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Imidazo[2,1-*b*]thiazole carbohydrate derivatives: Synthesis and antiviral activity against Junin virus, agent of Argentine hemorrhagic fever

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1. Introduction

ABSTRACT

Herein, we describe the syntheses of 3,5-disubstituted imidazo[2,1-*b*]thiazole. The cyclization step was performed in two different conditions by using either thermal or microwave heating. Comparing the results of both methodologies, we found that the microwave assistance is an improved alternative to obtain this family of heterocyclic compound. Compounds were first evaluated for cytotoxicity in Vero cells by MTT method and then, the antiviral activity was assayed by a virus yield inhibition assay in the range of concentrations lower than the corresponding CC_{50} , using JUNV strain IV4454 as the model system. The most active compounds (**3** and **4**), showed a level of antiviral activity against JUNV in monkey Vero cells better than the reference substance ribavirin. Then, they are promising lead compound for further analysis and characterization to establish their therapeutic potential against hemorrhagic fever viruses.

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Imidazo[2,1-*b*][1,3]-thiazole [1,2] derivatives occupy a prominent place in medicinal chemistry because of their therapeutic properties. Compounds containing this heterocyclic system have been reported as potential acetylcholinesterase and butyrylcholinesterase inhibitors [3] as well as antihelmintic, fungicide, herbicide, antitumor and cardiotonic agents [4,5].Anti-hypertensive, antiinflammatory, and immunosuppressive properties of this class of compounds were also reported together with their potent and balanced enzyme and cellular activity against insulin-like growth factor receptor and members of the epidermal growth factor family of receptor tyrosine kinases [6].

In a previous report, we described the synthesis and the antiviral activity against two hemorrhagic fever causing viruses, dengue virus type 2 (DENV-2) and Junin virus (JUNV) [7] of a series of nitrogenated heterocyclic compounds, including imidazothiazoles. JUNV belongs to the Arenaviridae family and is the etiological agent of Argentine hemorrhagic fever (AHF), an endemoepidemic disease recognized as a major public health

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problem in the richest agricultural zones of Argentina [8]. Although the evaluation of different types of compounds for inhibitory activity against JUNV has been reported [9], no specific and safe chemotherapy is currently available. Ribavirin is the only drug that has shown partial efficacy in the treatment of arenavirus hemorrhagic fever, but with a high level of undesirable adverse reactions [10]. The current therapy for AHF patients is the early administration of standardized doses of convalescent plasma, but this therapy is not effective when initiated after a week of illness and 10% of treated patients develop late neurological syndrome [10]. Other disadvantages of immune plasma therapy are the difficulties in maintaining adequate stocks of plasma and the risk of transfusion-borne diseases. Thus, there is a real need for active drugs against JUNV and other viruses producing hemorrhagic fever in humans.

In agreement with the potent bioactivities reported for compounds possessing the imidazothiazole core in their structure, in our earlier report antiviral activity against JUNV of the compound $3-(p-bromobenzoyl)-5-(1,2-O-isopropylidene-3-O-methyl-\alpha-D-xylo-furanos-5-ulo-5-yl)imidazo[2,1-b]thiazole was detected at concentrations not affecting cell viability [7]. These results encourage us to extend the study of the antiviral properties of imidazothiazoles. New derivatives were synthesized by changing the substituents and$

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- i) Thermal condition: dioxane (anh.) reflux: 16h, 2. 65 % [6]; 3. 41 % [6]; 4. 39 %; 5. 37 %; 6. 34 %; 7. 19 %; 8. 20 %; 9. 32 %.
 ii) Microwave condition: THF (anh.) 300W, 105°C, 90-150 min., 2. 61 %; 3. 39 %;
- ii) Microwave condition: THF (anh.) 300W, 105°C, 90-150 min., 2. 61 %; 3. 39 %; 4. 44 %; 5. 53 %; 6. 34 %; 7. 37 %; 8. 37 %; 9. 25 %.

Scheme 1.

their relative position in the heterocyclic ring and the anti-JUNV activity was analyzed.

low levels of cytotoxicity with CC_{50} values higher than 100 μ M, with compound **8** the only exception displaying CC_{50} of 70 μ M.

2. Results and discussion

2.1. Chemistry

Substituted imidazo[2,1-*b*]thiazoles (**2**–**7**) were obtained by a convergent synthetic pathway (Scheme 1), from either 6-bromo-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl- α -D-*xylo*-hexofuranos-5-ulose (**1a**) [7] or 6-bromo-6-deoxy-1,2-*O*-isopropylidene-3-*O*-acetyl- α -D-*xylo*-hexofuranos-5-ulose (**1b**).

Compound **1b** was obtained from 1,2-O-isopropylidene-3-O-acetyl- α -D-glucofuranose [11–13]. Selective replacement of the primary hydroxyl group by a bromine atom [14] following by the oxidation of the hydroxyl on C-5 using 1-hydroxy-1,2-benziodoxol-3(1*H*)-one-1-oxide (IBX) as oxidizing agent, led us to obtained compound **1b** in quantitative yield [15].

