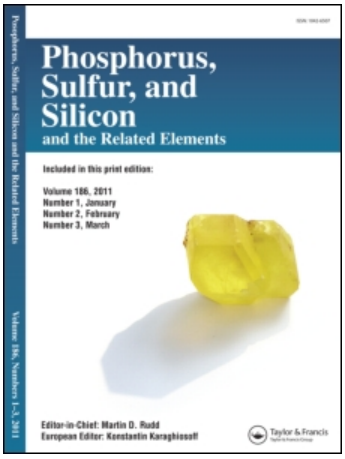

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The Computer-Aided Design, Synthesis, and Activity Prediction of New Leucine Aminopeptidase Inhibitors

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THE COMPUTER-AIDED DESIGN, SYNTHESIS, AND ACTIVITY PREDICTION OF NEW LEUCINE AMINOPEPTIDASE INHIBITORS

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The design, stereo-, and enantioselective synthesis and activity prediction of aminophosphonic acids as new leucine aminopeptidase inhibitors will be discussed.

Keywords: Aminopeptidase; aminophosphonic acids; enzyme inhibitors; leucine

INTRODUCTION

Aminophosphonic acids constitute a group of amino acid mimetics in which the carboxylic group is replaced by a phosphonic group. They reveal diverse and interesting biological and biochemical properties being applied as inhibitors of various enzymes, antibacterial agents, plant growth, and calcium metabolism regulators, as well as hypertensive and immunosupressive agents.

For years we were interested in the inhibitory activity of these compounds toward leucine aminopeptidase. Leucine aminopeptidase (LAP, E.C.3.4.11.1.) is a proteolytic enzyme, which catalyzes the removal of amino acids from the N-terminus of a peptide or protein. LAP has significant biological and medical importance resulting from its elevated activity observed in several pathological disorders, including cancer and eye lens cataracts. Moreover, LAP may play an important role in the early stages of HIV infection.[1]

In the case of cancer disorder, leucine aminopeptidase is involved in the degradation of anatomical barriers (tissues, membranes) and thus

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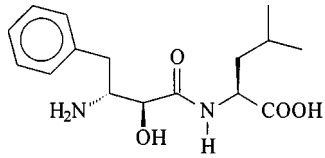
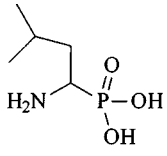
cancer development. Therefore, the design and enantioselective synthesis of the inhibitors of this enzyme represents a promising method of seeking for anticancer drugs. Additionally, such inhibitors might be helpful in understanding the mechanism of the reaction catalized by this enzyme.

Roughly, inhibitors of leucine aminopeptidase can be divided into two categories. First form analogues of amino acids which imitate the tetrahedral transition state after binding by the enzyme. This group includes aminoaldehydes and amino thiols, as well as phosphonic and boronic acid derivatives. Peptide mimetics form the second group of inhibitors, with bestatin **1** being the strongest so far known LAP inhibitor with K_i values being $2.8*10^{-8}$M for porcine kidney LAP – Zn^{+2} Zn^{+2} and $2.8*10^{-9}$ for bovine lens LAP – Zn^{+2} Zn^{+2}.[2]

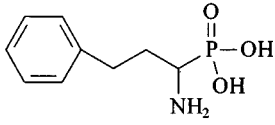


1 BESTATINE

The purpose of our work was to extend the family of aminophosphonic acids useful as inhibitors effective against leucine aminopaptidase. Previously described inhibitor, phosphonic acid analogue of L-leucine **2** (LeuP) of $K_i = 230$ nM for porcine kidney enzyme[3] was chosen as a lead compound. More effective inhibitors were designed by means of commercially available computer-aided LUDI procedure.



2 LeuP



3 hPheP

RESULTS AND DISCUSSION

Computational Methods

The Ligand Design (LUDI) was used in order to design potential inhibitors of leucine aminopeptidase (LAP) using crystal structure of bovine lens leucine aminopeptidase complexed with LeuP as a starting point.[4]

Synthetic Methods

The racemic mixtures of aminophosphonic acids were synthesized basing on well-known procedures described by Oleksyszyn – Soroka[5] and Mastalerz et al.[6] Enantiomerically pure mimetics were synthesized according to the two general methods described by Kafarski[7] and Walker.[8]

Because of the lack of general method of synthesis of pure enantiomers of aminophosphonic acids containing two chiral centers, we decided to apply the Walker method using chiral auxilliary. We have found that equimolar mixture of two diastereomers (SRS:SRR) of corresponding aminophosphonous acids have been obtained using S(−)–α-methylbenzyl amine and two other diastereomers (RSS:RSR) using R(+)–α-methylbenzyl amine as the auxilliary (Figure 1).

Reaction with bromine water resulted in simultaneous deprotection and oxidation of aminophosphonous acids and led to the equimolar pairs of desired aminophosphonic acids diastereoisomers—RS:RR and SS:SR—showing that the reaction cycle was fully stereoselective. Quite promising results were obtained while trying to resolve these diastereomers using ion-exchange resins and fractional crystallization.

Inhibitory Activity

The activity of synthesized aminophosphonic acids were performed using porcine kidney LAP (Sigma-Aldrich Chemical Co.) activated with

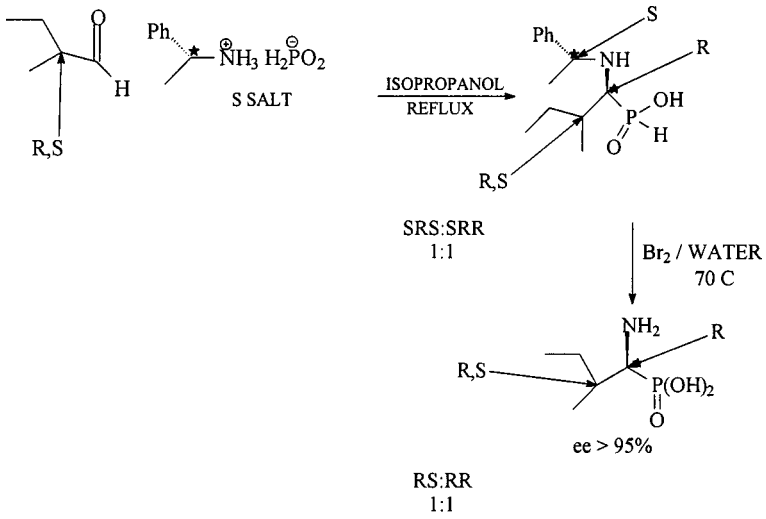


FIGURE 1 Synthesis of RS:RR pair of diastereomers of aminophosphonic acid using S(−)–α-methylbenzyl amine as chiral auxilliary.

22 mM triethanolamine hydrochloride solution, pH = 8.5, 1 mM $MnCl_2$, 2 h, 37°C.[9] L-Leucyl-4-nitroanilide at concentrations of 0.8—0.05 mM was used as substrate.

RESULTS

Based on the crystal structure of bovine lens leucine aminopeptidase complexed with its inhibitor, the phosphonic acid analogue of leucine (LeuP) and using LUDI programme, we have designed a group of new and effective inhibitors of LAP. They were synthesized as racemic mixtures, and in the case of potent inhibitors, obtained as enantiomers. Inhibitory tests show that phosphonic analogues of amino acids designed in this manner and containing modified side chain by introduction of more hydrophobic groups (as, for example, compound **3**) are few times better inhibitors of LAP than the lead compound LeuP itself.

Moreover, the experimental results of inhibition are in exceptionally good agreement with theoretical prediction. For example, for compound **3** (hPheP), the experimental $K_i = 140$ nM (RS mixture) is very close to theoretical value of $K_i = 290$ nM (S enantiomer).[10] This fact corresponds as well to the other synthesized and tested mimetics by us.

Comparing the K_i value of hPheP with LeuP, we can clearly see, that designed by us compounds are better inhibitors of LAP. Even as the racemic mixtures. This confirms the usefulness of the computational approach for the design of new and potent proteolytic enzyme inhibitors.

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