Scientific paper

Synthesis of New Regioisomers of 5-Nitro-1,4-Naphthoquinone, Evaluation of Antioxidant and Catalase Inhibition Activities

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Abstract

The studies on nitronaphthoquinone derivatives are rare in the literature, and 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. In the course of quinone derivatives, the new N(H)-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 ring substituted nucleophiles such as anilines, piperazines, or morpholines, according to a Michael 1,4-addition mechanism. Five NQ regioisomer couples having different functional group (2-chloro-isomers **3**, **5**, **7**, **9** and **13**; 3-chloro-isomers **2**, **4**, **6**, **8** and **12**) are reported here. All new synthesized compounds were characterized by spectroscopic methods and two-dimensional NMR techniques $^{1}H–^{1}H$ correlated spectroscopy (COSY).

The synthesized NQ regioisomers were evaluated for catalase enzyme inhibitory activities and antioxidant efficiency. The synthesized regioisomers were screened for their antioxidant capacity using the cupric-reducing antioxidant capacity (CUPRAC) method. 2-Chloro-3-((2,4-dimethoxyphenyl)amino)-5-nitronaphthalene-1,4-dione (**5**) showed the highest antioxidant capacity with a 1.80±0.06 CUPRAC-trolox equivalent antioxidant capacity (TEAC) coefficient. Compound **5** also showed strongest catalase enzyme inhibitory activity. The antioxidant capacity results of all 2-chloro regioisomers are higher than the 3-chloro regioisomers. Likewise, also catalase enzyme inhibitory activities results were determined in the same way, except for one regioisomer pair. The catalase was effectively inhibited by the newly synthesized compounds, with % inhibition values in the range of 0.71–0.86%. Some of these NQ compounds also showed remarkable antioxidant capacities.

Keywords: 5-Nitro-1,4-naphthoquinone; heterocyclic ring; CUPRAC method; Catalase inhibition activity

1. Introduction

Naphthoquinone derivatives have been used as antibacterial agents for several years already, there are many reports in the 1960s of chemical compounds synthesized with 1,4-naphthoquinone structure and having antibacterial properties.[1] Later, in the 1980s, there were studies on inhibition of bacteria, along with vitamin K and 1,4-naphthoquinone. It has been suggested that pharmaceuticals compete in the electronic transport system. Another suggestion was the production of ROS and radical semiquinone and cytotoxicity of naphthoquinone.[2,3] It has been shown that amino derivatives such as anilines, piperazines, or morpholines of naphthoquinone improve the biological properties of these derivatives.[4] 2,2′-[1-(2-Aminoethyl) piperazin-1-yl]-3,3′-dichloro-bis(1,4-naphthoquinone) has showed remarkable antioxidant capacity by using the cupric-reducing antioxidant capacity (CUPRAC) methods and cytotoxic activity against A549 (lung), MCF-7 (breast), DU145 (prostate), and HT-29 (colon) cancer cell lines.[4]

Furthermore, the amino-1,4-naphthoquinone derivative has been used as synthetic key intermediate or as starting material for synthesis of many compounds.[5–10] Some literature reports point to the pro-oxidative effect of 5-hydroxy-1,4-naphthoquinone (juglone).[11,12] Also it has been reported that pyridine, 4*H*-pyran and thiazolopyrimidine derivatives of 3-hydroxynaphthoquinone have high antioxidant activity.[13]

There are two main ways describing how to prepare the alkyl or arylamino naphthoquinone derivatives. In the first type, the reaction requires a Michael 1,4-addition reaction between the amino compound and 1,4-naphthoquinone ring to produce 2-amino-1,4-naphthoquinone. The second type involves a nucleophilic substitution reaction between the nucleophile with a mono or dihalogenated derivative of 1,4-naphthoquinone to generate the corresponding amino derivative.[14]

The studies on nitronaphthoquinone derivatives are rare in the literature, and 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 and 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.[15] Blackburn (2005) has treated 2,3-dichloro-5-nitro-1,4-naphthoquinone with linked resin amine to give very colorful products in high yields.[11] The resin under some reducing process and reacted with 2,3-dichloro-5-nitro-1,4-naphthoquinone in the presence of 2,6-di(*tert*-butyl)pyridine to give the red resin-quinone. Treatment with trifluoroacetic acid led to the rapid formation of regioisomeric mixtures of nitroquinones. As a comparison between the regioisomers the retention factor (R_f) of the 5-nitro isomer was found to be higher than for the 8-nitro isomer. Also, in the ^{1}H NMR, the proton signals of naphthoquinone ring for the first isomer are shifted more downfield than the naphthoquinone peaks of the second isomer.[15] In a later study, some derivatives of 5-nitro-2/3-aminonaphthalene-1,4-dione have been synthesized, studied and tested for biological activities by Samant *et al.* (2013).[16] They found that two regioisomers of nitronaphthoquinone derivatives, 3-chloro-5-nitro-2-((2-(trifluoromethyl)phenyl)amino)naphthalene-1,4-dione and 2-chloro-5-nitro-3-((2-(trifluoromethyl)phenyl)amino)naphthalene-1,4-dione demonstrated strong activity against the sleeping sickness disease (African human trypanosomiasis) with low cytotoxicity *in vitro*.[16] In addition to this, we have previously reported that some regioisomers of 5-nitro-1,4-naphthoquinone containing *N*-substituted group have been synthesized from 2,3-dichloro-5-nitro-1,4-naphthoquinone.[10]

Catalase (EC 1.11.1.6) as an antioxidant metalloenzyme capable of degradation of H_2O_2 is present in many cell types. Lack or malfunction of this class of enzyme may lead to severe disorders such as apoptotic cell death, anemia, some dermatological disorders, cardiovascular diseases, Wilson disease, hypertension and Alzheimer's diseases.[17] Some drugs bind to catalase and elicit enzyme inhibition; led to H_2O_2 accumulation and cytotoxicity in cancer cells.[18] So, it is important to measure catalase inhibition activity in the presence of new catalase inhibitor.

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 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 (FTIR), ^{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. Secondly, these compounds were also tested for their antioxidant capacity *in vitro* by CUPRAC method and catalase inhibition activities.

2. Experimental

2. 1. Materials and Methods

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 spectra were obtained using a Varian Unity Inova (500 MHz) spectrometer by using TMS as the internal standard and deuterated chloroform as the 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 60 F-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. CUPRAC Assay of Total Antioxidant Capacity

The CUPRAC total antioxidant capacity measurement method[19] depends on the oxidation of an antioxidant by cupric neocuproine complex (Cu(II)-Nc) generating yellow-orange colored product (cuprous chelate: Cu(I)-Nc). To a test tube 1 mL $CuCl_2{\cdot}2H_2O$ (10 mM), 1 mL Nc (7.5 mM), 1 mL NH_4Ac buffer solution (1.0 M, pH 7), x mL newly synthesized compound, and H_2O (1.1 – x mL) (total volume: 4.1 mL) were added in this order and mixed well. The absorbance at 450 nm was recorded

against a reagent blank using a Perkin–Elmer Lambda 35 UV–Vis spectrophotometer with a pair of matched quartz cuvettes of 1 cm thickness after 30 min incubation period at room temperature. The calibration graph was then constructed by plotting the final concentration of each compound against the absorbance values which were measured. The result of antioxidant efficiency was expressed as trolox equivalent antioxidant capacity (TEAC) coefficient, mean ±SD of three determinations.

2. 3. Catalase Enzyme Inhibition Activity

The catalase enzyme inhibition activity was evaluated by using a modified CUPRAC method described by Bekdeser *et al.*[20] To a test tube 0.5 mL H_2O_2 (1.0 mM), 1.8 mL H_2O, 0.1 mL catalase solution (3.691 U mL^{-1}), and 0.2 mL synthesized compound (1.0 mM, total volume 2.6 mL) were added in this order, mixed and incubated at room temperature for 30 min. After this period, the optical CUPRAC sensor (Cu(II)-Nc-impregnated nafion membrane) was taken out and immersed in a test tube consisting of 2 mL incubation mixture + 6.2 mL EtOH. After 30 min agitation period, the yellow-orange colored nafion membrane was taken out and its absorbance was measured at 450 nm against that of a blank membrane excluding analyte.

2. 4. Synthesis

2. 4. 1. General Synthesis Procedure 1 for 2,3-Dichloro-5-nitro-1,4-naphthoquinone (1)

2,3-Dichloro-5-nitro-1,4-naphthoquinone (**1**) was prepared *via* the following method.[21] First, a mixture of the isomer, in which nitro group is substituted at five and six positions, was obtained. These isomers were separated by using column chromatography. The physical properties and characterization methods have been described before.[21–23] A complete and unambiguous assignment of ^{1}H shifts was based on a combination of one- and two-dimensional techniques (^{1}H and ^{1}H–^{1}H correlated spectroscopy (COSY)), see Figures 2–4.

2. 4. 2. General Synthesis Procedure 2 for Regioisomeric Compounds 2–13

Regioisomeric compounds **2**–**13** were synthesized by a known previous method.[9] 2,3-Dichloro-5-nitro-1,4-naphthoquinone (**1**) and nucleophiles (anilines, piperazines, etc.) **1a**–**f** were stirred in 25 mL of absolute ethanol for 3–4 h in the presence of Na_2CO_3 at room temperature. The reaction mixture was monitoring by TLC to establish the end of the 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, the residue was then purified by column chromatography (Table 1). In the ^{1}H NMR spectra of compounds **1**–**13**, the signals for protons represented H_{1-3} belong to the naphthoquinone ring (Figure 1).

