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Cytotoxic effect of levoglucosenone and related derivatives against human hepatocarcinoma cell lines

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ABSTRACT

Levoglucosenone has been used as template for the synthesis of a wide variety of compounds with an impressive structural variability. However, scarce work has been done regarding the generation of new bioactive entities. Here we report the cytotoxic effect of levoglucosenone and some related derivatives against hepatocarcinoma cell lines. Compounds were obtained in only one synthetic step and one of them showed an activity within the range of IC_{50} values of cisplatin, a frequently administered chemotherapy drug.

It is generally recognized that the resources of the world are limited and sustainability has become a crucial point for the development of chemical products. These circumstances impose a great urgency to find renewable sources of raw material for their transformation into useful products including chemicals, fuels, and materials for replacing the enormous demand for fossil resources.

Biomass has received particular attention because it represents the only abundant source of renewable organic carbon. More importantly, its oxygenated nature, chemical diversity, and chirality render biomass a highly suitable raw material to manufacture a wide array of high-added-value compounds.[1] By far, carbohydrates are the leading annually renewable feedstocks from which to develop viable organic chemicals that can compete or eventually replace those derived from petrochemical industries.

The pyrolytic treatment of microcrystalline cellulose or cellulose-containing materials, such as waste paper, degrades the cellulose polymeric chain into a useful building block named levoglucosenone (1,6-anhydro-3,4-dideoxy-β-D-glycero-hex-3-enopyranos-2-ulose) (**1**). The highly functionalized structure of **1** makes it an attractive chiral synthon for the synthesis of a broad diversity of natural and unnatural compounds.[1]

Since the first report on the preparation of levoglucosenone about 40 years ago[2], different studies have been conducted in order to develop a wide variety of applications of this chiral template for the synthesis of enantiomerically pure compounds. The plethora of derivatives synthesized from levoglucosenone includes chiral catalysts and auxiliaries and natural products, many of them with known biological activities such as tetrodotoxin.[1] However, there was little investigation reported regarding the use of levoglucosenone as scaffold for the generation of novel bioactive molecular structures. Peri's group reported the synthesis of Ras protein inhibitors as potential antitumoral drugs[3] and employed a 1,3 dipolar addition to levoglucosenone's double bond as synthetic strategy. In this work, the authors showed that the aromatic moieties were highly relevant for the observed activity.

Although a result obtained more than 25 years ago showed that **1** could arrest cell cycle of hamsters' cells[4], surprisingly there has been no additional reports about their activity until now. Working with bio-oils from soybean hulls we recently observed that **1** was active against the pathogen *Salmonella enterica* but this compound resulted to be cytotoxic against CHO cells. In this context, we realized that the carbonyl group was important for the observed cytotoxic effect and the substitution of **1** with a bromine atom at C-3 highly improved the activity.[5]

Based on these data, we decided to reinvestigate the bioactivity of levoglucosenone **1** and its brominated derivative **2** against neoplastic cells. We also synthesized a set of thio-derivatives through Michael type additions to the α,β-unsaturated carbonyl system present in **1** and tested their activities. Levoglucosenone was obtained by pyrolysis of microcrystalline cellulose according with previous studies with an overall yield between 7% and 10%.[6] The reaction of **1** with bromine in CH_2Cl_2 at 0 °C followed by the

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addition of Et_3N afforded 3-bromolevoglucosenone (2)[7] in 92% yield (Scheme 1).

With respect to the thioconjugates derivatives, we carried out reactions between **1** with some representative thiols in the presence of a base (Scheme 2).[8] Table 1 showed the thiols and reaction conditions used to obtain the Michael adducts[9] which were then employed to assess the influence of the different types of substituents on the biological activity (Fig. 1, compounds **3**–**7**).

Additionally, we reduced compounds **4** and **6** in order to evaluate the role of the carbonyl group in the cytotoxic effect (Scheme 3).[10]

