Synthesis, Biological, and Computational Study of Naphthoquinone Derivatives Containing Heteroatoms

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Summary: A number of 2-(aryl/alkyl)thio-3-chloro-l,4-naphthoquinones (**3a-g**) and 2,3-(aryl/alkyl)thio-l,4-naphthoquinones (**4a-d**, **4f-g**) were obtained by the reactions of 2,3-dichloro-1,4-naphthoquinone (**1**) with some various thiols and subsequently used as building blocks for the synthesis of 2-(arylthio)-3-amino-1,4-naphthoquinone (**5a-c**, **5f**) derivatives. The substituted naphthoquinones were reacted with sodium azide in dimethylformamide and 2-arylthio-3-amino-1,4-naphthoquinone derivatives were obtained as the sole identifiable products except one cyclized compound. PASS prediction results and their analysis provided by PharmaExpert software were used for the studied compounds to explore pharmacotherapeutic potential, possible mechanism of action and drug-metabolising enzyme inhibition. Furthermore, *in vitro* antimicrobial potential was evaluated against seven bacterial strains (Gram-positive and Gram-negative bacteria) and one fungi. Among the tested compounds, **3c** was found to have the best level of antibacterial activity against *S. aureus* (MIC = 39.06 μg/mL). Molecular docking studies were applied to better clarify the action and binding modes.

Keywords: Naphthoquinones; Substituted quinones; Antibacterial activity; Antifungal activity; Docking study

Introduction

Since the discovery of their basic structure in 1838 [1], quinones have attracted great attention from scientists [2, 3]. The occurance of these derivatives in many natural compounds and their wide range of biological activities have also motivated researchers to synthesize new quinones in recent years [4, 5]. Naphthoquinones are among the most famous quinone derivatives with their significant pharmacological properties as antiviral, antifungal, antibacterial, and anti-tumour agents [6-10]. The most significant function of 1,4-naphthoquinones is their ability to adapt biological processes which arises from the redox reactions of the quinone system by carrying electrons to generate hydroquinone forms that can interplay with vital components such as DNA and proteins [11-13]. Oxidative stress that occurs with an increase in reactive oxygen species generation, along with an accompanying disruption in redox balance, leads to the proliferation of many types of cancer cells [14, 15]. Some specific redox catalysts containing naphthoquinone moieties among the different agents used to regulate the intracellular redox states of cells are also being used with a high degree in cancer treatment [16-19].

The synthesis of 2,3-bis(arylthio)- and 2-amino-l,4-naphthoquinone derivatives has been reported by a scientist Clark and tested inhibition against *Botrytis cinerea, Cladosporium fulvum* and *Venturiaina equalis*. The outcome of the study revealed that 2,3-bis(4-chlorophenylthio), 2-phenylthio-, 2-amino-, 2-amino-3-chloro-1,4-naphthoquinones showed a high level of activity against all three fungi [20, 21].

1,4-naphthoquinone derivatives containing sulfur or nitrogen atoms were synthesized by Tandon and evaluated for antiproliferative, antifungal, antiviral, and antibacterial activities. Most compounds showed *in vitro* antibacterial and antifungal activity and a few showed an inhibitory effect against RNA. Monoarylsubstituted-1,4-naphthoquinones showed antifungal activity compared to antifungal drugs (Fluconazole) against *Sporothrix schenckii* and Amphotericin-B against *Trichophyton mentagraphytes*. Bisarylsubstituted-1,4-naphthoquinones produced a high degree of

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antiproliferative and cell killing activity against human cervical cancer cells [22-25].

Recently, in 2014, Ravichandiran *et al.* synthesized new series of 2-(4-amino-benzosulfonyl)-5H-benzo[*b*]carbazole-6,11-dione derivatives by the reaction 1,4-naphthoquinone with 4-aminophenyl sulfone and their subsequent reactions and studied their cytotoxicity evaluation [26]. Another study in order to synthesize novel 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[*a*]phenoxazin-5-one derivatives has been published in addition to their molecular docking study with histone deacetylase (HDAC8) and cytotoxicity against cervical cancer cell line. Also, a number of novel heterocyclic naphthoquinone derivatives with phenothiazin-5-one moiety were prepared and used for their antibacterial and cytotoxicity behavior studies [27].

New bioactive naphthoquinones due to their biological importance have always fascinated and inspired synthetic organic chemists so it is no surprise that one of the today's favorite targets is to synthesize new agents having the 1,4-naphthoquinone moiety could display biological effects such as antiinflammatory, antiplatelet, cytotoxic, molluscidal, antiallergic, antiproliferative, antileishmanial, and antimalarial activities [2, 22, 28-33]. Such previous studies led us to explore some new and known naphthoquinone derivatives, which are cyclic structure containing heteroatoms such as sulfur and nitrogen and their structures, were analyzed by spectroscopic methods such as MS, FT-IR, NMR. Furthermore, the derivatives were screened for their *in vitro* antimicrobial activity against some organisms.

Experimental

All reagents were obtained from commercial supplier and used without further purification unless otherwise noted. Petroleum ether had a boiling range 40-60 ^{o}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 sized, 60-230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded either 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 7.26 and δ 77.0 ppm, respectively. 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 Thermo Scientific Nicolet 6700 spectrometer. Mass spectra were obtained on a ThermoFinnigan LCQ Advantage MAX MS/MS spectrometer equipped with an ESI (Electrospray ionization) sources. Melting points (mp) were determined with a Buchi B-540 melting point apparatus and were uncorrected.

