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RESEARCH ARTICLE

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Discovery of a new family of heterocyclic amine linked plastoquinone analogs for antimicrobial evaluation

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Abstract

A series of aminobenzoquinones, denoted as PQ analogs (**PQ1-13**), were synthesized by employing a green methodology approach using water as solvent developed by Tandon et al. Subsequently, *in vitro* antimicrobial potential of all PQ analogs was evaluated in a panel of seven bacterial strains (three gram positive and four gram negative bacteria) and three fungi. The antifungal profile of all PQ analogs indicated that four analogs (while **PQ2**, **PQ9**, and **PQ10** were effective against *Candida tropicalis*, **PQ11** is effective against *Candida albicans*) have potent antifungal activity. The results revealed that **PQ9** showed similar antibacterial activity against *Staphylococcus epidermidis* compared clinically prevalent antibacterial drugs cefuroxime. **PQ11** exhibited the highest antibacterial activity against *S. epidermidis*, which was about fourfold better than that of cefuroxime. Owing to their outstanding activities, **PQ9** and **PQ11** were chosen for a further investigation for biofilm and cytotoxicity evaluation. Based on the tests performed, there was a significant positive correlation between inhibition of the biofilm attachment and time. In addition, **PQ9** and **PQ11** showed cytotoxic effects at high concentrations on Balb/3T3, HaCaT, HUVEC, and NRK-52E cells (>24 and >18 μg/mL, respectively). Thus, two analogs (**PQ9** and **PQ11**) were identified as the hits with the strong antibacterial efficiency against the *S. epidermidis* with low MIC values.

KEYWORDS

aminobenzoquinone, antibacterial activity, antibiofilm activity, antifungal activity, cytotoxicity, green chemistry, piperazines, piperidines

1 | INTRODUCTION

Nosocomial infections that allude to healthcare-associated infections (HAIs) and hospital-acquired infections lead to inconvenience like pain, staying in the hospital for a long time, consistent disability, morbidity, and even mortality affecting hundreds of millions of patients in the world (ECDPC, 2014; WHO, 2011). According to the reports of European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), about 80,000 patients (one in 18 patients) in a European hospital approximately one in 31 patients in a U.S. hospital have at least one HAI on any single day (CDCP, 2016; ECDPC, 2013). *Staphylococcus epidermidis* and *Enterococcus faecalis* are two gram positive bacteria becoming major nosocomial pathogens. *S. epidermidis* is a primary cause of HAIs related to implanted prosthetic medical devices (i.e., heart valves, artificial joints, and cerebrospinal fluid shunt) due to the capable of forming biofilms and colonization in these devices (Namvar et al., 2014). *E. faecalis* is accounted for nosocomial infections such as bloodstream, surgical site, and urinary tract (Nallapareddy et al., 2006).

One of the two important mechanisms is that quinones can react with the thiol group of glutathione, which can act as electrophiles. The core structure of quinones could undergo one or two electron reduction to the corresponding semiquinones or hydroquinones, respectively. While a semiquinone occurs by a one-electron reduction, hydroquinone occurs by a two-electron reduction. The second one is the production of the semiquinones by a one-electron reduction (Castro, Mariani, Panek, Eleutherio, & Pereira, 2008; Gutierrez, 2000; Widhalm & Rhodes, 2016). Recent years have witnessed respectable progress in the development of biologically active 1,4-quinones based on natural compounds (Johnson-Ajinwo et al., 2018; Nain-Perez et al., 2017; Nain-Perez, Barbosa, Maltha, & Forlani, 2017). 1,4-Quinone moiety constitutes the fundamental framework of versatile natural or synthetic quinone molecules such as plastoquinone (PQ) (Kawamukai, 2018), mitomycin C (Begleiter, 2000; Sugiura, 1961), and thymoquinone (Collett et al., 2010; El-Dakhakhany, 1963; Glamoclija et al., 2018) since they have extensively found ample and numerous applications in the design and synthesis of pharmacologically active agents exhibiting a wide range of biological activities such as anticancer (Wellington, 2015; Wellington, Kolesnikova, Nyoka, & McGaw, 2019), antibacterial (Janeczko, Demchuk, Strzelecka, Kubinski, & Maslyk, 2016; Jordao et al., 2013), antifungal (Ryu, Oh, Choi, & Kang, 2014; Shrestha et al., 2017), anti-HIV (Alfadhli et al., 2016; Ilina et al., 2002), antimalarial (Carneiro et al., 2016; Pingaew et al., 2015), antiallergic (Lien, Huang, Teng, Wang, & Kuo, 2002), antiinflammatory (Tandon, Chhor, Singh, Rai, & Yadav, 2004), antithrombotic (Jin, Ryu, Moon, Cho, & Yun, 2004; Yuk et al., 2000), lipoxy-genase inhibitory (Richwien & Wurm, 2004), human monoamine oxidase (MOA) inhibitory (Cerqueira, Netz, Diniz, do Canto, & Follmer, 2011), and antiplatelet activities (Lien et al., 2002; Yuk et al., 2000). On the other hand, introducing amino substituents into the 1,4-quinone moiety, named aminoquinones, significantly improves biological properties which are of great importance in pharmaceutical chemistry (Egleton et al., 2014; Pingaew et al., 2015; Prachayasittikul et al., 2014; Xu et al., 2012). Aminoquinones are generally synthesized by the (a) reaction of hydroquinone and amines (Ikeda, Wakabayashi, & Nakane, 1991; Niedermeyer, Mikolasch, & Lalk, 2005), (b) nucleophilic addition reactions of 1,4-quinones with amines (Ryu et al., 2005; Valderrama et al., 2016), and (c) nucleophilic substitution reactions of the methoxy or halogen derivative of 1,4-quinones with amines in different media (Delarmelina et al., 2015; Kacmaz et al., 2018; Pingaew et al., 2015; Ryu, Nho, Jin, Oh, & Choi, 2014; Tandon, Kumar, Mishra, & Shukla, 2012; Tandon & Maurya, 2009).

A literature survey reveals that Tandon et al. have delineated on a number of heterocyclic amine core linked 1,4-quinones (**I-III** in Figure 1) with their antibacterial and antifungal activities by a green methodology approach using water as the solvent (Tandon, Maurya, Verma, Kumar, & Shukla, 2010). Davids et al. have discovered the heterocyclic amine core linked 1,4-quinones (**IV** and **V** in Figure 1) with their impressive antiproliferative activity against the three cancer cell lines (Davids et al., 2012). Abenquines are natural products containing amino acid residues and the first total synthesis of abenquines has

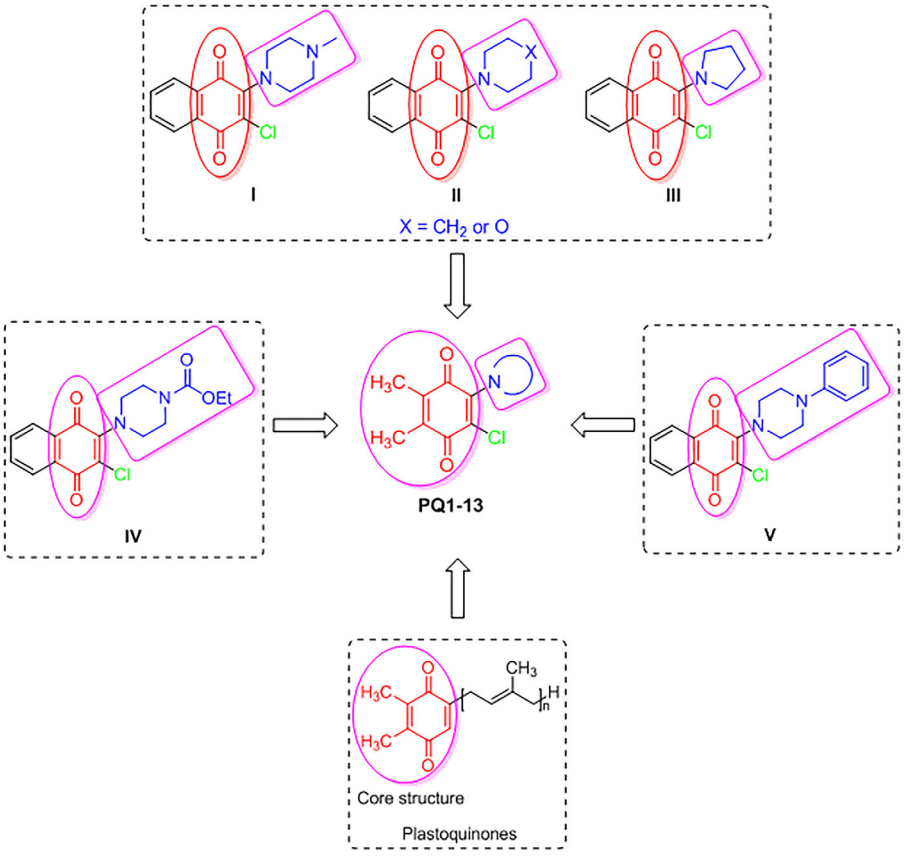


FIGURE 1 Design of target molecules (**PQ1-13**) based on pharmacologically interesting heterocyclic amine core linked 1,4-quinones from literature

been achieved by Nain-Perez and coworkers (Nain-Perez, Barbosa, Maltha, & Forlani, 2016). Nain-Perez et al. have shown abenquines and their analogs' cytotoxicity activities against six human tumor cell lines and nonmalignant mouse fibroblasts (Nain-Perez, Barbosa, Rodriguez-Hernandez, Kramell, et al., 2017). Additionally, the same group has also used this class of structures as a model for the synthesis of new herbicides targeting photosynthesis (Nain-Perez, Barbosa, Maltha, Giberti, & Forlani, 2017). Inspired by the above findings and as a part of our ongoing program for the generation of biologically active analogs, we thought of designing the newer heterocyclic amine linked PQ analogs by the introducing of heterocyclic amine core linked into the 1,4-quinone to ascertain their antimicrobial profiles. Some of the biologically active compounds containing 1,4-quinone moiety and heterocyclic amine, which provide the basic pharmacophore in designing our target compounds are shown in Figure 1.

