Derivatives (halogen, nitro and amino) of 8-hydroxyquinoline with highly potent antimicrobial and antioxidant activities

Rungrot Cherdtrakulkiat a, Somchai Boonpangrak b, Nujarin Sinthupoom a, Supaluk Prachayasittikul c, Somsak Ruchirawat d,e, Virapong Prachayasittikul a,*

a Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
b Center for Innovation Development and Technology Transfer, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
c Center of Data Mining and Biomedical Informatics, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
d Laboratory of Medicinal Chemistry, Chulabhorn Research Institute and Program in Chemical Biology, Chulabhorn Graduate Institute, Bangkok 10210, Thailand
e Center of Excellence on Environmental Health and Toxicology, Commission on Higher Education (CHE), Ministry of Education, Thailand

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8-Hydroxyquinoline (8HQ) compounds have been reported to possess diverse bioactivities. In recent years, drug repositioning has gained considerable attention in drug discovery and development. Herein, 8HQ (1) and its derivatives (2–9) bearing various substituents (amino, nitro, cyan and halogen) were investigated for their antimicrobial activity against 27 microorganisms (agar dilution method) and antioxidant (DPPH method) activities. The parent 8HQ (1) exerted a highly potent antimicrobial activity against Gram-positive bacteria including diploid fungi and yeast with MIC values in the range of 3.44–13.78 μM. Moreover, the halogenated 8HQ, especially 7-bromo-8HQ (4) and cloxyquin (6), displayed a high antigrowth activity against Gram-negative bacteria compared with the parent compound (1). Apparently, the derivatives with a relatively high safety index, e.g., nitroxoline (2), exhibited strong antibacterial activity against Aeromonas hydrophila (MIC = 5.26 μM) and selectively inhibited the growth of P. aeruginosa with the MIC value of 84.14 μM; cloxyquin (3) showed a strong activity against Listeria monocytogenes and Plesiomonas shigelloides with MIC values of 5.57 and 11.14 μM, respectively. Most compounds displayed an antioxidant activity. Specifically, 5-amino-8HQ (8) was shown to be the most potent antioxidant (IC50 = 8.70 μM) compared with the positive control (α-tocopherol) with IC50 of 13.47 μM. The findings reveal that 8HQ derivatives are potential candidates to be further developed as antimicrobial and antioxidant agents.

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1. Introduction

Nitrogen heterocycles constitute a large group of compounds with a vast array of pharmacological activities [1–3]. Specifically, quinoline is a privileged structure that is found in a variety of natural products and therapeutics. 8-Hydroxyquinoline (8HQ), a derivative of quinoline, has a strong metal chelating property [4]. The derivatives of 8HQ have been reported to have multifunctional uses as antimicrobial, antioxidant, anticancer, antiinflammatory and antineurodegenerative agents [5–8]. The halogenated 8HQs, such as cloxyquin (5-chloro-8HQ), cloxiqino (5-chloro-7-ido-8HQ or CQ), 7-bromo-8HQ and iodoquin (5,7-diiodo-8HQ), have been synthesized and are commercially available [9]. The cloxyquin was reported to show good anti-tubercular and antiamoebic activities [10]. CQ was also used for several years as an anti-diarrheal agent to treat amoebic infection. Then, it was banned from oral consumption in the 1960s because it can cause subacute myelo-optic neuropathy (SMON) [11]. However, the neurotoxicity of CQ can be solved by the recommended dosage control and vitamin supplementation. Nitroxoline (5-nitro-8HQ or NQ) has been used for the treatment and prophylaxis of acute and recurrent urinary tract infection [7]. In addition, NQ has been approved by the Food and Drug Administration (FDA), and is widely used as an anti-neurodegenerative drug to treat Alzheimer’s disease and cancer in humans [6]. Moreover, metal complexes in 8HQ have been reported to enhance 8HQ bioactivities [12]. The search for novel potent lead compounds and repositioning of the well-known compounds/drugs for therapeutic applications are the main challenges [13–16]. In recent years, drug repositioning or repurposing has attracted pharmaceutical companies because the possibility of using the approved or investigational drug in a new therapeutic area avoids the expensive and time-consuming pharmacokinetic and toxicity tests that are required for new drug candidates [17]. Currently, diverse bioactivities of the 8HQ derivatives have not been fully explored. Therefore, 8HQ and its derivatives that bear substituents (amino, halogen, nitro) at positions...
5 and/or 7 as well as a cyano group at 2-position were investigated for their antimicrobial and antioxidant activities as well as cytotoxic effect.