*General procedure for preparing mono- (**3a-g**) and disubstituted 1,4-naphthoquinones (**4a-d**, **4f-g**) from 2,3-dichloro-1,4-naphthoquinone* **(1)**

Mono- (**3a-g**) and disubstituted 1,4-naphthoquinones (**4a-d**, **4f-g**) were prepared from 2,3-dichloro-1,4-naphthoquinone (**1**) as previously described [25b]. 2,3-Dichloro-1,4-naphthoquinone was dissolved in ethanol (68.75 mL) and then into the resulting solution, thiol was added in small portions. The mixture was refluxed at 60 ^{o}C until completion of the reaction (TLC). The residue was extracted with chloroform. The organic layer was separated and washed with water (4 x 30 mL), and dried with Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel.

*2-Chloro-3-(p-tolylthio)naphthalene-1,4-dione (**3a**)* and *2,3-bis(p-tolylthio)naphthalene-1,4-dione (**4a**)* Compounds **3a** and **4a** were synthesized by the reaction of 0.50 g (2.2 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.27 g (2.2 mmol) *p*-tolylthiol.

3a: Orange powder, yield 0.21 g (30%), mp 140-141 ^{o}C. IR (ATR) (ν/cm^{-1}): 3030, 2956, 2921, 2850, 1660, 1587. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.08-8.06 (dd, *J* = 7.32, 1.46 Hz, 1H, $CH_{aromatic}$), 7.91-7.89 (dd, *J* = 7.81, 1.46 Hz, 1H, $CH_{aromatic}$), 7.67-7.60 (m, 2H, $CH_{aromatic}$), 7.31-7.29 (d, *J* = 8.3 Hz, 2H, $CH_{aromatic}$), 7.07-7.06 (d, *J* = 8.3 Hz, 2H, $CH_{aromatic}$), 2.28 (s, 3H, CH_3). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.9, 174.8, 147.0, 141.1, 137.9, 133.1, 133.0, 131.7, 131.4, 130.3, 129.0 127.0, 126.5, 126.3,. 20.3. MS: m/z 315.0 (M^+), $C_{17}H_{11}ClO_2S$ (M, 314.8).

4a: Red powder, yield 0.18 g (20%), mp 171-172 ^{o}C (Lit. mp 172-173 ^{o}C) [21]. IR (ATR) (ν/cm^{-1}): 3030, 2954, 2923, 2866, 1661, 1590.

*2-Chloro-3-((4-fluorophenyl)thio)naphthalene-1,4-dione (**3b**)* and *2,3-bis(4-fluorophenylthio)naphthalene-1,4-dione*

(4b) Compounds **3b** and **4b** were synthesized by the reaction of 0.50 g (2.2 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.28 g (2.2 mmol) 4-fluorobenzenethiol.

3b: Reddish-brown oil, yield 0.15 g (21%). IR (ATR) (ν/cm^{-1}): 3093, 3069, 1668, 1655, 1586. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.10-8.08 (d, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.92-7.91 (d, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.69-7.63 (m, 2H, $CH_{aromatic}$), 7.46-7.43 (m, 2H, $CH_{aromatic}$), 6.99-6.96 (t, *J* = 8.3 Hz, 2H, $CH_{aromatic}$). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.8, 174.8, 163.1, 161.1, 146.5, 141.4, 134.1, 134.0, 133.2, 133.1, 131.3, 130.2, 126.5, 126.4, 115.5, 115.4. MS: m/z 315.0 (M-3H), $C_{16}H_8ClFO_2S$ (M, 318.8).

4b: Orange powder, yield 0.37 g (41%), mp 176-177 ^{o}C (Lit. 174-175 ^{o}C) [21]. IR (ATR) (ν/cm^{-1}): 3053, 1664, 1586.

2-Chloro-3-((4-chlorophenyl)thio)naphthalene-1,4-dione (3c) and *2,3-bis(4-chlorophenylthio)naphthalene-1,4-dione (4c)* Compounds **3c** and **4c** were synthesized by the reaction of 0.50 g (2.2 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.16 g (1.1 mmol) 4-chlorobenzenethiol.

3c: Orange oil, yield 0.16 g (43%) [45]. IR (ATR) (ν/cm^{-1}): 3078, 1671, 1589. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.10-8.08 (dd, *J* = 7.32, 1.46 Hz, 1H, $CH_{aromatic.}$), 7.93-7.91 (dd, *J* = 7.81, 1.47 Hz, 1H, $CH_{aromatic}$), 7.70-7.63 (m, 2H, $CH_{aromatic}$), 7.36-7.34 (d, *J* = 8.79 Hz, 2H, $CH_{aromatic}$), 7.25-7.23 (d, *J* = 8.79 Hz, 2H, $CH_{aromatic}$). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.7, 174.7, 146.1, 142.1, 133.3, 133.2, 132.7, 131.3, 130.2, 129.1, 128.5, 126.6, 126.4. MS: m/z 333.3 (M-2H), $C_{16}H_8Cl_2O_2S$ (M, 335.2).

