Article

Journal of the Brazilian Chemical Society

https://dx.doi.org/10.21577/0103-5053.20240131

J. Braz. Chem. Soc. **2025**, *36*, 2, e-20240131, 1-13

Selenoacetylenes Protect against Beta-Amyloid Peptide-Induced Paralysis and Promote Longevity in *Caenorhabditis elegans*

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Selenium-containing compounds exhibit diverse biological activities, such as antioxidative, anti-inflammatory, and cancer preventive effects. Using *Caenorhabditis elegans* as a model organism, we assessed the toxicity, neuroprotective, antioxidant properties, and impact on longevity of 11 selenoacetylenes. Their toxicity and bioactivities varied based on molecular structure. Selenoacetylenes with butyl substituents were toxic to *Galleria mellonella* larvae. The presence of but-3-in-2-ol radical increased antiprotozoal activity against *Tetrahymena pyriformis*. Compared to the positive control (Nimitz® EC), selenoacetylenes were less toxic to nematode worms and eggs. Selenoacetylenes significantly reduced amyloid beta (Aβ) paralysis in *C. elegans* CL4176 worms, increased longevity by 18 to 22%, along with improving survival after oxidative or thermal stress. Galantamine, showed inferior results. These findings enhance our understanding of selenoacetylenes on neuroprotection, antioxidant activity, and longevity in *C. elegans*. Future mammalian studies will further elucidate mechanisms and explore the potential therapeutic use of selenoacetylenes in treating Alzheimer's disease and longevity.

Keywords: selenoacetylenes, neuroprotection, antioxidant activity, longevity, *Caenorhabditis elegans*, amyloid beta induced paralysis

Introduction

Aging is a natural and multifactorial process that induces molecular, cellular, and histological changes in living organisms. These alterations depend on physiological plasticity and time.[1,2] Aging is primarily caused by accumulation of time-dependent cellular damage.[3] This accumulation is reduced by joint action of the heat shock response complex (HSR),[4] the insulin signaling pathway (IGF-1),[5] the mitochondrial function,[6] and sirtuins,[7] which decline over time. Such decline renders individuals more susceptible and hampers their ability to self-adjust,[8] not to mention that it is a risk factor for neurodegenerative diseases.

Increased life expectancy in developed countries accounts for human population aging.[9-11] According to the World Population Prospects report,[12] the global population aged 65 or older in 2022 (771 million people) was approximately three times greater compared to 1980 (258 million).[12] The growth of younger age groups (0 to 14 years and 15 to 59 years) is being outpaced by the growth of groups aged 60 or older at approximately 3% *per* year.[13] As the global population ages, the incidence of neurodegenerative diseases is predicted to increase,[14] and the number of people with dementia is expected to triple by 2050, reaching 152 million up from the current 50 million.[13,15] In this scenario, neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and other forms of dementia are a growing global health setback affecting thousands of people worldwide.[16] AD is the most common form of dementia and accounts for 60-70% of the dementia cases in the world.[17] This progressive disease impairs mainly cognitive abilities and memory and interferes with the affected individual's quality of life.[18]

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Editor handled this article: Brenno A. D. Neto

In Brazil, the prevalence of dementia among individuals aged 65 or older resembles the prevalence in other Latin American countries, standing at approximately 7%.[19,20] Brazilians are undergoing rapid aging, which explains why Brazil ranked second in terms of age-standardized prevalence of AD and other dementias in 2016.[21]

The causes leading to AD onset and progression are multifactorial, which complicates treatment. Cholinergic deficiency,[22] amyloid beta peptide (Aβ) toxicity,[23] tau protein hyperphosphorylation,[24] synaptic dysfunction,[25] oxidative stress,[26] and neuroinflammation[27] are some factors that contribute to AD development. Extracellular Aβ deposits in senile plaques (SP) and formation of intracellular neurofibrillary tangles are the main AD neuropathological features.[28-31]

Despite advanced research, no pharmacological treatment can slow down or interrupt AD progression, damage, and neuron destruction.[32] Some medications like tacrine (1993),[33] donepezil (1996),[34] rivastigmine (2000),[35] and galantamine (2001)[36] can reduce cognitive and memory impairment through cholinergic inhibition or *N*-methyl D-aspartate (NMDA) receptor blockade and are recommended for AD.[37,38]

Efforts to develop treatments for AD have been focused on discovering molecules that continuously relieve symptoms by mitigating or avoiding abnormal Aβ accumulation and reducing oxidative damage.[39] Some authors[40] have suggested that donepezil, rivastigmine, and galantamine can reduce Aβ production and Aβ-induced toxicity.

