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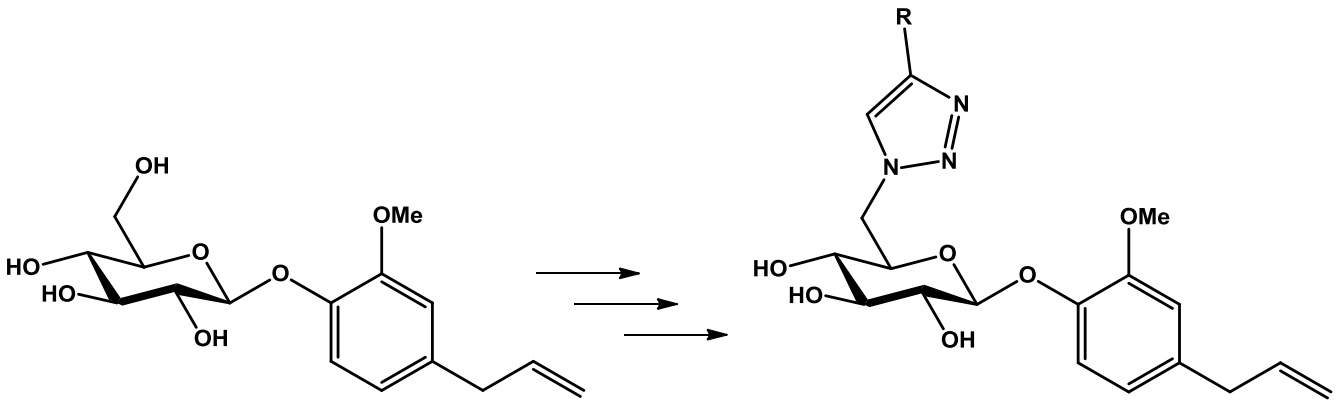

2

11: R= CH_2OH
12: R= C_6H_5
13: R= CH_2Cl
14: R= $COOCH_2CH_3$
15: R= C_6H_{11}

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

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Highlights

- First time synthesis of eugenol glucosides with different triazole moieties
- Impressive antimicrobial activities against *Salmonella typhimurium* and *Micrococcus luteus*
- Low cytotoxicity on normal host cells

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.

Keywords: eugenol glucosides; triazoles; antimicrobial activity

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]

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 (Figure 1) was the most potent of the synthesized compounds (IC_{50}: 3.8μM 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.

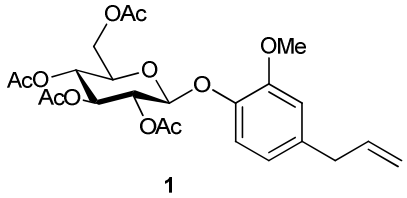

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Figure 1 Chemical strucutre of active peracetyl glucoside

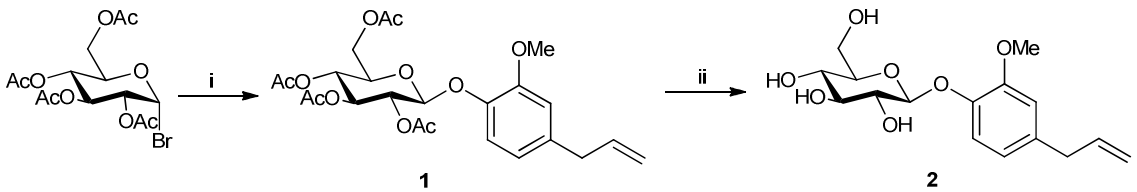
The most reactive sites of a monosaccharide, such as glucose, are the C1 and C6 positions. The anomeric position is substituted in the glycoside form; thus, the C6 position is more accessible for modification, as it differs from the other positions because it is a primary hydroxyl. The 1,2,3-triazole 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]

Scheme 1 Synthesis of eugenol glucoside **1**



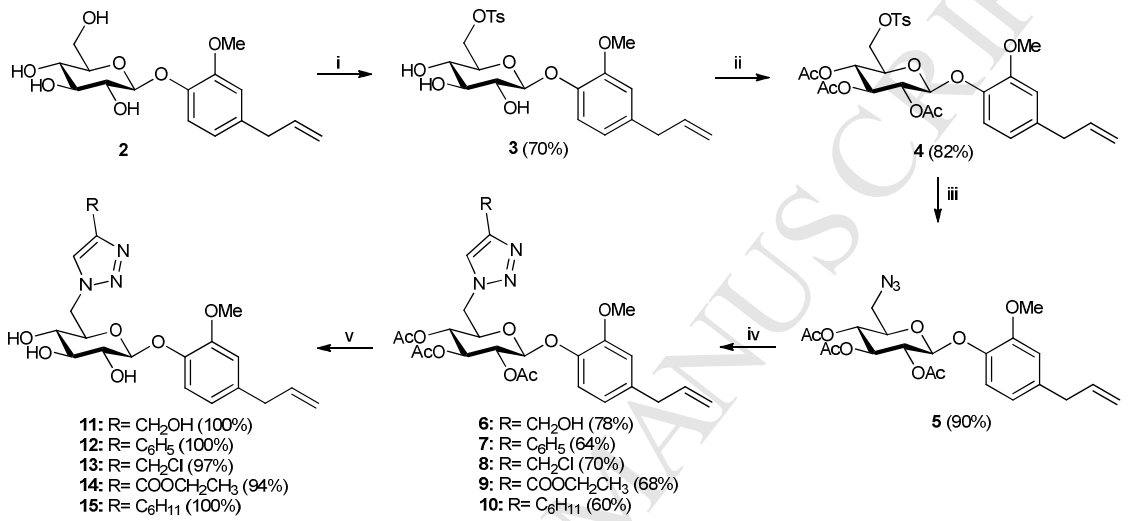
1 2

i: eugenol, LiOH, water, acetone, r.t.; ii: MeOH, KOH, 0°C

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.