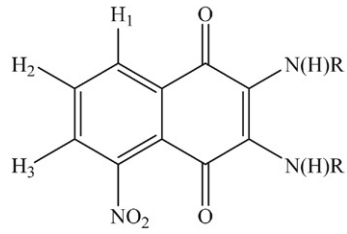


Figure 1. Characterization of quinonoid protons H_{1-3} of N(H)-substituted 5-nitro-1,4-naphthoquinones **1**–**13**

Synthesis of 2,3-Dichloro-5-nitro-1,4-naphthoquinone (1)

Dark yellow crystals, yield: 12 g (42%); R_f 0.3 (EtOAc/Hexane) (1:6 v/v). M.p. 151–152 °C (lit.[19] 156–157 °C); ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 7.81 (dd, H_1, J = 9.2, 0.98 Hz, 1H, H_{naphth}), 7.98 (t, H_2, J = 7.8 Hz, 1H, H_{naphth}), 8.42 (dd, H_3, J = 8.6, 0.98 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 121.95, 128.26, 130.04, 131.75, 135.53 (CH_{arom}, C_{arom}), 143.35 (=C-Cl), 143.82 (C-NO_2), 172.63, 174.29 (C=O). $C_{10}H_3Cl_2NO_4$ (M_w 272.04 g/mol). MS [+ESI]: m/z = 271.2 $[M]^+$.

Synthesis of 2-(4-(Benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-3-chloro-5-nitronaphthalene-1,4-dione (2)[9] and 3-(4-(Benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-2-chloro-5-nitronaphthalene-1,4-dione (3)[10]

Isomer compounds of **2** and **3** were obtained by reaction between 2,3-dichloro-5-nitro-1,4-naphthoquinone (**1**) and 1-piperonylpiperazine (**1a**) according to the general procedure 2. The mixture was purified by using column chromatography and mixture of ethyl acetate with hexane (1:3 ratio) was used as the mobile phase.

Isomer **2**: Red solid, R_f 0.80 (EtOAc /Hexane) (1:3 v/v). M.p. 131–132 °C.

Isomer **3**: Pink solid, R_f 0.73 (EtOAc /Hexane) (1:3 v/v). M.p. 89–91 °C.

Isomer **2**: Red solid, yield: 0.110 g (20%); R_f 0.80 (EtOAc/Hexane) (1:3 v/v). M.p. 131–132 °C (lit.[10] 131–132 °C); FTIR (cm^{-1}) ν 3094 (C-H_{arom}), 2912, 2809, 2772, 2659 (C-H_{aliph}), 1679, 1640 (C=O), 1590, 1555 (C=C), 1494, 1438 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 2.56 (br s, 4H, H_{piper}), 3.52 (s, 2H, CH_2), 3.64 (br s, 4H, H_{piper}), 5.96 (s, 2H, O-CH_2-O), 6.79–6.91 (m, 3H, CH_{arom}), 7.76–7.82 (m, 2H (H_1,H_2), H_{naphth}), 8.34 (dd, J = 9.27, 1.46 Hz, 1H (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 51.15, 53.31 (N-CH_2)$_{piper}$, 62.43 (CH_2), 101.12 (O-CH_2-O), 108.01, 109.56, 122.60, 127.07, 129.42, 130.49, 132.64, 134.17 (CH_{arom}, C_{arom}), 148.40 (C-NO_2), 150.40 (=C-N), 175.74, 179.07 (C=O). Anal. Calcd. for $C_{22}H_{18}N_3O_6Cl$ (M_w

455.85 g/mol): C, 57.97; H, 3.98; N, 9.22. Found: C, 58.31; H, 3.59; N, 9.12. MS [+ESI]: m/z = 456.0 $[M]^+$.

Isomer **3**: Pink solid, yield: 0.223 g (44 %); R_f 0.73 (EtOAc/Hexane) (1:3 v/v). M.p. 89–91°C (lit.[10] 89–91°C); FTIR (cm^{-1}) ν 3079 (C-H_{arom}), 2905, 2811, 2772 (C-H_{aliph}), 1676, 1644 (C=O), 1590, 1537 (C=C), 1492, 1439 (C_{arom}-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 2.52 (br s, 4H, H_{piper}), 3.62 (s, 2H, CH_2), 3.64 (br,s, 4H, H_{piper}), 5.97 (s, 2H, O-CH_2-O), 6.81–6.92 (m, 3H, CH_{arom}), 7.67 (dd, J = 9.2, 1.4 Hz, 1H (H_1), H_{naphth}), 7.81 (t, J = 7.8 Hz, 1H (H_2), H_{naphth}), 8.21 (dd, J = 9.2, 1.4 Hz, 1H (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 53.26 (N-CH_2), 62.39 (CH_2), 101.08 (O-CH_2-O), 108.07, 109.73, 122.67, 127.63, 129.16, 132.32, 133.70 (CH_{arom}, C_{arom}), 148.35 (C-NO_2), 149.33 (=C-N), 173.97, 179.84 (C=O). Anal. Calcd. for $C_{22}H_{18}N_3O_6Cl$ (M_w 455.85 g/mol): C, 57.97; H, 3.98; N, 9.22. Found: C, 58.21; H, 3.54; N, 9.15. MS [+ESI]: m/z = 456.0 $[M]^+$.

Synthesis of 3-Chloro-2-((2,4-dimethoxyphenyl)amino) -5-nitronaphthalene-1,4-dione (4) and 2-Chloro-3-((2,4-dimethoxyphenyl)amino)-5-nitronaphthalene-1,4 -dione (5)

According to the general procedure 2, 0.50 g (1.8 mmol) of 2,3-dichloro-5-nitro-1,4- naphthoquinone (**1**) was reacted with 0.30 g (2 mmol) of 2,4-dimethoxyaniline (**1b**) in 25 mL of ethanol at room temperature for 4 hours. The mixture was purified by column chromatography and mixture of ethyl acetate with hexane (1:4 ratio) was used as the mobile phase. Compounds **4** and **5** were obtained as a new regioisomer compounds.

Isomer **4**: Red solid, R_f 0.52 (EtOAc/Hexane) (1:4 v/v). M.p. 205–206 °C.

Isomer **5**: Red solid, R_f 0.35 (EtOAc/Hexane) (1:4 v/v). M.p. 198–199 °C.

Isomer **4**: Red solid, yield: 0.251 g (32%); R_f 0.52 (EtOAc/Hexane) (1:4 v/v). M.p. 205–206 °C; FTIR (cm^{-1}) ν 3316 (N-H), 3083 (C-H_{arom}), 2967, 2917 (C-H), 1675 (C=O), 1585, 1554 (C=C), 1534, 1370 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 3.84 (s, 6H, O-CH_3), 6.42–6.52 (m, 2H, H_{arom}), 6.93–7.02 (m, 2H, H_{arom}), 7.40 (s, 1H, N-H), 7.73 (d, J = 7.8 Hz, 1H, (H_1)H_{naphth}), 8.02 (t, J = 7.3 Hz, 1H, (H_2), H_{naphth}), 8.40 (d, J = 7.8 Hz, 1H, (H_3) H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 55.58 (O-CH_3), 98.65, 103.54 (CH_{arom}), 113.79 (=C-Cl), 119.17, 126.85, 128.30, 128.87, 130.88, 131.73, 135.60 (CH_{arom}, C_{arom}), 143.34 (C-NO_2), 148.93 (=C-N), 154.57, 159.64 (=**C**-OCH_3), 172.65, 174.28 (C=O). Anal. Calcd. for $C_{18}H_{13}ClN_2O_6$ (M_w 388.73 g/mol): C, 55.61; H, 3.37; N, 7.21. Found: C, 55.31; H, 3.49; N, 7.12. MS [+ESI]: m/z = 387.2 $[M–H]^+$.

Isomer **5**: Red solid, yield: 0.357 g (46%); R_f 0.35 (EtOAc/Hexane) (1:4 v/v). M.p. 198–199 °C; FTIR (cm^{-1}) ν 3308 (N-H), 3090 (C-H_{arom}), 2970 (C-H), 1679, 1640 (C=O), 1590, 1563 (C=C), 1455, 1339 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 3.82 (s, 6H, O-CH_3), 6.48 (dd, J = 6.9, 2.4 Hz, 2H, H_{arom}), 6.98–7.20 (m, 1H, H_{arom}), 7.54 (s, 1H, N-H), 7.69 (dd, J = 9.2, 1.4 Hz, 1H, (H_1), H_{naphth}), 7.87 (t, 7.8 Hz, 1H, (H_2), H_{naphth}), 8.29 (dd, J = 8.8, 1.2 Hz 1H, (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 55.68 (O-CH_3), 98.67, 103.60 (CH_{arom}), 113.34 (=C-Cl), 119.17, 123.71, 126.84, 128.95, 131.05, 133.37, 135.24 (CH_{arom}, C_{arom}), 142.86 (C-NO_2), 148.72 (=C-N), 154.11, 159.49 (=**C**-O-CH_3), 173.29, 178.67 (C=O). Anal. Calcd. for $C_{18}H_{13}ClN_2O_6$ (M_w 388.73 g/mol): C, 55.61; H, 3.37; N, 7.21. Found: C, 55.83; H, 3.71; N, 7.41. MS [+ESI]: m/z = 387.5 $[M–H]^+$.

