Acta Pharm. Sci. Vol 56 No: 4. 2018
DOI: 10.23893/1307-2080.APS.05622

An Efficient Approach to the Synthesis of Thymidine Derivatives Containing Various Acyl Groups: Characterization and Antibacterial Activities

Md Arifuzzaman[1], Md Mirajul Islam[1], Md Monjur Rahman[1], Mohammad Atiar Rahman[2], Sarkar M. A. Kawsar[1]*

1 Laboratory of Carbohydrate and Nucleoside Chemistry, Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong-4331, Bangladesh.

2 Department of Biochemistry and Molecular Biology, Faculty of Biological Science, University of Chittagong, Chittagong-4331, Bangladesh.

ABSTRACT

In search of new leads toward potent antibacterial agents; therefore, a series of thymidine analogues were synthesized by direct acylation method and furnished the 5´-*O*-acyl thymidine derivatives in good yield. A number of acyl derivatives were prepared in order to obtain a series of newer components for antibacterial screening experiments. The synthesized compounds were characterized by their FTIR, ^{1}HNMR spectral data and elemental analysis. These thymidine derivatives were evaluated for *in vitro* antibacterial screening studies against a number of human pathogenic microorganisms by disc diffusion method. The study revealed that most of the tested chemicals exhibited moderate to good antibacterial activities. It was also observed that the test chemical 2-bromobenzoyl derivative 11 very significantly inhibited the growth of all Gram-positive and Gram-negative bacterial strains used. For comparative studies, antibacterial activity of standard antibiotics, Azithromycin was also carried out against these microorganisms. Hence, these thymidine derivatives can be used to discover antibacterial agents that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Thymidine, synthesis, derivatives, ^{1}H-NMR, antibacterial

INTRODUCTION

Nucleosides are key compounds involved in major biological processes, such as nucleic acids and proteins synthesis, cell signaling, enzyme regulation, and metabolism. Nucleoside and their derivatives have emerged as molecules with

*Corresponding Author: Kawsar Sarkar M. A, e-mail: akawsarabe@yahoo.com
(Received 30 May 2018, accepted 23 June 2018)

potentially useful therapeutic properties that have gained considerable attention from both synthetic and medicinal chemists due to their versatile biological activities in various therapeutic areas. Zidovudine (3′-azido-3′-deoxythymidine) is the first approved drug for the treatment of HIV[1], marketed under the brand name Retrovir. Over the past few years, several derivatives of the nucleosides are known to possess antimicrobial[2], anticancer[3], anti-inflammatory[4] and antiviral activities[5-8].

Thymidine (=deoxythymidine) (Figure 1) is a pyrimidinedeoxy nucleoside. Deoxythymidine is the DNA nucleoside T, which pairs with deoxyadenosine (A) in double-stranded DNA. Since thymine nucleotides are precursors of DNA (but not RNA), the prefix "deoxy" is often left out, i.e., deoxythymidine is often just called thymidine. Since nucleosides and their analogues are of enormous importance. They are an established class of clinically useful medicinal agents possessing antiviral and anticancer activity; at the same time, they are one class of compounds worthy of further investigation as antibacterial agents since some derivatives have shown moderate to good activity against specific bacterial strains[9]. This has led to our interest in the search for new nucleoside i.e., thymidine derivatives that may be screened for broad-spectrum antibacterial activity.

It was revealed that a large number of biologically active compounds possess aromatic, heteroaromatic and acyl substituents[10]. Also, a wide variety of compounds having nitrogen, sulphur and halogen containing substituents possess effective biological activity[10-12]. It is also known that if an active nucleus is linked to another active nucleus, the resulting molecule may possess greater potential for biological activity[13]. From our previous works we also observed that in many cases the combination of two or more acyl substituents in a single molecular framework enhances the biological activity manyfold than their parent nuclei[14-18]. Encouraged by our own findings and also literature reports, we synthesised a series of thymidine derivatives deliberately incorporating a wide variety of anticipated biologically active components to the deoxyribose moiety. Antibacterial activities of these compounds were carried out using a variety of bacterial strains and the results are reported here as first time.

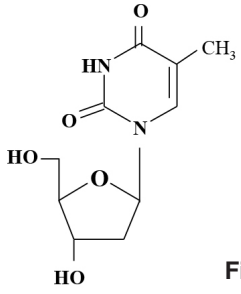


Figure 1. Thymidine (Compound 1)

METHODOLOGY

Chemistry

FTIR spectra were recorded by KBr disc at the Chemistry Department, University of Chittagong, Bangladesh, with an IR Affinity Fourier Transform Infrared Spectrophotometer (SHIMADZU, Japan). All reagents used were commercially available (Sigma-Aldrich) and were used as received, unless otherwise specified. Melting points were determined on an electro-thermal melting point apparatus (England) and are uncorrected. Evaporations were carried out under reduced pressure using VV-1 type vacuum rotary evaporator (Germany) with a bath temperature below 40°C. ^{1}H-NMR spectra (400 MHz) were recorded for solutions in deuteriochloroform ($CDCl_3$) (internal Me_4Si) with a Bruker DPX-40C spectrometer. Thin layer chromatography (t.l.c) was performed on Kieselgel GF_{254} and spots were detected by spraying the plates with 1% H2SO4 and heating at 150-200oC until coloration took place. Column chromatography was performed with silica gel G_{60}. Solvent system employed for TLC analyses was methanol-chloroform in different proportions.

Synthesis

Although studies for the synthesis of the nucleosides began[19] in 1948, the preparations of the nucleosides and their analogues are still a particularly challenging and attractive target for the synthetic community because of their promising pharmacological profiles.

5´-*O*-(Acetyl)thymidine (Compound 2)

A suspension of thymidine (1) (200 mg, 0.82 mmol) in dry pyridine (3 ml) was cooled to -50C where upon acetic anhydride (0.0848 ml, 1.1 molar eq.) was added to it. The mixture was stirred at this temperature for 6 hours and then stirred overnight at room temperature. The reaction progress was monitored by TLC (methanol-chloroform 1:5), which indicated full conversion of the starting material into a single product (R_f =0.52).

A few pieces of ice were then added to the reaction flask with constant shaking to destroy the excess reagent and the contents were extracted with chloroform (3x10 mL). The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The chloroform layer was dried with anhydrous magnesium sulphate ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to leave a syrup. The syrup was passed through a silica gel column chromatography and eluted with methanol-

chloroform (1:5) provided the acetyl chloride derivative (2) (141 mg) as semi solid. The compound was sufficiently pure for use in the next stage without further purification and identification.

