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Coumarin-Based Inhibitors of *Bacillus anthracis* and *Staphylococcus aureus* Replicative DNA Helicase: Chemical Optimization, Biological Evaluation, and Antibacterial Activities

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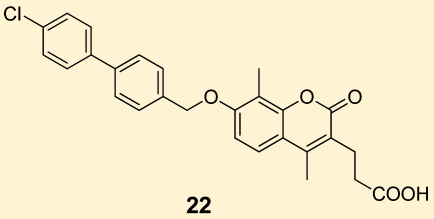
Supporting Information

ABSTRACT: The increasing prevalence of drug-resistant bacterial infections demands the development of new antibacterials that are not subject to existing mechanisms of resistance. Previously, we described coumarin-based inhibitors of an underexploited bacterial target, namely the replicative helicase. Here we report the synthesis and evaluation of optimized coumarin-based inhibitors with 9−18-fold increased potency against *Staphylococcus aureus* (*Sa*) and *Bacillus anthracis* (*Ba*) helicases. Compounds **20** and **22** provided the best potency, with IC_{50} values of 3 and 1 μM, respectively, against the DNA duplex strand-unwinding activities of both *B. anthracis* and *S. aureus* helicases without affecting the single strand DNA-stimulated ATPase activity. Selectivity index (SI = CC_{50}/MIC) values against *S. aureus* and *B. anthracis* for compound **20** were 33 and 66 and for compound **22** were 20 and 40, respectively. In addition, compounds **20** and **22** demonstrated potent antibacterial activity against multiple ciprofloxacin-resistant MRSA strains, with MIC values ranging between 0.5 and 4.2 μg/mL.



22

IC_{50} *Ba*: 1.5 μM MIC *Ba*: 0.625 μM

IC_{50} *Sa*: 1 μM MIC *Sa*: 1.25 μM

MIC *MRSA*: 0.5-2.1 μM

INTRODUCTION

Bacillus anthracis (Ba), the causative agent of anthrax, is considered an agent of biological warfare or terrorism because of its virulence, its stability in aerosol form, and its previous use in acts of terrorism.[1,2] While ciprofloxacin and doxycycline are effective antidotes if administered immediately after suspected contact with *B. anthracis*, there is little doubt that capable terrorists will develop forms of the bacterium resistant to these common antibiotics. Moreover, antibiotic resistant pathogens are not only a problem for biodefense but are also found increasingly in the clinic, e.g., community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). New antibiotics based on underexploited targets are critical components for treating drug-resistant pathogens both in the clinic and for biodefense because there will be no pre-existing target-based resistance mechanisms for such new agents. A key example of such an underexploited target is the bacterial replicative helicase, which catalyzes an essential rate-limiting step in DNA replication. Several features of the *B. anthracis* and *S. aureus* replicative DNA helicase make them particularly attractive as targets for the discovery of new antibacterial therapeutics for biodefense. First, they are members of a drug-validated pathway. While gyrase, topoisomerase IV, and DNA polymerase III have been targeted successfully, helicase remains an untapped vulnerability in the mechanism of bacterial DNA replication. Second, they are multifunctional proteins, providing multiple opportunities for antibacterial intervention.[3−9] Third, helicase activity is essential to bacteria.[10−14] Fourth, the primary structures of the *B. anthracis* and *S. aureus* replicative helicases differ significantly from those of their eukaryotic counterparts,[14,15] indicating that bacterial-specific inhibitors of helicase may be identified. The human replicative helicase was described recently as a complex of 11 proteins, namely Cdc45/MCM2−7/GINS ("CMG"), none of which have significant homology to the DnaB family of bacterial hexameric replicative helicases.[16,17] Accordingly, inhibitors of *B. anthracis* helicase are unlikely to demonstrate target-based toxicity vs mammalian hosts.

For all of the reasons described above, DnaB helicase from *Escherichia coli*, *S. aureus*, and *Pseudomonas aeruginosa* have been targeted previously in anti-infective screens. Screening assay readouts have included electrochemiluminescence,[18] fluorescence or FRET,[19−21] time-resolved FRET,[22] scintillation proximity (SPA),[23,24] and radiometric detection of ATPase inhibition,[25] but few hits have been described and none have progressed further in drug development. A triaminotriazine structure was recently shown to inhibit *P. aeruginosa* DnaB, but it displays significant cytotoxicity and is not selective in MMS studies.[20] A large antibacterial screening effort undertaken by GSK resulted in no hits for *S. aureus* replicative helicase.[26] While hits were obtained for another essential helicase (PcrA) in *S. aureus*, which is involved in DNA repair and plasmid replication, no lead compounds could be developed from these hits.[26] Inhibitors of the *E. coli* orthologue of PcrA, namely

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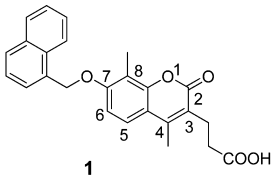
helicase IV, have also been described, but no information on cytotoxicity was provided and they do not appear to have progressed further.[25] Two investigators have described inhibition of *E. coli* helicases (DnaB and RepA) by flavones such as myricetin;[27,28] however, myricetin is quite promiscuous and cytotoxic. Similarly, intercalators and minor groove binders, which interact with DNA, are potent helicase inhibitors but they lack bacterial selectivity as well.[29]

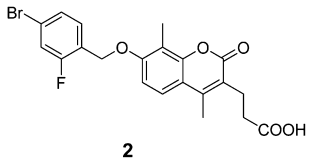
Recently, we reported the discovery and validation of five different chemotypes of *B. anthracis* and *S. aureus* helicase inhibitors in a high-throughput screening effort. The most potent inhibitors discovered in this campaign shared a coumarin scaffold as a common motif (Figure 1),[30] but they



1

IC_{50} *Ba*: 9 μM MIC *Ba*: 6 μM

IC_{50} *Sa*: 10 μM MIC *Sa*: >100 μM



2

IC_{50} *Ba*: 14 μM MIC *Ba*: 10 μM

IC_{50} *Sa*: 18 μM MIC *Sa*: >100 μM

Figure 1. Two coumarin-based helicase HTS hits.

did not inhibit gyrase or the binding of ATP to helicase. Preliminary SAR studies of the coumarin-based inhibitors indicated that the substituent at the 7-position dramatically affects the potency against *B. anthracis* and *S. aureus* helicases and that an ester functionality at the 3-position resulted in compounds that were inactive against both of the DNA helicases. Herein we report the chemical optimization, biological evaluation, and antibacterial activities of this coumarin-based series of *Bacillus anthracis* and *Staphylococcus aureus* DNA replicative helicase inhibitors.

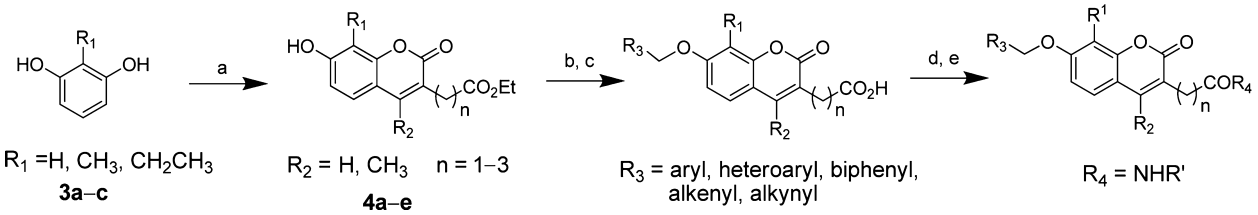
RESULTS AND DISCUSSION

Chemistry. The general synthesis of coumarin helicase inhibitors is illustrated in Scheme 1. The classic Pechmann condensation[31] of 2-ethylresorcinol (**3a**), 2-methylresorcinol (**3b**), or resorcinol (**3c**) with various β-keto esters provided 7-hydroxycoumarin intermediates **4a**–**e**, which were further derivatized with alkylating agents. Hydrolysis of coumarin esters provided the corresponding coumarin carboxylic acids. Amides were also prepared from selected coumarin carboxylic acids. Synthesis of biphenyl coumarin helicase inhibitors **24**–**27** is shown in Scheme 2. The 7-[(4-bromo)benzyloxy]coumarin compound **23** was produced by alkylation of the 7-hydroxycoumarin precursor **4a**. Biphenyl compounds **24**–**27** were prepared using Suzuki coupling reactions, followed by ester hydrolysis.

Structure–Activity Relationship (SAR) Studies. Synthesized coumarin analogues were evaluated in a fluorescence resonance energy transfer (FRET)-based assay to measure concentration-dependent inhibition of ATP-dependent DNA strand unwinding catalyzed by the *B. anthracis* and *S. aureus* DNA replicative helicases. To further optimize the potency of the coumarin-based inhibitors, we extended the preliminary SAR studies reported previously.[30] We maintained the essential carboxylic acid group attached to the 3-position through an alkyl linker and systematically investigated substitutents at the 7-position, which had been shown to influence antihelicase potency significantly. Specifically, we used alkenyl, alkynyl, aromatic, or heteroaromatic groups and explored substituent effects on the aromatic or heteroaromatic rings. As shown in Table 1, compounds **5** and **6** with an alkenyl or an alkynyl group at the R_3 position were inactive vs both helicases, while compound **7** with a phenyl group at the R_3 position exhibited weak inhibitory activity vs both helicases. However, with compound **8**, bearing a pyridyl group at the same position, inhibitory activity was undetectable. Compounds with quinolinyl- or isoquinolinyl-substitution at the R_3 position (**10**–**13**) exhibited weak or no inhibitory activity vs DNA helicases. Interestingly, 1-naphthyl, 2-naphthyl, or anthracenyl substitution at the R_3 position (compounds **1**, **9**, and **14**, respectively) provided moderate potency, with IC_{50} values ranging from 6 to 10 μM. Compounds with biphenyl substitution at the R_3 position were potent DNA helicase inhibitors, with 1,4-biphenyl substitution providing the best potency against both *B. anthracis* and *S. aureus* helicases (compound **20** (1,4-biphenyl) vs compounds **15** (1,2-biphenyl) and **18** (1,3-biphenyl) and compound **19** (1,4-biphenyl) vs compounds **16** (1,2-biphenyl) and **17** (1,3-biphenyl)). We next examined the effect of substituents on the biphenyl group. Substitutions with F, Cl, CF_3, and CN on the distal phenyl ring were all tolerated within a 2-fold range of potency compared to unsubstituted compound **20**. The most potent biphenyl compound was chloro compound **22**, which exhibited an IC_{50} value of 1.0 μM against *S. aureus* helicase and a comparable value against *B. anthracis* helicase (IC_{50} = 1.5 μM).

