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Proceeding Paper

New 1,2,4-Triazole Potential Inhibitors of Mycobacterial Imidazoleglycerol-Phosphate Dehydratase (IGPD) †

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Abstract: A number of new 5-aminomethyl-1,2,4-triazole-3-carboxamides, demonstrating high model affinity to the IGPD catalytic site comparable with that of native substrate, were synthesized. The derivatives obtained were tested for antimicrobial potential against the *M. smegmatis*, a related species of *M. tuberculosis*. During the initial antimicrobial activity evaluation in vitro some of compounds investigated inhibited microbial growth. Moreover, high binding energy values calculated for compounds/enzyme's catalytic site complexes correlate with the compounds' antimicrobial efficacy. This suggest that the probable biological action mechanism of new 5-aminomethyl-1,2,4-triazole-3-carboxamides may be based on binding to the catalytic site of IGPD, which provides prerequisites for further study of this class' compounds as potential IGPD inhibitors.

Keywords: 5-aminomethyl-1,2,4-triazole-3-carboxamides; imidazoleglycerol-phosphate dehydratase; IGPD inhibitors; anti-tuberculosis agents

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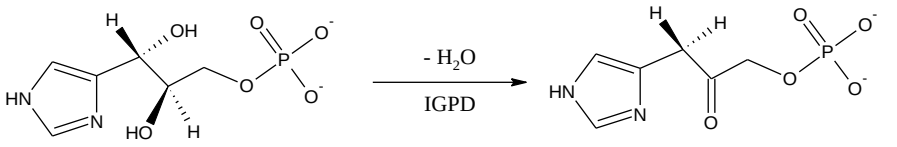
1. Introduction

Mycobacterium tuberculosis, especially its multi-resistant strains, poses a serious threat to human health, and therefore, the development of new anti-tuberculosis agents is an important direction in the search of new biologically active compounds. A number of macromolecular targets are known for *M. tuberculosis* [1–3], among which the enzyme catalyzing the sixth reaction of histidine bacterial biosynthesis–imidazole-glycerophosphate dehydratase (IGPD)—deserves special attention (Figure 1). This enzyme has no orthologs in the human body, and for that reason it can be considered as a selective target of anti-tuberculosis therapy [1,4].

1,2,4-Triazole derivatives show biological effect against various bacterial pathogens in in vitro and in vivo experiments. 1,2,4-Triazole is a privileged fragment of antibacterial drugs, including those demonstrating activity against the causative agent of tuberculosis [5–7]. Previously, it was shown that IGPD is inhibited in vitro by compounds containing a 1,2,4-triazole fragment [8,9]. According to the authors, 1,2,4-triazole is able to mimic the imidazole heterocycle of the IGPD substrate [10,11].

A number of new 5-aminomethyl-1,2,4-triazole-3-carboxamides, demonstrating high model affinity to the IGPD catalytic site comparable with that of the native substrate, were synthesized. At the same time, the derivatives discussed demonstrate a slightly different location within the catalytic site of the enzyme, therefore, the biological action study of such derivatives became the subject of our interest.



Imidazole glycerophosphate 3-(imidazol-4-yl)-2-oxopropyl phosphate

Figure 1. Sixth reaction of histidine bacterial biosynthesis, catalyzed by IGPD.

2. Results and Discussion

2.1. Design and In Silico Studies

To develop the design of potential inhibitors IGPD, we largely relied on the IGPD catalytic site structure, as well as on literature data describing known 1,2,4-triazole IGPD inhibitors. According to the literature, the structure of the enzyme's active center contains a number of key points important for substrate binding, as well as for binding of potential protein inhibitors. It was found that the Arg121 residue, being the part of the conservative loop formed by residues 118–121, has two different spatial positions that continuously replace each other in the free enzyme. When the substrate enters the binding pocket, Arg121 stabilizes in a conformation that covers the binding area [8]. It is also assumed that Arg99 and Lys184 can be involved in the substrate stabilization, namely in interaction with the phosphate group [10]. Based on the study of the tertiary IGPD A. thaliana structure, it is believed that Glu21 residue is conservative for *A. thaliana* and *M. tuberculosis* [13] and plays an important role in catalysis by participating in proton transfer [12]. Thus, Arg121, Arg99, Lys184 and Glu21 are recognized as important functional elements of IGPD *M. tuberculosis* active center (Figure 2).

