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Regioselective synthesis of novel 5-nitro-naphthoquinone derivatives: Electrochemistry and *in-situ* spectroelectrochemistry properties

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ABSTRACT

The novel *N-,O*-substituted-5-Nitro-1,4-naphthoquinones (NQ) as regioisomers were synthesized by reactions of 2,3-dichloro-5-nitro-1,4-naphthoquinone with some heterocyclic rings which were substituted with various nucleophiles according to a Michael 1,4-addition mechanism. All synthesized compounds were characterized by elemental analysis, electrospray ionization mass spectrometry (ESI-MS), Fourier transform infrared spectroscopy (FT-IR), ^{1}H-nuclear magnetic resonance (^{1}H NMR) and attached proton test nuclear magnetic resonance (APT-NMR). Two-dimensional techniques ^{1}H–^{1}H correlated spectroscopy (COSY) was used for characterization of compound **1a**. Cyclic and square wave voltammetric and *in situ* UV–Vis spectroelectrochemical characterizations of NQ derivatives were carried out to determine redox mechanism of these molecules. Although in-situ FT-IR spectroelectrochemical studies of these type compounds were frequently reported in the literature, this is the first study for the in-situ UV–Vis spectroelectrochemical studies of NQ compounds in the literature. All NQs illustrated two NQ based and one nitro-based reduction reactions. While NQ based reduction couples were electrochemically and chemically reversible, observation of nitro reduction at more negative potentials made all processes irreversible. Altering the substituents of the NQ derivatives slightly influenced the redox potentials and chemical reversibility of the processes. Isomers of different NQ derivatives almost showed similar voltametric responses. Distinct spectral and color changes were observed during the redox reactions which indicated possible usages of these molecules in display technologies.

1. Introduction

The several natural and synthetic heterocyclic naphthoquinones have important biological activities such as antitumoral, anti-protozoan, antibiotics, anticancer, antiproliferative [1–5]. In cancer chemotherapy, they are considered the second more important group [6]. After an initial bio-reduction step, their mode of action normally involves the generation of active oxygen species by redox cycling [6], intercalation in the DNA double helix [7] or alkylation of biomolecules [8]. As the bio-reduction of quinones is influenced by their redox properties, the understanding of how structural features of the quinones are related to these properties is an important step to comprehend their mechanism of action and predict modifications to improve their biological activity. Quinone-hydroquinone couples are the prototypical examples of organic redox systems and the research on the electrochemical behavior of these compounds has been actively pursued for many decades starting from the beginning of the twentieth century [9]. The electrochemical behavior associated with electron–proton transfer equilibrium and kinetics provides information on molecular structure and the environment of the basic process [10]. To find out their mode of action, evaluation of reaction mechanisms and determination of physicochemical parameters, studies on the reduction of these molecules under different conditions were carried out. Besides chemical aspects, quinones play important roles in the biochemistry of living cells [11].

The nitro group associated with the aromatic ring in the quinone system is known to increase the biological activity of naphthoquinone due to its electron-withdrawing properties. It has been reported that the 2,3-dichloro-5-nitro-1,4-naphthoquinone derivative is more active towards amines and the reaction provides a mixture of two regioisomers and the studies on nitro-naphthoquinone derivatives are rare in the literature. C. Blackburn (2005) has treated 2,3-dichloro-5-nitro-1,4-naphthoquinone with linked resin amine to give very colorful products at high yield [12]. The resin under some reducing process and reacted with 2,3-dichloro-5-nitro-1,4-naphthoquinone in presence of

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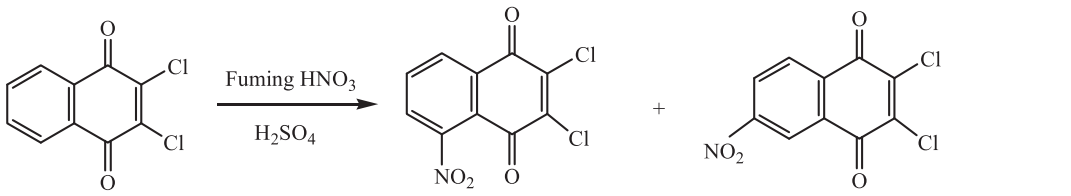


(1) 5-Nitro-2,3-dichloro-1,4-naphthoquinone **(1a)** 6-Nitro-2,3-dichloro-1,4-naphthoquinone **(1b)**

Scheme 1. The synthesis of mixture of the regioisomers **1a** and **1b** [26–27].

2,6-di-*tert*-butylpyridine to give the red resin-quinone. Treatment with trifluoracetic acid led to the rapid formation of regioisomeric mixtures of nitroquinones. In the ^{1}H NMR, the proton signal of naphthoquinone ring showed the first isomer are shifted more downfield than the naphthoquinone peaks of the second isomer [12].

Inspired by their electrochemical functionality in bio-electrochemical applications as redox acceptors, naphthoquinones (NQ) have chosen as found usability in many fields, such as corrosion inhibitor, antimicrobials, and sensors [13–16]. The functionality of NQ derivatives are particularly resulted from the multiple redox states accompanied by reversible reduced states (i.e. semiquinone radical anion ($SNQ^{\cdot-}$) and dianion (NQ^{2-}) [17–19]. The research on naphtaquinone-based compounds greatly has been concentrated on the control and monitoring of the redox activity which can be triggered with the suitable electron withdrawing and/or electron releasing substituents [20–24]. For this purpose there are many studies on the naphthoquinone derivatives [19]. For instance, E. Leyva et al. reported synthesis and characterizations of novel 2-(fluoroanilino)-1,4-naphthoquinones, which illustrated two reversible waves between −1.10 and −1.50 V vs. Fc/Fc^+ redox couple in acetonitrile. They indicated that he substitution of aniline and fluoroaniline increased electron density on the quinone structure and caused to negative shift of the redox processes [19]. In another study, Y. Hui and his coworkers reported electrochemical responses of phylloquinone (Vitamin K1) and they clearly indicated the significant influence of solvents and water content in the electrolyte to the redox mechanism of tis molecule [25]. In our previous study, we reported two well resolved reduction couples and influence of different substituents for many 1,4-naphthoquinone (NQ) derivatives [17]. The studies in the literature indicated that, the electron withdrawing and/or releasing nature of the substituents on the quinone derivatives manipulated their redox properties either facilitating or interfering with the charge transfer from the substituent to the quinone [19,25]. In continuation of our previous work on various NQ derivatives, here, we have carried out the synthesis, spectral, electrochemical and spectroelectrochemical characterization of novel NQ derivatives. Here we have proposed to increase redox richness of NQ derivatives by substituting with redox active nitro moieties and to tailor the redox mechanism with various electron releasing or withdrawing substituents.

In this study, new regioisomeric compounds of 5-nitro-1,4-naphthoquinone were synthesized by the reactions of 2,3-dichloro-5-nitro-1,4-naphthoquinone **1a** with some heterocyclic ring substituted nucleophiles such as amines, piperazines or morpholines according to a Michael 1,4-addition mechanism. Their structures were characterized by using Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR) and two-dimensional techniques (^{1}H–^{1}H correlated spectroscopy (COSY)), attached proton test nuclear magnetic resonance (APT-NMR), mass spectrometry (MS) and elemental analyses. The couple regioisomers (compounds **3**–**4**, **12**–**13** and **15**–**16**) were separated by column chromatography by using a different ratio of solvents. The obtained regioisomers have different color, melting point, and retention factor (R_f). Secondly, cyclic and square wave voltammetric and *in-situ* UV–Vis spectroelectrochemical characterizations of novel 5-nitro-1,4-naphthoquinone (NQ) **1a** and novel NQ derivatives (**3, 4, 6, 8, 10, 12, 13, 15, 16, 17**) were carried out in order to determine redox mechanism of these molecules. In-situ UV–Vis spectroelectrochemical studies were carried out to perform assignments of the redox reactions and to determine the spectra of the electrogenerated species of the compounds. Although in-situ FT-IR spectroelectrochemical studies of these type compounds were frequently reported in the literature, this is the first study for the in-situ UV–Vis spectroelectrochemical studies of 5-Nitro-*N,O*-Subtituted-1,4-naphthoquinone compounds in the literature.

2. Materials and methods

2.1. Apparatus

Melting points were measured on a Büchi B-540 melting point apparatus. FTIR spectra (cm^{-1}) were recorded as KBr pellets in nujol mulls on a Shimadzu IR Prestige 21 model Diamond spectrometer (ATR method). ^{1}H NMR and APT NMR spectrums were obtained using a Varian Unity Inova (500 MHz) spectrometer by using TMS as the internal standard and deuterated chloroform as solvent. Mass spectra were obtained on a Thermo Finnigan LCQ Advantage MAX LC/MS/MS spectrometer according to ESI probe. Elemental analyses were performed with a Thermo Finnigan Flash EA 1112 elemental analyzer. Products were isolated by column chromatography on silica gel (Fluka Silica gel 60, particle size 63–200 μm). Kieselgel 60F-254 plates (Merck) were used for thin layer chromatography (TLC). All chemicals were of reagent grade and were used without further purification. Moisture was excluded from the glass apparatus with $CaCl_2$ drying tubes. Solvents, unless otherwise specified, were of reagent grade and distilled once prior to use.

2.2. Synthetic procedures

General synthesis procedure for 2,3-dichloro-5-nitro-1,4-naphthoquinone (1a) [26–27].

