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Short communication

Synthesis and affinity to DNA of phenylbenzoimidazoles and benzoimidazo[1,2-*c*]quinazolines

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ABSTRACT

Novel *N*-(benzoimidazophenyl)dialkylaminoalkylamides and 6-dialkylaminoalkylbenzoimidazo[1,2-*c*]quinazolines were prepared as potential interferon inducers and antiviral agents. They were screened for the DNA affinity by the ethidium bromide displacement assay. It was shown that the lg K_a values of the compounds containing tetracyclic benzoimidazo[1,2-*c*]quinazoline fragment are approximately one order magnitude greater than those of the corresponding acyclic phenylbenzoimidazole derivatives.

1. Introduction

The fast spread of viral diseases and the ability of viruses to mutate into new dangerous forms are common obstacles in the search of reliable antiviral agents. For instance, the epidemic of severe acute respiratory syndrome (SARS), that took place 11.2002–07.2003 affected 8447 people, according to the WHO [1], resulting in 811 (9.6%) decease. The vaccine against coronavirus SARS was only found after the end of epidemic. Furthermore, application of chemotherapeutic agents, in particular, of ribavirin, is not usually effective in the regular therapeutic regimen, while an increase of its dose causes some serious side effects [2].

The need for the development of highly effective antiviral agents with a nonspecific protection is obvious. Such agents could provide a successful prophylaxis and therapy for viral diseases without preliminary identification of an infectious agent. In addition, nonspecific agents would eliminate the long procedure required for the creation and approval of a vaccine.

The natural factor providing nonspecific antiviral protection is the interferon system. It is well known [3,4] that interferons are effectively used for the treatment of viral, bacterial and oncological diseases. However, application of exogenous interferon has a number of limitations such as allergic reactions, formation of anti-interferonic antibodies after prolonged application [5,6], and the manifestation of side effects at overdose [7,8].

An alternative approach is to induce the synthesis of endogenous interferon. Several interferon inducers such as amizon [9], arbidol [10,11], primavir (cycloferon) [12,13] and amixine (tilorone) [14,15] were successfully introduced into clinical practice in Ukraine. Among these, tilorone showed a high preventive and therapeutic efficacy for a wide spectrum of infectious agents. It is known [16] that tilorone is capable of intercalating between the DNA base pairs with its planar tricyclic fragment [17,18], and it has been suggested that this intercalation into DNA is the most probable mechanism of interferon induction [19,20]. Significant interferon inducing properties have also been reported for intercalating derivatives of fluorene [20,21], acridine [22,23], anthraquinone [24] and phenanthroline [25]. Regarding the above, the search of interferon inducers among low-molecular weight polycyclic carbo- and heteroaromatic compounds, capable of forming complexes with nucleic acids due to intercalation is a perspective direction in the area of antiviral therapy.

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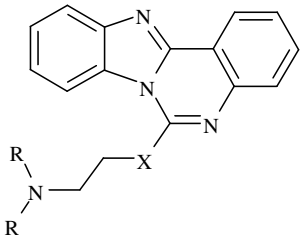
It prompted us to investigate the benzoimidazo[1,2-*c*]quinazolines **1** which have been previously shown, via ^{1}H NMR spectroscopy, to form intercalation complexes with DNA [26]. Nevertheless, quantitative characteristics of these compounds, in particular, the constants of association, were not published. Furthermore, the benzoimidazo[1,2-*c*]quinazoline derivatives have a low cytotoxicity ($IC_{50} = 0.45–19.2$ μM [26]) and moderate acute toxicity *in vivo* ($LD_{50} = 300–500$ mg/kg *i.p.* [27]).

On the other hand, there has been certain interest in the phenylbenzoimidazole derivatives, since this fragment is similar to that of the benzoimidazo[1,2-*c*]quinazolines. It is reported in most cases that crescent-shaped molecules containing one or several benzoimidazole motifs (Hoechst 33258, etc.) are well-known minor groove binding agents [28–36]. But then it was shown by Denny et al. [37], the short "round" substituted phenylbenzoimidazole derivatives **2** (R = CH_3, Cl, Ph) are also capable of intercalating into DNA (lg $K_a = 5.04–6.43$) as it demonstrated in ethidium bromide displacement assay studies.



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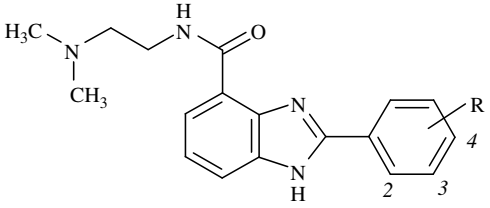


2

The present work is the first stage of our research aimed the synthesis, investigation of spectral characteristics, affinity of the *N*-(benzoimidazophenyl)dialkylaminoalkylamides and 6-dialkylaminoalkylbenzoimidazo[1,2-*c*]quinazolines to DNA, and "structure–affinity" relationships of these compounds.

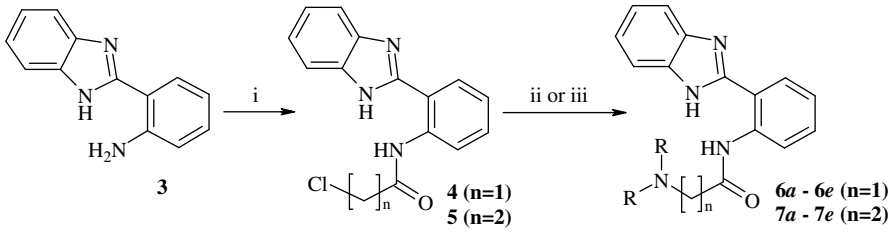
2. Results and discussion

2.1. Synthesis

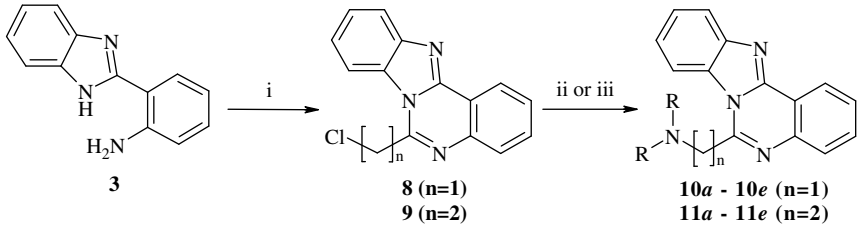
Initial 2-(2-aminophenyl)benzoimidazole **3** was prepared according to a literature method [38] in yield comparable to that of previously described [39].

Chloralkylamido derivatives **4** and **5** were obtained by the reaction of the amine **3** with corresponding chloroacylchlorides in glacial acetic acid in the presence of sodium acetate at room temperature for 30–40 min [40] (Scheme 1). Products **4** and **5** were isolated from the reaction mixture as chromatographically homogeneous compounds and then recrystallized from acetone. Treatment of **4** and **5** with the corresponding secondary amines afforded the compounds **6a–7e** (Scheme 1). Unlike the previous step, the methods of the dialkylaminoalkyl derivatives **6a–7e** preparation were dependent on the number of methylene units in the aliphatic moiety. Treatment of chloromethyl derivative **4** with a threefold excess of the secondary amines in DMF at room temperature for 24 h, followed by recrystallization from ethanol provided compounds **6a–6e** ($n = 1$) in good yield. This method was found unsuitable for the formation of similar compounds with **7a–7e** ($n = 2$), and initial alkylating agents were recovered from the reaction mixture. The synthesis of the dialkylaminoethyl derivatives **7a–7e** was accomplished by refluxing of compound **5** with a three- to fourfold excess of the secondary amine in ethanol for 1.5–2.0 h, followed by recrystallization from acetonitrile (Scheme 1).

