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Concise synthesis and antibacterial evaluation of novel 3-(1,4-disubstituted-1,2,3-triazolyl)uridine nucleosides

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Abstract

We report herein a simple and efficient synthesis of a new series of antibacterial uridine nucleosides. The strategy involved a sequential silylation/*N*-glycosylation/*N*-propargylation procedure of uracil **1** for preparing the dipolarophile **5** in good yield. A series of novel uridine-[1,2,3]triazole nucleosides **6a–j** were efficiently synthesized via the copper-catalyzed azide–alkyne cycloaddition (CuAAC) from dipolarophile **5** with different selected azides. The reactions were carried out under both conventional and ultrasonic irradiation conditions. In general, improvements were observed when reactions were carried out under sonication. Their antibacterial potential has been evaluated by means of a micro-dilution assay against either Gram-positive or Gram-negative bacteria. Compounds **6i** and **6j** have shown significant bactericidal activity against *Staphylococcus aureus* (MIC = 10 and 6 μM, respectively), and **6h** against *Escherichia coli* (MIC = 8 μM). Moreover, antibacterial kinetic assays showed that **6i** and **6j** significantly reduced the *S. aureus* growth rate at the MIC concentration, after 6 h, compared to their deprotected analogs, **6k** and **6l**, respectively. Compound **6h** also significantly reduced the growth of *E. coli*. These antibacterial effects may be related to the penetrating properties of these compounds, as revealed by the leakage of nucleic acids from the sensitive strains.

KEYWORDS
antibacterial activity, bactericidal action, click chemistry, microwaves, ultrasounds, uridine analogues, 1,2,3-triazoles

1 | INTRODUCTION

It is of utmost importance to identify novel antibacterial agents with original mechanisms of action due to the resistance of bacterial pathogens to current antibiotic drugs, and to the increasing emergence of multidrug-resistant bacteria.[1–3] Thus, numerous bioactive molecules have been synthesized and/or isolated from nature for their antibacterial effects.[4,5]

On the other hand, nucleoside analogues have emerged as important therapeutic agents for the treatment of a wide spectrum of pathologies.[6,7] They represent, for a long time, the cornerstone of modern scientific medical knowledge.[8] Over the last decades, many structural modifications have been studied, for the base and/or the sugar moieties, leading to novel derivatives with strengthened biological properties.[9–28] For example, uracil derivatives have been studied for the discovery of new therapeutic nucleoside agents,

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including antibacterial drugs (Figure 1).[29] In addition, uridine analogues have a potent wide range of pharmacological and biological activities that include antimicrobial, antiparasitic, antifungal, antiviral, anticancer, antinociceptive, antitubercular, anti-inflammatory, anti-convulsant and analgesic activities.[30]

Since the discovery of the anticancer agent 5-fluorouracil, and of the antiviral and antibacterial drugs, 5-ethyl-2-deoxyuridine (EdU) and zidovudine, the chemistry of uridine nucleosides has been well studied.[31] Nonetheless, we focused our interest on new modifications at position 3 of the nucleobase since the biological effect of such modifications has not been extensively studied.[6]

To this end, we decided to introduce at this position a 1,2,3-triazole core, a well-known pharmacophore.[32,33] Indeed, recent studies have revealed that triazole containing nucleosides exert a broad spectrum of pharmacological activities including antiviral,[34,35] antimicrobial,[36] antiproliferative,[37] and antitumor properties.[38–40]

In line with our previous studies directed at the development of new eco-compatible routes to nucleoside analogs,[7,41,42] we report herein a new synthetic pathway to novel modified uridine nucleosides, substituted with the 1,2,3-triazole core **6a–l** (Figure 1). All these compounds have been evaluated for their *in vitro* antibacterial activity against four bacterial strains (two Gram-positive bacteria, *Staphylococcus aureus* and *Listeria monocytogenes*, and two Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*).

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic route for the preparation of nucleosides **6a–j** is depicted in Scheme 1 and Table 1. Firstly, the commercially available uracil **1** reacted with an excess of hexamethyldisilazane (HMDS) **2** using $(NH_4)_2SO_4$ as catalyst. This reaction has been carried out using microwave irradiation or conventional heating. It occurred in a short reaction time when using microwave irradiation (120°C using the CEM Discover System, fixed power: 300 W). According to this protocol, the trimethylsilylated product **3** is obtained in 22 min only (similar conversion rate has been obtained within 5 h using conventional heating).[43] After cooling, the crude material was allowed to react with β-D-ribofuranose 1,2,3,5-tetraacetate without purification. This *N*-glycosylation was carried out according to the Vorbrüggen reaction,[44] using silylated uracil and electrophilic ribofuranose in the presence of 1.1 eq $SnCl_4$ as Friedel–Crafts catalyst. The reaction was performed under ultrasound irradiation at 5°C to afford the uridine **4** via the mechanism of the silyl Hilbert–Johnson reaction which involves a stereoselective nucleophilic attack at the anomeric position by the most nucleophilic nitrogen N(1).[45,46] The expected acetyl-protected uridine **4** was obtained in excellent yield, after only 15 min of sonication. Interestingly, ultrasound irradiation dramatically reduced the reaction times in comparison with magnetic stirring (15 min vs. 18 h, respectively).[46] Next, compound **4** reacted with propargyl bromide in the presence of K_2CO_3 in DMF at 70°C for 3 h, and the expected dipolarophile, the acetyl-protected *N*3-propynyl-ribouridine **5**, has been isolated in 87% yield. On the other hand, the aryl azides have been easily synthesized by adding sodium azide to a series of selected benzyl bromides in DMF at 70°C.

We then studied the cycloaddition reactions leading to triazoles.[41] Briefly, the cycloaddition reaction between the selected azide derivatives and the dipolarophile **5**, using copper(II) sulfate/sodium ascorbate or copper(I) iodide as catalyst in a mixture of *n*-butanol and water (1:1, v/v) afforded triazole nucleosides **6a–j** in good yields (73–95%, see Supporting Information). Interestingly, when these reactions were carried out under ultrasound irradiation the cycloadducts were obtained in almost quantitative yields (see Supporting Information). Moreover, reactions were achieved in short reaction times compared to those carried out without sonication (3–12 min vs. 4–5 h).

