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Synthesis of acyclo *C*-nucleosides: sterically controlled regiospecific heterocyclization of *aldehydo*-sugar {5-methyl-1,2,4-triazino[5,6-*b*]indol-3-yl}-hydrazones to 3-(alditol-1-yl)-10-methyl-1,2,4-triazolo[4′3′ : 2,3]1,2,4-triazino[5,6-*b*]-indoles

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Condensation of 3-hydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole (**1**) with monosaccharides **2a**–**g** gave the corresponding *aldehydo*-sugar hydrazones **3a**–**g** which were acetylated to the corresponding hydrazone acetates **4a**–**g**. The latter underwent sterically controlled regiospecific oxidative cyclization with bromine in acetic acid and in the presence of sodium acetate to the linearly annulated 3-(poly-*O*-acetyl-alditol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-*b*]indoles **8a**–**g** rather than to the angularly annulated regioisomers **6**. The regiospecific outcome of this heterocyclization has been discussed in terms of electronic and steric factors and the assigned structure **8** has been corroborated on the basis of chemical as well as spectral evidence. De-*O*-acetylation of **8a**–**g** with ammonium hydroxide in methanol gave the title acyclo *C*-nucleosides **9a**–**g**. Representative members of the prepared compounds were tested for antimicrobial activity.

1. Introduction

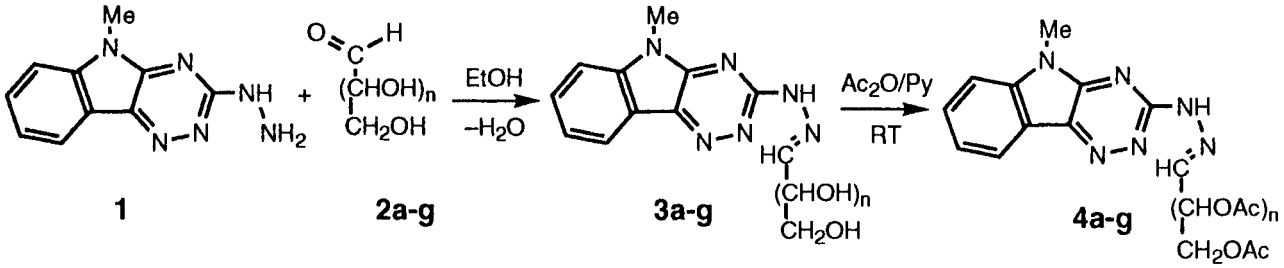
Acyclo *C*-nucleosides constitute a very important class of *C*-nucleoside analogs [1, 2], especially after the isolation of some naturally occurring members such as pyridindolol {1-[(1*R*)-2-dihydroxyethyl]-3-hydroxymethyl-9*H*-pyrido-[3,4-*b*]indole} from *Streptomyces alboverticillatus* [3, 4], the antibiotic CV-1 [5-hydroxy-4-(D-*arabino*-tetritol-1-yl)imidazolidin-2-one] from a strain of *Streptomyces* sp. II [5], and the antibiotic gualamycin {(2*R*, 3*S*, 4*S*)-2-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-α-D-galacto-pyranosyl]-2,3,4-trihydroxy-4-[(2*S*, 3*S*, 4*S*, 5*S*)]-3,4-dihydroxy-5-hydroxymethylpyrrolidin-2-yl]butanoic acid from *Streptomyces* sp. NK11687 [6–8]. Accordingly, substantial research work has been aimed at the synthesis of acyclo *C*-nucleosides carrying various types of heterocycles in order to study their biological activities [1, 2, 9–11].

Valuable medicinal applications and biological activities are known to be associated with the 1,2,4-triazino[5,6-*b*]indole [12–18] and 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indole [19–23] structures. In view of these highly desirable activities and applications and in continuation of our studies on the synthesis and biological activities of acyclo *C*-nucleosides [24–31], we report in the present article the synthesis and antimicrobial activity of the title compounds [32, 33]. In principle, the title acyclo *C*-nucleosides are expected to possess much more potent biological activities compared to their heterocyclic bases since the alditolyl chain would render the molecules more hydrophilic and, accordingly, ensure better pervasion into biological systems [34].

2. Investigations, results and discussion

Condensation of 3-hydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole (**1**) [12] with equimolar amounts of aldopentose and aldohexose monosaccharides, namely: D-arabinose (**2a**), L-arabinose (**2b**), D-ribose (**2c**), D-xylose (**2d**), D-galactose (**2e**), D-glucose (**2f**), and D-mannose (**2g**) gave the corresponding *aldehydo*-sugar {5-methyl-1,2,4-triazino[5,6-*b*]indol-3-yl}hydrazones **3a**–**g** (Scheme 1). IR spectra of these hydrazones showed absorptions at 3376–3191 (OH + NH) and 1591–1585 cm^{-1} (C=N) and their ^1H NMR revealed an exchangeable hydrazono proton (=N–NH) at δ 11.35–11.10, an azomethine proton (–CH=N–) and a benzo group with four aromatic protons at δ 8.20–7.10 in addition to the expected *N*-CH_3 protons at 4.25–3.50 ppm. In most cases, the alditolylidene group protons were associated with the solvent ab-

Scheme 1



n = 3; a = D-*arabino*-; b = L-*arabino*-; c = D-*ribo*-; d = D-*xylo*-

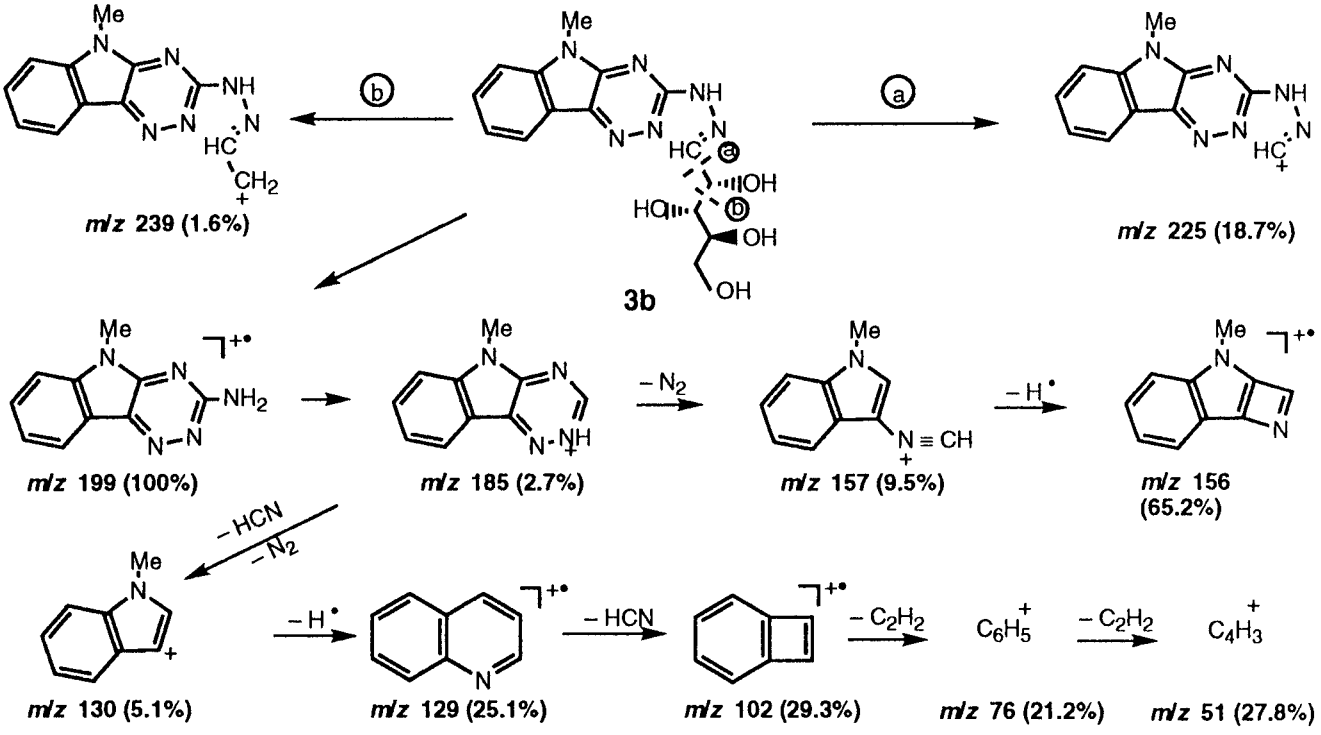
n = 4; e = D-*galacto*-; f = D-*gluco*-; g = D-*manno*-

