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ORIGINAL RESEARCH

Triphenylphosphonium conjugates of 1,2,3-triazolyl nucleoside analogues. Synthesis and cytotoxicity evaluation

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Abstract

A series of triphenylphosphonium (TPP) conjugates of 1,2,3-triazolyl analogues of several pyrimidine nucleosides was synthesized and evaluated for the in vitro cytotoxicity against human cancer cell lines M-HeLa, MCF-7, PANC-1, PC-3, DU145, SKOV-3, A275, and normal human cell line WI-38. In these TPP-conjugates triphenylphosphonium cation was attached via a tetramethylene chain to the *N*-3 atom of the heterocycle moiety (uracil, thymine, quinazoline-2,4-dione), which was coupled with the D-ribofuranosyl-1,2,3-triazol-4-yl fragment *via* methylene or tetramethylene linker. It was shown for the first time that the conjugation of 1,2,3-triazolyl derivatives of uridine, its analogues featuring quinazoline-2,4-dione fragment as well as uracil and thymine derivatives, having propargyl or a 1,2,3-triazolyl substituent at the *N*-1 atom, with a TPP-butyl cation endowed some of them with cytotoxic activity against human cancer cells. Among all human cancer cell lines used, DU-145 and A375 cells were the most sensitive to these TPP conjugates. At the same time, all tested compounds did not inhibit growth of normal cells WI-38. Propargyl containig TPP-conjugates of uracil **4f**, **4j**, and thymine **5f** showed the highest cytotoxicity with IC_{50} values in the low micromolar concentration range. The present findings suggest that TPP-conjugates of uracil and thymine derivatives would be promising for further development as an anticancer agent.

Keywords Nucleoside analogues · 1,2,3-Triazole · Triphenylphosphonium · TPP conjugates · Cytotoxicity

Introduction

The mitochondrion is a vital organelle in most eukaryotic cells. One of the main functions of mitochondria is the synthesis of adenosine triphosphate (ATP), an universal form of chemical energy in any living cell, via oxidative phosphorylation. Moreover, mitochondria play a key role in many other metabolic processes in a cell, such as modulating calcium homeostasis, the tricarboxylic acid and urea cycles, fatty acid oxidation, iron sulfur center and heme biosynthesis, amino acid metabolism, and redox signaling [1–4] Furthermore, mitochondria are critically involved in the regulation of programmed cell death, the intrinsic pathway of apoptosis [1–4]. Mitochondrial damage that disrupts ATP synthesis and calcium homeostasis is a key component of necrotic cell death [5]. For all these reasons, mitochondrial malfunction disrupts the function of cells, tissues, and organs and contributes to a remarkably wide range of diseases [2, 5]. Mitochondrial dysfunction has been implicated in neurodegenerative and neuromuscular disorders, ischemia-reperfusion injury,

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diabetes, obesity, inherited mitochondrial diseases and most importantly, cancer [2, 6]. In nearly all cases where mitochondrial dysfunction contributes to disease, a major cause of damage is reactive oxygen species (ROS) produced by mitochondria, either directly or as a secondary consequence of other malfunctions [5]. One approach to addressing these challenges is to target antioxidants to mitochondria by conjugation to a lipophilic cation, such as the triphenylphosphonium (TPP) cation [5]. The large membrane potential of 150–180 mV (negative inside) across the mitochondrial inner membrane is used to deliver lipophilic cations or molecules to mitochondria. Lipophilic TPP cation passes easily through lipid bilayers because its charge is dispersed over a large surface area and the potential gradient drives the accumulation of TPP cation into the mitochondrial matrix [7]. A wide range of antioxidants were targeted to mitochondria by conjugation to the TPP moiety, and those that have been employed to date include TPP-conjugated derivatives of ubiquinone, tocopherol, lipoic acid, spin traps, and the peroxidase mimetic ebselen [7–10]. Compared with normal cells, cancer cells exhibit a markedly higher mitochondrial transmembrane potential that provides the selective accumulation of the TPP cation inside cancer cell mitochondria. Hence, the bulky lipophilic TPP cations are assumed to be promising carriers for a drug accumulation in mitochondria [11, 12]. Notably, a conjugation of well-known anticancer agents (doxorubicin, salicylaldazine, chlorambucil, glycyrrhetinic acid, curcumin, etc) with TPP has resulted in both the targeting of mitochondria in cancer cells and the increase of cytotoxicity [8,13–15].

The literature has provided many examples of nucleoside analogues, which also displayed anticancer activity. Among them 2′-deoxy-2′,2′-difluorocytidine (Gemcitabine) was approved for the treatment of breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer, bladder cancer and 2′-deoxy-5-fluorouridine (Floxuridine) is an oncology drug to treat colorectal cancer [16]. 3′-(4-Chlorophenyl-1,2,3-triazolyl)thymidine exhibited high potential in bringing down tumor cell proliferation and induced their apoptosis [17]. Several nucleoside analogues containing substituted 1,2,3-triazolyl moieties at the C-5′ position of ribofuranose residue demonstrated significant cytotoxicity against cancer cell lines A549, HT-29, MCF-7, A-375 [18]. A 2′-deoxyuridine derivarive featuring perfluorodecyl substituted 1,2,3-triazole moiety attached to the C-5 position of the uracil ring exhibited significant growth inhibition of PC-3, MDA-MB-231, and ACHN cancer cells [19]. Surprisingly, there is only one publication reporting the synthesis of the TPP conjugate of a 1,2,3-triazolyl pyrimidine analog, that penetrated selectively the mitochondria of cancer cells [20].

Apparent scarcity of literature data regarding TPP-conjugates of 1,2,3-triazolyl nucleoside analogues prompted us to synthesize a series of 1,2,3-triazolyl pyrimidine analogues conjugated with a TPP cation and to study their cytotoxicity against a panel of human cancer cell lines. In these TPP-conjugates TPP cation was attached via a tetramethylene chain to the *N*-3 atom of the heterocycle moiety (uracil, thymine, quinazoline-2,4-dione), which was coupled with the D-ribofuranosyl-1,2,3-triazol-4-yl fragment via methylene or tetramethylene linker. In addition, a series of model compounds, namely derivatives of uracil and thymine containing ω-alkyne or a 1,2,3-triazolyl substituent at the *N*-1 atom as well as the TPP-butyl moiety at the *N*-3 atom of nucleobase was synthesized and evaluated for cytotoxicity against a panel of human cancer cells.

Material and methods

General chemistry

The ^{1}H NMR spectra were recorded on 400 MHz and 600 MHz Brucker Advance. ^{13}C NMR spectra were obtained in the above instruments operating at 100.6 MHz, ^{31}P NMR spectra at 161 MHz. Melting points were obtained on an Electrothermal IA 9000 instrument (Electrothermal, Great Britain). Mass spectra (MALDI) were recorded in a positive ion mode on a Bruker Ultraflex III TOF/TOF mass spectrometer for 10^{-3} mg/mL solutions. The ESI MS measurements were performed using an AmazonX ion trap mass spectrometer (Bruker Daltonic GmbH, Germany) in positive mode in the mass range of 70–3000. The capillary voltage was 3500 V, nitrogen drying gas 10 L/min, desolvation temperature 250 °C. A methanol/water solution (70:30) was used as a mobile phase at a flow rate of 0.2 mL/min by binary pump (Agilent 1260 chromatograph, USA). The sample was dissolved in methanol to a concentration of 10^{-6} g/L. Flash chromatography was performed on silica gel 60 (40–63 μm, Buchi, Sepacore). Thin-layer chromatography was carried out on plates with silica gel (Sorbfil, Russia). Spots of compounds were visualized by using ultraviolent fluorescence under a short wavelength (254 nm) followed by heating the plates (at ca. 150 °C) after immersion in a solution of 5% H_2SO_4 and 95% H_2O. All reactions sensitive to air and/or moisture were carried out under an argon atmosphere with anhydrous solvents, which were purified and dried (where appropriate) according to standard procedures.

1,2,3-Triazolyl pyrimidine analogues **1a**, **1d**, **1e**, **1h**, **2a**, **2d**, **3a**, **3d** and pyrimidines **4b**, **5b**, **4i** having ω-alkyne substituent at the *N*-1 atom were synthesized according a procedure earlier described [21]. Uracil and thymine derivatives **4g**, **5c**, **5g** were prepared by analogy with the

methods previously described [22–24]. 1-Azidopentane and (4-bromobutyl)TPP bromide were synthesized by known methods [25–27]. Uracil, thymine, quinazoline-2,4(1*H*,3*H*)-dione and propargyl bromide were purchased from Sigma-Aldrich.

General procedure for synthesis of triphenylphosphonium salts

A mixture of an uracil derivative (compounds **1a**, **1e**, **4b**, **4d**, **4g**, **4i**) or a thymine derivative (compounds **2a**, **5b**, **5d**, **5g**) or quinazoline-2,4-dione derivative **3a** (1 mmol), (4-bromobutyl)triphenylphosphonium bromide (1 mmol) and K_2CO_3 (3 mmol) in dimethylformamide (DMF) (3–5 ml) was stirred for 24 h in an argon atmosphere at the temperature 40 °C. The reaction mixture was filtered, and DMF was removed under reduced pressure at the temperature 40–50 °C. The residue was dissolved in CH_2Cl_2, washed with a saturated solution of NaCl and H_2O, dried with Na_2SO_4. The resulting mixture was concentrated under a vacuum to 3–5 mL, and 5 mL Et_2O was added. The product, after decanting the solvent, was dried in a high vacuum at 40–50 °C.

