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Original article

Synthesis, antimicrobial activity and possible mechanism of action of 9-bromo-substituted indolizinoquinoline-5,12-dione derivatives

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ABSTRACT

A series of 9-bromo-substituted indolizinoquinoline-5,12-dione derivatives was synthesized. Antimicrobial activity assessment indicates that compounds **1**, **26**, **27** and **28** exhibit strong activity against gram-positive bacterial strains, including *Beta-hemolytic streptococcus* CMCC32210, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Enterococcus faecalis* ATCC29212 and methicillin-resistant *S. aureus* ATCC43300 (MRSA). Compound **27** shows the best anti-MRSA activity with an MIC value of 0.031 μg/ml. To assess the mechanism of action, the inhibitory activities of compound **1** against DNA gyrase and DNA topoisomerase IV were also measured. The results indicate that compound **1** has strong inhibitory effects on the *Escherichia coli* DNA gyrase supercoiling activity and *S. aureus* Topo IV relaxing activity.

1. Introduction

Pathogen infections have threatened human's health for thousands years [1]. During past decades, with the abuse of antimicrobial agents, more and more drug-resistant pathogens were found. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) is a prominent pathogen, which causes a public health concern worldwide and associates with a high mortality [2–4]. Novel antimicrobial agents against MRSA have been introduced recently. However, the emergency of resistance and side effects for those agents raise the need for novel antimicrobial agents [5,6]. In this work, a series of 9-bromo-substituted indolizinoquinoline-5,12-dione derivatives was synthesized, and their antimicrobial activity was screened. The inhibitory activities against DNA gyrase and topoisomerase IV were also investigated.

2. Results and discussion

2.1. Chemistry

In our previous study of novel anticancer agents, the reaction of 6,7-dichloroquinoline-5,8-dione with active methylene agent and pyridine gave two main products, *N,N-syn* and *N,N-anti* indolizinoquinoline-5,12-dione derivatives [7]. According to the mechanism of heterocyclization proposed by Defant et al [8], four isomers, 9/7-bromo *N,N-syn* isomers and 9/7-bromo *N,N-anti* isomers, should be obtained theoretically if using 3-bromopyridine as material. In fact, only two main products, 7-bromo *N,N-syn* isomer and 7-bromo *N,N-anti* isomer, were obtained in our previous experiment [7]. In order to obtain the corresponding 9-bromo isomers, we examined the reaction conditions and have found that an increase of 3-bromopyridine amount and the reaction concentration improved the yield of 9-bromo products. When 12 equivalents of 3-bromopyridine was used, the reaction gave 9-bromo isomers as the main products, 9-bromo *N,N-syn* isomer (**1**) in 28% yield and 9-bromo *N,N-anti* isomer (**2**) in 16% yield, as well as 7-bromo *N,N-syn* isomer (**3**) in 20% yield and 7-bromo *N,N-anti* isomer (**4**) in 4% yield (Scheme 1). The NMR, MS spectra and melting point of compounds **3** and **4** are similar to our reported

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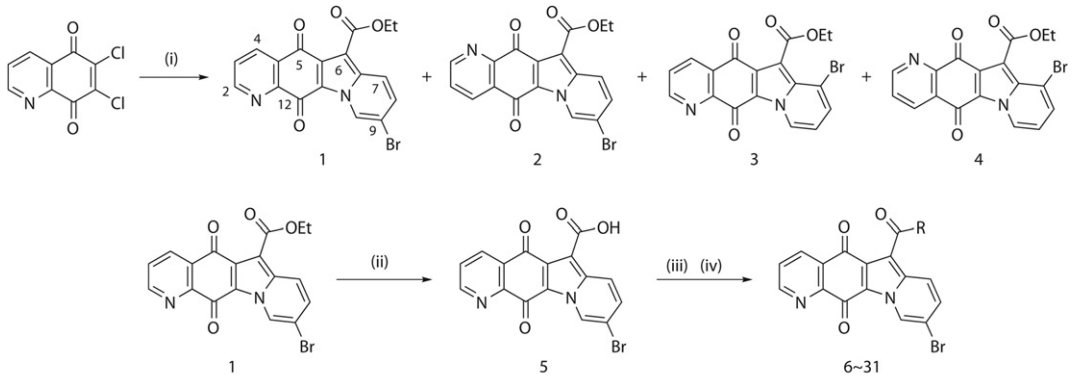


Scheme 1. Synthesis of compounds **1**–**31**.

results and confirm their structures [7]. HRMS spectrum of compound **1** and **2** shows they have the same formula, $C_{18}H_{11}N_2O_4Br$, as that of **3** and **4**. The HMBC spectrum of **1** shows a long-range hetero-correlation of C-5 at 178.8 ppm with 4-H at 8.57 ppm (dd, $J = 7.8$, 1.8 Hz). In the ^{1}H NMR spectrum, the signal at 10.10 ppm (dd, $J = 1.6$, 0.4 Hz) can be assigned to 10-H. As a consequence, the signals at 8.27 ppm and 7.56 ppm can be assigned to 7-H and 8-H, respectively. HRMS, 1D NMR and 2D NMR spectra of compound **1** prove its ethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate structure, also confirmed by X-ray single crystal analysis (Fig. 1). Similarly, compound **2** can be assigned as the 9-bromo *N*,*N*-*anti* isomer.

An antimicrobial screening indicates that compound **1** exhibits a strong activity against gram-positive bacterial strains, especially MRSA, but only a low solubility in water and organic solvent, such as dimethyl sulfoxide. This drawback pushed us to prepare its analogs to find more potent agents with higher antimicrobial activity and better solubility. The synthesis of these analogs, compounds **6**–**31**, is shown in Scheme 1. At first, we tried to convert compound **1** to its amide analogs according to Defant's method [9]. Unfortunately, it did not react with the corresponding amines under those conditions. Therefore, we hydrolyzed compound **1** in isopropanol with 15% K_2CO_3 aqueous solution to give a corresponding carboxylic acid **5** [10]. Following chlorination of compound **5** with thionyl chloride excess gave a corresponding acid chloride intermediate that was aminated or esterified to give the target products **6**–**31**. Their structures were elucidated by MS and NMR spectra.

2.2. Antimicrobial activity

The antimicrobial activity of compound **1** was firstly assessed by a standard two-fold microdilution assay in Mueller-Hinton broth against three gram-negative bacterial strains, one fungal strain, and sixteen gram-positive bacterial strains including twelve MRSA strains [11]. The minimum inhibition concentration (MIC) is summarized in Table 1. The results indicate that compound **1** exhibits a low activity against gram-negative bacterial strains and fungal strain. The MIC values are more than 64 μg/ml against *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), *S. paratyphi B* (CMCC50094) and *C. Albicans* (ATCC10231). However, compound **1** exhibits a strong activity against gram-positive bacterial strains, especially MRSA (ATCC43300) with a MIC value of 0.063 μg/ml, 16-fold superior to that of vancomycin. The activity of compound **1** against five clinical MRSA strains (72754, 071843, 82731, 071429 and 83160) is up to 0.016 μg/ml, 64-fold superior to that of vancomycin.

The antimicrobial activity of amide and ester derivatives **6**–**31** is summarized in Table 2. The results indicate that all products exhibit a low activity against fungal strain. Compared to compound **1**, its amide analogs **6**–**25** show a lower antimicrobial activity against bacterial and fungal strains, whereas ester analogs **26**–**31** exhibit a substantially higher activity against five gram-positive bacterial strains than its amide analogs. It is clear that alkoxycarbonyl group plays an important role in the antimicrobial activity of ester products. It is noteworthy that compound **27**, whose solubility in dimethyl sulfoxide is three times better than that of compound **1**, exhibits stronger anti-MRSA activity than compound **1**.

2.3. Inhibitory activities against DNA gyrase and DNA topoisomerase IV

To assess the mechanism of antimicrobial activity, DNA gyrase supercoiling assay was performed with compound **1** using relaxed pHOT1 DNA as a substrate. Ciprofloxacin (CFX) was used as

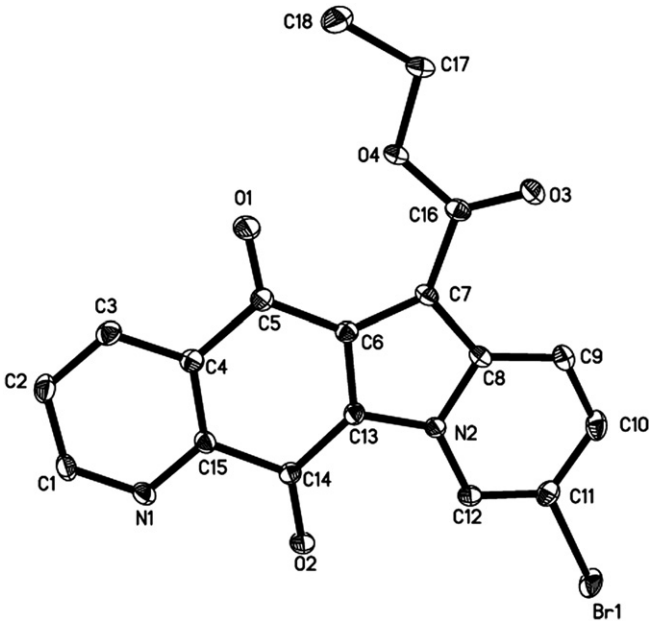


Fig. 1. Perspective ORTEP drawing of compound **1**.