2. Materials and methods

2.1. Compounds and chemical reagents

Nine tested compounds (1–9, Fig. 1) are commercially available. Specifically, 8HQ (1), NQ (2), cloxyquin (3), 7-bromo-8HQ (4), CQ (6), iodoquinol (7), and 5-amino-8HQ (8) were purchased from Sigma, USA. Compounds 5,7-dichloro-8HQ (5) and 8HQ-2-carbonitrile (2-CN-8HQ, 9) were purchased from Acros Organics. α-Tocopherol (vitamin E), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were supplied by Sigma, USA. Dimethyl sulfoxide (DMSO) was purchased from Merck, Germany.

2.2. Antimicrobial activity assay

Antimicrobial activity of 8HQ and its derivatives (1–9) was performed using the agar dilution method, as previously described [2]. Briefly, the tested compound was dissolved in DMSO and, then, was mixed with the Müller Hinton (MH) broth. The compound solution was two-fold diluted, and 1 mL of each dilution was mixed into the MH agar to obtain the final concentration range of 0.25–256 μg/mL. DMSO (0.5%) was added into the MH agar and was used as a reagent control. The microorganisms were cultured in the MH broth at 37 °C overnight and were diluted with a normal saline solution until the cell density was 0.5 McFarland standard (1.5 × 10⁸ CFU/mL). The microorganisms were inoculated onto the agar plates and were incubated at 37 °C for 24–48 h. A minimum inhibitory concentration (MIC) of the compounds was determined. Twenty-seven strains of the tested microorganisms were Gram-negative bacteria: Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Serratia marcescens ATCC 8100, Salmonella Typhimurium ATCC 13311, Salmonella Choleraesuis ATCC 10708, Salmonella Enteritidis, Shigella dysenteriae, Morganella morganii, Citrobacter freundii, Plesiomonas shigelloides, Aeromonas hydrophila, Pseudomonas aeruginosa ATCC 27853, Pseudomonas stutzeri ATCC 17587, Shewanella putrefaciens ATCC 8071, Acro- nomobacter xylosoxidans ATCC 27061; Gram-positive bacteria: Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC 12228, Micrococcus luteus ATCC 10240, Enterococcus faecalis ATCC 29212, Enterococcus faecalis ATCC 33186, Corynebacterium diptheriae NCTC 10356, Bacillus subtilis ATCC 6633, Listeria monocytogenes, Bacillus cereus; and diploid fungi and yeast: Candida albicans ATCC 90028 and Saccharomyces cerevisiae ATCC 2601.

2.3. Antioxidant activity assay

Compounds (1–9) were determined for their antioxidant properties using the DPPH assay [18]. DPPH (a stable purple color radical) reacts with an antioxidant to form a light-yellow diphenylpicrylhydrazine, which is the reduced product that can be detected using a spectrophotometer. The assay was initiated by adding a 1 mL solution of DPPH in methanol (0.1 mM) to a sample solution (0.45 mL, 1 mg/mL dissolved in DMSO). The reaction mixture was incubated for 30 min in a dark room. The absorbance at 517 nm was measured using a UV–visible spectrophotometer (UV-1610, Shimadzu), and the percentage of radical scavenging activity (RSA) was calculated using the following equation:

\[
\text{RSA} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100\%
\]

![Fig. 1. Chemical structures of 8HQ and its derivatives (1-9).](image-url)
R. Cherdtrakulkiat et al. / Biochemistry and Biophysics Reports 6 (2016) 135–141

RSA(%) = \left[ 1 - \frac{Abs_{\text{sample}}}{Abs_{\text{control}}} \right] \times 100

where $Abs_{\text{control}}$ is the absorbance of the control reaction, and $Abs_{\text{sample}}$ is the absorbance of the tested compound.

2.4. Cytotoxicity assay

Cytotoxicity of compounds (1–9) was performed using a normal embryonic lung cell line (MRC-5) [12]. Briefly, the MRC-5 cells were grown in a DMEM medium that was supplemented with 100 U/mL of penicillin-streptomycin and 10% FBS. Then, the cell lines were seeded in a 96-well plate at a density of 5,000-20,000 cells/well and were incubated at 37 °C under a humidified atmosphere (95% air and 5% CO₂) for 24 h. The cells were treated with the tested compounds at different concentrations and, then, incubated for 48 h. Cell viability was measured by staining with MTT. The MTT solution (10 μL/100 μL medium) was added to all assay wells and was incubated for 4 hours. After the incubation, DMSO was added to dissolve the resulting formazan using sonication. The plates were read on a microplate reader using a test wavelength of 550 nm and a reference wavelength of 650 nm. Cytotoxic activity of the tested compound was expressed as the compound concentration that inhibited the cell growth by 50% (IC50). A tested sample is the absorbance of the tested compound.

