New AChE inhibitors from microbial transformation of trachyloban-19-oic acid by *Syncephalastrum racemosum*

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A B S T R A C T

Trachyloban-19-oic acid (1) is a diterpene very abundant in nature and its structural modification can furnish new bioactive compounds. Biotransformation of 1 by fungus *Syncephalastrum racemosum* provided three derivatives, two hydroxylated products (2–3) and one product of rearrangement (4). Products 3 and 4 have never been reported so far, to the best of our knowledge. Structure of 3 was formed after oxidation and rearrangement of compound 2. Compounds 1–4 were evaluated for inhibition of acetylcholinesterase, enzyme linked to the symptomatic control of Alzheimer’s disease. All the compounds presented inhibitory activity higher than starting material 1, and product 3 presented IC_{50} = 0.06 μM, which is about six times higher than activity found for galanthamine (IC_{50} = 0.38 μM), the positive control used in this assay.

1. Introduction

Fungi are an inexhaustible source of natural products mainly due to their wide distribution in the nature, estimated to range from 1.5 to 5.1 million species in the world [1]. Secondary metabolites from fungi represent a substantial fraction of drugs and drug models in pharmaceutical industries, including antibiotics, statins and immunosuppressant [2,3]. Fungal biosynthetic routes used to produce secondary metabolites are also useful to undertake structural modifications in xenobiotic compounds.

Biotransformation is a tool that has been extensively used to prepare derivatives from trachylobane diterpenes. Trachyloban-19-oic acid (1), a natural diterpene found in plants of different genera such as *Croton* [4], *Xylopia* [5,6], *Arctopus* [7], *Iostephane* [8], and *Helianthus* [9], is a promisor substrate for preparing new bioactive derivatives.

Biotransformation by fungi has attracted great interest to the pharmaceutical, chemical and food industries due to numerous advantages, mainly the capacity of performing chemo-, regio- and enantioselective reactions [10,11]. In trachylobane diterpenes, bio-transformations most commonly leads to hydroxylation and skeleton rearrangement. Hydroxylation has been accomplished at several positions such as C-7β and C-17 [12] and C-11β [13] using *Rhizopus stolonifer*, C-1α and C-17 using *Rhizopus arrhizus* [14], C-19 using *Gibberella fujikuroi* [15], and at C-7β using *Mucor plumbeus* (Fig. 1) [16].

In addition, rearrangements of trachylobane into kauranes diterpenes by *R. stolonifer* were described [12,13]; in this case, the covalent bond between C-12 and C-16 is disrupted, with formation of a C-16 tertiary carbocation, which is subsequently hydrated. Thus, trachylobane skeleton is pointed as the precursor of ent-kaur-11-ene derivatives [9]. Another rearrangement found in the literature from trachylobane di-terpenes lead to the formation of trachylobagibberellins by *G. fujikuroi* [9,17]. Formation of trachylobagibberellins involves an oxidation of C-19 followed by hydroxylation at C-7 and contraction of ring B with C-7 extrusion. In the biotransformation of *ent*-trachyloban-18-oic acid by *R. arrhizus* another type of rearrangement was described, in which the bond between C-13 and C-16 was disrupted and a new bond was created between C-11 and C-13, followed by formation a double bond between C-15 and C-16 [14].

In a previous work [16], trachyloban-19-oic acid (1) and derivatives showed acetylcholinesterase inhibition, raising our interest to prepare further derivatives for biological screening, since new drug leads for treatment of Alzheimer’s disease are very welcome worldwide. Therefore, we report herein the biotransformation of trachyloban-19-oic acid (1) by *S. racemosum* into one known and two new products: 17-hydroxytrachyloban-19-oic acid (2), trachyloban-17,19-dioic acid (3) and *ent*-16β,17-dihydroxykaur-11-en-19-oic acid (4), respectively. S.
racemosum was chosen due to its fast growth and poor secondary metabolism, which are useful features in biotransformation experiments. Substrate 1 and products 2–4 were screened for acetylcholinesterase inhibitory activity.

2. Results and discussion

Incubation of trachyloban-19-oic acid (1) with the fungus S. racemosum led to the isolation of three compounds 2–4 (Fig. 2). These compounds were isolated by successive purifications by column chromatography, and their structures were identified by $^1$H and $^{13}$C NMR combined with 1D and 2D NMR techniques. Compound 2 was identified as a 17-hydroxytrachyloban-19-oic acid, which have already been obtained from biotransformation of 1 by R. stolonifer [12]. Compounds 3 and 4 are hydroxylation and rearrangement products, respectively, and to the best of our knowledge, these compounds have not been described before.

