Synthesis of new series of pyrimidine nucleoside derivatives bearing the acyl moieties as potential antimicrobial agents

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Abstract

Nucleoside derivatives are important therapeutic drugs and are the focal point in the ongoing search for novel, more potent drug targets. In this study, a new series of pyrimidine nucleoside i.e., uridine (1) derivatives were synthesized via direct method and evaluated for their antimicrobial potential activity. The title compound uridine (1) was treated with triphenylmethyl chloride in pyridine to give the 5´-O-(triphenylmethyl)uridine derivative (2), which was subsequently derivatized to create a series of 2´,3´-di-O-acyl analogs containing a wide variety of functionalities in a single molecular framework. In vitro antimicrobial functionality tests were determined against both human and plant pathogens by disc diffusion and food poisoned techniques. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral, analytical, physicochemical data. The antimicrobial results indicated that the synthesized derivatives exhibited moderate to good antibacterial and antifungal activity; in particular, they were found to be more effective against fungal phytopathogens than against human bacterial strains. Compounds 7, 9, and 14 were of particular interest as they exhibited noteworthy antifungal and antibacterial properties. In vitro MTT assays revealed that compound 9 was effective against Ehrlich’s ascites carcinoma (EAC) cells, resulting in 7.12% and 1.34% cell growth inhibition at concentrations of 200 and 6.25 µg/ml, respectively. The IC₅₀ value for compound 9 was rather high and found to be 1956.25 µg/ml. Structure-activity relationship (SAR) studies were also conducted to predict structural and pharmacokinetic properties. The findings of this study indicate that the different uridine derivatives are potentially useful antimicrobial agents for the advancement of future pharmaceutical research.

Keywords

uridine, synthesis, antimicrobial, anticancer, SAR studies
Introduction

Nucleosides and their analogs are an important class of clinically useful medicinal agents that possess antiviral and anticancer activities. Additionally, they have been shown to be effective antibacterial agents with moderate to good activity against certain bacterial strains (Rachakonda and Cartee 2004). For these reasons, they are of keen interest in the search for novel nucleoside derivatives with broad-spectrum biological activity.

Nucleosides belong to a class of organic compounds that possess a nitrogen-containing heterocyclic nucleobase and a C-5 sugar as ribose or deoxyribose in their structure. The nucleobase is bound to the C-5 sugar (anomeric carbon) via a β-N-glycosidic linkage (King 2006). Phosphorylation on the primary hydroxyl group of the sugar moiety results in the formation of nucleotides, which are the building blocks of DNA and RNA. Nucleosides and nucleotide derivatives are necessary for life as they are integral in a variety of cell metabolism and regulation processes. General nucleotide chemistry and investigations using both purine and pyrimidine nucleosides have contributed substantially to the discovery and elucidation of countless biological processes at the molecular level (Cooperwood et al. 2002; Damaraju et al. 2012; Kukhanova 2012).

The nucleoside, uridine (1) (Fig. 1) is an essential component in RNA synthesis and plays an important role in the synthesis of glycogen. In addition, it contributes to the synthesis of bio-membranes via the formation of pyrimidine-lipid conjugates. Upon digestion of foods containing RNA, uridine is released from the RNA molecule and is absorbed intact in the gut. Uridine acts as an antidepressant, alleviates asthmatic airway inflammation, and is key in hepatocyte proliferation (Murata et al. 2004; Xiao et al. 2014). Uridine is often administered as part of a cancer treatment regime to minimize the adverse effects of chemotherapy drugs like 5-fluorouracil (Groeningen et al. 1986). Furthermore, a combination of uridine and benzylcyclouridine was shown to reduce neurotoxicity and bone marrow toxicity related to the drug zidovudine in the treatment of HIV (Morris 1994). Despite these advances, the availability of novel, effective nucleoside derivatives for pharmaceutical purposes is still lacking, and research on this field remains of utmost importance (Jordheim et al. 2013). To this end, a number of fruitful and efficient methods for selective acylation have been reported, in which a variety of acylating agents and reaction conditions were utilized to attain the desired derivatives in good yields (Itoh et al. 1975; Tsuda and Haque 1983). Numerous methods for the acylation of carbohydrates and nucleosides have, so far, been developed and successfully employed (Ishii et al. 1980; Andary et al. 1982; Kabir et al. 2005).

Evaluation of the antimicrobial properties of nucleosides that had been subjected to selective acylation methods (Jesmin et al. 2017; Devi et al. 2019) has revealed that N-, S-, and X-containing substitution products showed markedly better antimicrobial and biological activity than their parent compound (Kawser et al. 2014; Kabir et al. 2004). Encouraged by literature reports and our own findings (Kawser et al. 2015; Mirajul et al. 2019; Shagir et al. 2016), we focused on synthesizing a series of uridine derivatives (Scheme 1) that deliberately incorporated a wide variety of biologically active components into the ribose moiety. This was done in the hope of finding new antibacterial and antifungal potential agents. The in vitro antimicrobial minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and Minimum fungicidal concentrations (MFC), antitumor, and SAR characteristics of these newly synthesized uridine derivatives are reported herein for the first time.

![Scheme 1. Reagents and conditions: (a) dry Py, (C,H,)OC,O, 5 °C, 6 h, (70%; R = 0.52); (b) dry Py, various acyl halides (3–14), 0 °C to rt, DMAP, 6 h.](image)

**Scheme 1.** Reagents and conditions: (a) dry Py, (C,H,)OC,O, 5 °C, 6 h, (70%; R = 0.52); (b) dry Py, various acyl halides (3–14), 0 °C to rt, DMAP, 6 h.