A mixture of 2,3-dichloro-1,4-naphthoquinone (**1**) and sulphuric acid was prepared by mixing 50.0 g of **1** with 65.0 mL of 98% H_2SO_4, then 130.0 mL of fuming HNO_3 added slowly with stirring. The temperature is increased after adding 31.0 mL of HNO_3, in this case, it was necessary to cooling the reaction and keep the temperature below 100 °C, (because at temperature above of 120 °C, the reaction can be decomposed). The remining of nitric acid was added drop-wise with keeping the temperature between (80–90 °C) for about 7 h and then the solution was transferred into ice when the yellow product precipitated. The precipitate was filtered and washed several times by water then stirred with 1 N Na_2CO_3 solution about 8 h. The solid formed was precipitated and washed several times by water, then dried and recrystallized with $CHCl_3$. At this stage the yellow product is a mixture of the isomer in which nitro group is substituted at position 5 (**1a**) and 6 (**1b**). The reaction showing the isomer formation of 5/6 nitro naphthoquinone from 2,3-dichloro-1,4-naphthoquinone **1** (Scheme 1).

Table 1
The some physical properties of the regoisomer couples (**3–4**, **12–13**, **15–16**).

Comp.	Yield (g / %)	Melting point (°C)	Color	Retention factor (R_f)
3	0.122 / 24	183–184	Red solid	0.37 (EtAc /Hexane) (1:3 v/v)
4	0.111 / 22	Oil	Pink oil	0.27 (EtAc/Hexane) (1:3 v/v)
12	0.350 / 46	148–149	Purple solid	0.61 (EtAc/PET) (1:2 v/v)
13	0.211 / 28	178–180	Red solid	0.45 (EtAc/PET) (1:2 v/v)
15	0.160 / 25	146–147	Pink solid	0.50 (EtAc/PET) (1:4 v/v)
16	0.110 / 17	155–156	Pink solid	0.40 (EtAc/PET) (1:4 v/v)

These regioisomers (**1a-b**) were separated by chromatographic column using Hexane/EtAc (6:1 v/v). Some physical properties and characterization methods have been described before [28–30]. A complete and unambiguous assignment of ^{1}H shifts was based in a combination of one- and two-dimensional techniques (^{1}H and ^{1}H–^{1}H correlated spectroscopy (COSY)). The two-dimensional technique ^{1}H–^{1}H correlated spectroscopy (COSY) was used for characterization of isomer formation **1a**.

General synthesis procedure 1 for regioisomeric compounds (3, 4, 6, 8, 10, 12, 13).

2,3-Dichloro-5-nitro-1,4-naphthoquinone (**1a**) and various amine nucleophiles (**2, 5, 7, 9, 11**) were stirred in 25.0 mL of absolute ethanol for 4 h at room temperature. The reaction mixture was monitoring by (TLC) to examine the end of reaction. 30.0 mL of chloroform was added to the reaction mixture. The organic layer was washed with water (3 × 30 mL), and dried with Na_2SO_4. Rotary evaporator system was used to remove the extra amount of solvent, then purified by using column chromatography.

General synthesis procedure 2 for regioisomeric compounds (15, 16, 17).

2,3-Dichloro-5-nitro-1,4-naphthoquinone (**1a**) and various amine nucleophile (**14**) were stirred in 25.0 mL of absolute ethanol with existence of Na_2CO_3 (0.20 g) for 8 h at room temperature. The reaction mixture was monitoring by (TLC) to examine the end of reaction. 30 mL of chloroform was added to the reaction mixture. The organic layer was washed with water (3 × 30 mL), and dried with Na_2SO_4. Evaporator system was used to remove the extra amount of solvent, then purified by using column chromatography.

2-(4-Benzhydrylpiperazine-1-yl)-3-chloro-5-nitronaphthalene-1,4-dion (**3**) *and 3-(4-Benzhydrylpiperazin-1-yl)-2-chloro-5-nitronaphthalene-1,4-dione* (**4**).

According to general procedure 1; 0.30 g (1.0 mmol) of the isolated isomer **1a** was reacted with 0.28 g (1 mmol) of 1-(diphenylmethyl) piperazine **2** in absolute ethanol at room temperature for 4 h.

Compound 3: Physical properties: (see Table 1); FT-IR (cm^{-1}): v = 3085 (C-H_{arom}), 2978, 2906, 2803, 2756 (C-H_{aliph}), 1679, 1644 (C = O), 1589, 1558, 1524 (C = C), 1446, 1285 (C-NO_2). ^{1}H NMR (ppm): δ = 2.64 (br, s, 4H, H_{piper}), 3.62 (br, s, 4H, H_{piper}), 4.34 (s, 1H, –CH <), 7.20–7.77 (m, 10*H*, H_{arom}) 7.80–7.82 (m,1H, H_{naphth}), 8.32 (t, *J* = 7.8 Hz, 1H, H_{naphth}), 8.39 (dd, *J* = 7.2, 1.8 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (ppm): δ = 45.8, 52.6, 56.1 (N-CH_2)$_{piper}$, 76.8 (–CH <), 110.0 (=C-Cl), 126.1, 127.1, 128.0, 128.8, 129.4, 130.0 (CH_{arom}, C_{arom}), 148.4 (=C-N), 175.7, 179.1 (C = O). $C_{27}H_{22}N_3O_4Cl$ (Mw = 487.94 g/mol). MS (+ESI): *m/z* = 487.9 $[M]^+$. Calcd., %: C 66.46; H 4.54; N 8.61. Found, %: C 66.31; H 4.49; N 9.12.

Compound 4: Physical properties: (see Table 1); FT-IR (cm^{-1}): v = 3084 (C-H_{arom}), 2979, 2905 (C–H), 1653, (C = O), 1590, 1532 (C = C), 1536, 1377(C-NO_2). ^{1}H NMR (ppm): δ = 2.56 (br, s, 4H, H_{piper}), 3.56 (br, s, 4H, H_{piper}), 4.32 (1H, s, –CH <), 7.11–7.67 (m, 10*H*, H_{arom}) 7.76–7.80 (m, 1H, H_{naphth}), 7.85 (t, 1H, *J* = 7.3, 1.2 Hz, H_{naphth}) 8.24 (dd, *J* = 7.2, 1.8 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (ppm): δ = 52.6, 56.2 (N-CH_2)$_{piper}$, 77.3 (–CH <), 110.2 (=C-Cl), 128.0, 129.0, 129.5, 130.1, 133.1 (CH_{arom}, Carom), 148.0 (=C-N), 179.6, 180.1 (C = O). $C_{27}H_{22}N_3O_4Cl$ (Mw = 487.94 g/mol). MS (+ESI): *m/z* = 487.9 $[M]^+$. Calcd., %: C 66.46; H 4.54; N 8.61. Found, %: C 66.41; H 4.44; N 8.72.

2-Chloro-3-(4-(2-((3-chloro-5-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)ethyl) piperazin-1-yl)-5-nitronaphthalene-1,4-dione (**6**).

According to general procedure 1, 0.54 g (2.0 mmol) of **1a** was reacted with 0.12 g (1.0 mmol) of 2-(1-piperazinyl)ethylamine **5** in absolute ethanol for 4 h.

Compound 6: Pink solid, yield: 0.122 g (40%), M.p.:143–144 °C; R_f: 0.57 (EtAc/Hexane) (1:3 v/v). FT-IR (cm^{-1}): v = 3258 (N–H), 3076 (C-H_{arom}), 2964, 2922 (C-H_{aliph}), 1676 (C = O), 1590, (C = C), 1534, 1447 (C-NO_2). ^{1}H NMR (ppm): δ = 2.64 (m, 2H, CH_2-N), 3.32 (2H, CH_2-NH) 3.50 (br, s, 4H, H_{piper}), 3.81 (br, s, 4H, H_{piper}), 7.50 (br, s, 1H, NH), 7.99 (dd, *J* = 7.1, 4.9 Hz, 1H, H_{naphth}), 8.01–8.03 (m, 1H, H_{naphth}), 8.05–8.08 (m, 1H, H_{naphth}), 8.11 (dd, *J* = 7.9, 1.1 Hz, 1H, H_{naphth}), 8.19–8.23(m, 2H, H_{naphth}). ^{13}C(APT) NMR (ppm): δ = 40.7 (CH_2-NH), 53.7, 55.2 (N-CH_2)$_{piper}$, 59.8 (CH_2-N), 98.4, 99.6 (=C-Cl), 127.2, 128.8, 129.4, 130.8, 132.6, 134.5 (CH_{arom}, C_{arom}), 148.5 (=C-N), 175.8, 179.0, 190.3 (C = O). $C_{26}H_{19}N_5O_8Cl_2$ (Mw = 600.36 g/mol). MS (+ESI): *m/z* = 600.0 $[M]^+$. Calcd., %: C 52.02; H 3.19; N 11.67. Found, %: C 52.14; H 3.36; N 11.72.

