ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
https://www.icf.ro/rrch/

Rev. Roum. Chim.,
2024, *69*(10–12), 635–642
DOI: 10.33224/rrch.2024.69.10-12.14

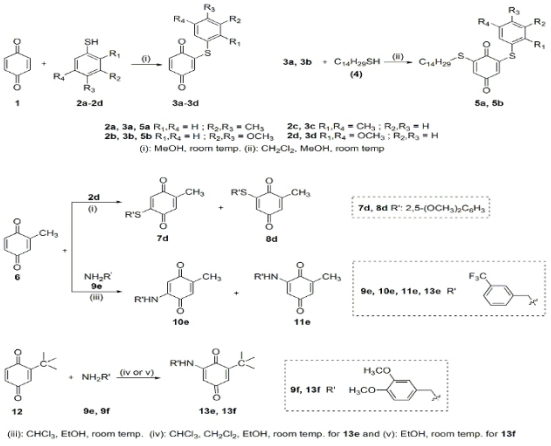
SYNTHESIS OF MONO(THIO)-, BIS(THIO)- AND MONO(AMINO)-SUBSTITUTED 1,4-BENZOQUINONES

Aysecik KACMAZ

Department of Chemistry, Division of Organic Chemistry, Istanbul University-Cerrahpasa, 34320, Avcılar, Istanbul, Turkey

Received August 22, 2024

Twelve thio- and amino- benzoquinones (**3a-d**, **5a**, **5b**, **7d**, **8d**, **10e**, **11e**, **13e** and **13f**) were synthesized from benzoquinones (*p*-benzoquinone **1**, *p*-toluquinone **6** and 2-*tert*-butyl-1,4-benzoquinone **12**) with thiols or primary amines. Among them, compounds **3a-3d** and **5a,b** have mono(thio)- and bis(thio)- substituted-benzoquinone structures, respectively. The compounds 2-(3,4-dimethylphenylthio)-6-(tetradecylthio)cyclohexa-2,5-diene-1,4-dione (**5a**) and 2-(3,4-dimethoxyphenylthio)-6-(tetradecylthio)cyclohexa-2,5-diene-1,4-dione, (**5b**) include two different thio- groups in each of them. Compounds **7d** and **8d** have 5-methyl- and 6-methyl- 2-thio-substituted-benzoquinone structures, respectively, while compounds **10e** and **11e** have 5-methyl- and 6-methyl- 2-benzylamino-substituted-benzoquinone structure, respectively. The compounds **13e** and **13f** include the 6-*tert*-butyl-2-benzylamino-substituted-1,4-benzoquinone skeleton. Twelve products were characterized, using FTIR, UV/Vis, MS(ESI), ^{1}H and ^{13}C NMR and elemental analysis. Antibacterial activity of compound **3d** has been evaluated against *Escherichia Coli* (E.coli).



INTRODUCTION

Quinone moieties are found in many natural and synthetic molecules that possess a variety of biological activities such as cytotoxic, antioxidant, antimalarial, antiviral, antibacterial, antifungal, anti-Alzheimer's disease etc. [1-3] Also, biological activities of quinones make them privileged structure in drug candidates.[1] Due to the broad biological and medical significance of quinones, studies on the synthesis of quinone derivatives is in demand. Among them, thio-, amino- or alkyl-/aryl-substituted quinones have attracted attention because of their enhanced activities.[4-7]

Among quinones, benzoquinones (natural or synthetic) are a remarkable class, based on the potential biological activities. Thus, the demand for synthesis of benzoquinone derivatives has continued to grow, and we can find many reports in the literature on the synthesis of benzoquinones, producing from reactions of *p*-benzoquinone with different groups, such as thiols,[8-12] amines,[4,13–16] arylsulfonylchlorides,[17] enamine,[18] 4-hydroxycoumarin,[19] vinyloxyalkylamines,[20] arylacetamides[21] etc.

Among these reactions, the formation of 2-(aryl/alkylthio)-1,4-quinones (or mono derivatives of quinonyl thioethers) have been reported,

* Corresponding author: kacmaz@iuc.edu.tr

obtaining from the reaction between *p*-benzoquinone and thiols under different reaction mediums such as in methanol,[11, 22] ethanol,[9,11] in the presence of CrO3 in DCM: H2O[23] etc. In this study, compounds **3a–3d** are mono derivatives of quinonyl thioethers, synthesized from *p*-benzoquinone (**1**) and thiols (**2a–2d**) in methanol medium. Additionally, when compounds **3a** and **3b** were treated with 1-tetradecanethiol (**4**), bis-thiolated products (**5a** and **5b**) were obtained, respectively, bearing with two different thio- groups in each of them, as shown Scheme 1.

p-Toluquinone (**6**) has been used before to produce potential bioactive compounds.[24,25] For example, Furukawa *et al*. reported anilino methylbenzoquinones from *p*-toluquinone and anilines, to produce carbazole-1,4-quinones[25] which are of interest in connection with the bioactive compounds. Chai *et al*. was carried out Pd (II)-mediated cyclization of anilino methylbenzoquinones to their respective carbazolediones and tested for cytotoxicity towards some cell lines.[24] In this study, the reaction between *p*-toluquinone (**6**) and amine (3-(trifluoromethyl)benzylamine, **9e**) in ethanol and chloroform medium produced two regioisomers (**10e ve 11e**), having amino-substituted methylbenzoquinone structures. As expected, we can also find similar regioisomers from the literature.[24–26] For example, Martinez-Cifuentes *et al*.[26] was carried out the reactions of *p*-toluquinone (**6**) with amines (1-phenylpiperazine or aniline), comparing H_2O or CH_2Cl_2 as reaction mediums, and yielded two/three regioisomers.

