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Synthesis and in vitro evaluation of (*R*), (*S*) and (*R*/*S*)-2-hexyne-1,4-diol, a natural product produced by fungus *Clitocybe catinus*, and related analogs as potential anticancer agents

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ABSTRACT

The search for natural products and related analogs as potential anticancer agents has seen a significant growth worldwide. Since small sized propargylic diols can be found in nature and chemically synthesized, their evaluation against cancer cells has been of great interest, being a topic of relevance to be investigated. For this purpose, a scalable approach aiming at the synthesis of several propargylic diols and their bioactivity against seven tumor cell lines were evaluated. Interestingly, when the compound **1a**, a natural product produced by fungus *Clitocybe catinus*, was tested in its racemic mixture a more effective activity was observed if compared when enantiopure *R*-**1a** or *S*-**1a** were tested separately.

Cancer remains one of the main global diseases, being responsible for about 15% of death worldwide.[1] Due to these expressive fatalities, several classes of compounds have been investigated as potential anticancer agents, including natural products. Examples of successfully employed compounds as anticancer agents are Taxol[2] and Discodermolide,[3] which possess IC_{50} in low nanomolar range. Although several natural products and related analogs have been exhaustively studied for this purpose, the search for new candidates which possess smaller side effects on cancer treatment remains of great interest.

Biologically active polyacetylenic metabolites are natural products containing a linear alkynylic moiety as main carbon backbone, differing from each other by the chain-length and/or the number of functional groups. Although these compounds are found in many organisms such algae, fungi, plants and sponges[4] and display a wide range of biochemical and ecological functions,[5] they are usually isolated in very small quantities.

Among several natural products containing an alkynylic moiety, propargylic diols are of great interest.[6] These substances are commonly isolated as polyacetylenes compounds which possess high cytotoxic behavior. Examples are the polyols such Osirisyne E (**4**), isolated from the sponge *Haliclonaosiri* (Chalinidae), that exhibit cytotoxic effect against human leukemia (LC_{50} = 25 μM)[7] and Nepheliosyne A (**5**), a cytotoxic metabolite produced by the marine sponge *Niphates* sp.[8] Although many studies focusing on macromolecules have been done, few examples related to small sized propargylic diols have been reported to date. Examples of small sized metabolites containing propargylic diols are the compounds **1**–**3**. These compounds are natural products produced by fungus *Clitocybe catinus*, being firstly isolated in their enantiomerically pure form by Pava and co-workers in 2000.[9] All the afore-mentioned substances and their related structures, which contain a propargylic diol backbone are shown in the Figure 1.

Therefore, due to our interest in synthesizing functionalized acetylenic natural products, we reported herein a scalable synthesis of natural and unnatural propargylic diols based on metabolites **1**–**2**, and an analysis of their potential anticancer properties again seven tumor cell lines, which has not yet been done.

For the synthesis of bis-substituted propargylic diols **1a**–**o** a literature modified method was employed.[10] Thereby, commercially available propargylic and homo propargylic alcohols were reacted with two equivalents of *n*-butyl lithium, in order to generate reactive bis-lithium salts in situ. Thereafter their reactivity was tested by reacting them with aldehydes, ketones and propylene oxide as appropriated electrophiles (Scheme 1).

After reacting the prepared bis-lithium salts using the conditions depicted in Scheme 1, the prepared diols **1a**–**o** were produced

