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ARTICLE

# Novel 5-substituted derivatives of 2′-deoxy-6-azauridine with antibacterial activity

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## Abstract

The emergence of new drug-resistant strains of bacteria necessitates the development of principally new antibacterial agents. One of the novel classes of antibacterial agents is nucleoside analogs. We have developed a fast and simple one-pot method for preparation of α- and β-anomers of 5-modified 6-aza- and 2-thio-6-aza-2′-deoxyuridine derivatives in high yields. 2-Thio derivatives demonstrated moderate activity against *Mycobacterium smegmatis* (MIC = 0.2–0.8 mM), *Staphylococcus aureus* (MIC = 0.03–0.9 mM) and some other Gram-positive bacteria. 2′-Deoxy-2-thio-5-phenyl-6-azauridine (**2b**) effectively suppressed the growth of Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 (MIC = 0.03 mM)—the one that causes diseases difficult to treat due to high resistance to antibiotics. 5′-Monophosphates of compounds **2a, b** and **3a, b** were docked into a binding site of *Mycobacterium tuberculosis* flavin-dependent thymidylate synthase (ThyX) enzyme. The molecular modeling demonstrates the possibility of binding of the 5-modified 2-thio-6-aza-2′-deoxyuridine 5′-monophosphates within the active site of the enzyme and thereby inhibiting the growth of the bacteria.

## Introduction

The discovery and introduction of antibiotics is among the most important achievements of the twentieth century. Their wide application in medical practice made it possible to significantly alleviate the course of illnesses and reduce mortality from infectious diseases [1, 2]. However, nowadays practically all pathogenic bacteria and viruses have developed resistance to most clinically important medicinal preparations [1, 2], and hence there is a need for new drugs acting on new targets and being active against resistant strains of pathogens.

The use of nucleoside derivatives as drugs is very attractive. Slight modifications at nucleic base or sugar moiety may provide an impact on the anti-pathogen activity. As a result, a lot of nucleoside derivatives are antiviral [3, 4] and anticancer agents [4, 5]. Some modified nucleosides have been in clinical use for over 50 years; however, these compounds have not been used against bacteria and only limited small-scale investigations into their antibacterial properties have been conducted [6]. A number of nucleosides have a significant impact on the most important processes of bacterial and fungal cells including nucleoside metabolism, DNA, RNA, protein as well as cell wall biosynthesis and some other cellular processes [7]. Despite the fact that currently there are a limited number of publications devoted to the study of the mechanisms of action of modified nucleosides on bacteria, it is obvious that nucleoside derivatives open up wide possibilities for creating fundamentally new drugs that affect drug-resistant strains of microorganisms [8]. Recently, several reports were published on modified nucleosides showing activity against various mycobacteria: *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium*, and *Mycobacterium smegmatis*

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(for review see refs. [6, 8–12]). A series of nucleoside phosphate prodrugs were synthesized and they demonstrated anti-tubercular activity of 200 μg $ml^{-1}$ [13].

The 6-azauracil-substituted nucleoside analogs have long been compounds of interest for anticancer activity [14]. Representatives of the family of 6-azapyrimidine 2'-deoxyribonucleosides, first synthesized in the 1960s, rarely exhibit significant antiviral activity. Among them, 5-(2-substituted vinyl)-6-aza-2'-deoxyuridines demonstrated in vitro activity against herpes simplex virus type 1 and 2 [15, 16] being potent and selective thymidine kinase inhibitors [15, 16]. At the same time, 6-azauridine and 2-thio-6-azauridine inhibited the growth of RNA viruses, including flaviviruses [17–19] and retroviruses [20].

At the beginning of the twenty-first century, a number of reports were published on C-5-modified nucleosides with extended alkyl substituents that exhibited in vitro antimycobacterial activity (for review see refs. [7, 9, 11]). The mechanism of their action is still unclear. Several microorganisms, including representatives of the genus *Helicobacter* and mycobacteria, contain the flavin-dependent thymidylate synthase (ThyX) enzyme as a potential target. The latter has no structural similarity to the ThyA enzyme of eubacteria and eukaryotes and catalyzes the formation of dTMP by a completely different mechanism [21]. Since ThyX is absent in humans, it can be considered as a convenient target for the development of specific inhibitors of ThyX in the fight against tuberculosis. Recently, 5'-monophosphates of 5-alkyl, 5-aryl-, and 5-alkyloxymethyl derivatives of 2'-deoxyuridine [22–24] have been identified as selective inhibitors of ThyX. 5'-Monophosphates of 5-modified 6-aza-2'-deoxyuridine demonstrated activity towards ThyX, but relatively weaker than 2'-deoxyuridine derivatives [25]. It can be assumed that the mechanism of action of 5-modified 2'-deoxyuridines can be partially associated with the inhibition of this enzyme [22–25]. The molecular modeling studies of 5'-monophosphates of 2-thio-5-modified-6-aza-2'-deoxyuridines performed by us demonstrate the possibility of their incorporation into the active site of the mycobacterial ThyX enzyme (see below) and, thereby, their potential ability to inhibit the growth of *M. tuberculosis*.

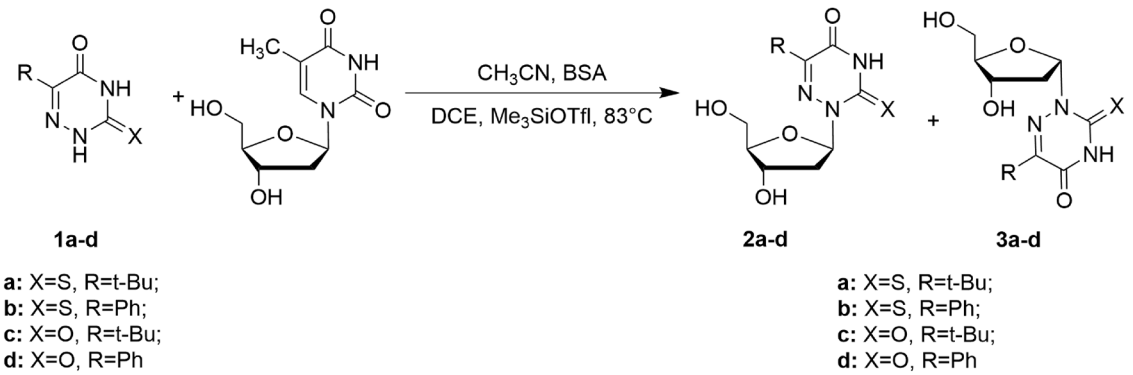
Based on the aforementioned facts, the aim of this study was to design and synthesize novel 6-aza derivatives of 2'-deoxyuridine with alkyl or aryl substituents at C-5 position of the nucleobase, as well as their 2-thio-analogs, and evaluate their activity against a number of microorganisms. The synthesis of the "natural" β-anomers was not of special interest since we previously showed that both α- and β-anomers of 5-substituted 2'-deoxyuridine can possess antimycobacterial activity of the same order [26].

