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Synthesis, Characterization, and Investigation of Antimicrobial Activities of New Naphthoquinone Compounds from 2-(butylthio)-3-chloronaphthalene-1,4-dione

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Research Article

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Abstract

In this study, the compound 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) was synthesized from the reaction of the compound 2,3-dichloro-1,4-naphthoquinone (**1**) with 1-butanethiol in alkaline medium. The synthesized compound 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) was used as the starting compound. As a result of the nucleophilic substitution of 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) with heterocyclic compounds containing nitrogen, sulfur, and oxygen atoms, a series of **10**, **11**, **12**, **13**, **14**, **15**, naphthoquinone derivative compounds were synthesized. The structures of the synthesized compounds were characterized by FTIR, ^{13}C-NMR, ^{1}H-NMR, and Mass Spectroscopy techniques. The antimicrobial properties of the synthesized compounds were examined by performing antimicrobial studies with Gram-positive and Gram-negative bacteria. The compound 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) shows the highest antimicrobial activity, whereas the compound 2-(butylthio)-3-((4,5-dihydrothiazol-2-yl)thio) naphhalene-1,4-dione (**10**) exhibits the lowest antimicrobial activity. Compounds **3**, **11**, **12**, **13**, **14**, and **15** exhibited enhanced activity against Gram-positive bacteria, such as *B. subtilis* and *S. aureus*, as well as Gram-negative *E. coli* and *K. pneumoniae*. In addition, compounds **11**, **12**, and **14** exhibited activity against Gram-positive *B. subtilis* and *S. aureus*, as well as Gram-negative *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The study aims to enhance existing literature research and create new unknown compounds through synthesis.

Keywords: Substituted quinones, nucleophilic substitution, antimicrobial activity

2-(Butiltiyo)-3-Kloronaftalen-1,4-Dion Bileşiğinden Yeni Naftakinon Bileşiklerin Sentezi, Karakterizasyonu ve Antimikrobiyal Aktivitelerinin İncelenmesi

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Öz

Bu çalışmada, 2,3-dikloro-1,4-naftakinon (**1**) bileşiğinin alkali ortamda 1-butantiyol ile reaksiyoundan 2-(butiltiyo)-3-kloronaftalen-1,4-dion (**3**) bileşiği sentezlendi. Sentezlenen 2-(butiltiyo)-3-kloronaftalen-1,4-dion (**3**) bileşiği başlangıç bileşiği olarak kullanıldı. 2-(butiltiyo)-3-kloronaftalen-1,4-dion (**3**) bileşiğin azot, kükürt ve oksijen atomu içeren heterosiklik bileşikler ile nükleofilik sübstitüsyonu sonucu bir seri **10**, **11**, **12**, **13**, **14**, **15**, naftakinon türevi bileşikler sentezlendi. Sentezlenen bileşiklerin yapıları FTIR, ^{13}C NMR, ^{1}H NMR ve Mass Spektroskopisi ile

karakterize edildi. Sentezlenen bileşiklerin Gram pozitif ve Gram negative bakteriler ile antimikrobiyal çalışması yapılarak antimikrobiyal özellikleri incelendi. 2-(butiltiyo)-3-kloronaftalen-1,4-dion (**3**) bileşiğinin antimikrobiyal aktivitesi en yüksek, 2-(butylthio)-3-((2,5-dihydrothiazol-5-yl) thio) naphthalene-1,4-dione (**10**) bileşiğinin antimikrobiyal aktivitesi en düşük olarak gözlenmiştir**. 3**, **11**, **12**, **13**, **14** ve **15** bileşikleri, Gram-pozitif *B. subtilis* ve *S. aureus* ile Gram-negatif *E. coli*, *K. pneumoniae* 'ye karşı daha iyi aktivite göstermiştir. Ayrıca, **11**, **12** ve **14** bileşikleri Gram-pozitif *B. subtilis* ve *S. aureus* ile Gram-negatif *E. coli*, *K. pneumoniae* ve *P. aeruginosa*'ya karşı aktivite gösterdi. Bu çalışma ile literatürde olan çalışmaların geliştirilmesi ve bilinmeyen yeni bileşiklerin sentezlenmesi amaçlanmıştır.

