

Efficient synthesis and antifungal investigation of nucleosides' quaternary ammonium salt derivatives

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Abstract: Quaternary ammonium salts are a group of compounds with diverse biological properties, the most important of which are their antiviral, antibacterial, and antifungal activities. The quaternization reactions of 5'-*O*-tosyl derivatives of uridine and thymidine with triethylamine, trimethylamine, 4-(*N,N*-dimethylamino)pyridine, 2-methylpyridine, and pyridine are described in this article. Two of the synthesized compounds are exceptional because they are first of this type that demonstrate concentration-dependent antifungal in vitro activity against two species of the genus *Candida* in minimal YNB-SG medium. The experimental results have been extended by adding full atom molecular dynamics simulations and substrates and products energies evaluation.

Key words: Quaternary ammonium salts, uridine-5'-ammonium derivatives, thymidine-5'-ammonium derivatives

1. Introduction

Nucleic acids DNA and RNA are built with natural nucleosides. They play very important roles in storing genetic information and transferring it to future generations.¹ Nucleotides are involved in many physiological processes through stimulating the purinergic and/or pyrimidine receptors.^{2,3} Nucleotides and nucleosides can come from food. Studies show that dietary nucleotides and nucleosides may be important for proper functioning of the immune system, small intestinal growth and development, lipid metabolism, and hepatic function.⁴ Most of the dietary nucleosides are dephosphorylated to nucleosides and subsequently rephosphorylated in enterocytes.⁵

Besides food, nucleotides can also be synthesized de novo. This synthesis consists of restoring nucleotides from purine and pyrimidine bases and nucleosides resulting, for example, from nucleic acid degradation processes.⁶

A very important family of pharmacologically active compounds comprises the nucleosides and their analogues. They have a wide range of properties, including cytotoxicity, antiviral activity, and immunosuppression. There are two major classes of nucleoside analogues: in the first, the purine or pyrimidine bases are modified,^{7–14} and in the second, the sugar part is altered.^{15–20} Listing pyrimidine analogues with a modified sugar molecule, it is worth mentioning cytarabine, used in treatment of acute leukemia, and capecitabine, used in treatment of colorectal and breast cancers (Figure 1). However, the best known pyrimidine analogue resulting from the modification of the sugar part is azidothymidine (AZT, Figure 2), used as antiretroviral medication in the treatment of HIV/AIDS.²¹

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Figure 1. The structures of cytarabine (A) and capecitabine (B).

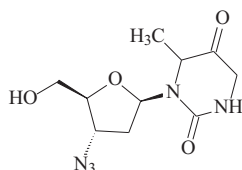


Figure 2. The structure of AZT.

AZT was obtained by modifying thymidine in a molecule whose C-3'-O bond was replaced by a C-3'-N bond. For many years, we have been working on the synthesis of sugar-containing ammonium salts with C-N bonds. Moreover, in these molecules, the nitrogen atom has a positive charge. There is much evidence that nucleoside analogues, as well as endogenous nucleosides or nucleotides using the same specific nucleoside transporters, come into the cells.^{22,23} Moreover, it is more likely that organic anion or cation transporters are involved in the cellular uptake of nucleoside antiviral analogues. Thus, we decided to synthesize and biologically test C-5' ammonium salt analogues of thymidine and uridine. Our results are presented in this article.

To explain the difficulties of the synthesis, we have additionally carried out molecular dynamics (MD) simulations of conformational changes in the studied compounds. MD analysis revealed preferred furanoid ring conformations and enabled the measurement of selected intramolecular distances within substrates and products of the synthesis described. For transition state complexes, energy evaluation calculations were performed, including heat of formation as well as their total and internal enthalpies and entropies. Results of these calculations were described and discussed.

Researchers worldwide are trying to modify nucleosides in search of new drugs, and the number of publications on this topic can be counted in the thousands each year. One of the most important turning points of this research is the synthesis and study of the biological properties of the nucleoside pentafuranosyl.²⁴ Operation of anticancer drugs is often disturbed by replication of the genetic material of tumor cells. One of the major directions of research is the synthesis of nucleoside analogues that can be used in the treatment of cancer, for antiviral therapies,^{25,26} and as nucleoside antibiotics.^{27,28} The functionalization of the nucleoside 5'-position is a separate interesting point in terms of biological action. Many of these derivatives have antiviral activity. An example would be a derivative of 5'-aminothymidine with an additional nitrogen atom^{29,30} that exhibits antiviral activity. Interestingly, the isomer of the above compound, i.e. 3'-aminothymidine synthesized by Horowitz,³¹ and Miller and Fox,³² no longer had antiviral properties or even showed cytotoxicity.

One of the biggest problems with the use of antibiotics and disinfectants, including quaternary ammonium salt (QAS), is the growing resistance of bacteria and fungi to them. That is why new compounds with greater activity, a wider spectrum of applications, and more environmentally friendly nature are still necessary. The antibacterial and antifungal activity depends mainly on the balance between the hydrophobic and the hydrophilic part of the molecule.³³ It is assumed that the combination of QAS with natural compounds such as sugars or amino acids can eliminate the limitations in the usage of this class of compounds. Such a combination may lead

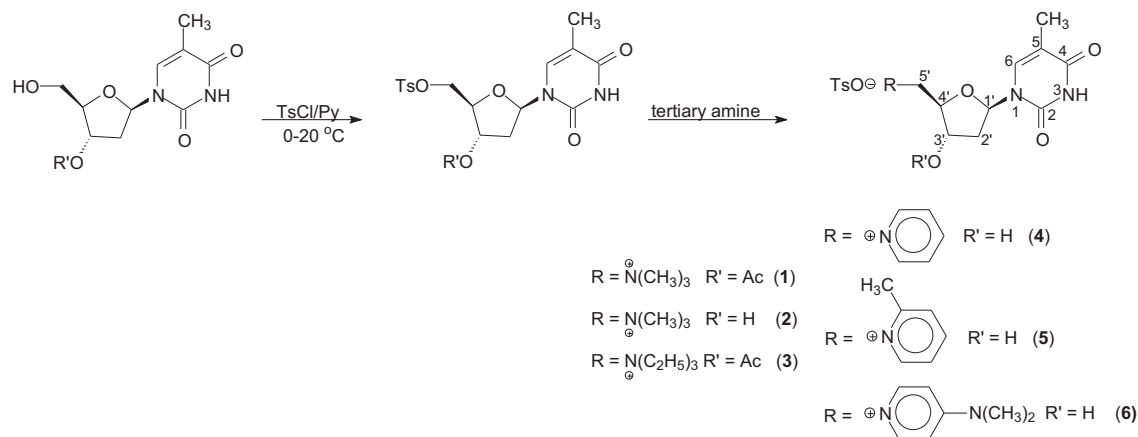
to a QAS with new properties. There are only a few reports on the synthesis and testing of sugar QAS in the literature.³⁴

In our opinion, nucleosides as compounds with commonly known biological activity after quaternization can give very interesting outcomes.

2. Results and discussion

2.1. Chemistry

The purpose of this study was to obtain quaternary ammonium salt derivatives of nucleosides. Studies were limited to derivatives of 2-deoxyribose and ribose. We selected five amines: triethylamine, trimethylamine, 4-(*N,N*-dimethylamino)pyridine (DMAP), 2-methylpyridine, and pyridine. Based on previous experiences with quaternary ammonium salt, it was decided to synthesize derivatives of thymidine tosylate (Scheme 1; Table 1) and derivatives of uridine tosylate (Scheme 2; Table 2).