## Results and discussion

### Chemistry

The 6-azauridine and its 5-substituted derivatives have passed a long way of glycosylation procedure optimizations since the pioneering works of Šorm and Keilova [27] and Pasternak and Handschumacher [28] in the 1960s. Herdewijn and co-workers [24] performed the coupling reactions between 6-azauracil moieties and the Hoffer's chloro sugar [29] in the presence of CuI as catalyst according to the method by Freskos [30]. Yields were usually good; however, in contrast to the method of Freskos [30], only a modest β/α-selectivity (usually ca. 60%, as determined by proton nuclear magnetic resonance ($^1$H NMR) spectroscopy) was observed [25].

Since our task was to obtain 2'-deoxy-6-azauridine derivatives for biological testing, to gain higher yields of the products we proposed an alternative way of synthesis via the Eckstein's reaction of transglycosylation [31] (Scheme 1). The major advantage is that protecting groups' strategy is unnecessary in transglycosylation reaction (in contrast to the previously used glycosylation procedures




**Scheme 1** Synthesis of 6-aza-nucleoside derivatives

[25]), and thus several steps are avoided. Higher yields were gained, besides, commercially available thymidine was used. As a result, we demonstrated for the first time that the Eckstein's method allows to synthesize 6-azauracil derivatives modified at the fifth position and containing a sulfur atom instead of the oxygen atom at the second position of the base in good yields.

As expected, in our case a product of interaction of the silylated base with thymidine in the presence of $(Me)_3SiTfl$ in dichloroethane resulted in a mixture of anomers. These compounds were isolated as separate anomers by column chromatography in chloroform-ethanol (100:1) eluting system.

Our attempts to change the anomeric ratio from ~5:4 to ~1:1 (α:β) for X = S and X = O, respectively, by setting the reaction in different solvents (dichloroethane to acetonitrile or their mixture) and varying temperature, time of reaction, and amounts of reagents did not lead to significant results. At room temperature none of the reactions succeeded. The use of dichloroethane as a solvent at elevated temperature gave the highest yields and the shortest time of reaction. The results of the reaction condition variations and their impact on the overall yield and anomeric ratio are summarized in the Supplementary file.

## Assignment of the anomers

The obtained compounds were characterized by $^{1}H$, $^{13}C$, and two-dimensional (2D) NMR, high-resolution mass spectrometry (HRMS), and ultraviolet (UV) spectra. The correct anomeric structure was proved by 2D nuclear Overhauser effect spectroscopy (NOESY) NMR spectroscopy (Fig. 1).

The corresponding β-anomer **2a** as a representative compound showed two significant cross-peaks via nuclear Overhauser effect (NOE): H-3'/*t*-Bu group and H-4'/H-1' (Fig. 1a). These peaks were not observed in the correlation spectroscopy (COSY) NMR spectrum. Thus, they are not in one spin system, but have close-dimensional location of the protons. Furthermore, in case of α-anomer, the opposite effect was expectedly found: corresponding NOESY correlations of H-3' with H-1' and H-4' with *t*-Bu group were observed—which confirmed the anomeric configuration.

The appropriate linkage of the N-1 atom of the nucleobase to the sugar moiety was confirmed by heteronuclear multiple bond correlation (HMBC) NMR spectroscopy using compound **3a** as a representative example. Figure 1b shows an intense cross-peak of H-1' with C-2 and a slight interaction of H-1' with C-5, but there is no interaction with C-4. Thus, H-1' is connected with N-1, but not with N-3 or N-6 atoms.

## Biology

Biological studies were performed for parental triazine bases (**1a–d**) [32], for synthesized 2'-deoxynucleosides (**2a–d** and **3a–d**), and also for the previously obtained ribonucleosides with 2-thio-5-(*tert*-butyl)-6-azauracil and 2-thio-5-phenyl-6-azauracil as nucleic bases (**4a** and **4b**, respectively) [33] for better comparison.

## Cytotoxicity

The toxicity of the synthesized compounds was estimated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [34] in human cell lines *Jurkat* (leukemic