Alkyl- or aryl- sulfanyl-toluquinones were reported by Georgian and Skaletzky,[27] Karrer and Dutta,[28] von Richthofen,[29] Alcalay[30] etc. In this study, compounds **7d** and **8d** have arylsulfanyl-toluquinone structures, synthesized from methyl-*p*-benzoquinone (*p*-toluquinone, **6**) and thiol (**2d**).

The reaction of 2-tert-butyl-1,4-benzoquinone (**12**) with amines was mentioned in literature.[31-33] For example, Jeremic *et al*. prepared two regioisomers of aminobenzoquinones from the reaction of **12** with alkyl and aralkylamines and tested their anticancer and antimicrobial activities.[31] In this study, compounds **13e** and **13f** have benzylamino- substituted tert-butyl- benzoquinone skeleton, obtained from the reaction between **12** and amine (**9e** and **9f**, respectively) at room temperature without a base.

The importance of 1,4-benzoquinone derivatives has motivated this work to obtain thio- and amino-substituted-1,4-benzoquinones. Thus, *p*-benzoquinone (**1**), *p*-toluquinone (**6**) and 2- tert-butyl-1,4-benzoquinone (**12**) were used as starting compounds, as shown Scheme 1. Spectroscopic techniques (^{1}H and ^{13}C NMR, MS, UV/Vis, FTIR) have been used to determine the structures of twelve products (**3a–3d**, **5a**, **5b**, **7d**, **8d**, **10e**, **11e**, **13e**, **13f**).

MATERIALS AND METHODS

Chemistry

Compounds (**1**, **4**, **6, 12**, **2a-2d**, **9e** and **9f**) were commercially purchased and were use das received. Buchi B-540 was used to determine melting points. FTIR spectra was taken with a JASCO- FTIR 4700 spectrometer. UV-Vis spectra of compounds recorded on Perkin Elmer Lambda 35. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on Varian Unity Inova instrument. Thin layer chromatography (TLC) was obtained with Kieselgel 60F 254 plates. The purification was performed by using column chromatography with silica gel. The masses of compounds were recorded with ThermoFinnigan LCQ AdvantageMAX system. The mass spectra were scanned a mass range from m/z 50-2000. Positive ion mode ESI-MS was used for the spectra of the $[M+H]^+$ ions of compounds. For examples, methanol was used for electrospray (ESI) as solvent, and 100 µl samples in chloroform/methanol were directly injected in each run, with a capillary temperature of 290 ^{o}C (for **3a**), 300 ^{o}C (for **5a**) and a source voltage of 8 kV (for **3a** and **5a**).

Antibacterial Activity

Antibacterial activity was done at Hitit University, Scientific Technical Application and Research Center. Only one compound (**3d**) was screened for its antibacterial activity against gram-negative bacterial starin (E. Coli). To determine the antibacterial activity, the stored cultures are adjusted to 0.5 McFarland (108 CFU/mL) after 24 hours of incubation at 37 ^{o}C for 18–24 hours in TSB (Tryptic Soy broth), absorbance values vary between 0.08 and 0.13. Following these steps, 100 µl of Müller hinton broth is added to the sterile,

U-bottom, 96-well plates to be used in the microdilution method. The prepared material is applied between 1000 µg – 15.5 µg. Then, the microorganisms adjusted to 0.5 McFarland are diluted 1/100 with physiological saline and 10 µl are added (The added microorganism concentration corresponds to 10^5). Sterility is checked by adding only MHB to the six wells of the plate used, and growth control is made by applying MHB medium containing microorganisms to six wells. The prepared test system is incubated at 37 °C for 24 hours. The first well without growth after incubation is determined as the MIC value. No bacterial growth was observed per 1000 µg, 500 µg and 250 µg. Bacterial growth was observed per 125 µg, 62.5 µg, 31.25 µg and 15.5 µg. MIC value is found 125 µg/ml for **3d**. Gentamicin sulphate was used as the reference standard (0.5 µg/ml).

Synthesis of Benzoquinone Derivatives

Compounds **3a-3d, 7d, 8d, 13f** were synthesized according to the previously described procedures.[11,22,34] Chloroform and/or dichloromethane were used as solvent to increase the solubility of reaction medium (for compounds **5a**, **5b**, **10e**, **11e** and **13e**). *p*-benzoquinone (**1**), *p*-toluquinone (**6**) and 2-*tert*-butyl-1,4-benzoquinone (**12**) were used as starting quinone compounds.

Synthesis of 2-(3,4-dimethylphenylthio) cyclohexa-2,5-diene-1,4-dione (**3a**).

