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Novel coumarin-thiazolyl ester derivatives as potential DNA gyrase Inhibitors: Design, synthesis, and antibacterial activity

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ABSTRACT

The design and synthesis of novel coumarin-thiazolyl ester derivatives of potent DNA gyrase inhibitory activity were the main aims of this study. All the novel synthesized compounds were examined for their antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella*. Compound **8p** exhibited excellent antibacterial activity against four bacteria strains with MIC values of 0.05, 0.05, 8, and 0.05 μg/mL, respectively. *In vitro* drug-resistant bacterial inhibition experiments indicated that compound **8p** exhibited the best bacteriostatic effect in the selected compounds and four positive control drugs with MIC values of 4 μg/mL. *In vitro* enzyme inhibitory assay showed that compound **8p** exhibited potent inhibition against DNA gyrase with IC_{50} values of 0.13 μM. The molecular docking model indicated that compounds **8p** can bind well to the DNA gyrase by interacting with amino acid residues. This study demonstrated that the compound **8p** can act as the most potent DNA gyrase inhibitor in the reported series of compounds and provide valuable information for the commercial DNA gyrase inhibiting bactericides.

1. Introduction

The spread of antibiotic-resistant bacteria poses a substantial threat to morbidity and mortality worldwide [1]. Deaths caused by microbial infections such as bacteria and fungi account for about one-fifth of the world's total deaths [2]. Regarding antibiotic-resistant bacteria, in the past two decades, the US Food and Drug Administration and the European Medicines Agency have disclosed that only two new antibiotic classes (lipopeptides and oxazolidinones) have been developed and approved [3,4]. As the basic nuclear enzyme of cells, DNA topoisomerases (DNA Topo) are involved in life processes such as cell replication, transcription, and mitosis [5]. The bacterial topoisomerases II (DNA gyrase) is a validated target for the development of novel antibacterial agents [6,7]. Since its discovery in 1962, the quinolones, one of the DNA gyrase inhibitors [8,9], gradually became helpless for many drug-resistant bacteria after extensive use and abuse [3]. In order to solve this problem, an effective method is to retain the existing antibiotic core scaffold and continuously modify the new compounds without reducing its antibacterial activity [10]. Based on our previous work [8], we had found that coumarin could be modified as a core scaffold to give compounds excellent antibacterial activity.

Azole compounds play an obvious and important role in combating bacterial infections. Among them, thiazole, by virtue of its unique structural features, is more conducive to the interaction of DNA, enzymes, and receptors in various biological cations, anions, small molecules and biological systems with respect to other azoles [11,12]. Accordingly, thiazole fragment has been widely used in the design of medicinal molecules [13]. Especially with the success of a series of thiazole compounds for clinical and agricultural production [14–17], such as the third *cephalosporins*, *tigemonam*, *aztreonam*, *sulfathiazole* and so on, had been widely used to deal with various diseases. These drugs indicated that thiazole derivatives had a large space for being developed as agents with excellent antibacterial activity, low toxicity, and fewer side effects [11,18].

Human use of antibacterial agents continues to cause pollution and residual toxicity in the natural environment [19,20]. In previous work [21], we found that when an ester group is introduced into a compound, it enhances the antibacterial activity of the compound and allows it to bind more tightly to the target enzyme [22]. In this work, we chose coumarin as a primer scaffold, introduced and bound the thiazolyl to coumarin with an ester group to generate target scaffold, there are 23 novel coumarin-thiazolyl ester derivatives targeting DNA gyrase designed and synthesized. All compounds were used to examined antibacterial activity against four bacteria strains. Moreover, molecular docking analysis and Lipinski's parameters [23] were performed and measured on all derivatives to fully discussed the structure-activity relationships.

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Table 1
MIC Values of Compounds 8a-8x against *S. aureus*, *L. monocytogenes*, *E. coli* and *Salmonella*.

Compd.	R^1	R^2	R^3	MIC (μg/mL)			
				S. a[a]	*L.m*[a]	*E.c*[a]	*S. e*[a]
8a	H	H	Phenyl	4	64	64	0.05
8b	H	H	*Para*-chlorophenyl	4	> 64	64	0.05
8c	H	H	Isobutyl	4	> 64	64	0.05
8d	H	H	Naphthyl	4	> 64	32	4
8e	H	H	*Meta*-chlorophenyl	4	4	8	0.05
8f	H	H	*Ortho*-chlorophenyl	0.05	32	8	4
8g	Me	H	*Meta*-chlorophenyl	0.05	0.05	4	4
8h	Br	H	*Para*-methylphenyl	4	> 64	4	> 64
8i	Br	H	Phenyl	0.05	0.05	4	0.05
8j	Br	H	*Para*-chlorophenyl	2	> 64	4	4
8k	Br	H	Isobutyl	4	0.05	4	0.05
8l	Br	H	Naphthyl	2	4	4	0.05
8m	Br	H	*Meta*-chlorophenyl	4	> 64	0.05	4
8n	NO_2	H	*Meta*-chlorophenyl	8	32	4	64
8o	Cl	H	Phenyl	4	32	4	32
8p	Cl	H	*Para*-chlorophenyl	0.05	0.05	8	0.05
8q	Cl	H	Naphthyl	0.05	> 64	4	> 64
8r	Cl	H	*Meta*-chlorophenyl	4	> 64	16	> 64
8s	H	$N(Et_2)$	*Para*-methylphenyl	4	64	8	> 64
8t	H	$N(Et_2)$	Phenyl	4	64	4	> 64
8u	H	$N(Et_2)$	Naphthyl	4	> 64	4	> 64
8v	H	$N(Et_2)$	*Meta*-chlorophenyl	2	32	4	> 64
8w	H	$N(Et_2)$	*Ortho*-chlorophenyl	8	64	4	> 64
8x	H	$N(Et_2)$	*Para*-chlorophenyl	4	0.05	4	0.05
CIP[b]	–	–	–	0.125	4	0.5	0.25
NB[c]	–	–	–	2	6.25	4	0.5
GAT[d]	–	–	–	0.5	4	8	4
LVX[e]	–	–	–	4	4	0.125	0.25

[a] Abbreviations: *Staphylococcus aureus* (ATCC-12600); *Listeria monocytogenes* (ATCC-15313); *Escherichia coli* (ATCC-25922); *Salmonella* (ATCC-9184).
[b] Ciprofloxacin.
[c] Novobiocin.
[d] Gatifloxacin.
[e] Levofloxacin.

