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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Nucleophilic substitution reactions of 1,4-naphthoquinone and biologic properties of novel *S*-, *S*,*S*-, *N*-, and *N*,*S*-substituted 1,4-naphthoquinone derivatives

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Abstract A novel series of *S*-, *S*,*S*-, *N*- and *N*,*S*-substituted 1,4-naphthoquinone derivatives were synthesized and evaluated for their antibacterial and antifungal activity. Among the synthesized compounds especially **10a** and **11b** have been discovered as an antibacterial or antifungal agents, and **15f** is the most effective compound against *M. luteum* as potent antifungal. The structures of the novel products were characterized by micro analysis, UV/Vis, ^{1}H NMR, ^{13}C NMR, and MS. The electrochemical properties of some of the novel 1,4-naphthoquinone derivatives were also investigated by cyclic voltammetry.

Keywords 1,4-Naphthoquinone · Amines · Thiols · Antibacterial activity · Antifungal activity

Introduction

Quinone structure is widespread in nature and synthetic compounds (Lien *et al*., 2002; Ibis *et al*., 2011; Oku *et al*., 2002; Ibis *et al*., 2013a). 1,4-Naphthoquinone is an important example of quinonoid structure and used from pharmaceuticals to dyes. The derivatives of 1,4-naphthoquinone have also many biological responses such as anti-inflammatory, antiallergic, antipruritic, and anticancer activities (Huang *et al*., 1998; Verma, 2006; Ngampong *et al*., 2003). The quinone structure undergoes redox cycling to generate reactive oxygen species that can destroy the tumor cells (Huang *et al*., 2000). Because of this important feature the electrochemistry of quinonoid structures have also been widely studied as an example of a simple two-step cathodic reduction (Guin *et al*., 2011; Kim *et al*., 2001; Pedersen *et al*., 1998).

In continuation of our previous work for the synthesis of biologically active quinones, we carried out the reactions of 2,3-dichloro-1,4-naphthoquinone **1** with sulfur or amine nucleophiles as biologic active agents and characterization of them with spectral methods.

Result and discussion

The novel 2,3-di(thio)substituted compounds **3a–d** were obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and thiol compounds **2a–d** in ethanol with sodium carbonate. In the mass spectrum of the compounds **3a** and **3b**, the molecular ion peaks were observed at *m*/*z* (%) 485 (100) $[M+Na]^+$ and 513 $[M+H]^+$, respectively. The carbon atom signals of carbonyl groups of compound **3c** were observed at 182.31 ppm as one peak only. The IR spectrum of compound **3d** showed characteristic carbonyl group's band at 1,665 cm^{-1} (Scheme 1).

The novel compounds **7**, **8** and known compound **6** (Ibis and Sahinler Ayla, 2011) were synthesized by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and the mixture of thiol compounds **4** and **5** in dimethylformamide with sodium carbonate. The compound **6** was synthesized before by our group (Ibis and Sahinler Ayla, 2011). In the IR

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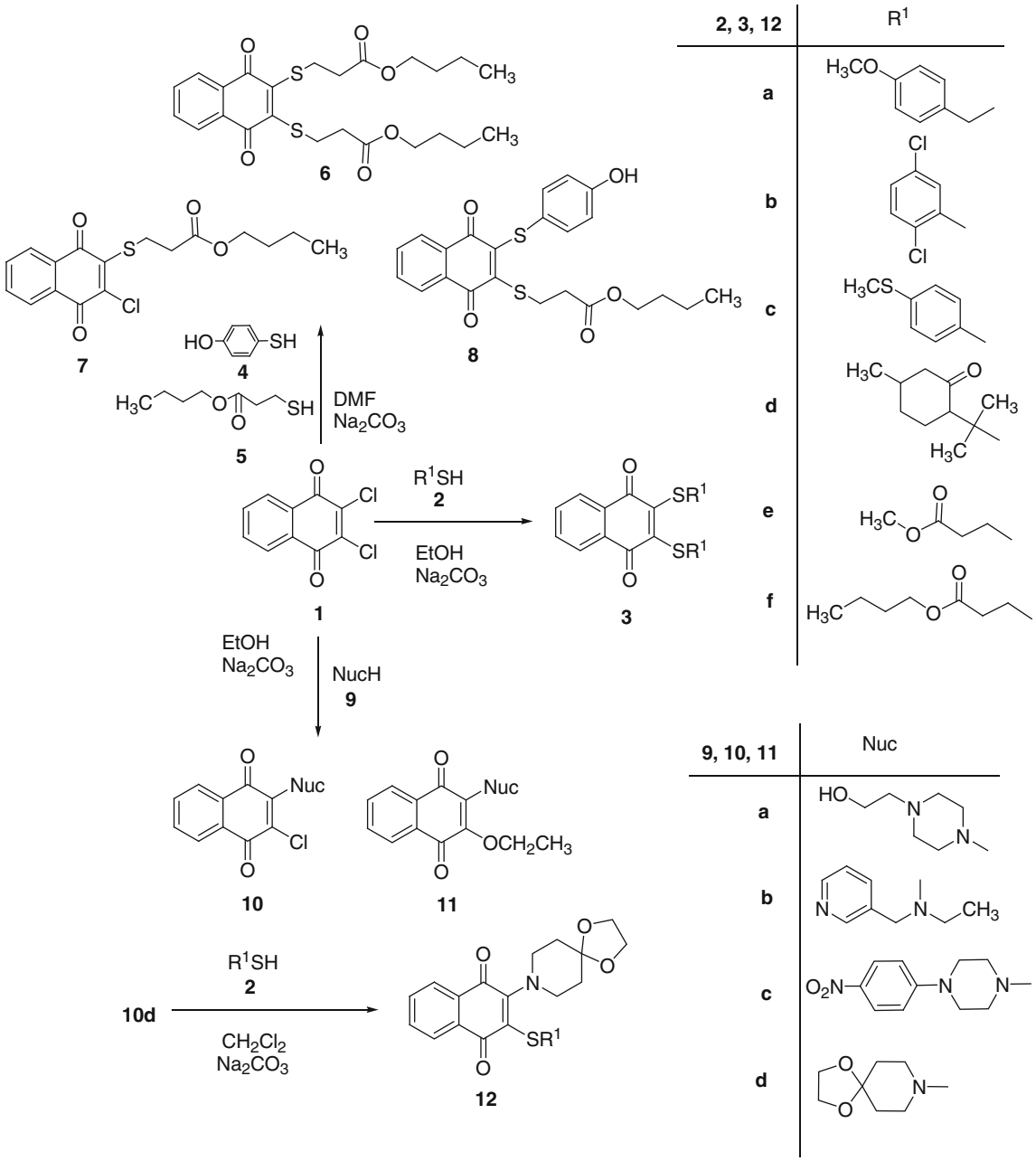


Scheme 1 Synthesis of novel naphthoquinone derivatives

spectra of compounds **7** and **8**, the quinone carbonyl group's band was observed at 1,670 and 1,664 cm^{-1}, while the ester carbonyl group's band was observed at 1,732 and 1,720 cm^{-1}, respectively.

