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Research Article

On the Reaction of 2-Alkanoylnaphthohydroquinones with Hydroxylamine: Access to Cytotoxic 2-(Hydroxyamino)-1,4-naphthoquinone and Their 3-(Hydroxyimino)alkyl Analogous

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Oximes are known for their anti-inflammatory, antimicrobial, antioxidant, and anticancer activities. Frequently, modification of biologically active carbonyl compounds into oximes leads to increased activity. The present study reports the reactivity of 2-alkanoylnaphthohydroquinones against hydroxylamine under aerial conditions. Results show that, depending on the structure of the hydroquinones, the reaction proceeds through two different chemical pathways to produce 2-(hydroxyamino)-1,4-naphthoquinone and their C-3 (hydroxyimino)alkyl derivatives. Both the formation of the quinoid compounds under aerial oxidation and C-C cleavage reactions of hemiaminal intermediates are discussed. In vitro screening of the substituted 1,4-naphthoquinones on a panel of cancer cells reveals moderate cytotoxic activities. Compound **19**, 2-(hydroxyamino)-1,4-naphthoquinone, stands out by its anticancer potency against prostate cancer cells as shown by the lowest IC_{50} value (8.08 μM) and the best selectivity index (3.90).

1. Introduction

2-Acylated-1,4-benzo- and 1,4-naphthohydroquinones **I** and **III** (Figure 1) are of current interest by their use as synthetic building blocks for carbo- and heterocyclic compounds endowed with cytotoxic and antiproliferative effects in various human cancer cells, as well as antifungal activities [1–8]. The synthetic advantage of these acylhydroquinones [9–14] emerges from the coexistence of the hydroquinone and the *ortho*-hydroxyacylarene fragments in their structures. Within this group of acetylhydroquinones, the simplest naturally occurring member named quinacetophenone stands out by its property to inhibit the growth of diverse myeloma cells [15] and by its extensive use as a synthetic precursor of organic molecules such as chalcones, flavonoids, chromones, coumarins, quinones, and psoralens, relevant in medicinal chemistry [16].

In previous studies performed in our laboratory, two series of cytotoxic active acyl-containing quinoid compounds, **V** and **VI,** were prepared from acetylhydroquinones

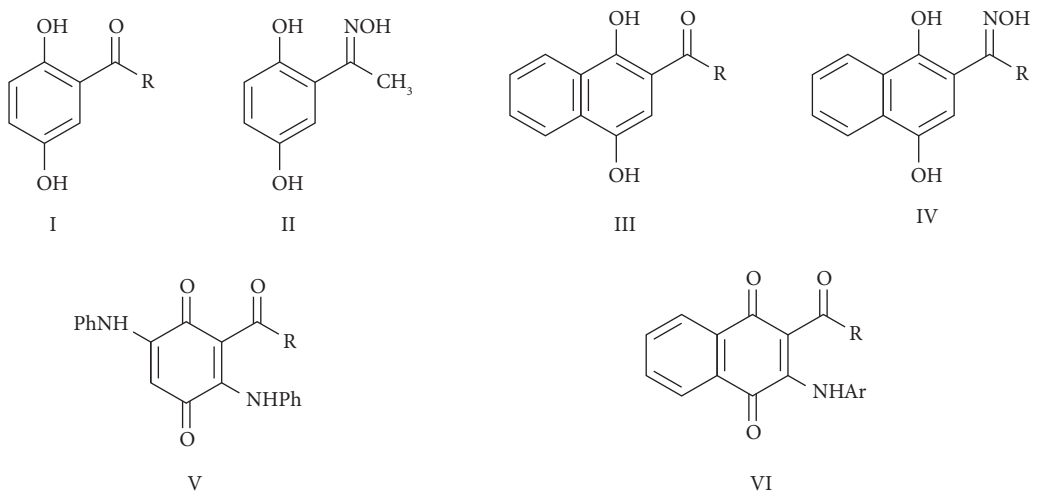


Figure 1: Structure of compounds **I–VI**.

types **I** and **III** (*R* = alkyl and aryl) [3, 4]. An interesting possibility to improve the cytotoxic activities of this series is to replace the corresponding acyl substituents with their oximes. Examples of this change in functionality to increase cytotoxic activity have been reported in cytotoxic acyl-containing natural products such as naringenin and flavonoid derivatives [17–20].

The abovementioned replacement approach requires precursors **II** and **IV**. Since access to oxime **II** from **I** ($R = CH_3$) has been reported [16], we focused our attention on the synthesis of oxime type **IV** from **III**.

