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Synthesis, antimicrobial activity and cytotoxicity of triphenylphosphonium (TPP) conjugates of 1,2,3-triazolyl nucleoside analogues

Irina Yu. Strobykina, Alexandra D. Voloshina *, Olga V. Andreeva, Anastasiia S. Sapunova, Anna P. Lyubina, Syumbelya K. Amerhanova, Mayya G. Belenok, Liliya F. Saifina, Vyacheslav E. Semenov, Vladimir E. Kataev

Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center, Russian Academy of Sciences, Arbuzov str. 8, Kazan 420088, Russian Federation

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ABSTRACT

Four new triphenylphosphonium (TPP) conjugates of 1,2,3-triazolyl nucleoside analogues were synthesized by coupling with 8-bromoctyl- or 10- bromdecyltriphenylphosphonium bromide and evaluated for the *in vitro* antibacterial activity against *S. aureus*, *B. cereus*, *E. faecalis*, two MRSA strains isolated from patients and resistant to fluoroquinolone antibiotic ciprofloxacin and β-lactam antibiotic amoxicillin, *E. coli*, antifungal activity against *T. mentagrophytes C. albicans* and cytotoxicity against human cancer cell lines M−HeLa, MCF-7, A549, HuTu-80, PC3, PANC-1 and normal cell line Wi-38. In these compounds a TPP cation was attached via an octyl or a decyl linker to the N 3 atom of the heterocycle moiety (thymine, 6-methyluracil, quinazoline-2,4-dione) which was bonded with 2′,3′,5′-tri- *O* - acetyl-greek beta-D-ribofuranose residue by the (1,2,3-triazol-4-il)methyl bridge. All synthesized compounds showed high antibacterial activity against *S. aureus* within the range of MIC values 1.2–4.3 greek muM, and three of them appeared to be bactericidal with respect to tis bacterium at MBC values 4.1–4.3 greek muM. Two lead compounds showed both high antibacterial activity against the MRSA strains resistant to Ciprofloxacin and Amoxicillin within the range of MIC values 1.0–4.3 greek muM and high cytotoxicity against human cancer cell lines HuTu-80 and MCF-7 within the range of IC_{50} values 6.4–10.2 greek muM. This is one of the few examples when phosphonium salts exhibited both antibacterial activity and cytotoxicity against human cancer cell lines. According to the results obtained the bactericidal effect of the lead compounds, unlike classical surfactants, was not caused by a violation of the integrity of the cytoplasmic membrane of bacteria and their cytotoxic activity is most likely associated both with the induction of apoptosis along the mitochondrial pathway and the arrest of the cell cycle in the G0/G1 phase.

1. Introduction

Based on the facts that bulky lipophilic cations penetrate the mitochondrial membrane [1,2] and the mitochondria of cancer cells have a more negative transmembrane potential compared to normal cells, a triphenylphosphonium (TPP) cation is widely used for the targeted delivery of antioxidants and cytostatics to the mitochondria of cancer cells [2–7]. The conjugation of such known drugs as doxorubicin, cisplatin, chlorambucil, camptothecin and others with a TPP cation has provided an increase in their cytotoxicity [5,8–10]. Besides, as a rule, phosphonium salts (especially TPP salts) with one, two or three alkyl chains exhibit high antimicrobial activity, including against bacteria of the ESKAPE group [11–15]. The activity increases both with an increase in the length of the alkyl chain and with an increase in the number of phosphonium groups [12,13,15,16]. As in the case of cytostatics, conjugation of known antibacterial agents with a TPP cation via an alkyl linker leads to an increase in antibacterial activity [14]. Previously, the mechanism of the antibacterial action of TPP salts was explained by their destructive integration into the membranes of pathogens [17–19]. However, evidence has recently been received that TPP-conjugates do not alter membrane permeability in some bacteria [20].

Since a TPP cation is capable of targeted penetration both into the mitochondria of cancer cells and into the membranes of bacteria, it can be assumed that TPP-conjugates should have dual activity - cytotoxic against cancer cells and antibacterial. The literature has provided just a few examples of such phenomenon. For example, the most active

* Corresponding author.
E-mail address: sobaka-1968@mail.ru (A.D. Voloshina).

