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Original article

1,2,4-Triazole D-ribose derivatives: Design, synthesis and antitumoral evaluation

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ABSTRACT

Herein we report the design, synthesis and characterization of novel 1,2,4-triazole D-ribose derivatives, as well as their synthetic precursors.

The antitumoral activity against T cell lymphoma cell line of these products was studied. Structures containing a 1,2,4-triazolic ring linked by sulfur to the carbohydrate moiety showed a moderate antiproliferative activity. The presence of the second heterocyclic ring did not show significant changes in their biological activity. Meanwhile, structures with 3-thiobenzyl-5-substituted-1,2,4-triazole ring linked by nitrogen leads to compounds with a biphasic behavior, stimulating cell proliferation at low concentrations and inhibiting it at higher ones. An increment in the polarity was associated with a decrease in the activity of the evaluated compounds.

A preliminary antitumoral screening pointed the 1,2,4-triazolic structures linked to protected sugars as promising leaders for further studies.

1. Introduction

The triazole derivatives and their nucleoside analogs have shown strong cytotoxicity against several human cancer cells [1]. For instance, Peng and col. reported two novel *N*-aryltriazoles as potent apoptosis-related antiproliferative activity against a drug-resistant pancreatic cancer cell line [2]. It has also been reported that the biological activity of compounds containing a triazolic ring in their structure is due to the high dipolar moment of this heterocycle which allows strong hydrogen bonding interaction with the biological target [3]. Furthermore, this heterocycle has been reported to be stable to acidic and basic hydrolysis as well as reductive/oxidative conditions [4].

On the other hand, it has been proved that the isoxazoline ring acts as inhibitor of tumor cells and enhances the antiviral activity of isocarbonucleosides [5]. In addition, Kamal et al. have recently described a new 3,5-diarylisoxazoline linked 2,3-dihydroquinazolinone as a good candidate to further develop potential anticancer agents [6].

Considering the potential antiviral and/or antitumoral activity of diheterocyclic compounds [7–9], we focused our attention in the design and synthesis of potential antitumoral structures containing a 1,2,4-triazole ring and an isoxazoline ring as the second pharmacophore in some of them. The antitumoral activity of the new triazole derivatives was performed on a T cell lymphoma cell line, which "*in vivo*" malignancy in relation to the invasive and the metastatic potential is known [10].

2. Results and discussion

2.1. Chemistry

Tosyl derivative (**1**) was obtained by treatment of allyl 2,3-*O*-cyclopentylidene-β-D-ribofuranoside [11] with tosyl chloride in pyridine. The synthetic strategy of this first step was the functionalization of the terminal hydroxyl group of the carbohydrate derivate with a good living group for subsequent nucleofilic displacements.

In the first case, compound **1** was treated with sodium 1,2,4-triazolyl-3-thiolate to obtain allyl 2,3-*O*-cyclopentylidene-5-deoxy-5-*S*-[3(1,2,4-triazolyl)]-β-D-ribofuranoside (**2**). This compound was subjected to a 1,3-dipolar cycloaddition reaction by treatment with 3,4,5-trimethoxy benzaldoxime in the presence of chloramine-T using *t*-butanol/water as a solvent. The diheterocyclic product (**4**), was obtained as a diasteroisomeric mixture in a ratio 1:1, estimated by ^{1}H-NMR spectroscopy.

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On the other hand, with the aim of obtaining triazoline thiones derivatives, compound **1** was also converted into the corresponding azido derivate (**6**) by nucleofilic displacement of the tosylate group with sodium azide. The azido derivative was transformed into the 1,2,4-triazoline-3-thione derivative (**9**) using the synthetic pathway that we reported in a previous work [12].

With the object to obtain a diheterocycle structure from compound **9**, the procedure described above for compound **2** was applied, but, in this case, the reaction produced several side-products and the expected diheterocycle could not be isolated. To avoid this problem, previously to 1,3-dipolar cycloaddition reaction, compound **9** was protected with a benzyl group to give the corresponding derivative **11**. From this compound, now the diheterocyclic **12** could be obtained satisfactorily (yield 78%).

Compounds **2**, **4**, **9** and **12** were subjected to deprotection reaction, by treatment with a solution of acetic acid 80% as are described in the experimental section [11].

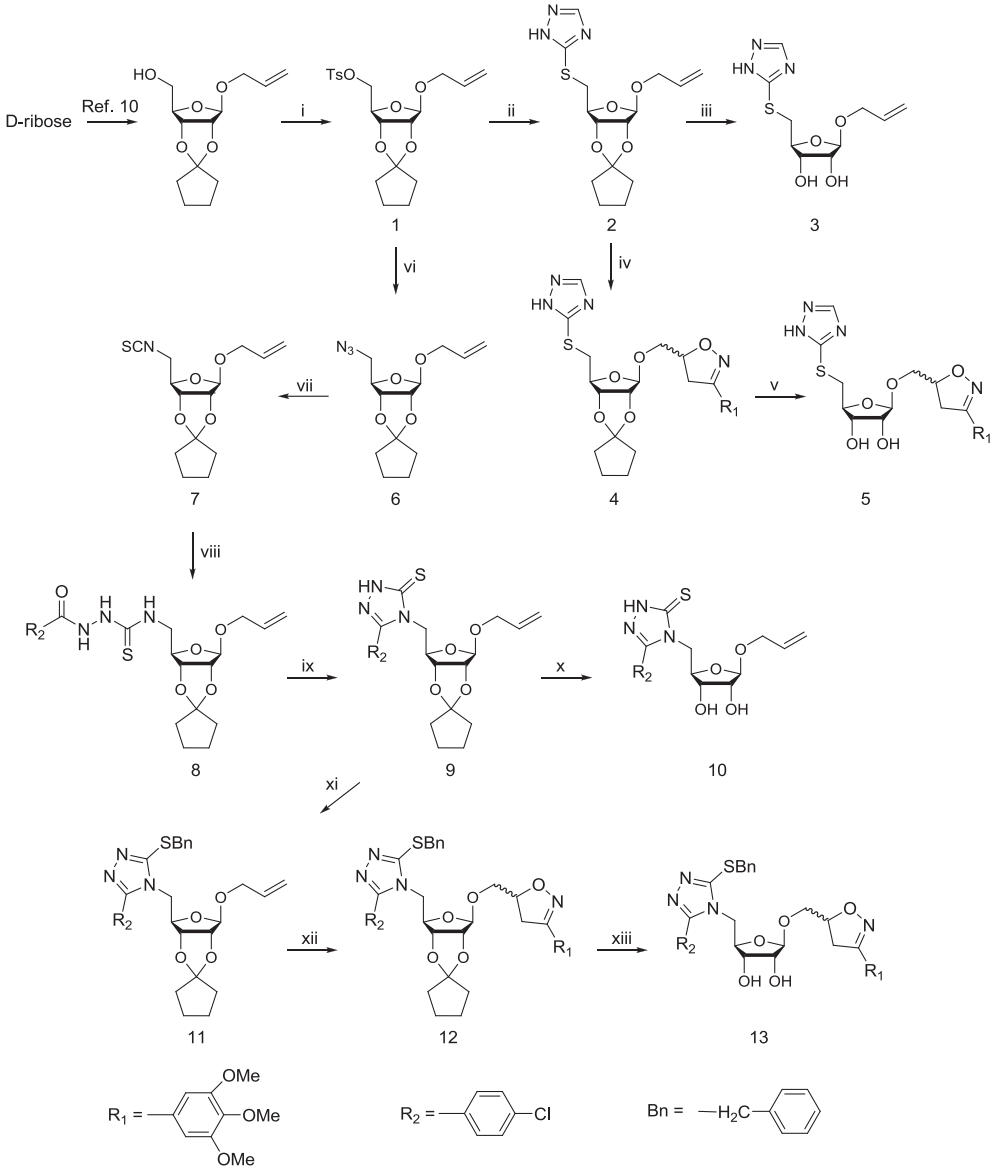
In Scheme 1 we show, the synthetic pathway applied to obtain the heterocyclic compounds. The physical and spectroscopical characterization of the final products and the corresponding precursors are shown in Section 4.

