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Research Article

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 IC_{50} 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

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PENSOFT

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*-glycoside 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 benzylacyclouridine 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 (Ishji et al. 1980; Andary et al. 1982; Kabir et al. 2005).

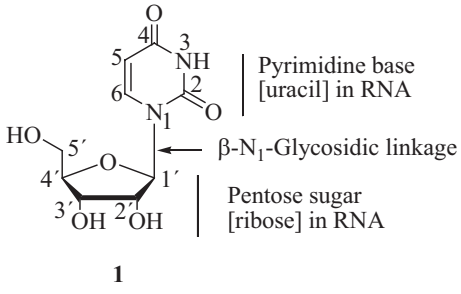
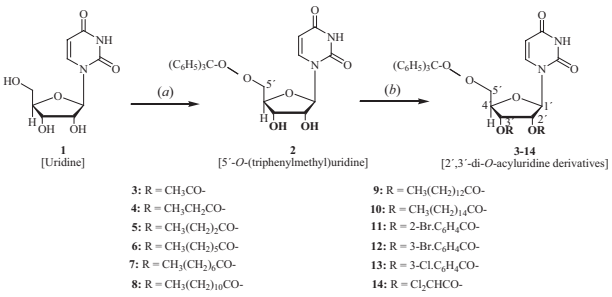


Figure 1. Structure of the uridine (**1**).

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 (Kawsar et al. 2014; Kabir et al. 2004). Encouraged by literature reports and our own findings (Kawsar 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), anticancer, and SAR characteristics of these newly synthesized uridine derivatives are reported herein for the first time.



Scheme 1. *Reagents and conditions:* (*a*) dry Py, $(C_6H_5)_3COCl$, –5 °C, 6 h, (70%; R_f = 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 GF_{254}, and the spots were visualized with a 1% H_2SO_4 solution, followed by heating to temperatures between 150 °C and 200 °C. Column chromatography was performed with silica gel G_{60} (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 triphenylmethyl chloride (2.44 g, 1.1 molar eq.) was added drop wise. The reaction mixture was continuously stirred for 6 h at 0 °C and then left overnight at room temperature under continuous stirring. The progress of the reaction was monitored via TLC ($CHCl_3/CH_3OH$, 15:1, v/v, R_f = 0.52) until full conversion of the starting material into a single product was detected. Next, the solution was quenched with ice water under constant stirring before extraction using $CHCl_3$ (3×10 ml). The combined $CHCl_3$ layers were successively washed with dilute HCl, saturated aqueous $NaHCO_3$ solution, and distilled H_2O. The organic layer was dried with $MgSO_4$ and filtered before the solvent was removed via evaporation. Purification via chromatography with $CHCl_3/CH_3OH$ (15:1, v/v) as the eluent furnished the triphenylmethyl derivative (**2**, 2.82 g) as a crystalline solid. Recrystallization in a methanol-chloroform solvent mixture gave the derivative **2** as needles.

5′-O-(*Triphenylmethyl*)*uridine* (***2***). Yield 83.51%, m.p. = 115–117 °C, R_f= 0.52 ($CHCl_3/CH_3OH$, 16:1, v/v). FTIR (ν_{max} cm^{-1}): 1701 (–CO), 3416–3468 (–OH). ^1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.87 (1H, s, –NH), 7.76 (1H, d, *J* = 7.8 Hz, H-6), 7.51 (6H, m, Ar-H), 7.31 (9H, m, Ar-H), 6.26 (1H, d, *J* = 5.8 Hz, H-1′), 6.10 (1H, s, 2′-OH), 5.93 (1H, dd, *J* = 2.2 and 12.2 Hz, H-5′a), 5.76 (1H, dd, *J* = 2.3 and 12.4 Hz, H-5′b), 5.70 (1H, d, *J* = 8.2 Hz, H-5), 5.20 (1H, s, 3′-OH), 4.75 (1H, dd, *J* = 2.3 and 5.6 Hz, H-4′), 4.25 (1H, d, *J* = 5.8 Hz, H-2′), 4.11 (1H, dd, *J* = 7.6 and 5.6 Hz, H-3′). LC-(ESI+)-MS/MS: *m/z* $[M+1]^+$ 487.10. Anal calcd for $C_{28}H_{26}O_6N_2$ (M.W.: 486.17 g/mol) calculated (%): C:69.14, H:5.02; found: C:69.16, H:5.37.

