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Synthesis and Inhibitory Activity of Novel Tri- and Tetracyclic Quinolines against Topoisomerases

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Abstract—A series of isoindolo[2,1-*a*]- and pyrrolo[1,2-*a*]quinolines were designed and synthesized for DNA-gyrase and topoisomerase-II inhibition studies. Some of the compounds showed significant activity against the enzymes.

Introduction

The antibacterial activity of the quinolones is due to the inhibition of the enzyme, DNA-gyrase, which belongs to a class of enzymes known as topoisomerases. DNA topoisomerases play a crucial role in catalyzing the biological interconversions necessary for DNA replication, transcription, and recombination in prokaryotic and eukaryotic cells. Although quinolones have become one of the most clinically useful classes of broad spectrum antibacterial drugs today, their usage is restricted in some cases because of their side effects, such as on the CNS and on the articular cartilage.[1–3] Since all the compounds possess a β-keto acid on a similar planar, rigid ring systems, it is very possible these common entities may be involved in the mechanisms of those side effects associated with this class of compounds.

Both prokaryotic and eukaryotic topoisomerase II inhibitors, developed as antibacterial or antitumor agents, respectively, share a similar mechanism of action. The formation of a ternary complex (DNA:topoisomerase II:drug) leading to the 'cleavable-complex' is required for activity with these inhibitors.[4–6] Specific binding sites and strand specificity for covalent catalysis leading to formation of the ternary drug complex are distinct for each of the prokaryotic and eukaryotic enzymes, even though both enzymes share a common tyrosine for the covalent catalysis.[7,8] These differences afford the possibility of finding agents that selectively inhibit the prokaryotic topoisomerase II, such as the antibacterial 4-quinolones,[9–11] or that inhibit the mammalian topoisomerase II, such as the antitumor 4-quinolones more recently reported.[6,10,12,13]

In our earlier study to identify novel inhibitors of DNA-gyrase lacking the β-keto acid moiety as potential antibacterials, the flavones were found to be bonafide inhibitors of the enzyme.[14] We then designed and synthesized a series of azaflavones **2** that exhibited comparable DNA-gyrase inhibitory activity as the flavones **1** (Figure 1).[15] In the studies on the azaflavones we noticed that substitution of the nitrogen atom with an alkyl group abolished activity. While such a substitution removes a donatable hydrogen and changes the electronic character in this region, loss of activity may be related to adding steric bulk either above or below the quinolinone ring thus destroying the planarity in this region. We now report a series of novel planar tricyclic and tetracyclic quinoline derivatives wherein steric bulk is added to this region, but confined to the quinolinone plane. In addition to identifying prokaryotic topoisomerase II (gyrase) inhibitors, we have also characterized the selectivity of these novel inhibitors against the eukaryotic topoisomerase II.

Key words: DNA-gyrase; topoisomerase II; isoindolo[2,1-*a*]-quinolines; pyrrolo[1,2-*a*]quinolines.
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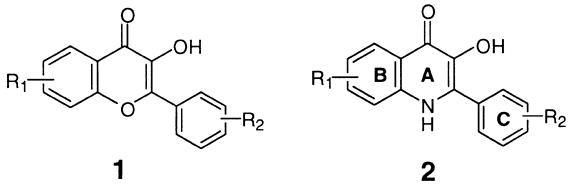


Figure 1.

Chemistry

The starting material 2-aminoacetophenones **3** were prepared by regiospecific acylation of the corresponding aromatic amines with 2-methoxy-acetonitrile catalyzed by boron trichloride using a similar procedure reported in the literature and employed in our previous studies.[15–17] The designed tricyclic and tetracyclic compounds were synthesized by reactions of **3** with phthalic or maleic anhydrides. All of the hydroxyl containing compounds were prepared by demethylation of the methoxy precursors.

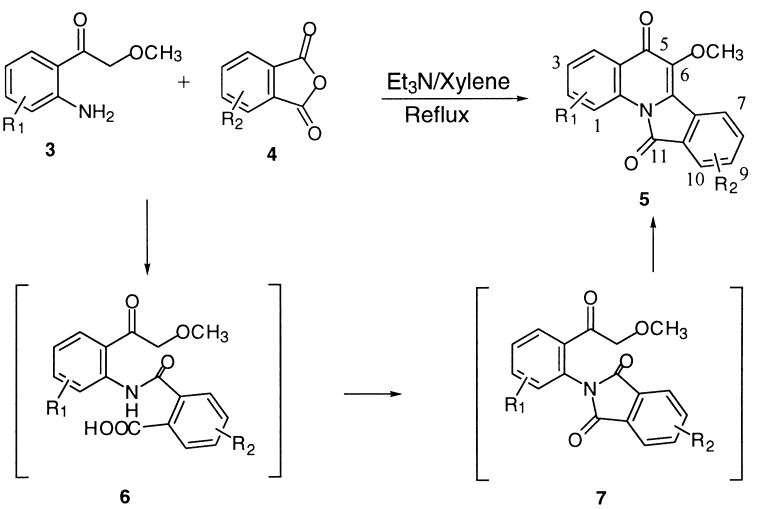
Phthalic anhydrides **4** without strong electron donating groups gave tetracyclic products **5** in fair to good yields by heating at reflux with the anilines **3** in xylene in the presence of triethylamine. A similar approach has been reported in the literature.[18] Only in the case of 2,4-dimethoxyphthalic anhydride was the imide intermediate **7** (R_1 = 2,4-OCH_3; R_2 = 4,5-OCH_3) isolated, because of the low reactivity of the carbonyl groups of the imide. This imide was converted to the tetracyclic compound by heating the reaction mixture at 200 °C in a Parr bomb (Scheme 1). The isolation of the imide **7** in one case confirmed that imide formation takes place prior to cyclization of the amide in **6** to form the quinoline ring. Due to the strong electron withdrawing effect of fluorine, the yield of **5g** was lower than **5a**. Compounds **5c** and **5d** were isolated in one to one ratio from the same reaction. The structures of **5c** and **5d** were determined by ^1H-^{13}C correlation studies. In **5d** a correlation between H-9 and C-11 can be clearly observed and in **5c** no such correlation appeared.

