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Preparation of 8-hydroxyquinoline derivatives as potential antibiotics against *Staphylococcus aureus*

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ABSTRACT

This work describes the preparation of quinoline compounds as possible anti-bacterial agents. The synthesized quinoline derivatives show anti-bacterial activity towards *Staphylococcus aureus*. It is interesting to observe that the synthetic 5,7-dibromo-2-methylquinolin-8-ol (**4**) shows a similar minimum inhibitory concentration of 6.25 μg/mL as compared to that of methicillin (3.125 μg/mL) against *Staphylococcus aureus*.

There is an increasing search for new antibiotics due to the rise of antibiotic-resistant microorganisms in society.[1–4] Quinine, chloroquine and amodiaquine were well known quinoline antimalarials.[5] Mitscher and co-workers found out that quaternary alkaloids isolated from *Ptelea trifoliate* showed antimicrobial activity towards *Staphylococcus aureus* and *Mycobacterium tuberculosis*.[6] Fournet reported that the presence of 2-substituted quinoline alkaloids, such as 2-propylquinoline and chimanine D, in the plant *Galipea longiflora* demonstrated activity against *Leishmania amazonensis*.[7] Heteroaromatic compounds containing quinoline moiety bearing various biological and pharmacological activities, such as antituberculosis,[8] antimalarial,[9] anti-inflammatory,[10] anticancer,[11–15] antibiotic,[16] antihypertensive,[17] antiHIV[18,19] as HIV integrase inhibitors (e.g., FZ41),[20,21] herbicidal,[22] as well as antibacterial[23] and antifungal properties.[24,25]

Musiol and co-workers[25] reported that the derivatives of quinolin-8-ol are important for the antifungal activity. In addition, quinolin-8-ol derivatives were also reported and studied for the treatment of neurodegenerative diseases such as Alzheimer's disease.[26] In addition, the reported derivatives also possess biological activities on the proliferation of rat mesenchymal stem cells (rMSCs).[27,28] In recent years, we reported the anti-tumor activities of quinoline type compounds.[12–14] In particular, we found out that 8-hydroxyquinoline derivatives are effective for anti-tumor agents.[12–14] Among the selected compounds, 8-hydroxy-2-quinolinecarbaldehyde showed the best in vitro cytotoxicity against the human cancer cell lines, including MDA231, T-47D, Hs578t, SaoS2, K562, SKHep1, Hep3B and normal fibroblast NIH3T3 with MTS_{50} range of 6.25–12.5 μg/mL.

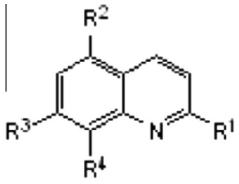

Figure 1. Quinoline derivatives for anti-bacterial study.

Here, we report the synthesis and characterization of eight quinoline compounds (Fig. 1). Their potential anti-bacterial effect against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were screened. In addition, their possible cellular cytotoxicity towards

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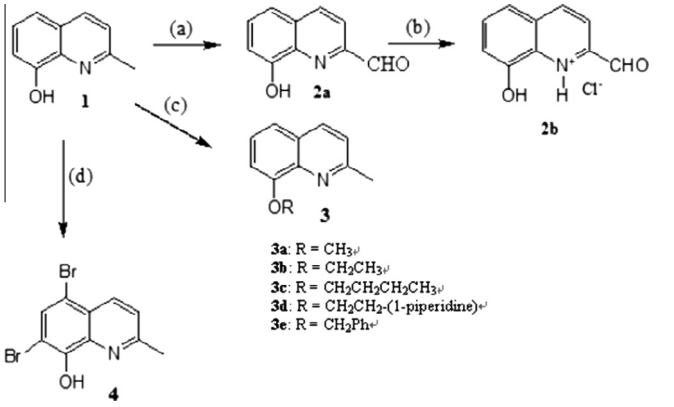


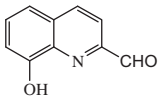
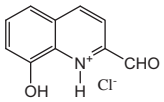
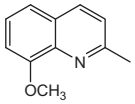
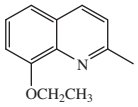
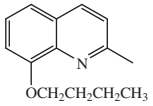
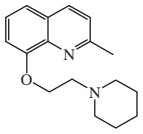
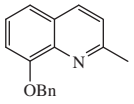
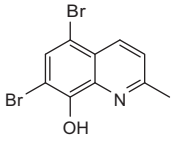
Scheme 1. Reagents: (a) SeO_2, dioxane/H_2O, reflux; (b) HCl (g), DCM; (c) RX (X = Cl or Br), K_2CO_3, DMF; (d) Br_2 (l), MeOH.

the human skin keratinocytes HaCaT and normal esophageal cells NE3 were performed.[29–31] Of which, compound **4**, (5,7-dibromo-2-methylquinolin-8-ol) showed an attractive anti-*S. aureus* activity with a mild cellular cytotoxicity in vitro.

