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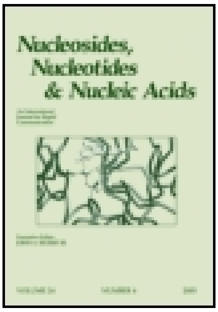

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis and Evaluation of Antimicrobial Activity of Some Pyrimidine Glycosides

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Version of record first published: 18 Aug 2008.

To cite this article: H. A. El-Sayed , A. H. Moustafa , A. Z. Haikal , I. M. Abdou & E. S. H. El-Ashry (2008): Synthesis and Evaluation of Antimicrobial Activity of Some Pyrimidine Glycosides, Nucleosides, Nucleotides and Nucleic Acids, 27:9, 1061-1071

To link to this article: http://dx.doi.org/10.1080/15257770802271805

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Nucleosides, Nucleotides and Nucleic Acids, 27:1061–1071, 2008
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ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802271805

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SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME PYRIMIDINE GLYCOSIDES

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□ *Reaction of ethyl 4-thioxo-3,4-dihydropyrimidine-5-carboxylate derivatives* ***1a,b*** *and ethyl 4-oxo-3,4-dihydropyrimidine-5-carboxylate* ***1c*** *with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in KOH or TEA afforded ethyl 2-aryl-4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio or/ oxy)-6-methylpyrimidine-5-carboxylate* ***6a-c****. The glucosides* ***6a*** *and* ***6b*** *were obtained by the reaction of* ***1a*** *and* ***1b*** *with peracetylated glucose****3*** *under MW irradiation. Mercuration of* ***1a*** *followed by reaction with acetobromoglucose gave the same product* ***6a****. The reaction of* ***1a-c*** *with peracetylated ribose* ***4*** *under MW irradiation gave ethyl 2-aryl-4-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosylthio)-6-methylpyrimidine-5-carboxylate* ***8a–c****. The deprotection of* ***6a–c*** *and* ***8a–****c in the presence of methanol and TEA/H_2O afforded the deprotected products* ***7a–c*** *and* ***9a–c****. The structure were confirmed by using ^1H and ^{13}CNMR spectra. Selected members of these compounds were screened for antimicrobial activity.*

Keywords Pyrimidin-4-one or/thione; pyrimidine glycosides and/ribosides; antimicrobial activity

INTRODUCTION

Pyrimidines, being an integral part of DNA and RNA, play an essential role in several biological processes and have considerable chemical and pharmacological importance. The pyrimidine ring can be found in antiviral nucleosides, antibiotics, antibacterials, antitumor, cardiovascular as well as agrochemical, veterinary products and antimycobacterial agents.[1–6] Some pyrimidine derivatives exhibit potent nanomolar activities against GSK-3β

Received 7 April 2008; accepted 22 May 2008.
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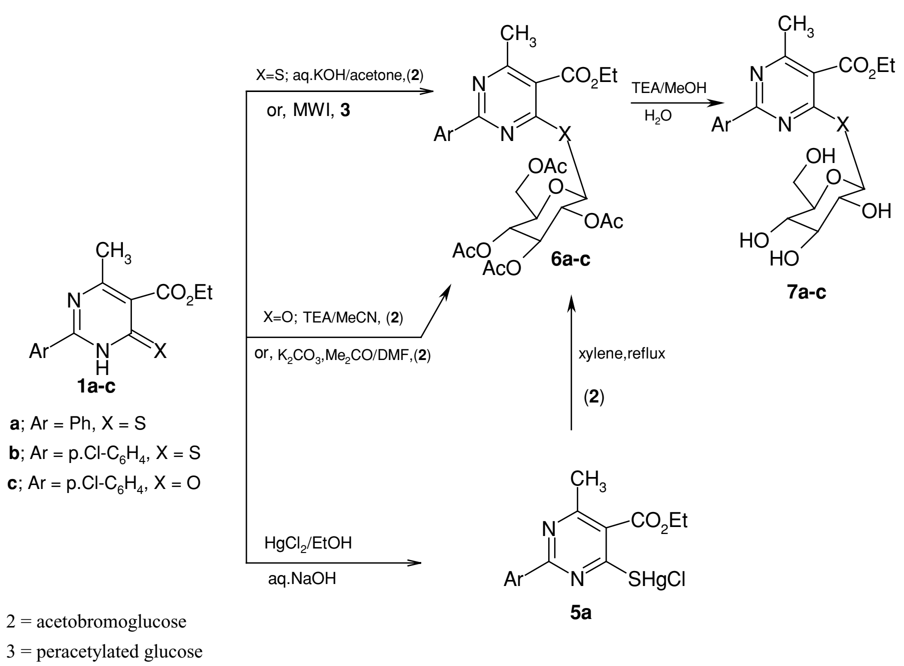
kinase as well as in an NF-$\kappa\beta$ reporter gene assay,[7] and anticancer activity against the MCF-7 cell line.[8] There are pyrimidine acyclic nucleosides that act as moderate viral replication inhibitors against HBV.[9]

Glycosylthio-heterocycles have attracted much attention because of their abilities to function as biological inhibitors,[10–17] inducers, and ligands[18] for affinity of chromatography of carbohydrate-process enzymes and proteins. For these reasons we synthesized several pyrimidine glycosides for evaluation of their biological activity.

