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Synthesis and antiviral activity of azoles obtained from carbohydrates

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ARTICLE INFO

Article history:
Received 21 November 2007
Received in revised form 1 June 2008
Accepted 28 June 2008
Available online xxxx

Keywords:
1,2,4-Triazolyl-3-thione
1,3,4-Oxadiazole
Imidazo[2,1-*b*]thiazole
Carbohydrates
Antiviral activity

ABSTRACT

Herein we describe the synthesis of 1,2,4-triazolyl-3-thione;1,3,4-oxadiazole, and imidazo[2,1-*b*]thiazole derivatives from carbohydrates. The antiviral activity of these compounds was tested against Dengue and Junin virus (the etiological agent of Argentine hemorrhagic fever). The 3-(*p*-bromobenzoyl)-5-(1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-ulos-5-yl)imidazo[2,1-*b*]thiazole was able to inhibit the replication of both viruses in Vero cells at concentration significantly lower than the CC_{50}.

Azoles are heterocyclic compounds containing at least two nitrogen atoms in their structure and are proved to be clinically useful agents against different kinds of diseases. They have been shown to possess a broad spectrum of biological activity depending on their particular structure. In fact, azoles constitute the largest group of antifungal compounds clinically in use. Antitumoral, antiviral, antibacterial, anti-inflammatory, and antihelminthic properties have also been reported.[1–5]

In an attempt to correlate structure with antiviral activity, herein we report the synthesis and antiviral evaluation (against Dengue and Junin virus) of 1,3,4-oxadiazoles, 1,2,4-triazolyl-3-thiones and imidazo[2,1-*b*]thiazoles substituted with a carbohydrate derivative and a halophenyl group. The choice of heterocyclic ring substituents was based on literature reports where the increase of biological activity in several compounds was related to the presence of asymmetric centers[6] and/or halogens in their structure.[7,8] On the other hand, according to the current tendency of using compounds that come from the renewable biomass, syntheses were carried out using D-glucose as starting material.

Substituted imidazo[2,1-*b*]thiazoles (**5a**/**b**) were obtained by a convergent synthetic pathway (Scheme 1), the synthesis of 6-bromo-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*xylo*-hexofuranos-5-ulose (**3**) being the key step of this synthetic approach.

Selective replacement of primary hydroxyl group in 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (**1**)[9] by a bromine atom, by using the *N*-bromosuccinimide/DMF system, led to **2** with acceptable yield as was previously described for related compounds.[10] Furthermore, an excellent primary versus secondary hydroxyl group selectivity was observed, since the dibromocarbohydrate derivate was not detected. These results reinforce those previously reported for pyranose forms, making this methodology a simple and versatile way to introduce a bromine atom into a monosaccharide structure.

Compound **3** was obtained after treating **2** with 1-hydroxy-1,2-benziodoxol-3(1*H*)-one-1-oxide (IBX) in DMSO as oxidizing agent.[11] The lack of the signal corresponding to H-5 in the ^{1}H NMR spectrum of **3**, and the observation of the carbonyl signal (198.8 ppm) in the ^{13}C NMR spectrum, allowed confirmation that oxidation had taken place. The 5,6-anhydro-1,2-isopropylidene-3-*O*-methyl-α-D-glucofuranose was detected by NMR as a secondary product (see Section 1).

The coupling between compounds **3** and **4a**/**b** led to the corresponding heterocyclic derivatives **5a**/**b**. In the ^{1}H NMR spectra the signals of the heterocyclic protons appeared at 8.94 and 8.51 ppm for both derivatives (**5a**/**b**), while in their ^{13}C NMR spectra the resonance corresponding to the heterocyclic carbons was observed in a range between 159 and 128 ppm. In addition, the appearance of aromatic signals and the absence of the methylenic ones confirmed that the transformation **3**→**5** had occurred (See Scheme 1).

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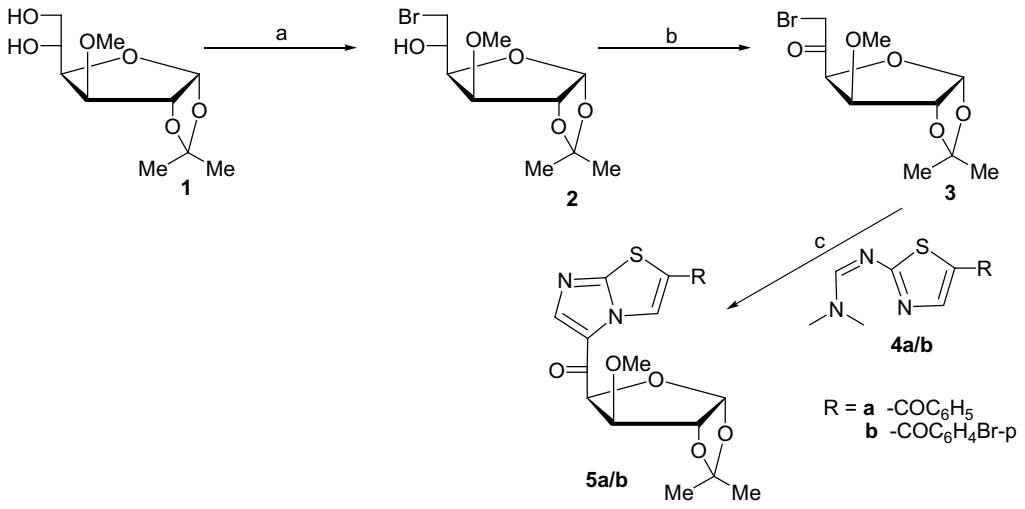
doi:10.1016/j.carres.2008.06.028



Scheme 1. Reagents and conditions: (a) NBS, PPh_3 (1:2 equiv)/DMF (anhyd), **2**: 72%; (b) IBX/DMSO (anhyd), **3**: 18%; (c) THF/Et, **5a**: 27%, **5b**: 30%.

In order to obtain other azole derivatives and taking into account our previous studies,[12] a series of 5-halophenyl-2-(1,2-*O*-isopropylidene-α-D-xylo-tetrafuranosyl)-1,3,4-oxadiazoles (**8a–f**) were synthesized (Scheme 2). Compounds **7a–f** were obtained by coupling 1,2-*O*-isopropylidene-α-D-xylopentadialdo-1,4-furanose (**6**)[13,14] with a series of benzoyl hidrazines. The intramolecular cyclization of **7a–f** gave the corresponding 1,3,4-oxadiazole derivatives **8a–f**.

In the ^{1}H NMR spectra of compounds **7a–f** the expected two broad singlets around 8 and 11 ppm corresponding to the NH groups were observed, while their ^{13}C NMR spectra showed signals of carbonyl groups around 170 ppm.