Both research groups of Fournet[7] and Musiol[25] has mentioned that the 2-substitution on the quinoline moiety is important to account for the biological activity. As a starting point, we selected several commercial available 2-substituted quinolines, such as 2-methylquinoline, 2-phenylquinoline, 2-chloroquinoline, 2-quinolinecarboxylic acid and 2-quinolinecarbonitrile. However, they did not exhibit significant activity against *S. aureus* and *E. coli*. As an extension of our previous work, we explore the anti-bacterial application of our quinoline derivatives. Several commercial available hydroxyquinoline compounds, such as 4-hydroxyquinoline, 6-hydroxyquinoline, 7-hydroxyquinoline and 8-hydroxyquinoline with hydroxyl group at position 4, 6, 7 and 8 were selected for our initialize study; however, only 8-hydroxylquinoline show anti-microbial activity. From our previous reported work,[14] we demonstrated that the hydroxyl group at 8-position of quinoline moiety is important for the significant biological activity. In addition, our screening test found out that the commercial available 8-hydroxyquinaldine (**1**) is effective for *S. aureus* and *E. coli* inhibition. Therefore, 8-hydroxyquinaldine (**1**) was employed as a starting chemical for this project. Scheme 1 shows the synthesis of quinoline derivatives.[13,14,32,33] The 8-hydroxy-2-quinolinecarbaldehyde (**2a**) which bears a carboxyaldehyde group on position 2 together with a hydroxyl group on position 8 and its corresponding salt 2-formyl-8-hydroxyquinolinium chloride (**2b**) exhibited relative low inhibitory effect on *S. aureus* and *E. coli*. The 8-alkoxy-substituted quinaldine, such as 8-methoxy-2-methylquinoline (**3a**), 8-ethoxy-2-methylquinoline (**3b**), 8-butoxy-2-methylquinoline (**3c**), 8-(2-(piperidin-1-yl)ethoxy)-2-methylquinoline (**3d**), as well as the 8-(benzyloxy)-2-methylquinoline (**3e**) showed a further decrease in activity. The results may suggest that the hydroxyl group is important for anti-bacterial activity. In our earlier findings, we found out that the presence of bromine on the 2,6-dimethoxylpyridine ring core is important to enhance the biological activity.[15] Herein, we will introduced the bromine group to the quinoline moiety and studied the corresponding anti-bacterial activity of 5,7-dibromo-2-methylquinolin-8-ol (**4**).

We have determined the minimum inhibitory concentration (MIC) of these quinolines against both *S. aureus* and *E. coli* respectively after 24 h of incubation.[34] As shown in Table 1, all the quinolines were found to be inactive towards the gram negative *E. coli* (MIC >50 μg/mL). When considering the gram positive *S. aureus*, it is found that **2a**, **2b** and **4** could inhibit the bacterial growth to a different extent. The MIC values of **2a** and **2b** were determined

Table 1
Minimum inhibitory concentration of nine quinolines towards *S. aureus* and *E. coli*

		MIC (μg/mL)	
		S. aureus	*E. coli*
2a		50	>50
2b		50	>50
3a		>50	>50
3b		>50	>50
3c		>50	>50
3d		>50	>50
3e		>50	>50
4		6.25	>50

MIC of methicillin towards *S. aureus* was 3.125 μg/mL while ampicillin towards *E. coli* was 7.5 μg/mL. Three independent experiments were performed and similar results were obtained.

to be 50 μg/mL which were considerably high while that of **4** was found to be 6.25 μg/mL. This MIC value was comparable to that of methicillin, which was found to be 3.125 μg/mL. For the remaining quinolines, their MIC values were found to be greater than 50 μg/mL. After determining the MIC values, **2a**, **2b** and **4** were further selected for zone of inhibition assays using *S. aureus*.[35] Figure 2 shows that the zone of inhibition (radius) from **2a** was greater than that of **2b** when they were loaded at 50 μg. Compound **4**, on the other hand, showed a better anti-*S. aureus* activity. When **4** was loaded at 15 μg, the zone of inhibition was comparable to that of **2** which was at 50 μg.

The possible cellular cytotoxicity of compounds **2a**, **2b** and **4** were tested using the human skin keratinocyte HaCaT and normal esophageal cells NE3 by the sulforhodamine B assay[36] (Fig. 3). After 24 h of incubation, all the three compounds had an average 50% inhibitory concentration between 18 and 22 μg/mL (Table 2).

