European Journal of Medicinal Chemistry 46 (2011) 794–798

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and biological activity of 7*H*-benzo[4,5]indolo[2,3-b]-quinoxaline derivatives

Marina O. Shibinskaya [a], Alexander S. Karpenko [a], Sergey A. Lyakhov [a,b,*], Sergey A. Andronati [a,b], Nadezhda M. Zholobak [c], Nikolay Ya. Spivak [c], Natalia A. Samochina [d], Lev M. Shafran [d], Mykhail Ju. Zubritsky [e], Valerij F. Galat [e]

[a] *A.V. Bogatsky Physico-Chemical Institute of NAS of Ukraine, Lyustdorfskaya Doroga 86, Odessa 65080, Ukraine*
[b] *Odessa National I.I. Mechnikov University, 2 Dvoryanskaya Street, Odessa 65026, Ukraine*
[c] *D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Zabolotnogo Str., 154, Kiev 03143, Ukraine*
[d] *Ukrainian Scientific and Research Institute of Medicine on Transport, 92 Kanatnaya Street, Odessa 65039, Ukraine*
[e] *L. M. Litvinenko Institute of Physical-Organic Chemistry and Coal Chemistry of the NAS of Ukraine, R. Luxemburg St., 70, Donetsk 83114, Ukraine*

ARTICLE INFO

Article history:
Received 31 August 2010
Received in revised form
9 November 2010
Accepted 23 November 2010
Available online 1 December 2010

Keywords:
7*H*-benzo[4,5]indolo[2,3-b]quinoxaline
Anti-viral activity
Interferon induction
DNA affinity

ABSTRACT

New 7-(2-aminoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxalines (**13**–**20**) were synthesized with high yields starting from 3*H*-benzo[e]indole-1,2-dione. These compounds were screened for the cytotoxicity, anti-viral activity, interferon inducing ability and DNA affinity compared with the corresponding 6-(2-aminoethyl)-6*H*-indolo[2,3-b]quinoxaline derivatives (**1**–**12**). It was shown, that compounds **13**–**20** bind to DNA stronger (lg K_a = 6.23–6.87) than compounds **1**–**12** (lg K_a = 5.57–5.89). Anti-viral activity is significantly reduced with annulations of benzene ring in Indoloquinoxaline moiety **13**–**20**.

1. Introduction

Earlier we have synthesized 6-(2-aminoethyl)-6*H*-indolo[2,3-b] quinoxaline derivatives (**1**–**12**) and shown that these compounds are low toxic potent interferon inducers and antivirals [1]. Interferon inducing ability of planar polycyclic compounds may be attributed to their DNA intercalating ability. This hypothesis, based on the spectrum of tilorone and its analogs activities [2], was confirmed by discovering of active interferon inducers and antivirals among some acridine and fluorene derivatives [3–8]. Furthermore, interferon inducing activity was demonstrated for different planar polycyclic compounds which intercalating ability was demonstrated independently [9,10], or may be supposed, regarding their planarity and presence of the positively charged aminoalkyl group. All above is rightful for the indoloquinoxaline derivatives **1**–**12**. Intercalation to DNA was shown for some indoloquinoxaline derivatives, synthesized and investigated by J. Bergman et al [11,12]. For the other ones it may be speculated according to structure similarity with investigated compounds. Dispersive interactions and π-stacking [13,14] as well as hydrophobic interactions play the major role in stabilization of the intercalative complex [15]. These interactions mainly, derived of the chromophore structure peculiarities, particularly its size and of the side groups positions. Regarding the above extending of indoloquinoxaline chromophore via annulation additive condensed benzene ring has led to the significant increase in DNA affinity of compounds. Importance of the size and nature of the intercalating chromophore for antitumor activity of intercalators was investigated carefully [16], but interferon inducing and anti-viral properties dependence from DNA affinity wasn't investigated till now on our knowledge.

That is why investigation of DNA affinity of **1**–**12** as well as their benzo homologs **13**–**20** (Fig. 1) and biological activity of the last ones became the aim of this work.

2. Results and discussion

2.1. Chemistry

Targeted compounds **13**–**20** were obtained starting from 3*H*-benzo[e]indole-1,2-dione (**21**), which synthesis was described

* Corresponding author. A.V. Bogatsky Physico-Chemical Institute of NAS of Ukraine, Lyustdorfskaya Doroga 86, Odessa 65080, Ukraine. Tel.: +38(048) 765 51 26; fax: +38(048) 765 96 02.
E-mail address: Sergey_A_Lyakhov@ukr.net (S.A. Lyakhov).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.11.040