A solution of **1** (1.56 g, 14.5 mmol) and 3,4-dimethylbenzenethiol (1 g, 7.23 mmol, **2a**) in MeOH(30 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **3a**. Rf = 0.56 ($CHCl_3$); Yield: 46% (0.8 g); Dark orange solid; m.p. 130-131 °C; UV($CHCl_3$) λ_{max} nm (log ε):258 (3.71), 436 (3.11); IR (ATR): 3064, 2968, 2859, 1661, 1642, 1606,1561; ^{1}H NMR ($CDCl_3$) δ: 7.21 (s, 1H, CH_{arom}), 7.20-7.16 (m, 2H, CH_{arom}), 6.78 (d, 1H, $CH_{quinone}$, *J*= 10.0 Hz), 6.64 (dd, 1H, $CH_{quinone}$, *J*= 10.0 Hz, 2.5 Hz), 5.86 (d, 1H, $CH_{quinone}$, *J*= 2.5 Hz), 2.28 (3H, CH_3), 2.25 (3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 184.6, 184.1 (C=O); 155.1, 139.8, 139.1, 137.4, 136.4, 135.9, 133.0, 131.6, 125.8, 123.3; 19.75 (Me), 19.71 (Me); MS (*m/z*) = 245.06 ($[M+H]^+$, 100%); Anal. calc. for $C_{14}H_{12}O_2S$ (244.31): C 68.83, H 4.95, S 13.12. Found: C 68.80, H 4.90, S 13.10.

Synthesis of 2-(3,4-dimethylphenylthio)-6-(tetradecylthio)cyclohexa-2,5-diene-1,4- dione (**5a**).

A solution of **3a** (0.15 g, 0.61 mmol) in CH_2Cl_2 and MeOH (60 mL) and 0.29 g (1.26 mmol) 1-tetradecanethiol (**4**) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction mixture was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **5a**. Rf = 0.83 ($CHCl_3$); Yield: 5% (0.14 g); Orange-red solid; m.p. 80-82 °C; IR (ATR): 2950, 2916, 2849, 1663, 1620, 1547, 1467, 1260; ^{1}H NMR ($CDCl_3$) δ: 7.24 (s, 1H, CH_{arom}), 7.21 (s, 2H), 6.25 (d,1H, $CH_{quinone}$, *J*= 2.4 Hz), 5.82 (d, 1H, $CH_{quinone}$, *J*= 2.4 Hz), 2.75 (t, 2H, -SCH_2, *J*= 7.4 Hz), 2.30 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 1.72 (p, 2H, $C\underline{H_2}$-CH_2S), 1.49-1.40 (m, 2H, $C\underline{H_2}$-CH_3), 1.26 (s, 20H, 10xCH_2), 0.88 (t, 3H, CH_3, *J*= 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 182.1, 181.3 (C=O); 154.4, 152.2, 139.9, 139.2, 136.6, 133.2, 131.7, 126.7, 125.5, 123.7, 32.1, 31.0, 29.83, 29.80, 29.76, 29.7, 29.6, 29.5, 29.23, 29.18, 27.5, 22.8, 19.9, 19.8, 14.3; MS (*m/z*) = 473.09 ($[M+H]^+$, 100%); Anal. calc. for $C_{28}H_{40}O_2S_2$ (472.75): C 71.14, H 8.53, S 13.57. Found: C 71.10, H 8.50, S 13.52.

Synthesis of 2-(3,4-dimethoxyphenylthio) cyclohexa-2,5-diene-1,4-dione (**3b**).

A solution of **1** (1.2 g, 11.1mmol) and 3,4-dimethoxythiophenol (0.95 g, 5.55 mmol, **2b**) in MeOH (30 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **3b**. Rf = 0.2 ($CHCl_3$);Yield: 95% (1.46 g); Reddish orange solid; m.p. 122-124 °C; UV($CHCl_3$) λ_{max} nm (log ε): 242(3.88), 431 (3.03); IR (ATR): 3069, 2963, 2834, 1663, 1637, 1561, 1504; ^{1}H NMR ($CDCl_3$) δ: 7.08 (dd, 1H, *J*= 8.3 Hz, 2.1 Hz), 6.97-6.93 (m, 2H), 6.80 (d, 1H, $CH_{quinone}$, *J*=10.1 Hz), 6.67 (dd, 1H, $CH_{quinone}$, *J*= 10.1 Hz, 2.5 Hz,), 5.89

(d, 1H, $CH_{quinone}$, *J*= 2.5 Hz), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ: 184.5, 184.1 (C=O); 155.2, 151.2, 150.2, 137.5, 135.9, 128.9, 125.9, 117.6, 117.1, 112.4, 56.1, 56.0; MS (*m/z*) = 277.06 ($[M+H]^+$, 100%); 246.2 ($[M-OCH_3]^+$, 76%); Anal. calc. for $C_{14}H_{12}O_4S$ (276.31): C 60.86, H 4.38, S 11.60. Found: C 60.81, H 4.36, S 11.57.

Synthesis of 2-(3,4-dimethoxyphenylthio)-6-(tetradecylthio)cyclohexa-2,5-diene- 1,4-dione **(5b)**.

