Synthesis and antitubercular evaluation of new 1,2,3-triazole derivatives of carbohydrates

Juliana Alves dos Santos¹, Roberta C. N. Reis Corrales¹, Fernando R. Pavan², Clarice Queico. F. Leite², Rafael Mafra de Paula Dias¹ and Adilson David da Silva¹*

¹Departamento de Química, I.C.E., Universidade Federal de Juiz de Fora, Campus Universitário, Juiz de Fora, Minas Gerais 36036-900, Brazil.
²Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, CEP 14801-902, Araraquara, SP, Brazil.

Abstract: We reported in this work the preparation of novel 1,4-disubstituted-1,2,3-triazoles derivatives from D-glucose and D-fructose and their in vitro antibacterial activity against Mycobacterium tuberculosis were evaluated. The chemical synthesis was performed based on the 1,3-dipolar cycloaddition reaction, and antimicrobial activity was determined based on Resazurin Microtiter Assay against Mycobacterium. None of the triazole glycoconjugates tested showed activity against these microorganisms.

Keywords: carbohydrate; glycoconjugates; 1,2,3-triazole; antitubercular activity.

Introduction

The chemical structures diversity of the 1,2,3-triazole family and their biological activities made these compounds to became attractive targets in synthetic organic chemistry¹. 1,2,3-triazole moiety does not occur in nature, although synthetic molecules containing 1,2,3-triazole have shown several biological activities including antibacterial, herbicidal, fungicidal, antiallergic and anti-HIV²,³. Literature has recently reported the preparation of triazole derivatives linked to carbohydrates and biological evaluation of their glycoconjugates, have been shown to stand out as HIV reverse transcriptase (HIV-RT) inhibitors¹, antitrypanosomal agents,²,⁴ inhibitors of α-glucosidases,³,⁵ antitubercular activity⁶,⁷ and antitumor agents⁴,⁸.

Moreover, carbohydrates bearing a 1,2,3-triazoles group have been explored as potential inhibitors of glycosidases and fucosyltransferases, as well as study model for substrate specificity of β-1,2-mannosyltransferases²,⁴.

The importance of the triazole core lies in the fact that they cannot be cleaved hydrolytically and are almost impossible to oxidize or reduce. 1,2,3-triazole may act as H-bond donors or acceptors, depending on their substitution. In 1,4-disubstituted 1,2,3-triazole, N-2 and N-3 act as H-bond acceptors. The strong dipole moment of triazole polarizes H-C-5 to a degree that it might function as a weak H-bond donor. Recent studies on 1,2,3-triazole revealed the hydrogen bonding and dipole interactions of the triazole core may favor their binding to biomolecular targets and may improve their solubility⁵,⁶. Carbohydrate-derivated compounds have also been studied for their antimicrobial action. In the last years the increase

*Corresponding author:
E-mail address: david.silva@ufjf.edu.br
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of the antimicrobial drug-resistant bacteria led international health organizations to issue the need of prospective studies regarding new potential substances to overcome the resistance phenomenon\textsuperscript{10-12}.

Two main approaches are currently being investigated for developing new drugs: (i) synthesis of analogues of existing drugs; and (ii) search for novel structures that the bacteria have never been presented to before\textsuperscript{12}. In this paper, we report the synthesis of a new series of 1,4-disubstituted 1,2,3-triazoles linked to derivatives of D-glucose and D-fructose using 1,3-dipolar cycloaddition of terminal acetylene and azides. The protecting groups on derivatives of D-fructose and the free hydroxyl from D-glucose derivatives allowed the evaluation of lipophilic glycoconjugates influence on biological activity\textsuperscript{5,9}.

Results and Discussion

**Anti-M. tuberculosis activity assay:**

The in vitro anti-MTB activities of the 1,2,3-triazoles derivatives from D-glucose and D-fructose were tested against MTB H\textsubscript{37}Rv ATCC 27294 and the MICs are reported in Table 1. The minimum inhibitory concentration (MIC) values of all the tested compounds were not reached because the assay limit with dilutions ranged from 0.15 to 250 µg/mL. On this basis, the results are presented higher than 250 µg/mL.

