Amino Acids (2021) 53:407–415
https://doi.org/10.1007/s00726-021-02958-0

ORIGINAL ARTICLE

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

Vera V. Musiyak[1] · Irina A. Nizova[1] · Evgeny N. Chulakov[1] · Liliya Sh. Sadretdinova[1] · Andrey A. Tumashov[1,2] · Galina L. Levit[1] · Victor P. Krasnov[1,2]

Received: 29 July 2020 / Accepted: 6 February 2021 / Published online: 18 February 2021

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; Krasnov 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; Krasnov et al. 2016, 2020), and other effects.

The modification of purine core at position N^9 leading to the analogs of natural nucleosides has been widely used (Wang et al. 2015; Mehellou 2016). At the same time, the synthesis of C^6-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 C^6 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

Handling editor: P. Meffre.

✉ Victor P. Krasnov
ca@ios.uran.ru

[1] Postovsky Institute of Organic Synthesis of RAS (Ural Branch), 22/20, S. Kovalevskoy/Akademicheskaya St., Ekaterinburg 620108, Russia

[2] Ural Federal University, 19, Mira St., Ekaterinburg 620002, Russia

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 α-amino acids, as well as the analogs in which the glycine fragment is replaced by residues of various ω-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 α-amino acids instead of glycine moiety. We used the following α-amino acids as simple structures without any additional functional groups: (*S*)-alanine, (*S*)-phenylalanine (common aromatic α-amino acid), and (*S*)-valine (common branched aliphatic α-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).

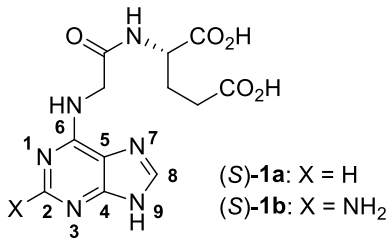
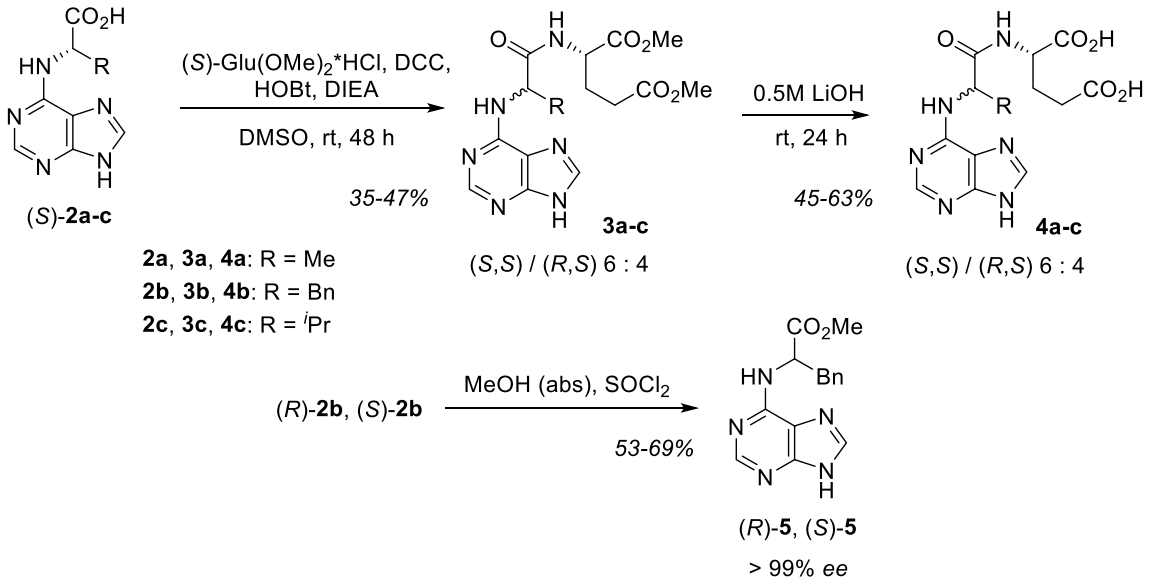


Fig. 1 Structures of the parent compounds (*S*)-**1a,b** (Krasnov et al. 2016)

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



Scheme 1 Synthesis of compounds **4a–c**, (*R*)-**5** and (*S*)-**5**

(*S*)-amino acids in aqueous Na_2CO_3 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.