Scheme 2 Synthesis of 1,2,3-triazoles



i: TsCl, pyridine, 0°C; ii: Ac_2O, pyridine, r.t.; iii: NaN_3, *N*,*N*-dimethylformamide, 80°C; iv: corresponding alkyne, sodium ascorbate, copper acetate, THF/H_2O, r.t.; v: MeOH, KOH, 0°C.

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*).

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]

Salmonella sp. are pathogenic bacteria that commonly cause foodborne infections. *Salmonella* sp. have demonstrated significant resistance to antibiotics. In addition, treatments that increase the susceptibility of immunocompromised patients to foodborne infections exist. According to the literature,[20] systemic infection by *S. typhimurium* has been described after tetracycline, ampicillin, oxacillin and gentamicin use. Cutaneous salmonellosis has also been described in compromised patients after sulfonamide, tetracycline, ampicillin and chloramphenicol use, with worsening clinical outcomes.[20]

Derivatives **3, 4, 5, 8, 11, 13** and **14** were active only against *M. luteus*, while derivatives **7**, **9, 10, 12** and **15** showed bacteriostatic activity only against *S. typhimurium*. Structure analyses suggest that bulky aromatic (phenyl) and aliphatic (cyclohexyl) groups linked to the triazole ring favor antimicrobial action in gram-negative strains. An exception to this trend is compound **9**, which bears an acetate group connected to the triazole ring but has a peracetylated glucoside ring, different from its derivative glucoside **14**, which exhibits bacteriostatic activity only against *M. luteus* (IC_{50} of 141.21 μM). Among the compounds that showed antimicrobial activity against *S. typhimurium*, derived phenyl-containing compound **7** presented the best antibacterial activity profile (IC_{50} of 49.73 μM).

Concerning *M. luteus*, peracetylated derivative **4**, which has a tosylate group in its structure, was the most active (IC_{50} of 42.89 μM). Azide derivative **5** was two-fold less potent than tosylate derivative **4**. Among the triazole derivatives, in general, only deprotected glucosides were active against *M. luteus*, except in case of compound **8**. This finding might indicate that hydrophilic triazoles enhance activity against this

bacterium. Moreover, compounds bearing bulky phenyl and cyclohexyl substituents were inactive, most likely due to steric effects.

Compounds containing hydroxymethyl (**11**) and chloromethyl (**8, 13**) groups connected to the triazole ring, which is considered a pharmacophoric group, presented bacteriostatic action against *M. luteus*.

According to our results, peracetylation of glucoside rings does not generate a significant effect on the selectivity and activity of compounds against the species of tested bacteria. Furthermore, the new series did not demonstrate significant activity against *Escherichia coli*, *Enterococcus feacalis* and *Staphylococcus aureus*.

Finally, the starting compounds (**1** and **2**) were inactive against all of the bacterial species evaluated, suggesting that the 1,2,3-triazole group is essential for the observed bacteriostatic activity of the eugenol glucosides synthesized. Further modifications of the ring substituents may lead to the continued discovery of new agents with antimicrobial activity.

The cytotoxic activities of all of the compounds were tested in spleen cells obtained from Swiss-Webster mice. Compounds **3**, **4**, **8**, **13** and **15** exhibited high toxicity in spleen cells, with CC_{50} values ranging from 64.28 to 67.93 μM, and **5**, **7**, **9**, **10**, **11**, **12** and **14** were less toxic, demonstrating CC_{50} values ranging from 118.89 to 234.94 μM (Table 2). The more toxic compounds carry a leaving group in their structures (**3** and **4** possess a tosyl group, and **8** and **13** have a chloromethylene group), except for derivative **15**. The less toxic compounds carry a 1,2,3-triazole group in their structures, except for derivative **5**, which possess an azide group in place of a triazole ring.

The selectivity indices of compounds **7**, **9**, **10** and **12** (especially **7**) suggest their potential as antibacterial agents to treat *Salmonella* sp. infections. These values also indicated a more discrete potential of the synthesized molecules herein to treat *M. luteus* infections. Among the seven compounds with antibacterial activity only against *M. luteus*, molecules **4**, **11** and **14** have selective indices of ≥ 1 (Table 2).

Table 1 *In vitro* antibacterial activity (μM) of the synthesized compounds.

Compound	*Salmonella typhimurium* ATCC 14028		*Escherichia coli* ATCC 25922		*Micrococcus luteus* ATCC 10240		*Enterococcus faecalis* ATCC 51299		*Staphylococcus aureus* ATCC 6538	
	IC_{50}	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**
1	-[a]	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	95.67	-	-	-	-	-
4	-	-	-	-	42.89	-	-	-	-	-
5	-	-	-	-	132.92	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	49.73	-	-	-	-	-	-	-	-	-
8	-	-	-	-	88.54	-	-	-	-	-
9	54.58	-	-	-	-	-	-	-	-	-
10	53.39	-	-	-	-	-	-	-	-	-
11	-	-	-	-	210.94	-	-	-	-	-
12	65.93	-	-	-	-	-	-	-	-	-
13	-	-	-	-	104.76	-	-	-	-	-
14	-	-	-	-	144.12	-	-	-	-	-
15	68.53	-	-	-	-	-	-	-	-	-
Chl	2.72	5.04	2.25	5.07	1.11	2.10	5.50	9.59	6.12	9.22

[a] No significant activity

Chl: Chloramphenicol

Table 2 Cytotoxic activity and selectivity index for the antibacterial compounds on tested microorganisms

Compound	CC_{50} (μM)	CC_{90} (μM)	Selectivity index	
			Salmonella typhimurium ATCC 14028	*Micrococcus luteus* ATCC 10240
1	ND	ND	ND	ND
2	ND	ND	ND	ND
3	60.93	122.17	ND	0.64
4	67.93	125.91	ND	1.58
5	112.68	319.33	ND	0.85
6	ND	ND	ND	ND
7	157.83	350.05	3.17	ND
8	63.77	132.88	ND	0.72
9	101.11	228.80	1.85	ND
10	108.39	237.60	2.03	ND
11	234.94	448.22	ND	1.11
12	111.89	213.72	1.70	ND
13	64.28	119.42	ND	0.61
14	166.39	336.65	ND	1.15
15	65.54	148.93	0.96	ND

CC_{50}: cytotoxic concentration for 50% of cells
CC_{90}: cytotoxic concentration for 90% of cells
ND: not done
Selectivity indexes: CC_{50}/IC_{50}.

