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Design, synthesis, characterization, and antimicrobial activity of novel piperazine substituted 1,4-benzoquinones

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ABSTRACT

Recently, amine substituted and halogen containing 1,4-benzoquinone molecules have attracted a significant attention because of the efficient biological activities. Thus, novel chlorinated/unchlorinated and piperazine substituted dimethyl-1,4-benzoquinone derivatives (**4a-h** and **5a-h**) were designed, synthesized and characterized in the present paper. Furthermore, antibacterial and antifungal activity performances of the new products were compared and evaluated employing the MIC (Minimum Inhibitory Concentrations) values of reference antimicrobial substances. The compounds of **4a** and **4b** were highly potent with the MIC values of 4.88 μg/mL and 78.12 μg/mL compared to Cefuroxime (MIC = 9.8 μg/mL against *S. epidermidis*) and Amikacin (MIC = 128.0 μg/mL against *E. faecalis*), respectively. The presence of the chlorine atom in the structure appeared essential, since most of the chlorinated compounds exhibited more improved activity in comparison to those of unchlorinated products. On the other side, an opposite tendency was observed for the antifungal activity that the MIC values of the unchlorinated derivatives were lower in most cases than those of chlorinated ones. According to the obtained results, while chlorinated derivatives, in particular **4a** and **4b**, can be proposed as potential antibacterial agents with nearly two fold lower MIC values compared to reference drugs, unchlorinated compounds might be suggested as a relatively active antifungal agents which are needed further improvements due to the higher MIC values than those of reference antifungal materials.

1. Introduction

Quinones are extensively appeared in nature. They are particularly obtained from animals, plants, bacteria and fungi in a direct way or synthesized indirectly as synthetic derivatives expecting the utilization in many research and industrial areas [1–8]. Quinones find a wide variety of application field in the following topics: chemosensor, reactive oxygen species generator, redox agent for batteries, dye, energy harvesting-storage material, catalyst and electron transfer agent for flow batteries [9–18]. Besides of many aforementioned properties, a great number of quinone molecules are biologically active compounds. Therefore, natural and/or synthetic benzoquinone, naphthoquinone and anthraquinone derivatives are members of a prominent family of commercial or possible drug molecules in medicinal chemistry and pharmaceutical industry. Quinone-core structured compounds exhibit diverse pharmacological properties such as antimicrobial [19–24], anticancer [25–29], antioxidant [30], anti-inflammatory [31], antiviral [32], antimalarial [33–35], antibiotic [36–38] and herbicidal [39] activities.

Phylloquinone, vitamin K, β-lapachone, lapachol, lawsone, plumbagin, adriamycin, daunomycin, mitoxantrone, juglomycin A, juglone, menadione and lambertellin can be given as examples (Fig. 1) for bioactive and natural molecules which consist of naphthoquinone or anthraquinone structures. Wellington et al. prepared some aminonaphthoquinone compounds bearing various electron withdrawing or donating groups (-F, -Cl, −CN, −OH, $-CH_3$) in different positions in the molecules. The compounds showed antibacterial, antifungal (against *C. albicans*) and anticancer (against the PC3 prostate cancer cell line) activity. They reported that a fluoro group in the ortho position of the aminobenzene ring structure provided an improved antifungal activity [40]. Tuyun et al. prepared a series of 2-arylamino-3-chloro-1,4-naphthoquinone derivatives and evaluated for their *in vitro* antibacterial and antifungal activities. Two of derivatives showed remarkable activity against both Gram-positive and Gram-negative bacteria and against the tested fungi (*C. albicans*). Some compounds

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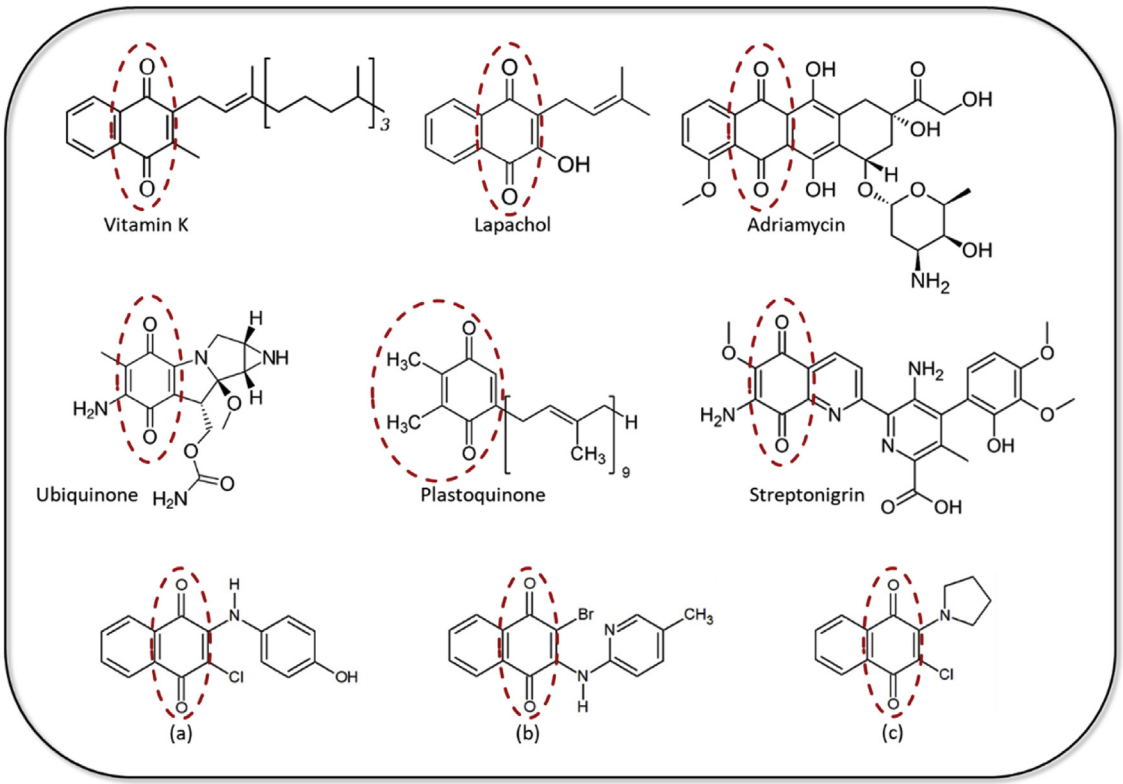

Fig. 1. Some examples of naturally occurring and synthetic bioactive quinone derivatives, a [47], b [48], c [49].