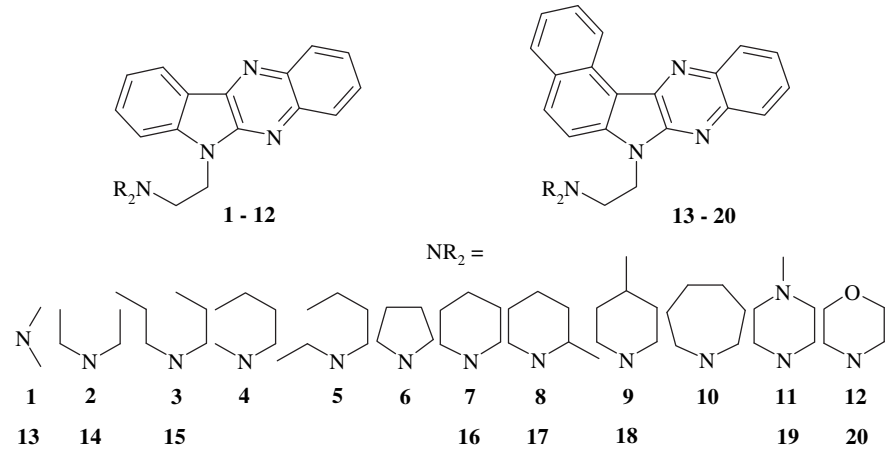
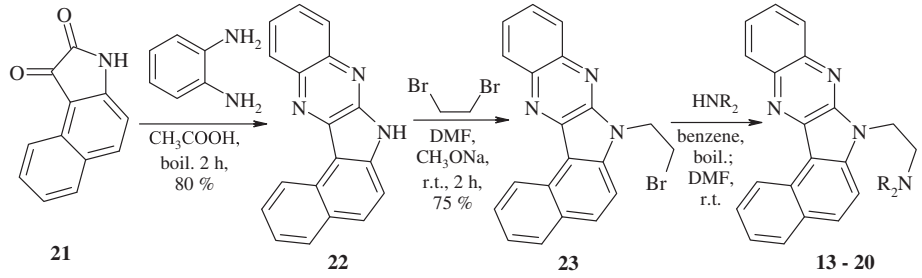


Fig. 1. Structures of early synthesized indoloquinoxalines (**1**–**12**) and new benzoindoloquinoxalines (**13**–**20**).



Scheme 1. Synthesis of 7-(2-aminoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxalines.

earlier [17]. 7*H*-Benzo[4,5]indolo[2,3-b]quinoxaline (**22**) was synthesized according to a described method for 6*H*-indolo[2,3-b] quinoxaline [18] by condensation of 3*H*-benzo[e]indole-1,2-dione (**21**) with 1,2-diaminobenzene under boiling in acetic acid with 80% yield (Scheme 1). Chromatographically pure product **22** was obtained after recrystallization from dimethylformamide. Further alkylation of **22** by excess of dibromoethane was carried out in dimethylformamide at room temperature in the presence of equimolar quantity of sodium methylate in methanol, the product **23** was purified by column chromatography. 7-(2-Aminoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxaline derivatives (**13**–**20**) were obtained by aminodebromination of **23** by excess secondary amines in boiling benzene (compounds **14**–**20**) or in dimethylformamide (compound **13**) at room temperature (Scheme 1) in 80–90% yields.

Table 1
The lg K_a and lg C_{50} values of compounds **1**–**12** and **13**–**20**.

Compound	lg K_a	lg C_{50}	$\pm\varepsilon$
1	5.93	−3.83	0.07
2	6.07	−3.97	0.07
3	6.09	−3.99	0.15
4	5.93	−3.82	0.08
5	6.01	−3.91	0.06
6	6.05	−3.95	0.06
7	6.01	−3.91	0.06
8	6.19	−4.08	0.08
9	5.98	−3.88	0.07
10	6.16	−4.06	0.15
11	5.87	−3.77	0.10
12	5.89	−3.78	0.09
13	6.94	−4.84	0.07
14	6.79	−4.69	0.12
15	6.91	−4.80	0.14
16	6.61	−4.51	0.12
17	6.83	−4.73	0.09
18	6.58	−4.48	0.10
19	6.94	−4.84	0.11
20	6.94	−4.84	0.09

The purity of compounds was controlled by thin-layer chromatography on pre-coated silica gel F_{254} plates using eluents of different composition.

The structure of the synthesized compounds was proved by mass-spectrometry, IR spectroscopy and NMR-spectroscopy.

Molecular ions peaks (MH^+) with intensity 100% are present in mass-spectra of compounds **13**–**20** with ionization method of fast atom bombardment (FAB). Fragment ions set correspond to suggested structures. Stretching vibrations of aromatic C–H bonds are observed in IR spectrums of compounds **13**–**20** at 3075–3030 cm^{-1}, aliphatic – 2980–2750 cm^{-1}. Double bonds vibrations of heterocyclic fragments exhibit a band set at 1620–1450 cm^{-1}. The bands at 1200–1030 cm^{-1} are corresponds to CH_2–N vibrations.

The signals of aromatic and aliphatic protons are observed in ^1H NMR-spectra of synthesized compounds **13**–**20**. The integral intensity and multiplicity of signals correspond to assigned structures.

