β-Carbolinedione Derivatives as Topoisomerase I Inhibitors☆

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Key Words: *Topoisomerase I inhibition; β-carbolinedione derivatives; microwave irradiation*

Summary

Pyrrolo[3,4-*c*]-β-carbolinedione dimers **5–14** were synthesized from furo[3,4-*c*]-β-carbolinediones and diamines by solvent-free $TaCl_5$/silica catalyzed reaction under microwave irradiation. The inhibitory property of these target compounds, the starting materials **2**, **31**, **32**, and the *N*-alkylated pyrrolo[3,4-*c*]-β-carbolinediones **16**, **17**, **20–30** was tested against the relaxation of supercoiled pRB322 DNA by calf thymus topoisomerases I and II. Some of these compounds, especially **7** and **23** proved to be selective inhibitors of topoisomerase I.

Introduction

Several types of substances showing polycyclic aza-aromatic structures as well as aminoalkyl moieties display antitumor activity [1–4]. Referred to their heterocyclic increments, these substances include isoquinolines, carbazoles, and carbolines (cf. A–D). Another classification could be made concerning the type of bonding of the aminoalkyl substituent either at the indole or at the imide *N*. Finally, bifunctional aminoalkyl moieties lead to compounds with dimeric structures (cf. B and D).

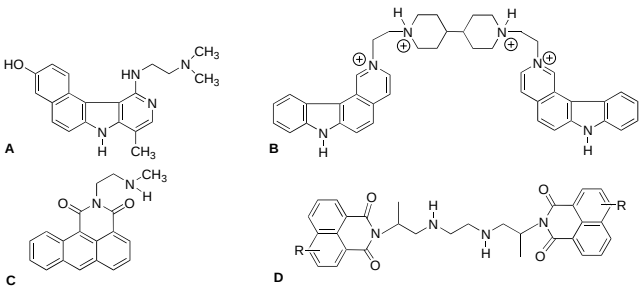


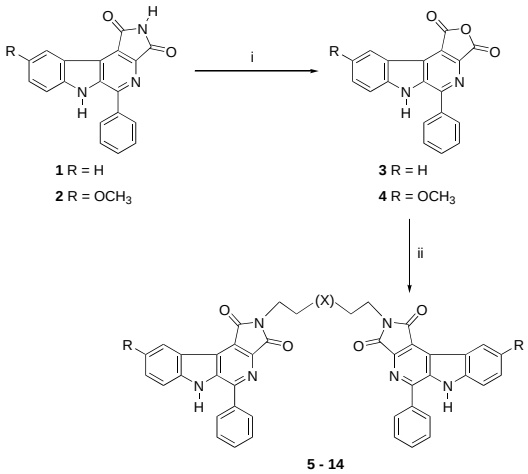
Figure 1. Antitumoral aminoalkyl heteroaromatics.

It was therefore our intention to synthesize derivates of structures including both an indolic and an imidic function. We used the pyrrolo[3,4-*c*]-β-carbolinediones **1**, **2** [5] as starting materials.

Topoisomerase inhibition has been recognized as a specific target for potential antitumor agents. DNA topoisomerases represent a class of enzymes capable of changing the topology and conformation of nuclear DNA, being able to cleave and rejoin one strand (topoisomerase I) of the phosphodiester backbone or both (topoisomerase II). One important feature common to several topoisomerase inhibitors seems to be their DNA-intercalating property[6–9].

Chemistry

The substituted β-carboline dimers **5–14** were synthesized by refluxing the corresponding β-carbolinedicarboxylic acid anhydrides **3**, **4**, prepared from the imides **1**, **2** analogously to the procedure of Teressa et. al.[1], and diamines in toluene (Scheme 1). This method, however, has some disadvantages. Since the anhydrides are not readily soluble in toluene we need relatively large amounts of solvent. The reaction is very slow and was not completed even after refluxing for two weeks. The yields could be slightly raised by an excess of anhydride. As an alternative to this method, we have found microwave irradiation to be useful. Thus, the β-carboline dimers were synthesized by irradiating the corresponding diamines and β-carboline anhydrides, adsorbed on activated silica gel in the presence of $TaCl_5$ in a microwave oven according to the method of Chandrasekar elaborated for *N*-alkyl imides[10]. This method affords improved yields; the reaction is finished within 45 min and can easily be followed due to the yellow colour of the dimers contrasting to the colourless starting materials.



Scheme 1. (X) is defined in Table 1 – Reagents: i) KOH in EtOH/H_2O (1:1); ii) method a: toluene, $H_2NCH_2CH_2(X)CH_2CH_2NH_2$, reflux, 14 days, or method b: $TaCl_5$-SiO_2, microwave, 450 W, 45 min.

Alkylation of the pyrrolo[3,4-*c*]-β-carbolinediones **1** and **2** should provide access to another class of products. At first, we tried to make use of the expectedly different reactivity of the indole and imide positions, caused by their unequal NH

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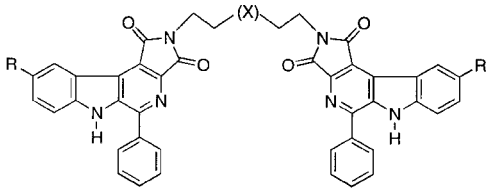
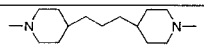
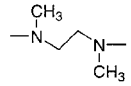
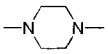
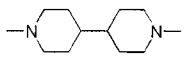
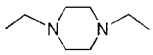
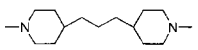
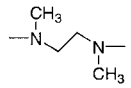
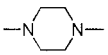
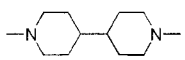
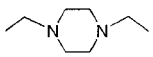
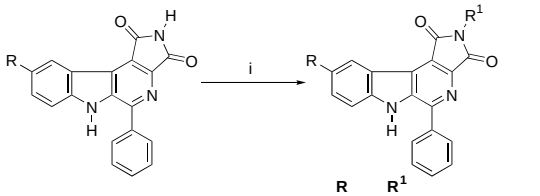
Table 1



