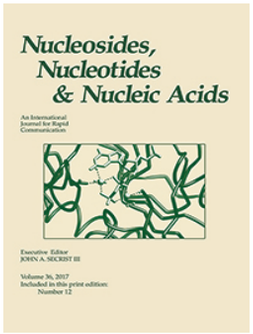

Nucleosides, Nucleotides and Nucleic Acids

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Nucleosides 9: Design and synthesis of new 8-nitro and 8-amino xanthine nucleosides of expected biological activity

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ABSTRACT

The coupling reaction of 1,3-dimethylxanthine (theophylline), 3-benzylxanthine and 3-benzyl-1-methylxanthine with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose afforded the corresponding protected nucleosides, respectively. Nitration of each of the theophylline and 3-benzy-1-methyllxanthine protected nucleosides yielded the corresponding 8-nitronucleosides derivatives, which were reduced to give the corresponding 8-aminonucleoside derivatives.

Debenzoylation of protected nucleosides formed by using methanolic sodium methoxide afforded the corresponding free N-nucleosides, respectively. The structures of products have been elucidated and reported and also some of the products were screened for their antimicrobial activity. Some of tested products showed moderate activity.

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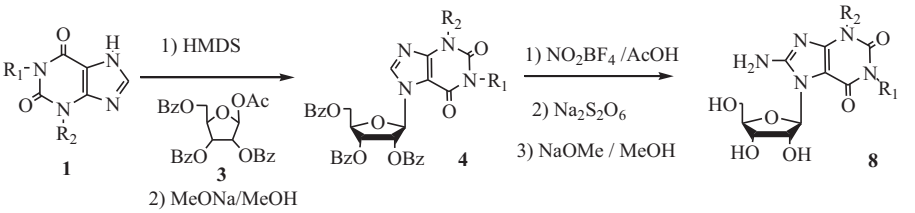
Synthesis; nucleoside; biological activity; xanthine; ribofuranose; xanthine nucleoside

GRAPHICAL ABSTRACT



Introduction

Nucleosides are originally glycosides applied to the pyrimidine or purine ribose derivatives obtained by alkaline hydrolysis of ribonucleic acid (RNA) isolated from yeast[2].

N-Heterocyclic nucleoside analogs[3] represent an important class of antiviral and anticancer agents[4–8] with antimicrobial and cholinesterase inhibitory

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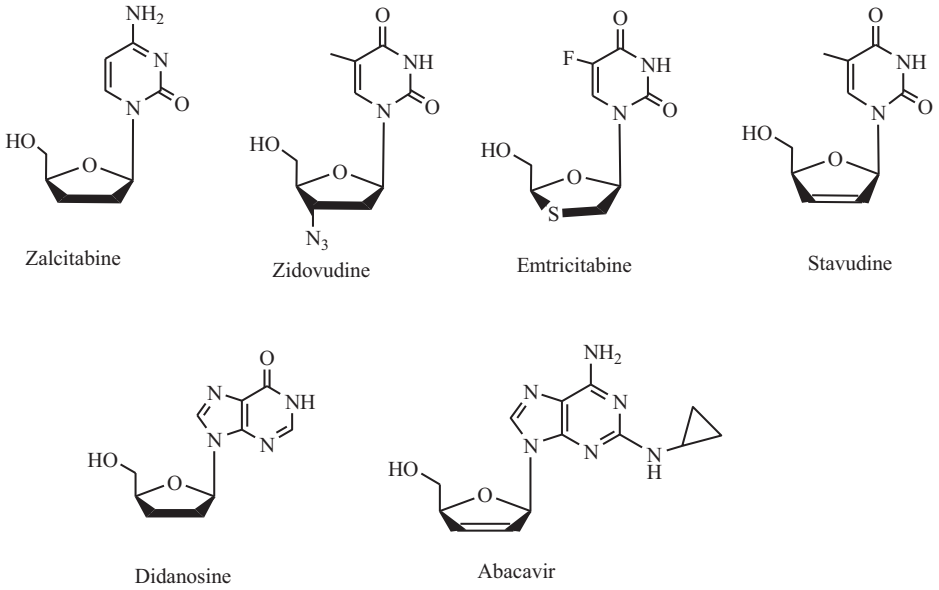


Figure 1. Structures of commonly used nucleoside drugs.

