

Alzheimer's Disease Prevention through Natural Compounds: Cell-Free, *In Vitro*, and *In Vivo* Dissection of Hop (*Humulus lupulus* L.) Multitarget Activity

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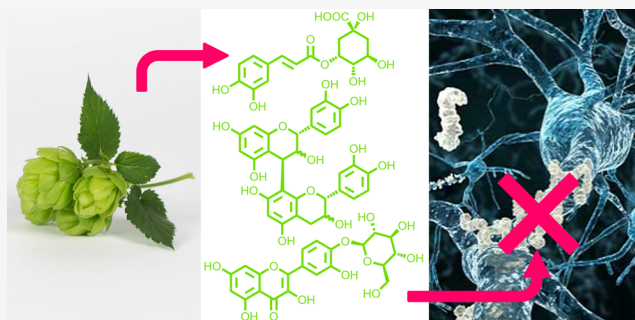
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ABSTRACT: The relevant social and economic costs associated with aging and neurodegenerative diseases, particularly Alzheimer's disease (AD), entail considerable efforts to develop effective preventive and therapeutic strategies. The search for natural compounds, whose intake through diet can help prevent the main biochemical mechanisms responsible for AD onset, led us to screen hops, one of the main ingredients of beer. To explore the chemical variability of hops, we characterized four hop varieties, *i.e.*, Cascade, Saaz, Tettnang, and Summit. We investigated the potential multitarget hop activity, in particular its ability to hinder A β 1–42 peptide aggregation and cytotoxicity, its antioxidant properties, and its ability to enhance autophagy, promoting the clearance of misfolded and aggregated proteins in a human neuroblastoma SH-SY5Y cell line. Moreover, we provided evidence of *in vivo* hop efficacy using the transgenic CL2006 *Caenorhabditis elegans* strain expressing the A β 3–42 peptide. By combining cell-free and *in vitro* assays with nuclear magnetic resonance (NMR) and MS-based metabolomics, NMR molecular recognition studies, and atomic force microscopy, we identified feruloyl and *p*-coumaroylquinic acids flavan-3-ol glycosides and procyanidins as the main anti-A β components of hop.

KEYWORDS: Alzheimer's disease, anti-A β compounds, hop, NMR, UPLC-HR-MS, *Caenorhabditis elegans*



1. INTRODUCTION

Aging is associated with the progressive accumulation of protein aggregates, which actively contribute to senescence and determine, at the central level, the onset of neurodegenerative diseases, of which Alzheimer's disease (AD) represents 60–70% of the cases.¹ One of the most peculiar features of this pathology concerns the time lag between the onset of the first symptoms and the triggering of the biochemical processes that cause it. The latter occurs many years in advance when the neuronal damage has already occurred,² making therapeutic interventions ineffective. Moreover, as AD is a multifactorial disease, single-targeted drugs are usually ineffective.³ It has several hallmarks, such as the aggregation of A β peptide and extracellular deposition of cytotoxic aggregates, the metal-ion dysregulation, triggering A β aggregation and cytotoxicity, the reduction of acetylcholine (ACh) levels in the brain, the formation of intracellular neurofibrillary tangles (NFTs), and a general increase in misfolded proteins and oxidative stress.⁴ In this scenario, the prevention of AD rather than treatment can represent an important strategy. Among the preventive interventions, diet is one of the most promising ones because

the intake of foods or nutraceuticals containing natural molecules can interfere with key biochemical events underlying aging in both physiological and pathological conditions. Thus, identifying food-derived compounds or compound mixtures showing multitarget anti-AD activity is mandatory.

Beer is one of the oldest beverages in the world and is the most widely consumed alcoholic beverage on Earth. It is the third most popular drink worldwide, behind water and tea. Hop (*Humulus lupulus* L.) is one of the main beer ingredients and shows several biological activities due to the wide variety of its chemical components.⁵ Recent studies suggested that intake of bitter hop acids improves cognitive function, attention, and mood in older adults.^{6,7} Moreover, Sasaoka and co-workers reported that long-term oral administration of

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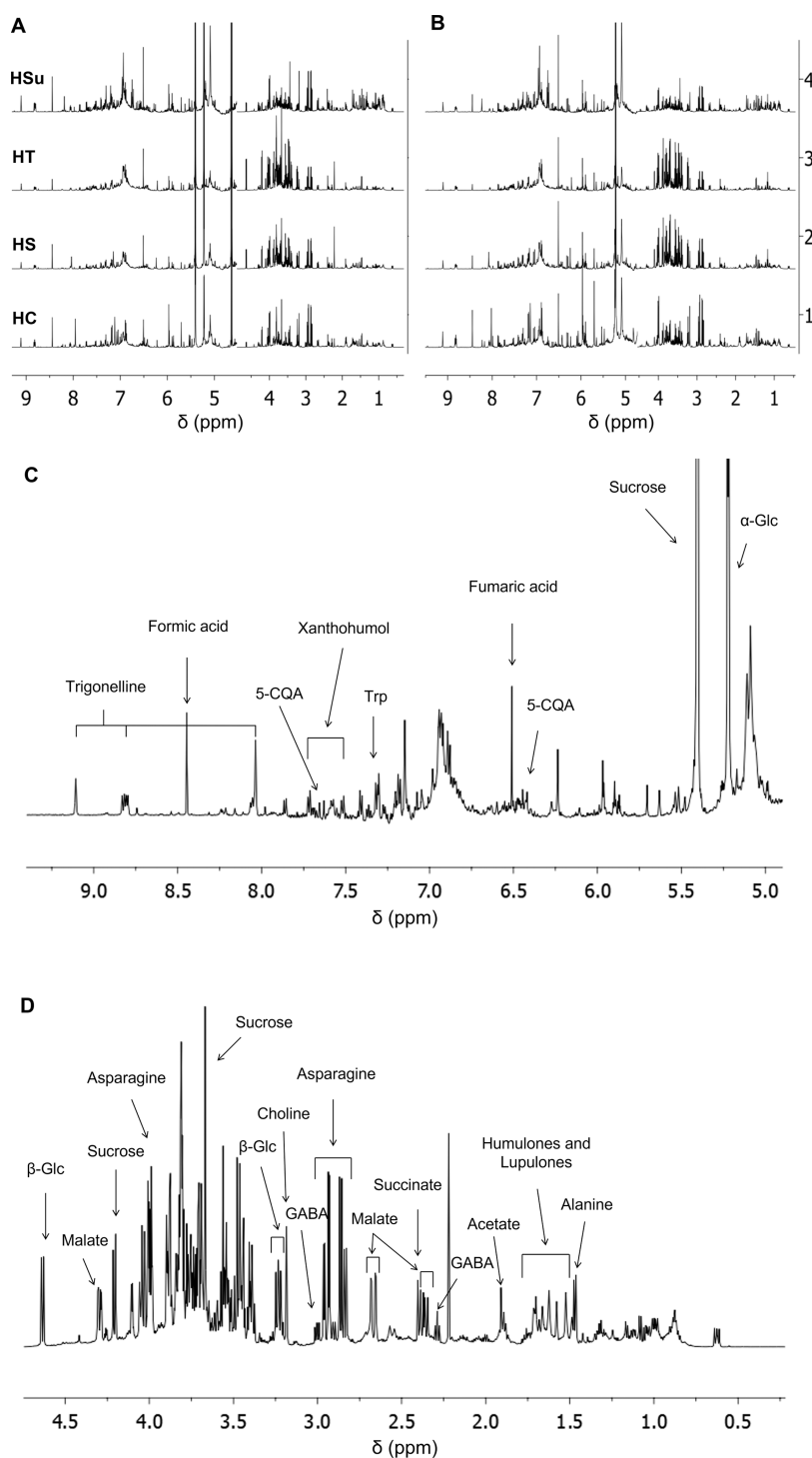


Figure 1. NMR metabolic profiling of hop extracts. Comparison of NMR metabolic profiling of the hops [1, Cascade (HC); 2, Saaz (HS); 3, Tettnang (HT); and 4, Summit (Hsu)] extracted with (A) boiling water or (B) H₂O/ethanol 9:1 solution, in 10 mM phosphate buffer (PB), pH 7.4 at 25 °C. (C, D) ¹H NMR spectrum of HT obtained by boiling water extraction, 22 mg/mL, 10 mM PB, 25 °C, 1 mM sodium trimethylsilylpropanesulfonate (DSS). The expanded region with assigned peaks for aromatic compounds from 9.1 to 5.1 ppm (C), bitter acids, and sugars from 4.7 to 0.8 ppm (D).

hop flower extracts mitigates AD phenotypes in mice, showing the capability to inhibit γ -secretase activity and A β production in cultured cells.⁸

This evidence prompted us to investigate other anti-AD activities of hop extracts, focusing our attention on their effect on the aggregation and toxicity of the synthetic amyloid β (A β)₁₋₄₂ peptide, their antioxidant capacity, and their ability

to enhance autophagy, promoting the clearance of amyloid aggregates. The capability of hop extracts to counteract the proteotoxic effect of A β *in vivo* was also investigated, using the transgenic CL2006 *Caenorhabditis elegans* strain constitutively expressing A β ₃₋₄₂ in the body-wall muscle cells. These *in vitro* and *in vivo* studies, together with extracts' metabolic profiling performed by nuclear magnetic resonance (NMR) spectroscopy