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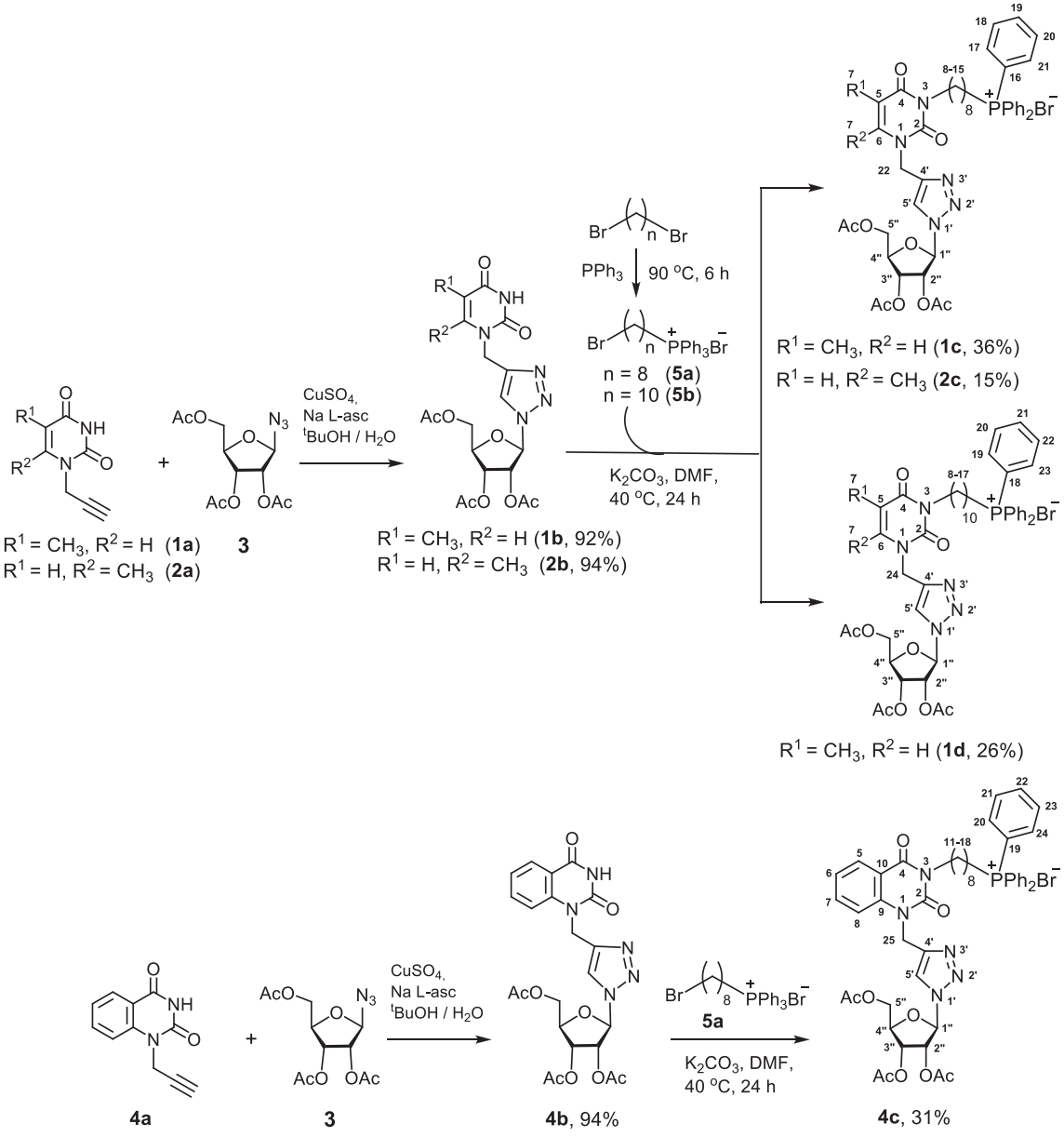
representatives of the series of 2-hydroxybenzylphosphonium salts exhibited both antimicrobial activity at the micromolar level against gram-positive bacteria *S. aureus*, *B. cereus* and fungi *T.mentagrophytes* and *C. ablicans* and selective cytotoxicity against human cancer cell line M−HeLa at the doxorubicin level, inducing mitochondrial apoptosis [20].

Recently, our group reported on the synthesis and cytotoxicity evaluation of the first TPP-conjugates of uracil, thymine and 1,2,3-triazolyl analogues of pyrimidine nucleosides in which the TPP cation was attached to the *N*3 atom of the pyrimidine moiety by a butyl linker [21]. In this short communication, we report on the first TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1c, d**; **2c**; **4c** possessing the TPP cation attached to the *N*3 atom of the pyrimidine moiety by an octyl or decyl linker which showed both high cytotoxicity against human cancer cell lines HuTu-80, MCF-7 and high antibacterial activity against MRSA strains resistant to ciprafloxacin and amoxicillin. The length of the linkers was chosen in accordance with the data that conjugation with a TPP cation by polymethylene linkers with n = 8, 10 or more provides higher antibacterial activity [15,19]. A full-scale study of the cytotoxicity and antibacterial activity of TPP-conjugates of 1,2,3-triazolyl nucleoside analogues with a wide range of alkyl chain lengths and both with acylated and unprotected hydroxyl groups of the sugar residue will soon follow this preliminary short communication.