FTIR (KBr) ν_{max} (cm^{-1}): 1684 (-CO), 3430 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 9. 01 (1H, s, -NH), 7.31 (1H, d, J=1.3 Hz, H-6), 6.29 (1H, m, H-1´), 4.22-4.20 (1H, m, H-3´), 4.18 (1H, dd, J=12.0 and 4.7 Hz, H-5´a), 4.11 (1H, dd, J=12.1 and 4.5 Hz, H-5´b), 4.05 (1H, ddd, J=3.6, 4.6 and 4.2 Hz, H-4´), 3.37 (1H, br s, 3´-OH), 2.35 (1H, ddd, J=13.7, 6.6 and 4.4 Hz, H-2´a), 2.25 (1H, ddd, J=13.6, 6.6 and 6.8 Hz, H-2´b), 2.11 (3H, s, CH_3CO-), 1.72 (3H, d, J = 1.3 Hz, 5-CH_3). Anal. calcd for $C_{11}H_{17}O_5N_2CO$ (289.26): C, 45.63; H, 5.87. Found: C, 45.65; H, 5.88.

General synthesis of thymidine derivatives

A solution of thymidine (1) (200 mg, 0.82 mmol) in anhydrous pyridine (3 ml) was cooled to 00C when pentanoyl chloride (0.1077 mL, 1.1 molar eq.), heptanoyl chloride (0.1336 mL, 1.1 molar eq.), octanoyl chloride (0.1534 mL, 1.1 molar eq.), decanoyl chloride (0.1846 mL, 1.1 molar eq.), myristoyl chloride (0.8256 mL, 1.1 molar eq.), pivaloyl chloride (0.1106 mL, 1.1 molar eq.), 2-chlorobenzoyl chloride (0.1092 mL, 1.1 molar eq.), 2-bromobenzoyl chloride (0.1163 mL, 1.1 molar eq.), 4-bromobenzoyl chloride (0.195 mL, 1.1 molar eq.) and cinnamoyl chloride (0.1482 mL, 1.1 molar eq.) were separately added to it, respectively. The mixture was stirred at 0°C for 6~7 hours and then overnight at room temperature. T.L.C. examination (methanol-chloroform, 1:5) showed complete conversion of reactant into a single product. A few pieces of ice were added to the reaction flask in order to destroy the excess reagent and the reaction mixture was processed as usual. Percolation of the resulting syrup by passage through a silica gel column with methanol-chloroform, (1:5), as eluant afforded the pentanoyl derivative (3) (148 mg) as a semi-solid mass which could not be crystallized. Similarly isolate the compound 4 (137 mg), compound 5 (162 mg), compound 6 (152 mg), compound 7 (156 mg), compound 8 (152 mg), compound 9 (159 mg), compound 10 (125 mg), compound 11 (138 mg), compound 12 (145 mg) and compound 13 (165 mg), successfully.

5´-*O*-(Pentanoyl)thymidine (Compound 3)

FTIR (KBr) ν_{max} (cm^{-1}): 1694 (-CO), 3432 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 9. 01 (1H, s, -NH), 7.27 (1H, d, J=1.3 Hz, H-6), 6.24 (1H, t, J=6.5 Hz, H-1´), 4.40-4.32 (1H, m, H-3´), 4.40 (1H, dd, J=12.0 and 4.5 Hz, H-5´a), 4.25 (1H, dd, J=12.0 and 3.5 Hz, H-5´b), 4.15 (1H, ddd, J=3.5, 4.5 and 3.9 Hz, H-4´), 3.47 (1H, br, 3´-OH), 2.38 (1H, ddd, J=13.5, 6.5 and 4.0 Hz, H-2´a), 2.36 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 2.25 (1H, ddd, J=13.5, 6.5 and 6.7 Hz, H-2´b),

1.92 (3H, d, J=1.3 Hz, 5-CH_3), 1.64 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.26 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.88 {3H, m, $CH_3(CH_2)_3CO$-}. Anal. calcd for $C_{15}H_{23}O_6N_2$ (326.346): C, 55.15; H, 7.11. Found: C, 55.18; H, 7.12.

5´-*O*-(Heptanoyl)thymidine (Compound 4)

FTIR (KBr) ν_{max} (cm^{-1}): 1706 (-CO), 3465 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 9. 00 (1H, s, -NH), 7.17 (1H, d, J=1.3 Hz, H-6), 6.82 (1H, t, J=6.5 Hz, H-1´), 4.30 (1H, m, H-3´), 4.27 (1H, dd, J=12.0 and 4.5 Hz, H-5´a), 4.20 (1H, dd, J=12.0 and 3.5 Hz, H-5´b), 4.01 (1H, ddd, J=3.5, 4.5 and 3.9 Hz, H-4´), 3.35 (1H, s, 3´-OH), 2.36 (1H, ddd, J=13.5, 6.5 and 4.0 Hz, H-2´a), 2.33 {2H, m, $CH_3(CH_2)_4CH_2CO$-}, 2.25 (1H, ddd, J=13.5, 6.5 and 6.7 Hz, H-2´b), 1.93 (3H, d, J=1.3 Hz, 5-CH_3), 1.64 {2H, m, $CH_3(CH_2)_3CH_2CH_2CO$-}, 1.28 {6H, m, $CH_3(CH_2)_3CH_2CH_2CO$-}, 0.89 {3H, m, $CH_3(CH_2)_5CO$-}. Anal. calcd for $C_{16}H_{23}O_5N_2CO$ (354.399): C, 54.17; H, 6.48. Found: C, 54.20; H, 6.51.

5´-*O*-(Octanoyl)thymidine (Compound 5)

FTIR (KBr) ν_{max} (c^{m-1}): 1731 (-CO), 3400 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 8. 98 (1H, s, -NH), 7.12 (1H, d, J=1.8 Hz, H-6), 6.80 (1H, t, J=6.6 Hz, H-1´), 4.43 (1H, m, H-3´), 4.33 (1H, dd, J=12.0 and 4.5 Hz, H-5´a), 4.28 (1H, dd, J=12.2 and 3.6 Hz, H-5´b), 4.10 (1H, m, H-4´), 3.41 (1H, br s, 3´-OH), 2.40 (1H, ddd, J=13.6, 6.6 and 4.6 Hz, H-2´a), 2.24 (1H, ddd, J=12.5, 6.1 and 6.2 Hz, H-2´b), 1.92 (3H, d, J=1.3 Hz, 5-CH_3), 2.36 {2H, m, $CH_3(CH_2)_5CH_2CO$-}, 1.61 {2H, m, $CH_3(CH_2)_4CH_2CH_2CO$-}, 1.24 {8H, m, $CH_3(CH_2)_4(CH_2)_2CO$-}, 0.86 {3H, m, $CH_3(CH_2)_6CO$-}. Anal. calcd for $C_{17}H_{29}O_5N_2CO$ (368.429): C, 55.37; H, 7.87. Found: C, 55.39; H, 7.90.