1-{[1-(2′′,3′′,5′′-Tri-*O*-acetyl-β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl]methyl}-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (1b)

Amorphous powder; yield: 0.17 g (57%); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.32$ (1H, s, H-5′), 7.83–7.65 (15H, m, 6H-12, 6H-13, 3H-14), 7.59 (1H, d, $J = 7.9$ Hz, H-6), 6.25 (1H, d, $J = 3.4$ Hz, H-1′′), 5.84–5.81 (1H, m, H-3′′), 5.63 (1H, t, $J = 5.5$ Hz, H-2′′), 5.57 (1H, d, $J = 7.9$ Hz, H-5), 5.10–5.00 (2H, m, H-15), 4.46–4.41 (1H, m, H-4′′), 4.37 (1H, dd, $J =$ 12.2, 3.3 Hz, H-5′′a), 4.17 (1H, dd, $J = 12.2$, 4.8 Hz, H-5′′ b), 3.93 (2H, t, $J = 6.3$ Hz, H-7), 3.88–3.77 (2H, m, H-10), 2.09, 2.09, 2.04 (3H, s, OAc), 2.03–1.97 (2H, m, H-8), 1.69–1.53 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 170.33, 169.37, 169.19 (C, CH_3CO), 163.12 (C, C-4), 151.22 (C, C-2), 142.87 (C, C-4′), 142.20 (CH, C-6), 135.04 (CH, d, $J = 2.9$ Hz, C-14), 133.59 (CH, d, $J =$ 10.1 Hz, C-12), 130.43 (CH, d, $J = 12.6$ Hz, C-13), 124.52 (C, C-5′), 118.04 (C, d, $J = 86.0$ Hz, C-11), 101.43 (CH, C-5), 89.82 (CH, C-1′′), 80.59 (CH, C-4′′), 74.13 (CH, C-3′′), 70.74 (CH, C-2′′), 62.94 (CH_2, C-5′′), 43.45, 39.28 (CH_2, C-7, C-15), 27.86 (CH_2, d, $J = 16.7$ Hz, C-8), 21.93 (CH_2, d, $J = 50.6$ Hz, C-10), 20.61, 20.38, 20.35 (CH_3, $\underline{C}H_3CO$), 19.52 (CH_2, d, $J = 3.8$ Hz, C-9); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 25.11$; MALDI MS *m/z*: 768.5 $[M\text{-}Br]^+$ (calcd. 768.3); Anal. Calcd. for $C_{40}H_{43}BrN_5O_9P$: C, 56.61; H, 5.11; Br, 9.42; N, 8.25; P, 3.65. Found: C, 56.72; H, 5.08; Br, 9.52; N, 8.15; P, 3.78%.

1-{[1-(2′′,3′′,5′′-Tri-*O*-acetyl-β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl]butyl}-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (1f)

Foam; yield: 0.20 g (65%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 7.90$–7.71 (16H, m, H-5′, 6H-12, 6H-13, 3H-14), 7.53 (1H, d, $J = 7.8$ Hz, H-6), 6.21 (1H, d, $J = 3.4$ Hz, H-1′′), 5.87–5.84 (1H, m, H-3′′), 5.67–5.60 (2H, m, H-5, H-2′′), 4.49–4.45 (1H, m, H-4′′), 4.36 (1H, dd, $J = 12.3$, 3.4 Hz, H-5′′a), 4.17 (1H, dd, $J = 12.2$, 4.4 Hz, H-5′′b), 3.95 (2H, t, $J = 6.6$ Hz, H-7), 3.79–3.72 (2H, m, H-15), 3.51–3.43 (2H, m, H-10), 2.77–2.70 (2H, m, H-18), 2.10, 2.09, 1.98 (3H, s, OAc), 1.89–1.82 (2H, m, H-8), 1.73–1.60 (6H, m, H-9, H-16, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.31$, 169.40, 169.26 (C, $CH_3\underline{C}O$), 163.29 (C, C-4), 151.25 (C, C-2), 147.45 (C, C-4′), 143.41 (CH, C-6), 135.09 (CH, d, J = 2.9 Hz, C-14), 133.57 (CH, d, $J = 9.9$ Hz, C-12), 130.44 (CH, d, $J = 12.5$ Hz, C-13), 121.18 (CH, C-5′), 117.97 (C, d, $J = 85.8$ Hz, C-11), 100.92 (CH, C-5), 89.65 (CH, C-1′′), 80.51 (CH, C-4′′), 74.10 (CH, C-3′′), 70.78 (CH, C-2′′), 62.98 (CH_2, C-5′′), 49.23 (CH_2, C-15), 39.10 (CH_2, C-7), 28.18 (CH_2, C-18), 27.82 (CH_2, d, $J = 16.5$ Hz, C-8), 25.84 (CH_2, C-16), 24.78 (CH_2, C-17), 21.91 (CH_2, d, $J =$ 51.0 Hz, C-10), 20.62, 20.41, 20.38 (CH_3, $\underline{C}H_3CO$), 19.42 (CH_2, d, $J = 3.7$, C-9); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta =$ 24.66; ESI MS *m/z*: 810.3 $[M\text{-}Br]^+$ (calcd. 810.3); Anal. Calcd. for $C_{43}H_{49}BrN_5O_9P$: C, 57.98; H, 5.54; Br, 8.97; N, 7.86; P, 3.48. Found: C, 57.90; H, 5.48; Br, 9.04; N, 7.78; P, 3.57.

5-Methyl-1-{[1-(2′′,3′′,5′′-tri-O-acetyl-β-D-ribofuranosyl)-1′H-1′,2′,3′-triazol-4-yl]methyl}-3-(n-butyltriphenylphosphonium)-2,4(1H,3H)-pyrimidinedione bromide (2b)

Amorphous powder; yield: 0.19 g (68%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 8.13$ (1H, s, H-5′), 7.90–7.70 (15H, m, 6H-12, 6H-13, 3H-14), 7.54 (1H, br. s, H-6), 6.26 (1H, d, $J = 3.3$ Hz, H-1′′), 5.86–5.82 (1H, m, H-3′′), 5.62 (1H, t, $J = 5.6$ Hz, H-2′′), 5.00 (2H, s, H-15), 4.51–4.46 (1H, m, H-4′′), 4.35 (1H, dd, $J = 12.4$, 3.2 Hz, H-5′′a), 4.17 (1H, dd, $J = 12.3$, 4.1 Hz, H-5′′b), 3.97 (2H, t, $J = 6.6$ Hz, H-7), 3.50–3.41 (2H, m, H-10), 2.09, 2.08, 1.95 (3H, s, OAc), 1.90–1.80 (5H, m, CH_3-5, H-8), 1.70–1.60 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.34$, 169.39, 169.22 (C, $CH_3\underline{C}O$), 163.84 (C, C-4), 151.17 (C, C-2), 142.34 (C, C-4′), 138.67 (CH, C-6), 135.01 (CH, d, $J =$ 2.6 Hz, C-14), 133.60 (CH, d, $J = 9.9$ Hz, C-12), 130.41 (CH, d, $J = 12.8$ Hz, C-13), 124.27 (CH, C-5′), 118.07 (C, d, $J = 85.8$ Hz, C-11), 109.76 (C, C-5), 89.86 (CH, C-1′′), 80.58 (CH, C-4′′), 74.15 (CH, C-3′′), 70.70 (CH, C-2′′), 62.93 (CH_2, C-5′′), 43.33, 39.58 (CH_2, C-7, C-15), 28.05

(CH_2, d, $J = 16.6$ Hz, C-8), 21.96 (CH_2, d, $J = 50.6$ Hz, C-10), 20.60, 20.38, 20.37 (CH_3, $\underline{C}H_3CO$), 19.65 (CH_2, d, $J =$ 4.0 Hz, C-9), 12.86 (CH_3, $\underline{C}H_3$-C-5); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 25.57$; MALDI MS *m/z*: 782.5 $[M\text{-}Br]^+$ (calcd. 782.3); Anal. Calcd. for $C_{41}H_{45}BrN_5O_9P$: C, 57.08; H, 5.26; Br, 9.26; N, 8.12; P, 3.59. Found: C, 56.92; H, 5.18; Br, 9.35; N, 8.20; P, 3.67.

1-{[1-(2″,3″,5″-Tri-*O*-acetyl-β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl]methyl}-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-quinazolinedione bromide (3b)

The product was purified by dry column chromatography with $CHCl_3$/MeOH from 100:0.1 to 100:2 solvent system to afford pure compound in 21% yield (0.08 g); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.16$ (1H, s, H-5′), 8.01 (1H, d, $J =$ 7.7 Hz, H-5), 7.81–7.60 (17H, m, H-7, H-8, 6H-16, 6H-17, 3H-18), 7.20–7.15 (1H, m, H-6), 6.25 (1H, d, $J = 3.0$ Hz, H-1″), 5.78–5.74 (1H, m, H-3″), 5.60 (1H, t, $J = 5.6$ Hz, H-2″), 5.38–5.27 (2H, m, H-19), 4.42–4.37 (1H, m, H-4″), 4.32 (1H, dd, $J = 12.2$, 3.0 Hz, H-5″a), 4.14–4.06 (3H, m, H-11, H-5″b), 3.89–3.80 (2H, m, H-14), 2.13–2.02 (8H, m, H-12, 2OAc), 1.96 (3H, s, OAc), 1.70–1.60 (2H, m, H-13); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.27$, 169.33, 169.19 (C, $CH_3\underline{C}O$), 161.80 (C, C-4), 150.63 (C, C-2), 142.66 (C, C-4′), 139.42 (C, C-9), 135.41, 128.55, 124.02 (CH, C-6, C-7, C-8), 134.90 (CH, d, $J = 2.7$ Hz, C-18), 133.59 (CH, d, $J = 10.0$ Hz, C-16), 130.33 (CH, d, $J = 12.7$ Hz, C-17), 123.04 (CH, C-5′), 118.06 (C, d, $J = 85.8$ Hz, C-15), 115.11 (C, C-10), 114.59 (CH, C-5), 89.80 (CH, C-1″), 80.48 (CH, C-4″), 74.14 (CH, C-3″), 70.63 (CH, C-2″), 62.88 (CH_2, C-5″), 40.10, 39.06 (CH_2, C-19, C-11), 28.19 (CH_2, d, $J = 17.0$ Hz, C-12), 21.99 (CH_2, d, $J = 50.9$ Hz, C-14), 20.51, 20.36, 20.34 (CH_3, $\underline{C}H_3CO$), 19.67 (CH_2, d, $J =$ 3.9 Hz, C-13); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 24.73$; MALDI MS *m/z*: 818.5 $[M\text{-}Br]^+$ (calcd. 818.3); Anal. Calcd. for $C_{44}H_{45}BrN_5O_9P$: C, 58.80; H, 5.05; Br, 8.89; N, 7.79; P, 3.45. Found: C, 58.95; H, 5.10; Br, 8.77; N, 7.68; P, 3.51.