Although diphenyl substituted maleic anhydride gave the tricyclic product (**10d**) in fair yield by the procedure used for the phthalic anhydrides (Method A, Scheme 2), maleic anhydride itself and 2,3-dichloromaleic anhydride polymerized under the same conditions. Even a large excess (10 equiv.) of the maleic anhydrides at lower temperature (refluxing toluene) gave only poor yields of tricyclic products. The polymerization might be related to the formation of the charge transfer complex of maleic anhydride and triethylamine, as reported in the literature,[19] since in the absence of an amine the polymerization was not observed at the same temperature. To avoid the polymerization we used a two step procedure (Method B, Scheme 2). Thus, the amines and the anhydrides were first subjected to reaction in dichloromethane at room temperature, followed by heating the resulting maleic acid monoamides in acetic anhydride in the presence of sodium acetate (Scheme 2). Several tricyclic products were prepared in satisfactory yields by this procedure (Table 1).

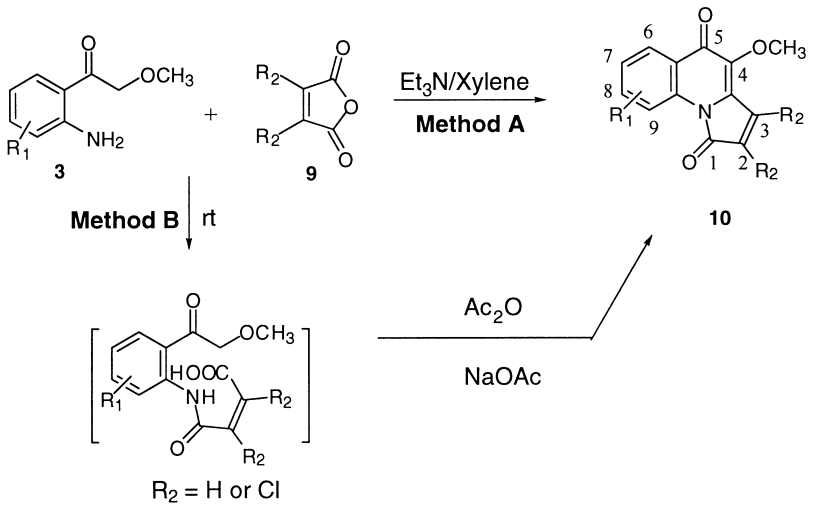
The demethylation of both the tricyclic and tetracyclic compounds was effected with boron tribromide or pyridine hydrochloride[20–22] as shown in Schemes 3 and 4.

Results and Discussion

Biological studies showed that these novel compounds are active against DNA-gyrase in the supercoiling assay



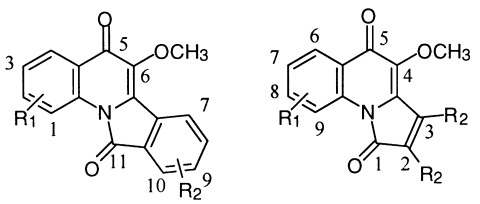
Scheme 1.



Scheme 2.

(Tables 2 and 3).[14] Some of them are more potent than nalidixic acid ($IC_{50} = 57\,\mu g/mL$ in the same assay system). All active compounds in both series contain a hydroxyl group at position 3 of the quinoline rings (position 6 for the tetracyclic and position 4 for the tricyclic derivatives). This indicates the importance of the hydroxyl group for the biological activity.

