Synthesis and Structure–Activity Relationship of Methyl-Substituted Indolo[2,3-*b*]quinolines: Novel Cytotoxic, DNA Topoisomerase II Inhibitors

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In furtherance of our SAR study on the chemistry and antitumor activity of fused nitrogen heteroaromatic compounds, a series of linear, methyl-substituted derivatives of 5*H*- and 6*H*-indolo[2,3-*b*]quinolines were synthesized according to the modified Graebe–Ullmann reaction. To establish the relationship between the physicochemical and biological activities of indolo-[2,3–*b*]quinolines, their lipophilic properties, cytotoxic and antimicrobial activity, and ability to induce topoisomerase II dependent pSP65 DNA cleavage *in vitro* were investigated. We found that the antimicrobial and cytotoxic activity of indolo[2,3-*b*]quinolines was strongly influenced by the position, and the number of methyl substituents and the presence of methyl group at pyridine nitrogen was essential for the cytotoxicity of these compounds. All indolo-[2,3-*b*]quinolines belonging to the 5*H* series, *i.e.*, bearing a methyl group on the pyridine nitrogen, showed significant activity against procaryotic and eucaryotic organisms. They inhibited the growth of Gram-positive bacteria and pathogenic fungi at MIC range 3×10^{-2} to 2.5×10^{-1} μmol/mL, displayed cytotoxicity against KB cells ID_{50} in the range 2×10^{-3} to 9×10^{-3} μmol/mL, and stimulated the formation of calf thymus topoisomerase II mediated DNA cleavage at concentration between 0.4 and 10 μM. None of the indolo[2,3-*b*]quinolines belonging to the 6*H* series, *i.e.*, lacking a methyl group on the pyridine nitrogen, was active in analogous tests. Of the investigated compounds, the most active was 2,5,9,11-tetramethyl-5*H*-indolo-[2,3-*b*]quinoline, a compound bearing the highest number of symmetrically distributed methyl groups. The interaction of indolo[2,3-*b*]quinolines with DNA was studied by measuring the increase of calf thymus DNA denaturating temperature (T_m). The ΔT_m values for the 5*H* series were found to be about 10 times as high as those for the 6*H* compounds. Indolo[2,3-*b*]quinolines with the highest number of methyl groups had the greatest contribution to the increase in the T_m of calf thymus DNA. The values of ΔT_m reached 19 °C and 1.6 °C for the most substituted compounds of both series.

Introduction

Our investigations on the cytotoxic and antitumor properties of synthetically obtained α-carboline derivatives have shown that, even though these species were not active *in vitro*, they became enzymatically activated. They are affected by adenosyl-*S*-methionine-dependent methyltransferases, thus yielding *N*-1-methyl derivatives. These are compounds of an iso-α-carboline structure, and the products of their further methylation of the nitrogen at position N-9 are carbolinium derivatives. The forming iso-α-carbolines showed cytotoxic properties with respect to KB cells (ID_{50} 3×10^{-2} to 2.4×10^{-1} μmol/mL), thus inhibiting the growth of Gram-positive bacteria and yeasts (MIC 2×10^{-1} to 2.5 μmol/mL). Within this group there was a distinct relationship between the cytotoxic properties and the position or character of the substituents.[1a] In our structure–activity relationship studies (SAR) we extended the tricyclic system (α-carbolines) into a tetracyclic one (indolo[2,3-*b*]quinolines). Within this tetracyclic system, the introduction of the methyl group into the position C-11 and C-5 yielded 5,11-dimethyl-5*H*-indolo[2,3-*b*]-quinoline, **10b**, its further methylation at N-6 led to quinolinium derivative,[1b,c,2] both compounds of potentiated cytotoxic properties. We found that **10b** displayed a strong antibacterial, antimycotic (MIC against Gram-positive bacteria and fungi 1×10^{-2} to 6×10^{-2} μmol/mL) and cytotoxic activity *in vitro* (ID_{50} against KB cells line 1×10^{-3} μmol/mL) as well as significant antitumor properties *in vivo*, thus prolonging the life span of leukemia P 388 and L 1210 and melanoma B 16 bearing animals up to 190%, 175%, and 240%, respectively.[2] Having a tetracyclic, planar, aromatic molecule with two heterocyclic nitrogen atoms and two methyl groups, this compound is structurally similar to ellipticine[3] (compound **11**) and meets all the requirements for DNA intercalation. It has been found that **10b**, as well as ellipticine, shows intercalating properties, but only **10b** is able to induce the topoisomerase II–DNA-mediated cleavable complex.[4] Such DNA topoisomerase II-dependent cleavable complexes are not formed in the presence of ellipticine.[3] Noteworthy, the cytotoxic and antitumor activity of **10b** makes this compound a potential antitumor agent.[2]

Our earlier results indicated the strong relationship between the molecule shape, as well as the position of

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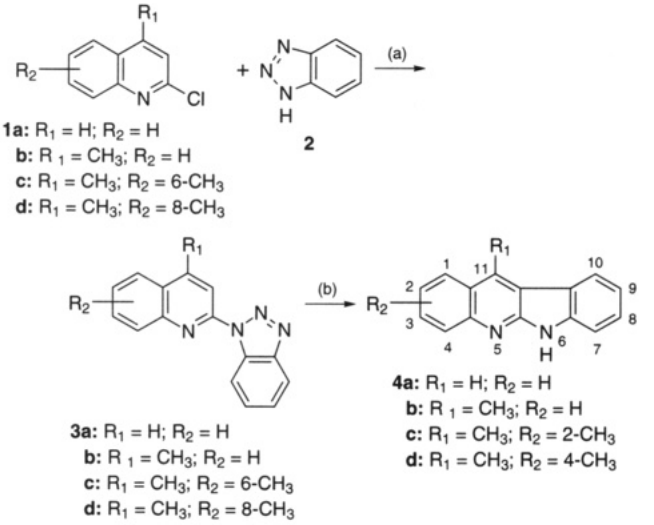
Scheme 1[a]



[a] (a) 110−120 °C; (b) polyphosphoric acid, 130−180 °C.