3. Results

3.1. Biological activities

3.1.1. Antimicrobial activity

All the tested compounds, 8HQ and its derivatives (1–9), were evaluated for their antimicrobial activities against twenty-seven strains of microorganisms using the agar dilution method. The results showed that these compounds exhibited the antimicrobial potency against both Gram-positive and Gram-negative bacteria (Table 1). 8HQ (1) showed a great inhibitory activity to Gram-positive bacteria such as S. aureus, E. epidermidis, M. luteus, E. faecalis, C. diphtheriae, B. cereus, S. subtilis and L. monocytogenes with the MICs in the range of 3.44–13.78 μM. The activity against Gram-negative bacteria, such as E. coli, K. pneumoniae, S. marcescens, Salmonella spp., S. dysenteriae, M. morganii, C. freundii, P. shigelloides, A. hydrophila, P. stutzeri, S. putrefaciens and A. xylosoxidans, were found to be 13.78–881.79 μM, except for P. aeruginosa ATCC 27853 (MIC > 1763.57 μM). Among the Gram-negative bacteria, the highest activity of compound 1 was noted for P. shigelloides with the lowest MIC value of 13.78 μM, followed by A. hydrophila (MIC = 110.22 μM). Additionally, the diploid fungi and yeast (C. albicans ATCC 90028 and S. cerevisiae ATCC 2601) were inhibited by the compound 1 with MICs of 13.78 μM. The 8HQ derivatives (2–9) have a wide range of MIC values that depend on the activity of each compound and bacterial group. The antimicrobial activity of NQ (2) against both the Gram-positive and Gram-negative bacteria was observed with MIC in the range of 5.26–84.14 μM. Particularly, NQ is the only compound that displayed the activity against P. aeruginosa ATCC 27853 with a MIC value of 84.14 μM, while the other derivatives (3–9), including 881.79 21.03 44.54 35.71 37.37 26.19 644.92 274.57 1504.38

S. marcescens ATCC 8100
700603
881.79 42.07 89.09 35.71 37.37 26.19 644.92 274.57 1504.38
K. pneumoniae ATCC 8104
881.79 42.07 89.09 35.71 37.37 26.19 644.92 274.57 1504.38
A. hydrophila
110.22 5.26 44.54 35.71 37.37 26.19 644.92 274.57 1504.38
P. aeruginosa ATCC 27853
700603
881.79 42.07 89.09 35.71 37.37 26.19 644.92 274.57 1504.38
K. pneumoniae ATCC 8104
881.79 42.07 89.09 35.71 37.37 26.19 644.92 274.57 1504.38
A. hydrophila
110.22 5.26 44.54 35.71 37.37 26.19 644.92 274.57 1504.38
P. aeruginosa ATCC 27853
> 1763.57 84.14 > 1412.60 > 1142.60 > 1195.98 > 837.97 > 644.92 > 1098.29 > 1504.38
P. stutzeri ATCC 17587
220.45 10.52 178.17 35.71 37.37 26.19 644.92 274.57 1504.38
S. putrefaciens ATCC 8071
220.45 10.52 178.17 35.71 37.37 26.19 644.92 274.57 1504.38
A. xylosoxidans ATCC 27061
55.11 21.03 89.09 35.71 37.37 26.19 644.92 549.14 1504.38
C. albicans ATCC 90028
13.78 42.07 178.17 35.71 37.37 26.19 644.92 1098.29 1504.38
S. cerevisiae ATCC 2601
13.78 42.07 178.17 35.71 37.37 26.19 644.92 1098.29 1504.38

Ampicillin (26.93 μM) was used as the control system of antimicrobial activity. It showed 100% inhibition against S. aureus ATCC 25923, S. aureus ATCC 29213, S. epidermidis ATCC 12228, M. luteus ATCC 10240, E. faecalis ATCC 29212, E. faecalis ATCC 33186, C. diphtheriae ATCC 10356, B. subtilis ATCC 6633, L. monocytogenes, S. Typhimurium ATCC 13311, S. Enteritidis, P. stutzeri ATCC 17587, S. putrefaciens ATCC 8071 and P. shigelloides.

