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Synthesis of carbohydrate-substituted isoxazoles and evaluation of their antitubercular activity

DOI 10.1515/hc-2016-0185
Received October 25, 2016; accepted May 3, 2017; previously published online June 14, 2017

Abstract: Eight new sugar-substituted isoxazoles were synthesized by a 1,3-dipolar cycloaddition reaction of aromatic nitrile oxides with carbohydrate-substituted alkynes. Products were screened for antimycobacterial activity against the Mycobacterium tuberculosis H37Rv strain. Four compounds, 5e–h, significantly inhibit growth of the bacterial strain with a minimum inhibitory concentration (MIC) of 3.125 μg/mL.

Keywords: aromatic nitrile oxides; 1,3-dipolar cycloaddition; isoxazole; Mycobacterium tuberculosis; propargyl O-glycoside.

Introduction

1,3-Dipolar cycloaddition is one of the most useful reactions for the synthesis of heterocyclic compounds [1]. Over the years, nitrile oxides have become important building blocks in organic synthesis. 1,3-Dipolar reactions of alkynes with nitrile oxides have been used to prepare isoxazoles [2]. Isoxazole derivatives represent an important class of biologically active heterocycles in drug discovery. They are clinically effective as antimicrobial, antibacterial, anticonvulsant, anticancer, antifungal, antiviral and antitubercular agents [3–12]. The synthesis of artificial enzymes containing isoxazole moieties is a dynamic area of research [13, 14]. Moreover, isoxazole derivatives have found applications as dyes, corrosion inhibitors and agrochemicals [15]. Many isoxazole derivatives have been synthesized to date [16–18]. Previously, we developed an efficient method for the synthesis of enantiopure isoxazoline derivatives based on the [3 + 2] cycloaddition between aromatic nitrile oxides and pyrrol-2-ones [19, 20]. In this paper, we report that chiral isoxazoles can be obtained by the [3 + 2] cycloaddition of aromatic nitrile oxides with propargyl O-glycoside derivatives, thereby providing a new synthetic route to carbohydrate-isoxazole conjugates.

Results and discussion

The propargyl O-glycosides 3a,b were synthesized beginning with alcohols, 1 (Scheme 1). Etherification of 1 using propargyl bromide in the presence of sodium hydride at 0°C generated the corresponding ether in a good yield [21]. The synthetic method to obtain the target isoxazoles 5a–h is shown in Scheme 2. The 1,3-dipolar cycloaddition of the propargyl O-glycoside derivatives 3a,b with aromatic nitrile oxides 4a–d at 110°C in toluene for 2 h furnished compounds 5a–h. The 1,3-dipolar cycloaddition between the propargyl O-glycoside derivatives 3 and aromatic nitrile oxides 4 gave cycloadducts 5 as single regioisomers [22]. The structures of the new isoxazoles 5a–h were established from their elemental analyses using Fourier transform infrared (FT-IR) and 1H nuclear magnetic resonance (NMR) and 13C NMR spectra. The IR spectra of all isoxazoles have a peak at 1635–1645 cm⁻¹, which is related to the C=N stretching of the isoxazole ring.

The structures of new isoxazoles 5a–h were by analysis of the 1H NMR and 13C NMR spectra. The relevant data are presented under the Experimental section. In particular, the 1H NMR spectrum of 5a shows that the sugar ring is strongly distorted from the regular conformation because of the presence of two fused five-membered isopropyldiene rings. Thus, unusual coupling constant values of 2.4 and 7.8 Hz are observed between trans (H-8a, H-8b) and cis (H-8a, H-5a)-coupled protons, respectively. In the heteronuclear multiple quantum coherence spectrum of 5a, the apparent singlet at 6.55 ppm correlates with the carbon C4′ (101.1 ppm), whereas it does not correlate with the carbon C5′ at 161.1 ppm. In the heteronuclear multiple
bond correlation (HMBC), the atom $H_{4}'$ is correlated with $C_{3}'$ and $C$-aromatic (Figure 1).