Here we describe the preparation of quinoline compounds and their potential anti-bacterial activity were screened using gram positive *S. aureus* and gram negative *E. coli*. None of them had significant inhibitory effect on *E. coli*. On the other hand, three of them showed anti-bacterial effect against *S. aureus*. For **2a** and **2b**, we observed that their MIC values were considerably high (50 μg/mL). However, we found that the synthetic 5,7-dibromo-2-methylquinolin-8-ol

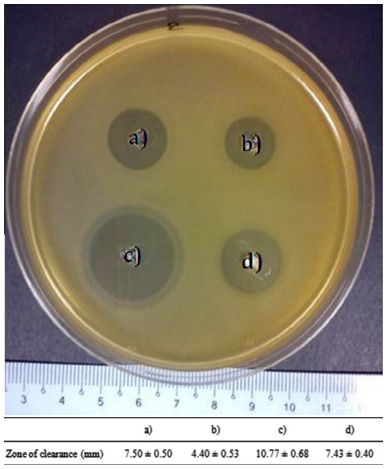


Figure 2. Zone of inhibition study. (a) **2a** at 50 μg/mL; (b) **2b** at 50 μg/mL; (c) methicillin at 5 μg/mL and (d) **4** at 15 μg/mL. Three independent experiments were conducted and representative results are shown. The zone of clearance was measured as the radius (mean ± SD from three results) where no bacterial growth could be found.

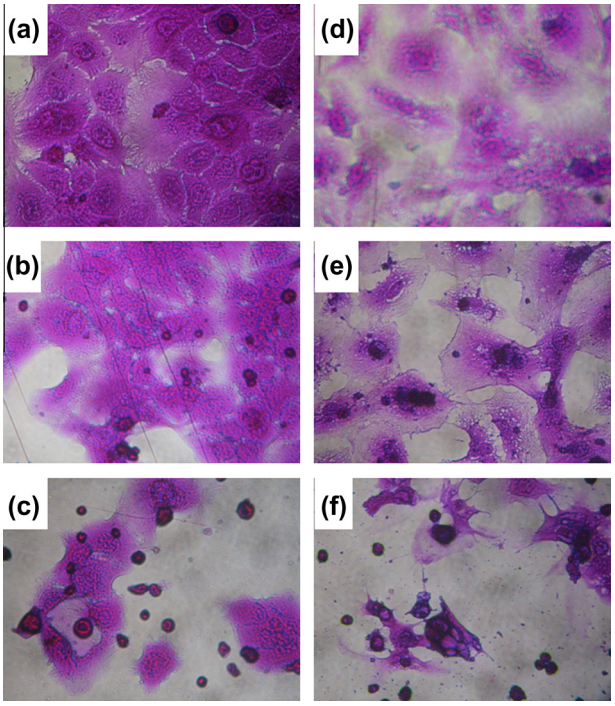


Figure 3. Representative cytotoxic effects of **4** and doxorubicin towards human skin keratinocytes HaCaT (a–c) and human normal esophageal cells NE3 (d–f). (a) and (d) were untreated control; (b) and (e) were 25 μg/mL of **4**; (c) was 4 μg/mL of doxorubicin and (f) was 2 μg/mL of doxorubicin. Certain degree of cell death morphology included cell shrinkage could be observed in (b) and (e) while significant cell death could be identified in (c) and (f).

Table 2
IC_{50} (μg/mL) of **2a**, **2b** and **4** on human skin keratinocytes HaCaT and human esophageal NE3 cells

	2a	2b	4	Doxorubicin
HaCaT	19.15 ± 1.26	22.53 ± 0.97	22.47 ± 1.44	3.26 ± 0.53
NE3	16.06 ± 0.92	18.14 ± 1.03	18.62 ± 0.84	1.89 ± 0.44

Doxorubicin was used as positive control. Three independent experiments were conducted. Each experiment was performed in triplicate and their mean values were calculated. Results are shown as mean ± SD from the three experiments.

(**4**) showed a similar minimum inhibitory concentration of 6.25 μg/mL as compared to that of methicillin (3.125 μg/mL) against *S. aureus* and simultaneously exhibited a mild cytotoxicity towards both human skin keratinocytes HaCaT and human esophageal NE3 cells with an average 50% inhibitory concentration between 18 and 22 μg/mL. Further chemical modification would be our next step to improve their anti-bacterial activity and simultaneously reduce their cellular toxicity in order to discover effective quinoline compounds as novel effective antibiotics.

