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Synthesis and antitrichinellosis activity of some bis(benzimidazol-2-yl)amines

Anelia Ts. Mavrova,[a,*] Pavletta Denkova,[b] Yordan A. Tsenov,[b] Kameliya K. Anichina[a] and Dimitar I. Vutchev[c]

[a]*Department of Organic Synthesis, University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria*
[b]*National Center of Infectious and Parasitic Diseases, 26 Yanko Sakazov Blvd., 1504 Sofia, Bulgaria*
[c]*Institute of Organic Chemistry, Bulgarian Academy of Science, 1113 Sofia, Bulgaria*

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Abstract—Novel bis(benzimidazol-2-yl)amines were synthesized using two methods and studied for antitrichinellosis activity. DFT calculations were performed in order to determine the geometry of molecules.
All derivatives of 2-aminobenzimidazole exhibited higher activity in vitro against *Trichinella spiralis larvae* in regard to the activity of albendazole, moreover compounds **4f–i** manifested antitrichinellosis effect, which surpassed five times the activity of albendazole. The in vivo screening of intestinal phase of the *T. spiralis* revealed 100% effectiveness of compounds **4g–i** at oral dosages of 50 and 100 mg/kg mw, while albendazole possesses 100% efficacy only at a dose of 100 mg/kg mw.

1. Introduction

The 2-aminobenzimidazoles are promising class of chemical compounds with different biological effects as immunotropic, diuretic, antihistaminic as well as highly selective p38α MAP inhibition properties.[1–5] The polyfunctionality of the 2-aminobenzimidazole molecule resulting from the cyclic guanidine moiety has made it a building block for the synthesis of a large number of benzimidazole derivatives of pharmacological interest.

Following enviradene and enviroxime, many other 2-aminobenzimidazole derivatives have been synthesized and evaluated for antiviral activity.[6,7] Moreover, a number of 2-aminobenzimidazoles have exhibited antiproliferative properties.[8,9] Different substituted 2-aminobenzimidazoles have been found to possess in vivo and in vitro growth inhibition activity against various strains of bacteria, fungi and yeasts.[10–12]

The demonstration of potent anthelmintic activity of the different substituted 2-aminobenzimidazoles as well as the development of various drugs as mebendazole, fenbendazole, oxbendazole and albendazole support further the importance of this heterocycle in generating better chemotherapeutical agents against parasitic diseases.[13–15] The presence of the 2-aminobenzimidazole moiety presumes that probably that fragment is responsible for the anthelmintic properties of these compounds. Unless the high efficacy of the above-mentioned drugs the definitive treatment of trichinellosis, one of the most disseminated tissue helminthosis, remains pending. Therefore, it is of pharmacological interest to synthesize some new derivatives of 2-aminobenzimidazole and to study their anthelmintic activity.

Continuing our studies aimed at developing new potent antitrichinellosis agents, 2-aminobenzimidazole derivatives were prepared. In this paper, we report the synthesis of some novel bis(benzimidazol-2-yl)amines as well as their pharmacological evaluation for antitrichinellosis activity. That set of compounds is structurally different from the already known antitrichinellosis drug albendazole because other benzimidazole cycle is bonded to the amino group at second position instead of a methoxycarbonyl group and an alkyl group is introduced to the imino group of the benzimidazole ring in some of the compounds. As 2-aminobenzimidazole compounds are usually associated with antiparasitic activity it may be expected the bis(benzimidazol-2-yl)amines to display

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* Corresponding author. Tel.: +359 28163207; fax: +359 2685488; e-mail: anmav@abv.bg

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potential biological activity against different parasites and physiological disorders.

2. Results

The synthesis of the compounds **4a–i** was accomplished using two reaction methods: substitution of the sulfonic group by fusion of 1-(un)substituted benzimidazol-2-yl-sulfonic acid **2a–d** with 1-alkyl-2-amino-benzimidazole **3a–d** (method A) and reaction of 1-alkyl-2-(methylthio)benzimidazoles **5a–c** with 1-alkyl-2-aminobenzimidazole **5a–c** (method B), as outlined in Figures 1 and 2.

The reaction of 1,2-diaminobenzene, carbon disulfide and sodium hydroxide in ethanol medium yielded compound **1a**.[16,17]

The starting compounds **1b–d** were obtained by fusion of the corresponding 1-alkylbenzimidazoles with sulfur at 180 °C for 30 min according to the cited procedure.[18] The oxidation of the 1-(un)substituted-1*H*-benzimidazol-2-yl-thiols **1a–d** with $KMnO_4$ in 25% water solution of sodium hydroxide led to the formation of 1-(un)substituted-1*H*-benzimidazol-2-yl-sulfonic acids **2a–d**.[19] The reaction of benzimidazol-2-yl-sulfonic acids **2a–d**, carried out with 25% ammonium hydroxide in welded ampoule at 145–150 °C for 5 h, resulted in 2-aminobenzimidazoles **3a–d**.[20]

The bis(benzimidazol-2-yl)amines **4a–d** were synthesized by heating of benzimidazol-2-yl-sulfonic acids **2a–d** and 2-aminobenzimidazoles **3a–d**.

