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Synthesis, cytotoxic activities and structure–activity relationships of topoisomerase I inhibitors: Indolizinoquinoline-5,12-dione derivatives

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Abstract—A series of indolizinoquinoline-5,12-dione derivatives (IQDs) are synthesized and evaluated for their cytotoxic activities toward human lung adenocarcinoma (GLC-82), large-cell lung carcinoma (NCI-H460), promyelocytic leukemia (HL-60) and breast carcinoma (MCF-7) cells by MTT method. Most of the IQDs show significant cytotoxic potency. In addition, the evaluation of structure–activity relationships indicated that the incorporation of electron-withdrawing substituents at the C or D ring will enhance the activities of the target compounds distinctly. The topoisomerase I inhibitory activity is also measured.

1. Introduction

DNA topoisomerase I (TOP1) controls the processive relaxation of supercoiled DNA by means of transient DNA strand breakage and religation,[1–4] and that can be used as a target to screen the anticancer agents.[5,6] Camptothecin (CPT) derivatives, the well-known TOP1 inhibitors, are successfully developed as anticancer drug.[6,7] Comptothecin derivatives contain indolizino[1,2-*b*]quinoline nucleus,[8] which is reported to be pharmacologically active moiety.[9] In accordance with Moore's theory,[10] the molecules contain a planar polycyclic aromatic ring and a conjugated quinone attend to be the DNA-intercalating TOP inhibitors.[11–13] In view of this reason, we designed and synthesized a series of indolizinoquinoline-5,12-dione derivatives (IQDs, Scheme 1) which combined the indolizine with the quinolinedione moiety.[12] The cytotoxic activities and the structure–activity relationships of the prepared compounds were studied. In general, these compounds exhibited potential cytotoxic activities. DNA-topoisomerase I relaxation assay indicated that the possible mechanism of their action was as a TOP1 inhibitor.

Keywords: Indolizinoquinoline-5,12-dione derivatives; Cytotoxic activity; Structure–activity relationship; Topoisomerase I.
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2. Results and discussion

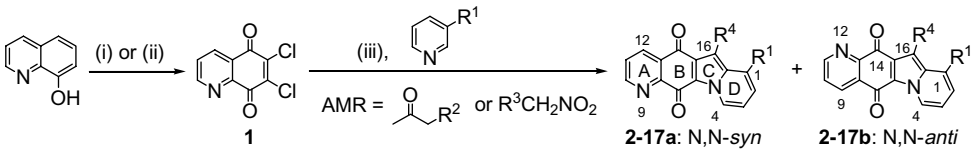
2.1. Chemistry

The indolizinoquinoline-5,12-dione derivatives were synthesized as shown in Scheme 1. 6,7-Dichloroquinoline-5,8-dione (**1**) was obtained by oxidizing 8-hydroxyquinoline in concentrated HCl solution with sodium chlorate or concentrated HNO_3.[12]

The heterocyclic skeleton was formed through a single-step reaction of substrate **1**, pyridine and an active methylene reagent (AMR).[14,15] Two regioisomers (*N*,*N*-*syn* and *N*,*N*-*anti* isomers) were obtained in the cyclization, which was different from Yanni's result.[14] The isomers were isolated in pure form by silicon gel flash column chromatography and fully characterized by mass spectrometry, elementary analysis, and NMR analysis.

The regioisomers, **2a** and **2b**, were obtained from the reaction of substrate **1** with ethyl acetoacetate and pyridine. The NMR spectra, ESI-MS spectrum, elementary analysis, and melting point were similar to Defant's result,[15] and confirmed their structure.

The cyclization reaction of 3-substituent pyridine, such as 3-fluoropyridine, with substrate **1** and ethyl acetoacetate gave the regioisomers **7a/b**. Mass spectra and elemental analysis showed that both had the same



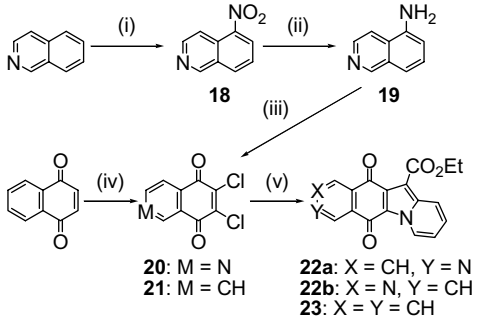
2a, b: R^1 = H, R^4 = R^2 = CO_2Et; **3a, b**: R^1 = H, R^4 = R^2 = COMe; **4a, b**: R^1 = H, R^4 = R^2 = CN; **5a, b**: R^1 = H, R^4 = R^3 = Me; **6a, b**: R^1 = H, R^4 = R^3 = H; **7a, b**: R^1 = F, R^4 = R^2 = CO_2Et; **8a, b**: R^1 = Cl, R^4 = R^2 = CO_2Et; **9a, b**: R^1 = Br, R^4 = R^2 = CO_2Et; **10a, b**: R^1 = Me, R^4 = R^2 = CO_2Et; **11a, b**: R^1 = OH, R^4 = R^2 = CO_2Et; **12a, b**: R^1 = NH_2, R^4 = R^2 = CO_2Et; **13a, b**: R^1 = F, R^4 = R^2 = COMe; **14a, b**: R^1 = Cl, R^4 = R^2 = COMe; **15a, b**: R^1 = Br, R^4 = R^2 = COMe; **16a, b**: R^1 = OH, R^4 = R^2 = COMe; **17a, b**: R^1 = NH_2, R^4 = R^2 = COMe;

Scheme 1. Synthesis of indolizinoquinoline-5,12-dione derivatives. Reagents and conditions: (i) $NaClO_3$, concd HCl, 50 °C; (ii) concd HNO_3, concd HCl, 90 °C; (iii) EtOH, pyridine derivatives, AMR, refluxing.

formula, $C_{18}H_{11}FN_2O_4$. The HMBC spectrum of **7a** indicated that the resonance at δ = 179.3 ppm was attributable to C-14 by long-range hetero-correlation with 12-H at δ = 8.54 ppm. As a consequence, the signal at δ = 172.8 ppm could be assigned to C-7. In the ^{1}H NMR spectrum, the signal at δ = 9.64 ppm (d, J = 6.8 Hz) could be assigned to H-4. Furthermore, ^{1}H–^{1}H COSY spectrum showed a correlation signal of H-4 with a hydrogen at δ = 7.06–7.16 ppm (m, 2H), which could be assigned to H-3 and H-2. Therefore, compound **7a** was assigned as the *N*,*N-syn* isomer, ethyl 7-fluoro-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate. Similarly, compound **7b** could be assigned as the *N*,*N-anti* isomer. These results mean that the cyclization of 3-substituent pyridine derivatives instead of pyridine would mainly give the 1-substituent target compounds.

In order to investigate the effects of the existence and the position of nitrogen atom in this heterocyclic skeleton on the biological activity, compounds **22a/b** and **23** (Scheme 2) were synthesized.

The mixture of regioisomers **22a** and **22b** were prepared through cyclization of 6,7-dichloroisoquinoline-5,8-dione (**20**), ethyl acetoacetate, and pyridine. The substrate **20** was obtained using isoquinoline as starting material, through nitration, hydrogenation, and oxidation-chlorination reactions. The pure compound, **22a** or **22b**, could not be obtained by flash column chromatography techniques and the inseparable mixture was used to determine the biological activities.