In order to improve the coupling step between compounds 1a/b and N'-(5-arylthiazol-2-yl)-N,N-dimethylformamidine (see Scheme 1), it was performed in two different conditions: i) by thermal heating, as reported in the literature and ii) by microwave assistance.

Although there were no significant differences in the yields of the final products between the two conditions, reactions times dropped from 16 h to 90 min by changing thermal for microwave condition. The drastic reduction in reaction time observed with the microwave assisted method provides an improved alternative for the synthesis of substituted imidazo[2,1-*b*]thiazoles.

In order to study the influence of the relative position of the substituent in the heterocyclic ring in the antiviral activity of the final products, the coupled position of the carbohydrate and the heterocylic moieties were inverted as shown in Scheme 2.

All products were characterized by physical and spectroscopic analysis and the results are shown in Section 4.

2.2. Biological studies

All compounds were first evaluated for cytotoxicity in Vero cells by MTT method. As seen in Table 1, most compounds exhibited very Then, the antiviral activity was assayed by a virus yield inhibition assay in the range of concentrations lower than the corresponding CC_{50} , using JUNV strain IV4454 as the model system. All derivatives were inhibitors of JUNV replication, but exhibited a wide range of antiviral effective concentrations (Table 1). Compounds **4** and **3** showed the highest inhibitory effect against JUNV with EC₅₀ values around 1 μ M, and, given the low toxicity of both azoles for uninfected cells, the selectivity index (SI:ratio CC₅₀/EC₅₀) was 463.7 and 268.2, respectively. Furthermore, both derivatives possessed greater antiviral activity and selectivity than ribavirin, the only drug in clinical use for arenavirus treatment which was evaluated in parallel as a reference substance.



Reagents : 1. i) Thermal condition: dioxane (anh.) reflux,16 h, 11. 70 %; 12. 40 %; 13. 33 %; 14. 59 %.
1. ii) Microwave condition: THF (anh.) 300W, 105°C, 90 min., 11. 83 %; 12. 43 %; 13. 40 %; 14. 77 %.

Scheme 2.

 Table 1

 Cytotoxicity and antiviral activity against IUNV of imidazothiazoles.

Compound	$CC_{50} \ [\mu M]^a$	$EC_{50}\pm SD[\mu M]^b$	SI ^c
2	>400	18.5 ± 6.8	>21.6
3	295	1.1 ± 0.7	268.2
4	371	0.8 ± 0.2	463.7
5	>400	78.3 ± 2.0	>5.1
6	>400	7.9 ± 1.9	>50.6
7	139	5.6 ± 2.1	24.8
8	70	7.5 ± 0.08	9.3
9	>400	4.9 ± 0.4	>81.6
11	>400	12.3 ± 0.01	>32.5
12	351	4.3 ± 0.7	81.6
13	>400	11.9 ± 0.2	>33.6
14	>400	2.9 ± 0.01	>137.9
Ribavirin	>400	18.5 ± 1.7	>21.6

Values are the mean of three determinations \pm standard deviation.

^a Cytotoxic concentration 50%: compound concentration required to reduce cell viability by 50%, as determined by MTT assay.

^b Effective concentration 50%: compound concentration required to reduce virus yield by 50%.

^c Selectivity index: ratio CC₅₀/EC₅₀.

The antiviral activity of the analyzed compounds decreased drastically when the methyl group of the carbohydrate residue was replaced by the more polar acetyl group. Beside, the higher values were observed when the carbohydrate residue was on position 5 of the heterocycle (Table 1).

To better evaluate the perspectives of the imidazothiazoles as inhibitors of a human hemorrhagic fever, the ability of compound **3** to inhibit JUNV replication in human cells was also analyzed. The values of CC₅₀ and EC₅₀ determined by MTT and JUNV yield inhibition assays, respectively, in the lung human cell line A549 were 274 μ M and 2.8 \pm 0.5 μ M, respectively, with similar effectiveness as in the monkey Vero cells (Table 1).

As a first approach to characterize the action of these compounds on arenaviruses, the possibility that they acted directly either on the virus particles or on the cells to be infected was investigated. To this end, virus suspensions or cell monolayers were pre-incubated with different compound concentrations before infection, as explained in Experimental Protocols. When cell monolayers were pre-incubated



Fig. 1. Effect of pre-treatment of cells or virus with compound **3**. Pretreatment of virus (\blacktriangle): suspensions of JUNV were incubated with different concentrations of compound for 1.5 h at 37 °C and then remaining infectivity was titrated by PFU. Pretreatment of cells (\blacksquare): Vero cells were pre-incubated with different concentrations of compound during 2 h at 37 °C; then, supernatants were removed and cells were infected with JUNV in absence of compound. Virus yields were determined at 48 h p.i. by PFU. Treatment during virus infection (\blacklozenge): Vero cells were infected with JUNV and compound was added and maintained during all period of incubation. Virus yields were measured at 48 h p.i. by PFU. Each value is the mean of duplicate assays.

with compound **3** during 2 h at 37 °C and then it was removed before JUNV infection, it was not effective in reducing virus yield at 48 h p.i. (Fig. 1). Similarly, following incubation of virus with compound for 2 h at 37 °C before cell infection reduction in remaining JUNV infectivity was only around 10% at the highest concentration tested of 50 μ M. By contrast, when the imidazothiazole derivative was added to cells simultaneously with virus inoculum (as in the screening assay shown in Table 1), virus titters in cell supernatants at 48 h p.i. was significantly reduced at concentrations 15–20-fold lower and in a dose dependent-manner (Fig. 1). Thus, the inhibitory effect against JUNV was entirely exerted through a blockade in virus multiplication during the infection process.