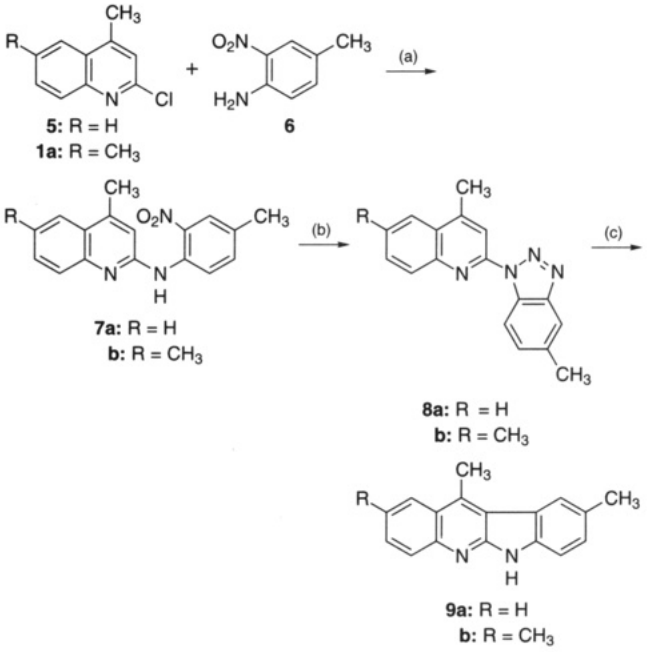
the methyl group and the cytotoxicity within the indoloquinolines.[2] In our SAR study on these compounds, the prime objective was to synthesize the basic system of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines, *i.e.*, compounds not bearing the bearing methyl substituent at pyridine nitrogen (position N-5), their *C*-methyl derivatives, and to assess the obtained compounds as potential cytotoxic agents.

This paper aims at presenting the method of synthesis and the physicochemical characterization of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines, both not having and having methyl groups at C-2, C-4, C-9, and C-11. Another objective of the study reported in this paper was to evaluate the cytotoxic properties of these species against KB cells, their antimicrobial activity against Gram-negative and Gram-positive bacteria and pathogenic fungi, as well as their ability to induce *in vitro* the formation of calf thymus DNA topoisomerase II-mediated cleavable complexes using pSP65 DNA as a substrate. The interaction of both 6*H*- and 5*H*-indolo[2,3-*b*]quinoline series with calf thymus DNA was studied using UV−vis spectroscopy. The stability of the indolo[2,3-*b*]quinoline−DNA complex obtained was estimated by the measurement of the differences in the temperature of calf thymus DNA denaturation in the presence of these derivatives.

Results

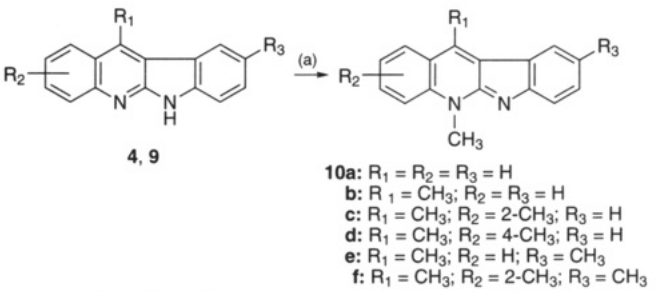
Chemistry. 6*H*-Indolo[2,3-*b*]quinolines **4** and **9** were obtained *via* the routes illustrated in Schemes 1 and 2. Triazoles **3** were prepared by heating the corresponding quinolines **1** with benzotriazole **2** at 110−120 °C. This sequence could not be used in the case of methyl-substituted triazoles **8**. Hence, compounds **8** were synthesized by condensation of quinolines **5** or **1a** with 4-methyl-3 nitroaniline **6** followed by the reduction of the nitro group in intermediate **7** and diazotization of the amine obtained. Decomposition of triazoles **3** and **8** in polyphosphoric acid (PPA) at 130−180 °C yielded indoloquinolines **4** and **9**, respectively. 6*H*-indoloquinolines **4** and **9** were converted into 5-methyl 5*H*-indolo[2,3-*b*]quinolines **10** by quaternization with dimethyl sulfate in toluene, followed by alkalization of the aqueous suspension of quarternary salts with 20% NaOH

Scheme 2[a]



[a] (a) 150−160 °C; (b) $SnCl_2$/HCl, then $NaNO_2$/HCl; (c) polyphosphoric acid, 130−180 °C.

Scheme 3[a]



[a] (a) $(CH_3)_2SO_4$/toluene, 150−160 °C, then 20% NaOH.

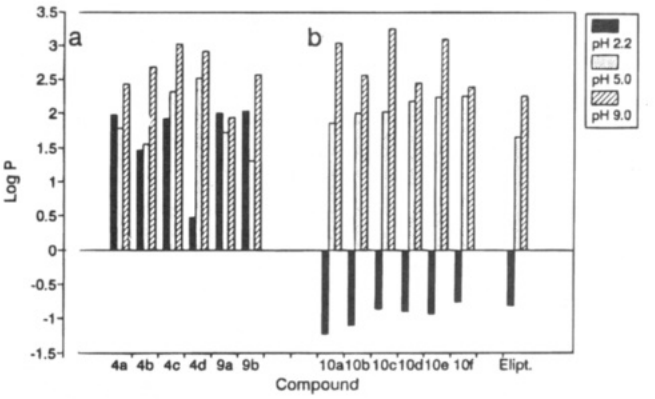


Figure 1. (a, b) Octanol−water partition coefficients of 5*H*- and 6*H*-indolo[2,3-*b*]quinolines. Methanol containing 0.01% H_2SO_4 was used as mobile phase (1.0 mL/min at 42 °C, 4.6 × 250 mm Supelcosil LC-18-DB column). All indolo[2,3-*b*]-quinolines had a retention time of 4.93 to 6.21; values of $k = (v_r - v_o)/v_o$ were **4a**, 1.78; **4b**, 1.86; **4c**, 1.82; **4d**, 1.84; **9a**, 1.9; **10a**, 2.19; **10b**, 2.24; **10c**, 2.12; **10d**, 2.12; **10e**, 2.22; **10f**, 2.12; and for elipticine, 2.40. v_o for 4.6 × 250 mm Supelcosil LC-C18-DB with precolumn filter was 2.77 mL.

(Scheme 3, Figure 1). Compounds **4a**, **4b**, **10a**, and **10b** were described in the literature.[2]

Lipophilicity. The hydrophobic parameter (log *P*) of 5*H*- and 6*H*-indolo[2,3-*b*]quinolines was estimated under acidic and basic conditions. We found that the

Table 1. Isosbestic Points and pK_a Values of 6*H*- and 5*H*-Indolo[2,3-*b*]quinolines

compd	isosbestic point (nm)	pK_a	(nm)[a]
4a	270	5.55	261
10a	271	7.24	281
4a	271	5.70	261
10b	272	7.45	280
4c	273	5.73	266
10c	274	7.60	282
4d	275	5.78	266
10d	276	7.62	284
9a	276	5.80	267
10e	280	7.65	268
9b	280	5.98	268
10f	282	7.68	287
Ellipticine[14]	293	7.40	284

[a] Determination of pK_a values was performed at indicated wavelength.