RESULTS AND DISCUSSION

We report in this work the results of our investigation into the utility of the reaction of previous reported pyrimidine[19,20] with α-halo and peracetylated sugars, for the synthesis of some glucosides and ribosides. Glucosides **6a** and **6b** could be obtained by the reaction of ethyl 2-aryl-4-mercapto-6-methyl-pyrimidine-5-carboxylate **(1a)** and **(1b)** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(2)** in the presence of aqueous potassium hydroxide[4] in 50 and 69% yields, respectively. The same glucosides **6a** and **6b** could be obtained in better yields by MW irradiation of **1a** and **1b** with peracetylated glucose **(3)** for 30 seconds using silica gel as a solid support.[21] On the other hand, glucoside **6a** was obtained in 15% yield by heating chloromercuric salt **5a** of **1a** with **2** in dry xylene at reflux (Scheme 1).



SCHEME 1

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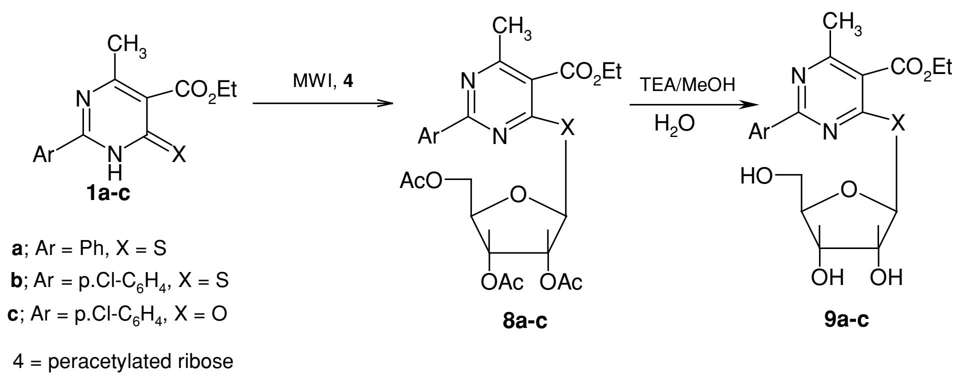
The structure of **6a** and **6b** were confirmed by using IR, UV, ^{1}H, and ^{13}C NMR spectra. The ^{1}H NMR spectra of **6a** and **6b** exhibited doublets at δ 6.22 and 6.40 with ($J_{1',2'}$ = 8.00 and 8.32 Hz), respectively, for the H-1′ protons characteristic for β-configuration. The ^{13}C NMR spectra revealed the absence of signals for (C = S) group, the presence of signals at δ 165.8 and 167.0 for C-4 atoms, and signals at δ 79.1 and 79.9 for the anomeric carbons, respectively.

Glucoside **6c** was obtained by the reaction of ethyl 2-(4-chlorophenyl)-4-oxo-6-methyl-3,4-dihydropyrimidine-5-carboxylate **(1c)** with glucosyl bromide **2** at room temperature in presence of triethylamine.[22] On the other hand, the same glucoside **6c** was obtained in 53% yield in the presence of acetone/DMF and potassium carbonate (Scheme 1). The ^{1}H NMR spectrum of **6c** showed the singlets at δ 1.89, 2.00, 2.01, and 2.04 characteristic for the acetoxy groups of the sugar and a doublet at δ 6.21 ($J_{1',2'}$ = 7.78 Hz) for the H-1′ proton which is characteristic for the β-configuration. The ^{13}C NMR spectrum of compound 6c showed four singlets at δ 20.4, 20.5, 20.9, and 22.6 corresponding to the four acetoxy methyl groups and also at δ 94.0 consistent with the anomeric carbon. The IR spectrum of **6c** showed the absence of an amide carbonyl band which indicates the formation of the *O*-glycoside and not the *N*-glycoside. Treatment of pyrimidines **1a** and **1b** with peracetylated ribose **(4)** under MW irradiation for 30 seconds using silica gel as a solid support[21] gave the corresponding ribosides **8a** and **8b** (Scheme 2).



SCHEME 2

The ^{1}H NMR spectra of **8a** and **8b** showed doublets at δ 6.46 and 6.25, respectively, characteristic for the anomeric protons. The ^{13}C NMR spectra of **8a** and **8b** showed the absence of C=S group and the presence of signals at δ 161.0 for C-4 and the presence of the anomeric carbons at δ 84.0 and 84.8, respectively.