The chemical structures of all new compounds were established by IR, ^{1}H NMR and ^{13}C NMR spectra as well as elemental analysis. The IR-spectral characteristics (all spectra taken in KBr) are quite similar and could be summarized in the range as follows: ν(N–H): 3159.67–3175.76 cm^{-1}; $\nu(CH_3)$: 2962.88 cm^{-1}; ν(—C=N—): 1627.35–1808.19 cm^{-1}; δ (Ar) 732.69–800.26 cm^{-1}. Detail assignment of the ^{1}H NMR and ^{13}C NMR spectra of the studied compounds is given in the Experimental part. The elemental analyses indicated by the symbols of the element were within ±0.4% of theoretical values (Table 1).

2.1. Computations

In order to determine the electronic properties and the geometry of the molecule of the synthesized compounds DFT computations were performed with standard Gaussian 98 program package. We employed the B3LYP hybrid functional with 6-31G basis set.[21]

2.2. Anthelmintic activity

The parasitological study in vitro showed that most of the tested compounds possess higher activity than both the activity of albendazole and the efficacy of ivermectin against *T. spiralis*. Moreover, the efficacy of compound **4g** was 98% and that of compounds **4h** and **4i** was 100%. The results are given in Table 2. In the control samples with physiological solution and the samples only in DMSO all *T. spiralis larvae* had spiral form, i.e., vital.

The in vivo screening of intestinal phase of the *T. spiralis* done by us revealed 100% effectiveness of compounds **4g–i** after a 3-day-treatment course with oral dosages of 50 and 100 mg/kg mw, beginning on the third day of infection. We obtained the same results using albendazole as standard at a concentration of 100 mg/kg mw.[22,23] White immature mice were used as experimental animals.

3. Discussion

From the large diversity of methods, described in the literature for the synthesis of the intermediate compounds **1–3**, we chose the methods marked by their performance, simplicity and the possibility of every one of the synthesized compounds being used as starting component in the next reaction.

It is well known that sulfonic group at the second position in the benzimidazole can be easily replaced with alkoxy, hydrazine, amino, alkyl, aryl and heteroamino-group.[18–20] On the base of these facts we used 1-(un)substituted benzimidazol-2-yl-sulfonic acids to synthesize bis(benzimidazol-2-yl)amines. The reaction between benzimidazol-2-yl-sulfonic acids **2** and 2-aminobenzimidazoles **3** (method A), as it is shown in Figure 1, was carried out at molar ratio of reagents 1:2 and temperature 180 °C for 30 min. The yields are in the range of 48–70%. It was ascertained that the yields of bis(benzimidazol-2yl)amines increased as the alkyl chain in the (*H*)-benzimidazol-2-yl-sulfonic acids was longer compared to the alkyl chain in the 2-aminobenzimidazoles. For compound **4h** the yield was 42% when compounds **2c** and **3d** were used, but the reaction between **2d** and **3c** led to an increase of the yield up to 70%. The same relations were observed for the yields of compounds **4a–c**, **4e** and **4f**.

We have investigated also the possibility to replace the starting benzimidazol-2-yl-sulfonic acids **2** with 2-(methylthio)benzimidazoles **5** (method B, Fig. 2). We have experimentally proved that the necessary temperature for the proceeding of the reaction is about 250 °C and that the duration of the process is 15 min, but the yields of bis(benzimidazol-2-yl)amines were lower in comparison to the yields obtained by means of method A.

Therefore, method A is the preferable reaction way. This method permits pure bis(benzimidazol-2-yl)amines to be obtained with reasonable yields. The basic advantages of the proposed method are its simplicity, lower reaction temperature, and the use of easily accessible and non-toxic raw materials.