2.2. Biological activity

The affinity of the earlier synthesized compounds **1**–**12** [1] and 7-(2-aminoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxalines (**13**–**20**) to calf thymus DNA was determined by ethidium bromide displacement assay [19]. The obtained association constants (lg K_a) and lg C_{50} values are shown in Table 1.

It was found that the lg K_a values of the pentacyclic derivatives **13**–**20** are approximately one order magnitude greater than those of the corresponding tetracyclic derivatives **1**–**12** ($P < 0.001$ using Kruskal–Wallis nonparametric test $H = 13.77$ vs $\chi^2 = 10.83$ [20]).

In vitro cytotoxicity, interferon inducing properties and anti-viral activity of synthesized compounds were tested using murine fibroblasts (L929) cells. The obtained results for synthesized

Table 2
Cytotoxicity of compounds **1**–**12** and **13**–**20**.

Compound	CC_{50}, μM[a]	− lg CC_{50}	$\pm\varepsilon$ [b]
1	91	4.04	0.05
2	645	3.19	0.03
3	218	3.66	0.05
4	562	3.25	0.06
5	173	3.76	0.04
6	76	4.12	0.06
7	177	3.75	0.03
8	234	3.63	0.04
9	575	3.24	0.05
10	144	3.84	0.09
11	97	4.01	0.02
12	1071	2.97	–
13	49.9	4.30	0.08
14	180	3.74	0.05
15	> 42.6	< 4.37	–
16	> 221	< 3.66	–
17	113	3.95	0.10
18	> 214	< 3.69	–
19	21.4	4.67	0.04
20	–	– [c]	–

[a] CC_{50} – concentration of tested compound, which leads to 50% cell monolayer destruction.
[b] Confidence interval value ($\pm\varepsilon$) was calculated using $P < 0.05$.
[c] CC_{50} value determination was failed because of low compound solubility.

compounds **13**–**20** as well as earlier published results [1] for compounds **1**–**12** are summarized in Table 2–4.

It should be noted that benzoindoloquinoxaline derivatives are low soluble in media therefore CC_{50} value determination for some compounds was failed; meanwhile the maximal achieved concentrations appeared as nontoxic. Compounds **13**, **14**, **17** and **19** showed higher cytotoxicity in comparison with corresponding indoloquinoxaline derivatives **1**, **2**, **8** and **11** (Table 2). Only piperidine derivative **16** appeared lower cytotoxicity than its analog **7**. In general, difference between these two groups of compounds (**1**–**12** and **13**–**20**) appeared as nonsignificant ($0.05 < P < 0.1$) according Kruskal–Wallis test ($H = 3.79$ *vs* $\chi^2 = 3.84$ and 2.71 correspondently).

Anti-viral activity was investigated against vesicular stomatitis virus (VSV) under preventive (the tested compounds were added to the cell monolayer 24 h before virus infection) and therapeutic (the tested compounds were added immediately after virus infection) models. The obtained results for compounds **13**–**20** and earlier published results [1] for compounds **1**–**12** are summarized in Table 3. It was found that the anti-viral activity is significantly reduced with the annulation of benzene ring in indoloquinixaline's molecule. Degree of the anti-viral protection for compounds **13**–**20** as in preventive model, as well as in therapeutic model mostly does not exceed 50%, while the concentration providing maximum protection increases almost one order of magnitude compared with the corresponding indoloquinoxalines **1**–**12**. It should be noted that anti-viral activity of the 7*H*-benzo[4,5]indolo [2,3-b] quinoxalines derivatives (**13**–**20**) is higher in therapeutic model ($E_{max} = 45–80\%$) than in preventive model ($E_{max} = 10–30\%$).

Table 3
Anti-viral activity of compounds **1**–**12** and **13**–**20**.

Compound	PE[a]		TE[b]	
	E_{max}[c]	C (E_{max})[d]	E_{max}	C (E_{max})
1	75	3.1	100	25
2	90	6.2	100	12.5
3	90	13	75	12.5
4	75	3.1	80	25
5	50	3.1	50	12.5
6	85	3.1	100	12.5
7	90	3.1	75	6.2
8	90	6.2	77	6.2
9	90	13	50	12.5
10	90	6.2	100	50
11	85	13	100	12.5
12	85	13	30	6.2
13	21	30	45	15
14	14	113	56	7.1
15	16	3.0	48	21.3
16	20	110	55	27.5
17	20	53	50	6.7
18	30	53	51	21.4
19	17	13	80	6.7
20	10	0.2	50	5.5

[a] TE-protective effect in therapeutic model (see text).
[b] PE-protective effect in preventive model (see text).
[c] E_{max}, %-maximal protection's degree of cell monolayer from VSV cytopatic action.
[d] C (E_{max}), μ-tested compound concentration (μM) at which E_{max} is observed.

Table 4
Interferon-inducing activity of compounds **1**–**12** and **13**–**20** at 1.1 μM.