The *N*-substituted compound **10a** was obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and compound **9a** in ethanol solution of sodium carbonate. The IR spectrum the compound **10a** showed broad band at 3,256 cm^{-1} for the OH stretching. The *N*-substituted compound **10c** was synthesized from the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and compound **9c** in ethanol solution of sodium carbonate. The molecular ion peak of compounds **10c** was identified at *m*/*z* 397 $[M]^+$ in the positive ion mode for ESI technique.

The ethoxy-amino-substituted compound **11b** was obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and compound **9b** in ethanol with sodium carbonate. In the ^1H NMR spectrum of compound **11b**, protons of methylene group (–O–CH_2–) were observed as a multiplet at 4.28 ppm.

The compound **10d** was synthesized before by our group (Ibis *et al.*, 2013a). The monoaminosubstituted compound **10d** (Ibis *et al.*, 2013a) was treated with

aliphatic thiols, and the *N*,*S*-substituted compounds **12e** and **12f** were obtained. The molecular ion peak of compounds **12e** and **12f** was identified at *m*/*z* (%) 441 (100) $[M+Na]^+$ and 460 $[M+H]^+$ in the positive ion mode for ESI technique, respectively (Scheme 2).

The *N*,*S*-substituted compounds **15a**–**g** were synthesized by the reactions of 2-chloro-3-((perchlorophenyl)thio)naphthalene-1,4-dione **13** (Ibis *et al*., 2013b) and amine compounds **14a**–**g** in dichloromethane solution of sodium carbonate. The target compounds were designed regarding the related nucleophile compound's biologic activity properties such as morpholine, thiomorpholine, methyl or butyl esters, and piperazine. (Levin *et al*., 2006; Milczarska *et al*., 2005; Olma *et al*., 2012; Ojima *et al*., 1997). It is also known that the sulfur or nitrogen containing naphthoquinone derivatives show important activity properties (Perez-Sacau *et al*., 2007, Corral *et al*., 2006).

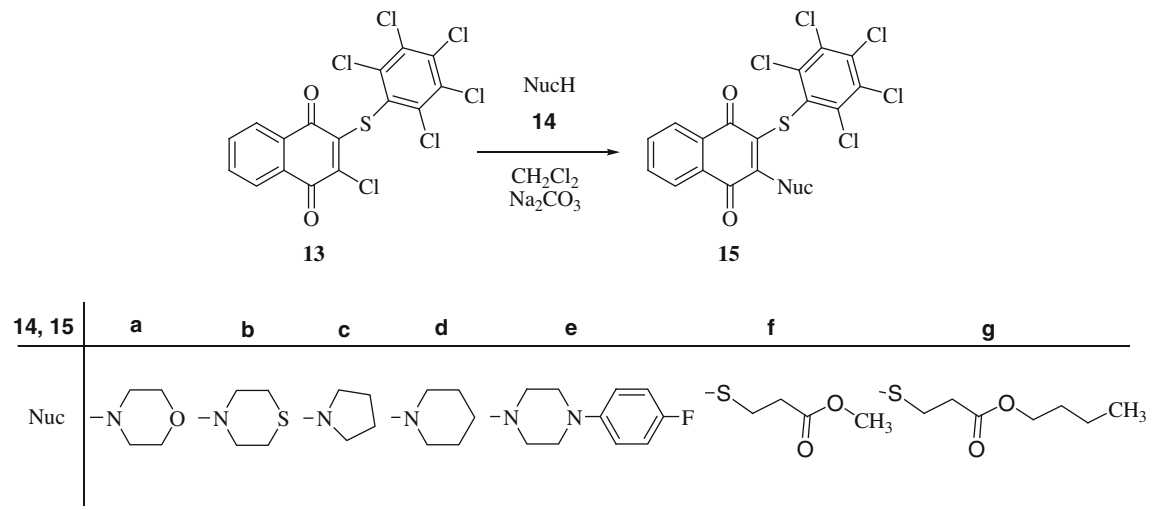
The molecular ion peak of compounds **15a** and **15b** was identified at *m*/*z* (%) 524 (100) $[M]^+$ and 540 $[M]^+$ in the positive ion mode for ESI technique, respectively. In the ^{1}H NMR spectrum of compounds **15c** and **15d**, protons of piperidine and pyrrolidine were observed as a multiplet at 3.99 and 3.60 ppm. In the ^{13}C NMR spectrum of compound **15e**, the carbon atoms of the carbonyl groups were gave signals at 180.48, 178.91 ppm. In the IR spectra of compounds **15f** and **15g**, the quinone carbonyl group's band was observed at 1,662 and 1,654 cm^{-1}, while the ester carbonyl group's band was observed at 1,736 and 1,666 cm^{-1}, respectively.

Biological study

The novel naphthoquinone derivatives were evaluated for their antifungal activity against fungi *Candida tenuis VKM Y-70* and *Aspergillus niger F-1119* by the diffusion method (Murray *et al.*, 1995) and serial dilution method (National Committee for Clinical Laboratory Standard, 1998) with a view to developing therapeutic agents having broad spectrum in antifungal activity. Antibacterial activity of synthesized compounds was elucidated against *Escherichia coli B-906*, *Staphylococcus aureus 209-P*, and *Mycobacterium luteum B-917* by the diffusion method and serial dilution method as shown in Tables 1 and 2. Their activities were compared with those of the known antibacterial agent vancomycin and the antifungal agent nystatin. Afterward, we have further synthesized and screened compounds of **10a** and **10c** antibacterial activity by the diffusion method as shown Table 1 on the basis of structure–activity relationship of antifungal activity of the (hetero) cyclic quinone derivatives.

The test-culture *E. coli* appeared not to be sensitive to any compounds except that **10a**. The *S. aureus* was moderately sensitive to compound **10a** by the diffusion method, whereas compound **3c** is nearly sensitive. The *M. luteum* strain was sensitive to compounds **3c**, **10a**, and **15f** at a concentration of 0.5 % (diameter of the inhibition zone was 11.0, 28.7, and 10.7 mm, respectively). Comparison of antibacterial activity with antibacterial drug vancomycin (at 0.1 % concentration) showed that **3c** (at 0.1 % concentration), **10a** (at 0.5 % concentration), and **15f** (at 0.5 % concentration) had good activity against *M. luteum*.