2. Results and discussion

2.1. Molecular design

In the beginning, we synthesized seven intermediates **7**. After measuring its MIC values against four bacteria (Table S1), we can see that the antibacterial activity of the intermediates **7** is not ideal (Table S2). So, we introduced the coumarin to get the target scaffold. The data in Table 1 indicated that the introduction of thiazolyl groups with the coumarin core greatly enhances the antibacterial activity of the compounds, which demonstrated the rationality of our design strategy. The compounds were designed based on Lipinski's "Rule of 5″ properties with computer aids [23]. The aim of computational analysis is to filter the compounds considered unsuitable for screening purposes. These parameters were calculated using Molinspiration software (https://www.molinspiration.com/) and the results (Table S3) showed that the compounds we designed and synthesized almost meet the requirements of the above parameters, making them play an important role in drug development.

2.2. Chemistry

The key reaction in the synthesis of this class of compounds was the esterification reaction. The synthetic routes of the **8a–8x** are outlined in Fig. 1. Intermediate **4** was easily prepared by the reaction of 2-hydroxybenzaldehydes **(1)** and Meldrum's Acid **(2)** with a catalytic amount of piperidinyl acetate refluxed in ethanol. This one-pot procedure is convenient and straightforward with simple product isolation. No recrystallization is needed in most cases. As shown in Fig. 1, the intermediates **7** were synthesized by stirring the mixture of 4,5-dimethylthiazole and a series of substituted aldehyde in the ultra-dry THF at −78 °C. Then the reaction was warmed to room temperature for 2 h, quenched with water and extracted with ethyl acetate to give the crude product. The crude product was purified by recrystallization (ethyl acetate/hexanes). The target compounds were prepared by mixing intermediate **4** and intermediate **7** with DCC and DMAP at 0 °C. The chemical structures of all synthesized compounds were determined by ^{1}H NMR, ^{13}C NMR, and MS. Compounds **8e** and **8i** have been characterized by single crystal X-ray crystallography. The check-cif files for compounds **8e** and **8i** are listed in Supporting Information, crystal data are shown in Fig. 2 and Table S4. Crystallographic data (excluding structure factors) for the structure were deposited with the Cambridge Crystallographic Data Center (CCDC) as No. CCDC-1908526 (**8e**) and No. CCDC-1908525 (**8i**).

2.3. Antibacterial activity and SAR discussion

The MIC values of the *in vitro* antibacterial activity screening of all compounds are presented in Table 1. Most of the derivatives exhibited moderate to potent antibacterial activities of four bacterial strains. For two gram-positive bacteria, in these derivatives, five compounds (i.e., **8f**, **8g**, **8i**, **8p**, and **8q**) displayed significant antibacterial activity against *S. aureus* compared with four positive control drugs (MIC = 0.125–4 μg/mL), with MIC values equal to 0.05 μg/mL. Five compounds (i.e., **8g**, **8i**, **8k**, **8p**, and **8x**) exhibited excellent antibacterial activity against *L. monocytogenes* compared with four positive control drugs (MIC = 4–8 μg/mL), with MIC values equal to 0.05 μg/mL. For two gram-negative bacteria, compound **8 m** was found to show improved antibacterial activity against *E. coli* compared with four positive control drugs (MIC = 0.125–8 μg/mL), with MIC values equal to 0.05 μg/mL. 9 compounds (i.e., **8a**, **8b**, **8c**, **8e**, **8i**, **8k**, **8l**, **8p** and **8x**) showed better antibacterial activity against *Salmonella* compared with four positive control drugs (MIC = 0.25–4 μg/mL), with MIC values equal to 0.05 μg/mL.

After analyzing biological activity data, we found that the antibacterial activities of the compounds were enhanced when the target compounds had a substituent on the coumarin core. Among them, bromine and chlorine substituents have more significant effect. For *S. aureus*, when the R^1 position of compounds was substituted with bromine or chlorine, its antibacterial activities were increased. When the R^2 position was substituted with diethylamino, only compound **8v** showed good antibacterial activity. For *L. monocytogenes*, compound **8v** exhibited excellent antibacterial activity, indicating that when both R^1 and R^3 were substituted by chlorine moiety, the enhancement of antibacterial activity is beneficial. However, when R^2 position was substituted with diethylamino, the antibacterial activity of the compound is poor, the same as when both R^1 and R^2 position were hydrogen. For *E. coli*, the overall antibacterial activities of compounds are not ideal except **8x**. For *Salmonella*, when R^1 position was substituted with bromine or chlorine, compounds **8m** and **8p** showed potent antibacterial activity compared with novobiocin and gatifloxacin. In summary, the results demonstrated that the improved antifungal activities of these compounds may be attributed to the substitution at the coumarin core and replacement of the phenyl in thiazolyl (Fig. 3).

2.4. Antibacterial effect of selected compounds on drug-resistant bacteria

In order to further verify whether these compounds have inhibitory effects on drug-resistant bacteria, we selected five compounds (**8i**, **8l**, **8p**, **8q**, and **8v**) to determine the minimum inhibitory concentration values for *MRSA* and *VRSA*. In terms of two drug-resistant bacteria strains, the selected compounds and the positive control drugs have a better antibacterial effect on MRSA. As can be seen from the data in

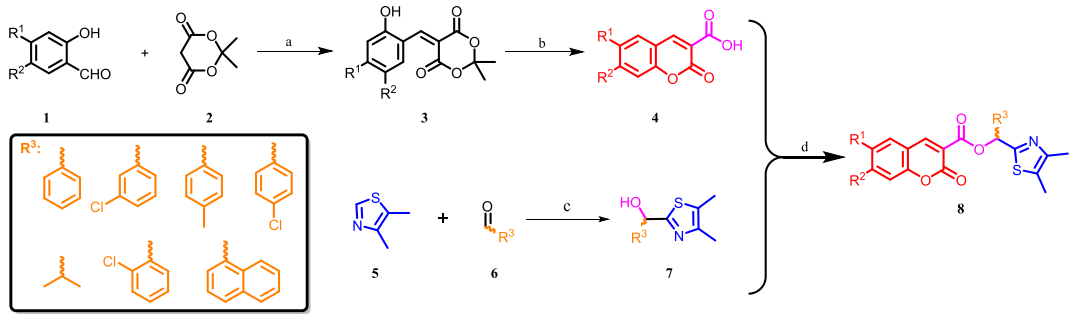

Fig. 1. Synthetic routes for intermediate **4, 7,** and target compounds. Reagents and conditions: (a) Piperidine, acetic acid, EtOH, 25 °C; (b) reflux, 2 h; (c) *n*-BuLi, THF (dry), −78 °C; (d) DCC, DMAP, DCM, 0 °C,10–15 h.