had moderate activity against *E. faecalis* with MIC values of between 312.5 and 1250 μg/mL compared to the reference antibiotic of Amikacin (128 μg/mL). Benzo [*b*]phenazine-6,11-dione derivatives were mostly active against Gram-positive bacteria. The change of the positions of sulfonic acid group (-SO_3H), sulfonamide group (-SO_2NH_2), trifluoromethyl group (-CF_3) and alkoxy group in the substituted phenyl ring caused some differences in the antimicrobial activity [41]. Bayrak synthesized, characterized and compared antimicrobial test results of a new family of 2-methylquinoline-5,8-dione compounds containing methoxy- or ethoxyphenylamino group and chlorine atom. Some of these novel molecules exhibited strong antibacterial activity against Gram-positive bacteria, *S. epidermidis* and *E. faecalis*. As the structure-activity relationship it was concluded that methoxy group(s) bearing phenyl substituted primary-amine introduction to azanaphthoquinone derivatives improved positively the antibacterial activity against tested pathogens [42]. Novais et al. synthesized a series of hydroxyl naphthoquinone derivatives and tested them against Gram-negative and Gram-positive bacteria strains. 2-hydroxy-3-phenylsulfanylmethyl-1,4-naphthoquinones were found promising structures against *E. coli* and *P. aeruginosa* strains and it was suggested a correlation between phenylsulfanymethyl ring with the antibacterial profile against Gram-negative strains [43]. Yıldırım et al. synthesized and characterized successfully sulfanyl and trifluoromethyl containing aryl amine substituted 1,4-naphthoquinones. The influences of the $-CF_3$ group position in aryl amine ring of the prepared compounds were clearly elucidated and the molecular docking studies have also supported the experimental results. A number of these compounds were reported as promising antibacterial and antimicrobial agents and it was suggested that the sec-butylthio and 2-hydroxypropylthio moieties with the additional effect of the position of $-CF_3$ are promising for the exploration of new antibacterial agents [44]. Halicki et al. evaluated the antibacterial activity of six 1,4-naphthoquinones derivatives against strains of *Mycobacterium tuberculosis*. Every compound was active against *M. tuberculosis* strains possessing various MIC values and it was found that tetrahydrofuran fused 1,4-naphthoquinones showed a better antimycobacterial activity against M. tuberculosis strains than that of tetrahydropyran fused 1,4-naphthoquinones. Tetrahydrofuran including compound represented also a reduced cytotoxicity [45]. Kacmaz et al. introduced bromine containing aminonaphthoquinones and amino-thio-substituted 1,4-naphthoquinones and investigated their electrochemical behavior and antifungal, antibacterial properties. The synthesized compounds exhibited as high and moderate activity against tested fungi (*M.canis* and *Trichopyton* sp.) in comparison to the reference antifungal molecule of Amphotericin B and they were also mostly bioactive against Gram negative bacteria (*E. coli*) [46].

1,4-benzoquinone is the simpliest member of the family of organic quinone compounds. Recently, numerous *p*-benzoquinone derivatives attract great attention due to their chemical and biological significance. Mitomycin, geldanamycin, streptonigrin, abenquine, thymoquinone, ubiquinone and plastoquinone compounds (Fig. 1) include also *p*-benzoquinone scaffold and they are exploited as well-known drug and drug-candidate of quinoid derivatives [50,51]. Johnson-Ajinwo et al. described the synthesis of thymoquinone analogues including different amine, alkyl chain and halogen substituents. The growth inhibition in three human ovarian cancer cell lines and immortalized human ovarian epithelial cell line (HOE) by determining their IC_{50} values using sulforhodamine B (SRB) cytotoxicity assay and antiproliferative activities against human malaria parasite were investigated for their biological activity potential. Certain analogues showed significant inhibitory activities that two-fold more than that of thymoquinone and also halogen substitution in some molecules led to an improvement against ovarian cancer cell lines [52]. Abenquine is

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one of the bioactive natural quinones having anticyanobacterial activity. Nain-Perez et al. designed and synthesized some synthetic abenquine analogues by substitution of the acetyl group by a benzoyl group in the quinone core and by replacement of the amino acid moiety with ethylpyrimidinyl or ethylpyrrolidinyl groups. This modification resulted in new analogues which exhibited 25-fold better activity than those of the natural abenquines [53]. Blunt et al. reported the first chemical synthesis of aulosirazole that shows selective antitumor cytotoxicity and synthesized pronqodine A analogues to be able to compare their results. Biological evaluation of the compounds was performed by targeting indoleamine-2,3-dioxygenase (IDO) enzyme. The isothiazoloquinone derivatives generate reactive oxygen species in the intracellular NQO1-dependent redox cycling. These compounds provide a benefit to change the ratio of intracellular oxidized to reduced pyridine nucleotides at lower proportions [54]. Embelin is a natural hydroxy benzoquinone with alkyl substitution and known to possess miscellaneous biological activities. Singh et al. carried out the reaction of embelin with various secondary amines in order to obtain a series of Mannich products. Antiproliferative, antimicrobial and cytotoxicity tests were conducted for the synthesized products. The benzyl-piperidine linked derivative indicated better antiproliferative activity in comparison to embelin family against a panel of cell lines including MIAPaCa-2, HCT-116, PC-3 and MCF-7. Besides that, dimethylamino- and piperidine-linked derivatives exhibited antibacterial activity against *Staphylococcus aureus*. While the Mannich derivatives did not show adequate solubility of aqueous, the aqueous solubility of their hydrochloride salts became better without any effect of biological activities [55]. Park et al. investigated antimicrobial activities of dimethoxy-, dimethyl- and dichloro-1,4-benzoquinones and evaluated their structure-activity relationships of against the seven food-borne bacteria. Whereas 2,6-Dimethoxy-1,4-benzoquinone showed activity against *Staphylococcus intermedius*, *Staphlococcus epidermidis*, *Shigella sonnei* and *Listeria monocytogenes*, 2,6-dichloro-1,4-benzoquinone was not bioactive compound against all tested food-borne bacteria. It was pointed out that the 2,6-dimethoxy-1,4-benzoquinone and its structural analogues can be beneficial by utilization of food supplemental additives [56]. Tandon et al. demonstrated the preparation of diverse thio- and aminobenzoquinone compounds by applying green methodology. Laundry detergent (surfactant) was used as a catalyst and the reactions was carried out in water. Sulfur atom containing alkyl and arylsulfanyl-*p*-benzoqinones gave better antifungal activity test results than drugs of Fluconazole and Flucytosine *in vitro* against *S. schenckii*, and *T. mentagraphytes*. A 2,5-diaminosubstituted-*p*-benzoquinone compound showed better antifungal activity compared to Fluconazole drug against *C. neoformans*, *S. schenckii*, *T. mentagraphytes* and *A. fumigatus*. The same molecule displayed a better antibacterial activity in comparison to Ampicillin against *E. coli*, *S. aureus* and *K. Pneumonia in vitro* and did not cause any toxicity towards mammalian cells L929 [57].

Plastoquinone is one of the most important naturally occurring 1,4-benzoquinone compounds and involved mostly in the electron transport chain of the light-dependent photosynthesis reactions. Its structural framework is consist of 2,3-dimethyl-1,4-benzoquinone with a polyprenyl side chain at the fifth position. There are several natural plastoquinones with side chains of different length (containing between six and nine isoprene units). Besides that, derivatives of plastoquinone molecule are also used for pharmacological purposes. Latterly, Yıldırım et al. prepared a number of thiolated plastoquinone analogues to investigate their antimicrobial activity. 2-Chloro-3-(2,4-dimethylphenylthio)-5,6-dimethyl-1,4-benzoquinone was the analog which showed the best *in vitro* antibacterial activity against *E. faecalis* compared to that of Amikacin. The same compund was also the most potent analog in the series against *C. albicans* and *C. tropicalis*. The chlorine atom of thiolated analogues were speculated to be essential for acceptable inhibitory activity. Whereas the methoxy group substituted thiol structure bearing analogues exhibited no inhibitory activity against the most of the microorganisms, methyl groups containing analogues were suggested as promising molecules as potent antimicrobial agents for further works [58].

The introduction of amine molecules into the 1,4-quinone structures yields aminoquinone derivatives and improves antimicrobial activity substantially [59–62]. Moreover, recently it has been pointed out prominently that presence or addition of a halogen atom, in particular chlorine, in the pharmacophore scaffold seems to be crucial for potential biological activity [26,40,44,58,63–65].

The above reported mini review reveal that a 1,4-quinone core containing quinone derivatives play an important role in diverse biologically active processes particularly such as in antibacterial and antifungal activities. Since antibiotic resistance has become worldwide a critical challenge in the health of public and the decline of the number of newly discovered and clinically approved antibiotics, an instant demand has arisen on the research and development of novel antimicrobial drugs in both academia and pharmaceutical industry. Therefore, it is reported in the present study the design, synthesis, characterization and antimicrobial activity of halogenated and non-halogenated plastoquinone-resembling derivatives containing variously substituted piperazine groups (Fig. 2). The structure-activity relationship (SAR) has been discussed for the novel dimetyhl-1,4-benzoqinone moiety bearing compounds by means of the diversity of the secondary amine groups, their methyl- and methoxy-group substitutions in different positions and presence or absence of chlorine atom.