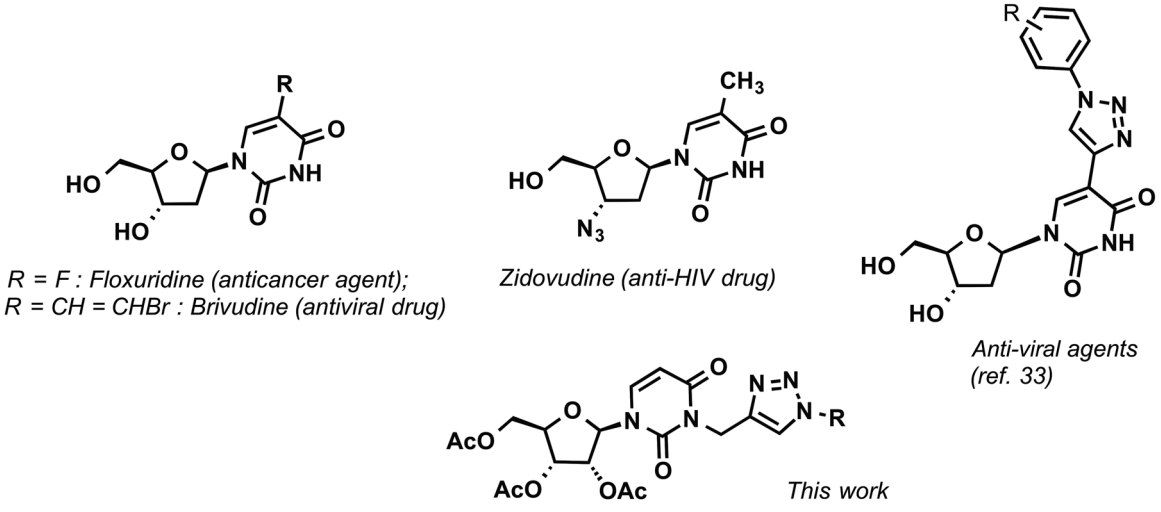
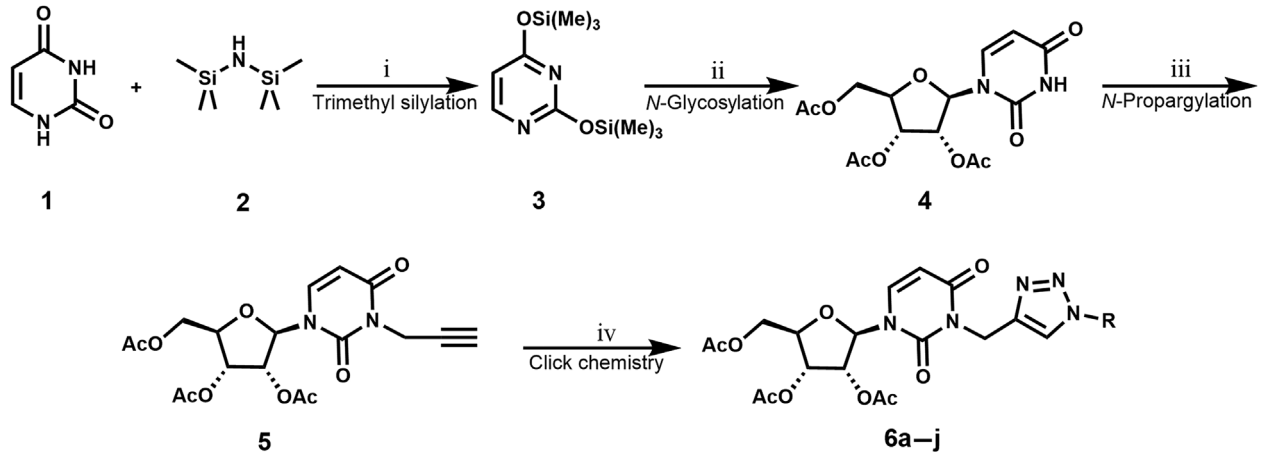
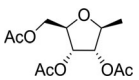
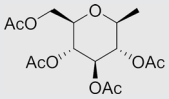
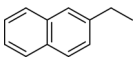
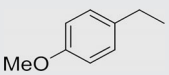
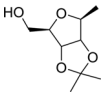
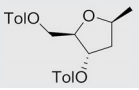
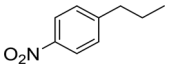
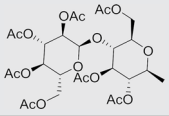


FIGURE 1 Representative example of bioactive nucleoside analogs, and general scaffold of the new series of 3-(1,4-disubstituted-1,2,3-triazolyl)uridine nucleosides



SCHEME 1 Reagent and reaction conditions: (i) trimethylsilylation: HMDS, $(NH_4)_2SO_4$, cat, reflux (120°C, 5 h) or MW (300 W, 120°C, 22 min); (ii) *N*-glycosylation: β-D-ribofuranose 1,2,3,4-tetraacetate, $SnCl_4$, acetonitrile, 5°C, 15 min (US) or 18 h (stirring); (iii) *N*-propargylation: K_2CO_3, DMF, propargyl bromide, 70°C, 3 h; (iv) click chemistry: R-N_3, CuI or $CuSO_4$ (0.2 eq), sodium ascorbate (0.2 eq), *n*-BuOH/H_2O (1:1, v/v), 3–12 min (US) or 4–5 h (stirring)

TABLE 1 Synthesis of the 3-(1,4-disubstituted-1,2,3-triazolyl)uridine nucleosides under CuAAC conditions

			Stirring		Ultrasonication	
Entry[a]	Compounds	R	Time (h)	Yield (%)	Time (min)	Yield (%)
1	**6a**	$C_{12}H_{25}$-	4.5	75	12	78
2	**6b**		4	83	5	81
3	**6c**		3.5	88	3	95
4	**6d**		3.5	89	5	94
5	**6e**		4	82	8	78
6	**6f**		4	93	8	91
7	**6g**		4.5	71	12	73
8	**6h**		4.5	73	11	77
9	**6i**		4	79	10	82
10	**6j**		4.5	75	12	74

[a]Reactions were carried out with 1 mmol dipolarophile **5**, 1 mmol corresponding azides, 0.2 mmol $CuSO_4$, 0.2 mmol sodium ascorbate at 20–25°C in tBuOH/H_2O (1:1, v/v).

2.2 | Antibacterial activity

These nucleoside analogs have been evaluated *in vitro* for their antibacterial activity against *L. monocytogenes* serovar 4b CECT 4032, *E. coli* K12, *S. aureus* MBLA, and *P. aeruginosa* IH. The minimum inhibitory concentrations (MICs), the minimum bactericidal concentrations (MBCs), and the antibacterial action have been measured and compared to those of two marketed antibiotics (chloramphenicol and erythromycin); all the results have been collected in Table 2.

