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Original article

Design, synthesis, DNA-binding and cytotoxicity evaluation of new potential combilexines

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Abstract

Combilexines, compounds in which a DNA intercalator is linked to a minor groove binding component, interact with the DNA in a sequence specific manner to yield in most cases compounds with anticancer activity. A series of new compounds closely related to netropsin in which the two components were linked by an amide group was synthesised as potential combilexines. As some of these compounds showed cytotoxic activity in vitro, an attempt was made to rationalise their mechanism of action. The DNA binding characteristics of the carboxamides were evaluated by thermal denaturation experiments and by ethidium bromide displacement assay. Their ability to inhibit the topoisomerase I was also determined. It was concluded that the new compounds were only weak DNA ligands although able in some cases to inhibit topoisomerase I.

Keywords: combilexines; DNA-binding; topoisomerase I inhibition; cytotoxic antitumor agents

1. Introduction

Cancer arises from normal cells which are transformed into tumour cells because of genetic alterations causing inhibition of tumour suppressor or activation of tumour promotor genes [1–3]. In recent years several compounds have been synthesized with the aim of controlling such oncogenes [4,5]. In particular, great interest has been shown in low molecular-weight agents interacting with double stranded DNA and which also show sequence selective binding. Distamycin A and netropsin (see Fig. 1), naturally occurring polyamide antibiotics, are among the first drugs discovered that bind sequence-selectively in the minor groove of the DNA [3,6,7]. The oligopyrrole carbamoyl framework of distamycin A and netropsin binds reversibly to the minor groove of the DNA with a strong preference for AT-rich sequences containing at least four AT-base pairs [6]. Such oligopyrrole derivatives, and the analogous oligohydroxypyrrole and oligoimidazole derivatives, are also called 'lexitropines' because of their ability to read special sequences on the DNA [8,9]. In this context they can also be used as sequence selective carriers for other cytotoxic active DNA ligands, such as intercalators [8]. Incorporation of intercalating heterocycles into the distamycin A–netropsin skeleton leads to compounds called combilexines which represent another drug series for oncogene inhibition [8]. A successful example of this approach is NetAmsa [8,10] (see Fig. 1). Starting from these promising candidates we report here the synthesis, biological evaluation and DNA binding of new combilexine compounds formally derived from netropsin and distamycin A.

2. Chemistry

Following the combilexine approach to DNA ligands, we synthesised a series of new compounds with differing indole or carbazole heterocycles or a nitro group as the left side building block (X in Figs. 2 and 3). These functions were linked via an amide group to pyrrole or oligopyrrole chains terminating with different polar or lipophilic functions (Y in Fig. 2).

The synthesis of the oligopyrrole-amides was carried out according to the method of Lown and Krowicki. [11]. The commercially available *N*-methylpyrrole

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carboxylic acid (**1**) was nitrated to compound **2,** which was then chlorinated with thionyl chloride and treated with propionitrile or *N*,*N*-dimethylamino propane-1,3-diamine to give rise to compounds **7** and **8**, respectively (yield: 79, 66%) [12]. The direct esterification of compound **2** to **6** with methanol–H_2SO_4 gave an unsatisfying yield. However, a better yield was achieved with another strategy: the pyrrole ester **5** was synthesised by condensation of nitromalone aldehyde **4** with glycine methylester **4a** in sodium hydroxide aquous solution and methylated to **6** (39%) [13–15]. Compound **6** was reduced to **6a** by the standard method and then acylated with **3** to give rise to the bis-pyrrole **9**. The tris-pyrrole amide building block **14** was synthesised by the same procedure.

The heterocyclic carbonic acids **10**–**12** were prepared by routine nitrogen alkylation with the appropriate halogen carbonic ester and subsequent hydrolysis [16–18] (see Fig. 4). The also used 2-(1*H*-1-indolyl)acetic acid and the 3-(1*H*-1-indolyl)propionic acid as coupling partners are commercially available.

For the final synthetic step to the respective combilexines the compounds **6**–**9** were used as lexine components. After reducing the pyrrolic nitro group of **6**–**9** the readily formed primary amine group was acylated with the respective heterocyclic acids (RCO_2H) using the DCC method [19], (see Fig. 5).

The constitution of the *N*-methyl-pyrrole derivates were unambigously clarified by the combination of several NMR techniques (for example, ^{1}H-NOE, proton decoupling experiments, ^{13}C-spin echo experiments).

3. Biochemical and biological results and discussion

3.1. Cytotoxicity assay

The new potential combilexines were submitted for cytotoxicity testing to the Developmental Therapeutics Program of the National Cancer Institute (USA) [19,20]. On the basis of structural novelty 11 of them—compounds **9**, **14**, **15**, **17**, **18**, **20-23**, **25**, **26**—were chosen initially for prescreening in a three-cell line panel consisting of the highly sensitive cell lines NCI-H460 (lung carcinoma), MCF-7 (breast carcinoma) and SF 268 (glioma). Results of the SRB-Assay for the determination of the cytotoxicity are reported in Table 1 [21].

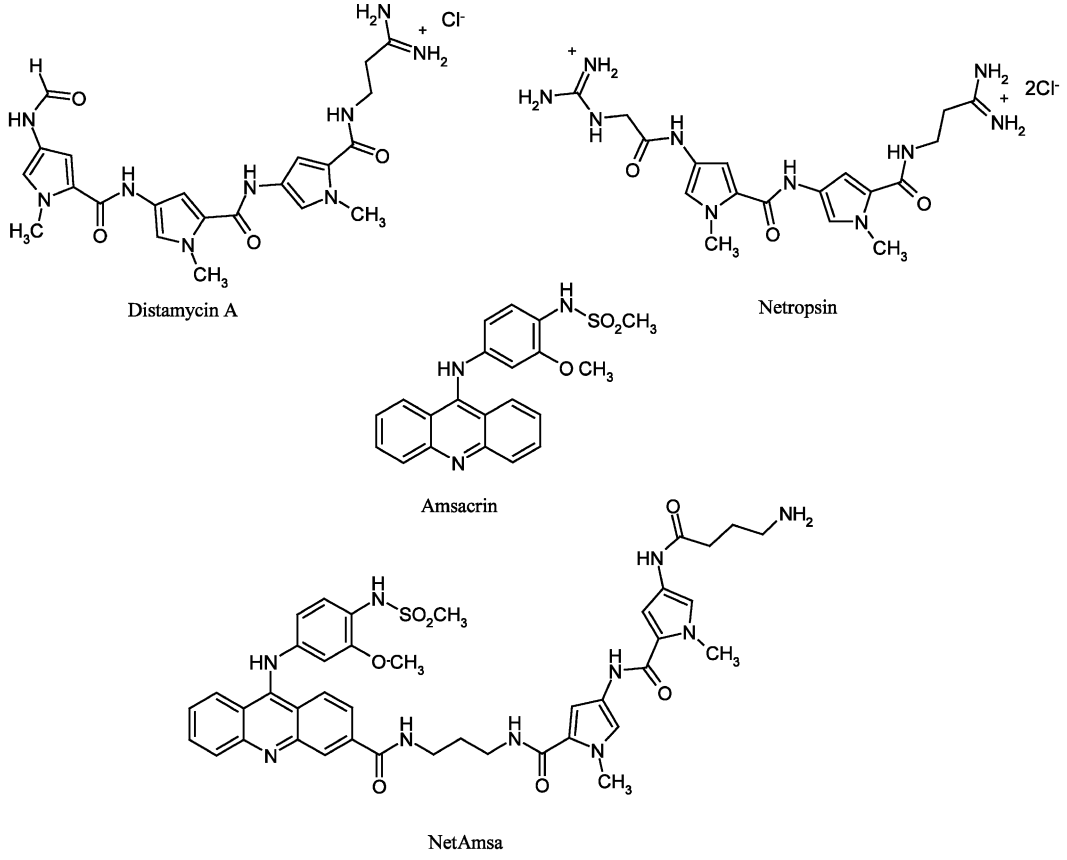
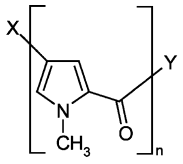


Fig. 1. The minor groove binding ligands distamycin A and netropsin, the intercalator amsacrin and as a typical example, the combilexine NetAmsa.



