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SUPPLEMENTARY MATERIAL TO

Synthesis and biological activity of alkylthio and arylthio derivatives of *tert*-butylquinone

JELENA ĐORĐEVIĆ[1,2], STOIMIR KOLAREVIĆ[3], JOVANA JOVANOVIĆ MARIĆ[3], MARIANA OALĐE PAVLOVIĆ[4], DUŠAN SLADIĆ[5], IRENA NOVAKOVIĆ[6]*# and BRANKA VUKOVIĆ-GAČIĆ[2]

[1]University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia, [2]University of Belgrade, Centre for Genotoxicology and Ecogenotoxicology, Faculty of Biology, Belgrade, Serbia, [3]University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, Belgrade, Serbia, [4]University of Belgrade, Chair of Plant Morphology and Systematics, Faculty of Biology, Belgrade, Serbia, [5]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia and [6]University of Belgrade, Institute for Chemistry, Technology and Metallurgy, Department for Chemistry, Belgrade, Serbia

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General information

All reagents used were commercial products purchased from Sigma-Aldrich or Merck, except for silver(I)-oxide which was synthesized. Column chromatography was performed on silica gel (0.063−0.200 mm, Merck). Analytical thin-layer chromatography (TLC) was performed on precoated aluminum-backed plates (silica gel 60 GF_{254}, Merck). ^{1}H- and ^{13}C-NMR spectra were recorded at 500 MHz (Bruker Avance III) in deuterated chloroform (CD_3Cl). Chemical shifts are reported in parts per milion (ppm), relative to tetramethylsilane. Coupling constants are reported in hertz (Hz). EI MS spectra were recorded on Agilent 7890A – 5975 inert XL EI CI. UV/Vis spectra were recoreded on a LLG-uniSPEC 2 Spectrophotometer in methanol.

*Synthesis of 2-*tert*-butyl-1,4-benzoquinone (TBQ)*

2-*tert*-Butyl-1,4-benzohydroquinone (2.13 g) was dissolved in 150 mL of diethyl ether in a 250 ml flask. After dissolution, 3.70 g of silver(I)-oxide was gradually added to the reaction mixture with constant stirring over 3.5 h at room temperature, after which the stirring was stopped, anhydrous sodium sulfate was added to the reaction mixture and the mixture were dried overnight. Oxidation was monitored by thin-layer chromatography in a toluene:ethyl acetate system (9:1). After 12 h, the mixture was filtered through a layer of celite and the solvent

* Corresponding author. E-mail: irenan@chem.bg.ac.rs

was evaporated under reduced pressure. Yellow crystals (m.p. 56-58 °C) of 2-*tert*-butyl-1,4-benzoquinonewere obtained. The yield was 2.05 g (98 %).

Synthesis of 2-tert-*butyl-5-(isopropylthio)-1,4-benzoquinone (**2**)*

2-*tert*-Butyl-1,4-benzoquinone (312 mg) was dissolved in 40 mL of EtOH/saturated $NaHCO_3$ solution (1:1). Then 498 mg (600 μL) of 2-propanethiol was added in one portion. The reaction was performed under an argon atmosphere, with constant stirring at 60 °C and lasted about 30 min. The aqueous-ethanolic solution was neutralized with 5 % HCl and then extracted with toluene. The organic solution was dried over anhydrous Na_2SO_4. Toluene was evaporated and a crude red product (444 mg) was applied to a column formed of 22 g of silica gel (0.063–0.200 mm). After elution with toluene, several fractions were obtained, the major fraction (136 mg) being rechromatographed on a 7 g silica gel column. An 87 mg mass fraction was obtained which was purified by preparative thin layer chromatography (silica gel 60 GF_{254}) with toluene as the eluent. An oily yellow colored product was obtained in an amount of 43 mg (19 %).

^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 6.62 (1H, *s*), 6.32 (1H, *s*), 3.26 (1H, septet, J = 6.5 Hz,), 1.37 (6H, *d*, J = 6.5 Hz), 1.26 (9H, s).

^{13}C-NMR (125 MHz, CDCl3, δ / ppm): 186.67 (C1), 185.92 (C4), 158.34 (C2), 151.73 (C5), 132.79 (C6), 129.01 (C3), 36.93 (C7), 35.78 (C11), 30.76 (C8, C9, C10), 23.70 (C12, C13).

UV/Vis, λ_{max} / nm (ε / dm^2 mol^{-1}): 252 (1.5×10^4), 290 (1.9×10^4), 420 (1.5×10^3).

EI MS, *m/z* (relative abundance, %): 238.2 (M^+, 100), 223.1 (100), 196.1 (80), 181.1 (50), 154.1 (55), 85.0 (70), 41.1 (55)

Synthesis of 2-tert-*butyl-5-(propylthio)-1,4-benzoquinone (**3**)*

2-*tert*-Butyl-1,4-benzoquinone (271 mg) was dissolved in 40 mL of EtOH/saturated $NaHCO_3$ solution (1:1) after which 368 mg (584 μL) of 1-propanethiol was added. The reaction was performed as with 2-propanethiol (with constant stirring at 60 °C under an argon atmosphere). The crude red product (432 mg) was applied to a column prepared from 22 g of silica gel (0.063–0.200 mm) and eluted with toluene. Multiple fractions were obtained and the selected fraction (133 mg) was purified by preparative thin layer chromatography (silica gel 60 GF_{254}) with toluene as the eluent. An oily orange compound was obtained in an amount of 63 mg (32 %).

^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 6.61 (1H, *s*), 6.27 (1H, *s*), 2.73 (2H, *t*, J = 4 Hz), 1.76 (2H, *sextet*, J = 6.5 Hz), 1.28 (9H, *s*), 1.07 (3H, *t*, J = 4 Hz).

^{13}C-NMR (125 MHz, $CDCl_3$, δ / ppm): 186.51 (C1), 185.71 (C4), 158.49 (C2), 152.47 (C5), 132.59 (C6), 128.28 (C3), 36.87 (C7), 33.64 (C11), 30.61 (C8, C9, C10), 22.18 (C12), 15.09 (C13).

UV/Vis, λ_{max} / nm (ε / dm^2mol^{-1}): 250 (1.8×10^4), 292 (1.4×10^4), 440 (1.4×10^3).