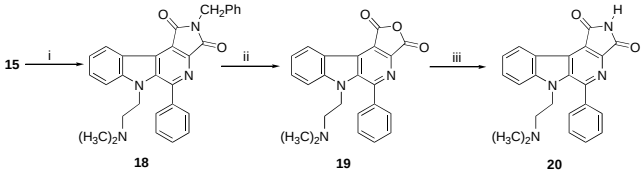
Compound	X	R	method a (14 days) % yield	method b (45 min) %yield
5		H	9	27
6		H	9	23
7		H	11	26
8		H	7	31
9		H	11	22
10		OCH_3	7	24
11		OCH_3	11	62
12		OCH_3	9	29
13		OCH_3	9	30
14		OCH_3	8	30



Scheme 2. Reagents: i) NaH, DMF, 0 °C, R^1Cl.

acidity. If NaH is used in a molar ratio of 1.1:1 to deprotonate **1** or **2**, respectively, in DMF at 0 °C, imide-substituted products **15**, **16**, and **17** are obtained with benzyl chloride, 2-chloroethyl-dimethylamine, and 3-chloropropyl-dimethylamine (the latter as their free bases) (Scheme 2). These results show the higher acidity of the imide NH as compared to that of the indole NH.

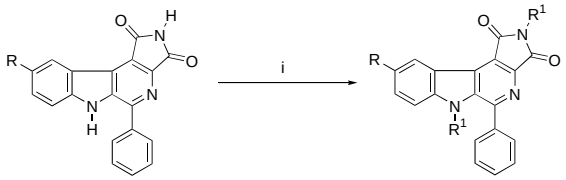
K_2CO_3 in refluxing acetone in the presence of 2-iodoethyl-dimethylamine hydroiodide produced **18**. Compounds of this type open a synthetic route to structures substituted at the



Scheme 3. Reagents: i) K_2CO_3, acetone, $(CH_3)_2NCH_2CH_2I \bullet HI$, reflux; ii) KOH in EtOH/H_2O (5:1); iii) NH_4OAc, melt.

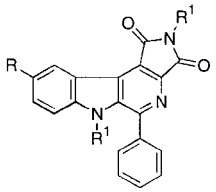
indole-*N*, while having a free imide NH. **18** could be converted, with loss of benzylamine, to the anhydride **19** with KOH in ethanol/H_2O, which in turn was melted with NH_4OAc without any further purification to yield the desired imide **20** (Scheme 3).

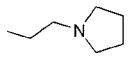
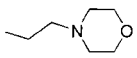
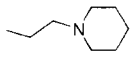
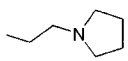
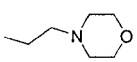
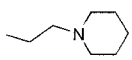
The reactions of **1** and **2** with the appropriate ω-chloroalkyl-dimethylamine hydrochlorides and K_2CO_3 in refluxing acetone lead to the bisaminoalkylated products **21–30** in good yields (Scheme 4).



Scheme 4. R and R^1 are defined in Table 2. Reagents: i) K_2CO_3, acetone, R^1-X, reflux.

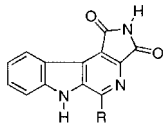
Table 2



Compound	R	R^1	% Yield
21	H	$CH_2CH_2N(CH_3)_2$	34
22	H	$CH_2CH_2CH_2N(CH_3)_2$	28
23	H		21
24	H		93
25	H		53
26	OCH_3	$CH_2CH_2N(CH_3)_2$	71
27	OCH_3	$CH_2CH_2CH_2N(CH_3)_2$	81
28	OCH_3		80
29	OCH_3		91
30	OCH_3		93

Topoisomerase Inhibition

Both the dimeric (**5–14**) as well as the bisaminoalkylated (**21–30**) and the monoaminoalkylated (**16**, **17**, **20**) β-carbolines were tested for their topoisomerase inhibition. As further samples, we included the monomeric β-carbolines **2**, **31**, and **32** bearing free NH groups[5].



31 R = 4-Me$_2$N-Ph

32 R = 3-OH-4-OMe-Ph

Table 3 shows the results of inhibition assays with calf thymus topoisomerases I and II[11] at supercoiled pBR 322 DNA. Also included are literature data for camptothecin, a topoisomerase I inhibitor[12] and etoposide[13], a topoisomerase II inhibitor.

Table 3

Compound	Concentration (μg/ml)	Inhibition (%) of topoisomerase	
		I	**II**
7	100	90	0
	50	60	0
22	>50	90	0
23	100	10	0
27	100	60	10
17	100	70	0
2	100	95	95
31	100	40	30
32	100	90	50
Camptothecin	50	100	0
	25	50	0
Etoposide	30	0	100
	6	0	50

Other tested samples did not show activity in both tests. These data indicate that aminoalkylation is able to enhance the specificity for inhibition of topoisomerase I (cpds. **7**, **22**, **27**, and **17**) in contrast to *N*-unsubstituted analogues (cpds. **2**, **31**, **32**), which inhibit both topoisomerases.

Experimental

Chemistry

Melting points were determined on a Reichert Thermovar 300419 hot-stage microscope and are uncorrected. Fourier-transform IR spectra (KBr) were recorded on a Nicolet 510 FTIR spectrometer. ^{1}H-NMR spectra were recorded on a Bruker AC250 (250 MHz) or Bruker ARX400 (400 MHz) spectrometer, all δ values (internal standard: TMS) are in the ppm scale. Mass spectra were obtained with Varian MAT 311A (EI) and Varian MAT 95 (FD) spectrometers. Elemental analyses were carried out at the Microanalytical Laboratory of the University of Regensburg. All reactions were performed in flame- or oven-dried vessels under nitrogen which had been dried over self-indicating silica gel, concentrated H_2SO_4 and KOH. The microwave oven was from Phillips, NN 5556/5506 and had 900 W. Thin layer chromatography was carried out on Al-sheets coated with 60F$_{245}$ silica. Column chromatography was carried out using Merck 60 (70–230 mesh ASTM) silica gel.