Table 1
In vitro antimicrobial activity of compound **1**.

Microorganism	MIC (μg/ml)[a]	
	1	Vancomycin
E. coli ATCC25922	>64.00	nd[b]
P. aeruginosa ATCC27853	64.00	nd
S. paratyphi B CMCC50094	>64.00	nd
H. streptococcus B CMCC32210	0.031	nd
S. aureus ATCC25923	0.063	nd
S. epidermidis ATCC12228	0.063	nd
E. faecalis ATCC29212	0.016	nd
C. albicans ATCC10231	>64.00	nd
MRSA		
ATCC43300	0.063	1.00
80754[c]	0.063	0.50
7365	0.063	2.00
0441	0.032	1.00
071410	0.032	0.50
72754	0.016	1.00
071843	0.016	1.00
82731	0.016	1.00
071429	0.016	1.00
83160	0.016	1.00
82896	0.032	1.00
82924	0.032	1.00

[a] The antimicrobial activity was expressed by the minimum inhibitory concentration, which was obtained in at least two independent experiments.
[b] "nd" means "not determined".
[c] These MRSA strains were clinically isolated from Guangzhou Liuhuaqiao Hospital, China.

a positive control. As shown in Fig. 2A, compound **1** shows a strong inhibition of the supercoiling activity of *E. coli* DNA gyrase but lesser than that of CFX. DNA gyrase supercoiling activity is inhibited over 78% and 98% at 1 μM and 100 μM, respectively. Likely to CFX, compound **1** does not exhibit a supercoiling inhibitory activity against *S. aureus* DNA gyrase at 125 μM (data not shown) [12].

DNA topoisomerase IV (Topo IV) relaxation assay was performed with compound **1** using supercoiled pBR322 DNA as a substrate. CFX was used as a positive control. As shown in Fig. 2B, likely to CFX, compound **1** exhibits a slight inhibitory activity against *S. aureus* Topo IV at 1 μM, and completely inhibits the relaxing activity at 25 μM. On the contrary, compound **1** is not effective against *E. coli* Topo IV at 125 μM (data not shown).

Topo IV decatenation assay was performed with compound **1** using catenated kDNA as a substrate. CFX was used as a positive control. As shown in Fig. 2C, the decatenation activity of *S. aureus* Topo IV is almost completely inhibited by CFX at 25 μM. Compound **1** has a lower inhibitory activity than CFX. There is 84% monomer circle DNA remained after being treated with compound **1** at 125 μM concentration.

3. Conclusion

In conclusion, we have synthesized a series of 9-bromo-substituted indolizinoquinoline-5,12-dione derivatives. The ester analogs exhibit strong antibacterial activity against gram-positive strains, especially MRSA. The MIC values of compounds **1**, **26** and **28** against MRSA are up to 0.063 μg/ml, 16-fold superior to that of vancomycin. Compound **27** shows the best activity with a MIC value of 0.031 μg/ml, 32-fold superior to that of vancomycin. DNA gyrase supercoiling assay and Topo IV inhibition assays indicate that compound **1** shows the mechanism of action similar to ciprofloxacin [12]. It effectively inhibits the supercoiling activity of *E. coli* DNA gyrase and the relaxation activity of *S. aureus* Topo IV, but has only a low decatenation inhibitory activity against *S. aureus* Topo IV. Our findings indicate that DNA gyrase and Topo IV might be the biological target for compounds under study.

4. Methods and materials

4.1. General experiments

The major chemical reagents for synthesis were purchased from Alfa Aesar or Sigma Aldrich Co. 6,7-Dichloroquinoline-5,8-dinone was prepared according to our reported method [7]. The common solvents were obtained from local commercial suppliers and used without further purification. Ciprofloxacin and vancomycin, used as positive control for biological assay, were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chemical reaction courses were monitored by silica gel GF_{254} thin layer chromatography. Melting points were determined in open capillary tubes on a MPA100 Optimelt Automated Melting Point System without being corrected. Nuclear magnetic resonance spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer using tetramethylsilane as an internal reference. Mass spectra were analyzed on an Agilent 6120 (Quadrupole LC-MS) mass spectrometer. The high-resolution mass spectra were analyzed on a SHIMADZU LCMS-IT-TOF mass spectrometer. UV spectra were recorded on a SHIMADZU UV-2501 PC spectrophotometer. All compounds tested for biological activity were analyzed by HPLC and their contents were more than 95%.

4.2. Synthesis of compounds *1–4*

To a yellow suspension of 6,7-dichloroquinoline-5,8-dione (1.14 g, 5.00 mmol) in absolute ethanol (25 ml), ethyl acetoacetate (1.20 ml, 10.00 mmol) and 3-bromopyridine (6.00 ml, 60.00 mmol) were added. The resultant solution was stirred and refluxed for 5 h. The reaction solution gradually became a dark red suspension. After completion of reaction, the reaction solution was cooled to room temperature. The precipitate was collected by filtration and purified by silica gel column chromatography with eluent CH_2Cl_2:AcOEt = 30:1. Four products, **1** (0.56 g, 28%), **2** (0.32 g, 16%), **3** (0.40 g, 20%) and **4** (0.08 g, 4%), were obtained. The structures of products **3** and **4** were identified by mass and NMR spectra, which were similar to those reported in our previous reference [7].

4.2.1. Ethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g] quinoline-6-carboxylate (*1*)

Orange solid, mp = 279.8–281.8 °C. ^{1}H NMR ($CDCl_3$) δ 10.10 (dd, J = 1.6, 0.4 Hz, 1H, 10-H), 9.05 (dd, J = 4.6, 1.8 Hz, 1H, 2-H), 8.57 (dd, J = 7.8, 1.8 Hz, 1H, 4-H), 8.27 (dd, J = 9.6, 0.4 Hz, 1H, 7-H), 7.67 (dd, J = 8.0, 4.8 Hz, 1H, 3-H), 7.56 (dd, J = 9.6, 1.6 Hz, 1H, 8-H), 4.52 (q, J = 7.1 Hz, 2H, $-OCH_2-$), 1.50 (t, J = 7.1 Hz, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$) δ 178.8 (C-5), 173.3 (C-12), 162.7 ($-COO-$), 154.2 (C-2), 149.2 (C-12a), 138.1 (C-6a), 135.5 (C-4), 131.9 (C-8), 131.0 (C-4a), 128.4 (C-10), 127.8 (C-5a), 127.2 (C-3), 122.8 (C-11a), 121.6 (C-7), 113.5 (C-6), 107.1 (C-9), 61.4 ($-CH_2-$), 14.3 ($-CH_3$). ESI-MS m/z: 401.0 (100%), 399.0 (88%) $[M+H]^+$. HRMS (ESI) m/z: 398.9988 $[M+H]^+$, calcd for $C_{18}H_{12}N_2O_4Br$ 398.9980. The structure of compound **1** was confirmed with 2D NMR spectra and single crystal analysis. Compound **1** was crystallized from $CHCl_3$/AcOEt as orange block crystals. Molecular formula = $C_{18}H_{11}N_2O_4Br$, molecular mass = 399.20, monoclinic, a = 7.1719(3) Å, b = 12.1221(5) Å, c = 17.0506(8) Å, β = 90.658(4)°, U = 1482.25(12) Å^3, T = 153, space group $P2_1/c$ (no. 14), Z = 4, μ (Cu Kα) = 4.044, 5836 reflections measured, 2698 unique (R_{int} = 0.0240) which were used in all calculations. The final R (reflections) = 0.0391, w$R2$ (reflections) = 0.1075. Crystallographic data for compound **1** has been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 828381. Copies of the data can be obtained, free of charge, on application to CCDC, 12

Table 2
Structures and *in vitro* antimicrobial activity of compounds **6**–**31**.