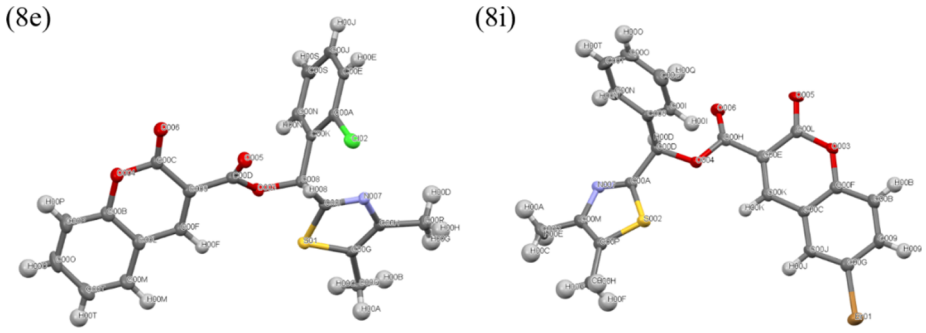

Fig. 2. The crystal structures of compound **8e** and **8i**.

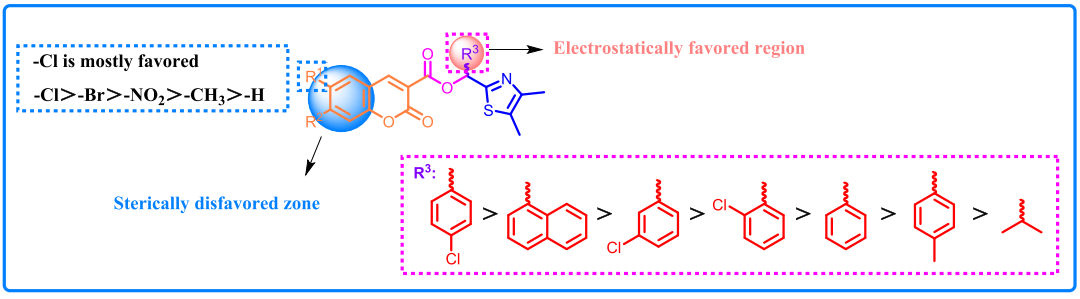

Fig. 3. Summarized SARs of target compounds.

Table 2, compound **8p** exhibited the best bacteriostatic effect in the selected compounds and four positive control drugs. Based on the above bioactivity experimental data, compound **8p** can act as the most potent DNA gyrase inhibitor in the reported series of compounds.

Table 2
MIC Values of selected compounds against MRSA and VRSA.

Compd.	MIC (μg/mL)	
	MRSA [a]	VRSA[b]
8i	8	> 64
8l	16	16
8p	4	4
8q	4	8
8v	8	32
CIP	4	16
NB	16	32
GAT	8	32
LVX	32	16

[a] *Methicillin-resistant S. aureus* (MRAS, N315).
[b] *Vancomycin-resistant S. aureus* (VRSA, Mu50).

2.5. Enzyme inhibitory activity

From the results of the *in vitro* biological assay, compound **8p** was identified as the most promising candidate for further study, and it's *in vitro* enzyme inhibitory activity against DNA gyrase isolated from *E. coli* was determined. As shown in Table 3, the inhibitory effect of compound **8p** on DNA gyrase is better than that of four control drugs, with an IC_{50} value of 0.13 μM. These results further indicated that the practical potential of this novel compound can put a great deal of pressure on the survival of the bacteria.

2.6. Molecular docking analysis

In order to gain a deep understanding of the molecular docking relationship between active and inactive compounds, the docking details of compound **8p** and **8a** with DNA gyrase are shown in Fig. 4. For compound **8p**, as shown in Fig. 3(A), Arg1122(D) established cation-π interaction with thiazolyl. In the coumarin core, Arg1122(B) formed a hydrogen bond (angle = 180°, distance = 2.29 Å) with chlorine substituent, and Dc11(E) formed a hydrogen bond (angle = 180°, distance = 2.33 Å) with a carbonyl group. Another hydrogen bond (angle = 180°,

Table 3
Inhibitory effects of selected compounds against DNA gyrase.

Compd.	IC_{50} (μM) DNA gyrase
8p	0.13
CIP	0.75
NB	0.82
GAT	10.66
LVX	22.14

distance = 2.44 Å) was formed between Ser1084(B) and chlorine substituent which attached to the benzene ring. For compound **8a**, the benzene ring established four π-π interactions with Dc11(E), Dc11(F), Dg10(E) and Dg10(F) respectively. Besides, it does not interact with other amino acid residues, which may account for its inactive in bioactivity. Moreover, we have found that the introduction of halogen substituents can form hydrogen bonds between compound and amino acid residues.

3. Conclusions

All the new coumarin-thiazolyl ester derivatives were synthesized and evaluated for their antibacterial activity against four bacteria strains. The experimental results indicated that most of the synthesized compounds showed moderate to potent antibacterial activities. Most surprisingly, compound **8p** exhibited excellent antibacterial activity against four bacteria strains with MIC values of 0.05, 0.05, 8 and 0.05 μg/mL, respectively. Among these compounds, **8i** and **8v** exhibited the best antibacterial activity against *S. aureus*, **8x** displayed the best antibacterial activity against *L. monocytogenes*. SAR and molecular docking analysis revealed that the promising antibacterial efficacy can be attributed to the substitution of chlorine on the benzene and modification of the coumarin core. Moreover, the rationality of the derivatives we designed was fully confirmed in experiments. The results of the present work showed that coumarin-thiazolyl ester derivatives can be potential bactericides for the discovery of DNA gyrase inhibitors and worth further study.

