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Original article

Synthesis, antitrichinnellosis and antiprotozoal activity of some novel thieno[2,3-*d*]pyrimidin-4(3H)-ones containing benzimidazole ring

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ABSTRACT

Some novel thieno[2,3-*d*]pyrimidin-4(3H)-ones containing benzimidazol-2-yl-thioethyl- and benzimidazol-2-yl-methanethioethyl moiety in second position of the pyrimidine ring were synthesized in order to determine their antitrichinellosis and antiprotozoal effects. The structures of the compounds were confirmed by IR, ^{1}H NMR and elemental analysis.

The antiparasitic screening showed that the benzimidazole derivatives of thieno[2,3-*d*]pyrimidin-4(3H)-ones exhibited higher activity against Trichinella spiralis in vitro in comparison albendazole. The most active compound, 2-[2-(5-nitro-1H-benzimidazol-1-yl)ethyl]-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4(3H)-one **22** revealed 95% activity at a dosage of 5 mg/kg mw after 24 h, while compounds **8** and **10** applied at the same dose showed efficacy of 90% after 48 h. The compound 2-{2-[(5(6)-nitro-1H-benzimidazol-2-yl)thio]ethyl}-5,6,7,8-tetrahydro[1]-benzothieno[2,3-*d*]pyrimidin-4(3H)-one **11** exhibited 90% efficacy after 24 h.

The pharmaco-therapeutic study in vivo on invaded with Lamblia muris white mice showed 100% effectiveness of the compounds **8**, **10**, **11**, **13**–**15** and **22**, **23** after five-days-treatment course.

1. Introduction

Thieno[2,3-*d*]pyrimidines are an important group of biological active compounds due to their diverse pharmacological properties as antitumour [1,2], antiviral [3], antibacterial [4] and antituberculosis agents [5] and their synthesis has been in focus of considerable attention in the synthetic organic chemistry as well as in the medicinal chemistry. Many thieno[2,3-*d*]pyrrimidin-4-ones were synthesized as potential inhibitors of Pneumocystis carinii and Toxoplasma gondii dihydrofolate reductase [6] as well as high inhibitory effect against FP-2 of Plasmodium falciparum [7].

Benzimidazole is another fused heteroaromatic system of great interest. Benzimidazole derivatives occupied unique place in medicinal chemistry. The demonstration of a potent biological activity from the different substituted benzimidazoles as antiviral [8,9], anticancer agents [10–11] support further the importance of benzimidazol-2-yl-thiol moiety as pharmacophore for generating new chemotherapeutical agents. Selected benzimidazole derivatives were shown to be active in vitro against protozoan parasites as Trichomonas vaginalis, Giardia lamblia, Entamoeba hystolica Leishmania major and Acanthamoeba polyphaga, others were active in vitro against Trypanosoma brucei rhodesience and Plasmodium Falciparum [12–15]. Following, mebendazole cambendazole, oxfendazole, oxibendazole and flubendazole many benzimidazole derivatives have been studied for their antiparasitic properties [16,17]. Regardless of the high efficacy of the above mentioned drugs the definitive treatment of trichinellosis, one of the most disseminated tissue helmintosis and as well as the problem with the medication of protozoa diseases, remains pending.

On the base of the above mentioned facts it is of a pharmacological interest to synthesized new derivatives of benzimidazole-2-thiols, containing thieno[2,3-*d*]pyrimidin-4-one moiety and to perform preliminary study of their antiparasitic properties. Probably the introduction of benzimidazole ring in the structure of a thienopyrimidinone molecule can contribute to its interaction with the biological target. In this paper we describe the synthesis of new thienopyrimidinone derivatives of 1H-benzimidazol-2-yl-thiole and their activity in vitro against Trichinella spiralis as well as their effect against Paramaecium caudatum and Lamblia muris.

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2. Chemistry

The synthesis of thieno[2,3-*d*]pyrimidin-4(3H)-one derivatives, containing benzimidazole ring is illustrated and outlined in Fig. 1.

The starting 3-ethoxycarbonyl-2-amino-thiophenes **3**–**4** were synthesized according to the method, described by Gewaldt et al. [18]. 2-(2-Chloroethyl)-thieno[2,3-*d*]pyrimidin-4(3H)-ones **5**–**6** were obtained by passing a stream of dry hydrogen chloride gas through a solution of the appropriate 2-amino-3-ethoxycarbonyl-thiophene and 3-chloro-propanonitrile.

The nucleophilic substitution between the thieno[2,3-*d*] pyrimidinones **5**–**6** and the appropriated benzimidazol-2-ylthiols in the presence of sodium hydroxide resulted in the formation of **7**–**17** and the reaction of **5**–**6** with benzimidazol-2-yl-methanethiols yielded in compounds **18**–**21**. The interaction between the starting thienopyrimidin-4-ones and 4-(un) substituted-1H-benzimidazoles was performed out in the presence of tetrabutylammonium bromide (TBAB) and dry potassium carbonate in acetonitrile as well as in the presence of sodium hydroxide and resulted to the formation of compounds **22**–**24**.

The structures of all new compounds were established by IR, ^{1}H NMR as well as elemental analysis. Detailed assignment of the ^{1}H NMR and some of the ^{13}C NMR spectra of the synthesized compounds is given in the Experimental. The elemental analyses indicated by the symbols of the element were within ±0.4% of the theoretical values.

3. Pharmacology

3.1. Antihelmintic activity

The parasitological study in vitro showed that most of the tested compounds possess higher activity than the activity of albendazole against T. spiralis. The results are given in Table 1.