2.2. Proliferation assays

The antiproliferative action on the lymphoma cell line of compounds **2**–**5**, **9**–**13** was analyzed. Since our aim was to perform a preliminary antitumoral screening some epimeric mixtures were included. The bioactivity of the analyzed compounds on cell lines BW 5147 is summarized in Table 1, as well as their lipophilic parameters.

From Table 1 it can be observed:

- Compounds **2**–**4** showed inhibitory activities over the range of measured concentrations.



Scheme 1. Reagents and conditions: i) TsCl pyridine; ii) triazolyl sodium tiolate, DMF, 50 °C, 18 h; iii) CH_3CO_2H 80%, 55 °C, 24 h; iv) $R_1HC{\equiv}NOH$, chloramine-T, EtOH, H_2O, 3 h; v) CH_3CO_2H 80%, 40 °C, 24 h; vi) NaN_3, DMF, 80 °C, 2 h; vii) CS_2. $(Ph)_3P$, dioxane, 24 h; viii) $R_1CONHNH_2$, dioxane, reflux, 3 h; ix) NaEtO, EtOH, reflux, 3 h; x) CH_3CO_2H, 80%, 40 °C, 24 h; xi) BnCl, K_2CO_3, acetone; xii) $R_1HC{\equiv}NOH$, chloramine-T, EtOH, H_2O, 4 h; xiii) CH_3CO_2H, 80%, 40 °C, 24 h.

Table 1
Effect of different compounds upon lymphoma cell line proliferation. Cells were incubated with different compounds in different concentrations during 24 h.

Comp.		Concentration (μg/mL)				logP	ClogP
		1	10	50	100		
2	%	−7 ± 0.8	−9 ± 1	−29 ± 2*	−43 ± 4**	1.95	0.78
	c	2.9	29.5	147.5	295.0		
3	%	−2.4 ± 0.2	−3 ± 0.2	−14 ± 1**	−38 ± 3**	−2.45	−0.95
	c	3.7	36.6	183.2	366.3		
4	%	−5 ± 0.5	−21 ± 2	−24 ± 1*	−45 ± 4**	0.90	1.60
	c	1.8	18.2	91.2	182.1		
5	%	93 ± 5**	51 ± 3**	43 ± 4*	29 ± 2	−0.52	−0.13
	c	2.1	20.7	103.7	207.0		
9	%	49 ± 4*	44 ± 4*	70 ± 5**	123 ± 10**	1.91	1.80
	c	2.2	22.2	111.2	222.2		
10	%	−1.5 ± 0.1	6 ± 0.5	4.4 ± 0.3	2 ± 0.2	0.18	−0.25
	c	2.6	26.1	130.4	260.8		
11	%	37 ± 3*	0	−13 ± 1	n/d	1.63	5.13
	c	1.85	18.5	92.7	184.8		
12	%	215 ± 2**	60 ± 6*	−18 ± 1.9	−45 ± 4**	1.97	5.96
	c	1.3	13.4	66.8	134.0		
13	%	143 ± 15**	210 ± 20**	−52 ± 2.6**	−67 ± 4.5**	1.17	3.91
	c	1.5	14.6	73.3	146.0		

Results were expressed as cell proliferation percentage of basal (%). Concentration expressed in μM (c). ClogP was calculated using ChemBio3D Ultra 11.0, logP data is experimental.
$^{*}p < 0.05$ and $^{**}p < 0.01$ are significant differences respect to basal accordingly to ANOVA + Dunnett's test.
n/d: not determined.

- Compound **5** showed an increment in cell proliferation, which decreased at higher concentrations. This stimulating effect could be related to an increment in the polarity (logP: −0.52) respect to compound **4** (log P: 0.90) or to a conformational change of the carbohydrate moiety.
- Compound **10** did not show differences significantly respect to basal percentage over all concentrations.
- Compounds **11**–**13** exerted a biphasic action, stimulating cell proliferation at low concentrations and inhibiting it at higher ones. Such behavior could be interpreted as evidence for the existence of separate modulatory drug binding sites.
- It is worth noting that compound **9** augmented cell proliferation (stimulation) as the concentration increased. This compound has a completely different behavior than its derivative **11**, likely due to the protection of thiol group which inhibits the tautomeric equilibrium between thiol and thione.

Compounds **2** and **4** exhibited moderate activity against the lymphoma cell line tested. The inhibitory pattern was similar between them and no stimulation activity was observed. The inhibitory action may be attributed to the 1,2,4-triazol ring linked through sulfur to C-5 of the carbohydrate moiety. Deprotection of compound **2** (logP 1.95) reduced its antiproliferative activity (see Table 1, compound **3**) may be due to the increase of the hydrophilicity (logP −2.45). Changes in the hydrophilicity could also justify the different behavior observed for compounds **3** and **5**, as well as for compound **9** and **10** (See Table 1).