According to TB Alliance, World Health Organization (OMS) and National Institutes of Health (NHI), new anti-TB candidates must show MIC values ≤ 6.25 µg/mL (or the molar equivalent) against standard MTB cultures\textsuperscript{13}. Therefore, according to these organizations guidelines, these compounds were not selected for further assays.

**Table 1**: Antitubercular activity (\textit{M. tuberculosis} H\textsubscript{37}Rv ATCC 27294) of compounds 5a-e and 6a-e.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC/(µg.mL\textsuperscript{-1})</th>
<th>Compound</th>
<th>MIC/(µg.mL\textsuperscript{-1})</th>
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<tbody>
<tr>
<td>5a</td>
<td>&gt;250</td>
<td>6a</td>
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<tr>
<td>5b</td>
<td>&gt;250</td>
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<td>&gt;250</td>
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<tr>
<td>isoniazid</td>
<td>0.03</td>
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</table>

Chemistry

The synthesis of derivatives of D-glucose and D-fructose (compounds 1 and 3) containing the terminal alkyne group has been achieved by alkylation of D-glucose and alkylation of diisopropylidene 2 with propargyl alcohol and propargyl bromide, respectively, as previously described\textsuperscript{14-16} (Scheme 1). The azides (4a-e) were prepared according to the literature\textsuperscript{17-19}. The 1,3-dipolar cycloaddition coupling was performed with azides (4a-e) (1.3 equiv) and the terminal alkynes (1 and 3) (1 equiv) to give the 1,2,3-triazoles (5a-e and 6a-e) as shown in scheme 2. CuSO\textsubscript{4}.5H\textsubscript{2}O (0.05 equiv) and sodium ascorbate (0.40 equiv) were used in the experiments for the \textit{in situ} generation of Cu(I) catalyst. Typically, the reactions were conducted at 25°C for 96h in DMSO/H\textsubscript{2}O 1:1, with the progress of the reaction being monitored by TLC. After completion of the reaction, solvent was removed by co-evaporation with toluene under reduced pressure and crude product was purified by column chromatography on silica gel using 5-10% MeOH/CH\textsubscript{2}Cl\textsubscript{2}.
All compounds were demonstrated to be of sufficient purity for use in biological assays (>95%) by $^{13}$C Nuclear Magnetic Resonance ($^{13}$C NMR). Compounds 2, 3, and 4a-e have been described in the literature.\textsuperscript{14-19}

Reagents and conditions - i: propargyl alcohol, H$_2$SO$_4$/silica, 6h, 65°C; ii: acetone, H$_2$SO$_4$, 48h, rt; iii: NaOH 50%, CH$_2$Cl$_2$, propargyl bromide, (Bu)$_4$NBr, 48h.

**Scheme 1**: Scheme of synthesis of terminal alkynes 1 and 3.

Reagents and conditions - i: H$_2$O/DMSO, CuSO$_4$·5H$_2$O, sodium ascorbate, rt, 96h.

**Scheme 2**: Scheme of synthesis of 1,2,3-triazoles 5a-e and 6a-e.

**Conclusion**

This work describes the preparation of novel 1,2,3-triazole derivatives of carbohydrates using the methodology azide-alkyne cycloaddition catalyzed Cu(I) - CuAAC. It is known that systems triazoles\textsuperscript{2} and those derived from carbohydrates\textsuperscript{20,21} have bacterial activity and, since the glucotriazoles result from the combination of these two units heterocyclic it was expected...
that the glycoconjugates synthesized were active against bacteria analyzed. But in this work we evaluated the biological activity of these glycoconjugates against MTB H37Rv ATCC 27294 and these compounds showed not activity, demonstrating once more that the combination between triazole and carbohydrate are not effective against Mycobacterium tuberculosis.6,7

Further studies in vitro and in vivo as HIV reverse transcriptase inhibitors, anti trypanosomal agents, inhibitors of α-glucosidases and antitumor agents are required to assess the biological properties of these glycoconjugates, to better understand its therapeutic value.