4c: Red powder, yield 0.15 g (31%), mp 151-152 ^{o}C (Lit. 151-152 ^{o}C) [21]. IR (ATR) (ν/cm^{-1}): 3078, 3051, 1668, 1660, 1589. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 7.91-7.90 (dd, *J* = 5.86, 3.42 Hz, 2H, $CH_{aromatic}$), 7.62-7.60 (dd, *J* = 5.86, 3.42 Hz, 2H, $CH_{aromatic}$), 7.25-7.24 (d, *J* = 8.30 Hz, 4H, $CH_{aromatic}$), 7.21-7.19 (d, *J* = 8.79 Hz, 4H, $CH_{aromatic}$). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.5, 146.9, 133.3, 133.0, 131.7, 131.6; 130.8, 128.4, 126.3.

2-((4-Bromophenyl)thio)-3-chloronaphthalene-1,4-dione (3d) and *2,3-bis(4-bromophenylthio)naphthalene-1,4-dione (4d)* Compounds **3d** and **4d** were synthesized by the reaction of 0.50 g (2.2 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.42 g (2.2 mmol) 4-bromobenzenethiol.

3d: Red powder, 0.25 g (30%), mp 157-159 ^{o}C [46]. IR (ATR) (ν/cm^{-1}): 3099, 1667, 1652, 1587. ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.5, 175.0, 146.9, 142.6, 133.6, 133.0, 131.8, 131.6, 131.4, 131.3, 126.3, 121.3.

4d: Reddish-brown powder, 0.43 g (37%), mp 173-175 ^{o}C [21]. IR (ATR) (ν/cm^{-1}): 3072, 1666, 1652, 1588. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 7.92-7.91 (dd, *J* = 5.86, 3.42 Hz, 2H, $CH_{aromatic}$), 7.63-7.61 (dd, *J* = 5.86, 3.42 Hz, 2H, $CH_{aromatic}$), 7.37-7.35 (d, *J* = 8.30 Hz, 4H, $CH_{aromatic}$), 7.18-7.17 (d, *J* = 8.30 Hz, 4H, $CH_{aromatic}$). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.5, 146.9, 133.0, 131.8, 131.6, 131.4, 131.3, 126.3, 121.3.

2-(Benzylthio)-3-chloronaphthalene-1,4-dione (3e) Compound **3e** was synthesized by the reaction of 1.0 g (4.4 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.55 g (4.4 mmol) benzylthiol.

3e: Orange powder, yield 0.98 g (71%), mp 140-141 ^{o}C [21, 46]. IR (ATR) (ν/cm^{-1}): 3099, 3063, 3031, 2942, 1660, 1589. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.04-7.99 (m, 2H, $CH_{aromatic}$), 7.65-7.63 (m, 2H, $CH_{aromatic}$), 7.28-7.27 (d, *J* = 7.32 Hz, 2H, $CH_{aromatic}$), 7.23-7.20 (t, *J* = 7.32 Hz, 2H, $CH_{aromatic}$), 7.18-7.16 (m, 1H, $CH_{aromatic}$), 4.56 (s, 2H, S-CH_2-). ^{13}CNMR (125 MHz, $CDCl_3$): δ 178.9, 174.1, 147.6, 139.3, 135.6, 133.1, 132.8, 131.6, 130.2, 128.2, 127.8, 126.8, 126.3, 126.2, 37.6. MS: m/z 314.9 (M^+), $C_{17}H_{11}ClO_2S$ (M, 314.8).

2-Chloro-3-((2-(hydroxymethyl)phenyl)thio)naphthalene-1,4-dione (3f) and *2,3-bis((2-(hydroxymethyl)phenyl)sulfanyl)naphthalene-1,4-dione (4f)* Compounds **3f** and **4f** were synthesized by the reaction of 1 g (4.4 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.62 g (4.4 mmol) (2-mercaptophenyl)methanol.

3f: Red powder, yield 0.29 g (20%), mp 192-193 ^{o}C (Lit. 190-191 ^{o}C) [47]. IR (ATR) (ν/cm^{-1}): 3072, 3040, 2957, 2919, 2848, 1661, 1649, 1589. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.02-7.98 (m, 2H, $CH_{aromatic}$), 7.62-7.60 (m, 2H, $CH_{aromatic}$), 7.47-7.45 (d, *J* = 7.8 Hz, 1H, $CH_{aromatic}$), 7.32-7.26 (m, 3H, $CH_{aromatic}$), 5.57 (s, 2H, O-CH_2-). ^{13}CNMR (125 MHz, $CDCl_3$): δ 181.7, 176.7, 156.1, 136.8, 133.9, 132.9, 132.8, 130.4, 130.0, 129.5, 129.0, 128.8, 127.7, 125.8, 125.6, 123.0, 71.9.

4f: Orange powder, yield 0.71 g (37%), mp 172-174 ^{o}C [48]. IR (ATR) (ν/cm^{-1}): 3316, 3054, 2916, 2857, 1667, 1590. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 7.83-7.81 (dd, *J* = 5.85,3.42 Hz, 2H, $CH_{aromatic}$), 7.57-7.55 (dd, *J* = 5.85, 3.42 Hz, 2H, $CH_{aromatic}$), 7.45-7.44 (d, *J* = 7.32 Hz, 2H, $CH_{aromatic}$), 7.36-7.34 (d, *J* = 7.81 Hz, 2H, $CH_{aromatic}$), 7.30-7.28 (t, *J* = 7.32, Hz, 2H, $CH_{aromatic}$), 7.17-7.16 (t, 2H, $CH_{aromatic}$), 4.87 (s, 4H, O-CH_2-). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.7, 147.9, 141.1, 132.9, 132.5, 131.8, 131.5, 128.8, 128.3, 127.8, 126.3, 63.2. MS: m/z 457.2 $(M+Na)^+$, $C_{24}H_{18}O_4S_2$ (M, 434.53).