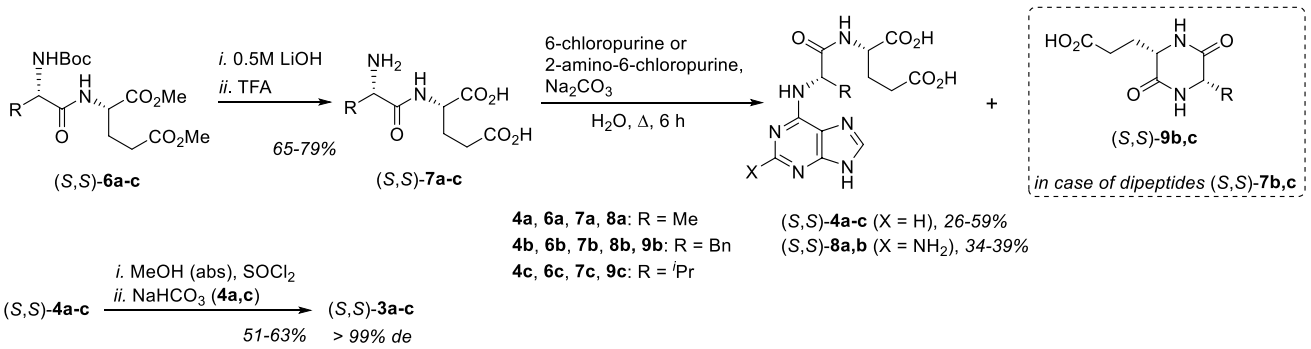
The coupling of *N*-(purin-6-yl)-(*S*)-amino acids (*S*)-**2a–c** to dimethyl (*S*)-glutamate was carried out in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIEA). We were surprised to observe double sets of signals in the ^{1}H and ^{13}C NMR spectra of products **3a–c** (according to ^{1}H NMR, in a 6:4 ratio). It should be noted that when conducting coupling of achiral carboxy component (*N*-(purin-6-yl) glycine and *N*-(purin-6-yl)-beta-alanine) to chiral amino component (methyl (*S*)-tyrosinate, methyl (*S*)-serinate, or dimethyl (*S*)-glutamate) in the presence of a DCC–HOBt mixture, we did not observe any racemization at the chiral centers of the target pyrinyl dipeptides (Musiyak et al. 2019). So, we can conclude that in the present case racemization occurs at the chiral center of the carboxy component, thus leading to the formation of a mixture of (*S*,*S*)- and (*R*,*S*)-diastereomers. Varying the reaction conditions (changing the order of adding the reagents, loading reagents at 0 °C, using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) instead of DCC/HOBt mixture) did not affect the diastereomeric composition of the products **3a–c**. Alkaline hydrolysis of diesters **3a–c** led to compounds **4a–c**, which were also mixtures of diastereomers in a 6:4 ratio (according to ^{1}H NMR spectroscopy).

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 Na_2CO_3 under reflux led to the products (*S*,*S*)-**4a–c** (Scheme 2). Diastereomeric purity of these compounds was confirmed by ^{1}H and ^{13}C 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 Na_2CO_3 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*)-**4b,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 ^{1}H and ^{13}C 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



Scheme 2 Synthesis of compounds (*S*,*S*)-**4a–c**, (*S*,*S*)-**8a,b** and (*S*,*S*)-**3a–c**

(Scheme 1). The diastereomerically pure dimethyl esters (*S*,*S*)-**3a–c** were obtained from conjugates (*S*,*S*)-**4a–c** (Scheme 2), which made it possible to assign corresponding peaks in HPLC chromatograms of diastereomeric mixtures **3a–c**. When comparing the HPLC and ^{1}H, ^{13}C NMR spectral data of individual diastereomers (*S*,*S*)-**3a–c** (> 99% *de*) with the data obtained for mixtures **3a–c**, we found that in all cases the coupling of compounds (*S*)-**2a–c** to dimethyl (*S*)-glutamate (Scheme 1) resulted in predominant formation of (*S*,*S*)-diastereomers; that is, products **3a–c** were mixtures of (*S*,*S*)-and (*R*,*S*)-diastereomers in a 6:4 ratio. Moreover, it has been shown that when *N*-(purin-6-yl)-(*R*)-phenylalanine [(*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*-(purin-6-yl)-(*S*)-phenylalanine (*S*)-**2b**. Thus, the configuration of chiral center of the starting *N*-(purin-6-yl)-α-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.