The test-cultures *C. tenuis and A. niger* appeared not to be sensitive to **15a**–**g** except that **15g** for *A. niger*. Antifungal activity against *C. tenuis* was observed for **10c** and **11b** at concentration of 0.5 % ($d = 15.7$ and 20.0 mm, respectively). *C. tenuis* was sensitive to compounds **3a**, **3b**, and **3d** at a concentration of 0.5 % (diameter of the inhibition zone was 11.7, 7.0, and 7.4 mm, respectively). Compounds **3b**, **8**, and **10a** have no antifungal activity against *A. niger* at 0.5 and 0.1 % evaluated concentrations



Scheme 2 Novel N,S- and S,S- substituted naphthoquinone compounds

Table 1 Antibacterial and antifungal activities of compounds by diffusion method

Compounds	Concentration (%)	Inhibition diameter of microorganism growth (mm)				
		Antibacterial activity			Antifungal activity	
		E. coli	*S. aureus*	*M. luteum*	*C. tenuis*	*A. niger*
3a	0.5	0	0	7.0	11.7	7.4
	0.1	0	0	0	7.4	0
3b	0.5	0	0	0	7.0	0
	0.1	0	0	0	0	0
3c	0.5	0	8.7	11.0	0	6.0
	0.1	0	0	8.7	0	0
3d	0.5	0	0	0	7.4	6.0
	0.1	0	0	0	0	0
6	0.5	0	0	0	0	6.0
	0.1	0	0	0	0	0
8	0.5	0	0	6.0	0	0
	0.1	0	0	0	0	0
10a	0.5	8.0	20.7	28.7	0	0
	0.1	6.0	11.4	16.0	0	0
10c	0.5	0	0	0	15.7	13.7
	0.1	0	0	0	8.5	0
11b	0.5	0	0	7.4	20.0	19.0
	0.1	0	0	0	12.4	8.0
12e	0.5	0	0	12.6	0	8.4
	0.1	0	0	0	0	0
12f	0.5	0	0	0	0	9.0
	0.1	0	0	0	0	7.0
15a	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
15b	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
15c	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
15d	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
15e	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
15f	0.5	0	0	10.7	0	0
	0.1	0	0	6.0	0	0
15g	0.5	0	0	0	0	6.0
	0.1	0	0	0	0	0
C[a]	0.1	14.0	15.0	18.0	19.0	20.0

[a] Vancomycin was used as a control in the tests of antibacterial activity, and nystatin was used in the tests of antifungal activity of the synthesized compounds

Table 2 Antibacterial and antifungal activities of compounds by serial dilution method

Compounds	Antibacterial activity			Antifungal activity	
	MIC (μg/mL)			MIC (μg/mL)	
	E. coli	*S. aureus*	*M. luteum*	*C. tenuis*	*A. niger*
3a	+	+	+	62.5	250.0
3b	+	+	+	500.0	+
3c	+	250.0	125.0	+	500.0
3d	+	+	500.0	+	500.0
6	+	+	+	+	+
8	+	+	+	+	+
10a	500.0	62.5	31.2	125.0	125.0
10c	+	+	+	31.2	31.2
11b	+	62.5	125.0	62.5	31.2
12e	+	+	250.0	+	500.0
12f	+	+	125.0	+	62.5
15a	+	+	+	+	+
15b	+	+	+	125.0	+
15c	+	+	+	+	+
15d	+	+	+	+	+
15e	+	+	125.0	+	+
15f	+	250.0	15.6	62.5	+
15g	+	+	125.0	+	+

+: Growth of microorganisms

by the diffusion method. Compounds **10c**, **12e** and **12f** were found to exhibit low antifungal activity against *A. niger* on comparison with antifungal drug nystatin evaluated by diffusion method. Compound **11b** has good antifungal activity against *A. niger* at 0.5 % concentration by the diffusion method.

The biological results of the compounds were classified as follows: The antibacterial activity was considered as significant when the MIC was 100 μg/mL or less; moderate, when the MIC was 100–500 μg/mL; weak, when the MIC was 500–1,000 μg/mL; and inactive, when the MIC was above 1,000 μg/mL. Evaluation of the antibacterial activity of synthesized compounds showed that **10a** has MIC (minimum inhibition concentration) 31.2 μg/mL for *M. luteum*, but **15f** was the most potent with MIC = 15.6 μg/mL for *M. luteum* (Table 2).

Notable activity for **10c** was observed against *C. tenuis* fungi at 31.2 μg/mL concentration. Evaluations of antifungal activity of compounds **3a**, **11b**, and **15f** showed MIC = 62.5 μg/mL against test-culture *C. tenuis*. MIC of **10a** and **15b** was observed at 125.0 μg/mL against test-culture *C. tenuis* (Table 2). Notable activity for **10c** and

11b was observed against *A. niger* fungi at 31.2 μg/mL concentrations. Evaluations of antifungal activity of compounds **12f** showed MIC = 62.5 μg/mL against test-culture *A. niger*. MIC of **10a** and **3a** was observed at 125.0 and 250.0 μg/mL against test-culture *A. niger*, respectively.

In conclusion, compounds especially **10a** and **11b** have been discovered as an antibacterial or antifungal agents. Synthesized compounds have been employed to prepare new antifungal agents with low MICs against *S. aureus*, *M. luteum* bacteria, and *C. tenuis* and *A. niger* fungi in comparison with controls. The some compounds (**3a–b**, **6**, **8**, and **15a–d**) did not show any significant antifungal and antibacterial activity against fungi and bacteria species. Among the tested compounds, **15f**, **10c**, and **11b** are the most effective compounds against *C. tenuis* as potent antifungal. **15f** is the most effective compound against *M. luteum* as potent antifungal.

Electrochemical study

Some of the novel naphthoquinone derivatives were studied by cyclic voltammetry in aprotic media (DMF) using tetrabutylammonium perchlorate (0.10 M) as supporting electrolyte at 100 mV s^{-1} on glassy carbon electrode. The electrochemical parameters, including cathodic peak potentials (E_{pc1} and E_{pc2}), the half-wave peak potentials ($E_{1,1/2}$), and the difference between the first oxidation and reduction processes (ΔE_p), are given at Table 3.

The cyclic voltammogram of compound **10c** showed in Fig. 1. The first and second peaks in Fig. 1 correspond to semiquinone (Q/Q^-) and dianion (Q^-/Q^{2-}) pairs, respectively (Fig. 1).

Table 3 Half-wave potentials (for the 1st wave) and electrochemical data for some of the novel naphthoquinone derivatives (10^{-3} M) in DMF/TBAP 0.1 M, $\upsilon = 100$ mV s^{-1}

Compound	E_p (Ic) (V)	E_p (IIc) (V)	ΔE^a_{p1} (mV)	$E^b_{1,1/2}$ (V)
1	−0.246	−1.052	211	−0.1405
8	−0.356	−0.937	76	−0.318
3b	−0.145	−0.786	75	−0.107
10a	−0.499	−1.154	82	−0.458
10c	−0.460	−1.154	72	−0.424
15a	−0.421	−0.765	–	–
15b	−0.398	–	–	–
15c	−0.599	−1.074	–	–
15e	−0.390	–	–	–
15f	−0.433	–	–	–

[a] $\Delta E_{p1} = E_{pa1} - E_{pc1}$

[b] $E_{1,1/2} = (E_{pa1} + E_{pc1})/2$

Experimental

General methods

Melting points were measured using a Buchi B-540 melting point apparatus and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. Infrared (IR) spectra were recorded in KBr pellets in Nujol mulls on a Perkin Elmer Precisely Spectrum One FTIR spectrometer. UV spectra were recorded in $CHCl_3$ on the UV–Vis spectrophotometer TU-1901. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ on a Varian Unity INOVA spectrometer. Mass spectra were obtained on a Thermo Finnigan LCQ Advantage MAX LC/MS/MS spectrometer using ESI technique.

