European Journal of Medicinal Chemistry 222 (2021) 113628

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Novel chalcone-conjugated, multi-flexible end-group coumarin thiazole hybrids as potential antibacterial repressors against methicillin-resistant *Staphylococcus aureus*

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ARTICLE INFO

Article history:
Received 8 April 2021
Received in revised form
3 June 2021
Accepted 3 June 2021
Available online 9 June 2021

Keywords:
Coumarin
Thiazole
Chalcone
Antibacterial
MRSA
DNA

ABSTRACT

The increasing resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) to antibiotics has led to a growing effort to design and synthesize novel structural candidates of chalcone-conjugated, multi-flexible end-group coumarin thiazole hybrids with outstanding bacteriostatic potential. Bioactivity screening showed that hybrid **5i**, which was modified with methoxybenzene, exerted a significant inhibitory activity against MRSA (MIC = 0.004 mM), which was 6 times better than the anti-MRSA activity of the reference drug norfloxacin (MIC = 0.025 mM). Compound **5i** neither conferred apparent resistance onto MRSA strains even after multiple passages nor triggered evident toxicity to human hepatocyte LO2 cells and normal mammalian cells (RAW 264.7). Molecular docking showed that highly active molecule **5i** might bind to DNA gyrase by forming stable hydrogen bonds. In addition, molecular electrostatic potential surfaces were developed to explain the high antibacterial activity of the target compounds. Furthermore, preliminary mechanism studies suggested that hybrid **5i** could disrupt the bacterial membrane of MRSA and insert itself into MRSA DNA to impede its replication, thus possibly becoming a potential antibacterial repressor against MRSA.

1. Introduction

The high frequency and rapid transmission of microbial infections, have inevitably become a significant challenge in modern medicine, and pose a serious threat to human health [1]. Even more concerning is the emergence of powerful drug-resistant strains from common strains, particularly, the lethal methicillin-resistant *Staphylococcus aureus* (MRSA), which makes infections more difficult to control [2]. Critically, MRSA exhibits varying degrees of resistance against numerous antibiotics, such as β-lactams, fluoroquinolones, macrolides and vancomycin, which have been clinically approved to treat MRSA infections, thereby making it extremely difficult to curb its spread [3,4]. All these concerns have laid the foundation for the exploration of new therapeutic strategies, and there is an urgent need to develop novel anti-MRSA agents with high efficiency and low cytotoxicity to treat the infections caused by such resistant strains [5].

Natural products have been invaluable in the discovery and development of medicines, and have been used to treat diseases for thousands of years. Coumarins are natural products that universally exist among plant secondary metabolites [6]. To date, more than 1800 different natural and synthetic coumarins with important

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https://doi.org/10.1016/j.ejmech.2021.113628
0223-5234/

biological activities, such as insecticidal, antimicrobial, anticancer, and anti-HIV activities, have been documented [7]. Notably, coumarins have been widely used as antibiotics owing to their active membrane penetration, e.g., chlorobiocin, novobiocin, and coumermycin A1 are frequently used in clinical practice [8]. In addition, coumarin agents can effectively inhibit the ATPase activity of DNA gyrase, to compete with the B subunit of the ATP-binding enzyme and exert their antimicrobial effects [9]. So far, more and more efforts have been devoted to the structural reconstruction of the coumarin skeleton, and the modified coumarin derivatives have been found to have satisfactory antibacterial potential [10]. Therefore, a series of novel coumarin molecules with high antimicrobial potential have been developed to reduce cytotoxicity and drug resistance by splicing and recombining clinical antimicrobial active fragments on coumarin scaffolds [11,12].

Chalcones are typically composed of two aromatic rings bridged by an α,β-unsaturated system of three carbons, which are commonly found in many medicinal plants, and have outstanding medicinal value owing to their antibacterial, antifungal, anticancer and anti-inflammatory properties [13]. Chalcones have large conjugated systems, which promote their photophysical properties and facilitate their binding ability with bioactive molecules (enzymes or DNA), and further stimulate a wave of the design and development of new drugs using chalcones as active fragments [14]. Remarkably, the strong potential of chalcones as antimicrobial agents is reflected in natural products. For example, licochalcone A, a natural chalcone product, shows significant antimicrobial ability against *Staphylococcus aureus* and *Micrococcus luteus* [15]. Additionally, in a large number of recent studies, many chalcone derivatives have been developed by introducing heterocyclic compounds, mainly azole compounds, into the chalcone skeleton, or by using the large conjugated systems of chalcones to splice the active ingredients of various antibacterial drugs to generate newly conjugated drug molecules [16,17]. Given this, there is a large space for the chalcone skeleton to be used in molecular modification, which is brought up to date for the construction of novel multicomponent chalcone hybrids that can be used as multi-target antimicrobial agents [18].

The frequent occurrence of azole heterocyclic compounds in various antimicrobial drugs has made azoles, which are alternative active fragments of drug recombination, attract much attention [19]. Significantly, thiazoles are aromatic heterocyclic compounds with a variety of covalent interactions, such as hydrogen bonds, van der Waals interactions, and π-π stacking. Therefore, the introduction of thiazole fragment may effectively improve the physicochemical properties of drug molecules and enhance their interactions with biological enzymes, as well as enhance the binding ability of functional targets [20]. To date, the clinical use of thiazole compounds has been considerable, especially in quinolones (cephalosporin, cefixime) and sulfonamides (sulfathiazole), wherein this pharmacophore is widely observed [21]. Hence, thiazoles have strong antibacterial potential and promising biological activity, which may provide a broad prospect for their further development and utilization as active fragments [22].

Hydroxyethyl fragments are frequently used in lots of clinical antimicrobial drugs such as antibacterial metronidazole and antifungal fluconazole, demonstrating that hydroxyethyl group is indeed beneficial for the improvement of antimicrobial activity [23–25]. Therefore, the excellent contribution of hydroxyethyl fragments in antimicrobial drug molecules and the strong vision of using them as flexible end groups support a more profound idea for exploring novel antimicrobial structures [26,27].

Based on all of these considerations, a series of new promising antimicrobial chalcone-conjugated structural splice recombinants with coumarin as the structural skeleton has been successfully developed, and the thiazole ring and two hydroxyethyl fragments have been incorporated into the reconstituted hybrids. These derivatives, which were chalcone conjugates containing different phenyl and indolyl groups, were obtained by condensing different aldehyde molecules, namely indole-aldehydes and aromatic aldehydes, and the effects of different substituents on the antimicrobial activity of these hybrids were measured (Fig. 1). The selection of different substituents for the target molecules was based on the following ideas: (1) Indole alkaloids are bacterial intercellular signaling molecules with unique characteristics of mediating a variety of biological processes. In addition, indole scaffolds may also exert potential biological activity through groove binding or insertion of DNA, especially the benign promotion of antimicrobial activity has attracted our great attention [28]; (2) Various benzyl groups can accelerate the regulation of cell membrane permeability and lipid solubility, and facilitate the absorption and mobility of novel heterozygous structures [20,25]; (3) Nitrogen and oxygen atoms can react well on multiple active sites in biological systems and may bind to diverse targets simultaneously [29].

2. Results and discussion

2.1. Chemistry

A series of novel chalcone-conjugated coumarin thiazole hybrids was designed and synthesized using the synthetic routes outlined in Schemes 1 and 2. Intermediate **2** was prepared from commercial resorcinol **1** and ethyl 4-chloroacetoacetate by stirring in concentrated sulfuric acid at 0 °C for 24 h with a yield of 71%. Thereafter, intermediate **2** and compound **7**, which had been prepared based on the literature, were reacted in acetonitrile in the presence of potassium carbonate at 60 °C for 8 h to obtain intermediate **3** with a yield of 47%. Intermediate **3** was then treated with diethanolamine in acetonitrile at 80 °C to obtain key intermediate **4** with a yield of 70%. Furthermore, compound **4** was condensed with different benzaldehydes (available in the market) and 3-formylindoles **11** (obtained using a synthesis route from the literature) in ethanol in the case of piperidine at 80 °C for 8 h to produce target chalcone-conjugated coumarin thiazoles **5** and **8** in yields ranging from 25% to 50%.

2.2. Antibacterial activity

In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, the two-fold serial dilution method was used to evaluate the antibacterial activity of newly synthesized coumarin thiazole derivatives *in vitro*. Norfloxacin, a widely used, clinically effective antimicrobial agent, was employed to compare the antibacterial activity for the series of coumarin thiazole hybrids. The antibacterial activity results were summarized in Table 1, in which the minimum inhibitory concentration (MIC) was the minimum concentration (mM) at which the target compounds could completely inhibit bacterial growth [30,31].

To further have a good knowledge of the structure-activity relationship (SAR) of these chalcone-conjugated coumarin thiazole hybrids, the effects of different active fragments and their various substituents on the antimicrobial activity of these compounds was studied. For the series of phenyl-derived coumarin thiazole hybrids, it could be observed that this series of derivatives exhibited obvious selectivity toward MRSA strains. Remarkably, methoxybenzene-modified hybrid 5i exhibited the strongest inhibition against MRSA compared with the other products, with a MIC value of 0.004 mM, which was 6 times better than that of the reference drug norfloxacin (0.025 mM). In addition, the inhibitory activity of compound 5i against *E. coli* was also noteworthy, with a