In the ^{13}C NMR spectra of compounds substituted in one of the two benzimidazole rings (compounds **4a–c**, R = H, see Fig. 3) we observed broadening of the signals for some carbon atoms from the unsubstituted benzimidazole ring. The respective carbons are

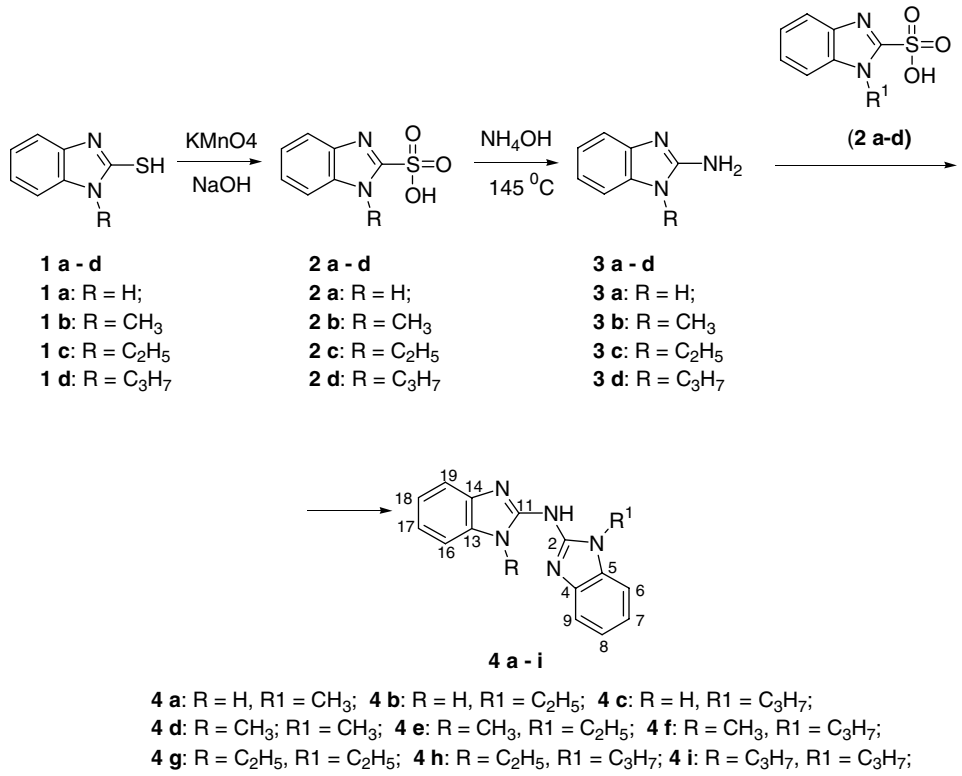

Figure 1. Synthesis of bis(benzimidazol-2-yl)amines (method A).

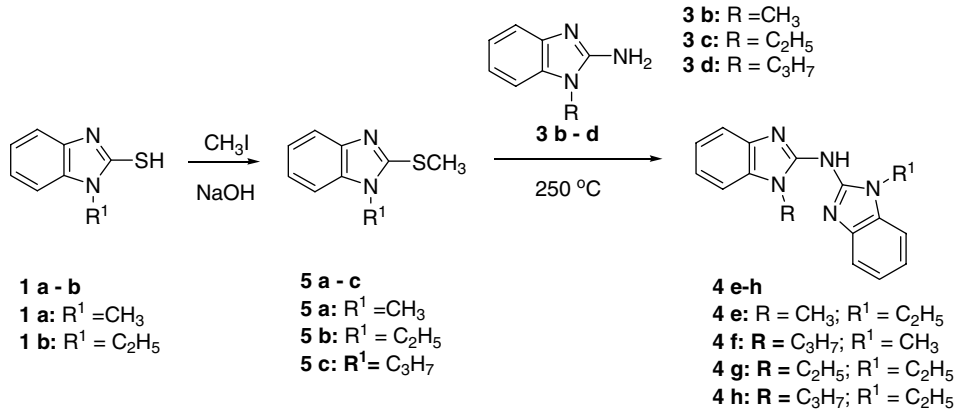

Figure 2. Synthesis of bis(benzimidazol-2-yl)amines (method B).

indicated as broad singlet (br s) in the description of the NMR spectra. These observations imply the presence of dynamic equilibrium between at least two possible tautomeric forms for the monosubstituted compounds. Each of the compounds **4a–c** has seven potential tautomeric forms. To determine the most favourable tautomeric structures of compounds **4a–c** we used ab initio calculations with complete optimization of the molecular geometry. We carried out the calculations with the standard Gaussian 98 package at RHF level using 6-31G basis set. The calculations performed in a gas phase show that the most favorable forms are those presented in Figure 3 with tautomer **B** being the more stable one. These results however are not consistent with the observed small chemical difference of carbon atoms C-11 and C-2 which is 0.2 ppm only. If the tautomeric form **B** is predominant larger chemical shift difference of the two atoms is expected because one of the C=N bonds is exocyclic, while the other one is in the benzimidazole ring. The small chemical shift difference of the two atoms suggests that both C=N bonds are included in the cyclic structure. It is well known that the tautomeric equilibrium is very sensitive to the type of the solvent. To account for the effect of the polar solvent we performed the theoretical calculations including two molecules of DMSO in the vicinity of the NH molecular fragments. The results show that in that case the tautomeric form **A** is more stable, which is in agreement with the chemical shifts of carbon atoms C-11 and C-2.