3. Conclusion

To summarize, we designed a synthetic pathway to obtain novel triazolic derivatives with or without an isoxazoline ring from D-ribose. Compounds **2**–**4**, **11**–**13** presented moderate inhibitory activity against BW 5147 lymphoma cell line at high concentrations but only compounds **2**–**4** showed inhibitory behavior in the range of measured concentrations. These results suggest that this kind of compounds may be considered as promising leader molecules for further synthetic and biological exploration.

4. Experimental protocols

4.1. Chemistry

4.1.1. General remarks

Synthesis of compounds **1**–**13** was carried out using reagents as purchased, without further purification. Solvents were reagent grade and, in most cases, dried and distillated before use according to standard procedures. Analytical TLC was conducted on Silica Gel 60G (Merck) on precoated plates and visualization was made by UV light and ethanol/sulfuric acid (10:1) or cerium molybdate followed by heating. Column-chromatographic separations were performed on Silica Gel (240–400 mesh, Merck). Elemental analysis was performed on an Exeter Analytical CE-440 elemental analyzer. Optical rotations were recorded at 20 °C on a Perkin Elmer 343 polarimeter, and melting points were uncorrected. ^{1}H, ^{13}C 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 ^{1}H and ^{13}C NMR spectra were confirmed with the aid of two dimensional techniques ^{1}H, ^{13}C (COSY, HSQC). Chemical shifts (δ) are reported in parts per million downfield from tetramethyl silane as internal standard. High-resolution mass spectra HRMS were obtained by Electrospray Ionization (ESI) and Q-TOF detection.

4.1.2. Allyl 2,3-O-cyclopentylidene-5-tosyl-β-D-ribofuranoside (*1*)

To a solution of 2.73 g of allyl 2,3-*O*-cyclopentylidene-β-D-ribofuranoside [11] dissolved in anhydrous pyridine (5 mL), 2.44 g of tosyl chloride was added with continuous stirring under Ar atmosphere. The mixture was kept at room temperature during one night. The crude product was extracted with methylene chloride and water, washed with hydrogen chloride (5%), then sodium bicarbonate (5%) and finally with water, then was purified by column chromatography (cyclohexane:acetone) affording compound **1** as white solid (3.25 g, 7.9 mmol, 69%); mp: 55–56 °C, $[\alpha]_D^{20}$ − 44.4 (*c* 1.1, chloroform). Anal. Calcd for $C_{20}H_{26}O_7S$: C, 58.52; H, 6.38. Found: C, 58.37; H, 6.54. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.78 (d, 2H, *J* 8.4 Hz, aromatic protons), 7.34 (d, 2H, *J* 8.0 Hz, aromatic protons), 5.77 (m, 1H, H-2′), 5.21 (m, 1H, H-3′b), 5.16 (m, 1H, H-3′a), 5.08 (s, 1H, H-1), 4.55 (dd, 1H, $J_{3,2}$ 6.0 Hz, $J_{3,4}$ 0.7 Hz, H-3), 4.51 (d,

1H, $J_{2,3}$ 6.0 Hz, H-2), 4.31 (ddd, 1H, $J_{4,5b}$ 7.7 Hz, $J_{4,5a}$ 6.7 Hz, $J_{4,3}$ 0.9 Hz, H-4), 4.03 (dd, 1H, $J_{5b,5a}$ 10.0 Hz, $J_{5b,4}$ 7.8 Hz, H-5b), 4.03 (m, 1H, H-1′b), 3.99 (dd, 1H, $J_{5a,5b}$ 10.0 Hz, $J_{5a,4}$ 6.7 Hz, H-5a), 3.87 (m, 1H, H-1′a), 2.44 (s, 3H, $\underline{CH}_3$), 1.92–1.62 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 145.2 (aromatic carbon), 133.4 (C-2′), 132.8, 130.0 and 128.1 (aromatic carbons), 122.4 (quaternary carbon of cyclopentylidene ring), 117.8 (C-3′), 107.1 (C-1), 84.9 (C-2), 83.4 (C-4), 81.3 (C-3), 69.4 (C-5), 68.2 (C-1′), 35.9, 35.8, 23.7, 23.2 (cyclopentylidene carbons), 21.8 ($\underline{CH}_3$).

4.1.3. Allyl 2,3-O-cyclopentylidene-5-deoxy-5-S-(1,2,4-triazol-3-yl)-β-D-ribofuranoside (2)

To a solution of sodium ethoxide in ethanol, prepared adding metallic sodium (approximately 243.9 mg) to ethanol (20 mL), was added 1,2,4-triazolyl-5-thiol (1.03 g). The reaction mixture was heated at 50 °C during 10 min and then the solvent was evaporated at reduced pressure. The resulting solid was added at a solution of compound **1** (1.04 g) in DMF (7 mL) and heated under argon atmosphere at 50 °C for 18 h. The solution was evaporated, extracted with dichloromethane and a solution of sodium chloride, dried with sodium sulfate, filtered and evaporated. The crude product was purified by flash column chromatography using cyclohexane:acetone, as eluent. Compound **2** was obtained as a crystalline solid (679.4 mg, 2.00 mmol, 79%), mp: 149–151 °C, $[\alpha]_D^{20}$ − 73.0 (*c* 3.0, acetone). Anal. Calcd for $C_{15}H_{21}N_3O_4S$: C, 53.08; H, 6.24; N, 12.38. Found: C, 53.05; H, 6.42; N, 12.23. ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 8.45 (s, 1H, NH), 8.37 (s, 1H, H-3 of triazolyl group), 5.93 (m, 1H, H-2′), 5.30 (d, 1H, $J_{3'b,2'}$ 17.2 Hz, H-3′b), 5.15 (d, 1H, $J_{3'a,2'}$ 10.6 Hz, H-3′a), 5.10 (s, 1H, H-1), 4.80 (d, 1H, $J_{3,2}$ 5.9 Hz, H-3), 4.64 (d, 1H, $J_{2,3}$ 5.9 Hz, H-2), 4.43 (t br, 1H, *J* 7.6 Hz, H-4), 4.25 (dd, 1H, $J_{1'b,1'a}$ 13.1 Hz, $J_{1'b,2'}$ 4.9 Hz, H-1′b), 4.01 (dd, 1H, $J_{1'a,1'b}$ 12.8 Hz, $J_{1'a,2'}$ 5.6 Hz, H-1′a), 3.38 (dd, 1H, $J_{5b,5a}$ 13.7 Hz, $J_{5b,4}$ 6.7 Hz, H-5b), 3.27 (dd, 1H, $J_{5a,5b}$ 13.9 Hz, $J_{5a,4}$ 9.1 Hz, H-5a), 1.84–1.62 (m, 8H, cyclopentylidene protons); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 157.1 (C-5 of heterocyclic ring), 146.5 (C-3 of heterocyclic ring), 133.5 (C-2′), 122.2 (quaternary carbon of cyclopentylidene ring), 117.8 (C-3′), 107.5 (C-1), 85.2 (C-2), 85.1 (C-4), 83.1 (C-3), 68.3 (C-1′), 35.8 (cyclopentylidene carbon), 35.7 (C-5 and cyclopentylidene carbon), 23.7 and 23.2 (cyclopentylidene carbons).

