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Synthesis, characterization, and in vitro antimicrobial investigation of novel pyran derivatives based on 8-hydroxyquinoline

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Abstract

Background: 8-Hydroxyquinoline derivatives are known for their extensive applications in the field of analytical chemistry and separation techniques; their complexes with transition metals also exhibit antibacterial and antifungal activity.

Results: In the present study, we synthesized a new series of pyranoquinoline derivatives and evaluated their antibacterial activities. The structures of the synthesized compounds *were* characterized by *Fourier transform infrared* (FT-IR), hydrogen-1 nuclear *magnetic resonance*, *carbon-13 nuclear magnetic resonance*, and *elemental analysis*. All the prepared compounds were evaluated in vitro as antimicrobial agents against Gram-positive and Gram-negative bacterial strains (*Escherichia coli* (ATCC35218), *Staphylococcus aureus* (ATCC29213), *Vibrio parahaemolyticus* (ATCC17802), and *Pseudomonas aeruginosa* (ATCC27853)). The screening test was determined by using the standard protocol of *disc diffusion method* (DDM).

Conclusion: We have synthesized new pyranic compounds bearing an 8-hydroxyquinoline moiety on their structure. The preliminary screening results showed that all the tested compounds have a remarkable inhibitory effect on the growth of the majority of the tested bacterial strains compared to the standard antibiotic (penicillin G), and the chlorinated compound (Q_1) is more active against Gram-positive bacteria than Gram-negative bacteria such as the *Staphylococcus aureus* strain which is the most sensitive. Gram-positive bacteria are responsible for a wide range of infectious diseases, and rising resistance in this group is causing increasing concern. Thus, this study develops novel heterocyclic compound derivatives of 8-hydroxyquinoline that have demonstrated good antibacterial activity against Gram-positive bacteria.

Keywords: Quinoline, Synthesis, Characterization, Antibacterial activity, In vitro, Bacterial strains

1 Background

Microbes are essential microorganisms which are needed for both humans and environment because of their vital role in almost all ecosystems. However, they can also be the cause of many infectious diseases [1], Some microorganisms like bacteria and yeasts can be pathogenic and cause diseases in humans, plants, or animals while pathogenic bacteria are responsible for several epidemic and pandemic diseases [2]. Therefore, the search for an anti-infectious substance has become a public health problem. From a series of observations and works by many researchers including Pasteur, Joubert, Duchesne, and Fleming, this quest has consequently led to the discovery of antibiotics [3].

This widespread use of antibiotics has also led in its turn to the appearance of strains of pathogenic microorganisms resistant to these drugs, together with the emergence of uncommon infections [4]. that compromise treatments with existing drugs. This phenomenon leads to an increase in morbidity and mortality [5]. In the face of these new barriers brought about by the use of available antimicrobials, it is essential to look for new, effective, and broad-spectrum substances.

On the other hand, the 8-hydroxyquinoline derivatives have long been known because of their widespread

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application in the field of analytical chemistry and separation techniques [6], since it plays a very important role for the extraction of metal ions; their complexes with various metal ions have been reported to be active against certain bacteria and fungi and whose power is proportional to their ionic chelation due to their lipid solubility [7]. On the other hand, some derivatives have also been reported as potential inhibitors of HIV-1 integrase. They have also been used as powerful agents for neuroprotection against Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases [8]. A work on antibacterial activity has recently been carried out in our laboratory on 8-hydroxyquinoline derivatives; these compounds have shown a good activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter ludwigii*, and *Bacillus subtilis* [9].

The preparation of pyranoquinoline derivatives consists in reacting equimolar amounts of 8-hydroxyquinoline, 4-alkylbenzaldehyde, and methyl-2-cyanoacetate in pure ethanol in the presence of calcium bicarbonate under magnetic stirring during 24 h.

The objective of this work is to synthesize and evaluate the antibacterial activity against Gram-positive and Gram-negative bacterial strains of new pyranoquinoline derivatives.