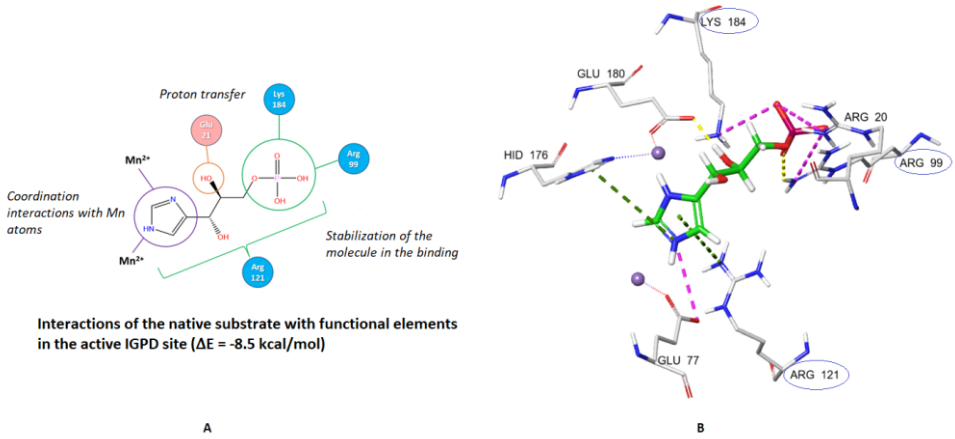


Figure 2. Models of native substrate in the IGPD enzyme catalytic center. (**A**)—image was created based on data from literary sources [8,10,12,13]. (**B**)—calculated binding of the native substrate.

We carried out a molecular docking for a number of 5-aminomethyl-1,2,4-triazole-3-carboxamides containing various amino acids radicals in fifth position of the 1,2,4-triazole ring. According to the results of the calculations, higher binding parameters were possessed by derivatives having an additional carrier of a positive charge in the structure (ones containing a residue of histidine, lysine and ornithine). The studied structures demonstrated a better affinity for the IGPD active site compared to the native substrate (−8.5 kcal/mol), while largely copying its location in the enzyme active center. Calculations also show that the corresponding compounds interact with amino acids considered to be important for catalysis and stabilization of the substrate—Arg99, Arg121, Lys184, Glu21, as well as with neighboring Glu77, Asp78, Glu180. Structures leading by binding energy value are presented on Figure 3.

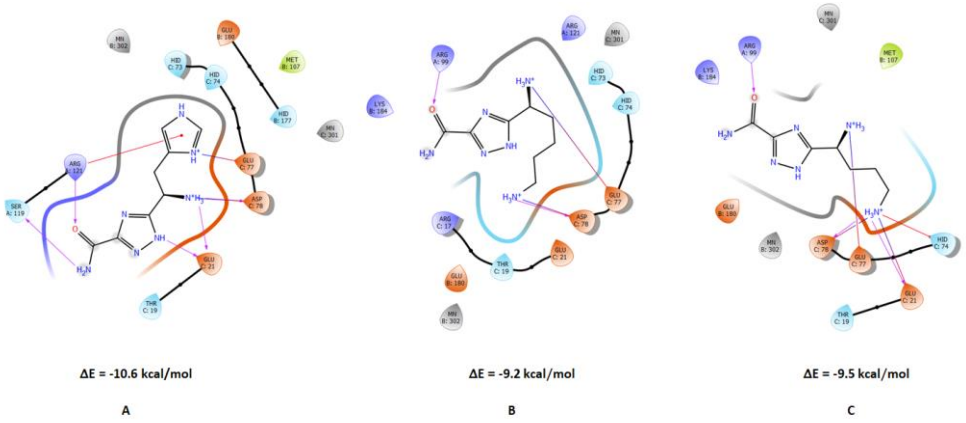


Figure 3. 5-Aminomethyl-1,2,4-triazole-3-carboxamides (**A**–**C**) in the active center of IGPD enzyme (model).