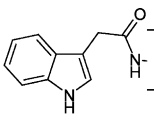
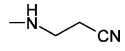
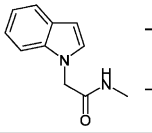
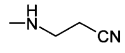
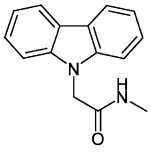
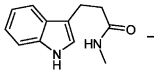
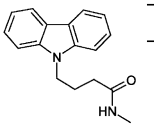
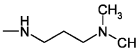
X	Y	n	Yield %	Compound
	-OCH_3	1	72	**15**
		1	42	**18**
	-OCH_3	2	39	**20**
	-OCH_3	1	25	**16**
		1	45	**19**
	-OCH_3	2	38	**21**
	-OCH_3	1	78	**17**
	-OCH_3	1	42	**22**
	-OCH_3	2	45	**23**
	-OCH_3	1	45	**24**
	-OCH_3	2	26	**25**
		1	17	**26**
O_2N-	-OCH_3	2	65	**9**
	-OCH_3	3	42	**14**
	-OH	2	86	**13**

Fig. 2. Structure of synthesised combilexines and lexins.

Compounds which in a 0.1 mM concentration limited cell growth to 32% or less of the untreated controls (compounds **14**, **15**, **17**, **20**, **26**) were submitted to the NCI 60-cell lines panel screening with the SRB-Assay in a concentration range from 10^{-4} to 10^{-8} M. The potency of the tested compounds in inhibiting cell growth was determined in terms of GI_{50}, the concentration at which growth is inhibited by 50% [21]. The mean values of GI_{50} for compounds **14**, **15**, **17**, **20** and **26** and for amsacrine, a commercially available anticancer drug, as a reference, is reported in Table 2. For all these compounds the GI_{50} value is above the limit for further biological screening at the NCI. Anyway compound **14** shows an interesting specific cytotoxicity on the ovarian cancer cell line IGROV1 (GI_{50} 1.47×10^{-8} M).

3.2. DNA-binding studies

We applied different methods to study the mechanistic principle of the cytotoxic activity of the synthesised compounds, starting by analysing the interactions with DNA as a possible molecular target. The general aim was to determine the occurrence of DNA-binding, the affinity of the substance for DNA and the mode of binding [22,23]. An established method for evaluation of the binding of a compound to DNA is the determination of the melting curve of dsDNA in the presence of the potential ligand [24,25]. Compounds which bind to dsDNA stabilise the double strand and raise the melting temperature (T_m) of the DNA. The ΔT_m-value, the difference between the melting temperature of the DNA in the presence and in the absence of the drug is related to the DNA binding affinity of the drug. However, none of the tested compounds showed a significant ΔT_m (> 5 °C) thus indicating a weak DNA affinity. This weak DNA binding was also confirmed by the ethidium bromide displacement assay, which comprises fluorimetric titration [26,27]. Adding a DNA binding drug to the fluorescent complex of DNA and ethidium bromide results in a decrease of the fluorescence of this complex. The concentration of a drug leading to 50% reduction in the fluorescence intensity of the ethidium–DNA complex is defined in the literature as the C_{50} value and is inversely proportional to the apparent binding constant value of the drugs [27]. The displacement assay was validated and evaluated in our group using test compounds whose affinity is known [27–29]. It leads to reliable results for qualitative comparisons of the DNA binding abilities of a series of related compounds. Anyway, the tested pyrrole carboxamides showed only a weak fluorescence reduction which may be attributed to quenching effects (Fig. 6) [30,31].

3.3. Topoisomerase I inhibition

Another interesting target of DNA binding ligands like distamycin A and netropsin is topoisomerase I [32,33]. Topoisomerase I introduces transient nicks in DNA at specific sites, leading to relaxation of the DNA helix. Incubation of the DNA–topoisomerase I mixture in the absence of and in the presence of the test drugs results in different population distributions of topoisomers which can be revealed by agarose gel electrophoresis [34]. Four of the five cytotoxic active compounds (see Table 1) showed inhibition of the catalytic activity of the topoisomerase I in different concentrations. Comparison of the obtained plasmid DNA forms (supercoiled or relaxed) in Fig. 7 indicated the ability of some of the oligopyrrole derivatives to inhibit the enzyme. Inhibition of topoisomerase I is revealed in lines 3 and 4 (camptothecin as a reference

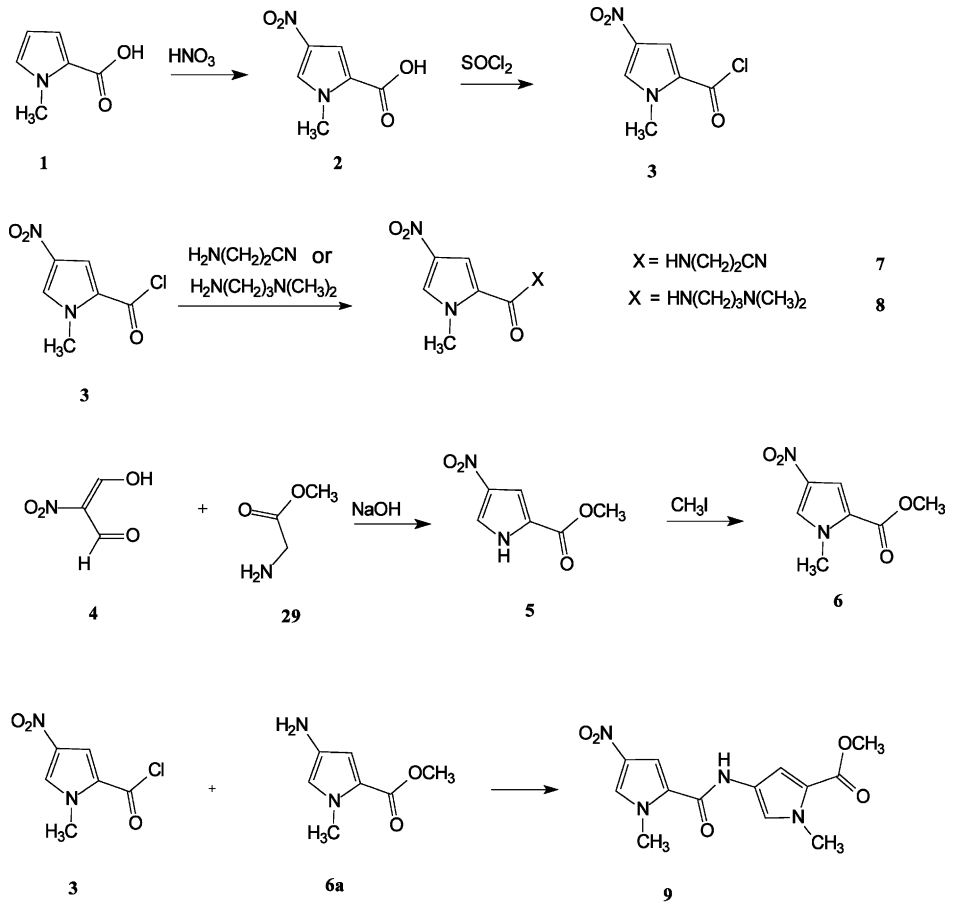


Fig. 3. Synthesis of the building block bis-pyrrole **9**.

compound), in line 7 (compound **26** at the concentration of 20 μM), in line 9 (compound **17** at the concentration of 5 μM), in line 13 (compound **20** at the concentration of 20 μM) and in line 18 (compound **14** at the concentration of 5 μM).

The cytotoxic active compound **15** showed no inhibition of topoisomerase I (results not reported).

4. Conclusion

None of the new compounds appeared to be real combilexins on the basis of the present DNA-binding studies. However, a variety of the new pyrrole carboxamides were probably able to inhibit the catalytic activity of the topoisomerase I. This characteristic seems to be in correlation with the cytotoxic activity determined at the NCI. Four of the five active compounds in the three cell lines preliminary screening showed topoisomerase I inhibition in concentrations from 5 to 50 μM. Due to the great structural differences in the active compounds, a rigorous determination of structure–activity relationships is not possible at the moment. However, it seems that cyanoalkyl side chains are deleterious for any activity (see compounds **18**, **19**). Moreover, the substitution of the aldehyde group of distamycin A with a nitro group as well as the substitution of the guanidine terminal with an ester prevent compound **14** from binding to the DNA, showing the importance of H-bond-donor and acceptor groups for DNA interactions.

Our research on these compounds will continue by modifying and developing the most promising cytotoxic active compounds. Moreover, a variety of new candidates are in synthetic development in our laboratory including also nucleobase-linked oligopyrroles for more systematic structure activity studies.

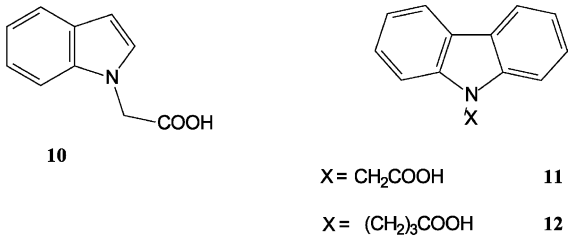


Fig. 4. Prepared acids as chromophore building blocks.