3. 3-Chloro-2-(mesitylamino)-5-nitronaphthalene-1,4-dione (8)

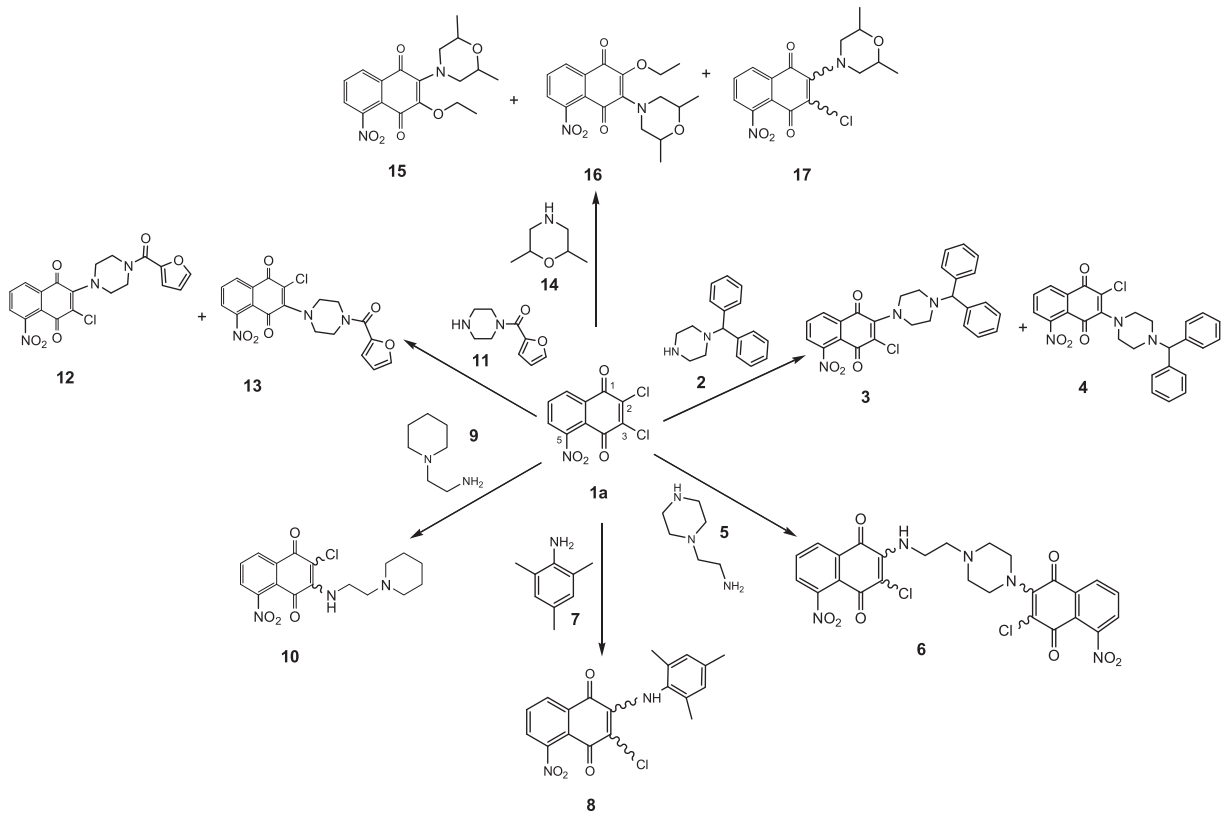
According to general procedure 2; 0.50 g (1.80 mmol) of (**1a**) was reacted with of 2,4,6-trimethylaniline **7** (0.26 g, 1.9 mmol) in absolute ethanol in the presence of Na_2CO_3 for 4 h.

Compound 8: Red solid, yield: 0.298 g (44%); M.p.:171–172 °C; R_f: 0.60 (EtAc/PET) (1:4 v/v). FT-IR (cm^{-1}): v = 3272 (N–H), 3091 (C-H_{arom}), 2982, 2904 (C–H), 1676, 1658 (C = O), 1588 (C = C), 1535, 1365 (C-NO_2). ^{1}H NMR (ppm): δ = 1.31–1.37 (m, 6H, CH_3), 2.04–2.12 (m, 3H, CH_3), 6.85 (s, 2H, H_{arom}) 5.20 (s, 1H, NH), 7.71–7.79 (m, 1H, H_{naphth}), 7.85–7.92 (m, 1H, H_{naphth}), 8.31–8.35 (m, 1H, H_{naphth}). ^{13}C (APT) NMR (ppm): δ = 15.9, 16.5 (CH_3), 111.9 (=C-Cl), 123.2, 126.7, 127.2, 129.0, 133.5, 134.9, 135.2 (CH_{arom}, C_{arom}), 148.6 (C-NO_2), 156.9 (=C-N), 176.4, 177.7 (C = O). $C_{19}H_{15}ClN_2O_4$ (Mw = 370.79 g/mol). MS (+ESI): *m/z* = 369.1 $[M-2H]^+$. Calcd., %: C 61.55; H 4.08; N 7.56. Found, %: C 61.72; H 4.34; N 7.77.

4. 2-Chloro-5-nitro-3-((2-(piperidin-1-yl)ethyl)amino) naphthalene-1,4-dione (10)

According to general procedure 1, 0.50 g (1.80 mmol) of **1a** was mixed with 0.24 g (1.80 mmol) of 2-(piperidin-1-yl)ethan-1-amine **9** in (25 mL) absolute ethanol about 4 h.

Compound **10:** Red solid, yield: 0.200 g (33%); M.p:184–185 °C; R_f: 0.28 (EtAc/Hexane) (1:2 v/v). FT-IR (cm^{-1}): v = 3277 (N–H), 3115 (C-H_{arom}), 2929, 2851 (C–H), 1683 (C = O), 1591, 1556 (C = C), 1520, 1336 (C-NO_2). ^{1}H NMR (ppm): δ = 1.45–1.60 (br, s, 2H, (CH_2)$_{piper}$), 1.62–1.81 (br, s, 4H, [CH_2-CH_2-CH_2)$_{piper}$], 2.52 [br, s, 4H, (CH_2-N-CH_2)$_{piper}$], 2.68 (br, s, 2H, CH_2-N), 3.88 (br, s, 2H, HN-CH_2), 7.16 (br, s, 1H, N–H), 7.69 (dd, *J* = 7.8, 1.2 Hz, 1H, H_{naphth}), 7.81 (t, *J* = 7.8 Hz, 1H, H_{naphth}), 8.34 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (ppm): δ = 23.9 (C-CH_2-C)$_{piper}$, 25.4 (C-CH_2-C)$_{piper}$, 41.0 (N-CH_2), 54.0 (CH_2-N-CH_2)$_{piper}$, 56.9 (HN-CH_2), 126.1, 129.0, 129.5, 133.5, 135.1 (C_{arom}, CH_{arom}), 148.4 (=C-N) 177.6 (C = O). $C_{17}H_{18}ClN_3O_4$ (Mw = 363.80 g/mol). MS (+ESI): *m/z* = 364.2 [M] +. Calcd., %: C 56.13; H 4.99; N 11.55. Found, %: C 56.41; H 4.74; N 11.72.



Scheme 2. The synthesis of *N-,O*-Substituted-5-Nitro-1,4-Naphthoquinones (**3, 4, 6, 8, 10, 12, 13, 15, 16, 17**).

5. 3-Chloro-2-(4-(furan-2-carbonyl)piperazin-1-yl)-5-nitronaphthalene-1,4-dione (12) and 2-Chloro-3-(4-(furan-2-carbonyl)piperazin-1-yl)-5-nitronaphthalene-1,4-dione (13):

According general procedure 1, 0.50 g (1.80 mmol) of **1a** was mixed with 0.33 g (1.80 mmol) of 1-(2-furoyl)piperazine **11** in (25 mL) absolute ethanol about 4 h.

Compound 12: Physical properties: (see Table 1); FT-IR (cm^{-1}): v = 3141, 3101 (C-H_{arom}), 2927, 2855 (C–H), 1724, 1677, 1648 (C = O), 1610, 1588 (C = C), 1522, 1416 (C-NO_2). ^{1}H NMR (ppm): δ = 3.62 (br, s, 4H, $(CH_2\text{-N-}CH_2)_{piper}$), 3.94 (br, s, 4H, CH_2-N-CH_2)piper, 6.50–6.56 (m, 1H, (CH_{furan}), 7.10 (m, 1H, CH_{furan}), 7.44–7.49 (m, 1H, CH_{furan}), 7.72–7.80 (m, 1H, H_{naphth}), 8.12–8.18 (m, 1H, H_{naphth}), 8.35 (dd, J = 7.8, 1.4 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (ppm): 40.7 $(CH_2\text{-N-}CH_2)_{piper}$, 51.4 $(CH_2\text{-N-}CH_2)_{piper}$, 111.5 (C-Cl), 111.9, 117.3, 147.6, 148.5 (C_{furan}, CH_{furan}), 122.6, 124.7, 128.0, 130.9, 135.0 (C_{arom}, CH_{arom}), 150.3 (=C-N) 159.2, 175.9, 179.8 (C = O). $C_{19}H_{14}ClN_3O_6$ (Mw = 415.79 g/mol). MS (+ESI): m/z = 413.2 $[M-2H]^+$. Calcd., %: C 54.89; H 3.39; N 10.11. Found, %: C 55.01; H 3.44; N 10.33.

Compound 13: Physical properties: (see Table 1); FT-IR (cm^{-1}): v = 3137, 3068 (C-H_{arom}), 2921 (C-H_{aliph}), 1724, 1682, 1646 (C = O), 1617, 1589 (C = C). 1538, 1428 (C-NO_2). ^{1}H NMR (ppm): δ = 3.64 (br, s, 4H, CH_2-N-$CH_2)_{piper}$, 3.90 (br, s, 4H, CH_2-N-$CH_2)_{piper}$, 6.42–6.48 (m, 1H, (CH_{furan}), 7.00 (m, 1H, CH_{furan}), 7.42–7.48 (m, 1H, CH_{furan}), 7.60–7.68 (m, 1H, H_{naphth}), 7.72–7.80 (m, 1H, H_{naphth}), 8.30 (dd, J = 7.8, 1.4 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (ppm): δ = 45.9 $(CH_2\text{-N-}CH_2)_{piper}$, 51.3 $(CH_2\text{-N-}CH_2\text{–})_{piper}$, 111.4 (=C-Cl), 111.5, 117.3, 147.6, 149.3 (C_{furan}, CH_{furan}), 122.5, 127.8, 129.3, 132.2, 134.4 (C_{arom}, CH_{arom}), 159.2 (=C-N) 167.7, 174.1, 179.8 (C = O). $C_{19}H_{14}ClN_3O_6$ (Mw = 415.79 g/mol). MS (+ESI): m/z = 413.2 $[M-2H]^+$. Calcd., %: C 54.89; H 3.39; N 10.11. Found, %: C 55.03; H 3.48; N 10.23.

2-(2,6-Dimethylmorpholino)-3-ethoxy-5-nitronaphthalene-1,4-dione ***(15)****, 3-(2,6-Dimethylmorpholino)-2-ethoxy-5-nitronaphthalene-1,4-dione* ***(16)*** *and 3-Chloro-2-(2,6-dimethylmorpholino)-5-nitronaphthalene-1,4-dione* ***(17)****:*

According to general procedure 2, 0.50 g (1.80 mmol) of **1a** was added to 0.21 g (1.80 mmol) of 2,6-dimethylmorpholine **14** in (25.0 mL) absolute ethanol in presence of Na_2CO_3 about 8 h.