4.1.4. Allyl 5-deoxy-5-S-(1,2,4-triazol-3-yl)-β-D-ribofuranoside (3)

Compound **2** (251.4 mg, 0.74 mmol) was dissolved in 80% acetic acid and heated to 55 °C during 24 h. Then, the solution was evaporated and the residue was purified by column chromatography using cyclohexane:acetone as eluent. Finally, compound **3** was obtained as a white solid (80.0 mg, 40%); mp: 122–123 °C, $[\alpha]_D^{20}$ − 36.6 (*c* 1.0, methanol). Anal. Calcd for $C_{10}H_{15}N_3O_4S$: C, 43.95; H, 5.53; N, 15.37. Found: C, 44.31; H, 5.32; N, 15.23. ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 8.30 (s, 1H, H-3 of triazolyl group), 5.92 (m, 1H, H-2′), 5.26 (m, 1H, H-3′b), 5.12 (m, 1H, H-3′a), 4.90 (br s, 1 H, H-1), 4.23–4.16 (m, 3H, H-2, H-4, H-1′b), 4.01 (dd, 1H, $J_{2,3}$ 4.7 Hz, $J_{2,1}$ 0.7 Hz, H-3), 3.94 (m, 1H, H-1′a), 3.50 (dd, 1H, $J_{5b,5a}$ 13.5 Hz, $J_{5b,4}$ 5.2 Hz, H-5b), 3.34 (dd, 1H, $J_{5a,5b}$ 13.5 Hz, $J_{5a,4}$ 6.6 Hz, H-5a); ^{13}C NMR (50 MHz, CD_3OD) δ: 158.3 (C-5 of heterocyclic ring), 147.8 (C-3 of heterocyclic ring), 135.5 (C-2′), 117.3 (C-3′), 107.9 (C-1), 82.7 (C-4), 76.5 (C-3), 75.3 (C-2), 69.2 (C-1′), 37.5 (C-5).

4.1.5. ((4R,S)-3-(3,4,5-trimethoxyphenyl)-isoxazolin-5-yl)methyl 5-deoxy-5-S-(1,2,4-triazol-3-yl)-2,3-O-cyclopentylidene-β-D-ribofuranoside (4)

To a solution of 3,4,5-trimethoxy benzaldoxime (194.3 mg) in *t*-buthanol:water 1:1, chloramine-T (195.1 mg) was added in small portions. This solution was slowly added to compound **2** (191.6 mg, 0.56 mmol) dissolved in *t*-buthanol:water 1:1 and the reaction mixture was heated at 45 °C for 2 h. Then, the solution was evaporated and the residue was purified by flash column chromatography using toluene:ethyl acetate, as eluent. Compound **4** was obtained as a diastereoisomeric pair as a 1:1 unresolved mixture of epimers (129.3 mg, 0.24 mmol, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.14, 8.12 (s, 1H, H-3 of triazolyl group), 6.89 (s, 2H, trimethoxyphenyl protons), 5.17, 5.16 (s, 1H, H-1), 4.92 (m, 1H, H-5″ isoxazoline ring), 4.67, 4.66 (d, 1H, $J_{3,2}$ 5.8 Hz, H-3), 4.64, 4.61 (d, 1H, $J_{2,3}$ 6.0 Hz, H-2), 4.47 (m, 1H, H-4), 4.00, 3.93 (dd, 1H, $J_{1'b,1'a}$ 10.8, 10.7 Hz, $J_{1'b,5''}$ 3.5, 6.0 Hz, H-1′b), 3.88, 3.87, 3.87 (s, 9H, methoxyl protons), 3.59, 3.58 (dd, 1H, $J_{1'a,1'b}$ 10.8, 10.7 Hz, $J_{1'a,5''}$ 3.9, 3.0 Hz, H-1′a), 3.43, 3.41 (dd, 1H, $J_{5b,5a}$ 13.3, 13.2 Hz, $J_{5b,4}$ 7.0, 7.4 Hz, H-5b), 3.41, 3.39 (dd, 1H, $J_{4''b,4''a}$ 16.5, 16.6, $J_{4''b,5''}$ 6.7, 6.9 Hz, H-4″b isoxazoline ring), 3.28, 3.26 (dd, 1H, $J_{5b,5a}$ 13.3,13.3 Hz, $J_{5b,4}$ 10.9, 11.3 Hz, H-5a), 3.23, 3.15 (dd, 1H, $J_{4''a,4''b}$ 16.6, 16.6 Hz, $J_{4''a,5''}$ 7.6, 7.0 Hz, H-4″a isoxazoline ring), 1.90–1.63 (m, 8H, cyclopentylidene protons); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 156.6, 156.5 (C-5 of triazolyl ring), 153.6 (C-3″ isoxazoline ring), 153.4, 140.0, 124.7, 104.2 (trimethoxyphenyl carbons), 147.5, 147.3 (C-3 of triazolyl ring), 122.3 (quaternary carbon of cyclopentylidene ring), 108.7, 108.7 (C-1), 85.3, 85.2, 85.0 (C-2, C-4), 83.1 (C-3), 79.6, 79.4 (C-5″ isoxazoline ring) 68.7 (C-1′), 61.0, 56.4 (OCH_3), 37.4, 37.1 (C-4″ isoxazoline ring), 36.0, 35.8, 35.7, 35.6 (C-5 and cyclopentylidene carbons), 23.7, 23.2 (cyclopentylidene carbons). HRMS (ESI) *m*/z (M + Na) calcd for $C_{25}H_{32}N_4NaO_8S$ 571.1833, found 571.1839; *m*/z (M + H) calcd for $C_{25}H_{33}N_4O_8S$ 549.2014, found 549.2026.