*2-Chloro-3-(phenethylthio)naphthalene-1,4-dione (**3g**)* and *2,3-bis(phenethylthio)naphthalene-1,4-dione (**4g**)* Compounds **3g** and **4g** were synthesized by the reaction of 1.0 g (4.4 mmol) 2,3-dichloro-1,4-naphthoquinone (**1**) with 0.61 g (4.4 mmol) 2-phenylethanethiol.

3g: Orange powder, yield 0.45 g (31%), mp 94-95 ^{o}C. IR (ATR) (ν/cm^{-1}): 3066, 3031, 2919, 2848, 1668, 1656, 1588. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.01-7.99 (m, 1H, $CH_{aromatic}$), 7.95-7.93 (m, 1H, $CH_{aromatic}$), 7.64-7.62 (m, 2H, $CH_{aromatic}$), 7.11-7.05 (m, 4H, $CH_{aromatic}$), 6.97-6.94 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 3.59-3.56 (t, *J* = 7.32 Hz, 2H, Ph-CH_2-), 2.91-2.90 (t, *J* = 7.32 Hz, 2H, SCH_2). ^{13}CNMR (125 MHz, $CDCl_3$): δ 178.7, 174.0, 147.8, 139.3, 138.1, 133.0, 132.7, 131.6, 130.2, 127.9, 127.5, 126.2, 126.0, 125.7, 36.3, 34.3. MS: m/z 328.9 (M^+), $C_{18}H_{13}ClO_2S$ (M, 328.8).

4g: Red oil, yield 0.90 g (47%). IR (ATR) (ν/cm^{-1}): 3061, 3026, 2921, 2850, 1652, 1589. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 7.92-7.90 (dd, *J* = 5.86, 3.42 Hz, 2H, $CH_{aromatic}$), 7.60-7.59 (dd, *J* = 5.86, 3.42 Hz, 2H, $CH_{aromatic}$), 7.12-7.11 (m, 10H, $CH_{aromatic}$), 3.48-3.45 (t, *J* = 7.81 Hz, 4H, CH_2), 2.91-2.88 (t, *J* = 7.81 Hz, 4H, SCH_2). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.9, 146.3, 138.6, 132.3, 127.8, 127.4, 125.7, 125.5, 36.2, 34.8, 28.7.

*General procedure for preparing 2-arythio-(3-amino)-1,4-naphthoquinone (**5a-c** and **5f**) and 2-phenylnaphtho[2,3-d]thiazole-4,9-dione (**6h**)*

2-Arylthio-(3-amino)-1,4-naphthoquinones (**5a**, **5c**) and 2-phenylnaphtho[2,3-*d*]thiazole-4,9-dione (**6h**) were prepared from monosubstituted 1,4-naphthoquinones as previously described [22]. **5b**, **5c**, and **5f** were also synthesized from disubstituted 1,4-naphthoquinones (**4b**, **4c**, and **4f**) an adaption of the procedure given for the monosubstituted 1,4-naphthoquinones [22]. Mono and disubstituted 1,4-naphthoquinones were dissolved mixture of DMF-water (68.75 mL) and then into the resulting solution, NaN_3 was added in small portions. The mixture was refluxed at 100 ^{o}C temperature until completion of the reaction (TLC), and then it was poured ice. The solvent was evaporated and the residue was purified by column chromatography on silica gel.

*2-Amino-3-(p-tolylthio)naphthalene-1,4-dione (**5a**)* Compound **5a** was synthesized by the reaction of 0.10 g (0.32 mmol) 2-(*p*-tolylthio)-3-chloronaphthalene-1,4-dione (**3a**) with 0.42 g (6.4 mmol) sodium azide.

5a: Red powder, yield 0.03 g (32%), mp 175-176 ^{o}C (Lit. 177-178 ^{o}C) [20]. IR (ATR) (ν/cm^{-1}): 3436, 3318, 3115, 3075, 3030, 2961, 2918, 2849, 1679, 1585. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.11-8.10 (d, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 8.02-8.00 (d, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 7.68-7.65 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.59-7.56 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.14-7.12 (d, J: 7.81 Hz, 2H, $CH_{aromatic}$), 6.98-6.97 (d, *J* = 7.81 Hz, 2H, $CH_{aromatic}$), 6.20-5.60 (br s, 2H, NH), 2.20 (s, 3H, CH_3). ^{13}CNMR (125 MHz, $CDCl_3$): δ 179.1, 178.6, 150.1, 135.4, 134.0, 132.7, 131.4, 129.7, 129.4, 129.0, 127.5, 126.2, 125.4, 20.0. MS: m/z 296.1 $(M+H^+)$, $C_{17}H_{13}NO_2S$ (M, 295.4).

*2-Amino-3-((4-fluorophenyl)thio)naphthalene-1,4-dione (**5b**)* Compound **5b** was synthesized by the reaction of 0.05 g (0.13 mmol) 2,3-bis(4-fluorophenylthio) naphthalene-1,4-dione (**4b**) with 0.17 g (2.6 mmol) sodium azide.

