

Amino Acid 1,2,4-Triazole Mimetics as Building Blocks of Peptides [†]

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Abstract

Therapeutic peptides are a unique drug class due to their high specificity binding with biological targets. However, low bioavailability of peptides, as well as the lack of enzymatic stability, imposes a number of limitations on their biomedical application. A good strategy to overcome the limitations is the use of peptidomimetics, which are able to imitate the binding and activity of peptides in vitro and in vivo. Peptidomimetics can be obtained by combining natural and synthetic amino acids in a peptide sequence. Various five-membered heterocycles are often used as structural fragments of peptide imitators in order to fix the chain in a certain conformation and increase proteolytic stability. The use of 5-aminomethyl-1,2,4-triazole-3-carboxylic acid derivatives as building blocks of peptidomimetic structures may be a very attractive strategy, in which the tautomeric 1,2,4-triazole fragment is capable of flexible forming of hydrogen bonds on protein surface of the target. At proposed work a number of ethyl 5-aminomethyl-1,2,4-triazole-3-carboxylates and their derivatives as mimetics of aliphatic amino acids were synthesized. Their use as building blocks for synthesizing of peptidomimetics was demonstrated. In addition, by use of a panel of pathogenic and model strains of microorganisms and fungi it was not shown any independent activity of the amino acid 1,2,4-triazole mimetics synthesized. This similarity of biological properties of the obtained mimetics and their natural analogues reveals in favor of their bioisosterism. Bioisosterism and geometric similarity of 1,2,4-triazole mimetics and natural amino acid encourages their further use as building blocks of therapeutic peptides.

Keywords: 1,2,4-triazole peptidomimetics; synthetic amino acid building blocks; 5-aminomethyl-1,2,4-triazole-3-carboxylic acid derivatives

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1. Introduction

Synthetic peptides are promising candidates for both diagnostic and therapeutic purposes in clinical and scientific research [1]. Therapeutic peptides are in demand in the treatment of infectious diseases, genetic disorders, oncology and in many other clinical

domains [2,3]. A number of advantages of peptide drugs, such as developed design strategies, the possibility of automated synthesis and proven modification protocols make them an excellent alternative to small molecule drugs [4]. At present the widespread use of native peptides is limited by their relatively high pyrogenicity, as well as extremely low stability in vivo due to rapid degradation by endogenous proteases and poor bioavailability [5,6]. Resistance to proteases depends on a wide range of factors, such as amino acid composition, secondary structure, flexibility and lipophilicity of the peptide molecule [1]. The use of natural peptides imitators—peptidomimetics—as therapeutic agents can help to circumvent the above problems. However, many modifications of the peptide structure cannot improve simultaneously both its proteolytic stability and biological activity. For example, the incorporation of D-amino acid usually increases the half-life of the peptide in plasma, but the structures modified in this way sometimes do not exhibit effective biological activity [7,8].

A common approach to obtaining peptidomimetics is the modification of peptide building blocks or peptide bonds with heterocyclic fragments, in particular triazole moiety [9]. In this case, 1,2,3-triazole acts as a stable isostere for metabolically labile amide bonds in the peptide backbone, making it possible to solve the problem of enzymatic instability of peptides [10,11]. Most likely, the popularity of such replacement is caused by the possibility of using click chemistry methods for 1,2,3-triazole peptidomimetics synthesis [12]. 1,2,4-Triazole is also in demand in the synthesis of peptidomimetics, however, not as an amide bond imitator, but as a component of peptide chain building blocks [13]. The inclusion of a 1,2,4-triazole moiety in the synthetic amino acid block of a peptidomimetic may lead to further advantages: the heterocycle can formation of additional hydrogen bonds, and due to the inherent tautomerism of 1,2,4-triazole, it has the ability to adapt to the amino acid environment of the active site of the target.