Herein, we report preliminary results about the unexpected reactivity of 2-acyl-1,4-naphthohydroquinones **III** (*R* = *n*-alkyl) against hydroxylamine leading to the formation of 2-(hydroxyamino)-1,4-naphthoquinone and C-3 (hydroxyamino) alkyl derivatives rather than the corresponding oximes **IV**. The isolated novel quinoid compounds were evaluated for their *in vitro* cytotoxic activities on a panel of three human-derived tumor cell lines. In order to get a range of selectivity, such cytotoxic activities were compared to those obtained on nontumorigenic HEK-293 (human embryonic kidney cells).

2. Materials and Methods

2.1. General Information. All the solvents and reagents were purchased from different companies, such as Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany), and were used as supplied. Melting points (mp) were determined on a Stuart Scientific SMP3 (Staffordshire, UK) apparatus and are uncorrected. The IR spectra were recorded on an FT IR Bruker spectrophotometer, model Vector 22 (Bruker, Rheinstetten, Germany), using KBr disks, and the wave numbers are given in cm^{-1}. ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker Ultrashield-300 instrument (Bruker, Ettlingen, Germany) in DMSO-d_6 at 300 and 75 MHz, respectively. Chemical shifts are expressed in ppm downfield relative to tetramethylsilane, and the coupling constants (*J*) are reported in Hertz. Data for the ^{1}H-NMR spectra are reported as follows: *s* = singlet, br *s* = broad singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, and the coupling constants (*J*) are in Hz. Bidimensional NMR techniques and distortion-less enhancement by polarization transfer (DEPT) were used for the signal assignment. Chemical shifts are expressed in ppm downfield relative to tetramethylsilane, and the coupling constants (*J*) are reported in Hertz. The HRMS data for all final compounds were obtained using an LTQ-Orbitrap mass spectrometer (Thermo-Fisher Scientific, Waltham, MA, USA) with the analysis performed using an atmospheric-pressure chemical ionization (APCI) source, operated in positive mode. Silica gel Merck 60 (70–230 mesh, from Merck) was used for preparative column chromatography and thin layer chromatography (TLC). Aluminum foil $60F_{254}$ was used for analytical thin layer chromatography. The acylbenzohydroquinones (**2**–**11**) were prepared according to a previously reported procedure [12].

2.2. General Procedure for the Reaction of 2-Alkanoylnaphthohydroquinones with Hydroxylamine. Suspensions of hydroxylamine hydrochloride (2-equiv.), sodium acetate (2 equiv.), and methanol (20 mL) were stirred for 1 h at room temperature. 2-Acylnaphthohydroquinones (1-equiv.) were added to the methanolic solutions and the mixtures were refluxed for 20 h. The solvents were removed at reduced pressure and the residues were column chromatographed over silica gel (1/1 petroleum ether/ethyl acetate) to give the corresponding substituted naphthoquinones **12**–**19**.

*2.2.1. 2-(Hydroxyamino)-3-(hydroxyimino)methyl-1,4-naphthoquinone **12**.* Prepared from **2** (200 mg, 0.99 mmol) and hydroxylamine (2-equiv.), isolated in 58% yield (140.3 mg, 0.57 mmol) as orange solid mp: 141–142°C; IR (KBr) ν_{max} cm^{-1}: 3467, 3416, 1638, 1618; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.16 (s, 1H, OH), 7.96 (d, 2H, J = 7.7 Hz, 2H, arom), 7.82 (t, 1H, J = 7.5 Hz, arom), 7.72 (t, 1H, J = 7.4 Hz, arom), 7.56 (br *s*, 2H, OH + NH), 2.09 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.5, 180.7,

153.9, 147.0, 135.0, 133.3, 132.5, 130.0, 125.8, 125.6, 109.5, 15.5. HRMS (APCI): $(M+H)^+$ Calcd for $C_{12}H_{10}N_2O_4$: 246.06406; found 246.07111.

*2.2.2. 2-(Hydroxyamino)-3-(hydroxyimino)propyl-1,4-naphthoquinone **13**.* Prepared from **3** (228 mg, 0.99 mmol) and hydroxylamine (2-equiv.), isolated in 60% yield (161.8 mg, 0.59 mmol) as orange solid mp: 148–149°C. IR (KBr) ν_{max} cm^{-1}: 3473 (OH), 3413 (NH), 1638 (C=O), 1618 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.07 (s, 1H, OH), 7.96 (d, 2H, J = 8.8 Hz, H-arom), 7.83 (t, 1H, J = 8.1 Hz, H-arom), 7.73 (t, 1H, J = 8.1 Hz, H-arom), 7.55 (br *s*, 2H, OH + NH), 2.63 (dd, J = 8.8, 6.7 Hz, 2H, HO-N=C-CH_2-CH_2-CH_3), 1.46 (h, 2H, J = 7.3 Hz, HO-N=C-CH_2-CH_2-CH_3), 0.84 (t, 3H, J = 7.4 Hz,, HO-N=C-CH_2-CH_2-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.49, 180.69, 157.01, 147.53, 135.03, 133.13, 132.46, 129.99, 125.79, 125.66, 109.12, 29.89, 19.03, 14.43. HRMS (APCI): $(M+H)^+$ Calcd for $C_{14}H_{14}N_2O_4$: 274.09536; found 274.09844.

