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Synthesis and antitrichinellosis activity of some 2-substituted-[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-ones

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Abstract—Some new thiazolo[3,2-*a*]benzimidazolone derivatives were synthesized using two methods. The structures of the synthesized compounds were proved by means of IR, ^{1}H NMR and mass spectral data. Ab initio computations were performed in order to determine the electronic structure and geometry of the investigated molecules and to compare it to the geometry of albendazole. Biologically, experiments in vitro and in vivo were accomplished in order to identify the efficacy of the obtained thiazolobenzimidazolones against *Trichinella spiralis*. The effectiveness of compounds **4a**–**c** in the intestinal phase of trichinellosis was 100% and in the muscle phase were 88% and 80% at a concentration of 100 mg/kg mw for the compounds **4a** and **4c**. The results of the hepatotoxicity test showed that the compounds **4a** and **4b** possess hepatotoxicity comparable to that of albendazole.

1. Introduction

The antihelmintic drugs derived from benzimidazole are the largest chemical family used to treat endoparasitic diseases in domestic animals and humans, characterized by high therapeutical index in the different helmintosis, polyvalent effect, and low toxicity. In spite of the significant features of these drugs, the definitive treatment of trichinellosis, one of the most disseminated tissue helmintosis, remains pending, and trichinosis continues to be a public-health concern throughout the world. Specifically, it has been estimated that 10 million people worldwide could be infected, and in the past 10 years an increase in occurrence of the infection has been reported among domestic pigs, horses, and wildlife, with a consequent increase among humans.

In the years since the first reports on the efficacy of benzimidazoles against *Trichinella spiralis* have appeared, many synthetic derivatives have been investigated under laboratory conditions for activity against this parasite. Only few of them, such as thiabendazole, cambendazole, oxfendazole, oxibendazole, and flubendazole, were thoroughly studied. The mode-of-action of the benzimidazole anthelmintics is the interruption of microtubular function. As a result of this interruption, egg hatching, larval development, and glucose transport are disturbed.[1] These compounds are effective against immature and mature intestinal phases and depress the productivity of the larvae in the muscles of the host, but only few of them, such as thiabendazole, mebendazole, and albendazole, are effective in relatively high doses and in prolonged treatment against capsulated muscle *T. spiralis* larvae.[2–7]

Another class of extremely potent and broad spectrum antiparasitic drugs is the class of macrocyclic lactones, which can be divided into two subclasses: the milbemycins and the avermectins. The mode-of-action of avermectins is affecting GABA-gated chloride channels. Ivermectin, which is a semisynthetic analog of avermectin, is widely used in the treatment of nematode infection in animals. In human medicine, it is used predominantly in treating onchocerciasis, strongyloidiasis, and filarial infection, especially *Wuchereria bancrofti*.[8–10] Ivermectin is highly

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effective in eliminating *T. spiralis* at the intestinal stage,[4] but is less effective against the larval muscle phase.[11–13]

Therefore, the development of new and more effective anthelmintics against trichinellosis is of pharmacological interest.

In our previous paper, we described the synthesis and antitrichinellosis activity of some 2-aryliden substituted thiazolo[3,2-*a*]benzimidazoles, which showed remarkably high activity against *T. spiralis*.[14]

Having in view the high activity of these compounds against *T. spiralis*, at the intestinal phase we undertook a synthesis of new 2-(indol-3-ylmethylene) and 2-(1,3-benzodioxole-5-ylmethylene)-thiazolo[2,3-*a*]benzimidazoles and in vitro investigation of their anthelmintic activity, as well as experimental therapeutic studies in vivo on the parasite in the muscle phase, in order to compare their activity with that of albendazole and ivermectin.

The structure of thiazolo[2,3-*a*]benzimidazol-3(2*H*)-ones gives the opportunity to realize changes by two ways: introduction of some substituents either in the benzene cycle of thiazolobenzimidazole or in the thiazole ring. This alteration in the structure of the main model compounds could be used to determine the influence of different substituents over the antitrichinellosis activity of the examined group of compounds. On the other hand, introduction of a condensed ring in the benzimidazole system may enhance the interaction of these molecules with the biological targets.

The choice of these structures was in accordance with the fact that the indole heterocycle is part of substances possessing anthelmintic activity.[15–20] The 1,3-benzodioxole moiety occurs in some biologically active compounds such as topomerase-II inhibitors (etoposide), ET_A–inhibitors (sitaxsentan), and HIV-protease inhibitors etc. [21] Therefore, it was of pharmacological interest to incorporate an indole or benzo-1,3-dioxole heterocycle in the structure of thiazolobenzimidazolones.

2. Results

The synthesis of 2-arylidene-thiazolo[3,2-*a*]benzimidazole-3(2*H*)-ones is illustrated and outlined in Schemes 1 and 2.

1*H*-Benzimidazole-2-thiols **1a–d** were prepared by refluxing ethanol–water solution of sodium hydroxide, carbon disulfide, and 4-(un)substituted-1,2-diaminobenzenes.[22]

The reaction between chloroacetic acid and 5-(un)substituted-1*H*-benzimidazole-2-thiols in the presence of sodium hydroxide led to compounds **2a–d**.[23]

1,3-Thiazolo[3,2-*a*]benzimidazol-3(2*H*)ones **3a–c** were obtained by heating (benzimidazol-2-ylthio)acetic acids **2a–c** and acetic anhydride in pyridine medium at 100 °C. For the preparation of compound **3d**, cyclization of (benzimidazol-2-ylthio)acetic acid **2d** was performed in the presence of dicyclohexyl carbodiimide (DCC) at room temperature.

The condensation between **3a–c** with 1,3-benzodioxole-5-carbaldehyde and indole-3-carbaldehyde, using pyridine as a catalyst resulted in products **4a–c** respectively (Method A).

The one-pot synthesis (Method B) carried out by a cyclocondensation (a Knoevenagel condensation, followed by cyclization) among 1*H*-benzimidazole-2-thiols **1a–d**, chloroacetic acid, aromatic or heteroaromatic aldehyde, acetic anhydride, and glacial acetic acid in the presence of sodium acetate (piperidine) led to 2-substituted thiazolo[2,3-*a*]benzimidazole-3(2*H*)-ones **4a–g** in good yields (51–93%). The compounds prepared were purified by re-crystallization and their physical constants were determined. The chemical structures of all the new compounds were established by IR, ^{1}H NMR and mass spectral data for the compounds **4a–d**. The analysis of the ^{1}H NMR spectra showed that the compounds **4c–g** present a mixture of two regioisomers— the 6- and 7-substituted derivatives in ratio 1:1. The results are presented in Section 5.