6-Chloroalkylbenzoimidazo[1,2-*c*]quinazolines **8** and **9** were obtained by acylation of compound **3** with the chloroacylclorides in glacial acetic acid at 60 °C (Scheme 2) as described previously [40]. Compound **8** was treated with various secondary amines in DMF at room temperature for 24 h to give the benzoimidazo[1,2-*c*]quinazolines **10a–10e** (Scheme 2). This method failed in case of the preparation of **11a–11e** ($n = 2$). On the other hand, the attempts to prepare **11a** by refluxing compound **9** with an excess of piperidine in ethanol resulted in the formation of significant amount of



Scheme 1. i: $Cl(CH_2)_nCOCl$, AcOH, AcONa, r.t.; ii: HNR_2, DMF, r.t., 24 h ($n = 1$); iii: HNR_2, EtOH, refl., 2 h ($n = 2$).

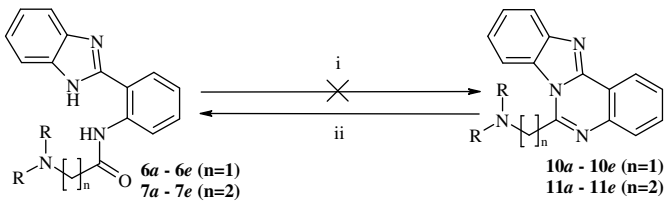


Scheme 2. i: $Cl(CH_2)_nCOCl$, AcOH, 60 °C; ii: HNR_2, DMF, r.t., 24 h ($n = 1$); iii: HNR_2, MeOH, refl. ($n = 2$).

NR_2=					
$n = 1$	**6a**	**6b**	**6c**	**6d**	**6e**
$n = 2$	**7a**	**7b**	**7c**	**7d**	**7e**
$n = 1$	**10a**	**10b**	**10c**	**10d**	**10e**
$n = 2$	**11a**	**11b**	**11c**	**11d**	**11e**

compounds **5** and **7a** (by TLC approx. <20% and approx. <60% respectively), though the desired product **11a** was also present in the reaction mixture. Similar results were observed under transformation of **9** to **11b**–**11e**.

It is interesting to note that the previously proposed method [26] of the synthesis for compounds **11a**–**11e** (Scheme 3) based on the treatment of compounds **7a**–**7e** with a mixture of pyridine-thionyl chloride in chloroform did not provide the targets **11a**–**11e** at room temperature. It was observed that refluxing of the chloride **9** with a three- to fourfold excess of the appropriate secondary amines in methanol provided the dialkylaminoethylbenzoimidazo[1,2-*c*]quinazolines **11a**–**11e** in high yield. The rate of this reaction exhibited a dependence on the nature of secondary amines. Reaction with 4-methyl-piperidine was completed in 10 min, reaction with piperidine or with 2-methylpiperidine within 30–40 min and reaction with 4-methyl-piperazine within 2.5–3 h. The reaction



Scheme 3. i: Pyridine-thionyl, $CHCl_3$, r.t.; ii: 6 M HCl, 80 °C.

with morpholine gave the product **11d** only after refluxing for 7 h, at which time less than 10% of the parent compound **9** remained unreacted, as detected by quantitative TLC.

Our attempt to obtain hydrochlorides of compounds **10a**–**10e** and **11a**–**11e** for testing was unsuccessful due to the appearance of **6a**–**6e** and **7a**–**7e** in the corresponding samples. It was found, that compounds **10a**–**11e** were not stable as hydrochloride salts, running hydrolysis in the presence of even traces of water. Furthermore, the 6-dialkylaminoalkylbenzimidazo[1,2-*c*]quinazolines **10a**–**11e** can be completely hydrolyzed by 6 M hydrochloric acid to the corresponding 2-(2-dialkylaminoalkyamidophenyl)benzoimidazoles **6a**–**7e** at 80 °C for 5 min (Scheme 3).

The use of acetic acid instead of hydrochloric acid allowed us to prepare the aqueous solution of acetates of **10a**–**11e** with no detectable hydrolysis to phenylbenzoimidazoles **6a**–**7e** during 10–12 h.

It was found that stability of compounds **10a**–**11e** in water solution substantially depends on pH. For the solutions of compounds **10a**–**11e** kept at room temperature and pH = 5.0–5.5, hydrolysis products **6a**–**7e** appeared within 15–45 min (<3%), reaching concentrations 25–40% in the following 10–12 h. Aqueous solutions of **10a**–**11e** were stable at pH = 5.7–7.0 with not more than 3% of the compound were hydrolyzed during 10–12 h, hence making them suitable for the DNA-binding assay. Additionally, the degree of hydrolysis of **10a**–**11e** depends on the character of tertiary amino group. Thus, the 2-methyl-piperidine derivative **10b**, in

Table 1
The lg K_a values of the synthesized compounds.

Compound	lgC_{50}	lg K_a	Compound	lgC_{50}	lg K_a
6a	−2.65	4.75	**10a**	−3.84	5.94
6b	−2.66	4.76	**10b**	−3.65	5.75
6c	−2.84	4.94	**10c**	−3.83	5.93
6d	−2.58	4.68	**10d**	−3.44	5.54
6e	−2.32	4.42	**10e**	−3.72	5.82
7a	−2.80	4.90	**11a**	−4.03	6.13
7b	−2.66	4.76	**11b**	−4.17	6.27
7c	−2.83	4.93	**11c**	−4.23	6.33
7d	−2.92	5.02	**11d**	−4.12	6.22
7e	−2.86	4.96	**11e**	−4.18	6.28
			Amixine (tilorone)	−4.20	6.30

a series of compounds **10a–10e** ($n = 1$), is the most stable product in aqueous acidic medium with stability decreasing in the order **10c** > **10a** > **10e** > **10d**. It is necessary to note that the benzoimidazo[1,2-*c*]quinazoline derivatives **11a–11e** ($n = 2$) are relatively more stable products in aqueous acids (by TLC < 1%, for 10–12 h). Therefore, compounds **10a–11e** were directly converted to corresponding acetates and **6a–7e** to hydrochlorides immediately before using them in the DNA-binding assay.

2.2. Biology

In this paper we have investigated the affinity of the synthesized compounds to DNA. As Denny had shown [37], that phenylbenzimidazoles **1** are also capable of intercalating into DNA, it is reasonable to evaluate the binding parameters of not only the tetracyclic compounds **10a–11e** but also their acyclic analogues **6a–7e**. The affinity of the synthesized compounds to calf thymus DNA was determined by ethidium bromide displacement assay [41] and the results are shown in Table 1.

The association constants of the resulting compounds exhibit a dependence on both the number of methylene units in the alkyl spacer as well as the nature of the intercalating fragment itself.