The effect of methyl substituents at the 4- and 8-positions (R_2 and R_1, Table 1) of the coumarin core on potency was examined by comparison with unsubstituted or ethyl-substituted counterparts. We found that removal of the methyl group from the 4-position of the coumarin core dramatically decreased the potency (**33** vs **20**), while unsubstituted 8-position compounds exhibited a decreased potency of about 2–3-fold (**15** vs **16**, **18** vs **17**, and **20** vs **19**). Ethyl substitution was well-tolerated at the 8-position, as evidenced by the low

Scheme 1. General Synthesis of Coumarin Helicase Inhibitors[a]



[a]Reagents and conditions: (a) $CH_3COCH(CO_2Et)(CH_2)_nCO_2Et$ (n = 1–3), H_2SO_4, 0 °C, or $HCOCH(CO_2Et)(CH_2)_2CO_2Et$, H_2SO_4, RT; (b) R_3CH_2X, Na_2CO_3, DMF, RT; (c) 2N NaOH, RT; (d) $(COCl)_2$, DMF, THF; (e) RNH_2.

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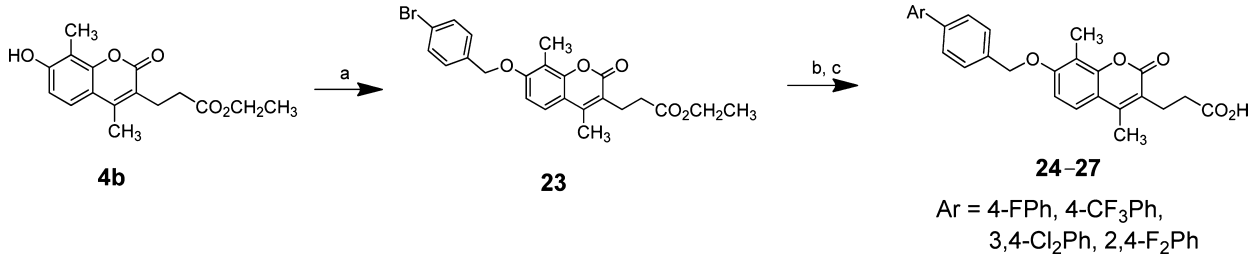
Scheme 2. Synthesis of Biphenyl Coumarin Helicase Inhibitors 24−27[a]



[a]Reagents and conditions: (a) 4-bromobenzyl bromide, Na_2CO_3, DMF, RT; (b) Ar−$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, 85 °C; (c) 2N NaOH, RT.

micromolar IC_{50} values for compounds **34** and **35**. We also varied the length of the aliphatic linker between the coumarin core and the carboxylic acid functionality at the 3-position. Compared to compound **20**, which bears an ethylene ($-CH_2CH_2-$) linker between the coumarin core and the carboxylic acid functionality, compound **31**, with a methylene ($-CH_2-$) linker, demonstrated 3−4-fold less potency against both DNA helicases. Interestingly, compounds **32** and **35**, bearing a propylene ($-CH_2CH_2CH_2-$) linker between the coumarin core and the carboxylic acid functionality, exhibited potency comparable to that of compound **20**.

In general, the carboxylic acid functionality plays an important role in helicase inhibition for the coumarin compounds, suggesting that the carboxylic acid makes favorable interactions with the bacterial DNA helicase target. Unlike the acid compounds, neutral amide compounds **28** and **29** were less active vs *B. anthracis* helicase (see Table 1: for **28**, IC_{50} (*Ba*) >100 μM; for **29**, IC_{50} (*Ba*) >100 μM) and exhibited a substantial reduction in potency against *S. aureus* helicase (see Table 1: for **28**, IC_{50} (*Sa*) = 38 μM; for **29**, IC_{50} (*Sa*) >100 μM). Surprisingly, amide compound **30**, with an additional flexible linker tethered to a tertiary amine moiety, exhibited even better helicase inhibitory activity than observed for compound **20**. This is an exception to the general SARs. In the absence of cocrystal structural information, it is not possible to provide a definitive explanation for the antihelicase potency of this compound. However, we propose that the positively charged amine moiety with a flexible long linkage may be extending into a solvent accessible region or a hydrophilic surface to make additional interactions with the enzyme, thus increasing affinity.

Antibacterial Activity of Helicase Inhibitors. Compounds which showed DNA helicase inhibitory activities were further evaluated in bacterial growth inhibition assays against *B. anthracis* and *S. aureus* (Table 2). Biphenyl compounds exhibited potent antibacterial growth activities, which correlated well with their helicase inhibitory activities. 1,4-Biphenyl compounds showed better antibacterial activities, especially for *S. aureus* growth inhibition, and less mammalian cytotoxicity than did 1,2- and 1,3-biphenyl isomeric compounds. For example, compound **20** exhibited MIC values of 1.25 and 2.5 μM versus *B. anthracis* and *S. aureus*, respectively, and the selectivity index (SI) values were 66 and 33, respectively.

The substituents on the biphenyl group dramatically affected the antibacterial activity of members of this series. Fluoro-, chloro-, or cyano-monosubstitution was generally tolerated (see compounds **21**, **22**, and **23**); however, compound **25**, bearing a trifluoromethyl group at the 4′-position, showed a higher MIC value vs *S. aureus* than did compound **20**. A 3′,4′-dichloro-substitution is tolerated, while 2′,4′-difluoro-substitution is detrimental to the antibacterial activities (see compound **27**).

Compound **34**, bearing an ethyl group at the 8-position of the coumarin ring, displayed antibacterial activities comparable to its 8-methyl analogue **20**, while compound **33**, without a substituent at the 4-position, displayed a higher MIC value vs *S. aureus*. Helicase inhibitors with different linkers at the 3-position of the coumarin core generally exhibited *Bacillus* growth inhibitory activity. However, compounds with a propylene linker exhibited much higher MIC values for *S. aureus* than did those with ethylene or methylene linkers (**32** and **35** vs **20** and **31**).

Several compounds, such as **9**, **14**, **25**, **32**, **33**, and **35**, exhibited much higher MIC values for *S. aureus* than they did for *B. anthracis*, even though they exhibited potent *S. aureus* helicase inhibitory activity. To explore whether the poor *S. aureus* growth inhibition was due to efflux, we added the efflux pump inhibitor reserpine to the growth inhibition assay (20 μg/mL).[32] The addition of reserpine did not affect the MIC values for compounds **9**, **14**, **32**, **33**, or **35**, suggesting that the higher MIC values vs *S. aureus* are due to influx deficiencies rather than to efflux. However, addition of reserpine did improve the MIC value for compound **25** by about 8-fold, indicating that efflux is a factor in the sensitivity of *S. aureus* cells to this compound.

Consistent with its lack of antihelicase activity, amide **28** exhibited no bacterial growth inhibitory activity. However, amide **30** exhibited potent MIC values comparable to that of **20**. Surprisingly, it also demonstrated significant HeLa cell cytotoxicity, suggesting that the carboxylic acid functionality may be necessary for maximizing the antibacterial activity and minimizing the cytotoxicity in the coumarin-type helicase inhibitors. In this regard, it is important to note that in general, antihelicase potency and cytotoxicity are not correlated. In fact, a scatter plot of the helicase strand unwinding inhibition potency (IC_{50}) vs the HeLa cytotoxicity in serum-free medium (CC_{50}) for the 21 coumarin analogues in Table 2 revealed no significant correlation (r^2 = 0.008) (Figure S1, Supporting Information). These results suggest that antihelicase potency can be optimized without concomitant increases in cytotoxicity. The structure−activity relationships (SARs) of the coumarin-based bacterial DNA helicase inhibitors for their antihelicase activities and antibacterial activities are summarized in Figure 2.

Mode of Helicase Inhibition by Coumarins. The effect of different concentrations of each of the two helicase substrates ATP and DNA on inhibitor IC_{50} values was used to assess the mode of inhibition of the most selective coumarin inhibitor **20** in the DNA duplex strand-unwinding assay. The IC_{50} values for compound **20** were only modestly affected by

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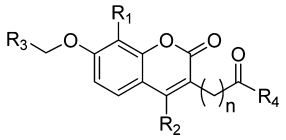
Table 1. *Bacillus anthracis* and *Staphyloccocus aureus* Helicase Inhibition of Coumarin Compounds



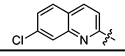
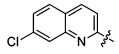
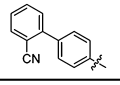
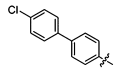
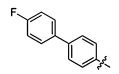
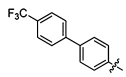
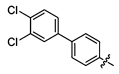
Compd. ID	R_1	R_2	R_3	R_4	n	IC_{50} *B. anthracis* Helicase (μM)	IC_{50} *S. aureus* Helicase (μM)
1	CH_3	CH_3	1-naphthyl	OH	2	6	10
5	CH_3	CH_3		OH	2	>100	>100
6	CH_3	CH_3		OH	2	>100	>100
7	CH_3	CH_3	phenyl	OH	2	85	60
8	CH_3	CH_3	4-pyridyl	OH	2	>100	>100
9	CH_3	CH_3	2-naphthyl	OH	2	9	8
10	CH_3	CH_3		OH	2	56	50
11	H	CH_3		OH	2	>100	70
12	CH_3	CH_3		OH	2	>100	>100
13	H	CH_3		OH	2	>100	>100
14	CH_3	CH_3	9-anthracenyl	OH	2	7.2	8.3
15	CH_3	CH_3	1,2-biphenyl	OH	2	10.2	9
16	H	CH_3	1,2-biphenyl	OH	2	19	12
17	H	CH_3	1,3-biphenyl	OH	2	15	15
18	CH_3	CH_3	1,3-biphenyl	OH	2	7.5	6
19	H	CH_3	1,4-biphenyl	OH	2	9.7	6
20	CH_3	CH_3	1,4-biphenyl	OH	2	3	2
21	CH_3	CH_3		OH	2	3.8	4.6
22	CH_3	CH_3		OH	2	1.5	1
24	CH_3	CH_3		OH	2	5.1	3.7
25	CH_3	CH_3		OH	2	6.1	3.3
26	CH_3	CH_3		OH	2	3	2.4

