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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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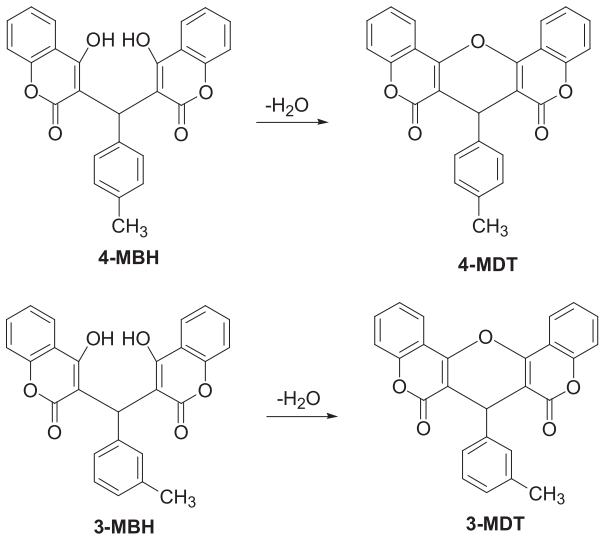

Fig. 1. Chemical structures of 4-MBH, 3-MBH, 4-MDT and 3-MDT.

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

Table 1
Crystal data, data collection and structure refinement.

Parameter	4-MBH	3-MBH	4-MDT	3-MDT
Formula	$C_{26}H_{18}O_6 \cdot CHCl_3$	$C_{26}H_{18}O_6$	$C_{26}H_{16}O_5$	$C_{26}H_{16}O_5$
M_r	545.77	426.4	408.39	408.39
Temperature (K)	113(2)	113(2)	113(2)	294(2)
Crystal size (mm)	0.20 × 0.20 × 0.16	0.20 × 0.10 × 0.01	0.18 × 0.16 × 0.14	0.22 × 0.18 × 0.16
Crystal system	Monoclinic	Monoclinic	Triclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P\bar{1}$	$P2_1/c$
a (Å)	13.1846(9)	9.881(4)	8.5390(10)	11.716(2)
b (Å)	10.5242(8)	10.094(4)	10.4500(15)	11.775(2)
c (Å)	18.6990(12)	20.535(7)	11.3367(18)	14.833(3)
α (°)	90	90	75.75(4)	90
β (°)	110.4520(10)	97.553(7)	73.44(2)	107.51(3)
γ (°)	90	90	84.73(4)	90
V (Å^3)	2431.1(3)	2030.4(13)	939.6(2)	1951.5(7)
Z	4	4	2	4
D_{calc} (g cm^{-3})	1.491	1.395	1.444	1.39
	0.42	0.1	0.823	0.097
μ(Mo Kα) (mm^{-1})	1.65–27.87	2.00–27.90	4.18–72.70	2.51–26.00
θ range (°)	24385	20751	10627	17224
Reflections collected	5763 [0.0391]	4847 [0.0425]	3517 [0.0677]	3842 [0.0519]
No. unique data [R(int)]	4037	3851	2699	2633
No. data with $I \geq 2\sigma(I)$	0.0596	0.0486	0.0714	0.0597
R_1	0.1755	0.1291	0.1466	0.155
ωR_2 (all data)	872211	843712		874730
CCDC			895385	

strain cultures to a final concentration of 128 μg/mL or 32 μg/mL. The strains were cultivated in the automated Bioscreen C system (Lab systems Helsinki, Finland) using MH broth culture medium. The working volume in the wells of the Bioscreen plate was 300 μL, comprising 150 μL of MH broth and 150 μL of drug solution. The temperature was controlled at 35 °C, and the optical density of the cell suspensions was measured automatically at 600 nm in regular intervals of 10 min for 20 h. Before each measurement, the culture wells were automatically shaken for 60 s. Statistical data for each experiment were obtained from at least two independent assays performed in duplicate.