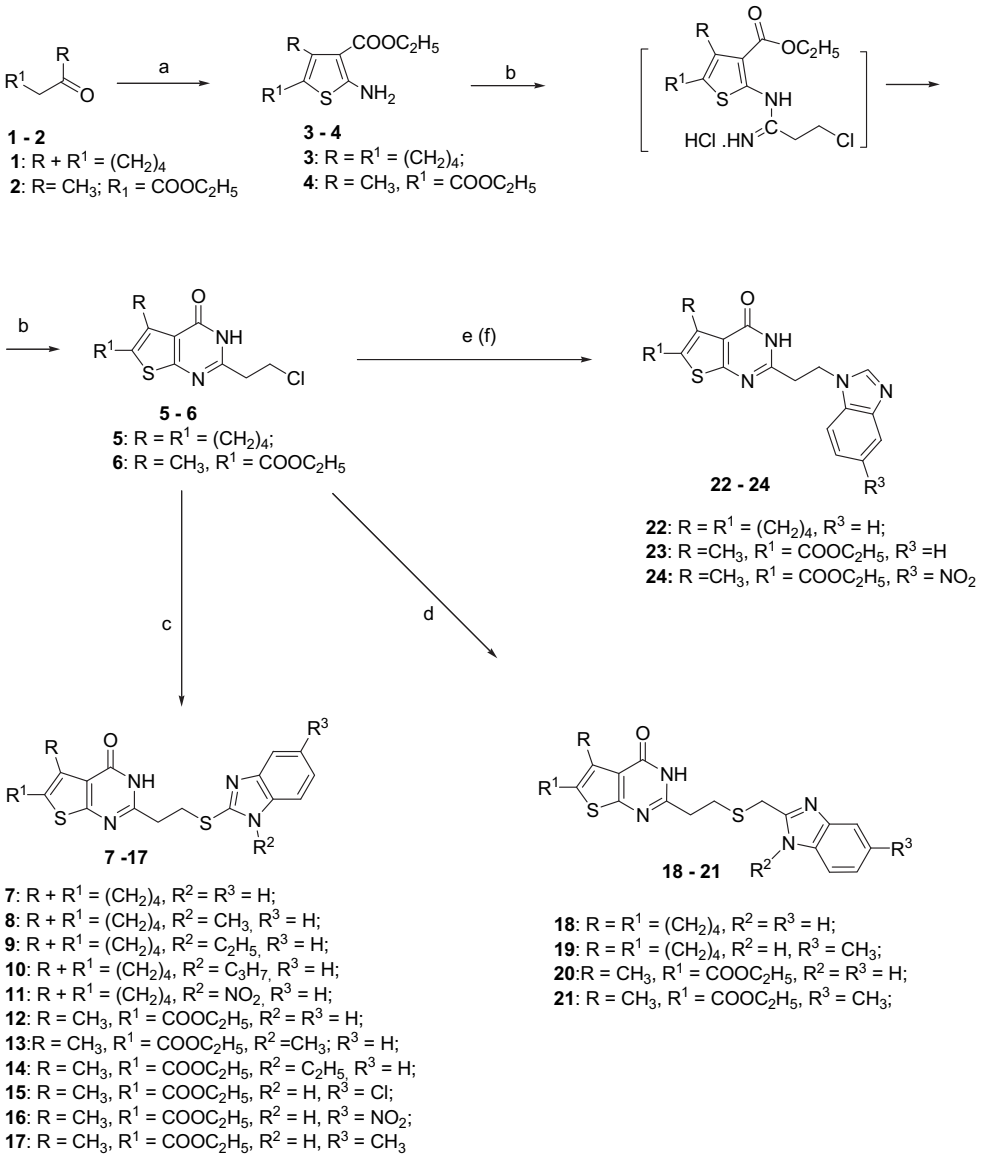

Fig. 1. Synthesis of benzimidazole derivatives of thieno[2,3-*d*]pyrimidin-4(3H)-ones. Regents and conditions: (a) ethyl cyanoacetate, sulfur, diethyl amine, 20 or 60 °C; (b) dioxan, 2-chloro-propionitrile, dry hydrogen chloride gas; 20 °C; (c) NaOH, benzimidazol-1-ylthiol; reflux; (d) NaOH, benzimidazol-2-ylmethanethiol, reflux; (e) ethanol, NaOH, benzimidazoles, reflux; (f) acetonitrile, benzimidazole, K_2CO_3, TBAB.

Table 1
Antihelminthicactivity of compounds **8**, **10**, **11**, **14**–**16** and **22**, **23** against Trichinella spiralis.

Comp.	Efficacy (%) [a] after 24 h [b]	Efficacy (%) after 48 h
	5 μg/ml	5 μg/ml
	(20 μg/ml) [c]	(20 μg/ml)
8	40.05	89.90
10	84.80	90.05
11	90.10	90.10
14	39.07	59.75
15	0.00	5.09
16	50.00	80.05
22	95.05	95.05
23	79.80	85.30
Albendazole	10.6	14.8

[a] Control − 96 parasites.
[b] $p < 0.05$.
[c] concentration of albendazole.

3.2. Antiprotozoal activity

An indicator test for antiprotozoal activity against Paramaecium caudatum was carried out. The response of Paramaecium caudatum was observed by means of electronic microscope.

3.3. Effect against Lamblia muris

The study was performed with white mice, invaded with Lamblia muris. The invaded mice were treated with the tested compounds and the mice faeces were studied for availability of Lamblia muris cysts.

4. Results and discussion

To synthesize thieno[2,3-*d*]pyrimidin-4(3H)-one containing benzimidazole moiety we use the nucleophillic substitution of different substituted benzimidazol-2-ylthiols as well as benzimidazol-2-yl-methanethiols with suitable substituted thienopyrimidinones. The reaction between compounds **5**–**6** and the respective benzimidazol-2-ylthiols was carried out in ethanol medium under reflux for 3–6 h to afford compounds **7**–**21**. The molar ratio thienopyrimidinone/benzimidazolthiols/sodium hydroxide was 1.0:1.0:1.8 and in the reaction of **5**–**6** and the benzimidazol-2-yl-methanethiols the molar ratio was 1.14:1.0:2.4. The yields of compounds **7**–**21** were in the range 49–80%, while that of compounds **22**–**24** were between 49 and 90%.