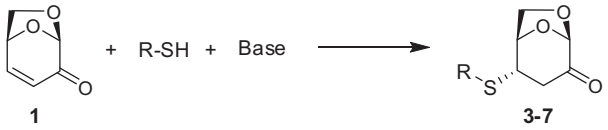
All derivatives were synthesized in very good to excellent yields with the exception of compound **5N** which was obtained in 57% in a serendipitous manner. The conjugate addition of 2-mercaptobenzoxazole was expected to afford adduct **5S** with an S–C linkage; however, its tautomer was the reacting species instead (Scheme 4). A careful analysis of the ^1H and ^{13}C NMR spectra revealed that the nucleophilic addition proceeded through a different pathway. The first spectral evidence for this outcome was the H-4 signal, which appeared at lower fields than the expected one. Also, its ^{13}C NMR spectrum showed a signal at 180 ppm attributed to C-2′, although a comparison with similar compounds indicated that the signal for this type of carbon usually appeared at 170 ppm.[11] These mismatching elements allowed us to suspect the possibility that the substitution had occurred through the N atom attack to yield compound **5N**. The HMBC correlation experiment showed a crosspeak a proton signal at 5.58 ppm with an aromatic C at 129.7 ppm which were assigned to H-4 and C-3′a. This correlation was only possible for the derivative with a C-N bond and not for that with a C–S bond (Scheme 4). These spectroscopic evidences allowed us to establish that the structure obtained was the N-substituted compound **5N** and not the expected adduct **5S**. At this point, we considered reasonable to test the biological behavior of compound **5N**.

The hepatocarcinoma or hepatocellular carcinoma (HCC) is one of the most common causes of cancer deaths worldwide. The HCC is highly resistant to systemic chemotherapy. Thus, the available and most commonly used chemotherapeutic agents, i.e., doxorubicin, cisplatin, and sorafenib show low effectiveness in clinical settings.[12] This situation highlights the need for searching new cytotoxic drugs against HCC.

Therefore, we decided to test the above mentioned compounds against two commonly used human HCC cell lines: Huh-7 and HepG2.[13] Cytotoxicity was assessed by methyl-thiazolyl tetrazolium bromide (MTT) quantitative colorimetric assay, as described.[14] In a preliminary screening, we found that compounds **1** and **2** were active but also derivatives **3**–**5** bearing an aromatic ring at C-4 showed cytotoxicity. In contrast, compounds **6**–**9** showed no apparent cytotoxicity. Based on this outcome, the IC_{50} values for compounds **1**–**5** were determined after 48 h of incubation with cells. The results of these assays are summarized in Table 2.

Table 2 showed that the activity of the new synthetic derivatives was better than the one observed with **1**. Compounds **2** and **5N** were even more active than Sorafenib and the first one was also comparable in activity with Cisplatin (entry 2 with Huh-7 cell line),[15] two of the drugs clinically used nowadays to treat HCC.

Scheme 1. Synthesis 3-bromolevoglucosenone **2**.



Scheme 2. Michael type additions.

Table 1
Reagents and reactions conditions

Entry	Reagent	Base/equiv	Yield (%)	Prod.
1	Thiophenol	Et_3N/0.035	78	**3**
2	*p*-Methyl thiophenol	Et_3N/0.035	75	**4**
3	2-Mercapto-benzoxazole	DIPEA/0.05	57	**5N**
4	1-Butanethiol	DIPEA/0.05	85	**6**
5	1-Hexanethiol	DIPEA/0.04	83	**7**

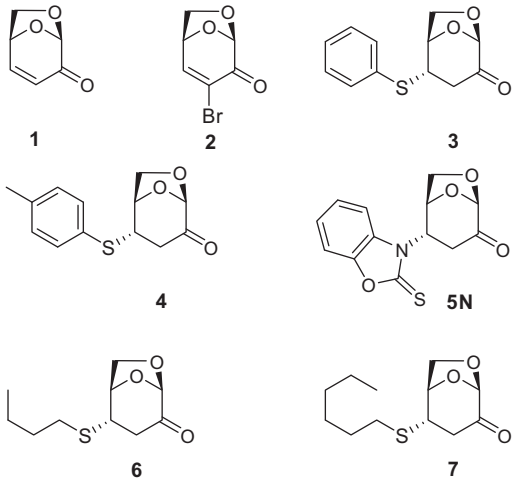
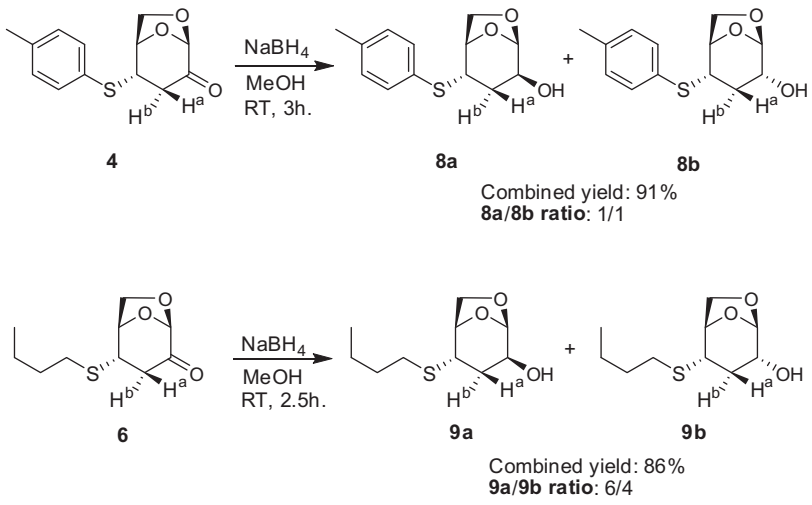


Figure 1.