A

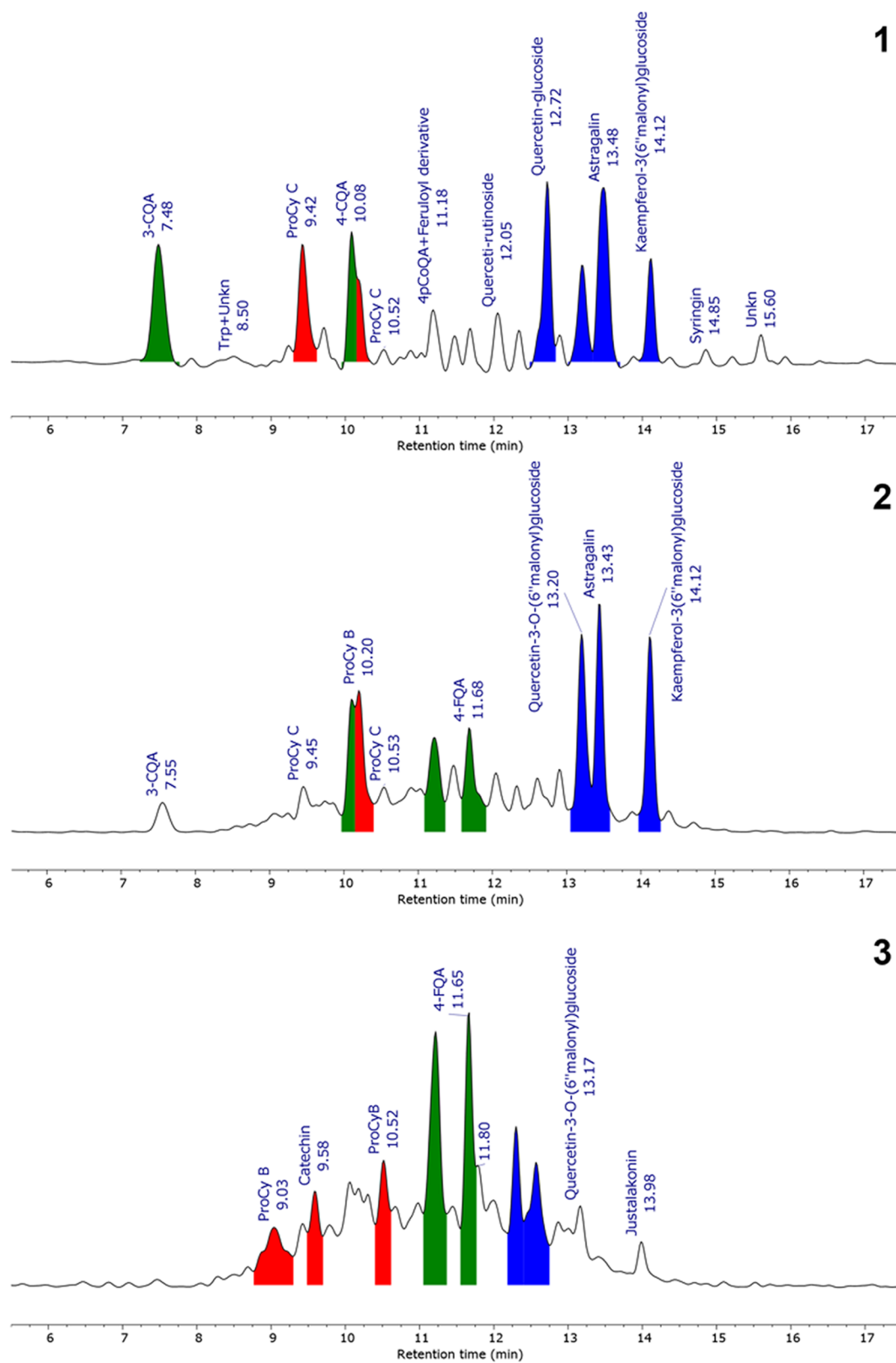


Figure 2. continued

B

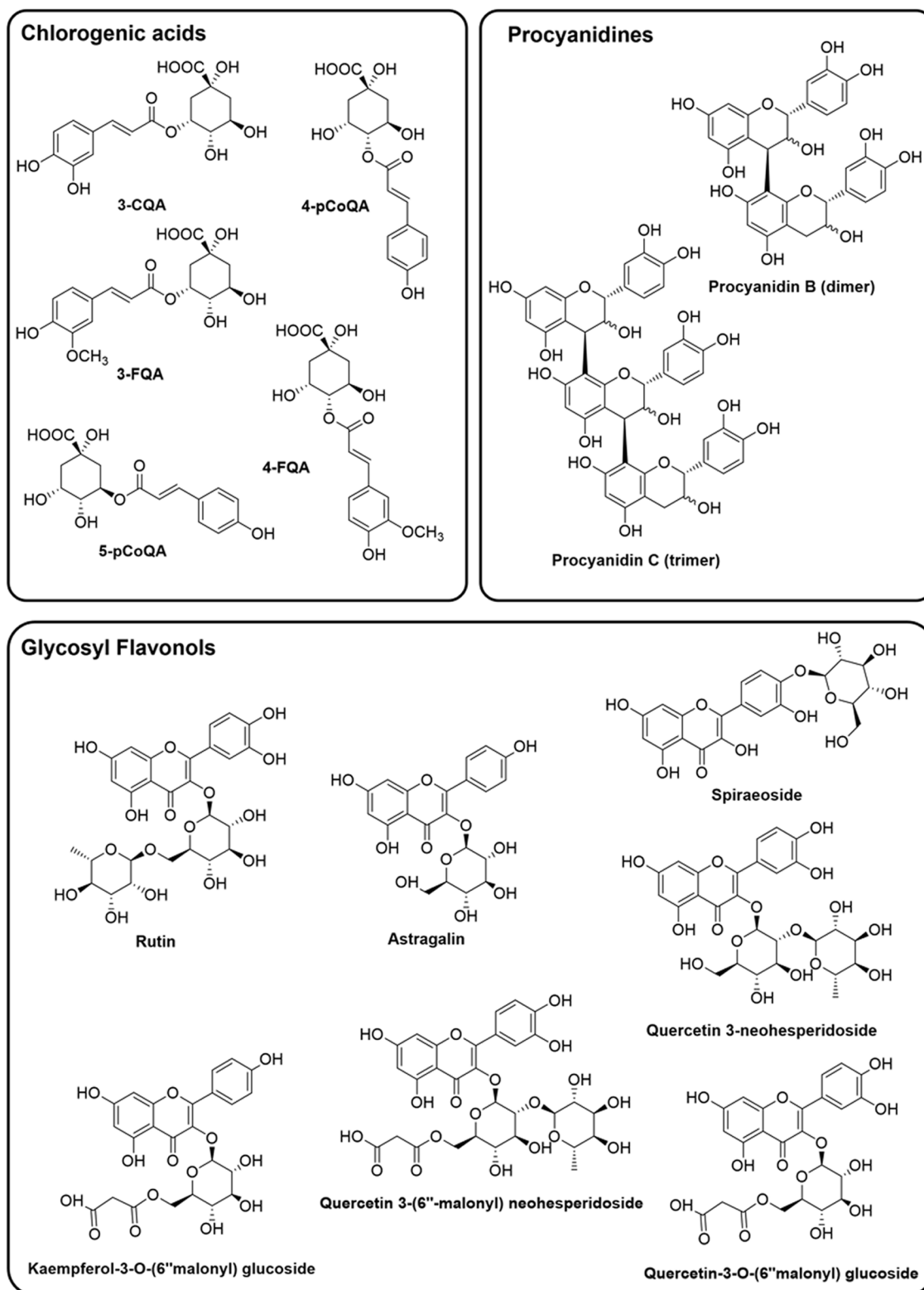


Figure 2. UPLC-PDA-HR-MS analysis of hop extracts HT and polyphenol-enriched fractions. (A) Chromatographic trace extracted at 325 nm was obtained from (A1) total extract, (A2) fraction B, and (A3) fraction B2 (see Section 2.3 for details), and (B) structures of the main polyphenolic compounds identified in the hop extract. Main peaks (with relative area >5%) are color-filled on the basis of their family: chlorogenic acids (green), procyanidines (red), and glycosyl flavonoids (blue). HT, Tettang. 3-CQA, 3-*O*-caffeoylquinic acid; 3-FQA, 3-*O*-feruloylquinic acid; 4-FQA, 4-*O*-feruloylquinic acid; 4-pCoQA, 4-*p*-coumaroylquinic acid; 5-pCoQA, 5-*p*-coumaroylquinic acid.

copy and UPLC coupled with high-resolution mass spectrometry (HR-MS), allowed the identification of the pool of

molecular components responsible for the multitarget protective activity of hop against the $A\beta$ -induced toxicity.

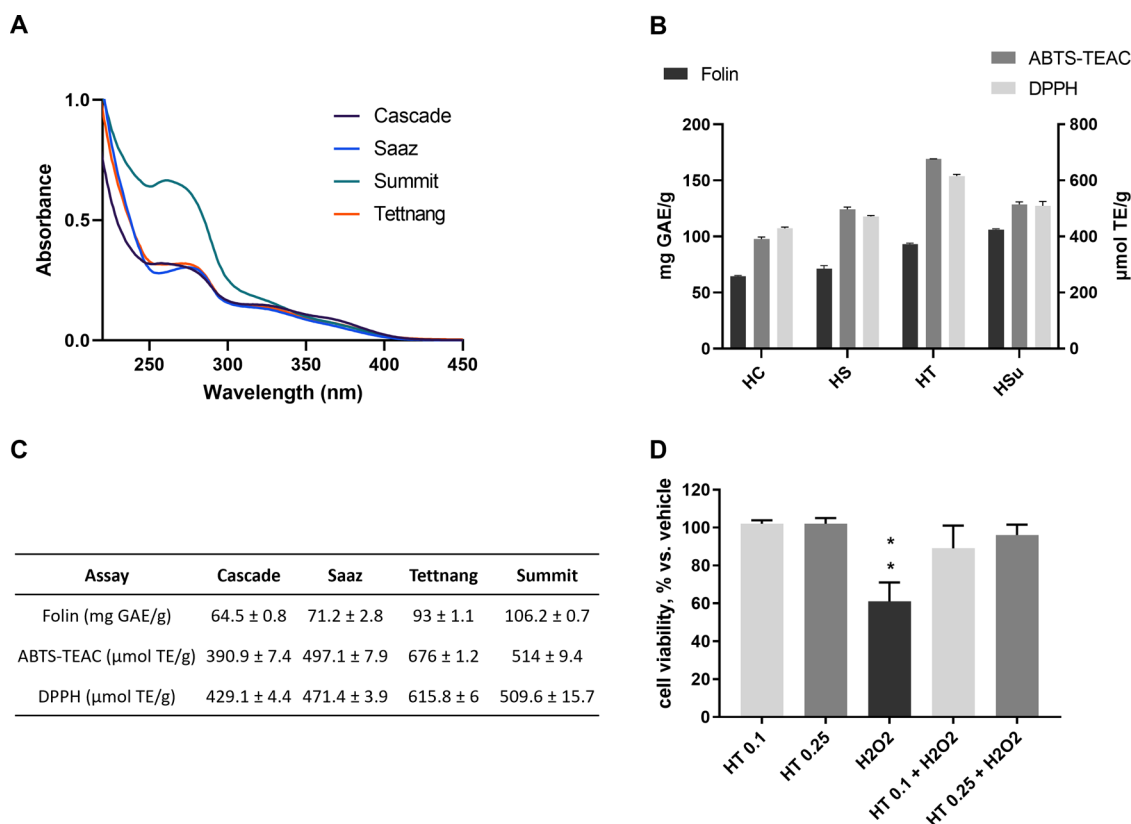


Figure 3. Evaluation of the antioxidant activity of hop extracts. (A) Absorption spectra recorded on hop extract solution at 80 $\mu\text{g}/\text{mL}$ concentration. (B) Comparison of the total reducing power (mg GAE/g) and radical scavenging activity ($\mu\text{mol TE/g}$) assessed on hop extracts by Folin Ciocalteu and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS)-Trolox equivalent antioxidant capacity (TEAC)/2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, respectively. (C) Values are reported as the mean ($\pm\text{SD}$) of a triplicate of three independent measurements. (D) Effect of HT extracts on hydrogen peroxide-induced cytotoxicity in human SH-SY5Y cells. Cell viability was assessed by the MTT assay after 1 h pretreatment with 0.25 or 0.1 mg/mL HT extracts followed by 24 h cotreatment with 100 μM hydrogen peroxide (H_2O_2). Values are expressed as % vs vehicle. Repeated-measures ANOVA test, followed by Dunnett's *post hoc* test; $**p < 0.01$ vs HT alone and vs HT + H_2O_2 .

2. RESULTS AND DISCUSSION

2.1. NMR and MS-Based Profiling of Hop Extracts and Their Antioxidant Activity. To investigate the anti-AD potential of the hop, we screened and compared the activities of four different varieties among the most employed in beer production, namely, Cascade (HC), Saaz (HS), Tettngang (HT), and Summit (Hsu), differing in the degree of concentration of α -acids and/or essential oils, which strongly correlates with their metabolomic composition. We optimized two solid-liquid extraction procedures mimicking hop addition during the brewing boiling phase (extraction with boiling water, 2 h) and to the cold beer (extraction with water/ethanol 9:1(v/v), 30 $^\circ\text{C}$, o. n.), the so-called “dry hopping”. The extracts were analyzed by NMR spectroscopy, and their metabolic profiles were compared (Figure 1). For each matrix, the profiles obtained with the two extraction procedures (Figure 1A,B) appeared very similar; however, extraction yields afforded with boiling water were significantly higher (yield range 28–32 vs 17–21% wt).

Therefore, this procedure was selected for the preparation of the samples for biological assays.

