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# Synthesis and Antioxidant Activity of Novel Series of Naphthoquinone Derivatives Attached to Benzothiophene Moiety

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**Abstract**

A series of aryl azonaphthoquinones 8a-e were obtained *via* coupling of the corresponding diazonium salts 7a-c with naphthoquinones 2 or 3 in pyridine. Moreover, treatment of 5 with 6a, b in ethanol containing potassium carbonate afforded the corresponding enaminones 9a, b, respectively. Furthermore, heating of 6a with 2-hydroxy-3-((piperidn-1-yl)methyl)naphthalene-1,4-dione 10 in EtOH/AcOHor sodium 3,4-dihydro-3,4-dioxonaphthalene-1-sulphonate 1 in MeOH/$H_2O$ afforded the corresponding enaminones 11 and 12, respectively. The newly synthesized compounds were screened for their antioxidant activity. Compounds 8b 89.87, 9a (89.93%) and 9b (95.97%) exhibited promising activities. On the other hand compounds 8b, 9a, 9b, 11 and 12 have the ability to protect DNA from the damage induced by bleomycin.

**Keywords:** Naphthoquinone; Thiophene; ABTS antioxidant; Bleomycin-dependent DNA damage

## Introduction

Naphthoquinone derivatives have attracted continuing interest over the years because of the use of its ring system as important core structure in many drug substances and reported to cover wide range of pharmacological applications [1,2]. Furthermore, quinones, particularly 1,4-naphthoquinones are widely distributed phenolic compounds in nature such naphthoquinones are reported to exhibit diverse pharmacological properties like antibacterial [3], antifungal, antiviral, anti-inflammatory antipyretic properties and anticancer activity [4]. These quinones have the ability to induce oxidative stress which is responsible for initiation of tissue damage selectively in tumor cells and this seems to be a promising approach for targeting cancer cells [5]. Moreover many biological properties for 2-aminothiophene derivatives have been reported, such as A1 adenosine receptor allosteric enhancers [6,7], antifungal [8], antibacterial [9], antiproliferative [8], anti-inflammatory [10], antitumor and anti-HIV activities [7]. In view of the above mention and as a part of our continuous efforts towards the development of more potent antioxidant agents [11-15], it was thought of interest to combine the above mentioned boilable rings together in a molecular framework to investigate the additive effect of these rings towards antioxidant activities.

## Experimental

### Instruments and methods

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Thin layer chromatography (TLC) was carried out on silica gel 60 $F_{254}$ precoated aluminum sheets. The IR spectra were recorded (KBr) on a Mattson 5000 FTIR Spectrophotometer ($\lambda$, $cm^{-1}$) at the Microanalytical Unit, Faculty of Science, Mansoura University. The $^1$H-NMR spectra were determined on a Varian XL 200 MHz in dimethylsulfoxide (DMSO) and chloroform as solvents using tetramethylsilane (TMS) as internal standard at the Microanalytical Center, Faculty of Science, Cairo University. $^{13}$C-NMR spectra were recorded on JEOL-ECA500 (National Research Center, Egypt). The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment at the Microanalytical Center, Cairo University, Giza, Egypt. Elemental analyses (C, H and N) were carried out at the Microanalytical Center, Cairo University, Giza, Egypt. The elemental analyses were found to agree favorably with the calculated values. Biological activities were carried at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

**General procedure for the synthesis of 3-[(3-substituted-4,5,6,7-tetrahydrobenzo[b] thiophen-2-yl)diazenyl]naphthalene-1,4-diones 8a-e:** A well stirred solution of 2-aminothiophens 6a-c (0.005 mole) in concentrated HCl (3.6 mL) and $H_2O$ (1 mL) was cooled in an ice-bath and diazotized with the solution of $NaNO_2$ (0.345 g, 0.005 mole) in $H_2O$ (2 mL). The cold diazonium solution was added slowly to a well stirred solution of 2-hydroxynaphthalene-1,4-dione (0.87 g, 0.005 mole) or 2-methoxynaphthalene-1,4-dione (0.94 g, 0.005 mole) in pyridine (20 mL). The reaction mixture was stirred for further 2 h. The crude product was filtered off, dried well and crystallized from the appropriate solvent to give compounds 8a-e.