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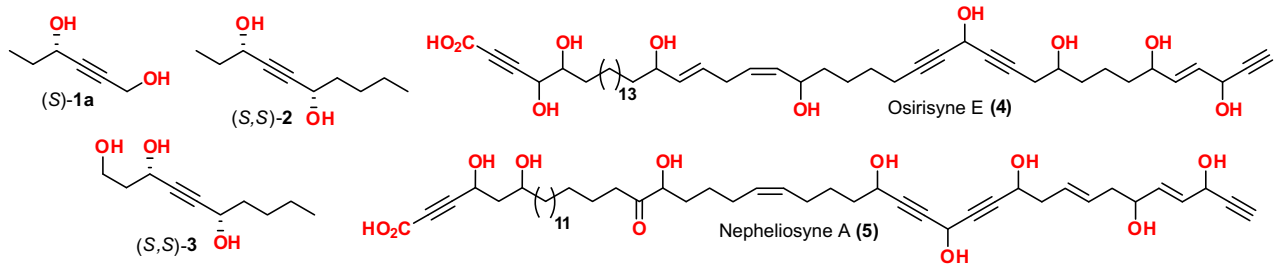
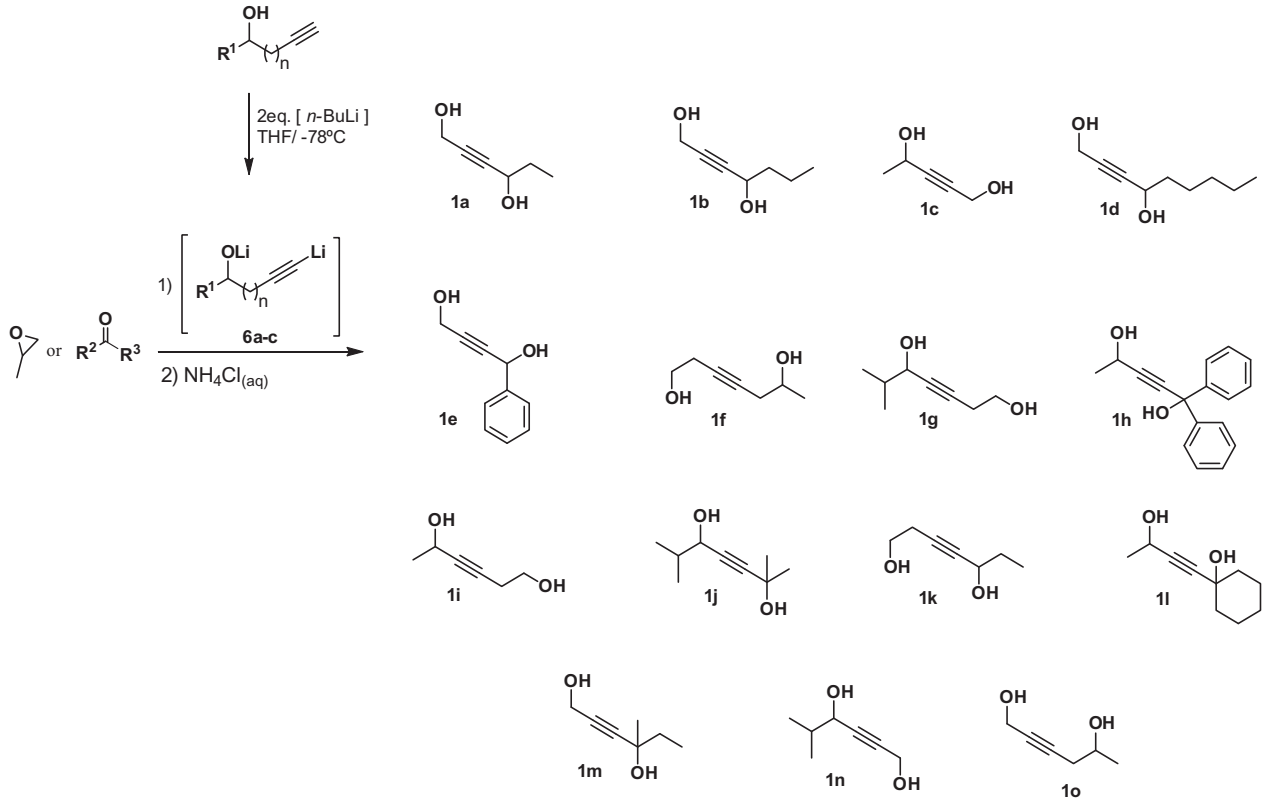


Figure 1. Naturally occurring propargylic diols.