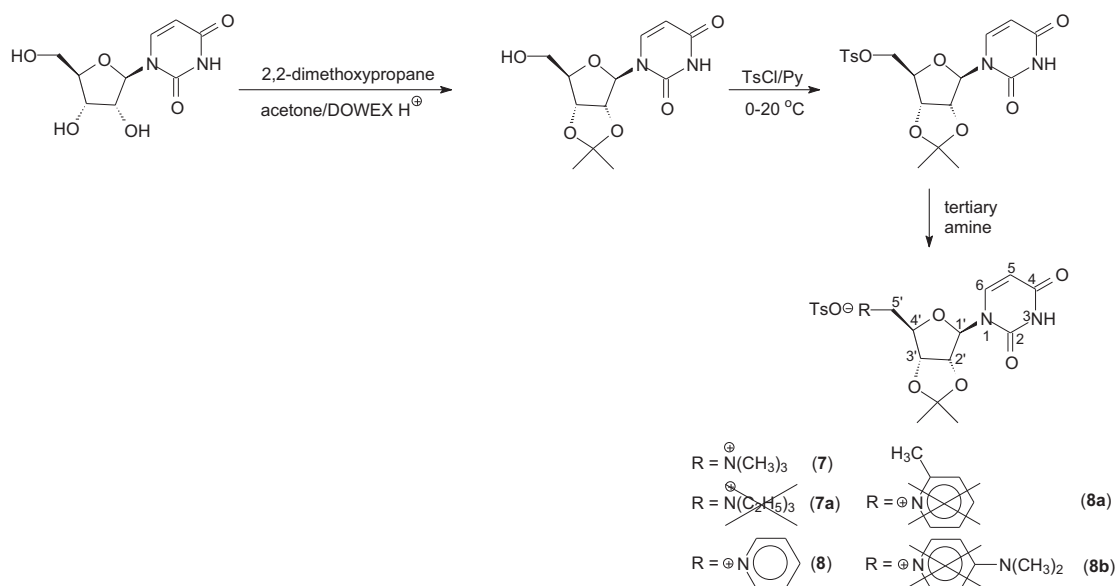
Scheme 1. Synthesis of quaternary ammonium salts from 5'-*O*-tosyl derivative of thymidine.

Table 1. The reaction conditions of the quaternization of 5'-*O*-tosylthymidine.

Amine	pK _b of amine*	Solvent	Time of reaction [h]	Temperature [°C]	Yield [%]	Product
Trimethylamine	4.20	Ethanol	3	70	87	2
Triethylamine	3.28	Triethylamine	92	70	59	3
Pyridine	8.83	Pyridine	13	70	71	4
2-Methylpyridine	8.03	2-Methylpyridine	120	70	79	5
DMAP	4.53	Acetonitrile	24	70	60	6

*pK_b values for bases at 25 °C, Physicochemical Manual, group work, Warsaw, Poland, 1974.

Commercially available thymidine was dissolved in pyridine and cooled to 0 °C. At this temperature, tosyl chloride was added according to the method of Michalson and Todd.³⁵ 5'-*O*-Tosylthymidine was obtained and was then *O*-acetylated to give 3'-*O*-acetyl-5'-*O*-tosylthymidine, which reacted with a tertiary amine. As a result of the reaction of 3'-*O*-acetyl-5'-*O*-tosylthymidine with 33% solution of trimethylamine in ethanol, *N*-[(3'-*O*-acetyl-5'-deoxythymidine)-5'-yl]-*N,N,N*-trimethylaminium tosylate (**1**) was obtained. The synthesis



Scheme 2. Synthesis of quaternary ammonium salts from 2',3'-*O*-isopropylidene-5'-*O*-tosyl derivative of uridine.

Table 2. The reaction conditions of the quaternization of 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine.

Amine	pK _b of amine*	Solvent	Time of reaction [h]	Temperature [°C]	Yield [%]	Product
Trimethylamine	4.20	Ethanol Ethanol	300 32	70 r.t.	No product 74	7
Triethylamine	3.28	Triethylamine	120 79	70 r.t.	No product Impure product	7a
Pyridine	8.83	Pyridine	140 63	70 r.t.	No product 43	8
2-Methylpyridine	8.03	2-Methylpyridine	120 79	70 r.t.	No product Impure product	8a
DMAP	4.53	Acetonitrile	120 79	70 r.t.	No product No product	8b

*pK_b values for bases at 25 °C, Physicochemical Manual, group work, Warsaw, Poland, 1974.

**The samples at r.t. were sonicated.

was carried out for 24 h at 70 °C. Compound **1** was recrystallized from a mixture of ethanol and ethyl acetate (mp 168–172 °C in 83% yield). Spectral analysis confirmed the structure. In the ¹H NMR spectrum, in addition to the signals from protons of thymidine (including the triplet at 6.23 ppm for the anomeric proton) and tosyl anion (two doublets at 7.71 ppm and 7.37 ppm corresponding to the four aromatic protons and a singlet at 2.41 ppm corresponding to the three protons of the methyl group), a singlet at 3.21 ppm was identified, corresponding to the protons of the nine residues of trimethylammonium, and the singlet at 2.17 ppm corresponded to the three protons of the *O*-acetyl group.

In a reaction of 5'-*O*-tosylthymidine and the 33% solution of trimethylamine in ethanol, after 3 h of

heating at the 70 °C the desired *N*-[(5'-deoxythymidine)-5'-yl]-*N,N,N*-trimethylammonium tosylate (**2**) was obtained with 87% yield. Comparing the results of both experiments, it was found that the presence of an *O*-acetyl group at the C-3' carbon atom has no effect on the yield of formation of the salt.

The reaction of 5'-*O*-tosylthymidine and triethylamine was performed for 92 h at 70 °C. Both the main product and the impurities were soluble in water, which made it impossible to purify it by extraction. Therefore, the water layer was concentrated and subjected to *O*-acetylation. After acetylation, the mixture was extracted with chloroform, in which the impurities dissolved and the main product remained in the aqueous layer, which was further purified by heating with activated carbon. Finally, *N*-[(5'-deoxythymidine)-5'-yl]-*N,N,N*-triethylammonium tosylate (**3**) was obtained with 59% yield, whose structure was confirmed by NMR analysis. In the proton spectrum there were signals of an anomeric proton at 6.23 ppm and a multiplet of six methylene protons at 3.40 ppm, and a multiplet of nine protons of the CH₃ group from triethylammonium residue at 1.28 ppm. The presence of the *O*-acetyl group confirmed a singlet at 2.18 ppm.

To obtain the *N*-[(5'-deoxythymidine)-5'-yl]pyridinium tosylate (**4**) from 5'-*O*-tosylthymidine and pyridine the reaction was conducted for 13 h at 70 °C. The resulting compound **4** was obtained as an oil with 71% yield. In the proton spectra in the range of 8.85 to 7.84 ppm two doublets of triplets corresponding to five protons of pyridine were present. At 6.11 ppm the signal of the anomeric proton was present in the form of a doublet of doublets as the result of coupling with two nonequivalent protons, H-2 and H-2'.