Scheme 2. Synthesis of compounds **22a/b** and **23**. Reagents and conditions: (i) H_2SO_4, KNO_3, 0–5 °C; (ii) EtOH, Pd/C, H_2; (iii) $NaClO_3$, concd HCl, 80 °C; (iv) $NaClO_3$, concd HCl, Bu_4NBr, ethyl acetate, 50 °C; (v) pyridine, ethylacetoacetate, ethanol, refluxing.

Ethyl 6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole 12-carboxylate (**23**) was obtained by cyclization of pyridine with ethyl acetoacetate and 2,3-dichloro-1,4-naphtoquinone (**21**).[16,17] Compound **21** was prepared by treating 1,4-naphthoquinone in concentrated HCl solution with sodium chlorate using Bu_4NBr as phase-transfer catalyst.

2.2. Cytotoxic activities

Evaluation of the synthetic IQDs for cytotoxic activity in incline was performed by MTT assay using four different human cancer cell lines, lung adenocarcinoma cell (GLC-82), large-cell lung carcinoma cell (NCI-H460), promyelocytic leukemia cell (HL-60) and breast carcinoma cell (MCF-7). The cultured cell lines were divided into multi-well microplate and treated with the synthesized compounds. The resulting solutions were detected and evaluated for their biological activities.

The assessments of cytotoxic activities were expressed as the concentration inhibiting 50% of cancer cell growth (IC_{50}). The results were summarized in Table 1.

According to Table 1, IQDs showed the significant cytotoxic activities with the different substituent groups against a wide variety of tumor cell lines. The substituent group at the C ring of heterocyclic skeleton had decisive affect on the IC_{50} value. Compounds with electron contributing groups at the C ring, such as **5a**, **5b**, **6a**, and **6b**, showed weaker cytotoxic activities (IC_{50} > 10 μM) against the four human cancer cell lines. When an electron-withdrawing substituent such as ethoxycarbonyl, carbonyl, or nitrile group was incorporated at the C ring, which would be increased activity.

Moreover, an enhancement of cytotoxic activities was observed by enhancing the substituent's electron-withdrawing properties at the D ring. Indeed, the halogen substituted derivatives showed more potent activity against the cancer cell lines than the corresponding the amino or hydroxyl substituted derivatives. Compound **8a** with chloro substitution showed a 95-fold activity

Table 1. Cytotoxic activities in vitro of IQDs, **22a/b** and **23**

Compound	Cytotoxic activities IC_{50}[a] (μM)			
	GLC-82	NCI-H460	HL-60	MCF-7
2a	<0.25	<0.25	0.88	0.96
2b	2.40	1.90	14.45	6.38
3a	<0.25	<0.25	0.86	7.92
3b	1.90	1.40	14.71	12.70
4a,b[b]	2.50	0.16	12.67	1.56
5a	14.20	12.20	12.46	>25.00
5b	21.50	>25.00	>25.00	>25.00
6a	11.50	15.60	16.97	>25.00
6b	16.70	12.30	14.68	20.20
7a	<0.25	<0.25	1.37	7.01
7b	1.70	1.20	5.51	6.20
8a	<0.25	2.10	6.52	7.37
8b	9.73	2.20	15.42	19.90
9a	<0.25	2.00	9.32	10.30
9b	1.10	2.40	11.47	20.80
10a	1.83	n.d.[c]	0.22	14.50
10b	9.27	n.d.	11.65	>25.00
11a	7.20	>25.00	>25.00	>25.00
11b	23.80	23.30	>25.00	>25.00
12a	23.41	17.58	16.43	20.90
12b	23.50	19.00	>25.00	>25.00
13a	5.26	<0.25	0.17	2.31
13b	12.70	6.75	1.39	9.28
14a	6.85	0.23	0.19	7.83
14b	16.10	1.50	2.20	16.10
15a	2.01	2.15	0.21	11.70
15b	4.68	0.20	1.56	12.40
16a	19.50	24.85	>25.00	17.60
16b	16.30	>25.00	>25.00	n.d.
17a	12.20	>25.00	22.30	>25.00
17b	10.30	10.29	15.80	>25.00
22a,b[b]	9.36	2.20	17.43	11.70
23	>25.00	n.d.	>25.00	>25.00

[a] IC_{50} values, defined as the concentration that inhibited growth by 50%. Every experiment was repeated at least three times.
[b] The mixture was used to determine the cytotoxic activities.
[c] n.d. means 'not determined'.

compared with compound **12a** with amino substitution against GLC-82 cell line.

Study on the structure–activity relationship of heterocyclic quinines containing nitrogen indicated that the position and number of nitrogen in the A ring was important for the cytotoxicity.[18] Compound **23** showed only a moderate activity, which probably implied that the existence of nitrogen in the A ring was necessary. The mixture of isoquinolinedione derivatives (**22a** and **22b**) showed lower activities than the corresponding pure quinolinedione derivative, **2a** or **2b**. Comparison of the two regioisomers, the *N*,*N-syn* isomer generally showed more potential activities than the *N*,*N-anti* isomer. The position of nitrogen atom made a moderate effect on the activity.

2.3. TOP1 inhibitory activity

The relative topoisomerase-targeting activities of IQDs were carried out by TOP1 relaxation assay.[19,20] The result indicated that some of IQDs strongly inhibited the catalytic activity of TOP1. Figure 1 (A) shows the conversion of supercoiled plasmid DNA by calf thymus TOP1 in the presence of selectively synthesized compound **8a**. CPT, a well-known TOP1 inhibitor, was used as a positive control (Fig. 1. B). Compound **8a** showed stronger inhibitory activity than CPT. There were 5.8% and 100% of supercoiled DNA after being treated with 0.2 μM and 125 μM of **8a**, respectively. CPT could inhibit 81.3% of TOP1's catalytic activity at 125 μM concentration.

3. Conclusion

Overall, the screening indicated that the synthesized IQDs showed significant cytotoxic activities against four human cancer cell lines. The incorporation of electron-withdrawing groups at C or D ring would enhance the activities of the resulting compound. Evaluation of structure–activity relationships indicated that the existence of nitrogen atom in the A ring was necessary. And the position of nitrogen atom made a variable, moderate effect on the activity. TOP1 relaxation assay indicated that the topoisomerase I might be a biological target of IQDs.

4. Experimental

4.1. General experimental

All chemicals and solvents were obtained from commercial suppliers and used without further purification. Calf thymus topoisomerase I and plasmid pBR322 were purchased from TakaRa Biotechnology (Dalian) Co., Ltd. Chemical reactions were monitored by thin layer chromatography using self-preparative silica gel GF_{254} plates. Flash column chromatography was performed with silica gel 200–300 meshes. Melting points were uncorrected and were determined using a XT-4 apparatus. ^1H NMR, ^{13}C NMR, ^1H–^{13}C HMQC, and HMBC spectra were measured on a Varian Mercury-Plus 300 MHz or Bruker UltraShield 400 MHz spectrometer. Chemical shifts were reported in parts per million (δ) relative to tetramethylsilane (TMS) as internal standard. For the electrospray (ESI) MS analysis a Finnigan LCQ Deca XP ion trap mass spectrometer equipped with a Microsoft Windows NT data system and an ESI interface was used. Elementary analysis was recorded on an Elementar Vario EL elementary analysis device. IR absorption was recorded on a Bruker TENSOR 37 spectrophotometer. UV absorption was recorded on a SHIMADZU UV-2501 PC spectrophotometer.

