Bioorganic & Medicinal Chemistry 20 (2012) 6680–6686

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# The synthesis and antituberculosis activity of 5′-nor carbocyclic uracil derivatives

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ARTICLE INFO

*Article history:*
Received 10 June 2012
Revised 6 September 2012
Accepted 11 September 2012
Available online 19 September 2012

*Keywords:*
Carbocyclic nucleosides
*Mycobacterium tuberculosis*
Enantiomers
Uracil derivatives

ABSTRACT

A series of new carbocyclic uracil derivatives were synthesized and evaluated as potential antituberculosis agents. Racemic 1-[4′-hydroxy-2′-cyclopenten-1′-yl]-5-tetradecynyluracil completely inhibited the growth of *Mycobacterium tuberculosis* H37Rv in vitro at a concentration of 10 μg/ml. Individual (+) and (−) isomers of the above uracil derivative were isolated and showed the same level of activity against two strains of *Mycobacterium tuberculosis*: laboratory sensitive (H37Rv) and clinical resistant to five top antituberculosis drugs (MS-115).



## 1. Introduction

Despite numerous efforts, tuberculosis still remains one of the main causes of morbidity and mortality of the world population. The number of people infected with tubercle bacillus all over the world is about two billion and the tendency toward the growth of tuberculosis active form is increasing. Annually the number of patients with tuberculosis is increased by eight million and the number of death achieves two million. Several factors can affect the development of tuberculous infection.[1] One of the reasons is the development of mycobacterial strains resistant to the current chemotherapy. Because of mutational and adaptive changes mycobacteria become resistant to the used drugs. New *Mycobacterium tuberculosis* strains, namely, XDR-TB (extensive drug resistant), which is nearly incurable, and MDR-TB (multidrug resistant) are indifferent to routine treatment. The treatment of drug resistant tuberculosis is a long, stressful, and high-cost although not always successful process.[1] A great challenge is a combination of tuberculosis and human immunodeficiency virus. HIV-infected patients are in the group of increased risk of TB development, whereas HIV-infected patients with TB are at the high risk of death. All these facts make it necessary to search for new antituberculosis (antiTB) agents. They should work by different mechanisms than currently used therapeutics for which resistance has developed. Thus this should render them active against TB resistant strains. This direction seems to be the most promising because drug resistance in the tuberculosis therapy achieved a critical point.[2] In this connection, not only new antibiotics, but also compounds from other classes are of interest because the structural difference can provide another mechanism of action and, hence, slow down the development of drug resistance. Nucleoside-based drugs are included in the drug lists for therapy of various viral infections and tumor diseases. However, among the current antiTB agents there are no compounds of the nucleoside nature.

Recently some reports on the modified nucleosides active against *M. tuberculosis*, *Mycobacterium bovis* and *Mycobacterium avium* have been published.[2–8] Particularly, some of the 5-alkynyl, 5-phenylalkynyl, and 5-pyridylalkynyl derivatives of pyrimidine nucleosides showed noticeable antiTB properties.[2–8] The best activities among analogues of uridine ($MIC_{90}$ 1–10 μg/ml) were found for 5-alkynyl (decynyl, dodecynyl, tetradecynyl, pyridylethynyl etc.) uracil derivatives bearing different sugar modification (see Fig. 1). Unfortunately the mechanisms of their action and target enzymes were unclear.[2,3,5–8]

Carbocyclic nucleosides are analogues of natural nucleic acid components in which the ring oxygen atom of the carbohydrate fragment is replaced by a methylene group. Compounds of this type are recognized by many receptors and enzymes due to their structural similarity to natural nucleosides. They display an increased stability to phosphorylase- and hydrolase-induced deglycosylation and possess a wide spectrum of biological activity,

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http://dx.doi.org/10.1016/j.bmc.2012.09.019

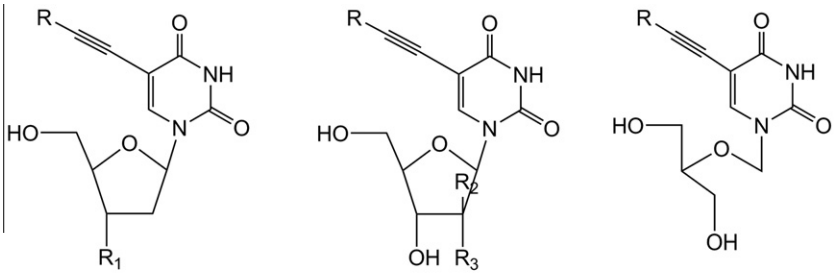



**Figure 1.** Chemical structures of 5-alkynyl uracil derivatives bearing different sugar modification.

particularly, antiviral and anticancer.[9] In an effort to overcome the cellular cytotoxicity of carbocyclic nucleosides that inhibit their target enzymes without preliminary phosphorylation (for example aristeromicyn and neplanocin A) 5′-nor carbocyclic nucleosides were designed. Due to the lack of a primary hydroxyl group they cannot be recognized by cell kinases and, therefore, are less toxic. We assumed that 5′-nor carbocyclic nucleosides with the 5-substituted uracil as a nucleic base can be promising antiTB agents. We also consider these compounds useful for the evaluation of the role of phosphorylation for inhibition of *M. tuberculosis* growth.

In this work we describe the synthesis of new 2′,3′-dideoxy-2′,3′-didehydro-5′-noruridine analogues **1**–**6** (Fig. 2) bearing 4′-hydroxy-2′-cyclopentenyl residues in place of the carbohydrate fragment as well as their inhibitory activities toward *M. tuberculosis*.

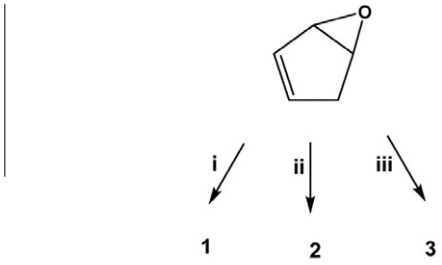
## 2. Results and discussion

### 2.1. Chemistry

The (−) enantiomer of compound **1** was earlier obtained by Schneller's group starting from the optically pure (−) 4-hydroxy-2-cyclopenten-1-yl acetate.[10,11] We obtained racemic carbocyclic nucleosides **1**–**3** by the Trost procedure[12] using the coupling of the other synthon, epoxy-cyclopentene, with uracil, 6-methyluracil or 5-iodouracil (Scheme 1).