A solution of **3b** (0.211 g, 0.76 mmol) in CH_2Cl_2 and MeOH (60 mL) and 0.35 g (1.52 mmol) 1-tetradecanethiol (**4**) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **5b**. Rf = 0.5 ($CHCl_3$);Yield: 36% (0.14 g); Red solid, m.p. 121-123 °C; UV($CHCl_3$) λmax nm (log ε): 241 (4.19), 377 (3.48), 488 (3.12); IR (ATR): 2916, 2849, 1662, 1617, 1582, 1550, 1506, 1465; ^1H NMR ($CDCl_3$) δ: 7.09 (dd, 1H, *J*= 8.3 Hz, 2.0 Hz), 6.96-6.92 (m, 2H), 6.26 (d, 1H, $CH_{quinone}$, *J*= 2.4 Hz), 5.84 (d, 1H, $CH_{quinone}$, *J*=2.3 Hz,), 3.93 (3H, OCH_3), 3.87 (3H, OCH_3), 2.75 (t, 2H, -CH_2S, *J*= 7.4 Hz), 1.77-1.67 (m, 2H, -C$\underline{H_2}$-CH_2S-), 1.49-1.39 (m, 2H, $CH_{2aliph.}$), 1.26 (s, 20H, 10 x CH_2), 0.88 (t, 3H, CH_3, *J*= 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 182.1, 181.4, 154.5, 152.2, 151.3,150.3, 129.1, 126.8, 125.5, 117.8, 117.6, 112.6, 56.3, 56.2, 32.1, 31.0, 29.83, 29.80, 29.76, 29.7, 29.6, 29.5, 29.24, 29.18, 27.5, 22.8, 14.3; MS (*m/z*) = 505.19 ($[M+H]^+$, 100%); Anal. calc. for $C_{28}H_{40}O_4S_2$ (504.75): C 66.63, H 7.99, S 12.71. Found: C 66.60, H 7.95, S 12.74.

Synthesis of 2-(2,5-dimethylphenylthio) cyclohexa-2,5-diene-1,4-dione (**3c**).

A solution of *p*-benzoquinone (1.56 g, 14.4 mmol, **1**) and 2,5-dimethylbenzenethiol (1g, 7.23 mmol, **2c**) in MeOH (50 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **3c**. Rf = 0.7 ($CHCl_3$);Yield: 34% (0.6 g); Dark brownish red solid; m.p. 118-119 °C; UV($CHCl_3$) λmax nm (log ε): 255(3.50), 434 (2.92); IR (ATR): 3049, 2960, 2917, 2849, 1658, 1634, 1604,1543; ^1H NMR ($CDCl_3$) δ: 7.30-7.24 (m, 2H, CH_{arom}, adjacented with the solvent signal of δ 7.26 ppm), 7.22 (dd, 1H, CH_{arom}, *J*= 7.7 Hz, 1.9 Hz), 6.84 (d, 1H, $CH_{quinone}$, *J*= 10.0 Hz), 6.69 (dd, 1H, $CH_{quinone}$, *J*= 10.1Hz, 2.5 Hz), 5.75 (d, 1H, $CH_{quinone}$, *J*= 2.5 Hz), 2.34 (6H, 2xCH_3); ^{13}C NMR ($CDCl_3$) δ: 184.6, 184.3, 153.5, 139.8, 137.8, 137.7, 137.0, 136.1, 132.1, 131.6, 125.7, 125.6, 20.8, 19.9; MS (*m/z*) = 245.07 ($[M+H]^+$, 100%); Anal. calc. for $C_{14}H_{12}O_2S$ (244.31): C 68.83, H 4.95, S 13.12. Found C 68.87, H 4.90, S 13.14.

Synthesis of 2-(2,5-dimethoxyphenylthio) cyclohexa-2,5-diene-1,4-dione (**3d**).

A solution of **1** (1.2g, 11.1 mmol) and 2,5-dimethoxythiophenol (1g, 5.87 mmol, **2d**) in MeOH (30 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **3d**. Rf = 0.4 ($CHCl_3$);Yield: 75% (1.22g); Dark red solid; m.p. 132-133; UV($CHCl_3$) λmax nm (log ε): 241 (4.21), 305(3.77), 432 (3.48); IR (ATR) 2952, 2890, 2829, 1662, 1637, 1561, 1492, 1285; ^1H NMR ($CDCl_3$) δ: 7.01 (dd, 2H, *J* = 7.3 Hz, 2.7 Hz), 6.95 – 6.92 (m, 1H), 6.79 (d, 1H, *J*= 10.0 Hz), 6.65 (dd, 1H, *J* = 10.0 Hz, 2.5 Hz), 5.83 (d, *J* = 2.5 Hz, 1H), 3.78 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ: 184.7, 184.3 (C=O); 154.2, 154.1, 152.3, 137.5, 136.0, 125.8, 122.0, 118.2, 114.8, 113.1, 110.1; 56.6, 56.0 (OCH_3); MS (*m/z*) = 277.07 ($[M+H]^+$, 100%); Anal. calc. for $C_{14}H_{12}O_4S$ (276.31): C 60.86, H 4.38, S 11.60. Found C 60.82, H 4.33, S 11.64.

Synthesis of 2-(2,5-dimethoxyphenylthio)-5-methylcyclohexa-2,5-diene-1,4-dione **(7d)** ***and 2-(2,5-dimethoxyphenylthio)-6-methylcyclohexa-2,5-diene-1,4-dione*** (**8d**).

