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Synthesis and Antiproliferative Activity of Some Ellipticine-Like 11*H*-Pyrido[*a*]carbazole Derivatives

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Some modified 11*H*-pyrido[*a*]carbazoles (11*H*-PyC) and their corresponding tetrahydro derivatives (11*H*-THPyC) were prepared. A common multistep pathway characterized by conventional reactions, including a Fischer-indole-type synthesis, yielded the tetracyclic compounds. To improve cytotoxicity, 11*H*-PyC and 11*H*-THPyC derivatives were endowed with a diethylaminoethyl side chain. The antiproliferative activity was assessed in three human tumor cell lines, and a number of derivatives showed a cytotoxic effect in agreement with their capacity to form a molecular intercalative complex with DNA and to interfere with the relaxation activity of DNA topoisomerase II. In contrast, three derivatives that exhibited significant antiproliferative efficacy, showed no inhibition of topoisomerase II, thus suggesting an unexpected and novel mode of action for these ellipticine-like compounds independent of topoisomerase II activity.

Introduction

Pyridocarbazoles (6*H*-, 7*H*- and 11*H*-PyC) form a class of compounds endowed with antitumor activity; 2-methyl-9-hydroxy-ellipticinium acetate, derived from ellipticine alkaloid, is particularly potent and is the only PyC in clinical use as an anticancer agent to date. In the recent past, this class of compounds has raised considerable attention because of their ability to intercalate double-stranded DNA and inhibit a specific enzyme. Structure–activity relationships (SARs) in the PyC series have been extensively studied in the context of topoisomerase II inhibition and cytotoxicity toward tumor cells. In general, compounds containing a PyC moiety equipped with a hydrophilic side chain were the most cytotoxic (Figure 1).[1–4a,b]

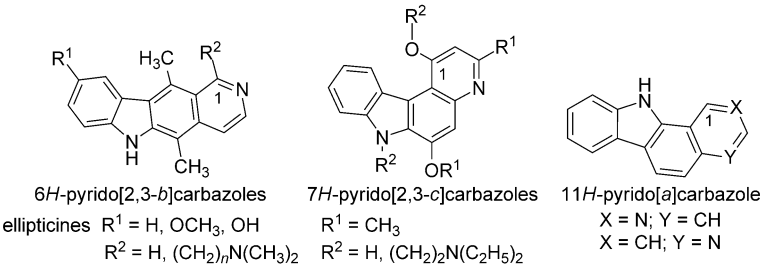

Figure 1. Structures of previously reported 6*H*-pyrido[2,3-*b*]carbazoles[4b] and 7*H*-pyrido-[2,3-*c*]carbazoles,[5] and the newly synthesized 11*H*-pyrido[*a*]carbazole described herein.

More recently, as a part of our ongoing research work in the field of the antiproliferative drugs, we synthesized and studied several branched 7*H*-PyC derivatives bearing one or two basic side chains on the tetracyclic nucleus (Figure 1). Some of these 7*H*-PyC derivatives displayed cytotoxicity in the low micromolar range, and one compound exhibited activity at nanomolar concentrations against numerous tumor cell lines, including some resistant ones. It was demonstrated that they act as intercalators and inhibitors of topoisomerase II.[5]

It is important to mention that closely related PyC derivatives with slight different substitutions have different biological activities. Moreover, the specific geometry of PyC derivatives was found to be a determinant factor for cytotoxicity, with activity decreasing in the order: 6*H* > 7*H* > 11*H*. In the latter series, none of the 11*H*-PyCs studied so far elicited measurable cytotoxicity, although some of them were able to form a complex with DNA.[6]

With the aim of gaining further SAR information useful for the identification of lead compounds with increased antiproliferative properties and to continue the study on PyC drugs,[7,8] we prepared and studied some new derivatives in the 11*H*-PyC series, both [3,4-*a*] and [3,2-*a*] derivatives bearing a chlorine or dimethylamine group at position 5, and according to previous work,[5] a hydrophilic basic side chain at 11*N*-pyrrole. Moreover, we synthesized a modified 11*H*-PyC derivative with an additional cyclic nitrogen atom, namely a piperidinopyrroloquinoline derivative. These basic motifs also turned out to be useful in making the derivatives more water soluble by preparing the corresponding hydrochloride salts or more simply improving their hydrophilicity under physiological conditions.

The in vitro antiproliferative activity of these derivatives was assessed in a panel of three human tumor cell lines belonging to different tumor types. Furthermore, their ability to complex DNA was studied by flow linear dichroism experiments, and their topoisomerase II inhibition was evaluated.

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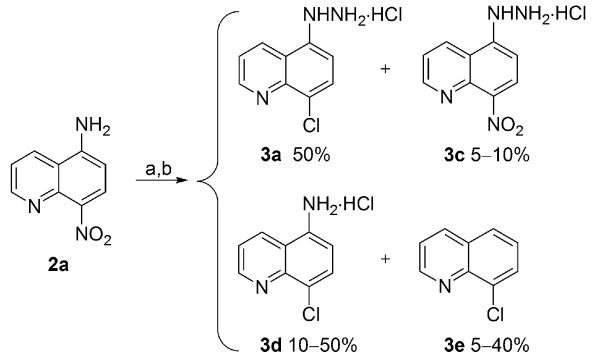
Results and Discussion

Chemistry

The synthesis of both 5-chloro-11*H*-pyrido[3,2-*a*] and [3,4-*a*]carbazoles was accomplished by conventional organic reactions (Scheme 1). Briefly, tetracyclic series **a** and **b** were synthesized from commercially available 8-nitroquinoline and 5-nitroisoquinoline, respectively. The use of hydroxylamine under alkaline conditions gave compounds **2a**,**b**, and these intermediates were then reacted with sodium nitrite in aqueous hydrochloric acid and tin(II) chloride at low temperature to obtain the corresponding 8- and 5-hydrazine derivatives. In both cases, a complex mixture formed where the major compounds were 8-chloro-5-hydrazine-quinoline and 5-chloro-8-hydrazine-isoquinoline **3a** and **3b** (50% yield), respectively. On working up the reaction mixture of **2a**, the formation of three other secondary products was observed, and these were identified as 5-hydrazino-8-nitroquinoline hydrochloride **3c**, 5-amino-8-chloroquinoline hydrochloride **3d**, and 8-chloroquinoline **3e** (Scheme 2). Evidently, a nucleophilic substitution reaction led to the replacement of the 8-nitro leaving group by a chloride substituent. On the basis of the observed separation of **3d** and **3e**, we propose that the replacement took place via the amine group of the 5-aminoquinoline hydrochloride derivative in a protonated state (see **3d**) or with the 5-diazonium salt derivative that, due to its unstable nature, released nitrogen gas (see **3e**).

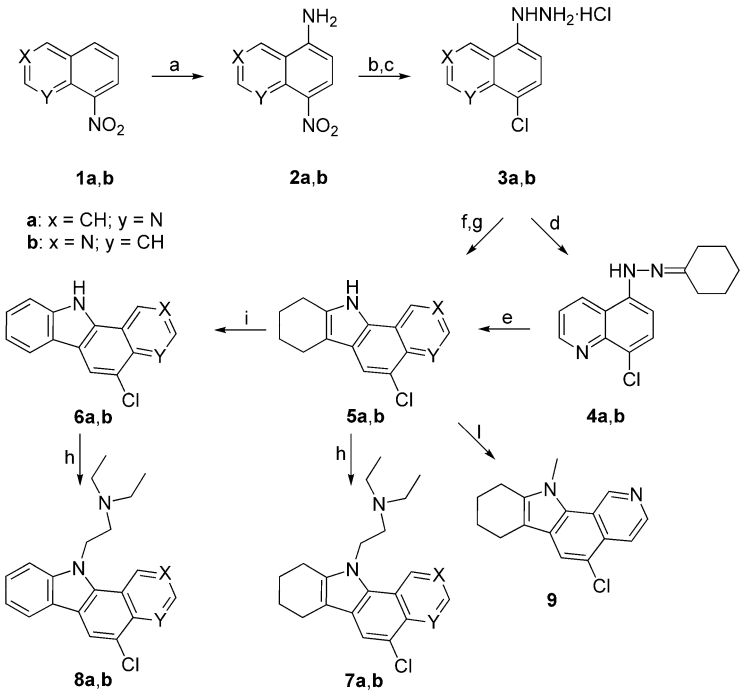


Scheme 2. Products formed from compound **2a** in the hydrazine reactions. *Reagents and conditions*: a) $NaNO_2$, HCl, H_2O, 0–4 °C, 1 h; b) $SnCl_2{\cdot}2H_2O$, HCl, 0–4 °C, 1 h.

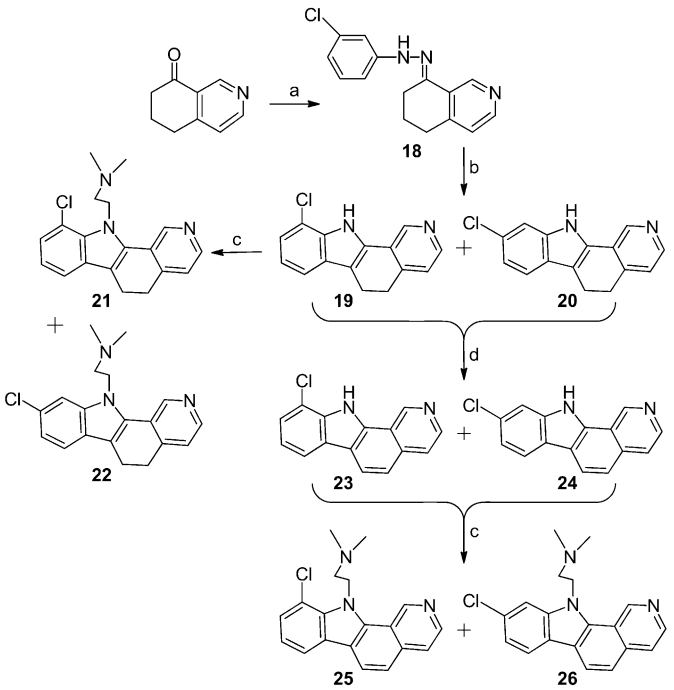
As shown in Scheme 1, bases **3a** and **3b** were treated with cyclohexanone in ethanol at reflux to give hydrazones **4a** and **4b**, which were easily cyclized to 5-chloro-PyCs **5a** and **5b** under acidic conditions (sulfuric acid/acetic acid). Compounds **5a** and **5b** could be directly obtained in good yields from **3a** and **3b**, respectively, by means of two methods: A) Reaction with cyclohexanone in methanol/ethanol at reflux in the presence of excess of triethylamine and molecular sieves; or B) Under acid conditions (sulfuric acid/acetic acid) by microwave irradiation (100 W) at 150 °C for 5 min. As often occurs, the latter method was the best: 90–97% yield after workup.[9]

Next, both tetrahydro compounds **5a** and **5b** were either oxidized using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in benzene to give PyCs **6a**,**b** or alkylated with diethylaminoethyl chloride to give compounds **7a**,**b**. Finally, fully aromatic derivatives **6a**,**b** were also alkylated at the *N*-pyrrole to give **8a**,**b** (KOH, acetone). Furthermore, the 11*N*-methyl derivative **9** was synthesized from **5b** by the same experimental method but using methyl iodide as the alkylating agent.