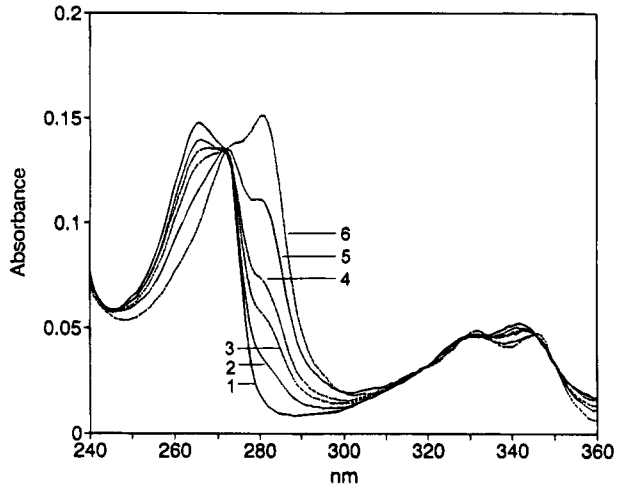


Figure 2. Ultraviolet absorbance spectra of **10b**. (1) pH 2.2–5.0, (2) pH 6.0; (3) pH 7.0, (4) pH 7.5; (5) pH 8.0, (6) pH 9.0.

determined partition coefficients covered a range of 4.0 log units (Figure 1a,b). The log *P* values were positive for all compounds at pH 5 and pH 9, but distinct differences were observed for both series at pH 2.2. The compounds belonging to the 6*H* series appeared to be much more hydrophobic, as indicated by the range of the log *P* values at pH 2.2 (Figure 1a). Under identical experimental conditions, 5*H*-indolo[2,3-*b*]quinolines were less hydrophobic, their log *P* values ranged between −1.23 and 3.25 (Figure 1b).

pK_a of Indolo[2,3-*b*]quinolines. The pK_a values of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines were deduced from their UV spectra at a fixed wavelength taken in buffers at a pH from 2.2 to 9.0 at a concentration of drugs of 3 μM. Measurements were taken at 37 °C, and the final concentration of DMSO was 5%. Under such conditions, the linearity of the absorbance *vs* drug concentration relationship was preserved. The pK_a values for 6*H*-indolo[2,3-*b*]quinolines were found to range from 5.55 to 5.98 with formation of an isosbestic point at 270–280 nm. The values of pK_a for 5*H*-indolo[2,3-*b*]quinolines were found to range from 7.24 to 7.68, and the isosbestic point was seen at 271–282 (Table 1). The pH-related changes in the UV absorbance spectrum of 5*H*-indolo[2,3-*b*]quinolines with the formation of a clear isosbestic point are shown in Figure 2, with compound **10b** as representative.

Antimicrobial Activity. 6*H*-Indolo[2,3-*b*]quinolines (compounds **4a-d** and **9a,b**) and 5*H*-indolo[2,3-*b*]quinolines (compounds **10a–f**) were tested against a variety of microorganisms. Table 2 summarizes the antimicrobial activity of the compounds (expressed as minimum inhibitory concentration values (MICs) against the representatives of Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*), and pathogenic fungi (clinical isolates) (*Candida albicans* and *Trichophyton mentagrophytes*). We found that any compound of the 6*H*-indolo[2,3-*b*]quinolines series displayed antimicrobial activity up to the concentration of 5 μmol/mL, whereas the derivatives belonging to the 5*H*-indolo[2,3-*b*]quinolines series showed significant activity against Gram-positive bacteria and fungi at the MIC range of 1.5×10^{-2} to 2.5×10^{-1} μmol/mL and 1.5×10^{-2} to 1.2×10^{-1} μmol/mL, respectively. No antimicrobial activity against Gram-negative bacteria was observed for the compounds of both series.

Cytotoxic Activity. Both 5*H*- and 6*H*-indolo[2,3-*b*]-quinolines were examined *in vitro* for cytotoxic activity against the KB cells line. It was found that of the compounds tested, only the 5*H* series (in addition to significant antimicrobial properties) displayed strong cytotoxicity against the KB cells line (Table 2). The values of ID_{50} for these compounds ranged from 2×10^{-3} to 9×10^{-3} μmol/mL, whereas those for ellipticine (used as a standard) amounted to 7×10^{-2} μmol/mL. Under identical experimental conditions, 6*H*-indolo[2,3-*b*]-quinolines showed poor ability to inhibit the growth of KB cells at concentrations much higher (1×10^{-1} to 6×10^{-1} μM/mL, for ID_{50}) than those for the 5*H* series.

DNA Thermal Denaturation Studies. The differences in DNA binding properties of both the 6*H* and the 5*H* series were estimated by examining the increase of calf thymus DNA denaturation temperature (ΔT_m) in the presence of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines. Measurements were taken at a drug/DNA ratio of 1:10 in the 5.0 mM Tris-HCl buffer. The ΔT_m values ranged from 0.3 °C to 1.6 °C for noncytotoxic compounds (6*H* series), whereas those for active 5*H*-indolo[2,3-*b*]quinolines were from 5.2 °C for compound **10a** to 19 °C for compound **10f**. For ellipticine and adriamycin (both used as a control) the ΔT_m values were found to be 9.8 °C and 13 °C, respectively (Table 3).

DNA Binding Studies. The interaction of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines with calf thymus DNA was examined by UV–vis spectroscopy. The binding of both 6*H*- and 5*H*-indolo[2,3-*b*]quinoline series to calf thymus DNA was accompanied by variations in the UV spectra, according to the pattern reported previously.[4] In the presence of increasing DNA amounts, the spectra of both 6*H*- and 5*H*-indolo[2,3-*b*]quinolines taken in the same buffer as those for ΔT_m determination (5.0 mM Tris-HCl, pH 7.0) showed a significant hypochromic and bathochromic shift accompanied by the appearance of the isosbestic point at range 345–348 and 350–354 nm for compounds of 6*H* series and 5*H* series, respectively (Table 3).

Topoisomerase II Assay. Our previous studies regarding the induction by compound **10b** double-strand DNA breaks mediated by topoisomerase II implied the furtherance of our enzymatic assay *in vitro* for both series of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines under

Table 2. Antimicrobial Activity and Cytotoxicity of 6*H*- and 5*H*-Indolo[2,3-*b*]quinolines

compd	MIC[a] (μmol/mL)						ID_{50}[b] (μmol/mL)
	1	2	3	4	5	6	
4a	–	–	–	–	–	–	0.6
10a	–	–	0.25	0.25	0.12	0.06	0.006
4b	–	–	–	–	–	–	0.2
10b	–	–	0.12	0.06	0.03	0.06	0.004
4c	–	–	–	–	–	–	0.1
10c	–	–	0.06	0.03	0.015	0.03	0.003
4d	–	–	–	–	–	–	0.1
10d	–	–	0.25	0.12	0.12	0.06	0.009
9a	–	–	–	–	–	–	0.1
10e	–	–	0.12	0.06	0.06	0.12	0.003
9b	–	–	–	–	–	–	0.1
10f	–	–	0.03	0.015	0.015	0.03	0.002
ellipticine	nd	nd	nd	nd	nd	nd	0.07

[a] (1) *Escherichia coli* PCM 271, (2) *Pseudomonas aeruginosa* PCM 499, (3) *Staphylococcus aureus* PCM 458, (4) *Micrococcus luteus* PCM 525, (5) *Candida albicans* (clinical isolate), (6) *Trichophyton mentagrophytes* (clinical isolate); MICs were determined at the concentration range of 0.01–5 μmol/mL. [b] Cytotoxicity expressed as ID_{50} values was determined *in vitro* against KB cell line.