Compound	T IFN[a]
1	16
2	64
3	8
4	16
5	8
6	32
7	64
8	32
9	8
10	64
11	4
12	8
13	2
14	2
15	2
16	8
17	10
18	2
19	6
20	2

[a] T IFN – titer of the induced IFN – maximal IFN-containing cultural medium dilution, under which it's ability to the cytopathic action of VSV inhibition remains.

The interferon-inducing activity of compounds **13**–**20** is also lower compared with compounds **1**–**12** (Table 4). The compounds containing piperidine (**16**) and 2-methylpiperidine (**17**) as terminal aminogroups induced highest titers of interferon among the 7*H*-benzo[4,5]indolo[2,3-b]quinoxalines derivatives. The interferon-inducing activity of compounds **1**–**12** and **13**–**20** is significantly lower ($P < 0.005$) using Kruskal–Wallis nonparametric test ($H = 9.38$ *vs* $\chi^2 = 7.88$).

3. Conclusions

In continuation of our investigation aimed to interferon inducing and anti-viral activity of indoloquinoxaline derivatives we have synthesized 7-(2-aminoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxaline derivatives (**13**–**20**) and investigated their above mentioned properties. Furthermore, comparative investigation of DNA affinity using ethidium bromide competition test also have been provided. According to the conventional conception about intercalation ability of polycyclic compounds, benzene ring annulation to the indiloquinoxaline structure leads to significant increase in DNA affinity of compounds. Annelation of the "additional" benzene ring leads to the increasing of DNA affinity and, simultaneously, to the decreasing of anti-viral and interferon inducing activity. Contrary to the above cytotoxicity doesn't change significantly. It's seemed that certain annulated cycle number and DNA affinity level are optimum for the

viral inhibition potency and interferon inducing ability; for indoloquinoxaline derivatives tetracyclic system is optimal as we think.

4. Experimental

4.1. Chemistry

Melting points are uncorrected. The ^{1}H NMR spectra of all compounds were recorded by a "Bruker Avance II" (400 MHz) spectrometer in $CDCl_3$ and DMSO-d6 solutions using TMS as an internal standard. The mass-spectra of electron impact were recorded using МЧ-1321 spectrometer with straight sample introduction. The ionization energy of electrons was 70 eV, the source temperature was 220 °C. The mass-spectra with the fast atom bombardment (FAB) ionization were recorded using VG 70-70 EQ spectrometer. Ionization was realized using beam of argon-atoms with energy 10 kV (the compounds were dissolved in 3-nitrobenzyl alcohol). Thin-layer chromatography was performed on pre-coated silica gel F_{254} plates (Merck). The ethidium bromide displacement assay was carried out on spectrofluorimeter Solar CM2203.

4.1.1. 7H-Benzo[4,5]indolo[2,3-b]quinoxaline (**22**)

A mixture of 3*H*-benzo[e]indole-1,2-dione (10 g, 0.05 mol) and o-phenylenediamine (5.4 g, 0.05 mol) in 200 ml acetic acid was refluxed with stirring for 2 h. The reaction mixture was then cooled to room temperature and the solid product was separated, washed with acetic acid 3 × 5 ml and recrystallised from dimethylformamide. Yield: 80% (10.8 g); m.p. > 300 C M.W. 269.31. Mass-spectrum (electron impact) – *m/z* (I, %): 269 (100) – M^+. ^{1}H NMR (DMSO-d6) *δ*: 7.53 (t, 1H, arom, *J* = 8.0 Hz); 7.73–7.83 (m, 4H, arom); 8.10 (d, 1H, arom, *J* = 8.0 Hz); 8.11 (d, 1H, *J* = 8.2 Hz); 8.21 (d, 1H, *J* = 8.8 Hz); 8.34 (d, 1H, *J* = 8.0 Hz); 9.39 (d, 1H, *J* = 8.4 Hz). Anal. Calcd for $C_{18}H_{11}N_3$: C 80.28; H 4.12; N 15.60. Found: C 80.41; H 4.05; N 15.42.

4.1.2. 7-(2-Bromoethyl)-7H-benzo[4,5]indolo[2,3-b]quinoxaline (**23**)