Synthesis of 3-Chloro-2-((4-methoxyphenyl)amino)-5-nitronaphthalene-1,4-dione (6) and 2-Chloro-3-((4-methoxyphenyl)amino)-5-nitronaphthalene-1,4-dione (7)

According to the general procedure 2, 0.50 g (1.8 mmol) of 2,3-dichloro-5-nitro-1,4- naphthoquinone (**1**) was reacted with 0.22 g (1.8 mmol) of 4-methoxyaniline (**1c**) in 25 mL of ethanol at room temperature for 4 hours. The mixture was purified by column chromatography and mixture of ethyl acetate with hexane (1:4 ratio) was used as the mobile phase. Compounds **6** and **7** were obtained as a new regioisomer compounds.

Isomer **6**: Red solid, R_f 0.51 (EtOAc/Hexane) (1:4 v/v). M.p. 167–168 °C.

Isomer **7**: Red solid, R_f 0.33 (EtOAc/Hexane) (1:4 v/v). M.p. 129–131 °C.

Isomer **6**: Red solid, yield: 0.157 g (24%); R_f 0.51 (EtOAc/Hexane) (1:4 v/v). M.p. 167–168 °C; FTIR (cm^{-1}) ν 3226 (N-H), 3084 (C-H_{arom}), 2919, 2850 (C-H), 1684, 1636 (C=O), 1590, 1563 (C=C), 1532, 1376 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 3.85 (s, 3H, O-CH_3), 5.38 (br s, 1H, NH), 6.85–6.94 (m, 2H, H_{arom}), 7.04–7.10 (m, 2H, H_{arom}), 7.91 (dd, J = 7.9, 1.2 Hz, 1H, (H_1), H_{naphth}), 7. 97 (t, J = 7.8 Hz, 1H, (H_2), H_{naphth}), 8.33–8.42 (m, 1H, (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 55.53 (O-CH_3), 111.70 (CH_{arom}), 113.36 (=C-Cl), 119.17, 123.17, 126.84, 127.14, 128.95, 131.37, 135.24 (CH_{arom}, C_{arom}), 144.86 (C-NO_2), 148.93 (=C-N), 158.64 (=C-OCH_3), 173.29, 178.91 (C=O). Anal. Calcd. for $C_{17}H_{11}ClN_2O_3$ (M_w 358.73 g/mol): C, 56.92; H, 3.09; N, 7.81. Found: C, 56.79; H, 2.93; N, 7.63. MS [+ESI]: m/z = 357.1 $[M–H]^+$.

Isomer **7**: Red solid, yield: 0.355 g (54%); R_f 0.33 (EtOAc/Hexane) (1:4 v/v). M.p. 129–131 °C; FTIR (cm^{-1}) ν 3289 (N-H), 3102, 3002 (C-H_{arom}), 2918, 2843 (C-H), 1680, 1644 (C=O), 1590, 1542 (C=C), 1500, 1329 (C -NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 3.86 (s, 3H, O-CH_3), 6.15 (s, 1H, N-H), 6.74–6.98 (m, 2H, H_{arom}), 7.06–7.17 (m, 2H, H_{arom}), 7.63–7.74 (m, 2H, (H_1,H_2), H_{naphth}), 8.26-8.41 (m, 1H, (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 55.49 (O-CH_3), 112.04 (=C-Cl), 113.66 (CH_{arom}), 123.12, 126.60, 128.46, 129.17, 131.90, 132.01, 134.60 (CH_{arom}, C_{arom}), 144.25 (C-NO_2), 148.23 (=C-N), 157.36 (=C-O-CH_3), 173.66, 178.92 (C=O). Anal. Calcd. for $C_{17}H_{11}ClN_2O_3$ (M_w 358.73 g/mol): C, 56.92; H, 3.09; N, 7.81. Found: C, 57.13; H, 3.25; N, 7.52. MS [+ESI]: m/z = 357.2 $[M–H]^+$.

Synthesis of 3-Chloro-2-((2-morpholinoethyl)amino)-5-nitronaphthalene-1,4-dione (8) and 2-Chloro-3-((2-morpholinoethyl)amino)-5-nitronaphthalene-1,4 -dione (9)

According to the general procedure 2, 0.50 g (1.8 mmol) of 2,3-dichloro-5-nitro-1,4- naphthoquinone (**1**) was reacted with 0.24 g (1.8 mmol) of 4-(2-aminoethyl) morpholine (**1d**) in 25 mL of ethanol at room temperature for 4 hours. The mixture was purified by column chromatography and mixture of ethyl acetate with hexane (1:2 ratio) was used as the mobile phase. Compounds **8** and **9** were obtained as a new regioisomer compounds.

Isomer **8**: Red solid, R_f 0.50 (EtOAc/Hexane) (1:2 v/v). M.p. 178–179 °C.

Isomer **9**: Red solid, R_f 0.33 (EtOAc/Hexane) (1:2 v/v). M.p. 173–174 °C.

Isomer **8**: Red solid, yield: 0.231 g (34%); R_f 0.50 (EtOAc/Hexane) (1:2 v/v). M.p. 178–179 °C; FTIR (cm^{-1}) ν 3292 (N-H), 3096 (C-H_{arom}), 2918, 2852, 2813 (C-H), 1690 (C=O), 1591, 1561 (C=C), 1519, 1338 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 2.50 (t, *J* = 6.1 Hz, 4H, (CH_2-N-CH_2)$_{morph}$), 2.58 (t, *J* = 6.0 Hz, 2H, N-CH_2CH_2), 3.75 (t, *J* = 6.1 Hz, 4H, CH_2-O-CH_2), 3.85 (q, *J* = 6.1 Hz, 2H, HN-CH_2), 6. 94 (br s, 1H, N-H), 7.65 (dd, *J* = 7.9, 0.98 Hz, 1H, (H_1) H_{naphth}), 7.86 (t, *J* = 7.8 Hz, 1H, (H_2) H_{naphth}), 8.36 (dd, *J* = 7.8, 1.5 Hz, 1H, (H_3) H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 40.87 (NH-**C**H_2-CH_2), 52.93 (CH_2-N-CH_2)$_{morph}$, 56.50 (NH-CH_2-**C**H_2), 66.94 (CH_2-O-CH_2)$_{morph}$, 110.36 (=C-Cl), 123.22, 126.09, 129.02, 133.54, 135.24 (CH_{arom}, C_{arom}), 152.16 (C-NO_2), 158.93 (=C-N), 178.69, 181.51 (C=O). Anal. Calcd. for $C_{16}H_{16}N_3O_5Cl$ (M_w 365.77 g/mol): C, 52.54; H, 4.41; N, 11.49. Found: C, 52.67; H, 4.70; N, 11.50. MS [+ESI]: *m/z* = 366.2 $[M]^+$.

Isomer **9**: Red solid, yield: 0.221 g (32%); R_f 0.33 (EtOAc/Hexane) (1:2 v/v). M.p. 173–174 °C; FTIR (cm^{-1}) ν 3275 (N-H), 3080 (C-H_{arom}), 2957, 2894, 2822 (C-H), 1679, 1640 (C=O), 1609, 1572 (C=C), 1535, 1330 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 2.62 (br s, 4H, (CH_2-N-CH_2)$_{morph}$), 2.73 (br s, 2H, N-CH_2-CH_2), 3.70 (br s, 4H, CH_2-O-CH_2), 3.80–3.94 (m, 2H, HN-CH_2), 7. 06 (br s, 1H, N-H), 7.66 (dd, *J* = 7.9, 0.98 Hz, 1H, (H_1), H_{naphth}), 7.81 (t, *J* = 7.8 Hz, 1H, (H_2), H_{naphth}), 8.23 (dd, *J* = 7.8, 1.5 Hz, 1H, (H_3), H_{naphth}). ^{13}C NMR (125.66 MHz, DMSO-d_6) δ 40.49 (NH-CH_2-CH_2), 53.28 (CH_2-N-CH_2)$_{morph}$, 57.13 (NH-CH_2-CH_2), 66.45 (CH_2-O-CH_2)$_{morph}$, 98.63 (=C-Cl), 122.98, 128.71, 129.50, 133.30, 134.24 (CH_{arom}, C_{arom}), 148.83 (C-NO_2), 160.45 (=C-N), 175.26, 176.76 (C=O). Anal. Calcd. for $C_{16}H_{16}N_3O_5Cl$ (M_w 365.77 g/mol): C, 52.54; H, 4.41; N, 11.49. Found: C, 52.83; H, 4.13; N, 11.08. MS [+ESI]: *m/z* = 366.5 $[M]^+$.

Synthesis of 3-Chloro-5-nitro-2-((2-(pyrrolidin-1-yl) ethyl)amino)naphthalene-1,4-dione (10) and 2-Chloro-5-nitro-3-((2-(pyrrolidin-1-yl)ethyl)amino)naphthalene-1,4-dione (11)

According to the procedure 2, 0.50 g (1.8 mmol) of 2,3-dichloro-5-nitro-1,4- naphthoquinone (**1**) was reacted with 0.24 g (1.8 mmol) of 2-(pyrrodin-1-yl)ethane-1-amine (**1e**) in 25 mL of ethanol at room temperature for 4 hours. The mixture was purified by column chromatography and mixture of ethyl acetate with hexane (1:2 ratio) was used as the mobile phase. Compounds **10** and **11** were obtained as a new regioisomer compounds.