Anahtar Kelimeler: Sübstitüe kinonlar, nükleofilik sübstitüsyon, antimikrobiyal aktivite

Introduction

Quinones are compounds with a cyclohexadiene dione structure. They are α, β-unsaturated ketones derived from the aromatic compounds dioxo derivatives. The first quinone compound was produced in 1838 by oxidizing Quinic acid. As a result, these compounds were named quinol [1, 2]. Compared to aromatics, quinones have two conjugated double bonds. They are electrophilic acceptors that are stabilized by conjugation. The reduction product of quinone can either aromatize or disrupt conjugation, depending on the structure of quinone and the position of the reduction [3]. It is better a series of oxidation and addition reactions form quinones. They can be synthesized through the oxidation of o-dihydroxy benzene to o-quinones in ether [2, 4], as well as through the oxidation of anilines and arenes [5]. Additionally, they can be produced through the Diels-Alder reaction of dienophiles and 1,3-dienes. Quinone compounds with four pi electrons in the ring exhibit similar properties to alpha and beta-unsaturated quinones [1, 6]. For several years, researchers have been studying compounds called 2-aryl(alkyl) amino-1,4-naphthoquinones. These compounds are formed when aryl amine and its derivatives react with 1,4-naphthoquinones [7]. 1,4-naphthoquinones can have a nitrogen atom [7, 8], sulfur [9], or an oxygen atom [10] at the 2-position or 2,3-position. The biological activity of these compounds has been analyzed by researchers. Antiproliferative activities of 2-chloro-3-arylsulfanyl-1,4-naphthoquinone, 2,3-bis-arylsulfanyl-1,4-naphthoquinone, and 12H-benzo[b] phenothiazine-6,11-diones and their analogs against cervical cancer cells were studied [9]. Reactions of 2,3-dichloro-1,4-naphthoquinone with heterocyclic compounds that contain nitrogen, sulfur, or oxygen are documented in literature. The number of studies on naphthoquinone-derived compounds has increased due to their biological activity. Novel compounds were synthesized using various methods to evaluate biological activity [11, 12]. Naphthoquinones are prevalent in the structure of vitamins, influencing their activities. Vitamin K1, which contains the 2-methyl-1,4-naphthoquinone structure and is found in plants, plays a vital role in maintaining the clotting properties of blood in humans. Vitamin K1 has various forms, such as K2 and K3, and the structure of these vitamins also contains quinones. The most well-known naphthoquinone is menadione. (also known as vitamin K_3 or 2-methyl-1,4-naphthoquinone). It is a redox cycler and acts as a "destructive substrate" for numerous flavoproteins [13, 14]. These compounds are

known to exhibit antiviral, antifungal, antibacterial, antiproliferative, and antitumor properties. It is known that many drugs have a 1,4-naphthoquinone ring structure [15-20]. There are pigments such as Thelephoric acid (permanganate color) and xylene in some phenanthrenequinones found in nature as animal and vegetable. Telephoric acid is found in fungi and lichens, and xylene is found as a green coating on overhanging branches of oaks and other hardwoods [16]. Quinones are essential compounds that occur naturally in plant and animal structures. Some of the quinones are used as bacteriostatic (phytocol), some against malaria (hydrolapacol), and some as stimulants (plumbagin). Structural formulas of quinone compounds are available in Figure 1.

