

DRUG SYNTHESIS

SYNTHESIS OF 6-SUBSTITUTED 6*H*-INDOLO[2,3-*b*]QUINOLINES
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Abstract: A systematic investigation into the impact of the substituents introduced into the indolo[2,3-*b*]quinoline system is described. The findings clearly demonstrate that the compounds bearing a methyl group or a longer aliphatic chain at the *N*-6 position are inactive against prokaryotic and eukaryotic cells. The introduction of alkyl-amino-alkyl substituent at the *N*-6 position of indolo[2,3-*b*]quinoline accounts for the appearance of the antimicrobial and cytotoxic properties. The cytotoxicity against oral epidermoid carcinoma KB (ID₅₀) is in the range from 2.0 to 9.0 μM, and the antimicrobial activity (MIC) falls between 0.03 and 0.50 mM. The structural relation within 6*H*-indolo[2,3-*b*]quinolines, concerning their antimicrobial and cytotoxic activity, corresponds well with their ability to bind DNA and to inhibit topoisomerase II activity.

Keywords: indolo[2,3-*b*]quinoline, cytotoxic agent, antimicrobial agent, topoisomerase II inhibitor.

Earlier investigations into indolo[2,3-*b*]quinolines show that compounds belonging to the 5*H*-series, display the strong antimicrobial and cytotoxic properties and are able to modulate topoisomerase II activity (1). In SAR study on indoloquinolines as cytotoxic agents, a series of 6*H*-indolo[2,3-*b*]quinolines bearing an alkyl or alkyl-amino-alkyl group at *N*-6 was synthesized. In this paper, the method of synthesis of 6*H*-indolo[2,3-*b*]quinoline derivatives is presented. These compounds were evaluated for their cytotoxicity against the KB cell line *in vitro* and for the antimicrobial activity against Gram-negative and Gram-positive bacteria and pathogenic fungi. The ability of indolo[2,3-*b*]quinoline derivatives to induce the formation of calf thymus DNA topoisomerase II mediated cleavable complexes *in vitro*, using pSP 65 DNA as a substrate, was also estimated. The interaction of these compounds with calf thymus DNA was studied by UV-VIS spectroscopy. The stability of the indolo[2,3-*b*]quinoline-DNA complexes obtained was determined by measuring the differences in the denaturation tem-

perature of calf thymus DNA in the presence of these compounds.

EXPERIMENTAL

Methods

Melting points (m.p.'s), determined on a Kofler-type apparatus were uncorrected. The IR spectra were recorded with a Perkin-Elmer 1640 FTIR spectrophotometer in KBr pellets. The ¹H NMR spectra were measured with a Varian Gemini 200 spectrometer in CDCl₃; the chemical shifts were expressed in ppm (δ) with TMS as an internal standard. The MS spectra were recorded with an Intetra AMD-604 apparatus (-70 eV). The elemental analyses were performed by the Analytical Laboratory, Institute of Organic Chemistry of Polish Academy of Sciences. UV absorption spectra were taken with Cary 3 UV-VIS Varian Spectrometer. Purity and identity of the products were checked by thin layer chromatography (TLC) with Merck DC-Alufolien Kieselgel 60 F₂₅₄. The chemicals and solvents were purchased from Aldrich

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Company. Column chromatography was performed on Merck silica gel 60 (230–400 mesh).

2-(Benzotriazol-1-yl)-6-fluoro-4-methylquinoline (**6**)

2-Chloro-6-fluoro-4-methylquinoline **5** (39.1 g, 0.02 mole) and benzotriazole **6** (23.8 g, 0.01 mole) were heated and stirred until the melted form and an exothermic reaction occurred (100–110°C). The heating mantle was then removed (at 140°C) and the mixture was stirred until solidified. The solid was recrystallized from DMF–water to afford triazole **6** in yield 50.0 g (90%); colorless crystals, m.p. 184–186°C; IR (n, cm⁻¹) 1495, 1240, 1050. Analysis: for C₁₆H₁₁N₄F (278.30), calcd: 69.06% C; 3.98% H; 20.13%N; found: 69.18% C; 3.81% H; 20.15% N.

2-(4-Fluoroanilino)-4-methylquinoline (**9**)

2-Chloro-4-methylquinoline **7** (8.9 g, 0.05 mole) and 4-fluoroaniline **8** (6.7 g, 0.06 mole) were heated and stirred until an exothermic reaction occurred (temperature increased from 120°C to 210°C). After that, the mixture was heated at 180–200°C for 10 min., cooled and alkalized with 10% ammonia. The product was extracted with chloroform (3 × 50 ml), dried over magnesium sulfate and evaporated. The residue was crystallized from toluene–hexane to give **9** in yield 11.5 g (91%); IR (n, cm⁻¹) 3430, 1615, 1535, 1500, 1410, 1200, 835, 745. Analysis: for C₁₆H₁₃N₂F (252.29), calcd: 76.17% C; 5.19% H; 11.10% N; found: 76.17% C; 5.16% H; 11.11% N.

2-(4-Fluoro-2-nitroanilino)-4-methylquinoline (**10**)

Anilinoquinoline **9** (11.5 g, 0.046 mole) was suspended in 30% HNO₃ (70 ml), NaNO₂ (100 mg) was added and the mixture was stirred on a steam bath for 1 h and left overnight at room temperature. Then, the solution was poured into water (200 ml), the precipitate was collected and chromatographed on a silica gel column with hexane–ethyl acetate 5:1. The first orange fraction was collected and evaporated to afford nitroderivative **10** in yield 11.7 g (66%); orange crystals, m.p. 191–192°C (from ethanol); IR (n, cm⁻¹) 3360, 1545, 1515, 1495. Analysis: for C₁₆H₁₂N₃FO₂ (297.30), calcd: 64.64% C; 4.07% H; 14.13% N; found: 64.71% C; 3.85% H; 13.55% N.