**(E)-Ethyl 2-((3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) diazenyl)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylate (8a)**: Yield (0.83 g, 96%); crystallization from ethanol; brown powder; m.p. 214°C. IR(KBr): ν/$cm^{-1}$= 3434(br,OH), 2933(C-H aliphatic), 1673 (br., 3CO), 1498 (N=N); $^1$H-NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.45 (t, 3H, $CH_3$, $J$ = 6.9 Hz), 1.78-1.82 (m, 4H, $C_5$-2H, $C_6$-2H), 2.73-2.85 (m, 4H, $C_4$-2H, $C_7$-2H), 4.52 (q, 2H, $CH_2$, $J$ = 6.9 Hz), 7.79-8.40 (m, 4H, Ar-H), 13.52 (br, 1H, OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) $\delta$ (ppm): 183.2, 181.1, 165.3, 155.8, 145.7, 135.0, 133.0, 132.6, 130.4, 129.3, 128.4, 126.9, 126.4, 120.4, 88.9, 59.8, 25.8, 24.1, 22. 3, 22.1, 13.8. MS (EI, 70 ev) *m/z* (%) = 408 ($M^+$-2, 0.4), 335 (3.1), 282 (1.9), 225 (9.7), 224 (62.5), 220 (31.3), 188 (59.2), 178 (100.0). Anal. Calcd. for $C_{21}H_{18}N_2O_5S$ (410.09): C, 61.45; H, 4.42; N, 6.83%. Found: C, 61.48; H, 4.37; N, 6.88%.

**(E)-2-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) diazenyl)-4,5,6,7-tetra-hydrobenzo[b]thiophene-3-carboxamide (8b):** Yield (0.35 g, 40%); crystallization from ethanol; brown powder; m.p 285°C. IR(KBr): ν/$cm^{-1}$= 3446 (br., OH), 3334 ($NH_2$), 2926 (C-

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H, aliphatic), 1673, 1639 (3CO), 1486 (N=N); $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$ (ppm): 1.77-1.90 (m, 4H, $C_5$-2H, $C_6$-2H), 2.35-2.51 (m, 4H, $C_4$-2H, $C_7$-2H), 3.41 (br., 2H, $NH_2$), 7.87-8.08 (m, 4H, Ar-H), 13.49 (br., 1H, OH). MS (EI, 70 ev) *m/z* (%) = 386 ($M^+$+5, 1.0), 359 (12.6), 345 (100.0). Anal. Calcd. for $C_{19}H_{15}N_3O_4S$ (381.08): C, 59.83; H, 3.96; N, 11.02%. Found: C, 59.79; H, 3.93; N, 11.06%.

**(E)-2-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) diazenyl)-4,5,6,7-tetra hydrobenzo[b]thiophene-3-carbonitrile (8c):** Yield (0.54 g, 62%); crystallization from ethanol; brown powder; m.p. >320°C. IR (KBr): ν/cm$^{-1}$= 3417 (br., OH), 2931, 2858 (C-H, aliphatic), 2189 (CN), 1672, 1629, (2CO), 1452 (N=N); $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$ (ppm): 1.71-1.80 (m, 4H, $C_5$-2H, $C_6$-2H), 2.68-2.75 (m, 4H, $C_4$-2H, $C_7$-2H), 7.21-8.23 (m, 4H, Ar-H), 13.50 (br., 1H, OH); $^{13}$C-NMR(125 MHz, DMSO-$d_6$) $\delta$ (ppm):183.2, 181.1, 159.4, 153.1, 135.1, 133.1, 132.7, 132.3, 129.3, 127.0 126.3, 120.5, 120.1, 88.6, 115.59 , 26.5, 25.5, 22.2, 22. 1. MS (EI, 70 ev) *m/z* (%)= 363 ($M^+$, 3.0), 337 (12.0), 310 (8.0), 280 (10.0), 266 (30.0), 240 (15.2), 223 (10.4), 211 (8.2), 199 (24.0), 188 (39.0), 173 (60.3), 161 (29.3), 150 (84.0), 145 (46.0), 134 (39.1), 99 (47.0), 98 (100.0). Anal. Calcd for $C_{19}H_{13}N_3O_3S$ (363.07): C, 62.80; H, 3.61; N, 11.56%. Found: C, 62.77; H, 3.56; N, 11.59%.

**(E)-Ethyl 2-((3-methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)diazenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (8d):** Yield (0.47 g, 50%); crystallization from ethanol; brown powder; m.p. 191°C. IR (KBr): ν/cm$^{-1}$= 2933 (C-H, aliphatic), 1714, 1673 (3CO), 1459 (N=N); $^1$H-NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.36 (t, 3H, $CH_3$, *J* = 7.2 Hz), 1.81-1.84 (m, 4H, $C_5$-2H, $C_6$-2H), 2.57-2.85 (m, 4H, $C_4$-2H, $C_7$-2H), 3.91 (s, 3H, $OCH_3$), 4.30 (q, 2H, $CH_2$, *J* = 7.2 Hz), 7.26-8.41 (m, 4H, Ar-H); MS (EI, 70 ev) *m/z* (%) = 426 ($M^+$+2, 0.2), 424 ($M^+$, 0.4), 343 (1.3), 287 (60.0), 258 (17.9), 241 (29.8), 224 (69.2), 221 (20.8), 188 (26.2), 178 (100.0). Anal. Calcd for $C_{22}H_{20}N_2O_5S$ (424.11): C, 62.25; H, 4.75; N, 6.60%. Found: C, 62.29; H, 4.70; N, 6.66%.