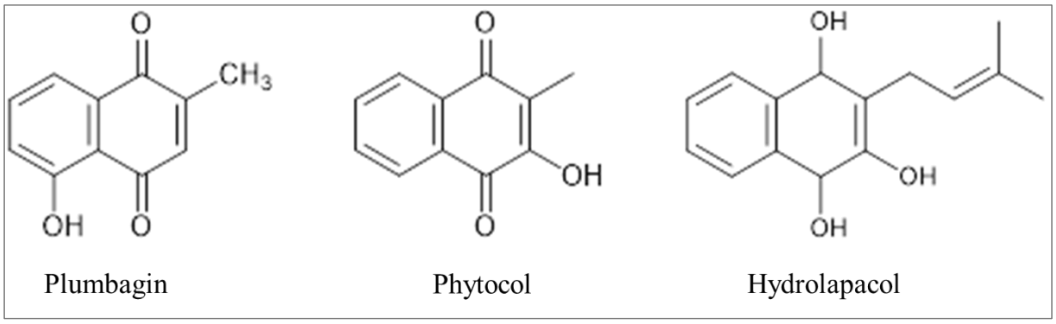

Figure 1*. Naturally occurring quinones*

Plastoquinone and ubiquinone are examples of biologically active quinones. Quinones catalyze biological reactions in some animals, plants, and microorganisms [21, 22]. In addition, the biological activities of certain quinones, such as fungicide, herbicide, nematocide, and insecticide properties, have been reported previously [16, 22, 23]. Daunomycin is an antibiotic that inhibits the development of various tumors. It is used to treat various tumors in animals. Streptonigrin is a compound known for its anticancer properties, while Mitosine is an antitumor antibiotic. Streptonigrin and Mitosine contain a benzoquinone ring, Plastoquinone and Ubiquinone are compounds with a hydroquinone structure, and Daunomycin contains an anthraquinone ring. Structural formulas are depicted in Figure 2. The bioactive properties of new naphthoquinone derivatives formed by naphthoquinones with groups containing nitrogen, sulphur, and oxygen atoms have increased the interest of organic chemists in naphthoquinones. Previous studies have shown that compounds derived from 1,4-naphthoquinone have cytotoxic, molluscicidal, antiallergic, antiproliferative, antileishmanial and antimalarial activities [24-27]. The compound 2-(butylthio)-3-chloronaphthalene-1,4-dione (2), a well-known substance based on existing studies, served as the starting material. New heterocyclic 1,4-naphthoquinone compounds were synthesized by nucleophilic substitution reaction of the starting material with heterocyclic compounds [15, 28], The structures of the new naphthoquinone compounds obtained were purified by column chromatography. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. Finally, the antimicrobial activities of the synthesised compounds against gram-positive and gram-negative bacteria were investigated.

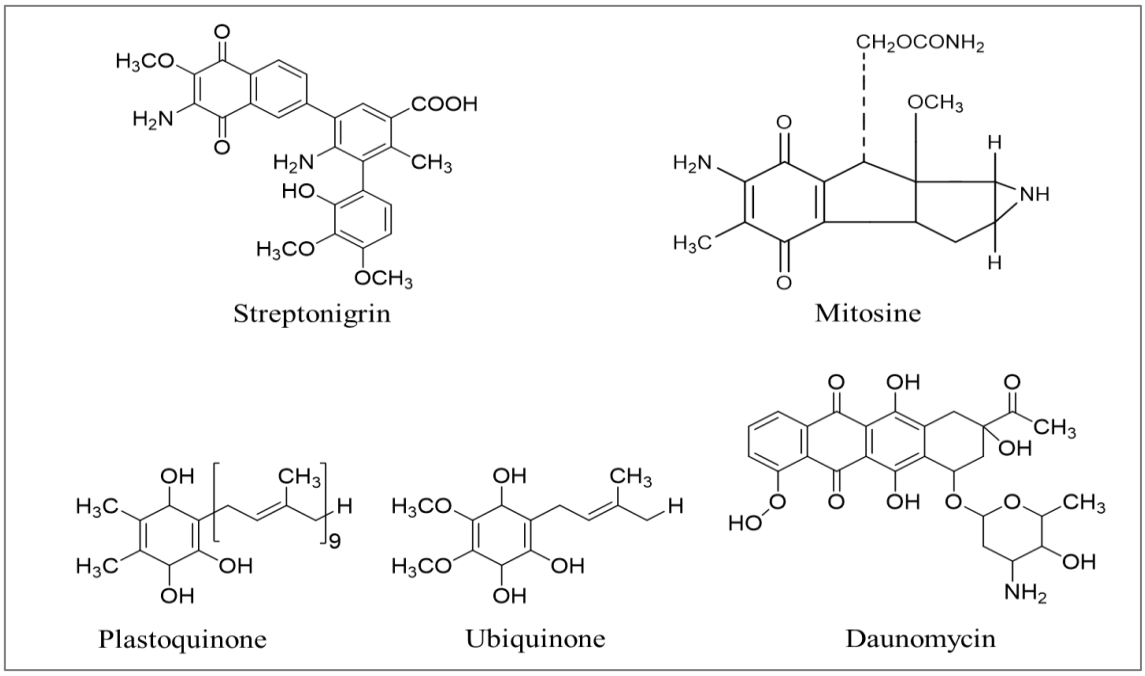

Figure 2*. Quinone structures with biological activity*

Experimental Section

Materials and Methods

The compounds used in the reactions are from Sigma Aldrich, with solvents purified by technical solvent and distillation, and using Na_2CO_3 and Acetonitrile from Merck. The progress of the reactions was monitored using thin-layer chromatography paper (TLC) purchased from Merck. Silica Gel 60 (Merck) was used for purification by column chromatography. Thin-layer chromatography paper was checked under ultraviolet light (254nm). There are various methods in the literature for the synthesis of new aryl group compounds from the compound 2,3-dichloro-1,4-naphthoquinone (**1**) [29]. ^{1}H NMR (400 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ on a Bruker BIOSPIN AVANCE III spectrometer. Mass spectra were obtained on a Thermo Scientific/TSQ Quantum Access Max LC/MS/MS spectrometer using the ESI technique. The IR studies were carried on a Thermo Scientific Nicolet 6700 FTIR spectrometer in the range of 400 to 4000 cm^{-1}. In this experiment, 2,3-dichloro-1,4-naphthoquinone (**1**) was dissolved in 50 mL of chloroform in a 100 mL reaction flask. Na_2CO_3 and 1-butanethiol were then added to the flask, and the mixture was stirred for 6 hours at room temperature. After completion of the reaction, 2-(butylthio)-3-chloronaphthalene-1,4-dione (**2**) was obtained [15, 28]. The resulting 2-(butylthio)-3-chloronaphthalene-1,4-dione (**2**) was the starting compound. Reactions of 2-(butylthio)-3-chloronaphthalene-1,4-dione (**2**) in chloroform/acetonitrile solvent with nucleophilic compounds containing (O-, N-, S-) at different molar ratios have been studied. After adding Na_2CO_3 to the reaction mixture at room temperature, they were stirred with a magnetic stirrer for 5-6 hours. The progress of the reactions was monitored by thin layer chromatography. After, they were extracted using chloroform and 200 mL of water (50 mL x 4). After treating the organic phase with sodium sulfate, it

