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Research paper

Substituted phosphonic analogues of phenylglycine as inhibitors of phenylalanine ammonia lyase from potatoes

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ABSTRACT

A series of phosphonic acid analogues of phenylglycine variously substituted in phenyl ring have been synthesized and evaluated for their inhibitory activity towards potato L-phenylalanine ammonia lyase. Most of the compounds appeared to act as moderate (micromolar) inhibitors of the enzyme. Analysis of their binding performed using molecular modeling have shown that they might be bound either in active site of the enzyme or in the non-physiologic site. The latter one is located in adjoining deep site nearby the to the entrance channel for substrate into active site.

1. Introduction

Phenylalanine ammonia lyase (PAL) catalyzes non-oxidative transformation of L-phenylalanine into *trans*-cinnamic acid and ammonia (Scheme 1A). This reaction is a first step for the channeling of carbon from primary metabolism into phenylpropanoid secondary metabolism in plants [1–3]. Since phenylpropanoids play a vital role in development and response to environmental stimuli this enzyme have been intensively studied with respect to: its role in plants, catabolic function in fungi, its mechanism of action, and three-dimensional structure [4–7]. It was also considered as a possible target in the search for new compounds of herbicidal activity, however, phenylopropanoids are so important to plants that blocking the activity of phenylalanine ammonia lyase launches their biosynthesis by alternative pathway [8–10]. Additionally, PAL natural ability to break down L-phenylalanine makes it a reliable treatment for the genetic condition phenylketonuria, an inherited disorder that increases the levels of phenylalanine in the blood [11,12]. Finally, reversed reaction catalyzed by PAL has been successfully utilized for the synthesis of structurally diverse analogues of phenylalanine, useful building blocks in medicinal chemistry [13–15].

Enzyme inhibitors are used as tools for studying mechanisms of enzymatic catalysis and as compounds for treating certain physiologic disorders. Phosphonic analogues of amino acids are well-recognized class of inhibitors of enzymes of variable activities and thus are believed to be valuable potential dugs and pesticides [16–18]. Such analogues of aromatic amino acids also rank amongst the most interesting inhibitors of phenylalanine ammonia lyase [19–23], with a strained analogue of substrate - 2-aminoindane-2-phosphonic acid (compound **1** in Scheme 1B) being the most potent [24–26] and with analogues of phenylglycine (compound **2**, Scheme 1B) being the simplest ones with micromolar inhibitory constants (value of which depends on enzyme source) [27]. Therefore, we have synthesized a series of analogues of phenylglycine bearing fluorine atoms in phenyl ring. For comparison, a series of variously substituted phenylglycine analogues was also obtained. All the compounds (see Table 1) were evaluated for their potency to inhibit activity of phenylalanine ammonia lyase isolated from potatoes.

Fluorination has become recently an increasingly popular strategy in medicinal chemistry and protein biochemistry. Replacement of phenylalanine, tyrosine or tryptophan by their fluorinated analogues has been recently described as a mean to study amino acid interactions within the protein fold [28,40], whereas the judicious introduction of fluorine into an inhibitor molecule can productively influence conformation, pK_a, intrinsic

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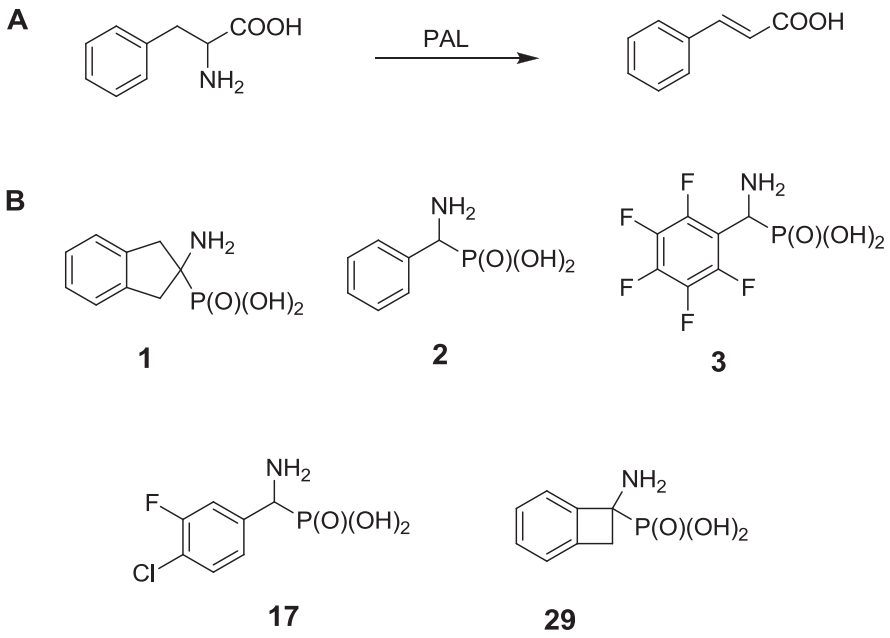