5b: Orange oil, 0.008 g (21%). IR (ATR) (ν/cm^{-1}): 3437, 3322, 1678, 1587, 1557. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.13-8.08 (d, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 8.05-8.00 (d, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 7.71-7.66 (t, *J* = 8.30 Hz, 2H, $CH_{aromatic}$), 7.61-7.56 (t, J: 7.32 Hz, 1H, $CH_{aromatic}$), 7.26-7.20 (m, 2H, $CH_{aromatic}$), 6.86-6.89 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 6.30-5.60 (br s, 2H, NH). ^{13}CNMR (125 MHz, $CDCl_3$): δ 179.1, 178.6, 150.1, 135.4, 134.0, 132.7, 131.4, 129.7, 129.4, 129.0, 127.5, 126.2, 125.4. MS: m/z 300.1 $(M+H^+)$, $C_{16}H_{10}FNO_2S$ (M, 299.3).

*2-Amino-3-((4-chlorophenyl)thio)naphthalene-1,4-dione (**5c**)* Compound **5c** was synthesized by the reaction of 0.15 g (0.45 mmol) 2-chloro-3-((4-chlorophenyl)thio) naphthalene-1,4-dione (**3c**) with 0.58 g (9mmol) sodium azide. It is also prepared from by the reaction of 0.05 g (0.11 mmol) 2,3-bis(4-

chlorophenylthio)naphthalene-1,4-dione (**4c**) with 0.15 g (2.2 mmol) sodium azide.

5c: Red powder, yield 0.03 g (21%) [20]. IR (ATR) (ν/cm^{-1}): 3437, 3318, 1682, 1587, 1561. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.14-8.08 (d, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 8.07-8.01 (d, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.72-7.66 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.63-7.57 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.14 (s, 4H, $CH_{aromatic}$), 6.30-5.60 (br s, 2H, NH). ^{13}CNMR (125 MHz, $CDCl_3$): δ 178.8, 178.4, 150.6, 134.2, 132.6, 132.1, 131.6, 129.3, 129.0, 128.3, 128.2, 126.3, 125.6. MS: m/z 316.1 (M^+), $C_{16}H_{10}ClNO_2S$ (M, 315.8).

2-Amino-3-((2-(hydroxymethyl)phenyl)thio) naphthalene-1,4-dione (5f) Compound **5f** was synthesized by the reaction of 0.05 g (0.12 mmol) 2,3-bis((2-(hydroxymethyl) phenyl)thio)naphthalene-1,4-dione (**4f**) with 0.16 g (2.4 mmol) sodium azide.

5f: Orange oil, yield 0.005 g (13%). IR (ATR) (ν/cm^{-1}): 3419, 3283, 2942, 1682, 1598, 1561, 1542. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.06-8.03 (d, *J* = 7.81 Hz, 2H, $CH_{aromatic}$), 8.03-8.01 (d, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 7.67-7.63 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.60-7.56 (t, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 7.32-7.30 (d, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.16-7.13 (t, *J* = 7.32 Hz, 1H, $CH_{aromatic}$), 7.09-7.05 (t, *J* = 7.81 Hz, 1H, $CH_{aromatic}$), 6.22 (br s, 2H, NH), 4.89 (s, 2H, CH_2). MS: m/z 311.9 ($M+H^+$), $C_{17}H_{13}NO_3S$ (M, 311.4).

2-Phenylnaphtho[2,3-d]thiazole-4,9-dione (6h) Compound **6h** was synthesized by the reaction of 0.5 g (1.59 mmol) 2-(benzylthio)-3-chloronaphthalene-1,4-dione (**3e**) with 2.07 g (31.8 mmol) sodium azide.

6h: Yellow oil, yield 0.03 g (32%) [49]. IR (ATR) (ν/cm^{-1}): 3046, 3025, 2961, 2918, 2849, 1674, 1658, 1593. ^{1}H NMR (499.74 MHz, $CDCl_3$): δ 8.30-8.25 (d, *J* = 6.34 Hz, 1H, $CH_{aromatic}$), 8.18-8.14 (d, *J* = 6.35 Hz, 1H, $CH_{aromatic}$), 8.10-8.05 (d, *J* = 6.83 Hz, 2H, $CH_{aromatic}$), 7.77-7.68 (m, 2H, $CH_{aromatic}$), 7.51-7.42 (m, 3H, $CH_{aromatic}$). ^{13}CNMR (125 MHz, $CDCl_3$): δ 177.3, 176.8, 174.1, 154.2, 133.4, 133.0, 132.1, 131.4, 131.1, 128.3, 127.8, 126.8, 125.9, 20.0. MS: m/z 292.3 ($M+H^+$), $C_{17}H_9NO_2S$ (M, 291.3).

Biological Assays

Antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231 were determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations [50, 51]. Mueller–Hinton broth for bacteria and RPMI-1640 medium for yeast strain were used as the test medium. Serial two-fold dilutions ranging from 5000 mg/L to 4.8 mg/L were prepared in medium. The inoculum was prepared by using a 4–6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 Mc Farland 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 in 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 a control, antimicrobial effects of the solvents were investigated against test microorganisms. According to the values of the controls, the results were evaluated. The MIC values of the compounds are given in Table-1.