Compound 15: Physical properties: (see Table 1); FT-IR (cm^{-1}): v = 3082 (C-H_{arom}), 2963, 2889, 2865 (C–H), 1680 (C = O), 1642, 1591 (C = C), 1526, 1370 (C-NO_2), 1219 (C-O). ^{1}H NMR (ppm): δ = 1.14 (s, 6H, $CH_3)_{morph}$, 1.31–1.33 (m, 3H, (O-CH_2CH_3), 3.00 (m, 4H, $(CH_2\text{-N-}CH_2)_{morph}$), 2.59–3.64 (m, 2H, $CH)_{morph}$., 3.74–3.80 (m, 2H, (O-CH_2CH_3), 7.76–7.85 (m, 2H, CH_{naphth}), 8.35 (dd, J = 7.8, 1.4 Hz, 1H, CH_{naphth}). ^{13}C(APT) NMR (ppm): δ = 17.4 $(CH_3)_{ethoxy}$, 18.6 $(CH_3)_{morph}$, 56.0 $(CH_2)_{ethoxy}$, 56.9 $(CH_2\text{-N-}CH_2)_{morph}$, 72.5 $(CH\text{-O-}CH)_{morph}$, 122.7, 127.2, 129.2, 130.9, 132.3, 134.2 (C_{arom}, CH_{arom}), 148.9 (=C-N) 174.0, 179.9 (C = O). $C_{18}H_{20}N_2O_6$ (Mw = 360.37 g/mol). MS (+ESI): m/z = 361.7 $[M + H]^+$. Calcd., %: C 59.99; H 5.59; N 7.77. Found, %: C 60.15; H 5.44; N 7.72.

Compound 16: Physical properties: (see Table 1); FT-IR (cm^{-1}): v = 3083 (C-H_{arom}), 2972, 2904 (C–H), 1679 (C = O), 1646, 1592 (C = C), 1527, 1370 (C-NO_2), 1219 (C-O). ^{1}H NMR (ppm): δ = 1.20 (s, 6H, $CH_3)_{morph}$, 1.32–1.35 (m, 3H, (O-CH_2CH_3), 3.23–3.27 (m, 4H, CH_2-N-$CH_2)_{morph}$, 3.46–3.67 (m, 2H, $CH)_{morph}$., 4.17–4.21 (m, 2H, O-CH_2CH_3), 7.79–7.84 (m, 2H, CH_{naphth}), 8.33 (dd, J = 7.4, 1.4 Hz, 1H, CH_{naphth}). ^{13}C (APT) NMR (ppm): δ = 17.6 $(CH_3)_{morph}$, 18.6 $(CH_3)_{ethoxy}$, 56.0 $(CH_2\text{-N-}CH_2)_{morph}$, 56.9 $(CH_2)_{ethoxy}$, 66.8 $(CH\text{-O-}CH)_{morph}$, 119.4, 125.2, 127.7, 129.5, 132.7, 134.2 (C_{arom}, CH_{arom}), 150.9 (=C-N) 176.6, 179.1 (C = O). $C_{18}H_{20}N_2O_6$ (Mw = 360.37 g/mol). MS (+ESI): m/z = 361.7 $[M + H]^+$. Calcd., %: C 59.99; H 5.59; N 7.77. Found, %: C 60.11; H 5.54; N 7.72.

Compound 17: Pink solid, yield: 0.143 g (23%); M.p.:159–160 °C; R_f: 0.35 (EtAc/PET) (1:4 v/v). FT-IR (cm^{-1}): v = 3093, 3035 (C-H_{arom}), 2972, 2927, 2900 (C–H), 1684 (C = O), 1632, 1590 (C = C), 1530, 1371 (C-NO_2). ^{1}H NMR (ppm): δ = 1.20 (s, 6H, $CH_3)_{morph,}$ 3.01, 3.66 (m, 4H, $(CH_2\text{-N-}CH_2)_{morph}$), 3.77–3.85 (m, 2H, $CH)_{morph}$.), 7.68 (dd, J = 7.8, 1.2 Hz 1H, CH_{naphth}), 7.79 (t, J = 7.8 Hz, 1H, CH_{naphth}), 8.22 (dd, J = 7.8, 1.4 Hz, 1H, CH_{naphth}). ^{13}C(APT) NMR (ppm): δ = 18.6 $(CH_3)_{morph}$, 56.6

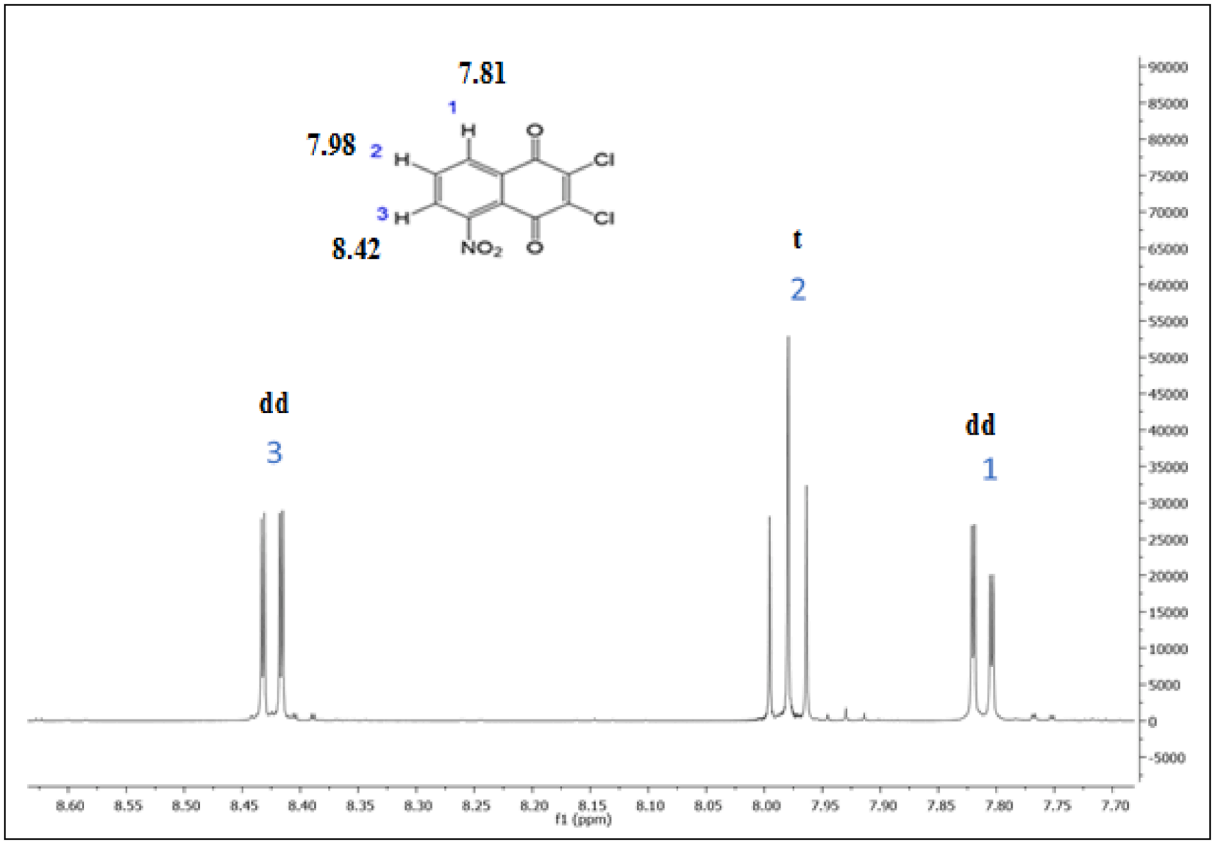


Fig. 1. ^{1}H NMR($CDCl_3$) spectrum concerning the isomer **1a**.

(CH_2-N-CH_2–)$_{morph}$, 72.4 (CH-O-CH)$_{morph}$, 111.2 (=C-Cl), 119.6, 124.9, 127.1, 129.4, 132.6, 134.2 (C_{arom}, CH_{arom}), 149.93 (=C-N) 175.8, 179.1 (C = O). $C_{16}H_{15}ClN_2O_5$ (Mw = 350.76 g/mol). MS (+ESI): m/z = 350.4 $[M-H]^+$. Calcd., %: C 54.79; H 4.31; N 7.99. Found, %: C 54.41; H 4.44; N 7.72.

5.1. Electrochemistry and in situ spectroelectrochemistry

The cyclic voltammetry (CV), square wave voltammetry (SWV) and spectroelectrochemical measurements were carried out with a Gamry Reference 600 Potentiostat/Galvanostat utilizing a three-electrode configuration at 25℃ by following the procedure conducted in our previous paper. GCE, Pt wire, and Ag/AgCl were served as the working, counter, and reference electrodes respectively. Tetrabuthylammonium perchlorate (TBAP) in dimethylsulfoxide (DMSO) was employed as the supporting electrolyte at a concentration of 0.10 moldm^{-3}. UV/Vis absorption spectra were measured by an OceanOptics QE65000 diode array spectrophotometer. In situ spectroelectrochemical measurements were carried out by utilizing a Pt tulle working electrode in the three-electrode configuration of thin-layer quartz spectroelectrochemical cell at 25℃.