5´-*O*-(Decanoyl)thymidine (Compound 6)

FTIR (KBr) ν_{max} (cm^{-1}): 1701 (-CO), 3399 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 8. 98 (1H, s, -NH), 7.17 (1H, d, J=2.3 Hz, H-6), 6.22 (1H, t, J=6.5 Hz, H-1´), 4.28 (1H, m, H-3´), 4.41 (1H, dd, J=11.8 and 4.6 Hz, H-5´a), 4.20 (1H, dd, J=12.1 and 4.5 Hz, H-5´b), 4.01 (1H, ddd, J=4.5, 5.5 and 4.9 Hz, H-4´), 3.41 (1H, s, 3´-OH), 2.36 (1H, ddd, J=13.0, 6.8 and 4.5 Hz, H-2´a), 2.32 {2H, m, $CH_3(CH_2)_7CH_2CO$-}, 2.20 (1H, ddd, J=13.6, 6.2 and 6.8 Hz, H-2´b), 1.90 (3H, d, J=1.3 Hz, 5-CH_3), 1.64 {2H, m, $CH_3(CH_2)_6CH_2CH_2CO$-}, 1.24 {12H, m, $CH_3(CH_2)_6CH_2CH_2CO$-}, 0.87 {3H, m, $CH_3(CH_2)_8CO$-}. Anal. calcd for $C_{19}H_{33}O_5N_2CO$ (396.479): C, 57.50; H, 8.32. Found: C, 57.52; H, 8.34.

5´-*O*-(Myristoyl)thymidine (Compound 7)

FTIR (KBr) ν_{max} (cm^{-1}): 1702 (-CO), 3398 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δH 8. 61 (1H, s, -NH), 7.27 (1H, d, J=1.3 Hz, H-6), 6.24 (1H, t, J=6.5 Hz, H-1´),

4.40-4.32 (1H, m, H-3´), 4.40 (1H, dd, J=12.0 and 4.5 Hz, H-5´a), 4.25 (1H, dd, J=12.0 and 3.5 Hz, H-5´b), 4.15 (1H, ddd, J = 3.5, 4.5 and 3.9 Hz, H-4´), 3.47 (1H, br, 3´-OH), 2.38 (1H, ddd, J = 13.5, 6.5 and 4.0 Hz, H-2´a), 2.34 {2H, m, $CH_3(CH_2)_{11}CH_2CO$-}, 2.25 (1H, ddd, J=13.5, 6.5 and 6.7 Hz, H-2´b), 1.92 (3H, d, J=1.3 Hz, 5-CH_3), 1.74-1.48 {2H, m, $CH_3(CH_2)_{10}CH_2CH_2CO$-}, 1.42-1.14 {20H, br m, $CH_3(CH_2)_{10}CH_2CH_2CO$-}, 0.86 {3H, t, J = 6.8 Hz, $CH_3(CH_2)_{12}CO$-}. Anal. calcd for $C_{23}H_{41}O_5N_2CO$ (452.589): C, 60.98; H, 9.05. Found: C, 60.99; H, 9.08.

5´-*O*-(Pivaloyl)thymidine (Compound 8)

FTIR (KBr) ν_{max} (cm^{-1}): 1708 (-CO), 3408 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 9. 00 (1H, s, -NH), 7.31 (1H, d, J=2.3 Hz, H-6), 6.25 (1H, t, J=6.6 Hz, H-1´), 4.20 (1H, m, H-3´), 4.41 (1H, dd, J=12.0 and 4.5 Hz, H-5´a), 4.25 (1H, m, H-5´b), 3.94 (1H, m, H-4´), 3.46 (1H, s, 3´-OH), 2.38 (1H, ddd, J=13.5, 6.5 and 4.0 Hz, H-2´a), 2.25 (1H, ddd, J=13.5, 6.5 and 6.7 Hz, H-2´b), 1.93 (3H, d, J=1.3 Hz, 5-CH_3), 1.22 {9H, s, $(CH_3)_3CCO$-}. Anal. calcd for $C_{14}H_{23}O_5N_2CO$ (326.349): C, 51.47; H, 7.04. Found: C, 51.49; H, 7.07.

5´-*O*-(Benzenesulphonyl)thymidine (Compound 9)

FTIR (KBr) ν_{max} (cm^{-1}): 1735 (-CO), 3378 (-OH), 1365 (-SO_2). ^{1}H-NMR (400 MHz, CDCl3): δ_H 8. 92 (1H, s, -NH), 7.88 (2H, m, Ar-H), 7.75 (1H, m, Ar-H), 7.60 (2H, m, Ar-H), 7.14 (1H, d, J=1.2 Hz, H-6), 6.82 (1H, t, J=6.4 Hz, H-1´), 4.41 (1H, m, H-3´), 4.22 (1H, dd, J=12.1 and 4.4 Hz, H-5´a), 4.19 (1H, dd, J=12.0 and 4.5 Hz, H-5´b), 3.96 (1H, ddd, J=3.4, 4.4 and 3.7 Hz, H-4´), 3.36 (1H, s, 3´-OH), 2.83 (1H, ddd, J=13.5, 6.5 and 4.0 Hz, H-2´a), 2.68 (1H, ddd, J=13.5, 6.5 and 6.7 Hz, H-2´b), 1.96 (3H, d, J=1.3 Hz, 5-CH_3). Anal. calcd for $C_{16}H_{19}O_5N_2SO_2$ (382.389): C, 50.21; H, 4.96. Found: C, 50.24; H, 4.99.

5´-*O*-(2-Chlorobenzoyl)thymidine (Compound 10)

FTIR (KBr) ν_{max} (cm^{-1}): 1701 (-CO), 3402 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 9. 01 (1H, s, -NH), 7.78 (1H, m, Ar-H), 7.61 (2H, m, Ar-H), 7.28 (1H, m, Ar-H), 7.15 (1H, d, J=1.7 Hz, H-6), 6.80 (1H, t, J=6.6 Hz, H-1´), 4.21 (1H, m, H-3´), 4.19 (1H, dd, J=12.1 and 4.7 Hz, H-5´a), 4.15 (1H, dd, J=11.9 and 3.8 Hz, H-5´b), 4.00 (1H, ddd, J=3.7, 4.8 and 4.2 Hz, H-4´), 3.35 (1H, s, 3´-OH), 2.86 (1H, m, H-2´a), 2.65 (1H, m, H-2´b), 1.72 (3H, d, J=1.6 Hz, 5-CH3). Anal. calcd for $ClC_{16}H_{17}N_2O_5CO$ (379.776): C, 50.60; H, 4.51. Found: C, 50.65; H, 4.54.

