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Synthesis and Anti-HBV Activity of Novel Substituted Pyrimidine Glycosides and Their Acyclic Analogues[1]

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Abstract—New aryl substituted uracil and thiouracil glycosides are synthesized by glycosylation at N^1 in the pyrimidine nucleus using glycopyranosyl halides in basic medium. In addition *C*-linked hydrazinyl acyclic sugar derivatives exhibiting different sugar moieties, attached at C^6, are also prepared. Antiviral activity of the newly synthesized compounds is studied against Hepatitis B virus (HBV). The antiviral tests data indicated high activity of compounds **6b, 6c** and **12a–12c** with mild cytotoxic effects. The influence of glycosyl moieties attached to the substituted pyrimidine system in addition to the substitution at the aryl fragment on activity is discussed.

Keywords*:* pyrimidines, glycosides, acyclic sugars, nucleosides, antiviral, HBV

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INTRODUCTION

A variety of methods of synthesis of pyrimidine and 4(3*H*)-pyrimidinone rings containing compounds and their diverse properties supported their applications in medicinal chemistry, including anticancer [1–6] and many other types of activity [7–12]. Sulfur modification of pyrimidine nucleosides by replacement of the natural carbonyl group with the thiocarbonyl is of high influence upon their biological activity [13–15] and practical applications [16–19]. It is recognized that the 2-thiocarbonyl group of 2-thiouracil nucleosides strongly influences their conformation properties and plays a key role in the modulation of base pair recognition [14]. On the other hand, thiated nucleosides exhibit antineoplastic properties: 4-thiouridine selectively inhibits growth of Ehrlich ascites and mouse L1210 leukemia cells, and 5-fluoro-4-thio-2'-deoxyuridine inhibits growth of L1210 cells and various hematopoietic human leukemia cells [20]. 2',3'-Dideoxy-3'-fluoro-4-thiopyrimidine [21] is determined to be a potent and selective inhibitor of the retrovirus HIV. Diarylpyrimidine (DAPYs) was among the earliest discovered NNRTI-s [22].

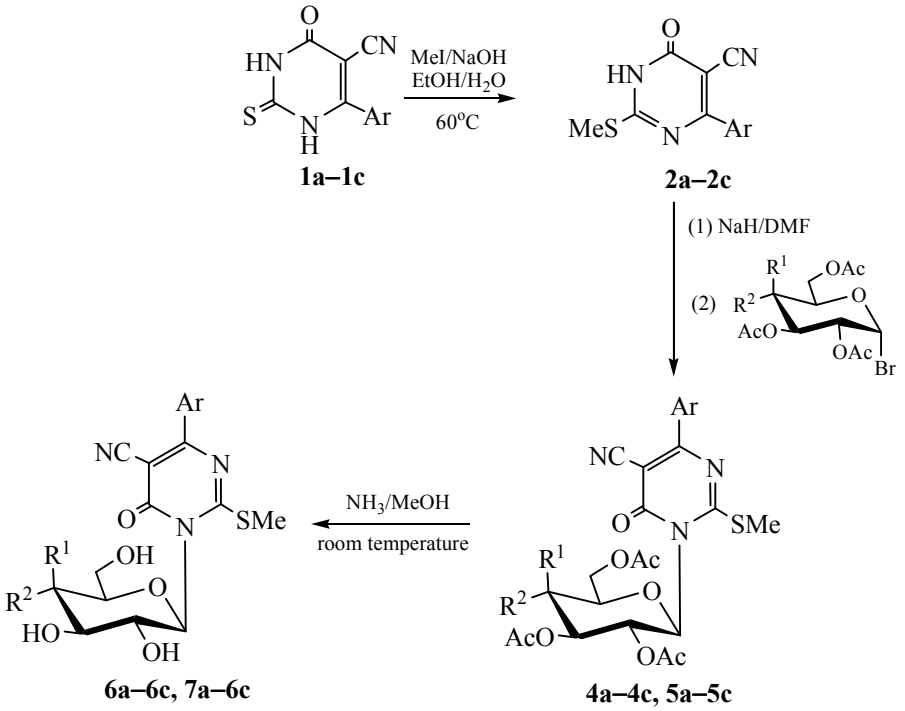
A variety of nucleosides and their analogs constitute an interesting class of biologically active compounds [23] that demonstrate antitumor [24, 25] and antiviral [26, 27] activities. Pyrimidine nucleosides and their derivatives are important molecular templates in the development of new and improved antiproliferative agents. Several structural changes in both the nitrogenated base and the furanose ring have been proposed for developing leading compounds or enhancing the antitumor activity of existing ones. As a result of these studies, a series of DNA bases and nucleoside analogues have been developed and used in treatment of various types of cancer. Among those are 5-fluorouracil, 6-mercaptopurine and cytarabine [28–30]. The above facts initiated our study of synthesis and antiviral activity study of functionalized pyrimidine glycosides and their hydrzainyl sugar derivatives as acyclic analogues of the glycoside skeletons.

RESULTS AND DISCUSSION

In the present study two types of substituted pyrimidine sugar derivatives, incorporating glycosyl and acyclic sugar chains, were synthesized. 6-Aryl-5-cyano-2-(methylsulfanyl)uracils (**2a–2c**) [31] were synthesized by treatment of 6-aryl-5-cyano-2-thiouracils **1a–1c** with methyl iodide and sodium hydroxide in water and ethanol at 60°C according to the

[1] The text was submitted by the authors in English.

Scheme 1. Synthesis of N^1-thiopyrimidine glycosides.



Ar = C_6H_5 (**1a, 2a**, **4a, 5a, 6a, 7a**), *p*-MeC_6H_4 (**1b, 2b**, **4b, 5b, 6b, 7b**), *p*-$MeOC_6H_4$ (**1c, 2c**, **4c, 5c, 6c, 7c**); R^1 = H (**3b, 4a–4c, 6a–6c**), OAc (**3a, 5a–5c**), OH (**7a–7c**); R^2 = OAc (**3b, 4a–4c**), H (**3a, 5a–5c, 7a–7c**), OH (**6a–6c**).