4. Materials and methods

4.1. General methods

All chemicals and reagents used in this work were purchased from Energy, Meryer. ^{1}H NMR and ^{13}C NMR spectra were collected on Agilent DD2 600 Hz spectrometer with $CDCl_3$ as the solvent, and the chemical shifts (δ) were recorded in parts per million (ppm). ESI-MS spectra were carried out on a Mariner System 5304 mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. Crystal data were collected with a Bruker D8 Venture diffractometer.

4.1.1. General procedure for the synthesis of coumarin-3-carboxylic acids [24]

2-hydroxybenzaldehydes **(1)** (0.02 mol) and Meldrum's Acid **(2)** (0.02 mol) were dissolved in ethanol, then hexahydropyridine and acetic acid were added as catalyst refluxed in ethanol. The crude product then crystallizes in the solvent. After filtration and washing with ethanol, intermediate **4** was obtained in excellent yield and purity.

4.1.2. General procedure for the synthesis of thiazole derivatives [25]

A flame-dried round bottom flask under N_2 was charged with 4,5-dimethylthiazole **(5)** (1 mL, 9.45 mmol) and THF and the mixture was cooled to −78 °C. Then *n*-Butyl lithium (1.9 M, 6 mL, 11.3 mmol) was added dropwise to the flask. The mixture was stirred for 45 min at −78 °C. The aldehyde **(6)** (28.4 mmol) was then added, and the reaction was stirred for 1 h at −78 °C. After that, the reaction was warmed to room temperature and reacted for 2 h. Finally, water was added to quench the reaction. The reaction mixture was extracted with ethyl acetate and water. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was purified by recrystallization (ethyl acetate/hexanes).

4.1.3. General procedure for the synthesis of coumarin-thiazolyl ester derivatives

Intermediate **4** (1 mmol) and the intermediate **7** (1 mmol) were dissolved in dichloromethane to the glass flask, then DCC and DMAP

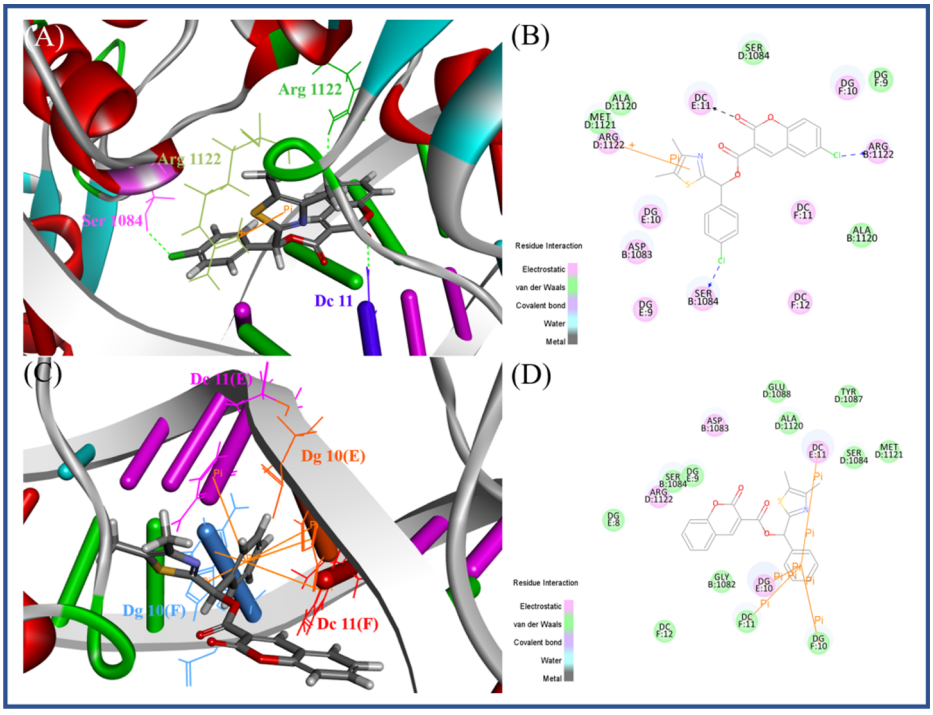

Fig. 4. Binding models of compound **8p(A&B) and 8a(C&D)** with DNA gyrase (3D&2D diagram).

are added as a condensing agent reacted at 0 °C for 10–15 h. After the thin layer chromatography (TLC) monitoring reaction was completed, the mixture was vacuum filtered and concentrated. Finally, the above crude product can be isolated and purified by column chromatography (Hexane/EtOAc = 4:1) to obtain target compounds **8**.

*4.1.4. (4,5-dimethylthiazol-2-yl)(phenyl)methyl 2-oxo-2H-chromene-3-carboxylate(**8a**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.62–7.54 (m, 3H), 7.42 (d, J = 7.4 Hz, 2H), 7.28 (dd, J = 11.3, 5.7 Hz, 4H), 7.23 (t, J = 7.3 Hz, 1H), 2.22 (dd, J = 30.7, 16.9 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170, 162.96, 156.71, 155.03, 148.62, 134.35, 129.54, 128.71, 128.42, 127.98, 127.20, 126.45, 124.86, 118.09, 117.79, 116.67, 116.64, 61.92, 14.20, 11.24. MS (ESI): 392.09($C_{22}H_{17}NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}NO_4S$: C, 67.50; H, 4.38; N, 3.58; O, 16.35; S, 8.19; Found: C, 67.48; H, 4.39; N, 3.57; O, 16.33; S, 8.20.