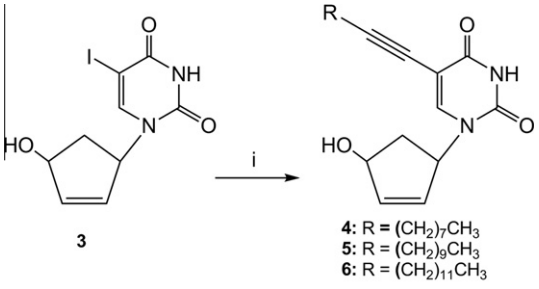
The target 5-alkynyl uracil derivatives **(±)-4-6** were synthesized by replacing the 5-iodine atom in 1-[4′-hydroxy-2′-cyclopenten-1′-yl]-5-iodouracil **(±)-3** for terminal alkynyl residues using the cross-coupling Sonogashira's reaction.[13] (Scheme 2)

The reactions were carried out at room temperature in the freshly distilled DMF under the argon atmosphere with TLC monitoring. The reactions were stopped in 2–4 h with 0.5 M EDTA/$H_2O$ to give the target compounds **(±)-4-6** in 24–32% yields. The corresponding bicyclic furanopurimidine carbocyclic derivatives **(±)-7-9** and 3,5-dialkynyl uracils **(±)-10-12** were obtained as side products under the same reaction conditions in the yields of 15 and 13%, respectively (Fig. 3). The formation of the similar side products for other uracil derivatives was described earlier.[2,6,7]




i) Uracil, $Pd(PPh_3)_4$, THF/DMF; ii) 6-methyluracil, $Pd(PPh_3)_4$, THF/DMF; iii) 5-iodouracil, $Pd(PPh_3)_4$, THF/DMF

**Scheme 1.** Synthesis of 5′-nor carbocyclic derivatives by the Trost procedure.




**Scheme 2.** Synthesis of 5-alkynyl uracil derivatives by the Sonogashira's coupling.

Enantiomers of analogue **(±)-6** were separated. The resolution of the racemic mixture was carried out by the enzyme-catalyzed reaction with Amano PS lipase according to the procedure described earlier.[15–17] The reaction was stopped when the 50% conversion was achieved. Filtration followed by standard work up procedure provided (−)-1-(4′-hydroxy-2′-cyclopenten-1′-yl)-5-iodouracil **(−)-3** and (+)-1-(4′-acethyloxy-2′-cyclopenten-1′-yl)-5-iodouracil

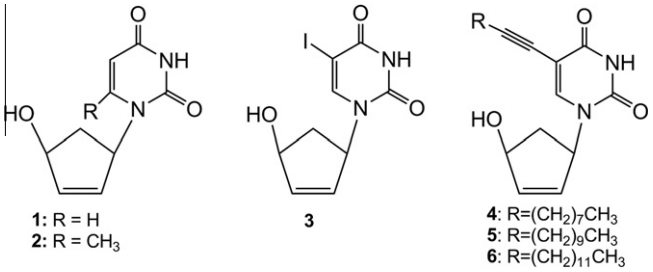



**Figure 2.** Chemical structures of 5′-nor carbocyclic uracil derivatives.

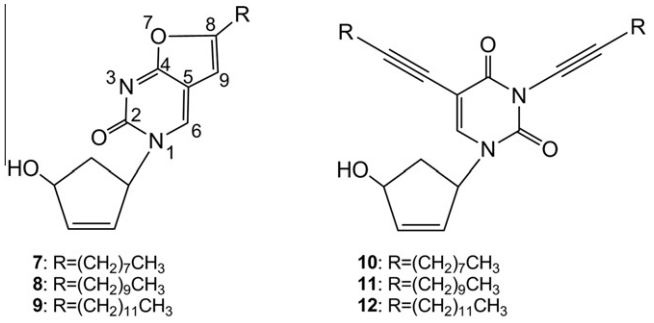
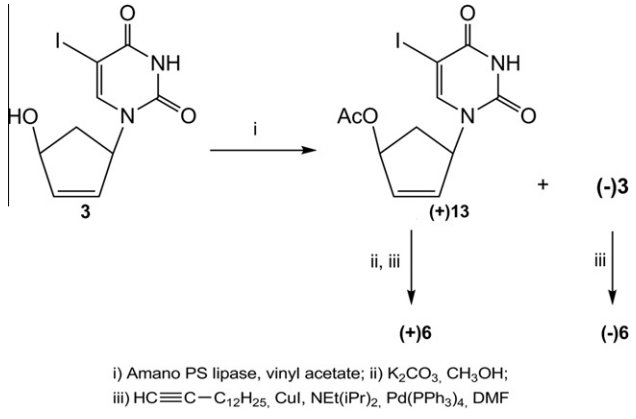



**Figure 3.** Chemical structures of 5′-nor carbocyclic derivatives of bicyclic furanopurimidine (7–9) and 3,5-dialkynyl (10–12) uracil.




**Scheme 3.** Enzyme-catalyzed resolution of enantiomers.

**(+)-13**. The treatment of 1-(4′-acethyloxy-2'-cyclopenten-1'-yl)-5-iodouracil **(+)-13** with $K_2CO_3$ in methanol gave (+)-1-(4′-hydroxy-2′-cyclopenten-1′-yl)-5-iodouracil **(+)-3**. (Scheme 3)

Compounds **(+)-3** and **(−)-3** were converted into the corresponding 5-tetradecynyl-uracil derivatives **(+)-6** and **(−)-6** similarly to the procedure described for **(±)-4-6**. The absolute configurations of target compounds **(+)-6** and **(−)-6** were confirmed by optical rotation $[\alpha]_D$ values and the data of circular dichroism.

## 2.2. Biological activity

The cytotoxicity of the synthesized compounds was evaluated in Vero cells. None of them were toxic in the concentration of 100 μg/ml.

The minimal inhibitory concentrations ($MIC_{99}$) of compounds **(±)-1-6** were determined using the proportion method in the BACTEC MGIT 960 system in the TB Exist mode.[14] For testing, a laboratory *M. tuberculosis* H37Rv strain was used, which is a virulent strain susceptible to all antiTB drugs. The solubility of the compounds under investigation in water was very low; therefore we developed a method for the preparation of stock solutions, preventing their precipitation when added to the culture medium. The use of Tween 80 (which is a part of Dubois medium) allowed us to prepare more concentrated stock solutions. It should be noted that this solvent mixture had no effect on *M. tuberculosis* growth. The compounds were tested at the concentrations of 2.5, 5, 10, 20 and 40 μg/ml. The results are shown in Table 1.