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Experimental Section

All chemicals were purchased as reagent grade and used without further purification. TLC was performed on precoated silica gel F254 plates (0.25 mm; E. Merck). Infrared spectra were recorded on Shimadzu 8400 series FTIR instrument. 1H NMR spectra were recorded on a Bruker AC-300 spectrometers at 300MHz and 13C NMR spectra were recorded on a Bruker AC-300 at 75 MHz. The [α]D results were taken on a digital Bellingham Stanley – ADP 410 polarimeter.

General procedure for cycloaddition (5a-e and 6a-e)
The alkene 1 or 2 (1 equiv) and the azide 4a-e (1.3 equiv) were dissolved in DMSO/H2O 1:1. To this solution, CuSO4.5H2O (0.05 equiv) and sodium ascorbate (0.40 equiv) were added. The reaction mixture was stirred for 96h at 25°C. Solvents were evaporated under reduced pressure and crude product was purified by column chromatography on silica gel using 5-10% MeOH/CH2Cl2 system to obtain 1,4-disubstituted-1,2,3-triazole 5a-e and 6a-e.

Synthesis of 2-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)-6-(hydroxymethyl)-2H-pyran-3,4,5-trioli (5a)
The compound 5a was obtained in 62% yield as a yellow oil; \( \nu_{\text{max}} \) (CsI) (cm\(^{-1}\)): 3370, 2946, 2833, 1648, 1450, 1020; 1H NMR (300 MHz, DMSO-\( d_6 \)), δ (ppm): 8.15 (s, 1H, H-9), 7.35-7.33 (m, 5H, Ph), 5.58 (s, 2H, -CH2Ph), 4.86 (m, 2H, H-7), 4.76-4.24 (m, 4H, H-2, H-5, H-6, H-6'), 3.31-3.07 (m, 2H, H-3, H-4); 13C NMR (75 MHz, DMSO-\( d_6 \)), δ (ppm): 144.2 (C-8), 136.1-128.0 (Ph), 124.4 (C-9), 102.3 (C-1), 98.1 (C-5), 76.7 (C-3), 73.3 (C-2), 70.3 (C-4), 61.6 (C-7), 61.0 (C-6), 52.9 (C13). [\( \alpha \])\( _D \) +21.8 (c 1.1, CH3OH).

Synthesis of 2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-6-(hydroxymethyl)-2H-pyran-3,4,5-trioli (5b)
The compound 5b was obtained in 80% yield as a brown oil; [\( \alpha \])\( _D \) +33.9 (c 0.53, CH3OH). Their characterizations are consistent with those described in literature.22

Synthesis of 2-((1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(hydroxymethyl)-2H-pyran-3,4,5-trioli (5c)
The compound 5c was obtained in 66% yield as a yellow oil; \( \nu_{\text{max}} \) (CsI) (cm\(^{-1}\)): 3356, 2946, 2833, 1457, 1020; 1H NMR (300 MHz, D2O), δ (ppm): 7.84 (s, 1H, H-9), 7.15-7.03 (m, 5H, Ph), 4.95 (m, 1H, H-1), 4.22 (s, 2H, H-7), 3.69-3.39 (m, 8H, -CH2CH2CH2Ph, H-2, H-3, H-4, H-5, H-6, H-6'), 2.43 (m, 2H, -CH2CH2CH2Ph), 2.03 (m, 2H, -CH2CH2CH2Ph); 13C NMR (75 MHz, D2O), δ (ppm): 141.6 (C-8), 129.3-129.2 (Ph), 126.9 (C-9), 102.3 (C-1), 98.7 (C-5),
76.8 (C-3), 73.8 (C-2), 72.0 (C-4), 70.2 (C-7), 61.0 (C-6), 50.5 (−CH₂CH₂CH₂Ph), 32.6 (−CH₂CH₂CH₃Ph), 31.5 (−CH₂CH₂CH₂Ph); [α]D +61.1 (c 0.36, CH₃OH).