2. Results and discussions

2.1. Chemistry

The target compounds **1c, d**; **2c**; **4c** were synthesized in 2 stages. At the first stage *N*1-propargyl derivatives of thymine **1a**, 6-methyluracil **2a**, quinazoline-2,4-dione **4a** and azide **3** prepared by the methods previously described [22] were involved in the Cu alkyne-azide cycloaddition (CuAAC) reaction which afforded 1,2,3-triazolyl analogues of pyrimidine nucleosides **1b**, **2b**, **4b** in very good yields (92%, 94%, and 94%, respectively). The spectral characteristics of **1b**, **2b**, **4b** corresponded to the literature ones [22] (Scheme 1). At the second stage 1,2,3-triazolyl nucleoside analogues **1b**, **2b**, **4b** were reacted with 8-bromooctyl- and 10-bromodecyltriphenylphosphonium bromides **5a** and



Scheme 1. Synthesis of the target TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1c**, **2c**, **1d**, **4c**.

5b obtained by heating 1,8-dibromoctane and 1,10-dibromodecane with triphenylphosphine without solvent at an oil bath temperature of 90 °C for 6 h similarly to the known procedure [23,24]. Target TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1c**, **2c**, **1d**, **4c** were obtained in 36%, 15%, 26%, 31% yields, respectively (Scheme 1). In the last 10 years, so-called prodrug forms of nucleoside analogues with protected polar groups were being synthesized to ensure better penetration into cells through lipid-rich cell membranes [25]. That is why, in this study we did not remove the acetyl protection of the hydroxyl groups of 1,2,3-triazolyl nucleoside analogues **1c**, **2c**, **1d**, **4c**.

Procedures for the synthesis of **1c**, **2c**, **1d**, **4c**, their characterizations including NMR spectra are presented in Supplementary data.

2.2. Biology

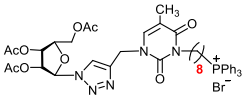
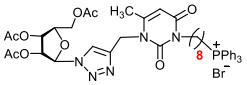
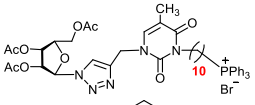
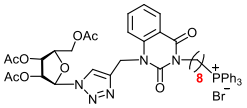
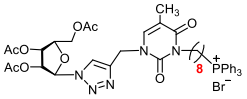
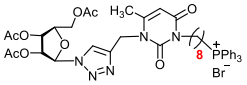
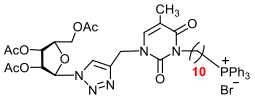
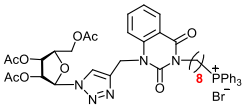
2.2.1. Antimicrobial activity

Synthesized TPP-conjugates **1c**, **2c**, **1d**, **4c** were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (*Sa*), *Bacillus cereus* (*Bc*), *Enterococcus faecalis* (*Ef*), MRSA-1 and MRSA-2 strains isolated from patients of the Republic Clinical Hospital (Kazan, Russian Federation), Gram-negative bacteria *Escherichia coli* (*Ec*) and *in vitro* antifungal activity against *Candida albicans* (*Ca*) and *Trichophyton mentagrophytes* var. *gypseum* (*Tm*). Both methicillin-resistant *S. aureus* strains possessed high drug resistance. The MRSA-1 strain was resistant to fluoroquinolone antibiotic ciprofloxacin and β-lactam antibiotic amoxicillin. The MRSA-2 strain was resistant only to amoxicillin. The resulting data expressed as minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) are presented in Table 1.

It can be seen that the TPP conjugates showed high selectivity for the Gram-positive bacteria used, including the MRSA strains, showing very low MIC values (1.0–4.3 μM). The lead compounds **1d** and **4c** demonstrated high antibacterial activity against Gram-positive bacteria *Sa*, *Bc*, MRSA-1, MRSA-2 (MIC = 1.0–2.0 μM) which exceeded the activity of the reference compound – drug norfloxacin by 12 times in the case of *Bc* and 250 times in the case of the resistant strain MRSA-1 (Table 1). TPP-conjugate **1c** also showed high antibacterial activity against Gram-positive bacteria *Sa*, *Ef*, MRSA-2 (MIC = 2.1, 8.5, 4.3 μM, respectively) which is comparable to the activity of the reference compound norfloxacin (MIC = 7.5 μM). As for the resistant strain MRSA-1, **1c** inhibited its growth at the concentration 63 times lower than norfloxacin (Table 1). It should be noted that TPP-conjugates **1c** and **1d** exhibited bactericidal action against *Sa*, MRSA-1, MRSA-2, and their MIC and MBC values did not differ by >4 times. TPP-conjugate **2c** was bactericidal only against *Sa* at MBC value of 4.3 μM (Table 1).