3. Results

3.1. Crystal structure description

The crystal structures of 4-MBH, 3-MBH, 4-MDT, and 3-MDT are presented in Fig. 2. Compounds 4-MBH and 3-MBH have similar structural features to those of dicoumarols. From the diagram of the asymmetric unit including the atomic numbering scheme of 4-MBH, we can see a chloroform solvent molecule incorporated in the asymmetric unit. In the crystal structure of 3-MBH, two crystallographically independent molecules are present in the asymmetric unit. Two 4-hydroxycoumarin fragments are linked by a methylene bridge, wherein one hydrogen atom is replaced with an *m*-methylphenyl residue with identical conformations between the two molecules. However, these fragments differ with respect to the reversed twist directions of the *m*-methylphenyl ring. In each compound, two classical asymmetrical intramolecular O–H···O hydrogen bonds [d(O1–O6)=2.677(3) Å, d(O3–O4)=2.631(3) Å for 4-MBH; d(O1–O6)=2.6729(15) Å, d(O3–O4)=2.6119(14) Å for 3-MBH] between a hydroxyl group of a coumarin fragment and a lacton carbonyl group of another coumarin fragment were used to stabilize the whole structure.

Compounds 4-MDT and 3-MDT were obtained from two hydroxyl groups forming an ether bond by the removal of the water molecule from the corresponding compounds 4-MBH and 3-MBH. The newly formed ether ring B (C1/C9/C10/C11/C19/O1) was essentially planar because the maximum deviation of the atoms in the skeleton from the molecular plane (C1–O1/C19) was only 0.2970 Å in 4-MDT and 0.3464 Å in 3-MDT. This phenomenon resulted in the formation of a large, essentially planar heterocyclic ring system with a 2.716(46)° dihedral angle between rings B and A (C8/C9/C1/C2/C7/O2) as well as a 3.822(44)° dihedral angle between rings B and C (C12/C11/C19/C18/C13/O5) in 4-MDT. The corresponding values in 3-MDT are 6.211(61)° and 3.295(59)°. The dihedral angles between the mean planes of the heterocyclic ring system and the 4-methylphenyl and 3-methylphenyl rings were 86.379(51)° and 84.686(65)°. In the two whole stabilized structures, the 4-methylphenyl and 3-methylphenyl groups "stand" on the plane of the heterocyclic ring system using a non-classical intramolecular hydrogen bond C25–H25···*Cg*. The former H-bond parameters are C25–H25: 0.95 Å, H25···*Cg*: 2.71 Å, and C25···*Cg*: 3.0785(17) Å, and the angle is 104°. The respective latter H-bond parameters are 0.93 Å, 2.65 Å, 3.033(2) Å, and 106° (*Cg* refers to the center of gravity of ring B).

3.2. MIC

3-MBH exerted potent bactericidal effects against almost all *S. aureus* tested including USA 300 (LAC), a highly virulent and widespread clinical isolate responsible for the recent epidemic of MRSA infections; most MIC values were around 64 μg/mL and 32 μg/mL (Table 2). By contrast, 4-MBH, 4-MDT and 3-MDT exerted no effect against *S. aureus*, with MIC values of more than 256 μg/mL for *S. aureus* (ATCC 29213) and the other three MRSA strains. The MIC values for levofloxacin, ceftazidime, ceftriaxone, gentamicin, vancomycin, and piperacillin against *S. aureus* (ATCC 29213) strains were <8 μg/mL, but were much higher against the resistant strains of varying degrees (Table 2).

3.3. Time–kill curves

The MICs showed 3-MBH had the most potent and broad-spectrum antibacterial activity on *S. aureus*. Thus, further investigations on the growth inhibitory and bactericidal effects of 3-MBH

