

## Abstract

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Title of Diploma Thesis: Characterisation of Gyrase inhibitors using ITC and enzymatic assay

This paper deals with *in vitro* evaluation of inhibitors of gyrase and topoisomerase IV - enzymes of type II topoisomerases family. These enzymes are essential for the proper function of bacterial cell and their inhibition leads to its destruction. The experimental part of the work had been developed at the Department of Medicinal Chemistry at the University of Ljubljana within Erasmus+ program. Tested novel inhibitors based on substituted pyrroleamide moiety with GyrB (ParE) mechanism of action were developed at the same department. The basic parameters of DNA topology, types and classification of topoisomerases, mechanism of action and structure of gyrase and topoisomerase IV and brief summary of inhibitors used in clinical practice were described. Enzymatic assay was used for evaluation in the experimental part, and half maximal inhibitory concentration  $IC_{50}$  was calculated. The activity against *E. coli* gyrase, *E. coli* topoisomerase IV, *S. aureus* gyrase and *S. aureus* topoisomerase IV was measured. Results were analyzed and described as structure-activity relationship (SAR) of given compounds. ITC (isothermal titration calorimetry) was carried out for some compounds as complementary analysis and thermodynamic characterization of binding was analyzed. Compounds KMG-15 KMG-17 and NAS-37 were evaluated as the most effective with dual targeting against all tested types of enzymes. Compounds TJL-19, TMK-16, 9-KMG and KMG-11 showed high activity against *E. coli* gyrase but were not tested against other enzymes. Further experiments will be performed in the future.

**Key words:** Topoisomerases, gyrase, topoisomerase IV, inhibitors, enzymatic assay, Isothermal Titration Calorimetry