As a result of the reaction of 5'-*O*-tosylthymidine with 2-methylpyridine, after 120 h of heating at the temperature of 70 °C, compound **5** was obtained with a 79% yield. It can be stated that the yields for the syntheses of compounds **4** and **5** were very similar. In syntheses described in our previous papers,^{36,37} the presence of the methyl substituent at the C-2 position of the pyridine ring resulted in a reduction in the yield of the salt-forming reaction. We have always interpreted this as an effect of the steric hindrance of the methyl group. In the case of the synthesis of compound **5**, this phenomenon was not observed. In the ¹H NMR spectrum in the range of 8.66 to 7.81 ppm a multiplet corresponding to four aromatic protons of the 2-methylpyridinium residues was present, and at 6.10 ppm the doublet of doublets of the anomeric proton. Additionally, the presence of 2-methylpyridinium residue confirmed a singlet of three protons of the methyl group at 2.86 ppm.

The reaction of 5'-*O*-tosylthymidine with 4-(*N,N*-dimethylamino)pyridine was carried out in THF, DMF, and acetonitrile. The highest yield was obtained in acetonitrile. The substrates were heated at 70 °C for 24 h and the expected salt **6** was obtained with 60% yield. Spectral analysis confirmed the structure of the *N*-[(5'-deoxythymidine)-5'-yl]-4-(*N,N*-dimethylamino)pyridinium tosylate (**6**). In ¹H NMR spectra in the range of 7.92 to 6.81 ppm two doublets corresponding to the four aromatic protons of the residue of 4-(*N,N*-dimethylamino)pyridine were present and a singlet at 3.17 ppm corresponded to six protons of -N(CH₃)₂. The signal of the anomeric proton in the form of a doublet of doublets was recorded at 6.15 ppm.

In the first step of forming salt derivatives of uridine, commercially available uridine was stirred with 2,2-dimethoxypropane in acetone in the presence of DOWEX (50 WX 8) ion exchange resin (H⁺) yielding 2',3'-*O*-isopropylideneuridine in 58% yield with mp = 159–160 °C, consistent with the literature values.^{38,39} Next, the 2',3'-*O*-isopropylidene derivative was dissolved in pyridine and tosyl chloride was added. After 24 h, 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine was obtained as an oil in 91% yield. This compound was the starting material for the quaternization reaction of amines (Scheme 2). Because heating at 70 °C in vials (similarly to the thymidine derivatives) did not bring the expected results, uridine derivatives were sonicated at room temperature.

The mixture of 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine and 33% ethanolic solution of trimethylamine was sonicated at room temperature and after 32 h the *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-*N,N,N*-trimethylammonium tosylate (**7**) was obtained in 74% yield as a colorless oil. Spectral methods confirmed its structure. In the ¹H NMR spectrum a doublet at 5.75 ppm corresponding to the anomeric proton and singlet at 3.10 ppm corresponding to nine methyl protons from the amine residue were present. Additionally, the two singlets of methyl groups of the *O*-isopropylidene group at 1.54 and 1.33 ppm were present.

In the mixture formed by heating 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine with triethylamine at 70 °C for 120 h, we did not find the expected product in the form of triethylammonium salt. Sonication of the analogous mixture at room temperature for 79 h resulted in a postreaction mixture containing *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-*N,N,N*-triethylammonium tosylate (**7a**). MALDI-TOF analysis of this mixture showed the presence of the signal *m/z* = 368.43 (consistent with the calculated). Unfortunately, despite a number of purification attempts (flash and RP HPLC chromatography), a clean product was not obtained.

Sonication of 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine with pyridine at room temperature for 63 h gave *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]pyridinium tosylate (**8**) in 43% yield. The signals of the ribose and uracil protons of *O*-isopropylidene groups in the ¹H NMR spectrum were present. In the range from 8.78 to 8.07 ppm, a multiplet of five pyridinium protons was recorded.

In the case of the reactions of 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine with 2-methylpyridine or DMAP, despite a number of purification attempts, clean products *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-2-methylpyridinium tosylate (**8a**) and *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-4-(*N,N*-dimethylamino)pyridinium tosylate (**8b**) were not obtained.

Preparation of salts of uridine derivatives turned out to be more difficult than that of the corresponding salts derived from thymidine. Only two pure salts were obtained: *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-*N,N,N*-trimethylammonium tosylate (**7**) and *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]pyridinium tosylate (**8**). This is probably due to the greater reactivity of the 2-deoxy-D-ribose present in thymidine. We have tried to explain the lower reactivity based on molecular calculations, which are presented in the next section.

2.2. Calculations

We performed a set of computational investigations to answer the question of why the synthesis of *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-*N,N,N*-triethylammonium tosylate (**7a**), *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-2-methylpyridinium tosylate (**8a**), and *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-4-(*N,N*-dimethylamino)pyridinium tosylate (**8b**) were not successfully synthesized and found it impossible to answer it.

The computational approach involved full parametrization of substrates and all products (even those not synthesized), as well as expected transition state complexes.

The parametrization was prepared for the AMBER⁴⁰ *ff03*⁴¹ and GAFF⁴² force field parameters. Partial atomic charges for the *ff03* force field were computed with the RESP protocol.⁴³ Full atom MD of substrates (for **1–6** these were 5'-*O*-tosylthymidine and 3'-*O*-acetyl-5'-*O*-tosylthymidine; for **7**, **8**, and three not synthesized salts (**7a**, **8a**, and **8b**) there was only one compound: 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine) was conducted with the continuum solvation model.⁴⁴

Initial equilibration with temperature adjustment to 300 K was carried out for 0.5 ns. Subsequently, all

complexes were submitted to 10 ns of isothermal-isobaric (300 K) MD in the AMBER suit of programs, then analyzed. For the MD we used the SHAKE algorithm⁴⁵ with default time step 0.001 ps, Berendsen⁴⁶ bath coupling (1 ps), and 12.0 Å cut-off for nonbonded interactions, with PME.^{47–49} During the MD run snapshots were taken every 200 steps and analyzed over the whole 10 ns of the run (50,000 snapshots from every complex simulated).

The analysis included measurement of the distances between center of mass of the substituting group and center of mass of the thymine (**1–6**) or uracil (**7–8b**), or between the substituting group and selected parts or center of mass of the furanoic ring.

Differences in resulting measurements were present, but they were inconclusive. The distances did not keep any particular trend, nor could they be divided into favorable or unfavorable from the point of view of the conducted syntheses.

For the substrate analysis, distances were measured between center of mass of the tosyl group and center of mass of thymine or uracil. Even though the medium distances in **1–6** were about 4% higher than in the case of **7–8b**, we cannot predicate that this influenced the synthesis in any of the investigated cases. This observation is in accordance with the dihedral angle measurement, where the C-3-C-4-C-5-O (Ts) dihedral angle has generally been on the same or a slightly higher (about 3–5°, especially for **8** and **8a**) level in the case of **7–8b** compared to **1–6**.

For the transition state complexes only energetic calculations were performed. All compounds were built in Avogadro,⁵⁰ then optimized using MMFF94s⁵¹ and the PM6⁵² method in MOPAC2016.⁵³ Computed energies involved heat of formation, total enthalpy of the products, total entropy of products, internal enthalpy, and internal entropy. None of these resulting values differentiated those syntheses that were successfully carried out from those that failed.

