Synthesis and Structural Analysis of Some New Sulfanyl Amino 1,4-Naphthoquinone Derivatives

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Abstract: In this study, some new sulfanyl-substituted amino 1,4-naphthoquinone derivatives which possess two electron-donating groups in the amino fragment were synthesized and their structures were analyzed by spectroscopic techniques. First, 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (3a) and 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (3b) were obtained from the reactions of dichloro-1,4-naphthoquinone (1) with 2,4-dimethoxyaniline and 3,5-dimethoxyaniline. In the following step, the compounds 3a,b were reacted with aliphatic nucleophiles; ethyl-, 1-propyl-, and 1-pentyl mercaptan. S-nucleophiles attacked the carbon atom of 1,4-naphthoquinone core and displaced the chloride atom to create target molecules; 2-arylamino-3-(ethylthio)naphthalene-1,4-dione (5a,b), 2-arylamino-3-(propylthio)naphthalene-1,4-dione (5c,d), 2-arylamino-3-(pentylthio)naphthalene-1,4-dione (5e,f) derivatives. The structures of the synthesized compounds were elucidated by utilizing 1D and 2D NMR techniques with additional spectroscopic data (mass and FTIR).

Keywords: Sulfanyl- and/or arylamine-substituted quinones, 1,4-naphthoquinone, spectroscopic analysis, HMQC/HMBC analysis.


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INTRODUCTION

Quinonic structures are quite valuable compounds because of having a wide range of applications. Such compounds allow electronic transition between molecules due to their reduction states. They have three oxidation states; one state is quinone, another state is semiquinone, which is one-electron reduced form of quinone, and the last one is also catechol or hydroquinone which is two-electron reduced form of quinone. These different oxidation states of quinone structures make them very crucial for chemical science (1). For instance, the reduction properties of quinones stand out in the field of energy storage. Quinones give reversible redox reactions at high potential. Although they are not practical because of their insolubility in electrolyte solutions, quinones are able to use as electron-active species in flow battery (2-5).

Quinones have not only some practices in the field of energy storage but also have biological effects such as antitumor, antifungal, antibacterial, and phytotoxic activities. Salinisporamycin and hygrocin A, containing 1,4-napthoquinone core, have antimicrobial and anticancer effects (6, 7). In particularly, since the test results of these napthoquinone derivatives presenting low cytotoxic effects, the interest of synthesis of new napthoquinone compounds has increased.

The amino 1,4-naphthoquinone structure plays a key role for synthesis of many bioactive molecules. It is possible to see amino and thioether derivatives of 1,4-naphthoquinone in the impressive bioactivity studies. They have a variety of pharmacological properties such as antibacterial, antifungal, antiviral, anti-inflammatory, and anti-cancer (1, 8-12). Some sulfanylamino-1,4-napthoquinone derivatives have been evaluated against a full panel of 60 primary human tumor cell lines derived from nine human cancer types. Among the tested compounds, it has been observed that the structure I affects the growth of two colon cancer cells, HCT116 and HCT15, and of two leukemia cells, MOLT-4 and SR. The structure II has also low cytotoxicity against the NCI-H23 cancer line (9) (Figure1)

**Figure 1.** Example structures of sulfanylamino-1,4-napthoquinones (I) and (II).

In previous studies, the effect of the electron-withdrawing groups on the biological activity of molecule has been investigated and the results of these studies have showed that amino- and/or
sulfanyl-derivatives of 1,4-naphthoquinone act as good antibacterial and antifungal agents against different pathogens. The sort of electron-withdrawing group(s) and also their position(s) in the amine ring have an influence upon the activity (8, 10, 13, 14). In this study, some novel sulfanylamino-1,4-naphthoquinone derivatives have been obtained from the nucleophilic substitution reactions of 1,4-napthoquinones possessing an aryl amine substituent which has two electron donating groups, in the 2,4- and 3,5-positions of phenyl ring, with alkyl mercaptans. The products have been purified by column chromatography and characterized by FTIR, 1D/2D NMR and mass spectroscopy.