**(E)-2-((3-Methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) diazenyl)-4,5,6,7-tetra hydrobenzo[b]thiophene-3-carboxamide (8e)**: Yield (0.43 g, 46%); crystallization from ethanol; brownish black powder; m.p. 225°C. IR (KBr): ν/cm$^{-1}$= 3440 (br., $NH_2$), 2925 (C-H aliphatic), 1652 (br., 3CO), 1454 (N=N); $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$ (ppm): 1.52-1.85 (m, 4H, $C_5$-2H, $C_6$-2H); 2.72-2.88 (m, 4H, $C_4$-2H, $C_7$-2H), 3.86 (s, 3H, $OCH_3$), 7.25-8.12 (m, 4H, Ar-H), 8.62 (br., 2H, $NH_2$); $^{13}$C-NMR(125 MHz, DMSO-$d_6$) $\delta$ (ppm): 183.2, 181.0, 158.7, 153.1, 135.1, 133.1, 132.6, 132.0, 129.3, 126.7, 126.2, 120.2, 120.1, 115.9, 87.2, 55.2, 24.1, 23.7, 23.3, 22.1. MS (EI, 70 ev) *m/z* (%) = 394 ($M^+$-1, 1.3), 348 (7.1), 329 (40.4), 313 (25), 255 (23.1), 196 (23.1), 191 (57.7), 188 (100.0). Anal. Calcd. for $C_{20}H_{17}N_3O_4S$ (395.09): C, 60.75; H, 4.33; N, 10.63%. Found: C, 60.72; H, 4.38; N, 10.61%.

**General procedure for the synthesis of 2-((3-substituted-4,5,6,7-tetrahydrobenzo[b] thiophen-2-yl)amino)-3-methoxynaphthalene-1,4-diones 9a, b:** To a solution of each of 2-bromo-3-methoxynaphthalene-1,4-dione (5) (1.33 g, 5 mmol) in ethanol (50 mL) and ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (6a) (1.12 g, 5 mmol) or 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (6b) (0.98 g, 5 mmol) and potassium carbonate (0.69 g, 5 mmol) were added. The reaction mixture was refluxed for 8 h and left to cool at room temperature. The obtained solid product was filtered off, dried and crystallized from ethanol to give compounds 9a, b, respectively.

**Ethyl 2-((3-methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) amino)-4,5,6,7-tetra-hydrobenzo[b]thiophene-3-carboxylate (9a):** Yield (0.89 g, 80%); crystallization from ethanol; brown powder; m.p. 101°C. IR (KBr): ν/cm$^{-1}$= 3297 (NH), 2935 (C-H, aliphatic), 1646 (br., 3CO); $^1$H-NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.34 (t, 3H, $CH_3$, *J* = 7.2 Hz), 1.73-1.79 (m, 4H, $C_5$-2H, $C_6$-2H), 2.57-2.69 (m, 4H, $C_4$-2H, $C_7$-2H), 3.94 (s, 3H, $OCH_3$), 4.25 (q, 2H, $CH_2$, *J* = 7.2 Hz), 7.27 (br., 1H, NH), 7.70-8.39 (m, 4H, Ar-H). MS (EI, 70 ev) *m/z* (%) = 390 ($M^+$-$OCH_3$, 0.5), 387 (4.1), 334 (1.9), 256 (0.2), 226 (10.1), 225 (70.7), 180 (20.3), 179 (100.0). Anal. Calcd. for $C_{22}H_{21}NO_5S$ (411.11): C, 64.22; H, 5.14; N, 3.40%. Found: C, 64.26; H, 5.05; N, 3.47%.