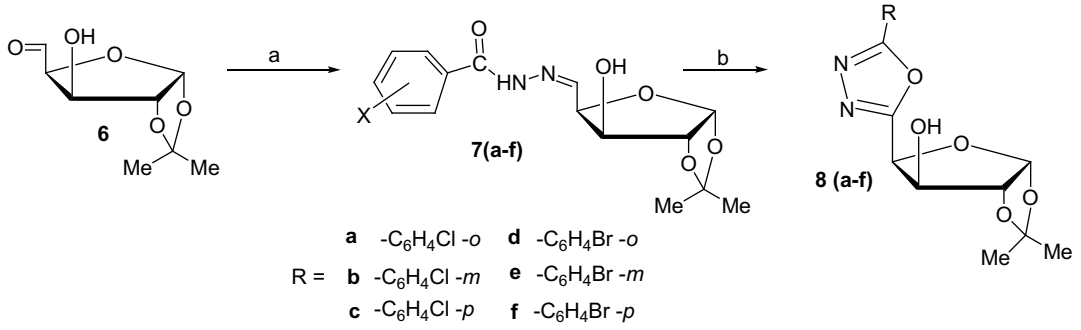
The ^{1}H and ^{13}C NMR spectra of **7a** and **7d** (*o*-halophenyl derivatives) showed the presence of a diastereomeric mixture (*syn* and *anti* isomers). Assignment of each isomer in the ^{1}H NMR spectrum was made taking into account data previously reported for related compounds.[15] In the NMR spectra of **7b**, **7c**, **7e**, and **7f** only one isomer was observed, and the resonance of C-5 around 149 ppm suggested the presence of the *anti* isomer.

In the ^{13}C NMR spectra of **8a–f** the signals of both heterocyclic carbons were observed between 162 and 164 ppm, confirming that the intramolecular cyclization of **7a**–**f** had occurred.

In order to extend the study to other azole derivates, substituted 1,2,4-triazoline-3-thiones were synthesized according to the synthetic pathway shown in Scheme 3. The 6-deoxy-6-isothiocyanate (**10**) was obtained from the 3,5-di-*O*-acetyl-6-deoxy-1,2-*O*-isopropylidene-6-azido-α-D-glucofuranose (**9**)[16,17] in quantitative yield. The chemical shift of the signal corresponding to the C-6 observed in the ^{13}C NMR spectrum was considered diagnostic of the conversion, since the replacement of the azido by an isothiocyanate group shifts the C-6 resonance upfield[18] (see Section 1).

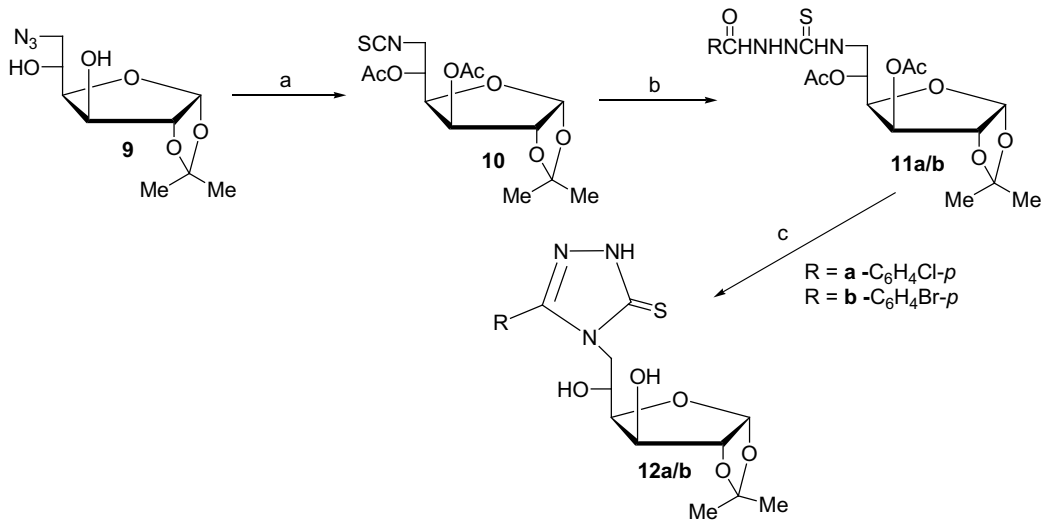
Reaction of **10** with either 4-chloro- or 4-bromobenzoic hydrazide led to thiosemicarbazides **11a** and **11b** in quantitative yield, which by a further intramolecular cyclization[19] gave the corresponding 1,2,4-triazoline-3-thiones (**12a**/**b**). The NMR spectra of **12a**/**b** showed the lack of the NH and carbonyl signals. In addition, in their ^{13}C NMR spectra the signal characteristic of a C=N bond was found at 152.9 ppm while that corresponding to the C=S bond was moved from 182.4 ppm (**11a/b**) to 169 ppm (**12a/b**).

The antiviral activity of the new compounds was evaluated against two viruses with an RNA genome, the Junin virus (JUNV) and the dengue virus type 2 (DENV-2). These viruses were chosen because they are human pathogenic agents of severe diseases such as dengue fever, dengue hemorrhagic fever, and Argentine hemorrhagic fever and because there is currently no specific antiviral chemotherapy for patient treatment. The toxicity for Vero cells was first investigated and taking into account the cytotoxicity results (Table 1), the antiviral activity of the azole derivatives was then evaluated at concentrations below 100 μM. As it can be seen in Table 1, the thiazole **5b** was able to inhibit the replication of both viruses in Vero cells with EC_{50} values of 12.0 and 29.9 μM against JUNV and DENV-2, respectively. Thus, the selectivity index (SI), that is, the relationship between antiviral activity and cytotoxicity, expressed as the ratio CC_{50}/EC_{50}, for this compound was 10.2 and 4.1, respectively. Although these values of SI are not very high, they are indicative of a specific inhibitory action of the compound



Scheme 2. Reagents and conditions: (a) *o*, *m*, *p*-bromo or chlorobenzoic hydrazide/THF, **7a**: 34%, **7b**: 63%, **7c**: 94%, **7d**: 76%, **7e**: 70%, **7f**: 86%; (b) PIDA/MeOH, **8a**: 40%, **8b**: 50%, **8c**: 40%, **8d**: 58%, **8e**: 54%, **8f**: 34%.

Please cite this article in press as: Barradas, J. S. et al., *Carbohydr. Res.* (2008), doi:10.1016/j.carres.2008.06.028



Scheme 3. Reagents and conditions: (a) (i) Ac_2O/Py; (ii) CS_2, $(Ph)_3P$/dioxane, **10**: 100%; (b) $RCONHNH_2$/THF, **11a**: 100%, **11b**: 100%; (c) (i) NaOH 2N (aq); (ii) HCl (aq), **12a**: 41%, **12b**: 55%.