6. Results and discussion

6.1. Chemistry

The new regioisomers (**3, 4, 6, 8, 10, 12, 13, 15, 16, 17**) were synthesized by the reactions of 2,3-dichloro-5-nitro-1,4-naphthoquinone **1a** with some amine nucleophiles (**2, 5, 7, 9, 11, 14**) according to a Michael 1,4-addition mechanism and reaction pathways of synthesizes were illustrated in Scheme 2. The couple regioisomers (compounds **3**–**4**, **12**–**13** and **15**–**16**) were separated by column chromatography by using a different ratio of solvents. The obtained regioisomers have different color, melting point and retention factor (R_f) and these values were showed in Table 1. The ^{1}H NMR spectra of the synthesized new regioisomers were indicated that, the peaks of the (2-*N*-substituted-3-chloro-5-nitro-naphthalene-1,4-dione) isomer of aromatic protons (H1-3) are shifted more down field than the aromatic protons of the (3-*N*-substituted-2-chloro-5-nitro-naphthalene-1,4-dione) isomer. The positions "2-*N*" and "3-*N*" were illustrated in Scheme 2 in the compound **1a**. Also, it has been found that, in the case of mixture regioisomers, the higher R_f component was shown to be the (2-*N*-substituted-3-chloro-5-nitro-naphthalene-1,4-dione) isomer and the lower R_f component the (3-*N*-substituted-2-chloro-5-nitro-naphthalene-1,4-dione) isomer. The comparison of R_f values is compatible with similar literatures [12,31–32].

The new regioisomers (**3, 4**) were synthesized from the reacting of **1a** and 1-(diphenylmethyl)piperazine **2** in ethanol at room temperature (Scheme 2). ^{1}H NMR spectrum of regioisomers **3** and **4**, the signals for protons of the naphthoquinone ring are observed as different pattern [31–33]. ^{1}H NMR of isomer **3**, signals concerning one proton of the naphthoquinone ring is shown in the range δ = 7.80 ppm as multiplet. The other proton detected at δ = 8.32 ppm as a triplet, last one is at δ = 8.39 ppm as doublet of doublet. Whereas ^{1}H NMR signal of the hydrogen atoms at naphthoquinone ring of isomer **4** is detected as multiplets, triplet and doublets of doublets at δ = 7.76, 7.85, and 8.24 ppm, respectively. The new compound **6** was obtained when 2-(1-piperazinyl) ethylamine **5** reacted with **1a** at room temperature using ethanol as medium. The proposed structure is the presence of nucleophile between two naphthoquinone rings as a bridge. In the IR spectra, (N–H) band was appeared at 3258 cm^{-1}. Piperazine ring were seen as a broad singlet at δ = 3.50, 3.81 ppm in ^{1}H NMR. The carbonyl groups (C = O) at δ = 175.8, 179.0, 190.3 ppm as were seen in ^{13}C(APT) NMR for compound **6**.

One new regioisomer **8** was obtained by refluxing the titled

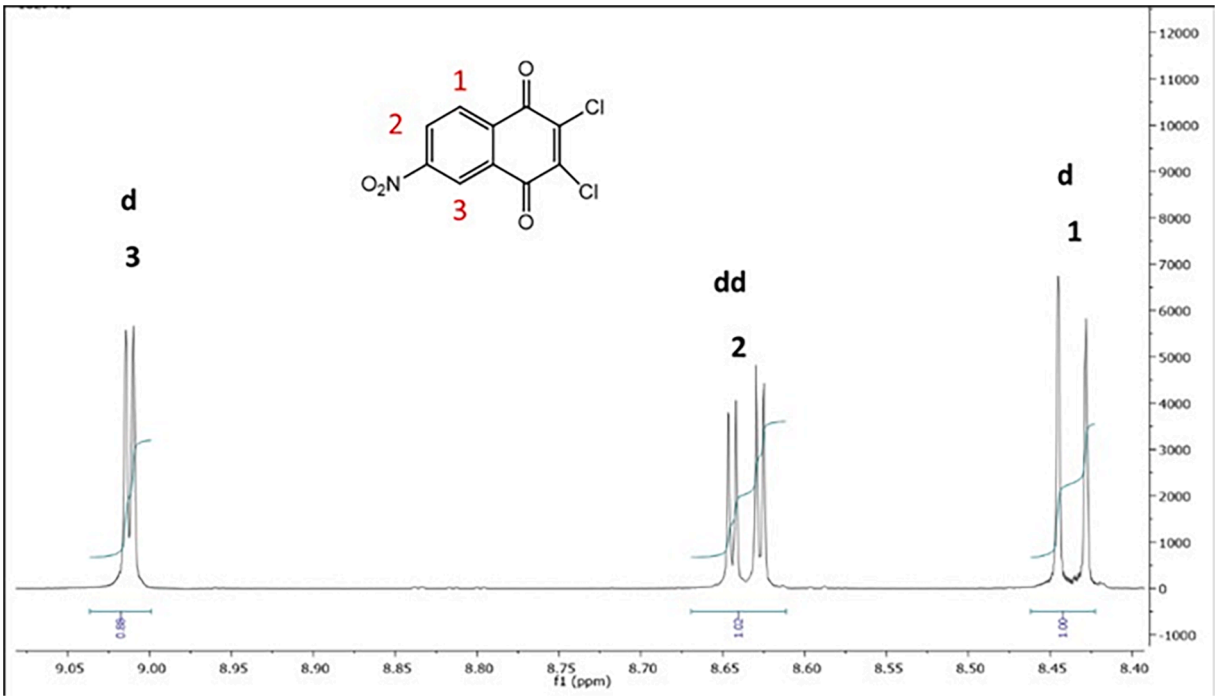


Fig. 2. ^{1}H NMR($CDCl_3$) spectrum concerning the isomer **1b**.

compound **1a** and 2,4,6-trimethylaniline **7** in ethanol and sodium carbonate (Scheme 2). The band of (N–H) was seen around $\nu = 3272$ cm^{-1} as was shown in the IR spectrum. Protons of methyl groups (CH_3) in ^{1}H NMR spectrum were appeared at $\delta = 1.31$–1.37 and 2.04–2.12 ppm as a multiplet. Two signals at $\delta = 176.4$, 177.7 ppm were related to carbonyl groups as was seen in the ^{13}C(APT) NMR spectrum. Similarly, the one new isomer **10** was obtained by reacting the titled compound **1a** with 2-(piperidin-1-yl)ethane-1-amine in ethanol. The band of (N–H) was seen around at $\nu = 3277$ cm^{-1} as was shown according to IR spectra. 5-Nitro naphthoquinone protons were detected around $\delta = 7.69$–8.34 ppm. A signal at $\delta = 177.6$ ppm was related to carbonyl group as was seen in the ^{13}C(APT) NMR spectrum.

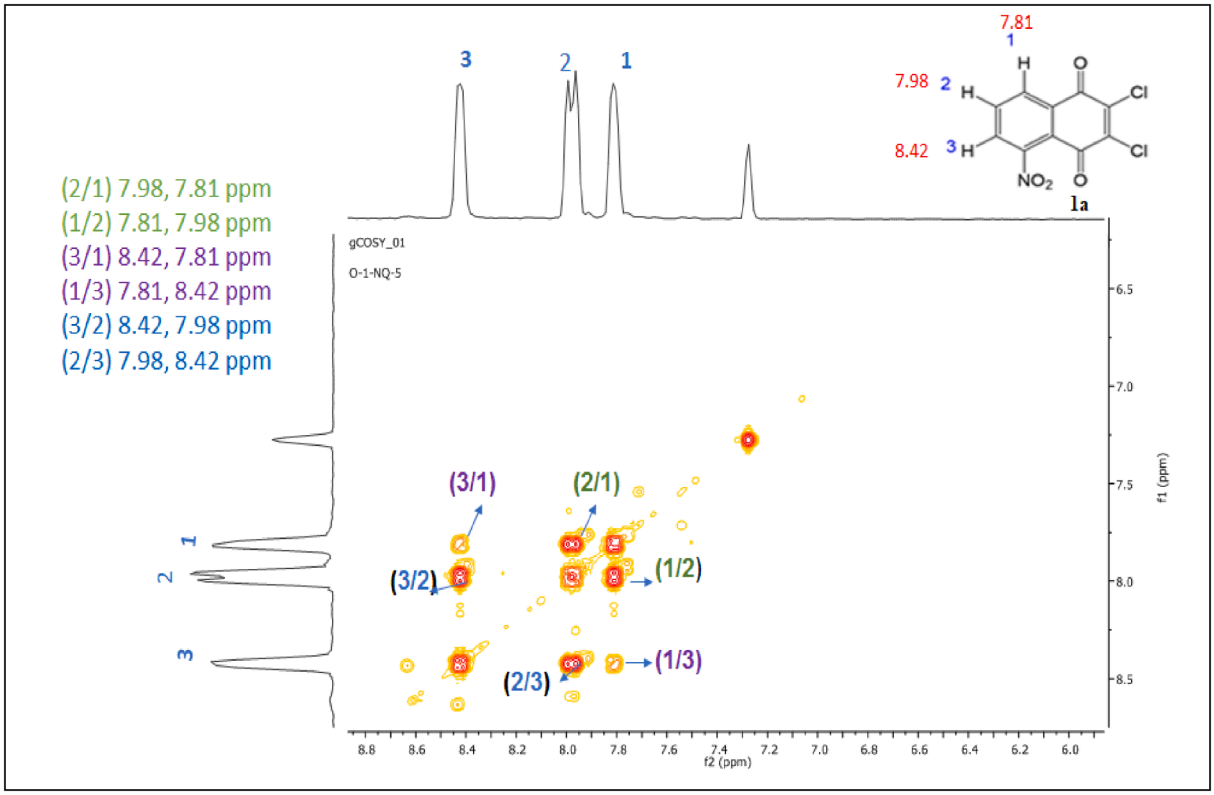


Fig. 3. ^{1}H–^{1}H COSY contour map of compound **1a**.

Table 2
Voltammetric data of **1a** and 5-Nitro-NQ derivatives (**3, 4, 6, 8, 10, 12–13, 15–17**).