Figure 1C,D reports the metabolic profile of hop Tettngang (HT); the assignment of the main resonances is reported (Figure 1C,D and Supporting Information, Table S1). Spectra assignment was afforded by combining ^1H monodimensional (Figure 1 and Supporting Information, Figure S1) and bidimensional (Supporting Information, Figures S2 and S3)

spectra, libraries of our laboratory,^{9–12} and the online databases Biological Magnetic Resonance Data Bank (BMRB, <http://www.bmrwisc.edu>), FooDB (<https://foodb.ca>), and Birmingham Metabolite Library (<http://www.bml-nmr.org>) and compared with reported assignments.^{13–16}

The aromatic regions of spectra of all hops showed broad and crowded resonances belonging to unassigned polyphenols (Figure 1C). Their identity was elucidated by ultraperformance liquid chromatography (UPLC) separation coupled with high-resolution mass spectrometry (HR-MS). Moreover, UPLC separation was monitored through a photodiode array (PDA) detector to reveal the characteristic polyphenol absorbances at 280 and 325 nm. The chromatogram extracted at 325 nm of crude hop extract was reported (Figure 2A1). Metabolites' annotation was manually supervised and performed by means of their measured accurate mass, fragmentation pattern, and spectrophotometric data in comparison with those previously reported in the literature¹⁶ and online databases (HMDB <https://hmdb.ca/>, ReSpect <http://spectra.psc.riken.jp/menta.cgi/respect/index>, MassBank <https://massbank.eu/MassBank/>). Spectrometric data and structures of major identified compounds have been reported in the Supporting Information, Table S2 and Figure 2B, respectively. Overall, we found 42 compounds, mainly belonging to the family of chlorogenic acids (CGAs), proanthocyanins, and glycosyl flavonoids. In particular, we observed the presence of the caffeoyl-, feruloyl-, and p-

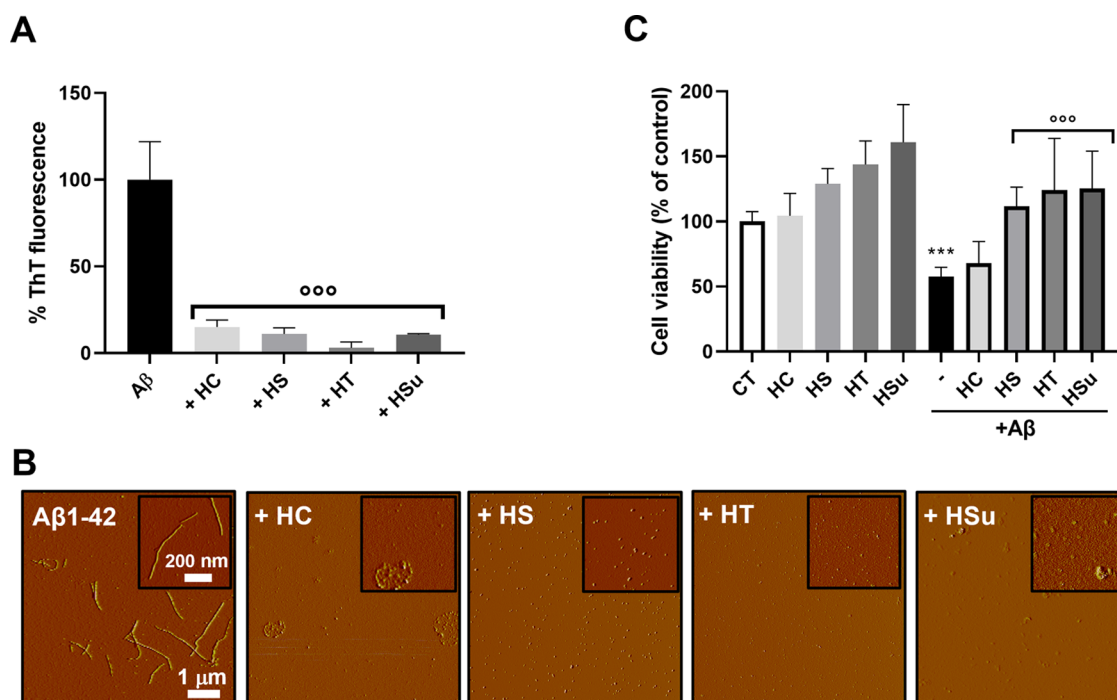


Figure 4. Effects of hop extracts on A β 1-42 aggregation and toxicity on the human neuroblastoma SH-SY5Y cell line. (A) The effect of incubation (24 h at 37 °C) of HC, HT, HS, or HSu extracts (0.25 mg/mL) on A β 1-42 (2.5 μ M) aggregation was determined by the ThT fluorescence assay. Data were expressed as the mean \pm SD ($N = 3$), calculated by subtracting the relative control solutions (fraction alone) and were expressed as the percentage reduction of A β 1-42 aggregation, $^{\circ\circ\circ}p < 0.001$ vs A β alone, one-way ANOVA and Dunnett's *post hoc* test. (B) AFM images were acquired after 24 h incubation at 37 °C of the A β 1-42 peptide (2.5 μ M) with or without 0.25 mg/mL HC, HT, HS, or HSu extracts. (C) Human neuroblastoma SH-SY5Y cells were treated with 10 μ M A β 1-42 peptide in the absence or presence of 0.5 mg/mL HC, HT, HS, or HSu extracts for 24 h, and the toxicity was evaluated by the MTT assay. Control cells were treated with the vehicle (CT). Data are the mean \pm SD of the percentage of viable cells ($N = 6$). $^{***}p < 0.001$ A β vs the respective control and $^{\circ\circ\circ}p < 0.001$ A β + hop vs A β alone, according to one-way ANOVA and Dunnett's *post hoc* test. HC, Cascade; HS, Saaz; HT, Tettngang; Hsu, Summit.

coumaroylquinic acid derivatives and several glycosyl-flavonols, including rutin, astragalins, and spiraeosides, being quercetin and kaempferol the most representative aglycones. Hop extracts are also rich in flavan-3-ols in monomeric and oligomeric forms, including catechins, A-type and B-type procyanidin dimers, and C-type procyanidin trimers.

The antioxidant activity of hop extracts was then determined. A β species induce oxidative stress both *in vitro* and *in vivo*, leading to cell damage and death. Thus, molecules showing antioxidant properties can counteract this effect, significantly preventing proteomic changes due to A β -mediated oxidative stress.¹⁷ We evaluated the total reducing power (or total polyphenolic content) and radical scavenging capacity of the different hop extracts by spectrophotometric method assays (Figure 3).^{18,19}

The observation of the Ultraviolet (UV–visible) absorption spectra of hop extracts showed an intense broad absorption band centered at 280 nm and a minor broad absorption between 325 and 370 nm; the latter supports a remarkable polyphenolic content in all of the extracts. The evaluation of the antioxidant activity indicated that all hop extracts exert relevant activity in terms of the total reducing power and against free radicals in *in vitro* assays. In particular, the HT extract showed 93 mg GAE/g and 616–676 μ mol TE/g (Figure 3B,C).

The antioxidant potential of hop extracts was also verified in a human neuroblastoma cell line (SH-SY5Y). Based on the results reported (Figure 3C), HT was identified as the extract endowed with the most effective antioxidant and radical

scavenging activities and was chosen for these experiments. Cells were pretreated for 1 h with HT (0.25 or 0.1 mg/mL) before exposing them to 100 μ M hydrogen peroxide, a well-known oxidative stress donor, for 24 h. No cytotoxic effect was evidenced after exposure to the HT extract alone, and, as expected, a significant 40% reduction in cell viability was observed in hydrogen peroxide-treated cells. Both concentrations of HT extract were able to significantly counteract hydrogen peroxide-induced cell death (Figure 3D), suggesting that hop extracts possess a considerable antioxidant activity on these neuronal-like cells.

These results demonstrated that hop extracts have remarkable antioxidant power and radical scavenging activity.

2.2. Hop Extracts Hinder A β 1-42 Aggregation and Protect from A β -Induced Toxicity *In Vitro*. The ability of hop extracts to hinder the aggregation of A β 1-42 peptide was investigated by the Thioflavin T (ThT) assay, commonly used to monitor the formation of amyloid fibrils and the effect of anti-amyloidogenic compounds.²⁰ To this end, 2.5 μ M A β 1-42 peptide was incubated at 37 °C with 20 μ M ThT in the absence or presence of 0.25 mg/mL of each extract, and the fluorescence was monitored for 24 h (Supporting Information, Figure S4). The results indicated that hop extracts were all effective in inhibiting peptide aggregation, with slightly different potencies, HT being the most potent and HC the least (Figure 4A).

These findings were further supported by atomic force microscopy (AFM) analysis (Figure 4B), showing that the coinubation of A β 1-42 with hop extracts strongly reduced the

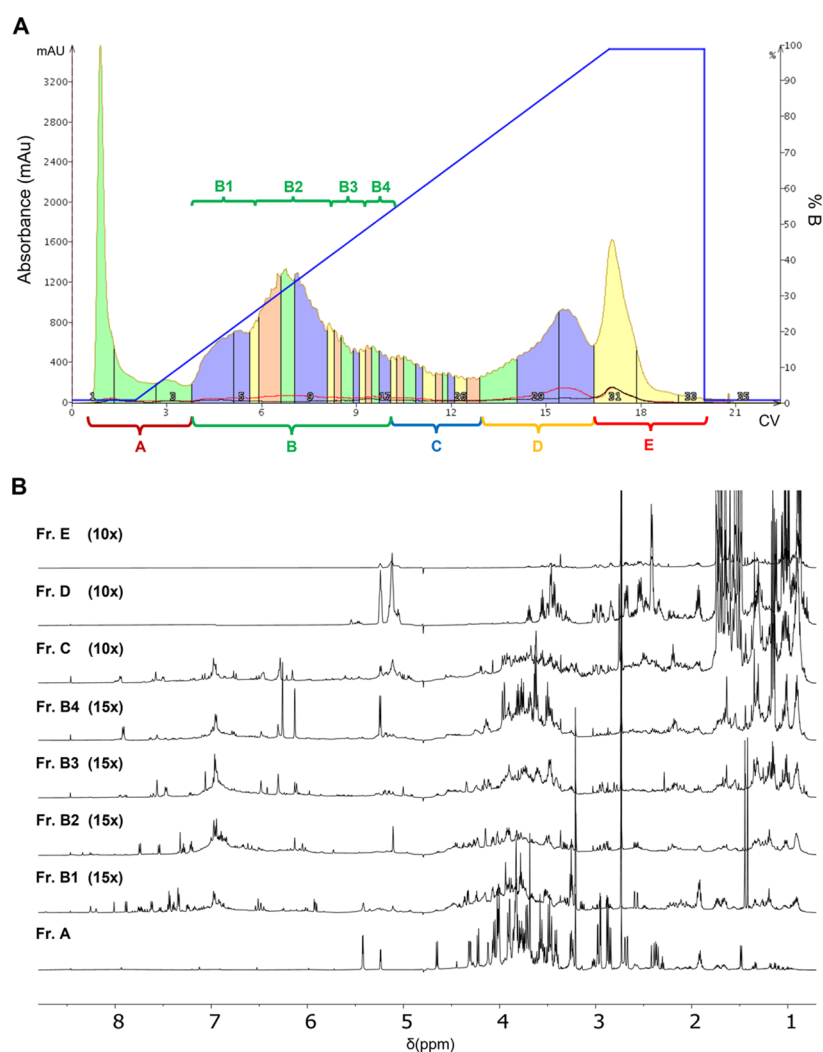


Figure 5. Hop extracts' fractionation. (A) Chromatographic profile of the separation of the HT extract obtained by reverse-phase C18 chromatography (linear elution gradient from 2 to 100% MeOH in 15 CV). (B) ^1H NMR spectra of the chromatographic fractions A–E. ^1H NMR spectra were recorded on 2 mg/mL samples dissolved in D_2O , 25 $^\circ\text{C}$, at 600 MHz. The intensity ratios with respect to spectrum A, which has the highest signal-to-noise ratio, are shown in brackets. CV, column volume.

peptide's ability to aggregate. Once again, HT proved to be the most effective extract as no aggregates were visible after treatment with this sample. Nevertheless, also the other hops exhibit significant inhibitory activity since, when present, the small quantity of aggregates had an amorphous morphology. Fibrils or protofibrils were not found in any of the samples when the $\text{A}\beta$ 1-42 peptide was incubated with hop extracts. The incubation with HS produced small aggregates ranging from 5 to 10 nm. The presence of the HT extract afforded the formation of a carpet of unstructured material over the entire surface of the mica and of rare aggregates with dimensions <5 nm. When $\text{A}\beta$ 1-42 was incubated in the presence of HSu, the AFM analysis showed the formation of a hydrated amorphous material formed of rare clusters of dimensions within the range of 20–100 nm but without a defined structure. Moreover, in the presence of HC, we also observed clusters of 200 nm in the absence of structured materials.

We then investigated whether the antiaggregating property of hop extracts translated into a protective effect against $\text{A}\beta$ 1-42 toxicity *in vitro* using the human neuroblastoma SH-SY5Y cell line. To this end, cells were treated for 24 h with 10 μM $\text{A}\beta$ 1-42 peptide in the absence or presence of different

concentrations of hop extracts (Supporting Information, Figure S5). Cell viability was reduced by $\sim 50\%$ by the $\text{A}\beta$ 1-42 peptide, and HS, HT, and HSu counteracted the $\text{A}\beta$ -induced toxicity in a dose-dependent manner. Figure 4C reports the comparison of extracts' effect at 0.5 mg/mL, showing that HC proved significantly less effective than the others.