2. Materials and methods

2.1. Chemicals and apparatus

All reagents, solvents, and compounds were commercially obtained from commercial supplier with a minimum purity of 95%

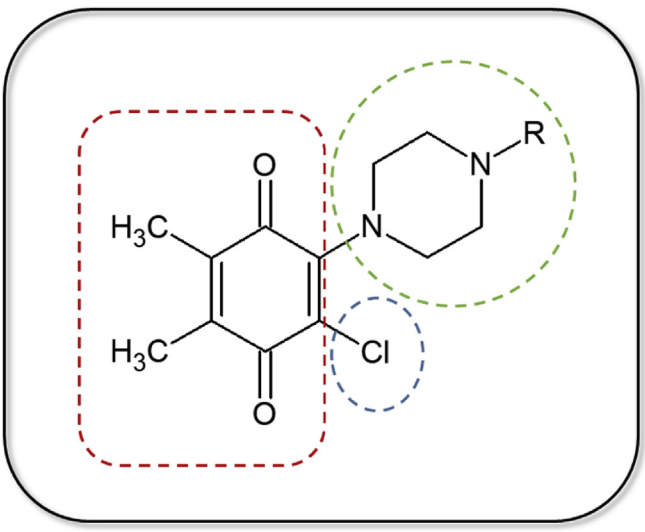

Fig. 2. Design of desired plastoquinone derivatives based on literature survey: i) 2,3-dimethyl-1,4-benzoquinone moiety of plastoquinone as a core structure, ii) substitution of the side chain with diversely substituted amines, iii) chlorine atom at the second position of the 1,4-benzoquinone moiety to increase the inhibitory activity, iv) methyl- and methoxy-substitution at different positions and presence or absence of chlorine atom to investigate structure-activity relationship of the novel molecules.

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and used without further purification unless specified otherwise. A Stuart SMP-10 melting point apparatus was used to determine the melting points (mp) that were uncorrected. For column chromatography, silica gel 60 (Merck, 63–200 μm particle sized, 60–230 mesh) was used as the stationary phase. Thin layer chromatography (TLC) was purchased from Merck KGaA (silica gel 60 F254) based on Merck DC-plates (aluminum based). Visualization of TLC plates was performed by means of UV light (254 nm). Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were obtained on a Varian^{UNITY} INOVA spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) in $CDCl_3$ refer to the solvent signal centre at δ 7.19 and δ 76.0 ppm. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are reported in Hz. Multiplicities were described using the following abbreviations: s (singlet), bs (broad singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were obtained with a BRUKER Microflex LT by MALDI (Matrix Assisted Laser Desorption Ionization)-TOF technique via addition of 1,8,9-anthracenetriol (DIT, dithranol) or 2,5-dihydroxybenzoic acid (DHB) as matrix. Infrared spectrums were recorded as ATR on a PerkinElmer Spectrum 100 Optical FT-IR Spectrometer.

2.2. X-ray diffraction analysis

The single-crystal data of the some compounds were obtained with Bruker APEX II QUAZAR three-circle diffractometer. Crystal structure validations and geometrical calculations were performed using the Platon software [66]. Mercury software [67] was used for visualization of the. cif files. Each of the structures has been solved and refined using the Bruker SHELXTL Software Package [68]. Indexing was performed using APEX2 [69]. Data integration and reduction were carried out with SAINT [70]. Absorption correction was performed by multi-scan method implemented in SADABS [71]. Aromatic and aliphatic H atoms bonded to C atoms were positioned geometrically and refined using a riding mode. Details of data collection and crystal structure determinations are given in Table 1. The selected bond lengths and bond angles are given in Tables 2 and 3. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre and CCDC reference numbers are 1941240 for the compound **5d**, 1941244 for the compound **5e**, and 1941241 for the compound **5f**. The data can be obtained available free of charge from http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 (0) 1223336033; email: nndeposit@ccdc.cam.ac.uk.

2.3. General methods for the synthesis of compounds

2.3.1. Method A for the synthesis of the piperazine and chlorine substituted dimethyl-1,4-benzoquinones (**4a-h**) [49]

To a suspension of the 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone (0.1025 g, 0.50 mmol) in H_2O (10 mL), a suspension of the appropriate piperazine (1.10 mmol, 2.2 equiv.) was added dropwise and stirred at 50–60 °C for 5–10 h until consumption of the 1,4-benzoquinone. The reaction mixture was cooled to room temperature. After the solvent evaporation, the crude product was dissolved in chloroform, and the solution was washed with distilled water. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. Column chromatography on silica gel was conducted for the residue to obtain separated and purified target compounds.

2.3.2. Method B for the synthesis of the piperazine substituted dimethyl-1,4-benzoquinones (**5a-h**) [72]

An appropriate substituted piperazine (1.448 mmol, 2 equiv.) and 2,3-dimethylhydroquinone (0.100 g, 0.724 mmol) were suspended in MeOH (5 mL) in a round-bottom flask. After that, sodium iodate (2.172 mmol, 3 equiv.) was added in water (5 mL) to the stirred solution at room temperature for 5–10 h. The reaction was checked by TLC until the spot of starting compounds disappeared under UV light. After the reaction was completed, the mixture of reaction in solution was extracted by using chloroform and the organic phase was washed with water. At the end, the organic phase was dried with Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel to achieve desired pure products.

2.3.3. 2-Chloro-5,6-dimethyl-3-(4-(isopropyl)piperazin-1-yl)-1,4-benzoquinone (**4a**)

The method A was implemented to synthesize the title compound which was obtained from the reaction of 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone (**2**) with 1-isopropylpiperazine (**3a**). The crude product was purified by column chromatography to furnish (**4a**) as a brown solid. Yield: 16%, mp > 250 °C. FTIR (ATR) υ (cm^{-1}): 2964, 2922, 2848 ($CH_{aliphatic}$), 1659 (>C=O). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.09–1.47 (m, 6H, $CH_{3isopropyl}$), 2.01–2.07 (m, 6H, CH_3), 2.16–2.23 (m, 1H, $CH_{isopropyl}$), 2.69–2.88 (m, 4H, $CH_{2piperazine}$), 3.55–3.73 (m, 4H, $CH_{2piperazine}$). ^{13}C NMR ($CDCl_3$) δ (ppm): 12.6, 13.1, 17.4 (CH_3), 29.6, 48.8 ($CH_{2piperazine}$), 56.9 ($CH_{isopropyl}$), 139.0, 141.5, 147.1 ($C_{quinone}$), 180.0, 183.7 (>C=O). MS MALDI TOF (*m/z*): 296 $[M]^+$. Anal. Calcd. for $C_{15}H_{21}ClN_2O_2$ (296.13).