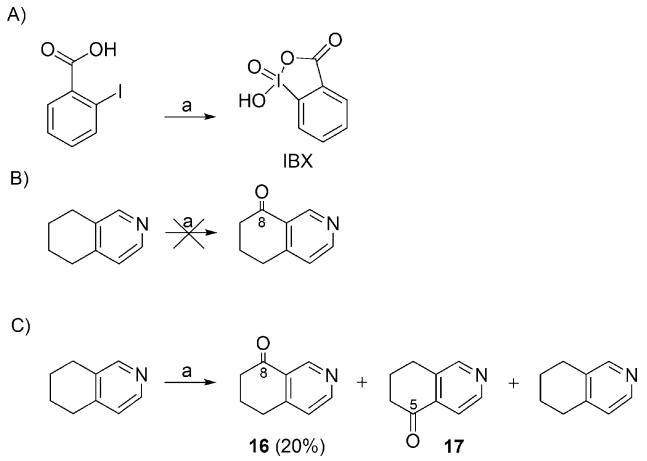
Scheme 3 describes the pathway leading to tetrahydropyrido[3,2-*a*]carbazole **15**. It was obtained from commercially available 8-hydroxy-5-nitroquinoline that, when treated with an excess of phosphorus oxychloride in *N*,*N*-dimethylformamide (DMF) yielded a mixture of compounds **10** and **11**. However, when treated with five equivalents of phosphorus oxychloride and an excess of DMF, 8-dimethylamino derivative **11** was obtained as the only reaction product. Subsequently, the latter was catalytically reduced (H_2, 10% Pd/C) at atmosphere pressure to 5-aminoquinoline **12**, which was transformed into the corresponding hydrazine derivative **13** via the known procedure ($NaNO_2$/HCl, $SnCl_2{\cdot}2H_2O$). Reaction of **13** with cyclohexanone (see method A in the Experimental Section) directly gave tetracyclic compound **14**, which was alkylated at *N*-pyrrole with diethylaminoethyl chloride hydrochloride under alkaline conditions (KOH, acetone) to yield **15**.



Scheme 1. Synthesis of 5-chloro-substituted 11*H*-pyrido[*a*]carbazole derivatives **5a**,**b**–**8a**,**b** and **9**. *Reagents and conditions*: a) $NH_2OH{\cdot}HCl$, KOH, EtOH, 50 °C, 2 h; b) $NaNO_2$, HCl, H_2O, 0–4 °C, 1 h; c) $SnCl_2{\cdot}2H_2O$, HCl, 0–4 °C, 1 h; d) cyclohexanone, EtOH, reflux, 3 h; e) CH_3CO_2H, H_2SO_4, reflux, 15 min; f) cyclohexanone, absolute EtOH, TEA, H_2SO_4), reflux,15 h; g) cyclohexanone, CH_3CO_2H/H_2SO_4 (3:1), microwave, 150 °C, 3 min; h) $(C_2H_5)_2NCH_2CH_2Cl{\cdot}HCl$, KOH, acetone, RT, 2.5 h; i) DDQ, benzene, reflux, 30 h; l) CH_3I, KOH, acetone, RT, 2 h.



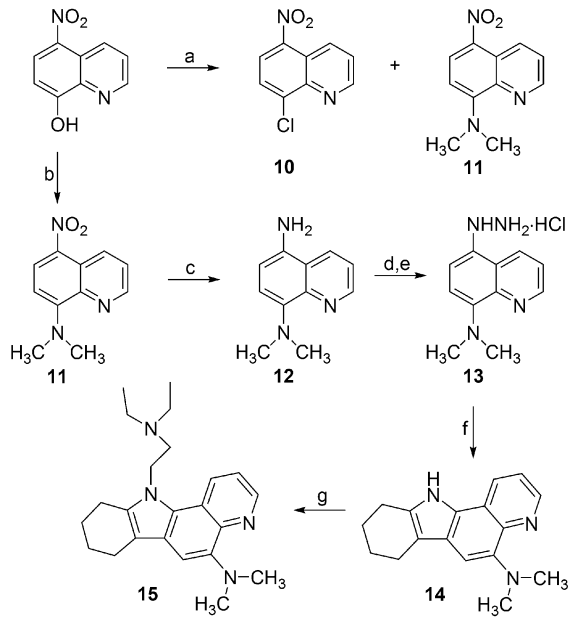
Scheme 5. An alternative synthetic route to substituted 11*H*-pyrido[3,4-*a*]carbazoles. *Reagents and conditions*: a) *m*-chlorophenylhydrazine·HCl, EtOH, reflux, 4–5 h; b) CH_3CO_2H, H_2SO_4, reflux, 15–20 min; c) $(C_2H_5)_2NCH_2CH_2Cl$·HCl, KOH, acetone, RT, 1.5 h; d) DDQ, benzene, reflux, 24 h.



Scheme 4. Synthesis of 6,7-dihydroisoquinolin-8(5*H*)-one **16**. A) Synthesis of iodoxybenzoic acid (IBX)[11]: *Reagents and conditions*: a) Oxone ($2KHSO_5·KHSO_4·K_2SO_4$), fluorobenzene/DMSO (2:1), reflux, 12–24 h. B) Oxidation of 5,6,7,8-tetrahydroisoquinoline by IBX.[10] *Reagents and conditions*: a) IBX, H_2O, 3 h. C) Oxidation of 5,6,7,8-tetrahydroisoquinoline.[12] a) $KMnO_4$, H_2O/CH_3CO_2H (33:1), RT, 20 min.

Next, in order to optimize the synthesis of the pyrido[3,4-*a*]carbazole nucleus by means of a Fischer method, for which the step involving the preparation of isoquinoline hydrazine significantly reduces the overall yields (see the Experimental Section), we synthesized 11*H*-pyrido[3,4-*a*]carbazoles starting from commercially available phenyl hydrazine and 6,7-dihydroisoquinolin-8(5*H*)-one (Scheme 5). The latter starting material is not commercially available and so was prepared following two previously described methods, both consisting of an oxidation reaction starting from 5,6,7,8-tetrahydroisoquinoline (Scheme 4). The first applied method involved the use of iodoxybenzoic acid (IBX), an oxidant agent that should have furnished the 8-oxo isomer only, as previously described.[10] Given that this method was unsuccessful (Scheme 4B) using either commercial IBX or IBX prepared by us (Scheme 4A),[11] we returned to the older procedure using potassium permanganate in aqueous acetic acid at room temperature.[12] This second procedure gave a mixture of unreacted starting material, 8-oxo **16** and 5-oxo-dihydroisoquinoline **17**; unfortunately, we were only able to separate the 8-oxo isomer **16** in pure form (Scheme 4C), the exact structure of which was confirmed by one-dimensional and two-dimensional NMR experiments (see Supporting Information).

In Scheme 5, a test synthesis of 11*H*-pyrido[3,4-*a*]carbazole is shown starting from 8-oxodihydroisoquinoline **16** and *m*-chlorophenylhydrazine. The condensation reaction was straightforward and yielded hydrazone **18** in high yield. The hydrazone was easily cyclized at reflux in CH_3COOH in the presence of H_2SO_4 to furnish a mixture of the expected chloro isomers **19** and **20**. As these compounds were not separable in our hands,

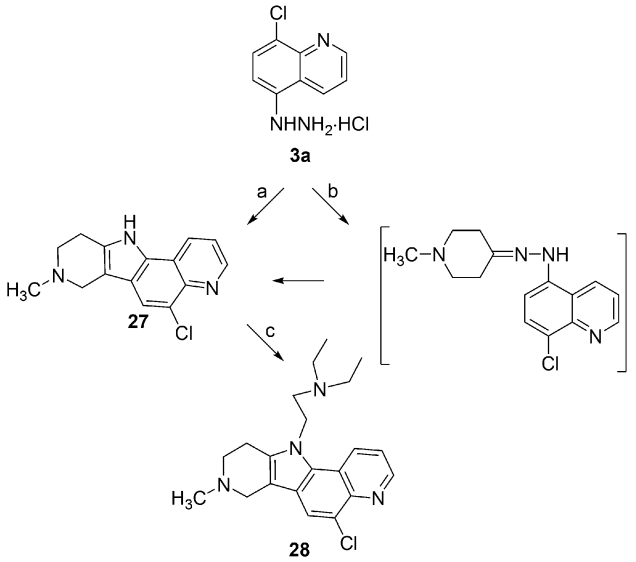


Scheme 3. Synthesis of 5-dimethylamino-substituted derivatives **14** and **15**. *Reagents and conditions*: a) $POCl_3$ (excess), cat DMF, 55 °C, 20–30 h; b) $POCl_3$, DMF (excess), 55 °C, 20–30 h; c) Pd/C (10%), EtOAc, 50 °C, 3 h; d) $NaNO_2$, HCl, H_2O, 0–4 °C, 1 h; e) $SnCl_2·2H_2O$, HCl, 1 h; f) cyclohexanone, absolute ETOH, TEA, H_2SO_4, reflux, 15 h; g) $(C_2H_5)_2NCH_2CH_2Cl$·HCl, KOH, acetone, RT, 1.5 h.

they were functionalized at the 11*N* position with 2-chloro-*N,N*-dimethylethanamine hydrochloride as above to give a mixture of compounds **21** and **22**, from which only compound **22** was separated pure in good yield. The mixture of compounds **19** and **20** was also oxidized with DDQ in benzene to give an inseparable mixture of the two fully aromatic tetracycles **23** and **24**. Finally, this mixture was alkylated at the pyrrole nitrogen with 2-chloro-*N,N*-dimethylethanamine hydrochloride in the

same conditions described above to give isomers **25** and **26**, from which only compound **25** was isolated pure in good yield.

Scheme 6 describes the synthetic route to PyC analogous **27**, in which the carbazole moiety is significantly modified by the presence of another cyclic nitrogen atom in order to increase basicity. Starting from hydrazine **3a**, it was impossible to obtain the hydrazone by reaction with 1-methylpiperidin-4-one in ethanol/methanol at reflux; the desired compound formed and hydrolyzed upon work up. In contrast, the condensation reaction under acidic conditions (Fischer carbazole synthesis) gave the 5-chloro-substituted tetracycle **27** directly in good yield. The latter, as before, was then transformed in to the corresponding 11-*N*-dimethylethanamine compound **28**.



Scheme 6. Synthesis of 8*N*-methyl-7,8,9,10-tetrahydro-pyrido[3′,4′,4,5]pyrrolo-[2,3-*f*]quinoline derivatives **27** and **28**. *Reagents and conditions*: a) 1-methylpiperidin-4-one, CH_3CO_2H, H_2SO_4, reflux, 15–20 min; b) 1-methylpiperidin-4-one, EtOH/MeOH (3:1), TEA), reflux, 15 h; c) $(C_2H_5)_2NCH_2CH_2Cl{\cdot}HCl$, KOH, acetone, RT, 1.5 h.

Table 1. Cell growth inhibition of three tumor cell lines by test compounds and ellipticine as a reference agent.

Compd	IC_{50} [μM][a]		
	A-431	HL-60	HeLa
5a	18.9 ± 2.9	> 30	> 30
6a	> 30	> 30	> 30
7a	23.5 ± 1.5	> 30	> 30
8a	5.5 ± 1.3	13.4 ± 1.0	> 30
5b	19.5 ± 1.5	> 30	> 30
6b	24.9 ± 1.1	> 30	> 30
7b	18.6 ± 1.4	22.1 ± 0.6	> 30
8b	> 30	> 30	> 30
9	11.3 ± 0.6	14.9 ± 1.3	24.3 ± 1.5
14	5.7 ± 1.7	7.4 ± 1.8	21.7 ± 1.7
15	6.5 ± 0.8	8.1 ± 0.6	25.4 ± 1.3
19, **20**	> 30	> 30	> 30
22	13.4 ± 1.7	n.d.	17.9 ± 1.5
23, **24**	> 30	> 30	> 30
25	12.5 ± 1.3	n.d.	4.9 ± 0.9
27	7.6 ± 1	25.5 ± 2.6	27.8 ± 1
28	8.6 ± 0.9	> 30	> 30
Ellipticine	0.43 ± 0.02	0.64 ± 0.03	0.32 ± 0.01

[a] Values represent the mean ± SD of five independent experiments; n.d.: not determined; A-431: skin carcinoma squamous cell line; HL-60: human myeloid leukemic cell line; HeLa: human cervix adenocarcinoma cell line.