**2-((3-Methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxamide (9b)**: Yield (0.52 g, 53%); crystallization from ethanol; brown powder; m.p. 136°C. IR (KBr): ν/cm$^{-1}$= 3342, 3322, 3271 ($NH_2$, NH), 2931 (C-H, aliphatic), 1640, 1646, 1668 (3CO); $^1$H-NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.74-1.97 (m, 4H, $C_5$-2H, $C_6$-2H), 2.66-2.75 (m, 4H, $C_4$-2H, $C_7$-2H), 3.91 (s, 3H, $OCH_3$), 6.18 (br., 1H, NH), 7.26 (br., 2H, $NH_2$), 7.73-8.40 (m, 4H, Ar-H);$^{13}$C-NMR(125 MHz, DMSO-$d_6$) $\delta$ (ppm) 183.2, 180.9, 167.5, 160.3, 155.8, 134.8, 134.5, 133.2, 131.8, 120.3, 120.0, 117.5, 116.4, 116.0, 112.0, 55.9, 22.1, 23.3, 23.7, 24.1 MS (EI, 70 ev) *m/z* (%) = 382 ($M^+$, 0.2), 329 (4.1), 302 (0.9), 255 (1.6), 224 (9.4), 192 (8.4), 187 (100.0). Anal. Calcd. for $C_{20}H_{18}N_2O_4S$ (382.1): C, 62.81; H, 4.74; N, 7.33%. Found: C, 62.85; H, 4.68; N, 7.35%.

**Synthesis of ethyl 2-(((3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl) amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (11):** To a suspension of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (6a) (1.12 g, 5 mmol) in a mixture ethanol / acetic acid (25 mL, 4:1 V) and 2-hydroxy-3-((piperidin-1-yl)methyl)naphthalene-1,4-dione (10) (1.35 g, 5 mmol) was added. The reaction mixture was refluxed for 6 h then left to cool at room temperature. The obtained solid product was filtered off, dried and crystallized from ethanol to give compound 11.

Yield (0.82 g, 73%); crystallization from ethanol; brown powder; m.p. > 320°C. IR (KBr): ν/cm$^{-1}$= 3428 (br., OH), 3280 (NH), 2935 (C-H, aliphatic), 1712, 1664, 1659 (3CO); $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$ (ppm): 1.35 (t, 3H, $CH_3$, *J* = 6.9 Hz), 1.68-1.90 (m, 4H, $C_5$-2H, $C_6$-2H), 2.54- (s, 2H, $CH_2$), 2.47-2.60 (m, 4H, $C_4$-2H, $C_7$-2H), 3.74 (q, 2H, $CH_2O$, *J* = 6.9 Hz), 7.50 (br., 1H, NH), 7.60-8.23 (m, 4H, Ar-H), 11.28 (br., 1H, OH); $^{13}$C-NMR(125 MHz, DMSO-$d_6$) $\delta$ (ppm) 183.0, 180.6, 167.3, 160.1, 156.2, 134.6, 133.3, 132.2,131.6, 129.9, 125.7, 120.5, 119.2 115.6, 113.1,62.3, 45.1, .24.1,23.7,23.3 ,22.1,15.0. MS (EI, 70 ev) *m/z* (%) = 413 ($M^+$+2, 0.3), 360 (12.5), 359 (100.0), 340 (36.1), 330 (13.3), 314 (21.4), 313 (88.4), 257 (7.1), 200 (4.9), 188 (19.6), 174 (73.6), 146 (23.1), 118 (6.3), 99 (75.8), 98 (28.9), 82 (16.8), 73 (31.6). Anal. Calcd. for $C_{22}H_{21}NO_5S$ (411.11): C, 64.22; H, 5.14; N, 3.40%. Found: C, 64.28; H, 5.08; N, 3.36%.

**Synthesis of ethyl 2-((3,4-dioxo-3,4-dihydronaphthalen-1-yl) amino)-4,5,6,7-tetra-hydrobenzo[b]thiophene-3-carboxylate (12):** A mixture of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (6a) (1.12 g, 5 mmol) and sodium 3,4-dihydro-3,4-dioxonaphthalene-1-sulfonate (1) (1.3 g, 5 mmol) in a mixture of methanol / $H_2O$ (20 mL, 3:1 V) was refluxed for 1 h. The reaction mixture was left to cool at room temperature and the formed precipitate was crystallized from ethanol to give compound 12.

Yield (1 g, 98%); dark brown powder; crystallization from ethanol; m.p. 158°C. IR (KBr): ν/cm$^{-1}$= 3270 (NH), 2931 (C-H, aliphatic), 1689 (CO), 1654 (2CO); $^1$H-NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.31 (t, 3H, $CH_3$, *J* = 6.9 Hz), 1.77-1.92 (m, 4H, $C_5$-2H, $C_6$-2H); 2.70-2.82 (m, 4H, $C_4$-2H, $C_7$-2H); 4.24 (s, 1H, C-H); 4.27 (q, 2H, $CH_2O$, *J* = 6.9 Hz); 6.77 (br., 1H, NH); 7.27-8.25 (m, 4H, Ar-H); $^{13}$C-NMR (125 MHz, DMSO-$d_6$) $\delta$ (ppm) 179.8, 165.2, 163.7, 160.2, 150.1, 134.5, 1330, 132.3, 132.2, 130.2, 125.6, 125.5, 120.5, 115.7, 115.4, 113.3, 62.3, 25.1, 23.8, 23.5, 22,1, 14.9. MS (EI, 70 ev) *m/z* (%) = 381 ($M^+$, 0.3), 367 (10.9), 336 (1.6), 320 (18.5), 305 (4.6), 290 (2.2), 267 (2.2), 224 (71.4), 179 (18.9), 178