was filtered to remove any remaining impurities. The crude products were purified using column chromatography after solvent recovery in the rotavapor. The obtained products were dried and made ready for the necessary analysis for characterization. [30, 31]. The Figure 3 shows the availability of naphthoquinone compounds (**10-15**) containing (O-, N-, S-) groups.

Microbial strains and in vitro antimicrobial activity studies

In the study of antimicrobial activity, various types of bacteria and fungi were used, including Gram-positive bacteria *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, Gram-negative bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Pseudomonas aeruginosa* ATCC 27853, as well as fungus *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The broth microdilution method in 96 multi-well microtiter plates was utilized to determine the minimal inhibitory concentrations (MICs) of the tested compounds against the strains [32]. To assess the compound's antimicrobial activity, it was dissolved in DMSO to a concentration of 8192 µg/mL. The bacterial strains were incubated at 37°C in nutrient broth for 24 hours before obtaining the cultures. After being incubated for 24 hours at 28°C, the fungi were kept in nutrient broth. Suspensions of bacteria and fungi with a turbidity of approximately 10^6 cells/mL were prepared. Control wells were also set up with pure microorganisms, pure media, and DMSO. In each well, 100 mL of the microorganism suspension and 100 mL of the compound being tested were added. Table 1. shows the minimum inhibitory concentration (MIC) in µg/mL, which was determined by observing the microplate with no microbial growth. Amoxicillin and Tetracycline served as a reference standards for antibacterial and Ketoconazole for antifungal activity.

Synthesis and Analysis

2-(butylthio)-3-chloronaphthalene-1,4-dione (3) [28]

2-(butylthio)-3-chloronaphthalene-1,4-dione ***(3)*** was synthesized from 2,3-dichloro-1,4-naphthoquinone (1) (1g, 4.4mmol) and 1-butanethiol (2) (0,39 g, 4.3 mmol) using a literature method. Red viscous oil. Yield: 0.88 g (% 71). R_f: 0.59 [(1:1) Petroleum Ether-CH_2Cl_2]; IR (ATR): ν = 3006 (C-H_{arom}), ν = 2977, 2959, 2923 (C-H), 1672, 1662 (C=O), 1589, 1520 (C=C); 813 (C-Cl).

2-(butylthio)-3-((4,5-dihydrothiazol-2-yl) thio) naphthalene-1,4-dione (10)

2-(butylthio)-3-((4,5-dihydrothiazol-2-yl) thio) naphthalene-1,4-dione **(10)** was synthesized from the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione **(3)** (0.25g, 0.89 mmol) with 2-thiazoline-2-thiole **(4)** (0.11g, 0.92mmol) using a literature method. Red viscous oil. Yield: 0.09 g (%28). R_f =0.43 [2:1) $CHCl_3$-Hexane]; IR (ATR): ν = 3072 (C-H_{arom}), ν = 2957, 2926 (C-H), 1652 (C=O), 1590, 1539 (C=C), 1271 (C-N); ^{1}H-NMR (499.74 MHz, $CDCl_3$): δ = 8.15, 7.81 (m, 4H, CH_{arom}), 4.55 (m, 2H, -NCH_2), 3.28, 3.25, 2.98 (m, 4H, SCH_2-), 1.47(s, 2H, -CH_2-), 1.00 (m, 2H, -CH_2-CH_3), 0.94 (m, 3H, CH_3); ^{13}C-NMR (125.66 MHz, CDCl3): δ = 174.52, 169.65 (C=O), 148.34 ($C_{quinone}$-S-), 138.89, 134.30,

129.38, 126.56, 116.66 (C_{napht}), 64.29 (NC), 45.82, 39.76 (S*C*), 33.88, 30.04, 21.74, 14.54 (SC-*C*-), (CH_3-*C*), 13.95 (CH_3-); MS (+ESI): *m/z* 362.62 $[M]^+$. $C_{17}H_{17}NO_2S_3$ (M, 363.5 g/mol).