First, it is noteworthy that the newly synthesized nucleoside analogs exhibit antibacterial effects against the different bacteria strains assayed. Moreover, three molecules (**6i**, **6j**, and **2h**) appear significantly more efficient in killing several types of bacteria than chloramphenicol and erythromycin. Indeed, compounds **6i** and **6j** show an important antibacterial activity either against *S. aureus* (MIC = 10 and 6 μM, respectively) and *L. monocytogenes* (MIC = MBC = 21 and 23 μM, respectively), a bacterium responsible for serious diseases including meningitis. In addition, **6i** exerts also a bactericidal effect against *E. coli* (MIC = MBC = 42 μM). On the other hand, **6h** inhibits significantly *E. coli* growth (MIC = MBC = 8 μM) and *S. aureus* (MIC = MBC = 16 μM). Interestingly, in these assays, the MICs are very close or equal to the MBCs, proving a bactericidal action of these three compounds at the MICs.

Furthermore, the chemical nature of the "R" group encompassed by compounds **6h** (protected deoxyribose), **6i** (nitroaromatic derivative), and **6j** (polysaccharide) differ strongly, suggesting a complex structure–activity relationship. Nevertheless, in this study, the protected nucleosides appear more efficient in killing bacteria than their unprotected counterparts, as underlined by the comparison of the MICs and MBCs obtained with **6h** and **6j** on the one hand versus **6k** and **6l** on the other hand.

Next, we studied the effects of **6i**, **6j**, and **6h** on bacterial growth kinetics (Figure 2). To this end, *S. aureus* has been grown in the presence of **6i** and **6j** and *E. coli* has been grown in the presence of **6h**. For each kinetic, three different concentrations of nucleoside have been assayed (2MIC, MIC, and MIC/2).

As anticipated, the three compounds exert a significant action on the growth kinetics, in a dose-dependent manner. Compound **6i** decreases by more than 50% *S. aureus* growth in 4 h of incubation when used at the MIC; in addition, this molecule exerts a complete bactericidal action within 6 h at the 2MIC concentration. Remarkably, at the same dose (2MIC), **6j** kills all the bacteria within 4 h of incubation only; moreover, at the MIC level, this bactericidal action is completed in 10 h. On the other hand, **6h** decreases dramatically the growth of *E. coli* within 6 h at the 2MIC concentration, while at the MIC level, this molecule reduces by more than 60% the bacteria growth in 8 h.

We next focused our attention on the bactericidal mechanism exerted by the three hits. To this end, we studied the kinetics of nucleic acids leakage issued from bacteria grown under treatment with **6i**, **6j** (*S. aureus*), and **6h** (*E. coli*). As shown in Figure 3, the three compounds induced in a dose-dependent manner the nucleic acids leakage. Importantly, this leakage may be related to the lysis of the bacteria membranes. Moreover, **6i** exerts dramatic membrane damages after 4 h incubation when used at the 2MIC concentration, while similar effects are also observed after 8 h for bacteria grown with same levels of **6j** and **6h**.

3 | CONCLUSIONS

In summary, we reported herein an efficient synthesis of 12 new 3-(1,4-disubstituted-1,2,3-triazolyl)uridine nucleoside analogues through microwave and ultrasound irradiation. All these compounds have been purified and fully characterized by spectral analyses, and finally assayed for their potential antibacterial activity against Gram-positive and Gram-negative bacteria. By comparison with the marketed antibiotics chloramphenicol and erythromycin, the nucleoside analogs **6i** and **6j** proved a better antibacterial activity against *S. aureus* (MIC and MBC in the 10 μM range) and *L. monocytogenes* (MIC and MBC in the 20 μM range), and the analog **6h** exhibited a promising antibacterial effect against *E. coli* (MIC = MBC = 8 μM). Our preliminary results suggest that the bactericidal mechanism of these compounds may be due to irreversible damages caused to bacteria membranes. However, studies aiming at the precise comprehension of this mechanism are currently under way, and their results will be reported in due course.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

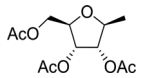
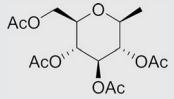
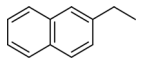
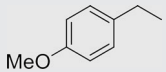
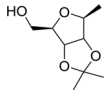
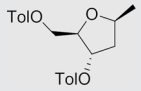
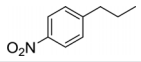
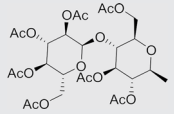
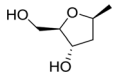
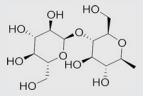
All organic solvents were purchased from commercial sources and used as received or dried using standard procedures; all chemicals were purchased from Aldrich, Merck, or Alfa Aesar and used without further purification.

Analytical thin layer chromatographies (TLC) have been performed on pre-coated silica gel plates (Kieselgel 60 F_{254}, E. Merck, Germany), and chromatograms were visualized by UV-light irradiation (254 and 365 nm), then by staining with ninhydrin, *p*-anisaldehyde or H_2SO_4/EtOH. Purifications by column chromatography have been performed by Isco Combiflash system using combiflash column 12 g with a flow rate of 30 mL/min.

NMR spectroscopies were recorded in dry deuterated solvent (DMSO or chloroform) on a Bruker AC 200, or on a Bruker AC 400 spectrometers at 200 or 400 MHz for ^{1}H NMR and 50 or 100 MHz for ^{13}C NMR; δ is expressed in ppm related to TMS (0 ppm) as internal standard (see Supporting Information for the original spectra). Splitting patterns are designated as follow: s (singlet), d (doublet), t (triplet), m (multiplet). Coupling constants (*J* values) are listed in Hertz (Hz). Mass spectra (ESI-MS) were recorded on a Bruker Daltonics Esquire 3000+, and the samples were diluted in methanol.

The microwave-assisted reactions were carried out in a CEM Discover, single mode cavity with focused MW heating (MW power supply 0–300 W in 1 W increments, IR temperature sensor, open or closed vessel mode, pressure range 0–20 bar, 10-mL or 80-mL vials).