2-(2-Amino-4-fluoroanilino)-4-methylquinoline (**11**)

Nitro-compound **10** (11.7 g, 0.039 mole) was added portionwise to the stirred solution of SnCl₂ (60 g) in 36% HCl (120 ml) and ethanol (20 ml). The mixture was then heated on a steam bath for 1 h, cooled and the precipitate of the complex

collected. The complex was dissolved in water (100 ml), alkalized with 20% NaOH (pH = 14) and extracted with ether (5 × 100 ml). The extract was dried over magnesium sulfate and evaporated to give amine **11** in yield 9.7 g % (92%); colorless crystals, m.p. 137–139°C (from aqueous ethanol); IR (n, cm⁻¹) 3400–3200, 1620, 1510. Analysis: for C₁₆H₁₄N₃F (267.30), calcd: 71.89% C; 5.28% H; 15.72% N; found: 72.03% C; 5.25% H; 15.64% N.

2-(5-Fluorobenzotriazol-1-yl)-4-methylquinoline (**12**)

Amine **11** (8.5 g, 0.032 mole) was dissolved in a mixture: 36% HCl, water, ethanol 1:1:1 (100 ml), cooled to 5°C and the solution of NaNO₂ (3.1 g, 0.045 mole) in water (50 ml) was dropped in with stirring. The mixture was then stirred overnight at room temperature and the precipitate of **12** collected; colorless crystals, m.p. 190–191°C (from ethanol), yield 8.0 g (90%); IR (n, cm⁻¹) 1605, 1495, 1310, 1100, 1050, 915. Analysis: for C₁₆H₁₁N₄F (278.30), calcd: 69.06% C; 3.98% H; 20.13% N; found: 69.41% C; 3.68% H; 20.56% N.

2- and 9-Fluoro-11-methyl-6H-indolo[2,3-*b*]quinolines **1i**, **1j**

General procedure.

Tiazole **6** or **12** (5.56 g, 0.02 mole) was suspended in PPA (60 ml) and heated at 140–200°C for 30 min. and at 200–250°C for 20 min., until gas evolution ceased. The solution was then cooled, diluted with ethanol (50 ml), poured into the solution of picric acid (5 g) in 70% aqueous ethanol (150 ml) and heated for 15 min. on a steam bath. The precipitate was collected, washed with water (50 ml) and heated on a steam bath with 8% NaOH (250 ml). The resulting solid was again collected, washed with water (50 ml) and 15% HCl (20 ml) and recrystallized to give desired product.

2-Fluoro-11-methyl-6H-indolo[2,3-*b*]quinoline **1i**

Yield 1.64 g (33%); creamy crystals, m.p. 293–295°C (from ethanol); IR (n, cm⁻¹) 3210–2640, 1400, 1225, 1185, 815; ¹H NMR (CF₃COOH): 8.30 (d, 1H, J=8 Hz); 8.05 (d-d, 1H, J=7–2Hz); 8.01 (d-d, 1H, J=8–5 Hz); 7.76–7.59 (m., 3H); 7.55–7.46 (m., 1H), 3.28 (s, 3H); MS (m/e, rel. int.) 250 (M⁺, 100). Analysis: for C₁₆H₁₁N₂F (250.28), calcd: 76.78% C; 4.43% H; 11.19% N; found: 76.70% C; 4.17% H; 11.31% N.

9-Fluoro-11-methyl-6H-indolo[2,3-*b*]quinoline **1j**

Yield 2.05 g (41%); creamy crystals, m.p. 297–301°C (from ethanol); IR (n, cm⁻¹) 3200–2800, 1620, 1495, 1475, 745; ¹H NMR (CF₃COOH): 8.43

(d, 1H, *J*=8 Hz); 8.03–7.96 (m., 3H); 7.84–7.75 (m., 1H); 7.62 (d–d, 1H, *J*=8–4.5 Hz); 7.38 (t–d, 1H, *J*=9–2.5 Hz), 3.33 (s, 3H); MS (*m/e*, rel. int.) 250 (M^+ , 100). Analysis: for $C_{16}H_{11}N_2F$ (250.28), calcd: 76.78% C; 4.43% H; 11.19% N; found: 76.49% C; 4.23% H; 11.08% N.

Synthesis of 6-substituted indoloquinolines **3** General procedure.

Indoloquinoline **1** (0.01 mole) was suspended in toluene (20 ml), 50% NaOH (10 ml), TBAB (0.5 g) and 1-chloro-*w*-dialkylaminoalkyl hydrochloride **2** ($X=Cl$; $R_4=N(CH_3)_2$, $N(C_2H_5)_2$, 4(3-chlorophenyl)-1-piperidynyl; *n*=2,3) or iodo(bromo)alkyl ($R=alkyl$) **2** (0.012 mole) were added and the mixture was refluxed with vigorous stirring for 2–4 hrs (TLC monitoring). After cooling, the mixture was diluted with water (50 ml) and toluene (50 ml), stirred at room temperature for 5 min. and an organic layer was separated, washed with water (4 × 10 ml) and evaporated. The product **3** was then purified by chromatography on silica gel column with hexane–acetone 5:1 and crystallized from hexane.

6,11-Dimethyl-6*H*-indolo[2,3-*b*]quinoline **3a**: ($R_2=R_3=H$; $R_1=R_4=CH_3$; $n=O$)

Obtained in a reaction of **1b** with methyl sulfate **2a**; m.p. 138–139°C; yield 1.90 g (76%); IR (*n*, cm^{-1}) 1605, 1480, 1400, 760, 740; 1H NMR: 8.27 (d, 1H, *J*=8 Hz); 8.23 (d–m., 1H, *J*=8); 8.14 (d–m., 1H, *J*=8 Hz); 7.72 (d–d–d, 1H, *J*=8–6.5–1.5 Hz); 7.58 (d–d–d, 1H, *J*=9–7–1.5 Hz); 7.48 (d–d–d, 1H, *J*=8–7–1.5 Hz); 7.41 (d, 1H, *J*=8 Hz), 7.32 (d–d–d, 1H, *J*=8–7.5–1); 3.97 (s, 3H); 3.18 (s, 3H). MS (*m/e*, rel. int.) 246 (M^+ , 100), 231 (14). Analysis: for $C_{17}H_{14}N_2$ (246.31); calcd: 82.90% C; 5.73% H; 11.37% N; found: 82.61% C; 5.54% H; 11.09% N.