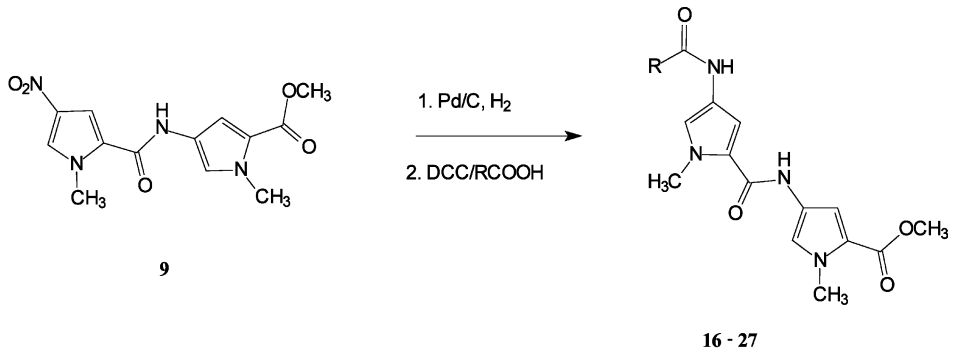


Fig. 5. Preparation of the potential combilexins **16**–**27** starting from compound **9**.

5. Experimental

5.1. Chemistry

Melting points were measured with a Büchi 510 and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1310 infrared-spectrometer using potassium bromide pellets (v in cm^{-1}). ^1H NMR, ^{13}C NMR and NOE- spectra were recorded on a Bruker AC-300 apparatus (300 MHz). The samples were dissolved in DMSO-d_6. The chemical shift values are reported in parts per million (ppm, δ units) and spin–spin coupling J were listed in Hz. EI-mass spectra were performed with a Mascom 311-A apparatus. C, H, N-analysis were performed. Analyses indicated by the symbols of the elements or functions were within $\pm 0.4\%$ of the theoretical values.

5.1.1. 1-Methyl-4-nitropyrrole-2-carboxylic acid (2) [11]

Acetic anhydride (8 mL) was treated with 1.6 mL of 70% nitric acid and the mixture cooled to -25 °C and slowly added to a suspension of 2 g (16 mmol) 1-methylpyrrole-2-carboxylic acid (**1**) in 12 mL of acetic anhydride. The mixture was stirred for 0.5 h, then the temperature was allowed to rise to the ambient and stirring was continued for 20 min. The mixture was cooled again to -25 °C and the resulting precipitate collected in a funnel cooled in dry ice. The solid was washed with a small quantity of cold acetic anhydride (-25 °C) and then with acetic anhydride–CCl_4 1:1 (-25 °C) and then CCl_4 and hexane. The obtained residue was purified on silica gel (petroleum ether–ethyl acetate (1:1)). Yellow amorphous compound was obtained (6.5 mmol, 1.1 g) (41%), melting point (m.p.) 204 °C (lit. m.p. 201–201.5 °C); IR (KBr) (cm^{-1}): 3140, 3090, 3010, 2870, 1700, 1580, 1520, 1450, 1420, 1400, 1360, 1320, 1270, 1200, 1130, 1090, 850, 825, 750, 610, 480; ^1H NMR (200 MHz) ($CDCl_3$) δ: 3.9 (s, 3H, CH_3), 7.23 (d, 1H, $^4J = 2.0$ Hz, pyrrole-H3), 8.22 (d, 1H, $^4J = 2.0$ Hz, pyrrole-H5). MS m/z: 170 [M^+].

5.1.2. 1-Methyl-4-nitropyrrole-2-carboxylic acid methyl ester (6) [12]

Compound **2** (5 g, 29.4 mmol) was dissolved in MeOH (50 mL) and concentrated H_2SO_4; the mixture was heated under reflux for 12 h. Water was added and the mixture was extracted with $CHCl_3$. The residue was chromatographed on silica gel (petroleum ether–ethyl acetate (3:1)). Yellow needles were obtained (2.1 g) (11.5 mmol) (39%), m.p: 121 °C (lit, m.p.: 120 °C); IR (KBr) (cm^{-1}): 3200, 3110, 3100, 3040, 1710, 1510, 1485, 1470, 1405, 1395, 1320, 1260, 1240, 1200, 1190, 1050, 1010, 905, 880, 780; ^1H NMR (200 MHz) ($CDCl_3$) δ: 3.82 (s, 3H, CH_3), 4.04 (s, 3H, CH_3), 7.31 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H 3), 8.05 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5). MS m/z: 184 [M^+].

5.1.3. Nitromalonic aldehyde (4) [13]

Mucochloric acid (33.8 g, 200 mmol) dissolved in ethanol (100 mL) was added to a solution of sodium nitrite (55.2 g) in water (100 mL) during a period of 25

Table 1
Cytotoxic activity of compounds **9**, **14**, **15**, **17**, **18**, **20-23**, **25**, **26** in the primary three cell lines NCI-H460 (lung carcinoma), MCF-7 (breast carcinoma) and SF 268 (glioma) assay

Compound 0.1 mM	Growth percentages		
	NCI-H460	MCF7	SF-268
9	104	74	97
14 [a]	14	62	59
15 [a]	31	24	41
17 [a]	14	31	7
18	86	53	76
20 [a]	−2	12	23
21	109	90	93
22	102	91	62
23	113	86	83
25	93	84	96
26 [a]	10	13	36

The values are reported as cell growth percentages.
[a] Further screening.

Table 2
Mean GI_{50} of compounds **14**, **15**, **17**, **20**, **26** and of amsacrine for comparison, expressed as $\log_{10}$ of the molar concentration

Compound	**14**	**15**	**17**	**20**	**26**	Amsacrine
Mean GI50	−4.27	−4.19	−4.36	−4.47	−4.52	−6.28

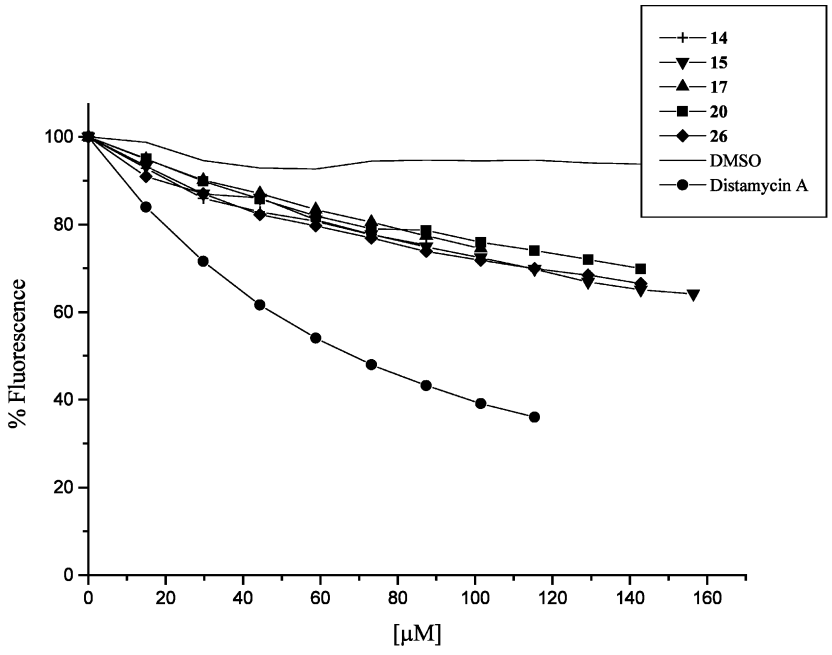


Fig. 6. Ethidium bromide displacement effect of the cytotoxic active compounds **14**, **15**, **17**, **20**, **26** and distamycin A in relation to DMSO.