Docking Studies

The molecules were built using Chem Sketch 12.0.1 and converted to 3D structure from the 2D structures, and further 3D structures were energetically minimized and saved as MDL Mol File (*.mol2). Docking can be performed by Molegro Virtual Docker 2010.4.1.0 program [44]. Cytochrome 14 a-sterol demethylase (*Candida* P450DM PDB ID: 1EA1) and DNA gyrase subunit A *Eschericnia coli* (PDB ID: 1AB4) were taken as target enzyme for rationalization of antifungal and antibacterial activity, respectively [42, 52]. Polar hydrogen atoms, partial atomic charges and solvation parameters were added, MolDock optimization search algorithm with a maximum of 100 runs was used through the calculations, with all other parameters kept as defaults. All the poses were examined manually, and the best pose was retained.

Results and Discussion

Chemistry

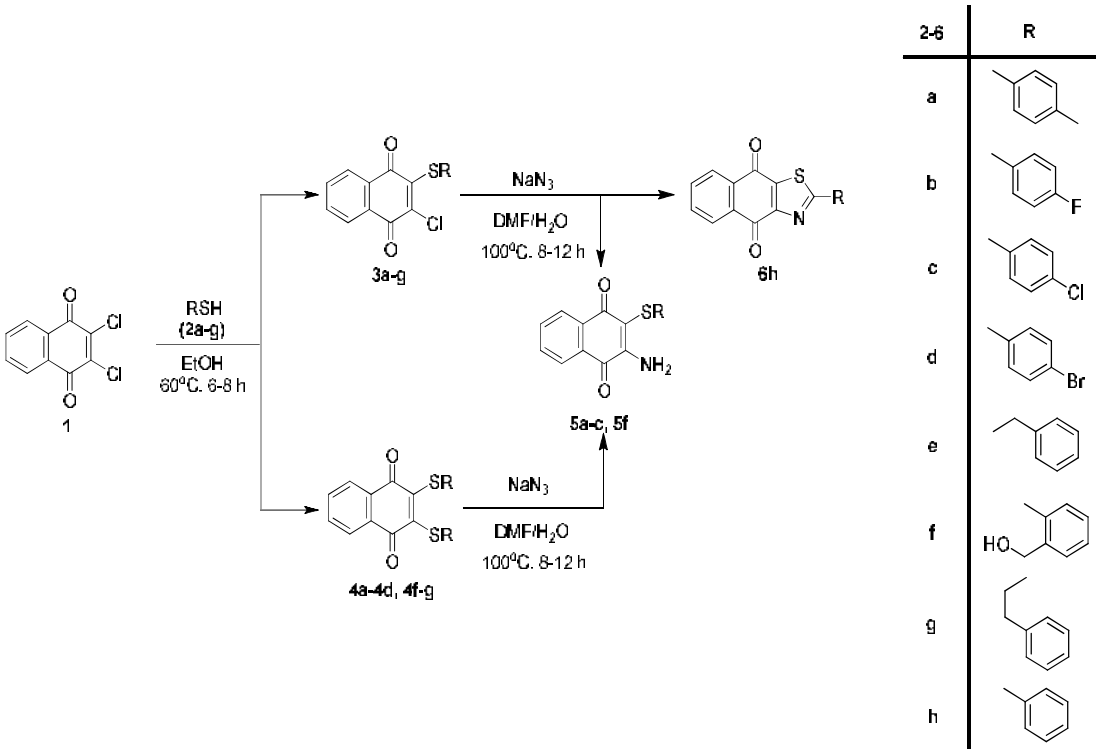
As a perusal of literature, the ring closure reactions of 2-arylamino-3-chloro-l,4-naphthoquinones were carried out by Vanallan *et al*. in 1963 and it is reported 2-arylamino-3-amino-l,4-napthoquinones as the secondary product beside

benzophenazine-quinones. Some of their reactions by the treatment of 2-arylamino-3-chloro-l,4-naphthoquinones with sodium azide (NaN_3) in dimethylformamide (DMF) at elevated temperature gave only 2-(arylamino)-3-amino-1,4-naphthoquinones and none of the heterocyclic quinones [34]. On the other side, Tandon *et al.* described the synthesis of 2-chloro-3-arylsulfanyl-1,4-naphthoquinones and 2,3-bis-arylsulfanyl-1,4-naphthoquinones obtained from the reactions of 2,3-dichloro-1,4-naphthoquinone with aryl thiols. However, the nucleophilic displacement reaction of 2-chloro-3-arylsulfanyl-1,4-naphthoquinones with NaN_3 in DMF-H_2O afforded benzophenothiazine-quinones as the only isolated products [22].

To achieve the target compounds, we first decided to perform reaction of 2,3-dichloro-1,4-naphthoquinone (**1**) with some various thiols (**2a-g**) to give 2-(aryl/alkyl)thio-3-chloro-l,4-naphthoquinones (**3a-g**) and 2,3-(aryl/alkyl)thio-l,4-naphthoquinones (**4a-d**, **4f-g**) according to the standard procedures [25b]. Owing to the fact that the reaction of 2-arylthio-3-chloro-1,4-naphthoquinones with NaN_3 was sensitive to the structures of arylthio or substituents in the arylthio moiety, it is possible to observe 2-arylthio-3-amino-1,4-naphthoquinones and/or benzothiophenazine as products in these reactions. We also chosed some different compounds to further investigate about the reaction products. Then, we firstly reacted 2-(aryl/alkyl)thio-3-chloro-l,4-naphthoquinones (**3a**, **3c**, and **3e**) with NaN_3 in DMF at a high temperature according to the reported literature [34]. We obtained 2-(arylthio)-3-amino-1,4-naphthoquinones (**5a**, **5c**) as the sole identifiable products except one heterocyclic quinone (**6h**) in these reactions. We also afforded the reactions of these compounds (**4b**, **4c**, and **4f**) with sodium azide under the same conditions and observed that the formation of 2-(arylthio)-3-amino-1,4-naphthoquinones (**5b**, **5c**, and **5f**).

