Stereochemical aspects in the synthesis of novel N-(purin-6-yl) dipeptides as potential antimycobacterial agents

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Abstract
The synthesis of purine conjugates with natural amino acids is one of the promising directions in search for novel therapeutic agents, including antimycobacterial agents. The purpose of this study was to synthesize N-(purin-6-yl)dipeptides containing the terminal fragment of (S)-glutamic acid. To obtain the target compounds, two synthetic routes were tested. The first of them is based on coupling of N-(purin-6-yl)-(S)-amino acids to dimethyl (S)-glutamate in the presence of carbodiimide coupling agent followed by the removal of ester groups. However, it turned out that this coupling process was accompanied by racemization of the chiral center of N-(purin-6-yl)-α-amino acids and in all cases led to mixtures of (S,S)- and (R,S)-diastereomers (6:4). Individual (S,S)-diastereomers were obtained using an alternative approach based on the nucleophilic substitution of chlorine in 6-chloropurine or 2-amino-6-chloropurine with corresponding dipeptides as nucleophiles. The enantiomeric purity of the target compounds was confirmed by chiral HPLC. To test the assumption that racemization of the chiral center of N-(purin-6-yl)-α-amino acids occurs with the participation of nitrogen atoms of the imidazole ring via the stage of formation of a chirally labile intermediate, we obtained such structural analogs of N-(purin-6-yl)-(S)-alanine as N-(9-benzylpurin-6-yl)-(S)-alanine and N-(7-deazapurin-6-yl)-(S)-alanine. It was found that coupling of these compounds to dimethyl (S)-glutamate was also accompanied by racemization. This indicates that the imidazole fragment does not play a crucial role in this process. When testing the antimycobacterial activity of some of the obtained compounds, conjugates with moderate activity against the laboratory Mycobacterium tuberculosis H37Rv strain (MIC 3.1–6.25 μg/mL) were identified.

Keywords Dipeptides · Racemization · Purine · Coupling · Nucleophilic substitution · Antimycobacterial activity

Introduction
The synthesis of purine conjugates with natural amino acids is one of the promising directions in search for novel efficient therapeutic agents. These compounds are known to be highly active against herpes viruses (Beauchamp et al. 1992; Krashov et al. 2019), HIV (McGuigan et al. 2005, 2006), hepatitis C (McGuigan et al. 2010; Chang et al. 2011; Al-Harbi and Abdel-Rahman 2012), and influenza viruses (Pautus et al. 2013). It was also reported about purinyl amino acids exhibiting antitumor (Ward et al. 1961), antimycobacterial (Voynikov et al. 2014; Stavrakov et. al. 2016; Gruzdev et al. 2017, 2018; Krashov et al. 2016, 2020), and other effects.

The modification of purine core at position N9 leading to the analogs of natural nucleosides has been widely used (Wang et al. 2015; Mehellou 2016). At the same time, the synthesis of C6-substituted derivatives is also an important area of the purine chemistry (Legraverend and Grierson 2006; Legraverend 2008). The most common synthetic route to purine conjugates bearing an amino acid fragment at position C6 is the nucleophilic substitution of the chlorine atom in 6-chloropurine (Ward et al. 1961; Niu et al. 2012; Boerema et al. 2016). Depending on the chosen synthetic strategy, reaction conditions and the amino acid structure, the
target compounds may be obtained in enantiomerically pure form or as a mixture of enantiomers (Vigorov et al. 2014).

The search for novel antimycobacterial agents is an urgent task. According to the World Health Organization, tuberculosis (TB) was diagnosed in 10 million people worldwide and 1.5 million people died of TB in 2018 (WHO 2019). Moreover, at present during the COVID-19 pandemic, there is an increased risk for unfavorable outcome in TB patients (Motta et al. 2020; WHO 2020). TB treatment is often complicated because of the emergence of mycobacterium strains resistant to clinically used anti-TB drugs such as isoniazid and rifampicin (Tripathi et al. 2005; de Wet et al. 2019). Design of novel antimycobacterial agents differing in the mechanism of action from known drugs may be the solution to the problem of multidrug resistance (MDR).