2.2. Synthetic Section

The literature describes several approaches to the synthesis of 5-substituted 1,2,4-triazole-3-carboxylates, which may be used as precursors of the 5-substituted 1,2,4-triazole-3-carboxamides. We selected a method based on the thermocyclization of N-acylated amidrazones [14], which is presented on Figure 4. for the derivatives **4a–c**.

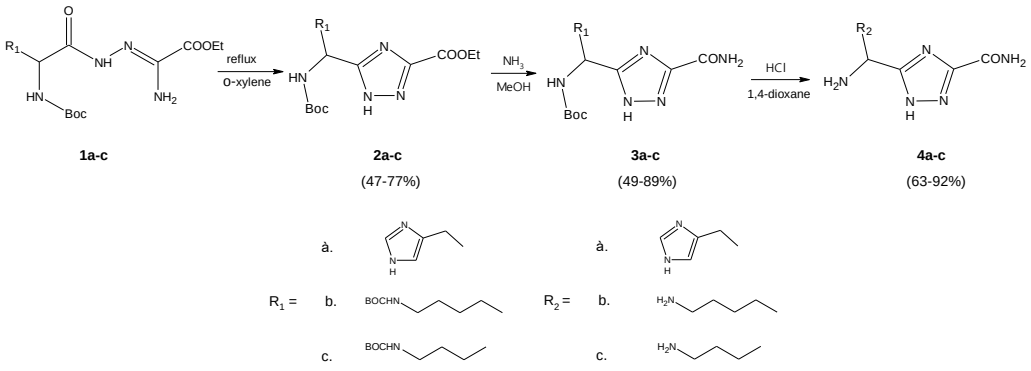


Figure 4. 5-Substituted 1,2,4-triazole-3-carboxamides derivatives **4a–c** synthesis.

Based on the methods in silico, a series design was proposed and a number 5-substituted 1,2,4-triazole-3-carboxylates **2** were obtained using a method based on thermal cyclization with yields 47–89%. New 5-substituted 1,2,4-triazole-3-carboxamides **4** were obtained by ammonolysis of **2** esters followed by removal of protective groups from the radical's amino component in the fifth position of 1,2,4-triazole.

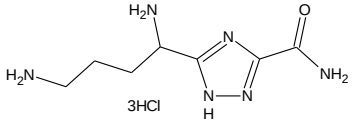
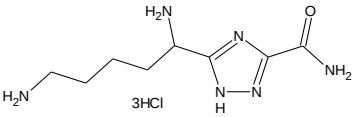
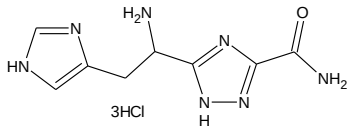
For the initial study of biological properties compounds were synthesized as a mix of enantiomers. Further investigation suggests synthesis of individual enantiomers only in case of active compounds detection.

2.3. Biology Section

The obtained derivatives were tested for their antimicrobial potential against the *M. tuberculosis*-related species *M. smegmatis*. Non-pathogenic *M. smegmatis* was used as a model microorganism due to the high conservatism of IGPD in the genus *Mycobacterium*. In addition, this species of mycobacteria is designated as a generally accepted model used in preliminary screening of compounds for anti-tuberculosis activity detection due to its high genetic similarity (more than 90%) with *M. tuberculosis* and the presence of common genes involved in stress adaptation processes [15]. According to the initial in vitro antimicrobial evaluation results, the inhibition of microorganism growth was revealed in the

presence of derivatives **4a–c** that showed higher binding energy values calculated within enzyme's active center.

Table 1. *M. smegmatis* growth inhibition by synthesized compounds.

Compounds Structures	Time, h	Inhibition Growth *M. tuberculosis*, % *
		10 mM
	24	34 ± 3
	48	50 ± 4
	24	30 ± 4
	48	36 ± 5
	24	44 ± 2
	48	74 ± 4

*—for the comparison drug isoniazid at a concentration of 30 μM—47 ± 3% (24 h) and 80 ± 4% (48 h).