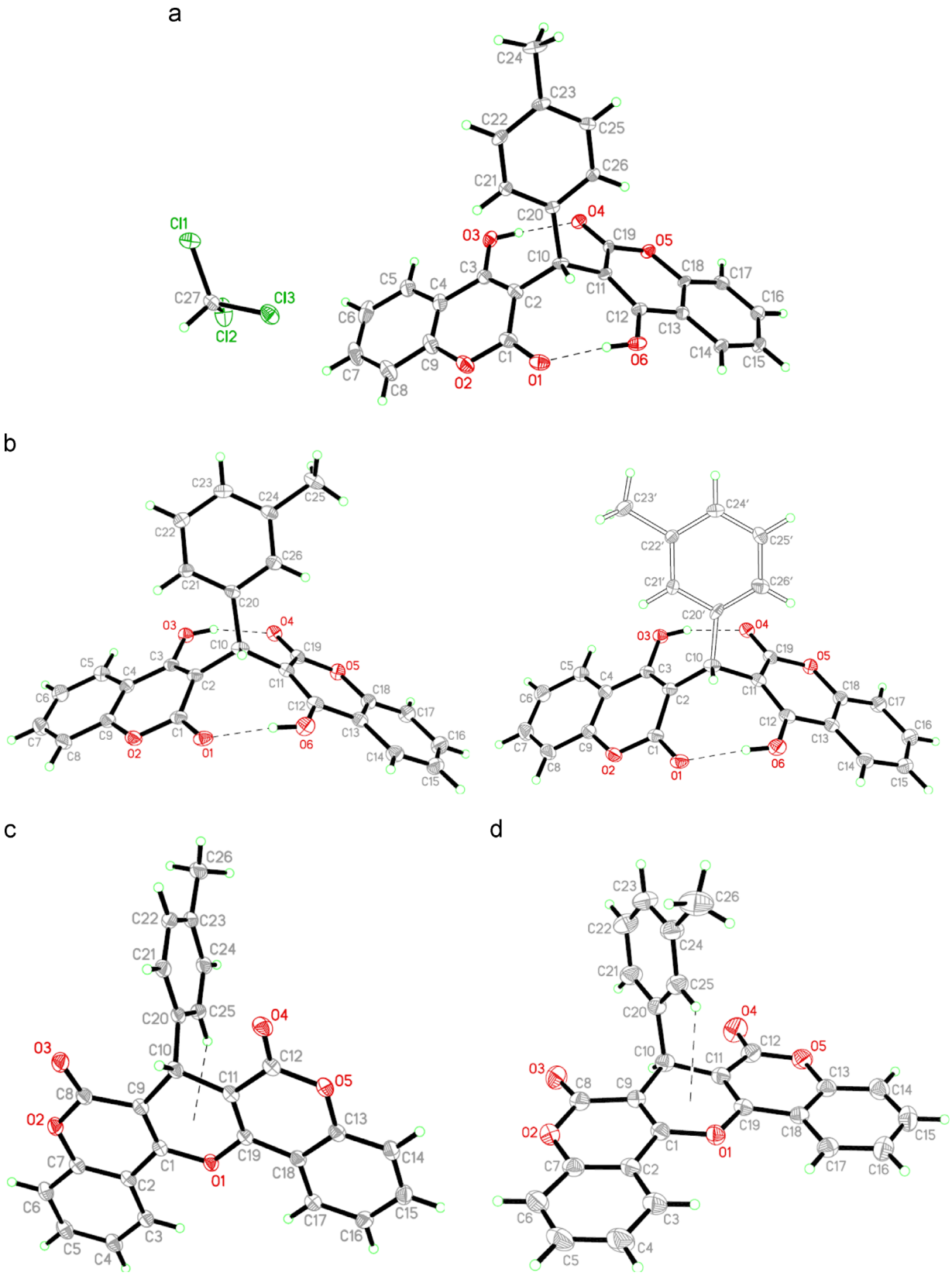

Fig. 2. Crystal structures of 4-MBH (a), 3-MBH (b), 4-MDT (c) and 3-MDT (d).

were conduct. To evaluate the growth inhibitory effects on *S. aureus* ATCC 29213 (Fig. 3), MRSA XJ 75302 (Fig. 4), Mu50 (Fig. 5) and MRSA USA 300 LAC (Fig. 6), 4-MBH, 3-MBH, 4-MDT and 3-MDT were added to cultures at 128 μg/mL or 32 μg/mL, respectively. As shown in Figs. 3–6, 3-MBH inhibited the growth of these pathogens and exhibited almost completely growth inhibition on these pathogens at 128 μg/mL or 32 μg/mL. This finding is consistent with the result of MIC, 4-MBH, 4-MDT and 3-MDT had

Table 2
MIC of 4-MBH、3-MBH、4-MDT、3-MDT and antibiotics in Mueller–Hinton broth culture.