The compounds can be divided into four groups with intercalating ability increasing in the following sequence: **6a–6e** ($n = 1$) ≤ **7a–7e** ($n = 2$) ≪ **10a–10e** ($n = 1$) ≤ **11a–11e** ($n = 2$) (Fig. 1).

In order to determine whether the lg K_a values of the phenylbenzoimidazoles **6a–7e** and benzoimidazo[1,2-*c*]quinazolines **10a–11e** belong to the same sample, we have carried out the Mann–Whitney–Wilcoxon *U*-test [42,43]. The obtained *U* value ($U = 0$, $U_{tab.} = 16$, $U \ll U_{tab.}$) is statistically significant ($\alpha = 0.01$) and shows that the observed difference among these samples is also significant. However, similar application of this rank criterion within

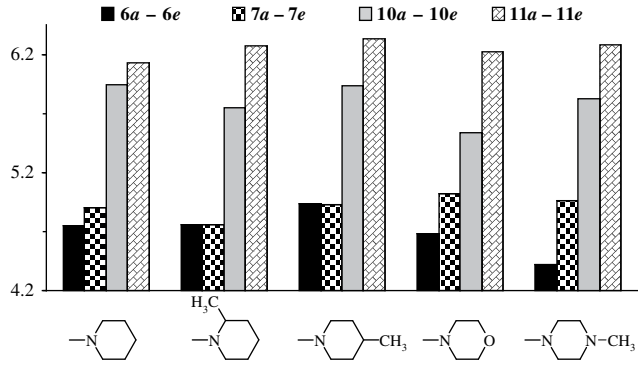


Fig. 1. The affinity of the synthesized compounds to CT DNA.

phenylbenzoimidazoles **6a–6e** ($n = 1$) and **7a–7e** ($n = 2$) indicates that the difference between lg K_a values within the series of these compounds is not significant ($U = 3$, $U_{tab.} = 1$, $U > U_{tab.}$, $\alpha = 0.01$). On the other hand, the lg K_a values of benzoimidazo[1,2-*c*]quinazolines **10a–10e** ($n = 1$) are significantly different from **11a–11e** ($n = 2$) ($U = 0$, $U_{tab.} = 1$, $U < U_{tab.}$, $\alpha = 0.01$).

The observed lg K_a values for the ligands **6a–11e** are specific to monointercalators 4.42–6.33 (Table 1). Additionally, there is a significant difference in the values of constants for the phenylbenzoimidazoles (lg $K_a = 4.42–5.02$) and the benzimidazo[1,2-*c*]quinazolines (lg $K_a = 5.54–6.33$).

3. Conclusion

Thus, the lgK_a values of the compounds **10a–11e** containing tetracyclic benzoimidazo[1,2-*c*]quinazoline fragment are approximately one order magnitude greater than those of the corresponding acyclic phenylbenzoimidazole derivatives **6a–7e.** On the one hand, these differences are in agreement with the Denny's conception about minimal intercalators, based on the terms of planar area size. According to this conception planar part of **10a–11e** is really larger than that of **6a–7e** [37]. Though, in case of planar conformation realization of the **6a–7e**, these compounds have even more number of the "heavy atoms" (C, N, O) coplanar with benzoimidazole ring than tetracyclic **10a–11e**. On the other hand, possibility of rotation about Ph-BIm single bond in 2-phenylbenzoimidazole allows realization of a set of non-planar conformations in non-bound by DNA molecule, and only planar conformation can be realized in the intercalative complex. Decreasing of the possible conformations number of **6a–7e** under intercalation has to decrease the enthropic increment in the total intercalation free energy. The last one also may be regarded as a reason for the affinity decreasing of 2-phenylbenzoimidazoles **6a–7e** comparatively to benzoimidazo-quinazolines **10a–11e**. Further clarification of the observed difference in DNA affinity of 2-phenylbenzoimidazoles and benzoimidazo-quinazolines needs additional investigations, which are in progress.

4. Experimental

4.1. General

TLC: silica gel plates (MERK, UV-254), eluent A ($CHCl_3$:$(CH_3)_2CO$ = 10:1); eluent B (C_6H_6:$(C_2H_5)_3N$ = 10:1). Mp: Electrothermal IA9100 digital melting-point apparatus, uncorrected. IR spectra were recorded on spectrometer Shimadzu FTIR-8400 in tablets KBr. Mass spectra (FAB) were written on VG 70-70EQ. Mass spectra (electron stroke) were written on MH-1321. ^{1}H NMR spectra are obtained on Varian VXR-300 at 300 MHz (the internal standard – TMS). The ethidium bromide displacement assay was carried out on spectrofluorimeter Solar CM2203.

4.2. 2-(1H-Benzoimidazol-2-yl)-phenylamine (**3**)

A mixture of anthranilic acid (28.1 g, 0.2 mol) and *o*-phenylenediamine (21.6 g, 0.2 mol) in 400 ml polyphosphoric acid was heated at 250 °C for 4 h with stirring. Then the reaction mixture was cooled up to 100 °C, poured under stirring onto 2000 ml of cold distilled water and solution was led up to pH = 8–9 by addition of 50% NaOH solution. The resulting precipitate was filtered and washed with H_2O up to neutral pH. The crude product was recrystallized from ethanol–water mixture with a small amount of activated charcoal. A yield: 13.2 g (31%). $C_{13}H_{11}N_3$. M.W. 209.25. M.p.: 214.5–215.0 °C. (Lit. M.p.: 213–214 °C [38]). $R_f = 0.36$ (eluent A); $R_f = 0.55$ (eluent B), at UV-254 spot fluoresces. Mass spectrum

(electron stroke), *m/z* (%): 209 (100) $[M]^+$, 182 (12), 118 (7), 104 (17).

4.3. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-2-chloro-acetamide* (**4**)

Solution of anhydrous sodium acetate (1 g in 10 ml of glacial acetic acid) was added at a stretch to the solution of 2-(1H-benzoimidazol-2-yl)-phenylamine 3 (4.2 g, 0.02 mol) in 40 ml glacial acetic acid at room temperature under intensive stirring, following by chloracetylchloride (3.2 ml, 4.5 g, 0.04 mol) addition dropwise. A reaction mixture was stirred at room temperature 40 min and put in solution of sodium acetate (10 g in 150 ml of distilled water). The resulting precipitate was filtered and properly washed by distilled water. The product was recrystallized from acetone or ethanol. A yield: 4.3 g (75%). $C_{15}H_{12}ClN_3O$. M.W. 285.74. M.p.: 307 °C. $R_f = 0.27$ ($CHCl_3$). Mass spectrum (FAB), *m/z* (%): 286 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 285 (0.8) $[M]^+$, 267 (100); 249 (68), 232 (57), 220 (34), 194 (19). ^{1}H NMR (DMSO-d_6): 4.51 (s, 2H), 7.30–7.36 (m, 3H), 7.51–7.57 (m, 1H), 7.66–7.67 (n/r m, 2H), 8.13 (d, 1H), 8.64 (d, 1H), 13.56 (br s, 1H).