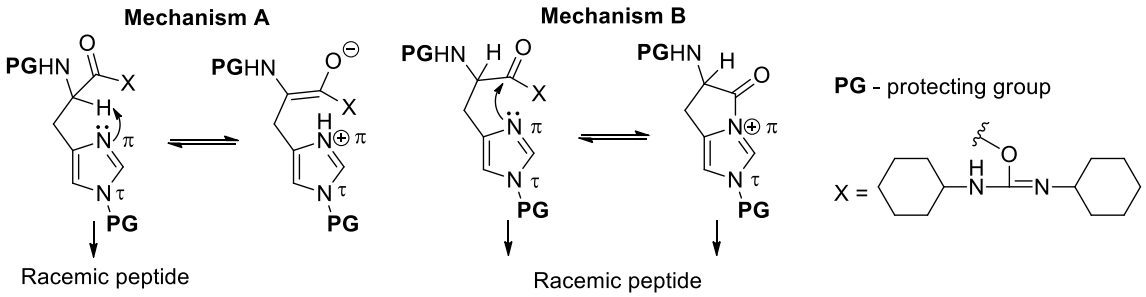
It is known that histidine (imidazole-substituted amino acid), which can be considered as a structural analog of *N*-(purin-6-yl)-α-amino acids, is racemized while DCC treatment (Scheme 3) (Jones et al. 1980). Histidine is supposed to form the corresponding *O*-acylisourea while interaction with DCC. Racemization of the chiral center occurs with the participation of the imidazole N^{π} 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^{τ}-protecting group is not a hindrance for this process; at the same time, in the case of N^{π}-protected histidine racemization does not occur.

We suppose that the racemization of the chiral center of *N*-(purin-6-yl)-α-amino acids occurs with the participation of the imidazole fragment, as in the case of histidine. Therefore, we obtained *N*-(9-benzylpurin-6-yl)-(*S*)-alanine ((*S*)-**11a**) and *N*-(7-deazapurin-6-yl)-(*S*)-alanine ((*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 Na_2CO_3 under reflux (Scheme 4).

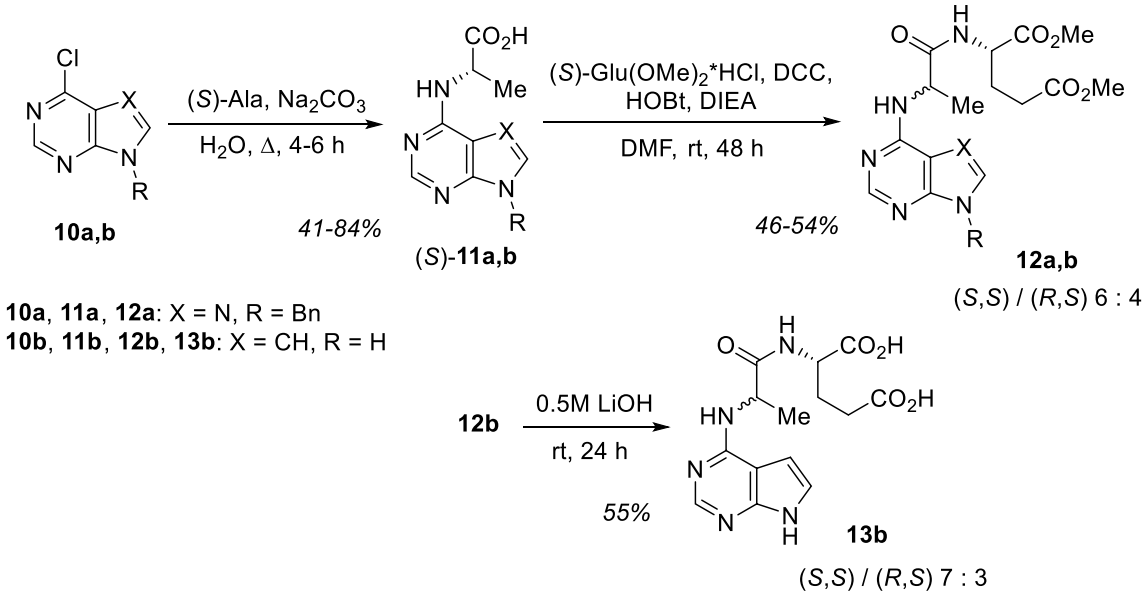
It has been found that the coupling of *N*-(9-benzylpurin-6-yl)-(*S*)-alanine (*S*)-**11a** to dimethyl (*S*)-glutamate in the presence of DCC, HOBt, and DIEA is accompanied by racemization and leads to the product **12a** that is a mixture of diastereomers in a 6:4 ratio (Scheme 4), as in the case of N^{9}-unsubstituted analogs (Scheme 1). In the case of *N*-(7-deazapurin-6-yl)-(*S*)-alanine (*S*)-**11b**, racemization also occurred, despite the absence of the N^{7} atom (analog of N^{π} 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 ^{1}H NMR data (Scheme 4). The results obtained indicate that the imidazole nitrogen atoms of *N*-(purin-6-yl)-α-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*-(purin-6-yl)-α-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 HCl (Liu and Robins 2007), nor while using DMF as a solvent (Terrier 2013), nor without any solvent (Novosjolova et al. 2015).

