ANTIVIRAL ACTIVITY AND STRUCTURAL INSIGHTS INTO BENZOTHIAZOLE ANALOGUES AGAINST CHIKUNGUNYA VIRUS

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ABSTRACT

Background: Chikungunya virus (CHIKV) is an arthropod-borne virus transmitted by *Aedes* sp. mosquitoes, leading to a disease primarily marked in the acute phase by symptoms that include joint pain, fever, headaches, myalgia, cutaneous rash and arthralgia. Some patients may evolve into a chronic phase, manifesting as chronic rheumatic syndrome (CRS), which can persist for months or even years. Currently, there is no specific antiviral treatment available for this condition. **Objectives:** The aim of this study was to evaluate the antiviral activity of novel benzothiazole analogues designed against CHIKV *in vitro* and analyze their structure-activity relationships. **Methods:** Initially, 19 previously synthesized benzothiazole analogs (over 98% purity) were tested in cytotoxicity assays by treating Vero E6 cells (2×10^4 cells/well in a 96-well plate) with different concentrations ranging from 200 to 50 µM for 48 h (37 °C, 5% CO_2). Cell viability was evaluated via an MTT colorimetric assay, and CC_{50} values were obtained via nonlinear regression. Antiviral assays were performed via CHIKV inoculation into Vero E6 cells at an MOI of 0.01 for 2 h. After this period, the cells were treated with serial dilutions of the compounds from 50 to 6.25 µM for 48 h, and viral inhibition was evaluated via the MTT assay. The EC_{50} was also determined via nonlinear regression, and the selectivity index (SI) was calculated as the CC_{50}/EC_{50}. Time-of-drug addition was performed to evaluate antiviral activity at different times before or after infection. To confirm antiviral activity, intracellular flow cytometry was conducted via an antiviral assay as previously described. After 48 h, the cells were obtained and stained with an anti-CHIKV monoclonal antibody and goat anti-mouse IgG conjugated with AlexaFluor488. The samples were acquired and analyzed on a BD FACSVia flow cytometer. To understand the activity of the compounds, the structure–activity relationship was determined by comparing the chemical substitutes and their impacts on the activity of the compounds. **Results and discussion:** Of the 19 tested benzothiazoles, 11 were noncytotoxic at 50 µM and were subjected to antiviral assays. All 11 compounds showed significant antiviral activity against CHIKV *in vitro*, and the highest SI values obtained were >20.55 for EdCHIK10, >30.58 for EdCHIK39 and >20.87 for EdCHIK82. Time-of-drug-addition experiments revealed slight but significant effects of pretreatment with EdCHIK10 and EdCHIK39; however, none of the compounds inhibited viral infection when CHIKV was added simultaneously, and there was significant inhibition after treatment. Flow cytometry analysis revealed a reduction in infection from a mean of 40.96% ± 9.84% (untreated) to 8.40% ± 1.81% (EdCHIK10), 11.13% ± 0.35% (EdCHIK39), and 21.86% ± 12.04% (EdCHIK82). A structure–activity relationship study revealed that the most promising compound, EdCHIK39, was the only long-chain electron donor, and EdCHIK10 and EdCHIK82, with methoxyl and hydroxyl groups, respectively, presented the best SI. Interestingly, these best compounds present substitute groups at the 6^{th} position of the benzothiazole. **Conclusion:** In summary, these novel benzothiazole compounds demonstrated promising antiviral activity against CHIKV *in vitro*. Further investigations are needed to elucidate the underlying molecular mechanisms and help with the development of new biomolecules against this arbovirus.