**Experimental part**

**Materials and methods**

Uridine and all reagents used in this study are commercially available from Sigma-Aldrich, and they were used as received, unless otherwise specified. Melting points were determined on an electrothermal melting point apparatus (England) and were uncorrected. Evaporations were carried out under reduced pressure using a VV-1 type vacuum rotary evaporator (Germany), and the temperature of the evaporator’s water bath was kept below 40 °C during our experiments. FTIR spectra were recorded on KBr disks at the Chemistry Department, University of Chittagong, Bangladesh, using an IR Affinity Fourier Transform Infrared Spectrophotometer (Shimadzu, Japan). Spectroscopic data were recorded at Wazed Miah Science Research Centre (WMSRC), Jahangirnagar University,
Bangladesh. Mass spectra of the synthesized compounds were measured via liquid chromatography electrospray ionization-tandem mass spectrometry in positive ionization mode (LC-(ESI+)-MS/MS) using a JASSO system. Thin layer chromatography (TLC) was performed on Kieselgel GF₅₄, and the spots were visualized with a 1% H₂SO₄ solution, followed by heating to temperatures between 150 °C and 200 °C. Column chromatography was performed with silica gel G₂₅ (Sigma-Aldrich).

Chemistry

Synthesis of uridine derivatives

Synthesis of nucleosides and their analogs began in 1948 by Davoll et al because of their biological importance (Davoll et al. 1948). Our laboratory has already synthesized nucleoside derivatives bearing various acyl groups to explore their antimicrobial properties (Kawsar et al. 2015; Jesmin et al. 2017).

A solution of uridine (1) (200 mg, 0.82 mmol) in anhydrous pyridine (3 mL) was cooled to 0 °C before the addition of the respective acyl chlorides (3–14) (234 mg) as a semi-solid mass that could not be crystallized; propionyl chloride (1.1 mL, 3.5 mol eq.), compound 3 (133 mg); butyryl chloride (0.13 mL, 3.5 mol eq.), compound 4 (133 mg); heptanoyl chloride (1.2 mL, 3.5 mol eq.), compound 5 (142.76 mg); octanoyl chloride (0.24 mL, 3.5 mol eq.), compound 6 (81.3 mg); nonanoyl chloride (0.37 mL, 3.5 mol eq.), compound 7 (148.5 mg); lauroyl chloride (0.37 mL, 3.5 mol eq.), compound 8 (184.6 mg); myristoyl chloride (0.48 mL, 3.5 mol eq.), compound 9 (193.8 mg); palmitoyl chloride (0.43 mL, 3.5 mol eq.), compound 10 (158.8 mg); 3-chlorobenzoyl chloride (0.26 mL, 3.5 mol eq.), compound 11 (169 mg); 3-bromobenzoyl chloride (0.19 mL, 3.5 mol eq.), compound 12 (92 mg); 3-chloro benzyl chloride (0.21 mL, 3.5 mol eq.), compound 13 (150 mg); and dichloroacetyl chloride (0.19 mL, 3.5 mol eq.), compound 14 (234 mg). The purity of compounds was checked with TLC (Lu et al. 2014).

To a solution of the diol, the triphenylmethyl derivative 2 (270 mg, 0.555 mmol) in anhydrous pyridine (3 mL) was cooled to 0 °C before the addition of the respective acyl chloride (3.5 mol eq.), followed by a catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred at 0 °C for 6 to 7 h and then left overnight at room temperature under constant stirring. TLC analysis (CHCl₃/CH₂OH, 5:1, v/v) indicated that there was complete conversion of the starting material into a single product. A few pieces of ice were added to the reaction flask to quench the reaction, and the mixture was processed as usual. Percollation of the resulting syrup through a silica gel column with CHCl₃/CH₂OH (5:1, v/v) as the eluent afforded the respective derivative. The acyl chlorides used in this synthetic route and their respective products are listed: acetyl chloride (0.14 mL, 3.5 mol eq.) produced the acetyl derivative 3 (234 mg) as a semi-solid mass that could not be crystallized; propionyl chloride (1.1 mL, 3.5 mol eq.), compound 4 (133 mg); butyryl chloride (0.13 mL, 3.5 mol eq.), compound 5 (142.76 mg); heptanoyl chloride (1.2 mL, 3.5 mol eq.), compound 6 (81.3 mg); octanoyl chloride (0.24 mL, 3.5 mol eq.), compound 7 (148.5 mg); lauroyl chloride (0.37 mL, 3.5 mol eq.), compound 8 (184.6 mg); myristoyl chloride (0.48 mL, 3.5 mol eq.), compound 9 (193.8 mg); palmitoyl chloride (0.43 mL, 3.5 mol eq.), compound 10 (158.8 mg); 3-chlorobenzoyl chloride (0.26 mL, 3.5 mol eq.), compound 11 (169 mg); 3-bromobenzoyl chloride (0.19 mL, 3.5 mol eq.), compound 12 (92 mg); 3-chloro benzyl chloride (0.21 mL, 3.5 mol eq.), compound 13 (150 mg); and dichloroacetyl chloride (0.19 mL, 3.5 mol eq.), compound 14 (234 mg).
CH₂OH, 16.1%, v/v). FTIR (ν (max cm⁻¹): 1738 (–CO). ¹H NMR (CDCl₃, 400 MHz, δ in ppm): 9.02 (1H, s, –NH), 7.74 (1H, d, J = 7.6 Hz, H-6), 7.53 (6H, m, Ar-H), 7.31 (9H, m, Ar-H), 6.31 (1H, d, J = 5.6 Hz, H-´1), 5.97 (1H, dd, J = 2.4 and 12.4 Hz, H-5´a), 5.88 (1H, dd, J = 2.2 and 12.4 Hz, H-5´b), 5.75 (1H, d, J = 7.9 Hz, H-5´), 5.58 (1H, d, J = 5.4 Hz, H-2´), 4.69 (1H, dd, J = 7.8 and 5.8 Hz H-3´), 4.57 (1H, m, H-4´), 2.87 (4H, m, 2 × CH₂CH₂CH₂CO–), 1.73 (4H, m, 2 × CH₂CH₂CH₂CO–), 1.02 (6H, d, 2 × CH₂(CH₃)₂CO–), 0.93 (6H, m, 2 × CH₂(CH₃)₂CO–). LC-ESI+-MS/MS: m/z [M+H⁺] 627.01. Anal calc for C₇₀H₆₆N₃O₇S (M.W.: 626.07 g/mol) calculated (%): C:69.00, H:6.07; found: C:69.05, H:6.10.