4.4. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-3-chloro-propionamide* (**5**)

This compound was prepared from **3** according to the method mentioned under the synthesis of **4** in 66% yield. $C_{16}H_{14}ClN_3O$. M.W. 299.76. M.p.: 210–211 °C (decomp.). $R_f = 0.58$ (eluent A), $R_f = 0.55$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 300 (100) $[M+H]^+$. ^{1}H NMR (DMSO-d_6): 3.03 (t, 2H); 4.00 (t, 2H); 7.26–7.35 (m, 3H); 7.49–7.54 (n/r t, 1H); 7.60 (n/r d, 1H); 7.77 (n/r d, 1H); 8.13 (d, 1H); 8.70 (d, 1H); 13.20 (s, 1H); 13.23 (s, 1H).

4.5. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-2-piperidin-1-yl-acetamide* (**6a**)

To a solution of **4** (0.57 g, 0.002 mol) in DMF (3 ml) was added piperidine (0.6 ml, 0.006 mol). The reaction mixture was kept at room temperature overnight, then poured out in 150 ml of distilled water and led up to pH = 7 by 10% solution HCl. The resulting precipitate was filtered, washed with H_2O to neutral pH, dried and recrystallized from isopropanole. A yield: 0.45 g (67%) $C_{20}H_{22}N_4O$. M.W. 334.42. M.p.: 186–187 °C. $R_f = 0.22$ (eluent A), $R_f = 0.20$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 335 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 334 (3) $[M]^+$, 251 (3), 236 (100), 233 (4), 209 (2), 98 (86). ^{1}H NMR ($CDCl_3$): 1.41–1.49 (m, 2H, $-N(CH_2CH_2)_2CH_2$); 1.63–1.70 (m, 4H, $-N(CH_2CH_2)_2CH_2$); 2.52 (t, 4H, $-N(CH_2CH_2)_2CH_2$); 3.22 (s, 2H, $COCH_2N$); 6.99–7.04 (m, 1H); 7.21–7.32 (m, 3H); 7.59–7.67 (m, 3H); 8.68 (d, 1H); 12.49 (s, 1H). ^{1}H NMR (DMSO-d_6): 1.41–1.47 (m, 2H, $-N(CH_2CH_2)_2CH_2$); 1.61–1.68 (m, 4H, $-N(CH_2CH_2)_2CH_2$); 2.49 (t, 4H, $-N(CH_2CH_2)_2CH_2$); 3.17 (s, 2H, $COCH_2N$); 7.23–7.31 (m, 3H); 7.45–7.50 (m, 1H); 7.67 (n/r m, 2H); 8.01 (d, 1H); 8.82 (d, 1H); 12.82 (s, 1H); 12.97 (br s, 1H).

4.6. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-2-(2-methyl-piperidin-1-yl)-acetamide* (**6b**)

This compound was prepared from **4** according to the method mentioned under the synthesis of **6a**, recrystallized from EtOH–H_2O (2:1) in 52% yield. $C_{21}H_{24}N_4O$. M.W. 348.45. M.p.: 166–167 °C. $R_f = 0.11$ (eluent A), $R_f = 0.46$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 349 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 348 (3) $[M]^+$, 251 (6), 249 (3), 236 (77), 233 (6), 112 (100), 98 (28). ^{1}H NMR (DMSO-d_6): 1.02 (d, 3H, $-N(CH_2CH_2)_2CHCH_3$); 1.27–1.37 (n/r m, 2H); 1.41–1.55 (n/r m, 2H); 1.62–1.66 (m, 2H); 2.21–2.30 (m, 1H); 2.36–2.40 (m, 1H); 2.80–2.84 (m, 1H); 2.97 (d, 1H, $COCH_2N$); 3.40 (d, 1H, $COCH_2N$); 7.22–7.30 (m, 3H); 7.44–7.50 (m, 1H); 7.66 (n/r m, 2H); 8.00 (d, 1H); 8.76 (d, 1H); 12.72 (s, 1H); 13.02 (br s, 1H).

4.7. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-2-(4-methyl-piperidin-1-yl)-acetamide* (**6c**)

This compound was prepared from **4** according to the method mentioned under the synthesis of **6a**, recrystallized from acetonitrile in 74% yield. $C_{21}H_{24}N_4O$. M.W. 348.45. M.p.: 191–192 °C. $R_f = 0.19$ (eluent A), $R_f = 0.50$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 349 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 348 (3) $[M]^+$, 249 (22), 236 (100), 233 (14), 220 (11), 112 (79), 98 (6). ^{1}H NMR (DMSO-d_6): 0.83 (d, 3H, $-N(CH_2CH_2)_2CHCH_3$); 1.27–1.34 (n/r m, 3H); 1.49–1.52 (n/r m, 2H); 2.01–2.08 (m, 2H); 2.82 (d, 2H); 3.17 (s, 2H, $COCH_2N$); 7.23–7.30 (m, 3H); 7.44–7.49 (m, 1H); 7.68 (n/r m, 2H); 7.99 (d, 1H); 8.81 (d, 1H); 12.80 (s, 1H).

4.8. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-2-morpholin-4-yl-acetamide* (**6d**)

This compound was prepared from **4** according to the method mentioned under the synthesis of **6a**, recrystallized from the mixture acetonitrile–water (2:1) in 72% yield. $C_{19}H_{20}N_4O_2$. M.W. 336.40. M.p.: 209–210 °C. $R_f = 0.26$ (eluent A), $R_f = 0.42$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 337 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 334 (3) $[M]^+$, 236 (100), 233 (4), 100 (28). ^{1}H NMR (DMSO-d_6): 2.49 (t, 4H, $N(CH_2CH_2)_2O$); 3.23 (s, 2H, $COCH_2N$); 3.68 (t, 4H, $N(CH_2CH_2)_2O$); 7.22–7.37 (m, 3H); 7.44–7.60 (m, 1H); 7.67–7.80 (n/r m, 2H); 8.00 (d, 1H); 8.80 (d, 1H); 10.64 (s, 1H).

4.9. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-2-(4-methyl-piperazin-1-yl)-acetamide* (**6e**)

This compound was prepared from **4** according to the method mentioned under the synthesis of **6a**, recrystallized from benzene in 70% yield. $C_{20}H_{23}N_5O$. M.W. 349.44. M.p.: 199–200 °C. $R_f = 0.02$ (eluent A), $R_f = 0.23$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 350 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 349 (2) $[M]^+$, 306 (2), 293 (1), 279 (3), 266 (1), 249 (6), 236 (100), 220 (2), 210 (6), 113 (100). ^{1}H NMR (DMSO-d_6): 2.13 (s, 3H, $-N(CH_2CH_2)_2NCH_3$); 2.40 (n/r m, 4H, $-N(CH_2CH_2)_2NCH_3$); 2.50 (n/r m, 4H, $-N(CH_2CH_2)_2NCH_3$); 3.21 (s, 2H, $COCH_2N$); 7.23–7.32 (m, 3H); 7.44–7.50 (m, 1H); 7.73 (n/r m, 2H); 7.99 (d, 1H); 8.79 (d, 1H); 12.82 (s, 1H).