A solution of methyl-*p*-benzoquinone (0.24 g, 1.96 mmol, **6**) and 2,5-dimethoxythiophenol (0.34g, 2.0 mmol, **2d**) in MeOH (30 mL) was solution with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction mixture was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure products **7d** and **8d**.

7d: Rf = 0.3 ($CHCl_3$); Yield: 28% (0.16 g); Red solid; m.p. 127-128°C; UV($CHCl_3$) λ_{max} nm (log ε):

288 (3.92), 431 (3.29); IR (ATR): 2953, 2831, 1640, 1618, 1558, 1271; ^{1}H NMR ($CDCl_3$) δ: 7.02 (dq, 2H, *J* = 6.3 Hz, 3.1 Hz,), 6.96-6.93 (m, 1H), 6.64 (q, 1H, *J* = 1.6 Hz), 5.82 (s, 1H), 3.78 (d, 6H, 2xOCH_3, *J*= 10.2 Hz), 2.02 (d, 3H, CH_3, *J*= 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ:185.1, 184.5 (C=O); 154.3, 154.2, 152.3, 147.2, 132.9, 125.9, 122.1, 118.2, 115.2, 113.2; 56.7, 56.0 (OCH_3); 15.9 (Me); MS (*m/z*) = 290.95 ($[M+H]^+$, 100%); 260.2 ($[M-OCH_3]^+$, 20%). Anal. calc. for $C_{15}H_{14}O_4S$ (290.33): C 62.05, H 4.86, S 11.04. Found: C 62.01, H 4.82, S 11.00.

8d: Rf = 0.3 ($CHCl_3$); Yield: 2% (0.012 g); Dark red solid; m.p. 149-151 ºC; UV($CHCl_3$) λ_{max} nm (log ε): 242 (4.03), 289 (4.00), 433 (3.41); IR (ATR): 2961, 2918, 2830, 1661, 1620, 1560, 1493, 1261; ^{1}H NMR ($CDCl_3$) δ: 7.03 (dd, 2H, *J*= 7.4 Hz, 2.8 Hz), 6.98-6.94 (m, 1H), 6.50 (dq, 1H, *J*= 3.2 Hz, 1.6 Hz), 5.80 (d, 1H, *J*= 2.5 Hz), 3.80 (d, 6H, 2xOCH_3, *J*= 10.2 Hz,), 2.08 (d, 3H, CH_3, *J*= 1.6 Hz,); ^{13}C NMR ($CDCl_3$) δ: 184.9, 184.8 (C=O); 154.1, 154.0, 152.1, 145.3, 134.0, 125.9, 122.0, 118.0, 115.2, 113.0; 56.5, 55.9 (OCH_3); 15.83 (Me); MS (*m/z*) = 291.00 ($[M+H]^+$, 100%); 260.2 ($[M-OCH_3]^+$, 31%). Anal. calc. for $C_{15}H_{14}O_4S$ (290.33): C 62.05, H 4.86, S 11.04. Found: C 62.00, H 4.84, S 11.09.

***Synthesis of 2-(3-trifluoromethyl)benzylamino)-5-methylcyclohexa-2,5-diene-1,4- dione* (10e) *and 2-(3-trifluoromethyl)benzylamino)-6-methylcyclo hexa-2,5-diene-1,4- dione* (11e).**

A solution of **6** (0.60 g, 4.9 mmol) and 3-(trifluoromethyl)benzyl amine (**9e**, 0.86 g, 4.9 mmol) in $CHCl_3$ (30 ml) and EtOH (20 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure products **10e** and **11e**.

10e: Rf = 0.3 ($CHCl_3$); Yield: 17% (0.25 g); Dark red solid; m.p. 145-147 ºC; UV($CHCl_3$) λ_{max}nm (log ε): 273 (4.00), 346 (3.52), 466 (3.26); IR (ATR): 3242, 2920, 1672, 1638, 1575, 1488,1325; ^{1}H NMR ($CDCl_3$) δ: δ 7.60 (d, 1H, CH_{arom}, *J*= 7.4 Hz), 7.54-7.44 (m, 3H, CH_{arom}, 6.51 (q, 1H, $CH_{quinone}$, *J*= 1.6 Hz), 5.94 (sb, 1H, NH), 5.48 (s, 1H, $CH_{quinone}$), 4.38 (d, 2H, -CH_2-, *J*= 5.9 Hz), 2.08 (d, 3H, CH_3, *J*= 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 185.8, 183.5 (C=O); 150.0, 146.2, 137.0, 130.7, 129.5, 129.2, 124.99, 124.95, 124.15, 124.12, 99.51; 46.15 (CH_2); 16.52 (CH_3); MS (*m/z*) = 296.21 ($[M+H]^+$, 100%); Anal. calc. for $C_{15}H_{12}F_3NO_2$ (295.26): C 61.02, H 4.10, N 4.74. Found C 61.06, H 4.13, N 4.72.