The characteristic absorption bands at 1680–1720 cm^{-1} for thiazolobenzimidazolone carbonyl and at 1580–1630 cm^{-1} for C=N bond were observed in the IR spectra.

2.1. Computations

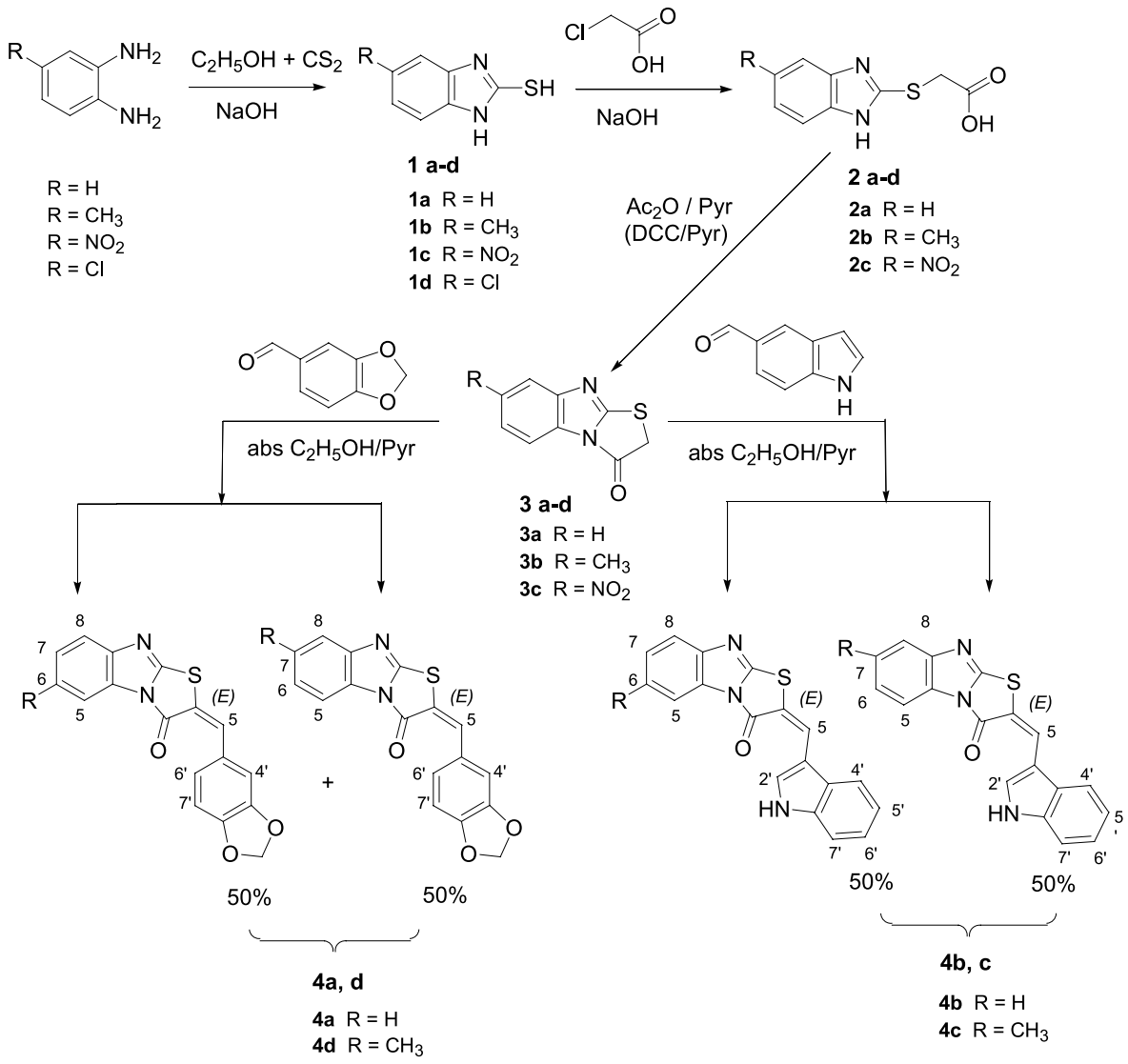
The ab initio computations were performed with standard Gaussian 98 (AIX Version 1998) at the RHF level, using the 6-31G basis set.[24]

It was found out that total energy of **4a** was $E = -1380.0981587$ Å and the total energies of **4b** and **4c** were $E = -1323.3565016$ Å, -1326.37765335 Å. The atomic charges by the oxygen (14O) from the carbonyl group of the thiazolobenzimidazolone cycle and by the hydrogen (31H) in sixth position of 1,3-benzodioxole in the compound **4a** were -0.593111 and 0.324999; the charges by 23O and 33H in indole cycle in **4b** were -0.352894 and 0.294352, whereas the charges by14O and 32H in **4c** were -0.620421 and 0.342756. The distance between 31H and 14O in **4a** 23O and 33H in **4b** and 14O and 32H in **4c** were 2.02 and 2.08 Å, respectively.

The total energy of albendazole was -1173.4120662 Å, the charge by 12O was -0.618868 and 22H was 0.443716. The distance between 12O and 22H was 2.157 Å.

2.2. Antihelmintic activity

The parasitological study in vitro showed that most of the tested compounds exhibited higher activity than albendazole against *T. spiralis*. In comparison to ivermectin, compound **4a** showed higher effectiveness after 24 h against encysted *T. spiralis* but exhibited the same activity as ivermectin after 48 h. The results are given in Tables 1 and 2. In the control samples with



Scheme 1. Synthesis of 2-substituted-[1,3]thiazolo[3,2-*a*]benzimiazol-3(2*H*)-ones (Method A).

physiological solution and the samples only in DMSO, all *T. spiralis* larvae had spiral form, that is, vital.

The obtained results promoted to study further the three compounds—**4a**–**4c** for parasitocide effect in vivo using white mice infected with *T. spiralis*.

The in vivo screening of intestinal phase of *T. spiralis* done by us showed 100% effectiveness of the compounds **4a**–**c** after a 3-day treatment course with an oral dosage of 100 mg/kg mw, beginning on the third day of infection. During the control microscopic study of intestinal content after autopsy of all tested animals, no larvae were found. This fact indicated that all the three compounds possess a remarkable antitrichinellosis efficacy. We obtained the same results using albendazole as a standard.