3 Conclusion

This work described the synthesis and antimicrobial activity of a series of 1,2,3-triazole eugenol glucosides. This series was prepared in five steps from known eugenol glucoside in good overall yields. Several compounds exhibited relevant activity against *Salmonella typhimurium* and *Micrococcus luteus*. Compounds **7**, **9**, **10**, **12** and **15** were active against *Salmonella typhimurium*, while **3**, **4**, **5**, **8**, **11**, **13** and **14** demonstrated bacteriostatic activity against *Micrococcus luteus*. Structure-activity relationship studies showed that the nature of the triazole substituent is important for the observed activity. Concerning *Salmonella typhimurium*, the introduction of a bulky group (phenyl or cyclohexyl) is generally favorable. However, small substituents (CH_2Cl, CH_2OH and CO_2Et) seem to enhance the activity against *Micrococcus luteus*. Apparently, glucoside ring peracetylation does not generate a significant effect on the selectivity and activity of compounds against the types of strains evaluated. Considering potency and selectivity, we consider compound **7** to be a good candidate for improving antimicrobial activity in future studies.

4 Experimental

4.1 Physical measurements

Melting point of the compounds was determined on Microquímica MOAs 301 apparatus and was uncorrected. IR spectroscopy was performed by Shimadzu a FTIR-Affinity-1 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker AC-300 spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C spectra) in deuterated chloroform, methanol or dimethylsulfoxide. Chemical shifts (δ) were reported in parts per million (ppm) with reference to tetramethylsilane (TMS) as internal standard and coupling constants (J) were reported in Hertz (Hz). The following abbreviations were used for the ^{1}H multiplicities: singlet (s), doublet (d), triplet (t), quartet (qr), quintet (qt), double doublet (dd), triple doublet (td), multiplet (m) and broad signal (br s). The specific optical rotation $[\alpha]_D$ were measured on Perkin Elmer 341 polarimeter, at 20°C. High resolution mass spectra were acquired using a LCMS-IT-TOF mass spectrometer and the samples were solubilized in MeOH + 0.1% formic acid, following manual injection. Reaction courses and product mixtures were monitored by thin-layer chromatography (TLC) on commercial silica gel 60 plates. For chromatography, column grade silica gel (0.040–0.063 mm mesh size) was employed.

4.2 Synthesis of the compounds

4.2.1 Synthesis of 4-allyl-2-methoxyphenyl-(6-*O*-toluenesulfonyl-β-D-glucopyranoside) (3)

To a solution of **2** (1.53 mmol) in pyridine (2.5 mL) was added a solution of tosyl chloride (3.84 mmol) in pyridine (2.5 mL) at 0° C, and the reaction was stirred at this temperature for 3 hours and monitored by TLC. 100 mL of ethyl acetate was added to the mixture, which was washed with 4M HCl (3x30mL) and water until pH 7. Organic phase was dried with Na_2SO_4 and concentrated, providing a colorless oily crude product (70% yield) which was purified by chromatography (dichloromethane/methanol 98:2); $[\alpha]_D$ -44º (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3373, 2914, 1594, 1508, 1448, 1172. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.70 (2H, d, J_3 = 7.8 Hz, Ar-H), 7.17 (2H, d, J_3 = 7.8, Ar-H), 6.87 (1H, d, J_3 = 7.6 Hz, Ar-H), 6.67 (1H, s, Ar-H),

6.61 (1H, d, J_3 = 7.8 Hz, Ar-H), 5.98-5.84 (1H, m, allylic), 5.10-5.04 (2H, m, allylic), 4,64 (1H, br s, sugar), 4,31-4.19 (7H, m, sugar, OH), 3.74 (3H, s, OCH_3), 3.57-3.51 (2H, br s, sugar, OH), 3.30 (2H, d, J_3 = 6.1 Hz, allylic), 2.32 (3H, s, $C_6H_5C\underline{H_3}$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 149.8 (1C, ar.), 145.2 (1C, ar.), 144.7 (1C, ar.), 137.7 (1C, allylic), 136.2 (1C, ar.), 133.0 (1C, ar.), 130.2 (2C, ar.), 128.4 (2C, ar.), 121.4 (1C, ar.), 118.8 (1C, ar.), 116.4 (1C, allylic), 112.9 (1C, ar.), 102,4 (1C, sugar), 76.3 (1C, sugar), 74.04 (1C, sugar), 73.5 (1C, sugar), 69.9 (1C, sugar), 69.4 (1C, sugar), 56.4 (1C, OCH_3), 40.3 (1C, allylic), 22.0 (1C, $C_6H_5\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{23}H_{28}O_9S$ $(M+Na)^+$: 503.1346; found: 503.1278.