Previously, 5-aminomethyl 1,2,4-triazole-3-carboxylic acid was proposed as a building block for peptidomimetics [14]. However, the incorporation of this unit as a C-terminal fragment caused significant difficulties due to the instability of this acid [15]. Using the ethyl ester of 5-aminomethyl 1,2,4-triazole-3-carboxylic acid as a model, we wanted to develop a synthesis in solution of peptidomimetics with terminal 1,2,4-triazole-containing amino acid blocks.

2. Results and Discussion

We choose ethyl 5-aminomethyl-1,2,4-triazole-3-carboxylates as non-canonical amino acid blocks based on 1,2,4-triazole for peptidomimetics synthesis. Previously, several representatives of this structural class have already been obtained as monomer units for the synthesis of peptide mimetics [14,16]. Despite the attractiveness of 5-aminomethyl-1,2,4-triazole-3-carboxylic acids using as monomer units for peptides synthesis, their individual biological activity has not been studied yet. We expanded the synthetic series of 5-aminomethyl-1,2,4-triazole-3-carboxylic acid derivatives obtained from various α -amino acids and showed the absence of their individual antimicrobial activity on a panel of pathogenic strains of gram-positive and gram-negative microorganisms and fungi: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, as well as model strains of microorganisms: *Micrococcus luteus* and *Mycobacterium smegmatis*. The antimicrobial activity was assessed by measuring the zone of inhibition of microorganism growth (agar diffusion method) in comparison with known antibiotics: isoniazid, ciprofloxacin and amphotericin. For all compounds at a concentration of 50 mM, the absence of microorganism growth inhibition zones was observed. The lack of biological activity may be an additional advantage for these structures, since these derivatives are also not expected to have antimicrobial properties similar to natural amino acids.

In this work, the possibility of synthesis of peptidomimetic with terminal 1,2,4-triazole-containing synthetic units was demonstrated on example of ethyl 5-aminomethyl-1,2,4-triazole-3-carboxylate (Figure 1).

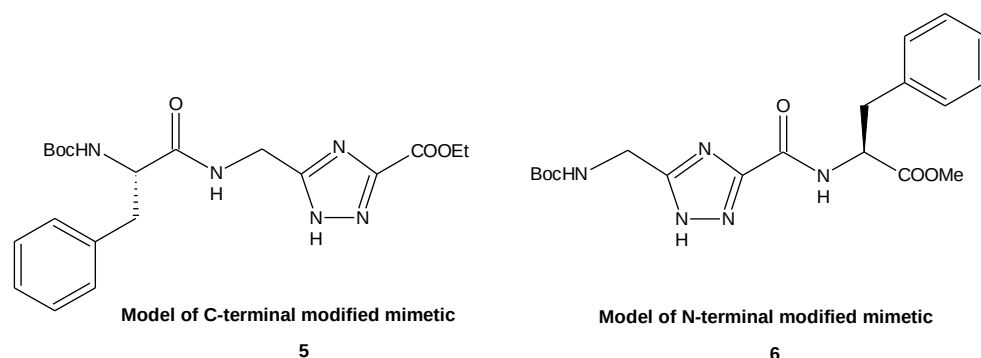
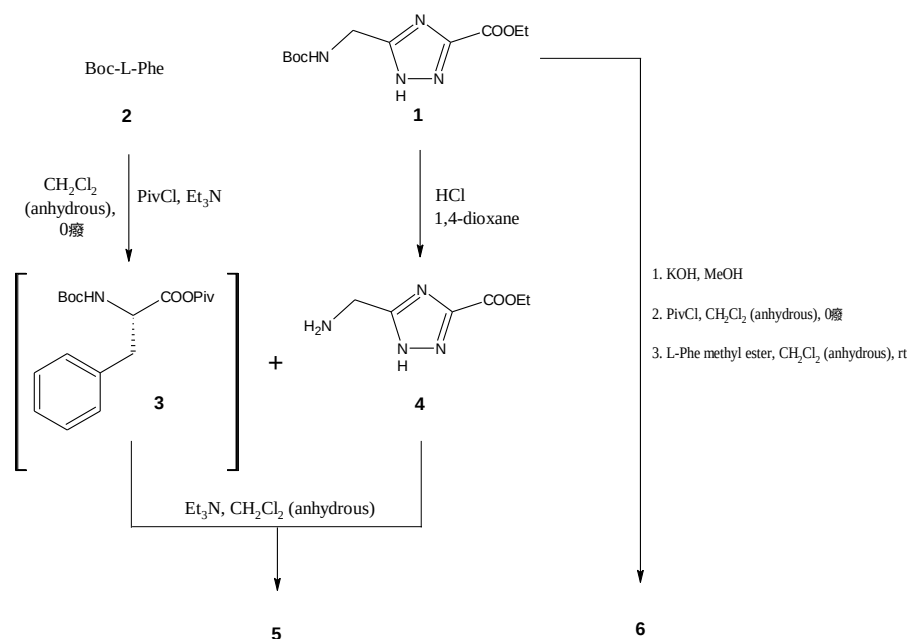