MATERIALS AND METHODS

All reagents were commercially obtained from commercial suppliers and used without further purification unless otherwise noted. The purity of reaction products was routinely monitored by thin-layer chromatography on analytical thin layer chromatography (TLC), which was purchased from Merck KGaA (silica gel 60 F_{254}) based on Merck DC-plates (aluminum-based). Visualization of the chromatogram was performed by UV light (254 nm). Column chromatographic separations were carried out using silica gel 60 (Merck, 63–200 μm particle size, 60–230 mesh). All NMR spectra were acquired using a Varian UNITY INOVA 500 MHz spectrometer equipped with AutoX PFG probe operating at a proton observation frequency of 499.7 MHz and a carbon observation frequency 125.7 MHz at 25 °C in CDCl₃ as the solvent. ¹H NMR spectra and ¹³C NMR spectra in CDCl₃ referred to the solvent signal center at δ 7.25 and δ 77.0 ppm, respectively. Standard abbreviations indicating multiplicity were used as follows: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), td (triplet of doublets), q (quartet) and m (multiplet). The one-bond J(CH) coupling constant was 146 Hz and the long range J(CH) coupling constant was 8 Hz for 2D spectra. FTIR spectra were recorded using Jasco FT/IR-4700 spectrometer with high resolution 0.4 cm⁻¹ using ATR accessory. Mass spectra were obtained on a Thermo Finnigan LCQ Advantage MAX MS/MS spectrometer equipped with an ESI (Electrospray ionization) sources. Melting points (mp) were determined with a Buchi B-540 melting point apparatus and were uncorrected.

**General Procedure:**

In this study, two types of synthetic procedures were used to obtain substituted 1,4-naphthoquinone derivatives.

**General Procedure for Preparation of the 2-arylamino 1,4-naphthoquinone Derivatives (3a-b)**

2-Chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (3a) and 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (3b) were obtained from the reactions of 2,3-dichloro-1,4-naphthoquinone (1) with 2,4-dimethoxyaniline (2a) and 3,5-dimethoxyphenyl amine (2b) according to previous literature reported (1, 13, 15).
General Procedure for Preparation of the 2-arylamino-3-sulfanyl-1,4-naphthoquinone Derivatives (5a-f)

2-Arylamino-3-chloro-1,4-naphthoquinone derivatives (3a,b) (1 mmol) and the aliphatic thiols (4a-c) (1.5 mmol) in the presence of Et₃N were stirred in CHCl₃ (5 mmol) at room temperature for 6-8 hours. The resulting solution was extracted with 100 mL chloroform then washed with water (3x100 mL) and dried over calcium chloride. The solvent was removed in vacuo. The purification of product was subjected to column chromatography on silica gel using a proper solvent mixture such as chloroform-petroleum ether (2:1 or 3:1, v/v).

2-(2,4-Dimethoxyphenylamino)-3-(ethylthio)naphthalene-1,4-dione 5a was synthesized from 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (3a) and ethanethiol (4a) as a dark purple viscous product by using the general procedure. Yield: 0.088 g, 81%. FTIR (ATR) ν(cm⁻¹): 3312 (-NH), 3060 (CH₆arom.), 2924, 2833 (CH₆aliph.), 1662 (C=O), 1590 (C=C). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.16 dd, J: 7.65, 0.93 Hz, 1H (-CH₆arom.); 8.04 dd, J: 7.65, 0.92 Hz, 1H (-CH₆arom.); 7.77 bs, 1H (-NH); 7.72 td, J: 7.60, 1.40 Hz, 1H (-CH₆arom.); 7.63 td, J: 7.50, 1.34 Hz, 1H (-CH₆arom.); 6.93 d, J: 8.41 Hz, 1H (-CH₆arom.); 6.50 t, J: 2.70 Hz, 1H (-CH₆arom.); 6.48 dd, J: 8.43, 2.63 Hz, 1H (-CH₆arom.); 3.84 s, 3H (-OCH₃); 3.82 s, 3H, (-OCH₃); 2.58 q, J: 7.40 Hz, 2H (-SCH₂); 1.07 t, J: 7.40 Hz, 3H (CH₃). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 180.7, 180.5 (C=O), 158.3, 153.3, 146.9, 134.4, 133.7, 132.4, 130.9, 126.7, 126.4, 124.7, 120.9, 113.9, 103.1, 98.8 (-CH₆arom. and C₆) 55.7, 55.5 (OCH₃), 28.0 (-SCH₂), 14.6 (CH₃). MS (GC-MS): m/z (%) 370.1 (100, [M]+). Anal. Calcd. for C₂₀H₁₉NO₄S (369.43).