Synthesis of 2-(4-((3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetic acid (5d)
The compound 5d was obtained in 60% yield as a yellow oil; νmax (CsI) (cm⁻¹): 3508, 2926, 1732, 1645, 1614, 1450, 1016; ¹H NMR (300 MHz, D₂O), δ (ppm): 8.02 (s, 1H, H-9), 5.05-5.03 (m, 3H, H-1, -CH₂COOH), 3.73-3.17 (m, 6H, H-2, H-3, H-4, H-5, H-6, H-6’); ¹³C NMR (75 MHz, D₂O), δ (ppm): 175.8 (COOH), 126.9 (C-9), 102.3 (C-1), 98.6 (C-2), 76.7 (C-3), 73.7 (C-5), 70.9 (C-4), 62.6 (C-6), 61.1 (C-7), 53.8 (−CH₂COOH); [α]D +10.5 (c 0.57, CH₃OH).

Synthesis of 2-((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(hydroxymethyl)-2H-pyran-3,4,5-triol (5e)
The compound 5e was obtained in 64% yield as a yellow oil; [α]D +58.3 (c 0.72, CH₃OH). Their characterization are consistent with those described in literature²³.

Synthesis of 1-benzyl-4-((2,2,7,7-tetramethyl-3H-bis[1,3]dioxol-[4,5;4′,5′]pyran-3-yl)methoxy)methyl)-1H-1,2,3-triazole (6a)
The compound 6a was obtained in 68% yield as a yellow oil; νmax (CsI) (cm⁻¹): 2923, 2854, 1072; ¹H NMR (300 MHz, CDCl₃), δ (ppm): 7.36-7.18 (m, 6H, H-9, Ph), 5.42 (s, 2H, -CH₂Ph), 4.80 (m, 2H, H-3, H-4), 4.20 (m, 3H, H-5, H-7), 3.91 (m, 1H, H-6’), 3.79 (m, 1H, H-6), 3.54 (m, 2H, H-1, H-1’), 1.41-1.22 (4s, 12H, 4x CH₃); ¹³C NMR (75 MHz, CDCl₃), δ (ppm): 145.7 (C-8), 134.8 (C-9), 129.3-122.5 (Ph), 109.1, 108.6 (2x C iso), 102.7 (C-2), 74.0 (C-3), 72.1 (C-5), 71.1 (C-4), 70.1 (C-1), 65.7 (C-7), 61.2 (C-6), 54.3 (−CH₂Ph), 26.7-24.2 (4x CH₃); [α]D -114.8° (c 0.07, CHCl₃).

Synthesis of 1-phenylpropyl-4-((2,2,7,7-tetramethyl-3H-bis[1,3]dioxol-[4,5;4′,5′]pyran-3-yl)methoxy)methyl)-1H-1,2,3-triazole (6b)
The compound 6b was obtained in 80% yield as yellow oil; νmax (CsI) (cm⁻¹): 2987, 2935, 1674, 1080; ¹H NMR (300 MHz, CDCl₃), δ (ppm): 7.25-7.06 (m, 6H, H-9, Ph), 4.97 (d, 1H, H-3, JH₃-H₄= 12.0 Hz), 4.72 (dd, 1H, H-4, JH₄-H₅= 9.0 Hz, JH₄-H₃= 12.0 Hz), 4.55 (m, 4H, H-7, -CH₂CH₂Ph), 4.18 (d, 1H, H-5, JH₅-H₄= 9.0 Hz), 4.18 (d, 1H, H-1’, JH₁,H₁= 9.0 Hz), 4.09 (m, 1H, H-1), 3.75 (dd, 1H, H-6’, JH₆,H₆= 12.0 Hz, JH₆,H₆= 9.0 Hz), 3.58 (d, 1H, H-6, JH₆,H₆= 3.0 Hz), 3.17 (t, 2H, -CH₂CH₂Ph), J= 9.0 Hz, J = 6.0 Hz), 1.50-1.29 (5s, 12H, 4x CH₃); ¹³C NMR (75 MHz, CDCl₃), δ (ppm): 145.7 (C-8), 137.5 (C-9), 129.4-123.3 (Ph), 74.4 (C-3), 72.3 (C-5), 71.6 (C-4), 70.7 (C-1), 65.9 (C-7), 60.8 (C-6), 52.2 (−CH₂CH₂Ph), 37.3 (−CH₂CH₂Ph), 28.8-24.7 (4x CH₃); [α]D -53.2° (c 0.60, CHCl₃).