Scheme 1. Preparation of propargylic diols **1**.

in considerable amounts and good overall yields, through large-scale reactions.

As is well known, isomers of specific molecules can exhibit different biological activities. Consequently, in order to identify lead compounds for development, it is crucial to establish the cytotoxic profiles of both racemates and optical pure enantiomers. As we were particularly interested in synthesizing the compound **1a** in their enantiopure form from (*R*/*S*)-**1a**, it was subjected to a lipase-catalyzed resolution, in order to achieve *R* and *S* enantiomers in high ee. For this, a lipase-screened test was performed using two equivalents of vinyl acetate as acyl donor and dry *n*-hexane as organic solvent. For this, a literature-modified method was employed,[11] where the follow isolated lipases were tested for the initial study: immobilized *Candida antarctica* lipase B, supported on acrylic resin (CAL-B) and the powder free enzymes: Amano Lipase G from *Penicillium camemberti* (PCL-G); Lipase A amano12 (A12L-A); lipase from *Pseudomonas stutzeri* (AE07) and lipase from *Alcaligenes* spp. (A2AE011). The results from the enzymatic kinetic resolution studies upon (*R*/*S*)-**1a** are depicted in the Figure 2.

Analyzing the result from the lipase screening tests, the immobilized CAL-B has proved to be the more efficient catalyst on the

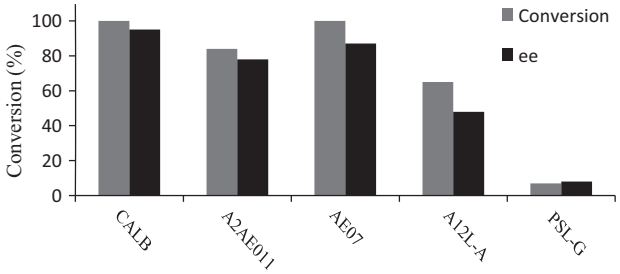
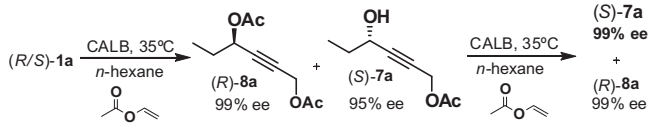
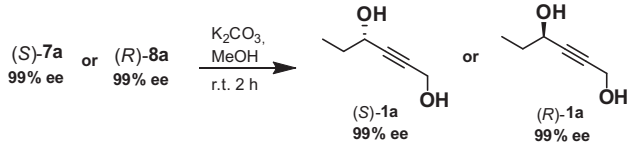


Figure 2. Screening based on conversion of (*R*/*S*)-**1a**. Conditions: (*R*/*S*)-**1a** (0.1 mmol), Enzyme (10 mg)/[5 mg/mL], vinyl-acetate (0.1 mL), *n*-hexane (2.0 mL), 150 rpm, 35 °C.

enantioselective biotransformation, due to this it was chosen as the lipase of choice. Thus, after an enzymatic kinetic resolution of racemic **1a**, the enantiomer (*S*)-**7a** and (*R*)-**8a** could be obtained in 95% and 99% ee, respectively, after a quantitative conversion of (*R*/*S*)-**1a**. Although other lipases such AE07 and A2AE011 have shown similar degree of conversion, (*S*)-**7a** was obtained in moderate selectivity (78–87% ee).



Scheme 2. CALB-catalyzed resolution of (*R*/*S*)-**1a**.



Scheme 3. Synthesis of enantiopure (*R*)- and (*S*)-**1a**.

Although (*S*)-**1a** has been achieved in 95% ee, for an accurate in vitro evaluation we decided to re-subject it to an additional asymmetric acetylation upon CALB. Thereby, both (*S*)-**7a** and (*R*)-**8a** were achieved in their enantiopure form (Scheme 2).