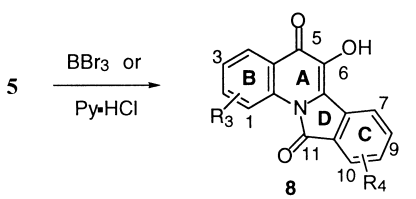
Table 1. Yields of the cyclization reactions



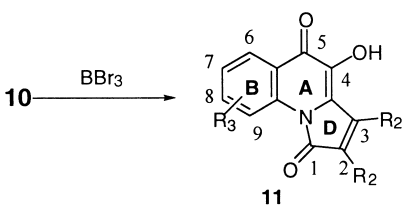
Compd	R_1	R_2	Yield (%)	
5a	2,4-$(OMe)_2$	H	59	
5b	2,4-$(OMe)_2$	8,9-Cl_2	74	
5c	2,4-$(OMe)_2$	9-*t*-Bu	21*	
5d	2,4-$(OMe)_2$	8-*t*-Bu	16*	
5e	2,4-$(OMe)_2$	8,9-Me_2	35	
5f	2,4-$(OMe)_2$	8,9-$(OMe)_2$	65	
5g	2,4-F	H	35	
			Yield (method)	
10a	6,8-$(OMe)_2$	Cl	5(A)	39(B)
10b	6,8-$(OMe)_2$	Me	14(A)	—
10c	7,8-$(OMe)_2$	Cl	0(A)	46(B)
10d	6,8-$(OMe)_2$	Ph	37(A)	—
10e	6,8-$(OMe)_2$	H	3(A)	79(B)

*Total yield: 37%.

It has been suggested that the toxicity of classic quinolones are related to their β-keto acid structure.[23–27] Recent studies have shown that the toxicity of quinolone drugs maybe associated with their ability to form chelate complexes with divalent cations.[28] The formation and the stability of the potential complex with a metal ion (M) are different for the classic quinolones and the compounds we synthesized as illustrated in Figure 2 with ofloxacin and **8f**. In the case of the classic quinolones, a six-membered ring is formed versus a five-membered ring in the compounds studied here. Furthermore, replacement of the carboxylic group with the hydroxy group changes the pK_a approximately five units (from four to nine) and renders this region charge neutral. Ab initio calculations of the pyridinone systems analogous to ofloxacin and **8f** show that the geometric disposition

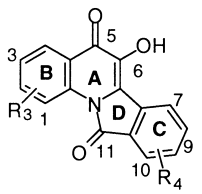


Scheme 3.



Scheme 4.

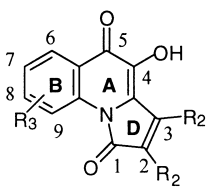
Table 2. Biological activities of the tetracyclic derivatives



Compd	R_3	R_4	IC_{50} (μg/mL) Gyrase	IC_{50} (μg/mL) Topo-II
8a	2,4-OH	H	> 500	—
8b	2,4-OH	8,9-Cl	82	13
8c	2,4-OH	9-*t*-Bu	> 500	3.1
8d	2,4-OH	8-*t*-Bu	> 500	3.7
8e	2,4-OH	8,9-Me	130	> 500
8f	2,4-OH	8,9-OH	22	0.29
8g	2,4-F	H	> 500	> 500
Nalidixic acid			57	—
Ellipticine			—	5.1

of quinolino oxygen and an oxygen of the R-6 substituent, which are presented to the DNA strand as hydrogen bond acceptors according to the Shen model,[29] decreases from 2.77 Å for the carboxylate to 2.56 Å for the hydroxyl group. While this would be expected to have an imperceptable effect on the hydrogen bond iteractions with the DNA strand, the absence of the charged carboxylate translates into a greater than 200 kcal/mol decrease in Mg^{2+} binding to the analogue of **8f**. Therefore, this modification effectively designs out optimal interactions with the divalent cations, while retaining the structural features required for interaction with the DNA strand in the ternary complex.

Table 3. Biological activities of the tricyclic derivatives



Compd	R_2	R_3	IC_{50} (μg/mL) Gyrase	IC_{50} (μg/mL) Topo-II
11a	Cl	6,8-OH	32	0.53
11b	Me	6,8-OH	210	—
11c	Cl	7,8-OH	70	> 500
11d	Ph	6,8-OH	> 500	0.71
Nalidixic acid			57	—
Ellipticine			—	5.1

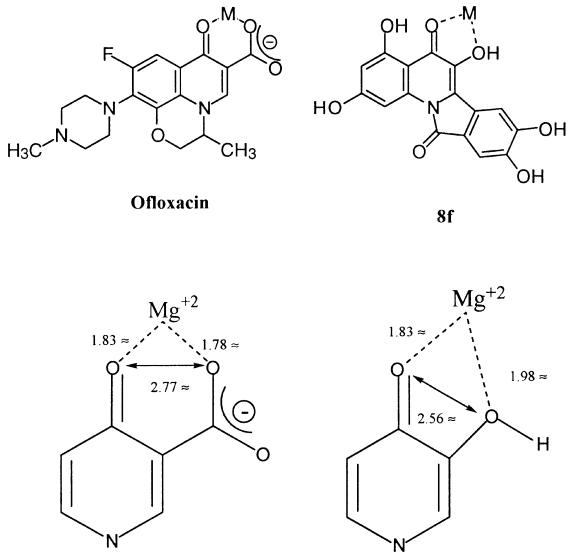


Figure 2.