4.1. Antihelminthic activity

The parasitological experiment in vitro ascertained that the tested compounds possessed a different antihelminthic effect expressed in suppressing the motor activity of Trichinella larvae and in losing of the spiral form, which is a mark for not viability. In control samples both in physiological solution and in DMSO practically all Trichinella larvae were in spiral form namely 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 **8**, **10**–**11**, **14**–**16** and **22**–**23** proved the efficacy of the tested compounds (excluding compound **15**). It deserves to be underlined that the studied compounds revealed higher activity than albendazole at concentration of 5 μg/ml, while albendazole was used at concentration of 20 μg/ml.

The compounds **8**, **10**–**11** and **22** containing tetrahydrobenzothienopyrimidin-4-one cycle showed higher antihelminthic activity in comparison to the compounds **14**–**16** and **23**. The compound **22** comprising unsubstituted benzimidazole ring, but not benzimidazolthiols moiety was distinguished for the highest activity − 95.1%, while compound **23** exhibited 85.3% larvocide effect. From the thienopyrimidinones, containing benzimidazolthioethyl group the highest activity after 24 h revealed compound **11** − 90.1%, while compounds **8** and **10** possessed the same effect after 48 h.

Due to the obtained results for the antirichinellosis activity, further research is currently in progress for the antirichinellosis activity in intestinal and muscle phase.

4.2. Antiprotozoal activity in vitro

Six of the new synthesized compounds, **8**, **10**–**11**, **14**–**16** and **22**–**23** were subjected to a preliminary test for antiprotozoal activity in vitro on Paramaecium caudatum. Immediately after the treatment, the motions of the Paramaecia became more intensively and chaotically, but after 2 min they began to grow weaker and slower. After 15–20 min the paramaecia cells were motionless and the cell content becomes turbid. A multitude of drop formations of the cell content, permeated through the cell membrane appeared on membrane outside, probably due to cell membrane lysis. That phenomenon was an indicator for the antiprotozoal activity of the tested compounds.

4.3. Antiprotozoal activity in vivo

The compounds mentioned above demonstrated high efficacy in the implemented study for antiprotozoal activity.

The screening in vivo was performed using immature white mice, infected at equal conditions with Lamblia muris. The mice faeces were examined stained with Lugol's solution and observed under microscope for the availability of Lamblia cysts. The invaded animals were separated from the other mice in a 40-member group.

Thirty mice were divided in six groups, each of them of five mice. The six groups of mice were treated *p. o*. with each one of the compounds **8**, **10**–**11**, **14**–**16** and **22**–**23** at a dose of 0.5 μg/ml *pro die* for 5 days cure course. Five mice were not treated and were used as control samples. One group of five mice was treated only with DMSO. In the control study, accomplished five-days after treatment no Lamblia muris cysts were observed in each group of the treated with the compounds mice. That indicated a significant antiprotozoal activity. In the control group and in the group treated with DMSO an increasing of Lamblia muris was determined.

5. Conclusion

New benzimidazole derivatives of thieno[2,3-*d*]pyrimidin-4-ones were synthesized and studied for their antiparasitic activity.

The in vitro screening for antinematode effect showed that the compounds exhibit significant antiparasitic activity at concentration of 5 μg/ml. All tested benzimidazole derivatives of 4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine excluding compound **15** exhibited higher activity against Trichinella spiralis larvae in comparison to albendazol, furthermore some of the compounds demonstrated antitrichinellosis activity, which surpassed many times that of albendazole.

The indicator test on Paramaecium caudatum revealed antiprotozoal activity of all tested compounds. The pharmaco-therapeutic study *in vivo* on invaded with Lamblia muris white mice, showed 100% effectiveness of compounds after five-days-treatment course. During the control microscopic study of mice faeces no Lamblia muris cysts were found. That fact indicated that all studied compounds possess remarkable antiprotozoal efficacy.

These results confirmed also the hypothesis that the introduction of a1H-benzimidazolyl-2-thiol as well as 1-H-benzimidazole moiety in the structure of thieno[2,3-*d*]pyrimidine ring is favourable to the interaction of these molecules with the biological targets.

6. Experimental part

Melting points (mp) were determined on an Electrothermal AZ 9000 3MK4 apparatus and were uncorrected. The thin layer chromatography (TLC, R_f values) was performed on F_{254} or silica gel plates F_{254} (Merck, 0.2 mm thick) and visualization was effected with ultraviolet light. IR spectra were recorded on a Bruker Equinox 55 spectrophotometer as potassium bromide discs. All NMR spectra were recorded on a Bruker Avance DRX 250 spectrometer (Bruker, Faelanden, Switzerland) operating at 250.13 MHz for ^1H and 62.89 MHz for ^{13}C, using a dual 5 mm $^1H/^{13}C$ probehead. Chemical shifts were expressed relative to tetramethylsilane (TMS) and were reported as δ (ppm). The measurements were carried out at ambient temperature (300 K). The microanalyses for C, H, N and S were performed on Perkin–Elmer elemental analyzer.

The 2-amino-thiophenes (**3**–**4**) were synthesized by condensation of cyclohexanone or ethyl acetoacetate with ethyl cyanoacetate and sulfur in the presence of diethyl amine according to [18].

The 1-(un)substituted-2-mercapto-benzimidazoles were synthesized in good yields through two methods, namely by fusing 1-alkylbenzimidazoles with sulfur at 260 °C and by the reaction of 4-(un) substituted-1,2-diamino-benzene with potassium ethyl-dithiocarbonate in the presence of sodium hydroxide in ethanol medium [19]. Benzimidazole, 4-nitro-benzimidazole and 4-chloro-benzimidazole are commercially available.