All hops' samples showed a remarkable trophic effect quite common for natural extracts rich in sugars and other nutrients. To better evaluate their biological activity and to discern the contribution of the different molecular components, we fractionated the total extracts, obtaining samples enriched in the different classes of compounds.

2.3. Extracts' Fractionation and Identification of Polyphenols as the Most Potent Anti- $\text{A}\beta$ Activity. The four extracts were fractionated by C18-reverse-phase chromatography. The example of HT fractionation is depicted in Figure 5A.

After obtaining the chromatographic profile (Figure 5A), fractions A–E were collected and concentrated on the bases of absorbance and then characterized by NMR spectroscopy (Figure 5B and Supporting Information, Figure S6). The

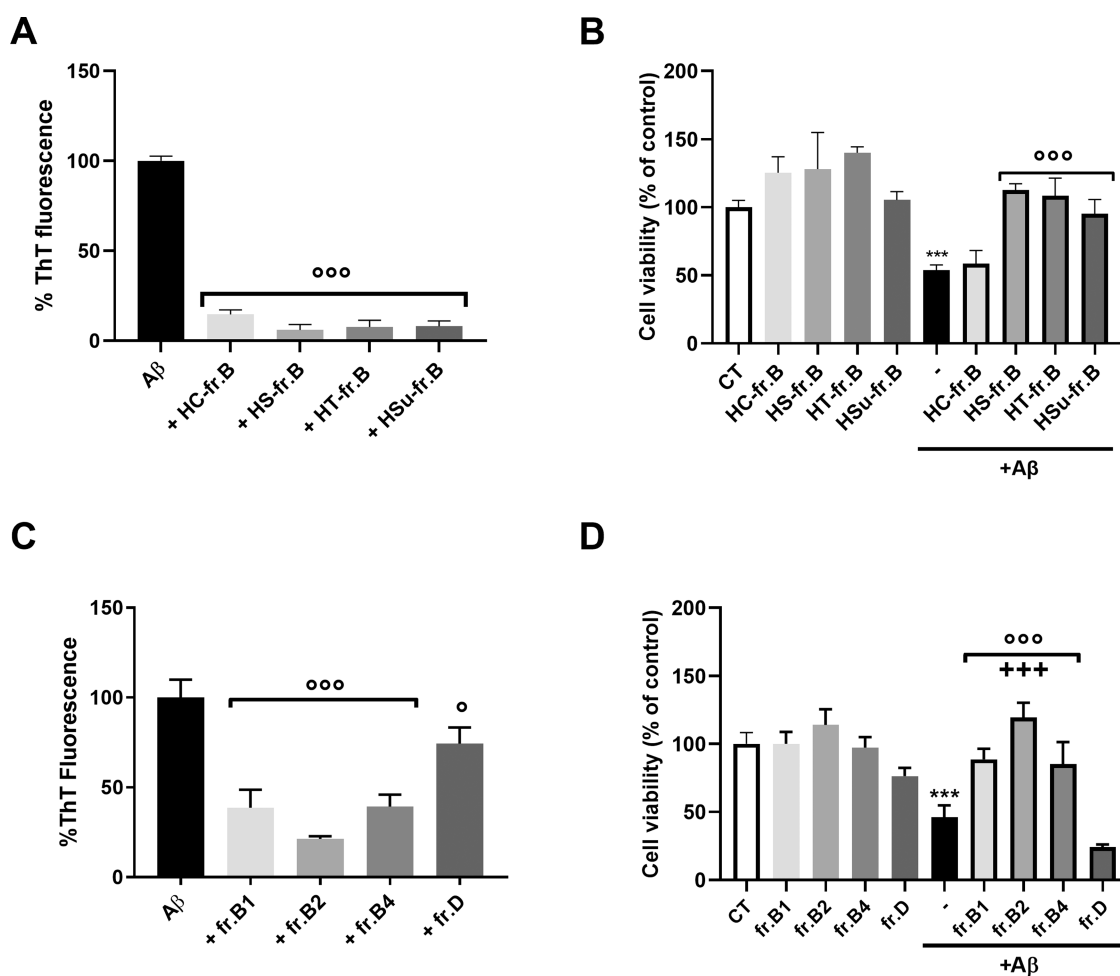


Figure 6. Effect of hop fractions on A β 1-42 aggregation and *in vitro* toxicity. (A, C) Coincubation (24 h) of (A) fr.B from HC, HT, HS, or Hsu (0.03 mg/mL) or (C) HT fr. B1, B2, B4, or D (0.0125 mg/mL) with A β 1-42 (2.5 μ M) reduced the fibrillation determined by the ThT fluorescence assay. Data were expressed as mean \pm SD ($N = 3$), calculated by subtracting the relative control solutions (fraction alone), and were expressed as the percentage reduction of A β 1-42 aggregation, $^{\circ\circ\circ}p < 0.001$ vs A β alone, one-way ANOVA and Dunnett's *post hoc* test. (B, D) Human neuroblastoma SH-SY5Y cells were treated with the A β 1-42 peptide (10 μ M) in the absence or presence of (B) fr. B (0.03 mg/mL) from HC, HT, HS, or Hsu or (D) HT fr. B1, B2, B4, or D (0.125 mg/mL). Control cells were treated with vehicle (CT). Cell viability was determined 24 h later by the MTT assay. Data are the mean \pm SD of the percentage of viable cells ($N = 3$). $^{***}p < 0.001$ A β vs the respective control, $^{\circ\circ\circ}p < 0.001$ A β + fr. B vs A β alone and $^{+++}p < 0.001$ B2 + hop vs B1, B4, and D according to one-way ANOVA and Dunnett's *post hoc* test. HC, Cascade; HS, Saaz; HT, Tettang; Hsu, Summit.

metabolic profiling, in agreement with data reported in specific databases (see Section 2.1) and in the literature^{13–15} about the chemical shift range expected for the different compound classes, suggested that fraction A was enriched in sugars, amino acids, and small organic acids, B in aromatic compounds, D in bitter acids, while C was a mixture of aromatic molecules and bitter acids. Fraction E components show a low solubility in aqueous media, according to its elution with a high percentage of methanol, and we can postulate that this fraction contains mainly resins.

In the light of previous data,^{19,21–28} we speculated that the polyphenolic portion of hop extracts could be the most active one. Therefore, we further fractionated fraction B to ease the identification of the compounds mainly responsible for the antioxidant as well as antiaggregating activity, affording fractions B1–B4. ¹H NMR spectra of all of the HT fractions collected are reported in Figure 5B. The ability of fraction B (fr. B) prepared from the four hops to counteract A β 1-42 aggregation and toxicity was evaluated applying the same experimental approach used to characterize the total extracts

(see Section 2.2). Fr. B, at 0.03 mg/mL, resulted effectively in inhibiting the peptide aggregation (Figure 6A).

Moreover, they protected SH-SY5Y cells from the toxicity induced by 10 μ M A β 1-42 peptide. Similar to what was observed for the HC total extract, HC-fr. B too resulted in the lowest activity, exerted only for a concentration of 0.25 mg/mL (Figure 6B and Supporting Information, Figure S7). Noteworthy, fr. B was more effective than the total hop extracts, being the dose required for the complete recovery of cell vitality lower. In the case of HT, a fr. B concentration of 0.03 mg/mL gave a total inhibition of peptide toxicity vs 0.25 mg/mL for the total extract, confirming that fraction B was enriched in anti-amyloidogenic compounds.

Similar experiments were then performed using HT sub-fr. B1, B2, and B4. Fr. B3 was not considered because its ¹H NMR profile revealed a significant overlapping with B2 and B4 fractions. Fr. D was also assayed to verify the biological activity of hop α -acids. Fr. B1, B2, and B4, at 0.125 mg/mL, hindered both peptide aggregation and cytotoxicity, being fr. B2 the most potent one (Figure 6C,D). Although fr. D, and thus hop

α -acids contained therein, prevented peptide aggregation, albeit to a lesser extent compared to the other fractions, it did not counteract the toxic effect of $A\beta$, probably due to its cytotoxic effect (Figure 6C,D). Notably, fr. B2 reduced the toxicity of the $A\beta$ 1-42 peptide already at the dose of 4 μ g/mL (Supporting Information, Figure S8), which was at a significantly lower concentration than the total HT extract (Figure 4C and Supporting Information, Figure S5). Based on the detailed characterization of fr. B2's chemical composition, we verified that 4-*O*-feruloylquinic acid, 5-*O*-*p*-coumaroylquinic acid, rutin, quercetin-3(6''malonyl)-neohesperioside, and B-type procyanidin dimers are the main molecular components of this fraction. The anti- $A\beta$ activity described so far can therefore be mainly ascribed to these classes of molecules. Notably, fr. B2 as well as the total fr. B also showed an increase in the *in vitro* antioxidant activity (Supporting Information, Figure S9) when compared with the total extract (Figure 3).

Together, these data indicated polyphenols depicted in Figure 2B as the most potent antiamyloidogenic components of hop extracts, also endowed with a remarkable antioxidant power.

2.4. Main Polyphenolic Components of Hop Extracts Directly Interact with $A\beta$ 1-42 Oligomers. We investigated whether the protective activity of hop extracts can be related to the ability of polyphenols to directly interact with $A\beta$ performing ligand–receptor interaction studies. To this end, saturation-transfer difference (STD) NMR,^{29,30} a very powerful and versatile technique employed for the screening of $A\beta$ ligands, was used. We have already applied it to the screening of pure compounds,^{23,31} small compound libraries,³² or complex mixtures.^{19,21,22,24–26,31} Thus, we carried out STD experiments on a mixture containing the $A\beta$ 1-42 peptide and fr. B2 from HT (Figure 7).

The presence of $A\beta$ oligomers in the NMR sample was assured by dissolving the $A\beta$ 1-42 peptide in an aqueous PB (see ref 31 for details). The sample was irradiated at -1.00 ppm (on-resonance frequency) to selectively saturate some aliphatic protons of $A\beta$ oligomers. The $A\beta$ ligand(s) in solution received magnetization from the receptor, and ligand(s) NMR signals appeared in the STD spectrum (Figure 7, spectrum D). Aromatic compounds' signals appearing in the STD spectrum (Figure 7, spectrum D) supported their direct interaction with $A\beta$ 1-42 oligomers. A blank experiment was acquired under the same experimental conditions on a sample containing only fr. B2 to confirm that signals in the STD spectrum were due to real ligand binding events (Figure 7, spectrum B).

Due to signal overlapping, the univocal assignment of compound resonances was not possible and thus the unambiguous identification of $A\beta$ 1-42 oligomers' ligands. However, the STD experiment suggested that CGAs and several flavonoids, also in glycosylated and polymeric forms (procyanidins), bind $A\beta$ oligomers. Some of their resonances are labeled in Figure 7D and were assigned after comparison with STD spectra obtained in previous works on pure flavonoids²³ or complex mixtures from natural extracts.^{19,22,24–26,28} The presence of these species in our sample is supported by MS analysis (Figure 2 and Supporting Information, Table S2).

Collectively, NMR binding studies and biological assays suggest that the species responsible for the antiamyloidogenic

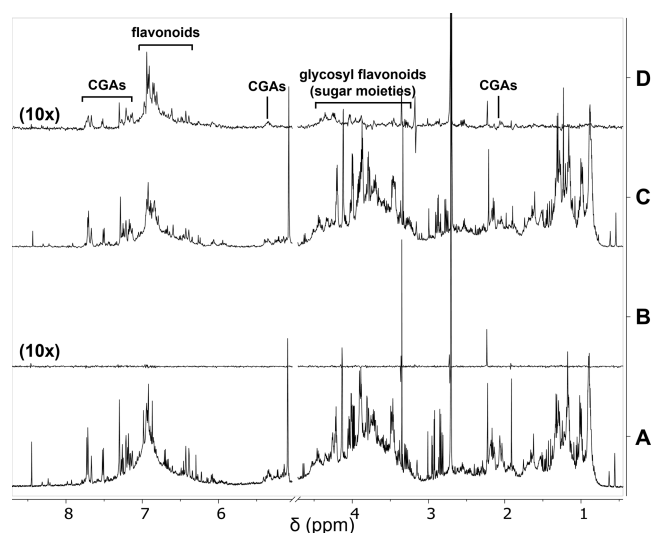


Figure 7. NMR binding studies with $A\beta$ 1-42. (A) Off-resonance NMR spectrum of a solution containing HT extract fr. B2 (4 mg/mL). (B) STD NMR spectrum of the same sample of A. (C) Off-resonance NMR spectrum of a solution containing HT extract fr. B2 (4 mg/mL) and the $A\beta$ 1-42 peptide (120 μ M). (D) STD NMR spectrum of the same sample of C. Samples were dissolved in deuterated PB, pH 7.4. STD spectra were acquired with 1024 scans and 2 s saturation time at 600 MHz, 25 °C. The intensity ratios with respect to spectrum A, which has the highest signal-to-noise ratio, are shown in brackets. HT, Tettwang.

activity of hop extracts are glycosylated flavonoids, procyanidins, and CGAs.