Riboside **8c** was obtained by the reaction of ethyl 2-(4-chlorophenyl)-6-methyl-4-oxo-3,4-dihydropyrimidine-5-carboxylate **(1c)** with peracetylated ribose **(4)** under MW irradiation for 30 seconds using silica gel as a solid support.[21] (See Scheme 2.) The ^{1}H NMR spectrum of **8c** showed three singlets at δ 1.86, 2.06, and 2.12 for three acetoxy groups and a doublet at δ 6.28 characteristic for the anomeric proton. The IR spectrum of **8c** showed the absence of an amide carbonyl band which indicates the formation of the *O*-glycoside and not the *N*-glycoside.

The deprotected glucosides **7a–c** and ribosides **9a–c** were obtained by treatment of the acetylated compounds **6a–c** and **8a–c**, respectively, and the reaction mixture was stirred overnight at room temperature. The structures of these products were confirmed by their spectral data (IR, ^{1}H and ^{13}C NMR). The ^{1}H NMR spectra revealed the disappearance of the acetoxy proton signals and the appearance of OH proton signals which were exchangeable with D_2O. The IR spectra showed absorption bands at 3381–3434 cm^{-1} for OH groups.

In summary, the pyrimidine glycosides were synthesized by reaction of substituted pyrimidines with glucosyl bromide (2), peracetylated glucose (3) and peracetylated ribose (4), respectively.

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ANTIMICROBIAL ACTIVITY

Glucosides **7a–c** and ribosides **8b** and **9a** were evaluated for antibacterial activity against Gram (−ve) bacteria (*Pseudomonas aeruginosa*) and Gram (+ve) bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) using a cup plate agar diffusion method.[24] Ampicillin was used as a reference to evaluate the potency of tested compounds. Riboside **8b** showed higher antibacterial activity than the standard drug (ampicillin). Glucoside **7a** and riboside **9a** did not show any activity against tested micro-organisms. Glucosides **7b** and **7c** showed higher activity against Gram (+ve) bacteria than the standard drug but were inactive against Gram (–ve) bacteria. The results of the biological activities encourage further work on such a ring system.

TABLE 1 Antimicrobial activity of tested compounds

Compound No.	*P. aeruginosa*	*S. aureus*	*B. subtilis*
7a	—	—	—
7b	—	10	13
7c	—	8	20
8b	25	9	11
9a	—	—	—
Ampicillin	23	7	6

Inhibition zones (mm), minimum inhibitory concentration(μg/mL).

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EXPERIMENTAL

All melting points are uncorrected and were measured using an Electro thermal IA 9100 apparatus. TLC was performed on Merck Silica Gel $60F_{254}$ with detection by UV light and by the charring with H_2SO_4; IR spectra (KBr disc) were recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The UV. Spectra were recorded by UV-160A, UV-visible recording spectrometer Shimadzu using DMSO (dimethyl-solphoxid) as a solvent. The ^{1}H and ^{13}C NMR spectra were determined with JEOL-JNM-LA 200, 300, 400, or 500 MHz spectrometers. The chemical shifts are expressed on the δ (ppm) scale using TMS as the standard. Elemental analyses determined on a Perkin Elmer 240 (microanalysis).

General Methods for Preparation of Glucosides and Ribosides

Method A

To a solution of pyrimidinethione **1a** and **1b** (0.01 mol) in aqueous KOH [0.01 mol in distilled water (6 mL)] was added a solution of glucosyl bromide **2** (0.011 mol) in acetone (30 mL); the reaction mixture was stirred at room temperature for 5 hours and the reaction followed by TLC till the reaction was finished, the reaction mixture was evaporated and the residue was washed with distilled water to remove potassium bromide formed. The product was dried and crystallized from an appropriate solvent.

Method B

A mixture of pyrimidin-4-one or/thione **1a–c** (0.001 mol) and (0.001 mol) of peracetylated glucose (3) or ribose (4), respectively, were dissolved in a mixture of methylene chloride/methanol (80/20) then 1 g of silica gel (200–400 mesh) was added, the solvent was removed by evaporation, the dried residue was transferred into a glass beaker and irradiated for (0.5–3 minutes) in a domestic microwave oven. The product was extracted with methylene chloride, decolorized with charcoal and crystallized from an appropriate solvent or chromatographed on a silica gel column.

Method C

To a solution of glucosyl bromide **2** (0.011 mol) in dry xylene a solution of chloromercuric salt (0.01 mol) of the pyrimidinethione **1a** in dry xylene was added. The reaction mixture was refluxed, and followed by TLC till all starting material was consumed (4 hours), then the solvent was evaporated under reduced pressure and the residue was extracted by chloroform, the chloroform evaporated and the residue was crystallized from ethanol.