3. Conclusions

In conclusion, a series of imidazo[2,1-*b*]thiazole substituted in position 3 and 5 with a carbohydrate moiety and different halobenzoyl groups was synthesized. All the new compounds were characterized by NMR spectra, optical rotation as well as elemental analyses.

The most active imidazothiazole derivatives evaluated in the present study, compounds **4** and **3**, showed a level of antiviral activity against JUNV in monkey Vero cells better than the reference substance ribavirin. Compound **3**, taken as model of this type of derivatives, exerted the antiviral effect only during the intracellular multiplication cycle of JUNV. Furthermore, it was also an effective JUNV inhibitor in human cells. Then, they are promising lead compounds for further analysis and characterization to establish their therapeutic potential against hemorrhagic fever viruses.

In addition, an improvement methodology to obtain these promising compounds was also reported.

4. Experimental protocols

4.1. Chemistry

4.1.1. General remarks

Research chemicals were purchased from Sigma-Aldrich Co and used without further purification in the reactions or were prepared according to procedures described in the literature [7]. For microwave reactions a CEM Discover with 300 Watts of power was used, using the mode Power Max ON and constant agitation. Reactions were monitored by thin layer chromatography (TLC) on silica gel plated (60 F₂₅₄; Merck) visualizing with ultraviolet light or by spraying with sulphuric acid in ethanolic solution. Column chromatographic separations were performed on silica gel 60 (240-400 mesh, Merck). Melting points were measured on a Thomas Hoover melting point apparatus and are uncorrected, and the optical rotation, $[\alpha]_D$, measurements were made using a 343 Perkin Elmer Polarimeter. Solvents were reagent grade and, in most cases, dried and distillated before use according to standard procedures. ¹H, ¹³C NMR spectra were recorded on a Bruker AC-200 spectrometer, operating at 200, 50 MHz respectively; or a Bruker AMX-500 spectrometer, operating at 500, 125 MHz respectively. Assignments of the ¹H and ¹³C NMR spectra were confirmed with the aid of two dimensional techniques ¹H, ¹³C (HSQC, HMBC). Chemical shifts (δ) are reported in parts per million downfield from tetramethyl silane as internal standard. Elemental analyses were performed on a Carlo Erba EA 1108 CHN elemental analyzer.

4.1.2. 3-O-Acetyl-6-bromo-6-deoxy-1,2-O-isopropylidene- α -D-xylo-hexofuranos-5-ulose (**1b**)

IBX (38.4 mmol) was added to a solution of 3-O-acetyl-6-bromo-6-deoxy-1,2-O-isopropylidene- α -D-glucofuranose (7.68 mmol) in acetonitrile (50 mL). The reaction mixture was refluxed during 16 h and then cooled, filtered, concentrated and purified by flash column chromatography, using cyclohexane: acetone as eluent (90:10), affording pure **1b** (89.2% yield) as a syrup; $[\alpha]_D - 72.6$ (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.33 (s, 3H, CH₃), 1.51 (s, 3H, CH₃), 2.04 (s, 3H, CH₃CO), 4.26 (s, 2H, H-6), 4.57 (d, 1H, H-2, $J_{2,1} = 3.6$ Hz), 4.96 (d, 1H, H-4, $J_{4,3} = 3.5$ Hz), 5.47 (d, 1H, H-3, $J_{3,4} = 3.5$ Hz), 6.06 (d, 1H, H-1, $J_{1,2} = 3.6$ Hz); ¹³C NMR (CDCl₃): δ 20.7 (CH₃CO), 26.2 [C(CH₃)₂], 26.8 [C(CH₃)₂], 33.8 (C-6), 77.5 (C-3), 82.6 (C-2), 82.9 (C-4), 105.6 (C-1), 113.1 [C(CH₃)₂], 157.4 (C-5), 169.2 (C=O). *Anal. Calcd.* for C₁₁H₁₅BrO₆: C, 40.89; H, 4.68. Found: C, 40.39; H, 4.91.

4.1.3. General procedure for the synthesis of compounds 2–9

Procedure 1 (*Thermal Heating*): A mixture of 6-bromo-6-deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylo-hexofuranos-5-ulose (**1a**) (6 mmol) [7] (to obtain compounds **2**–**5**) or 6-bromo-6-deoxy-1,2-O-isopropylidene-3-O-acetyl-α-D-xylo-hexofuranos-5-ulose (**1b**) (6 mmol) (to prepare products **6**–**9**) and N'-(5-benzoylthiazol-2-yl)-N,N-dimethylformamidine (0.75 mmol) in anhydrous dioxane (20 mL) was refluxed for 16 h under argon atmosphere. Et₃N (1.2 mmol) was added and the mixture was stirred for 12 h at room temperature. The crude product was purified by column chromatography on silica gel G (cyclohexane: acetone 95:5 and then 80:20) affording the products **2–9**.

