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Synthesis, crystal structures, and anti-drug-resistant *Staphylococcus aureus* activities of novel 4-hydroxycoumarin derivatives

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ABSTRACT

Four novel 4-hydroxycoumarin derivatives (4-MBH, 3-MBH, 4-MDT and 3-MDT) were successfully synthesized and their structures were verified by single-crystal X-ray crystallography. All target compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* (*S. aureus* ATCC 29213), methicillin-resistant *S. aureus* (MRSA XJ 75302), vancomycin-intermediate *S. aureus* (Mu50 ATCC 700699), and USA 300 (Los Angeles County clone, LAC). The minimum inhibitory concentration and time–kill curves were obtained for the test compounds and antibiotics. Among the tested compounds, 3-MBH showed the most potent antibacterial activities.

1. Introduction

Staphylococcus aureus, an important pathogen widely presents in the natural environment, can cause a variety of human tissue and organ infections (Dukic et al., 2013). Methicillin-resistant *S. aureus* (MRSA) emerged in the 1960s and has been widely disseminated since then. MRSA is the major source of nosocomial infections worldwide, causing ≥50% of the hospital-acquired *S. aureus* infections in several countries (Sandora and Goldmann, 2012; Kinnevey et al., 2013; Blomfeldt et al., 2013; Babakir-Mina et al., 2012). The prevalence rates of MRSA in hospitals in some Asian countries, such as Taiwan, China, Japan, and South Korea, range from 70% to 80% (Song et al., 2011). Although the predominant clinical manifestations of the pathogen are skin and soft tissue infections (SSTIs), severe life-threatening infections, such as necrotizing fasciitis, necrotizing pneumonia, and severe sepsis, have been reported (Miller et al., 2005). In the 48 contiguous states of the USA, community-associated MRSA skin and soft tissue infections are predominantly caused by the MRSA strain USA 300 (Amini and Salzman, 2013; Moran et al., 2006).

Vancomycin, a representative of the glycopeptide class of clinical antibiotics for serious Gram-positive bacterial infections, is widely used to treat MRSA infection. However, vancomycin is losing its effectiveness against MRSA and the prevention of MRSA infections at the surgical site (Haill et al., 2013). Thus, *S. aureus* continues to challenge surgeons as an adaptable pathogen that can defy all treatment efforts (Pitz et al., 2011; Koyama et al., 2012). To prevent the spread of both methicillin-sensitive *S. aureus* and MRSA, antibiotics that can effectively eradicate this pathogen need to be developed urgently.

4-Hydroxycoumarin is an important component of numerous synthetic and natural products with wide-ranging biological activities, including anticoagulant, insecticidal, anthelminthic, hypnotic, antifungal, phytoalexin, and HIV protease inhibition (Jung and Oh, 2011; Su et al., 2006). These special properties of 4-hydroxycoumarin have stimulated considerable interest in this class of compounds, and various biscoumarins and epoxydicoumarins have been synthesized. Epoxydicoumarins are a derivative of biscoumarins resulting from the removal of a water molecule. Epoxydicoumarins and biscoumarins possess versatile activities through chemical modifications (different substituents on the aromatic ring). Single-crystal X-ray diffraction, which can reveal molecular conformation, intramolecular and intermolecular interactions in the solid state, is among the most versatile techniques used to study coumarins (Khan et al., 2004; Hamdi et al., 2008).