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Ethyl 2-phenyl-4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosylthio)-6-methylpyrimidine-5-carboxylate (6a). Method A: 50% yield; method B: 67% yield; method C: 15% yield, as colorless crystals from ethanol; m.p. 178–180°C; $R_f = 0.42$ (eluent: $CHCl_3$/MeOH, 9.9 : 0.1); UV $\lambda_{max.}$ 302.1 nm; IR (KBr) 1760 cm^{-1}, 1731 cm^{-1} (C=O, acetoxy) and 1701 cm^{-1} (C=O, ester); ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ 1.34 (t, 3 H, J = 7.60 Hz $\underline{CH_3}CH_2$), 1.94, 1.98, 1.99 and 2.10 (4s, 12 H, 4 $\underline{CH_3}CO$), 2.61 (s, 3 H, $\underline{CH_3}$-6), 3.98 (m, 2 H, H-5′ and H-6″), 4.38 (m, 3 H, H-6′ and 2 H for $CH_3\underline{CH_2}$), 4.90 (t, 1 H, J = 8.10 Hz, H-4′), 5.13 (t, 1 H, J = 8.05 Hz, H-2′), 5.68 (t, 1 H, J = 8.10 Hz, H-3′), 6.22 (d, 1 H, $J_{1',2'}$ = 8.00 Hz, H-1′) and 7.57–8.50 (m, 5 H, Ar-H). ^{13}C NMR (300 MHz, DMSO-d_6) δ 13.7, 20.1, 20.1, 20.2, 20.2 and 23.6 (6 CH_3), 61.9 ($CH_3\underline{C}H_2$), 61.9 (C-6′), 68.3 (C-4′), 68.9 (C-3′), 72.9 (C-2′), 74.9 (C-5′), 79.1(C-1′), 128.6, 128.7, 129.0, 131.7, 135.6, 161.8, 164.8, 165.8, 165.9, 169.1, 169.2, 169.4 and 169.6 (Ar-C, 2 C=N and **5 acetyl C=O**). Anal. Calcd for $C_{28}H_{32}N_2O_{11}S$ (604.63): C, 57.60; H, 5.46; N, 4.63. Found: C, 57.43; H, 5.62; N, 4.63.

Ethyl 2-(4-chlorophenyl)-4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosylthio)-6-methylpyrimidine-5-carboxylate (6b). Method A: 69% yield; method B: 70% yield, as colorless crystals from ethanol; m.p. 150–152åC; $R_f = 0.38$ (eluent: $CHCl_3$/MeOH; 9.9 : 0.1); UV $\lambda_{max.}$ 305 and 223 nm; ^{1}H NMR spectrum (500 MHz, DMSO-d_6) δ 1.30 (t, 3 H, J = 7.60 Hz, $\underline{CH_3}CH_2$), 1.85, 1.95, 2.00, and 2.50 (4s,12 H, 4 $\underline{CH_3}CO$), 2.63 (s, 3 H, $\underline{CH_3}$-6), 3.95 (m, 2 H, H-5′ and H-6″), 4.4 (m, 3 H, H-6′ and 2 H,$CH_3\underline{CH_2}$),4.90 (t, 1 H, J = 8.6 Hz, H-4′), 5.05 (t, 1 H, J = 8.3 Hz, H-2′), 5.65 (t, 1 H, J = 8.65 Hz, H-3′), 6.25 (d, 1 H, $J_{1',2'}$ = 8.3 Hz, H-1′), 7.60 (d, 2 H, J = 9.6 Hz, Ar-H) and 8.55 (d, 2 H, J = 9.6 Hz, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 14.6, 20.7, 21.1, 21.2, 24.7 and 30.4 (6 CH_3), 62.8 ($CH_3\underline{C}H_2$), 62.9 (C-6′), 69.2 (C-4′), 69.7 (C-3′), 73.8 (C-2′), 75.8 (C-5′), 79.9 (C-1′), 122.0, 129.7, 131.4, 135.4, 137.6, 161.8, 165.7, 167.0, 167.1, 170.1, 170.2, 170.4 and 170.5 (Ar-C, 2 C=N and 5 acetyl C=O). Anal. Calcd for $C_{28}H_{31}ClN_2O_{11}S$ (638.07): C, 52.62; H, 4.89; N, 4.38. Found: C, 52.45; H, 4.92; N, 4.24.

Ethyl 2-(4-chlorophenyl)-4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-6-methylpyrimidine-5-carboxylate (6c).

Method D

Glucosyl bromide **2** (0.0011 mol) was added to a solution of ethyl 4-oxo-3,4-dihydropyrimidine-5-carboxylate **1c** (0.001 mol) in dry acetonitrile (5 mL); triethylamine (0.2 mL, 0.0014 mol) was added, the reaction mixture was stirred overnight at room temperature, then cooled in an ice bath and acidified with acetic acid. The precipitate was collected by filtration, dried and finally crystallized from ethanol.

Method E

A mixture of ethyl 4-oxo-3,4-dihydropyrimidine-5-carboxylate **1c** (0.001 mol) and 0.001 mol potassium carbonate was stirred in acetone or a mixture of acetone/DMF (15 mL) for 1 hour, then (0.0011 mol) glucosyl bromide **2** was added, the stirring was continued overnight then the mixture was heated at reflux for 3 hours, filtered, the solvent was evaporated under vacuum, and the residue was crystallized from ethanol.