4.1.6. ((4R,S)-3-(3,4,5-trimethoxyphenyl)-isoxazolin-5-yl)methyl 5-deoxy-5-S-(1,2,4-triazol-3-yl)-β-D-ribofuranoside (5)

Compound **4** (230.0 mg, 0.42 mmol) was dissolved in 80% acetic acid and heated to 40 °C for 48 h. Then, the solution was evaporated and the residue was purified by column chromatography using cyclohexane:acetone as eluent, giving **5** as a white solid (114.3 mg, 0.24 mmol, 57%). Anal. Calcd for $C_{20}H_{26}N_4O_8S$: C, 49.78; H, 5.43; N, 11.61. Found: C, 49.39; H, 5.59; N, 11.26. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.31 (br s, 1H, H-3 of triazolyl group), 7.01 (s, 2H, trimethoxyphenyl protons), 4.96 (s, 1H, H-1), 4.94–4.87(m, 1H, H-5″ isoxazoline ring), 4.22–4.17 (m, 2H, H-2, H-4), 4.00, 3.98 (dd, 1H, $J_{3,2}$ 4.2 Hz, $J_{3,4}$ 0.8 Hz, H-3), 3.88, 3.87, 3.77 (s, 9H, methoxyl protons), 3.83, 3.80 (dd, 1H, $J_{1'b,1'a}$ 10.9, 10.6 Hz, $J_{1'b,5''}$ 4.8, 4.6 Hz, H-1′b), 3.62–3.57 (m, 1H, H-1′a), 3.55, 3.54 (dd, 1H, $J_{5b,5a}$ 13.7, 13.6 Hz, $J_{5b,4}$ 4.9, 4.7 Hz, H-5b), 3.51, 3.49 (dd, 1H, $J_{4''b,4''a}$ 16.9, 16.8, $J_{4''b,5''}$ 4.4, 4.5 Hz, H-4″b isoxazoline ring), 3.44–3.39 (m, 1H, H-5a), 3.35, 3.29 (dd, 1H, $J_{4''a,4''b}$ 16.8, 16.9 Hz, $J_{4''a,5''}$ 7.6, 7.4 Hz, H-4″a isoxazoline ring); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 157.0, 157.0 (C-5 of heterocyclic ring), 154.4, 142.4, 140.8, 126.4, 126.3, 105.1 (trimethoxyphenyl carbons), 147.2 (C-3 of triazolyl ring), 108.6 (C-1), 83.1, 83.0, (C-4), 80.7, 80.6 (C-5″ isoxazoline ring), 76.3 (C-3), 75.0, 74.9 (C-2), 69.6, 69.1 (C-1′), 60.6, 56.6 (OCH_3), 38.0, 37.7 (C-4″ isoxazoline ring), 36.7, 36.7 (C-5).

4.1.7. Allyl 2,3-O-cyclopentylidene-5-deoxy-5-azide-β-D-ribofuranoside (6)

To a solution of compound **1** (318.5 mg) in DMF (8 mL) we added sodium azide (312.5 mg). The reaction mixture was heated under argon atmosphere at 80 °C for 3 h. Then it was filtered and washed with ethyl ether. The product was evaporated under reduced pressure, suspended in water and extracted with ethyl ether. The extract was dried with sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography using cyclohexane:acetone, as eluent. Compound **6** was obtained as a transparent syrup (161.9 mg, 0.58 mmol, 74%); $[\alpha]_D^{25}$ − 65.4 (*c* 1.7, chloroform). Anal. Calcd for $C_{13}H_{19}N_3O_4$: C, 55.50; H, 6.81. Found: C, 55.53; H, 6.74. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.88 (m, 1H, H-2′), 5.29 (m, 1H, H-3′b), 5.21 (m, 1H, H-3′a), 5.15 (s, 1H, H-1), 4.60 (d, 1H, $J_{2,3}$ 6.1 Hz, H-2), 4.56 (dd, 1H, $J_{3,2}$ 6.0 Hz, $J_{3,4}$ 1.1 Hz, H-3), 4.31 (ddd, 1H,

$J_{4,5b}$ 7.8 Hz, $J_{4,5a}$ 6.8 Hz, $J_{4,3}$ 1.1 Hz, H-4), 4.21 (m, 1H, H-1′b), 3.99 (m, 1H, H-1′a), 3.47 (dd, 1H, $J_{5b,5a}$ 12.6 Hz, $J_{5b,4}$ 7.9 Hz, H-5b), 3.28 (dd, 1H, $J_{5a,5b}$ 12.5 Hz, $J_{5a,4}$ 6.7 Hz, H-5a), 1.94–1.70 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 133.6 (C-2′), 122.4 (quaternary carbon of cyclopentylidene ring), 117.8 (C-3′), 107.6 (C-1), 85.3 (C-2), 85.2 (C-4), 82.0 (C-3), 68.2 (C-1′), 53.9 (C-5), 36.0, 35.9, 23.7 and 23.3 (cyclopentylidene carbons).

4.1.8. Allyl 2,3-O-cyclopentylidene-5-deoxy-5-isothiocyanate-β-D-ribofuranoside (7)

To a solution of compound **6** (158.2 mg), carbon disulfide (0.4 mL) in dioxane anhydrous (5 mL) was added 169.0 mg of triphenylfosfine. The reaction mixture was stirred at room temperature during 24 h, under Ar atmosphere. Then the solvent was evaporated and the crude product was extracted with ethyl ether. The extract was dried with sodium sulfate, filtered and concentrated, dissolved in cyclohexane and filtered off. The solvent was evaporated. The residue was purified by flash column chromatography using cyclohexane:acetone, as eluent. Compound **7** was obtained as a transparent syrup (131.6 mg, 0.44 mmol, 79%); $[\alpha]_D^{25}$ − 21.6 (*c* 1.0, chloroform). Anal. Calcd for $C_{14}H_{19}NO_4S$: C, 56.55; H, 6.44; N, 4.70. Found: C, 56.47; H, 6.51; N, 4.86. IR (KBr, cm^{-1}): 2207 and 2089 (N═C═S). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.86 (m, 1H, H-2′), 5.29 (m, 1H, H-3′b), 5.20 (m, 1H, H-3′a), 5.15 (s, 1H, H-1), 4.60 (d, 1H, $J_{2,3}$ 6.0 Hz, H-2), 4.55 (dd, 1H, $J_{3,2}$ 6.0 Hz, $J_{3,4}$ 0.7 Hz, H-3), 4.37 (ddd, 1H, $J_{4,5b}$ 7.8 Hz, $J_{4,5a}$ 7.0 Hz, $J_{4,3}$ 0.9 Hz, H-4), 4.20 (m, 1H, H-1′b), 3.99 (m, 1H, H-1′a), 3.68 (dd, 1H, $J_{5b,5a}$ 14.3 Hz, $J_{5b,4}$ 7.9 Hz, H-5b), 3.55 (dd, 1H, $J_{5a,5b}$ 14.3 Hz, $J_{5a,4}$ 7.0 Hz, H-5a), 1.92–1.65 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 133.4 (C-2′), 122.6 (quaternary carbon of cyclopentylidene ring), 118.0 (C-3′), 107.5 (C-1), 85.1 (C-2), 84.5 (C-4), 81.7 (C-3), 68.5 (C-1′), 48.1 (C-5), 35.9, 35.8, 23.7, 23.2 (cyclopentylidene carbons).