3. Conclusions

A number of new biologically active heterocycles, namely derivatives of 5-aminomethyl-1,2,4-triazole-3-carboxamide, have been synthesized. The compounds were evaluated for their biological properties that may be applied to the *M. tuberculosis* related species *M. smegmatis*. Compounds 4a-c showed antibacterial activity correlating with their high affinity to the IGPD catalytic site in silico. There is reason to believe that this class of structures has potential use as potential anti-tuberculosis agents, but a strategy to increase the lipophilicity of 5-aminomethyl-1,2,4-triazole-3-carboxamide may lead to better penetration through the cell wall and thereby increase the antibacterial activity of the studied class of compounds.

4. Materials and Methods

4.1. Materials

All the chemicals were obtained from commercial sources (Merck KGaA, Darmstadt, Germany) and were used without further purification. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Silica gel 60 (Merck KGaA, Darmstadt, Germany) was used for column chromatography. Analytical TLC was performed on Sorbfil PTX-AF-A-UV silica gel plates (Russia).

^{1}H and ^{13}C 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.

4.2. Synthesis

General procedure for compounds 2a–c synthesis

Cyclization of amidrazones **1** was carried out in o-xylene (20 mL of o-xylene per 1 g of amidrazone) at the boiling point of the reaction mixture until complete conversion of amidrazone, monitored by TLC (10% methanol in chloroform). O-xylene was evaporated. The reaction product was isolated by column chromatography on silica gel in a chloroform-methanol solvent system (with a methanol gradient from 0 to 20%).

General procedure for amides 3a–c synthesis

A solution of ammonia in methanol (12 M) was added to ethyl ester **2** (1 mL for every 100 mg of ester **2**). The reaction mixture was stirred at boiling until complete conversion of the starting ester **7** (TLC control). Every two hours 0.5 mL of ammonia solution in methanol (12 M) was added. After completion of the reaction, volatile components were evaporated. The product was isolated by column chromatography on silica gel in a methanol-chloroform solvent system with a methanol gradient from 0 to 30%.

General procedure for target compounds 4a–c synthesis

HCl/1,4-dioxane (3.42 M) was added to carboxamide **3** to form a solution. The reaction mass was stirred at room temperature under anhydrous conditions. The transformation of compounds **3** was monitored by TLC. The precipitate was filtered and washed with 10 mL of anhydrous diethyl ether. The crystals were dried on a filter for 1 h.

4.3. In Silico studies

The Schrödinger Suite 2020 software (Schrödinger, Inc., USA) was used to carry out the simulation. Ligand preparation was carried out using the Schrödinger Maestro LigPrep module. Preparation of the protein structure (removal of water molecules, protonation, addition of partial charges, energy minimization, etc.) was carried out using the Schrödinger Maestro Protein Preparation Wizard module. The creation of the grid box and setting of the simulation parameters were carried out using the Schrödinger Maestro Receptor grid generation module. Molecular docking was performed using the Schrödinger Maestro Ligand docking (Glide) module in Extra precision (XP) mode. The results were evaluated and visualized in Schrödinger Maestro.

4.4. Antibacterial Activity

A 48-h culture of *M. smegmatis* grown on peptone agar medium (Fizlabpribor, Moscow, Russia) at 37 °C was aseptically suspended in PBS buffer with glass beads. The suspension, with a turbidity of 0.5 McFarland units, was added to a sterile medium up to 10%. The tested substances were dissolved in a sterile medium and sterilized by filtration through membrane filters with a pore size of 0.22 microns. Cultivation was carried out in a 96-well plate with the test substances at concentrations of 100, 10, and 1 mM. The positive control was isoniazid at 300, 30, and 3 μM, and the negative control was the culture without additives. The experiment was triplicated. Cultivation was carried out at 37 °C and 300 rpm for 48 h. Percent inhibition was determined by optical density at the wavelength 620 nm.

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Conflicts of Interest:

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