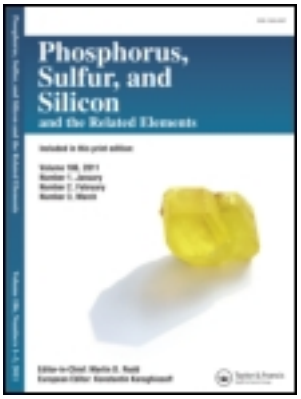

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Synthesis and Antibacterial and Antifungal Properties of Novel S-, N-, N,S-, and S,O-Substituted 1,4-Naphthoquinone Derivatives

Cemil Ibis [a], Amac Fatih Tuyun [b], Zeliha Ozsoy-Gunes [c], Sibel Sahinler Ayla [a], Maryna V. Stasevych [d], Rostyslav Ya. Musyanovych [d], Olena Komarovska-Porokhnyavets [d] & Volodymyr Novikov [d]

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SYNTHESIS AND ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF NOVEL *S-, N-, N,S-*, AND *S,O*-SUBSTITUTED 1,4-NAPHTHOQUINONE DERIVATIVES

Cemil Ibis,[1] Amac Fatih Tuyun,[2] Zeliha Ozsoy-Gunes,[3] Sibel Sahinler Ayla,[1] Maryna V. Stasevych,[4] Rostyslav Ya. Musyanovych,[4] Olena Komarovska-Porokhnyavets,[4] and Volodymyr Novikov[4]

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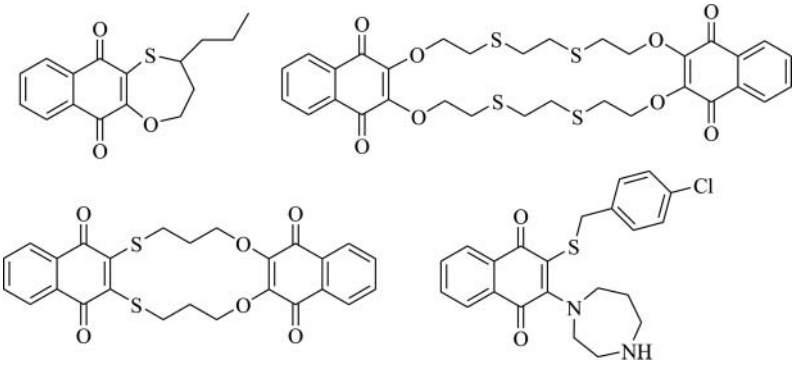
GRAPHICAL ABSTRACT



Abstract *A novel series of substituted 1,4-naphthoquinone derivatives were synthesized and evaluated for their antibacterial and antifungal activity. The structures of the novel products were characterized by spectroscopic methods. Among the tested compounds, 2,2′,3,3′-alkoxy*

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substituted naphthoquinones, S,O-*substituted naphthoquinone, and* N,S-*substituted* naphthoquinone *derivatives are the most potent antifungals against* C. tenuis. *2,3-Thio-2′,3′-alkoxy substituted naphthoquinones are the most effective antifungal compounds against* A. niger.

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.*

Keywords *N*,*S*-substituted 1,4-naphthoquinones; heterocyclic ethers with quinone group; antibacterial activity; antifungal activity

INTRODUCTION

The 1,4-naphthoquinone moiety represents an important structural motive with quinonoid structure which exists in many natural or synthetic intermediates from dyes to pharmaceuticals. 1,4-Naphthoquinone derivatives show important biological properties such as antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory effects.[1–5] Thio or amino derivatives of 1,4-naphthoquinones have been studied widely because of these important biological properties.[6–8] The biological activity of 1,4-naphthoquinone is due to the presence of two conjugated carbonyl groups which have the ability to accept one or two electrons to form the corresponding radical anion or dianion species.[9] The important activity properties of piperazine and homopiperazine analogs are also known from the literature.[10]

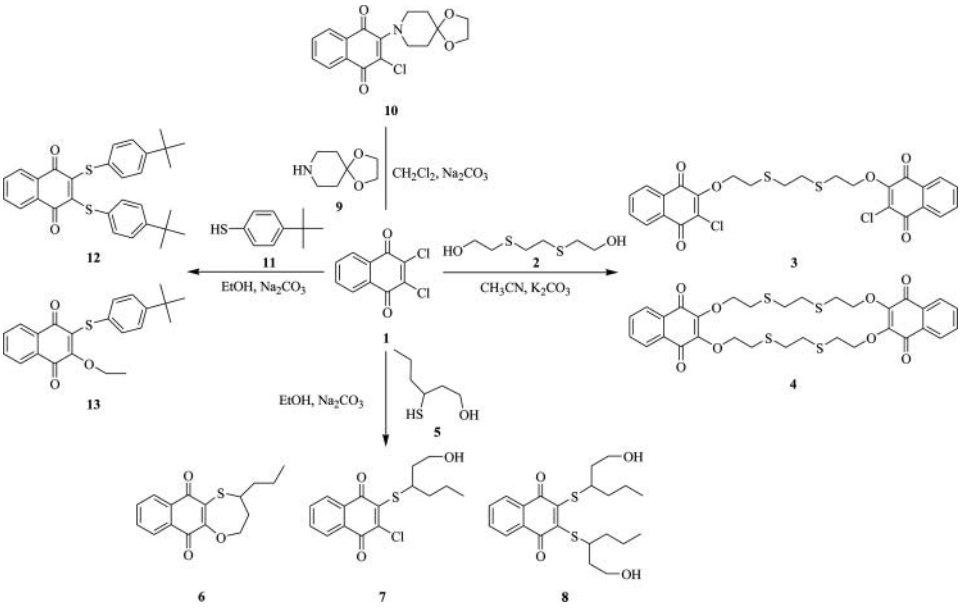
The synthesis of novel nitrogen and sulfur containing naphthoquinone compounds as biologically active agents and their spectroscopic characterization is the aim of this study.

