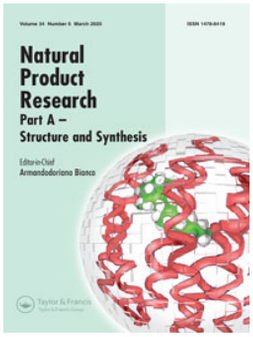

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SHORT COMMUNICATION

Continental and Antarctic Lichens: isolation, identification and molecular modeling of the depside tenuiorin from the Antarctic lichen *Umbilicaria antarctica* as tau protein inhibitor

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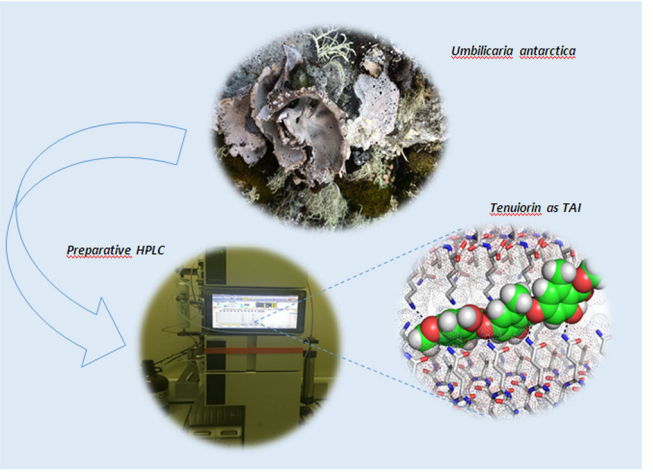
ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia involving Aβ and tau protein. So far, AD cure remains elusive, but considering that AD progresses throughout tau pathology, which turns tau protein an appropriate target, besides tau is also included in other neurodegenerative disorders named as tauopathies. Here, we have isolated seventeen compounds belonging to six lichens species. Due to scarce of spectroscopic data of the compound 5,7-dihydroxy-6-methylphthalide, we explained their structural elucidation based on NMR data. In this study, we show that only tenuiorin from *Umbilicaria antarctica* inhibited 50% of tau 4R at 100 μM. Then, we shown that molecular interactions of tenuiorin with the steric zipper model of the hexapeptide ^{306}VQIVYK^{311} were studied by docking calculations and the results suggested that tenuiorin forms both hydrogen bonds with lysine and glutamine side chains and forms several hydrophobic interactions with valine and lysine from ^{306}VQIVYK^{311} motif.



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1. Introduction

Lichens are complex symbiotic associations between fungi and algae. They are producers of secondary metabolites known as lichen substances, which are a result of these associations. Lichens have a varied chemistry and produce many compounds, including several phenolics such as dibenzofurans, depsides, depsidones, depsones, lactones, anthraquinones and pulvinic acid derivatives with a plethora of biological activities (Shukla et al. 2010; Boustie et al. 2011). Alzheimer's disease (AD) is the most common form of dementia. There are two main protein involved, β-amyloid protein and microtubule-associated tau protein. Those proteins are characterized by the deposition of plaques and neurofibrillary tangles, respectively (Panza et al. 2016). Therapeutic approaches with different mechanisms of actions directed against β-amyloid have been published and subjected to clinical trials (Yamada et al. 2015; Panza et al. 2016). Since tau is also included into AD and it is part of neurodegenerative disorders such as tauopathies, we considering important to focus in to develop or find new naturally occurring compounds as tau inhibitors, since there is no cure for AD or tauopathies. Continuing our search for tau targeting agents, here we describe the isolation of major secondary metabolites from the six-lichen species together with the tau aggregation inhibitory activity of their isolated metabolites. In addition, we provide structure-based models of complex between the fibril-forming motif ^{306}VQIVYK^{311} of tau and tenuiorin to explain the mechanism of its tau anti-aggregation capacity.