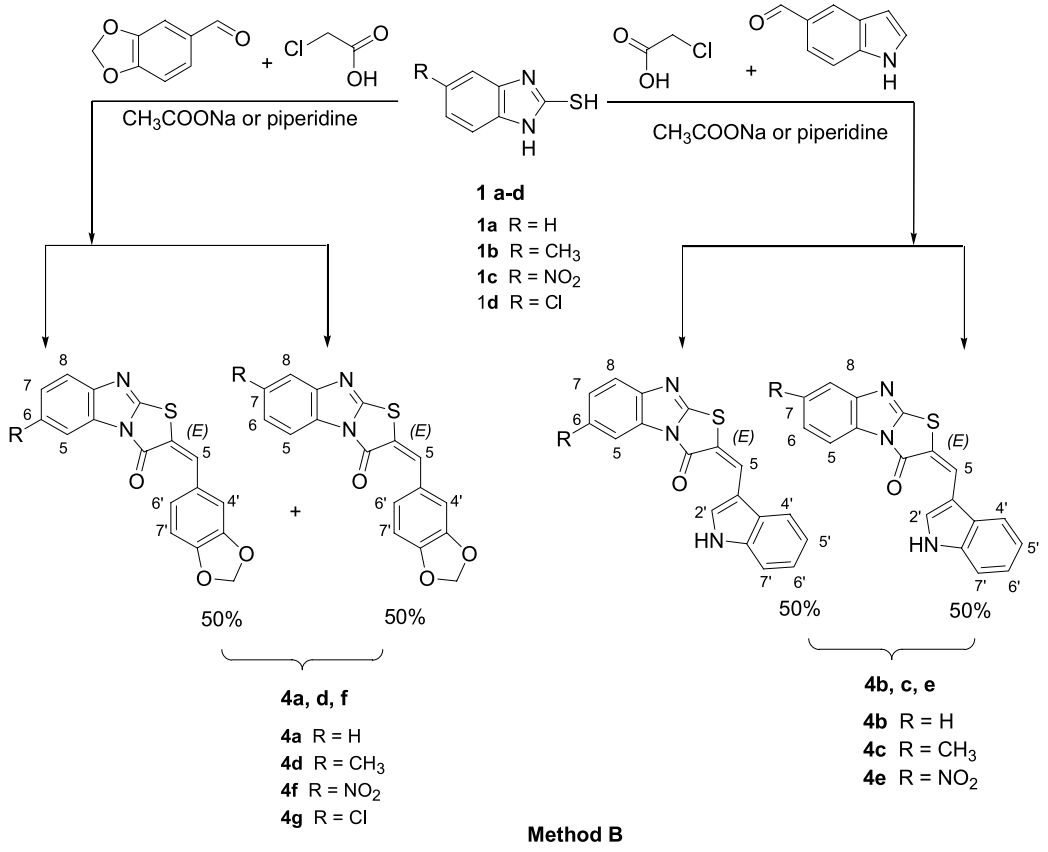
The results obtained by the pharmaco-therapeutic experiment on the antitrichinellosis activity in muscle stage (capsulated larvae) in 60 white mice pointed out that the compounds **4a** and **4c** exhibit, 88% and 80% efficacy respectively, whereas albendazole possessed 65% activity at a concentration of 100 mg/kg mw with respect to the control group of untreated animals. Ivermectin, administered as a dosage of 0.2 mg/kg mw exhibited 28% efficacy after 2 days of treatment (Table 3).

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

Preliminary investigation in muscle phase by using four guinea pigs showed 86% activity of **4a** and 80% of **4c**.

2.3. Hepatotoxicity in vitro

The results from a hepatotoxicity test in vitro of the thiazolobenzimidazole derivatives on the cell viability



Scheme 2. Synthesis of 2-substituted-[1,3]thiazolo[3,2-*a*]benzimiazol-3(2*H*)-ones (Method B).

Table 1. In vitro biological activity of compounds **4a**–**g** against *Trichinella spiralis* larvae[a]

Compound	Efficacy[b] (%)/24 h						
	20 (μg/mL)	50 (μg/mL)	100 (μg/mL)	200 (μg/mL)	400 (μg/mL)	800 (μg/mL)	1600 (μg/mL)
4a	42.5	60.5	80.1	85.2	88.5	92.5	95.0
4b	30.0	38.2	40.2	43.0	52.1	62.2	65.2
4c	38.5	49.5	55.2	61.2	63.0	68.6	73.5
4d	18.3	20.0	22.5	26.1	48.2	51.3	58.3
4e	5.1	6.6	11.5	13.4	16.7	21.3	25.8
4f	9.7	10.2	10.7	14.1	27.8	29.4	39.6
4g	3.7	4.3	6.3	9.9	19.2	24.6	27.5
Albendazole	9.3	10.6	10.7	13.3	14.2	23.1	28.9
Ivermectin	38.2	45.3	48.8	54.0	69.5	79.3	82.0

[a] Control—96 parasites.
[b] $p < 0.05$.

Table 2. In vitro biological activity of compounds **4a**–**g** against *Trichinella spiralis* larvae[a]

Compound	Efficacy [b] (%)/48 h						
	20 (μg/ml)	50 (μg/ml)	100 (μg/ml)	200 (μg/ml)	400 (μg/ml)	800 (μg/ml)	1600 (μg/ml)
4a	53.0	72.0	84.5	88.4	92.5	95.5	98.0
4b	33.3	39.8	44.9	54.1	59.2	68.3	72.0
4c	40.5	53.5	60.2	74.5	77.1	80.2	88.3
4d	28.0	31.7	36.0	42.3	49.8	62.4	73.6
4e	8.5	11.0	18.2	22.1	25.0	32.1	36.8
4f	18.0	20.7	21.5	21.6	27.3	36.7	42.0
4g	4.1	6.8	10.5	14.3	26.5	27.8	32.0
Albendazole	12.0	14.8	15.1	17.4	18.2	25.6	33.4
Ivermectin	40.0	62.1	78.5	88.2	92.3	97.0	98.1

[a] Control: 87 parasites.
[b] $p < 0.05$.