Table 1
Cytotoxicity and antiviral activity

Compound	CC_{50} (μM)[a]	EC_{50} (μM)[b]	
		JUNV	DENV-2
5a	137.4	>100	>100
5b	122.6	12.0	29.9
8a	547.2	>100	>100
8b	343.9	>100	>100
8c	364.4	>100	>100
8d	692.0	>100	>100
8e	221.0	>100	>100
8f	251.0	>100	64.6
12a	210.3	>100	>100
12b	245.3	>100	>100

[a] CC_{50} (cytotoxic concentration 50%): concentration required to reduce 50% of the number of viable Vero cells.
[b] EC_{50} (effective concentration 50%): concentration required to reduce virus yield in Vero cells by 50%.

against virus multiplication at concentrations significantly lower than the CC_{50}. The oxadiazole **8f** also exhibited antiviral action against DENV-2 with an EC_{50} value of 64.6 μM, but with selectivity lower than that of **5b** (Table 1). No virucidal activity was detected in any compound. When comparing the antiviral activity of **5a** and **5b**, we observed an increased activity in the latter that could only be attributed to the presence of a bromine atom, showing the convenience of including halogens in the antiviral drugs designed.

1. Experimental

1.1. General methods

NMR spectra were recorded on either a Bruker AC-200 or a Bruker AMX-500 spectrometer. Assignments were confirmed with the aid of two-dimensional techniques ^1H, ^1H (COSY 45) and ^1H, ^{13}C (HSQC). For the assignment of the ^{13}C NMR spectra, DEPT experiments were also conducted. Analytical TLC was conducted on Silica Gel 60G (Merck) on precoated plates. Optical rotations were measured with a Perkin–Elmer Model 343 Polarimeter. Column-chromatographic separations were performed on Silica Gel (240–400 mesh, Merck). Solvents were reagent grade and, in most cases, dried, and distillated before use according to standard procedures.

1.2. 6-Bromo-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (2)

NBS (1.77 g, 10.08 mmol) in DMF was added to a stirred solution of triphenylphosphine (2.62 g, 9.97 mmol) and **1**[9] (1.25 g, 5.34 mmol) in dry DMF under an argon atmosphere.[10] The mixture was stirred for 1 h at room temperature and quenched by addition of MeOH. The solvent was evaporated, and the residue was extracted with CH_2Cl_2/water and dried (Na_2SO_4). The crude product was purified by column chromatography on silica gel (80:20 cyclohexane/acetone) affording the 6-bromo derivative (**2**) as pallid yellow oil (1.14 g, 72% yield); $[\alpha]_D^{25}$ −44.5 (*c* 3.4, chloroform). Anal. Calcd for $C_{10}H_{17}BrO_5$: C, 40.42; H, 5.77. Found: C, 40.45; H, 5.75. ^1H NMR (500 MHz, $CDCl_3$) δ: 5.82 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.54 (d, 1H, $J_{2,1}$ 3.7 Hz, H-2), 3.84 (d, 1H, $J_{3,4}$ 3.0. Hz, H-3), 4.06 (dd, 1H, $J_{4,3}$ 2.9 Hz, $J_{4,5}$ 8.3 Hz, H-4), 4.92 (ddd, 1H, $J_{5,4}$ 8.3 Hz, $J_{5,6a}$ 6.1 Hz; $J_{5,6b}$ 3.0 Hz, H-5), 3.53 (dd, 1H, $J_{6a,5}$ 6.1 Hz, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.67 (dd, 1H, $J_{6b,5}$ 3.0 Hz, $J_{6b,6a}$ 10.7 Hz, H-6b), 3.41 (s, 3H, O*Me*), 1.43 (s, 3H, C*Me*$_2$); 1.27 (s, 3H, C*Me*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.1 (C-1), 81.5 (C-2), 83.8 (C-3), 80.7 (C-4), 68.0 (C-5), 38.3 (C-6), 111.9 (*C* Me$_2$), 58.0 (O*Me*), 26.9 (C*Me*$_2$), 26.3 (C*Me*$_2$).

1.3. 6-Bromo-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylo-hexofuranos-5-ulose (3)

1-Hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX) (1.55 g, 5.53 mmol) was added to a solution of **2** (1.09 g, 3.69 mmol) in DMSO, and the mixture was stirred for 72 h at room temperature and extracted with NaOH (aq)/$CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel (90:10 cyclohexane/acetone). Although TLC analyses of the product showed one spot, a secondary product was detected in the sample by NMR. Further purification of the mixture on silica gel (98:2 CH_2Cl_2/acetone) led to pure compound **3** as a pallid yellow oil (143.56 mg, 18% yield); $[\alpha]_D^{25}$ −76.9 (*c* 3.6, chloroform). Anal. Calcd for $C_{10}H_{15}BrO_5$: C, 40.70; H, 5.12. Found: C, 40.72; H, 5.09. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.05 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.60 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.08 (d, 1H,

$J_{3,4}$ 3.7 Hz, H-3), 4.96 (d, 1H, $J_{4,3}$ 3.7 Hz, H-4), 4.36 (d, 1H, $J_{6a,6b}$ 15.7 Hz, H-6a), 4.23 (d, 1H, $J_{6b,6a}$ 15.7 Hz, H-6b), 3.50 (s, 3H, O*Me*), 1.51 (s, 3H, C*Me*$_2$), 1.31 (s, 3H, C*Me*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 106.6 (C-1), 81.0 (C-2), 85.9 (C-3), 84.7 (C-4), 198.8 (C-5), 35.5 (C-6), 112.6 (*C*Me$_2$), 58.03 (O*Me*), 26.9 (C*Me*$_2$), 26.3 (C*Me*$_2$).

The secondary product was identified as 5,6-anhydro-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.92 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.62 (d, 1H, $J_{2,1}$ 3.6 Hz, H-2), 3.86 (d, 1H, $J_{3,4}$ 3.2 Hz, H-3), 3.72 (dd, 1H, $J_{4,3}$ 3.2 Hz, $J_{4,5}$ 7.1 Hz, H-4), 3.24 (ddd, 1H, $J_{5,4}$ 6.9 Hz, $J_{5,6a}$ 3.9 Hz, $J_{5,6b}$ 2.6 Hz, H-5), 2.91 (dd, 1H, $J_{6a,5}$ 3.9 Hz, $J_{6a,6b}$ 5.1 Hz, H-6a), 2.77 (dd, 1H, $J_{6b,5}$ 3.2 Hz, $J_{6b,6a}$ 5.1 Hz, H-6b), 3.45 (s, 3H, O*Me*), 1.47 (s, 3H, C*Me*$_2$), 1.32 (s, 3H, C*Me*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.2 (C-1), 81.9 (C-2), 84.3 (C-3), 81.5 (C-4), 47.9 (C-5), 46.8 (C6), 111.8 (*C*Me$_2$), 58.2 (O*Me*), 26.7 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.4. *N*′-(5-Benzoylthiazol-2-yl)-*N*,*N*-dimethylformamidine and *N*′-(5-*p*-bromobenzoylthiazol-2-yl)-*N*,*N*-dimethylformamidine (4a/b)

Compounds **4a**/**b** were synthesized from *N*,*N*′-bis(dimethylaminomethylene)thiourea as was previously described for **4b**.[20]

Compound **4a**: (light yellow crystals, 451.2 mg, 65% yield); mp: 114 °C. Anal. Calcd for $C_{13}H_{13}N_3OS$: C, 60.21; H, 5.05; N, 16.20; S, 12.37. Found: C, 60.10; H, 5.22; N, 16.01; S, 12.30. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.3 (s, 1H), 7.79 (s, 1H), 7.75–7.43 (m, 5H, aromatic protons), 3.11 (s, 3H), 3.08 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 187.3, 180.9, 156.5, 149.2, 138.4–128.5, 41.2, 45.3.