*2.2.3. 2-(Hydroxyamino)-3-(hydroxyimino)butyl-1,4-naphthoquinone **14**.* Prepared from **4** (244 mg, 1.0 mmol) and hydroxylamine (2-equiv.), isolated into 58%, yield (167.2 mg, 0.58 mmol) as orange solid mp: 157–158°C. IR (KBr) ν_{max} cm^{-1}: 3452 (OH), 3450 (NH), 1638 (C=O), 1619 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.06 (s, 1H, OH), 7.96 (d, 2H, J = 8.9 Hz, H-arom), 7.83 (t, 1H, J = 7.7 Hz, H-arom), 7.73 (t, 1H, J = 7.4 Hz, H-arom), 7.53 (br *s*, 2H, OH + NH), 2.65 (m, 2H, HO-N=C-$\underline{CH_2}$-CH_2-CH_2-CH_3), 1.41 (q, 2H, J = 7.3 Hz, HO-N=C-CH_2-$\underline{CH_2}$-CH_2-CH_3), 1.24 (dq, 2H, J = 14.4, 7.1 Hz, HO-N=C-CH_2-CH_2-$\underline{CH_2}$-CH_3), 0.82 (t, 3H, J = 7.2 Hz, HO-N=C-CH_2-CH_2-CH_2-$\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.51, 180.69, 157.10, 147.55, 135.05, 133.12, 132.48, 130.00, 125.80, 125.68, 109.14, 27.72, 27.60, 22.64, 13.91. HRMS (APCI): $(M+H)^+$ Calcd for $C_{15}H_{16}N_2O_4$: 288.1110; found 288.1010.

*2.2.4. 2-(Hydroxyamino)-3-(hydroxyimino)pentyl-1,4-naphthoquinone **15**.* Prepared from **5** (258 mg, 0.99 mmol) and hydroxylamine (2-equiv.), isolated in 65% yield (193.5 mg, 0.64 mmol) as orange solid mp: 160–161°C. IR (KBr) ν_{max} cm^{-1}: 3473 (OH), 3413 (NH), 1638 (C=O), 1617 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.06 (s, 1H, OH), 7.96 (d, 2H, J = 7.7 Hz, H-arom), 7.83 (t, 1H, J = 7.5 Hz, H-arom), 7.73 (t, 1H, J = 7.5 Hz, H-arom), 7.53 (br *s*, 2H, OH + NH), 2.64 (m, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_3), 1.44 (q, 2H, J = 6.9 Hz, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_3), 1.22 (m, 4H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_3), 0.80 (t, 3H, J = 6.6 Hz, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.51, 180.71, 157.17, 147.57, 135.08, 133.14, 132.50, 130.00, 125.82, 125.70, 109.12, 31.68, 27.78, 25.15, 21.96, 13.97. HRMS (APCI): $(M+H)^+$ Calcd for $C_{16}H_{18}N_2O_4$: 302.12666; found 302.12123.

*2.2.5. 2-(Hydroxyamino)-3-(hydroxyimino)hexyl-1,4-naphthoquinone **16**.* Prepared from **6** (272 mg, 1.0 mmol) and hydroxylamine (2-equiv.), isolated in 55% yield (174.1 mg, 0.55 mmol) as orange solid mp. 162–163°C. IR (KBr) ν_{max} cm^{-1}: 3473 (OH), 3414 (NH), 1638 (C=O), 1619 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.05 (s, 1H, OH), 7.96 (d, 2H, J = 8.8 Hz, H-arom), 7.82 (td, 1H, J = 7.7; 1.1 Hz, H-arom), 7.72 (td, 1H, J = 7.7; 1.1 Hz, H-arom), 7.54 (br *s*, 2H, OH + NH), 2.64 (m, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.41 (q, J = 7.4, 6.9 Hz, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.18 (m, 6H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 0.78 (t, J = 6.6 Hz, 3H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.50, 180.69, 157.17, 147.54, 135.05, 133.13, 132.48, 129.99, 125.80, 125.67, 109.14, 31.09, 29.09, 27.78, 25.45, 22.07, 13.97. HRMS (APCI): $(M+H)^+$ Calcd for $C_{17}H_{20}N_2O_4$: 316.14231; found 316.14799.