4.2. Preparation of compounds 1 and 20

6,7-Dichloroquinoline-5,8-dinone (**1**) was prepared according to Shaikh method,[12] by treating 8-hydroxyquinoline in concentrated HCl solution with sodium chlorate or concentrated HNO_3. The reaction residue was recrystallized in methanol to obtain the light yellow solid **1**. Yield 18% or 39%, respectively; mp = 221–223 °C (lit.[12] 221–223 °C); ^1H NMR (300 MHz, DMSO-d_6) δ: 9.08 (dd, 1H, J = 4.7, 1.7 Hz), 8.49 (dd,

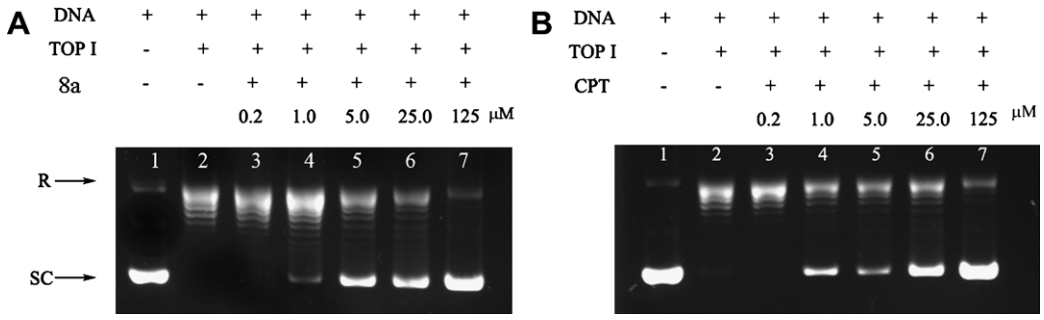


Figure 1. Inhibition of topoisomerase I relaxation activities of **8a** (A) and CPT (B). SC, supercoiled DNA; R, relaxed DNA. The lane 1 was DNA control without enzyme and drugs. The lane 2 was the control of DNA and enzyme without drugs. The rest lanes contained calf thymus TOP1, DNA and serially diluted drugs from 0.2 to 125 μM.

1H, J = 7.8, 1.5 Hz), 7.93 (dd, 1H, J = 8.0, 4.7 Hz);[18] ESI-MS m/z: 228.3, 230.2 $[M+1]^+$.

6,7-Dichloroisoquinoline-5,8-dinone (**20**) was obtained by treating 5-aminoisoquinoline (**19**) in concentrated HCl solution with sodium chlorate. Mp = 177–179 °C (lit.[15] 178–180 °C).

4.3. General preparation of IQDs

The heterocyclic skeleton was cyclized according to a published method with a slight modification.[17,18] Substrate **1** (2.2 mmol) was dissolved in absolute ethanol (50 mL). The solution was heated to 60 °C. Then, the AMR (2.6 mmol) and pyridine derivative (6.8 mmol) were gradually added to the solution. The reaction solution was refluxed for 16 h, to give a dark-reddish suspension, and concentrated in vacuum. The residue was subsequently subjected to flash column chromatography packed manually with silica gel to give the corresponding target compound.

4.3.1. Ethyl 5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 2a. Orange solid, yield 31%; mp = 226–227 °C (lit.[15] 225–226 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.92 (d, 1H, J = 7.2 Hz), 9.01 (d, 1H, J = 3.9 Hz), 8.54 (d, 1H, J = 7.5 Hz), 8.34 (d, 1H, J = 9.0 Hz), 7.64 (dd, 1H, J = 7.5, 4.5 Hz), 7.49 (t, 1H, J = 8.0 Hz), 7.24 (t, 1H, J = 6.9 Hz), 4.52 (q, 2H, J = 7.1 Hz), 1.51 (t, 3H, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 179.1, 173.1, 162.9, 154.1, 149.5, 139.9, 135.4, 130.9, 128.6, 128.5, 128.1, 126.9, 122.9, 121.1, 117.9, 106.3, 61.1, 14.3; IR ν_{max} (KBr): 1678.6, 1641.8, 1571.3, 1487.6, 1383.1 cm^{-1}; UV/vis λ_{max} (EtOH): 250, 322, 337, 356, 468 nm; ESI-MS m/z: 321.2 $[M+1]^+$, 343.2 $[M+Na]^+$, 663.0 $[2M+Na]^+$; $C_{18}H_{12}N_2O_4$, calcd: C, 67.50%; H, 3.78%; N, 8.75%; O, 19.98%; found: C, 67.52%; H, 3.77%; N, 8.71%. The structure was confirmed through HMQC and HMBC.[15]

4.3.2. Ethyl 5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 2b. Orange solid, yield 12%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.78 (d, 1H, J = 6.9 Hz), 8.99 (d, 1H, J = 3.6 Hz), 8.55 (d, 1H, J = 7.8 Hz), 8.38 (d, 1H, J = 9.0 Hz), 7.65 (dd, 1H, J = 7.8, 4.5 Hz), 7.47 (ddd, 1H, J = 8.7, 6.9, 0.9 Hz), 7.20 (td, 1H, J = 6.9, 1.2 Hz), 4.50 (q, 2H, J = 7.2 Hz), 1.52 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.9, 173.2, 162.9, 153.2, 149.2, 139.7, 134.0, 130.2, 128.2, 128.1, 127.8, 126.9, 121.3, 120.6, 117.7, 106.4, 60.8, 13.8; IR ν_{max} (KBr): 1675.8, 1637,4, 1488.3, 1383.4, 1318.6 cm^{-1}; UV/vis λ_{max} (EtOH): 248, 321, 355, 466 nm; ESI-MS m/z: 321.1 $[M+1]^+$, 343.2 $[M+Na]^+$; $C_{18}H_{12}N_2O_4$, calcd: C, 67.50%; H, 3.78%; N, 8.75%; O, 19.98%; found: C, 67.51%; H, 3.80%; N, 8.74%. The structure was confirmed through HMQC and HMBC.[15]

4.3.3. 6-Acetylindolizino[2,3-*g*]quinoline-5,12-dione 3a. Red solid, yield 13%; mp (deg.) = 252 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.93 (d, 1H, J = 6.9 Hz), 9.03 (d, 1H, J = 3.6 Hz), 8.53 (d, 1H, J = 7.8 Hz), 8.46 (d, 1H, J = 9.3 Hz), 7.66 (dd, 1H, J = 7.7, 4.7 Hz), 7.51 (t, 1H, J = 7.8 Hz), 7.25 (t, 1H, J = 7.5 Hz), 2.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.7, 180.9, 173.1, 154.4, 149.7, 139.8, 135.3, 130.6, 129.5, 128.4, 126.9, 126.8, 122.3, 121.8, 118.6, 114.9, 31.9; IR ν_{max} (KBr): 2962.9, 1642.3, 1491.9, 1367.0, 1312.4 cm^{-1}; UV/vis λ_{max} (EtOH): 245, 327, 361, 480 nm; ESI-MS m/z: 291.3 $[M+1]^+$, 313.1 $[M+Na]^+$, 602.9 $[2M+Na]^+$; $C_{17}H_{10}N_2O_3$, calcd: C, 70.34%; H, 3.47%; N, 9.65%; O, 16.54%; found: C, 70.31%; H, 3.51%; N, 9.63%.