As it was reported previously, the antibacterial effect of quaternized ammonium compounds and phosphonium salts is associated with disruption of the bacterial cell membrane [17–19]. To examine whether

Table 1
In vitro antimicrobial activity of TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1c**,**2c**,**1d**,**4c**.

Compound	Structure	Minimal inhibitory concentration (MIC, μM)							
		[a]*Sa*	[b]*Bc*	[c]*Ef*	[d]*MRSA-1*	[e]*MRSA-2*	[f]*Ec*	[g]*Tm*	[h]*Ca*
1c		2.1 ± 0.1	17.0 ± 1.3	8.5 ± 0.7	4.3 ± 0.3	4.3 ± 0.3	136 ± 12	na	na
2c		4.3 ± 0.3	34 ± 2.3	272 ± 22	17 ± 1.4	8.5 ± 0.7	272 ± 20	na	na
1d		**1.0 ± 0.08**	**2.0 ± 0.1**	8.3 ± 0.6	**1.0 ± 0.08**	**1.0 ± 0.06**	132 ± 10	na	na
4c		**1.0 ± 0.09**	**2.0 ± 0.1**	8.2 ± 0.7	**1.0 ± 0.06**	**1.0 ± 0.07**	na	na	na
Norfloxacin		7.5 ± 0.5	24.4 ± 2.1	7.5 ± 0.5	250 ± 21	7.5 ± 0.5	4.7 ± 0.02	na	na
Ketoconazol		nd	nd	nd	nd	nd	nd	7.3 ± 0.5	7.3 ± 0.5
		Minimal bactericidal concentration (MBC, μM)						Minimal fungicidal concentration (MFC, μM)	
1c		**4.3 ± 0.3**	34.0 ± 2.6	68.0 ± 5.7	**4.3 ± 0.3**	**4.3 ± 0.3**	272 ± 21	na	na
2c		**4.3 ± 0.3**	68.0 ± 5.5	na	17.0 ± 1.4	17.0 ± 1.3	na	na	na
1d		**4.1 ± 0.3**	na	33.0 ± 2.6	**2.0 ± 0.1**	**4.1 ± 0.2**	132 ± 11	na	na
4c		16.4 ± 1.3	131 ± 21	131 ± 19	**1.0 ± 0.08**	**1.0 ± 0.07**	131 ± 12	na	na
Norfloxacin		7.5 ± 0.6	24.4 ± 2.1	7.5 ± 0.6	nd	7.5 ± 0.5	24.4 ± 2.1	nd	nd
Ketoconazol		nd	nd	nd	nd	nd	nd	7.3 ± 0.5	7.3 ± 0.5

[a]*Sa* - *Staphylococcus aureus*; [b]*Bc* - *Bacillus cereus*; [c]*Ef* - *Enterococcus faecalis*; [d]*MRSA-1* – methicillin-resistant *S. aureus* resistant to fluoroquinolones and β-lactam antibiotics; [e]*MRSA-2* - methicillin-resistant *S. aureus* resistant to β-lactam antibiotics; [h]*Ec* - *Escherichia coli*; [g]*Tm* - *Trichophyton mentagrophytes*; [h]*Ca* - *Candida albicans*; *na* - non active; *nd* - not determined. The experiments were repeated 3 times.

TPP-conjugates **1c**, **2c**, **1d**, **4c** can alter the integrity of the cell membrane of *S. aureus* or not, its integrity has been verified by measuring the absorption of hydrophobic crystal violet (CV) dye. The data obtained (Supplementary data) allow to conclude that the mechanism of the antibacterial effect of TPP-conjugates **1c**, **2c**, **1d**, **4c** differs from that of classical surfactants [17–19], that is, it is not associated with a violation of the integrity of the cytoplasmic membrane of bacteria.

