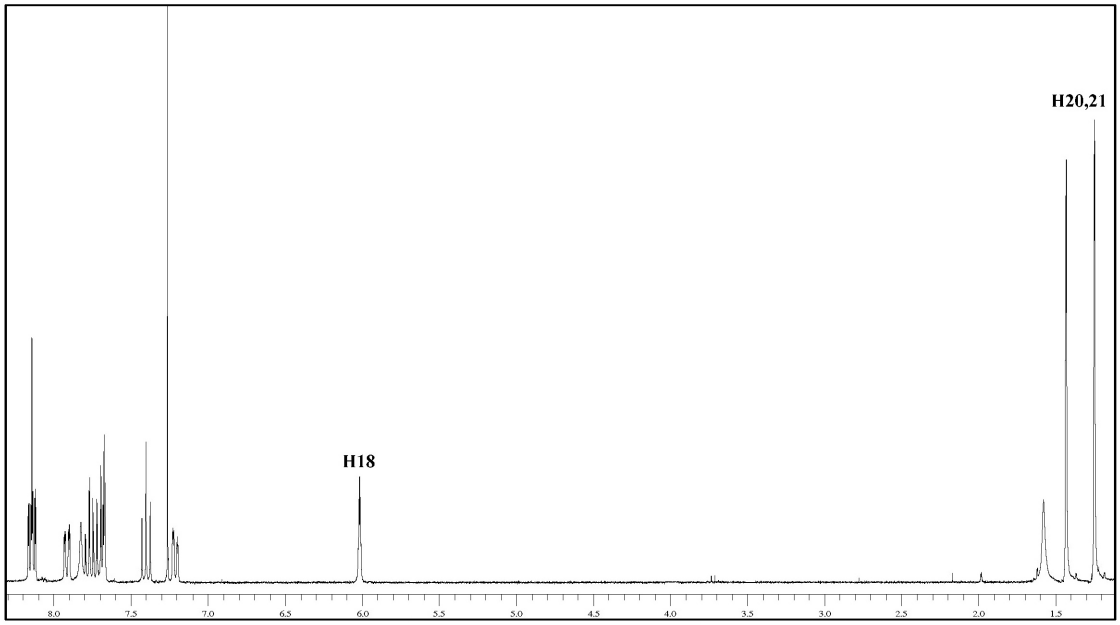


Figure S24. ^{1}H NMR spectrum of 2-(3-nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2k**).

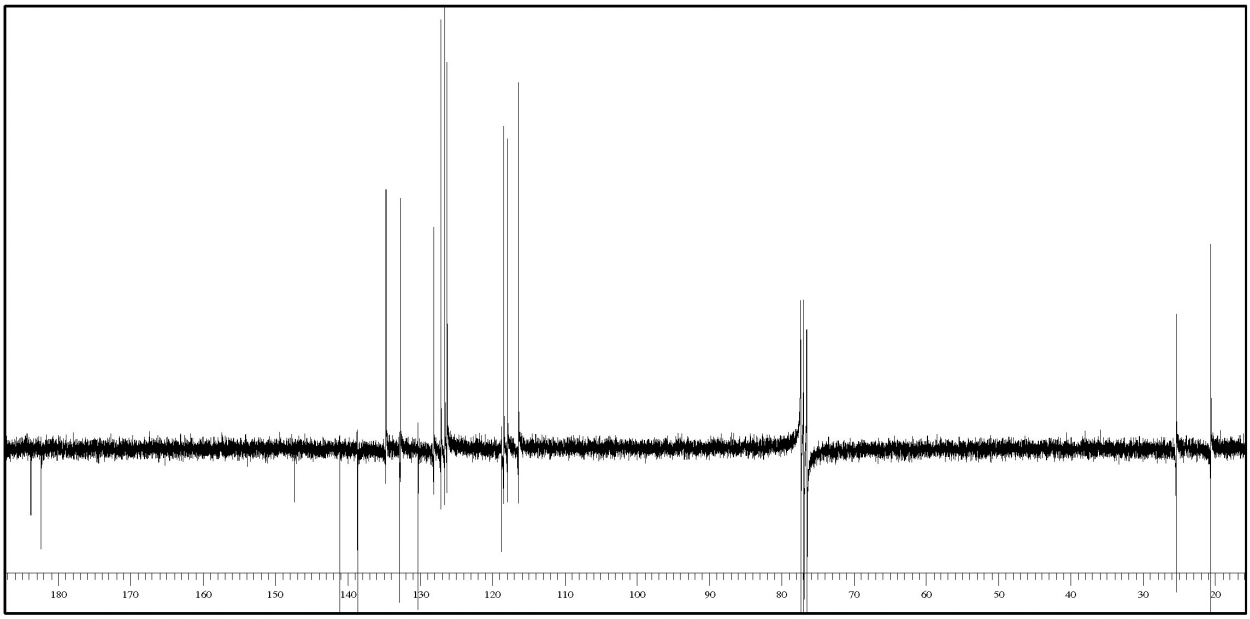


Figure S25. APT spectrum of 2-(3-nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2k**).