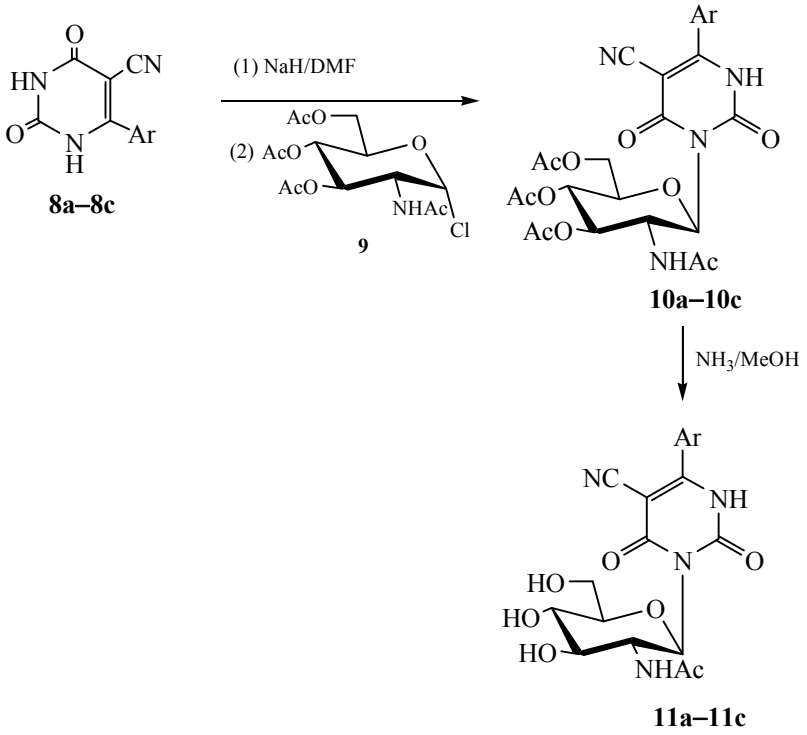
earlier developed method [32]. Condensation of sodium salts of 6-aryl-5-cyano-2-(methylsulfanyl)uracils (**2a–2c**) with glycosyl halides, 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or galactopyranosyl bromide (**3a, 3b**), in dry DMF at room temprature afforded 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)-6-aryl-5-cyano-2-(methylsulfanyl) pyrimidin-4(1*H*)-ones **4a–4c** and **5a–5c** with high yields (Scheme 1).

In ^{1}H NMR spectra of **4a–4c** and **5a–5c** signals of the anomeric protons were recorded as doublets (5.33–6.44 ppm, J = 7.4–8.4 Hz), that corresponded to a diaxial orientation of $H^{1'}$ proton which was indicative of the β-configuration of the glycone part. For compounds **5a–5c** containing the galactopyranosyl moeitey, the axial protons ($H^{4'}$) of the galactose moieties were recorded as doublets of doublets at 5.30, 5.45 and 5.48 ppm. ^{13}C NMR spectra demonstrated the signals assigned to sugar moietey carbons in addition to the acetyl, aryl groups and pyrimidyl ring carbons.

Deacetylation of the glycosides **4a–4c** and **5a–5c** in a mixture with methanol and ammonium hydroxide (25%) (1 : 1) at room temperature gave free nucleosides **6a–6c** and **7a–7c**, respectively, with high yields (Scheme 1). The hydroxyl group bands were recorded in IR spectra of the latter free nucleoside analogs. ^{1}H NMR spectra of the deprotcted nucleosides **6a–6c** and **7a–7c** demonstrated the signals assigned to the anomeric protons as doublets in the range of 5.69–5.78 ppm (J = 7.5–7.7 Hz), clearly indicating that these compounds were also fromed in the β-configuration.

The ease of accessibility and the biological significance of 2-acetamido-2-deoxy-D-glucose have prompted us to use this aminosugar as a starting material in the glycosylation reaction according to the developed earlier method [33]. 6-Aryl-5-cyanouracils **8a–8c** [33] were synthesized by base-catalyzed condensation cyclization of ethyl cyanoacetate with urea and aromatic aldehydes with 40-45% yields. Glycosylation of sodium salts of the substituted cyanouracil derivatives **2a–2c** with 2-acetamido-1-chloro-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucose (**9**) in dry DMF at 90°C gave the desired 1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-aryl-5-cyanopyrimidin-4(1*H*)-ones (**10a–10c**) with 75–80% yields (Scheme 2). ^{1}H NMR spectra of **10a–10c** demonstrated the signals of anomeric protons as doublets at 5.89, 5.88 and 5.90 ppm (J = 9.3, 9.4 and 9.2 Hz, respectively), assigned to the diaxial orientation of $H^{1'}$ and $H^{2'}$

Scheme 2. Synthesis of N^1-pyrimidine aminoglycosides.



Ar = C_6H_5 (**8a, 10a**, **11a**), *p*-MeC_6H_4 (**8b, 10b**, **11b**), *p*-$MeOC_6H_4$ (**8c, 10c**, **11c**).

protons that were indicative of the β-configuration. ^{13}C NMR spectra of **10b** and **10c** demonstrated the anomeric carbons at 97.3 and 95.1 ppm.

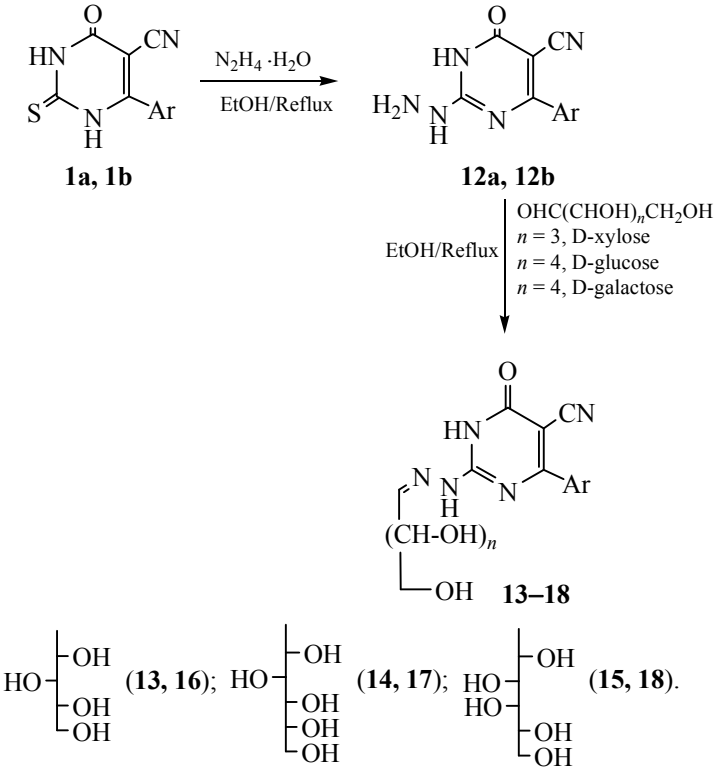
Deacetylation of the modified nucleoside analogues **10a–10c** in a mixture of methanol and ammonium hydroxide (25%) (1 : 1) at room temperature afforded free nucleosides **11a–1c** in 83–90% yields (Scheme 2). In ^1H NMR spectra of free tetrahydropyrimidine nucleosides **11a–1c** the signals corresponging to the anomeric protons were recorded as doublets at 5.76, 5.78 and 5.80 (J = 9.4, 9.6 and 9.5 Hz, respectively) indicating their β-conformation as well.

The substituted thiouracil derivatives **1a, 1b** were alkylated with formation of the corresponding alkylthio analogues. The latter were refluxed with hydrazine hydrate in ethanol to give the corresponding hydrazine derivatives **12a, 12b** [34]. The resulting hydrazinyl pyrimidines reacted with D-xylose, D-glucose or D-galactose in an aqueous ethanol solution in presence of the catalytic amount of acetic acid. The corresponding sugar hydrazinyl uracil derivatives **13–18** were obtained with 70–78% yield (Scheme 3).