To a stirred suspension of 7*H*-benzo[4,5]indolo[2,3-b]quinoxaline (10g, 0.037 mol) in 200 ml dimethylformamide was added 7.4 ml 5M solution of sodium methylate. The obtained solution was stirred for 10 min and 1,2-dibromoetane (139 g, 0.74 mol, 64 ml) was then added. After stirring at room temperature for 2 h the reaction mixture was evaporated under reduced pressure, the residue was washed with water, filtered and dried. The product was purified by silica gel column chromatography using benzene as eluent. Yield: 75% (10.4 g); m.p. 181–182 °C M.W. 376.26. Mass-spectrum (electron impact) – *m/z* (I, %): 377 (**50**), 375 (**50**) – M^+. ^{1}H NMR ($CDCl_3$) *δ*: 3.89 (t, 2H, Br*CH*$_2$CH$_2$N, *J* = 7.2 Hz); 4.92 (t, 2H, BrCH$_2$*CH*$_2$N, *J* = 6.8 Hz); 7.52 (t, 1H, arom, *J* = 7.6 Hz); 7.66 (d, 1H, arom, *J* = 8.6 Hz); 7.72–7.82 (m, 3H, arom); 7.967 (d, 1H, arom, *J* = 8.0 Hz); 8.07 (d, 1H, arom, *J* = 8.8 Hz); 8.15 (d, 1H, arom, *J* = 8.0 Hz); 8.39 (d, 1H, arom, *J* = 8.0 Hz); 9.55 (d, 1H, arom, *J* = 8.4 Hz). Anal. Calcd for $C_{20}H_{14}BrN_3$: C 63.85; H 3.75; N 11.17. Found: C 63.95; H 3.93; N 11.20.

4.1.3. [2-(7H-benzo[4,5]indolo[2,3-b]quinoxalin-7-yl)ethyl] dimethylamine (**13**)

A 33% water solution of dimethylamine (1.07 ml, 0.008 mol) was added to a solution of 7-(2-bromoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxaline (1.00 g, 0.00265 mol) in 50 ml dimethylformamide. The reaction mixture was stirred at room temperature until the reaction is complete (TLC control, $CHCl_3$). The reaction mixture was then evaporated under reduced pressure. The residue was dissolved in 25 ml benzene and extracted with 10% acetic acid (3 × 20 ml). This extract was neutralized with saturated solution of sodium carbonate to pH = 8–9. The obtained precipitate was collected, washed with water (3 × 5 ml) and dried. The product was purified by silica gel column chromatography using benzene-triethylamine (20:1) as eluent. Yield: 80% (0.72 g); m.p. 159–161 °C M.W. 340.43. Mass-spectrum (FAB) – *m/z* (I, %): 341 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) *δ*: 2.40 (s, 6H, N(*CH*$_3$)$_2$); 2.89 (t, 2H, *CH*$_2$N(CH$_3$)$_2$, *J* = 6.9 Hz); 4.69 (t, 2H, *CH*$_2$CH$_2$N(CH$_3$)$_2$, *J* = 5.6 Hz); 7.51 (t, 1H, arom, *J* = 8.0 Hz); 7.66–7.81 (m, 4H, arom); 7.96 (d, 1H, arom, *J* = 7.6 Hz); 8.06 (d, 1H, arom, *J* = 8.8 Hz); 8.16 (dd, 1H, arom, *J* = 8.0 Hz, *J* = 1.6 Hz); 8.40 (dd, 1H, arom, *J* = 8.0 Hz, *J* = 1.2 Hz); 9.56 (d, 1H, arom, *J* = 8.0 Hz). Anal. Calcd for $C_{22}H_{20}N_4$: C 77.62; H 5.92; N 16.46. Found: C 77.79; H 5.97; N 16.61.

4.1.4. [2-(7H-benzo[4,5]indolo[2,3-b]quinoxalin-7-yl)ethyl] diethylamine (**14**)

Diethylamine (0.58 g, 0.008 mol) was added to a solution of 7-(2-bromoethyl)-7*H*-benzo[4,5]indolo[2,3-b]quinoxaline (1.00 g, 0.00265 mol) in 50 ml benzene. The reaction mixture was refluxed with stirring until the reaction is complete (TLC control, $CHCl_3$). After cooling the precipitate was filtered and washed with benzene 3 × 5 ml. Filtrate was evaporated under reduced pressure, the residue was dissolved in 25 ml benzene and extracted with 10% acetic acid (3 × 20 ml). This acetous extract was neutralized with saturated solution of sodium carbonate to pH = 8–9. The obtained precipitate was collected, washed with water (3 × 5 ml) and dried. The product was purified by silica gel column chromatography using benzene-triethylamine (20:1) as eluent. Yield: 82% (0.80 g); m.p. 168–170 °C M.W. 368.49. Mass-spectrum (FAB) – *m/z* (I, %): 369 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) *δ*: 1.01 (t, 6H, N(CH$_2$*CH*$_3$)$_2$, *J* = 5.6 Hz); 2.68 (q, 4H, N(*CH*$_2$CH$_3$)$_2$, *J* = 3.9 Hz); 2.99 (t, 2H, *CH*$_2$N(C$_2$H$_5$)$_2$, *J* = 6.9 Hz); 4.68 (t, 2H, *CH*$_2$CH$_2$N(C$_2$H$_5$)$_2$, *J* = 6.8 Hz); 7.51 (t, 1H, arom, *J* = 6.8 Hz); 7.68–7.82 (m, 4H, arom); 7.98 (d, 1H, arom, *J* = 8.0 Hz); 8.07 (d, 1H, arom, *J* = 8.8 Hz); 8.16 (d, 1H, arom, *J* = 8.0 Hz); 8.41 (d, 1H, arom, *J* = 8.0 Hz); 9.58 (d, 1H, arom, *J* = 8.4 Hz). Anal. Calcd for $C_{24}H_{24}N_4$: C 78.23; H 6.57; N 15.20. Found: C 78.42; H 6.76; N 15.32.

