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Research Article

Synthesis and *In Vitro* Biological Evaluation of Aminonaphthoquinones and Benzo[*b*]phenazine-6,11-dione Derivatives as Potential Antibacterial and Antifungal Compounds

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A series of 2-arylamino-3-chloro-1,4-naphthoquinone derivatives (**3a**–**h**) by the reaction of 2,3-dichloro-1,4-naphthoquinone with aryl amines (**2a**–**h**) and benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) by the treatment of 2-arylamino-3-chloro-1,4-naphthoquinone derivatives (**3a**–**h**) with sodium azide were synthesized and tested for their *in vitro* antibacterial and antifungal activities. The results suggest that compounds **3d** and **3g** had potent antifungal activity against *Candida albicans* (MIC = 78.12 μg/mL). All synthesized compounds (**3a**–**h**, **4a**–**c**) possessed activity against *E. faecalis* with MIC values of between 312.5 and 1250 μg/mL. Benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) were mostly active against Gram-positive bacteria. The structures of the new members of the series were established on the basis of their spectral properties (IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry).

1. Introduction

Quinones are active compounds used widely as raw materials in pharmaceuticals and agrochemical industries. Particularly, (hetero)cyclic quinone moieties not only exist in many natural products and pharmaceutical compounds, but also are well-known and versatile building blocks for the synthesis of quinones derived from benzoquinone, naphthoquinone, or anthracenequinone condensed with five-membered heterocycles [1–3] such as isoxazoles [4], six-membered heterocycles [3, 5], and seven-membered heterocycles [6, 7] such as 1,4-benzodiazepines [8] in order to evaluate their important bioactivities. Therefore, among the bioactive quinones, 1,4-naphthoquinones have been extensively studied since those ones contain two ketone groups as a crucial pharmacophore for their bioactivities because of their ability to accept electrons [9]. A considerable number of natural and synthetic 1,4-naphthoquinones have shown an interesting variety of biological properties, such as antimalarial [10–12], antibacterial [13–16], antifungal [17–20], antitumor [21, 22], anti-inflammatory [23, 24], and antiallergic [25, 26] activities, due to their redox potentials [27]. Some important compounds shown in Figure 1 are good examples for emphasizing their important biological properties [28, 29]. All findings showed that the position and the number of nitrogen atoms in the structure could improve the redox potential of the quinone system for biological properties [30]. It has been reported that, generally, increase of the number of the nitrogen atoms and the rings enhances the activities [19].

The reactions of amines and their derivatives with 1,4-naphthoquinones to give 2-aryl(alkyl)amino-1,4-naphthoquinone derivatives have been known for several years [31]. A lot of studies related to 1,4-naphthoquinones containing a nitrogen [32, 33], sulfur [34], or an oxygen atom [35] in

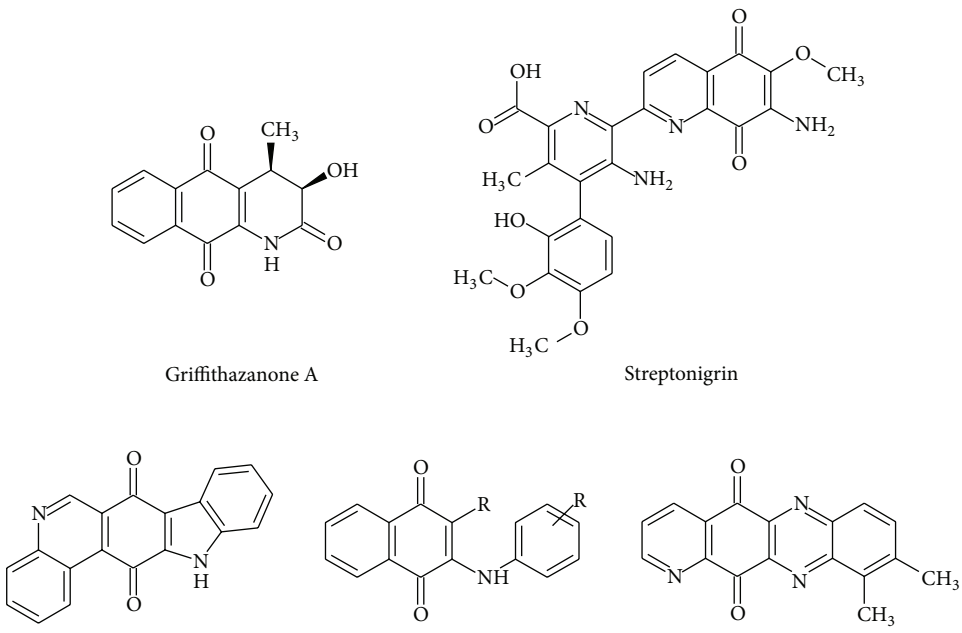


Figure 1: Examples of bioactive aminonaphthoquinones.