Comp.	[a]NQ/NQ$^{\cdot-}$ reduction couple			[a]NQ$^{\cdot-}$/NQ^{2-} reduction couple			NO_2/NO_2^{1-} reduction couple		
	$E_{1/2}$	[b]Δ E_p	I_{pa}/I_{pc}	$E_{1/2}$	[b]Δ E_p	I_{pa}/I_{pc}	[a]$E_{1/2}$	[b]Δ E_p	I_{pa}/I_{pc}
1a	−0.11	63	0.97	−0.83	65	0.92	−1.65	170	0.24
3	−0.28	62	0.95	−0.98	83	0.90	−1.55	100	0.22
4	−0.26	65	0.93	−0.94	80	0.92	−1.50	95	0.27
6	−0.27	64	0.91	−0.96	87	0.95	−1.52	110	0.33
8	−0.27	69	0.97	−1.01	77	0.92	−1.82	130	0.18
10	−0.24	74	0.94	−0.98	82	0.94	−1.80	105	0.25
12	−0.17	61	0.97	−0.81	82	0.92	−1.45	138	0.20
13	−0.19	63	0.94	−0.86	88	0.97	−1.48	108	0.29
15	−0.33	64	0.90	−0.96	67	0.93	−1.61	107	0.24
16	−0.34	59	0.99	−0.98	63	0.96	−1.64	110	0.17
17	−0.30	68	0.95	−0.99	66	0.97	−1.58	97	0.30

([a]) = $E_{1/2}$ values were derived from the CVs recorded between 0.0 and −1.20 V. [b]: $\Delta E_p = |E_{pa} - E_{pc}|$.
All potentials were given vs. Ag/AgCl (V).

In the scheme 2; the new isomer compounds (**12, 13**) obtained by the reaction between **1a** and 1-(2-furoyl)piperazine in ethanol at room temperature. As was shown in IR spectra for compounds (**12, 13**) isomers; asymmetric and symmetric stretching of (C-NO_2) appeared at ν = 1522, 1416 cm^{-1} and 1538, 1428 cm^{-1} respectively. The piperazine ring (CH_2NCH_2) observed at δ = 3.62, 3.94, 3.58–3.64 and 3.90 ppm as a broad singlet for two isomers respectively. As were shown in APT-NMR; three expected carbonyl signals were observed for two isomers.

The new isomer compounds (**15, 16**) and compound **17** obtained by reaction between **1a** and 2,6-dimethylmorpholine in ethanol (Scheme 2). In this reaction, the solvent reacted as a nucleophile (O-CH_2CH_3) and substituted with one chlorine, while the other chlorine replaced with morpholine to form an isomer (**15, 16**). According to IR spectra for these isomers, C-O bands were appeared at ν = 1219 cm^{-1}. Signals at δ = 7.76–7.85 and 8.35 ppm are corresponding to naphthoquinone protons for first isomer and at δ = 7.79–7.84 and 8.34 ppm for second isomer. As were shown in the ^{13}C(APT) NMR, an expected carbonyl signals were observed for two isomers. In compound **17**, stretching (asymmetric, symmetric) bands for (C-NO_2) were appeared at 1530 and 1371 cm^{-1}. Two methyl groups (CH_3) in morpholine ring were observed at δ = 1.20 ppm as a singlet however, signal corresponding to carbonyl groups (C = O) were detected at δ = 175.8 and 179.1 ppm.

6.2. Study of ^{1}H–^{1}H correlated spectroscopy (COSY) of one regioisomer (1a)

The structure of 5-nitro-1,4-naphthoquinone **1a** was elucidated by using one- and two-dimensional NMR techniques in which the differences of positions of nitro group on the naphthalene ring were detected. The three hydrogen signals at the quinone ring of 2,3-dichloro-5-nitro-1,4-naphthoquinone **1a** were assigned in the ^{1}H NMR spectrum (Fig. 1) and confirmed by the ^{1}H–^{1}H COSY (Fig. 3). In the ^{1}H NMR spectrum of compound **1a**, a doublet of doublet (dd) at 7.81 ppm corresponding to H1 that couple to H2 (t, 7.98, 1H, 3JH, H 9.27), and to H3 (dd, 8.42, 1H, 3JH, H 9.27) (Fig. 1). According to the ^{1}H NMR spectrum of **1b** isomer; three protons of nitro-naphthoquinone ring appeared at δ = 8.44 ppm as a doublet, 8.63 ppm as doublet of doublet and last proton at δ = 9.01 ppm as a doublet too (Fig. 2).

All these hydrogens of compound **1a** were assigned based on the ^{1}H–^{1}H COSY spectrum, where can be observed that H1 is coupled to H2 and H3, H2 to H1 and H3, and H3 to H2 and H1. From ^{1}H–^{1}H COSY contour maps these hydrogens are coupling to each other (Fig. 3).

6.3. Electro- and in-situ spectroelectrochemical studies of 5-nitro-N,O-subtituted-1,4-naphthoquinones

It is well documented that; NQ derivatives undergo two successive 1e$^-$ reduction processes with two distinct cathodic redox couples. These couples are assigned to the formation of the NQ$^{\cdot-}$ radical anion and the NQ^{2-} formation [17–19,25]. The studies clearly represented the influence of the type and polarity of the solvent, type of conducting electrolyte, or presence of water or acid which facilitate intra- and intermolecular hydrogen bonding, significantly influenced the reduction reactions of NQs. Here we aimed to illustrate influence of redox active nitro and redox inactive chloride substituents to the redox mechanism of NQs. For these purposes, voltametric and *in-situ* UV–vis spectroelectrochemical characterizations of *N,O*-Substituted-5-nitro-1,4-Naphthoquinones (NQ) (**3, 4, 6, 8, 10, 12, 13, 15, 16, 17**) were carried out in DMSO/TBAP electrolyte on GCE. In addition to the CV and SWV measurements, repetitive CV analyses were conducted with different vertex potential to illustrate influence of each redox process to the hole mechanism.

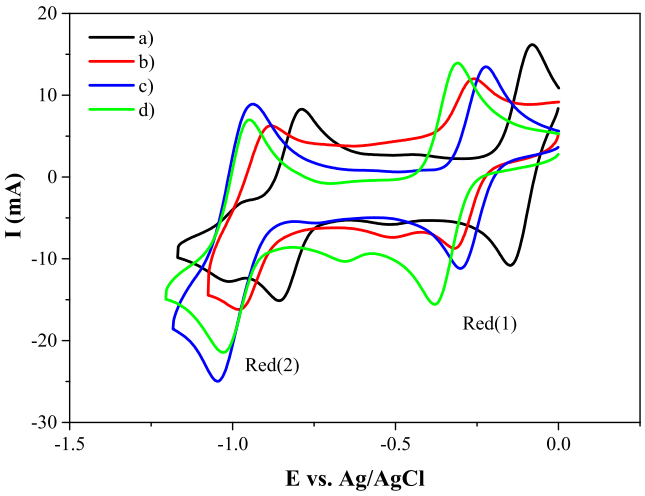


Fig. 4. CV responses of all NQs recorded between 0 and −1.20 V at 100 mVs^{-1} scan rate on GCE in DMSO/TBAP; **a**) Compound **1a**, **b**) Compound **3**, **c**) Compound **8**, **d**) Compound **16**.

Redox parameters of all NQs derived from the CVs and SWVs are tabulated in Table 2 for comparison with each other and with the literature. When the potential scans are shifted just after the second reduction wave, all NQs illustrates two reversible reduction couples with one electron transfer for each process (Fig. 4). Δ E_p values at around 60 mV and unity of the I_{pa}/I_{pc} ratios indicate the electrochemical and chemical reversibility of the couples of NQs. Due to the different electron releasing/withdrawing ability of the substituents small potential shifts are observed with respect to that of unsubstituted **1a**.

Compound **1a** having two chloride substituents on 2 and 3 positions of the 5-nitro-1,4-naphthoquinone is the most easily reduced substance, while compound **16** having dimethyl morpholine at 3 and ethoxy at 2 position of 5-nitro-1,4-naphthoquinone was the most difficult to reduce.

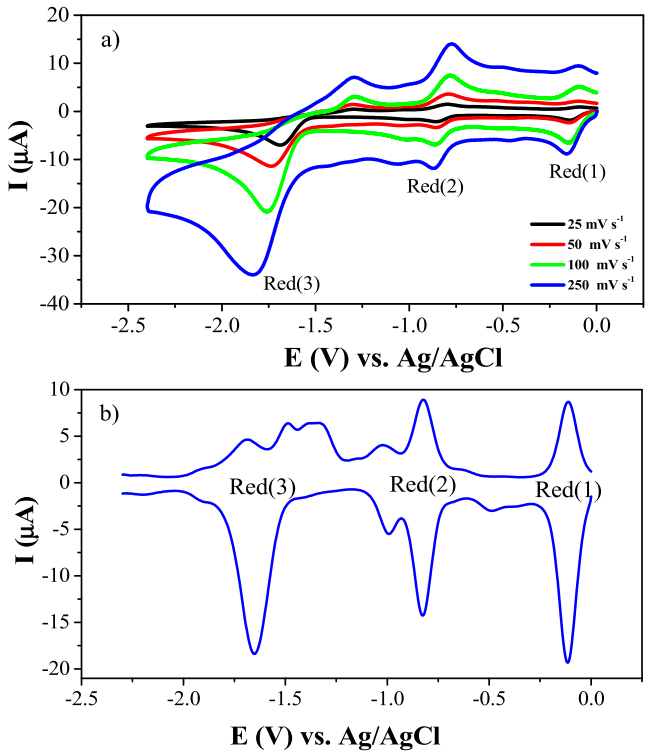


Fig. 5. CV and SWV responses of compound **1a** at various scan rates on GCE in DMSO/TBAP.