*4.1.5. (4-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 2-oxo-2H-chromene-3-carboxylate(**8b**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.66–7.58 (m, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.40–7.26 (m, 4H), 7.15 (s, 1H), 2.27 (dd, J = 19.4, 11.3 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.95, 161.71, 155.40, 153.17, 149.54, 136.31, 134.67, 129.66, 128.92, 128.67, 127.90, 127.74, 124.84, 117.78, 117.56, 116.85, 116.05, 75.32, 14.66, 11.22. MS (ESI): 426.05($C_{22}H_{16}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{16}ClNO_4S$: C, 62.05; H, 3.79; Cl, 8.32; N, 3.29; O, 15.03; S, 7.53; Found: C, 62.04; H, 3.80; Cl, 8.31; N, 3.28; O, 15.01; S, 7.51.

*4.1.6. 1-(4,5-dimethylthiazol-2-yl)-2-methylpropyl 2-oxo-2H-chromene-3-carboxylate(**8c**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.61 (dd, J = 15.8, 7.6 Hz, 2H), 7.34–7.28 (m, 2H), 5.95 (d, J = 6.6 Hz, 1H), 2.28 (d, J = 2.8 Hz, 6H), 2.25 (s, 1H), 0.98 (d, J = 6.8 Hz, 3H), 0.93 (dd, J = 20.5, 6.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.26, 163.24, 162.06, 156.22, 155.25, 148.80, 134.43, 129.59, 126.48, 125.82, 124.77, 117.80, 116.73, 79.04, 33.33, 18.64, 14.63, 11.16. MS (ESI): 358.10($C_{19}H_{19}NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}NO_4S$: C, 63.85; H, 5.36; N, 3.92; O, 17.90; S, 8.97; Found: C, 63.83; H, 5.37; N, 3.91; O, 17.91; S, 8.99.

*4.1.7. (4,5-dimethylthiazol-2-yl)(naphthalen-1-yl)methyl 2-oxo-2H-chromene-3-carboxylate(**8d**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.98–7.90 (m, 2H), 7.87 (d, J = 8.1 Hz, 2H), 7.67–7.61 (m, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.56–7.46 (m, 3H), 7.38–7.29 (m, 2H), 2.29 (d, J = 16.4 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.37, 161.73, 156.27, 155.37, 149.48, 148.50, 134.53, 133.83, 133.57, 130.48, 129.64, 129.58, 128.77, 128.15, 126.67, 125.89, 125.39, 125.37, 124.76, 123.72, 117.83, 116.79, 73.43, 14.70, 11.25. MS (ESI): 442.10($C_{26}H_{19}NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{19}NO_4S$: C, 70.73; H, 4.34; N, 3.17; O, 14.50; S, 7.26; Found: C, 70.72; H, 4.32; N, 3.18; O, 14.51; S, 7.25.

*4.1.8. (3-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 2-oxo-2H-chromene-3-carboxylate(**8e**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.81 (d, J = 7.7 Hz, 1H), 7.67–7.59 (m, 2H), 7.57 (s, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.38–7.28 (m, 4H), 2.31 (d, J = 4.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.63, 161.52, 156.23, 155.35, 149.46, 148.94, 133.18, 129.94, 129.71, 129.66, 128.78, 127.27, 124.81, 117.80, 117.59, 116.81, 72.29, 14.75, 11.26. MS (ESI): 426.05($C_{22}H_{16}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{16}ClNO_4S$: C, 62.05; H, 3.79; Cl, 8.32; N, 3.29; O, 15.03; S, 7.53; Found: C, 62.03; H, 3.77; Cl, 8.31; N, 3.30; O, 15.05; S, 7.51.

*4.1.9. (2-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 2-oxo-2H-chromene-3-carboxylate(**8f**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.80 (d, J = 6.8 Hz, 1H), 7.66–7.59 (m, 2H), 7.56 (s, 1H), 7.38 (d, J = 7.7 Hz, 1H), 7.31 (dt, J = 23.3, 7.8 Hz, 4H), 2.30 (d, J = 4.5 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.62, 161.50, 156.23, 155.33, 149.47, 135.59, 133.16, 129.94, 129.68, 128.77, 128.13, 127.27, 118.02, 117.78, 117.54, 116.78, 72.27, 14.74, 11.25. MS (ESI): 426.05($C_{22}H_{16}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{16}ClNO_4S$: C, 62.05; H, 3.79; Cl, 8.32; N, 3.29; O, 15.03; S, 7.53; Found: C, 62.03; H, 3.78; Cl, 8.30; N, 3.28; O, 15.04; S, 7.55.

*4.1.10. (3-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 6-methyl-2-oxo-2H-chromene-3-carboxylate(**8g**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.79 (dd, J = 7.7, 1.4 Hz, 1H), 7.55 (s, 1H), 7.43 (dd, J = 8.5, 1.3 Hz, 1H), 7.38 (d, J = 7.7 Hz, 2H), 7.30 (ddt, J = 9.0, 7.5, 4.0 Hz, 2H), 7.22 (d, J = 8.5 Hz, 1H), 2.39 (s, 3H), 2.30 (d, J = 5.0 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.69, 161.59, 156.52, 153.49, 149.54, 148.91, 135.83, 134.71, 129.93, 129.69, 129.29, 128.77, 127.28, 117.53, 117.24, 116.48, 72.21, 20.63, 14.75, 11.27. MS (ESI): 440.06($C_{23}H_{18}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{18}ClNO_4S$: C, 62.80; H, 4.12; Cl, 8.06; N, 3.18; O, 14.55; S, 7.29; Found: C, 62.81; H, 4.13; Cl, 8.04; N, 3.17; O, 14.53; S, 7.28.

*4.1.11. (4,5-dimethylthiazol-2-yl)(p-tolyl)methyl 6-bromo-2-oxo-2H-chromene-3-carboxylate(**8h**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.75 (d, J = 2.2 Hz, 1H), 7.72 (dd, J = 8.8, 2.3 Hz, 1H), 7.47 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.9 Hz, 1H), 7.21–7.15 (m, 3H), 2.34 (s, 3H), 2.30 (d, J = 10.5 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.53, 161.18, 155.50, 154.12, 148.57, 147.69, 138.80, 137.14, 134.73, 131.65, 129.43, 127.26, 126.49, 119.26, 118.54, 117.33, 76.12, 21.19, 14.68, 11.22. MS (ESI): 484.01($C_{23}H_{18}BrNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{18}BrNO_4S$: C, 57.03; H, 3.75; Br, 16.50; N, 2.89; O, 13.21; S, 6.62; Found: C, 57.01; H, 3.76; Br, 16.51; N, 2.88; O, 13.20; S, 6.63.