2 Materials and method

2.1 Chemicals and apparatus

The reagents and solvents used in this study were purchased from Acros or Sigma-Aldrich companies. All tests were done on a Kofler bench (infrared (IR)). *Melting points were determined* on a *Banc Kofler* apparatus and are uncorrected. The recording of nuclear magnetic resonance spectra was performed on a Bruker Advanced 300 WB at 300 MHz for solutions in $Me_2SO\text{-}d_6$, and chemical shifts are given in δ_{ppm} with reference to tetramethylsilane (TMS) as an internal standard. Infrared spectra were recorded in a FT-IR Nicolet 400D Spectrophotometer using potassium bromide (KBr) pellets. *The elemental composition (carbon, hydrogen and nitrogen) was determined* on a Perkin-Elmer Model 240 CHN Analyzer. The progress of the reaction is followed by thin-layer chromatography (TLC) of silica 60 F254 (E. Merck).

2.2 Bacterial strains

Four bacteria have been selected for this study to test the antibacterial activity of synthesized products. These bacteria are *Escherichia coli* that cause food poisoning and infections [10]; *Staphylococcus aureus* that causes life-threatening complications such as infection in blood [11], bones, or lungs; *Pseudomonas aeruginosa* is considered a human pathogen more often responsible for nosocomial infections [12]; and *Vibrio parahaemolyticus*, which represents a serious and global threat to human health [13]. The bacterial isolates *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are of clinical origin. However, *Vibrio parahaemolyticus* is a major cause of food poisoning. They were all provided by the Laboratory of Nutrition, Health and Environment, Department of Biology, Faculty of Sciences, University Ibn Tofaïl - Kenitra, Morocco. Each bacterium was inoculated on the Ageller Mueller-Hinton culture medium.

2.3 Culture medium and solvent

The medium used in this study is Mueller-Hinton agar standardized by the World Health Organization (WHO) to determine the susceptibility of bacteria to antibiotics. The Mueller-Hinton agar medium composition is shown in Table 1. *Dimethyl sulfoxide* (DMSO) was used as a solvent to solubilize the tested compounds, and the physiological solution (9 g of sodium chloride (NaCl) in 1 L of distilled water) was used to regulate the physiological metabolisms of the bacteria under the experimental conditions (denaturing condition).

3 Experimental part

3.1 Chemical synthesis and characterization (See Additional file 1)

3.1.1 General procedure for the synthesis of compounds

A mixture of the substituted *p*-benzaldehyde (0.01 eq), methyl 2-cyanoacetate (0.01 eq), and calcium carbonate (0.01 eq) in absolute ethanol (30 ml) was stirred at room temperature for 6 h. To this mixture was added 8-hydroxyquinoline (HQ) (0.01 eq), and the reaction mixture was refluxed under magnetic stirring for 18 h. The reaction mixture was filtered while hot, and the filtrate was allowed to cool for 30 min until the expected product was precipitated. The formed solid was collected by filtration, washed with hexane, purified by silica column chromatography using a mixture of acetone/hexane (85: 15, v/v), and then recrystallized from absolute ethanol to afford the desired compounds (Fig. 1).

The chemical structures, names, and abbreviations of the synthesized compounds are given in Table 2.

Table 1 Composition of Mueller-Hinton agar culture medium

Casein hydrolysate	17.5 g
Starch	1.5 g
Agar	10.0 g
Distilled water	1000.0 ml
Final pH	7.4

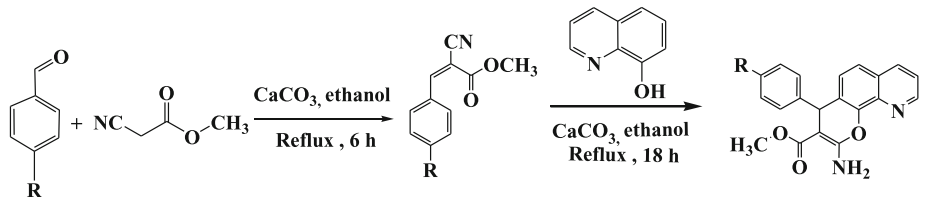


Fig. 1 Synthetic route for the preparation of pyranoquinoline derivatives

3.2 Antibacterial test

The antibacterial activity of our tested compounds was determined using the agar disc diffusion assay, while a 24-h bacterial culture was spread on the surface of the Mueller-Hinton agar plate. A disc of sterile 6-mm filter paper was saturated with 10 μl of solution of the studied compounds in dimethyl sulfoxide. After 1 h of diffusion, the Petri dishes were incubated at 37 °C for 24 h and the diameter of the zone of inhibition was measured and compared with that of the penicillin G reference disc.

