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Keywords: Cucurbitacin; cucurbitacin glycosides; triazole derivatives; cycloaddition reaction; 1,3-dipolar cycloaddition.

Graphical abstract

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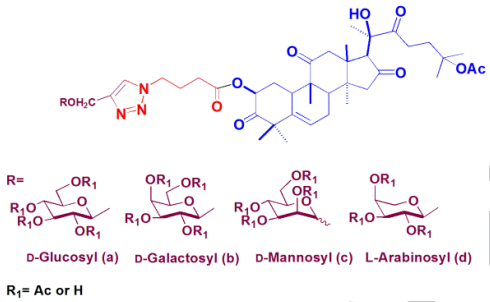

Abstract

Cucurbitacins are natural triterpenoids to which several biological activities are attributed. These molecules can be isolated as free aglycones or as glycosides, despite cucurbitacin glycosides are difficult to be obtained from natural sources. In this work, we report the synthesis of a new 2-β-*O*- galactoside of dihydrocucurbitacin B and also the synthesis of eight new glycosides containing triazole moiety between the cucurbitane skeleton and the monosaccharidic unit.

1. Introduction

Cucurbitacins are natural compounds of great interest in medicinal chemistry, mainly due to their previously reported biological activities. Presently, there are over one hundred cucurbitacins described, to which several activities have been attributed, such as laxative, anticancer, antibacterial, antiviral, anti-inflammatory and antitumoral[1-2]. These highly functionalized triterpenes are found in abundance in plants of the family Cucurbitaceae and may be found as free aglycone or as glycosylated forms, although the glycosides are difficult to obtain from vegetable sources, due to the action of β-glucosidases[3-4].

Cucurbitacin glycosides presented interesting reports regarding biological activities such as cytotoxic, anti-inflammatory and antiparasitic [1,5-6]. Some authors suggest selectivity of these compounds against certain cancer cell lines and against *P. falciparum* [6-7].

Our research group has been working with isolation and molecular modifications by semi-synthesis in several cucurbitacins, mainly cucurbitacin B (**1**) and dihydrocucurbitacin B (**2**) (figure 1) [8-9]. Both are isolated in high yields from fruits of *Luffa opperculata* and roots of *Wilbrandia ebracteata* respectively [9-10]. There are few studies regarding semi-synthesis of cucurbitacin derivatives. All works in literature involve reactions such as oxidation, reduction, acetylation, formation of esters and ethers, etc [9,11-12]. In addition, there is one work which describes the total synthesis of cucurbitacins B and D [11].

Figure 1

Our previous results encouraged us to synthesize new cucurbitacin glycosides, as there are no reports of synthesis of glycosylated derivatives other than the publication from our research group [13]. These new derivatives may possibly improve biological activity and will enrich SAR studies of this class of compounds.

2. Results and discussion

2.1. Molecular Planning

In an earlier work, the isolation and semi-synthesis of novel derivatives of dihydrocucurbitacin B (**1**) and cucurbitacin B (**2**) were reported. The reactions were carried out mainly targeting the C-2, C-16 positions and the side chain. The evaluation of the *in vitro* cytoxicity of the new derivatives in non-small-lung cancer cells (A549) showed that the substituents in positions C-2 and C-16 had strong influence on the biological activity. The study also demonstrated that the derivative 16-oxo-dihydrocucurbitacin B (**3**) presented higher cytotoxic activity than its precursor **1**. Modification in C-2 increased or decreased activity, depending on the substituent. For example, addition of acetoxy group or carbamate substituent reduced the cytotoxic activity, while Br, NH_2 or aminothiazole substituent at C-2 showed promising cytotoxic activity. Facing these results, there is still a necessity to further study the role of the substituents at C-2 of dihydrocucurbitacin B and cucurbitacin B, in several biological activities [9,14].

In previous reports, a serie of modified cucurbitacins were synthesized, including three novel glycosides (figure 2). The compounds **3**, **4** and **5** were prepared via classical *O*-glycosylation reactions, such as Köenigs-Knorr and Schmitd

glycosylation [13]. However, these glycosides were obtained in low yields, possibly due to the low nucleophilicity of the hydroxyl group in C-2. This lack of reactivity may be influenced by the ketone present in C-3, forming the α-ketol system.