Method D: 40% yield; method E : 53% yield, as colorless crystals from ethanol; m.p. 156–157°C; $R_f = 0.47$ (eluent: CH_2Cl_2/MeOH; 9.9 : 0.1), UV λ_{max} 271 and 242.5 nm; IR (KBr) 1758 and 1731 cm^{-1} (C=O, ester); ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 1.37 (t, 3 H, J = 7.12 Hz, $C\underline{H}_3CH_2$), 1.89, 2.00, 2.01 and 2.04 (4s, 12 H, 4 $C\underline{H}_3CO$), 2.57 (s, 1 H, CH_3_6), 4.00 (m, 1 H, H-5′), 4.14(dd, 1H, $J_{5',6''} = 2.10$, $J_{6',6''} = 12.3$ Hz, H-6″), 4.22 (dd, 1 H, $J_{5',6'} = 5.69$, $J_{6',6''} = 12.3$ Hz, H-6′), 4.39 (q, 2 H, J = 7.12 Hz, $CH_3C\underline{H}_2$),5.15 (t, 1 H, $J_{3',4'} = 8.97$, $J_{4',5'} = 9.13$ Hz, H-4′), 5.31 (t, 1 H, $J_{1',2'} = 7.7$, $J_{2',3'} = 9.22$ Hz, H-2′), 5.36 (t, 1 H, $J_{2',3'} = 9.2$, $J_{3',4'} = 8.78$ Hz, H-3′), 6.21 (d, 1 H, $J_{1',2'} =$ 7.78 Hz, H-1′), 7.39 (d, 2 H, J = 8.53 Hz, Ar-H), 8.33 (d, 2 H, J = 8.53 Hz, Ar-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 14.0, 20.4, 20.5, 20.5, 22.6 and 29.6 (6 CH_3), 62.0 ($CH_3\underline{C}H_2$), 62.1 (C-6′), 68.5 (C-4′), 70.3 (C-3′), 72.7 (C-2′), 72.9(C-5′), 94.0 (C-1′), 128.8, 128.9, 129.0, 135.0, 137.7, 162.4, 164.6, 164.9, 167.5, 169.0, 169.3, 170.0 and 170.4 (Ar-C, 2 C=N and 5 acetyl C=O). Anal. Calcd for $C_{28}H_{31}Cl\ N_2O_{12}$ (622.16): C, 53.98; H, 5.02; N, 4.50. Found: C, 53.76; H, 5.12; N, 4.57.

Ethyl 2-phenyl-4-(β-D-glucopyranosylthio)-6-methylpyrimidine-5-carboxylate (7a). *General method for deacetylation***:** Triethylamine (1 mL) was added to a solution of glucosides **6a–c** or ribosides **8a–c** (0.001 mol) in (10 mL MeOH and 3 drops of water). The mixture was stirred overnight at room temperature, evaporated under reduced pressure and the residue was co-evaporated with MeOH until the triethylamine was removed. The residue was crystallized from ethanol/water to give colorless crystals; 85% yield; m.p. 190–191°C; $R_f = 0.32$ (eluent: CH_2Cl_2/MeOH; 9.6 : 0.4); IR (KBr) 3411 cm^{-1} (broad, 4 OH) and 1723 cm^{-1} (CO, ester); ^1H NMR spectrum (300 MHz, DMSO-d_6) δ 1.34 (t, 3 H, J = 6.93 Hz, $C\underline{H}_3CH_2$), 2.56 (s, 3 H, CH_3_6), 3.12–3.50 (m, 6 H, H-6′, H-6″, H-5′, H-4′, H-3′ and H- 2′), 4.38 (q, 2 H, J = 6.93 Hz, for $CH_3C\underline{H}_2$), 4.51 (t, 1 H, J = 3.32 Hz, OH-6′), 5.08 (d, 1 H, J = 4.36 Hz, OH-4′), 5.23 (d, 1 H, J = 3.84 Hz, OH-3′), 5.47 (d, 1 H, J = 4.70 Hz, OH-2′), 5.63 (d, 1 H, $J_{1',2'} = 7.86$ Hz, H-1′), 7.55–8.39 (m, 5 H, Ar-H). ^{13}C-NMR (300 MHz, DMSO-d_6) δ 13.9 and 23.4 (2 CH_3), 60.6 ($CH_3\underline{C}H_2$), 61.9 (C-6′), 69.0 (C-2′), 63 (C-2′), 71.8 (C-3′), 78.6 (C-4′), 81.7 (C-5′), 82.8 (C-1′), 121.4, 128.8, 131.6, 135.2, 136.2, 161.9, 164.7, 165.4 and 167.3 (Ar-C, 2 C = N and C=O). Anal. Calcd for $C_{20}H_{24}\ N_2O_7S$ (436.48): C, 55.04; H, 5.70; N, 6.40. Found: C, 54.90; H, 6.03; N, 6.27.