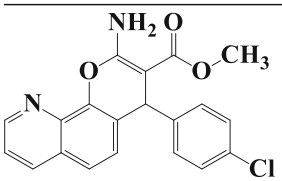
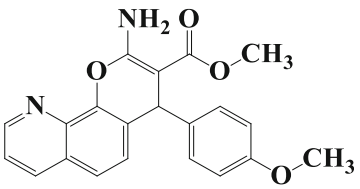
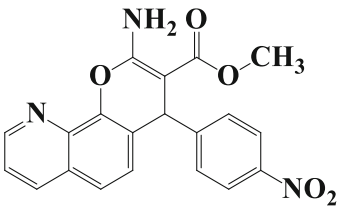
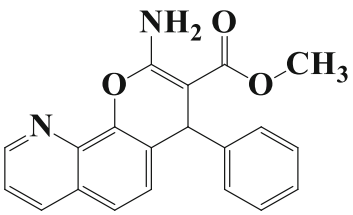
4 Result

4.1 Synthesis and spectral data

4.1.1 Synthesis of methyl 2-amino-4-(4-chlorophenyl)-4H-pyrano[3,2-h]quinoline-3-carboxylate (Q_1)

It was synthesized from 4-chlorobenzaldehyde with methyl 2-cyanoacetate and 8-hydroxyquinoline (HQ)

Table 2 Chemical structures, names, and abbreviations of the *synthesized compounds*

Product	Name	Abbreviation
	Methyl 2-amino-4-(4-chlorophenyl)-4H-pyrano[3,2-h]quinoline-3-carboxylate	Q_1
	Methyl 2-amino-4-(4-methoxyphenyl)-4H-pyrano [3,2-h] quinoline-3-carboxylate	Q_2
	Methyl 2-amino-4-(4-nitrophenyl)-4H-pyrano [3,2-h] quinoline-3-carboxylate	Q_3
	Methyl 2-amino-4-phenyl-4H-pyrano [3,2-h] quinoline-3-carboxylate	Q_4

adopting the general procedure: yield 91%, aspect white solid, M_p 133–135 °C, R_f value 0.78 (n-hexane/dichloromethane: 5/5, (v/v)).

IR (KBr, in cm^{-1}): 2071.88 (C=N), 1387.17 (C=C), 3444.60 (NH_2), 1606.20 (C=O).

^1H (300 MHz, DSMO-d_6): δ_{ppm} = 8.73 (S, 2H, NH_2), 5.62 (S, 1H, CH_{pyran}), 3.35 (CH_3), 7.50-7.76-8.05-8.39-8.74 (m, 5H, Ar- quinoline), 7.27-7.30-7.58 (m, 4H, benzene ring).

^{13}C (300 MHz, DSMO-d_6): δ_{ppm} = 47.93 (CH_3), 152.37 (C-NH_2), 153.74 (C=O), 111.04-127.94-148.21 (ArCH of quinoline), 119.74-122.09-133.27 (ArC of quinoline), 127.73-129.23 (ArCH of benzene ring), 139.31 (ArC of benzene ring).

4.1.1.1 Microanalysis

Calculated: C, 65.49%; H, 4.12%; N, 7.64%.

Obtained: C, 65.15%; H, 4.64%; N, 7.56%.

4.1.2 Synthesis of methyl 2-amino-4-(4-methoxyphenyl)-4H-pyrano[3,2-h]quinoline-3-carboxylate (Q_2)

It was synthesized from 4-methoxybenzaldehyde with methyl 2-cyanoacetate and 8-hydroxyquinoline (8-HQ) adopting the general procedure: yield 81%, aspect yellow solid, M_p 141–143 °C, R_f value 0.90 (n-hexane/dichloromethane: 5/5, (v/v)).

IR (KBr, in cm^{-1}): 2686.20 (C=N), 1469.89 (C=C), 3444.60 (NH_2), 1629.08 (C=O).