5´-*O*-(2-Bromobenzoyl)thymidine (Compound 11)

FTIR (KBr) ν_{max} (cm^{-1}): 1702 (-CO), 3399 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 8. 71 (1H, s, -NH), 7.81 (1H, d, J=7.8 Hz, Ar-H), 7.27 (1H, d, J=1.3 Hz, H-6), 7.67 (2H, m, Ar-H), 7.42 (1H, m, Ar-H), 6.28 (1H, t, J=6.7 Hz, H-1´), 4.43-4.29 (1H,

m, H-3´), 4.54 (1H, dd, J=12.1 and 4.6 Hz, H-5´a), 4.25 (1H, dd, J=12.1 and 3.7 Hz, H-5´b), 4.19 (1H, ddd, J=3.5, 4.6 and 4.1 Hz, H-4´), 3.47 (1H, br s, 3´-OH), 2.42 (1H, ddd, J=13.6, 6.5 and 4.2 Hz, H-2´a), 2.25 (1H, ddd, J=13.5, 6.7 and 6.8 Hz, H-2´b), 1.92 (3H, d, J=1.6 Hz, 5-CH_3). Anal. calcd for $BrC_{16}H_{18}O_5N_2CO$ (425.229): C, 45.15; H, 4.23. Found: C, 45.18; H, 4.26.

5´-*O*-4-(Bromobenzoyl)thymidine (Compound 12)

FTIR (KBr) ν_{max} (cm^{-1}): 1698 (-CO), 3396 (-OH). ^{1}H-NMR (400 MHz, $CDCl_3$): δH 9. 00 (1H, s, -NH), 7.91 (2H, m, Ar-H), 7.61 (2H, m, Ar-H), 7.21 (1H, d, J=1.8 Hz, H-6), 6.82 (1H, t, J=6.6 Hz, H-1´), 4.52 (1H, m, H-3´), 4.39 (1H, dd, J=11.8 and 4.3 Hz, H-5´a), 4.18 (1H, dd, J=12.0 and 3.6 Hz, H-5´b), 4.10 (1H, ddd, J=3.6, 4.7 and 4.2 Hz, H-4´), 3.36 (1H, s, 3´-OH), 2.74 (1H, ddd, J=13.1, 6.4 and 4.1 Hz, H-2´a), 2.12 (1H, ddd, J=13.2, 6.3 and 6.6 Hz, H-2´b), 1.78 (3H, d, J=1.6 Hz, 5-CH_3). Anal. calcd for $BrC_{16}H_{18}O_5N_2CO$ (425.231): C, 45.15; H, 4.23. Found: C, 45.19; H, 4.25.

5´-*O*-(Cinnamoyl)thymidine (Compound 13)

FTIR (KBr) ν_{max} (cm^{-1}): 1735 (-CO), 3400 (-OH), 1628 (-CH=CH-). ^{1}H-NMR (400 MHz, $CDCl_3$): δH 9. 31 (1H, s, -NH), 7.75 (1H, d, J=12.0 Hz, PhCH=CHCO-), 7.51 (2H, m, Ar-H), 7.40 (1H, d, J=1.8 Hz, H-6), 7.11 (3H, m, Ar-H), 6.82 (1H, d, J=12.1 Hz, PhCH=CHCO-), 6.44 (1H, t, J=6.4 Hz, H-1´), 4.45 (1H, m, H-3´), 4.23 (1H, dd, J=12.1 and 4.5 Hz, H-5´a), 4.18 (1H, dd, J=12.0 and 3.6 Hz, H-5´b), 3.96 (1H, ddd, J=3.8, 4.8 and 3.8 Hz, H-4´), 3.35 (1H, br s, 3´-OH), 2.44 (1H, ddd, J=13.4, 6.4 and 4.4 Hz, H-2´a), 2.15 (1H, ddd, J=13.5, 6.6 and 6.8 Hz, H-2´b), 1.90 (3H, d, J=1.6 Hz, 5-CH_3). Anal. calcd for $C_{18}H_{21}O_5N_2CO$ (372.369): C, 58.0; H, 5.64. Found: C, 58.03; H, 5.66.

Tested Bacterial pathogens

Test tube cultures of bacterial pathogens were obtained from the Department of Biochemistry and Molecular Biology, University of Chittagong. The synthesized test compounds (Scheme 1 & 2) were subjected to antibacterial screening against two Gram-positive (*Bacillus subtilis* BTCC 17 & *Bacillus cereus* BTCC 19) and two Gram-negative (*Escherichia coli* ATCC 25922 & *Salmonella paratyphi* AE 14612) bacterial strains (Table 1).

Table 1. List of used bacteria

Types of bacteria	Name of tested bacteria	Strain no.
Gram +Ve	*Bacillus subtilis* *Bacillus cereus*	BTCC 17 BTCC 19
Gram -Ve	*Escherichia coli* *Salmonella paratyphi*	ATCC 25922 AE 146313

Antibacterial assay

The *in vitro* antibacterial spectrum of the newly synthesized thymidine derivatives (2-13) was done by disc diffusion method[20] with little modification[21]. Sterilized paper discs of 4 mm in diameter and Petri dishes of 150 mm in diameter were used throughout the experiment. The autoclaved Mueller-Hinton agar medium, cooled to 45˚C, was poured into sterilized Petri dishes to a depth of 3 to 4 mm and after solidification of the agar medium; the plates were transferred to an incubator at 37˚C for 15 to 20 minutes to dry off the moisture that developed on the agar surface. The plates were inoculated with the standard bacterial suspensions (as McFarland 0.5 standard) followed by spread plate method and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 50 μg dry weight/disc from 2% solution (in $CHCl_3$) of each test chemical using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A control plate was also maintained in each case without any test chemical. These plates were kept for 4-6 hours at low temperature (4-6˚C) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at 35±2˚C for 24 hours to allow maximum growth of the organisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions in millimeter. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic ampicillin (20 μg/disc, BEXIMCO Pharm Bangladesh Ltd).