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Table 1. continued

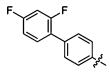
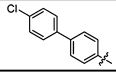
Compd. ID	R_1	R_2	R_3	R_4	n	IC_{50} *B. anthracis* Helicase (μM)	IC_{50} *S. aureus* Helicase (μM)
27	CH_3	CH_3		OH	2	9	4.9
28	CH_3	CH_3		$NHCH_3$	2	>100	38
29	CH_3	CH_3	1,4-biphenyl	$NHCH_2CH_2NHAc$	2	>100	>100
30	CH_3	CH_3	1,4-biphenyl	$NHCH_2CH_2N(CH_3)_2$	2	1.8	1.7
31	CH_3	CH_3	1,4-biphenyl	OH	1	9	8.6
32	CH_3	CH_3	1,4-biphenyl	OH	3	2.0	2.2
33	CH_3	H	1,4-biphenyl	OH	2	21	14
34	CH_2CH_3	CH_3	1,4-biphenyl	OH	2	2	2
35	CH_2CH_3	CH_3	1,4-biphenyl	OH	3	5	1.8

Table 2. Antibacterial Activity of Coumarin-Based Helicase Inhibitors

compd ID	MIC *B. anthracis* (μM)	MIC *S. aureus* (μM)[a]	HeLa CC_{50} in SFM (μM)	SI (CC_{50}/MIC) *Ba*	SI (CC_{50}/MIC) *Sa*
1	65	>100	6	0.09	<0.06
9	5	>100 (>100)	38	7.6	<1
14	2.5	20 (50)	8.3	3.3	0.4
15	2.5	10	4.4	1.8	0.4
16	5	12.5	7.8	1.6	0.6
17	2.5	10	10.1	4.0	1.0
18	0.625	20	5.6	9.0	0.3
19	2.5	3.125	21.2	8.5	6.8
20	1.25	2.5	82	66	33
21	1.25	2.5	19.5	15.6	7.8
22	0.625	1.25	25	40	20
24	<0.8	3	19.2	>24	6.4
25	<0.8	50 (6.3)	20.3	>25.4	3.2[a]
26	3	3 (6.3)	19.2	6.4	6.4
27	>100	>100			
28	>100	>100	90.7	0.91	0.91
30	2.5	5	1.1	0.44	0.22
31	2.5	8.2 (12.5)	115	46	14
32	1.25	>100 (>100)	31	25	<0.8
33	2.5	>100 (>100)	>100	>40	2.5
34	1.25	5.0	42	34	8.4
35	2.5	>100 (>100)	15.4	6.2	3.1

[a]MIC value (μM) with efflux pump inhibitor reserpine.

27-fold variations in the oligonucleotide concentrations (IC_{50} = 1.8−2.2 μM) (Figure 3A) or by 16-fold variations in ATP concentration (IC_{50} = 1.5−4.1 μM) (Figure 3B). As described by Wei et al.,[33] insensitivity of IC_{50} values to variations in the substrates is indicative of a noncompetitive mode of inhibition for compound **20** vs both substrates. Consistent with these results, a fit of the initial velocities to standard, nonlinear four-parameter curves demonstrated that the data were best represented by a noncompetitive mode of inhibition, with K_i values of 4.0 and 2.4 μM for compound **20** vs oligonucleotide and ATP, respectively.

We also examined the effect of compounds **20** and **22** on the single-strand DNA stimulated ATPase activity of *S. aureus* helicase. Neither compound inhibited the ATPase activity significantly at a concentration that is 25−50-fold greater than its IC_{50} in the strand-unwinding assay (6% and 8% inhibition by compounds **20** and **22** at 50 μM, respectively; see Table 3). Taken together, these results indicate that the coumarin-type helicase inhibitors bind at sites distinct from both the DNA and ATP substrates, and the mechanism of strand-unwinding inhibition does not involve reduction of the energy supply (ATP hydrolysis) for translocation and strand unwinding. This mechanism is distinct from that of the known DNA helicase

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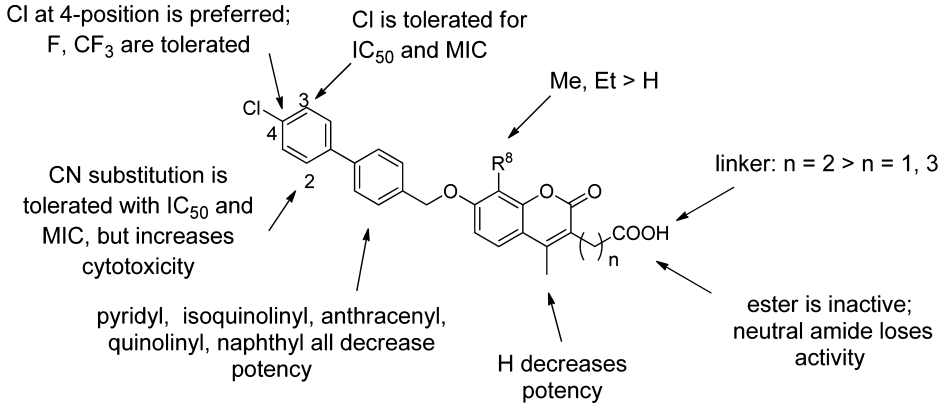

Figure 2. Summary of SARs of the coumarin-based bacterial DNA helicase inhibitors.

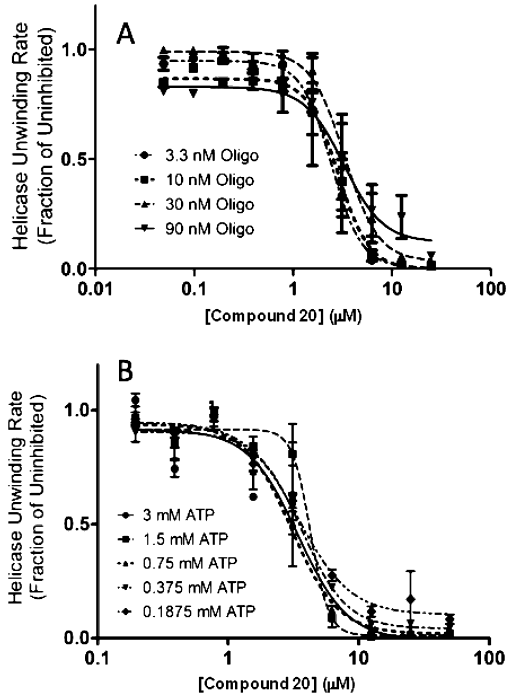

Figure 3. Dependence of IC_{50} values for compound **20** on concentrations of substrates. The concentration dependence of compound **20** inhibition of *S. aureus* helicase was determined in the FRET-based DNA duplex strand-unwinding assay in the presence of various concentrations of the two substrates, DNA (A) and ATP (B) as noted. RFU/min values were normalized to the slope of the uninhibited control. Lines were drawn based on four-parameter nonlinear curve fitting (GraphPad Prizm 5.0).

Table 3. Effect of Helicase Strand Unwinding Inhibitors on the Helicase ATPase

reaction	average A_{630} ± Stdev	% inhibition
complete	0.75 ± 0.01	
−ATP	0.09 ± 0.01	88
−PhiX174	0.19 ± 0.06	75
−helicase	0.15 ± 0.05	80
complete + 50 μM compd **20**	0.70 ± 0.01	6
complete + 50 μM compd **22**	0.69 ± 0.02	8

inhibitor myricetin, which also inhibits ATPase activity in a noncompetitive manner.[27] No clear mechanism of inhibition information is available for other inhibitors of bacterial DnaB type replicative helicases.

Effect of Coumarin Helicase Inhibitor 20 on Bacterial Viability. *S. aureus* cells were incubated with various concentrations of compound **20** or the control bactericidal antibiotic ciprofloxacin, and aliquots of the culture were diluted and plated on rich medium without inhibitors to determine the number of viable cells capable of forming colonies. The resulting killing curve (Figure 4) confirmed the cidality of

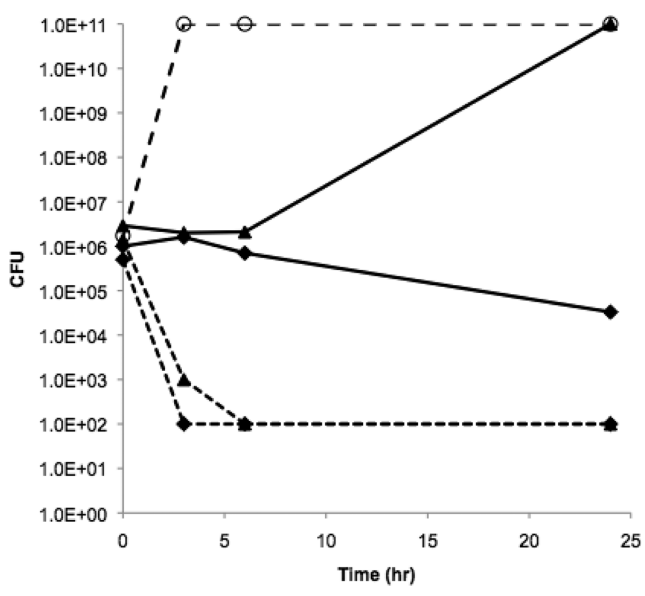

Figure 4. Effect of compound **20** and ciprofloxacin on the viability of *S. aureus* cells. *S. aureus* (ATCC 25923) cells were incubated for the indicated time with no drug (open circles), 1× MIC (triangles), or 4× MIC (diamonds) of compound **20** (solid lines) or ciprofloxacin (dashed lines) and spread on LB agar plates at various times indicated on the abscissa to determine the number of viable colony-forming units (CFU). For ciprofloxacin, 1× MIC was 0.5 μg/mL, and 4× MIC was 2.0 μg/mL; for compound **20**, 1× MIC was 2.5 μM and 4× MIC was 10 μM (Table 2).

ciprofloxacin at concentrations of both 1× and 4× MIC (>3 log decrease in viability over 24 h). However, compound **20** was bacteriostatic at concentrations of 1× and 4× its MIC value, and some regrowth was observed at 24 h in 1× compound **20**, possibly due to cellular metabolism of the inhibitor or mutations in the population. While it is somewhat surprising that a DNA synthesis inhibitor should appear bacteriostatic, the

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potency of compound **20** may simply be too low, and much higher concentrations or a more potent helicase inhibitor could be bactericidal. Nevertheless, many bacteriostatic agents, such as the tetracycline, oxazolidinone, and macrolide classes of protein synthesis inhibitors, are very useful antibiotics. So, further development of coumarin-type helicase inhibitors may provide novel antibiotics with important clinical applications.