^1H (300 MHz, DSMO-d_6): δ_{ppm} = 8.37 (S, 2H, NH_2), 5.72 (S, 1H, CH_{pyran}), 2.05 (CH_3), 6.71-7.19-7.22-7.60-7.61-8.38 (m, 5H, Ar-quinoline), 7.59-7.22-7.58 (m, 4H, benzene ring).

^{13}C (300 MHz, DSMO-d_6): δ_{ppm} = 57.78 (CH_3), 149.16 (C-NH_2), 150.95 (C=O), 113.27-114.04-128.86-129.39-146.18 (ArCH of quinoline), 122.43-124.72-131.03-145.53 (ArC of quinoline), 122.67-128.95

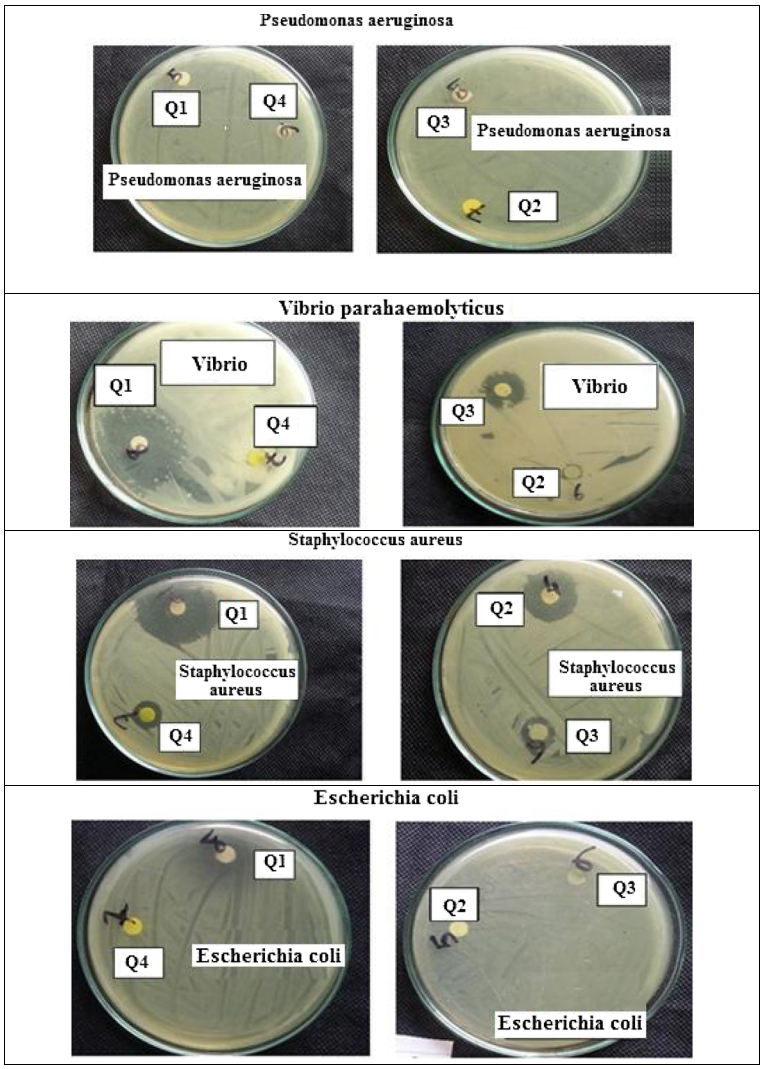


Fig. 2 Antibacterial activity of the pyranoquinoline derivatives against bacteria after 24 h of incubation at 37 °C

(ArC1H of benzene ring), 129.74-129.32 (ArC of benzene ring).

4.1.2.1 Microanalysis

Calculated: C, 69.60%; H, 5.01%; N, 7.73%.
Obtained: C, 69.65%; H, 5.62%; N, 7.54%.

4.1.3 Synthesis of methyl 2-amino-4-(4-nitrophenyl)-4H-pyrano[3,2-h]quinoline-3-carboxylate (Q_3)

It was synthesized from 4-nitrobenzaldehyde, methyl 2-cyanoacetate and 8-hydroxyquinoline (HQ) by adopting the general procedure: yield 93%, aspect red solid, M_p 137–139 °C, R_f value 0.25 (n-hexane/dichloromethane: 5/5, (v/v)).

