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Short communication

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

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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

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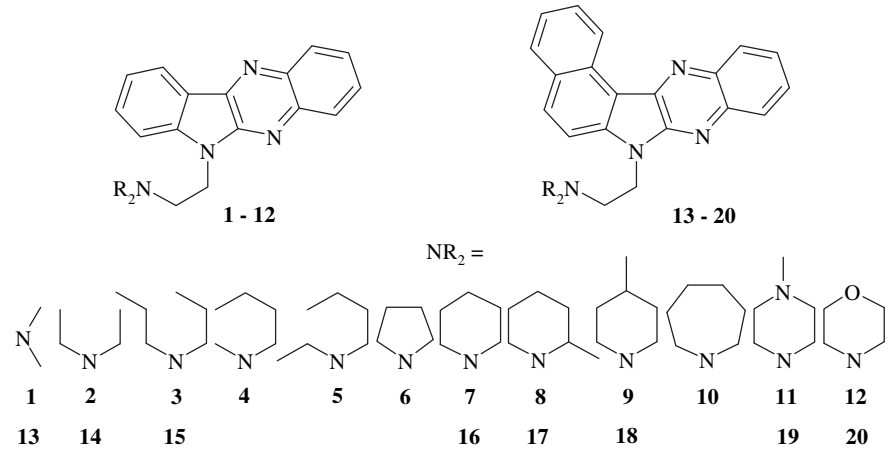
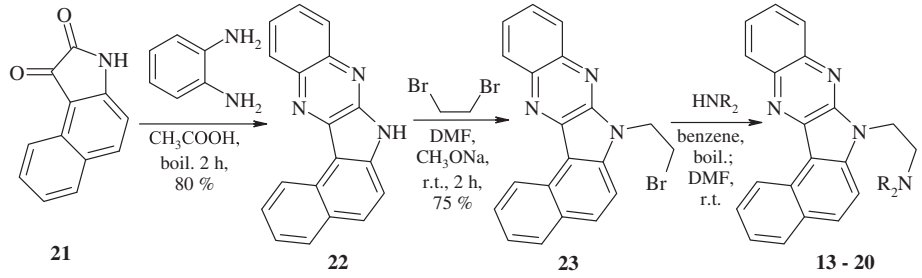


Fig. 1. Structures of early synthesized indoloquinoxalines (**1**–**12**) and new benzoindoloquinoxalines (**13**–**20**).



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