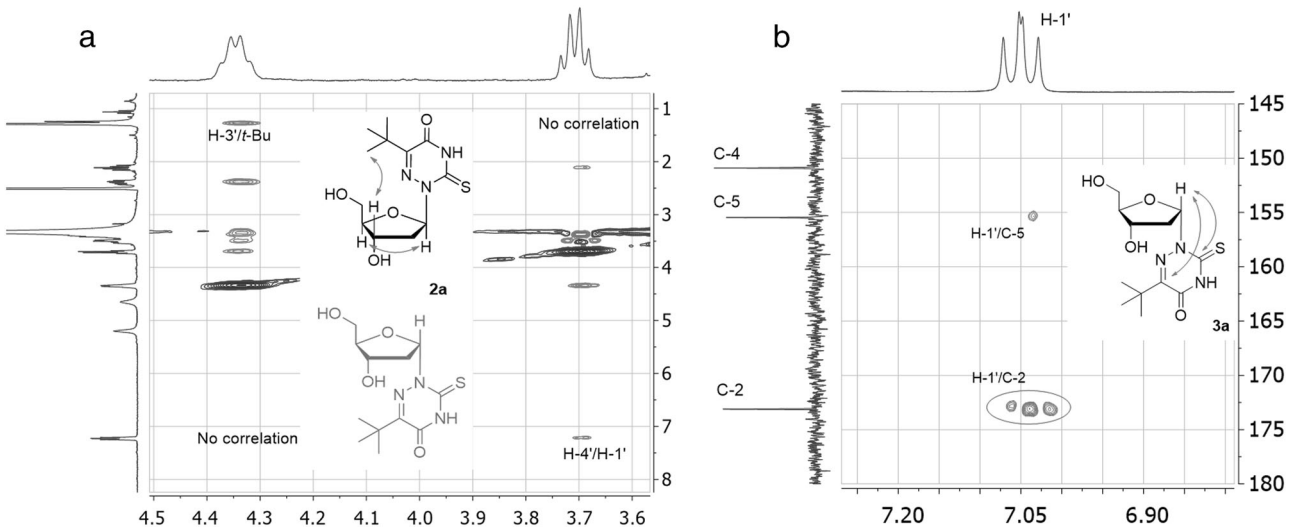



**Fig. 1** The two-dimensional nuclear magnetic resonance (2D NMR) spectra of the synthesized compounds. **a** Nuclear Overhauser effect spectroscopy (NOESY) NMR spectrum fragment of compound **2a**. **b** Heteronuclear multiple bond correlation (HMBC) NMR spectrum fragment of compound **3a**

T lymphocyte) and A549 (pulmonary adenocarcinoma) as well as *Vero* cell culture (kidney cells of the green monkey) by Trypan blue assay [35]. All compounds showed no cytotoxic effect at the concentrations up to 200 μg $ml^{-1}$ (about 0.6 mM), which is a typical value for currently used antimicrobial drugs.

## Bacterial growth inhibition

Antibacterial effect of the obtained compounds was studied as described earlier by their ability to inhibit the growth in vitro of a number of microorganisms [36]: Gram-positive bacteria were: *Bacillus subtilis* ATCC 6633, methicillin-resistant *Staphylococcus aureus* (MRSA) strain INA 00761 MRSA (MRSA strains occur widely and cause intrahospital infections that resist the modern antibiotic therapy); streptococcus-like bacteria *Leuconostoc mesenteroides* (strain VKPM B-4177 distinguished by a high native resistance to glycopeptide antibiotics of the vancomycin group, which often appear effective towards pathogenic bacteria with multidrug resistance); two strains of mycobacteria *M. smegmatis*: $mc^2155$ and VKPM Ac 1339 (which are used for the preliminary assessment of the activity followed by the analysis of promising compounds against the strains of *M. tuberculosis*—the causative agent of tuberculosis). Gram-negative bacteria were: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (opportunistic human pathogen, causing difficulty in the treatment of nosocomial infection); baking yeast *Saccharomyces cerevisiae* INA 01129 and fungal test culture *Aspergillus niger* INA 00760.

Previously both α- and β-anomers of 5'-thiourea-substituted thymidine and 5-dodecyloxymethyl derivative of 2'-deoxyuridine were shown to inhibit *M. tuberculosis* and *M. bovis* [26, 37]. Hence, we tested both anomers of our products. The results are summarized in Table 1 below.

The majority of the examined compounds, with the exception of 2'-deoxy-5-phenyl- and 5-(*tert*-butyl)-6-azauridines (**2c, d** and **3c, d**), suppressed the growth of Gram-positive bacteria. In all cases, the antibacterial effect of 2-thio derivatives was higher than of 2-oxo derivatives. They showed moderate activity towards *M. smegmatis* MIC = 0.2–0.8 mM for VKPM Ac 1339 strain and 0.6 mM for $mc^2$ 155 strain (MIC is the lowest concentration of antimicrobial that inhibits the visible growth of a microorganism after overnight incubation). Moreover, both α-anomers (**3a** and **3b**) showed considerably higher activities compared with the β-counterparts (Table 1).

The data obtained are comparable with the activities of a number of antibiotics in medical use. For example, $MIC_{99}$ against *M. tuberculosis* of widely used antibiotics are: 0.8 mM of pyrazinamide (one of the first-line anti-tuberculosis drugs) [38] and 0.05 mM of amikacin (Table 1). The

**Table 1** Antibacterial effect of the obtained compounds (mM)

| Bacterial strains | Tested compounds | | | | | | | | | | | Antibiotics in medical use |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Parental triazine bases | | | | 2'-Deoxynucleosides | | | | | Ribonucleosides | | |
| | **1a** | **1b** | **1c** | **1d** | **2a** | **2b** | **3a** | **3b** | **2c, d, 3c, d** | **4a** | **4b** | |
| *B. subtilis* ATCC 6633 | 1.46 | 0.43 | 1.24 | 1.43 | 0.96 | 0.9 | 0.96 | 0.9 | >2 | 1.04 | 0.98 | AN (0.007) |
| *L. mesenteroides* VKPM B-4177 | 1.46 | 1.32 | 1.3 | 1.43 | 0.93 | 0.12 | 0.23 | 0.9 | >2 | 0.12 | 0.05 | CIP (0.006); VA (>0.275) |
| *S. aureus* INA 00761 | 0.8 | 0.43 | >2 | 1.75 | 0.3 | 0.03 | 1.1 | 0.32 | >2 | 1.04 | 0.98 | AN (0.05); CIP (0.012); OX (0.08) |
| *M. smegmatis* VKPM Ac 1339 | 0.54 | 0.28 | >2 | >2 | 0.8 | 0.8 | 0.23 | 0.2 | >2 | 0.19 | 0.27 | AN (0.05); CIP (0.012); INZ (0.002); RFP (0.01) |
| *M. smegmatis* $mc^2$ 155 | 1.46 | 1.1 | >2 | 1.43 | 0.66 | 0.6 | 0.66 | 0.6 | >2 | 0.6 | >2 | AN (0.05); CIP (0.012); INZ (0.029); RFP (0.005) |
| *E. coli* ATCC 25922 | 1.46 | 0.8 | 1.3 | 1.43 | 0.93 | 0.03 | 0.23 | 0.9 | >2 | 1.04 | 0.53 | CIP (0.015) |
| *P. aeruginosa* INA 00760 | >2 | 0.8 | 0.97 | 1.75 | >2 | 0.03 | 0.55 | 0.5 | >2 | 1.04 | 0.98 | CIP (0.015) |