IR (KBr, in cm^{-1}): 2382.08 (C=N), 1400.90 (C=C), 3436.18 (NH_2), 1628.16 (C=O).

^1H (300 MHz, DSMO-d_6): δ_{ppm} = 8.28 (S, 2H, NH_2), 5.75 (S, 1H, CH_{pyran}), 3.36 (CH_3), 7.20-7.21-7.22-7.60-7.62-7.63 (m, 5H, Ar-quinoline), 7.48-7.49-7.50 (m, 4H, benzene ring).

^{13}C (300 MHz, DSMO-d_6): δ_{ppm} = 44.88 (CH_3), 153.61 (C-NH_2), 169.18 (C=O), 110.34-110.92-127.08-129.14-148.52 (ArCH of quinoline), 121.91-123.48-132.40-139.12 (ArC of quinoline), 122.43-127.48 (ArCH of benzene ring), 132.02-131.38 (ArC of benzene ring).

4.1.3.1 Microanalysis

Calculated: C, 63.66%; H, 4.01%; N, 11.14%.
Obtained: C, 63.54%; H, 4.16%; N, 11.78%.

4.1.4 Synthesis of methyl 2-amino-4-phenyl-4H-pyrano[3,2-h]quinoline-3-carboxylate (Q_4)

It was synthesized from *benzaldehyde* with methyl 2-cyanoacetate and 8-hydroxyquinoline (HQ) according to the general procedure: yield 92 %, aspect white solid, M_p 147–149 °C, R_f value 0.76 (n-hexane/dichloromethane: 5/5, (v/v)).

IR (KBr, in cm^{-1}): 2022.38 (C=N), 1636.29 (C=C), 3496.01 (NH_2), 1387.48 (C=O).

^1H (300 MHz, DSMO-d_6): δ_{ppm} = 8.21 (S, 2H, NH_2), 3.33 (S, 3H, CH_3), 5.69 (S, 1H, CH_{pyran}), 7.50-7.56-7.85-7.92-8.55 (m, 5H, Ar- quinoline), 7.22-7.24-7.34 (m, 5H, benzene ring).

^{13}C (300 MHz, DSMO-d_6): δ_{ppm} = 21.17 (CH_3), 164.15 (C-NH_2), 114.88-127.80-148.34 (ArCH of quinoline), 115.41-117.56-138.19 (ArC of quinoline), 127.21-129.28 (ArCH of benzene ring), 139.12 (ArC of benzene ring).

4.1.4.1 Microanalysis

Calculated: C, 72.28%; H, 4.85%; N, 8.43%.
Obtained: C, 72.87%; H, 4.77%; N, 8.66%.

4.2 Antibacterial test

The synthesized products were evaluated and screened in vitro for their antibacterial activity on four bacterial strains. The bacterial growth inhibition results are recorded in Figs. 2, 3 and Table 3.