* MIC is the lowest concentration that inhibits the growth of microorganisms.
8HQ, exhibited antigrowth activity against this microorganism with the MIC value greater than 644.92 μM. The halogenated 8HQ (3-6) inhibited Gram-positive bacteria with the MIC range of 5.57-89.09 μM. Whereas, compound 7 showed a weaker antimicrobial activity (MIC ≥ 644.92 μM) against most of the bacterial strains, except for M. luteus ATCC 10240 and B. subtilis ATCC 6633 (MIC = 322.45 and 80.61 μM, respectively). In addition, 2-CN-8HQ (9) exerted the antimicrobial activity with the MIC value higher than 1504.38 μM against most of the tested microorganisms. The derivatives 2-6 exhibited activity against diploid fungi and yeasts with MIC values of 26.19-178.17 μM, while compounds 7-9 had the MIC values ≥ 644.92 μM.

3.1.2. Antioxidant activity

The radical scavenging activity (RSA) of compounds (1-9) was performed using the DPPH assay. The results (Table 2) demonstrated that 5-amino-8HQ (8) exhibited the highest percentage of RSA with an IC50 value of 8.71 μM, while the parent compound 8HQ (1) had the IC50 value of 614.77 μM. The halogenated 8HQ (3-6) displayed the antioxidant activity with IC50 in the range of 335.90-1050.67 μM, whereas 5,7-dihalo-8HQ (7) was shown to be an inactive antioxidant. In addition, inactive antioxidants were noted for the 5-nitro (NQ, 2) and 2-cyano (9) derivatives of 8HQ.

3.1.3. Cytotoxicity

The cytotoxic activity of compounds (1-9, Table 3) was tested against normal cells (MRC-5) using the MTT assay. It was found that compounds 7 and 9 were non-cytotoxic toward the normal cells. Cytotoxic effect was observed for the compounds 2, 3, and 6 (IC50 76.17-88.14 μM), compounds 4, 5, and 8 (IC50 22.09-27.98 μM), and 8HQ (1) with IC50 value of 6.27 μM. Selectivity index (SI) of potent antimicrobials (1-3 and 6) was determined. The result (Table 4) showed that these compounds had SI values in the range of 0.91–16.67.