2-(3,5-Dimethoxyphenylamino)-3-(ethylthio)naphthalene-1,4-dione 5b was synthesized from 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (3b) and ethanethiol (4a) as a dark red solid product by using the general procedure. Yield: 0.06 g, 56%; mp 136-138 °C. FTIR (ATR) ν(cm⁻¹): 3295 (-NH), 3064 (CH₆arom.), 2834 (CH₆aliph.), 1648 (C=O), 1584 (C=C). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.15 dd, J = 7.81, 0.98 Hz, 1H (-CH₆arom.); 8.08 dd, J = 7.81, 0.98 Hz, 1H (-CH₆arom.); 7.76 bs, 1H (-NH); 7.73 td, J = 7.32, 1.46 Hz, 1H (-CH₆arom.); 7.66 td, J = 7.32, 0.98 Hz, 1H (-CH₆arom.); 6.26 t, J = 1.96 Hz, 1H (-CH₆arom.); 6.17 d, J = 2.44 Hz, 2H (-CH₆arom.); 3.79 s, 6H (-OCH₃); 2.69 q, J = 7.32 Hz, 2H (-SCH₂); 1.08 t, J = 7.32 Hz, 3H (-SCH₂). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 181.1, 180.3 (C=O), 160.7, 144.8, 140.4, 134.5, 133.5, 132.8, 130.6, 126.8, 126.6, 119.2, 100.6, 96.8 (-CH₆arom. and C₆) 55.4 (OCH₃), 28.0 (-SCH₂), 14.6 (CH₃). MS (ESI+) m/z (%): 370.1 (100, [M]+). Anal. Calcd. for C₂₂H₁₉NO₄S (369.43).

2-(2,4-Dimethoxyphenylamino)-3-(propylthio)naphthalene-1,4-dione 5c was synthesized from 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (3a) and propane-1-thiol (4b) as a dark purple viscous product by using the general procedure. Yield: 0.097 g, 87%. FTIR (ATR) ν(cm⁻¹): 3311 (-NH), 2929, 2831 (CH₆aliph.), 1663 (C=O), 1590 (C=C). ¹H NMR (500 MHz,
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2-(3,5-Dimethoxyphenylamino)-3-(propylthio)naphthalene-1,4-dione 5d was synthesized from 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (3b) and propan-1-thiol (4b) as a dark red-colored solid product by using the general procedure. Yield: 0.088 g, 79%; mp 73-75 °C. FTIR (ATR) ν(cm⁻¹): 3293 (-NH), 2961, 2920 (CH₃, CH₂), 1666, 1633 (C=O), 1591, 1552 (C=C). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.15 dd, J: 7.62, 1.0Hz, 1H (-CH₃); 8.08 dd, J: 7.59, 1.0Hz, 1H (-CH₃); 7.77 bs, 1H (-NH); 7.73 td, J: 7.55, 1.40Hz, 1H (-CH₃); 7.66 td, J: 7.52, 1.34Hz, 1H (-CH₃); 6.27 t, J: 2.17 Hz, 1H (-CH₃); 6.18 d, J: 2.09 Hz, 2H (-CH₃); 3.79 s, 6H (-OCH₃); 2.65 t, J: 7.40 Hz, 2H (-SCH₂); 1.44 q, J: 7.34 Hz, 2H(-CH₂); 0.86 t, J: 7.35 Hz, 3H (-CH₃). ¹³C NMR (125 MHz, CDCl₃) δ(ppm): 181.1, 180.3 (C=O), 160.7, 144.9, 140.5, 134.5, 133.5, 132.8, 130.6, 126.8, 126.6, 119.4, 100.7, 96.8 (-CH₃, and C₆), 55.4 (OCH₃), 35.7 (-SCH₂), 23.0 (-CH₂), 13.3 (CH₃). MS (ESI+) m/z (%): 384.1 (100, [M]+). Anal. Calcd. for C₂₁H₂₃NO₄S (383.46).