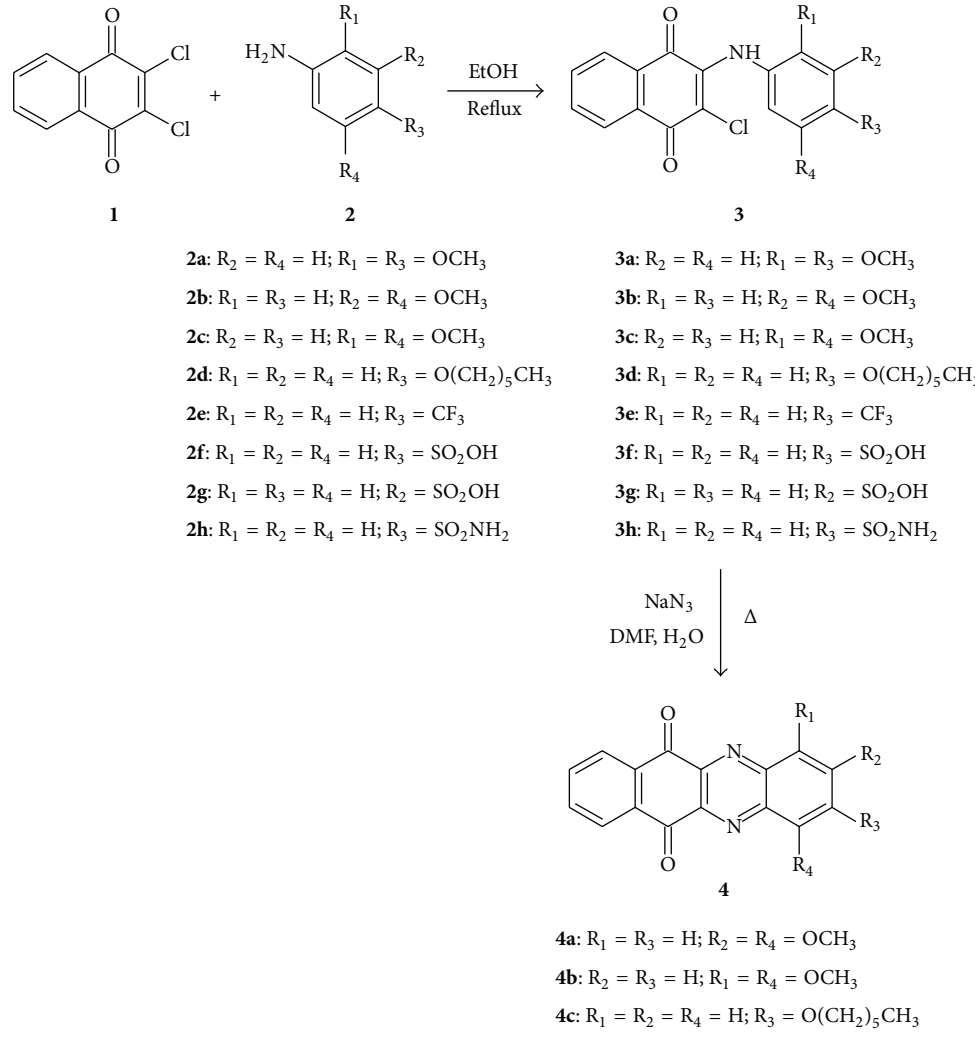
the 2-position or 2,3-positions have been reported up to now because of their use in a variety of medical and biological applications as mentioned above. The reactions of 2-arylamino-3-chloro-1,4-naphthoquinone derivatives with sodium azide to give heterocyclic phenazine derivatives have been described previously [29, 36, 37]. Analogously, the reactions of 2-sulfanyl-3-chloro-1,4-naphthoquinone derivatives with sodium azide to give heterocyclic phenothiazine derivatives have been also reported [17, 34]. Additionally, the cyclization of 2-arylamino-1,4-naphthoquinones to benzo[*b*]phenazine-6,11-dione 5-oxides by the treatment with nitrosylsulfuric acid as a new group of tetracyclic diazaquinones has been recently reported [38].