All tests were performed in at least three independent experiments

*AN* Amikacin, *CIP* Ciprofloxacin, *INZ* Isoniazid, *RF* Rifampicin, *OX* Oxacillin, *VA* Vancomycin

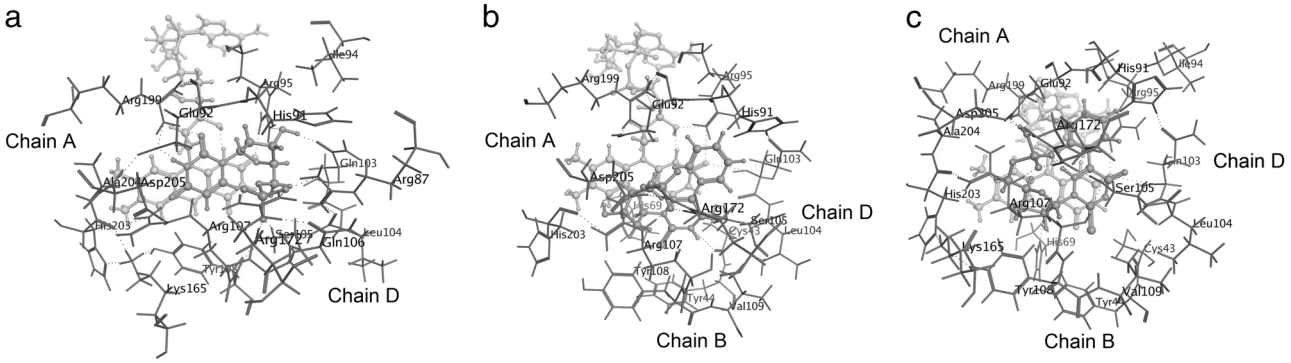



**Fig. 2** The binding mode of 5'-monophosphate of 2'-deoxyuridine (dUMP) substrate (**a**) and compounds 2-thio-5-phenyl-6-aza-β-dUMP (**b**), or 5'-phosphate of 2-thio-5-phenyl-6-aza-α-dUMP (**c**) within thymidylate synthase (ThyX) active site. Flavin cofactor fragment is shown in green

latter antibiotic also inhibit the growth of *S. aureus* ($MIC_{99} = 0.05$ mM), which does not have ThyX enzyme, thus suggesting that it is not the unique target for its antibacterial activity.

The synthesized 2-thio nucleosides also blocked the growth of *S. aureus* strain INA 00761 MRSA (MIC = 0.03–0.9 mM); β-anomer of 2'-deoxy-2-thio-5-phenyl-6-azauridine (**2a**) demonstrated the best activity with MIC = 0.03 mM, comparable to the activity of antibiotics used in medical practice.

2'-Deoxy-2-thio-5-phenyl-6-azauridine (**2b**) was the only one to effectively suppress the growth of *P. aeruginosa* ATCC 27853 (MIC = 0.03 mM) and *Escherichia coli* ATCC 25922, comparable to the activity of ciprofloxacin. The antibacterial activity of the other examined compounds towards Gram-negative bacteria was rather low if any.

Test strains of fungi *A. niger* INA 00760 and *S. cerevisiae* RIA 259 are resistant to the action of the compounds. An exception is compound **4a**, which exhibits weak activity.

It is well known that many antibiotics act not on one, but on several targets [39]. In our study, we have found out that, on one hand, 5-modified pyrimidine nucleosides effectively suppress the growth of *M. tuberculosis* [26, 40, 41], presumably by the ThyX enzyme inhibition [24], and on the other, they demonstrate destruction of cell wall of mycobacteria, suggesting that the mechanism of action for these compounds may be related to their interactions with bacteria cell walls [40].

## Molecular modeling

As mentioned above, we have found out that 2'-deoxy-2-thio-5-substituted-6-azauridines (**2a, b** and **3a, b**) showed activity against *M. smegmatis* strains. The latter are widely used for the preliminary assessment of the activity followed by the analysis of promising compounds against the strains of the causative agent of tuberculosis, *M. tuberculosis*.

To test the possibility of binding of the obtained modified nucleosides within the active site of this enzyme we performed in silico molecular modeling (Fig. 2). The natural substrate of *M. tuberculosis* ThyX enzyme is 5'-monophosphate of 2'-deoxyuridine (dUMP). The compounds 2-thio-5-phenyl-6-aza-α-dUMP, 2-thio-5-phenyl-6-aza-β-dUMP, 2-thio-5-*tert*-butyl-6-aza-α-dUMP, and 2-thio-5-*tert*-butyl-6-aza-β-dUMP, as well as the substrate, dUMP, were docked in the 2.01 Å resolution structure of the ThyX complex with 5-Br-dUMP (Protein Data Bank (PDB) file 2AF6) [42] using the Molecular Operating Enviroment program (MOE) [43] and AMBER 99 [44]. The active center of ThyX enzyme is the site where the substrate interacts with the polypeptide chains of the A- and D-subunits.

We conducted MMFF94x mixed force field method job (parametrization data can be found in the Supplementary file) to evaluate the free energy and optimize geometry of the studied compounds on their own and in complex with the enzyme [45, 46]. The optimization process was run until the energy gradient of the system reached 0.001 kcal $mol^{-1}$; further iterations led to no significant changes in energy and geometry minima.

Before discussing the results of optimizations, it should be noted that there are at least two tautomers. This fact is unambiguously seen when comparing tautomers in the UV spectra and quantum-mechanical calculations of these spectra (see Supplementary file pages S33–S39). As there are two tautomeric forms, the energy binding was calculated for both of them.

All the synthesized compounds are stronger bound in the active site than in natural substrate (dUMP) and, therefore, have good inhibitory properties. 5-Phenyl derivatives appeared to have better interaction with α-deoxyribose residue, while 5-*tert*-butyl derivatives possess better interaction with β-deoxyribose; nevertheless, this effect may be speculated due to complexity of hydrophobic interaction of *tert*-butyl moiety in MMFF94x parametrization.