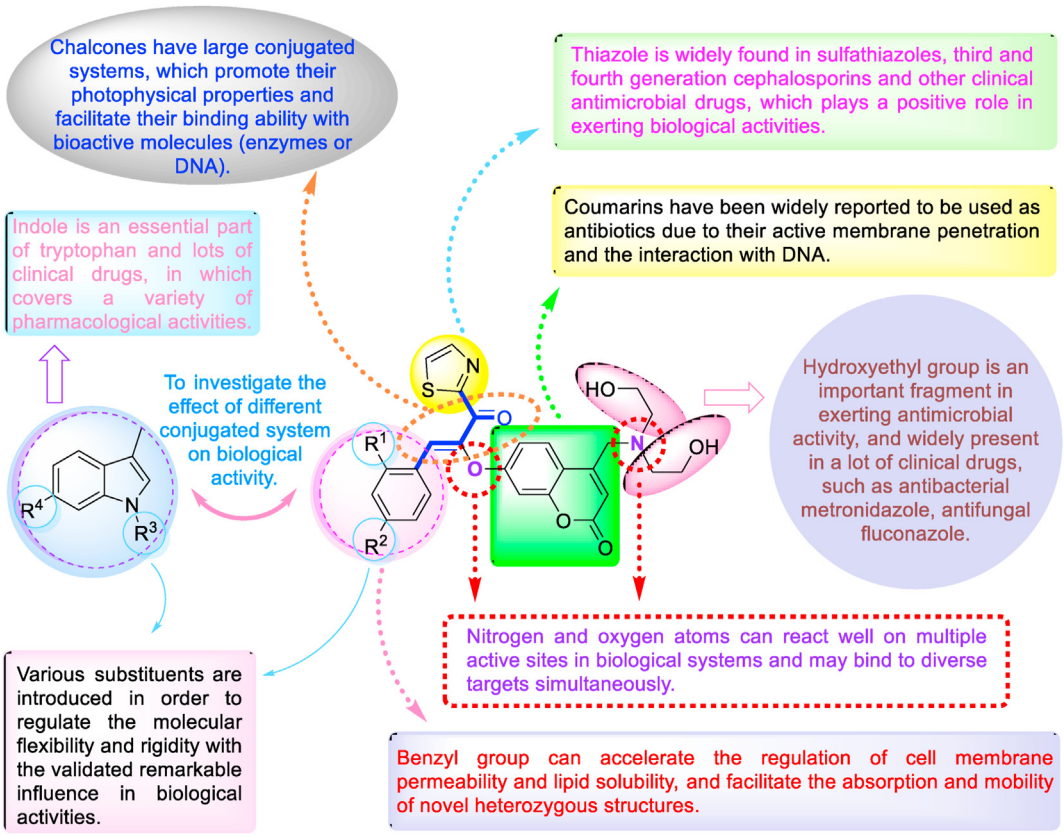
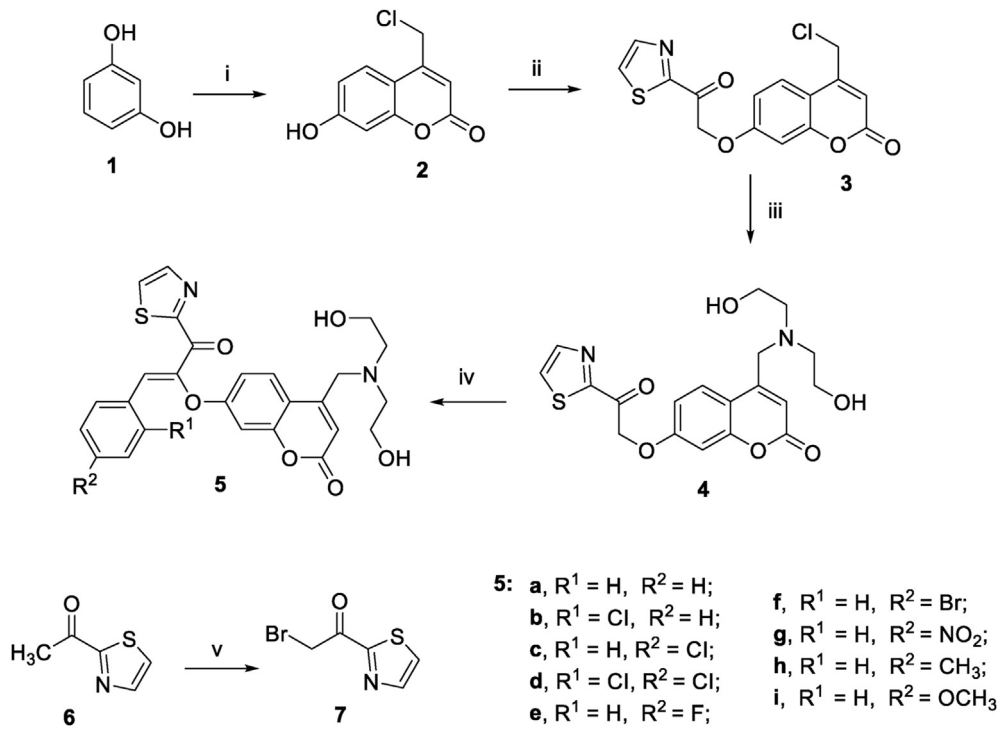
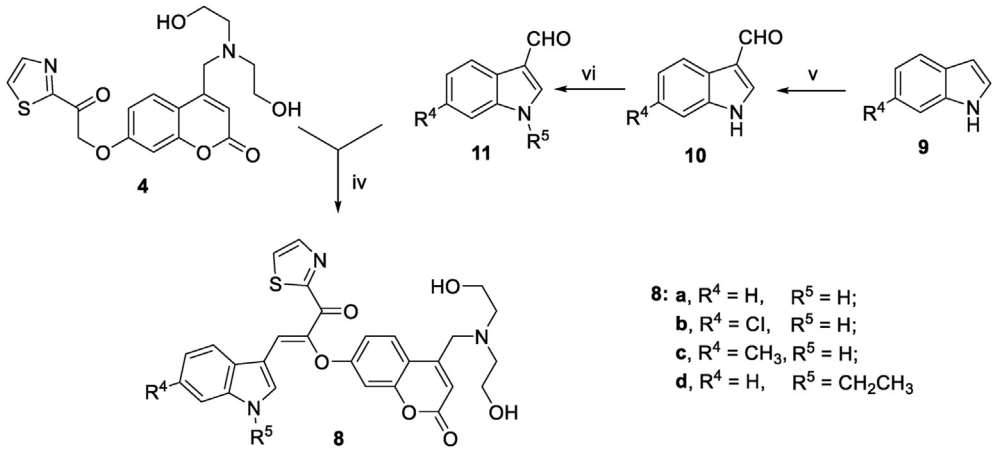


Fig. 1. Design of novel chalcone-conjugated coumarin thiazole hybrids.



Scheme 1. Synthetic route of phenyl-derived chalcone-conjugated coumarin thiazole hybrids.
Reagents and conditions: (i) ethyl 4-chloroacetoacetate, sulfuric acid, 0 °C, 24 h, 71%; (ii) 2-bromoacetylthiazole, potassium carbonate, acetonitrile, 60 °C, 8 h, 48%; (iii) diethanolamine, acetonitrile, 80 °C, 8 h, 70%; (iv) aryl aldehyde, piperidine, ethanol, 80 °C, 8 h, 25–50%; (v) bromine, acetic acid, 50 °C, 2 h, 78%.



Scheme 2. Synthetic route of indole-derived chalcone-conjugated coumarin thiazole hybrids.
Reagents and conditions: (iv) 3-formylindole, piperidine, ethanol, 80 °C, 8 h, 40–45%; (v) dimethylformamide, phosphorus oxychloride, 0 °C, 5 h, 81%; (vi) bromoethane, potassium carbonate, acetonitrile, 60 °C, 5 h, 78%.

Table 1
In vitro antibacterial data as MIC (mM) for coumarin thiazole hybrids **4**, **5** and **8**.[a,b].

Compds	Structure	Gram-positive bacteria					Gram-negative bacteria					
	R	MRSA	*E. f.*	*S. a.*	*S. a.* 25923	*S. a.* 29213	*K. p.*	*E. c.*	*P. a.*	*A. b.*	*P. a.* 27853	*E. c.* 25922
4	–	0.020	0.316	0.316	0.633	1.266	0.316	0.316	0.633	0.633	1.266	0.633
5a		0.130	0.130	0.260	1.040	1.040	1.040	1.040	0.520	1.040	0.520	0.260
5b		0.061	0.121	0.121	0.486	0.486	0.972	0.972	0.486	0.486	0.243	0.243
5c		0.121	0.243	0.243	0.243	0.243	0.486	0.486	0.061	0.243	0.243	0.486
5d		0.007	0.228	0.228	0.456	0.456	0.228	0.228	0.114	0.114	0.228	0.456
5e		0.125	0.125	0.125	0.251	0.251	0.251	1.003	1.003	0.251	0.251	0.251
5f		0.136	0.136	0.068	0.272	0.272	1.086	1.086	0.272	0.543	1.086	0.543
5g		0.007	0.119	0.119	0.060	0.119	0.238	0.119	0.476	0.238	0.238	0.119
5h		0.063	0.063	0.126	0.063	0.126	0.063	0.016	0.253	0.253	0.505	0.126
5i		0.004	0.061	0.061	0.122	0.122	0.061	0.015	0.122	0.244	0.244	0.244
8a		0.015	0.060	0.120	0.120	0.120	0.060	0.241	0.241	0.482	0.482	0.241
8b		0.057	0.057	0.113	0.057	0.057	0.057	0.226	0.226	0.113	0.113	0.226
8c		0.117	0.015	0.029	0.007	0.117	0.235	0.235	0.117	0.117	0.059	0.235
8d		0.114	0.014	0.029	0.007	0.057	0.229	0.229	0.114	0.057	0.057	0.229
Norfloxacin	–	0.025	0.025	0.006	0.003	0.006	0.013	0.050	0.025	0.025	0.003	0.025

[a] Minimum inhibitory concentrations were determined by micro broth dilution method for microdilution plates.
[b] MRSA, methicillin-resistant *Staphylococcus aureus* N315; *E. f.*, *Enterococcus faecalis*; *S. a.*, *Staphylococcus aureus*; *S. a.* 25923, *Staphylococcus aureus* ATCC 25923; *S. a.* 29213, *Staphylococcus aureus* ATCC 29213; *K. p.*, *Klebsiella pneumonia*; *E. c.*, *Escherichia coli*; *P. a.*, *Pseudomonas aeruginosa*; *A. b.*, *Acinetobacter baumanii*; *P. a.* 27853, *Pseudomonas aeruginosa* ATCC 27853; *E. c.* 25922, *Escherichia coli* ATCC 25922.

MIC value of 0.015 mM, which was more effective than the reference drug norfloxacin (MIC = 0.05 mM). Furthermore, compounds 5d and 5g, modified with dichlorobenzene and nitrobenzene, respectively, showed satisfactory inhibitory effect on MRSA, and their MIC values were 0.007 mM. Otherwise, we also found that derivative 5h, modified with methylbenzene, had slightly better antibacterial activity (MIC = 0.015 mM) against *E. coli* than norfloxacin. Therefore, based on the antibacterial activity results of the series of phenyl-derived coumarin thiazoles, it could be seen that the inhibitory effect of the target hybrids was diversified due to the different substituents on the phenyl group. Numerous studies have pointed out that the introduction of nitro group as an active functional group was beneficial for the antibacterial activity of the target structures, and our activity analysis results of the series of

derivatives were consistent with this [29]. However, a few reports stated that the activity of target molecules modified by methoxy group and di-halogen atoms significantly improved, and it is precisely because of this that these fragments were of unique value as functional group candidates for further research and development of antibacterial agents with novel structural frameworks.

Subsequently, a series of indole-derived coumarin thiazole hybrids was derived by replacing the introduced phenyl ring with an indole ring. However, in this series of derivatives, only compound 8a showed slightly better anti-MRSA activity (MIC = 0.015 mM) than the reference drug norfloxacin. Notably, although these structural modifications did not induce the expected anti-MRSA activity, they did show unforeseen sensitivity to *E. faecalis*. Specifically, indole derivatives 8c and 8d, modified with methyl and ethyl groups respectively, showed significantly better antibacterial activity against *E. faecalis* than the reference drug (MIC = 0.025 mM), with MIC values of 0.015 and 0.014 mM, respectively. These results indicated that the introduction of indole rings with different substituents contributed to the improvement of the antibacterial properties of the target molecules. In addition to the positive effect of the indole ring itself on the inhibitory activity against bacteria, the introduction of alkyl chains also broadened the antibacterial spectrum of indole-derived coumarin thiazoles to some extent.

In general, various phenyl- and indole-modified chalcone-bridged coumarin thiazole hybrids showed effectively improved inhibitory effects against bacteria, especially their selective inhibition advantage of MRSA should not be underestimated. Besides, some of the modified structures were also sensitive to *E. coli* and *E. faecalis* and displayed relatively low MIC values. In particular, highly active compound **5i**, derived from the introduction of a methoxybenzene group, had great potential as a new repressor against MRSA, and provided a reliable case for the subsequent screening of antimicrobial drug candidates [32].

2.3. Analysis of Clog P values

The lipophilic/hydrophilic properties of target active compounds are generally reflected indirectly by the theoretical calculated value of the partition coefficient (*Clog P*). In general, the appropriate range of *Clog P* values is conducive to the distribution and transportation of target molecules in biological systems. The *Clog P* values of all target coumarin thiazole derivatives were summarized in Table 2 [33]. Obviously, the *Clog P* values of coumarin thiazole hybrids **5** and **8** varied with the type and the number of substituents on the benzene and indole rings, ranging from 2.86 to 4.54.

To analyze the correlation between the *Clog P* value and the antibacterial efficacy against MRSA, their relationship was established, as shown in Fig. 2. It could be observed that dichlorobenzene-modified hybrid **5d** had the highest lipophilicity (*Clog P* = 4.54) of all target molecules, but did not show the best expected anti-MRSA activity (MIC = 0.007 mM). Surprisingly, methoxybenzene-modified compound **5i** showed the most significant antimicrobial activity (MIC = 0.004 mM), which was better than that of the

Table 2
Clog P values of coumarin thiazole hybrids (Mean ± SD, n = 3).[a]

Compds	*Clog P*	Compds	*Clog P*	Compds	*Clog P*
5a	3.11	**5f**	3.98	**8b**	3.99
5b	3.83	**5g**	2.86	**8c**	3.60
5c	3.83	**5h**	3.61	**8d**	4.10
5d	4.54	**5i**	3.03	-	-
5e	3.26	**8a**	3.10	-	-

[a] *Clog P* values were calculated by ChemDraw Ultra 14.0.

