Journal of Molecular Structure 1097 (2015) 117–123

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Crystal structure and activities of three biscoumarin derivatives against *Staphylococcus aureus*

Fen Li [a,1], Chang-wei Lv [b,1], Zi-dan Zhang [c,1], Jing Li [d], Zheng Hou [a], Xiao-hui Yang [d], Jiang-tao Li [d], Xiao-xing Luo [a,*], Ming-kai Li [a,*]

[a] Department of Pharmacology, School of Pharmacy, The Fourth Military Medical University, Xi'an, China
[b] Department of Orthopaedics, Xijing Hospital, The Fourth Military Medical University, Xi'an, China
[c] Department of Physics, School of Science, Tianjin University, Tianjin, China
[d] College of Chemistry and Chemical Engineering, The Key Laboratory for Surface Engineering and Remanufacturing in Shaanxi Province, Xi'an University, Xi'an, China

HIGHLIGHTS

- Three new biscoumarin derivatives were successfully synthesized.
- Their structures were verified by single crystal X-ray crystallography.
- The antibacterial activities of the three compounds were further investigated.

ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 8 May 2015
Accepted 11 May 2015
Available online 19 May 2015

Keywords:
Biscoumarin
Crystallography
DFT
S. aureus

ABSTRACT

Three new biscoumarin derivatives, namely, 3,3′-[(4-nitrophenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (NBH), 3,3′-[(4-methoxyphenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (MBH) and 3,3′-[(4-chloromethylphenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (CBH) were successfully synthesized and their structures were verified by single crystal X-ray crystallography. In their structures, there are two intramolecular H-bonds and the corresponding H-bond energies were calculated by DFT method. The antibacterial activities of NBH, MBH and CBH in vitro against drug-sensitive *Staphylococcus aureus* (ATCC 29213) and methicillin-resistant *S. aureus* (isolated MRSA strains) were further investigated.

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most important pathogens that leads to various illnesses in humans, including wound infections, pneumonia, sepsis, and toxic shock [1–3]. Methicillin-resistant *S. aureus* (MRSA) is the cause of most antibiotic resistant healthcare associated infections, because it spreads rapidly and cause illness more severe, which further leads to mortality and morbidity rates of patients affected by MRSA are high [4,5], and the related proportion of such cases increased significantly [6,7]. In addition, the emergence of MRSA with decreased susceptibility to vancomycin, which is customarily used as the most effective antibiotic to treat for MRSA, induced to the urgent necessity of developing new antimicrobials.

Coumarin (2*H*-chromen-2-one) derivatives, containing aromatic δ-lactones system, are an important class of heterocycles that have attracted significant importance in the field of organic and natural product chemistry [8–11]. For example, biscoumarin and its derivatives have received considerable attention in the past few years for their versatile biological and medical activities because of the ease of fine-tuning the aromatic ring with different substituents on leading to multiple chemical modifications and activities such as antioxidant, anti-inflammatory, antibacterial and anticancer [12–14]. Recognizing the considerable importance of the compounds, the researchers focused on the synthesis of biscoumarin derivatives [15].

In this study, we successfully synthesized three novel biscoumarin derivatives, namely, 3,3′-[(4-nitrophenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (NBH), 3,3′-[(4-methoxyphenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (MBH) and 3,3′-[(4-chloromethylphenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (CBH) (Fig. 1), tested their anti-bacterial activities on drug-sensitive and drug-resistant *S. aureus* in vitro, and then

* Corresponding authors.
E-mail addresses: xxluo3@fmmu.edu.cn (X.-x. Luo), mingkai@fmmu.edu.cn (M.-k. Li).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.molstruc.2015.05.021
0022-2860/

calculated their total HB energies by density functional theory (DFT) method.

Experimental

Apparatus and materials

IR spectra were measured using a Brucker Equinox-55 spectrophotometer. ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectra were tested using the Varian Inova-400 spectrometer, Bruker Avance III spectrometer and micrOTOF-Q II mass spectrometer, respectively. The melting points were determined on a XT-4 micro melting apparatus.

S. aureus strain (ATCC 29213) and isolated MRSA strains (1-3) were obtained from Chinese National Center for Surveillance of Antimicrobial Resistance and Xijing Hospital (Xi'an, China), respectively. Antibiotics including levofloxacin, ceftazidime, ceftriaxone, gentamicin and piperacillin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals and solvents were of analytical grade.

General procedure for preparation of biscoumarin derivatives NBH, MBH and CBH

NBH, MBH and CBH were prepared from a reported procedure [16]. In a 100 mL round bottom flask, a mixture of 4-nitrobenzaldehyde (4-methoxybenzaldehyde or 4-chloromethylbenzaldehyde) (10 mmol) and 4-hydroxycoumarin (20 mmol) were placed over a magnetic stirrer and the contents were stirred. To this stirred mixture, a few drops of piperidine were added. The reaction mixture was heated at 90 °C for 3–5 h and the progress was monitored by TLC using hexane–chloroform–ethyl acetate mixture (1:1:1) as eluent. After completion of the reaction, the reaction mixture was allowed to cool in room temperature (25 °C) until precipitation occurred. The precipitates were filtered and then washed with ethanol to get pure products.