The IR spectra of the latter products, that contained acyclic sugar chains, demonstrated the characteristic absorption bands attributed to the hydroxy groups in the region of 3377–3428 cm^{-1}. ^1H NMR spectra contained signals assigned to sugar chain protons in addition to the sugar hydroxyl groups. The H^1 methine proton (originally the aldehydic hydrogen) was recorded as a doublet in the range of 7.30–7.70 ppm. The observed relatively high chemical shifts assigned to H^1 provided an evidence for the acyclic structure of the sugar moieties incorporated in sugar hydrazinyl uracil products. For compounds containing the cyclic sugar structures, the anomeric proton (H^1 in the glycosyl moiety, an sp^3 hydrogen) should be recorded at a lower chemical shift.

Antiviral activity. Hepatitis B virus (HBV) is a DNA virus that causes acute hepatitis and leads to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [35]. The potential target for antiviral chemotherapy is the reverse transcription step in HBV life cycle. The minus strand of HBV is synthesized by reverse transcription of the pregenome using the endogenous viral reverse transcriptase. It is determined that reverse transcriptase enzyme leads to incorporated nucleotide analogues more efficiency than cellular DNA polymerase [36]. These nucleotide analogues are competitive inhibitors of the reverse transcriptase with

Scheme 3. Synthesis of pyrimidine hydrazinyl acyclic sugars derivatives.



Ar = C_6H_5 (**1a, 12a**, **13–15**), *p*-MeC_6H_4 (**1b, 12b**, **16–18**).

the nucleosides pool in the cells cytoplasm in minus strand synthesis. The recent development of heterocyclic analogues initiated the research in selective antiviral activities. Among these agents, Lamivudine acts as a retroviral inhibitor [37]. It demonstrated activity against HBV replication both in vitro and in vivo.

The results of the viral screening against HBV of selected compounds indicated that the products **6b, 6c, 11a–11c** demonstrated viral replication inhibition and mild cytotoxicity with selective index > 151 > 625 > 370 > 500, and > 400, respectively. On the other hand, compounds **6a**, **7a–7c** exhibited very low inhibition and high cytotoxicity with selective index > 80 > 64 > 73 and > 84, respectively (Tables 1, 2).

The accumulated data revealed that attachment of a certain glycosyl moiety or acyclic sugar unit chain to the substituted pyrimidinone ring system led to more active compounds. Derivatives incorporating the glucopyranosyl fragment demonstrated highar activity than the galactopyranosyl analogue. The results also indicated that substitution in the phenyl substituent at the position 5 in the pyrimidinone ring led to more active derivatives. The attachment of free hydroxy glycosyl moities to the substituted pyrimidine nucleus demonstrated high inhibition activity. The activity of N-glycosides of pyrimidine was higher than that of compounds containing the pyrimidine system attached to acyclic sugar moities via the hydrazinyl linkage.

Table 1. Cytotoxic effect (CC_{50}), inhibitory concentration (IC_{50}) and selective index (SI) for selected compounds

Compound	HBV DNA IC_{50}, μM	Hep G2 2.2.15 CC_{50}, μM	SI
Lamivudine		>100	>1000
6a	1.25	>100	>80
6b	0.66	>100	>151
6c	0.16	>100	>625
7a	1.56	>100	>64
7b	1.37	>100	>73
7c	1.19	>100	>84
11a	0.27	>100	>370
11b	0.20	>100	>500
11c	0.25	>100	>400

Table 2. Results of inhibition of HBV replication by selected compounds

Compound	Concentration, μM	HBV DNA in supernatant	Compound	Concentration, μM	HBV DNA in supernatant
Lamivudine	1.0	0.25	**7b**	1.0	0.18
	10.0	0.18		10.0	0.16
	100.0	0.15		100.0	0.12
6a	1.0	0.21	**7c**	1.0	0.22
	10.0	0.18		10.0	0.20
	100.0	0.15		100.0	0.17
6b	1.0	0.37	**11a**	1.0	0.68
	10.0	0.31		10.0	0.61
	100.0	0.21		100.0	0.56
6c	1.0	0.80	**11b**	1.0	0.78
	10.0	0.76		10.0	0.72
	100.0	0.69		100.0	0.70
7a	1.0	0.15	**11c**	1.0	0.73
	10.0	0.13		10.0	0.70
	100.0	0.11		100.0	0.63

EXPERIMENTAL

Melting points were determined on a Kofler block apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1720 FTIR spectrophotometer using KBr disks. ^{1}H NMR spectra were measured on a Varian Gemini spectrometer (300 MHz) in DMSO-d_6 using TMS as the internal standard. Mass spectra were measured on a CC 2010 Shimadzu Gas chromatographer (70 eV). The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F245. Microanalysis was carried out at the Microanalytical unit, Tokyo University, Japan. Anticancer activity of the synthesized compounds was tested at the National Cancer Institute (NCI), Cairo, Egypt. Antiviral activity against HBV was tested at the Liver Institute, Menoufia University, Shebin El-Koam Egypt.

Synthesis of 6-aryl-5-cyano-2-(methylsulfanyl)-uracils (2a–2c). A mixture of substituted 2-thiouracils **1a–1c** (0.05 mole) with methyl iodide (3.11 mL, 0.05 mol) and sodium hydroxide (2 g, 0.05 mole) in water (50 mL) and ethanol (100 mL) was stirred at 60°C for 1 h. A white solid began to precipitate upon cooling. The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol to give the corresponding compound **2a–2c** in 88–92% yields. Physical data of compounds **2a–2c** were in full agreement with the reported ones.

Synthesis of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glycol-pyranosyl)-6-aryl-5-cyano-2-(methylsulfanyl)pyrimidin-4(1*H*)-ones (4a–4c, 5a–5c). 2-(Methylsulfanyl)-uracil derivatives **2a–2c** (5 mmol) were suspended in 25 mL of dry DMF at room temperature. To this suspension, NaH (50%, 0.26 g, 5 mmol) was added and the mixture was stirred at room temprature for 0.5 h. 2,3,4,6-Tetra-*O*-acetyl-α-D-gluco- or galacto-pyranosyl bromide (**3a, 3b**) (5.5 mmol) was added, and the mixture was stirred at room temperature for 4 h until the starting material was consumed (TLC). The residue precipitated upon evaporation of the filtrate under reduced pressure was purified by recrystallization from absolute ethanol to afford a compound **4a–4c** or **5a–5c**, respectively.

2-(Methylthio)-6-oxo-4-phenyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,6-dihydropyrimidine-5-carbonitrile (4a). Yield 78%, mp 166–168°C. IR spectrum, ν, cm^{-1}: 2224 (C≡N), 1738, 1663 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.02, 2.03, 2.04, 2.07 4s (12H, 4OAc), 2.47 s (3H, SCH_3), 4.09–4.15 m (2H,

$H^{6,6'}$), 4.22 m (1H, $H^{5'}$), 5.06 t (1H, $J_{3',4'}$ = 9.5 Hz, $H^{4'}$), 5.16 t (1H, $J_{2',3'}$ = 9.2 Hz, $H^{2'}$), 5.58 t (1H, $J_{2',3'}$ = 9.3 Hz, $H^{3'}$), 6.42 d (1H, $J_{1',2'}$ = 8.2 Hz, $H^{1'}$), 7.35–7.40 m (5H, Ar-H). Found, %: C 54.23, H 4.61, N 7.25. $C_{26}H_{27}N_3O_{10}S$. Calculated, %: C 54.44, H 4.74, N 7.32.