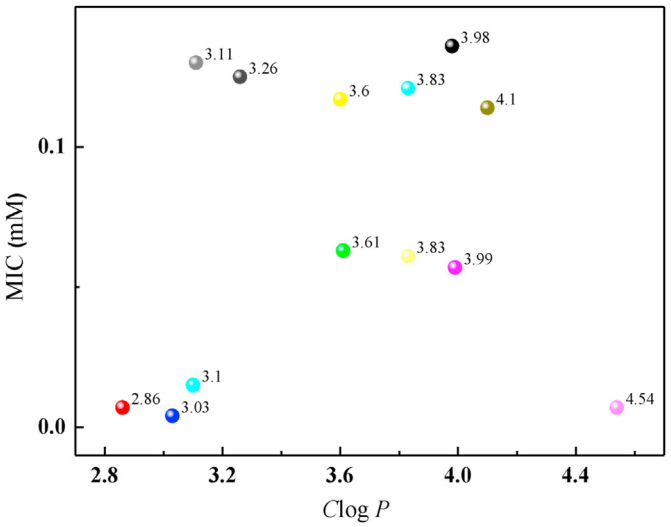


Fig. 2. The calculated partition coefficients of coumarin thiazole hybrids **5** and **8** vs antibacterial effect against MRSA.

reference drug norfloxacin (MIC = 0.021 mM), with a *Clog P* value of 3.03. Furthermore, MRSA was relatively sensitive to derivatives **5g** (MIC = 0.007 mM) and **8a** (MIC = 0.015 mM), and their *Clog P* values were 2.86 and 3.10, respectively. However, no clear correlation was observed between the *Clog P* values and MIC values. These results indicated that when the *Clog P* values of the target compounds were distributed between 2.86 and 4.54, it might be beneficial to inhibit the growth of MRSA strains, and could promote the transport of the target coumarin thiazoles and their release at the active site in the organism [34].

2.4. Bactericidal kinetics

In order to further verify whether highly active coumarin thiazole derivative **5i** had an effective bactericidal ability against MRSA, a time-kill kinetic experiment was carried out (Fig. 3). Notably, at a reference concentration (4 × MIC), the viable cells number was less than 10^3 CFU/mL after 2 h. These results showed that hybrid **5i** had a strong killing ability against MRSA and could quickly inactivate the bacteria in a short time [35,36].

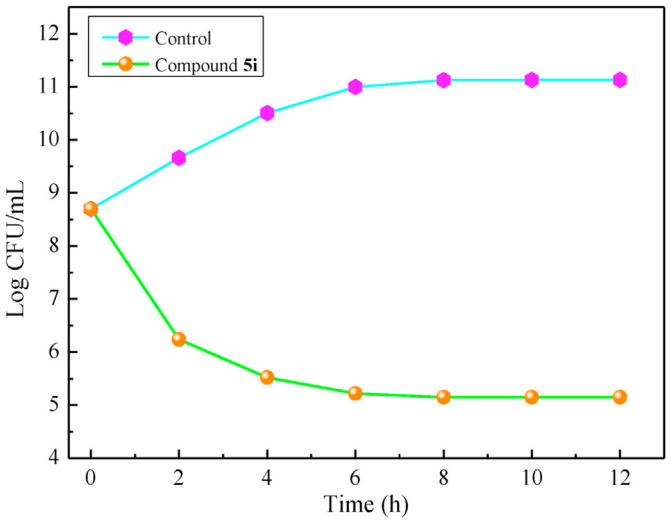


Fig. 3. Bactericidal kinetics of compound **5i** (4 × MIC) against MRSA.

2.5. Drug resistance development

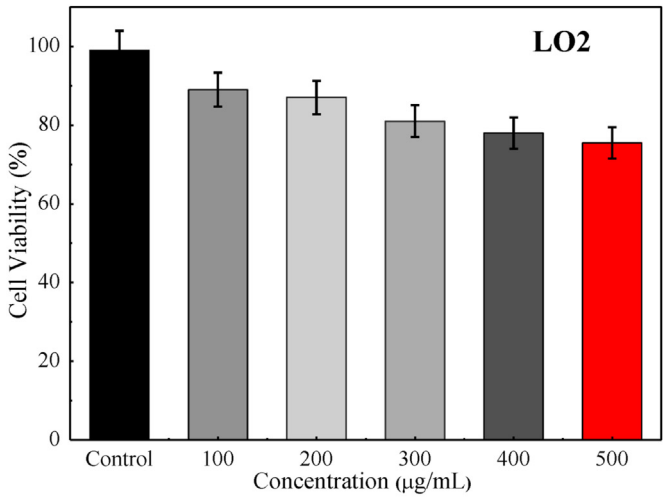
The continuous rise in bacterial resistance was a huge obstacle to drug development, hence, it was important to evaluate whether newly synthesized coumarin thiazole derivatives could induce bacterial resistance. In this study, highly active compound **5i** was selected to determine its ability to confer resistance onto MRSA, and norfloxacin was used as a positive control. The results in Fig. 4 showed that MRSA remained almost unchanged for 7 consecutive passages and produced slow resistance to molecule **5i** compared with the reference drug norfloxacin. These results indicated that it was more difficult for the hybrid **5i** to induce the development of MRSA resistance than the control drug norfloxacin. Therefore, this kind of modification of coumarin thiazole hybrids might yield a promising low-resistance candidate structure against MRSA [37,38].

2.6. Cytotoxicity

Cytotoxicity has long been used as an indicator to determine whether novel and potentially modified drug molecules can be employed as candidates for antimicrobial agents. The cytotoxic effects of bioactive compound **5i** on human hepatocyte LO2 cells and normal mammalian cells (RAW 264.7) were assessed *in vitro* using the MTT assay. The cytotoxicity results (Fig. 5) showed that no less than 75% of living cells were present even when the hybrid concentration was increased to 500 μg/mL. This result indicated that the selected drug molecule **5i** had low cytotoxicity, which was very beneficial for its therapeutic potential, and its harmfulless characteristic was also consistent with the screening criteria for active drug candidate molecules [39,40].

2.7. Bacterial membrane permeabilization

Membrane-active drug molecules can penetrate bacterial membranes to increase drug delivery rates, which is expected to reduce bacterial resistance, due to their strong destructive power. In addition, propidium iodide (PI) can successfully cross the cell membrane of damaged bacteria and fluoresce when it binds to DNA. Therefore, PI was used as a probe to determine the destructive ability of hybrid **5i** toward the MRSA membrane. As shown in Fig. 6, the fluorescence intensity of PI with compound **5i** showed a trend



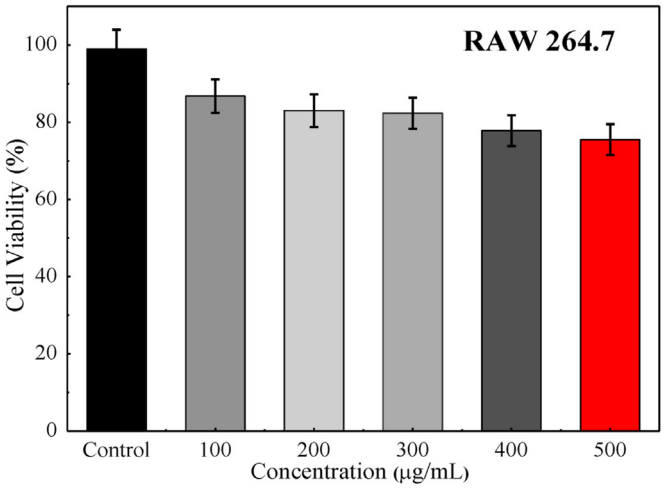


Fig. 5. Cytotoxic assay of target compound **5i** on human hepatocyte LO2 cells and normal mammalian cells (RAW 264.7) tested by MTT methodology.

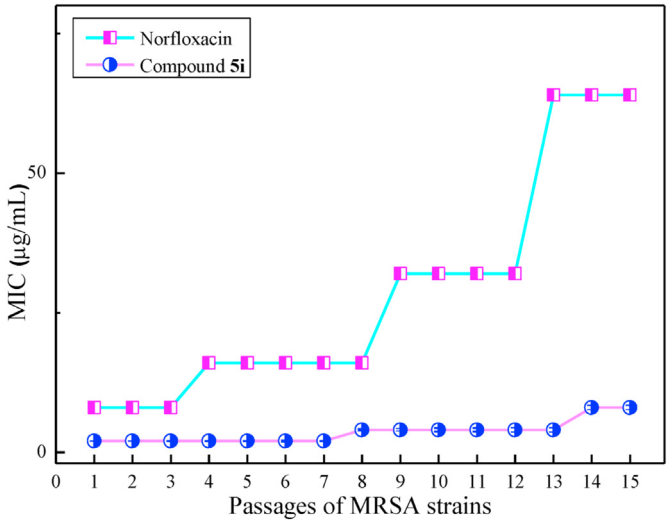


Fig. 4. Drug resistance development of compound **5i** against MRSA.

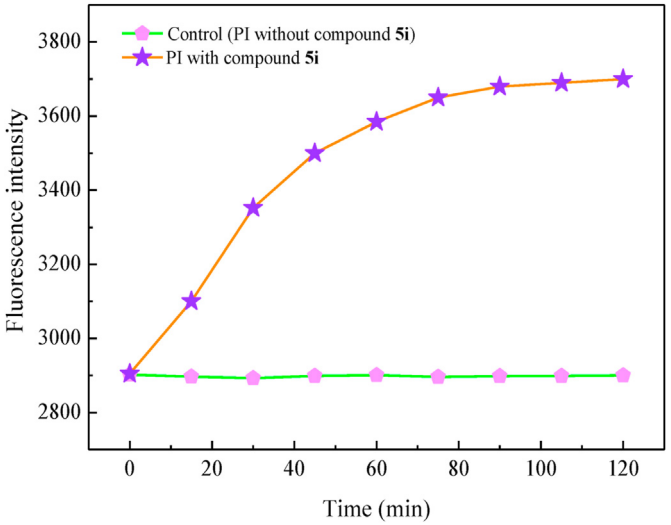


Fig. 6. Membrane permeabilization of MRSA toward compound **5i** (12 × MIC).

of rapid increase, and then gradually became flat after 60 min, whereas the relative change in the control group was not obvious. These results indicated that at the reference concentration (12 × MIC), molecule **5i** could destroy the membrane of MRSA and form a PI-DNA complex, which might further penetrate the MRSA membrane to achieve its effective antibacterial purpose [41,42].

2.8. Molecular docking

Flexible ligand-receptor docking of highly active compound **5i** was successfully conducted to rationalize antibacterial activity as much as possible and to understand the possible mechanisms of this type of coumarin thiazole derivatives. Crystal structure data (gyrase-DNA complex) were obtained from the protein data bank (PDB code: 2XCS), which was a potential target for antimicrobial research as previously reported [8,43]. The molecular docking results (Fig. 7) indicated that the oxygen atom on the hydroxyl group in hybrid **5i** was adjacent to DC-11, forming a hydrogen bond at a distance of 2.1 Å. Furthermore, hybrid **5i** might also form hydrogen bonds with the ARG-1122 residue *via* the nitrogen atom of thiazole fragment and the oxygen atom on the carbonyl group, with distances of 2.0 Å and 2.7 Å. These stable existence of these hydrogen bonds was beneficial to maintaining the stability of the coumarin thiazole hybrid **5i**-DNA complex, which might help explain why compound **5i** had a good inhibitory ability against MRSA.

