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Referee report on PhD Thesis

Fluorinated phosphonate analogues of phenylalanine – synthesis, structural studies and reactivity

by

Joanna Marianna Kwiczak

1. The subject of the thesis

Aminophosphonic acid derivatives constitute an important class of organophosphorous compounds on account of their versatile biological activity. The low level toxicity of these compounds to mammals makemade them attractive for use in agriculture and medicine.

Aminophosphonates are esters of the aminophosphonic acids of amino acids and are classified as α, β and γ-aminophosphonates. They are considered to be structural analogues of amino acids and transition state mimics of peptide hydrolysis.

α-aminophosphonates are probably the most important substitutes for the corresponding amino acids in biological systems. α- aminophosphonates are considered phosphorous analogues to α-amino acids where the planar carboxylic group is replaced by tetrahedral phosphonate functionality. Therefore phosphorous geometry serves as a useful tool in medicinal chemistry.

α-aminophosphonates have been the focus of interest in last several years because their structural analogy to corresponding amino acids heterocyclic phosphonates and aminophosphonates.

Being the structural analogues of amino acids aminophosphonates act as an antagonist and compete with the carboxylic counterparts for the active site of enzymes or other cell receptors. Therefore, they exert various biological activities as enzyme and plant growth inhibitors.

I see the thesis by Joanna M. Kwiczak in the range of chemical biology. Chemical biology is a new, rapidly emerging branch of chemistry that represents all aspects of chemical endeavor, devoted to understanding the way biology works at the molecular level. Chemical biology is in fact inter-disciplinary, and essentially problem driven and not discipline driven.

Organic, physical, inorganic and analytical chemistry all contribute towards this discipline. Chemical biology is about chemistry-trained researchers taking a fundamental interest in the way biology works. The focus is on the molecular and the quantitative, where molecular properties are studied and then gradually linked to macromolecular and cellular behavior where possible. This is a fundamentally a 'bottom-up' approach to understanding biology in keeping with the chemist's natural enthusiasm and appreciation for molecular structure and function. This branch of natural science means the application of chemistry to study of molecular events in biological systems. Novel chemical tools and technologies developed at the interface of chemistry and biology are enabling unprecedented understanding of biological function and regulation as well as opportunities for the creation of translational technologies and molecules in medicine, materials and energy, as well as to develop new concepts and principles for understanding biological phenomena to invent new molecular probes ranging from small molecules to genetic circuits.

Chemical biology aims to leverage the tools and principles of chemistry to understand complex biological systems at the molecular level. Current research efforts span the fields of cell biology, cancer biology, medicine, synthetic biology, genomics and materials science. Bioactive natural products or synthetic compounds play key roles in biological studies. Chemical biologists generate and utilize chemical tools to explore biological systems.

α-aminophosphonates as small synthetic molecules provide an extremely rich source for chemical biology and drug discovery. They have found different applications in various areas of chemical biology due to their biological and physical properties. Some antibiotics, enzyme inhibitors and pharmacological agents are α-aminophosphonate derivatives. They are found as constituents of natural products. Some substituted phosphonates exhibit antifungal, antibacterial and antiviral activities as well as antitumor effects. Some aminophosphonic acids can be used also as antioxidants. Several methods have been developed for their synthesis. The efficient generation (synthesis) of structurally diverse small molecules is one of the key starting points for chemical biology and drug discovery, which enables us to explore more chemical space and identify biologically meaningful molecules.

For many years natural products have been a leading source for the majority of the approved drugs and their structures are a valuable source of inspiration for molecular medicine.

Interestingly, only ca 1/3 of new chemical entities discovered in 1981-2010 have been developed without inspiration from a natural product.

2. The goals of the thesis

Increasing importance and interest of α-aminophosphonates as phosphorous analogoues of natural amino acids and particularly their properties as efficient enzyme inhibitors, prompted the Author to:

2.1. develop the organic synthesis methods and structural studies of novel phosphonate analogues of phenylalanine containing variable number of fluorine substituent's in aromatic ring,

2.2. develop new methods for mono-, and full deprotection of phosphonates which can be used in peptide synthesis,

2.3. synthesize of different para substituted fluorinated analogues of phenylalanine with various nucleophiles as potential aminopeptidase inhibitors.

One should to stress that introduction of fluorine in order to improve the pharmacological properties of a drug is a modern trend in molecular medicine and chemical biology. Currently, there are about 200 fluorinated drugs on the market (1/5 of all pharmaceuticals) with even higher figures for agrochemicals (about 30%).

3. Characteristics of the PhD thesis

This thesis represents a good piece of work. They have been done at Chemistry Department of Adam Mickiewicz University and partially at Institut Charles Gerhardt de Montpellier, France in the frame of Erasmus + programme.

The PhD thesis is written in English. It is composed of three chapters: literature review, results and discussion and experimental. There is also a very extended list of references (198 pos.) and attachments (15).