4.1.9. Allyl 5-(p-clorobenzohydrazinecarbothionyl) amino-2,3-O-cyclopentylidene-5-deoxy-β-D-ribofuranoside (8)

A suspension of *p*-chloro-benzoic hydrazide (275.3 mg, 1.61 mmol) in dry dioxane was heated until dissolution and compound **7** was added (432.6 mg, 1.45 mmol). The resulting solution was kept at room temperature for 24 h under Ar atmosphere. The solvent was evaporated and the crude product was purified by flash chromatography using cyclohexane:acetone as eluent. The *p*-chloro substituted thiosemicarbazide (**8**) was obtained as white crystals (623.6 mg, 1.33 mmol, 92%); mp: 119–121 °C, $[\alpha]_D^{25}$ − 49.2 (*c* 1.0, chloroform). Anal. Calcd for $C_{21}H_{26}ClN_3O_5S$: C, 53.90; H, 5.60; N, 8.98. Found: C, 53.68; H, 5.64; N, 8.95. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.10 (NH), 8.72 (NH), 7.82 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.59 (NH), 7.45 (d, 2H, *J* 8.6 Hz, aromatic protons), 5.62 (m, 1H, H-2′), 5.14 (dd, 1H, $J_{3'b,2'}$ 17.2 Hz, $J_{3'b,3'a}$ 1.3 Hz, H-3′b), 5.10 (dd, 1H, $J_{3'a,2'}$ 10.3 Hz, $J_{3'a,3'b}$ 1.2 Hz, H-3′a), 5.07 (s, 1H, H-1), 4.62 (d, 1H, $J_{2,3}$ 6.4 Hz, H-3), 4.53 (d, 1H, $J_{3,2}$ 6.2 Hz, H-2), 4.37 (br signal, 1H, $J_{4,5b}$ 5.8 Hz, $J_{4,5a}$ 4.6 Hz, H-4), 4.11 (br signal, 1H, H-5b), 4.04 (dd, 1H, $J_{1'b,1'a}$ 12.8 Hz, $J_{1'b,2'}$ 5.4 Hz, H-1′b), 3.88 (dd, 1H, $J_{1'a,1'b}$ 12.9 Hz, $J_{1'a,2'}$ 6.4 Hz, H-1′a), 3.63 (br d, 1H, $J_{5b,5a}$ 14.2 Hz, H-5a), 1.88–1.63 (m, 8H, cyclopentylidene protons); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 182.6 (CS), 166.2 (CO), 139.2 (aromatic carbon), 133.0 (C-2′), 129.4, 129.3 and 129.1 (aromatic carbons), 122.1 (quaternary carbon of cyclopentylidene ring), 118.5 (C-3′), 107.3 (C-1), 85.3 (C-2), 85.0 (C-4), 81.6 (C-3), 68.6 (C-1′), 47.7 (C-5), 35.8, 35.7, 23.6, 23.1 (cyclopentylidene carbons).

4.1.10. Allyl 5-deoxy-5-[(5-p-chlorophenyl-3-thionyl)-1,2,4-triazolin-4-yl]-2,3-O-cyclopentylidene-β-D-ribofuranoside (9)

Thiosemicarbazide (**8**) (401.3 mg, 0.86 mmol) was dissolved in ethanol and sodium metallic was added (79.8 mg, 3.47 mmol). The reaction mixture was refluxed, with continuous stirring, during 24 h. The solution was neutralized with HCl 1 N, evaporated and the crude residue was further purified by flash column chromatography using cyclohexane:acetone as eluent, affording compound **9** as white crystals (312.9 mg, 0.70 mmol, 81%); mp: 53–54 °C, $[\alpha]_D^{25}$ − 54.0 (*c* 1.0, chloroform). Anal. Calcd for $C_{21}H_{24}ClN_3O_4S$: C, 56.06; H, 5.38. Found: C, 56.07; H, 5.69. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.60 (d, 2H, *J* 8.8 Hz, aromatic protons), 7.53 (d, 2H, *J* 8.7 Hz, aromatic protons), 5.83 (m, 1H, H-2′), 5.27 (m, 1H, H-3′b), 5.19 (m, 1H, H-3′a), 5.08 (s, 1H, H-1), 4.93 (dd, 1H, $J_{3,2}$ 6.0 Hz, $J_{3,4}$ 0.7 Hz, H-3), 4.68 (dd, 1H, $J_{5b,5a}$ 14.3 Hz, $J_{5b,4}$ 9.3 Hz, H-5b), 4.57 (d, 1H, $J_{2,3}$ 6.0 Hz, H-2), 4.36 (ddd, 1H, $J_{4,5b}$ 9.3 Hz, $J_{4,5a}$ 4.6 Hz, $J_{4,3}$ 0.8 Hz, H-4), 4.07 (m, 1H, H-1′b), 4.05 (dd, 1H, $J_{5a,5b}$ 14.4 Hz, $J_{5a,4}$ 4.5 Hz, H-5a), 3.90 (m, 1H, H-1′a), 1.85–1.61 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.4 (CS) and 150.9 (CN) (heterocyclic carbons), 137.7 (aromatic carbon), 133.4 (C-2′), 130.1, 129.8 and 124.1 (aromatic carbons), 122.4 (quaternary carbon of cyclopentylidene ring), 117.5 (C-3′), 107.4 (C-1), 84.9 (C-2), 83.7 (C-4), 81.7 (C-3), 68.4 (C-1′), 46.8 (C-5), 35.9, 35.8, 23.6, 23.1 (cyclopentylidene carbons).