Biological Activity Spectra Prediction

In this study, biological activity spectra prediction analysis could be performed by using the PASS and PharmaExpert: http://www.pharmaexpert.ru/PASSOnline/ of the synthesized compounds provides a good example of *in silico* study of chemical compounds before their *in vivo* or *in vitro* experimental [35-43].



Scheme-1: The synthesis of naphthoquinone derivatives containing heteroatoms.

The biological activity spectrum is represented by a list of predictable biological activity for which the probability to be revealed (Pa) and the probability not to be revealed (Pi) are calculated. Independent Pa and Pi values range from between 0 to 1. In process of planning experiments not only the results of the prediction are important, but also some other factors such as special attention to different kinds of activity, favorable originality, available facilities for experimentals, etc should be noted. There is a negative correlation between Pa value and probability of false positiveness. By default, in PASS, Pa = Pi value is chosen as a threshold; if Pa > Pi, the compounds are considered to be active. The statistical and "activity–activity" relationship analysis of PASS was performed by means of PharmaExpert. PASS prediction results of the compound (**3c**) are summarized as follows:

Pa	Pi	Activity
0.860	0.005	Glycosylphosphatidylinositol phospholipase D inhibitor
0.724	0.012	IgA-specific serine endopeptidase inhibitor
0.683	0.031	Glutamyl endopeptidase II inhibitor
0.535	0.025	Anti-fungal
0.316	0.090	Anti-infective
0.273	0.092	Para amino benzoic acid antagonist
0.222	0.063	Antiseptic
0.169	0.018	Lanosterol 14 alpha demethylase inhibitor
0.180	0.068	Deoxyribonuclease I inhibitor
0.111	0.051	Cyclooxygenase 1 inhibitor
0.136	0.083	Anti-bacterial, ophthalmic
0.106	0.060	Antibiotic Anthracycline-like
0.102	0.064	Cytochrome-c3 hydrogenase inhibitor
0.053	0.035	Anti-fungal (Pneumocystis)

It has been found that the compounds having **Pa** nearly equal to 0.5 for the antifungal activity experimentally also showed good results which means they are new chemical entity. These results were quite encouraging in further experimental studies.

Antimicrobial Activity

The synthesized compounds 2-(aryl/alkyl)thio-3-chloro-1,4-naphthoquinones (**3a-e**, **3g**), 2,3-bis(aryl/alkyl)thio-1,4-naphthoquinones (**4a-d**, **4f-g**), 2-(arylthio)-3-amino-1,4-naphthoquinone (**5a-c**, **5f**) and 2-phenylnaphtho[2,3-*d*]thiazole-4,9-dione (**6h**) were evaluated for their *in vitro* antifungal activity against a yeast *Candida albicans* ATCC 10231. The antibacterial activity was tested against four Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153) and three Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212). All of the synthesized and screened antimicrobial assay results are given in Table-1.

In general, all tested compounds had an activity against *E. faecalis* with MIC values of between 625–1250 µg/mL. Among the tested compounds, **3c** was found to have the best level of antibacterial activity against *S. aureus* (MIC = 39.06 µg/mL). In addition to *E. faecalis*, all compounds apart from the compound **5a**, possessed activity against *P. aeruginosa*. The Gram-negative bacteria *E. coli* were resistant against to synthesized compounds except for only four compounds. Taking into consideration the antifungal activity of the synthesized compounds showed that **3c** and **3e** were the most potent with MIC 78.12 µg/mL for *C. albicans* (Table 1). Results also reveal that compound **3c** was the most potent compound with the antibacterial and the antifungal activities. From another point of view, it might be mentioned that tested compounds were mostly active against Gram positive microorganisms like *E. faecalis*, *S. aureus*.

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

Microorganisms	MIC Values (µg/mL)							
	Gram-Positive Bacteria			Gram-Negative Bacteria				Fungi
	S. aureus	*S. epidermidis*	*E. faecalis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*C. albicans*
3a	1250	1250	1250	1250	1250	-	-	625
3b	156.2	312.5	1250	1250	-	-	-	312.5
3c	39.06	78.12	1250	1250	-	1250	1250	78.12
3d	625	625	1250	1250	-	1250	1250	156.2
3e	625	1250	625	1250	1250	-	-	78.12
3g	1250	1250	1250	1250	1250	-	-	156.2
4a	1250	-	1250	1250	-	-	-	-
4b	1250	1250	1250	1250	-	-	-	-
4c	1250	1250	1250	1250	1250	-	-	312.5
4d	1250	1250	625	1250	-	-	-	625
4f	156.2	156.2	625	1250	-	1250	1250	312.5
4g	312.5	312.5	1250	1250	-	1250	1250	-
5a	1250	1250	1250	-	-	-	-	-
5b	1250	1250	1250	625	-	1250	-	-
5c	-	-	1250	1250	-	1250	-	-
5f	625	1250	625	1250	-	1250	-	-
6h	156.2	1250	1250	1250	-	1250	-	-