11-Methyl-6-propyl-6*H*-indolo[2,3-*b*]quinoline **3b**: ($R_2=R_3=H$; $R_1=CH_3$; $R_4=CH_2CH_2CH_3$)

Obtained in a reaction of **1b** with propyl iodide **2b**, m.p. 109–110°C, yield 2.25 g (82%); IR (*n*, cm^{-1}) 1605, 1490, 1475, 1410, 750; 1H NMR: 8.30 (d, 1H, *J*=8 Hz); 8.25 (d–d, 1H, *J*=8–1.5 Hz); 8.13 (d–d, 1H, *J*=8–1.5 Hz); 7.72 (d–d–d, 1H, *J*=8–7–1.5 Hz); 7.57 (d–d–d, 1H, *J*=8–7–1.5 Hz); 7.51–7.42 (m., 2H); 7.31 (d–d–d, 1H, *J*=8–7–1.5 Hz); 7.31 (d–d–d, 1H, *J*=8–7–1.5 Hz); 4.50 (t, 2H, *J*=7 Hz); 3.20 (s, 3H); 2.00 (sextet, 2H, *J*=7 Hz); 1.02 (t, 3H, *J*=7 Hz); MS (*m/e*, rel. int.) 274 (M^+ , 42), 259 (4), 245 (40), 232 (100). Analysis: for $C_{19}H_{18}N_2$ (274.36); calcd: 83.18% C; 6.61% H; 10.21% N; found: 83.09% C; 6.50% H; 10.29% N.

6-Isopentyl-11-methyl-6*H*-indolo[2,3-*b*]quinoline **3c**: ($R_1=R_3=H$; $R_1=CH_3$; $R_4=CH(CH_3)_2$; $n=2$)

Obtained in a reaction of **1b** with isopentyl bromide **2c**; m.p. 110–111°C, yield 3.00 g (95%); IR (*n*, cm^{-1}) 1605, 1495, 1480, 1415, 1385, 745; 1H NMR: 8.33 (d, 1H, *J*=8 Hz); 8.26 (d–d, 1H, *J*=8–1.5 Hz); 8.15 (d–d, 1H, *J*=8–1.5 Hz); 7.70 (d–d–d, 1H, *J*=8–7–1.5 Hz); 7.60–7.25 (m., 4H); 4.51 (t, 2H, *J*=7 Hz); 3.22 (s, 3H); 2.00 (m., 2H); 1.00 (m., 7H); MS (*m/e*, rel. int.) 302 (M^+ , 29); 287 (3), 259 (4), 246 (100), 232 (52). Analysis: for $C_{21}H_{22}N_2$ (302.42); calcd: 83.40% C; 7.33% H; 9.26% N; found: 83.27% C; 7.25% H; 9.31% N.

11-Methyl-6-[2-(dimethylamino)ethyl]-6*H*-indolo[2,3-*b*]quinoline **3d**: ($R_2=R_3=H$; $R_1=CH_3$, $R_4=N(CH_3)_2$; $n=2$)

Obtained in a reaction of **1b** with 2-chloroethyl(dimethyl)amine hydrochloride **2d**; m.p. 97–98°C, yield 2.87 g (95%); IR (*n*, cm^{-1}) 1610, 1495, 1480, 1410, 755, 745; 1H NMR: 8.28 (d, 1H, *J*=8.5 Hz); 8.23 (d–d, 1H, *J*=8.5–1 Hz); 8.12 (d–d, 1H, *J*=8.5–1 Hz); 7.70 (d–d–d, 1H, *J*=8.5–7–1 Hz), 7.56 (d–d–d, 1H, *J*=8.5–7–1 Hz); 7.51–7.43 (m, 2H); 7.30 (d–d–d, 1H, *J*=8.5–7–1 Hz); 4.64 (t, 2H, *J*=7.5 Hz); 3.17 (s, 3H); 2.81 (t, 2H, *J*=7.5 Hz); 2.40 (s, 6H); MS (*m/e*, rel. int.) 303 (M^+ , 4), 245 (8), 232 (72), 58 (100). Analysis: for $C_{20}H_{21}N_3$ (303.41); calcd: 79.17% C; 6.98% H; 13.85% N; found: 79.36% C; 6.97% H; 13.89% N.

6-[2-(Diethylamino)ethyl]-11-methyl-6*H*-indolo[2,3-*b*]quinoline **3e**: ($R_2=R_3=H$; $R_1=CH_3$; $R_4=N(C_2H_5)_2$; $n=2$)

Obtained in a reaction of **1b** with 2-chloroethyl(diethyl)amine hydrochloride **2e**; m.p. 43–45°C, yield 3.0 g (91%); IR (*n*, cm^{-1}) 1605, 1490, 1405, 745, 735; 1H NMR: 8.30–8.20 (m, 2H); 8.11 (d–d, 1H, *J*=8.5–1 Hz); 7.70 (d–d–d, 1H, *J*=8.5–7–1.5 Hz); 7.56 (d–d–d, 1H, *J*=8.5–7–1.5 Hz); 7.51–7.43 (m, 2H); 7.29 (d–d–d, 1H, *J*=8.5–7–1.5 Hz); 4.60 (t, 2H, *J*=7.5 Hz); 3.18 (s, 3H); 2.93 (t, 2H, *J*=7.5 Hz); 2.70 (q, 4H, *J*=7.5 Hz); 1.10 (t, 6H, *J*=7.5 Hz); MS (*m/e*, rel. int.) 331 (M^+ , 2), 259 (3), 245 (10), 232 (39), 86 (100). Analysis: for $C_{22}H_{25}N_3$ (331.46); calcd: 79.72% C; 7.60% H; 12.68% N; found: 80.02% C; 7.71% H; 12.73% N.

11-Methyl-6-[3-(dimethylamino)propyl]-6*H*-indolo[2,3-*b*]quinoline **3f**: ($R_2=R_3=H$; $R_1=CH_3$; $R_4=N(CH_3)_2$; $n=3$)

Obtained in a reaction of **1b** with 3-chloropropyl(dimethyl)amine hydrochloride **2f**; m.p. 76–78°C, yield 1.85 g (58%); IR (*n*, cm^{-1}) 1600, 1575, 1485, 1470, 1400, 735; 1H NMR: 8.28 (d, 1H, *J*=8 Hz); 8.23 (d–d, 1H, *J*=8.5–1 Hz); 8.12

(d-d, 1H, J=8.5–1 Hz); 7.70 (d-d-d, 1H, J=8–7–1.5 Hz); 7.59–7.42 (m, 3H); 7.29 (d-d-d, 1H, J=8–6.5–1.5 Hz); 4.56 (t, 2H, J=7 Hz); 3.17 (s, 3H); 2.37 (t, 2H, J=7 Hz), 2.24 (s, 6H); 2.10 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 317 (M⁺, 22), 302 (2), 259 (22), 246 (100). Analysis: for C₂₁H₂₃N₃ (317.43); calcd: 79.46% C; 7.30% H; 13.24% N; found: 79.45% C; 7.30% H; 12.47% N.