Cyclic voltammetry measurements were performed in a conventional three-electrode cell using a computer controlled system of a Gamry Reference 600 Model potentiostat/galvanostat. A glassy carbon disk was used as a working electrode. The surface of the working electrode was polished with alumina before each run. A platinum wire served as the counter electrode. The reference electrode was an Ag/AgCl electrode. Electrochemical grade tetrabutylammonium perchlorate (TBAP) in extra pure DMF was employed as the supporting electrolyte at a concentration of 0.10 M. Prior to each run, solutions were purged with nitrogen. Measurements were taken over a potential range between 1 and −2 V with a step rate of 0.1 V s^{-1}.

Products were isolated by column chromatography on silica gel (Fluka silica gel 60, particle size 63–200 μm). Thin-layer chromatography (TLC) was performed on Merck silica gel plates ($60F_{254}$), and detection was carried out with ultraviolet light (254 nm). All chemicals were reagent grade and used without further purification.

General procedures

General procedure 1: synthesis of disulfanyl derivatives of 1,4-naphthoquinones

Potassium carbonate (1.52 g) was dissolved in ethanol (65 mL). 2,3-Dichloro-1,4-naphthoquinone (**1**) and thiol (**2**) were added to the solution, respectively. Without heating, the mixture was stirred for 6–8 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. After the reaction mixture was filtered, filtrate was extracted in a Soxhlet extractor with dichloromethane. After recovery of the solvent, the crude product was purified by column chromatography.

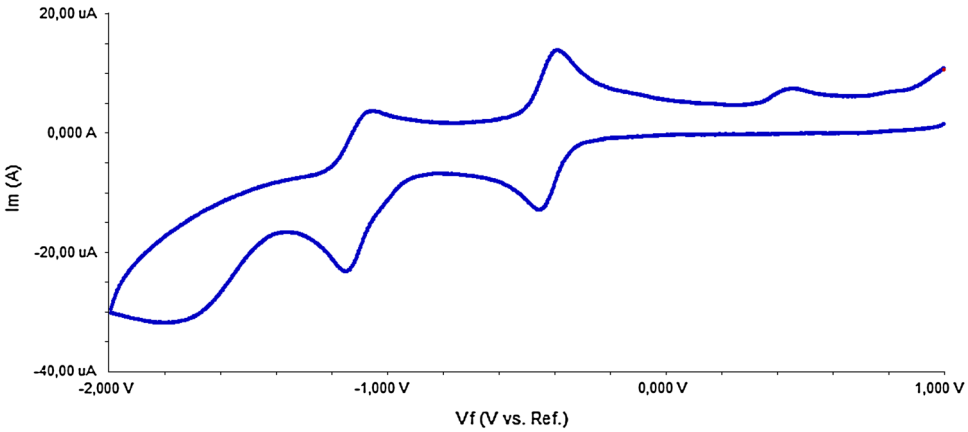


Fig. 1 Cyclic voltammogram of compound **10c** in DMF + 0.1 M TBAP on glassy carbon electrode at 0.1 V s^{-1}

*2,3-Bis((4-methoxybenzyl)thio)naphthalene-1,4-dione (**3a**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **2a** (0.68 g, 4.38 mmol) by using general procedure 1* Orange solid. M.p.: 194–195 °C. Yield: 0.65 g (38.69 %). R_f : 0.9 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): υ 2,925(C–H), 1,655(C=O), 1,592 (C=C). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 252 (4.84), 276 (4.93), 443 (3.88). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 3.38 (s, 6H, O–CH_3), 4.39(s, 4H, –S–CH_2), 6.79 (d, 4H, H_{arom}), 7.15(d, 4H, H_{arom}). 7.78–7.84(m, 2H, H_{arom}) 7.90–7.99(m, 2H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 40.23 (–S–CH_3), 55.26 (O–CH_3), 158.66, 147.16, 134.05, 132.66, 130.43, 126.64, 114.10 (C_{arom}, CH_{arom}), 178.77 (C=O). MS [+ESI]: *m/z* 485 $[M+Na]^+$. Anal. Calcd. for $C_{26}H_{22}O_4S_2$ (M, 462.58): C, 67.51; H, 4.79; S, 13.86 %. Found: C, 67.15; H, 4.20; S, 14.30 %.

*2,3-Bis((2,5-dichlorophenyl)thio)naphthalene-1,4-dione (**3b**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **2b** (0.79 g, 4.38 mmol) by using general procedure 1* Orange solid. M.p.: 88–89 °C. Yield: 0.72 g (40.26 %). R*f*: 0.85 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): υ 2,919(C–H), 1,661(C=O), 1,593 (C=C). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 260 (4.87), 438 (3.99); ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 7.14–7.18 (dd, 4H, H_{arom}), 7.26 (s, 2H $_{Harom}$), 7.64–7.68 (m, 2H, H_{arom}), 7.94–7.96 (m, 2H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 145.64, 133.14, 132.78, 132.06, 131.28, 131.13, 129.93, 128.37, 126.46 (C_{arom}, CH_{arom}), 176.79 (C=O). MS [+ESI]: *m/z* 513 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{10}O_2S_2Cl_4$ (M, 512.26): C, 51.58; H, 1.97; S, 12.52 %. Found: C, 51.28; H, 1.55; S, 13.50 %.

*2,3-Bis((4-(methylthio)phenyl)thio)naphthalene-1,4-dione (**3c**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **2c** (0.69 g, 4.38 mmol) by using general procedure 1* Red solid. M.p.: 80–81 °C. Yield: 0.54 g (31.99 %). R*f*: 0.75 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): υ 2,921(C–H), 1,662(C=O), 1,590 (C=C). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 280 (5.08), 477 (3.92). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 2.42–2.72 (m, 6H, –S–CH_3), 7.13–7.18 (dd, 8H, H_{arom}), 7.78–7.81 (m, 4H, H_{arom}), 7.93–7.95 (m, 2H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 15.20 (–CH_3), 127.30, 127.37, 127.70 129.81, 132.51, 139.46 (C_{arom}, CH_{arom}), 182.31 (C=O). MS [+ ESI]: *m/z* 466 $[M]^+$. Anal. Calcd. for $C_{24}H_{18}O_2S_4$ (M, 466.66): C, 61.77; H, 3.89; S, 27.48 %. Found: C, 61.65; H, 3.75; S, 28.11 %.