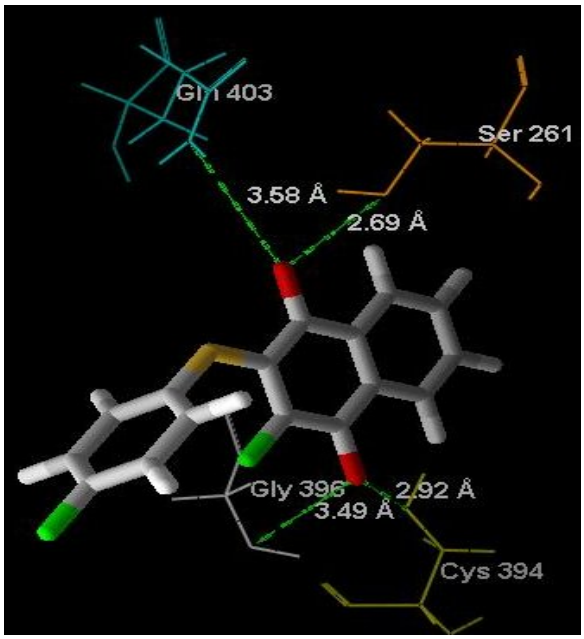


Fig. 1: Binding mode of compound **3c** in 14 a-sterol demethylase cavity [PDB ID: 1EA1].

Docking Studies

Significant antibacterial and antifungal activity of compound **3c** led us to apply molecular docking study which further helps understanding the protein-ligand interactions. The docking was performed with Molegro Virtual Docker 2010.4.1.0 program into the active site of *Candida* P450DM (PDB ID: 1EA1) for antifungal and DNA gyrase subunit A *Escherichia coli* (PDB ID: 1AB4) for antibacterial activity in order to predict the binding mode and support the biological results [42, 44]. In case of antifungal activity, compound **3c** reveals the MolDock score of -69.19 and form four interactions shown as green dotted lines (Figure 1) showing two hydrogen bonds O of C=O (naphthoquinone) moiety at position 1 with Gln 403 and Ser 261 of distances 3.59 Å and 2.69 Å, respectively and two hydrogen bonds between O of C=O (naphthoquinone) moiety at position 4 with Gly 396 and Cys 394 of distances 3.49 Å and 2.92 Å, respectively.

However, in case of antibacterial activity, compound **3c** reveals the MolDock score of -41.04 and forms three interactions as indicated in Figure 2 showing two hydrogen bonds O of C=O (naphthoquinone) moiety at position 1 with Asn 269 and Gly 114 of distances 2.58 Å and 2.87 Å respectively and one hydrogen bond between O of C=O (naphthoquinone) moiety at position 4 with Ser 97 of distance 2.97 Å.

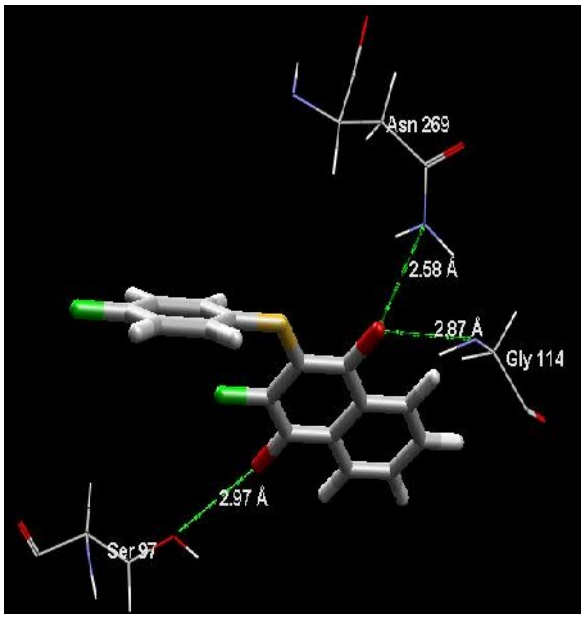


Fig. 2: Binding mode of compound **3c** in DNA gyrase subunit A *Escherichia coli* [PDB ID: 1AB4].

From the docking studies, it has been confirmed that O atom of C=O at position 1 and 4 of naphthoquinone is essential antimicrobial activity and the interactions with amino acid residues.

Conclusion

The combination of naphthoquinone core with heteroatoms or aromatic structures leads to important biological profile. For that reason, we aimed to discover antifungal and antibacterial compounds that are known and unknown cyclic 1,4-naphthoquinone derivatives containing heteroatoms. The structures of the synthesized compounds have been confirmed by means of different spectroscopic methods. All the compounds were subjected to PASS analysis and PharmaExpert software for prediction of the therapeutic potential besides docking study through Molegro virtual Docker. On the basis of the obtained data for synthesized compounds, the *in vitro* antimicrobial activities were evaluated against clinically important Gram-positive and Gram-negative bacterial strains, as well as a fungal strain. It has been found that the compounds **3c** and **3e** exhibited as potent antibacterial effect. The results also reveal that compound **3c** was the most potent; it has antibacterial and antifungal activities. On the other hand, it was seemed that synthesized compounds were mostly active against Gram positive microorganisms.

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