*1,3-Dihydro-5-phenyl-6H-furo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**3**)*

1 (5.00 g, 15.96 mmol) was refluxed in a solution of KOH (150 g, 2.67 mol) in of 50% aqueous ethanol (600 ml) for 1 h. The mixture was cooled in an ice bath and acidified with concd. HCl. The precipitate occurring on addition of of H_2O (3 l) was filtered, washed with ice water, and dried under oil pump vacuum to yield 2.44 g (7.76 mmol, 49%) of pale yellow crystals, mp 312 °C (ethanol).– Anal. ($C_{19}H_{10}N_2O_3$).– IR (KBr): ν = 3030–2875 (CH) cm^{-1}; 1770, 1710 (C=O); 1625, 1600, 1570, 1485 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 7.35–8.18 (m, 9H, aromatic H), 12.04 (s; 1H, NH indole, D$_2$O exchange); MS (EI, 70 eV): *m/z* (%) = 314 (100) [M$^{+\bullet}$].

*1,3-Dihydro-9-methoxy-5-phenyl-6H-furo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**4**)*

4 was prepared as described for **3**. Yield 2.30 g (6.69 mmol, 42%) pale brown solid (no purification possible), mp 295 °C.– IR (KBr): ν = 3380 (NH) cm^{-1}; 3065–2910 (CH); 1775, 1720 (C=O); 1620, 1575, 1495 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 3.82 (s, 3H, OCH$_3$), 7.26–8.64 (m, 8H, aromatic H), 11.91 (s, 1H, NH indole, D$_2$O exchange).– MS (EI, 70 eV): *m/z* (%) = 344 (45) [M$^{+\bullet}$].

*1,3-Bis{N-[2-(5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)ethyl]-1-piperidine-4-yl}propane (**5**)*

Method a) A mixture of the corresponding amines (0.31 mmol) and β-carboline anhydrides **3** or **4** (0.62 mmol) in dry toluene (250 ml) was refluxed under N$_2$ for 14 d. Toluene was removed *in vacuo* and the residue was purified by column chromatography on silica gel eluting with ethyl acetate/dichloromethane/methanol 4:4:1.

Method b) The corresponding amine (0.1 mmol) and anhydride **3** or **4** (0.2 mmol) were dissolved in dry methanol/chloroform (1:1; 5 ml) under N$_2$. Then activated (dried overnight above 100 °C) silica gel (1 g) was added to this solution, the suspension was evaporated carefully *in vacuo* and dried (0.1 Torr) at room temp. for 30 min. After shaking vigorously for 1 h, TaCl$_5$–silica gel[10] (75 mg) was admixed thoroughly. This mixture was irradiated in a microwave oven at 450 W for 45 min, cooled, transferred onto a silica gel column and eluted by ethyl acetate/dichloromethane/methanol 4:4:1.

Yield a) 26 mg (0.029 mmol, 9%), b) 24 mg (0.027 mmol, 27%) yellow crystals, mp 257 °C (ethanol).– Anal. ($C_{55}H_{52}N_8O_4$).– IR (KBr): ν = 3300 (NH) cm^{-1}; 3050–2925 (CH); 1755, 1715 (C=O); 1640, 1595, 1480, 1450 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.28–1.67 (m, 32H), 7.39–8.80 (m, 18H, aromatic H), 12.27 (s, 2H, 2 × NH indole, D$_2$O exchange).– MS (FD): *m/z* (%) = 889 [M$^{+\bullet}$].

Compounds **6**–**14** were prepared analogously.

*N,N′-Bis[2-(5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)-ethyl]-N,N′-dimethyl-1,2-diaminoethane (**6**)*

Yield a) 22 mg (0.028 mmol, 9%), b) 18 mg (0.023 mmol, 23%) yellow crystals, mp 168 °C (ethanol).– Anal. ($C_{46}H_{38}N_8O_4$).– IR (KBr): ν = 3450 (NH), 3050–2925 (CH) cm^{-1}; 1765, 1710 (C=O); 1630, 1580, 1495, 1460 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 2.20 (s, 6H, 2 × CH$_3$), 2.55 (t, *J* = 6.3 Hz, 4H, 2 × CH$_2$), 3.35–3.51 (m, 4H, 2 × CH$_2$), 3.77 (t, *J* = 6.3 Hz, 4H, 2 × CH$_2$), 7.21–8.64 (m, 18H, aromatic H), 12.11 (s, 2H, 2 × NH indole, D$_2$O exchange).– MS (FD): *m/z* (%) = 766 [M$^{+\bullet}$].

*1,4-Bis[2-(5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)-ethyl]piperazine (**7**)*

Yield a) 29 mg (0.037 mmol, 11%), b) 20 mg (0.026, 26%) yellow crystals, mp 160 °C (ethanol).– Anal. ($C_{46}H_{36}N_8O_4$).– IR (KBr): ν = 3270 (NH) cm^{-1}; 3050–2925 (CH); 1765, 1710 (C=O); 1630, 1580, 1495, 1460 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.31–1.64 (m, 16H), 7.23–8.81 (m, 18H, aromatic H), 11.68 (s, 2H, 2 × NH indole, D$_2$O exchange).– MS (FD): *m/z* (%) = 764 [M$^{+\bullet}$].

*1,1-Bis[2-(5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)-ethyl]-4,4′-bipiperidine (**8**)*

Yield a) 21 mg (0.024 mmol, 7%), b) 26 mg (0.031, 31%) yellow crystals, mp 288 °C (ethanol).– Anal. ($C_{52}H_{46}N_8O_4$).– IR (KBr): ν = 3460 (NH) cm^{-1}; 3050–2925 (CH); 1765, 1710 (C=O); 1630, 1580, 1495, 1460 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.33–1.62 (m, 26H), 7.23–8.81 (m, 18H, aromatic H), 12.35 (s, 2H, 2 × NH indole, D_2O exchange).– MS (FD): *m/z* (%) = 846 [$M^{+\bullet}$].