Procedure 2 (*Microwave Heating*): A mixture of 6-bromo-6deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylo-hexofuranos-5ulose (**1a**) (6 mmol) [7] or 6-bromo-6-deoxy-1,2-O-isopropylidene-3-O-acetyl-α-D-xylo-hexofuranos-5-ulose (**1b**)(6 mmol) and *N'*-(5benzoylthiazol-2-yl)-*N*,*N*-dimethylformamidine (0.75 mmol) in anhydrous THF (6 mL) was heated under microwave irradiation (300 W, 105 °C), for 90 min (compounds **2–5**) or 150 min (Compounds **6–9**). The reaction mixture was diluted with anhydrous THF (20 mL) and Et₃N (1.2 mmol) was added under argon atmosphere. After the addition of triethylamine the procedure was the same as described for thermal conditions.

4.1.3.1. 3-(*p*-*Chlorobenzoyl*)-5-(1,2-O-isopropylidene-3-O-methyl-α-*D*-xylofuranos-5-ulo-5-yl)imidazo[2,1-b]thiazole (**4**). Compound **4** was obtained as yellow amorphous solid: m.p. 77–80 °C; $[\alpha]_D$ - 115.6 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.40 (s, 3H, CH₃), 1.56 (s, 3H, CH₃), 3.33 (s, 3H, CH₃O), 4.20 (d, 1H, H-3, $J_{3,4} = 3.6$ Hz), 4.68 (d, 1H, H-2, $J_{2,1} = 3.6$ Hz), 5.06 (d, 1H, H-4, $J_{4,3} = 3.6$ Hz), 6.19 (d, 1H, H-1, $J_{1,2} = 3.6$ Hz), 7.57 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.86 (d, 2H, *J* 8.6 Hz, aromatic protons), 8.52 (s, 1H, imidazo[2,1-*b*]thiazole proton), 8.94 (s, 1H, imidazo[2,1-*b*]thiazole proton); ¹³C NMR (CDCl₃): δ 26.3 [C(CH₃)₂], 26.9 [C(CH₃)₂], 58.3 (CH₃O), 80.8 (C-2), 84.9 (C-4), 86.1 (C-3), 105.8 (C-1), 112.6 [C(CH₃)₂], 129.4, 130.2, 134.7, 140.0 (aromatic carbons), 127.0, 128.2, 134.3, 146.8, 156.3 (imidazo [2,1-*b*]thiazole carbons), 185.0, 186.0 (C=O). Anal. Calcd. for C₂₁H₁₉ClN₂O₆S: C, 54.49; H, 4.14; N, 6.05. Found: C, 54.87; H, 4.25; N, 5.77.

4.1.3.2. 3-(*p*-Fluorobenzoyl)-5-(1,2-O-isopropylidene-3-O-methyl-α-*p*-xylofuranos-5-ulo-5-yl)imidazo[2,1-b]thiazole (**5**). Compound **5** was obtained as white amorphous solid: m.p. 88–91 °C; $[\alpha]_D$ - 125.9 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.39 (s, 3H, CH₃), 1.55 (s, 3H, CH₃), 3.32 (s, 3H, CH₃O), 4.20 (d, 1H, H-3, $J_{3,4} = 3.6$ Hz), 4.68 (d, 1H, H-2, $J_{2,1} = 3.6$ Hz), 5.06 (d, 1H, H-4, $J_{4,3} = 3.6$ Hz), 6.18 (d, 1H, H-1, $J_{1,2} = 3.6$ Hz), 7.26 (m, 2H, aromatic protons), 7.95 (m, 2H, aromatic protons), 8.51 (s, 1H, imidazo [2,1-*b*] proton), 8.93 (s, 1H, imidazo [2,1-*b*] proton); ¹³C NMR (CDCl₃): δ 26.3 [C(CH₃)₂], 26.9 [C(CH₃)₂], 58.3 (CH₃O), 80.7 (C-2), 84.8 (C-4), 86.1 (C-3), 105.8 (C-1), 112.6 [C (CH₃)₂], 116.3, 131.5, 132.7, 165.5 (aromatic carbons), 127.0, 128.1, 134.4, 147.0, 156.4 (imidazo [2,1-*b*] carbons), 184.9, 185.6 (C=O). Anal.

Calcd. for C₂₁H₁₉FN₂O₆S: C, 56.50; H, 4.29; N, 6.27. Found: C, 56.85; H, 4.45; N, 5.92.