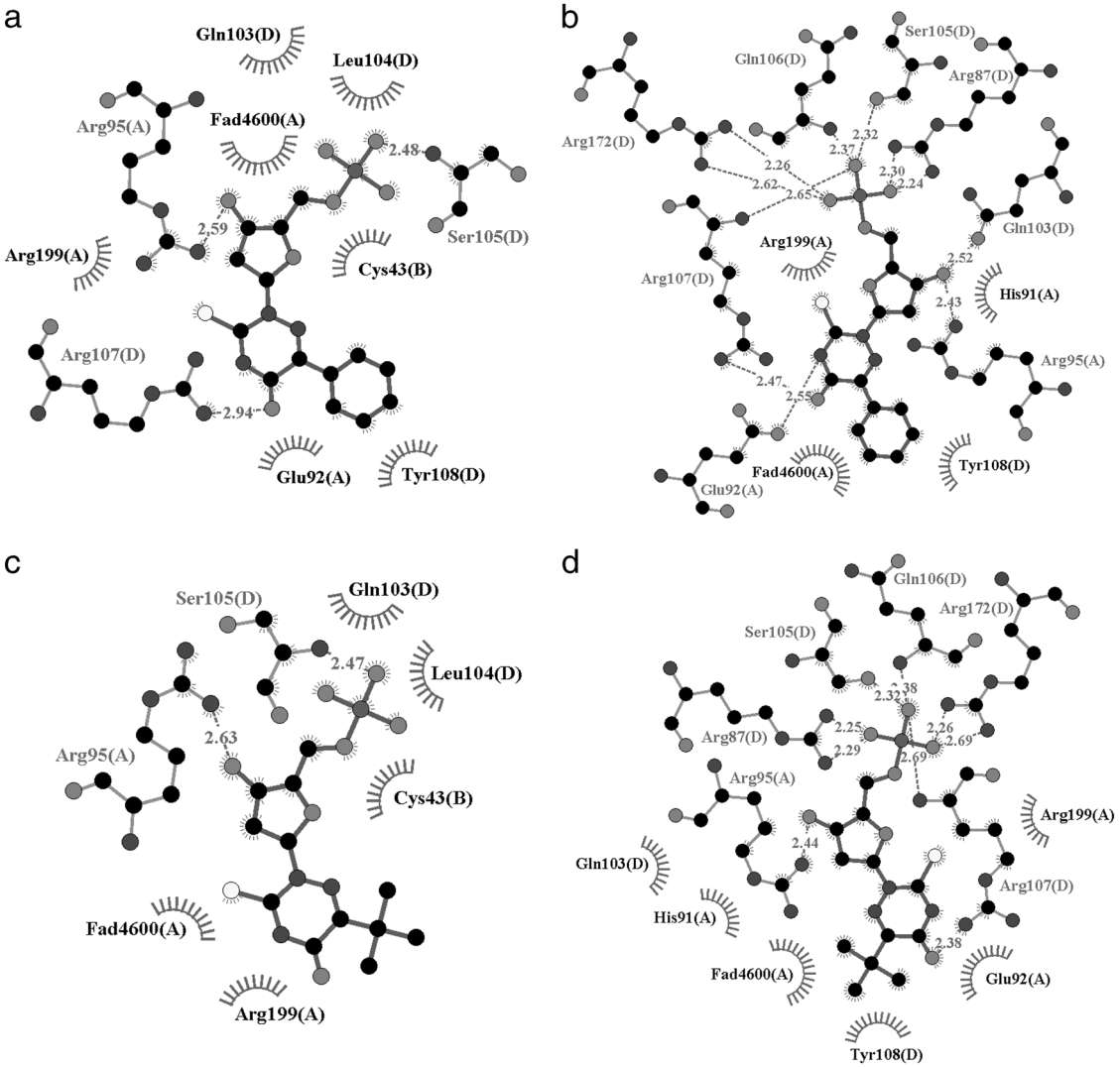



**Fig. 3** Main contacts of 2-thio-5-phenyl-6-aza-dUMP ((**a**) α-deoxyribose isomer and (**b**) β-deoxyribose isomer) and 2-thio-5-*tert*-butyl-6-aza-dUMP ((**c**) α-deoxyribose isomer and (**d**) β-deoxyribose isomer). The figure is created using LigPlus program [47]. Flavin contacts are fully taken into account

Figure 3 demonstrates the main possible contacts between the nucleoside and amino acid residues of the ThyX active site (more detailed summary can be found in the Supplementary file, page S40).

It can be figured out that the most conservative residues are chain A amino acids. Seven out of eight amino acids contact both with the substrate and the inhibitors. The exception is Ile208, which contacts only with the phosphate group of 2-thio-5-*tert*-butyl-6-aza-α-dUMP. Out of 12 amino acids of the chain D, 6 (Gln103, Leu104, Ser105, Arg107, Tyr108, and Arg172) contact both with the substrate and all the inhibitors; remaining amino acids have different specificities.

Amino acids of the chain B are also involved in binding with the inhibitors, in addition to chains A and D. There are several points of interaction: Cys43 polarizes the S(2) atom of Ura or makes hydrogen bonding with other heteroatoms of Ura; Tyr44 is involved in van der Waals interactions with Ura moiety. In the case of β-deoxyribose derivatives, His69 facilitates hydrogen or polar bond with S(2) Ura, while it makes van der Waals bonding with Ura of the α-deoxyribose derivatives.

All the above described connections of the compounds and the substrate in the active site of ThyX are additionally stabilized by hydrogen bonding of the neighboring amino acids (see Table 3 of the Supplementary file), forming a well-developed network between themselves, compounds, and flavin. The results of molecular modeling demonstrate the possibility of binding of our derivatives with flavin-dependent ThyX of *M. tuberculosis* as a possible target for the anti-tuberculosis action of the 5-modified 2'-deoxy-2-thio-6-azauridines.