2.3.4. 2-Chloro-5,6-dimethyl-3-(4-(cyclohexyl)piperazin-1-yl)-1,4-benzoquinone (**4b**)

The method A was implemented to synthesize the title compound which was obtained from the reaction of 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone (**2**) with 1-cyclohexylpiperazine (**3b**). The crude product was purified by column chromatography to furnish (**4b**) as a dark brown solid. Yield: 37%, mp 180–182 °C. FTIR (ATR) υ (cm^{-1}): 2926, 2852, 2811 ($CH_{aliphatic}$), 1659 (>C=O). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.04–1.30 (m, 5H, $CH_{2cyclohexyl}$), 1.59–1.72 (bs, 1H, $CH_{2cyclohexyl}$), 1.77–1.87 (m, 2H, $CH_{2cyclohexyl}$), 1.87–1.97 (m, 2H, $CH_{2cyclohexyl}$), 2.00 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.32–2.42 (bs, 1H, $CH_{cyclohexyl}$), 2.70–2.80 (bs, 4H, $CH_{2piperazine}$), 3.53–3.61 (bs, 4H, $CH_{2piperazine}$). ^{13}C NMR ($CDCl_3$) δ (ppm): 12.6, 13.1 (CH_3), 25.7, 26.0, 28.4 ($CH_{2cyclohexyl}$), 49.6, 50.7 ($CH_{2piperazine}$), 64.2 ($CH_{cyclohexyl}$), 138.7, 141.3, 147.7 ($C_{quinone}$), 180.0, 183.8 (>C=O). MS MALDI TOF (*m/z*): 336 $[M]^+$. Anal. Calcd. for $C_{18}H_{25}ClN_2O_2$ (336.16).

2.3.5. 2,3-Dimethyl-5-(4-(isopropyl)piperazin-1-yl)-1,4-benzoquinone (**5a**)

The method B was implemented to synthesize the title compound which was obtained from the reaction of 2,3-dimethylhydroquinone (**1**) with 1-isopropylpiperazine (**3a**). The crude product was purified by column chromatography to furnish (**5a**) as a dark red oil. Yield: 11%. FTIR (ATR) υ (cm^{-1}): 2965, 2930 ($CH_{aliphatic}$), 1659 (>C=O). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.08 (d, J = 6.5 Hz, 6H, $CH_{3isopropyl}$), 1.99 (d, J = 3.4, 6H, CH_3), 2.64–2.69 (m, 4H, $CH_{2piperazine}$), 2.72–2.78 (m, 1H, $CH_{isopropyl}$), 3.36–3.42 (m, 4H, $CH_{2piperazine}$), 5.74 (s, 1H, $CH_{quinone}$). ^{13}C NMR ($CDCl_3$) δ (ppm): 12.3, 12.4, 18.4 (CH_3), 48.2, 48.9 ($CH_{2piperazine}$), 54.6 ($CH_{isopropyl}$), 109.1, 139.2, 141.1, 152.4 ($C_{quinone}$), 185.1, 186.2 (>C=O). MS MALDI TOF (*m/z*): 262 $[M]^+$. Anal. Calcd. for $C_{15}H_{22}N_2O_2$ (262.17).

2.3.6. 2,3-Dimethyl-5-(4-(cyclohexyl)piperazin-1-yl)-1,4-benzoquinone (**5b**)

The method B was implemented to synthesize the title compound which was obtained from the reaction of 2,3-dimethylhydroquinone (**1**) with 1-cyclohexylpiperazine (**3b**). The crude product was purified by column chromatography to furnish

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Table 1
Crystallographic data for compounds **5d, 5e**, and **5f**.

Identification code	5d	5e	5f
Chemical formula	$C_{19}H_{22}N_2O_2$	$C_{19}H_{22}N_2O_2$	$C_{19}H_{22}N_2O_3$
Formula weight (g mol^{-1})	310.38	310.38	326.38
Temperature (K)	296 (2)	296 (2)	296 (2)
Radiation, λ (Å)	0.71073	0.71073	0.71073
Crystal system	monoclinic	orthorhombic	orthorhombic
Space groups, Z	P1 21/c 1	P b c a	P b c a
Unit cell dimensions (Å)	a = 10.101 (2) b = 20.911 (6) c = 8.0122 (19) (α, γ = 90° β = 104.075 (15)°)	a = 11.9340 (19) b = 7.7876 (19) c = 35.739 (7) (α, β, γ = 90°)	a = 11.8686 (14) b = 7.9234 (10) c = 35.924 (4) (α, β, γ = 90°)
Volume ($Å^3$)	1641.5 (7)	3321.5 (12)	3378.3 (7)
Crystal sizes (mm)	0.125 × 0.167 × 0.269	0.140 × 0.157 × 0.324	0.098 × 0.198 × 0.273
dcalc (g cm^{-3})	1.256	1.241	1.283
Absorption coefficient (mm^{-1})	0.082	0.081	0.087
Absorption correction, T_{min}, T_{max}	multi-scan, 0.9900, 0.9780	multi-scan, 0.9890, 0.9740	multi-scan, 0.9910, 0.9770
θ_{max}, deg	25.0		
Goodness-of-fit on F^2	1.045	1.030	1.028
Index ranges	−11 ≤ h<=12, −23 ≤ k<=20, −9 ≤ l<=9	−13 ≤ h<=14, −9 ≤ k<=5, −41 ≤ l<=42	−12 ≤ h<=13, −9 ≤ k<=9, −42 ≤ l<=42
Reflections collected	8171	14860	23046
Independent reflections	2807 [R (int) = 0.0641]	2912 [R (int) = 0.0963]	2898 [R_{int} = 0.0679]
Final R indices [I > 2σ(I)]	1595 data; R1 = 0.0687, w*R2* = 0.1808	1592 data; R1 = 0.0960, w*R2* = 0.2344	1684 data; R1 = 0.0546, w*R2* = 0.1293
R indices (all data)	R1 = 0.1252, w*R2* = 0.2278	R1 = 0.1587, w*R2* = 0.2773	R1 = 0.1035, w*R2* = 0.1605
Refinement method	Full-matrix least-squares on F^2		
Scan mode	ω/ϕ		
Data/restraints/parameters	2807/0/212	2912/0/211	2898/0/220
$\Delta\rho_{max}$, $\Delta\rho_{min}$ ($eÅ^{-3}$)	0.289, −0.249	0.456, −0.297	0.163 and −0.211

Table 2
Selected bond lengths (Å) for compounds **5d**, **5e**, and **5f**.

5d		5e		5f	
O2–C14	1.221 (4)	C6–O2	1.221 (6)	C13–O2	1.219 (3)
O1–C19	1.222 (4)	C1–O1	1.220 (6)	C18–O3	1.234 (3)
C17–C15	1.329 (4)	C2–C4	1.323 (7)	C13–C14	1.478 (3)
C12–C13	1.346 (4)	C4–C6	1.496 (8)	C14–C16	1.333 (4)
C17–C19	1.493 (4)	C6–C7	1.446 (7)	C12–C13	1.490 (4)
C12–C19	1.495 (4)	C7–C8	1.339 (7)	C12–C19	1.346 (3)
N2–C12	1.382 (3)	C8–N1	1.376 (6)	C12–N2	1.376 (3)
N2–C9	1.468 (4)	C12–N1	1.459 (6)	C11–N2	1.458 (3)
N1–C7	1.417 (3)	C11–C12	1.499 (6)	C10–C11	1.508 (3)
C7–C1	1.395 (4)	C13–N2	1.414 (5)	C5–N1	1.427 (3)
C9–C8	1.513 (4)	C13–C19	1.380 (6)	C5–C6	1.386 (3)

Table 3
Selected bond angles (°) for compounds **5d**, **5e**, and **5f**.