4.1.3.3. 3-(*Benzoyl*)-5-(3-O-acetyl-1,2-O-isopropylidene-α-*D*-xylofuranos-5-ulo-5-yl)imidazo[2,1-*b*]thiazole (**6**). Compound **6** was obtained as white amorphous solid: m.p. 147–149 °C; $[\alpha]_D$ - 75.9 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.39 (s, 3H, CH₃), 1.59 (s, 3H, CH₃), 1.90 (s, 3H, CH₃CO), 4.64 (d, 1H, H-2, $J_{2,1}$ = 3.6 Hz), 5.17 (d, 1H, H-4, $J_{4,3}$ = 3.3 Hz), 5.63 (d, 1H, H-3, $J_{3,4}$ = 3.3 Hz), 6.20 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 7.59–7.92 (m, 5H, aromatic protons), 8.55 (s, 1H, imidazo[2,1-*b*]thiazole proton), 8.91 (s, 1H, imidazo[2,1-*b*]thiazole proton); ¹³C NMR (CDCl₃): δ 20.7 (CH₃CO), 26.2 [C(CH₃)₂], 26.8 [C (CH₃)₂], 77.5 (C-3), 82.4 (C-2), 83.2 (C-4), 105.5 (C-1), 113.0 [C (CH₃)₂], 128.9, 129.1, 133.5, 136.3 (aromatic carbons), 126.6, 128.0, 135.1, 147.0, 156.8 (imidazo[2,1-*b*]thiazole carbons), 169.3, 183.4, 187.1 (C=O). Anal. Calcd. for C₂₂H₂₀N₂O₇S: C, 57.89; H, 4.42; N, 6.14. Found: C, 57.74; H, 4.40; N, 5.91.

4.1.3.4. 3-(*p*-Bromobenzoyl)-5-(3-O-acetyl-1,2-O-isopropylidene-α-*D*-xylofuranos-5-ulo-5-yl)imidazo[2,1-b]thiazole (**7**). Compound **7** was obtained as yellow amorphous solid: m.p. 164–165 °C; $[\alpha]_D$ - 54.4 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.39 (s, 3H, CH₃), 1.58 (s, 3H, CH₃), 1.90 (s, 3H, CH₃CO), 4.63 (d, 1H, H-2, *J*_{2,1} = 3.6 Hz), 5.16 (d, 1H, H-4, *J*_{4,3} = 3.3 Hz), 5.62 (d, 1H, H-3, *J*_{3,4} = 3.3 Hz), 6.20 (d, 1H, H-1, *J*_{1,2} = 3.6 Hz), 7.74 (d, 2H, *J* 8.5 Hz, aromatic protons), 7.78 (d, 2H, *J* 8.5 Hz, aromatic protons), 7.78 (d, 2H, *J* 8.5 Hz, aromatic protons), 8.55 (s, 1H, imidazo[2,1-b]thiazole proton), 8.89 (s, 1H, imidazo[2,1-b]thiazole proton); ¹³C NMR (CDCl₃): δ 20.7 (CH₃CO), 26.2 [C(CH₃)₂], 26.8 [C(CH₃)₂], 77.5 (C-3), 82.4 (C-2), 83.2 (C-4), 105.5 (C-1), 113.1 [C(CH₃)₂], 128.8, 130.3, 132.5, 135.0 (aromatic carbons), 126.6, 127.9, 134.7, 146.8, 156.6 (imidazo [2,1-b]thiazole carbons), 169.3, 183.5, 186.0 (C=O). Anal. Calcd. for C₂₂H₁₉BrN₂O₇S: C, 49.36; H, 3.58; N, 5.23. Found: C, 49.32; H, 3.69; N, 4.84.

4.1.3.5. 3-(*p*-Chlorobenzoyl)-5-(3-O-acetyl-1,2-O-isopropylidene-α-*p*-xylofuranos-5-ulo-5-yl)imidazo[2,1-b]thiazole (**8**). Compound **8** was obtained as yellow amorphous solid: m.p. 166–168 °C; $[\alpha]_D$ - 89.2 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.30 (s, 3H, CH₃), 1.50 (s, 3H, CH₃), 1.81 (s, 3H, CH₃CO), 4.55 (d, 1H, H-2, *J*_{2,1} = 3.6 Hz), 5.10 (d, 1H, H-4, *J*_{4,3} = 3.3 Hz), 5.53 (d, 1H, H-3, *J*_{3,4} = 3.3 Hz), 6.11 (d, 1H, H-1, *J*_{1,2} = 3.6 Hz), 7.48 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.78 (d, 2H, *J* 8.6 Hz, aromatic proton); ¹³C NMR (CDCl₃): δ 20.6 (CH₃CO), 26.2 [C(CH₃)₂], 26.8 [C(CH₃)₂], 77.7 (C-3), 82.4 (C-2), 83.2 (C-4), 105.5 (C-1), 113.1 [C(CH₃)₂], 129.6, 130.2, 134.7, 140.1 (aromatic carbons), 126.6, 127.9, 134.6, 147.0, 156.7 (imidazo [2,1-*b*]thiazole carbons), 169.3, 183.4, 185.8 (C=O). Anal. Calcd. for C₂₂H₁₉ClN₂O₇S: C, 53.83; H, 3.90; N, 5.71. Found: C, 54.27; H, 3.93; N, 5.44.