*2.2.6. 2-(Hydroxyamino)-3-(hydroxyimino)heptyl-1,4-naphthoquinone **17**.* Prepared from **7** (287 mg, 1.0 mmol) and hydroxylamine (2-equiv.). Isolated in 72% yield (234.6 mg, 0.71 mmol) as orange solid mp. 165–166°C. IR (KBr) ν_{max} cm^{-1}: 3470 (OH), 3416 (NH), 1638 (C=O), 1618 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.11 (s, 1H, OH), 7.95 (d, 2H, J = 7.9 Hz, H-arom), 7.81 (td, 1H, J = 7.6, 1.3 Hz, H-arom), 7.71 (td, 1H, J = 7.5, 1.4 Hz, H-arom), 7.48 (br s, 2H, OH + NH), 2.63 (m, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.40 (m, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.15 (m, 8H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 0.75 (t, J = 6.8 Hz, 3H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.66, 180.93, 157.35, 147.67, 135.27, 133.24, 132.69, 130.12, 125.96, 125.86, 109.35, 31.38, 29.46, 28.64, 27.86, 25.60, 22.25, 14.13; HRMS (APCI): $(M+H)^+$ Calcd for $C_{18}H_{22}N_2O_4$: 330.15796; found 330.16101.

*2.2.7. 2-(Hydroxyamino)-3-(hydroxyimino)octyl-1,4-naphthoquinone **18**.* Prepared from **8** (300 mg, 0.99 mmol) and hydroxylamine (2-equiv.) to yield quinone **18** (192.8 mg, 0.56 mmol, 57%), orange solid mp. 169–170°C. IR (KBr) ν_{max} cm^{-1}: 3472 (OH), 3416 (NH), 1638 (C=O), 1618 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.11 (s, 1H, OH), 7.95 (d, 2H, J = 7.7 Hz, H-arom), 7.82 (td, 1H, J = 7.6, 1.1 Hz, H-arom), 7.72 (td, 1H, J = 7.5, 1.2 Hz, H-arom), 7.47 (br *s*, 2H, OH + NH), 2.63 (m, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.40 (m, 2H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.14 (m, 10H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 0.76 (t, J = 6.5 Hz, 3H, HO-N=C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.67, 180.95, 157.36, 147.68, 135.30, 133.25, 132.73, 130.13, 125.98, 125.88, 109.37, 31.41, 29.44, 28.89, 28.77, 27.80, 25.56, 22.28, 14.14. HRMS (APCI): $(M+H)^+$ Calcd for $C_{19}H_{24}N_2O_4$: 344.17361; found 344.17398.

*2.2.8. 2-(Hydroxyamino)-1,4-naphthoquinone **19**.* Prepared from **10** (328 mg, 0.99 mmol) and hydroxylamine (2-equiv.), isolated in 72% yield (134.3 mg, 0.71 mmol) as orange solid mp. 142–143°C. IR (KBr) ν_{max} cm^{-1}: 3467, 3417, 1638, 1618;

^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.92 (ddd, 2H, J = 13.9, 7.6, 1.4 Hz, arom), 7.80 (td, 1H, J = 7.5, 1.1 Hz, arom), 7.70 (td, 1H, J = 7.4, 1.5 Hz, arom), 7.22 (br *s*, 2H, OH + NH), 5.81 (s, 1H, 2-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.0, 181.8, 150.5, 134.7, 133.2, 132.2, 130.4, 125.8, 125.3, 102.3. HRMS (APCI): $(M + H)^+$ Calcd for $C_{10}H_7NO_3$: 189.04259; found 189.04899.

2.3. Cell Lines and Cell Cultures. Briefly, human cancer cell lines DU-145 (prostate), MCF-7 (breast), T-24 (bladder), and nontumor HEK-293 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). They were cultured at a density of 1-2 x 10^5 cells/mL in high-glucose Dulbecco's modified Eagle medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). All cultures were kept at 37°C in 95% air/5% CO_2 at 100% humidity. The medium was changed at 48–72 h intervals. Phosphate-buffered saline (PBS) was purchased from Gibco. Cells were incubated for the indicated times at 37°C in the absence or the presence of quinones at various concentrations.

2.4. Cytotoxic Assays. The cytotoxicity of quinones was assessed by following the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan blue [21]. Cells were seeded into 96-well plates at a density of 10,000 cells/well for 24 h and then further incubated for 48 h, with or without the quinone derivatives. Doxorubicin was used as a standard chemotherapeutic agent (positive control). Cells were then washed twice with warm PBS and incubated with MTT (0.5 mg/mL) for 2 hours at 37°C. Blue formazan crystals were solubilized by adding 100 μL DMSO/well, and the optical density of the colored solutions was subsequently read at 550 nm. Results are expressed as percentage of MTT reduction, compared to untreated control conditions. The IC_{50} values were calculated using the GraphPad Prism software (San Diego, CA, USA).