*2,3-Bis((2-(4-methyl-2-oxocyclohexyl)propan-2-yl)thio) naphthalene-1,4-dione (**3d**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **2d** (0.82 g, 4.38 mmol) by using general procedure 1* Red solid. M. p.: 114–114.6 °C. Yield: 0.76 g (42 %). R*f*: 0.6 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): υ 2,925(C–H), 1,665(C=O), 1,589 (C=C). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 262 (5.06); ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 1.17–1.25 (m, 6H, CH-CH_3), 1.73 (s, 12H, C-CH_3), 1.87–2.37(m, 16H, –CH–CH_2), 7.43–8.16 (m, 4H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 26.93, 28.09, 33.38, 36.31, 37.65 (–CH_2), 55.74, 63.76 (O=C–CH_2), 120.96, 128.59, 130.43, 131.85, 132.84, 136.66, 137.00, 146.03 (C_{arom}, CH_{arom}), 182.86, 207.99 (C=O). MS [−ESI]: *m/z* 526 $[M-H]^-$. Anal. Calcd. for $C_{30}H_{38}O_4S_2$ (M, 526.75): C, 68.40; H, 7.27; S, 12.17 %. Found: C, 68.66; H, 7.57; S, 12.70 %.

General procedure 2: for the reactions of mixture of two thiols with 1,4-naphthoquinones

2,3-Dichloro-1,4-naphthoquinone was dissolved in DMF as reaction media (50 mL). Subsequently, the mixture of two thiols (**4** and **5**) and Na_2CO_3 (1.52 g) were added to the solution, respectively. Without heating, the mixture was stirred for 4–6 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. The reaction mixture was extracted in a Soxhlet extractor with dichloromethane. After recovery of the

solvent, the crude product was purified by column chromatography.

*Dibutyl 3,3′-((1,4-dioxo-1,4-dihydronaphthalene-2,3-diyl)bis (sulfanediyl))dipropanoate (**6**) (Ibis and Sahinler Ayla, 2011), butyl 3-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio) propanoate (**7**), and butyl 3-((3-((4-hydroxyphenyl)thio)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)propanoate (**8**) were synthesized by the reaction of 1 (2 g, 8.76 mmol) and the mixture of 4 (0.35 g, 2.19 mmol)and 5 (1.43 g, 8.76 mmol) by using general procedure 2* **6** (Ibis and Sahinler Ayla, 2011): Red oil; Yield 0.25 g (6 %); R*f*: 0.24 with $CHCl_3$ as an eluent; IR (KBr, cm^{-1}): 2,959, 2,933, 2,873 (C–H), 1,659 (quinone C=O), 1,734 (ester C=O), 1,591 (C=C); MS (+ESI): m/z 479 $(M + H)^+$; $C_{24}H_{30}O_6S_2$ (M, 478.62). Calcd. C, 60.23; H, 6.32; S, 13.40. Found C, 60.12; H, 6.15; S, 12.90 %.

7: Red oil; Yield 0.20 g (7 %); R*f*: 0.4 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): 2,959, 2,932, 2872 (C–H), 1670 (quinone, C=O), 1,732 (ester C=O); UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 263 (4.94), 431 (l4.02). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 0.85 (m, 3H, –CH_3), 1.18–1.56 (m, 4H, –CH_2), 3.55 (t, $J = 7.3$ Hz, 2H, –SCH_2), 4.03 (t, $J = 6.8$ Hz, 2H, –OCH_2), 2.69 (t, $J = 6.8$ Hz, 2H, –C=O–CH_2), 8.01–8.09 (m, 2H, CH_{napht}), 7.68 (m, 2H, CH_{napht}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 12.67 (–CH_3); 29.58, 28.69, 18.10 (–CH_2); 63.87 (–OCH_2); 34.54 (–SCH_2); 126.27, 131.56, 132.93 (CH_{napht}, C_{napht}), 147.25 (–C–S), 174.04 (–C–Cl), 170.30 (–O–C=O), 178.76 (quinone C=O). MS (−ESI): m/z 352 $[M]^-$. Anal. Calcd. for $C_{17}H_{17}ClO_4S$ (M, 352.83): C, 57.87; H, 4.86; S, 9.09 %. Found: C, 57.80; H, 4.45; S, 10.05 %.

8: Red solid; M.p.: 114–115 °C; Yield 0.40 g (11 %); R*f*: 0.10 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): 3,404 (–OH), 2,956, 2,931, 2,873 (C–H), 1,664 (quinone C=O), 1,720 (ester C=O); UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 256 (4.48), 443 (3.59). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 0.83 (m, 3H, –CH_3), 1.18–1.57 (m, 4H, –CH_2), 3.44 (t, $J = 7.3$ Hz, 2H, –SCH_2), 4.01 (t, $J = 6.8$ Hz, 2H, –OCH_2), 2.65 (t, $J = 7.3$ Hz, 2H, –C=O–CH_2), 5.72 (s, 1H, –OH), 6.67–7.99 (m, 8H, CH_{napht}, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): 12.68 (–CH_3); 28.96, 18.10 (–CH_2); 63.95 (–OCH_2); 34.53 (–SCH_2); 115.29, 115.31, 122.75, 126.02, 126.06, 131.74, 131.94, 132.56, 132.77, 132.82, 132.88 (CH_{napht}, C_{napht}, CH_{arom}, C_{arom}), 147.90 (–C–S), 170.73 (–O–C=O), 178.63, 177.30 (quinone C=O). MS (+ESI): m/z 442 $(M)^+$; $C_{23}H_{22}O_5S_2$ (M, 442.55). Hesaplanan C, 62.42; H, 5.01; S, 14.49. Bulunan C, 62.22; H, 5.10; S, 13.60 %.

General procedure 3: for amination of 1,4-naphthoquinones

Sodium carbonate (1.52 g) was dissolved in ethanol as reaction media (50 mL). 2,3-Dichloro-1,4-naphthoquinone (**1**) amine (**9**) was added to the solution, respectively. Without heating, the mixture was stirred for 4–6 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. The reaction mixture was extracted in a Soxhlet extractor with dichloromethane. After recovery of the solvent, the crude product was purified by column chromatography.

*2-Chloro-3-(4-(2-hydroxyethyl)piperazin-1-yl)naphthalene-1,4-dione (**10a**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **9a** (0.566 g, 4.38 mmol) by using general procedure 3* Orange solid. M.p.: 225.2–225.8 °C. Yield: 0.51 g (32.6 %). R_f: 0.8 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): υ 3,256 (O–H), 2,924 (C–H_{arom}), 1,676 (C=O), 1,553 (C=C), 1,445 (C–N). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 249 (4.49), 275 (4.45), 478 (3.64). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 1.2 (s, 1H, O–H), 2.48–2.69 (m, 10H, –N–CH_2), 3.56–3.57(m, 2H, –CH_2), 7.78–7.80 (m, 2H, $H_{quinone}$). 7.91–7.94 (m, 2H, $H_{quinone}$). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 51.16, 54.30, 58.58, 60.55 (–CH_2), 121.56, 126.41, 131.61, 132.07 (C_{arom}, CH_{arom}), 150.92 (=C–N), 177.82, 181.72 (C=O). MS [+ESI]: *m/z* 321 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{17}ClO_3N_2$ (M, 320.77): C, 59.91; H, 5.34; N, 8.73 %. Found: C, 59.65; H, 5.67; N, 8.49 %.