The compounds **15**–**20** were obtained in a similar manner.

4.1.5. [2-(7H-benzo[4,5]indolo[2,3-b]quinoxalin-7-yl)ethyl] dipropylamine (**15**)

Yield: 83% (0.87 g); m.p. 101–102 °C M.W. 396.54. Mass-spectrum (FAB) – *m/z* (I, %): 397 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) *δ*: 0.80 (t, 6H, N(CH$_2$CH$_2$*CH*$_3$)$_2$, *J* = 7.2 Hz); 1.42 (m, 4H, N(CH$_2$*CH*$_2$CH$_3$)$_2$); 2.53 (t, 4H, N(*CH*$_2$CH$_2$CH$_3$)$_2$, *J* = 6.8 Hz); 2.99 (t, 2H, *CH*$_2$N(C$_3$H$_7$)$_2$, *J* = 7.2 Hz); 4.68 (t, 2H, *CH*$_2$CH$_2$N(C$_3$H$_7$)$_2$, *J* = 6.8 Hz); 7.52 (t, 1H, arom, *J* = 7.2 Hz); 7.69–7.82 (m, 4H, arom); 7.98 (d, 1H, arom, *J* = 8.4 Hz); 8.09 (d, 1H, arom, *J* = 8.8 Hz); 8.15 (d, 1H, arom, *J* = 8.0 Hz); 8.41 (dd, 1H, arom, *J* = 7.2 Hz, *J* = 1.6 Hz); 9.57 (d, 1H, arom, *J* = 8.4 Hz). Anal. Calcd for $C_{26}H_{28}N_4$: C 78.75; H 7.12; N 14.13. Found: C 78.67; H 7.31; N 14.09.

4.1.6. 7-(2-(Piperidin-1-ylethyl)-7H-benzo[4,5]indolo[2,3-b] quinoxaline (**16**)

Yield: 87% (0.88 g); m.p. 161–162 °C M.W. 380.50. Mass-spectrum (FAB) – *m/z* (I, %): 381 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) *δ*: 1.42 (m, 2H, N(CH$_2$CH$_2$)$_2$*CH*$_2$); 1.58 (m, 4H, N(CH$_2$*CH*$_2$)$_2$CH$_2$); 2.58 (m, 4H, N(*CH*$_2$CH$_2$)$_2$CH$_2$); 2.88 (t, 2H, *CH*$_2$N(CH$_2$CH$_2$)$_2$CH$_2$, *J* = 6.8 Hz); 4.74 (t, 2H, *CH*$_2$CH$_2$N(CH$_2$CH$_2$)$_2$CH$_2$, *J* = 7.2 Hz); 7.51 (t, 1H, arom, *J* = 8.0 Hz); 7.68–7.82 (m, 4H, arom); 7.97 (d, 1H, arom, *J* = 8.4 Hz); 8.08 (d, 1H, arom, *J* = 8.8 Hz); 8.15 (dd, 1H, arom, *J* = 8.4 Hz, *J* = 1.6 Hz); 8.40 (dd, 1H, arom, *J* = 8.0 Hz, *J* = 1.6 Hz); 9.57 (d, 1H, arom, *J* = 8.4 Hz). Anal. Calcd for $C_{25}H_{24}N_4$: C 78.92; H 6.36; N 14.72. Found: C 79.02; H 6.34; N 14.89.

4.1.7. 7-[2-(2-Methylpiperidin-1-yl)ethyl]-7H-benzo[4,5]indolo [2,3-b]quinoxaline (**17**)

Yield: 84% (0.88 g); m.p. 158–159 °C M.W. 394.52. Mass-spectrum (FAB) – *m/z* (I, %): 395 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) *δ*: 1.03 (d,

3H, $CHCH_3$, J = 6.3 Hz); 1.15–1.34 (m, 2H, $CHCH_2(CH_2)_2CH_2$); 1.47–1.48 (m, 4H, $CHCH_2(CH_2)_2CH_2$); 2.34–2.54 (m, 2H, $CH(CH_2(CH_2)_2CH_2)$); 2.77–2.94 (m, 1H, $CHCH_3$); 3.09–3.22 (m, 2H, $CH_2N(CH(CH_3)CH_2(CH_2)_2)CH_2$); 4.62–4.81 (m, 2H, $CH_2CH_2NCH(CH_3)CH_2(CH_2)_2)CH_2$); 7.52 (t, 1H, arom, J = 7.2 Hz); 7.68–7.82 (m, 4H, arom); 7.99 (d, 1H, arom, J = 8.4 Hz); 8.09 (d, 1H, arom, J = 8.8 Hz); 8.16 (dd, 1H, arom, J = 8.4 Hz, J = 1.6 Hz); 8.41 (dd, 1H, arom, J = 8.0 Hz, J = 1.6 Hz); 9.58 (d, 1H, arom, J = 8.4 Hz). Anal. Calcd for $C_{26}H_{26}N_4$: C 79.16; H 6.64; N 14.20. Found: C 79.14; H 6.78; N 14.12.