The only successful theoretical investigation involved statistical analysis of the collected snapshot from the MD trajectory. In this analysis the dihedral angles O-C-1-C-2-C-3 and C-2-C-3-C-4-O were controlled during MD progress. The collected data (Figure 3) show that there are two centers around which other possible conformations group. These are with the values of 0° and –30° or 30° and 0° for the named angles. For the former pair, the furanoic ring takes the form of a flat to twisted ring or E₄ envelope, which results in the withdrawal of the substituent group from the vicinity of the aromatic base and furanoic surroundings. The latter pair of preferred torsional values (conformation E₃) shows the preferred isopropylidene ring approaching to the center of mass of the furanoic ring.

The same trend is present and visible in the measured virtual-bond angle between C5, C4, and the center of mass of the furanoic ring (Figure 4). All measured virtual-bond angles in compounds **1–6** were identified with values between 128° and 135°, while compounds **7–8b** were identified with values between 135° and 142°. Unfortunately, no other differentiation in the latter group (between **7**, **8** and **7a**, **8a**, **8b**) could be identified using this methodology.

2.3. Antifungal activity of QAS

The QAS derivatives of thymidine and uridine (**1–8**) were tested for antifungal activity against three yeast species belonging to the genus *Candida*, namely *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*. The growth inhibitory effect was quantified by the serial dilution microtiter plate method in two growth media, RPMI-1640 and YNB-SG, and compared to that of known antifungals fluconazole and amphotericin B. Only two compounds, *N*-[(5'-deoxythymidine)-5'-yl]trimethylammonium tosylate (**2**) and *N*-[(5'-deoxythymidine)-5'-



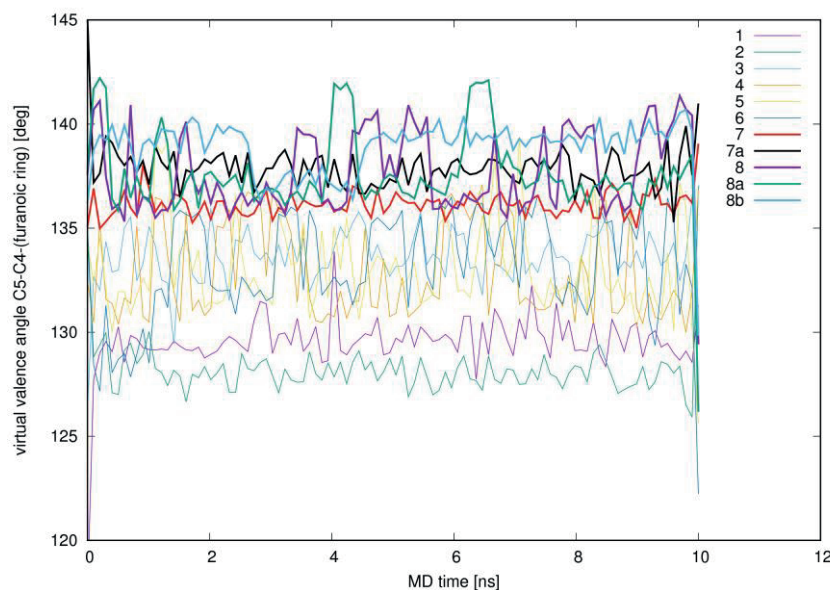


Figure 3. O-C1-C2-C3 vs C2-C3-C4-O dihedral angles' distribution.

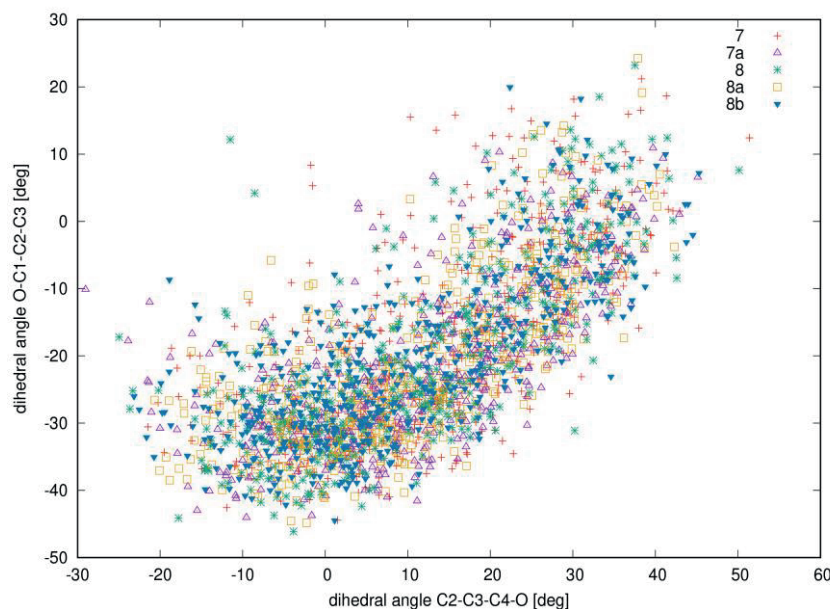


Figure 4. C5-C4-(center of mass of furanico ring) virtual-bond angle analysis.

yl]pyridinium tosylate (**4**), exhibited minimal activity. The results are presented in Table 3 and Figures 5 and 6. Compounds **2** and **4** exhibited concentration-dependent growth inhibitory effect against *C. albicans* and *C. tropicalis* but not *C. glabrata* in YNB-SG medium, with 50% inhibition observed at 0.3–1.0 mg/mL and MIC values as shown in Table 3. These values were remarkably higher than those of fluconazole and amphotericin B. Both compounds were inactive in the RPMI-1640 medium.

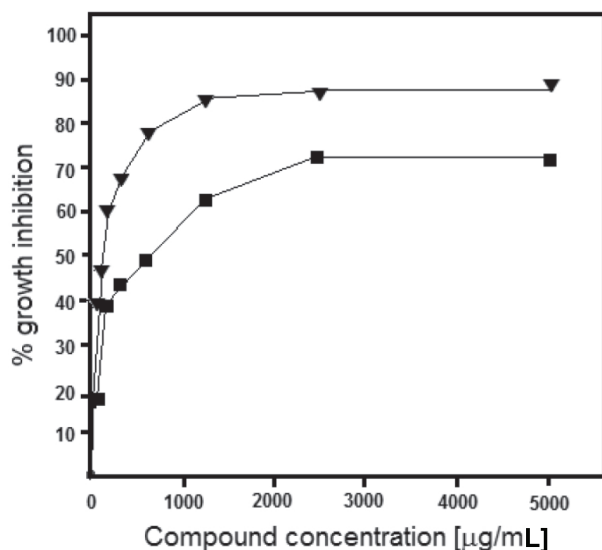


Figure 5. Growth inhibitory curve of compounds **2** (▼) and **4** (■) against *C. albicans* ATCC 10231. Cells were grown in YNB-SG medium.