2-(2,4-Dimethoxyphenylamino)-3-(pentylthio)naphthalene-1,4-dione 5e was synthesized from 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (3a) and pentan-1-thiol (4c) as a dark purple-colored viscous product by using the general procedure. Yield: 0.08 g, 67%. FTIR (ATR) ν(cm⁻¹): 3323 (-NH), 2924, 2844 (CH₃, CH₂), 1664 (C=O), 1590, 1547 (C=C). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.15 dd, J: 7.81, 1.47Hz, 1H (-CH₃); 8.04 dd, J: 7.81, 1.47Hz, 1H (-CH₃); 7.76 bs, 1H (-NH); 7.71 td, J: 7.32, 1.46Hz, 1H (-CH₃); 7.63 td, J: 7.32, 1.46Hz, 1H (-CH₃); 6.92 d, J: 8.3 Hz, 1H (-CH₃); 6.48 dd, J: 7.56, 2.68Hz, 1H (-CH₃); 6.48 dd, J: 16.10, 2.68Hz, 1H (-CH₃); 3.83 s, 3H (-OCH₃); 3.82 s,3H (-OCH₃); 2.52 t, J: 7.32 Hz, 2H (-SCH₂); 1.41 -1.34 m, 2H (-CH₂); 1.23 -1.18 m, 4H(-CH₂-); 0.83 -0.80 m, 3H(-CH₃). ¹³C NMR (125 MHz, CDCl₃) δ(ppm): 180.7, 180.6 (C=O), 158.2, 153.2, 146.5, 134.3, 133.7, 132.3, 130.8, 126.7, 126.4, 124.6, 120.7, 114.4, 103.0, 98.8 (-CH₃, and C₆), 55.6, 55.5 (OCH₃), 33.7 (-SCH₂), 30.9, 29.1, 22.2 (-CH₂), 13.9 (CH₃). MS (ESI+) m/z (%): 412.2 (100, [M]+). Anal. Calcd. for C₂₃H₂₅NO₄S (411.51).

2-(3,5-Dimethoxyphenylamino)-3-(pentylthio)naphthalene-1,4-dione 5f was synthesized from 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (3b) and propan-1-thiol (4c) as a dark red solid product by using the general procedure. Yield: 0.07 g, 58%; mp 125-127
RESULTS AND DISCUSSION

The general pathway of the synthesis of 2-arylamino-3-sulfanyl-1,4-naphthoquinones (5a–f) was summarized in Scheme 1. The first step was to obtain the starting compounds, 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (3a) and 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (3b), by the nucleophilic substitution of 2,3-dichloro-1,4-naphthoquinone (1) reported in the literature (1, 13, 15). In the next step, new sulfanyl-substituted 1,4-naphthoquinones (5a–f) containing arylamino substituent with two methoxy groups were obtained and characterized by some spectroscopic methods.

Scheme 1. The synthesized 2-arylamino-3-sulfanyl-1,4-naphthoquinone derivatives.
The reactions of compounds 3a, b with 1-ethyl-, 1-propyl- and 1-pentyl mercaptan took place via nucleophilic substitution. It is known that the addition of a base into the reaction medium reduced the reaction time and also increased the yields of products.

Carbonyl groups are observed at about 180-181 ppm as two peaks which prove being two different substituents in the $^{13}$C NMR spectra. The methoxy groups are in the 2,4- or 3,5-positions of the amine ring. Although some compounds (5a, 5c, 5e) which have two methoxy groups in the 2,4- position are at about 55.6 and 55.5 ppm as two bifurcate peaks, other compounds (5b, 5d, 5f) having two methoxy groups in the 3,5- position of amine ring are at 55.4 ppm as only one bifurcate peak. It can be inferred that their distances to amine group (-NH) have an effect on the NMR shifts. In the $^1$H NMR spectra of compounds 5a, 5c, 5e, methoxy groups are at between 3.84-3.81 ppm as two singlet peaks even though they are seen at about 3.79 ppm as only one singlet peak in the $^1$H NMR spectra of compounds (5b, 5d, 5f).