Synthesis of 1-(3-phenylpropyl)-4-((2,2,7,7-tetramethyl-3H-bis[1,3]dioxol-[4,5;4′,5′]pyran-3-yl)methoxy)methyl)-1H-1,2,3-triazole (6c)
The compound 6c was obtained in 80% yield as yellow oil; νmax (CsI) (cm⁻¹): 2987, 2935, 1674, 1080; ¹H NMR (300 MHz, CDCl₃), δ (ppm): 7.65 (s, 1H, H-9), 7.36-7.21 (m, 5H, Ph), 4.90-4.82 (m, 1H, H-3), 4.64 (dd, 1H, H-4, JH₄-H₅= 3.0 Hz, JH₃-H₄= 12.0 Hz), 4.43-4.39 (m, 3H, -CH₂CH₂CH₂PH), JH₇= 4.28 (d, 1H, H-5, JH₅-H₄= 6.0 Hz), 4.17-4.09 (m, 2H, H-1’, H-7), 3.96 (dd, 1H, H-6’, JH₆,H₆= 12.0 Hz, JH₆,H₆= 3.0 Hz), 3.79 (d, 1H, H-6, JH₆,H₆= 12.0 Hz), 3.73-3.64 (m, 1H, H-1), 2.70 (t, 2H, -CH₂CH₂CH₂Ph), 2.31 (q, 2H, -CH₂CH₂CH₂Ph), 1.62-1.37 (7s, 12H, 4x CH₃); ¹³C NMR (75 MHz, CDCl₃), δ (ppm): 140.2 (C-8), 128.8-126.5 (Ph), 109.0 (C iso), 102.7 (C-2), 74.0 (C-3), 72.1 (C-5), 70.3 (C-4), 65.6 (C-1), 61.2 (C-7), 60.4 (C-6), 49.7 (−CH₂CH₂CH₂Ph), 32.6 (−CH₂CH₂CH₂Ph), 31.8 (−CH₂CH₂CH₂Ph), 28.3-24.2 (4x CH₃); [α]D -111.1° (c 0.12, CHCl₃).
Synthesis of 2-(4-(((2,2,7,7-tetramethyl-3H-bis[1,3]dioxolono[4,5:4’,5’]pyran-3-yl) methoxy)methyl)-1H-1,2,3-triazol-1-yl)acetic acid (6d)

The compound 6d was obtained in 77% yield as a brown oil; $\nu_{max}$ (CsI) (cm$^{-1}$): 3398, 2935, 1750, 1080; $^1$H NMR (300 MHz, CDCl$_3$), $\delta$ (ppm): 7.68 (s, 1H, H-9), 5.12 (s, 2H, -CH$_2$-COOH), 4.80 (m, 1H, H-3), 4.34-4.20 (m, 2H, H-7), 4.04 (m, 2H, H-4, H-5), 3.85 (m, 1H, H-6’), 3.65-3.56 (m, 1H, H-6), 3.45 (s, 2H, H-1 e H-1’), 1.54, 1.46 (2s, 6H, 2x CH$_3$), 1.35 (s, 6H, 2x CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$), $\delta$ (ppm): 168.6 (COOH), 145.6 (C-9), 124.3 (C-8), 109.3, 112.2 (C$_{isop}$), 102.7 (C-2), 76.8 (C-3), 74.0 (C-5), 71.9 (C-4), 70.3 (C-1), 65.1 (C-7), 60.4 (C-6), 51.3 (-CH$_2$-COOH), 24.2-28.3 (4x CH$_3$).