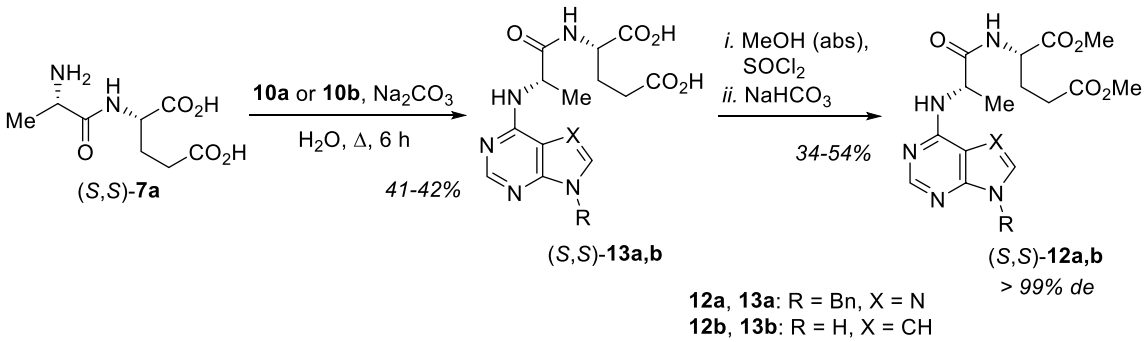
Diastereomerically pure compounds (*S*,*S*)-**13a,b** were converted to dimethyl esters (*S*,*S*)-**12a,b** (Scheme 5). This made it possible to assign corresponding peaks in HPLC chromatograms of diastereomeric mixtures **12a,b**. When comparing the HPLC data (Fig. 2) and ^{1}H, ^{13}C NMR spectra of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



Scheme 3 Proposed mechanisms of the histidine racemization (Jones et al. 1980)



Scheme 4 Synthesis of compounds (*S*)-**11a,b**, **12a,b** and **13b**



Scheme 5 Synthesis of diastereomerically pure compounds (*S*,*S*)-**13a,b** and (*S*,*S*)-**12a,b**

the data of mixtures **12a,b**, we found that the coupling of *N*-(9-benzylpurin-6-yl)-(*S*)-alanine and *N*-(7-deazapurin-6-yl)-(*S*)-alanine (compounds (*S*)-**11a** and (*S*)-**11b**) to dimethyl (*S*)-glutamate (Scheme 5) also led to predominant formation of (*S*,*S*)-diastereomers as it was observed for the coupling reaction of *N*-(purin-6-yl)-(*S*)-amino acids ((*S*)-**2a–c**) (Scheme 1).