With the rapid emergence of resistant bacteria in the world, the effectiveness of antibiotics saving millions of lives is endangered. Research and development of novel antimicrobial drugs are the vital roles that must be adopted to fight the emergence and spread of antibiotic resistance in worldwide. In this context, our laboratory is dedicated to synthesizing pharmacologically active substances based on 1,4-quinone moiety (Bayrak et al., 2017; Tuyun et al., 2015; Yildirim et al., 2017). Encouraged by all these facts and in continuation of our previous work on the discovery of pharmacologically important PQ analogs employing 2,3-dimethyl-1,4-benzoquinone moiety of PQ as a core structure linked with heterocyclic amines, in this article, we report the design, synthesis, antimicrobial evaluation, antibiofilm evaluation, cytotoxicity, and structure–activity relationship (SAR) of heterocyclic amine linked PQ analogs.

2 | RESULTS AND DISCUSSION

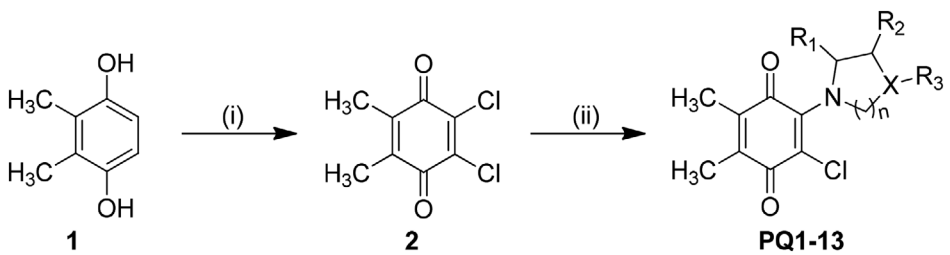
2.1 | Chemical synthesis

1,4-Quinones react with various amines to generate amino-1,4-quinone compounds in different media with nucleophilic substitution reactions or nucleophilic addition reactions by oxidative addition pathway. However, this conversion has already been affected from the reaction medium and it is found that the reaction takes place smoothly in water with high yields as a solvent instead of other solvents. When the precursor (**2**) was stirred with different heterocyclic amines (2.2 equivalent) viz. pyrrolidine, piperidine, hexamethyleneimine, 2-methylpiperidine, 3-methylpiperidine, 4-methylpiperidine, 4-piperidinecarboxamide, 1,4-dioxa-8-azaspiro [4.5]decane, 4-benzylpiperidine, morpholine, thiomorpholine, ethyl piperazine-1-carboxylate, 1-phenylpiperazine, and 1-(4-nitrophenyl) piperazine at around 50–60 °C for 6–12 hr using water as solvent, target molecules, namely PQ analogs (**PQ1-13**), were obtained as shown in Scheme 1 according to the developed method by Tandon et al. (2010). Among used heterocyclic amines, substitution reaction of the precursor (**2**) with 2-methylpiperidine failed to produce any product. Futile efforts by changing the reaction conditions did not yield the desired product possibly due to steric hindrance of the bulky *ortho* substituent as the spatial arrangement of atoms or functional groups near the reacting site hindered the reaction from proceeding. **PQ1**, **PQ2**, **PQ9**, and **PQ10** were prepared before by our group (Tuyun & Yıldız, 2018), while the other PQ analogs are novel molecules.

The structures of all PQ analogs (**PQ1-13**, Table 1) were determined on the basis of their analytical and spectroscopic data (FTIR, ^{1}H NMR, ^{13}C NMR, and MS). ^{13}C NMR spectra of the PQ analogs showed the downfield shifts of two quaternary carbon in the region between 180 and 184 ppm indicated the nonequivalent carbonyl carbons within the quinone moiety. In ^{13}C NMR spectra, two carbon atoms in the methyl groups of quinone moiety (C-5 and C-6) were displayed at around 12 ppm. The protons of methyl groups attached to quinone moiety appeared at around 2 ppm. In the mass spectra of all PQ analogs, the molecular ions were observed with the base peaks corresponding to the molecular weights of the analogs. Further, in the FTIR, the absorption signal at around 1,650 cm^{-1} indicated the presence of the carbonyl group. Additionally, the structures of the **PQ7** (1894854) and **PQ12** (1894852) were further confirmed by the single crystal diffraction (Figure 2) (for details, please see the Supplementary file).

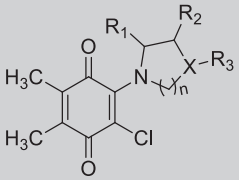
2.2 | Determination of the in vitro antibacterial and antifungal activity

After synthesis and characterization, we studied the in vitro antimicrobial activity of PQ analogs (**PQ1-13**) against three gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 29212), four gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, and *Proteus mirabilis* ATCC 14153), and three fungi (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750) by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI, 1997; CLSI, 2006). The



SCHEME 1 Synthesis of the chlorinated PQ analogs (**PQ1-13**): (i) HNO_3/HCl, 10 min, 90 °C; (ii) substituted heterocyclic amines, H_2O, 50–60 °C, 6–18 hr

TABLE 1 In vitro antibacterial activity results of the PQ analogs (**PQ1-13**)



PQ1-13

ID	*n*	X	R_1	R_2	R_3	Microorganisms: Gram positive bacteria (MIC, μg/mL)		
						Staphylococcus aureus	*Staphylococcus epidermidis*	*Enterococcus faecalis*
PQ1	1	CH	H	H	H	–	312.5	625
PQ2	2	CH	H	H	H	–	78.12	312.5
PQ3	3	CH	H	H	H	625	1,250	625
PQ4	2	CH	H	CH_3	H	9.76	1,250	625
PQ5	2	CH	H	H	CH_3	78.12	1,250	–
PQ6	2	CH	H	H	$CONH_2$	9.76	625	625
PQ7	2	C	H	H	OCH_2CH_2O	–	19.53	625
PQ8	2	CH	H	H	CH_2Ph	19.53	625	–
PQ9	2	O	H	H	–	625	9.76	156.25
PQ10	2	S	H	H	–	–	19.53	156.25
PQ11	2	N	H	H	COOEt	625	2.44	–
PQ12	2	N	H	H	Ph	–	625	–
PQ13	2	N	H	H	4-NO_2-Ph	–	–	–
Amikacin						–	–	128
Cefuroxime						–	9.8	–
Cefuroxime-Na						1.2	–	–

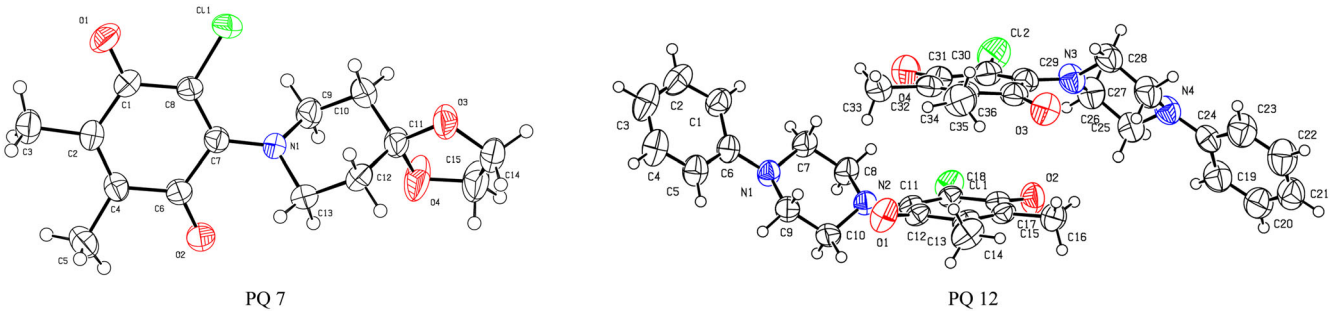
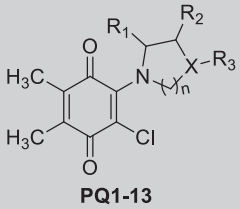


FIGURE 2 ORTEP drawings of **PQ7** and **PQ12**

MIC (minimum inhibitory concentration) values were determined by comparison with standard agents.