6-[(3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl)-1-methyl-6H-indolo[2,3-*b*]quinoline **3g**: (R₂=R₃=H; R₁=CH₃; R=4-(3-chlorophenyl)piperazin-1-yl; n=3)

Obtained in a reaction of **1b** with 3-[4-(3-chlorophenyl)piperazin-1-yl]propyl chloride **2g**, m.p. 134–135°C, yield 2.0 g (43%); IR (n, cm⁻¹) 1600, 1490, 1475, 1405, 1240, 740; ¹H NMR: 8.32–8.24 (m., 2H), 8.11 (d, 1H, J=9 Hz), 7.72 (d-d-d, 1H, J=8–7–1 Hz), 7.57–7.45 (m., 3H), 7.35–7.25 (m., 1H), 7.16 (t, 1H, J=8 Hz), 6.88–6.73 (m., 3H), 4.63 (t, 2H, J=7 Hz), 3.20 (m., 7H), 2.60–2.45 (m., 6H), 2.20 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 468 (M⁺, 9), 452 (2), 427 (2), 302 (100). Analysis: for C₂₉H₂₉N₄Cl (469.04); calcd: 74.26% C; 6.23% H; 11.95% N; found: 74.16% C; 6.01% H; 11.91% N.

6-[(3-Dimethylamino)propyl]-6H-indolo[2,3-*b*]quinoline **3h**: (R₁=R₂=R₃=H; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1a** with **2f**; m.p. 76–78°C, yield 2.96 g (98%); IR (n, cm⁻¹) 1610, 1490, 1475, 1415, 1400, 740; ¹H NMR: 8.76 (s, 1H), 8.18–8.11 (m., 2H), 8.01 (d-d, 1H, J=81.5 Hz), 7.71 (d-d-d, 1H, J=8.5–7–1.5 Hz), 7.58 (d-d-d, 1H, J=8.5–7–1.5 Hz), 7.53 (m., 1H), 7.45 (d-d-d, 1H, J=8.5–7–1.5 Hz), 7.29 (d-d-d, 1H, J=8.5–7–1.5 Hz), 4.58 (t, 2H, J=7 Hz), 2.41 (t, 2H, J=7 Hz), 2.26 (s, 6H), 2.14 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 303 (M⁺, 27), 245 (20), 232 (100). Analysis: for C₂₀H₂₁N₃ (303.41); calcd: 79.17% C; 6.98% H; 13.85% N; found: 13.85% C; 6.89% H; 13.54% N.

2,11-Dimethyl-6-[3-(dimethylamino)propyl]-6H-indolo[2,3-*b*]quinoline **3i**: (R₁=CH₃; R₅=2CH₃; R₃=H; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1c** with **2f**; m.p. 107–109°C; yield 3.10 g (94%); IR (n, cm⁻¹) 1600, 1485, 1470, 1400, 730; ¹H NMR: 8.28 (d, 1H, J=8 Hz), 8.02 (d, 1H, J=8 Hz), 7.98 (s, 1H), 7.60–7.46 (m., 3H), 7.28 (d-d-d, 1H, J=7.5–6.5–1.5 Hz), 4.55 (t, 2H, J=7 Hz), 3.17 (s, 3H), 2.60 (s, 3H), 2.44 (t, 2H, J=7 Hz), 2.26 (2, 6H), 2.14 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 331 (M⁺, 17), 316 (1), 273 (22), 260 (100). Analysis: for C₂₂H₂₅N₃ (331.46); calcd: 79.72% C;

7.60% H; 12.68% N; found: 79.62% C; 7.64% H; 12.67% N.

4,11-Dimethyl-6-[3-(dimethylamino)propyl]-6H-indolo[2,3-*b*]quinoline **3j**: (R₁=CH₃; R₂=4-CH₃; R₃=H; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1d** with **2f**; yellow oil; yield 2.71 g (82%); IR (n, cm⁻¹) 1605, 1490, 1470, 1405, 1375, 770, 745; ¹H NMR: 8.29 (d, 1H, J=8 Hz), 8.11 (d-d, 1H, J=8–1 Hz), 7.60–7.47 (m., 3H), 7.42–7.26 (m., 2H), 4.58 (t, 2H, J=7 Hz), 3.18 (s, 3H), 2.90 (s, 3H), 2.43 (t, 2H, J=7 Hz), 2.25 (s, 6H), 2.15 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 331 (M⁺, 19), 273 (22), 260 (100). Analysis: for C₂₂H₂₅N₃ (331.46); calcd: 79.72% C; 7.60% H; 12.68% N; found: 79.66% C; 7.80% H; 12.61% N.

9,11-Dimethyl-6-[3-(dimethylamino)propyl]-6H-indolo[2,3-*b*]quinoline **3k**: (R₁=R₃=CH₃; R₂=H; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1e** with **2f**; m.p. 80–82°C; yield 3.30 g (quant.); IR (n, cm⁻¹) 1615, 1580, 1490, 1460, 1400, 800, 760; ¹H NMR: 8.24 (d-d, 1H, J=8–1 Hz), 8.13–8.08 (m., 2H), 7.69 (d-d-d; 1H, J=8–7–1.5 Hz), 7.47 (d-d-d, 1H, J=8–7–1.5 Hz), 7.38 (m., 2H), 4.55 (t, 2H, J=7 Hz), 3.19 (s, 3H), 2.57 (s, 3H), 2.39 (t, 2H, J=7 Hz), 2.24 (s, 6H), 2.14 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 331 (M⁺, 18), 273 (21), 260 (100). Analysis: for C₂₂H₂₅N₃ (331.46); calcd: 79.72% C; 7.60% H; 12.68% N; found: 79.80% C; 7.60% H; 12.52% N.