RESULTS AND DISCCUSSION

The novel compounds **3** and **4** were obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and diol **2** with potassium carbonate in acetonitrile. Compound **3** is a novel intramolecular compound with ether structure (Scheme 1). This reaction consists of a substitution with concomitant Michael-Type addition followed by chloride elimination.[8,11] The novel compound **4** is also an interesting heterocyclic compound that is formed by the same reaction type as **3**. Two carbon atom signals of the carbonyl groups were observed at 179.79 and 178.55 ppm in the ^{13}C NMR spectra of **3**. However, the carbon atom signals of the two carbonyl groups of **4** were observed at 177.74 ppm as one peak only.

The novel compounds **6**, **7**, and **8** were synthesized by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and thioalcohol **5** in ethanol solution of sodium carbonate. The IR spectra of the monothio-substituted naphthoquinone **7** and the dithio-substituted naphthoquinone **8** showed broad bands at 3512 and 3390 cm^{-1} for the OH stretching, respectively, but compound **6** did not show any band at this region of the IR spectrum because of the ring closure.

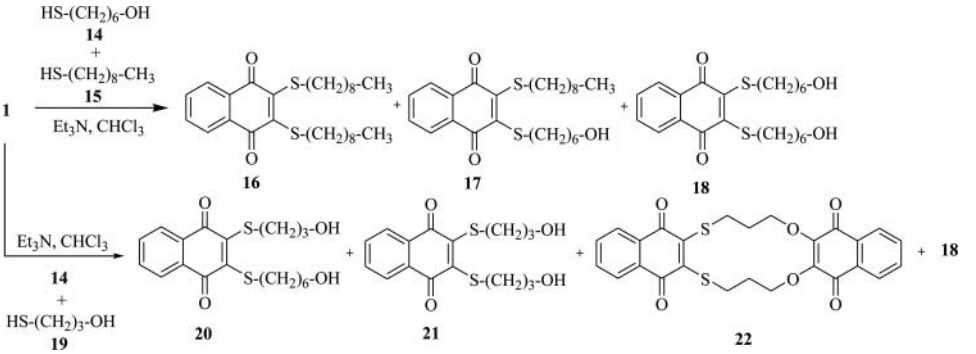
The *N*-substituted naphthoquinone **10** was obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and compound **9** with sodium carbonate in dichloromethane. The molecular ion peak of **10** was identified at *m*/*z* 334 $[M]^+$ in the positive ion mode for ESI technique. The dithio-substituted compound **12** and the ethoxy-monothio-substituted compound **13** were obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and compound **11** with sodium carbonate in ethanol. The molecular ion peak of compounds **12** and **13** was identified at *m*/*z* 487 $[M]^+$ and 367 $[M]^+$ in the positive ion mode for ESI



Scheme 1

technique, respectively. In the ^{1}H NMR spectrum of **13**, the methylene protons (OCH_2) were observed at 4.19 ppm as a multiplet.

The dithio-substituted compounds **16**, **17**, and **18**[12] were obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and a mixture of **14** and **15** with triethylamine in chloroform (Scheme 2). No band was observed in OH region of the IR spectra of compound **16**. However, compounds **17** and **18**[12] showed broad bands at 3369 and 3382 cm^{-1} for the OH stretching.

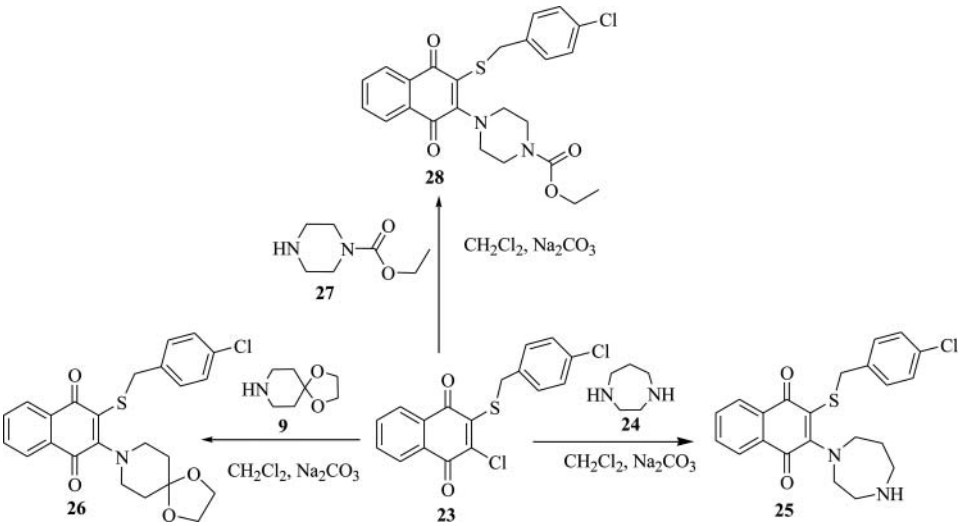


Scheme 2

The carbonyl carbon atom signals were observed at 179.1 ppm in the ^{13}C NMR spectrum of **17**. The novel compounds **20**, **21**, **22**, and the known compound **18**[12] were obtained by the reaction of 2,3-dichloro-1,4-naphthoquinone **1** and a mixture of **14** and **19** with triethylamine in chloroform. Compound **18** was synthesized by our group recently.[12]

The IR spectra of compounds **20** and **21** showed broad bands at 3349 and 3357 cm^{-1}, respectively, for the OH stretching. The carbonyl carbon atom signals in the ^{13}C NMR spectrum of **22** were observed at 178.40 and 183.30 ppm.

The *N*,*S*-substituted compounds **25**, **26**, and **28** were obtained from the reactions of compound **23**[6] (Scheme 3). Compound **25** is a monosubstituted homopiperazine derivative and was formed by an addition–elimination reaction. The IR spectrum of **25** showed characteristic amine band (NH) at 3411 cm^{-1}. The ^{13}C NMR spectra of **28** exhibited a carbonyl carbon signal of the ester groups at 155.82 ppm while the carbonyl carbon signals of the quinone moiety were observed at 182.04 and 181.85 as two peaks.