*2-Chloro-3-(4-(4-nitrophenyl)piperazin-1-yl)naphthalene-1,4-dione (**10c**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **9c** (0.91 g, 4.38 mmol) by using general procedure 3* Brown solid. M.p.: 219–220 °C. Yield: 1.20 g (69 %). R_f : 0.68 with $CHCl_3$ as an eluent IR (KBr): υ (cm^{-1}) 3,110, 3,059 (C–H_{arom}), 2,920, 2,844 (C–H_{aliph}), 1,589, 1,557 (C=C), 1,678, 1,631 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 284 (4.39), 394 (4.39), 485 (3.51). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 3.55 (t, 4H, $J = 4.88$, N–CH_2), 3.71 (t, 4H, $J = 5.86$, N–CH_2–), 6.75–6.85 (m, 4H, CH_{arom}), 7.59–7.69 (m, 2H, CH_{arom}), 8.06–8.15 (m, 2H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 49.59, 47.10 (N–CH_2–), 152.99, 133.01, 131.20, 129.99, 130.54, 126.84, 124.96, 112.19 (C_{arom}), 111.38 (=C–Cl), 153.74 (=C–S), 180.80, 177.00 (C=O). MS [ESI]: *m/z* 397 $[M]^+$. Anal. Calcd. for $C_{20}H_{16}ClN_3O_4$ (M, 397.812): C, 60.38; H, 4.05; N, 10.56 %. Found: C, 60.75; H, 4.39; N, 10.12 %.

*2-Ethoxy-3-(ethyl(pyridin-4-ylmethyl)amino)naphthalene-1,4-dione (**11b**) was synthesized by the reaction of **1** (1 g, 4.38 mmol) and **9b** (0.60 g, 4.38 mmol) by using general procedure 3.* Red solid. M.p.: 128–129 °C. Yield: 0.45 g (28.12 %). R_f: 0.85 with $CHCl_3$ as an eluent. IR (KBr, cm^{-1}): υ 2,925(C–H_{arom}), 1,680(C=O), 1,603 (N–H), 1,575 (C=C UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 244 (4.85), 276 (5.13), 471 (4.18). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 1.18–1.21 (m, 6H, –CH_3), 3.29(s 2H, N–CH_2–CH), 3.73–3.76

(m, 2H, N–CH_2–CH_3), 4.27–4.28 (q, 2H, O–CH_2), 7.39–7.41 (d, 2H, H_{arom}) 7.70–7.82 (m, 4H, H_{arom}) 7.95–7.96 (d, 2H, H_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 17.21, 16.21 (–CH_3), 39.61, 38.79, 69.80 (–CH_2), 148.04, 135.59, 134.63, 133.28, 132.80, 131.28, 127.18, 126.49 (C_{arom}, CH_{arom}), 180.92 (C=O). MS [+ESI]: *m/z* 339 $[M+2H]^+$. Anal. Calcd. for $C_{20}H_{20}O_3N_2$ (M, 336.38): C, 71.41; H, 5.99; N, 8.33 %. Found: C, 71.15; H, 5.47; N, 7.84 %.

General procedure 4: for the synthesis of sulfanyl derivatives of 2-amino-1,4-naphthoquinones

Potassium carbonate (1.52 g) was dissolved in dichloromethane as reaction media (50 mL). 2-Chloro-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)naphthalene-1,4-dione (**10d**) (Ibis *et al.*, 2013a) and thiol (**2**) were added to the solution, respectively. Without heating, the mixture was stirred for 6–8 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. After the reaction mixture was filtered, filtrate was extracted in a Soxhlet extractor with dichloromethane. After recovery of the solvent, the crude product was purified by column chromatography.

*Methyl 3-((1,4-dioxo-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-1,4-dihydronaphthalen-2-yl)thio)propanoate (**12e**) was synthesized by the reaction of **10d** (Ibis et al., 2013a) (0.26 g, 0.78 mmol) and **2e** (0.09 mL, 0.86 mmol) by using general procedure 4* Dark red oil. Yield: 0.28 g (85 %). R_f : 0.18 with $CHCl_3$ as an eluent. IR (KBr): υ (cm^{-1}) 3,057 (C–H_{arom}), 2,962, 2,826 (C–H_{aliph}), 1,591, 1,538 (C=C), 1,737, 1,667 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 287 (3.66), 517 (2.94). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 1.86 (t, 4H, $J = 5.86$, CH_{2piper}), 2.52 (t, 2H, $J = 7.32$, (C=O)–CH_2–), 3.06 (t, 2H, $J = 7.32$, S–CH_2), 3.55 (t, 4H, $J = 5.86$, CH_{2piper}), 3.57 (s, 3H, O–CH_3), 3.95 (s, 4H, O–CH_2–CH_2–O), 7.56–7.63 (m, 2H, CH_{arom}), 7.92–7.94 (dd, 1H, CH_{arom}), 7.99–8.01 (dd, 1H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 29.63 ((C=O)–CH_2–), 36.20 (S–CH_2), 51.11 (O–CH_3), 35.01, 51.18, 126.56 (CH_{2piper}), 64.64 (O–CH_2–CH_2–O), 133.09, 132.38, 126.88, 124.03 (C_{arom}), 107.06 (=C–S), 155.56 (=C–N), 182.01, 172.32 (C=O). MS [ESI]: *m/z* 441 $[M+Na]^+$. Anal. Calcd. for $C_{21}H_{23}NO_6S$ (M, 417.475): C, 60.42; H, 5.55; N, 3.36; S, 7.68 %. Found: C, 60.46; H, 5.74; N, 3.15; S, 7.40 %.

*Butyl 3-((1,4-dioxo-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-1,4-dihydronaphthalen-2-yl)thio)propanoate (**12f**) was synthesized by the reaction of **10d** (Ibis et al., 2013a) (0.27 g, 0.79 mmol) and **2f** (0.15 mL, 0.87 mmol) by using general procedure 4* Dark red oil. Yield: 0.32 g (86 %). R_f : 0.29 with $CHCl_3$ as an eluent. IR (KBr): υ (cm^{-1}) 3,064 (C–H_{arom}), 2,961, 2,924, 2,853 (C–H_{aliph}), 1,591, 1,557 (C=C), 1,729, 1,667 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 250 (4.06), 300 (4.01), 517 (3.26). ^{1}H NMR (499.74 MHz, $CDCl_3$): $\delta = 0.84$ (t, 3H, $J = 7.32$, CH_3), 1.26 (m, 2H, O–CH_2–CH_2), 1.54 (m, 2H, CH_2–CH_3), 1.85 (t, 4H, $J = 5.86$, CH_{2piper}), 2.51 (t, 2H, $J = 7.32$, (C=O)–CH_2–), 3.06 (t, 2H, $J = 7.32$, S–CH_2), 3.55 (t, 4H, $J = 5.86$, CH_{2piper}), 3.94 (s, 4H, O–CH_2–CH_2–O), 3.96 (t, 2H, $J = 6.83$, O–CH_2), 7.55–7.62 (m, 2H, CH_{arom}), 7.92–7.94 (dd, 1H, CH_{arom}), 7.99–8.00 (dd, 1H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 12.67 (CH_3), 18.10, 28.41 (CH_2), 28.70 ((C=O)–CH_2–), 34.00 (S–CH_2), 34.98, 49.80, 125.64 (CH_{2piper}), 63.66 (O–CH_2), 64.64 (O–CH_2–CH_2–O), 132.67, 131.88, 125.31, 123.03 (C_{arom}), 105.83 (=C–S), 154.16 (=C–N), 180.81, 180.76, 170.70 (C=O). MS [ESI]: *m/z* 460 $[M+H]^+$. Anal. Calcd. for $C_{24}H_{29}NO_6S$ (M, 459.555): C, 62.73; H, 6.36; N, 3.05; S, 6.98 %. Found: C, 62.55; H, 5.92; N, 2.84; S, 6.18 %.