4.1.8. 7-[2-(4-Methylpiperidin-1-yl)ethyl]-7H-benzo[4,5]indolo [2,3-b]quinoxaline (**18**)

Yield: 90% (0.94 g); m.p. 152–153 °C M.W. 394.52. Mass-spectrum (FAB) − m/z (I, %): 395 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 0.90 (d, 3H, $CHCH_3$, J = 6.4 Hz); 1.24–1.35 (m, 3H, $N(CH_2CH_2^A)_2CH$); 1.60–1.63 (m, 2H, $N(CH_2CH_2^B)_2CH$); 2.08–2.22 (m, 2H, $N(CH_2^ACH_2)_2CH$); 2.82–2.96 (m, 2H, $N(CH_2^BCH_2)_2CH$); 3.00–3.13 (m, 2H, $CH_2N(CH_2CH_2)_2CH$); 4.65–4.82 (m, 2H, $CH_2CH_2N(CH_2CH_2)_2CH$); 7.50 (t, 1H, arom, J = 6.8 Hz); 7.67–7.81 (m, 4H, arom); 7.97 (d, 1H, arom, J = 8.0 Hz); 8.06 (d, 1H, arom, J = 8.4 Hz); 8.15 (dd, 1H, arom, J = 8.0 Hz, J = 1.6 Hz); 8.40 (dd, 1H, arom, J = 8.0 Hz, J = 1.6 Hz); 9.56 (d, 1H, arom, J = 8.0 Hz). Anal. Calcd for $C_{26}H_{26}N_4$: C 79.16; H 6.64; N 14.20. Found: C 79.06; H 6.83; N 14.24.

4.1.9. 7-[2-(4-Methylpiperazin-1-yl)ethyl]-7H-benzo[4,5]indolo [2,3-b]quinoxaline (**19**)

Yield: 86% (0.90 g); m.p. 184–185 °C M.W. 395.51. Mass-spectrum (FAB) − m/z (I, %): 396 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.25 (s, 3H, NCH_3); 2.32–2.48 (m, 4H, $N(CH_2CH_2)_2NCH_3$); 2.58–2.75 (m, 4H, $N(CH_2CH_2)_2NCH_3$); 2.85–2.91 (m, 2H, $CH_2N(CH_2CH_2)_2N$); 4.67 (t, 2H, $CH_2CH_2N(CH_2CH_2)_2N$, J = 6.8 Hz); 7.51 (t, 1H, arom, J = 8.0 Hz); 7.61–7.81 (m, 4H, arom); 7.97 (d, 1H, arom, J = 8.4 Hz); 8.06 (d, 1H, arom, J = 8.8 Hz); 8.13 (dd, 1H, arom, J = 8.4 Hz, J = 1.6 Hz); 8.39 (dd, 1H, arom, J = 7.6 Hz, J = 1.6 Hz); 9.56 (d, 1H, arom, J = 8.0 Hz). Anal. Calcd for $C_{25}H_{25}N_5$: C 75.92; H 6.37; N 17.71. Found: C 75.91; H 6.40; N 17.89.

4.1.10. 7-(2-Morpholin-4-ylethyl)-7H-benzo[4,5]indolo[2,3-b] quinoxaline (**20**)

Yield: 88% (0.89 g); m.p. 199–201 °C M.W. 382.47. Mass-spectrum (FAB) − m/z (I, %): 383 (100) $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.52–2.70 (m, 4H, $N(CH_2CH_2)_2O$); 2.83–2.98 (m, 2H, $CH_2N(CH_2CH_2)_2O$); 3.53–3.69 (m, 4H, $N(CH_2CH_2)_2O$); 4.66–4.81 (m, 2H, $CH_2CH_2N(CH_2CH_2)_2O$); 7.53 (t, 1H, arom, J = 8.0 Hz); 7.69–7.83 (m, 4H, arom); 7.99 (d, 1H, arom, J = 8.0 Hz); 8.10 (d, 1H, arom, J = 8.8 Hz); 8.14 (d, 1H, arom, J = 8.0 Hz); 8.42 (dd, 1H, arom, J = 7.6 Hz, J = 1.6 Hz); 9.59 (d, 1H, arom, J = 8.0 Hz). Anal. Calcd for $C_{24}H_{22}N_4O$: C 75.37; H 5.80; N 14.65. Found: C 75.39; H 5.80; N 14.84.