2-Methoxy-11-methyl-6-[3-(dimethylamino)propyl]-6H-indolo[2,3-*b*]quinoline **3l**: (R₁=CH₃; R₂=2-OCH₃; R₃=H; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1f** with **2f**; m.p. 93–94°C; yield 3.40 g (98%); IR (n, cm⁻¹) 1635, 1610, 1480, 1410, 1250, 1235, 820, 750; ¹H NMR: 8.29 (d, 1H, J=8 Hz), 8.04 (d, 1H, J=9 Hz), 7.59–7.37 (m., 4H), 7.27 (d-d-d, 1H, J=8–6.5–1.5 Hz), 4.55 (t, 2H, J=7 Hz), 3.98 (s, 3H), 3.14 (s, 3H), 2.38 (t, 2H, J=7 Hz), 2.24 (s, 6H), 2.11 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 347 (M⁺, 27), 289 (25), 276 (100). Analysis: for C₂₂H₂₅N₃O (347.46); calcd: 76.05% C; 7.25% H; 12.09% N; found: 76.13% C; 7.43% H; 12.16% N.

9-Methoxy-11-methyl-6-[3-(dimethylamino)propyl]-6H-indolo[2,3-*b*]quinoline **3m**: (R₁=CH₃; R₂=H; R₃=OCH₃; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1g** with **2f**; m.p. 90–91°C; yield 3.35 g (96%); IR (n, cm⁻¹) 1620, 1585, 1490, 1465, 1300, 1215, 1180, 1050, 745; ¹H NMR: 8.23 (d-d, 1H, J=8.5–1.5 Hz), 8.10 (d-d, 1H, 8.5–1.5 Hz), 7.84 (d, 1H, J=2.5 Hz), 7.69

(d-d-d, 1H, J=8.5-7.5-1.5 Hz), 7.45 (d-d-d, 1H, J=8.5-7.5-1.5 Hz), 7.42 (d, 1H, J=8.5 Hz), 7.19 (d-d, 1H, J=8.5-2.5 Hz), 4.58 (t, 2H, J=7 Hz), 3.95 (s, 3H), 3.20 (s, 3H), 2.39 (t, 2H, J=7 Hz), 2.25 (s, 6H), 2.10 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 347 (M⁺, 28), 289 (27), 276 (100). Analysis: for C₂₂H₂₅N₃O (347.46); calcd: 76.05% C; 7.25% H; 12.09% N; found: 75.95% C; 7.28% H; 11.78% N.

11-Methyl-6-[3-(dimethylamino)propyl]-9-nitro-6*H*-indolo[2,3-*b*]quinoline **3n**: (R₁=CH₃; R₂=H; R₃=NO₂; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1h** with **2f**; m.p. 141-142°C; yield 0.72 g (20%); IR (n, cm⁻¹) 1600, 1515, 1475, 1210, 755; ¹H NMR: 8.51 (d-d, 1H, J=8-1 Hz), 8.28 (d-d, 1H, J=8-1 Hz), 8.16 (dd, 1H, J=8.5-1 Hz), 8.02 (d-d, 1H, J=8.5-1 Hz), 7.76 (d-d-d, 1H, J=8.5-6.5-1 Hz), 7.55 (d-dd, 1H, J=8.5-6.5-1 Hz), 7.34 (t, 1H, J=8 Hz), 4.75 (t, 2H, J=7 Hz), 3.21 (s, 3H), 2.21 (t, 2H, J=7 Hz), 2.15 (s, 6H), 1.90 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 362 (M⁺, 3), 332 (2), 318 (45), 58 (100). Analysis: for C₂₁H₂₂N₄O₂ (362.44); calcd: 69.59% C; 6.12% H; 15.46% N; found: 69.54% C; 6.21% H; 15.08% N.

2-Fluoro-11-methyl-6-[3-(dimethylamino)propyl]-6*H*-indolo[2,3-*b*]quinoline **3o**: (R₁=CH₃; R₂=2-F; R₃=H; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1i** with **2f**; m.p.

140-141°C; yield 3.21 g (96%); IR (n, cm⁻¹) 1610, 1490, 1475, 1405, 735; ¹H NMR: 8.30 (d, 1H, J=8 Hz), 8.09 (d-d, 1H, J=9-5.5 Hz), 7.84 (d-d, 1H, J=10.5-3 Hz), 7.62-7.44 (m, 3H), 7.31 (d-d-d, 1H, J=8-6.5-1.5 Hz), 4.57 (t, 2H, J=7 Hz), 3.15 (s, 3H), 2.42 (t, 2H, J=7 Hz), 2.28 (s, 6H), 2.12 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 335 (M⁺, 20), 278 (23), 264 (100). Analysis: for C₂₁H₂₂FN₃ (335.43); calcd: 75.20% C; 6.61% H; 12.53% N; found: 74.92% C; 6.72% H; 12.44% N.

9-Fluoro-11-methyl-6-[3-(dimethylamino)propyl]-6*H*-indolo[2,3-*b*]quinoline **3p**: (R₁=CH₃; R₂=H; R₃=F; R₄=N(CH₃)₂; n=3)

Obtained in a reaction of **1j** with **2f**; m.p. 98-100°C; yield 3.12 g (93%); IR (n, cm⁻¹) 1610, 1480, 1400, 1285, 1170, 810, 740; ¹H NMR: 8.23 (d-d, 1H, J=8.5-1 Hz), 8.11 (d-d, 1H, J=8.5 Hz), 7.95 (d-d, 1H, J=9.5-2.5 Hz), 7.71 (d-d-d, 1H, J=8-7-1.5 Hz), 7.52-7.39 (m, 2H), 7.28 (t-d, 1H, J=9-2.5 Hz), 4.55 (t, 2H, J=7 Hz), 3.14 (s, 6H), 2.35 (t, 2H, J=7 Hz), 2.24 (s, 3H), 2.10 (q, 2H, J=7 Hz); MS (m/e, rel. int.) 335 (M⁺, 19), 277 (24), 264 (100). Analysis: for C₂₁H₂₂FN₃ (335.43); calcd: 75.20% C; 6.61% H; 12.53% N; found: 75.15% C; 6.77% H; 12.50% N.

Antimicrobial activity study

The MIC values were determined by routine serial dilution method (4).