In this context, compounds containing the essential trace element selenium are promising, particularly because human selenoproteins, including thioredoxin reductases (TrxR), glutathione peroxidases (GPx), and thyroid hormone deiodinases (DIO), participate in redox regulation of intracellular signaling[30,41] and help to regulate neurodegenerative disorders.[31]

Advances in AD treatment require a model system that recapitulates the AD hallmark features.[42] *Caenorhabditis elegans* is a free-living nematode that has proven an excellent model organism in various areas of knowledge,[43-46] even in the study of intricate neurological diseases like AD.[47,48] Molecular conservation of neuronal signaling pathways in this invertebrate has allowed related pathways to be identified in vertebrate models and drugs to be cost-effectively assessed *in vivo*.[49]

The numerous biological activities associated with selenium derivatives have made these compounds prominent in the field of medicinal chemistry.[50] Selenium-containing molecules are considered key elements in cancer prevention[51-55] and anti-inflammatory effects.[56,57] Nevertheless, merely having biological activity does not suffice for a molecule to be used in living organisms, their toxicity must be determined to ensure that they are safe for both living organisms and the environment.

Toxicity assays demand that organisms from different taxonomic groups be employed.[58] *Tetrahymena pyriformis* cells (protozoan),[59] *Galleria mellonella* larvae[60] (arthropod),[61] and *C. elegans* (nematode)[62] are some examples of models that are used to assess bioactive compound toxicity. This approach allows one to evaluate several effects qualified and quantified on the basis of parameters such as mortality, growth, and physiological, molecular, or reproductive effects.[63]

In this study, we have employed *C. elegans*, recognized as a model organism to study complex neurological diseases, including AD,[48,64] to investigate whether 11 selenoacetylene derivatives can reduce Aβ-induced toxicity and improve longevity. We have also determined their antioxidant capacity in nematodes and their toxicity in different biological models (insect larvae, protozoan cells, and nematodes).

Experimental

Synthesis and characterization of selenoacetylenes

The selenoacetylenes were synthesized by using the methodology described by Bieber *et al.*[65] All reagents and materials were supplied by Sigma-Aldrich (São Paulo, Brazil). Diorganoyl diselenide (1 mmol L^{-1}), dimethyl sulfoxide (DMSO), and copper iodide (CuI, 0.1 mmol L^{-1}) were combined in a test tube containing terminal alkyne (2 mmol L^{-1}) (Figure 1). The solution was stirred at 25 °C for 24 h. After incubation, the solution was washed with 10 mL of an aqueous NH_4Cl solution (0.3 mol L^{-1}).

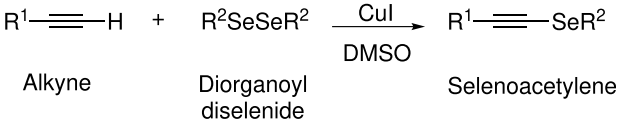


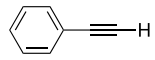
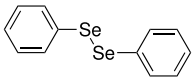
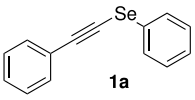
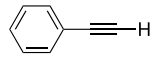
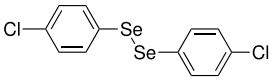
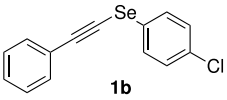
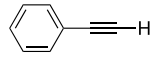
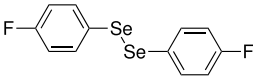
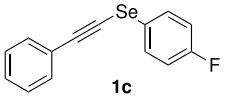
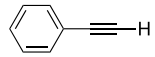
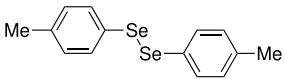
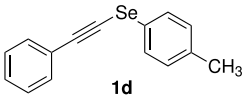
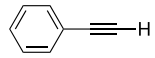
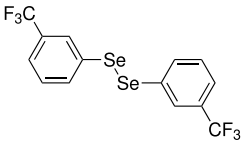
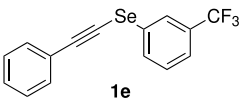
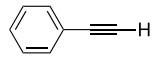
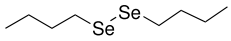
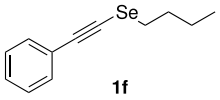
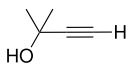
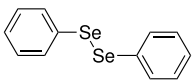
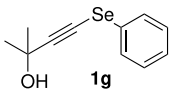
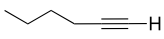
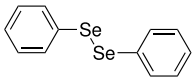
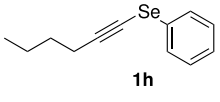
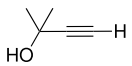
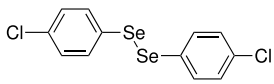
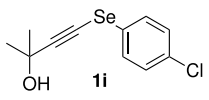
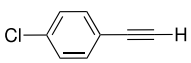
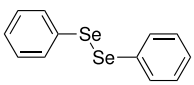
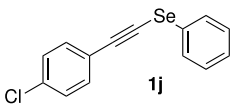
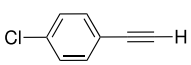
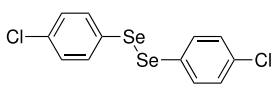
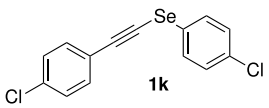
Figure 1. General scheme of selenoacetylene synthesis.