1-[(*n*-Pentyl-1′*H*-1′,2′,3′-triazol-4′-yl)methyl]-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (4e)

Amorphous powder; yield: 0.19 g (59%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 7.96$ (1H, s, H-5′), 7.90–7.71 (15H, m, 6H-12, 6H-13, 3H-14), 7.65 (1H, d, $J = 7.9$ Hz, H-6), 5.69 (1H, d, $J = 7.8$ Hz, H-5), 5.00 (2H, s, H-15), 4.36 (2H, t, $J = 7.2$ Hz, H-16), 3.95 (2H, t, $J = 6.7$ Hz, H-7), 3.51–3.42 (2H, m, H-10), 1.91–1.81 (4H, m, H-8, H-17), 1.72–1.60 (2H, m, H-9), 1.39–1.22 (4H, m, H-18, H-19), 0.88 (3H, t, $J = 7.1$ Hz, H-20); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 165.31$ (C, C-4), 152.78 (C, C-2), 145.16 (C, C-4′), 143.57 (CH, C-6), 136.33 (CH, d, $J = 2.6$ Hz, C-14), 134.88 (CH, d, $J = 9.9$ Hz, C-12), 131.57 (CH, d, $J =$ 12.5 Hz, C-13), 125.10 (CH, C-5′), 119.83 (C, d, $J =$ 86.2 Hz, C-11), 102.04 (CH, C-5), 51.46 (CH_2, C-16), 44.99 (CH_2, C-15), 40.76 (CH_2, C-7), 30.95 (CH_2, C-17), 29.64 (CH_2, C-18) 29.34 (CH_2, d, $J = 17.3$ Hz, C-8), 23.09 (CH_2, C-19), 22.49 (CH_2, d, $J = 51.4$ Hz, C-10), 20.83 (CH_2, d, $J = 3.7$ Hz, C-9), 14.23 (CH_3, C-20); ^{31}P NMR (CD_3OD, 161 MHz): $\delta = 23.98$; ESI MS *m/z*: 580.4 $[M\text{-}Br]^+$ (calcd. 580.3); Anal. Calcd. for $C_{34}H_{39}BrN_5O_2P$: C, 61.82; H, 5.95; Br, 12.10; N, 10.60; P, 4.69. Found: C, 61.85; H, 5.81; Br, 12.24; N, 10.51; P, 4.77.

1-Propargyl-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (4f)

Foam; yield: 0.15 g (27%); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.86$–7.65 (15H, m, 6H-12, 6H-13, 3H-14), 7.58 (1H, d, $J = 7.9$ Hz, H-6), 5.64 (1H, d, $J = 7.9$ Hz, H-5), 4.57 (2H, d, $J = 2.5$ Hz, H-15), 3.95 (2H, t, $J = 6.3$ Hz, H-7), 3.88–3.78 (2H, m, H-10), 2.45 (1H, t, $J = 1.9$ Hz, H-17), 2.07–1.96 (2H, m, H-8), 1.66–1.55 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 162.95$ (C, C-4), 150.90 (C, C-2), 141.41 (CH, C-6), 135.03 (CH, d, $J = 2.9$ Hz, C-14), 133.71 (CH, d, $J = 9.9$ Hz, C-12), 130.46 (CH, d, $J =$ 12.6 Hz, C-13), 118.15 (C, d, $J = 86.0$ Hz, C-11), 101.74 (CH, C-5), 76.48 (C, C-16), 75.18 (CH, C-17), 39.47, 38.06 (CH_2, C-7, C-15), 27.84 (CH_2, d, $J = 16.5$ Hz, C-8), 22.01 (CH_2, d, $J = 50.7$ Hz, C-10), 19.56 (CH_2, d, $J =$ 3.8 Hz, C-9); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 24.60$; MALDI MS, *m/z*: 467.5 $[M\text{-}Br]^+$ (calcd. 467.2); Anal. Calcd. for $C_{29}H_{28}BrN_2O_2P$: C, 63.63; H, 5.16; Br, 14.60; N, 5.12; P, 5.66. Found: C, 63.75; H, 5.09; Br, 14.72; N, 5.05; P, 5.58.

1-Methyl-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (4h)

Foam; yield: 0.20 g (26%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 7.90$–7.72 (15H, m, 6H-12, 6H-13, 3H-14), 7.50 (1H, d, $J = 7.8$ Hz, H-6), 5.65 (1H, d, $J = 7.9$ Hz, H-5), 3.96 (2H, t, $J = 6.7$ Hz, H-7), 3.51–3.43 (2H, m, H-10), 3.31 (3H, s, CH_3-1), 1.91–1.83 (2H, m, H-8), 1.71–1.61 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 163.46$ (C, C-4), 151.66 (C, C-2), 144.13 (CH, C-6), 135.10 (CH, C-14), 133.61 (CH, d, $J = 10.0$ Hz, C-12), 130.47 (CH, d, $J = 12.4$ Hz, C-13), 118.01 (C, d, $J = 75.3$ Hz, C-11), 100.84 (CH, C-5), 39.13 (CH_2, C-7), 36.97 (CH_3, CH_3-N-1), 27.81 (CH_2, d, $J = 16.6$ Hz, C-8), 22.01 (CH_2, d, $J = 50.9$ Hz, C-10), 19.43 (CH_2, C-9); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 24.64$; MALDI MS *m/z*: 443.4 $[M\text{-}Br]^+$ (calcd. 443.2); Anal. Calcd. for $C_{27}H_{28}BrN_2O_2P$: C, 61.96; H, 5.39; Br, 15.27;

N, 5.35; P, 5.92. Found: C, 62.10; H, 5.32; Br, 15.34; N, 5.28; P 5.86.

1-(Hex-5′-yn-1′-yl)-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (4j)

Foam; yield: 0.19 g (41%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 7.92–7.72$ (15H, m, 6H-12, 6H-13, 3H-14), 7.55 (1H, d, $J = 7.9$ Hz, H-6), 5.67 (1H, d, $J = 7.9$ Hz, H-5), 3.96 (2H, t, $J = 6.7$ Hz, H-7), 3.75 (2H, t, $J = 7.3$ Hz, H-15), 3.52–3.43 (2H, m, H-10), 2.25–2.17 (3H, m, H-18, H-20), 1.91–1.83 (2H, m, H-8), 1.79–1.61 (4H, m, H-9, H-17), 1.53–1.44 (2H, m, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 163.23$ (C, C-4), 151.24 (C, C-2), 143.13 (CH, C-6), 135.02 (CH, d, $J = 2.9$ Hz, C-14), 133.60 (CH, d, $J = 9.9$ Hz, C-12), 130.43 (CH, d, $J = 12.5$ Hz, C-13), 118.05 (C, d, $J = 85.8$ Hz, C-11), 101.04 (CH, C-5), 83.52 (C, C-19), 68.97 (CH, C-20), 49.05 (CH_2, C-15), 39.20 (CH_2, C-7), 27.96 (CH_2, C-18), 27.79 (CH_2, d, $J = 16.9$ Hz, C-8), 25.06 (CH_2, C-16), 21.86 (CH_2, d, $J = 42.6$ Hz, C-10), 19.47 (CH_2, d, $J = 3.7$ Hz, C-9), 17.94 (CH_2, C-17); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 24.68$; ESI MS *m/z*: 509.4 $[M\text{-}Br]^+$ (calcd. 509.2); Anal. Calcd. for $C_{32}H_{34}BrN_2O_2P$: C, 65.20; H, 5.81; Br, 13.55; N, 4.75; P, 5.25. Found: C, 65.12; H, 5.8; Br, 13.63; N, 4.68; P, 5.19.

1-[(*n*-Pentyl-1′*H*-1′,2′,3′-triazol-4′-yl)methyl]-3-(*n*-butyltriphenylphosphonium)-5-methyl- 2,4(1*H*,3*H*)-pyrimidinedione bromide (5e)

Amorphous powder; yield: 0.30 g (63%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 7.99$ (1H, s, H-5′), 7.90–7.71 (15H, m, 6H-12, 6H-13, 3H-14), 7.53 (1H, s, H-6), 4.98 (2H, s, H-15), 4.36 (2H, t, $J = 7.2$ Hz, H-16), 3.97 (2H, t, $J = 6.7$ Hz, H-7), 3.55–3.44 (2H, m, H-10), 1.92–1.85 (4H, m, H-8, H-17), 1.84 (3H, d, $J = 1.0$ Hz, CH_3-5), 1.71–1.60 (2H, m, H-9), 1.37–1.22 (4H, m, H-18, H-19), 0.88 (3H, t, $J = 7.2$ Hz, H-20); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 165.75$ (C, C-4), 152.79 (C, C-2), 143.80 (C, C-4′), 141.15 (CH, C-6), 136.36 (CH, d, $J = 2.9$ Hz, C-14), 134.88 (CH, d, $J = 10.3$ Hz, C-12), 131.57 (CH, d, $J = 12.5$ Hz, C-13), 125.02 (CH, C-5′), 119.86 (C, d, $J = 86.2$ Hz, C-11), 110.72 (C, C-5), 51.47 (CH_2, C-16), 44.75 (CH_2, C-15), 40.99 (CH_2, C-7), 30.95 (CH_2, C-17), 29.65 (CH_2, C-18), 29.46 (CH_2, d, $J = 16.9$ Hz, C-8), 23.10 (CH_2, C-19), 22.53 (CH_2, d, $J = 52.7$ Hz, C-10), 20.88 (CH_2, d, $J = 3.7$ Hz, C-9), 14.24 (CH_3, C-20), 13.01 (CH_3, CH3-C-5); ^{31}P NMR (CD_3OD, 161 MHz): $\delta = 24.51$; ESI MS *m/z*: 594.4 $[M\text{-}Br]^+$ (calcd. 594.3); Anal. Calcd. for $C_{35}H_{41}BrN_5O_2P$: C, 62.31; H, 6.13; Br, 11.84; N, 10.38; P, 4.59. Found: C, 62.50; H, 6.08; Br, 12.05; N, 10.29; P, 4.71.