## Experimental section

Commercial reagents were purchased from Acros, Aldrich, and Fluka. Column chromatography was performed on silica gel 60 0.040–0.063 mm (Merck, Germany). Thin-layer chromatography was performed on silica gel 60 $F_{254}$ plates (Merck, Germany). NMR spectra were registered on an AMX III-400 spectrometer (Bruker, USA) with the working frequency of 400 MHz for $^1$H NMR ($Me_4Si$ as an internal standard for organic solvents) and 100.6 MHz for $^{13}$C NMR (with the carbon–proton interaction decoupling). UV spectra were recorded on a UV-2401PC spectrophotometer (Shimadzu, Japan) in ethanol. High-resolution mass spectra were recorded on a Bruker Daltonics micrOTOF-Q II device by electrospray ionization mass spectrometry (ESI-MS). Measurements were carried out in positive ion mode under the following conditions: spray capillary voltage 4500 V; the mass scanning range *m/z* 100–3000 Da; external calibration (Electrospray Calibrant Solution, Fluka); nebulizer pressure 0.8 bar; flow rate 3 μl · min$^{-1}$; nebulizer gas–nitrogen (4 l · min$^{-1}$); and interface temperature 190 °C. Samples were injected to the spray chamber of the mass spectrometer from an Agilent 1260 liquid chromatograph equipped with an Agilent Poroshell 120 EC-C18 column (3.0 × 50 mm; 2.7 μm); the flow rate was 0.2 ml · min$^{-1}$; the samples of compounds were loaded to a high-performance liquid chromatograph from acetonitrile–water solution 1:1 (5 μl).

Starting 2-thio-5-(*tert*-butyl)-6-azauracil (**1a**), 2-thio-5-phenyl-6-azauracil (**1b**), 5-(*tert*-butyl)-6-azauracil (**1c**), and 5-phenyl-6-azauracil (**1d**), were obtained as previously reported [32], 2-thio-5-(*tert*-butyl)-6-azauridine (**4a**) and 2-thio-5-phenyl-6-azauridine (**4b**) were synthesized as previously reported [33].

### A general method for transglycosylation

Thymidine (121 mg, 0.5 mmol) and 2,5-substituted-6-azauracil (1.0 mmol) were dissolved in acetonitrile (10 ml) and then *N*,*O*-bis(trimethylsilyl)acetamide (0.7 ml, 2.7 mmol) was added. The mixture was stirred at 82 °C for 15 min. A solution of trimethylsilyl triflate (100 μl, 0.6 mmol) in dichloroethane (5 ml) was added. The mixture was refluxed for 45 min, cooled to room temperature, and neutralized with pyridine (5 ml). Anomers (60–70% overall) were isolated by column chromatography in $CHCl_3$: EtOH, 100:1 (v/v) eluting system.

### 2′-Deoxy-2-thio-5-(tert-butyl)-6-azauridine, β-anomer (2a)

$^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.29 (9 H, s, *t*-Bu), 2.07-2.16 (1 H, m, H-2'b), 2.36-2.44 (1 H, ddd, $J = 4$, 6 and 13 Hz, H-2'a), 3.48-3.54 (2 H, dd, $J = 9$ and 12 Hz, $H_2$-5'), 3.68-3.73 (1 H, dd, $J = 5$ and 10 Hz, H-4'), 4.32-4.37 (1 H, m, H-3'), 4.65 (1 H, s, OH-5'), 5.20 (1 H, s, OH-3'), 7.21-7.25 (1 H, dd, $J = 4$ and 7 Hz, H-1'). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): δ 27.21 (($CH_3$)$_3$-, (*t*-Bu)), 37.05 (C, *t*-Bu), 38.54 (C-2'), 62.12 (C-5'), 70.24 (C-3'), 87.47 (C-4'), 88.90 (C-1'), 151.12 (C-4), 155.42 (C-5), 174.24 (C-2). UV: $\lambda_{max}$ 272.1 nm (ε 15035). HRMS (ESI) calcd $[M + Na]^+$ for $C_{12}H_{19}N_3O_4S$: *m/z* 324.0988, found: *m/z* 324.0990. Yield: 50.2 mg (31%).

### 2′-Deoxy-2-thio-5-(tert-butyl)-6-azauridine, α-anomer (3a)

$^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.30 (9 H, s, *t*-Bu), 2.14-2.23 (1 H, m, H-2'b), 2.55-2.61 (1 H, m, H-2'a), 3.38-3.40 (1 H, m, H-5'a), 3.58-3.61 (1 H, dd, $J = 6$ and 12 Hz, H-5'b), 3.98-4.08 (2 H, m, H-4' + H-3'), 4.68 (1 H, s, OH-5'), 5.14 (1 H, s, OH-3'), 7.04-7.07 (1 H, dd, $J = 6$ and 7 Hz, H-1'). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): δ 27.22 (($CH_3$)$_3$-, *t*-Bu), 36.91 (C, *t*-Bu), 38.15 (C-2'), 61.02 (C-5'), 69.08 (C-3'), 86.42 (C-4'), 88.53 (C-1'), 150.90 (C-4), 155.30 (C-5), 173.19 (C-2). UV: $\lambda_{max}$ 272.1 nm (ε 15035). HRMS (ESI) calcd $[M + Na]^+$ for $C_{12}H_{19}N_3O_4S$: *m/z* 324.0988, found: *m/z* 324.0988. Yield: 63.2 mg (39%).

### 2′-Deoxy-2-thio-5-phenyl-6-azauridine, β-anomer (2b)

$^1$H NMR (400 MHz, DMSO-$d_6$): δ 2.24-2.30 (1 H, m, H-2'b), 2.62-2.69 (1 H, dt, $J = 7$ and 14 Hz, H-2'a), 3.42-3.46 (1 H, dd, $J = 5$ and 12 Hz, H-5'a), 3.60-3.64 (1 H, dd, $J = 2$ and 12 Hz, H-5'b), 4.10-4.15 (2 H, m, H-4' + H-3'), 7.14-7.17 (1 H, dd, $J = 5$ and 7 Hz, H-1'), 7.46-7.54 (3 H, m, *o*-Ph + *p*-Ph), 8.08-8.11 (2 H, m, *m*-Ph), 13.34 (1 H, s, H-3). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): δ 38.45 (C-2'), 62.24 (C-5'), 67.72 (C-3'), 69.32 (C-4'), 85.64 (C-1'), 128.19 (*m*-Ph), 128.25 (*o*-Ph), 130.46 (*p*-Ph), 131.28 (*i*-Ph), 144.95 (C-5), 151.42 (C-2), 173.19 (C-4). UV: $\lambda_{max}$ 282.1 nm (ε 24000). HRMS (ESI) calcd $[M + H]^+$ for $C_{14}H_{15}N_3O_4S$: *m/z* 322.0856, found: *m/z* 322.0851; calcd $[M + NH_4]^+$ for $C_{14}H_{15}N_3O_4S$: *m/z* 339.1122, found: *m/z* 339.1117; calcd $[M + Na]^+$ for $C_{14}H_{15}N_3O_4S$: *m/z* 344.0675, found: *m/z* 344.0669. Yield: 61.4 mg (36%).