The reaction product was extracted with ethyl acetate (in three steps, 10 mL in each step), dried with magnesium sulfate ($MgSO_4$) (Sigma-Aldrich, São Paulo, Brazil) to remove moisture and concentrated under reduced pressure to remove the solvent. The resulting products, labeled **1a**-**1k** (Table 1), were purified by silica gel chromatography; hexane was used as eluent. Subsequently, the products were characterized by hydrogen-1 nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic

resonance (^{13}C NMR) spectroscopy on a Bruker Ascend 500™ spectrometer (Billerica, Massachusetts, USA).

The samples were analyzed using a high-performance liquid chromatography (HPLC) system (LC-20A Prominence, Shimadzu®, Kyoto, Japan) equipped with two quaternary pumps (LC-20AD), degasser (DGU-20A3), autosampler (SIL-20A), oven (CTO-20A), diode array detector (SPD-M20A) and a communication module (CBM-20A). For separation, a reversed phase column (Shim-pack VP-ODS, Shimadzu®, Kyoto, Japan) was used, with 250 × 4.6 mm internal diameter, with a particle size of 4.6 μm and porosity of 12 nm. The mobile phases were 0.1% (v/v) formic acid in ultrapure water (A) and methanol (B). The elution condition applied was 0-5 min, linear gradient of 90-100% B, 5-15 min in isocratic mode with 100% B followed by reconditioning the column in 15-25 min of 100-90% B, with flow of 1 mL min^{-1}. The injection volume was 10 μL. All reagents used in the analysis were HPLC grade and deionized water was obtained from a Milli-Q water purification system

Table 1. Molecular structure of selenoacetylene derivatives

Alkyne	Diorganoyl diselenide	Product
		 **1a**
		 **1b**
		 **1c**
		 **1d**
		 **1e**
		 **1f**
		 **1g**
		 **1h**
		 **1i**
		 **1j**
		 **1k**

(Millipore Corporation®, Watford, United Kingdom). LabSolutions software (version 5.3, Japan) was used for data acquisition and processing.

The NMR spectra were recorded with chemical shifts (δ) adjusted in parts *per* million (ppm), referenced to the residual solvent peak of tetramethylsilane (TMS, used as internal standard for proton spectra) in $CDCl_3$ (Sigma-Aldrich, São Paulo, Brazil). The multiplicity of each peak is designated by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sext (sextet), and m (multiplet). Coupling constants (*J*) are reported in hertz (Hz).

Caenorhabditis elegans strains and maintenance

All the *C. elegans* strains used in this study are described in Table 2. The *C. elegans* worms were maintained at 15 °C on solid nematode growth medium (NGM) seeded with *Escherichia coli* OP50 as a food source, following Brenner's protocol.[66]

Preparation of stock selenoacetylene solutions for toxicity and bioactivity assays

Each selenoacetylene was dissolved at 20 g L^{-1} in pure DMSO (Sigma-Aldrich, São Paulo, Brazil) or methanol (MeOH) (Sigma-Aldrich, São Paulo, Brazil) and stored at –4 °C. At the time of the assays, diluted selenoacetylene solutions were prepared at the desired concentration. The final DMSO or methanol concentration used in negative controls was 10% (v/v). In the assays conducted with heat-killed bacteria, a concentrated suspension of *E. coli* OP50 cells was previously prepared and killed by heat in an autoclave.[67]