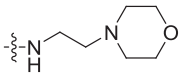
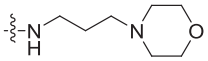
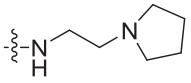
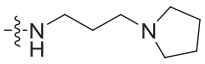
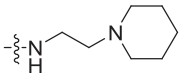
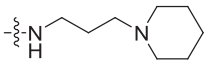
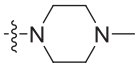
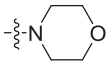
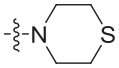
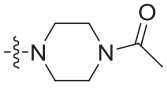
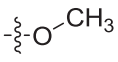
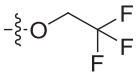
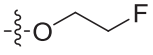
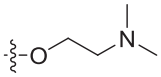
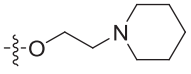
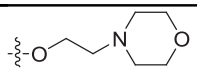
Comp	*R*	MIC (μg/ml)[a]								
		E. coli ATCC25922	*P. aeruginosa* ATCC27853	*S. paratyphi B* CMCC50094	*H. streptococcus B* CMCC32210	*S. aureus* ATCC25923	*S. epidermidis* ATCC12228	*E. faecalis* ATCC29212	MRSA ATCC43300	*C. albicans* ATCC10231
6	$-NH(CH_2)_2CH_3$	–[b]	–	–	–	–	–	–	–	–
7	$-NHCH(CH_3)_2$	–	–	–	–	–	–	–	–	–
8	$-NH(CH_2)_3CH_3$	–	–	–	–	–	–	–	–	–
9	$-NEt_2$	>64.00	>64.00	>64.00	8.00	8.00	16.00	16.00	16.00	>64.00
10	$-NHCH_2Ph$	–	–	–	–	–	–	–	–	–
11	$-NH(CH_2)_2NMe_2$	>64.00	>64.00	>64.00	0.50	4.00	2.00	1.00	4.00	>64.00
12	$-NH(CH_2)_3NMe_2$	>64.00	>64.00	>64.00	1.00	4.00	8.00	4.00	4.00	>64.00
13	$-NH(CH_2)_2NEt_2$	>64.00	>64.00	>64.00	0.50	4.00	2.00	4.00	4.00	>64.00
14	$-NH(CH_2)_3NEt_2$	>64.00	>64.00	>64.00	1.00	8.00	4.00	4.00	8.00	>64.00
15		64.00	64.00	>64.00	0.50	1.00	4.00	4.00	8.00	>64.00
16		>64.00	>64.00	>64.00	1.00	8.00	4.00	4.00	4.00	>64.00
17		>64.00	>64.00	>64.00	0.50	4.00	1.00	2.00	4.00	>64.00
18		>64.00	>64.00	>64.00	1.00	8.00	4.00	4.00	4.00	>64.00
19		64.00	64.00	64.00	0.50	1.00	1.00	2.00	2.00	>64.00
20		>64.00	>64.00	>64.00	1.00	2.00	8.00	2.00	4.00	>64.00
21		>64.00	>64.00	>64.00	16.00	8.00	32.00	>64.00	64.00	>64.00
22		>64.00	>64.00	>64.00	8.00	16.00	32.00	>64.00	32.00	>64.00
23		>64.00	>64.00	>64.00	16.00	16.00	32.00	>64.00	64.00	>64.00
24		>64.00	>64.00	>64.00	64.00	>64.00	>64.00	>64.00	64.00	>64.00
25		>64.00	>64.00	>64.00	16.00	64.00	>64.00	>64.00	>64.00	>64.00
26		64.00	>64.00	>64.00	0.031	0.031	0.016	0.031	0.063	>64.00
27		>64.00	>64.00	>64.00	0.031	0.031	0.016	0.016	0.031	>64.00
28		>64.00	>64.00	>64.00	0.031	0.031	0.016	0.016	0.063	>64.00
29		2.00	>64.00	1.00	0.031	0.13	0.13	0.13	0.13	>64.00
30		4.00	>64.00	16.00	0.031	0.063	0.063	0.031	0.50	>64.00

Table 2 (*continued*)

Comp	*R*	MIC (μg/ml)[a]								
		E. coli ATCC25922	*P. aeruginosa* ATCC27853	*S. paratyphi B* CMCC50094	*H. streptococcus B* CMCC32210	*S. aureus* ATCC25923	*S. epidermidis* ATCC12228	*E. faecalis* ATCC29212	MRSA ATCC43300	*C. albicans* ATCC10231
31		32.00	>64.00	>64.00	0.063	0.25	0.25	0.13	0.25	>64.00
Pen[c]		64.00	>64.00	2.00	<0.031	<0.031	16.00	2.00	>64.00	nd[d]

[a] The MIC values represent the results obtained in at least two independent experiments.
[b] "-" means "not determined" because of poor solubility of the compound.
[c] "Pen" means the standard penicillin.
[d] "nd" means "not determined".

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4.2.2. Ethyl 8-bromo-5,12-dioxo-5,12-dihydroindolizino[3,2-g] quinoline-11-carboxylate (**2**)

Orange solid, mp = 266.1–268.9 °C. ^{1}H NMR ($CDCl_3$) δ 9.98 (dd, J = 1.6, 0.8 Hz, 1H), 9.03 (dd, J = 4.4, 1.6 Hz, 1H), 8.57 (dd, J = 8.0, 1.6 Hz, 1H), 8.30 (dd, J = 9.6, 0.8 Hz, 1H), 7.69 (dd, J = 8.0, 4.4 Hz, 1H), 7.54 (dd, J = 9.6, 1.6 Hz, 1H), 4.50 (q, J = 7.2 Hz, 2H), 1.52 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 177.9, 174.0, 163.1, 154.0, 149.6, 138.3, 134.4, 131.7, 130.3, 128.1, 127.3, 121.6, 113.4, 107.6, 61.5, 14.1. ESI-MS *m/z*: 399.0 (100%), 401.0 (98%) $[M + H]^+$. HRMS (ESI) *m/z*: 420.9794 $[M + Na]^+$, calcd for $C_{18}H_{11}N_2O_4BrNa$ 420.9800.

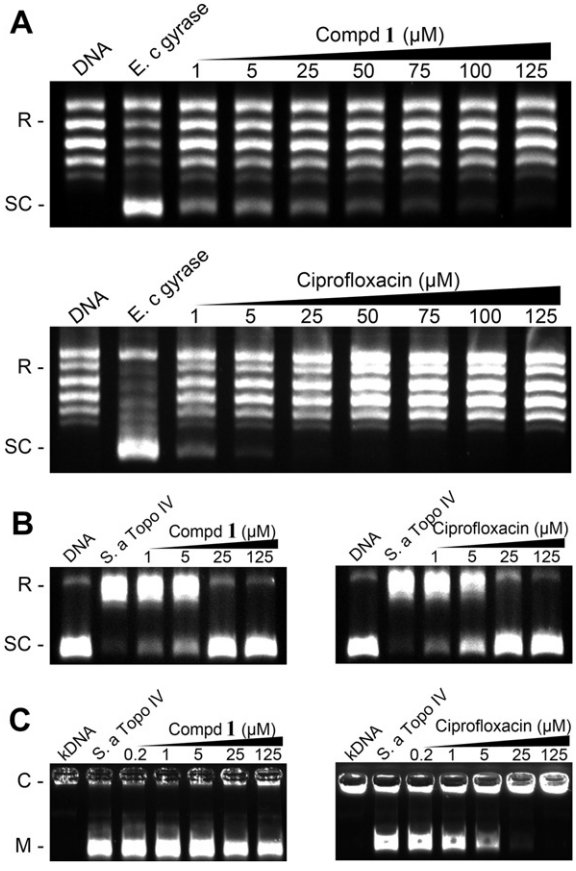

Fig. 2. (A) Inhibition of the supercoiling activity of *E. coli* gyrase by compound **1** (Upper panel) or ciprofloxacin (Bottom panel). Lane 1, relaxed pHOT1 DNA only. Lane 2, relaxed pHOT1 DNA and enzyme. Lane 3–9, relaxed pHOT1 DNA, enzyme and compound at various concentrations. (B) Inhibition of *S. aureus* Topo IV-catalyzed DNA relaxation by compound **1** (Left panel) or ciprofloxacin (Right panel). Lane 1, pBR322 DNA only. Lane 2, pBR322 DNA and enzyme. Lane 3–6, pBR322 DNA, enzyme and compound at various concentrations. (C) Inhibition of *S. aureus* Topo IV-catalyzed decatenation by compound **1** (Left panel) or ciprofloxacin (Right panel). Lane 1, kDNA only. Lane 2, kDNA and enzyme. Lane 3–6, kDNA, enzyme and compound at various concentrations. Key: R, relaxed DNA; SC, supercoiled DNA; C, catenated kDNA; M, monomer circle DNA.