2.5. Hop Extracts Potentiate Autophagy in SH-SY5Y Cells. Accumulating evidence indicates that, among other mechanisms, impaired autophagy, including bulk and selective autophagy, plays a crucial role in AD pathogenesis.³³ Age-dependent increase of $A\beta$ levels reduces the expression of several autophagic proteins and causes autophagy defects.³⁴ These defects, in turn, are responsible for an abnormal accumulation of neurotoxic proteins, including $A\beta$, which cannot be correctly degraded through autophagy in a deleterious self-amplifying vicious cycle.

As the upregulation of autophagy represents a promising therapeutic strategy to potentiate the clearance and avoid the accumulation of toxic proteins, identifying new compounds potentially able to induce autophagy is of great interest in AD prevention and therapy. For this reason, in this study, we investigated in SH-SY5Y cells a possible influence of hop extracts on the two main autophagic pathways involved in $A\beta$ clearance, macroautophagy (bulk autophagy) and chaperone-mediated autophagy (CMA, selective autophagy). To this aim, SH-SY5Y cells were exposed to 0.1 mg/mL HT extract for 24 h, and gene and protein levels of key macroautophagy (beclin-1, LC3, and p62) and CMA (lamp2A and hsc70) markers were quantified (Figure 8A–C). Figure 8A shows that a 24 h exposure to HT extract significantly activates the transcription of genes involved in both macroautophagy (increased mRNA levels of beclin-1, LC3, and p62) and CMA (increased mRNA levels of the CMA receptor lamp2A); no effect of the HT extract was evidenced on the expression of the CMA carrier hsc70HT.

Extract exposure also activates the transcription of the neurotrophic factor brain-derived neurotrophic factor (BDNF), further confirming the protective effect of the tested

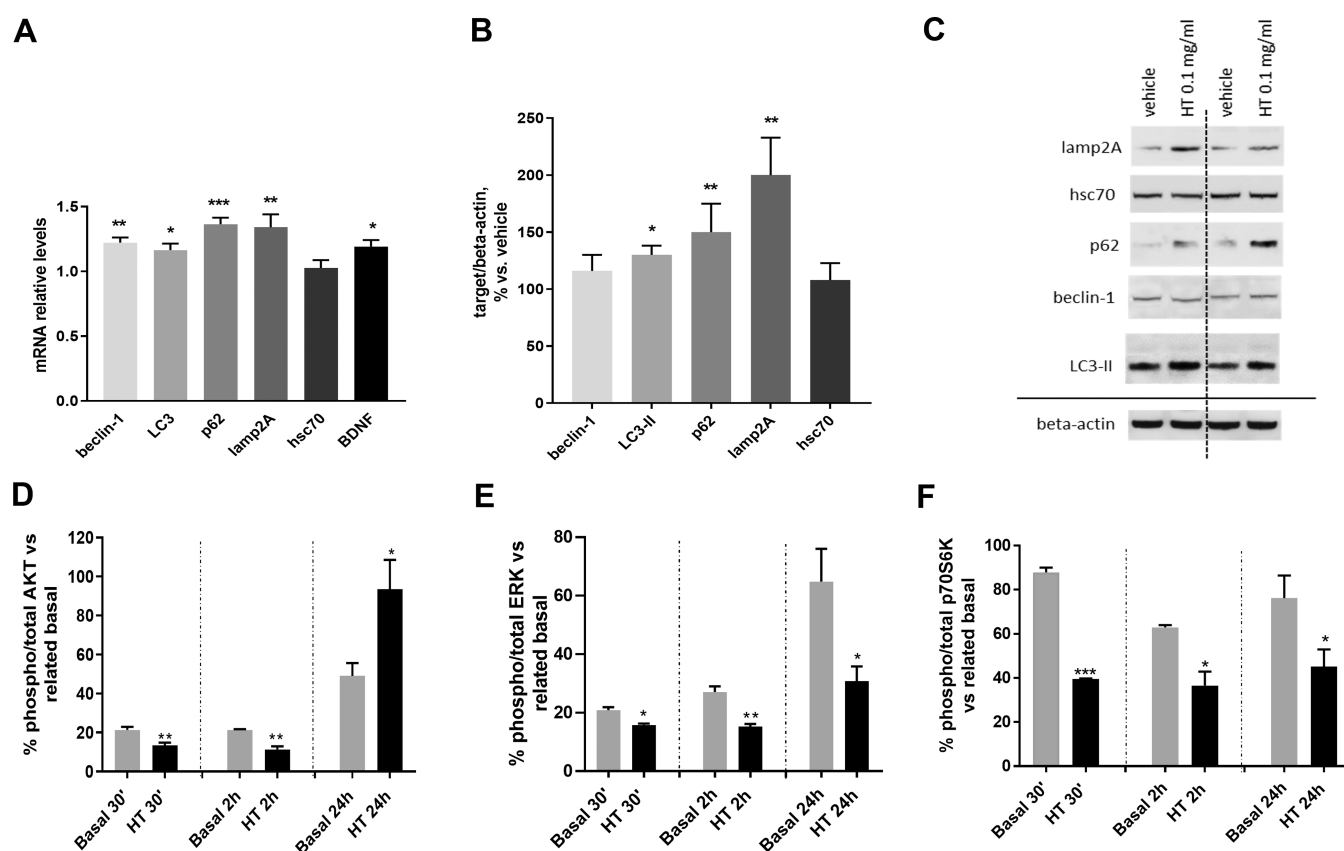


Figure 8. Effect of HT extracts on autophagy markers and related kinase regulatory pathways (PI3K/AKT and ERK1/2) in human SH-SY5Y cells. (A) Relative quantification, calculated as the ratio to β -actin, of mRNA levels of macroautophagy (beclin-1, LC3, and p62) and CMA (lamp2A, hsc70) markers and BDNF after 24 h treatment with 0.1 mg/mL HT extract. Two-tailed paired *t*-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs vehicle. (B) Protein expression of macroautophagy (beclin-1, LC3-II, and p62) and CMA (lamp2A, hsc70) markers after 24 h treatment with 0.1 mg/mL HT extract and (C) representative Western blot image showing immunoreactivity for the target proteins and the corresponding β -actin, used as the internal standard. Two-tailed paired *t*-test; * $p < 0.05$, ** $p < 0.01$ vs vehicle. Time course of the phosphorylation status of AKT (D), ERK1/2 (E), and p70S6K (F) kinases after 30 min, 2 h, and 24 h treatment with 0.1 mg/mL HT extract. Values represent the percentage of the ratio between the phosphorylated and total kinase levels. Student's *t*-test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs related basal.

extracts. Exposure to this extract leads to a significant increase in autophagic protein levels (increased LC3-II, p62, and lamp2A), as displayed in Figure 8B,C. Collectively, these results indicate that hop extracts induce the expression of multiple autophagic genes and increase autophagic proteins. The elevation of autophagic proteins can activate or facilitate the autophagic processes in cells, contributing to preventing proteotoxicity and, as a consequence, the onset or progression of neurodegeneration.

To further deepen the intracellular mechanisms involved in the observed hop-induced autophagy upregulation, the phosphorylation levels of the main kinase pathways (PI3K/AKT and ERK1/2) involved in autophagy regulation were analyzed. The phosphorylation of p70S6K was also evaluated. p70S6K is a downstream effector of PI3K/AKT and ERK1/2, an mTOR-dependent autophagy hallmark that correlates with autophagy inhibition.³⁵

Considering that the phosphorylation status of kinases can change in a time-dependent way, SH-SY5Y cells were exposed to HT extracts (0.1 mg/mL) for different time points (30 min, 2 h and 24 h). Short-term HT treatments (30 min and 2 h) reduced the phosphorylation of AKT ($p < 0.01$) (Figure 8D), ERK ($p < 0.05$) (Figure 8E), and p70S6K (Figure 8F) ($p < 0.001$ for 30 min; $p < 0.05$ for 2 h). The downmodulation of these signaling pathways results in the decrease of the mTOR

inhibitor effect on the macroautophagy, thus causing its induction. Furthermore, p-AKT downregulation is also able to stimulate chaperone mediated autophagy (CMA).³⁶ Following 24 h HT treatment, p-ERK (Figure 8E) and p-p70S6K (Figure 8F) were also decreased ($p < 0.05$), confirming the autophagy induction. On the other hand, HT exposure for 24 h increased the p-AKT (Figure 8D, $p < 0.05$); this might result in apoptosis downregulation because AKT is also involved in cell survival.

Thus, we can speculate that, in addition to the direct effect on $A\beta$ aggregation, hop might protect against $A\beta$ neurotoxicity through the regulation of ERK1/2 and PI3K/AKT cell signaling, promoting $A\beta$ catabolism through autophagy activation.

2.6. Hop Extract Protected from $A\beta$ -Induced Toxicity *In Vivo*. The anti-AD activity of hop was characterized by testing their effectiveness *in vivo* in the model organism *C. elegans*. Transgenic nematodes expressing human $A\beta$ are widely employed to investigate the ability of compounds to counteract the proteotoxic activity before planning preclinical studies in vertebrate animals.^{37,38} The protective effect of HT on $A\beta$ -induced toxicity was evaluated by employing CL2006 transgenic worms, in which the paralysis phenotype is caused by the deposition of both oligomeric and fibrillar $A\beta$ 3-42 in the body muscle cells.³⁷ The worms, in the L3 larval stage, were treated

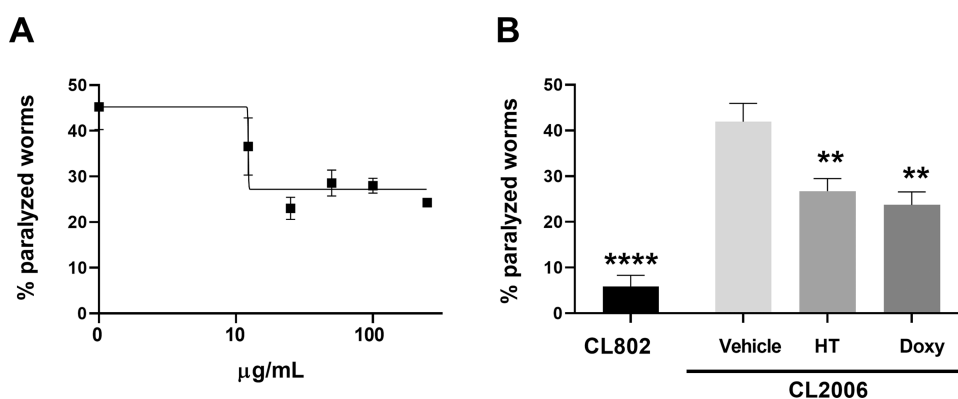


Figure 9. HT protected CL2006 worms from paralysis caused by $A\beta$ expression. (A) Dose-response effect of HT on the paralysis of CL2006 worms. Synchronized CL2006 worms were treated in the L3 larval stage with different concentrations (10–250 $\mu\text{g/mL}$) of HT dissolved in water. Control worms were treated with the same volume of water only (Vehicle). Paralysis was scored 120 h after treatment. (B) Synchronized CL2006 worms were treated in the L3 larval stage with 50 $\mu\text{g/mL}$ HT dissolved in water. CL2006 worms were treated in the same experimental conditions with 100 μM doxycycline (Doxy) dissolved in water as a positive control. CL802 and CL2006 worms were treated with the same volume of water only (CL802 and Vehicle, respectively) as negative controls. Paralysis was scored 120 h after treatment. **** $p < 0.0001$ and ** $p < 0.1$ vs CL2006 treated with Vehicle according to one-way ANOVA and Bonferroni's *post hoc* test. HT, Tettang.

with different concentrations (10–250 $\mu\text{g/mL}$) of HT dissolved in water, and their paralysis was scored 120 h later. Control worms were treated with the same volume of water only (Vehicle). We compared the effect of HT to that of doxycycline (Doxy), a tetracycline with a known antibiotic activity that also possesses pleiotropic effects against various amyloidogenic proteins and has already been described to be able to protect CL2006 worms against $A\beta$ -induced toxicity.³⁷ HT protected CL2006 worms from $A\beta$ -induced paralysis in a dose-dependent manner starting from 10 $\mu\text{g/mL}$, and the IC50 value was calculated to be 12.37 $\mu\text{g/mL}$ (Figure 9A).