The synthesis of 5(6)-(un)substituted-benzimidazol-2-yl-methanethiols was performed by refluxing the respective 1,2-diaminobenzene hydrochloride with mercaptoacetic acid according to the procedure given in [20].

6.1. General procedure for the preparation of ***5***–***6***

A stream of dry hydrogen chloride gas was passed through a solution of 0.002 mol of compound **3** or **4** and 2.15 g (0.002 mol) of 3-chloro-propanonitrile in dry dioxane for 5 h at ambient temperature. At the beginning of the reaction a formation of amidine hydrocloride has been observed, which is turned later in the reaction solution, forming the pyrimidinone ring. The reaction solution was poured in ice-water (40 ml) and basified with 10% ammonium hydroxide. The obtained precipitate was filtered and crystallized from ethanol.

6.1.1. 2-(2-chloroethyl)-5,6,7,8-tetrahydrobenzothieno[2,3-d] pyrimidin-4(3H)-one (***5***)

Yield: 85%; mp: 300–302 °C (decomp); re-crystallized with ethanol; R_f = 0.62; mobile phase: chloroform/heptane/ethanol – 3:2:1; ^1H NMR (DMSO): 1.77 (m, 4H, $(CH_2)_2$–Bzth); 2.72 (bt, 2H, CH_2); 2.85 (bt, 2H, CH_2); 3.08 (t, 2H, CH_2–Pyr); 4.01 (t, 2H, CH_2–Cl); 12.33 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc.: C, 53.63; H, 4.88; N, 10.42; S, 11.93; Found: C, 53.59; H, 4.92; N, 10.39; S, 11.90.

6.1.2. 2-(2-chloroethyl)-6-ethoxycarbonyl-5-methyl-thieno[2,3-d] pyrimidin-4(3H)-one (***6***)

Yield: 74.2%; Mp: 271–272 °C; re-crystallized with ethanol; R_f = 0.65, mobile phase: chloroform/heptane/ethanol – 3:2:1; ^1H NMR (DMSO): 1277 (t, 3H, CH_3); 2.78 (s, 3H, CH_3); 3.08 (t, 2H, CH_2–Pyr); 4.232 (q, 2H, –CH_2–O); 4.41 (t, 2H, CH_2–Cl); 12.33 (s, 1H, NH, exchangeable with D_2O). Analysis: Calc.: C, 47.92; H, 4.36; N, 9.31; S, 10.66; Found: C, 47.97; H, 4.31; N, 9.38; S, 10.69.

6.2. General procedure for preparation of compounds ***7***–***17***

To a solution of 0.13 g (3.3 mmol) sodium hydroxide in ethanol (5 ml), (1.8 mmol) of 1,5-(un)substituted-2-mercapto-benzimidazole **3a**–**g** was added. The solution was refluxed 1 h and 1.8 mmol of thieno[2,3-*d*]pyrimidin-4(3H)-one (**2a**–**b**) was added and the mixture was heated by reflux for two-three hours. The reaction solution was concentrated under reduced pressure. To the dry residue was added diethyl ether and the obtained precipitate was filtered.

6.2.1. 2-[2-(1H-benzimidazol-2-ylthio)ethyl]-5,6,7,8-tetrahydro[1] benzothieno[2,3-d]pyrimidin-4(3H)-one (***7***)

Yield: 66.4%; Mp: 213–215 °C; re-crystallized with ethanol; R_f = 0.55, mobile phase: chloroform/heptane/ethanol – 3:2:0.5; ^1H NMR (DMSO): 1.74 (m, 4H, $(CH_2)_2$–Bzth); 2.7 (bt, 2H, CH_2); 2.85 (bt, 2H, CH_2); 3.08 (t, 2H, CH_2–Pyr); 4.25(t, 2H, CH_2–S); 7.23–7.48(m, 4H, Ar); 12.29 (s, 1H, NH, exchangeable with D_2O); Analysis: C, 59.66; H, 4.74; N, 14.65; S, 16.77; Found: C, 59.69; H, 4.69; N, 14.55; S, 16.81.

6.2.2. 2-{2-[(1-methyl-1H-benzimidazol-2-yl)thio]ethyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (***8***)

Yield: 68.6%; Mp: 228–230 °C, re-crystallized with ethanol; R_f = 0. 53, mobile phase: chloroform/heptane/ethanol – 3:2:0.5; ^1H NMR (DMSO): 1.75 (m. 4H. $2(CH_2)_2$–Bzth); 2.63 (bt, 2H, CH_2–Bzth); 2 (dt, 4H, $2(CH_2)_2$–Bzth, CH_2–Pyr); 3.69 (s, 3H, CH_3); 4.26 (t, 2H, CH_2–S); 7.02 (m, 2H, Ar); 7.45 (m, 2H, Ar); 12.30 (s, 1H, NH, exchangeable with D_2O); Analysis: C, 60.58; H, 5.08; N, 14.13; S, 16.17; Found: C, 60.55; H, 5.11; N, 14.08; S, 16.21.

6.2.3. 2-{2-[(1-ethyl-1H-benzimidazol-2-yl)thio]ethyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (***9***)

Yield: 62.50%; Mp: 247–249 °C; R_f = 0.55, mobile phase: chloroform/heptane/ethanol – 3:2:0.5; ^1H NMR (DMSO): 1. 25 (t, J = 7.1 Hz, 3H, CH_3); 1.75(m, 4H, 4H, $(CH_2)_2$–Bzth); 2.65 (bt, 2H, CH_2–Bzth); 2.86(t, 2H, CH_2–Bzth); 2.95(t, 2H, CH_2–Pyr); 4.31 (q, J = 7.1 Hz, 2H, CH_2–N); 4.64 (t, 2H, CH_2–S); 7.22 (m, 2H, Ar); 7.51 (m, 2H, Ar); 12.31 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO): 12.7(CH_3), 22.2(CH_2), 22.6(CH_2), 24.4(CH_2), 25.5(CH_2), 34.0(CH_2–Pyr), 38.8(CH_2–N), 42.1(CH_2–S), 109.2(Ar-CH), 109.8(Ar-CH), 119.8(C–Bzth), 122.5(Ar-CH), 122.6(Ar-CH), 127.7(C–Bzth), 130.3(Ar-C), 130.8(Ar-C), 131.2(S–C=N), 158.1(S–C–N–Bzth), 163.5 (N–C=N–Bzth), 167.7(C=O); Analysis: C, 61.43; H, 5.40; N, 13.65; S, 15.62; Found: C, 61.48; H, 5.36; N, 13.60; S, 15.58.