*1,4-Bis[3-(5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)-propyl]piperazine (**9**)*

Yield a) 29 mg (0.036 mmol, 11%), b) 17 mg (0.022, 22%) yellow crystals, mp 327 °C (ethanol). Anal. ($C_{48}H_{40}N_8O_4$).– IR (KBr): ν = 3350 (NH) cm^{-1}; 3130–2925 (CH); 1775, 1710 (C=O); 1625, 1590, 1500, 1460 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.28–1.67 (m, 20 H), 7.39–8.80 (m, 18H, aromatic H), 12.26 (s, 2H, 2 × NH indole, D_2O exchange).– MS (FD): *m/z* (%) = 792 [$M^{+\bullet}$].

*1,3-Bis{N-[2-(9-methoxy-5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)ethyl]piperidine-4-yl}propane (**10**)*

Yield a) 21 mg (0.021 mmol, 7%), b) 23 mg (0.024 mmol, 24%) yellow powder, mp > 300 °C (ethanol).– Anal. ($C_{57}H_{56}N_8O_6$).– IR (KBr): ν = 3395 (NH) cm^{-1}; 2925 (CH); 1765, 1720 (C=O).– ^{1}H-NMR/400 MHz ([D$_5$]pyridine): δ = 1.02–1.32 (m, 12H), 1.56 (d, *J* = 12 Hz, 4H, 2 × CH_2), 1.99 (t, *J* = 11 Hz, 4H, 2 × CH_2), 2.85 (t, *J* = 6.5 Hz, 4H, 2 × CH_2), 3.14 (d, *J* = 10 Hz, 4H, 2 × CH_2), 4.00 (s, 6H, 2 × OCH_3), 4.14 (t, *J* = 6.5 Hz, 4H, 2 × CH_2), 7.30–8.90 (m, 16H, aromatic H), 13.22 (s; 2H, 2 × NH indole, CD_3OD exchange).– MS (FD): *m/z* (%) = 948 [$M^{+\bullet}$].

*N,N′-Bis[2-(9-methoxy-5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)ethyl]-N,N′-dimethyl-1,2-diaminoethane (**11**)*

Yield a) 28 mg (0.034 mmol, 11%), b) 51 mg (0.062 mmol, 62%) yellow powder, mp > 300 °C (ethanol).– Anal. ($C_{48}H_{44}N_8O_6$).– IR (KBr): ν = 3320 (NH) cm^{-1}; 3060–2945 (CH); 1765, 1715 (C=O).– ^{1}H-NMR/400 MHz ([D$_5$]pyridine): δ = 2.35 (s, 6H, 2 × CH_3), 2.64 (s, 4H, 2 × CH_2), 2.84 (t, *J* = 6.7 Hz, 4H, 2 × CH_2), 3.95 (s, 6H, 2 × OCH_3), 4.03 (t, *J* = 6.7 Hz, 4H, 2 × CH_2), 7.33–8.78 (m, 16H, aromatic H) 13.17 (s, 2H, 2 × NH indole, CD_3OD exchange).– MS (FD): *m/z* (%) = 828 [$M^{+\bullet}$].

*1,4-Bis[2-(9-methoxy-5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)ethyl]piperazine (**12**)*

Yield a) 23 mg (0.028 mmol, 9%), b) 24 mg (0.029 mmol, 29%) yellow powder, mp > 300 °C (ethanol).– Anal. ($C_{48}H_{30}N_8O_6$).– IR (KBr): ν = 3370 (NH) cm^{-1}; 2980 (CH); 1770, 1720 (C=O).– ^{1}H-NMR/400 MHz ([D$_5$]pyridine): δ = 2.78 (t, *J* = 6.4 Hz, 4H, 2 × CH_2), 3.88 (t, *J* = 6.0 Hz, 4H, 2 × CH_2), 4.01 (s, 6H, 2 × OCH_3), 4.06–4.37 (m, 8H, 4 × CH_2), 7.34–9.05 (m, 16H, aromatic H), 13.23 (s, 2H, 2 × NH indole, CD_3OD exchange).– MS (FD): *m/z* (%) = 814 [$M^{+\bullet}$].

*1,1′-Bis[2-(9-methoxy-5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)ethyl]-4,4′-bipiperidine (**13**)*

Yield a) 25 mg (0.028 mmol, 9%), b) 27 mg (0.030 mmol, 30%) yellow powder, mp >300 °C (ethanol).– Anal. ($C_{54}H_{50}N_8O_6$).– IR (KBr): ν = 3670, 3555 (NH) cm^{-1}; 3055–2945 (CH); 1765, 1715 (C=O).– ^{1}H-NMR/400 MHz ([D$_5$]pyridine): δ = 1.06–1.49 (m, 6H), 1.49 (d, *J* = 13.0 Hz, 4H, 2 × CH_2), 1.90 (t, *J* = 12 Hz, 4H, 2 × CH_2), 2.81 (t, *J* = 6.5 Hz, 4H, 2 × CH_2), 3.12 (d, *J* = 11.0 Hz, 4H, 2 × CH_2), 4.00 (s, 6H, 2 × OCH_3), 4.11 (t, *J* = 6.5 Hz, 4H, 2 × CH_2), 7.37–8.95 (m, 16H, aromatic H), 13.23 (s, 2H, 2 × NH indole, CD_3OD exchange).– MS (FD): *m/z* (%) = 906 [$M^{+\bullet}$].

*1,4-Bis[3-(9-methoxy-5-phenylpyrrolo[3,4-c]pyrido[3,4-b]indole-1,3(2H)dion-2-yl)propyl]piperazine (**14**)*

Yield a) 21 mg (0.024 mmol, 8%) b) 26 mg (0.03 mmol, 30%) yellow powder, mp > 300 °C (ethanol).– Anal. ($C_{50}H_{54}N_8O_6$).– IR (KBr): ν = 3670, 3330 (NH) cm^{-1}; 2930 (CH); 1765, 1715 (C=O).– ^{1}H-NMR NMR/400 MHz ([D$_5$]pyridine): δ = 1.97 (t, *J* = 6.7 Hz, 4H, 2 × CH_2), 2.35–2.38 (m, 12H, 6 × CH_2), 4.02 (s, 6H, 2 × OCH_3), 4.06 (t, *J* = 7.0 Hz, 4H, 2 × CH_2), 7.36–8.87 (m, 16H, aromatic H), 13.27 (s, 2H, 2 × NH indole, CD_3OD exchange).– MS (FD): *m/z* (%) = 862 [$M^{+\bullet}$].