*4.1.12. (4,5-dimethylthiazol-2-yl)(phenyl)methyl 6-bromo-2-oxo-2H-chromene-3-carboxylate(**8i**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.79–7.67 (m, 2H), 7.58 (d, J = 7.3 Hz, 2H), 7.35 (dt, J = 28.0, 7.0 Hz, 3H), 7.24–7.17 (m, 2H), 2.29 (d, J = 8.7 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.28, 161.27, 155.50, 154.09, 148.57, 147.85, 137.62, 137.19, 131.68, 128.82, 128.73, 127.22, 119.23, 118.75, 118.52, 117.34, 76.14, 14.68, 11.23. MS (ESI): 470.00($C_{22}H_{16}BrNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{16}BrNO_4S$: C, 56.18; H, 3.43; Br, 16.99; N, 2.98; O, 13.61; S, 6.82; Found: C, 56.17; H, 3.42; Br, 16.97; N, 2.96; O, 13.60; S, 6.83.

*4.1.13. (4-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 6-bromo-2-oxo-2H-chromene-3-carboxylate(**8j**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.77–7.71 (m, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 7.27 (s, 1H), 7.25 (s, 1H), 7.16 (s, 1H), 2.31 (d, J = 13.6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.69, 161.27, 155.49, 154.14, 148.75, 148.04, 137.32, 136.12, 134.82, 131.69, 128.97, 128.67, 119.18, 118.64, 118.56, 117.42, 75.50, 14.68, 11.24. MS (ESI): 503.96($C_{22}H_{15}BrClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{15}BrClNO_4S$: C, 52.35; H, 3.00; Br, 15.83; Cl, 7.02; N, 2.77; O, 12.68; S, 6.35; Found: C, 52.36; H, 3.02; Br, 15.84; Cl, 7.03; N, 2.78; O, 12.67; S, 6.33.

*4.1.14. 1-(4,5-dimethylthiazol-2-yl)-2-methylpropyl 6-bromo-2-oxo-2H-chromene-3-carboxylate(**8k**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.72 (dd, J = 8.8, 2.2 Hz, 1H), 7.24 (s, 1H), 5.96 (d, J = 6.6 Hz, 1H), 2.44 (dq, J = 13.5, 6.7 Hz, 1H), 2.31 (d, J = 4.1 Hz, 6H), 1.09 (d, J = 6.7 Hz, 3H), 1.00 (d, J = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.97, 161.68, 155.56, 154.04, 148.14, 147.29, 137.07, 131.61,

126.57, 119.26, 118.53, 117.32, 79.33, 33.35, 18.66, 14.70, 11.23. MS (ESI): 436.01($C_{19}H_{18}BrNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{18}BrNO_4S$: C, 52.30; H, 4.16; Br, 18.31; N, 3.21; O, 14.67; S, 7.35; Found: C, 52.31; H, 4.15; Br, 18.30; N, 3.22; O, 14.65; S, 7.34.

*4.1.15. (4,5-dimethylthiazol-2-yl)(naphthalen-1-yl)methyl 6-bromo-2-oxo-2H-chromene-3-carboxylate(**8l**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.23 (d, J = 8.5 Hz, 1H), 7.94 (s, 1H), 7.89 (dd, J = 17.6, 7.6 Hz, 3H), 7.73 (dd, J = 4.8, 2.0 Hz, 1H), 7.71 (d, J = 4.2 Hz, 1H), 7.69–7.65 (m, 1H), 7.57–7.47 (m, 3H), 2.29 (d, J = 17.3 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.13, 161.30, 155.58, 154.11, 148.59, 147.95, 137.17, 133.82, 133.38, 131.66, 130.90, 130.43, 129.68, 128.81, 128.25, 126.73, 125.94, 125.36, 123.64, 119.25, 118.83, 118.52, 117.34, 73.64, 14.72, 11.27. MS (ESI): 520.01($C_{26}H_{18}BrNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{18}BrNO_4S$: C, 60.01; H, 3.49; Br, 15.35; N, 2.69; O, 12.30; S, 6.16; Found: C, 60.02; H, 3.47; Br, 15.36; N, 2.68; O, 12.31; S, 6.15.

*4.1.16. (3-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 6-bromo-2-oxo-2H-chromene-3-carboxylate(**8m**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.79–7.69 (m, 3H), 7.55 (s, 1H), 7.40 (dd, J = 7.6, 1.0 Hz, 1H), 7.36–7.28 (m, 2H), 7.24 (d, J = 8.8 Hz, 1H), 2.31 (d, J = 5.3 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.39, 161.09, 155.54, 154.09, 149.01, 147.94, 137.21, 131.68, 130.03, 129.74, 128.74, 127.29, 119.22, 118.68, 118.53, 117.37, 72.47, 14.76, 11.28. MS (ESI): 503.96($C_{22}H_{15}BrClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{15}BrClNO_4S$: C, 52.35; H, 3.00; Br, 15.83; Cl, 7.02; N, 2.77; O, 12.68; S, 6.35; Found: C, 52.36; H, 3.02; Br, 15.82; Cl, 7.01; N, 2.75; O, 12.67; S, 6.37.

*4.1.17. (3-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 6-nitro-2-oxo-2H-chromene-3-carboxylate(**8n**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.56 (s, 1H), 8.71 (d, J = 2.7 Hz, 1H), 8.34 (dd, J = 9.2, 2.8 Hz, 1H), 7.63–7.60 (m, 1H), 7.47 (dd, J = 5.2, 3.9 Hz, 1H), 7.37–7.34 (m, 2H), 7.22 (d, J = 9.2 Hz, 1H), 7.07 (s, 1H), 2.34 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.26, 163.13, 161.17, 149.21, 142.25, 133.10, 130.63, 130.47, 130.13, 127.90, 127.77, 124.67, 114.98, 77.15, 14.75, 11.38. MS (ESI): 471.03($C_{22}H_{15}ClN_2O_6S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{15}ClN_2O_6S$: C, 56.12; H, 3.21; Cl, 7.53; N, 5.95; O, 20.39; S, 6.81; Found: C, 56.10; H, 3.20; Cl, 7.52; N, 5.93; O, 20.38; S, 6.82.