1.5. General procedure for the synthesis of imidazo[2,1-*b*]-thiazoles (5a,b)

Compound **3** (140.71, 0.48 mmol) in THF was added to a solution of **4a** (125.43 mg, 0.48 mmol) or **4b** (163.34 mg, 0.48 mmol) in dry THF. The reaction mixture was heated at reflux for 48 h, 2 equiv of Et_3N were added, and the mixture was stirred for 24 h at room temperature and evaporated. The crude product (**5a** or **5b**) was purified by column chromatography on silica gel (75:25 cyclohexane/acetone).

1.5.1. 3-Benzoyl-5-(1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-ulo-5-yl)imidazo[2,1-*b*]thiazole (5a)

White waxy appearance, 55.52 mg, 27% yield, $[\alpha]_D^{25}$ −99.2 (*c* 2.8, chloroform). Anal. Calcd for $C_{21}H_{20}N_2O_6S$: C, 58.87; H, 4.70; N, 6.54; S, 7.48. Found: C, 58.70; H, 4.60; N, 6.60; S, 7.50. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.18 (d, 1H, $J_{1,2}$ 3.60 Hz, H-1), 4.67 (d, 1H, $J_{2,1}$ 3.60 Hz, H-2), 4.19 (d, 1H, $J_{3,4}$ 3.60 Hz, H-3), 5.06 (d, 1H, $J_{4,3}$ 3.60 Hz, H-4), 7.91–7.55 (m, 5H, aromatic protons), 8.94 (s, 1H, imidazo[2,1-*b*] proton), 8.51 (s, 1H, imidazo[2,1-*b*] proton), 3.31 (s, 3H, O*Me*), 1.54 (s, 3H, C*Me*$_2$), 1.38 (s, 3H, C*Me*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.8 (C-1), 80.7 (C-2), 86.1 (C-3), 84.8 (C-4), 136.4–128.8 (aromatic carbons), 156.5, 146.9, 134.6, 128.3 (imidazo[2,1-*b*] carbons), 187.2 (*C*O), 184.8 (*C*O), 112.5 (*C*Me$_2$), 58.3 (O*Me*), 26.9 (C*Me*$_2$), 26.2 (C*Me*$_2$).

1.5.2. 3-(*p*-Bromobenzoyl)-5-(1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-ulo-5-yl)imidazo[2,1-*b*]thiazole (5b)

White waxy appearance, 73.07 mg, 30% yield, $[\alpha]_D^{25}$ −120.5 (*c* 1.2, chloroform). Anal. Calcd for $C_{21}H_{19}BrN_2O_6S$: C, 49.71; H, 3.77; N, 5.52; S, 6.32. Found: C, 49.80; H, 3.70; N, 5.60; S, 6.40. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.18 (d, 1H, $J_{1,2}$ 3.60 Hz, H-1), 4.67 (d, 1H, $J_{2,1}$ 3.60 Hz, H-2), 4.19 (d, 1H, $J_{3,4}$ 3.60 Hz, H-3), 5.06 (d, 1H, $J_{4,3}$ 3.60 Hz, H-4), 7.85 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.56 (d, 2H, *J* 8.6 Hz, aromatic protons), 8.94 (s, 1H, imidazo[2,1-*b*] proton), 8.51 (s, 1H, imidazo[2,1-*b*] proton), 3.33 (s, 3H, O*Me*), 1.54 (s, 3H, C*Me*$_2$), 1.39 (s, 3H, C*Me*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 106.0 (C-1), 80.9 (C-2), 86.3 (C-3), 85.0 (C-4), 140.1, 130.4, 129.6, 127.2 (aromatic carbons), 156.5, 147.2, 134.9, 129.0, 128.4 (imidazo[2,1-*b*] carbons), 186.1 (*C*O), 185.1 (*C*O), 112.8 (*C*Me$_2$), 58.5 (O*Me*), 27.0 (C*Me*$_2$), 26.4 (C*Me*$_2$).

1.6. General procedure for the synthesis of benzoylhidrazones (7a–f)

A solution of **6**[13,14] and the corresponding benzoylhydrazide in dry THF (1:1 molar ratio) was refluxed with continuous stirring for 2 h. The mixtures were concentrated, and from these solutions compounds **7(a–f)** crystallized.

1.6.1. 1,2-*O*-Isopropylidene-α-D-xylopentadialdo-1,4-furanose (*o*-chlorobenzoyl)hydrazone (7a)

34% yield as a mixture of *anti* and *syn* isomers (*anti*:*syn* 58:42). Anal. Calcd for $C_{15}H_{17}ClN_2O_5$: C, 52.87; H, 5.03. Found: C, 52.90; H, 4.98. ^{1}H NMR (500 MHz, CD_3COCD_3) *Anti* isomer δ: 5.93 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.56 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.32 (d, 1H, $J_{3,4}$ 2.9, H-3), 4.67 (dd, 1H, $J_{4,3}$ 3.0, $J_{4,5}$ 6.4, H-4), 7.52–7.36 (m, 5H, H-5 + aromatic protons), 1.44 (s, 3H, C*Me*$_2$), 1.26 (s, 3H, C*Me*$_2$), 10.82 (s, 1H, NH) 3.01, (s, 1H, OH); *Syn* isomer δ: 5.88 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 4.50 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.11 (d, 1H, $J_{3,4}$ 2.9 Hz, H-3), 4.35 (dd, 1H, $J_{4,3}$ 3.0 Hz, $J_{4,5}$ 7.1, H-4), 7.71 (d, 1H $J_{5,4}$ 6.4 Hz, H-5), 7.52–7.36 (m, 4H, aromatic protons), 1.31 (s, 3H, C*Me*$_2$), 1.21 (s, 3H, C*Me*$_2$), 10.73 (s, 1H, NH), 3.01, (s, 1H, OH); ^{13}C NMR (125 MHz, CD_3COCD_3) *Anti* isomer δ: 107.0 (C-1), 87.3 (C-2), 78.2 (C-3), 82.1 (C-4), 149.4 (C-5), 137.5–128.2 (aromatic carbons), 164.3 (*C*O), 112.9 (*C*Me$_2$), 27.9 (C*Me*$_2$), 27.2 (C*Me*$_2$); δ: *Syn* isomer δ 106.9 (C-1), 87.1 (C-2), 78.1 (C-3), 81.8 (C-4), 145.6 (C-5), 137.5–128.2 (aromatic carbons), 164.3 (*C*O), 112.7 (*C*Me$_2$), 27.8 (C*Me*$_2$), 26.9 (C*Me*$_2$).