*2,3-Dihydro-2-benzyl-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**15**)*

A solution of 100 mg (0.32 mmol) **1** in 0.5 ml of dry DMF was cooled to 0 °C, 10.5 mg (0.35 mmol) NaH (80% in paraffin) were added portionwise, and the mixture was stirred for 1 h at 0 °C. Benzyl chloride (0.037 ml; 0.35 mmol) was added, and the reaction mixture was stirred at 0 °C overnight, poured onto 5 ml of satd. $NaHCO_3$ solution and extracted with dichloromethane (2 × 10 ml). The organic phase was washed with water (2 × 5 ml) and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* and purification of the residue by column chromatography on silica gel eluting with dichloromethane/ethyl acetate 10:1 yielded 75 mg (0.19 mmol, 58%) **15** as yellow crystals, mp 269 °C (ethanol).– Anal. ($C_{26}H_{17}N_3O_2$).– IR (KBr): ν = 3405 (NH) cm^{-1}; 3050–2980 (CH); 1765, 1705 (C=O); 1630, 1605, 1585, 1570 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 4.90 (s, 2H, CH_2), 7.30–7.55 (m, 6H, 5H benzyl + 1 aromatic H), 7.63–7.85 (m, 5H phenyl), 8.05 (m, 2H, aromatic H), 8.74 (d, *J* = 8.0 Hz, 1H, aromatic H), 12.25 (s, 1H, NH indole, D_2O exchange).– MS (EI, 70 eV): *m/z* (%) = 403 (82) [$M^{+\bullet}$], 375 (6) [M–CO]$^{+\bullet}$, 244 (100) [M–159]$^+$, 91 (21) [C_7H_7]$^+$.

16 and **17** were prepared analogously.

*2,3-Dihydro-2-[2-(N,N-dimethylamino)ethyl]-5-phenyl-1H,6H-pyrrolo-[3,4-c]pyrido[3,4-b]indole-1,3-dione (**16**)*

Yield 37 mg (0.10 mmol, 30%) yellow crystals, mp 233 °C (ethanol).– Anal. ($C_{23}H_{20}N_4O_2$).– IR (KBr): ν = 3450 (NH) cm^{-1}; 3050–2970 (CH); 1770, 1710 (C=O); 1585, 1500 (C=C).– ^{1}H-NMR/250 MHz ($CDCl_3$): δ = 2.32 (s, 6H, $N(CH_3)_2$), 2.70 (t, *J* = 6.4 Hz, 2H, CH_2), 4.25 (t, *J* = 6.4 Hz, 2H, CH_2), 7.36–8.85 (m, 9H, aromatic H), 9.36 (s, 1H, NH indole, CD_3OD exchange).– MS (EI, 70 eV): *m/z* (%) = 384 (5) [$M^{+\bullet}$], 58 (100) [$(CH_3)_2N{=}CH_2$]$^+$.

*2,3-Dihydro-2-[3-(N,N-dimethylamino)propyl]-9-methoxy-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**17**)*

Yield 0.53 g (1.23 mmol, 77%) yellow crystals, mp 227 °C (ethanol).– Anal. ($C_{25}H_{24}N_4O_3$).– IR (KBr): ν = 3415 (NH) cm^{-1}, 3055–2865 (CH); 1765, 1705 (C=O); 1630, 1570, 1490 (C=C).– ^{1}H-NMR/400 MHz (CD_3OD): δ = 1.95 (quin, *J* = 7.4 Hz, 2H, CH_2), 2.27 (s, 6H, $N(CH_3)_2$), 2.47 (t, *J* = 7.4 Hz, 2H, CH_2), 3.78 (t, *J* = 7.3 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 7.22 (dd, J_o = 9.0 Hz, J_m = 2.6 Hz, 1H, 8-H), 7.48 (dd, J_o = 8.9 Hz, *J* = 0.5 Hz, 1H, 7-H), 7.56–7.98 (m, 5H phenyl), 8.17 (d, J_m = 2.4 Hz, 1H, 10-H).– MS (EI, 70 eV): *m/z* (%) = 428 (11) [$M^{+\bullet}$], 58 (100) [$(H_3C)_2N{=}CH_2$]$^+$.

*2,3-Dihydro-2-benzyl-6-[2-(N,N-dimethylamino)ethyl]-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**18**)*

15 (2.00 g, 4.96 mmol), K_2CO_3 (1.71 g, 12.39 mmol), and 2-iodoethyl-dimethylamine hydroiodide (1.78 g, 5.45 mmol) were refluxed in dry acetone (50 ml) for 24 h. Work-up according to **22** yielded 0.54 g (1.14 mmol, 23%) as yellow crystals, mp 249 °C (ethanol).– Anal. ($C_{30}H_{26}N_4O_2$).– IR (KBr): ν = 3085–2870 (CH) cm^{-1}; 1765, 1705 (C=O); 1625, 1595, 1590, 1490 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.80 (s, 6H, $N(CH_3)_2$), 2.10 (t, *J* = 7.3 Hz, 2H, CH_2), 4.25 (t, *J* = 7.3 Hz, 2H, CH_2), 4.91 (s, 2H, CH_2), 7.05–8.93 (m, 14H, aromatic H).– MS (EI, 70 eV): *m/z* (%) = 474 (0.23) [$M^{+\bullet}$], 58 (100) [$(CH_3)_2N{=}CH_2$]$^+$.