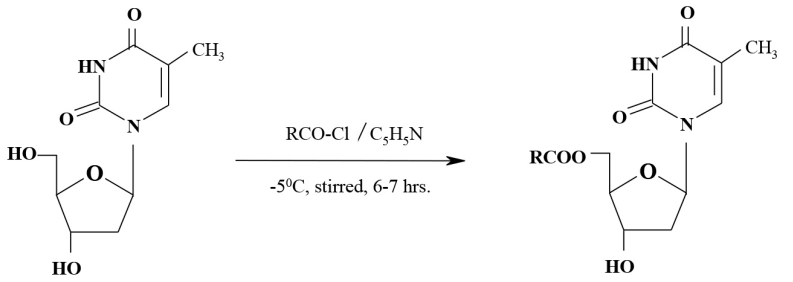
RESULTS AND DISCUSSION

Synthesis and spectroscopic characterization

In the present study, selective acylation of thymidine (1) was performed using the direct method. The structure of the acylated products were ascertained by analyzing their FTIR and ^{1}H-NMR spectra[22-24]. The reaction pathways have been summarized in the Scheme 1- 2 and Table 2.

Our initial effort was reacted thymidine (1) with unimolecular amount of acetic anhydride as acylating agent in dry pyridine at freezing temperature, followed by removal of solvent and silica gel column chromatographic purification, furnished the 5′-*O*-acetyl derevative (2). The FTIR spectrum of compound 2 showed the following absorption bands: 1684 cm^{-1} (due to -CO), 3430 cm^{-1} and (due to –OH) and stretchings. In its ^{1}H-NMR spectrum one three-proton singlet at ● 2.11 was due to the methyl protons of one acetyloxy group. The downfield shifts of H-5/ to δ 4.18 (as dd, J = 12.0 and 4.7 Hz, H-5´a) and 4.11 (as, dd, J = 12.1 and 4.5 Hz, H-5´b) as compared to the usual values indicated the attachment of the acetyl

group at position 5′. Complete analysis of the rest of the FTIR and 1H-NMR spectra was in supported the structure ascertained as 5′-*O*-(acetyl)thymidine (2). This finding was in conformity with the mechanism proposed by Parang et al.[25] based on similar thymidine derivatives.



Scheme 1. Synthetic pathway followed for the preparation of thymidine derivatives

Compound no.	R	Compound no.	R
2	CH_3CO-	**8**	$(CH_3)_3CCO$-
3	$CH_3(CH_2)_3CO$-	**9**	$C_6H_5SO_2$-
4	$CH_3(CH_2)_5CO$-	**10**	2-Cl.C_6H_4CO-
5	$CH_3(CH_2)_6CO$-	**11**	2-Br.C_6H_4CO-
6	$CH_3(CH_2)_8CO$-	**12**	4-Br.C_6H_4CO-
7	$CH_3(CH_2)_{12}CO$-	**13**	C_6H_5CH=$CHCO$-

Scheme 2. Structure of thymidine derivatives (Compounds 2-13)

Further reaction was achieved by its conversion to the pentanoate derivative **3.** In its ^{1}H-NMR spectrum, the resonance peaks three two-proton multiplets at δ 2.36 {$CH_3(CH_2)_2CH_2CO$-}, δ 1.64 {$CH_3CH_2CH_2CH_2CO$-} and δ 1.24 {$CH_3CH_2(CH_2)_2CO$-} and one three-proton multiplet at δ 0.88 {$CH_3(CH_2)_3CO$-} showed the presence of one pentanoyl group in the compound. The deshielding of C-5/ protons to δ 4.40 (as dd, J = 12.0 and 4.5 Hz, H-5′a) and 4.25 (as dd, J = 12.0 and 3.5 Hz, H-5′b) indicated the introduction of the one pentanoyl group at position 5′. Heptanoylation of the thymidine **(1)** with heptanoyl chloride in dry C_5H_5N using the conventional work-up and purification procedure provided the heptanoate derivative **(4)**. The FTIR, ^{1}H-NMR and elemental analysis enabled us to assign the structure of the heptanoyl derivative as 5′-*O*-(heptanoyl)thymidine **(4).** The same thymidine **1** was then converted to the octanoyl and decanoyl derivatives **(5 & 6)**. The structure of the octanoyl and decanoyl derivatives **(5**

& 6) were confidently established by completely analyzing their FTIR, ^{1}H-NMR and elemental data as 5/-*O*-(octanoyl)thymidine **(5)** and 5/-*O*-(decanoyl) thymidine **(6).**

Table 2. Physicochemical properties of the synthesized of thymidine derivatives (2-13).

Compound no	RT (h)	Rf	Yield (%)	Physical State
2	6.0	0.52	70.5	semi solid
3	6.5	0.50	74.0	semi solid
4	6.0	0.52	68.5	liquid syrup
5	6.0	0.51	81.0	thick syrup
6	6.0	0.50	76.0	needles, m.p. 120-1250°C
7	6.5	0.52	78.0	semi solid
8	7.0	0.53	76.0	liquid
9	6.0	0.51	79.5	pasty mass
10	6.5	0.52	62.5	pasty mass
11	6.5	0.51	69.0	pasty mass
12	6.0	0.49	72.5	semi solid mass
13	6.0	0.51	82.5	semi solid

Thymidine **(1)** on treatment with myristoyl chloride, afforded compound **7** and its FTIR spectrum the absorption bands at 1702 cm^{-1} for C=O stretching and 3398 cm^{-1} for -OH stretching. The ^{1}H-NMR spectrum displayed a two two-proton multiplet at δ 2.34 {$CH_3(CH_2)_{11}CH_2CO$-}, and δ 1.74-1.48 {$CH_3(CH_2)_{10}CH_2CH_2CO$-}, a twenty-proton multiplet at δ 1.42-1.14 {$CH_3(CH2)_{10}CH_2CH_2CO$-}, and a three-proton triplet at δ 0.86 {$CH_3(CH_2)_{12}CO$-} suggested the attachment of one myristoyl group in the compound. The rest of the ^{1}H-NMR spectrum was in conformity with the structure accorded to it. The formation of compound **7** may be explained by assuming that myristoyl chloride attaches with the most reactive and less sterically hindered primary hydroxyl group of the ribose moiety at 5/ position, thereby forming the 5´-*O*-(myristoyl) thymidine **(7)** as the sole product.