The antimicrobial assay results of all the PQ analogs (**PQ1-13**) are given in Tables 1 and 2. Concerning the antibacterial activity, the gram positive bacteria were more susceptible to PQ analogs than the gram negative ones. Generally, the results showed that some compounds displayed varying effects on the growth of the tested gram positive bacterial strains. The test-cultures *E. coli*, *K. pneumoniae*, and *P. mirabilis* were appeared as resistant to the most synthesized compounds between the studied concentrations at 2500–1.22 μg/mL. The results showed that the analogs (**PQ1**, **PQ5**, and **PQ8-13**) exhibited no antibacterial activity against the gram negative bacteria. **PQ2-4**, **PQ6**, and **PQ7** had a weak activity against *P. aeruginosa* with the MIC value at 625 μg/mL. Additionally, **PQ3** and **PQ4** had the same inhibitory activity against *P. mirabilis* and *E. coli* with the MIC value at 625 μg/mL, respectively. Some of the PQ analogs exhibited moderate activity against gram positive bacteria. **PQ7** and **PQ10** showed good activity against *S. epidermidis* with the MIC value of 19.53 μg/mL (twofold less potent compared reference standard cefuroxime). As shown in Table 1, among the all PQ analogs (**PQ1-13**), the activity of the **PQ11** was the best and most potent activity against *S. epidermidis* which was about fourfold better than that of cefuroxime. Notably, **PQ11**, completely inhibited the growth of *S. epidermidis*, was tested at the MIC level of 2.44 μg/mL. **PQ9** had the same inhibitory activity against *S. epidermidis* as that of cefuroxime (MIC = 9.76 μg/mL). **PQ9** and **PQ10** possessed moderate activity against *E. faecalis* which had the MIC values

TABLE 2 In vitro antifungal activity results of the PQ analogs (**PQ1-13**)



PQ1-13

						Microorganisms		
						Fungi (MIC, μg/mL)		
ID	***n***	**X**	**R_1**	**R_2**	**R_3**	*Candida albicans*	*Candida parapsilosis*	*Candida tropicalis*
PQ1	1	CH	H	H	H	78.12	156.25	39.06
PQ2	2	CH	H	H	H	39.06	78.12	9.76
PQ3	3	CH	H	H	H	156.25	78.12	78.12
PQ4	2	CH	H	CH_3	H	–	312.5	156.25
PQ5	2	CH	H	H	CH_3	–	312.5	312.5
PQ6	2	CH	H	H	$CONH_2$	625	625	312.5
PQ7	2	C	H	H	OCH_2CH_2O	78.12	156.25	156.25
PQ8	2	CH	H	H	CH_2Ph	625	78.12	156.25
PQ9	2	O	H	H	–	78.12	78.12	19.53
PQ10	2	S	H	H	–	78.12	78.12	19.53
PQ11	2	N	H	H	COOEt	19.53	156.25	156.25
PQ12	2	N	H	H	Ph	–	–	312.5
PQ13	2	N	H	H	4-NO_2-Ph	156.25	–	625
Amphotericin B						–	0.5	1
Clotrimazole						4.9	–	–

156.25 μg/mL very close to that of Amikacin. The most potent analogs against *S. aureus* in the series were **PQ4** and **PQ6**; but these were eight-fold less potent (MIC = 9.76 μg/mL) than reference standard cefuroxime-Na. Evaluation of the antifungal activity of the PQ analogs exhibited that most of the PQ analogs (**PQ1-13**) possessed activity against *C. albicans*, *C. parapsilosis*, and *C. tropicalis* with MIC values of between 9.76 and 625 μg/mL. **PQ2** was the most potent analog against *C. tropicalis* (MIC = 9.76 μg/mL) while **PQ11** was the most potent analog against *C. albicans* (MIC = 19.53 μg/mL). **PQ9** and **PQ10** were also effective against *C. tropicalis* at the MIC level of 19.53 μg/mL (Table 2). According to the results, two analogs (**PQ9** and **PQ11**) were the most effective analogs and chosen for further investigation against the standard *S. epidermidis* and *C. albicans*. For that reason, we investigated the potential antimicrobial activity against each of 20 clinically obtained strains of *Candida* spp. and *Staphyloccocus* spp. Susceptibility testing demonstrated that the MIC ranges for **PQ9** and **PQ11** were 78.12–625 and 39.06–625 μg/mL for *Candida* spp. and 19.53–>2,500 and 9.76–>2,500 μg/mL for *Staphyloccocus* spp., respectively.

2.3 | Structure–activity relationship study for antibacterial and antifungal activity

The aim of using versatile heterocyclic amines was to investigate the effects of the structural features of the PQ analogs on the antimicrobial activity. A structure–activity relationship (SAR) of this class of the PQ analogs was investigated by analyzing the structural features that are essential for activity. The results obtained from the antimicrobial activity study are quite interesting since they present some correlation against *S. epidermidis*. **PQ1** with the 5-membered ring containing only one nitrogen atom had low antibacterial activity against *S. epidermidis* (MIC = 312.5 μg/mL). With the ring expansion from a 5-membered to a 7-membered ring containing only one nitrogen atom, the best MIC value was for a 6-membered ring in the selected **PQ1-3** against *S. epidermidis*. When a methyl or an amide group was added in the 3- or 4-position of the 6-membered ring (**PQ4-6**), the activity dramatically decreased. Leading the remarkable increase has been noticed for PQ analogs containing ethylenedioxy group in the 4-position of the 6-membered ring (**PQ7**). For the analogs by the introduction of the second heteroatom into the heterocyclic amine such as sulfur or oxygen to afford **PQ9** or **PQ10**, **PQ9** had the same activity with the control drug against *S. epidermidis*. **PQ11**, both replacing the heteroatom with a nitrogen atom and having an ester group in the 4-position on the 6-membered ring, had the best activity against *S. epidermidis*. It is thus evident that PQ analogs containing 6-membered ring with additional heteroatom favor the inhibitory activity against *S. epidermidis*.

2.4 | Determination of the in vitro antibiofilm activity

Because of the only biofilm which susceptible to studied active molecules were *S. epidermidis* and *C. albicans*, we performed both biofilm

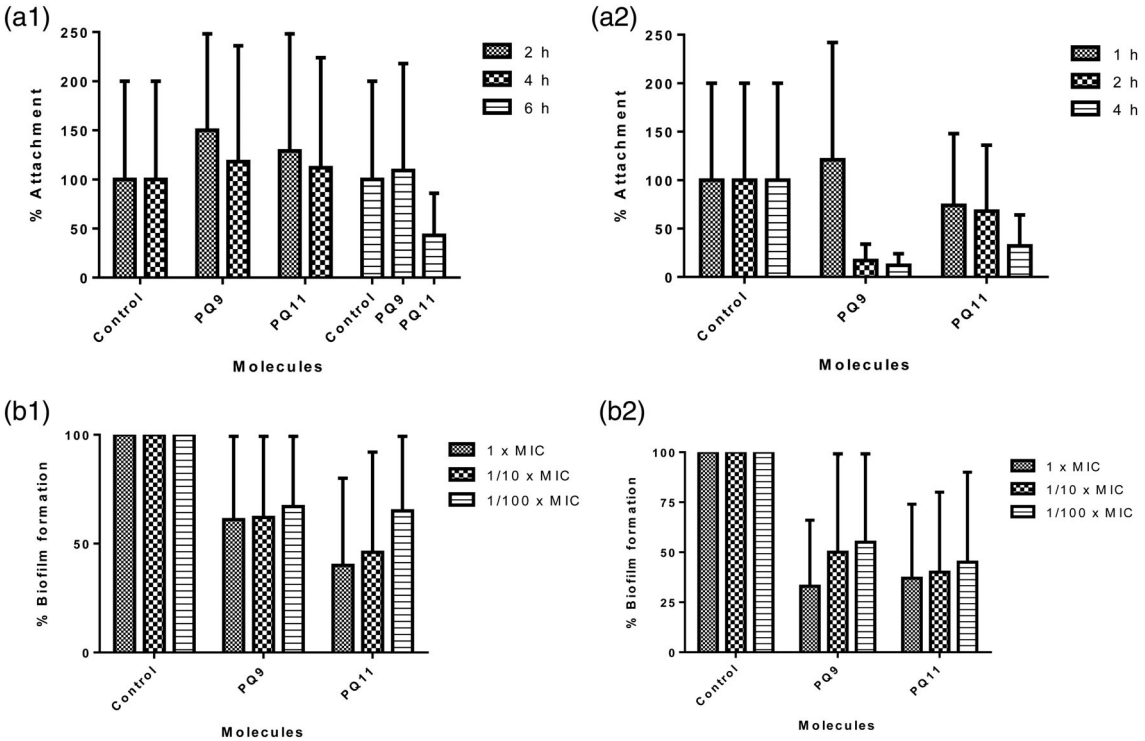


FIGURE 3 Inhibition of *Candida albicans* and *Staphylococcus epidermidis*: (a) Surface attachment to the wells contained 1/10 × MIC of molecules and an inoculum of 1×10^7 cfu/200 μL, incubated for 2, 4, or 6 hr for *C. albicans* (a1) and for *S. epidermidis* (a2) incubated for 1, 2, or 4 hr at 37 °C; (b) Biofilm formation in each well contained 1 ×, 1/10 ×, or 1/100 × MIC of molecules and an inoculum of 5×10^5 cfu/200 μL, incubated for 24 hr at 37 °C (b1 for *C. albicans* and b2 for *S. epidermidis*). Control bars indicate microorganisms without molecules, accepted as 100%. Six wells were used for each molecule. Each experiment is representative of two independent tests, and the error bars indicate the standard deviations. All differences between the control and the molecules treated biofilms were statistically significant ($p < .001$)

attachment and inhibition of biofilm formation assays with them. Based on the tests performed, there was a significant positive correlation between inhibition of the biofilm attachment and time for both analogs (**PQ9** and **PQ11**), especially for *S. epidermidis* for **PQ9**. In addition, they showed inhibitory activity against biofilm formation at 24 hr according to their concentrations (Figure 3). Importantly, the results showed that both analogs (**PQ9** and **PQ11**) are able to reduce biofilm mass, ranging from around 30% to 60%, at 1 × MIC, 1/10 × MIC, and 1/100 × MIC, respectively (Figure 3, b1 and b2).