*2,3-Dihydro-6-[2-(N,N-dimethylamino)ethyl]-5-phenyl-1H,6H-pyrrolo-[3,4-c]pyrido[3,4-b]indole-1,3-dione (**20**)*

18 (0.35 mg, 0.74 mmol) and aqueous 5N KOH (3 ml) were stirred in ethanol (17 ml) for 1 h at room temp. Concd. HCl was added until a white precipitate occurred. The mixture was diluted with water, the precipitate was filtered, washed with water and dried at the oil pump. Crude **19** was heated with NH_4OAc (5 g) at 140 °C for 1 h. After cooling to room temp., satd. $NaHCO_3$ solution (100 ml) was added, and the aqueous layer was extracted

with dichloromethane (2 × 30 ml). The organic layer was dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. Purification of the residue by column chromatography on silica gel eluting with dichloromethane/methanol 10:1 yielded 11 mg (0.03 mmol, 4%) **20** as pale yellow crystals, mp 212 °C (ethanol).– Anal. ($C_{23}H_{20}N_4O_2$).– IR (KBr): ν = 3405 (NH) cm^{-1}, 3075–2965 (CH); 1765, 1705 (C=O); 1625, 1600, 1575, 1490 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.80 (s, 6H, N(CH$_3$)$_2$), 2.06 (t, *J* = 7.5 Hz, 2H, CH$_2$), 4.23 (t, *J* = 7.5 Hz, 2H, CH$_2$), 7.33–8.31 (m, 10 H, 9 aromatic H + NH imide, partly D$_2$O exchange).– MS (EI, 70 eV): *m/z* (%) = 384 (1.26) [$M^{+\bullet}$], 58 (100) [$(CH_3)_2N{=}CH_2]^+$.

*2,3-Dihydro-2,6-bis[2-(N,N-dimethylamino)ethyl]-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**21**)*

1 (0.50 g, 1.60 mmol), K_2CO_3 (1.15 g, 7.98 mmol), and 2-chloroethyl-dimethylamine hydrochloride (0.57 g, 3.99 mmol)were refluxed in dry acetone (20 ml) for 18 h. After cooling to room temp., H_2O (100 ml) was added, and the aqueous layer was extracted with dichloromethane (3 × 30 ml). After drying over Na_2SO_4, the solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel eluting with dichloromethane/methanol 9:1, yielding **21** (0.25 g, 0.55 mmol, 34%) as yellow crystals, mp 179 °C (ethanol).– Anal. ($C_{27}H_{29}N_5O_2$).– IR (KBr): ν = 3085–2865 (CH) cm^{-1}; 1765, 1705 (C=O); 1625, 1600, 1575, 1490 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.81 (s, 6H, N(CH$_3$)$_2$), 2.11 (t, *J* = 7.3 Hz, 2H, CH$_2$), 2.21 (s, 6H, N(CH$_3$)$_2$), 2.58 (t, *J* = 6.3 Hz, 2H, CH$_2$), 3.81 (t, *J* = 6.3 Hz, 2H, CH$_2$), 4.24 (t, *J* = 7.3 Hz, 2H, CH$_2$), 7.47–8.94 (m, 9H, aromatic H).– MS (EI, 70 eV): *m/z* (%) = 455 (3) [$M^{+\bullet}$], 58 (100) $[(CH_3)_2N{=}CH_2]^+$.

Diamines **22**–**30** were prepared analogously.

*2,3-Dihydro-2,6-bis[3-(N,N-dimethylamino)propyl]-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**22**)*

Yield 0.22 g (0.44 mmol, 28%) yellow crystals, mp 188 °C (ethanol).– Anal. ($C_{29}H_{33}N_5O_2$).– IR (KBr): ν = 3250–2940 (CH) cm^{-1}; 1770, 1710 (C=O); 1560, 1520, 1460 (C=C).– ^{1}H-NMR/250 MHz ($CDCl_3$): δ = 1.44–4.11 (m, 12H), 2.01 (s, 6H, N(CH$_3$)$_2$), 2.11 (s, 6H, N(CH$_3$)$_2$), 7.29–9.10 (m, 9H, aromatic H).– MS (EI, 70 eV): *m/z* (%) = 483 (21) [$M^{+\bullet}$], 425 (3) $[M–58]^+$, 399 (4) $[M–84]^+$, 84 (43) $[H_2C{=}CH{-}CH{=}N(CH_3)_2]^+$, 58 (100) $[(CH_3)_2N{=}CH_2]^+$.

*2,3-Dihydro-5-phenyl-2,6-bis[2-(1-pyrrolidinyl)ethyl]-1H,6H-pyrrolo-[3,4-c]pyrido[3,4-b]indole-1,3-dione (**23**)*

Yield 0.17 g (0.33 mmol, 21%) yellow crystals, mp 192 °C (ethanol).– Anal. ($C_{31}H_{33}N_5O_2$).– IR (KBr): ν = 3030–2875 (CH) cm^{-1}; 1770, 1710 (C=O); 1625, 1600, 1570, 1485 (C=C).– ^{1}H-NMR/250 MHz ($CDCl_3$): δ = 1.62 (m, 4H, β-CH$_2$), 1.76 (m, 4H, α-CH$_2$), 2.10 (m, 4H, β-CH$_2$), 2.38 (t, *J* = 8.0 Hz, 2H, CH$_2$), 2.64 (m, 4H, α-CH$_2$), 2 .86 (t, *J* = 6.8 Hz, 2H, CH$_2$), 3.99 (t, *J* = 6.8 Hz, 2H, CH$_2$), 4.30 (t, *J* = 8.0 Hz, 2H, CH$_2$), 7.53–9.10 (m, 9H, aromatic H).– MS (EI, 70 eV): *m/z* (%) = 507 (2) [$M^{+\bullet}$], 422 (0.86) $[M–C_5H_{10}N]^+$, 84 (100) $[C_5H_{10}N]^+$.