4.10. N-*[2-(1H-Benzoimidazol-2-yl)-phenyl]-3-piperidin-1-yl-propionamide* (**7a**)

Piperidine (0.6 ml, 0.51 g, 0.006 mol) was added to a solution of 5 (0.6 g, 0.002 mol) in ethanol (10 ml). The reaction mixture was boiled for 2 h, evaporated to 1/3 of volume in vacuum and poured in distilled water (100 ml). In case precipitate not formes, mixture have been saturated with NaCl then the resulting precipitate was filtered, washed with H_2O and recrystallized from acetonitrile. A yield 0.49 g (70%). $C_{21}H_{24}N_4O$. M.W. 348.45. M.p.: 195–196 °C. $R_f = 0.01$ (eluent A), $R_f = 0.11$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 349 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 348 (0.6) $[M]^+$, 264 (2), 244 (2), 236 (15), 221 (3), 210 (18), 98 (100). ^{1}H NMR (DMSO-d_6): 1.27–1.30 (m, 2H,

–N(CH_2CH_2)$_2CH_2$); 1.33–1.40 (m, 4H, –N(CH_2CH_2)$_2CH_2$); 2.36–2.38 (m, 4H, –N(CH_2CH_2)$_2CH_2$); 2.60–2.64 (m, 2H, COCH_2CH_2N); 2.69–2.73 (m, 2H, COCH$_2CH_2$N); 7.21–7.31 (m, 3H); 7.44–7.49 (m, 1H); 7.63–7.67 (m, 2H); 8.09 (d, 1H); 8.66 (d, 1H); 13.00 (s, 1H).

4.11. N-[2-(1H-Benzoimidazol-2-yl)-phenyl]-3-(2-methyl-piperidin-1-yl)-propionamide (**7b**)

This compound was prepared from **5** according to the method mentioned under the synthesis of **7a**, recrystallized from acetonitrile in 58% yield. $C_{22}H_{26}N_4O$. M.W. 362.48. M.p.: 176–177 °C. $R_f = 0.05$ (eluent A), $R_f = 0.12$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 363 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 362 (0.4) $[M]^+$, 347 (6), 263 (21), 244 (16), 236 (74), 219 (10), 209 (25), 112 (100), 84 (84). ^{1}H NMR (DMSO-d_6): 1.00 (d, 3H, –N(CH_2CH_2)$_2$CHCH_3); 1.05–1.53 (n/r m, 6H); 2.17–2.26 (m, 1H); 2.30–2.35 (m, 1H); 2.55–2.60 (t, 2H, COCH_2CH_2N); 2.76–2.85 (m, 2H, COCH$_2CH_2$N), 3.00–3.08 (m, 1H); 7.21–7.31 (m, 3H); 7.44–7.49 (m, 1H); 7.62–7.65 (m, 2H); 8.09 (d, 1H); 8.66 (d, 1H); 13.03 (s, 1H).

4.12. N-[2-(1H-Benzoimidazol-2-yl)-phenyl]-3-(4-methyl-piperidin-1-yl)-propionamide (**7c**)

This compound was prepared from **5** according to the method mentioned under the synthesis of **7a**, recrystallized from acetonitrile in 71% yield. $C_{22}H_{26}N_4O$. M.W. 362.48. M.p.: 202–204 °C. $R_f = 0.01$ (eluent A), $R_f = 0.12$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 363 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 362 (1.2) $[M]^+$, 264 (3), 244 (4), 236 (11), 221 (4), 210 (20), 112 (100). ^{1}H NMR (DMSO-d_6): 0.74 (d, 3H, –N(CH_2CH_2)$_2$CHCH_3); 0.90–1.03 (m, 2H); 1.19 n/r m, 1H); 1.41 (d, 2H); 1.85 (t, 2H); 2.59–2.64 (m, 2H, COCH_2CH_2N); 2.70–2.74 (m, 2H, COCH$_2CH_2$N); 2.84 (n/r d, 2H); 7.20–7.31 (m, 3H); 7.43–7.49 (m, 1H); 7.63–7.66 (m, 2H); 8.09 (d, 1H); 8.67 (d, 1H); 13.01 (s, 1H).

4.13. N-[2-(1H-Benzoimidazol-2-yl)-phenyl]-3-morpholin-4-yl-propionamide (**7d**)

This compound was prepared from **5** according to the method mentioned under the synthesis of **7a**, recrystallized from acetonitrile in 76% yield. $C_{20}H_{22}N_4O_2$. M.W. 350.42. M.p.: 210–212 °C (decomp.). $R_f = 0.04$ (eluent A), $R_f = 0.48$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 351 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 350 (1) $[M]^+$, 332 (8), 320 (6), 307 (3), 281 (8), 264 (6), 236 (100), 222 (9), 209 (75), 100 (70). ^{1}H NMR (DMSO-d_6): 2.43–2.45 (m, 4H, –N(CH_2CH_2)$_2$O); 2.64–2.69 (m, 2H, COCH_2CH_2N); 2.74–2.79 (m, 2H, COCH$_2CH_2$N); 3.43 (t, 4H, –N(CH_2CH_2)$_2$O); 7.23–7.31 (m, 3H); 7.46–7.51 (m, 1H); 7.61 (n/r m, 1H); 7.72 (n/r m, 1H); 8.10 (d, 1H); 8.66 (d, 1H); 13.00 (s, 1H); 13.14 (br s, 1H).

4.14. N-[2-(1H-Benzoimidazol-2-yl)-phenyl]-3-(4-methyl-piperazin-1-yl)-propionamide (**7e**)

This compound was prepared from **5** according to the method mentioned under the synthesis of **7a**, recrystallized from acetonitrile in 56% yield. $C_{21}H_{25}N_5O$. M.W. 363.47. M.p.: 278–279 °C. $R_f = 0$ (eluent A), $R_f = 0.02$ (eluent B), at UV-254 spot fluoresces. Mass spectrum (FAB), *m/z* (%): 364 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 363 (3) $[M]^+$, 320 (6), 307 (10), 293 (31), 275 (11), 264 (18), 244 (7), 236 (51), 222 (27), 210 (47), 113 (71). ^{1}H NMR (DMSO-d_6): 2.04 (s, 3H, –N(CH_2CH_2)$_2$NCH_3); 2.17 (n/r m, 4H, –N(CH_2CH_2)$_2$NCH$_3$); 2.43 (n/r m, 4H, –N(CH_2CH_2)$_2$NCH$_3$); 2.61–2.65 (m, 2H, COCH_2CH_2N); 2.72–2.76 (m, 2H, COCH$_2CH_2$N); 7.21–7.31 (m, 3H); 7.44–7.49 (m, 1H); 7.64–7.67 (m, 2H); 8.09 (d, 1H); 8.66 (d, 1H); 13.00 (s, 1H).