2.9. Electronic properties

Molecular electrostatic potential (MEP) surfaces can effectively highlight the distribution trend of electrons carried by the target structures, and enable further analysis of the region formed by hydrogen bonds, which have a positive impact on the interpretation of the electrostatic interactions between the target molecules and the active sites in an organism. Herein, MEP simulation images of target compounds **5d**, **5g** and **5i** were recorded and summarized in Table 3 Through a comparison of the following three maps, we might roughly observe such a phenomenon. In the three selected hybrids, the positively charged regions (in blue) were mainly located on the hydroxyl group of the diethanolamine fragment and the negatively charged regions (in red) were mainly concentrated on the oxygen atom of the coumarin skeleton. This result precisely confirmed that hydrogen bonds were formed because of such electron sharing, and the formed hydrogen bonds were conducive to the hydrophilic ability of the coumarin thiazole hybrids modified

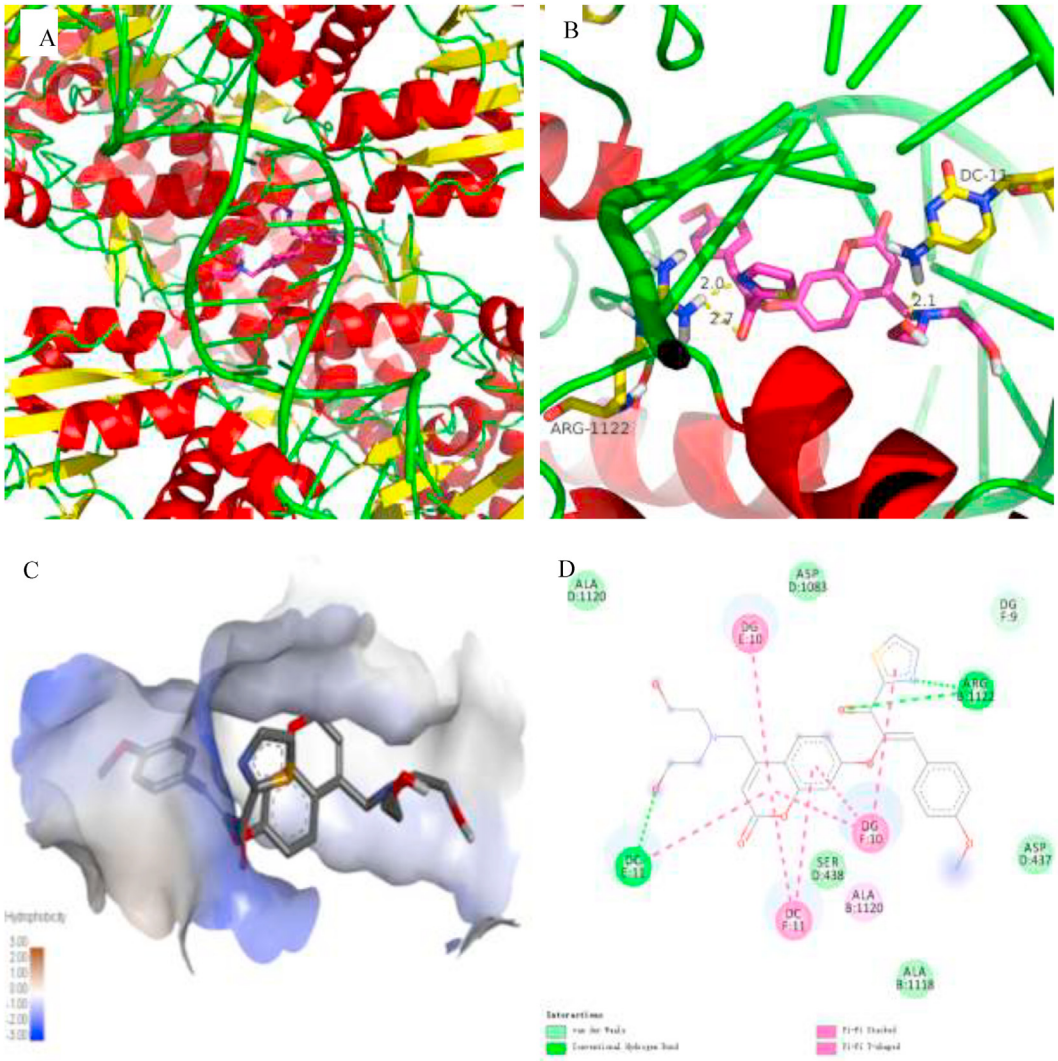
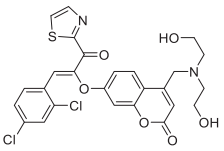
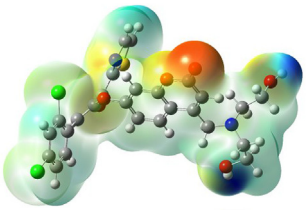
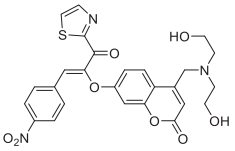
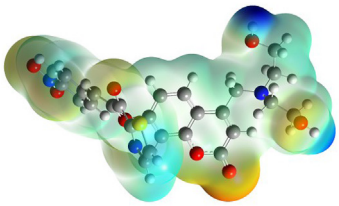
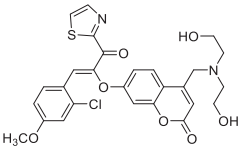
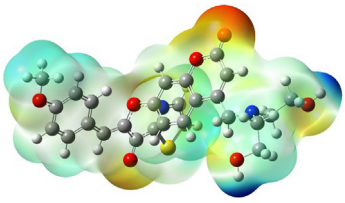


Fig. 7. (A and B) Stereoview and three-dimensional conformations of hybrid **5i** docked in gyrase DNA complex (PDB code: 2XCS); (C) Visualization of hybrid **5i** interacting with the hydrophobic residues at the active sites of gyrase DNA complex; (D) Two-dimensional conformation of hybrid **5i** docked in gyrase DNA complex.

Table 3
MEP surfaces of coumarin thiazole hybrids **5d**, **5g** and **5i**.

Compds	Structures	MEPs
5d		
5g		
5i		

with hydroxyethyl fragments, which thus made it easy to interact with diverse enzymes and receptors in biological systems. This conclusion, obtained from the study of electronic properties, was consistent with the favorable mode of action indicated by the aforementioned molecular docking research [34,44].

2.10. Interactions of compound ***5i*** with MRSA DNA

DNA, the deliverer of genetic instructions, has played an important role in the target construction of many antimicrobial drugs, which provides a significant direction for the exploration of novel potential DNA-targeting antimicrobial molecules [45]. Hence, coumarin thiazole molecule **5i** was selected as a sample for its highly selective inhibition of MRSA strains, and its antibacterial mechanism was preliminarily explored by reacting it with MRSA DNA *in vitro*, and the interaction was monitored *via* UV−vis spectroscopy. Herein, the tested DNA was isolated from the MRSA strains, and neutral red (NR) dye as the spectroscopic probe was used, and two spectral characteristics, hyperchromism and hypochromism, were used to distinguish the variations in the DNA double-helix structure. The UV−vis spectra (Fig. 8) showed that the maximum absorption peak (260 nm) of MRSA DNA increased along with the increasing content of active compound **5i**. In addition, the absorption of the **5i**-DNA complex was significantly lower than that of the single sum of free hybrid **5i** and MRSA DNA. This hypochromic phenomenon might be related to the strong interaction caused by the embedding of the aromatic chromophore of compound **5i** into the DNA helix structure [46]. The corresponding equation and plot of the binding constant were shown in Fig. S2 (Supporting information).

2.10.1. Interactions of compound ***5i***, NR and MRSA DNA

As could be seen from Fig. 9, the maximum absorption peak (530 nm) of the DNA-NR complex decreased with a gradual increase in compound concentration, whereas the trend of free NR

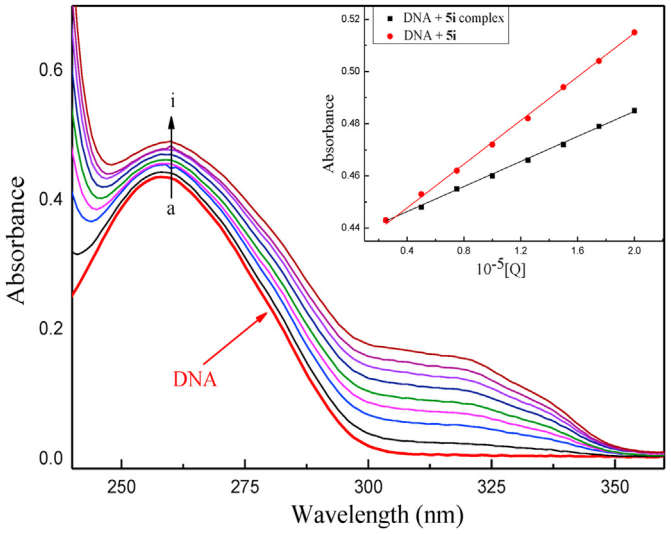


Fig. 8. Different concentrations of coumarin thiazole hybrid **5i** and DNA (pH = 7.4, T = 298 K). Inset: comparison of absorption of the **5i**-DNA complex at 260 nm and the sum of free DNA and hybrid **5i**. c (DNA) = 1.86×10^{-4} mol/L, and c (hybrid **5i**) = 0–1.60×10^{-5} mol/L for curves a–i respectively, at increment 0.20×10^{-5}.

absorbance was opposite to an increase in DNA content. These obvious spectral changes suggested that highly active hybrid **5i** might compete with NR and effectively embed into the DNA double-helix structure to block DNA replication and further exert its highly efficient antibacterial activity [47].

2.11. Drug combination

Drug combination is an important means to improve the cure rate of multiple diseases by combining potential drug candidates

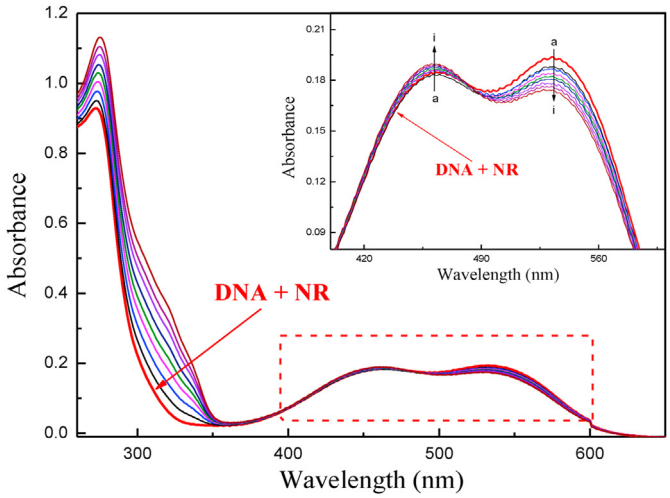


Fig. 9. Competitive reaction of coumarin thiazole hybrid 5i and neutral red with DNA. c (DNA) = 1.86 × 10^{-4} mol/L, c (NR) = 2 × 10^{-5} mol/L, and c (compound 5i) = 0–4.8 × 10^{-5} mol/L for curves a–i respectively, at increment 0.6 × 10^{-5}. Inset: in the wavelength range of 380–620 nm, with the increase of compound 5i content, the absorption spectra of the system reacted competitively with compound 5i, NR and DNA.

with clinical drugs. It is believed to minimize the occurrence of side effects and the development of bacterial resistance [20,23]. In this study, highly active molecule **5i** was combined with clinical norfloxacin against the test bacteria *in vitro*, and the effect of the combination was verified by calculating the fractional inhibitory concentration (FIC) index.

The results in Table 4 showed that the combination uses had a significantly better inhibitory effect on bacteria than their single use of either component, which was reflected by their final MIC values. It was worth noting that compound **5i** exhibited a significantly improved inhibitory effect against MRSA after being combined with norfloxacin, showing an expected synergistic effect (FIC = 0.45). In addition, hybrid **5i** combined with norfloxacin showed different degrees of additive effects against *K. pneumonia* and *E. coli*, with FIC values of 0.58 and 0.51, respectively. However, contrary to expectations, the combination of compound **5i** and norfloxacin had an indifferent effect on *E. faecalis*. These results showed that the combination of coumarin thiazole molecule **5i** and clinical drugs could maximize the antibacterial efficiency, and might also provide a feasible clue for broadening the antibacterial spectrum and reducing drug resistance [25].