(100.0), 160 (17.9), 150 (34.0), 136 (1.8), 130 (8.3), 105 (1.0), 84 (7.9), 73 (7.3). Anal. Calcd. for $C_{21}H_{19}NO_4S$ (381.1): C, 66.12; H, 5.02; N, 3.67%. Found: C, 66.16; H, 4.97; N, 3.71%.

## Antioxidant activity

**ABTS screening assay:** Antioxidant activity were evaluated from the bleaching of ABTS derived radical cations [16]. The radical cation derived from ABTS [2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid)] was prepared by reaction of ABTS (60 mL) with $MnO_2$ (3 mL, 25 mg/mL) in (5 mL) aqueous buffer solution (pH 7). After shaking the solution for a few minutes, it was centrifuged and filtered. The Absorbance (A control) of the resulting green-blue solution (ABTS radical solution) was recorded at $\lambda_{max}$ 734 nm. The absorbance (A test) was measured upon the addition of (20 mL of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The inhibition ratio (%) was calculated using the following formula:

(%) Inhibition = [A (control) – A (test) /A (control)] x 100

Ascorbic acid (20 mL, 2 mM) solution was used as a standard antioxidant (positive control). Blank sample was run using solvent without ABTS (Table 1).

**Bleomycin-dependent DNA-damage:** The assay was done according to Aeschbach et al. [17], Chan and Tang [18] with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), and $MgCl_2$ (5 mM), $FeCl_3$ (50 mM) and the samples were dissolved in DMSO at concentration (20 mL of 1 mg/mL). L-Ascorbic acid was used as a positive control. The mixture was incubated at 37°C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding thiobarbituric acid (TBA) (0.5 mL) (1%, w/v) and HCl (0.5 mL) (25%, v/v) followed by heating at 80°C for 10 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm (Table 2).

# Results and Discussion

## Chemistry

The target compounds were synthesized as outlined in Schemes 1, 2, 3 and 4. Detailed synthetic procedures for all compounds are described in the experimental section. Briefly, the starting compounds 2-methoxynaphthalene-1,4-dione (2), 2-hydroxynaphthalene-1,4-dione (3), 2-bromo-3-hydroxynaphthalene-1,4-dione (4) and 2-bromo-3-methoxynaphthalene-1,4-dione (5) were prepared from sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate according to the reported procedure [19,20] (Scheme 1).

**Scheme 1:** Furthermore, 2-aminothiophenes 6a-c were prepared according to a reported procedure [21] and allowed to react with sodium nitrite in hydrochloric acid to produce the corresponding diazonium salts 7a-c which coupled with compounds 2 or 3 (Nga and Dao, 2009) in pyridine to give the corresponding naphthoquinone derivatives 8a-e (Scheme 2).

**Scheme 2**: Structures of 8a-c were confirmed on the basis of analytical and spectral data. The IR spectra of 8a-c displayed absorption bands at $\upsilon$ = 3446-3417, 1673-1629 and 1498-1452 $cm^{-1}$ due to the stretching vibration of hydroxy, carbonyl and azo groups, respectively. In addition, the IR spectra of compounds 8d, e showed bands at $\upsilon$ = 1714-1652 and 1459-1454 $cm^{-1}$ due to stretching vibration of carbonyl and azo groups, respectively. The $^1$H NMR spectra of 8a-c displayed characteristic signals at $\delta$ = 1.71-1.90 (m, 4H) , 2.35-2.85 ppm (m, 4H and 13.49-13.52 ppm (br, 1H) due to cycohexene and