As it is seen in the table, compound **(±)-1** was the least toxic against the H37Rv strain. It inhibited the culture growth at none of the tested concentrations but, nevertheless, affected the culture by impeding the culture growth start if to compare with the control. Compounds **(±)-2** and **(±)-4** bearing in the nucleic base 6-methyl and 5-decynyl groups, respectively, were more active ($MIC_{99}$ of 40 μg/ml). At a concentration of 20 μg/ml these compounds negatively affected the culture: the culture growth started 2 and 3 days later, respectively, than in the control culture (Fig. 4). The activities of compounds **(±)-3** and **(±)-5** were similar and their $MIC_{99}$ achieved 20 μg/ml. The most active compound in our experiments with the H37Rv strain was **(±)-6**, whose $MIC_{99}$ was 10 μg/ml.

Compound **6**, as racemic mixture, as well as its enantiomers were tested against *M. tuberculosis* MDR strain (MS-115), which is resistant to isoniazide, rifampicin, streptomycin, ethambutol and pyrazinamide. The results are shown in Table 2. The activity of the (−)-isomer against H37Rv was found to be the highest ($MIC_{99}$ 5 μg/ml). However the racemic mixture and both isomers demonstrated a similar activity of 10 μg/ml against the MDR strain.

The results of testing allow us to reveal some structure-activity relationship. Particularly, the presence of substituents in uracil positions 5 or 6 supported antiTB activity. Compound **(±)-3** is the first example of the inhibitor of *M. tuberculosis* growth with 5-iodo substituent in the uracil residue. Although 5-iodouracil derivatives were normally used for the synthesis of the corresponding 5-alkynyluracils, their antiTB activity has not been described.[2,3,5–8]

For compounds bearing alkynyl fragments, the longer was the substituent residue, the higher was the antiTB activity. Particularly, $MIC_{99}$ of compound **(±)-4** containing an octynyl residue was 40 μg/ml, whereas for compounds **(±)-5** and **(±)-6** with decynyl and dodecynyl substituents in the uracil position 5 these values rose to 20 and 10 μg/ml, respectively. These results agree with the previous date on antiTB activities of 5-alkynyl uracil derivatives with different substituents at $N^1$ position.[2,3,5–8]

The most active compound proved to be (−) enantiomer of 5-tetradecynyluracil derivative **6**, which completely inhibited the growth of *M. tuberculosis* culture at a concentration of 5 μg/ml

**Table 1**
In vitro antimycobacterial activity of carbocyclyc uracil derivatives on *M. tuberculosis* H37Rv stain[a]

| Compound | % Inhibition (concentration μg/ml) | $MIC_{99}$[c] (μg/ml) | $CC_{50}$[d], (μg /ml) |
|---|---|---|---|
| **1** | 75 (40) | >40 | >200 |
| **2** | 100 (40) | 40 | >200 |
| **3** | 100 (40), 99 (20) | 20 | >200 |
| **4** | 100 (40) | 40 | >125 |
| **5** | 99 (20, 40) | 20 | >125 |
| **(±)6** | 100 (40, 20), 99 (10) | 10 | 125 |
| **std**[b] | 100 (1) | 1 | >500 |

[a] Antimycobacterial activity was determined at concentrations 40, 20, 10, 5 and 2.5 μg/ml.
[b] Positive control drugs; **std** = rifampicin.
[c] Concentration of compounds exhibiting 99% inhibition in mycobacterial growth.
[d] Concentration of compounds required to inhibit 50% cell growth on Vero cells.

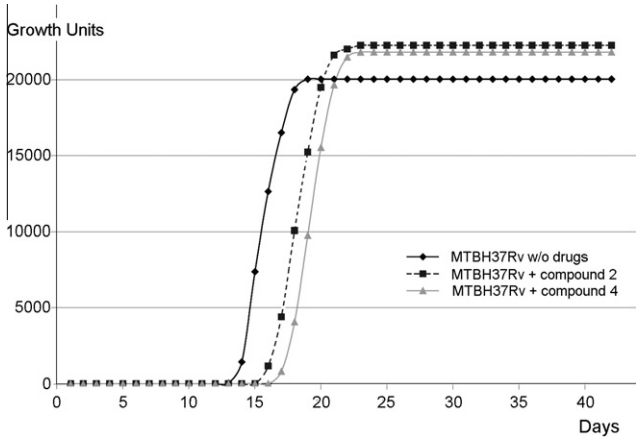



**Figure 4.** Kinetics of *Mtb* growth in presence of compounds **2** and **4** at concentration 20 μg/ml.

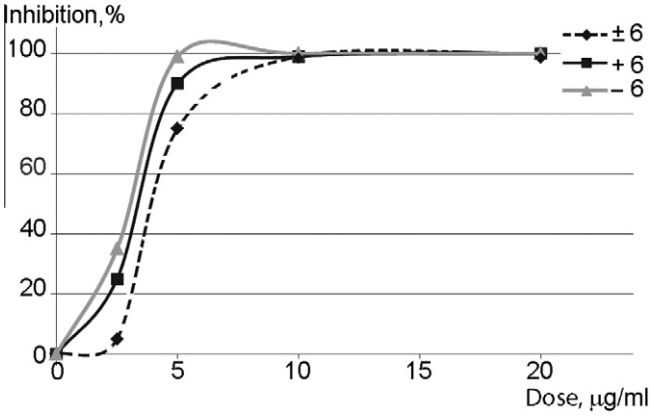



**Figure 5.** Dose-response curve for the compound **6**.

(Fig. 5) to compare with 0.1–2 μg/ml for approved antiTB drugs isoniazid, ofloxacin and rifampicin.

The capacity of compound **6** (both as a racemic mixture and individual (+) and (−) enantiomers) to suppress the growth of *M. tuberculosis* MS-115 MDR strain resistant to isoniazide, rifampicin, streptomycin, ethambutol and pyrazinamide implies that it has another mechanism of action. This makes carbocyclic compounds of this type interesting for future studies.