2³-Di-O-heptanoyl-5´-O-(triphenylmethyl)uridine (6). Yield 76.42%, m.p. = 108–109 °C, Rf = 0.54 (CHCl₃/CH₂OH, 19:1, v/v). FTIR (ν (max cm⁻¹): 1708 (–CO). ¹H NMR (CDCl₃, 400 MHz, δ in ppm): 9.0 (1H, s, –NH), 7.65 (1H, d, J = 7.8 Hz, H-6), 7.50 (6H, m, Ar-H), 7.29 (9H, m, Ar-H), 6.22 (1H, d, J = 5.6 Hz, H-1´), 5.64 (1H, dd, J = 2.0 and 12.0 Hz, H-5´a), 5.70 (1H, dd, J = 2.0 and 12.0 Hz, H-5´b), 5.50 (1H, d, J = 5.5 Hz, H-2´), 4.67 (1H, dd, J = 7.6 and 5.6 Hz H-3´), 4.60 (1H, m, H-4´), 2.84 (4H, m, 2 × CH₂(CH₃)₂CO–), 1.70 (4H, m, 2 × CH₂CH₂CH₂CO–), 1.51 (12H, m, 2 × CH₂(CH₃)₂CO–), 0.96 (6H, m, 2 × CH₂(CH₃)₂CO–). LC-ESI+-MS/MS/MS: m/z [M+H⁺] 711.0. Anal calc for C₇₀H₆₆N₃O₇S (M.W.: 710.09 g/mol) calculated (%): C:70.99, H:7.04; found: C:71.01, H:7.08.

2³,3³-Di-O-octanoyl-5´-O-(triphenylmethyl)uridine (7). Yield 75.34%, m.p. = 144–145 °C, Rf = 0.53 (CHCl₃/CH₂OH, 20:1, v/v). FTIR (ν (max cm⁻¹): 1684 (–CO). ¹H NMR (CDCl₃, 400 MHz, δ in ppm): 8.72 (1H, s, –NH), 7.56 (1H, d, J = 7.7 Hz, H-6), 7.48 (6H, m, Ar-H), 7.29 (9H, m, Ar-H), 6.20 (1H, d, J = 5.6 Hz, H-1´), 7.60 (1H, m, H-5´a), 5.81 (1H, m, H-5´b), 5.70 (1H, d, J = 8.2 Hz, H-5´), 5.56 (1H, m, H-2´), 4.84 (1H, m, H-3´), 4.65 (1H, m, H-4´), 2.38 (4H, m, 2 × CH₂(CH₃)₂CO–), 1.66 (4H, m, 2 × CH₂(CH₂CH₂CO–), 1.27 (16H, m, 2 × CH₂(CH₃)₂CO–), 0.93 (6H, m, 2 × CH₂(CH₃)₂CO–). LC-ESI+-MS/MS/MS: m/z [M+H⁺] 739.1. Anal calc for C₇₀H₆₆N₃O₇S (M.W.: 738.16 g/mol) calculated (%): C:71.55, H:7.32; found: C:71.57, H:7.35.

2³,3³-Di-O-lauroyl-5´-O-(triphenylmethyl)uridine (8). Yield 81.61%, m.p. = 125–127 °C, Rf = 0.52 (CHCl₃/CH₂OH, 17:1, v/v). FTIR (ν (max cm⁻¹): 1710 (–CO). ¹H NMR (CDCl₃, 400 MHz, δ in ppm): 9.02 (1H, s, –NH), 7.84 (2H, m, Ar-H), 7.54 (4H, m, Ar-H), 7.49 (6H, m, Ar-H), 7.33 (9H, m, Ar-H), 7.31 (2H, m, Ar-H), 7.28 (1H, d, J = 7.6 Hz, H-6), 6.18 (1H, d, J = 5.6 Hz, H-1´), 6.08 (1H, m, H-5´a), 5.75 (1H, dd, J = 2.0 and 12.0 Hz, H-5´b), 5.65 (1H, d, J = 8.2 Hz, H-5´), 5.50 (1H, m, H-2´), 4.65 (1H, m, H-3´), 4.35 (1H, m, H-4´). LC-ESI+-MS/MS/MS: m/z [M+H⁺] 852.81. Anal calc for C₇₀H₆₆N₃O₇S (M.W.: 851.87 g/mol) calculated (%): C:59.18, H:3.79; found: C:59.18, H:3.79.

2³,3³-Di-O-(2-bromobenzoyl)-5´-O-(triphenylmethyl)uridine (II). Yield 84.21%, m.p. = 117–119 °C, Rf = 0.51 (CHCl₃/CH₂OH, 21:1, v/v). FTIR (ν (max cm⁻¹): 1718 (–CO). ¹H NMR (CDCl₃/TMS, 400 MHz, δ in ppm): 8.90 (1H, s, –NH), 7.84 (2H, m, Ar-H), 7.54 (4H, m, Ar-H), 7.49 (6H, m, Ar-H), 7.33 (9H, m, Ar-H), 7.31 (2H, m, Ar-H), 7.28 (1H, d, J = 7.6 Hz, H-6), 6.18 (1H, d, J = 5.6 Hz, H-1´), 6.08 (1H, m, H-5´a), 5.75 (1H, dd, J = 2.0 and 12.0 Hz, H-5´b), 5.65 (1H, d, J = 8.2 Hz, H-5´), 5.50 (1H, m, H-2´), 4.65 (1H, m, H-3´), 4.35 (1H, m, H-4´). LC-ESI+-MS/MS/MS: m/z [M+H⁺] 852.80. Anal calc for C₇₀H₆₆N₃O₇S (M.W.: 851.87 g/mol) calculated (%): C:59.17, H:3.76; found: C:59.20, H:3.77.