1.6.2. 1,2-*O*-Isopropylidene-α-D-xylopentadialdo-1,4-furanose (*m*-chlorobenzoyl)hydrazone (7b)

63% yield; $[\alpha]_D^{25}$ −48.6 (*c* 0.9, chloroform). Anal. Calcd for $C_{15}H_{17}ClN_2O_5$: C, 52.87, H, 5.03. Found: C, 52.76; H, 5.08. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 5.98 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.60 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.35 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.68 (dd, 1H, $J_{4,3}$ 2.7 Hz, H-4), 7.71 (d, 1H $J_{5,4}$ 6.4 Hz, H-5), 7.93–7.78 (m, 3H, H-5 + aromatic protons), 7.63–7.48 (m, 2H, aromatic protons), 1.42 (s, 3H, C*Me*$_2$), 1.32 (s, 3H, C*Me*$_2$), 11.16 (s, 1H, NH), 2.99 (s, 1H, OH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 106.3 (C-1), 86.7 (C-2), 77.4 (C-3), 81.4 (C-4), 149.4 (C-5), 136.9–128.7 (aromatic carbons), 163.0 (*C*O), 112.2 (*C*Me$_2$), 27.1 (C*Me*$_2$), 26.4 (C*Me*$_2$).

1.6.3. 1,2-*O*-Isopropylidene-α-D-xylopentadialdo-1,4-furanose (*p*-chlorobenzoyl)hydrazone (7c)

94% yield; $[\alpha]_D^{25}$ −56.5 (*c* 0.6, chloroform). Anal. Calcd for $C_{15}H_{17}ClN_2O_5$: C, 52.87; H, 5.03. Found: C, 52.77; H, 5.15. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 5.96 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.57 (d, 1H, $J_{2,1}$ 3.6 Hz, H-2), 4.31 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.68 (dd, 1H, $J_{4,3}$ 2.7 Hz, H-4), 7.81 (d, 1H, *J* 6.4 Hz, H-5), 7.96 (d, 2H, *J* 7.9 Hz, aromatic protons), 7.48 (d, 2H, *J* 8.2 Hz, aromatic protons), 1.42 (s, 3H, C*Me*$_2$), 1.26 (s, 3H, C*Me*$_2$), 11.3 (s, 1H, NH), 3.09 (s, 1H, OH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 106.9 (C-1), 87.2 (C-2), 78.2 (C-3), 82.1 (C-4), 149.8 (C-5), 138.9, 133.3, 131.0, 130.1 (aromatic carbons), 164.1 (*C*O), 112.9 (*C*Me$_2$), 27.9 (C*Me*$_2$), 27.1 (C*Me*$_2$).

1.6.4. 1,2-*O*-Isopropylidene-α-D-xylopentadialdo-1,4-furanose (*o*-bromobenzoyl)hydrazone (7d)

76% yield as a mixture of *anti* and *syn* isomers (*anti*:*syn* 61:49). Anal. Calcd for $C_{15}H_{17}BrN_2O_5$: C, 46.87; H, 4.45. Found: C, 47.01; H, 4.47. ^{1}H NMR (500 MHz, CD_3COCD_3) *Anti* isomer δ: 5.93 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 4.55 (d, 1H, $J_{2,1}$ 3.3 Hz, H-2), 4.29 (d, 1H, $J_{3,4}$ 2.9 Hz, H-3), 4.66 (dd, 1H, $J_{4,3}$ 2.8 Hz, $J_{4,5}$.6.3 Hz, H-4), 7.63–7.33 (m, 5H, H-5 + aromatic protons), 1.42 (s, 3H, C*Me*$_2$), 1.24 (s, 3H, C*Me*$_2$),

10.90 (s, 1H, NH) 3.10, (s, 1H, OH); *Syn* isomer δ: 5.87 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 4.49 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.10 (d, 1H, $J_{3,4}$ 2.6 Hz, H-3), 4.29 (m, 1H, H-4), 7.70 (d, 1H, $J_{5,4}$ 6.3 Hz, H-5), 7.63–7.33 (m, 4H, aromatic protons), 1.35 (s, 3H, CMe_2), 1.20 (s, 3H, CMe_2), 10.80 (s, 1H, NH), 3.10, (s, 1H, OH); ^{13}C NMR (125 MHz, CD_3COCD_3) *Anti* isomer δ: 106.4 (C-1), 86.7 (C-2), 77.5 (C-3), 81.5 (C-4); 148.9 (C-5), 134.1–128.6 (aromatic carbons), 164.7 (*C*O), 112.4 (*C*Me$_2$), 27.3 (C*Me*$_2$), 26.5 (C*Me*$_2$); δ: *Syn* isomer δ 106.3 (C-1), 86.5 (C-2), 77.5 (C-3), 81.2 (C-4), 145.2 (C-5), 134.1–128.6 (aromatic carbons), 164.7 (*C*O), 112.1 (*C*Me$_2$), 27.2 (C*Me*$_2$), 26.4 (C*Me*$_2$).

1.6.5. 1,2-*O*-Isopropylidene-α-D-xylopentadialdo-1,4-furanose (*m*-bromobenzoyl)hydrazone (7e)

70% yield; $[\alpha]_D^{25}$ −59.3 (*c* 1.1, chloroform). Anal. Calcd for $C_{15}H_{17}BrN_2O_5$: C, 46.87; H, 4.45. Found: C, 46.90; H, 4.40. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 5.98 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.60 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.35 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.68 (dd, 1H, $J_{4,3}$ 2.7 Hz, $J_{4,5}$ 6.4 Hz, H-4), 7.81 (d, 1H, $J_{5,4}$ 6.5 Hz, H-5), 8.09 (s, 1H, aromatic proton), 7.92 (d, 1H, *J* 7.5 Hz, aromatic proton), 7.74 (d, 1H, *J* 7.9 Hz, aromatic proton), 7.43 (t, 1H, *J* 7.8, aromatic proton), 1.44 (s, 3H, C*Me*$_2$), 1.27 (s, 3H, C*Me*$_2$), 11.32 (s, 1H, NH), 4.93 (s, 1H, OH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 106.3 (C-1), 86.5 (C-2), 77.5 (C-3), 81.4 (C-4), 149.3 (C-5), 136.2–122.8 (aromatic carbons), 163.0 (*C*O), 112.2 (*C*Me$_2$), 27.2 (C*Me*$_2$), 26.5 (C*Me*$_2$).