6.2.4. 2-{2-[(1-propyl-1H-benzimidazol-2-yl)thio]ethyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (***10***)

Yield: 64.2% Mp: 254–256 °C (decomp); R_f = 0.59, mobile phase: chloroform/heptane/ethanol – 3:2:0.5; ^1H NMR (DMSO): 0.89 (t, 3H, CH_3); 1.68–1.79 (m, 6H, $(CH_2)_2$–Bzth, $\underline{CH_2}$–CH_3); 2.66 (bt, 2H, CH_2–Bzth); 2.86 (bt, 2H, CH_2–Bzth); 2.96 (t, 2H, CH_2–Pyr); 4.23 (t, 2H, CH_2–N); 4.65 (t, 2H, CH_2–S); 7.17–72 (m, 2H, Ar); 7.47–7.53(m, 2H, Ar); 12.34 (s, 1H, NH, exchangeable with D_2O); Analysis: C, 62.23; H, 5.70; N, 13.20; S, 15.10; Found: C, 62.18; H, 5.768; N, 13.25; S, 15.08.

6.2.5. 2-{2-[(5(6)-nitro-1H-benzimidazol-2-yl)thio]ethyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (***11***)

Yield: 38.47%; Mp: 212–214 °C; re-crystallized with ether–chloroform; R_f = 0.53, mobile phase: ethyl acetate/heptane – 3:2; ^1H NMR (DMSO): 1.74 (m, 4H, $2CH_2$); 2.72 (m, 4H, $2CH_2$); 2.84 (t. 2H, CH_2); 3.01 (t. 2H, CH_2); 7.68 (d, 1H, Ar, J^3 = 11.37 Hz); 7.79 (m, 2H, Ar); 11.55 (bs, 2H, 2NH, exchangeable with D_2O). ^{13}C NMR (DMSO): 21.7(CH_2), 22.4(CH_2), 24.3(CH_2), 25.2(CH_2), 32.8(CH_2–Pyr), 40.5(CH_2–S), 103.4

(Ar-CH), 109.5(Ar-CH), 116.4(Ar-CH), 131.1(Ar-C), 139.2(Ar-C), 140.8 (Ar-C), 157.0(S–C=N), 158.5(S–C–N–Bzth), 162.6(N–C=N–Bzth), 176.4(C=O); Analysis: Calc.: C, 53.38; H, 4.01; N, 16.38; S, 15.00; Found: C, 53.31; H, 4.06; N, 16.33; S, 15.06.

*6.2.6. Ethyl 2-[2-(1H-benzimidazol-2-ylthio)ethyl]-5-methyl-4-oxo-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxylate (**12**)*

Yield: 68.0%; Mp: 251–253 °C; R_f = 0.50, mobile phase: chloroform/heptane/ethanol – 3:2:0.5; ^{1}H NMR: 1.27 (t, 3H, CH_3); 2.79 (s, 3H, CH_3); 2.88 (t, 2H, CH_2–Pyr); 4.26 (q, 2H, –CH_2–O); 4.6 (t, 2H, –CH_2–S); 6.99–7.48 (m. 3H, Ar); 12.23 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc.: C, 55.05; H, 4.38; N, 13.52; S, 15.47; Found: C, 55.09; H, 4.40; N, 13.50; S, 15.43.

*6.2.7. Ethyl 5-methyl-2-{2-[(1-methyl-1H-benzimidazol-2-yl)thio] ethyl}-4-oxo-3,4-dihydro-thieno[2,3-d]pyrimidine-6-carboxylate (**13**)*

Yield: 64.5%; Mp: 244–248 °C (decomp), re-crystallized with ethanol; R_f = 0.68, mobile phase: chloroform/heptane/ethanol = 3:2:1; ^{1}H NMR (DMSO): 1.27 (t, 3H, CH_3); 2.81 (s, 3H, CH_3); 2.87 (t, 2H, CH_2); 3.71 (s, 3H, CH_3); 4.21 (q, 2H, CH_2); 4.64 (t, 2H, 2H, CH_2); 7.22 (m, 2H, Ar); 7.43 (m, 2H, Ar); Analysis: Calc. C, 56.06; H, 4.70; N, 13.07; S, 14.97; Found: C, 56.09; H, 4.68; N, 13.05; S, 14.94.

*6.2.8. Ethyl 5-methyl-2-{2-[(1-ethyl-1H-benzimidazol-2-yl)thio] ethyl}-4-oxo-3,4-dihydrothieno-[2,3-d]pyrimidine-6-carboxylate (**14**)*

Yield: 52.5%; Mp: 236–239 °C, re-crystallization with diethyl ether/chloroform; R_f = 0.48, mobile phase: ethyl acetate/heptane 3:2; ^{1}H NMR (DMSO): 1.274 (dt, 3H, 2CH_3); 2.781(s, 3H, CH_3); 2.830 (t, 2H, CH_2–Pyr); 4.237 (q, 2H, –CH_2–N); 4.347 (q, 2H, –CH_2–O); 4.634 (t, 2H, –CH_2–S); 7.115–7.248 (m. 2H, Ar); 7.434–7.483(m. 2H, Ar); 12.312 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO): 12.7(CH_3), 14.2(CH_3), 15.4(CH_3), 36.0(CH_2–Pyr), 38.8(CH_2–N), 42.6 (CH_2–S), 60.0(CH_2O), 109.2(Ar-CH), 109.7(Ar-CH), 122.0(Ar-CH), 122.0(Ar-CH), 120.4(C–Bzth), 130.8(C–Bzth), 131.4(Ar-C), 141.2(Ar-C), 143.8(Ar-C), 158.0(S–C=N), 158.6(S–C–N–Bzth), 159.4(O–C=O), 163.0(N–C=N–Bzth), 167.7(C=O); Analysis: Calc.: C, 56.99; H, 5.01; N, 12.66; S, 14.49; Found: C, 56.96; H, 5.05; N, 12.63; S, 14.51.