Isomer **10**: Red solid, R_f 0.71 (EtOAc/Hexane) (1:2 v/v). M.p. 146–147 °C

Isomer **11**: Red solid, R_f 0.33 (EtOAc/Hexane) (1:2 v/v). M.p. 132–133 °C

Isomer **10**: Red solid, yield: 0.280 g (46%); R_f 0.71 (EtOAc/Hexane) (1:2 v/v). M.p. 146–147 °C; FTIR (cm^{-1}) ν 3281 (N-H), 3112 (C-H_{arom}), 2958, 2851 (C-H), 1681 (C=O), 1591, 1559 (C=C), 1525, 1335 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 1.72 (br s, 4H, (CH_2-CH_2)$_{pyrro}$), 2.51 (br s, 4H, (CH_2-N-CH_2)$_{pyrro}$), 2.61–2.70 (m, 2H, CH_2-N), 3.82 (br s, 2H, HN-CH_2), 6.96 (br s, 1H, N-H), 7.61 (d, *J* = 7.8 Hz, (H_1), 1H, H_{naphth}), 7.79 (t, *J* = 7.8 Hz, 1H, (H_2), H_{naphth}), 8.32 (d, *J* = 7.8 Hz, 1H, (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 23.34 (CH_2-CH_2)$_{pyrro}$, 43.24 (**N**-CH_2), 54.11 (CH_2-N-CH_2)$_{pyrro}$, 55.32 (HN-CH_2), 109.98 (=C-Cl), 126.02, 128.88, 130.91, 133.43, 135.37 (C_{arom}, CH_{arom}), 148.23 (=C-N) 173.95, 177.32 (C=O). Anal. Calcd. for $C_{16}H_{16}ClN_3O_4$ (M_w 363.80 g/mol): C, 54.94; H, 4.61; N, 12.01. Found: C, 54.64; H, 4.39; N, 11.77. MS [+ESI]: *m/z* = 349.5 $[M]^+$.

Isomer **11**: Red solid, yield: 0.220 g (35%); R_f 0.33 (EtOAc/Hexane) (1:2 v/v). M.p. 132–133 °C. FTIR (cm^{-1}) ν 3241 (N-H), 3072 (C-H_{arom}), 2968 (C-H), 1681 (C=O), 1590 (C=C), 1531, 1372 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 1.50 (br s, 4H, CH_2-CH_2)$_{pyrro}$, 2.48 (br s, 4H, CH_2-N-CH_2)$_{pyrro}$, 2.58–2.69 (m, 2H, (CH_2-N), 3.60 (br s, 2H, HN-CH_2), 6.73 (br s, 1H, N-H), 7.47 (d, *J* = 7.8 Hz, 1H, (H_1), H_{naphth}), 7.76 (t, *J* = 7.8 Hz, 1H, (H_2), H_{naphth}), 8.29 (d, *J* = 7.8 Hz, 1H, (H_3), H_{naphth}). ^{13}C NMR (125.66 MHz, DMSO-d_6) δ 23.83 (**C**H_2-**C**H_2)$_{pyrro}$, 45.17 (**N**-CH_2), 54.05 (**C**H_2-N-**C**H_2)$_{pyrro}$, 60.90 (HN-**C**H_2), 103.93 (=C-Cl), 122.02, 126.05, 128.16, 131.35, 136.42 (C_{arom}, CH_{arom}), 148.03 (=C-N), 166.72 (C-NO_2), 172.46, 181.90 (C=O). Anal. Calcd. for $C_{16}H_{16}ClN_3O_4$ (M_w 349.77 g/mol): C, 54.94; H, 4.61; N, 12.01. Found: C, 55.03; H, 4.81; N, 12.29. MS [+ESI]: *m/z* = 348.5 $[M]^+$.

Synthesis of 2-(4-Benzylpiperidin-1-yl)-3-chloro-5-nitronaphthalene-1,4-dione (12) and 3-(4-Benzylpiperidin-1-yl)-2-chloro-5-nitronaphthalene-1,4-dione (13)

According to the general procedure 2, 0.50 g (1.8 mmol) of 2,3-dichloro-5-nitro-1,4- naphthoquinone (**1**) was reacted with 0.32 g (1.8 mmol) of 4- benzylpiperidine (**1f**) in 25 mL of ethanol at room temperature for 4 hours. The mixture was purified by column chromatography and mixture of chloroform with petroleum ether (1:1 ratio) was used as the mobile phase. Compounds **12** and **13** were obtained as a new regioisomer compounds.

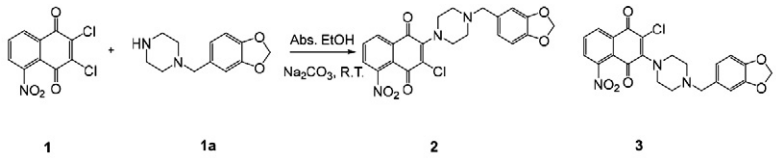
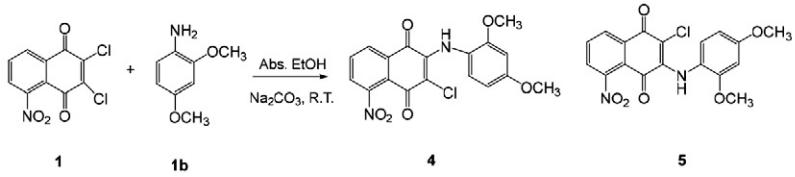
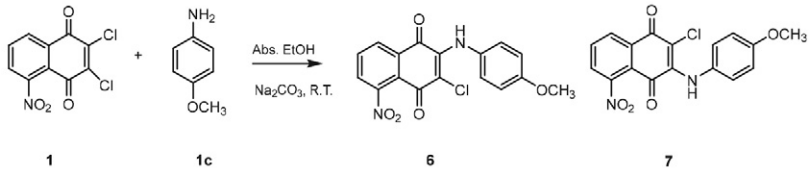
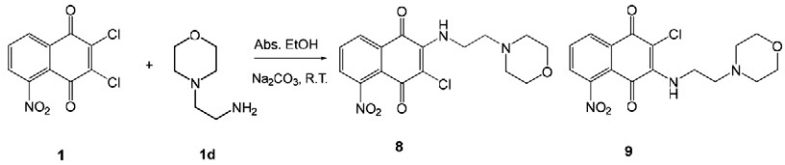
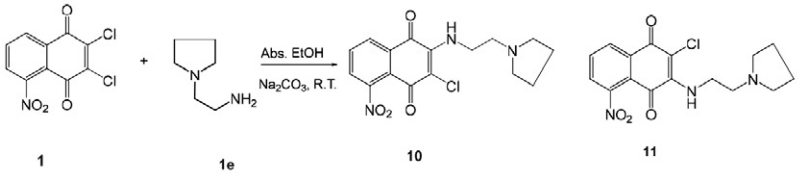
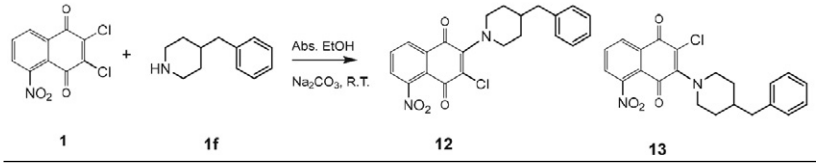
Isomer **12**: Red solid, R_f 0.46 ($CHCl_3$/PE) (1:1 v/v). M.p. 153–154 °C.

Isomer **13**: Red oil, R_f 0.37 ($CHCl_3$/PE) (1:1 v/v).

Isomer **12**: Red solid, yield: 0.332 g (44 %); R_f 0.46 ($CHCl_3$/PE) (1:1 v/v). M.p. 153–154 °C; FTIR (cm^{-1}) ν 2952, 2920, 2853 (C-H), 1718 (C=O), 1606, 1594 (C=C), 1453, 1375 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 1.46–1.59 (m, 2H, $(CH_2)_{piperi}$), 1.66–1.93 (m, 3H, $(CH_2$-$CH)_{piperi}$), 2.53–2.71 (m, 2H, (CH_2), 3.12–3.19 (m, 2H, (N-$CH_2)_{piperi}$), 3.69–3.81 (m, 2H, (N-$CH_2)_{piperi}$, 7.07–7.25 (m 5H, H_{arom}), 7.70–7.74 (m, 2H, (H_1,H_2), H_{naphth}), 8.26–8.37 (dd, J = 2.4, 6.8 Hz, 1H, H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 33.06 ($\mathbf{C}H_2$-CH-$\mathbf{C}H_2)_{piperi}$, 37.65 (CH_2-$\mathbf{C}H$-$CH_2)_{piperi}$, 42.95 (CH_2), 52.20 (CH_2-N-$CH_2)_{piperi}$, 119.98 (=C-Cl), 125.79, 126.95, 128.27, 129.08, 130.02, 132.80, 135.51, 140.00 (C_{arom}, CH_{arom}), 151.10 (=C-N), 175.69, 179.36 (C=O). Anal. Calcd. for $C_{22}H_{19}ClN_2O_4$ (M_w 410.85 g/mol): C, 64.32; H, 4.66; N, 6.82. Found: C, 64.64; H, 5.02; N, 6.72. MS [+ESI]: m/z = 411.1 $[M]^+$, 433.2 $[M+Na]^+$.