TABLE 2 Determination of the minimum inhibitory concentration and minimum bactericidal concentration of compounds **6a–l**

		E. coli			*S. aureus*			*P. auruginosa*			*L. monocytogenes*		
Molecules	R	MIC	MBC	Action	MIC	MBC	Action	MIC	MBC	Action	MIC	MBC	Action
6a	$C_{12}H_{25}$-	161	>161	Bactericidal	161	>161	Bacteriostatic	>161	>161	Nd	81	>161	Bacteriostatic
6b		23	46	Bacteriostatic	92	92	Bactericidal	92	184	Bacteriostatic	92	92	Bactericidal
6c		141	141	Bactericidal	35	141	Bacteriostatic	>141	>141	Nd	35	70	Bacteriostatic
6d		64	>128	Bacteriostatic	32	64	Bacteriostatic	>128	>128	Nd	64	64	Bactericidal
6e		42	169	Bactericidal	42	84	Bacteriostatic	84	>169	Bacteriostatic	84	84	Bactericidal
6f		44	175	Bacteriostatic	44	44	Bactericidal	175	>175	Bacteriostatic	88	88	Bacteriostatic
6g		20	20	Bactericidal	40	40	Bactericidal	160	>160	Bacteriostatic	80	80	Bactericidal
6h		8	8	Bactericidal	16	16	Bactericidal	64	>129	Bacteriostatic	32	64	Bacteriostatic
6i		42	42	Bactericidal	10	21	Bacteriostatic	>166	>166	Nd	21	21	Bactericidal
6j		23	46	Bacteriostatic	6	6	Bactericidal	93	93	Bactericidal	23	23	Bactericidal
6k[a]		28	56	Bacteriostatic	28	57	Bacteriostatic	113	226	Bacteriostatic	113	>226	Bacteriostatic
6l[a]		38.5	38.5	Bactericidal	38.5	38.5	Bactericidal	154	154	Bactericidal	38.5	77	Bacteriostatic
	Erythromycin	22	43	Bacteriostatic	43	87	Bacteriostatic	87	174	Bacteriostatic	43	87	Bacteriostatic
	Chloramphenicol	25	99	Bacteriostatic	25	99	Bacteriostatic	198	198	Bactericidal	50	99	Bacteriostatic

MIC, minimum inhibitory concentration (μM); MBC, minimum bactericidal concentration (μM); final bacterial density was around 10^6 CFU/mL.

[a]Compounds **6h** and **6j** have been deprotected using K_2CO_3/MeOH for 4 h at r.t. to give the corresponding unprotected *N*-nucleosides **6k** and **6l**.

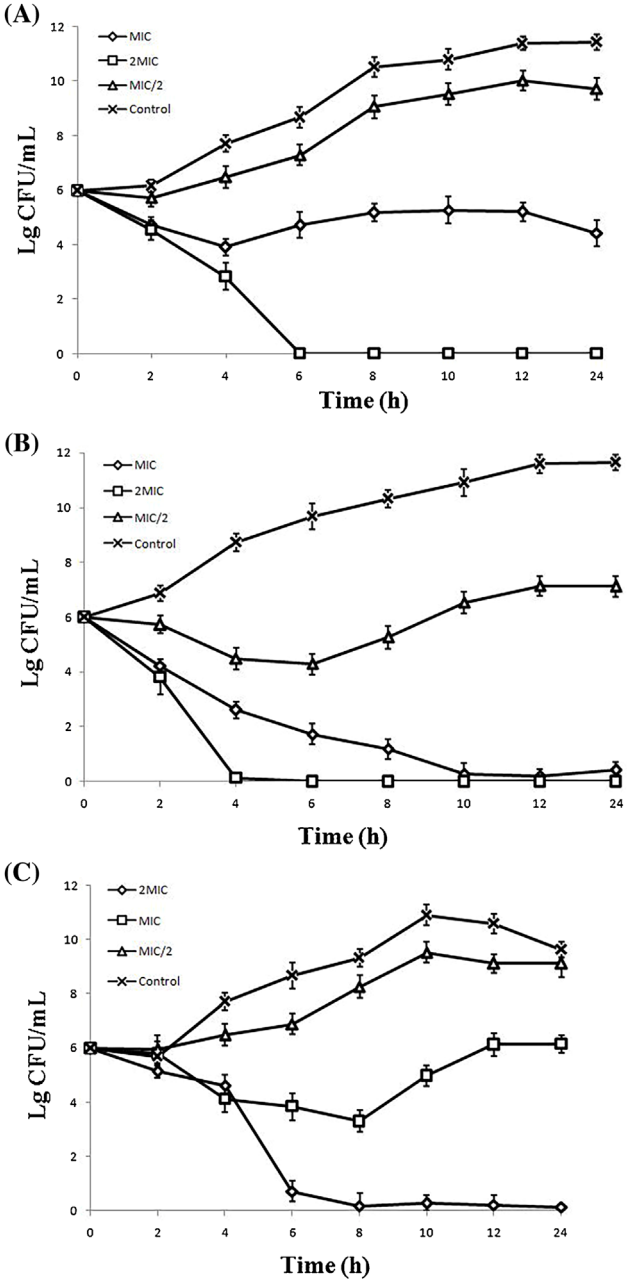


FIGURE 2 Bacterial growth kinetics under treatment with nucleosides analogues **6i**, **6j**, and **6h**. (A and B) *S. aureus* is grown with **6i** (A) or **6j** (B); (C) *E. coli* is grown with **6h**. For each kinetics, bacteria have been grown in absence (negative control) or in presence of the selected nucleoside (three different concentrations have been studied: MIC, MIC/2, and 2MIC)