A number of coumarin derivatives (novobiocin and analogs) have proven to be highly active antibiotics. Among synthetic coumarin derivatives, several antibacterial 4-hydroxycoumarins have been described (Lin et al., 2012). However, the effects of biscoumarins and epoxydicoumarins on bacteria, especially drug-resistant *S. aureus*,

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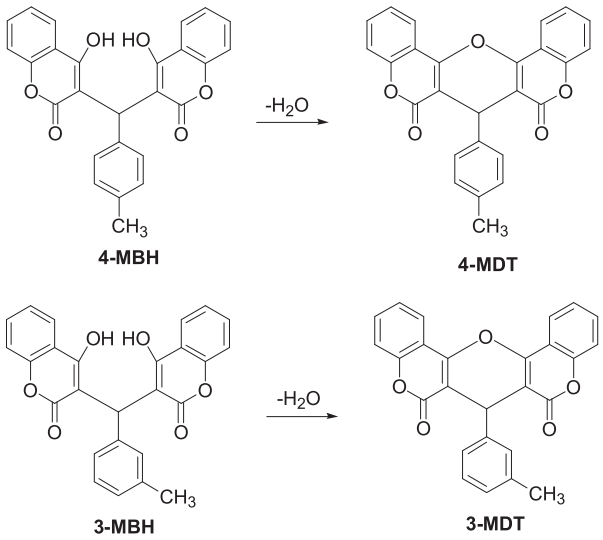

Fig. 1. Chemical structures of 4-MBH, 3-MBH, 4-MDT and 3-MDT.

remain unclear. In this work, a new series of biscoumarins and epoxydicoumarins (Fig. 1) were synthesized and their corresponding crystal structures were successfully obtained. Furthermore, the antibacterial properties of the compounds were also investigated. A possible relationship between the spatial structure and antibacterial function of these kinds of compound was then proposed.

2. Materials and methods

2.1. Chemicals and instruments

All antibiotics used were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals and solvents were analytical grade.

MRSA (XJ 75302) was isolated from cultures of sputum samples from patients in Xijing Hospital (Xi′an, China). *S. aureus* strain (ATCC 29213) was purchased from the Chinese National Center for Surveillance of Antimicrobial Resistance. Mu50 (ATCC 700699) and USA 300 (LAC) were purchased from MicroBiologics (MN, USA).

IR spectra (400–4000 cm^{-1}) were measured on a Brucker Equinox-55 spectrophotometer. ^1H NMR spectra were obtained using a Varian Inova-400 spectrometer (at 400 MHz). Mass spectra were recorded on a micrOTOF-Q II mass spectrometer. Melting points were taken on a XT-4 micro melting apparatus; the thermometer was uncorrected.

2.2. Synthesis and characterization of 4-MBH, 3-MBH, 4-MDT and 3-MDT

4-MBH and 3-MBH were synthesized according to a previous report (Kontogiorgis and Hadjipavlou-Litina, 2005; Kidwai et al., 2007). A mixture of 4-methylbenzaldehyde (or 3-methylbenzaldehyde) (10 mmol) and 4-hydroxycoumarin (20 mmol) was dissolved in 100 mL of EtOH. A few drops of piperidine were added, and the mixture was stirred for 4 h at room temperature. After reaction completion as determined by TLC, water was added until precipitation occurred. After filtering the precipitates, they were sequentially washed with ice-cooled water and ethanol and then dried under a vacuum.

3,3′-(4-Methylbenzylidene)-bis-(4-hydroxycoumarin) (4-MBH): m.p. 291–292 °C. IR (KBr pellet cm^{-1}): 1670, 1618, 1564, 1352, 1095, 906, 763 cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.521 (s, 1H), 11.296 (s, 1H), 8.000–8.082 (q, 2H), 7.615–7.649 (m, 2H), 7.408–7.425 (d, 4H), 7.099–7.145 (q, 4H), 6.076 (s, 1H), 2.342 (s, 3H). HRMS (ESI^+): *m*/*z*: calcd for $C_{26}H_{18}O_6$: 449.0996 [$M+Na^+$]; found: 449.0941.

3,3′-(3-Methylbenzylidene)-bis-(4-hydroxycoumarin) (3-MBH): m.p. 237–238 °C. IR (KBr pellet cm^{-1}): 1674, 1604, 1560, 1348, 1101, 763 cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.528 (s, 1H), 11.285 (s, 1H), 8.009–8.088 (q, 2H), 7.623–7.654 (t, 2H), 7.415–7.432 (d, 4H), 7.206–7.236 (t, 1H), 7.082–7.097 (d, 1H), 7.012–7.042 (t, 2H), 6.080 (s, 1H), 2.312 (s, 3H). HRMS (ESI^+): *m*/*z*: calcd for $C_{26}H_{18}O_6$: 449.0996 [$M+Na^+$]; found: 449.0985.

