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COMBINED IN VITRO EVALUATION OF ANTIPARASITIC AND PROTECTIVE EFFECT TOWARDS BIOLOGICALLY IMPORTANT MOLECULES OF NEW 1*H*-BENZIMIDAZOL-2-YL HYDRAZONES

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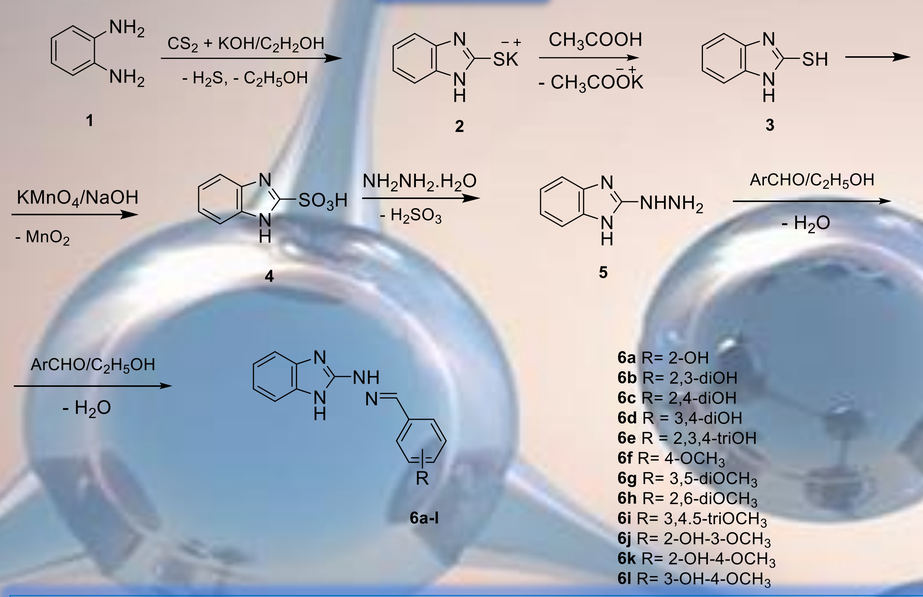
Trichinellosis is a severe and sometimes deadly parasitic disease in carnivorous mammals and people, caused by infection with nematodes of the genus *Trichinella*. The main source in humans is infected wild or domestic pigs, whose meat and meat products are used for consumption without good heat treatment. Benzimidazole broad-spectrum anthelmintics as albendazole and mebendazole are widely used in the chemotherapy of human trichinellosis [1].

The benzimidazole nucleus is a heteroaromatic system of great interest for the medicinal chemistry. The various biological activities related to this pharmacophore (antineoplastic, antibacterial, antiviral, antioxidant etc.) support its importance for generating new therapeutical agents [2]. Furthermore, many benzmidazole derivatives were designed and synthesized as antiparasitic agents [3]. The mode of action of the compounds is by blocking the microtubule function [4].