Spectrum of Antibacterial Activity. The antibacterial spectra of the two most potent helicase inhibitors **20** and **22** were examined. The results shown in Table 4 revealed considerable breadth across the *Bacillus* and *Staphylococcus* genera, with detectable but reduced potency versus species of the enterococcus genus. Importantly, both **20** and **22** showed potent antibacterial activity against multiple ciprofloxacin-resistant MRSA strains, with MIC values ranging between 0.5 and 4.2 μg/mL.

Table 4. Average MIC (μg/mL) Values vs Gram(+) Bacterial Strains for Compounds 20, 22, and Ciprofloxacin

	MIC (μg/mL)		
bacterial strain	compd **20**	compd **22**	ciprofloxacin
Bacillus subtilis BD54	4.2	3.1	0.078
B. anthracis ΔANR	0.65	0.65	0.078
B. thuringiensis ATCC 10792	0.65	0.52	0.078
B. anthracis Sterne	0.52	0.17	0.039
B. lichenformis ATCC 14580	0.39	0.098	0.039
E. faecalis ATCC 29212	5.2	6.3	0.625
VRE faecalis ATCC 51575	>25	25	0.625
VRE faecalis 51299	18.8	4.2	0.313
VRE faecalis 700802	>25	16.7	0.469
VRE faecalis F118	16.7	3.6	>20
E. faecium ATCC 19434	2.6	12.5	10.0
VRE faecium B42762	14.6	9.4	>20
VRE faecium 1644	2.1	10.4	>20
S. aureus ATCC 25923	1.56	0.78	0.235
MRSA 1234522733	2.6	0.78	10.0
MRSA 1234543349	4.2	1.56	>20.0
MRSA 1234544081	1.56	0.65	0.313
MRSA 1234547263	2.1	0.65	>20
MRSA 1234549404	1.56	0.52	0.469
MRSA 1234558336	3.1	1.04	>20
MRSA 1094	12.5	2.1	15.0

CONCLUSIONS

The replicative DNA helicase catalyzes a rate-limiting step in DNA replication and is essential for bacterial growth. However, the DNA helicase is an underexploited biological target for the discovery of new antibacterial therapeutics for biodefense and clinical use, and very few small molecule inhibitors have been identified that can potently inhibit helicase activity and bacterial growth. In a previous high-throughput screening campaign, we discovered several coumarin-based inhibitors that inhibit both *Bacillus anthracis* and *Staphylococcus aureus* DNA helicases. Chemical optimization of the coumarin helicase inhibitor series identified several derivatives exhibiting more potency and selectivity. The structure−activity relationships are responsive to alterations in the scaffold and exhibit clear trends, such as preferences for a hydrophobic group at position 7, an acid group attached to position 3 through an ethylene linker, and for a methyl group at position 4. Biphenyl coumarins **20** and **22** are the most potent compounds, with IC_{50} values of 3 and 1 μM, respectively, versus both *B. anthracis* and *S. aureus* helicases. Selectivity index (SI = CC_{50}/MIC) values were 20−66. Moreover, compounds **20** and **22** demonstrated potent antibacterial activity against multiple ciprofloxacin-resistant MRSA strains with MIC values ranging between 0.5 and 4.2 μg/mL. Enzyme kinetic studies demonstrated that these coumarin-based helicase inhibitors act noncompetitively with both the DNA and ATP substrates to inhibit the DNA duplex strand-unwinding activity of helicase, but they do not inhibit the single-strand stimulated ATPase activity of helicase, which is the energy source for translocation and unwinding. These results indicate that further optimization of coumarin-based helicase inhibitors may provide a new class of antibacterial agents.

EXPERIMENTAL SECTION

General Procedures. All commercially obtained solvents and reagents were used as received. Melting points were determined in open capillary tubes with an EZ-Melt (Stanford Research Systems) apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Bruker 300 MHz instrument. Chemical shifts are given in δ values referenced to the internal standard tetramethylsilane. LC-MS analyses were performed using a Shimadzu LC-10 AD VP HPLC, with Waters micromass Quattro Ultima triple quad MS. Analytical HPLC analyses were performed with a Gilson LC system. Elemental analyses were performed by Columbia Analytical Services. All of the compounds tested in vitro showed >95% purity either by LC-MS or HPLC.

*Ethyl 3-(8-Ethyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)-propanoate (**4a**).* Dry HCl gas was passed through a solution of 2-ethylresorcinol (**3a**) (2.00 g, 0.0150 mol) and diethyl 2-acetylglutarate (3.33 g, 0.0150 mol) in absolute ethanol (40 mL) at 0 °C for 1 h. The reaction mixture was stirred at room temperature for 24 h, the volume was reduced to about 20 mL and the mixture was poured into water. The resulting precipitate was collected by filtration, washed with water, and dried at 50 °C under vacuum overnight to give an off-white solid (3.92 g, 86% yield) of the desired product, mp = 156−157 °C, R_f = 0.21 (1:99 MeOH:DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, *J* = 9.0 Hz, 1H), 6.79 (d, *J* = 9.0 Hz, 1H), 6.56 (br, 1H), 4.14 (q, *J* = 6.0 Hz, 2H), 2.97 (t, *J* = 9.0 Hz, 2H), 2.85 (q, *J* = 9.0 Hz, 2H), 2.63 (t, *J* = 9.0 Hz, 2H), 2.41 (s, 3H), 1.25 (t, *J* = 6.0 Hz, 3H), 1.18 (t, *J* = 9.0 Hz, 3H). LC/MS *m*/*z* 304.8 (M + H$^+$).

*Ethyl 3-(7-Hydroxy-4,8-dimethyl-2-oxo-2H-chromen-3-yl)-propanoate (**4b**).* 2-Methylresorcinol (**3b**) was treated with diethyl 2-acetylglutarate as above. Compound **4b** was obtained as an off-white powder, mp = 169−170 °C, R_f = 0.29 (2:98 MeOH:DCM). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.45 (d, *J* = 8.7 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 1H), 4.05 (q, *J* = 7.2 Hz, 2H), 2.79 (t, *J* = 7.5 Hz, 2H), 2.47 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.15 (s, 3H), 1.16 (t, *J* = 7.2 Hz, 3H). LC/MS *m*/*z* 290.9 (M + H$^+$).

*Ethyl 3-(7-Hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)-propanoate (**4c**).* 2-Resorcinol (**3c**) was treated with diethyl 2-acetylglutarate as above. Compound **4c** was obtained as an off-white powder, mp = 118−119 °C, R_f = 0.71 (5:95 MeOH:DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.41 (s, 1H), 7.59 (d, *J* = 9.0 Hz, 1H), 6.79 (dd, *J* = 1.8, 8.7 Hz, 1H), 6.68 (d, *J* = 1.8 Hz, 1H), 4.06 (q, *J* = 6.9 Hz, 2H), 2.79 (t, *J* = 7.2 Hz, 2H), 2.48 (t, *J* = 7.5 Hz, 2H), 2.36 (s, 3H), 1.17 (t, *J* = 6.6 Hz, 3H). LC/MS *m*/*z* 276.9 (M + H$^+$).

*Ethyl 2-(7-Hydroxy-4,8-dimethyl-2-oxo-2H-chromen-3-yl)acetate (**4d**).* 2-Methyl resorcinol (**3b**) was treated with diethyl 2-acetylsuccinate as above. Compound **4d** was obtained as an off-white powder, 86% yield, mp = 199−200 °C, R_f = 0.31 (1:9 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 1H), 7.51 (d, *J* = 9.0 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 1H), 4.08 (q, *J* = 7.2 Hz, 2H), 3.64 (s, 2H), 2.34 (s, 3H), 2.16 (s, 3H), 1,18 (t, *J* = 6.9 Hz, 3H). LC/MS *m*/*z* 276.9 (M + H$^+$).

*Ethyl 4-(8-Ethyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)-butanoate (**4e**).* 2-Ethylresorcinol (**3a**) was treated with diethyl 2-acetylhexanoate as above. Compound **4e** was obtained as an off-white

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powder, 74% yield, mp =130−131 °C, R_f = 0.13 (1:4 EtOAc:hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.45 (d, J = 8.7 Hz, 1H), 6.84 (d, J = 8.7 Hz, 1H), 4.03 (dd, J = 3.0, 7.2 Hz, 2H), 2.72 (dd, J = 6.9, 14.4 Hz, 2H), 2.60−2.51 (dd, J = 7.2, 15.3 Hz, 2H), 2.36 (s, 3H), 1,73−1.68 (m, 2H), 1.16 (t, J = 7.2 Hz, 3H), 1.09 (t, J = 7.2 Hz, 3H). LC/MS m/z 318.9 (M + H$^+$).

*Methyl 4-(7-Hydroxy-4,8-dimethyl-2-oxo-2H-chromen-3-yl)-butanoate (**4f**).* 2-Methyl resorcinol (**3b**) was treated with diethyl 2-acetylhexanoate as above. Compound **4e** was obtained as an off-white powder, 95% yield, mp = 152−153 °C, R_f = 0.23 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, J = 9.0 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 4.14 (q, J = 7.2 Hz, 2H), 2.70 (t, J = 6.6 Hz, 2H), 2.44−2.39 (m, 5H), 2.32 (s, 3H), 1.92−1.82 (m, 2H), 1.26 (t, J = 7.2 Hz, 3H). LC/MS m/z 304.8 (M + H$^+$).