11e: Rf = 0.1 ($CHCl_3$); Yield: 4% (0.06 g); Dark red solid; m.p. 154-156 ºC; ^{1}H NMR ($CDCl_3$)δ: 7.59 (d, 1H, CH_{arom}, *J*=7.4 Hz), 7.54-7.44 (m, 3H, CH_{arom}), 6.47 (dq, 1H, $CH_{quinone}$, *J*= 3.2 Hz, 1.5 Hz), 5.95 (s, 1H, NH), 5.43 (d, 1H, $CH_{quinone}$, *J*= 2.5 Hz), 4.37 (d, 2H, -CH_2-, *J*= 5.9 Hz), 2.04 (d, 3H, CH_3, *J*= 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 186.0, 184.1 (C=O); 146.1, 141.2, 137.0, 136.3, 130.69, 130.68, 129.5, 125.0, 124.1, 99.5; 46.3 (CH_2); 15.3 (CH_3); MS (*m/z*) = 296.21 ($[M+H]^+$); Anal. calc. for $C_{15}H_{12}F_3NO_2$ (295.26): C 61.02, H 4.10, N 4.74. Found C 61.05, H 4.14, N 4.70.

***Synthesis of 2-(3-trifluoromethyl)benzylamino)-6-tert-butylcyclohexa-2,5-diene-1,4-dione* (13e).**

A solution of **12** (0.60 g, 3.65 mmol) and 3-(trifluoromethyl)benzyl amine (**9e**, 0.64 g, 3.65 mmol) in a mixture of $CHCl_3$, CH_2Cl_2 and EtOH (total 50 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using chloroform to give the pure product **13e**. Rf = 0.3 ($CHCl_3$); Yield: 20% (0.25 g); Red solid; m.p. 153–155 ºC; UV($CHCl_3$) λ_{max} nm (log ε): 241 (3.80), 280 (4.00), 464 (3.42); IR (ATR): 3350, 2970,2872, 1668, 1621, 1571, 1485, 1331, 1165; ^{1}H NMR ($CDCl_3$) δ: 7.60 (d, 1H, CH_{arom}, *J*= 7.3 Hz), 7.54 (s, 1H, CH_{arom}), 7.53–7.46 (m, 2H, CH_{arom}), 6.47 (d, 1H, *J*= 2.4 Hz), 6.02 (s, 1H, NH), 5.44 (d, 1H, *J*= 2.4 Hz), 4.35 (d, 2H, -C$\underline{H_2}$-NH-, *J*= 5.8 Hz), 1.28 (9H, 3xCH_3); ^{13}C NMR ($CDCl_3$) δ: 186.4, 183.2, 151.8, 147.3, 137.2, 134.9, 131.7, 131.0, 130.9, 129.7, 125.2, 124.5, 98.7; 46.6 (-$\underline{C}H_2$-NH-); 35.0 (-$\underline{C}$-$(CH_3)_3$); 29.2 ($3CH_3$); MS (*m/z*) = 338.22 ($[M+H]^+$, 100%); Anal. calc. for $C_{18}H_{18}F_3NO_2$ (337.34): C 64.09, H 5.38, N 4.15. Found C 64.11, H 5.34, N 4.12.

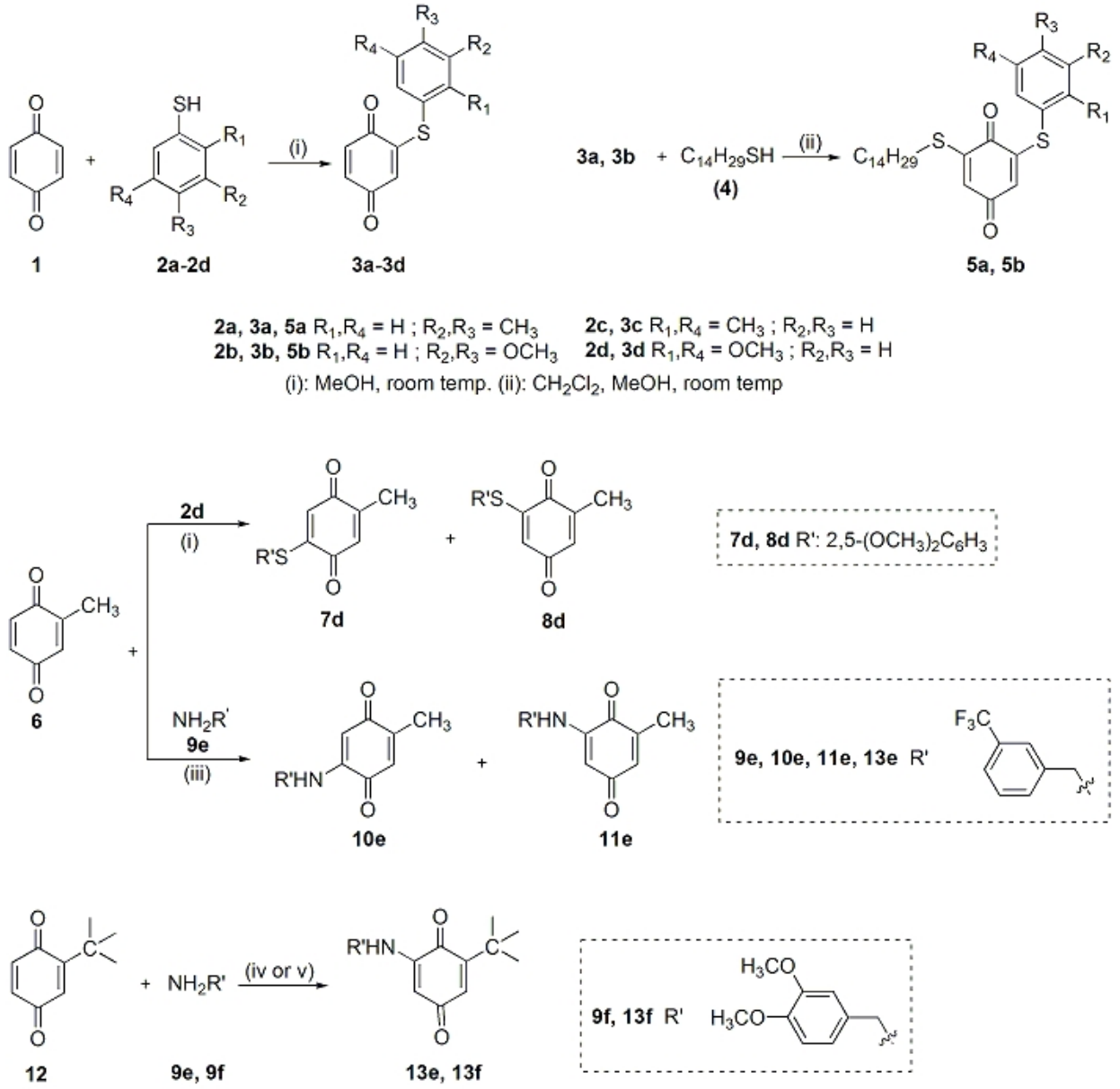
***Synthesis of 2-(3,4-dimethoxybenzylamino)-6-tert-butylcyclohexa-2,5-diene-1,4-dione* (13f).**