sorption [$(CD_3)_2SO$] forming a broad signal at δ 3.65–3.45. The ^{13}C NMR spectrum of **3e** revealed signals at δ 158.30 (alditolylidene C1′), 148.64, 147.75, 140.72, 137.57, 128.75, 122.07, 119.78, 118.17 and 110.21 (triazino-indole C3, C4a, C9b, C5a, C8, C9, C7, C9a, and C6 respectively), 71.29, 70.96, 70.60, 69.54, 63.91 (alditolylidene C2′, C3′, C4′, C5′ and C6′ respectively) and δ 26.99 ppm (*N*-CH_3). These ^{13}C NMR data were assigned on the basis of comparing the data obtained for **3e** with those reported in the literature for 1,2,4-triazine [35–37], indole [38, 39] and 1,2,4-triazino[5,6-*b*]indole [40] ring systems. MS of **3b**, **3e** and **3g** did not show their molecular ion peaks, yet revealed the characteristic fragments depicted in Scheme 2.

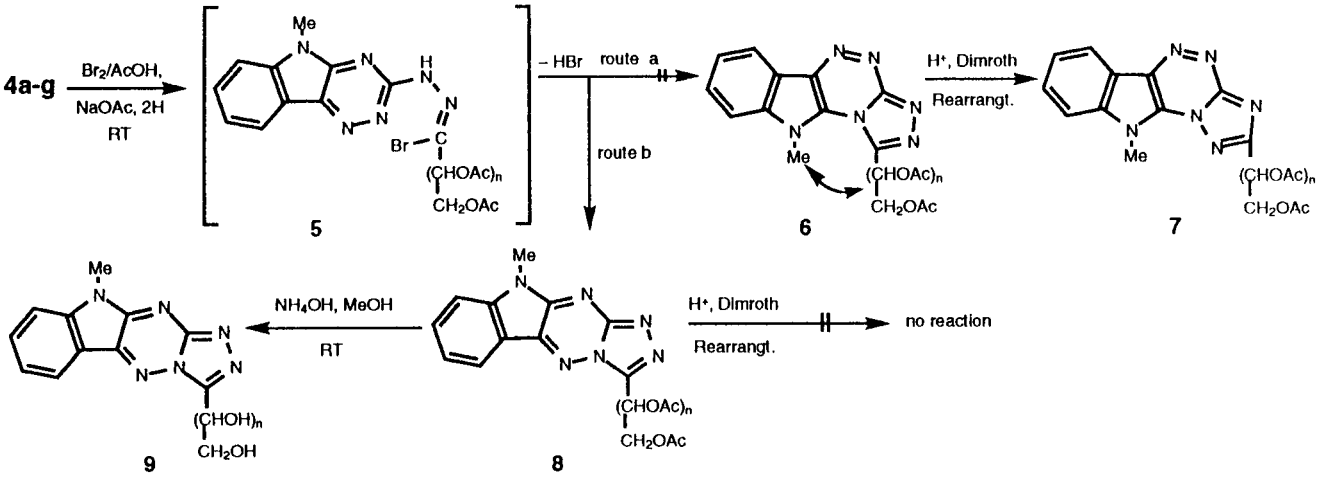
Acetylation of the *aldehydo*-sugar hydrazones **3a–g** with acetic anhydride in the presence of pyridine at room temperature gave the corresponding poly-*O*-acetyl-*aldehydo*-sugar {5-methyl-1,2,4-triazino[5,6-*b*]indol-3-yl}hydrazones **4a–g** which showed the expected IR absorptions (OAc and C=N) as well as ^1H NMR signals of four or five *O*-acetyl groups (Scheme 1).

Subjecting the hydrazone acetates **4a–g** to oxidative cyclization with bromine in acetic acid in the presence of anh. sodium acetate afforded a single crystalline product in each case that lacked the NH absorption and the ^1H NMR azomethine proton (−CH=N) signal characteristic of the parent hydrazone acetate. The cyclization products were analyzed for two hydrogens less than the parent hydrazone acetates and may, accordingly, be assigned the linearly annulated 3-(poly-*O*-acetyl-alditol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-*b*]indole (**8a–g**) or the angularly annulated 1-(poly-*O*-acetyl-alditol-1-yl)-10-methyl-1,2,4-triazolo[3′,4′ : 3,4]1,2,4-triazino[5,6-*b*]indole (**6**) structures (Scheme 3). Formation of either **6** or **8** took place, most probably, through the intermediate hydrazonoyl bromide intermediates **5** [41] as a result of nucleophilic attack of the triazine ring N4 (route A) or N2 (route B) respectively on the hydrazonoyl bromide carbon. Previous results on heterocyclization of 5-hydrazino-1,2,4-triazino[5,6-*b*]indoles with one-carbon cyclizing reagents suggest that it may take place according to route A [13, 42–46] or route B [19, 20, 47–53], depending on electronic factors that enhance the nucleophilicity of the triazine rings N4 or N2 [54–64]. Surprisingly, steric factors which may surmount electronic factors in determining the regioselective (or regiospecific) outcome of this cyclization have been overlooked. Inspection of molecular models and computer optimized geometries showed that N5-unsubstituted-3-hydrazino-1,2,4-triazino[5,6-*b*]indoles would cyclize with aldehydes as well as with carboxylic acids or their derivatives to afford the linearly annulated 3-substituted-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-*b*]indoles and/or angularly annulated regioisomeric 1-substituted-1,2,4-triazolo[3′,4′ : 3,4]1,2,4-triazino[5,6-*b*]indoles; both structures are free of unfavorable steric interactions. Results reported in the literature did assign products of this cyclization, either the linear [42, 50–53] or the angular [13, 42, 45, 46] structures on the basis of electronic grounds. Not in one case has the formation of a mixture of both regioisomers been documented. Molecular models and computer optimized geometries showed, on the other hand, that cyclization of N5-substituted-3-hydrazino-1,2,4-triazino[5,6-*b*]indoles, such as **1**, with aldehydes or carboxylic acids [65] would be sterically controlled to preferably produce the linearly annulated 3,10-disubstituted regioisomer such as **8** rather than the angularly annulated 1,10-disubstituted regioisomer such as **6**; the former (**8**) is free of adverse steric interactions (Fig. 1c) in contrast to the latter (**6**) which suffers crowding of the C1 and N10 substituents (Fig. 1a, b). This argument is taken to favour, beyond reasonable doubt, the assignment of the linearly annulated structure **8** to the cyclization products of the acetylated hydrazones **4**. This assignment is in harmony with electronic factors that also favour cyclization at the triazine ring N2, rather than N4, due to its enhanced nucleophilicity [54–61]. It seems that the electronic and ster-