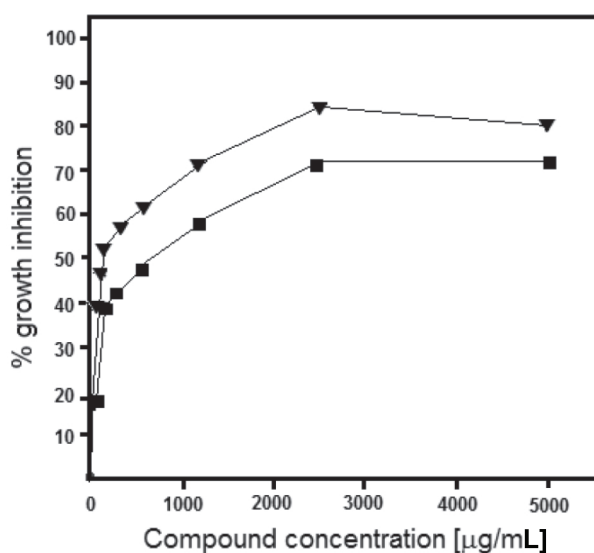


Figure 6. Growth inhibitory curve of compounds **2** (▼) and **4** (■) against *C. tropicalis* KKP 334. Cells were grown in YNB-SG medium.

Table 3. Anticandidal in vitro activity of compounds **2** and **4**. The MIC values [µg/mL] were determined in YNB-SG and RPMI-1640 growth media.

	<i>C. albicans</i>		<i>C. glabrata</i>		<i>C. tropicalis</i>	
	YNB-SG	RPMI-1640	YNB-SG	RPMI-1640	YNB-SG	RPMI-1640
Fluconazole	0.78	0.63	50	12.5	0.39	0.125
Amphotericin B	0.25	0.125	0.25	0.125	0.5	0.25
2	625	>5000	>5000	>5000	2500	>5000
4	2500	>5000	>5000	>5000	2500	>5000

2.4. Conclusion

Six new salt derivatives of thymidine and two *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]aminium salts were obtained. The yields of the preparation of the salts ranged from 59% to 86%. The salt formation reactions were carried out until the state in which no more expected product was formed. Comparing the results of Table 1, it is clear that the steric factor was significant. Despite the much longer quaternization reaction time, use of triethylamine proceeded with less efficiency than in the case of trimethylamine. A similar effect is apparent in the case of pyridine and 2-methylpyridine.

The comparison of the results of Tables 1 and 2 confirms the fact known in the literature that 2-deoxysugars are more reactive than the corresponding sugars.⁵⁴ The presence of 2-deoxy-D-ribose in thymidine makes its derivatives more reactive. Under conditions analogous to those for thymidine derivatives, in the case of uridine derivatives we have not been able to achieve any product. However, in this case, the influence of steric effect was also visible. Only in the case of trimethylamine and pyridine were the desired salts obtained by sonication.

Compounds **2** and **4** exhibited concentration-dependent antifungal in vitro activity against two species of the genus *Candida* in minimal YNB-SG medium. Although this activity was much lower than that of established

antifungal drugs fluconazole and amphotericin B, this is the first report of the defined antifungal activity of compounds of this type.

3. Experimental

3.1. Materials and methods

Commercial thymidine and uridine (Sigma-Aldrich) were used.

All reactions were monitored by thin-layer chromatography on Kieselgel 60 F₂₅₄ silica gel plates (Merck, 0.20 mm thickness). The spots were detected by spraying with 5% ethanolic H₂SO₄ and charring. ¹H NMR and ¹³C NMR spectra were recorded at 25 °C with a Varian Mercury spectrometer at 400 and 100 MHz, respectively, with Me₄Si as the internal standard. Assignments were made on the basis of homonuclear decoupling experiments and homo- and heteronuclear correlation. Optical rotations were measured with a PerkinElmer 343 polarimeter. High-resolution mass spectrometry (HRMS) data were acquired with an Agilent 6550 (Q-TOF) mass spectrometer using a Zorbax Extended C18 column (2.1 × 50 mm; 1.8 μm) at 25 °C; the mobile phase employed water and acetonitrile (95:5) at a flow rate of 0.400 mL/min.

3.2. Chemistry

3.2.1. General procedure for synthesis of QAS derivatives of thymidine und uridine

O-Tosyl substrate (5'-*O*-tosylthymidine or 3'-*O*-acetyl-5'-*O*-tosylthymidine or 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine) was dissolved in a tertiary amine. The solution was stored in a screw-capped ampoule at 70 °C, after which it was evaporated to dryness. The residue was dissolved in H₂O, then extracted with CHCl₃. The aqueous layer was evaporated with reduced pressure at a temperature below 40 °C, and the oil was dried over P₂O₅ in a vacuum desiccator to yield quaternary ammonium compounds, which were crystallized from 2-butanone or ethanol:ethyl acetate. *R*_f = 0 (CHCl₃-MeOH 3:1).

3.2.2. General procedure for exhaustive *O*-acetylation

QAS was dissolved in Py (3 mL) and Ac₂O (3 mL). After 24 h the solutions were evaporated to a dense oil, dissolved in H₂O (2 mL), and extracted with CHCl₃ (2 mL). The aqueous layer was concentrated under reduced pressure at a temperature below 40 °C, and the oil was dried over P₂O₅.

N-[(3'-*O*-Acetyl-5'-doxythymidine)-5'-yl]-*N,N,N*-trimethylammonium tosylate (**1**) was synthesized by heating of the mixture of 3'-*O*-acetyl-5'-*O*-tosylthymidine (34 mg, 0.78 mmol) and 33% ethanolic solution of trimethylamine (0.1 mL) at 70 °C for 24 h. After evaporation of the excess of amine and solvent, the product was obtained almost quantitatively (99% yield) as oil. The reaction products were *O*-acetylated. The aqueous layer, concentrated under reduced pressure and crystallized from ethanol:ethyl acetate, gave the title compound as a white solid: **1** (321 mg, 83% yield); mp 168.2–172.6 °C; [α]_D²⁰ 2.2 (*c* 0.45; H₂O); ¹H NMR (D₂O): δ 7.71–7.37 (2d, 4H, Ph); 7.49 (*bs*, 1H, H-6); 6.23 (*t*, 1H, H-1', *J*_{1',2a'} 7.0 Hz); 5.20 (*q*, 1H, H-3', *J*_{3',4'} 4.0 Hz); 4.62 (*m*, 1H, H-4'); 3.88 (*m*, 1H, H-5a'); 3.80 (*m*, 1H, H-5b'); 2.64 (*m*, 1H, H-2a'); 2.49 (*m*, 1H, H-2b'); 3.21 (*s*, 9H, 3 × CH₃ of N(CH₃)₃); 2.41 (*s*, 3H, PhCH₃); 2.17 (*s*, 3H, OAc); 1.90 (*s*, 3H, CH₃-C5), ¹³C NMR (D₂O): 173.86 (1C, C=OCH₃); 166.40 (C-4); 151.64 (C-2); 142.64–125.59 (6C, Ph); 138.59 (C-6); 111.84 (C-5); 87.32 (C-1'); 77.72 (C-4'); 75.94 (C-3'); 67.88 (C-5'); 54.25 (3C, N(CH₃)₃); 34.64 (C-2'); 20.68 (1C, COCH₃); 20.43

(1C, PhCH₃); 11.63 (CH₃-C5); HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₅H₂₄O₅N₃: 326.1710; found: 326.1709.