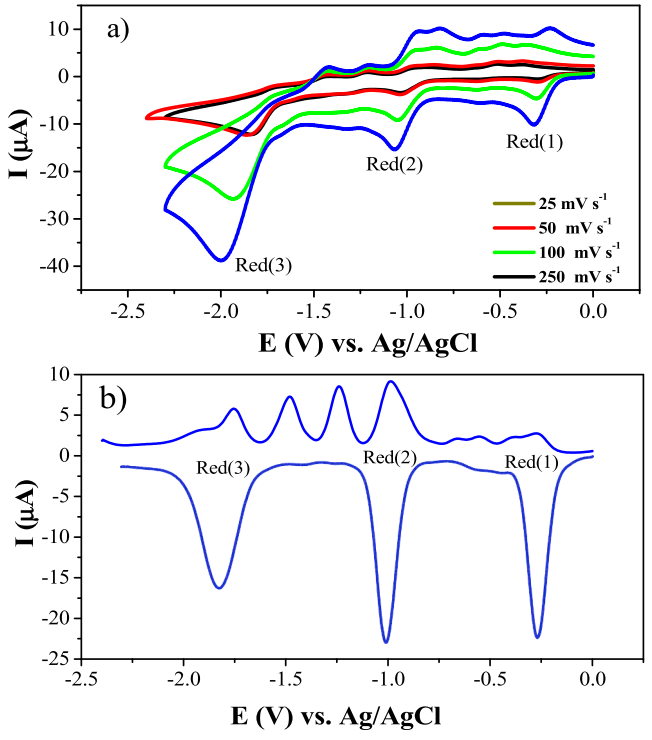


Fig. 6. CV and SWV responses of compound **8** at various scan rates on GCE in DMSO/TBAP.

When compared with the similar studies in the literature, the NQs based couples are shifted towards the positive potentials due to the electron withdrawing nature of the nitro moieties on all molecules. When the vertex potential is switched from more negative potentials, all NQs

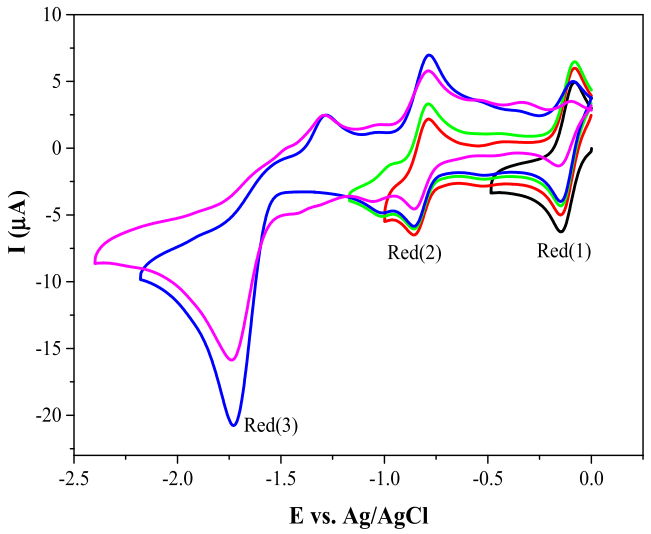


Fig. 7. Repetitive CV responses of compound **1a** at 100 mVs^{-1} scan rate on GCE in DMSO/TBAP.

illustrate an extra irreversible reduction process at more negative potentials, which is easily attributed to the reduction of nitro moieties on NQs. Moreover, nitro reduction process considerable influence the reversibility of the previous NQ based reduction processes. Controlled potential coulometric studies indicated one-electron transfer nature of the three reduction processes. CV and SWV responses of compounds **1a** (Fig. 5) and **8** (Fig. 6) at various scan rates on GCE in DMSO/TBAP as examples. As shown in Fig. 5, NO_2 substituent based reduction process is observed at −1.65 V in addition to the NQ based process at −0.11 and −0.83 V. While electrochemically and chemically reversible Red (1) and Red(2) couples are observed with short potential window (between 0.0 and −1.20 V; Fig. 4), these couples get chemically irreversible as shown in Fig. 5. Due to the possible chemical reactions succeeding the Red(3) process, new oxidation waves are observed, while the reverse waves of Red(1) and Red(2) couples get smaller during the reverse potential scans. These voltametric responses indicate decomposition of the molecule after the Red(3) process, and the decomposition products oxidize during the reverse potential scans. Moreover, while the reverse waves of Red(1) and Red(2) couples completely disappeared at slow scan rates, these wave can be observed at high scan rates, which is

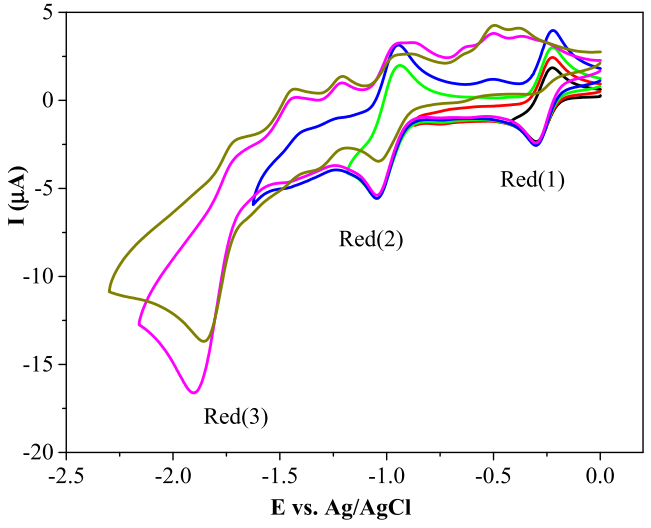


Fig. 8. Repetitive CV responses of compound **8** at 100 mVs^{-1} scan rate on GCE in DMSO/TBAP.

$$NO_2@NQ + e^- \overset{Red(1)}{\Longleftrightarrow} NO_2@NQ^{\cdot -}$$

$$NO_2@NQ^{\cdot -} + e^- \overset{Red(2)}{\Longleftrightarrow} NO_2@NQ^{-2}$$

$$NO_2@NQ^{-2} + e^- \overset{Red(3)}{\Longleftrightarrow} (NO_2)^{-1}@NQ^{-2}$$

$$(NO_2)^{-1}@NQ^{-2} \xrightarrow{Chemical\ reactions} Products$$

Scheme 3. Redox mechanism of NQ derivatives.

resulted from the slow rate of the proposed chemical reactions.

Compound **8** illustrates similar CV and SWV responses with those of **1a** with small potential shifts and difference in the peak currents. As shown in Fig. 6, nitro based wave is observed at −1.82 V (Red(3)) in addition to the NQ reduction couples at −0.27 and −1.01 V. Due to the chemical reactions after the Red(3) process, the Red(1) and Red(2) couples get chemically irreversible and new oxidation waves at around −1.25 and −1.50 V during the reverse scan. When compared with compound **1a**, influence of the chemical reactions is more pronounced for compound **8** due to the different rate of the chemical reactions. All other NQs illustrates similar voltametric responses with those of **1a** and **8** with small potential shift and peak current differences.

In order to more clarify the influence of the chemical reactions, repetitive CV responses of the molecules are recorded with variating of the vertex potentials. The Fig. 7 represents repetitive CV responses of compound **1a** at 100 mVs^{-1} scan rate on GCE in DMSO/TBAP. While reversible Red(1) and (Red(2) couples are observed with the potential scan between 0.0 and −1.20 V, these couples get chemically irreversible and new oxidation waves are observed during the reverse scans when the potential is scan until −2.20 V due to the chemical reaction succeeding the Red(3) process. Similar CV responses are observed with the compound **8** given in Fig. 8. Differently more dominant new waves are observed for compound **8** with respect to those of **1a** due to the faster chemical reaction of the anionic species of compound **8**. All NQs illustrates similar responses, thus repetitive CV responses of compounds **3, 12** and **16** are given in the supplementary file for the comparisons. As a result of the voltametric analyses of the NQs, the mechanism given in Scheme 3 is proposed.

There are few studies the *in situ* UV–Vis spectroelectrochemical studies of NQ derivatives [25,34–35]. For instance, S. Záliš et.al reported the spectra of the neutral, monoanionic and dianionic NQ species [35–37]. In another study, Y. Hui and coworkers investigated the spectral changes during the reduction reactions of substituted naphthoquinones. Here, *in situ* UV–Vis spectroelectrochemical studies of NQs were studied to investigate the colors and spectra of the electrogenerated species. Compound **1a** illustrates different spectral responses than the others. Fig. 9 show *in situ* UV–Vis spectral changes during the reduction reactions of NO_2. Under open circuit potential, compound **1a** gives two bands at 300 and 400 nm. During the first reduction reaction at −0.50 V, while the band at 300 nm decreases and the band at 400 nm increases with a shift to 420 nm, two new bands are enhanced at 500 and