1.6.6. 1,2-*O*-Isopropylidene-α-D-xylopentadialdo-1,4-furanose (*p*-bromobenzoyl)hydrazone (7f)

86% yield; $[\alpha]_D^{25}$ −22.2 (*c* 1.5, chloroform). Anal. Calcd for $C_{15}H_{17}BrN_2O_5$: C, 46.87; H, 4.45. Found: C, 46.91; H, 4.46. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 5.96 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.59 (d, 1H, $J_{2,1}$ 3.6 Hz, H-2), 4.35 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.66 (dd, 1H, $J_{4,3}$ 2.7 Hz, $J_{4,5}$ 6.5, H-4), 7.78 (d, 1H, $J_{5,4}$ 6.6 Hz, H-5), 7.66 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.87 (d, 2H, *J* 8.1, aromatic protons), 1.45 (s, 3H, C*Me*$_2$), 1.28 (s, 3H, C*Me*$_2$), 11.21 (s, 1H, NH), 3.10 (s, 1H, OH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 106.5 (C-1), 86.7 (C-2), 77.7 (C-3), 81.6 (C-4), 149.1 (C-5), 132.6–130.6 (aromatic carbons), 163.4 (*C*O), 112.4 (*C*Me$_2$), 27.4 (C*Me*$_2$), 26.7 (C*Me*$_2$).

1.7. General procedure for the synthesis of l,3,4-oxadiazoles (8a–f)

Sodium acetate trihydrate and PIDA were added in a 1.2:1 molar proportion to a stirred solution of **7a–f** in MeOH. The mixture was stirred at room temperature, and the reaction was followed by TLC (80:20 cyclohexane/ethyl acetate). The reaction medium was evaporated and extracted with CH_2Cl_2/water the organic layers were evaporated and the residue was purified by flash chromatography (80:20 cyclohexane/ethyl acetate), giving the products as white crystals.

1.7.1. 2-(1,2-*O*-Isopropylidene-α-D-xylo-tetrafuranos-4-yl)-5-(*o*-chlorophenyl)-l,3,4-oxadiazole (8a)

40% yield; mp: 110–112 °C; $[\alpha]_D^{25}$ +1.5 (*c* 2.8, chloroform). Anal. Calcd for $C_{15}H_{15}ClN_2O_5$: C, 53.19; H, 4.46. Found: C, 53.03; H, 4.44. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.16 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.74 (d, 2H, H-2 + H-3), 5.43 (d, 1H, $J_{3,4}$ 2.7 Hz, H-4), 7.93–7.31 (m. 4H, aromatic protons), 1.56 (s, 3H, C*Me*$_2$), 1.36 (s, 3H, C*Me*$_2$), 4.45 (s, 1H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.6 (C-1), 84.4 (C-2), 74.6 (C-3), 76.1 (C-4), 133.1–122.1 (aromatic carbons), 163.6, 162.7 (oxadiazole carbons), 112.4 (*C*Me$_2$), 26.9 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.7.2. 2-(1,2-*O*-Isopropylidene-α-D-xylo-tetrafuranos-4-yl)-5-(*m*-chlorophenyl)-l,3,4-oxadiazole (8b)

50% yield; mp: 135–136 °C; $[\alpha]_D^{25}$ +4.7 (*c* 2.9, chloroform). Anal. Calcd for $C_{15}H_{15}ClN_2O_5$: C, 53.19; H, 4.46. Found: C, 53.08; H, 4.43. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.17 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.73 (d, 2H, H-2 + H-3), 5.43 (d, 1H, $J_{3,4}$ 2.4 Hz, H-4), 7.85–7.24 (m, 4H, aromatic protons), 1.57 (s, 3H, C*Me*$_2$), 1.38 (s, 3H, C*Me*$_2$), 4.61 (s, 1H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.7 (C-1), 84.6 (C-2), 74.7 (C-3), 76.1 (C-4), 135.1–124.4 (aromatic carbons), 164.3, 162.7 (oxadiazole carbons), 112.6 (*C*Me$_2$), 26.9 (C*Me*$_2$), 26.2 (C*Me*$_2$).

1.7.3. 2-(1,2-*O*-Isopropylidene-α-D-xylo-tetrafuranos-4-yl)-5-(*p*-chlorophenyl)-l,3,4-oxadiazole (8c)

40% yield; mp: 193–195 °C; $[\alpha]_D^{25}$ +3.2 (*c* 3.1, chloroform). Anal. Calcd for $C_{15}H_{15}ClN_2O_5$: C, 53.19; H, 4.46. Found: C, 52.87; H, 4.44. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.15 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.74 (d, 2H, H-2 + H-3), 5.39 (1H, $J_{3,4}$ 2.6 Hz, H-4), 7.91 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.43 (d, *J* 8.8 Hz, 2H, aromatic protons), 1.58 (s, 3H, C*Me*$_2$), 1.38 (s, 3H, C*Me*$_2$), 4.31 (s, 1H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.7 (C-1), 84.4 (C-2), 74.4 (C-3), 76.1 (C-4), 138.6–121.4 (aromatic carbons), 164.7, 162.5 (oxadiazole carbons), 112.6 (*C*Me$_2$), 26.9 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.7.4. 2-(1,2-*O*-Isopropylidene-α-D-xylo-tetrafuranos-4-yl)-5-(*o*-bromophenyl)-l,3,4-oxadiazole (8d)

58% yield; mp: 69–71 °C; $[\alpha]_D^{25}$ −1.7 (*c* 1.8, chloroform). Anal. Calcd for $C_{15}H_{15}BrN_2O_5$: C, 47.02; H, 3.90. Found: C, 46.99; H, 3.87. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.07 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.65 (d, 2H, H-2 + H-3), 5.36 (d, 1H, $J_{3,4}$ 2.7 Hz, H-4), 7.79–7.21 (m, 4H, aromatic protons), 1.53 (s, 3H, C*Me*$_2$), 1.39 (s, 3H, C*Me*$_2$), 4.35 (s, 1H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.6 (C-1), 84.5 (C-2), 74.5 (C-3), 76.0 (C-4), 134.4–121.6 (aromatic carbons), 164.1–162.7 (oxadiazole carbons), 112.4 (*C*Me$_2$), 26.8 (C*Me*$_2$), 26.0 (C*Me*$_2$).

1.7.5. 2-(1,2-*O*-Isopropylidene-α-D-xylo-tetrafuranos-4-yl)-5-(*m*-bromophenyl)-l,3,4-oxadiazole (8e)

54% yield; mp: 84–85 °C; $[\alpha]_D^{25}$ +3.1 (*c* 1.9, chloroform). Anal. Calcd for $C_{15}H_{15}BrN_2O_5$: C, 47.02; H, 3.90. Found: C, 46.92; H, 3.88. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.16 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.74 (d, 2H, H-2 + H-3), 5.39 (d, 1H, $J_{3,4}$ 2.2 Hz, H-4), 8.16–7.32 (m, 4H, aromatic protons), 1.58 (s, 3H, C*Me*$_2$), 1.39 (s, 3H, C*Me*$_2$), 4.54 (s, 1H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.8 (C-1), 84.4 (C-2), 74.2 (C-3), 76.2 (C-4), 135.2–123.2 (aromatic carbons), 164.2–162.7 (oxadiazole carbons), 112.7 *C*Me$_2$, 26.9 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.7.6. 2-(1,2-*O*-Isopropylidene-α-D-xylo-tetrafuranos-4-yl)-5-(*p*-bromophenyl)-l,3,4-oxadiazole (8f)