N-[(5'-Doxythymidine)-5'-yl]-*N,N,N*-trimethylammonium tosylate (**2**) was synthesized by heating of the mixture of 5'-*O*-tosylthymidine (61 mg, 0.15 mmol) and 33% ethanolic solution of trimethylamine (0.1 mL) at 70 °C for 3 h. Crystallization attempts were unsuccessful. We achieved the title product as yellow oil (61.5 mg; 87%): [α]_D²⁰ 11.40 (*c* 0.088, H₂O); ¹H NMR (D₂O): δ 7.72–7.37 (2d, 4H, Ph); 7.49 (d, 1H, H-6, *J*_{H6,CH3} 0.8 Hz); 6.23 (dd, 1H, H-1', *J*_{1',2a'} 5.4 Hz, *J*_{1',2b'} 2.4 Hz); 4.41 (dt, 1H, H-3'); 4.31 (m, 1H, H-4'); 3.75 (m, 2H, H-5a', H-5b'); 3.23 (s, 9H, 3 × CH₃ of N(CH₃)₃); 2.54 (m, 1H, H-2a'); 2.41 (s, 3H, PhCH₃); 2.37 (m, 1H, H-2b'); 1.90 (s, 3H, CH₃-C5), ¹³C NMR (D₂O): 142.65–125.59 (6C, Ph); 138.55 (C-6); 111.79 (C-5); 86.52 (C-1'); 78.90 (C-4'); 72.05 (C-3'); 68.03 (C-5'); 54.25 (3C, N(CH₃)₃); 37.11 (C-2'); 20.68 (PhCH₃); 11.64 (CH₃-C5); HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₃H₂₂O₄N₃: 284.1605; found: 284.1605.

N-[(3'-*O*-Acetyl-5'-doxythymidine)-5'-yl]-*N,N,N*-triethylammonium tosylate (**3**) was synthesized by heating of the mixture of 5'-*O*-tosylthymidine (50 mg, 0.12 mmol), triethylamine (90.5 mL), and acetonitrile (0.8 mL) at 70 °C for 92 h. The reaction products were *O*-acetylated. The aqueous layer was evaporated and dried over P₂O₅ to give oil of compound **3** (39.7 mg, 59%) as light oil: [α]_D²⁰ 10.0 (*c* 0.22; H₂O); ¹H NMR (D₂O): δ 7.71–7.38 (2d, 4H, Ph); 7.52 (d, 1H, H-6, *J*_{H6,CH3} 1.2 Hz); 6.23 (t, 1H, H-1', *J*_{1',2a'} 7.4 Hz); 5.20 (q, 1H, H-3', *J*_{3',4'} 4.4 Hz); 4.55 (m, 1H, H-4'); 3.72 (m, 2H, H-5a', H-5b'); 3.40 (m, 6H, 3 × CH₂ of N(CH₂CH₃)₃); 2.66 (m, 1H, H-2a'); 2.49 (m, 1H, H-2b'); 2.41 (s, 3H, PhCH₃); 2.18 (s, 3H, OAc); 1.91 (s, 3H, CH₃-C5); 1.28 (m, 9H, 3 × CH₃ of N(CH₂CH₃)₃), ¹³C NMR (D₂O): 173.94 (1C, COCH₃); 166.65 (C-4); 151.64 (C-2); 142.65–125.60 (6C, Ph); 138.52 (C-6); 111.91 (C-5); 87.10 (C-1'); 77.12 (C-4'); 76.05 (C-3'); 58.27 (C-5'); 53.86 (3C, N(CH₂CH₃)₃); 34.51 (C-2'); 20.69 (COCH₃); 20.42 (PhCH₃); 11.61 (CH₃-C5); 6.89 (3C, N(CH₂CH₃)₃); HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₈H₃₀O₅N₃: 368.2180; found: 368.2178.

N-[(5'-Doxythymidine)-5'-yl]pyridinium tosylate (**4**) was synthesized by heating of the mixture of 5'-*O*-tosylthymidine (41 mg, 0.10 mmol) and pyridine (0.3 mL) at 70 °C for 13 h. Crystallization attempts were unsuccessful. We achieved the title product as a light oil (34.8 mg; 71%): [α]_D²⁰ 56.80 (*c* 0.074, H₂O); ¹H NMR (D₂O): δ 8.85–8.07 (m, 5H, Py); 7.69–7.36 (2d, 4H, Ph); 7.27 (d, 1H, H-6, *J*_{H6,CH3} 1.2 Hz); 6.11 (dd, 1H, H-1', *J*_{1',2a'} 5.2 Hz, *J*_{1',2b'} 2.8 Hz); 5.07 (dd, 1H, H-5a', *J*_{5a',5b'} 10.8 Hz, *J*_{5a',4'} 3.2 Hz); 4.90 (dd, 1H, H-5b', *J*_{4',5b'} 7.4 Hz); 4.51 (q, 1H, H-3', *J*_{2b',3'} 5.8 Hz); 4.34 (m, 1H, H-4'); 2.66 (m, 1H, H-2a'); 2.44 (m, 1H, H-2b'); 2.40 (s, 3H, PhCH₃); 1.86 (s, 3H, CH₃-C5), ¹³C NMR (D₂O): 166.65 (C-4); 151.64 (C-2); 146.62–128.40 (5C, Py); 142.64–125.58 (6C, Ph); 138.96 (C-6); 111.61 (C-5); 87.32 (C-1'); 83.16 (C-4'); 70.47 (C-3'); 61.85 (C-5'); 37.03 (C-2'); 20.68 (PhCH₃); 11.58 (CH₃-C5); HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₅H₁₈O₄N₃: 304.1292; found: 304.1291.

N-[(5'-Doxythymidine)-5'-yl]-2-methylpyridinium tosylate (**5**) was synthesized by heating of the mixture of 5'-*O*-tosylthymidine (47 mg, 0.12 mmol) and 2-methylpyridine (0.4 mL) at 70 °C for 120 h. Crystallization attempts were unsuccessful. We achieved the title product as a light yellow oil (45.9 mg; 79%): [α]_D²⁰ 68.00 (*c* 0.25, H₂O); ¹H NMR (D₂O): δ 8.66–7.84 (m, 4H, Py); 7.67–7.33 (2d, 4H, Ph); 7.31 (d, 1H, H-6, *J*_{H6,CH3} 1.2 Hz); 6.10 (dd, 1H, H-1', *J*_{1',2a'} 5.2 Hz, *J*_{1',2b'} 2.4 Hz); 5.03 (dd, 1H, H-5a', *J*_{5a',5b'} 11.6 Hz, *J*_{5a',4'} 3.0 Hz); 4.90 (dd, 1H, H-5b', *J*_{4',5b'} 7.4 Hz); 4.53 (q, 1H, H-3', *J*_{2b',3'} 6.6 Hz); 4.30 (m, 1H, H-4'); 2.86 (s, 3H, CH₃Py); 2.64 (m, 1H, H-2a'); 2.44 (m, 1H, H-2b'); 2.38 (s, 3H, PhCH₃); 1.86 (s, 3H, CH₃-C5), ¹³C NMR (D₂O): 166.65 (C-4); 156.27–125.64 (5C, Py); 151.58 (C-2); 142.60–125.58 (6C, Ph); 138.61 (C-6); 111.71 (C-5);

86.85 (C-1'); 82.77 (C-4'); 70.76 (C-3'); 58.13 (C-5'); 37.23 (C-2'); 20.68 (PhCH₃); 20.21 (CH₃-Py); 11.59 (CH₃-C5); HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₆H₂₀O₄N₃: 318.1448; found: 318.1448.