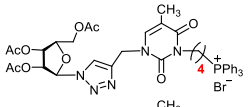
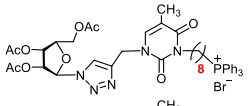
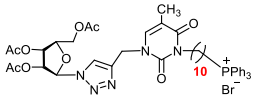
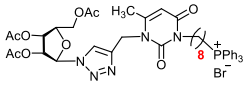
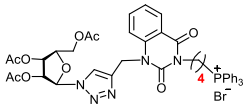
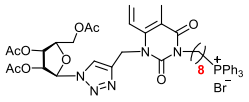
To assess the effect of TPP-conjugates **1c**, **2c**, **1d**, **4c** on the *S. aureus* plasma membrane we used Molecular Probe LIVE/DEAD® BacLightTM Bacterial Viability Kit method which is widely used for the qualitative and quantitative analysis of viable and damaged cells after exposure to antimicrobial agents [26]. The data obtained (Supplementary data) confirmed the results of the absorption of crystal violet (CV) dye, namely, TPP-conjugates **1c**, **2c**, **1d**, **4c** within the range of their MIC and MBC values (1.0–16.4 μM) have no significant effect on the permeability of the cytoplasmic membrane of *S. aureus*. So, we can confidently suppose that the mechanism of bacteriostatic and bactericidal action of TPP-conjugates **1c**, **2c**, **1d**, **4c** on *S. aureus* is not associated with damage to the bacterial cell wall and cytoplasmic membrane.

2.2.2. Cytotoxicity

TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1c**, **2c**, **1d, 4c** were also evaluated for *in vitro* cytotoxicity against six human cancer cell lines: M−HeLa cervical epitheloid carcinoma, MCF-7 breast adenocarcinoma, A549 pulmonary adenocarcinoma, HuTu-80 duodenal adenocarcinoma, PC3 prostate adenocarcinoma, PANC-1 pancreatic carcinoma as well as a diploid human cell strain WI-38 composed of fibroblasts. The resulting data expressed as concentrations causing the inhibition of the growth of 50% of cells in the experimental population (IC_{50}) and selectivity index (SI) which is the ratio IC_{50}(normal cell)/IC_{50}(cancer cell) are presented in Table 2 and Table 3. As one can see in Table 2, the tested TPP-conjugates demonstrated high activity against some human cancer cell lines and moderate cytotoxicity against normal Wi-38 cells of the lung embryo. The most significant results were obtained for TPP-conjugates **1c**, **4c** and **1d**. The first two compounds showed selective cytotoxicity against HuTu-80 duodenal adenocarcinoma cell line (IC_{50} = 8.3, 10.2 μM, respectively) and MCF-7 breast adenocarcinoma cell line (IC_{50} = 10.2, 7.3 μM, respectively). The lead compound **1d** showed high selective cytotoxicity against HuTu-80 cell line (IC_{50} = 6.4 μM, SI > 16, Table 2, Table 3). Thus, TPP-conjugates **1c**, **1d**, **4c** showed cytotoxicity against HuTu-80 cell line which corresponded to the cytotoxicity of the reference drug doxorubicin and exceeded the cytotoxicity of the reference compound – drug fluorouracil against the same cancer cell lines by 7–10 times. It should be emphasized that the selectivity index of the lead compound **1d** (SI > 16) is 23 times higher than the selectivity index of fluorouracil (SI = 0.7) and 40 times higher than the selectivity index of doxorubicin (SI = 0.4) (Table 3). And, besides, TPP-conjugates **1c** and **4c** showed cytotoxicity against MCF-7 cell line which corresponded to the cytotoxicity of the reference drug doxorubicin and surpassed the cytotoxicity of the reference drug tamoxifen against the same cancer cell line by 2–3 times (Table 2).

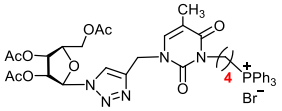
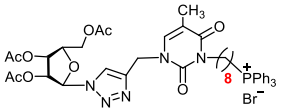
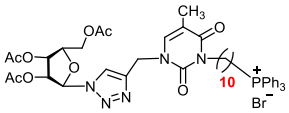
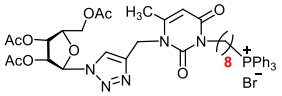
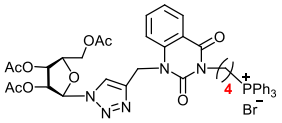
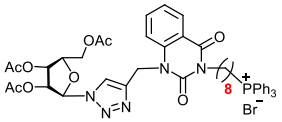
In addition to the already mentioned high selective cytotoxicity of TPP-conjugates **1c**, **1d**, **4c**, the analysis of Table 2 leads to two more important conclusions. First, TPP-conjugate **2c** with the 6-methyluracil moiety was significantly less active against the used human cancer cell lines than TPP-conjugates **1c**, **1d** with the thymine moiety and TPP-conjugate **4c** with the quinazoline-2,4-dione moiety. Second, the increase in the length of the polymethylene chain linking the TPP cation with the *N*3 atom of the heterocyclic moiety of TPP-conjugates of 1,2,3-triazolyl nucleoside analogues is accompanied by a significant increase in their cytotoxicity. This can be seen by comparing the cytotoxicity of TPP-conjugates **1e**, **1c**, **1d** against cancer cell lines M−HeLa, PC3, PANC-1 presented in Table 2. In the series of compounds **1e**, **1c**, **1d** with the increase in the length of the polymethylene chain (n = 4, 8, 10,