Keeping in mind that 1,4-naphthoquinones are involved in a wide range of biological studies and the presence of nitrogen atoms would improve the bioactivity, herein, a series of 2-arylamino-1,4-naphthoquinone derivatives (**3a**–**h**) were synthesized by using the standard procedure [16, 17, 28] via nucleophilic displacement reaction of 2,3-dichloro-1,4-naphthoquinone (**1**) with aryl amines (**2a**–**h**) as shown in Scheme 1. Subsequently, the final compounds, new benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**), were synthesized via intramolecular cyclization with sodium azide of 2-arylamino-1,4-naphthoquinone derivatives (**3a**–**h**) in accordance with the literature [29, 36, 37]. Finally, synthesized compounds were investigated for their antimicrobial activity against both Gram-positive and Gram-negative bacteria, in addition to fungi. Structures of the synthesized new compounds were determined by using FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

2. Results and Discussion

2.1. Chemistry. It is known that the reactions of 2,3-dichloro-1,4-naphthoquinone (**1**) with aryl and alkyl amines proceed by nucleophilic substitution [15–17, 28, 39–43]. A series of 2-arylamino-1,4-naphthoquinone derivatives (**3a**–**h**) were synthesized via the nucleophilic substitution reaction of 2,3-dichloro-1,4-naphthoquinone (**1**) by appropriate aryl amines (**2a**–**h**) in refluxing ethanol as shown in Scheme 1. The aminonaphthoquinones (**3a**–**h**) were obtained in around 55–60% yields as dark orange, red, dark red, and purple solid. Structures of the aminonaphthoquinone derivatives were confirmed by spectroscopic methods comprising ^{1}H and ^{13}C NMR, IR, and MS. In the MS of aminonaphthoquinones, the molecular ion peaks of compounds **3a**, **3f**, and **3g** were observed at 343 $[M]^+$, 362 $[M–H]^+$, and 362 $[M–H]^+$, respectively. Some of the IR spectra of aminonaphthoquinones revealed the absorption bands of the N–H group at around 3300 cm^{-1} and of >C=O moiety at 1683 cm^{-1}. The ^{1}H NMR spectra exhibited aromatic protons at around 6.50–8.00 ppm. The methylene protons of alkoxy groups in **3a** appeared at around 3.5–4 ppm as a singlet. The methylene protons of compound **3d** (–OCH_2–) were observed as a triplet at 3.96 ppm. The singlet peak at around 8-9 ppm was assigned to the NH proton in aminonaphthoquinones. In addition, aromatic protons of aminonaphthoquinones are displayed at 6.50–8.00 ppm. In the ^{13}C NMR spectra, characteristic signals of two carbonyl carbons of aminonaphthoquinones were visible at chemical shift at around 175 and 182 ppm.

Further reactions of 2-arylamino-1,4-naphthoquinone derivatives (**3a**–**h**) with sodium azide for cyclization in DMF



1 **2** **3**

2a: $R_2 = R_4 = H$; $R_1 = R_3 = OCH_3$
2b: $R_1 = R_3 = H$; $R_2 = R_4 = OCH_3$
2c: $R_2 = R_3 = H$; $R_1 = R_4 = OCH_3$
2d: $R_1 = R_2 = R_4 = H$; $R_3 = O(CH_2)_5CH_3$
2e: $R_1 = R_2 = R_4 = H$; $R_3 = CF_3$
2f: $R_1 = R_2 = R_4 = H$; $R_3 = SO_2OH$
2g: $R_1 = R_3 = R_4 = H$; $R_2 = SO_2OH$
2h: $R_1 = R_2 = R_4 = H$; $R_3 = SO_2NH_2$

3a: $R_2 = R_4 = H$; $R_1 = R_3 = OCH_3$
3b: $R_1 = R_3 = H$; $R_2 = R_4 = OCH_3$
3c: $R_2 = R_3 = H$; $R_1 = R_4 = OCH_3$
3d: $R_1 = R_2 = R_4 = H$; $R_3 = O(CH_2)_5CH_3$
3e: $R_1 = R_2 = R_4 = H$; $R_3 = CF_3$
3f: $R_1 = R_2 = R_4 = H$; $R_3 = SO_2OH$
3g: $R_1 = R_3 = R_4 = H$; $R_2 = SO_2OH$
3h: $R_1 = R_2 = R_4 = H$; $R_3 = SO_2NH_2$