Isomer **13**: Red oil, yield: 0.226 g (30 %); R_f 0.37 ($CHCl_3$/PE) (1:1 v/v). FTIR (cm^{-1}) ν 3060 (C-H_{arom}), 2970, 2919 (C-H), 1717, 1664 (C=O), 1592, 1561 (C=C), 1452, 1329 (C-NO_2). ^{1}H NMR (499.74 MHz, $CDCl_3$) δ 1.38–1.43 (m, 2H, $(CH_2)_{piper.}$), 1.69–1.81 (m, 3H, $(CH_2$-$CH)_{piper.}$), 2.50–2.55 (m, 2H, (CH_2), 3.12–3.18 (m, 2H, (N-$CH_2)_{piper.}$), 3.77–3.80 (m, 2H, (N-$CH_2)_{piper.}$, 7.05–7.25 (m 5H, H_{arom}), 7.62–7.70 (m, 1H, (H_1), H_{naphth}), 7.75–7.85 (m, 1H, $(H_2)H_{naphth}$) 8.22 (d, J = 7.8 Hz, 1H, (H_3), H_{naphth}). ^{13}C(APT) NMR (125.66 MHz, $CDCl_3$) δ 33.05 (CH_2-CH-$CH_2)_{piperi}$, 37.60 (CH_2-$\mathbf{C}H$-$CH_2)_{piper.}$, 42.92 (CH_2), 52.20 (CH_2-N-$CH_2)_{piperi}$, 119.90 (=C-Cl), 122.70, 126.08, 127.01, 128.33, 129.63, 133.61, 134.14, 139.87 (C_{arom}, CH_{arom}), 148.23 (C-NO_2), 151.11 (=C-N), 173.89, 179.35 (C=O).

Table 1. The reaction pathway and obtained regioisomeric products **2**–**13**

Reactions and conditions	**Colour, R_f, yield (%), m.p. (°C)**
 **1** **1a** **2** **3**	**(2):** Red solid, R_f: 0.80, yield 20%, m.p. 131–132 **(3):** Pink solid, R_f: 0.37, yield 44%, m.p. 89–91
 **1** **1b** **4** **5**	**(4):** Red solid, R_f: 0.52, yield 32%, m.p. 205–206 **(5):** Red solid, R_f: 0.35, yield 46%, m.p. 198–199
 **1** **1c** **6** **7**	**(6):** Red solid, R_f: 0.51, yield 24%, m.p. 167–168 **(7):** Red solid, R_f: 0.33, yield 54%, m.p. 129–131
 **1** **1d** **8** **9**	**(8):** Red solid, R_f: 0.50, yield 34%, m.p. 178–179 **(9):** Red solid, R_f: 0.33, yield 32%, m.p. 173–174
 **1** **1e** **10** **11**	**(10):** Red solid, R_f: 0.71, yield 46%, m.p.146–147 **(11):** Red solid, R_f: 0.33, yield 35%, m.p.132–133
 **1** **1f** **12** **13**	**(12):** Red solid, R_f: 0.46, yield 44%, m.p.153–154 **(13):** Red oil R_f: 0.37, yield 30%

Anal. Calcd. for $C_{22}H_{19}ClN_2O_4$ (M_w 410.85 g/mol): C, 64.32; H, 4.66; N, 6.82. Found: C, 64.60; H, 4.66; N, 7.16. MS [+ESI]: m/z = 411.2 $[M]^+$, 433.1 $[M+Na]^+$.

3. Results and Discussion

3. 1. Chemistry and Spectral Study

The new regioisomers **2**–**13** were synthesized by the reactions of 2,3-dichloro-5-nitro-1,4-naphthoquinone (**1**) with some nucleophiles such as amines, piperazines, or morpholines (**1a**–**f**) according to a Michael 1,4-addition mechanism and reaction pathways of synthesizes are illustrated in Table 1. The regioisomers were separated by column chromatography by using a different ratio of solvents. The obtained regioisomers have different color, melting point, retention factor (R_f) and chemical shifts of naphthoquinone ring protons in ^{1}H NMR spectra.

The ^{1}H NMR spectra of the synthesized new regioisomers indicate that the peaks of the 2-N-substituted-3-chloro-5-nitro-naphthalene-1,4-dione isomer of aromatic protons (H_{1-3}) are shifted more downfield than the aromatic protons of the 3-N-substituted-2-chloro-5-nitro-naphthalene-1,4-dione isomer. Also, it has been found that in the case of mixture of 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 values published in the literature.[15–16, 24] As a comparison between the ^{1}H NMR spectra of the synthesized new regioisomers **4** and **5**; we found that the protons of the naphthoquinone (H_{1-3}) peaks (δ 7.73–8.40 ppm) for the first isomer 3-chloro-2-((2,4-dimethoxyphenyl)amino)-5-nitronaphthalene-1,4-dione (**4**) are shifted more downfield than the naphthoquinone peaks (δ 7.69–8.29 ppm) of the second isomer 2-chloro-3-((2,4-dimethoxyphenyl)amino)-5-nitronaphthalene-1,4-dione (**5**). Also, it was found that the isomer **4** has a higher R_f component than the isomer **5**. The comparison of R_f values is consistent with the related literature.[15] In APT-NMR spectra signals of methoxy group (CH_3-O) and carbonyl group for isomer **4** were detected at δ 55.58 and δ 172.65, 174.28 ppm, while in isomer **5** at δ 55.68 and δ 173.29, 178.67 ppm, respectively.

3. 2. ^{1}H–^{1}H Correlated Spectroscopy (COSY)

The structures of these 5-nitro-1,4-naphthoquinone (**1**) and regioisomers of 5-nitro-1,4-naphthoquinone (**2** and **3**) were 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 (**1**) were assigned in the ^{1}H NMR spectrum (Figure 2) and confirmed by the ^{1}H–^{1}H COSY (Figure 3). In the ^{1}H NMR spectrum of compound **1**, a doublet of doublet (dd) at 7.81 ppm corresponding to H_1 that are coupled to H_2 (t, 7.98, 1H, $^3J_{H,H}$ = 9.27 Hz), and to H_3 (dd, 8.42, 1H, $^3J_{H,H}$ = 9.27 Hz) (Figure 2).

All these hydrogens were assigned on the basis of the ^{1}H–^{1}H COSY spectrum, where can be observed that H_1 is coupled to H_2 and H_3, H_2 to H_1 and H_3, and H_3 to H_2 and H_1. From ^{1}H–^{1}H COSY contour map these hydrogens are coupled to each other (Figure 3).

The hydrogen signals at the quinone and piperonylpiperazine ring of compounds **2** and **3** were assigned in the ^{1}H NMR spectrum (Figures 4 and 7) and also confirmed by the ^{1}H–^{1}H COSY spectrum (Figures 5, 6 and 8,

