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Synthesis and antimicrobial activity of 6-triazolo-6-deoxy eugenol glucosides

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ABSTRACT

A new series of 1,2,3-triazole eugenol glucosides were synthesized. The new compound structures were confirmed by MS, ^{1}H NMR and ^{13}C NMR. All of the synthesized compounds were screened for antimicrobial and cytotoxic activity. Five compounds exerted significant activity against the Gram-negative bacteria *Salmonella typhimurium* with low IC_{50} values (49.73–68.53 μM), and seven compounds were active against the Gram-positive bacteria *Micrococcus luteus* (42.89–210.94 μM). In vitro cytotoxicity on mouse spleen cells was also evaluated. One compound bearing a phenyl substituent at the triazole ring showed good activity against *Salmonella typhimurium* (49.73 μM) and low toxicity to normal cells (CC_{50}=157.83 μM). Thus, the compounds herein can be considered for further modification for improving their antibacterial activity or obtaining novel antibacterial drug candidates.

1. Introduction

Microbial resistance to antibiotics has become a serious threat to human health.[1] Since the development of sulfonamides and penicillin, many new classes of antibacterial compounds have been developed. Most antibiotics used today were discovered approximately 70 years ago, and newer versions have mainly been generated by chemical modifications based on these compounds. However, only two new classes of antibiotic (daptomycin and linezolid) have been discovered and approved for use since the early 1960s. The low level of new antibiotic class discovery and the emergence of antibiotic resistance in several microorganisms have a significant impact on patient management.[2]

The incidence of fungal infections has increased, and has been associated with high mortality in immunocompromised patients.[3] Azole antifungals have emerged as vanguard drugs for the treatment and prophylaxis of many systemic mycoses. Fluconazole was first introduced and serves an excellent drug for treating superficial and invasive fungal infections. The other triazoles available for clinical use include itraconazole, voriconazole and posaconazole.[4] The mechanism of action of azoles is based on fungal cytochrome P450 14-α-sterol demethylase and 24-methylene dihydrolanosterol demethylase inhibition, which are key enzymes of ergosterol biosynthesis.[5]

The ideal antimicrobial agent should exhibit a high biological activity (fungicidal or bactericidal activity), reluctance to induce resistance, broad spectrum of action and low toxicity. None of the current clinical antimicrobial agents possess all of these requirements. Therefore, the discovery of new, more effective, less toxic and safe antimicrobial agents with novel mechanisms of action is urgently required.[6]

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Recently, we described the synthesis and antifungal evaluation of eugenol glycosides.[7] Eugenol is a phenolic compound found in Indian clove, basil and cinnamon, which are widely used as flavoring agents and preservatives in food, beverages and cosmetics. Eugenol is a widespread antiseptic and anesthetic used in dentistry[8] and possesses many pharmacological activities, such as antifungal, antibacterial[9] and anti-inflammatory.[10] We observed that the peracetylated eugenol glucoside (Fig. 1) was the most potent of the synthesized compounds (IC_{50}: 3.8 μM against *Candida glabrata*). As result of our continuous search for new antifungal agents, in particular, glycosides, we describe here new eugenol glucosides with a triazole group at the sugar C6 position.

The most reactive sites of a monosaccharide, such as glucose, are the C1 and C6 positions. The anomeric position is substituted in the glycoside form; thus, the C6 position is more accessible for modification, as it differs from the other positions because it is a primary hydroxyl. The 1,2,3-triazole ring can be rapidly and selectively synthesized from alkyne and azide compounds,[11] and its introduction can enhance eugenol glycoside antimicrobial activity, improve water solubility due to its hydrophility and increase its basicity.[12] In this context, we take advantage of active eugenol peracetyl glucoside (**1**) and the remarkable antifungal activity of triazole groups and describe the synthesis of new triazole eugenol glucoside derivatives and evaluate their antifungal, antibacterial and cytotoxicity activities.

2. Results and discussion

2.1. Chemistry

Glucoside **2** was synthesized following a previously reported protocol,[7] as shown in Scheme 1. Briefly, this synthesis was accomplished by glycosylating eugenol with peracetylglucosyl bromide in an alkaline acetone/water solution, followed by the deacetylation of peracetylglucoside in a methoxide/methanol solution, an adaptation of Zemplén's transesterification method that originally employs sodium/methanol.[13]