General procedure for the synthesis of triphenylmethyluridine derivatives (3–14)

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 molar 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 ($CHCl_3/CH_3OH$, 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. Percolation of the resulting syrup through a silica gel column with $CHCl_3/CH_3OH$ (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 molar 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 molar eq.), compound **4** (133 mg); butyryl chloride (0.13 mL, 3.5 molar eq.), compound **5** (142.76 mg); heptanoyl chloride (1.2 mL, 3.5 molar eq.), compound **6** (81.3 mg); octanoyl chloride (0.24 mL, 3.5 molar eq.), compound **7** (148.5 mg); lauroyl chloride (0.37 mL, 3.5 molar eq.), compound **8** (184.6 mg); myristoyl chloride (0.48 mL, 3.5 molar eq.), compound **9** (193.8 mg); palmitoyl chloride (0.43 mL, 3.5 molar eq.), compound **10** (158.8 mg); 2-bromobenzoyl chloride (0.26 mL, 3.5 molar eq.), compound **11** (169 mg); 3-bromobenzoyl chloride (0.19 mL, 3.5 molar eq.), compound **12** (92 mg); 3-chlorobenzoyl chloride (0.21 mL, 3.5 molar eq.), compound **13** (150 mg); and dichloroacetyl chloride (0.19 mL, 3.5 molar eq.), compound **14** (234 mg). The purity of compounds was checked with TLC (Lu et al. 2014).

2′,3′-Di-O-acetyl-5′-O-(*triphenylmethyl*)*uridine* (***3***). Yield 87.12%, m.p. = 104–106 °C, R_f 0.53 ($CHCl_3/CH_3OH$, 18:1, v/v). FTIR (ν_{max} cm^{-1}): 1738, 1710, 1684 (–CO). ^1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.71 (1H, s, –NH), 7.38 (1H, d, *J* = 7.6 Hz, H-6), 7.35 (6H, m, Ar-H), 7.31 (9H, m, Ar-H), 5.75 (1H, d, *J* = 5.5 Hz, H-1′), 5.68 (1H, dd, *J* = 2.1 and 12.1 Hz, H-5′a), 5.64 (1H, d, *J* = 2.0 and 12.1 Hz, H-5′b), 5.60 (1H, d, *J* = 7.8 Hz, H-5), 5.30 (1H, d, *J* = 5.4 Hz, H-2′), 5.01 (1H, dd, *J* = 7.2 and 5.1 Hz, H-3′), 4.42 (1H, m, H-4′), 2.15, 2.10 (2 × 3H, 2 × s, 2 × CH_3CO–). LC-(ESI+)-MS/MS: *m/z* $[M+1]^+$ 571.08. Anal calcd for $C_{32}H_{30}O_8N_2$ (M.W.: 570.02 g/mol) calculated (%): C:67.37, H:5.26; found: C:67.39, H:5.27.

2′,3′-Di-O-propionyl-5′-O-(*triphenylmethyl*)*uridine* (***4***). Yield 72.21%, m.p. = 109 °C–110 °C, R_f = 0.51 ($CHCl_3/CH_3OH$, 19:1, v/v). FTIR (ν_{max} cm^{-1}): 1738 (–CO). ^1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 9.05 (1H, s, –NH), 7.78 (1H, d, *J* = 7.8 Hz, H-6), 7.50 (6H, m, Ar-H), 7.34 (9H, m, Ar-H), 6.02 (1H, d, *J* = 5.8 Hz, H-1′), 5.87 (1H, dd, *J* = 2.4 and 12.4 Hz, H-5′a), 5.78 (1H, dd, *J* = 2.2 and 12.4 Hz, H-5′b), 5.74 (1H, d, *J* = 7.9 Hz, H-5), 5.50 (1H, d, *J* = 5.4 Hz, H-2′), 4.62 (1H, dd, *J* = 7.8 and 5.8 Hz H-3′), 4.51 (1H, m, H-4′), 2.90, 2.84 {2 × 2H, 2 × q, 2 × CH_3CH_2CO–}, 1.23, 1.11 {2 × 3H, 2 × t, 2 × CH_3CH_2CO–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 599.04. Anal calcd for $C_{34}H_{34}O_8N_2$ (M.W.: 598.01 g/mol) calculated (%): C:68.23, H:5.69; found: C:68.26, H:5.71.

2′,3′-Di-O-butyryl-5′-O-(*triphenylmethyl*)*uridine* (***5***). Yield 86.1%, m.p. = 118 °C–119 °C, R_f = 0.54 ($CHCl_3$/

CH_3OH, 16:1, v/v). FTIR (ν_{max} cm^{-1}): 1738 (–CO). ^1H NMR ($CDCl_3$, 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 × $CH_3CH_2CH_2CO$–}, 1.73 (4H, m, 2 × $CH_3CH_2CH_2CO$–), 1.02 {6H, m, 2 × $CH_3(CH_2)_2CO$–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 627.01. Anal calcd for $C_{36}H_{38}O_8N_2$ (M.W.: 626.07 g/mol) calculated (%): C:69.00, H:6.07; found: C:69.05, H:6.10.