A solution of **12** (0.75 g, 4.56 mmol) and 3,4-dimethoxybenzylamine (**9f**, 0.76 g, 4.56 mmol) in EtOH (30 mL) was stirred with magnetic stirring until consumption of starting compounds, monitoring by TLC. Reaction solution was extracted three times with $CHCl_3$ and H_2O. The

organic layers were dried, concentrated under vacum, and the residue was subjected to column chromatography using EtOAc:$CHCl_3$ (1:9) to give the pure product **13f**. Rf = 0.13 ($CHCl_3$): Yield: 98% (1.47 g); Dark red solid; m.p. 138-140 °C; UV($CHCl_3$) λ_{max} nm (log ε): 241 (4.27), 280 (4.25), 471 (3.58); IR (ATR): 3313, 2956, 2872, 2836, 1670, 1628, 1567, 1490, 1443, 1252; ^{1}H NMR ($CDCl_3$) δ: 6.85 (s, 2H), 6.79 (s, 1H), 6.46 (d, 1H, *J*= 2.3 Hz), 5.93 (sb, 1H, NH), 5.49 (s, 1H), 4.18 (d, 2H, -C<u>H</u>$_2$-NH-, *J*= 5.4 Hz), 3.88 (d, 6H, 2xOCH_3, *J*= 1.3 Hz), 1.26 (d, 9H, 3xCH_3, *J*= 1.7 Hz); ^{13}C NMR ($CDCl_3$) δ: 186.2, 183.2 (C=O); 151.4, 149.4, 148.9, 147.2, 134.9, 128.4, 120.3, 111.4, 111.0, 97.9; 56.0 (OCH_3); 46.9 (-<u>C</u>H$_2$-NH-); 34.8 (C_{tert}); 29.0 (Me); MS (*m/z*) = 329.95 ([M+H]$^+$, 100%); Anal. calc. for $C_{19}H_{23}O_4N$ (329.39): C 69.28, H 7.04, N 4.25. Found C 69.25, H 7.00, N 4.21.

RESULTS AND DISCUSSION

Scheme 1 describes the synthetic outlines for new thio- or amino- substituted benzoquinones (**3a–3d**, **5a**, **5b**, **7d**, **8d**, **10e**, **11e**, **13e**, **13f**). Detailed methods of these compounds can be found in Experimental section.



Scheme 1 – Synthesis of compounds of series **3a–d**, **5a–b**, **7d**, **8d**, **10e**, **11e**, **13e, 13f**.

The reaction of *p*-benzoquinone (**1**) with thiols (**2a–d**) in methanol leads to formation of mono(thio)- substituted benzoquinones (**3a–3d**) (34–95% yields). Compounds **3a–3d** have presented three benzoquinone protons at about δ 6.79 ppm (d, *J*= 10 Hz), δ 6.65 ppm (dd, *J*= 10, 2.5 Hz), δ 5.80 ppm (d, *J*= 2.5 Hz), and also CH_3/OCH_3 protons, bearing with thio- group, at δ 2.25–3.92 ppm in the proton NMR spectrum. Also, **3a-3d** showed UV (241–258 and 431–436 nm) and IR (1634–1642 cm^{-1}) absorptions characteristic of benzoquinones.[22,35]