3. Results and Discussion

Since the synthesis of acetylhydroquinone oxime **II** from hydroquinone **I** (R = CH_3) and hydroxylamine has been reported with high yield [16] and based on standard procedures to prepare acetophenone oximes [22], the reactivity of acetylhydroquinones **III** leading to the corresponding oximes **IV** was first explored. In this context, Scheme 1 shows the synthesis of the required precursor 2-acetylnaphthohydroquinone **2** from 1,4-naphthoquinone **1** and acetaldehyde, according to our previously reported procedure based on the solar photoacylation Friedel–Crafts reaction [12].

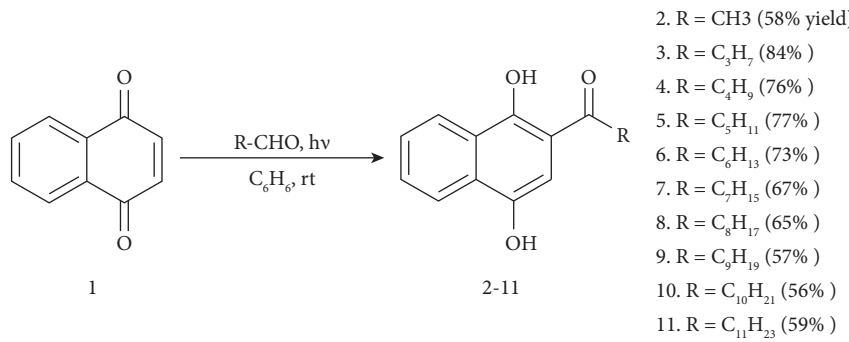
In this preliminary assay, acetylnaphthohydroquinone **2** was reacted with NH_2OH in boiling methanol (Scheme 2). Workup of the reaction mixture provides an orange crystalline product, m.p. 141–142°C. The ^{1}H-NMR spectrum displays, at 11.16 ppm, a singlet (D_2O exchangeable) for the hydroxyl proton of a ketoxime group [23]. The characteristic A_2B_2 pattern signals for the aromatic protons of the 1,4-naphthoquinone system appeared at 7.82 and 7.72 ppm. At *ca*. 7.56 ppm, there was a broad singlet signal for two protons (D_2O exchangeable) assignable to the NHOH group and a singlet signal at 2.09 ppm for the proton of a methyl group. The ^{13}C NMR displays signals at 181.5 and 181.7 ppm for quinoid carbonyl carbons and for ketoxime carbon at 153.9 ppm [23]. Based on these spectral properties, HMBC correlation, and the high-resolution mass spectroscopy (HRMS), structure **12** was fully established for the new compound isolated in 58% yield.

This interesting and unexpected reaction of **2** with hydroxylamine to give **12** leads us to change our primary objective to prepare compounds type **IV** towards the substrate scope of the previously unreported one-pot reaction as potential general access to novel 2-(hydroxyamino)-3-(hydroxyimino)alkyl-1,4-naphthoquinones. In this context, compounds **3–12** were prepared, just like compound **2**, by solar photoacylation of 1,4-naphthoquinone **1** with C_3-C_{11} linear aliphatic aldehydes according to Scheme 1.

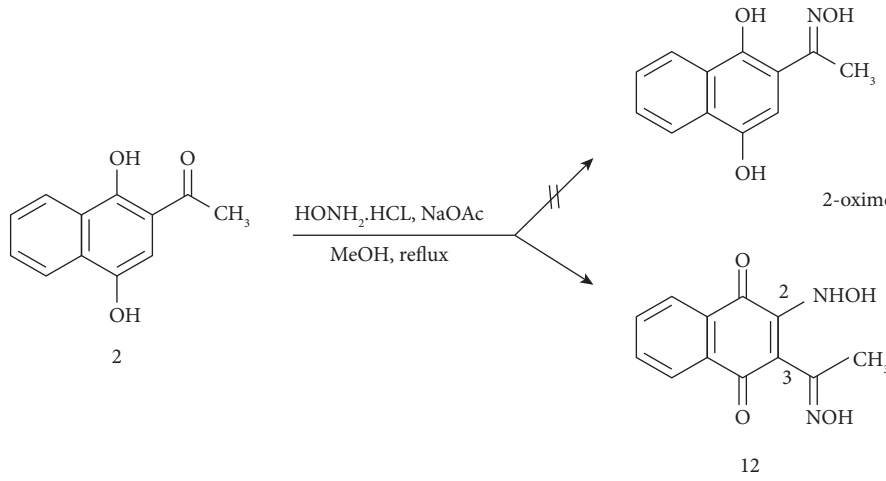
The synthesized 2-acylnaphthohydroquinones **3–11** were reacted with hydroxylamine according to the aforementioned reaction conditions, and the products were isolated by column chromatography over silica gel. The results of the assays are summarized in Scheme 3. The structures of the new compounds **13–19** were fully established by ^{1}H and ^{13}C nuclear magnetic resonance (NMR), bidimensional nuclear magnetic resonance (2D-NMR), and high-resolution mass spectrometry (HRMS).