4.2. Biological assays

4.2.1. DNA affinity assay

The solution "A" containing calf thymus DNA ($C = 2.12 \times 10^{-5}$ M); ethidium bromide ($C = 2.54 \times 10^{-5}$ M); NaCl ($C = 3.73 \times 10^{-2}$ M); sodium acetate ($C = 8.00 \times 10^{-3}$ M, acetate buffer solution with pH = 5.5); EDTA ($C = 4.93 \times 10^{-4}$ M) was 2-folds diluted with distilled water giving solution "B". Investigated compound (10–20 mg) was dissolved in 10 ml of distilled water giving solution "C". Solution "A" (3 ml) was added to solution "C" (3 ml) giving solution "D". From solution "B" and solution "D" by double logarithmic dilution was prepared series of solutions with constant contents of all components but variable contents of investigated compound. Solution contained DNA and ethidium bromide in the same concentrations and in the same buffer was used as control. Fluorescence spectra were registered in the 550–730 nm range using 535 nm excitation.

References

[1] M.O. Shibinskaya, S.A. Lyakhov, A.V. Mazepa, S.A. Andronati, A.V. Turov, N.M. Zholobak, N.Ya. Spivak, Eur. J. Med. Chem. 45 (2010) 1237–1243.
[2] S.A. Lyakhov, Abstract Book of 6th International Symposium on Molecular Aspects of Chemoterapy, Gdansk, Poland, 1997. p. 137.
[3] Y.I. Suveyzdis, S.A. Lyakhov, L.A. Litvinova, S.A. Andronati, S.L. Rybalko, S.T. Dyadyun, Pharmazie 55 (2000) 733–736.
[4] S.A. Lyakhov, L.A. Litvinova, S.A. Andronati, L.K. Berezina, B.N. Galkin, V.E. Osetrov, T.O. Filippova, N.Ya. Golovenko, Ukr. Biokhim. Zh 73 (2001) 108–113.
[5] S.A. Lyakhov, L.A. Litvinova, Y. Suveyzdis, S.A. Andronati, S.L. Rybalko, S.T. Dyadyun, Pharm. Chem. J. 34 (2000) 474–475.
[6] S. Alcaro, A. Arena, S. Neri, R. Ottana, F. Ortuso, B. Pavone, M.G. Vigorita, Bioorg. Med. Chem. 12 (2004) 1781–1791.
[7] A. Arena, N. Arena, R. Ciurleo, A. de Gregorio, R. Maccari, R. Ottana, B. Pavone, A. Tramice, A. Trincone, M.G. Vigorita, Eur. J. Med. Chem. 43 (2008) 2656–2664.
[8] S. Alcaro, A. Arena, R. Di Bella, S. Neri, R. Ottana, F. Ortuso, B. Pavone, M.G. Vigorita, Arkivoc 5 (2004) 334–348.
[9] A.S. Karpenko, I.V. Dorovskikh, S.A. Lyakhov, S.A. Andronati, N.M. Zholobak, N.Ya. Spivak, J.V. Nekhoroshkova, L.M. Shafran, Ukrainica Bioorganica Acta 6 (2008) 42–48.
[10] K.E. Miller, J.M. Grace, T.L. Macdonald, Bioorg. Med. Chem. Lett. 4 (1994) 1643–1648.
[11] J. Harmenberg, A. Åkesson-Johansson, A. Gräslund, T. Malmfors, J. Bergman, B. Wahren, S. Åkerfeldt, L. Lundblad, S. Cox, Antivir. Res. 15 (1991) 193–204.
[12] L.M. Wilhelmsson, N. Kingi, J. Bergman, J. Med. Chem. 51 (2008) 7744–7750.
[13] F. Gago, Methods 14 (1998) 277–292.
[14] D. Reha, M. Kabelac, F. Ryjacek, J. Sponer, J.E. Sponer, M. Elstner, S. Suhai, P. Hobza, J. Am. Chem. Soc. 124 (2002) 3366–3376.
[15] V.V. Kostjukov, N.M. Khomytova, M.P. Evstigneev, Biopolymers 91 (2009) 773–790.
[16] W.A. Denny, Acridine-4-carboxamides and the concept of minimal DNA. in: M. Demeunynck, C. Bailly, W.D. Wilson (Eds.), DNA and RNA Binders from Small Molecules to Drugs. Wiley-VCH, 2002, pp. 482–503.
[17] A.S. Karpenko, M.O. Shibinskaya, N.M. Zholobak, Z.M. Olevinskaya, S.A. Lyakhov, L.A. Litvinova, N.Ya. Spivak, S.A. Andronati, Pharm. Chem. J. 40 (2006) 595–602.
[18] E. Schunck, L. Marchlewski, Chem. Ber. 28 (1895) 2525–2531.
[19] I. Antonini, P. Polucci, L.R. Kelland, E. Menta, N. Pescalli, S. Martelli, J. Med. Chem. 42 (1999) 2535–2541.
[20] S.A. Glantz, Primer of Biostatistics, fourth ed. McGraw-Hill Inc., New York, 1997.