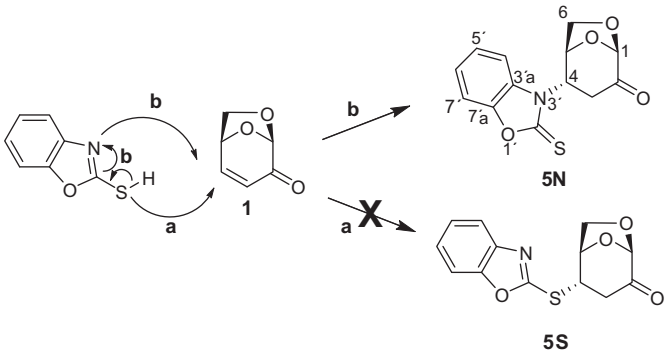
Another observation derived from these experiments was that the Huh-7 cell line resulted to be more susceptible to the in vitro therapy than HepG2 cell line. Although the reason for this effect is still unknown, it might be related to the fact that Huh-7 cells possess a mutation in the p53 tumor suppressor gene while HepG2 cells, conversely, has a normal p53. Interestingly, mutations of the p53 tumor suppressor are present in the majority of tumors, including HCC.[16]

Meanwhile, during the development of this research, a new report in literature showed that some thio-sugars derivates obtained from Michael type additions to **1** were active against some tumoral cell lines, although the activities were relatively low.[17] The authors mentioned the relevance of C-S-C bond angle in activity determination. In our examples this assumption could be true for compounds **3** and **4** but in the case of compound **5-N** which has a C–N bond it could not be applied. On the other hand, aliphatic analogs **6** and **7** which possess a C–S–C bond similar to **3** and **4** were inactive. A similar lost of activity was observed when the carbonyl group was reduced to the corresponding alcohols **8** and **9**. Based on the data collected with this small library of compounds we could infer that in our case, the two main features that were essential for the observed cytotoxic effect were the aromatic moiety and the carbonyl group.

In summary, in this work we demonstrated the potential antitumor activity for levoglucosenone, a glucose-related derivative firstly obtained nearly 4 decades ago. With this starting material, we synthesized a set of structurally related compounds in only



Scheme 3. Ketone reduction.



Scheme 4. Addition of 2-mercaptobenzoxazole tautomers.

Table 2
Cytotoxic assays against hepatocarcinoma cell lines

Entry	Compound	IC_{50} (μM)*	
		Huh-7	HepG2
1	**1**	12.94	39.39
2	**2**	4.68	13.96
3	**3**	11.02	17.00
4	**4**	12.79	27.53
5	**5N**	8.79	22.20
6	Sorafenib[15a]	11.30	12.00
7	Cisplatin[15b]	3.09	7.28
8	Doxorubicin[15b]	0.24	0.68
9	5-FU[15b]	390.00	205.19

* See graphics in Supplementary data.

one simple step with very good yields and some of them showed a very interesting activity against two HCC cell lines. We also noted the key role exerted by the carbonyl group in the biological activity. Finally, this report shows a new horizon in the field of levoglucosenone's chemistry, allowing the synthesis of a novel class of bioactive compounds with original structural features.

Acknowledgments

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Supplementary data

Supplementary data (cytotoxic activity graphics, experimental procedures and spectroscopic characterizations data of the compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.007.

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9. *General procedure for the conjugated addition:* Levoglucosenone was dissolved in anhydrous chloroform (5.5 mL) under Ar atmosphere, followed by the sequential addition of the corresponding nucleophile and base. The reaction was stirred at room temperature until the TLC showed complete consumption of the Michael acceptor. Solvent was evaporated under reduced pressure. The crude product obtained was purified by column chromatography.
10. *General procedure for reduction:* Ketones **4** and **6** were dissolved in MeOH and $NaBH_4$ 98% was then added. The reaction was stirred at room temperature until the TLC showed complete consumption of the substrate. Acetone (0.5 mL) was then added and the mixture was filtered through a celite pad. Solvent was evaporated under reduced pressure. The crude product was purified by column chromatography to separate the epimeric alcohols.
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