4

4a: $R_1 = R_3 = H$; $R_2 = R_4 = OCH_3$
4b: $R_2 = R_3 = H$; $R_1 = R_4 = OCH_3$
4c: $R_1 = R_2 = R_4 = H$; $R_3 = O(CH_2)_5CH_3$

Scheme 1: Preparation of 2-arylamino-3-chloro-1,4-naphthoquinones (**3a**–**h**) and benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**).

at 90–100°C overnight afforded the expected benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**). The reaction is believed to proceed via the formation of the unstable intermediate compound (2-arylamino-3-azido-1,4-naphthoquinones) as mentioned in the literature [29]. The mass spectra of benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) showed molecular ion peak at 321 $[M+H]^+$, 343 $[M+Na]^+$, and 361 $[M+H]^+$, respectively. The formation of benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) was confirmed by the absence of both a singlet at 8-9 ppm attributable to the NH proton in the ^1H NMR spectra and absorption bands of the N–H group at around 3300 cm^{-1} in the IR spectra. One of the methoxy derivatives of benzo[*b*]phenazine-6,11-dione (**4a**) was obtained from both 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3a**, 21%) and 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3b**, 51%).

2.2. Antimicrobial Activity. All the synthesized 2-arylamino-3-chloro-1,4-naphthoquinones (**3a**–**h**) and benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) were evaluated for their *in vitro* antibacterial activities against three Gram-positive (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 29212) and four Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, and *Proteus mirabilis* ATCC 14153). The antifungal activity was tested against a yeast *Candida albicans* ATCC 10231. All the synthesized and screened antimicrobial assay results of 2-arylamino-3-chloro-1,4-naphthoquinones (**3a**–**h**) and benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) are given in Table 1.

Results shown in Table 1 reveal that compounds **3d** and **3g** have exhibited moderate activity against both Gram-positive and Gram-negative bacteria, except *E. coli* for **3d**. All of the synthesized compounds (**3a**–**h**, **4a**–**c**) possessed activity against *E. faecalis* with MIC values of between 312.5 and 1250 μg/mL. In addition to *E. faecalis*, all of the compounds, except **4b**, possessed activity against *P. aeruginosa* with MIC values of between 625 and 1250 μg/mL. The synthesized compounds, except **3e** and **4a**, also showed good antibacterial activity against *S. epidermidis*. The test-culture *E. coli* appeared

Table 1: *In vitro* antibacterial and antifungal activity of the synthesized compounds.

Microorganisms	MIC values (μg/mL)											
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**4a**	**4b**	**4c**	Reference antimicrobials
Fungi												
C. albicans	—	—	—	78.12	—	—	78.12	—	—	—	—	4.9 (Clotrimazole)
Gram-positive bacteria												
S. aureus	—	—	1250	312.5	—	625	312.5	—	1250	1250	1250	1.2 (Cefuroxime-Na)
S. epidermidis	1250	1250	1250	312.5	—	1250	312.5	1250	—	1250	156.2	9.8 (Cefuroxime)
E. faecalis	312.5	1250	1250	625	1250	1250	312.5	1250	1250	1250	1250	128 (Amikacin)
Gram-negative bacteria												
P. aeruginosa	1250	1250	1250	1250	625	1250	1250	1250	1250	—	1250	2.4 (Ceftazidime)
E. coli	—	—	—	—	—	—	625	—	—	—	—	4.9 (Cefuroxime-Na)
K. pneumoniae	—	—	—	1250	1250	—	1250	—	—	—	1250	4.9 (Cefuroxime-Na)
P. mirabilis	—	—	1250	1250	—	—	1250	—	—	—	—	2.4 (Cefuroxime-Na)

not to be susceptible to synthesized compounds except that **3g**. Evaluation of the antifungal activity of the synthesized compounds showed that **3d** and **3g** were the most potent with MIC (minimum inhibition concentration) 78.12 μg/mL for *C. albicans* (Table 1). The results also reveal that compounds **3d** and **3g** were the most active among the synthesized compounds; they have both antibacterial and antifungal activities. On the other hand, benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) were mostly active against Gram-positive bacteria.