1,2,3-Triazole derivatives **11**–**15** were prepared following a seven-step synthetic route and are described here for the first time (Scheme 2); their synthetic pathway initially employed the selective tosylation of the less hindered, primary 6-hydroxyl group of glucoside **2**. As this hydroxyl group is more reactive than the others, monotosyl derivative **3** was expected to be the first and major product, which is in fact what was observed. Thus, to avoid tosylating other positions, the reaction was stopped as soon as TLC analysis indicated product formation. Derivative **3** was obtained in high yield as a colorless oil after column chromatography. Analyses of the ^{1}H and ^{13}C NMR spectra showed a pair of doublets at δ 7.70 and 7.17 ppm, typical of a *para*-disubstituted tosyl group. The remaining hydroxyl groups of the saccharide unit of derivative **3** were then acetylated. According to the literature,[14] the formation of 3,6-anhydro derivatives is quite common when working with unprotected glycosides with a good leaving group at C6, especially in reactions under heating, as would be expected in the tosyl-azide conversion step. Thus, we protected the hydroxyl groups with an acetyl group to prevent this undesired nucleophilic displacement and also to obtain peracetylated analogues of the final products for a biological activity comparison. Derivative **4** was obtained in an 82% yield as a pure, white and crystalline solid after reaction with **3** and acetic anhydride in pyridine. Treatment of **4** with sodium azide in dimethylformamide at 90 °C smoothly afforded azide derivative **5** in 90% yield. This compound is a key intermediate for 1,2,3-triazole synthesis, and its identity was confirmed by a sharp IR stretching band at 2100 cm^{-1} and a typical methylene carbon-azide chemical shift at δ 51.1 ppm. The click reaction of **5** with different alkynes afforded peracetylated triazole glucosides **6**–**10**. This method, 1,3-dipolar cycloaddition between alkynes and alkyl azides in the presence of copper acetate and sodium ascorbate, generating the reactive catalyst Cu(I) in situ, has been widely used to obtain 1,2,3-triazole derivatives.[15,16] Click reactions were conducted under room temperature by stirring reagents in a THF/water mixture, and after 1 h, all reactions were complete, as evidenced by TLC analyses. The five 1,2,3-triazole derivatives were obtained in good yields and high purity after column chromatography. Compounds **6**–**10** were then deacetylated by being stirred in a potassium hydroxide/methanol solution for 30 min, affording 1,2,3-triazoles **11**–**15**. Acetylated and deacetylated 1,2,3-triazole derivatives were characterized by infrared, ^{1}H and ^{13}C NMR spectroscopies and high-resolution mass spectrometry. In the ^{13}C NMR spectra of the peracetylated compounds, carbonyl peaks were recorded in the δ 170.0–169.2 ppm range, and these signals were not observed for corresponding deacetylated derivatives. The typical H-5′ singlet of the 1,2,3-triazole ring was observed in the δ 8.31–7.49 ppm range and in the C4′ and C5′ peaks between δ 150–139 ppm and δ 129–121 ppm in the NMR spectra of all triazole derivatives, confirming heterocycle formation.[17] The different substituents on the C4′ position of the triazole ring also showed consistent IR and NMR signs for all compounds.

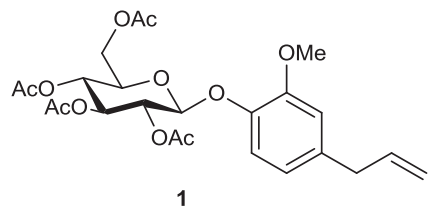

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Fig. 1. Chemical strucutre of active peracetyl glucoside.

2.2. In vitro assays

All of the synthesized 1,2,3-triazoles and synthetic intermediate compounds were inactive against the evaluated fungi species: *Candida albicans* (*C. albicans*), *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. glabrata*. The results were estimated using the inhibitory concentration that was able to inhibit microbial growth at 50% (IC_{50}). These assays were performed in duplicate, and the results obtained from the replicates were comparable.

However, when the antibacterial activity against different bacteria species was evaluated, some of the synthesized compounds showed bacteriostatic action. The results indicate that all of the synthesized compounds, with the exception of derivative **6**, showed bacteriostatic activity for one species. Optimal results were observed against *Micrococcus luteus* (*M. luteus*) and *Salmonella typhimurium* (*S. typhimurium*) (Table 1).

M. luteus is part of the normal flora of the skin or microbiota of mammals. This bacterium is also found in various environments, such as soil, air and water, and is resistant to severe environmental conditions, including a high salt concentration and dry substrates. *M. luteus* is not considered to be the general cause of bacterial infections in humans; however, several reports have shown that endocarditis induced by this strain can result in death.[18] Furthermore, this bacterium is a source of nosocomial infections in immunocompromised individuals and can cause complications in pre-existing infections, such as meningitis, pneumonia and urinary tract infections. *M. luteus* can also become resistance to antibiotics, which is caused by the presence of extrachromosomal genetic elements. In some cases, *M. luteus* has exhibited resistance to multiple drugs.[19]