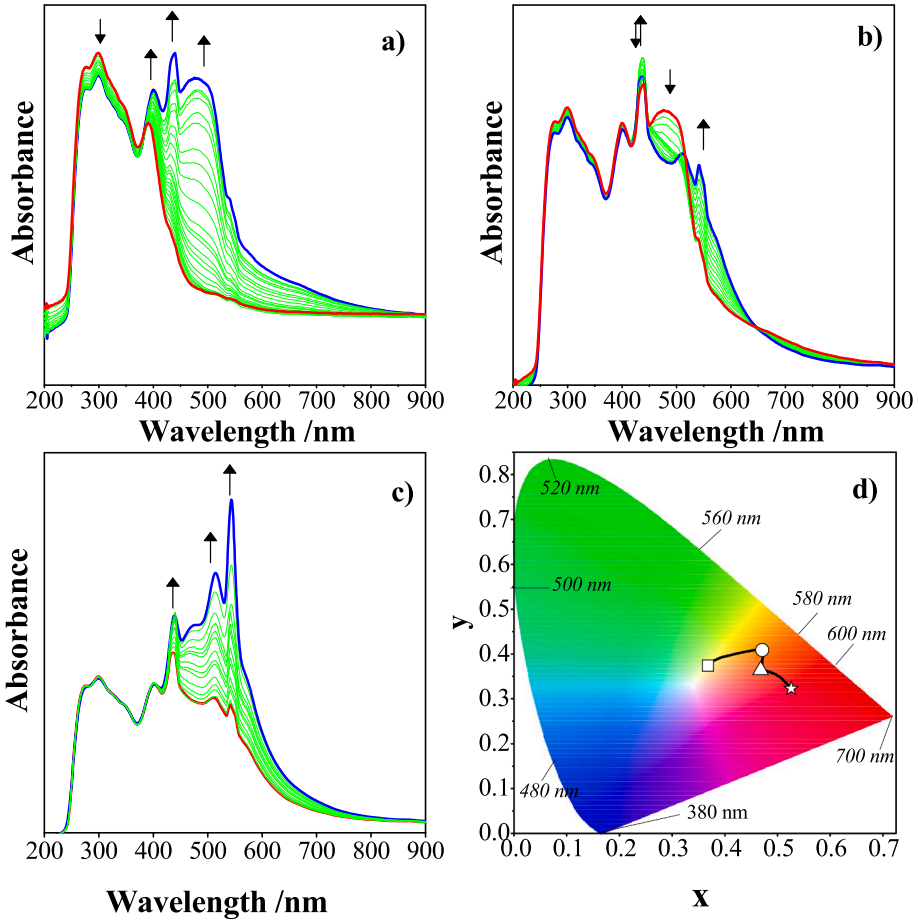


Fig. 9. UV–vis spectral changes of compound **1a** recorded during *in-situ* spectroelectrochemical measurements at applied potentials of a) E_{app} = -0.50 V, b) E_{app} = -1.25 V, c) E_{app} = -2.00 V in DMSO/TBAP electrolyte system (changing of the spectrum during the redox reactions were represented with the arrow directions), and d) color of the species (Neutral:; Red(1):; Red(2):; Red(3):).

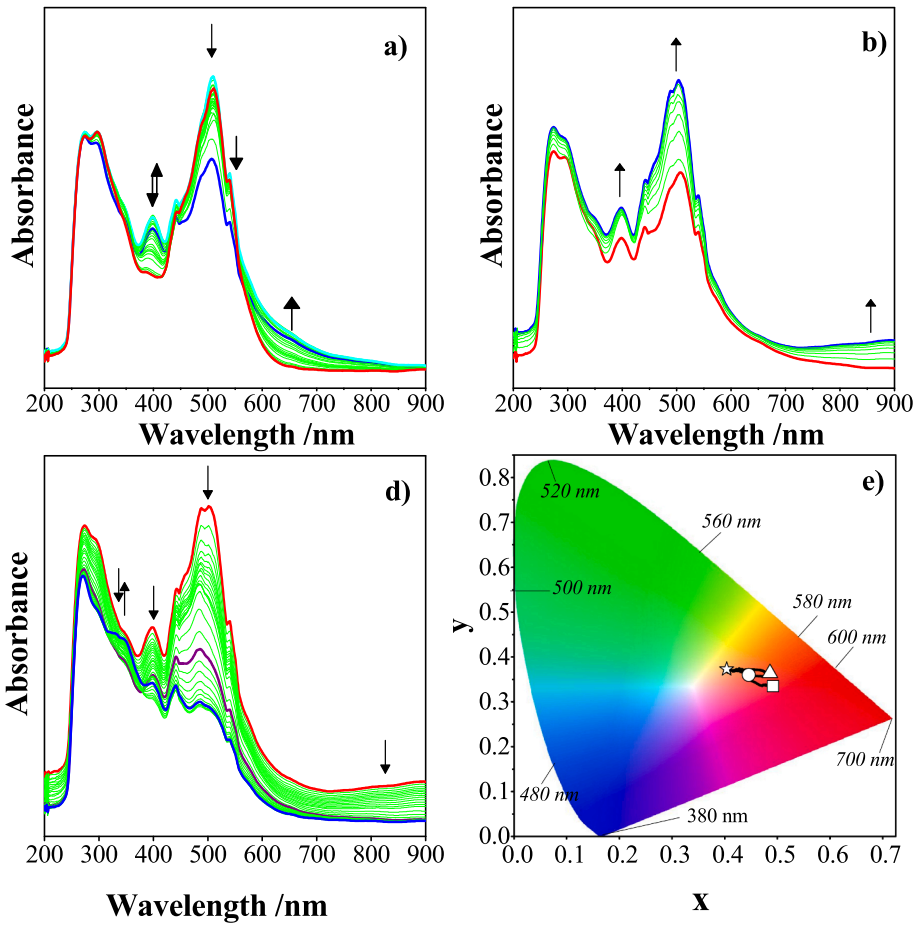


Fig. 10. UV–vis spectral changes of compound **8** recorded during *in-situ* spectroelectrochemical measurements at applied potentials of a) E_{app} = -0.50 V, b) E_{app} = -1.25 V, c) E_{app} = -2.00 V in DMSO/TBAP electrolyte system (changing of the spectrum during the redox reactions were represented with the arrow directions), and d) color of the species (Neutral:; Red(1):; Red(2):; Red(3):).

550 nm (Fig. 9a). These spectral changes cause a color (point □; x = 0.368 and y = 0.374) changes from yellow to orange (point ○; x = 0.471 and y = 0.409) (Fig. 9d). During the Red(2) process, while the band at 550 nm decreases in intensity, a new band is observed at 600 nm (Fig. 9b). Red color (point △; x = 0.468 and y = 0.364) is observed for the dianinoic NQ species as shown in Fig. 9d. Due to the enhancement of a new sharp band at 540 nm and increasing the intensity of the band at 550 nm (Fig. 9c), the red color of the dianinic species tuns to deep red (point ☆; x = 0.526 and y = 0.323) as shown in (Fig. 9d).

All substituted quinone compounds illustrated similar spectral changes, thus the *in situ* UV–Vis spectral responses of compound **8** are illustrated in Fig. 10 as an example. As shown in Fig. 10a, spectrum of the neutral **8** is slightly different than that of compound **1a**. When −0.50 V is applied, while the band at 500 nm decreases a new band is observed at 400 nm and the intensity of the region at around 600 nm increases (Fig. 10a). During the second reduction reaction (Red(2)), the band at 500 nm increases again with the increase of the band at 400 nm as shown in Fig. 10b. During the third reduction processes all bands decreased in intensity with an observation of a small band at 350 nm at the beginning of the process and then it disappears again (Fig. 10c). These spectral changes observed during the reduction processes cause to color changes from deep red (point □; x = 0.492 and y = 0.335) to red (point ○; x = 0.436 and y = 0.360), deep red (point △; x = 0.486 and y = 0.364) and then to yellow (point ☆; x = 0.404 and y = 0.373) respectively as shown in Fig. 10d. The distinct color differences between the electrogenerated NQ species indicate their possible usage in cathodic electrochromic applications.

7. Conclusions

The nitro group associated with the aromatic ring in the quinone system is known to increase the biological activity of naphthoquinone due to its electron-withdrawing properties. For the investigation of effect of nitro group substituted quinone ring to electrochemistry and *in situ* spectroelectrochemistry properties 2,3-dichloro-5-nitro-1,4-naphthoquinone **1a** was choosed as the starting material. During ten regioisomeric compounds of 5-nitro-1,4-naphthoquinone (**3–4, 6, 8, 10, 12–13, 15–17**) were synthesized by the reactions of 2,3-dichloro-5-nitro-1,4-naphthoquinone **1a** with some amine nucleophiles according to a Michael 1,4-addition mechanism. The couple regioisomers (compounds **3–4**, **12–13** and **15–16**) were separated by column chromatography by using a different ratio of solvents. The obtained regioisomers have different color, melting point and retention factor (R_f). All synthesized compounds were characterized by using elemental analysis, electrospray ionization mass spectrometry (ESI-MS), Fourier transform infrared spectroscopy (FT-IR), ^{1}H-nuclear magnetic resonance (^{1}H NMR), attached proton test nuclear magnetic resonance (APT-NMR). The two-dimensional technique ^{1}H–^{1}H correlated spectroscopy (COSY) was used for characterization of isomer formation **1a**.

Electrochemical features of NQs supported the proposed structure of the molecules. Three well resolved reduction processes were observed for all NQs. Although NQS based reduction processes had reversible character, succeeding chemical reactions after the nitro based third reduction process made all processes chemically irreversible. In situ spectroelectrochemical responses represents pronounced spectral and color changes during the reduction reactions.

8. Author's Contribution Statement

Nahide Gulsah Deniz*: Corresponding author. The regioselective synthesis, purification and characterization of novel 5-nitro naphthoquinone derivatives by using spectroscopic techniques.

Aesha F.SH. Abdassalam: Co-Author. The regioselective synthesis, purification and characterization of novel 5-nitro naphthoquinone derivatives by using spectroscopic techniques.

Cigdem Sayil**: Corresponding author. The regioselective synthesis, purification and characterization of novel 5-nitro naphthoquinone derivatives by using spectroscopic techniques.

Ozlem Uguz: Co-Author. The determination of Electrochemistry and *in-situ* spectroelectrochemistry properties of novel 5-nitro naphthoquinone derivatives.

Atif Koca: Co-Author. The determination of Electrochemistry and *in-situ* spectroelectrochemistry properties of novel 5-nitro naphthoquinone derivatives.

CRediT authorship contribution statement

Nahide Gulsah Deniz: Conceptualization, Funding acquisition, Data curation, Writing – original draft, Investigation, Methodology, Supervision. **Aesha F.SH. Abdassalam:** Software, Methodology, Formal analysis, Data curation. **Cigdem Sayil:** Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ozlem Uguz:** Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Atif Koca:** Conceptualization, Writing – review & editing, Formal analysis, Resources, Writing – original draft, Supervision, Software, Methodology, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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