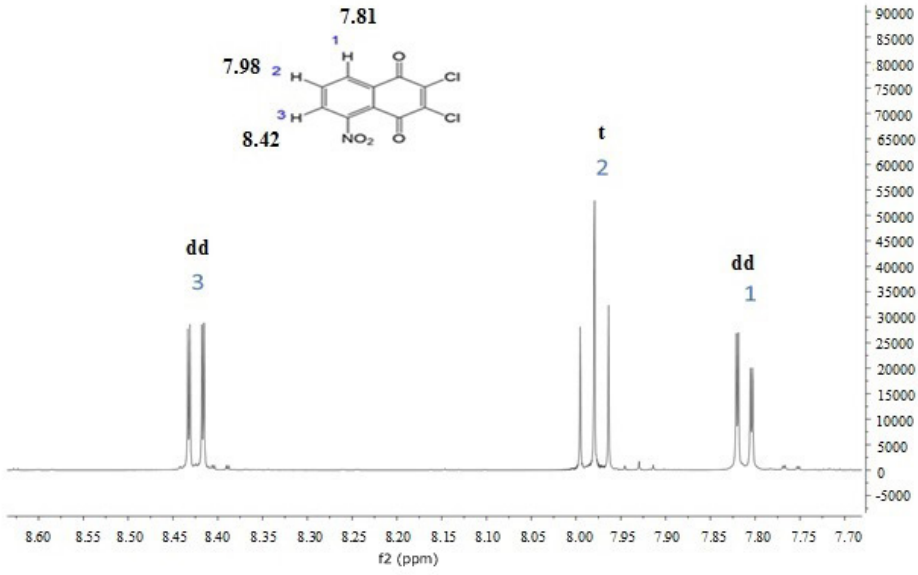


Figure 2. ^{1}H NMR spectrum of compound **1**

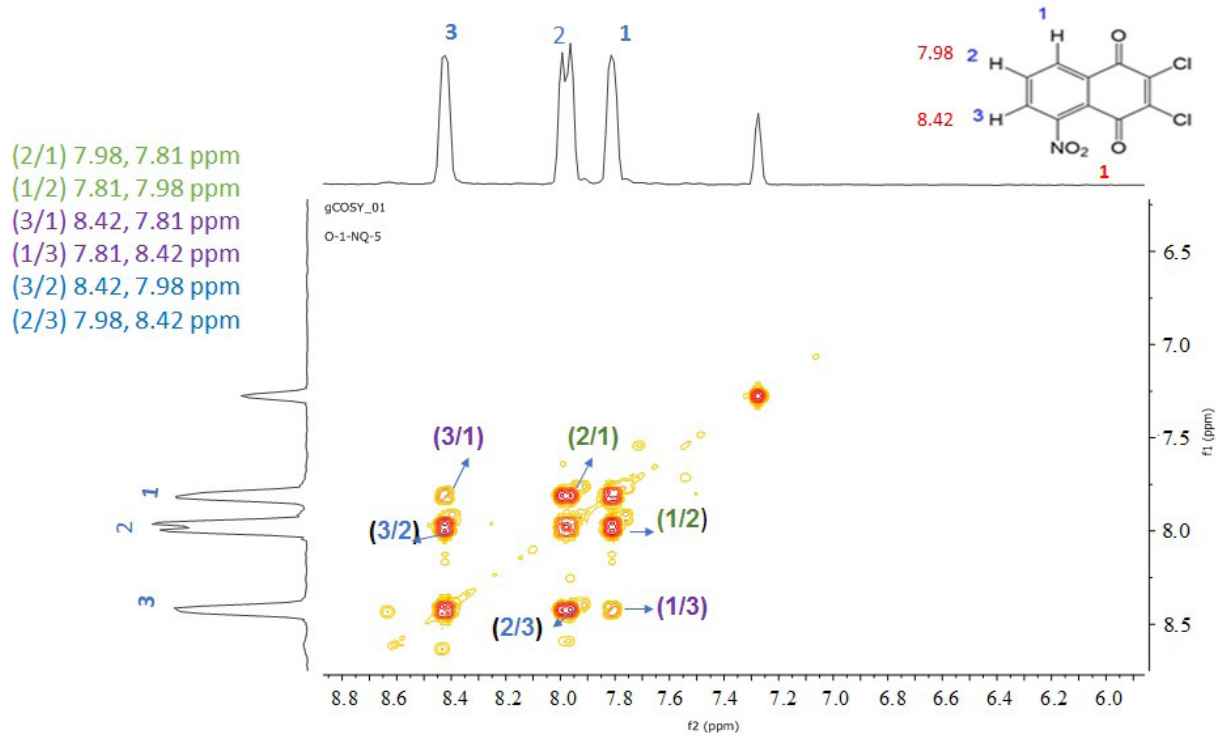


Figure 3. ^{1}H–^{1}H COSY contour map of compound **1**

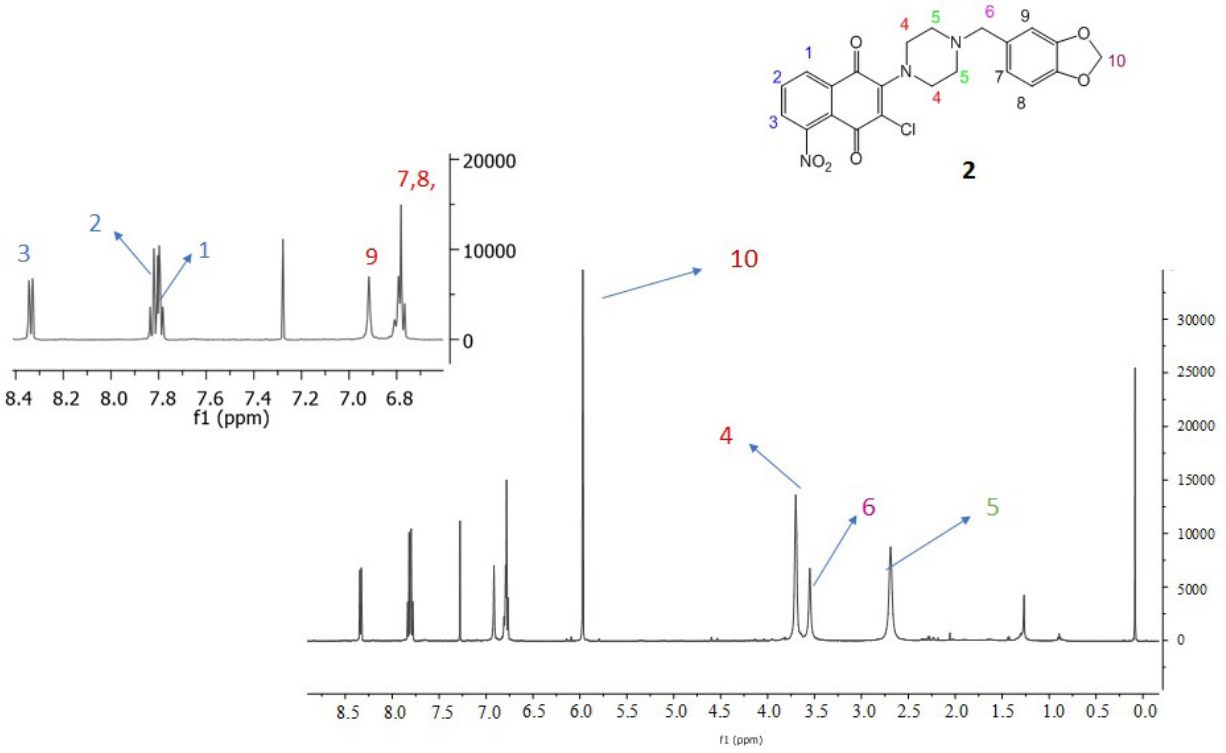


Figure 4. The hydrogen signals at the quinone and piperonylpiperazine ring of compound **2** in ^{1}H NMR spectrum

9). For compound **2** a multiplet at 7.76–7.82 corresponding to H_1 and H_2 that are coupled to H_3 (8.34, dd, $^{3}J_{H,H}$ = 9.27 Hz). As expected, H_4 and H_5 at piperizine ring appear as broad singlets at 3.64 and 2.56 ppm, respectively. A multiplet at 6.79–6.91 ppm corresponds to H_7 and H_8, respectively. In the ^{1}H NMR spectrum of compound **3**, a doublet of doublet (dd) at 7.67 ppm corresponding to H_1 that is coupled to H_2 (t, 7.81, 1H, $^{3}J_{H,H}$ = 7.8 Hz), and to H_3 (dd,

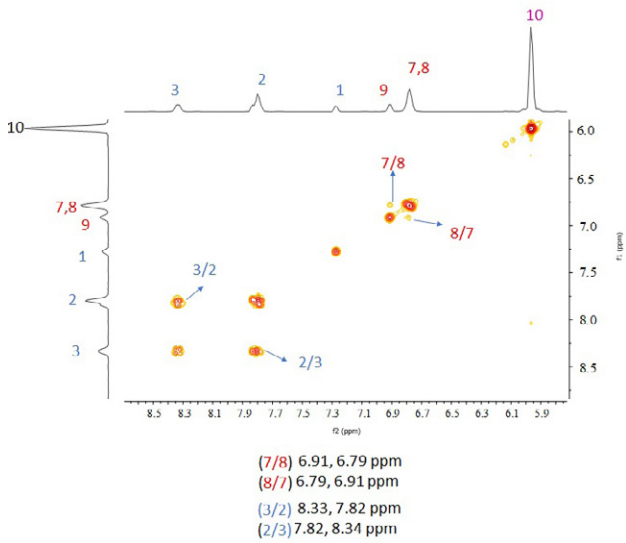


Figure 5. ^{1}H–^{1}H COSY contour map of aromatic protons of compound **2**

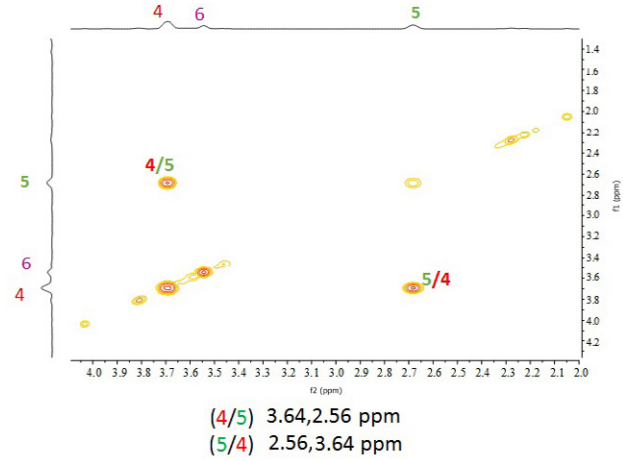


Figure 6. ^{1}H–^{1}H COSY contour map of piperazine ring protons of compound **2**

8.21, 1H, $^{3}J_{H,H}$ = 9.20 Hz) was observed. H_4 and H_5 appear as broad singlets at 3.64 and 2.52 ppm, respectively. A multiplet at 6.81–6.92 ppm corresponds to H_7 and H_8, respectively (Figures 4 and 7).

The interaction of hydrogens of 1, 2, 3, 7, 8 and 4, 5 in compound **2** and **3** were observed on the basis of the ^{1}H–^{1}H COSY spectrums, as can be seen from Figures 5, 8 and Figures 6, 9. H_3 is coupled to H_2, H_2 to H_3, and H_7 is coupled to H_8, H_8 to H_7 and H_4 is coupled to H_5, H_5 to H_4 for compound **2** (Figures 5, 6). As can be observed from Figure 8, H_3 is coupled to H_1 and H_2, H_2 to H_3 and H_1, and H_1 is coupled to H_2 and H_3 also H_7 coupled to H_8, H_8 to H_7. As can be seen in Figure 9, H_4 is coupled to H_5 and H_5 to H_4 for compound **3**. Chemical shifts in ppm of the above mentioned hydrogens are indicated in the figures.