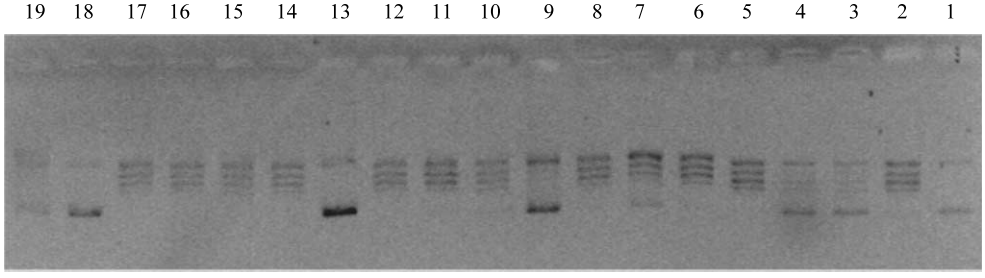


Fig. 7. Topoisomerase I inhibition assay for compounds **14**, **17**, **20**, **23**, **26**. Line 1: Form I DNA, Line 2: Form IV DNA, Lines 3, 4: Camptothecin 20 μM; Lines 5, 6, 7: **26**; Lines 8, 9, 10: **17**; Lines 11, 12, 13: **20**; Lines 14, 15, 16: **23**; Lines 17, 18, 19: **14**. Each compound was tested at 0.5, 5 and 20 μM concentration.

min. Some external cooling was necessary to keep the temperature from rising above 60 °C. After stirring for a further 35 min and cooling overnight, the formed solid was filtered and extracted with boiling ethanol (70 mL). After rotating in vacuum the total yield was 3.2 g. Orange amorphous powder (2.8 g, 24 mmol, 12%), m.p: 124 °C (lit. m.p.: 124 °C); IR (KBr) (cm^{-1}): 3510, 3440, 3370, 3290, 1650, 1600, 1570, 1530, 1480, 1380, 1350, 1270, 930, 820, 760, 530, 490; ^1H NMR (300 MHz) (DMSO-d^6) δ: 8.4 (s, 1H, enole-H), 9.7 (s, 1H, aldehyde-H), 12.0 (1H, s, OH); MS m/z: 116 [M^+].

*5.1.4. 4-Nitro-pyrrole-2-carboxylic methyl ester (**5**) [14]*

Equimolar quantities of sodium nitromalonic aldehyde (**4**) (1.3 g, 11.15 mmol) and of glycine methyl ester (**4a**, 1.4 g, 11.15 mmol) were dissolved in 6–7 mL of warm of 65% ethanol. 40 drops of a 20% sodium hydroxide solution were added and the red reaction mixture set aside for half an hour at a constant temperature of 50 °C. Then the mixture was added to water and the resulting yellow precipitate was extracted several times with ether. The diethylether-phase was dried and concentrated in vacuum. The residue was chro-

matographed on silica gel (petroleum ether–ethyl acetate (3:1)). Yellow amorphous powder was obtained (1.35 g) (7.94 mmol) (71%); m.p. 197 °C (lit. mp. 197 °C); IR (KBr) (cm^{-1}): 3280, 3260, 3140, 1700, 1560, 1500, 1450, 1440, 1410, 1385, 1340, 1325, 1260, 1215, 1180, 1145, 1090, 980, 960, 930, 850, 840, 820, 800, 780, 750; ^{1}H NMR (200 MHz) ($CDCl_3$) δ: 3.85 (s, 3H, CH_3), 7.28 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 8.96 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5); MS m/z: 170 [M^+].

5.1.5. 1-Methyl-4-nitropyrrole-2-carboxylic methyl ester (**6**) [15]

A solution of **5** (300 mg, 1.75 mmol) in dry THF (15 mL) was added dropwise to a suspension of hot THF (20 mL) and potassium (200 mg, 5.1 mmol). After stirring the mixture on reflux for 1 h, iodomethane (250 mg, 141.91 mmol) (0.1 mL) in dry THF (2 mL) was added dropwise. The reaction mixture was stirred under reflux for another 1 h. Methanol was added to destroy the potassium; the THF was removed in vacuum and then soluted in diethylether. The diethylether was dried over magnesium sulfate and removed in vacuum. The residue was chromatographed on silica gel (petroleum ether–ethyl acetate (3:1)). Yellow amorphous powder was obtained (231 mg, 1.25 mmol, 72%); m.p: 121 °C (lit. m.p.: 120 °C); IR (KBr) (cm^{-1}): 3150, 1710, 1570, 1510, 1500, 1445, 1420, 1400, 1370, 1340, 1320, 1260, 1200, 1120, 1090, 985, 945, 860, 830, 800, 765, 750; ^{1}H NMR (200 MHz) ($CDCl_3$) δ: 3.82 (s, 3H, CH_3), 4.04 (s, 3H, CH_3), 7.31 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 8.05 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5); MS m/z: 184 [M^+].

5.1.6. 1-Methyl-4-nitropyrrole-2-carboxamide propionitrile (**7**) [11]

A solution of 7 g (41.14 mmol) of **2** and a slight excess of $SOCl_2$ in 20 mL of dry THF was heated under reflux for 5 min. The excess of $SOCl_2$ and the solvent were removed under reduced pressure and the evaporation was repeated with some anhydrous THF. The residue was dissolved in THF and cooled to -20 °C. A solution of 3.12 mL (2.97 g, 42.37 mmol) of aminopropionitrile and 7.8 mL of Hünig's base in 10 mL of THF was added. The temperature was allowed to rise to the ambient, then the solvent was removed in vacuo, water was added, and the resulting crystalline solid collected. A yellow amorphous powder was obtained (7.22 g, 32.5 mmol, 79%), m.p.: 132 °C (lit. m.p.: 132–133 °C); IR (KBr) (cm^{-1}): 3310, 3115, 2875, 2240, 1640, 1550, 1525, 1410, 1320, 1140, 1110, 1070, 855, 750; ^{1}H NMR (200 MHz) ($CDCl_3$) δ: 2.65 (t, 2H, $^3J = 6.5$ Hz, CH_2–CN), 3.5 (2H, q, $^3J = 6{,}5$ Hz), 3.9 (s, 3H, CH_3), 7.25 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 7.8 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5), 8.05 (s, 1H, NH); MS m/z: 222 [M^+].

5.1.7. 1-Methyl-4-nitropyrrole-2-carboxamido (-3-(N,N-dimethyl))propanamine (**8**) [11]

A solution of 0.7 g (4.1 mmol) **2** and a slight excess of $SOCl_2$ in dry THF was heated under reflux for 5 min. The excess of $SOCl_2$ and the solvent were removed under reduced pressure and the evaporation was repeated with some dry THF. The residue was dissolved in THF and cooled to -20 °C. A solution of 0.6 mL (490 mg, 4.8 mmol) *N*,*N*-dimethyl-1,3-propandiamine and 0.8 mL Hünig's base in 10 mL THF was added dropwise. The temperature was allowed to rise to the ambient and then the solvent was removed in vacuo.

The residue was chromatographed on neutral aluminium oxide (methanol–ethyl acetate (1:4)). Colourless crystals were obtained (688 mg, 2.7 mmol) (66%), m.p. 125 °C (lit. m.p: 125.5–127 °C). IR (KBr) (cm^{-1}): 3420, 2720, 2340, 1650, 1540, 1530, 1490, 1470, 1410, 1305, 1265, 1215, 1035, 820, 755; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 1.6 (m, 2H, $^3J = 6.5$ Hz, CH_2), 2.05 (s, 6H, 2 CH_3), 2.2 (t, 2H, $^3J = 6.7$ Hz, $CH_2N(Me)_2$), 3.18 (q, 2H, $^3J = 6.7$ Hz, CH_2N), 3.77 (s, 3H, CH_3), 7.38 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 8.09 (1H, d, $^4J = 1.6$ Hz, pyrrole-H5), 8.40 (t, 1H, $^3J = 7.4$ Hz, NH); MS m/z: 254 [M^+].

5.1.8. Methyl-1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylate (**9**) [12]

A solution of **6** (200 mg) (1.08 mmol) was hydrogenated over 60 mg of 10% Pd on charcoal in 10 mL of dry methanol at atmospheric pressure and room temperature (r.t.). After 3 h of reduction the catalyst was removed by filtration, the solvent evaporated under reduced pressure, and then 0.5 mL of Hünig's base in 3 mL of dry THF was added. The mixture was cooled to -20 °C and treated with a solution of 1-methyl-4-nitropyrrole-2-carboxylic acid chloride (**3**). Compound **3** was prepared as above by heating 200 mg (1.18 mmol) of compound **2** under reflux with $SOCl_2$ and dissolved in THF. After 30 min of stirring at r.t. the mixture was evaporated to dryness. The residue was chromatographed on silica gel (methanol–ethyl acetate (1:4)). A yellow amorphous solid was obtained. (228 mg, 0.75 mmol) (69%), m.p: 225 °C (lit. m.p.: 225 °C); IR (KBr) (cm^{-1}): 3370, 3140, 2980, 2500, 1690, 1660, 1565, 1500, 1480, 1440, 1390, 1320, 1250, 1210, 1120, 1100, 845, 815, 780, 750; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 3.72 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 3.96 (s, 3H, CH_3), 6.86 (1H, d, $^4J = 1.8$ Hz, pyrrole-H3), 7.45 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H5), 7.53 (d, 1H, $^4J = 1.8$Hz, pyrrole-H3′), 8.18 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H5′), 10.28 (s, 1H, NH); MS m/z: 306 [M^+]; Anal. $C_{13}H_{14}N_4$ (C, H, N).