activities[9–13] and are commonly used to treat hepatitis B virus[14,15], hepatitis C virus[16,17], herpes simplex[18,19], human immunodeficiency virus (HIV) and neoplasms[20,21]. Zalcitabine (2′,3′-dideoxycytidine, ddc), Zidovudine (azidothymidine, AZT), Emtricitabine (FTC), Stavudine (2′,3′-didehydro-2′,3′-didehydro-2′,3′-dideoxythymidine, d4T), Didanosine (2′,3′-dideoxyinosine, ddI) and Abacavir (Figure 1) are among the essential antiretroviral nucleoside analogue reverse transcriptase inhibitors (NRTIs) that are used to treat HIV/Acquired Immune Deficiency Syndrome (AIDS) infections[22].

Moreover, alkylxanthines are used as therapeutic agents acting, among other ways, as stimulants of the nervous system[23,24]. The well-known caffeine, present in coffee and tea and various soft beverages, is 1,3,7-trimethylxanthine. It is also worth noticing that 2′-deoxyxanthosine has been used in the extension of the genetic alphabet by purine pairing with a 2,4-diaminopyrimidine nucleoside, which has a hydrogen bonding pattern complementary to 2′-deoxyxanthosine[25].

Xanthine and its nucleosides are involved in a variety of intracellular metabolic pathways as substrates and/or intermediates of numerous enzymes or enzyme systems. Xanthine for example, a substrate of both xanthine oxidase and xanthine dehydrogenase, is an intermediate in the formation of urate, from hypoxanthine[26].

For these biological and medicinal purposes and others it is important to develop the field of the synthesis of nucleosides. As a part our research program dealing with the chemistry of azoles and azines nucleosides[27–35], and in continuation of this work, it was considered worthwhile to study the coupling reaction of xanthine

derivatives with l-*O*-acety1-2,3-5-tri-*O*-benzoyl-β-D-ribofuranose to obtain new xanthine nucleosides and also to synthesize their 8-nitro and 8-aminoderivatives.

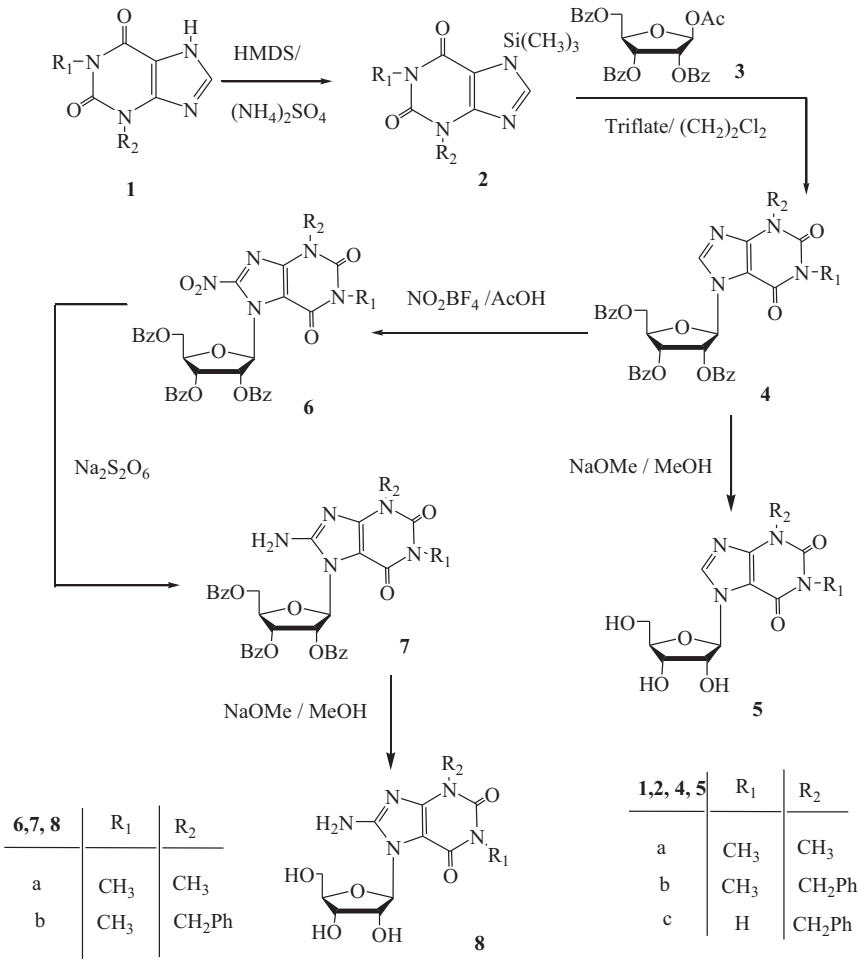
Furthermore, the biological activities of some selected new prepared nucleosides were tested using some of microorganisms.