3. Conclusion

A series of novel chalcone-conjugated, multi-flexible end-group coumarin thiazole derivatives was successfully developed through efficient and productive synthetic methods. *In vitro* antibacterial evaluation displayed that the bacteriostatic ability of the prepared hybrids was better than or equal to that of the reference drug norfloxacin against some tested strains. Remarkably, methoxybenzene-modified coumarin thiazole hybrid **5i** showed strong selectivity and significant inhibitory activity against MRSA (MIC = 0.004 mM), which was 6 times superior than that of norfloxacin (MIC = 0.025 mM). When the *Clog P* values of the target compounds were in the range of 2.63–4.54, it was beneficial to the development of their physicochemical and pharmacokinetic properties. In addition, compound **5i** did not produce apparent cytotoxicity or drug resistance. Molecular docking and MEP surface studies have shown that highly active molecule **5i** could facilitate molecular interactions with various enzymes and receptors in biological systems by forming stable hydrogen bonds. Moreover, further research into the antibacterial mechanism revealed that hybrid **5i** might damage the MRSA cell membrane and could also be inserted into MRSA DNA to inhibit its replication and growth. Based on these research facts, the modification of this type of coumarin thiazole derivatives could offer enormous possibilities for developing more novel antibacterial repressors and might also provide an alternative for the synthesis of biologically active hybrids, in addition to revealing their therapeutic targets and potential antibacterial mechanism.

4. Experimental protocols

4.1. General methods

Unless otherwise stated, all materials are commercially available without any additional processing. The mass was measured on a micro-balance. Thin layer UV chromatography (254 nm) (TLC) was employed. Silica gel (500–600 mesh) was used for column chromatography. Melting point (mp) was determined with an X-6 melting point meter. ^{1}H NMR and ^{13}C NMR spectra of coumarin thiazole hybrids were recorded by Bruker AVANCE III spectrometer (600 MHz). High resolution mass spectra (HRMS) was tested by mass spectrometer (FT-ICR). The following abbreviations were used to express the signal of NMR spectra: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. The coupling constant (*J*) was applied to Hertz unit (Hz). Abbreviations for the following were used to denote structural fragments: Coumarin = Cou; Ph = Phenyl.

4.1.1. Synthesis of intermediates *2* and *7*

The intermediates **2** and **7** were prepared according to the literature procedures [8,48].

4.1.2. Synthesis of intermediates *10* and *11*

The intermediates **10** and **11** were prepared according to the literature procedures [28].

4.1.3. Synthesis of 4-(chloromethyl)-7-(2-oxo-2-(thiazol-2-yl) ethoxy)-2H-chromen-2-one (*3*)

To a solution of intermediate **2** (2.10 g, 10.0 mmol), potassium carbonate (1.70 g, 12.0 mmol) in acetonitrile (50 mL) was added intermediate **7** (2.47 g, 12.0 mmol), the mixture was stirred at 60 °C for 8 h. After completion of the reaction, solvent was evaporated

Table 4
Drug combinations of compound **5i** with antibacterial drugs Norfloxacin.[a]

Bacteria	MRSA	*E. faecalis*	*K. pneumonia*	*E. coli*
Compound **5i** MIC/10^{-3} mM	0.125	1.906	0.953	0.234
Compound **N** MIC/10^{-3} mM	0.781	0.781	0.203	0.781
FIC index	0.45	1.34	0.58	0.51
Effect	synergistic	indifferent	additivism	additivism

[a] N, Norfloxacin; MRSA, Methicillin-Resistant Staphylococcus aureus N315; E. faecalis, Enterococcus faecalis; K. pneumonia, Klebsiella pneumonia; E. coli, Escherichia coli.

under reduced pressure and further purified by silica gel column chromatography (eluent, dichloromethane/petroleum ether (10/1, V/V)) to afford the desired compound **3** as white solid. Yield: 48%; mp: 190–192 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.32 (d, J = 3.0 Hz, 1H, thiazole-5-*H*), 8.24 (d, J = 3.0 Hz, 1H, thiazole-4-*H*), 7.78 (d, J = 8.8 Hz, 1H, Cou-5-*H*), 7.12 (m, J = 9.0, 3.2 Hz, 2H, Cou-6, 8-*H*), 6.52 (s, 1H, Cou-3-*H*), 5.82 (s, 2H, OCH_2), 5.00 (s, 2H, CH_2Cl) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 187.6, 164.1, 161.6, 160.4, 155.5, 151.2, 145.8, 128.7, 127.0, 126.9, 113.0, 112.8, 111.5, 102.6, 70.5, 55.4 ppm.

4.1.4. Synthesis of 4-((bis(2-hydroxyethyl)amino)methyl)-7-(2-oxo-2-(thiazol-2-yl)ethoxy)-2H-chromen-2-one (**4**)

To a solution of intermediate **3** (336 mg, 1.00 mmol) in acetonitrile (25 mL) was added diethanol amine (212 mg, 2.00 mmol), the mixture was stirred at 80 °C for 8 h. After completion of the reaction, solvent was evaporated under reduced pressure and further purified by silica gel column chromatography (eluent, dichloromethane/methanol (3/1, V/V)) to afford the desired compound **4** as brown liquid. Yield: 70%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.33 (d, J = 2.9 Hz, 1H, thiazole-5-*H*), 8.24 (d, J = 2.9 Hz, 1H, thiazole-4-*H*), 7.87 (d, J = 8.9 Hz, 1H, Cou-5-*H*), 7.07 (d, J = 2.3 Hz, 1H, Cou-8-*H*), 7.01 (dd, J = 8.9, 2.4 Hz, 1H, Cou-6-*H*), 6.51 (s, 1H, Cou-3-*H*), 5.80 (s, 2H, CH_2CO), 4.49 (m, 2H, 2O*H*), 3.89 (s, 2H, Cou-4-CH_2), 3.51–3.48 (m, 4H, 2CH$_2$CH_2OH), 2.64 (t, J = 5.8 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 187.7, 164.1, 161.1, 160.9, 155.3, 155.0, 145.8, 128.7, 126.9, 112.9, 112.6, 111.4, 102.2, 70.4, 59.7, 57.4, 56.3, 55.4 ppm.

4.1.5. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((3-oxo-1-phenyl-3-(thiazol-2-yl)prop-1-en-2-yl) oxy)-2H-chromen-2-one (**5a**)

To a solution of intermediate **4** (404 mg, 1.00 mmol), benzaldehyde (107 mg, 1.00 mmol) in ethanol (25 mL) was added piperidine (17 mg, 0.20 mmol), the mixture was stirred at 80 °C for 8 h. After completion of the reaction, solvent was evaporated under reduced pressure and further purified by silica gel column chromatography (eluent, dichloromethane/petroleum ether (1/1, V/V)) to afford the desired compound **5a** as yellow liquid. Yield: 25%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.79 (s, 1H, C=C*H*), 8.27 (s, 2H, thiazole-4,5-*H*), 7.92 (d, J = 8.6 Hz, 1H, Cou-5-*H*), 7.79 (d, J = 8.8 Hz, 2H, Ph-2,6-*H*), 7.06 (d, J = 1.7 Hz, 2H, Ph-3,5-*H*), 7.03 (m, J = 6.2, 2.7 Hz, 3H, Ph-4-*H*, Cou-6,8-*H*), 6.55 (s, 1H, Cou-3-*H*), 4.47 (m, 2H, 2O*H*), 3.88 (s, 2H, Cou-4-CH_2), 3.51 (d, J = 5.4 Hz, 4H, 2CH$_2$CH_2OH), 2.65 (d, J = 6.3 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.2, 170.8, 166.6, 162.1, 160.6, 159.3, 155.2, 145.6, 142.6, 136.46, 133.8, 132.1, 129.1, 128.5, 127.4, 124.9, 115.3, 114.2, 112.1, 103.1, 102.2, 59.7, 57.5, 55.9 ppm; HRMS (ESI) calcd. for $C_{26}H_{24}N_2O_6S$ [M+H]$^+$, 493.1428, found, 493.1426.

4.1.6. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(2-chlorophenyl)-3-oxo-3-(thiazol-2-yl) prop-1-en-2-yl)oxy)-2H-chromen-2-one (**5b**)

Compound **5b** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *o*-chlorobenzaldehyde (140 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5b** was obtained as yellow liquid. Yield: 40%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.19 (s, 1H, C=C*H*), 8.33 (d, J = 11.0 Hz, 2H, thiazole-4,5-*H*), 7.98 (d, J = 7.7 Hz, 1H, Ph-6-*H*), 7.91 (d, J = 8.8 Hz, 1H, Cou-5-*H*), 7.63 (d, J = 7.9 Hz, 1H, Ph-2-*H*), 7.47 (t, J = 7.4 Hz, 1H, Ph-3-*H*), 7.39 (t, J = 7.4 Hz, 1H, Ph-5-*H*), 7.11 (s, 1H, Cou-8-*H*), 7.05 (d, J = 8.6 Hz, 1H, Cou-6-*H*), 6.56 (s, 1H, Cou-3-*H*), 4.49 (m, 2H, 2O*H*), 3.88 (s, 2H, Cou-4-CH_2), 3.50 (s, 4H, 2CH$_2$CH_2OH), 2.63 (t, J = 5.3 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.3, 166.1, 160.6, 159.1, 155.1, 154.7, 145.9, 145.3, 134.9, 132.6, 131.3, 130.9, 130.5, 130.4, 129.3, 128.3, 127.5, 114.4, 112.3, 112.2, 103.5, 59.7, 57.3, 56.2 ppm; HRMS (ESI) calcd. for $C_{26}H_{23}ClN_2O_6S$ [M+H]$^+$, 527.1038, found, 527.1043.

4.1.7. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(4-chlorophenyl)-3-oxo-3-(thiazol-2-yl) prop-1-en-2-yl)oxy)-2H-chromen-2-one (**5c**)

Compound **5c** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *p*-chlorobenzaldehyde (140 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5c** was obtained as yellow liquid. Yield: 42%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.71 (s, 1H, C=C*H*), 8.32 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 8.30 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 7.93 (d, J = 8.8 Hz, 1H, Cou-5-*H*), 7.83 (d, J = 8.7 Hz, 2H, Ph-2,6-*H*), 7.53 (d, J = 8.6 Hz, 2H, Ph-3,5-*H*), 7.10 (d, J = 2.5 Hz, 1H, Cou-8-*H*), 7.07 (dd, J = 8.8, 2.5 Hz, 1H, Cou-6-*H*), 6.58 (s, 1H, Cou-3-*H*), 4.51 (m, 2H, 2O*H*), 3.89 (s, 2H, Cou-4-CH_2), 3.52 (d, J = 5.2 Hz, 4H, 2CH$_2$CH_2OH), 2.65 (t, J = 6.1 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.6, 166.1, 160.6, 158.9, 155.2, 154.7, 145.8, 144.5, 136.0, 134.4, 133.0, 131.3, 129.7, 129.0, 127.5, 114.4, 112.3, 112.1, 103.4, 59.7, 57.3, 56.2 ppm; HRMS (ESI) calcd. for $C_{26}H_{23}ClN_2O_6S$ [M+H]$^+$, 527.1038, found, 527.1043.