Table 2
In vitro cytotoxicity of synthesized TPP-conjugates of 1,2,3-triazolyl nucleoside analogues against human cancer and human normal cell lines (IC_{50} values in μM with standard errors).

Compound	Structure	Cancer cell lines						Normal cell line
		[a]M−HeLa	[b]MCF-7	[c]A549	[d]HuTu-80	[e]PC3	[f]PANC-1	[g]Wi-38
1e		81 ± 6.2[h]	>100[h]	nd	nd	>100[h]	>100[h]	>250[h]
1c		50.2 ± 4.5	**10.2 ± 0.8**	57.9 ± 4.5	**8.3 ± 0.6**	>100	>100	55.5 ± 4.8
1d		19.7 ± 1.6	48.7 ± 3.8	39.9 ± 2.7	**6.4 ± 0.4**	30.8 ± 2.3	60 ± 5.4	**>100**
2c		73.2 ± 6.3	55.4 ± 4.6	>100	35.7 ± 2.9	>100	>100	54.5 ± 4.6
4d		>100[h]	>100[h]	nd	nd	>100[h]	>100[h]	>100[h]
4c		22.8 ± 1.9	**7.3 ± 0.5**	>100	**10.2 ± 0.9**	25.7 ± 1.7	27.5 ± 1.9	54.2 ± 4.3
Tamoxifen		28 ± 2.5	25 ± 2.2	nd	nd	nd	nd	45 ± 3.8
Fluorouracil		nd	nd	42.5 ± 3.6	65.2 ± 5.5	43 ± 3.6	39.3 ± 2.7	48 ± 4.1
Doxorubicin		3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0.1	7.0 ± 0.6	1.3 ± 0.1

[a]M−Hela is a human cervix epitheloid carcinoma; [b]MCF-7 is a human breast adenocarcinoma (pleural fluid); [c]A549 is a human lung carcinoma; [d]HuTu-80 is a human duodenal adenocarcinoma; [e]PC3 is a human prostate adenocarcinoma; [f]PANC-1 is a human pancreatic carcinoma; [g]Wi-38 is a diploid human embryo lung; [h]previously published data [21]; *ns* no selectivity; *nd* not determined; the experiments were repeated 3 times.

Table 3
Selectivity indices of TPP-conjugates **1c**, **1d**, **2c**, **4c** synthesized in this work and TPP-conjugates **1e**, **4d** previously published [21].

Compound	Structure	Cancer cell lines					
		[a]M-HeLa	[b]MCF-7	[c]A549	[d]HuTu-80	[e]PC3	[f]PANC-1
1e		>3[g]	ns[g]	nd	nd	ns[g]	ns[g]
1c		1.1	5.4	0.9	6.7	ns	ns
1d		>5	>2	>3	**>16**	>3	>1.7
2c		0.7	1.0	ns	1.5	ns	ns
4d		ns[g]	ns[g]	nd	nd	ns[g]	ns[g]
4c		2.4	7.4	ns	5.3	2.1	2.0
Tamoxifen		1.6	1.8	nd	nd	nd	nd
Fluorouracil		nd	nd	1.1	0.7	1.1	1.2
Doxorubicin		0.4	0.4	0.4	0.4	0.4	0.2

[a]M-Hela is a human cervix epitheloid carcinoma; [b]MCF-7 is a human breast adenocarcinoma (pleural fluid); [c]A549 is a adenocarcinomic human alveolar basal epithelial cell line; [d]HuTu-80 is a duodenal adenocarcinoma; [e]PC3 is a human prostate adenocarcinoma; [f]PANC-1 is a human pancreatic cancer cell line isolated from a pancreatic carcinoma of ductal cell origin; [g]previously published data [21]; *ns* no selectivity; *nd* not determined.