Scheme 3

The mass spectra of compounds **25**, **26**, and **28** in the positive ion mode for ESI technique confirmed the proposed structure: the molecular ion peaks were identified at *m/z* 413$[M]^+$, 478 $[M+Na]^+$, and 493 $[M+Na]^+$, respectively.

Biological Study

Herein, we have synthesized different (hetero)cyclic naphthoquinones and evaluated their antifungal activity against fungi *Candida tenuis VKM Y-70* and *Aspergillus niger F-1119* by the diffusion method[13] and the serial dilution method.[14] The antibacterial activity of the compounds was elucidated against *Escherichia coli B-906*, *Staphylococcus aureus 209-P*, and *Mycobacterium luteum B-917* by the diffusion method and the serial dilution method as shown in Tables S1 and S2 (Supplemental Materials are available online). Their activities were compared with those of the known antibacterial agent Vancomycine and the antifungal agent Nystatin.

Afterwards, on the basis of structure–activity relationship of antifungal activity of the (hetero)cyclic quinone derivatives, we have further synthesized and screened compounds of **10**, **20**, and **25** for antibacterial and antifungal activity by the diffusion method as shown Table S1.

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

Compound	E_p(Ic) (V)	E_p(IIc) (V)	ΔEp_1[a] (mV)	$Ep_{1,1/2}$[b] (V)
3	−0.468	−0.867	108	−0.4140
7	−0.654	−0.923	94	−0.6070
10	−0.594	−1.146	84	−0.5520
12	−0.430	−1.084	102	−0.3790
13	−0.571	−1.266	117	−0.5125
17	−0.542	−1.118	114	−0.4850
20	−0.546	−1.144	108	−0.3920
22	−0.693	−1.324	137	−0.6245

[a]$\Delta Ep_1 = Epa_1 - Epc_1$.
[b]$E_{1,1/2} = (Epa_1 + Epc_1)/2$.

Evaluation of the antibacterial activity of the synthesized compounds showed that the MIC (minimum inhibitory concentration) of **25** is 15.6 μg/mL for *S. aureus*, but **20** was the most potent with MIC = 7.8 μg/mL for *M. luteum* (Table S2).

Notable antifungal activities against *C. tenuis* at 3.9 μg/mL were observed for **4**, **13**, and **25**. Evaluation of antifungal activities against test-culture *C. tenuis* of **8**, **20**, and **22** showed MIC = 15.6 μg/mL.

The MIC of **3**, **6**, and **10** against test-culture *C. tenuis* were determined at 31.2 μg/mL. A notable activity of **22** was observed against *A. niger* fungi at 3.9 μg/mL. Evaluations of antifungal activity of **10** showed MIC = 7.8 μg/mL against test-culture *A. niger*. MIC of **6** and **13** were determined at 15.6 μg/mL against test-culture *A. niger*.

We have synthesized compound **23** with a slight antifungal activity in our previous study.[6] The antifungal activity significantly increased for the derivatives of compound **23** after the substitution with a homopiperazine group, as determined by the diffusion and the serial dilution method.

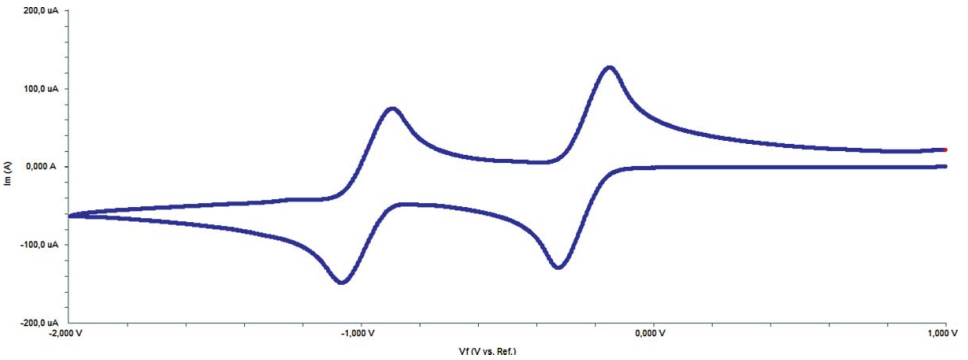


Figure 1 Cyclic voltammogram of compound **22** in DMF+0.1 M TBAP on Glassy Carbon Electrode at 0.1 V s^{-1} (Color figure available online).

Electrochemical Study

Some of the novel naphthoquinone derivatives were studied by cyclic voltammetry in aprotic media (DMF) using tetrabutylammonium perchlorate (TBAP, 0.10 M) as supporting electrolyte at 100 mVs^{-1}.[15] The electrochemical parameters, including cathodic peak potentials (Epc_1 and Epc_2), the half-wave peak potentials ($E_{1,\frac{1}{2}}$), and the difference between the first oxidation and reduction processes (ΔEp) are given in Table 1.

The cyclic voltamogram of **22** is shown in Figure 1 for DMF + 0.1 M TBAP on Glassy Carbon Electrode at 0.1 Vs^{-1}.

CONCLUSION

A convenient synthetic route for the preparation of 2- and 2,3-substituted naphthalene-1,4-diones from 2,3-dichloro-1,4-naphthoquinone has been reported. The compounds, especially **13**, **22**, and **25**, have been shown to exhibit antibacterial and antifungal properties. Some further compounds (**3**, **6**, **7**, **16**, **17**, and **28**) did not show any significant antifungal or antibacterial activity against fungi and bacteria species. Among the tested compounds **4**, **13**, and **25** are the most effective antifungal compounds against *C. tenuis* whereas **22** is the most potent antifungal compound against *A. niger*.