2′,3′-Di-O-heptanoyl-5′-O-(*triphenylmethyl*)*uridine* (***6***). Yield 76.42%, m.p. = 108–109 °C, R_f = 0.54 ($CHCl_3$/CH_3OH, 19:1, v/v). FTIR (ν_{max} cm^{-1}): 1708 (–CO). ^1H NMR ($CDCl_3$, 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′), 5.84 (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* = 7.5 Hz, H-5), 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 × $CH_3(CH_2)_4CH_2CO$–}, 1.70 {4H, m, 2 × $CH_3(CH_2)_3CH_2CH_2CO$–}, 1.51 {12H, m, 2 × $CH_3(CH_2)_3CH_2CH_2CO$–}, 0.96 {6H, m, 2 × $CH_3(CH_2)_5CO$–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 711.0. Anal calcd for $C_{42}H_{50}O_8N_2$ (M.W.: 710.09 g/mol) calculated (%): C:70.99, H:7.04; found: C:71.01, H:7.08.

2′,3′-Di-O-octanoyl-5′-O-(*triphenylmethyl*)*uridine* (***7***). Yield 75.34%, m.p. = 144 °C–145 °C, R_f = 0.53 ($CHCl_3$/CH_3OH, 20:1, v/v). FTIR (ν_{max} cm^{-1}): 1684 (–CO). ^1H NMR ($CDCl_3$, 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′), 6.10 (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 × $CH_3(CH_2)_5CH_2CO$–}, 1.66 {4H, m, 2 × $CH_3(CH_2)_4CH_2CH_2CO$–}, 1.27 {16H, m, 2 × $CH_3(CH_2)_4(CH_2)_2CO$–}, 0.93 {6H, m, 2 × $CH_3(CH_2)_6CO$–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 739.11. Anal calcd for $C_{44}H_{54}O_8N_2$ (M.W.: 738.16 g/mol) calculated (%): C:71.55, H:7.32; found: C:71.57, H:7.35.

2′,3′-Di-O-lauroyl-5′-O-(*triphenylmethyl*)*uridine* (***8***). Yield 81.61%, m.p. = 125 °C–127 °C, R_f = 0.52 ($CHCl_3$/CH_3OH, 17:1, v/v). FTIR (ν_{max} cm^{-1}): 1710 (–CO). ^1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 9.02 (1H, s, –NH), 7.87 (1H, d, *J* = 7.8 Hz, H-6), 7.51 (6H, m, Ar-H), 7.31 (9H, m, Ar-H), 6.22 (1H, d, *J* = 5.6 Hz, H-1′), 5.86 (1H, m, H-5′a), 5.70 (1H, m, H-5′b), 5.58 (1H, d, *J* = 8.1 Hz, H-5), 5.44 (1H, d, *J* = 5.6 Hz, H-2′), 4.81 (1H, dd, *J* = 7.6 and 5.6 Hz, H-3′), 4.61 (1H, m, H-4′), 2.33 {4H, m, 2 × $CH_3(CH_2)_9CH_2CO$–}, 1.61 {4H, m, 2 × $CH_3(CH_2)_8CH_2CH_2CO$–}, 1.26 {32H, m, 2 × $CH_3(CH_2)_8CH_2CH_2CO$–}, 0.88 {6H, m, 2 × $CH_3(CH_2)_{10}CO$–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 851.17. Anal calcd for $C_{52}H_{70}O_8N_2$ (M.W.: 850.11 g/mol) calculated (%): C:73.41, H:8.24; found: C:73.43, H:8.25.

2′,3′-Di-O-myristoyl-5′-O-(*triphenylmethyl*)*uridine* (***9***). Yield 77.18%, m.p. = 125–127 °C, R_f = 0.54 ($CHCl_3$/CH_3OH, 22:1, v/v). FTIR (ν_{max} cm^{-1}): 1708 (–CO). ^1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.72 (1H, s, –NH), 7.70 (1H, d, *J* = 7.6 Hz, H-6), 7.35 (6H, m, Ar-H), 7.22 (9H, m, Ar-H), 6.14 (1H, d, *J* = 5.6 Hz, H-1′), 6.08 (1H, dd, *J* = 2.0 and 12.0 Hz, H-5′a), 5.78 (1H, dd, *J* = 2.1 and 12.1 Hz, H-5′b), 5.61 (1H, d, *J* = 7.6 Hz, H-5), 5.45 (1H, d, *J* = 5.5 Hz, H-2′), 4.75 (1H, m, H-3′), 4.45 (1H, dd, *J* = 2.1 and 5.6 Hz, H-4′), 2.36 {4H, m, 2 × $CH_3(CH_2)_{11}CH_2CO$–}, 1.65 {4H, m, 2 × $CH_3(CH_2)_{10}CH_2CH_2CO$–}, 1.28 {40H, m, 2 × $CH_3(CH_2)_{10}CH_2CH_2CO$–}, 0.93 {6H, m, 2 × $CH_3(CH_2)_{12}CO$–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 907.15. Anal calcd for $C_{56}H_{78}O_8N_2$ (M.W.: 906.10 g/mol) calculated (%): C:74.17, H:8.61; found: C:74.18, H:8.63.