The in vitro antimycobacterial activity of conjugates **4a–c**, (*S*,*S*)-**4a**, (*S*,*S*)-**8a**, and **13b** against *Mycobacterium tuberculosis* H37Rv strain was studied in the Ural Research Institute of Phthisiopulmonology (National Medical Research Center, Ministry of Healthcare of the Russian Federation, Ekaterinburg, Russia). The minimum inhibitory concentrations (MIC) of these compounds were determined by the previously described procedure (Krasnov et al. 2016). Among tested compounds, alanine derivative **4a** was found to exhibit the highest inhibitory activity (MIC 3.1 μg/mL), while phenylalanine (**4b**) and valine (**4c**) derivatives were not significantly active (MIC 6.2–12.5 μg/mL). Moreover, individual diastereomer (*S*,*S*)-**4a** exhibits the same antimycobacterial activity as the mixture **4a** of (*S*,*S*)- and (*R*,*S*)-diastereomers in 6:4 ratio (MIC 3.1 μg/mL). It has been also demonstrated that the modification of structure **4a** by introducing an amino group at position C^2 of the purine core ((*S*,*S*)-**8a**) or by replacement of the imidazole moiety with pyrrole fragment (**13b**) leads to the decreased inhibitory activity (MIC 6.2–12.5 μg/mL). In general, these results show that the replacement of the glycine fragment in structures (*S*)-**1a,b** with other α-amino acid residues leads to a decrease in antimycobacterial activity.

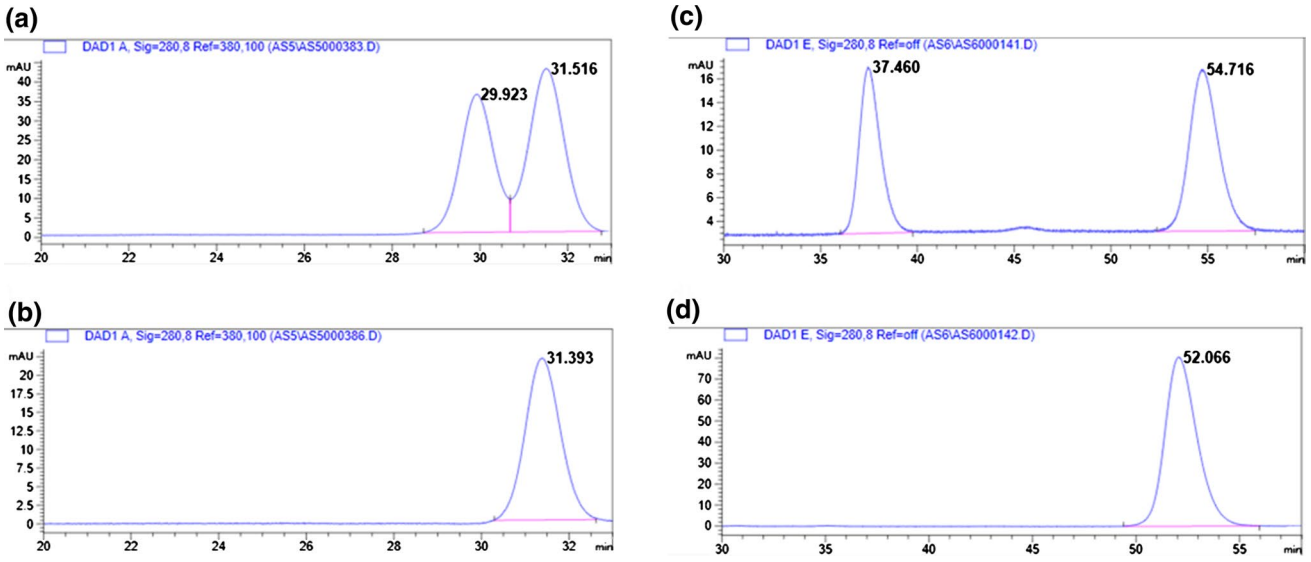


Fig. 2 HPLC chromatograms of compounds: **a 3a**,**b** (*S*,*S*)-**3a** (S,S-Whelk-O1, 0.025 N aqueous AcONa–MeOH, 55:45, 0.75 mL/min, detection at 280 nm); **c 12b**,**d** (*S*,*S*)-**12b** (*S*,*S*-Whelk-O1, H_2O–MeOH, 55:45, 0.8 mL/min, detection at 280 nm)

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-benzylpurin-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 ^{1}H NMR spectra were recorded on Bruker DRX-400 (400 MHz) or Bruker Avance 500 (500 MHz) (Bruker, Germany); the ^{13}C NMR spectra were recorded on Bruker Avance 500 (125 MHz); TMS was used as internal reference. CHN elemental microanalyses were performed using Perkin Elmer 2400 II analyser (Perkin Elmer, USA). Analytical TLC was performed using Sorbfil 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: Chiralcel 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 dm^3/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 $NaHCO_3$ (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 $MgSO_4$ before evaporation. In all cases the residue was purified by flash column chromatography on silica gel ($CHCl_3$–EtOH as an eluent).