5d		5e		5f	
N2–C12–C19	116.5 (3)	N1–C8–C1	117.2 (4)	N2–C12–C13	117.7 (2)
C13–C12–N2	125.3 (3)	C7–C8–N1	124.8 (5)	C19–C12–N2	125.2 (2)
C12–N2–C9	117.4 (3)	C8–N1–C12	116.9 (4)	C12–N2–C11	117.20 (19)
C12–N2–C10	118.5 (2)	C8–N1–C9	117.4 (4)	C12–N2–C9	117.75 (19)
C15–C17–C19	119.8 (3)	C4–C2–C1	120.6 (5)	C16–C14–C13	119.5 (2)
C17–C15–C14	120.6 (3)	C2–C4–C6	119.3 (5)	C14–C16–C18	119.3 (2)
O1–C19–C17	119.6 (3)	O1–C1–C2	119.2 (5)	O2–C13–C14	119.1 (2)
O1–C19–C12	121.3 (3)	O1–C1–C8	121.7 (4)	O2–C13–C12	120.8 (2)
C9–N2–C10	110.3 (2)	C12–N1–C9	111.0 (4)	C11–N2–C9	110.75 (18)
C1–C7–N1	119.0 (3)	C19–C13–N2	123.1 (4)	C6–C5–N1	122.9 (2)
C6–C7–N1	122.5 (3)	C14–C13–N2	120.2 (4)	C2–O1–C1	118.0 (2)

(**5b**) as a dark red oil. Yield: 15%. FTIR (ATR) υ (cm^{-1}): 2927, 2853 ($CH_{aliphatic}$), 1659 (>C=O). ^{1}H NMR (*CDCl*$_3$) δ (ppm): 1.05–1.35 (m, 6H, $CH_{2cyclohexyl}$), 1.73–1.93 (m, 4H, $CH_{2cyclohexyl}$), 1.95–2.11 (m, 6H, CH_3), 2.23–2.40 (m, 1H, $CH_{cyclohexyl}$), 2.65–2.76 (m, 4H, $CH_{2piperazine}$), 3.32–3.43 (m, 4H, $CH_{2piperazine}$), 5.73 (s, 1H, $CH_{quinone}$). ^{13}C NMR (*CDCl*$_3$) δ (ppm): 12.3, 12.5 (CH_3), 25.8, 26.2, 28.8 ($CH_{2cyclohexyl}$), 48.5, 49.1 ($CH_{2piperazine}$), 63.6 ($CH_{cyclohexyl}$), 109.0, 139.2, 141.1, 152.4 ($C_{quinone}$), 185.1, 186.2 (>C=O). MS MALDI TOF

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(*m/z*): 302 $[M]^+$. Anal. Calcd. for $C_{18}H_{26}N_2O_2$ (302.19).

In the present section, the experimental data of **4a-4b** and **5a-5b** are given comprehensively. However, for all data, spectra, and analysis results of other novel compounds (**4c-4h**) and (**5c-5h**), please refer to the supplementary material of this publication.

2.4. Antimicrobial activity

2.4.1. Determination of Minimum Inhibitory Concentrations (MIC)

Antimicrobial activities 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, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750 were determined by the microbroth dilution technique using the Clinical Laboratory Standards Institute (CLSI) recommendations [73,74]. Mueller-Hinton broth for bacteria and RPMI-1640 medium for the yeast strain were used as the test media. Serial two-fold dilutions ranging from 2500 μg/mL to 1.2 μg/mL were prepared in the media. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent of a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 cfu/mL for bacteria and 0.5×10^3 to 2.5×10^3 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. The results were evaluated according to the values of the controls.

3. Results and discussions

3.1. Chemical synthesis

The nucleophilic substitution reactions of 1,4-quinones with amines, thiols and alcohols are well-recognized and fast way to obtain possible biologically active molecules. In this study, some amine groups linked dimethyl-1,4-benzoquinone compounds were prepared from the reactions of various secondary amines with 1,4-hydroquinone to achieve unchlorinated products or with 1,4-quinone to achieve chlorinated products. Since a halogen atom presence in quinone structure is of an important influence on biological activity as mentioned in the introductory part, chlorinated target compounds were initially attempted for this purpose. Thus, commercially available 2,3-dimethylhydroquinone (**1**) compound was firstly oxidized and chlorinated to the corresponding quinone structure, 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone (**2**), in HNO_3/HCl medium at 90 °C (Scheme 1) according to a formerly published preparation method [75].

After preparation of starting compound in quinoid structure, the reactions were carried out to achieve the target products. In order to conduct these experiments, 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone (**2**) was stirred with diverse piperazine compounds (1-isopropylpiperazine (**3a**), 1-cyclohexylpiperazine (**3b**), 1-(2-methylphenyl)piperazine (**3c**), 1-(3-methylphenyl)piperazine (**3d**), 1-(4-methylphenyl)piperazine (**3e**), 1-(4-methoxyphenyl)piperazine (**3f**), 1-(3-methoxyphenyl)piperazine (**3g**), 1-(2-methoxyphenyl)piperazine (**3h**)) by applying a method from literature [49] at 50–60 °C in the absence of a base using water as solvent. As a result of that mono piperazine substituted products were obtained in yield of 16–58% as nominated from **4a** to **4h** (Scheme 2).

After preparation of chlorinated compounds, unchlorinated compounds were also synthesized to be able to check, compare and discuss the structure-activity relationship about the presence and absence of chlorine atom in newly obtained quinone molecules. For this purpose, various piperazine compounds (**3a-h**) mentioned above reacted in a single step way with 2,3-dimethylhydroquinone (**1**) compound according to a previously present method [72] at room temperature in the presence of $NaIO_3$ using mixture of water and methanol as solvent to yield desired unchlorinated and mono piperazine substituted 1,4-benzoquinone derivatives in yield of 11–57% assigning the codes of **5a** to **5h** (Scheme 3).

The novel dimethyl-1,4-benzoquinone compounds (**4a-h** and **5a-h**) were purified by utilization of silica gel column chromatography applying mixture of solvents as a mobile phase. The aforenamed products were elucidated on the basis of FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The FTIR spectra of the derivatives showed a characteristic carbonyl (C=O) signals between 1645 and 1666 cm^{-1}, aliphatic (C–H) signals between 2811 and 2965 cm^{-1}, aromatic (C–H) signals between 2976 and 3074 cm^{-1}. Whereas the ^{13}C NMR spectra exhibited the peaks of (-CH_3) carbons bonded to $(C{=}C)_{quinone}$ around 12.3–13.1 ppm, the (-CH_3) carbons of isopropyl- and methylphenyl-substituted piperazines gave signals between 17.4 and 21.8 ppm. Besides that, the (-CH_3) carbons which are adjacent to oxygen atom showed peaks around 55 ppm and the signals of $(C{-}H)_{isopropyl}$ and $(C{-}H)_{cyclohexyl}$ carbon atoms were detected at 54.6, 56.9 ppm and 63.6, 64.2 ppm, respectively. While the $(CH_2)_{piperazine}$ carbon peaks were observed mostly around 48–50 ppm, the $(CH_2)_{cyclohexyl}$ carbon signals were determined between 25.7 and 28.8 ppm. The carbon atoms of $(C{=}C)_{quinone}$ and $(C{-}H)_{aromatic}$ were depicted peaks between 102.7 and 160.8 ppm and the typical signals of all (C=O) carbons were observed around 180 and 183 ppm for the chlorinated compounds (**4a-h**), 185 and 186 ppm for the unchlorinated compounds (**5a-h**). The ^{1}H NMR spectra showed the peaks of (-CH_3) protons linked to $(C{=}C)_{quinone}$ between 1.97 and 2.10 ppm. While the signals of (-CH_3) protons bonded to phenyl moiety were detected around 2.30 ppm, the (-CH_3) proton peaks of isopropyl group were observed between 1.09 and 1.47 ppm. On the other hand, the (-CH_3) protons attached to the oxygen atoms were shown around 3.80 ppm. The (C–H) protons of isopropyl group gave signals between 2.72 and 2.92 ppm and also the peaks of cyclohexyl (C–H) protons were seen around 2.32 ppm. The ^{1}H NMR spectra exhibited the peaks of (C–H) protons of unchlorinated quinone products (**5a-h**) around 5.80 ppm, whereas the aromatic (C–H) proton signals were determined between 6.47 and 7.23 ppm. The (CH_2) proton peaks of cyclohexyl group were depicted between 1.11 and 1.93 ppm. Besides that, the (CH_2) protons of piperazine moiety were characterized by the signals between 2.64 and 3.73 ppm. The structure of the new compounds were also characterized and supported by mass spectroscopy results as given in the following: 4a, 4b, 4c-e, 4f-h (296 $[M]^+$), (336 $[M]^+$), (344 $[M]^+$), (360 $[M]^+$) and 5a, 5b, 5c-e, 5f-h (262 $[M]^+$), (302 $[M]^+$), (310 $[M]^+$), (326 $[M]^+$), respectively. Moreover, the structures of the **5d** (1941240), **5e** (1941244) and **5f** (1941241) were further approved by the diffraction analysis of a single crystal obtained by slow evaporation of the ethanol solution (Fig. 3). The crystallographic data of molecules (**5d**, **5e** and **5f**) are summarized in Tables 1–3 For details about characterization results, please see the supplementary file.