Figure 2

Continuing the study of Machado and co-workers, the synthesis of *O*-glycosides **6a-d** was proposed by direct *O*-glycosylation at C-2 position of the derivative 16-oxo-dihydrocucurbitacin B, for the study of these modifications facing biological activities (figure 3).

Figure 3

Additionally, spacer groups can be added between the monosaccharide moiety and cucurbitane scaffold as a tool of junction between two blocks. Since triazole rings have been included in many compounds which presented several biological activities, such as anticancer, antifungal and antibacterial, the 1,2,3 triazole groups were used as spacer in this work. Similarly to the monosaccharides, the triazole spacer could play some interesting interaction with biological targets.

A chain extension at C-2 containing terminal bromine enabled the synthesis of various derivatives through classic S_N2 reactions. The replacement of the bromine with azide allows the synthesis of a series of glycosylated dihydrocucurbitacin B derivatives (**7a-d**) via cycloaddition reaction with four different monosaccharides containing terminal alkynes. Microwave reactor was used for a Click Chemistry strategy to obtain these proposed derivatives. This methodology offers advantages over conventional

methods, as it allowed the obtention of the products in few minutes, in good yields and using small amount of solvent (figure 4).

Figure 4

A series of protected triazole derivatives was designed in view of *in vitro* studies of cytotoxic activity against tumor cell lines, including a QSAR study performed by Bartalis and co-workers, which reported a correlation between increased lipophilicity and increased cytotoxic activity [11].

2.2. Synthesis

The dihydrocucurbitacin B was obtained from roots of *Wilbrandia ebracteata* using methodology previously described [8]. The derivative **6** was obtained after oxidation with pyridinium chlorochromate and $BaCO_3$ which gave the desired product oxidized at C-16 and the compound **8** (Scheme 1)[9].

Scheme 1

Compound **8** is formed as a result of the oxidation in C-2 position, which is the most stable tautomer form in basic medium, generating the diosphenol system. This derivative has been described by Lang and co-workers and showed low cytotoxic activity, compared with its precursor [9]. However, it is obtained in low yields and does not hinder the proposed synthetic route.

As continuation of a previous work [13], different *O*-glycosylaton reactions were performed as an attempt to obtain new glycosides of dihydrocucurbitacin B. Thereby, bromine and trichloroacetimidate glycosidic donors were prepared starting from D-glucose, D-galactose, D-mannose and L-arabinose, accordingly to literature [15,16,17,18]. Several *O*-glycosylation methodologies were tested, varying catalysts [Ag_2O [19], $HgBr_2$ [20], $Hg(CN)_2$ [21,22], Montmorillonite K10 [23], NaH [24], BF_3OEt_2 [25,26,27], AgOTf [28], monosaccharidic donors and conditions. Nonetheless, even after changing the equivalence relation of the reagents and varying reaction time, it was not possible to obtain glycosylated derivatives of **6**.

Finally, the *O*-glycosylated derivative **6b** was obtained by Schmidt's *O*-glycosylation reaction between donor trichloroacetimidate **9b** and derivative 16-oxo-dihydrocucurbitacin B (**6**), promoted by TMSOTf (Scheme 2) [29]. In nature, cucurbitacin glycosides are mainly found as β isomers [5]. For this reason, the choice of acetyl as protecting group was performed in order to promote the effect of the neighboring group, which favors the formation of β-glycosides. The galactoside **6b** was obtained with 3% yield and could not be deprotected because of the small amount of product. The ^1H NMR spectra showed five singlets in δ 2 which were referred to the acetyl groups of the galactose unit and from the side chain of di-hydrocucurbitacin B. Moreover, the doublet which is referred to the anomeric hydrogen has a coupling constant of J= 8,0 Hz, once it is a β-galactoside. This product is novel in literature. No glycosylated derivatives were obtained with glucose, mannose and arabinose trichloroacetimidate donors.