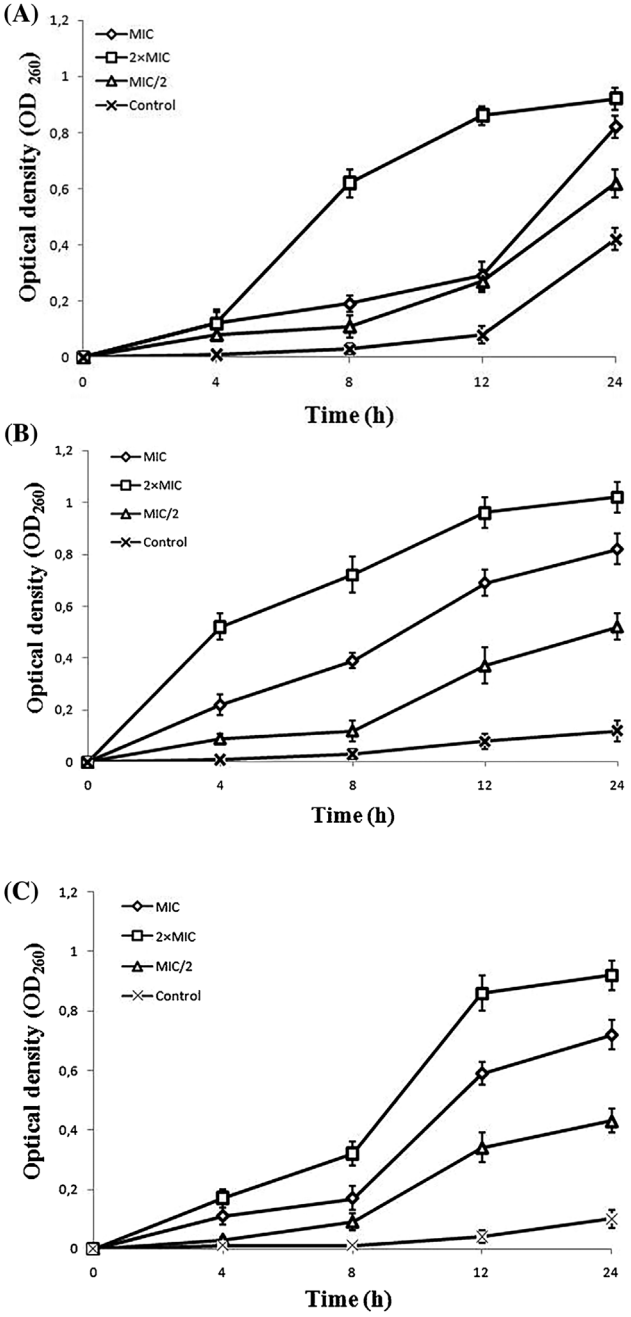


FIGURE 3 Mechanistic studies: Kinetics of nucleic acids leakage issued from bacteria stains grown with the nucleosides derivatives measured by UV-absorbance at 260 nm. (A and B) *S. aureus* is grown with **6i** (A) or **6j** (B); (C) *E. coli* is grown with **6h**. For each assay, bacteria have been grown in absence (negative control) or in presence of the selected nucleoside (three different concentrations have been studied: MIC, MIC/2, and 2MIC)

The ultrasound-assisted reactions were carried out in an ultrasonic processor Vibra-Cell™ model 75022 with Titanium alloy Ti-6Al-4 V probe (20 kHz, 130 W) with 4 mm tip diameter; all reactions were carried out by using 60% of Pmax. Vials of 10–50 mL were used and the sonotrode was dipped in the solution in a way to reach the maximum energy.

The InChI codes of the investigated compounds together with some biological data are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of compound 3: Trimethylsilylation of uracil

Classical method

In a round-bottom flask equipped with a magnetic stirred bar, uracil **1** (1 mmol), $(NH_4)_2SO_4$ (5%), HDMS (10 mL) were added. The resulting

mixture was refluxed for 4–5 h until getting a clear solution. Upon completion, the excess of HMDS was removed under pressure. The crude product was engaged in the next step without any previous purification.

Microwave heating method

In a round-bottom flask equipped with a magnetic stirred bar, uracil **1** (0.161 g, 1 mmol), $(NH_4)_2SO_4$ (5%), HMDS (0.627 mL, 3 mmol) were added. The resulting mixture was heated under microwave irradiation at 120°C for 22 min in an open vessel mode at 300 W. Upon completion, the crude product was engaged in the next step without any previous purification.

4.1.3 | General procedure for the synthesis of compound 4: *N*-Glycosylation

Classical and sonication methods

The previously prepared trimethylsilylated uracil **3**, 1,2,3,4-tetraacetate-β-D-ribofuranose (0.318 g, 1 mmol), $SnCl_4$ (1.1 mmol), acetonitrile (8 mL) were added. The resulting mixture was sonicated or stirred at 5°C for 15 min (under ultrasound) or 18 h (stirring). After completion (TLC), 5 mL of pyridine was added to quench the reaction, filter the salt, the liquid phase was concentrated under pressure. The residue was dissolved in DCM (40 mL), washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the crude product. The crude product was subjected to purification by Isco Combiflash system using flash column 12 g with a flow rate of 30 mL/min of AcOEt/cyclohexane (1:1) as eluent to give pure **4**; yield 88%. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 9.39 (s, 1H), 7.34 (d, J = 8.2 Hz, 1H), 5.98 (d, J = 4.9 Hz, 1H), 5.74 (d, J = 8.1 Hz, 1H), 5.28 (d, J = 3.3 Hz, 3H), 4.29 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 171.15, 170.13, 169.63, 162.82, 150.22, 139.29, 103.40, 87.44, 79.90, 72.69, 70.16, 63.13, 20.76, 20.49, 20.39; MS (ESI$^+$): m/z = 371 $[M+H]^+$.

4.1.4 | General procedure for the synthesis of compound 5: *N*3-Propargylation of uridine

In a round-bottom flask equipped with a magnetic stirred bar, uridine **4** (0.5 g, 1.35 mmol), propargyl bromide (0.167 mL, 1.62 mmol), potassium carbonate (0.56 mg, 4.05 mmol), DMF (15 mL) were added. The resulting mixture was stirred and heated at 70°C. The completion of the reaction was monitored by TLC. Upon completion, the reaction mixture was concentrated under pressure. The residue was dissolved in DCM (40 mL), washed with water, brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum to afford the crude product. The crude product was subjected to purification by Isco Combiflash system using flash column 12 g with a flow rate of 30 mL/min of AcOEt/cyclohexane (1:1) as eluent to give pure **5** (0.494 g, yield 89.5%). Brown oil; ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.34 (d, J = 8.2 Hz, 1H), 5.95 (d, J = 4.7 Hz, 1H), 5.79 (d, J = 8.2 Hz, 1H), 5.32 (dd, J = 5.8, 4.7 Hz, 1H), 5.29–5.23 (m, 1H), 4.61 (qd, 2H), 4.29 (s, 4H), 2.12 (t, J = 2.4 Hz, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 170.12, 169.56, 169.54, 161.11, 150.11, 137.72, 102.67, 88.82, 79.73, 77.66, 72.96, 70.91, 69.85, 62.84, 30.30, 20.78, 20.47, 20.44; MS (ESI$^+$): m/z = 409 $[M+H]^+$.

4.1.5 | General procedure for the synthesis of 1,2,3-triazoles derivatives compounds *6a–j*: Click chemistry

Classical and sonication methods

In a round-bottom flask equipped with a magnetic stirred bar, **5** (100 mg, 0.25 mmol), azide derivatives (0.25 mmol), $CuSO_4$ (0.05 mmol) or CuI (0.05 mmol), sodium ascorbate (0.05 mmol), *n*-BuOH/H_2O (1:1, v/v) (8 mL) were added. The resulting mixture was sonicated or stirred at room temperature for 3–12 min (under ultrasound) or 4–5 h (stirring). The completion of the reaction was monitored by TLC. Upon completion a solution of NH_4Cl was added and the reaction mixture was extracted with DCM (3 × 30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum to afford the crude product. The crude product was subjected to purification by Isco Combiflash system using flash column 12 g with a flow rate of 30 mL/min of AcOEt/cyclohexane (1:1) as eluent to give pure **6a–j**.