Results and discussion

The first required starting materials in Scheme 1, are theophylline (1,3-dimethylxanthine) **(1a)**, which was obtained commercially,1-methyl-3-benzylxanthine **(1b)** and 3-benzylxanthine **(1c)**, which were prepared by literature methods[36, 39]. Ribosylation of each of **1a-c** was carried out by the silylation method according to Vorbruggen[37] by refluxing of **1a-c** in hexamethyldisilazane (HMDS) with ammonium sulfate as a catalyst and then stirring of the silylated product (**2a-c**), respectively with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (**3**) in dry 1,2-dichloroethane and trimethylsilane triflate; $CF_3SO_2OSiMe_3$] at room temperature for 48 hours. This method yielded the benzoylated nucleosides **4a-c**, in about 65% yields (see Scheme). The structures of the products **4a-c** were established and confirmed on the bases of their elemental analyses and spectral data (^{1}H & ^{13}C NMR and MS) (see Experimental), which were consistent with the structure of nucleosides **4a-c**. Thus, the spectral data for **(4a)** as a typical example, revealed in its ^{1}H NMR spectrum a doublet at $\delta = 6.5$–6.6 assigned to the anomeric proton of the ribose moiety with a coupling constant equal to 4.5 Hz that corresponds to a diaxial orientation for the 1′- and 2′-H protons, i.e., the β-configuration[27–30]. ^{13}C NMR spectrum of **4a** revealed the chemical shifts of C- ribose moiety.

Deprotection of the nucleosides **4a-c** was carried out by reaction of each of **4a-c** with methanolic sodium methoxide. Stirring at room temperature at 15 h (TLC), yielded the corresponding free *N*-nucleoside **5a**, which was reported in literature[38] and **5b,c**, which hitherto have not been reported in literature yet, respectively (see Scheme). The ^{1}H NMR of **5a** showed the expected base moiety protons in addition to the sugar moiety protons; however no signal for aromatic protons appeared.

Nitration of the protected nucleosides, **4a** and **4b** by using nitronium tetrafluoroborate (NO_2BF_4) afforded 8-nitro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl) 1,3-disubstituted-xanthine nucleosides **(6a)** and **(6b)**, respectively (Scheme). The structures of products **6a**, and **6b** were confirmed by spectral [^{1}H NMR and MS] data (see Experimental). The ^{1}H NMR spectra of **6a** and **6b** showed no 8-CH signal at δ 8.1 and their mass spectra revealed molecular peak ions (M^+) at m/z = 669 (33%) and 745 (20%), respectively. Reduction of 8-nitro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3-disubstituted-xanthine nucleosides **(6a)** and **(6b)** by using sodium dithionite at 90°C afforded 8-amino-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3-disubstituted xanthine nucleosides **(7a)** and **(7b)** (Scheme 1), respectively. The structure of **7a** and **7b** were established by their elemental and spectral analyses (see Experimental).



Scheme 1. Synthesis of xanthine, 8-nitro and 8-aminoxanthine nucleoside derivatives.

Furthermore the reaction of 8-amino-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3-disubstitutedxanthine nucleosides **(7a)** and **(7b)** with methanolic sodium methoxide at room temperature yielded the corresponding pure free 8-amino-7-(β-D-ribofuranosy1)-1,3-disubstitutedxanthine nucleosides **(8a)**[38] and **(8b),** which hitherto have not been reported in literature, in 52% and 50% yield, respectively (Scheme 1). The structures of **8a** and **8b** were confirmed also by ′H NMR, where the deuterium exchangeable signal at δ 6.8 assigned to the amino group was observed as well as by mass spectra, m/z = 327 (M^+) for **8a** and m/z = 403 (M^+) for **8b**.