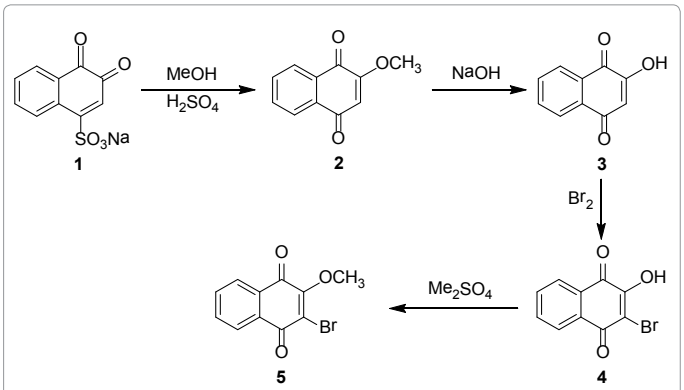
| Compound No | Absorbance of samples (λ) | % inhibition |
|---|---|---|
| Control of ABTS[a] | 0.474 | 0% |
| Ascorbic acid | 0.048 | 89.87% |
| **8a** | 0.466 | 6.23% |
| **8b** | 0.050 | 89.93% |
| **8c** | 0.358 | 27.96% |
| **8d** | 0.418 | 15.89% |
| **8e** | 0.370 | 25.55% |
| **9a** | 0.020 | 95.97% |
| **9b** | 0.102 | 79.47% |
| **11** | 0.183 | 63.17% |
| **12** | 0.171 | 65.59% |

[a]ABTS: The method used for antioxidant activity
(%) Inhibition = [A (control) – A (test) /A (control)] x 100

**Table 1:** ABTS Antioxidant activity assay of compounds 8a-e, 9a,b, 11 and 12.

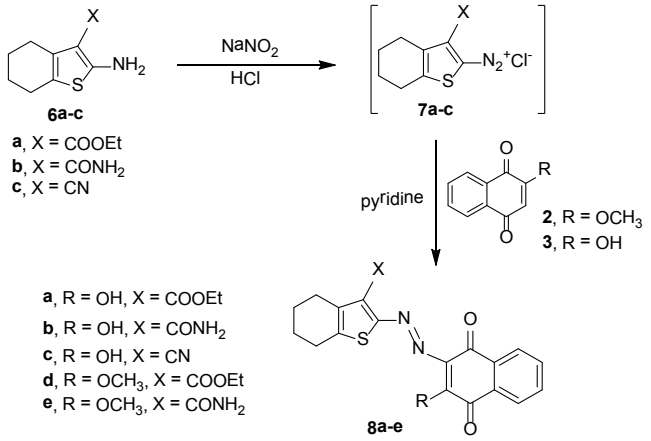
| Compound No. | Absorbance of samples |
|---|---|
| **Ascorbic-acid** | 0.097 |
| **8a** | 0.145 |
| **8b** | 0.098 |
| **8c** | 0.126 |
| **8d** | 0.138 |
| **8e** | 0.140 |
| **9a** | 0.082 |
| **9b** | 0.089 |
| **11** | 0.093 |
| **12** | 0.099 |

**Table 2:** Bleomycin dependent-DNA damage of the investigated compounds.




**Scheme 1:** Synthesis of 2-bromo-3-methoxynaphthalene-1,4-dione (5).

OH proton respectively . Furthermore, the $^1$H NMR spectra of 8d and 8e revealed singlet signals at $\delta$ 3.91 and 3.86 ppm (s, 3H) due to methoxy protons, respectively, in addition to cyclohexene signals at $\delta$ 1.52-1.85 (m, 4H) and 2.57-2.88 ppm (m, 4H). The mass spectrum of 8a showed the molecular ion peak at *m/z* 408 ($M^+$-2) which is in agreement with the molecular formula ($C_{21}H_{18}N_2O_5S$), in addition to the base peak at *m/z* 178 which is related to $M^+$ - [$C_{10}H_5O_3$ + OEt] moiety. Also, the mass spectrum of 8b displayed the molecular ion peak at *m/z* 386 ($M^+$+5) which in agreement with the molecular formula ($C_{19}H_{15}N_3O_4S$), in addition to the base peak at *m/z* 345 corresponding to ($M^+$-[$H_2O$+$NH_2$]). Furthermore, the mass spectrum of 8c showed the molecular ion peak at *m/z* 363 ($M^+$) which is in agreement with the molecular formula ($C_{19}H_{13}N_3O_3S$) in addition to the base peak at *m/z* 98 due to thiophen-2-yl-methene moiety. The mass spectrum of 8d showed two molecular ion peaks at *m/z* 426 ($M^+$+2) and 424 ($M^+$) which are in agreement with the molecular formula ($C_{22}H_{20}N_2O_5S$) in addition to the base peak at *m/z* 178 due to $M^+$ - [$C_{10}H_5O_3$ + OEt]




**Scheme 2:** Synthesis of 3-[(3-substituted-4,5,6,7-tetrahydrobenzo[*b*] thiophen-2-yl)diazenyl] naphthalene-1,4-diones 8a-e

moiety. Also, the mass spectrum of 8e showed the molecular ion peak at *m/z* 394 ($M^+$-1) which is in agreement with the molecular formula ($C_{20}H_{17}N_3O_4S$) in addition to the base peak at *m/z* 188 corresponding to 2-methoxynaphthoquinone moiety.

