ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Bc. Barbora Fiklíková

Supervisor: Vladimír Wsól, Ph.D.

Title of diploma thesis: The influence of branebrutinib on the activity of selected reductases from AKR and SDR superfamilies

Acute myeloid leukaemia (AML), along with other cancer diseases, is a widespread health problem not just in the Czech Republic but in the entire world. There is no reliable cure, and millions of people die of cancer every year.

Anthracycline antibiotics (ANT) such as daunorubicin have been used as the cure for many years in combination with other cytostatics. However, even these drugs have their issues. One of the problems is toxicity for the healthy cells (especially cardiomyocytes) caused by the inhibition of topoisomerase 2β and by the reactive oxygen species (ROS) formation. The other problem is the increasing resistance of the ANT to cancer cells.

ANTs are metabolized by carbonyl reducing enzymes to appropriate alcohols with lower effects against cancer cells and more severe toxicity for the heart cells. The inhibition of these enzymes could be used to achieve better therapeutic results.

One of the potential inhibitors could be branebrutinib (BRA). It inhibits Bruton tyrosine kinase and is used to cure immune-mediated diseases and inflammatory diseases, and B-cells malignancies, for example, rheumatoid arthritis, lupus, and others.

The aim of this work was to find out whether BRA inhibits any of the selected reductases, determine IC50, kinetic parameters, and a type of inhibition of the most inhibited enzyme.

BRA was most effectively inhibiting the enzyme AKR1C3. The inhibition reached 77.20% for 10 µM BRA and 91.79% for 50 µM BRA. Somewhat less inhibited was AKR1B10. The inhibition reached 62.97% for 10 µM BRA and 81.75 % for 50 µM BRA. Other tested enzymes displayed low inhibitory potential to BRA, so there was no need to continue in their following experiments.

The IC50 for AKR1C3 was 2.65 µM, and the inhibitory constant Ki was 3.08 µM. Based on the Lineweaver-Burk plot, the noncompetitive inhibition type was observed. The tight binding inhibitor was not proven, and BRA is reversibly bound to AKR1C3. The average IC50 for AKR1B10 was 5.62 µM, and the inhibitory constant Ki was 6.40 µM. The type of inhibition was also noncompetitive, and BRA was bound to AKR1B10 reversibly.

Based on the results, the BRA could increase the therapeutic effect of daunorubicin due to AKR1C3 and AKR1B10 inhibition. However, further research with the cell lines is necessary to complete the in vitro observations with recombinant proteins.