Table 1. 6*H*-indolo[2,3-*b*]quinolines tested (substituents are numbered as shown in Figure 1)

| Compound | Substituents | | | |
|---------------------|------------------|--------------------|-------------------|---|
| | R ₁ | R ₂ | R ₃ | R ₄ |
| 3a | -CH ₃ | -H | -H | -CH ₃ |
| 3b | -CH ₃ | -H | -H | -(CH ₂) ₂ CH ₃ |
| 3c | -CH ₃ | -H | -H | -(CH ₂) ₂ CH(CH ₃) ₂ |
| 3d | -CH ₃ | -H | -H | -(CH ₂) ₂ N(CH ₃) ₂ |
| 3e | -CH ₃ | -H | -H | -(CH ₂) ₂ N(CH ₂ CH ₃) ₂ |
| 3f | -CH ₃ | -H | -H | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3g | -CH ₃ | -H | -H | 3-[4-(3-chlorophenyl)-piperazin-1-yl]propyl |
| 3h | -H | -H | -H | -(CH ₂) ₂ N(CH ₃) ₂ |
| 3i | -CH ₃ | 2-CH ₃ | -H | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3j | -CH ₃ | 4-CH ₃ | -H | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3k | -CH ₃ | -H | -CH ₃ | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3l | -CH ₃ | 2-OCH ₃ | -H | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3m | -CH ₃ | -H | -OCH ₃ | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3n | -CH ₃ | -H | -NO ₂ | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3o | -CH ₃ | 2-F | -H | -(CH ₂) ₃ N(CH ₃) ₂ |
| 3p | -CH ₃ | -H | -F | -(CH ₂) ₃ N(CH ₃) ₂ |
| DiMIQ ¹⁾ | -CH ₃ | -H | -H | N-5-CH ₃ |

¹⁾ Reference compound: 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline (DiMIQ).^{1,3}

Table 2. Antimicrobial, antifungal and cytotoxic activities of *N*-6-substituted 6*H*-indolo[2,3-*b*]quinolines

| No. of compd. | MIC[mM] ^{a)} | | | | | | ID ₅₀ [M] ^{b)} |
|------------------|-----------------------|-------------|-------------|-------------|-------------|-------------|------------------------------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | KB |
| 3a | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> |
| 3b | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> |
| 3c | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> |
| 3d | <i>n.a.</i> | <i>n.a.</i> | 0.25 | 0.25 | 0.12 | 0.12 | 6.0 ± 0.2 |
| 3e | <i>n.a.</i> | <i>n.a.</i> | 0.25 | 0.25 | 0.12 | 0.12 | 6.0 ± 0.2 |
| 3f | <i>n.a.</i> | <i>n.a.</i> | 0.12 | 0.06 | 0.06 | 0.06 | 4.0 ± 0.3 |
| 3g | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> | <i>n.a.</i> |
| 3h | <i>n.a.</i> | <i>n.a.</i> | 0.50 | 0.50 | 0.25 | 0.25 | 8.0 ± 0.2 |
| 3i | <i>n.a.</i> | <i>n.a.</i> | 0.06 | 0.06 | 0.06 | 0.06 | 2.0 ± 0.1 |
| 3j | <i>n.a.</i> | <i>n.a.</i> | 0.25 | 0.50 | 0.12 | 0.25 | 9.0 ± 0.2 |
| 3k | <i>n.a.</i> | <i>n.a.</i> | 0.06 | 0.06 | 0.06 | 0.06 | 2.0 ± 0.15 |
| 3l | <i>n.a.</i> | <i>n.a.</i> | 0.06 | 0.06 | 0.03 | 0.06 | 2.0 ± 0.1 |
| 3m | <i>n.a.</i> | <i>n.a.</i> | 0.06 | 0.06 | 0.06 | 0.03 | 2.0 ± 0.05 |
| 3n | <i>n.a.</i> | <i>n.a.</i> | 0.25 | 0.25 | 0.06 | 0.06 | 4.0 ± 0.1 |
| 3o | <i>n.a.</i> | <i>n.a.</i> | 0.12 | 0.12 | 0.06 | 0.06 | 2.0 ± 0.05 |
| 3p | <i>n.a.</i> | <i>n.a.</i> | 0.12 | 0.12 | 0.06 | 0.06 | 2.0 ± 0.1 |
| DiMIQ | <i>n.a.</i> | <i>n.a.</i> | 0.12 | 0.06 | 0.03 | 0.06 | 4.0 ± 0.2 |

^{a)} Strains used: 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); the MICs were determined at the concentration range from 0.01 to 5 mM; *n.a.* –no activity (MIC > 5 mM).

^{b)} Cytotoxic activity *in vitro* against oral epidermoid carcinoma KB.

Table 3. Interaction of 6*H*-indolo[2,3-*b*]quinolines with calf thymus DNA and DNA topoisomerase II

| No. of compd. | CT-DNA binding | | | TopoII-induced breaks | |
|------------------|---|---|----------|--------------------------|---------------------------|
| | $\Delta T_m \pm SD$ ^{a)} [°C] | K_{app} ^{b)} [M ⁻¹] | n [-] | %CC ^{c)} [-] | CC _{max} [μM] |
| 3a | 0.3 ± 0 | 2.20 × 10 ⁶ | 0.32 | – | – |
| 3b | 0.3 ± 0 | 2.91 × 10 ⁶ | 0.77 | – | – |
| 3c | 0.4 ± 0.1 | 4.13 × 10 ⁶ | 0.90 | – | – |
| 3d | 8.0 ± 0.2 | 1.49 × 10 ⁶ | 4.02 | 10 | 15 |
| 3e | 9.0 ± 0.1 | 4.01 × 10 ⁶ | 2.02 | 10 | 15 |
| 3f | 11.0 ± 0.1 | 3.25 × 10 ⁶ | 1.22 | 15 | 10 |
| 3g | <i>n.d.</i> | 4.71 × 10 ⁶ | 1.59 | – | – |
| 3h | 5.0 ± 0.2 | 3.62 × 10 ⁶ | 2.03 | 10 | 20 |
| 3i | 14.0 ± 0.1 | 8.75 × 10 ⁶ | 3.13 | 20 | 5 |
| 3j | 12.0 ± 0.2 | 7.64 × 10 ⁶ | 3.55 | 15 | 5 |
| 3k | 14.0 ± 0.1 | 7.83 × 10 ⁶ | 1.90 | 25 | 5 |
| 3l | 15.0 ± 0.2 | 8.27 × 10 ⁶ | 3.17 | 25 | 5 |
| 3m | 15.0 ± 0.2 | 6.98 × 10 ⁶ | 2.47 | 20 | 5 |
| 3n | 11.0 ± 0.2 | 8.01 × 10 ⁶ | 3.84 | 10 | 10 |
| 3o | 13.0 ± 0.1 | 9.33 × 10 ⁶ | 3.30 | 18 | 5 |
| 3p | 13.5 ± 0.2 | 11.1 × 10 ⁶ | 3.29 | 20 | 5 |
| DiMIQ | 11.0 ± 0.2 | 1.43 × 10 ⁶ | 3.36 | 25 | 5 |

^{a)} The increase (ΔT_m) of calf-thymus DNA denaturation temperature (*n.d.* – not determined). ^{b)} The binding constant values (K_{app}) and n –values (nucleotide/binding site) were calculated from Scatchard plot using McGhee and von Hippel model of DNA–ligand interaction.