Regarding the control bars, we could say that microorganisms in biofilm could alter physiological properties from their planktonic counterparts. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated (Donlan, 2001; Ghannoum & O'Toole, 2004; Harrison, Ceri, Stremick, & Turner, 2004; Sanchez et al., 2019). So, in these kinds of studies, variation in the results could be observed. For this reason, six wells for each molecule in each experiment and two repetitions were performed.

2.5 | Cytotoxic evaluation with MTT assay

Due to their significant inhibitory activity potentials against *S. epidermidis*, the cytotoxic effects of **PQ9** and **PQ11** analogs were determined by using MTT assay on Balb/3T3 (mouse embryonic fibroblasts), HUVEC (Human umbilical vein endothelial cells), HaCaT (human keratinocytes), and NRK-52E (normal rat kidney cells) after 24 hr exposure. Our results demonstrated that the analog **PQ9** did not affect cell viability at tested concentrations below 12.5 μg/mL and showed significant concentration-dependent cytotoxic activity in the concentration range of 12.5–100 μg/mL after 24 hr exposure on tested cell lines ($p < .05$), (Figure 4a). While the analog **PQ11** did not show cytotoxic effects tested concentrations below 6.25 μg/mL and exhibited significant concentration-dependent cytotoxic activity in the concentration range of 6.25–100 μg/mL after 24 hr exposure ($p < .05$), (Figure 4b). Based on the IC_{50} values, the analog **PQ11** is found to have greater cytotoxic activity than the analog **PQ9** for all of the tested cell lines ($p < .05$). HaCaT were the most sensitive cells to both of the compounds with IC_{50} values of 24.36 μg/mL of **PQ9** and 18.14 μg/mL of **PQ11**. While Balb/3T3 were the least affected cell line with IC_{50} values of 51.76 μg/mL of **PQ9** and 33.91 μg/mL of **PQ11**. IC_{50} values of **PQ9** and **PQ11** were 36.76 and 26.41 μg/mL on HUVEC, respectively. **PQ9** and **PQ11** affected the cell viability of NRK-52E cells similarly to the other cell lines with IC_{50} values of 36.17 μg/mL and 22.73 μg/mL, respectively. Taken together, these provide the foundation for further investigation on whether the analogs **PQ9** and **PQ11** could be used

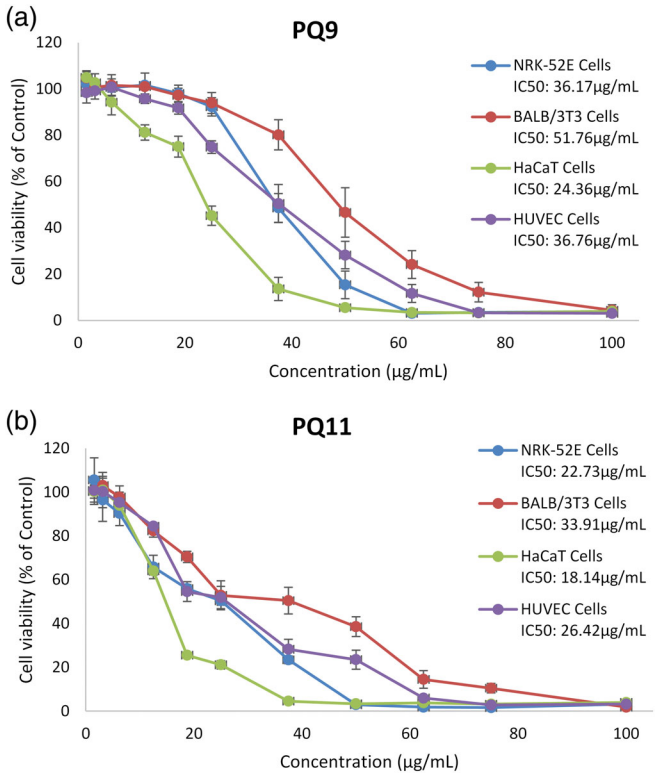


FIGURE 4 Cytotoxicity of **PQ9** and **PQ11** compounds on Balb/3T3 (mouse embryonic fibroblasts), HUVEC (human umbilical vein endothelial cells), HaCaT (human keratinocytes), and NRK-52E (normal rat kidney cells) after 24 hr exposure with MTT assay. Cells were treated with **PQ9** and **PQ11** at 1.5625, 3.125, 6.25, 12.5, 18.75, 25, 37.5, 50, 62.5, 75, and 100 μg/mL concentrations. Data are presented as the mean ± *SD* (*n* = 3)

for their antibacterial properties at the concentrations without substantial cytotoxicity.

3 | CONCLUSIONS

In the present study, the synthesis and *in vitro* the antibacterial and antifungal activity profile of a library of heterocyclic amine linked PQ analogs have been reported. Two analogs (**PQ7** and **PQ10**) had the inhibitory activity against *S. epidermidis*, these were twofold less potent than the reference standard of cefuroxime. Among the most promising antibacterial compounds, **PQ11** showed better antibacterial activity than clinically prevalent antibacterial drug cefuroxime against *S. epidermidis*, while **PQ9** had similar activity. These two analogs (**PQ9** and **PQ11)** effectively decreased biofilm formations against *C. albicans* and *S. epidermidis* and had a better profile than control at different concentrations and time. Thus, SAR study showed that the introduction of the second heteroatom, such as nitrogen or oxygen atom, on the six-membered ring played an important role in the antibacterial activity. Evaluation of cytotoxicity with nontumor cell lines showed that IC_{50} values are >24 μg/mL for **PQ9** while >18.14 μg/mL for **PQ11**. Similar to their antibacterial activities, **PQ11** exhibited more cytotoxic potential than **PQ9** for all of the cell lines that we used. Taken together, these findings provide the foundation for further investigation on whether **PQ9** and **PQ11** can be used for their antibacterial properties at the concentrations without substantial cytotoxicity. The analogs are more selective for the antibacterial activity, especially for gram positive bacteria than fungi. PQ analogs (**PQ9** and **PQ11**) appear as the lead molecules as potent antibacterial agents for further studies, that is, improving the biological activity and the mechanism of action of these analogs against bacterial and fungal strains. Therefore, these analogs could be applied in the future to control *S. epidermidis* infections one of the major HAIs.

4 | EXPERIMENTAL SECTION

4.1 | Chemistry

Melting points (mp) were determined using capillary tubes on a Buchi B-540 melting point apparatus and were uncorrected. All corresponding substituted heterocyclic amines, reagents and solvents employed in the synthesis process were obtained from commercial suppliers and used without further purification unless otherwise noted. Merck DC-plates (aluminum based, silica gel 60 F254) were used for analytical thin layer chromatography (TLC) purchased from Merck KGaA. Plates were viewed by UV light (254 nm). Silica gel 60 (Merck, 63–200 μm particle sized, 60–230 mesh) was used for column chromatographic separations. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were obtained on a Varian^{UNITY} INOVA spectrometers (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) in $CDCl_3$ refer to the solvent signal center at δ 7.19 and δ 76.0 ppm. The chemical shifts (δ) and coupling constants (*J*) are expressed in parts per million (ppm) and hertz (Hz), respectively. Standard abbreviations indicating multiplicity were used as follows: *s* (singlet), *br s* (broad singlet), *d* (doublet), *t* (triplet), *q* (quartet), *dt* (doublet of triplets), *td* (triplet of doublets), *qd* (quartet of doublets), and *m* (multiplet). FTIR spectra were recorded as ATR on either a Thermo Scientific Nicolet 6700 spectrometer or an Alpha T FTIR spectrometer. Mass spectra were run by a BRUKER Microflex LT by MALDI (Matrix Assisted Laser Desorption Ionization)-TOF technique via the addition of 1,8,9-anthracenetriol (DIT, dithranol) as the matrix. Data for the single crystal compounds were obtained with Bruker APEX II QUAZAR three-circle diffractometer. Indexing was performed using APEX2 (APEX2, 2014). Data integration and reduction were carried out with SAINT (SAINT, 2013). Absorption correction was performed by a multi-scan method implemented in SADABS (SADABS, 2012). The Bruker SHELXTL (SHELXTL, 2000) software package was used for structures solution and structures refinement. Aromatic C-bound and N-bound hydrogen atoms were positioned geometrically and refined using a riding mode. Crystal structure validations and geometrical calculations were performed using the Platon software (Spek, 2009). Mercury software (Macrae et al., 2006) was used for visualization of the .cif files. The precursor (**2**) was synthesized using the reported method in the literature (Ryu &

Lee, 2006). **PQ1**, **PQ2**, **PQ9**, and **PQ10** have been prepared according to the literature (Tuyun & Yıldız, 2018).