2³,3³-Di-O-(3-chlorobenzoyl)-5´-O-(triphenylmethyl)uridine (13). Yield 72.51%, m.p. = 129–131 °C, Rf = 0.52 (CHCl₃/CH₂OH, 21:1, v/v). FTIR (ν (max cm⁻¹): 1739 (–CO). ¹H NMR (CDCl₃/TMS, 400 MHz, δ in ppm): 8.84 (1H, s, –NH), 7.84 (2H, d, J = 7.5 Hz, Ar-H), 7.58 (2H, s, Ar-H),
Antimicrobial screening studies

Antibacterial and antifungal activity tests were conducted against standard strains. The American Type Culture Collection (ATCC) and NCTC strains of the microorganisms used in this study were obtained and maintained at the Department of Microbiology, University of Chittagong. The following reference strains were used for testing antimicrobial activity: Gram-positive bacteria: Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 6538, Gram-negative bacteria: Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Salmonella aborty NCTC 6017 and Fungus: Candida albicans ATCC 10231. The test compounds were subjected to antibacterial and antifungal screening studies as shown in Scheme 1.

Screening of antibacterial activity

The disk diffusion method was used to check in vitro sensitivity of bacteria to the test materials (Bauer et al. 1966). Mueller Hinton agar media was distributed in sterilized petri dishes. A bacterial suspension (0.1 mL) and about 15 to 20 mL of agar media were added to the petri dish. Paper disks (5 mm in diameter) that had been soaked with the test chemicals (20 µL/disk) were set aside for antibacterial analysis. To perform the sensitivity spectrum analysis, the agar medium plates were uniformly selected with the test organisms, and the disks were prepared with a given amount of the test chemicals. A disk containing each of the solvent systems was used as the experimental control (C). These plates are then kept at a low temperature (4 °C) for 2 to 4 h to allow for maximum diffusion of the compounds. During this time, the dried disks absorbed water from the surrounding media. The test materials then went into solution and were diffused throughout the media. Diffusion occurred according to the physical laws that govern the diffusion of molecules through agar gels. The plates were then incubated at 37 °C for 24 h in an inverted position to allow for maximum growth of the microorganisms. After incubation, the notable “Zones of Inhibition” (i.e., distinct zones surrounding the disks that contained no microbial growth) were observed and measured. The diameter of the transparent scale included the diameter of the disks and of each experiment itself, and the experimentation was done in triplicates. All of the results were compared against the standard antibiotic azithromycin (Beximco Pharmaceuticals Ltd., Bangladesh).

Determination of MIC and MBC

The minimum inhibition concentrations (MIC) and minimum bactericidal concentrations (MBC) of the compounds that showed activity against the aforementioned organisms were determined by applying different concentrations of the compounds alongside the same bacterial loads in a nutrient broth. MIC and MBC were determined via the broth microdilution method (Amsterdam 2005).

Screening of the mycelial growth

The “poisoned food” technique (Grover RK, Moore 1962) was used to screen for antifungal activity in which potato dextrose agar (PDA) was used as the culture medium. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to a 1% (w/v) concentration. From this, a sterilized pipette was used to transfer 0.1 mL (containing 1 mg of the respective compound being tested) to a sterile petri dish, after which 20 mL of the medium was poured into the petri dish and allowed to solidify. Inoculation was performed at the center of each petri dish with a 5-mm mycelium block of each fungus. The mycelium block was prepared by applying a corkscrew to the growing area of a 5-day-old culture of the test fungi on PDA. The blocks were placed at the center of each petri dish in an inverted position to maximize contact between the mycelium and the culture medium. The inoculation plates were incubated at 25 °C ± 2 °C, and the experiment was conducted in triplicate. A control sample (i.e., PDA without any test chemicals applied) was also maintained under the same conditions. After 5 days of incubation, the diameter of the fungal radial mycelia growth was measured. The average of three measurements was taken as the radial mycelia growth of the fungus in mm. The percentage inhibition of mycelia growth of the test fungus was calculated as follows:

\[ I = \left( \frac{C - T}{C} \right) \times 100 \]

where I is the percentage of inhibition, C represents the diameter of the fungal colony in the control (DMSO), and T is the diameter of the fungal colony during treatment. The results obtained were compared with those of the standard antifungal agent nystatin.

Determining MFC

MFC were also assessed by testing various concentrations of the derivatives against fungal cultures.
Screening of anticancer activity

MTT colorimetric assay

In this study, adult Swiss albino mice were obtained from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Cells were harvested from the mice, and their viability was checked using the trypan blue exclusion assay. In *vivo* proliferation of Ehrlich’s ascites carcinoma (EAC) cells was performed according to the method reported by Ahmed (Ahmed et al. 2017) MTT colorimetric assay was used to detect the *in vitro* proliferation of EAC cells. Viable EAC cells (5 × 10⁵ in 200 μL RPMI-1640 media) were placed in a 96-well flat-bottom culture plate in the presence and absence of different concentrations (12.5–200 μg/mL) of uridine derivatives under investigation and incubated at 37 °C in a CO₂ incubator for 24 h. After removal of the aliquot from each well, 10 mM of PBS (180 μL) and MTT (20 μL, 5 mg/mL MTT in PBS) was added, and the plate was incubated at 37 °C for 4 h. The aliquot was removed again, and 200 μL of acidic isopropanol was added to each well. The plate was agitated for 5 min and incubated at 37 °C for 1 h before absorbance values were measured at 570 nm using a titer plate reader. The cell proliferation inhibition ratio was calculated as follows:

Proliferation inhibition ratio (%) = [(A – B) × 100]/A

where A is the OD₅₇₀ nm of the cellular homogenate (control) without the derivative and B is the OD₅₇₀ nm of the cellular homogenate with the derivative added.

Structure-activity relationship (SAR) studies

Structure-activity relationship (SAR) studies can be used to predict a biological activity from the molecular structure of a pharmaceutical target. This powerful technology is often used in drug discovery processes to guide the acquisition or synthesis of desirable new compounds and to characterize existing molecules. Here SAR assays were performed according to the Kim (Kim et al. 2007) and Hunt (Hunt 1975) membrane permeation concept.