4.1.11. Allyl 5-deoxy-5-[(5-p-chlorophenyl-3-thionyl)-1,2,4-triazolin-4-yl]-β-D-ribofuranoside (10)

Compound **9** (95.9 mg, 0.21 mmol) was dissolved in 80% acetic acid and heated to 40 °C for 24 h. Then, the solution was evaporated (as described for compound **3**) and the residue was purified by column chromatography using cyclohexane:acetone as eluent, giving **10** as a white solid (50.8 mg, 0.13 mmol, 62%); mp: 149–152 °C (dec), $[\alpha]_D^{20}$ − 15.3 (*c* 1.0, methanol). Anal. Calcd for $C_{16}H_{18}ClN_3O_4S$: C, 50.06; H, 4.73; N, 10.95. Found: C, 50.00; H, 5.07; N, 10.63. ^{1}H NMR (200 MHz, CD_3OD) δ: 7.73 (d, 2H, *J* 8.6 Hz, aromatic protons), 7.54 (d, 2H, *J* 8.6 Hz, aromatic protons), 5.73 (m, 1H, H-2′), 5.14 (m, 1H, H-3′b), 5.10 (m, 1H, H-3′a), 4.72 (s, 1H, H-1), 4.58 (dd, 1H, $J_{5b,5a}$ 17.4 Hz, $J_{5b,4}$ 7.3 Hz, H-5b), 4.34–4,20 (m, 2H, H-4 and H-5a), 4.07 (dd, 1H, $J_{3,2}$ 4.6 Hz, $J_{3,4}$ 7.0 Hz, H-3), 3.81 (d, 1H, $J_{2,3}$ 4.6 Hz, H-2), 3.70 (m, 1H, H-1′b), 3.58 (m, 1H, H-1′a); ^{13}C NMR (50 MHz, CD_3OD) δ: 169.7 (CS) and 153.2 (CN) (heterocyclic carbons), 138.3 (aromatic carbon), 135.7 (C-2′), 132.2, 130.7 and 126.9 (aromatic carbons), 117.3 (C-3′), 108.3 (C-1), 80.3 (C-4), 76.3 (C-3), 74.6 (C-2), 69.6 (C-1′), 49.7 (C-5).

4.1.12. Allyl 5-deoxy-5-[(5-p-chlorophenyl-3-thiobenzyl)-1,2,4-triazol-4-yl]-2,3-O-cyclopentylidene-β-D-ribofuranoside (11)

A mixture of compound **9** (501.3 mg, 1.1 mmol), benzyl bromide (0.2 mL) and anhydrous potassium carbonate (153.6 mg) in acetone was stirred at room temperature for 17 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was purified by flash column chromatography using toluene:ethyl acetate as eluent, affording compound **11** as a syrup (567.6 mg, 1.05 mmol, 94%); $[\alpha]_D^{25}$ − 55.7 (*c* 1.0, methanol). Anal. Calcd for $C_{28}H_{30}ClN_3O_4S$: C, 62.27; H, 5.60; N, 7.78. Found: C, 61.89; H, 6.00; N, 7.93. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45–7.20 (9H, aromatic protons), 5.72 (m, 1H, H-2′), 5.16 (m, 1H, H-3′b), 5.12 (m, 1H, H-3′a), 4.92 (s, 1H, H-1), 4.40 (d, $J_{12.8}$ Hz, thiobenzyl proton), 4.37 (d, *J* 12,8 Hz, thiobenzyl proton), 4.30 (d, *J* 6.0 Hz, H-2), 4.17 (dd, 1H, $J_{3,2}$ 6.0 Hz, $J_{3,4}$ 0.9 Hz, H-3), 4.01 (ddd, 1H, $J_{4,5b}$ 9.2 Hz, $J_{4,5a}$ 5.3 Hz, $J_{4,3}$ 0.8 Hz, H-4), 3.92 (dd, 1H, $J_{5b,5a}$ 14.5 Hz, $J_{5b,4}$ 9.3 Hz, H-5b), 3.92 (m, 1H, H-1′b), 3.76 (m, 1H, H-1′a), 3.74 (dd, 1H, $J_{5a,5b}$ 14.5 Hz, $J_{5a,4}$ 5.3 Hz, H-5a), 1.74–1.44 (m, 8H, cyclopentylidene protons); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 154.6, 151.3 (CN), 133.4 (C-2′), 136.8, 136.5, 130.2, 129.6, 129.3, 128.8, 128.0, 125.7 (aromatic carbons), 122.6 (quaternary carbon of cyclopentylidene ring), 117.7 (C-3′), 107.4 (C-1), 84.9 (C-2), 83.8 (C-4), 81.1 (C-3), 68.6 (C-1′), 46.8 (C-5), 39.0 (CH_2 benzyl carbon), 35.8, 35.8, 23.6, 23.2 (cyclopentylidene carbons).

*4.1.13. ((4R,S)-3-(3,4,5-trimethoxyphenyl)-isoxazolin-5-yl)methyl 5-deoxy-5-[(5-p-chlorophenyl-3-thiobenzyl)-1,2,4-triazol-4-yl]-2,3-O-cyclopentylidene-β-D-ribofuranoside (**12**)*

To a homogeneous solution of compound **11** (393.2 mg, 0.73 mmol) and 3,4,5-trimethoxy benzaldoxime (320.0 mg, 1.45 mmol) in ethanol/water, chloramine-T (665.9 mg) were slowly added. The reaction mixture was kept at room temperature for 5 h. Then, the solution was evaporated, the crude was dissolved in acetone and filtered off. The residue was purified by flash column chromatography using toluene:ethyl acetate, as eluent. Compound **12** was obtained as a diastereoisomeric pair as a 1:1 unresolved mixture of epimers (425.3 mg, 0.57 mmol, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.53–7.20 (m, 9H, aromatic protons), 6.85 (s, 1 H, trimethoxyphenyl proton), 6.82 (s, 1H, trimethoxyphenyl proton), 4.94, 4.93 (s, 1H, H-1) 4.82–4.74 (m, 1H, H-5″ isoxazoline ring), 4.45, 4.45 (d, 1H, *J* 12.7 Hz, thiobenzyl proton), 4.39, 4.37 (d, 1H, *J* 12.7 Hz, thiobenzyl proton), 4.23–4.00 (m, 4H, H-2, H-3, H-4, H-5b), 3.89 (s, 9H, methoxy protons), 3.94, 3.85 (dd, 1H, $J_{5b,5a}$ 14.2, 14.7 Hz, $J_{5b,4}$ 4.0, 4.8 Hz, H-5a), 3.85–3.61 (m, 1H, H-1′b), 3.42, 3.39 (dd, 1H, $J_{1'a,1'b}$ 11.1, 10.7 Hz, $J_{1'a,4''}$ 4.2, 3.6 Hz, H-1′a), 3.32, 3.25 (dd, 1H, $J_{4''b,4''a}$ 16.5, 16.4, $J_{4''b,5''}$ 10.8, 11.0 Hz, H-4″b isoxazoline ring), 3.17, 2.95 (dd, 1H, $J_{4''a,4''b}$ 16.4 Hz, 16.5 Hz, $J_{4''a,5''}$ 8.5, 7.4 Hz, H-4″a isoxazoline ring), 1.69–1.49 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 156.4, 156.1 (C-3″ isoxazoline ring), 154.5, 153.6, 151.7, 151.5 (triazolyl carbons), 153.5, 140.2–124.7, 104.2 (aromatic carbons) 122.6, 122.5 (quaternary carbon of cyclopentylidene ring), 108.5, 108.4 (C-1), 84.7, 84.7 (C-2), 84.0, 83.9 (C-4), 81.0 (C-3), 79.6, 79.4 (C-5″ isoxazoline ring), 69.3, 68.2 (C-1′), 61.1, 61.1, 56.5, 56.4 (OCH_3), 46.4, 46.4 (C-5), 38.6, 38.4 (thiobenzyl carbon), 37.4, 36.3 (C-4″ isoxazoline ring), 35.7, 35.6, 23.6, 23.1, 23.1 (cyclopentylidene carbons). HRMS (ESI) *m/z* (M + Na) calcd for $C_{38}H_{41}ClN_4NaO_8S$ 771.2226, found 771.2235.