EI MS, *m/z* (relative abundance, %): 238.2 (M^+, 100), 223.1 (100), 196.1 (80), 181.1 (50), 154.1 (55), 85.0 (70), 41.1 (55).

*Synthesis of 2-*tert*-butyl-5,6-(ethylenedithio)-1,4-benzoquinone (**4**)*

2-*tert*-Butyl-1,4-benzoquinone (303 mg) was dissolved in 40 mL of EtOH/saturated $NaHCO_3$ aqueous solution (1: 1) after which 92 mg (82 μL) of ethylenedithiol was added to the resulting solution. The reaction was performed under an argon atmosphere, with constant stirring at 60 °C and lasted about 50 min. 412 mg of crude product was obtained and applied to a column of 20 g of silica gel (0.063–0.200 mm). The solvent was eluted with a mixture of petroleum ether (30-50 °C)−benzene (3:1), and the chromatography was followed by thin layer chromatography. An oily purple substance, 39 mg (17 %), was obtained.

^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 6.57 (1H, *s*), 3.23 (4H, *dd*, J_1 = 5 Hz, J_2 = 10 Hz,), 1.28 (9H, *s*).

^{13}C-NMR (125 MHz, $CDCl_3$ δ / ppm): 181.44 (C1), 180.57 (C4), 156.17 (C2), 139.61 (C6), 136.54 (C5), 131.41 (C3), 35.45 (C7), 29.11 (C8, C9, C10), 27.06 (C12), 26.34 (C11).

UV/Vis, λ_{max} / nm (ε / dm^2 mol^{-1}): 230 (8.9×10^4), 275 (4.1×10^4), 535 ($1.3x10^3$).

EI MS, *m/z* (relative abundance, %): 254.1 (M^+, 100), 239.1 (30), 210.9 (40), 193.0 (25), 183.0 (20), 88.0 (15), 67.1 (15), 41.1 (20).

*Synthesis of 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquinone (**5**) and 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquinone (**6**)*

2-*tert*-Butyl-1,4-benzoquinone (313 mg) and 55 mg (51 μL) of thiophenol were mixed. The reaction was performed under an air atmosphere in 96 % ethanol at 60 °C for 30 min. The dark red crude product (466 mg) was applied to a column of 24 g silica gel (0.063–0.200 mm). It was eluted with toluene and the chromatography was evaluated by TLC. Two products were obtained: 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquinone **(5)** in an amount of 29 mg (11 %) and 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquinone **(6)** in amount of 51 mg (19 %).

(5) ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.48 (5H, *m*), 6.49 (1H, *s*), 5.82 (1H, *s*), 1.31 (9H, *s*).

^{13}C-NMR (125 MHz, $CDCl_3$, δ / ppm): 186.96 (C1), 185.71 (C4), 157.80 (C6), 156.99 (C2), 137.23 (C5), 134.00 (C3), 132.01 (C11), 131.86 (C12, C16), 129.27 (C13, C15), 126.62 (C14), 36.88 (C7), 30.58 (C8, C9, C10).

UV/Vis, λ_{max} / nm (ε / dm^2 mol^{-1}): 253 (2.1×10^4), 290 (1.4×10^4), 443 (5.8×10^3).

EI MS, *m/z* (relative abundance, %): 272.1 (M^+, 100), 257.1 (20), 229.1 (30), 188.1 (20), 134.1 (20), 109.1 (20), 95.1 (20), 67.1 (20).

(6) ^{1}H-NMR (500 MHz, $CDCl_3$) δ / ppm: 7.47 (5H, *m*), 6.48 (1H, *d*, J = 5 Hz,), 5.80 (1H, *d*, J = 5 Hz), 1.30 (9H, *s*)

^{13}C-NMR (125 MHz, $CDCl_3$, δ / ppm): 186.74 (C1), 185.45 (C4), 157.69 (C6), 156.99 (C2), 137.16 (C5), 133.85 (C3), 132.38 (C11), 131.79 (C12, C16), 129.18 (C13, C15), 126.57 (C14), 36.87 (C7), 30.36 (C8, C9, C10)

UV/Vis, λ_{max} / nm (ε / dm^2 mol^{-1}): 250 (6.1×10^3), 298 (4.9×10^3), 440 (1.5×10^3);

EI MS, *m/z* (relative abundance, %): 272.1 (M^+, 100), 257.1 (20), 229.1 (30), 188.1 (20), 134.1 (20), 109.1 (20), 95.1 (20), 67.1 (20)

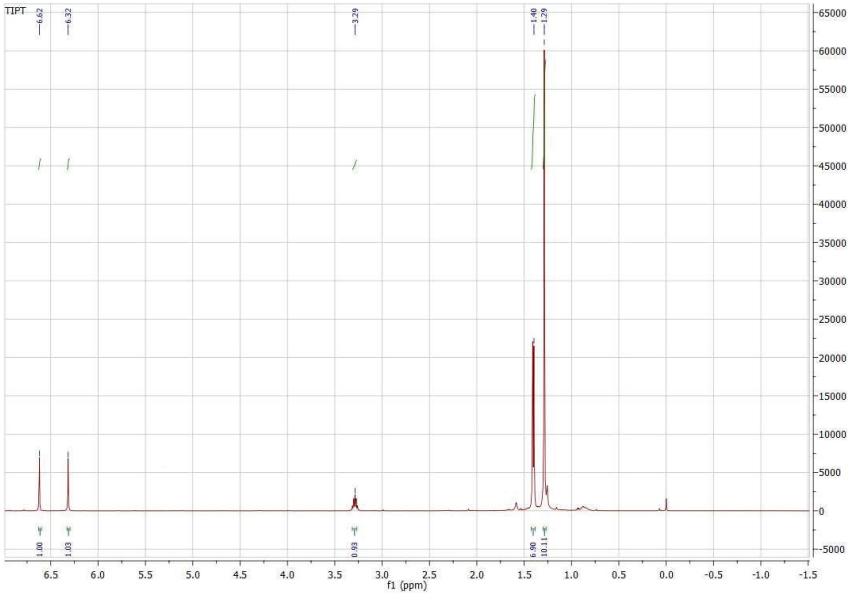

Fig. S-1. ^{1}H-NMR spectrum of 2-*tert*-butyl-5-(isopropylthio)-1,4-benzoquionone.