3. 3. CUPRAC Antioxidant Capacities

NQ compounds were assayed using the normal CUPRAC assay (at 25 °C) against trolox (TR) as the reference standard.[18] $TEAC_{CUPRAC}$ coefficients are defined as the ratio of the slope of the curve of the tested compounds to that of TR (Table 2). LODs for synthesized compounds with respect to CUPRAC method were established between 0.74–7.37 μM (n = 10) and RSD% were found to be

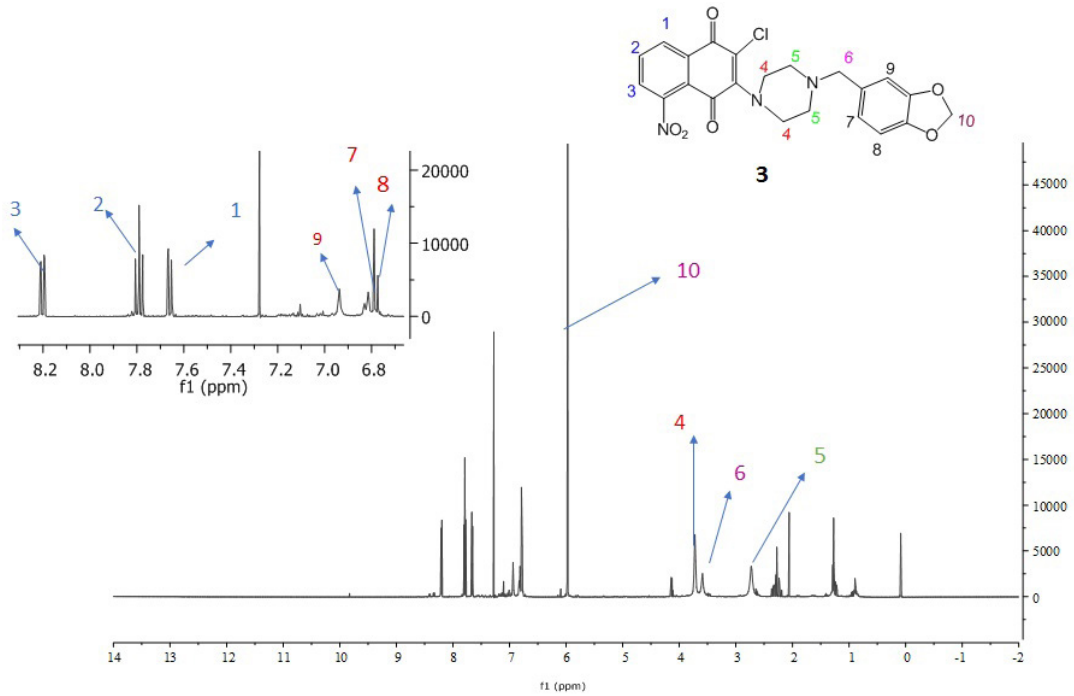


Figure 7. The hydrogen signals at the quinone and piperonylpiperazine ring of compound **3** in ^{1}H NMR spectrum

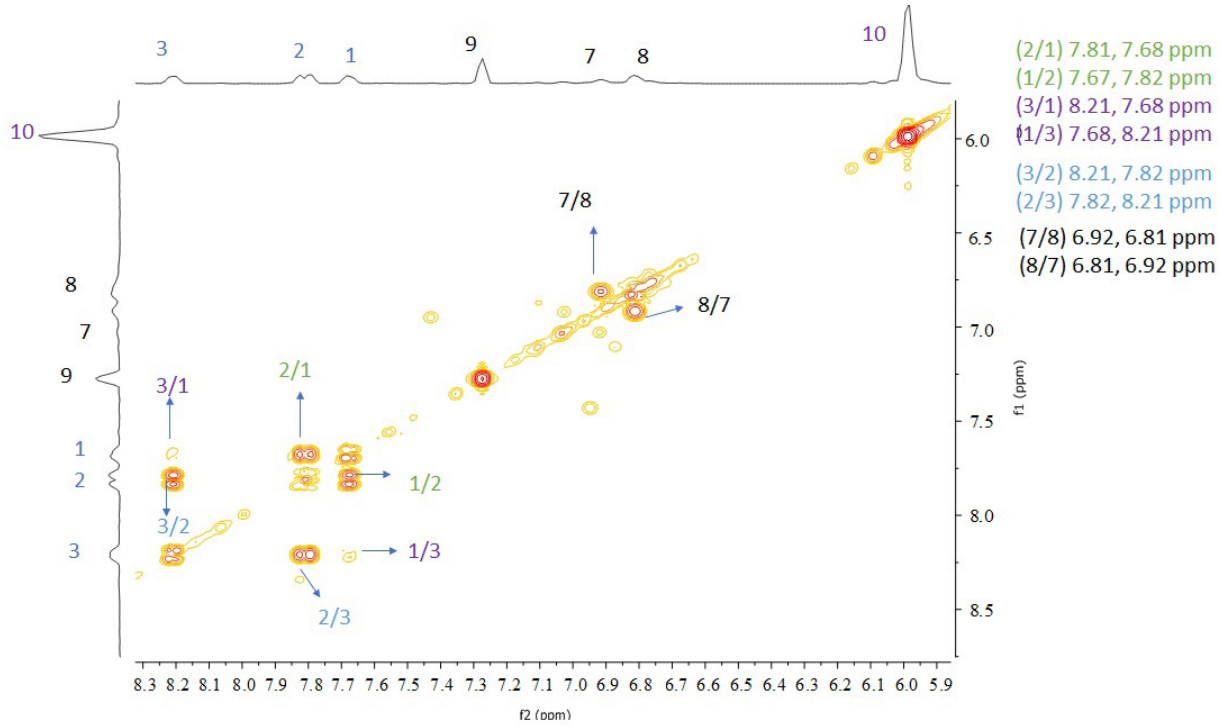


Figure 8. ^{1}H–^{1}H COSY contour map of aromatic protons of compound **3**

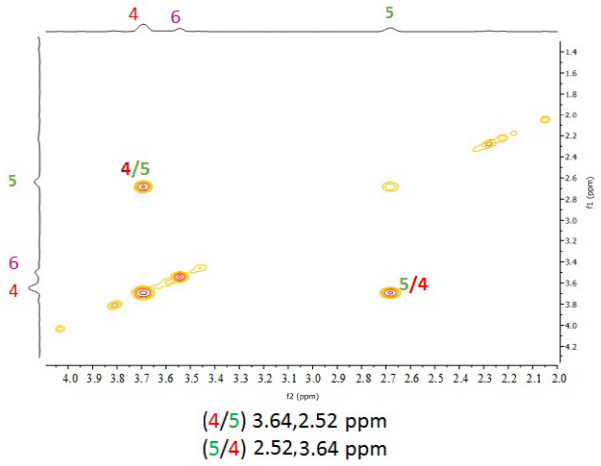


Figure 9. ^{1}H–^{1}H COSY contour map of piperazine ring protons of compound **3**

less than 4%. Among all the compounds synthesized, compound **5** showed the highest antioxidant potency ($TEAC_{CUPRAC}$ = 1.80±0.06). The $TEAC_{CUPRAC}$ coefficient of compound **5** was also higher than unity ($TEAC_{TR}$ = 1.0). Since $TEAC_{CUPRAC}$ coefficient of ascorbic acid (for comparison)[19] and compound **7** is close to 1.0, their antioxidant power are approximately equal to that of TR.

Five NQ regioisomer couples having different functional group (2-chloro-isomers **3**, **5**, **7**, **9** and **13**, as well as 3-chloro-isomers **2**, **4**, **6**, **8** and **12**) are reported here (Table 2). Although all isomer couples are just regioisomers, very interesting and dramatic differences in biological activities have been observed. Antioxidant capacity result of isomers showed that it is directly related to the bonding of N-nucleophiles at the 2 or 3 position on the naphthoquinone ring. As can be seen from the Table 2, the capacity results of all 2-chloro regioisomers are higher than the 3-chloro regioisomers. Surprisingly, two isomer pairs having –OCH_3 functional group also attracted attention in these results. Interestingly, despite having the same functional group, isomer **6** and **7**, if we compare the antioxidant result of the two isomers, we see that the difference is more than two times. Likewise, there is a remarkable difference in capacity result of isomers **4** and **5**. As shown in Table 2, **4** and **5** had stronger antioxidant capacity than **6** and **7**. These results may be related to that the larger the number of the –OCH_3 groups in the same structure, the higher is the antioxidant capacity of a molecule.[25]

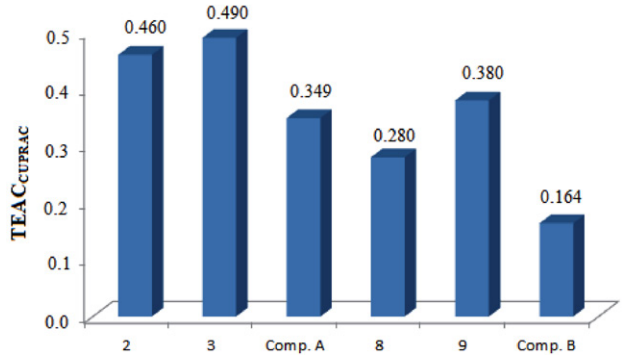


Figure 10. The comparison of TEAC coefficients of derivatives with and without NO_2 substituents (**2**, **3**, compound **A**, **8**, **9** and compound **B**)

Table 2. The linear calibration equations, correlation coefficients, linear concentration ranges, and TEAC coefficients of the NQ compounds using CUPRAC method.