The reactions of 1-tetradecanethiol (**4**) with **3a** and **3b** gave compounds **5a** (5% yield) and **5b** (36% yield), respectively. These compounds (**5a** and **5b**) include two different thio- groups in their benzoquinoid structures, which was agreement with the spectral analyses. For example, thiolation of **3b** yielded red solid of **5b**, (36%). Compound **5b** exhibited close similarity to **3b** in the ^{1}H-NMR spectrum: presence of two methoxy groups' protons at δ 3.93 and 3.87 ppm, benzoquinoid proton at δ 5.84 (d, *J*= 2.3 Hz) and aromatic protons at δ 7.09 (dd, *J*= 8.3, 2.0 Hz) and δ 6.96–6.92 (m). But, the presence of aliphatic -CH_2- protons at δ 2.75 (t, *J*= 7.4 Hz), δ 1.77–1.67 (m), δ 1.49–1.39 (m), δ 1.26 (s), and of methyl protons at δ 0.88 (t, *J*=7.0 Hz) are an indication that the thio group ($C_{14}H_{29}S$-) have been introduced to benzoquinone skeleton to produce **5b**, including two different thio-groups in it. The location of these thio- groups on the compound **5b** was elucidated based on ^{1}H-NMR spectrum at δ 6.26 ppm and δ 5.84 ppm with *J* values of 2.4 Hz and 2.3 Hz, respectively. Hence, **5b** was deduced to be an 2,6-thiosubstituted 1,4-benzoquinone.

The reaction between methyl-*p*-benzoquinone (**6**) and thiol (2,5-dimethoxylthiophenol, **2d**) in 1:1 molar ratio, using methanol as solved, resulted two isomers 5-methyl- and 6-methyl- 2-thio-1,4-benzoquinones (**7d** and **8d**), respectively. The separation of isomers (**7d** and **8d**) was performed by column chromatography; the 2-(2,5-dimethoxyphenylthio)-5-methylcyclohexa-2,5-diene-1,4-dione (**7d**) (28%), m.p. 127–128 ºC, and 2-(2,5-dimethoxyphenylthio)-6-methylcyclohexa-2,5-diene-1,4-dione (**8d**) (2%), m.p. 149–151 ºC, were isolated. Also, the position of thio- group (SR´) in the isomers **7d** and **8d** was elucidated on the basis of the ^{1}H NMR spectra; the quinoid protons of **7d** resonated at δ 6.64 (q) with a coupling constant *J*= 1.6 Hz and at δ 5.82 (s) in the ^{1}H NMR spectrum, while **8d** resonated at δ 6.50 (dq) with two coupling constants *J*= 3.2 Hz and 1.6 Hz, and at δ 5.80 (d) with a coupling constant *J*= 2.5 Hz.

Methyl-*p*-benzoquinone (**6**) was reacted with **9e** to produce 5-methyl- and 6-methyl-2- benzylamino-1,4-benzoquinones, **10e** and **11e**, respectively. Similarly, the reaction of 2-tert- butyl-1,4-benzoquinone (**12**) with **9e** gave compound 2-(3-trifluoromethyl) benzylamino)-6-*tert*-butylcyclohexa-2,5-diene-1,4-dione **13e**. The assignment of location of -NHR' group (at C-5 or C-6) on the 1,4-benzoquinone moiety was determined by protons δ 6.51 (q) with *J*=1.6 Hz and δ 5.48 ppm (s) for compound **10e**; δ 6.47 ppm (dq) with *J*=3.2, 1.5 Hz and δ 5.43 ppm (d) with *J*=2.5 Hz for compound **11e**; δ 6.47 ppm (d) with *J*= 2.4 Hz and δ 5.44 ppm (d) with *J*= 2.4 Hz for compound **13e**, similar values of *J* were observed previously.[25,26,31] The carbon NMR spectra of compounds **10e**, **11e**, **13e** and **13f** revealed signals for two characteristic C=O carbons of benzoquinones at about δ 186 and δ 183 ppm, -$\underline{C}H_2$-NH- carbons at about δ 46 ppm, methyl carbons at about δ 16 ppm (for **10e** and **11e**) and δ 29 ppm (for **13e** and **13f**), *tert*- butyl carbons (-$\underline{C}(CH_3)_3$) at about δ 35 ppm (for **13e** and **13f**) and methoxy carbon (OCH_3) at δ 56.0 ppm (for **13f**), as expected. Also, **10e**, **13e** and **13f** showed UV (273–280 and 464–471 nm) and IR (at about 3300, 1670, 1630 cm^{-1}) absorptions characteristic of aminobenzoquinones.[26, 31]

CONCLUSION

In conclusion, the synthesis of mono(thio)- and bis(thio)- substituted benzoquinones (**3a-d** and **5a-b**), mono(thio)- and mono(amino)-substituted toluquinones (**7d**, **8d** and **10e**, **11e**) and mono(amino)-substituted *tert*-butylbenzoquinones (**13e** and **13f**) have been reported, carrying out the reaction between different benzoquinones (**1**, **6** and **12**) with thiols/amines (**2a-d**, **4**, **9e**, **9f**). Among them, 2,5-thio- and 2,6-thio- substituted-benzoquinones (**5a** and **5b)** have two different thio- groups in each of them. Antibacterial activity of compound **3d** has been evaluated against *Escherichia Coli* and its MIC value is found 125 µg/ml. The compounds were characterized using UV/Vis, IR, NMR or mass spectroscopy studies.

Acknowledgements: We thank for the financial support (Project Number: FBA-2021-31554, the Research Fund of Istanbul University-Cerrahpasa).

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