2. Results and discussion

From a methanolic extract of *Umbilicaria antarctica* the known compounds tenuiorin (1), usnic acid (2), gyrophoric acid (3), methyl orsellinate (4) and lobaric acid (5) were isolated. The compounds usnic acid (2), atranorin (6), and methyl orsenillate (4) were isolated from the lichen *Stereocaulon ramulo*sum. From the lichen *Everniopsis tru*lla: methyl haematommate (7), ethyl haematommate (8), atranol (9), usnic acid (2), atranorin (6), chloroatranorin (10) and gyrophoric acid (3) were isolated. From *Usnea antarcti*ca was isolated usnic acid (2), 5,7-dihydroxy-6-methylphthalide (11), fumarprotocetraric acid (**12b**), and lobaric acid (5). From *Catillaria corimbo*sa was isolated atranorin (6) and protolichesterinic acid (**13b**). Finally from *Usnea barb*ata: usnic acid (2), galbinic acid (**14**), norstictic acid (**15**) divaricatic acid (**16**), and salazinic acid (**17**), were isolated (Figure S1). In addition, due to scarce of spectroscopic data published for compound **11** (Huneck and Yoshimura 1996), we explained here its structural elucidation. 5,7-dihydroxy-6-methylphthalide (11), was isolated as a colourless gum, and showed a molecular formula of $C_9H_8O_4$ according to NMR and HRESIMS data. The ^{1}H NMR revealed an aromatic proton at δ_H 6.59 (1H, *s*, H-4), a benzylic proton at δ_H 5.19 (2H, *s*, H-3) and a methyl group attached to an aromatic ring at δ_H 2.05 (3H, *s*, H-8). The ^{13}C NMR spectrum showed resonances for 9 carbons. DEPT 135 analysis indicated the presence of one methyl, one methylene, one methine, and six non-hydrogenated carbons compared to the ^{13}C NMR spectrum. HMQC and HMBC correlations corroborated all connectivities and thus compound **11** was established as 5,7-dyhydroxy-6-methylphthalide.

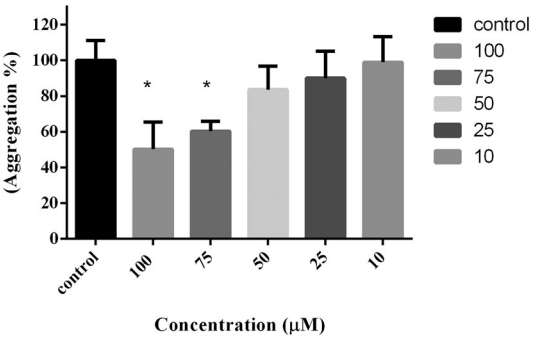

Figure 1. Tau aggregation process inhibited by tenuiorin (1) from *Umbilicaria antarctica*. Black and grey bars represent positive control (aggregation) and inhibition respectively. A paired-*t*-test was conducted in order to compare control (aggregation) and tenuiorin. There was a significant differences for tenuiorin $t(4) = 3.746$, $p < 0.05$ (data are represented as Mean ± SEM).

Regarding lichen substances as tau inhibitors, all compounds (**1**–**17**) were tested in ThT fluorescence assay and only tenuiorin (1) showed activity. Tenuiorin inhibits aggregation process of tau in a range between 75 μM to 100 μM (Figure 1). Several small molecules are considered as alternative on drug discovery for AD and tauopathies, therefore the identification of tau-aggregation inhibitors appears as a valid alternative strategy for the development of new treatment for AD. For example, natural polyphenols, anthraquinones, phenothiazines, porphyrins, thioxothiazolidinones, *N*-phenylamines, phenylthiazole-hydrazides, and aminothienopyridazines were reported as tau aggregation inhibitors (Pickhardt et al. 2005). The anthraquinone parietin isolated from the Antarctic lichen *Ramalina terebrata* reported as tau inhibitor (Cornejo et al. 2016). On the other hands, phenolic diterpenoids (carnosol, carnosic acid, rosmanol and epiisorosmanol) and the phenolic rosmarinic acid isolated from the plant *Rosmarinus officinalis* have recently demonstrated to inhibit *in vitro* fibrillization of tau (Cornejo et al. 2017). It is also important to mention that nitrocatechols (tolcapone and entacapone) and oleocanthal exhibit both anti-aggregation properties against Aβ, α-synuclein and tau (Monti et al. 2012; Mohamed et al. 2013).

There are previous evidences that negatively charged molecules such as orange-G bind specifically to the lysine residues of tau fibril-forming motifs ^{306}VQIVYK^{311} (Landau et al. 2011). Considering that tenuiorin has groups with a negative charge density, we proposed that its activity against aggregation process of tau is due to molecular interactions with fibril-forming motifs. In the complex between orange-G and ^{306}VQIVYK^{311}, the fragment of tau has a β-sheet form with the dye binding between two sheets. Considering this information, we constructed the possible tridimensional structure of tenuiorin in interaction with the tau ^{306}VQIVYK^{311} motif using docking. Recent works have used the information of the ^{306}VQIVYK^{311}-orange-G crystals to propose interactions between ligands that prevent tau aggregation and the hexapeptide ^{306}VQIVYK^{311} using docking method (Mohamed et al. 2013; Cornejo et al. 2016). We propose the possible interaction based on the only structural information available for tau. To explain a possible interaction of tenuiorin with the ^{306}VQIVYK^{311} two tau fiber structure models were prepared for docking: model A contains 24 units of the hexapeptide forming a cavity, and model B contains 12 units of the