Compounds	Linear range (mol L^{-1})	Calibration equation	r	$TEAC_{CUPRAC}$[a]
2	3.28×10^{-5} – 1.05×10^{-4}	$A = 7.60\times10^{3}\ c + 0.12$	0.992	0.46±0.02
3	1.23×10^{-6} – 1.42×10^{-4}	$A = 8.10\times10^{3}\ c + 0.05$	0.988	0.49±0.02
4	4.09×10^{-6} – 4.75×10^{-5}	$A = 2.44\times10^{4}\ c + 0.04$	0.989	1.46±0.07
5	1.26×10^{-6} – 3.51×10^{-5}	$A = 3.16\times10^{4}\ c + 0.09$	0.992	1.80±0.06
6	2.89×10^{-6} – 1.63×10^{-4}	$A = 6.90\times10^{3}\ c + 0.07$	0.990	0.41±0.01
7	4.45×10^{-6} – 6.88×10^{-5}	$A = 1.57\times10^{4}\ c + 0.06$	0.991	0.94±0.04
8	2.13×10^{-6} – 2.47×10^{-4}	$A = 4.68\times10^{3}\ c + 0.04$	0.989	0.28±0.01
9	2.05×10^{-5} – 1.61×10^{-4}	$A = 6.32\times10^{3}\ c + 0.18$	0.991	0.38±0.02
10	3.77×10^{-6} – 1.40×10^{-4}	$A = 7.95\times10^{3}\ c + 0.08$	0.990	0.48±0.03
12	1.39×10^{-5} – 2.54×10^{-4}	$A = 4.29\times10^{3}\ c + 0.11$	0.988	0.26±0.01
13	1.76×10^{-6} – 2.03×10^{-4}	$A = 5.69\times10^{3}\ c + 0.04$	0.991	0.34±0.02

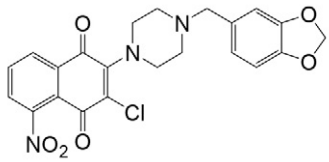
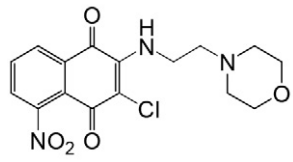
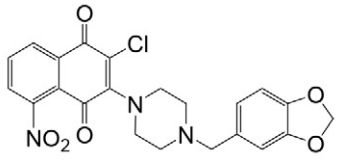
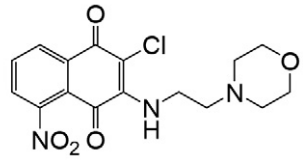
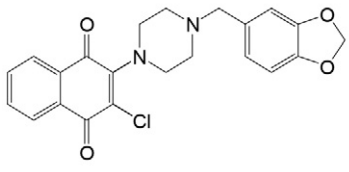
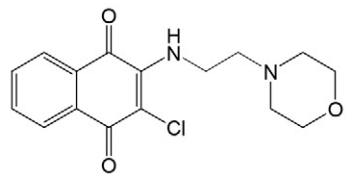
[a] $TEAC_{compound} = \varepsilon_{compound} / \varepsilon_{TR}$ $\varepsilon_{TR} = 1.67\times10^{4}$ $Lmol^{-1}cm^{-1}$ (DMSO).

A few years ago, our research group synthesized two derivatives not substituted with nitro groups on the aromatic skeleton (compounds **A** and **B**) and reported their antioxidant results.[4] Comparing these results with those for the regioisomers **2**, **3** and **8**, **9** (Table 3 and Figure 10), it is evident that the effect of the electron-withdrawing nitro group in the system is directly reflected on the antioxidant results and higher antioxidant capacities were obtained for nitro derivatives. Regiosomers **8** and **9** showed approximately twice as high antioxidant activity, while regioisomers **2** and **3** showed approximately half times higher antioxidant activity than compounds **B** and **A**, respectively.

3. 4 Catalase Activity

The screening of NQ compounds against the catalase revealed that most of the compounds have moderate inhibition activity of this enzyme (> 0.7 U/mL, Figure 11). Enzyme activity results were determined in accordance with the antioxidant activity results. As can be seen in Figure 11, generally the enzyme inhibitory activities results of 2-chloro regioisomers are higher than for the 3-chloro regioisomers except for one regioisomer pair and compounds **5** and **7** revealed significant inhibition activity.

Table 3. Comparison of antioxidant results of substituted NO_2 and unsubstituted NO_2 derivatives of NQ according to CUPRAC method

Compounds	$TEAC_{CUPRAC}$[a]	Compounds	$TEAC_{CUPRAC}$[a]
 **2**	0.46±0.02	 **8**	0.28±0.01
 **3**	0.49±0.02	 **9**	0.38±0.02
 Compound **A**[22]	0.349±0.02[22]	 Compound **B**[22]	0.164±0.02[22]

[a] $TEAC_{compound} = \varepsilon_{compound} / \varepsilon_{TR}$ $\varepsilon_{TR} = 1.67\times10^{4}$ $Lmol^{-1}cm^{-1}$ (DMSO).

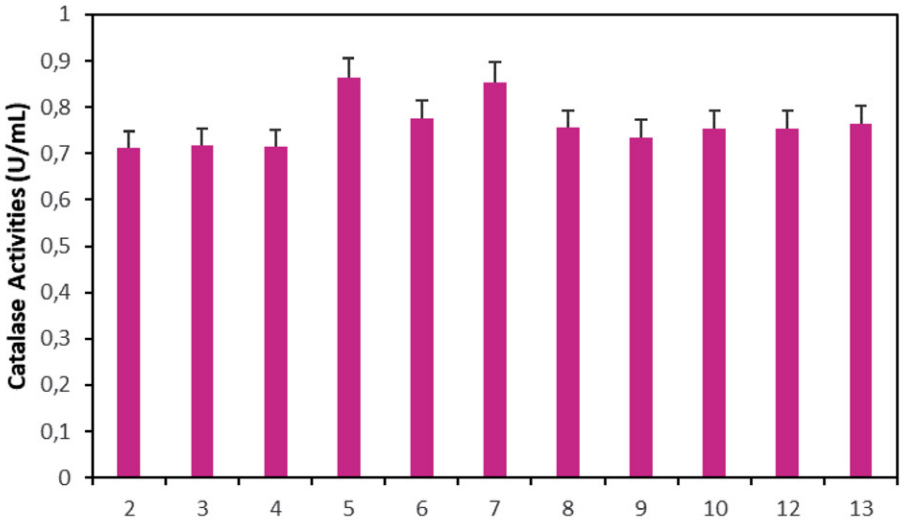


Figure 11. Catalase enzyme activities (U mL^{-1}) of the novel NQ compounds.

4. Conclusions

The studies on nitronaphthoquinone derivatives are rare in the literature and 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 this reason, 2,3-dichloro-5-nitro-1,4-naphthoquinone (**1**) was used as the starting material in this study. The new regioisomeric compounds of 5-nitro-1,4-naphthoquinone **2**–**13** were synthesized by the reactions of 2,3-dichloro-5-nitro-1,4-naphthoquinone with some heterocyclic ring substituted nucleophiles according to a Michael 1,4-addition mechanism. All newly synthesized compounds were characterized by electrospray ionisation mass spectrometry (ESI-MS), Fourier transform infrared spectroscopy (FTIR), ^{1}H nuclear magnetic resonance (^{1}H NMR), attached proton test nuclear magnetic resonance (APT-NMR). Two-dimensional technique ^{1}H–^{1}H correlated spectroscopy (COSY) was used for characterization of compound **1** and regioisomers **2** and **3**. Their *in vitro* antioxidant capacity and catalase enzyme inhibition activity were investigated. The effect of the electron-withdrawing nitro group in the system was directly reflected on the antioxidant results and higher antioxidant capacities were obtained. The compounds **4** and **5** showed comparable antioxidant potency to ascorbic acid. The antioxidant capacity results of all 2-chloro regioisomers are higher than for the 3-chloro regioisomers. Likewise, also catalase enzyme inhibitory activities results were determined in the same way, except for one regioisomer pair.

Supplementary Information (SI)

Supplementary information for this article is available at the journal web site.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

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Povzetek

Raziskave nitronaftakinonskih derivatov so v literaturi redke, čeprav je znano, da prisotnost nitro skupine na aromatskem obroču zaradi svojih elektron-privlačnih lastnosti skupaj s kinonskim sistemom poveča biološko aktivnost tovrstnih naftokinonskih sistemov. Z reakcijo med 2,3-dikloro-5-nitro-1,4-naftokinonom in različnimi nukleofili, substituiranimi s heterocikličnimi fragmenti, kot so anilini, piperazini in morfolini, smo s pomočjo Michaelove 1,4-adicije sintetizirali nove regioizomere N(H)-substituiranih-5-nitro-1,4-naftokinonov (NQ). Poročamo o petih regioizomernih parih NQ z različnimi funkcionalnimi skupinami, ki se ločijo po položajih klorovega substituenta (2-kloro izomeri **3**, **5**, **7**, **9** in **13** ter 3-kloro izomeri **2**, **4**, **6**, **8** in **12**). Vse nove spojine smo karakterizirali s spektroskopskimi metodami in dvodimenzionalno NMR tehniko ^{1}H–^{1}H korelacijske spektroskopije (COSY).

Pripravljenim NQ regioizomerom smo določili inhibitorne aktivnosti na encimu katalaza. S pomočjo metode bakrove redoks antioksidativne kapacitete (CUPRAC) smo določili njihovo antioksidativno delovanje. 2-Kloro-3-((2,4-dimetoksifenil)amino)-5-nitronaftalen-1,4-dion (**5**) se je izkazal z najvišjo antioksidativno kapaciteto in sicer je koeficient CUPRAC-troloks antioksidativne kapacitete (TEAC) znašal 1.80±0.06. Spojina **5** je izkazala tudi najmočnejšo inhibitorno aktivnost na encim katalaza. Ugotovili smo, da je antioksidativna kapaciteta za vse 2-kloro regioizomere večja kot za 3-kloro regioizomere. Za vse spojine, razen za en regioizomerni par, so bili analogni tudi rezultati za inhibitorno aktivnost na encim katalaza. Nove spojine so učinkovito inhibirale katalazo, odstotek inhibicije je bil v območju 0.71–0.86 %. Nekatere izmed teh NQ spojin so pokazale precejšnje antioksidativne kapacitete.