4.3.4. 11-Acetylindolizino[3,2-*g*]quinoline-5,12-dione 3b. Red solid, yield 2.5%; mp (deg.) = 234 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.80 (d, 1H, J = 6.8 Hz), 8.99 (d, 1H, J = 3.6 Hz), 8.56 (dd, 1H, J = 7.8, 1.5 Hz), 8.46 (d, 1H, J = 9.0 Hz), 7.67 (dd, 1H, J = 7.7, 4.7 Hz), 7.49 (ddd, 1H, J = 8.6, 6.9, 1.2 Hz), 7.23 (td, 1H, J = 6.9, 1.2 Hz), 2.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.9, 180.1, 173.7, 153.7, 149.4, 139.9, 134.4, 130.8, 129.4, 128.1, 127.6, 127.5, 122.0, 121.1, 118.5, 115.5, 31.9; IR ν_{max} (KBr): 2962.6, 1681.3, 1634.2, 1580.2, 1485.1, 1312.3 cm^{-1}; UV/vis. λ_{max} (EtOH): 244, 325, 361, 480 nm; ESI-MS m/z: 291.3 $[M+1]^+$, 313.2 $[M+Na]^+$, 603.0 $[2M+Na]^+$; $C_{17}H_{10}N_2O_3$, calcd: C, 70.34%; H, 3.47%; N, 9.65%; O, 16.54%; found: C, 70.32%; H, 3.50%; N, 9.63%.

Pure products **4a** and **4b** could not be obtained by the flash column chromatographic techniques. **4a** (red solid mixture with **4b**): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.77 (d, 1H, J = 7.2 Hz), 9.00 (dd, 1H, J = 4.8, 1.5 Hz),

8.51 (dd, 1H, J = 7.8, 1.5 Hz), 7.90 (d, 1H, J = 9.0 Hz), 7.84 (dd, 1H, J = 7.8, 4.8 Hz), 7.56 (td, 1H, J = 6.9, 1.2 Hz), 7.39 (td, 1H, J = 6.9, 0.9 Hz). **4b** (red solid mixture with **4a**): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.51 (d, 1H, J = 7.2 Hz), 8.91 (dd, 1H, J = 4.8, 1.5 Hz), 8.48 (dd, 1H, J = 7.8, 1.5 Hz), 7.91 (d, 1H, J = 9.0 Hz), 7.78 (dd, 1H, J = 7.8, 4.8 Hz), 7.40 (td, 1H, J = 6.9, 1.2 Hz), 7.28 (td, 1H, J = 6.9, 0.9 Hz). ESI-MS m/z(mixture): 274.4 $[M+1]^+$.

4.3.5. 6-Methylindolizino[2,3-*g*]quinoline-5,12-dione 5a. Red solid, yield 15%; mp = 238 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.77 (d, 1H, J = 6.6 Hz), 8.99 (d, 1H, J = 3.0 Hz), 8.49 (d, 1H, J = 7.5 Hz), 7.67 (d, 1H, J = 9.0 Hz), 7.57 (dd, 1H, J = 7.4, 4.8 Hz), 7.27 (t, 1H, J = 7.4 Hz), 7.11 (t, 1H, J = 6.6 Hz), 2.70 (s, 3H); IR ν_{max} (KBr): 2925.8, 1731.7, 1659.3, 1633.0, 1574.7, 1477.9, 1318.9 cm^{-1}; UV/vis λ_{max} (EtOH): 253, 340, 380, 504 nm; ESI-MS m/z: 263.3 $[M+1]^+$, 247.0 $[2M+Na]^+$; $C_{16}H_{10}N_2O_2$, calcd: C, 73.27%; H, 3.84%; N, 10.68%; O, 12.20%; found: C, 73.27%; H, 3.85%; N, 10.66%.

4.3.6. 11-Methylindolizino[3,2-*g*]quinoline-5,12-dione 5b. Red solid, yield 19%; mp (deg.) = 233 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.64 (d, 1H, J = 6.6 Hz), 8.94 (d, 1H, J = 2.6 Hz), 8.58 (d, 1H, J = 7.5 Hz), 7.69 (d, 1H, J = 9.0 Hz), 7.63 (dd, 1H, J = 7.4, 4.8 Hz), 7.26 (t, 1H, J = 7.4 Hz), 7.10 (t, 1H, J = 6.6 Hz), 2.74 (s, 3H); IR ν_{max} (KBr): 2927.5, 1731.1, 1677.4, 1619.7, 1576.8, 1478.3, 1315.7 cm^{-1}; UV/vis λ_{max} (EtOH): 252, 336, 378, 507 nm; ESI-MS m/z: 263.3 $[M+1]^+$; $C_{16}H_{10}N_2O_2$, calcd: C, 73.27%; H, 3.84%; N, 10.68%; O, 12.20%; found: C, 73.25%; H, 3.82%; N, 10.71%.

4.3.7. Indolizino[2,3-*g*]quinoline-5,12-dione 6a. Red solid, yield 13%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.74 (d, 1H, J = 5.7 Hz), 9.02 (s, br, 1H, J = 4.5 Hz), 8.51 (d, 1H, J = 6.9 Hz), 7.71 (d, 1H, J = 7.5 Hz), 7.50–7.75 (m, 1H), 7.25–7.32 (m, 2H), 7.10 (s, 1H);[15] IR ν_{max} (KBr): 2925.4, 1726.7, 1619.9, 1535.8, 1493.4, 1383.2, 1342.4 cm^{-1}; ESI-MS m/z: 249.3 $[M+1]^+$, 518.9 $[2M+Na]^+$; $C_{15}H_8N_2O_2$, calcd: C, 72.58%; H, 3.25%; N, 11.28%; O, 12.89%; found: C, 72.60%; H, 3.28%; N, 11.24%.

4.3.8. Indolizino[3,2-*g*]quinoline-5,12-dione 6b. Red solid, yield 8%; ^{1}H NMR (300 MHz, DMSO-d6, TMS) δ: 9.49 (d, 1H, J = 6.9 Hz), 8.91 (d, br, 1H, J = 1.8 Hz), 8.48 (d, 1H, J = 7.5 Hz), 7.93 (d, 1H, J = 9.3 Hz), 7.79 (dd, 1H, J = 7.5, 4.5 Hz), 7.42 (t, 1H, J = 7.5 Hz), 7.30 (t, 1H, J = 6.9 Hz), 7.19 (s, 1H); IR ν_{max} (KBr): 2961.6, 1736.7, 1678.2, 1620.6, 1577.7, 1488.0, 1391.6, 1337.2 cm^{-1}; UV/vis λ_{max} (EtOH): 251, 326, 363, 481 nm; ESI-MS m/z: 249.2 $[M+1]^+$; $C_{15}H_8N_2O_2$, calcd: C, 72.58%; H, 3.25%; N, 11.28%; O, 12.89%; found: C, 72.58%; H, 3.27%; N, 11.29%.

4.3.9. Ethyl 7-fluoro-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 7a. Orange solid, yield 12%; mp = 197–200 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.64 (d, 1H, J = 6.8 Hz), 9.06 (dd, 1H, J = 4.8, 1.6 Hz), 8.54 (dd, 1H, J = 8.0, 1.6 Hz), 7.67 (dd, 1H, J = 8.0, 4.8 Hz), 7.16–7.06 (m, 2H), 4.54 (q, 2H, J = 7.2 Hz), 1.47 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 179.3, 172.8, 163.2, 154.5d(J^1_{C-F} = 254.4 Hz), 154.4, 153.8, 135.2, 134.7, 130.5, 128.9d(J^2_{C-F} = 32.8 Hz), 127.4, 127.1, 126.6, 124.8d(J^4_{C-F} = 5.2 Hz), 122.6, 117.1d(J^3_{C-F} = 6.8 Hz), 110.5d(J^2_{C-F} = 17.1 Hz), 62.3, 14.1; IR ν_{max} (KBr): 1725.2, 1674.9, 1639.1, 1619.1, 1575.9, 1494.0, 1404.5 cm^{-1}; ESI-MS m/z: 339.0 $[M+1]^+$, 698.9 $[2M+Na]^+$; $C_{18}H_{11}FN_2O_4$, calcd: C, 63.91%; H, 3.28%; F, 5.62%; N, 8.28%; O, 18.92%; found: C, 63.90%; H, 3.31%; F, 5.62%; N, 8.26%. The structure was confirmed through HMQC and HMBC.