Selenoacetylene toxicity to *Galleria mellonella* larvae and *Tetrahymena pyriformis* cells

Selenoacetylene toxicity was assessed in *G. mellonella* larvae as described by Ramarao *et al.*[68] Ten (10) *G. mellonella* larvae weighing between 0.2 and 0.3 g were treated with 10 µL of a selenoacetylene solution (4 g L^{-1}) prepared in saline solution (0.8%). The negative control was carried out with saline solution. After that, the larvae were kept in the dark at room temperature. Larval mortality was assessed daily for seven days. All the assays were performed in triplicate, and the results are expressed as the mean and standard deviation of the percentage of mortality.[68,69]

The *T. pyriformis* cells were cultured according to Maurya *et al.*[70] protocol. Approximately 1×10^6 cells of *T. pyriformis* mL^{-1} were added to 96-well plates containing a selenoacetylene. The plates were kept at 28 °C for 24 h. Then, the number of cells was counted by using a microscope. The negative control was performed with 10% DMSO.

Selenoacetylene toxicity to nematodes

The nematicidal activity of the selenoacetylenes was estimated in *C. elegans* N2 in a population previously synchronized in the L4 stage.[71] After synchronization, L4 worms (n = 30) or eggs (n = 20) were transferred to 96-well plates containing different concentrations of the evaluated compounds diluted in K medium.[72] The plates were kept in a biological oxygen demand (BOD) incubator model TE-402/240L Tecnal (Piracicaba, SP) at 20 °C for 24 h. After that, the mortality of L4 individuals or the hatching percentage was counted with the aid of a magnifier. *M. incognita* eggs were obtained from infected tomato plants and kept in a greenhouse, so that the J2 infective form would be obtained according to Nitao *et al.*[73] The assays were conducted in 96-well plates as described for *C. elegans*; *M. incognita* eggs (n = 20) or juveniles (J2, n = 20) were used. With the aid of a magnifier, the number of hatched eggs and dead J2 individuals were counted. The commercial nematicide Nimitz® EC and DMSO were employed as positive and negative control, respectively. The obtained data were used to estimate the concentrations that were able to kill 50% (LC_{50}) and 90% (LC_{90}) of the population. LC_{50} and LC_{90} were estimated by using the R software[74] and the calculate.lc function.[75]

Table 2. *C. elegans* strains used in this study

Strain	Transgene	Phenotype
N2	–	wild type
CL4176	dvIs27 [myo-3p::A-Beta (1-42)::let-851 3'UTR) + rol-6(su1006)]	expression of human amyloid-β in muscle cell walls
CL802	smg-1 (cc546) I; rol-6 (su1006) II.	standard control for CL4176
BA17	fem-1 (hc17) IV	population feminization at 25 °C
LD1171	Is[gcs-1p::GFP]	expression of phase II detoxification gene gamma-glutamine cysteine synthetase-1 (GCS-1)

Selenoacetylene ability to inhibit Aβ-induced paralysis in *C. elegans*

The populations of *C. elegans* CL4176 and its control strain CL802 were obtained through synchronization. The worms were kept at 15 °C until they reached the third larval stage (L3). Then, they were transferred (n = 20) to 12-well plates containing NGM seeded with heat-inactivated *E. coli* OP50 bacterial cells and a selenoacetylene (at 0.1 or 0.05 g L^{-1}). Next, the plates were then moved to an incubator model TE-402/240L Tecnal (Piracicaba, SP) at 25 °C. After incubation at 25 °C for 20 h, paralyzed worms were counted every 2 h for a total of 8 h. Worms were considered paralyzed if they did not respond to repeated stimuli or if a bacterial "halo" was found around their heads, indicating that they were unable to move their bodies.[76] MeOH and galantamine at 0.05 g L^{-1} were used as negative and positive controls, respectively. The results are expressed as the mean values along with the standard deviation.

For the subsequent assays, the selenoacetylenes at 0.05 g L^{-1} that reduced *C. elegans* paralysis by over 80% were evaluated.

Longevity assay

To evaluate whether the lifespan was extended, the *C. elegans* BA17 strain was employed.[77,78] Synchronized L3 stage larvae were obtained from eggs hatched at 25 °C. Twenty worms were transferred to 12-well plates containing NGM seeded with heat-inactivated *E. coli* OP50 bacterial cells and a selenoacetylene. The lifespan was assessed daily until all the individuals were dead. The worms that showed no spontaneous movement during evaluation were considered dead. Dead worms displaying internally hatched progeny, extruded gonads, or desiccation caused by crawling out of the agar well boundaries were excluded from the data. Galantamine at 0.05 g L^{-1} and MeOH were used as positive and negative controls, respectively. The data were obtained from three independently conducted assays, and the results are expressed as the mean and standard deviation.