N-[(5'-Doxythymidine)-5'-yl]-4-(*N,N*-dimethylamino)pyridinium tosylate (**6**) was synthesized by heating of the mixture of 5'-*O*-tosylthymidine (61 mg, 0.15 mmol) 4-(*N,N*-dimethylamine)pyridine (66 mg) and 0.8 mL acetonitrile at 70 °C for 24 h. Crystallization attempts were unsuccessful. We achieved the title product as a light oil (48.0 mg; 60%): $[\alpha]_D^{20}$ 21.90 (*c* 0.694, H₂O); ¹H NMR (D₂O): δ 7.91–6.81 (2d, 4H, Py); 7.67–7.33 (2d, 4H, Ph); 7.05 (*bs*, 1H, H-6); 6.15 (dd, 1H, H-1', *J*_{1',2a'} 4.0 Hz, *J*_{1',2b'} 3.6 Hz); 4.51 (dd, 1H, H-5a', *J*_{5a',5b'} 11.6 Hz, *J*_{5a',4'} 3.4 Hz); 4.40 (m, 1H, H-5b'); 4.37 (m, 1H, H-3'); 4.20 (m, 1H, H-4'); 3.17 (s, 6H, (CH₃)₂Py); 2.56 (m, 1H, H-2a'); 2.49 (m, 1H, H-2b'); 2.38 (s, 3H, PhCH₃); 1.75 (s, 3H, CH₃-C5), ¹³C NMR (D₂O): 167.41 (C-4); 156.44–142.68, 107.20 (5C, Py); 152.82 (C-2); 142.53–125.56 (6C, Ph); 137.23 (C-6); 111.31 (C-5); 85.52 (C-1'); 82.91 (C-4'); 69.34 (C-3'); 56.58 (C-5'); 39.65, (2C, (CH₃)₂NPy); 39.42 (C-2'); 20.68 (PhCH₃); 11.58 (CH₃-C5); HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₇H₂₃O₄N₃: 347.1714; found: 347.1712.

For *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]-*N,N,N*-trimethylaminium tosylate (**7**), 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine (47 mg, 0.11 mmol) and 33% ethanolic solution of NMe₃ (0.20 mL) were placed in an ultrasonic bath at room temperature. After 32 h the solution was evaporated to a dense oil, dissolved in H₂O (2 mL), and extracted with CHCl₃ (2 mL). The aqueous layer was concentrated under reduced pressure at a temperature below 40 °C, and the colorless oil was dried over P₂O₅ in a vacuum desiccator. Crystallization attempts were unsuccessful. The yield of compound **7** was 40 mg (74%); $[\alpha]_D^{20}$ 33.00 (*c* 0.240, H₂O); ¹H NMR (D₂O): δ 7.61–7.28 (2d, each 2H, Ph); 7.55 (d, 1H, H-6, *J*_{5,6} 7.8); 5.76 (d, 1H, H-5); 5.75 (d, 1H, H-1', *J*_{1',2'} 1.8); 5.12 (dd, 1H, H-2', *J*_{2',3'} 4.8); 4.82 (t, 1H, H-3', *J*_{3',4'} 1.2); 4.53 (m, 1H, H-4'); 3.69 (m, 2H, H-5a' and H-5b'); 3.10 (s, 9H, N(CH₃)₃); 2.31 (s, 3H, PhCH₃); 1.54 and 1.33 (2s, each 3H, C(CH₃)₂); ¹³C NMR (H₂O) δ 166.70 (C-4); 151.37 (C-2); 145.18 (C-6); 142.61–125.61 (6C, Ph); 116.11 (C(CH₃)₂); 102.28 (C-5); 95.26 (C-1'); 83.35 (C-2'); 82.51 (C-3'); 81.24 (C-4'); 67.91 (C-5'); 54.24 (3C, N(CH₃)₃); 26.19 and 24.52 (2C, C(CH₃)₂); 20.70 (PhCH₃), HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₅H₂₄O₅N₃: 326.1716; found: 326.1709.

For *N*-[(5'-deoxy-2',3'-*O*-isopropylideneuridine)-5'-yl]pyridinium tosylate (**8**), 2',3'-*O*-isopropylidene-5'-*O*-tosyluridine (67 mg, 0.15 mmol) was dissolved in dry pyridine (0.5 mL). The reaction mixture was kept in an ultrasonic bath at room temperature. After 63 h the mixture was evaporated. The aqueous layer was the title salt (34.0 mg; 43%); $[\alpha]_D^{20}$ 94.88 (*c* 0.254, H₂O); ¹H NMR (D₂O): δ 8.78–8.07 (5H, Py); 7.69–7.36 (2d, each 2H, Ph); 7.62 (d, 1H, H-6, *J*_{5,6} 7.8); 5.86 (d, 1H, H-5); 5.73 (d, 1H, H-1', *J*_{1',2'} 1.6); 5.30 (dd, 1H, H-2', *J*_{2',3'} 4.8); 5.09 (dd, 1H, H-5a', *J*_{5a',5b'} 10.8 Hz, *J*_{5a',4'} 3.2 Hz); 5.06 (m, 1H, H-4'); 5.04 (q, 1H, H-3', *J*_{3',4'} 1.2); 4.96 (dd, 1H, H-5b'); 2.40 (s, 3H, PhCH₃); 1.62 and 1.43 (2s, each 3H, C(CH₃)₂); ¹³C NMR (H₂O) δ 166.75 (C-4); 151.32 (C-2); 146.68–128.44 (5C, Py); 145.18 (C-6); 142.60–125.61 (6C, Ph); 115.87 (C(CH₃)₂); 102.29 (C-5); 95.68 (C-1'); 85.24 (C-2'); 83.92 (C-3'); 80.79 (C-4'); 62.23 (C-5'); 26.26 and 24.58 (2C, C(CH₃)₂); 20.69 (PhCH₃), HRMS (ESI): m/z ([M-Ts]⁺) calcd. for C₁₇H₂₀O₅N₃: 346.1403; found: 346.1400.

3.3. Microorganisms and growth conditions

Antifungal activity was tested against *Candida albicans* ATCC 10231, *Candida glabrata* DSM 11226, and *Candida tropicalis* KKP 334. Strains were grown at 30 °C in YPD medium (1% yeast extract, 2% peptone, and 2% glucose) and stored on YPD plates containing 2% agar. Susceptibility testing was performed in RPMI-1640

medium (RPMI-1640 w/o sodium bicarbonate, with L-glutamine + 2% glucose + 3.45% MOPS, pH adjusted to 7.0) and YNB-SG medium (yeast nitrogen base w/o amino acids and ammonium sulfate + 2% glucose + 0.2 g L⁻¹ sodium glutamate).

3.4. Determination of antifungal in vitro activity

The in vitro growth inhibitory activity of antifungals was quantified by determination of MIC values by the serial two-fold dilution method, using 96-well microtiter plates in RPMI-1640 and YNB-SG growth media. Conditions of the RPMI-1640-based assay were the same as outlined in the CLSI recommendations (Clinical Laboratory Standards Institute, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Third Edition (M27-A3); Wayne, PA. USA, 2008), except for the end-point readout that was done by spectrophotometric determination of cell density at 531 nm. Turbidity in individual wells was measured with a microplate reader (Victor³; PerkinElmer). MIC was defined as the lowest drug concentration that gave at least a 70% decrease in turbidity, relative to that of the drug-free growth control. The 96-well microtiter plates were also used for determination of in vitro growth inhibitory activity in YNB-SG medium. Individual wells were inoculated with 5×10^3 cfu (colony forming units) mL⁻¹ of fungal cells from the overnight culture in YPD medium. The inoculated plates were incubated 37 °C for 24 h and then turbidity was measured with a microplate reader at 531 nm, as described above for the RPMI-1640-based assay. The readouts collected from individual wells were used for the construction of growth inhibitory curves.