In the ^{1}H-NMR spectrum of the compound **8** showed a nine-proton singlet at δ 1.22 {$(CH_3)_3CCO$-} was due to the methyl protons of pivaloyl group which indicated the introduction of a pivaloyl group. The downfield shift of H-5/ proton to δ 4.41 (1H, dd, J = 12.0 and 4.5 Hz, H-5´a), and δ 4.25 (as m) from their precursor value and these δ values showed the attachment of the pivaloyl group at position 5/. The rest of the FTIR and 1H-NMR spectra was compatible with the structure assigned as 5/-*O*-(pivaloyl)thymidine **(8).**

Thus, treatment of compound **1** with benzenesulfonyl chloride in dry pyridine followed by usual work-up procedure. The FTIR spectrum of this compound showed the following absorption bands: 1735 cm^{-1} (C=O stretching), 3378 cm^{-1} (-OH stretching) and 1365 cm^{-1} (-SO_2 stretching). In its ^{1}H-NMR spectrum the peaks at δ 7.88 (2H, m), δ 7.75 (1H, m) and δ 7.60 (2H, m) corresponded the protons of one phenyl group. The downfield shift of H-5′ to δ 4.22 (as dd, J = 12.1 and 4.4 Hz, H-5´a) and δ 4.19 (as dd, J = 12.0 and 4.5 Hz, H-5´b) from their usual values ascertained the attachment of benzenesulfonyl group at position 5′. By complete analysis of the FTIR, 1H-NMR and elemental data, the structure of the compound was ascertained as 5′-*O*-(benzenesulphonyl)thymidine **(9).**

Encouraged by the results obtained, we performed 2-chlorobenzoylation of compound **1** using similar procedures and isolated compound **10** in (125 mg, 62.5%) as a pasty mass. The FTIR and ^{1}H-NMR spectrum was in accord with the structure of this compound assigned as 5´-*O*-(2-chlorobenzenzoyl) thymidine **(10).** The formation of a monosubstitution product **11** was clearly revealed by its ^{1}H-NMR spectrum which showed one one-proton doublet at δ 7.81 (as d, J = 7.8 Hz), one two-proton multiplet at δ 7.67 (2H, m) and one one-proton multiplet at δ 7.42 (1H, m) corresponding to the aromatic ring protons of one 2-bromobenzoyl group in the molecule. Complete analysis of the FTIR, ^{1}H-NMR of this compound was in agreement with the structure accorded as 5′-*O*-(2-bromobenzoyl)thymidine **(11).** As same as reaction of compound **1** with 4-bromobenzoyl chloride in dry C_6H_5N, as usual procedure and purification gave the 4-bromobenzoyl derivative **(12).**

Finally, we have carried out cinnamoylation of **1** with an excess of cinnamoyl chloride in pyridine as same work-up and purification techniques, we isolated compound **(13)** in (165 mg, 82.5%) as a semi solid. FTIR spectrum showed absorption bands at 1735 cm^{-1} (for -CO stretching), 3400 cm^{-1} (for -OH-stretching) and 1628 cm^{-1} (for -CH=CH- stretching). In the ^{1}H-NMR spectrum one one-proton doublet at δ 7.75 (as d, J = 12.0 Hz, PhCH=CHCO-) and also one one-proton doublet at δ 6.82 (as d, J = 12.1 Hz, PhCH=C*H*CO-) due to the presence of one cinnamoyl group in the molecule. In addition a two-proton multiplet at δ 7.51 (as m, Ar-H) and a three-proton multiplet at δ 7.11 (as, m, Ar-H) due to the one aromatic ring protons. The downfield shift of C-5 to δ 4.23 (as dd) and δ 4.18 (as dd) from their usual values in the precursor compound 1 and the resonances of other protons in their anticipated positions, showed the presence of the cinnamoyl group at position 5´. The rest of the FTIR and ^{1}H-NMR was in accord with the structure of this compound assigned as *5´-O*-(cinnamoyl)thymidine **(13)**. Thus, selective acylation of thymidine **(1)**

with a number of acylating agents by using the direct method was carried out successfully. The study was found to be very promising since all the reactions; a single monosubstitution product was isolated in reasonably high yield.

Antibacterial activity

The results of antibacterial screening of the test chemicals and the standard antibiotic, Azithromycin are furnished in Table 3 and Figure 2-3. The results revealed that most of the derivatives were prone to antibacterial action against most of the Gram-positive and Gram-negative bacteria. The results exhibited that the test compounds **9** and **13** were highly active towards the growth of all the Gram-positive bacteria. Compound **10** and **11** were completely insensitive towards any of the Gram-positive bacteria.

Table 3. Zone of inhibition observed against Gram+Ve and Gram-Ve test organisms by the thymidine derivatives.

Compound no.	Zone of inhibition (mm) at 200 µg dw/disc			
	Gram +Ve bacteria		Gram -Ve bacteria	
	B. subtilis	*B. cereus*	*E. coli*	*S. paratyphi*
2	NF	5	NF	NF
3	*14	8	*15	7
4	8	7	8	*11
5	10	9	10	8
6	*12	6	*13	9
7	6	*12	6	*12
8	5	6	7	7
9	*13	*14	*12	*11
10	NF	NF	5	NF
11	NF	NF	NF	NF
12	7	8	8	6
13	*12	10	9	9
** Azithromycin	*22	*18	*22	*20

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.

The results showed that except the test chemicals **2, 11** all other test chemicals were found to be effective towards the different Gram-negative bacteria in different degrees. The test chemical **3, 6** and **9** very significantly inhibited the growth of all Gram-negative bacterial strains used.

The inhibition of *E. coli* by **3** (15 mm), **6** (13 mm), **9** (12 mm), of *S. paratyphi* by **4** (11 mm), **7** (12 mm), **9** (11 mm), were remarkable. The inhibitions of growth of

bacteria were very remarkable in many cases which were in conformity with our previous work[26-29]. However, compound 11 was found insensitive towards all the Gram-positive and Gram-negative bacteria.

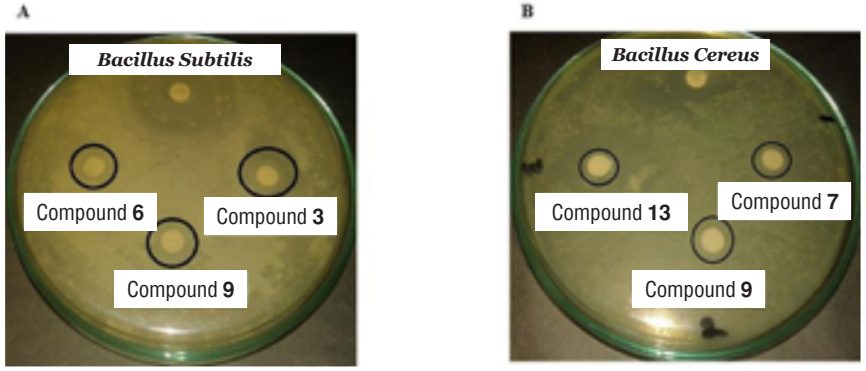


Figure 2. % Zone of inhibition of the compounds 3, 6 and 9 against *B. subtilis* (A) and the compounds 7, 9 and 13 against *B. cereus* (B).