4.2 | General procedure for the preparation of heterocyclic amine linked plastoquinone analogs (PQ1-13) (Tandon et al., 2010)

A suspension of the appropriate heterocyclic secondary amines (1.10 mmol, 2.2 equiv) and 2,3-dichloro-5,6-dimethyl-1,4-benzoquinone **2** (0.1025 g, 0.50 mmol) in H_2O (10 mL) was stirred at 50–60 °C for 6–18 hr until consumption of the 1,4-benzoquinone. The reaction mixture was cooled to ambient temperature. After evaporation of the solvent, the residue was dissolved with $CHCl_3$ (50 mL), and the solution was washed sequentially with water (3 × 30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure, and the residue was purified by means of column chromatography on silica gel to give target compounds.

4.2.1 | 2-Chloro-5,6-dimethyl-3-(pyrrolidin-1-yl)-1,4-benzoquinone (PQ1)

The title analog (**PQ1**) was prepared according to the literature and identified by comparing its spectral properties to those reported for the analog in the literature (Tuyun & Yıldız, 2018).

4.2.2 | 2-Chloro-5,6-dimethyl-3-(piperidin-1-yl)-1,4-benzoquinone (PQ2)

The title analog (**PQ2**) was prepared according to the literature and identified by comparing its spectral properties to those reported for the analog in the literature (Tuyun & Yıldız, 2018).

4.2.3 | 2-(Azepan-1-yl)-3-chloro-5,6-dimethyl-1,4-benzoquinone (PQ3)

Following general procedure by applying hexamethyleneimine (0.1091 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ3** as a dark purple oil. Yield: 24%. FTIR (ATR) υ (cm^{-1}): 2,924, 2,854 ($CH_{aliphatic}$), 1,660 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.59–1.63 (m, 4H, CH_2), 1.69–1.74 (m, 4H, CH_2), 1.92 (t, *J* = 1.5 Hz, 3H, CH_3), 1.98 (t, *J* = 1.5 Hz, 3H, CH_3), 3.50–3.54 (m, 4H, NCH_2). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 12.7, 13.1 (CH_3), 27.4, 29.1, 29.7, 54.4 ($CH_{2Azepan}$), 117.9, 138.7, 140.9, 150.4 (C_q), 180.1, 184.9 (>C=O). MS (MALDI TOF) *m*/*z*: 267 $[M]^+$. Anal. Calcd. for $C_{14}H_{18}ClNO_2$ (267.75).

4.2.4 | 2-Chloro-5,6-dimethyl-3-(3-methylpiperidin-1-yl)-1,4-benzoquinone (PQ4)

Following general procedure by applying 3-methylpiperidine (0.1091 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ4** as a dark purple oil. Yield: 35%. FTIR (ATR) υ (cm^{-1}): 2,927, 2,848 ($CH_{aliphatic}$), 1,659 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 0.82 (d, *J* = 6.3 Hz, 3H, $CH_{3Piperidine}$), 1.03–1.12 (m, 1H, $CH_{Piperidine}$), 1.62–1.69 (m, 2H, $CH_{2Piperidine}$), 1.71–1.81 (m, 2H, $CH_{2Piperidine}$), 1.92 (d, *J* = 2.44 Hz, 3H, CH_3), 1.98 (d, *J* = 2.44 Hz, 3H, CH_3), 2.69–2.75 (m, 1H, $NCH_{2Piperidine}$), 3.02–3.08 (m, 1H, $NCH_{2Piperidine}$), 3.48–3.57 (m, 2H, $NCH_{2Piperidine}$). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 12.6, 13.1 (CH_3), 19.0, 26.2, 32.1, 32.7, 52.0, 59.1 ($CH_{2Piperidine}$), 118.4, 138.5, 141.2, 148.7 (C_q), 180.1, 184.1 (>C=O). MS (MALDI TOF) *m*/*z*: 267 $[M]^+$. Anal. Calcd. for $C_{14}H_{18}ClNO_2$ (267.75).

4.2.5 | 2-Chloro-5,6-dimethyl-3-(4-methylpiperidin-1-yl)-1,4-benzoquinone (PQ5)

Following general procedure by applying 4-methylpiperidine (0.1091 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ5** as a purple solid. Yield: 32%, mp 76–78 °C. FTIR (ATR) υ (cm^{-1}): 2,945, 2,904, 2,863 ($CH_{aliphatic}$), 1,655 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 0.91 (d, *J* = 6.8 Hz, 3H, $CH_{3Piperidine}$), 1.31 (qd, *J* = 12.7 and 3.9 Hz, 2H, $CH_{2Piperidine}$), 1.49–1.59 (m, 1H, $CH_{Piperidine}$), 1.61–1.66 (m, 2H, $CH_{2Piperidine}$), 1.92 (d, *J* = 1.5 Hz, 3H, CH_3), 1.98 (d, *J* = 1.0 Hz, 3H, CH_3), 3.10 (td, *J* = 13.2 and 2.4 Hz, 2H, $NCH_{2Piperidine}$), 3.59 (dt, *J* = 13.2 and 2.4 Hz, 2H, $NCH_{2Piperidine}$). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 12.6, 13.1 (CH_3), 21.9, 30.6, 35.1, 51.9 ($CH_{2Piperidine}$), 118.5, 138.5, 141.2, 148.8 (C_q), 180.1, 184.1 (>C=O). MS (MALDI TOF) *m*/*z*: 267 $[M]^+$. Anal. Calcd. for $C_{14}H_{18}ClNO_2$ (267.75).

4.2.6 | 1-(2-Chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)piperidine-4-carboxamide (PQ6)

Following general procedure by applying 4-piperidinecarboxamide (0.1410 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ6** as a dark purple solid. Yield: 38%, mp 175–176 °C. FTIR (ATR) υ (cm^{-1}): 3,423, 3,315, 3,215 (NH_{amide}), 2,956, 2,917, 2,849 ($CH_{aliphatic}$), 1,660 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.80–1.93 (m, 4H, $CH_{2Piperidine}$), 1.93 (d, *J* = 1.0 Hz, 3H, CH_3), 1.99 (d, *J* = 1.0 Hz, 3H, CH_3), 2.31–2.39 (m, 1H, $CH_{Piperidine}$), 3.15 (td, *J* = 13.7 and 2.4 Hz, 2H, $NCH_{2Piperidine}$), 3.66 (d, *J* = 13.7 Hz, 2H, $NCH_{2Piperidine}$), 5.55 (br s, 1H, NH), 5.70 (br s, 1H, NH). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 12.6, 13.1 (CH_3), 29.7, 42.2, 50.9 ($CH_{2Piperidine}$), 120.0, 138.8, 141.2, 148.4 (C_q), 176.8, 180.2, 183.9 (>C=O). MS (MALDI TOF) *m*/*z*: 267 $[M\text{-}2CH_3]^+$. Anal. Calcd. for $C_{14}H_{17}ClN_2O_3$ (296.75).

4.2.7 | 2-Chloro-5,6-dimethyl-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-1,4-benzoquinone (PQ7)

Following general procedure by applying 1,4-dioxa-8-azaspiro[4.5]decane (0.1575 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ7** as a dark red solid. Yield: 50%, mp 94–95 °C. FTIR (ATR) υ (cm^{-1}): 2,949, 2,919, 2,863 ($CH_{aliphatic}$), 1,654 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.77 (t, *J* = 5.9 Hz, 4H,

CH_2), 1.93 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 3.46 (t, J = 5.4 Hz, 4H, NCH_2), 3.92 (s, 4H, OCH_2). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 11.6, 12.1 (CH_3), 35.0 (CH_2), 48.4 ($NCH_{2piperidine}$), 63.4 (OCH_2), 105.7 ($C_{piperidine}$), 118.6, 137.7, 140.2, 147.5 (C_q), 179.2, 182.9 (>C=O). MS (MALDI TOF) *m/z*: 312 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{18}ClNO_4$ (311.76).

4.2.8 | 2-(4-Benzylpiperidin-1-yl)-3-chloro-5,6-dimethyl-1,4-benzoquinone (PQ8)

Following general procedure by applying 4-benzylpiperidine (0.1928 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ8** as a dark purple oil. Yield: 33%. FTIR (ATR) υ (cm^{-1}): 3,026 ($CH_{aromatic}$), 2,918, 2,848 ($CH_{aliphatic}$), 1,659 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.38 (qd, J = 12.2 and 3.9 Hz, 2H, $CH_{2Piperidine}$), 1.60–1.66 (m, 2H, $CH_{2Piperidine}$), 1.66–1.74 (m, 1H, $CH_{Piperidine}$), 1.89–1.92 (m, 3H, CH_3), 1.95–1.97 (m, 3H, CH_3), 2.50 (d, J = 6.8 Hz, 2H, CH_2), 3.05 (td, J = 13.2 and 2.0 Hz, 2H, $NCH_{2Piperidine}$), 3.56–2.62 (m, 2H, $NCH_{2Piperidine}$), 7.06–7.09 (m, 2H, $CH_{Aromatic}$), 7.09–7.13 (m, 1H $CH_{Aromatic}$), 7.21 (t, J = 7.8 Hz, 2H $CH_{Aromatic}$). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 12.6, 13.1 (CH_3), 33.1, 37.8, 43.2, 51.8 ($CH_{2Piperidine}$ and CH_2), 118.7, 125.9, 128.2, 129.2, 138.5, 140.1, 141.2, 148.7 ($CH_{aromatic}$ and C_q), 180.1, 184.1 (>C=O). MS (MALDI TOF) *m/z*: 343 $[M]^+$. Anal. Calcd. for $C_{20}H_{22}ClNO_2$ (343.85).