### 2′-Deoxy-2-thio-5-phenyl-6-azauridine, α-anomer (3b)

$^1$H NMR (400 MHz, DMSO-$d_6$): δ 2.16-2.22 (1 H, ddd, $J =$ 7, 7 and 13 Hz, H-2'b), 2.44-2.49 (1 H, m, H-2'a), 3.40-3.45 (1 H, dd, $J = 5$ and 11 Hz, H-5'a), 3.52-3.56 (1 H, dd, $J = 5$ and 11 Hz, H-5'b), 3.75-3.80 (1 H, dd, $J = 5$ and 5 Hz, H-4'), 4.39-4.43 (1 H, d, $J = 5$ Hz, H-3'), 4.64 (1 H, s,

OH-5'), 5.22 (1 H, s, OH-3'), 7.28-7.30 (1 H, dd, $J = 4$ and 7 Hz, H-1'), 7.46-7.54 (3 H, m, *o*-Ph + *p*-Ph), 7.95-7.98 (2 H, m, *m*-Ph), 13.34 (1 H, s, H-3). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): $\delta$ 37.99 (C-2'), 61.75 (C-5'), 70.22 (C-3'), 87.67 (C-4'), 89.36 (C-1'), 128.17 (*m*-Ph), 128.36 (*o*-Ph), 130.35 (*p*-Ph), 131.43 (*i*-Ph), 145.76 (C-5), 151.42 (C-2), 173.83 (C-4). UV: $\lambda_{max}$ 282.1 nm (ε 24000). HRMS (ESI) calcd $[M + H]^+$ for $C_{14}H_{15}N_3O_4S$: *m/z* 322.0856, found: *m/z* 322.0855; calcd $[M + NH_4]^+$ for $C_{14}H_{15}N_3O_4S$: *m/z* 339.1122, found: *m/z* 339.1121; calcd $[M + Na]^+$ for $C_{14}H_{15}N_3O_4S$: *m/z* 344.0675, found: *m/z* 344.0673. Yield: 62.4 mg (36%).

### 2′-Deoxy-5-(tert-butyl)-6-azauridine β-anomer (2c)

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.28 (9 H, s, *t*-Bu), 2.03-2.11 (1 H, m, H-2'b), 2.39-2.47 (1 H, m, H-2'a), 3.33-3.41 (1 H, dd, $J = 6$ and 12 Hz, H-5'a), 3.45-3.50 (1 H, dd, $J = 6$ and 12 Hz, H-5'b), 3.66-3.71 (1 H, dd, $J = 5$ and 11 Hz, H-4'), 4.29-4.35 (1 H, dd, $J = 6$ and 11 Hz, H-3'), 6.32-6.36 (1 H, dd, $J = 5$ and 7 Hz, H-1'). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): 27.94 ($(CH_3)_3$-, *t*-Bu), 37.18 (C-, *t*-Bu), 37.34 (C-2'), 62.65 (C-5'), 70.93 (C-3'), 84.91 (C-4'), 87.69 (C-1'), 149.18 (C-2), 150.80 (C-4), 155.94 (C-5). UV: $\lambda_{max}$ 272.1 nm (ε 15035). HRMS (ESI) calcd $[M-H]^-$ for $C_{12}H_{19}N_3O_5$: *m/z* 284.1241, found: *m/z* 284.1247. Yield: 50.7 mg (36%).

### 2′-Deoxy-5-(*tert*-butyl)-6-azauridine, α-anomer (3c)

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.29 (9 H, s, *t*-Bu), 2.26-2.35 (1 H, ddd, $J = 6$, 8 and 13 Hz, H-2'b), 2.44-2.51 (1 H, ddd, $J = 4$, 6 and 13 Hz, H-2'a), 3.34-3.40 (1 H, dd, $J = 9$ and 12 Hz, H-5' a), 3.57-3.62 (1 H, dd, $J = 9$ and 12 Hz, H-5' b), 3.82-3.87 (1 H, dd, $J = 5$ and 10 Hz, H-3'), 4.00-4.07 (1 H, dd, $J = 5$ and 10 Hz, H-4'), 6.19-6.24 (1 H, dd, $J = 4$ and 7 Hz, H-1'), 12.00 (1 H, s, H-3). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): $\delta$ 27.96 ($(CH_3)_3$- (*t*-Bu)), 37.06 (*t*-Bu), 38.54 (C-2'), 62.12 (C-5'), 70.30 (C-3'), 84.17 (C-4'), 85.99 (C-1'), 149.03 (C-2), 150.75 (C-4), 155.84 (C-5). UV: $\lambda_{max}$ 272.1 nm (ε 15035). HRMS (ESI) calcd $[M-H]^-$for $C_{12}H_{19}N_3O_5$: *m/z* 284.1241, found: *m/z* 284.1240. Yield: 50.1 mg (35%).