Table 3. Antitrichinosis activity of compounds **4a–c** in muscle phase

Compounds	Number of parasites in 1 g muscle mice mass	Efficacy (%)
4a	300 ± 71.8[a]	88
4b	1000 ± 169	60
4c	500 ± 122	80
Albendazole	900 ± 73	64
Ivermectin	1800 ± 145	28
Control	2–500 ± 901	

[a] $p < 0.05$.

Table 4. Effects of thiazolo[3,2-*a*]benzimidazole derivatives on the viability of isolated hepatocytes (vs control)

Group	Cell viability	Effect % versus control	Effect % versus albendazole
Control	93 ± 3.5[a]	100	
Albendazole	41 ± 2.0[a]	↓56	
4a	37 ± 3.9	↓60	↓10
4b	47 ± 4.2[a,b]	↓49	↑15
4c	20 ± 3.1[a]	↓78	↓51
4d	19 ± 2.5[a]	↓80	↓54
4e	17 ± 5.4[a]	↓82	↓59
4f	15 ± 3.7[a]	↓84	↓63

[a] $p \leqslant 0.05$ versus control.
[b] $p \leqslant 0.05$ versus albendazole.

of isolated rat hepatocytes (vs control) are presented in Table 4.

After addition of the examined compounds (250 μM) to the incubation medium, a statistically significant decrease in cell viability was observed. Compounds **4a** and **4b** had lowest toxicity in comparison to the control. Compound **4b** preserved the cell viability (15%) compared with albendazole ($p \leqslant 0.05$).

3. Discussion

The yields of 2-arilyden-thiazolo[3,2-*a*]benzimidazol-3(2*H*)-ones obtained by using Method A were found to be in the range of 61–68% after the third reaction stage of the three-step synthesis, but compared to the starting 1*H*-benzimidazole-2-thiols, the yields varied in the range of 26–30%.

Method B is a one-pot synthesis permitting the preparation of thiazolo[3,2-*a*]benzimidazol-3(2*H*)-ones in good yields (49–62%). Nevertheless, in order to achieve better results, we investigated the possibility of replacing the sodium acetate with another catalyst, namely piperidine. In that case, the yield of product **4a** increased to 78%, and that of **4c** and **4d** increased to 93% and 89%, respectively. Therefore, the compounds **4e–g** were synthesized by using the efficient and simple procedure of Method B.

The prototropic properties of the benzimidazole are well known. By the spectral assignment and multiplet analysis of ^{1}H NMR spectra, it was observed that the compounds **4c–g** present a mixture of the 6 and 7-substituted derivatives in ratio 1:1. The presence of the methyl group in the sixth position of **4d** leads to an upfield shift of the signal of H-5 proton by 7.855 ppm, while the signal of H-5 proton in the 7-substituted isomer is shifted downfield by 7.896 ppm (for description of chemical shifts, see the numbering of the H-atoms in Schemes 1). If the signals of H-8 protons in both the isomers are taken into consideration, it might be pointed out that the signal of H-8 in the 6-methyl-substituted thiazolobenzimidazolone is shifted downfield by 7.55 ppm and in the 7-substituted isomer by 7.47 ppm. The chlorine atom exerts on the signals of the H-5 protons in both the isomers, **4g** similar influence, by 7.588 ppm for the 7-chloro-substituted isomer and by 7.670 ppm for the 6-chloro-isomer. However, the nitro group at C-6 position of the thiazolobenzimidazolone cycle (**4f**) causes deshielding of the signal of the proton H-5 by 8.941 ppm, the nitro group at C-7 position shift upfield the signal of the H-5 by 7.773 ppm, and the signals for chemical shift of H-8 protons are by 8.16 and 8.57 ppm. The signals of H-5 protons in the isomers **4c** and **4e** overlap.

On the basis of computational results, a planar geometry (dihedral angle 180°) of the compounds **4a–c** was identified (Fig. 1). The possibility of H-bond formation between the carbonyl oxygen and the hydrogen atom in the sixth position of 1,3-benzodioxole cycle and the

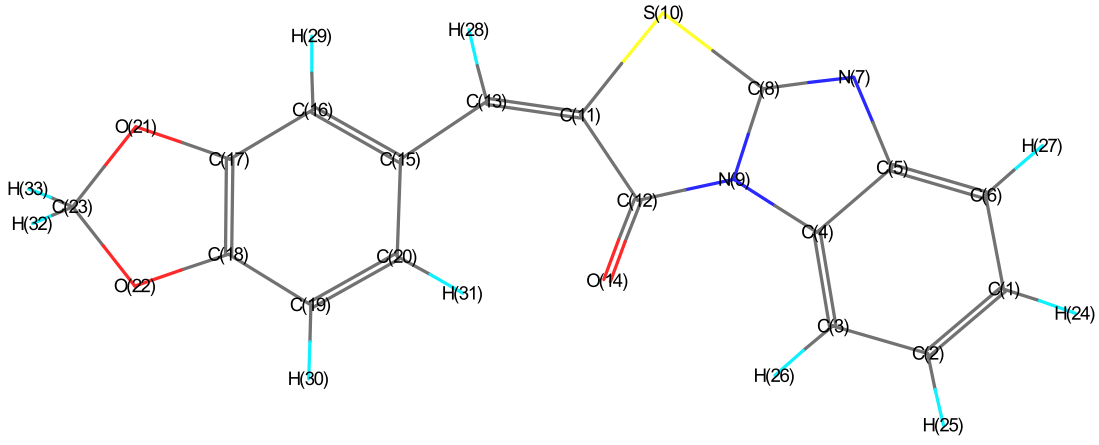


Figure 1. Planar geometry of compound **4a**.

hydrogen in the second position of indole was likely. Probably, the presence of H-bond allows the formation of seven-member-pseudo-cycle, which additionally stabilizes the planar geometry. The albendazole molecule also possessed a planar geometry and the formation of amid H-bonds is obvious (Fig. 2).