1-Propargyl-3-(*n*-butyltriphenylphosphonium)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione bromide (5f)

Amorphous powder; yield: 0.15 g (44%); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.86–7.66$ (15H, m, 6H-12, 6H-13, 3H-14), 7.30 (1H, d, $J = 0.9$ Hz, H-6), 4.52 (2H, d, $J = 2.5$ Hz, H-15), 3.97 (2H, t, $J = 6.4$ Hz, H-7), 3.95–3.89 (2H, m, H-10), 2.44 (1H, t, $J = 3.0$ Hz, H-17), 2.06–1.99 (2H, m, H-8), 1.86 (3H, d, $J = 1.0$ Hz, CH_3-5), 1.66–1.57 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 163.66$ (C, C-4), 150.89 (C, C-2), 137.25 (CH, C-6), 134.98 (CH, d, $J = 3.1$ Hz, C-14), 133.66 (CH, d, $J = 9.9$ Hz, C-12), 130.41 (CH, d, $J = 12.5$ Hz, C-13), 118.17 (C, d, $J = 86.0$ Hz, C-11), 110.06 (C, C-5), 76.75 (C, C-16), 74.81 (CH, C-17), 39.67, 37.63 (CH_2, C-7, C-15), 27.98 (CH_2, d, $J = 16.7$ Hz, C-8), 21.87 (CH_2, d, $J = 50.5$ Hz, C-10), 19.59 (CH_2, d, $J = 3.8$ Hz, C-9), 12.95 (CH_3, CH3-C-5); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 24.69$; MALDI MS *m/z*: 481.1 $[M\text{-}Br]^+$ (calcd. 481.2); Anal. Calcd. for $C_{30}H_{30}BrN_2O_2P$: C, 64.18; H, 5.39; Br, 14.23; N, 4.99; P, 5.52. Found: C, 64.02; H, 5.40; Br, 14.38; N, 5.00; P, 5.44.

1,5-Dimethyl-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (5h)

Foam; yield: 0.22 g (31%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 7.92–7.72$ (15H, m, 6H-12, 6H-13, 3H-14), 7.38 (1H, d, $J = 1.2$ Hz, H-6), 3.98 (2H, t, $J = 6.7$ Hz, H-7), 3.53–3.45 (2H, m, H-10), 3.29 (3H, s, CH_3-1), 1.90–1.84 (2H, m, H-8), 1.84 (3H, d, $J = 1.0$ Hz, CH_3-5), 1.70–1.60 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 164.09$ (C, C-4), 151.60 (C, C-2), 140.06 (CH, C-6), 135.06 (CH, C-14), 133.62 (CH, d, $J = 9.9$ Hz, C-12), 130.43 (CH, d, $J = 12.4$ Hz, C-13), 118.05 (C, d, $J = 86.0$ Hz, C-11), 109.07 (C, C-5), 39.37 (CH_2, C-7), 36.62 (CH_3, CH_3-N-1), 27.97 (CH_2, d, $J = 16.6$ Hz, C-8), 22.05 (CH_2, d, $J = 50.9$ Hz, C-10), 19.51 (CH_2, d, $J = 2.2$ Hz, C-9), 12.75 (CH_3, CH3-C-5); ^{31}P NMR ($CDCl_3$, 161 MHz): $\delta = 24.60$; MALDI MS *m/z*: 457.5 $[M\text{-}Br]^+$ (calcd. 457.2); Anal. Calcd. for $C_{28}H_{30}BrN_2O_2P$: C, 62.58; H, 5.63; Br, 14.87; N, 5.21; P, 5.76. Found: C, 62.62; H, 5.57; Br, 15.05; N, 5.12; P, 5.83.

General procedure for synthesis of unprotected triphenylphosphonium salts of 1,2,3-triazolyl nucleoside analogues 1b, 1f, 2b, 3b

The protected TPP salts of 1,2,3-triazolyl nucleoside analogues **1b, 1f, 2b, 3b** were dissolved in anhydrous MeOH at room temperature and the pH was adjusted to 9.0 using a solution of 0.1 N MeONa/MeOH. The deacetylation procedure was monitored by TLC and upon its completion the pH adjusted to 7.0 with acidic ion-exchange resin Amberlyst 15. After filtration, the filtrate

was concentrated under reduced pressure to afford the corresponding target compounds.

1-{[1-(β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl] methyl}-3-(*n*-butyltriphenylphosphonium)-2,4 (1*H*,3*H*)-pyrimidinedione bromide (1c)

Foam; yield: 0.05 g (68%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 8.27$ (1H, s, H-5′), 7.90–7.70 (15H, m, 6H-12, 6H-13, 3H-14), 7.67 (1H, d, $J = 7.8$ Hz, H-6), 6.01 (1H, d, $J =$ 4.2 Hz, H-1″), 5.68 (1H, d, $J = 7.9$ Hz, H-5), 5.02 (2H, s, H-15), 4.48 (1H, t, $J = 4.6$ Hz, H-3″), 4.29 (1H, t, $J =$ 5.0 Hz, H-2″), 4.13–4.09 (1H, m, H-4″), 3.94 (2H, t, $J =$ 6.8 Hz, H-7), 3.77 (1H, dd, $J = 12.2$, 3.2 Hz, H-5″a), 3.67 (1H, dd, $J = 12.2$, 4.1 Hz, H-5″b), 3.52–3.42 (2H, m, H-10), 1.92–1.82 (2H, m, H-8), 1.72–1.59 (2H, m, H-9); δ ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 165.32$ (C, C-4), 152.79 (C, C-2), 145.17 (CH, C-6), 143.87 (C, C-4′), 136.34 (CH, s, C-14), 134.86 (CH, d, $J = 10.0$ Hz, C-12), 131.58 (CH, d, $J =$ 12.7 Hz, C-13), 123.96 (C, C-5′), 119.79 (C, d, $J = 86.3$ Hz, C-11), 102.10 (CH, C-5), 94.48 (CH, C-1″), 87.37 (CH, C-4″), 77.11 (CH, C-3″), 71.92 (CH, C-2″), 62.80 (CH_2, C-5″), 44.97, 40.81 (CH_2, C-7, C-15), 29.34 (CH_2, d, $J =$ 16.5 Hz, C-8), 22.54 (CH_2, d, $J = 51.7$ Hz, C-10), 20.84 (CH_2, s, C-9); ^{31}P NMR (CD_3OD, 161 MHz): $\delta = 25.15$; MALDI MS *m/z*: 642.5 $[M\text{-}Br]^+$ (calcd. 642.2); Anal. Calcd. for $C_{34}H_{37}BrN_5O_6P$: C, 56.52; H, 5.16; Br, 11.06; N, 9.69; P, 4.29. Found: C, 56.68; H, 5.08; Br, 11.15; N, 9.58; P, 4.34%.

1-{[1-(β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl] butyl}-3-(*n*-butyltriphenylphosphonium)-2,4(1*H*,3*H*)-pyrimidinedione bromide (1g)

A foam; yield: 0.05 g (63%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 8.34$ (1H, s, H-5′), 7.95–7.71 (15H, m, 6H-12, 6H-13, 3H-14), 7.58 (1H, d, $J = 7.7$ Hz, H-6), 6.06 (1H, d, $J =$ 4.0 Hz, H-1″), 5.66 (1H, d, $J = 7.7$ Hz, H-5), 4.51 (1H, t, $J = 4.4$ Hz, H-3″), 4.30 (1H, t, $J = 5.0$ Hz, H-2″), 4.18–4.13 (1H, m, H-4″), 3.95 (2H, t, $J = 6.8$ Hz, H-7), 3.84-3.75 (3H, m, H-15 and H-5″a), 3.70 (1H, dd, $J = 12.1$, 3.9 Hz, H-5″b), 3.54–3.44 (2H, m, H-10), 2.86–2.78 (2H, m, H-18), 1.92–1.82 (2H, m, H-8), 1.75–1.60 (6H, m, H-9, H-16, H-17); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 165.46$ (C, C-4), 152.94 (C, C-2), 147.46 (C, C-4′), 145.58 (CH, C-6), 136.36 (CH, C-14), 134.88 (CH, d, $J = 10.2$ Hz, C-12), 131.59 (CH, d, $J = 12.7$ Hz, C-13), 123.64 (C, C-5′), 119.83 (C, d, $J =$ 86.6 Hz, C-11), 101.68 (CH, C-5), 95.56 (CH, C-1″), 87.79 (CH, C-4″), 77.31 (CH, C-3″), 71.78 (CH, C-2″), 62.55 (CH_2, C-5″), 50.22 (CH_2, C-15), 40.74 (CH_2, C-7), 29.40 (CH_2, d, $J = 16.6$ Hz, C-8), 29.13 (CH_2, C-18), 26.70 (CH_2, C-16), 25.00 (CH_2, C-17), 22.50 (CH_2, d, $J = 51.9$ Hz, C-10), 20.89 (CH_2, s, C-9); ^{31}P NMR (CD_3OD, 161 MHz): $\delta = 24.58$; MALDI MS *m/z*: 684.6 $[M\text{-}Br]^+$ (calcd. 684.3); Anal. Calcd. for $C_{37}H_{43}BrN_5O_6P$: C, 58.12; H, 5.67; Br, 10.45; N, 9.16; P, 4.05. Found: C, 58.31; H, 5.58; Br, 10.54; N, 9.08; P, 3.98.

1-{[1-(β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl] methyl}-3-(*n*-butyltriphenylphosphonium)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione bromide (2c)

Foam; yield: 0.06 g (75%); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 8.26$ (1H, s, H-5′), 7.90–7.70 (15H, m, 6H-12, 6H-13, 3H-14), 7.54 (1H, s, H-6), 6.00 (1H, d, $J = 4.0$ Hz, H-1″), 4.99 (2H, s, H-15), 4.47 (1H, t, $J = 4.6$ Hz, H-3″), 4.29 (1H, t, $J = 5.0$ Hz, H-2″), 4.13–4.08 (1H, m, H-4″), 3.96 (2H, t, $J = 6.6$ Hz, H-7), 3.77 (1H, dd, $J = 12.1$, 3.2 Hz, H-5″a), 3.66 (1H, dd, $J = 12.3$, 4.2 Hz, H-5″b), 3.52–3.43 (2H, m, H-10), 1.91–1.79 (5H, m, CH_3-5, H-8), 1.70–1.59 (2H, m, H-9); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 165.74$ (C, C-4), 152.79 (C, C-2), 144.37 (C, C-4′), 141.15 (CH, C-6), 136.34 (CH, d, $J = 3.3$ Hz, C-14), 134.86 (CH, d, $J =$ 9.9 Hz, C-12), 131.57 (CH, d, $J = 12.5$ Hz, C-13), 123.88 (C, C-5′), 119.82 (C, d, $J = 86.5$ Hz, C-11), 110.76 (C, C-5), 94.45 (CH, C-1″), 87.34 (CH, C-4″), 77.10 (CH, C-3″), 71.92 (CH, C-2″), 62.80 (CH_2, C-5″), 44.75 (CH_2, C-15), 41.01 (CH_2, C-7), 29.46 (CH_2, d, $J = 16.9$ Hz, C-8), 22.55 (CH_2, d, $J = 51.7$ Hz, C-10), 20.87 (CH_2, d, $J = 4.0$ Hz, C-9), 13.00 (CH_3, $\underline{C}H_3$-C-5); ^{31}P NMR (CD_3OD, 161 MHz): $\delta = 25.45$; MALDI MS *m/z*: 656.5 $[M\text{-}Br]^+$ (calcd. 656.3); Anal. Calcd. for $C_{35}H_{39}BrN_5O_6P$: C, 57.07; H, 5.34; Br, 10.85; N, 9.51; P, 4.21. Found: C, 57.20; H, 5.27; Br, 10.98; N, 9.46; P, 4.14.