Ethyl 2-(4-chlorophenyl)-4-(β-D-glucopyranosylthio)-6-methylpyrimidine -5-ca rboxylate (7b). As for 7a; crystallized from ethanol/water to give colorless crystals; 86% yield; m.p. 194–196°C; $R_f = 0.3$ (eluent: CH_2Cl_2/MeOH; 9.6 : 0.4); IR (KBr) 3434 cm^{-1} (broad, 4 OH) and 1717 cm^{-1} (CO, ester); ^1H

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NMR spectrum (300 MHz, DMSO-d_6) δ 1.33 (t, 3 H, J = 7.0 Hz, $C\underline{H}_3CH_2$), 2.56 (s, 3 H, CH_3-6), 3.13–3.64 (m, 6 H, H-6′, H-6″, H-5′, H-4′, H-3′ and H-2′), 4.38 (q, 2 H, J = 7.0 Hz, $CH_3C\underline{H}_2$), 4.5 (t, 1 H, J = 3.56 Hz, OH-6′), 5.09 (d, 1 H, J = 4.34 Hz, OH-4′), 5.22 (d, 1 H, J = 4.20 Hz, OH-3′), 5.47 (d, 1 H, J = 4.96 Hz, OH-2′), 5.57 (d, 1 H, $J_{1',2'}$ = 7.79 Hz, H-1′), 7.59 (d, 2 H, J = 8.3 Hz, Ar-H) 8.38 (d, 2 H, J = 8.3 Hz, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6) δ 13.9 and 23.3 (2 CH_3), 60.7 ($CH_3\underline{C}H_2$), 62.0 (C-6′), 69.6 (C-2′), 71.6 (C-3′), 78.5 (C-4′), 81.8(C-5′), 82.8 (C-1′), 121.5, 128.9, 130.2, 135.0, 136.5, 160.9, 164.83, 165.3 and 167.6 (Ar-C, 2 C=N and C=O). Anal. Calcd for $C_{20}H_{23}ClN_2O_7S$ (470.92): C, 56.17; H, 6.17; N, 4.9. Found: C, 56.10; H, 6.40; N, 5.07.

Ethyl 2-(4-chlorophenyl)-4-(β-D-glucopyranosyloxy)-6-methylpyrimidin-5-ca-rboxylate (7c). As for 7a; crystallized from ethanol/water to give colorless crystals; 85% yield; m.p. 180–182°C; R_f = 0.42 (eluent: CH_2Cl_2/MeOH; 9.1 : 0.9); ^{1}H NMR spectrum (200 MHz, DMSO-d_6/D_2O) δ 1.39 (t, 3 H, J = 7.2 Hz, $C\underline{H}_3CH_2$), 2.58 (s, 3 H, CH_3-6), 3.20–3.61 (m, 6 H, H-6′, H-6″, H-5′, H-4′, H-3′ and H-2′), 4.42 (q, 2 H, J = 7.2 Hz, $CH_3C\underline{H}_2$), 6.11 (d, 1 H, $J_{1',2'}$ = 7.2 Hz, H-1′), 7.67 (d, 2 H, J = 8.8 Hz, Ar-H), 8.46 (d, 2 H, J = 8.8 Hz, Ar-H). Anal. Calcd for $C_{20}H_{23}Cl\ N_2O_8$ (454.11): C, 52.81; H, 5.10; N, 6.16. Found: C, 53.10; H, 5.22; N, 5.86.

Ethyl 2-phenyl-4-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylthio)-6-methyl-pyrimidine-5-carboxylate (8a). Method B: 82% yield, as Colorless crystals from methanol; m.p. 79–80°C; R_f = 0.43 (eluent: CH_2Cl_2 /MeOH; 9.8 : 0.2); UV $\lambda_{max.}$ 298 nm; ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 1.43 (t, 3 H, J = 5.20 Hz, $C\underline{H}_3CH_2$), 2.06, 2.07 and 2.15 (3s, 9 H, 3 $C\underline{H}_3CO$), 2.70 (s, 3 H, CH_3-6), 4.11(dd, 1 H, $J_{4',5'}$ = 3.4, $J_{5',5''}$ = 11.6 Hz, H-5′), 4.16 (dd, 1 H, $J_{4',5''}$ = 3.6, $J_{5',5''}$ = 11.6 Hz, H-5″), 4.42 (m, 3 H, $CH_3C\underline{H}_2$ and H-4′), 5.54 (dd, 1 H, $J_{2',3'}$ = 2.7, $J_{3',4'}$ = 6.6 Hz, H-3′), 5.63 (dd, 1 H, $J_{1',2'}$ = 2.6, $J_{2',3'}$ = 2.80 Hz, H-2′), 6.46 (d, 1 H, $J_{1',2'}$ = 2.4 Hz, H-1′), 7.45–8.40 (m, 5 H, Ar-H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 13.0, 19.4, 19.4, 19.7 and 23.2 (5 CH_3), 61.1 ($CH_3\underline{C}H_2$), 61.1 (C-5′), 69.0 (C-3′), 74.4 (C-2′), 77.9 (C-4′), 84.0 (C-1′), 127.3, 127.6, 127.9, 128.1, 131.3, 134.8, 160.7, 161.0, 164.1, 168.3, 168.5 and 169.0 (Ar-C, 2 C=N and 4 acetyl C=O). Anal. Calcd for $C_{25}H_{28}N_2O_9S$ (532.38): C, 56.38; H, 5.30; N, 5.26. Found: C, 56.42; H, 5.21; N, 5.33.