The parasitological experiment in vitro ascertained that the tested compounds possessed a different antihelmintic effect expressed in suppressing the motor activity of *T. spiralis* larvae and in losing the spiral form, which is a mark of non-viability. In the control samples, both in physiological solution and in DMSO, practically all *T. spiralis* larvae were in spiral form, i.e., vital. The observed difference between the antitrichinellosis efficacy of the compounds and that of albendazole was statistically significant ($p < 0.05$). The results obtained by the in vitro test for the activity of the compounds **4a**–**g** and albendazole proved that the efficacy of the compounds **4a**–**d** was higher than the activity of albendazole. The efficacy of the compounds **4e**–**g** was also comparable with the activity of albendazole. The most active compounds towards albendazole proved to be compound **4a**–98% efficacy—followed by **4c** (88%) and **4b** (75%). In a concentration of 1600 μg/mL, ivermectin exhibited 82% and 98.1% larvocide effect after 24 and 48 h, respectively. It deserves to be emphasized that at the same concentration (1600 μg/mL) the larvocide effect of the compound **4a** was 95% and 98%, while the compound **4c** was less active—73.5% and 88.3%, respectively.

The flat dose–response curve in the experiment in vitro can be explained with the biological characteristics of *T. spiralis* larvae. On the other hand, obtaining a good dose–response relationship depends on how much is previously known about the safety and the biological activity of the tested compounds. To obtain an acceptable dose–response curve, additional experiments using higher or lower concentrations are required.

Some anthelmintics from the benzimidazole group are more effective against immature *Trichinella*, whereas others are more effective against the adult worms. The antitrichinellosis activity of albendazole in intestinal phase, according to the data given by McCracken, is as follows: albendazole is 100% effective for 2 h with a dosage of 12.5 mg/kg in mice, but only 73% active for 72 h with a dosage of 50 mg/kg. If the data reported by the McCrackens for the efficacy of thiabendazole, are taken into consideration, it might be pointed out that in intestinal phase in mice, a single dose of thiabendazole at 50 mg/kg is highly effective against 2-h old *Trichinella*, but not against 20-h old worms. However, a dosage of 150 mg/kg is highly effective against 24-h old worms. Against mature worms, a dosage of 150 mg/kg is ineffective, and for 72 h, even a dosage of 500 mg/kg fails to remove the worms.[25]

Our results obtained in the in vivo screening of intestinal phase of *T. spiralis* in mice showed 100% effectiveness of the compounds **4a**–**c** and albendazole after a 3-days treatment course with an oral dosage of 100 mg/kg mw. The last effectiveness was established through microscopic observation of small intestine of the animals. Ivermectin was not used in that test, but according to Campbell W.C. et al., avermectins are highly active against intestinal stage.[4]

Campbell et al.[4] reported that in the early muscle phase, albendazole is 67% effective at 5×50 mg/kg and that larvae encysted in the muscles could be reduced by a dosage of 100 mg/kg.[4]

As it can be seen, the results obtained by the test (Table 3) in muscle phase indicated that the compounds **4a** and **4c** exhibited, respectively, 88% and 80% efficacy, respectively, against capsulated muscle *T. spiralis* larvae, while albendazole showed only 65% activity at a dosage of 100 mg/kg mw after 10 days of treatment. The test performed with ivermectin at a concentration of 0.200 mg/kg mw, which is the usually administrated dose in nemathode infections, pointed out 28% effectiveness of ivermectin. The obtained results agree with the low capacity of ivermectin to eliminate the capsulated muscle *T. spiralis* larvae as shown by Criado-Fornelio et al.[11], Ros-Moreno et al.[12], and Blackhall et al.[13]. Statistically significant differences in the level of parasites in both control and experimental groups in muscle phase were determined ($p \leqslant 0.05$). All these facts confirm the significance of the new compounds.

The preliminary results indicated also that the substituent at position 6(7) of the thiazolobenzimidazole deriva-

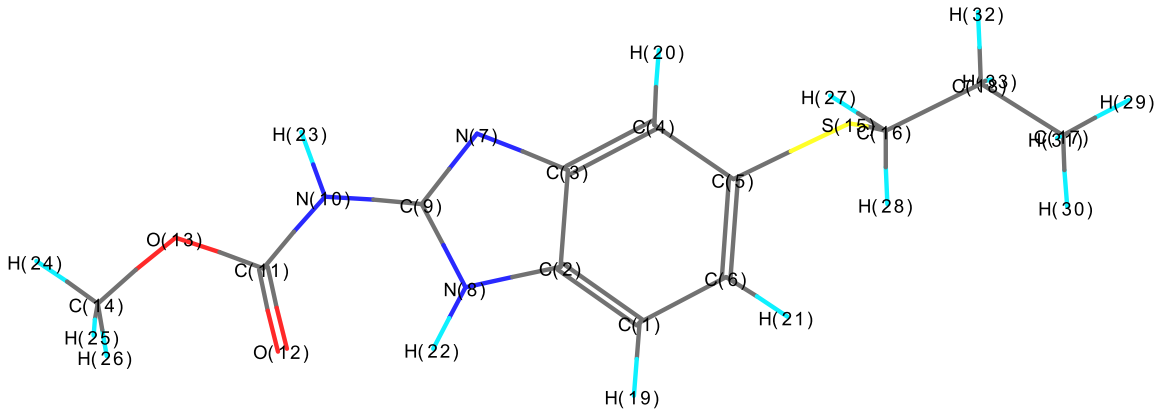


Figure 2. Planar geometry of albendazole.

tives played a significant role in their biological activity. As it can be seen from Table 1 that the most active compounds **4a–d** were not substituted or substituted with a methyl group, while the presence of chlorine atom or nitro group at that place was unessential; the corresponding derivatives **4e–g** were inactive. As mentioned above, the compounds **4c–g** present a mixture of two regioisomers—substituted at positions 6 and 7.

Compound **4a** containing 1,3-benzodioxole moiety at the second position of the thiazolobenzimidazole heterocycle was more active than that having an indolydene residue, for instance **4a** (R = H) was more active than **4b** (R = H). From the compounds containing an indolydene residue, the most active was compound **4c**, i.e., with substituent methyl group at 6-th and 7-th positions while **4a** (R = H) was more effective than **4d** ($R = CH_3$).