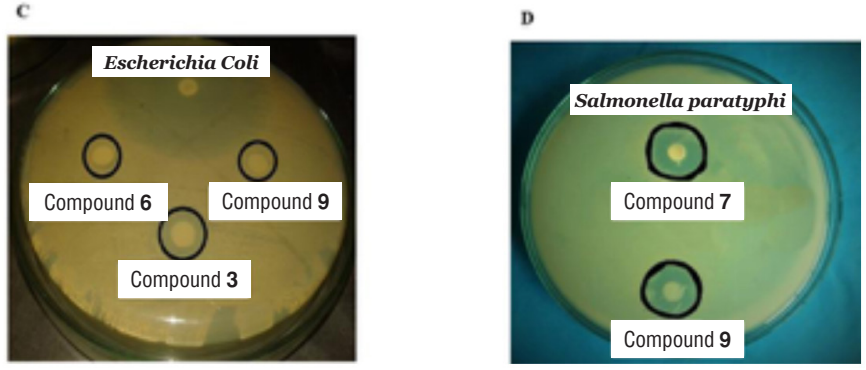


Figure 3. % Zone of inhibition of the compounds 3, 6 and 9 against *E. coli* (C) and the compounds 7 and 9 against *S. paratyphi* (D).

In general, it has been noticed that antibacterial results of the selectively acylated thymidine derivatives obtained by using various acylating agents follow the order for Gram positive organisms: **3 > 9 > 6 = 13 > 5 > 4 > 12 > 7 > 8** and Gram negative bacteria follow the order: **3 > 6 > 9 > 5 > 13 > 12 > 4 > 7.**

In a word this series of test chemicals was found to show very good antibacterial activity, particularly the presence of different acyl groups e.g. pentanoyl, decanoyl, benzenesulphonyl, 2-bromobenzoyl, cinnamoyl, groups improved the

antibacterial activity by a very good margin which was in accordance with our previous work[14]. We believe that a similar hydrophobic interaction might occur between the acyl chains of uridine accumulated in the lipid like nature of the bacteria membranes. As a consequence of their hydrophobic interaction, bacteria lose their membrane permeability, ultimately causing death of the bacteria[30-32].

CONCLUSION

We report an efficient one-pot synthesis of a small library of novel thymidine derivatives by direct method. We show that this one-pot three component reaction appears to be favorable for the preparation of variously substituted thymidine derivatives in moderate to good yields and opens the way for preparation of libraries of other nucleoside derivatives as potential biologically active molecules. Preliminary antibacterial in vitro screening against human pathogens showed that some of the prepared thymidine derivatives possess promising antibacterial activity. In addition, the antifungal, anticancer and antiviral activities of the thymidine derivatives thereof are currently under investigation and will be published in due course.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Education (MOE), Government of the People's Republic of Bangladesh for providing financial support *(Ref.: 37-01.0000.078.02.018.13-50, 2014-2015)* to carrying out this research project. We are also indebted to the Chairman, Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka, for providing the ^{1}H-NMR spectra.

REFERENCES

1. Yarchoan, R.; Mitsuya, H.; Broder, S. AIDS Therapies. *Sci. Am.* **1988,** *259,* 110-9.

2. Cao, S.; Sun, L. Q.; Wang, M. Antimicrobial Activity and Mechanism of Action of Nu-3, a Protonated Modified Nucleotide. *Ann. Clin. Microbiol. Antimicrob.* **2011,** *10,* 01-10.

3. Murata, D.; Endo, Y.; Obata, T.; Sakamoto, K.; Syouji, Y.; Kadohira, M.; Matsuda, A.; Sasaki, T. A Crucial Role of Uridine/Cytidine Kinase 2 in Antitumor Activity of 3/-Ethynyl Nucleosides. *Drug Metab. Dispos.* **2004,** *32,* 1178-1182.

4. Xiao, P.; Huang, H.; Chen, J.; Li, X. In Vitro Antioxidant and Anti-inflammatory Activities of Radix Isatidis Extract and Bioaccessibility of Six Bioactive Compounds After Simulated Gastro-intestinal Digestion. *J. Ethnopharmacol.* **2014,** *57,* 55-61.

5. Schettler, C. H. Virus Hepatitis of Geese 3. Properties of the Causal Agent. *Avian Pathol.* **1973,** *2,* 179-193.

6. Sterzycki, R. Z.; Ghazzouli, I.; Brankovan, V.; Martin, J. C.; Mansuri, M. M. Synthesis and Anti-HIV Activity of Several 2′-fluoro-Containing Pyrimidine Nucleosides. *J. Med. Chem.* **1990,** *33,* 2150-2157.

7. Smith, D. B.; Kalayanov, G.; Sund, C.; Winqvist, A.; Pinho, P. ; Maltseva, T.; Morisson, V.; Leveque, V.; Rajyaguru, S.; Pogam, S. L.; Najera, I.; Benkestock, K.; Zhou, X. X.; Maag, H.; Cammack, N.; Martin, J. A.; Swallow, S.; Johansson, N. G.; Klumpp, K.; Smith, M. The Design, Synthesis, and Antiviral Activity of 4′-Azidocytidine Analogues Against Hepatitis C Virus Replication: the Discovery of 4′-Azidoarabinocytidine. *J. Med. Chem.* **2009,** *52,* 219-223.

8. Krol, E.; Wandzik, I.; Szeja, W.; Grynkiewicz, G.; Szewczyk, B. In Vitro Antiviral Activity of Some Uridine Derivatives of 2-Deoxy Sugars Against Classical Swine Fever Virus. *Antiviral Res.* **2010,** *86,* 54-162.

9. Kimura, K.; Bugg, T. D. H. Recent Advances in Antimicrobial Nucleoside Antibiotics Targeting Cell Wall Biosynthesis. *Nat. Prod. Rep.* **2003,** *20,* 252-273.

10. Ghorab, M. M.; Ismail, Z. H.; Gaward, S. M. A.; Aziem, A. A. Antimicrobial Activity of Amino Acid, Imidazole and Sulfonamide Derivatives of Pyrazolo[3,4-d]pyrimidine. *Heteroatom Chem.* **2004,** *15,* 57-62.

11. Kabir, A. K. M. S.; Kawsar, S. M. A.; Bhuiyan, M. M. R.; Islam, M. R.; Rahman, M. S. Biological Evaluation of Some Mannopyranoside Derivatives. *Bull. Pure Appl. Sci.* (India), **2004,** *23C,* 83-91.