Antiproliferative activity

The antiproliferative activity of the final compounds was evaluated by an in vitro assay on three human tumor cell lines: A-431 (epithelial carcinoma), HL-60 (promyelocytic leukemia) and HeLa (cervix adenocarcinoma). Results are expressed as IC_{50} values (Table 1)—the concentration at which a compound causes 50% cell death with respect to a control culture. Ellipticine was used as a reference compound.

Regarding the antiproliferative effect of 11*H*-PyC **6a**,**b** and **8a**,**b** and the corresponding 11*H*-THPyC **5a**,**b** and **7a**,**b**, the A-431 cell line was the most sensitive to these derivatives, while in HeLa cells, all compounds were practically inactive. In detail, compound **8a** exhibits the most interesting antiproliferative activity with IC_{50} values of 5.5 and 13.4 μM on A-431 and HL-60 cell lines, respectively. From a structural point of view, this 11*H*-PyC derivative is characterized by a dimethylaminoethyl side chain in position 11 and by an unsaturated pyridocarbazole chromophore. As previously reported for other intercalating topoisomerase II inhibitors,[5] both these structural features appear to play an important role in cytotoxicity. Indeed, the lack of the protonable dialkylamino side chain (**6a**) completely abolishes the cytotoxic effect, while the partial hydrogenation of the carbazole nucleus, giving 11*H*-THPyC **7a**, causes a significant decrease in activity (A-431: $IC_{50} = 23.5$ μM; HL-60: $IC_{50} > 30$ μM).

The quinoline ring also seems to be crucial for cytotoxicity as compound carrying the isoquinoline moiety (compound **8b**) does not show any appreciable antiproliferative effects on tumor cell lines compared with the corresponding isomer (**8a**). Nevertheless, among the isoquinoline derivatives, an unexpected weak activity was observed for derivative **9**. In this derivative, the 11-dimethylaminoethyl chain is replaced by a methyl group that seems to be essential for cytotoxic activity because its absence (**5a**) is accompanied by a significant increase in IC_{50} values.

Regarding the presence of a chlorine substituent on the PyC core, a relationship is derived from a comparison between **8b** and **25** in the isoquinoline series, which have the halogen in position 5 and 10, respectively. Specifically, moving the chlorine substituent from the 5 position (**8b**) to position 10 (**25**) causes a significant antiproliferative activity that appears also when the halogen substituent is moved to position 9 (**22**). Thus, compounds **25** and **22** show comparable IC_{50} values.

Furthermore, a significant antiproliferative effect on all test cell lines occurs when the chlorine inserted in position 5 is replaced by a protonable alkylamino group (compare **5a** and **7a**

with **14** and **15**). Interestingly, for compounds **14** and **15**, the IC_{50} values are comparable suggesting that the cytotoxic effect is mainly related to the dimethylamino side chain in position 5 rather than the diethylaminoethyl in position 11. Finally, for the quinoline derivatives **27** and **28**, an increase in biological activity was observed with respect to the corresponding 11*H*-THPyC analogues **5a** and **7a**, suggesting that the *N*-methylpiperidine is a positive structural moiety for the cytotoxicity.

Interaction with DNA

To investigate the molecular mechanism responsible for the antiproliferative activity exerted by 11*H*-PyC and 11*H*-THPyC derivatives, and taking into consideration the ability of PyCs to intercalate double-stranded DNA,[5] we evaluated the ability of the most active compounds to form a complex with the macromolecule using linear flow dichroism (LD) technique. For this purpose, LD experiments were performed in the presence of DNA from salmon testes and test compounds at different [drug]/[DNA] ratios (see spectra in Figure 2–4).

Figure 2a shows the spectrum of DNA solution in the absence (trace a) and in the presence of compound **8a** at 0.08 and 0.16 [drug]/[DNA] ratios (traces b and c, respectively). The DNA spectrum shows the typical negative dichroic signal at 260 nm, while in the presence of test compound, a further negative dichroic signal appears at higher wavelengths (300–400 nm) where only the PyC chromophore absorbs. The occurrence of this latter signal is indicative of an intercalative mode of binding, where the planar PyC chromophore can insert between DNA base pairs. Figure 2b reports the spectra of compounds **8a**, **6a** and **8b** observed under the same experimental conditions. Comparison between the LD spectra of **8a** (trace b) and **6a** (trace c), indicate that **6a** is less able to intercalate double-stranded DNA. As previously proposed, the presence of a protonable side chain can significantly improve the intercalative process of a planar chromophore by promoting the interaction between the charged compound and nucleic acid.[13] This interaction could also be responsible for the difference in intercalative ability between quinoline derivatives **8a** and **6a**. Interestingly, the inactive isoquinoline isomer **8b** (trace d) appears practically unable to form a complex with DNA. It is noteworthy that the weaker ability of both **6a** and **8b** to intercalate DNA can be retained in agreement with the absence of cytotoxicity (Table 1).

Correlation between the cytotoxicity and complexation with the macromolecule can be traced out also for the other derivatives. Indeed, a noticeable concentration-dependent ability to intercalate between base pairs was exhibited by compound **9**, in line with its antiproliferative effect (traces b and c in Figure 2c). Figure 3a shows a significant dichroic negative signal for both the 11*H*-PyC derivatives **22** (trace b) and **25** (trace c) characterized by a comparable activity in cells.

Finally, compounds **27** and **28** show a different complexation ability with DNA (trace b and c, respectively, in Figure 3b). Furthermore, in disagreement with their antiproliferative activity, 11*H*-THPyC **14** and **15** showed a modest ability to intercalate DNA (Figure 4a and b, respectively), suggesting the improbable participation of DNA in the intracellular events responsible for their cytotoxicity.

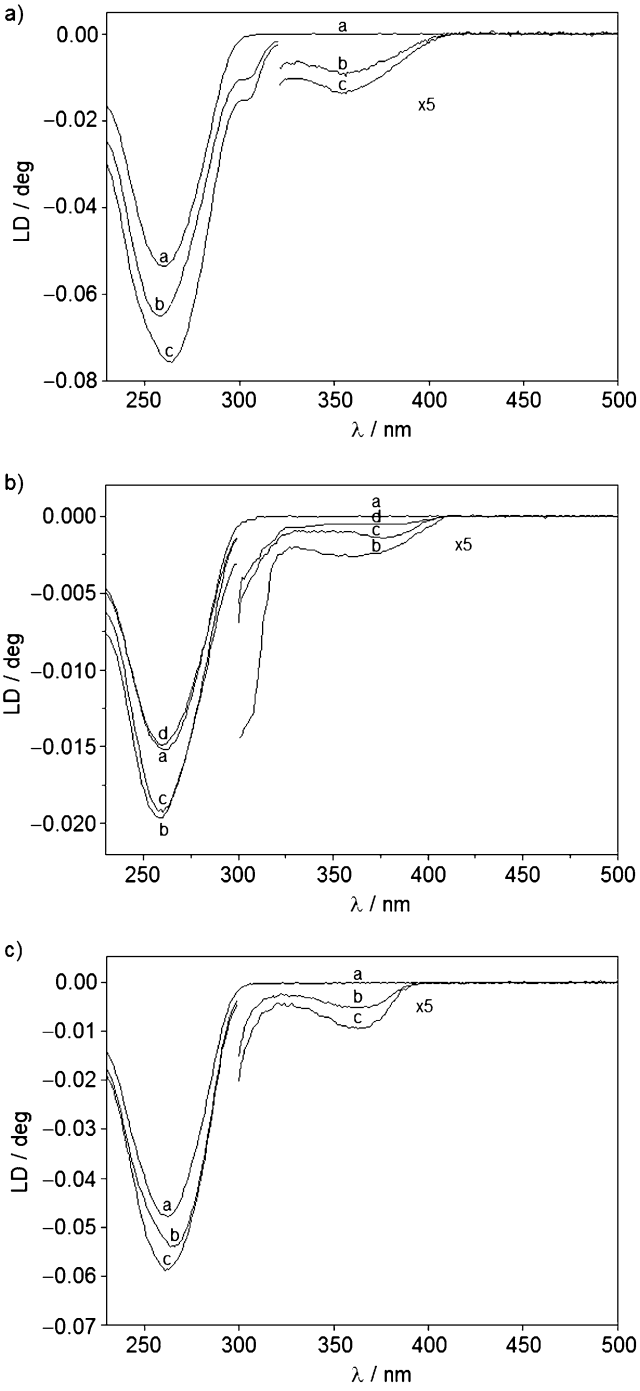


Figure 2. Linear flow dichroism (LD) spectra for a) compound **8a** at different [drug/DNA] ratios (line a: 0; line b: 0.08; line c: 0.16); b) compounds **8a**, **6a** and **8b** at [drug/DNA] ratios of 0 (line a) and 0.16 (lines b–d); c) compound **9** at different [drug/DNA] ratios (line a: 0; line b: 0.08; line c: 0.16). [DNA] = 1.9×10^{-3} M.

Topoisomerase activity

Many intercalating and active anticancer drugs, like doxorubicin, mitoxantrone and *m*-AMSA, target topoisomerase II.[14] The

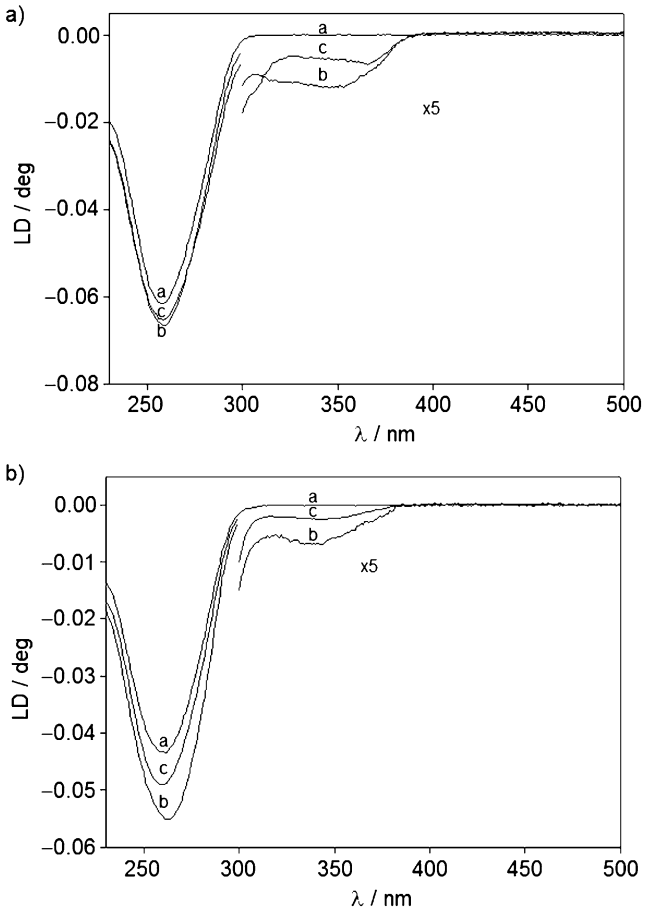


Figure 3. Linear flow dichroism (LD) spectra for a) compounds **22** and **25** at [drug/DNA] ratios of 0 (line a) and 0.08 (lines b–c); b) compounds **27** and **28** at [drug/DNA] ratios of 0 (line a) and 0.08 (lines b–c). [DNA] = 1.9×10^{-3} M.