We found that replacing the sulfonic acid group ($-SO_3H$) position in 2-arylamino-3-chloro-1,4-naphthoquinones from the meta position to the para position led to activity loss. Additionally, replacing the sulfonic acid group ($-SO_3H$) at the para position by a sulfonamide group ($-SO_2NH_2$) and trifluoromethyl ($-CF_3$) did not show any progress against *C. albicans* but showed an increase in activity against some of the Gram-negative bacteria. By contrast, replacing this sulfonic acid group ($-SO_3H$) at the para position by a hexyloxy group ($-O(CH_2)_5CH_3$) led to an increase in activity against *C. albicans* and no significant increase in activity against some of the Gram-positive and Gram-negative bacteria. Changing this hexyloxy group ($-O(CH_2)_5CH_3$) by the methoxy groups at different positions, unfortunately, led to activity loss again.

3. Experimental Section

3.1. Materials and Equipment. All reagents were commercially obtained from commercial supplier and used without further purification unless otherwise noted. Petroleum ether had a boiling range of 40–60°C. Analytical thin layer chromatography (TLC) was purchased from Merck KGaA (silica gel 60 F254) based on Merck DC-plates (aluminum based). Visualization of the chromatogram was performed by UV light (254 nm). Column chromatographic separations were carried out using silica gel 60 (Merck, 63–200 μm particle size, 60–230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian^{UNITY} INOVA spectrometers with 500 MHz frequency for ^{1}H and 125 MHz frequency for ^{13}C NMR in ppm (δ). ^{1}H NMR spectra and ^{13}C NMR spectra in $CDCl_3$ refer to the solvent signal center at δ 7.19 and δ 76.0 ppm, respectively. Other solvents are as follows: DMSO-d_6: 2.49, 3.30 ppm (^{1}H), 40.27 ppm (^{13}C). Standard abbreviations indicating multiplicity were used as follows: s (singlet), br s (broad singlet), d (doublet), t (triplet), and m (multiplet). Coupling constants J are given in Hz. IR spectra were recorded as ATR on either Thermo Scientific Nicolet 6700 spectrometer or Alpha T FTIR spectrometer. Mass spectra were obtained on either a Thermo Finnigan LCQ Advantage MAX MS/MS spectrometer equipped with ESI (electrospray ionization) sources or GC-MS Shimadzu QP2010 Plus. Melting points (mp) were determined with an Electrothermal IA9000 series and were uncorrected.

*3.2. General Procedure for the Preparation of 2-Arylamino-3-chloro-1,4-naphthoquinones (**3a**–**h**).* Compounds **3a**–**h** were prepared using the following general procedure according to the reported literature [10, 16, 17, 28, 39–43]: aryl amine (2.42 mmol) was added to the solution of 2,3-dichloro-1,4-naphthoquinone (2.20 mmol) in ethanol (100 mL) and refluxed. Then the reaction mixture was cooled, and the precipitate was filtered. The filtered precipitate was isolated after purification either by column chromatography on silica gel or recrystallized from ethanol.

*3.2.1. 2-Chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3a**).* It was synthesized from 2,3-dichloro-1,4-naphthoquinone and 2,4-dimethoxyaniline. Yield: 63%. Mp: 156–158°C. IR (ATR) ν (cm^{-1}): 3252 (NH); 3010 (Ar–H); 1669, 1636 (C=O); 1592, 1563 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.68 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.50 (dd, J = 8.78, 2.44 Hz, 1H, $CH_{aromatic}$), 6.57 (d, J = 2.44 Hz, 1H, $CH_{aromatic}$), 7.08 (d, J = 8.78 Hz, 1H, $CH_{aromatic}$), 7.71–7.80 (td, J = 7.81, 1.46 Hz, 1H, $CH_{aromatic}$), 7.80–7.88 (td, J = 7.32, 1.47 Hz, 1H, $CH_{aromatic}$), 7.94–8.03 (td, J = 8.78, 0.98 Hz, 2H, $CH_{aromatic}$), 8.77 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 56.1, 56.3 (OCH_3), 99.2, 104.8, 121.4, 126.7, 127.1, 127.9, 130.7, 132.7, 133.7, 135.6, 144.9, 159.4 ($C_{aromatic}$), 111.8 (C=C–Cl), 155.2 (C=C–NH), 176.7, 180.5 (>C=O). MS (GC-MS), (m/z %): 343 (100, $[M]^+$). Anal. Calcd for $C_{18}H_{14}ClNO_4$ (343.76).