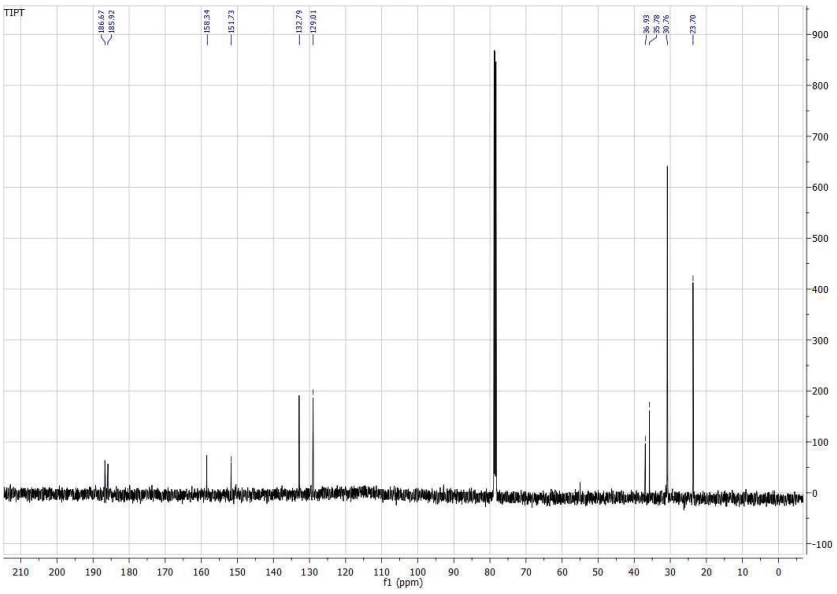

Fig. S-2. ^{13}C-NMR spectrum of 2-*tert*-butyl-5-(isopropylthio)-1,4-benzoquionone.

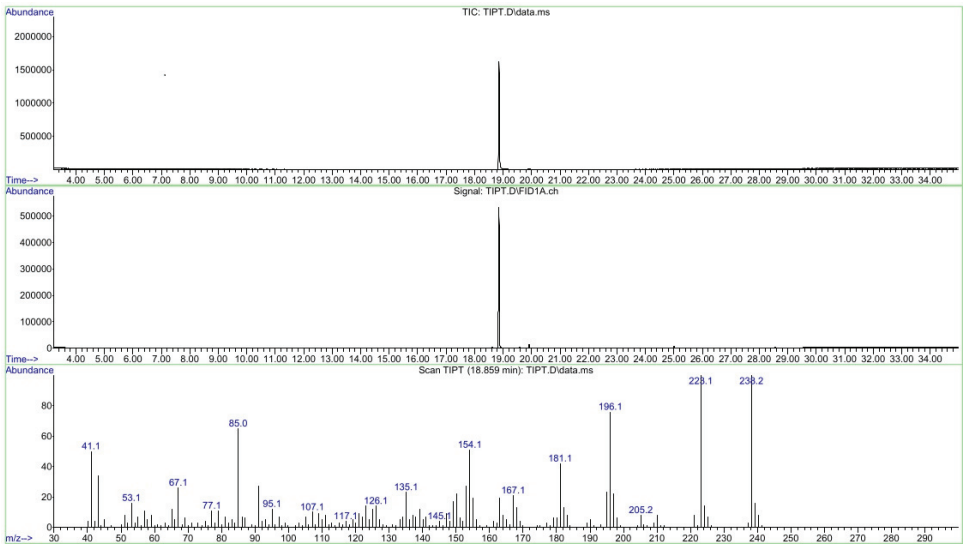

Fig. S-3. EI MS spectrum of 2-*tert*-butyl-5-(isopropylthio)-1,4-benzoquionone.

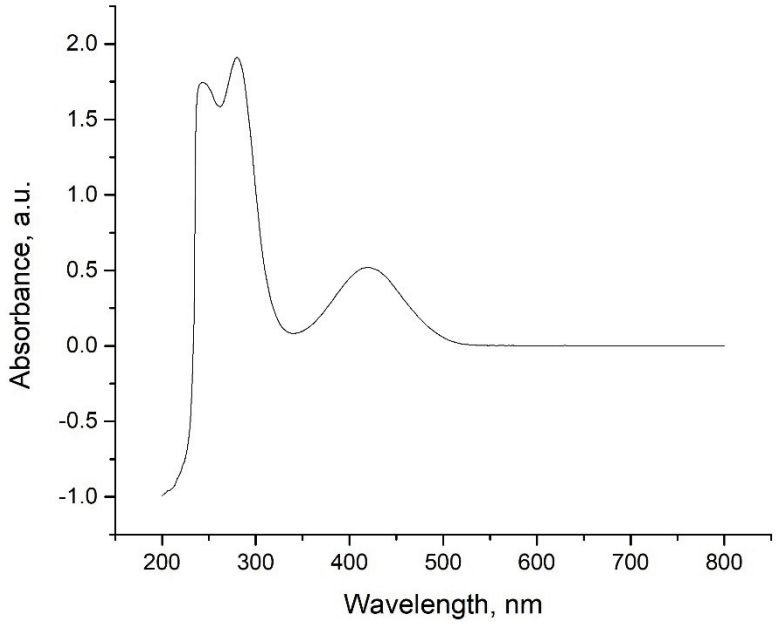

Fig. S-4. UV/Vis spectrum of 2-*tert*-butyl-5-(isopropylthio)-1,4-benzoquionone.