respectively), the IC_{50} values decrease (Table 2). So, if TPP-conjugate **1e** in which the TPP cation is attached to the *N*3 atom of the thymine moiety by the butyl chain was inactive against PC3 and PANC-1 cancer cell lines and showed moderate cytotoxicity (IC_{50} = 81 μM) against M−HeLa, then TPP-conjugate **1d** with decyl chain between the TPP cation and the *N*3 atom of the thymine moiety demonstrated moderate to high cytotoxicity against these cancer cell lines with IC_{50} values of 30.8, 60, and 19.7 μM, respectively (Table 2). Similarly, if TPP-conjugate **4d** in which the TPP cation is attached to the *N*3 atom of the quinazoline-2,4-dione moiety via the butyl chain was inactive against M−HeLa, MCF-7, PC3, and PANC-1 cancer cell lines, then TPP-conjugate **4c** possessing the decyl chain between the TPP cation and the quinazoline-2,4-dione moiety showed moderate cytotoxicity against these cancer cell lines with IC_{50} values of 22.8, 7.3, 25.7, and 27.5 μM, respectively (Table 2). So, the lengthening of the polymethylene linker between the TPP cation and the pyrimidine moiety in the studied TPP-conjugates considerably increases not only antimicrobial activity but also cytotoxicity against some human cancer cell lines.

Currently, apoptosis is one of the key mechanisms used in the development of new anticancer agents. In this regard, it was of considerable interest to study the ability of the lead compound **1d** to induce apoptosis in HuTu-80 cells. According to the data of flow cytometry, it can be seen that as a result of 24 h incubation of HuTu-80 cells in the presence of **1d**, a dose-dependent induction of the apoptosis process is observed (Fig. 1, Fig. 2). Moreover, apoptotic effects were manifested only at the stage of early apoptosis.

There are two mechanisms for the induction of apoptosis: the external apoptotic pathway through death receptors and the internal apoptotic pathway (mitochondria-dependent apoptosis). The external pathway triggers apoptosis in response to extrinsic stimuli, during which specific ligands bind to death receptors on the cell membrane surface, belonging to the superfamily of tumor necrosis factor receptor (TNFR), with their respective protein TNF family ligands [27]. In the case of mitochondrial apoptosis, cell death occurs as a result of irreparable damage to DNA. For this reason, the cell starts an internal apoptotic cascade. The internal pathway of apoptosis induction is accompanied by the destruction of the mitochondrial membrane, which leads to a decrease in its potential, which is a key indicator of the state of cells [28]. The results obtained with the help of flow cytometry using JC-10 fluorescent dye from the Mitochondria Membrane Potential Kit showed that the lead compound **1d** induced apoptosis along the mitochondrial pathway (Supplementary data).

The mechanism of action of cytotoxic agents, as a rule, is associated with a violation of the passage of cells of the cell cycle, leading to synchronization and slowing down of proliferation of rapidly multiplying cells. Cell cycle analysis by quantifying the DNA content of a cell is a reliable method to assess at which phase the cell cycle has been stopped. We have carried out such investigation using fluorescent dye propidium iodide, which binds in proportion to the amount of DNA present in the cell. The results of the analysis of the cell cycle of a cell line HuTu-80 after its treatment with the lead compound **1d** for 24 h (Supplementary data) showed that the mechanism of its action is most likely associated not only with the induction of apoptosis along the mitochondrial pathway but also with the arrest of the cell cycle in the G0/G1 phase.

3. Conclusion

Four new triphenylphosphonium (TPP) conjugates of 1,2,3-triazolyl nucleoside analogues were synthesized and evaluated for the *in vitro* antibacterial activity against *S. aureus*, *B. cereus*, *E. faecalis*, two MRSA

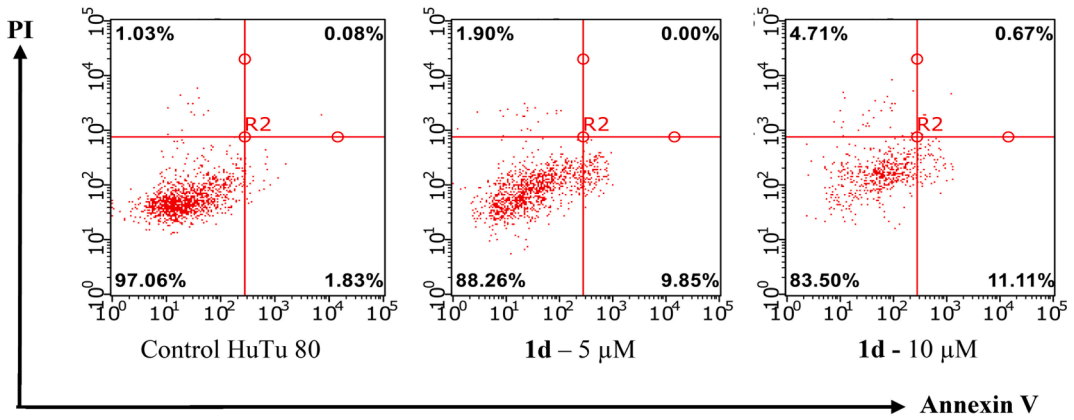


Fig. 1. Apoptosis induction in HuTu-80 cells incubated with 5 μM and 10 μM of compound **1d** for 24 h. Quantification of dot plots expressed as percentage of total cells (mean ± SD (n = 3); (*) P < 0.01 compared to control).