At the optimal concentration of 50 $\mu\text{g/mL}$, HT reduced the CL2006 worms' paralysis by 36.3% ($41.9 \pm 4.1\%$ of paralyzed worms for vehicle-fed CL2006 and $26.7 \pm 2.7\%$ for HT-fed worms) (Figure 9B).

Although the extract did not restore the percentage of CL2006 paralyzed worms at a level comparable to that scored for CL802 worms that did not express $A\beta$ ($5.80 \pm 1.22\%$ of paralyzed worms for vehicle-fed CL802), its protective effect was comparable to that of 100 μM Doxy, which reduced the paralysis of CL2006 of 43.1% (Figure 9B). HT at 50 $\mu\text{g/mL}$ had no effects in transgenic CL802 worms that were used as control strains (data not shown).

3. EXPERIMENTAL SECTION

3.1. Preparation of Hop Extracts. Hop (*H. lupulus* L.) pellet samples of four different varieties (Cascade, Saaz, Tettang, and Summit) were selected according to their widespread use in brewing production and obtained from a local beer producer (Menaresta Brewery, Carate Brianza, Italy). Hop pellets (2 g) were extracted in (a) boiling water (200 mL) for 2 h or (b) hydroalcoholic solution (10% ethanol in water) at 30 °C for 24 h under magnetic stirring. The suspension was filtered on a cotton and paper filter (Whatman grade 1, pore size 11 μm) and, finally, the permeate was concentrated under reduced pressure. Residues were freeze-dried, and samples were stored at -20 °C until use.

3.2. Preparation of Polyphenol-Enriched Fractions.
3.2.1. Preparative Reverse-Phase Column Chromatography. Automated flash chromatography was performed on a Biotage Isolera Prime system equipped with a Spektra package (Biotage AB, Uppsala, Sweden). A solution of the extract sample (260 mg in 2.5 mL of MeOH) was loaded in a precolumn sample SNAP-C18 (1 g) and left for air-drying overnight. Column chromatography was performed on a

SNAP KP-C18-HS (12 g) cartridge using water (solvent A) and methanol (solvent B) as eluent solvents. A linear elution gradient was applied (2% B for 2 CV, 2 to 100% of B in 12 CV, and 100% B for 3 CV) at a flow rate of 12 mL/min. The eluate was automatically collected in fractions based on the photodiode array detector signal (range 200–400 nm, monitor $\lambda_1 = 280$ nm, $\lambda_2 = 320$ nm). Fractions were pooled in homogeneous groups, the organic solvent was removed under reduced pressure, and residues were freeze-dried, obtaining fractions A, B1–B4, C, D, and E.

3.3. Extracts' Chemical Characterization.
3.3.1. NMR Spectroscopy. Freeze-dried samples were suspended in deuterated phosphate buffer d-PB 10 mM at a final concentration of 25 mg/mL, sonicated (37 kHz, 10 min, Elmasonic P 30H, Elma Schmidbauer GmbH, Singen, Germany), and centrifuged (15,000 rpm, 15 min, 20 °C, ScanSpeed 1730R Labogene, Lyngø, Sweden). 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS, final concentration 0.5 mM) was added to the supernatant as an internal reference for concentrations and chemical shift. The pH of each sample was verified with a microelectrode (Mettler Toledo, Columbus, OH) and adjusted to 7.4 with NaOD or DCl. All pH values were corrected for the isotope effect. All spectra were acquired on an Avance III 600 MHz NMR spectrometer (Bruker, Billerica, MA) equipped with a QCI (^1H , ^{13}C , $^{15}\text{N}/^{31}\text{P}$, and ^2H lock) cryogenic probe. ^1H NMR spectra were recorded with *noesygppr1d*, *cpmgpr1d*, and *ledbpgppr2s1d* pulse sequences in the Bruker library, with 256 scans, spectral width 20 ppm, and relaxation delay 5 s. The acquisition temperature was 25 °C. They were processed with 0.3 Hz line broadening, automatically phased, and baseline-corrected. Chemical shifts were internally calibrated to the DSS peak at 0.0 ppm. The ^1H , ^1H -TOCSY (Total Correlation Spectroscopy) spectra were acquired with 48 scans and 512 increments, a mixing time of 80 ms, and a relaxation delay of 2 s. ^1H , ^{13}C -HSQC (Heteronuclear Single Quantum Coherence) spectra were acquired with 32 scans and 512 increments, with a relaxation delay of 2 s. NMR spectra processing and peak peaking were done using the MNova software package of Mestrelab (MestReNova v 14.2.1, 2021, Mestrelab Research, Santiago de Compostela, Spain).

3.3.2. UPLC Coupled with ESI-HR-MS Spectrometry. UPLC/ESI-HR-MS analysis was carried out by coupling an Acquity UPLC separation module (Waters, Milford, MA) with an in-line photodiode array (PDA) e λ detector (Waters) to a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer and a HESI-II probe for electrospray ionization (Thermo Scientific, San Jose, CA). The ion source and interface conditions were spray voltage $+3.5/-3.5$ kV, sheath gas flow 35 arbitrary units, auxiliary gas flow 15 arbitrary units, vaporizer temperature 300 °C, and capillary temperature 350 °C. Positive mass calibration was performed with Pierce LTQ ESI Positive Ion

Calibration Solution (Thermo Scientific Pierce, Rockford, IL), containing caffeine, the tetrapeptide MRFA, and Ultramark 1621. Negative mass calibration was performed with the Pierce ESI Negative Ion Calibration Solution (Thermo Scientific Pierce), containing sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621. A sample quantity of 2 μL (2 mg/mL crude extract or 1 mg/mL enriched fraction diluted in water) was separated using a Waters Acquity BEH C18 column (150 mm \times 2.1 mm, 1.7 μm , 130 Å) (Waters, Milford, MA) kept at 40 °C and using 0.1 mL of 100 mL⁻¹ formic acid in H₂O_{MilliQ} (solvent A) and 0.1 mL of 100 mL⁻¹ formic acid in acetonitrile (solvent B). For UPLC separation, a linear elution gradient was applied (isocratic 5% B for 5 min and then 5 to 50% of solvent B in 15 min) at a flow rate of 0.2 mL/min. The LC eluate was analyzed by full MS and data-dependent tandem MS analysis (dd-MS²) of five of the most intense ions (Top 5). The resolutions were set at 70,000 and 17,500 and the AGC targets were 1 \times 10⁶ and 1 \times 10⁵ for full MS and dd-MS² scan types, respectively. The maximum ion injection times were 50 ms. The MS data were processed using Xcalibur software (Thermo Scientific) and Mnova MS plug-in (MestreNova 14.2.1, Mestrelab). Metabolites were determined according to their calculated exact mass and absorption spectra. Their structures were confirmed by high-resolution tandem MS (HR-MS/MS) compared to reported assignments in the literature or databases.

3.3.3. Antioxidant Activity. The antioxidant activity of the extracts was evaluated as the mean of the total polyphenols (or total reducing power) and radical scavenging ability and measured by three spectrophotometric methods. Preliminarily, the UV–vis absorbance profile was determined. Each extract was dissolved at 80 $\mu\text{g/mL}$ in bidistilled water, and the spectra were recorded at room temperature. Absorbance was measured with a Varian Cary 50 Scan UV–visible spectrophotometer (Agilent, Santa Clara, CA) using disposable polymethyl methacrylate (PMMA) or quartz semimicro 10 mm cuvettes relative to a blank solution.

The total polyphenol content (or total reducing power) was determined with the Folin Cioaltea assay, as previously reported.³⁹ Briefly, 80 μL of diluted samples (or standards/blank) and 40 μL of Folin's reagent (Sigma-Aldrich, St. Louis, MO) were dispensed in a cuvette containing 400 μL of MilliQ water. Then, 480 μL of Na₂CO₃ 10.75% (w/v) solution was added, and after 30 min of incubation at room temperature, the absorbance was read at 760 nm. Samples were diluted to 1 mg/mL, and standard solutions (0–200 $\mu\text{g/mL}$) of gallic acid were used for calibration (linear fitting $R^2 > 0.99$, $N = 7$). Results were expressed as mg of gallic acid equivalent (GAE eq) per g of freeze-dried hop extract.

The radical scavenging ability of the extract was determined by ABTS-TEAC and DPPH assays. The ABTS-TEAC assay is based on the evaluation of the scavenging capacity of an antioxidant to the long-life colored cation ABTS⁺.⁴⁰ Briefly, a 7 mM stock solution of ABTS⁺ was produced by mixing equal amounts of a 14 mM ABTS solution and a 4.9 mM K₂S₂O₈ solution in MilliQ water (final concentrations 7.00 and 2.45 mM, respectively). The mixture was left at room temperature in the dark for at least 12–16 h before use and stored at 4 °C for 7 days. A working solution of ABTS⁺ was prepared daily by diluting the stock solution (1:50), reaching 0.70 \pm 0.05 absorbance at 734 nm. A total of 50 μL of the sample (or standards) was added in a cuvette containing 950 μL of ABTS⁺ solution, and the absorbance at 734 nm was read after 30 min of incubation at room temperature. Samples were diluted to 1 mg/mL, and standard solutions (0–500 μM) of Trolox were used for calibration (linear fitting $R^2 > 0.99$, $n = 7$).

The DPPH assay is based on the scavenging of the stable free-radical 2,2-diphenyl-1-picrylhydrazyl, according to the literature.⁴¹ Briefly, 950 μL of a diluted solution of DPPH in buffered MeOH (100 μM in a mixture of 60% MeOH and 40% acetate buffer, pH 4.5, Abs 0.70 \pm 0.05) and 50 μL of a diluted sample (or standard) were placed in a cuvette. The absorbance at 517 nm was read after 30 min of incubation at room temperature. Samples were diluted to 1 mg/mL, and standard solutions (0–500 μM) of Trolox were used for calibration (linear fitting $R^2 > 0.99$, $n = 7$). Results of both radical

scavenging assays were expressed as μmol of Trolox equivalent (TE) per g of freeze-dried hop extract.

3.3.4. A β Peptide Synthesis. A β 1-42 (DAEFRHDS-GYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA) was prepared by solid-phase peptide synthesis (SPPS) on a 433A Syro I synthesizer (Applied Biosystems, Foster City, CA) using Fmoc-protected L-amino acid derivatives, NovaSyn-TGA resin (Novabiochem, Sigma-Aldrich, St. Louis, MO), and a 0.1 mM scale. The peptide was cleaved from the resin as previously described⁴² and purified by reverse-phase HPLC on a semipreparative C4 column (Waters, Milford, MA) using a water/acetonitrile gradient elution. Peptide identity was confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis (model Reflex III, Bruker, Billerica, MA). The purity of peptides was always above 95%.

3.3.5. Thioflavin T Binding Assay. A β 1-42 was dissolved in 10 mM NaOH, H₂O, and 50 mM PB (1:1:2) to 2.5 μM with or without a defined concentration of hop extracts (0.25 mg/mL) or enriched fractions (0.03 mg/mL for fraction B and 0.0125 mg/mL for fractions B1, B2, B4, and D) and were incubated at 37 °C in 20 μM ThT (Sigma-Aldrich, St. Louis, MO) in 96-well black plates (Isoplate, Perkin Elmer, Waltham, MA). The ThT fluorescence was monitored for 24 h with a plate reader (Infinite F500 Tecan: excitation 448 nm, emission 485 nm, 37 °C). Data were expressed as the mean of three replicates, calculated by subtracting the relative control solutions (extract or fraction alone), and were expressed as the percentage reduction of A β 1-42 aggregation.