4.1.3.6. 3-(*p*-Fluorobenzoyl)-5-(3-O-acetyl-1,2-O-isopropylidene-α-*D*-xylofuranos-5-ulo-5-yl)imidazo[2,1-b]thiazole (**9**). Compound **9** was obtained as white amorphous solid: m.p. 152–153 °C; $[\alpha]_D$ - 88.6 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.39 (s, 3H, CH₃), 1.59 (s, 3H, CH₃), 1.90 (s, 3H, CH₃CO), 4.64 (d, 1H, H-2, $J_{2,1}$ = 3.6 Hz), 5.17 (d, 1H, H-4, $J_{4,3}$ = 3.3 Hz), 5.63 (d, 1H, H-3, $J_{3,4}$ = 3.3 Hz), 6.20 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 7.28 (m, 2H, aromatic protons), 7.96 (m, 2H, aromatic protons), 8.56 (s, 1H, imidazo[2,1-b]thiazole proton); ¹³C NMR (CDCl₃): δ 20.6 (CH₃CO), 26.2 [C(CH₃)₂], 26.8 [C(CH₃)₂], 77.5 (C-3), 82.4 (C-2), 83.2 (C-4), 105.5 (C-1), 113.1 [C(CH₃)₂], 116.4, 116.6, 131.5, 131.6, 132.5, 132.6, 165.0, 167.0 (aromatic carbons), 126.6, 127.7, 134.9, 146.8, 156.6 (imidazo[2,1-b]thiazole carbons), 169.3, 183.5, 185.5 (C=O). Anal. Calcd. for C₂₂H₁₉FN₂O₇S: C, 55.69; H, 4.04; N, 5.90. Found: C, 55.46; H, 3.98; N, 5.64.

4.1.4. General procedure for the synthesis of 3-(1,2-O-isopropylidene-3-O-methyl- α -D-xylofuranos-5-ulo-5-yl)-5-benzoylimidazo[2,1-b] thiazole and 3-(1,2-O-isopropylidene-3-O-methyl- α -D-xylofuranos-5-ulo-5-yl)-5-(p-halobenzoyl)-imidazo[2,1-b]thiazole (11-14) 4.1.4.1. N,N-dimethylformamidyl-5-(1,2-O-isopropylidene-3-Omethyl- α -D-xylofuranos-5-ulo-5-yl)-1.3-thiazole (**10**). 6-Bromo-6deoxy-1.2-O-isopropylidene-3-O-methyl-a-p-xylo-hexofuranos-5ulose (1.85 mmol) was added to a solution of N.N'-bis(dimethylaminomethtlene)thiourea (2.77 mmol) in anhydrous dicloromethane (25 mL), and refluxed for 3 h under argon atmosphere. Et₃N (3.7 mmol) was added and the mixture was stirred for 12 h at room temperature. The crude product was purified by flash column chromatography, using cyclohexane: acetone as eluent (90:10 to 75:25) to obtain compound **10** as a waxy solid; $[\alpha]_D$ - 24.3 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.37 (s, 3H, CH₃C), 1.52 (s, 3H, CH₃C), 3.13 (s, 3H, CH₃N), 3.17 (s, 3H, CH₃N), 3.32 (s, 3H, CH₃O), 4.18 (d, 1H, H-3, $J_{3,4} = 3.6$ Hz), 4.62 (d, 1H, H-2, $J_{4,3} = 3.7$ Hz), 5.05 (d, 1H, H-4, $J_{3,4} = 3.6$ Hz), 6.14 (d, 1H, H-1, $J_{1,2} = 3.6$ Hz), 8.35 (s, 1H, thiazole proton), 8.39 (s, 1H, -HC=N); ¹³C NMR (CDCl₃): δ 26.9 [C(<u>CH</u>₃)₂], 27.0 [C(CH₃)₂], 35.2 [(CH₃)₂N], 41.2 [(CH₃N], 58.6 (CH₃O), 81.3 (C-2), 85.1 (C-4), 86.3 (C-3), 105.7 (C-1), 112.5 [C(CH₃)₂], 130.7, 148.8, 181.0 (thiazole carbons), 156.6 (C=N), 187.8 (C=O). Anal. Calcd. for C15H21N3O5S: C, 50.69; H, 5.96 N, 11.82. Found: C, 51.01; H, 5.70 N, 11.24

Procedure 1 (Thermal heating): A mixture of N,N-dimethylformamidyl-5-(1,2-O-isopropylidene-3-O-methyl-α-d-xylofuranos-5-ulo-5yl)-1,3-thiazole (0.45 mmol) and the corresponding α-carbonylated bromide compound (0.68 mmol) in anhydrous dioxane (20 mL) was refluxed for 16 h under argon atmosphere. Et₃N (0.9 mmol) was added and the mixture was stirred for 12 h at room temperature. Compounds **11–14** were obtained and, after being purified as was described for compounds **4–9**, pure compounds were afforded.

Procedure 2 (*Microwave heating*): A mixture of *N*,*N*-*dimethylformamidyl*-5-(1,2-*O*-*isopropylidene*-3-*O*-*methyl*-α-*d*-*xylofuranos*-5-*ulo*-5-*yl*)-1,3-*thiazole* (0.45 mmol) and the corresponding α-carbonylated bromide compound (0.68 mmol) in anhydrous THF (6 mL) was heated under microwave irradiation (300 W, 100 °C), for 90 min. The reaction mixture was diluted with anhydrous THF (20 mL) and Et₃N (0.9 mmol) was added under argon atmosphere, continuing with the procedure as was described for thermal conditions.