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References

1. Li, N.; Smith, T. J.; Zong, M. H. *Biotechnol. Adv.* **2010**, *28*, 348-366.
2. Ralevic, V.; Burnstock G. *Pharmacol. Rev.* **1998**, *50*, 413-492.
3. Jacobson, K. A.; Jarvis, M. F.; Williams, M. *J. Med. Chem.* **2002**, *45*, 4057-4093.
4. Young, J. F.; Therkildsen, M.; Ekstrand, B.; Che, B. N.; Larsen, M. K.; Oksbjerg, N.; Stagsted, J. *Meat. Sci.* **2013**, *95*, 904-911.
5. Uauy, R.; Quan, R.; Gil, A. *J. Nutr.* **1994**, *124*, 1436S-1441S.
6. Micheli, V.; Camici, M.; Tozzi, M. G.; Ipata, P. L.; Sestini, S.; Bertelli, M.; Pompucci, G. *Curr. Top. Med. Chem.* **2011**, *11*, 923-947.
7. Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875-1916.
8. Robins, M. J. In *Nucleoside Analogues: Chemistry, Biology, and Medical Applications*; Walker, R. T.; DeClercq, E.; Eckstein, F., Eds. Plenum Press: New York, NY, USA, 1979, pp. 165-192.
9. Townsend, L. B. In *Nucleoside Analogues: Chemistry, Biology, and Medical Applications*; Walker, R. T.; DeClercq, E.; Eckstein, F., Eds. Plenum Press: New York, NY, USA, 1979, pp. 193-223.
10. Lee, S. E.; Sidorov, A.; Gourelain, T.; Mignet, N.; Thorpe, S. J.; Brazier, J. A.; Dickman, M. J.; Hornby, D. P.; Grasby, J. A.; Williams, D. M. *Nucleic Acids Res.* **2001**, *29*, 1565-1573.

11. Sakthivel, K.; Barbas, C. F. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2872-2875.
12. Ruth, J. L. In *Oligonucleotides and Analogues*; Eckstein, F., Ed. OUP: New York, NY, USA, 1991, pp. 255-281.
13. Anderson, J. P.; Angerer, B.; Loeb, L. A. *Biotechniques* **2005**, *38*, 257-264.
14. Abdoli, M.; Mirjafary, Z.; Saeidian, H.; Kakanejadifard, A. *RSC Adv.* **2015**, *5*, 44371-44389.
15. Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99-134.
16. Eaton, B. E.; Pieken, W. A. *Annu. Rev. Biochem.* **1995**, *64*, 837-863.
17. Pankiewicz, K. W. *Carbohydr. Res.* **2000**, *327*, 87-105.
18. Sproat, B. S.; Lamond, A. I. In *Oligonucleotides and Analogues*. Eckstein, F., Ed. OUP: New York, NY, USA, 1991, pp. 49-86.
19. Beigelman, L.; Haerberli, P.; Sweedler, D.; Karpeisky, A. *Tetrahedron* **2000**, *56*, 1047-1056.
20. Robins, M. J.; Wilson, J. S.; Madej, D.; Low, N. H.; Hansske, F.; Wnuk, S. F. *J. Org. Chem.* **1995**, *60*, 7902-7908.
21. Rao, A. V. R.; Gurjar, M. K.; Lalitha, S. V. S. *J. Chem. Soc. Chem. Commun.* **1994**, 1255-1256.
22. Cano-Soldado, P.; Pastor-Anglada, M. *Med. Res. Rev.* **2012**, *32*, 428-457.
23. Minuesa, G.; Huber-Ruano, I.; Pastor-Anglada, M.; Koepsell, H.; Clotet, B.; Martinez-Picado, J. *Pharmacol. Ther.* **2011**, *132*, 268-279.
24. Pathak, T. *Chem. Rev.* **2002**, *102*, 1623-1668.
25. Pastor-Anglada, M.; Felipe, A.; Casado, F. J. *Trends Pharmacol. Sci.* **1998**, *19*, 424-430.
26. Connolly, G. P.; Duley, J. A. *Trends Pharmacol. Sci.* **1999**, *20*, 218-225.
27. Koyama, G.; Maeda, K.; Umezawa, H. *Tetrahedron Lett.* **1966**, *6*, 597-602.
28. Krugh, T. R. *J. Am. Chem. Soc.* **1973**, *95*, 4761-4762.
29. Horowitz, J. P.; Tomson, A. J.; Urbański, J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3045-3048.
30. Lin, T. S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109-112.
31. Horowitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076-2078.
32. Miller, N.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 1772-1776.
33. Kuca, K.; Kivala, M.; Dohnal, V. *J. Appl. Biomed.* **2004**, *2*, 195-198.
34. Quagliotto, P.; Viscardi, G.; Barolo, C.; D'Angelo, D.; Barni, E.; Compari, C.; Duce, E.; Fiscaro, E. *J. Org. Chem.* **2005**, *70*, 9857-9866.
35. Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1955**, 816-823.
36. Skorupa, E.; Dmochowska, B.; Pellowska-Januszek, L.; Wojnowski, W.; Chojnacki, J.; Wiśniewski, A. *Carbohydr. Res.* **2004**, *339*, 2355-2362.
37. Pellowska-Januszek, L.; Dmochowska, B.; Skorupa, E.; Chojnacki, J.; Wojnowski, W.; Wiśniewski, A. *Carbohydr. Res.* **2004**, *339*, 1537-1544.
38. Fromageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *23*, 2315-2331.
39. Shuman, A.; Robins, R. K.; Robins, M. J. *J. Am. Chem. Soc.* **1969**, *91*, 3391-3392.
40. Case, D. A.; Darden, T. A.; Cheatham, T. E. 3rd; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Walker, R. C.; Zhang, W.; Merz, K. M. et al. *AMBER 12*; University of California: San Francisco, CA, USA, 2012.
41. Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T. et al. *J. Comput. Chem.* **2003**, *24*, 1999-2012.
42. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004**, *25*, 1157-1174.
43. Cieplak, P.; Cornell, W. D.; Bayly, C.; Kollman, P. A. *J. Comput. Chem.* **1995**, *16*, 1357-1377.

44. Onufriev, A.; Bashford, D.; Case, D. A. *J. Phys. Chem. B* **2000**, *104*, 3712-3720.
45. Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327-341.
46. Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684-3690.
47. Ewald, P. P. *Ann. Phys.* **1921**, *369*, 253-287.
48. Darden, T.; Perera, L.; Li, L.; Pedersen, L. *Structure* **1999**, *7*, R55-R60.
49. Salomon-Ferrer, R.; Götz, A. W.; Poole, D.; Le Grand, S.; Walker, R. C. *J. Chem. Theory Comput.* **2013**, *9*, 3878-3888.
50. Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. *J. Cheminform.* **2012**, *4*, 17.
51. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720-729.
52. Stewart, J. J. P. *J. Mol. Model.* **2007**, *13*, 1173-1213.
53. Stewart, J. J. P. *MOPAC2016*; Stewart Computational Chemistry: Colorado Springs, CO, USA, 2016.
54. Newth, F. H.; Phillips, G. O. *J. Chem. Soc.* **1953**, 2904-2909.