EXPERIMENTAL

Melting points were measured using a Büchi B-540 melting point apparatus and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra (ν/cm^{-1}) were recorded as KBr pellets or Nujol mulls on a Perkin Elmer Precisely Spectrum One FTIR spectrometer. ^1H NMR (499.74 MHz) and ^{13}C NMR (125.66 MHz) spectra (δ/ppm) were recorded in CD_3OD, $CDCl_3$, or DMSO-d_6 on a Varian Unity INOVA spectrometer. Mass spectra were obtained on a Thermo Finnigan LCQ Advantage MAX LC/MS/MS spectrometer using the ESI technique. 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.

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 disc 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 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 made over a potential range between +1 and −2 V with a step rate of 0.1 Vs^{-1}.

General Procedure 1: Synthesis of Alkoxy-1,4-Naphthoquinones

Potassium carbonate (1.52 g) was dissolved in acetonitrile (65 mL). 2,3-Dichloro-1,4-naphthoquinone (**1**) and alcohol (**2**) were added to the solution. 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 filtration of the reaction mixture, the filtrate was extracted in a Soxhlet extractor with CH_2Cl_2. After recovery of the solvent, the crude product was purified by column chromatography.

3,3′-{[(Ethane-1,2-diylbis(sulfanediyl)]bis(ethane-2,1-diyl)bis(oxy)bis(2-chloro-1,4-naphthoquinone} **(3)** and **7,8,10,11,13,14,23,24,26,27,29,30-dodecahydrodinaphtho[2,3-b:2′,3′-n][1,4,7,10,13,16,19,22]tetraoxatetrathiacyclotetracosine-5,16,21,32-tetraone (4)** were synthesized from **1** (1 g, 4.38 mmol) and **2** (0.80 mL, 4.38 mmol) by use of the general procedure 1.

3: Yellow solid. Yield: 1.21 g (49%). M.p. 93.5–95 °C. R_f: 0.61 ($CHCl_3$). IR: 3309, 3061 (CH_{arom}), 2975, 2937, 2919 (CH_{aliph}), 1596, 1570 (C=C), 1678, 1659 (C=O). ^{1}H NMR ($CDCl_3$): 2.80 (t, 4H, SCH_2CH_2S), 2.91 (t, 4H, SCH_2), 4.62 (t, 4H, OCH_2), 7.61–7.65 (m, 4H, CH_{arom}), 7.93–7.94 (m, 2H, CH_{arom}), 7.97–7.99 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 32.38 (SCH_2), 32.93 (SCH_2CH_2S), 73.68 (OCH_2), 134.61, 134.18, 131.20, 130.92, 129.20, 127.19, 127.08, 110.00 (C_{arom}), 127.75 (=CCl), 156.38 (=C−O), 179.79, 178.55 (C=O). MS [+ESI]: *m/z* = 585 $[M+Na]^+$. Anal. Calcd. for $C_{26}H_{20}Cl_2O_6S_2$ (563.469): C, 55.42; H, 3.58; S, 11.38. Found C, 53.98; H, 3.52; S, 10.27.

4: Red oil. Yield: 1.12 g (45%). R_f: 0.1 (CH_2Cl_2). IR: 3391 (CH_{arom}), 2924 (CH_{aliph}), 1589, 1539 (C=C), 1679 (C=O). ^{1}H NMR ($CDCl_3$): 3.16 (t, 4H, SCH_2CH_2S), 3.20 (t, 4H, SCH_2), 4.65 (t, 4H, OCH_2), 7.46–7.60 (m, 4H, CH_{arom}), 7.83–7.92 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 34.24 (SCH_2), 32.25 (SCH_2CH_2S), 61.27 (OCH_2), 125.75, 131.31, 134.09 (C_{arom}), 167.68 (=C−O), 177.74 (C=O). MS [ESI]: *m/z* = 674 $[M+H]^+$. Anal. Calcd. for $C_{32}H_{32}O_8S_4$ (672.852): C, 57.12; H, 4.79; S, 19.06. Found C, 57.96; H, 4.78; S, 18.54.

General Procedure 2: Synthesis of Monosulfanyl-, Disulfanyl-, and Ethoxy-Sulfanyl-1,4-Naphthoquinones

Sodium carbonate (1.52 g) was dissolved in ethanol (65 mL). 2,3-Dichloro-1,4-naphthoquinone (**1**) and thiol (**5** and **11**) were added to the solution. 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. The reaction mixture was extracted in a Soxhlet extractor with CH_2Cl_2. After recovery of the solvent, the crude product was purified by column chromatography.

4-Propyl-3,4-dihydro-2*H*-naphtho[2,3-b][1,4]oxathiepine-6,11-dione (6), **2-chloro-3-[(1-hydroxyhexan-3-yl)thio]-1,4-naphthoquinone (7)**, and **2,3-bis[(1-hydroxyhexan-3-yl)-thio]-1,4-naphthoquinone (8)** were synthesized from **1** (1 g, 4.38 mmol) and **5** (0.61 mL, 4.38 mmol) by use of the general procedure 2.

6: Orange solid. Yield: 0.51 g (40%). M.p. 146.5–148 °C. R_f: 0.71 ($CHCl_3$). IR: 3307, 3068 (CH_{arom}), 1587, 1551 (C=C), 1663 (C=O). ^{1}H NMR ($CDCl_3$): 0.86 (t, 3H, CH_3), 1.37–1.74 [m, 4H, $(CH_2)_2$], 2.43–2.51 (m, 1H, SCH), 4.51–4.55 (m, 2H, OCH_2CH_2), 4.90–4.95 (m, 2H, OCH_2), 7.58–7.63 (m, 2H, CH_{arom}), 7.96–8.01 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 13.94 (CH_3), 20.29, 34.61 [$(CH_2)_2$], 37.53 (SCH_2), 71.47 (OCH_2), 45.03 (OCH_2CH_2), 133.89, 131.95, 131.17, 126.86, 126.66 (C_{arom}), 129.74 (=C–S), 156.50 (=C−O), 183.34, 178.50 (C=O). MS [+ESI]: *m/z* = 311 $[M+Na]^+$. Anal. Calcd. for $C_{16}H_{16}O_3S$ (288.361): C, 66.64; H, 5.59; S, 11.12. Found C, 66.92; H, 5.70; S, 12.17.