*6.2.9. Ethyl 5-methyl-2-{2-[(5(6)-chloro-1H-benzimidazol-2-yl) thio]ethyl}-4-oxo-3,4-dihydro-thieno [2,3-d]pyrimidine-6-carboxylate (**15**)*

Yield: 54.0%; Mp. 167–169 °C re-crystallized with diethyl ether/chloroform; R_f = 0.56, mobile phase ethyl acetate/heptane – 3:2; ^{1}H NMR(DMSO): 1.26 (t, 6H, 2CH_3); 2.80 (s, 3H, CH_3); 2.88(t, 2H, CH_2–Pyr); 4.24 (q, 2H, –CH_2–O); 4.60 (t, 2H, –CH_2–S); 7.00–7.50 (m. 3H, Ar); 12.23 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO): 14.2(CH_3), 15.1(CH_3), 28.2(CH_2–Pyr), 42.4(CH_2–S), 60.5 (CH_2O), 109.0(Ar-CH), 110.7(Ar-CH), 119.8(Ar-CH), 121.8(C–Bzth), 122.2(C–Bzth), 130.2(C–Bzth), 132.2(Ar-C), 143.9(Ar-C), 144.1(Ar-C), 162.2(S–C=N), 162.4(S–C–N–Bzth), 162.5(O–C=O), 166.9 (N–C=N–Bzth), 169.1(C=O). Analysis: Calc.: C, 50.83; H, 3.82; Cl, 7.90; N, 12.48; S, 14.28; Found: C, 50.85; H, 3.80; Cl, 7.92; N, 12.47; S, 14.29.

*6.2.10. Ethyl 5-methyl-2-{2-[(5(6)-nitro-1H-benzimidazol-2-yl) thio]ethyl}-4-oxo-3,4-dihydro thieno-[2,3-d]pyrimidine-6-carboxylate (**16**)*

Yield: 55.8%; Mp. 241–243 °C; R_f = 0.49, mobile phase ethyl acetate/heptane – 3:2; ^{1}H NMR(DMSO): 1.27 (t, J = 7.0 Hz, 3H, CH_3); 2.76 (s, 3H, CH_3); 2.86 (t, 2H, CH_2–Pyr); 4.26 (q, J = 7.0 Hz, 2H, –CH_2–O); 4.65 (t, 2H, –CH_2–S); 7.73–8.10 (m. 3H, Ar); 12.29 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO): 14.6(CH_3), 15.2 (CH_3), 32.5(CH_2–Pyr), 42.2(CH_2–S), 61.5(CH_2O), 106.4(Ar-CH), 110.3 (Ar-CH), 119.4(Ar-CH), 121.4(C–Bzth), 122.4(C–Bzth), 132.0 (C–Bzth), 136.6(Ar-C), 143.5(Ar-C), 143.6(Ar-C), 158.2(S–C=N), 158.4(S–C–N–Bzth), 159.4(O–C=O), 162.4(N–C=N–Bzth), 166.3 (C=O); Analysis: Calc.: C, 49.66; H, 3.73; N, 15.24; S, 13.96; Found: C, 49.68; H, 3.70; N, 15.26; S, 13.96.

*6.2.11. Ethyl 5-methyl-2-{2-[(5(6)-methyl-1H-benzimidazol-2-yl) thio]ethyl}-4-oxo-3,4-dihydrothieno [2,3-d]pyrimidine-6-carboxylate (**17**)*

Yield: 92%; Mp 237–239 °C; R_f = 0.58, mobile phase: chloroform/heptane/ethanol – 3:2:1; ^{1}H NMR (DMSO): 1.29 (t, 3H, CH_3); 2.49 (s, 3H, CH_3); 2.78 (s, 3H, CH_3); 2.89 (t, 2H, CH_2); 4.24 (q, 2H, CH_2); 4.58 (t, 2H, CH_2); 6.97 (m, 2H, Ar); 7.32 (m, 1H, Ar); Analysis: Calc.: C, 56.06; H, 4.70; N, 13.07; S, 14.97; Found: C, 56.04; H, 4.73; N, 13.11; S, 14.99.

*6.3. General procedure for preparation of compounds **18**–**21***

A solution of 0.46 g (0.012 mol) sodium hydroxide in absolute ethanol and 0.8 g (4.9 mmol) benzimidazol-2-yl-methylthiol was refluxed for 1 h. After that 5.6 mmol of the thieno[2,3-*d*]pyrimidin-4(3H)-one **5** or **6** was added and the solution was heated by reflux for 2 h. After cooling the obtained precipitate was filtered and crystallized from ethanol.

*6.3.1. 2-(benzimidazol-2-yl)-methylthioethyl-5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidin-4(3H)-one (**18**)*

Yield: 72.4%; Mp-196–198 °C; R_f = 0.48, mobile phase chloroform/heptane/ethanol – 3:2:1; ^{1}H NMR: 1.76 (m, 6H, 4H, $(CH_2)_2$–Bzth, CH_2–Pyr); 2.52 (bt, 2H, CH_2–Bzth); 2.69(bt, 2H, CH_2–Bzth); 2.90 (t, 2H, CH_2–S); 3.94 (s, 2h, S–CH_2–Bzi); 7.31–7.43 (m, 4H, Ar); 12.33 (s, 1H, NH, exchangeable with D_2O.) Analysis: Calc.: C, 60.27; H, 5.56; N, 14.06; S, 16.09; Found: C, 60.21; H, 5.58; N, 14.09; S, 16.04.

