Abstract

α-Aminophosphonates are important representatives of organophosphorus compounds. Their structural analogy to natural α-amino acids makes them exhibit a range of biological activities, such as enzyme inhibition, antimicrobial, antiviral and anticancer effects. One of the enzymes whose elevated levels lead to serious consequences in the body is urokinase. Overexpression of this protein is associated with an increase in the course of cancer manifested, among other things, by the formation of metastases. Urokinase represents an interesting therapeutic target, as inhibition of its activity can result in an improved prognosis for patients. The introduction of a fluorine into the molecules of chemical compounds is a strategy for modulating the chemical and physical properties of these compound. Among other things, it allows improving lipophilicity, which is a very important parameter for substances with potential medical applications. The aim of the research carried out within the framework of the doctoral project was the synthesis of new fluorinated α-aminophosphonates, structural studies of the obtained molecules, as well as biological activity studies to assess their anticancer cytotoxicity and urokinase enzyme inhibition.

The title compounds were obtained by hydrophosphonylation of imines. Due to the great importance of Schiff bases in the synthesis, a new mechanochemical method for their preparation was developed, which fits in with the principles of green chemistry. Using this method, a series of imines were synthesized using fluorinated benzaldehydes derivatives and aniline derivatives containing different substituents at different positions in the aromatic ring for the reaction. The structures of the imines were confirmed by performing ^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra and crystallographic analyses. In addition, HRMS analyses were performed for compounds not yet described in the literature. For the synthesis of the title α-aminophosphonates, chiral amines with a specific absolute configuration were used, so that the products were obtained as diastereoisomeric mixtures. All compounds were characterized in detail by both spectroscopic (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, ^{31}P NMR) and spectrometric (HRMS) methods. Using 2D NMR and X-ray diffraction techniques, the absolute configuration of new chirality center generated in the main diastereoisomeric product during the addition of phosphite to imine was determined.

The obtained α-aminophosphonates were subjected to biological activity studies. First, *in silico* pharmacokinetic analysis was carried out to evaluate the compounds as drug-like substances. All synthesized α-aminophosphonates met the so-called “Lipinski’s Rule of Five”,

which is the most popular criterion for determining the similarity of substances to drugs. The obtained compounds were tested *in vitro* to evaluate their cytotoxicity. The tests were performed against ten human cancer cell lines from different organs and against one normal cell line. To determine the long-term effects of the compounds, clonogenic assays were performed. The α-aminophosphonates were also subjected to enzyme inhibition tests, during which their ability to inhibit urokinase was examined. Enzymatic inhibition tests against urokinase were also conducted using molecular docking. This made it possible to find out the detailed relationships between the inhibition shown and the structure of the molecule.