

Double substituted thiosemicarbazides as potent and selective compounds for inhibition of *Toxoplasma gondii in vitro* proliferation

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Infection with the protozoan parasite *Toxoplasma gondii* has a worldwide distribution. This obligate intracellular parasite can infect all warm-blooded animals, including mammals and birds. Since its first description in the common gundi (*Ctenodactylus gundi*), a rodent from North Africa, by Nicolle and Manceaux in 1908, the parasite was progressively recognized as the agent of a widespread zoonosis. Nevertheless, its entire life cycle was definitively understood only in the late 1960s when discovered the important role of the animal from Felidae family as a definitive host. In the same period of time, it was classified in the phylum Apicomplexa, and well characterized the infectivity of the three parasitic stages: tachyzoite, bradyzoite, and sporozoite. The true importance of toxoplasmosis in humans remained unknown until the first reports of cases of congenital toxoplasmosis. The growing role of *Toxoplasma* infection in immunocompromised patients was acknowledged in the mid-1970s, and the concept of the reactivation of infection was thereafter extensively explored by immunologists. During the last decade, the development of new genotyping tools and the multiplication of field studies have led to breakthroughs in the comprehension of the phylogenetic evolution of *T. gondii* in the world. Currently, we only know few treatments available against this parasite, but it has low effectiveness and high risk of side effects. The strategy in the development of new therapies in the treatment of toxoplasmosis is the identification of new non-toxic small molecules with high specificity to *T. gondii*. Therefore, in our study we synthesised new double substituted thiosemicarbazides (Fig. 1) and tested them as potential anti-*T. godnii* drugs.

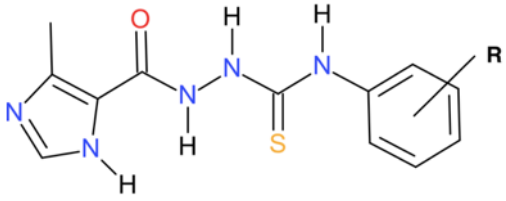

Figure 1. Structures of double substituted thiosemicarbazides.

Firstly, we established cytotoxicity of our compounds by common MTT assay using L929 cell line and determined the cytotoxic concentration [according to ISO 10993-5:2009]. In next step,

using highly virulent RH strain, we measured anti-*T. godnii* activity of our new compounds by [5,6-3H] uracil incorporation assay and due to inhibitory concentration was calculated. Furthermore, we established the drug susceptibility of our RH strain, especially to sulfadiazine which are the most common drug using to anti-*T. gondii* therapy. Also, we determined the physicochemical properties of our thiosemicarbazide derivatives important for central nervous system (CNS) penetration. The best double substituted thiosemicarbazides, at concentrations that are non-toxic to the host cells, showed much higher potency when compared to sulfadiazine, indicating a high selectivity of their anti-toxoplasma activity. All derivatives possess suitable properties for CNS penetration.