7: Red oil. Yield: 0.29 g (20%). R_f: 0.22 ($CHCl_3$). IR: 3512 (OH), 3071 (CH_{arom}), 2957, 2931, 2871 (CH_{aliph}), 1591, 1549 (C=C), 1664, 1655 (C=O). ^{1}H NMR ($CDCl_3$): 0.85 (t, 3H, CH_3), 1.37–1.95 (m, 4H, $(CH_2)_2$), 4.50–4.54 (m, 2H, OCH_2CH_2), 2.42–2.49 (m, 1H, SCH), 4.88–4.94 (m, 2H, OCH_2), 7.57–7.61 (m, 2H, CH_{arom}), 7.93–7.98 (m, 2H, CH_{arom}).

^{13}C NMR ($CDCl_3$): 13.92 (CH_3), 20.26, 34.58 [$(CH_2)_2$], 37.5 (SCH_2), 45.02 (OCH_2CH_2), 71.46 (OCH_2), 133.89, 131.90, 131.11, 129.73, 126.82, 126.62 (C_{arom}), 127.51 (=C–Cl), 156.46 (=C–S), 183.31, 178.47 (C=O). MS [+ESI]: *m/z* = 305 $[M\text{-}OH]^+$. Anal. Calcd. for $C_{16}H_{17}ClO_3S$ (324.822): C, 59.16; H, 5.28; S, 9.87. Found C, 60.49; H, 5.95; S, 9.79.

8: Red oil. Yield: 0.28 g (15%). R_f: 0.12 ($CHCl_3$). IR: 3390 (OH), 3070 (CH_{arom}), 2957, 2931, 2872 (CH_{aliph}), 1591 (C=C), 1660, 1652 (C=O). ^{1}H NMR (CD_3OD): 0.93 (t, 3H, CH_3), 1.49–1.87 (m, 4H, $[CH_2)_2]$, 3.3 (s, 2H, OH), 3.71 (m, 2H, OCH_2CH_2), 1.91 (m, 1H, SCH), 4.15 (m, 2H, $HOCH_2$), 7.35–7.76 (m, 2H, CH_{arom}), 8.00–8.03 (m, 2H, CH_{arom}). ^{13}C NMR (CD_3OD): 13.11 (CH_3), 19.83, 38.67 $[(CH_2)_2]$, 38.51 (SCH_2), 46.03 ($HOCH_2CH_2$), 59.39 (HOC), 133.59, 133.40, 126.66 (C_{arom}), 149.21 (=C–O), 179.12 (C=O). MS [+ESI]: *m/z* = 445 $[M+Na]^+$. Anal. Calcd. for $C_{22}H_{30}O_4S_2$ (422.601): C, 62.53; H, 7.16; S, 15.18. Found C, 60.50; H, 7.09; S, 14.75.

2,3-Bis[(4-*tert*-butylphenyl)thio]-1,4-naphthoquinone (12) and **2-[(4-*tert*-butyl phenyl)-thio]-3-ethoxy-1,4-naphthoquinone (13)** were synthesized from **1** (1 g, 4.38 mmol) and **11** (0.73 g, 4.38 mmol) by use of the general procedure 2.

12: Red solid. Yield: 0.61 g (28%). M.p. 78.1–79.7 °C. R_f: 0.50 (Petroleum ether/CH_2Cl_2 1:1). IR: 3070 (CH_{arom}), 2961, 2903, 2867 (CH_{aliph}), 1665 (C=O), 1590 (C=C). ^{1}H NMR ($CDCl_3$): 1.21 (s, 18H, C*H*$_3$), 7.16–7.25 (m, 8H, *CH*$_{arom}$), 7.54–7.56 (m, 2H, *CH*$_{arom}$), 7.87–7.89 (m, 2H, *CH*$_{arom}$). ^{13}C NMR ($CDCl_3$): 30.2, 33.6 (*C*, *C*H_3), 125.1, 126.1, 129.2, 130.1, 131.8, 132.6, 147.4, 149.9 (*CH*$_{arom}$, *C*$_{arom}$), 177.8 (*C*=O). MS [+ESI]: *m/z* = 487 $[M]^+$. Anal. Calcd. for $C_{30}H_{30}O_2S_2$ (486.70): C, 74.04; H, 6.21; S, 13.18. Found C, 72.98; H, 6.34; S, 12.60.

13: Red solid. Yield: 0.19 g (12%). M.p. 64.9–65.7 °C. R_f: 0.40 (Petroleum ether/CH_2Cl_2 1:1). IR: 3065 (CH_{arom}), 2955, 2903, 2867 (CH_{aliph}), 1661 (C=O), 1591, 1544, 1560 (C=C). ^{1}H NMR ($CDCl_3$): 0.99 (t, 3H, C*H*$_3$), 1.19 (s, 9H, C*H*$_3$), 4.19 (q, 2H, OC*H*$_2$), 7.19–7.22 (m, 2H, *CH*$_{arom}$), 7.26–7.28 (m, 2H, *CH*$_{arom}$), 7.56–7.58 (m, 2H, *CH*$_{arom}$), 7.92–7.95 ppm (m, 2H, *CH*$_{arom}$). ^{13}C NMR ($CDCl_3$): 14.3, 30.2, 33.5 (*C*, *C*H_3), 68.8 (O*C*H_2), 124.9, 125.4, 125.7, 128.9, 130.2, 130.4, 131.1, 131.3, 132.5, 132.8, 149.7 (*CH*$_{arom}$, *C*$_{arom}$), 157.5 (O–*C*–C=O), 178.6, 181.1 (*C*=O). MS [+ESI] *m/z* = 367 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{22}O_3S$ (366.48): C, 72.10; H, 6.05; S, 8.75. Found C, 71.79; H, 6.05; S, 8.57.