^{c)} The percent of topoisomerase II – indolo[2,3-*b*]quinoline induced DNA cleavages (%CC) at indicated IQ (CC_{max}) concentration.

Cell cytotoxicity assay

The KB cell line was grown in an opti-MEM medium supplemented with 5% fetal calf serum,

penicillin (50 U/ml), streptomycin (50 μg/ml) and fresh L-glutamine (2 mM). All cell lines were incubated at 37°C in a humidified atmo-

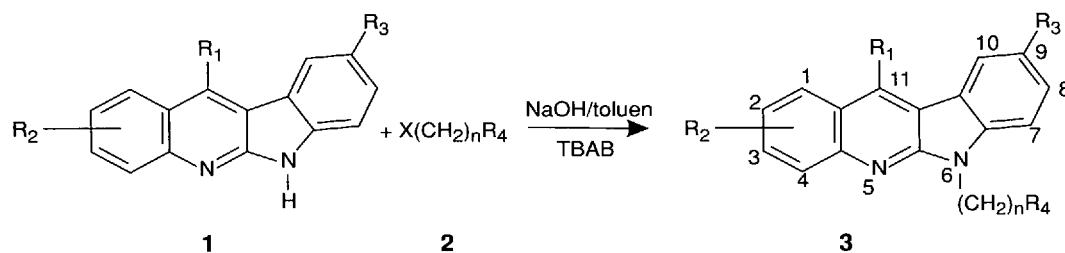


Figure 1. General scheme of synthesis of 6*H*-indolo[2,3-*b*]quinolines. TBAB-tetrabutylammonium bromide; DMF-dimethylformamide.

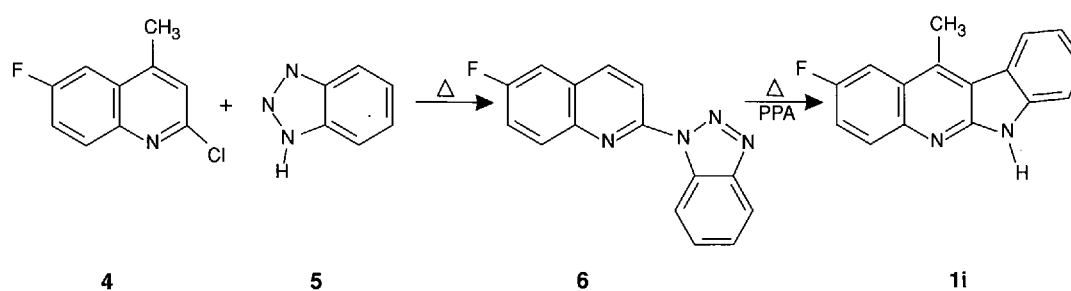


Figure 2. Scheme of synthesis of 2-fluoro-11-methyl-6*H*-indolo[2,3-*b*]quinolines. PPA-polyphosphoric acid.

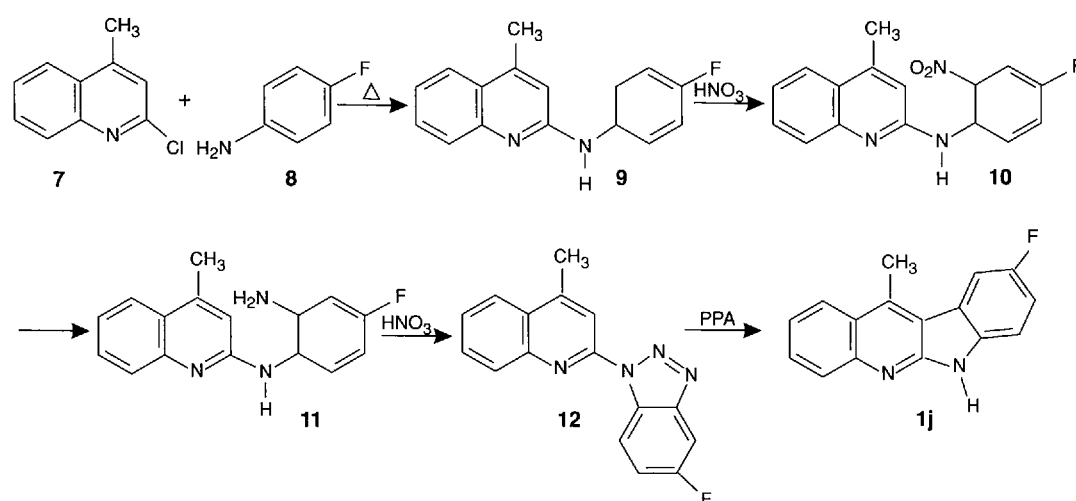


Figure 3. Scheme of synthesis of 9-fluoro-11-methyl-6*H*-indolo[2,3-*b*]quinolines. PPA-polyphosphoric acid.

here of 95% air and 5% CO₂. The concentrations of the drugs causing 50% growth inhibition (ED₅₀) were determined according to the MTT

procedure (5). The ID₅₀ values, presented here, are the mean of nine experiments (3 samples per plate, 3 times).

Determination of ΔT_m

The experiments were carried out, using a Cary 3 Varian spectrometer equipped with a DNA Thermal Application Program according to the Cory method (6). The presented values are the mean of five experiments. The measurements were taken in a 5.0 mM Tris-HCl buffer of pH = 7.0 containing 50 μ M calf thymus DNA (Boeringer-Mannheim, average molecular weight 1.2×10^6 D), 50 μ M EDTA, 5.0 μ M drug, and 5% DMSO.