Scheme 2

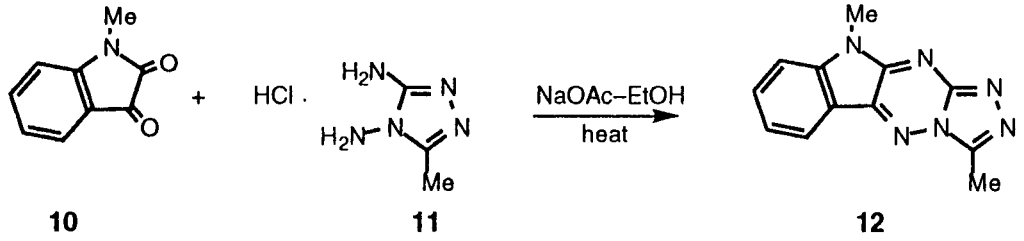


Scheme 3



ic factors controlling this cyclization operate synergistically towards the regiospecific production of the linear isomer **8**. Experimental evidence in favour of the assigned linear structure **8** are: (a) attempted acid-induced Dimroth-like rearrangement of the obtained products by heating with acetic acid gave the unchanged compounds. This result concords with the 1,2,4-triazolo[4,3-*b*]1,2,4-triazine type of fusion (such as that present in the linear structure **8**) known to be incapable of undergoing such a rearrangement and contrasts the facile rearrangement of the 1,2,4-triazolo[3,4-*c*]1,2,4-triazine type of fusion (such as that present in the angular structure **6**) to 1,2,4-triazolo[5,1-*c*] 1,2,4-triazine type of fusion (such as that present in structure 7) [56–58] (Scheme 3) and (b) the UV spectra of the obtained cyclization products **8a–g** are very similar to the spectrum of 3,10-dimethyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-*b*]indole (**12**) unequivocally prepared by cyclocondensation of 1-methylisatin (**10**) and 3,4-diamino-5-methyl-1,2,4-triazole (**11**) [59] (Scheme 4). Compounds **8a–g** and **12** showed two absorption maxima at λ 320–317 and 269–268 nm and a shoulder at 264–263 nm (Fig. 2). X-ray crystal structure analysis would be the tool of choice to differentiate between structures **6** and **8** [59]. Unfortunately, attempts to obtain well developed crystals suitable for this type of analysis were unsuccessful. The ^{13}C NMR of **8g** showed signals at δ 170.62, 169.90, 169.78, 169.48, 169.28 (5 OO*C*CH$_3$), 148.39, 147.08, 146.82, 142.11, 139.82, 134.08, 123.58, 123.03, 115.99,

Scheme 4



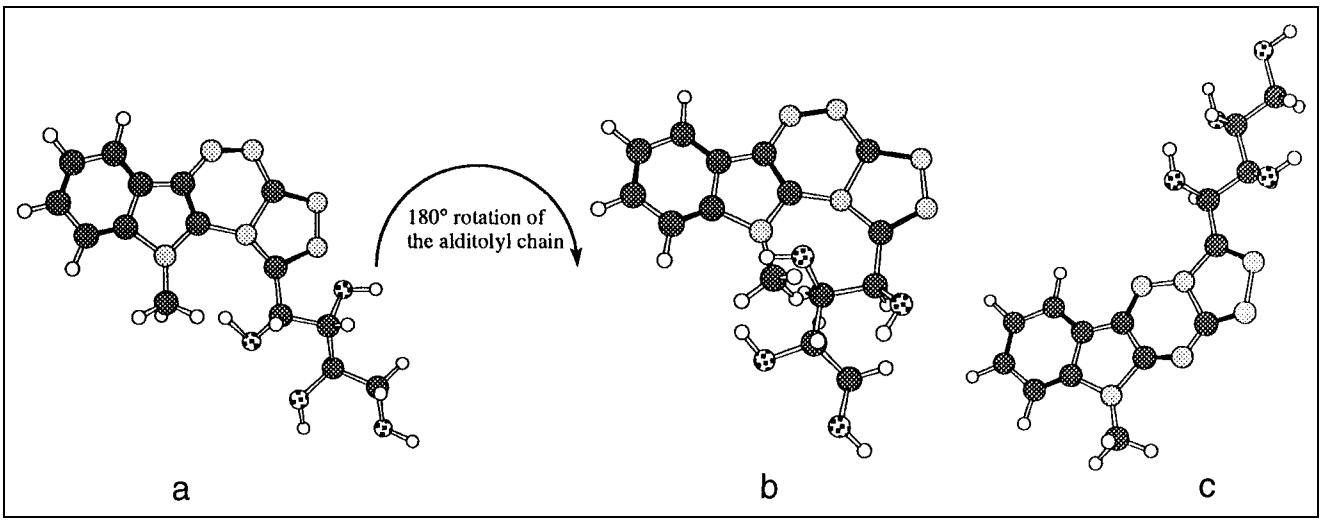


Fig. 1: Computer optimized geometries of **6a** (a, b) and **8a** (c)

110.37 (C3, C11a, C10a, C5a, C9a, C7, C6, C8, C5b, C9 respectively), 68.18, 68.06, 67.38, 64.02, 62.02 (C1′, C2′, C3′, C4′, C5′ respectively), 27.71 (*N*-CH_3), 21.05, 20.73, and 20.25 ppm (5 OO*C*CH_3). The MS of **8b** showed its M + 1 and M^+ peaks at m/z 513 and 512 respectively in addition to the characteristic fragments shown in Scheme 5; the base peak appeared at m/z 253 (100%) and corresponds to the protonated formyl heterocylic moiety. The latter fragment is known to be diagnostic of *C*-nucleoside structures [66].

Compounds **8a**–**g** were also prepared alternatively by one-pot oxidative cyclization/acetylation of **3a**–**g** with bromine in acetic acid in the presence of anh. sodium acetate followed by treatment with acetic anhydride.

De-*O*-acetylation of **8a**–**g** with ammonium hydroxide in methanol afforded the corresponding acyclo *C*-nucleosides namely: 3-(alditol-1-yl)-10-methyl-1,2,4-triazolo[4′, 3′: 2,3]1,2,4-triazino[5,6-*b*]indoles **9a**–**g** (Scheme 3). IR spectra of the latter compounds showed absorptions at 3375–3253 (OH) and 1605–1600 cm^{-1} (C=N) and their UV spectra revealed two maxima at 318–317 and 269 nm and a shoulder at 265–264 nm. These maxima also correspond to those of the unequivocally prepared **12**. The ^{1}H NMR of **9a**–**g** showed the expected aromatic, *N*-CH_3