4.2.2 Synthesis of 4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-*O*-toluenesulfonyl-β-D-glucopyranoside) (4)

To a solution of **3** (0.31 mmol) in pyridine (5 mL) was added dropwise acetic anhydride (3.1 mmol) and the reaction was monitored by TLC. After 2 hours, 10 mL of water and concentrated HCl was added to the reaction at 0^{o}C to give pH 1. The mixture was extracted with dichloromethane (3x20 mL) and the organic layer obtained was washed with water until pH 7, dried and concentrated, affording the interest product as a white solid (82% yield); m.p. 106-107^{o}C; $[\alpha]_D$ -16 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 2923, 1753, 1597, 1515, 1503, 1172; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.74 (2H, d, J_3 = 8.1 Hz, Ar-H), 7.29 (2H, d, J_3 = 8.1, Ar-H), 6.91 (1H, d, J_3 = 8.0 Hz, Ar-H), 6.71 (1H, s, Ar-H), 6.66 (1H, dd, J_3 = 8.1 Hz, J_4 = 1.8 Hz, Ar-H), 5.98-5.87 (1H, m, allylic), 5.23-4.98 (5H, m, sugar, allylic), 4,85 (1H, d, J_3 = 7.5 Hz,), 4,15-4.04 (2H, m, sugar), 3.78 (3H, s, OCH_3), 3.80-3.74 (1H, m, sugar), 3.34 (2H, d, J_3 = 6.6 Hz, allylic), 2.41 (3H, s, $C_6H_5C\underline{H_3}$), 2.05-2.00 (9H, 3s, $OCOCH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.67-169.78 (3C, ester C=O), 150.9 (1C, ar.), 145.6 (1C, ar.), 144.6 (1C, ar.), 137.6 (1C, allylic), 137.3 (1C, ar.), 132.8 (1C, ar.), 130.3 (2C, ar.), 128.5 (2C, ar.), 121.1 (1C, ar.), 120.7 (1C, ar.), 116.4 (1C, allylic), 113.4 (1C, ar.), 101,2 (1C, sugar), 72.8 (1C, sugar), 72.1 (1C, sugar), 71.5 (1C, sugar), 69.0 (1C, sugar), 68.1 (1C, sugar), 56.4 (1C, OCH_3), 40.4 (1C, allylic), 22.1 (1C, $C_6H_5\underline{C}H_3$), 21.0 (3C, $OCO\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{29}H_{34}O_{12}S$ $(M+Na)^+$: 629.1663; found: 629.1650.

4.2.3 Synthesis of 4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside) (5)

To a solution of **4** (0.33 mmol) in 15 mL of *N*,*N*-dimethylformamide was added sodium azide (3.3 mmol) and the mixture was heated to 90°C. After the end of reaction, noted by TLC after 3 hours, the solvent was removed and the product obtained was partitioned between water/ethyl acetate. The aqueous phase was extracted with ethyl acetate (3x20 mL) and the organic layer dried with Na_2SO_4 and concentrated, providing the compound as a white solid (90% yield); m.p. 129-130°C; $[\alpha]_D$ -36 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 2942, 2100, 1743, 1594, 1510, 1470; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.06 (1H, d, J_3 = 7.8 Hz, Ar-H), 6.71-6.69 (2H, m, Ar-H), 6.00-5.87 (1H, m, allylic), 5.27-5.24 (2H, m, allylic), 5.10-5.05 (3H, m, sugar), 4.95 (1H, d, J_3 = 7.7 Hz, sugar), 3.80 (3H, s, OCH_3), 3.70-3.63 (1H, m, sugar), 3.42-3.24 (4H, m, sugar, allylic), 2.08-2.02 (9H, 3s, $OCOCH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.2-169.3 (3C, ester C=O), 150.6 (1C, ar.), 144.0 (1C, ar.), 137.2 (1C, ar.), 137.1 (1C, allylic), 120.9 (1C, ar.), 120.7 (1C, ar.), 115.9 (1C, allylic), 112.8 (1C, ar.), 101,0 (1C, sugar), 73.3 (1C, sugar), 72.4 (1C, sugar), 71.2 (1C, sugar), 69.5 (1C, sugar), 55.8 (1C, OCH_3), 51.1 (1C, sugar), 39.9 (1C, allylic), 20.6 (3C, $OCO\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{22}H_{27}O_9N_3$ $(M+Na)^+$: 500.1640; found: 500.1549.

4.2.4 General procedure for synthesis of 1,2,3-triazole derivatives 6-10

To a solution of **5** (0.209 mmol) in tetrahydrofuran (0.5 mL) was added the corresponding acetylene (0.229 mmol, 1.1 equiv) and water (0.5 mL). To this mixture was added sodium ascorbate (0.6 equiv) and copper acetate (0.5 equiv) solubilized in 0.5 ml of water. The reaction was monitored by TLC and 1 hour after its completion was observed. Then was added 30 mL of water to the mixture, which was extracted with dichloromethane (3x20mL), the organic layer dried with Na_2SO_4 and concentrated, providing pure **6-10** derivatives after purification by CCS.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-methanol (**6**): this product was obtained in 78% yield as white crystals after purified by chromatography (hexane/ethyl acetate 4:6); m.p. 160-161°C; $[\alpha]_D$ -24 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 2933, 1748, 1592, 1508, 1465, 1249; ^{1}H-NMR