4.15. 6-Chloromethyl-benzo[4,5]imidazo[1,2-c]quinazoline (**8**)

To the stirred solution of **3** (7.0 g, 0.035 mol) in 100 ml glacial acetic acid was added dropwise chloracetylchloride (4.4 ml, 6.2 g, 0.055 mol). Solution was heated up on the water bath (not above 60 °C) for 15 min, cooled and poured out in cool water. The resulting precipitate was filtered, washed with H_2O to neutral pH and dried. After recrystallization from acetone product was obtained with yield 6.3 g (68%). $C_{15}H_{10}ClN_3$. M.W. 267.72. M.p.: 237–238 °C. $R_f = 0.33$ ($CHCl_3$). IR ν_{max} cm^{-1}: 2970–2990; 1595; 1665; 690. Mass spectrum (FAB), *m/z* (%): 268 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 267 (100) $[M]^+$, 232 (55). ^{1}H NMR (DMSO-d_6): 5.49 (s, 2H); 7.55–7.66 (m, 2H); 7.77–7.83 (m, 1H); 7.87–7.93 (m, 1H); 7.97–8.02 (m, 2H); 8.25 (d, 1H); 8.60 (d, 1H).

4.16. 6-(2-Chloro-ethyl)-benzo[4,5]imidazo[1,2-c]quinazoline (**9**)

This compound was prepared from **3** according to the method mentioned under the synthesis of **8** in 97% yield. $C_{16}H_{12}ClN_3$. M.W. 281.75. M.p.: 165–166 °C (decomp.). $R_f = 0.69$ (eluent A), $R_f = 0.80$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 282 (100) $[M+H]^+$. ^{1}H NMR ($CDCl_3$): 3.77 (t, 2H); 4.20 (t, 2H); 7.38–7.42 (m, 1H); 7.47–7.51 (m, 1H); 7.57–7.63 (m, 1H); 7.70–7.74 (m, 1H); 7.80–7.83 (m, 1H); 7.88–7.95 (m, 2H); 8.62 (d, 1H).

4.17. 6-Piperidin-1-ylmethyl-benzo[4,5]imidazo[1,2-c]quinazoline (**10a**)

To a suspension of **8** (0.54 g, 0.002 mol) in DMF (10 ml) at room temperature was added piperidine (0.6 ml, 0.51 g, 0.006 mol), thus a suspension passed in solution. The reaction mixture was kept at room temperature for 24 h, then poured in 100 ml of distilled water. The resulting precipitate was filtered, washed with H_2O to neutral pH and dried in the exsiccator above alkali within the night. After recrystallization from heptane product was obtained with yield 0.58 g (61%). $C_{20}H_{20}N_4$. M.W. 316.41. M.p.: 145–146 °C. $R_f = 0.27$ (eluent A), $R_f = 0.52$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 317 (100) $[M+H]^+$. ^{1}H NMR (DMSO-d_6): 1.43–1.45 (m, 6H); 2.59 (n/r m, 4H); 4.15 (s, 2H, ArCH_2N); 7.48–7.60 (m, 2H); 7.71–7.77 (m, 1H); 7.82–7.88 (m, 1H); 7.93–7.96 (m, 2H); 8.09 (d, 1H); 8.57 (d, 1H).

4.18. 6-(2-Methyl-piperidin-1-ylmethyl)-benzo[4,5]imidazo[1,2-c]quinazoline (**10b**)

This compound was prepared from **8** according to the method mentioned under the synthesis of **10a**, recrystallized from heptane in 62% yield. $C_{21}H_{22}N_4$. M.W. 330.44. M.p.: 134–135 °C. $R_f = 0.41$ (eluent A), $R_f = 0.52$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 331 (100) $[M+H]^+$. ^{1}H NMR (DMSO-d_6): 1.20 (d, 3H, –N(CH_2CH_2)$_2$CHCH_3); 1.37 (n/r m, 4H); 1.58 (n/r m, 2H); 2.46–2.56 (m, 1H); 2.65–2.71 (m, 1H); 2.85 (n/r m, 1H); 4.09 (d, 1H, ArCH_2N); 4.54 (d, 1H, ArCH_2N); 7.47–7.60 (m, 2H); (m, 1H); 7.81–7.87 (m, 1H); 7.91–7.95 (m, 2H); 8.32 (d, 1H); 8.57 (d, 1H).

4.19. 6-(4-Methyl-piperidin-1-ylmethyl)-benzo[4,5]imidazo[1,2-c]quinazoline (**10c**)

This compound was prepared from **8** according to the method mentioned under the synthesis of **10a**, recrystallized from heptane in 68% yield. $C_{21}H_{22}N_4$. M.W. 330.44. M.p.: 142–144 °C. $R_f = 0.52$ (eluent A), $R_f = 0.52$ (eluent B), at UV-254 spot absorbs. Mass

spectrum (FAB), *m/z* (%): 331 (100) $[M+H]^+$, 259, 233, 219, 112. Mass spectrum (electron stroke), *m/z* (%): 233 (100), 98 (25). ^{1}H NMR (DMSO-d_6): 0.81 (d, 3H, N(CH_2CH_2)$_2$CHCH_3); 0.98–1.06 (m, 2H); 1.36 (n/r m, 1H, N(CH_2CH_2)$_2$$CH$$CH_3$); 1.56 (d, 2H); 2.17 (t, 2H); 2.95 (d, 2H); 4.16 (s, 2H, COCH_2N); 7.47–7.58 (m, 2H); 7.70–7.76 (m, 1H); 7.81–7.89 (m, 1H); 7.92–7.95 (m, 2H); 8.07 (d, 1H); 8.57 (d, 1H).

4.20. 6-Morpholin-4-ylmethyl-benzo[4,5]imidazo[1,2-c]quinazoline (**10d**)

This compound was prepared from **8** according to the method mentioned under the synthesis of **10a**, recrystallized from heptane in 82% yield. $C_{19}H_{18}N_4O$. M.W. 318.38. M.p.: 191–192 °C. $R_f = 0.38$ (eluent A), $R_f = 0.40$ (eluent B), at UV-254 spot absorbs. IR ν_{max} cm^{-1}: 2770–2990; 1600, 1665. Mass spectrum (FAB), *m/z* (%): 319 (100) $[M+H]^+$, 233, 100. Mass spectrum (electron stroke), *m/z* (%): 233 (98), 83 (100). ^{1}H NMR (DMSO-d_6): 2.63 (t, 4H, N(CH_2CH_2)$_2$O); 3.52 (t, 4H, N(CH_2CH_2)$_2$O); 4.22 (s, 2H, COCH_2N); 7.48–7.60 (m, 2H); 7.71–7.76 (m, 1H); 7.81–7.87 (m, 1H); 7.93–7.97 (m, 2H); 8.10 (d, 1H); 8.56 (d, 1H).

4.21. 6-(4-Methyl-piperazin-1-ylmethyl)-benzo[4,5]imidazo[1,2-c]quinazoline (**10e**)

This compound was prepared from **8** according to the method mentioned under the synthesis of **10a**, recrystallized from heptane in 54% yield. $C_{20}H_{21}N_5$. M.W. 331.42. M.p.: 164–165 °C. $R_f = 0.01$ (eluent A), $R_f = 0.15$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 332 (100) $[M+H]^+$, 289, 261, 233, 220. ^{1}H NMR (DMSO-d_6): 2.08 (s, 3H, N(CH_2CH_2)$_2$NCH_3); 2.25 (n/r m, 4H, N(CH_2CH_2)$_2$NCH$_3$); 2.62 (n/r m, 4H, N(CH_2CH_2)$_2$NCH$_3$); 4.17 (s, 2H, COCH_2N); 7.47–7.58 (m, 2H); 7.70–7.75 (m, 1H); 7.80–7.85 (m, 1H); 7.92–7.94 (m, 2H); 8.05 (d, 1H); 8.55 (d, 1H).