4. Discussion

8HQ and its derivatives have been reported to be antimicrobial agents against many parasites, viruses, fungi and bacteria including Mycobacterium tuberculosis [2,5,19]. Moreover, CQ was used as the antineurodegenerative drug for Alzheimer’s disease. Other 8HQ derivatives exhibited anticancer and anti-inflammatory activities [5,11]. Previously, the antimicrobial activity of 8HQ and its transition metal complexes against many bacterial strains was reported [2]. In this study, 8HQ was diluted until a 1.72 μM concentration was reached. The result showed that 8HQ exerted a great antimicrobial activity (MIC = 3.44–13.78 μM) against most Gram-positive bacteria and diploid fungi. Resistant pathogens have been found among both the Gram-positive and Gram-negative bacteria. Particularly, S. aureus is the most common cause of nosocomial infection [20], for example, in patients with severe burns [21,22]. However, most Gram-negative bacteria, except for P. shigeloides (MIC = 13.78 μM), were inhibited by 8HQ with a higher MIC range of 110.22–881.79 μM. Apparently, 8HQ displayed a higher activity against Gram-positive than Gram-negative.
bacteria. This occurs possibly because the cell wall of Gram-negative bacteria has high lipophilicity. Thus, the compounds with a more hydrophobic effect are required to enhance absorption at the site of action and to exert a higher activity. Therefore, most of the 8HQ derivatives with lipophilic substituents at 5-position (NO₂, Cl), 7-position (Br) and 5,7-positions (dichloro) exhibited an improved activity against Gram-negative bacteria compared with the parent un-substituted 8HQ (1). 7-Bromo-8HQ (4) exhibited a higher antibacterial activity against Gram-negative bacteria than cloyxyquin (3), except for P. shigelloides. In addition, substitution of the chloro group at the 7-position of compound 3 gave compound 5 (5,7-dichloro-8HQ) with a higher activity compared with compound 3 against most of the tested microorganisms. Substitution of 7-bromo and 5,7-dichloro groups on the 8HQ core structure afforded compounds 4 and 5 with an almost comparable activity. When the 7-position of compound 3 was substituted with iodoo group, CQ (6, 5-chloro-7-iodo-8HQ) was obtained with a lower antibacterial activity against certain microorganisms such as S. Enteritidis, S. dysenteriae, M. morganii, C. freundii and P. stutzeri ATCC 17587 compared with compound 5 (5,7-dichloro-8HQ). It was found that 5,7-diodo-8HQ (7) displayed a weak activity against Gram-negative bacteria with MIC values ≥ 644.92 μM. It should be noted that the derivatives of 8HQ, which displayed a higher potency against Gram-negative bacteria, required lipophilic halogen substituents at 5-position and/or 7-position. Specifically, the 5-position was substituted with a small halogen atom (Cl) but not a large atom (I), and 7-position can be either chloro, bromo or iodoo groups. This could be due to the size of chloro group at the 5-position of 8HQ being appropriate for the compound to interact with the lipophilic area at the site of action. Thus far, CQ (6) and iodoquinol (7) are the known drugs with antiamoebic activities and are commonly used to treat intestinal infection [10]. Notably, NQ (2) with a strong electron withdrawing effect of NO₂ group at the 5-position of 8HQ exerted the most potent activity against almost all of the Gram-negative bacteria (MIC = 5.26–84.14 μM). Such electronic effects of the NO₂ and halogen groups may enhance the chelating effect of 8HQ in exerting antibacterial activity [23]. However, the electron donating effect of polar NH₂ group at the 5-position (8) exhibited a weaker activity (MIC = 1098.29 μM) against most of the Gram-negative bacteria compared with the nitro and halogen derivatives of 8HQ (2–6). In case of the CN group substituted at the 2-position of 8HQ, compound 9 displayed a weak activity (MIC ≥ 1504.38 μM) against all of the tested microorganisms. This can be attributed to the electron withdrawing substituent (CN) at the 2-position that deactivated the chelating effect of the N atom in the quinoline ring. Considering the activity against Gram-positive bacteria, most of the 8HQ derivatives displayed a weaker activity than the 8HQ, particularly, compounds 7 and 9. NQ (2) and halogenated-8HQ (4-6) exhibited a comparable activity (MIC = 26.19–42.07 μM) against most of the Gram-positive bacteria except for M. luteus (MIC = 10.52 μM) and B. cereus (MIC = 21.03 μM), while cloxyquin (3) displayed an activity with the MIC range of 5.57–89.09 μM. It