Moreover, reaction of 5 with 6a, b in ethanol containing potassium carbonate afforded the corresponding enaminnones 9a, b, respectively (Scheme 3). Structures 9a, b were elucidated based on analytical and spectral data. The IR spectra of 9a, b showed absorption bands at $\upsilon$ = 3297 and 3271 $cm^{-1}$ due to stretching vibration of NH groups, respectively. The $^1$H NMR spectra of 9a and 9b revealed signals due to cyclohexene at $\delta$ = 1.73-1.97 (m, 4H) and 2.57-2.75 ppm (m, 4H) and displayed signals at $\delta$ = 3.91-3.94 (s, 3H) and 6.18-7.27 ppm (br, 1H) due to methoxy and NH groups, respectively. The mass spectrum of 9a showed the molecular ion peak at *m/z* 390 ($M^+$-$OCH_3$) which is equivalence with the molecular formula ($C_{22}H_{21}NO_5S$) in addition to the base peak at *m/z* 179 due to 2-imino-3-carbonyl-tetrahydrobenzothiophene moiety. On the other hand, the mass spectrum of 9b displayed the molecular ion peak at *m/z* 382 ($M^+$) which is in agreement with the molecular formula ($C_{20}H_{18}N_2O_4S$) in addition to the base peak at *m/z* 187 that is related to 2-methoxynaphthoquinone moiety.

**Scheme 3**: Finally, heating of 6a with 2-hydroxy-3-((piperidn-1-yl)methyl) naphthalene-1,4-dione (10) [22] in EtOH/AcOH or sodium 3,4-dihydro-3,4-dioxonaphthalene-1-sulphonate 1 in MeOH / $H_2O$ afforded the corresponding enaminones 11 and 12, respectively (Scheme 4).

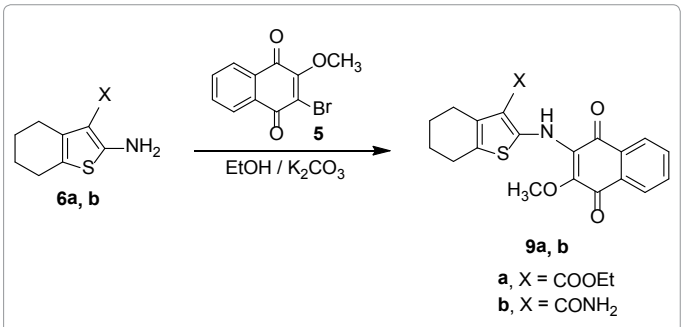
**Scheme 4**: Structures 11 and 12 were assigned on the basis of analytical and spectral data. The IR spectra of 11 and 12 exhibited absorption bands at 3280-3270 and 1712-1659 $cm^{-1}$ due to stretching vibrations of NH and CO, respectively, Furthermore, the $^1$H-NMR spectrum of 11 showed characteristic signals at $\delta$ 1.33 (t, 3H, $CH_3$, *J* = 6.9 Hz), 3.74 (q, 2H, $CH_2O$, *J* = 6.9 Hz), 7.50 (br, 1H, NH), and 11.28 ppm (br, 1H, OH). Furthermore, the $^1$H-NMR spectrum of compound 12 revealed signals at $\delta$ 1.31 (t, 3H, $CH_3$, *J* = 6.9 Hz), 4.24 (s, 1H, C-H), 4.27 (q, 2H, $CH_2O$) and 6.77 (br, 1H, OH). The mass spectrum of 11 showed the molecular ion peak at *m/z* 413 ($M^+$+2) which is in agreement with the molecular formula ($C_{22}H_{21}NO_5S$) in addition to the base peak at *m/z* 359 due to ($M^+$-[EtOH+2$H_2$]) fragment. Moreover, the mass spectrum of 12 displayed the molecular ion peak at *m/z* 381 ($M^+$) which is in agreement with the molecular formula ($C_{21}H_{19}NO_4S$) in addition to the base peak at *m/z* 178 that is related to *N*-ethenyl-2-amino-tetrahydrobenzothiophene moiety [22].