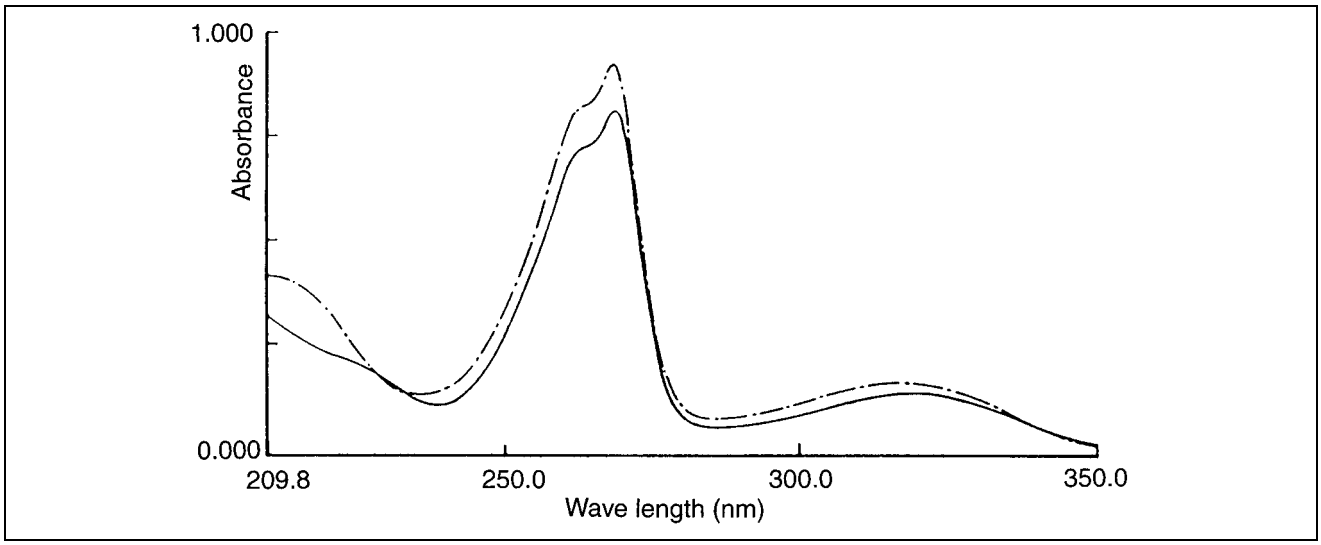
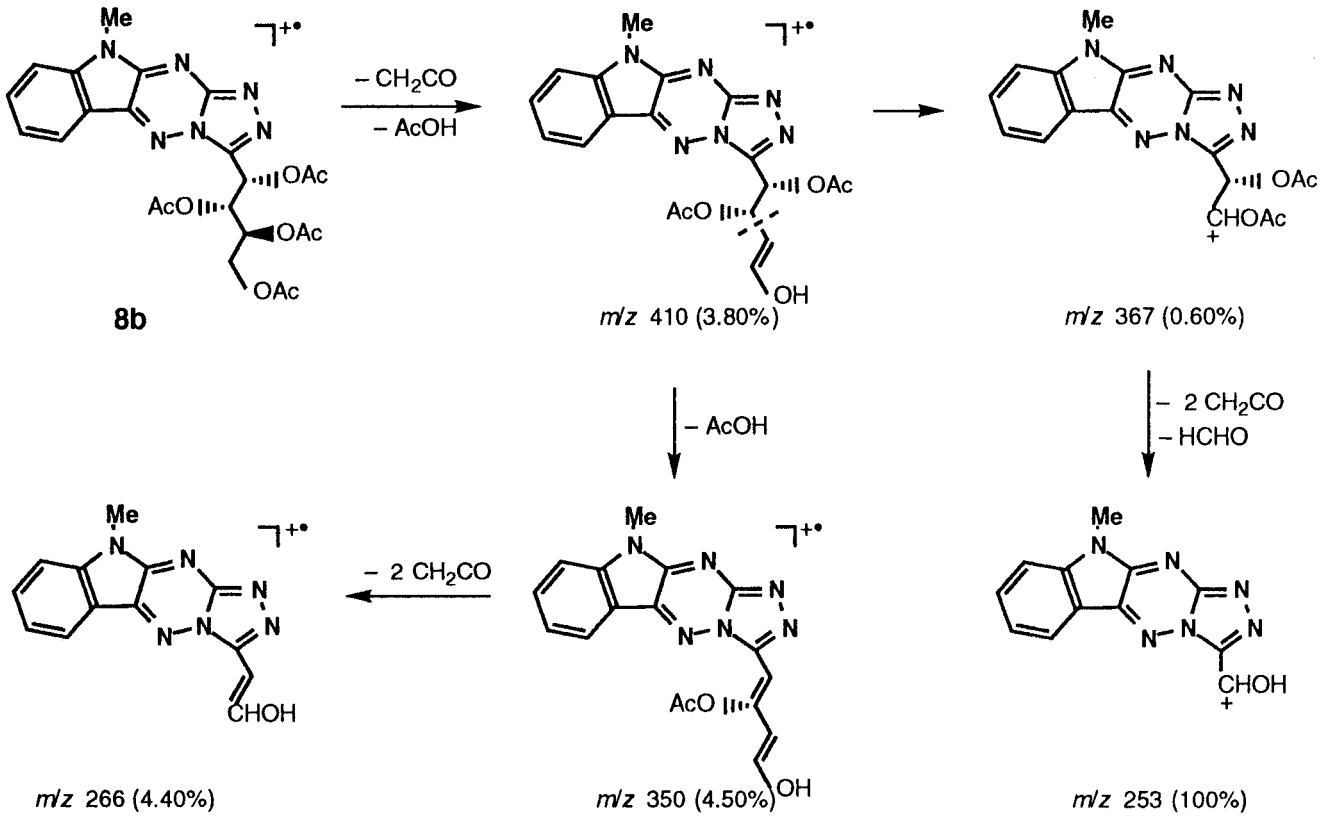
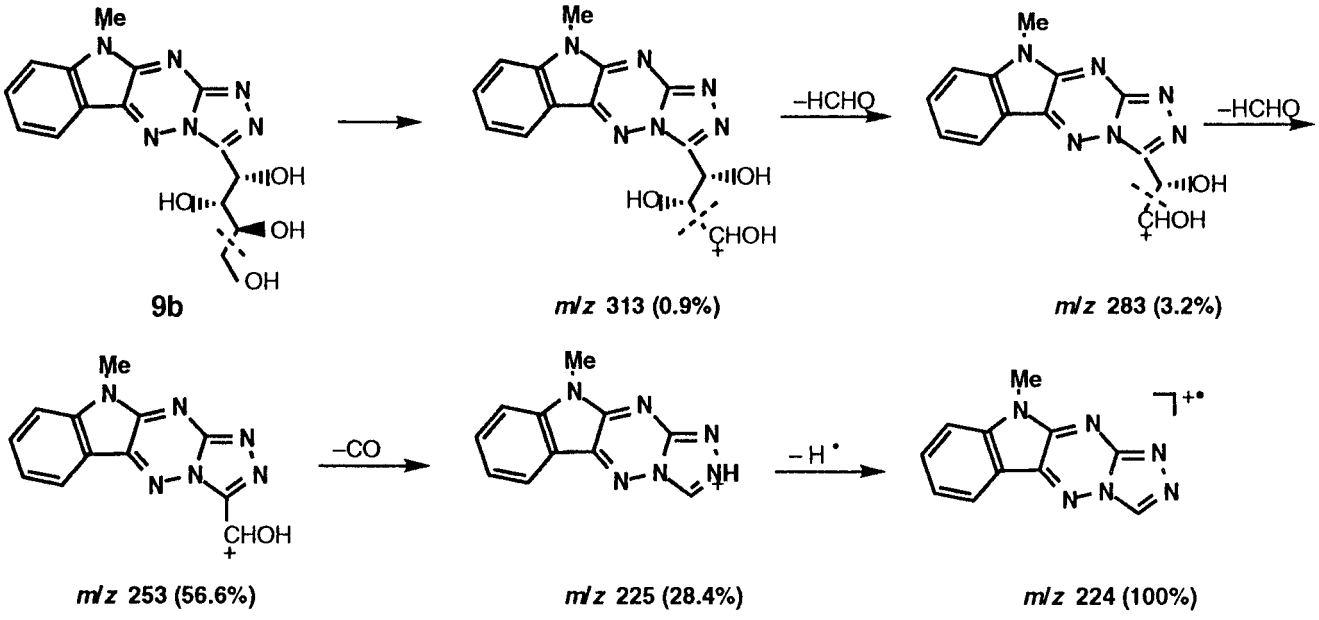


Fig. 2: UV spectra of solutions of **8a** (– – –) (1.17×10^{-5} M) and **12** (– · – · –) (8.40×10^{-6} M)

Scheme 5



Scheme 6



group protons in addition to the alditolyl protons which were, in most cases, associated with the solvent [$(CD_3)_2SO$] to give broad signals between δ 3.55–3.35. The MS of **9b** (Scheme 6) revealed its M + 1 and M^+ peaks at m/z 345 and 344 respectively.

Compounds **3c**, **3f**, **9c** and **9f** were screened for their antibacterial activity *in vitro* against the Gram negative bacterium *Escherichia coli* and the two Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* as well as for antifungal activity against *Candida albicans* and *Aspergillus niger* using the agar diffusion method [67]. They showed (Table) weak antibacterial activity against *E. coli* and *B. subtilis* and were devoid of activity against *S. aureus*. They possessed weak antifungal activity against *C. albicans* and *A. niger*.