34% yield; mp: 182–184 °C; $[\alpha]_D^{25}$ −0.6 (*c* 1.9, chloroform). Anal. Calcd for $C_{15}H_{15}BrN_2O_5$: C, 47.02; H, 3.90. Found: C, 46.89; H, 3.87. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.16 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.73 (d, 2H, H-2 + H-3), 5.39 (d, 1H, $J_{3,4}$ 2.6 Hz, H-4), 7.84 (d, 2H, *J* 8.8 Hz, aromatic protons), 7.60 (d, 2H, *J* 8.8 Hz, aromatic protons), 1.57 (s, 3H, C*Me*$_2$), 1.37 (s, 3H, C*Me*$_2$), 4.32 (s, 1H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.7 (C-1), 84.4 (C-2), 74.3 (C-3), 76.1 (C-4), 132.5–121.8 (aromatic carbons), 164.8, 162.6 (oxadiazole carbons), 112.6 (*C*Me$_2$), 26.9 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.8. 3,5-Di-*O*-acetyl-6-deoxy-1,2-*O*-isopropylidene-6-isothiocyanate-α-D-glucofuranose (10)

Compound **10** was obtained as a syrup (1.10 g, 3.18 mmol, 84.9%) from **9**[16,17] (1.23 g, 3.74 mmol), as previously described for similar derivatives;[21] $[\alpha]_D^{25}$ −50.2 (*c* 2.1, chloroform). Anal. Calcd for $C_{14}H_{19}NO_7S$: C, 48.69; H, 5.55; N, 4.06; S, 9.28. Found: C, 48.90; H, 5.47; N, 3.74; S, 9.23. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.91 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.51 (d, 1H, $J_{2,1}$ 3.6 Hz, H-2), 5.37 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 4.43 (dd, 1H, $J_{4,3}$ 2.9 Hz, $J_{4,5}$ 9.3 Hz, H-4), 5.13 (dt, 1H, $J_{5,4}$ 9.5 Hz, $J_{5,6a}$ 3.5 Hz, $J_{5,6b}$ 3.8 Hz, H-5), 3.84 (m, 2H, H-6a + H-6b), 2.08 (s, 3H, *Me*CO), 2.06 (s, 3H, *Me*CO), 1.56 (s, 3H, C*Me*$_2$), 1.32 (s, 3H, C*Me*$_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 105.1

(C-1), 83.3 (C-2), 76.7 (C-3), 74.5 (C-4), 67.4 (C-5), 46.5 (C-6), 169.5, 169.3 (*C*O), 112.8 (*C*Me$_2$), 20.7 (*Me*CO), 20.6 (*Me*CO), 26.8 (C*Me*$_2$), 26.2 (C*Me*$_2$).

1.9. General procedure for the synthesis of compounds (11a,b)

A solution of *p*-chloro- or *p*-bromobenzoic hydrazide (2.84 mmol) and **10** (946.61 mg, 2.84 mmol) in dry THF was refluxed for 2 h, and the solvent was evaporated, affording the *p*-chloro and *p*-bromo substituted thiosemicarbazides (**11a** and **11b**) in quantitative yield.

1.9.1. 3,5-di-*O*-acetyl-6-deoxy-1,2-isopropylidene-6-[(*p*-clorobenzohydrazinecarbothionyl)amino]-α-D-glucofuranose (11a)

White crystals, mp: 86 °C; $[\alpha]_D^{25}$ +2.7 (*c* 2.6, chloroform). Anal. Calcd for $C_{21}H_{26}ClN_3O_8S$: C, 48.88; H, 5.08; N, 8.14; S, 6.21. Found: C, 49.20; H, 5.08; N, 8.04; S, 6.05. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.85 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.44 (d, 1H, $J_{2,1}$ 3.7 Hz, H-2), 5.29 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.31 (dd, 1H, $J_{4,3}$ 2.8 Hz, $J_{4,5}$ 8.9, H-4), 5.14 (ddd, 1H, $J_{5,4}$ 8.8 Hz, $J_{5,6a}$ 3.4, $J_{5,6b}$ 7.8, H-5), 4.05 (d (w), 1H, $J_{6a,6b}$ 13.9 Hz, H-6a), 3.84 (m, 1H, H-6b), 7.83 (d, 2H, *J* 8.5 Hz, aromatic protons), 7.38 (d, 2H, *J* 8.5 Hz, aromatic protons), 2.00 (s, 3H, *Me*CO), 1.90 (s, 3H, *Me*CO), 1.45 (s, 3H, C*Me*$_2$), 1.27 (s, 3H, C*Me*$_2$), 9.71 (s, 1H, NH), 8.57 (s, 1H, NH), 7.50 (s(w), 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 105.0 (C-1), 83.0 (C-2), 74.9 (C-3), 78.3 (C-4), 68.2 (C-5), 46.5 (C-6), 138.8–128.5 (aromatic carbons), 170.8 (*C*O), 169.6 (*C*O), 182.4 (*C*S), 112.4 (*C*Me$_2$), 20.9 (*Me*CO), 20.7 (*Me*CO), 26.6 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.9.2. 3,5-di-*O*-acetyl-6-deoxy-1,2-isopropylidene-6-[(*p*-bromobenzohydrazinecarbothionyl)amino]-α-D-glucofuranose (11b)

White crystals, mp: 83 °C; $[\alpha]_D^{25}$ +3.1 (*c* 2.7, chloroform). Anal. Calcd for $C_{21}H_{26}BrN_3O_8S$: C, 45.01; H, 4.68; N, 7.50; S, 5.72. Found: C, 45.40; H, 5.10; N, 7.41; S, 5.52. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.86 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.45 (d, 1H, $J_{2,1}$ 3.7 Hz, H-2), 5.30 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.32 (dd, 1H, $J_{4,3}$ 2.9 Hz, $J_{4,5}$ 8.9, H-4), 5.15 (ddd, 1H, $J_{5,4}$ 8.8 Hz, $J_{5,6a}$ 3.5, $J_{5,6b}$ 7.7, H-5), 4.06 (d (w), 1H, $J_{6a,6b}$ 13.9 Hz, H-6a), 3.84 (m, 1H, H-6b), 7.75 (d, 2H, *J* 8.8 Hz, aromatic protons), 7.58 (d, 2H, *J* 8.8 Hz, aromatic protons), 2.00 (s, 3H, *Me*CO), 1.96 (s, 3H, *Me*CO), 1.47 (s, 3H, C*Me*$_2$), 1.28 (s, 3H, C*Me*$_2$), 9.50 (s, 1H, NH), 9.03 (s, 1H, NH), 7.45 (s(w), 1H, NH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 105.1 (C-1), 83.1 (C-2) 75.0 (C-3), 78.4 (C-4), 68.4 (C-5), 46.6 (C-6), 132.1–127.6 (aromatic carbons), 171.0 (*C*O), 169.6 (*C*O), 182.3 (*C*S), 112.5 (*C*Me$_2$), 20.9 (*Me*CO), 20.7 (*Me*CO), 26.6 (C*Me*$_2$), 26.1 (C*Me*$_2$).