Scheme 2

Alternatively, new glycosides containing a spacer group between the cucurbitacin and the monosaccharide were synthesized. Thus, chain extension of di-hydrocucurbitacin B was prepared by reaction with 4-bromobutyryl chloride catalyzed by N, N-dimethylamimopyridine and pyridine. The intermediate **10** was obtained with 61% yield, and further submitted to reaction with sodium azide. In this step, the azide derivative **7** was achieved by seven minutes irradiation in microwave, with 78% yield (Scheme 3). The alkyne derivatives of the monosaccharidic units were obtained from trichloroacetimidate donors, by *O*-glycosylation reaction with propargyl alcohol, accordingly to literature [30].

Scheme 3

The cycloaddition reaction was performed using the derivative **7** with the protected alkynes **11a-d** with $CuSO_4$ as catalyst (Scheme 4). Furthermore, protected sugars can show improved absorption across cell membranes, and could be a way to bypass pharmacokinetic problems presented by more polar sugars. The protected products **7a-d** were obtained after a ten-minute reaction under microwave irradiation with $CuSO_4$ and sodium ascorbate, with 15-54% yield. The ^{1}H NMR spectra of **7a-d** derivatives showed a singlet in the region of δ 7.0 which is attributed to the aromatic hydrogen of the triazole ring. Additionally, five singlets were observed in the region of δ 2 for the derivatives **7a-c** and four singlets for derivative **7d**, indicating the methyl from the acetyl groups.

Scheme 4

Deprotection of the monosaccharide units from **7a-d** were performed with NaOMe 0,1 mol.L.$^{-1}$ in methanol. However, the hydrolysis of the ester at C-2 were observed, even carefully keeping pH between 9 and 10. Other classical methodologies were performed in an effort to obtain the unprotected triazole derivatives, including ammonolysis [31] and acidic conditions with HCl 0,1 N [32]. All the attempts gave the products of hydrolysis of the ester in C-2, except the acidic hydrolysis, which gave a very complex mixture of products, observed by TLC analysis.

Based on these results, deprotection of the alkyne derivatives were performed before the cycloaddition reaction, with NaOMe, as previously described. The reaction was kept overnight, neutralized to pH 7 with DOWEX 50WX8-200 resin and filtered, to give the unprotected alkynes in quantitative yields.

The action of esterases could perform the breaking of the C-2 ester in the unprotected triazole derivatives, which could possibly acting as prodrugs. The improvement of the solubility properties of dihydrocucurbitacin B by addition of the triazole ring and the monosaccharide units would improve the distribution of the molecule along the body. In this case, the dihydrocucurbitacin B would be released to perform activities already assigned to it, such as anti-inflammatory and antitumor activity [33,34,35,36,37]. Thus, the cycloaddition reaction was performed as previously described between the azide derivative **7** and the unprotected alkynes **12a-d**. The new derivatives **13a-d** were obtained with 14-39% yield (Scheme 5). In the ^{1}H NMR spectra of the unprotected derivatives **13a-d**, only one singlet corresponding to the acetyl group of the dihydrocucurbitacin B side chain could be observed.

Scheme 5

3. Conclusion

Eight new cucurbitacin glycosides, the protected triazoles **7a-d** and unprotected triazoles **13a-d,** were obtained using a simple synthetic approach. The *O*-glycosylation using TMSOTf as promoter gave the galactoside **6b**. All these compounds are new in literature and will enable the studies of substituents in C-2 position of dyhidrocucurbitacin B in several biological activities.