In the chapter I e.g., Literature review, the Author has described synthesis and chemical properties of some fluorinated aminophosphonates. Effects of those compounds on cancer cells and activity of selected enzymes have been reviewed. Furthermore major methods of α-aminophosphonates and their fluorinated derivatives synthesis are shortly presented. The third topic discussed in this chapter is nucleophylic aromatic substitution. The literature review encompasses a wide range of key issues in aminophosphonates research, in an authoritative, critical and well-informed manner.

In the chapter II, Results and discussion, the Author's original data are described and deeply discussed. The rationale for the study itself is explained clearly, and the investigation is carried out with a high degree of rigour, in accordance with current standards of good practice for research in this field. Appropriate conclusions and discussion are included. New fluorinated phosphonate analogues of phenylalanine are characterized with NMR spectroscopy. The spectra

are analyzed in detail. The reaction conditions are described. For some α-aminophosphonates crystal structures have been obtained with X-ray diffraction measurements. Different conditions for hydrolysis of the ester are described. Mono deprotection and full protection reaction are deeply discussed.

Final part of chapter II concerns nucleophylic aromatic substitution. Reaction conditions and yield have been described. Products have been identified with NMR spectroscopy. The thesis is well-written and well presented – throughout, complex material is handled in a mature and succinct way.

4. The main results of the thesis

4.1. The new two steps method of synthesis of phosphonate analogues of phenylalanine was developed based on reduction of fluorinated phenylacetic acids to alcohols in the presence of $LiAlH_4$ followed by oxidation of alcohols to aldehydes.

4.2. The chemical stability of fluorinated phenyl ethanol's depends on position of fluorine substituent in the phenyl ring.

4.3. The one-pot two steps method for effective synthesis of α-aminophosphonates was invented.

4.4. Several novel α-aminophosphonates, analogues of phenylalanine we obtained.

4.5. Synthetic cyclic side products were isolated and a mechanism of intramolecular nucleophylic aromatic substitution was identify.

4.6. Novel approach for monodeprotection of phosphonates with LiI was invented.

4.7. Nucleophylic aromatic substitution reactions with perfluorinated phosphonate analogues of phenylalanine take place in the presence of soft nucleophiles and para substituted products were obtained, only.

The research reported in the thesis is groundbreaking and innovative, and I would urge the Author to move toward publication, in a leading peer reviewed journal, as soon as possible.

In the thesis new fluorine substituted aminophosphonates were synthesized, characterized and extensively discussed. Obviously it broadens our current knowledge on this group of very interesting compounds which show many important properties in the living cell. These new data also contribute to a discussion of very famous question why nature chose phosphates put forward by Frank Westheimer 40 years ago (Ref. 8). It would be favorable to have the Author's comments on that.

5. Remarks and comments

5.1. In literature review chapter the Author discusses the effects of aminophosphonates on selected enzymes and a crystal structure is shown from PDB (e.g. Figs. 1.11; 1.12; 1.14;

1.15; 1.16). What is a purpose of that? From that presentation it is obvious that there is no possibility to identify the active center of the enzyme and interactions with inhibitor.

5.2. Description of selected enzymes affected by aminophosphonates is very poor.

5.3. The quality of Fig. 2.21 showing electron density map is low and it is difficult to follow structural changes.

5.4. The main goal of the doctoral work was to synthesize phosphonate analogues of phenylalanine and its fluorinated derivatives. Several new compounds have been obtained. These have been done. I am wondering why the Author of the thesis didn't take an efforts to evaluate biological properties of the compounds obtained in the work. They have a big potential for anticancer or antiviral treatments and most probably they could be epigenetic drugs.

6. Remarks on editing of the thesis text

Some mistakes or errors have been found

6.1. page 132 is followed by page 134,

6.2. reference citation in the text by journal name and year and in addition by numbers (p. 134, 135).

7. Final conclusions

The submitted dissertation is clearly written, demonstrates extensive knowledge and high skills in solving problems in solving synthetic and structural problems of α-aminophosphonates. The aims of the thesis are achieved fully at a high scientific level. The fact that the obtaining new compounds are well described and have a biological potential, increases the significance of the reviewed thesis.

In my opinion the thesis fulfils are requirements posed on theses aimed for obtaining Phd degree according to Polish law from March 14, 2003 (Dz. U. z 2003 r., Nr 65, poz. 595 with latter changes Dz. U. z 2005 r., Nr 164, poz. 1365, Dz. U. z 2010 r., Nr 96, poz. 620, Dz. U. z 2010, Nr 182, poz. 1228).

It is without hesitation that I fully recommend the scientific Board of the Chemistry Department of Adam Mickiewicz University to proceed will all necessary steps to confer Joanna Marianna Kwiczak a PhD degree.

Taking into account the significance of the studies and their potential, interesting and innovative approach to the experimental problems solving, and high level of the experimental work and discussion also consider thus thesis as a meritorious.