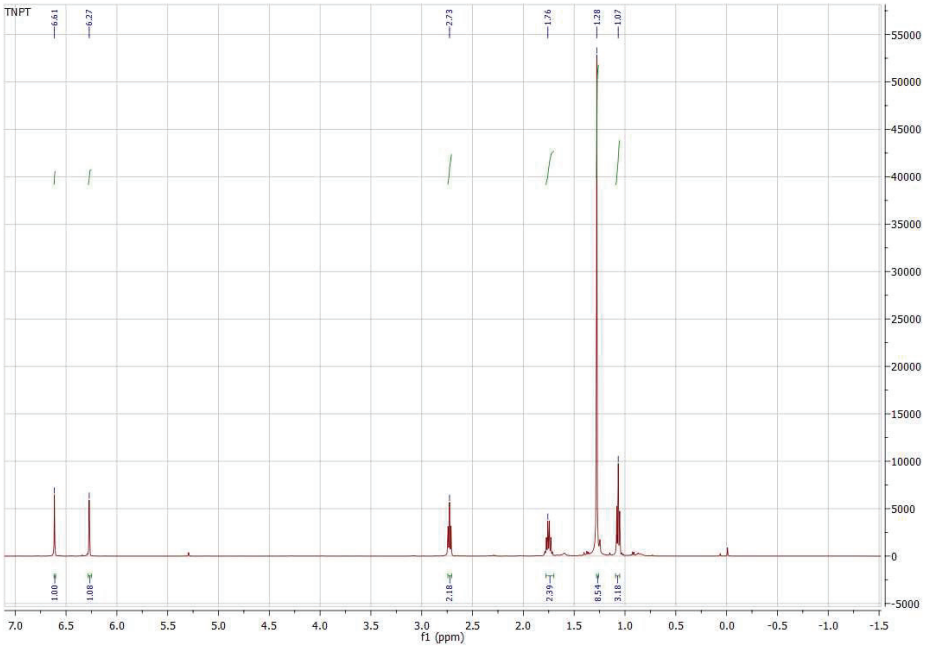

Fig. S-5. ^{1}H-NMR spectrum of 2-*tert*-butyl-5-(propylthio)-1,4-benzoquionone.

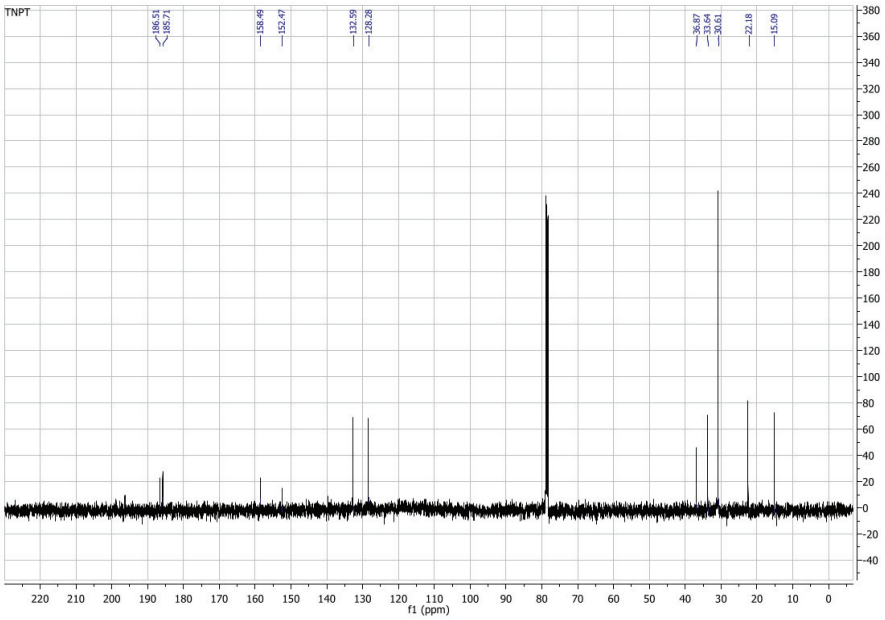

Fig. S-6. ^{13}C-NMR spectrum of 2-*tert*-butyl-5-(propylthio)-1,4-benzoquionone.

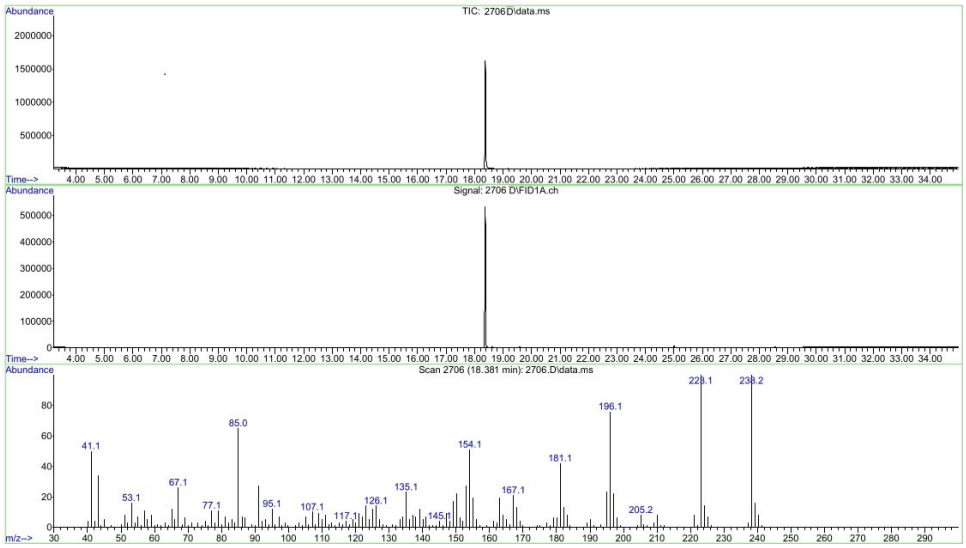

Fig. S-7. EI MS spectrum of 2-*tert*-butyl-5-(propylthio)-1,4-benzoquionone.