The achievement of the absolute configuration for the unreacted enantiomer in the enzymatic kinetic resolution was done, comparing the experimental optical rotation value obtained for (*S*)-**7a** $[\alpha]_D^{22} = -4.1$ (c 1.0, $CHCl_3$), 99% ee with the literature, where $[\alpha]_D^{20} = -3.4$ (*c* 1.0, $CHCl_3$).[10] Then, after (*S*)-**7a** and (*R*)-**8a** have been obtained in their enantiopure form, they were treated with $CaCO_3$ in MeOH, in order to hydrolyze de acetyl group, which furnished the diols (*R*)-**1a** and (*S*)-**1a** (Scheme 3). Regarding to antiproliferative activity tests all the in vitro assays contained herein were accomplished submitting the compounds (*R*)-**1a**, (*S*)-**1a** and (*R*/*S*)-**1a**–**o** against HEp-2, NCI-H292, MCF-7, K562, HL-60, J774.A1 e RAW 264.7 cell lines, that were obtained from the Cell Bank of Rio de Janeiro, Brazil.

Thus, a cytotoxic activity was evaluated using a colorimetric method (MTT),[12] where the bioassay screening was conducted to determine the cytotoxic potential of the compounds **1a**–**o**, at 100 μg/ml as the final concentration.

As shown in Table 1, the compounds *R*/*S*-**1a**, **1b**, **1c** and **1d** demonstrated cytotoxic activity with 100% inhibiting against the cancer and normal cells lines.

Although compounds **1** possess similar structure, very different results were obtained after their evaluation against the seven tumor cell lines.

By checking the structure of the more active compounds, as potential cytotoxic agents, it becomes clear that the cytotoxic behavior could be due to the presence of both a terminal hydroxyl group and a non-ramified alkylic side-extended chain, as end groups. It can be seeing comparing the structures of **1a**–**d** with compounds **1e**–**o**.

From a broader analysis, the highest active compounds in the screening assays such diols **1a**–**d** must be highlighted due to their good in vitro antitumor activity, exhibiting a total growth inhibition against all of the tested cells lines. Thus, the fixed concentration (100 μg/mL) was property converted and the total growth inhibition values were found as follows; **1a** 0.84 mM, **1b** 0.76 mM, **1c** 1.00 mM and **1d** 0.64 mM.

Surprisingly, when enantiopure (*R*)-**1a** and (*S*)-**1a** were separately subjected to the in vitro assays, both of them showed to be far less effective than racemic **1a**. Therefore, comparing the results it is possible to see that for the HL-60 (*R*/*S*)-**1a** has shown to be 70 times more effective than (*S*)-**1a** (entry 3), while if compared with the (*R*)-**1a** for the cell such J774.A1 the racemate was 500 times more toxic.

We also observed compounds **1e** and **1i**–**o** exhibited low, or no, cytotoxicity. Analyzing the results from compounds such **1f**–**g**, despite they have shown moderate activity upon HEp-2 cells they provided no encouraging results for other cell lines. However, analyzing the results upon diol **1h** (entry 10), which has a tertiary dibenzylic alkynol as feature, was possible to observe a wider range of cytotoxicity, being liable for the following growth inhibitions HEp-2 (84%), NCI-H292 (91%), HL-60 (96%), J774.A1 (93%), K562 (80%) and RAW 264.7 (95%).

Although these results being less significant if compared with the more complex structural natural product Osirisyne E (**4**), that exhibit cytotoxicity against human leukemia cell line (LC_{50} = 25 μM), this communication shows the possibility of these compounds be structurally modified into more cytotoxic derivatives. Therefore, new opportunities aiming at the synthesis of small to medium sized compounds containing a 1,4-alkynylic portion as target can be evaluated.

Among all synthesized molecules, (*R*/*S*)-**1a**–**d** have shown to be very active compounds against all the cancer cell lines tested.