Recently P. Herdewijn with co-workers found 5-substituted 2′-deoxyuridine monophosphate analogues to be effective inhibitors of *M. tuberculosis* flavin-dependent thymidilate synthase (Thy-X).[18] The authors did not focus on anti-TB activity, but rather, they synthesized monophosphates of uridine analogs, for some of which anti-TB activity had been shown earlier.[2,3] We can assume that one of the mechanisms of action of 5-substituted 2′-deoxyuridine derivatives can involve intracellular phosphorylation followed by inhibition of Thy-X by the resulting monophosphates. This hypothesis is indirectly supported by the inactivity of 5-alkynyluracils ($C_5$–$C_{14}$) which do not bear carbohydrate fragments.[8] Obviously, this mechanism cannot be applied to 5′-norcarbocyclic nucleosides which lack a 5′-primary hydroxyl group and therefore cannot be phosphorylated.[19,20] However, the activities of the tested 5′-norcarbocyclic nucleosides were close enough to those of 5-substituted deoxyuridine derivaties.[2–8] The results of our studies demonstrate that 5-substituted uracil derivaties should have an alternative mechanism of *M. tuberculosis* growth inhibition. The target enzyme of antiTB action of 5′-norcarbocyclic nucleosides with 5-substituted uracil as a base is the subject of future investigation.

## 3. Materials and methods

### 3.1. General

The reactions were performed with the use of commercial reagents (Acros, Aldrich and Fluka); anhydrous solvents were purified according to the standard procedures. Column chromatography was performed on Silica Gel 60 0.040–0.063 mm (Merck, Germany) columns (elution with 95:5 $CHCl_3$/MeOH, 9:1 $CHCl_3$/MeOH or 3:2 Hexane/EtOAc). Thin layer chromatography (TLC) was performed on Silica Gel 60 $F_{254}$ aluminium-backed plates (Merck, Germany). Melting points were measured on Mel-Temp (Barnstead International, USA) and uncorrected. 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 carbon–proton interaction decoupling). UV spectra were recorded on a UV-2401PC spectrophotometer (Shimadzu, Japan) in ethanol at pH 7.0. High resolution mass spectra were measured on Bruker micrOTOF II or maXis (Bruker, Germany) instruments using electrospray ionization (HRESIMS).[21] The measurements were done in a positive ion mode (interface capillary voltage −4500 V) in a mass range from *m*/*z* 50 to *m*/*z* 3000 Da; external or internal calibration was done with Electrospray Calibrant SolutionTM (Fluka,

**Table 2**
The influence of racemic mixture and individual enantiomers of compound **6** on the growth of laboratory sensitive and clinical resistant strains of *M. tuberculosis*[a]

| Compound | *M. tuberculosis* H37Rv | | *M.tuberculosis* MS-115 (MDR strain) | | $CC_{50}$[c], (μg/ml) |
|---|---|---|---|---|---|
| | % Inhibition (concentration μg/ml) | $MIC_{99}$[c] (μg/ml) | % Inhibition (concentration μg/ml) | $MIC_{99}$[b](μg/ml) | |
| **(±)6** | 100 (20), 99 (10) | 10 | 100 (20, 10) | 10 | 125 |
| **(+)6** | 100 (20,10), 25 (5) | 10 | 100 (20), 99 (10) | 10 | >100[d] |
| **(−)6** | 100 (20,10), 99 (5) | 5 | 100 (20), 99 (10) | 10 | >100[d] |
| Rifampicin | 100 (1) | 1 | 0 (1) | — | >500 |
| Isoniazid | 100 (0.1) | 0.1 | 0 (0.1) | — | ND[e] |
| Streptomycin | 100 (0.1) | 0.1 | 0 (0.1) | — | ND[e] |
| Ethambutol | 100 (5) | 5 | 0 (5) | — | ND[e] |
| Pyrazinamide | 100 (100) | 100 | 0 (100) | — | ND[e] |
| Ofloxacin | 100 (2) | 2 | 100 (2) | 2 | ND[e] |

[a] Antimycobacterial activity was determined at concentrations 20, 10, 5 and 2.5 μg/ml, the reaction was conducted in anorexic conditions.
[b] Concentration of compounds exhibiting 99% inhibition in mycobacterial growth.
[c] Concentration of compounds required to inhibit 50% cell growth on Vero cells.
[d] Compound precipitation was detected at higher compound concentration.
[e] ND = not determined.

Switzerland). A syringe injection was used for solutions in acetonitrile (flow rate 3 μl/min). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C. Optical rotations were measured on a 341 Polarimeter (Perkin–Elmer, USA) at 21 ± 2 °C. Circular dichroism (CD) spectra were recorded on a Jasco J-715 spectropolarimeter (Jasco, Japan) in a 1 cm cell at 20 °C in $CHCl_3$/MeOH (95:5). The results were expressed as the molar circular dichroic absorbtion $\Delta\varepsilon = \theta/(32980Cl)$ $cm^2$/mol, where θ was the measured ellipticity in degrees, C—the molar concentration (mol/l), and l—the optical path in centimeters.

### 3.2. Synthesis

#### 3.2.1. (±)-1-(4′-Hydroxy-2′-cyclopenten-1′-yl)-uracil (1)

Epoxy-cyclopentene (0.9 g, 10 mmol) in freshly distilled THF (15 ml) and tetrakis(triphenylphosphine)palladium (0) [$Pd(PPh_3)_4$] (308 mg; 0.26 mmol) were added to a stirred solution of uracil (1 g; 8.9 mmol) in anhydrous DMF (100 ml). The reaction mixture was stirred at room temperature for 18 h. The mixture was concentrated in vacuum, and the residue was dissolved in a 98:2 $CHCl_3$/MeOH mixture (5 ml) and loaded onto a silica gel column eluted with the same mixture to give compound **(±)-1** as yellow powder (520 mg, 28%): mp 190.5–191 °C, $R_f$ 0.26 (elution with 95:5 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 266.2 nm (ε 9200).$^1$H NMR ($CD_3OD$): 7.57–7.55 (1H, d, $J$ = 7.9 Hz, H-6), 6.23–6.22 (1H, m, H-2′), 5.86-5.84 (1H, m, H-3′), 5.71–5.69 (1H, d, $J$ = 7.9 Hz, H-5), 5.53–5.49 (1H, m, H-1′), 4.56–4.50 (1H, m, H-4′), 2.93–2.85 (1H, m, H-a5′), 1.51–1.47 (1H, m, H-b5′). $^{13}$C NMR ($CD_3OD$): 166.31, 152.79 (C-4, C-2), 143.59 (C-6), 140.98, 132.10 (C-2′, C-3′), 102.92 (C-5), 73.34 (C-1′), 60.43 (C-4′), 41.27 (C-5′). HRESIMS: found $m/z$ 195.0763, calcd for $C_9H_{10}N_2O_3$ $[M+H]^+$ 195.0764; found $m/z$ 217.0580; calcd for $C_9H_{10}N_2O_3$ $[M+Na]^+$ 217.0584.