Determination of binding constants (K_{app})

The measurements were taken with a Cary 3 Varian spectrometer in a 5 cm pathlength cuvette, at indicated wavelength, by subsequent adding of 5 μ l of 1.33 mM DNA (in 0.025 M acetate buffer, pH=5.0) to 10.0 ml of 10 μ M solution of the drug (in 0.025 M acetate buffer pH=5.0, and 5% of DMSO). The binding constant values (K_{app}) and n -values (nucleotide/binding site) were calculated from the Scatchard plot, using the McGhee and von Hippel model of the DNA-ligand interaction (7). The binding constants were evaluated in terms of the Marquardt procedure of non linear regression, using $K_{app} = 1.0 \times 10^6$ [M^{-1}] and $n=2.0$ as starting values, and $\Delta ss = 1.0 \times 10^{-8}$ as a final parameter of optimization. The presented values are the mean of five experiments.

Determination of topoisomerase II inhibitory activity

The measurements were performed using a previously elaborated procedure (%CC – percent of generated pSP65 DNA cleavable complexes vs. total pSP65 DNA quantity) (8). The presented values are the mean of five experiments.

RESULTS AND DISCUSSION

N-6 substituted 6*H*-indolo[2,3-*b*]quinoline derivatives (IQ) were synthesized according to the procedure shown in Figure 1. Some of the starting indoloquinolines were synthesized as described in the following references: IQ ($R_1=R_2=R_3=H$) **1a** (2); 11-methyl-IQ ($R_1=CH_3$, $R_2=R_3=H$) **1b** (2); 2, 11-dimethyl-IQ ($R_1=CH_3$, $R_2=2-CH_3$, $R_3=H$) **1c** (1); 4, 11-dimethyl-IQ ($R_1=CH_3$, $R_2=4-CH_3$, $R_3=H$) **1d** (1); 9, 11-dimethyl-IQ ($R_1=CH_3$, $R_2=H$, $R_3=CH_3$) **1e** (1); 2-methoxy-11-methyl-IQ ($R_1=CH_3$, $R_2=H$, $R_3=CH_3$, $R_4=2-OCH_3$, $R_5=H$) **1f** (2); 9-methoxy-11-methyl-IQ ($R_1=CH_3$, $R_2=H$, $R_3=OCH_3$) **1g** (3), and 9-nitro-11-methyl-IQ ($R_1=CH_3$, $R_2=H$, $R_3=NO_2$) **1h** (2).

2-Fluoro-11-methyl-IQ (**1h**) and 9-fluoro-11-methyl-IQ (**1i**) were synthesized as shown in Figure 2 and Figure 3.

The cytotoxic activity of compounds **3a-p** (Table 1) was evaluated *in vitro* using oral epidermoid carcinoma KB. The antimicrobial properties of 6*H*-indolo[2,3-*b*]quinolines were estimated against the representatives of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*), and pathogenic fungi (*Candida albicans*) and *Trichophyton mentagrophytes*.

The ability of 6*H*-indolo[2,3-*b*]quinolines to induce topoisomerase II-dependent DNA cleavable complexes (using a pSP65 DNA substrate and calf thymus topoisomerase II) was estimated *in vitro* and their interaction with DNA by examining the changes in their UV-VIS spectra in the presence of calf thymus DNA was studied. The influence of indoloquinolines on the calf thymus DNA thermal denaturation was investigated.

It was found that indolo[2,3-*b*]quinolines bearing an alkyl substituent at *N*-6 (compounds **3a-c**) did not show any cytotoxic or antimicrobial properties (Table 2). Although they bind to DNA as it was indicated by their K_{app} values (which ranged from 2.2 to 4.14×10^6 M^{-1}), they did not increase the DNA melting temperature (ΔT_m 0.3 to 0.4°C), and did not modulate the activity of DNA topoisomerase II.

6*H*-Indolo[2,3-*b*]quinolines substituted with an alkyl-amino-alkyl chain at *N*-6 (compounds **3d-f**, **3h-p**) displayed cytotoxic (also in the range from 2.0 to 9.0 μ M) and antimicrobial properties against Gram-positive bacteria and pathogenic fungi (the MIC values fell between 0.03 and 0.5 mM, Table 2). They bind to DNA (K_{app} 1.49 to 11.1×10^6 M^{-1}) and account for a marked rise in the denaturation temperature of calf thymus DNA (ΔT_m fell in the range from 5.0 to 15.0°C). *In vitro*, these compounds produce calf thymus DNA-topoisomerase II cleavable complexes (Table 3).

The compound **3g**, which bears as large a substituent as the 3-[4-(chlorophenyl)piperazin-1-yl]-propyl chain, lacked the capability for stimulating the formation of topoisomerase II mediated cleavable complexes and did not show any cytotoxic or antimicrobial properties, in spite of its ability to bind to DNA.

The following observations emerge from our SAR study: (i) all the 6*H*-indolo[2,3-*b*]quinolines obtained, bind to DNA as manifested by their K_{app} values but there is no clear correlation between the DNA affinity and the biological properties; (ii) the introduction of an alkyl substituent at *N*-6 results in the formation of non-cytotoxic compounds; these derivatives do not form thermally stable complexes

with DNA and do not affect the topoisomerase II activity; (iii) all indolo[2,3-*b*]quinolines, bearing an alkyl-amino-alkyl substituent at *N*-6, are cytotoxic, show antimicrobial properties and inhibit topoisomerase II activity; they also account for a marked rise in the denaturation temperature of calf thymus DNA; (iv) the activity of 6*H*-indolo[2,3-*b*]quinolines depends on the length of the alkyl-amino-alkyl chain (the number of -CH₂- units) and the presence of substituents at C-2 and C-9 position; (v) the introduction of a bulky-end substituent at *N*-6 (bearing piperazine and chlorophenyl at the propyl chain results in the lack of biological activity).

These results indicate that the presence and the character of the substituent, introduced in indolo[2,3-*b*]quinoline core, contribute significantly to the creation of the cytotoxic activity.

In the presented study (which is a part of a comprehensive research of the SAR of indoloquinolines) it was demonstrated that cytotoxicity is concomitant with the presence of the alkyl-amino-alkyl chain at *N*-6 position. Further investigation, concentrated on optimizing length of this chain (the number of nitrogen atoms and -CH₂- units), are in progress.

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