*4.1.18. (4,5-dimethylthiazol-2-yl)(phenyl)methyl 6-chloro-2-oxo-2H-chromene-3-carboxylate(**8o**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.59 (dd, J = 9.7, 4.3 Hz, 4H), 7.38 (t, J = 7.4 Hz, 2H), 7.36–7.28 (m, 2H), 7.20 (s, 1H), 2.30 (d, J = 8.8 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.29, 161.21, 155.64, 153.63, 148.58, 148.03, 137.59, 134.45, 130.19, 128.86, 128.77, 128.61, 127.23, 118.69, 118.30, 76.15, 14.72, 11.28. MS (ESI): 426.05($C_{22}H_{16}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{16}ClNO_4S$: C, 62.05; H, 3.79; Cl, 8.32; N, 3.29; O, 15.03; S, 7.53; Found: C, 62.04; H, 3.78; Cl, 8.31; N, 3.28; O, 15.04; S, 7.55.

*4.1.19. (4-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 6-chloro-2-oxo-2H-chromene-3-carboxylate(**8p**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.62–7.58 (m, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.34 (dd, J = 20.7, 8.5 Hz, 3H), 7.16 (s, 1H), 2.31 (d, J = 14.0 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.71, 161.30, 155.53, 153.68, 148.72, 148.11, 136.13, 134.81, 134.50, 130.24, 128.96, 128.67, 124.33, 123.42, 118.71, 118.68, 118.29, 75.49, 14.66, 11.23. MS (ESI): 460.01($C_{22}H_{15}Cl_2NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{15}Cl_2NO_4S$: C, 57.40; H, 3.28; Cl, 15.40; N, 3.04; O, 13.90; S, 6.96; Found: C, 57.41; H, 3.29; Cl, 15.41; N, 3.03; O, 13.91; S, 6.97.

*4.1.20. (4,5-dimethylthiazol-2-yl)(naphthalen-1-yl)methyl 6-chloro-2-oxo-2H-chromene-3-carboxylate(**8q**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.24 (d, J = 8.5 Hz, 1H), 8.12 (s, 1H), 7.94 (s, 1H), 7.91 (d, J = 7.0 Hz, 1H), 7.88 (d, J = 7.9 Hz, 3H), 7.72 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.9 Hz, 2H), 2.30 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.06, 162.66, 158.44, 153.48, 153.09, 152.11, 146.95, 139.44, 134.06, 132.71, 130.45, 128.38, 127.85, 119.08, 118.24, 118.18, 108.68, 62.17, 14.15, 14.05. MS (ESI): 476.06($C_{26}H_{18}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{18}ClNO_4S$: C, 65.61; H, 3.81; Cl, 7.45; N, 2.94; O, 13.45; S, 6.74; Found: C, 65.60; H, 3.80; Cl, 7.46; N, 2.96; O, 13.43; S, 6.75.

*4.1.21. (3-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 6-chloro-2-oxo-2H-chromene-3-carboxylate(**8r**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.72 (s, 1H), 7.58 (d, J = 7.9 Hz, 2H), 7.55–7.50 (m, 2H), 7.32 (s, 1H), 7.30 (s, 1H), 1.94 (d, J = 11.5 Hz, 3H), 1.83 (d, J = 12.7 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.30, 162.65, 158.45, 156.12, 154.48, 153.09, 152.10, 146.98, 139.45, 134.07, 132.72, 130.45, 128.38, 127.85, 119.07, 118.24, 118.19, 62.18, 14.15, 14.06. MS (ESI): 460.01($C_{22}H_{15}Cl_2NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{15}Cl_2NO_4S$: C, 57.40; H, 3.28; Cl, 15.40; N, 3.04; O, 13.90; S, 6.96; Found: C, 57.41; H, 3.27; Cl, 15.41; N, 3.03; O, 13.92; S, 6.95.

*4.1.22. (4,5-dimethylthiazol-2-yl)(p-tolyl)methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate(**8s**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.66 (s, 1H), 7.35 (d, J = 8.9 Hz, 1H), 7.28 (s, 1H), 6.59 (d, J = 8.8 Hz, 2H), 6.47 (d, J = 5.6 Hz, 2H), 6.42 (s, 1H), 3.51 (s, 4H), 2.41(s, 3H), 2.31–2.30(d, J = 6 Hz, 6H), 1.25–1.22(t, J = 6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.13, 164.23, 160.17, 158.45, 158.19, 156.89, 153.62, 152.83, 151.62, 149.09, 142.03, 130.94, 129.88, 117.96, 109.45, 109.14, 107.69, 107.33, 97.05, 96.76, 61.10, 14.33, 12.40, 12.38. MS (ESI): 477.18($C_{27}H_{28}N_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{28}N_2O_4S$: C, 68.05; H, 5.92; N, 5.88; O, 13.43; S, 6.73; Found: C, 68.04; H, 5.91; N, 5.87; O, 13.41; S, 6.74.

*4.1.23. (4,5-dimethylthiazol-2-yl)(phenyl)methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate(**8t**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.67 (s, 1H), 7.35 (d, J = 12.1 Hz, 1H), 7.29–7.27 (m, 1H), 7.25 (s, 1H), 6.60 (s, 2H), 6.47 (s, 2H), 6.43 (s, 1H), 3.48–3.44(m, 4H), 2.41–2.39(t, J = 6 Hz, 6H), 1.25–1.22(m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.03, 162.10, 156.15, 153.32, 150.75, 147.43, 141.68, 128.59, 128.28, 126.52, 124.60, 123.79, 119.07, 115.79, 108.04, 105.46, 92.07, 73.65, 49.16, 14.53, 14.06, 11.26. MS (ESI): 463.16($C_{26}H_{26}N_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{26}N_2O_4S$: C, 67.51; H, 5.67; N, 6.06; O, 13.84; S, 6.93; Found: C, 67.50; H, 5.66; N, 6.05; O, 13.85; S, 6.94.