Similar to the azaflavone, topoisomerase inhibitors (**2**).[15] In most cases, the hydroxy groups were maintained on the B-ring of the tetracyclic and tricyclic inhibitors. When R_4 is a hydrogen (Table 2), the compound **8a** showed no activity, nor did its counterpart in the azaflavone series. Compound **8b** is equipotent to the azaflavone derivative with the same substitution pattern (IC_{50} = 82 μg/mL). However, compound **8c** is inactive while its counterpart in the azaflavone series has an IC_{50} of 67 μg/mL. This can be explained by the fact that the phenyl ring which bears the *t*-butyl group in the azaflavone can rotate to a certain extent to accommodate binding to the enzyme, and in the tetracyclic series this rotation is impossible because of the carbonyl group between the A-ring and C-ring. These results, combined with the fact that the azaflavone analogue of the most potent compound **8f** was also the most potent compound in that series, suggest that the binding modes of the tetracyclic derivatives are probably similar to those of the azaflavones.

To investigate whether the C-ring in **8** is necessary for the DNA-gyrase inhibitory activity, we designed several truncated compounds (**11**) by eliminating this aromatic ring. The biological results (Table 3) showed that they possess comparable activity as the tetracyclic analogues. This finding opened a new avenue to design DNA-gyrase inhibitors with lower molecular weight. In the tricyclic derivatives **11**, when the D-ring is substituted with a chlorine (**11a**), the potency is greater than that of the methyl substituted compound **11b**. The bulky phenyl substituent abolished activity (**11d**). These results seem to be consistent with the SAR of the tetracyclic derivatives (vide supra).

Several analogues possess potent activity against human topoisomerase II. Some of them (e.g. **8f**, **11a**, and **11d**) are more potent than ellipticine (IC_{50} = 5.1 μg/mL in the same assay system). In the tricyclic series, the 6-hydroxy group seems to be essential for the topoisomerase-II inhibitory activity. When the 6-hydroxy was replaced with a 7-hydroxy group the activity was abolished. The biological results showed that it is possible to design compounds in this series that are selective against human topoisomerase II or against bacterial DNA-gyrase. As examples, **8c**, **8d**, and **11d** showed very good activity in the topoisomerase II assay but no activity in the gyrase assay. On the other hand, **8e** and **11c** are active against gyrase but inactive against topoisomerase II.

Conclusion

A convenient synthetic route to novel tri- and tetracyclic quinolones was established. The designed compounds showed inhibitory activities against bacterial DNA-gyrase and human topoisomerase II. Some of the derivatives are more potent than nalidixic acid in the DNA-gyrase assay, and others are more potent than ellipticine against human topoisomerase II. The activities against DNA-gyrase and topoisomerase are separable, which enables further design of selective inhibitors.

Experimental

Melting points were determined with a Thomas capillary melting point apparatus and are uncorrected. Satisfactory NMR and IR spectra were obtained for all compounds. Proton NMR spectra were recorded at 300 MHz on a QE-300 instrument. Infrared spectra were run on a Perkin-Elmer instrument. Mass spectra (CI) were measured with a INCOS 50 spectrometer. Elemental analysis results were within ±0.4% of theoretical values.

2,4,6-Trimethoxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (5a, a general procedure for preparation of 5). To a solution of 2′-amino-2,4′,6′-trimethoxy-acetophenone **3a** (2.09 g, 10 mmol) in xylenes (30 mL) under nitrogen was added phthalic anhydride (1.8 g, 12 mmol). Triethylamine (1 mL) was then added to the stirring solution. The reaction mixture was stirred at reflux for 19 h, and water was removed with a Dean–Stark apparatus. After cooling, the precipitate was filtered and washed with hexanes. Recrystallization from ethyl acetate and dichloromethane gave yellow crystals. Yield: 2.08 g (59%); mp: 224–226 °C. IR (KBr) 1736, 1667, 1624 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.95 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 4.18 (s, 3 H, OCH_3), 6.39 (d, J = 2 Hz, 1 H, H-3), 7.55 (dt, J = 1 Hz, 7 Hz, 1 H, H-8), 7.70 (dt, J = 1 Hz, 7 Hz, 1 H, H-9), 7.91 (d, J = 7 Hz, 1 H, H-7), 8.17 (d, J = 7 Hz, 1 H, H-10), 8.46 (d, J = 2 Hz, 1 H, H-1). MS (CI): 338, (M + 1). Anal. $C_{19}H_{15}NO_5$.

8,9-Dichloro-2,4,6-trimethoxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (5b). From 5.0 g (22.2 mmol) of **3a** and 5.77 g (26.6 mmol) of 4,5-dichlorophthalic anhydride a yellow solid was obtained. Yield: 6.71 g (74%); mp: 300–301 °C. IR (KBr) 1740, 1638, 1601 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.19, 4.34, 4.36 (3s, 3×3 H, OCH_3), 6.94 (d, J = 2 Hz, 1 H, H-3), 8.21 (s, 1 H, H-7), 8.49 (s, 1 H, H-10), 8.64 (d, J = 2 Hz, 1 H, H-1); MS (CI): 405, (M^+), 407 (M + 2), 409 (M + 4). Anal. $C_{19}H_{13}Cl_2NO_5$.