General Procedure 3. Reactions of Mixtures of Two Thiols with 1,4-Naphthoquinones

2,3-Dichloro-1,4-naphthoquinone (1.0 g, 4.38 mmol) was dissolved in $CHCl_3$ (50 mL). Subsequently, the mixture of two thiols (**8**) (4.38 mmol) and triethylamine (catalytic amount) was added to the solution. 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 CH_2Cl_2. After recovery of the solvent, the crude product was purified by column chromatography.

2,3-Bis(nonylthio)-1,4-naphthoquinone (16), **2-[(6-hydroxyhexyl)thio]-3-(nonylthio)-1,4-naphthoquinone (17)**, and **2,3-bis[(6-hydroxyhexyl)thio]-1,4-naphthoquinone (18)**[12] were synthesized from **1** (0.5 g, 2.19 mmol), **14** (0.3 mL, 2.19 mmol), and **15** (0.35 g, 2.19 mmol) by use of the general procedure 3.

16: Red oil. Yield: 0.13 g (26%). R_f: 0.78 ($CHCl_3$). IR: 2954, 2924, 2854 (CH_{aliph}), 1660, 1652 (C=O), 1592 (C=C). ^{1}H NMR ($CDCl_3$): 0.79 (t, 6H, C*H*$_3$), 1.17–1.20 (m, 20H,

CH_2), 1.31–1.36 (m, 4H, CH_2), 1.52–1.57 (m, 4H, CH_2), 3.19 (t, $J = 7.32$ Hz, 4H, SCH_2), 7.58–7.60 (m, 2H, CH_{arom}), 7.95–7.97 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 13.1, 21.6, 27.7, 28.1, 28.2, 28.4, 29.5, 30.8 (CH_2, CH_3), 33.9 (SCH_2), 125.8, 132.1, 132.3 (CH_{arom}, C_{arom}), 146.8 (S−C−C=O), 177.9 ppm (C=O). MS [-ESI] $m/z = 474$ $[M]^-$. Anal. Calcd. for $C_{28}H_{42}O_2S_2$ (474.76): C, 70.84; H, 8.92; S, 13.51. Found C, 70.90; H, 8.27; S, 12.76.

17: Red oil. Yield: 0.16 g (32%). R_f: 0.43 ($CHCl_3$). IR 3369 (OH), 3069 (CH_{arom}), 2923, 2853 (CH_{aliph}), 1658 (C=O), 1591 (C=C). ^{1}H NMR ($CDCl_3$): 0.77 (t, $J = 6.8$ Hz, 3H, CH_3), 1.15–1.19 (m, 10H, CH_2), 1.28–1.37 (m, 6H, CH_2), 1.38–1.49 (m, 2H, CH_2), 1.50–1.59 (m, 4H, CH_2), 1.95 (s, 1H, OH), 3.18 (t, 4H, SCH_2), 3.53 (t, 2H, OCH_2), 7.58–7.59 (m, 2H, CH_{arom}), 7.93–7.94 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 14.3, 22.8, 25.5, 28.7, 28.9, 29.3, 29.4, 29.6, 30.5, 30.6, 32.0, 32.7 (CH_2, CH_3), 35.0, 35.2 (SCH_2), 62.9 (OCH_2), 126.9, 133.2, 133.6 (CH_{arom}, C_{arom}), 147.7, 148.2 (S−C−C=O), 179.1 (C=O). MS [+ESI] $m/z = 471$ $[M+Na]^+$. Anal. Calcd. for $C_{25}H_{36}O_3S_2$ (448.69): C, 66.92; H, 8.09; S, 14.29. Found C, 66.35; H, 7.90; S, 14.20.

18[12]: Red solid. Yield: 0.11 g (22%). M.p. 70.3–70.6 °C. R_f: 0.3 (CH_2Cl_2/EtAc 3:1). IR: 3382, 3304 (OH), 2922, 2854 (CH_{aliph}), 1654 (C=O), 1589 (C=C). Anal. Calcd. for $C_{22}H_{30}O_4S_2$ (422.61): C, 62.53; H, 7.16; S, 15.17. Found C, 62.79; H, 7.05; S, 15.57.

2,3-Bis[(6-hydroxyhexyl)thio]-1,4-naphthoquinone (18)[12], **2-[(6-hydroxyhexyl) thio]-3-[(3-hydroxypropyl)thio]-1,4-naphthoquinone (20)**, **2,3-bis[(3-hydroxypropyl) thio]-1,4-naphthoquinone** (**21**), and **8,9,19,20-tetrahydrodinaphtho[2,3-b:2′,3′-i] [1,4,8,11]dioxadithia-cyclotetradecine-5,11,16,22(7*H*,18*H*)-tetraone (22)** were synthesized from **1** (0.5 g, 2.19 mmol), **14** (0.3 mL, 2.19 mmol), and **19** (0.38 mL, 2.19 mmol) by use of the general procedure 3.

18[12]: Red solid. Yield: 0.11 g (22%). M.p. 70.3–70.6 °C. R_f: 0.3 (CH_2Cl_2/EtAc 3:1). IR: 3382, 3304 (OH), 2922, 2854 (CH_{aliph}), 1654 (C=O), 1589 (C=C). Anal. Calcd. for $C_{22}H_{30}O_4S_2$ (422.61): C, 62.53; H, 7.16; S, 15.17. Found C, 62.79; H, 7.05; S, 15.57.