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Supplementary data

Supplementary data (representative experimental procedures, characterization data and copies of NMR) associated with this article can be found in the online version, at

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List of Legends:

Figure 1: Cucurbitacin B (**1**) and dihydrocucurbitacin B (**2**) isolated from *Luffa operculata* and *Wilbrandia ebracteata*, respectively

Figure 2: Novel semi-synthetic glycosides of di-hydrocucurbitacin B obtained by Machado and co-workers[13]

Figure 3: Proposal of new glycosylated dihydrocucurbitacin B derivatives

Figure 4: Proposal of new glycosides of dihydrocucurbitacin B using spacer group between glycosidic unit and the cucurbitacin

Scheme 1: Oxidation reaction of dihydrocucurbitacin B

Scheme 2: *O*-glycosylation reaction between **3** and **9b** promoted by TMSOTf

Scheme 3: Proposed steps for the synthesis of the azide derivative **7**

Scheme 4: Synthesis of triazole derivatives **7a-d**, via Click Chemistry

Scheme 5: Synthesis of unprotected triazole derivatives **13a-d**, via Click Chemistry

List of Figures

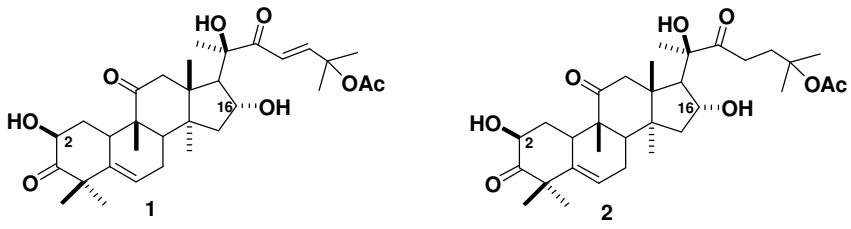


Figure 1

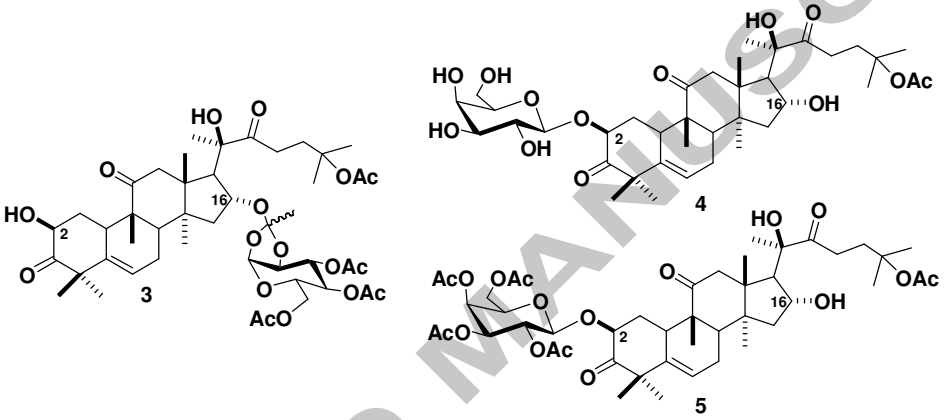
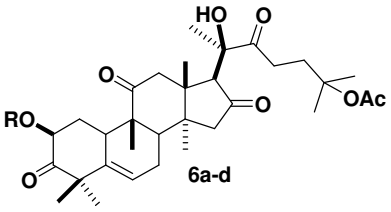
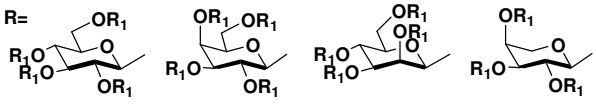