9-*t*-Butyl-2,4,6-trimethoxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione and 8-*t*-butyl-2,4,6-trimethoxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (5c and 5d). From 4.18 g (20 mmol) of **3a** and 4.9 g (24 mmol) of 4-*t*-butylphthalic anhydride two yellow solids were isolated by column chromatography (silica gel, hexane:ethyl acetate, 1:1). **5c**: Yield: 2.0 g (21%); mp: 160–161 °C. IR (KBr) 1738, 1632, 1603 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H, CCH_3), 3.92, 3.93, 4.15 (3s, 3×3 H, OCH_3), 6.33 (d, J = 2 Hz, 1 H, H-3), 7.73 (dd, J = 2 Hz, 8 Hz, 1 H, H-8), 7.91 (d, J = 8 Hz, 1 H, H-10), 8.05 (d, J = 8 Hz, 1 H, H-7), 8.41 (d, J = 2 Hz, 1 H, H-1); MS (CI): 394 (M^+). Anal. $C_{23}H_{23}NO_5 \cdot 0.1\ H_2O$. **5d**: Yield: 1.5 g (16%). mp: 239–240 °C. IR (KBr) 1740, 1628 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9 H, CCH_3), 3.91/3.92/4.18 (3s, 3×3 H, OCH_3), 6.31 (d, J = 2 Hz, 1 H, H-3), 7.59 (dd, J = 8 Hz, 1 H, H-9), 7.78 (d, J = 8 Hz, 1 H, H-10), 8.20 (d, J = 1 Hz, 1 H, H-7), 8.39 (d, J = 2 Hz, 1 H, H-1); MS (CI): 394 (M^+). Anal. $C_{23}H_{23}NO_5$.

8,9-Dimethyl-2,4,6-trimethoxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (5e). From 1.28 g (5.67 mmol) of **3a** and 1.20 g (6.8 mmol) of 4,5-dimethylphthalic anhydride a yellow solid was obtained. Yield: 0.72 g (35%); mp: 260–262 °C. IR (KBr) 1734, 1634, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39, 2.42 (2s, 2×3 H, CH_3), 3.95, 3.96, 4.16 (3s, 3×3 H, OCH_3), 6.39 (d, J = 2 Hz, 1 H, H-3), 7.65 (s, 1 H, H-7), 7.91 (s, 1 H, H-10), 8.46 (d, J = 2 Hz, 1 H, H-1); MS (CI): 366 (MH^+). Anal. $C_{21}H_{19}NO_5 \cdot 0.2\ H_2O$.

***N*-[3,5-Dimethoxy-(2-methoxyacetyl)phenyl]-3,4-dimethoxy-phthalimide (7: R_1 = 2,4-OCH_3; R_2 = 4,5-OCH_3) and 8,9-dimethoxyl-2,4,6-trimethoxy-5*H*,11*H*-isoindolo[2,1-*a*]-quinolin-5,11-dione (5f).** From 1.1 g (4.80 mmol) of **3a** and 1.2 g (5.76 mmol) of 4,5-dimethoxyphthalic anhydride a white solid (**7:** R_1 = 2,4-OCH_3; R_2 = 4,5-OCH_3) was obtained. Yield: 0.42 g (21%). mp > 250 °C. IR (KBr) 1775, 1603, 1501 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.38, 3.86, 3.91, 3.98, 4.00 (5s, 5×3 H, OCH_3), 4.57, (s, 2 H, CH_2), 6.48, 6.56 (2d, J = 2 Hz, 2 H, Hs of acetophenone), 7.35 (s, 2 H, Hs of phthalimide); MS (CI):

416, (MH^+). Anal. $C_{21}H_{21}NO_8$. When the reaction mixture was heated at reflux for 72 h and then heated in a reaction bomb at 250 °C for 4 h. Compound **5f** was isolated as a yellow solid. Yield: 1.24 g (65%). mp > 250 °C. IR (KBr) 1725, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.95, 3.96, 3.99, 4.03, 4.17 (5s, 5×3 H, OCH_3), 6.37 (d, J = 2 Hz, 1 H, H-3), 7.32 (s, 1 H, H-7), 7.59 (s, 1 H, H-10), 8.41 (d, J = 2 Hz, 1 H, H-1); MS (CI): 398 (MH^+). Anal. $C_{21}H_{19}NO_7$.

2,4-Difluoro-6-methoxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (5g). From 1.0 g (7.46 mmol) of 2′-amino-4′,6′-difluoro-2-methoxyacetophenone and 3.0 g (20 mmol) of phthalic anhydride a yellow solid was obtained. Yield: 0.55 g (35%); mp: 228–229 °C. IR (KBr) 1753, 1648, 1487 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.20 (s, 3 H, OCH_3), 6.82 (dt, J = 2 Hz, 9 Hz, 1 H, H-3), 7.63 (dt, J = 1 Hz, 8 Hz, 1 H, H-8), 7.76 (dt, J = 1 Hz, 8 Hz, 1 H, H-9), 7.97 (d, J = 8 Hz, 1 H, H-7), 8.22 (d, J = 8 Hz, 1 H, H-10), 8.82 (td, J = 11 Hz, 2 Hz, 1 H, H-1); MS (CI): 314 (MH^+). Anal. $C_{17}H_9F_2NO_3 \cdot 0.2\ H_2O$.