(300 MHz, $CDCl_3$) δ: 7.53 (1H, s, triazole), 6.69-6.61 (3H, m, Ar-H), 5.96-5.87 (1H, m, allylic), 5.31-5.20 (2H, m, allylic), 5.09-4.93 (3H, m, sugar), 4.88 (1H, d, J_3 = 7.6 Hz, sugar), 4.66-4.60 (3H, m, C$\underline{H}_2$OH, sugar), 4.33 (1H, dd, J_2 = 14.3 Hz, J_3 = 8.9 Hz, sugar), 3.89-3.82 (1H, m, sugar), 3.76 (3H, s, OCH_3), 3.31 (2H, d, J_3 = 6.7 Hz, allylic), 2.91 (1H, br s, OH), 2.09-2.02 (9H, 3s, $OCOCH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.0-169.3 (3C, ester C=O), 150.4 (1C, ar.), 147.6 (1C, ar.), 143.5 (1C, triazole), 137.1 (1C, ar.), 137.0 (1C, allylic), 123.6 (1C, triazole) 120.4 (1C, ar.), 120.0 (1C, ar.), 116.0 (1C, allylic), 113.0 (1C, ar.), 100.3 (1C, sugar), 72.6 (1C, sugar), 72.1 (1C, sugar), 71.1 (1C, sugar), 69.6 (1C, sugar), 56.1 (1C, sugar), 55.8 (1C, OCH_3), 50.8 (1C, CH_2OH), 39.8 (1C, allylic), 20.6-20.5 (3C, OCO$\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{25}H_{31}O_{10}N_3$ $(M+H)^+$: 534.2082; found: 534.2020.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-benzene (**7**): this product was obtained in 64% yield as white crystals after purified by chromatography (hexane/ethyl acetate 55:45); m.p.: 180-181°C; $[\alpha]_D$ -64 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3081, 1736, 1592, 1510, 1458; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.77 (1H, s, triazole), 7.71 (2H, dd, J_3 = 7.3 Hz, J_4 = 1.4 Hz, Ar-H), 7.39-7.32 (3H, m, Ar-H), 6.64 (1H, d, J_3 = 8.1 Hz, Ar-H), 6.60 (1H, d, J_3 = 1.9 Hz, Ar-H), 6.34 (1H, dd, J_3 = 8.1 Hz, J_4 = 2.0 Hz, Ar-H), 5.78-5.69 (1H, m, allylic), 5.31-5.27 (2H, m, allylic), 5.06-4.87 (4H, m, sugar), 4.70 (1H, dd, J_2 = 14.3 Hz, J_3 = 2.3 Hz, sugar), 4.36 (1H, dd, J_2 = 14.3 Hz, J_3 = 9.2 Hz, sugar), 3.95-3.87 (1H, m, sugar), 3.72 (3H, s, OCH_3), 3.13 (2H, d, J_3 = 6.7 Hz, allylic), 2.11-2.03 (9H, 3s, $OCOCH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.0-169.3 (3C, ester C=O), 150.1 (1C, ar.), 147.8 (1C, ar.), 143.5 (1C, ar.), 136.9 (1C, allylic), 130.2 (1C, ar.), 128.7 (2C, ar.), 128.1 (1C, ar.), 125.7 (2C, ar.), 121.5 (1C, triazole), 120.6 (1C, ar.), 119.4 (1C, ar.), 115.9 (1C, allylic), 113.0 (1C, ar.), 100.3 (1C, sugar), 72.8 (1C, sugar), 72.1 (1C, sugar), 71.1 (1C, sugar), 69.8 (1C, sugar), 55.8 (1C, OCH_3), 50.9 (1C, sugar), 39.6 (1C, allylic), 20.6-20.5 (3C, OCO$\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{30}H_{33}O_9N_3$ $(M+H)^+$: 580.2290; found: 580.2252.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-chloromethylene (**8**): this product was obtained in 70% yield as white crystals after purified by chromatography (hexane/ethyl acetate 6:4); m.p. 162-

163°C; $[\alpha]_D$ -24 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 2928, 1748, 1592, 1508, 1249; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.55 (1H, s, triazole), 6.72-6.64 (3H, m, Ar-H), 5.99-5.87 (1H, m, allylic), 5.27-5.25 (2H, m, allylic), 5.10-4.90 (4H, m, sugar), 4.65 (1H, dd, J_2 = 14.4 Hz, J_3 = 1.9 Hz, sugar), 4.57 (2H, d, J_4 = 2.0 Hz, CH_2Cl), 4.32 (1H, dd, J_2 = 14.4 Hz, J_3 = 9.0 Hz, sugar), 3.88-3.83 (1H, m, sugar), 3.78 (3H, s, OCH_3), 3.32 (2H, d, J_3 = 6.7 Hz, allylic), 2.10-2.03 (9H, 3s, $OCOCH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.0-169.3 (3C, ester C=O), 150.4 (1C, ar.), 144.6 (1C, ar.), 143.5 (1C, triazole), 137.2 (1C, ar.), 136.9 (1C, allylic), 124.5 (1C, triazole) 120.6 (1C, ar.), 120.0 (1C, ar.), 116.1 (1C, allylic), 113.1 (1C, ar.), 100.4 (1C, sugar), 72.6 (1C, sugar), 72.1 (1C, sugar), 71.1 (1C, sugar), 69.7 (1C, sugar), 55.8 (1C, OCH_3), 50.9 (1C, sugar), 39.8 (1C, allylic), 35.8 (1C, CH_2Cl), 20.6-20.5 (3C, $OCO\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{25}H_{30}O_9N_3Cl$ $(M+H)^+$: 552.1743; found: 552.1685.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-ethyl formate (**9**): this product was obtained in 68% yield as white crystals after purified by chromatography (hexane/ethyl acetate 6:4); m.p. 165-166°C; $[\alpha]_D$ -44º (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 2933, 1741, 1714, 1592, 1542, 1510, 1254; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.13 (1H, s, triazole), 6.69-6.56 (3H, m, Ar-H), 5.93-5.84 (1H, m, allylic), 5.29-5.25 (2H, m, allylic), 5.06-4.96 (3H, m, sugar), 4.83 (1H, d, J_3 = 7.5 Hz, sugar), 4.71 (1H, dd, J_2 = 14.3 Hz, J_3 = 2.2 Hz, sugar), 4.43-4.34 (3H, m, $CO_2C\underline{H}_2CH_3$, sugar), 3.87 (1H, td, J_3 = 9.5 Hz, J_4 = 2.3 Hz, sugar), 3.79 (3H, s, OCH_3), 3.29 (2H, d, J_3 = 6.6 Hz, allylic), 2.10-2.03 (9H, 3s, $OCOCH_3$), 1.38 (3H, t, J_3 = 7.1 Hz, $CO_2CH_2C\underline{H}_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.0-169.2 (3C, ester C=O), 160.3 (1C, ester C=O), 150.4 (1C, ar.), 143.5 (1C, triazole), 140.3 (1C, ar.), 137.2 (1C, ar.), 137.0 (1C, allylic), 129.3 (1C, triazole), 120.4 (1C, ar.), 120.1 (1C, ar.), 115.9 (1C, allylic), 113.0 (1C, ar.), 100.9 (1C, sugar), 72.3 (1C, sugar), 72.0 (1C, sugar), 71.1 (1C, sugar), 69.7 (1C, sugar), 61.2 (1C, $CO_2\underline{C}H_2CH_3$), 55.8 (1C, OCH_3), 51.5 (1C, sugar), 39.8 (1C, allylic), 20.5 (3C, $OCO\underline{C}H_3$), 14.3 (1C, $CO_2CH_2\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{27}H_{33}O_{11}N_3$ $(M+H)^+$: 576.2188; found: 576.2138.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-cyclohexane (**10**): this product was obtained in 60% yield as white crystals after purified by chromatography (hexane/ethyl acetate 7:3); m.p. 152-153°C; $[\alpha]_D$ -32 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 2923, 1738, 1592, 1510, 1448, 1256;