2′,3′-Di-O-palmitoyl-5′-O-(*triphenylmethyl*)*uridine* (***10***). Yield 80.51%, m.p. = 135–136 °C, R_f = 0.51 ($CHCl_3$/CH_3OH, 16:1, v/v). FTIR (ν_{max} cm^{-1}): 1702 (–CO). ^1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 9.01 (1H, s, –NH), 7.66 (1H, d, *J* = 7.5 Hz, H-6), 7.50 (6H, m, Ar-H), 7.30 (9H, m, Ar-H), 6.20 (1H, d, *J* = 5.6 Hz, H-1′), 6.10 (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), 5.60 (1H, d, *J* = 8.1 Hz, H-5), 5.50 (1H, d, *J* = 5.5 Hz, H-2′), 5.40 (1H, m, H-3′), 4.65 (1H, m, H-4′), 2.33 {4H, m, 2 × $CH_3(CH_2)_{13}CH_2CO$–}, 1.24 {52H, m, 2 × $CH_3(CH_2)_{13}CH_2CO$–}, 0.90 {6H, m, 2 × $CH_3(CH_2)_{14}CO$–}. LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 963.08. Anal calcd for $C_{60}H_{86}O_8N_2$ (M.W.: 962.09 g/mol) calculated (%): C: 74.84, H: 8.94; found: C: 74.86, H: 8.96.

2′,3′-Di-O-(*2-bromobenzoyl*)*-5′-O-*(*triphenylmethyl*)*uridine* (***11***). Yield 84.21%, m.p. = 117–119 °C, R_f = 0.51 ($CHCl_3$/CH_3OH, 21:1, v/v). FTIR (ν_{max} cm^{-1}): 1718 (–CO). ^1H NMR ($CDCl_3$/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: *m/z* $[M+H]^+$ 852.81. Anal calcd for $C_{42}H_{32}O_8N_2Br_2$ (M.W.: 851.87 g/mol) calculated (%): C:59.17, H:3.76; found: C:59.18, H:3.79.

2′,3′-Di-O-(*3-bromobenzoyl*)*-5′-O-*(*triphenylmethyl*)*uridine* (***12***). Yield 81.43%, m.p. = 119–121 °C, R_f = 0.55 ($CHCl_3$/CH_3OH, 24:1, v/v). FTIR (ν_{max} cm^{-1}): 1713 (–CO). ^1H NMR ($CDCl_3$/TMS, 400 MHz, δ in ppm): 8.10 (1H, s, –NH), 7.92 (2H, d, *J* = 7.6 Hz, Ar-H), 7.90 (2H, s, Ar-H), 7.83 (1H, d, *J* = 7.6 Hz, H-6), 7.78 (2H, d, *J* = 7.5 Hz, Ar-H), 7.51 (6H, m, Ar-H), 7.31 (9H, m, Ar-H), 7.47 (2H, t, *J* = 7.5 Hz, Ar -H), 6.19 (1H, d, *J* = 6.5 Hz, H-1′), 5.58 (1H, m, H-5′a), 5.83 (1H, m, H-5′b), 5.79 (1H, d, *J* = 7.8 Hz, H-5), 4.78 (1H, d, *J* = 5.2 Hz, H-2′), 4.72 (1H, dd, *J* = 7.6 and 5.5 Hz, H-3′), 4.36 (1H, m, H-4′). LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 852.80. Anal calcd for $C_{42}H_{32}O_8N_2Br_2$ (M.W.: 851.87 g/mol) calculated (%): C:59.17, H:3.76; found: C:59.20, H:3.77.