Table 3. Interaction of 6*H*- and 5*H*-Indolo[2,3-*b*]quinolines with Calf Thymus DNA

compd	ΔT_m (°C)[a] (±SD, °C)	isosbestic point[b] (nm)
4a	0.3 (±0)	345
10a	5.2 (±0.1)	350
4b	0.7 (±0)	345
10b	11.0 (±0.2)	350
4c	1.4 (±0.1)	346
10c	13.3 (±0.2)	351
4d	1.1 (±0.1)	346
10d	10.4 (±0.3)	352
9a	0.9 (±0.1)	347
10e	13.0 (±0.3)	353
9b	1.6 (±0.1)	348
10f	19.0 (±0.4)	354
ellipticine	9.8 (±0.2)	310[c]
adriamycin	13.0 (±0.1)	nd

[a] Measurements were taken in 5.0 mM, pH 7.0 Tris-HCl buffer containing 50 μM calf thymus DNA (Boehringer Mannheim, average molecular weight 1.2×10^6 D) 50 μM EDTA, 5.0 μM drug, 5% DMSO. [b] Measurements were taken in buffer as given above (footnote *a*) in 5 cm pathlength cuvette, 37 °C, fixed amounts of drugs (solution 3 μM, final concentration of DMSO, 5%) were progressively titrated by increasing amounts of DNA (starting from molar ratio drug/DNA, 1) to obtain the spectrum of fully bound drugs in the presence of a large excess of DNA. [c] Lit. data 308 nm.[22]

conditions established earlier.[4] We found that only cytotoxic 5*H*-indolo[2,3-*b*]quinolines stimulated efficiently DNA cleavage, producing a maximum of complexes cleavable at a concentration of 0.4–10 μM when purified calf thymus topoisomerase II and circular pSP65 DNA as substrate were used (Figure 3a,b). The alterations in the chromophore structure due to the introduction of methyl substituents at carbons greatly affected 5*H*-indolo[2,3-*b*]quinolines action on topoisomerase II. The position and number of the methyl groups at carbons play a crucial role in the induction of the calf thymus topoisomerase II-mediated complexes cleavable of pSP65 DNA. Compound **10f**, with the highest number of methyl groups (at C-2, C-9, and C-11), appeared to be the most active of all the derivatives tested, providing the most efficient induction of calf thymus topoisomerase II-mediated DNA cleavage at the concentration of 0.4 μM, whereas compound **10a**, the unsubstitued one, was the least active, inducing the formation of complexes cleavable at 10 μM. In the same experiment, compound **10b** (used as standard[4]) bearing the methyl group at C-11 induced the maximum of topoisomerase II-mediated DNA breaks at a concentration of 5 μM. Its monomethyl derivatives, isomeric compounds **10c–e** having methyl group at C-2, C-4 or C-9, were found to be slightly less active, inducing DNA breaks at 0.6, 1.0, and 0.8 μM, respectively. The least active within this series was compound **10d** having the methyl group at C-4, *i.e.*, in close vicinity to the methyl

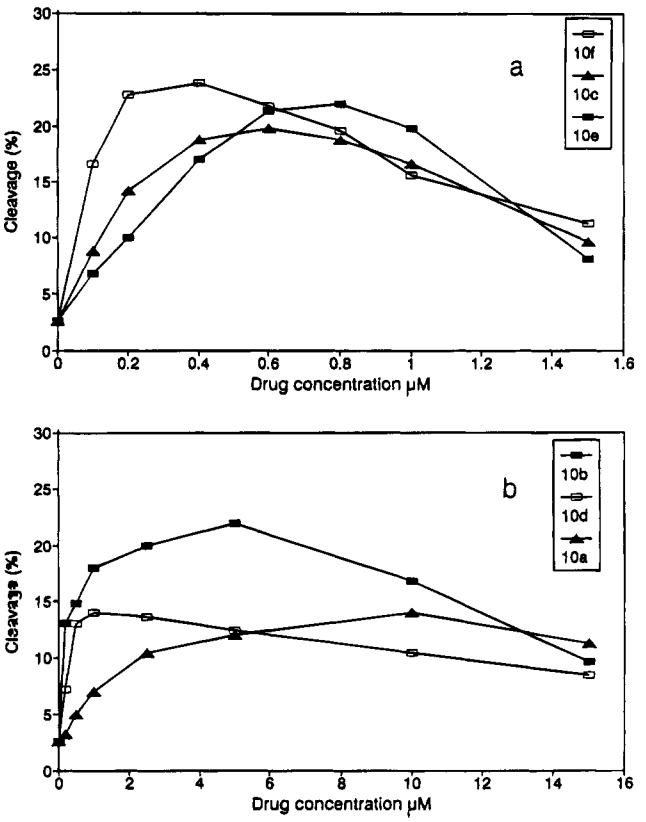


Figure 3. (a, b) Topoisomerase II-induced cleavage by 5*H*-indolo[2,3-*b*]quinolines (compounds **10a-f**). The cleavable complex was formed by incubation at 37 °C for 15 min of 100 ng of pSP65 DNA with 200 ng of calf thymus topoisomerase II in 15 μL of 60 mM KCl, 7.5 mM $MgCl_2$, 0.5 mM Na_3–EDTA, 0.5 mM dithiotreitol, 20 mM Tris-HCl, pH 7.0, 0.1 mg/mL bovine serum, and drug at indicated concentration. Compound **10b** was used as standard.[4] After addition of 2 μL of 3% sodium dodecyl sulfate and 0.75 mg/mL proteinase K (Boehringer Mannheim), the mixture was incubated at 55 °C for 45 min and loaded on a 1.2% agarose gel. Electrophoresis was carried out in the presence of 0.45 μg/mL ethidium bromide in Tris-borate buffer. DNA bands were photographed under UV light using Ilford FP4 film, and their intensity was measured with the Chromoscan 3 densitometer.

group at pyridine nitrogen (N-5). None of the derivatives of the 6*H*-indolo[2,3-*b*]quinoline series nor ellipticine itself produced detectable amounts of pSP65 DNA breaks mediated by calf thymus topoisomerase II at concentrations between 1 and 100 μM.