4.2.9 | 2-Chloro-5,6-dimethyl-3-morpholino-1,4-benzoquinone (PQ9)

The title analog (**PQ9**) was prepared according to the literature and identified by comparing its spectral properties to those reported for the analog in the literature (Tuyun & Yıldız, 2018).

4.2.10 | 2-Chloro-5,6-dimethyl-3-thiomorpholino-1,4-benzoquinone (PQ10)

The title analog (**PQ10**) was prepared according to the literature and identified by comparing its spectral properties to those reported for the analog in the literature (Tuyun & Yıldız, 2018).

4.2.11 | Ethyl 4-(2-chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)piperazine-1-carboxylate (PQ11)

Following general procedure by applying ethyl piperazine-1-carboxylate (0.1740 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ11** as a purple oil. Yield: 60%. FTIR (ATR) υ (cm^{-1}): 2,915, 2,848 ($CH_{aliphatic}$), 1,659, 1,649 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.21 (t, J = 7.3 Hz, 3H, CH_3), 1.93 (d, J = 1.0 Hz, 3H, CH_3), 1.98 (d, J = 1.5 Hz, 3H, CH_3), 3.35–3.37 (m, 4H, NCH_2), 3.52–3.54 (m, 4H, NCH_2), 4.10 (q, J = 7.3 Hz, 2H, OCH_2). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 11.6, 12.1, 13.7 (CH_3), 28.7, 49.8 ($NCH_{2piperidine}$), 60.6 (OCH_2), 119.7, 138.0, 140.3, 147.0 (C_q), 154.5, 179.1, 182.8 (>C=O). MS (MALDI TOF) *m/z*: 326 $[M]^+$. Anal. Calcd. for $C_{15}H_{19}ClN_2O_4$ (326.78).

4.2.12 | 2-Chloro-5,6-dimethyl-3-(4-phenylpiperazin-1-yl)-1,4-benzoquinone (PQ12)

Following general procedure by applying 1-phenylpiperazine (0.1785 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ12** as a dark brown solid. Yield: 70%, mp 118–120 °C. FTIR (ATR) υ (cm^{-1}): 2,959, 2,917, 2,841 ($CH_{aliphatic}$), 1,658 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.93 (d, J = 1.0 Hz, 3H, CH_3), 1.98 (d, J = 1.0 Hz, 3H, CH_3), 3.22 (t, J = 4.9 Hz, 4H, NCH_2), 3.57 (t, J = 4.9 Hz, 4H, NCH_2), 6.79–6.82 (m, 1H, $CH_{aromatic}$), 6.80 (t, J = 7.3 Hz, 1H, $CH_{aromatic}$), 6.86 (d, J = 7.8 Hz, 2H, $CH_{aromatic}$), 7.17–7.22 (m, 2H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 11.6, 12.1 (CH_3), 49.2, 49.9 ($CH_{2piperidine}$), 115.5, 119.3, 128.2, 137.8, 140.3, 146.8, 150.1 ($CH_{aromatic}$ and C_q), 179.0, 182.8 (>C=O). MS (MALDI TOF) *m/z*: 331 $[M + H]^+$. Anal. Calcd. for $C_{18}H_{19}ClN_2O_2$ (330.81).

4.2.13 | 2-Chloro-5,6-dimethyl-3-(4-(4-nitrophenyl) piperazin-1-yl)-1,4-benzoquinone (PQ13)

Following general procedure by applying 1-(4-nitrophenyl)piperazine (0.2280 g, 1.10 mmol), the crude residue was purified by column chromatography to furnish **PQ13** as a dark purple solid. Yield: 67%, mp 184–185 °C. FTIR (ATR) υ (cm^{-1}): 2,915 ($CH_{aliphatic}$), 1,661 (>C=O). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 1.95 (d, J = 1.0 Hz, 3H, CH_3), 2.00 (s, 3H, CH_3), 3.47–3.49 (m, 4H, NCH_2), 3.57–3.61 (m, 4H, NCH_2), 6.79 (d, J = 9.3 Hz, 2H, $CH_{aromatic}$), 8.07 (d, J = 9.3 Hz, 2H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 11.6, 12.1 (CH_3), 47.0, 49.3 ($CH_{2piperidine}$), 112.1, 119.9, 124.9, 138.0, 140.4, 146.5, 153.8 ($CH_{aromatic}$ and C_q), 179.0, 182.8 (>C=O). MS (MALDI TOF) *m/z*: 375 $[M]^+$. Anal. Calcd. for $C_{18}H_{18}ClN_3O_4$ (375.81).

4.3 | Antimicrobial activity

4.3.1 | 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 (CLSI, 1997; CLSI, 2006). Mueller–Hinton broth for bacteria and RPMI-1640 medium for the yeast strain were used as the test media. Serial twofold dilutions ranging from 2,500 μg/mL to 1.2 μg/mL were prepared in the media. The inoculum was prepared using a 4–6 hr broth culture of each bacteria and 24 hr 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 hr while the trays containing RPMI-1640

medium were incubated at 35 °C for 46–50 hr. 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.

According to the antimicrobial activity results, we also studied in vitro activities of the **PQ9** and **PQ11** against each of 20 clinically obtained strains of *Candida* spp. and *Staphyloccocus* spp. by the microbroth dilution technique as described by the CLSI recommendations (CLSI, 1997; CLSI, 2006).

4.3.2 | Determination of antibiofilm activities

Biofilm attachment and inhibition of biofilm formation assays were performed as previously described method with some modifications (Mataraci & Dosler, 2012). 1/10 × MIC concentrations of molecules were added to the 24 hr biofilm and plates were incubated 1, 2, and 4 hr for *S. epidermidis* and 2, 4, and 6 hr for *C. albicans* at 37 °C; molecules at 1× 1/10 × and 1/100 × MIC concentrations were added to the 24 hr biofilm and plates were incubated 24 hr at 37 °C, respectively. Six wells were used for each molecule. The positive controls were microorganisms in TSB-glucose without molecules. After the incubation, wells were washed with PBS solutions and measured at OD_{595} nm.

4.3.3 | Statistical analysis

All experiments were performed in two independent assays. One way ANOVA-Bonferroni's multiple comparison test was used to compare differences between control and antimicrobials treated biofilms. *p* value <.001 was considered as statistically significant.

4.4 | Cytotoxicity

4.4.1 | Cell cultures

Balb/3T3 mouse embryonic fibroblasts, HUVEC Human umbilical vein endothelial cells, HaCaT human keratinocytes, and NRK-52E normal rat kidney cells were purchased from American Type Culture Collection (ATCC). All of the cells were incubated in Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO Laboratories) and 1% antibiotic/antimycotic solution (Invitrogen Carlsbad, CA) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were passaged routinely when they reached 80% confluency.

4.4.2 | Cell treatments and MTT assay

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to determine cytotoxic effects of compounds **PQ9** and **PQ11** after 24 hr exposure following the protocol described by van Meerloo et al. (van Meerloo, Kaspers, and Cloos, 2011) with minor modifications. 1×10^4 cells/well for Balb/3T3 (mouse embryonic fibroblasts), HaCaT (human keratinocytes), HUVEC (Human umbilical vein endothelial cells) and 5×10^3 cells/well for NRK-52E (normal rat kidney cells) were plated into 96 microplates and incubated overnight before to start treatments. Compound stocks were dissolved in dimethyl sulfoxide (DMSO) (Bioshop Canada, Ontario, Canada) and stocks were diluted with the medium before to use. After changing medium, compounds were added into wells at different concentrations (1.5625–100 μg/mL). DMSO concentration was 0.25% in the final culture medium and negative control cells received 0.25% DMSO. Positive control wells received 1% TritonX-100. After 24 hr exposure, cells were incubated with 5 mg/mL MTT (Santa Cruz Biotechnology, Santa Cruz, CA) containing medium for 3 hr at 37 °C in the dark. Then the medium was discarded and MTT formazan dissolved in 100 μL DMSO. Optical density (OD) of the solutions was measured at 590 nm using a microplate reader (Biotek, Epoch, Winooski, VT). All experiments were made as three independent experiments and performed in triplicates. Cell viability calculated according to the following formula:

Cell viability (percentage of negative control) = $[(OD_{sample} - OD_{positive\ control})/(OD_{negative\ control} - OD_{positive\ control})] \times 100$.