Table 1
Antiproliferative activity of diols **1a**–**o** against Hep-2; NCI-H292; MCF-7; K562; HL-60; J774.A1; RAW 264.7; cells

Entry	Compound	Percentage of cell death (%)						
		HEp-2	NCI-H292	MCF-7	K562	HL-60	J774.A1	RAW 264.7
1	(*R*/*S*)-**1a**	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
2	(*R*)-**1a**	38.8 (0.0)	55.7 (8.5)	52.5 (2.7)	40.9 (3.1)	12.1 (0.0)	0.2 (0.5)	9.6 (3.4)
3	(*S*)-**1a**	31.3 (0.0)	85.0 (9.0)	51.6 (4.6)	27.0 (5.0)	1.4 (2.2)	1.7 (1.4)	19.5 (7.3)
4	**1b**	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (2.7)	100 (0.0)	100 (0.0)
5	**1c**	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
6	**1d**	100 (0.0)	100 (9.1)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
7	**1e**	28.1 (2.7)	86.9 (2.9)	68.1 (0.0)	3.8 (0.0)	29.5 (2.3)	17.1 (2.7)	16.0 (0.8)
8	**1f**	84.1 (0.0)	71.5 (10.7)	69.5 (0.0)	0.0 (0.0)	9.6 (5.0)	15.7 (5.9)	19.3 (2.3)
9	**1g**	85.7 (0.0)	49.9 (1.6)	54.1 (1.2)	0.0 (0.0)	0.0 (4.9)	30.4 (5.5)	17.8 (0.8)
10	**1h**	84.0 (1.1)	91.0 (3.0)	27.3 (5.5)	80.0 (7.0)	96.1 (1.1)	93.1 (0.2)	95.0 (3.1)
11	**1i**	2.2 (0.0)	0.3 (1.4)	17.8 (0.8)	25.1 (3.1)	0.0 (0.0)	16.5 (3.5)	9.4 (3.7)
12	**1j**	0.0 (0.0)	21.5 (1.9)	40.7 (0.0)	9.7 (6.7)	7.3 (0.0)	55.3 (0.2)	0.0 (0.0)
13	**1k**	12.9 (0.0)	15.5 (3.6)	34.3 (5.5)	0.0 (0.0)	5.1 (9.8)	42.6 (10.8)	6.1 (8.6)
14	**1l**	9.3 (0.0)	6.6 (2.6)	40.8 (3.3)	16.7 (4.3)	8.9 (1.6)	57.5 (3.8)	4.2 (3.7)
15	**1m**	0.0 (0.0)	5.0 (0.2)	27.2 (0.6)	16.0 (9.8)	13.3 (4.3)	61.2 (8.2)	0.0 (0.0)
16	**1n**	42.7 (0.0)	69.8 (10)	28.7 (3.2)	20.9 (9.6)	0.0 (0.3)	2.0 (8.9)	17.8 (1.3)
17	**1o**	14.8 (0.0)	20.2 (4.8)	0.0 (6.3)	0.0 (1.2)	11.0 (0.0)	60.8 (5.4)	8.5 (8.3)
18	DOX	79,4 (2,6)	94.1 (2.0)	74.8 (2.1)	91.7 (1.4)	92.9 (0.6)	96.0 (0.1)	96.5 (0.8)

Results are expressed as means (standard deviations) by MTT assay after 72 h of incubation.
DOX = Doxorubicin was the positive control.

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Analyzing the molar concentration, compound (*R*/*S*)-**1d** has proved to be most active compounds, using a fixed concentration of 100 μg/mL, needing 0.16 mM solution for total cell growth inhibition.

Thus, the present communication, for which small sized propargylic diols were prepared and had their cytotoxicity evaluated, should be property expanded and used as a guide for the investigation of others acetylenic target molecules featuring biochemical relevance. For this, the current short chemoenzymatic approach will be combined with others synthetic strategies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.060.

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