Discussion

Quinoline derivatives are known for their DNA binding properties. There is evidence that antimalaric drugs, such as chloroquine, quinacrine, or quinine, can interact with DNA as intercalators,[5] and quinolone antibiotics are known as inhibitors of bacterial gyrase[6,13] and mammalian DNA topoisomerase II.[7,8]

The nature of quinoline derivatives binding to DNA is still far from being well-understood. References in literature to quinoline methanolamines[9] show that two types of interaction are involved in DNA complex formation: (1) the electrostatic attraction between the amino groups of the side chain protonated at physiological pH and the phosphate group of DNA and (2) a more specific interaction between the aromatic system of quinoline and nucleotide bases. It has been postulated that the protonated aminoquinoline ring should be able to intercalate between the base pair in the DNA helix.[10] There are also several indications that the DNA–quinoline derivatives interaction, as well as the antimicrobial and/or cytotoxic properties of the quinoline derivatives, is strongly influenced by the position and type of the substituents introduced into the quinoline nucleus.[10–13]

Our current findings corroborate the earlier observation that there exists a distinct relationship between the shape of the molecule, the position of the substituents, and cytotoxicity. Under the SAR study we synthesized the 6*H*- and 5*H*-indolo[2,3-*b*]quinolines, *i.e*, compounds with and without methyl group at pyridine nitrogen (position N-5) and also with methyl groups introduced at various positions at carbons of the aromatic area of both (6*H* and 5*H*) series. Consequently, we estimated their basicity by measuring their pK_a and their lipophilicity by determining their log *P* values, and we evaluated their cytotoxicity and antimicrobial properties. We also examined their ability to induce topoisomerase II-dependent DNA cleavable complexes *in vitro* (using pSP 65 DNA as substrate and calf thymus topoisomerase II) and studied their interaction with DNA by examining the changes in their UV–vis spectra due to the presence of calf thymus DNA. The influence of both 6*H*- and 5*H*-indolo[2,3-*b*]quinoline series on the calf thymus DNA thermal denaturation was also estimated. In our SAR study we made use of a synthetically obtained basic system of 6*H*-indolo[2,3-*b*]quinoline represented by nonsubstituted compound **4a**, its C-11 monomethyl derivative **4b**, dimethyl derivatives **4c**, substituted at C-2 and C-11, **4d**, having substituents at C-4 and C-11, **9a**, with substituents at C-9 and C-11, and three-methyl-bearing derivative **9b** having substituents at C-2, C-9, and C-11. These compounds were converted into 5*H*-indolo[2,3-*b*]quinolines (*i.e.*, compounds **10a–f**) by introducing the methyl group at the pyridine nitrogen.

It became obvious that the presence of the methyl group at the pyridine nitrogen in the indolo[2,3-*b*]-quinolines increased their basic properties. Thus, the compounds of the 6*H* series have pK_a values in the range 5.55–5.98, whereas pK_a values for 5*H*-indolo[2,3-*b*]quinolines were found to be in the range 7.24–7.68. This indicates that 5*H*-indolo[2,3-*b*]quinolines are partially protonated under physiological conditions (pH 7.4). At low pH values, these compounds occurred in the form of salts, and due to a better water solubility, their log *P* values became negative.

Some distinct difference in the antimicrobial and cytotoxic activity of both 6*H*- and 5*H*-indolo[2,3-*b*]-quinoline series were observed. They can be definitely attributed to the presence of the methyl group at the pyridine nitrogen. While none of the compounds of the 6*H* series was active in reasonable concentration, the members of the 5*H* series were strongly cytotoxic against KB cells, showing ID_{50} values in the range 2×10^{-3} to 9×10^{-3} μmol/mL, and they inhibited the growth of Gram-positive bacteria and pathogenic fungi at MIC 3×10^{-2} to 2.5×10^{-1} μmol/mL. Apart from the strong influence of the methyl group at N-5 on the cytotoxicity and antimicrobial properties of indolo[2,3-*b*]quinolines, the contribution of the methyl substituents at carbons of the aromatic area within indolo[2,3-4b]quinolines was also observed. The highest activity with respect to both microorganisms and KB cells was that of the **10f** compound from the 5*H* series, bearing four symmetrically distributed methyl groups. In the 6*H* series, none of the derivatives (even compound **9b** bearing the same pattern of methyl groups distribution in the aromatic area of **10f**) was effective against microorganisms and KB cells. Our observations are generally in agreement with the findings of Schenkman *et al.*[11] Although the cytotoxic and antimicrobial activity of both 5*H*- and 6*H*-indolo[2,3-*b*]quinolines varies considerably, the UV absorption spectra of both series in the presence of the calf thymus DNA show similar changes. We observed that the titration of these compounds with calf thymus DNA induced hypochromic and bathochromic effects and the appearance of the isosbestic point in the range of 345–348 nm and 350–354 nm, for 6*H*- and 5*H*-indolo-[2,3-*b*]quinolines, respectively. The differences in the basic properties of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines, as well as their cytotoxic and antimicrobial properties, were reflected in the stability of their calf thymus DNA complex. In our experiments the interaction of both 5*H*- and 6*H*-indolo[2,3-*b*]quinoline series with DNA was established in terms of their effect on the rise in the denaturation temperature of calf thymus DNA. Measurements were carried out under standard conditions[21] at pH 7.0 at which the quinolinium form in the 5*H* series is predominant.[4] We found that all 5*H*-indolo[2,3-*b*]-quinolines (*i.e.*, compounds characterized by methyl group substitution at pyridine nitrogen stabilizing their positive charge) accounted for a marked rise in the denaturation temperature of calf thymus DNA. No such differences in ΔT_m were observed for the 6*H* series showing lower pK_a values than those for the 5*H* derivatives. The contribution of the position and number of the methyl groups substituted into the aromatic area to the properties of indolo[2,3-*b*]quinolines also became distinct. Thus, in the 5*H* series, ΔT_m rose from 5.2 °C to 19 °C for unsubstituted **10a** and four-methyl-group-substituted **10f**. Analogous compounds, **4a** and **9b**, belonging to the inactive 6*H* series, accounted for a markedly smaller increase of ΔT_m, which was only from 0.3 °C to 1.6 °C, respectively.

The structural relation between 5*H*- and 6*H*-indolo[2,3-*b*]quinolines concerning their antimicrobial, cytotoxic activity and interaction with DNA corresponds well with their ability to modulate calf thymus topoisomerase II activity *in vitro*. Although none of the 6*H*-indolo[2,3-*b*]quinolines was able to generate *in vitro* a linear form of pSP65 DNA in the presence of calf thymus topoisomerase II, the compounds belonging to the 5*H* series stimulated efficiently pSP65 DNA cleavage in analogous tests, as it has been illustrated by the bell-shaped dose–response curves (the shape of the curves seems to corroborate the intercalative manner of 5*H*-indolo[2,3-*b*]quinolines binding to DNA[3,4,15]). In that case compound **10f** producing the highest number of pSP65 DNA breaks at 0.4 μM was also the most active.