Interestingly, the results reveal that the reaction of compounds **3–11** with hydroxylamine proceed, as that described for analogue **2**, to yield the respective 2-(hydroxyamino)-3-(hydroxyimino)alkyl-1,4-naphthoquinones **13–18**. Nevertheless, the reaction of compounds **9–11**, with hydroxylamine, holding longer hydrocarbon chains than analogue **8**, takes place in a quite different manner to give, in all the cases, the same product 2-(hydroxyamino)-1,4-naphthoquinone **19**.

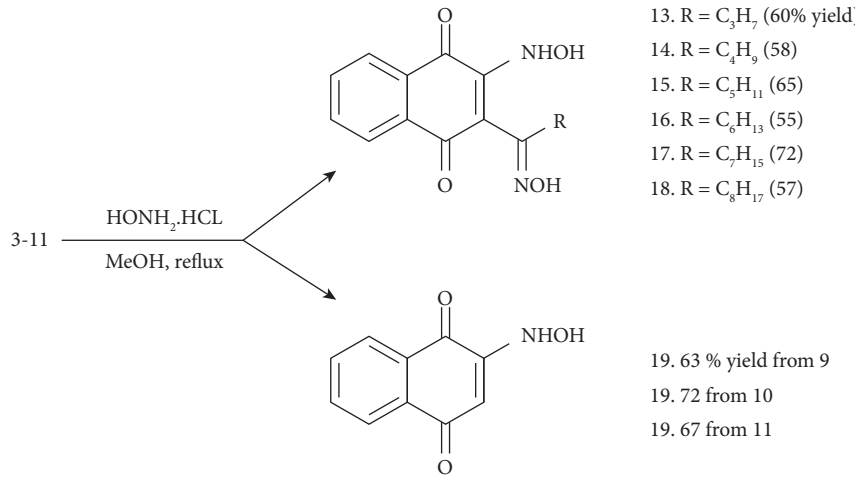
A tentative explanation was proposed for the reaction of compounds **2–8** with hydroxylamine to yield the respective disubstituted 1,4-naphthoquinones **12–18**. Indeed, the course reaction explaining the formation of **12** from **2** is depicted in Scheme 4. To build such a hypothesis, the following points were taken into account: (a)Aerobic oxidation is chemically inert to acylnaphthohydroquinones 2–11. This assumption was confirmed by doing an experiment where compound **2** remained unchanged after boiling it for 20 hours in methanol under aerial conditions. This is likely because the hydroquinone system is strongly stabilized through the internal hydrogen bond between the carbonyl and 2-hydroxy groups [24]. (b) The oxime formation process, involved in the reaction of carbonyl compounds with hydroxylamine, is reversible and occurs via a hemiaminal intermediate (tetrahedral intermediate) [25]. (c) The aerial oxidation of the hemiaminal and/or oxime intermediates prevents the reversal of the equilibria



Scheme 1: Synthesis of 2-acetylnaphthohydroquinones **2**–**11**.



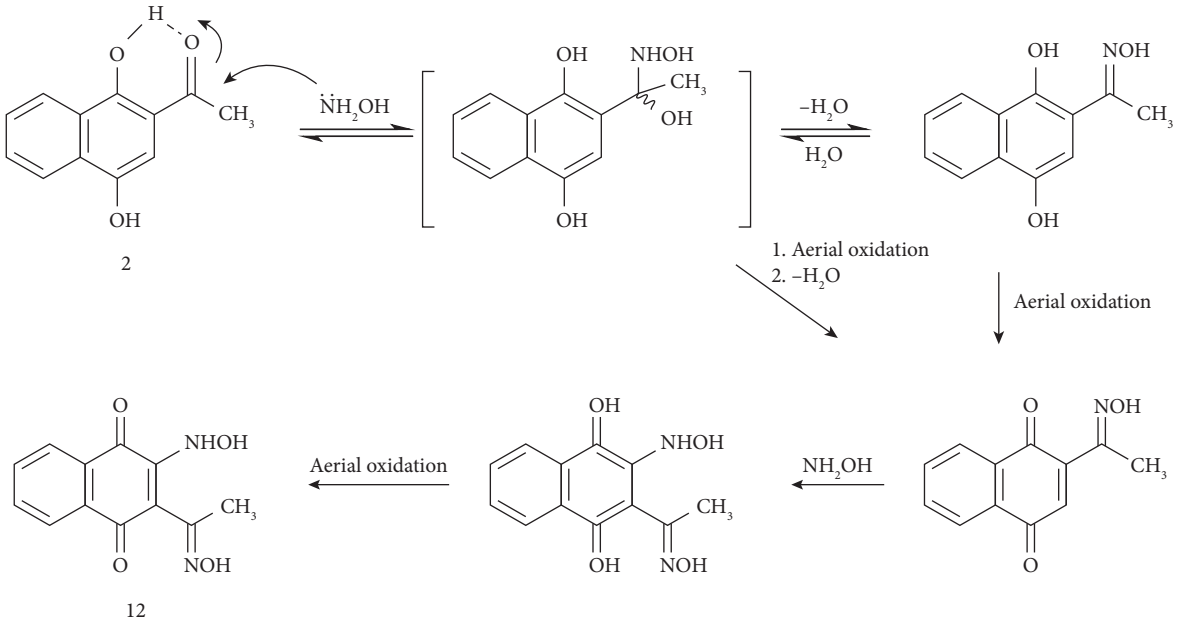
Scheme 2: Reaction of 2-acetylhydroquinone **2** with NH_2OH.