4.22. 6-(2-Piperidin-1-yl-ethyl)-benzo[4,5]imidazo[1,2-c]quinazoline (**11a**)

To a solution of **9** (0.56 g, 0.002 mol) in methanol (50 ml) at warming was added piperidine (0.6 ml, 0.006 mol). The reaction mixture was boiled for 30–40 min, evaporated to 10 ml and poured in 100 ml of distilled water. The resulting precipitate was filtered, washed with H_2O and dried. After recrystallization from isopropanol product was obtained with yield 0.49 g (74%). $C_{21}H_{22}N_4$. M.W. 330.44. M.p.: 119–120 °C. $R_f = 0.01$ (eluent A), $R_f = 0.48$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 331 (100) $[M+H]^+$, 307, 246, 220, 98. ^{1}H NMR (DMSO-d_6): 1.41–1.43 (m, 2H); 1.53–1.59 (m, 4H); 2.50 (n/r m, 4H); 2.93 (t, 2H, ArCH$_2$$CH_2$N); 3.57 (t, 2H, Ar$CH_2CH_2$N); 7.46–7.57 (m, 2H); 7.63–7.68 (m, 1H); 7.76–7.86 (m, 2H); 7.92 (d, 1H); 8.14 (d, 1H); 8.50 (d, 1H).

4.23. 6-[2-(2-Methyl-piperidin-1-yl)-ethyl]-benzo[4,5]imidazo[1,2-c]quinazoline (**11b**)

This compound was prepared from **9** according to the method mentioned under the synthesis of **11a**, recrystallized from isopropanol in 64% yield. $C_{22}H_{24}N_4$. M.W. 344.46. M.p.: 162–163 °C. $R_f = 0.06$ (eluent A), $R_f = 0.48$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 345 $[M+H]^+$, 113 (100). Mass spectrum (electron stroke), *m/z* (%): 344 (0.3) $[M]^+$, 244 (16), 112 (100). ^{1}H NMR (DMSO-d_6): 1.00 (d, 3H, –N(CH_2CH_2)$_2$CHCH_3); 1.16–1.35 (n/r m, 2H); 1.44–1.49 (n/r m, 1H); 1.56–1.63 (m, 3H); 2.28–2.37 (m, 1H); 2.41–2.45 (m, 1H); 2.93–3.01 (m, 2H, ArCH$_2$$CH_2$N); 3.34–3.40 (m, 1H); 3.56–3.62 (m, 2H, ArCH_2CH$_2$N); 7.48–7.60 (m, 2H); 7.65–7.71 (m, 1H); 7.78–7.88 (m, 2H); 7.94 (d, 1H); 8.18 (d, 1H); 8.53 (d, 1H).

4.24. 6-[2-(4-Methyl-piperidin-1-yl)-ethyl]-benzo[4,5]imidazo[1,2-c]quinazoline (**11c**)

This compound was prepared from **9** according to the method mentioned under the synthesis of **11a**, recrystallized from isopropanol in 68% yield. $C_{22}H_{24}N_4$. M.W. 344.46. M.p.: 108–110 °C. $R_f = 0.07$ (eluent A), $R_f = 0.48$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 345 $[M+H]^+$, 112 (100). Mass spectrum (electron stroke), *m/z* (%): 344 (0.5) $[M]^+$, 244 (9), 112 (100). ^{1}H NMR (DMSO-d_6): 0.91 (d, 3H, N(CH_2CH_2)$_2$CHCH_3); 1.16–1.29 (m, 2H); 1.38 (n/r m, 1H, N(CH_2CH_2)$_2$$CH$$CH_3$); 1.62–1.66 (m, 2H); 2.06–2.13 (m, 2H); 3.00–3.04 (m, 4H); 3.60 (t, 2H); 7.45–7.57 (m, 2H); 7.62–7.67 (m, 1H); 7.75–7.80 (m, 1H); 7.83 (d, 1H); 7.91 (d, 1H); 8.16 (d, 1H); 8.53 (d, 1H).

4.25. 6-(2-Morpholin-4-yl-ethyl)-benzo[4,5]imidazo[1,2-c]quinazoline (**11d**)

This compound was prepared from **9** according to the method mentioned under the synthesis of **11a**, recrystallized from isopropanol in 81% yield. $C_{20}H_{20}N_4O$. M.W. 332.41. M.p.: 190–191 °C. $R_f = 0.12$ (eluent A), $R_f = 0.31$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 333 (100) $[M+H]^+$. Mass spectrum (electron stroke), *m/z* (%): 332 (0.9) $[M]^+$, 314 (1.5), 245 (7), 233 (1.4), 100 (100). ^{1}H NMR (DMSO-d_6): 2.56 (t, 4H, N(CH_2CH_2)$_2$O); 3.00 (t, 2H, ArCH$_2$$CH_2$N); 3.61–3.65 (m, 6H═4H (N(CH_2CH_2)$_2$O) + 2H (ArCH_2CH$_2$N)); 7.46–7.56 (m, 2H); 7.64–7.70 (m, 1H); 7.77–7.87 (m, 2H); 7.93 (d, 1H); 8.17 (d, 1H); 8.51 (d, 1H).

4.26. 6-[2-(4-Methyl-piperazin-1-yl)-ethyl]-benzo[4,5]imidazo[1,2-c]quinazoline (**11e**)

This compound was prepared from **9** according to the method mentioned under the synthesis of **11a**, recrystallized from isopropanol in 78% yield. $C_{21}H_{23}N_5$. M.W. 345.45. M.p.: 97–98 °C. $R_f = 0$ (eluent A), $R_f = 0.13$ (eluent B), at UV-254 spot absorbs. Mass spectrum (FAB), *m/z* (%): 346 $[M+H]^+$, 275, 246, 113 (100). Mass spectrum (electron stroke), *m/z* (%): 345 (2) $[M]^+$, 303 (12), 289 (5), 275 (22), 244 (20), 233 (8), 113 (81), 70 (100). ^{1}H NMR (DMSO-d_6): 2.18 (s, 3H, N(CH_2CH_2)$_2$NCH_3); 2.37 (n/r m, 4H, N(CH_2CH_2)$_2$NCH$_3$); 2.58 (n/r m, 4H, N(CH_2CH_2)$_2$NCH$_3$); 3.00 (t, 2H, ArCH$_2$$CH_2$N); 3.60 (t, 2H, Ar$CH_2CH_2$N); 7.47–7.59 (m, 2H); 7.66–7.71 (m, 1H); 7.78–7.88 (m, 2H); 7.94 (t, 1H); 8.17 (d, 1H); 8.52 (d, 1H).

4.27. General procedure of benzimidazo[1,2-c]quinazolines dissolution

To a suspension of benzimidazo[1,2-*c*]quinazoline (25–30 mg) in methanol (0.5 ml) was added glacial acetic acid (5 ml) and distilled water (9.5 ml). It was obtained initial solution of a ligand "L". The prepared solution is necessary for using (to bring to the buffer with pH = 7 or on medium of culture) during 10–12 h.