To identify highly effective anti-TB agents, we carried out a screening of a large group of novel purine conjugates with amino acids and short peptides. Among them, \(N\)-(purin-6-yl)glycyl-(\(S\))-glutamic acid ((\(S\))-1a, Fig. 1) and \(N\)-(2-aminopurin-6-yl)glycyl-(\(S\))-glutamic acid ((\(S\))-1b) were the most active and exhibited high antimycobacterial activity against both the standard laboratory strain (\(M.\) tuberculosis H37Rv) and the MDR-TB strain (Krasnov et al. 2016).

It has been found that the analogs of compounds (\(S\))-1a,b in which the (\(S\))-glutamic acid fragment is replaced by residues of other \(\alpha\)-amino acids, as well as the analogs in which the glycine fragment is replaced by residues of various \(\alpha\)-amino acids, do not exhibit substantial antimycobacterial activity (Musiyak et al. 2019). The purpose of this work was to obtain the analogs of compounds (\(S\))-1a,b containing fragments of natural \(\alpha\)-amino acids instead of glycine moiety. We used the following \(\alpha\)-amino acids as simple structures without any additional functional groups: (\(S\))-alanine, (\(S\))-phenylalanine (common aromatic \(\alpha\)-amino acid), and (\(S\))-valine (common branched aliphatic \(\alpha\)-amino acid). Moreover, special attention was paid to monitoring the enantiomeric purity of the target compounds, since stereo configuration is known to significantly affect the biological activity of substances (Islam et al. 1997; Melchert and List 2007; Lin et al. 2011).

**Results and discussion**

Initially, synthesis of purine conjugates containing (\(S\))-alanine (4a), (\(S\))-phenylalanine (4b) or (\(S\))-valine (4c) fragments instead of glycine residue was carried out by coupling of \(N\)-(purin-6-yl)-(\(S\))-amino acids (\(S\))-2a–c to dimethyl (\(S\))-glutamate followed by alkaline hydrolysis (Scheme 1). The starting compounds, \(N\)-(purin-6-yl)-(\(S\))-amino acids (\(S\))-2a–c, were synthesized by the nucleophilic substitution of chlorine in 6-chloropurine with the corresponding...
(S)-amino acids in aqueous Na₂CO₃ under reflux according to the described procedure (Ward et al. 1961). It should be noted that in the original work (Ward et al. 1961) there was no information about enantiomeric purity of the purinyl amino acids obtained under rather drastic conditions. So, we decided to check enantiomeric purity of compounds 2a–c by the example of phenylalanine derivatives 2b, thereby confirming the absence of racemization at the stage of nucleophilic substitution. To do this, we converted compounds (R)-2b and (S)-2b to methyl esters (R)-5 and (S)-5, respectively (Scheme 1), and then performed analysis of their enantiomeric purity (> 99% ee) by chiral HPLC. The coupling of N-(purin-6-yl)-(S)-amino acids (S)-2a–c to dimethyl (S)-glutamate was carried out in the presence of N,N′-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and N,N-diisopropylethylamine (DIEA). We were surprised to observe double sets of signals in the 1H NMR to dimethyl (S,S)-meric purity (> 99%)-philic substitution. To do this, we converted compounds (R)-2b and (S)-2b to methyl esters (R)-5 and (S)-5, respectively (Scheme 1), and then performed analysis of their enantiomeric purity (> 99% ee) by chiral HPLC. To obtain the target conjugates 4a–c in diastereomerically pure form, we used an alternative approach that was based on the nucleophilic substitution of chlorine in 6-chloropurine with dipeptides (S,S)-7a–c (Scheme 2). The protected dipeptides (S,S)-6a–c obtained according to the known procedure (Deng et al. 2008) were used as the starting compounds. The subsequent deprotection of ester protecting groups by alkaline hydrolysis followed by Boc-group cleavage via treatment with trifluoroacetic acid led to dipeptides (S,S)-7a–c, their characteristics were identical to those described in the literature (McGregor and Carpenter 1961; Gray and Khoujah 1969; Deng et al. 2008). The interaction of dipeptides (S,S)-7a–c with 6-chloropurine in aqueous Na₂CO₃ under reflux led to the products (S,S)-4a–c (Scheme 2). Diastereomeric purity of these compounds was confirmed by ¹H and ¹³C NMR spectral data.