4-((3-(butylthio)-1,4-dioxo-1,4-dihydronaphthalen-2-yl) oxy) benzaldehyde (11)

4-((3-(butylthio)-1,4-dioxo-1,4-dihydronaphthalen-2-yl) oxy) benzaldehyde **(11)** was synthesized from the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione **(3)** (0.3g, 1.1 mmol) with 4-hydroxybenzaldehyde **(5)** (0.13g, 1.1 mmol) using a literature method. Dark red viscous oil. Yield: 0.08 g (%20). R_f =0.57 [$CHCl_3$]; IR (ATR): ν = 3065, 3015 (C-H_{arom}), ν = 2958, 2927 (C-H), 1764, 1633 (C=O), 1590, 1538 (C=C); ^{1}H-NMR (499.74 MHz, $CDCl_3$): δ = 8.11, 7.75 (m, 8H, CH_{arom}), 9.78 (s, 1H,$CH_{aldehid}$), 3.21 (m, 2H, SCH_2-), 1.62 (m, 2H, -*CH_2*-), 1.44 (m, 2H, -*CH_2*-CH_3), 0.96 (m, 3H, CH_3); ^{13}C-NMR (125.66 MHz, CDCl3): δ = 178.62, 170.70 (C=O), 182.11 (C=$O_{aldehyd}$), 154.62 (*C_{napht}*-O), 134.79, 133.34, 127.20, 126.53, 122.34 (*C_{benzen}*, C_{napht}), 32.36, 29.70, 21.79 (*C*H_2), 13.64 (CH_3); MS (+ESI): *m/z* 277,2 $[M-C_4H_9S]^+$. $C_{21}H_{18}O_4S$ (M, 366.4 g/mol).

2-(butylthio)-3-(naphthalen-1-ylamino) naphthalene-1,4-dione (12)

2-(butylthio)-3-(naphthalen-1-ylamino) naphthalene-1,4-dione **(12)** was synthesized from the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione **(3)** (0.32g, 1.1 mmol) with 1-naphthylamine **(6)** (0.16g, 1.1 mmol) using a literature method. Dark red viscous oil. Yield: 0.12 g (%28). R_f =0.39 [(1:1) Petroleum Ether -CH_2Cl_2]; IR (ATR): ν = 3074, 3009 (C-H_{arom}), ν = 2958, 2926, 2885, 2856 (C-H), 1737, 1667 (C=O), 1592 (C=C), 1174 (C-N); ^{1}H-NMR (499.74 MHz, $CDCl_3$): δ = 8.14, 8.10, 8.05, 7.78, 7.68, 7.55 (m, 11H, CH_{arom}), 4.03 (s, 1H, NH), 2.40 (m, 2H, SCH_2-), 1.71 (m, 2H, -*CH_2*-), 1.26 (m, 2H, -*CH_2*-CH_3), 0.91 (m, 3H, CH_3); ^{13}C-NMR (125.66 MHz, CDCl3): δ = 173.56 (C=O), 151.56 (C_{napht}-N), 139.24, 128.41, 126.82, 124.46, 117.02, 114.07 (*C_{benzen}*, C_{napht}), 66.79 (S-C), 38.72, 33.99, 28.91 (*C*H_2), 14.04 (CH_3); MS (+ESI): *m/z* 387,87 $[M]^+$. $C_{24}H_{21}NO_2S$ (M, 387.5 g/mol).

6-(butylthio)-10-methyl-5H-benzo[a]phenoxazin-5-one (13)

6-(butylthio)-10-methyl-5H-benzo[a]phenoxazin-5-one **(13)** was synthesized from the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione **(3)** (0.33g, 1.1 mmol) with 2-amino-4-methyl phenol **(7)** (0.15g, 1.2 mmol) using a literature method. Red viscous oil. Yield: 0.17 g (%42.5). R_f =0.43 [$CHCl_3$]; IR (ATR): ν = 3070, 3033 (C-H_{arom}), ν = 2958, 2929, 2875(C-H), 1736 (C=O), 1636 (C=C), 1594 (C=N), 1280 (C-O), 1221 (C-N); ^{1}H-NMR (499.74 MHz, $CDCl_3$): δ = 8.63, 8.29, 8.25, 7.73, 7.55, 7.23 (m, 7H, CH_{arom}), 3.97 (t, 2H, SCH_2-), 3.10, 2.33 (m, 4H, -*CH_2*-), 1.28, 0.86 (m, 6H, CH_3); ^{13}C-NMR (125.66 MHz, CDCl3): δ = 173.47 (C=O), 150.28 (C_{napht}=N), 145.87 (*C_{napht}*-S), 142.21 (*C_{napht}*-O), 135.34, 131.51, 129.47, 124.55, 115.41 (*C_{benzen}*, C_{napht}), 66.74 (S-C), 38.70, 33.95 (*C*H_2), 22.94 (C_{phenyl}-CH_3), 14.01 (CH_3); MS (+ESI): m/z 349,57 $[M]^+$, 371,80 $[M+Na]^+$. $C_{21}H_{19}NO_2S$ (M, 349.4 g/mol).

