CHEMISTRY

SYNTHESIS AND ANTI-TRICHINELLA SPIRALIS ACTIVITY IN VITRO OF SOME NEW 1H-BENZIMIDAZOLES

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Abstract. *Novel derivatives of 1H-benzimidazole which combine into a single molecule two pharmacophores – the benzimidazole and piperazine rings possessing antihelmintic activity have been synthesized. Their structures were confirmed by IR, 1H NMR, 13C NMR and elemental analysis techniques.*

The compounds exhibited remarkable effect on the viability of isolated Trichinella spiralis muscle larvae in in vitro model at a dose of 100 μg/ml after 24 h. The results obtained by the hepatotoxicity test showed that compound ***4*** *had the lowest hepatotoxity.*

Keywords: *Benzimidazoles; Antitrichinellosis activity; Hepatotoxicity.*

Trichinellosis is a severe and sometimes deadly parasitic disease in carnivorous mammals and people, caused by infection with *Trichinella* species (commonly *Trichinella spiralis*) after consumption of infected raw and semi-rawer (with insufficient heat treatment) meat and/or meat products. Due to the predominantly zoonotic importance of infection, the main efforts in the developed countries have focused on elimination of Trichinella from the food chain through enhanced veterinary control [1].

Contemporary drug treatment on the human and animal trichinellosis was performed with the benzimidazole anthelmintics as albendazole and mebendazole [1-4]. Regardless of the high efficacy and low toxicity of the above mentioned drugs the definitive treatment of the trichinellosis remains pending, especially when the parasite is encapsulated in the muscle cells of the host. The enteral (intestinal) phase of human trichinellosis is curable, but patients are usually diagnosed later when the parasitic larvae have already reached the muscle cells, where the penetration of the drug very low.

Therefore, the development of new and more effective anti-*Trichinella* drug on the basis of benzimidazole heterocyclic structure is of special pharmacological interest.

In our recently published papers, we have described the synthesis some 2-aryliden substituted thiazolo[3,2-a]benzimidazoles [5] and 5(6)-(un)substituted-1H-benzimidazol-2-ylthioacetylpiperazines [6]. Same of the tested compounds exhibited higher activity than albendazole against *Trichinella spiralis.*

The aim of this work was to synthesize new derivatives of 1H-benzimidazole that combine two pharmacophores with antihelmintic activity - benzimidazole and piperazine ring into a single molecule, in order to evaluate *in vitro* against nematode *Trichinella spiralis* muscle larvae (ML) and to investigate the in vitro effects of the compounds on isolated rat hepatocytes.

The synthesis of the two groups of piperazine-containing benzimidazoles is illustrated in Fig. 1.

The first step of the synthesis involved preparation of 5(6)-methyl-1H-benzoimidazol-2-ylthio)acetic acid **2** as reported in [7], which cyclizes in the presence of acetic anhydride in pyridine medium at 100 ºC to 6(7)-methyl[1,3]thiazolo[3,2-a]benzimidazol-3(2H)one **3** [5]. The hydrolysis of the thiazolone **3** with 1-methylpiperazine or 1-benzylpiperazine in ethanol under refluxing afforded 5(6)-methyl-1H-benzimidazol-2-ylthioacetylpiperazine derivatives **4** and **5**, respectively.

The synthesis of the second group compounds **7a-b** and **8a-b** was accomplished by heating of benzimidazol-2-yl-sulfonic acids **6a-b,** previously obtained by oxidation of the 1H-benzimidazol-2-yl-

thiols **1a-b** with $KMnO_4$ in 50 % water solution of sodium hydroxide [8] and 1-methylpiperazine or 1-benzylpiperazine.

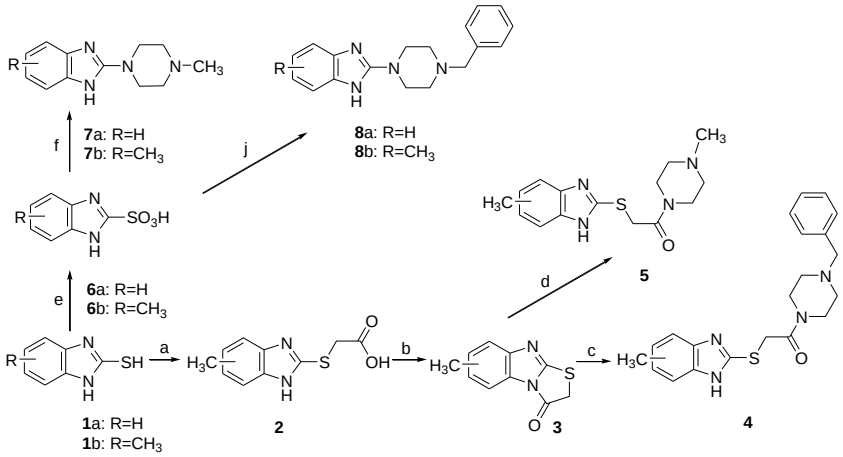

Fig. 1. Synthesis of 1H-benzimidazol-2-ylthioacetylpiperazine derivatives 4, 5 and 1H-benzimidazol-2-piperazines 7a-b, 8a-b.

The synthesis of the second group compounds **7a-b** and **8a-b** was accomplished by heating of benzimidazol-2-yl-sulfonic acids **6a-b,** previously obtained by oxidation of the 1H-benzimidazol-2-yl-thiols **1a-b** with $KMnO_4$ in 50 % water solution of sodium hydroxide [8] and 1-methylpiperazine or 1-benzylpiperazine.

The compounds prepared were purified by re-crystallization and their chemical structures were established by IR, 1H NMR and 13C NMR spectra as well as elemental analysis.

The targeted benzimidazole derivatives **4**, **5**, **7a**, **7b**, **8a** and **8b** were evaluated *in vitro* for their antihelmintic activity against isolated *T. spiralis* larvae (100 specimens for 1 mL physiological solution) in concentrations 50 μg/ml and 100 μg/ml, dissolved in DMSO. 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 [3, 9].

The parasitological study showed that all tested compounds exhibited higher activity against *T. spiralis* comparable to that of albendazole used as standard drug. The substances demonstrated 50-78% activities, at a dose of 100 μg/ml after 24 h, and 70-100% efficacy at the same dose after 48h expressed in suppressing motor activity of larvae and opening of their spiral form.

The piperazine derivatives of benzimidazole in concentration 250 μM were tested and in vitro for toxicity on isolated rat hepatocytes. Male Wistar rats (body weight 200–250 g) were used. In situ liver perfusion and cell isolation were performed as described by Fau et al. [10], with modifications [11].

The results obtained by the study showed that compound **4** had the lowest hepatotoxity followed by the methylpiperazine analoque **5**.

Due to the obtained good results for the antitrichinellosis effect in vitro, a research is currently in progress for estimation of the antitrichinellosis activity in vivo.

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