1-{[1-(β-D-ribofuranosyl)-1′*H*-1′,2′,3′-triazol-4-yl] methyl}-3-(*n*-butyltriphenylphosphonium)-2,4 (1*H*,3*H*)-quinazolinedione bromide (3c)

Foam; yield: 0.06 g (74%); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.26$ (1H, s, H-5′), 8.03 (1H, d, $J = 7.7$ Hz, H-5), 7.85–7.65 (16H, m, H-7, 6H-16, 6H-17, 3H-18), 7.53 (1H, d, $J = 8.4$ Hz, H-8), 7.26 (1H, t, $J = 7.5$ Hz, H-6), 5.98 (1H, d, $J = 4.0$ Hz, C-1″), 5.40 (2H, s, C-19), 4.45 (1H, t, $J =$ 4.4 Hz, C-3″), 4.27 (1H, t, $J = 5.0$ Hz, C-2″), 4.13 (2H, t, $J = 7.5$ Hz, C-11), 4.10–4.06 (1H, m, C-4″), 3.73 (1H, dd, $J = 12.5$, 3.3 Hz, H-5″a), 3.63 (1H, dd, $J = 12.3$, 4.2 Hz, H-5″b), 3.57–3.47 (2H, m, H-14), 2.02–1.92 (2H, m, H-12), 1.74–1.63 (2H, m, H-13); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 163.54$ (C, C-4), 152.36 (C, C-2), 144.23 (C, C-4′), 140.99 (C, C-9), 136.68, 129.59, 124.41 (CH, C-6, C-7, C-8), 136.27 (CH, d, $J = 2.9$ Hz, C-18), 134.85 (CH, d, $J =$ 10.2 Hz, C-16), 131.52 (CH, d, $J = 12.5$ Hz, C-17), 123.67 (CH, C-5′), 119.79 (C, d, $J = 85.8$ Hz, C-15), 116.54 (C, C-10), 115.88 (CH, C-5), 94.43 (CH, C-1″), 87.28 (CH, C-4″), 77.05 (CH, C-3″), 71.85 (CH, C-2″), 62.74 (CH_2, C-5″),

41.38, 40.08 (CH_2, C-11, C-19), 29.61 (CH_2, d, $J = 16.1$ Hz, C-12), 22.60 (CH_2, d, $J = 51.3$ Hz, C-14), 20.83 (CH_2, d, $J = 4.4$ Hz, C-13); ^{31}P NMR (CD_3OD, 161 MHz): $\delta =$ 24.65; MALDI MS *m/z*: 692.6 $[M\text{-}Br]^+$ (calcd. 692.3); Anal. Calcd. for $C_{38}H_{39}BrN_5O_6P$: C, 59.07; H, 5.09; Br, 10.34; N, 9.06; P, 4.01. Found: C, 59.18; H, 5.00; Br, 10.26; N, 9.12; P, 4.13.

General procedure for synthesis of compounds 4c, 4d, 5d

To a solution of equimolar amounts of *N*-1-propargyl pyrimidine-2,4-dione **4b** (or **5b**) and trimethylsilyl azide (or 1-azidopentane) in a mixture of 1:1 tert-butanol/water was added freshly prepared solution of equimolar amounts of sodium ascorbate in 2 mL of water and $CuSO_4 \times 5\ H_2O$ in 2 mL of water. The reaction mixture was stirred at 40 °C for 48 h, then was concentrated under reduced pressure. The residue was taken up in methylene chloride, washed successively with water, dried over anhydrous sodium sulfate, and concentrated under a vacuum to provide the desired compounds.

1-[(2′*H*-1′,2′,3′-Triazol-4′-yl)methyl]-2,4(1*H*,3*H*)-pyrimidinedione (4c)

Trimethylsilyl azide (1.5 ml, 11.3 mmol) was added to a solution of *N*-1-propargyl uracil **4b** (0.2 g, 1.33 mmol) in anhydrous toluene (5 mL). The mixture was heated in an oil bath (120 °C) for 35 h under argon and volatiles were distilled off. The residue was dissolved in dichloromethane and was purified by flash chromatography using petroleum ether–ethyl acetate 10:1–1:1 as eluent. White crystal; yield: 0.06 g (20%); mp 237.2–237.8 °C; ^1H NMR (DMSO, 400 MHz): $\delta = 11.29$ (1H, s, NH), 7.80 (1H, s, H-5′), 7.67 (1H, d, $J = 7.7$ Hz, H-6), 5.59 (1H, d, $J = 7.7$ Hz, H-5), 4.94 (2H, s, H-7); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 164.56$ (C, C-4), 151.21 (C, C-2), 146.13 (CH, C-6), 142.07 (C, C-4′), 128.81 (CH, C-5′), 101.71 (CH, C-5), 42.66 (CH_2, C-7); ESI MS *m/z*: 194.2 $[M + H]^+$ (calcd. 194.1); Anal. Calcd. for $C_7H_7N_5O_2$: C, 43.53; H, 3.65; N, 36.26. Found: C, 43.45, H, 3.79, N, 36.31%.

1-[(*n*-Pentyl-1′*H*-1′,2′,3′-triazol-4′-yl)methyl]-2,4(1*H*,3*H*)-pyrimidinedione (4d)

Amorphous powder; yield: 0.28 g (72%); ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 10.05$ (1H, s, H-3), 7.70 (1H, s, H-5′), 7.53 (1H, d, $J = 7.9$ Hz, H-6), 5.68 (1H, d, $J = 7.9$ Hz, H-5), 4.98 (2H, s, H-7), 4.30 (2H, t, $J = 7.3$ Hz, H-8), 1.92–1.83 (2H, m, H-9), 1.36–1.22 (4H, m, H-10, H-11), 0.86 (3H, t, $J =$ 7.0 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 163.89$ (C, C-4), 151.13 (C, C-2), 144.35 (CH, C-6), 141.68 (C, C-4′), 123.51 (CH, C-5′), 102.62 (C, C-5), 50.45 (CH_2, C-8), 43.13 (CH_2, C-7), 29.76, 28.48 (CH_2, C-9, C-10), 21.93 (C-11), 13.70 (CH_3, C-12); ESI MS *m/z*: 264.1 $[M + H]^+$ (calcd. 264.1); Anal. Calcd. for $C_{12}H_{17}N_5O_2$: C, 54.74; H, 6.51; N, 26.60. Found: C, 54.91; H, 6.40; N, 26.48.

1-[(*n*-Pentyl-1′*H*-1′,2′,3′-triazol-4′-yl)methyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (5d)

Amorphous powder; yield: 0.28 g (80%); ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 9.70$ (1H, s, H-3), 7.70 (1H, s, H-5′), 7.34 (1H, d, $J = 1.1$ Hz, H-6), 4.95 (2H, s, H-7), 4.30 (2H, t, $J =$ 7.3 Hz, H-8), 1.93–1.83 (5H, m, H-9, CH_3-5), 1.37–1.23 (4H, m, H-10, H-11), 0.87 (3H, t, $J = 7.0$ Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 164.28$ (C, C-4), 151.16 (C, C-2), 142.01 (C, C-4′), 140.18 (CH, C-6), 123.47 (CH, C-5′), 111.22 (C, C-5), 50.48 (CH_2, C-8), 42.96 (CH_2, C-7), 29.80, 28.53, 21.98 (CH_2, C-9, C-10, C-11), 13.73 (CH_3, C-12), 12.20 (CH_3, $\underline{C}H_3$ - C-5); ESI MS *m/z*: 278.1 $[M + H]^+$ (calcd. 278.2); Anal. Calcd. for $C_{13}H_{19}N_5O_2$: C, 56.30; H, 6.91; N, 25.25. Found: C, 56.41; H, 6.80; N, 25.19.

X-ray analysis

The X-ray diffraction experiments for **4c** was carried out on a Bruker KAPPA APEX II CCD diffractometer (graphite-monochromated Mo Kα (0.71073 Å) radiation). Data collection: images were indexed, integrated, and scaled using the APEX2 data reduction package and corrected for absorption using SADABS program for empirical X-ray absorption correction. The structure of **4c** was solved by the direct methods and refined by anisotropic (isotropic for all H atoms) full-matrix least-squares method against F^2 of all reflections using SHELX programs. The positions of the hydrogen were calculated geometrically and refined in riding model.

Crystallographic data for **4c**: $C_7H_7N_5O_2$, *M* 193.18, monoclinic, $P2_1/n$, *a* 4.1712(3), *b* 17.0328(13), *c* 11.4114 (9) Å, *β* 90.091(4)°, *V* 810.75(11) $Å^3$, *Z* 4, D_{calcd} 1.583 $g{\cdot}cm^{-3}$, μ(Mo-*K*α) 0.122 mm^{-1}, F(000) 400, (θ 3.0–28.0°, completeness 99.3%), T 296(2) K, colorless prism, (0.05 × 0.05 × 0.2) mm^3, 10349 measured reflections in index range $-5 <= h <= 5$, $-22 <= k <= 22$, $-14 <= l <= 15$, 1948 independent (R_{int} 0.068), 127 parameters, $R_1 = 0.0567$ (for 1491 observed $I > 2\sigma(I)$), $wR_2 = 0.2047$ (all data), GOOF 0.82, largest diff. peak and hole 0.46 and −0.32 e.A^3.

Crystallographic data for **4c** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 2033250. Copy of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44 122 3336033 or e-mail: deposit@ccdc.cam.ac.uk; internet: www.ccdc.cam.ac.uk).