5.1.9. 2-(1H-1-Indolyl)acetic acid (**10**) [16]

Molten indole (22 g, 188 mmol) was stirred with finely powdered potassium hydroxide (17 g, 425.1

mmol) in a fractionating flask (200 mL capacity) inserted deeply in a salt bath kept at 200–250 °C. The stirring was continued until no more water has been formed (6–8 h). The flask was removed from the bath and allowed to cool a little. Dry chloroacetate (36 g) (285 mmol) was added and the flask was heated at 140–150 °C for 4 h with occasional shaking, and cooled. A solution of potassium hydroxide (25 g) in water (75 mL) was added, the mixture heated on a waterbath for 3 h and then poured into water (250 mL). The formed crystalline product was separated and cooled, washed, dried, dissolved in ether, and the solution filtered through a short column of aluminium oxide and then evaporated to dryness. The crude product acid was dissolved in hot ethanol; on cooling the acid light brown needles were obtained (3.95 g, 22.56 mmol, 12%) m.p: 175 °C (lit. mp.: 174–179 °C);. IR (KBr) (cm^{-1}): 3420, 3200, 3110, 3100, 3040, 1710, 1610, 1510, 1485, 1470, 1405, 1395, 1340, 1320, 1260, 1240, 1200, 1200, 1190, 1125, 1050, 1010, 905, 880, 780, 740; ^{1}H NMR (200 MHz) ($CDCl_3$) δ: 4.63 (s, 2H, CH_2), 6.48 (d, 1H, $^3J = 7.8$ Hz, indole-H3), 7.0 (pt, 1H, $^3J = 7.6$ Hz, 1H, indole-H 5 or 6), 7.1 (pt, 1H, $^3J = 7.7$ Hz, indole-H 5 or 6), 7.32 (d, 1H, $^3J = 7.6$ Hz, indole-H2), 7.34 (d, 1H, $^3J = 7.7$ Hz, indole-H7), 7.58 (d, 1H, $^3J = 7.9$ Hz, indole-H2); MS m/z: 175 [M^+].

*5.1.10. 2-(9H-9-Carbazolyl)acetic acid (**11**) [17]*

Molten carbazole (20 g, 119.76 mmol) was stirred with fine powdered potassium hydroxide (15 g, 441.3 mmol) until no more steam was generated. After cooling and powdering the solid, dry ethyl-2-chloroacetate was added. The mixture was heated at 180 °C for 4 h. Then water was added (100 mL); HCl (1 N) was added dropwise until precipitation occurred; the obtained solid was dissolved in CH_2Cl_2 and evaporated to dryness a few times. Brown amorphous solid (3.77 g, 16.77 mmol, 14%); m.p.: 192 °C (lit. mp.: 193–194 °C); IR (KBr) (cm^{-1}): 3480, 3400, 2800, 2350, 1600, 1480, 1450, 1425, 1400, 1305, 1215, 1070, 1000, 750, 725; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 4.6 (s, 2H, CH_2), 7.1 (pt, 2H, $^3J = 7.2$ Hz, carbazole-H1,8), 7.3 (m, 4H, $^3J = 7.3$ Hz, carbazole-H2,3,6,7), 8.1 (pt, 2H, $^3J = 7.3$ Hz, carbazole-H4,5); MS m/z: 225 [M^+].

*5.1.11. 4-(9H-9-Carbazolyl)butanoic acid (**12**) [18]*

A mixture of carbazole (20 g, 119.76 mmol) and potassium hydroxide (10 g, 294.2 mmol) was finely powdered and made molten (260 °C). The solid carbazole-potassium was again powdered after cooling. Then γ-butyrolactone (300 g, 3.488 mol) was added and heated (180–200 °C) for 10 h. The mixture was cooled in ice, precipitated carbazole was filtered off and the product was chromatographed on neutral aluminium oxide (petroleum ether–ethylacetate–methanol (10:10:1)); brown needles were obtained, (5.15 g, 20.36 mmol, 17%); m.p.: 150 °C (lit. m.p.: 150 °C); IR (KBR) (cm^{-1}): 3100, 2930, 2860, 2330, 1680, 1565, 1460, 1430, 1380, 1350, 1320, 1300, 1230, 1210, 1190, 1150, 1120, 110, 900, 725, 700; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 1.95 (m, 2H, $^3J = 6.5$ Hz, CH_2), 2.25 (t, 2H, $^3J = 6.4$ Hz, CH_2), 4.4 (t, 2H, $^3J = 6.6$ Hz, CH_2), 7.2 (d, 2H, $^3J = 6.3$ Hz, carbazole-H1,8), 7.4 (pt, 2H, $^3J = 6.9$ Hz, carbazole-H2,7), 7.6 (pt, 2H, $^3J = 6.9$ Hz, carbazole-H3,6), 8.1 (d, 2H, $^3J = 7.0$ Hz, carbazole-H4,5), 11.5 (s, 1H, COOH); MS m/z: 253 [M^+].

*5.1.12. 1-Methyl-4-{[(1-methyl-4-nitro-1H-2-propyl)-carbonyl] aminoc-1-H-2-pyrrole carboxylic acid (**13**) [11]*

To 1 g of **9** (3.27 mmol) dissolved in methanol (30 mL) a solution of 50% potassium hydroxide (10 mL) was added, and the mixture was heated under reflux for 12 h; HCl (conc.) was added dropwise, until pH was 1. The precipitate was filtered, washed with water, soluted in CH_2Cl_2 and evaporated a few times. A yellow amorphous solid was obtained, (0.82 g, 2.8 mmol)(86%); m.p.: 231 °C (lit. m.p.: 233–234 °C); IR (KBr) (cm^{-1}): 3040, 3010, 1640, 1550, 1480, 1430, 1400, 1300, 1280, 1230, 1190, 1100, 800; 740; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 3.9 (s, 3H, CH_3), 3.95 (s, 3H, CH_3), 6.9 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H3), 7.4 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H5), 7.5 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H3′), 8.1 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H5′), 10.25 (s, 1H, COOH); MS m/z: 292 [M^+]; Anal. $C_{12}H_{12}N_4O_5$ (C, H, N).

*5.1.13. Methyl-1-methyl-4-{[(1-methyl-4-{[(1-methyl-4-nitro-1H-2-pyrrol)carbonyl]amino}-1H-2-pyrrolyl)-carbonyl]amino}-1H-2-pyrrole carboxylate (**14**) [12]*

A solution of **9** (612 mg) (2 mmol) in dry methanol (10 mL) was hydrogenated over 200 mg of Pd on charcoal. After **9** was reduced, the catalyst was removed by filtration, the solvent evaporated under reduced pressure, and then 1 mL of Hünig's base in 3 mL dry THF was added. The mixture was cooled to −20 °C and treated with a solution of the acid chloride from **2** (340 mg) (2 mmol) in 5 mL of dry THF. After 30 min of stirring at r.t. the mixture was evaporated to dryness. The residue was chromatographed on silica gel (petroleum ether–ethyl acetate (1:1). A yellow amorphous solid was obtained (360 mg, 0.84 mmol, 42%), m.p: 228 °C; IR (KBr) (cm^{-1}): 3030, 2970, 1700, 1640, 1550, 1430, 1390, 1300, 1250, 1200, 1100, 1050, 790; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 3.7 (s, 3H, CH_3), 3.8 (s, 3H, CH_3), 3.9 (s, 3H, CH_3), 4.1 (s, 3H, CH_3), 6.9 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H), 7.05 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H), 7.3 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H), 7.4 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H), 7.6 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H), 8.1 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H), 9.9 (s, 1H, NH), 10.25 (s, 1H, NH), 10.4 (s, 1H, NH); MS m/z: 428 [M^+]; Anal. $C_{19}H_{20}N_6O_6$ (C, H, N).

5.1.14. General procedure for the synthesis of the pyrrole- and the oligopyrrole carboxamides

Four mmol of the carboxylic acid were dissolved in 40 mL CH_2Cl_2. Then 4.4 mmol of the pyrrole amine (prepared from the nitropyrrole analogues by hydrogenation in methanol over Pd on charcoal) and 0.1 mmol (12 mg) of dimethylaminopyridin (DMAP) were added. The mixture was cooled to −15 °C. A second solution of 4.8 mmol (1.2 g) dicyclohexylcarbodiimide (DCC) in 10 mL CH_2Cl_2 was added dropwise. After stirring for 24 h the precipitate a dicyclohexyl urea was removed by filtration. The solution was evaporated to dryness, and the residue was chromatographed on silica gel (petroleum ether–ethyl acetate (1:1)).