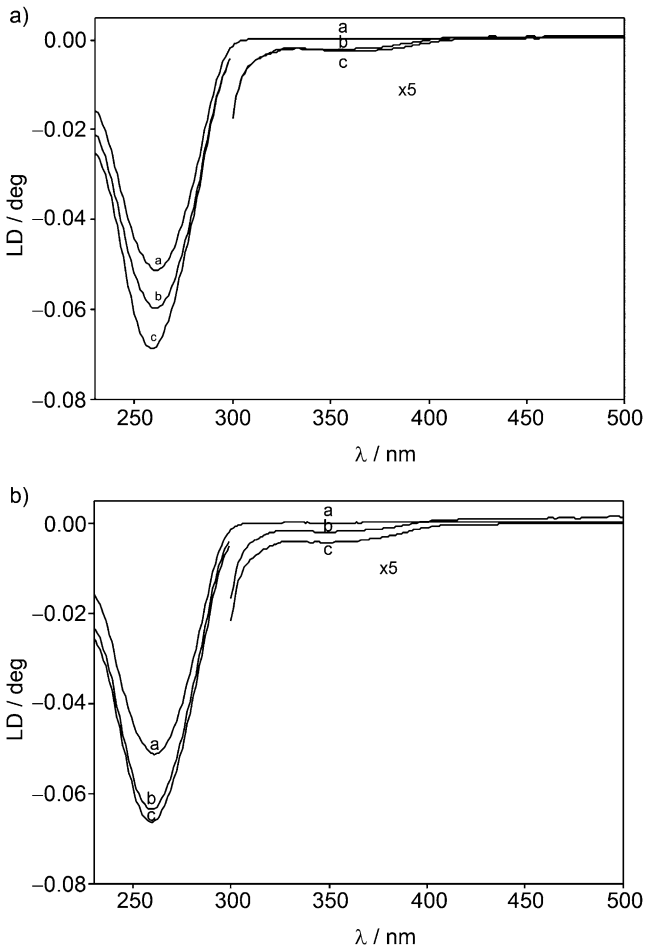


Figure 4. Linear flow dichroism (LD) spectra for compounds a) **14** and b) **15** at different [drug/DNA] ratios (line a: 0; line b: 0.08; line c: 0.16). [DNA] = 1.6×10^{-3} M.

known biological functions of this nuclear enzyme are expanding and include DNA replication, transcription and chromosome segregation.[15] Topoisomerase II solves the topological problems that arise during the molecular processes described above by introducing transient double-strand breaks in DNA. Drugs targeting topoisomerase II can be divided into two classes: poisons and inhibitors. Poisons stabilize the covalent DNA–topoisomerase II complex and cause DNA strand breaks. Inhibitors do not induce an increase in cleavable complexes because they inhibit the enzyme activity acting on other steps of the catalytic cycle. These latter compounds induce cell death through the impairment of the essential activity of topoisomerase II.[14]

With the aim to determine whether the antiproliferative activity shown by the new derivatives can be attributed to the inhibition of topoisomerase II, we evaluated their effect on the relaxation of supercoiled pBR322 DNA, mediated by the enzyme (Figures 5–9; see tables S3–S7 in the Supporting Information for the corresponding DNA quantification). 11*H*-PyC derivative **8a** shows concentration-dependent inhibition, and at 50 μM concentration, this effect becomes comparable to that of the reference drug *m*-AMSA at 8 μM (Figure 5). These results suggest that compound **8a** exerts its biological activity via a mechanism of action related to the capacity of the planar chromophore to intercalate DNA. Thus, the compound hinders the catalytic activity of topoisomerase II.

Surprisingly, a lack of correlation between cytotoxicity and topoisomerase II inhibition was noted for the other 11*H*-THPyC derivatives **9**, **14** and **15**. In fact, despite their antiproliferative effect, derivatives **9** (Figure 6) and **14** and **15** (Figure 7) do not

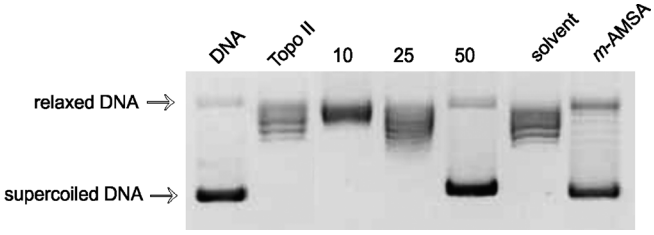


Figure 5. Effect of compound **8a** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topoisomerase II (Topo II) in the absence and presence of test compound at indicated concentrations (μM); *m*-AMSA (8 μM) was used as a reference compound.

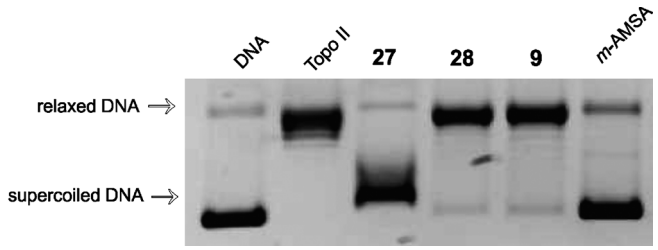


Figure 6. Effect of compounds **9**, **27**, and **28** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topoisomerase II (Topo II) in the absence and presence of test compound at 50 μM; *m*-AMSA (8 μM) was used as a reference compound.

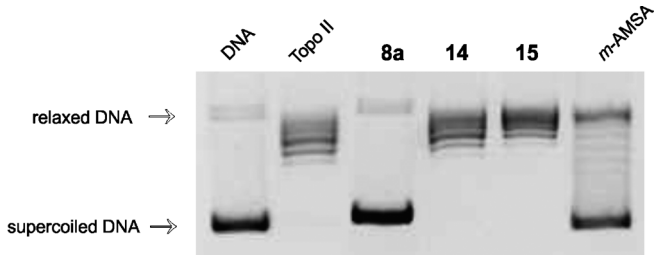


Figure 7. Effect of compounds **8a**, **14**, and **15** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topoisomerase II (Topo II) in the absence and presence of test compound at 50 μM; *m*-AMSA (8 μM) was used as a reference compound.

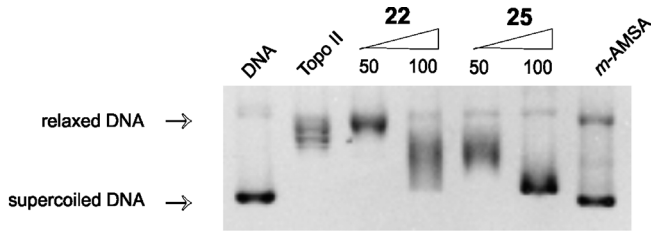


Figure 8. Effect of compounds **22** and **25** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topoisomerase II (Topo II) in the absence and presence of test compound at 50 and 100 μM; *m*-AMSA (8 μM) was used as a reference compound.

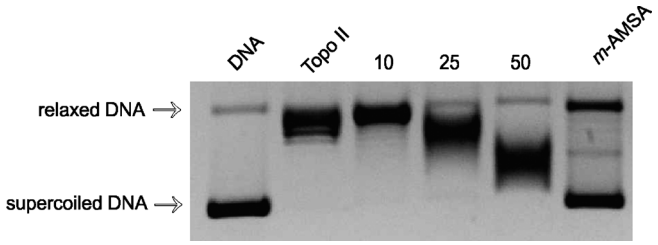


Figure 9. Effect of compound **27** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topoisomerase II (Topo II) in the absence and presence of test compound at indicated concentrations (μM); *m*-AMSA (8 μM) was used as a reference compound.

cause any inhibition of the relaxation activity of the enzyme at 50 μM. For compounds **14** and **15**, this behavior appears to be in agreement with the above-reported weak ability to form a complex with DNA (Figure 4a and b). These two derivatives share a common structural feature, a dimethylamino side chain inserted in position 5 of the chromophore, that could hinder insertion between base pairs and thereby compromise an effective complexation with DNA through an intercalative mode of binding. Compound **9** intercalates between base pairs (Figure 2c), however, this ability seems insufficient to affect the topoisomerase II relaxation process. These results suggest that 11*H*-THPyC **9**, **14** and **15** exert their cytotoxicity by affecting a molecular target other than DNA and/or topoisomerase II.

11*H*-PyC derivatives **22** and **25** showed a concentration-dependent ability to interfere with the relaxation activity of topoisomerase II. The observed effect is higher for derivative **25**, which completely inhibits the enzyme at 100 μM (Figure 8), while compound **22** shows the presence of a series of topoisomers, indicative of a partial inhibition at the same concentration. Comparison of compounds **27** and **28** at 50 μM (Figure 6) shows that **28** is unable to exert any inhibition of the catalytic activity. This result, notwithstanding its agreement with the intercalative ability, does not explain the similar cytotoxicity between the two derivatives. The concentration-dependent effect of **27** on topoisomerase II is shown in Figure 9. Comparison of derivatives **27** and **8a** provides evidence for the weaker inhibitory capacity of 11*H*-THPyC with respect to **8a**, and this behavior is in accordance with the different cytotoxic effect exerted by the two compounds (c.f. Figures 5 and 9).

Many topoisomerase II poisons show anticancer activity, causing cell damage by inducing an increase in covalent DNA–topoisomerase II complexes that affect important cell functions. The presence of the cleavage complex can be evidenced by the enzyme-mediated formation of linear DNA from supercoiled DNA. Experiments performed with the most active 11*H*-PyCs and 11*H*-THPyCs demonstrate the inability of these agents to induce any increase in covalent topoisomerase II–DNA complexes, thus leading to the conclusion that these derivatives are not topoisomerase II poisons (see figures S1 and S2 in the Supporting Information).

Conclusions

Some modified ellipticine analogues were synthesized: 11*H*-pyrido[3,4-*a*] and 11*H*-pyrido[3,2-*a*]carbazoles (11*H*-PyC) and their corresponding tetrahydro derivatives (11*H*-THPyC). The results obtained from the evaluation of their antiproliferative activity, the complexation with DNA, and the interference with the relaxation activity of topoisomerase II, elucidated some interesting structure–activity relationships.

Topoisomerase II may be a molecular target for 11*H*-PyC **8a**, **22** and **25**. In all cases, the planar chromophore carrying a protonable aminoalkyl side chain could allow the formation of an effective intercalative complex with DNA able to interfere with

the relaxation activity of DNA topoisomerase II, thus leading to the cytotoxic effect.

Despite the significant antiproliferative effect, 11*H*-THPyC **9**, **14** and **15** are unable to cause detectable levels of topoisomerase II inhibition, even though compound **9** maintains the capacity to intercalate between base pairs, while both derivatives **14** and **15** lack this ability. Compound **27**, characterized by a methylpiperidine moiety fused to a pyrroloisoquinoline nucleus, intercalates DNA and inhibits topoisomerase II, but to a lesser extent compared with compound **8a**. The presence of a saturated ring in the PyC chromophore, even though it might allow complexation with DNA (compounds **9** and **27**), causes a decrease (**27**) or completely abolishes (**9**) the ability of these derivatives to inhibit topoisomerase II. Most importantly, insertion of a dimethylamino side chain in position 5 prevents both complexation with DNA and the compound's ability to interfere with the enzymatic activity (compounds **14** and **15**). An extensive planarity, a protonable aminoalkyl side chain in position 11, and a lack of steric hindrance in position 5, are the structural requirements for the 11*H*-PyC nucleus to effectively intercalate and interact with the enzyme.

In conclusion, the most interesting structure–activity relationships reported here for the 11*H*-PyC derivatives are related to compounds **9**, **14** and **15**, which show significant antiproliferative effects that are not due to interaction with topoisomerase II and/or DNA. Therefore, certain modifications and appropriate substitutions on the PyC scaffold might give rise to novel small molecules that act via a cellular mechanism different to that of the lead compound, ellipticine. With this in mind, compounds **9**, **14** and **15** can be considered interesting substructures worthy of further development and investigation.