*3-(7-(Allyloxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**5**).* A mixture of compound **4b** (0.2 g, 0.69 mmol), allyl bromide (0.125 g, 1 mmol), and potassium carbonate (0.19 g, 1.4 mmol) in acetone (25 mL) was heated at reflux for 24 h. The volume of acetone was reduced by 50%, and the solution was poured into water. The resulting precipitate was collected by filtration and dried at 50 °C under vacuum overnight to give 0.286 g (89%) of the coumarin ester intermediate, mp 112−113 °C, R_f = 0.58 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (d, J = 9.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.15−6.02 (m, 1H), 5.43 (dd, J = 1.8, 17.4 Hz, 1H), 5.29 (dd, J = 1.5, 10.5 Hz, 1H), 4.70 (d, J = 5.1 Hz, 2H), 4.05 (q, J = 7.2 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 2.21 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 330.9 (M + H$^+$). A mixture of the coumarin ester intermediate (0.15g g, 0.45 mmol) and NaOH (0.020 g, 0.50 mmol) in water (2 mL) and ethanol (8 mL) was heated at reflux for 1 h, cooled to room temperature, diluted with water (10 mL), and then neutralized to pH 7 with 1N HCl. The resulting precipitate was collected by filtration, washed with water, and dried at 50 °C overnight to give a white powder (0.109 g, 80% yield), mp = 181−182 °C, R_f = 0.23 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.15−6.02 (m, 1H), 5.43 (dd, J = 1.8, 17.3 Hz, 1H), 5.29 (dd, J = 1.5, 10.7 Hz, 1H), 4.70 (dd, J = 1.8, 3.6 Hz, 2H), 2.79 (t, J = 7.5 Hz, 2H), 2.43−2.38 (m, 5H), 2.21 (s, 3H). LC/MS m/z 303.0 (M + H$^+$). Anal. ($C_{17}H_{18}O_5$): C, 67.54; H, 6.00. Found: C, 67.31; H, 5.94.

*3-(4,8-Dimethyl-2-oxo-7-(prop-2-ynyloxy)-2H-chromen-3-yl)-propanoic Acid (**6**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white solid, 98% yield, mp = 153−154 °C, R_f = 0.59 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.66 (d, J = 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 4.96 (d, J = 2.1 Hz, 2H), 4.05 (q, J = 7.2 Hz, 2H), 3.61 (t, J = 2.1 Hz, 1H), 2.83 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.41 (s, 3H), 2.19 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 328.9 (M + H$^+$). Compound **6** was obtained as a beige powder after NaOH hydrolysis of the ester intermediate, 87% yield, mp = 190−191 °C, R_f = 0.23 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 4.96 (d, J = 2.1 Hz, 2H), 3.61 (t, J = 2.1 Hz, 1H), 2.79 (t, J = 7.5 Hz, 2H), 2.43−2.38 (m, 5H), 2.19 (s, 3H). LC/MS m/z 300.9 (M + H$^+$). Anal. ($C_{17}H_{16}O_5$·0.2H_2O): C, 67.19; H, 5.44. Found: C, 67.03; H, 5.28.

*3-(7-(Benzyloxy)-4, 8-dimethyl-2-oxo-2H-chromen-3-yl)-propanoic Acid (**7**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: off-white powder, 91% yield, mp = 116−117 °C, R_f = 0.62 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (d, J = 9.0 Hz, 1H), 7.49−7.31 (m, 5H), 7.22 (d, J = 9.0 Hz, 1H), 5.26 (s, 2H), 4.04 (q, J = 6.9 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.39 (s, 3H), 2.23 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 381.2 (M + H$^+$). Compound **7** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 85% yield, mp = 232−233 °C, R_f = 0.23 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.49−7.34 (m, 5H), 7.11 (d, J = 9.0 Hz, 1H), 5.26 (s, 2H), 2.79 (t, J = 7.5 Hz, 2H), 2.43−2.38 (m, 5H), 2.24 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{21}H_{21}O_5$ (M +H)$^+$ 353.1389, found 353.1385.

*3-(4,8-Dimethyl-2-oxo-7-(pyridin-4-ylmethoxy)-2H-chromen-3-yl)propanoic Acid (**8**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: light-brown powder, 64% yield, mp = 154−155 °C, R_f = 0.59 (5:95 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (d, J = 6.0 Hz, 2H), 7.42 (d, J = 9.0 Hz, 1H), 7.37 (d, J = 5.7 Hz, 2H), 6.81 (d, J = 9.0 Hz, 1H), 5.2 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 2.97 (t, J = 7.5 Hz, 2H), 2.61 (t, J = 7.8 Hz, 2H), 2.43 (s, 3H), 2.41 (s, 3H), 1.24 (t, J = 7.2 Hz, 3H). LC/MS m/z 382.0 (M + H$^+$). Compound **8** was obtained as a light-brown powder after NaOH hydrolysis of the ester intermediate, 71% yield, mp = 277−278 °C, R_f = 0.19 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.19 (br s, 1H), 8.59 (dd, J = 1.5, 4.5 Hz, 2H), 7.62 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 5.7 Hz, 2H), 7.06 (d, J = 9.0 Hz, 1H), 5.35 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.39 (m, 5H), 2.29 (s, 3H). LC/MS m/z 353.9 (M + H$^+$). Anal. ($C_{20}H_{19}NO_5$·0.2H_2O): C, 67.29; H, 5.48; N, 3.92. Found: C, 67.07; H, 5.30; N, 3.95.

*3-(4,8-Dimethyl-7-(naphthalen-2-ylmethoxy)-2-oxo-2H-chromen-3-yl)propanoic Acid (**9**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 94% yield, mp = 145−146 °C, R_f = 0.60 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.00−7.92 (m, 4H), 7.60 (dd, J = 2.4, 10.5 Hz, 2H), 7.55−7.52 (m, 2H), 7.16 (d, J = 8.7 Hz, 1H), 5.43 (s, 2H), 4.04 (q, J = 7.2 Hz, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.47 (t, J = 7.5 Hz, 2H), 2.37 (s, 3H), 2.27 (s, 3H), 1.15 (t, J = 7.2 Hz, 3H). LC/MS m/z 431.1 (M + H$^+$). Compound **9** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 70% yield, mp = 205−206 °C, R_f = 0.27 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 1H), 8.00−7.91 (m, 4H), 7.61 (d, J = 9.0 Hz, 2H), 7.55−7.52 (m, 2H), 7.17 (d, J = 9.0 Hz, 1H), 5.44 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.43−2.39 (m, 5H), 2.28 (s, 3H). LC/MS m/z 403.2 (M + H$^+$). Anal. ($C_{25}H_{22}O_5$): C, 74.61; H, 5.51. Found: C, 74.47; H, 5.30.

*3-(7-((7-Chloroquinolin-2-yl)methoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**10**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 89% yield, mp = 204−205 °C, R_f = 0.47 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6 + TFA-d) δ 8.73 (d, J = 8.4 Hz, 1H), 7.96 (s, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.76 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 6.74 (d, J = 9.0 Hz, 1H), 5.43 (s, 2H), 3.85 (q, J = 7.2 Hz, 2H), 2.71 (t, J = 6.9 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 0.88 (t, J = 7.2 Hz, 3H). LC/MS m/z 466.5 (M + H$^+$). Compound **10** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 57% yield, mp = 249−250 °C (decomposed), R_f = 0.31 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6 + TFA-d) δ 8.55 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 8.7 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 5.55 (s, 2H), 2.82 (t, J = 6.0 Hz, 2H), 2.46−2.39 (m, 5H), 2.34 (s, 3H). LC/MS m/z 438.0 (M + H$^+$).

*3-(7-((7-Chloroquinolin-2-yl)methoxy)-4-methyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**11**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 93% yield, mp = 148−149 °C, R_f = 0.19 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 9.5 Hz, 2H), 7.66 (dd, J = 2.1, 8.7 Hz, 1H), 7.11−7.07 (m, 2H), 5.49 (s, 2H), 4.04 (q, J = 7.2 Hz, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.47 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 1.15 (s, 3H). LC/MS m/z 452.2 (M + H$^+$). Compound **11** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 25% yield, mp = 216−217 °C, R_f = 0.25 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.19 (br s, 1H), 8.49 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 9.0 Hz, 2H), 7.73 (dd, J = 2.7, 9.0 Hz, 2H), 7.67 (dd, J = 2.1, 8.7 Hz, 1H), 7.10−7.07 (m, 2H), 5.49 (s, 2H), 2.78 (t, J = 7.5 Hz, 2H), 2.39 (m, 5H). LC/MS m/z 424.2 (M + H$^+$). Anal. ($C_{23}H_{18}ClNO_5$): C, 65.18; H, 4.28; N, 3.30. Found: C, 65.01; H, 4.38; N, 3.26.

*3-(7-(Isoquinolin-1-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**12**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 93% yield, mp = 130−131 °C, R_f = 0.54 (3:97 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (d, J = 5.7 Hz, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 5.7 Hz, 1H), 7.84−7.71 (m, 2H), 7.62 (d, J = 9.0 Hz, 1H), 7.30 (d, J = 9 Hz, 1H), 5.84 (s, 2H), 4.04 (q, J = 7.2 Hz, 2H), 2.80 (t, J = 7.5 Hz, 2H), 2.47 (t, J = 7.5 Hz, 2H), 2.38 (s, 3H), 2.14 (s, 3H), 1.15 (t, J = 6.9 Hz, 3H). LC/MS m/z 432.1 (M + H$^+$). Compound **12** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 99% yield, mp = 206−207 °C, R_f = 0.32 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (d, J = 5.7 Hz, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 5.7 Hz, 1H), 7.84−7.71 (m, 2H), 7.59 (d, J = 9.0 Hz, 1H), 7.28 (d, J = 9.0 Hz, 1H), 5.82 (s, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.37 (s, 3H), 2.29 (t, J = 8.1 Hz, 2H), 2.13 (s, 3H). LC/MS m/z 404.1 (M + H$^+$). Anal. ($C_{24}H_{21}NO_5$·0.4H_2O): C, 70.20; H, 5.35; N, 3.41. Found: C, 69.96; H, 5.01; N, 3.40.

*3-(7-(Isoquinolin-1-ylmethoxy)-4-methyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**13**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 94% yield, mp = 99−100 °C, R_f = 0.56 (3:97 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (d, J = 5.7 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 5.7 Hz, 1H), 7.85−7.67 (m, 3H), 7.17 (d, J = 2.4 Hz, 1H), 7.06 (dd, J = 2.4, 9.0 Hz, 1H), 5.80 (s, 2H), 4.05 (q, J = 7.2 Hz, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.38 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 418.4 (M + H$^+$). Compound **13** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 74% yield, mp = 195−196 °C, R_f = 0.32 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (d, J = 5.7 Hz, 1H), 8.33 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 5.7 Hz, 1H), 7.85−7.69 (m, 3H), 7.18 (d, J = 2.4 Hz, 1H), 7.07 (dd, J = 2.7, 9.0 Hz, 1H), 5.8 (s, 2H), 2.78 (t, J = 7.5 Hz, 2H), 2.43−2.37 (m, 5H). LC/MS m/z 390.1 (M + H$^+$). Anal. ($C_{23}H_{19}NO_5$·0.2H_2O): C, 70.29; H, 4.98; N, 3.56. Found: C, 70.33; H, 4.94; N, 3.50.

