10th Brazilian Conference on Natural Products
XXXVI RESEM
4-7 November 2025, Belo Horizonte, MG, Brazil
Section: 3

AFFINITY SELECTION MASS SPECTROMETRY (AS-MS) ASSAY FOR THE IDENTIFICATION OF HUMAN BUTYRYLCHOLINESTERASE LIGANDS FROM HYPOMONTAGNELLA MONT EXTRACT

Nícolas Matheus Matias[1*], Vitor Eduardo Narciso do Reis[1], Fernando Cassas[2], Alana Evangelista Honório[3], Lucas Henrique Silva Moura[4], Ariele Maria Morelli[4], Dulce Helena Siqueira Silva[4], Carmen Lúcia Cardoso[1]

nicolas.m.matias@usp.br;

1- Department of Chemistry, Group of Bioaffinity Chromatography and Natural Products (GCBPN), Faculty of Philosophy, Science and Letters at Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil; 2- Separare -Núcleo de Pesquisa em Cromatografia, Federal University of São Carlos, São Carlos-SP, Brazil. 3- EMS Farmacêutica, Rodovia Jornalista Francisco Aguirre Proença, Km 8, Hortolândia, SP. 4- NUBBE - Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, Instituto de Química, Universidade Estadual Paulista UNESP, Araraquara -SP, Brazil.

The human butyrylcholinesterase enzyme (*hu*BChE) is part of the cholinergic system, which is responsible for neurotransmission through the hydrolysis of acetylcholine. In advanced stages of Alzheimer's disease (AD), increased *hu*BChE activity has been observed, contributing to dysregulation of the cholinergic system and progression of the disease[1]. In this context, identifying selective inhibitors of this enzyme holds significant therapeutic potential. In this study, "an affinity-based assay Affinity Selection Mass Spectrometry (AS-MS)" was employed to screen for bioactive ligands. This method involves immobilizing the target enzyme on a solid support, incubating it with the sample (S0), conducting washing steps to remove non-specific ligands (S1–S3), desorbing high-affinity ligands (S4), and subsequently structural characterization[2]. *hu*BChE was immobilized on magnetic nanoparticles (MPs) functionalized with amino groups via an amine-glutaraldehyde reaction, yielding *hu*BChE-MPs. This platform was used to investigate ligands present in an extract of *Hypomontagnella monticulosa* Mont., a microorganism associated with the marine algae *Dichotomaria marginata*, which had exhibited inhibitory activity against *hu*BChE. Immobilization efficiency and the kinetic parameters of *hu*BChE-MPs were consistent with those reported in the literature. Mass spectra from the S4 fraction were analyzed using the Global Natural Products Molecular Networking (GNPS) platform to annotate metabolites potentially responsible for the observed inhibitory activity. Using this platform, 40 metabolites were annotated. Among these, ligands with an affinity ratio (AR) > 1.0 were selected as strong binders for *hu*BChE: dehydroeburicoic acid (AR=1.39); pimaric acid derivative (AR=1.33); grisowen (AR=1.32); sucrose oleate, (AR=1.14); and a related cytochalasin, (AR=1.04).

The authors thank FAPESP (2022/00432-7; 2014/502995), CNPq (307108-2021-0; 172986/2023-0) and CAPES (financial code 001) agencies for financial support and fellowship.

Keywords: *Affinity-based assay, AS-MS, butyrylcholinesterase ligands, huBChE, Hypomontagnella monticulosa*