4. Variable Temperature ^{1}H NMR Spectra of Compound 2a

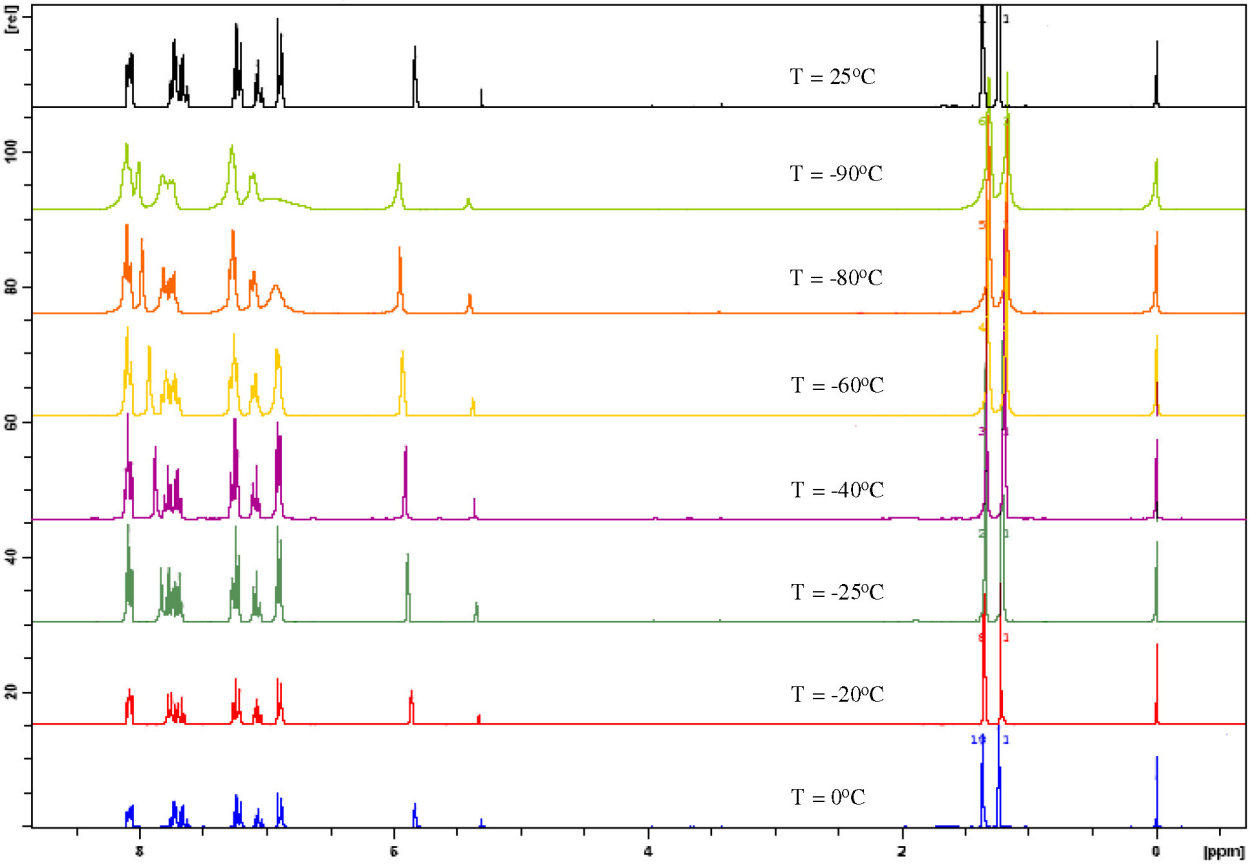


Figure S26. VT ^{1}H NMR spectra of compound **2a** (range: 25ºC to –90 ºC) in CD_2Cl_2.