Experimental Section

Chemistry

Melting points (mp) were determined on a Gallenkamp MFB 595 010 M/B capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin–Elmer 1760 FTIR spectrometer using pressed disks of KBr; values are expressed in reciprocal centimeters (cm^{-1}). UV/Vis spectra were recorded on a Perkin–Elmer Lambda UV/Vis spectrometer. NMR spectra were recorded on a Bruker Spectrospin spectrometer (^1H: 300 MHz; ^{13}C: 300 MHz) using the indicated solvents. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) as the internal reference. Coupling constants (J) are given in Hertz (Hz). In the case of multiplets (m), the chemical shift quoted is approximately the center of the peak range. Integrals corresponded satisfactorily to those expected on the basis of compound structure. Elemental analyses were performed for each final compound in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova (Italy), on a model 240B Perkin–Elmer Elemental Analyzer; the results in a tabulated format are available in the Supporting Information Mass spectra were obtained on a Mat 112 Varian Mat Bremen (70 eV) mass spectrometer and an Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Flash column chromatography was carried out on Merck silica gel (250–400 mesh ASTM); reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates. Microwave irradiation was performed in a Discover monomode reactor (IR detector for temperature from CEM Corp.). Starting materials were purchased from Aldrich Chimica, Acros and Riedel-de Haen, and solvents from Carlo Erba, Fluka and Lab-scan. Anhydrous DMSO was obtained by distillation in a vacuum and stored over molecular sieves.

General procedure for the synthesis of 8-nitroquinoline and 5-nitroisoquinoline amino derivatives 2a,b: Compound **1a** or **1b** (3 g, 17.23 mmol) and $NH_2OH{\cdot}HCl$ (7.5 g, 108 mmol) were dissolved in abs EtOH (90 mL) at 40–60 °C with stirring and KOH (60% in EtOH, 100 mL) was added dropwise over 1 h. After ~90 min, the yellow solution changed to an orange suspension. Upon completion (TLC, $CHCl_3$/MeOH 9:1), the mixture was cooled to RT, ice/water was added, and the mixture was left at 4 °C overnight. The precipitate was collected, washed with water and dried.

5-Amino-8-nitroquinoline (2a): Yield 87%; R_f = 0.35 ($CHCl_3$/MeOH 9:1); mp: 240–241 °C; ^1H NMR (300 MHz, [D_6]DMSO): δ = 6.73 (d, 1H, J = 8.9 Hz, 6-H), 7.33 (s, 2H, NH_2), 7.55 (dd, 1H, J = 4.0, 8.4 Hz, 3-H), 8.21 (d, 1H, J = 8.9 Hz, 7-H), 8.68 (dd, 1H, J = 1.4, 4.0 Hz, 4-H), 8.99 ppm (dd, 1H, J = 1.4, 8.4 Hz, 2-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_8N_3O_2$: 190.0572, found: 190.0743.

8-Amino-5-nitro-isoquinoline (2b): Yield 90%; R_f = 0.39 ($CHCl_3$/MeOH 9:1); mp: 280–330 °C (dec); ^1H NMR (300 MHz, [D_6]DMSO): δ = 6.75 (d, 1H, J = 9.2 Hz, 7-H), 8.13 (brs, 2H, NH_2), 8.53 (d, 1H, J = 9.2 Hz, 6-H), 8.65 (d, 1H, J = 6.2 Hz, 4-H), 8.68 (dd, 1H, J = 0.7, 6.2 Hz, 3-H), 9.62 ppm (d, 1H, J = 0.7 Hz, 1-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_8N_3O_2$: 190.0572, found: 190.0496.

General procedure for the synthesis of 8-chloroquinoline and 5-chloroisoquinoline hydrazino derivatives 3a,b and 13: A solution of **2a** or **2b** (1.5 g, 7.93 mmol) in 37% HCl/H_2O (2:1, 50 mL) was heated at reflux to obtain a clear solution. In the case of **2a**, the starting orange suspension became a red solution and remained so even after cooling; in the case of **2b**, an orange precipitate formed on cooling. Next, aq $NaNO_2$ (8 mL, 1.5 M) was added dropwise at 0–4 °C to form the diazo derivatives. The reaction mixture was left to stand for ~1 h at 0 °C before the dropwise addition of a cooled solution of $SnCl_2{\cdot}2H_2O$ (23.76 mmol, 5.36 g) in 37% HCl (5 mL). In the case of **3b**, a precipitate formed but it was contaminated with tin salts. For this reason, the precipitate was dissolved in water, and the pH was adjusted to 12 with 20% aq NaOH. The aqueous solution was extract with EtOAc, and the combined organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo to give the hydrazine.

8-Chloro-5-hydrazinoquinoline hydrochloride (3a): Yield 50%; R_f = 0.75 ($CHCl_3$/MeOH 9:1); mp: 170 °C (dec); ^1H NMR (300 MHz, [D_6]DMSO): δ = 7.01 (d, 1H, J = 8.4 Hz, 6-H), 7.69 (dd, 1H, J = 4.2, 8.6 Hz, 3-H), 7.91 (d, 1H, J = 8.4 Hz, 7-H), 8.59 (dd, 1H, J = 1.5, 8.6 Hz, 4-H), 9.04 (dd, 1H, J = 1.5, 4.2 Hz, 2-H), 10.27 ppm (s, 3H, NH_3).

8-Chloro-5-hydrazinoquinoline (3a): Yield 50%; R_f = 0.76 ($CHCl_3$/MeOH 9:1); mp: 206–207 °C; ^1H NMR (300 MHz, [D_6]DMSO): δ = 4.40 (brs, 2H, NH_2), 7.02 (d, 1H, J = 8.8 Hz, 6-H), 7.48 (dd, 1H, J = 4.0, 8.4 Hz, 3-H), 7.74 (d, 1H, J = 8.8 Hz, 7-H), 7.89 (s, 1H, N-H), 8.63 (dd, 1H, J = 8.6, 1.3 Hz, 4-H), 8.92 ppm (dd, 1H, J = 4.2, 1.3 Hz, 2-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_9ClN_3$: 194.0485, found: 194.0511.

5-Chloro-8-hydrazino-isoquinoline (3b): Yield 18%; R_f = 0.63 ($CHCl_3$/MeOH 9:1); mp: 215–217 °C (dec); ^1H NMR (300 MHz, [D_6]DMSO): δ = 3.87 (brs, 2H, NH_2), 7.07 (d, 1H, J = 8.4 Hz, 7-H), 7.99 (d, 1H, J = 8.4 Hz, 6-H), 8.04 (d, 1H, J = 6.1 Hz, 4-H), 8.70 (d,

1H, $J=6.1$ Hz, 3-H), 9.54 (s, 1H, N-H), 9.58 ppm (s, 1H, 1-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_9ClN_3$: 194.0485, found: 194.0423.

Furthermore, it was possible to identify three by-products (**3c–e**) formed in the acid reaction mixture from **2a**:

5-Hydrazino-8-nitroquinoline hydrochloride (3c): Yield 8–10%; $R_f=0.53$ ($CHCl_3$/MeOH 9:1); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_9N_4O_2$: 205.0726, found: 205.0832.

5-Amino-8-chloroquinoline hydrochloride (3d): Yield 10–50%; $R_f=0.66$ ($CHCl_3$/MeOH 9:1); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_9H_8ClN_2$: 179.0376, found: 179.0273.

8-Chloroquinoline (3e). Yield 5–40%; $R_f=0.45$ ($CHCl_3$/MeOH 9:1); HRMS (ESI): m/z $[M+H]^+$ calcd for C_9H_7ClN: 164.0267, found: 164.0868.

5-Hydrazinyl-*N,N*-dimethylquinolin-8-amine hydrochloride (13): Yield 50%; $R_f=0.90$ ($CHCl_3$/MeOH 9:1); mp: 170 °C (dec); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=3.32$ (s, 6H, $2CH_3$), 7.12 (d, 1H, $J=8.6$ Hz, 7-H), 7.81 (dd, 1H, $J=8.6$, 4.2 Hz, 3-H), 8.27 (d, 1H, $J=8.6$ Hz, 6-H), 8.72 (dd, 1H, $J=1.5$, 8.6 Hz, 4-H), 9.12 ppm (dd, 1H, $J=4.2$, 1.6 Hz, 2-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{11}H_{15}N_4$: 203.1297, found: 203.1256:

General procedure for the synthesis of 8-chloroquinoline and 5-chloroisoquinoline hydrazones 4a,b: A solution of hydrazine **3a** or **3b** (12–13 mmol) in abs EtOH (50 mL) was treated with cyclohexanone (1–1.5 mL, d = 0.947, 12–13 mmol) and drierite (300 mg) was added. The mixture was held at reflux for 3 h. After cooling, the solution was filtered, and the filtrate was evaporated to dryness to give an orange solid that was purified by recrystallization from MeOH to give the desired product.

2-Cyclohexylidene-1-(8-chloroquinolin-5-yl)hydrazine (4a): Yield 75%; $R_f=0.52$ ($CHCl_3$/MeOH 9:1); mp: 132 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.72$ (m, 6H, $(CH_2)_3$), 2.53 (m, 4H, $(CH_2)_2$), 7.01 (d, 1H, $J=9.0$ Hz, 6-H), 7.48 (dd, 1H, $J=4.3$, 8.7 Hz, 3-H), 7.70 (d, 1H, $J=9.0$ Hz, 7-H), 8.59 (dd, 1H, $J=1.4$, 4.3 Hz, 4-H), 8.91 (dd, 1H, $J=1.4$, 8.7 Hz, 2-H), 10.33 ppm (br s, 1H, N-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{17}ClN_3$: 274.1111, found: 274.1109.

2-Cyclohexylidene-1-(5-chloroisoquinolin-8-yl)hydrazine (4b): Yield 70%; $R_f=0.49$ ($CHCl_3$/MeOH 9:1); mp: 133 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.72$ (m, 6H, $(CH_2)_3$), 2.56 (m, 4H, $(CH_2)_2$), 6.94 (d, 1H, $J=8.6$ Hz, 7-H), 7.52 (d, 1H, $J=8.6$ Hz, 6-H), 7.62 (d, 1H, $J=5.9$ Hz, 4-H), 8.11 (s, 1H, N-H), 8.38 ppm (d, 1H, $J=5.9$ Hz, 3-H), 9.35, (s, 1H, 1-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{17}ClN_3$: 274.1111, found: 274.1115.

General procedures for the synthesis of 11*H*-pyridocarbazole derivatives 5a,b, 14, 19, 20 and 27 from hydrazines: Method A: Hydrazine **3a,b** or **13** (10–11 mmol) was dissolved in abs EtOH (200–250 mL) and treated with cyclohexanone (1.17 mL, $d=0.947$, 13 mmol) and TEA ($d=0.726$, 13.8 mmol, 1.93 mL). The mixture was held at reflux for 12 h in the presence of 4 Å molecular sieves. After this time, concd H_2SO_4 (a few drops) was added as a catalyst. Upon completion (TLC $CHCl_3$/MeOH 9:1), the reaction was cooled to RT, filtered, and the orange filtrate was concentrated. The red residue was recrystallized from EtOH/MeOH to give the desired product **5a** (yield 65%) or **5b** (yield 61%).

Method B:[9] Cyclohexanone ($d=0.947$, 0.96 mmol, 0.1 mL), hydrazine **3a** or **3b** (equimolar ratio), acetic acid (9 mL) and 2–3 drops of concd H_2SO_4 were placed in a 10 mL vial closed with a silicon septum and fitted with a magnetic stirring bar. The vial was subjected to microwave irradiation (100 W) at 150 °C for 3 min. Next, the reaction mixture was poured into cold water, neutralized with 20% $NaHCO_3$ and extracted with EtOAc. Organic layers were dried and concentrated to give the desired products **5a** (yield 97%) or **5b** (yield 90%) in high purity.