3.3.6. Atomic Force Microscopy (AFM). A β 1-42 was dissolved as previously described to 2.5 μM with or without a hop extract (0.25 mg/mL) and incubated in quiescent conditions at 37 °C for 24 h. After the incubation, 30 μL of samples was spotted onto a freshly cleaved Muscovite mica disk and incubated for 7 min. The excess sample on the disk was washed with 10 mL of MilliQ water and dried under a gentle nitrogen stream. Samples were mounted onto a Multimode AFM with a NanoScope V system (Veeco/Digital Instruments, Plainview, NY) operating in the tapping mode, and measurements were made using 0.01–0.025 Ω/cm antimony-doped silicon probes (T: 3.5–4.5 μm , L: 115–135 μm , W: 30–40 μm , k: 20–80 N/m, f₀: 323–380 kHz; Bruker AFM probes) with a scan rate in the 0.5–1.2 Hz range, proportional to the area scanned. Measurements confirmed all of the topographic patterns in at least four separate areas. To exclude interference from any artifacts, freshly cleaved mica DISCS soaked with 30 μL of PB 50 mM were also analyzed as controls. Samples were analyzed with the Scanning Probe Image Processor (SPIP Version 5.1.6 released April 13, 2011) data analysis package.

3.4. NMR Interaction Studies. To obtain samples containing A β oligomers, lyophilized A β 1-42 was dissolved in 10 mM NaOD and then diluted 1:1 with 20 mM deuterated PB (pH 7.4) to a final concentration of 120 μM and in the presence of hop extract-enriched fractions (4 mg/mL). The pH of each sample was measured with a Microelectrode (InLab Micro, Mettler Toledo, Columbus, OH) and adjusted to pH 7.4 with NaOD and/or DCl. All pH values were corrected for the isotope effect. Experiments were run on an AVANCE III 600 MHz NMR spectrometer (Bruker, Billerica, MA) equipped with a QCI (¹H, ¹³C, ¹⁵N/³¹P, and ²H lock) cryogenic probe. A basic sequence from the Bruker library was employed for the STD experiments. A train of Gaussian-shaped pulses of 50 ms each was employed to saturate the protein envelope selectively; the total saturation time of the protein envelope was adjusted to the number of shaped pulses and set at 2 s. On- and off-resonance spectra were acquired in an interleaved mode with the same number of scans. The STD NMR spectrum was obtained by subtracting the on-resonance spectrum from the off-resonance spectrum.

3.5. In Vitro Studies. **3.5.1. Cytotoxicity Assay.** Human neuroblastoma SH-SY5Y cell line was grown in Dulbecco's modified Eagle's medium (DMEM, Lonza, Basel, Switzerland) supplemented with L-glutamine (2 mM, Gibco, Invitrogen, Waltham, MA), antibiotics (penicillin/streptomycin 10,000 U, Lonza, Basel, Switzerland), and 10% heat-inactivated fetal calf serum (FCS, Gibco, Invitrogen, Waltham, MA).

To assess $A\beta$ -induced cytotoxicity, SH-SY5Y cells were seeded in 96-well plates (10^5 cell/mL) and incubated overnight (37°C , in a humidified 5% CO_2 atmosphere). The medium was then replaced with 1% FCS in DMEM to reduce cell growth. $A\beta$ 1-42 was dissolved in 10 mM NaOH, H_2O , and phosphate-buffered saline (PBS) (1:1:2) and added to the hop extract or fractions to obtain a final concentration of 10 μM for $A\beta$ 1-42 in the well. Cytotoxicity was evaluated after 24 h incubation using the MTT reduction assay. Tetrazolium solution (20 μL of 5 mg/mL, Sigma-Aldrich, St. Louis, MO) was added to each well and incubated for 4 h. The medium was replaced with acidified isopropanol (0.04 M HCl) to dissolve the purple precipitate, and the absorbance intensity was measured at 570 nm using a plate reader (Infinite M200, Tecan, Männedorf, Switzerland). Data were expressed as control (Vehicle) percentages for three separate replicates.

3.5.2. Assessment of Autophagy Markers. Gene and protein expressions of autophagy markers were assessed by real-time quantitative PCR (qPCR) and Western blot, respectively, at the conditions recently published.²⁶ Briefly, for qPCR assays, after extraction, total RNA (2 μg) was retrotranscribed into cDNA and amplified (50 ng for beclin-1, LC3, p62, Hsc70, and BDNF and 100 ng for Lamp2A) in triplicate in the ABI Prism 7500 HT Sequence detection system (Applied Biosystems) using the primers listed in Table 1.

Table 1. Sequences of Primers Used (Sigma-Aldrich)

target		sequence
beclin-1	F	ATCTCGAGAAGGTCCAGGCT
	R	CTGTCCACTGTGCCAGATGT
LC3	F	CAGCATCCAACCAAAATCCC
	R	GTTGACATGGTCAGGTACAAG
p62	F	CCAGAGAGTTCAGCACAGA
	R	CCGACTCCATCTGTTCTCA
lamp2A	F	GCAGTGCAGATGAAGACAAC
	R	AGTATGATGGCGCTTGAGAC
Hsc70	F	CAGGTTTATGAAGGCGAGCGTGCC
	R	GGGTGCAGGAGGTATGCCTGTGA
BDNF	F	TGGCTGACACTTTCGAACAC
	R	AGAAGAGGAGGCTCCAAAGG
β -actin	F	TGTGGCATCCACGAAACTAC
	R	GGAGCAATGATCTTGATCTTCA

The comparative CT method was used to quantify mRNA levels of each target vs β -actin, used as the housekeeping gene.

For Western blot analysis, after denaturation, samples were separated by SDS-PAGE in 4–12% tris glycine gels (Invitrogen) and transferred to nitrocellulose. After blocking, the membranes were incubated overnight at 4°C with specific primary antibodies (beclin-1, Cell Signaling, 1:1000 dilution; LC3B, Cell Signaling, 1:500 dilution; p62, Cell Signaling, 1:1000 dilution, Lamp2A, Abcam, 1:900 dilution; Hsc70, Abcam, 1:3000 dilution) and then with HRP-linked antimouse or antirabbit IgG antibody (Sigma-Aldrich) for 1 h. Signals were revealed by chemiluminescence, detected using the ImageQuant 800 (Amersham) imaging system, and quantified using ImageJ software. Protein expression was calculated as the ratio between optical densities of the target protein and the internal standard (β -actin, Sigma-Aldrich, 1:40,000 dilution) and expressed as a percentage vs the vehicle-treated cells.

3.5.3. Total- and Phospho-ELISA for ERK, AKT, and p70S6K. To detect and quantify the levels of AKT (total/phosphor), ERK1/2 (total/phosphor), and p70S6K (total/phosphor) in protein lysates, we used immunoassay kits (InstantOne ELISA kit Invitrogen Carlsbad, California) according to the manufacturer's instructions. Cytosol protein extractions were performed in a cell extraction buffer (Biosource Thermo Fisher Scientific, Waltham, MA), containing 1 mM PMSF, protease, and phosphatase inhibitor cocktail (1:200 and 1:100, respectively; Sigma-Aldrich St. Louis, Missouri) for 30 min, on

ice. Then, lysates were centrifuged at 12,000 g for 10 min at 4°C . Protein concentration was determined by the Bradford assay at 595 nm. Protein lysates were diluted 1:20. The results were expressed as the ratio between the phosphorylated/total kinase status. The absorbance was determined by plate reading at 450 nm Fluo Star OMEGA (BMG Labtech, Germany).

3.6. In Vivo Studies. **3.6.1. Mobility Assay in *C. elegans*.** The transgenic *C. elegans* CL2006 produced $A\beta$ 3-42 in the body-wall muscles and contained the dominant mutant collagen [rol-6 (su 1066)] as the morphological marker. CL802 worms were used as control strains. Nematodes were obtained from the Caenorhabditis Genetic Center (CGC, University of Minnesota) and were propagated at 16°C on a solid nematode growth medium (NGM) seeded with *Escherichia coli* (OP50) for food (obtained from CGC). To prepare age-synchronized animals, the nematodes were transferred to fresh NGM plates on reaching maturity at 3 days of age and allowed to lay eggs overnight. Isolated hatchlings from the synchronized eggs (day 1) were cultured on fresh NGM plates (50 worms/plate) at 16°C . In the L3 larval stage, the worms were fed with 10–250 $\mu\text{g}/\text{mL}$ HT dissolved in water (50 $\mu\text{L}/\text{plate}$), and paralysis was evaluated 120 h later. In the same experimental conditions, 100 μM doxycycline (Doxy), dissolved in water, was administered to worms as positive controls.³⁷ CL802 and CL2006 worms were treated with water only as controls (50 $\mu\text{L}/\text{plate}$).

3.7. Statistical Analysis. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software). Data are shown as mean \pm standard deviation (SD). For *in vitro* experiments, a two-tailed *t*-test was used to assess the significance of differences between two groups. Repeated-measures ANOVA, followed by Dunnett's multiple comparison test, was used to assess the significance of differences among more than two groups. The effects of Vehicle and extracts on *in vivo* experiments were compared by the one-way ANOVA test and the Bonferroni *post hoc* test. The IC50 value was determined using GraphPad Prism 8. A *p* < 0.05 was considered statistically significant.

4. CONCLUSIONS

The identification of natural compounds or natural mixtures, such as nutraceuticals, exploitable for the development of preventive strategies against AD (and other NDs) appears as a better alternative to the treatment of symptoms, as the neuronal damage associated with the disease is irreversible.² Different biochemical hallmarks of AD can be targeted, among which are $A\beta$ peptides and their amyloid aggregates, oxidative stress, and the accumulation of misfolded peptides and proteins.

Given the precocity concerning the onset of symptoms with which preventive treatments must take place, diet can represent a very effective approach. For this reason, we are exploring food matrices in search of multitarget molecules capable of simultaneously interfering with the processes reported above.

Here, we report the screening of four different hop varieties, Cascade, Saaz, Tettnang, and Summit, whose relevance from a nutraceutical point of view derives from the use of this ingredient in the preparation of beer, as well as herbal teas and infusions. The analysis and comparison of the four varieties increased the chemical space explored, raising the possibility of identifying natural compounds with the biological activities of interest.

To dissect the neuroprotective effects of hops and their main constituents, we fractionated the extracts to identify a pool of molecular components mainly responsible for their neuroprotective action. According to our data, they are feruloyl and *p*-coumaroylquinic acids, flavan-3-ol glycosides, and procyanidins. These molecules are $A\beta$ oligomer ligands, hindering peptide fibrillation and neurotoxicity through their direct

interaction with the target. Moreover, hop extracts prevented cell death due to oxidative stresses and induced autophagic pathways.

Finally, we demonstrated the antiamyloidogenic hop activity *in vivo*, exploiting the transgenic *C. elegans* CL2006 strain constitutively producing A β 3-42. Our experiments showed that hop possesses a protective effect comparable to that of 100 μ M Doxy, a compound already described for its efficacy *in vivo*.

Our results show that hop is a source of bioactive molecules with synergistic and multitarget activity against the early events underlying AD development. We can therefore think of its use for the preparation of nutraceuticals useful for the prevention of this pathology.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acschemneuro.2c00444>.