Figure 2





R_1= Ac or H

Figure 3

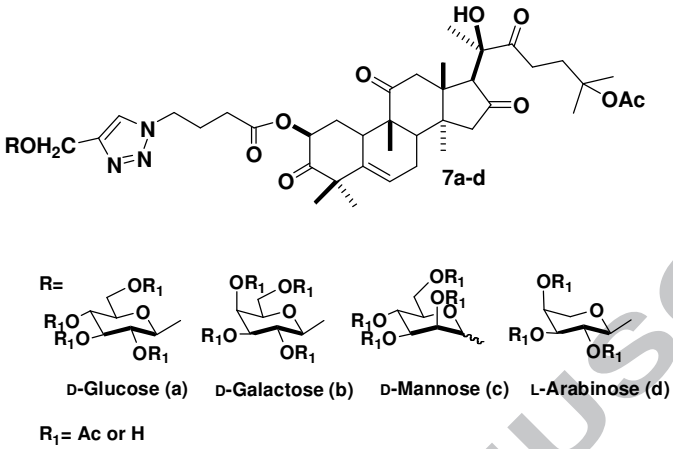
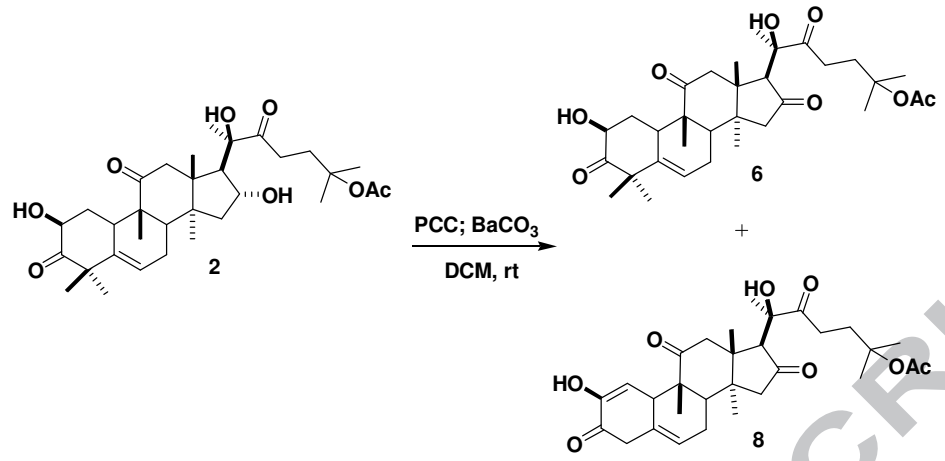
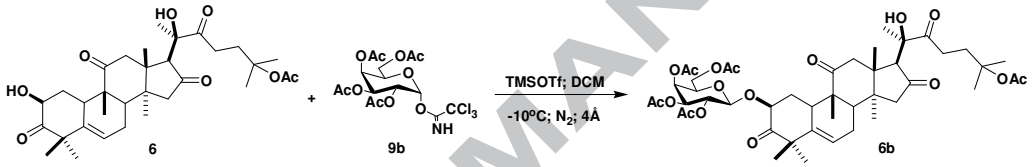


Figure 4

List of Schemes



Scheme 1



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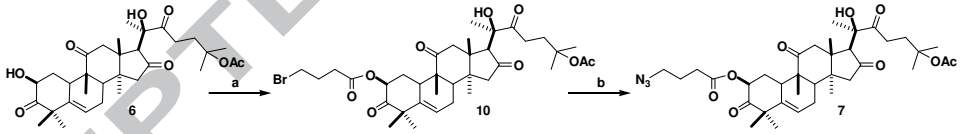
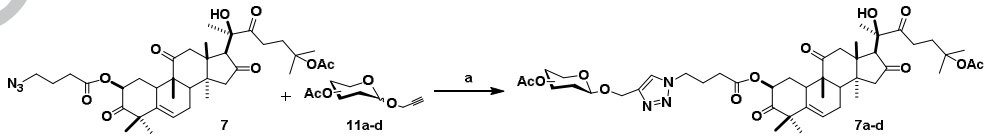


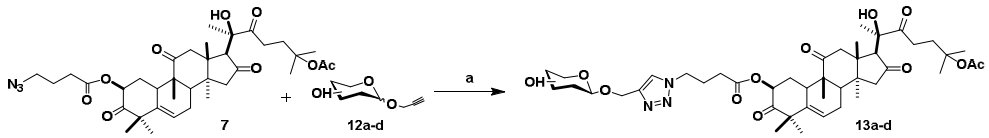
a) 4-bromobutyryl chloride, py, DMAP, DCM, rt, 3h; b) NaN_3, DMF, MW, 7'; P= 150W; 70°C

Scheme 3



a) $CuSO_4$; Sodium ascorbate; DMF; MW; P = 150W; 70 °C; 10'

Scheme 4



a) $CuSO_4$; Sodium ascorbate; DMF; MW; P = 150W; 70 °C; 10'

Scheme 5