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AUTHOR CONTRIBUTIONS

A.F.T. designed the research, and A.F.T., M.Y., N.B., and H.Y. performed the synthetic work. E.M.K. and B.Ö.Ç. coordinated the biological research, E.M.K. and A.T.J. undertook the main biological experiments. All authors discussed, edited, and approved the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Alfadhli, A., Mack, A., Harper, L., Berk, S., Ritchie, C., & Barklis, E. (2016). Analysis of quinolinequinone reactivity, cytotoxicity, and anti-HIV-1 properties. *Bioorganic & Medicinal Chemistry*, *24*(21), 5618–5625.

APEX2, version 2014.1-1, Bruker, 2014, Bruker AXS Inc., Madison, WI.

Bayrak, N., Yildirim, H., Tuyun, A. F., Kara, E. M., Celik, B. O., Gupta, G. K., … Nasiri, H. R. (2017). Synthesis, computational study, and evaluation of in vitro antimicrobial, antibiofilm, and anticancer activities of new sulfanyl aminonaphthoquinone derivatives. *Letters in Drug Design & Discovery*, *14*(6), 647–661.

Begleiter, A. (2000). Clinical applications of quinone-containing alkylating agents. *Frontiers in Bioscience*, *5*, E153–E171.

Carneiro, P. F., Pinto, M. C. R. F., Marra, R. K. F., da Silva, F. D., Resende, J. A. L. C., Silva, L. F. R. E., … Ferreira, V. F. (2016). Synthesis and antimalarial activity of quinones and structurally-related oxirane derivatives. *European Journal of Medicinal Chemistry*, *108*, 134–140.

Castro, F. A. V., Mariani, D., Panek, A. D., Eleutherio, E. C. A., & Pereira, M. D. (2008). Cytotoxicity mechanism of two naphthoquinones (Menadione and Plumbagin) in *Saccharomyces cerevisiae*. *PLoS One*, *3*(12), e3999.

Cerqueira, E. C., Netz, P. A., Diniz, C., do Canto, V. P., & Follmer, C. (2011). Molecular insights into human monoamine oxidase (MAO) inhibition by 1,4-naphthoquinone: Evidences for menadione (vitamin K3) acting as a competitive and reversible inhibitor of MAO. *Bioorganic & Medicinal Chemistry*, *19*(24), 7416–7424.

Clinical and Laboratory Standards Institute (CLSI), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard–Second Edition, Wayne, PA, 1997.

Clinical and Laboratory Standards Institute (CLSI), Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Wayne, PA, 2006.

Collett, N. P., Amin, A. R. M. R., Bayraktar, S., Pezzuto, J. M., Shin, D. M., Khuri, F. R., … Kucuk, O. (2010). Cancer prevention with natural compounds. *Seminars in Oncology*, *37*(3), 258–281.

Davids, H., Theunissen, R., Chakravorty, S., Mohammed, R., Frost, C., van Otterlo, W. A. L., & de Koning, C. B. (2012). Aminonaphthoquinones as potential anti-breast cancer agents. *African Journal of Pharmacy and Pharmacology*, *6*(45), 3102–3112.

Delarmelina, M., Daltoe, R. D., Cerri, M. F., Madeira, K. P., Rangel, L. B. A., Lacerda, V., … Greco, S. J. (2015). Synthesis, antitumor activity and docking of 2,3-(substituted)-1,4-naphthoquinone derivatives containing nitrogen, oxygen and sulfur. *Journal of the Brazilian Chemical Society*, *26*(9), 1804–1816.

Donlan, R. M. (2001). Biofilm formation: A clinically relevant microbiological process. *Clinical Infectious Diseases*, *33*(8), 1387–1392.

Egleton, J. E., Thinnes, C. C., Seden, P. T., Laurieri, N., Lee, S. P., Hadavizadeh, K. S., … Russell, A. J. (2014). Structure–activity relationships and colorimetric properties of specific probes for the putative cancer biomarker human arylamine N-acetyltransferase 1. *Bioorganic & Medicinal Chemistry*, *22*(11), 3030–3054.

El-Dakhakhany, M. (1963). Studies on the chemical constitution of Egyptian *N. sativa* L. seeds. *Planta Medica*, *11*, 465–470.

European Centre for Disease Prevention and Control (ECDPC). 2013. Retrieved from https://ecdc.europa.eu/en/publications-data/point-prevalence-survey-healthcare-associated-infections-and-antimicrobial-use-0.

European Centre for Disease Prevention and Control (ECDPC). 2014. Antimicrobial resistance and healthcare-associated infections', in: *Annual epidemiological report* 2014.

Ghannoum, M., & O'Toole, G. A. (2004). *Microbial biofilms*. Washington, DC: ASM Press.

Glamoclija, U., Padhye, S., Spirtovic-Halilovic, S., Osmanovic, A., Veljovic, E., Roca, S., … Završnik, D. (2018). Synthesis, biological evaluation and docking studies of benzoxazoles derived from thymoquinone. *Molecules*, *23*(12), Article Number: 3297.

Gutierrez, P. L. (2000). The metabolism of quinone-containing alkylating agents: Free radical production and measurement. *Frontiers in Bioscience*, *5*, D629–D638.

Harrison, J. J., Ceri, H., Stremick, C., & Turner, R. J. (2004). Differences in biofilm and planktonic cell mediated reduction of metalloid oxyanions. *FEMS Microbiology Letters*, *235*(2), 357–362.

Ikeda, T., Wakabayashi, H., Nakane, M. (1991). *Benzoquinone antiallergy and antiinflammatory agents*. Patent.

Ilina, T. V., Semenova, E. A., Pronyaeva, T. R., Pokrovskii, A. G., Nechepurenko, I. V., Shults, E. E., … Tolstikov, G. A. (2002). Inhibition of HIV-1 reverse transcriptase by aryl-substituted naphto- and anthraquinones. *Doklady Biochemistry and Biophysics*, *382*, 56–59.

Janeczko, M., Demchuk, O. M., Strzelecka, D., Kubinski, K., & Maslyk, M. (2016). New family of antimicrobial agents derived from 1,4-naphthoquinone. *European Journal of Medicinal Chemistry*, *124*, 1019–1025.

Jin, Y. R., Ryu, C. K., Moon, C. K., Cho, M. R., & Yun, Y. P. (2004). Inhibitory effects of j78, a newly synthesized 1,4-naphthoquinone derivative, on experimental thrombosis and platelet aggregation. *Pharmacology*, *70*(4), 195–200.

Johnson-Ajinwo, O. R., Ullah, I., Mbye, H., Richardson, A., Horrocks, P., & Li, W. W. (2018). The synthesis and evaluation of thymoquinone analogues as anti-ovarian cancer and antimalarial agents. *Bioorganic & Medicinal Chemistry Letters*, *28*(7), 1219–1222.

Jordao, A. K., Novais, J., Leal, B., Escobar, A. C., dos Santos, H. M., Castro, H. C., & Ferreira, V. F. (2013). Synthesis using microwave irradiation and antibacterial evaluation of new N,O-acetals and N,S-acetals derived from 2-amino-1,4-naphthoquinones. *European Journal of Medicinal Chemistry*, *63*, 196–201.

Kacmaz, A., Acar, E. T., Atun, G., Kaya, K., Sigirci, B. D., & Bagcigil, F. (2018). Synthesis, electrochemistry, DFT calculations, antimicrobial properties and X-ray crystal structures of some NH- and/or S-substituted-1,-4-quinones. *ChemistrySelect*, *3*(30), 8615–8623.

Kawamukai, M. (2018). Biosynthesis and applications of prenylquinones. *Bioscience Biotechnology and Biochemistry*, *82*(6), 963–977.

Lien, J. C., Huang, L. J., Teng, C. M., Wang, J. P., & Kuo, S. C. (2002). Synthesis of 2-alkoxy 1,4-naphthoquinone derivatives as antiplatelet, antiinflammatory, and antiallergic agents. *Chemical & Pharmaceutical Bulletin*, *50*(5), 672–674.

Macrae, C. F., Edgington, P. R., McCabe, P., Pidcock, E., Shields, G. P., Taylor, R., … van De Streek, J. (2006). Mercury: Visualization and analysis of crystal structures. *Journal of Applied Crystallography*, *39*, 453–457.

Mataraci, E., & Dosler, S. (2012). In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin-resistant *Staphylococcus aureus* biofilms. *Antimicrobial Agents and Chemotherapy*, *56*(12), 6366–6371.

Nain-Perez, A., Barbosa, L. C. A., Maltha, C. R. A., & Forlani, G. (2016). First total synthesis and phytotoxic activity of *Streptomyces* sp. metabolites abenquines. *Tetrahedron Letters*, *57*(16), 1811–1814.

Nain-Perez, A., Barbosa, L. C. A., Maltha, C. R. A., & Forlani, G. (2017). Natural abenquines and their synthetic analogues exert algicidal activity against bloom-forming cyanobacteria. *Journal of Natural Products*, *80*(4), 813–818.

Nain-Perez, A., Barbosa, L. C. A., Maltha, C. R. A., Giberti, S., & Forlani, G. (2017). Tailoring natural abenquines to inhibit the photosynthetic electron transport through interaction with the D1 protein in photosystem II. *Journal of Agricultural and Food Chemistry*, *65*(51), 11304–11311.