Table: Antimicrobial activity

Compd.	Inhibition zones (mm)*					
	Concn. (mg/ml)	*E. coli*	*B. subtilis*	*S. aureus*	*C. albicans*	*A. niger*
3c	0.24	7	8	–	8	9
3f	0.24	8	10	–	8	9
9c	0.24	–	8	–	8	9
9f	0.24	8	–	–	–	9
Ampicillin	0.24	21	21	17	–	–
Streptomycin	0.24	30	35	22	–	–
DMF	–	6.5	6.5	–	–	9

* Inhibition zones of less than 10 mm in diameter are considered to indicate weak activity

3. Experimental

Melting points were determined on MEL-TEMP II apparatus in open glass capillaries and are uncorrected. The (UV) spectra were recorded on a Perkin-Elmer Lambda 4B UV/VIS spectrophotometer. The (IR) spectra were recorded for potassium bromide discs on a Pye-Unicam SP1025 spectrophotometer. NMR spectra were carried out at ambient temperature (~25 °C) with a Varian EM-390 or with a Bruker AC-250 spectrometers using tetramethylsilane (TMS) as an internal standard. MS were performed at 70 eV on an analytical system consisting of a Du Pont 21-419 mass spectrometer interfaced with a Du Pont 492-094 data acquisition station or on a Hewlett-Packard 5995 GC/MS system. Structure geometries were optimized using molecular mechanics (program Chem 3D Plus, version 2.0.1). Homogeneity of the products and follow up of the reactions were checked by ascending TLC on plates precoated with silica gel G (E. Merck; layer thickness 0.25 mm), used without pretreatment. All ratios of the used solvent systems were volume to volume (V/V); the distance of the solvent travel was 5 cm and the spots were visualized by exposure to iodine vapour for a few minutes. Elemental microanalyses were performed at the Microanalytical Unit, Cairo University, Cairo, Egypt. The prepared compounds gave satisfactory elemental analyses.

3.1. General procedure for the preparation of aldehydo-sugar {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazones **3a–g**

A solution of **1** (5 mmol) in ethanol (30 ml) was added to the appropriate sugar (**2a–g**, 5 mmol) in water (2 ml) and heated in a boiling water-bath for 15 min, kept at room temperature for 24 h and the crystalline product which separated, was filtered, washed with ethanol, and recrystallized from H_2O/EtOH. The following hydrazones were prepared:

3.1.1. aldehydo-D-Arabinose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(3a)**

Pale yellow crystals; Yield; 84%; m.p.: 195 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.51; IR 3290 (OH + NH) and 1591 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.35 (s, 1 H, exchangeable, =N–NH–), 8.15 (d, 1 H, aromatic H), 7.75–7.15 (m, 4 H, –CH–=N– + 3 aromatic H), 4.95 (d, 1 H, exchangeable, OH) 4.55 [m, 2 H; an exchangeable H (OH) + alditolylidene H], 4.35 (m, 2 H, alditolylidene H), and 3.75 ppm (s, 3 H, *N*-CH_3).
$C_{15}H_{18}N_6O_4 \cdot 1\frac{1}{2}\ H_2O$ (373)

3.1.2. aldehydo-L-Arabinose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(3b)**

Pale yellow crystals; Yield: 77%; m.p.: 188 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.68; IR: 3286 (OH + NH) and 1591 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.1 (s, 1 H, exchangeable, =N–NH–), 8.10 (d, 1 H, aromatic H), 7.80–7.10 (m, 4 H, –CH=N– + 3 aromatic H), 4.95 (m, 1 H, alditolylidene H), 4.60 [m, 2 H; an exchangeable H (OH) + alditolylidene H], 4.40 (m, 3 H, alditolylidene H), and 3.70 ppm (s, 3 H, *N*CH_3).
$C_{15}H_{18}N_6O_4 \cdot H_2O$ (364)

3.1.3. aldehydo-D-Ribose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(3c)**

Pale yellow crystals; Yield: 80%; m.p.: 124 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.66; IR: 3338 (OH + NH) and 1585 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.25 (s, 1 H, exchangeable, =N–NH–), 8.20 (d, 1 H, aromatic H), 7.80–7.20 (m, 4 H, –CH=N– + 3 aromatic H), 5.30 (m, 1 H, exchangeable, OH), 5.10–4.82 (m, 1 H, alditolylidene H), 4.82–4.50 (m, 2 H, alditolylidene H), and 4.25 ppm (s, 3 H, *N*-CH_3).
$C_{15}H_{18}N_6O_4 \cdot H_2O$ (364)

3.1.4. aldehydo-D-Xylose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(3d)**

Pale yellow crystals; Yield: 81%; m.p.: 153 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.60; IR: 3214 (OH + NH) and 1586 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.20 (s, 1 H, exchangeable, =N−NH−), 8.15 (d, 1 H, aromatic H), 7.75–7.20 (m, 4 H, −CH=N− + 3 aromatic H), 5.15 (m 1 H, exchangeable, OH), 4.85 (m, 1 H, exchangeable, OH), 4.70–4.30 (m, 3 H, alditolylidene H), 4.30–4.10 (m, 2 H, alditolylidene H), and 3.70 ppm (s, 3 H, *N*-CH_3).
$C_{15}H_{18}N_6O_4 \cdot 1\frac{1}{2}\ H_2O$ (373)

3.1.5. aldehydo-D-Galactose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}-hydrazone **(3e)**

Pale yellow crystals; Yield: 88%; m.p.: 158 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.82; IR: 3376 (OH + NH) and 1588 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.10 (s, 1 H, exchangeable, =N−NH−), 8.15 (d, 1 H, aromatic H), 7.80–7.20 (m, 4 H, −CH=N− + 3 aromatic H), 4.85 (m, 1 H, exchangeable, OH), 4.50 [m, 2 H; an exchangeable, H (OH) + alditolylidene H), 4.30 [m, 2 H, alditolylidene H], 4.10 (m, 1 H, exchangeable, OH), 3.90 (m, 1 H, exchangeable, OH), and 3.50 ppm (s, 3 H, *N*-CH_3). ^{13}C NMR [$(CD_3)_2SO$]: δ 158.30 (alditolylidene C1′), 148.64, 147.75, 140.72, 137.57, 128.75, 122.07, 119.78, 118.17, 110.21 (C3, C4a, C9b, C5a, C8, C9, C7, C9a, and C-6 respectively), 71.29, 70.96, 70.60, 69.54, 63.91, (alditolylidene C2′, C3′, C4′, C5′ and C6′ respectively), and 26.99 ppm (*N*-CH_3).
$C_{16}H_{20}N_6O_5 \cdot 2\ H_2O$ (412)

3.1.6. aldehydo-D-Glucose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(3f)**

Pale yellow crystals; Yield; m.p.: 152 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.67; IR: 3373 (OH + NH) and 1585 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.30 (s, 1 H, exchangeable, =N−NH−), 8.10 (d, 1 H, aromatic H), 7.70–7.15 (m, 4 H, −CH=N− + 3 aromatic H), 5.20 (m, 1 H, exchangeable, OH), 4.75 (m, 1 H, alditolylidene H), 4.35 [m, 5 H; two exchangeable H (2 OH) + alditolylidene 3 H], and 3.70 ppm (s, 3 H, *N*-CH_3).
$C_{16}H_{20}N_6O_5 \cdot \frac{1}{2}\ H_2O$ (385)

3.1.7. aldehydo-D-Mannose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(3g)**

Pale yellow crystals; Yield: 80%; m.p.: 180 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.65; IR: 3191 (OH + NH) and 1587 cm^{-1} (C=N); ^1H NMR [$(CD_3)_2SO$]: δ 11.35 (s, 1 H, exchangeable, =N−NH−), 8.20 (d, 1 H, aromatic H), 7.85–7.25 (m, 4 H, −CH=N− + 3 aromatic H), 5.35 (m, 1 H, exchangeable, OH), 4.75 (m, 1 H, alditolylidene H), 4.40 [m, 5 H; two exchangeable H (2 OH) + alditolylidene 3 H], and 3.80 ppm (s, 3 H, *N*-CH_3).
$C_{16}H_{20}N_6O_5 \cdot H_2O$ (394)

3.2. General procedure for the preparation of poly-O-acetyl-aldehydo-sugar {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazones **(4a–g)**