20: Red oil. Yield: 0.13 g (16%). R_f: 0.17 ($CHCl_3$/EtAc 2:1). IR: 3349 (OH), 3065 (CH_{arom}), 2930, 2856 (C–H_{aliph}), 1657 (C=O), 1591 (C=C). ^{1}H NMR ($CDCl_3$): 1.19 (s, 1H, OH), 1.29–1.33 (m, 2H, CH_2), 1.37–1.43 (m, 2H, CH_2), 1.45–1.51 (m, 2H, CH_2), 1.54–1.61 (m, 2H, CH_2), 1.79–1.84 (m, 2H, CH_2), 1.93 (s, 1H, OH), 3.22 (t, $J = 7.3$ Hz, 2H, SCH_2), 3.29 (t, 2H, SCH_2), 3.53–3.56 (m, 2H, OCH_2), 3.71 (t, 2H, OCH_2), 7.59–7.63 (m, 2H, CH_{arom}), 7.94–7.97 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 24.3, 27.3, 29.3, 30.5, 31.5 (CH_2), 31.9, 33.9 (SCH_2), 59.9, 61.6 (OCH_2), 125.9, 131.9, 132.5 (CH_{arom}, C_{arom}), 146.1, 147.8 (S−C−C=O), 177.9, 178.0 (C=O). MS [+ESI] $m/z = 404$ $[M+Na]^+$. Anal. Calcd. for $C_{19}H_{24}O_4S_2$ (380.53): C, 59.97; H, 6.36; S, 16.85. Found C, 60.77; H, 7.19; S, 15.92.

21: Orange solid. Yield: 0.11 g (15%). R_f: 0.10 ($CHCl_3$/EtAc 2:1). IR: 3357 (OH), 2967, 2924, 2853 (CH_{aliph}), 1658 (C=O), 1590 (C=C). ^{1}H NMR ($CDCl_3$): 1.81–1.90 (m, 4H, CH_2), 2.75 (t, 2H, SCH_2), 3.32 (t, $J = 6.8$ Hz, 2H, SCH_2), 3.70 (t, 2H, OCH_2), 3.74 (t, $J = 5.9$ Hz, 2H, OCH_2), 7.62–7.64 (m, 2H, CH_{arom}), 7.97–7.99 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 31.9 (CH_2), 35.4 (SCH_2), 61.3 (OCH_2), 127.2, 133.2, 133.9 (CH_{arom}, C_{arom}), 148.3 (S−C−C=O), 179.2 (C=O). MS [–ESI] $m/z = 338$ $[M]^-$. Anal. Calcd. for $C_{16}H_{18}O_4S_2$ (338.45): C, 56.78; H, 5.36; S, 18.95. Found C, 56.83; H, 6.16; S, 17.42.

22: Orange solid. Yield: 0.6 g (47%). M.p. 165.2–165.6 °C. R_f: 0.71 ($CHCl_3$/EtAc 2:1). IR: 3076 (CH_{arom}), 2966, 2942, 2925, 2845 (CH_{aliph}), 1666, 1643 (C=O), 1589, 1546 (C=C). ^{1}H NMR ($CDCl_3$): 2.17–2.22 (m, 4H, CH_2), 3.24 (t, 4H, SCH_2), 4.77 (t, $J = 5.9$ Hz, 4H, OCH_2), 7.58–7.61 (m, 2H, CH_{arom}), 7.93–7.98 (m, 2H, CH_{arom}). ^{13}C NMR ($CDCl_3$):

27.7 (CH_2), 30.0 (SCH_2), 71.9 (OCH_2), 126.6, 126.9, 130.6, 131.1, 131.8 (CH_{arom}, C_{arom}), 133.9 (S–*C*–C=O), 156.8 (O–*C*–C=O), 178.4, 183.3 (*C*=O). MS [+ESI] *m/z* = 517 $[M+Na]^+$. Anal. Calcd. for $C_{26}H_{20}O_6S_2$ (492.57): C, 63.40; H, 4.09; S, 13.02. Found C, 63.76; H, 4.25; S, 13.98.

General Procedure 4. Amination of 1,4-Naphthoquinones

Na_2CO_3 (1.52 g) was dissolved in CH_2Cl_2 (50 mL). 2,3-Dichloro-1,4-naphthoquinone (**1**) or 2-chloro-3-[(4-chlorobenzyl)thio]-1,4-naphthoquinone (**23**) and a secondary cyclic amine were added to the solution. 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 CH_2Cl_2. After recovery of the solvent, the crude product was purified by column chromatography.

2-Chloro-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-1,4-naphthoquinone (10) was synthesized from **1** (0.4 g, 1.15 mmol) and **9** (0.163 g, 2.30 mmol) by use of the general procedure 4.

10: Red solid. Yield: 1.28 g (87%). M.p. 148–149.5 °C. R_f: 0.59 ($CHCl_3$). IR: 3072 (CH_{arom}), 2954, 2900, 2860 (CH_{aliph}), 1589, 1548 (C=C), 1677, 1640 (C=O). ^{1}H NMR ($CDCl_3$): 1.83 (t, 4H, $CH_{2piperidine}$), 3.57 (t, 4H, $CH_{2piperidine}$), 3.93 (s, 4H, OCH_2CH_2O), 7.54–7.61 (m, 2H, CH_{arom}), 7.89–7.91 (d, 1H, CH_{arom}), 7.98–8.00 (d, 1H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 36.23, 49.94 ($CH_{2piperidine}$), 64.65 (OCH_2CH_2O), 134.20, 133.24, 131.83, 131.68, 126.71, 123.51 (C_{arom}), 106.90 (=CCl), 150.68 (=C–N), 181.98, 178.19 (C=O). MS [+ESI]: *m/z* = 334 $[M]^+$. Anal. Calcd. for $C_{17}H_{16}ClNO_4$ (333.766): C, 61.18; H, 4.83; N, 4.20. Found C, 61.31; H, 5.08, N, 4.23.

2-[(4-Chlorobenzyl)thio]-3-(1,4-diazepan-1-yl)-1,4-naphthoquinone (25) was synthesized from **23**[6] (0.6 g, 1.72 mmol) and **24** (0.18 g, 1.72 mmol) by use of the general procedure 4.