#### 3.2.2. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-6-methyl-uracil (2)

Compound **(±)-2** was obtained according to the procedure described for **(±)-1**, starting from 6-methyluracil. Purification by column chromatography on silica gel (98:2 $CHCl_3$/MeOH) provided the product as a yellow powder (480 mg, 38%): mp 167.5–168.5 °C, $R_f$ 0.39 (elution with 95:5 $CHCl_3$:MeOH). UV: $\lambda_{max}$ 263.0 nm (ε 8900). $^1$H NMR (DMSO-$d_6$): 11.03 (1H, s, NH), 5.80–5.79 (1H, m, H-2′), 5.75–5.73 (1H, m, H-3′), 5.59–5.57 (1H, m, H-1′), 5.43 (1H, s, H-5), 4.74 (1H, s OH), 4.58–4.56 (1H, m, H-4′), 2.50–2.49 (1H, m, H-a5′), 2.01 (3H, m, $CH_3$), 1.89–1.86 (1H, m, H-b5′). $^{13}$C NMR (DMSO-$d_6$): 163.09, 151.37, 151.24 (C-4, C-6, C-2), 134.36, 131.97 (C-2′, C-3′), 98.55 (C-5), 74.13 (C-1′), 53.93 (C-4′), 37.66(C-5′), 17.82 ($CH_3$). HRESIMS: found $m/z$ 209.0933, calcd for $C_{10}H_{12}N_2O_3$ $[M+H]^+$ 209.0921; found $m/z$ 231.0741; calcd for $C_9H_9IN_2O_3$ $[M+Na]^+$ 231.0740.

#### 3.2.3. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-iodouracil (3)

Compound **(±)-3** was obtained according to the procedure described for **(±)-1**, starting from 5-iodouracil. Purification by column chromatography (98:2 $CHCl_3$/MeOH) provided the product as an off-white powder (325 mg, yield 24%): mp 189.5–190.5 °C, $R_f$ 0.32 (elution with 95:5 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 291.1 nm (ε 9050). $^1$H NMR (DMSO-$d_6$): 11.60 (1H, s, NH), 7.84 (1H, s, H-6), 6.17–6.16 (1H, m, H-2′), 5.38–5.36 (1H, m, H-3′), 5.35–5.34 (1H, m, H-1′), 5.25 (1H, d, $J$ = 6.04 Hz, OH), 5.28–5.26 (1H, m, H-4′), 2.68–2.66 (1H, m, H-a5′), 1.43–1.39 (1H, m, H-b5′). $^{13}$C NMR (DMSO-$d_6$): 160.48, 150.49 (C-4, C-2), 146.12 (C-6), 140.38, 130.69 (C-2′, C-3′), 73.24 (C-5), 68.99 (C-1′), 58.59 (C-4′), 38.8(C-5′). HRESIMS: found $m/z$ 320.9728, calcd for $C_9H_9IN_2O_3$ $[M+H]^+$ 320.9731; found $m/z$ 342.9545; calcd for $C_9H_9IN_2O_3$ $[M+Na]^+$ 342.9550.

#### 3.2.4. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-decynyl-uracil (4)

To a stirred solution of compound **(±)-3** (100 mg; 0.3 mmol) in freshly distilled DMF (15 ml), copper (I) iodide (30 mg; 0.15 mmol), *N*,*N*-diisopropil ethylamine (200 μl; 1.2 mmol), $Pd(PPh_3)_4$ (52 mg; 0.045 mmol) and 1-decyn (110 μl; 0.6 mmol) were added. The reaction mixture was stirred at room temperature under argon atmosphere. The progress of the reaction was monitored by TLC in 95:5 $CHCl_3$/MeOH. After 2 h, 0.3 ml of 0.5 M EDTA/$H_2O$ (pH 8.0) was added to the reaction mixture and then the mixture was concentrated in vacuo. The product was purified by column chromatography on a silica gel column (10 × 40 mm) eluted with 98:2 $CHCl_3$/MeOH and repurified on a similar column eluted with EtOAc/hexane (4:1) to give the target compound as a white powder (32 mg, 32%): mp 135.5–137 °C, $R_f$ 0.34 (elution with 98:2 $CHCl_3$:MeOH). UV: $\lambda_{max}$ 297.1 nm (ε 10200). $^1$H NMR ($CD_3OD$): 7.70 (1H, s, H-6), 6.24–6.23 (1H, m, H-2′), 5.87–5.85 (1H, m, H-3′), 5.54–5.50 (1H, m, H-1′), 4.54–4.50 (1H, m, H-4′), 2.89–2.86 (1H, m, H-a5′), 2.37 (2H, m, $CH_2$-α), 1.58–1.56 (2H, m, $CH_2$-β), 1.54–1.52 (1H, m, H-b5′), 1.50–1.48 (2H, m, $CH_2$-γ), 1.45–1.42 (8H, s, $(CH_2)_6$), 1.35 (3H, m, $CH_3$). $^{13}$C NMR ($CD_3OD$): 164.76, 150.05 (C-4, C-2), 145.20, 141.18 (C-2′, C-3′), 132.09 (C-6), 101.66 (≡C), 95.47 (C-5), 75.35 (C-1′), 72.39 (C≡), 60.72 (C-4′), 41.25 (C-α), 32,99 (C-β), 29.73 (C-5′, C-δ, C-ε, C-ζ, C-η), 23,68 (C-γ), 14,40 ($CH_3$). HRESIMS: found $m/z$ 331.2023, calcd for $C_{19}H_{26}N_2O_3$ $[M+H]^+$ 331.2016; found $m/z$ 353.1839; calcd for $C_{19}H_{26}N_2O_3$ $[M+Na]^+$ 353.1836.