2-(Methylthio)-6-oxo-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-(*p*-tolyl)-1,6-dihydropyrimidine-5-carbonitrile (4b). Yield 79%, mp 166–168°C. IR spectrum, ν, cm^{-1}: 2220 (C≡N), 1738, 1660 (C=O). ^1H NMR spectrum, δ, ppm: 1.99, 2.02, 2.04, 2.06 4s, (12H, 4OAc), 2.28 s (3H, CH_3), 2.53 s (3H, SCH_3), 4.11–4.17 m (2H, $H^{6,6'}$), 4.29–4.32 m (1H, $H^{5'}$), 5.11–5.13 m (1H, $H^{4'}$), 5.25–5.28 m (1H, $H^{2'}$), 5.61–5.64 m (1H, $H^{3'}$), 6.44 d (1H, $J_{1',2'}$ = 8.4 Hz, $H^{1'}$), 7.20 d (2H, J = 10.3 Hz, Ar-H), 7.40 d (2H, J = 10.3 Hz, Ar-H). ^{13}C NMR spectrum, δ, ppm: 17.5, 20.8, 20.9, 21.1, 22.2 ($5CH_3$), 61.7 (C^6), 67.9 (C^4), 69.2 (C^3), 70.5 (C^2), 72.2 (C^5), 91.3 (C^1), 116.4 (CN), 133.1–160.9 (Ar-C and pyrimidine $C^{2,5}$), 163.4, 169.5, 169.7, 170.0, 170.1, 170.5, 171.8 (C=O, pyrimidine $C^{4,6}$). Found, %: C 54.98, H 4.81, N 7.05. $C_{27}H_{29}N_3O_{10}S$ (587.60). Calculated, %: C 55.19, H 4.97, N 7.15.

4-(4-Methoxyphenyl)-2-(methylthio)-6-oxo-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,6-dihydropyrimidine-5-carbonitrile (4c). Yield 83%, mp 189–191°C. IR spectrum, ν, cm^{-1}: 2221 (C≡N), 1738, 1662 (C=O). ^1H NMR spectrum, δ, ppm: 1.98, 2.02, 2.05, 2.08 4s (12H, 4OAc), 2.53 s (3H, SCH_3), 3.48 s (3H, OCH_3), 4.12–4.17 m (2H, $H^{6,6'}$), 4.32–4.35 m (1H, $H^{5'}$), 5.21–5.30 m (2H, $H^{2'}$, $H^{4'}$), 5.66–5.68 m (1H, $H^{3'}$), 6.43 d (1H, $J_{1',2'}$ = 8.4 Hz, $H^{1'}$), 7.25 d (2H, J = 10.3 Hz, Ar-H), 7.43 d 2H (J = 10.3 Hz, Ar-H). ^{13}C NMR spectrum, δ, ppm: 14.1, 20.7, 20.9, 21.1, 21.5 ($5CH_3$), 55.4 (OCH_3), 62.1 (C^6), 68.5 (C^4), 68.9 (C^3), 71.1 (C^2), 73.3 (C^5), 98.3 (C^1), 114.9 (CN), 132.1–154.9 (Ar-C and pyrimidine $C^{2,5}$), 165.8, 169.5, 169.8, 170.0, 170.5, 171.1, 172.5 (C=O, pyrimidine $C^{4,6}$). Found, %: C 53.60, H 4.80, N 7.08. $C_{27}H_{29}N_3O_{11}S$ (603.60). Calculated, %: C 53.72, H 4.84, N 6.96.

2-(Methylthio)-6-oxo-4-phenyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,6-dihydropyrimidine-5-carbonitrile (5a). Yield 71%, mp 143–144°C. IR spectrum, ν, cm^{-1}: 2218 (C≡N), 1740, 1662 (C=O). ^1H NMR spectrum, δ, ppm: 2.02, 2.03, 2.07, 2.17 4s (12H, 4OAc), 2.51 s (3H, SCH_3), 3.97–4.01 m (1H, $H^{5'}$), 4.16–4.23 m (2H, $H^{6,6'}$), 4.77–4.81 m (1H, $H^{3'}$), 5.30 d.d (1H, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.1 Hz, $H^{4'}$), 5.33 d (1H, $J_{1',2'}$ = 7.8 Hz, $H^{1'}$), 5.76 m (1H, $H^{2'}$), 7.30–7.40 m (5H, Ar-H). Found, %: C 54.27, H 4.51, N 7.20. $C_{26}H_{27}N_3O_{10}S$ (573.57). Calculated, %: C 54.44, H 4.74, N 7.32.

2-(Methylthio)-6-oxo-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-(*p*-tolyl)-1,6-dihydropyrimidine-5-carbonitrile (5b). Yield 69%, mp 163–164°C. IR spectrum, ν, cm^{-1}: 2218 (C≡N), 1736, 1665 (C=O). ^1H NMR spectrum, δ, ppm: 2.00, 2.03, 2.05, 2.14 4s (12H, 4OAc), 2.33 s (3H, CH_3), 2.50 s (3H, SCH_3), 3.96–4.00 m (1H, $H^{5'}$), 4.20–4.27 m (2H, $H^{6,6'}$), 4.75–4.77 m (1H, $H^{3'}$), 5.45 d.d (1H, $J_{3',4'}$ = 3.3 Hz, $J_{4',5'}$ = 1.0 Hz, $H^{4'}$), 5.47 d (1H, $J_{1',2'}$ = 7.5 Hz, $H^{1'}$), 5.75 t (1H, $J_{2',1'}$ = 7.5 Hz, $H^{2'}$), 7.21 d (2H, J = 10.3 Hz, Ar-H), 7.41 d (J = 10.3 Hz, 2H, Ar-H). Found, %: C 54.02, H 4.77, N 7.00. $C_{27}H_{29}N_3O_{10}S$ (587.60). Calculated, %: C 55.19, H 4.97, N 7.15.