6-(butylthio)-10-chloro-5H-benzo[a]phenoxazin-5-one (14)

6-(butylthio)-10-chloro-5H-benzo[a]phenoxazin-5-one **(14)** was synthesized from the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione **(3)** (0.33g, 1.2 mmol) with 2-amino-chlorophenol **(8)** (0.17g, 1.2 mmol) using a literature method. Red solid: 0.19 g (% 30). R_f =0.58 [CH_2Cl_2]; Melting point. 146-148°C; IR (ATR): ν = 3078, 3054, 3007 (C-H_{arom}), ν = 2959, 2929, 2871, 2859(C-H), 1736 (C=O), 1640 (C=C), 1596 (C=N), 1260 (C-O), 1173 (C-N), 782 (C-Cl); ^{1}H-NMR (499.74 MHz, $CDCl_3$): δ = 8.57, 8.25, 7.70, 7.40, 7.28 (m, 7H, CH_{arom}), 3.14 (t, 2H, SCH_2-), 1.62, 1.47 (m, 4H, -*CH_2*-), 0.92 (m, 3H, CH_3); ^{13}C-NMR (125.66 MHz, CDCl3): δ = 180.74 (C=O), 149.24 (C_{napht}=N), 147.00 (*C_{napht}*-S), 142.78 (*C_{napht}*-O), 133.16, 132.12, 130.93, 126.38, 124.70, 116.67 (*C_{benzen}*, C_{napht}), 32.94 (S-C), 32.24, 21.90 (*C*H_2), 13.71 (CH_3); MS (+ESI): *m/z* 369,52 $[M]^+$. $C_{20}H_{16}ClNO_2S$ (M, 369.9 g/mol).

2-((4-aminophenyl) amino)-3-(butylthio) naphthalene-1,4-dione (15)

2-((4-aminophenyl) amino)-3-(butylthio) naphthalene-1,4-dione **(15)** was synthesized from the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione **(3)** (0.25g, 0.9 mmol) with phenylenediamine **(6)** (0.1g, 0.9 mmol) using a literature method. Purple viscous oil: 0.11 g (%39). R_f =0.53 [(1:1)EtAc- Petroleum Ether]; IR (ATR): ν = 3086, 3035(C-H_{arom}), ν = 3453, 3318 (N-H, NH_2), ν = 2957, 2925, 2854 (C-H), 1664, 1628 (C=O), 1591, 1546 (C=C), 1284, 1268 (C-O), 1015 (C-N), 826 (N-H)); ^{1}H-NMR (499.74 MHz, $CDCl_3$): δ = 8.16, 7.73, 6.91, 6.89, 6.68 (m, 8H, CH_{arom}), 7.28 (s, 2H, C_{phenyl}-NH), 4.29 (s, 2H, NH_2), 2.61 (t, 2H, SCH_2-), 1.35, 1.26, (m, 4H, -*CH_2*), 0.83 (t, 3H, CH_3); ^{13}C-NMR (125.66 MHz, CDCl3): δ = 180.80, 180.73 (C=O), 146.14 (C_{napht}-N), 143.95 (*C_{napht}*-S), 134.52, 132.44, 130.65, 129.83, 126.55, 114.91 (*C_{phen}*, C_{napht}), 33.82 (S-C), 31.55, 21.87 (*C*H_2), 13.65 (CH_3); MS (+ESI): m/z 352,65 $[M]^+$. $C_{20}H_{20}N_2O_2S$ (M, 352.5 g/mol).

Antimicrobial activities

The MIC values of the tested compounds against two Gram-positive and three Gram-negative bacterial strains, as well as two fungal strains, were determined and are provided in Table 1.

Table 1. *The minimum inhibition concentrations (MIC's) of the synthesized compound*

Sample	Minimum inhibition concentration (µg/mL)						
	Gram-staining-positive			Gram-staining-negative		Fungi	
	B. subtilis	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. niger*	*C.albicans*
3	4	32	8	64	8	2	4
10	64	256	256	1024	1024	512	1024
11	128	256	512	512	512	256	128
12	256	128	256	256	512	256	128
13	64	128	256	256	64	128	256
14	128	256	512	512	256	256	512
15	64	128	64	512	256	512	512
Amoxicillin	>1024	>1024	>1024	>1024	>1024	-	-
Tetracycline	4	64	64	64	64	-	-
Ketoconazole	-	-	-	-	-	1	2

Compound 3 displayed superior antimicrobial activity against all tested strains, while compound 10 exhibited the lowest activity. Table 1 shows that compounds 3, 11, 12, 13, 14, and 15 were more effective against *B. subtilis, S. aureus, E. coli, K. pneumoniae*, and *P. aeruginosa* than Amoxicillin. The 11, 12 and 14 compounds showed activity against the Gram-positive *B. subtilis* and *S. aureus*, and the Gram-negative *E. coli, K. pneumoniae* and *P. aeruginosa*, but at levels lower than the Tetracycline reference used. The synthesized compounds showed activity against fungi, but lower than the ketoconazole reference used.