Compound 2 (13 mg) was purified as an amorphous, colourless powder. The molecular formula C$_{20}$H$_{30}$O$_{4}$ was established by HRESIMS (Fig. S11) and corroborated with $^1$H and $^{13}$C NMR spectroscopic data (Table 1). IR spectrum showed bands for hydroxyl (3433 cm$^{-1}$) and carbonyl (1686 cm$^{-1}$) groups. $^{13}$C NMR spectrum exhibited twenty signals, being two methyl groups at $\delta_{C}$ 29.6 and 13.3 (C-18 and C-20, respectively), and two carboxyl groups at $\delta_{C}$ 180.4 and 177.7 (C-19 and C-17, respectively). In addition, the spectrum showed sixteen signals of non-oxigenated carbons. DEPT 135 spectrum showed that, from the twenty carbon atoms, eight were methylene, two were methyl, four were methine and six were quaternary. $^1$H NMR spectrum showed a signal at $\delta_{H}$ 1.98–2.01. Although its shape and integral points to a methyl group (Fig. S3), HSQC indicated the overlap of signals of hydrogens at C-6$\beta$, C-12 and C-13.

HMBC spectrum showed correlations between the signal of H-1$\alpha$ and C-2, C-9, C-10 and C-20; H-9 and C-7, C-11, C-14 and C-20; H-14b and C-7, C-9, C-13 and C-15; and H-15$\alpha$a and C-7, C-9, C-16 and C-17. Some HMBC correlations for compound 3 are shown in Fig. 3. NOESY spectrum showed correlations between H-1$\beta$ with H-9; H-3$\beta$ with H-5; H-6$\alpha$ with H-20; H-9 with H-1$\beta$, H-5 and H-15$\beta$; and between H-18 with H-3$\beta$, H-5 and H-6$\beta$. All correlations in HMBC and NOESY corroborated with the maintenance of the original skeleton. Structure of compound 3, trachyloban-17,19-dioic acid, was elucidated as a new trachylobane diterpene. Proposed structure of 3 was confirmed by extensive heteronuclear-2D-correlations experiments HMBC, HSQC and NOESY (Table S1, supporting information).

Compound 4 (16 mg) was purified as an amorphous, colourless powder. Molecular formula C$_{20}$H$_{30}$O$_{4}$ was established by HRESIMS (Fig. S19) associated with the $^1$H and $^{13}$C NMR spectroscopic data (Table 1). IR spectrum showed bands for hydroxyl (3420 cm$^{-1}$) and carbonyl (1686 cm$^{-1}$) groups. $^{13}$C NMR spectrum exhibited twenty signals of carbon atoms, being two of methyl groups at $\delta_{C}$ 29.7 and 16.4 (C-18 and C-20, respectively), and five signals of quaternary carbon atoms, including a carboxyl group at $\delta_{C}$ 180.4 (C-19). In addition, there were observed two signals of olefinic carbon atoms at $\delta_{C}$ 128.0 and 133.1 (C-11 and C-12, respectively), and two signals of carbinolic carbon atoms at $\delta_{C}$ 67.9 and 87.2 (C-17 and C-16, respectively). DEPT 135 spectrum showed eight methylene, two methyllic, five are methine and five quaternary carbon atoms. $^1$H NMR spectrum showed two signals of hydrogen atoms in oxygenated carbons, one at $\delta_{H}$ 4.01 ($J = 10.6$ Hz) and other at $\delta_{H}$ 4.17 ($J = 10.6$ Hz), both were assigned to H-17a and H-17b. The overlap of the signal of H-17 with H-11 and H-17b. Also, there were observed two singlet of methyl hydrogen atoms at $\delta_{H}$ 0.83 (1H, d, $J = 10.6$ Hz) and other at $\delta_{H}$ 0.89. Compound 4 has two signals of methyl groups were present, one of them was hydroxylated (H-17). Through the HMBC correlations, compound 4 was determined to have a kaurane diterpene skeleton, due to a rearrangement that took place in compound 2. Some HMBC correlations for compound 4 are shown in Fig. 3. Compound 4 presents NOESY correlations between H-17 with H-11 and H-12, indicating that stereochemistry of C-17 must be $\beta$. The structure of compound 4, ent-16$\beta$,17-dihydroxykaur-11-en-19-oic acid, was elucidated as being a new compound. Proposed structure of 4 was confirmed by extensive heteronuclear-2D-correlation experiments HMBC, HSQC and NOESY (Table S2, supporting information).

Compounds 1–4 were submitted to bioassay of acetylcholinesterase (AChE) inhibitory activity to evaluate possible improvement of activity after structural modifications performed by biotransformation. Currently, anti-AChE drugs are the main drugs used to control Alzheimer’s disease [18]. Discovery of new compounds with activity against AChE may be the key to relief patients with this disease that affects more than 24 millions of people around the world [19]. Activity of the substrate 1 was lower than 50% at the highest concentration used in this assay and, therefore, IC$_{50}$ was not determined for substrate 1. The results for the derivatives obtained by biotransformation of 1 showed that modification of trachyloban-19-oic acid skeleton improved the power of inhibiting AChE (IC$_{50}$ values varying from 0.06 to