2,4,6-Trihydroxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8a), a general procedure for preparation of 8 and 11. A solution of **5a** (500 mg, 1.48 mmol) and BBr_3 (1 M in CH_2Cl_2, 10 mL) in CH_2Cl_2 (10 mL) was heated at reflux for 36 h. Dichloromethane was removed in vacuum and water (100 mL) was added. The suspended solid was then filtered and dried in vacuum. Column chromatography (silica gel, ethyl acetate:methanol, 10:1) gave **8a**. Yield: 310 mg (71%); mp: > 300 °C. IR (KBr) 3287, 1703, 1678, 1636 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.19 (d, J = 2 Hz, 1 H, H-3), 7.63 (t, J = 7 Hz, 1 H, H-8), 7.82 (t, J = 7 Hz, 1 H, H-9), 7.93 (d, J = 7 Hz, 1 H, H-7), 8.02 (td, J = 1 Hz, 1 H, H-1), 8.14 (d, J = 7 Hz, 1 H, H-10), 10.72, 10.92, 13.28 (3s, 3×1 H, OH); MS (CI): 296 (MH^+). Anal. $C_{16}H_9NO_5 \cdot 0.6\ H_2O$.

8,9-Dichloro-2,4,6-trihydroxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8b). A mixture of **5b** (1.0 g, 2.46 mmol) and pyridine hydrochloride (12 g, 104 mmol) was heated at 220 °C for 7.5 h and then poured into ice water. The mixture was stirred for 1 h. After filtration, the solid was recrystallized from ethyl acetate and methanol. Red crystals were obtained. Yield: 56.9 mg (6.4 %); mp: > 300 °C. IR (KBr) 3208, 1711, 1593 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.19 (s, 3 H, OCH_3), 4.34 (s, 3 H, OCH_3), 4.36 (s, 3 H, OCH_3), 6.09, 6.56, 8.45, 8.89 (4s, 4×1 H, aromatic), 10.45, 10.55, 11.75 (3s, 3×1 H, OH); MS (CI): 364 (MH^+). Anal. $C_{16}H_7Cl_2NO_5$.

9-*t*-Butyl-2,4,6-trihydroxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8c). From 500 mg (1.3 mmol) of **5c** and 10 mL of BBr_3 (1.0 M in CH_2Cl_2) using the procedure for **8a**, a red solid was obtained. Yield: 170 mg (37%); mp: > 300 °C. IR (KBr) 3305, 1701, 1676 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 (s, 9 H, CCH_3), 6.19 (d, J = 1 Hz, 1 H, H-3), 7.89 (s, 1 H, H-10), 7.91 (d, J = 8 Hz, 1 H, H-8), 8.01 (d, J = 1 Hz, 1 H, H-1), 8.05 (d, J = 8 Hz, 1 H, H-7), 10.58, 10.88, 13.30 (3s, 3×1 H, OH); MS (CI): 352 (MH^+). Anal. $C_{20}H_{17}NO_5 \cdot 0.1\ H_2O$.

8-*t*-Butyl-2,4,6-trihydroxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8d). From 500 mg (1.3 mmol) of **5d** and 10 mL of BBr_3 (1.0 M in CH_2Cl_2) using the procedure for **8a**, a red solid was obtained. Yield: 150 mg (33%); mp: > 300 °C. IR (KBr) 3288, 1701, 1674 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 (s, 9 H, CCH_3), 6.17 (d, J = 2 Hz, 1 H, H-3), 7.71 (d, J = 8 Hz, 1 H, H-10), 7.86 (d, J = 8 Hz, 1 H, H-8), 8.01 (d, J = 1 Hz, 1 H, H-1), 8.15 (d, J = 8 Hz, 1 H, H-7), 10.65, 10.85, 13.28 (3s, 3×1 H, OH); MS (CI): 352 (MH^+). Anal. $C_{20}H_{17}NO_5$.

8,9-Dimethyl-2,4,6-trihydroxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8e). From 183 mg (0.5 mmol) of **5e** and 1.16 g (10 mmol) of pyridine hydrochloride, using the procedure for **8b**, a yellow solid was obtained. Yield: 132 mg (82%); mp: > 250 °C. IR (KBr) 3187, 1699, 1651 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.41, 2.47 (2s, 2×3 H, CH_3), 6.7 (d, J = 2 Hz, 1 H, H-3), 6.58, 8.05 (2s, 2×1 H, H-7/H-10), 8.40 (d, J = 2 Hz, 1 H, H-1), 10.50, 11.75, 13.80 (3s, 3×1 H, OH); MS (CI): 324 (MH^+). Anal. $C_{18}H_{13}NO_5 \cdot H_2O$.