4-(4-Methoxyphenyl)-2-(methylthio)-6-oxo-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,6-dihydropyrimidine-5-carbonitrile (5c). Yield 66%, mp 205–206°C. IR spectrum, ν, cm^{-1}: 2217 (C≡N), 1737, 1660 (C=O). ^1H NMR spectrum, δ, ppm: 2.01, 2.03, 2.05, 2.16 4s (12H, 4OAc), 2.49 s (3H, SCH_3), 3.50 s (3H, OCH_3), 3.98–4.06 m (1H, $H^{5'}$), 4.20–4.29 m (2H, $H^{6,6'}$), 4.79–4.82 m (1H, $H^{3'}$), 5.48 d.d 1H ($J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ = 1.2 Hz, $H^{4'}$), 5.50 d (1H, $J_{1',2'}$ = 7.4 Hz, $H^{1'}$), 5.50 m (1H, $H^{2'}$), 7.22 d (2H, J = 10.3 Hz, Ar-H), 7.40 d (2H, J = 10.3 Hz, Ar-H). ^{13}C NMR spectrum, δ, ppm: 14.0, 20.7, 20.8, 21.0, 21.5 ($5CH_3$), 56.4 (OCH_3), 62.3 (C^6), 68.8 (C^4), 69.3 (C^3), 71.2 (C^2), 72.8 (C^5), 98.3 (C^1), 114.3 (CN), 135.1-156.6 (Ar-C and pyrimidine $C^{2,5}$), 162.6, 169.5, 169.8, 170.0, 170.5, 171.2, 172.5 (C=O, pyrimidine $C^{4,6}$). Found, %: C 53.63, H 4.77, N 6.88. $C_{27}H_{29}N_3O_{11}S$ (603.60). Calculated, %: C 53.72, H 4.84, N 6.96.

Synthesis of 1-(β-D-Glycopyranosyl)-6-aryl-5-cyano-2-(methylsulfanyl)-pyrimidin-4(1*H*)-ones (6a–6c, 7a–7c). Solution of a compound **4a–4c** or **5a–5c** (0.5 g) in a 1 : 1 mixture (50 mL) with methanol was treated with conc. ammonia (25%) and stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. The residue was recrystallized from methanol to give a compound **6a–6c** or **7a–7c**, respectively.

2-(Methylthio)-6-oxo-4-phenyl-1-(β-D-glucopyranosyl)-1,6-dihydropyrimidine-5-carbonitrile (6a). Yield 92%, mp 170–171°C. IR spectrum, ν, cm^{-1}: 3440–3425 (OH), 2225 (C≡N), 1660 (C=O). ^1H NMR spectrum, δ, ppm: 2.51 s (3H, SCH_3), 3.28–3.37 m (4H, $H^{6''}$, $H^{6'}$, $H^{5'}$, $H^{4'}$), 3.50–3.53 m (1H, $H^{3'}$), 3.84–3.87 m (1H, $H^{2'}$), 4.50–4.54 br.s (1H, 6'-OH), 5.00–

5.02 br.s (1H, 4'-OH), 5.45–5.51 m (2H, 2'-OH, 3'-OH), 5.74 d (1H, $J_{1',2'}$ = 7.8 Hz, $H^{1'}$), 7.30–7.40 m (5H, Ar-H). Found, %: C 53.09, H 4.63, N 10.17. $C_{18}H_{19}N_3O_6S$ (405.42). Calculated, %: C 53.32, H 4.72, N 10.36.

1-(β-D-Glucopyranosyl)-2-(methylthio)-6-oxo-4-(*p*-tolyl)-1,6-dihydropyrimidine-5-carbonitrile (6b). Yield 90 %, mp 198–199°C. IR spectrum, ν, cm^{-1}: 3465–3440 (OH), 2220 (C≡N), 1655 (C=O). ^1H NMR spectrum, δ, ppm: 2.38 s (3H, CH_3), 2.50 s (3H, SCH_3), 3.35–3.47 m (5H, $H^{3'}$, $H^{4'}$, $H^{5'}$, $H^{6,6'}$), 3.79–3.83 m (1H, $H^{2'}$), 4.52 br.s (1H, 6'-OH), 5.07 br.s (1H, 4'-OH), 5.50–5.59 m (2H, 2'-OH, 3'-OH), 5.78 d (1H, $J_{1',2'}$ = 7.5 Hz, $H^{1'}$), 7.25 d (2H, *J* = 10.3 Hz, Ar-H), 7.45 d (2H, *J* = 10.3 Hz, Ar-H). Found, %: C 54.28, H 4.89, N 9.89. $C_{19}H_{21}N_3O_6S$ (419.45). Calculated, %: C 54.40, H 5.04, N 10.01.

1-(β-D-Glucopyranosyl)-4-(4-methoxyphenyl)-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6c). Yield 88%, mp 218–220°C. IR spectrum, ν, cm^{-1}: 3448-3430 (OH), 2225 (C≡N), 1668 (C=O). ^1H NMR spectrum, δ, ppm: 2.50 s (3H, SCH_3), 3.39–3.52 m (7H, $H^{4'}$, $H^{5'}$, $H^{6,6'}$, OCH_3), 3.75–3.81 m (2H, $H^{3'}$, $H^{2'}$), 4.48 br.s (1H, 6'-OH), 5.05 br.s (1H, 4'-OH), 5.55 m (2H, 2'-OH, 3'-OH), 5.77 d (1H, $J_{1',2'}$ = 7.7 Hz, $H^{1'}$), 7.25 d (2H, *J* = 10.3 Hz, Ar-H), 7.47 d (2H, *J* = 10.3 Hz, Ar-H). ^{13}C NMR spectrum, δ, ppm: 15.8, 58.1 (OCH_3), 62.2 (C^6), 66.8 (C^4), 70.4 (C^3), 74.5 (C^2), 78.8 (C^5), 98.5 (C^1), 108.9 (CN), 132.1–154.8 (Ar-C and pyrimidine $C^{2,5}$), 171.9, 172.1 (pyrimidine $C^{4,6}$). Found, %: C 52.27, H 4.80, N 9.53. $C_{19}H_{21}N_3O_7S$ (435.45). Calculated, %: C 52.40, H 4.86, N 9.64.

1-(β-D-Galactopyranosyl)-2-(methylthio)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (7a). Yield 80%, mp 210–211°C. IR spectrum, ν, cm^{-1}: 3460–3445 (OH), 2225 (C≡N), 1665 (C=O). ^1H NMR spectrum, δ, ppm: 2.50 s (3H, SCH_3), 3.42–3.49 m (3H, $H^{6'}$, $H^{6''}$, $H^{5'}$), 3.65–3.71 m (2H, $H^{3',4'}$), 3.98–4.01 m (1H, $H^{2'}$), 4.70 br.s (1H, 6'-OH), 5.05 br.s (1H, 4'-OH), 5.50 m (2H, 2'-OH, 3'-OH), 5.70 d (1H, *J* = 7.8 Hz, $H^{1'}$), 7.30–7.40 m (5H, Ar-H). Found, %: C 53.22, H 4.60, N 10.24. $C_{18}H_{19}N_3O_6S$ (405.42). Calculated, %: C 53.32, H 4.72, N 10.36.