Ethyl 2-(4-chlorophenyl)-4-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylthio)-6-methylpyrimidine-5-carboxylate (8b). Method B: 79% yield, as colorless crystals from methanol; m.p. 129–130°C; R_f = 0.38 (eluent: CH_2Cl_2 /MeOH; 9.8: 0.2); UV λ_{max} 318 and 269.5 nm; ^{1}H NMR spectrum (200 MHz, DMSO-d_6) δ 1.40 (t, 3 H, J = 7.5 Hz, $C\underline{H}_3CH_2$), 2.10, 2.15 and 2.23 (3s, 9 H, 3 $C\underline{H}_3CO$), 2.69 (s, 3 H, CH_3-6), 4.10, (dd, 1 H, $J_{4',5'}$ = 4.8, $J_{5',5''}$ = 11.2 Hz, H-5′), 4.17 (dd, 1 H, $J_{4',5''}$ = 4.6, $J_{5',5''}$ = 11.2 Hz, H-5″), 4.50 (m, 3 H, $CH_3C\underline{H}_2$ and H-4′), 5.48 (dd, 1 H, $J_{2',3'}$ = 3.1, $J_{3',4'}$ = 6.8 Hz, H-3′), 5.59 (dd, 1 H, $J_{1',2'}$ = 2.4, $J_{2',3'}$ = 3.0 Hz, H-2′), 6.44 (d, 1 H, H-1′, $J_{1',2'}$ = 2.4 Hz), 7.60 (d, 2

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H, J = 8.4 Hz, Ar-H), 8.35 (d, 2 H, J = 8.4 Hz, Ar-H); ^{13}C NMR (200 MHz, DMSO-d_6) δ 13.8, 20.2, 20.5, 23.9 and 24.0 (5 CH_3), 62.0 ($CH_3\underline{C}H_2$), 62.1 (C-5′), 69.9 (C-3′), 75.1 (C-2′), 78.9 (C-4′),84.8 (C-1′), 128.9, 129.0, 129.5, 129.9, 134.6, 136.8, 160.7, 161.0, 164.9, 166.0, 169.4 and 169.8 (Ar-C, 2 C=N and 4 acetyl C=O). Anal. Calcd for $C_{25}H_{27}Cl\ N_2O_9S$ (566.11): C, 52.96; H, 4.80; N, 4.94. Found: C, 52.93; H, 4.74; N, 4.86.

Ethyl 2-(4-chlorophenyl)-4-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyloxy)-6-meth- ylpyrimidine-5-carboxylat (8c). Method B: 08% yield, as colorless crystals chromatographied by using CH_2Cl_2 as eluent; m.p. 96–97°C; R_f = 0.3 (eluent: CH_2Cl_2 /MeOH; 9.8 : 0.2); IR (KBr) 1735 cm^{-1} (C=O,ester); ^{1}H NMR spectrum (300 MHz; DMSO-d_6) δ 1.40 (t, 3 H, J = 7.5 Hz, $C\underline{H}_3CH_2$), 1.86, 2.06 and 2.12 (3s, 9 H, 3 $C\underline{H}_3CO$), 2.5 (s, 3 H, CH_3_6), 4.12 (m, 2 H, H-5′,5″), 4.38 (m, 3 H, $CH_3C\underline{H}_2$ and H-4′), 5.46 (m, 2 H, H-2′ and H-3′), 6.28 (d, 1 H, $J_{1',2'}$ = 3.4 Hz, H-1′), 7.63(d, 2 H, J = 6.9 Hz, Ar-H), 8.04 (d, 2 H, J = 6.9 Hz, Ar-H). Anal. Calcd for $C_{25}H_{27}Cl\ N_2O_{10}$ (550.14) : C, 54.50; H, 4.94; N, 5.08. Found: C, 54.72; H, 5.10; N, 5.00.