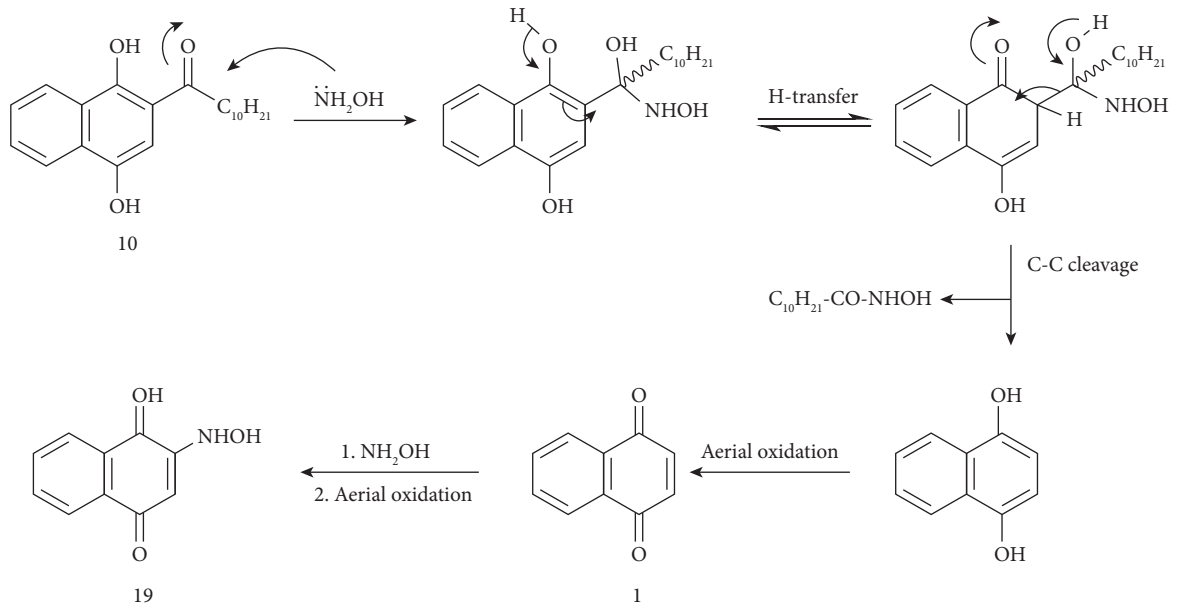
Scheme 3: Reaction of compounds **3**–**11** with hydroxylamine.

process to precursor **2**, thus allowing the further oxidative amination of naphthoquinone intermediates to produce **12**.

Regarding the formation of aminonaphthoquinone **19** from compounds **9**–**11** and hydroxylamine, a tentative course reaction is outlined in Scheme 5, starting from compound **10**. The observed C-C cleavage is likely taking place through a retroaldol-type reaction, releasing the internal energy of the sterically crowded hemiaminal intermediate, giving rise to aminoquinone **19,** along with hydroxamic acid $C_{10}H_{21}CONHOH$. The last step (1–**19**) of this reaction mechanism was suggested to occur



Scheme 4: Plausible course reaction of **2** with NH_2OH to **12**.



Scheme 5: Plausible course reaction of **10** with NH_2OH to **19**.

through an oxidative amination of intermediate naphthoquinone **1** with hydroxylamine. Such an assumption was confirmed through an experiment where naphthoquinone **I** was reacted with hydroxylamine, in methanol at room temperature, to give aminoquinone **19** in 88% yield.