General procedure 5: for the reactions of 2-sulfanyl-1,4-naphthoquinones with nucleophiles

Potassium carbonate (1.52 g) was dissolved in dichloromethane as reaction media (50 mL). 2-Sulfanyl-3-chloro-1,4-naphthoquinone (**13**) (Ibis *et al.*, 2013b) and nucleophile (**14**) were added to the solution, respectively. Without heating, the mixture was stirred for 6–8 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. After the reaction mixture was filtered, filtrate was extracted in a Soxhlet extractor with dichloromethane. After recovery of the solvent, the crude product was purified by column chromatography.

*2-Morpholino-3-((perchlorophenyl)thio)naphthalene-1,4-dione (**15a**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.36 g, 0.76 mmol) and **14a** (0.13 g, 1.52 mmol) by using general procedure 5* Brown solid. M.p.: 196.5–198 °C. Yield: 0.32 g (80 %). R_f : 0.15 with petroleum ether: $CHCl_3$/2:1. 0.15. IR (KBr): υ (cm^{-1}) 2,970, 2,898, 2,847 (C–H_{aliph}), 1,588, 1,525 (C=C), 1,655 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 284 (5.42), 519 (4.64). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 3.56 (t, 4H, N–CH_{2morph}), 3.82 (t, 4H, O–CH_{2morph}), 7.55–7.61 (m, 2H, CH_{arom}), 7.81–7.83 (d, 1H, CH_{arom}), 7.93–7.95 (d, 1H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 51.79 (O–CH_2), 66.65 (N–CH_2), 135.20, 133.06, 132.99, 132.66, 132.24, 131.18, 131.15, 130.80, 124.26 (C_{arom}), 125.66 (=C–S), 151.61 (=C–N), 180.46, 178.95 (C=O). MS [ESI]: *m/z* 524 $[M]^+$. Anal. Calcd. For $C_{20}H_{12}Cl_5NO_3S$ (M, 523.644): C, 45.87; H, 2.31; N, 2.67; S, 6.12 %. Found: C, 45.51; H, 2.14; N, 2.59; S, 6.22 %.

*2-((Perchlorophenyl)thio)-3-thiomorpholinonaphthalene-1,4-dione (**15b**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.36 g, 0.76 mmol) and **14b** (0.16 g, 1.52 mmol) by using general procedure 5* Black solid. M.p: 191–192.5 °C. Yield: 0.37 g (90 %). R_f : 0.35 with Petroleum ether: $CHCl_3$/2:1. IR (KBr): υ (cm^{-1}) 2,958, 2,907, 2,847 (C–H_{aliph}), 1,587, 1,525 (C=C), 1,657 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 247 (4.86), 287 (4.67), 514 (4.00). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 2.80 (t, 4H, S–$CH_{2thiomorph}$), 3.68 (t, 4H, N–$CH_{2thiomorph}$), 7.56–7.61 (dd, 1H, CH_{arom}), 7.80–7.81 (dd, 1H, CH_{arom}), 7.93–7.95 (d, 2H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 27.37 (S–$CH_{2thiomorph}$), 53.62 ($CH_{2thiomorph}$), 135.31, 132.92, 132.77, 132.37, 131.18, 131.08, 130.83, 127.03 (C_{arom}), 125.80 (=C–S), 152.48 (=C–N), 180.33, 179.11 (C=O). MS [ESI]: *m/z* 540 $[M]^+$. Anal. Calcd. for $C_{20}H_{12}Cl_5NO_3S$ (M, 539.710): C, 44.51; H, 2.24; N, 2.60; S, 11.88 %. Found: C, 45.38; H, 2.86; N, 2.21; S, 10.40 %.

*2-((Perchlorophenyl)thio)-3-(pyrrolidin-1-yl)naphthalene-1,4-dione (**15c**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.33 g, 0.70 mmol) and **14c** (0.099 g, 1.40 mmol) by using general procedure 5* Brown solid. M.p.: 176–177.5 °C. Yield: 0.29 g (83 %). R_f : 0.37 with Petroleum ether: $CHCl_3$/2:1. IR (KBr): υ (cm^{-1}) 2,962, 2,924, 2,853 (C–H_{aliph}), 1,512 (C=C), 1,670, 1,618 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 249 (4.19), 283 (4.18), 493 (3.38). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 1.91 (m, 4H, N–CH_2–$CH_{2pyrrolidine}$), 3.99 (t, 4H, N–$CH_{2pyrrolidine}$), 7.45–7.55 (dd, 1H, CH_{arom}), 7.76–7.78 (dd, 1H, CH_{arom}), 7.85–7.87 (m, 2H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 24.61 (N–CH_2–$CH_{2pyrrolidine}$), 54.11 (N–$CH_{2pyrrolidine}$), 134.99, 134.87, 133.12, 132.07, 131.04, 130.71, 124.87, 104.40 (C_{arom}), 125.16 (=C–S), 156.71 (=C–N), 182.91, 178.16 (C=O). MS [ESI]: *m/z* 508 $[M]^+$. Anal. Calcd. for $C_{20}H_{12}Cl_5NO_2S$ (M, 507.645): C, 47.32; H, 2.38; N, 2.76; S, 6.32 %. Found: C, 47.67; H, 2.50; N, 3.09; S, 5.46 %.