Statistical analysis

For each parameter investigated, experimental results were presented as mean ± standard error for three replicates. Two-tailed Student’s *t*-tests were used as appropriate for statistical analysis. Only *p* values that were less than 0.05 were considered to be statistically significant.

Results and discussion

Synthesis

Although research into the synthesis of nucleosides started in the middle of the nineteenth century (Davoll et al. 1948), their preparation is still a particularly challenging and attractive target for the pharmaceutical community because of their promising pharmacological profiles. The main aim of the research presented in this paper was to carry out selective derivatization of uridine (1) with the appropriate acyl halide using a direct method (Scheme 1).

Synthesis of 5´-O-(triphenylmethyl)uridine (2)

Uridine was initially converted to the 5´-O-(triphenylmethyl)uridine derivative 2 via treatment with triphenylmethyl chloride. After the usual workup and purification procedures were performed, compound 2 was obtained in 83.51% yield as needles, m.p. 115–117 °C. The structure of the triphenylmethyl derivative 2 was established by analyzing its elemental data, FTIR, ¹H-NMR, and mass spectra. Compound 2 was deemed sufficiently pure for use in the next step without the need for additional purification procedures. The following characteristic peaks were observed in the FTIR spectrum: 1701 cm⁻¹ (–CO) and between 3416 and 3468 cm⁻¹ (br. –OH str.). In its ¹H-NMR spectrum, two characteristic six-proton multiplets at δ 7.51 (Ar-H) and the nine proton multiplets observed at δ 7.31 (Ar-H) were attributed to three phenyl protons of the trityl group of the molecule. The downfield shift of C-5´ proton to δ 5.93 (as dd, *J* = 2.2 and 12.2 Hz, 5´a) and 5.76 (as dd, *J* = 2.3 and 12.4, 5´b) from their usual values (Kabir et al. 1997) in the precursor compound 1 and the resonances of the other protons in their anticipated positions were indicative of the presence of the triphenylmethyl group at position 5´. The formation of 5´-O-(triphenylmethyl)uridine (2) might be due to increased reactivity of the sterically less hindered primary hydroxyl group of the ribose moiety on uridine (1). The reactivity of the–OH groups follows the sequence 5-OH > 2-OH ≥ 3-OH. The mass spectrum of compound 2 contained a molecular ion peak at *m/z* [M+H]⁺ 487.10 that corresponded to the same molecular formula C₂₄H₂₂O₅N₂. After analysis of elemental and spectral data, the structure of this compound was assigned as 5´-O-(triphenylmethyl)uridine (2).

Synthesis of 2´,3´-di-O-acetyl derivatives (3–14) of 5´-O-(triphenylmethyl) uridine (2)

Several derivatives of the triphenylmethylated product were also prepared for structure elucidation purposes and to obtain novel derivatives of synthetic and biological importance. The two free –OH groups at C-2 and C-3 were subjected to further acylation, which provided further confirmation of the structure of 5´-O-(triphenylmethyl) uridine (2). Treatment of compound 2 with acetic anhydride in anhydrous pyridine in the presence of the catalyst DMAP, followed by removal of the solvent and subsequent column chromatography, provided the di-O-acetyl derivative (3) in 87.12% yield as a crystalline solid. The FTIR spectrum revealed that three carbonyl (–CO) stretching absorption bands were observed at 1738, 1710, and 1652 cm⁻¹.
In its ¹H NMR spectrum, the two three-proton singlets at δ 2.15 and δ 2.10 were due to the methyl protons of two acetyloxy groups. Downfield shifts of H-2’ and H-3’ to δ 5.30 (as d, J = 5.4 Hz) and δ 5.01 (as dd, J = 7.2 and 5.1 Hz), when compared to the precursor compound 2 (δ 4.25, d, J = 5.8 Hz, H-2’; δ 4.11, dd, J = 7.6 and 5.6 Hz, H-3’), indicated the attachment of the acetyl groups at positions 2’ and 3’. Mass spectrometry provided a molecular ion peak at m/z [M+H]+ 571.08, which corresponded to the aforementioned molecular formula. The structure of the acetyl derivative was confidently established as 2’,3’-di-O-acetyl-5’-O-(triphenylmethyl)uridine (3) by analyzing the accompanying FTIR, ¹H NMR, mass spectra, and elemental data.

Direct propionylation of compound 2 using propionyl chloride in anhydrous pyridine furnished the propionyl derivative 4 in good yield as needles, m.p. 109–110°C. The presence of two two-proton quartets at δ 2.90 and δ 2.84 [2 × CH₂CH₂CO–] as well as two three-proton triplets at δ 1.23 and δ 1.11 [2 × CH₃CH₂CO–] in its ¹H NMR spectrum indicated the presence of two propionyl groups in the molecule. The considerable deshielding effect exerted on the H-2’, and H-3’ protons that led to a shift to δ 5.50 (as d, J = 5.4 Hz) and δ 4.62 (as dd, J = 7.8 and 5.8 Hz) from their precursor 2 values (δ 4.25) and (δ 4.11) was indicative of the introduction of two propionyl groups at the 2’ and 3’ positions.

Similarly, derivatization of triphenylmethylate 2 using fatty acid chlorides, such as butyryl chloride, heptanoyl chloride, octanoyl chloride, lauroyl chloride, myristoyl chloride, and palmitoyl chloride, led to the corresponding acyl derivatives (5–10) in good yields. Analysis of their elemental and spectra provided confirmation that the corresponding 2’,3’-di-O-substitution products had been formed. Finally, the triphenylmethyl derivative 2 was easily transformed into 2’,3’-di-O-(2-bromobenzoate) 11, 2’,3’-di-O-(3-bromobenzoate) 12, 2’,3’-di-O-(3-chlorobenzoate) 13, and 2’,3’-di-O-dichloroacetyl 14. Spectroscopic and elemental data for these compounds supported the proposed structures for these derivatives.