Nain-Perez, A., Barbosa, L. C. A., Rodriguez-Hernandez, D., Kramell, A. E., Heller, L., & Csuk, R. (2017). Natural abenquines and synthetic analogues: Preliminary exploration of their cytotoxic activity. *Bioorganic & Medicinal Chemistry Letters*, *27*(5), 1141–1144.

Nallapareddy, S. R., Singh, K. V., Sillanpaa, J., Garsin, D. A., Hook, M., Erlandsen, S. L., & Murray, B. E. (2006). Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. *The Journal of Clinical Investigation*, *116*, 2799–2807.

Namvar, A. E., Bastarahang, S., Abbasi, N., Ghehi, G. S., Farhadbakhtiarian, S., Arezi, P., … Chermahin, S. G. (2014). Clinical

characteristics of *Staphylococcus epidermidis*: A systematic review. *GMS Hygiene and Infection Control*, *9*(3), Doc23.

Niedermeyer, T. H. J., Mikolasch, A., & Lalk, M. (2005). Nuclear amination catalyzed by fungal laccases: Reaction products of p-hydroquinones and primary aromatic amines. *The Journal of Organic Chemistry*, *70*(6), 2002–2008.

Pingaew, R., Prachayasittikul, V., Worachartcheewan, A., Nantasenamat, C., Prachayasittikul, S., Ruchirawat, S., & Prachayasittikul, V. (2015). Novel 1,4-naphthoquinone-based sulfonamides: Synthesis, QSAR, anticancer and antimalarial studies. *European Journal of Medicinal Chemistry*, *103*, 446–459.

Prachayasittikul, V., Pingaew, R., Worachartcheewan, A., Nantasenamat, C., Prachayasittikul, S., Ruchirawat, S., & Prachayasittikul, V. (2014). Synthesis, anticancer activity and QSAR study of 1,4-naphthoquinone derivatives. *European Journal of Medicinal Chemistry*, *84*, 247–263.

Richwien, A., & Wurm, G. (2004). Influence of 2-aryl-3-halogen/3--hydroxy-1,4-naphthoquinones with salicylic and cinnamic acid partial structures on the arachidonic acid cascade. *Pharmazie*, *59*(3), 163–169.

Ryu, C. K., & Lee, J. Y. (2006). Synthesis and antifungal activity of 6-hydroxycinnolines. *Bioorganic & Medicinal Chemistry Letters*, *16*(7), 1850–1853.

Ryu, C. K., Lee, S. K., Han, J. Y., Jung, O. J., Lee, J. Y., & Jeong, S. H. (2005). Synthesis and antifungal activity of 5-arylamino-4,7-dioxobenzo[b]thiophenes. *Bioorganic & Medicinal Chemistry Letters*, *15*(10), 2617–2620.

Ryu, C. K., Nho, J. H., Jin, G., Oh, S. Y., & Choi, S. J. (2014). Synthesis of benzofuro [6,7-d]thiazoles, benzofuro[7,6-d]thiazoles and 6-arylaminobenzo[d]thiazole-4,7-diones as antifungal agent. *Chemical & Pharmaceutical Bulletin*, *62* (7), 668–674.

Ryu, C. K., Oh, S. Y., Choi, S. J., & Kang, D. Y. (2014). Synthesis of antifungal evaluation of 2H-[1,2,3]Triazolo[4,5-g]isoquinoline-4,9-diones. *Chemical & Pharmaceutical Bulletin*, *62*(11), 1119–1124.

SADABS, version 2012/2, Bruker, 2012, Bruker AXS Inc., Madison, WI.

SAINT, version 8.34A, Bruker, 2013, Bruker AXS Inc., Madison, WI.

Sanchez, M. C., Romero-Lastra, P., Ribeiro-Vidal, H., Llama-Palacios, A., Figuero, E., Herrera, D., & Sanz, M. (2019). Comparative gene expression analysis of planktonic *Porphyromonas gingivalis* ATCC 33277 in the presence of a growing biofilm versus planktonic cells. *BMC Microbiology*, *19*(1), 58.

SHELXTL, version 6.14, Bruker, 2000, Bruker AXS Inc., Madison, WI.

Shrestha, J. P., Baker, C., Kawasaki, Y., Subedi, Y. P., de Paul, N. N. V., Takemoto, J. Y., & Chang, C. W. T. (2017). Synthesis and bioactivity investigation of quinone-based dimeric cationic triazolium amphiphiles selective against resistant fungal and bacterial pathogens. *European Journal of Medicinal Chemistry*, *126*, 696–704.

Spek, A. L. (2009). Structure validation in chemical crystallography. *Acta Crystallographica Section D*, *65*, 148–155.

Sugiura, K. (1961). Antitumor activity of mitomycin C. *Cancer Chemotherapy Reports*, *13*, 51–65.

Tandon, V. K., Chhor, R. B., Singh, R. V., Rai, S. J., & Yadav, D. B. (2004). Design, synthesis and evaluation of novel 1,4-naphthoquinone derivatives as antifungal and anticancer agents. *Bioorganic & Medicinal Chemistry Letters*, *14*(5), 1079–1083.

Tandon, V. K., Kumar, S., Mishra, N. N., & Shukla, P. K. (2012). Micelles catalyzed chemo- and regio-selective one pot and one step synthesis of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-1,4-benzoquinones and 2,5-diaminosubstituted-1,4-benzoquinones "in-water" and their biological evaluation as antibacterial and antifungal agents. *European Journal of Medicinal Chemistry*, *56*, 375–386.

Tandon, V. K., & Maurya, H. K. (2009). 'On water': Unprecedented nucleophilic substitution and addition reactions with 1,4-quinones in aqueous suspension. *Tetrahedron Letters*, *50*(43), 5896–5902.

Tandon, V. K., Maurya, H. K., Verma, M. K., Kumar, R., & Shukla, P. K. (2010). 'On water' assisted synthesis and biological evaluation of nitrogen and sulfur containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents. *European Journal of Medicinal Chemistry*, *45*(6), 2418–2426.

The Centers for Disease Control and Prevention (CDCP), 2016. Retrieved from https://www.cdc.gov/hai/data/portal/progress-report.html.

Tuyun, A. F., Bayrak, N., Yildirim, H., Onul, N., Kara, E. M., & Celik, B. O. (2015). Synthesis and in vitro biological evaluation of aminonaphthoquinones and benzo[b]phenazine-6,11-dione derivatives as potential antibacterial and antifungal compounds. *Journal of Chemistry*, *2015*, 1–8.

Tuyun, A. F., & Yıldız, M. (2018). Synthesis and characterization of new cyclic aminobenzoquinones. *Journal of the Turkish Chemical Society Section A: Chemistry*, *5*(2), 371–380.

Valderrama, J. A., Delgado, V., Sepulveda, S., Benites, J., Theoduloz, C., Calderon, P. B., & Muccioli, G. G. (2016). Synthesis and cytotoxic activity on human cancer cells of novel Isoquinolinequinone-amino acid derivatives. *Molecules*, *21*(9), Article Number: 1199.

van Meerloo, J., Kaspers, G. J., & Cloos, J. (2011). Cell sensitivity assays: The MTT assay. *Methods in Molecular Biology*, *731*, 237–245.

Wellington, K. W. (2015). Understanding cancer and the anticancer activities of naphthoquinones—A review. *RSC Advances*, *5*(26), 20309–20338.

Wellington, K. W., Kolesnikova, N. I., Nyoka, N. B. P., & McGaw, L. J. (2019). Investigation of the antimicrobial and anticancer activity of aminonaphthoquinones. *Drug Development Research*, *80*, 138–146.

World Health Organization (WHO). (2011). *Report on the burden of endemic health care-associated infection worldwide*. Geneva, Switzerland: World Health Organization.

Widhalm, J. R., & Rhodes, D. (2016). Biosynthesis and molecular actions of specialized 1,4-naphthoquinone natural products produced by horticultural plants. *Horticulture Research*, *3*, 16046.

Xu, K., Xiao, Z. Y., Tang, Y. B., Huang, L., Chen, C. H., Ohkoshi, E., & Lee, K. H. (2012). Design and synthesis of naphthoquinone derivatives as antiproliferative agents and 20S proteasome inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *22*(8), 2772–2774.

Yildirim, H., Bayrak, N., Tuyun, A. F., Kara, E. M., Celik, B. O., & Gupta, G. K. (2017). 2,3-Disubstituted-1,4-naphthoquinones containing an arylamine with trifluoromethyl group: Synthesis, biological evaluation, and computational study. *RSC Advances*, *7*(41), 25753–25764.

Yuk, D. Y., Lee, H. Y., Ryu, C. K., Hong, J. T., Kang, W. S., Yoo, H. S., & Yun, Y. P. (2000). Studies on the antithrombotic and antiplatelet activities of NQ304, a newly synthesized naphthoquinone derivative. *Arzneimittel-Forschung*, *50*(3), E254–E259.

Yuk, D. Y., Ryu, C. K., Hong, J. T., Chung, K. H., Kang, W. S., Kim, Y., … Yun, Y. P. (2000). Antithrombotic and antiplatelet activities of 2-chloro-3-[4-(ethylcarboxy)-phenyl]-amino-1,4-naphthoquinone (NQ12), a newly synthesized 1,4-naphthoquinone derivative. *Biochemical Pharmacology*, *60*(7), 1001–1008.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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