Ethyl 2-phenyl-4-(β-D-ribofuranosylthio)-6-methylpyrimidin-5–carboxylate (9a). As for 7a; crystallized from ethanol to give colorless crystals; 85% yield; m.p. 148–150°C; R_f = 0.2 (eluent: CH_2Cl_2/MeOH; 9.6 : 0.4); IR (KBr) 3381 cm^{-1} (broad, 3 OH), 1704 cm^{-1} (CO, ester); ^{1}H NMR spectrum (200 MHz, DMSO-d_6/D_2O) δ 1.41 (t, 3 H, J = 7.00 Hz, $C\underline{H}_3CH_2$), 2.64 (s, 3 H, CH_3_6), 3.50–3.60 (m, 2 H, H-5′ and H-5″), 3.95 (m, 1 H, H-4′), 4.10 (dd, 1 H, $J_{3',4'}$ = 4.8, $J_{2',3'}$ = 4.6 Hz, H-3′), 4.23 (dd, 1 H, $J_{1',2'}$ = 3.83 Hz, $J_{2',3'}$ = 4.60 Hz, H-2′), 4.47 (q, 2 H, J = 7.00 Hz, $CH_3C\underline{H}_2$), 6.23 (d, 1 H, $J_{1',2'}$ = 3.8 Hz, H-1′), 7.6–8.51 (m, 5H, Ar-H). Anal. Calcd for $C_{19}H_{22}N_2O_6S$ (406.12): C, 56.15; H, 5.46; N, 6.89. Found: C, 55.90; H, 5.57; N, 6.72.

Ethyl 2-(4-chlorophenyl)-4-(β-D-ribofuranosylthio)-6-methylpyrimidin-5-carboxylate (9b). As for **7a**; crystallized from ethanol/water to give colorless crystals; 87% yield; m.p. 138–140°C; R_f = 0.38 (eluent: CH_2Cl_2/MeOH; 9.2 : 0.8); IR (KBr) 3421 cm^{-1} (broad, 3 OH), 1716 cm^{-1} (CO, ester); ^{1}H NMR spectrum (300 MHz, DMSO-d_6/D_2O) δ 1.32 (t, 3 H, J = 7.2 Hz, $C\underline{H}_3CH_2$), 2.54 (s, 3 H, CH_3 -6), 3.54 (m, 2 H, H-5′ and H-5″), 3.86 (m, 1 H, H-4′), 4.00 (t, 1 H, J = 3.67 Hz, H-3′), 4.12 (t, 1 H, J = 3.6 Hz, H-2′), 4.36 (q, 2 H, J = 7.2 Hz, $CH_3C\underline{H}_2$), 6.12 (d, 1 H, $J_{1',2'}$ = 3.6 Hz, H-1′), 7.59 (d, 2 H, J = 8.7 Hz, Ar-H) 8.43 (d, 2 H, J = 8.7 Hz, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6) δ 20.6 and 20.7 (2 CH_3) 62.3 ($CH_3\underline{C}H_2$), 63.2 (C-5′), 73.0 (C-3′), 75.3 (C-2′), 75.8 (C-4′), 80.6 (C-1′), 116.0, 129.1, 131.0, 131.3, 134.0, 137.2, 169.0, 169.7 and 169.9 (Ar-C, 2 C=N and C=O). Anal. Calcd for $C_{19}H_{21}Cl\ N_2O_6S$ (440.08): C, 51.76; H, 4.8; N, 6.53. Found: C, 51.77; H, 4.76; N, 6.38.

Ethyl 2-(4-chlorophenyl)-4-(β-D-ribofuranosyloxy)-6-methylpyrimidin-5-carb-oxylate (9c). As for 7a; crystallized from ethanol/water to give colorless crystals; 86% yield; m.p. 115–117°C; R_f = 0.43 (eluent: CH_2Cl_2/MeOH; 9.2 : 0.8); ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ 1.32 (t, 3 H, J = 7.0 Hz,

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$C\underline{H}_3CH_2$), 2.49 (s, 3 H, CH_3_6), 3.98–4.07 (m, 5 H, H-5′, H-5″, H-4′, H-3′ and $\underline{H}$-2′), 4.36 (q, 2 H, J = 7.0 Hz, $CH_3C\underline{H}_2$), 4.2 (t, 1 H, J = 5.3 Hz, OH-5′), 4.26 (d, 1 H, J = 4.87 Hz, OH-3′), 4.3 (d, 1 H, J = 5.0 Hz, OH-2′), 6.03 (d, 1 H, $J_{1',2'}$ = 3.60 Hz, H-1′),7.55 (d, 2 H, J = 8.90 Hz, Ar-H), 7.9 (d, 2 H, J = 8.90 Hz, Ar-H). Anal. Calcd for $C_{19}H_{21}Cl\ N_2O_7$ (424.10): C, 53.72; H, 4.98; N, 6.59. Found: C, 53.54; H, 4.73; N, 6.60

ANTIBACTERIAL SCREENING

The antimicrobial activities of some synthesized compounds were screened for their antibacterial activity against three species of bacteria, namely (*Pseudomonas aeruginosa*) as Gram (−ve), (*Staphylococcus aureus*), and (*Bacillus subtilis*) as Gram (+ve) using a cup agar diffusion method.[23] The tested compounds were dissolved in dimethyl sulfoxide to get a solution of 1 mg/mL concentration. The inhibition zone were measured in mm at the end of an incubation period of 48 hours at 37°C. Dimethyl sulfoxide showed no inhibition zones. Ampicllin was used as a reference.

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