2,4,6,8,9-Pentahydroxy-*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8f). From 200 mg (0.5 mmol) of **5f** and 1.16 g (10 mmol) of pyridine hydrochloride, using the procedure for **8b**. The crude solid was dissolved in 0.1 N NaOH, extracted with ether and the aqueous layer was acidified with acetic acid. The precipitate was filtered, washed with water, and dried under vacuum. A yellow solid was obtained. Yield: 120 mg (73%); mp: > 250 °C. IR (KBr) 3172, 1696, 1653 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.05, 6.64, 7.66, 8.00 (4s, 4×1 H, aromatic), 10.52, 11.75, 13.97 (3s, 3×1 H, OH); MS (CI): 328 (MH^+). Anal. $C_{16}H_9NO_7 \cdot 0.7CH_3COOH$.

2,4-Difluoro-6-hydroxy-5*H*,11*H*-isoindolo[2,1-*a*]quinolin-5,11-dione (8g). From 110 mg (0.35 mmol) of **5g** and 2 mL of BBr_3 (1.0 M in CH_2Cl_2) in CH_2Cl_2 (40 mL), using the procedure for **8a**, a yellow solid was obtained. Yield: 98 mg (94%); mp: > 250 °C. IR (KBr) 3854, 1743, 1675 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.35 (dt, J = 2 Hz, 9 Hz, 1 H, H-3), 7.66 (dt, J = 1 Hz, 8 Hz, 1 H, H-8), 7.85 (dt, J = 1 Hz, 8 Hz, 1 H, H-9), 7.97 (d, J = 8 Hz, 1 H, H-7), 8.14 (d, J = 8 Hz, 1 H, H-10), 8.75 (td, J = 11 Hz, 2 Hz, 1 H, H-1), 10.78 (s, 1 H, OH); MS (CI): 300, (MH^+). Anal. $C_{16}H_7F_2NO_3$.

2,3-Dichloro-4,6,8-trimethoxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (10a). Method A. A mixture of **3a** (1.85 g, 8.21 mmol) and 2,3-dichloromaleic anhydride (13.7 g,

82.1 mmol) in xylene (100 mL) and triethylamine (2 mL) was heated under reflux for 16 h. After cooling the black precipitate was removed by filtration and the solvent in the filtrate was evaporated. Column chromatography (twice, silica gel, hexane:ethyl acetate, 1:1) gave a yellow solid. Yield: 90 mg (3%); mp: 200–201 °C. Method B. A solution of **3a** (900 mg, 4 mmol) and 2,3-dichloromaleic anhydride (668 mg, 4 mmol) in dichloromethane (40 mL) was stirred at rt for 2 h. A white precipitate was formed. After filtration and washing with dichloromethane the white solid was heated with sodium acetate (328 mg, 4 mmol) in acetic anhydride (10 mL) to 80 °C for 4 h. 1 N NaOH was added, and the mixture was extracted with dichloromethane and dried with magnesium sulfate. Column chromatography (silica gel, CH_2Cl_2:ethyl acetate, 9:1) gave the title compound as a yellow solid. Yield: 550 mg (39%). mp: 200–201 °C. IR (KBr) 2963, 1728, 1649, 1603 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.93, 3.95, 4.12 (3s, 3×3 H, OCH_3), 6.37 (d, $J=2$ Hz, 1 H, H-7), 8.19 (d, $J=2$ Hz, 1 H, H-9); MS (CI): 356 (MH^+), 358 (MH + 2), 360 (MH + 4). Anal. $C_{15}H_{11}Cl_2NO_5$.

2,3-Dimethyl-4,6,8-trimethoxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (10b). From **3a** (2.09 g, 10 mmol), 2,3-dimethylmaleic anhydride (1.40 g, 11 mmol), triethylamine (1 mL) and xylene (30 mL) using Method A the title compound was obtained as a yellow solid. Yield: 450 mg (14%); mp: 209–210 °C. IR (KBr) 2952, 1723, 1630, 1599 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.89, 2.29 (2s, 2×3 H, CH_3), 3.84, 3.86, 3.87 (3s, 3×3 H, OCH_3), 6.48 (d, $J=2$ Hz, 1 H, H-7), 8.12 (d, $J=2$ Hz, 1 H, H-9); MS (CI): 316 (MH^+). Anal. $C_{17}H_{17}NO_5$.

2,3-Dichloro-4,7,8-trimethoxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (10c). From **3c** (225 mg, 1 mmol) and 2,3-dichloromaleic anhydride (175 mg, 1.05 mmol) using Method B the title compound was obtained as a yellow solid. Yield: 162 mg (46%); mp: >250 °C. IR (KBr) 2948, 1730, 1626, 1602 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.97, 4.02, 4.14 (3s, 3×3 H, OCH_3), 7.56 (s, 1 H, H-9), 8.39 (s, 1 H, H-6); MS (CI): 356 (MH^+). Anal. $C_{15}H_{11}Cl_2NO_5$.

