Phosphonic Acid Analogues of Aromatic Amino Acids bearing Fluorine Atoms in the Phenyl Ring – Inhibitors of Chosen Enzymes

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Abstract

Aminophosphonic acids are defined as phosphorus mimetics of amino acids, where carboxylic group is replaced by phosphonic moiety. They constitute one of the most extensively studied families of organophophorus compounds used to manipulate biological pathways in living organisms. The introduction of the phosphonic group significantly affects the properties of the whole molecule. From medicinal chemistry point of view, the most important modification is the tetrahedral shape of the phosphoric fragment in comparison to the planar carboxylic group, which determines their ability to mimic transition state of hydrolysis of peptides and ester bonds. In consequence, the phosphonic analogues are effectively bound to the catalytic centers of hydrolytic enzymes and blocks their catabolic functions. That similarity plays a crucial role in the searching for new inhibitors towards proteinases and esterases with the capability to regulate the metabolic pathways, in which they participate [1,2]. The phosphonic analogues of naturally occurring amino acids and their derivatives are distinguished by a variety of biological applications. Their high activity determines the number of uses especially in medicinal chemistry and agrochemistry - they exhibit antitumor [3,4], antibacterial [5,6], antiviral [7], anti-inflammatory [8] and antifungal [9] properties, and can be used as pesticides and herbicides [10].

The introduction of fluorine atoms into the bioactive molecules turns out to be a relatively new, but more and more popular trend in the pharmaceutical industry. Statistical data show, that in 1970 fluorinated drugs accounted for only about 2% of total drug sales. However, in 2011 the number of medicaments and substances acting as dietary supplements containing fluorine increased to 25% [11]. That growing tendency is conditioned by the development of unique properties of the modified molecule. Additionally, the fluorine atom is visible in ^{19}F NMR spectra and can be used as a molecular probe to monitor the mechanisms of action of physiologically active molecules [12]. Thus, the research on organophosphorus compounds containing one or more fluorine atoms is gaining increasing attention. In recent years, it has been shown that fluorinated aminophosphonates possess inhibitory activity against many enzymes, as well as cytotoxic and antibacterial properties [13,14].

Compounds with a well-known broad spectrum of biological activity, resulting from their ability to inhibit the activity of many enzymes - α-aminophosphonic acids, were selected for the

research in this dissertation. Thus, the aim of the study was the synthesis of phosphonic mimetics of aromatic amino acids including fluorine atoms in their phenyl rings. It was assumed that compounds bearing in the structures two pharmacophores with the possibility of their detection by NMR (^{19}F and ^{31}P NMR) should constitute molecular probes used to monitor the structure of binding sites and changes in the structure of the enzyme during reaction. The results of enzymatic studies have been supplemented by analysis mode of the binding of the obtained inhibitors using molecular modeling.

The Thesis presents the synthesis of three series of α-aminophosphonic acids: phenylglycine, phenylalanine and homophenylalanine analogues, and the examination of their ability to inhibit catalytic functions against selected enzymes.

Phosphonic acid analogues of phenylglycine containing fluorine and other halogen substituents in the aromatic ring were obtained by using amidoalkylation of trivalent phosphorus compounds (condensation of aldehyde, amide and phosphorus trichloride) [15,16]. The study of their inhibitory activity against *L*-phenylalanine ammonia-lyase (isolated from potato tubers) indicated that these compounds are able to effectively inhibit the enzyme-catalyzed reaction when were applied at the micromolar concentration. The molecular modeling showed, that the affinity of these compounds for the enzyme is closely related to the position and type of substituents in the aromatic ring. Inhibitory activity of the structurally compact library of phenylglycine mimetics were also examined against two aminopeptidases - isolated from barley seeds and commercially available porcine aminopeptidase N.

The second part of the Thesis was the synthesis of the fluorinated phosphonic acid derivatives of phenylalanine and the evaluation of their inhibitory properties against porcine and human aminopeptidase N. Human aminopeptidase N is considered as a target for the design of anticancer compounds and screening studies are mostly carried out porcine enzyme because of its higher availability. The phosphonic acid mimetics of phenylalanine were obtained from carboxylic acids counterparts by using multistep reaction starting from suitable carboxylic acids, which was named „*on line*" and was characterized by lack of separation of unstable intermediates [17,18,19].

The final part of the dissertation considers the synthesis of the fluorinated phosphonic acid analogues of homophenylalanine and phenylalanine. The later ones consider those, which were unavailable by previously elaborated procedures. The commercially available homophenylacetic and phenylacetic acid precursors, substituted by fluorine and bromine atoms in the aromatic ring were converted to corresponding aldehydes, following by their reaction with benzyl carbamate and triphenyl phosphite [20,21,22]. The reductive deprotection of

blocking group gave the desired phosphonic acid analogues. The inhibitory potencies of the obtained compounds were examined, once more, towards porcine and human aminopeptidase N.

The structural characterization of the final compounds was done by NMR (one and two-dimensional), HRMS and analytical HPLC studies. Additionally, these structures were determined by X-Ray crystallography method.

The analyses of the inhibitory properties of the studied compounds against selected aminopeptidases have been supplemented by computational techniques – molecular modelling, which enabled to understand modes of their binding to the chosen enzymes.