12. Kabir, A. K. M. S.; Dutta, P.; Anwar, M. N. Synthesis of Some Derivatives of D-Mannose for Biological Studies. *Bull. Pure Appl. Sci.* (India), **2003,** *22C,* 119-127.

13. Gupta, R.; Paul, S.; Gupta, A. K.; Kachroo, P. L.; Bani, S. Synthesis and Biological Activities of Some Substituted Phenyl-3-(3-alkyl/aryl-5,6-dihydro-s-triazolo[3,4-b][1,3,4]thiazol-6-yl) Indoles. *Indian J. Chem.* **1997.** *36,* 707-710.

14. Kawsar, S. M. A.; Hamida, A. A.; Sheikh, A. U.; Hossain, M. K.; Shagir, A. C.; Sanaullah, A. F. M.; Manchur, M. A.; Imtiaj, H.; Ogawa, Y.; Fujii, Y.; Koide, Y.; Ozeki, Y. Chemically Modified Uridine Molecules Incorporating Acyl Residues to Enhance Antibacterial and Cytotoxic Activities. *Int. J. Org. Chem.* **2015,** *5,* 232-245.

15. Kawsar, S. M. A.; Faruk, M. O.; Rahman, M. S.; Fujii, Y.; Ozeki, Y. Regioselective Synthesis, Characterization and Antimicrobial Activities of Some New Monosaccharide Derivatives. *Sci. Pharm.* **2014,** 82, 1-20.

16. Kawsar, S. M. A.; Sharif, U.; Manchur, M. A.; Fujii, Y.; Ozeki, Y. Acylation of D-Glucose Derivatives Over C_5H_5N: Spectral Characterization and *In Vitro* Antibacterial Activities. *Int. J. Biol. Chem.* **2015,** *9,* 269-282.

17. Kawsar, S. M. A.; Nishat, S. S. B. S.; Manchur, M. A.; Ozeki, Y. Benzenesulfonylation of Methyl α-D-Glucopyranoside: Synthesis, Characterization and Antibacterial Screening. *Int. Lett. Chem. Phys. Astron.* **2016,** *64,* 95-105.

18. Kawsar, S. M. A.; Sharif, U.; Nishat, S. S. B. S.; Manchur, M. A.; Ozeki, Y. Synthesis, Characterization and Antibacterial Susceptibility of Some Benzenesulfonyl and *N*-acetylsulfanilyl Derivatives of Methyl α-D-Glucopyranoside. *Curr. Res. Chem.* **2015,** *7,* 21-33.

19. Davoll, J.; Lythgoe, B.; Todd, A. R. Experiments on the Synthesis of Purine Nucleosides: A Synthesis of Guanosine. *J. Chem. Soc.* **1948,** 1685-1687.

20. Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disc Method. *Am. J. Clin. Pathol.* **1966,** *45,* 439-476.

21. Miah, M. A. T.; Ahmed, H. U.; Sharma, N. R.; Ali, A.; Miah, S. A. Antifungal Activity of Some Plant Extracts. *Bang. J. Bot.* **1990,** *19,* 05-10.

22. Loss, A.; Lutteke, T. Using NMR data on Glycosciences.de. *Method Mol. Biol.* **2015,** *1273,* 87-95.

23. Brauer, B.; Pincu, M,; Buch, V.; Bar, I.; Simons, J. P.; Gerber, R. B. Vibrational Spectra of α-Glucose, β-Glucose, and Sucrose: Anharmonic Calculations and Experiment. *J. Phys. Chem. A*. **2011,** *115,* 5859-5872.

24. Kawsar, S. M. A.; Khaleda, M.; Asma, R.; Manchur, M. A.; Koide, Y.; Ozeki, Y. Infrared, ^{1}H-NMR Spectral Studies of some Methyl 6-O-Myristoyl-α-D-Glucopyranoside Derivatives: Assessment of Antimicrobial Effects. *Int. Lett. Chem. Phys. Astron.* **2015,** *58,* 122-136.

25. Parang, K.; Wiebe L. I.; Knaus, E. E. Syntheses and Biological Evaluation of 5'-O-myristoyl Derivatives of Thymidine Against Human Immunodeficiency Virus. *Antiv. Chem. Chemother.* **1997,** *8,* 417-427.

26. Kawsar, S. M. A.; Faruk, M. O.; Mostafa, G.; Rahman, M. S. Synthesis and Spectroscopic Characterization of Some Novel Acylated Carbohydrate Derivatives and Evaluation of Their Antimicrobial Activities. *Chem. Biol. Interface,* **2014,** *4,* 37-47.

27. Kawsar, S. M. A.; Hasan, T.; Chowdhury, S. A.; Islam, M. M.; Hossain, M. K.; Manchur, M. A. Synthesis, Spectroscopic Characterization and *in vitro* Antibacterial Screening of Some D-glucose Derivatives. *Int. J. Pure Appl.* Chem. **2013,** *8,* 125-135.

28. Kawsar, S. M. A.; Kabir, A. K. M. S.; Bhuiyan, M. M. R.; Siddiqa, A; Anwar, M. N. Synthesis, Spectral and Antimicrobial Screening Studies of Some Acylated D-glucose Derivatives. *Rajiv Gandhi Univ. Health Sci. (RGUHS) J. Pharm. Sci.* **2012,** *2,* 107-115.

29. Kabir, A. K. M. S.; Kawsar, S. M. A.; Bhuiyan, M. M. R.; Rahman, M. S.; Banu, B. Biological Evaluation of Some Octanoyl Derivatives of Methyl 4,6-O-cyclohexylidene-a-D-glucopyranoside. *Chittagong Univ. J. Biol. Sci.* **2008,** *3,* 53-64.

30. Kim, Y. M.; Farrah, S.; Baney, R. H. Structure–Antimicrobial Activity Relationship for Silanols, a New Class of Disinfectants, Compared with Alcohols and Phenols. *Int. J. Antimicrob. Agents,* **2007,** *29,* 217-222.

31. Hunt, W. A. The Effects of Aliphatic Alcohols on the Biophysical and Biochemical Correlates of Membrane Function. *Adv. Exper. Med. Biol.* **1975,** *56,* 195-210.

32. Judge, V.; Narasimhan, B.; Ahuja, M.; Sriram, D.; Yogeeswari, P.; Clercq, E. D.; Pannecouque, C.; Balzarini, J. Synthesis, Antimycobacterial, Antiviral, Antimicrobial Activity and QSAR Studies of N2-acyl isonicotinic Acid Hydrazide Derivatives. *Med. Chem.,* **2013,** *9,* 53-76.