Table 1. Bis(benzimidazol-2-yl)amines **4a–i**

Compound	R	R′	Mol. Form. (MW)	Calculated (%)			Found (%)			R_f	Yield (%)
				C	H	N	C	H	N		
4a	H	CH_3	$C_{15}H_{13}N_5$	68.42	4.98	26.60	68.21	5.14	26.48	0.58	14
	CH_3	H	(263.30)								40
4b	H	C_2H_5	$C_{16}H_{15}N_5$	69.29	5.45	25.25	69.45	5.34	25.41	0.56	20
	C_2H_5	H	(277.13)								49
4c	H	C_3H_7	$C_{17}H_{17}N_5$	70.08	5.88	24.02	70.31	5.67	23.98	0.34	28
	C_3H_7	H	(291.35)								55
4d	CH_3	CH_3	$C_{16}H_{15}N_5$ (277.13)	69.29	5.45	25.25	69.19	5.61	25.30	0.66	38
4e	CH_3	C_2H_5	$C_{17}H_{17}N_5$	70.08	5.88	24.04	70.23	5.68	24.33	0.47	34
	C_2H_5	CH_3	(291.35)							0.47	47
4f	CH_3	C_3H_7	$C_{18}H_{19}N_5$	70.08	6.27	22.93	70.21	6.38	22.74	0.70	37
	C_3H_7	CH_3	(305.38)							0.70	64
4g	C_2H_5	C_2H_5	$C_{18}H_{19}N_5$	70.08	6.27	22.93	70.21	6.38	22.74	0.71	35
4h	C_2H_5	C_3H_7	$C_{19}H_{21}N_5$	71.45	6.63	21.93	71.60	7.06	22.09	0.76	42
	C_3H_7	C_2H_5	(319.40)							0.76	70
4i	C_3H_7	C_3H_7	$C_{20}H_{23}N_5$	72.04	6.95	21.00	72.23	7.09	20.89	0.78	50

Table 2. In vitro efficacy of compounds **4a–i** against *Trichinella larvae*

Compound	Efficacy (%) after 24 h			Compound	Efficacy (%) after 48 h		
	50 μg/ml	100 μg/ml	200 μg/ml		50 μg/ml	100 μg/ml	200 μg/ml
4a	9.1	13.5	30.2	**4a**	17.2	28.3	58.3
4b	15.7	20.0	38.2	**4b**	25.3	38.6	65.0
4c	12.0	17.6	67.7	**4c**	20.0	34.5	78.0
4d	8.8	17.3	45.7	**4d**	14.0	18.3	67.9
4e	35.3	65.7	76.3	**4e**	65.3	73.0	84.0
4f	33.3	73.8	80.0	**4f**	43.4	80.0	88.4
4g	45.8	92.0	94.0	**4g**	66.3	97.5	98.0
4h	42.0	84.0	96.0	**4h**	46.0	92.1	100
4i	45.9	81.2	100	**4i**	58.6	95.3	100
Albendazole	10.6	10.7	13.3	Albendazole	14.8	15.1	17.4
Ivermectin	45.5	48.6	54.3	Ivermectin	62.4	78.6	88.2

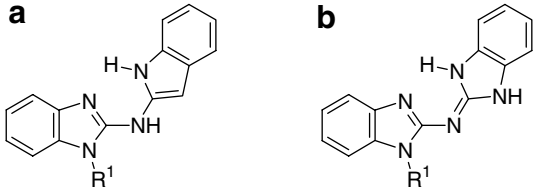

Figure 3. The most favourable tautomeric forms of compounds **4a–c** as determined by ab initio calculations.

The ab initio computations performed for compounds **4d–i** indicated that they possess nonplanar geometry of molecule in contrast to the other broad spectrum benzimidazole anthelmintics. The dihedral angle between both cycles increases and is 45° for compound **4i**. It may be supposed that the nonplanar structure facilitates the interaction with the biological targets.

3.1. Antitrichinellosis activity

Most of the tested bis(benzimidazol-2-yl)amines exhibited significant activity against *T. spiralis larvae*. In parasitological experiment in vitro for antitrichinellosis activity it was ascertained that the investigated compounds **4a–i** exert different anthelmintic effect, expressed in the suppressing of larvae motor activity and the opening of their spiral form, which is a mark of nonviability. In the first control group with physiological solution and in the second one with DMSO practically all larvae were in spiral form.