4.3. Synthesis of 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g] quinoline-6-carboxylic acid (**5**)

To a yellow suspension of compound **1** (1.60 g, 4.00 mmol) in isopropanol (250 ml), an aqueous solution of K_2CO_3 (15%, 30 ml) was added. The yellow suspension was stirred at room temperature for 15 min, and then refluxed for 24 h to give a red suspension. The suspension was cooled to room temperature. The solvent was removed in vacuo. The residue was dissolved in water (2 L), filtered to remove the undissolved precipitate. The filtrate was adjusted to pH 3 with 2 M aqueous HCl to give abundant aubergine precipitate. The resultant precipitate was collected by filtration and washed with water, and then dried in vacuo to give an aubergine solid **5** (1.40 g), yield 94%, mp > 300 °C. ^{1}H NMR ($CDCl_3$) δ 9.93 (br.s, 1H), 8.95 (d, J = 4.4 Hz, 1H), 8.46 (d, J = 7.6 Hz, 1H), 8.21 (d, J = 9.6 Hz, 1H), 7.54 (dd, J = 7.6, 4.4 Hz, 1H), 7.34 (d, J = 9.6 Hz, 1H). ESI-MS *m/z*: 370.9 $[M+H]^+$.

4.4. General procedure for the synthesis of compounds **6-31**

At room temperature, to a red solution of compound **5** (0.19 g, 0.50 mmol) and triethylamine (0.14 ml, 1.00 mmol) in chloroform (40 ml), thionyl chloride (2.5 ml) was added dropwise. The mixture was stirred and refluxed for 5 h. The mixture gradually became a red solution. The reaction solution was then cooled to room temperature. The solvent was evaporated under reduced pressure. The residue was contained under reduced pressure for a period to get rid of most of the residual $SOCl_2$ to give an orange solid residue. 4-(Dimethylamino)pyridine (0.07 g, 0.6 mmol) and different amine or alcohol derivative (1.80 mmol) in $CHCl_3$ (30 ml) were added dropwise to the resultant residue. The reaction mixture instantaneously became a red solution. The reaction mixture was refluxed for 5 h, and cooled to room temperature. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography.

4.4.1. N-Propyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g] quinoline-6-carboxamide (**6**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:AcOEt = 11:1 to give an aubergine solid **6** (0.20 g), yield 96%, mp = 280.2–283.1 °C. ^{1}H NMR ($CDCl_3$) δ 10.19 (t, J = 5.6 Hz, 1H), 10.10 (br.s, 1H), 9.06 (dd, J = 4.8, 1.2 Hz, 1H), 9.04 (d, J = 9.6 Hz, 1H), 8.58 (dd, J = 7.8, 1.2 Hz, 1H), 7.69 (dd, J = 7.8, 4.6 Hz, 1H), 7.53 (dd, J = 9.6, 1.5 Hz, 1H), 3.50–3.45 (m, 2H), 1.76 (sext,

J = 7.4 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 183.0, 172.5, 162.2, 155.0, 149.5, 139.2, 135.7, 131.7, 130.2, 127.7, 127.0, 124.3, 124.1, 121.9, 114.8, 111.3, 41.4, 22.7, 11.7. UV/vis λ_{max} ($CHCl_3$, ε): 259 (26,100), 334 (8500), 346 (9100), 367 (5300), 503 (4400) nm. ESI-MS *m/z*: 414.1 (100%), 412.1 (97%) $[M + H]^+$. HRMS (ESI) *m/z*: 412.0289 $[M + H]^+$, calcd for $C_{19}H_{15}N_3O_3Br$ 412.0297.

4.4.2. N-Isopropyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**7**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:AcOEt = 8:1 to give a bright red solid **7** (0.19 g), yield 92%; mp = 258.4–260.2 °C. ^{1}H NMR ($CDCl_3$) δ 10.16–10.08 (m, 2H), 9.09–9.02 (m, 2H), 8.59 (d, J = 8.0 Hz, 1H), 7.69 (dd, J = 7.8, 4.6 Hz, 1H), 7.54 (dd, J = 9.6, 1.6 Hz, 1H), 4.33–4.25 (m, 1H), 1.39 (d, J = 6.6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 183.0, 172.5, 161.4, 155.0, 149.5, 139.3, 135.7, 131.7, 130.2, 127.7, 127.0, 124.3, 124.1, 121.9, 114.8, 111.7, 41.6, 22.7. UV/vis λ_{max} ($CHCl_3$, ε): 260 (16,400), 345 (5700), 504 (2800) nm. ESI-MS *m/z*: 412.0 (100%), 414.0 (97%) $[M + H]^+$. HRMS (ESI) *m/z*: 412.0310 $[M + H]^+$, calcd for $C_{19}H_{15}N_3O_3Br$ 412.0297.

4.4.3. N-Butyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**8**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:AcOEt = 11:1 to give a red solid **8** (0.19 g), yield 87%, mp = 264.8–267.1 °C. ^{1}H NMR ($CDCl_3$) δ 10.16 (t, J = 4.6 Hz, 1H), 10.07 (br.s, 1H), 9.06 (d, J = 4.4 Hz, 1H), 9.01 (d, J = 9.6 Hz, 1H), 8.57 (d, J = 7.6 Hz, 1H), 7.68 (dd, J = 7.6, 4.4 Hz, 1H), 7.52 (dd, J = 9.6, 1.4 Hz, 1H), 3.52–3.47 (m, 2H), 1.72 (quint, J = 7.3 Hz, 2H), 1.52 (sext, J = 7.4 Hz, 2H), 1.02 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 182.9, 172.5, 162.1, 155.0, 149.4, 139.2, 135.7, 131.7, 130.2, 127.7, 127.0, 124.2, 124.1, 121.9, 114.8, 111.3, 39.4, 31.5, 20.4, 13.9. UV/vis λ_{max} ($CHCl_3$, ε): 259 (17,500), 346 (6000), 366 (3500), 504 (2900) nm. ESI-MS *m/z*: 426.0 (100%), 428.0 (98%) $[M + H]^+$. HRMS (ESI) *m/z*: 426.0473 $[M + H]^+$, calcd for $C_{20}H_{17}N_3O_3Br$ 426.0453.

4.4.4. N,N-Diethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**9**)

The product was purified by silica gel column chromatography with eluent CH_2Cl_2:AcOEt:MeOH = 60:3:1 to give a red solid **9** (0.20 g), yield 93%, mp = 281.9–284.3 °C. ^{1}H NMR ($CDCl_3$) δ 9.92 (dd, J = 1.6, 0.8 Hz, 1H), 9.04 (dd, J = 4.8, 1.6 Hz, 1H), 8.51 (dd, J = 7.8, 1.6 Hz, 1H), 7.64 (dd, J = 7.8, 4.8 Hz, 1H), 7.59 (dd, J = 9.2, 0.8 Hz, 1H), 7.41 (dd, J = 9.2, 1.6 Hz, 1H), 3.95–3.86 (m, 1H), 3.59–3.51 (m, 1H), 3.40–3.21 (m, 2H), 1.42 (t, J = 7.0 Hz, 3H), 1.02 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 180.1, 172.3, 163.2, 154.4, 150.5, 135.0, 134.9, 130.3, 130.2, 128.2, 126.8, 125.1, 121.0, 120.1, 113.4, 113.0, 43.2, 39.6, 14.5, 12.8. UV/vis λ_{max} (MeCN, ε): 257 (32,900), 334 (9500), 479 (6400) nm. ESI-MS *m/z*: 426.1 (100%), 428.0 (92%) $[M + H]^+$. HRMS (ESI) *m/z*: 426.0473 $[M + H]^+$, calcd for $C_{20}H_{17}N_3O_3Br$ 426.0453.

4.4.5. N-Benzyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**10**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:AcOEt = 8:1 to give an aubergine solid **10** (0.19 g), yield 81%, mp = 264.6–266.2 °C. ^{1}H NMR ($CDCl_3$) δ 10.62 (t, J = 5.2 Hz, 1H), 10.10 (br.s, 1H), 9.07–9.04 (m, 2H), 8.53 (dd, J = 8.0, 1.6 Hz, 1H), 7.65 (dd, J = 8.0, 4.6 Hz, 1H), 7.54 (dd, J = 9.8, 1.6 Hz, 1H), 7.47–7.45 (m, 2H), 7.40–7.36 (m, 2H), 7.32–7.27 (m, 1H), 4.72 (d, J = 5.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 183.0, 172.6, 162.3, 155.1, 149.4, 139.4, 138.5, 135.8, 131.9, 130.2, 128.7, 127.8, 127.7, 127.3, 127.1, 124.4, 124.1, 122.0, 114.8, 110.9, 43.6. UV/vis λ_{max} ($CHCl_3$, ε): 260 (52,500), 334 (16,600), 344 (17,800), 366 (10,400), 501 (8400) nm. ESI-MS *m/z*: 460.1 (100%), 462.1 (96%) $[M + H]^+$. HRMS (ESI) *m/z*: 460.0297 $[M + H]^+$, calcd for $C_{23}H_{15}N_3O_3Br$ 460.0297.