A solution of the respective *aldehydo*-sugar hydrazone (**3a–g**, 4 mmol) in pyridine (3 ml) was treated with acetic anhydride (15 ml) for 24 h at ambient temperature. The reaction mixture was poured onto ice and H_2O and extracted with $CHCl_3$ (3 × 20 ml) and the $CHCl_3$ extract was washed with 10% $NaHSO_4$ solution (2 × 20 ml), H_2O, and dried (Na_2SO_4). The solvent was evaporated and the obtained residue was crystallized from MeOH. The following title compounds were prepared as just described:

3.2.1. 2,3,4,5-Tetra-O-acetyl-aldehydo-D-arabinose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(4 a)**

Yellow crystals; Yield: 65%; m.p.: 102 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.77; IR: 3463 (NH), 1747 (OAc), and 1587 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.35 (d, 1 H, aromatic H), 7.80–7.20 (m, 4 H, −CH=N− + 3 aromatic H), 6.25 (d, 1 H, alditolylidene H-1), 5.70 (dd, 1 H, alditolylidene H-2), 5.50–4.80 (m, 2 H, alditolylidene H-3 + H-4), 4.05 (dd, 1 H, alditolylidene H-4′), 3.80 (s, 3 H, *N*-CH_3), and 2.00 ppm (s, 12 H, 4 OAc).
$C_{23}H_{26}N_6O_8$ (514)

3.2.2. 2,3,4,5-Tetra-O-acetyl-aldehydo-L-arabinose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(4b)**

Yellow crystals; Yield; 64%; m.p.: 78 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.75; IR: 3464 (NH), 1746 (OAc), and 1692 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.40 (d, 1 H, aromatic H), 7.85–7.25 (m, 4 H, −CH=N− + 3 aromatic H), 6.28 (d, 1 H, alditolylidene H-1), 5.75 (dd, 1 H, alditolylidene H-2), 5.10 (m, 1 H, alditolylidene H-3), 4.40–4.00 (m, 2 H, alditolylidene H-4 + H-4′), 3.85 (s, 3 H, *N*-CH_3), 2.15 (s, 3 H, OAc), and 2.10 ppm (s, 9 H, 3 OAc).
$C_{23}H_{26}N_6O_8$ (514)

3.2.3. 2,3,4,5-Tetra-O-acetyl-aldehydo-D-ribose {5-methyl-1,2,4-triazino-[5,6-b]indol-3-yl}hydrazone **(4c)**

Yellow crystals; Yield: 58%; m.p.: 85 °C; TLC in 9 : 1 $CHCl_3$/MeOH R_f: 0.88; IR: 3427 (NH), 1737 (OAc), and 1588 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.40 (d, 1 H, aromatic H), 7.85–7.35 (m, 4 H, −CH=N− + 3 aromatic H), 6.40 (d, 1 H, alditolylidene H-1), 6.80 (dd, 1 H, alditolylidene H-2), 5.68–5.55 (m, 1 H, alditolylidene H-3), 5.30 (dd, 1 H, alditolylidene H-4), 4.15 (dd, 1 H, alditolylidene H-4′), 3.85 (s, 3 H, *N*-CH_3), 2.10 (s, 6 H, 2 OAc), 2.05, and 1.95 ppm (2 s, 3 H each, 2 OAc).
$C_{23}H_{26}N_6O_8$ (514)

3.2.4. 2,3,4,5-Tetra-O-acetyl-aldehydo-D-xylose {5-methyl-1,2,4-triazino-[5,6-b]indol-3-yl]hydrazone **(4d)**

Yellow crystals; Yield: 60%; m.p.: 85 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.65; IR: 3432 (NH), 1749 (OAc), and 1585 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.50 (d, 1 H, aromatic H), 7.90–7.30 (m, 4 H, −CH=N− + 3 aromatic H), 6.00 (d, 1 H, alditolylidene H-1), 5.70–5.10 (m, 2 H, alditolylidene H-2 + H-3), 4.50–4.05 (m, 2 H, alditolylidene H-4 + H-4′), 3.90 (s, 3 H, *N*-CH_3), and 2.20 ppm (s, 12 H, 4 OAc).
$C_{23}H_{26}N_6O_8$ (514)

3.2.5. 2,3,4,5,6-Penta-O-acetyl-aldehydo-D-galactose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(4e)**

Yellow crystals; Yield: 66%; m.p.: 92 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.70; IR: 3429 (NH), 1746 (OAc), and 1581 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.45 (d, 1 H, aromatic H), 7.95–7.40 (m, 4 H, −CH=N− + 3 aromatic H), 6.05 (d, 1 H, alditolylidene H-1), 5.75 (dd, 1 H, alditolylidene H-2), 5.35–5.00 (m, 2 H, alditolylidene H-3 + H-4), 4.40–4.10 (m, 2 H, alditolylidene H-5 + H-5′), 3.95 (s, 3 H, N−CH_3), 2.50, 2.20 (2 s, 3 H each, 2 OAc), 2.15 (s, 6 H, 2 OAc), and 2.05 ppm (s, 3 H, OAc).
$C_{26}H_{30}N_6O_{10}$ (586)

3.2.6. 2,3,4,5,6-Penta-O-acetyl-aldehydo-D-glucose{5-methyl-1,2,4-triazino-[5,6-b]indol-3-yl]hydrazone **(4f)**

Yellow crystals; Yield: 63%; m.p.: 99 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.75; IR: 3427 (NH), 1749 (OAc), and 1585 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.40 (d, 1 H, aromatic H), 7.85–7.25 (m, 4 H, −CH=N− + 3 aromatic H), 6.05 (d, 1 H, alditolylidene H-1), 5.30 (dd, 1 H, alditolylidene H-2), 5.20–4.85, 4.35–4.00 (2 m, 2 H each, alditolylidene H-3 + H-4, H-5 + H-5′), 3.80 (s, 3 H, *N*-CH_3), 2.40 (s, 3 H, OAc), and 2.05 ppm (s, 12 H, 4 OAc).
$C_{26}H_{30}N_6O_{10}$ (586)

3.2.7. 2,3,4,5,6-Penta-O-acetyl-aldehydo-D-mannose {5-methyl-1,2,4-triazino[5,6-b]indol-3-yl}hydrazone **(4g)**

Yellow crystals; Yield: 64%; m.p.: 93 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.76; IR: 3447 (NH), 1747 (OAc), and 1587 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 8.35 (d, 1 H, aromatic H), 7.80–7.25 (m, 4 H, −CH=N− + 3 aromatic H), 6.32 (d, 1 H, alditolylidene H-1), 6.10, 5.65 (2 dd, 1 H each, alditolylidene H-2, H-3), 5.45–4.85 (m, 1 H, alditolylidene H-4), 4.35–4.00 (m, 2 H, alditolylidene H-5 + H-5′), 3.85 (s, 3 H, *N*-CH_3), 2.20, 2.10, 2.05, 2.00, and 1.95 ppm (5 s, 3 H each, 5 OAc).
$C_{26}H_{30}N_6O_{10}$ (586)

3.3. General procedure for the preparation of 3-(poly-O-acetyl-alditol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indoles **(8a–g)**

Method A: A solution of bromine (2 mmol) in glacial acetic acid (5 ml) was gradually added at ambient temperature to a stirred mixture of the appropriate hydrazone acetate (**4a–g**, 2 mmol) and anh. sodium acetate (6 mmol) in glacial acetic acid (15 ml). Stirring was continued for two additional hours and the mixture was evaporated under reduced pressure. The $CHCl_3$− soluble portion of the residue was evaporated and the product was crystallized from MeOH.
Method B: A solution of bromine (3 mmol) in glacial acetic acid (5 ml) was added dropwise at ambient temperature to a stirred mixture of the respective hydrazone (**3a–g**, 3 mmol) and anh. sodium acetate (9 mmol) in glacial acetic acid (15 ml). The reaction mixture was stirred for two additional hours and then treated with acetic anhydride (15 ml). The reaction mixture was kept for 24 h at ambient temperature and evaporated to dryness. The $CHCl_3$− soluble portion of the obtained residue was evaporated and the product was crystallized from CH_3OH.