General procedure for alkaline hydrolysis (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

$SOCl_2$ (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 Et_2O, 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 $MgSO_4$ 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 $NaHCO_3$ 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*)-11a,b, and (*S,S*)-13a,b)

Na_2CO_3 (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).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00726-021-02958-0.

Acknowledgements We are grateful to Dr. Marionella A. Kravchenko [Ural Research Institute of Phthisiopulmonology (National Medical Research Center, Ministry of Healthcare of the Russian Federation, Ekaterinburg, Russia)] for conducting biological trials. Analytical studies were carried out using equipment of the Centre for Joint Use "Spectroscopy and Analysis of Organic Compounds" at the Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences.

Funding The work was financially supported by the Russian Science Foundation (Grant 19-13-00231).

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.

References

Al-Harbi RAK, Abdel-Rahman AAH (2012) Synthesis and antiviral evaluation of α-amino acid esters bearing *N*6-benzyladenine side chain. Acta Pol Pharm 69:917–925

Beauchamp LM, Orr GF, de Miranda P, Burnette T, Krenitsky TA (1992) Amino acid ester prodrugs of acyclovir. Antiviral Chem Chemother 3:157–164. https://doi.org/10.1177/095632029200300305

Boerema DJ, Tereshko VA, Zhangc J, Kent SBH (2016) Chemical synthesis and enzymatic properties of RNase A analogues designed to enhance second-step catalytic activity. Org Biomol Chem 14:8804–8814. https://doi.org/10.1039/C6OB01163B

Chang W, Bao D, Chun BK, Naduthambi D, Nagarathnam D, Rachakonda S, Reddy PG, Ross BS, Zhang HR, Bansal S, Espiritu CL, Keilman M, Lam AM, Niu C, Steuer HM, Furman PA, Otto MJ, Sofia MJ (2011) Discovery of PSI-353661, a novel purine nucleotide prodrug for the treatment of HCV infection. ACS Med Chem Lett 2:130–135. https://doi.org/10.1021/ml100209f

de Wet TJ, Warner DF, Mizrahi V (2019) Harnessing biological insight to accelerate tuberculosis drug discovery. Acc Chem Res 52:2340–2348. https://doi.org/10.1021/acs.accounts.9b00275

Deng G, Liu Z, Ye F, Luo X, Zhu W, Shen X, Liu H, Jiang H (2008) Tryptophan-containing dipeptide derivatives as potent PPARγ antagonists: design, synthesis, biological evaluation, and molecular modeling. Eur J Med Chem 43:2699–2716. https://doi.org/10.1016/j.ejmech.2008.01.032

Gray SG, Khoujah AM (1969) Selective cleavage of the t-butoxycarbonyl protecting group. Tetrahedron Lett 10:2647–2650. https://doi.org/10.1016/S0040-4039(01)88233-2

Gruzdev DA, Chulakov EN, Levit GL, Kravchenko MA, Krasnov VP, Charushin VN (2017) Synthesis and antimycobacterial activity of novel purin-6-yl and 2-aminopurin-6-yl conjugates with (*S*)-aspartic and (*S*)-glutamic acids. Mendeleev Commun 27:547–549. https://doi.org/10.1016/j.mencom.2017.11.002

Gruzdev DA, Musiyak VV, Levit GL, Krasnov VP, Charushin VN (2018) Purine derivatives with antituberculosis activity. Russ Chem Rev 87:604–618. https://doi.org/10.1070/RCR4772

Islam MR, Mahdi JG, Bowen ID (1997) Pharmacological importance of stereochemical resolution of enantiomeric drugs. Drug Saf 17:149–165. https://doi.org/10.2165/00002018-199717030-00002

Jones JH, Ramage WI, Witty MJ (1980) Mechanism of racemisation of histidine derivatives in peptide synthesis. Int J Peptide Protein Res 15:301–303. https://doi.org/10.1111/j.1399-3011.1980.tb02581.x