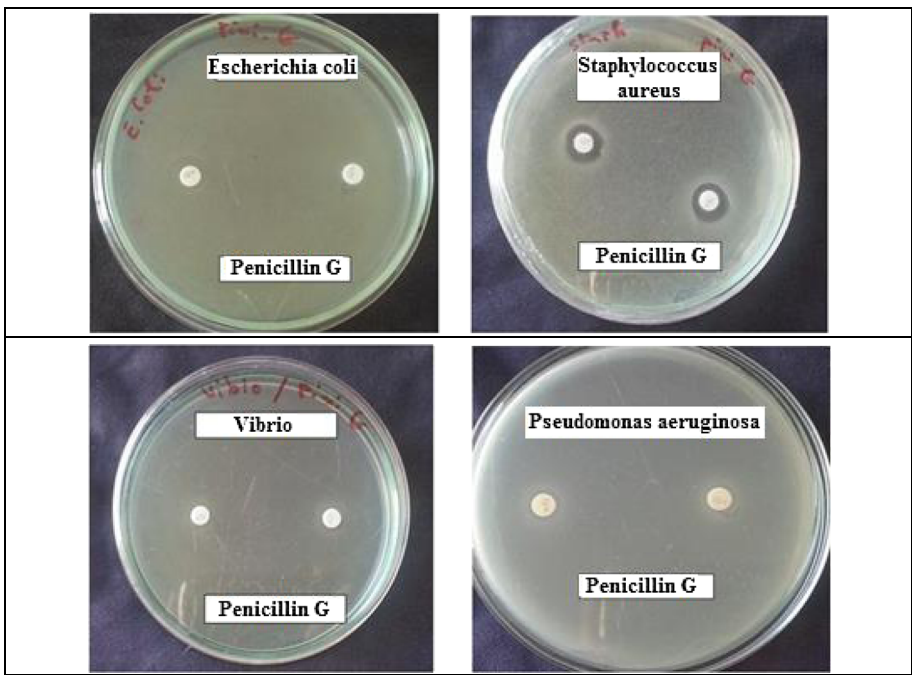


Fig. 3 The *antibacterial activity* of *penicillin G* against bacteria after 24 h of incubation at 37 °C

Table 3 Inhibition zone (in mm) of the synthesized compounds compared with standard antibiotic penicillin G against Gram-positive and Gram-negative bacteria at 10^{-3} g/ml

Compound	Inhibition zone diameter (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	Staphylococcus aureus	*Vibrio parahaemolyticus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Q_1 (R = Cl)	35	30	18	–
Q_2 (R = OCH_3)	17	10	–	–
Q_3 (R = NO_2)	13	10	–	–
Q_4 (R = H)	11	5	–	–
Penicillin G	11	5	10	9

5 Discussion

All the tested compounds Q_1, Q_2, Q_3, and Q_4 show outstanding antibacterial activity against Gram-positive and Gram-negative strains compared to the standard antibiotic (penicillin G). This activity is higher against Gram-positive bacteria than Gram-negative bacteria. We note that for the strain *Pseudomonas aeruginosa* (ATCC 27853) all these compounds have no effect. According to the literature, these poor results against Gram-negative bacteria can be explained by the presence of another membrane which reduces the transfer of the compounds Q_1, Q_2, Q_3, and Q_4 through the cytoplasmic membrane, these results are in agreement with those of Himmi et al. [14], who showed that in vitro 8-hydroxyquinoline derivatives were more active against Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC 8739), and Gram-negative *Pseudomonas aeruginosa* (CIP 82118) and *Clostridium sporogenes* (ATCC 31793).

The results obtained in the present work show that the tested compounds have a remarkable inhibitory effect on the growth of the majority of bacterial strains tested. These observed antibacterial activities are also explained by the presence of the quinoline nucleus, which has already shown an antibacterial effect in other research work [15].

Therefore, in order to improve the antibacterial activity of our compounds, we have bound the quinoline ring with a pyranic ring and a benzene ring bearing electron-donor substituents such as –Cl, $–OCH_3$, and $–OC_2H_5$. Furthermore, it has been shown that heterocyclic compounds bearing electron-withdrawing substituents (nitro, acid function, etc.) have shown lower antimicrobial activity against Gram-positive and Gram-negative bacteria than compounds carrying electron-donor substituents such as *O*-alkyl, *O*-aryl, and chlorophenyl [16]. In our study, we note that compounds bearing electron-donor substituents on the benzene ring linked to the pyranic nucleus exert a positive antimicrobial activity against some Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Escherichia coli*.

6 Conclusion

The analysis of the results obtained by the diffusion method of the disc shows that the compound whose benzene nucleus bears chlorine is therefore the most active compared to the other compounds and also compared to the blank of penicillin G. The antibacterial activity of the chlorinated compound is more important against Gram-positive bacteria than Gram-negative bacteria such as the *Staphylococcus aureus* strain which is the most sensitive.

7 Additional file

Additional file 1: Spectral data of the synthetized compounds. (DOCX 1047 kb)

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Authors' contributions

MR contributed in the synthesis of the tested compounds and identifying their structures, participated in the antibacterial activity evaluation, and wrote the manuscript. AH contributed in the evaluation of the antibacterial activities. OB performed the antibacterial tests. YL participated in the preparation of the manuscript and the verification of the English language. KO contributed in the conception and verification of the biological part. BL contributed in the design of the organic synthesis part, manuscript preparation, and the verification of the English language. All authors read and approved the final manuscript.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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