Figure 1. Model peptidomimetic structures with terminal 1,2,4-triazole-containing synthetic units.

The most common method in the literature for 1,2,4-triazole ring synthesis is thermal cyclization of β -*N*-acyloxalamidrazones [14–16]. Based on this method, we previously obtained ethyl 5-(tert-butoxycarbonyl)-aminomethyl-1,2,4-triazole-3-carboxylate **1** [17]. In order to demonstrate the possibility of ethyl 5-aminomethyl-1,2,4-triazole-3-carboxylate to act as a building block of a peptide mimetic, we synthesized two variants of the peptide mimetic structure **5** and **6** (Scheme 1).



Scheme 1. Scheme of 1,2,4-triazole-containing peptide mimetics synthesis.

The incorporation of a 1,2,4-triazole-containing amino acid mimetic at the N-terminus of the peptide was demonstrated using the example of the compound **5** synthesis. To achieve **5**, Boc-L-phenylalanine **2** was treated with pivaloyl chloride in the presence of triethylamine to obtain mixed anhydride **3**. Previously deprotection of **1** by standard methods gives dihydrochloride of ethyl 5-aminomethyl-1,2,4-triazole-3-carboxylate **4** which was acylated by intermediate **3**. Thus, model N-terminus peptidomimetic **5** modified with a 1,2,4-triazole-containing amino acid was obtained with a total yield of 59%.

The difficulty of model mimetic **6** obtaining is due to the tendency of 1,2,4-triazole-3-carboxylic acid to decarboxylation. Therefore, the ester group of the compound **1** was hydrolyzed to the potassium salt of acid and then without isolation it was activated with PivCl. L-phenylalanine methyl ester was then added to the resulting activated derivative. This resulted in model mimetic **6**, modified at the C-terminus, with a yield of 17%.

The low yield of our first attempts to use PivCl for activation C- components may be due to a non-optimized protocol of 1,2,4-triazole-containing peptide mimetics synthesis and isolation. Further we will try to adjust the reaction time, reagents excess and other conditions, or also to vary the isolation protocol.

3. Conclusions

5-Aminomethyl-1,2,4-triazole-3-carboxylic acid derivatives are structural amino acid mimetics useful as building blocks that can replace amino acid residues in modified peptides. 5-Aminomethyl-1,2,4-triazole-3-carboxylic acid derivatives have been shown to lack antimicrobial activity against a panel of model pathogenic and nonpathogenic microorganisms and fungi, enabling their use as building blocks for peptidomimetics of antimicrobial peptides. However, the use of 1,2,4-triazole-3-carboxylic acid derivatives to replace amino acids in peptides has significant limitations due to the chemical properties of 1,2,4-triazole-3-carboxylic acid. In particular, the use of free 1,2,4-triazole-3-carboxylic acid as the C-component is impossible due to its decarboxylation. We demonstrated the feasibility of introducing the 5-aminomethyl-1,2,4-triazole-3-carboxylic acid ester into the model peptide chain as both a C- and N-component. The yields of the resulting model mimetics **5** and **6** have not yet been optimized, and their preparation requires the selection of an optimal method for 1,2,4-triazole component activation. After optimizing the step of introducing 5-aminomethyl-1,2,4-triazole-3-carboxylic acid as the C-component into the peptide chain, we plan to move on to the design of 1,2,4-triazole peptidomimetics and study their biological potential.