Summing up, we arrived at the following findings: (1) both N-5 methyl-unsubstituted (series 6*H*) and N-5 methyl-substituted (series 5*H*) indolo[2,3-*b*]quinolines differ considerably between each other in the values of pK_a which range from 5.55 to 5.98 and from 7.24 to 7.68 for 6*H* and for 5*H*, respectively; (2) the log *P* values are lower for 5*H*-indolo[2,3-*b*]quinolines, amounting to −1.23; (3) all compounds comprising the 5*H* system showed antimicrobial activity against Gram-positive bacteria and pathogenic fungi, displayed cytotoxic activity against KB cells, and were able to generate *in vitro* cleavable complexes in pSP65 DNA through the interaction with calf thymus topoisomerase II; (4) none of the representatives of the 6*H*-indolo[2,3-*b*]quinoline series was active in analogous tests; (5) indolo[2,3-*b*]quinoline derivatives belonging to the 5*H* series increased the T_m calf thymus DNA to 19 °C, but no such increase was observed within the 6*H* series; (6) UV absorption spectra of both 6*H*- and 5*H*-indolo[2,3-*b*]quinolines showed that the titration of these compounds with calf thymus DNA produced similar changes in every instance: hypochromic effect, bathochromic shift, and appearance of an isosbestic point. Analogous changes in UV spectra caused by the presence of DNA have been reported for compound **10b**,[4] ellipticine,[13] and several other DNA-binding compound.[5,12,15]

On comparing the properties of cytotoxic 5*H*-indolo[2,3-*b*]quinolines (particularly these of **10b**, regarded as our "leading compound") with the properties of ellipticine, as well as taking into account relevant literature data, we arrived at the following conclusions: (1) the lipophilic properties of 5*H*-indolo[2,3-*b*]quinolines (including **10b**) and ellipticine are similar, as it may be inferred from their log *P* values; (2) the pK_a values for 5*H*-indolo[2,3-*b*]quinolines, **10b**,[4] and ellipticine[14] fall in the same range, pH 7–8; (3) cytotoxic activity of **10b** and other 5*H*-indolo[2,3-*b*]quinolines against KB cells (expressed in ID_{50}) is about 10 times as high as that of ellipticine; (4) each compound of the 5*H*-indolo[2,3-*b*]-quinoline series (including **10b**[4]) produces *in vitro* calf thymus topoisomerase II-induced cleavable complexes of pSP65 DNA at concentrations ranging between 0.4 and 10 μM. Under similar conditions, ellipticine shows no ability to induce cleavable complexes mediated by topoisomerase II,[3] even though ellipticine derivatives do (in the ellipticine series, the ability to induce cleavable complexes is always associated with the electron-donating properties of the phenolic group[3]); (5) the ΔT_m values found for **10b** and ellipticine are similar, amounting to 11 °C and 9.8 °C, respectively, the ΔT_ms of other 5*H*-indolo[2,3-*b*]quinolines are in the range 5.2–19 °C; (6) titration of the solution of 5*H*-indolo[2,3-*b*]quinolines, **10b**,[4] and ellipticine[14] with calf thymus DNA modulates the UV absorption spectra in a similar manner, producing the hypochromic effect and bathochromic shift and contributing to the appearance of the isosbestic point.

In summation, from the data reported in the literature and from the results obtained in our study, it may be inferred that the analogy between 5*H*-indolo[2,3-*b*]-quinolines and ellipticines is only apparent. Although the representative compound **10b** (which bears the greatest structural similarity to ellipticine) and ellipticine itself are intercalators of DNA[3,4] and even though their physicochemical properties are similar, the difference in the cytotoxic activity between the two species is striking. The compound **10b** shows a cytotoxic activity which is about 10 times as high as that of ellipticine (the same holds for the other methyl derivatives of the 5*H* series). Compound **10b** (and the others of the 5*H* series) is also able to induce *in vitro* calf thymus topoisomerase II-mediated cleavable complexes,[4] but ellipticine does not.[9] Our results clearly show that even a slight variation in the structure of indolo[2,3-*b*]quinolines may lead to remarkable changes in their activity. The same holds for ellipticines, anthracyclines and other antitumor DNA-binding.[3,5,15]

In this paper we provide only preliminary data on the interaction of indolo[2,3-*b*]quinolines with DNA. Further investigations on these and several other derivatives comprising such a system are underway, and the results will be reported in a separate paper. We hope that these easy-to-synthesize 5*H*-indolo[2,3-*b*]quinoline-based compounds should serve as the basic system for the molecular modeling[16] of novel and more efficient topoisomerase II inhibitors.

Experimental Section

Methods. Melting points were taken in open capillary tubes on a Kofler type apparatus and were uncorrected. ^{1}H NMR spectra were recorded on JEOL JNM-4H-100 (100 MHz) or Varian Gemini 200 (200 MHz) spectrometers. Chemical shifts are expressed in ppm using TMS as an internal standard. The IR spectra were taken in KBr pellets using Beckman 4240 spectrometer. The UV spectra were measured in dioxane with a Beckman Acta M-VI spectrometer. The MS spectra were obtained with an LKB-9000a apparatus (−70 eV). Elemental microanalyses were performed by the Analytical Laboratory of the Institute of Organic Chemistry (only symbols of the elements analyzed are given; the elements were within 0.4% of the theoretical values). Column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh). The purity and identity of the compounds was checked out by TLC using Merck DC-Alufolien Kieselgel F_{254} and by HPLC analysis.

Abbreviations: DMSO = dimethyl sulfoxide, DMF = dimethylformamide, MTT = 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium blue).

Synthesis of 2-(1-Benzotriazolo)quinolines 3. General Procedure. The mixture of chloroquinoline **1**[17] (0.1 mol) and benzotriazole **2** (12.0 g, 0.1 mol) was heated until exothermic reaction occurred (internal temperature 110–120 °C). When the reaction stopped, the solid was crystallized from DMF/H_2O to afford pure compound **3**.

2-(1-Benzotriazolo)quinoline (3a):[2] yield 68%; mp 148–150 °C.

2-(1-Benzotriazolo)-4-methylquinoline (3b):[2] yield 73%; mp 163–165 °C.

2-(1-Benzotriazolo)-4,6-dimethylquinoline (3c): yield 71%; mp 165–167 °C; IR ν 1595, 1485, 1040, 740 cm^{-1}. Anal. ($C_{17}H_{14}N_4$): C, H, N.

2-(1-Benzotriazolo)-4,8-dimethylquinoline (3d): yield 65%; mp 147–149 °C; IR ν 1305, 1050, 870, 765, 745 cm.$^{-1}$ Anal. ($C_{17}H_{14}N_4$): C, H, N.