*4.1.14. ((4R,S)-3-(3,4,5-trimethoxyphenyl)-isoxazolin-5-yl)methyl 5-deoxy-5-[(5-p-chlorophenyl-3-thiobenzyl)-1,2,4-triazol-4-yl]-β-D-ribofuranoside **(13)***

Compound **12** (138.6 mg, 0.18 mmol) was dissolved in 80% acetic acid and heated to 40 °C for 48 h. Then, the solution was evaporated and the residue was purified by column chromatography using cyclohexane:acetone as eluent, giving **13** as a white solid (74.6 mg, 0.11 mmol, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.76–7.13 (m, 9H, aromatic protons), 7.02, 7.00 (s, 1 H, trimethoxyphenyl proton), 4.81 (s, 1H, H-1), 4.76–4.70 (m, 1H, H-5″ isoxazoline ring), 4.46–4.42 (m, thiobenzyl protons), 4.29–4.25 (m, 1H, H-5b), 4.17, 4.13 (dd, 1H, $J_{5b,5a}$ 15.0, 15.1 Hz, $J_{5b,4}$ 7.7, 7.8 Hz, H-5a), 3.83, 3.78 (d, 1H, $J_{3,2}$ 3.5, 4.5 Hz, H-3), 4.00–3.96 (m, 2H, H-2, H-4), 3.88, 3.87, 3.76, 3.76 (s, 9H, methoxy protons), 3.55, 3.48 (dd, 1H, $J_{1'b,1'a}$ 10.9, 10.7 Hz, $J_{1'b,2'}$ 4.8, 4.1 Hz, H-1′b), 3.42, 3.35 (dd, 1H, $J_{1'a,1'b}$ 11.9, 10.6 Hz, $J_{1'a,4''}$ 3.7, 6.4 Hz, H-1′a), 3.42, 3.39 (dd, 1H, $J_{4''b,4''a}$ 16.9, 16.8 Hz, $J_{4''b,5''}$ 10.9, 11.0 Hz, H-4″b isoxazoline ring), 3.10, 3.09 (dd, 1H, $J_{4''a,4''b}$ 16.9, 16.8 Hz, $J_{4''a,5''}$ 7.7, 7.9 Hz, H-4″a isoxazoline ring); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 157.0 (C-3″ isoxazoline ring), 155.5, 152.0 (triazolyl carbons), 154.5, 138.4–126.3, 105.2, 105.2 (aromatic carbons), 108.8, 108.7 (C-1), 81.5, 81.3 (C-4), 80.7, 80.5 (C-5″ isoxazoline ring), 75.5 (C-3), 73.6 (C-2), 69.9, 69.5 (C-1′), 60.7, 56.6 (OCH_3), 49.0, 48.7 (C-5), 38.8 (thiobenzyl carbon), 37.8, 37.5 (C-4″ isoxazoline ring). HRMS (ESI) *m/z* (M + Na) calcd for $C_{33}H_{35}ClN_4NaO_8S$ 705.1756, found 705.1770.

4.2. Pharmacological studies

4.2.1. Cell culture conditions

The tumor cell line BW 5147 (Institute fur Virologie und Immunobiologie der Universitat Wurzburg, Germany) is a T cell lymphoma cell line that expresses the H-2 k haplotype, is $CD3^+$ and has a TCRαβ as determined by flow cytometric analysis. Cells were cultured at optimal concentrations of 3×10^5 cells/mL in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine and antibiotics [13]. Cells were cultured at a final volume of 0.2 mL in 96-well flat-bottom microtiter plates (Nunc).

4.2.2. Analyzed compounds

The compounds analyzed were **2**–**5**, **9**–**13**. Compound **13** was dissolved in dimethyl sulfoxide (DMSO) and the others in different concentrations of ethanol:water mixtures. Compound **2** was dissolved in ethanol 12%, compound **4** in ethanol 15%, and compounds **3**, **5**, **9**–**12** in ethanol 10%. The final ethanol concentration in wells was 0.5% and DMSO 1%.

4.2.3. Proliferation assays

The effects of different compounds on tumoral lymphocytes proliferation was evaluated by the uptake of tritiated thymidine [^{3}H]TdR. Cells were cultured during 24 h in presence or absence (basal) of different concentrations of compounds **2**–**5**, **9**–**13** (1, 10, 50 and 100 μg/mL) and then pulsed with 0.75 μCi/well of [^{3}H]TdR ($S = 25$ Ci/mmol) for the last 16 h previously to culture sacrifice by freezing as previously described [14]. Blanks corresponding to ethanol 0.5% and DMSO 1% were assayed.

Results were expressed as cpm or as cell proliferation (% of basal): [cpm basal − cpm treated/cpm basal × 100]. Data represent the mean ± SEM of three experiments performed in triplicate. It is important to note that the blanks did not modify cell proliferation by their self (proliferation cpm (mean ± SEM): Basal: 5487 ± 453, Ethanol 0.5%: 5520 ± 400; DMSO 1%: 5500 ± 500).

4.2.4. Statistical analysis

Data was analyzed using one way analysis of variance and Dunnett's test. Significant difference was determined when $p \leq 0.05$.

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