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32. *Preparation of quinoline derivatives (**2**–**4**).* All reactions were monitored by thin layer chromatography (TLC) on Merck aluminum-coated plates of silica gel 60 F 254 and observed under ultra-violet light (λ = 254 nm). NMR spectra were recorded on a Varian Oxford AS 500 MHz Fourier transform spectrometer using $CDCl_3$ as solvent unless otherwise specified. Residual protic solvents in $CDCl_3$ (δ_H 7.26 ppm, s; δ_C 77.7 ppm, t) was used as internal reference for ^1H NMR and ^{13}C NMR spectra. Coupling constants were recorded in hertz (Hz) and multiplicities were recorded as the following abbreviations: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass analyses were performed utilizing a Micromass Q-Tof-2 spectrometer. As reported in previous work,[14] 8-hydroxyl-2-methylquinoline (**2a**) was prepared by oxidation of 8-hydroxyquinaldine (**1**) with selenium dioxide in dioxane/water mixture under reflux overnight; and its corresponding salt, 2-formyl-8-hydroxyquinolinium chloride (**2b**), was then prepared by bubbling the in-situ generated HCl gas into dichloromethane solution of 8-hydroxyl-2-methylquinoline (**2a**). 8-Alkoxyquinoline derivatives (**3a**–**e**) were prepared by simple substitution of 8-hydroxyquinaldine (**1**) with alkyl halide under basic condition. 5,7-dibromo-2-methylquinolin-8-ol (**4**) was synthesized by dropwisely addition of bromine in methanol solution to 8-hydroxyquinaldine (**1**).[13,14] Unless otherwise specified, the reaction was generally run at room temperature and monitored by TLC until the reaction was completed. The crude products were extracted by using either dichloromethane or ethyl acetate; and purified by silica gel column chromatography using ethyl acetate/hexane mixture as eluting solvent.
33. *Preparation of 5,7-dibromo-2-methylquinolin-8-ol (**4**).* 2-Methyl-8-quinolinol (1.6 g, 10 mmol) was dissolved in 150 mL MeOH. 1 mL bromine liquid in MeOH was added into the solution dropwise. When the reaction completed, Na_2SO_3 was added and the product was extracted with DCM to give a crude product. The crude product was purified by silica gel column chromatography to give a pure product as a pink solid. ^1H NMR (500 MHz, $CDCl_3$): δ 2.75 (s, 3H), 7.39 (d, 1H, J = 8.5 Hz), 7.79 (s, 1H), 8.26 (d, 1H, J = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.41, 104.24, 110.65, 124.61, 125.48, 133.30, 136.65, 138.64, 149.77, 159.47; HRMS (ESI): Calcd for $C_{10}H_8NOBr_2$ $[M+H]^+$, 315.8973; found 315.8981; Melting Point = 126.9–128.5 °C; Yield = 64%.
34. *Determination of MICs.* The concentration of *S. aureus* and *E. coli* were adjusted to 1×10^6 cell/mL. The MIC values of synthesized compounds were determined by the LB broth dilution method. Various concentrations of compounds were added from a starting concentration of 50 μg/mL (stocks were prepared as 50 mg/mL in dimethylsulfoxide, DMSO) and they were diluted serially in the 96 wells microtitre plate. Methicillin was used as a positive control for *S. aureus* (started at 50 μg/mL) and ampicillin was used as positive control for *E. coli* (started at 30 μg/mL) while 0.1% DMSO was used as a vehicle control. They were then incubated at 37 °C for 24 h. The minimum concentrations of compounds that induced a complete growth inhibition will be recorded as MIC values.
35. *Zone of inhibition study. S. aureus* was used to study the anti-bacterial activity of compounds **2a**, **2b** and **4** against its grown in culture. *S. aureus* was diluted with broth medium and plated on the nutrient agar plate and the holes were created on the agar using a sterile transfer pipette. The tested compounds and methicillin (as positive control) were placed in the holes of agar. The plates were then incubated at 37 °C for 24 h and the inhibition zones (mm, in terms of radius) of bacteria on the agar plates were recorded.
36. *Cytotoxicity assay.* Human cells including HaCaT skin keratinocytes and normal esophageal cells NE3 were removed from 75 mL sterile cell culture flasks with trypsin and neutralized with fetal bovine serum. After washing with phosphate buffered saline and centrifugation, skin cells were re-suspended in complete cell culture medium at a concentration of approximately 1×10^5 cells/mL and counted manually using a haematocytometer under an inverted microscope. Human cells seeded in the 96 wells microtitre plates for 24 h were prepared for the screening of our compounds. Compounds **2a**, **2b** and **4** with the starting concentration of 50 μg/mL and 4 μg/mL doxorubicin (as positive control) were incubated with cells for a further period of 24 h. 0.1% DMSO was used as vehicle control. The cytotoxicity assessment was conducted by sulforhodamine B protein staining method. Skin cells were fixed with trichloroacetic acid, washed with deionized water and stained with sulforhodamine B. Afterwards, cells were washed again with acetic acid. Photomicrographs of skin keratinocytes were captured and optical absorptions were then measured at 575 nm after dissolving in Tris-base buffer using a a Perkin Elmer Victor V multiwell plate reader.