### Antimicrobial evaluation of uridine derivatives

### Determining the antibacterial activity

The results of the in vitro antibacterial screening of the test compounds and a standard antibiotic, namely, azithromycin, are listed in Table 1 as well as in Fig. 2.

From the results, it was evident that compound 14 showed the maximum inhibitory activity against both B. subtilis (22 ± 0.3 mm) and S. aureus (14 ± 0.37 mm). The activity of compound 7 was also quite similar to that of compound 14 against these microbes. Compounds 1, 2, 3, 10, 12, and 13 were inactive, whereas compounds 4, 8, and 9 showed increased potency against Gram-positive microorganisms. In accordance with the results obtained from the primary screening data for the test compounds, compound 9 showed the most extensive inhibition against E. coli and S. abony (18 ± 0.39 and 20 ± 0.41 mm, respectively). Compound 6 was found to have the lowest activity of the lot. On the other hand, antibacterial activity test results for the Gram-negative bacterium P. aeruginosaa indicated that the greatest activity was observed for compound 14 at 17 ± 0.42 mm. A significant and reasonable amount of inhibition phenomena was observed for compounds 3, 5, 7, and 10. However, no inhibition was seen against the tested pathogens for compounds 11 and 13. Compounds 7 and 10 exhibited moderate inhibition against P. aeruginosaa, which was comparable to that of the azithromycin standard. It should also be noted that the obtained inhibition results were in line with our previous research works (Devi et al. 2019; Kawsar et al. 2018).

Compounds which had greater zones of inhibition, namely, 7, 9, and 14, were subjected to further analyses (determination of MIC, MBC, and MFC) to test their activity against other commonly occurring microbes. These results are presented in Figs 3, 4. From the MIC values, it can be seen that compound 7 had the highest MIC values (2.50 mg/ml) against S. abony but the lowest value against E. coli, namely, 0.625 mg/ml. Compound 9 had a small MIC value of 0.625 mg/mL against B. subtilis. Since

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### Table 1. Zone of inhibition of the synthesized compounds 2–14 against Gram-positive and Gram-negative bacteria.

| Compound | B. subtilis (+ve) | S. aureus (+ve) | E. coli (-ve) | S. abony (-ve) | P. aeruginosaa (-ve) |
|----------|------------------|-----------------|---------------|----------------|----------------------|
|          | Diameter of zone of inhibition (mm) |               |               |                |                      |
| 2        | NI               | NI              | 12 ± 0.41     | NI             | 11 ± 0.35            |
| 3        | NI               | NI              | 10 ± 0.34     | 9 ± 0.28       | 12 ± 0.42            |
| 4        | 10 ± 0.5         | 8 ± 0.3         | 13 ± 0.35     | NI             | NI                   |
| 5        | NI               | 7 ± 0.25        | 10 ± 0.33     | 9 ± 0.27       | 10 ± 0.19            |
| 6        | NI               | 11 ± 0.31       | 9 ± 0.23      | 10 ± 0.34      | 8 ± 0.38             |
| 7        | 21 ± 0.41        | 13 ± 0.31       | 11 ± 0.29     | 13 ± 0.31      | *16 ± 0.39           |
| 8        | 14 ± 0.26        | 10 ± 0.23       | NI            | 7 ± 0.18       | NI                   |
| 9        | 10 ± 0.33        | 11 ± 0.36       | *18 ± 0.39    | *20 ± 0.41     | NI                   |
| 10       | NI               | NI              | 11 ± 0.31     | 12 ± 0.34      | *16 ± 0.37           |
| 11       | 13 ± 0.4         | NI              | NI            | NI             | NI                   |
| 12       | NI               | NI              | 10 ± 0.27     | 10 ± 0.4       | NI                   |
| 13       | NI               | NI              | NI            | NI             | NI                   |
| 14       | 22 ± 0.3         | 14 ± 0.37       | 12 ± 0.39     | 13 ± 0.35      | *17 ± 0.42           |

Azithromycin 19 ± 0.4 18 ± 0.31 17 ± 0.39 19 ± 0.38 17 ± 0.39

Data are presented as mean ± SD. Values are represented for triplicate experiments. Statistically significant inhibition (p < 0.05) is marked with an asterisk (*) for test compounds and a double asterisk (**) for the reference antibiotic azithromycin.

NI = No inhibition, (+ve) = Gram-positive, (-ve) = Gram-negative.
compound 9 did not show any inhibition activity in the primary screening test against *P. aeruginosa*, no MIC data could be determined. For compound 14, significant MIC values (0.625 mg/ml) were noted for *B. subtilis*, *S. aureus*, and *S. abony*, whereas the lowest value (0.3125 mg/ml) was seen for *E. coli*.

The MBC result data in Fig. 4 revealed that compounds 7 and 9 had the highest MBC values against *S. abony* (5 mg/mL). On the other hand, both compounds had similar MBC values (2.5 mg/mL) against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*. Compound 14 showed the greatest activity against *S. abony* since it had the smallest MBC value (1.25 mg/mL). The MBC values reported were the same for all of the tested organisms, except *P. aeruginosa*.

From the MIC and MBC data analysis, it can be inferred that compounds 7, 9, and 14 could be used as antibacterial drugs against the aforementioned microbes. However, further investigation is needed to ascertain their mode of action and possible associated side effects.

**Determining antifungal activity**

The antifungal screening test results (Table 2 and Fig. 5) indicate that compound 7 showed maximum mycelial growth inhibition against *A. niger* (99% ± 1.9%) and C.
**Figure 3.** MIC values for compounds 7, 9, and 14.

**Figure 4.** MBC data for compounds 7, 9, and 14.

**Figure 5.** Percentage zone of mycelial growth inhibition for compounds 3 and 11 against *A. niger* (A and B, respectively). DMSO represents the negative control, whereas nystatin is the positive control.