1-(β-D-Galactopyranosyl)-2-(methylthio)-6-oxo-4-(*p*-tolyl)-1,6-dihydropyrimidine-5-carbonitrile (7b). Yield 80%, mp 237–239°C. IR spectrum, ν, cm^{-1}: 3455–3440 (OH), 2220 (C≡N), 1667 (C=O). ^1H NMR spectrum, δ, ppm: 2.33 s (3H, CH_3), 2.52 s (3H, SCH_3), 3.52–3.69 m (4H, $H^{4'}$, $H^{5'}$, $H^{6'',6'}$), 3.92–3.95 m (1H, $H^{3'}$), 3.99–4.01 m (1H, $H^{2'}$), 4.74 br.s (1H, 6'-OH), 5.15 br.s (1H, 4'-OH), 5.52–5.57 m (2H, 2'-OH, 3'-OH), 5.69 d (1H, *J* = 8.2 Hz, $H^{1'}$) 7.24 d (2H, *J* = 10.3 Hz, Ar-H), 7.44 d (2H, *J* = 10.3 Hz, Ar-H). ^{13}C NMR spectrum, δ, ppm: 14.3, 22.8, 23.7 ($2CH_3$), 61.1 (C^6), 67.6 (C^4), 72.5 (C^3), 74.2 (C^2), 77.4 (C^5), 88.1 (C^1), 110.3 (CN), 124.8-156.5 (Ar-C and pyrimidine $C^{2,5}$), 164.4, 167.5 (pyrimidine $C^{4,6}$). Found, %: C 54.32, H 4.95, N 9.94. $C_{19}H_{21}N_3O_6S$ (419.45). Calculated, %: C 54.40, H 5.04, N 10.01.

1-(β-D-Galactopyranosyl)-4-(4-methoxyphenyl)-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (7c). Yield 78%, mp 243–245°C. IR spectrum, ν, cm^{-1}: 3465–3440 (OH), 2222 (C≡N), 1665 (C=O). ^1H NMR spectrum, δ, ppm: 2.51 s (3H, SCH_3), 3.50–3.65 m (4H, $H^{4'}$, $H^{5'}$, $H^{6'',6'}$), 3.88 s (3H, OCH_3), 3.93–3.95 m (1H, $H^{3'}$), 3.99–4.02 m (1H, $H^{2'}$), 4.77 br.s (1H, 6'-OH), 5.19 br.s (1H, 4'-OH), 5.59 m (2H, 2'-OH, 3'-OH), 5.71 d (1H, *J* = 7.8 Hz, $H^{1'}$), 7.21 d (2H, *J* = 10.3 Hz, Ar-H), 7.45 d (2H, *J* = 10.3 Hz, Ar-H). Found, %: C 52.33, H 4.77, N 9.49. $C_{19}H_{21}N_3O_7S$ (435.45). Calculated, %: C 52.40, H 4.86, N 9.64.

Synthesis of 1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-aryl-5-cyanopyrimidin-4(1*H*)-ones (10a–10c). A mixture of one of the compounds of 6-aryl-5-cyanouracils **8a–8c** (5 mmol) with 50% oil-immersed sodium hydride (0.24 g, 5 mmol) in DMF (30 mL) was stirred at 70–80°C for 1 h and then cooled to room temperature. α-Chloroacetamido sugar **10** (1.83 g, 5 mmol) was added to the mixture, and it was stirred at 90°C for 3–5 h. The mixture was evaporated to dryness under reduced pressure and the residue was recrystallized from absolute ethanol to give the corresponding compound **11a–11c**.

3-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4-dioxo-6-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (10a). Yield 78%, mp 150–151°C. IR spectrum, ν, cm^{-1}: 3305 (NH), 2218 (C≡N), 1742, 1663 (C=O). ^1H NMR spectrum, δ, ppm: 1.75 s (3H, NHAc), 1.94, 1.99, 2.00 3s (9H, 3OAc), 3.95–4.11 m (3H, $H^{5'}$, $H^{6'',6'}$), 4.54–4.47 m (1H, $H^{2'}$), 5.15–5.17 m (1H, $H^{4'}$), 5.30–5.32 m (1H, $H^{3'}$), 5.89 d (1H, *J* = 9.3 Hz, $H^{1'}$), 7.77 d (1H, *J* = 7.5 Hz, NHAc), 7.37–7.47 m (5H, Ar-H), 9.18 br (1H, NH). Found, %: C 55.21, H 4.77, N 10.13. $C_{25}H_{26}N_4O_{10}$ (542.50). Calculated, %: C 55.35, H 4.83, N 10.32.

1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4-dioxo-6-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (10b). Yield 80%,

mp 242–243°C. IR spectrum, ν, cm^{-1}: 3312 (NH), 2224 (C≡N), 1740, 1660 (C=O). ^1H NMR spectrum, δ, ppm: 1.76 s (3H, NHAc), 1.97, 2.00, 2.05 3s (9H, 3 OAc), 2.36 s (3H, CH_3), 4.25–4.45 m (4H, $H^{6'',6'}$, $H^{5'}$, $H^{2'}$), 5.30–5.32 m (1H, $H^{4'}$), 5.43–5.46 m (1H, $H^{3'}$), 5.88 d (1H, *J* = 9.4 Hz, $H^{1'}$), 7.21 d (2H, *J* = 10.3 Hz, Ar-H), 7.39 d (2H, *J* = 10.3 Hz, Ar-H), 7.80 d (1H, *J* = 7.5 Hz, NHAc), 9.21 br (1H, NH). ^{13}C NMR spectrum, δ, ppm: 20.7, 20.8, 20.9, 21.1, 23.6 ($6CH_3$), 62.1 (C^6), 67.7 (C^4), 71.1 (C^3), 72.5 (C^2), 73.8 (C^5), 97.3 (C^1), 116.4 (CN), 133.1-133.2 (Ar-C and pyrimidine C^5), 161.0, 163.4, 169.5, 169.7, 170.0, 170.5, 171.6 (C=O, pyrimidine $C^{2,4,6}$). Found, %: C 56.00, H 4.89, N 9.97. $C_{26}H_{28}N_4O_{10}$ (556.52). Calculated, %: C 56.11, H 5.07, N 10.06.

1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,4-dioxo-6-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (10c). Yield 75%, mp 263–264°C. IR spectrum, ν, cm^{-1}: 3295 (NH), 2220 (C≡N), 1738, 1662 (C=O). ^1H NMR spectrum, δ, ppm: 1.78 s (3H, NHAc), 1.96, 1.99, 2.02 3s (9H, 3OAc), 3.53 s (3H, OCH_3), 4.07–4.19 m (3H, $H^{5'}$, $H^{6'',6'}$), 4.33–4.35 m (1H, $H^{2'}$), 5.32–5.35 m (1H, $H^{4'}$), 5.50–5.58 t (1H, *J* = 9.5 Hz, $H^{3'}$), 5.90 d (1H, *J* = 9.2 Hz, $H^{1'}$), 7.19 d (2H, *J* = 10.3 Hz, Ar-H), 7.33 d (2H, *J* = 10.3 Hz, Ar-H), 7.79 d (1H, *J* = 7.5 Hz, NHAc), 9.24 br (1H, NH). ^{13}C NMR spectrum, δ, ppm: 20.1, 20.2, 20.3, 20.4 ($4CH_3$), 56.2 (OCH_3), 62.0 (C^6), 68.1 (C^4), 70.1 (C^3), 70.4 (C^2), 71.8 (C^5), 95.1 (C^1), 104.2 (CN), 129.1–134.5 (Ar-C and pyrimidine C^5), 168.8, 169.1, 170.0, 170.1, 170.2, 170.9 (C=O, pyrimidine $C^{2,4,6}$). Found, %: C 54.32, H 4.81, N 9.57. $C_{26}H_{28}N_4O_{11}$ (572.52). Calculated, %: C 54.54, H 4.92, N 9.78.