4.4.6. N-(2-(Dimethylamino)ethyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**11**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH = 12:1 to give a red solid **11** (0.20 g), yield 93%, mp = 234.8–236.4 °C. ^{1}H NMR ($CDCl_3$) δ 10.25 (t, J = 5.2 Hz, 1H), 10.12 (br.s, 1H), 9.07 (dd, J = 4.8, 1.6 Hz, 1H), 9.03 (d, J = 10.0 Hz, 1H), 8.59 (dd, J = 7.6, 1.6 Hz, 1H), 7.68 (dd, J = 7.6, 4.8 Hz, 1H), 7.54 (dd, J = 9.6, 1.6 Hz, 1H), 3.66 (q, J = 6.1 Hz, 2H), 2.69 (t, J = 6.6 Hz, 2H), 2.40 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 182.9, 172.6, 162.4, 155.0, 149.5, 139.2, 135.7, 131.7, 130.2, 127.8, 127.0, 124.5, 124.0, 121.9, 114.7, 111.1, 58.3, 45.5, 37.7. UV/vis λ_{max} (MeCN, ε): 259 (34,500), 332 (11,100), 364 (6,300), 491 (5,000) nm. ESI-MS *m/z*: 441.0 (100%), 443.0 (98%) $[M + H]^+$. HRMS (ESI) *m/z*: 441.0557 $[M + H]^+$, calcd for $C_{20}H_{18}N_4O_3Br$ 441.0562.

4.4.7. N-(3-(Dimethylamino)propyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**12**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH = 14:1 to give a red solid **12** (0.20 g), yield 87%, mp = 165.6–169.1 °C. ^{1}H NMR ($CDCl_3$) δ 10.21 (t, J = 5.2 Hz, 1H), 10.13 (dd, J = 1.6, 0.8 Hz, 1H), 9.07 (dd, J = 4.8, 1.8 Hz, 1H), 9.04 (dd, J = 9.6, 0.8 Hz, 1H), 8.58 (dd, J = 7.8, 1.8 Hz, 1H), 7.68 (dd, J = 8.0, 4.4 Hz, 1H), 7.54 (dd, J = 9.6, 1.6 Hz, 1H), 3.58 (q, J = 6.4 Hz, 2H), 2.54 (t, J = 7.4 Hz, 2H), 2.36 (s, 6H), 1.96 (quint, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 183.1, 172.6, 162.4, 155.1, 149.6, 139.3, 135.7, 131.8, 130.3, 127.8, 127.1, 124.4, 124.1, 122.0, 114.8, 111.2, 57.2, 45.3, 37.6, 27.3. UV/vis λ_{max} (MeCN, ε): 259 (50,000), 339 (15,500), 489 (6600) nm. ESI-MS *m/z*: 457.1 (100%), 455.1 (98%) $[M + H]^+$. HRMS (ESI) *m/z*: 455.0698 $[M + H]^+$, calcd for $C_{21}H_{20}N_4O_3Br$ 455.0719.

4.4.8. N-(2-(Diethylamino)ethyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**13**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH = 15:1 to give a red solid **13** (0.21 g), yield 91%, mp = 200.0–202.6 °C. ^{1}H NMR ($CDCl_3$) δ 10.28 (t, J = 5.2 Hz, 1H), 10.10 (s, 1H), 9.07 (d, J = 4.0 Hz, 1H), 9.00 (d, J = 10.0 Hz, 1H), 8.57 (d, J = 8.0 Hz, 1H), 7.69 (dd, J = 7.6, 4.8 Hz, 1H), 7.54 (d, J = 9.6 Hz, 1H), 3.70 (q, J = 6.3 Hz, 2H), 2.91 (t, J = 6.8 Hz, 2H), 2.79 (q, J = 7.1 Hz, 4H), 1.18 (t, J = 7.2 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 182.6, 172.6, 162.5, 155.0, 149.5, 139.2, 135.7, 131.8, 130.2, 127.8, 127.1, 124.5, 124.0, 121.9, 114.7, 110.9, 51.5, 47.1, 37.2, 11.2. UV/vis λ_{max} (MeCN, ε): 259 (43,200), 332 (13,700), 364 (7,700), 494 (6,300) nm. ESI-MS *m/z*: 469.1 (100%), 471.1 (88%) $[M + H]^+$. HRMS (ESI) *m/z*: 469.0881 $[M + H]^+$, calcd for $C_{22}H_{22}N_4O_3Br$ 469.0875.

4.4.9. N-(3-(Diethylamino)propyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**14**)

The product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH = 15:1 to give a red solid **14** (0.23 g), yield 94%, mp = 243.3–244.9 °C. ^{1}H NMR ($CDCl_3$) δ 10.24 (t, J = 5.5 Hz, 1H), 10.05 (d, J = 1.2 Hz, 1H), 9.02 (dd, J = 4.6, 1.8 Hz, 1H), 8.88 (d, J = 10.0 Hz, 1H), 8.50 (dd, J = 8.0, 2.0 Hz, 1H), 7.64 (dd, J = 8.0, 4.8 Hz, 1H), 7.48 (dd, J = 10.0, 1.8 Hz, 1H), 3.56 (q, J = 6.0 Hz, 2H), 3.10–2.96 (m, 6H), 2.23–2.12 (m, 2H), 1.31 (t, J = 7.2 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 182.2, 171.7, 161.8, 154.2, 148.4, 138.2, 134.8, 131.0, 129.2, 126.8, 126.3, 123.4, 122.7, 121.0, 113.8, 109.4, 48.5, 45.6, 36.0, 23.4, 8.1. UV/vis λ_{max} (MeCN, ε): 260 (30,800), 332 (10,000), 340 (9800), 489 (4400) nm. ESI-MS *m/z*: 485.2 (100%), 483.2 (93%) $[M + H]^+$. HRMS (ESI) *m/z*: 483.1051 $[M + H]^+$, calcd for $C_{23}H_{24}N_4O_3Br$ 483.1032.

4.4.10. N-(2-Morpholinoethyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**15**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH:Et_3N = 30:1:0.04 to give a red solid **15** (0.20 g) as, yield 81%, mp = 227.5–228.4 °C. ^{1}H NMR

($CDCl_3$) δ 10.32 (t, J = 5.2 Hz, 1H), 10.14 (d, J = 1.6 Hz 1H), 9.08 (dd, J = 4.8, 1.6 Hz, 1H), 9.04 (d, J = 9.6 Hz, 1H), 8.58 (dd, J = 8.0, 1.6 Hz, 1H), 7.70 (dd, J = 8.0, 4.4 Hz, 1H), 7.55 (dd, J = 9.6, 1.6 Hz, 1H), 3.82 (t, J = 4.4 Hz, 4H), 3.67 (q, J = 6.0 Hz, 2H), 2.71 (t, J = 6.2 Hz, 2H), 2.62 (s, br. 4H). ^{13}C NMR ($CDCl_3$) δ 182.7, 172.6, 162.3, 155.0, 149.5, 139.2, 135.6, 131.7, 130.2, 127.8, 127.1, 124.4, 124.0, 121.9, 114.7, 111.0, 67.0, 57.2, 53.5, 36.6. UV/vis λ_{max} (MeCN, ε): 259 (34,400), 331 (11,000), 365 (6200), 492 (4900) nm. ESI-MS *m/z*: 483.1 (90%), 485.1 (100%) $[M + H]^+$. HRMS (ESI) *m/z*: 483.0647 $[M + H]^+$, calcd for $C_{22}H_{20}N_4O_4Br$ 483.0668.

4.4.11. N-(3-Morpholinopropyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**16**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH = 25:1 to give a bright red solid **16** (0.23 g), yield 92%, mp = 230.1–232.8 °C. ^{1}H NMR ($CDCl_3$) δ 10.22 (t, J = 5.2 Hz, 1H), 10.11 (dd, J = 1.8, 0.8 Hz, 1H), 9.07 (dd, J = 4.8, 1.6 Hz, 1H), 9.04 (dd, J = 9.6, 0.8 Hz, 1H), 8.56 (dd, J = 8.0, 1.6 Hz, 1H), 7.69 (dd, J = 8.0, 4.4 Hz, 1H), 7.54 (dd, J = 9.6, 2.0 Hz, 1H), 3.74 (t, J = 4.6 Hz, 4H), 3.60–3.55 (m, 2H), 2.58–2.46 (m, 6H), 1.93 (quint, J = 7.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 183.1, 172.6, 162.3, 155.1, 149.5, 139.3, 135.6, 131.8, 130.2, 127.8, 127.1, 124.3, 124.1, 122.0, 114.8, 111.2, 67.1, 56.4, 53.8, 37.7, 26.4. UV/vis λ_{max} (MeCN, ε): 258 (39,400), 332 (12,600), 365 (7100), 496 (5500) nm. ESI-MS *m/z*: 499.1 (100%), 497.1 (92%) $[M + H]^+$. HRMS (ESI) *m/z*: 497.0827 $[M + H]^+$, calcd for $C_{23}H_{22}N_4O_4Br$ 497.0824.