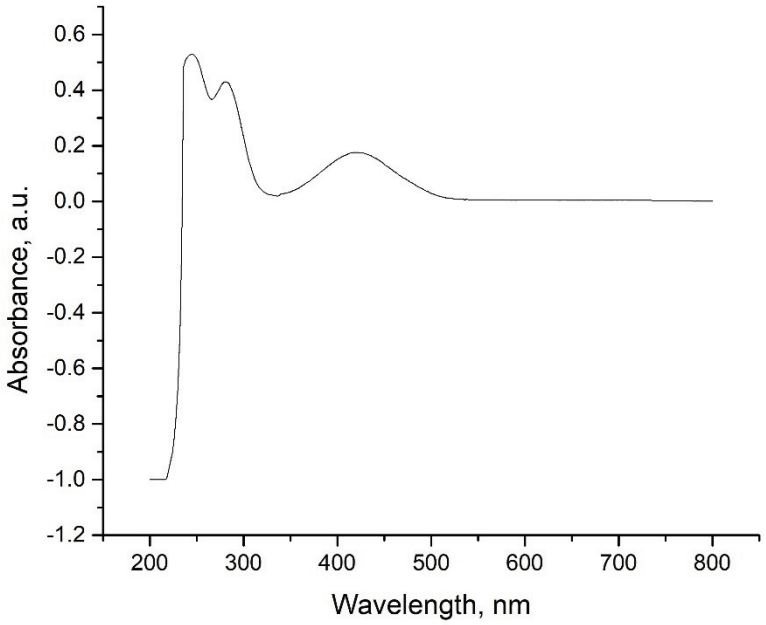

Fig. S-8. UV/Vis spectrum of 2-*tert*-butyl-5-(propylthio)-1,4-benzoquionone.

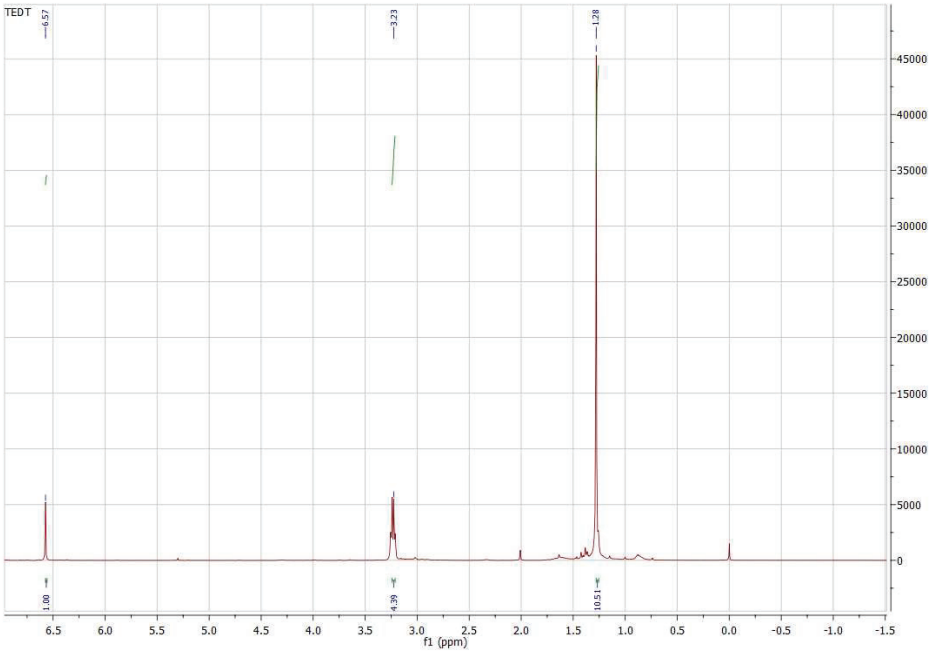

Fig. S-9. ^{1}H-NMR spectrum of 2-*tert*-butyl-5,6-(ethylenedithio)-1,4-benzoquionone.

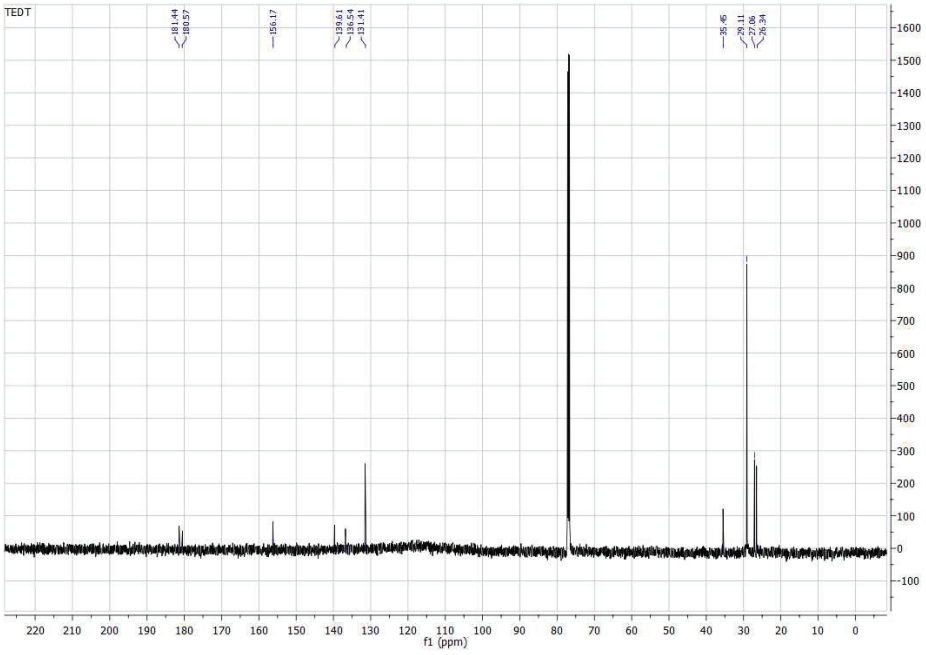

Fig. S-10. ^{13}C-NMR spectrum of 2-*tert*-butyl-5,6-(ethylenedithio)-1,4-benzoquionone.