The bond lengths between carbonyl carbon atoms and oxygen atoms of **5d**, **5e** and **5f** compounds are around 1.22 Å. The average values of C–N bond lengths of **5d**, **5e** and **5f** are around 1.38–1.508 Å. Regarding the bond angles among N atom and carbonyl carbon and vinylic carbon atoms, it is seen that they support the arrangement shown in Tables 2 and 3 The C–C–C and

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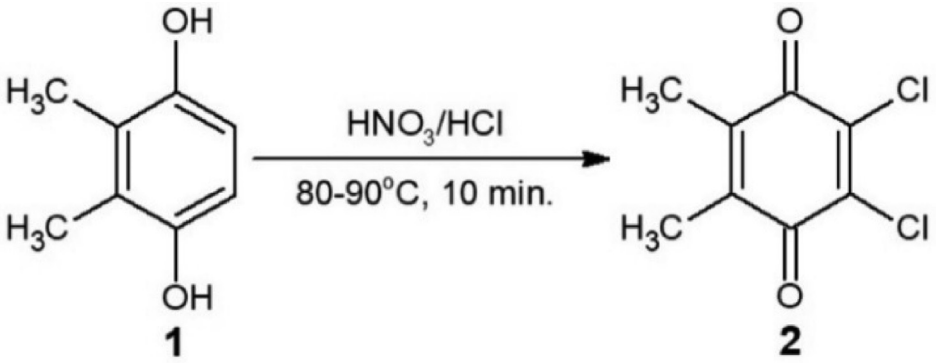
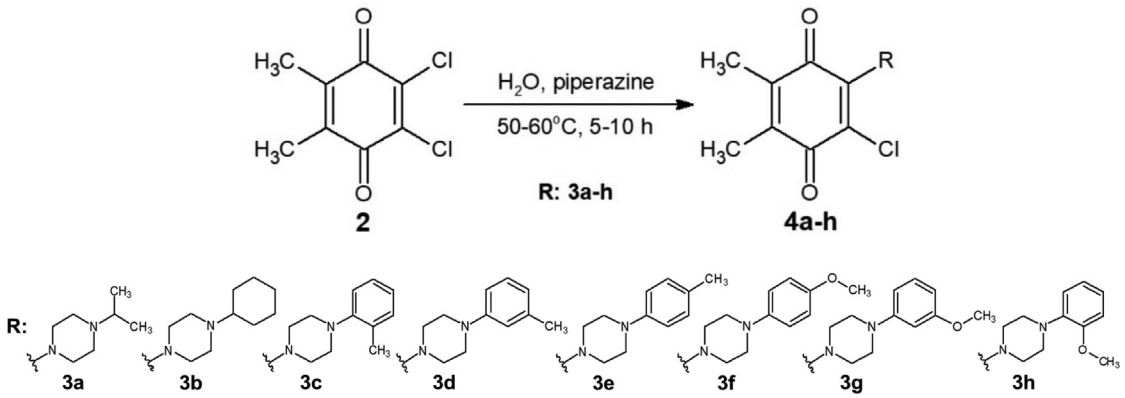
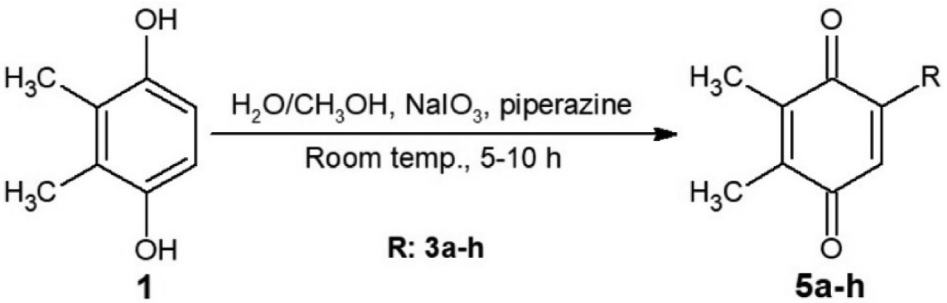


Scheme 1. Preparation way of chlorinated dimethyl-1,4-benzoquinone adopted from Ref. [75].



Scheme 2. Synthesis of piperazine linked target products and the codes of the substituted piperazines. Synthesis method was adopted from Ref. [49].



Scheme 3. Synthesis of piperazine linked unchlorinated derivatives. Synthesis method was adopted from Ref. [72]. R groups can be seen in Scheme 2 above.

C–C–O angles of the compounds **5d**, **5e**, and **5f** are close to 120.8° which proves the structures involving sp^2 hybridized atoms.

3.2. Antimicrobial activity

In vitro antimicrobial activity of the chlorinated/unchlorinated piperazine substituted dimethyl-1,4-benzoquinone derivatives (**4a-h** and **5a-h**) was investigated against four Gram-negative bacteria (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, and *P. mirabilis* ATCC 14153), three Gram-positive bacteria (*E. faecalis* ATCC 29212, *S. epidermidis* ATCC 12228, *S. aureus* ATCC 29213) and two fungi (*C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019) by comparing them with the activity results of the known reference antimicrobials used as drug product. The micro-broth dilutions technique was used by applying the Clinical Laboratory Standards Institute (CLSI) recommendations [73,74]. The MIC (minimum inhibitory concentration) values were calculated by a comparison with standard agents. Table 4 shows the antimicrobial test results of all newly synthesized compounds (**4a-h** and **5a-h**). Codes **a** to **h** stands for the groups of isopropyl, cyclohexyl, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 4-methoxyphenyl, 3-methoxyphenyl, 2-methoxyphenyl and codes **4** and **5** represent the chlorinated and unchlorinated piperazine substituted dimethyl-1,4-benzoquinones, respectively.

Generally speaking, most chlorinated derivatives exhibited more or less activity against tested Gram-positive and Gram-negative bacteria. However, most of these compounds except **4c** and **4f**, which represented clearly no activity, possessed activity against *E. faecalis* with the MIC values around 300 and 1250 μg/mL among Gram-positive bacteria. Furthermore, compound **4b** showed an excellent activity against *E. faecalis* with **78.12** μg/mL MIC value better than that of Amikacin (MIC = 128 μg/mL)