Synthesis of 3-(4-(((2,2,7,7-tetramethyl-3H-bis[1,3]dioxolono[4,5:4’,5’]pyran-3-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (6e)

The compound 6e was obtained in 55% yield as yellow oil; $\nu_{max}$ (CsI) (cm$^{-1}$): 3398, 2987, 2935, 1637, 1080, 1078. $^1$H NMR (300 MHz, CDCl$_3$), $\delta$ (ppm): 7.64 (s, 1H, H-9), 5.03 (d, 1H, H-3, $J_{H,3-H,4}=12.0$ Hz), 4.75 (m, 1H, H-4), 4.45 (m, 3H, H-7, -CH$_2$CH$_2$CH$_2$OH), 4.33 (s, 1H, H-7), 4.21 (d, 1H, H-5, $J_{H,5-H,4}=6.0$ Hz), 4.03 (m, 1H, H-1’), 3.70 (m, 2H, H-6 e H-6’), 3.60 (m, 3H, H-1’-CH$_2$CH$_2$CH$_2$OH), 2.10 (q, 2H, -CH$_2$CH$_2$CH$_2$OH), 1.54-1.31 (6s, 12H, 4x CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$), $\delta$ (ppm): 123.2 (C-9), 112.0 (C$_{isop}$), 105.8 (C-2), 72.0 (C-3), 70.9 (C-5), 70.0 (C-4), 65.7 (C-7), 63.9 (C-1), 61.2 (C-6), 58.7 (-CH$_2$CH$_2$CH$_2$OH), 41.1 (-CH$_2$CH$_2$CH$_2$OH), 32.7 (-CH$_2$CH$_2$CH$_2$OH), 24.2-29.9 (4x CH$_3$); $[\alpha]_{D}$ -50.0º (c 0.24, CHCl$_3$).

Anti-M. tuberculosis (MTB) activity assay:

The anti-MTB activity of the compounds was determined by the Resazurin Microtiter Assay (REMA)\textsuperscript{24}. Stock solutions of the test compounds were prepared in dimethyl sulfoxide (DMSO) and diluted in Middlebrook 7H9 broth (Difco), supplemented with oleic acid, albumin, dextrose and catalase (OADC enrichment - BBL/Becton Dickinson, Sparks, MD, USA), to obtain final drug concentration ranges from 0.15 to 250 µg/mL. The serial dilutions were realized in Precision XS Microplate Sample Processor (Biotektm). The isoniazid was dissolved in distilled water, according to the manufacturers recommendations (Difco laboratories, Detroit, MI, USA), and used as a standard drug. MTB H$_3$Rv ATCC 27294 was grown for 7 to 10 days in Middlebrook 7H9 broth supplemented with OADC, plus 0.05% Tween 80 to avoid clumps. Cultures were centrifuged for 15 min at 3,150 x g, washed twice and resuspended in phosphate-buffered saline and aliquots were frozen at -80°C. After 2 days the number of CFU was determined. MTB H$_3$Rv (ATCC 27294) was thawed and added together with the test compounds, yielding a final testing volume of 200 µL with 2x10$^4$ CFU/mL. Microplates were incubated for 7 days at 37°C, after which resazurin was added for the reading. Wells that turned from blue to pink, with the development of fluorescence, indicated growth of bacterial cells while maintenance of the blue colour indicated bacterial inhibition\textsuperscript{24}. The fluorescence was read (530 nm excitation filter and 590 nm emission filter) in a SPECTRAfluor Plus (Tecan\textsuperscript{®}) microfluorimeter. The MIC was defined as the lowest concentration resulting in 90% inhibition of growth of MTB\textsuperscript{24}. As a standard test, the MIC of isoniazid was determined on each microplate. The acceptable range of isoniazid MIC is from 0.015 to 0.06 µg/mL\textsuperscript{12,24}. Each test was set up in triplicate.

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