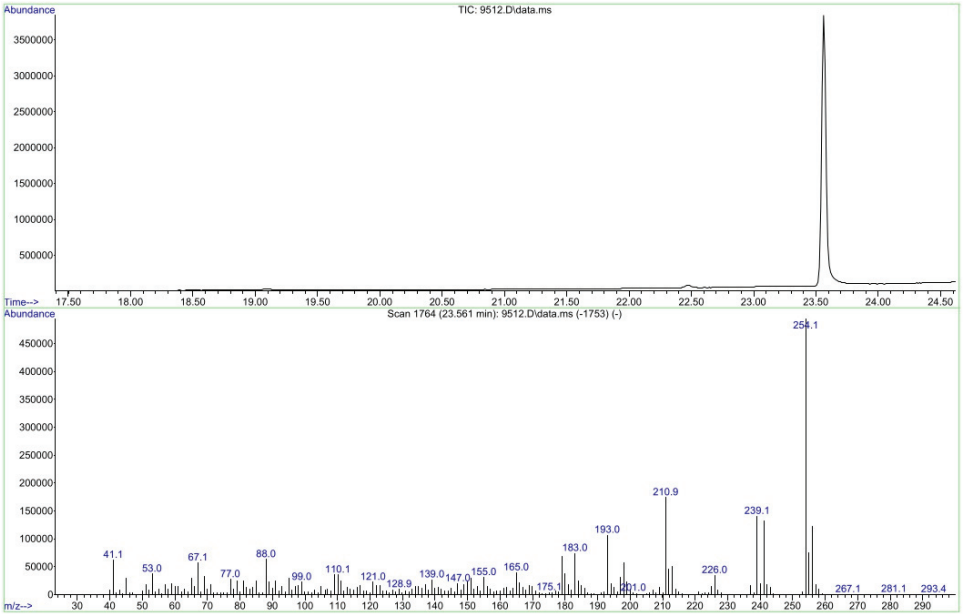

Fig. S-11. EI MS spectrum of 2-*tert*-butyl-5,6-(ethylenedithio)-1,4-benzoquionone.

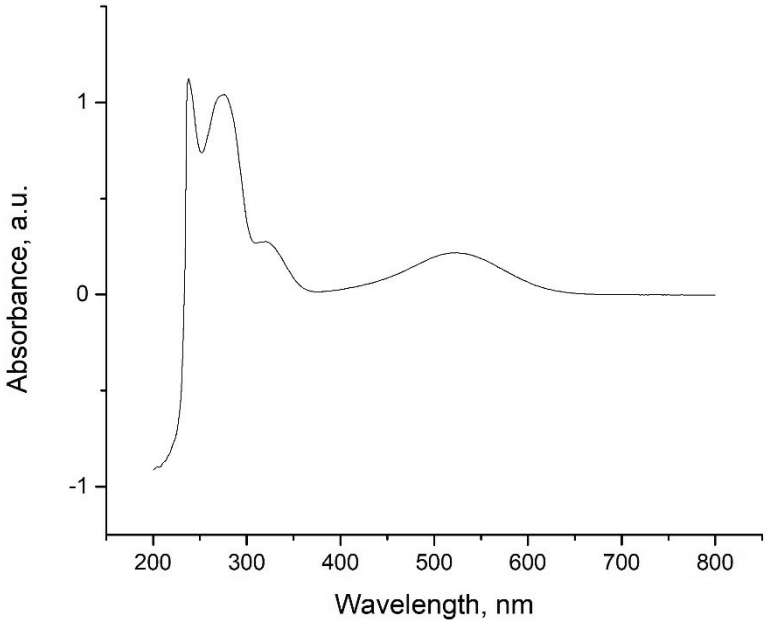

Fig. S-12. UV/Vis spectrum of 2-*tert*-butyl-5,6-(ethylenedithio)-1,4-benzoquionone.

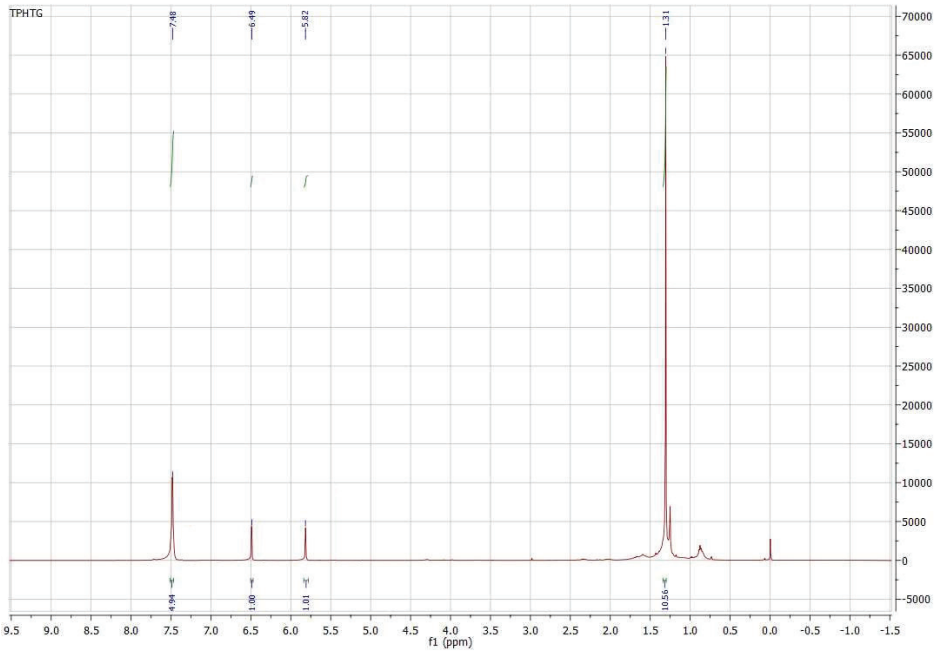

Fig. S-13. ^{1}H-NMR spectrum of 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquionone.