*3-(7-(Anthracen-9-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**14**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 15% yield, mp = 187−188 °C, R_f = 0.60 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.39 (d, J = 8.4 Hz, 2H), 8.16 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 9.0 Hz, 1H), 7.64−7.54 (m, 5H), 6.19 (s, 2H), 4.05 (q, J = 7.2 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.49 (t, J = 7.2 Hz, 2H), 2.41 (s, 3H), 1.97 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 481.4 (M + H$^+$). Compound **14** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 77% yield, mp = 190−191 °C, R_f = 0.27 (80:18 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.25 (s, 1H), 8.72 (s, 1H), 8.39 (d, J = 8.7 Hz, 2H), 8.16 (dd, J = 1.5, 9.3 Hz, 2H), 7.69 (d, J = 9.0 Hz, 1H), 7.64−7.54 (m, 5H), 6.19 (s, 2H), 2.80 (t, J = 7.2 Hz, 2H), 2.45−2.39 (m, 5H), 1.97 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{29}H_{25}O_5$ (M + H)$^+$ 453.1702, found 453.1697.

*3-(7-(Biphenyl-2-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**15**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 90% yield, mp = 133−134 °C, R_f = 0.44 (1:9 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65−7.62 (m, 1H), 7.54 (d, J = 9.0 Hz, 1H), 7.47−7.33 (m, 8H) 6.87 (d, J = 9.0 Hz, 1H), 5.12 (s, 2H), 4.04 (q, J = 6.9 Hz, 2H), 2.81 (t, J = 7.2 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 2.36 (s, 3H), 2.11 (s, 3H), 1.15 (t, J = 7.2 Hz, 3H). LC/MS m/z 480.5 (M + H$^+$). Compound **15** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 90% yield, mp = 87−88 °C, R_f = 0.27 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65−7.62 (m, 1H), 7.52 (d, J = 9.0 Hz, 1H), 7.47−7.33 (m, 8H), 6.85 (d, J = 9.0 Hz, 1H), 5.11 (s, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.39−2.34 (m, 5H), 2.11 (s, 3H). LC/MS m/z 429.5 (M + H$^+$). Anal. ($C_{27}H_{24}O_5$·0.3H_2O): C, 74.74; H, 5.71. Found: C, 74.44; H, 5.69.

*3-(7-(Biphenyl-2-ylmethoxy)-4-methyl-2-oxo-2H-chromen-3-yl)-propanoic Acid (**16**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: gummy material, 81% yield, R_f = 0.70 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61−7.26 (m, 10H), 6.82 (dd, J = 2.4, 9.0 Hz, 1H), 6.70 (d, J = 2.7 Hz, 1H), 4.99 (s, 2H), 4.11 (q, J = 6.9 Hz, 2H), 2.94 (t, J = 7.5 Hz, 2H), 2.58 (t, J = 7.8 Hz, 2H), 2.41 (s, 3H), 1.23 (t, J = 7.2 Hz, 3H). LC/MS m/z 443.5 (M + H$^+$). Compound **16** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 94% yield, mp = 172−173 °C, R_f = 0.27 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.68 (d, J = 9.6 Hz, 1H), 7.62 (dd, J = 7.8, 1.8 Hz, 1H), 7.48−7.34 (m, 8H), 6.92 (d, J = 2.4 Hz, 1H), 6.89 (s, 1H), 5.04 (s, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.42−2.37 (m, 5H). LC/MS m/z 415.6 (M + H$^+$). Anal. ($C_{26}H_{22}O_5$): C, 75.35; H, 5.35. Found: C, 75.28; H; 5.37.

*3-(7-(Biphenyl-3-ylmethoxy)-4-methyl-2-oxo-2H-chromen-3-yl)-propanoic Acid (**17**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 87% yield, mp = 81−82 °C, R_f = 0.86 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63−7.31 (m, 10H), 6.93 (dd, J = 2.7, 8.7 Hz, 1H), 6.86 (d, J = 2.4 Hz, 1H), 5.15 (s, 2H), 4.11 (q, J = 7.2 Hz, 2H), 2.94 (t, J = 7.5 Hz, 2H), 2.58 (t, J = 7.2 Hz, 2H), 2.39 (s, 3H), 1.22 (t, J = 7.2 Hz, 3H). LC/MS m/z 443.4 (M + H$^+$). Compound **17** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 70% yield, mp = 156−157 °C, R_f = 0.34 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (s, 1H), 7.77−7.63 (m, 5H), 7.53−7.46 (m, 4H), 7.41−7.36 (m, 1H), 7.09−7.04 (m, 2H), 5.30 (s, 2H), 2.78 (t, J = 7.5 Hz, 2H), 2.42−2.37 (m, 5H). LC/MS m/z 415.5 (M + H$^+$). Anal. ($C_{26}H_{22}O_5$): C, 75.35; H, 5.35. Found: C, 75.19; H; 5.23.

*3-(7-(Biphenyl-3-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**18**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 90% yield, mp = 126−127 °C, R_f = 0.86 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.66−7.36 (m, 10H), 6.91 (d, J = 9.0 Hz, 1H), 5.24 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 2.97 (t, J = 7.8 Hz, 2H), 2.60 (t, J = 7.8 Hz, 2H), 2.42 (s, 3H), 2.39 (s, 3H), 1.23 (t, J = 7.2 Hz, 3H). LC/MS m/z 457.6 (M + H$^+$). Compound **18** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 94% yield, mp = 200−201 °C, R_f = 0.34 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.77 (s, 1H), 7.69−7.62 (m, 4H), 7.53−7.46 (m, 4H), 7.41−7.36 (m, 1H), 7.16 (d, J = 9.0 Hz, 1H), 5.35 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.43−2.38 (m, 5H), 2.26 (s, 3H). LC/MS m/z 429.4 (M + H$^+$). Anal. ($C_{27}H_{24}O_5$·0.2H_2O): C, 75.05; H, 5.69. Found: C, 74.98; H; 5.44.

*3-(7-(Biphenyl-4-ylmethoxy)-4-methyl-2-oxo-2H-chromen-3-yl)-propanoic Acid (**19**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 85% yield, mp = 102−103 °C, R_f = 0.84 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64−7.33 (m, 10H), 6.95 (dd, J = 2.7, 8.9 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 5.16 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 2.96 (t, J = 7.5 Hz, 2H), 2.59 (t, J = 8.1 Hz, 2H), 2.43 (s, 3H), 1.23 (t, J = 7.2 Hz, 3H). LC/MS m/z 443.3 (M + H$^+$). Compound **19** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 81% yield, mp = 219−220 °C, R_f = 0.34 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.19 (s, 1H), 7.74−7.66 (m, 5H), 7.56 (d, J = 8.4 Hz, 2H), 7.47 (t, J = 7.5 Hz, 2H), 7.39−7.35 (m, 1H), 7.08−7.03 (m, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.43−2.38 (m, 5H). LC/MS m/z 415.3 (M + H$^+$). Anal. ($C_{26}H_{22}O_5$): C, 75.35; H, 5.35. Found: C, 75.39; H; 5.16.

*3-(7-(Biphenyl-4-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**20**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 91% yield, mp = 139−140 °C, R_f = 0.84 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63−7.33 (m, 10H), 6.89 (d, J = 8.7 Hz, 1H), 5.21 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 2.97 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 7.8 Hz, 2H), 2.42 (s, 3H), 2.39 (s,

3H), 1.23 (t, J = 7.2 Hz, 3H). LC/MS m/z 457.4 (M + H$^+$). Compound **20** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 90% yield, mp = 221−222 °C, R_f = 0.34 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 7.72−7.55 (m, 7H), 7.47 (t, J = 6.9 Hz, 2H), 7.39−7.35 (m, 1H), 7.15 (d, J = 9.3 Hz, 1H), 5.32 (s, 2H), 2.79 (t, J = 7.5 Hz, 2H), 2.43−2.38 (m, 5H), 2.26 (s, 3H). LC/MS m/z 429.3 (M + H$^+$). Anal. ($C_{27}H_{24}O_5$·0.35H_2O): C, 74.59; H, 5.73. Found: C, 74.63; H; 5.74.

*3-(7-((2′-Cyanobiphenyl-4-yl)methoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**21**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 81% yield, mp = 158−159 °C, R_f = 0.34 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.96 (d, J = 7.5 Hz, 1H), 7.83−7.78 (m, 1H), 7.66−7.57 (m, 7H), 7.17 (d, J = 9.0 Hz, 1H), 5.37 (s, 2H), 4.05 (q, J = 7.2 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 2.49 (t, J = 7.5 Hz, 2H), 2.40 (s, 3H), 2.28 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 482.2 (M + H$^+$). Compound **21** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 14% yield, mp = 223−224 °C, R_f = 0.23 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.83−7.78 (m, 1H), 7.67−7.57 (m, 7H), 7.17 (d, J = 9.0 Hz, 1H), 5.37 (s, 2H), 2.79 (t, J = 9.0 Hz, 2H), 2,43−2.38 (m, 5H), 2.28 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{28}H_{24}NO_5$ (M + H)$^+$ 454.1654, found 454.1651.

*3-(7-((4′-Chlorobiphenyl-4-yl)methoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**22**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 82% yield, mp = 139−140 °C, R_f = 0.49 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (d, J = 1.8 Hz, 4H), 7.69−7.50 (m, 5H), 7.14 (d, J = 9.0 Hz, 1H), 5.32 (s, 2H), 4.04 (q, J = 6.9 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 2.25 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 491.0 (M + H$^+$). Compound **22** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 94% yield, mp = 260−261 °C, R_f = 0.32 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.71 (d, J = 8.4 Hz, 4 H), 7.63−7.50 (m, 5H), 7.13 (d, J = 9.0 Hz, 1H), 5.32 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.42−2.37 (m, 5H), 2.25 (s, 3H). LC/MS m/z 463.0 (M + H$^+$). Anal. ($C_{27}H_{23}ClO_5$): C, 70.05; H, 5.01. Found: C, 70.13; H; 4.89.