Selenoacetylene ability to protect *C. elegans* against oxidative and thermal stress

The ability of selenoacetylenes to reduce oxidative stress in *C. elegans* was assessed by using worms at the L1 stage. The worms were treated with a selenoacetylene (0.05 g L^{-1}) at 20 °C until they reached the L4 stage. After treatment, adult worms (20 worms *per* group) were added to NGM plates containing 0.003 mmol L^{-1} H_2O_2 to induce oxidative stress. The number of living and dead animals was assessed every 30 min. The assay was repeated three times with negative (MeOH) and positive (galantamine at 0.05 g L^{-1}) controls.[79]

The thermal stress assay was conducted according to the previously reported method. After treatment, the worms were transferred to NGM plates for thermal stress assessment. The number of dead worms was recorded every 6 h after the worms were transferred from a 20 °C culture environment to a 35 °C culture environment.

In vivo antioxidant activity

The LD1171 strain (gcs-1p::GFP) was used to study how the selenoacetylenes affected the phase II detoxification gene glutamine cysteine synthetase-1 (gcs-1). In K medium, synchronized worms of the LD1171 strain were treated with each selenoacetylene at 0.05 g L^{-1} or the vehicle MeOH (in volumes proportional to the volumes used in the treatments) at 20 °C for 72 h. Galantamine at 0.05 g L^{-1} was employed as positive control. The worms were visualized under a fluorescence microscope with a 10× objective. The ImageJ software[80] was used to measure the intestinal fluorescence in each image. Only the fluorescence intensity detected in the green channel was used for quantifying GCS-1 in the intestinal area.[81]

Statistical analyses

All the results were evaluated for normality and homogeneity by using the Shapiro-Wilk test and Levene's test, respectively. Groups with normally distributed data were compared by using Student's *t*-test; one-way analysis of variance (ANOVA) with Dunnett's *post hoc* test was performed to compare multiple groups. GraphPad Prism 5.0[82] was used to plot the graphs and to determine significant differences between survival curves by means of log-rank tests (Mantel-Cox).

Results

Synthesis and characterization of selenoacetylenes

It was obtained 11 selenoacetylenes as products of coupling reactions in up to 92% yield (Table 3). These compounds were characterized by ^{1}H NMR and ^{13}C NMR, and the purity was determined by HPLC (Figures S1-S31, Supplementary Information (SI) section). All compounds have a purity greater than 90% by HPLC.

Table 3. Yield and purity of selenoacetylene derivatives after synthesis

Compound	Name	Yield / %	Purity / %
1a	1-phenylseleno-2-phenylacetylene	92	96
1b	1-(4'-chlorophenyl)seleno-2-phenylacetylene	72	98
1c	1-(4'-fluorophenyl)seleno-2-phenylacetylene	82	91
1d	1-(4'-methylphenyl)seleno-2-phenylacetylene	91	96
1e	1-(3'-trifluoromethylphenyl)seleno-2-phenylacetylene	74	99
1f	1-butylseleno-2-phenylacetylene	62	98
1g	2-methyl-4-(phenylsulfanyl) but-3-in-2-ol	78	97
1h	1-phenylseleno-2-butylacetylene	64	99
1i	2-methylseleno-4-(4'-chlorophenylsulfanyl) but-3-in-2-ol	59	99
1j	1-phenylseleno-2-(4'-chlorophenylacetylene)	78	94
1k	1-(4'-chlorophenylseleno)-2-(4'-chlorophenylacetylene)	65	95

Toxicity bioassays

The toxicity of the selenoacetylene derivatives to insect larvae, protozoa, and nematodes (worms and eggs) (Table 4) were examined. Only compound **1f** was toxic to *G. mellonella* larvae (mortality: 70 ± 10%); galantamine did not kill *G. mellonella* larvae (0% mortality). The toxicity of the selenoacetylene derivatives to protozoa and nematodes (worms and eggs) (Table 4) were examined.