5-Chloro-7,8,9,10-tetrahydro-11*H*-pyrido[3,2-*a*]carbazole (5a): Prepared using method A. Yield 70%; $R_f=0.48$ ($CHCl_3$/MeOH 9:1); mp: 270–271 °C (MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.80$ (m, 4H, 8-CH_2 and 9-CH_2), 2.70 (m, 4H, 7-CH_2 and 10-CH_2), 2.84 (t, 2H, $J=4.3$, 6.1 Hz, 7-CH_2), 2.84 (t, 2H, $J=6.1$, 4.3 Hz, 10-CH_2), 7.60 (dd, 1H, $J=4.3$, 8.2 Hz, 2-H), 7.98 (s, 1H, 6-H), 8.73 (dd, 1H, $J=8.2$, 1.7 Hz, 1-H), 8.82 (dd, 1H, $J=4.3$, 1.7 Hz, 3-H), 11.94 ppm (br s, 1H, N-H); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta=20.9$, 22.4, 23.3, 24.8, 108.9, 118.8, 120.6, 120.7, 122.1, 124.9, 127.0, 128.3, 135.4, 141.6, 146.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{14}ClN_2$: 257.0846, found: 257.0634; Anal. calcd for $C_{15}H_{13}ClN_2$: C 70.18, H 5.10, N 10.91, found: C 70.45, H 5.21, N 10.52.

5-Chloro-7,8,9,10,-tetrahydro-11*H*-pyrido[3,4-*a*]carbazole (5b): Prepared using method A. Yield 68%; $R_f=0.43$ ($CHCl_3$/MeOH 9:1); mp: 287–288 °C (MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.86$ (m, 4H, 8-H_2 and 9-H_2), 2.71 (m, 4H, 7-H_2 and 10-H_2), 2.84 (t, 2H, $J=5.2$, 6.3 Hz, 7-H_2), 7.95 (dd, 1H, $J=5.2$, 0.6 Hz, 3-H), 8.01 (s, 1H, 6-H), 8.52 (d, 1H, $J=5.2$ Hz, 4-H), 9.76 (d, 1H, $J=0.6$ Hz, 1-H), 12.15 ppm (br s, 1H, N-H); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta=20.8$, 23.5, 23.5, 24.6, 109.9, 117.3, 126.8, 129.6, 131.4, 132.9, 134.2, 134.6, 144.2, 148.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{14}ClN_2$: 257.0846, found: 257.0622; Anal. calcd for $C_{15}H_{13}ClN_2$: C 70.18, H 5.10, N 10.91, found: C 71.34, H 5.12, N 10.65.

5-Dimethylamino-7,8,9,10-tetrahydro-11*H*-pyrido[3,2-*a*]carbazole (14): Prepared using method A. Yield 64%; $R_f=0.57$ ($CHCl_3$/MeOH 8/2); mp: 254 °C; ^{1}H NMR ($CDCl_3$): $\delta=1.92$ (m, 4H, $J=5.5$ Hz, 8-H_2 and 9-H_2), 2.78 (m, 4H, $J=4.6$ Hz, 7-H_2 and 10-H_2), 3.03 (s, 6H, $2CH_3$), 7.31 (dd, 1H, $J=8.4$, 4.2 Hz, 2-H), 7.36 (s, 1H, 5-H), 8.28 (dd, 1H, $J=8.4$, 1.7 Hz, 1-H), 8.79 (dd, 1H, $J=4.2$, 1.7 Hz, 3-H), 8.88 ppm (s, 1H, N-H); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta=21.0$, 23.2, 23.3, 24.8, 45.3, 108.9, 111.1, 118.6, 119.7, 123.5, 123.9, 128.3, 135.4, 141.3, 143.7, 144.5 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{20}N_3$: 266.1657, found: 266.1598; Anal. calcd for $C_{17}H_{20}N_3$: C 76.66, H 7.57, N 15.78, found: C 76.83, H 7.48, N 15.48.

5-Chloro-8*N*-methyl-7,8,9,10-tetrahydro-11*H*-pyrido[3′,4′,4,5]pyrrolo[2,3-*f*]quinoline (27): Prepared using method A. Yield 95%; $R_f=0.40$ ($CHCl_3$/MeOH 8:2); mp: 190 °C; ^{1}H NMR ($CDCl_3$): $\delta=2.46$ (s, 3H, N-CH_3), 2.79 (t, 2H, $J=5.53$ Hz, 9-H_2), 2.93 (t, 2H, $J=5.5$ Hz, 10-H_2), 3.64 (s, 2H, 7-H_2) 7.61 (dd, 1H, $J=4.2$, 8.6 Hz, 2-H), 7.99 (s, 1H, 6-H), 8.74 (dd, 1H, $J=1.7$, 8.6 Hz, 1-H), 8.84 (dd, 1H, $J=1.7$, 4.2 Hz, 3-H), 12.11 ppm (s, 1H, N-H); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta=21.0$, 30.0, 45.2, 45.3, 109.2, 118.8, 120.6, 121.7, 122.1, 124.8, 127.0, 128.8, 134.3, 141.6, 146.6 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{15}ClN_3$: 272.0955, found: 272.0931; Anal. calcd for $C_{15}H_{14}ClN_3$: C 66.30, H 5.19, N 15.46, found: C 66.48, H 5.03, N 14.52.

General procedures for the synthesis of 11*H*-pyridocarbazole derivatives 5a,b, 19 and 20 from hydrazones: A solution of hydrazone **4a,b** or **18** (12-12.5 mmol) in acetic acid/concd H_2SO_4 (3:1) was heated at reflux for 15–20 min. After cooling, the mixture was poured into cold water and the solution was neutralized using 10% aq $NaHCO_3$. The resultant suspension was stirred for 2–3 h at RT, and the precipitate was then collected by filtration, washed with water, and dried in a desiccator in vacuo. Products **5a,b** and the mixture of **19**/**20** were obtained in high purity.

9- and 10-Chloro-6,11-dihydro-5*H*-pyrido[3,4-*a*]carbazoles 19 and 20 (mixture): Prepared from hydrazone **18**. Yield 77%; R_f = 0.38 (EtOAc); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.42 (m, 4H, 6- and 6′-CH$_2$), 3.28 (m, 2H, 5- and 5′-CH$_2$), 7.14 (m, 2H, J = 8.6, 8.4 Hz, 7- and 7′-H), 7.23 (m, 1H, J = 8.0 Hz, 8-H), 7.45 (dd, 1H, J = 8.0 Hz, 9-H), 7.58 (m, 1H, 10′-H), 7.61 (d, 1H, J = 8.6 Hz, 8′-H), 7.90 (m, 1H, 4- and 4′-H), 8.68 (d,1H, 3′-H), 8.09 (s, 2H, 1- and 1′-H), 9.80 ppm (s, 2H, NH and NH′); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{12}ClN_2$: 255.0689, found: 255.0610; Anal. calcd for $C_{15}H_{11}ClN_2$: C 70.73, H 4.35, N 11.00, found: C 71.12, H 4.28, N 11.33.

General procedure for the synthesis of 5-chloro-pyridocarbazoles 6 a,b, 23 and 24: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 2 equiv for **6 a** and **6 b**; 1 equiv for **23** and **24**) was added to a solution of **5 a,b**, **23** or **24** (1.1–1.2 g, 4.1–4.2 mmol) in benzene (35 mL). The mixture was heated at reflux for 24 h under a N_2 atmosphere (completion monitored by TLC: $CHCl_3$/MeOH 9:1). The reaction was filtered, the filtrate was concentrated in vacuo, and the residue was purified by flash chromatography ($CHCl_3$/MeOH 9:1) to give the desired products.

5-Chloro-11*H*-pyrido[3,2-*a*]carbazole (6 a): Yield 35%; R_f = 0.45 ($CHCl_3$/MeOH 9:1); mp: 236–240 °C (dec); ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 7.31 (m, 1H, J = 8.2, 1.4 Hz, 8-H), 7.46 (m, 1H, J = 8.2, 1.4 Hz, 9-H), 7.66 (dq, 1H, J = 8.2, 1.4 Hz, 0.77 Hz, 10-H), 7.69 (dd, 1H, J = 4.2, 8.6 Hz, 2-H), 8.27 (td, 1H, J = 8.2, 1.4, 0.8 Hz, 7-H), 8.65 (s, 1H, 6-H), 8.94 (dd, 1H, J = 1.7, 8.6 Hz, 1-H), 9.02 (dd, 1H, J = 1.7, 4.2 Hz, 3-H), 11.59 (s, 1H, N-H); ^{13}C NMR (300 MHz, [D$_6$]acetone) δ = 111.8, 112.6, 120.2, 120.8, 121.7, 122.2, 122.8, 124.3, 126.7, 127.6, 132.6, 142.9, 145.3, 150.3 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{10}ClN_2$: 253.0533, found: 253.0456; Anal. calcd for $C_{15}H_9ClN_2$: C 71.29, H 3.59, N 11.09, found: C 71.05, H 3.49, N 10.86.

5-Chloro-11*H*-pyrido[3,4-*a*]carbazole (6 b): Yield 41%; R_f = 0.73 ($CHCl_3$/MeOH 9:1); mp: 255 °C (dec); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.46 (m, 1H, J = 8.6, 1.6 Hz, 8-H), 7.61 (m, 1H, J = 8.6, 1.6 Hz, 9-H), 7.66 (td, 1H, J = 8.6, 1.6, 0.8 Hz, 10-H), 7.72 (d, 1H, J = 5.5 Hz, 4-H), 8.27 (td, 1H, J = 8.6, 1.6, 0.8 Hz, 7-H), 8.60 (s, 1H, 6-H), 8.65 (d, 1H, J = 5.9 Hz, 3-H), 9.7 (s, 1H, 1-H), 12.78 ppm (br s, 1H, N-H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 111.1, 119.8, 117.3, 119.8, 121.4, 121.7, 123.3, 129.5, 131.4, 132.8, 134.2, 134.5, 141.3, 144.2, 148.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{10}ClN_2$: 253.0533, found: 253.0429; Anal. calcd for $C_{15}H_9ClN_2$: C 71.29, H 3.59, N 11.09, found: C 70.72, H 3.42, N 10.79.

9- and 10-Chloro-11*H*-pyrido[3,4-*a*]carbazoles 23 and 24 (mixture): Yield 25%; R_f = 0.50 ($CHCl_3$/MeOH 9:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.34 (m, 2H, J = 8.6, 8.4 Hz, 5 and 5′-H), 7.48 (m, 1H, J = 8.4 Hz, 8-H), 7.70 (m, 4H, J = 8.2 Hz, 7-, 7′-,10′-, 9-H), 7.91 (m, 2H, 4- and 4′-H), 8.23 (d, 1H, J = 8.4 Hz, 8′-H), 8.47 (m, 1H, 6′-H) 8.68 (d, 2-H, J = 5.2, 3- and 3′-H), 8.81 (d, 1H, J = 8.4, 6-H), 9.78 (s, 1H, 1-H), 9.98 (s, 1H, 1′-H), 12.83 (s, 1H, N-H), 13.02 ppm (s, 1H, NH′); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{10}ClN_2$: 253.0533, found: 253.0532; Anal. calcd for $C_{15}H_9ClN_2$: C 71.29, H 3.59, N 11.09, found: C 70.82, H 3.51, N 10.75.