The studied compounds can be regarded as thiabendazole derivatives. Rey-Grobellet et al.[26] found that thiabendazole in rabbit hepatocytes showed an activation of cytochrome P450 enzymes and supposed that the metabolites of thiabendazole might be responsible for the observed toxicity.[21]

According to Mirfazaelian A. et al.,[28] albendazole is a benzimidazole carbamate used as the drug of choice in treatment of echinococcosis. After an oral administration, the drug is quickly oxidized by CYP3A4 to its pharmacologically active metabolite albendazole sulfoxide. Some pharmacokinetic studies indicate that albendazole sulfoxide is responsible for both antihelmintic and toxic effects of albendazole.[19,20]

The purpose of our study, using isolated hepatocytes was to compare whether the new compounds showed more toxic effect than albendazole. Because, it is known that albendazole exerted hepatotoxic effect, we used it as a control, versus which, to trace the effect of the new compounds (more toxic/less toxic). We investigated the effect of the compounds on cell viability by using a trypan blue test,[29] which showed that with lowest toxicity on the cell viability was **4a** and **4b** and most toxic versus control was **4f**. Compound **4b** preserved the cell viability 15%, with respect to albendazole and the compound **4a** had hepatotoxicity comparable to that of albendazole ($p \leqslant 0.05$).

The different effect of the compounds viability of hepatocytes might be explained and connected with the differences in their structure (different substituents at positions 2 and 6(7)). The unsubstituted compounds **4a** and **4b** were less toxic in contrast to the compounds containing methyl-, nitro-group or chlorine atom as a substituent at position 6(7) of the benzimidazole ring.

4. Conclusion

The 2-substituted-[1,3]thiazolo[3,2-*a*]benzimidazole-3(2*H*)-ones were synthesized by two different methods and tested for activity against *T. spiralis*. Ab initio computations indicated that the investigated compounds possess a planar geometry as all other broad spectrum benzimidazole antihelmintics. The spectral assignment and multiplet analysis of ^{1}H NMR spectra showed that the obtained compounds **4c–g** present a mixture from 6- and 7-substituted derivatives in ratio 1:1.

The in vitro and in vivo screening showed that the compounds exhibit remarkable antiparasitic activity. They were more effective both against encysted *T. spiralis* larvae in vitro and against muscle phase of *T. spiralis* in vivo in comparison with albendazole. With respect to ivermectin, compound **4a** had the same activity as ivermectin in vitro, but against muscle phase all three compounds**4a–c** demonstrated much higher effectiveness than ivermectin. The most active compounds possess hepatotoxicity comparable to that of albendazole.

These results confirmed also the hypothesis that the introduction of a condensed ring in the benzimidazole system is favorable 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 were uncorrected. Thin layer chromatography (R_f values) was performed on silica gel 60 plates F_{254} (Merck, 0.2-mm thick) using mobile phase benzene–methanol, 3:1 and benzene–acetone, 8:1, and visualization was effected with ultraviolet light. IR spectra were recorded on a Specord 71 IR spectrophotometer as potassium bromide discs. ^{1}H NMR spectra were obtained on a Bruker Avance DRX 250 MHz spectrometer (Bruker, Faelanden, Switzerland) using a dual 5-mm probe head and $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts were expressed relative to tetramethylsilan 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 Scheme 1. Typical condition for 1-D ^{1}H spectra were pulse width 30°, FT size 32 K and digital resolution 0.2 Hz per point. Mass spectrometric measurements were performed using a LCQ DECA instrument (Thermo Finnigan, Palo Alto, CA, USA). Compounds were solubilized in CH_3OH. Compounds (10^{-6} M solution) were prepared and directly infused into the ESI source. The ions were produced using spray voltage, capillary voltage and capillary temperature of 4 kV, 8 kV and 220 °C, respectively.

5.1. Chemistry

5.1.1. General procedure for compounds 1a–d. 4-(Un)substituted-1,2-diamino-benzene (0.019 mol) and water (3 mL) were added to 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 is then added cautiously; and after the mixture has been refluxed for 10 min, the charcoal was removed by filtration. The filtrate is heated to 60–70 °C and quenched with warm water (70 °C, 20 mL), and then 50% acetic acid (9 mL) was added by thorough

stirring. The product was separated, and after cooling in the refrigerator for 3 h, the crystallization was completed.

5.1.2. General procedure for compounds 2a–d. Solution of sodium hydroxide (0.012 mol) in ethanol (14 mL) and 5-(un)substituted-1*H*-benzimidazole-2-thiol (0.0067 mol) was refluxed for 1 h. After cooling, chloroacetic acid (0.0067 mol) was added and the refluxing was continued for another 1–5 h. Then, the reaction mixture was cooled, poured into water, and acidified with diluted acetic acid. The crystallized product was filtrated, carefully washed with water, and re-crystallized.

5.1.3. General procedures for compounds 3a–c. *Method A*: solution of 5(6)-(un)substituted-(1*H*-benzimidazol-2ylthio)acetic acid (0.104 mol) and acetic anhydride (19.5 mL) in dry pyridine (65 mL) was heated by reflux for 10 min. Products were crystallized as pale orange sediment after cooling and adding water (100 mL) in small portions by stirring.

Method B: DCC (0.0144 mol) in pyridine (10 mL) was added dropwise to the compounds **2a**–**c** (0.0144 mol) diluted in dry pyridine (60 mL). The solution was stirred for 10–12 h at 5–10 °C. The precipitate of dicyclohexylurea was removed, and the filtrate was evaporated under reduced pressure to dryness. The residue was dissolved in chloroform and filtrated after heating with charcoal. After cooling and filtering, the products were re-crystallized.

[1,3]Thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **3a**: Yield, 61% (Method A); 70% (Method B); mp 179–181 °C, re-crystallized with ethanol–benzene 1:2; $R_f = 0.69$, mobile phase benzene:methanol = 3:1; IR: 1740–*v* C=O; 1620–*v* C=N; ^{1}H NMR ($CDCl_3$): 7.94 (m, 1H, C5-H); 7.62 (t, 1H, C8-H); 7.41−7.27 (m, 2H, C6-H, C7-H), 4.36 (s, 2H, CH_2).

6(7)-Methyl-[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **3b**: Yield, 64% (Method A); 71% (Method B); mp 192–195 °C re-crystallized with ethanol; $R_f = 0.50$, mobile phase benzene:acetone = 8:1; ^{1}H NMR ($CDCl_3$): 7.76 (m, 1H, C5-H); 7.49 (d, 1H, C8-H); 7.16 (m, 1H, C7-H); 4.36 (s, 2H, CH_2); 2.47 (s, 3H, CH_3).

6(7)-Nitro-[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **3c**: Yield, 52% (Method B); mp 197 °C (destr.), re-crystallized with benzene; $R_f = 0.69$, mobile phase benzene:methanol = 3:1; ^{1}H NMR ($CDCl_3$): 8.84 (d, 1H, C5-H); 8.33 (m, 1H, C8-H); 7.73 (d, 1H, C7-H); 4.47 (s, 2H, CH_2).