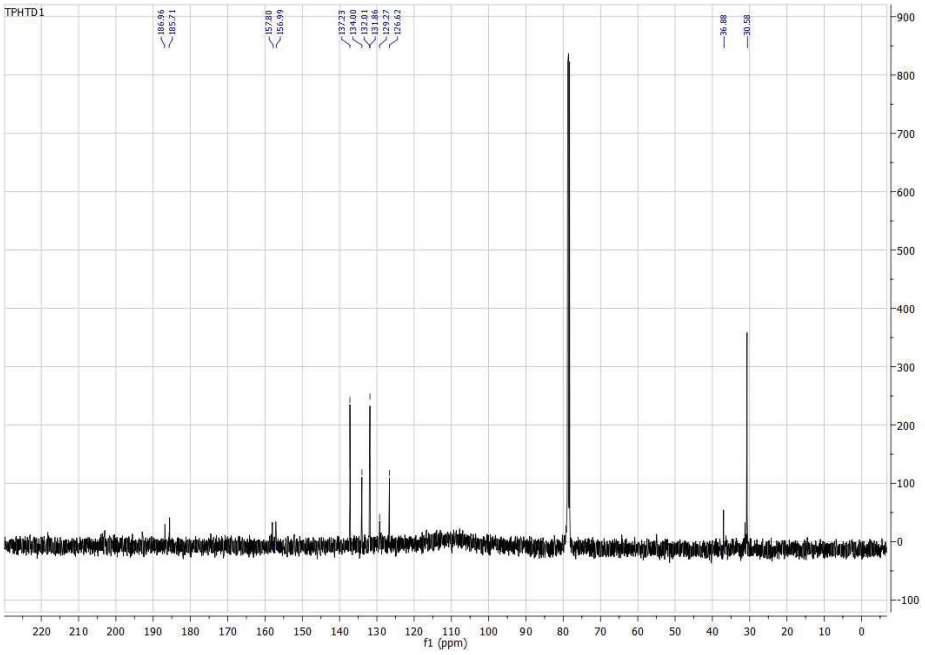

Fig. S-14. ^{13}C-NMR spectrum of 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquionone.

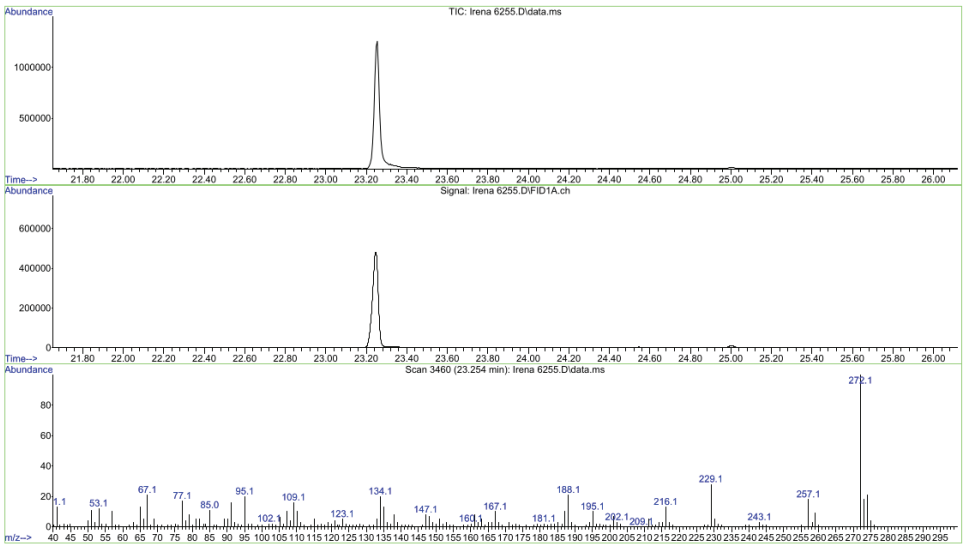

Fig. S-15. EI MS spectrum of 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquionone.

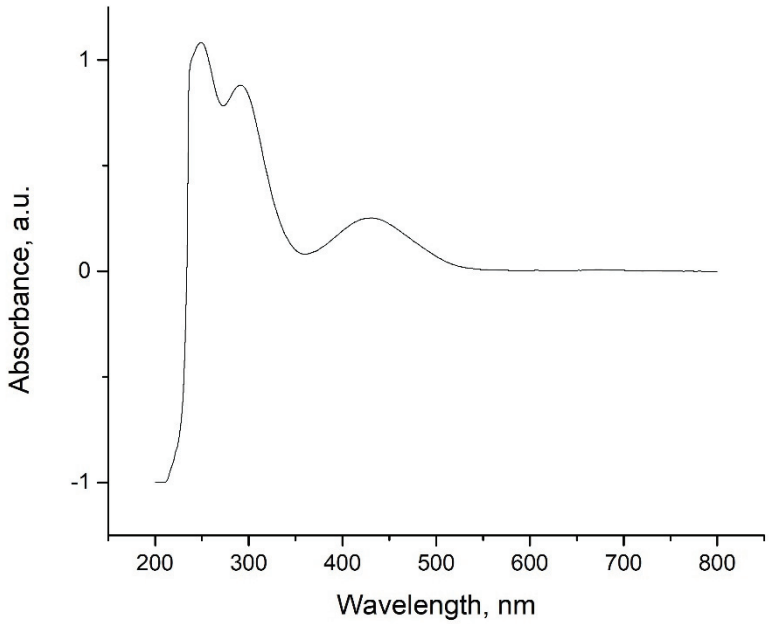

Fig. S-16. UV/Vis spectrum of 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquionone.

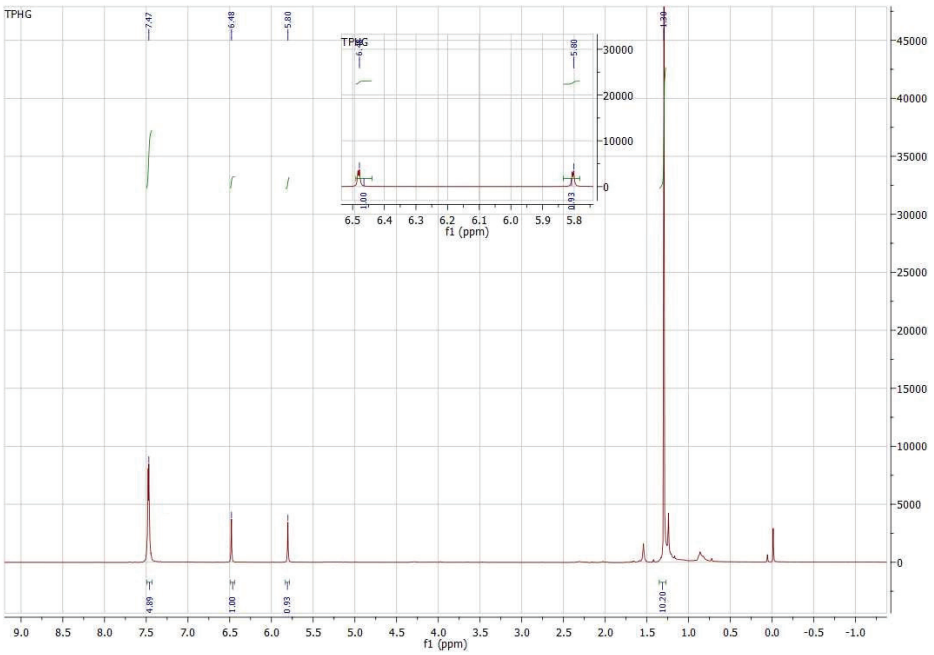

Fig. S-17. ^{1}H-NMR spectrum of 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquionone.