This approach was also applied for the synthesis of 2-aminopurine derivatives, the structural analogs of compound (S)-1b. Interaction of dipeptides (S,S)-7a,b with 2-amino-6-chloropurine (ACP) in aqueous Na₂CO₃ under reflux afforded the target diastereomerically pure products (S,S)-8a,b (Scheme 2). It has been found that part of the starting ACP does not react under these conditions. In the case of the synthesis of compound (S,S)-8a, 6% of unreacted ACP was isolated; taking this into account, the yield of product (S,S)-8a was 34%. In the case of compound (S,S)-8b, significantly more ACP (39%) was isolated from the reaction mixture, and the product yield was 28%, relative to reacted ACP.

During the synthesis of conjugates (S,S)-4a,c and (S,S)-8b, we observed unexpected intramolecular cyclization of dipeptides (S,S)-7b,c with the formation of diketopiperazines (S,S)-9b,c (Scheme 2). Because of this side process, the yield of target products was decreased, and their purification was rather laborious. The structure of compounds (S,S)-9b,c was confirmed by ¹H and ¹³C NMR spectroscopy and CHN elemental microanalysis; the characteristics of (S)-phenylalanine derivative (S,S)-9b were identical to those described in the literature (Kleinsmann and Nachtsheim 2013).

Compounds (S,S)-4a–c were used for identification of the diastereomers formed via the coupling of N-(purin-6-yl)-α-amino acids (S)-2a–c to dimethyl (S)-glutamate.
The diastereomerically pure dimethyl esters \((S,S)-3a\textendash}c\) were obtained from conjugates \((S,S)-4a\textendash}c\) (Scheme 2), which made it possible to assign corresponding peaks in HPLC chromatograms of diastereomeric mixtures \(3a\textendash}c\). When comparing the HPLC and \(^1H, \(^13C\) NMR spectral data of individual diastereomers \((S,S)-3a\textendash}c\) (>99% de) with the data obtained for mixtures \(3a\textendash}c\), we found that in all cases the coupling of compounds \((S,S)-2a\textendash}c\) to dimethyl \((S)-\)glutamate (Scheme 1) resulted in predominant formation of \((S,S)\)-diastereomers; that is, products \(3a\textendash}c\) were mixtures of \((S,S)\)-and \((R,S)\)-diastereomers in a 6:4 ratio. Moreover, it has been shown that when \(-N-(\text{purin-6-yl})-(R)\)-phenylalanine \([\text{(R-2b)}]\) was used as a carboxy component, exactly the same mixture of diastereomers \([(S,S)-3b/(R,S)-3b 6:4]\) was formed as in the case of \(-N-(\text{purin-6-yl})-(S)\)-phenylalanine \((\text{S-2b})\). Thus, the configuration of chiral center of the starting \(-N-(\text{purin-6-yl})\)-\(\alpha\)-amino acid does not affect the diastereomeric composition of products. So, we suppose that \((S,S)\)- and \((R,S)\)-diastereomers are formed from the same chirally labile intermediate.

It is known that histidine (imidazole-substituted amino acid), which can be considered as a structural analog of \(-N-(\text{purin-6-yl})\)-\(\alpha\)-amino acids, is racemized while DCC treatment (Scheme 3) (Jones et al. 1980). Histidine is supposed to form the corresponding \(O\)-acylisourea while interaction with DCC. Racemization of the chiral center occurs with the participation of the imidazole \(N^\tau\) atom that acts as a proton acceptor (mechanism A) or as a nucleophile interacting with a carbonyl fragment (mechanism B). It is noted that the presence of \(N^\pi\)-protecting group is not a hindrance for this process; at the same time, in the case of \(N^\pi\)-protected histidine racemization does not occur.

We suppose that the racemization of the chiral center of \(-N-(\text{purin-6-yl})\)-\(\alpha\)-amino acids occurs with the participation of the imidazole fragment, as in the case of histidine. Therefore, we obtained \(-N-(\text{9-benzylpurin-6-yl})-(S)\)-alanine \((\text{S-11a})\) and \(-N-(\text{7-deazapurin-6-yl})-(S)\)-alanine \((\text{S-11b})\) by the interaction of 9-benzyl-6-chloropurine \((10a)\) (Kelley et al. 1988) or the commercially available 7-deaza-6-chloropurine \((10b)\) with \((S)\)-alanine in aqueous \(\text{Na}_2\text{CO}_3\) under reflux (Scheme 4).