Biological assays

Human cell culture

M-Hela human cervix epitheloid carcinoma cells, human breast adenocarcinoma cells (MCF-7), PANC-1 human pancreatic cancer cells, and Wi-38 human fibroblast-like fetal lung cells were presented by the Institute of Cytology (Russian Academy of Sciences) and cultured in a standard Eagle's nutrient medium manufactured at the Chumakov Institute of Poliomyelitis and Virus Encephalitis (PanEco company). DU145 human prostate epithelial carcinoma cells derived from brain metastatic site (HTB-81™) were cultured with Eagle's Minimum Essential Medium. PC-3 human prostate epithelial adenocarcinoma grade IVcells, derived from bone metastatic site (CRL-1435™) were cultured with Ham's F-12K (Kaighn's) Medium. SKOV-3 human ovary: ascites epithelial adenocarcinoma cells (HTB-77™) were cultured with McCoy's 5a Modified Medium. All cancer cell cultures were performed in media contained 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 µg/mL) at 37 °C under a 5% CO_2 humidified atmosphere.

Cell toxicity MTT assay

The cells were seeded in 96-well plates (Eppendorf, Cat. № 0030730119), 200 µL of cell suspension in culture media (Gibco) with 10% fetal bovine serum (FBS, Gibco) per well at a density of 5×10^3 cells per well. Cells were incubated in a humidified atmosphere with 5% CO_2. Stock solutions of test compounds were prepared in dimethylsulfoxide (DMSO, Sigma). Next day (overnight incubation) cells were treated for 48 h with test compounds titrated in Dulbecco's modified eagle medium (DMEM) media. Doxorubicin (Sigma-Aldrich) and tubercidin (Sigma-Aldrich) served as a positive control. The cytotoxicity was determined by the colorimetric cell proliferation assay MTT (Thiazolyl Blue Tetrazolium Bromide, Sigma). For that 20 µL of MTT-reagent in Dulbecco's phosphate-buffered saline was added per each well (final concentration of 0.5 mg/mL). The plates were incubated at 37 °C for 2–3 h under a 5% CO_2 humidified atmosphere. The absorbance was recorded at 570 nm using a CLARIO star microplate reader (BMG LABTECH). Experiments for all compounds were repeated three times. IC_{50} values were determined using GraphPad Prism 6 software.

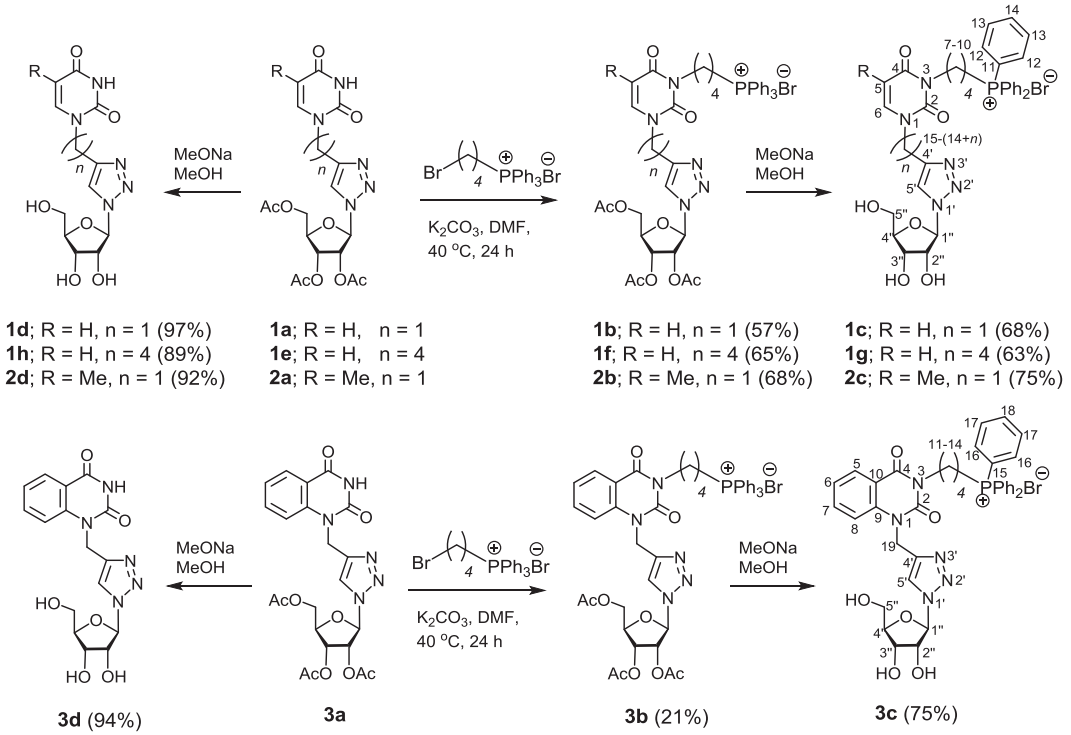
Phenotypic sea urchin embryo assay [27, 28]

Adult sea urchins, *Paracentrotus lividus* L. (Echinidae), were collected from the Mediterranean Sea on the Cyprus coast and kept in an aerated seawater tank. Gametes were obtained by intracoelomic injection of 0.5 M KCl. Eggs were washed with filtered seawater and fertilized by adding drops of diluted sperm. Embryos were cultured at room temperature under gentle agitation with a motor-driven plastic paddle (60 rpm) in filtered seawater. The embryos were observed with a Biolam light microscope (LOMO, St. Petersburg, Russia). For treatment with the test compounds, 5 mL aliquots of embryo suspension were transferred to six-well plates and incubated as a monolayer at a concentration up to 2000 embryos/mL. Stock solutions of compounds were prepared in DMSO at 10–20 mM concentration followed by a tenfold dilution with 96% EtOH. This procedure enhanced the solubility of the test compounds in the salt-containing medium (seawater), as evidenced by microscopic examination of the samples. The maximal tolerated concentrations of DMSO and EtOH in the in vivo assay were determined to be 0.05% and 1%, respectively. Higher concentrations of either DMSO (~0.1%) or EtOH (>1%) caused nonspecific alteration and retardation of the sea urchin embryo development independent of the treatment stage. The antimitotic activity was assessed by exposing fertilized eggs (8–15 min after fertilization, 45–55 min before the first mitotic cycle completion) to twofold decreasing concentrations of the compound. Normally at the minimal effective (threshold) concentration a tested compound caused 100% cleavage alteration and embryo death before hatching, whereas at twofold lower concentration the compound failed to produce any effect. Cleavage alteration and arrest were observed at 2.5–5.5 h after fertilization, when control embryos reached 8-cell and early blastula stages, respectively. Experiments with the sea urchin embryos fulfill the requirements of biological ethics. The artificial spawning does not cause animal death, embryos develop outside the female organism, and both post spawned adult sea urchins and the excess of intact embryos are returned to the sea, their natural habitat.

Results and discussion

Chemistry

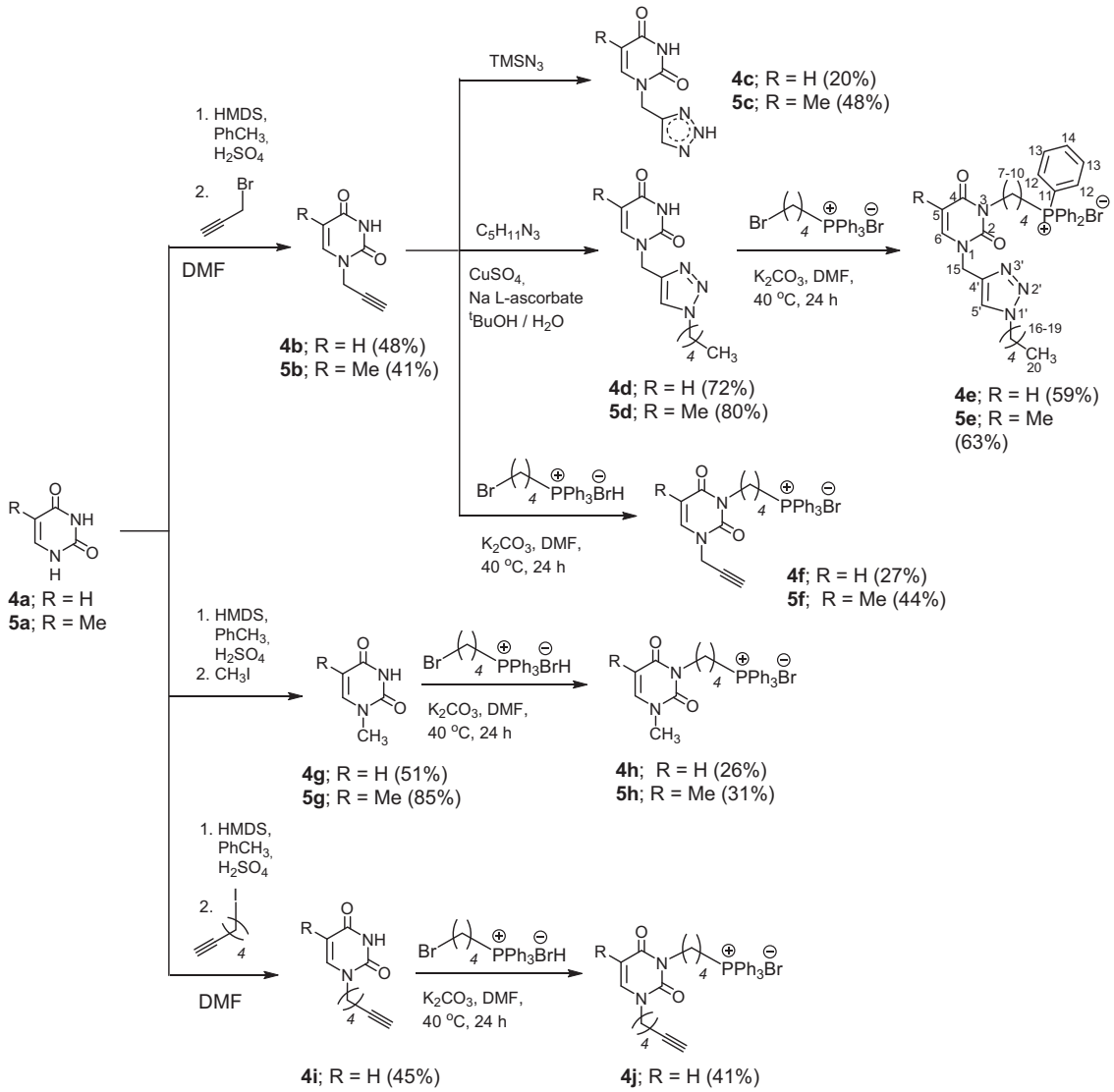
The synthetic route of TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1b, c, f, g**; **2b, c**; **3b, c** is outlined in Scheme 1. Starting material (**1a, e**; **2a**; **3a**) was synthesized as has been reported [21]. (4-Bromobutyl)triphenylphosphonium bromide (TPP-butyl bromide) was prepared by the reaction of triphenylphosphine with fivefold excess of 1,4-dibromobutane under heating in an oil bath at 90 °C without any solvent similarly to a procedure described [26]. Then it was reacted with equimolar amounts of starting compounds **1a, e**; **2a**; **3a** in the presence of K_2CO_3 in according to the method previously described [29] to afford the target TPP-conjugates **1b, f**; **2b**; **3b** in 57%, 65%, 68%,