## Biological evaluation

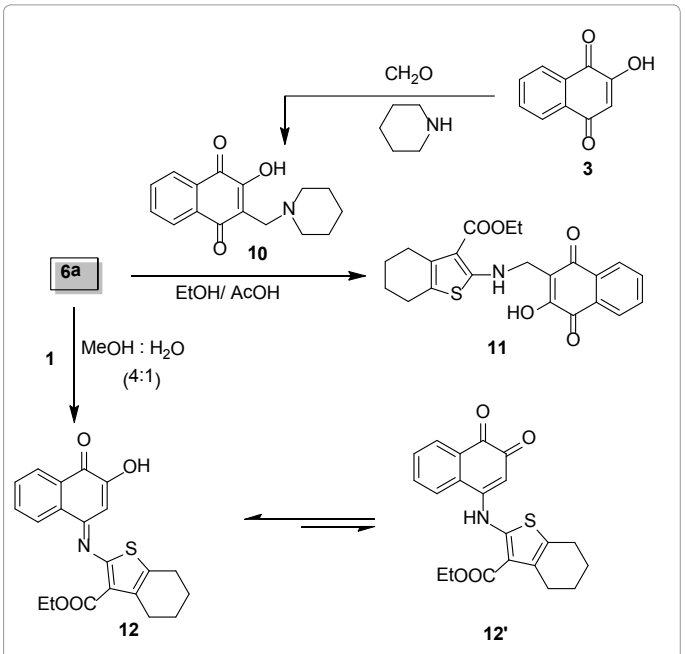
**Antioxidant activity assay:** Ten thiophenes were screened for their antioxidant activity as reported [16]. The data showed clearly that compounds 8b, 9a and 9b exhibited good activities, while compounds 11 and 12 exhibited moderate activities compared with ascorbic acid. On the other hand, the rest of compounds exhibited weak activities (Table 1).

**Bleomycin-dependent DNA-damage:** Ten thiophenes were selected for bleomycin-dependent DNA-damage testing (Table 2). Damage of DNA in the presence of a bleomycin-Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents [23]. If the samples to be tested are able to reduce the bleomycin-$Fe^{+3}$ to bleomycin-$Fe^{+2}$, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to malondialdehyde (MDA). L-Ascorbic acid was used as a reducing agent can reduce $Fe^{+3}$ to $Fe^{+2}$.

Results in Table 2 showed that compounds 8b, 9a, 9b, 11 and 12 have an ability to protect DNA from the induced damage by bleomycin. On the other hand, the rest of compounds exhibited weak activities. Thus,




**Scheme 3:** Synthesis of 2-((3-substituted-4,5,6,7-tetrahydrobenzo[*b*] thiophen-2-yl)amino)-3-methoxynaphthalene-1,4-diones 9a, b.




**Scheme 4:** Synthesis of ethyl 2-substituted-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate derivatives 11 and 12.

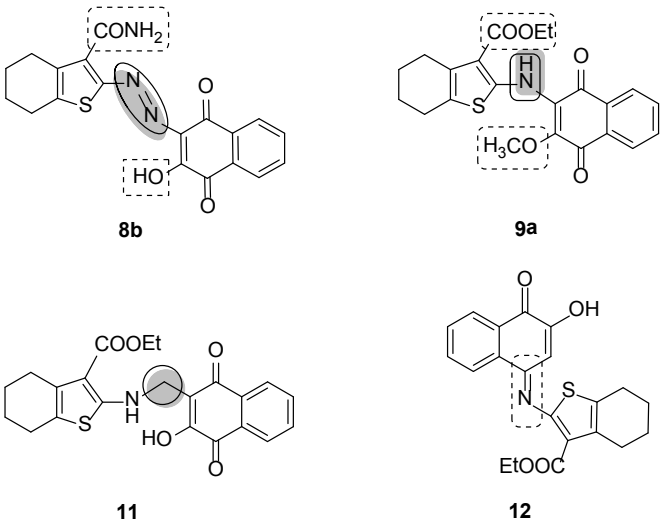



**Figure 1:** Structure activity relationship's (SAR's) of the more potent compounds.

it would appear generally that introducing of naphthoquinone moiety enhances the antioxidant properties of 2-aminothiophene compounds. By comparing the results obtained of the investigated compounds to their structures the following structure activity relationships (SAR's) were postulated: (*i*) naphthoquinones 8b and 9a are more potent than 5-florouracil (5-Fu) which may be attributed to the presence of thiophene and naphthoquinone moiety. (*ii*) Compound 8b is less potent than 9a which may be due to replacement of $CONH_2$, OH and N=N moieties into COOEt, OMe and NH, respectively. (*iii*) Compound 11 is more potent than the thiophene 9a which may be attributable to the presence of $CH_2$ moiety (*iv*) Compound 12 is more potent than 11 which may be due to the presence of C=N moiety (Figure 1).

## Conclusion

The objective of the present study was to synthesize and evaluate the antioxidant activity of some novel naphthoquinone derivatives with the hope of discovering new structure serving as antioxidant gent. The data showed clearly that compounds 8b, 9a, 9b, 11 and 12 have the ability to protect DNA from the induced damage by bleomycin.

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