Antimicrobial activity

Twelve products, namely **4a-c**, **5a-c**, **7a,b** and **8a,b** were evaluated for their antibacterial and antifungal activities against two bacteria species namely, *Escherichia coli* EC, and *Staphylococcus aureus* SA as well as two fungal species, namely *Aspergillus flavus* AF, and *Candida albicans* CA.

Table 1. Antibacterial and antifungal activities of some of the synthesized compounds.

Compound No.	(EC) G^-	(SA) G^+	(AF) Fungus	(CA) Fungus
Control: DMSO	0.0	0.0	0.0	0.0
Ampicillin Antibacterial agent	22+++	18+++	–	–
Amphotericin B Antifungal agent	–	–	17++	19++
4a	0.0–	0.0–	0.0–	0.0–
4b	0.0–	0.0–	0.0–	0.0–
4c	13++	13++	0.0–	10++
5a	9+	9+	0.0–	0.0–
5b	13++	13++	0.0–	12++
5c	0.0–	0.0–	0.0–	0.0–
6a	0.0–	0.0–	0.0–	0.0–
6b	12++	13++	0.0–	11++
7a	9+	9+	0.0–	0.0–
7b	0.0–	0.0–	0.0–	0.0–
8a	12++	11++	0.0–	0.0–
8b	0.0–	0.0–	0.0–	0.0–

Inhibition Zone Diameter (IZD*) (mm/mg Compound Tested)
*IZD = 2–9 mm beyond control = + (low activity).
IZD = 10–20 mm beyond control = ++ (moderate activity).
IZD = 20–30 mm beyond control = +++ (high activity)

The antibacterial and antifungal activities were carried out in the Microbiology Division of Microanalytical Center of Cairo university, using the diffusion plate method[40,41] a bottomless cylinder containing a measured quantity (1mI, mg/mL) of the sample is placed on (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism.

Most of the compounds were tested *in vitro* against gram negative bacteria [Escherichia coli (EC) and gram positive bacteria [Staphylococcus aureus (SA)] and antifungal activity against Aspergillus flavus (AF) and Candida albicans (CA). The reference antibiotics Ampicillin (antibacterial agent) and Amphotericin B (antifungal agent) were used as references to evaluate the potency of the tested compounds under the same condition. The test results are depicted in Table 1 on the following basis:

The solvent used was dimethylsulfoxide and concentration of the sample in 100 µg/ml.

The test results revealed that the compounds, **4c**, **5a**, **5b**, **6b**, **7a** and **8a** exhibited moderate activity against the two bacterial species and all compounds showed no activity against all fungal species against aspergillus flavus (AF), while compounds, **4c, 5b** and **6b** showed also moderate activity against candida albicans (CA).

Experimental

All evaporations were carried out under reduced pressure at 60°C. TLC was carried out on aluminum sheet silica gel 60 (Fluka) and detected by UV light. All melting points were measured on an electrothermal melting point apparatus and are

uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulphoxide (DMSO-d_6) at 300 MHz on a Varian Mercury VXR-300 NMR spectrometer (Cairo University), 600 MHz Bruker NMR spectrometer (King Abdelaziz University). Chemical shifts were related to that of the solvent. The infrared spectra were recorded in potassium bromide discs on a Pye -Unicam, SP300 and Shimadzu, FT IR 8101 PC infrared spectrophotometers,Taif University. Biological activity was carried out at the Microanalytical Center of Cairo University, Cairo, Egypt. Mass spectra were recorded on a Shimadzu GC MS-QP 1000 EX mass spectrometer at 70 e.V. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt.

Synthesis of 7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl-N-substituted xanthine nucleosides (4a-c)

General procedure

A mixture of each of theophylline (1,3-dimethylxanthine) **(1a)** or 1-methyl-3-benzylxanthine **(1b)** or 3-benzylxanthine **(1c)** (10 mmol) and dry hexamethyldisilazane (20 ml) was heated under reflux for 24 h with a catalytic amount of ammonium sulfate. After the solution cooled, it was evaporated to dryness under anhydrous condition to give the corresponding silylated derivative **(2)**, which was dissolved in 15 ml of dry 1,2-dichloroethane. To this was added a solution of l-*O*-acety1-2,3-5-tri-*O*-benzoyl-*β*-D-ribofuranose **(3)** (4.80 g, 9.8 mmol) dissolved in dry 1,2-dichloroethane (15 ml), and the mixture was treated with trimethylsilyl trifluoromethane sulfonate (2.00 ml, l0 mmol) as catalyst. After the solution had been stirred for 48 h (TLC) at room temperature, and then diluted with chloroform (30 ml), washed with a saturated solution of aqueous sodium bicarbonate (100 ml), water (3 × 20 ml) and dried over anhydrous sodium sulfate. Separation of the pure product was achieved to silica gel column chromatography with chloroform and acetone (9:1) as eluent. On evaporation of the main fraction and recrystallisation from n-hexane, **4a** or **4b** or **4c** was obtained as a white crystals, respectively.