*6.3.2. 2-{2-[(5(6)-methyl1-1H-benzimidazol-2-ylmethyl)thio] ethyl}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (**19**)*

Yield 83%; Mp-168–170 °C; R_f = 0.52, mobile phase chloroform/heptane/ethanol – 3:2:1; ^{1}H NMR: 1.77(m, 6H, 4H, $(CH_2)_2$–Bzth, CH_2–Pyr); 2.51 (bt, 2H, CH_2–Bzth); 2.69 (bt, 2H, CH_2–Bzth); 2.88 (t, 5H, CH_2–S, CH_3); 3.93 (s, 2H, S–CH_2–Bzi); 7.31–7.43 (m, 3H, Ar); 12.31 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc.; C, 61.43; H, 5.40; N, 13.65; S, 15.62; Found: C, 61.41; H, 5.43; N, 13.62; 3; S, 15.64.

*6.3.3. Ethyl 2-{2-[(1H-benzimidazol-2-ylmethyl)thio]ethyl}-5-methyl-4-oxo-3,4-dihydro-thieno[2,3-d]pyrimidine-6-carboxylate (**20**)*

Yield 49%; Mp-178–180 °C; R_f = 0.50, mobile phase chloroform/heptane/ethanol – 3:2:1; ^{1}H NMR: 1.30 (t, 3H, CH_3); 2.78 (s, 3H, CH_3); 2.88 (t, 2H, CH_2–Pyr); 2.98 (s, 3H, CH_3); 3.33 (t, 2H, –CH_2–S); 4.1 (s, 2H, S–CH_2); 4.32 (q, 2H, –CH_2–O); 7.31–7.44 9(m, 3H, Ar); 12.31 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc: C, 55.79; H, 5.15; N, 13.01; S, 14.90; Found: C, 55.81; H, 5.18; N, 13.04; S, 14.88.

*6.3.4. Ethyl 2-{2-[(5(6)-methyl-1H-benzimidazol-2-ylmethyl)thio] ethyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate (**21**)*

Yield: 49%; Mp: 163–165 °C; R_f = 0.55, mobile phase chloroform/heptane/ethanol – 3:2:1; ^{1}H NMR: 1.30 (t, 3H, CH_3); 2.79 (s, 3H, CH_3); 2.88 (t, 2H, CH_2–Pyr); 2.98 (s, 3H, CH_3); 3.33 (t, 2H, –CH_2–S); 4.08 (s, 2H, S–CH_2); 4.32 (q, 2H, –CH_2–O); 7.31–7.44 9(m, 3H, Ar); 12.32 (s, 1H, NH, exchangeable with D_2O); Analysis:

Calc.: C, 56.73; H, 5.44; N, 12.60; S, 14.43; Found: C, 56.70; H, 5.41; N, 12.64; S, 14.41.

6.4. General procedure for preparation of compounds **22**–**24**

Method A: To a solution of 0.1 g (2.6 mmol) sodium hydroxide in 40 ml ethanol, 1.7 mmol of the appropriated 1H-benzimidazole was added and the mixture was refluxed for 1 h. After that 3.4 mmol of thieno[2,3-*d*]pyrimidinone **2a** or **2b** was added and the solution was refluxed for 5 h more. The ethanol was removed under reduced pressure. The remaining product was dissolved in chloroform and the organic layer was washed with water and dried. The chloroform was removed under reduced pressure and the obtained residue crystallized.

Method B: To a solution of 0.01 mol of the respective benzimidazole in acetonitrile (50 ml), 2.7 g (0.02 mol) anhydrous K_2CO_3, 0.9 g (0.03 mol) thieno[2,3-*d*]pyrimidinone **2a** or **2b** was added. The mixture was stirred at ambient temperature and monitored by TLC over the reaction period. After completion of the reaction, the mixture was filtered to separate the solid K_2CO_3, the organic solvent was evaporated under reduced pressure and the residue crystallized.

6.4.1. 2-[2-(5(6)-nitro-1H-benzimidazol-1-yl)ethyl]-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one, **22**

Yield: 53% (method A), 74% (method B); Mp: 196–198 °C; $R_f = 0.50$, mobile phase: benzene/methanol – 2:1. ^{1}H NMR (DMSO): 1.77 (t, 4H, $2CH_2$); 2.73 (t, 2H, CH_2); 2.87 (t, 2H, CH_2); 3.06 (t, 2H, CH_2); 3.99 (t, 2H, CH_2); 7.54 (m, 4H, Bz); 12.35 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc.: C, 57.71; H, 4.33; N, 17.71; O, 12.14; S, 8.11; Found: C, 57.73; H, 4.30; N, 17.69; S, 8.15.

6.4.2. Ethyl 2-[2-(1H-benzimidazol-1-yl)ethyl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate, **23**

Yield 75% (method A), 85% (method B); Mp-231–233 °C, benzene/methanol 2:1, R_f = 0,68, mobile phase: benzene/methanol = 2:1 ^{1}H NMR (DMSO): 1.37 (t, 3H, CH_3); 2.85 (s, 3H, CH_3); 3.28 (t, 2H, CH_2); 4.35 (q, 2H, O–CH_2); 4.75 (t, 2H, N–CH_2); 7.49–8.36 (m, 5H, CH–Bzi), 12.33 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc.: C, 59.67; H, 4.74; N, 14.65; S, 8.38; Found: C, 59.69; H, 4.71; N, 14.66; S, 8.35.