4. Cyclic Voltammograms (100mV) of Compounds 2a-k

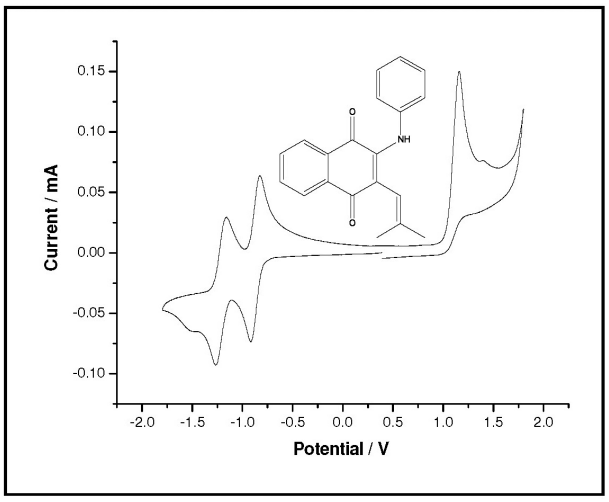


Figure S27. CV of 2-(phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2a**).

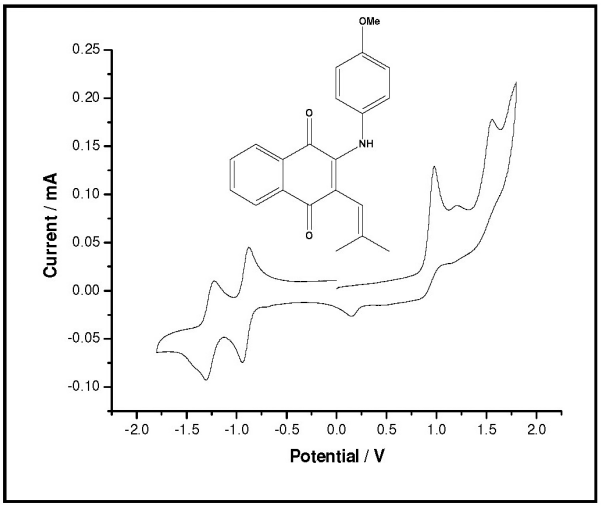


Figure S28. CV of 2-(4-methoxy-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2b**).

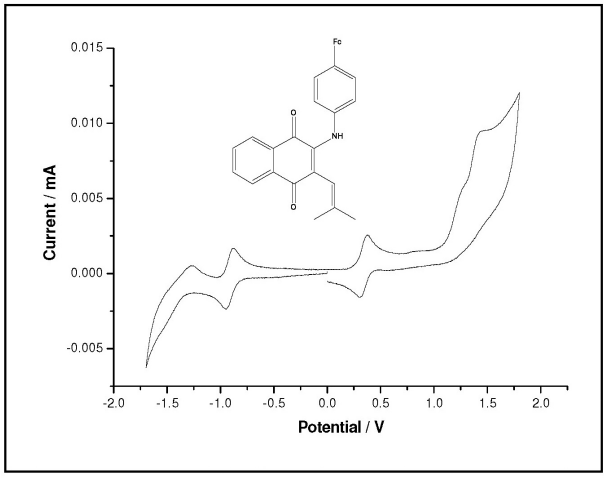


Figure S29. CV of 2-(4-ferrocenyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2c**).

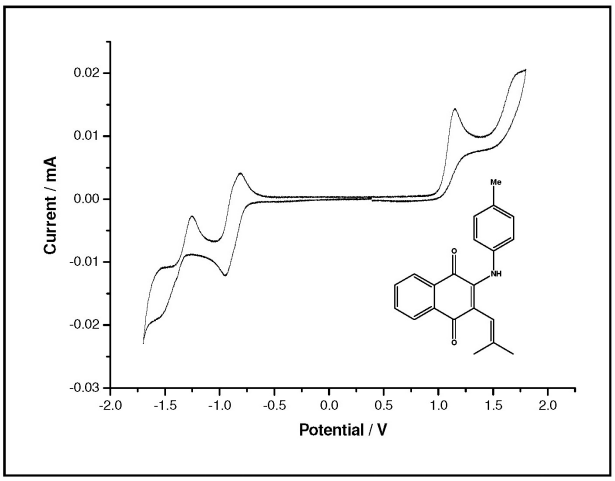


Figure S30. CV of 2-(4-methyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2d**).