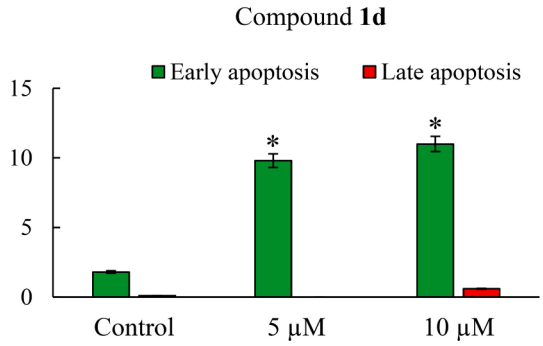


Fig. 2. Representative histograms for the number of cells (% of the total) at the early and late stages of apoptosis for the control and experimental groups after treatment with **1d**. The values are presented as the mean ± SD (n = 3); (*) P < 0.01 compared to control.

strains isolated from patients and resistant to fluoroquinolone antibiotic ciprofloxacin and β-lactam antibiotic amoxicillin, *E. coli*, antifungal activity against *T. mentagrophytes*, *C. albicans* and cytotoxicity against human cancer cell lines M−HeLa, MCF-7, A549, HuTu-80, PC3, PANC-1 and normal cell line Wi-38. All synthesized compounds showed high antibacterial activity against *S. aureus* within the range of MIC values 1.2–4.3 μM, and three of them appeared to be bactericidal with respect to this bacterium at MBC values of 4.1–4.3 μM. Moreover, TPP-conjugates **1c** and **1d** in which the TPP cation was attached to the *N*3 atom of the thymine moiety via an octyl (or a decyl) linker respevtively and TPP-conjugate **4c** in which the TPP cation was attached to the *N*3 atom of quinazoline-2,4-moiety via an octyl linker showed both high antibacterial activity against the MRSA strains resistant to ciprofloxacin and amoxicillin within the range of MIC values 1.0–4.3 μM and high cytotoxicity against human cancer cell lines HuTu-80 and MCF-7 within the range of IC_{50} values 6.4–10.2 μM. This is one of the few examples when phosphonium salts exhibited both antibacterial activity and cytotoxicity against human cancer cell lines. According to the results of crystal violet dye assay and LIVE/DEAD BacLightTM Bacterial Viability Method the synthesized TPP-conjugates within the range of their MIC and MBC values, unlike classical surfactants, do not effect the integrity of the cytoplasmic membrane of *S. aureus*. Therefore, one can suppose that the mechanism of antibacterial action of these TPP-conjugates is significantly different from that of classical antibacterial agents i.e. is not associated with damage to the bacterial cell wall and cytoplasmic membrane. It was found that cytotoxic activity of the lead compound **1d** is due both to induction of apoptosis proceeding along the mitochondrial pathway and the arrest of the cell cycle in the G0/G1 phase.

A full-scale study of the cytotoxicity and antibacterial activity of TPP-conjugates of 1,2,3-triazolyl nucleoside analogues with a wide range of alkyl chain lengths and both with acylated and unprotected hydroxyl groups of the sugar residue will soon follow this preliminary short communication.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

This Appendix includes the data about the examination of the ability of TPP-conjugates **1c**, **2c**, **1d**, **4c** to alter the integrity of the cell membrane of *S. aureus*; the data about the qualitative and quantitative analysis of viable and damaged cells *S. aureus* after exposure to TPP-conjugates **1c, d; 2c, 4c**; the data about the estimation of the possibility of the lead compound **1d** to induce apoptosis in a cell line HuTu-80 along the mitochondrial pathway; the data about the analysis of the cell cycle of a cell line HuTu-80 after its treatment with the lead compound **1d**; general procedures for the synthesis of TPP-conjugates **1c, 2c, 1d, 4c** and theit characterizations including ^{1}H, ^{13}C and ^{31}P spectra; detailes of all biological experiments. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.105328.

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