All derivatives of 2-aminobenzimidazole revealed higher activity against *T. spiralis larvae* in regard to the activity of albendazole, moreover compounds **4f–i** manifested antitrichinellosis effect, which surpassed five times the activity of albendazole. The compounds **4e–i** demonstrated higher effect against *T. spiralis larvae* than ivermectin after 24 h. The efficacy of compounds **4g–i** was also higher than the efficacy of ivermectin after 48 h.

Statistically significant differences in the level of parasites in both control and experimental groups were determined ($p \leqslant 0.05$).

The obtained results prompted us to study further three of the compounds—**4g–i** for parasitocide effect in vivo using white mice, infected with *T. spiralis*.

As a result of the in vivo screening of intestinal phase of the *T. spiralis* in white mice, 100% effectiveness of compounds **4g–i** was established after a 3-day-treatment course with oral dosages of 50 and 100 mg/kg mw, beginning on the third day of infection. Diversions in the behaviour of the experimental mice during the treatment were not observed. By means of microscopic control study of the intestinal content after autopsy of all tested animals no larvae were found. That fact indicated that all three compounds possess remarkable antitrichinellosis efficacy while albendazole exhibits 100% activity at a dose of 100 mg/kg mw.

4. Conclusion

New derivatives of 2-amino-benzimidazole were synthesized by use of two methods and studied for their anthelmintic activity. The ab initio computations indicated that the investigated compounds **4a–c** possess planar geometry of molecule, while for the disubstituted compounds **4d–i** nonplanar structure was determined in contrast to the other broad spectrum benzimidazole anthelmintics.

The in vitro screening revealed significant antiparasitic activity of the tested compounds. All benzimidazole derivatives of 2-aminobenzimidazole showed higher activity against *T. spiralis larvae* in comparison to albendazole, furthermore, some of the compounds (**4g–i**) demonstrated antitrichinellosis activity, which surpassed five times the activity of albendazole. In regard to ivermectin compounds **4c–d** and **4g–i** demonstrated higher effect against *T. spiralis larvae* at concentration 200 μg/ml after 24 h. The results obtained from the in vivo screening indicated that compounds **4g–i** possess 100% effectiveness at a concentration of 50 mg/kg mw.

These above results confirmed also the hypothesis that the introduction of a second benzimidazole ring in the structure of 2-aminobenzimidazoles and the nonplanar geometry are auspicious to the interaction of these molecules with the biological targets.

5. Experimental

Melting points (mp) were determined on an Electrothermal AZ 9000 3MK4 apparatus and are uncorrected. The thin layer chromatography (TLC, R_f values) was performed on silica gel 60 plates F_{254} (Merck, 0.2 mm thickness) using mobile phase *n*-heptane/ethyl acetate −2:1 and visualization was effected with ultraviolet light. IR spectra were recorded on a HP-ST-IR spectrophotometer as potassium bromide discs. NMR spectra were recorded on a Bruker Avance DRX 250 spectrometer (Bruker, Faelanden, Switzerland), with working frequency for ^{1}H 250 MHz, using a dual ^{1}H/^{13}C 5 mm probehead. $CDCl_3$ and DMSO-d_6 were used as solvents. Chemical shifts were expressed relative to tetramethylsilane (TMS) as internal standard and were reported as δ (ppm). The measurements were carried out at ambient temperature (300 K). The atom numbering used for description of the spectra is shown in Figure 1. The microanalyses for C, H and N were performed on Perkin-Elmer elemental analyzer.

5.1. Chemistry

5.1.1. General procedure for compound 1a. 1,2-Diaminobenzene (0.019 mol) and water (3 ml) were added to a solution of sodium hydroxide (0.022 mol) in ethanol (20 ml) and carbon disulfide (0.022 mol). The mixture was heated under reflux for 3 h. Charcoal was added cautiously and removed by filtration after the mixture has been refluxed for 10 min more. The filtrate was heated to 60–70 °C and quenched with warm water (70°, 20 ml), and then 50% acetic acid (9 ml) was added by good stirring. The product was separated and after cooling in refrigerator for 3 h the crystallization was completed.

5.1.2. General procedure for compounds 1b–d. 1-Alkylbenzimidazole (0.01 mol) and sulfur (0.01 mol) were heated at 260 °C for 30 min. About 10% water solution of sodium hydroxide was added after cooling of the fusion. The 1-alkyl-benzimidazole-2-thiol crystallized by neutralization of the obtained solution with acetic acid. After filtration the compound was washed with water and re-crystallized with ethanol.