2′,3′-Di-O-(*3-chlorobenzoyl*)*-5′-O-*(*triphenylmethyl*)*uridine* (***13***). Yield 72.51%, m.p. = 129–131 °C, R_f = 0.52 ($CHCl_3$/CH_3OH, 21:1, v/v). FTIR (ν_{max} cm^{-1}): 1739 (–CO). ^1H NMR ($CDCl_3$/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),

7.53 (1H, d, *J* = 7.5 Hz, H-6), 7.46 (2H, d, *J* = 7.6 Hz, Ar-H), 7.46 (6H, m, Ar-H), 7.40 (9H, m, Ar-H), 7.38 (2H, t, *J* = 7.6 Hz, Ar-H), 6.01 (1H, d, *J* = 6.5 Hz, H-1′), 5.89 (1H, m, H-5′a), 5.81 (1H, m, H-5′b), 5.78 (1H, d, *J* = 7.8 Hz, H-5), 5.18 (1H, d, *J* = 5.2 Hz, H-2′), 5.11 (1H, dd, *J* = 7.6 and 5.5 Hz, H-3′), 4.30 (1H, m, H-4′). LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 764.01. Anal calcd for $C_{42}H_{32}O_8N_2Cl_2$ (M.W.: 763.0.07 g/mol) calculated (%): C:66.06, H:4.19; found: C:66.07, H:4.21.

2′,3′-Di-O-dichloroacetyl-5′-O-(triphenylmethyl)uridine (***14***). Yield 93.11%, m.p. = 112–114 °C, R_f = 0.53 ($CHCl_3$/CH_3OH, 20:1, v/v). FTIR (ν_{max} cm^{-1}): 1719 (–CO). ^1H NMR ($CDCl_3$/TMS, 400 MHz, δ in ppm): 9.04 (1H, s, –NH), 7.54 (6H, m, Ar-H), 7.31 (9H, m, Ar-H), 7.38 (1H, d, *J* = 7.5 Hz, H-6), 6.15, 6.13 (2 × 1H, 2 × s, 2 × Cl_2CH-CO–), 6.00 (1H, d, *J* = 6.5 Hz, H-1′), 5.89 (1H, m, H-5′a), 5.81 (1H, m, H-5′b), 5.78 (1H, d, *J* = 7.8 Hz, H-5), 5.13 (1H, d, *J* = 5.2 Hz, H-2′), 5.02 (1H, dd, *J* = 7.6 and 5.5 Hz, H-3′), 4.17 (1H, m, H-4′). LC-(ESI+)-MS/MS: *m/z* $[M+H]^+$ 709.01. Anal calcd for $C_{32}H_{26}O_8N_2Cl_4$ (M.W.: 708.07 g/mol) calculated (%): C:54.24, H:3.67; found: C:54.27, H:3.70.

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: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NCTC 6017 and Fungus: *Aspergillus niger* ATCC 16404 and *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 (5×10^5 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 CO_2 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:

$$\text{Proliferation inhibition ratio (\%)} = \{(A - B) \times 100\}/A$$

where A is the OD_{570} nm of the cellular homogenate (control) without the derivative and B is the OD_{570} 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 ρ 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, ^{1}H-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 it's ^{1}H-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 $C_{28}H_{26}O_6N_2$. 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*-acyl derivatives (3–14) of 5′-*O*-(triphenylmethyl) uridine (2)

Several derivatives of the triphenylmethylation 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

1684 cm^{-1}. In its ^{1}H-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, ^{1}H-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 × CH_3CH_2CO–} as well as two three-proton triplets at δ 1.23 and δ 1.11 {2 × CH_3CH_2CO–} in its ^{1}H 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*-dichloroacetylate **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. aeruginosa* 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. aeruginosa*, 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

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

Compound	Diameter of zone of inhibition (mm)				
	B. subtilis (+*ve*)	***S. aureus*** (+*ve*)	***E. coli*** (-*ve*)	***S. abony*** (-*ve*)	***P. aeruginosa*** (-*ve*)
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	NI	10 ± 0.27	10 ± 0.4
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.