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.26 (1H, s, triazole), 6.69-6.57 (3H, m, Ar-H), 5.96-5.83 (1H, m, allylic), 5.29-5.25 (2H, m, allylic), 5.09-4.96 (3H, m, sugar), 4.87 (1H, d, J_3 = 7.8 Hz, sugar), 4.62 (1H, dd, J_2 = 14.4 Hz, J_3 = 2.3 Hz, sugar), 4.27 (1H, dd, J_2 = 14.4 Hz, J_3 = 9.2 Hz, sugar), 3.9 (1H, td, J_3 = 9.5 Hz, J_3 = 2.3 Hz sugar), 3.77 (3H, s, OCH_3), 3.30 (2H, d, J_3 = 6.7 Hz, allylic), 2.71-2.63 (1H, m, CH), 2.10-2.03 (9H, 3s, $OCOC\underline{H}_3$), 2.00-1.92 (2H, m, CH_2), 1.81-1.70 (4H, m, sp^3, CH_2), 1.40-1.30 (4H, m, sp^3, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.0-169.3 (3C, ester C=O), 153.6 (1C, ar.), 150.3 (1C, triazole), 143.9 (1C, ar.), 136.9 (1C, allylic), 136.8 (1C, ar.), 121.2 (1C, triazole) 120.6 (1C, ar.), 119.3 (1C, ar.), 116.0 (1C, allylic), 113.0 (1C, ar.), 100.4 (1C, sugar), 72.8 (1C, sugar), 72.1 (1C, sugar), 71.1 (1C, sugar), 69.8 (1C, sugar), 55.8 (1C, OCH_3), 50.8 (1C, sugar), 39.8 (1C, allylic), 35.3 (1C, CH), 32.9 (1C, CH_2), 32.8 (1C, CH_2), 29.6 (1C, CH_2), 26.1 (1C, CH_2), 25.9 (1C, CH_2), 20.6-20.5 (3C, $OCO\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{30}H_{39}O_9N_3$ $(M+H)^+$: 586.2759; found: 586.2746.

4.2.5 General procedure for synthesis of 1,2,3-triazole derivatives 11-15

The triacetylated 1,2,3-triazoles (1 mmol) were solubilized in a solution of KOH in MeOH (20 mL, 1.0 mol/L) and stirred at 0^{o}C for 30 min. After the completion of the reaction, noticed by TLC, the mixture was neutralized with IRA-120 resin. The resin was filtered off and washed with methanol. The collected filtrate was concentrated in vaccum to afford deacetylated 1,2,3-triazoles **11-15**.

{1-[4-allyl-2-methoxyphenyl-(6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-methanol (**11**): this product was obtained in 100% yield as white crystals; m.p. 205-206°C; $[\alpha]_D$ -48 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3311, 2928, 2856, 1640, 1592, 1510, 1259; ^{1}H-NMR (300 MHz, DMSO) δ: 7.66 (1H, s, triazole), 6.76 (1H, d, J_4 = 1.2 Hz, Ar-H), 6.50 (2H, br s, Ar-H), 5.99-5.86 (1H, m, allylic), 5.49 (1H, d, J_3 = 5.6 Hz, OH), 5.32 (1H, d, J_3 = 4.6 Hz, OH), 5.24 (1H, d, J_3 = 3.9 Hz, OH), 5.14 (1H, br s, OH), 5.08 (2H, s, $C\underline{H}_2OH$), 5.05-5.01 (2H, m, sugar), 4.74-4.69 (2H, m, allylic), 4.45-4.32 (3H, m, sugar), 3.71 (3H, s, OCH_3), 3.65 (1H, dd, J_2 = 9.2 Hz, J_3 = 1.9 Hz, sugar), 3.27 (2H, d, J_3 = 6.5 Hz, allylic), 3.15-3.07 (1H, m, sugar); ^{13}C-NMR (75 MHz, DMSO) δ: 149.1 (1C, ar.), 147.6 (1C, ar.), 144.3 (1C, ar.), 137.8 (1C, allylic), 134.0 (1C, ar.), 123.6 (1C, triazole), 120.2 (1C, ar.), 116.3 (1C, ar.), 115.7 (1C, allylic), 112.9 (1C, ar.),

100.6 (1C, sugar), 76.3 (1C, sugar), 74.4 (1C, sugar), 73.2 (1C, sugar), 71.2 (1C, sugar), 55.6 (1C, OCH_3), 54.9 (1C, sugar), 50.7 (1C, CH_2OH), 39.1 (1C, allylic); HRMS-ESI: m/z calcd. for $C_{19}H_{25}O_7N_3$ $(M+H)^+$: 408.1765; found: 408.1604.