Drugs	MIC (μg/mL)			
	S. aureas (ATCC 29213)	MRSA (XJ 75302)	Mu50 (ATCC 700699)	LAC (USA 300)
3-MBH	64	64	32	32
3-MDT	> 256	> 256	> 256	> 256
4-MBH	> 256	> 256	> 256	> 256
4-MDT	> 256	> 256	> 256	> 256
Levofloxacin	< 0.125 (S)	4 (R)	4 (R)	8 (R)
Ceftazidime	8 (S)	> 256 (R)	256 (R)	64 (R)
Ceftriaxone	2 (S)	> 256 (R)	256 (R)	32 (R)
Gentamicin	0.12 (S)	64 (R)	32 (R)	0.25 (S)
Vancomycin	0.5 (S)	128 (R)	8(I)	0.5 (S)
Piperacillin	2 (S)	> 128 (R)	> 128 (R)	8 (R)

S means drug susceptibility, *R* means drug resistance, and *I* means intermediate resistance. Levofloxacin, ceftazidime, ceftriaxone, gentamicin, vancomycin, and piperacillin as control antibiotics exert antibacterial effects on the drug-susceptible *S. aureas* strain (ATCC 29213). MRSA (XJ 75302) and Mu50 (ATCC 700699) are resistant to all of the control antibiotics, whereas USA 300 is susceptible to vancomycin and gentamicin but resistant to the other control antibiotics. Compared with 3-MDT, 4-MBH, and 4-MDT, 3-MBH has more potent effects on the bacterials listed above.

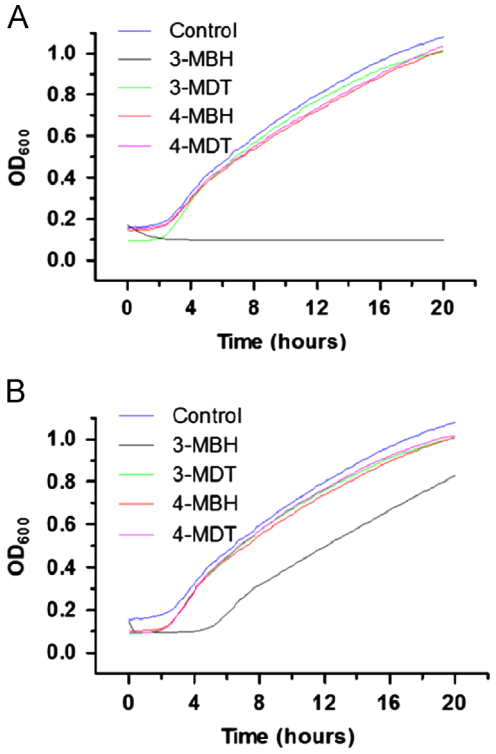

Fig. 3. Concentration-dependent inhibition of compounds on the growth of *S. aureus* ATCC 29213. The cells were cultured in liquid culture medium and treated with different concentrations of 4-MBH, 3-MBH, 4-MDT and 3-MDT. The growth curves for *S. aureus* ATCC 29213 were measured using a Bioscreen C™ instrument in the absence (blue line) and presence (black line) of 3-MBH, 3-MDT (green line), 4-MBH (red line), and 4-MDT (purple line). The sample frequency was 10 min. The responses to 128 μg/mL compound (A) and 32 μg/mL compound (B) treatments of *S. aureus* ATCC 29213 were investigated. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

no inhibitory effects on these pathogens at 128 μg/mL or 32 μg/mL. *S. aureus* growth in MH broth without any compound as the control did not show any significant growth inhibitory effect.