2-(Acetoxymethyl)-5-(3-((1-dodecyl-1*H*-1,2,3-triazol-4-yl)-methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6a)

Yield 78%. Yellow oil; R_f = 0.39 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.50 (s, 1H), 7.30 (d, J = 8.2 Hz, 1H), 5.96 (d, J = 4.7 Hz, 1H), 5.76 (d, J = 8.1 Hz, 1H), 5.31–5.22 (m, 2H), 5.16 (q, 2H), 4.27 (d, J = 4.5 Hz, 3H), 4.22 (t, J = 7.4 Hz, 2H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.80 (t, 3H), 1.21 (d, J = 23.2 Hz, 18H), 0.81 (t, J = 6.8 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.10, 169.59, 169.55, 161.83, 150.54, 142.67, 137.43, 123.20, 102.75, 88.65, 79.71, 72.97, 69.92, 62.88, 50.34, 36.21, 31.90, 30.29, 29.70, 29.60, 29.52, 29.37, 29.33, 29.01, 26.53, 22.68, 20.77, 20.48, 20.46, 14.12; MS (ESI$^+$): m/z = 620.3 $[M+H]^+$, 1239.3 $[2M+H]^+$.

2-(Acetoxymethyl)-5-(3-((1-benzyl-1*H*-1,2,3-triazol-4-yl)-methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6b)

Yield 81%. Colorless oil; R_f = 0.30 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.45 (s, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.27 (d, J = 6.8 Hz, 2H), 7.19 (dd, J = 7.4, 2.1 Hz, 2H), 5.94 (d, J = 4.6 Hz, 1H), 5.73 (d, J = 8.1 Hz, 1H), 5.40 (s, 2H), 5.31–5.22 (m, 3H), 5.12 (d, J = 2.1 Hz, 2H), 4.27 (d, J = 1.6 Hz, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.11, 169.58, 169.55, 161.78, 150.53, 143.19, 137.51, 134.60, 129.05, 128.66, 128.14, 123.29, 102.71, 88.64, 79.73, 72.95, 69.94, 62.91, 54.09, 36.17, 20.76, 20.48, 20.41; MS (ESI$^+$): m/z = 542.2 $[M+H]^+$, 1083 $[2M+H]^+$.

(3*R*,4*R*)-2-(Acetoxymethyl)-5-(3-((1-((2*R*,3*S*,4*S*,5*S*)-3,4-diacetoxy-5-(acetoxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6c)

Yield 95%. Colorless oil; R_f = 0.35 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.74 (s, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 6.06 (d, *J* = 4.0 Hz, 1H), 5.96 (d, *J* = 4.8 Hz, 1H), 5.76 (d, *J* = 8.1 Hz, 1H), 5.71 (dd, *J* = 5.2, 4.0 Hz, 1H), 5.53 (t, *J* = 5.2 Hz, 1H), 5.30–5.23 (m, 2H), 5.19 (q, 2H), 4.38 (dd, *J* = 8.2, 4.2 Hz, 1H), 4.32 (dd, *J* = 12.3, 3.2 Hz, 1H), 4.28 (q, *J* = 1.6 Hz, 3H), 4.15 (dd, *J* = 12.3, 4.2 Hz, 1H), 2.06 (s, 3H), 2.04 (d, *J* = 1.1 Hz, 6H), 2.03 (s, 6H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.48, 170.11, 169.62, 169.55, 169.40, 169.20, 161.72, 150.54, 143.30, 137.46, 122.79, 102.72, 89.97, 88.49, 80.98, 79.78, 74.40, 72.92, 70.83, 69.96, 62.93, 62.90, 35.99, 20.78, 20.70, 20.49, 20.44, 20.42; MS (ESI$^+$): *m/z* = 710.1 $[M+H]^+$.

(2*R*,3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-6-(4-((3-((3*R*,4*R*)-3,4-diacetoxy-5-(acetoxymethyl)tetrahydrofuran-2-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (6d)

Yield 94%. Colorless oil; R_f = 0.41 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.79 (s, 1H), 7.34 (dd, *J* = 14.2, 8.2 Hz, 1H), 5.98 (dd, *J* = 14.3, 4.9 Hz, 1H), 5.82–5.73 (m, 1H), 5.67 (d, *J* = 9.4 Hz, 1H), 5.38–5.29 (m, 1H), 5.30 (d, *J* = 5.4 Hz, 1H), 5.29–5.20 (m, 1H), 5.22–5.12 (m, 2H), 5.08 (dd, *J* = 18.2, 14.7 Hz, 1H), 4.28 (d, *J* = 2.3 Hz, 3H), 4.20 (td, *J* = 13.1, 5.1 Hz, 1H), 4.15–4.02 (m, 1H), 3.96–3.87 (m, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.77 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.54, 170.12, 169.92, 169.59, 169.32, 168.82, 168.43, 161.64, 150.57, 143.60, 137.44, 122.13, 102.73, 88.67, 85.66, 79.78, 75.13, 73.02, 72.67, 70.25, 69.92, 67.70, 62.89, 61.60, 35.96, 20.77, 20.72, 20.55, 20.51, 20.45, 20.34, 20.13; MS (ESI$^+$): *m/z* = 782.1 $[M+H]^+$.

2-(Acetoxymethyl)-5-(3-((1-(naphthalen-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,4-dioxo-3,4 dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6e)

Yield 78%. Yellow oil; R_f = 0.28 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.76–7.73 (m, 3H), 7.66 (s, 1H), 7.47 (s, 1H), 7.45–7.39 (m, 2H), 7.28 (t, *J* = 8.2 Hz, 2H), 5.93 (d, *J* = 4.6 Hz, 1H), 5.71 (d, *J* = 8.3 Hz, 1H), 5.55 (s, 2H), 5.29–5.18 (m, 3H), 5.11 (s, 2H), 4.25 (s, 3H), 2.02 (s, 7H), 1.93 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.10, 169.57, 169.54, 161.76, 150.54, 143.29, 137.49, 133.19, 133.15, 131.98, 129.08, 127.97, 127.75, 127.45, 126.63, 125.46, 123.38, 102.71, 88.59, 79.73, 72.93, 69.95, 62.91, 54.29, 53.50, 36.19, 20.76, 20.48, 20.36; MS (ESI$^+$): *m/z* = 592.3 $[M+H]^+$.

2-(Acetoxymethyl)-5-(3-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6f)

Yield 91%. Colorless oil; R_f = 0.24 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.41 (s, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 5.94 (s, 1H), 5.73 (d, *J* = 8.2 Hz, 1H), 5.33 (s, 2H), 5.26 (s, 2H), 5.14–5.08 (m, 2H), 4.27 (s, 3H), 3.72 (s, 3H), 2.04 (s, 6H), 1.98 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.11, 169.57, 169.54, 161.78, 159.84, 150.51, 143.08, 137.50, 129.72, 126.58, 123.04, 114.41, 102.71, 88.65, 79.72, 72.94, 69.93, 62.90, 55.31, 53.63, 36.17, 20.76, 20.47, 20.40; MS (ESI$^+$): *m/z* = 572.3 $[M+H]^+$, 1143 $[2M+H]^+$.