4a: Yield 3.3 g, (52%); m.p. 92°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δH: 3.30 (s, 3H, 1-CH_3), 3.7 (s, 3H, 3-CH_3), 4.7-4-9 (m, 3H, 4′ H & 5′ H), 6.0–6.6 ((m, 2H, 2′ H & 3′ H), 6.5–6.6 (d, 1H, 1′ H, $J_{1',2'} = 4.5$ Hz), 7.29–8.0 (m, 15 H, 3 Ph), 8.11 (s, 1H, 8-H);^{13}C NMR ($CDCl_3$, 75 MHz), δC: 29.0, 32.1, 62.1, 74.0, 77.0, 77.5, 77.9, 106.5, 128.7–133.1, 144.8, 151.2, 151.4, 154.9, 165.9, 166.4, 166.7; MS: m/z = 624 (10%). Anal. calcd. for $C_{33}H_{28}N_4O_9$ (624.60); C, 63.46; H, 4.52; N, 8.97. Found: C, 63.20; H, 4.40; N, 8.80%.

4b: Yield, 1.90 g (70%); m.p. 119–121°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δH: 3.30 (s, 3H, 1-CH_3), 4.62–4.80 (m, 3H, 4′ H & 5′ H), 5.20 (s, 2H, N3-CH_2), 5.93–6.01 (m, 2H, 2′ H & 3′ H), 6.51–6.53 (d, 1H, 1′ H, $J_{1',2'} = 4.5$ Hz), 7.18–7. 90 (m, 20 H, 4 Ph), 8.1 (s, 1H, 8-H);^{13}C NMR ($CDCl_3$, 75 MHz), δC: 29.0, 59.0, 62.1, 74.0, 77.0, 77.5, 77.9, 106.5, 128.7–133.1, 144.8, 151.2, 151.4, 154.9, 165.5, 165.8, 166.2, 166.5; MS:

m/z = 700 (25%). Anal. calcd. for $C_{39}H_{32}N_4O_9$ (700.68) C, 66.85; H,4.60; N,8.00. Found: C, 66.60, H, 4.70; N, 7.86%.

4c: Yield, 3.2 g (47%); m.p. 101°C; IR (KBr): $\bar{\upsilon}$ = 3430 (NH), 1722, 1602 (CO); ^{1}H NMR ($CDCl_3$, 600 MHz), δH: 3.6–3.7 (d, 1H, J = 4.5, 4′-H), 4.62- 4.70 (m, 2H, 5′ H), 5.6 (s, 2H, N3-CH_2), 5.67 (m, 2H, 2′ H & 3′ H), 5.7–5.8 (d, 1H, 1′ H, $J_{1',2'}$ = 4.59 Hz), 7.2–7. 90 (m, 20 H, 4 Ph), 8.00 (s, 1H, 8-H), 11.5 (s, 1H, NH);^{13}C NMR ($CDCl_3$, 75 MHz), δC: 59.0, 64.18, 65.12, 71.7, 71.9, 72.4, 76.2, 76.7, 77.35, 79.6, 95.8, 100.5, 127.4.7–133.49, 165.3, 165.5; MS: m/z = 686 (45%). Anal. calcd. for $C_{38}H_{30}N_4O_9$ (686.67). C, 66.47; H, 4.40; N, 8.16. Found: C, 66.50, H, 4.30, N, 8.10%.

Deprotection of 7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-substitutedxanthine nucleosides (4a-c): Synthesis of 7-(β-D-ribofuranosyl)-1,3-disubstitutedxanthine nucleosides (5a-c)

General procedure

A mixture of each of protected nucleoside **4a-c** (0.5 mmol) in absolute methanol (10 ml) and sodium methoxide (27 mg, 0.5 mmol) was stirred at room temperature for 24 h. Evaporation of the solvent under vacuum gave colorless solid, which was dissolved in hot water and neutralized with acetic acid. The precipitate was filtered and dried, which was TLC pure [TLC (chloroform/ ethyl acetate) (9:1)] to give **5a-c** respectively.