4.4.12. N-(2-(Pyrrolidin-1-yl)ethyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**17**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH:Et_3N = 25:1:0.25 to give a red solid **17** (0.20 g), yield 85%, mp = 225.1–226.3 °C. ^{1}H NMR ($CDCl_3$) δ 10.31 (t, J = 4.6 Hz, 1H), 10.16 (d, J = 1.8 Hz, 1H), 9.09–9.04 (m, 2H), 8.60 (dd, J = 8.0, 1.6 Hz, 1H), 7.69 (dd, J = 8.0, 4.4 Hz, 1H), 7.55 (dd, J = 9.8, 1.8 Hz, 1H), 3.72 (q, J = 6.4 Hz, 2H), 2.88 (t, J = 6.2 Hz, 2H), 2.72 (br.s, 4H), 1.87 (br.s, 4H). ^{13}C NMR ($CDCl_3$) δ 182.8, 172.6, 162.4, 155.0, 149.5, 139.2, 135.7, 131.7, 130.2, 127.7, 127.0, 124.5, 124.0, 121.9, 114.7, 111.1, 55.0, 54.2, 38.8, 23.6. UV/vis λ_{max} (MeCN, ε): 259 (36,400), 331 (11,500), 492 (5100) nm. ESI-MS *m/z*: 469.1 (100%), 467.2 (68%) $[M + H]^+$. HRMS (ESI) *m/z*: 467.0708 $[M + H]^+$, calcd for $C_{22}H_{20}N_4O_3Br$ 467.0719.

4.4.13. N-(3-(Pyrrolidin-1-yl)propyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**18**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH:Et_3N = 12:1:0.25 to give a red solid **18** (0.22 g), yield 90%, mp = 195.7–196.9 °C. ^{1}H NMR ($CDCl_3$) δ 10.26 (t, J = 5.2 Hz, 1H), 10.13 (dd, J = 1.8, 0.8 Hz, 1H), 9.08 (dd, J = 4.8, 1.6 Hz, 1H), 9.05 (dd, J = 9.8, 0.8 Hz, 1H), 8.59 (dd, J = 8.0, 1.6 Hz, 1H), 7.70 (dd, J = 8.0, 4.8 Hz, 1H), 7.55 (dd, J = 9.8, 1.8 Hz, 1H), 3.59 (q, J = 6.2 Hz, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.60 (br.s, 4H), 1.98 (quint, J = 7.2 Hz, 2H), 1.88–1.75 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 183.0, 172.6, 162.3, 155.0, 149.6, 139.3, 135.7, 131.7, 130.3, 127.8, 127.1, 124.4, 124.1, 122.0, 114.8, 111.3, 54.3, 54.1, 37.9, 28.8, 23.5. UV/vis λ_{max} (MeCN, ε): 259 (40,600), 340 (12,600), 361 (7400), 491 (5400) nm. ESI-MS *m/z*: 481.1 (100%), 483.1 (98%) $[M + H]^+$. HRMS (ESI) *m/z*: 481.0872 $[M + H]^+$, calcd for $C_{23}H_{22}N_4O_3Br$ 481.0875.

4.4.14. N-(2-(Piperidin-1-yl)ethyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**19**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH:Et_3N = 20:1:0.05 to give a red solid **19** (0.23 g), yield 96%, mp = 237.8–239.6 °C. ^{1}H NMR ($CDCl_3$) δ 10.28 (t, J = 4.6 Hz, 1H), 10.15 (dd, J = 1.8, 0.8 Hz, 1H), 9.09–9.05 (m, 2H), 8.59 (dd, J = 8.0, 1.6 Hz, 1H), 7.70 (dd, J = 8.0, 4.4 Hz, 1H), 7.55 (dd, J = 9.8, 1.8 Hz, 1H), 3.68 (q, J = 6.2 Hz, 2H), 2.69 (t, J = 6.6 Hz, 2H), 2.56 (br.s, 4H), 1.70–1.65 (m, 4H), 1.53–1.45 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 182.7, 172.6, 162.3, 155.0, 149.5, 139.3, 135.6, 131.7, 130.3, 127.8, 127.1, 124.5, 124.1, 121.9, 114.7, 111.2, 57.7, 54.6, 37.1, 26.0, 24.5. UV/vis λ_{max} (MeCN, ε): 259 (30,900), 333 (9900), 491 (4400) nm. ESI-MS *m/z*: 483.1 (100%), 481.1 (73%) $[M + H]^+$. HRMS (ESI) *m/z*: 481.0877 $[M + H]^+$, calcd for $C_{23}H_{22}N_4O_3Br$ 481.0875.

4.4.15. N-(3-(Piperidin-1-yl)propyl)-9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxamide (**20**)

The crude product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH = 20:1 to give a red solid **20** (0.21 g), yield 85%, mp = 213.5–214.5 °C. ^{1}H NMR ($CDCl_3$) δ 10.20 (t, J = 5.2 Hz, 1H), 10.08 (dd, J = 1.8, 0.6 Hz, 1H), 9.06 (dd, J = 4.8, 1.6 Hz, 1H), 9.01 (dd, J = 9.8, 0.6 Hz, 1H), 8.56 (dd, J = 7.8, 1.8 Hz, 1H), 7.69 (dd, J = 7.8, 4.6 Hz, 1H), 7.53 (dd, J = 9.8, 1.8 Hz, 1H), 3.54 (q, J = 6.4 Hz, 2H), 2.56–2.40 (m, 6H), 1.95 (quint, J = 7.3 Hz, 2H), 1.63–1.60 (m, 4H), 1.50–1.40 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 182.9, 172.5, 162.2, 155.0, 149.4, 139.2, 135.7, 131.7, 130.2, 127.7, 127.1, 124.3, 124.0, 121.9, 114.8, 111.2, 56.8, 54.6, 37.9, 26.7, 25.9, 24.4. UV/vis λ_{max} (MeCN, ε): 260 (44,900), 337 (12,700), 360 (7400), 489 (5300) nm. ESI-MS *m/z*: 495.1 (100%), 497.1 (87%) $[M + H]^+$. HRMS (ESI) *m/z*: 495.1018 $[M + H]^+$, calcd for $C_{24}H_{24}N_4O_3Br$ 495.1032.

4.4.16. 9-Bromo-6-(pyrrolidine-1-carbonyl)indolizino[2,3-g]quinoline-5,12-dione (**21**)

The crude product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH = 15:1 to give a red solid **21** (0.20 g), yield 96%, mp > 300 °C. ^{1}H NMR ($CDCl_3$) δ 9.95 (dd, J = 1.6, 0.8 Hz, 1H), 9.06 (dd, J = 4.6, 1.8 Hz, 1H), 8.51 (dd, J = 8.0, 2.0 Hz, 1H), 7.73 (dd, J = 9.6, 0.8 Hz, 1H), 7.65 (dd, J = 8.0, 4.8 Hz, 1H), 7.43 (dd, J = 9.4, 1.8 Hz, 1H), 3.93–3.82 (m, 1H), 3.81–3.71 (m, 1H), 3.50–3.37 (m, 1H), 3.23–3.14 (m, 1H), 2.12–1.80 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 180.3, 172.3, 162.1, 154.4, 150.3, 135.4, 135.1, 130.31, 130.29, 128.1, 126.9, 125.0, 121.1, 120.7, 113.5, 113.4, 47.5, 46.1, 25.9, 24.5. UV/vis λ_{max} (MeCN, ε): 256 (32,200), 334 (9800), 479 (6100) nm. ESI-MS *m/z*: 426.1 (100%), 424.1 (92%) $[M + H]^+$. HRMS (ESI) *m/z*: 424.0287 $[M + H]^+$, calcd for $C_{20}H_{15}N_3O_3Br$ 424.0297.