3.3.1. 3-(1,2,3,4-Tetra-O-acetyl-D-arabino-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole **(8a)**

Yellow crystals; Yield: method A: 76%; method B: 73%; m.p.: 175 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.64; IR: 1750 (OAc) and 1607 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 318 (4.08), 269 (4.84), and 263 (sh); ^1H NMR ($CDCl_3$); δ 8.20 (d, 1 H, aromatic H), 7.70 (m, 1 H, aromatic H), 7.35 (m,

2 H, aromatic H), 6.75 (d, 1 H, alditolyl H-1), 5.85 (m, 1 H, alditolyl H-2), 5.50 (m, 1 H, alditolyl H-3), 4.30 (m, 2 H, alditolyl H-4 + H-4′), 3.75 (s, 3 H, *N*-CH_3), 2.20, 2.15 (2 s, 3 H each, 2 OAc), and 2.05 ppm (s, 6 H, 2 OAc); MS: m/z (%) 513 (2.87%) (M + 1).
$C_{23}H_{24}N_6O_8$ (512)

3.3.2. 3-(1,2,3,4-Tetra-O-acetyl-L-arabino-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(8b)**

Yellow crystals; Yield: method A: 74%; method B: 70%; m.p.: 185 °C; TLC in 9:1 $CHCl_3$/MeOH, R_f: 0.60; IR: 1750 (OAc) and 1607 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 317 (4.19), 269 (4.91), and 263 (sh); ^1H NMR ($CDCl_3$); δ 8.25 (d, 1 H, aromatic H), 7.75 (m, 1 H, aromatic H), 7.35 (m, 2 H, aromatic H), 6.75 (d, 1 H, alditolyl H-1), 5.80, 5.45 (2 m, 1 H each, alditolyl H-2, H-3), 4.30 (m, 2 H, alditolyl H-4 + H-4′), 3.80 (s, 3 H, *N*-CH_3), 2.20, 2.15 (2 s, 3 H each, 2 OAc), and 2.05 ppm (s, 6 H, 2 OAc); MS: m/z (intensity %) 513 (0.6%) (M + 1) and 512 (1.1%) (M^+).
$C_{23}H_{24}N_6O_8$ (512)

3.3.3. 3-(1,2,3,4-Tetra-O-acetyl-D-ribo-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(8c)**

Yellow crystals; Yield: method A: 68%; method B: 65%; m.p.: 122 °C; TLC in 9:1 $CHCl_3$/MeOH, R_f: 0.68; IR: 1753 (OAc) and 1608 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 319 (4.23), 269 (4.98), and 264 (sh); ^1H NMR ($CDCl_3$); δ 8.05 (m, 1 H, aromatic H), 7.65 (m, 1 H, aromatic H), 7.25 (m, 2 H, aromatic H), 6.68 (d, 1 H, alditolyl H-1), 5.95 (t, 1 H, alditolyl H-2), 5.50 (m, 1 H, alditolyl H-3), 4.35 (m, 2 H, alditolyl H-3), 4.35 (m, 2 H, alditolyl H-4 + H-4′), 3.75 (s, 3 H, *N*-CH_3), and 2.15 ppm (s, 12 H, 4 OAc).
$C_{23}H_{24}N_6O_8$ (512)

3.3.4. 3-(1,2,3,4-Tetra-O-acetyl-D-xylo-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(8d)**

Yellow crystals; Yield: method A: 71%; method B: 67%; m.p.: 110 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.53; IR: 1748 (OAc) and 1601 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 319 (4.00), 269 (4.74), and 263 (sh); ^1H NMR ($CDCl_3$); δ 8.15 (d, 1 H, aromatic H), 7.70 (m, 1 H, aromatic H), 7.35 (m, 2 H, aromatic H), 6.70 (d, 1 H, alditolyl H-1), 6.10, 5.30 (2 m, 1 H each, alditolyl H-2, H-3), 4.35 (m, 2 H, alditolyl H-4 + H-4′), 3.80 (s, 3 H, *N*-CH_3), 2.20 (s, 3 H, OAc), and 2.10 ppm (s, 9 H, 3 OAc).
$C_{23}H_{24}N_6O_8$ (512)

3.3.5. 3-(1,2,3,4,5-Penta-O-acetyl-D-galacto-pentitol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(8e)**

Yellow crystals; Yield: method A: 78%; method B: 77%; m.p.: 228 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.70; IR: 1750 (OAc) and 1606 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 319 (4.39), 269 (5.12), and 264 (sh); ^1H NMR ($CDCl_3$); δ 8.25 (d, 1 H, aromatic H), 7.75 (t, 1 H, aromatic H), 7.40 (t, 2 H, aromatic H), 6.60 (s, 1 H, alditolyl H-1), 5.70 (s, 2 H, alditolyl H-2 + H-3), 5.40 (m, 1 H, alditolyl H-4) 3.80 (s, 3 H, *N*-CH_3), 4.35, 4.00 (2 dd, 1 H each, alditolyl H-5, H-5′), 2.15 (s, 6 H, 2 OAc), 2.10 (s, 3 H, OAc), and 2.10 ppm (s, 6 H, 2 OAc).
$C_{26}H_{28}N_6O_{10}$ (584)

3.3.6. 3-(1,2,3,4,5-Penta-O-acetyl-D-gluco-pentitol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(8f)**

Yellow crystals; Yield: method A: 70%; method B: 66%; m.p.: 210 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.70; IR: 1749 (OAc) and 1603 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 320 (4.89), 268 (5.60), and 263 (sh); ^1H NMR ($CDCl_3$); δ 8.10 (d, 1 H, aromatic H), 7.70 (m, 1 H, aromatic H), 7.30 (m, 2 H, aromatic H), 6.55 (d, 1 H, alditolyl H-2), 6.15 (dd, 1 H, alditolyl H-2), 5.15 (m, 2 H, alditolyl H-3 + H-4), 4.05 (m, 2 H, alditolyl H-5, H-5′), 3.75 (s, 3 H, *N*-CH_3), 2.10 (s, 9 H, 3 OAc), 2.00, and 1.90 ppm (2 s, 3 H each, 2 OAc).
$C_{26}H_{28}N_6O_{10}$ (584)

3.3.7. 3-(1,2,3,4,5-Penta-O-acetyl-D-manno-pentitol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(8g)**

Yellow crystals; Yield: method A: 72%; method B: 66%; m.p.: 202 °C; TLC in 9 : 1 $CHCl_3$/MeOH, R_f: 0.77; IR: 1747 (OAc) and 1605 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 319 (3.66), 269 (4.45) and 263 (sh); ^1H NMR ($CDCl_3$); δ 8.31 (d, 1 H, aromatic H), 7.79 (t, 1 H, aromatic H), 7.43 (t, 2 H, aromatic H), 6.53 (d, 1 H, alditolyl H-1), 6.21 (t, 1 H, alditolyl H-2), 5.81 (d, 1 H, alditolyl H-3), 5.21 (m, 1 H, alditolyl H-4), 4.28, 4.19 (2 dd, 1 H each, alditolyl H-5, H-5′), 3.82 (s, 3 H, N−CH_3), 2.19, 2.13, 2.10, 2.00, and 1.81 ppm (5 s, 3 H each, 5 OAc); ^{13}C NMR ($CDCl_3$): δ 170.62, 169.90, 169.78, 169.48, 169.28 (5 OO*C*CH_3), 148.39, 147.08, 146.82, 142.11, 139.82, 134.08, 123.58, 123.03, 115.99, 110.37 (C3, C11a, C10a, C5a, C9a, C7, C6, C8, C5b, C9 respectively), 68.18, 68.06, 67.38, 64.02, 62.02 (C1′, C2′, C3′, C4′, C5′, respectively), 27.71 (*N*-CH_3), 21.05, 20.73, and 20.25 ppm (5 OO*C*CH_3).
$C_{26}H_{28}N_6O_{10}$ (584)