**Table 2.** Mycelial growth inhibition (%) by the test compounds.

| Compound | *Aspergillus niger* | Candida albicans |
|----------|--------------------|-----------------|
| 2        | NI                 | NI              |
| 3        | 99.0 ± 1.9         | 70.0 ± 1.4      |
| 4        | 44.28 ± 0.9        | NI              |
| 5        | NI                 | NI              |
| 6        | 55.17 ± 1.7        | NI              |
| 7        | *66.67 ± 1.8       | NI              |
| 8        | 48.05 ± 0.9        | 45.0 ± 0.9      |
| 9        | NI                 | 65.0 ± 1.2      |
| 10       | 53.57 ± 1.2        | 52.0 ± 1.1      |
| 11       | 58.33 ± 1.6        | 53.0 ± 1.3      |
| 12       | *70.37 ± 1.8       | 59.0 ± 1.3      |
| 13       | 51.54 ± 1.1        | NI              |
| 14       | 52.50 ± 1.2        | *65.0 ± 1.5     |
| Nystatin | **66.4 ± 1.8**     | **63.1 ± 1.9**  |

Data are presented as mean ± SD. Values are represented for triplicate experiments. Statistically significant inhibition (*p* < 0.05) is marked with an asterisk (*) for test compounds and a double asterisk (**) for the reference antibiotic nystatin. NI = No inhibition.

*albicans* (70% ± 1.4%), which far exceeded the results seen for the standard. However, compound 3 (66.67 ± 1.8) and 12 (70.37 ± 1.8) exhibited moderate inhibition against *A. niger* while compound 6, 8, 10, 11, and 13 displayed a significant inhibition. The compound 14 (65.0 ± 1.5) exhibited reasonable potentiality, compounds 9, 10, 11, and 12 reported great inhibition on the other hand compound 8 revealed lowest inhibitions. No inhibition was observed for compounds 2 and 5 against both fungal pathogens.

Since compounds 7 and 14 showed greater mycelial growth inhibition against *C. albicans*, these two compounds were subjected to further MIC and MFC screening to evaluate their performance against *C. albicans* (Fig. 6). According to the MIC and MFC data above, both compounds 7 and 14 exhibited similar levels of potency, 0.625 mg/mL and 2.5 mg/mL. From the primary screening, MIC, and MBC data analyses, it could be said that compounds 7 and 14 might be used as potential antifungal therapeutics. However, further investigation into the associated side effects as well as major safety concerns is needed. In summary, the synthesized compounds exhibited very good antimicrobial activity. Especially the presence of different acyl moieties, including the 3-bromobenzoyl, and 2,6-dichlorobenzoyl groups, significantly improved the antimicrobial activity, being in line with our previous studies (Arifuzzaman et al. 2018; Misbah et al. 2020).
Mammalian tumor cells provide a model for the properties of drug resistance. The resistance mechanisms can be categorized as: (a) those that increase the intracellular concentration of the drug, (b) those that reduce the accessibility of the drug to the target, and (c) those that alter the drug’s mechanism of action. The MRU was used to assess the effect of the test compounds on the cellular viability of the A549 cells. The IC₅₀ values of the test compounds were found to be 25.75, 25.75, and 25.75 µg/ml for compounds 7, 9, and 14, respectively. The results showed that the test compounds had a significant inhibitory effect on the viability of the A549 cells.

**Conclusion**

A very simple route for the synthesis of novel uridine derivatives containing the alkyl or aryl halide groups was conducted in an effort to find a versatile synthon for selective reactions. Antimicrobial screening indicated that the introduction of octanoyl-, 3-chlorobenzoyl,
and dichloro-substituents on the ribose ring led to more bioactive compounds. Moreover, variations in the antimicrobial activity of the uridine derivatives may be associated with the nature of microorganisms against which they were tested as well as the chemical structure of the tested compounds themselves. Antimicrobial screening indicated that the tested compounds (namely 7, 9, and 14) possessed promising biological activity and were a potential source of antimicrobial agents at least in the field of agriculture. This research, in our opinion, has created an opportunity for further investigation using these test chemicals with the hope of ultimately developing more effective anticancer pharmaceutical drugs.

### References

Ahmed FRS, Amin R, Hasan I, Asaduzzaman AKM, Kabir SR (2017) Antitumor properties of a methyl-β-D-galactopyranoside specific lectin from Kaempferia rotunda against Ehrlich ascites carcinoma cells. International Journal of Biological Macromolecules 102: 952–959. https://doi.org/10.1016/j.ijbiomac.2017.04.109

Amsterdam D (2005) Susceptibility testing of antimicrobials in liquid media. In: Lorian V (Ed.) Antibiotics in laboratory medicine (5th ed.). L. Williams & Wilkins, Philadelphia, 61 pp. https://www.academia.edu/32438978/Antibiotics_in_Laboratory_Medicine_5th_Edition

Andary C, Wylde R, Laffite C, Privat G, Winternitz F (1982) Structures of uracil- and thymine-guanine; synthesis of guanosine. Journal of Chemical Society, Perkin Transactions 2: 1685–1687. https://doi.org/10.1039/jp9480001685

Devi SR, Jesmin S, Rahman M, Manchur MA, Fujii Y, Kanaly RA, Ozeki Y, Kawsar SMA (2019) Microbial efficiency and two step synthesis of uridine derivatives with potential antitumor activity. ACTA Pharmaceutica Scientia 78: 276–286. https://doi.org/10.1038/s13358-019-00265-5

Groener Small, L. Chemical Society 21: 39–45. https://doi.org/10.1039/j1cs20003a00394

Itoh M, Hagiwara D, Notani J (1975) A simple and mild esterification method for carboxylic acids using sulfonate -type coupling reagents. Synthesis 1975: 456–457. https://doi.org/10.1055/s-1975-23804

Jesmin S, Devi SR, Rahman M, Islam M, Canaly RA, Fuji Y, Hayashi N, Ozeki Y, Kawsar SMA (2017) An efficient synthesis and spectroscopic characterization of some uridine derivatives. Journal of Bangladesh Chemical Society 29: 12–20.