5.1.4. General procedure for compounds 4a–f. *Method A*: 0.0105 mol of the relevant aldehyde was added to the solution of 0.01 mol **3a**–**d** in 50 mL absolute ethanol and 3–4 drops of pyridine. After several hours of reflux, the solution was cooled and the precipitate obtained was washed with ethanol, followed by double re-crystallization from ethanol. *Method B*: Mixture of 0.01 mol 1*H*-benzimidazole-2-thiol **1**, 0.015 mol chloroacetic acid, 2 g sodium acetate, 0.01 mol aromatic aldehyde, and glacial acetic acid was refluxed for 6 h. After cooling, the product obtained was re-crystallized from glacial acetic acid and washed with water. An additional re-crystallization was performed with ethanol.

2-(1,3-Benzodioxol-5-ylmethylene)[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **4a** (R = H, Ar = 1,3-benzodioxol-5-yl): Yield, 62%; (method A); 51% (method B, sodium acetate as a catalyst); 78% (Method B, piperidine as catalyst); mp 247–248 °C; $R_f = 0.744$; IR (KBr, cm^{-1}): 1710—*v* C=O; 1580—*v* N=C; MH^+ *m*/*z* = 323(45), $[MH+MeOH]^+$ *m*/*z* 355(100); ^{1}H NMR (250 MHz, $CDCl_3$, δ ppm): 8.06 (m, 1H, C8-H); 7.97 (s, 1H, C=CH); 7.68 (m, 1H, C5-H); 7.34–7.42 (m, 2H, C7-H, C6-H); 7.17 (dd, 1H, C6′-H, *J* = 1.79 and 8.13 Hz); 7.09 (d, 1H, C4′-H, *J* = 1.763 Hz); 6.95 (d, 1H, C7′-H, *J* = 8.13 Hz); 6.09 (s, 2H, CH_2).

2-(Indol-3-ylmethylene)[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **4b**: Yield, 65% (Method A); Mp 192–195 °C; $R_f = 0.67$; IR (KBr cm^{-1}): 3110—*v* NH; 1630—*v* C=O; 1600—*v* N=C; MH^+ *m*/*z* = 318(100), $[MH+MeOH]^+$ *m*/*z* 350(15); ^{1}H NMR (250 MHz, DMSO, δ ppm): 12.16 (s, 1H, NH, D_2O exchangeable); 9.93 (s, 1H, C=CH); 8.30–8.29 (m, 2H, C2′-H, C4′-H); 8.10–8.07 (dd, 2H, C8-H, C5-H, *J* = 8.32 and7.89); 7.53–7.49 (m, 2H, C7-H, C7′-H , *J* = 8.32 Hz); 7.21–7.27 (m, 3H, C6′-H, C5′-H, C6-H).

2-(Indol-3-ylmethylene)-6(7)-methyl[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **4c**: (R = CH_3) Yield, 68% (method A), 93% (method B, piperidine as a catalyst); mp > 298 °C; $R_f = 7.07$; IR: 3100—*v* NH; 1700—*v* C=O; 1580—*v* N=C; MH^+ *m*/*z* = 332 (100) $[MH+MeOH]^+$ *m*/*z* 364(55); ^{1}H NMR ($CDCl_3$+DMSO, δ ppm):11.90 (s, 1H, NH, D_2O exchangeable); 8.08 (s, 1H, –C=CH); 7.60–7.54 (m, 3H, C4′-H, C5-H, C2′-H); 7.23–7.15 (m, 2H, C7′-H, C8- H); 6.85–6.99 (m, 3H, C7-H, C6′-H, C5′-H).

2-(1,3-Benzodioxol-5-ylmethylene)-6(7)-methyl[1,3]thiazolo[3,2*a*]benzimi-dazol-3(2*H*)-one **4d**: Yield, 63% (method B, sodium acetate as a catalyst), 89% (method B, piperidine as a catalyst); mp = 215–218 °C; $R_f = 0.817$; IR: 1700—*v* C=O; MH^+ *m*/*z* = 337 (90) $[MH+MeOH]^+$ *m*/*z* 369(100); ^{1}H NMR (250 MHz, $CDCl_3$).

2-(1,3-Benzodioxol-5-ylmethylene)-(7)-methyl[1,3]thiazolo[3,2*a*]benzimi-dazol-3(2*H*)-one: 7.95 (s, 1H, C=CH); 7.89 (d, 1H, C5-H, *J* = 8.124 Hz); 7.47 (br s, 1H, C8-H); 7.20 (dd, 1H, C6-H, *J* = 1.086 Hz); 7.16 (d, 1H, C6′-H, *J* = 8.211 Hz); 7.09 (s, 1H, C4′-H); 6.95 (d, 1H, C7′-H); 6.09 (s, 2H, CH_2); 2.50 (s, 3H, CH_3).

2-(1,3-Benzodioxol-5-ylmethylene)-(6)-methyl[1,3]thiazolo[3,2*a*]benzimi-dazol-3(2*H*)-one: 7.95 (s, 1H, C=CH); 7.85 (s, 1H, C5-H); 7.55 (d, 1H, C8-H); 7.20 (dd, 1H, C7-H, *J* = 1.086 and 10.596 Hz); 7.16 (d, 1H, C6′-H); 7.09 (s, 1H, C4′-H); 6.95 (d, 1H, C7′-H, *J* = 8.09 Hz); 6.09 (s, 2H, CH_2); 2.49 (s, 3H, CH_3).

2-(Indol-3-ylmethylene)-6(7)-nitro[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one **4e**: Yield, 62% (Method B, sodium acetate); mp °C; $R_f = 0.613$; IR (KBr, cm^{-1}): 3100—

v NH; 1630—*v* C=O; 1330—*v* NO_2; ^{1}H NMR (250 MHz, $CDCl_3$): 10.08 (s, 1H, C5-H); 8.80 (br s, NH, D_2O exchangeable); 8.33 (m, 1H, C8-H); 7.86 (d, 2H, C4′-H, C2′-H); 7.47–7.41 (m, 2H, C7-H, C=CH)] 7.37–7.33 (m, 3H, C7′-H, C6′-H, C5′-H).