2-(Acetoxymethyl)-5-(3-((1-((4*R*,6*S*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6g)

Yield 73%. Orange oil; R_f = 0.32 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.78 (s, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 6.00 (s, 1H), 5.91 (d, *J* = 4.6 Hz, 1H), 5.76 (d, *J* = 8.1 Hz, 1H), 5.31 (t, *J* = 5.3 Hz, 1H), 5.27–5.24 (m, 1H), 5.15 (s, 3H), 4.94 (d, *J* = 5.8 Hz, 1H), 4.44 (s, 1H), 4.28 (d, *J* = 1.7 Hz, 3H), 3.71 (d, *J* = 12.5 Hz, 1H), 3.56 (d, *J* = 12.4 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.51 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.16, 169.78, 169.62, 161.74, 150.51, 137.79, 123.70, 113.57, 102.65, 95.23, 89.02, 88.94, 85.64, 81.85, 79.72, 73.03, 69.94, 63.23, 62.87, 60.37, 35.93, 27.01, 25.09, 20.76, 20.47, 20.44; MS (ESI$^+$): *m/z* = 624.1 $[M+H]^+$, 1247.1 $[2M+H]^+$.

2-(Acetoxymethyl)-5-(2,4-dioxo-3-((1-((2*R*,4*R*,5*S*)-5-(2-oxo-2-(*p*-tolyloxy)ethyl)-4-((*p*-tolyloxy)carbonyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6h)

Yield 77%. Colorless oil; R_f = 0.60 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.86 (d, *J* = 7.0 Hz, 3H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.18 (t, *J* = 8.4 Hz, 4H), 6.38 (d, *J* = 6.7 Hz, 1H), 5.92 (d, *J* = 4.7 Hz, 1H), 5.74 (d, *J* = 8.1 Hz, 1H), 5.56 (d, *J* = 6.9 Hz, 1H), 5.28 (t, *J* = 5.4 Hz, 1H), 5.21 (d, *J* = 13.3 Hz, 2H), 5.09 (d, *J* = 14.4 Hz, 1H), 4.71 (s, 1H), 4.52 (qd, *J* = 12.2, 3.8 Hz, 2H), 4.26 (s, 3H), 3.05 (d, *J* = 15.0 Hz, 1H), 3.01–2.88 (m, 1H), 2.35 (s, 3H), 2.33 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.08, 169.54, 169.53, 166.12, 166.01, 161.70, 150.54, 144.35, 144.18, 142.95, 137.35, 129.78, 129.69, 129.30, 126.73, 126.20, 121.86, 102.71, 89.78, 88.66, 84.48, 79.70, 74.40, 72.99, 69.90, 63.93, 62.87, 38.63, 36.11, 21.74, 21.72, 20.77, 20.47, 20.42; MS (ESI$^+$): *m/z* = 804.2 $[M+H]^+$.

2-(Acetoxymethyl)-5-(3-((1-(4-nitrophenethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3,4-diyl diacetate (6i)

Yield 82%. White oil; R_f = 0.30 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.03 (d, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.24 (s, 1H), 7.12 (d, *J* = 6.6 Hz, 2H), 5.90 (d, *J* = 4.5 Hz, 1H), 5.72 (d, *J* = 8.2 Hz, 1H), 5.38–5.23 (m, 2H), 5.11 (q, 2H), 4.52 (t, *J* = 6.9 Hz, 2H), 4.29 (s, 3H), 3.24 (t, *J* = 7.2 Hz, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.18, 169.63, 169.60, 161.66, 150.42, 147.13, 144.58, 142.85, 137.82, 129.68, 123.97, 123.96, 102.53, 89.10, 79.78, 76.74, 72.96, 69.97, 62.89, 50.75, 36.43, 36.01, 20.79, 20.47, 20.47; MS (ESI$^+$): *m/z* = 601.3 $[M+H]^+$, 1201.1 $[2M+H]^+$.

(2*S*,3*R*,4*S*,5*R*,6*R*)-6-(Acetoxymethyl)-5-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4-diacetoxy-6-(acetoxymethyl)-5-(4-((3-(3,4-diacetoxy-5-(acetoxymethyl)tetrahydrofuran-2-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2,3,4-triyl triacetate (6j)

Yield 73%. White solid; mp: 130–132°C; R_f = 0.39 (cyclohexane/AcOEt 1:4); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): δ 7.73 (s, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 5.95 (d, *J* = 4.7 Hz, 1H), 5.78 (t, *J* = 8.7 Hz, 2H), 5.41–5.34 (m, 2H), 5.34–5.27 (m, 2H), 5.25 (d, *J* = 9.5 Hz, 2H), 5.13 (q, 2H), 5.00 (t, *J* = 9.9 Hz, 1H), 4.82 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.40 (dd, *J* = 12.4, 2.4 Hz, 1H), 4.28 (s, 3H), 4.18 (ddd, *J* = 12.3, 6.0, 4.3 Hz, 2H), 4.10–4.01 (m, 1H), 3.99 (dd, *J* = 12.5, 2.3 Hz, 1H), 3.91 (ddt, *J* = 7.2, 5.1, 2.8 Hz, 2H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 6H), 2.02 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 170.54, 170.49, 170.37, 170.10, 169.92, 169.88, 169.56, 169.50, 169.41, 169.11, 161.67, 150.53, 137.51, 102.68, 95.91, 88.70, 79.75, 75.28, 75.09, 72.98, 72.50, 70.87, 69.99, 69.89, 69.24, 68.73, 67.93, 62.86, 62.60, 61.47, 35.93, 20.80, 20.79, 20.76, 20.69, 20.59, 20.47, 20.44, 20.18; MS (ESI$^+$): *m/z* = 1070.1 $[M+H]^+$.

4.1.6 | General procedure for nucleoside deprotection

In a round-bottom flask equipped with a magnetic stirred bar, 3-(1,4-disubstituted-1,2,3-triazolo)uridine nucleoside (1 mmol), potassium carbonate (3 mmol), and methanol (8 mL) were added, the resulting mixture was stirred at room temperature for 3–4 h. After the completion of the reaction (TLC), the solvent was removed under pressure. The residue was dissolved in DCM (40 mL) washed with water, brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum to afford the crude product. The crude product was subjected to purification by Isco Combiflash system using flash column 12 g with a flow rate of 30 mL/min of AcOEt/cyclohexane (1:4) as eluent to give pure **6k** and **6l**.