Synthesis of 2-(5-Methyl-1-benzotriazolo)quinolines 8. General Procedure. The mixture of chloroquinoline **5** or **1a** (0.1 mol) and 4-methyl-2-nitroaniline (**6**) (15.2 g, 0.1 mol) was heated at 150–160 °C for 10 h, and the resulting solid was recrystallized from ethanol containing 10% of ammonia to give pure compound **7a** or **7b**.

4-Methyl-2-(4-methyl-2-nitroanilino)quinoline (7a): yield 75%; mp 151–153 °C; IR ν 3330, 1525 cm^{-1}. Anal. ($C_{17}H_{15}N_3O_2$): C, H, N.

4,6-Dimethyl-2-(4-methyl-2-nitroanilino)quinoline (7b): yield 97%; mp 191–193 °C; IR ν 3310, 1595, 1570, 1515, 1440, 1345, 1215, 1210, 1140 cm^{-1}. Anal. ($C_{18}H_{17}N_3O_2$) C, H, N.

Compound **7** (0.1 mol) was added portionwise to a stirred and cooled solution of $SnSl_2{\cdot}2H_2O$ (0.5 mol) in a 36% HCl–ethanol (1:2) mixture (360 mL). The whole was heated for 30 min on a steam bath and refrigerated overnight. The precipitate was collected, added to a 20% KOH solution (250 mL), and stirred at ambient temperature for 1 h. The product was filtered off, washed with H_2O, dissolved in a 20% HCl–ethanol (1:1) mixture (300 mL) and diazotized at 5–10 °C, with a solution of $NaNO_2$ (0.11 mol) in H_2O (75 mL). The precipitate was collected, washed with H_2O, and recrystallized from DMF to give triazole **8**.

4-Methyl-2-(5-methyl-1-benzotriazolo)quinoline (8a): yield 75%; mp 150–152 °C; IR ν 1600, 1425, 1050, 910 cm^{-1}. Anal. ($C_{17}H_{14}N_4$): C, H, N.

4,6-Dimethyl-2-(5-methyl-1-benzotriazolo)quinoline (8b): yield 95%; mp 181–182 °C; IR ν 1600, 1590, 1490, 1085, 1040, 810 cm^{-1}. Anal. ($C_{18}H_{16}N_4$): C, H, N.

Decomposition of Triazoles 3 and 8. General Procedure. Triazole **3** or **8** (0.1 mol) was mixed with polyphosphoric acid (150 mL), and the mixture was heated until gas evolution ceased and then heated to 180 °C. After cooling, the syrup was poured into iced water (2 L), and the precipitate was collected, washed with H_2O, heated on a steam bath with 25% ammonia (100 mL), and filtered off. The product was then washed with H_2O and crystallized from pyridine to yield indoloquinoline **4** or **9**.

***6H*-Indolo[2,3-*b*]quinoline (4a)**:[2] yield 30%; mp above 300 °C.

11-Methyl-6*H*)-indolo[2,3-*b*]quinoline (4b):[2] yield 40%; mp 270–272 °C.

2,11-Dimethyl-6*H*-indolo[2,3-*b*]quinoline (4c): yield 32%; mp 287–289 °C; NMR (100 MHz, CF_3COOH) δ 8.30–8.15 (m, 2H), 7.85 (m, 2H), 7.60 (m, 3H), 3.33 (s, 3H), 2.70 (s, 3H); IR ν 3120–2650, 1615, 1305, 735 cm^{-1}; MS (*m/e*, rel intensity) 246 (100, M$^{\bullet+}$). Anal. ($C_{17}H_{14}N_2$): C, H, N.

4,11-Dimethyl-6*H*-indolo[2,3-*b*]quinoline (4d): yield 43%; mp 236–238 °C; NMR (100 MHz, CF_3COOH) δ 8.36–8.21 (m, 2H), 7.93–7.45 (m, 5H), 3.34 (s, 3H), 2.83 (s, 3H); IR ν 3130–2700, 1605, 1385, 1365, 1245, 715 cm^{-1}; MS (*m/e*, rel intensity) 246 (100, M$^{\bullet+}$), 231 (21). Anal. ($C_{17}H_{14}N_2$) C, H, N.

9,11-Dimethyl-6*H*-indolo[2,3-*b*]quinoline (9a): yield 25%; mp above 300 °C; NMR (100 MHz, CF_3COOH) δ 8.41 (d, 1H, J = 7 Hz), 8.03–7.62 (m, 4H), 7.37 (m, 2H), 3.28 (s, 3H), 2.53 (s, 3H); IR ν 3150–2850, 1595, 750 cm^{-1}; MS (*m/e*, rel intensity) 246 (100, M$^{\bullet+}$), 231 (6). Anal. ($C_{17}H_{14}N_2$): C, H, N.

2,9,11-Trimethyl-6*H*-indolo[2,3-*b*]quinoline (9b): yield 18%; mp above 300 °C; NMR (200 MHz, DMSO-d_6) δ 11.42 (bs, 1H), 8.10 (d, 2H, J = 7 Hz), 7.84 (d, 1H), 7.53 (dd, 1H, J = 8.5, 1.5 Hz), 7.37 (d, 1*H*, H = 8 Hz), 7.32 (dd, 1H, J = 8, 1.5 Hz), 3.35 (s, 3H), 3.15 (s, 3H), 2.55 (s, 3H); IR ν 3150–2860, 1630, 1605, 1590, 820 cm^{-1}; MS (*m/e*, rel intensity) 260 (100, M$^{\bullet+}$), 245 (16). Anal. ($C_{18}H_{16}N_2$): C, H, N.

5-Methyl-5*H*-indolo[2,3-*b*]quinolines (10). General Procedure. Compound **4** or **9** (0.01 mol) and dimethyl sulfate (1.5 mL) in toluene (20 mL) were heated in a sealed tube at 150–160 °C for 12 h. After cooling, the precipitate was filtered off, washed with acetone, suspended in H_2O (100 mL), and alkalized with 20% NaOH. The mixture was extracted with $CHCl_3$, and the extract was dried over $MgSO_4$ and chromatographed on silica gel column. The orange product **10** was eluted with $CHCl_3$, the solvent evaporated, and the residue recrystallized.

5-Methyl-5*H*-indolo[2,3-*b*]quinoline (10a):[2] yield 42%; mp 104–108 °C (from hexane).

5,11-Dimethyl-5*H*-indolo[2,3-*b*]quinoline (10b):[2] yield 65%; mp 222–223 °C (from benzene).

2,5,11-Trimethyl-5*H*-indolo[2,3-*b*]quinoline (10c): yield 67%; mp 149–151 °C (from benzene/hexane); NMR (100 MHz, $CDCl_3$) δ 7.90 (d, 1H, J = 8 Hz), 7.71–7.64 (m, 2H), 7.51–7.04 (m, 4H), 4.03 (s, 3H), 2.76 (s, 3H), 2.47 (s, 3H); IR ν 1575, 1495, 1285 cm^{-1}; UV (λ_{max}, log ϵ) 281 nm (4.76); MS (*m/e*, rel intensity) 260 (100, M$^{\bullet+}$), 245 (30). Anal. ($C_{18}H_{16}N_2$): C, H, N.