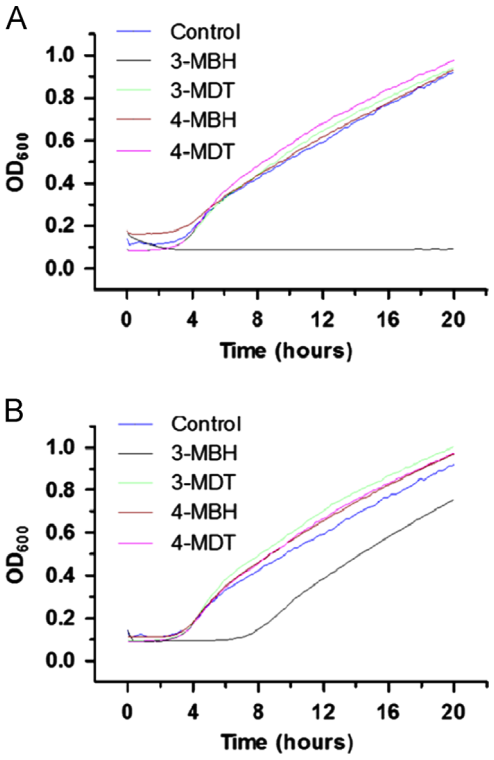

Fig. 4. Concentration-dependent inhibition of compounds on the growth of MRSA XJ 75302. The cells were cultured in liquid culture medium and treated with different concentrations of 4-MBH, 3-MBH, 4-MDT and 3-MDT. The growth curves for MRSA XJ 75302 were measured using a Bioscreen C™ instrument in the absence (blue line) and presence of 3-MBH (black line), 3-MDT (green line), 4-MBH (red line), and 4-MDT (purple line). The sample frequency was 10 min. The responses to 128 μg/mL compound (A) and 32 μg/mL compound (B) treatments of MRSA XJ 75302 were investigated. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

4. Discussion

As a result of the emergence, spread, and rapid evolution of resistance genes developing among pathogens, MRSA is the cause of major outbreaks and epidemics among hospitalized patients, with high mortality and morbidity rates. Recently, some schemes have led to both a dramatic rise in the total numbers of cases of *S. aureus* bacteremia reported annually and an increase in the proportion of such cases that involve MRSA (from 2% in 1990 to > 40% in the early 2000s) (Murray et al., 2008; Brennan et al., 2013). However, the emergence of vancomycin-resistant *S. aureus* and treatment failure of MRSA infections urgently require developing new antimicrobials.

In this work, 4-hydroxycoumarin derivatives were demonstrated as able to inhibit the growth of multi-drug resistant *S. aureus* significantly. The MIC values showed the efficacy of 3-MBH at around 32 μg/mL or 64 μg/mL to *S. aureus* (ATCC 29213), MRSA (XJ 75302), Mu50, and USA 300. To prove the anti-MRSA activity of 3-MBH, the growth inhibition rate of bacterial using time–kill curves was evaluated. The concentration-dependent inhibition of 3-MBH on the growth of *S. aureus* (ATCC 29213), MRSA (XJ 75302), Mu50, and USA 300 was consistent with the MIC results, in which 128 or 32 μg/mL 3-MBH significantly inhibited the growth of MRSA. However, 4-MBH, 4-MDT and 3-MDT have no effect on any *S. aureus* in the MIC or growth inhibition rate experiments.

X-ray structural analysis showed that the biscoumarins 4-MBH and 3-MBH have two classical intramolecular O–H···O hydrogen bonds in their structures whereas the epoxydicoumarins 4-MDT and 3-MDT have non-classical intramolecular hydrogen bonds in their structures. The formation of intramolecular hydrogen bonds is considered as an

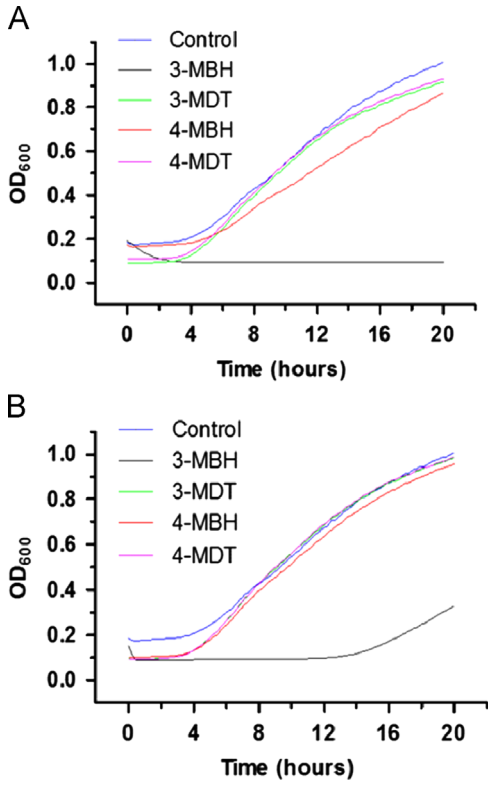

Fig. 5. Concentration-dependent inhibition of compounds on the growth of Mu50 ATCC 700699. The cells were cultured in liquid culture medium and treated with different concentrations of 4-MBH, 3-MBH, 4-MDT and 3-MDT. The growth curves for Mu50 ATCC 700699 were measured using a Bioscreen C™ instrument in the absence (blue line) and presence of 3-MBH (black line), 3-MDT (green line), 4-MBH (red line), and 4-MDT (purple line). The sample frequency was 10 min. The responses to 128 μg/mL compound (A) and 32 μg/mL compound (B) treatments of Mu50 ATCC 700699 were investigated. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