Scheme 1. Reaction catalyzed by L-phenylalanine ammonia lyase (A) and structures of the chosen inhibitors of the enzyme (B).

potency, membrane permeability, metabolic pathways, and pharmacokinetic properties of new drug candidate [29–33]. Although a C-F bond (1.34 Å for an sp^3 carbon) is about 20% longer than a C-H bond (1.09 Å), fluorination has been shown as being well tolerated by a variety of proteins without introducing much steric perturbation to the parent structure.

2. Results and discussion

2.1. Synthesis

Synthesis of the series of racemic ring-substituted 1-aminobenzylphosphonates, analogues of phenylglycine, was performed by applying three-component amidoalkylation reaction designed by Oleksyszyn and Soroka [34–36]. This procedure is simple and straightforward and provided the desired compounds in satisfactory yields.

2.2. Inhibitory activity

Inhibitory potency of the obtained compounds was evaluated using phenylalanine ammonia lyase isolated from potatoes (*Solanum tuberosum* L.) and compared with literature data for buckwheat (*Fagopyrum esculentum*) enzyme [27]. Results presented in Table 1 indicate that, with exception of compounds **3**, **15**, **25** and **26**, which are inactive, all analogues of phenylglycine appeared to be weak or moderate inhibitors of PAL. Generally, analogues of phenylglycine exhibit inhibitory activity towards buckwheat enzyme higher than towards potato PAL. They are also equipotent with or even more active than aminobenzylphosphonic acid **2**, a formal analogue of phenylglycine, against parsley (*Petroselinum crispum*) enzyme [21,23]. Additionally, the most active compounds: **5**, **6**, **17**, **18** and **20** were significantly less active than cyclic derivative **29** (Scheme 1B) towards buckwheat enzyme [22].

It is worth to note that total replacement of hydrogen atoms in phenylglycine analogue **2** leading to perfluorinated compound **3** (Scheme 1B) resulted in total abolishment of inhibitory activity. When analyzing single substitution of phenyl ring it is seen that substitution of parent compound **2** in *para*-position if of choice with bromine, chlorine and methyl group being the most suitable (compounds **5**, **6** and **20**). This suggests that steric effects are playing the most important role here. Analogues substituted with nitrile moiety appeared to be completely inactive showing that there is a certain limit of *para*-substitution.

Moreover, the introduction of fluorine as an additional substituent in position 3 usually resulted in enhanced inhibitory activity (see compounds **16** and **17**), an effect, which is in opposition to introduction of this atom in position 2 of aromatic ring (compounds **14** and **21**). Summing up, introduction of one fluorine atom into phenyl ring of phosphonic analogues did not affect significantly activity of these compounds against PAL.

Thus, despite of descriptive analysis, there is no possibility to build-up the simple structure-activity relationship for phosphonic analogues of phenylalanine also because modeling studies (see next paragraph) indicate that they might be bound in two neighboring binding sites of the enzyme – either in the active site (subunit A) or in site, which has no physiological meaning located nearby to the entrance channel of substrate phenylalanine (subunit B).

2.3. Docking of most potent inhibitors to Petroselinum crispum PAL

Docking studies might contribute to understanding of mode of binding of phenylglycine analogues by the enzyme and to identification of amino acids vital for this process. Quite surprisingly, docking studies on parsley enzyme indicated that analogues of phenylalanine could be bound in two subunits, namely in the active site (subunit A marked as green in Fig. 1) and, unexpectedly, in adjoining deep site located in subunit B (marked in red in Fig. 1). The second site is located nearby the to the channel, which forms an entrance for substrate into active site in unit A. Modeling predicts binding of compounds: **5, 6** and **10** (only *S* isomers), and both isomers of compounds: **7**, **11**, **20** and **22** in this site, whereas remaining ones are bound, as expected, in subunit A. It is worth to mention, that in the case of aminophosphonic acids *R*-isomers are mimicking *S*-amino acids. From modeling it is clearly seen that Arg354 plays a major role for binding in site A forming strong electrostatic interactions with phosphonic moiety, while in site B similar role play Lys 336 and Lys345.