It has been found that the coupling of \(-N-(\text{9-benzylpurin-6-yl})-(S)\)-alanine \((\text{S-11a})\) to dimethyl \((S)-\)glutamate in the presence of DCC, HOBt, and DIEA is accompanied by racemization and leads to the product \(12a\) that is a mixture of diastereomers in a 6:4 ratio (Scheme 4), as in the case of \(N^\pi\)-unsubstituted analogs (Scheme 1). In the case of \(-N-(\text{7-deazapurin-6-yl})-(S)\)-alanine \((\text{S-11b})\), racemization also occurred, despite the absence of the \(N^\pi\) atom (analog of \(N^\pi\) atom in histidine) in the purine fragment, resulting in product \(12b\) as a 6:4 mixture of diastereomers. The alkaline hydrolysis of compound \(12b\) led to the product \(13b\) as a 7:3 mixture of diastereomers, according to the \(^1H\) NMR data (Scheme 4). The results obtained indicate that the imidazole nitrogen atoms of \(-N-(\text{purin-6-yl})\)-\(\alpha\)-amino acids either are not involved in the racemization process during the coupling reaction in the presence of DCC at all, or do not play a key role in this process. In general, the question of the mechanism of \(-N-(\text{purin-6-yl})\)-\(\alpha\)-amino acid racemization requires further study.

To identify diastereomers in mixtures \(12a\) and \(12b\), diastereomerically pure conjugates \((S,S)-13a,b\) were synthesized by the nucleophilic substitution of chlorine in 9-benzyl-6-chloropurine \((10a)\) or in 7-deaza-6-chloropurine \((10b)\) with dipeptide \((S,S)-7a\) (Scheme 5). It should be noted that the conversion of compound \(10b\) was only about 50%; so, the yield of the product \((S,S)-13b\) was 42% relative to reacted \(10b\). To find more efficient synthetic approach to \((S,S)-13b\), we varied the reaction conditions. Unfortunately, we did not observe the product formation neither in aqueous \(\text{HCl}\) (Liu and Robins 2007), nor while using \(\text{DMF}\) as a solvent (Terrier 2013), nor without any solvent (Novosjolova et al. 2015).

Diastereomerically pure compounds \((S,S)-13a,b\) were converted to dimethyl esters \((S,S)-12a,b\) (Scheme 5). This made it possible to assign corresponding peaks in HPLC chromatograms of diastereomeric mixtures \(12a,b\). When comparing the HPLC data (Fig. 2) and \(^1H, \(^13C\) NMR spectra of individual diastereomers \((S,S)-12a,b\) (>99% de) with

![Scheme 3 Proposed mechanisms of the histidine racemization (Jones et al. 1980)](image-url)
Stereochemical aspects in the synthesis of novel N-(purin-6-yl)dipeptides as potential...
Conclusion

In summary, we developed the synthetic approaches to novel purine and 2-aminopurine conjugates with dipeptides bearing the (S)-glutamic acid terminal fragment, and methods for monitoring their enantiomeric and diastereomeric composition. It has been shown that approach based on the nucleophilic substitution of chlorine in 6-chloropurine and 2-amino-6-chloropurine with dipeptides is racemization-free and leads to the diastereomerically pure target products. It has been found that another approach based on the coupling of N-(purin-6-yl)-(S)-amino acids to dimethyl (S)-glutamate is accompanied by racemization of the carboxy component chiral center and leads to the mixtures of (S,S)- и (R,S)-diastereomers in a 6:4 ratio.

To verify the hypothesis that the racemization of the chiral center of N-(purin-6-yl)-α-amino acids proceeds with the participation of the imidazole nitrogen atoms via the stage of formation of a chirally labile intermediate, we synthesized N-(purin-6-yl)-(S)-alanine analogs differing in the structure of the imidazole fragment: N-(9-benzyl-purin-6-yl)-(S)-alanine and N-(7-deazapurin-6-yl)-(S)-alanine. Since the coupling of these compounds to dimethyl (S)-glutamate was also accompanied by racemization; it can be assumed that the imidazole fragment does not play a crucial role in the course of this process.

Some of the obtained compounds were found to exhibit moderate activity against M. tuberculosis H37Rv.