*3.2.2. 2-Chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3b**).* It was prepared from 2,3-dichloro-1,4-naphthoquinone and 3,5-dimethoxyaniline as described in the literature reported previously [39]. Mp: 175–177°C (lit. 169–171°C [39] and 185–185.5°C [39]).

*3.2.3. 2-Chloro-3-[(2,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3c**).* It was prepared from 2,3-dichloro-1,4-naphthoquinone and 2,5-dimethoxyaniline as described in the literature reported previously [28, 40]. Mp: 145-146°C (lit. 146.7–146.9°C [40] and 146–149°C [28]).

*3.2.4. 2-Chloro-3-{[4-(hexyloxy)phenyl]amino}naphthalene-1,4-dione (**3d**).* It was synthesized from 2,3-dichloro-1,4-naphthoquinone and 4-(hexyloxy)aniline as described in the literature reported recently [28]. Yield: 58%. Mp: 125–127°C (lit. [28] 130–133°C). IR (ATR) ν (cm^{-1}): 3219 (NH); 3069 (Ar–H); 2924, 2853 (Aliphatic-CH); 1675, 1631 (C=O); 1595, 1561 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 0.88 (t, J = 6.83 Hz, 3H, CH_3), 1.26–1.36 (m, 4H, CH_2–CH_2), 1.37–1.46 (m, 2H, CH_2), 1.71 (q, J = 6.84 Hz, 2H, CH_2), 3.96 (t, J = 6.34 Hz, 2H, OCH_2), 6.87 (d, J = 8.79 Hz, 2H, $CH_{aromatic}$), 7.07 (d, J = 8.79 Hz, 2H, $CH_{aromatic}$), 7.77–7.81 (td, J = 7.81, 1.47 Hz, 1H, $CH_{aromatic}$), 7.84–7.87 (td, J = 7.81, 1.47 Hz, 1H, $CH_{aromatic}$), 8.01–8.04 (m, 2H, $CH_{aromatic}$), 9.18 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 14.6 (CH_3), 22.8, 25.9, 29.4, 31.7 ((CH_2)$_4$), 68.3 (OCH_2), 114.4, 126.7, 127.2, 127.8, 130.8, 132.2, 132.8, 133.7, 135.5, 144.1 ($C_{aromatic}$), 112.9 (C=C–Cl), 156.8 (C=C–NH), 177.2, 180.9 (>C=O).

*3.2.5. 2-Chloro-3-{[4-(trifluoromethyl)phenyl]amino}naphthalene-1,4-dione (**3e**).* It was prepared from 2,3-dichloro-1,4-naphthoquinone and 4-(trifluoromethyl)aniline as described in the literature reported previously [41].

*3.2.6. 4-[(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino]benzenesulfonic Acid (**3f**).* It was synthesized from 2,3-dichloro-1,4-naphthoquinone and 4-aminobenzenesulfonic acid as described in the general procedure reported previously [10, 42]. Yield: 60%. IR (ATR) ν (cm^{-1}): 3419 (OH); 3231 (NH); 3070 (Ar–H); 1673, 1645 (C=O); 1591, 1565 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.06 (s, 1H, OH), 7.05 (d, J = 8.30 Hz, 2H, $CH_{aromatic}$), 7.52 (d, J = 8.30 Hz, 2H, $CH_{aromatic}$), 7.80 (t, J = 7.32 Hz, 1H, $CH_{aromatic}$), 7.85 (t, J = 7.32 Hz, 1H, $CH_{aromatic}$), 8.02 (d, J = 7.32 Hz, 2H, $CH_{aromatic}$), 9.30 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 123.4, 126.0, 126.8, 127.2, 131.0, 132.6, 133.9, 135.5, 139.6, 143.8 ($C_{aromatic}$), 115.7 (C=C–Cl), 144.7 (C=C–NH), 177.4, 180.8 (>C=O). MS (−ESI), (*m/z* %): 364 (37, $[M+H]^+$), 363 (22, $[M]^+$), 362 (100, $[M–H]^+$). MS2 (−ESI, 362), (*m/z* %): 326 (100, $[M–Cl]^+$), 282 (58, $[M–SO_3H]^+$). Anal. Calcd for $C_{16}H_{10}ClNO_5S$ (363.77).