*5.1.14.1. Methyl-4-{[2-(1H-3-indolyl)acetyl]amino}-1-methyl-1H-2-pyrrole carboxylate (**15**).* Brown needles (896 mg, 2.88 mmol, 72%), m.p.: 155 °C; IR (KBr) (cm^{-1}): 3400, 3330, 3270, 3110, 3040, 2900, 2870, 1700, 1650, 1575, 1480, 1450, 1400, 1350, 1315, 1260, 1215, 1195, 1140, 1100, 1060, 1015, 750; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 3.67 (s, 2H, CH_2), 3.75 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 6.72 (d, 1H, $^4J = 1.88$ Hz, pyrrole-H3), 6.9 (pt, 1H, $^3J = 7$ Hz, indole-H5 or 6), 7.0 (pt, 1H, $^3J = 6.9$ Hz, indole-H5 or 6), 7.2 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H5), 7.3 (d, 1H, $^3J = 7.9$ Hz, indole-H7), 7.34 (s, 1H, indole-H2), 7.5 (d, 1H, $^3J = 7.9$ Hz, indole-H4), 10.0 (s, 1H, NH), 10.8 (s, 1H, indole-NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 33.0 (s), 36.5 (p), 51.3 (p), 108.0 (t), 109.5 (q), 111.8 (t), 118.9 (t), 119.0 (t), 120.6 (t), 121.5 (t), 123.6 (q), 124.3 (t), 127.4 (q), 136.8 (q), 160.9 (q), 168.8 (q); MS m/z: 311 [M^+]; Anal. $C_{17}H_{17}N_3O_3$ (C, H, N).

*5.1.14.2. Methyl-4-{[2-(1H-1-indolyl)acetyl]amino}-1-methyl-1H-2-pyrrole carboxylate (**16**).* Orange amorphous solid (310 mg, 1 mmol, 25%), m.p. 160 °C; IR (KBr) (cm^{-1}): 3560, 3480, 3400, 3340, 3270, 3195, 3120, 3050, 2980, 2950, 1700, 1650, 1590, 1505, 1260, 1445, 1400, 1365, 1330, 1310, 1261, 1240, 1200, 1140, 1100, 1060, 1010, 960, 825, 790; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 3.70 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 4.9 (s, 2H, CH_2), 6.4 (d, 1H, $^4J = 2.7$ Hz, pyrrole-H3), 6.7 (d, 1H, $^3J = 7.7$ Hz, indole-H3), 7.0 (pt, 1H, $^3J = 7.3$ Hz, indole-H5 or 6), 7.1 (pt, 1H, $^3J = 7.8$ Hz, indole-H5 or 6), 7.35 (d, 1H, $^4J = 3.0$ Hz, pyrrole-H5), 7.38 (d, 1H, $^3J = 7.2$, indole-H2), 7.5 (d, 1H, $^3J = 7.4$ Hz, indole-H7), 7.54 (d, 1H, $^3J = 7.7$ Hz, indole-H7), 10.3 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 36.0 (p), 49.3 (s), 51.1 (p), 105.3 (t), 113.0 (t), 115 (t) 121.6 (q), 122.1 (t), 124.2 (t) 125.1 (t), 125.3 (t), 127.0 (q), 133.1 (q), 135.8 (t) 141.1 (q), 166.3 (q), 171.0 (q); MS m/z: 311 [M^+]; Anal. $C_{17}H_{17}N_3O_3$ (C, H, N).

*5.1.14.3. Methyl-4-{[2-(9H-9-carbazolyl)acetyl]amino}-1-methyl-1H-2-pyrrole carboxylate (**17**).* Brown amorphous solid (1.13 g, 3.62 mmol, 78%), m.p.: 195 °C; IR (KBr) (cm^{-1}): 3380, 3300, 3050, 3010, 2910, 2860, 2800, 1710, 1660, 1630, 1610, 1605, 1595, 1580, 1490, 1460, 1450, 1405, 1380, 1350, 1330, 1265, 1240, 1210, 1200, 1150, 1130, 1120, 1100, 1060, 750; ^{1}H NMR (75 MHz) DMSO-d^6): 3.70 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 6.7 (d, 1H, $^4J = 2.0$ Hz, pyrrole-H3), 7.20 (pt, 2H, $^3J = 7.8$ Hz, carbazole-H3,6), 7.29 (d, 1H, $^4J = 2.8$ Hz, pyrrole-H5), 7.42 (pt, 2H, $^3J = 7.5$ Hz, carbazole-H2,7), 7.5 (d, 2H, $^3J = 8.2$ Hz, carbazole-H1,8), 8.1 (d, 2H, $^3J = 7.7$ Hz, carbazole-H4,5), 10.4 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 36.5 (p), 44.5 (s), 52.8 (p), 108.0 (t), 109.8 (2t), 119.4 (t), 119.7 (2t), 120.5 (2t), 122.5 (4q), 126.1 (2t), 141.2 (q), 141.3 (q), 164.9 (q), 169.6 (q); MS m/z: 361 [M^+]; Anal. $C_{21}H_{19}N_3O_3$ (C, H, N).

*5.1.14.4. N2-(2-Cyanoethyl)-4-{[2-(1H-3-indolyl)acetyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**18**).* Orange amorphous solid (586 mg, 1.68 mmol, 42%), m.p.: 126 °C; IR (KBr) (cm^{-1}): 3400, 3330, 3270, 3100, 3040, 2920, 2850, 2790, 2400, 2220, 1720, 1650, 1620, 1570, 1520, 1430, 1400, 1310, 1290, 1270, 1240, 1215, 1150, 1085, 1060, 1005, 890, 800, 780, 740; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 2.7 (t, 2H, $^3J = 6.4$ Hz, CH_2), 3.39 (q, 2H, $^3J = 6.4$ Hz, CH_2), 3.67 (s, 2H, CH_2), 3.75 (s, 3H, CH_3), 6.7 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H3), 6.96 (pt, 1H, $^3J = 7.1$ Hz, indole-H5 or 6), 7.04 (pt, 1H, $^3J = 6.9$ Hz, indole-H5 or 6), 7.1 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H5), 7.2 (s, 1H, indole-H2), 7.32 (d, 1H, $^3J = 7.8$ Hz, indole-H7), 7.5 (d, 1H, $^3J = 7.7$ Hz, indole-H4), 9.9 (s, 1H, NH), 10.8 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 32.0 (s), 32.4 (s), 35.2 (s), 37.4 (p), 107.9 (t), 113.2 (q), 116.1 (t), 123.1 (t), 123.8 (t), 124.0 (q), 126.3 (2t), 127.0 (q), 128.9 (t), 132.5 (q), 140.6 (q), 165.1 (q), 168.1 (q); MS m/z: 349 [M^+]; Anal. $C_{19}H_{19}N_5O_2$ (C, H, N).

*5.1.14.5. N2-(2-Cyanoethyl)-4-{[2-(1H-1-indolyl)acetyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**19**).* Brown amorphous solid (628 mg, 1.8 mmol, 45%), m.p.: 109 °C; IR (KBr) (cm^{-1}): 3600, 2550, 3450, 3380, 3320, 3010, 2920, 2850, 2770, 2680, 2300, 2220, 1960, 1900, 1720, 1650, 1620, 1570, 1510, 1440, 1400, 1360, 1310, 1270, 1240, 1200, 1150, 1085, 1010, 960, 890, 850, 805, 780, 760, 750, 710; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 2.68 (t, 2H, $^3J = 6.4$ Hz, CH_2), 3.37 (q, 2H, $^3J = 6.4$ Hz, CH_2), 3.75 (s, 3H, CH_3), 4.9 (s, 2H, CH_2), 6.42 (d, 1H, $^4J = 3.1$ Hz, pyrrole-H3), 6.76 (d, 1H, $^4J = 1.6$ Hz, indole-H3), 7.0 (pt, 1H, $^3J = 7.5$ Hz, indole-H5 or 6), 7.1 (pt, 1H, $^3J = 6.9$ Hz,indole-H5 or 6), 7.34 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H5), 7.36 (d, 1H, $^3J = 8.1$ Hz, indole-H2), 7.39 (d, 1H, $^3J = 7.6$ Hz, indole-H7) 7.54 (d, 1H, $^3J = 7.9$ Hz, indole-H4), 8.34 (s, 1H, NH), 10.25 (s, 1H, NH); ^{13}C NMR (75 MHz) (cm^{-1}),: 32.0 (s), 32.8 (s), 35.1 (s), 44.1 (s), 107.9 (t),

113.2 (q), 116.1 (t), 123.1 (t), 123.8 (t), 124.0 (q), 126.3 (2t), 127.0 (q), 128.9 (t), 132.5 (q), 140.6 (q), 165.1 (q), 168.1 (q); MS *m*/*z*: 349 [M$^+$]; Anal. $C_{19}H_{19}N_5O_2$ (C, H, N).