*2-((Perchlorophenyl)thio)-3-(piperidin-1-yl)naphthalene-1,4-dione (**15d**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.37 g, 0.78 mmol) and **14d** (0.133 g, 1.56 mmol) by using general procedure 5* Purple solid. M.p.: 160.5–162 °C. Yield: 0.36 g (88 %). R_f : 0.87 with $CHCl_3$ as an eluent. IR (KBr): υ (cm^{-1}) 2927, 2,849 (C–H_{aliph}), 1,588, 1,540 (C=C), 1663 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 246 (4.30), 526 (3.38). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 1.65 (m, 2H, $CH_{2piperidine}$), 1.74 (m, 4H, $CH_{2piperidine}$), 3.60 (t, 4H, $CH_{2piperidine}$), 7.50–7.54 (m, 1H, CH_{arom}), 7.76–7.80 (m, 1H, CH_{arom}), 7.88–7.91 (m, 2H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 23.15 (N–CH_2–CH_2–$CH_{2piperidine}$), 26.08 (N–CH_2–$CH_{2piperidine}$), 53.45 (N–$CH_{2piperidine}$), 135.30, 133.84, 132.83, 132.24, 131.83, 130.99, 125.47, 120.93 (C_{arom}), 125.68 (=C–S), 153.85 (=C–N), 180.83, 178.82 (C=O). MS [ESI]: *m/z* 522 $[M]^+$. Anal. Calcd. for $C_{21}H_{14}Cl_5NO_2S$ (M, 521.671): C, 48.35; H, 2.70; N, 2.68; S, 6.15 %. Found: C, 48.53; H, 2.93; N, 2.83; S, 5.26 %.

*2-(4-(4-Fluorophenyl)piperazin-1-yl)-3-((perchlorophenyl)thio)naphthalene-1,4-dione (**15e**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.35 g, 0.74 mmol) and **14e** (0.27 g, 1.48 mmol) by using general procedure 5* Black solid. M.p.: 168–170 °C. Yield: 0.35 g (78 %). R_f : 0.60 with $CHCl_3$ as an eluentIR (KBr): υ (cm^{-1}) 3056 (C–H_{arom}), 2958, 2923, 2825 (C–H_{aliph}), 1547, 1509 (C=C), 1666 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 256 (5.03), 287 (4.77), 517 (3.99). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 2.91–2.95 (m, 4H, $CH_{2piperazine}$), 2.99–3.13 (m, 4H, $CH_{2piperazine}$), 6.49–6.70 (m, 4H, CH_{arom}), 6.72–6.92 (m, 4H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 49.66, 44.60 ($CH_{2piperazine}$), 159.77, 157.30, 155.39, 147.17, 135.21, 133.18, 131.15, 130.82, 118.08, 118.02, 117.35, 117.17, 116.76, 116.70, 114.64, 124.26 (C_{arom}), 125.79 (=C–S), 152.00 (=C–N), 180.48, 178.91 (C=O). MS [ESI]: *m/z* 617 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{16}Cl_5FN_2O_2S$ (M, 615.758): C, 50.63; H, 2.61; N, 4.54; S, 5.20 %. Found: C, 51.05; H, 2.74; N, 4.07; S, 5.86 %.

*Methyl 3-((1,4-dioxo-3-((perchlorophenyl)thio)-1,4-dihydronaphthalen-2-yl)thio)propanoate (**15f**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.33 g, 0.69 mmol) and **14f** (0.092 g, 0.76 mmol) by using general procedure 5* Red solid. M.p.: 184–185.5 °C. Yield: 0.28 g (72 %). R_f : 0.62 with $CHCl_3$ as an eluent. IR (KBr): υ (cm^{-1}) 3069 (C–H_{arom}), 2983, 2955, 2929 (C–H_{aliph}), 1593 (C=C), 1736, 1662 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 249 (5.14), 450 (3.85). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 2.67 (t, 2H, J = 7.32, (C=O)–CH_2–), 2.96 (t, 2H, J = 7.32, S–CH_2), 3.63 (s, 3H, O–CH_3), 7.61–7.64 (m, 2H, CH_{arom}), 7.95–7.99 (m, 2H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 26.84 ((C=O)–CH_2–), 32.88 (S–CH_2), 50.87 (O–CH_3), 137.06, 135.65, 134.35, 132.73, 132.60, 131.99, 131.28, 125.62 (C_{arom}), 125.45, 157.00 (=C–S), 181.64, 177.87, 171.09 (C=O). Anal. Calcd. for $C_{20}H_{11}Cl_5O_4S_2$ (M, 556.694): C, 43.15; H, 1.99; S, 11.52 %. Found: C, 43.67; H, 1.74; S, 11.85 %.

*Butyl 3-((1,4-dioxo-3-((perchlorophenyl)thio)-1,4-dihydronaphthalen-2-yl)thio)propanoate (**15g**) was synthesized by the reaction of **13** (Ibis et al., 2013b) (0.36 g, 0.76 mmol) and **14g** (0.14 g, 0.84 mmol) by using general procedure 5* Red oil. Yield: 0.38 g (83 %). R_f : 0.6 with $CHCl_3$ as an eluentIR (KBr): υ (cm^{-1}) 3072 (C–H_{arom}), 2961, 2927, 2870 (C–H_{aliph}), 1591, 1491 (C=C), 1666, 1654 (C=O). UV–Vis ($CHCl_3$) λ_{max} (nm) (logε): 244 (4.53), 444 (3.45). ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 0.84 (t, 3H, J = 7.32, CH_3), 1.30 (m, 2H, O–CH_2–CH_2), 1.53 (m, 2H, CH_2–CH_3), 2.60 (t, 2H,

$J = 7.32$, (C=O)–CH_2–), 3.35 (t, 2H, $J = 7.32$, S–CH_2), 4.03 (t, 2H, $J = 6.83$, O–CH_2), 7.59–7.64 (m, 2H, CH_{arom}), 7.95–7.99 (m, 2H, CH_{arom}). ^{13}C NMR (125.66 MHz, $CDCl_3$): δ 12.68 (CH_3), 18.11 (S–CH_2–CH_2), 29.59 (CH_2–CH_3), 29.62 ((C=O)–CH_2–), 33.15 (S–CH_2), 63.69 (O–CH_2–), 137.08, 132.71, 132.60, 132.13, 131.43, 131.31, 130.41, 125.62 (C_{arom}), 125.44, 156.99 (=C–S), 181.65, 177.90, 170.74 (C=O). Anal. Calcd. for $C_{23}H_{17}Cl_5O_4S_2$ (M, 598.774): C, 46.14; H, 2.86; S, 10.71 %. Found: C, 46.26; H, 2.61; S, 10.49 %.

Antifungal and antibacterial evaluation

Diffusion technique

Antibacterial activity of compounds was evaluated by diffusion in peptone on nutrient medium (meat-extract agar for bacteria; wort agar for fungi). The microbial loading was 10^9 cells (spores)/1 mL. The required incubation periods were as: 24 h at 35 °C for bacteria and 48–72 h at 28–30 °C for fungi. The results were recorded by measuring the zones surrounding the disk. Control disk contained vancomycin (for bacteria) or nystatin (for fungi) as a standard.

Serial dilution technique

Testing was performed in a flat-bottomed 96-well tissue culture plate. The tested compounds were dissolved in DMSO and arriving the necessary concentration. The exact volume of solution of compounds is brought in nutrient medium. The inoculum of bacteria and fungi was inoculated in nutrient medium (meat-extract agar for bacteria; wort agar for fungi). The duration of incubation was at 37 °C for bacteria and 30 °C for fungi during 24–72 h. The results were estimated according to the presence or absence of microorganism growth.

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