25: Red oil. Yield: 0.60 g (84%). IR: 3411 (NH), 2962, 2822 (CH_{aliph}), 1592, 1556 (C=C), 1632 (C=O). ^{1}H NMR (CD_3OD): 1.97 (s, 1H, NH), 2.16–2.30 (m, 2H, $CH_{2homopiper.}$), 3.40–3.50 (m, 4H, $CH_{2homopiper.}$), 3.60 (t, ^3J = 8.30 Hz, 2H, $CH_{2homopiper.}$), 3.77 (t, ^3J = 4.88 Hz, 2H, $CH_{2homopiper.}$), 4.02 (s, 2H, SCH_2), 7.14–7.21 (m, 4H, CH_{arom}), 7.67–7.73 (m, 2H, CH_{arom}), 7.90–7.92 (d, ^3J = 7.32 Hz, 1H, CH_{arom}), 7.94–7.96 (d, ^3J = 7.32 Hz, 1H, CH_{arom}). ^{13}C NMR (CD_3OD): 52.98, 51.92, 45.52, 25.83, 16.64 ($CH_{2homopiper.}$), 37.64 (SCH_2), 136.80, 132.88, 132.60, 132.11, 130.71, 130.54, 128.52, 128.43, 128,35, 126.54 (C_{arom}), 133.54 (=C–S), 155.33 (=C–N), 182.71, 182.31 (C=O). MS [+ESI]: *m/z* = 413 $[M]^+$. Anal. Calcd. for $C_{22}H_{21}ClN_2O_2S$ (412.932): C, 63.99; H, 5.13; N, 6.78; S, 7.77. Found C, 64.07; H, 4.99, N, 6.56; S, 7.59.

2-[(4-Chlorobenzyl)thio]-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-1,4-naphthoquinone (26) was synthesized from **23**[6] (0.48 g, 1.38 mmol) and **9** (0.39 g, 2.76 mmol) by use of the general procedure 4.

26: Purple oil. Yield: 0.53 g (84%). R_f: 0.47 ($CHCl_3$). IR: 3054 (CH_{arom}), 2958, 2928, 2881 (CH_{aliph}), 1591, 1536 (C=C), 1668, 1636 (C=O). ^{1}H NMR ($CDCl_3$): 1.76 (t, ^3J = 5.86 Hz, 4H, $CH_{2piperidine}$), 3.38 (t, ^3J = 5.37 Hz, 4H, $CH_{2piperidine}$), 3.93 (s, 4H, OCH_2CH_2O), 3.97 (s, 2H, SCH_2), 7.01–7.04 (d, ^3J = 8.30 Hz, 2H, CH_{arom}), 7.06–7.08 (d, ^3J = 8.30 Hz, 2H, CH_{arom}), 7.54–7.63 (m, 2H, CH_{arom}), 7.87–7.89 (d, ^3J = 7.32 Hz, 1H, CH_{arom}), 8.00–8.02 (d, ^3J = 7.32 Hz, 1H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 35.89, 51.05, 106.71 ($C_{piperidine}$), 64.40 (OCH_2CH_2O), 38.27 (SCH_2), 136.60, 132.86, 132.77, 131.99, 130.24,

128.39, 126.65, 126.24, 126.20 (C_{arom}), 122.33 (=C–S), 156.24 (=C−N), 181.79, 181.73 (C=O). MS [+ESI]: *m/z* = 478 $[M+Na]^+$. Anal. Calcd. for $C_{24}H_{22}ClNO_4S$ (455.954): C, 63.22; H, 4.86; N, 3.07; S, 7.03. Found C, 60.91; H, 4.70, N, 3.28; S, 6.49.

Ethyl 4-{3-[(4-Chlorobenzyl)thio]-1,4-dioxo-1,4-dihydronaphthalen-2-yl}piperazine-1-carboxylate (28) was synthesized from **23**[6] (0.4 g, 1.15 mmol) and **27** (0.36 g, 2.30 mmol) by use of the general procedure 4.

28: Purple oil. Yield: 0.47 g (87%). R_f: 0.33 ($CHCl_3$). IR: 3056 (CH_{arom}), 2979, 2925 (CH_{aliph}), 1592, 1536 (C=C), 1699, 1669, 1639 (C=O). ^{1}H NMR ($CDCl_3$): 1.22 (t, ^3J = 7.32 Hz, 3H, CH_3), 3.26 (t, ^3J = 4.88 Hz, 4H, $CH_{2piperazine}$), 3.50 (t, ^3J = 5.37 Hz, 4H, $CH_{2piperazine}$), 4.02 (s, 2H, SCH_2), 4.11 (q, 2H, OCH_2), 7.02–7.04 (d, ^3J = 8.79 Hz, 2H, CH_{arom}), 7.08–7.11 (d, ^3J = 8.78 Hz, 2H, CH_{arom}), 7.57–7.65 (m, 2H, CH_{arom}), 7.88–7.90 (d, ^3J = 7.81 Hz, 1H, CH_{arom}), 8.01–8.03 (d, ^3J = 7.32 Hz, 1H, CH_{arom}). ^{13}C NMR ($CDCl_3$): 14.89 (CH_3), 52.63, 29.93 ($CH_{2piperazine}$), 61.87 (OCH_2), 136.74, 133.21, 133.03, 132.13, 130.48, 128.74, 127.48, 126.95, 126.60 (C_{arom}), 124.48 (=C–S), 155.72 (=C−N), 182.04, 181.85, 155.82 (C=O). MS [+ESI]: *m/z* = 493 $[M+Na]^+$. Anal. Calcd. for $C_{24}H_{23}ClN_2O_4S$ (470.968): C, 61.21; H, 4.92; N, 5.95; S, 6.81. Found C, 58.42; H, 4.92, N, 5.34; S, 5.41.

Antifungal and Antibacterial Evaluation

Diffusion Technique. The antibacterial activities were 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 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 Vancomycine (for bacteria) or Nystatine (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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