5a:Yield, 80 mg, (52%); m.p 195–197°C (lit. m.p. 190–191^{0}C[36].^{1}H NMR (DMSO-d_6, 300 MHz), δH: 3.35 (s, 3H, 1-CH_3), 3.45 (s, 3H, 3-CH_3), 3.58 (m, 2H, 5′H), 3.85 (m,1H, 4′ H), 4.10–4.26 (m, 2H, 2′ H & 3′ H), 5.1 (t, 1H, 5′-OH), 5.3 (d, J = 3.8 Hz,1H, 1′-H), 5.45 (d, J = 4 Hz,1H, 3′-OH), 6.1 (d, J = 3.8 Hz, 1H, 2′-OH), 8.00 (s, 1H, 8-H).^{13}C NMR (DMSO-d_6, 75 MHz), δ C: 29.1, 32.3, 61.0, 74.0, 76.5, 77.0, 77.2, 80.5, 84.1, 110.0, 144.5, 151.1, 151.8, 155.0; MS: m/z = 312 (50%). Anal. calcd. for $C_{12}H_{16}N_4O_6$ (312.29), C, 46.15; H, 5.16; N, 17.94. Found: C, 46.00; H, 5.10; N, 17.70%.

5b: Yield, 105 mg (54%); m.p. 213–214°C; ^{1}H NMR (DMSO-d_6, 300 MHz), δH: 3.35 (s, 3H, 1-CH_3), 3.6 (m, 2H, 5′ H), 3.9 (dd, J = 4 Hz, 1H, 4′ H), 4.0–4.3 (m, 2H, 2′ H & 3′ H), 5.0 (t, 1H, 5′-OH), 5.17 (d, J = 4 Hz,1H, 1′-H), 5.5 (d, J = 4 Hz,1H, 3′-OH), 6.2 (d, J = 4 Hz, 1H, 2′-OH), 7.18–7. 80 (m, 5 H, Ph), 8.00 (s, 1H, 8-H).^{13}C NMR (DMSO-d_6, 75 MHz), δ C: 29.1, 58.6, 61.0, 80.1, 81.5, 81.7.0, 82.0, 109.0, 127.0–129.0, 144.5, 151.1, 151.8, 155.0; MS: m/z = 388 (20%). Anal. calcd. for $C_{18}H_{20}N_4O_6$ (388.36) C, 55.67; H, 5.19; N, 14.43. Found: C, 55.47; H, 5.21; N, 14.25%.

5c: Yield, 230 mg (61%); m.p. 255°C; IR (KBr) $\bar{\upsilon}$ = 3438 (NH); ^{1}H NMR (DMSO-d_6, 300 MHz), δH: 3.6 (m, 2H, 5′,5″-H), 3.9 (m, 1H, 4′ H), 4.0–4.3 (m, 2H, 2′ H & 3′ H), 5.0 (t, 1H, 5′-OH), 5.15 (d, J = 4 Hz, 1′-H), 5.5 (m,3H, 3′-OH & N-CH_2), 6.0 (d, J = 4 Hz, 1H, 2′-OH), 7.2–7. 80 (m, 5 H, Ph), 8.2 (s, 1H, 8-H), (s, 1H, NH); MS: m/z = 374 (42%). Anal. calcd. for $C_{17}H_{18}N_4O_6$ (374.35) C, 54.54; H, 4.85; N, 14.97. Found: C, 54.60; H, 5.10; N, 14.70%.

Nitration of 7-(2,3,5-tri-O-benzoyl – β- D- ribofuranosyl-1,3-disubstituted xanthine nucleosides (4a,b): Synthesis of 8-nitro-7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) substituted xanthine nucleosides (6a,b)

General procedure

To a solution of nitronium tetrafluoroborate (0.79 g, 6 mmol) in glacial acetic acid (15 ml), was added 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1,3-disubstituted-xanthine nucleosides **(4a)** 0r **(4b)** (5 mmol) with stirring. The mixture was heated at 90° for 1 hour, then evaporated to dryness. The residue was treated with 0.5 N sodium bicarbonate (40 ml) and finally extracted with chloroform (3 × 50ml). The chloroform extract was dried over sodium sulfate and evaporated. The residue was triturated with methanol (5 ml) and the solid formed was collected. Recrystallization from methanol gave yellow crystals of **6a** or **6b**, respectively.