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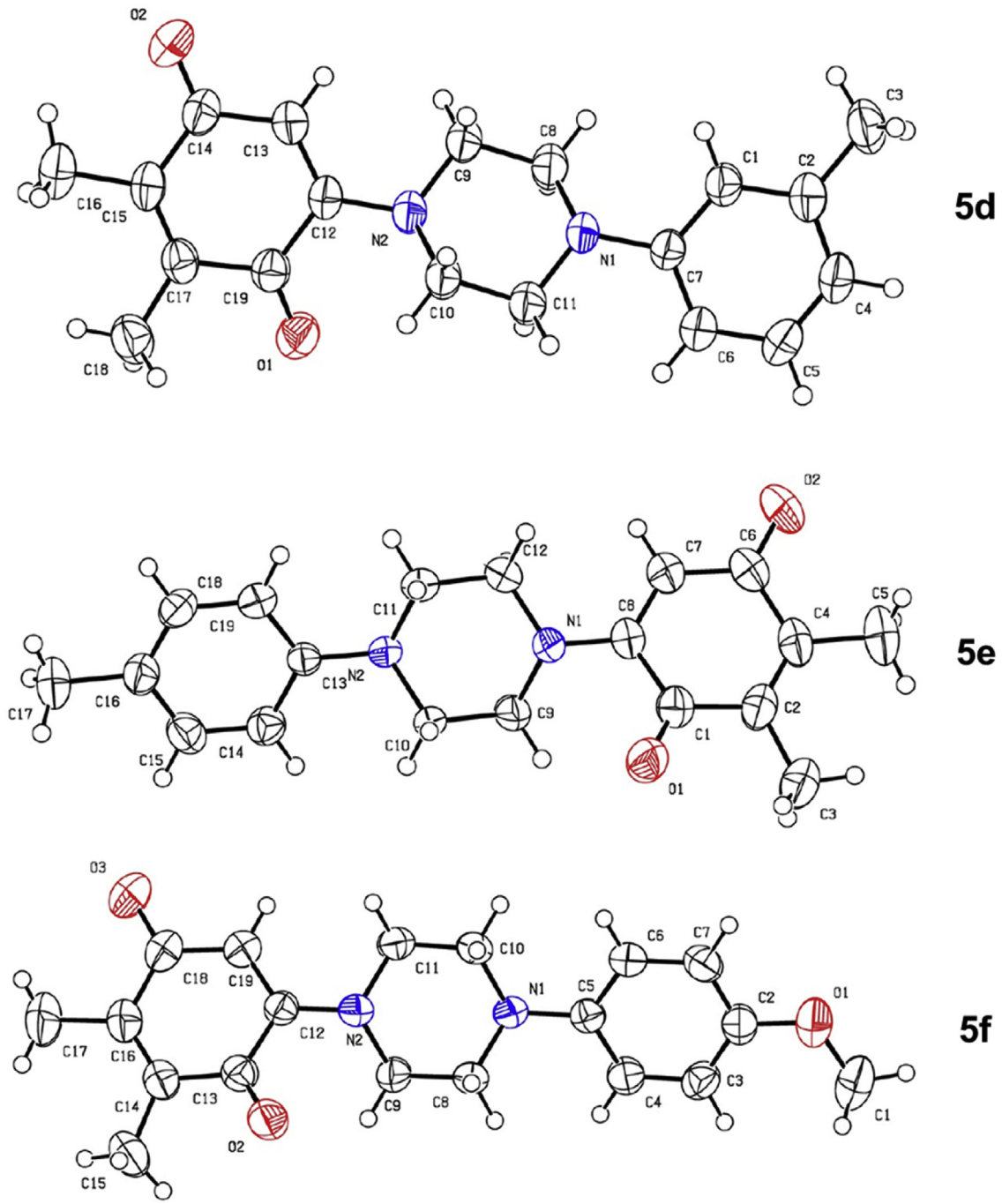

Fig. 3. ORTEP drawings of **5d**, **5e** and **5f**.

reference material. Moreover, the antibacterial activity of compound **4a** against *S. epidermidis* was also outstanding performance at **4.88** μg/mL MIC value which is nearly two-fold better considering the result of Cefuroxime drug (MIC = 9.8 μg/mL) used as reference. Besides that, other compounds, except **4b** that showed activity in a moderate level (MIC = 78.12 μg/mL), displayed lessened performance between the MIC values of 312.5–1250 μg/mL for the *S. epidermidis*. For *S. aureus*; performances of the **4a** (19.53 μg/mL), **4b**, **4c**, **4e** (78.12 μg/mL), **4d** (312.5 μg/mL) and **4f-h** (1250 μg/mL) can be classified as remarkable for the **4a-c**, **4e** and poor for the **4d**, **4f-h** products. Besides that, while nearly all chlorinated compounds shown activity had very low performance at the MIC values of 1250 μg/mL against Gram-negative bacteria than those of reference drugs, **4f** and **4g** were evidently not active for any Gram-negative bacterium. On the other hand, unchlorinated compounds showed hardly any activity against the Gram-negative bacteria except **5a** compound which had even very low activity against *K. pneumoniae* compared to that of Ceftazidime. Besides that, these molecules were active for Gram-positive bacteria but once again with very low level such as around 150, 300, 600 and 1250 μg/mL of MIC values. However, **5a** and **5b** displayed a moderate activity against *E. faecalis* and *S. epidermidis* with the MIC values of 625 and 39.06 μg/mL in comparison to those of Amikacin and Cefuroxime.

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Table 4
In vitro antimicrobial activity results of the chlorinated/unchlorinated piperazine substituted dimethyl-1,4-benzoquinone derivatives (**4a-h** and **5a-h**) and determined MIC values in μg/mL.

Code	Microorganism								
	Gram-negative Bacteria[a]				Gram-positive Bacteria[b]			Fungi[c]	
	PA	EC	KP	PM	EF	SE	SA	CA	CP
4a	1250	1250	1250	625	312.5	**4.88**	19.53	625	312.5
5a	–	–	625	–	625	625	312,5	312,5	156,2
4b	1250	1250	1250	1250	**78.12**	78.12	78.12	625	312.5
5b	–	–	–	–	–	39,06	156,2	156,2	156,2
4c	1250	1250	–	–	–	1250	78.12	1250	625
5c	–	–	–	–	–	1250	–	–	312,5
4d	1250	1250	1250	1250	1250	312.5	312.5	1250	625
5d	–	–	–	–	–	1250	–	–	
4e	1250	1250	1250	1250	1250	156.2	78.12	1250	625
5e	–	–	–	–	–	1250	1250	625	–
4f	–	–	–	–	–	1250	1250	–	–
5f	–	–	–	–	–	1250	1250	625	–
4g	–	–	–	–	1250	–	1250	–	–
5g	–	–	–	–	–	625	1250	312,5	312,5
4h	1250	1250	1250	1250	1250	1250	1250	–	–
5h	–	–	–	–	–	1250	1250	312,5	312,5
Reference Antimicrobial	2.4 Ceftazidime	4.9 Cefuroxime-Na	4.9 Cefuroxime-Na	2.4 Cefuroxime-Na	128.0 Amikacin	9.8 Cefuroxime	1.2 Cefuroxime-Na	4.9 Clotrimazole	0.5 Amphotericin B

[a] Abbreviations and full forms of the screening Gram-negative bacteria: PA; *Pseudomonas aeruginosa* (ATCC 27853), EC; *Escherichia coli* (ATCC 25922), KP; *Klebsiella pneumoniae* (ATCC 4352), PM; *Proteus mirabilis* (ATCC 14153).
[b] Abbreviations and full forms of the screening Gram-positive bacteria: EF; *Enterococcus faecalis* (ATCC 29212), SE; *Staphylococcus epidermidis* (ATCC 12228), SA; *Staphylococcus aureus* (ATCC 29213).
[c] Abbreviations and full forms of the screening Fungi: CA; *Candida albicans* (ATCC 10231), CP; *Candida parapsilosis* (ATCC 22019).

From the point of view of the antifungal activity, the test-cultures *C. albicans* and *C.* parapsilosis were definitely appeared as resistant to the **4f**-**h** and to the **5c**-**d**, **5d**-**f**, respectively. The MIC values of the rest compounds were between 156.2 and 1250 μg/mL for *C. albicans* and 156.2–625 μg/mL for *C. parapsilosis* which indicates substantially lower inhibitory activity by taking into account the MIC values of Clotrimazole (MIC = 4.9 μg/mL) and Amphotericin B (MIC = 0.5 μg/mL).