All the products 5a–h were screened against *Mycobacterium tuberculosis H37RV* (ATCC27294) using agar dilution method [23]. Their antimycobacterial activity was evaluated in terms of minimum inhibitory concentration (MIC) values. Four compounds, 5e–h, are potent antitubercular agents with an MIC of 3.125 μg/mL. The MIC of those compounds is comparable with the MIC value of the standard drug, ethambutol. Compounds 5a–d show a moderate inhibitory activity with MIC of 12.5 μg/mL.

**Conclusion**

New sugar-isoxazole conjugates were obtained regio- and stereoselectively by the [3+2] cycloaddition of aromatic nitrile oxides with propargyl $O$-glycoside derivatives. The antimycobacterial activity of these compounds is
significant; hence, they could potentially serve as antitubercular agents.

**Experimental**

IR spectra were recorded in KBr pellets on a Perkin-Elmer IR-197 spectrometer. Mass spectra were recorded in the electrospray ionization (ESI) mode. NMR spectra were obtained in CDCl₃ on a Bruker AC 300 spectrometer operating at 300 MHz for ¹H and at 75 MHz for ¹³C. Melting points were recorded in open capillaries and are uncorrected. Optical rotations were measured at 589 nm. Thin-layer chromatography (TLC) was performed on precoated plates (0.2 mm, silica gel 60 F254). All solvents were distilled and purified as necessary.

**General procedure for the synthesis of propargyl \( \text{O-glycosides 3a,b} \)**

NaH (29 mg, 1.2 mmol) was added to a solution of protected sugar 2 (300 mg, 1.15 mmol) in dry DMF (10 mL) at 0°C. The mixture was stirred for 20 min and then treated slowly with propargyl bromide (300 mg, 1.15 mmol) in dry DMF (10 mL) at 0°C. The mixture was stirred for 20 min and then treated slowly with propargyl bromide. Then, a solution of triethylamine (0.39 mmol) in toluene (20 mL) was added dropwise. The resultant precipitate of triethylamine chloride was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was subjected to a silica gel column chromatography eluting with hexanes/EtOAc (3:1) to afford compounds 5a–h.

**(3AR, 8bR, 8aS, 5aS, 5R)-3'-[4-Methoxypheny1]-5-(2,2,7,7-tetra- methylytetrahydro-bis[1,3]dioxolo[4,5-b:4,5'-d']pyran-5-ylmethoxy)isoxazole (5a)** Yellow oil; [α]D+ = 55 (c 1, CHCl₃), [α]D+ = 0.15 (cyclohexane/AcOEt, 2:8); yield 72%; IR: \( \nu_{\text{max}} \) 3300 cm⁻¹; ¹H NMR: \( \delta \) 7.95–7.45 (AA′BB′, \( J_{\text{AA}} = 6.5 \) Hz, 4H, Harom), 4.61 (d, \( J_{\text{HH}} = 2.1 \) Hz, 1H, H₅), 4.10 (m, 2H, H₆), 3.95 (m, 2H, H₂), 3.67 (m, 1H, H₃), 2.07 (t, \( J_{\text{HH}} = 2.1 \) Hz, 2H, H₄), 1.40, 1.38, 1.31, 1.26 (s, 3H, CH₃); ¹³C NMR: \( \delta \) 121.1, 108.6, 104.3, 84.4, 80.0, 78.9, 74.2, 72.4, 66.2, 53.5, 26.3, 25.4, 23.4, HRMS. Calcd for C₂₃H₂₉NO₈: m/z 495.0890. Anal. Calcd for C₂₃H₂₉NO₈: C, 57.14, H, 5.67, N, 6.06. Found: C, 57.17, H, 5.64, N, 6.02.

**General procedure for synthesis of isoxazoles 5a–h**

A mixture of alkyne 3a,b (0.33 mmol) and chloroxime 4a–d (0.63 mmol) was dissolved in 10 mL of toluene and the mixture was stirred at 110°C. Then, a solution of triethylamine (0.39 mmol) in toluene (10 mL) was added dropwise. The resultant precipitate of triethylammonium chloride was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was subjected to a silica gel column chromatography eluting with hexanes/EtOAc (3:1) to afford compound 5a–h.
Antituberular studies

A standard methodology recommended by the National Committee for Clinical Laboratory Standards, USA, for the determination of MIC was used. The assays were conducted in triplicate.

References


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