4. Materials and Methods

4.1. Materials

All the chemical reagents were obtained from commercial suppliers (Acros organics, USA and Macklin, China) and used without further purification. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). PTX-AF-A-UV silica gel plates (Sorbfil, Russia) were used for thin-layer chromatography. The substances visualization on thin-layer chromatograms were carried out using UV irradiation at 254 nm and iodine followed by phosphomolybdic acid or ninhydrin. Column chromatography was carried out on silica gel Kieselgel 60 0.040–0.063 mm (Merck, Germany).

¹H and ¹³C NMR spectra were recorded on a Bruker DPX-300 instrument (300 and 75 MHz, respectively). High-resolution mass spectra (HRMS) were recorded on the Agilent 6224 using electron spray ionization (ESI). HPLC measurements were carried out on the Agilent 1200 Series. High-resolution mass spectra (HRMS) were recorded on the Agilent 6224 instrument using the electron spray ionization (ESI) method.

4.2. Synthesis

Ethyl 5-aminomethyl-1,2,4-triazole-3-carboxylate dihydrochloride (**4**)

5 mL of HCl in anhydrous 1,4-dioxane (4.52 M) were added to 316 mg (1.17 mmol) of compound **1**. The reaction mixture was stirred at room temperature. The reaction was monitored by complete conversion of compound **1** (TLC in a system of 3% MeOH in

CHCl₃). After conversion of compound **1**, the volatile components was evaporated. Yield: 283 mg (99%), as hygroscopic solid.

¹H NMR (DMSO-d₆) 1.25 (t, 3H, J = 7.31, -CH₃); 3.51 (s, 2H, N-CH₂-); 4.29 (q, 2H, J = 7.31, -CH₂-CH₃); 8.83 (s, 3H, NH₃⁺). The ¹H NMR spectra are similar to the data obtained in the literature [14].

¹³C NMR (DMSO-d₆) 14.2; 34.7; 61.6; 151.6; 154.3; 158.7.

HRMS (ESI), m/z: 171.0885 [M + H]⁺ (calc. for C₆H₁₁N₄O₂, m/z: 171.0882), m/z: 169.0728 [M - H]⁻ (calc. for C₆H₁₁N₄O₂, m/z: 169.0725).

Ethyl 5-(N-Boc-phenylalanyl)-aminomethyl-1,2,4-triazole-3-carboxylate (**5**)

To a suspension of Boc-L-phenylalanine 310 mg (1.17 mmol) in 3 mL of anhydrous methylene chloride a triethylamine 0.57 mL and then 0.14 mL (1.17 mmol) of pivaloyl chloride at 0 °C were added. The reaction mixture was stirred for 20 min. Then a suspension of 283 mg of compound **2** (1.17 mmol) in 3 mL of anhydrous methylene chloride added to the mixture. The reaction mixture was stirred at room temperature for 2 h. After complete conversion of compound **2** (TLC monitoring, 30% CH₃OH in CHCl₃ + 0.5% NH₃ in CH₃OH system), the volatile components were evaporated. Then 10 mL of H₂O were added to the residue and the resulting suspension was extracted with methylene chloride (2 × 20 mL). The organic phases were combined, dried over anhydrous Na₂SO₄, filtered off and evaporated. The product was isolated by column chromatography on silica gel, eluent 0.5%NH₃ in CH₃OH-CHCl₃. Yield 488 mg (59%).

¹H NMR (DMSO-d₆) δ: 1.05–1.31 (m, 12H, -O-CH₂-CH₃ and -C(-CH₃)); 2.65–3.03 (m, 2H, -CH₂-C₆H₅); 4.13–4.25 (m, 1H, -NH-CH-C(O)); 4.31 (q, 2H, J = 7.04 Hz, -O-CH₂-CH₃); 4.41 (d, 2H, J = 5.20 Hz, -NH-CH₂-Tr); 6.91 (d, 1H, J = 8.21 Hz, -NH-CH-); 7.11–7.33 (m, 5H, -C₆H₅); 8.55–8.74 (m, 1H, -NH-CH₂-).