4.4.17. 9-Bromo-6-(4-methylpiperazine-1-carbonyl)indolizino[2,3-g]quinoline-5,12-dione (**22**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH:Et_3N = 25:1:0.05 to give a red solid **22** (0.18 g) as, yield 81%, mp = 248.3–250.1 °C. ^{1}H NMR ($CDCl_3$) δ 9.93 (dd, J = 1.6, 0.8 Hz, 1H), 9.05 (dd, J = 4.8, 1.6 Hz, 1H), 8.52 (dd, J = 7.8, 1.8 Hz, 1H), 7.70–7.62 (m, 2H), 7.44 (dd, J = 9.6, 1.6 Hz, 1H), 4.13–4.04 (m, 1H), 3.95–3.84 (m, 1H), 3.52–3.42 (m, 1H), 3.41–3.34 (m, 1H), 2.79–2.71 (m, 1H), 2.53–2.42 (m, 2H), 2.35 (s, 3H), 2.22–2.12 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 180.0, 172.4, 162.3, 154.5, 150.3, 135.4, 135.1, 130.5, 130.3, 128.2, 126.9, 125.2, 121.1, 120.3, 113.5, 111.5, 55.2, 54.6, 46.8, 46.0, 42.1. UV/vis λ_{max} (MeCN, ε): 256 (32,700), 330 (10,200), 477 (5700) nm. ESI-MS *m/z*: 455.1 (100%), 453.1 (90%) $[M + H]^+$. HRMS (ESI) *m/z*: 453.0582 $[M + H]^+$, calcd for $C_{21}H_{18}N_4O_3Br$ 453.0562.

4.4.18. 9-Bromo-6-(morpholine-4-carbonyl)indolizino[2,3-g]quinoline-5,12-dione (**23**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH:Et_3N = 30:1:0.3 to give a red solid **23** (0.19 g), yield 86%, mp > 300 °C. ^{1}H NMR ($CDCl_3$) δ 9.95 (dd, J = 1.6, 0.8 Hz, 1H), 9.06 (dd, J = 4.6, 1.8 Hz, 1H), 8.52 (dd, J = 7.8, 1.8 Hz, 1H), 7.73–7.64 (m, 2H), 7.45 (dd, J = 9.2, 1.6 Hz, 1H), 4.06–3.89 (m, 3H), 3.84–3.72 (m, 2H), 3.54–3.45 (m, 2H), 3.38–3.26 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 180.1, 172.3, 162.5, 154.5,

150.2, 135.6, 135.1, 130.6, 130.2, 128.2, 127.0, 125.2, 121.1, 120.4, 113.6, 110.9, 66.8, 47.3, 42.6. UV/vis λ_{max} (MeCN, ε): 257 (32 500), 334 (9600), 478 (5900) nm. ESI-MS *m/z*: 440.0 (100%), 442.0 (99%) $[M + H]^+$. HRMS (ESI) *m/z*: 462.0057 $[M + Na]^+$, calcd for $C_{20}H_{14}N_3O_4BrNa$ 462.0065.

4.4.19. 9-Bromo-6-(thiomorpholine-4-carbonyl)indolizino[2,3-g]quinoline-5,12-dione (**24**)

The crude product was purified by silica gel column chromatography with eluent $CHCl_3$:MeOH:Et_3N = 130:7:0.2 to give an orange solid **24** (0.22 g), yield 96%, mp > 300 °C. ^1H NMR ($CDCl_3$) δ 9.94 (dd, J = 1.6, 0.8 Hz, 1H), 9.06 (dd, J = 4.6, 1.8 Hz, 1H), 8.52 (dd, J = 7.8, 1.8 Hz, 1H), 7.67 (dd, J = 7.8, 4.6 Hz, 1H), 7.64 (dd, J = 9.2, 0.8 Hz, 1H), 7.44 (dd, J = 9.2, 1.6 Hz, 1H), 4.22 (t, J = 4.9 Hz, 2H), 3.70–3.65 (m, 2H), 2.96–2.90 (m, 1H), 2.84–2.75 (m, 1H), 2.69–2.61 (m, 1H), 2.45–2.36 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 180.1, 172.3, 162.8, 154.5, 150.3, 135.2, 135.1, 130.6, 130.3, 128.6, 127.0, 125.1, 121.2, 120.1, 113.6, 111.3, 49.7, 44.7, 28.1, 27.5. UV/vis λ_{max} (MeCN, ε): 257 (33,600), 334 (9900), 481 (6100) nm. ESI-MS *m/z*: 456.1 (100%), 458.1 (92%) $[M + H]^+$. HRMS (ESI) *m/z*: 477.9851 $[M + Na]^+$, calcd for $C_{20}H_{14}N_3O_3SBrNa$ 477.9837.

4.4.20. 6-(4-Acetylpiperazine-1-carbonyl)-9-bromoindolizino[2,3-g]quinoline-5,12-dione (**25**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH = 30:1 to give a isomers mixture **25** (0.21 g, red solid), yield 89%, mp > 300 °C. ^1H NMR ($CDCl_3$) δ 9.95 (dd, J = 1.2, 0.8 Hz, 1H), 9.07 (dd, J = 4.8, 1.6 Hz, 1H), 8.50 (d, J = 7.2 Hz, 1H), 7.67 (m, 2H), 7.46 (dd, J = 9.2, 1.2 Hz, 1H), 4.07–3.86 (m, 3H), 3.80–3.70 (m, 2H), 3.65–3.58 (m, 1H), 3.49–3.43 (m, 1H), 3.36–3.29 (m, 1H), 1.65 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 180.2, 172.34, 172.31, 169.3, 169.1, 163.0, 162.6, 154.6, 150.2, 135.8, 135.6, 130.7, 130.2, 128.2, 128.1, 127.0, 125.2, 125.1, 121.2, 121.1, 120.4, 120.3, 113.8, 113.7, 110.8, 110.6, 46.9, 46.6, 46.3, 45.9, 42.3, 42.1, 41.5, 41.1, 21.4. UV/vis λ_{max} (MeCN, ε): 257 (35 200), 334 (10 500), 478 (6400) nm. ESI-MS *m/z*: 481.1 (100%), 483.1 (86%) $[M + H]^+$. HRMS (ESI) *m/z*: 503.0317 $[M + Na]^+$, calcd for $C_{22}H_{17}N_4O_4BrNa$ 503.0331.

4.4.21. Methyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxylate (**26**)

The crude product was purified by silica gel column chromatography with eluent $CHCl_3$:AcOEt = 5:1 to give an orange solid **26** (0.12 g), yield 53%, mp = 285.7–286.9 °C. ^1H NMR ($CDCl_3$) δ 10.08 (br.s, 1H), 9.04 (dd, J = 4.4, 1.6 Hz, 1H), 8.55 (dd, J = 8.0, 1.6 Hz, 1H), 8.26 (dd, J = 9.6, 0.8 Hz, 1H), 7.68 (dd, J = 8.0, 4.4 Hz, 1H), 7.55 (dd, J = 9.6, 1.6 Hz, 1H), 4.04 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 178.8, 173.3, 163.1, 154.3, 149.2, 138.2, 135.5, 132.0, 130.9, 128.3, 127.8, 127.2, 122.9, 121.6, 113.6, 106.5, 52.3. UV/vis λ_{max} (MeCN, ε): 259 (34,200), 326 (9200), 459 (4900) nm. ESI-MS *m/z*: 384.9 (100%), 386.9 (96%) $[M + H]^+$. HRMS (ESI) *m/z*: 384.9812 $[M + H]^+$, calcd for $C_{17}H_{10}N_2O_4Br$ 384.9824.

4.4.22. 2,2,2-Trifluoroethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxylate (**27**)

The crude product was purified by silica gel column chromatography with eluent $CHCl_3$:AcOEt = 5:1 to give an orange solid **27** (0.15 g), yield 67%, mp = 262.1–264.9 °C. ^1H NMR ($CDCl_3$) δ 10.15 (br.s Hz, 1H), 9.07 (d, J = 3.2 Hz, 1H), 8.60(dd, J = 7.6, 0.8 Hz, 1H), 8.26 (dd, J = 9.6, 0.4 Hz, 1H), 7.71 (dd, J = 7.8, 4.6 Hz, 1H), 7.64 (dd, J = 9.6, 1.6 Hz, 1H), 4.84 (q, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 178.5, 173.5, 160.7, 154.4, 149.0, 138.5, 135.7, 132.9, 130.9, 128.6, 128.5, 127.4, 123.4, 121.3, 113.7, 104.1, 60.8 (q, J_{C-F} = 36.6 Hz), 29.7. UV/vis λ_{max} (MeCN, ε): 261 (40,400), 325 (10,900), 357 (6300), 453 (5500) nm. ESI-MS *m/z*: 453.0 (100%), 455.0 (94%) $[M + H]^+$. HRMS (ESI) *m/z*: 452.9708 $[M + H]^+$, calcd for $C_{18}H_9N_2O_4F_3Br$ 452.9698.