Kelley JL, Krochmal MP, Linn JA, McLean EW, Soroko FE (1988) 6-(Alkylamino)-9-benzyl-9H-purines. A new class of anticonvulsant agents. J Med Chem 31:606–612. https://doi.org/10.1021/jm00398a019

Kleinsmann AJ, Nachtsheim BJ (2013) Phenylalanine-containing cyclic dipeptides—the lowest molecular weight hydrogelators based on unmodified proteinogenic amino acids. Chem Commun 49:7818–7820. https://doi.org/10.1039/C3CC44110E

Krasnov VP, Vigorov AYu, Musiyak VV, Nizova IA, Gruzdev DA, Matveeva TV, Levit GL, Kravchenko MA, Skornyakov SN, Bekker OB, Danilenko VN, Charushin VN (2016) Synthesis and antimycobacterial activity of *N*-(2-aminopurin-6-yl) and *N*-(purin-6-yl) amino acids and dipeptides. Bioorg Med Chem Lett 26:2645–2648. https://doi.org/10.1016/j.bmcl.2016.04.017

Krasnov VP, Musiyak VV, Vozdvizhenskaya OA, Galegov GA, Andronova VL, Gruzdev DA, Chulakov EN, Vigorov AYu, Ezhikova MA, Kodess MI, Levit GL, Charushin VN (2019) *N*-[ω-(Purin-6-yl)aminoalkanoyl] derivatives of chiral heterocyclic amines as promising anti-herpesvirus agents. Eur J Org Chem 2019:4811–4821. https://doi.org/10.1002/ejoc.201900727

Krasnov VP, Levit GL, Musiyak VV, Gruzdev DA, Charushin VN (2020) Fragment-based approach to novel bioactive purine derivatives. Pure Appl Chem. https://doi.org/10.1515/pac-2019-1214

Legraverend M (2008) Recent advances in the synthesis of purine derivatives and their precursors. Tetrahedron 64:8585–8603. https://doi.org/10.1016/j.tet.2008.05.115

Legraverend M, Grierson DS (2006) The purines: Potent and versatile small molecule inhibitors and modulators of key biological targets. Bioorg Med Chem 14:3987–4006. https://doi.org/10.1016/j.bmc.2005.12.060

Lin GQ, You QD, Cheng JF (2011) Chiral drugs. Wiley, Hoboken

Liu J, Robins MJ (2007) S_NAr Displacements with 6-(fluoro, chloro, bromo, iodo, and alkylsulfonyl) purine nucleosides: synthesis, kinetics, and mechanism. J Am Chem Soc 129:5962–5968. https://doi.org/10.1021/ja070021u

McGregor WH, Carpenter FH (1961) Synthesis of L-leucyl-L-alanyl-L-valyl-L-glutamic acid and intermediate peptides. J Org Chem 26:1849–1854. https://doi.org/10.1021/jo01065a039

McGuigan C, Harris SA, Daluge SM, Gudmundsson KS, McLean EW, Burnette TC, Marr H, Hazen R, Condreay LD, Johnson L, De Clercq E, Balzarini J (2005) Application of phosphoramidate pronucleotide technology to Abacavir leads to a significant enhancement of antiviral potency. J Med Chem 48:3504–3515. https://doi.org/10.1021/jm0491400

McGuigan C, Hassan-Abdallah A, Srinivasan S, Wang Y, Siddiqui A, Daluge SM, Gudmundsson KS, Zhou H, McLean EW, Peckham JP, Burnette TC, Marr H, Hazen R, Condreay LD, Johnson L, Balzarini J (2006) Application of phosphoramidate ProTide technology significantly improves antiviral potency of carbocyclic adenosine derivatives. J Med Chem 49:7215–7226. https://doi.org/10.1021/jm060776w

McGuigan C, Madela K, Aljarah M, Gilles A, Brancale A, Zonta N, Chamberlain S, Vernachio J, Hutchins J, Hall A, Ames B, Gorovits E, Ganguly B, Kolykhalov A, Wang J, Muhammad J, Patti JM, Henson G (2010) Design, synthesis and evaluation of a novel double pro-drug: INX-08189. A new clinical candidate for hepatitis C virus. Bioorg Med Chem Lett 20:4850–4854. https://doi.org/10.1016/j.bmcl.2010.06.094