2,3-Diphenyl-4,6,8-trimethoxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (10d). From **3a** (3.75 g, 16.65 mmol), 2,3-diphenylmaleic anhydride (5 g, 20 mmol) using Method A the title compound was obtained as a yellow solid. Yield: 2.7 g (37%); mp: 224–225 °C. IR (KBr) 2948, 1717, 1626, 1601 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.37, 3.86, 3.91 (3s, 3×3 H, OCH_3), 6.55 (d, $J=2$ Hz, 1 H), 7.30–7.42 (m, 10 H, phenyl), 8.27 (d, $J=2$ Hz, 1 H, H-9); MS (CI): 440 (MH^+). Anal. $C_{27}H_{21}NO_5$.

4,6,8-Trimethoxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (10e). From **3a** (1.35 g, 6 mmol) and maleic anhydride (588 mg, 6 mmol) using Method B the title compound was obtained as a yellow solid. Yield: 1.36 g (79%); mp: 209–210 °C. IR (KBr) 3129, 2961, 1712, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.92, 3.95, 4.12 (3s, 3×3 H, OCH_3), 6.24, 6.33 (2d, $J=7$ Hz, 2×1 H), 7.67 (d, $J=2$ Hz, 1 H), 8.17 (d, $J=2$ Hz, 1 H, H-9); MS (CI): 288 (MH^+). Anal. $C_{15}H_{13}NO_5$.

2,3-Dichloro-4,6,8-trihydroxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (11a). From **10a** (175 mg, 0.49 mmol) and BBr_3 (1.0 M in CH_2Cl_2, 5 mL) in CH_2Cl_2 (80 mL), using the procedure for **8a**, column chromatography (hexane:ethyl acetate:acetic acid, 8:2:0.1) gave **11a**. Yield: 25 mg (16%); mp: >250 °C. IR (KBr) 3278, 1701, 1636 cm^{-1}; ^1H NMR (Acetone-d_6) δ 6.24 (s, 1 H, H-7), 7.92 (s, 1 H, H-9); MS (CI): 314 (MH^+), 316 (MH + 2), 318 (MH + 4). Anal. $C_{12}H_5Cl_2NO_5 \cdot 0.3CH_3COOH$.

2,3-Dimethyl-4,6,8-trihydroxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (11b). From **10b** (200 mg, 0.63 mmol) and BBr_3 (1.0 M CH_2Cl_2, 10 mL) using the procedure for **8a**, the title compound was obtained as a yellow solid. Yield: 189 mg (95%); mp: >300 °C. IR (KBr) 3272, 1688, 1634 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.86, 2.30 (2s, 2×3 H, CH_3), 6.08 (d, $J=2$ Hz, 1 H, H-7), 7.77 (d, $J=2$ Hz, 1 H, H-9), 10.38, 10.86, 13.03 (3s, 3×1 H, OH); MS (CI): 316 (MH^+). Anal. $C_{14}H_{11}NO_5 \cdot 0.3H_2O$.

2,3-Dichloro---4,7,8-trihydroxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (11c). From **10c** (610 mg, 1.71 mmol) and BBr_3 (1.0 M CH_2Cl_2, 13.7 mmol) using the procedure for **8a**, the title compound was obtained as a red solid. Yield: 505 mg (94%); mp: >250 °C (ethanol). IR (KBr) 3222, 1691, 1587 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.36 (s, 1 H, H-9), 8.24 (s, 1 H, H-6), 9.68, 10.76, 11.15 (3s, 3×1 H, OH); MS (CI): 314 (MH^+). Anal. $C_{12}H_5Cl_2NO_5 \cdot 0.1CH_3CH_2OH$.

2,3-Diphenyl-4,6,8-trihydroxy-1*H*,5*H*-pyrrolo[1,2-*a*]quinolin-1,5-dione (11d). From **10d** (500 mg, 1.14 mmol) and BBr_3 (1.0 M in CH_2Cl_2, 6.84 mL), the title compound was obtained as a red solid. Yield: 250 mg (55%); mp: 283–284 °C. IR (KBr) 3295, 1715, 1634 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.17 (d, $J=2$ Hz, 1 H, H-7), 7.23–7.40 (m, 10 H, phenyl), 7.98 (d, $J=2$ Hz, 1 H, H-9), 10.30, 11.03, 13.00 (3s, 3×1 H, OH); MS (CI): 398 (MH^+). Anal. $C_{27}H_{15}NO_5 \cdot 0.3H_2O$.

DNA-gyrase supercoiling assay was conducted as described in our previous reports.[14,30] Topoisomerase inhibitory activity was measured by quantitating the formation of the cleavable complex[31] on pYRG plasmid DNA by mammalian topoisomerase II. The cleavable complex product, linear DNA, was quantitated by densitometric analyses.

All molecular modeling was performed on an SGI Indigo II workstation using the Spartan molecular

orbital package.[32] Structures were geometry optimized with and without the Mg^{2+} divalent cation optimized using the default 3-21G* basis set.

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