4.3.10. Ethyl 10-fluoro-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 7b. Orange solid, yield 18%; mp = 200–202 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.49 (dd, 1H, J = 6.3, 1.2 Hz), 9.00 (dd, 1H, J = 4.7, 1.7 Hz), 8.57 (dd, 1H, J = 7.8, 1.8 Hz), 7.68 (dd, 1H, J = 7.8, 4.5 Hz), 7.14–7.03 (m, 2H), 4.54 (q, 2H, J = 7.2 Hz), 1.48 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.3, 173.5, 163.2, 154.6d(J^1_{C-F} = 254.9 Hz), 153.8, 149.3, 134.7, 131.2, 128.9d(J^2_{C-F} = 32.4 Hz), 127.4, 127.3, 124.4d(J^4_{C-F} = 5.3 Hz), 121.5, 117.0d(J^3_{C-F} = 6.7 Hz), 110.3d(J^2_{C-F} = 17.0 Hz), 62.4, 14.0; IR ν_{max} (KBr): 1722.1, 1680.4, 1634.8, 1577.7, 1547.5, 1488.3, 1403.1 cm^{-1}; UV/vis λ_{max} (EtOH): 244, 319, 463 nm; ESI-MS m/z: 339.0 $[M+1]^+$; $C_{18}H_{11}FN_2O_4$, calcd: C, 63.91%; H, 3.28%; F, 5.62%; N, 8.28%; O, 18.92%; found: C, 63.87%; H, 3.32%; F, 5.61%; N, 8.27%. The structure was confirmed through HMQC and HMBC.

4.3.11. Ethyl 7-chloro-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 8a. Red solid, yield 16%; mp = 185–190 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.58 (d, 1 H, J = 6.9 Hz), 8.98 (d, 1H, J = 4.5 Hz), 8.57 (d, 1H, J = 7.5 Hz), 7.67 (dd, 1H, J = 7.8, 4.5 Hz), 7.36 (d, 1H, J = 7.5 Hz), 7.07 (t, 1H, J = 7.2 Hz), 4.56 (q, 2H, J = 7.2 Hz), 1.49 (t, 3H, J = 7.2 Hz); IR ν_{max} (KBr): 2924.9, 1733.1,1680.9, 1645.3, 1578.3, 1514.6, 1497.3, 1299.9 cm^{-1}; UV/vis λ_{max} (EtOH): 246, 329, 468 nm; ESI-MS m/z: 355.2, 357.2 $[M+1]^+$; $C_{18}H_{11}ClN_2O_4$, calcd: C, 60.94%; H, 3.13%; Cl, 9.99%; N, 7.90%; O, 18.04%; found: C, 60.90%; H, 3.15%; Cl, 9.98%; N, 7.87%.

4.3.12. Ethyl 10-chloro-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 8b. Red solid, yield 12%; mp (deg.) = 195 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.72 (d, 1H, J = 6.3 Hz), 9.03 (d, br, 1H, J = 3.3 Hz), 8.52 (d, 1H, J = 7.2 Hz), 7.65 (dd, 1H, J = 7.8, 4.6 Hz), 7.38 (d, 1H, J = 7.2 Hz), 7.09 (t, 1H, J = 7.2 Hz), 4.55 (q, 2H, J = 7.2 Hz), 1.47 (t, 3H, J = 7.2 Hz); IR ν_{max} (KBr): 2924.9, 1731.1, 1670.5, 1639.4, 1618.5, 1498.4, 1457.1 cm^{-1}; UV/vis λ_{max} (EtOH): 254, 324, 359, 472 nm; ESI-MS m/z: 355.2, 357.2 $[M+1]^+$; $C_{18}H_{11}ClN_2O_4$, calcd: C, 60.94%; H, 3.13%; Cl, 9.99%; N, 7.90%; O, 18.04%; found: C, 60.93%; H, 3.14%; Cl, 9.98%; N, 7.89%.

4.3.13. Ethyl 7-bromo-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 9a. Orange solid, yield 18%; mp = 210–215 °C; ^{1}H NMR (300 MHz, $CDCl_3$)

δ: 9.63 (d, 1H, J = 6.9 Hz), 8.98 (dd, 1H, J = 4.5, 1.5 Hz), 8.55 (dd, 1H, J = 7.8, 1.8 Hz), 7.67 (dd, 1H, J = 7.8, 4.5 Hz), 7.55 (d, 1H, J = 7.5 Hz), 6.99 (t, 1H, J = 7.2 Hz), 4.57 (q, 2H, J = 7.2 Hz), 1.50 (t, 3H, J = 7.2 Hz); IR ν_{max} (KBr): 2925.2, 1727.0, 1679.5, 1640.6, 1575.3, 1494.2, 1379.3 cm^{-1}; UV/vis λ_{max} (EtOH): 250, 326, 467 nm; ESI-MS *m/z*: 399.0, 401.0 $[M+1]^+$; $C_{18}H_{11}BrN_2O_4$, calcd: C, 54.16%; H, 2.78%; Br, 20.02%; N, 7.02%; O, 16.03%; found: C, 54.15%; H, 2.80%; Br, 20.00%; N, 7.01%.

4.3.14. Ethyl 10-bromo-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 9b. Orange solid, yield 16%; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.79 (d, 1H, J = 6.6 Hz), 9.02 (d, br, 1H, J = 1.8 Hz), 8.51 (d, 1H, J = 7.5 Hz), 7.65 (dd, 1H, J = 7.8, 4.5 Hz), 7.57 (d, 1H, J = 6.9 Hz), 7.02 (t, 1H, J = 6.9 Hz), 4.59 (q, 2H, J = 7.2 Hz), 1.49 (t, 3H, J = 7.2 Hz); ESI-MS *m/z*: 399.0, 401.0 $[M+1]^+$, 421.1, 423.1 $[M+Na]^+$; $C_{18}H_{11}BrN_2O_4$, calcd: C, 54.16%; H, 2.78%; Br, 20.02%; N, 7.02%; O, 16.03%; found: C, 54.13%; H, 2.81%; Br, 20.01%; N, 7.02%.

4.3.15. Ethyl 7-methyl-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 10a. Yellow solid, yield 21%; mp (deg.) = 165–170 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.73 (d, br, 1H, J = 4.2 Hz), 9.03 (dd, 1H, J = 4.8, 1.5 Hz), 8.56 (dd, 1H, J = 7.8, 1.5 Hz), 8.23 (d, 1H, J = 9.0 Hz), 7.67 (dd, 1H, J = 7.8, 4.5 Hz), 7.34 (dd, 1H, J = 9.0, 1.5 Hz), 4.50 (q, 2H, J = 6.9 Hz), 2.49 (s, 3H), 1.50 (t, 3H, J = 6.9 Hz); IR ν_{max} (KBr): 1726.5, 1674.9, 1638.1, 1573.3, 1482.0, 1382.0 cm^{-1}; ESI-MS *m/z*: 335.0 $[M+1]^+$, 357.1 $[M+Na]^+$; $C_{19}H_{14}N_2O_4$, calcd: C, 68.26%; H, 4.22%; N, 8.38%; O, 19.14%; found: C, 68.25%; H, 4.22%; N, 8.37%.