4.1.8. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(2,4-dichlorophenyl) -3-oxo-3-(thiazol-2-yl)prop-1-en-2-yl) oxy)-2H-chromen-2-one (**5d**)

Compound **5d** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), 2, 4-dichlorobenzaldehyde (175 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5d** was obtained as yellow liquid. Yield: 40%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.11 (s, 1H, C=C*H*), 8.34 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 8.31 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 7.98 (d, J = 8.7 Hz, 1H, Ph-6-*H*), 7.91 (d, J = 8.9 Hz, 1H, Cou-5-*H*), 7.81 (d, J = 2.1 Hz, 1H, Ph-5-*H*), 7.50 (dd, J = 8.6, 2.1 Hz, 1H, Ph-3-*H*), 7.10 (d, J = 2.5 Hz, 1H, Cou-8-*H*), 7.04 (dd, J = 8.8, 2.5 Hz, 1H, Cou-6-*H*), 6.56 (s, 1H, Cou-3-*H*), 4.50 (m, 2H, 2O*H*), 3.88 (s, 2H, Cou-4-CH_2), 3.50 (s, 4H, 2CH$_2$CH_2OH), 2.64 (t, J = 6.0 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.2, 165.9, 160.5, 158.9, 155.1, 154.7, 145.9, 145.6, 136.3, 135.8, 132.4, 130.1, 129.7, 129.4, 128.6, 127.5, 114.5, 112.4, 112.2, 103.6, 59.6, 57.3, 55.3 ppm; HRMS (ESI) calcd. for $C_{26}H_{22}Cl_2N_2O_6S$ [M+H]$^+$, 561.0648, found, 561.0651.

4.1.9. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(4-fluorophenyl)-3-oxo-3-(thiazol-2-yl) prop-1-en-2-yl)oxy)-2H-chromen-2-one (**5e**)

Compound **5e** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *p*-fluorobenzaldehyde (124 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5e** was obtained as yellow liquid. Yield: 50%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.74 (s, 1H, C=C*H*), 8.31 (dd, J = 7.1, 3.1 Hz, 2H, thiazole-4,5-*H*), 7.92–7.87 (m, 3H, Ph-2,6-*H*, Cou-5-*H*), 7.32 (s, 2H, Ph-3,5-*H*), 7.11 (d, J = 2.5 Hz, 1H, Cou-8-*H*), 7.07 (dd, J = 8.8, 2.6 Hz, 1H, Cou-6-*H*), 6.57 (s, 1H, Cou-3-*H*), 4.52 (m, 2H, 2O*H*), 3.89 (s, 2H, Cou-4-CH_2), 3.51 (t, J = 5.4 Hz, 4H, 2CH$_2$CH_2OH), 2.65 (t, J = 6.0 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.6, 166.2, 164.9, 162.5, 160.6, 159.0, 155.2, 154.8, 145.8, 143.8, 134.8, 134.08, 128.98, 127.58, 116.98, 116.88, 114.4, 112.1, 103.3, 59.6, 57.3, 56.14 ppm; HRMS (ESI) calcd. for $C_{26}H_{23}FN_2O_6S$ [M+H]$^+$, 511.1334, found, 511.1339.

*4.1.10. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(4-bromophenyl)-3-oxo-3-(thiazol-2-yl) prop-1-en-2-yl)oxy)-2H-chromen-2-one (**5f**)*

Compound **5f** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *p*-bromobenzaldehyde (184 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5f** was obtained as yellow liquid. Yield: 40%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.68 (s, 1H, C═C*H*), 8.32 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 8.30 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 7.92 (d, J = 8.9 Hz, 1H, Cou-5-*H*), 7.74 (d, J = 8.6 Hz, 2H, Ph-2,6-*H*), 7.66 (d, J = 8.6 Hz, 2H, Ph-3,5-*H*), 7.09 (d, J = 2.5 Hz, 1H, Cou-8-*H*), 7.06 (dd, J = 8.8, 2.5 Hz, 1H, Cou-6-*H*), 6.56 (s, 1H, Cou-3-*H*), 4.49 (m, 2H, 2O*H*), 3.89 (s, 2H, Cou-4-CH_2), 3.51 (d, J = 5.2 Hz, 4H, 2CH$_2$CH_2OH), 2.64 (t, J = 6.1 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.6, 166.1, 160.5, 158.9, 155.2, 154.7, 145.8, 144.6, 134.5, 133.2, 132.7, 131.6, 129.0, 127.5, 124.9, 114. 4, 112.3, 112.2, 103.4, 59.7, 57.3, 56.2 ppm; HRMS (ESI) calcd. for $C_{26}H_{23}BrN_2O_6S$ [M+H]$^+$, 571.0533, found, 571.0538.

*4.1.11. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(4-nitrophenyl)-3-oxo-3-(thiazol-2-yl) prop-1-en-2-yl)oxy)-2H-chromen-2-one (**5g**)*

Compound **5g** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *p*-nitrobenzaldehyde (151 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5g** was obtained as yellow liquid. Yield: 45%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.71 (s, 1H, C═C*H*), 8.32 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 8.30 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 7.93 (d, J = 8.8 Hz, 1H, Cou-5-*H*), 7.83 (d, J = 8.7 Hz, 2H, Ph-2,6-*H*), 7.53 (d, J = 8.6 Hz, 2H, Ph-3,5-*H*), 7.10 (d, J = 2.5 Hz, 1H, Cou-8-*H*), 7.07 (dd, J = 8.8, 2.5 Hz, 1H, Cou-6-*H*), 6.58 (s, 1H, Cou-3-*H*), 4.51 (m, 2H, 2O*H*), 3.89 (s, 2H, Cou-4-CH_2), 3.52 (d, J = 5.2 Hz, 4H, 2CH$_2$CH_2OH), 2.65 (t, J = 6.1 Hz, 4H, 2CH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.6, 166.1, 160.6, 158.9, 155.2, 154.7, 145.8, 144.5, 136.0, 134.4, 133.0, 131.3, 129.7, 129.0, 127.5, 114.4, 112.3, 112.1, 103.4, 59.7, 57.3, 56.2 ppm; HRMS (ESI) calcd. for $C_{27}H_{26}N_2O_7S$ [M+H]$^+$, 538.1279, found, 538.1285.

*4.1.12. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((3-oxo-3-(thiazol-2-yl)-1-(p-tolyl)prop-1-en-2-yl) oxy)-2H-chromen-2-one (**5h**)*

Compound **5h** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *p*-tolualdehyde (120 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5h** was obtained as yellow liquid. Yield: 40%; ^{1}H NMR (600 MHz, DMSO-d_6) ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.75 (s, 1H, C═C*H*), 8.29 (s, 2H, thiazole-4,5-*H*), 7.91 (d, J = 8.7 Hz, 1H, Cou-5-*H*), 7.71 (d, J = 8.1 Hz, 2H, Ph-2,6-*H*), 7.27 (d, J = 8.1 Hz, 2H, Ph-3,5-*H*), 7.08−7.04 (m, 2H, Cou-6,8-*H*), 6.55 (s, 1H, Cou-3-*H*), 4.48 (m, 2H, 2O*H*), 3.88 (s, 2H, Cou-4-CH_2), 3.51 (dd, J = 11.1, 5.6 Hz, 4H, 2CH$_2$CH_2OH), 2.64 (t, J = 5.9 Hz, 4H, 2NCH_2CH$_2$OH), 2.32 (s, 3H, Ph-CH_3) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 178.5, 166.4, 160.6, 159.2, 155.2, 154.8, 145. 7, 143.6, 141.9, 136.3, 131.6, 130.3, 129.6, 128.7, 127. 5, 114.2, 112.3, 112.1, 103.1, 59.7, 57.3, 56.2, 21.6 ppm; HRMS (ESI) calcd. for $C_{27}H_{26}N_2O_6S$ [M+H]$^+$, 507.1584, found, 507.1585.

*4.1.13. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(4-methoxyphenyl)-3-oxo-3-(thiazol-2-yl) prop-1-en-2-yl) oxy)-2H-chromen-2-one (**5i**)*

Compound **5i** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), *p*-anisaldehyde (136 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **5i** was obtained as yellow liquid. Yield: 50%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.74 (s, 1H, C═C*H*), 8.30 (q, J = 3.1 Hz, 2H, thiazole-4,5-*H*), 7.92 (d, J = 8.8 Hz, 1H, Cou-5-*H*), 7.81 (dd, J = 6.6, 3.0 Hz, 2H, Ph-2,6-*H*), 7.46 (d, J = 2.8 Hz, 2H, Ph-3,5-*H*), 7.09 (d, J = 2.5 Hz, 1H, Cou-8-*H*), 7.07 (dd, J = 4.4, 1.7 Hz, 1H, Cou-6-*H*), 6.55 (s, 1H, Cou-3-*H*), 4.48 (m, 2H, 2O*H*), 4.04 (s, 2H, Cou-4-CH_2), 3.89 (d, J = 9.3 Hz, 3H, OCH_3), 3.50 (d, J = 5.5 Hz, 4H, 2CH$_2$CH_2OH), 2.65−2.64 (m, 4H, 2NCH_2CH$_2$OH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 187.7, 178.7, 170.8, 166.2, 164.1, 160.6, 159.2, 155.2, 145.7, 144.2, 135.9, 131.5, 129.6, 128.9, 127.5, 114.3, 112.6, 112.2, 112.1, 103.2, 102.2, 59.7, 57.5, 56.2, 53.5 ppm; HRMS (ESI) calcd. for $C_{27}H_{26}N_2O_7S$ [M+H]$^+$, 523.1533, found, 523.1532.

*4.1.14. Synthesis of (Z)-7-((1-(1H-inden-3-yl)-3-oxo-3-(thiazol-2-yl)prop-1-en-2-yl)oxy)-4-((bis(2-hydroxyethyl) amino)methyl)-2H-chromen-2-one (**8a**)*

Compound **8a** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), compound **11a** (145 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **8a** was obtained as yellow liquid. Yield: 40%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.10 (s, 1H, N*H*), 9.47 (s, 1H, C═C*H*), 8.32 (d, J = 3.0 Hz, 1H, thiazole-5-*H*), 8.25 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 7.94 (d, J = 2.8 Hz, 1H, Cou-5-*H*), 7.90 (d, J = 8.9 Hz, 1H, indole-2-*H*), 7.84 (dd, J = 5.8, 3.0 Hz, 1H, indole-4-*H*), 7.51 (dd, J = 6.0, 2.8 Hz, 1H, indole-5-*H*), 7.26 (dd, J = 5.9, 3.0 Hz, 2H, indole-6,7-*H*), 7.11 (d, J = 2.3 Hz, 1H, Cou-8-*H*), 7.07 (dd, J = 8.9, 2.3 Hz, 1H, Cou-6-*H*), 6.53 (s, 1H, Cou-3-*H*), 4.48 (m, 2H, 2O*H*), 3.88 (s, 2H, Cou-4-CH_2), 3.49 (dd, J = 10.5, 5.1 Hz, 4H, 2CH$_2$CH_2OH), 2.63 (t, J = 5.4 Hz, 4H, 2NCH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 176.3, 167.7, 160.7, 159.4, 155.3, 154.9, 145.5, 140.6, 136.6, 132. 5, 130.7, 127.8, 127.5, 127.3, 123.5, 121.9, 118.7, 113.9, 113.1, 111.9, 111.8, 108.9, 102.9, 59.7, 57.3, 56.2 ppm; HRMS (ESI) calcd. for $C_{28}H_{25}N_3O_6S$ [M+H]$^+$, 532.1537, found, 532.1538.