5.1.3. General procedure for compounds 2a–d. To a boiling solution of 1-(un)substituted-1*H*-benzimidazol-2-ylthiol (0.04 mol) in water (25 ml) and 50% sodium hydroxide (15 ml), a solution of potassium permanganate (0.08 mol) in 200 ml was added in small portions by stirring. After the complete addition of potassium permanganate the reaction solution was refluxed for 30 min more. The formed manganese dioxide was filtered. The filtrate was treated with hydrochloric acid to pH 1 by cooling. The obtained precipitate of 1-(un)substituted-1*H*-benzimidazol-2-yl-sulfonic acid was filtered and washed with water.

5.1.4. General procedure for compounds 3a–d. 1-(Un)substituted-1*H*-benzimidazol-2-sulfonic acid (0.002 mol) and 25% ammonium hydroxide (1 ml) were heated in welded ampoule for 5 h at 145–150 °C. After cooling the formed crystals of 2-aminobenzimidazole were filtered and washed with ammonium hydroxide (0.3 ml) and water (4 ml) and re-crystallized with ethanol.

5.1.5. General procedure for compounds 4a–d. Method A: 1-(un)substituted-1*H*-benzimidazol-2-sulfonic acid (1.55 mmol) and the corresponding 2-aminobenzimidazole (3.1 mmol) were heated at 180 °C for 30 min. After cooling the fusion was diluted with ethanol. The obtained sediment was filtered and re-crystallized with ethanol.

Method B: 1-(un)substituted-2-(methylthio)benzimidazole (1.4 mmol) and 1-(un)substituted-2-amino-benzimidazoles **3a–c** were heated at 250 °C for 15 min. The fusion was cooled and minimum quantity of ethanol was added. The obtained precipitate was re-crystallized with ethanol.

5.1.5.1. *N*-1*H*-Benzimidazol-2-yl-1-methyl-1*H*-benzimidazol-2-amine (4a). Method A: yield −40% (starting reagents **2b** and **3a**); 14% (starting reagent **2a** and**3b**); mp 240–242 °C; $R_f = 0.58$; ^{1}H NMR (DMSO, δ ppm): 3.61 (s, 3H, N–CH_3), 7.34–7.04 (m, 8H, Ar–Bz); ^{13}C NMR (DMSO, δ ppm): 18.9 (N–CH_3), 108.4 (CH-6), 111.5 (CH-16, br s), 111.8 (CH-19, br s), 113.8 (CH-9), 120.7 (CH-8), 120.9 (CH-18), 121.2 (CH-17), 121.3 (CH-7), 133.2 (C-5), 133.7 (C-14, br s), 134.8 (C-13, br s), 136.6 (C-4), 154.6 (C-2), 154.8 (C-11).

5.1.5.2. *N*-1*H*-Benzimidazol-2-yl-1-ethyl-1*H*-benzimidazol-2-amine (4b). Method A: yield 49% (starting reagents **2c** and **3a)** resp. 20% (starting reagent **2a** and **3c**); mp 269–272 °C; $R_f = 0.56$; ^{1}H NMR ($DMSO_3$, δ ppm): 1.30 (t, 3H, –N–CH_2–CH_3, $J = 6.95$ Hz), 4.18 (q, 2H, –N–CH_2–CH_3, $J = 6.95$ Hz), 7.51–7.02 (m, 8H, Ar–Bz); ^{13}C NMR: 13.8 (–N–CH_2–CH_3), 36.0 (–N–CH_2–CH_3), 108.1 (CH-6), 111.0 (CH-19, CH-16, br s), 113.4 (CH-9), 120.4 (CH-8), 120.9 (CH-7, CH-17, CH-18), 131.7 (C-5), 133.1 (C-13, C-14, br s), 136.1 (C-4), 153.6 (C-2), 154.2 (C-11).

5.1.5.3. *N*-1*H*-Benzimidazol-2-yl-1-propyl-1*H*-benzimidazol-2-amine (4c). Method A: yield 55% (starting reagents **2d** and **3a)** resp. 28% (**2a** and **3d**); mp 209–211 °C; $R_f = 0.34$; ^{1}H NMR (DMSO, δ ppm): 0.91 (t, 3H, N–CH_2–CH_2–CH_3, $J = 7.35$ Hz), 1.78 (m, 2H, N–CH_2–CH_2–CH_3), 4.09 (t, 2H, N–CH_2–CH_2–CH_3, $J = 7.05$ Hz), 7.49–7.04 (m, 8H, Ar–Bz); ^{13}C NMR: 11.2 (–N–CH_2–CH_2–CH_3), 21.7 (N–CH_2–CH_2–CH_3), 42.7 (N–CH_2–CH_2–CH_3), 108.2 (CH-6), 110.9 (CH-16, CH-19, br s), 113.5 (CH-9), 120.2 (CH-8), 120.7 (CH-7), 120.8 (CH-17, CH-18), 132.3 (C-5), 132.9 (C-13, br s), 134.2 (C-14, br s), 136.3 (C-4), 154.1 (C-2), 154.2 (C-11).