#### 3.2.5. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-dodecynyl-uracil (5)

Compound **(±)-5** was obtained similarly to the procedure described for **(±)-4**, with 1-dodecyne as a terminal alkyne. Purification and repurification by column chromatography on silica gel (98:2 $CHCl_3$/MeOH; 4:1 EtOAc/hexane) afforded the title compound as a white powder (47 mg, 28%): mp 138–139.5 °C, $R_f$ 0.32 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 297.4 nm (ε 10400). $^1$H NMR (DMSO-$d_6$): 11.52 (1H, s, NH), 7.66 (1H, s, H-6), 6.22–6.20 (1H, m, H-2′), 5.38–5.36 (1H, m, H-3′), 5.43–5.42 (1H, m, H-1′), 5.32–5.31 (1H, s OH), 4.67 (1H, m, H-4′), 2.77–2.75 (1H, m, H-a5′), 2.41–2.39 (2H, m, $CH_2$-α), 1.53–1.49 (2H, m, $CH_2$-β), 1.45–1.43(1H, m, H-b5′), 1.41–1.39 (2H, m, $CH_2$-γ), 1.30 (12H, s, $(CH_2)_6$), 0.92–0.89 (3H, m, $CH_3$). $^{13}$C NMR (DMSO-$d_6$): 161.99, 150.05 (C-4, C-2), 144.02 (C-6), 140.51, 130.79 (C-2′, C-3′), 99.28 (C-5), 93.67 (≡C), 73.47 (C-1′), 72.82 (C≡), 58.76 (C-4′), 31.41 (C-α), 29.05 (C-5′, C-δ, C-ε, C-ζ, C-η, C-θ, C-λ), 22,21 (C-β), 18,89 (C-γ), 14,06 ($CH_3$). HRESIMS: found $m/z$ 359.2310, calcd for $C_{21}H_{30}N_2O_3$ $[M+H]^+$ 359.2317; found $m/z$ 381.2134; calcd for $C_{21}H_{30}N_2O_3$ $[M+Na]^+$ 381.2149.

#### 3.2.6. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-tetradecynyl-uracil (6)

Compound **(±)-6** was obtained similarly to the procedure described for **(±)-4**, with the 1-tetradecyne as a terminal alkyne. Purification and repurification by column chromatography on silica gel (98:2 $CHCl_3$/MeOH; 4:1 EtOAc/hexane) afforded the title compound as a white powder (43 mg, 24%): mp 143–144°C, $R_f$ 0.3 (elution with 98:2 $CHCl_3$:MeOH). UV: $\lambda_{max}$ 297.2 (ε 9900). $^1$H NMR ($CDCl_3$): 8.53 (1H, s, NH), 7.46 (1H, s, H-6), 6.2–6.18 (1H, m, H-2′), 5.77–5.75 (1H, m, H-3′), 5.43–5.42 (1H, m, H-1′), 4.82–4.8 (1H, m, H-4′), 2.87–2.85 (1H, m, H-a5′), 2.3 (2H, m, $CH_2$-α), 1.58–1.5 (1H, m, H-b5′), 1.51–1.48 (2H, m, $CH_2$-β), 1.33–1.3 (2H, m, $CH_2$-γ), 1.24 (16H, s, $(CH_2)_8$), 0.87–0.83 (3H, m, $CH_3$). $^{13}$C NMR ($CDCl_3$): 161.79, 149.89 (C-4, C-2), 143.52, 140 (C-2′, C-3′), 131.36 (C-6), 101.38 (≡C), 95.74 (C-5), 74.84 (C-1′), 70.91 (C≡), 60.24 (C-4′), 40.38 (C-α), 31.99 (C-5′), 29.72 (C-δ, C-ε, C-ζ, C-η,

C-θ, C-λ, C-ν, C-μ), 22.76 (C-β), 19.72(C-γ), 14.19 ($CH_3$). HRESIMS: found *m*/*z* 387.2620, calcd for $C_{23}H_{34}N_2O_3$ $[M+H]^+$ 387.2642; found *m*/*z* 409.2438; calcd for $C_{23}H_{34}N_2O_3$ $[M+Na]^+$ 409.2462.

### 3.2.7. (−)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-iodouracil (3) and (+)-1-[4′-acethyloxy-2′-cyclopenten-1′-yl]-5-iodouracil (13)

To a stirred solution of **(±)-3** (133 mg; 0.41 mmol) in vinyl acetate (25 ml) lipase (Amano PS lipase) (100 mg) was added. The reaction mixture was stirred virgously during 18 h. After this time the mixture was concentrated in vacuo. The residue was dissolved in 5 ml $CHCl_3$ and purified on silica gel using $CHCl_3$ as an eluent to give the product **(−)-3** as a off-white powder (68 mg, 51%) and product **(+)-13** as a yellow powder (69 mg, 46%). For compound **(−)-3**: $[\alpha]_D$ −66.4° (*c* 0.035, $CHCl_3$), CD −10.92 $mol^{-1}\times cm^{-1}$; The $R_f$, $^1H$ and $^{13}C$ NMR spectral data were identical with those for **(±)3**. For compound **(+)-13**: $R_f$ 0.55 (elution with $CHCl_3$). $^1H$ NMR ($CDCl_3$): 8.71 (1H, s, NH), 7.60 (1H, s, H-6), 6.29–6.27 (1H, m, H-2′), 5.93–5.91 (1H, m, H-3′), 5.60–5.58 (2H, m, H-1′, H-4′), 2.90–2.81 (1H, m, H-a5′), 2.05 (1H. s, $CH_3$), 1.65–1.63 (1H, m, H-b5′). HRESIMS: found *m*/*z* 362.9832, calcd for $C_{11}H_{11}IN_2O_4$ $[M+H]^+$ 362.9836; found *m*/*z* 384.9661; calcd for $C_{11}H_{11}IN_2O_4$ $[M+Na]^+$ 384.9656.

### 3.2.8. (+)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-iodouracil (3)

To a stirred solution of compound **(+)-13** (163 mg; 0.45 mmol) in MeOH (10 ml) $K_2CO_3$ (155 mg; 1.13 mmol) was added. The progress of the reaction was monitored by TLC in 95:5 $CHCl_3$/MeOH. After 18 h the reaction mixture was concentrated in vacuo. The residue was purified on silica gel column using 98:2 $CHCl_3$–MeOH as an eluent to give compound **(+)-3** as a white powder (108 mg, 75%): $[\alpha]_D$ +65.7° (*c* 0.04, $CHCl_3$), CD 10.89 $mol^{-1}\times cm^{-1}$. The $R_f$, $^1H$ and $^{13}C$ NMR spectral data were identical with those for **(±)-3.**

### 3.2.9. (+)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-tetradecynyluracil (6)

Compound **(+)-6** was prepared using the procedure described for **6**, starting from **(+)-3**. Purification by column chromatography on silica gel (98:2 $CHCl_3$/MeOH) afforded the title compound as a white powder (25 mg, 46%): $[\alpha]_D$ +42. 9° (*c* 0.05, $CH_2Cl_2$), CD 2.68 $mol^{-1}\times cm^{-1}$. The $R_f$, $^1H$ and $^{13}C$ NMR spectral data were identical with those for **(±)-6.**

### 3.2.10. (−)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-5-tetradecynyluracil (6)

Compound **(−)-6** was prepared as described for **(±)6** starting from **(−)-3.** Purification by column chromatography on silica gel (98:2 $CHCl_3$/MeOH) afforded the title compound as a white powder (30 mg, 49%): $[\alpha]_D$ −77.7° (*c* 0.038, $CH_2Cl_2$), CD −2.61 $mol^{-1}\times cm^{-1}$. The $R_f$, $^1H$ and $^{13}C$ NMR spectral data were identical with those for **(±)-6**.