Results and Discussion

Compounds **3**, **10**, **11**, **12**, **13**, **14**, and **15** were obtained via nucleophilic substitution of 2,3-dichloro-1,4-naphthoquinone (**1**) with heterocyclic compounds containing nitrogen, sulphur, and oxygen atoms in an appropriate environment (Figure 3). Compounds **10**, **11**, **12**, and **13** were prepared by reacting compound **3** with compounds **4**, **5**, **6**, and **7** in chloroform solvent using Na_2CO_3 as a catalyst. On the other hand, compounds **14** and **15** were synthesized by reacting compound **8** with compounds **8** and **9** in the presence of acetonitrile solvent and Na_2CO_3 as a catalyst. Based on the analysis, it was concluded that compounds **3**, **10**, **13**, and **14** have a red, viscous, oily physical property. On the other hand, compounds **11** and **12** have a dark red, viscous, oily physical property, while compound **15** has a purple-colored viscous, oily physical property. The synthesized compounds' structure was confirmed by taking its mass spectrum. Further, ^{13}C NMR, ^{1}H NMR, and FTIR spectroscopy were employed to determine the positions of the substituent groups in the structure and the atoms to which they were connected. The chemical shift of the NCH_2 proton in the thiazole ring of compound **10**, formed by the reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) with 2-thiazoline-2-thiol, was around 4.55 ppm and the molecular ion mass was found to be 362.62 $[M]^+$ confirms. The ion peaks of molecules **11**, **12**, **13**, **14**, and **15** are observed at 277.2 $[M\text{-}C_4H_9S]^+$, 387.87 $[M]^+$, 349.57 $[M]^+$, 369.52 $[M]^+$ and 352.65 $[M]^+$ respectively. The structure is confirmed by the chemical shift of two carbonyl groups belonging to the naphthoquinone compound at 178.62 and 170.70 ppm in ^{13}C NMR, and a carbonyl group belonging to one aldehyde group at 182.11 ppm in the molecule formed by the binding of the aldehyde compound in compound **11** to naphthoquinone. The absorption stretch band in the (C-N) group formed by the binding of the amine group in compound **12** to naphthoquinone shows a chemical shift of 1174 cm^{-1} in FTIR and 4.03 ppm singlet in the NH proton ^{1}H NMR. The NH stretching band of compound **12** could not be detected in FTIR spectroscopy. The NH stretching band of secondary amines is weak and may overlap with aromatic bands. The FTIR absorption band of compound **13** shows the presence of C-N and C=N groups at 1221 and 1594 cm^{-1}, respectively. In addition, a shift of one carbonyl group at 173.47 ppm in ^{13}C NMR confirms the structure. Similarly, the presence of C-N and C=N groups in compound **14** at 1173 and 1596 cm^{-1} absorption bands in FTIR and a shift of one carbonyl group at 180.74 ppm in ^{13}C-NMR confirms the structure. Compounds **14** and **15** were found to undergo cyclization. The NH_2 group

absorption band formed by the bonding of the amine group in compound **15** to naphthoquinone is 3413 cm^{-1}, and the absorption stretch band in the (C-N) group is around 1015 cm^{-1} in FTIR, and the shift of two carbonyl groups at 180.80 and 180.73 ppm in ^{13}C NMR confirms the structure. The biological activity of the synthesized compounds was studied, and it was found that three compounds (**3**,**13**,**15**) had the highest antimicrobial activity, while **10** compounds had the lowest antimicrobial activity. Although compound 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) had high antimicrobial activity, the attachment of the 2-thiazoline-2-thiol group significantly decreased its activity. Other aromatic compounds that bind to 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) did not significantly reduce its antimicrobial activity. Compounds **3**, **11**, **12**, **13**, **14**, and **15** exhibited superior activity against Gram-positive bacteria *B. subtilis* and *S. aureus*, as well as Gram-negative *E. coli* and K. *pneumoniae*. Compounds **11**, **12**, and **14** also showed activity against Gram-staining-positive *B. subtilis* and *S. aureus* and Gram-staining-negative *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Studies have also shown that the compounds formed due to vinylic substitution reactions have dyestuff properties, which can be utilized for fabric dyeing [33, 34]. The synthesis process and resulting chemical compounds are depicted in Figure 3.