{1-[4-allyl-2-methoxyphenyl-(6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-benzene (**12**): this product was obtained as white crystals in quantitative yield; m.p. 223-224°C; $[\alpha]_D$ -56 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3335, 2918, 2856, 1637, 1592, 1510, 1259; ^{1}H-NMR (300 MHz, DMSO) δ: 8.25 (1H, s, triazole), 7.74-7.71 (2H, m, Ar-H), 7.46-7.31 (3H, m, Ar-H), 6.69 (1H, s, Ar-H), 6.44 (1H, d, J_3 = 8.1 Hz, Ar-H), 6.14 (1H, d, J_3 = 8.1 Hz, Ar-H), 5.75-5.61 (1H, m, allylic), 5.54 (1H, d, J_3 = 4.6 Hz, OH), 5.31 (1H, br s, OH), 5.26 (1H, br s, OH), 3.80-3.74 (1H, m, sugar), 3.68 (3H, s, OCH_3), 3.37-3.19 (6H, m, sugar), 3.05 (2H, d, J_3 = 5.6 Hz, sugar); ^{13}C-NMR (75 MHz, DMSO) δ: 148.8 (1C, ar.), 146.1 (1C, triazole), 144.1 (1C, ar.), 137.4 (1C, allylic), 133.6 (1C, ar.), 130.8 (1C, ar.), 128.8 (2C, ar.), 127.7 (1C, ar.), 125.1 (2C, ar.), 122.4 (1C, triazole), 119.9 (1C, ar.), 115.8 (1C, ar.), 115.6 (1C, allylic), 112.7 (1C, ar.), 100.1 (1C, sugar), 76.4 (1C, sugar), 74.4 (1C, sugar), 73.1 (1C, sugar), 71.4 (1C, sugar), 55.5 (1C, OCH_3), 51.1 (1C, sugar), 38.9 (1C, allylic); HRMS-ESI: m/z calcd. for $C_{24}H_{27}O_6N_3$ $(M+H)^+$: 454.1973; found: 454.1888.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-chloromethylene (**13**): this product was obtained as white crystals, 97% yield; m.p. 168-169°C; $[\alpha]_D$ -44 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3335, 2952, 2918, 1635, 1592, 1510, 1460; ^{1}H-NMR (300 MHz, MeOH) δ: 7.69 (1H, s, triazole), 6.74 (1H, s, Ar-H), 6.58-6.50 (2H, m, Ar-H), 5.95-5.81 (1H, m, allylic), 5.02-4.95 (2H, m, allylic), 4.84-4.71 (5H, m, sugar), 4.54 (2H, d, J_4 = 3.0 Hz, CH_2Cl), 4.36 (1H, dd, J_2 = 14.4 Hz, J_3 = 9.2 Hz, sugar), 3.73 (3H, s, OCH_3), 3.42 (2H, d, J_3 = 8.1 Hz, allylic), 3.63 (1H, td, J_3 = 9.4 Hz, J_4 = 2.0 Hz, allylic); ^{13}C-NMR (75 MHz, MeOH) δ: 149.6 (1C, ar.), 144.1 (1C, ar.), 143.9 (1C, ar.), 137.4 (1C, allylic), 135.5 (1C, ar.), 124.9 (1C, triazole), 120.5 (1C, ar.), 117.2 (1C, ar.), 114.6 (1C, allylic), 112.7 (1C, ar.), 101.2 (1C, sugar), 76.0 (1C, sugar), 74.5 (1C, sugar), 73.4 (1C, sugar), 71.4 (1C, sugar), 55.2 (1C, OCH_3), 51.2 (1C, sugar), 39.3 (1C, allylic), 34.9 (1C, CH_2Cl); HRMS-ESI: m/z calcd. for $C_{19}H_{24}O_6N_3Cl$ $(M-H)^+$: 424.1281; found: 424.1381.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-ethyl formate (**14**): this product was obtained in 94% yield, white crystals; m.p. 130-132°C; $[\alpha]_D$ -48 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3464, 2923, 2856, 1721, 1637, 1592, 1513; ^{1}H-NMR (300 MHz, DMSO) δ: 8.34 (1H, s, triazole), 6.78 (1H, s, Ar-H), 6.52-6.44 (2H, m, Ar-H), 5.97-5.88 (1H, m, allylic), 5.07-4.99 (2H, m, allylic), 4.93-4.50 (6H, m, sugar), 4.36 (2H, qt, J_3 = 6.8 Hz, $CO_2C\underline{H}_2CH_3$), 3.82 (3H, s, OCH_3), 3.76-3.73 (1H, m, sugar), 3.50 (2H, d, J_3 = 5.6 Hz, allylic), 1.35 (3H, t, J_3 = 6.5 Hz, J_3 = 7.0 Hz, $CO_2CH_2C\underline{H}_3$); ^{13}C-NMR (75 MHz, DMSO) δ: 160.5 (1C, ester C=O), 149.5 (1C, ar.), 144.1 (1C, ar.), 139.1 (1C, allylic), 137.4 (1C, allylic), 135.3 (1C, ar.), 129.7 (1C, triazole), 120.3 (1C, ar.), 116.9 (1C, ar.), 114.6 (1C, allylic), 112.6 (1C, ar.), 101.4 (1C, sugar), 76.0 (1C, sugar), 74.1 (1C, sugar), 73.3 (1C, sugar), 71.5 (1C, sugar), 60.8 (1C, $CO_2\underline{C}H_2CH_3$), 55.1 (1C, OCH_3), 51.4 (1C, sugar), 39.3 (1C, allylic), 13.2 (1C, $CO_2CH_2\underline{C}H_3$); HRMS-ESI: m/z calcd. for $C_{21}H_{27}O_8N_3$ $(M+H)^+$: 450.1871; found: 450.1771.