General procedure for the synthesis of 11-*N*-substituted pyridocarbazoles 7 a,b, 8 a,b, 9, 15, 21, 22, 25, 26 and 28: A cooled solution of pyridocarbazole (100–300 mg, 0.22–0.66 mmol) in acetone (15–60 mL) was treated with powdered KOH (200–600 mg, 11.22–33.66 mmol) and stirred at 0 °C until a color change from brown to green was observed (15–20 min). 2-Chloro-*N*,*N*-diethylethylamine·HCl (200–600 mg, 1.16 mmol; for **9**, CH_3I was used 1.16 mmol) was then added and, after 30 min, the temperature was increased to reflux under an inert atmosphere. Upon completion (TLC $CHCl_3$/MeOH/NH_3 90:1:0.2), toluene was added and the resultant precipitate was removed by filtration. The filtrate was dried (Na_2SO_4), filtered and concentrated in vacuo, and the solid residue was purified by flash chromatography ($CHCl_3$/MeOH/NH_3 90:1: 0.1).

2-(5-Chloro-7,8,9,10-tetrahydro-11*H*-pyrido[3,2-*a*]carbazol-11-yl)-*N*,*N*-diethylethanamine (7 a): Yield 91%; R_f = 0.45 ($CHCl_3$/MeOH 9:1); mp: 114–116 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.85 (t, 6H, J = 7.3 Hz, 2CH$_3$), 1.80 (m, 2H, J = 5.5, 1.5 Hz, 8-H$_2$), 1.90 (m, 2H, J = 5.5, 1.5 Hz, 9-H$_2$), 2.46 (q, 4H, J = 7.3 Hz, 2CH$_2$), 2.71 (t, 4H, J = 6.5 Hz, 7-H$_2$ and 10-H$_2$), 2.84 (t, 2H, J = 6.3 Hz, CH$_2$), 4.51 (t, 2H, J = 6.9 Hz, N-CH$_2$), 7.64 (dd, 1H, J = 4.2, 8.6 Hz, 2-H), 8.04 (s, 1H, 6-H), 8.78 (dd, 1H, J = 1.7, 8.6 Hz, 1-H), 8.8 ppm (dd, 1H, J = 1.7, 4.2 Hz, 3-H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 11.5, 20.9, 22.4, 23.3, 24.8, 47.5, 52.1, 108.9, 118.8, 120.6, 120.7, 122.1, 124.9, 127.0, 128.3, 135.4, 141.6, 146.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{27}ClN_3$: 356.1894, found: 356.1939; Anal. calcd for $C_{21}H_{26}ClN_3$: C 70.87, H 7.36, N 11.81, found: C 71.50, H 7.59, N 11.44.

2-(5-Chloro-7,8,9,10-tetrahydropyrido[3,4-*a*]carbazol-11-yl)-*N*,*N*-diethylethanamine (7 b): Yield 62%; R_f = 0.33 ($CHCl_3$/MeOH/NH_3 90:1:0.2); mp: 117–118 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.80 (t, 6H, 2CH$_3$), 1.85 (m, 4H, 8-H$_2$ and 9-H$_2$), 2.68 (m, 4H, 2CH$_2$), 2.71 (t, 2H, J = 6.5 Hz, 10-H$_2$) 2.88 (t, 2H, 7-H$_2$), 4.87 (t, 2H, J = 7.6 Hz, CH$_2$), 7.3 (s, 1H, 5-H), 8.6 (d, 1H, J = 5.5 Hz, 4-H), 8.9 (d, 1H, J = 8.6 Hz, 3-H), 9.9 ppm (s, 1H, 1-H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 11.5, 20.8, 23.5, 23.5, 24.6, 47.5, 52.1, 109.9, 117.3, 126.8, 129.6, 131.4, 132.9, 134.2, 134.6, 144.2, 148.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{27}ClN_3$: 356.1894, found: 356.1935; Anal. calcd for $C_{21}H_{26}ClN_3$: C 70.87, H 7.36, N 11.81, found: C 71.92, H 7.58, N 11.51.

5-Chloro-11-methyl-7,8,9,10-tetrahydro-11*H*-pyrido[3,4-*a*]carbazole (9): Yield 59%; R_f = 0.55 ($CHCl_3$/MeOH 9:1); mp: 245–247 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.73 (t, 4H, J = 4.6, 4.8 Hz, 8-H$_2$ and 9-H$_2$), 2.74 (t, 2H, J = 5.7 Hz, 7-H$_2$), 2.82 (t, 2H, J = 6.3, 5.0 Hz, 10-H$_2$), 4.13 (s, 3H, CH$_3$), 8.06 (t, 2H, J = 5.7, 2.3 Hz, 6-H, 4-H), 8.58 (d, 1H, J = 5.5 Hz, 3-H), 9.95 ppm (s, 1H, 1-H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 29.8, 20.8, 22.5, 23.5, 24.6, 108.6, 117.3, 126.8, 129.6, 131.4, 132.9, 134.2, 135.6, 144.2, 148.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{16}ClN_2$: 271.1002, found: 271.1164; Anal. calcd for $C_{16}H_{15}ClN_2$: C 70.98, H 5.58, N 10.35, found: C 71.12, H 5.43, N 10.02.

2-(5-Chloro-pyrido[3,2-*a*]carbazol-11-yl)-*N*,*N*-diethylethanamine (8 a): Yield 30%; R_f = 0.41 ($CHCl_3$/MeOH 9:1); mp: > 300 °C; ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 0.91 (t, 6H, J = 7.3 Hz, 2CH$_3$), 2.59 (q, 4H, J = 7.3 Hz, 2CH$_2$), 3.02 (t, 2H, J = 6.3 Hz, CH$_2$), 5.00 (t, 2H, J = 6.3 Hz, N-CH$_2$), 7.35 (m, 1H, J = 8.2, 1.4 Hz, 8-H), 7.56 (m, 1H, J = 8.2, 1.4 Hz, 9-H), 7.74 (dd, 1H, J = 4.2, 8.6 Hz, 2-H), 7.80 (dq, 1H, J = 8.2, 1.4, 0.8 Hz, 7-H) 8.30 (dq, 1H, J = 8.2, 1.4, 0.8 Hz, 10-H), 8.70 (s, 1H, 6-H), 9.03 (dd, 1H, J = 1.7, 4.2 Hz, 3-H), 9.21 ppm (dd, 1H, J = 1.7, 8.6 Hz, 1-H); ^{13}C NMR (300 MHz, [D$_6$]acetone): δ = 12.5, 45.9, 49.0, 53.0, 111.8, 112.6, 120.2, 120.8, 121.7, 122.2, 122.8, 124.3, 126.7, 127.6, 132.6, 142.9, 145.3, 150.3 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{23}ClN_3$: 352.1581, found: 352.1498; Anal. calcd for $C_{21}H_{22}ClN_3$: C 71.68, H 6.30, N 11.94, found: C 72.08, H 6.50, N 11.64.

2-(5-Chloro-11*H*-pyrido[3,4-*a*]carbazol-11-yl)-*N*,*N*-diethylethanamine (8 b): Yield 25%; R_f = 0.72 ($CHCl_3$/MeOH 9:1); mp: > 300 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.85 (t, 6H, 2CH$_3$), 2.68 (m, 4H, 2CH$_2$), 2.88 (t, 2H, J = 7.6, CH$_2$), 4.87 (t, 2H, J = 7.6, CH$_2$), 7.32 (s, 1H, 6-H), 7.44 (m, 1H, J = 8.6, 8.4 Hz, 8-H), 7.46 (d, 1H, J = 8.8 Hz, 7-H), 7.78 (d, 1H, J = 5.5 Hz, 4-H), 8.61 (d, 1H, J = 5.5 Hz, 3-H), 8.95 (d, 1H, J = 8.6 Hz,10-H), 9.9 ppm (s, 1H, 1-H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 12.9, 45.9, 49.0, 53.0, 110.3, 120.8, 119.3, 121.8,

122.7, 123.3, 129.5, 131.4, 132.8, 134.2, 134.5, 141.3, 144.2, 148.7 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{23}ClN_3$: 352.1581, found: 352.1495; Anal. calcd for $C_{21}H_{22}ClN_3$: C 71.68, H 6.30, N 11.94, found: C 72.32, H 6.49, N 11.71.

5-Dimethylamino-7,8,9,10-tetrahydro-11*H*-pyrido[3,2-*a*]carbazole-*N,N*-diethylethanamine (15): Yield 63%; R_f=0.45 ($CHCl_3$/MeOH 8:2); mp: 168 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.06 (t, 6H, J=7.3 Hz, $2CH_3$), 1.89 (m, 2H, J=5.2, 1.5 Hz, 8-H), 1.97 (m, 2H, J=5.2, 1.5 Hz, 9-H), 2.65 (t, 4H, J=7.3 Hz, $2CH_2$), 2.79 (m, 6H, J=5.7 Hz, 7-H_2, 10-H_2, J=6.3 Hz, CH_2), 3.02 (s, 6H, $2CH_3$), 4.49 (t, 2H, J=6.3 Hz, N-CH_2), 7.35 (s, 1H, 5-H), 7.40 (dd, 1H, J=8.6, 4.2 Hz, 2-H), 8.65 (dd, 1H, J=8.6, 1.5 Hz, 1-H), 8.83 ppm (dd, 1H, J=4.2, 1.7 Hz, 3-H); ^{13}C NMR (300 MHz, $CDCl_3$): δ=11.7, 21.0, 23.2, 23.3, 24.8, 45.3, 47.5, 52.2, 108.9, 111.1, 118.6, 119.7, 123.5, 123.9, 128.3, 135.4, 141.3, 143.7, 144.5 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{43}N_4$: 365.2705, found: 365.2673; Anal. calcd for $C_{23}H_{42}N_4$: C 75.57, H 9.10, N 15.33, found: C 76.60, H 8.81, N 15.73.

2-(10-Chloro-5,6-dihydro-11*H*-pyrido[3,4-*a*]carbazol-11-yl)-*N,N*-dimethylethanamine (22): Prepared by alkylation of a mixture of **19** and **20**; only compound **22** was isolated pure by flash chromatography. Yield 14%; R_f=0.45 ($CHCl_3$/MeOH 9:1); mp: >280 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.82 (s, 6H, $2CH_3$), 2.92 (t, 2H, J=7.2 Hz, CH_2), 3.12 (m, 4H, 5-H_2 and 6-H_2), 4.89 (t, 2H, J=7.3 Hz, CH_2), 7.41 (d, 1H, J=8.4 Hz, 8-H), 7.72 (d, 1H, J=4.8 Hz, 4-H), 7.89 (d, 1H, J=8.4 Hz, 7-H), 8.01 (m, 1H, 10-H), 8.71 (d, 1H, J=4.8 Hz, 3-H), 9.24 ppm (s, 1H, 1-H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ=23.4, 30.6, 45.9, 49.0, 53.0, 111.3, 119.3, 120.8, 121.8, 122.7, 123.3, 129.5, 131.4, 132.8, 134.2, 141.3, 144.2, 148.7 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{21}ClN_3$: 326.1424, found: 326.1418; Anal. calcd for $C_{19}H_{20}ClN_3$: C 70.04, H 6.19, N 12.90, found: C 69.70, H 5.91, N 12.63.

2-(10-Chloro-11*H*-pyrido[3,4-*a*]carbazol-11-yl)-*N,N*-dimethylethanamine (25): Prepared by alkylation of a mixture of **25** and **26**; only compound **25** was isolated pure by flash chromatography. Yield 13%; R_f=0.40 ($CHCl_3$/MeOH 9:1); mp: >300 °C;^{1}H NMR ($CDCl_3$): δ=2.88 (t, 2H, J=8.0 Hz, CH_2), 4.04 (m, 6H, J=7.1 Hz, $2CH_3$), 4.87 (t, 2H, J=8.0 Hz, CH_2), 7.62 (m, 2H, J=7.8 Hz, 5- and 6-H), 7.84 (m, 2H, J=8.7 Hz, 8-H), 7.58 (d, 1H, J=8.6 Hz, 9-H), 8.18 (d, 1H, J=5.5 Hz, 4-H), 8.57 (d, 1H, J=5.5, 3-H), 8.91 (d, 1H, J=8.6 Hz, 10-H) 9.93 ppm (s, 1H, 1-H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ=45.9, 49.0, 53.0, 111.3, 115.6, 119.3, 120.8, 121.5, 122.7, 123.3, 126.4, 129.5, 130.8, 136.2, 141.3, 144.2, 148.7 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{19}ClN_3$: 324.1268, found: 324.1204; Anal. calcd for $C_{19}H_{18}ClN_3$: C 70.47, H 5.60, N 12.98, found: C 71.98, H 5.79, N 13.21.