6a: Yield, 1.5 g (45%); m.p. 198°C; ^{1}H NMR ($CDCl_3$, 600 MHz), δH: 3.49 (s, 3H, 1-CH_3), 3.66 (s, 3H, 3-CH_3), 4.7–4-9 (m, 3H, 4′ H & 5′ H), 6.0–6.4 ((m, 2H, 2′ H & 3′ H), 6.5 (d, 1H, 1′ H, $J_{1',2'} = 4.59$ Hz), 7.26–8.05 (m, 15 H, 3 Ph); MS: m/z = 669 (33%). Anal. calcd. for $C_{33}H_{27}N_5O_{11}$ (669.59); C,59.19; H,4.06; N,10.46. Found: C, 58.80; H, 4.00; N, 10.20%.

6b: Yield, 1.5 g (43%); m.p. 116°C; ^{1}H NMR ($CDCl_3$, 600 MHz), δH: 3.4 (s, 3H, 1-CH_3), 4.6-4-8 (m, 3H, 4′ H & 5′ H), 5.15 (s, 2H, N3-CH_2), 6.0–6.2 (m, 2H, 2′ H & 3′ H), 6.5–6.6 (d, 1H, 1′ H, $J_{1',2'} = 4.59$ Hz), 7.15–7. 90 (m, 20 H, 4 Ph); MS: m/z = 745 (20%). Anal. calcd. for $C_{39}H_{31}N_5O_{11}$ (745.69), C, 62.82; H, 4.19; N, 9.39. Found: C, 62.50, H, 4.00; N, 9.30%.

Reduction of 8-nitro-7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,3-substituted xanthine nucleosides (6a,b): Synthesis of 8-amino-7-(2,3,5-tri-O-benzoyl-β-D -ribofuranosyl)-1,3-disubstitutedxanthine nucleosides (7a,b)

General procedure

A suspension of 8-nitro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3-disubstitutedxanthine nucleosides **(6a)** or **(6b)** (0.5 mmol) in H_2O (10 ml) was treated at 90°C under stirring with sodium dithionite, $Na_2S_2O_4$ (0.9 g) by gradual addition. After stirring 1 h, the solution was cooled overnight. The solid formed was filtered and dried to give buff-white crystals of product **(7a)** or **(7b)**, respectively.

7a: Yield, 200 mg (60%); m.p 178°C; TLC [chloroform/ethanol (9:1)]; IR (KBr): $\bar{\upsilon}$ = 3435, 3121 (NH_2), 1715, 1667 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), δH: 3.3 (s, 3H, 1-CH_3), 3.4 (s, 3H, 3-CH_3), 4.5- 4.7 (m, 3H, 4′ H & 5′ H), 6.0–6.3 ((m, 2H, 2′ H & 3′ H), 6.5 (d, 1H, 1′ H, $J_{1',2'} = 4.5$ Hz), 6.69 (s, 2H, NH_2), 7.2–8.1 (m, 15 H, 3 Ph); MS: m/z = 639 (30%). Anal. calcd. for $C_{33}H_{29}N_5O_9$ (639.61); C, 61.97; H, 4.57; N, 10.95. Found: C, 61.70; H, 4.50, N, 10.80%.

7b: Yield, 70 mg (77%); m.p 146°C; TLC [chloroform/ethanol (9:1)]; IR (KBr): $\bar{\upsilon}$ = 3348, 3446, 3150 (NH_2), 1706, 1642 (CO) cm^{-1} ^{1}H NMR ($CDCl_3$, 600 MHz), δH: 3.3 (s, 3H, 1-CH_3), 4.5-4-7 (m, 3H, 4′ H & 5′ H), 5.2 (s, 2H, N-CH_2), 6.0–6.3

((m, 2H, 2′ H & 3′ H), 6.5 (d, 1H, 1′ H, $J_{1',2'} = 4.5$ Hz), 6.7 (s, 2H, NH_2), 7.2–8.1 (m, 15 H, 3 Ph); MS: m/z = 715 (15%). Anal. calcd. for $C_{39}H_{33}N_5O_9$ (715.71); C, 65.45; H, 4.65; N, 9.79. Found: C, 65.30; H, 4.40, N, 10.00%.