5.1.5.4. 1-Methyl-*N*-(1-methyl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazol-2-amine (4d). Yield 38%: Method A; mp 200–202 °C; $R_f = 0.66$; ^{1}H NMR ($CDCl_3$, δ ppm): 3.66 (s, 6H, 2 N–CH_3), 7.19–7.08 (m, 6H, Ar–Bz), 7.44–7.37 (m, 2H, Ar–Bz), 9.78 (br s, 1H, NH); ^{13}C NMR: 27.9 (2 N–CH_3), 107.7 (CH-6, CH-16), 113.0 (CH-9, CH-19), 120.8 (CH-8, CH-18), 121.4 (CH-7, CH-17), 132.8 (C-5, C-13), 135.2 (C-14, C-4), 154.5 (C-11, C-2).

5.1.5.5. 1-Ethyl-*N*-(1-methyl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazol-2-amine (4e). Method A: yield 47% (starting reagents **2c** and **3b)** resp. 34% (**2b** and **3c**); Method B: 31%; mp 147–148 °C; $R_f = 0.47$; ^{1}H NMR ($CDCl_3$, δ ppm): 1.42 (t, 3H, N–CH_2–CH_3, $J = 7.21$ Hz), 3.68 (s, 3H, N–CH_3), 4.22 (q, 2H, N–CH_2–CH_3, $J = 7.21$ Hz), 7.20–7.13 (m, 6H, Ar–Bz), 7.43–7.40 (m, 2H, Ar–Bz), 8.92 (br s, 1H, NH); ^{13}C NMR: 13.8 (N–CH_2–CH_3), 28.0 (N–CH_3), 36.6 (N–CH_2–CH_3), 107.7 (CH-16), 107.8 (CH-6), 113.1 (CH-9, CH-19), 120.8 (CH-8, CH-18), 121.2 (CH-7), 121.3 (CH-17), 131.8 (C-5), 132.9 (C-13), 135.2 (C-14), 135.4 (C-4), 153.9 (C-2), 154.7 (C-11).

5.1.5.6. 1-Methyl-*N*-(1-propyl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazol-2-amine (4f). Method A: yield 64% (starting reagents **2d** and **3b**) resp. 37% (**3d** and **2b**); Method B: 42%; mp 136–138 °C; $R_f = 0.69$; ^{1}H NMR ($CDCl_3$, δ ppm): ^{1}H NMR: 0.99 (t, 3H, N–CH_2–CH_2–CH_3, $J = 7.34$ Hz), 1.89 (m, 2H, N–CH_2–CH_2–CH_3, $J = 7.34$ Hz, $J = 7.09$ Hz), 3.67 (s, 3H, N–CH_3), 4.15 (t, 2H, N–CH_2–CH_2–CH_3, $J = 7.09$ Hz), 7.17–7.09 (m, 6H, Ar–Bz), 7.45–7.40 (m, 2H, Ar–Bz), 8.63 (br s, 1H, NH); ^{13}C NMR: 11.6 (N–CH_2–CH_2–CH_3), 22.0 (N–CH_2–CH_2–CH_3), 28.0 (N–CH_3), 43.5 (N–CH_2–CH_2–CH_3), 107.6 (CH-16), 108.0 (CH-6), 112.9 (CH-9), 113.0 (CH-19), 120.8 (CH-8, CH-18), 121.2 (CH-7), 121.3 (CH-17), 132.2 (C-5), 132.9 (C-13), 135.0 (C-14), 135.4 (C-4), 154.2 (C-2), 154.6 (C-11).

5.1.5.7. 1-Ethyl-*N*-(1-ethyl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazol-2-amine (4g). Yield: Method A: 64%; Method B: 48%; mp 156–158 °C; $R_f = 0.71$; ^{1}H NMR ($CDCl_3$, δ ppm): ^{1}H NMR: 1.41 (t, 6H, 2 N–CH_2–CH_3, $J = 7.10$ Hz), 4.22 (q, 4H, 2 N–CH_2–CH_3, $J = 7.10$ Hz), 7.16–7.11 (m, 6H, Ar), 7.43–7.40 (m, 2H, Ar), 9.40 (br s, 1H, NH); ^{13}C NMR: 13.8 (2 N–CH_2–CH_3), 36.6 (2 N–CH_2–CH_3), 107.7 (CH-6, CH-16), 113.1 (CH-9, CH-19), 120.7 (CH-8, CH-18), 121.1 (CH-7, CH-17), 131.8 (C-5, C-13), 135.4 (C-4, C-14), 154.0 (C-2, C-11).