4.3.16. Ethyl 10-methyl-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 10b. Brown solid, yield 7%; mp = 160 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.52 (d, 1H, J = 6.9 Hz), 8.94 (dd, 1H, J = 4.8, 1.5 Hz), 8.55 (dd, 1H, J = 7.8, 1.8 Hz), 7.65 (dd, 1H, J = 7.8, 4.5 Hz), 7.11 (d, 1H, J = 6.9 Hz), 7.05 (t, 1H, J = 7.2 Hz), 4.54 (q, 2H, J = 7.2 Hz), 2.54 (s, 3H), 1.48 (t, 3H, J = 7.2 Hz); IR ν_{max} (KBr): 2921.2, 1674.8, 1637.5, 1472.3, 1315.7 cm^{-1}; ESI-MS *m/z*: 335.0 $[M+1]^+$, 357.1 $[M+Na]^+$, 690.9 $[2M+Na]^+$; $C_{19}H_{14}N_2O_4$, calcd: C, 68.26%; H, 4.22%; N, 8.38%; O, 19.14%; found: C, 68.23%; H, 4.23%; N, 8.38%.

4.3.17. Ethyl 7-hydroxy-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 11a. Red solid, yield 24%; mp = 221–223 °C; ^1H NMR (300 MHz, DMSO-d_6) δ: 9.16 (d, 1H, J = 6.6 Hz), 8.96 (dd, 1H, J = 4.5, 1.5 Hz), 8.38 (dd, 1H, J = 7.8, 1.5 Hz), 7.75 (dd, 1H, J = 7.8, 4.5 Hz), 7.19 (t, 1H, J = 7.2 Hz), 6.73 (d, 1H, J = 7.8 Hz), 4.35 (q, 2H, J = 6.9 Hz), 1.33 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.7, 171.3, 164.2, 153.9, 150.2, 150.0, 134.4, 129.9, 129.5, 127.0, 124.1, 122.0, 121.1, 119.5, 107.9, 107.8, 61.1, 13.9; UV/vis λ_{max} (EtOH): 253, 339, 360, 516 nm; ESI-MS *m/z*: 335.2 $[M-1]^-$; $C_{18}H_{12}N_2O_4$, calcd: C, 64.29%; H, 3.60%; N, 8.33%; O, 23.79%; found: C, 64.29%; H, 3.61%; N, 8.31%.

4.3.18. Ethyl 10-hydroxy-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 11b. Red solid, yield 13%; mp = 220–223 °C; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.3 (s, 1H), 9.09 (d, 1H, J = 6.6 Hz), 8.91 (d, br, 1H, J = 3.9 Hz), 8.47 (dd, 1H, J = 7.8, 0.9 Hz), 7.80 (dd, 1H, J = 7.8, 4.7 Hz), 7.19 (t, 1H, J = 7.2 Hz), 6.73 (d, 1H, J = 7.8 Hz), 4.36 (q, 2H, J = 6.9 Hz), 1.33(t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.6, 172.0, 164.2, 152.9, 150.0, 148.7, 134.2, 131.6, 129.7, 127.8, 125.3, 119.8, 119.5, 119.0,108.5, 107.8; IR ν_{max} (KBr): 1681.4, 1681.8, 1479.9, 1385.1 cm^{-1}; ESI-MS *m/z*: 335.2 $[M-1]^-$; $C_{18}H_{12}N_2O_4$, calcd: C, 64.29%; H, 3.60%; N, 8.33%; O, 23.79%; found: C, 64.30%; H, 3.61%; N, 8.30%.

4.3.19. Ethyl 7-amino-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline 6-carboxylate 12a. Purple solid, yield 24%; mp = 198–200 °C; ^1H NMR (300 MHz, DMSO-d_6) δ: 9.16 (d, 1H, J = 6.6 Hz), 8.95 (d, 1H, J = 3.6 Hz), 8.38 (d, 1H, J = 7.8 Hz), 7.75 (dd, 1H, J = 7.8, 3.6 Hz), 7.18 (t, 1H, J = 6.9 Hz), 6.70 (d, 1H, J = 7.5 Hz), 6.04 (s, br, 2H), 4.41 (q, 2H, J = 6.9 Hz), 1.38 (t, 3H, J = 6.9 Hz); IR ν_{max} (KBr): 1718.2, 1673.5, 1621.3, 1573.6, 1494.1, 1392.8 cm^{-1}; UV/vis λ_{max} (EtOH): 266, 360, 557 nm; ESI-MS *m/z*: 336.2 $[M+1]^+$; $C_{18}H_{13}N_3O_4$, calcd: C, 64.47%; H, 3.91%; N, 12.53%; O, 19.09%; found: C, 64.44%; H, 3.93%; N, 12.51%.

4.3.20. Ethyl 10-amino-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline 11-carboxylate 12b. Purple solid, yield 24%; mp = 323 °C; ^1H NMR (300 MHz, DMSO-d_6) δ: 9.11 (d, 1H, J = 6.6 Hz), 8.92 (dd, 1H, J = 4.5, 1.8 Hz), 8.46 (dd, 1H, J = 7.8, 1.5 Hz), 7.81 (dd, 1H, J = 7.8, 4.8 Hz), 7.19 (dd, 1H, J = 7.5, 6.6 Hz), 6.71 (d, 1H, J = 7.5 Hz), 4.42 (q, 2H, J = 7.1 Hz), 1.38 (t, 3H, J = 7.1 Hz); IR ν_{max} (KBr): 1693.1, 1676.3, 1620.3, 1578.8, 1548.4, 1462.2, 1421.6, 1303.2 cm^{-1}; UV/vis λ_{max} (EtOH): 266, 361, 564 nm; ESI-MS *m/z*: 336.2 $[M+1]^+$, 358.1 $[M+Na]^+$, 692.9 $[2M+Na]^+$; $C_{18}H_{13}N_3O_4$, calcd: C, 64.47%; H, 3.91%; N, 12.53%; O, 19.09%; found: C, 64.46%; H, 3.92%; N, 12.52%.

4.3.21. 6-Acetyl-7-fluoro-indolizino[2,3-*g*]quinoline-5,12-dione 13a. Yellow solid, yield 18%; mp = 143–145 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.92 (d, br, 1H, J = 2.1 Hz), 9.04 (d, 1H, J = 4.5 Hz), 8.54 (d, 1H, J = 7.8 Hz), 8.49 (d, 1H, J = 9.6 Hz), 7.68 (dd, 1H, J = 7.5, 4.5 Hz), 7.39 (t, 1H, J = 9.6 Hz), 2.90 (s, 3H); IR ν_{max} (KBr): 2963.5, 1638.2, 1618.5, 1577.5, 1417.2, 1261.2 cm^{-1}; ESI-MS *m/z*: 331.0 $[M+Na]^+$; $C_{17}H_9FN_2O_3$, calcd: C, 66.24%; H, 2.94%; F, 6.16%; N, 9.09%; O, 15.57%; found: C, 66.24%; H, 2.95%; F, 6.14%; N, 9.06%.

4.3.22. 11-Acetyl-10-fluoro-indolizino[3,2-*g*]quinoline-5,12-dione 13b. Yellow solid, yield 11%; mp = 141–143 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.64 (d, 1H, J = 6.6 Hz), 9.06 (d, 1H, J = 4.2 Hz), 8.53 (d, 1H, J = 7.5 Hz), 7.67 (dd, 1H, J = 7.8, 4.5 Hz), 7.17–7.05 (m, 2H), 2.81 (s, 3H); IR ν_{max} (KBr): 2963.7, 1637.3, 1617.3, 1558.5, 1418.4, 1261.4 cm^{-1}; ESI-MS *m/z*: 308.0 $[M+1]^+$, 331.0 $[M+Na]^+$; $C_{17}H_9FN_2O_3$, calcd: C, 66.24%; H, 2.94%;

F, 6.16%; N, 9.09%; O, 15.57%; found: C, 66.28%; H, 2.96%; F, 6.13%; N, 9.07%.