### 3.2.11. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-6-octynyl-3H-furo[2,3-d]-pyrimidine-2-one (7) and (±)-1-[4′-hydroxy-2′-cyclopenten-1′-yl]-3,5-didecynyl-uracil (10)

The title compounds were obtained as side products in the synthesis of **(±)-4**. The purification described for compound **(±)-4** gave product **(±)-7** as yellow powder (13 mg, 13%): mp 164–165.5°C, $R_f$ 0.3 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 335.6 (ε 5000), $\lambda_{max}$ 246.1 (ε 8400). $^1H$ NMR ($CDCl_3$): 7.99 (1H, s, H-6), 6.29–6.28 (1H, m, H-2′), 6.06 (1 H, s H-9) 5.89–5.87 (1H, m, H-3′), 5.83–5.81 (1H, m, H-1′), 4.93–4.92 (1H, m, H-4′), 3.01–2.97 (1H, m, H-a5′), 2.63–2.59 (2H, m, $CH_2$-α), 1.65 (1H, m, H-b5′), 1.60–1.58 (2H, m, $CH_2$-β), 1.25 (10H, s, $(CH_2)_5$), 0.86 (3H, m, $CH_3$). $^{13}C$ NMR ($CDCl_3$): 171.74 (C-9), 160.34, 155.78 (C-4, C-2), 139.81 (C-6), 136.97, 132.32 (C-2′, C-3′), 108.58 (C-8), 98.81 (C-5), 75.13 (C-5′), 62.60 (C-1′), 41.21 (C-4′), 31.95 (C- α), 29.35 (C-β), 29.29, 29.18, 28.44, 26.96, 22.77 (5 × C), 14.20 ($CH_3$). HRESIMS: found *m*/*z* 331.2010, calcd for $C_{19}H_{26}N_2O_3$ $[M+H]^+$ 331.2016; found *m*/*z* 353.1825; calcd for $C_{19}H_{26}N_2O_3$ $[M+Na]^+$ 353.1836.

The same purification procedure gave product **(±)-10** as syrup (22 mg, 16%). $R_f$ 0.32 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 342.2 (ε 6200). $^1H$ NMR ($CDCl_3$): 7.94 (1H, s, H-6), 6.31–6.29 (1H, m, H-2′), 5.9–5.88 (1H, m, H-3′), 5.75–5.72 (1H, m, H-1′), 4.93–4.91 (1H, m, H-4′), 3.05–3.01 (1H, m, H-a5′), 2.71–2.69 (2H, m, N-$CH_2$-α), 2.40–2.38 (2H, m, C-$CH_2$-α), 1.7–1.68 (2H, m, N-$CH_2$-β), 1.58–1.56 (2H, m, C-$CH_2$-β), 1.42 (1H, m, H-b5′), 1.51–1.18 (20H, m, 2×$(CH_2)_5$), 0.88 (6H, m, 2-$CH_3$). HRESIMS: found *m*/*z* 467.3308, calcd for $C_{29}H_{42}N_2O_3$ $[M+H]^+$ 467.3315; found *m*/*z* 489.3178; calcd for $C_{29}H_{42}N_2O_3$ $[M+Na]^+$ 489.3193.

### 3.2.12. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-6-decynyl-3H-furo[2,3-d]-pyrimidine-2-one (8) and (±)-1-[4′-hydroxy-2′-cyclopenten-1′-yl]-3,5-didodecynyl-uracil (11)

The title compounds were obtained as side products in the synthesis of **(±)-5**. The purification described for compound **(±)-5** gave product **(±)-8** as pale yellow powder (16 mg, 15%): mp 166–168°C, $R_f$ 0.28 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 335.9 (ε 5400), $\lambda_{max}$ 245.6 (ε 9000). $^1H$ NMR ($CDCl_3$): 7.96 (1H, s, H-6), 6.24–6.22 (1H, m, H-2′), 6.01 (1 H, s H-9) 5.82–5.80 (1H, m, H-3′), 5.77–5.75 (1H, m, H-1′), 4.87–4.86 (1H, m, H-4′), 2.97–2.95 (1H, m, H-a5′), 2.57–2.53 (2H, m, $CH_2$-α), 1.61 (1H, m, H-b5′), 1.59–1.57 (2H, m, $CH_2$-β), 1.19 (14H, s, $(CH_2)_7$), 0.82–0.79 (3H, m, $CH_3$). $^{13}C$ NMR ($CDCl_3$): 171.63 (C-9), 160.24, 155.74 (C-4, C-2), 139.83 (C-6), 137.01, 132.13 (C-2′, C-3′), 108.52 (C-8), 98.77 (C-5), 74.96 (C-5′), 62.48 (C-1′), 41.13 (C-4′), 31.94 (C- α), 29.61 (C-β), 29.55, 29.34, 29.31, 29.10, 28.35, 26.88, 22.72 (7 × C), 14.14 ($CH_3$). HRESIMS: found *m*/*z* 359.2323, calcd for $C_{21}H_{30}N_2O_3$ $[M+H]^+$ 359.2329; found *m*/*z* 381.2143; calcd for $C_{21}H_{30}N_2O_3$ $[M+Na]^+$ 381.2149.

The same purification procedure gave product **(±)-11** as syrup (27 mg, 17%). $R_f$ 0.3 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 342 (ε 6500). $^1H$ NMR ($CDCl_3$): 7.91–7.89 (1H, s, H-6), 6.4–6.37 (1H, m, H-2′), 6.0–5.98 (1H, m, H-3′), 5.79–5.77 (1H, m, H-1′), 4.91–4.87 (1H, m, H-4′), 3.21–3.18 (1H, m, H-a5′), 2.83–2.78 (2H, m, N-$CH_2$-α), 2.45–2.4 (2H, m, C-$CH_2$-α), 1.76–1.74 (2H, m, N-$CH_2$-β), 1.61–1.57 (2H, m, C-$CH_2$-β), 1.48–1.46 (1H, m, H-b5′), 1.44–1.23 (28H, m, 2×$(CH_2)_7$), 0.84 (6H, m, 2-$CH_3$). HRESIMS: found *m*/*z* 523.3904, calcd for $C_{33}H_{50}N_2O_3$ $[M+H]^+$ 523.3911; found *m*/*z* 545.3717; calcd for $C_{33}H_{50}N_2O_3$ $[M+Na]^+$ 545.3721.