*Ethyl 3-(7-((4-Bromobenzyl)oxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoate (**23**).* The synthetic procedure used was the same as described for compound **5**. White powder, 100% yield, mp = 182−183 °C, R_f = 0.59 (1:99 MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63−7.59 (m, 3H), 7.44 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 9.0 Hz, 1H), 5.25 (s, 2H), 4.04 (q, J = 7.2 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.39 (s, 3H), 2.23 (s, 3H), 1.15 (t, J = 7.2 Hz, 3H). LC/MS m/z 459.0 (M + H$^+$).

General Procedure for Suzuki Coupling Used in Scheme 2 for Synthesis of Compounds 24−27. A mixture of compound **23** (200 mg, 0.44 mmol, 1 equiv), the corresponding boronic acid (1.5 equiv), tetrakis(triphenylphosphine)palladium(0) (0.1 equiv), and sodium carbonate (3 equiv) in 1:1 dimethoxyethane:water (6 mL) was heated at 85 °C for 4 h, and the solvents were evaporated to dryness. The residue was sonicated for 15 min with 50 mL of EtOAc and filtered. Silica gel (1 g) was added to the filtrate, and the solvents were evaporated to dryness. The residue was applied to a silica gel column and eluted using EtOAc−hexane as the mobile phase to give the biphenyl coumarin ester intermediates.

*3-(7-((4′-Fluorobiphenyl-4-yl)methoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**24**).* The ester intermediate: white powder, 51% yield, mp = 156−157 °C, R_f = 0.22 (1:4 EtOAc:hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95−7.54 (m, 6H), 7.29 (t, J = 8.7 Hz, 1H), 7.21−7.11 (m, 2H), 5.29 (s, 2H), 4.05 (q, J = 6.9 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.8 Hz, 2H), 2.38 (s, 3H), 2.24 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 474.9 (M + H$^+$). Compound **24** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 74% yield, mp = 248−249 °C, R_f = 0.23 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 7.74−7.55 (m, 7H), 7.29 (t, J = 9.0 Hz, 2H), 7.15 (d, J = 9.3 Hz, 1H), 5.32 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.43−2.38 (m, 5H), 2.26 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{27}H_{24}FO_5$ (M + H)$^+$ 447.1608, found 447.1604.

*3-(4,8-Dimethyl-2-oxo-7-((4′-trifluoromethylbiphenyl-4-yl)-methoxy)-2H-chromen-3-yl)propanoic Acid (**25**).* The ester intermediate: white powder, 41% yield, mp = 172−173 °C, R_f = 0.38 (1:3 EtOAc:hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89 (d, J = 8.1 Hz, 2H), 7.82−7.76 (m, 4H), 7.61 (d, J = 8.4 Hz, 3H), 7.13 (d, J = 9.0 Hz, 1H), 5.32 (s, 2H), 4.05 (q, J = 7.2 Hz, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.38 (s, 3H), 2.25 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 525.0 (M + H$^+$). Compound **25** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 89% yield, mp = 240−241 °C, R_f = 0.21 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.84−7.77 (m, 4H), 7.63 (t, J = 6.6 Hz, 3H), 7.15 (d, J = 9.0 Hz, 1H), 5.35 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.43−2.38 (m, 5H), 2.27 (s, 3H). LC/MS m/z 497.1 (M + H$^+$).

*3-(7-((3′,4′-Dichlorobiphenyl-4-yl)methoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**26**).* Coumarin ester intermediate: light-yellow powder, 30% yield, mp = 241−242 °C, R_f = 0.77 (1:1 EtOAc:hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.84−7.63 (m, 7H), 7.21 (d, J = 8.4 Hz, 1H), 5.40 (s, 2H), 4.10 (q, J = 7.2 Hz, 2H), 2.88 (t, J = 6.6 Hz, 2H), 2.51 (t, J = 6.6, 2H), 2.46 (s, 3H), 2.32 (s, 3H), 1.22 (t, J = 6.6 Hz, 3H). LC/MS m/z 525.1 (M + H$^+$). Compound **26** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 73% yield, mp = 241−242 °C, R_f = 0.01 (1:5 EtOAc:hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 7.98 (s, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.71 (s, 2H), 7.64−7.57 (m, 3H), 7.14 (d, J = 1H), 5.33 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.40−2.37 (m, 5H), 2.26 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{27}H_{23}Cl_2O_5$ (M + H)$^+$ 497.0923, found 497.0925.

*7-[(2′,4′-Difluorobiphenyl-4-yl)methoxy]-4,8-dimethyl-3-(3-carboxypropyl)-2-oxo-2H-chromene (**27**).* The ester intermediate: white powder, 37% yield, mp = 151−152 °C, R_f = 0.39 (1:3 EtOAc:hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.64−7.57 (m, 6H), 7.36 (dt, J = 2.4, 10.2 Hz, 1H), 7.23−7.13 (m, 2H), 5.32 (s, 2H), 4.05 (q, J = 7.2 Hz, 2H), 2.82 (t, J = 7.2 Hz, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 2.26 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 492.9 (M + H$^+$). Compound **27** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 70% yield, mp = 225−226 °C, R_f = 0.21 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.65−7.54 (m, 6H), 7.37 (dt, J = 2.1, 10.0 Hz, 1H), 7.24−7.14 (m, 2H), 5.34 (s, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.50−2.38 (m, 5H), 2.27 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{27}H_{23}F_2O_5$ (M + H)$^+$ 465.1514, found 465.1509.

*7-[(4′-Chlorobiphenyl-4-yl)methoxy]-4,8-dimethyl-3-[3-(methylamino)-3-oxopropyl]-2-oxo-2H-chromene (**28**).* 3-(7-((4′-Chlorobiphenyl-4-yl)methoxy)-4,8-dimethyl-2-oxo-2*H*-chromen-3-yl)-propanoic acid (**22**) (217 mg, 0.47 mmol) was suspended in dry THF (3 mL) and oxalyl chloride (0.12 mL, 0.14 mmol) was added followed by 2 drops of DMF. The reaction mixture was stirred at room temperature for 18 h and evaporated to dryness. The residue was dried at 50 °C under vacuum for 1 h, dissolved in dry THF (5 mL), and cooled to 0 °C in an ice bath. Methylamine (29 mg, 0.94 mmol) was added into the solution and stirred at room temperature for 3 h. The reaction mixture was then diluted with water, and the resulting precipitate was collected by filtration, washed with water, and dried at 50 °C under vacuum for 24 h to the crude product, which was purified by recrystallization from DMSO. The crystals were washed with ethyl acetate and dried at 50 °C under vacuum for 24 h to give a white powder **28** (45 mg, 20% yield), mp = 166−167 °C, R_f = 0.25 (CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71 (d, J = 8.4 Hz, 4H), 7.64−7.50 (m, 5H), 7.14 (d, J = 8.7 Hz, 1H), 5.32 (s, 2H), 3.59 (s, 3H), 2.82 (t, J = 7.5 Hz, 2H), 2.52 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 2.25 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{28}H_{27}ClNO_4$ (M + H)$^+$ 476.1629, found 476.1623.

*N-(2-Acetylaminoethyl)-3-(7-(biphenyl-4-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)propanamide, TFA Salt (**29**).* The synthetic procedure used was the same as described for compound **27**:

Compound **29** was obtained as a white powder after HPLC purification, 14% yield, mp = 230−231 °C (decomposed), R_f = 0.94 (5:95 CH_3OH:CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6) δ 7.90 (br s, 1H), 7.83 (br s, 1H), 9.69 (t, J = 8.4 Hz, 4H), 7.62 (d, J = 9.0 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.47 (t, J = 7.5 Hz, 2H), 7.38 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 9.3 Hz, 1H), 5.32 (s, 2H), 3.03 (s, 4H), 2.78 (t, J = 7.2 Hz, 2H), 2.38 (s, 3H), 2.27 (s. 5H), 1.76 (s, 3H). HRMS (ESI-TOF) m/z calcd for $C_{31}H_{33}N_2O_5$ (M + H)$^+$ 513.2389, found 513.2384.

*3-(7-(Biphenyl-4-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)-N-(2-(dimethylamino)ethyl)propanamide, Trifluoroacetic Acid Salt (**30**).* The synthetic procedure for the used was the same as described for compound **27**: Compound **30** was obtained as a colorless film after HPLC purification, 5% yield, R_f = 0.71 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.14 (t, J = 6.0 Hz, 1H), 7.72−7.62 (m, 5H), 7.57 (d, J = 9.0 Hz, 2H), 2.78 (s, 8H), 2.39 (s, 3H), 2.34−2.27 (m, 5H). LC/MS m/z 499.3 (M + H$^+$).

*2-(7-([1,1′-Biphenyl]-4-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)acetic Acid (**31**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 79% yield, mp = 155−156 °C, R_f = 0.69 (1:99 CH_3OH:CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6) δ 7.69−7.63 (m, 5H), 7.55 (d, J = 7.8 Hz, 2H), 7.47−7.42 (m, 2H), 7.37−7.32 (m, 1H), 7.15 (d, J = 9.3 Hz, 1H), 5.31 (s, 2H), 4.07 (q, J = 6.6 Hz, 2H), 3.64 (s, 2H), 2.35 (s, 3H), 2.24 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 443.4 (M + H$^+$). Compound **31** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 91% yield, mp = 215−216 °C, R_f = 0.29 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ 7.70−7.63 (m, 5H), 7.56 (d, J = 8.4 Hz, 2H), 7.48−7.43 (m, 2H), 7.38−7.33 (m, 1H), 7.16 (d, J = 9.0 Hz, 1H), 5.32 (s, 2H), 3.57 (s, 2H), 2.35 (s, 3H), 2.25 (s, 3H). LC/MS m/z 415.3 (M + H$^+$). Anal. ($C_{26}H_{22}O_5$·0.2H_2O): C, 74.70; H, 5.40. Found: C, 74.44; H; 5.15.