4,5,11-Trimethyl-5*H*-indolo[2,3-*b*]quinoline (10d): yield 7%; mp 189–191 °C (from hexane); NMR (100 MHz, $CDCl_3$) δ 7.95 (d, 1H, J = 8 Hz), 7.84–705 (m, 5H), 4.29 (s, 3H), 2.85 (s, 3H), 2.77 (s, 3H); IR ν 1485, 1445, 1425, 1240, 760, 725 cm^{-1}; UV (λ_{max} log ϵ) 285 nm (4.79); MS (*m/e*, rel intensity) 260 (84, M$^{\bullet+}$), 245 (100). Anal. ($C_{18}H_{16}N_2$) C, H, N.

5,9,11-Trimethyl-5*H*-indolo[2,3-*b*]quinoline (10e): yield 36%; mp 211–213 °C (from benzene–hexane); NMR (100 MHz, $CDCl_3$) δ 8.00 (d, 1H, J = 8 Hz), 7.76 (d, 1H, J = 5 Hz), 7.56 (d, 1H J = 8 Hz), 7.52 (s, 1H), 7.42–7.23 (m, 3H), 4.57 (s, 3H), 2.86 (s, 3H), 2.48 (s, 3H); IR ν 1495, 1470, 1460, 1290, 1245, 745 cm^{-1}; UV (λ_{max}, log ϵ) 284 nm (4.78); MS (*m/e*, rel intensity) 260 (100, M$^{\bullet+}$) 245 (23). Anal. ($C_{18}H_{16}N_2$): C, H, N.

2,5,9,11-Tetramethyl-5*H*-indolo[2,3-*b*]quinoline (10f): yield 40%; mp 166–168 °C (from benzene–hexane); NMR (200 MHz, $CDCl_3$) δ 7.93–7.90 (m, 2H), 7.63 (d, 1H, J = 8.5 Hz), 7.56–7.51 (m, 2H), 7.34 (dd, 1H, J = 8.0, 1.5 Hz), 4.26 (s, 3H), 3.06 (s, 3H), 2.56 (s, 3H), 2.54 (s, 3H), IR ν 1645, 1500, 1465, 1285, 1240, 1180, 810 cm^{-1}; UV (λ_{max}, log ϵ) 287 nm (4.69); MS (*m/e*, rel intensity) 274 (100, M$^{\bullet+}$), 259 (37). Anal. ($C_{19}H_{18}N_2$): C, H, N.

Determination of Octanol–Water Partition Coefficients. Lipophilicity of 5*H*- and 6*H*-indolo[2,3-*b*]quinolines was determined by estimation of log P for individual compounds, using the microscale-flask method combined with HPLC analysis of both water (buffer) and octanol phase.[18] A sample (20 μL) of each indolo[2,3-*b*]quinoline (0.01M solution in DMSO) was dissolved in 0.5 mL of octanol saturated with appropriate buffer (0.025 M citric buffer, pH 2.2 and pH 5.0, or 0.025 M bicarbonate buffer, pH 9.0). The solution was mixed thoroughly with 0.5 mL of an appropriate buffer saturated with octanol in 1.5 mL Eppendorf tube at 22 °C for 2 h. After standing for 15 min the phases were separated by contrifugation at 14000*g* for 5 min, and the concentration of indolo[2,3-*b*]quinolines in each phase was determined by HPLC analysis. For injection into HPLC column, a 20 μL volume sample was used. The partition coefficient was calculated by dividing the absorbance area of an appropriate integrated peak from the octanol phase by that of the area from the buffer phase. Chromatography of indolo[2,3-*b*]-quinolines was performed, using a 4.6 × 250 mm Supelcosil LC-18-DB deactivated reversed-phase column. The column was eluted with 0.01% sulfuric acid in methanol at 42 °C at a flow rate of 1.0 mL/min. The presence of indolo[2,3-*b*]quinolines was detected with a Hewlett-Packard 1050 variable-wavelength detector at the wavelength of their isosbestic points. Peak identity was determined from coincidence of retention time with standards and by comparing of-the-fly UV spectra obtained with a Waters 990+ diode-array detector. Peak areas were measured with a Hewlett-Packard 3396A computing integrator.

Determination of pK_a. The values of pK_a of both 6*H*- and 5*H*-indolo[2,3-*b*]quinolines were deduced from their UV absorption spectra taken with a Cary 3 UV–vis Varian spectrometer, by the same method as reported for ellipticine.[14] The spectra of drugs (dissolved initially in DMSO) at concentration of 3 μM were recorded in 0.025 M buffers of various pH values (pH 2.2–4 citric buffer, pH 5–6 acetate buffer, pH 7.0–8.0 phosphate buffer, pH 9 borate buffer) in 5 cm pathlength cuvette at 37 °C. The final concentration of DMSO was 5%.

Antimicrobial Susceptibility Testing. Minimum inhibitory concentrations (MICs) of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines were determined according to routine procedure.[19] The

tested compounds were dissolved in DMSO and serially diluted 2-fold in sterile distilled water.

Cytotoxicity. The effect of 6*H*- and 5*H*-indolo[2,3-*b*]-quinolines on the growth inhibition of the KB cells was performed according to the MTT procedure.[20]

Determination of ΔT_m. Measurements were carried out using a Cary 3 Varian spectrometer equipped with DNA Thermal Application Program according to the Cory method.[21] Ellipticine and adriamycin (Sigma) were used as standards. The experiments were run in six parallel cuvettes equipped with an internal temperature probe and calf thymus DNA as a control. Measurements at the ratio of drug/DNA (1:10) were taken in the temperature range of 40–95 °C with a speed of 0.3 °C/min at pH 7.0.

DNA Binding Experiments. The interaction of 6*H*- and 5*H*-indolo[2,3-*b*]quinolines with calf thymus DNA was studied by UV–vis spectrometry using a Cary 3 Varian spectrometer. Experiments were run in 5-cm pathlength cuvettes at 37 °C. The effect of the increasing amounts of DNA on the UV absorption spectra of the 3 μM 5*H*- and 6*H*-indolo[2,3-*b*]-quinolines was studied in the buffer as used for the DNA thermal denaturation study (5.0 mM Tris-HCl, pH 7.0). The final concentration of DMSO was 5%.

Topoisomerase II Activity. The activity of the 5*H*- and 6*H*-indolo[2,3-*b*]quinolines was determined *in vitro* using the calf thymus topoisomerase II and pSP65 as substrate DNA under conditions established earlier.[4]

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