Triazole-Based Compound as a Candidate To Develop Novel Medicines To Treat Toxoplasmosis

Katarzyna Dzitko, Agata Paneth, Tomasz Plech, Jakub Pawelczyk, Lidia Węglińska, Piotr Paneth

Department of Immunoparasitology, University of Lodz, Lodz, Poland; Department of Organic Chemistry, Medical University of Lublin, Lublin, Poland; Institute of Applied Radiation Chemistry, Technical University of Lodz, Lodz, Poland; Institute for Medical Biology of the Polish Academy of Sciences, Lodz, Poland

This article reports anti-Toxoplasma gondii activity of 3-(thiophen-2-yl)-1,2,4-triazole-5-thione. The compound displayed significant and reproducible antiparasitic effects at nontoxic concentrations for the host cells, with an experimentally determined 50% inhibitory concentration (IC50) at least 30 times better than that of the known chemotherapeutic agent sulfadiazine. Purine nucleoside phosphorylase was defined as the probable target for antitoxoplasmosis. Toxoplasma gondii infection of 50% of concentration of tested compounds that was required for inhibition of $50\% \text{IC}_{50}$ at least 30 times better than that of the known chemotherapeutic agent sulfadiazine. Purine nucleoside phosphorylase was defined as the probable target for anti-Toxoplasma activity. The third class of anti-Toxoplasma drugs, which are only occasionally used as a potential substitute, comprises electron transport inhibitors such as atovaquone (1). In all of these situations, drug resistance, high cost, limited efficacy, and side effects of these drugs often result in discontinuation of therapy (2–5). Therefore, new agents with better activity profiles and that are less expensive are needed. One possible class of drugs are s-triazole derivatives, and in this article, we present a newly found triazole-based candidate to develop novel medicines for more effective treatment of toxoplasmosis.

The search for agents that are potent and selective against Toxoplasma continues in several laboratories. Numerous inhibitors with activities in the nanomolar range with no appreciable in vitro toxicity to human cells have been identified. Examples are pyrimidines, oryzalidinones, berberines, tryptanthines, thiocyanates, and bisphosphonates (6, 7). Our attention has been focused on the role of s-triazole series as potential new toxoplasmosis therapeutics. We found that 3-(thiophen-2-yl)-1,2,4-triazole-5-thione (compound 1) showed a potent and reproducible antiparasitic effect with no appreciable toxicity to human cells, while 4-ethyl-3-(4-methyl-1,2,3-thiadiazol-5-yl)-1,2,4-triazole-5-thione (compound 2) was inactive.

The procedure for synthesis of 3-(thiophen-2-yl)-1,2,4-triazole-5-thione (compound 1) and 4-ethyl-3-(4-methyl-1,2,3-thiadiazol-5-yl)-1,2,4-triazole-5-thione (compound 2), the effects of tested compounds and sulfadiazine on the viability of L929 cells, and inhibition of Toxoplasma (RH strain; ATCC no. 50174) growth were described elsewhere (8). The effect of tested compounds on the intensity of Toxoplasma gondii proliferation (%) was measured by 2 methods: incorporation of $[^{3}\text{H}]\text{uracil}$ (Fig. 1) into the T. gondii DNA (9) and quantitative real-time PCR (qRT-PCR) (Fig. 1) (8). The 50% inhibitory concentration (IC50 [μg/ml]) represents the concentration of tested compounds that was required for inhibition of $50\%$ of T. gondii proliferation on the cell lines used. The cytotoxic effect of the tested compound on the mouse L929 fibroblasts (percentage of viable cells) (Fig. 2) was measured using the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay according to the international standard ISO 10993-5:2009(E). To calculate the reduction of cell viability compared to viability in the untreated blank, the following equation was used: % viability = 100 × (sample OD570/blank OD570), where sample OD570 is the mean value of the measured optical density at 570 nm of the tested samples and “blank OD570” is the mean value of the measured OD570 of the untreated cells. The 30% cytotoxic concentration (CC50 [μg/ml]) represents the concentration of tested compounds that was required for a 30% cytotoxic effect of the tested compounds on L929 cells. Selectivity refers to the ratio of the CC50 value for L929 fibroblasts to the IC50 for T. gondii proliferation of 50% on the cell lines used. The

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The receptor for FlexX was prepared using the de-gram (version 6.04). The results of the experiments are shown as determined based on the plotted curves using GraphPad Prism program together with the IC50s is shown in Fig. 1. As can be seen, compound 1 and the control drug, sulfadiazine, IC50 (42.46 or 37.33 p/mg/ml), depending on which method was used for measuring the intensity of parasite proliferation ([3H]uracil incorporation or qRT-PCR, respectively). In the case of the HeLa cells, the IC50 (58.5 µg/ml) was also lower (30.6-fold) than that with IC50 (1,790.0 µg/ml) for sulfadiazine using the [3H]uracil incorporation assay. Compound 1 showed evident anti-Toxoplasma activity and exhibited more than 4× greater selectivity than sulfadiazine (Fig. 2). It should be mentioned that the drug susceptibility of the RH strain measured by incorporation of [3H]uracil (Fig. 2) and exhibited more than 4-fold selectivity (CC30/sulfadiazine (1,790.0 µg/ml) was also lower (30.6-fold) than that with IC50 (1,790.0 µg/ml) for sulfadiazine using the [3H]uracil incorporation assay. Compound 1 showed evident anti-Toxoplasma activity and exhibited more than 4× greater selectivity than sulfadiazine (Fig. 2). It should be mentioned that the drug susceptibility of the RH strain measured by incorporation of [3H]uracil (Fig. 2) (1-deoxy-D-xylulose 5-phosphate reductoisomerase), 4M84 (calmodulin-domain protein kinase 1), 3MB8 (purine nucleoside phosphorylase), 1LII (adenosine kinase), 2O2S (enoyl-acyl carrier reductase) (18). Based on the structures deposited in the Protein Data Bank, we analyzed the binding affinities of the active compound 1 and inactive compound 2 s-triazoles to the active sites of the aforementioned enzymes. We have excluded the model of the dihydrofolate reductase binding site (PDB ID no. 4EIL) because this enzyme is also present in humans, so treatment with DHRF inhibitors may induce a folate deficiency, which is possibly responsible for severe hematological pathologies and embryopathies. The largest difference in docking score has been observed for the enzyme model 3AU9, followed by 4M84 and 3MB8 (Fig. 3). Given the difference in bioactivities of compounds 1 and 2, these three enzymes seem to be potential targets. In all cases, the apparent main source of the differentiation is the size exclusion; active sites are tight and deeply buried in the protein, making access for the bulkier compound much harder or impossible. In fact, the active site is so tight in the case of 3AU9 that it was not possible to dock inactive s-triazole (compound 2) using the “hard” docking protocol, and only after loosening the “maximum allowed overlap volume” to 100 Å³ (i.e., “soft” docking) did it become possible (Fig. 3). Nearly no preference in binding to the active site was observed in case of 1LII, while in case of 2O2S, the docking pref-
ference is reversed, with strong binding of the inactive s-triazole (compound 2), which was in clear disagreement with experimental observations. Out of the chosen three proteins, the strongest binding is expected for 3AU9, and this enzyme seems to be the most probable target. The binding mode of the active ligand (compound 1) in its active site is illustrated in Fig. 3.

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