1.10. General procedure for the synthesis of 1,2,4-triazoline-3-thiones (12a,b)

Thiosemicarbazides (**11a** and **11b**) (0.60 mmol) were suspended in 1 M NaOH and refluxed for 2 h. Solutions were neutralized, evaporated, and dissolved in acetone, and residual mineral salts were filtrated off. Evaporation of acetone yielded the crude products, which were further purified by flash column chromatography (90:10 cyclohexane: acetone) affording **12a** and **12b** as white crystals.

1.10.1. 5-(*p*-Clorophenyl)-4-(1,2-*O*-isopropylidene-α-D-glucofuranos-6-yl)-1,2,4-triazoline-3-thione (12a)

94.98 mg, 41%; mp: 224 °C (dec); $[\alpha]_D^{25}$ +3.2 (*c* 1.4, MeOH). Anal. Calcd for $C_{16}H_{18}ClN_3O_4S$: C, 49.34; H, 4.87. Found: C, 49.72; H, 4.88. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 5.89 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.49 (d, 1H, $J_{2,1}$ 3.8 Hz, H-2), 4.25 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 3.99 (dd, 1H, $J_{4,3}$ 2.8 Hz, $J_{4,5}$ 7.8 Hz, H-4), 4.67 (ddd, 1H, $J_{5,4}$ 7.8 Hz, $J_{5,6a}$ 3.0 Hz, $J_{5,6b}$ 9.8 Hz, H-5), 4.47 (dd, 1H, $J_{6a,5}$ 3.1 Hz, $J_{6a,6b}$ 14.2 Hz, H-6a), 4.15 (dd, 1H, $J_{6b,5}$ 9.8 Hz, $J_{6b,6a}$ 14.2 Hz, H-6b), 8.04 (d, 2H, *J* 8.5 Hz, aromatic protons), 7.58 (d, 2H, *J* 8.5 Hz, aromatic protons), 1.41 (s, 3H, C*Me*$_2$), 1.27 (s, 3H, C*Me*$_2$), 12.85 (s, 1H, NH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 106.1 (C-1), 86.0 (C-2), 75.3 (C-3), 83.0 (C-4), 66.5 (C-5), 49.5 (C-6), 152.9 (heterocyclic carbon), 136.9–126.5 (aromatic carbons), 169.5 (*C*S), 111.8 (*C* Me$_2$), 27.1 (C*Me*$_2$), 26.4 (C*Me*$_2$).

1.10.2. 5-(*p*-Clorophenyl)-4-(1,2-*O*-isopropylidene-α-D-glucofuranos-6-yl)-1,2,4-triazoline-3-thione (12b)

142.11 mg, 55%; mp: 236 °C (dec); $[\alpha]_D^{25}$ +5.0 (*c* 2.5, MeOH). Anal. Calcd for $C_{16}H_{18}BrlN_3O_4S$: C, 44.55; H, 4.40. Found: C, 44.95; H, 4.65. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 5.88 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.48 (d, 1H, $J_{2,1}$ 3.5 Hz, H-2), 4.23 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 3.93 (dd, 1H, $J_{4,3}$ 2.8 Hz, $J_{4,5}$ 7.8, H-4), 4.65 (dt, 1H, $J_{5,4}$ 3.1 Hz, $J_{5,6a}$ 3.1 Hz, $J_{5,6b}$ 9.7 Hz, H-5), 4.45 (dd, 1H, $J_{6a,5}$ 3.1 Hz, $J_{6a,6b}$ 14.8 Hz, H-6a), 4.13 (dd, 1H, $J_{6b,5}$ 9.7 Hz, $J_{6b,6a}$ 14.8 Hz, H-6b), 7.84 (d, 2H, *J* 8.5 Hz, aromatic protons), 7.75 (d, 2H, *J* 8.5 Hz, aromatic protons), 1.39 (s, 3H, C*Me*$_2$), 1.26 (s, 3H, C*Me*$_2$), 12.81 (s, 1H, NH); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 106.1 (C-1), 85.2 (C-2), 74.5 (C-3), 82.2 (C-4), 67.7 (C-5), 48.8 (C-6), 132.7–125.3 (aromatic carbons), 152.9 (heterocyclic carbon), 169.6 (*C*S), 111.8 (*C*Me$_2$), 26.6 (C*Me*$_2$), 26.3 (C*Me*$_2$).

1.11. Antiviral studies

1.11.1. Cells and viruses

Vero (African green monkey kidney) cells were grown in Eagle's minimum essential medium (MEM) supplemented with 5% inactivated calf serum and 50 μg/ml gentamicin. For maintenance medium (MM), the serum concentration was reduced to 1.5%.

The C6/36 HT mosquito cell line from *Aedes albopictus* was cultured at 33 °C in L-15 medium (Leibovitz) supplemented with 0.3% tryptose phosphate broth, 0.02% glutamine, 1% MEM non-essential amino acids solution, and 5% fetal calf serum.

The IV4454 strain of virus Junín (JUNV) and the NGC strain of dengue virus type 2 (DENV-2) were used. DENV-2 stocks were prepared in C6/36 HT cells and titrated by plaque formation in Vero cells. JUNV stocks were prepared and titrated by plaque formation in Vero cells.

1.11.2. Biological assays

Vero cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma–Aldrich, USA) method. Confluent cultures in 96-well plates were exposed to twofold dilutions of the compounds, three wells for each concentration, and incubated for 48 h at 37 °C. Then, 10 μl of MM containing MTT (final concentration, 0.5 mg/mL) was added to each well. After 2 h of incubation at 37 °C, the supernatant was removed, 200 μl of ethanol was added to each well to solubilize the formazan crystals, and absorbance was measured in a microplate reader at 595 nm. The cytotoxic concentration 50% (CC_{50}) was calculated as the compound concentration required to reduce cell viability by 50%. The antiviral activity was determined by a virus yield inhibition assay. Vero cells grown in 24-well plates were infected at a multiplicity of infection of 0.1 PFU/cell of JUNV or DENV-2. After 1 h adsorption at 37 °C, cells were washed and refed with MM containing different concentrations of the compounds (two wells per concentration). After 48 h of incubation at 37 °C, supernatant cultures were harvested and extracellular virus yields were determined by a plaque assay. The effective concentration 50% (EC_{50}) was calculated as the concentration required to reduce virus yield by 50% in the compound-treated cultures compared with untreated ones. Finally, virucidal activity was determined by incubating a virus suspension of 10^6 PFU of JUNV

or DENV-2 with an equal volume of MM either with or without different concentrations of each compound for 1.5 h at 37 °C. The samples were then diluted in cold MM to determine residual infectivity in a plaque formation assay using Vero cells. The ratios of virus titer in compound-treated samples with respect to virus titer in control samples were calculated.

Acknowledgments

Financial support from CONICET (PIP 5011, 5513), ANPCyT (PICT 13922, 14124), and UBA is gratefully acknowledged.

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