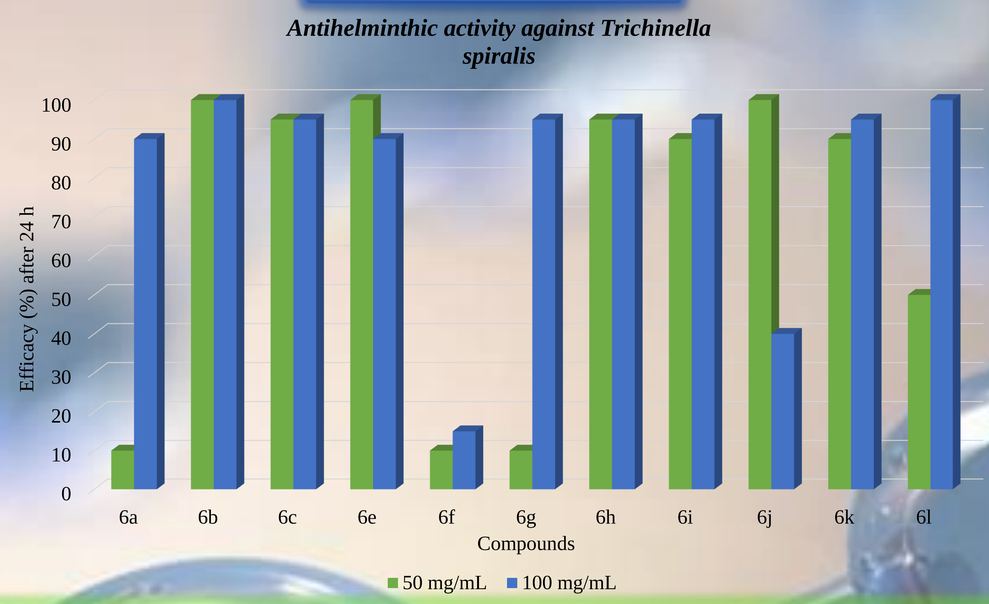
I. Synthesis



6a R= 2-OH
6b R= 2,3-diOH
6c R= 2,4-diOH
6d R = 3,4-diOH
6e R = 2,3,4-triOH
6f R= 4-OCH_3
6g R= 3,5-diOCH_3
6h R= 2,6-diOCH_3
6i R= 3,4.5-triOCH_3
6j R= 2-OH-3-OCH_3
6k R= 2-OH-4-OCH_3
6l R= 3-OH-4-OCH_3

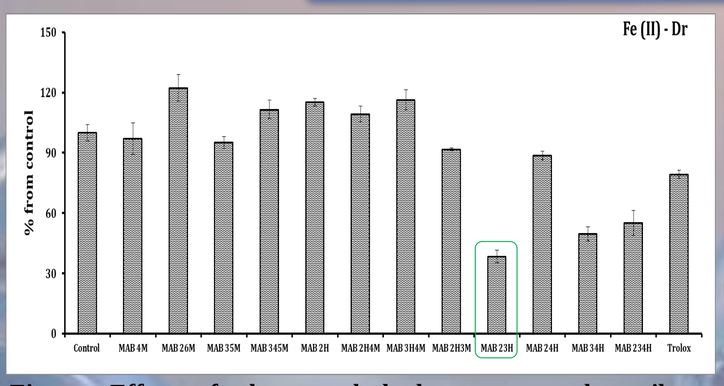
1H-benzimidazol-2-yl-thiol **2** was prepared by refluxing ethanol-water solution of potassium hydroxide, carbon disulfide and 1,2-diamino-benzene.
The reaction of benzimidazol-2-yl-sulfonic acid **4**, obtained by oxidation of the thiols with KMnO4 in 50 % water solution of sodium hydroxide, in excesses of hydrazine hydrate under refluxing conditions for 3h resulted in 2-hydrazino-1*H*-benzimidazole **5**.
Target compounds **6a-l** were prepared through condensation of **5** with the corresponding aldehyde in ethanol.

II. Antitrichinellosis activity



The anthelmintic activity of the 1*H*-benzimidazolyl hydrazone derivatives was evaluated *in vitro* against *Trichinella spiralis larvae*. The compounds containing two or three hydroxyl groups **6b**, **6c** and **6e** demonstrated the strongest larvocide effect ranging from 100% to 90% in comparison to the compounds containing methoxyl groups **6f-i** at concentration of 100 μg/mL after 24 h incubation period at 37°C. From the benzimidazoles possessing hydroxyl group at second or third place and methoxyl substituent at third or fourth place **6j-l** showed 100% efficacy at concentration of 50 μg/ml after 24 hours.