*3.2.7. 3-[(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino]benzenesulfonic Acid (**3g**).* It was synthesized from 2,3-dichloro-1,4-naphthoquinone and 3-aminobenzenesulfonic acid as described in the general procedure. Yield: 62%. IR (ATR) ν (cm^{-1}): 3384 (OH); 3255 (NH); 1671, 1639 (C=O); 1591, 1566 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.24 (s, NH), 4.75 (br s, OH), 7.67–7.69 (m, 2H, $CH_{aromatic}$), 7.73–7.76 (m, 2H, $CH_{aromatic}$), 8.01–8.03 (m, 1H, $CH_{aromatic}$), 8.07–8.09 (m, 1H, $CH_{aromatic}$), 8.12–8.15 (m, 2H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 126.0, 126.8, 129.9, 130.1, 132.9, 133.3, 133.7, 142.6, 175.1 ($C_{aromatic}$), 125.9 (C=C–Cl), 155.8 (C=C–NH), 177.6, 178.7 (>C=O). MS (−ESI), (*m/z* %): 364 (38, $[M+H]^+$), 363 (18, $[M]^+$), 362 (100, $[M–H]^+$). MS2 (−ESI, 362), (*m/z* %): 326 (100, $[M–Cl]^+$), 282 (9, $[M–SO_3H]^+$). Anal. Calcd for $C_{16}H_{10}ClNO_5S$ (363.77).

*3.2.8. 4-[(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino]benzenesulfonamide (**3h**).* It was prepared from 2,3-dichloro-1,4-naphthoquinone and 4-aminobenzenesulfonamide as described in the literature reported previously [10, 43]. Mp > 300°C (lit. >300°C [10, 43]).

*3.3. General Procedure for the Preparation of Benzo[b]phenazine-6,11-dione Derivatives (**4a**–**c**).* Compounds **4a**–**c** were prepared using the following general procedure according to the reported literature [29, 36, 37]: to a solution of the corresponding 2-arylamino-3-chloro-1,4-naphthoquinone (**3a**–**d**) in DMF (15 mL), sodium azide (20 mmol), suspended in 2.5 mL water, was added and refluxed. The reaction mixture was diluted with dichloromethane and the organic phase was washed twice with water and then dried over $CaCl_2$. After evaporating the solvent, the crude product was purified by column chromatography on silica gel to yield the corresponding benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**).

*3.3.1. 1,3-Dimethoxybenzo[b]phenazine-6,11-dione (**4a**).* It was synthesized from 2-chloro-3-[(2,4-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3a**). Yellow crystals. Yield: 21%. Mp > 300°C. IR (ATR) ν (cm^{-1}): 3108, 3054 (Ar–H); 1686, 1614 (C=O); 1588, 1565. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.96 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.79 (s, 1H, $CH_{aromatic}$), 7.26 (s, 1H, $CH_{aromatic}$), 7.82–7.83 (m, 2H, $CH_{aromatic}$), 8.40–8.43 (m, 2H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 55.4, 55.6 (OCH_3), 98.9, 104.1, 127.1, 132.7, 133.1, 133.2, 133.7, 134.1, 139.6, 143.7, 145.8, 156.1, 164.1 ($C_{aromatic}$), 179.5, 180.7 (>C=O). MS (+ESI), (*m/z* %): 343 (38, $[M+Na]^+$), 321 (100, $[M+H]^+$). Anal. Calcd for $C_{18}H_{12}N_2O_4$ (320.30).

Alternatively, **4a** was prepared from 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3b**) by using the general procedure. Yield: 51%. Spectroscopic data are in accordance with **4a** which is prepared from 2-chloro-3-[(3,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3a**).

*3.3.2. 1,4-Dimethoxybenzo[b]phenazine-6,11-dione (**4b**).* It was synthesized from 2-chloro-3-[(2,5-dimethoxyphenyl)amino]naphthalene-1,4-dione (**3c**). Yellow crystals. Yield: 29%. Mp > 300°C. IR (ATR) ν (cm^{-1}): 3075 (Ar–H); 1686, 1613 (C=O); 1587, 1486. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.05 (s, 6H, 2OCH_3), 7.15 (s, 2H, $CH_{aromatic}$), 7.83–7.85 (m, 2H, $CH_{aromatic}$), 8.42–8.43 (m, 2H, $CH_{aromatic}$). ^{13}C NMR

(125 MHz, $CDCl_3$) δ (ppm): 55.5 (OCH_3), 110.2, 127.2, 133.0, 134.1, 135.7, 141.9, 148.9 ($C_{aromatic}$), 179.6 (>C=O). MS (+ESI), (*m/z* %): 343 (100, $[M+Na]^+$). Anal. Calcd for $C_{18}H_{12}N_2O_4$ (320.30).