*5.1.14.6. Methyl-4-{[4-{[2-(1H-3-indolyl)acetyl]amino}-1- methyl-1H-2-pyrrolyl)carbonyl}-1-methyl-1H-2-pyrrole carboxylate (**20**).* Yellow needles (675 mg, 1.56 mmol, 39%), m.p.: 173 °C; IR (KBr) (cm^{-1}): 3600, 3450, 3380, 3320, 3240, 3160, 3080, 3000, 2930, 2850, 2780, 2300, 2100, 2000, 1700, 1650, 1620, 1570, 1530, 1435, 1400, 1350, 1310, 1250, 1200, 1150, 1105, 1080, 1060, 1005, 960, 890, 820, 795, 755, 745, 660; ^{1}H NMR (300 MHz) (cm^{-1}) δ: 3.63 (s, 2H, CH_2), 3.71 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 5.5 (d, 1H, $^3J = 8.0$ Hz, indole-H2), 6.8 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H3), 6.9 (pt, 1H, $^3J = 7.9$ Hz, indole-H5 or 6), 7.0 (pt, 1H, $^3J = 7.9$ Hz, indole-H5 or 6), 7.1 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H5), 7.2 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3′), 7.32 (d, 1H, $^3J = 8.0$ Hz, indole-H4), 7.4 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5′), 7.5 (d, 1H, $^3J =$ 7.9Hz, indole-H7), 9.9 (s, 1H, NH), 10.8 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 33.6 (s), 36.4 (p), 47.8 (p), 51.2 (p), 104.4 (t), 108.6 (t) 109.2 (q), 111.6 ((t), 115.8 (t), 118.5 ((t), 118.7 (t), 121.2 (t), 121.3 (t), 122.4 (q), 122.8 (q), 124.0 (t), 127.5 (q), 136.4 (q), 158.7 (q), 161.1 (q), 168.3 (q); MS *m*/*z*: 433 [M$^+$]; Anal. $C_{23}H_{23}N_5O_4$ (C, H, N).

*5.1.14.7. Methyl-4-{[4-{[2-(1H-1-indolyl)acetyl]amino}-1-methyl-1H-2-pyrrolyl)carbonyl}-1-methyl-1H-2-pyrrole carboxylate (**21**).* Brown amorphous solid (658 mg, 1.52 mmol, 38%), m.p.: 152 °C; IR (KBr) (cm^{-1}): 3550, 3480, 3400, 3330, 2970, 1740, 1625, 1590, 1425, 1310, 1215, 1190, 1120, 1090, 755, 725, 600; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 3.7 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.9 (s, 2H s, CH_2), 6.4 (d, 1H, $^3J = 7.8$ Hz, indole-H3), 6.8 (d, 1H, $^4J = 1.7$Hz, pyrrole-H3), 7.0 (pt, 1H, $^3J = 7.9$ Hz, indole-H5 or 6), 7.1 (pt, 1H, $^3J = 7.9$ Hz, indole-H5 or 6), 7.15 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5), 7.30 (d, 1H, $^3J = 7.8$ Hz, indole-H2), 7.32 (d, 1H, $^3J = 7.9$, indole-H7), 7.38 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H3′), 7.4 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H5′), 7.45 (d, 1H, $^3J = 7.8$ Hz, indole-H4), 9.9 (s, 1H, NH), 10.35 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 36.8 (p), 46.9 (p), 51.8 (p), 58.9 (s),104.4 (t), 108.9 (t) 109.3 (q), 111.6 (t), 115.8 (t), 118.5 (t), 118.7 (t), 120.6 (t), 121.1 (t), 122.9(t), 123.3 (q), 124.0 (t), 127.9(q), 135.9 (q), 158.6 (q), 161.2 (q), 168.7 (q); MS *m*/*z*: 433 [M$^+$]; Anal. $C_{23}H_{23}N_5O_4$ (C, H, N).

*5.1.14.8. Methyl-4-{[3-(1H-3indolyl)propanoyl]amino}-1-methyl-1H-2-pyrrole carboxylate (**22**).* Brown needles (546 mg, 1.68 mmol, 42%), m.p.: 116 °C; IR (KBr) (cm^{-1}): 3350, 3260, 3190, 3100, 3040, 2980, 2800, 1640, 1570, 1550, 1450, 1400, 1350, 1260, 1190, 1130, 1100, 1020, 850, 780, 720, 650; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 2.7 (t, 2H, $^3J = 7.4$ Hz), 2.9 (t, 2H, $^3J = 7.5$ Hz, CH_2), 3.8 (s, 3H, CH_3), 3.9 (s, 3H, CH_3), 6.8 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H3), 6.9 (pt, 1H, $^3J = 7.8$ Hz, indole-H5 or 6), 7.05 (pt, 1H, $^3J = 7.9$ Hz, indole-H5 or 6), 7.15 (s, 1H, indole-H2), 7.25 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H5), 7.3 (d, 1H, $^3J = 7.9$ Hz, indole-H7), 7.5 (d, 1H, $^3J = 7.8$ Hz, indole-H4), 10.3 (s, 1H, NH), 10.8 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 25.8 (s), 33.2 (s), 36.5 (p), 51.3 (p), 108.0 (t), 109.4 (q), 111.6 (t), 115.1 (q), 118.9 (t), 119.1 (t), 120.5 (t), 121.5 (t), 123.8 (q), 124.5 (t), 127.5 (q), 136.9 (q), 160.6 (q), 171.4 (q); MS *m*/*z*: 325 [M$^+$]; Anal.: $C_{18}H_{19}N_3O_3$ (C, H, N).

*5.1.14.9. Methyl 4-[2-(4-{[3-(1H-indolyl)propynolyl]-amino}-1-methyl-1H-2-pyrrolyl)-2-oxoethyl]-1-methyl-1H-2-pyrrole carboxylate (**23**).* Orange amorphous solid (805 mg, 1.8 mmol, 45%), m.p.: 163 °C; IR (KBr) (cm^{-1}): 3350, 3260, 1700, 1640, 1600, 1550, 1440, 1400, 1350, 1250, 1200, 1100, 750; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 2.7 (t, 2H, $3J = 7.4$ Hz, CH_2), 2.9 (t, 2H, $^3J = 7.5$ Hz, CH_2), 3.7 (s, 3H, CH_3), 3.8 (s, 3H, CH_3), 3.9 (s, 3H, CH_3), 6.8 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H3), 6.9 (pt, 1H, $^3J = 7.8$ Hz, indole-H5 or 6), 7.05 (pt, 1H, $^3J = 7.9$ Hz, indole-H5 or 6), 7.15 (s, 1H, indole-H2), 7.3 (d, 1H, $^3J = 7.9$ Hz, indole-H7), 7.45 (d, 1H, $^4J =$ pyrrole-H5), 7.5 (d, 1H, $^3J = 7.8$ Hz, indole-H4), 7.55 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H3′), 8.1 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H5′), 9.8 (s, 1H, NH), 10.3 (s, 1H, NH), 10.9 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 25.4 (s), 33.0 (s), 36.1 (p), 47.1 (p), 51.0 (p), 104.6 (t), 108.1 (t), 108.3 (q), 111.2 (q), 111.6 (t), 113.9 (q), 115.8 (t), 118.0 (q), 118.7 (t), 119.1 (t), 120.4 (t), 121.6 (t), 124.6 (t), 125.6 (q), 127.4 (q), 135.3 (q), 158.5 (q), 165.4 (q), 170.1 (q); MS *m*/*z*: 447 [M$^+$]; Anal.: $C_{24}H_{25}N_5O_4$ (C, H, N).