is presumed that the most potent activity of the un-substituted 8HQ (1) resulted from its lower lipophilicity, compared with the 8HQ derivatives that bear halogen and nitro substituents. Thus, lower lipophilicity enhances better absorption to the Gram-positive bacteria that contain hydrophilic polysaccharides and charged amino acids in the peptidoglycan [24]. CQ (6) is a well-known commercial drug for treating human cancer. However, a recent study has revealed that NQ (2) exerts a higher anticancer potency than CQ [9]. The present study shows that NQ (2) possesses higher antibacterial activity than CQ (6) against some Gram-positive bacteria (M. luteus and B. cereus), and Gram-negative bacteria (E. coli ATCC 25922, K. pneumoniae ATCC 700603, S. marcescens ATCC 8100, S. typhimurium ATCC 13311, S. Choleraesuis ATCC 10708, S. Enteritidis, S. dysenteriae, M. morganii, C. freundii, A. hydrophila, P. aeruginosa ATCC 27853, P. stutzeri ATCC 17587, S. putrefaciens ATCC 8071, and A. xylosoxidans ATCC 27061). Interestingly, NQ (2) was the only compound that selectively inhibited P. aeruginosa ATCC 27853 with the MIC value of 84.14 μM, while the other derivatives including the parent 8HQ displayed MIC values that were greater than 644.92 μM. Currently, P. aeruginosa, which is the common causative agent of nosocomial infection, has been reported to generate multi-drug resistance because of its biofilm synthesis [25]. Furthermore, Sobke et al. also reported the same result, specifically, that NQ can be used as an anti-biofilm agent for P. aeruginosa with the MIC range of 84.14–168.28 μM [26]. NQ (2) and 7-bromo-8HQ (4) inhibited K. pneumoniae ATCC 700603 and S. Choleraesuis ATCC 10708 with the MIC values of 84.14 and 71.41 μM, respectively, whereas the other derivatives (5–9) showed their MICs > 644.92 μM. Moreover, K. pneumoniae was found to be a causative agent of pneumonia, and was also found to be a pathogen of septicemia in patients [27]. Recently, it has been reported worldwide that the spread of K. pneumoniae carbapenemase (KPC) emerged in many countries [28]. In addition, S. Typhimurium, and S. Enteritidis were inhibited by NQ (2), 7-bromo-8HQ (4) and 5,7-dichloro-8HQ (5) with MICs of 21.03, 35.71 and 37.37 μM, respectively. Salmonella species are well-known human food-borne pathogen of salmonellosis [29]. Clearly, S. Enteritidis, which is the most leading cause of salmonellosis, was inhibited by compounds 2, 4 and 5 with the lower MICs when compared with S. Choleraesuis. In addition, L. monocytogenes is an important food-borne pathogen that can contaminate both raw and processed food products. Furthermore, L. monocytogenes is a board-range causative agent of septicemia, meningitis, encephalitis and pneumonia [30]. However, this study showed that L. monocytogenes was completely inhibited by the parent 8HQ (1) and cloxyquin (3) at MICs as low as 3.44 and 5.57 μM, respectively. However, compound 3 had a higher selectivity index (SI = 14.67, Table 4) than the 8HQ (1, SI = 1.82) against L. monocytogenes. Additionally, compound 3 showed a high growth inhibition against P. shigelloides with the MIC value of 11.14 μM. Among the Gram-negative bacteria, the NQ (2) displayed the most potent activity (MIC = 5.26 μM) and had the highest SI value (16.76) against A. hydrophila that can cause an opportunistic infection in humans. Moreover, NQ (2) also exerted the lowest cytotoxicity (IC₅₀ = 88.14 ± 2.11 μM) compared with the other compounds (1, 3–6 and 8). A relatively low cytotoxicity was noted for compounds 3 (IC₅₀ = 81.74 ± 0.96 μM) and 6 (IC₅₀ = 76.17 ± 0.23 μM). Previously, 8HQ and its copper-complexes were reported to inhibit Gram-positive bacteria, such as S. aureus, E. faecalis and L. monocytogenes, and Gram-negative bacteria such as E. coli, K. pneumoniae and P. aeruginosa [2]. CQ was shown to display activity against Gram-positive bacteria (S. aureus and B. subtilis) and against Gram-negative bacteria such as S. marcescens, P. aeruginosa and E. coli [31]. NQ was documented to inhibit E. coli with the MIC value of 42.07 μM [32]. As a result, the 8HQ derivatives with a relatively high safety index [i.e., the NQ (2, SI = 16.76) and cloxyquin (3, SI = 14.67)] can be selected as effective compounds to inhibit pathogens both in Gram-positive (L. monocytogenes) and Gram-negative (A. hydrophila and P. shigelloides) bacteria, whereas CQ (6) should be selected to inhibit Gram-positive bacteria. Additionally, the non-cytotoxic compounds (7 and 9) displayed a weak antimicrobial activity.