*2,3-Dihydro-2,6-bis[2-(1-morpholinyl)ethyl]-5-phenyl-1H,6H-pyrrolo-[3,4-c]pyrido[3,4-b]indole-1,3-dione (**24**)*

Yield 0.80 g (0.15 mmol, 93%) yellow crystals, mp 177 °C (ethanol).– Anal. ($C_{31}H_{33}N_5O_4$).– IR (KBr): ν = 3085–2870 (CH) cm^{-1}; 1765, 1705 (C=O); 1625, 1595, 1580, 1490 (C=C).– ^{1}H-NMR/250 MHz ($CDCl_3$): δ = 2.01 (t, *J* = 4.6 Hz, 4H, CH_2NCH_2), 2.27 (t, *J* = 7.6 Hz, 2H, CH$_2$), 2.57 (t, *J* = 4.4 Hz, 4H, CH$_2$), 2.73 (t, *J* = 6.3 Hz, 2H, CH$_2$), 3.46 (t, *J* = 4.6 Hz, 4H, CH$_2$), 3.65 (t, *J* = 4.6 Hz, 2H, CH$_2$), 3.98 (t, *J* = 6.4 Hz, 2H, CH$_2$), 2.73 (t, *J* = 7.6 Hz, 2H, CH$_2$), 7.44–8.90 (m, 9H, aromatic H).– MS (EI, 70 eV): *m/z* (%) = 539 (1) [$M^{+\bullet}$], 100 (100) $[C_5H_{10}NO]^+$.

*2,3-Dihydro-5-phenyl-2,6-bis[2-(1-piperidinyl)ethyl]-1H,6H-pyrrolo-[3,4-c]pyrido[3,4-b]indole-1,3-dione (**25**)*

Yield 0.45 g (0.84 mmol, 53%) yellow crystals, mp 146 °C (ethanol).– Anal. ($C_{33}H_{37}N_5O_2$).– IR (KBr): ν = 3050–2855 (CH) cm^{-1}; 1765, 1705 (C=O); 1625, 1695, 1580, 1490 (C=C).– ^{1}H-NMR/250 MHz ($CDCl_3$): δ = 1.32–1.73 (m; 12H, 2 × β- and 2 × α-CH$_2$), 1.93–1.95 (m; 4H), 2.22 (t, *J* = 7.7 Hz, 2H, CH$_2$), 2.48–2.53 (m, 4H), 2.69 (t, *J* = 6.8 Hz, 2H, CH$_2$), 3.97 (t, *J* = 6.9 Hz, 2H, CH$_2$), 4.24 (t, *J* = 7.7 Hz, 2H, CH$_2$), 7.42–9.10 (m, 9H, aromatic H).– MS (EI, 70 eV): *m/z* (%) = 535 (2) [$M^{+\bullet}$], 98 (100) $[C_6H_{12}N]^+$.

*2,3-Dihydro-2,6-bis[2-(N,N-dimethylamino)ethyl]-9-methoxy-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**26**)*

Yield 0.55 g (1.14 mmol, 71%) yellow crystals, mp 197 °C (ethanol).– Anal. ($C_{28}H_{31}N_5O_3$).– IR (KBr): ν = 3055–2945 (CH) cm^{-1}; 1765, 1710 (C=O); 1630, 1565, 1490 (C=C).– ^{1}H-NMR/250 MHz ([D$_6$]DMSO): δ = 1.79 (s, 6H, N(CH$_3$)$_2$), 2.08 (t, *J* = 7.2 Hz, 2H, CH$_2$), 2.21 (s, 6H, N(CH$_3$)$_2$), 2.56 (t, *J* = 6.3 Hz, 2H, CH$_2$), 3.80 (t, *J* = 6.4 Hz, 2H, CH$_2$), 3.93 (s, 3H, OCH$_3$), 4.20 (t, *J* = 7.3 Hz, 2H, CH$_2$), 7.44 (dd, J_o = 9.1 Hz, J_m = 2.6 Hz, 1H, 8-H), 7.61–7.71 (m, 5H phenyl), 7.80 (d, J_o = 9.2 Hz, 1H, 7-H), 8.41 (d, J_m = 2.5 Hz, 1H, 10-H).– MS (EI, 70 eV): *m/z* (%) = 485 (1) [$M^{+\bullet}$], 58 (100) $[(H_3C)_2N{=}CH_2]^+$.

*2,3-Dihydro-2,6-bis[3-(N,N-dimethylamino)propyl]-9-methoxy-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**27**)*

Yield 0.64 g (1.26 mmol, 81%) yellow crystals, mp 166 °C (ethanol).– Anal. ($C_{30}H_{35}N_5O_3$).– IR (KBr): ν = 3065–2860 (CH) cm^{-1}; 1770, 1710 (C=O); 1635, 1605, 1570, 1490 (C=C).– ^{1}H-NMR/400 MHz ([D$_6$]acetone): δ = 1.50 (quint, *J* = 7.2 Hz, 2H, CH$_2$), 1.70 (t, *J* = 6.6 Hz, 2H, CH$_2$), 1.91 (s, 6H, N(CH$_3$)$_2$), 2.19 (s, 6H, N(CH$_3$)$_2$), 2.40 (t, *J* = 6.8 Hz, 2H, CH$_2$), 2.83 (m, 2H, CH$_2$), 3.84 (t, *J* = 7.4 Hz, 2H, CH$_2$), 4.00 (s, 3H, OCH$_3$), 4.19 (t, *J* = 7.4 Hz, 2H, CH$_2$), 7.41 (dd, J_o = 9.1 Hz, J_m = 2.6 Hz, 1H, 8-H), 7.61–7.76 (m, 6H, 7-H + 5H phenyl), 8.58 (d, J_m = 2.6 Hz, 1H, 10-H).– MS (EI, 70 eV): *m/z* (%) = 513 (26) [$M^{+\bullet}$], 58 (100) $[(H_3C)_2N{=}CH_2]^+$.

*2,3-Dihydro-9-methoxy-5-phenyl-2,6-bis[2-(1-pyrrolidinyl)ethyl]-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**28**)*

Yield 0.69 g (1.28 mmol, 80%) yellow crystals, mp 183 °C (ethanol).– Anal. ($C_{32}H_{35}N_5O_3$).– IR (KBr): ν = 3060–2875 (CH) cm^{-1}; 1770, 1715 (C=O); 1635, 1605, 1570, 1490 (C=C).– ^{1}H-NMR/400 MHz ([D$_6$]acetone): δ = 1.50–1.53 (m, 4H, β-CH$_2$), 1.69–1.72 (m, 4H, α-CH$_2$), 2.07–2.10 (m, 4H, β-CH$_2$), 2.39 (t, *J* = 7.4 Hz, 2H, CH$_2$), 2.57–2.62 (m, 4H, α-CH$_2$), 2.83 (t, *J* = 6.4 Hz, 2H, CH$_2$), 3.92 (t, *J* = 6.6 Hz, 2H, CH$_2$), 3.99 (s, 3H, OCH$_3$), 4.32 (t, *J* = 7.4 Hz, 2H, CH$_2$), 7.42 (dd, J_o = 9.1 Hz, J_m = 2.7 Hz, 1H, 8-H), 7.61–7.78 (m, 6H, 7-H + 5H phenyl), 8.58 (d, J_m = 2.6 Hz, 1H, 10-H).– MS (EI, 70 eV): *m/z* (%) = 537 (1) [$M^{+\bullet}$], 84 (100) $[C_4H_8N{=}CH_2]^+$.