Synthesis of 1-(2-acetamido-β-D-glycopyranosyl)-2,4-dioxo-6-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (11a–11c). Solution of a compound **11a–11c** (0.5 g) in a 1 : 1 mixture (50 mL) of methanol with conc. ammonia (25 %) was stirred at room temperature for 8 h. The reaction mixture was concentrated under reduced pressure. Thus obtained residue was recrystallized from methanol to give one of the corresponding compounds **12a–12c**.

1-(2-Acetamido-β-D-glucopyranosyl)-2,4-dioxo-6-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (11a). Yield 90%, mp 276–277°C. IR spectrum, ν, cm^{-1}: 3310 (NH), 2225 (C≡N), 1745, 1662 (C=O). ^1H NMR spectrum, δ, ppm: 1.68 s (3H, NHAc), 3.32–3.50 m (4H, $H^{6,6''}$, $H^{5'}$, $H^{4'}$), 3.77–4.88 m (2H, $H^{2'}$, $H^{3'}$), 5.16–5.26 m (3H, 3OH), 5.76 d (1H, *J* = 9.4 Hz, $H^{1'}$), 7.30–7.40 m (5H, Ar-H), 7.90 d (1H, *J* = 8.5 Hz, NHAc), 9.24 br (1H, NH). Found, %: C 54.60, H 4.71, N 13.37. $C_{19}H_{20}N_4O_7$ (416.39). Calculated, %: C 54.80, H 4.84, N 13.45.

1-(2-Acetamido-β-D-glucopyranosyl)-2,4-dioxo-6-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (11b). Yield 88%, mp 288–289°C. IR spectrum, ν, cm^{-1}: 3305 (NH), 2222 (C≡N), 1748, 1665 (C=O). ^1H NMR spectrum, δ, ppm: 1.69 s (3H, NHAc), 2.38 s (3H, CH_3), 3.19–3.29 m (4H, $H^{6,6''}$, $H^{5'}$, $H^{4'}$), 3.70–3.80 m (2H, $H^{2'}$, $H^{3'}$), 5.15–5.21 m (3H, 3OH), 5.78 d (1H, *J* = 9.6 Hz, $H^{1'}$), 7.22 d (2H, *J* = 10.3 Hz, Ar-H), 7.41 d (2H, *J* = 10.3 Hz, Ar-H), 7.88 d (1H, *J* = 8.5 Hz, NHAc), 9.22 br (1H, NH). ^{13}C NMR spectrum, δ, ppm: 21.7 (OCH_3), 61.0 (C^6), 69.4 (C^4), 71.4 (C^3), 78.5 (C^2), 81.5 (C^5), 84.9 (C^1), 111.9 (CN), 128.5-155.7 (Ar-C and pyrimidine C^5), 159.9, 160.2, 161.8, 171.5 (C=O, pyrimidine $C^{2,4,6}$). Found, %: C 55.68, H 5.04, N 12.92. $C_{20}H_{22}N_4O_7$ (430.41). Calculated, %: C 55.81, H 5.15, N 13.01.

1-(2-Acetamido-β-D-glucopyranosyl)-2,4-dioxo-6-(*p*-methoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (11c). Yield 83%, mp 290–292°C. IR spectrum, ν, cm^{-1}: 3290 (NH), 2225 (C≡N), 1738, 1668 (C=O). ^1H NMR spectrum, δ, ppm: 1.73 s (3H, NHAc), 3.39–3.58 m (8H, $H^{3'}$, $H^{4'}$, $H^{5'}$, $H^{6'}$, OCH_3), 3.88 m (1H, $H^{2'}$), 5.20–5.30 m (3H, 3OH), 5.80 d (1H, *J* = 9.5 Hz, $H^{1'}$), 7.21 d (2H, *J* = 10.3 Hz, Ar-H), 7.39 d (2H, *J* = 10.3 Hz, Ar-H), 7.90 d (1H, *J* = 8.5 Hz, NHAc), 9.23 br (1H, NH). Found, %: C 53.60, H 4.83, N 12.33. $C_{20}H_{22}N_4O_8$ (446.41). Calculated, %: C 53.81, H 4.96, N 12.55.

Synthesis of hydrazinyl sugar derivatives 13–18. The mixture of 2-hydrazinyluracils **12a, 12b** (2.79 mmol) in ethanol (70 mL) with the appropriate pentose or hexose sugar derivative (2.79 mmol) in water (10 mL) and acetic acid (0.4 mL) was refluxed for 5–6 h. The excess ethanol was removed under reduced pressure, and the residue was triturated with diethyl ether (15 mL), filtered off, washed with ether, and recrystallized from ethanol to give the corresponding compound **13–18**.

6-Oxo-4-phenyl-2-[2-(D-xylotetritolylidene)hydrazinyl]-1,6-dihydropyrimidine-5-carbonitrile (13). White powder, yield 75%, mp 210–212°C. IR spectrum, ν, cm^{-1}: 3445–3411 (OH and NH), 2220 (C≡N), 1663 (C=O). ^1H NMR spectrum, δ, ppm: 3.35–3.42 m (3H, $H^{5',5''}$, $H^{4'}$), 3.76–3.80 m (1H, $H^{3'}$), 4.20–

4.25 m (2H, $H^{2'}$ and OH), 4.98–5.05 m (2H, 2OH), 5.12–5.14 m (1H, OH), 7.30 d (1H, *J* = 7.4 Hz, $H^{1''}$), 7.35–7.72 m (5H, Ar-H), 7.94–8.02 br.s (2H, 2NH). Found, %: C 53.32; H 4.90; N 19.24. $C_{16}H_{17}N_5O_5$ (359.12). Calculated, %: C 53.48; H 4.77; N 19.49.

2-[2-(D-Glucopentitolylidene)hydrazinyl]-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (14). White powder, yield 78%, mp 188–190°C. IR spectrum, ν, cm^{-1}: 3418 (OH and NH), 2221 (C≡N), 1664 (C=O). ^1H NMR spectrum, δ, ppm: 3.37–3.45 m (3H, $H^{6',6''}$, $H^{5'}$), 3.78–3.84 m (2H, $H^{4'}$, $H^{3'}$), 4.22–4.28 m (2H, $H^{2'}$ and OH), 5.02–5.08 m (2H, 2OH), 5.14–5.19 m (2H, 2OH), 7.33 d (H, *J* = 7.4 Hz, $H^{1''}$), 7.38–7.70 m (5H, Ar-H), 7.98–8.05 br.s (2H, 2NH). Found, %: C 52.32; H 4.85; N 18.10. $C_{17}H_{19}N_5O_6$ (389.13). Calculated, %: C 52.44; H 4.92; N 17.99.