4.3.23. 6-Acetyl-7-chloro-indolizino[2,3-*g*]quinoline-5,12-dione 14a. Red solid, yield 9%; mp (deg.) = 128–130 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.88 (d, br, 1H, J = 1.8 Hz), 9.04 (d, br, 1H, J = 3.9 Hz), 8.54 (d, 1H, J = 7.8 Hz), 8.42 (d, 1H, J = 9.6 Hz), 7.69 (dd, 1H, J = 7.5, 4.5 Hz), 7.39 (dd, 1H, J = 9.6, 1.8 Hz), 2.88 (s, 3H); IR ν_{max} (KBr): 2963.6, 1637.8, 1618.7, 1559.2, 1417.9, 1261.5 cm^{-1}; ESI-MS *m*/*z*: 324.0, 326.0 [M+1]$^+$, 347.0, 348.2 [M+Na]$^+$; $C_{17}H_9ClN_2O_3$, calcd: C, 62.88%; H, 2.79%; Cl, 10.92%; N, 8.63%; O, 14.78%; found: C, 62.87%; H, 2.80%; Cl, 10.90; N, 8.62%.

4.3.24. 11-Acetyl-10-chloro-indolizino[3,2-*g*]quinoline-5,12-dione 14b. Red solid, yield 15%; mp = 135–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.60 (d, 1H, J = 6.9 Hz), 8.99 (d, 1H, J = 3.6 Hz), 8.57 (d, 1H, J = 7.5 Hz), 7.68 (dd, 1H, J = 7.5, 4.5 Hz), 7.34 (d, 1H, J = 7.2 Hz), 7.07 (t, 1H, J = 7.2 Hz), 2.82 (s, 3H); IR ν_{max} (KBr): 1637.4, 1618.9, 1558.3, 1541.8, 1418.6, 1260.1 cm^{-1}; ESI-MS *m*/*z*: 324.0, 326.0 [M+1]$^+$; $C_{17}H_9ClN_2O_3$, calcd: C, 62.88%; H, 2.79%; Cl, 10.92%; N, 8.63%; O, 14.78%; found: C, 62.88%; H, 2.80%; Cl, 10.89; N, 8.61%.

4.3.25. 6-Acetyl-7-bromo-indolizino[2,3-*g*]quinoline-5,12-dione 15a. Red solid, yield 12%; mp = 165–168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.65 (d, 1H, J = 6.9 Hz), 9.03 (d, 1H, J = 3.9 Hz), 8.56 (d, 1H, J = 7.8 Hz), 7.65 (d, 1H, J = 7.5 Hz), 7.53 (dd, 1H, J = 7.5, 4.5 Hz), 6.99 (t, 1H, J = 7.2 Hz), 2.87 (s, 3H); IR ν_{max} (KBr): 1702.7, 1684.8, 1636.2, 1557.9, 1508.3, 1489.2, 1399.3, 1250.9 cm^{-1}; ESI-MS *m*/*z*: [M+1]$^+$; $C_{17}H_9BrN_2O_3$, calcd: C, 55.31%; H, 2.46%; Br, 21.64%; N, 7.59%; O, 13.00%; found: C, 55.31%; H, 2.47%; Br, 21.63%; N, 7.57%.

4.3.26. 11-Acetyl-10-bromo-indolizino[3,2-*g*]quinoline-5,12-dione 15b. Red solid, yield 17%; mp = 180–182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.67 (d, 1H, J = 6.9 Hz), 8.91 (d, 1H, J = 4.5 Hz), 8.56 (d, 1H, J = 8.1 Hz), 7.86 (dd, 1H, J = 7.5, 4.5 Hz), 7.71 (d, 1H, J = 7.5 Hz), 7.13 (t, 1H, J = 7.2 Hz), 2.80 (s, 3H); IR ν_{max} (KBr): 2963.2, 1637.3, 1611.6, 1558.2, 1541.8, 1418.4, 1261.3 cm^{-1}; ESI-MS *m*/*z*: [M+1]$^+$; $C_{17}H_9BrN_2O_3$, calcd: C, 55.31%; H, 2.46%; Br, 21.64%; N, 7.59%; O, 13.00%; found: C, 55.30%; H, 2.50%; Br, 21.61%; N, 7.56%.

4.3.27. 6-Acetyl-7-hydroxy-indolizino[2,3-*g*]quinoline-5,12-dione 16a. Purple solid, yield 5%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.42 (s, 1H), 9.58 (d, 1H, J = 6.6 Hz), 9.09 (d, br, 1H, J = 2.4 Hz), 8.56 (dd, 1H, J = 7.8, 1.8 Hz), 8.12 (d, br, 1H, J = 5.1 Hz), 7.94 (dd, br, 1H, J = 8.7, 1.2 Hz), 7.72 (dd, 1H, J = 8.7, 5.7 Hz), 2.98 (s, 3H); ESI-MS *m*/*z*: 307.1 [M+1]$^+$; $C_{17}H_{10}N_2O_4$, calcd: C, 66.67%; H, 3.29%; N, 9.15%; O, 20.90%; found: C, 66.65%; H, 3.32%; N, 9.14%.

4.3.28. 11-Acetyl-10-hydroxy-indolizino[3,2-*g*]quinoline-5,12-dione 16b. Purple solid, yield 28%; mp = 200–203 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.33 (s, 1H), 9.71 (d, 1H, J = 6.6 Hz), 9.04 (dd, 1H, J = 4.5, 1.5 Hz), 8.51 (dd, 1H, J = 7.8, 1.5 Hz), 7.67 (dd, 1H, J = 7.8, 4.5 Hz), 7.23 (dd, 1H, J = 7.5, 6.6 Hz), 7.01 (d, 1H, J = 7.5 Hz), 2.96 (s, 3H); IR ν_{max} (KBr): 3415.2, 1963.6, 1637.7, 1618.3, 1576.5, 1541.7, 1456.8, 1261.8 cm^{-1}; ESI-MS *m*/*z*: 307.1 [M+1]$^+$; $C_{17}H_{10}N_2O_4$, calcd: C, 66.67%; H, 3.29%; N, 9.15%; O, 20.90%; found: C, 66.66%; H, 3.31%; N, 9.13%.

4.3.29. 6-Acetyl-7-amino-indolizino[2,3-*g*]quinoline-5,12-dione 17a. Purple solid, yield 11%; mp = 155–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.48 (d, 1H, J = 6.6 Hz), 9.01 (d, 1H, J = 4.5 Hz), 8.50 (d, 1H, J = 7.8 Hz), 7.62 (dd, 1H, J = 7.8, 4.5 Hz), 7.06 (t, 1H, J = 6.9 Hz), 6.59 (d, 1H, J = 7.5 Hz), 2.86 (s, 3H); IR ν_{max} (KBr): 3419.3, 1733.4, 1716.3, 1668.9, 1617.9, 1542.3, 1489.0, 1396.7 cm^{-1}; ESI-MS *m*/*z*: 306.0 [M+1]$^+$, 328.0 [M+Na]$^+$; $C_{17}H_{11}N_3O_3$, calcd: C, 66.88%; H, 3.63%; N, 13.76%; O, 15.72%; found: C, 66.86%; H, 3.65%; N, 13.77%.