III. Peroxidation of biologically important molecules



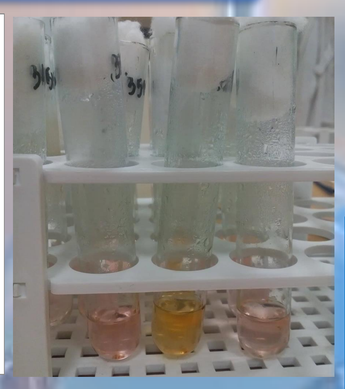


Figure: **Effect of the tested hydrazones on deoxyribose molecular damage.**

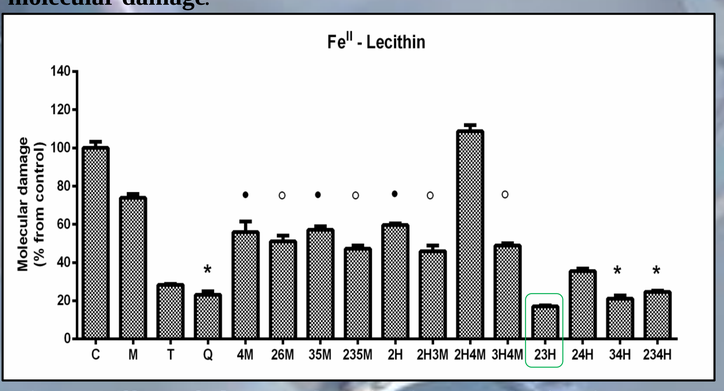


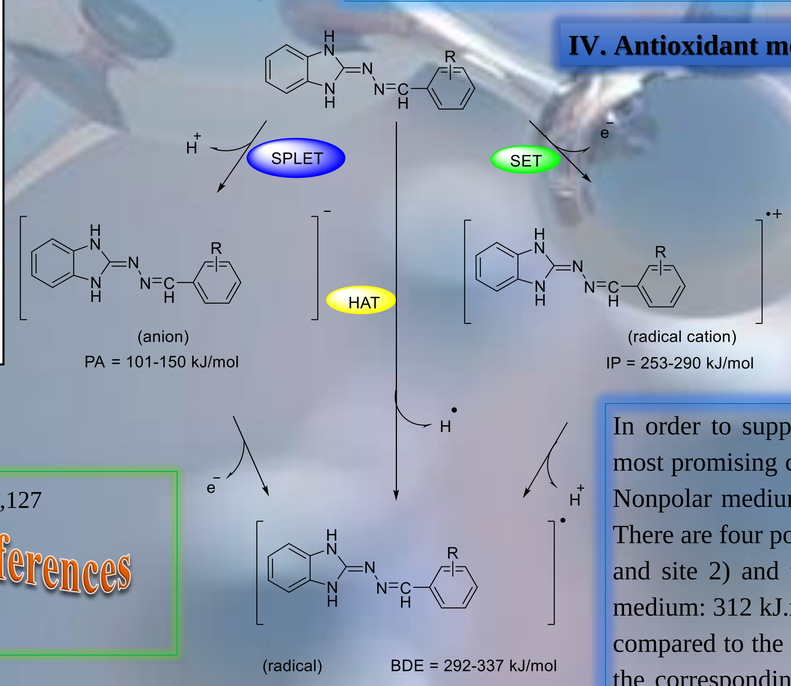
Figure: **Effect of the tested derivatives on the in vitro Fe(II) induced oxidative degradation in lecithin containing model system**

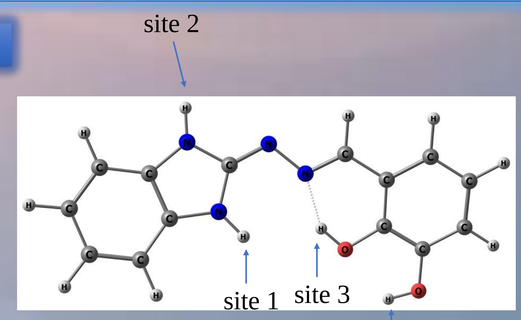
III. Antiprotozoal activity in vitro

The new synthesized compounds 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 to 5 min they began to grow weaker and slower at concentration of 100 μg/mL and 50 μg/mL. After 15-20 min the paramaecia cells were motionless and the cell content becomes turbid. The hydrazones containing hydroxyl and methoxy groups **6j-l** exhibited high efficacy by immediate extinction of the paramaecia cells at the same concentrations.

All tested compounds except **6k**, which extent of molecular damage was similar to the control, demonstrated protection effect in the lecithin model system of ferrous iron induced oxidative damage. The observed extent of molecular damage for the rest of the compounds was lower compared to the reference Melatonin. Compounds substituted with one methoxy or hydroxyl group, several methoxy groups, or one hydroxyl combined with one methoxy group (except **6k**) have demonstrated similar protection activity. Compounds substituted with several hydroxyl groups **6b-e** have better protection capability compared to the previously mentioned groups. Their extent of molecular damage was identical to the strong antioxidant quercetin (**6d** and **6e)** and even better (**6b**).

IV. Antioxidant mechanism





Gaussian 16
Expanding the limits of computational chemistry
B3LYP/6-311++G**

In order to support the assignments of the pharmacological activities, the radical-scavenging mechanisms of the most promising compound **6b** was evaluated. The BDE, IP and PA were evaluated for the most stable imino isomer. Nonpolar medium was represented in the calculations by benzene and gas phase, while polar medium - by water. There are four possible sites for hydrogen atom abstraction: the amine groups from the benzimidazole nucleus (site 1 and site 2) and the hydroxyl groups (site 3 and site 4). The BDE values for the amide N-H group in nonpolar medium: 312 $kJ.mol^{-1}$ (gas phase) and 315 $kJ.mol^{-1}$ (benzene), show that it can more easily transfer a hydrogen atom compared to the hydroxyl groups: 330 $kJ.mol^{-1}$ (gas phase) and 340 $kJ.mol^{-1}$ (benzene). In polar medium, *i.e.* water, the corresponding values of BDE and ionization potential are much higher than the proton affinity 125 $kJ.mol^{-1}$. Therefore, the SPLET mechanism is the preferred one in water.

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