hexapeptide without forming a cavity. Thorough models A and B, the interactions between tenuiorin and the ^{306}VQIVYK^{311} fiber structure were evaluated considering the presence and absence of a cavity. Docking was performed inside the cavity of model A (Figure S2A) and at the surface of model B (Figure S2B) using the software Glide (see Figure S2). Docking results for tenuiorin inside the cavity of the model A shows that the ligand forms an extensive hydrogen bond network due to interactions of its phenolic, methoxy group and ester oxygens with different ^{306}VQIVYK^{311} lysine or glutamine side chains. At the same time, methyl groups of the ligand establish hydrophobic interactions with Val^{309} or side chain CH_2 groups of several lysine residues. On the other hand, docking results for tenuiorin at the surface of the model B shows similar orientation of the ligand forming less hydrogen bond interactions with lysine and glutamine side chains and hydrophobic interactions with Val^{309}. Taken together, docking experiments predict a possible interaction of tenuiorin with ^{306}VQIVYK^{311} with orientation and chemical interactions like the ones reported for orange-G.

Further synthetic derivatives from tenuiorin are needed, such as polar or apolar derivatives in order to improve inhibition capacity. Since the best inhibition activity in this study was tenuiorin, the evaluation of new compounds will make clear whether the inhibition observed is due to either phenolic, or carboxylic moieties, or the effect of both functional groups. These chemical aspects are being comtemplated for future works of our group.

4. Conclusions

Our results show tenuiorin (1) had moderate inhibitory activity against the aggregation process of tau protein and it also diminish β-sheet content, while the compounds **2**–**17** were inactive. Molecular docking showed tenuiorin forms both hydrogen bonds with lysine and glutamine side chains from ^{306}VQIVYK^{311} motif and the hydrophobic interactions with valine and lysine.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Boustie J, Tomasi S, Grube M. 2011. Bioactive lichen metabolites: alpine habitats as an untapped source. Phytochem Rev. 10:287–307.

Cornejo A, Salgado F, Caballero J, Vargas R, Simirgiotis M, Areche C. 2016. Secondary metabolites in ramalina terebrata detected by UHPLC/ESI/MS/MS and identification of parietin as tau protein inhibitor. Int J Mol Sci. 17:E1303.

Cornejo A, Aguilar F, Caballero L, Machuca L, Muñoz P, Caballero J, Perry G, Ardiles A, Areche C. 2017. Rosmarinic acid prevents fibrillization and diminishes vibrational modes associated to β sheet in tau protein linked to Alzheimer's disease. J Enzyme Inhib Med Chem. 32:945–953.

Huneck S, Yoshimura I. 1996. Data of lichen substances. In: Identification of lichen substances. Berlin (Germany): Springer Verlag; p. 125–446.

Landau M, Sawaya MR, Faull KF, Laganowsky A, Jiang L, Sievers SA, Liu J, Barrio JR, Eisenberg D. 2011. Towards a pharmacophore for amyloid. PLoS Biol. 9:e1001080.

Mohamed T, Hoang T, Jelokhani-Niaraki M, Rao PP. 2013. Tau-derived-hexapeptide 306VQIVYK311 aggregation inhibitors: nitrocatechol moiety as a pharmacophore in drug design. ACS Chem Neurosci. 4:1559–1570.

Monti MC, Margarucci L, Riccio R, Casapullo A. 2012. Modulation of tau protein fibrillization by oleocanthal. J Nat Prod. 75:1584–1588.

Panza F, Seripa D, Solfrizzi V, Imbimbo BP, Santamato A, Lozupone M, Capozzo R, Prete C, Pilotto A, Greco A, Logroscino G. 2016. Tau aggregation inhibitors: the future of Alzheimer's pharmacotherapy? Expert Opin Pharmacother. 17:457–461.

Pickhardt M, Gazova Z, von Bergen M, Khlistunova I, Wang Y, Hascher A, Mandelkow EM, Biernat J, Mandelkow E. 2005. Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments in vitro and in cells. J Biol Chem. 280:3628–3635.

Shukla V, Joshi G, Rawat MSM. 2010. Lichens as a potential natural source of bioactive compounds: a review. Phytochem Rev. 9:303–314.

Yamada M, Ono K, Hamaguchi T, Noguchi-Shinohara M. 2015. Natural Phenolic compounds as therapeutic and preventive agents for cerebral amyloidosis. Adv Exp Med Biol. 863:79–94.