4.1.4.2. 3-(1,2-O-Isopropylidene-3-O-methyl-α-*D*-xylofuranos-5-ulo-5-yl)-5-benzoyl-imidazo[2,1-b]thiazole) (**11**). Compound **11** was obtained as white amorphous solid: m.p. 84–87 °C; $[α]_D$ - 80.8 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.39 (s, 3H, CH₃), 1.55 (s, 3H, CH₃), 3.35 (s, 3H, CH₃O), 4.24 (d, 1H, H-3, $J_{3,4} = 3.7$ Hz), 4.69 (d, 1H, H-2, $J_{2,1} = 3.7$ Hz), 5.11 (d, 1H, H-4, $J_{4,3} = 3.7$ Hz), 6.28 (d, 1H, H-1, $J_{1,2} = 3.7$ Hz), 5.11 (d, 1H, H-4, $J_{4,3} = 3.7$ Hz), 6.28 (d, 1H, H-1, $J_{1,2} = 3.7$ Hz), 7.54–7.91 (m, 5H, aromatic protons), 8.00 (s, 1H, imidazo[2,1-b]thiazole proton), 9.49 (s, 1H, imidazo[2,1-b]thiazole proton); ¹³C NMR (CDCl₃): δ 26.3 [C(<u>CH₃</u>)₂], 26.9 [C(<u>CH₃</u>)₂], 58.5 (CH₃O), 80.9 (C-2), 85.7 (C-4), 86.5 (C-3), 106.1 (C-1), 112.8 [<u>C</u> (CH₃)₂], 128.7, 128.8, 132.7, 137.7 (aromatic carbons), 127.1, 129.5, 133.0, 145.8, 156.7 (imidazo[2,1-b]thiazole carbons), 183.0, 189.8 (C=O). Anal. Calcd. for C₂₁H₂₀N₂O₆S: C, 58.87; H, 4.70; N, 6.54. Found: C, 59.02; H, 4.76; N, 6.65.

4.1.4.3. 3-(1,2-O-Isopropylidene-3-O-methyl-α-*D*-xylofuranos-5-ulo-5yl)-5-(*p*-bromobenzoyl)-imidazo[2,1-b]thiazole (**12**). Compound **11** was obtained as yellow amorphous solid: m.p. 77–79 °C; $[\alpha]_D$ - 42.5 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.40 (s, 3H, CH₃), 1.55 (s, 3H, CH₃), 3.35 (s, 3H, CH₃O), 4.24 (d, 1H, H-3, $J_{3,4}$ = 3.6 Hz), 4.69 (d, 1H, H-2, $J_{2,1}$ = 3.6 Hz), 5.11 (d, 1H, H-4, $J_{4,3}$ = 3.7 Hz), 6.27 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 7.70 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.78 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.98 (s, 1H, imidazo[2,1-b]thiazole proton), 9.46 (s, 1H, imidazo[2,1-*b*]thiazole proton); ¹³C NMR (CDCl₃): δ 26.3 [C(CH₃)₂], 26.9 [C(CH₃)₂], 58.6 (CH₃O), 80.9 (C-2), 85.7 (C-4), 86.5 (C-3), 106.1 (C-1), 112.5 [C(CH₃)₂], 127.7, 130.1, 132.1, 136.4 (aromatic carbons), 126.8, 129.4, 133.2, 145.5, 156.9 (imidazo[2,1-*b*] thiazole carbons), 181.8, 190.0 (C=O). *Anal. Calcd.* for C₂₁H₁₉BrN₂O₆S: C, 49.71; H, 3.77; N, 5.52. Found: C, 49.32; H, 3.69; N, 4.84.

4.1.4.4. $3-(1,2-O-Isopropylidene-3-O-methyl-\alpha-D-xylofuranos-5-ulo-5-yl)-5-(p-chlorobenzoyl)-imidazo[2,1-b]thiazole (13). Compound 10 was obtained as yellow amorphous solid: m.p. 73–76 °C; [α]_D – 72.7 (c 1, CHCl₃); ¹H NMR (CDCl₃): <math>\delta$ 1.40 (s, 3H, CH₃), 1.55 (s, 3H, CH₃), 3.35 (s, 3H, CH₃O), 4.24 (d, 1H, H-3, $J_{3,4} = 3.7$ Hz), 4.69 (d, 1H, H-2, $J_{2,1} = 3.6$ Hz), 5.11 (d, 1H, H-4, $J_{4,3} = 3.7$ Hz), 6.27 (d, 1H, H-1, $J_{1,2} = 3.6$ Hz), 7.53 (d, 2H, J8.6 Hz, aromatic protons), 7.86 (d, 2H, J8.6 Hz, aromatic protons), 7.98 (s, 1H, imidazo[2,1-b]thiazole proton), 9.46 (s, 1H, imidazo[2,1-b]thiazole proton), 9.46 (s, 1H, imidazo[2,1-b]thiazole proton); 1³C NMR (CDCl₃): δ 26.3 [C(CH₃)₂], 26.9 [C(CH₃)₂], 58.5 (CH₃O), 80.9 (C-2), 85.7 (C-4), 86.5 (C-3), 106.1 (C-1), 112.5 [C (CH₃)₂], 129.2, 130.1, 135.9, 139.2 (aromatic carbons), 126.8, 129.4, 133.2, 145.4, 156.9 (imidazo[2,1-b]thiazole carbons), 181.6, 190.0 (C= O). *Anal. Calcd.* for C₂₁H₁₉ClN₂O₆S: C, 54.49; H, 4.14; N, 6.05. Found: C, 54.96; H, 4.30; N, 5.92.