The antioxidant properties of 8HQ and its derivatives resulting from their metal chelating ability have been reported [3]. 8HQ was shown to be a strong iron chelator with an antioxidant activity [33]. Recently, Kharadi [31] reported the antioxidant activity of CQ using the ferric-reducing antioxidant power (FRAP) method. In this study, the antioxidant activity of the parent 8HQ (1) and its derivatives (2–9) were evaluated via the DPPH assay using α-tocopherol as a positive control. It was found that 8HQ (1) exhibited
the activity with the IC_{50} value of 614.77 μM. Previously, 8HQ was reported to display the SOD (superoxide dismutase) activity with the IC_{50} of 91.83 μM [34]. Interestingly, 5-amino-8HQ (8) was shown to be the most potent antioxidant with the IC_{50} value of 8.71 μM compared with the positive control (IC_{50} = 13.47 μM). In addition, compound 8 displayed cytotoxic effect against normal cells with a higher IC_{50} value (22.14 ± 1.02 μM) compared with its antioxidant activity (IC_{50} = 8.71 μM). This indicated that the antioxidant compound 8 had a safety index with SI value of 2.54.

Among the halogenated 8HQ, compound 5 was the best antioxidant (IC_{50} = 335.90 μM), whereas the IC_{50} values of other derivatives (3, 4 and 6) were 1050.67, 597.46 and 366.02 μM, respectively. However, NQ (2) and 2-CN-8HQ (9) were found to be inactive antioxidants. The antioxidant activity of compounds (1–9) may be attributed to the chelating property and electronic effects of substituent groups on the 8HQ scaffold. Specifically, compound 8 with an electron donating group (NH_2) at the 5-position of 8HQ can stabilize the phenoxyl radical (derived from the phenolic moiety) in enhancing the radical scavenging activity. However, the total loss of antioxidant activity was noted for compound 2 (NQ) with a strong electron withdrawing group (NO_2) at the 5-position of 8HQ. Together, the 8HQ derivative with an electron withdrawing group (NO_2) as noted for NQ (2) exerted better anti-microbial activity against Gram-negative bacteria compared with the 8HQ (1) but had no antioxidant activity. In addition, the absence of antioxidant activity was observed for derivative (9) that contained an electron withdrawing group (CN) at the 2-position of 8HQ. Compound 8 with an electron donating effect (NH_2) at the 5-position of 8HQ exhibited the most potent antioxidant effect.

Currently, there are many drug-resistant strains that are generated in Gram-positive bacteria, such as methicillin-resistant S. aureus (MRSA), vancomycin-resistant Enterococci (VRE) and penicillin-resistant S. pneumoniae (PRSP), and in Gram-negative bacilli, such as extended spectrum β-lactamase (ESBL), AmpC β-lactamase and carbapenemase-producing Enterobacteriaceae (CPE). Therefore, it is urgent to discover new and potent antimicrobial drugs because of the increase in the number of multi-drug resistant strains. This finding reveals that 8HQ derivatives, especially NQ (2) and the halogenated 8HQ (3–6), are possible candidates to be further developed as antimicrobial agents to treat these multi-drug resistant bacteria. In addition, 5-amino-8HQ (8) is a promising compound with a highly potent antioxidant activity. The property and position of substituents on the 8HQ pharmacophore provide insight into further development of antimicrobial agents.

Competing interests
None declared.

Ethical approval
Not required.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jbbrep.2016.03.014.

References

[1] R. Pinkaew, S. Prachayasitikul, S. Ruchirawat, V. Prachayasitikul, Synthesis and cytotoxicity of novel 4-[(4’-substituted)-1H-1,2,3-triazol-1-yl]-N-phenethylbenzenesulfonamides, Med. Chem. Res. 23 (2014) 1758–1780.
[2] S. Srisung, T. Sukrachavikul, S. Prachayasitikul, S. Ruchirawat, V. Prachayasitikul, Antimicrobial activity of 8-hydroxyquinoline and transition metal complexes, Int. J. Pharmacol. 9 (2013) 170–175.
[3] J.M. Tanzer, A.M. Slee, B. Kamay, E. Scheere, Activity of three 8-hydroxyquinoline derivatives against multi-viral plaque, Antimicrob. Agents Chemother. 13 (6) (1978) 1044–1045.
[4] J. Kos, I. Zadrazilova, E. Nevin, M. Soral, T. Gonce, P. Kollar, et al., Ring-substituted 8-hydroxyquinoline-2-carboxanilides as potential antimycobacterial agents, Bioorganic Med. Chem. 23 (15) (2015) 4188–4196.
[5] V. Prachayasitikul, S. Prachayasitikul, S. Ruchirawat, V. Prachayasitikul, 8-Hydroxyquinolines: a review of their metal chelating properties and medicinal applications, Drug. Des. Dev. Ther. 7 (2013) 1157–1178.
[6] J. Lazovic, L. Guo, J. Nakashima, L. Mirsadraei, W. Yong, H.J. Kim, et al., Nitroxline induces apoptosis and slows gloma growth in vivo, Neuro-Oncol. 17 (1) (2015) 53–62.
[7] K.G. Naber, H. Niggemann, G. Stein, Review of the literature and individual patients’ data meta-analysis on efficacy and tolerance of nitroxline in the treatment of uncomplicated urinary tract infections, BMJ Infect. Dis. 14 (2014) 628.
[8] W. Chan-On, N.T.B. Huyen, N. Songtaweeg, W. Suwanjang, S. Prachayasitikul, V. Prachayasitikul, Quinoline-based cloquionol and nitroxline exhibit anti-cancer activity inducing FOXM1 inhibition in cholangiocarcinoma cells, Drug Des. Dev. Ther. 9 (2015) 2033–2047.
[9] H. Jiang, J.E. Taggart, X. Zhang, D.M. Benbrook, S.E. Lind, W.Q. Ding, Nitroxline (8-hydroxy-5-nitroquinoline) is a more potent anti-cancer agent than cloquionol (5-chloro-7-ido-8-quinoline), Cancer Lett. 312 (11) (2011) 11–17.
[10] P. Hongmanee, K. Rukseere, B. Bubat, B. Somri, P. Patilapongarnpim, In vitro activities of cloxyquin (5-chloroquinolin-8-ol) against Mycobacterium tu- berculosis, Antimicrob. Agents Chemother. 51 (3) (2007) 1105–1108.
[11] S.R. Bareegi, U. Cornelli, Cloquionol: review of its mechanisms of action and clinical uses in neurodegenerative disorders, CNS Neurosci. Ther. 18 (1) (2012) 41–46.
[12] S. Prachayasitikul, R. Pinkaew, C. Nantasenamat, S. Prachayasitikul, S. Ruchirawat, V. Prachayasitikul, Investigation of aromatase inhibitory activity of metal complexes of 8-hydroxyquinoline and uracil derivatives, Drug Des. Dev. Ther. 8 (2014) 1089–1096, Epub 2014/08/26.
[13] K. Barot, S. Jain, L. Kremer, S. Singh, M. Ghate, Recent advances and therapeutic journey of coumarins: current status and perspectives, Med. Chem. Res. 24 (7) (2015) 2771–2798.
[14] E. Li, J. Subramanian, S. Anderson, D. Thomas, J. McKinley, L.A. Jacobs, Development of biosimilars as an end of oncologic drug shortages, Drug Des. Dev. Ther. 9 (2015) 3247–3255.
[15] R.A. Borchardt, K.V. Rolston, Antibiotic shortages: effective alternatives in the treatment of uncomplicated urinary tract infections, BMC Infect. Dis. 14 (2014) 628.
[16] D.J. Diekema, M.A. Pfaller, F.J. Schmitz, J. Smayevsky, J. Bell, R.N. Jones, et al., In vitro antimicrobial activity and in vitro antimicrobial susceptibility of isolates collected in the United States, Ca-