[2-(5-Chloro-8*N*-methyl-7,8,9,10-tetrahydropyrido[3′,4′,4,5]pyrrolo[2,3-*f*]quinolin-11-yl)ethyl]diethylamine (28): Yield 36%; R_f=0.35 ($CHCl_3$/MeOH 8:2); mp: 136 °C (dec); ^{1}H NMR ($CDCl_3$): δ=0.98 (t, 6H, J=7.3 Hz, $2CH_3$), 2.37 (s, 3H, N-CH_3), 2.69 (t, 4H, J=7.3 Hz, $2CH_2$), 2.76 (t, 2H, J=7.6 Hz, CH_2), 2.90 (t, 2H, J=5.5 Hz, 8-H_2), 2.97 (t, 2H, J=5.5 Hz, 9-H_2), 3,71 (s, 2H, 7-H_2), 4.46 (t, 2H, J=7.6 Hz, N-CH_2), 7.48 (dd, 1H, J=4.2, 8.6 Hz, 2-H), 7.89 (s, 1H, 6-H), 8.67 (dd, 1H, J=1.7, 8.6 Hz, 1-H), 8.91 ppm (dd, 1H, J=1.7, 4.2 Hz, 3-H); ^{13}C NMR (300 MHz, $CDCl_3$): δ=11.6, 30.0, 45.2, 45.3, 47.6, 52.1, 109.2, 118.8, 120.6, 121.7, 122.1, 124.8, 127.0, 128.8, 134.3, 141.6, 146.6 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{28}ClN_4$: 371.2002, found: 371.1912; Anal. calcd for $C_{21}H_{27}ClN_4$: C 68.00, H 7.34, N 15.10, found: C 69.65, H 7.53, N 14.79.

General procedure for the synthesis of 8-chloro-5-nitroquinoline and *N,N*-dimethyl-5-nitroquinolin-8-amine 10 and 11: In a three necked flask, 8-hydroxy-5-nitroquinoline (2 g, 10.52 mmol) was suspended in $POCl_3$ (9.8 mL, d=1.675, 107.05 mmol) and heated at 90 °C with stirring. At this temperature, DMF (for **10**: 4 mL; for **11**: 12 mL) was added dropwise and then the temperature was decreased to 55 °C for the remainder of the reaction period (20–30 h, TLC $CHCl_3$/MeOH 9:1). The mixture was cooled and poured into water/ice (20 g), the aqueous solution was then made alkaline with 30% aq NaOH (pH 12) and extracted with EtOAc (4×50 mL). The combined organic extracts were washed with brine and water, dried (Na_2SO_4), filtered and concentrated to dryness to give the desired product as a yellow solid.

8-Chloro-5-nitroquinoline (10): Yield 61%; R_f=0.88 ($CHCl_3$/MeOH 9:1); mp: 122 °C; ^{1}H NMR ($CDCl_3$): δ=6.76 (d, 1H, J=8.2 Hz, 7-H), 7.43 (dd, 1H, J=8.6, 4.2 Hz, 3-H), 7.62 (d, 1H, J=8.2 Hz, 6-H), 8.20 (dd, 1H, J=1.7, 8.6 Hz, 4-H), 9.03 ppm (dd, 1H, J=4.2, 1.7 Hz, 2-H); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_9H_6O_2N_2Cl$: 209.0118, found: 209.0103.

***N,N*-Dimethyl-5-nitroquinolin-8-amine (11)**: Yield 75%; R_f=0.95 ($CHCl_3$/MeOH 9:1); mp: 172 °C; ^{1}H NMR ($CDCl_3$): δ=2.95 (s, 6H, $2CH_3$), 7.63 (dd, 1H, J=8.4, 4.2 Hz, 3-H), 7.91 (d, 1H, J=8.2 Hz, 6-H), 8.13 (dd, 1H, J=1.7, 8.4 Hz, 4-H), 8.73 (d, 1H, J=8.2 Hz, 7-H), 8.88 ppm (dd, 1H, J=4.2, 1.7 Hz, 2-H); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{12}N_3O_2$: 218.0930, found: 218.0943.

Synthesis of *N,N*-dimethylquinoline-5,8-diamine (12): A solution of nitroquinoline derivative **11** (1.20 g, 5.52 mmol) in 400 mL EtOH was dropped into a suspension of 10% Pd/C (125 mg) saturated with H_2 in EtOH 200 mL. The mixture was stirred at the temperature of 50 °C and hydrogen at atmospheric pressure for a 3 h period. The catalyst was filtered off and the solution evaporated under reduced pressure to give the corresponding aminoquinoline **12** as a greenish solid. Yield 89%; R_f=0.45 ($CHCl_3$/MeOH 9:1); mp: 188 °C; ^{1}H NMR ($CDCl_3$): δ=2.95 (s, 6H, $2CH_3$), 3.94 (s, 2H, NH_2), 6.71 (d, 1H, J=8.2 Hz, 7-H), 7.03 (d, 1H, J=8.2 Hz, 6-H), 7.30 (dd, 1H, J=8.4, 4.2 Hz, 3-H), 8.13 (dd, 1H, J=1.7, 8.4 Hz, 4-H), 8.88 ppm (dd, 1H, J=4.2, 1.7 Hz, 2-H); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{14}N_3$: 188.1188, found: 188.1210.

6,7-Dihydroisoquinolin-8-(5*H*)-one 16 and 6,7-dihydroisoquinolin-5-(8*H*)-one (17): An ice-cold stirred solution of 5,6,7,8-teyrahydroisoquinoline (1 g, 6.79 mmol) in glacial acetic acid (1 mL) and water (33 mL) was treated portionwise with K_2MnO_4 (2.67 g, 13.54 mmol) over 10 min. After stirring for 20 min, the resultant black slurry was filtered through a fritted glass funnel, and the filtrate was extracted with $CHCl_3$ (5×10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude material as an oil. Purification by flash chromatography (Et_2O) gave the two products, but only one was obtained pure. Its exact structure was determined by 1-D and 2-D NMR experiments (see Supporting Information).

6,7-Dihydroisoquinolin-8(5*H*)-one (16): Yield 20% (oil); R_f=0.32 (Et_2O); ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.14 (m, 2H, J=5.9, 6.3, 6.1 Hz, 6-H), 2.65 (m, 2H, J=5.9, 6.3, 6.1 Hz, 5-H), 2.93 (m, 2H, J=5.9, 6.3, 6.1 Hz, 7-H), 7.16 (d, 1H, J=5.2 Hz, 4-H), 8,57 (d, 1H, J=5.2 Hz, 3-H), 9.11 ppm (s, 1H, 1-H); ^{13}C NMR (300 MHz, $CDCl_3$): δ=21.9, 29.3, 38.9, 125.6, 129.4, 146.5, 148.4, 157.4, 195.2 ppm (CO); IR (NaCl support): $\tilde{\nu}$=1688 cm^{-1} (C=O); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_9H_{10}NO$: 148.0762, found: 148.0814.

(*E,Z*)-8-(2-(3-Chlorophenyl)hydrazono)-5,6,7,8-tetrahydroisoquinoline (18): A solution of **16** (0.190 g, 1.29 mmol) in 10 mL of abs EtOH was added dropwise to a solution of 3-chloro-phen]quinhydrazine hydrochloride (0.232 g, 1.29 mmol) in 20 mL EtOH. The

mixture was held at reflux in the presence of drierite for 4–5 h. At the end (TLC EtOAc), the reaction mixture was concentrated and a precipitate formed which was collected, washed with cooled EtOH and dried. Yield 33%; $R_f = 0.58$ (EtOAc); mp: 235.5–237 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 2.18$ (m, 2H, 6-H$_2$), 2.76 (m, 2H, 7-H$_2$), 3.00 (m, 2H, 5-H$_2$), 7.36 (m, 1H, $J = 5.2$ Hz, 14-H), 7.51 (m, 1H, $J = 5.9$, 5.2 Hz, 13-H), 7.98 (d, 1H, $J = 5.3$ Hz, 12-H), 8.48 (d, 1H, $J = 5.3$ Hz, 4-H), 8.50 (m, 1H, 10-H), 8.74 (d, 1H, $J = 5.3$ Hz, 3-H), 9.05 (s, 1H, 1-H), 9.80 ppm (s, 1H, N-H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{15}ClN_3$: 272.0955, found: 272.0757.

Biological assays

Cell cultures: HL-60 (human myeloid leukemic cells) were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 15% heat-inactivated fetal calf serum (FCS; Biological Industries). HeLa (human cervix adenocarcinoma cells) and A-431 (skin carcinoma squamous cells) were grown in Ham's F-12 nutrient mixture (Sigma Chemical Co.) and Dulbecco's modified Eagle's medium, respectively, supplemented with 10% heat-inactivated FCS. Penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) and amphotericin B (0.25 μg mL^{-1}; Sigma Chemical Co.) were added to the media. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Inhibition growth assay: Cells (4×10^4) were seeded into each well of a 24-well plate. After incubation for 24 h, various concentrations of the test agents were added to HL-60, while for HeLa and A-431 cell lines, the test agents were added after the replacement of media with an equal volume of fresh medium. The cells were incubated under standard conditions for a further 72 h. After staining with trypan blue, the cell number was determined using a Bürker counting chamber. Cytotoxicity data are expressed as IC_{50} values—the concentration of test agent required to induce a 50% reduction in cell number compared with control culture.

Nucleic acids: DNA from salmon testes was purchased from Sigma Chemical Co. Its hypochromicity, determined according to the procedure described by Marmur and Doty,[16] was found to be over 35%. pBR322 DNA was purchased from Fermentas Life Sciences.

Linear flow dichroism: Linear dichroism (LD) measurements were performed on a Jasco J500A circular dichroism spectropolarimeter converted for LD and equipped with an IBM computer and a Jasco J interface. LD is defined by Equation (1), where $A_{\parallel}$ and $A_{\perp}$ correspond to the absorbances of the sample when polarized light is oriented parallel or perpendicular to the flow direction, respectively. The orientation is produced by a device designed by Wada and Kozawa[17] at a shear gradient of 500–700 rpm, and each spectrum was accumulated four times. A solution of salmon testes DNA (1.9×10^{-3} M) in ETN buffer (containing 10 mM TRIS, 10 mM NaCl, and 1 mM EDTA, pH 7) was used. Spectra were recorded at 25 °C at different [drug]/[DNA] ratios.

$$LD_{(\lambda)} = A_{\parallel(\lambda)} - A_{\perp(\lambda)} \quad (1)$$

Topoisomerase II-mediated DNA relaxation: Supercoiled pBR322 plasmid DNA (0.25 μg) was incubated with 1 U topoisomerase II (human recombinant topoisomerase II α; USB Corp., Cleveland, OH, USA) and test compounds as indicated for 60 min at 37 °C in 20 μL reaction buffer. Reactions were stopped by adding 4 μL stop buffer (5% sodium dodecyl sulfate (SDS), 0.125% bromophenol blue and 25% glycerol), 50 μg mL^{-1} proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel at RT. The gels were stained with ethidium bromide (1 μg mL^{-1}) in TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA), transilluminated by UV light, and the fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus. The data were analyzed using Quantity One v. 4.0.6 software (BioRad Laboratories).

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