Deprotection of 8-amino-7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,3-disubstitutedxanthine nucleosides (7a,b): Synthesis of 8-amino-7-(β-D-ribofuranosyl)-1,3-disubstitutedxanthine nucleosides (8a,b)

General procedure

A mixture of compound **7a** or **7b** (1 mmol) in absolute methanol (10 ml) and sodium methoxide (54 mg, 1 mmol) was stirred at room temperature for 24 h. Evaporation of the solvent under vacuum gave colorless solid, which was dissolved in hot water and neutralized with acetic acid. The precipitate formed was filtered and dried to give **8a** or **8b**, respectively, as pure colorless crystals [TLC (chloroform/ ethyl acetate) (9:1)].

8a: Yield, 170 mg (52%); m.p 227–230°C (lit. m.p. 223–224 ^{0}C[36]). IR (KBr): $\bar{\upsilon}$ = 3450 (OH), 3410, 3161 (NH_2), 1705, 1645 (CO);^{1}H NMR (DMSO-d_6, 600 MHz), δH: 3.0 (s, 3H, 1-CH_3), 3.25 (s, 3H, 3-CH_3), 3.58 (m, 2H, 5′H), 3.85 (m,1H, 4′ H), 3.98–4.3 (m, 2H, 2′ H & 3′ H), 5.0–5.4 (m, 3H, 5′-OH, 2′- & 3′-OH), 5.8 (d, $J = 7.2$ Hz, 1H, 1′-H), 6.8 (s, 2H, NH_2, exchangeable by D_2O).^{13}C NMR (DMSO-d_6, 150 MHz), δ C: 24.9, 26.7, 63.0, 64.0, 67.45, 69.0, 80.34, 104.4, 138,9, 146.4, 149.0, 152.29; MS: m/z = 327 (41%). Anal. calcd. For $C_{12}H_{17}N_5O_6$ (327.29); C,44.04; H, 5.24; N, 21.40. Found: C, 44.10; H, 5.00, N, 21.10%.

8b: Yield, 200 mg (50%); m.p. 218°C; IR (KBr): $\bar{\upsilon}$ = 3455 (OH), 3405, 3150 (NH_2), 1710, 1635 (CO); ^{1}H NMR (DMSO-d_6, 600 MHz), δH: 3.1 (s, 3H, 1-CH_3), 3.53 (m, 2H, 5′H), 3.83 (m,1H, 4′ H), 3.9–4.3 (m, 2H, 2′ H & 3′ H), 5.0–5.4 (m, 5H, N-CH_2, 5′-OH, 2′- & 3′-OH), 5.8 (d, $J = 7.2$ Hz, 1H, 1′-H), 6.8 (s, 2H, NH_2, exchangeable by D_2O), 7.3–7.5 (s, 5H, Ph);^{13}C NMR (DMSO-d_6, 150 MHz), δ C: 24.9, 26.7, 60.5, 63.0, 64.0, 67.45, 69.0, 80.34, 104.4, 138,9, 146.4, 149.0, 152.29 MS: m/z = 403 (20%). Anal. calcd. for $C_{18}H_{21}N_5O_6$ (403.39); C, 53.59; H, 5.25; N, 17.36. Found: C, 53.30; H, 5.00, N, 17.10%.

Antimicrobial assay

Cultures of two bacterial species namely, *Escherichia coli* **EC**, and *Staphylococcus aureus* **S**A as well as well as two fungal species, namely *Aspergillus flavus* **AF**, and *Candida albicans* **CA** were used to investigate the antimicrobial activity of twelve products, namely **4a-c**, **5a-c**, **7a,b** and **8a,b** were evaluated for The antimicrobial activity was assayed biologically using the diffusion plate technique. The latter technique was carried out by pouring a spore suspension of the fungal species (1cm^3 of sterile water contains approximately 108 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer is allowed to set for 30 min. A solution of the test compounds (1.0 g = cm^3) in DMSO was placed onto sterile 5mm filter

paper discs and allowed to dry, then the discs were placed on the centre of the malt agar plate and incubated at optimum incubation temperature 28± 2°C. The bactericide Ampicillin and the fungicide Amphotericin B were used as standards under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria. The results are summarized in Table.

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