### 2′-Deoxy-5-phenyl-6-azauridine, β-anomer (2d)

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.10-2.18 (1 H, ddd, $J =$ 6, 7 and 13 Hz, H-2'b), 2.47-2.56 (4 H, m, H-2'a + DMSO-$d_5$), 3.38-3.44 (1 H, dd, $J = 6$ and 12 Hz, H-5'a), 3.48-3.55 (1 H, dt, $J = 5$ and 10 Hz, H-5'b), 3.71-3.76 (1 H, dd, $J = 5$ and 10 Hz, H-4'), 4.36-4.43 (1 H, m, H-3'), 4.62-4.66 (1 H, t, $J = 6$ Hz, OH-5'), 5.19-5.21 (1 H, d, $J = 5$ Hz, OH-3'), 6.42-6.46 (1 H, dd, $J = 4$ and 7 Hz, H-1'), 7.45-7.50 (3 H, m, *o*-Ph + *p*-Ph), 7.90-7.94 (2 H, m, *m*-Ph), 12.24 (1 H, s, H-3). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): $\delta$ 37.75 (C-2'), 62.37 C-5', 70.81 (C-3'), 85.29 (C-4'), 87.78 (C-1'), 128.61 (*m*-Ph), 128.66 (*o*-Ph), 130.19 (*p*-Ph), 132.69 (*i*-Ph), 141.96 (C-2), 148.93 (C-4), 156.57 (C-5). UV: $\lambda_{max}$ 282.1 nm (ε 24000). HRMS (ESI) calcd $[M-H]^-$ for $C_{14}H_{15}N_3O_5$: *m/z* 304.0928, found: *m/z* 304.0920. Yield: 54.7 mg (36%).

### 2′-Deoxy-5-phenyl-6-azauridine, α-anomer (3d)

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.32-2.41 (1 H, ddd, $J =$ 6, 7 and 13 Hz, H-2'b), 2.55-2.62 (1 H, m, H-2'a), 3.38-3.44 (1 H, dd, $J = 6$ and 12 Hz, H-5'a), 3.56-3.63 (1 H, ddd, $J = 3$, 5 and 12 Hz, H-5'b), 3.93-3.96 (1 H, m, H-4'), 4.06-4.15 (1 H, m, H-3'), 4.67-4.71 (1 H, t, $J = 6$ Hz, OH-5'), 5.17-5.18 (1 H, d, $J = 6$ Hz, OH-3'), 6.32-6.36 (1 H, dd, $J = 6$ and 7 Hz, H-1'), 7.46-7.50 (3 H, m, *o*-Ph + *p*-Ph), 7.98-8.04 (2 H, m, *m*-Ph), 12.27 (1 H, s, H-3). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): $\delta$ 37.89 (C-2'), 61.69 (C-5'), 70.51 (C-3'), 84.81 (C-4'), 86.55 (C-1'), 128.63 (*m*-Ph + *o*-Ph), 130.23 (*p*-Ph), 132.64 (*i*-Ph), 141.70 (C-2), 148.80 (C-4), 156.57 (C-5). UV: $\lambda_{max}$ 282.1 nm (ε 24000). HRMS (ESI) calcd $[M-H]^-$ for $C_{14}H_{15}N_3O_5$): *m/z* 304.0928, found: *m/z* 304.0918. Yield: 53.2 mg (35%).

### Cell cultures

The African green monkey kidney cell line *Vero* E6 (ATCC no. CCL-1587) was obtained from the State Collection of Cell Cultures (Ivanovskii Research Institute of Virology, Gamaleya Federal Research Center for Epidemiology and Microbiology, Ministry of Public Health and Social Development of Russia). The lines of lung carcinoma A549 (ATCC no. CCL-185) and T lymphocyte cells Jurkat (ATCC no. CRL-2676) were obtained from the collection of the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences. Adherent Vero E6 cells being passaged were cultured in EAGLE'S medium containing 5% fetal calf serum, 2 mM glutamine, and 100 units $ml^{-1}$ of penicillin at 37 °C in an atmosphere of 5% $CO_2$ at a 90% humidity. The cytotoxicity of compounds was quantitatively estimated from the intensity of the staining of dead cells by Trypan blue [35]. The $CD_{50}$ value is the concentration of a compound at which the cell survival was 50%.

Adherent A549 cells being passaged were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 2 mM glutamine at 37 °C in an atmosphere of 5% $CO_2$ at a humidity of 90%. A suspension culture of Jurkat cells was cultured in medium RPMI containing 10% fetal calf serum and 2 mM glutamine under the same conditions. Cytotoxicity of the compounds was determined by the MTT test [34]. The $CC_{50}$ value is the

concentration of a compound required to inhibit 50% cell culture growth.

### Study of the antibacterial effect

The following test strains were used: Gram-positive bacteria: *B. subtilis* ATCC 6633, *S. aureus* INA 00761 (MRSA), *L. mesenteroides* VKPM B-4177, *M. smegmatis* $mc^2$ 155, and *M. smegmatis* VKPM Ac 1339; Gram-negative bacteria: *P. aeruginosa* ATCC 27853; and fungi: *A. niger* and *S. cerevisiae* INA 01129 from the collection of the Gause Institute of New Antibiotics. Test strains were incubated in modified Gause's nutrient medium no. 2 containing (in percent): glucose 1, peptone 0.5, tryptone 0.3, NaCl 0.5, and tap water; pH of medium 7.2–7.4. The level of infection with test cultures was $10^6$ cells per ml. A compound being tested was dissolved in a water–dimethyl sulfoxide (DMSO)–Tween-80 mixture (50:45:5) or 50% aq methanol. Ten volume percent of tested was added to the nutrient medium. Samples without the addition of substances and samples of medium supplemented with a mixture of solvents served as controls of the test culture growth. Fungal test cultures and *L. mesenteroides* were incubated at 28 °C, and all other strains were incubated at 37 °C.

### Molecular modeling

Jobs were calculated using MOE 2009.10 program [43], the algorithm of calculation of binding energies has been previously described in detail [22].

## Conclusions

In summary, we have demonstrated a fast and simple method for preparation of α- and β-anomers of the 5-modified 6-aza- and 2-thio-6-aza-2'-deoxyuridine derivatives that leads to high yields. 2-Thio derivatives showed moderate activity against *M. smegmatis*, *S. aureus*, and some other Gram-positive bacteria. None of the obtained compounds was active against Gram-negative bacteria and fungi with the exception of β-2'-deoxy-2-thio-5-phenyl-6-azauridine (**2b**), which effectively suppressed the growth of *P. aeruginosa* ATCC 27853. The convenient method for the synthesis of 5-modified 6-aza- and 2-thio-6-aza-2'-deoxyuridine derivatives will make it possible to obtain novel series of effective inhibitors of bacterial replication. We assume that modified 2'-deoxy-2-thio-6-azauridines are likely to act on other than ThyX targets as well.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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