The compounds 4-MBH and 3-MBH were dissolved by heating in anhydride acetic. The reaction mixture was heated to reflux under magnetic stirring for 4 h. Then, the solution was cooled to room temperature, and the separated white solid was filtered off. The solid was subsequently recrystallized from ethanol to obtain 4-MDT and 3-MDT.

9-(4-Methylphenyl)-1,8-dioxo-9*H*-dibenzo[*c*,*h*]-2,7,10-trioxanthene (4-MDT): m.p. 319–320 °C. IR (KBr pellet cm^{-1}): 1735, 1668, 1606, 1456, 1365, 1182, 1058, 887, 763 cm^{-1}. ^1H NMR (DMSO-d_6, δ, ppm): 8.394–8.417 (q, 2H), 7.750–7.793 (m, 2H), 7.506–7.573 (m, 4H), 7.253–7.273 (d, 2H), 7.066–7.086 (d, 2H), 4.848 (s, 1H), 2.208–2.223 (d, 3H). HRMS (ESI^+): *m*/*z*: calcd for $C_{26}H_{16}O_5$: 431.0890 [$M+Na^+$]; found: 431.0838.

9-(3-Methylphenyl)-1,8-dioxo-9*H*-dibenzo[*c*,*h*]-2,7,10-trioxanthene (3-MDT): m.p. 333–334 °C. IR (KBr pellet cm^{-1}): 1735, 1669, 1608, 1456, 1365, 1178, 1058, 887, 759 cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 8.088–8.106 (d, 2H), 7.621–7.657 (t, 2H), 7.441–7.478 (t, 2H), 7.370–7.390 (d, 2H), 7.218–7.238 (d, 2H), 7.140–7.178 (t, 1H), 7.005–7.023 (d, 1H), 5.113 (s, 1H), 2.287 (s, 3H). HRMS (ESI^+): *m*/*z*: calcd for $C_{26}H_{16}O_5$: 431.0890 [$M+Na^+$]; found: 431.0899.

2.3. X-ray crystallography

Single crystals of 4-MBH, 3-MBH, 4-MDT and 3-MDT for X-ray diffraction experiments were grown from methanol. The X-ray diffraction data were collected on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromated Mo Kα radiation (λ=0.71073 Å) using ω–2θ scan technique at room temperature. The structure was solved by direct methods with 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 (Sheldrick, 1997). Hydrogen atoms were generated geometrically. The crystal data, details concerning data collection and structure refinement are given in Table 1. Molecular illustrations were prepared using the XP package. Parameters in CIF format are available as Electronic Supplementary Publication from Cambridge Crystallographic Data Center.

2.4. Bacterial susceptibility assays

According to the CLSI broth microdilution method, the minimum inhibitory concentrations (MICs) were determined by microdilution assay performed in sterilized 96-well polypropylene microtiter plates (Sigma–Aldrich) in a final volume of 200 μL. Bacteria were grown overnight in nutrient broth. Mueller–Hinton (MH) broth (100 μL) containing bacteria (5×10^5 CFU/mL) was added to 100 μL of culture medium containing the test compound (0.12–256 μg/mL in serial two-fold dilutions). The plates were incubated at 37 °C for 20 h in an incubator. About 50 μL of 0.2% triphenyl tetrazolium chloride (TTC), a colorimetric indicator, was added to each well of microter plates and incubated at 35 °C for 1.5 h. The TTC-based MIC was determined as the lowest concentration of oxacillin that showed no red color change indicating complete growth inhibition.

To obtain time–kill curves for methicillin-susceptible *S. aureus* and MRSA, the synthetic compounds and antibiotics were added to