Mehellou Y (2016) The ProTides Boom. ChemMedChem 11:1114–1116. https://doi.org/10.1002/cmdc.201600156

Melchert M, List A (2007) The thalidomide saga. Int J Biochem Cell Biol 39:1489–1499. https://doi.org/10.1016/j.biocel.2007.01.022

Motta I, Centis R, D'Ambrosio L, García-García JM, Goletti D, Gualano G, Lipani F, Palmieri F, Sánchez-Montalvá A, Pontali E, Sotgiu G, Spanevello A, Stochino C, Tabernero E, Tadolini M, van den Boom M, Villa S, Visca D, Migliori GB (2020) Tuberculosis, COVID-19 and migrants: Preliminary analysis of deaths occurring in 69 patients from two cohorts. Pulmonology 26:233–240. https://doi.org/10.1016/j.pulmoe.2020.05.002

Musiyak VV, Nizova IA, Matveeva TV, Levit GL, Krasnov VP, Charushin VN (2019) Synthesis of new purine derivatives containing α- and ω-amino acid fragments. Russ J Org Chem 55:762–770. https://doi.org/10.1134/S1070428019060046

Niu HY, Bai SX, Wu S, Qu GR, Guo HM (2012) Synthesis of chiral *N*-(Purin-6-yl)amino acid derivatives by using natural amino acids as starting materials. Asian J Org Chem 1:238–244. https://doi.org/10.1002/ajoc.201200081

Novosjolova I, Bizdēna E, Turks M (2015) Synthesis and applications of azolylpurine and azolylpurine nucleoside derivatives. Eur J Org Chem 2015:3629–3649. https://doi.org/10.1002/ejoc.201403527

Pautus S, Sehr P, Lewis J, Fortune A, Wolkerstorfer A, Szolar O, Guilligay D, Lunardi T, Decout JL, Cusack S (2013) New 7-methylguanosine derivatives targeting the influenza polymerase PB2 cap-binding domain. J Med Chem 56:8915–8930. https://doi.org/10.1021/jm401369y

Stavrakov G, Valcheva V, Voynikov Y, Philipova I, Atanasova M, Konstantinov S, Peikov P, Doytchinova I (2016) Design, synthesis, and antimycobacterial activity of novel theophylline-7-acetic acid derivatives with amino acid moieties. Chem Biol Drug Des 87:335–341. https://doi.org/10.1111/cbdd.12676

Terrier F (2013) The S_NAr reactions: mechanistic aspects. In: Terrier F (ed) Modern nucleophilic aromatic substitution. Wiley-VCH Verlag GmbH & Co, Weinheim, pp 1–84

Tripathi RP, Tewari N, Dwivedi N, Tiwari VK (2005) Fighting tuberculosis: an old disease with new challenges. Med Res Rev 25:93–131. https://doi.org/10.1002/med.20017

Vigorov AYu, Krasnov VP, Gruzdev DA, Men'shikova AA, Demin AM, Levit GL, Charushin VN (2014) Novel synthetic routes to *N*-(2-amino-9H-purin-6-yl)-substituted amino acids. Mendeleev Commun 24:35–36. https://doi.org/10.1016/j.mencom.2013.12.011

Voynikov Y, Valcheva V, Momekov G, Peikov P, Stavrakov G (2014) Theophylline-7-acetic acid derivatives with amino acids as antituberculosis agents. Bioorg Med Chem Lett 24:3043–3045. https://doi.org/10.1016/j.bmcl.2014.05.026

Wang C, Song Z, Yu H, Liu K, Ma X (2015) Adenine: an important drug scaffold for the design of antiviral agents. Acta Pharm Sin B 5:431–441. https://doi.org/10.1016/j.apsb.2015.07.002

Ward DN, Wade J, Walborg EF Jr, Osdexe TS (1961) The Synthesis of *N*-(6-Purinyl)amino acids. Amino acids with a single reactive amino group. J Org Chem 26:5000–5005. https://doi.org/10.1021/jo01070a054

WHO (2019) Global Tuberculosis Report 2019. https://www.who.int/tb/global-report-2019. Accessed 30 June 2020

WHO (2020) COVID-19: Considerations for tuberculosis (TB) care. https://www.who.int/docs/default-source/documents/tuberculosis/infonote-tb-covid-19.pdf?sfvrsn=b5985459_18. Accessed 20 July 2020