¹³C NMR (DMSO-d₆) δ: 14.1; 28.1; 34.9; 37.4; 55.6; 61.0; 78.1; 126.1; 128.0; 129.2; 138.1; 152.9; 155.3; 156.4; 159.4; 172.0.

HRMS (ESI), m/z: 418,2022 [M + H]⁺ (calc. for C₂₀H₂₇N₅O₅, m/z: 418,2020), m/z: 416.1868 [M - H]⁻ (calc. for C₂₀H₂₇N₅O₅, m/z: 416.1865).

N-(5-(N-Boc)-aminomethyl-1,2,4-triazole-3-carboxyl)-phenylalanine methyl ester (**6**)

To 100 mg (0.37 mmol) of compound **1** 0.51 mL (0.78 mmol) of an alcoholic KOH solution was added and stirred at 40 °C. After complete conversion of compound **1** (control by TLC, 3% CH₃OH in CHCl₃ system), the volatile components were evaporated. To a suspension of the result residue in 1 mL of anhydrous methylene chloride pivaloyl chloride 0.13 mL (1.11 mmol) was added at 0 °C and stirred for 40 min. Then a suspension of 198 mg (1.11 mmol) of phenylalanine methyl ester in 2 mL of anhydrous methylene chloride was added to the resulting mixture, and the reaction mixture was stirred at room temperature for 12 h. Then the volatile components of the reaction were evaporated and 2 mL H₂O was added to the residue and extracted with methylene chloride (3 × 4 mL). The organic phases were combined, dried over anhydrous Na₂SO₄, filtered off and evaporated. The reaction product was isolated by column chromatography on silica gel, eluent chloroform-methanol with a methanol gradient from 0 to 5%. Yield 25 mg (17%).

¹H NMR (DMSO-d₆) δ: 1.35 (s, 9H, -C(-CH₃)); 3.13–3.17 (m, 2H, -CH₂-C₆H₅); 3.75 (s, 3H, -CH₃); 4.54 (br.s, 2H, -CH₂-Tr); 4.97–5.04 (m, 1H, -NH-CH-); 6.91 (d, 1H, J = 8.21 Hz, -NH-CH-); 7.21–7.35 (m, 5H, -C₆H₅); 8.63–8.76 (m, 1H, -NH-CH₂-).

¹³C NMR (DMSO-d₆) δ: 28.1; 37.6; 39.7; 50.4; 53.4; 80.5; 126.1; 128.9; 129.2; 139.0; 146.1; 152.9; 156.4; 159.8; 172.1.

HRMS (ESI), m/z: 404,1858 [M + H]⁺ (calc. for C₁₉H₂₅N₅O₅, m/z: 404,1858), m/z: 402,1703 [M - H]⁻ (calc. for C₁₉H₂₅N₅O₅, m/z: 402,1703).

4.3. Antibacterial Activity

Antimicrobial activity was determined with standard agar wells method by measuring the diameter of the inhibition zones [18]. The cultures grown at 35 °C on the following media: Mueller-Hinton agar (*Staphylococcus aureus* INA 00985, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853), Sabouraud agar (*Candida albicans* ATCC 14053) for 24 h before assay setting. For *Mycobacterium smegmatis* ATCC 607, a 48-h culture grown on peptone agar (OOO Fizlabpribor, Moscow, Russia) at 37 °C was used. Preparation of inoculum: the cells of the bacterial were suspended in sterile saline to a turbidity of 0.5 McFarland by shaking on a vortex mixer by 10–15 s and applied to petri dishes with Mueller-Hinton agar (Mueller-Hinton agar with 2% glucose for *Candida albicans*). Dishes were incubated at 35 °C. Growth inhibition zones size were measured after 24 h of incubation.

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Conflicts of Interest:

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