5.1.5.8. 1-Ethyl-*N*-(1-propyl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazol-2-amine (4h). Method A: yield: 70% (starting reagents **2d** and **3c**) resp. 42% (**2c** and **3d**); Method B: 52%; mp 145–146 °C; $R_f = 0.76$; ^{1}H NMR ($CDCl_3$, δ ppm): ^{1}H NMR: 0.99 (t, 3H, N–CH_2–CH_2–CH_3, $J = 7.34$ Hz), 1.41 (t, 3H, N–CH_2–CH_3, $J = 7.36$ Hz), 1.89 (m, 2H, N–CH_2–CH_2–CH_3, $J = 7.34$ Hz, $J = 6.85$ Hz), 4.12 (t, 2H, N–CH_2–CH_3, $J = 6.85$ Hz), 4.23 (q, 2H, N–CH_2–CH_2–CH_3, $J = 7.36$ Hz), 7.19–7.11 (m, 6H, Ar), 7.46–7.41 (m, 2H, Ar), 8.25 (br s, 1H, NH); ^{13}C NMR: 11.6 (N–CH_2–CH_2–CH_3), 13.8 (N–CH_2–CH_3), 22.0 (N–CH_2–CH_2–CH_3), 36.7 (N–CH_2–CH_3), 43.4 (N–CH_2–CH_2–CH_3), 107.7 (CH-16), 107.9 (CH-6), 113.0 (CH-9, CH-19), 120.8 (CH-8, CH-19), 121.2 (CH-7, CH-17), 131.8 (C-13), 132.3 (C-5), 135.2 (C-4), 135.3 (C-14), 153.8 (C-2) 154.3 (C-11).

5.1.5.9. 1-Propyl-*N*-(1-propyl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazol-2-amine (4i). Method A: yield: 50%; mp 132–134 °C; $R_f = 0.78$; ^{1}H NMR ($CDCl_3$, δ ppm): ^{1}H NMR: 0.99 (t, 6H, 2 N–CH_2–CH_2–CH_3, $J = 7.38$ Hz), 1.89 (m, 4H, 2 N–CH_2–CH_2–CH_3, $J = 7.38$ Hz, $J = 7.02$ Hz), 4.13 (t, 4H, 2 N–CH_2–CH_2–CH_3, $J = 7.02$ Hz), 7.18–7.09 (m, 6H, Ar), 7.45–7.40 (m, 2H, Ar), 9.22 (br s, 1H, NH); ^{13}C NMR: 11.6 (2 N–CH_2–CH_2–CH_3), 22.0 (2 N–CH_2–CH_2–CH_3), 43.5 (2 N–CH_2–CH_2–CH_3), 107.9 (CH-6, CH-16), 113.0 (CH-9, CH-19), 120.7 (CH-8, CH-18), 121.1 (CH-7, CH-17), 132.3 (C-5, C-13), 135.3 (C-4, C-14), 154.4 (C-2, C-11).

5.2. Biological screening

5.2.1. Antitrichinellosis activity in vitro. The parasitological pharmaco-therapeutic experiments in vitro and in vivo for antitrichinellosis activity of the tested

compounds were carried out according to Campbell's method.[22,23]

Encapsulated *T. spiralis larvae* were used in the parasitological pharmaco-therapeutic experiment in vitro, 150 specimens for 1 ml physiological solution. The tested benzimidazole derivatives were dissolved in DMSO–water. The used concentrations are given in Table 2. The samples were incubated in 'humid' chamber with thermostat at 37 °C. The microscopic control study for vitality of the *Trichinella larvae* was carried out 24 as well as 48 h after treatment using stereomicroscope MBC-9.5.

5.2.2. Antitrichinellosis activity in vivo. The biological test for antitrichinellosis activity in intestinal phase in vivo was accomplished using 45 white immature mice, infected under same conditions with 150 *Trichinella larvae*. The tested animals were divided in six groups of five mice for treatment with each one of the compounds as well as in two control groups of five mice one of them without treatment and one control group treated with albendazole, applied at dose of 100 mg/mw. The treatment course was carried out during 3 days at doses of 50 and 100 mg/kg mice-weight per os, beginning on the third day after infection. The compounds were used as DMSO solutions in a volume of 0.5 ml and introduced in the oesophagus of each mouse by means of thin metallic probe. The results of the performed test were estimated through microscopic observation of small intestines of the animals.

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