6.4.3. Ethyl 5-methyl-2-[2-(5(6)-nitro-1H-benzimidazol-1-yl) ethyl]-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate, **24**

Yield: 51% (method A), 71.5% (method B); Mp – 248–250 °C; R_f = 0.52, mobile phase: benzene/methanol = 2:1; ^{1}H NMR (DMSO): 1.31 (t, 3H, CH_3); 2.88 (s, 3H, CH_3); 3.27 (2H, CH_2); 4.25 (q, 2H, CH_2); 4.83 (t, 2H, CH_2); 7.86 (m 2H, CH–Bzi); 8.22 (m, 1H, CH–Bzi); 8.66 (m, 1H, CH–Bzi); 12.48 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc.: C, 53.39; H, 4.01; N, 16.38; S, 7.50; Found: C, 53.36; H, 4.05; N, 16.36; S, 7.53.

6.5. Biological screening

6.5.1. Antitrichinellosis activity in vitro

The parasitological pharmaco-therapeutic experiments in vitro for antitrichinellosis activity of the tested compounds were carried out according to Campbell's method [21,22].

Encapsulated Trichinella 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 used concentration was 5 μg/ml. The samples were incubated in "humid" chamber with thermostat at 37 °C. The microscopy control for vitality of the Trichinella larvae was carried out 24 as well as 48 h after treatment using stereomicroscope MBC-9.

6.5.2. Indicator test for antiprotozoal activity on Paramaecium caudatum

Culture of Paramaecium caudatum, obtained through macerating of dry hay was used. The tested compounds were dissolved in DMSO at concentration 1 μg/ml. To a drop from the solution of each compound in a Petri dish, 2–3 drops of the Paramecia culture were added. The reaction of Paramecia was observed stereomicroscopically.

6.5.3. Antiprotozoal activity in vivo against Lamblia muris

Thirty white mice, divided in six groups were treated *p. o*. with each one of the compounds **8**, **10**–**11**, **14**–**16** and **22**–**23**. The tested compounds were introduced in the oesophagus of each mouse by means of thin metallic probe in a single time dose of 0.5 μg/ml *pro die* as a five-days-treatment course. The compounds were used as DMSO solutions in volume of 0.5 ml. The first control group was without treatment and the mice in the second control group were given only the solvent (DMSO) during five days. After treatment during five days the mice faeces were studied microscopic for the availability of Lamblia muris cysts.

References

[1] A. Gangjee, W. Li, R.L. Kisliuk, et al., Med. Chem. 52 (15) (2009) 4892–4902.
[2] Y.D. Wang, S. Johnson, D. Powell, et al., Bioorg. Med. Chem. Lett. 15 (16) (2005) 3763–3766.
[3] T. Horiuchi, J. Chiba, K. Koto, et al., Bioorg. Med. Chem. Lett. 19 (2) (2009) 305–308.
[4] A.S. Aly, A.B.A. El-Gazzar, H.A.R. Hussein, Phosphorus Sulfur Silicon Relat Elem 182 (1) (2007) 35–56.
[5] S. Ananthan, E.R. Faaleolea, R.C. Goldman, et al., Tuberculosis 89 (5) (2009) 334–353.
[6] A. Rosowsky, A.P. Papoulis, S.F. Queener, J. Med. Chem. 40 (22) (1997) 3694–3699.
[7] J. Zhu, T. Chen, J. Liu, et al., Molecules 14 (2009) 785–797.
[8] K. Starčević, M. Kralj, K. Ester, et al., Bioorg. Med. Chem. 15 (13) (2007) 4419–4426.
[9] M.M. Ramla, M.A. Omar, A.-M.M. El-Khamry, et al., Bioorg. Med. Chem. 14 (2006) 7324.
[10] M.T. Omar, H.H. Fahmy, H.S. Mohamed, et al., Egypt. J. Pharm Sci. 37 (1–6) (1996) 37(1-4), 606–620.
[11] M. Goebel, B. Staels, T. Unger, et al., Chem. Med. Chem. 4 (7) (2009) 1136–1142.
[12] S.K. Katiyar, V.R. Gordon, G.L. McLaughlin, et al., Antimicrob. Agents Chemother. 38 (9) (1994) 2086–2090.
[13] J. Valdez, R. Cedillo, A. Hernández-Campos, et al., Bioorg. Med. Chem. Lett. 12 (16) (2002) 2221–2224.
[14] G. Navarrete-Vázquez, M.M. Rojano-Vilchis, L. Yépez-Mulia, et al., Eur. J. Med. Chem. 41 (1) (2006) 135–141.
[15] L. MacDonald, A. Armson, A.R.C. Thompson, et al., Mol. Biochem. Parasitol. 138 (1) (2004) 89–96.
[16] G. Navarrete-Vázquez, L. Yépez, A. Hernández-Campos, et al., Bioorg. Med. Chem. 11 (21) (2003) 4615–4622.
[17] A. Ts, K.K. MavrovaAnichina, D.I. Vuchev, et al., Bioorg. Med. Chem. 13 (2005) 5550–5559.
[18] K. Gewald, E. Schinke, H. Boettcher, Chem. Ber. 99 (1966) 94–96.
[19] A. Mavrova, P. Denkova, Y. Tsenov, et al., Bioorg. Med. Chem. 15 (18) (2007) 6291–6297.
[20] A. Chimiri, M.A. Monforte, P. Monforte, et al., Heterocycles 53 (3) (2000) 613–620.
[21] W.C. Campbell, D.A. Denham, Chemotherapy. in: W.C. Campbell (Ed.), Trichinella and Trichinosis. Plenum Press, USA and London, UK, 1983, pp. 340–355.
[22] W.C. Campbell, in: R. Rew (Ed.), Chemotherapy of Parasitic Diseases, Plenum Press, New York, 1986, p. 655.