{1-[4-allyl-2-methoxyphenyl-(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranosid-6-yl)]-1*H*-1,2,3-triazol-4-yl}-cyclohexane (**15**): this product was obtained in quantitative yield as white crystals; m.p. 216-217°C; $[\alpha]_D$ -56 (*c* 0.005, DMSO); IR ($\bar{\upsilon}$/cm^{-1}): 3473, 2923, 2856, 1640, 1592, 1510; ^{1}H-NMR (300 MHz, DMSO) δ: 7.49 (1H, s, triazole), 6.76 (1H, s, Ar-H), 6.45 (2H, m, Ar-H), 5.96-5.82 (1H, m, allylic), 5.51 (1H, br s, OH), 5.32 (1H, br s, OH), 5.25 (1H, br s, OH), 5.08-5.01 (2H, m, allylic), 4.77-4.69 (2H, m, sugar), 4.24 (1H, dd, J_2 = 14.3 Hz, J_3 = 9.6 Hz, sugar), 3.70 (3H, s, OCH_3), 3.72-3.28 (4H, m, sugar), 3.48 (2H, d, J_3 = 6.7 Hz, allylic), 3.12 (1H, br s, CH), 1.85-1.79 (2H, m, CH_2), 1.35-1.15 (8H, m, CH_2); ^{13}C-NMR (75 MHz, DMSO) δ: 152.1 (1C, ar.), 149.0 (1C, triazole), 144.4 (1C, ar.), 137.7 (1C, allylic), 133.9 (1C, ar.), 121.4 (1C, triazole) 120.1 (1C, ar.), 115.8 (1C, allylic), 115.6 (1C, ar.), 112.9 (1C, ar.), 100.2 (1C, sugar), 76.3 (1C, sugar), 74.7 (1C, sugar), 73.2 (1C, sugar), 71.5 (1C, sugar), 55.7 (1C, OCH_3), 51.0 (1C, sugar), 39.2 (1C, allylic), 34.7 (1C, CH), 32.6 (2C, CH_2), 25.8 (3C, CH_2); HRMS-ESI: m/z calcd. for $C_{24}H_{33}O_6N_3$ $(M+H)^+$: 460.2442; found: 460.2326.

4.3 *In vitro* bioassays

4.3.1 Antifungal activity evaluation

The compounds were evaluated *in vitro* for their antifungal activity using Mueller Hinton Broth and the microdilution method following methodology and interpretative criteria proposed by document M27A3.[21] The stock solutions of all the compounds were prepared in DMSO 1% and tested at concentrations (µg/mL) 100; 60; 30; 15; 7.5; 3.75; 1.875; 0.468; 0.23; 0.06. The standard drug fluconazole was applied as control of fungistatic action at concentrations (µg/mL) 64; 32; 16; 8; 4; 2; 1; 0.5; 0.25; 0.125. The microplates were incubated at 37^{o}C for 24 h. Results were visualized and analyzed at 530 nm in an Anthos Zenyth 200rt Microplate Reader. The inhibitory concentrations of microbial growth of the compounds were determined by reduction of 50% in the absorbance values compared to the absorbance value of maximum growth of the isolates (IC_{50}) in µg/mL. The tests were done in duplicates.

4.3.2 Antibacterial activity evaluation

The antibacterial activity of the products was evaluated against strains of American Type Culture Collection, including Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella Typhimurium* ATCC 14028) and Gram-positive (*Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 51299, *Micrococcus luteus* ATCC 10240). A microdilution protocol using Mueller Hinton Broth (Imedia, Curitiba, PR, Brazil) in 96-well colourless microplates was performed according to the standardised method of the Clinical Laboratories Standardization Institute by the document M07-A9. [22] All analysis were done in triplicate. Chloramphenicol (positive control) and the stock solutions of all the synthesized compounds were prepared in DMSO (dimethylsulfoxide) and tested at concentrations (µg/mL) 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19. The microplates were incubated at 37°C/24h for *S. aureus* ATCC 6538 and 37°C/16-20h for the other strains. The microbial growth was quantified by spectrometric measurement at 530 nm in Anthos Zenyth 200rt Microplate Reader. The results were expressed as IC_{50} (concentration of the substance that was able to inhibit 50% of bacteria growth) and IC_{90} (concentration of the substance that was able to inhibit 90% of bacteria growth).

4.3.3 Cytotoxicity assay

The cytotoxicity of the compounds (200 to 1.5 μg/mL) to mouse spleen cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method.[7] The spleen cells from Swiss Webster mice were prepared by gently disrupting spleens using forceps in 5 ml of RPMI 1640 medium with L-glutamine (Sigma Aldrich, USA) and then isolated via centrifugation (500 g). The cell pellet was incubated for 4 min with red blood cells lysis buffer (one part of 0.17 mol/L Tris to nine parts of 0.16 mol/L ammonium chloride). The cells were washed again and suspended to a concentration of 2.4×10^6 cells/mL in RPMI 1640 supplemented with 1% fetal calf serum and antibiotics (100 μg/mL penicillin and 100 μg/mL streptomycin). The cell suspension of spleen cells at a concentration of 2.4×10^5 cells/ml was distributed in a 96-well plate, 90 μL in each well with 10 μL of test compounds at different concentrations, incubated at 37 °C in an incubator at 5% CO_2 for 48 h. After, 10 μL of MTT dye (5 mg/mL) was added and the cells were incubated again for an additional 4 h period. Then, the medium was carefully removed and added to 100 μL of DMSO for solubilization of formazan crystals. The plates were shaken for 5 min and absorbance for each sample was measured in a spectrophotometric microplate reader at 560 nm. The percentage of cytotoxicity was calculated as [(A-B)/Ax100)], where A and B are the absorbances of control and treated cells, respectively. Data were analyzed using linear regression to obtain values for CC_{50} and CC_{90} (cytotoxic concentration for 50% and 90% of cells, respectively). Selectivity indexes were expressed as the ratio CC_{50}/IC_{50}.

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6. Conflict of interest

The authors declare no conflict of interest.

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