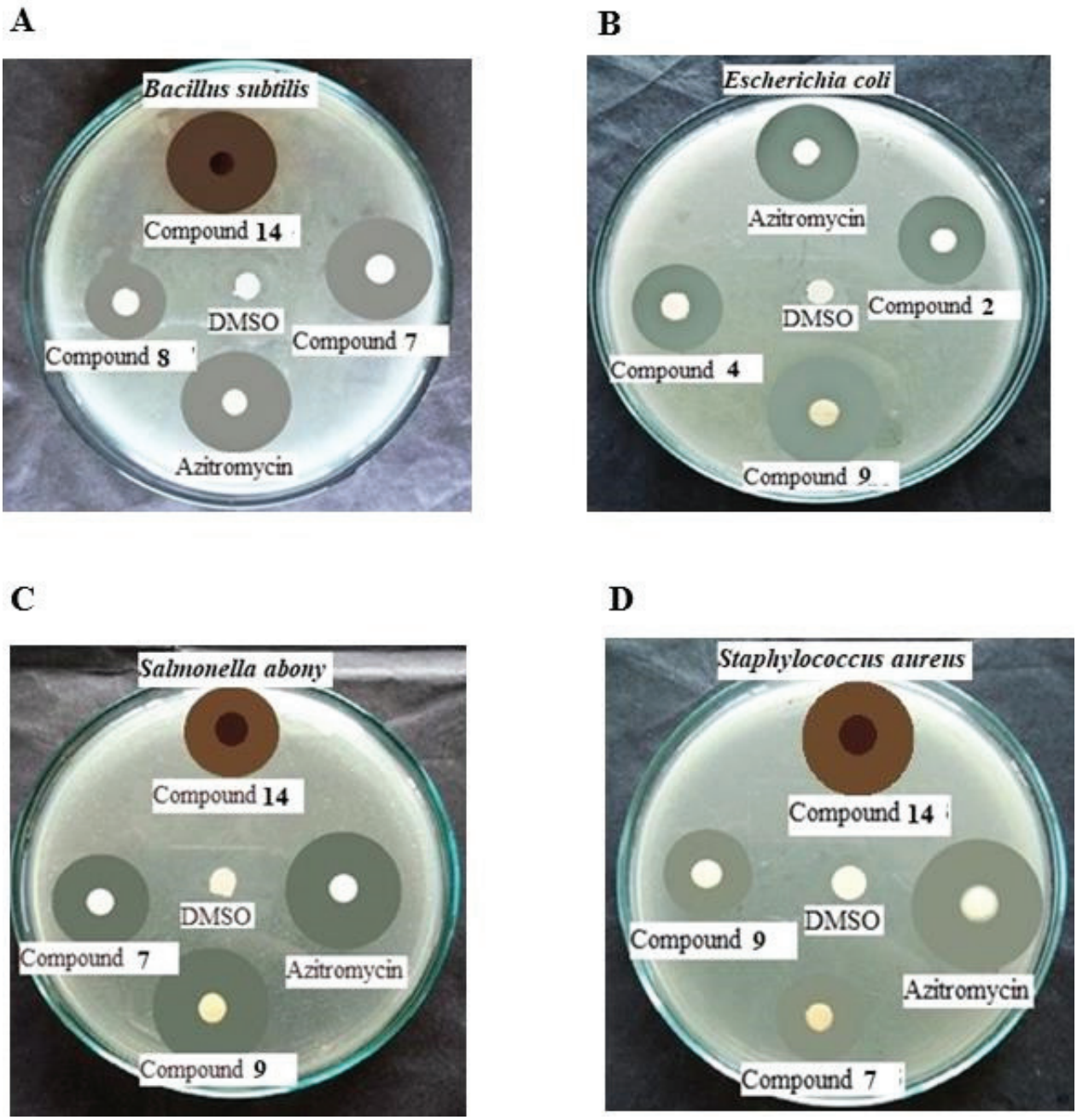


Figure 2. Percentage of inhibition observed for A) *B. subtilis* by compounds **7**, **9**, and **8**; B) *E. coli* by compounds **2**, **4**, and **9**; C) *S. abony* by compounds **7**, **9**, and **14**; and D) *S. aureus* by compounds **7**, **9**, and **14**. DMSO was the negative control, whereas azithromycin represented the positive control.

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 their 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.*

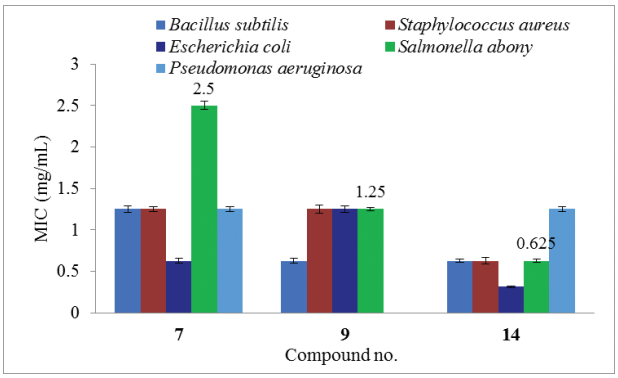


Figure 3. MIC values for compounds **7**, **9**, and **14**.