***3.4. General procedure for the preparation of 3-(alditol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indoles* 9a–g**

A solution of the appropriate acetate (**8a–g**, 2 mmol) in methanol (50 ml) was treated with 20% aqueous NH_3 solution (10 ml) and kept at ambient temperature for 24 h. Evaporation of the solvents under reduced pressure gave a residue which crystallized from a H_2O/MeOH-mixture. The following title compounds were prepared as just described:

3.4.1. 3-(D-Arabino-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(9a)**

Yellow crystals; Yield: 62%; m.p.: 215 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.67; IR: 3253 (OH) and 1605 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 317 (4.10), 269 (4.77), and 265 (sh): ^1H NMR [$(CD_3)_2SO$]: δ 7.95 (d, 1 H, aromatic H), 7.65–7.05 (m, 3 H, 3 aromatic H), 5.35 (d, 1 H, alditolyl H), and 4.00 ppm (s, 3 H, *N*-CH_3).
$C_{15}H_{16}N_6O_4$ (344)

3.4.2. 3-(L-Arabino-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole **(9b)**

Yellow crystals; Yield: 64%; m.p.: 203 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.68; IR: 3256 (OH) and 1604 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 318 (3.87), 269 (4.65) and 265 (sh); ^1H NMR [$(CD_3)_2SO$]: δ 8.00 (d, 1 H, aromatic H), 7.80–7.20 (m, 3 H, 3 aromatic H), 5.40 [m, 2 H; an exchangeable H (OH) + alditolyl H], 4.75, 4.55, 4.25 (3 m, 1 H each, exchangeable, 3 OH), 4.05 (m, 1 H, alditolyl H), and 3.65 ppm (s, 3 H, *N*-CH_3), MS: m/z (%) 345 (0.8%) (M + 1) and 344 (0.8%) (M^+).
$C_{15}H_{16}N_6O_4$ (344)

3.4.3. 10-Methyl-3-(D-ribo-tetritol-1-yl)-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole **(9c)**

Yellow crystals; Yield: 57%; m.p.: 260 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.72; IR: 3375 (OH) and 1602 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 318 (3.96), 269 (4.72), and 265 (sh); ^1H NMR [$(CD_3)_2SO$]: δ 8.00 (d, 1 H, aromatic H), 7.55–7.20 (m, 3 H, 3 aromatic H), 5.80 (d, 1 H, exchangeable, OH), 5.25 (m, 1 H, alditolyl H), 4.70 (m, 2 H, exchangeable, 2 OH), 4.35 (m, 2 H, alditolyl 2 H), and 3.60 ppm (s, 3 H, *N*-CH_3).
$C_{15}H_{16}N_6O_4$ (344)

3.4.4. 10-Methyl-3-(D-xylo-tetritol-1-yl)-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indoles **(9d)**

Yellow crystals; Yield: 60%; m.p.: 246 °C; TLC in 1:1 $CHCl_3$/MeOH, R_f: 0.66; IR: 3361 (OH) and 1605 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 318 (4.65), 269 (4.82), and 264 (sh); ^1H NMR [$(CD_3)_2SO$]: δ 7.95 (d, 1 H, aromatic H), 7.70–7.10 (m, 3 H, 3 aromatic H), 5.65 (m, 1 H, exchangeable, OH), 5.35 (d, 1 H, alditolyl H), 4.65 (m, 1 H, exchangeable, OH), 4.35 (m, 4 H, two exchangeable H, 2 OH + alditolyl 2 H), and 3.70 ppm (s, 3 H, *N*-CH_3).
$C_{15}H_{16}N_6O_4$ (344)

3.4.5. 3-(D-Galacto-pentitol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ :2,3]1,2,4-triazino[5,6-b]indole **(9e)**

Yellow crystals; Yield: 68%; m.p.: 257 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.59; IR: 3364 (OH) and 1603 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 318 (3.72), 269 (4.49), and 264 (sh); ^1H NMR [$(CD_3)_2SO$]: δ 8.00 (d, 1 H, aromatic H), 7.50–7.15 (m, 3 H, 3 aromatic H), 5.35 (m, 1 H, alditolyl H), 4.65 (d, 1 H, exchangeable, OH), 4.40 (t, 1 H, alditolyl H), 4.20 (m, 2 H, alditolyl H), and 3.65 ppm (s, 3 H, *N*-CH_3).
$C_{16}H_{18}N_6O_5$ (374)

3.4.6. 3-(D-Gluco-pentitol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole **(9f)**

Yellow crystals; Yield: 64%; m.p.: 247 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.52; IR: 3324 (OH) and 1603 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 317 (3.86), 269 (4.63), and 264 (sh); ^1H NMR [$(CD_3)_2SO$]: δ 8.00 (d, 1 H, aromatic H), 7.55 (m, 2 H, aromatic H), 7.20 (t, 1 H, aromatic H), 5.60 (d, 1 H, exchangeable, OH), 5.25 (m, 1 H, alditolyl H), 4.60 [m, 2 H; an exchangeable H (OH) + alditolyl H], 4.20 (m, 3 H, exchangeable, 3 OH), and 3.65 ppm (s, 3 H, *N*-CH_3).
$C_{16}H_{18}N_6O_5$ (374)

3.4.7. 3-(D-Manno-pentitol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′:2,3]1,2,4-triazino[5,6-b]indole **(9g)**

Yellow crystals; Yellow Crystals; Yield: 62%; m.p.: 288 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.52; IR: 3372 (OH) and 1600 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 318 (3.88), 269 (4.65), and 265 (sh), and 209.9 (4.76); ^1H NMR [$(CD_3)_2SO$]: δ 8.15 (d, 1 H, aromatic H), 7.55 (m, 2 H, 2 aromatic H), 7.25 (t, 1 H, aromatic H), 5.75 (d, 1 H, exchangeable, OH), 5.25 (m, 1 H, alditolyl H), 4.45 [m, 6 H; four exchangeable H (4 OH) + alditolyl 2 H], and 3.80 ppm (s, 3 H, *N*-CH_3).
$C_{16}H_{18}N_6O_5$ (374)

3.5. 3,10-Dimethyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole (**12**)

A mixture of 1-methylisatin (**10**, 1 mmol), 3,4-diamino-5-methyl-1,2,4-triazole hydrochloride [59] (**11**, 1 mmol) and sodium acetate (1 mmol) in 25% aqueous EtOH (25 ml) was heated at reflux for 1 hrs. Acetic acid (0.2 ml) was added and heating was continued for two additional hours. The product which separated after attaining ambient temperature was filtered and crystallized from EtOH in yellow crystals (62%); m.p.: 338 °C, lit. [47], m.p.: 332 °C; TLC in 9:1 $CHCl_3$/MeOH, R_f: 0.37; IR: 1609 cm^{-1} (C=N); λ_{max}^{MeOH} nm (log ε): 317 (4.29), 268 (5.03), and 263 (sh).
$C_{12}H_{10}N_6$ (238)

3.6. Attempted acid-catalyzed Dimroth rearrangement of 3-(1,2,3,4-tetra-O-acetyl-L-arabino-tetritol-1-yl)-10-methyl-1,2,4-triazolo[4′,3′ : 2,3]1,2,4-triazino[5,6-b]indole (**8b**)

A solution of **8b** (1 mmol) in acetic acid (10 ml) was refluxed for 2 h and then evaporated under reduced pressure. The residue was crystallized from EtOH. TLC, m.p., mixed m.p., and UV spectrum of the obtained product were identical to those of the starting compound **8b**.

3.7. Antimicrobial screening

Sterile nutrient agar plates (100 ml) were separately inoculated with a 24 h broth culture (1 ml) of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. Solutions (60 ml) of the tested compounds (0.24 mg) in DMF (1 ml) were placed in wells (6 mm diam.) cut in the agar media and the plates were incubated at 37 °C in the case of bacteria and 25 °C in the case of yeast. The diameters of the resulting inhibition zones were measured after 28 h for bacteria and 96 h for the yeast [67].

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