2-[2-(D-Galactopentitolylidene)hydrazinyl]-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (15). White powder, yield 75%, mp 218–220°C. IR spectrum, ν, cm^{-1}: 3428 (OH and NH), 2220 (C≡N), 1647 (C=O). ^1H NMR spectrum, δ, ppm: 3.37–3.50 m (3H, $H^{6',6''}$, $H^{5'}$), 3.77–3.83 m (2H, $H^{4'}$, $H^{3'}$), 4.27–4.34 m (2H, $H^{2'}$ and OH), 5.09–5.15 m (2H, 2OH), 5.17–5.25 m (2H, 2OH), 7.35 d (1H, *J* = 7.6 Hz, $H^{1''}$), 7.39–7.74 m (5H, Ar-H), 8.12–8.17 br.s (2H, 2NH). Found, %: C 52.28; H 4.71; N 18.12. $C_{17}H_{19}N_5O_6$ (389.13). Calculated, %: C 52.44; H 4.92; N 17.99.

6-Oxo-4-(*p*-tolyl)-2-[2-(D-xylotetritolylidene)-hydrazinyl]-1,6-dihydropyrimidine-5-carbonitrile (16). White powder, yield 70%, mp 200–202°C. IR spectrum, ν, cm^{-1}: 3378 (OH and NH), 2221 (C≡N), 1678 (C=O). ^1H NMR spectrum, δ, ppm: 2.33 s (3H, CH_3), 3.38–3.60 m (3H, $H^{5',5''}$, $H^{4'}$), 3.78–3.83 m (1H, $H^{3'}$), 4.53–4.59 m (2H, $H^{2'}$ and OH), 5.09–5.20 br.s (3H, 3OH), 7.50 d (2H, *J* = 7.6 Hz, Ar-H), 7.66 d (1H, *J* = 7.5 Hz, $H^{1''}$), 7.76 d (2H, *J* = 7.6 Hz, Ar-H), 9.95–10.02 br.s (2H, 2NH). Found, %: C 54.82; H 5.22; N 18.58. $C_{17}H_{19}N_5O_5$ (373.14). Calculated, %: C 54.69; H 5.13; N 18.76.

2-[2-(D-Glucopentitolylidene)hydrazinyl]-6-oxo-4-(*p*-tolyl)-1,6-dihydropyrimidine-5-carbonitrile (17). White powder, yield 72%, mp 222–224°C. IR spectrum, ν, cm^{-1}: 3377 (OH and NH), 2223 (C≡N), 1673 (C=O). ^1H NMR spectrum, δ, ppm: 2.30 s (3H, CH_3), 3.38–3.46 m (3H, $H^{6',6''}$, $H^{5'}$), 3.78–3.84 m (2H, $H^{4'}$, $H^{3'}$), 4.22–4.28 m (2H, $H^{2'}$ and OH), 5.02–5.08 m (2H, 2OH), 5.14–5.19 m (2H, 2OH), 7.50 d (2H, *J* = 7.2 Hz, Ar-H), 7.70 d (1H, *J* = 7.4 Hz, $H^{1''}$), 7.82 d (2H, *J* = 7.2 Hz, Ar-H), 10.25–10.31 br.s (2H, 2NH). Found, %: C 53.32; H 4.98; N 17.27. $C_{18}H_{21}N_5O_6$ (403.15). Calculated, %: C 53.59; H 5.25; N 17.36.

2-[2-(D-Galactopentitolylidene)hydrazinyl]-6-oxo-4-(*p*-tolyl)-1,6-dihydropyrimidine-5-carbonitrile (18). White powder, yield 71%, mp 206–208°C. IR spectrum, ν, cm^{-1}: 3403, 3221 (OH and NH), 2222 (C≡N), 1672 (C=O). ^1H NMR spectrum, δ, ppm: 2.27 s (3H, CH_3), 3.39–3.48 m (3H, $H^{6',6''}$, $H^{5'}$), 3.77–3.83 m (2H, $H^{4'}$, $H^{3'}$), 4.26–4.32 m (2H, $H^{2'}$ and OH), 5.00–5.08 m (2H, 2OH), 5.19–5.24 m (2H, 2OH), 7.48 d (2H, *J* = 7.2 Hz, Ar-H), 7.68 d (1H, J =7.4 Hz, $H^{1''}$), 7.75 d (2H, *J* = 7.2 Hz, Ar-H), 10.05–10.12 br.s (2H, 2NH). Found, %: C 54.31; H 5.18; N 17.49. $C_{18}H_{21}N_5O_6$ (403.15). Calculated, %: C 53.59; H 5.25; N 17.36.

Antiviral activity. *Preparation and culture of Hep G2 2.2.15 cells.* The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw) [38]. The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/mL nystatin and 380 μg/ mL G418 (geneticin). The transferred HEP G2-2.2.15 cell line was stored in tissue culture flask at 37°C + 5%CO_2. Subcultures were set up after a week by aspiration of the media from culture flask and washing the cells twice by PBS. A 10% versene/trypsin was added and the cells were incubated for 1 min at 37°C.

The drug Lamivudine, which is a potent selective inhibitor of HBV replication [39], has been used as a standard for the comparative studies.

DNA extraction. HBV-DNA extraction was carried out by mixing 10 μL of diluted supernatant (1 : 5 with PBS) in a reaction tube with 10 μL of 0.2 M NaOH and incubated at 37°C for 1h. Carefully, 9.6 μL of 0.2 M HCl was added followed by addition of 90 μL of TE buffer solution.

PCR-Ellisa. The PCR reaction mixture contained 14 μL extracted supernatant, 4 mmol/l $MgCl_2$, 10 μmol/L DIG-11-dUTP, 190 μmol/L dTTP, 200 μmol/L dATP, dGTP, dCTP, 1.5 U Taq polymerase, 20 mmol/l HCl (pH 8.4), 50 mmol/l KCl, 1 μmol/L HCID-1 primer (5'GGA AAG AAG TCA GAA GGC A3') and 1 μmol/L HCID-2 (5'TTG GGG GAG GAG ATT AGG TT3'), in total volume 50 μL. PCR reaction conditions were 32 cycles of 1 min at 94°C, 30 s at 58°C and 30 s at 72°C + 3 s for each cycle in a thermal circler as described in literature [39].

Cytotoxicity assay. A colorimetric assay for living cells utilized the colorless substrate of 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) that was modified to a colored product by any living cells, but not by dead cells or tissue culture medium. The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds using a MTT-assay [40].

Calculation of IC_{50}, CC_{50}, and SI. The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation of the plots of amount of DNA copies versus antiviral drug concentration. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50} [40].

CONCLUSIONS

New substituted pyrimidine N^1-gycosides and C-linked hydrazinyl acyclic sugars were synthesized and charactarized. Their bological tests indicated that incorporation of glycosyl moiety or acyclic sugar unit in the substituted pyrimidinone ring system afforded more active antiviral derivatives against HBV.

CONFLICT OF INTERESTS

No conflict of interest was declared by the authors.

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