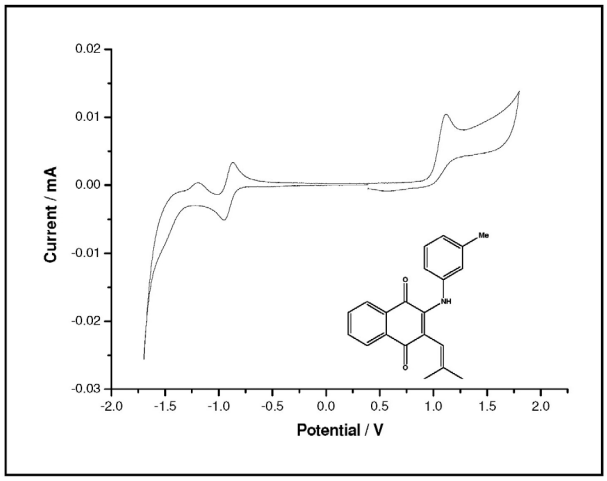


Figure S31. CV of 2-(3-methyl-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2e**).

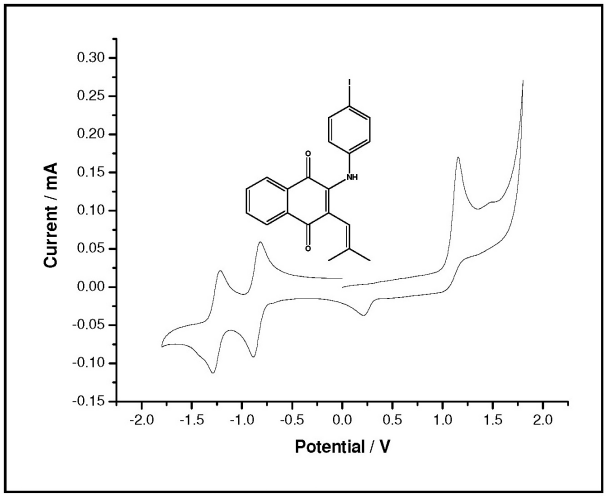


Figure S32. CV of 2-(4-iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2f**).

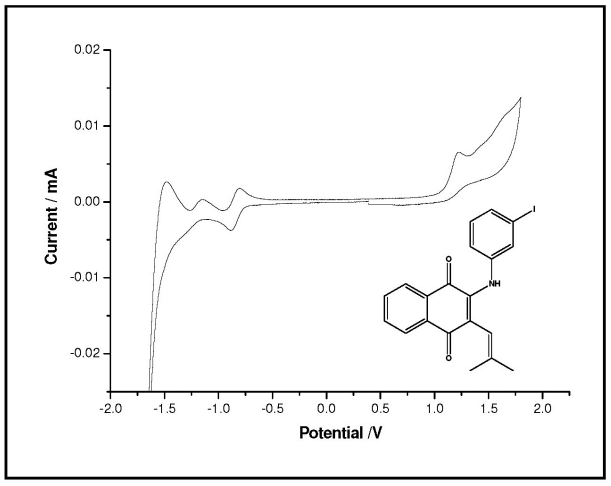


Figure S33. CV of 2-(3-iodo-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2g**).

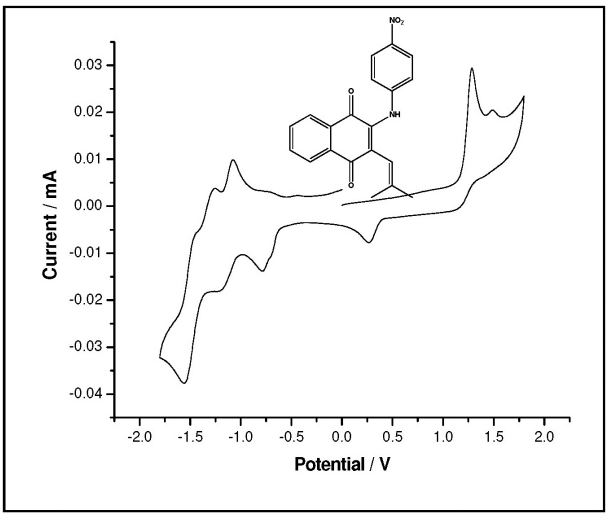


Figure S36. CV of 2-(4-nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2j**).