The main purpose of the preparation of chlorinated and unchlorinated dimethylbenzoquinone series was to investigate the influence of the halogen atom in the quinoid skeletone on the antimicrobial activity of the novel compounds. When the test results of the newly synthesized derivatives against the Gram-negative bacteria were analyzed, it was clearly seen that unchlorinated compounds (**5a**-**h**) did not show any activity against all four tested bacteria strains. On the other hand, most of the chlorinated compounds were active against the Gram-negative bacteria, even in a very low level. The **4c** compound did not reveal any activity against *K. pneumoniae* and *P. mirabilis*.

Nearly the same trend about the halogen effect on the activity is also prevailing for the Gram-positive bacteria as determined for the Gram-negative bacteria. Chlorine atom in quinone moiety either improve the existing poor activity or gain to the inactive molecule an antibacterial activity. Particularly, some great improvements were distinctly observed for the potent molecules of **4a** and **4b** by addition of chlorine atom to **5a** and **5b** compounds. Besides that, it is noteworthy to say that, the activities of **4c**, **4f**, **5c** and **5f** against *E. faecalis*, of **4h** and **5h** against *S. epidermidis*, of **4f**-**h** and **5f**-**h** against *S. aureus* remained constant at the MIC value of 1250 μg/mL and were not influenced from the presence or absence of chlorine atom. Only **5b** and **5g** compounds were outlier within this present trend, because their activities were better than those of chlorinated products against *S. epidermidis*. Independently from the chlorine atom in the structure, the Gram-positive and Gram-negative bacteria were resistant against **4c**, **4f**, **4g**, **5c** and **5f** and no activity was detected for these compounds.

The effect of chlorine atom on the antifungal activity displays a contrary trend compared to that of the antibacterial activity. For *C. albicans*, most unchlorinated compounds were superior to the chlorinated derivatives in terms of the antifungal activity except **4c**-**4d** and **5c**-**5d** analogues which maintained the same promotive halogen influence for the **4c** and **5c**. Besides that, while presence of chlorine provided a positive effect on the activity for **4b**–**5b**, **4d**-**5d**, **4e**-**5e**, absence of chlorine atom enhanced the activity of **4a**-**5a**, **4c**-**5c**, **4g**–**5g** and **4h**–**5h** analogues against *C. parapsilosis* with the exception of completely inactive **4f**-**5f** analogues.

Another discussion on the structure-activity relationship can be made for **4a**-**h** and **5a**-**h** series by comparing the influences of the different substitutions in piperazine groups on the activities. For the chlorinated compounds, there was no connection between the activity results against Gram-negative bacteria which showed mostly MIC values at 1250 μg/mL and isopropyl, cyclohexyl, methylphenyl and methoxyphenyl substituted piperazines moieties. In addition to that, 4-methoxyphenyl substituted (**4f**) and 3-methoxyphenyl substituted (**4g**) molecules were completely inactive against all Gram-negative bacteria. The isopropyl or cyclohexyl groups containing piperazine substituted chlorinated derivatives seemed in particular more potent against Gram-positive bacteria than methyl or methoxyphenyl groups bearing piperazine substituted compounds. While exactly the same MIC values were determined against *E. faecalis* for methylphenyl and methoxyphenyl substituted piperazines including quinones, methylphenylpiperazine containing derivatives were comparatively more active that methoxyphenylpiperazine linked compounds. No activity was observed for methoxyphenylpiperazine substituted quinones against both *C. albicans* and *C. parapsilosis*. However, the methylphenyl containing derivatives possessed relatively worse activity in comparison to those of isopropyl or cyclohexyl carrying compounds. Since hardly any unchlorinated derivative of dimethyl-1,4-benzoquinone exhibited almost no activity against Gram-negative bacteria and *E. faecalis*, a detection of any correlation between different substituents and activities was indeed not possible.

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Furthermore, the same tendency with chlorinated derivatives in structure-activity relationship can be seen for the unchlorinated compounds against *S epidermidis* and *S. aureus*. Namely, the isopropyl or cyclohexyl bearing molecules were comparatively more effective than methyl- or methoxyphenyl including substances. The activity against Gram-positive bacteria was almost not influenced from differently positioned electron donating groups (-CH_3 or $-OCH_3$) in the phenyl ring. The unchlorinated products revealed also either no activity or very high MIC numbers which are also undesired against tested two different fungi. Nevertheless, the isopropyl or cyclohexyl group containing derivatives were comperatively more potent compared to the other unchlorinated compounds against fungi as determined evidently for Gram-positive bacteria. Notwithstanding, the two methoxyphenylpiperazine linked quinones (**5g** and **5h**) were relatively efficient rather than other substituted phenyl ring bearing compounds against *C. albicans* or *C. parapsilosis*.

4. Conclusions

In the present work, 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone (**2**) was prepared by oxidation and chlorination of commercial 2,3-dimethylhydroquinone (**1**) fast but at relatively high temperature according to a previously present method [75]. The chlorinated and piperazine substituted novel 2,3-dimethyl-1,4-benzoquinone molecules (**4a-h**) were obtained from the reaction of the compound (**2**) with various piperazines at moderate temperature and in water by applying a formerly reported procedure in literature [49]. In order to examine the influence of the chlorine atom on the activity, unchlorinated and piperazine linked novel 2,3-dimethyl-1,4-benzoquinone molecules (**5a-h**) were also synthesized from the commercial 2,3-dimethylhydroquinone (**1**) in the presence of $NaIO_3$ and diverse piperazines via a single-step method published in a patent [72]. The characterizations of the novel compounds were performed by applying IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic methods. Besides that, the structures of the some new compounds (**5d**, **5e** and **5f**) were obviously confirmed via the X-ray single crystal method. *In vitro* antimicrobial activity tests of all new compounds (**4a-h** and **5a-h**) were carried out against microbiologic references. The hit molecules among the novel products were **4a** (MIC = 4.88 μg/mL) and **4b** (MIC = 78.12 μg/mL) against *S. epidermidis* and *E. faecalis*, respectively. The both compounds were nearly two-fold more potent than those of standard Cefuroxime (MIC = 9.8 μg/mL) and Amikacin (MIC = 128.0 μg/mL) reference drugs. On the other hand, while unchlorinated compounds possessed almost no activity, chlorinated compounds exhibited activity against Gram-negative bacteria even if they had too high MIC values compared to reference materials. Furthermore, taking into consideration the activity results of the all new compounds against Gram-positive bacteria the chlorine atom in the quinone structure improved the antibacterial activity excluding the exceptions and seemed to be essential for a good and/or comparable antimicrobial activity. The results in the present study about the chlorine effect on the antimicrobial activity were consistent with the previously elucidated results in literature [40,44,65]. The antifungal activity was influenced contrariwise from the presence of chlorine atom in the structure, since the unchlorinated products possessed comparatively lower MIC values that those of chlorinated compounds. While isopropyl or cyclohexyl group containing piperazine substituted compounds revealed a good antimicrobial activity independent of chlorine presence against investigated bacteria and fungi, electron donating methyl or methoxy group including piperazine linked molecules showed either no activity or too high MIC numbers in comparison to those of standard references and this was clearly an evidence that the activity was not effected from the substitution of phenyl ring. Eventually, isopropyl or cyclohexyl group and chlorine atom improved the antimicrobial activities of the 2,3-dimethyl-1,4-benzoquinone derivatives, particularly **4a** and **4b**, and the evaluated results promise room for further improvement to develop potential antimicrobial agents.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2019.127422.

Author contributions

Mahmut Yıldız: Literature investigation, Conceptualization, Design, Experimental work, Writing-Original draft preparation, Writing-Reviewing and Editing, Writing-Revision.

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