**Figure 1.** Expanded one-bond $^1$H-$^{13}$C couplings(δC/δH 10–140/0.5–8.5 ppm): HSQC of 5e
Figure 2. Expanded long-range $^1$H-$^{13}$C couplings($\delta$C/$\delta$H 95–165/6.3–8.2 ppm): HMBC spectrum of 5e

To better understand the structure of compounds, we should look at the 2D $^1$H-$^{13}$C correlation NMR spectra. The HSQC (heteronuclear single quantum correlation) of 5e spectra presented in the Figure 1 shows the region $\delta$C/$\delta$H 10–140 / 0.5–8.5 ppm. The aliphatic protons labeled a, b, c and d are linked to aliphatic carbons labeled 1, 2, 4, 3, and 5, respectively. The methyl carbon attached to the oxygen atom signals at $\delta$C 55.5 ppm (6) and $\delta$C 55.6 ppm (7) which have two bifurcate peaks correlate to e ($\delta$H 3.82 ppm) and f ($\delta$H 3.83 ppm) proton signals. In addition, it is clearly picked out that the aromatic carbons 8 ($\delta$C 98.8 ppm), 9 ($\delta$C 103.0 ppm), 12 ($\delta$C 124.6 ppm), 13 ($\delta$C 126.4 ppm), 14 ($\delta$C 126.7 ppm), 16 ($\delta$C 132.3 ppm), 18 ($\delta$C 134.3 ppm) are bound to proton h ($\delta$H 6.48 ppm), g ($\delta$H 6.47 ppm), i ($\delta$H 6.92 ppm), m ($\delta$H 8.04 ppm), n ($\delta$H 8.15 ppm), j ($\delta$H 7.63 ppm) and k ($\delta$H 7.71 ppm) respectively. In Figure 2, expanded long-range heteronuclear coupling spectrum of 5e is seen. In the HMBC (Heteronuclear Multiple Bond Correlation) spectra, proton signal g ($\delta$H 6.47 ppm) correlates to carbon signals 8 ($\delta$C 98.8 ppm), 9 ($\delta$C 103.0 ppm), and 21 ($\delta$C 158.2 ppm) while proton signal h ($\delta$H 6.48 ppm) correlates to carbon signals 9 ($\delta$C 103.0 ppm), 11 ($\delta$C 120.7 ppm), 20 ($\delta$C 153.2 ppm) and 21 ($\delta$C 158.2 ppm). The methyl protons attached to the oxygen atom e ($\delta$H 3.82 ppm) and f ($\delta$H 3.83 ppm) also correlate to carbon signals 20 ($\delta$C 153.2 ppm) and 21 ($\delta$C 158.2 ppm) which means protons g and h are adjacent to each other in the phenyl ring. Proton i ($\delta$H 6.92 ppm) correlates to the carbon 8 ($\delta$C 98.8 ppm), 11 ($\delta$C 120.7 ppm), 20 ($\delta$C 153.2 ppm) and 21 ($\delta$C 158.2 ppm) because of belonging to the same ring.
In Figure 3, the differences between the methoxy signals of compound 5e and 5b can be observed. Methoxy groups of compound 5e and 5b are in the 2,4-position and 3,5-position of the phenyl ring in order; therefore, there are two cross peaks in the Figure 3 (a) and there is only one cross peak in the Figure 3 (b).

The mass spectra of the new synthesized compounds shows the molecular ion peak as expected.

**CONCLUSION**

In this study, the first step was to synthesize the known amino-substituted 1,4-naphthoquinones (3a,b) from the reactions of 2,3-dichloro 1,4-naphthoquinone (1) with 2,4-dimethoxyphenyl amine and 3,5-dimethoxyphenyl amine as described in the literature (1, 13, 15). After that, these compounds were used as starting materials to obtain novel sulfanyl-substituted amino 1,4-naphthoquinone derivatives (5a-f). The newly synthesized compounds were acquired in good yields. The structures of these new naphthoquinone derivatives (5a-f) were elucidated by one- and two-dimensional NMR techniques in which the differences of positions of methoxy groups on the phenyl ring were detected. In addition, mass spectroscopy and FTIR data helped to specify the structures of new compounds.

The amino and thioether derivatives of naphthoquinones have a wide range of biological activities. Therefore, it is predicted that these novel derivatives of 1,4-naphthoquinone (5a-f) will most probably play a significant role in many biological applications.

**REFERENCES**