¹H NMR spectra, ¹H, ¹H-TOSCY spectrum, and ¹³C, ¹H-HSQC spectrum of HT obtained by boiling water extraction (Figures S1–S3); NMR chemical shift assignments for HT obtained by boiling water extraction (Table S1); UPLC-HR-MS of hop extract and polyphenol-enriched fractions (Table S2); kinetics of inhibition of hop extracts or fractions on A β 1-42 aggregation by the ThT binding assay (Figure S4); effects of different concentrations of hop extracts on A β 1-42 toxicity on the human neuroblastoma SH-SY5Y cell line (Figure S5); ¹H NMR spectra of the chromatographic fractions A–E (Figure S6); effects of different concentrations of hop fraction B on A β 1-42 toxicity on the human neuroblastoma SH-SY5Y cell line (Figure S7); effects of different concentrations of hop fractions B2 on A β 1-42 toxicity on the human neuroblastoma SH-SY5Y cell line (Figure S8); and evaluation of the antioxidant activity of hop fractions (Figure S9) (PDF)

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Author Contributions

A.P. and C.A. designed the project. V.A. and C.Br. performed extractions, chromatography, and the analysis of NMR data under the guidance of C.A. P.A. performed MS analysis with the support of I.D.N., and UV analysis. A.P. and G.S. characterized extracts' antioxidant activity. A.D.L. performed MTT and ThT assays. L.C. carried out AFM analysis. C.Ba. and C.P.Z. performed ELISA experiments. M.I. performed C. elegans experiments under the supervision of L.D. C.A., P.A., V.M., C.Br., A.D.L., G.S., and L.D. analyzed and interpreted the experiments. C.A. drafted the manuscript, and C.A., A.P., G.S., and L.D. reviewed the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Scheltens, P.; De Strooper, B.; Kivipelto, M.; Holstege, H.; Chételat, G.; Teunissen, C. E.; Cummings, J.; van der Flier, W. M. Alzheimer's disease. *Lancet* **2021**, *397*, 1577–1590.
- (2) McDade, E.; Bateman, R. J. Stop Alzheimer's before it starts. *Nature* **2017**, *547*, 153–155.
- (3) Long, J. M.; Holtzman, D. M. Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. *Cell* **2019**, *179*, 312–339.
- (4) Knopman, D. S.; Amieva, H.; Petersen, R. C.; Chételat, G.; Holtzman, D. M.; Hyman, B. T.; Nixon, R. A.; Jones, D. T. Alzheimer disease. *Nat. Rev. Dis. Primers* **2021**, *7*, No. 33.
- (5) Bolton, J. L.; Dunlap, T. L.; Hajirahimkhan, A.; Mbachou, O.; Chen, S. N.; Chadwick, L.; Nikolic, D.; van Breemen, R. B.; Pauli, G. F.; Dietz, B. M. The Multiple Biological Targets of Hops and Bioactive Compounds. *Chem. Res. Toxicol.* **2019**, *32*, 222–233.
- (6) Fukuda, T.; Ohnuma, T.; Obara, K.; Kondo, S.; Arai, H.; Ano, Y. Supplementation with Matured Hop Bitter Acids Improves Cognitive Performance and Mood State in Healthy Older Adults with Subjective Cognitive Decline. *J. Alzheimer's Dis.* **2020**, *76*, 387–398.
- (7) Ayabe, T.; Fukuda, T.; Ano, Y. Improving Effects of Hop-Derived Bitter Acids in Beer on Cognitive Functions: A New Strategy for Vagus Nerve Stimulation. *Biomolecules* **2020**, *10*, No. 131.
- (8) Sasaoka, N.; Sakamoto, M.; Kanemori, S.; Kan, M.; Tsukano, C.; Takemoto, Y.; Kakizuka, A. Long-Term Oral Administration of Hop Flower Extracts Mitigates Alzheimer Phenotypes in Mice. *PLoS One* **2014**, *9*, No. e87185.
- (9) Airolidi, C.; Ciaramelli, C.; Palmioli, A. *SMA Libraries for Metabolite Identification and Quantification in Cocoa Extracts*, Mendeley Data; v1, 2020.
- (10) Airolidi, C.; Palmioli, A.; Ciaramelli, C. *SMA Libraries for Metabolite Identification and Quantification in Beers*, Mendeley Data; v1, 2019.
- (11) Airolidi, C.; Ciaramelli, C.; Palmioli, A. *SMA Libraries for Metabolite Identification and Quantification in Coffee Extracts*, Mendeley Data; v1, 2018.
- (12) Airolidi, C.; Ciaramelli, C.; Palmioli, A. *SMA Libraries for Metabolite NMR-Based Identification and Quantification in Cinnamon Extracts*, Mendeley Data; v1, 2021.
- (13) Farag, M. A.; Porzel, A.; Schmidt, J.; Wessjohann, L. A. Metabolite profiling and fingerprinting of commercial cultivars of *Humulus lupulus* L. (hop): a comparison of MS and NMR methods in metabolomics. *Metabolomics* **2012**, *8*, 492–507.
- (14) Bertelli, D.; Brighenti, V.; Marchetti, L.; Reik, A.; Pellati, F. Nuclear magnetic resonance and high-performance liquid chromatography techniques for the characterization of bioactive compounds from *Humulus lupulus* L. (hop). *Anal. Bioanal. Chem.* **2018**, *410*, 3521–3531.
- (15) Hoek, A. C.; Hermans-Lokkerbol, A. C. J.; Verpoorte, R. An improved NMR method for the quantification of α -Acids in hops and hop products. *Phytochem. Anal.* **2001**, *12*, 53–57.
- (16) McCallum, J. L.; Nabuurs, M. H.; Gallant, S. T.; Kirby, C. W.; Mills, A. A. S. Phytochemical Characterization of Wild Hops (*Humulus lupulus* ssp. *lupuloides*) Germplasm Resources From the Maritimes Region of Canada. *Front. Plant Sci.* **2019**, *10*, No. 1438.
- (17) Cheignon, C.; Tomas, M.; Bonnefont-Rousselot, D.; Faller, P.; Hureau, C.; Collin, F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biol.* **2018**, *14*, 450–464.
- (18) Amigoni, L.; Stuknytė, M.; Ciaramelli, C.; Magoni, C.; Bruni, I.; De Noni, I.; Airolidi, C.; Regonesi, M. E.; Palmioli, A. Green coffee extract enhances oxidative stress resistance and delays aging in *Caenorhabditis elegans*. *J. Funct. Foods* **2017**, *33*, 297–306.
- (19) Ciaramelli, C.; Palmioli, A.; De Luigi, A.; Colombo, L.; Sala, G.; Riva, C.; Zoia, C. P.; Salmona, M.; Airolidi, C. NMR-driven identification of anti-amyloidogenic compounds in green and roasted coffee extracts. *Food Chem.* **2018**, *252*, 171–180.
- (20) Hawe, A.; Sutter, M.; Jiskoot, W. Extrinsic Fluorescent Dyes as Tools for Protein Characterization. *Pharm. Res.* **2008**, *25*, 1487–1499.
- (21) Airolidi, C.; Sironi, E.; Dias, C.; Marcelo, F.; Martins, A.; Rauter, A. P.; Nicotra, F.; Jimenez-Barbero, J. Natural Compounds against Alzheimer's Disease: Molecular Recognition of A β 1–42 Peptide by *Salvia sclareoides* Extract and its Major Component, Rosmarinic Acid, as Investigated by NMR. *Chem. - Asian J.* **2013**, *8*, 596–602.
- (22) Sironi, E.; Colombo, L.; Lompo, A.; Messa, M.; Bonanomi, M.; Regonesi, M. E.; Salmona, M.; Airolidi, C. Natural Compounds against Neurodegenerative Diseases: Molecular Characterization of the Interaction of Catechins from Green Tea with A β 1–42, PrP106–126, and Ataxin-3 Oligomers. *Chem. - Eur. J.* **2014**, *20*, 13793–13800.
- (23) Guzzi, C.; Colombo, L.; Luigi, A. D.; Salmona, M.; Nicotra, F.; Airolidi, C. Flavonoids and Their Glycosides as Anti-amyloidogenic Compounds: A β 1–42 Interaction Studies to Gain New Insights into Their Potential for Alzheimer's Disease Prevention and Therapy. *Chem. - Asian J.* **2017**, *12*, 67–75.
- (24) Palmioli, A.; Bertuzzi, S.; De Luigi, A.; Colombo, L.; La Ferla, B.; Salmona, M.; De Noni, I.; Airolidi, C. bioNMR-based identification of natural anti-A β compounds in *Peucedanum ostruthium*. *Bio-org. Chem.* **2019**, *83*, 76–86.
- (25) Ciaramelli, C.; Palmioli, A.; De Luigi, A.; Colombo, L.; Sala, G.; Salmona, M.; Airolidi, C. NMR-based Lavado cocoa chemical characterization and comparison with fermented cocoa varieties: Insights on cocoa's anti-amyloidogenic activity. *Food Chem.* **2021**, *341*, No. 128249.
- (26) Ciaramelli, C.; Palmioli, A.; Angotti, I.; Colombo, L.; De Luigi, A.; Sala, G.; Salmona, M.; Airolidi, C. NMR-Driven Identification of Cinnamon Bud and Bark Components With Anti-A β Activity. *Front. Chem.* **2022**, *10*, No. 553.
- (27) Martinez Pomier, K.; Ahmed, R.; Melacini, G. Catechins as Tools to Understand the Molecular Basis of Neurodegeneration. *Molecules* **2020**, *25*, No. 3571.
- (28) Ahmed, R.; Huang, J.; Lifshitz, R.; Martinez Pomier, K.; Melacini, G. Structural determinants of the interactions of catechins with A β oligomers and lipid membranes. *J. Biol. Chem.* **2022**, *298*, No. 101502.
- (29) Mayer, M.; Meyer, B. Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy. *Angew. Chem., Int. Ed.* **1999**, *38*, 1784–1788.
- (30) Ciaramelli, C.; Palmioli, A.; Airolidi, C. Chapter 6 NMR-based Ligand–Receptor Interaction Studies under Conventional and Unconventional Conditions. In *NMR Spectroscopy for Probing Functional Dynamics at Biological Interfaces*; The Royal Society of Chemistry, 2022; pp 142–178.
- (31) Airolidi, C.; Colombo, L.; Manzoni, C.; Sironi, E.; Natalello, A.; Doglia, S. M.; Forloni, G.; Tagliavini, F.; Del Favero, E.; Cantù, L.; Nicotra, F.; Salmona, M. Tetracycline prevents A β oligomer toxicity through an atypical supramolecular interaction. *Org. Biomol. Chem.* **2011**, *9*, 463–472.
- (32) Airolidi, C.; Cardona, F.; Sironi, E.; Colombo, L.; Salmona, M.; Silva, A.; Nicotra, F.; La Ferla, B. cis-Glyco-fused benzopyran compounds as new amyloid- β peptide ligands. *Chem. Commun.* **2011**, *47*, 10266–10268.
- (33) Zhang, Z.; Yang, X.; Song, Y. Q.; Tu, J. Autophagy in Alzheimer's disease pathogenesis: Therapeutic potential and future perspectives. *Ageing Res. Rev.* **2021**, *72*, No. 101464.
- (34) Reddy, P. H.; Oliver, D. M. Amyloid Beta and Phosphorylated Tau-Induced Defective Autophagy and Mitophagy in Alzheimer's Disease. *Cells* **2019**, *8*, No. 488.
- (35) Datan, E.; Shirazian, A.; Benjamin, S.; Matassov, D.; Tinari, A.; Malorni, W.; Lockshin, R. A.; Garcia-Sastre, A.; Zakeri, Z. mTOR/p70S6K signaling distinguishes routine, maintenance-level autophagy from autophagic cell death during influenza A infection. *Virology* **2014**, *452–453*, 175–190.

(36) Arias, E.; Koga, H.; Diaz, A.; Mocholi, E.; Patel, B.; Cuervo, A. M. Lysosomal mTORC2/PHLPP1/Akt Regulate Chaperone-Mediated Autophagy. *Mol. Cell* **2015**, *59*, 270–284.

(37) Diomedede, L.; Cassata, G.; Fiordaliso, F.; Salio, M.; Ami, D.; Natalello, A.; Doglia, S. M.; De Luigi, A.; Salmona, M. Tetracycline and its analogues protect *Caenorhabditis elegans* from β amyloid-induced toxicity by targeting oligomers. *Neurobiol Dis.* **2010**, *40*, 424–431.

(38) Natale, C.; Barzago, M. M.; Diomedede, L. *Caenorhabditis elegans* Models to Investigate the Mechanisms Underlying Tau Toxicity in Tauopathies. *Brain Sci.* **2020**, *10*, No. 838.

(39) Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In *Methods in Enzymology*; Elsevier, 1999; Vol. 299, pp 152–178.

(40) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(41) Sharma, O. P.; Bhat, T. K. DPPH antioxidant assay revisited. *Food Chem.* **2009**, *113*, 1202–1205.

(42) Manzoni, C.; Colombo, L.; Messa, M.; Cagnotto, A.; Cantù, L.; Del Favero, E.; Salmona, M. Overcoming synthetic $A\beta$ peptide aging: a new approach to an age-old problem. *Amyloid* **2009**, *16*, 71–80.

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