Scheme 1 Synthesis of triphenylphosphonium conjugates of 1,2,3-triazolyl nucleoside analogues. The numbering of atoms of target compounds is shown

21% yields, respectively. The attachment of the TPP-butyl moiety to starting compounds **1a, e**; **2a**; **3a** was indicated by the ^{31}P, ^{1}H, and ^{13}C NMR spectra of compounds **1b, f**; **2b**; **3b**. In the ^{31}P NMR spectra of these compounds the signal for the P^{+} atom appeared as a singlet within the range 23.98–25.57 ppm. The protons of the P^{+}-CH_2 moiety in the ^{1}H NMR spectra of compounds **1b, f**; **2b**; **3b** resonated as multiplets within the range 3.40–3.95 ppm. The carbon of the P^{+}-CH_2 bond in the ^{13}C NMR spectra of TPP-conjugates **1b, f**; **2b**; **3b** resonated as a doublet at 22 ppm with a coupling constant within the range 50.6–52.7 Hz. The phenyl rings of these compounds were observed in the ^{1}H NMR spectra as multiplets within the range 7.65–7.95 ppm and as four doublets within the range 120–135 ppm in the ^{13}C NMR spectra. The CH_2 unit at the *N*-1 atom of the pyrimidine rings of TPP-conjugates **1b, f**; **2b** occurred in their ^{1}H NMR spectra as a triplet within the range 3.93–3.97 ppm ($^{3}J = 6.3$–6.6 Hz) and a singlet within the range 39–40 ppm in their ^{13}C NMR spectra. The same unit of the quinazoline's conjugate **3b** resonated in its ^{1}H NMR spectrum as a triplet at 4.13 with a vicinal coupling constant of 7.5 Hz and a singlet at 39 ppm in its ^{13}C NMR spectrum. All spectral data were in full accordance with the spectral features of TPP-conjugates of nitrogen containing heterocyclic compounds [29–32]. The ^{1}H NMR spectra of TPP-conjugates of 1,2,3-triazolyl nucleoside analogues **1b, 1f**; **2b**; **3b** displayed the anomeric protons as doublets at 6.25, 6.21, 6.26, 6.25 ppm with a vicinal constant of 3.0–3.4 Hz, respectively, that confirmed a β-orientation of the glycosidic bonds [21]. Finally, the removal of the *O*-acetyl protective groups of **1b, f**; **2b**; **3b** with 0.1 N MeONa/MeOH solution furnished TPP-conjugates **1c, g**; **2c**; **3c** bearing unprotected OH groups in yields of 76–68%. Similarly, the removal of the *O*-acetyl protection of the starting 1,2,3-triazolyl nucleoside analogues **1a, e**; **2a**; **3a** afforded their derivatives **1d, h**; **2d**; **3d** possessing unprotected OH groups with good yields (89–98%).

In addition, a series of model compounds, namely derivatives of uracil and thymine containing ω-alkyne or a 1,2,3-triazolyl substituent at the *N*-1 atom as well as the TPP-butyl moiety at the *N*-3 atom was synthesized (Scheme 2). *N*-1-Propargyl derivatives of uracil **4b** and thymine **5b** were synthesized in 48% and 41% yields, respectively, by an alkylation of monosodium salts of uracil **4a** and thymine **5a** prepared by their reaction with NaH according to known procedure [21]. The *N*-1-ω-alkyne derivative of uracil **4i** was prepared in two steps similarly to a method previously described [21]. At first, uracil **4a** was reacted with an excess of hexamethyldisilazane (HMDS) in toluene in the presence of H_2SO_4 to afford a bissilylated derivative followed by alkylation with 1-iodo-hex-5-yne to obtain **4i** in 45% yield. Then the Cu alkyne-azide cycloaddition reaction of the alkyne derivatives **4b** and **5b** with trimethylsilylazide ($TMSN_3$) afforded *N*-1-(1,2,3-triazol-4-yl) derivatives **4c** and **5c** and this reaction with *n*-pentyl azide furnished *N*-1-(pentyl-1*H*-1,2,3-triazol-4-yl) derivatives **4d** and **5d** (Scheme 2).



Scheme 2 Synthesis of triphenylphosphonium conjugates of uracil and thymine. The numbering of atoms of target compounds is shown

The structure of **4c** was confirmed by single crystal X-ray diffraction analysis (Fig. 1). Attaching the TPP-butyl moiety to **4d** and **5d** in the presence of K_2CO_3 in DMF by analogy with a procedure described [29] afforded TPP-conjugates **4e** and **5e**. Similarly, TPP-butyl moiety has been attached to the *N*-3 atom of the *N*-1-propargyl derivatives of uracil **4b**, thymine **5b** and *N*-1-ω-alkyne derivative of uracil **4i** to obtain TPP-conjugates **4f**, **5f**, **4j** (Scheme 2). Besides, uracil **4a** and thymine **5a** were preliminary alkylated at the *N*-1 position in two steps in order to provide the attachment of the TPP-butyl moiety to the *N*-3 atom. At first, treatment of **4a** and **5a** with hexamethyldisilazane (HMDS) in toluene in the presence of H_2SO_4 afforded bissilylated derivatives which then were alkylated at the *N*-1 position with CH_3I to obtain pyrimidines **4g** and **5g** (Scheme 2). Then, **4g** and **5g** were alkylated with TPP-butyl bromide to obtain TPP-conjugates **4h** and **5h**.

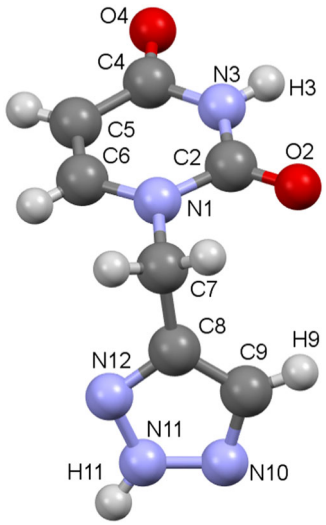
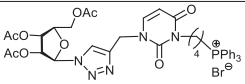
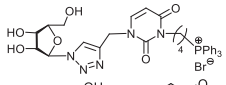
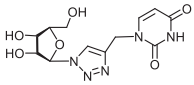
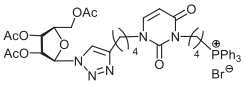
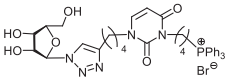
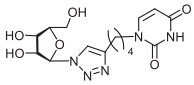
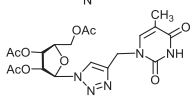
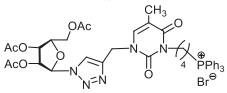
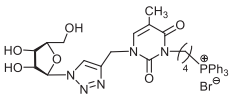
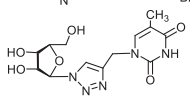
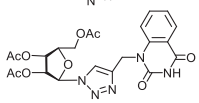
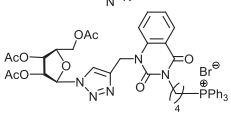
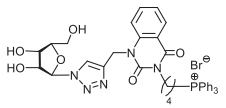
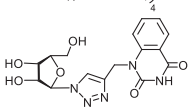


Fig. 1 X-ray crystal structure of compound **4c**

Table 1 Cytotoxic activity of several 1,2,3-triazolyl nucleoside analogues against human cancer and human normal cell lines (IC_{50} values in μM with standard errors)

Compound	Structure	IC_{50} (μM)							
		Cancer cell lines							Normal cell line
		[a]M-HeLa	[b]MCF-7	[c]PANC-1	[d]PC-3	[e]DU145	[f]A375	[g]SKOV-3	[h]WI38
1b		>100	>100	>100	>100	>250	>50	>50	>100
1c		>100	>100	>100	>100	16.4 ± 1.10	>250	>250	>100
1d		>100	>100	>100	>100	>250	>50	>50	>100
1f		>100	>100	>100	>100	68.7 ± 3.87	>100	>100	>100
1g		100	>100	>100	>100	44.6 ± 2.52	>250	>250	>100
1h		>100	>100	>100	>100	nd	nd	nd	nd
2a		92.5 ± 7.3	>100	>100	>100	>250	>250	>250	>250
2b		81 ± 6.2	>100	>100	>100	>250	>250	>250	>250
2c		>100	>100	>100	>100	>250	>250	>250	>250
2d		>100	>100	>100	>100	nd	>50	>50	>100
3a		>100	>100	>100	>100	nd	nd	nd	>100
3b		>100	>100	>100	>100	49.8 ± 2.36	20.5 ± 1.08	62.6 ± 2.76	>100
3c		>100	>100	>100	>100	nd	nd	nd	>100
3d		>100	>100	>100	>100	nd	nd	nd	>100
Doxorubicin		3.0 ± 0.2	3.0 ± 0.1	0.09 ± 0.01	1.09 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	nd
Tubercidin		0.64 ± 0.02	0.82 ± 0.01	nd	0.77 ± 0.01	0.89 ± 0.01	0.59 ± 0.01	0.8 ± 0.01	nd

nd not determined

[a]M-Hela is a human cervix epitheloid carcinoma

[b]MCF-7 is a human breast adenocarcinoma (pleural fluid)

[c]PANC-1 is a human pancreatic cancer cell line isolated from a pancreatic carcinoma of ductal cell origin

[d]PC-3 is a human prostate cancer cell line

[e]DU-145 is a human prostate cancer cell line

[f]A375 is a human malignant melanoma

[g]SKOV-3 is a human ovarian adenocarcinoma

[h]Wi-38 is a diploid human cell strain composed of fibroblasts derived from lung tissue of a 3-months gestation aborted female fetus

Biological activity

In vitro cytotoxicity of the TPP-conugates of 1,2,3-triazolyl nucleoside analogues as well as alkyne and 1,2,3-triazolyl derivatives of uracil and thymine