1-((2*S*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-3-((1-((2*S*,4*R*,5*S*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)-methyl)pyrimidine-2,4(1*H*,3*H*)-dione (6k)

Yield 93%. Colorless oil; R_f = 0.11 (cyclohexane/AcOEt 1:9); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.59 (s, 1H), 7.41 (d, *J* = 10.9 Hz, 1H), 6.03 (d, *J* = 6.9 Hz, 1H), 5.95 (t, *J* = 7.7 Hz, 1H), 5.47 (d, *J* = 10.9 Hz, 1H), 4.65–4.53 (m, 3H), 4.40 (dt, *J* = 7.1, 5.8 Hz, 1H), 3.95–3.89 (m, 1H), 3.88–3.82 (m, 2H), 3.77 (dd, *J* = 12.4, 5.4 Hz, 2H), 3.52 (dd, *J* = 12.3, 5.4 Hz, 2H), 2.64–2.52 (m, 1H), 2.35–2.26 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 162.22, 149.80, 141.96, 139.12, 124.14, 102.99, 89.00, 88.76, 88.28, 83.98, 71.01, 70.93, 70.50, 62.09, 61.50, 40.19, 32.55. MS (ESI$^+$): *m/z* = 442.1 $[M+H]^+$.

1-((2*S*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3-((1-((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4-dihydroxy-6-(hydroxymethyl)-5-(((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)-methyl)pyrimidine-2,4(1*H*,3*H*)-dione (6l)

Yield 95%. Colorless oil; R_f = 0.09 (cyclohexane/AcOEt 1:9); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.67 (s, 1H), 7.38 (d, *J* = 10.9 Hz, 1H), 5.95 (d, *J* = 6.9 Hz, 1H), 5.70 (d, *J* = 6.9 Hz, 1H), 5.47 (d, *J* = 10.9 Hz, 1H), 4.60 (d, *J* = 7.3 Hz, 1H), 4.59–4.55 (m, 3H), 4.47–4.34 (m, 1H), 3.90 (t, *J* = 6.9 Hz, 2H), 3.85 (t, *J* = 7.1 Hz, 1H), 3.80 (d, *J* = 6.8 Hz, 1H), 3.79–3.74 (m, 3H), 3.72–3.68 (m, 3H), 3.62–3.58 (m, 1H), 3.52 (dd, *J* = 12.5, 4.0 Hz, 3H), 3.30 (t, *J* = 6.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 162.22, 149.80, 142.07, 139.12, 123.73, 102.99, 101.45, 89.00, 86.58, 83.98, 79.91, 76.86, 76.81, 74.65, 74.51, 73.06, 71.01, 70.93, 70.50, 69.79, 62.55, 62.01, 61.50, 32.55. MS (ESI$^+$): *m/z* = 650.2 $[M+H]^+$.

4.2 | Biology

4.2.1 | Bacteria strains and growth conditions

The antibacterial activity of synthesized molecules was evaluated against the following bacteria: *L. monocytogenes* serovar 4b CECT 4032 (Spanish Type Culture Collection: CECT), *E. coli* K12 and *S. aureus* MBLA (Laboratory of Food Microbiology, UCL, Belgium: MBLA), and *P. aeruginosa* IH (Institute of Hygiene, Rabat, Morocco: IH). Strains are maintained on an inclined agar medium at 4°C. Before use, the bacteria were revived by two subcultures in an appropriate culture medium: Lysogeny broth (LB) (Biokar Diagnostics, Beauvais, France) at 37°C for 18–24 h. For the test, final inoculums concentrations of 10^6 CFU/mL bacteria were used according to the National Committee for Clinical Laboratory Standards, USA (NCCLS 1999).

4.2.2 | MIC

MICs were determined using the broth micro-dilution assay as described.[47,48] Briefly, agar at 0.15% (w/v) was used as a stabilizer of the extract–water mixture and resazurin as a bacterial growth indicator. Fifty microliter of bacteriological agar (0.15% w/v) was distributed from the second to the eighth well of a 96-well polypropylene microtiter plate. The dilutions of synthesized molecules were prepared in Mueller-Hinton broth supplemented with bacteriological agar (0.15% w/v), to reach a final concentration of 200 µg/mL; 100 µL of these suspensions was added to the first test well of each microtiter line, and then 50 µL of scalar dilution was transferred from the second to the eighth well. The eighth well was considered as growth control, because no essential oil was added. We then added 50 µL of a bacterial suspension to each well at a final concentration of approximately 10^6 CFU/mL. The final concentration of the molecule was between 100 and 3.125 µg/mL. Plates were incubated at 37°C for 18 h. After incubation, 10 µL of resazurin was added to each well to assess bacterial growth. After further incubation at 37°C for 2 h, the MIC was determined as the lowest molecule concentration that prevented a change in resazurin color. Bacterial growth was detected by reduction in blue dye resazurin to pink resorufin. A control was carried out to ensure that, at the concentrations tested, the molecule did not induce a color change in the resazurin. Experiments were performed in triplicate.

4.2.3 | Determination of MBC

The MBC corresponded to the lowest concentration of the molecule yielding negative subcultures after incubation at appropriate temperature for 24 h. It is determined in broth dilution tests by sub-culturing 10 µL from negative wells on plate count agar (PCA) medium. All the tests were performed in triplicate.[47,48]

4.2.4 | Kinetics of bacterial growth

The growth curve assay was used to investigate the bactericidal effects of the essential oil. Inoculums were prepared by inoculating medium LB with an overnight culture of *S. aureus* and *E. coli* incubating for 3 h. One milliliter of aliquot of inoculums was added to 9 mL of medium of LB containing 0.15% of agar. The compounds **6h–j** were added to each tube to achieve final concentrations of compound of 2MIC, MIC, and MIC/2. The bacterial culture used without compounds was considered as negative control. The tubes were incubated at 37°C. At selected time intervals, the OD_{600} of supernatants were determined by UV-Vis spectrophotometer. All measurements were carried out in triplicate. Through the assay above, the growth curve of *E. coli* and *S. aureus* was ordered, the time as the horizontal axis, the OD_{600} of supernatant as the vertical axis.[49]

4.2.5 | Integrity of cell membrane by leakage of DNA and RNA through the membrane of bacteria

The integrity of cell membrane could be monitored by the release of cytoplasmic constituents of the cell.[50] The experiments were designed as follows: The bacteria were incubated in NB medium at 37°C for 12 h. Logarithmic growth phase cells of bacteria were treated with the essential oil at 1 × MIC, 2 × MIC value except the control. Then the samples were incubated at 37°C for 1, 4, 8, and 24 h, respectively. For detecting genetic material (DNA and RNA) released from the cytoplasm, 1 mL sample of each tube was centrifuged at 1200×*g* for 5 min to remove all trace of bacteria. The supernatant was re-suspended in PBS and used to measure UV 260 nm absorption by a visible spectrophotometer at each time point. We used untreated bacteria as negative control.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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