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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Received 24 May 2022; Revised 29 July 2022; Accepted 4 August 2022; Published 23 August 2022

Academic Editor: Liviu Mitu

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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 IC50 value (8.08 μM) and the best selectivity index (3.90).

1. Introduction

2-Acylated-1,4-benzo- and 1,4-naphthoquinones 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-hydroxyacarylarene 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...
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 = CH₃) 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-naphthoquinones III (R = n-alkyl) against hydroxylamine leading to the formation of 2-(hydroxyamino)-1,4-naphthoquinone and C-3 (hydroxylamino) 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. ¹H- and ¹³C NMR spectra were recorded on a Bruker Ultrashield-300 instrument (Bruker, Ettingen, Germany) in DMSO-d₆ 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 ¹H-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 60F₂₅₄ 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-Alkanoylnaphthoquinones 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-Acynaphthoquinones (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) νmax cm⁻¹: 3467, 3416, 1638, 1618; ¹H-NMR (300 MHz, DMSO-d₆): 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, CH₃); ¹³C NMR (75 MHz, DMSO-d₆): 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_{2}O_{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 hydroxyamine (2-equiv.) in 60% yield (161.8 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); ^1H-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}), 1.41 (q, (J = 7.4, 6.9 Hz, 2H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}CH_{2}), 1.18 (m, 6H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 0.78 (t, J = 6.6 Hz, 3H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 13C NMR (75 MHz, DMSO-d_{6}): δ 181.50, 180.69, 175.17, 174.54, 135.05, 133.13, 132.48, 129.99, 125.80, 125.67, 109.14, 31.09, 29.78, 27.58, 25.45, 22.07, 13.97. HRMS (APCI): (M + H)^+ Calcd for C_{17}H_{20}N_{2}O_{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 hydroxyamine (2-equiv.) 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); ^1H-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}), 1.40 (m, 2H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2)-CH_{2}), 1.15 (m, 7H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 0.75 (t, J = 6.8 Hz, 3H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 13C NMR (75 MHz, DMSO-d_{6}) δ 181.66, 180.93, 175.35, 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_{2}O_{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 hydroxyamine (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); ^1H-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}), 1.40 (m, 2H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 1.14 (m, 10H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 0.76 (t, J = 6.5 Hz, 3H, HO-N=C=CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}-CH_{2}), 13C 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, 27.77, 27.80, 25.56, 22.28, 14.14. HRMS (APCI): (M + H)^+ Calcd for C_{19}H_{23}N_{2}O_{4}: 344.17361; found 344.17398.

2.2.8. 2-(Hydroxyamino)-1,4-naphthoquinone 19. Prepared from 10 (328 mg, 0.99 mmol) and hydroxyamine (2-equiv.) 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;
Scheme 2. In this preliminary assay, acetylhydroquinone 2 was reacted with NH₂OH in boiling methanol. The reaction mixture was worked up, and the product displays a singlet signal at 2.09 ppm for the hydroxyl proton of a keto group [23]. The characteristic A₂B₂ pattern 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 (D₂O exchangeable) assignable to the NHOH group and a singlet signal at 2.09 ppm for the proton of a methyl group. The 13C 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 high-resolution mass spectrometry (HRMS), structure 12 was fully established for the new compound isolated in 58% yield.

Interestingly, the results reveal that the reaction of 2 with hydroxylamine gave 12, leading 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)-alkyl-1,4-naphthoquinones. In this context, compounds 3–12 were prepared, just like compound 2, by solar photoacetylation of 1,4-naphthoquinone 1 with C₅H₁₁ linear aliphatic aldehydes according to Scheme 1.

The synthesized 2-acylnaphthoquinones 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 1H and 13C nuclear magnetic resonance (NMR), bidimensional nuclear magnetic resonance (2D-NMR), and high-resolution mass spectrometry (HRMS).

3. Results and Discussion

Since the synthesis of acetylhydroquinone oxime II from hydroquinone 1 (R = CH₃) and hydroxylamine has been reported with high yield [16] and based on standard procedures to prepare acetonaphthene oximes [22], the reactivity of acetylnaphthoquinones III leading to the corresponding oximes IV was first explored. In this context, Scheme 1 shows the synthesis of the required precursor 2-acyl hydroquinone 2 from 1,4-naphthoquinone 1 and acetaldehyde, according to our previously reported procedure based on the solar photoacetylation of Friedel–Crafts reaction [12].

In this preliminary assay, acetylhydroquinone 2 was reacted with NH₂OH in boiling methanol (Scheme 2). Workup of the reaction mixture provided an orange crystalline product, m.p. 141–142°C. The 1H-NMR spectrum displays, at 11.16 ppm, a singlet (D₂O exchangeable) for the hydroxyl proton of a keto group [23]. The characteristic A₂B₂ 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 (D₂O exchangeable) assignable to the NHOH group and a singlet signal at 2.09 ppm for the proton of a methyl group. The 13C 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 high-resolution mass spectrometry (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)-alkyl-1,4-naphthoquinones. In this context, compounds 3–12 were prepared, just like compound 2, by solar photoacetylation of 1,4-naphthoquinone 1 with C₅H₁₁ linear aliphatic aldehydes according to Scheme 1.

The synthesized 2-acylnaphthoquinones 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 1H and 13C 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 acynaphthoquinones 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 (tetravalent intermediate) [25]. (c) The aerial oxidation of the hemiaminal and/or oxime intermediates prevents the reversal of the equilibria
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 C10H21CONHOH. The last step (1–19) of this reaction mechanism was suggested to occur

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\begin{align*}
\text{Scheme 1: Synthesis of 2-acetylnaphthohydroquinones 2–11.}
\end{align*}
\]

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\begin{align*}
\text{Scheme 2: Reaction of 2-acetylhydroquinone 2 with NH}_2\text{OH.}
\end{align*}
\]

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\begin{align*}
\text{Scheme 3: Reaction of compounds 3–11 with hydroxylamine.}
\end{align*}
\]
through an oxidative amination of intermediate naphthoquinone 1 with hydroxylamine. Such an assumption was confirmed through an experiment where naphthoquinone 1 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 1 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
The length of the linear alkyl substituent (Cn) of the hydroquinone substrates since over C8, the reaction of 2-alkanoylnaphthohydroquinones such as 9–11 with hydroxyamine 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.

**Acknowledgments**

The authors thank Dra. Angélica Guerrero (School of Pharmacy, San Sebastian University) for biological evaluation.

**Supplementary Materials**

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

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