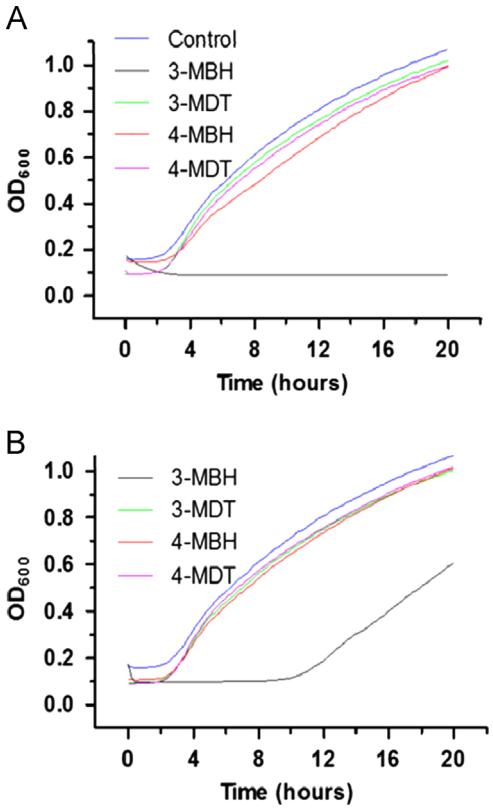

Fig. 6. Concentration-dependent inhibition of compounds on the growth of USA 300 LAC. The cells were cultured in liquid culture medium and treated with different concentrations of 4-MBH, 3-MBH, 4-MDT and 3-MDT. The growth curves for USA 300 LAC were measured using a Bioscreen C™ instrument in the absence (blue line) and presence (black line) of 3-MBH, 3-MDT (green line), 4-MBH (red line), and 4-MDT (purple line). The sample frequency was 10 min. The responses to 128 μg/mL compound (A) and 32 μg/mL compound (B) treatments of USA 300 LAC were investigated. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

important factor in assisting the molecule to attain the correct configuration for biological activity. In biological systems, strong intramolecular hydrogen bonds stabilize and hold the compounds in a suitable configuration for binding to an enzyme. Weak, non-classical intramolecular hydrogen bonds in the structures of 4-MDT and 3-MDT caused their weaker structural stability compared with those of 4-MBH and 3-MBH. Thus, 4-MDT and 3-MDT have weaker antibacterial activity than 4-MBH and 3-MBH. The antibacterial activity of 4-MBH has almost no effect compared with 3-MBH, although both have the same substituent groups. Differences in antibacterial effects may be due to different positions (*para* and *meta*) of these substituent groups.

Since the antimicrobial activity of some antimicrobial agents is related to the rate of bacterial growth, this may be a major limitation of in vitro models. As an example, β-lactams are known to display bactericidal activity that is dependent on the rate of growth. Thus, this class of antibacterial agents may be expected to be more effective in vitro than in vivo. The present study shows that the novel biscoumarin derivative 3-MBH has potent antibacterial effects using the two different but equally important in vitro methods of MIC determination and time–kill curve analysis. Future applications of the present work are expected to ultimately provide insights into the development of treatments for antibiotic-resistant infectious diseases.

5. Conclusion

With the emergence of methicillin-resistant, vancomycin-intermediate resistant, or multi-drug resistant *S. aureus*, more appropriate antibiotics have to be developed to treat infected patients. Our results confirmed that the novel biscoumarin derivative 3-MBH has excellent antibacterial efficiency. However, additional studies that aim to define the mechanism underlying the antibacterial activity of the derivative and evaluate correlations with its drug efficacy in vivo are necessary.

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