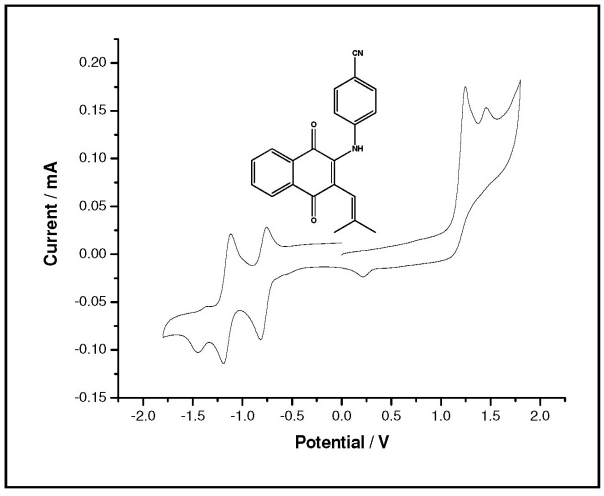


Figure S34. CV of 2-(4-ciano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2h**).

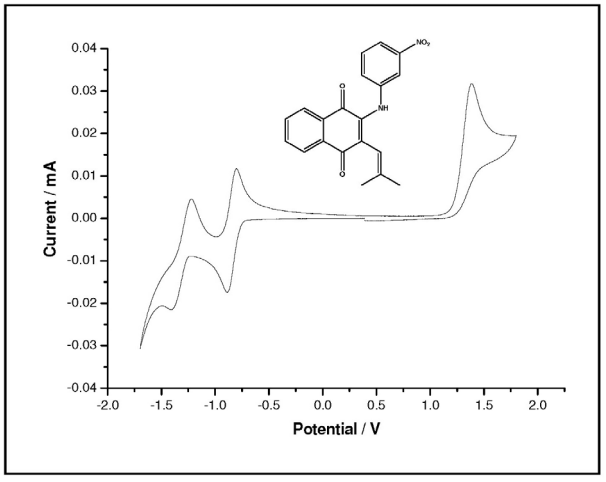


Figure S37. CV of 2-(3-nitro-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2k**).

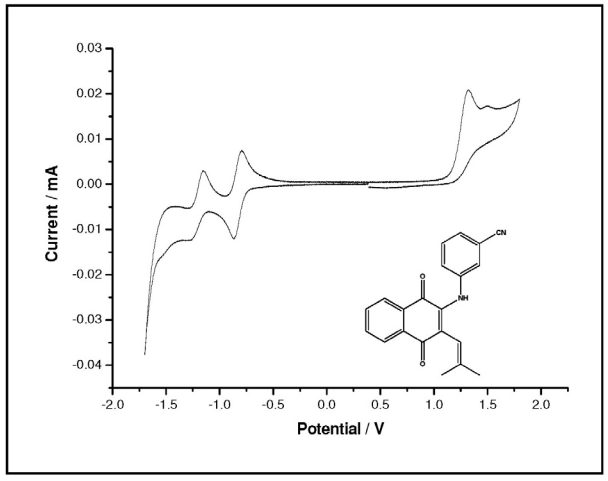


Figure S35. CV of 2-(3-ciano-phenyl)amino-3-(2-methylpropenyl)-1,4-naphthoquinone (**2i**).

5. 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 assay[ref] and the results are summarized in Table S6.

Table S6. Screening of the cytotoxic activity (growth inhibition %) of compounds **2a-k** and **1**

Compounds	SF295	GI%	HCT-8	GI%	MDA-MB435	GI%
	Average	SD	Average	SD	Average	SD
2a (H)	68.20	4.80	73.75	1.68	54.20	5.00
2b (4-OMe)	-11.01	11.89	-2.95	13.01	24.54	9.15
2c (4-Fc)	2.57	7.43	5.45	6.24	**102.31**	1.09
2d (4-Me)	12.20	3.71	12.33	2.76	17.21	3.58
2e (3-Me)	58.59	0.40	71.52	1.01	43.86	3.54
2f (4-I)	-4.93	23.88	10.29	2.13	-7.63	3.42
2g (3-I)	2.49	2.20	15.92	4.49	25.14	13.42
2h (4-CN)	-5.79	0.56	2.65	0.84	10.72	0.93
2i (3-CN)	-12.56	2.48	6.73	6.36	-20.51	4.35
2j (4-NO_2)	8.78	0.90	17.00	1.44	-18.20	8.24
2k (3-NO_2)	-7.40	4.20	17.03	2.02	4.01	7.68
1	41.09	15.08	27.01	3.12	**106.05**	0.16
DOX	96.19	0.63	94.85	3.99	**95.38**	2.23

DOX = positive control, doxorubicin.