*4-(7-([1,1′-Biphenyl]-4-ylmethoxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl)butanoic Acid (**32**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 89% yield, mp = 127−128 °C, R_f = 0.22 (1:4 EtOAc:hexanes). ^1H NMR (300 MHz, DMSO-d_6) δ 7.69 (t, J = 7.8 Hz, 4H), 7.58 (t, J = 7.5 Hz, 3H), 7.47 (t, J = 7.8 Hz, 2H), 7.38 (d, J = 6.9 Hz, 1H), 7.13 (d, J = 9.0 Hz, 1H), 5.31 (s, 2H), 4.02 (q, J = 6.9 Hz, 2H), 2.58 (t, J = 6.9 Hz, 2H), 2.37−2.33 (m, 5H), 2.25 (s, 3H), 1.76−1.67 (m, 2H), 1.16 (t, J = 7.2 Hz, 3H). LC/MS m/z 470.8 (M + H$^+$). Compound **32** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 91% yield, mp = 238−239 °C, R_f = 0.47 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ 12.04 (s, 1H), 7.69 (t, J = 8.1 Hz, 4H), 7.61 (d, J = 9.0 Hz, 1H), 7.57 (d, J = 8.1 Hz, 2H), 7.47 (t, J = 7.2 Hz, 2H), 7.38 (d, J = 7.2 Hz, 1H), 7.14 (d, J = 9.0 Hz, 1H), 5.32 (s, 2H), 2.58 (t, J = 7.2 Hz, 2H), 2.39 (s, 3H), 2.30−2.26 (m 5H), 1.73−1.64 (m, 2H). LC/MS m/z 442.9 (M + H$^+$). Anal. ($C_{28}H_{26}O_5$): C, 76.00; H, 5.92. Found: C, 76.02; H, 5.92.

*3-(7-(Biphenyl-4-ylmethoxy)-8-methyl-2-oxo-2H-chromen-3-yl)-propanoic Acid (**33**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 81% yield, mp = 209−210 °C, R_f = 0.21 (1:4 EtOAc:hexanes). ^1H NMR (300 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.69 (t, J = 8.1 Hz, 4H), 7.57 (d, J = 8.1 Hz, 2H), 7.47 (t, J = 8.1 Hz, 3H), 7.38 (d, J = 6.9 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 5.30 (s, 2H), 4.05 (q, J = 6.9 Hz, 2H), 2.73−2.59 (m, 4H), 2.25 (s, 3H), 1.15 (t, J = 6.9 Hz, 3H). LC/MS m/z 443.0 (M + H$^+$). Compound **33** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 96% yield, mp = 224−225 °C, R_f = 0.20 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.69 (t, J = 8.4 Hz, 4H), 7.57 (d, J = 8.1 Hz, 2H), 7.47 (t, J = 8.1 Hz, 3H), 7.38 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 5.30 (s, 2H), 2.67 (t, J = 6.9 Hz, 2H), 2.55 (t, J = 6.3 Hz, 2H), 2.26 (s, 3H). LC/MS m/z 415.0 (M + H$^+$). Anal. ($C_{26}H_{22}O_5$·0.2H_2O): C, 74.70; H, 5.40. Found: C, 74.66; H; 5.28.

*3-(7-(Biphenyl-4-ylmethoxy)-8-ethyl-4-methyl-2-oxo-2H-chromen-3-yl)propanoic Acid (**34**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: white powder, 27% yield, mp = 133−134 °C, R_f = 0.25 (1:4 EtOAc:hexanes). ^1H NMR (300 MHz, $CDCl_3$) δ 7.64−7.36 (m, 10H), 6.91 (d, J = 9.0 Hz, 1H), 5.23 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 3.00−2.93 (m, 4H), 2.61 (t, J = 7.8 Hz, 2H), 2.42 (s, 3H), 1.26−1.19 (m, 6H). LC/MS m/z 470.9 (M + H$^+$). Compound **34** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 95% yield, mp = 224−225 °C, R_f = 0.32 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.69 (t, J = 8.0 Hz, 4H), 7.63 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 2H), 7.47 (t, J = 7.0 Hz, 2H), 7.37 (t, J = 7.0 Hz, 1H), 7.16 (d, J = 9.0 Hz, 1H), 5.33 (s, 2H), 2.83−2.77 (m, 4H), 2.44−2.40 (m, 5H), 1.14 (t, J = 9.0 Hz, 3H). LC/MS m/z 442.9 (M + H$^+$). Anal. ($C_{28}H_{26}O_5$); C, 76.00; H, 5.92. Found: C, 75.82; H, 5.80.

*4-(7-([1,1′-Biphenyl]-4-ylmethoxy)-8-ethyl-4-methyl-2-oxo-2H-chromen-3-yl)butanoic Acid (**35**).* The synthetic procedure used was the same as described for compound **5**. 7-Hydroxycoumarin ester intermediate: light-yellow powder, 26% yield, mp = 112−113 °C, R_f = 0.20 (1:4 EtOAc:hexanes). ^1H NMR (300 MHz, $CDCl_3$) δ 7.61 (t, J = 8.4 Hz, 4H), 7.52−7.36 (m, 6H), 6.90 (d, J = 9.0 Hz, 1H), 5.23 (s, 2H), 4.13 (q, J = 7.2 Hz, 2H), 2.97 (q, J = 7.5 Hz, 2H), 2.70 (dd, J = 7.5, 9.9 Hz, 2H), 2.43−2.39 (m, 5H), 1.92−1.84 (m, 2H), 1.26 (t, J = 7.2 Hz, 3H), 1.22 (q, J = 7.5 Hz, 3H). LC/MS m/z 485.1 (M + H$^+$). Compound **35** was obtained as a white powder after NaOH hydrolysis of the ester intermediate, 50% yield, mp = 217−218 °C, R_f = 0.48 (80:18:2 $CHCl_3$:CH_3OH:CH_3NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ 12.04 (s, 1H), 7.70 (t, J = 8.4 Hz, 4H), 7.62 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.47 (t, J = 7.2 Hz, 2H), 7.38 (m, 1H), 7.16 (d, J = 9.0 Hz, 1H), 5.33 (s, 2H), 3.31 (s, 3H), 2.79 (q, J = 7.2 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 2.78 (t, J = 7.5 Hz, 2H), 1.73−1.66 (m, 2H), 1.14 (t, J = 7.5 Hz, 3H). LC/MS m/z 456.9 (M + H$^+$).

Bacterial Strains. The bacterial strains and plasmids used in this study and their sources are as described earlier.[30] Standard strains used for profiling analogues and their sources are as follows: *B. anthracis* Sterne 34F2 (Colorado Serum Co.), *S. aureus* ATCC 25923 (ATCC).

Fluorescent Resonance Energy Transfer (FRET) Assays of Helicase DNA Duplex Strand-Unwinding Activity. The FRET-based helicase activity assay was performed essentially as previously described[20,21] using labeled annealed oligodeoxynucleotides Hel-5′FAM:Hel-3′BHQ.[30] The assay is based on the helicase-mediated dissociation of two annealed oligonucleotides, one with a fluorescent label, the other bearing a quencher moiety. The replicative helicases of *B. anthracis* and *S. aureus* were prepared as previously described.[30] Mode of inhibition studies and K_i value determinations were done in triplicate, and data were analyzed using GraphPad Prizm 5.0 for four-parameter nonlinear curve fitting.

Malachite Green Assays of *S. aureus* Helicase ATPase Activity. The single-strand DNA stimulated ATPase activity of *S. aureus* helicase was measured with a colorimetric assay for the release of inorganic phosphate, essentially as developed by Baykov et al. (1988).[34] Samples were incubated in clear 96-well microplates in 50 μL comprised of 30 mM Tris-HCl(pH 8.0), 25 mM NaCl, 2 mM $MgCl_2$, 0.01% Triton X-100, 2.5 mM ATP, *S. aureus* helicase, and 22 nM phiX174 single-stranded DNA. Samples were incubated for 30 min at 37 °C. Then, 150 μL of a 0.45 μm filtered 3:1 mixture of 0.045% (w/w) Malachite Green (carbinol hydrochloride salt):4.2% (w/w) ammonium molybdate plus 0.2% Brij 35 (w/w) was added. After 10 min incubation at room temperature, the absorbency at 630 nm was measured for each sample in a Molecular Devices SpectraMax plate reader. ATPase activity was dependent on the presence of ATP, *S. aureus* helicase, and phiX174 DNA. About 20% of the ATP was hydrolyzed under these conditions, yielding a signal/background ratio of 4−5.

Minimum Inhibitory Concentration (MIC) and Bactericidal Assays. MIC values were determined by the broth microdilution method described in the CLSI (formerly NCCLS) guidelines.[35] MIC values were expressed in μM to facilitate comparisons with IC_{50} and CC_{50} values (Table 2) or in μg/mL for comparison to ciprofloxacin

(Table 4) and were determined in duplicate using a 10-point curve consisting of 2-fold dilutions of inhibitory compound from 100 to 0.2 μM. For bactericidality tests, inhibitors were examined in a standard method of LB broth culture of *S. aureus* ATCC 25923 cells followed by plating on LB agar media and counting colony-forming units.[36]

Determination of Mammalian Cytotoxicity. The cytotoxic concentration (CC_{50}) of compounds versus mammalian cells (HeLa) cultured in serum-free medium was determined as the concentration of compound that inhibits 50% of the conversion of MTS to formazan.[37,38] Values were determined in duplicate using a 10-point curve consisting of 2-fold dilutions of inhibitory compound from 100 to 0.2 μM. The "selectivity index" (SI) of a given agent is defined as the ratio of its mammalian cell cytotoxicity to its MIC value against *B. anthracis* or *S. aureus* (e.g., CC_{50}/MIC).

■ ASSOCIATED CONTENT

Supporting Information

Scatter plot of potency vs cytotoxicity for 21 coumarin-type helicase inhibitors. Scatter plot of anti-helicase potency vs cytotoxicity for 21 coumarin-type inhibitor analogues. Data are from Tables 1 (IC_{50}) and 2 (CC_{50}). Compound numbers are shown to the right of each data point. No significant correlation is observed between these two variables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Sa, *Staphylococcus aureus*; Ba, *Bacillus anthracis*; CA-MRSA, community-acquired methicillin-resistant *S. aureus*; FRET, fluorescence resonance energy transfer; SPA, scintillation proximity; SAR, structure−activity relationship; MIC, minimal inhibitory concentration; SI, selectivity index

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