3,3′-[(4-Nitrophenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (NBH): Yield: 60%. mp 251–252 °C. ν_{max}(KBr): 1658, 1616, 1564, 1521, 1348, 1109, 763 cm^{-1}. ^{1}H NMR ($CDCl_3$): 11.57(s, 1H), 11.38(s, 1H), 8.18–8.20(d, J = 8.8 Hz, 2H), 8.00–8.10(q, J = 7.6 Hz, J = 7.6 Hz, 2H), 7.67–7.69(t, 2H), 7.40–7.44(t, 6H), 6.12(s, 1H). ^{13}C NMR (CD_3Cl) δ: 169.1, 167.0, 166.4, 164.9, 152.6, 152.3, 146.9, 143.4, 133.4, 127.6, 125.2, 125.2, 124.5, 124.5, 123.9, 116.8, 116.8, 116.7, 116.2, 104.8, 103.3, 36.5. HRMS (ESI$^+$): m/z: calcd for $C_{25}H_{15}NO_8$: 480.0690 [M + Na$^+$]; found: 480.0489.

3,3′-[(4-Methoxyphenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (MBH): Yield: 66%. mp 269–270 °C. ν_{max}(KBr): 1670, 1604, 1560, 1510, 1350, 1256, 1093, 769 cm^{-1}. ^{1}H NMR ($CDCl_3$): 11.51(s, 1H), 11.30(s, 1H), 7.99–8.08(q, J = 6.8 Hz, J = 6.8 Hz, 2H), 7.61–7.65(t, 2H), 7.40–7.42(d, J = 8.0 Hz, 4H), 7.12–7.14(d, J = 8.4 Hz, 2H), 6.84–6.87(d, J = 8.8 Hz, 2H), 6.05(s, 1H), 3.80(s, 3H). ^{13}C NMR (CD_3Cl) δ: 158.4, 132.8, 127.6, 126.9, 124.9, 124.4, 116.6, 114.0, 55.3, 35.5. HRMS (ESI$^+$): m/z: calcd for $C_{26}H_{18}O_7$: 465.0945 [M + Na$^+$]; found: 465.0967.

3,3′-[(4-Chloromethylphenyl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (CBH): Yield: 63%. mp 254–255 °C. ν_{max}(KBr): 1666, 1616, 1567, 1354, 1095, 908, 767 cm^{-1}. ^{1}H NMR ($CDCl_3$): 11.54(s, 1H), 11.32(s, 1H), 8.00–8.09(q, J = 6.4 Hz, J = 6.4 Hz, 2H), 7.63–7.66(m, 2H), 7.35–7.44(m, 6H), 7.22–7.23(d, J = 6.0 Hz, 2H), 6.08(s, 1H), 4.59(s, 2H). ^{13}C NMR (CD_3Cl) δ: 169.2, 166.9, 165.9, 164.6, 152.5, 152.3, 136.1, 135.7, 133.0, 128.9, 128.0, 126.9, 126.5, 125.0, 124.4, 116.9, 116.7, 116.4, 105.4, 103.8, 45.8, 36.3. HRMS (ESI$^+$): m/z: calcd for $C_{26}H_{17}ClO_6$: 483.0606 [M + Na$^+$]; found: 483.0601.

X-ray crystallography

For compounds NBH, MBH and CBH, three white crystals with approximate dimensions of 0.22 × 0.20 × 0.16 mm^3, 0.20 × 0.20 × 0.12 mm^3, and 0.20 × 0.18 × 0.10 mm^3 were selected for data collection respectively. The X-ray diffraction data were collected on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromated Mo Ka radiation (λ = 0.71073 A) by using ω–2θ scan technique at room temperature. The structures were solved by direct methods (SHELXS-97) and refined using the full-matrix leastsquares method on F^2 with anisotropic thermal parameters for all non-hydrogen atoms using SHELXL-97 [17]. The hydrogen atoms were placed in calculated positions and refined using the riding model. The crystal data, details concerning data collection and structure refinement are given in Table 1. Molecular illustrations were prepared using the XP package.

Quantum chemical calculations

Theoretical studies based on density functional theory (DFT) calculations using the Gaussian 09 package [18–20]. In order to obtain precise results that are in good agreement with experimental results, three level of theories have been carried out, they are B3LYP/6-31G*, B3LYP/6-31 + G** and B3LYP/6-311G*, respectively [21–23].

Bacterial susceptibility testing

The minimum inhibitory concentration (MIC) values were determined according to the previously reported method [24], *S. aureus* strains were grown in 100 μL of nutrient Mueller–Hinton (MH) broth in the concentration of 5 × 10^5 CFU/mL, then 100 μL of MH medium containing the NBH, MBH or CBH (0.12 – 256 μg/mL in serial two-fold dilutions) was added to the wells of plates. After they were incubated at 37 °C for 20 h, the lowest concentration of compound without visible bacterial growth in the wells was taken as the MIC value. The experiments were done independently five times, using duplicate samples each time.

Bacterial growth rate assay

The effect of NBH, MBH and CBH to the growth rate of *S. aureus* and MRSA were determined as reported method [25], *S. aureus* and MRSA were cultured in the 150 μL MH broth each well using automated Bioscreen C system (Lab systems Helsinki, Finland), then 150 μL coumarin derivatives solution were added to culture medium at 32 or 128 μg/mL in 35 °C, then the culture medium were shaken for 1 min. As the optical density of each sample, OD600 was obtained in regular intervals of 10 min for 20 h at a wavelength of 600 nm to estimate the concentration of bacterial.

Cytotoxicity assay

For NBH, MBH and CBH, the cytotoxicity with respect to the human umbilical vein endothelial cells (HUVECs) were investigated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Firstly, HUVECs cells (5 × 10^3/well) were grown for 24 h at 37 °C to confluence in 96-well plates in DMEM media with 20% fetal bovine serum, then treated with NBH, MBH or CBH at different concentrations (6.25, 12.5, 25, 50 and 100 μg/mL) for 24 h. After that, 0.5% MTT solution was added into the cultured HUVECs and incubated for 4 h. Finally, the supernatant was removed and 150 μL DMSO was added to each well, the absorbance was read at 490 nm.