*4.1.15. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(6-chloro-1H-indol-3-yl) -3-oxo-3-(thiazol-2-yl)prop-1-en-2-yl)oxy)-2H-chromen-2-one (**8b**)*

Compound **8b** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), compound **11b** (179 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **8b** was obtained as yellow liquid. Yield: 43%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.13 (s, 1H, N*H*), 9.37 (s, 1H, C═C*H*), 8.31 (d, J = 2.7 Hz, 1H, thiazole-5-*H*), 8.26 (d, J = 2.8 Hz, 1H, thiazole-4-*H*), 7.97 (s, 1H, Cou-5-*H*), 7.90 (d, J = 8.9 Hz, 1H, indole-2-*H*), 7.83 (d, J = 8.5 Hz, 1H, indole-4-*H*), 7.56 (s, 1H, indole-5-*H*), 7.27 (d, J = 8.4 Hz, 1H, indole-7-*H*), 7.10 (s, 1H, Cou-8-*H*), 7.06 (d, J = 8.8 Hz, 1H, Cou-6-*H*), 6.53 (s, 1H, Cou-3-*H*), 4.46 (m, 2H, 2O*H*), 3.88 (s, 2H, Cou-4-CH_2), 3.49 (d, J = 4.8 Hz, 4H, 2CH$_2$CH_2OH), 2.64 (d, J = 12.7 Hz, 4H, 2NCH_2CH$_2$OH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 176.5, 167.5, 160.7, 159.3, 155.3, 154.8, 145.5, 141.1, 137.0, 133.2, 130. 0, 128.1, 127.9, 127.4, 126.2, 122.1, 120.3, 114.0, 112.8, 111.9, 111.8, 109.0, 102.9, 59.7, 57.3, 56.2 ppm; HRMS (ESI) calcd. for $C_{28}H_{24}ClN_3O_6S$ [M+H]$^+$, 566.1142, found, 566.1148.

*4.1.16. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(6-methyl-1H-indol-3-yl)-3-oxo-3- (thiazol-2-yl)prop-1-en-2-yl)oxy)-2H-chromen-2-one (**8c**)*

Compound **8c** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), compound **11c** (159 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **8c** was obtained as yellow liquid. Yield: 45%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.96 (s, 1H, N*H*), 9.42 (s, 1H, C═C*H*), 8.31 (d, J = 2.9 Hz, 1H, thiazole-5-*H*), 8.24 (d, J = 2.9 Hz, 1H, thiazole-4-*H*), 7.90 (d, J = 8.9 Hz, 1H, Cou-5-*H*), 7.86 (s, 1H, indole-2-*H*), 7.71 (d, J = 8.1 Hz, 1H, indole-4-*H*), 7.29 (s, 1H, indole-5-*H*), 7.08 (d, J = 6.7 Hz, 2H, indole-7-*H*, Cou-8-*H*), 7.06 (d, J = 8.8 Hz, 1H, Cou-6-*H*), 6.52 (s, 1H, Cou-3-*H*), 4.46 (m, 2H, 2O*H*),

3.88 (s, 2H, Cou-4-CH_2), 3.50 (d, J = 5.2 Hz, 4H, 2CH$_2$CH_2OH), 2.64 (dd, J = 11.6, 6.2 Hz, 4H, 2NCH_2CH$_2$OH), 2.43 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 176.2, 167.8, 160.7, 159.4, 155.3, 154.9, 145.4, 140.5, 137.0, 132.9, 132.1, 130.9, 127.7, 127.3, 125.4, 123.5, 118.5, 113.9, 112.8, 111.9, 111.7, 109.0, 102.9, 59.7, 57.4, 56.2, 21.7 ppm; HRMS (ESI) calcd. for $C_{29}H_{27}N_3O_6S$ $[M+H]^+$, 546.1693, found, 546.1695.

*4.1.17. Synthesis of (Z)-4-((bis(2-hydroxyethyl)amino)methyl)-7-((1-(1-ethyl-1H-indol-3-yl)-3-oxo- 3-(thiazol-2-yl)prop-1-en-2-yl) oxy)-2H-chromen-2-one (**8d**)*

Compound **8d** was prepared according to the procedure described for compound **5a**, starting from intermediate **4** (404 mg, 1.00 mmol), compound **11d** (173 mg, 1.00 mmol) and piperidine (17 mg, 0.20 mmol). The pure product **8d** was obtained as yellow liquid. Yield: 45%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.43 (s, 1H, C=CH), 8.32 (d, J = 3.1 Hz, 1H, thiazole-5-H), 8.25 (d, J = 3.1 Hz, 1H, thiazole-4-H), 8.05 (s, 1H, Cou-5-H), 7.90 (d, J = 8.9 Hz, 1H, indole-2-H), 7.86 (d, J = 7.1 Hz, 1H, indole-5-H), 7.61 (d, J = 7.2 Hz, 1H, indole-5-H), 7.33–7.28 (m, 2H, indole-6,7-H), 7.12 (d, J = 2.5 Hz, 1H, Cou-8-H), 7.08 (dd, J = 8.9, 2.5 Hz, 1H, Cou-6-H), 6.54 (s, 1H, Cou-3-H), 4.48 (m, 2H, 2OH), 4.29–4.26 (m, 2H, NCH_2CH$_3$), 3.88 (s, 2H, Cou-4-CH_2), 3.52–3.49 (m, 4H, 2CH$_2$CH_2OH), 2.64 (t, J = 6.1 Hz, 4H, 2NCH_2CH$_2$OH), 1.33 (t, J = 7.2 Hz, 3H, NCH$_2$CH_3) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 176.2, 167.7, 160.7, 159.4, 155.2, 154.9, 145.4, 140.7, 136.3, 134.5, 130.1, 128.1, 127.8, 127.2, 123.5, 122.1, 119.1, 113.9, 112.1, 111.7, 111.5, 108.3, 103.1, 59.7, 57.4, 56.2, 41.8, 15.5 ppm; HRMS (ESI) calcd. for $C_{30}H_{29}N_3O_6S$ $[M+H]^+$, 560.1850, found, 560.1852.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21905069), the Shenzhen Science and Technology Innovation Committee (JCYJ20180507183907224, KQTD20170809110344233), Economic, Trade and Information Commission of Shenzhen Municipality through the Graphene Manufacture Innovation Center (201901161514), and Guangdong Province Covid-19 Pandemic Control Research Fund (2020KZDZX1220). The authors would like to thank Zhou Qian from Shiyanjia Lab (www.shiyanjia.com) for Molecular docking, He Daijia from Ceshigo Research Service (www.ceshigo.com) for NMR analysis, Geng Huanran from the testing technology center of materials and devices, Tsinghua Shenzhen international graduate school (https://mdtc.sz.tsinghua.edu.cn) for the NMR analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113628.

References

[1] (a) E.D. Brown, G.D. Wright, Antibacterial drug discovery in the resistance era, Nature 529 (2016) 336–343;
(b) I. Levin-Reisman, I. Ronin, O. Gefen, I. Braniss, N. Shoresh, N.Q. Balaban, Antibiotic tolerance facilitates the evolution of resistance, Science 355 (2017) 826–830.
[2] S.L. Silver, Challenges of antibacterial discovery, Clin. Microbiol. Rev. 24 (2011) 71–109.
[3] (a) B. Mishra, G.S. Wang, Ab initio design of potent anti-MRSA peptides based on database filtering technology, J. Am. Chem. Soc. 134 (2012) 12426–12429;
(b) S.M. Lin, J.J. Koh, T.T. Aung, W.L.W. Sin, F.H. Lim, L. Wang, R. Lakshminarayanan, L. Zhou, D.T.H. Tan, D.R. Cao, R.W. Beuerman, L. Ren, S.P. Liu, Semisynthetic flavone-derived antimicrobials with therapeutic potential against methicillin-resistant *Staphylococcus aureus* (MRSA), J. Med. Chem. 60 (2017) 6152–6165.
[4] T.L. Harris, R.J. Worthington, C. Melander, Potent small-molecule suppression of oxacillin resistance in methicillin-resistant *Staphylococcus aureus*, Angew. Chem. Int. Ed. 51 (2012) 11254–11257.
[5] J.M. Stokes, C.R. MacNair, B. Ilyas, S. French, J.P. Cote, C. Bouwman, E.D. Brown, Pentamidine sensitizes Gram negative pathogens to antibiotics and overcomes acquired colistin resistance, Nat. Microbiol. 2 (2017) 17028.
[6] (a) M.E. Riveiro, K.N. De, A. Moglioni, R. Vazquez, F. Monczor, C. Shayo, C. Davio, Coumarins: old compounds with novel promising therapeutic perspectives, Curr. Med. Chem. 17 (2010) 1325–1338;
(b) M.A. Farha, E.D. Brown, Strategies for target identification of antimicrobial natural products, Nat. Prod. Rep. 33 (2016) 668–680.
[7] (a) H. Liu, Z.L. Ren, W. Wang, J.X. Gong, M.J. Chu, Q.W. Ma, J.C. Wang, X.H. Lv, Novel coumarin-pyrazole carboxamide derivatives as potential topoisomerase II inhibitors: design, synthesis and antibacterial activity, Eur. J. Med. Chem. 157 (2018) 81–87;
(b) W. Li, Y.N. Sun, X.T. Yan, S.Y. Yang, E.J. Kim, H.K. Kang, Y.H. Kim, Coumarins and lignans from zanthoxylum schinifolium and their anticancer activities, J. Agric. Food Chem. 61 (2013) 10730–10740.
[8] C.F. Hu, P.L. Zhang, Y.F. Sui, J.S. Lv, M.F. Ansaria, N. Battinia, S. Li, C.H. Zhou, R.X. Geng, Ethylenic conjugated coumarin thiazolidinediones as new efficient antimicrobial modulators against clinical methicillin-resistant *Staphylococcus aureus*, Bioorg. Chem. 94 (2020) 103434.
[9] X.M. Peng, K.V. Kumar, G.L.V. Damu, C.H. Zhou, Coumarin-derived azolyl ethanols: synthesis, antimicrobial evaluation and preliminary action mechanism study, Sci. China Chem. 59 (2016) 878–894.
[10] Y. Hu, Y.F. Shen, X.H. Wu, X. Tu, G.X. Wang, Synthesis and biological evaluation of coumarin derivatives containing imidazole skeleton as potential antibacterial agents, Eur. J. Med. Chem. 143 (2018) 958–969.
[11] X.M. Peng, G.L.V. Damu, C.H. Zhou, Coumarin-derived azolyl ethanols: synthesis, antimicrobial evaluation and preliminary action mechanism, Curr. Pharmaceut. Des. 19 (2013) 3884–3930.
[12] (a) B. Li, R. Pai, M. Di, D. Aiello, M.H. Barnes, M.M. Butler, T.F. Tashjian, N.P. Peet, T.L. Bowlin, D.T. Moir, Coumarin-based inhibitors of bacillus anthracis and staphylococcus aureus replicative DNA helicase: chemical optimization, biological evaluation, and antibacterial activities, J. Med. Chem. 55 (2012) 10896–10908;
(b) E. Jameel, T. Umar, J. Kumar, N. Hoda, Coumarin: a privileged scaffold for the design and development of antineurodegenerative agents, Chem. Biol. Drug Des. 87 (2016) 21–38.
[13] H.B. Liu, L. Gopala, S.R. Avula, P. Jeyakkumar, X.M. Peng, C.H. Zhou, R.X. Geng, Chalcone-benzotriazole conjugates as new potential antimicrobial agents: design, synthesis, biological evaluation and synergism with clinical drugs, Chin. J. Chem. 35 (2017) 483–496.
[14] B.T. Yin, C.Y. Yan, X.M. Peng, S.L. Zhang, S. Rasheed, R.X. Geng, C.H. Zhou, Synthesis and biological evaluation of a-triazolyl chalcones as a new type of potential antimicrobial agents and their interaction with calf thymus DNA and human serum albumin, Eur. J. Med. Chem. 71 (2014) 148–159.
[15] N. Tadigoppula, V. Korthikunta, S. Gupta, P. Kancharla, T. Khaliq, A. Soni, R.K. Srivastava, K. Srivastava, S.K. Puri, K.S.R. Raju, Wahajuddin, P.S. Sijwali, V. Kumar, I.S. Mohammad, Synthesis and insight into the structure-activity relationships of chalcones as antimalarial agents, J. Med. Chem. 56 (2013) 31–45.
[16] V. Tomar, G. Bhattacharjee, Kamaluddin, A. Kumar, Synthesis and antimicrobial evaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety, Bioorg. Med. Chem. Lett 17 (2007) 5321–5324.
[17] N. Selvakumar, G.S. Kumar, A.M. Azhagan, G.G. Rajulu, S. Sharma, M.S. Kumar, J. Das, J. Iqbal, S. Trehan, Synthesis, SAR and antibacterial studies of novel chalcone oxazolidinone hybrids, Eur. J. Med. Chem. 42 (2007) 538–543.
[18] H.B. Liu, W.W. Gao, V.K.R. Tangadanchu, C.H. Zhou, R.X. Geng, Novel aminopyrimidinyl benzimidazoles as potentially antimicrobial agents: design, synthesis and biological evaluation, Eur. J. Med. Chem. 143 (2018) 66–84.
[19] (a) R.D. Taylor, M. MacCoss, A.D.G. Lawson, Rings in drugs, J. Med. Chem. 57 (2014) 5845–5859;
(b) L. Zhang, X.M. Peng, G.L.V. Damu, R.X. Geng, C.H. Zhou, Comprehensive review in current developments of imidazole-based medicinal chemistry, Med. Res. Rev. 34 (2014) 340–437.
[20] Y.Y. Hu, J. Wang, T.J. Li, R.R.Y. Bheemanaboina, M.F. Ansari, Y. Cheng, C.H. Zhou, An unexpected discovery toward novel membrane active sulfonyl thiazoles as potential MRSA DNA intercalators, Future Med. Chem. 12 (2020) 1709–1727.
[21] (a) P. Sharma, T.S. Reddy, D. Thummuri, K.R. Senwar, N.P. Kumar, V.G.M. Naidu, S.K. Bhargava, N. Shankaraiah, Synthesis and biological evaluation of new benzimidazole-thiazolidinedione hybrids as potential cytotoxic and apoptosis inducing agents, Eur. J. Med. Chem. 124 (2016) 608–621;
(b) Y. Cheng, S.R. Avula, W.W. Gao, D. Addla, V.K.R. Tangadanchu, L. Zhang, J.M. Lin, C.H. Zhou, Multi-targeting exploration of new 2-aminothiazolyl quinolones: synthesis, antimicrobial evaluation, interaction with DNA, combination with topoisomerase IV and penetrability into cells, Eur. J. Med. Chem. 124 (2016) 935–945.
[22] J.P. Chen, N. Battini, M.F. Ansari, C.H. Zhou, Membrane active 7-thiazoxime