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Grover RK, Moore JD (1962) Toximetric Studies of fungicides against the brown rot organisms, Sclerotinia fructicola and S. lata. Phytopathology 52: 876–879.

Hunt WA (1975) The effects of aliphatic alcohols on the biophysical and biochemical correlates of membrane function. Advances in Experimental Medicine and Biology 56: 195–210. https://doi.org/10.1007/978-1-4684-7529-6_9

Ishii H, Nakamura M, Seo S, Tori K, Toyo T, Yoshimura Y (1980) Isolation, characterization, and nuclear magnetic resonance spectra of new saponins from the roots of Bupleurum falcatum L. Chemical and Pharmaceutical Bulletin 28: 2367–2373. https://doi.org/10.1248/cpb.28.2367

Kabir AKMS, Kawsar SMA (2018) An efficient approach to the synthesis of thymidine derivatives containing various acyl groups: characterization and antibacterial activities. ACTA Pharmaceutica Scientia 56: 7–22. https://doi.org/10.1038/s13358-019-00265-5

Kabir AKMS, Rabin NM, Islam MS, Rahman MS, Fujii Y, Ozeki Y, Kawsar SMA (2019) Microbial efficacy and two step synthesis of uridine nucleosides; synthesis of guanosine. Journal of Chemical Society, Perkin Transactions 2: 1685–1687. https://doi.org/10.1039/jp9480001685

Kabir AKMS, Dutta P, Anwar MN (2005) Synthesis of some new monosaccharide derivatives. Scientific Research Centre (WMSRC), Jahangirnagar University, Savar, Dhaka, for providing spectroscopic data.
Bulbul MZH et al.: Synthesis of pyrimidine nucleoside derivatives as potential antimicrobial agents

Kawsar SMA, Hamida AA, Sheikh AU, Hossain MK, Shagir AC, Sanaullah AFM, Manchur MA, Imtiaj H, Ogawa Y, Fuji Y, Koide Y, Ozeki Y (2015) Chemically modified uridine molecules incorporating acyl residues to enhance antibacterial and cytotoxic activities. International Journal of Organic Chemistry 5: 232–245. https://doi.org/10.4236/ijoc.2015.54023

Kawsar SMA, Nishat SSBS, Manchur MA, Ozeki Y (2016) Benzenesulfonylation of Methyl α-D-Glucopyranoside: Synthesis, Characterization and Antibacterial Screening. International Letters of Chemistry Physics and Astronomy 64: 95–105. https://doi.org/10.18052/www.scipress.com/ILCPA.64.95

Kawsar SMA, Islam M, Jesmin S, Manchur MA, Hasan I, Rajia S (2018) Evaluation of the antimicrobial activity and cytotoxic effect of some uridine derivatives. International Journal of Bioscience 12: 211–219.

Kim YM, Farrah S, Baney RH (2007) Structure-antimicrobial activity relationship for silanols, a new class of disinfectants, compared with alcohols and phenols. International Journal of Antimicrobial Agents 29: 217–222. https://doi.org/10.1016/j.ijantimicag.2006.08.036

King MW (2006) Medical Biochemistry-IU School of Medicine. http://www.miking.iupui.edu

Kukhanova MK (2012) Anti-HIV nucleoside drugs: A retrospective view into the future. Molecular Biology 46: 768–779. PMID: 23350232 https://doi.org/10.1134/S002689331206012X

Kumaresan S, Senthilkumar V, Stephen A, Balakumar BS (2015) GC-MS analysis and pass-assisted prediction of biological activity spectra of extract of Phomopsis sp. isolated from Andrographis paniculata. World Journal of Pharmaceutical Research 4: 1035–1053. www.wjpr.net

Lu C, Tang K, Li Y, Li P, Lin Z, Yin D, Chen X, Huang H (2014) Design, synthesis and evaluation of novel diaryl urea derivatives as potential antitumor agents. European Journal of Medicinal Chemistry 77: 351–360. https://doi.org/10.1016/j.ejmech.2014.03.020

Misbah MMH, Ferdous J, Bulbul MZH, Chowdhury TS, Dey S, Hasan I, Kawsar SMA (2020) Evaluation of MIC, MBC, MFC and anticancer activities of acylated methyl β-D-galactopyranoside esters. International Journal of Bioscience 16: 299–309.

Morris DJ (1994) Adverse effects and drug interactions of clinical importance with antiviral drugs. Drug Safety 10: 281–291. https://doi.org/10.2165/00002018-199410040-00002

Murata D, Endo Y, Obata T, Sakamoto K, Syouji Y, Kadohira M, Matsuda A, Sasaki TA (2004) A crucial role of uridine/cytidine kinase 2 in antitumor activity of 3′-ethynyl nucleosides. Drug Metabolism & Disposition 32: 1178–1182. https://doi.org/10.1124/dmd.104.000737

Rachakonda S, Cartee L. (2004) Challenges in antimicrobial drug discovery and the potential of nucleoside antibiotics. Current Medicinal Chemistry 11: 775–793. https://doi.org/10.2174/0929867043455774

Shagir AC, Bhuiyan MMR, Ozeki Y, Kawsar SMA (2016) Simple and rapid synthesis of some nucleoside derivatives: structural and spectral characterization. Current Chemistry Letters 5: 83–92. https://doi.org/10.5267/j.ccl.2015.12.001

Tsuda Y, Haque ME (1983) Regioselective introduction of p-coumaroyl group to α-L-arabinono-pyranosides. Total synthesis of undinoside-G and undinoside-D1. Chemical and Pharmaceutical Bulletin 31: 1437–1439. https://doi.org/10.1248/cpb.31.1437

Xiao P, Huang H, Chen J, Li X (2014) In vitro antioxidant and anti-inflammatory activities of Radix isatidis extract and bioaccessibility of six bioactive compounds after simulated gastro-intestinal digestion. Journal of Ethnopharmacology 157: 55–61. https://doi.org/10.1016/j.jep.2014.09.005