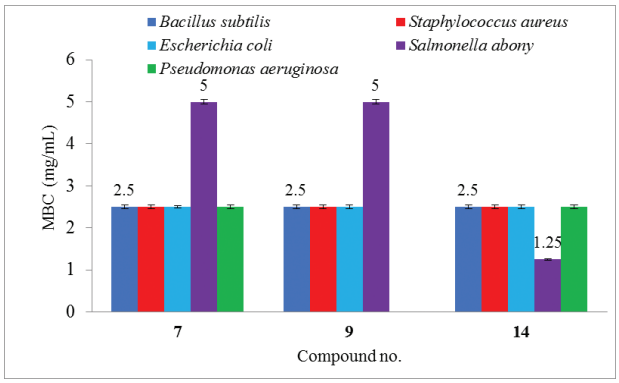


Figure 4. MBC data for compounds **7**, **9**, and **14**.

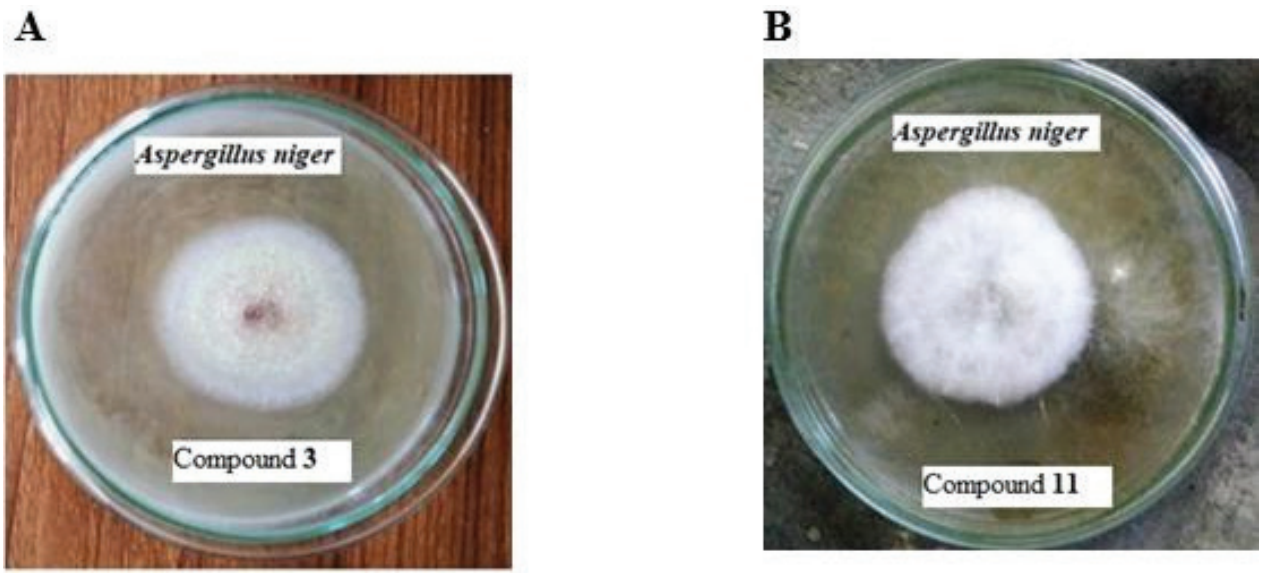


Figure 5. Percentage zone of mycelial growth inhibition for compounds **3** and **11** against *A. niger* (**A** and **B**, respectively). DMSO represents the negative control, whereas nystatin is the positive control.

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

Compound	% Inhibition of fungal mycelial growth	
	Aspergillus niger	*Candida albicans*
2	NI	NI
3	*66.67 ± 1.8	NI
4	44.28 ± 0.9	NI
5	NI	NI
6	55.17 ± 1.7	NI
7	*99.0 ± 1.9	*70.0 ± 1.4
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** reveled 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).

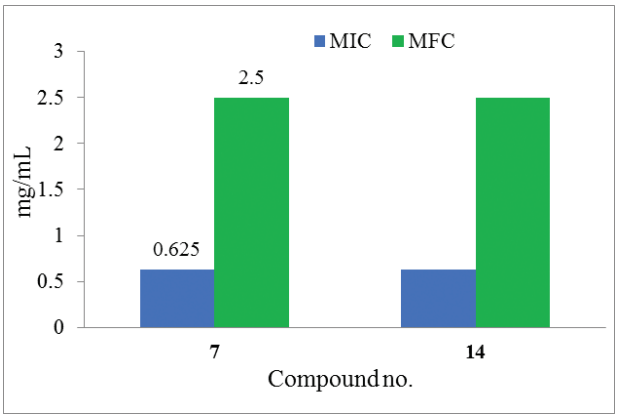


Figure 6. MIC and MFC values for the compounds **7** and **14**.