### 3.2.13. (±)-1-[4′-Hydroxy-2′-cyclopenten-1′-yl]-6-dodecynyl-3H-furo[2,3-d]-pyrimidine-2-one (9) and (±)-1-[4′-hydroxy-2′-cyclopenten-1′-yl]-3,5-ditetradecynyl-uracil (12)

The title compounds were obtained as side products in the synthesis of **(±)-6**. The purification described for compound **(±)-6** gave product **(±)-9** as yellow powder (17 mg, 15%): mp 156.5–158.5°C, $R_f$ 0.26 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 335.4 (ε 4900), $\lambda_{max}$ 245.7 (ε 8900). $^1H$ NMR ($CDCl_3$): 7.92 (1H, s, H-6), 6.23–6.22 (1H, m, H-2′), 6.0 (1 H, s H-9) 5.83–5.82 (1H, m, H-3′), 5.77–5.75 (1H, m, H-1′), 4.87–4.86 (1H, m, H-4′), 2.98–2.96 (1H, m, H-a5′), 2.55–2.54 (2H, m, $CH_2$-α), 1.61 (1H, m, H-b5′), 1.60–1.58 (2H, m, $CH_2$-β), 1.18 (18H, s, $(CH_2)_9$), 0.80 (3H, m, $CH_3$). $^{13}C$ NMR ($CDCl_3$): 172.03 (C-9), 161.10, 156.12 (C-4, C-2), 140.29 (C-6), 135.08, 132.25 (C-2′, C-3′), 108.64 (C-8), 98.69 (C-5), 74.92 (C-5′), 63.50 (C-1′), 42.35 (C-4′), 32.65 (C- α), 30.50 (C-β), 29.43, 29.27, 29.12, 28.97, 28.78, 28.35, 26.68, 25.54, 22.17 (9×C), 14.21 ($CH_3$). HRESIMS: found *m*/*z* 387.2637, calcd for $C_{23}H_{34}N_2O_3$ $[M+H]^+$ 387.2642; found *m*/*z* 409.2458; calcd for $C_{23}H_{34}N_2O_3$ $[M+Na]^+$ 409.2462.

The same purification procedure gave product **(±)-12** as syrup (29 mg, 17%). $R_f$ 0.28 (elution with 98:2 $CHCl_3$/MeOH). UV: $\lambda_{max}$ 342.2 (ε 6300). $^1H$ NMR ($CDCl_3$): 7.87 (1H, s, H-6), 6.25–6.23 (1H,

m, H-2′), 5.84–5.82 (1H, m, H-3′), 5.69–5.66 (1H, m, H-1′), 4.87–4.85 (1H, m, H-4′), 3.02–2.94 (1H, m, H-a5′), 2.67–2.63 (2H, m, N-$CH_2$-α), 2.37–2.33 (2H, m, C-$CH_2$-α), 1.65–1.63 (2H, m, N-$CH_2$-β), 1.55–1.54 (2H, m, C-$CH_2$-β), 1.54–1.53 (1H, m, H-b5′), 1.51–1.18 (36H, m, 2×$(CH_2)_9$), 0.81 (6H, m, 2-$CH_3$). HRESIMS: found *m*/*z* 579.4509, calcd for $C_{37}H_{58}N_2O_3$ $[M+H]^+$ 579.4520; found *m*/*z* 601.4332; calcd for $C_{37}H_{57}N_2O_3$ $[M+Na]^+$ 601.4340.

### 3.3. Cytotoxicity

Vero cell culture (green monkey kidney epithelial cells, ATCC No CRL-1586) was obtained from Laboratory of tissues, Ivanovskii Institute of Virology, RAMS (Moscow). The Vero cell culture was grown in Dulbecco's modified Eagle's media (D-MEM) with 10% fetal calf serum (HyClone, USA) at 37°C in a 96-well plate in the atmosphere of 5% $CO_2$. Cytotoxicity ($CD_{50}$) was estimated by MTT-assay in the presence of tested compounds at the concentrations of 0 to 250 μg/ml after 72 h incubation with uninfected cells and calculated as compound concentration, at which 50% cells died.[22] The tested compounds were dissolved in DMSO at a concentration of 40 mg/ml (stock solutions).

### 3.4. Antituberculosis Tests

#### 3.4.1. Mycobacterial strains

The laboratory *M. tuberculosis* H37Rv strain susceptible to antiTB drugs and a MS-115 clinical strain resistant to five first line antiTB drugs (rifampicin, isoniazid, streptomycin, ethambutol, and pyrazinamide) were used. The mycobacteria were transformed into a suspension of single cells at the same growth phase and normalized by CFU.[23] The enriched Dubois medium (Difco, USA) was used.

#### 3.4.2. Activity tests

The influence of the tested compounds on the growth of the bacterial strains was studied using the automated BACTEC MGIT 960 system (BD, USA). The tested compounds were dissolved in DMSO at a concentration of 1 mg/ml (stock solutions). Water and Tween 80 were added to the stock solutions to give a 5:0.5:4.5 ratio of DMSO: Tween 80: $H_2O$. The mycobacterial suspension (500 μl) was inoculated in the culture medium (7.9 ml) up to the final concentration of $10^6$ CFU/ml. Each sample including the control samples lacking the tested compound were studied in triplicate. The antibacterial activity was evaluated by the proportion method with the TB Exist software.[24] The method is based on the comparison of the bacterial growth in the samples containing the tested compounds and the control samples diluted 100× if compared with the initially inoculated culture. The culture is susceptible (that is, the compound is active) if the optical density of the control sample is 400 GU (growth units) in and that of the tested sample is less than 100 GU. In addition, using the absolute concentrations method a delay in the bacterial growth of the tested sample was measured in comparison with the control samples, which reflected a negative effect of the tested compounds at the concentrations lower than MIC on the bacterial viability. The measurements were carried out automatically every one hour and registered with the Epicenter software (BD, USA).

## Acknowledgments

The work was supported by the Russian Foundation for Basic Research (projects No. 11-04-00603, 12-04-31355-mol-a and 11-04-12035-ofi-m) and by the Presidium of the Russian Academy of Sciences (Programme 'Molecular and Cellular Biology')

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