Materials and methods

General

N-(Purin-6-yl)-(S)-alanine (S)-2a (Ward et al. 1961), dimethyl N-Boc-(S)-phenylalaninyl-(S)-glutamate (S,S)-6b (Deng et al. 2008) and 9-benzyl-6-chloropurine 10a (Kelley et al. 1988) were obtained as previously described. N-(Purin-6-yl)-α-amino acids (S)-2b,c, (R)-2b (Ward et al. 1961), dimethyl N-Boc-(S)-alaninyl-(S)-glutamate (S,S)-6a, and dimethyl N-Boc-(S)-valyl-(S)-glutamate (S,S)-6c (Deng et al. 2008) were synthesized by analogy with the known methods. Other reagents are commercially available and were purchased from Alfa Aesar (UK) and Sigma-Aldrich (Germany).

The solvents were purified according to traditional methods and used freshly distilled. Melting points were obtained on a SMP3 apparatus (Barloworld Scientific, UK). Optical rotations were measured on a Perkin Elmer 341 polarimeter (Perkin Elmer, USA). The 1H NMR spectra were recorded on Bruker DRX-400 (400 MHz) or Bruker Avance 500 (500 MHz) (Bruker, Germany); the 13C NMR spectra were recorded on Bruker Avance 500 (125 MHz); TMS was used as internal reference. CHN elemental micro-analyses were performed using Perkin Elmer 2400 II analyser (Perkin Elmer, USA). Analytical TLC was performed using Sorbil plates (Imid, Russia). Flash-column chromatography procedures were performed using Silica gel 40 (230–400 mesh) (Alfa Aesar, UK). For ion exchange resin
purification, we used Amberlite IR-120(H) resin (Alfa Aesar, UK). Analytical chiral HPLC of compounds 3a, (S,S)-3a, 12b, (S,S)-12b was performed on a Agilent 1100 instrument (Agilent Technologies, USA) using a S,S-Whelk-O1 column (250 × 4.6 mm, 5 μm) (Phenomenex, USA); analytical chiral HPLC of compounds 3b, (S,S)-3b, 3c, (S,S)-3c, (R)-5, (S)-5, 12a, (S,S)-12a was performed on Knauer Smartline-1100 instrument (Knauer, Germany) using the following columns: Chiralsel OD-H (250 × 4.6 mm, 5 μm) (Daicel, Japan) for compounds 3b, (S,S)-3b, 12, (S,S)-12; Chiralpak AD (250 × 4.6 mm, 5 μm) (Daicel, Japan) for compounds 3c, (S,S)-3c, or S,S-Whelk-O1 (250 × 4.6 mm, 5 μm) (Phenomenex, USA) for compounds (R)-5, (S)-5. Preparative HPLC of compounds (S,S)-4b, (S,S)-4c, (S,S)-8b, (S,S)-9b was performed on a Shimadzu Prominence LC-20 instrument (Shimadzu, Japan) using a Phenomenex Luna C18(2) column (250 × 21.2 mm, 5 μm) (Phenomenex, USA). The high-resolution mass spectra were obtained on a Bruker maXis Impact HD mass spectrometer (Bruker, Germany), electrospray ionization with direct sample inlet (4 dm³/min flow rate).

**General procedure for the coupling reactions (synthesis of compounds 3a–c, 12a,b)**

DIEA (345 μL, 1.98 mmol), HOBt (0.27 g, 1.98 mmol), and DCC (0.41 g, 1.98 mmol) were successively added to a suspension of compound (S)-2a–c, (R)-2b, or (S)-11a,b (1.98 mmol) in DMSO or DMF (10 mL) under stirring at room temperature. After 15 min, dimethyl (S)-glutamate hydrochloride (0.42 g, 1.98 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 48 h. The resulting precipitate was filtered off and washed with DMSO or DMF (3 × 0.5 mL), the filtrate and washings were poured in cold water (150 mL) and extracted with n-BuOH (4 × 25 mL). The combined organic layers were washed with 5% aqueous NaHCO₃ (3 × 20 mL), saturated aqueous NaCl (3 × 20 mL), then evaporated to dryness under reduced pressure. In the case of (S)-11a, EtOAc (4 × 25 mL) was used for extraction; combined organic layers were dried over MgSO₄ before evaporation. In all cases the residue was purified by flash column chromatography on silica gel (CHCl₃–EtOH as an eluent).