2-(1,3-Benzodioxol-5-ylmethylene)-6(7)-nitro[1,3]thiazolo-[3,2-*a*]benzimidazol-3(2*H*)-one **4f**: Yield, 58% (method B, sodium acetate); mp = 260 °C (destruction); R_f = 0.789; IR (KBr, cm^{-1}): 1710—*v* C=O; 1580—*v* C=N; 1330—*v* NO_2; ^{1}H NMR (250 MHz, $CDCl_3$).

2-(1,3-Benzodioxol-5-ylmethylene)-7-nitro[1,3]thiazolo-[3,2-*a*]benzimidazol-3(2*H*)-one: 8.59 (s, 1H, C8-H); 8.31 (dd, 1H, C6-H); 8.08 (s, 1H, C=CH); 7.77 (d, 1H, C5-H, *J* = 8.95 Hz); 7.19 (m, 1H, C6′-H); 7.11 (br s, 1H, C4′-H); 6.98 (d, 1H, C7′-H, *J* = 8.114 Hz); 6.12 (s, 2H, CH_2).

2-(1,3-Benzodioxol-5-ylmethylene)-6-nitro[1,3]thiazolo-[3,2-*a*]benzimidazol-3(2*H*)-one: 8.94 (d, 1H, C5-H); 8.35 (dd, 1H, C7-H); 8.16 (d, 1H, C8-H, *J* = 8.80 Hz); 8.05 (s, 1H, C=CH); 7.19 (m, 1H, C6′-H); 7.11 (br s, 1H, C4′-H); 6.98 (d, 1H, C7′-H, *J* = 8.11 Hz); 6.12 (s, 2H, CH_2).

2-(1,3-Benzodioxol-3-ylmethylene)-6(7)-chloro[1,3]thiazolo-[3,2-*a*]benzimidazol-3(2*H*)-one **4g**: Re-crystallized; Yield, 51%; mp = 240 °C (destr.); R_f = 0.772; **IR** (KBr, cm^{-1}):1700—*v* C=O; 1580—*v* C=N; ^{1}H NMR (250 MHz, $CDCl_3$).

2-(1,3-Benzodioxol-3-ylmethylene)-7-chloro[1,3]thiazolo-[3,2-*a*]benzimidazol-3(2*H*)-one: 7.97 (br s, 1H, C=CH); 7.49 (dd, 1H, C8-H, *J* = 2.547 and 8.171 Hz); 7.67 (dd, 1H, C5-H, *J* = 2.12 Hz); 7.32 (dd, 1H, C6-H, *J* = 1.89 and 8.47 Hz); 7.17 (dd, 1H, C6′-H, *J* = 1.83 and 8.15 Hz); 7.09 (d, 1H, C4′-H, *J* = 1.81 Hz); 6.96 (d, 1H, C7′-H, *J* = 8.14 Hz); 6.10 (s, 2H, CH_2).

2-(1,3-Benzodioxol-3-ylmethylene)-6-chloro[1,3]thiazolo-[3,2-*a*]benzimidazol-3(2*H*)-one: 8.05 (dd, 1H, C8-H); 7.99 (s, 1H, C=CH); 7.59 (dd, 1H, C5-H, *J* = 0.38 and 8.59 Hz); 7.36 (dd, 1H, C7-H, *J* = 2.053 and 8.59 Hz); 7.17 (dd, 1H, C6′-H, *J* = 1.828 and 8.152 Hz); 7.09 (d, 1H, C4′-H, *J* = 1.815 Hz); 6.96 (d, 1H, C7′-H, *J* = 8.12 Hz); 6.10 (s, 2H, CH_2).

5.2. Biological screening

The parasitological experiments in vitro as well as in vivo for the intestinal phase of trichinellosis were carried out according to Campbell's method.[3]

Encapsulated *T. spiralis* larvae were used in the parasitological experiment in vitro, 100 specimens for 1 mL physiological solution. The tested benzimidazole derivatives were dissolved in DMSO. The concentrations used are given in Table 1. The samples were incubated in 'humid' chamber with thermostat at 37 °C. The microscopy control for vitality of *T. spiralis* larvae was carried out after 24 h as well as 48 h after treatment, using stereomicroscope MBC-9.

The biological test for trichinellocide activity by intestinal phase in vivo was accomplished using 50 white immature mice, infected by equal conditions with 150 *Trichinella* larvae. The tested animals were divided into three groups of 10 species for treatment with each one of the compounds as well as one control group of 10 mice without treatment and one control group of 10 mice treated with albendazole. The treatment course was carried out during 3 days in doses of 100 mg/kg mice weight *per os*, beginning on the third day after infection. The compounds were used in the form of a 1% suspension in starch. The results of the performed test were estimated through microscopic observation of small intestine of the animals.

The preliminary pharmaco-therapeutic experiment for antihelmintic activity in muscles phase—capsulated larvae was accomplished on 60 white mice and four guinea pigs, invaded 40 days early with 1000 *T. spiralis* larvae each. Thirty mice and three of the experimental animals were treated *per os* with each one of compounds **4a–c** in doses 100 mg/kg mw pro die for 10 days cure course. Ten mice and one of the guinea pigs were not treated and were used as control samples. Two groups, each of 10 mice were treated with albendazole and ivermectin.

To estimate the effect of the chemical substances, the tested animals were post-mortem examined 10 days after the last treatment. The intensity of invasion in mice was established by compressive trichinelloscopy of 1 g skeleton muscle mass taken from each one of the mice. The intensity of invasion for each guinea pig was determined through artificial grind of 10 g from the skeleton muscles (intercostal and motor muscles). The number of *T. spirallis* larvae in 1 g muscles mass from the treated and control animals were determined by digestion of the invaded muscles in pepsin–hydrochloric acid.

5.3. Hepatotoxicity test

Rat hepatocytes were used to examine the hepatotoxicity of the synthesized compounds. The rat was anaesthetized with sodium pentobarbital. In situ liver perfusion and cell isolation were performed (Fau et al.,[27]). The cells were counted under microscope and the viability was estimated by trypan blue (0.05%) exclusion. The initial viability averaged 90%. Incubations were carried out in 25-mL Erlenmeyer flasks. The cells were diluted with Krebs–Ringer-carbonate buffer, pH = 7.4. Each flask contained 3 mL of the cell suspension (i.e., 9×10^6 hepatocytes) and the corresponding compound in concentration 250 μM. The incubation was performed under carbogen atmosphere.

The significance of differences between groups was determined by Student's *t*-test.

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