quinolones as novel DNA binding agents to decrease the genes expression and exert potent anti-methicillin-resistant *Staphylococcus aureus* activity, Eur. J. Med. Chem. 217 (2021) 113340.
[23] Y.Y. Hu, R.R.Y. Bheemanaboina, N. Battini, C.H. Zhou, Sulfonamide-derived four-component molecular hybrids as novel DNA-targeting membrane active potentiators against clinical *Escherichia coli*, Mol. Pharm. 16 (2019) 1036–1052.
[24] Y. Zhang, V.K.R. Tangadanchu, Y. Cheng, R.G. Yang, J.M. Lin, C.H. Zhou, Potential antimicrobial isopropanol-conjugated carbazole azoles as dual targeting inhibitors of *Enterococcus faecalis*, ACS Med. Chem. Lett. 9 (2018) 244–249.
[25] Y.Y. Hu, G.X. Pan, Z.X. Yang, T.J. Li, J. Wang, M.F. Ansari, C.F. Hu, R.R.Y. Bheemanaboina, Y. Cheng, C.H. Zhou, J.H. Zhang, Novel Schiff base-bridged multi-component sulfonamide imidazole hybrids as potentially highly selective DNA-targeting membrane active repressors against methicillin-resistant *Staphylococcus aureus*, Bioorg. Chem. 107 (2021) 104575.
[26] (a) N.A. Turner, B.K. Sharma-Kuinkel, S.A. Maskarinec, E.M. Eichenberger, P.P. Shah, M. Carugati, T.L. Holland, V.G. Fowler Jr., Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research, Nat. Rev. Microbiol. 17 (2019) 203–218;
(b) G.B. Zhang, S.K. Maddili, V.K.R. Tangadanchu, L. Gopala, W.W. Gao, G.X. Cai, C.H. Zhou, Discovery of natural berberine-derived nitroimidazoles as potentially multi-targeting agents against drug-resistant *Escherichia coli*, Sci. China Chem. 61 (2018) 557–568.
[27] W. Kim, W.P. Zhu, G.L. Hendricks, D.V. Tyne, A.D. Steele, C.E. Keohane, N. Fricke, A.L. Conery, S. Shen, W. Pan, K. Lee, R. Rajamuthiah, B.B. Fuchs, P.M. Vlahovska, W.M. Wuest, M.S. Gilmore, H.J. Gao, F.M. Ausubel, E. Mylonakis, A new class of synthetic retinoid antibiotics effective against bacterial persisters, Nature 556 (2018) 103–107.
[28] Z.Z. Li, V.K.R. Tangadanchu, N. Battini, R.R.Y. Bheemanaboina, Z.L. Zang, S.L. Zhang, C.H. Zhou, Indole-nitroimidazole conjugates as efficient manipulators to decrease the genes expression of methicillin-resistant *Staphylococcus aureus*, Eur. J. Med. Chem. 179 (2019) 723–735.
[29] Z.Z. Li, S.K. Maddili, V.K.R. Tangadanchu, R.R.Y. Bheemanaboina, J.M. Lin, R.G. Yang, G.X. Cai, C.H. Zhou, Researches and applications of nitroimidazole heterocycles in medicinal chemistry, Sci. Sin. Chim. 49 (2019) 230–255.
[30] W.W. Gao, S. Rasheed, V.K.R. Tangadanchu, Y. Sun, X.M. Peng, Y. Cheng, F.X. Zhang, J.M. Lin, C.H. Zhou, Design, synthesis and biological evaluation of amino organophosphorus imidazoles as a new type of potential antimicrobial agents, Sci. China Chem. 60 (2017) 769–785.
[31] Y. Zhang, V.K.R. Tangadanchu, R.R.Y. Bheemanaboina, Y. Cheng, C.H. Zhou, Novel carbazole-triazole conjugates as DNA-targeting membrane active potentiators against clinical isolated fungi, Eur. J. Med. Chem. 155 (2018) 579–589.
[32] P. Andriollo, C.K. Hind, P. Picconi, K.S. Nahar, S. Jamshidi, A. Varsha, M. Clifford, J.M. Sutton, K.M. Rahman, C8-linked pyrrolobenzodiazepine monomers with inverted building blocks show selective activity against multidrug resistant Gram-positive bacteria, ACS Infect. Dis. 4 (2018) 158–174.
[33] S.F. Cui, D. Addla, C.H. Zhou, Novel 3-aminothiazolquinolones: design, synthesis, bioactive evaluation, SARs, and preliminary antibacterial mechanism, J. Med. Chem. 59 (2016) 4488–4510.
[34] L.L. Wang, N. Battini, R.R.Y. Bheemanaboina, S.L. Zhang, C.H. Zhou, Design and synthesis of aminothiazolyl norfloxacin analogues as potential antimicrobial agents and their biological evaluation, Eur. J. Med. Chem. 167 (2019) 105–123.
[35] D. Li, R.R.Y. Bheemanaboina, N. Battini, V.K.R. Tangadanchu, X.F. Fang, C.H. Zhou, Novel organophosphorus aminopyrimidines as unique structural DNA-targeting membrane active inhibitors towards drug-resistant methicillin-resistant *Staphylococcus aureus*, Med. Chem. Commun. 9 (2018) 1529–1537.
[36] Y.N. Wang, R.R.Y. Bheemanaboina, W.W. Gao, J. Kang, G.X. Cai, C.H. Zhou, Discovery of benzimidazole-quinolone hybrids as new cleaving agents toward drug-resistant *Pseudomonas aeruginosa* DNA, ChemMedChem 13 (2018) 1004–1017.
[37] (a) L.L. Ling, T. Schneider, A.J. Peoples, A.L. Spoering, I. Engels, B.P. Conlon, A. Mueller, T.F. Schäberle, D.E. Hughes, S. Epstein, M. Jones, L. Lazarides, V.A. Steadman, D.R. Cohen, C.R. Felix, K.A. Fetterman, W.P. Millett, A.G. Nitti, A.M. Zullo, C. Chen, K. Lewis, Erratum: a new antibiotic kills pathogens without detectable resistance, Nature 517 (2015) 455–459;
(b) Y.F. Sui, D. Li, J. Wang, R.R.Y. Bheemanaboina, M.F. Ansari, L.L. Gan, C.H. Zhou, Design and biological evaluation of a novel type of potential multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles, Bioorg. Med. Chem. Lett 30 (2020) 126982.
[38] J. Kang, L. Gopala, V.K.R. Tangadanchu, W.W. Gao, C.H. Zhou, Novel naphthalimide nitroimidazoles as multitargeting antibacterial agents against resistant *Acinetobacter baumannii*, Future Med. Chem. 10 (2018) 711–724.
[39] Y.Y. Chen, L. Gopala, R.R.Y. Bheemanaboina, H.B. Liu, Y. Cheng, R.X. Geng, C.H. Zhou, Novel naphthalimide aminothiazoles as potential multitargeting antimicrobial agents, ACS Med. Chem. Lett. 8 (2017) 1331–1335.
[40] H.Z. Zhang, S.C. He, Y.J. Peng, H.J. Zhang, L. Gopala, V.K.R. Tangadanchu, L.L. Gan, C.H. Zhou, Design, synthesis and antimicrobial evaluation of novelbenzimidazole-incorporated sulfonamide analogues, Eur. J. Med. Chem. 136 (2017) 165–183.
[41] H. Sun, M.F. Ansari, N. Battini, R.R.Y. Bheemanaboina, C.H. Zhou, Novel potential artificial MRSA DNA intercalators: synthesis and biological evaluation of berberinederived thiazolidinediones, Org. Chem. Front. 6 (2019) 319–334.
[42] P. Jeyakkumar, H.B. Liu, L. Gopala, Y. Cheng, X.M. Peng, R.X. Geng, C.H. Zhou, Novel benzimidazolyl tetrahydroprotoberberines: design, synthesis, antimicrobial evaluation and multi-targeting exploration, Bioorg. Med. Chem. Lett 27 (2017) 1737–1743.
[43] W.W. Gao, L. Gopala, R.R.Y. Bheemanaboina, G.B. Zhang, S. Li, C.H. Zhou, Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*, Eur. J. Med. Chem. 146 (2018) 15–37.
[44] L.L. Wang, N. Battini, R.R.Y. Bheemanaboina, M.F. Ansari, J.P. Chen, Y.P. Xie, G.X. Cai, S.L. Zhang, C.H. Zhou, A new exploration towards aminothiazolquinolone oximes as potentially multi-targeting antibacterial agents: design, synthesis and evaluation acting on microbes, DNA, HSA and topoisomerase IV, Eur. J. Med. Chem. 179 (2019) 166–181.
[45] Y. Shimazaki, J. Tanaka, Y. Kohara, M. Kamahori, T. Sakamoto, Parallel evaluation of melting temperatures of DNAs in the arrayed droplets through the fluorescence from DNA intercalators, Anal. Chem. 89 (2017) 6305–6308.
[46] H.H. Gong, K. Baathulaa, J.S. Lv, G.X. Cai, C.H. Zhou, Synthesis and biological evaluation of Schiff base-linked imidazolyl naphthalimides as novel potential anti-MRSA agents, Med. Chem. Commun. 7 (2016) 924–931.
[47] X.Y. Liang, N. Battini, Y.F. Sui, M.F. Ansari, L.L. Gan, C. H Zhou, Aloe-emodin derived azoles as a new structural type of potential antibacterial agents: design, synthesis, and evaluation of the action on membrane, DNA, and MRSA DNA isomerase, RSC Med. Chem. (2021), https://doi.org/10.1039/d0md00429d.
[48] A. Rouf, C. Tanyeli, Bioactive thiazole and benzothiazole derivatives, Eur. J. Med. Chem. 97 (2015) 911–927.