1,2,3-Triazolyl pyrimidine analogues **1d**, **h**; **2a**, **d**; **3a**, **d** as well as their conjugates with *n*-butyltriphenylphosphonium bromide (TPP-conjugates) **1b**, **c**, **f**, **g**; **2b**,**c**; **3b**,**c** were evaluated for in vitro cytotoxicity against seven human cancer cell lines: M-HeLa cervical epitheloid carcinoma, MCF-7 breast adenocarcinoma, PANC-1 pancreatic carcinoma, PC-3 and DU145 prostate carcinoma, SKOV-3 ovarian adenocarcinoma, A375 malignant melanoma, as well as a diploid human cell strain WI-38 composed of fibroblasts. The results are presented in Table 1. As shown in the table, all tested 1,2,3-triazolyl nucleoside analogues were inactive against all human cancer cell lines, whereas some of their TPP-conjugates exhibited cytotoxicity against human cancer cell lines M-HeLa, DU145, A375, and SKOV-3. Thus, a weak inhibition of cell growth was demonstrated by TPP-conjugates **1f**, **1g**, and **3b** in DU145 (IC_{50} values of 68.7 μM, 44.6 μM, and 49.8 μM, respectively), TPP-conjugate **2b** in M-HeLa ($IC_{50} = 81$ μM), and TPP-conjugate **3b** in SKOV-3 ($IC_{50} = 62.6$ μM). The most potent compounds, TPP-conjugates **1c** and **3b**, exhibited cytotoxicity against DU145 and A375 cells with $IC_{50} =$ 16.4 μM and 20.5 μM, respectively (Table 1). These results suggested that attachment of the TPP moiety to the 1,2,3-triazole pyrimidine analogues endowed some of them with cytotoxic activity.

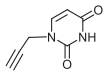
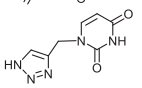
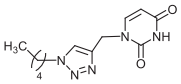
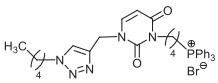
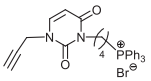
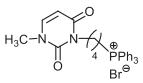
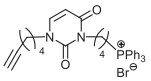
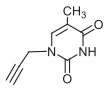
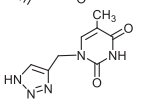
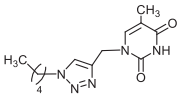
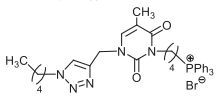
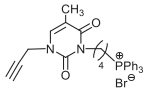
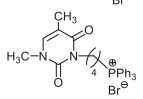
To identify the pharmacophore essential for cytotoxicity, a series of model compounds (**4b–j**, **5b–h**) was synthesized (Scheme 2), and their ability to inhibit the in vitro growth of human cancer cell lines M-HeLa, MCF-7, PANC-1, PC-3, DU145, A375, and SKOV-3 as well as normal human cell line WI-38 was evaluated. The results are presented in Table 2. As anticipated, uracil **4a** and thymine **5a** themselves were inactive. The addition of a propargyl fragment to the *N*-1 atom of these nucleobases resulted in moderate cytotoxicity against M-HeLa. Namely, compounds **4b** and **5b** inhibited the growth of the cancer cell line M-HeLa with IC_{50} values of 26 μM and 32.4 μM, respectively (Table 2). The derivatives of uracil (compound **4c**) and thymine (compound **5c**) with the 1,2,3-triazol-4-yl-methyl fragment at the *N*-1 atom showed weak cytotoxicity against cell lines DU145 and M-HeLa with IC_{50} values of 56.8 μM and 75.8 μM, respectively. The attachment of a pentyl substituent to the *N*-1′ atom of the 1,2,3-triazole moiety of compounds **4c** and **5c** (i.e., the transition to compounds **4d** and **5d**) has caused activity loss (Table 2). Similarly to 1,2,3-triazolyl nucleoside analogues, attachment of the TPP-butyl moiety to the *N*-3 atom of the pyrimidine fragment of inactive compounds **4d** and **5d** (i.e., the transition to compounds **4e** and **5e**) resulted in the appearance of the ability to inhibit the growth of cancer cell lines DU145, A375, and SKOV-3, with the TPP conjugate **5e** being the most potent. This compound inhibited the in vitro growth of cancer cell lines DU145, A375, and SKOV-3 with IC_{50} values of 14.5 μM, 13.2 μM, and 16.1 μM, respectively. Therefore, the coupling of uracil and thymine with the TPP moiety afforded cytotoxic molecules exemplified by uracil derivative **4h** and thymine derivative **5h** (Table 2). Moreover, the TPP conjugates of *N*-1-ω-alkyne derivatives of uracil **4f** and thymine **5f** showed considerable cytotoxicity against DU145 with IC_{50} values of 6.1 μM and 9.3 μM, respectively. In addition, **4j** inhibited the growth of A375 cancer cells with the IC_{50} value of 5.1 μM.

Note, the growth of MCF-7, PANC-1, and PC-3 cells was not suppressed by the tested molecules at 100 μM concentration. It should be emphasized that all tested compounds failed to induce cytotoxic effects on normal WI-38 fibroblasts.

In vivo sea urchin embryo tests

All compounds synthesized were evaluated for antimitotic activity using in vivo phenotypic screening on a sea urchin embryo model [27, 28]. This simple organism provides rapid and highly reproducible assessment of both antiproliferative/antimitotic effect and/or systemic toxicity together with information about mode of action, in particular, targeting tubulin and microtubules. Multiple advantages of the sea urchin embryo allows for its consideration as a relevant model organism for biomedical research [33, 34]. It was found that all tested molecules failed to cause developmental abnormalities of sea urchin embryos when applied to fertilized eggs. The results suggest that uracil and thymine derivatives including their TPP conjugates were nontoxic toward this animal model.

Table 2 Cytotoxic activity of several N-1-alkyne and N-1-1,2,3-triazolyl derivatives of uracil and thymine as well as their TPP-conjugates (IC_{50} values in μM with standard errors)

Compound	Structure	IC_{50} (μM)							
		Cancer cell lines							Normal cell lines
		[a]M-Hela	[b]MCF-7	[c]PANC-1	[d]PC-3	[e]DU145	[f]A375	[g]SKOV-3	[h]WI38
4a		>100	>100	>100	>100	>250	>250	>250	>100
4b		26 ± 2.0	>100	>100	>100	nd	>50	>50	>100
4c		>100	>100	>100	>100	58.6 ± 5.8	>250	>250	>100
4d		>100	>100	>100	>100	>250	>250	>250	>100
4e		>100	>100	>100	>100	24.8 ± 0.87	60.2 ± 3.65	54.6 ± 4.98	>100
4f		>100	>100	>100	>100	6.1± 0.09	>50	>50	>100
4h		>100	>100	>100	>100	53.6 ± 4.0	84.0 ± 7.17	75.7 ± 3.34	>100
4j		>100	>100	>100	62.8 ± 5.1	13.4 ± 0.22	5.1 ± 0.19	20.9 ± 0.38	>100
5a		>100	>100	>100	>100	>250	>250	>250	>100
5b		32.4 ± 2.6	>100	>100	>100	nd	>50	>50	>100
5c		75.8 ± 6.2	>100	>100	>100	>250	>250	>250	>100
5d		100	>100	>100	>100	>250	>250	>250	>100
5e		>100	>100	>100	>100	14.5 ± 0.28	13.2 ± 0.40	16.1 ± 1.13	>100
5f		>100	>100	>100	>100	9.3 ± 0.21	17.8 ± 0.12	23.4 ± 0.14	>100
5h		87.7 ± 7.2	>100	>100	>100	29.6 ± 1.14	21.2 ± 2.09	62.3 ± 2.83	>100
Doxorubicin		3.0 ± 0.2	3.0 ± 0.1	0.09 ± 0.01	1.09 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	nd
Tubercidin		0.64 ± 0.02	0.82 ± 0.01	nd	0.77 ± 0.01	0.89 ± 0.01	0.59 ± 0.01	0.8 ± 0.01	nd

nd not determined

[a]M-Hela is human cervix epitheloid carcinoma

[b]MCF-7 is human breast adenocarcinoma (pleural fluid)

[c]PANC-1 is human pancreatic cancer cell line isolated from a pancreatic carcinoma of ductal cell origin

[d]PC-3 is human prostate cancer cell line

[e]DU-145 is human prostate cancer cell line

[f]A375 is human malignant melanoma

[g]SKOV-3 is human ovarian adenocarcinoma

[h]Wi-38 is diploid human cell strain composed of fibroblasts derived from lung tissue of a 3-months gestation aborted female fetus

Conclusion

A series of TPP conjugates of 1,2,3-triazolyl analogues of several pyrimidine nucleosides was synthesized and evaluated for the in vitro cytotoxicity against human cancer cell lines M-HeLa, MCF-7, PANC-1, PC-3, DU145, SKOV-3, A275 and normal human cell line WI-38. In these TPP-conjugates TPP cation was attached via a tetramethylene linker to the *N*-3 atom of the heterocycle moiety (uracil, thymine, and quinazoline-2,4-dione), which was coupled with the D-ribofuranosyl-1,2,3-triazol-4-yl fragment via methylene or tetramethylene linker. It was shown for the first time that the conjugation of 1,2,3-triazolyl derivatives of uridine as well as its analogues featuring quinazoline-2,4-dione fragment with a TPP-butyl cation endowed some of them with cytotoxic activity against DU145 human cancer cells. The most potent compound in this series, namely, the TPP conjugate of 1,2,3-triazolyl analogue of uridine **1c**, showed cytotoxicity against DU145 with $IC_{50} = 16.4\ \mu M$. Similarly, the attachment of the TPP-butyl moiety to the *N*-3 atom of the uracil derivatives having propargyl or a 1,2,3-triazolyl substituent at the *N*-1 atom (compounds **4b** and **4d**) and to the respective thymine derivatives **5b** and **5d** afforded compounds **4e, f, j** and **5e, f** that inhibited the growth of cancer cell lines DU145 and A375 with IC_{50} values in the range of 5.1–9.3 μM. Propargyl derivatives **4f**, **4j**, and **5f** showed the highest cytotoxicity in the series against a DU145 cell line with IC_{50} values of 6.1 μM, 13.4 μM, and 9.3 μM, respectively. In addition, **4j** was the most potent against A375 melanoma cells ($IC_{50} = 5.1\ \mu M$).

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Compliance with ethical standards

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

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