4.3.30. 11-Acetyl-10-amino-indolizino[3,2-*g*]quinoline-5,12-dione 17b. Purple solid, yield 18%; mp = 138–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.37 (d, 1H, J = 6.3 Hz), 8.97 (d, 1H, J = 3.9 Hz), 8.58 (d, 1H, J = 8.1 Hz), 7.67 (dd, 1H, J = 8.1, 4.8 Hz), 7.05 (t, 1H, J = 7.5 Hz), 6.58 (d, 1H, J = 7.8 Hz), 2.89 (s, 3H); IR ν_{max} (KBr): 3414.7, 1716.3, 1637.1, 1618.2, 1541.9, 1457.1 cm^{-1}; ESI-MS *m*/*z*: 328.0 [M+Na]$^+$; $C_{17}H_{11}N_3O_3$, calcd: C, 66.88%; H, 3.63%; N, 13.76%; O, 15.72%; found: C, 66.88%; H, 3.66%; N, 13.75%.

4.4. Preparation of 5-aminoisoquinoline 19

Isoquinoline (3.0 g, 0.023 mol) was dissolved in concentrated H_2SO_4 (15 mL), a considerable amount of heat being evolved, and the solution was then cooled to 0 °C and stirred mechanically while KNO_3 (2.4 g, 0.024 mol) in H_2SO_4 (170 mL) was added dropwise over 2 h. After being kept below 50 °C for 4 h, the reaction was poured into ice and neutralized with cold aqueous ammonia. Filtration and recrystallization from ethanol gave the yellow solid 5-nitroisoquinoline **18**: 1.9 g, yield 48%; mp = 108–111 °C (lit.[21] 108–110 °C).

5-Nitroisoquinoline **18** (2.5 g, 0.014 mol) and palladium charcoal (10%, 0.13 g) in EtOH (35 mL) was stirred at room temperature for 4 h in hydrogen atmosphere. The catalyst was removed by filtration. The filtrate was evaporated under vacuum, and the residue was purified by silica gel chromatography to give **19**: 2.0 g, yield 96.6%; mp = 128–129 °C (lit.[22] 128–129 °C).

4.5. Preparation of 2,3-dichloro-1,4-naphtoquinone 21

1,4-Naphthoquinone (5.0 g, 0.030 mol) was dissolved in ethyl acetate (150 mL) at 50 °C. The solution was added with concentrated HCl (150 mL) and Bu_4NBr (0.5 g), and then stirred. Sodium chlorate (10.6 g, 0.10 mol) was added to the solution over 15 min, and stirred for 20 h. The precipitate was removed by filtration, and the filtrate was washed with water. The organic layer was concentrated under vacuum. The residue was crys-

tallized in BuOH (20 mL) to give the yellow solid **21**: 5.3 g, yield 73%; mp = 194–195 °C (lit.[23] 195 °C).

4.6. Preparation of regioisomers 22a and 22b

According to the general cyclization procedure (**Step 4.3**), using 6,7-dichloro isoquinoline-5,8-dione as material instead of substrate **1**, the reaction gave insperative regioisomers.[15] **22a** (red solid mixture with **22b**): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.71 (d, 1H, J = 7.2 Hz), 9.34 (s, 1H), 8.91 (d, 1H, J = 5.1 Hz), 8.23 (d, 1H, J = 9.0 Hz), 7.89 (d, 1H, J = 5.1 Hz), 7.38 (t, 1H, J = 8.1 Hz), 7.12 (t, 1H, J = 6.6 Hz), 4.51 (q, 2H, J = 7.2 Hz), 1.50 (t, 3H, J = 7.2 Hz). **22b** (red solid mixture with **22a**): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.68 (d, 1H, J = 7.2 Hz), 9.31 (s, 1H), 8.91 (d, 1H, J = 5.1 Hz), 8.23 (d, 1H, J = 9.0 Hz), 7.91 (d, 1H, J = 4.8 Hz), 7.40 (t, 1H, J = 7.5 Hz), 7.14 (t, 1H, J = 6.0 Hz), 4.51 (q, 2H, J = 7.2 Hz), 1.50 (t, 3H, J = 7.2 Hz). ESI-MS m/z (mixture): 321.0 $[M+1]^+$.

4.7. Preparation of Ethyl 6,11-dioxo-6,11-dihydrobenzo[*f*]-pyrido[1,2-*a*]indole 12-carboxylate 23

According to the general cyclization procedure (**Step 4.3**), using 2,3-dichloro-1,4-naphtoquinone as material instead of substrate **1**, the reaction gave pure **23**. Orange-brown solid, yield 27%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.82 (d, 1H, J = 7.2 Hz), 8.29 (d, 1H, J = 9.0 Hz), 8.21–8.18 (m, 2H), 7.70 (d, 1H, J = 5.7 Hz), 7.69 (d, 1H, J = 7.2 Hz), 7.41 (t, 1H, J = 8.1 Hz), 7.14 (td, 1H, J = 7.2, 0.9 Hz), 4.51 (q, 2H, J = 7.2 Hz), 1.50 (t, 3H, J = 7.2 Hz);[24] IR ν_{max} (KBr): 1673.3, 1638.2, 1591.8, 1509.8, 1321.3 cm^{-1}; ESI-MS m/z: 320.1 $[M+1]^+$, 342.2 $[M+Na]^+$, 660.9 $[2M+Na]^+$.

4.8. Cell culture and drug treatment

Four different human cancer cell lines, lung adenocarcinoma cell (GLC-82), large-cell lung carcinoma cell (NCI-H460), promyelocytic leukemia cell (HL-60) and breast carcinoma cell (MCF-7), were cultured on RPMI-1640 medium supplemented with fetal bovine serum (10%), penicillin (100 U/mL) and streptomycin (100 μg/mL) in 25-cm^2 culture flasks at 37 °C in a humidified atmosphere with 5% CO_2. All cells to be tested in the following assays had a passage number of 3–6. For the drug treatment experiments, the cancer cells were harvested from the culture during the exponential growth phase, and seeded into multiwell culture plates at $5 \times 10^4 - 1 \times 10^5$ cells/ml in fresh medium. After overnight growth, the cells were treated with the compounds (predissolved in DMSO) at selected concentrations for a period of 2 days.

4.9. MTT assay of cell viability

At the end of the drug treatment period, MTT solution (50 μL, 1 mg/mL) in PBS (PBS without MTT as the blank) was fed to each well of the culture plate (containing 100 μL medium). After 4 h incubation, the formazan crystal formed in the well was solubilized with 100 μL DMSO (100 μL solvent including 10% SDS, 5% isopropanol and 0.01 mol/L HCl as for HL-60) for optical density reading at 492 nm.

4.10. TOP1 inhibitory assay

The topoisomerase I relaxation assay was performed as mentioned in the reported method.[19,20] Briefly, relaxation of 0.5 μg plasmid pBR322 was performed in 20 μL of topoisomerase I relaxing buffer (20 mM Tris pH 7.5, 0.1 mM EDTA, 10 mM $MgCl_2$, 50 μg/mL acetylated BSA, 100 mM KCl) in the presence or the absence of the test drugs previously dissolved in DMSO solution. Reactions were started by the addition of DNA, control groups were either DNA alone or DNA treated with TOP1. The drugs and DNA in the presence of TOP1 was incubated for 30 min at 37 °C. The reaction was terminated by the addition of 4 μL 6× loading buffer. Products were then run in a 1% agarose gel in 45 mM Tris–borate (pH 8.0), 1 mM EDTA at 5 V/cm. Gels were stained with ethidium bromide and visualized with a UV transilluminator. Images were acquired and quantified through AlphaEaseFC software.

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