Establishing anticancer activity by MTT assay

MTT assay was used to investigate the effect of tested uridine derivatives on EAC cells *in vitro*. Among 13 derivatives under investigation, only myristoyl derivative **9** was found to be potentially active (Fig. 7), with an inhibitory effect of 7.12% at 200 µg/ml, 6.49% at 100 µg/ml, 6.16% at 50 µg/ml, 4.17% at 25 µg/ml, 2.83% at 12.5 µg/ml, and 1.34% at 6.25 µg/ml. This meant that the inhibitory activity of compound **9** was affected when its concentration was gradually decreased. The IC_{50} value for compound **9** was rather high, determined to be 1956.25 µg/ml.

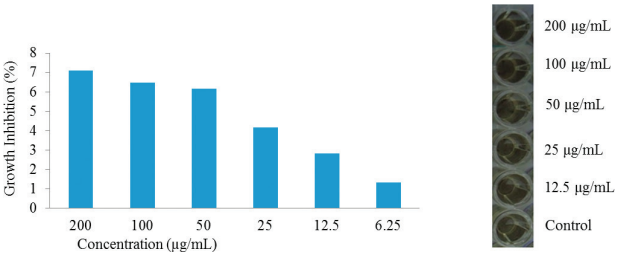


Figure 7. Percentage growth inhibition observed for various concentrations of compound **9**. Right figure 96-well flat-bottom experiment.

Structure-activity relationship (SAR) studies

The theory that the chemical structure of a therapeutic is interrelated with its biological activity has experienced significant focus over the past few years (Kumaresan et al. 2015). Taking this into account, SAR studies were conducted for the synthesized compounds. It can be seen in Fig. 8 that introducing the triphenylmethyl group at C-5´ position, the octanoyl group at C-2´ and C-3´, and the presence of both the myristoyl and dichloroacetal groups at C-2´ and C-3´ led to enhanced antimicrobial activity for compounds **7**, **9**, and **14**. Compound **7** was found to be equally active against bacteria and fungi alike, whereas compound **14** showed no inhibition against *A. niger*. Incorporation of the myristoyl group at C-2´ and C-3´ while C-5´ still contained its triphenylmethyl group led to increased anticancer efficacy and moderate inhibition activity against the tested microorganisms.

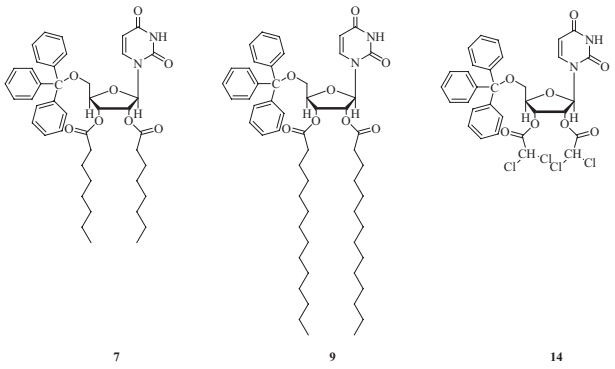


Figure 8. Structures of compounds **7** (octanoyl derivative), **9** (myristoyl derivative), and **14** (dichloroacetyl derivative).

This series of test chemicals showed very good antimicrobial activity, particularly the presence of groups such as octanoyl, myristoyl, and dichloroacetyl. This led to improved antimicrobial activity when compared to previous studies (Devi et al. 2019; Kabir et al. 2004; Mirajul et al. 2019). In addition, the introduction of these functional groups successively increased the hydrophobicity of the tested compounds, a parameter that is essential for establishing bioactivity, toxicity, or altering membrane integrity because it is directly related to membrane permeation (Kumaresan et al. 2015). Hunt (1975) suggested that the potency of aliphatic alcohols was directly related to their lipid solubility through the hydrophobic interactions taking place between the acyl chains of the alcohol and the lipid region in the membrane. In addition, Kawsar et al. reported that halogen-containing molecules had greater antimicrobial activity when compared to their counterparts (Kawsar et al. 2014). It is safe to assume that similar types of hydrophobic interactions might occur between acyl chains of uridine residues that have accumulated in bacterial membranes. Thus, membrane permeability is lost because of their lipid-like nature. As a result of this hydrophobic interaction, apoptosis is inevitable for the bacterial cells (Judge et al. 2013).

Direct, selective acylation of uridine (**1**) with a number of acylating agents was found to be very promising since a mono-substituted derivative was isolated in reasonably high yields as the sole product in all cases. These newly synthesized products may be used as important precursors for the derivatization and optimization of uridine, which provides a pathway for further development of pesticides and potential pharmaceutical targets.

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.

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