Taking into account that the reaction of compounds **9**–**11** with hydroxylamine yields aminoquinone **19**, resulting from C-C cleavage reactions and the efficient and single access to **19** from naphthoquinone **I** and hydroxylamine**,** no efforts were made in order to get additional pieces of evidence on these degradation reactions.

In light of our results, providing access to a new class of compounds **12**–**19** containing the oxime group and the anticancer aminonaphthoquinone pharmacophore as well [26, 27], the *in vitro* cytotoxic evaluation of quinones was undertaken by using a panel of three human-derived tumor cell lines (Table 1). In order to get a range of selectivity, such cytotoxic activities were compared to those obtained on nontumorigenic HEK-293 (human embryonic kidney cells).

Cells were seeded at a density of 10 000 cells/well for 24 h into 96-well plates and then incubated for 48 h in the absence or presence of compounds. After washing, cells were further incubated with MTT (0.5 mg/mL) for 2 hours at 37°C. Blue

Table 1: *In vitro* cytotoxic activity of **12–19** on DU-145 (prostate), MCF-7 (breast), T-24 (bladder) cancer cell lines, and HEK-293 human embryonic kidney cells (nontumor fibroblasts).

$IC_{50} \pm SEM$ (μM)[a]				
No.	DU-145	MCF-7	T-24	HEK-293
12	24.92 ± 0.03	26.30 ± 0.34	18.47 ± 0.80	32.38 ± 1.65
13	32.37 ± 1.27	48.55 ± 3.25	30.24 ± 0.90	48.57 ± 1.92
14	84.25 ± 1.66	58.60 ± 6.18	35.30 ± 3.40	33.32 ± 3.71
15	65.62 ± 4.50	91.02 ± 3.68	62.45 ± 3.38	81.39 ± 2.18
16	>100	>100	69.52 ± 6.50	>100
17	60.30 ± 3.89	90.01 ± 2.26	86.06 ± 1.19	62.92 ± 0.68
18	>100	>100	70.03 ± 6.57	>100
19	8.08 ± 0.42	13.59 ± 1.03	15.89 ± 3.04	31.53 ± 7.94
D [b]	0.93 ± 0.06	0.33 ± 0.05	0.65 ± 0.07	4.27 ± 0.34

formazan crystals were solubilized by adding 100 μl DMSO/well, and the optical density of colored solutions was subsequently read at 550 nm. Results are expressed as % of MTT reduction compared to untreated control conditions. The IC_{50} values were calculated using the GraphPad Prism software (San Diego, CA, USA). In table, [a]data represent the mean average values ± SEM for three separate experiments. [b]*D*, doxorubicin.

Table 1 shows moderate antiproliferative activities of 2-(hydroxyamino)naphthoquinone **19** and, to a lesser extent, its hydroxyimino-ethylnaphthoquinone analogue **12**. Indeed, **19** was the most active compound in all cancer cell lines, but its effects are lower than those of doxorubicin. It should be underlined that doxorubicin IC_{50} values are in agreement with those recently reported in the literature [28–30]. Regarding the mechanism of action of compound **19**, it is unlike that which is the same as doxorubicin. Indeed, the cytotoxicity of the latter involves miscellaneous ways such as DNA adduct formation, DNA intercalation, inhibition of topoisomerase II, oxidative stress by ROS formation, and lipid peroxidation [31]. Moreover, the comparison of the cytotoxic activities of analogues **12–18** shows that the longer the linear aliphatic chain, the lower their potencies. It is also observed that the enlargement of the aliphatic chain from **12** to **18** somehow reduces the selectivity, given that cytotoxicity was similar in both nontumorous and cancer cells. Last, although the observed anticancer activities of the **12-18** series were rather low, it appears that bladder cancer cells were slightly more sensitive to that series when compared with breast cancer cells.

4. Conclusions

In summary, we report a novel and straightforward route to antiproliferative 2-(hydroxyamino)-3-(hydroxyimino)alkyl-1,4-naphthoquinones **12–18** and 2-(hydroxyamino)-1,4-naphthoquinone **19** from 2-alkanoylnaphthohydroquinones **2–11** and hydroxylamine. The study demonstrates that the access to 2,3-disubstituted 1,4-naphthoquinones depends on the length of the linear alkyl substituent (C_n) of the hydroquinone substrates since over C_8, the reaction of 2-alkanoylnaphthohydroquinones such as **9–11** with hydroxylamine yields 2-(hydroxyamino)-1,4-naphthoquinone **19**. This compound has moderate anticancer activity. Studies on suitable methods to prepare 2-(hydroxyimino)alkyl-naphthohydroquinones are in progress.

Data Availability

The data used to support this study are available from the corresponding author upon request and are included within supplementary materials.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

The NMR spectra of the synthesized compounds are incorporated as supplementary information. . (*Supplementary Materials*)

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