*4.1.24. (4,5-dimethylthiazol-2-yl)(naphthalen-1-yl)methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate(**8u**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (d, J = 7.8 Hz, 1H), 8.29 (t, J = 8.1 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.90 (t, J = 7.3 Hz, 1H), 7.86–7.82 (m, 2H), 7.53–7.45 (m, 3H), 7.32 (t, J = 8.4 Hz, 1H), 6.57 (d, J = 14.6 Hz, 1H), 6.43 (d, J = 7.7 Hz, 1H), 3.46–3.39 (m, 4H), 2.27 (t, J = 8.0 Hz, 6H), 1.25–1.19 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.20, 162.28, 158.67, 157.82, 153.09, 149.73, 148.33, 133.81, 131.22, 130.62, 128.64, 126.52, 125.75, 125.45, 125.37, 123.95, 109.50, 108.14, 107.73, 96.73, 72.59, 45.06, 14.69, 12.40, 11.23. MS (ESI): 513.18($C_{30}H_{28}N_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{30}H_{28}N_2O_4S$: C, 70.29; H, 5.51; N, 5.46; O, 12.48; S, 6.25; Found: C, 70.28; H, 5.50; N, 5.48; O, 12.46; S, 6.24.

*4.1.25. (3-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate(**8v**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.58 (s, 1H), 7.50 (d,

J = 7.0 Hz, 1H), 7.36 (d, J = 8.9 Hz, 1H), 7.31–7.28 (m, 2H), 7.15 (s, 1H), 6.60 (d, J = 8.9 Hz, 1H), 6.45 (s, 1H), 3.45 (q, J = 7.1 Hz, 4H), 2.31 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.59, 162.54, 158.74, 157.75, 153.22, 149.77, 148.53, 140.34, 134.46, 131.30, 129.88, 128.61, 128.37, 127.41, 127.33, 125.51, 124.69, 109.59, 107.72, 96.75, 74.41, 45.10, 14.67, 12.41, 11.21. MS (ESI): 497.12($C_{26}H_{25}ClN_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{25}ClN_2O_4S$: C, 62.83; H, 5.07; Cl, 7.13; N, 5.64; O, 12.88; S, 6.45; Found: C, 62.81; H, 5.06; Cl, 7.11; N, 5.65; O, 12.89; S, 6.44.

4.1.26. (2-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate(**8w**)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.81 (d, J = 7.7 Hz, 1H), 7.54 (s, 1H), 7.38 (d, J = 7.9 Hz, 1H), 7.34 (d, J = 9.0 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 6.59 (dd, J = 8.9, 2.1 Hz, 1H), 6.44 (s, 1H), 3.44 (q, J = 7.1 Hz, 4H), 2.30 (d, J = 6.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.51, 162.47, 158.68, 157.80, 153.12, 149.74, 148.72, 136.20, 133.17, 131.23, 129.62, 129.60, 128.88, 128.18, 127.73, 127.16, 109.53, 107.73, 96.74, 71.56, 45.07, 14.73, 12.41, 11.23. MS (ESI): 497.12($C_{26}H_{25}ClN_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{25}ClN_2O_4S$: C, 62.83; H, 5.07; Cl, 7.13; N, 5.64; O, 12.88; S, 6.45; Found: C, 62.84; H, 5.09; Cl, 7.11; N, 5.65; O, 12.90; S, 6.44.

4.1.27. (4-chlorophenyl)(4,5-dimethylthiazol-2-yl)methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate(**8x**)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.33 (dd, J = 14.3, 8.7 Hz, 3H), 7.14 (s, 1H), 6.59 (dd, J = 8.9, 2.2 Hz, 1H), 6.43 (d, J = 1.8 Hz, 1H), 3.44–3.40 (m, 4H), 2.30–2.27 (m, 6H), 1.22 (t, J = 6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.87, 162.66, 158.71, 156.87, 153.20, 149.79, 148.48, 136.94, 134.34, 131.31, 129.91, 128.79, 128.69, 127.33, 109.62, 107.67, 96.67, 74.49, 45.11, 14.70, 12.41, 10.93. MS (ESI): 497.12($C_{26}H_{25}ClN_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{25}ClN_2O_4S$: C, 62.83; H, 5.07; Cl, 7.13; N, 5.64; O, 12.88; S, 6.45; Found: C, 62.85; H, 5.06; Cl, 7.12; N, 5.65; O, 12.89; S, 6.46.

4.2. Biological assay

4.2.1. Minimum inhibitory concentration (MIC) [8,26]

The *in vitro* antibacterial activity for intermediates **4&7** and target compounds **8a-8x** were evaluated using the agar-dilution method [27]. The test bacterial strains, *S. aureus, L. monocytogenes, E. coli, Salmonella*, *methicillin-resistant S. aureus* and *vancomycin-resistant S. aureus* were provided by the Laboratory of Plant Disease Control, Anhui Agricultural University. 2-fold serial dilutions of the compounds and positive control drugs (ciprofloxacin, novobiocin, gatifloxacin, and levofloxacin) were prepared in LB-Broth-Agar-Medium. Compounds and drugs (10.0 mg) were dissolved in DMSO (1 mL), then the solution was diluted with water (9 mL). Further progressive double dilution with melted LB-Broth-Agar-Medium was performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.05 μg/mL. The MIC values were calculated separately. The MIC values were calculated separately.

4.2.2. Enzyme inhibitory activity assay

The *in vitro* enzyme inhibitory activity of the selected compound was carried out by the literature [28].

4.3. 3D(Three-dimensional) quantitative structure activity relationships

The 3D QSAR simulation processes were performed with Discovery Studio (version 3.5, Supporting Information), using genetic function algorithm (GFA) [29].

4.4. Molecular docking

The crystal structure of DNA gyrase (PDB ID:2xcs) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/). The molecular docking procedures were performed by the literature [30]. First, we clean the receptor protein, such as water removal and hydrogenation. Second, we define the active site with the position of GSK299423 and a radius of 9.0. All target compounds are then given a force field as prepared small ligands. Finally, the docking work can be carried out by using DS_CDOCKER protocol for receptor–ligand interactions section of Discovery Studio 3.5. All compounds generated a total of 960 docking poses with the target protein.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103907.

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