*3.3.3. 2-(Hexyloxy)benzo[b]phenazine-6,11-dione (**4c**).* It was synthesized from 2-chloro-3-{[4-(hexyloxy)phenyl]amino}naphthalene-1,4-dione (**3d**). Yellow crystals. Yield: 56%. Mp: 167–169°C. IR (ATR) ν (cm^{-1}): 3066 (Ar–H); 2952, 2919, 2859 (Aliphatic-CH); 1681, 1607 (C=O); 1517, 1484. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.85 (t, J = 6.83 Hz, 3H, CH_3), 1.23–1.34 (m, 4H, CH_2–CH_2), 1.39–1.48 (m, 2H, CH_2), 1.78–1.97 (m, 2H, CH_2), 4.11 (t, J = 6.59 Hz, 2H, OCH_2), 7.53 (dd, J = 9.28, 2.93 Hz, 1H, $CH_{aromatic}$), 7.58 (d, J = 2.93 Hz, 1H, $CH_{aromatic}$), 7.78–7.84 (m, 2H, $CH_{aromatic}$), 8.22 (d, J = 9.27, 1H, $CH_{aromatic}$), 8.36–8.40 (m, 2H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 13.0 (CH_3), 21.5, 24.6, 27.7, 30.5 ((CH_2)$_4$), 68.4 (OCH_2), 106.7, 127.1, 127.7, 131.0, 132.7, 132.9, 133.9, 134.1, 139.8, 140.9, 143.2, 145.3, 162.4 ($C_{aromatic}$), 180.1, 180.5 (>C=O). MS (+ESI), (*m/z* %): 361 (100, $[M+H]^+$). Anal. Calcd for $C_{22}H_{20}N_2O_3$ (360.40).

3.4. Biological Assays. Antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231 was determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations [44, 45]. Mueller-Hinton broth for bacteria and RPMI-1640 medium for yeast strain were used as the test medium. Serial twofold dilutions ranging from 5000 mg/L to 4.8 mg/L were prepared in medium. The inoculum was prepared using a 4–6 h broth culture of each bacteria type and 24 h culture of yeast strains adjusted to a turbidity equivalent to 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 cfu/ml for bacteria and 5×10^3 cfu/mL for yeast in the test tray. The trays were covered and placed into plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35°C for 18–20 h while the trays containing RPMI-1640 medium were incubated at 35°C for 46–50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. As control, antimicrobial effects of the solvents were investigated against test microorganisms. According to values of the controls, the results were evaluated. The MIC values of the compounds are given in Table 1.

4. Conclusions

In conclusion, we have synthesized a series of 2-arylamino-3-chloro-1,4-naphthoquinone derivatives (**3a**–**h**) and benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) which were given by reacting 2-arylamino-3-chloro-1,4-naphthoquinone derivatives (**3a**–**d**) with sodium azide through known chemical routes. The structures of the five new compounds (**3a**, **3g**, and **4a**–**c**) have been confirmed by means of different spectroscopic methods. These new compounds possess high solubility in various organic solvents such as chloroform and dichloromethane while they are insoluble in water and these compounds have shown good stability. On the basis of screening data for the presented compounds, the *in vitro* antimicrobial activities were evaluated against different Gram-positive and Gram-negative bacterial strains in addition to the antifungal activities. The test-culture *E. coli* appeared not to be susceptible to most of the synthesized compounds. Results revealed that compounds **3d** and **3g** have remarkable activity against both Gram-positive and Gram-negative bacteria and against the tested fungi (*C. albicans*), while all of the synthesized compounds (**3a**–**h**, **4a**–**c**) possessed activity against *E. faecalis* with MIC values of between 312.5 and 1250 μg/mL. Benzo[*b*]phenazine-6,11-dione derivatives (**4a**–**c**) were mostly active against Gram-positive bacteria.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Amaç Fatih Tuyun, Nilüfer Bayrak, Hatice Yıldırım, Nihal Onul, Emel Mataraci Kara, and Berna Ozbek Celik contributed equally to this work.

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