4.4.23. 2-Fluoroethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxylate (**28**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:AcOEt:MeOH = 60:3:1 to give an orange solid **28** (0.12 g), yield 61%, mp = 265.4–267.3 °C. ^1H NMR ($CDCl_3$) δ 10.11 (dd, J = 1.6, 0.8 Hz, 1H), 9.05 (dd, J = 4.4, 1.6 Hz, 1H), 8.57 (dd, J = 8.0, 1.6 Hz, 1H), 8.28 (dd, J = 9.6, 0.8 Hz, 1H), 7.68 (dd, J = 8.0, 4.8 Hz, 1H), 7.58 (dd, J = 9.6, 1.6 Hz, 1H), 4.92–4.88 (m, 1H), 4.80–4.77 (m, 1H), 4.75–4.72 (m, 1H), 4.68–4.65 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 178.7, 173.4, 162.2, 154.3, 149.2, 138.3, 135.6, 132.3, 131.0, 128.4, 128.1, 127.2, 123.1, 121.6, 113.6, 106.0, 81.3 (d, J_{C-F} = 169.5 Hz), 64.0 (d, J_{C-F} = 19.8 Hz). UV/vis λ_{max} (MeCN, ε): 260 (43,300), 326 (11,300), 358 (6100), 462 (6200) nm. ESI-MS *m/z*: 417.0 (100%), 419.0 (96%) $[M + H]^+$. HRMS (ESI) *m/z*: 416.9900 $[M + H]^+$, calcd for $C_{18}H_{11}N_2O_4FBr$ 416.9886.

4.4.24. 2-(Dimethylamino)ethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxylate (**29**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH = 20:1 to give an orange solid **29** (0.16 g), yield 73%, mp = 182.2–184.7 °C. ^1H NMR ($CDCl_3$) δ 10.09 (br.s, 1H), 9.05 (dd, J = 4.4, 1.6 Hz, 1H), 8.56 (dd, J = 7.6, 1.4 Hz, 1H), 8.36 (d, J = 9.6 Hz, 1H), 7.67 (dd, J = 7.8, 4.6 Hz, 1H), 7.55 (dd, J = 9.6, 1.6 Hz, 1H), 4.56 (t, J = 5.8 Hz, 2H), 2.83 (t, J = 5.8 Hz, 2H), 2.38 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 178.8, 173.3, 162.5, 154.3, 149.2, 138.2, 135.5, 131.9, 131.0, 128.3, 127.9, 127.2, 122.8, 121.8, 113.5, 107.0, 62.8, 57.7, 45.7. UV/vis λ_{max} (MeCN, ε): 259 (54,400), 326 (11,500), 358 (6300), 463 (6100) nm. ESI-MS *m/z*: 442.0 (100%), 444.0 (98%) $[M + H]^+$. HRMS (ESI) *m/z*: 442.0404 $[M + H]^+$, calcd for $C_{20}H_{17}N_3O_4Br$ 442.0402.

4.4.25. 2-(Piperidin-1-yl)ethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxylate (**30**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH = 25:1 to give an orange solid **30** (0.18 g), yield 76%, mp = 188.1–189.2 °C. ^1H NMR ($CDCl_3$) δ 10.10 (dd, J = 2.0, 0.8 Hz, 1H), 9.04 (dd, J = 4.8, 1.6 Hz, 1H), 8.57 (dd, J = 8.0, 1.6 Hz, 1H), 8.52 (dd, J = 9.6, 0.8 Hz, 1H), 7.67 (dd, J = 7.8, 4.6 Hz, 1H), 7.54 (dd, J = 9.6, 2.0 Hz, 1H), 4.57 (t, J = 5.8 Hz, 2H), 2.82 (t, J = 5.8 Hz, 2H), 2.54 (br.s, 4H), 1.68–1.58 (m, 4H), 1.52–1.44 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 178.8, 173.2, 162.2, 154.2, 149.2, 138.2, 135.5, 131.8, 130.9, 128.2, 127.9, 127.2, 122.9, 122.2, 113.5, 107.2, 62.2, 57.1, 54.7, 26.0, 24.2. UV/vis λ_{max} (MeCN, ε): 260 (44,700), 327 (12,700), 358 (7400), 463 (7400) nm. ESI-MS *m/z*: 482.1 (100%), 484.1 (96%) $[M + H]^+$. HRMS (ESI) *m/z*: 482.0692 $[M + H]^+$, calcd for $C_{23}H_{21}N_3O_4Br$ 482.0715.

4.4.26. 2-Morpholinoethyl 9-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-g]quinoline-6-carboxylate (**31**)

The crude product was purified by silica gel column chromatography with eluent CH_2Cl_2:MeOH = 25:1 to give an orange solid **31** (0.17 g), yield 69%, mp = 138.4–139.7 °C. ^1H NMR ($CDCl_3$) δ 10.10 (dd, J = 1.6, 0.8 Hz, 1H), 9.05 (dd, J = 4.6, 1.8 Hz, 1H), 8.56 (dd, J = 7.8, 1.8 Hz, 1H), 8.43 (dd, J = 9.6, 0.8 Hz, 1H), 7.68 (dd, J = 7.8, 4.6 Hz, 1H), 7.55 (dd, J = 9.6, 1.6 Hz, 1H), 4.58 (t, J = 5.8 Hz, 2H), 3.80–3.70 (m, 4H), 2.87 (t, J = 5.8 Hz, 2H), 2.67–2.57 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 178.8, 173.3, 162.3, 154.3, 149.2, 138.2, 135.5, 131.9, 130.9, 128.3, 127.9, 127.2, 122.9, 121.9, 113.5, 106.9, 67.0, 61.8, 56.9, 53.7. UV/vis λ_{max} (MeCN, ε): 260 (30,000), 326 (9500), 461 (5400) nm. ESI-MS *m/z*: 486.1 (100%), 484.1 (92%) $[M + H]^+$. HRMS (ESI) *m/z*: 484.0519 $[M + H]^+$, calcd for $C_{22}H_{19}N_3O_5Br$ 484.0508.

4.5. In vitro antimicrobial activity evaluation by MIC assay

The MIC values were determined using a broth dilution method [11]. The starting concentrations of tested compounds were 64 μg/

ml. The solution of compound in DMSO (15 μl) was added to 285 μl of bacterial culture (5×10^5 cells/ml) at the first well of flat-bottomed 96-well tissue culture plates (JET BIOFIL®, JET BIOCHEMICALS Intl., Inc, CANADA). The solution was then double-diluted. Bacterial culture solution containing appropriate compound (150 μl) was discarded at the last well in order to ensure 150 μl volume of bacterial culture in every well. The plate was incubated at 37 °C overnight in electro-heating standing-temperature cultivator before the measurement of the absorbance value. The optical density values at 600 nm were measured using a multifunction microplate reader (PowerWave™ XS2, BioTek® Instruments Inc, USA).

4.6. DNA gyrase supercoiling assay

DNA gyrase supercoiling assay was performed with relaxed pHOT1 DNA (TopoGEN, USA) as a substrate according to the manufacturer's protocol. Briefly, 1 U of DNA gyrase (TopoGEN, USA) from *E. coli* or *S. aureus* and 0.5 μg of relaxed pHOT1 DNA in 30 μl supercoiling assay buffer (35 mM Tris–HCl, pH 7.5, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM Spermidine, 1 mM ATP, 6.5% glycerol and 0.1 mg/ml BSA) were incubated with or without the compound at 37 °C for 60 min. After incubation, the reactions were terminated by the addition of 3 μl of 2 mg/ml proteinase K. 5 μl of the reaction solution was mixed with 1 μl of 6 × DNA loading buffer (30 mM EDTA, 36% glycerol, 0.05% xylene cyanol FF, 0.05% bromophenol blue), and then analyzed by electrophoresis on 1% agarose gel at 3 V/cm in 1 × TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.5). The gel was stained with 1 × Gel Red, visualized with a UV transilluminator and analyzed by AlphaEaseFC software.

4.7. Topoisomerase IV relaxation assay and decatenation assay

Topo IV relaxation assay or decatenation assay was performed according to a slightly modified method [13]. 1 U of Topo IV (TopoGEN, USA) was incubated with 0.5 μg of supercoiled pBR322 DNA (for relaxation assay, Takara Biotechnology Co., Ltd.) or 200 ng of catenated kDNA (for decatenation assay, TopoGEN, USA) in 30 μl reaction buffer (20 mM HEPES–KOH, pH 7.6, 50 mM potassium glutamate, 5 mM magnesium acetate, 5 mM dithiothreitol, 1 mM ATP and 25 μg/ml BSA) at 37 °C for 30 min. The reaction was stopped by the addition of 30 μl of chloroform/iso-amyl alcohol (24:1). 5 μl of the reaction solution was mixed with 1 μl of 6 × DNA loading buffer, and then analyzed by electrophoresis on 1% agarose gel at 5 V/cm (for relaxation assay) or 10 V/cm (for decatenation assay) in 1 × TAE buffer. The gel was stained with 1 × Gel Red, visualized with a UV transilluminator and analyzed by AlphaEaseFC software.

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