*2,3-Dihydro-9-methoxy-2,6-bis[2-(1-morpholinyl)ethyl]-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**29**)*

Yield 0.83 g (1.46 mmol, 91%) yellow crystals, mp 185 °C (ethanol).– Anal. ($C_{32}H_{35}N_5O_5$).– IR (KBr): ν = 3055–2860 (CH) cm^{-1}; 1760, 1705 (C=O); 1630, 1605, 1565, 1490 (C=C).– ^{1}H-NMR/400 MHz ([D$_6$]acetone): δ = 2.01 (t, *J* = 4.7 Hz, 4H, CH$_2$ ring), 2.28 (t, *J* = 7.2 Hz, 2H, CH$_2$ chain), 2.51–2.54 (m, 4H, CH$_2$ ring), 2.70 (t, *J* = 6.4 Hz, 2H, CH$_2$ chain), 3.29 (t, *J* = 4.5 Hz, 4H, CH$_2$ ring), 3.56 (t, *J* = 4.6 Hz, 4H, CH$_2$ ring), 3.93 (t, *J* = 6.0 Hz, 2H, CH$_2$ chain), 4.00 (s, 3H, OCH$_3$), 4.33 (t, *J* = 7.0 Hz, 2H, CH$_2$ chain), 7.43 (dd, J_o = 9.1 Hz, J_m = 2.7 Hz, 1H, 8-H), 7.61–7.80 (m, 5H phenyl), 7.79 (d, J_o = 9.6 Hz, 1H, 7-H), 8.59 (d, J_m = 2.5 Hz, 1H, 10-H).– MS (EI, 70 eV): *m/z* (%) = 569 (3) [$M^{+\bullet}$], 469 (0.4) $[M–C_5H_{10}NO]^+$, 100 (100) $[OC_4H_8N{=}CH_2]^+$.

*2,3-Dihydro-9-methoxy-5-phenyl-2,6-bis[2-(1-piperidinyl)ethyl]-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione (**30**)*

Yield 0.84 g (1.49 mmol, 93%) yellow crystals, mp 172 °C (ethanol).– Anal. ($C_{34}H_{39}N_5O_3$).– IR (KBr): ν = 3060–2850 (CH) cm^{-1}; 1770, 1715 (C=O); 1630, 1605, 1570, 1490 (C=C).– ^{1}H-NMR /400 MHz ([D$_6$]acetone): δ = 1.21–1.47 (m, 4H, γ-CH$_2$), 1.48–1.51 (m, 4H, β-CH$_2$), 1.94–1.97 (m, 4H, α-CH$_2$), 2.22 (t, *J* = 7.1 Hz, 2H, CH$_2$), 2.47–2.50 (m, 4H, β-CH$_2$), 2.65 (t, *J* = 6.6 Hz, 2H, CH$_2$), 2.79–2.83 (m, 4H, α-CH$_2$), 3.90 (t, *J* = 6.6 Hz, 2H, CH$_2$), 4.00 (s, 3H, OCH$_3$), 4.29 (t, *J* = 7.0 Hz, 2H, CH$_2$), 7.42 (dd, J_o = 9.1 Hz, J_m = 2.7 Hz, 1H, 8-H), 7.60–7.78 (m, 5H phenyl), 7.75 (d, J_o = 9.1 Hz, 1H, 7-H), 8.59 (d, J_m = 2.6 Hz, 1H, 10-H).– MS (EI, 70 eV): *m/z* (%) = 565 (3) [$M^{+\bullet}$], 467 (0.4) $[M–C_6H_{12}N]^+$, 98 (100) $[C_5H_{10}N{=}CH_2]^+$.

Topoisomerase Inhibition Assay

Topoisomerases I and II were isolated from calf thymus glands according to Strausfeld and Richter[11]. Enzymes were detected by their DNA relaxation activity. In a standard assay, 0.5 μg supercoiled pRB322 DNA was assayed in a total volume of 20 μl containing 20 mM Tris-HCl, pH 7.8, 150 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 50% glycerol, 100 μg/ml bovine serum albumine and, in case of topoisomerase II, 1 mM ATP. The amount of enzyme that relaxes 0.5 μg supercoiled DNA in 30 min is defined as one unit. Substances to be tested as enzyme inhibitors were employed in a concentration range usually from 1 to 100 μg/ml. The indicated amounts of inhibitors were preincubated with the DNA for 10 min in the standard assay. Then one unit of enzyme was added and the incubation was continued for 10 min at 37 °C. The reaction was terminated by 5 μl of stopper solution containing 0.5% sodium dodecylsulfate, 1 mg/ml proteinase K and 1 mM $CaCl_2$ in 50 mM Tris-HCl, pH 7.9 and incubation at 50 °C for 15 min. 5 μl of 0.005% bromophenolblue in 50% glycerol were then added and the samples analyzed on 1% agarose gels in TAE buffer (50 mM Tris-acetate, pH 8.0, 20 mM Na-acetate, 2 mM EDTA, 18 mM NaCl). Electrophoresis was carried out at 2 Vcm^{-1} overnight. The gels were stained with ethidium bromide (0.5 μg/ml) and photographed under UV illumination. The inhibitory effect of the substances on the relaxation activities of topoisomerases was estimated by comparison with the complete relaxation activity observed in the absence of any inhibitor.

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