4.1.4.5. 3-(1,2-O-Isopropylidene-3-O-methyl-α-D-xylofuranos-5-ulo-5yl)-5-(p-fluorobenzoyl)-imidazo[2,1-b]thiazole (**14**). Compound **14** was obtained as yellow amorphous solid : m.p. 72–74 °C; $[\alpha]_D$ - 92.5 (c 1, CHCl₃); ¹H NMR (CDCl₃): δ 1.31 (s, 3H, CH₃), 1.47 (s, 3H, CH₃), 3.27 (s, 3H, CH₃O), 4.16 (d, 1H, H-3, $J_{3,4}$ = 3.6 Hz), 4.61 (d, 1H, H-2, $J_{2,1}$ = 3.6 Hz), 5.03 (d, 1H, H-4, $J_{4,3}$ = 3.7 Hz), 6.19 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 7.14–7.20 (m, 2H, *J* 8.6 Hz, aromatic protons), 7.85–7.88 (m, 2H, *J* 8.6 Hz, aromatic protons), 7.87 (s, 1H, imidazo[2,1-b]thiazole proton), 9.39 (s, 1H, imidazo[2,1-b]thiazole proton); ¹³C NMR (CDCl₃): δ 26.3 [C(CH₃)₂], 26.9 [C(CH₃)₂], 58.6 (CH₃O), 80.9 (C-2), 85.7 (C-4), 86.5 (C-3), 106.1 (C-1), 112.5 [C(CH₃)₂], 116.0, 116.1, 131.2, 134.0, 164.5, 166.5 (aromatic carbons), 181.5, 189.8 (C=O). Anal. Calcd. for C₂₁H₁₉FN₂O₆S: C, 56.50; H, 4.29; N, 6.27. Found: C, 56.39; H, 4.32; N, 6.04.

4.2. Biological activity

4.2.1. Cells and viruses

The cell lines Vero (African green monkey kidney) and A549 (lung carcinoma human cells) were grown in Eagle's minimum essential medium (MEM) (GIBCO, USA) supplemented with 5% and 10% inactivated calf serum, respectively, and 50 μ g/mL gentamycin. For maintenance medium (MM), the serum concentration was reduced to 1.5%.

The attenuated strain IV4454 of JUNV was used. Virus stocks were propagated and titrated by plaque forming units (PFU) method in Vero cells.

4.2.2. Cytotoxicity assay

Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma–Aldrich, USA) method [16] in Vero cells. Confluent cultures in 96-well plates were exposed to different concentrations of the compounds, with three wells for each concentration, and incubated for 48 h at 37 °C. Then 10 μ l 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 and 200 μ l of ethanol was added to each well to solubilize the formazan crystals. After vigorous shaking, absorbance was measured in a microplate reader at 595 nm. The cytotoxic concentration 50% (CC₅₀) was calculated as the compound concentration required to reduce the MTT signal by 50% compared to untreated controls. All determinations were performed twice and each in duplicate.

4.2.3. Antiviral assay

Antiviral activity was determined by a virus yield inhibition assay [17]. Vero and A549 cells grown in 24-well plates were infected at a multiplicity of infection (MOI) of 0.1 PFU/cell. After 1 h adsorption at 37 °C, cells were washed and re-fed with MM containing or not serial two-fold dilutions of each compound. Ribavirin (Sigma–Aldrich, USA) was used as a reference anti-arenavirus substance. After 48 h of incubation at 37 °C, supernatant cultures were harvested and extracellular virus yields were determined by plaque assay. The effective concentration 50% (EC₅₀) was calculated as the concentration required to reduce virus yield by 50% in the compound-treated cultures compared with untreated ones. All determinations were performed twice and each in duplicate.

4.2.4. Effect of pre-treatment of virus or cells with compound prior to infection

Virus and cell pre-treatment assays were performed as previously described [18]. For virus pre-treatment, equal volumes of a virus suspension containing approximately 1×10^6 PFU of virus and various concentrations of compound in MM were mixed and incubated for 1.5 h at 37 °C. A virus control was also performed by incubation of the virus suspension with MM under the same conditions. Then, samples were chilled and diluted further with MM before being placed on Vero cell cultures for plaque assay, to assess that titer reduction was only due to cell-free virion inactivation.

In cell pre-treatment assay, Vero cell monolayers were preincubated with MM containing different compound concentrations during 2 h at 37 °C. Then, supernatants were removed; cells were thoroughly washed with PBS, and infected with JUNV at a MOI of 0.1 in the absence of compound. After 1 h incubation at 37 °C, inocula were discarded and MM was added. Virus yields were determined at 48 h p.i. by plaque formation in Vero cells.

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