*5.1.14.10. Methyl4-{[4-(9H-9-carbazolyl)butanolyl]amino}-1-methyl-1H-2-pyrrole carboxylate (**24**).* Yellow amorphous solid (700 mg, 1.8 mmol, 45%), m.p.: 240 °C; IR (KBr) (cm^{-1}): 3320, 2930, 2850, 1700, 1650, 1620, 1570, 1480, 1450, 1400, 1350, 1260, 1190, 1140, 1100, 780, 750; ^{1}H NMR (300 MHz) (cm^{-1}) δ: 2.0 (t, 2H, $^3J = 6.5$ Hz), 2.3 (q, 2H, $^3J = 6.9$ Hz, CH_2), 3.7 (s, 3H, CH_3), 3.8 (s, 3H, CH_3), 4.4 (t, 2H, $^3J = 6.7$ Hz, CH_2), 6.6 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 7.2 (pt, 2H, $^3J = 7.3$ Hz, carbazole-H2,7), 7.3 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5), 7.4 (pt, 2H, $^3J = 7.5$ Hz, carbazole-H3,6), 7.6 (d, 2H, $^3J = 8.1$ Hz, carbazole-H1,8), 8.1 (d, 2H, $^3J = 7.6$ Hz, carbazole-H4,5), 9.8 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 24.6 (s), 32.6 (s), 36.4 (p), 42.0 (s), 51.2 (p), 108.0 (t), 109.4, (t), 109.5 (t), 118.9 (q), 118.9 (t), 119.0 (t), 120.5 (t), 120.7 (t), 122.3 (q), 122.4 (q) 123.0 (q), 125.8 (t), 125.9 (t), 126.0 (t), 140.2 (q), 140.3 (q), 161.0 (q), 169.1 (q); MS *m*/*z*: 389 [M$^+$]; Anal. $C_{23}H_{23}N_3O_3$ (C, H, N).

*5.1.14.11. Methyl-4-{[-(9H-carbazolyl)butanoyl]amino}-1-methyl-1H-2-pyrrolyl)-2-oxoethyl]-1-methyl-1H-2-pyrrole carboxylate (**25**).* Yellow amorphous solid (531 mg, 1.04 mmol, 26%), m.p.: 104 °C; IR (KBr) (cm^{-1}): 3410, 3350, 1640, 1560, 1300, 1215, 820; ^{1}H NMR (300 MHz) (DMSO-d^6) δ: 1.9 (t, 2H, $^3J = 6.4$ Hz, CH_2), 2.3 (q, 2H, $^3J = 6.9$ Hz, CH_2), 3.7 (s, 3H, CH_3), 3.8 (s, 3H, CH_3), 3.9 (s, 3H, CH_3), 4.4 (t, 2H, $^3J = 6.7$ Hz, CH_2), 6.8 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 7.2 (pt, 2H, $^3J =$ 7.3 Hz, carbazole-H2,7), 7.35 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5), 7.4 (pt, 2H, $^3J = 7.5$ Hz, carbazole-H3,6), 7.45 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H3′), 7.6 (d, 2H, $^3J = 8.1$ Hz, carbazole-H1,8), 8.0 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5′), 8.1 (d, 2H, $^3J = 7.6$ Hz, carbazole-H4,5), 9.8 (s, 1H, NH), 10.4 (s, 1H, NH), 10.8 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 24.3 (s), 33.0 (s), 36.5 (p), 39.6 (p), 42.0 (s), 51.5 (p), 104.7 (t), 108.0 (t), 109.1 (t), 109.6 (t), 111.2 (q), 113.9 (q), 115.6 (t), 118.8 (q), 118.9 (t), 119.0 (t), 120.3 (t), 120.6 (t), 122.6 (q), 122.7 (q), 123.0 (q), 126.0 (t), 126.1 (t), 126.2 (t), 140.7 (q), 140.8 (q), 158.9 (q), 161.0 (q), 169.0 (q); MS m/z: 511 [M^+]; Anal. $C_{29}H_{29}N_5O_4$ (C, H, N).

*5.1.14.12. N2-[3-(Dimethylamino)propyl]-4-{[4-(9H-9-carbazolyl)butanolyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**26**).* Brown amorphous solid (312 mg, 0.68 mmol, 17%), m.p.: 118 °C; IR (KBr) (cm^{-1}): 3320, 2920, 2845, 1700, 1650, 1550, 1450, 1350, 1260, 780, 750; ^{1}H NMR (300 MHz) (DMSO-d^6) δ:1.6 (m, 2H, $^3J = 6.5$ Hz), 1.9 (m, 2H, $^3J = 6.5$ Hz, CH_2), 2.0 (s, 6H, $2CH_3$), 2.2 (t, 2H, $^3J = 6.8$ Hz, CH_2), 2.3 (t, 2H, $^3J =$ 7.6 Hz, CH_2), 3.1 (q, 2H, $^3J = 6.7$ Hz, CH_2), 3.8 (s, 3H, CH_3), 4.4 (t, 2H, $^3J = 6.8$ Hz, CH_2), 7.2 (pt, 2H, $^3J = 7.3$ Hz, carbazole-H2,7), 7.3 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H3), 7.4 (pt, 2H, $^3J = 7.5$ Hz, carbazole-H3,6), 7.6 (d, 2H, $^3J = 8.1$ Hz, carbazole-H1,8), 8.05 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H5), 8.1 (d, 2H, $^3J = 7.6$ Hz, carbazole-H4,5), 8.4 (t, 1H, $^3J = 7.4$ Hz, NH), 9.9 (s, 1H, NH); ^{13}C NMR (75 MHz) (DMSO-d^6) δ: 24.5 (s), 28.0 (s), 32.6 (s), 36.4 (p), 38.1 (s), 42.0 (s), 45.0 (p), 45.1 (p), 59.2 (s), 108.0 (t), 109.3 (t), 109.4 (t), 118.0 (q), 119.0 (t), 119.1 (t), 120.1 (t), 120.6 (t), 122.3 (q), 122.6 (q), 123.0 (q), 125.8 (t), 126.0 (t), 126.1 (t), 140.0 (q), 140.3 (q), 160.3 (q), 171.0 (q); MS m/z 459 [M^+]; Anal. $C_{27}H_{33}N_5O_2$ (C, H, N).

5.2. DNA-binding methods

5.2.1. Melting experiments

Melting curves were measured using an Hitachi U-3200-spectrophotometer coupled to a Julabo thermostat. The measurements were performed in BPE buffer, pH 7 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) with a drug–DNA ratio of 1:1, using [Poly(-dAdT). Poly(dAdT)] as DNA (Amersham Pharmacia). The temperature inside the cuvette was increased over the range 25–90 °C with a heating rate of 1.5 °C min^{-1}. The absorption data were registered and plotted against the temperature. The melting curve was analysed using the program ORIGIN for data analysis and technical graphics. The melting temperature (T_m) was taken as the midpoint of the hyperchromic transition [25].

5.2.2. Ethidium bromide displacement assay

All fluorescence measurements were conducted on an Hitachi F-2000 spectrofluorometer. Calf thymus DNA (Sigma, Type I; 1.0×10^{-5} M in base pairs) was added in small aliquots to ethidium bromide (Invitrogen; 5.0×10^{-6} M) resulting in a 2:1 ratio of basepair–ethidium in 2 mL of a 10 mM Tris–HCl (pH 7.4), 75 mM NaCl buffer solution. The fluorescence of the DNA–ethidium buffer solution was calibrated at r.t. to 100% fluorescence and that of the ethidium buffer solution to 0% fluorescence, respectively. The premixed DNA–ethidium solution was titrated with 3 μL aliquots of the stock solution of the test substances (3 mM drug in DMSO) and stirred at r.t. for 30 min prior to each fluorescence measurement. The fluorescence was measured with $\lambda = 545$ nm excitation and 595 nm emission with a slit width of 10 nm. The binding affinity was determined at 50% ethidium bromide displacement, measured as a drop in fluorescence to 50%. Results were calculated with the Data Analysis and Graphics Program GRAFIT and with the graphical program ORIGIN. Distamycin A as reference was tested in the same way [28].

5.3. Topoisomerase I Inhibition

Plasmid DNA (pUC 19, Invitrogen; 0.033 μg μL^{-1}) was incubated for 15 min at 35 °C with different concentrations of the tested drugs (5, 20 and 50 μM) in 1 × topoisomerase I reaction buffer (50 mM Tris–HCl pH 7.5, 50 mM KCl, 10 mM MgCl, 0.5 mM DTT, 0.1 mM EDTA, 30 μg mL^{-1} BSA) to ensure equilibration. The reaction was initiated by adding topoisomerase I (Topogen; 0.25 U μL) and the samples were reincubated for 15 min at 35 °C. Reactions were stopped by addition of SDS to a final concentration of 0.25% and proteinase K to 250 μg mL^{-1}, followed by incubation for 30 min at 50 °C. After addition of 2 μL denaturing loading buffer (Invitrogen) samples were loaded onto a 1% agarose gel in TBE Buffer. Electrophoresis was conducted at 120 V for 2 h.

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