4.28. General procedure of phenylbenzimidazoles dissolution

To a suspension of phenylbenzimidazole (50–65 mg) in methanol (0.5 ml) was added 0.1 M solution of HCl (4 ml) and distilled water (5.5 ml). The prepared solution is necessary for using during 24–48 h.

4.29. Procedure of preparation of initial solution for studying an affinity into CT DNA by the ethidium bromide displacement assay

The capable of intercalating into DNA was studied by ethidium bromide displacement assay [41]. It was prepared initial solution

"A", keeping the following components in the concentrations: 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); trilon B ($C = 4.93 \times 10^{-4}$ M); $pH = 5.50 \pm 0.05$. From initial solution "A" and solution "L" by double logarithmic dilution it was prepared series of solutions with the constant contents of all components, including DNA ($C = 1.06 \times 10^{-5}$ M) and ethidium bromide ($C = 1.26 \times 10^{-5}$ M) and the variable contents of ligand. Solution contained DNA and ethidium bromide in the same concentrations in the same buffer was used as control.

Fluorescence spectra were registered in the 560 – 800 nm range while 535nm excitation, using standard 1sm cell. The peak of ethidium bromide ($\lambda_{max} = 615$ nm) was marked out. Intensity of working solutions' fluorescence **I** was expressed in percentage from intensity of control solution fluorescence. On the obtained data dependence **I**, % – lgC was build.

Obtained values were approximated by sigmoid curve. The lgC_{50} point was determined as inflection point, and the confidence interval – as width of 50% displacement errors corridor. From lgC_{50} value was determined C_{50}, and further [41] calculated K_a.

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References

[1] http://whqlibdoc.who.int/searo/2003/SEA_ARI_5.pdf.
[2] G. Koren, S. King, S. Knowles, E. Phillips, CMAJ 168 (2003) 1289–1292.
[3] M. Peters, Semin. Liver Dis. 9 (1989) 235–239.
[4] C.E. Samuel, Virology 183 (1991) 1–11.
[5] M. Milella, G. Antonelli, et al., Liver 13 (1993) 146–150.
[6] K. Bendtzen, M.B. Hansen, M. Diamant, C. Ross, J. Interferon Res. 14 (1994) 157–159.
[7] U.B. Wandl, M. Nagel-Hiemke, et al., Clin. Immunol. Immunopathol. 65 (1992) 70–74.
[8] J.M. Bottomley, J.L. Toy, Interferon 4: In Vivo and Clinical Studies, Elsevier, Amsterdam–N.Y.–Oxford, 1984.
[9] V.M. Frolov, L.M. Vinnikova, V.O. Ter'oshyn, D.I. Klokol, Lik. Sprava 3 (2001) 135–138.
[10] L. Shi, H. Xiong, J. He, H. Deng, Q. Li, et al., Arch. Virol. 152 (2007) 1447–1455.
[11] Y.S. Boriskin, E.I. Pecheur, S. Polyak, Virol. J. 3 (2006) 56.
[12] V.V. Zarubaev, A.V. Slita, V.Z. Krivitskaya, et al., Antiviral Res. 58 (2003) 131–137.
[13] T.M. Sokolova, L.V. Uryvaev, E.B. Tazulakhova, et al., Vopr. Virusol. 50 (2005) 32–36.
[14] S.A. Liakhov, L.A. Litvinova, S.A. Andronati, et al., Ukr. Biokhim. Zh. 73 (2001) 108–113.
[15] L. Litvinova, A. Ptiashko, Sci. Innov. 3 (2007) 75.
[16] P. Chandra, F. Zunino, A. Zaccara, et al., FEBS Lett. 23 (1972) 145–148.
[17] T. Nishimura, T. Okobira, A.M. Kelly, et al., Biochemistry 46 (2007) 8156–8163.
[18] K. Geller, K.E. Reinert, W. Schulze, Biomed. Biochim. Acta 44 (1985) 1095–1103.
[19] S.A. Lyakhov. Abstract Book of 6th International Symposium on Molecular Aspects of Chemoterapy, Gdansk, Poland, 1997. p. 137.
[20] S. Alcaro, A. Arena, S. Neri, et al., Bioorg. Med. Chem. 12 (2004) 1781–1791.
[21] D.A. Stringfellow, L.A. Glasgow, Antimicrob. Agents Chemother. 2 (1972) 73–78.
[22] E.T. Glaz, E. Szolgay, I. Stoger, M. Talas, Antimicrob. Agents Chemother. 3 (1973) 537–544.
[23] S.A. Lyakhov, L.A. Litvinova, Y.I. Syveyzdis, et al., Khim.-Farm. Zh. 34 (2000) 20–21.
[24] D.A. Stringfellow, S.D. Weed, G.E. Underwood, Antimicrob. Agents Chemother. 15 (1979) 111–118.
[25] M. Fikus, T. Golas, A.D. Inglot, et al., Chem. Biol. Interact. 62 (1987) 25–43.
[26] M.F. Brana, J.M. Castellano, et al., Anticancer Drug Des 9 (1994) 527–538.
[27] L.N. Vostrova, T.A. Voronina, T.L. Karaseva, et al., Khim.-Farm. Zh. 20 (1986) 690–693.
[28] M. Tanada, S. Tsujita, S. Sasaki, J. Org. Chem. 71 (2006) 125–134.
[29] L. Wang, A. Kumar, D.W. Boykin, C. Bailly, W.D. Wilson, J. Mol. Biol. 317 (2002) 361–374.
[30] K.D. Goodwin, M.A. Lewis, et al., J. Am. Chem. Soc. 128 (2006) 7846–7854.
[31] P.M. Reddy, T.C. Bruice, J. Am. Chem. Soc. 126 (2004) 3736–3747.
[32] S. Mallena, M.P. Lee, C. Bailly, S. Neidle, et al., J. Am. Chem. Soc. 126 (2004) 13659–13669.
[33] M.A. Marques, R.M. Doss, S. Foister, P.B. Dervan, J. Am. Chem. Soc. 126 (2004) 10339–10349.
[34] P.M. Reddy, P.T. Jindra, et al., J. Am. Chem. Soc. 125 (2003) 7843–7848.
[35] J. Mann, A. Baron, Y. Opoku-Boahen, et al., J. Med. Chem. 44 (2001) 138–144.
[36] J. Bunkenborg, C. Behrens, J.P. Jacobsen, Bioconjugate Chem. 13 (2002) 927–936.
[37] W.A. Denny, G.W. Rewcastle, B.C. Baguley, J. Med. Chem. 33 (1990) 814–819.
[38] D.W. Hein, R.J. Alheim, J.J. Leavitt, J. Am. Chem. Soc. 79 (1957) 427–429.
[39] G.C. Helsley. US Patent. 3642778, 1972.
[40] A.V. Bogatsky, E.I. Ivanov, L.N. Vostrova, et al., Ukr. Khim. Zhurn. 45 (1979) 225–230.
[41] I. Antonini, P. Polucci, L.R. Kelland, et al., J. Med. Chem. 42 (1999) 2535–2541.
[42] F. Wilcoxon, Biometr. Bull. 1 (1945) 80–83.
[43] H.B. Mann, D.R. Whitney, Ann. Math. Stat. 18 (1947) 50–60.