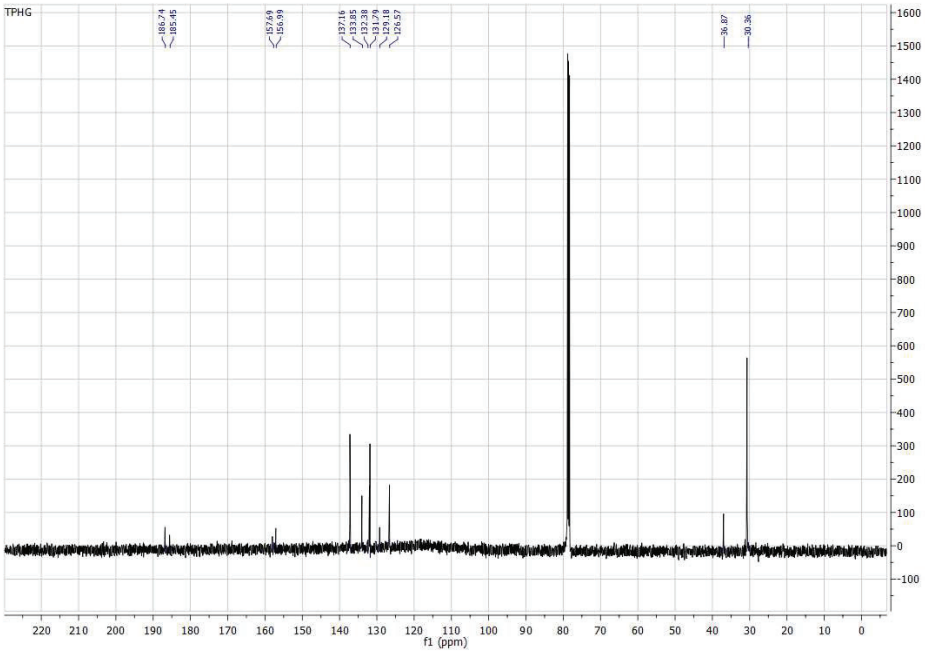

Fig. S-18. ^{13}C-NMR spectrum of 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquionone.

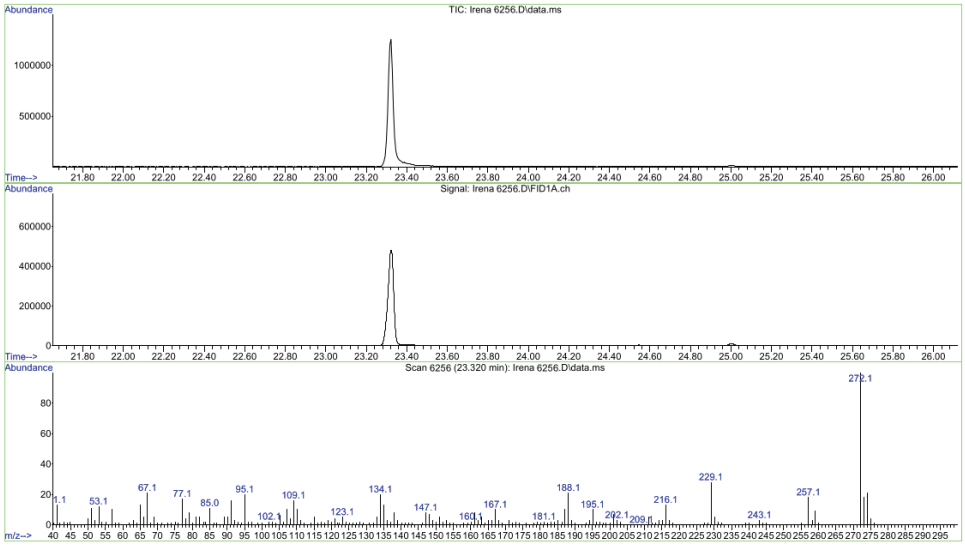

Fig. S-19. EI MS spectrum of 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquionone.

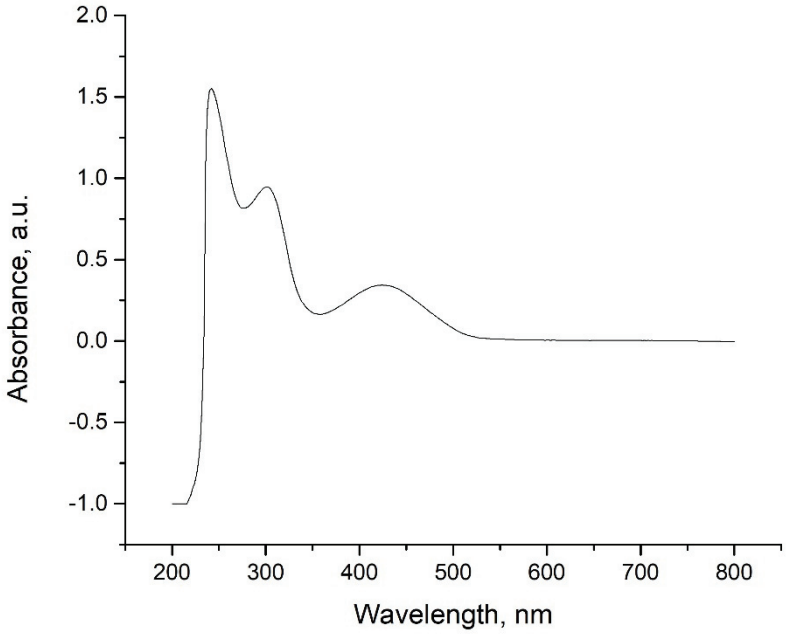

Fig. S-20. UV/Vis spectrum of 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquionone.