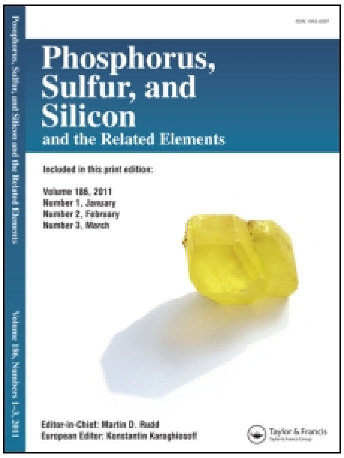

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STRUCTURE-BASED DESIGN AND SYNTHESIS OF DIPEPTIDE ANALOGUES AS NEW INHIBITORS OF LEUCINE AMINOPEPTIDASE

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The structure-based design, synthesis and activity prediction of new leucine aminopeptidase inhibitors, the phosphonamidate and phosphinate dipeptide analogues are presented.

Keywords: Drug design; leucine aminopeptidase; phosphinic pseudodipeptides; phosphonamidates; protease inhibitors

INTRODUCTION

Leucine aminopeptidase (LAP, E.C.3.4.11.1) is a zinc-containing metalloprotease, which catalyzes the hydrolysis of amino acids from the N-terminus of a peptide or protein.[1] Its altered activity is observed in human eye lens aging and cataracts, hepatic diseases, cancer, and HIV infection.[2] Since this enzyme appears to be involved in many important biological processes, the compounds that inhibit LAP are of medical and therapeutic significance.[2]

The most active inhibitors of the enzyme are naturally occurring peptide analogues: bestatin ($K_i = 0.0006\ \mu M$) and amastatin, ($K_i = 0.03\ \mu M$).[3,4] Among the amino acid analogues, L-leucinal ($K_i = 0.06\ \mu M$),[5] the phosphonic acid analogue of L-leucine (LeuP, $K_i = 0.23\ \mu M$),[6] and the boronic acid analogue of L-leucine ($K_i = 0.13\ \mu M$)[7] play a major role in leucine aminopeptidase inhibition. All of them are considered as transition state analogues and bind in a similar way to

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LAP active site, which has been confirmed by the X-ray crystal structures of bovine lens leucine aminopeptidase with L-leucinal[8] (1lan code in PDB), LeuP[9] (1lcp code in PDB), and amastatin[10] (1bll code in PDB).

The crystal structure of the complex; LAP-LeuP, was used for structure-based design of new enzyme inhibitors. LeuP was chosen as a lead for finding new enzyme inhibitors, as it is the most active LAP inhibitor among the compounds containing phosphorus atom in the structure.[6] Unlike the boronic analogue of leucine and leucinal, which are also potent inhibitors of serine proteases,[11] LeuP is selective toward metalloproteases.

One of the most promising methods for designing enzyme inhibitors and predicting their activity, implemented in the LUDI program, was proposed by Böhm.[12] We applied this approach in order to generate new LAP inhibitors and to analyze their interactions with the enzyme. Moreover, the activity prediction of designed inhibitors by using theoretical methods (e.g., Ludi scoring function) facilitate the selection of the most promising compounds for synthesis.

Thus, the main purpose of our work consists of generation of new leucine aminopeptidase inhibitors by modification of LeuP structure, with the focus on finding the substituents bound in S1′ enzyme pocket, analysis of the interactions of designed inhibitors with LAP binding site, and prediction their binding affinities toward leucine aminopeptidase.

METHODS

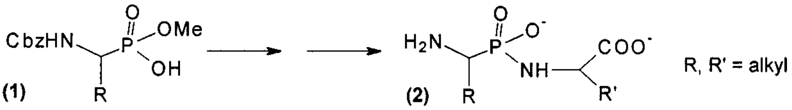
Computational Methods

The coordinates of leucine aminopeptidase and LeuP were obtained from the refined 1.65 Å X-ray structure of bovine lens leucine aminopeptidase complexed with phosphonic analogue of leucine.[9] The hydrogen atoms were added using the Insight 97.0 (MSI) program.[13]

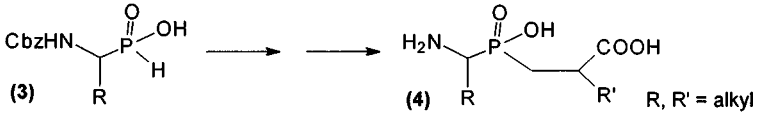
The computer program LUDI[14] was applied to design new LAP inhibitors. A systematic search of LUDI's fragment library, containing about 1000 structural fragments, was performed. The LeuP structure was fixed and Link mode of the program was used to generate new LAP inhibitors. The structures of designed inhibitors were obtained by the replacement of the hydroxyl group of LeuP by the structural fragments bound in LAP S1′ pocket, which were generated by the program.

Synthesis

The synthesis of the designed phosphonamidates started with preparation of the appropriate *N*-substituted phosphonate monoester (**1**), which was converted into its phosphonochloridate by treatment with thionyl chloride. An amino ester reacts readily with the chloridate in the presence of a base yielding the desired phosphonamidates. Removal of the blocking groups was achieved in a two-step procedure: hydrogenation-hydrolysis yielding the target compounds (**2**).



Phosphinic compounds were obtained by Michael addition of appropriate methyl acrylates to α-(N-benzyloxycarbonylamino)alkylphosphinic acids (**3**) preactivated to their trivalent silyl esters.[15] The series of acrylates were synthesized by multistep procedure with use of malonate chemistry. Final phosphinic dipeptides (**4**) were separated after standard removal of benzyloxycarbonyl and methyl terminal blocking groups.



RESULTS AND DISCUSSION

The procedures implemented in the LUDI program allowed us to generate the numerous potential inhibitors of leucine aminopeptidase. There are two groups of designed compounds: the phosphonamidate dipeptide analogues, with P—N bond (Figure 1), and phosphinate dipeptide analogues, with P—C bond.

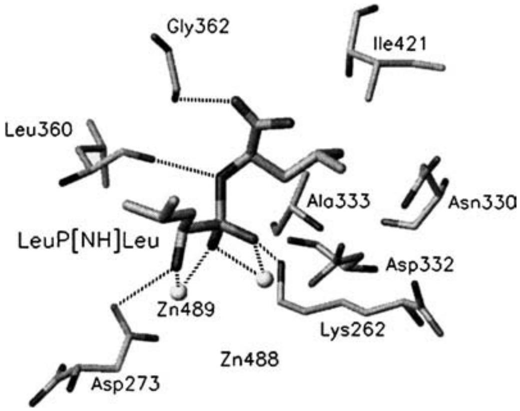


FIGURE 1 The modeled binding mode of LeuP[NH]Leu with LAP.

TABLE I Predicted by LUDI K_i values for new potential LAP inhibitors

Designed inhibitor	Predicted K_i value [nM]
LeuP	210
LeuP[NH]Leu	5
LeuP[NH]Gly	46
LeuP[CH_2]Leu	48
LeuP[CH_2]Gly	250

These compounds represent new classes of potential LAP inhibitors for which the binding affinity toward the enzyme has not been determined thus far. The novel substituents placed at the P1′ position, instead of hydroxyl group of LeuP, were designed to fulfill steric and electrostatic complementarity to LAP active site and S1′ pocket. This pocket is mainly hydrophobic in its nature, and both aryl and alkyl substituents were found as able to interact with its residues. The phosphonamidates seem to be more interesting as LAP inhibitors, as they are able to form a hydrogen bond with Leu360, which is absent for phosphinate dipeptide analogues. Both groups are able to form a hydrogen bond with Gly362, similarly to LAP dipeptide substrates and bestatin. The analogues containing side chain at P1′ position interact with residues of S1′ pocket: Ile421, Ala333, Asn330, Asp332 (Figure 1).

The binding affinities predicted by the application of Ludi empirical scoring function[14] for the designed inhibitors (Table I) suggest that the binding affinity towards LAP should be higher that for LeuP.

Thus, these compounds exhibit higher theoretical activity than known so far for LAP inhibitors containing phosphorus atom in the structure. Some of them have been synthesized (Table I), and their binding affinities towards LAP will be determined soon by *in vitro* kinetic studies.

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