...
A. Sobke, M. Klinger, B. Hermann, S. Sachse, S. Nietzsche, O. Makarewicz, et al., The urinary antibiotic 5-nitro-8-hydroxyquinoline (Nitroxoline) reduces the formation and induces the dispersal of Pseudomonas aeruginosa biofilms by chelation of iron and zinc, Antimicrob. Agents Chemother. 56 (11) (2012) 6021–6025.

S. Ahmad, A. Abulhamd, Phenotypic and molecular characterization of nosocomial K. pneumoniae isolates by ribotyping, Adv. Med. Sci. 60 (1) (2015) 69–75.

A. Lerner, A. Adler, J. Abu-Hanna, S. Cohen Percia, M. Kazma Matalon, Y. Carmeli, Spread of KPC-producing carbapenem-resistant Enterobacteriaceae: the importance of super-spreaders and rectal KPC concentration, Clin. Microbiol. Infect. 21 (5) (2015) 470.e1–470.e7.

J. Campos, M. Pichel, T.M.I. Vaz, A.T. Tavechio, S.A. Fernandes, N. Muñoz, et al., Building PulseNet Latin America and Caribbean Salmonella regional database: first conclusions of genetic subtypes of S. Typhi, S. Typhimurium and S. Enteritidis circulating in six countries of the region, Food Res. Int. 45 (2) (2012) 1030–1036.

C. Roed, F.N. Engsig, L.H. Omland, P. Skinhoj, N. Obel, Long-term mortality in patients diagnosed with Listeria monocytogenes meningitis: a Danish nationwide cohort study, J. Infect. 64 (1) (2012) 34–40.

G.J. Kharadi, Effect of substituent of terpyridines on the in vitro antioxidant, antitubercular, biocidal and fluorescence studies of copper(II) complexes with clioquinol, Spectrochim. Acta A Mol. Biomol. Spectrosc. 117 (2014) 662–668.

B. Murugasu-Oei, T. Dick, In vitro activity of the chelating agents nitroxoline and oxine against Mycobacterium bovis BCG, Int. J. Antimicrob. Agents 18 (6) (2001) 579–582.

H. Zheng, L.M. Weiner, O. Bar-Am, S. Epsztejn, Z.I. Cabantchik, A. Warshawsky, et al., Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases, Bioorganic Med. Chem. 13 (3) (2005) 773–783.

S. Prachayasittikul, A. Worachatcheewan, R. Pingaew, T. Suksrichavalit, C. Isarankura-Na-Ayudhya, S. Ruchirawat, et al., Metal complexes of uracil derivatives with cytotoxicity and superoxide scavenging activity, Lett. Drug Des. Discov. 9 (3) (2012) 282–287.