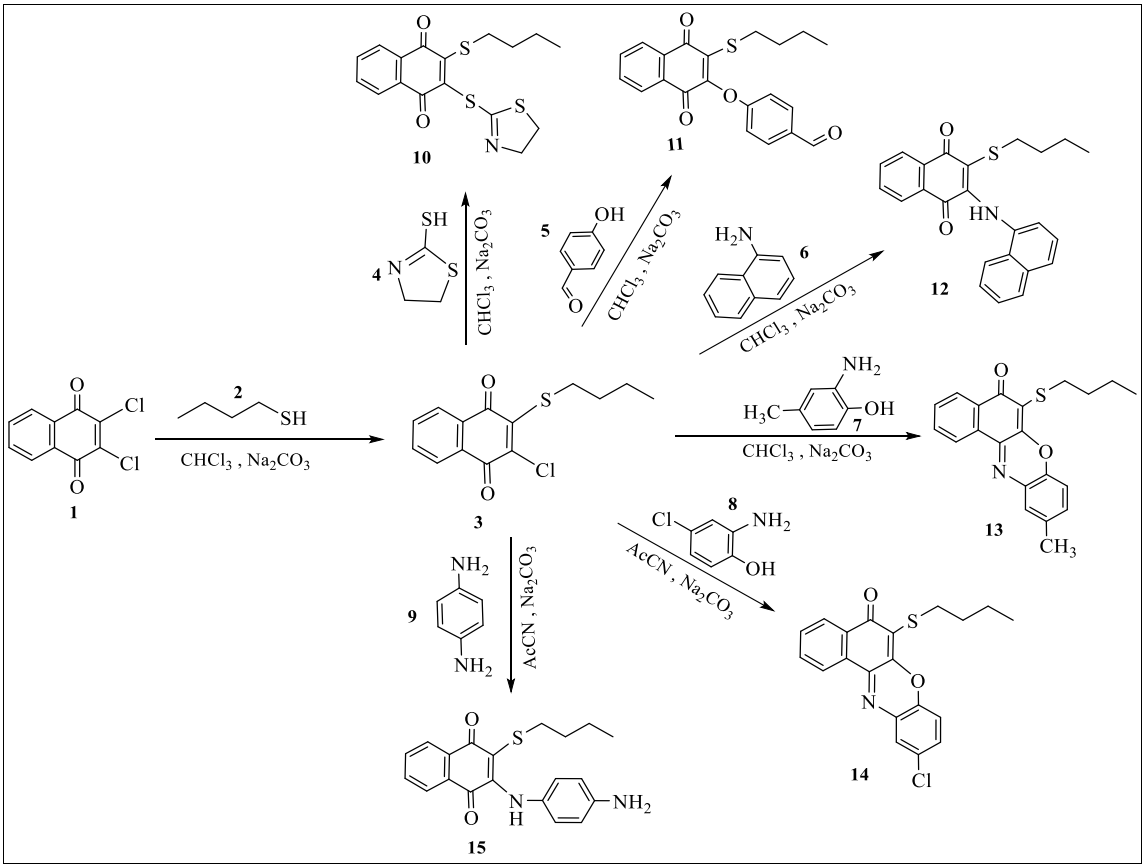

Figure 3*. Compounds synthesized from 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) [28]*

Conclusion

The compound 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) was synthesized from the reaction of the compound 2,3-dichloro-1,4-naphthoquinone (1) with 1-butanethiol in alkaline medium, resulting in the displacement of chloro atom number 1 in its structure by a nucleophilic substitution reaction. The synthesized compound was used as a starting material in subsequent reactions. Compounds **10**-**15** were obtained by reaction of 2-(butylthio)-3-chloronaphthalene-1,4-dione (**3**) with heterocyclic compounds containing nitrogen, sulphur, and oxygen. The resulting compounds were subjected to four different characterisation methods, including FTIR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy. Antimicrobial activity was evaluated for all compounds against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The study revealed that three compounds were found to have the highest antimicrobial activity, while ten compounds had the lowest. Replacing the chlorine atom in the naphthoquinone molecule with aromatic and heteroatoms changes its antimicrobial activity. While the binding of naphthoquinone to thiazoline decreased its activity, the binding of aromatic compounds increased the activity. Although the newly synthesized naphthoquinone compounds are soluble in organic solvents such as chloroform and dichloromethane, their solubility in water is low due to their dense and oily nature. The scientists aimed to improve the existing knowledge on the production of naphthoquinone, a compound with significant applications in various fields.

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