**General procedure for the coupling reactions (synthesis of compounds 4a–c, 13b)**

Compound 3a–c or 12b (0.500 mmol) was dissolved in 0.5 M aqueous LiOH (3 mL, 1.50 mmol). The reaction mixture was stirred at room temperature for 24 h, and then cooled to 0 °C (ice bath); the pH was adjusted to 3 by concentrated HCl. In the cases of 3a,b and 12b, the resulting precipitate was filtered off and washed with cold water (3 × 0.5 mL). In the case of 3c, the resulting solution was treated with ion-exchange resin Amberlite IR-120(H); the resin was washed with water; then the target compound was eluted with a water–pyridine 9:1 mixture; the eluate was evaporated to dryness under reduced pressure.

**General procedure for the synthesis of methyl esters (R)-5, (S)-5 and dimethyl esters (S,S)-3a–c, (S,S)-12a,b**

SOCl₂ (38 μL, 0.330 mmol) for (R)-2b, (S)-2b), 76 μL, 0.660 mmol for (S,S)-4b and (S,S)-13a), 101 μL, 0.880 mmol for (S,S)-4a,c) or 114 μL, 0.990 mmol for (S,S)-13b) was added to a suspension of compound (R)-2b, (S)-2b, (S,S)-4a–c or (S,S)-13a,b (0.220 mmol) in absolute MeOH (3 mL) under stirring at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 24 h. The solvent was evaporated to dryness under reduced pressure; the residue was treated with cold Et₂O, and the resulting precipitate was filtered off.

**General procedure for the synthesis of dipeptides (S,S)-7a–c**

0.5 M aqueous LiOH (40 mL, 25.1 mmol) was added to a solution of compound (S,S)-6a–c (8.37 mmol) in EtOH (10 mL) at to 0 °C. The reaction mixture was stirred at 0 °C for 30 min, then at room temperature for 4 h. The reaction mixture was washed with EtOAc (2 × 10 mL), acidified with 4 M HCl to pH 3 and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over MgSO₄ and evaporated to dryness under reduced pressure. The residue was dissolved in trifluoroacetic acid (20 mL); then the reaction mixture was stirred at room temperature for 1 h and evaporated under reduced pressure. The residue was dissolved in water (20 mL); the solution was alkalized with 5% aqueous NaHCO₃ to pH 5. The resulting solution was treated with ion-exchange resin Amberlite IR-120(H); the resin was washed with water; then the target compound was eluted with a water–pyridine 9:1 mixture. The eluate was evaporated to dryness under reduced pressure.

**General procedure for the nucleophilic substitution reactions (synthesis of compounds (S,S)-4a–c, (S,S)-8a,b, (S,S)-11a,b, and (S,S)-13a,b)**

Na₂CO₃ (0.16 g, 1.52 mmol) and 6-chloropurine, 2-amino-6-chloropurine or compounds 10a,b (1.02 mmol) were successively added to a solution of compound (S,S)-7a–c or (S)-alanine (2.03 mmol) in water (5 mL). The reaction mixture was refluxed for 4–6 h; the resulting solution was cooled to 0 °C and acidified with concentrated HCl to pH...
3. Further operations are described for each compound in Electronic supplementary material.

Analytical data for all compounds can be found in Electronic supplementary material.

Assessment of antimycobacterial activity

The inhibitory effect of compounds 4a–c, (S,S)-4a, (S,S)-8a and 13b was studied against *M. tuberculosis* H37Rv strain. The minimal inhibitory concentration (MIC) for each compound was determined by the micro broth dilution method (Krasnov et al. 2016). All compounds tested were dissolved in DMSO and their 1/2 dilutions were added in tubes with 5 ml of the Löwenstein–Jensen medium. Each compound was tested at six concentrations 12.5, 6.25, 3.1, 1.5, 0.7, and 0.35 μg/mL. Tubes with Löwenstein–Jensen medium (5 mL) containing tested compounds and those without them (controls) were inoculated with a suspension of *M. tuberculosis* H37Rv strain and incubated at 37 °C for 10 days. The mycobacterial growth was assessed by the standart procedure: the appearance of zones of mycobacterial growth delay (more than 10 mm) indicated the presence of antimycobacterial properties of tested compounds at the studied concentrations. The value of growth retardation zone (in mm) was proportional to the antimycobacterial activity of the compounds. Growth delay (100 mm or more) was considered as a complete mycobacteria growth inhibition. Isoniazid (Alfa Aesar, UK) was used as a reference drug (MIC 0.1 μg/mL).

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Data availability The authors declare that the data supporting the findings of this study are available within the article and its supplementary information files.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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