As for the population of the ciliated protozoan *T. pyriformis*, it responded differently depending on the selenoacetylene. Compounds **1i** (100% cell death) and **1g** (3.68 ± 0.07 log cell mL^{-1}) were more toxic to *T. pyriformis* cells, while compounds **1a** (4.76 ± 0.15 log cell mL^{-1}) and **1b** (4.71 ± 0.15 log cell mL^{-1}) showed lower toxicity, including induced protozoan growth compared to the control (4.32 ± 0.10 log cell mL^{-1}). Galantamine was not toxic to *T. pyriformis* cells.

We observed that *C. elegans* and *M. incognita* worms and eggs were sensitive to the selenoacetylenes (Table 4). Toxicity varied according to the nematode species and developmental stage and to the selenoacetylene structure. Compounds **1c** and **1k** were more toxic to nematode worms (L4 and J2) and displayed the lowest LC_{50}: 0.78 mmol L^{-1} (0.64-0.95 mmol L^{-1}) and 1.40 mmol L^{-1} (NaN-NaN, where NaN means not a number), respectively, whereas compounds **1b** and **1f** were the least toxic 5.70 and 5.90 mmol L^{-1}, respectively.

Table 4. Nematicidal and ovicidal activity of selenoacetylenes (**1a**-**1k**) for *C. elegans* and *M. incognita* populations and for ***T. pyriformis*** cell

Compound	*C. elegans*				*M. incognita*				*T. pyriformis* / (log cell mL^{-1})
	L4		Eggs		J2		Eggs		
	LC_{50}[a] (lw-up)	LC_{90}[b] (lw-up[c])	LC_{50} (lw-up)	LC_{90} (lw-up)	LC_{50} (lw-up)	LC_{90} (lw-up)	LC_{50} (lw-up)	LC_{90} (lw-up)	
1a	2.10 (1.86-2.35)	4.25 (3.81-4.93)	2.16 (1.71-2.61)	6.82 (5.85-8.33)	2.27 (1.60-2.94)	6.48 (5.05-11.03)	0.15 (0.11-0.19)	1.13 (1.05-1.22)	4.76 ± 0.15***
1b	5.70 (5.20--6.42)	9.24 (8.49-10.42)	5.96 (5.25-6.68)	11.24 (10.10-13.05)	5.69 (5.19-6.19)	7.83 (7.21-9.18)	0.22 (0.14-0.30)	2.33 (2.16-2.52)	4.71 ± 0.15***
1c	0.78 (0.64-0.95)	2.13 (1.90-2.67)	4.62 (3.85-5.38)	10.62 (9.23-12.93)	5.59 (4.78-6.40)	10.45 (9.15-12.95)	0.12 (0.04-0.19)	1.75 (1.61-1.90)	4.61 ± 0.01**
1d	1.91 (1.70-2.11)	3.27 (2.95-3.79)	4.58 (4.10-5.05)	9.96 (9.04-11.27)	4.44 (3.88-5.00)	7.41 (6.60-9.02)	1.28 (1.21-1.28)	3.06 (2.91-3.25)	4.56 ± 0.04*
1e	1.48 (1.27-1.70)	3.31 (2.91-3.95)	2.32 (2.04-2.59)	5.23 (4.69-6.03)	3.79 (3.45-4.14)	5.07 (4.69-5.94)	0.47 (0.45-0.50)	0.98 (0.93-1.04)	4.59 ± 0.10**
1f	1.14 (1.01-1.27)	2.33 (2.10-2.68)	3.05 (2.61-3.49)	7.81 (6.87-9.21)	5.90 (5.30-6.50)	8.67 (7.91 -10.18)	0.34 (0.25-0.44)	2.82 (2.62-3.05)	4.65 ± 0.13***
1g	1.20 (1.09-1.31)	2.02 (1.85-2.30)	2.95 (2.60-3.30)	4.54 (4.08-5.50)	3.66 (3.12-4.21)	6.69 (5.86-8.37)	1.05 (0.99-1.12)	2.81 (2.66-3.00)	3.68 ± 0.07***
1h	0.81 (0.63-0.99)	2.67 (2.26-3.34)	3.35 (2.89-3.81)	5.52 (4.88-6.91)	4.84 (4.23-5.46)	8.08 (7.21-9.78)	2.05 (1.94-2.16)	4.59 (4.35-4.86)	3.79 ± 0.06***
1i	3.07 (2.89-3.25)	3.99 (3.78-4.35)	3.93 (3.48-4.39)	5.95 (5.37-7.21)	4.38 (3.94-4.82)	6.10 (5.52-8.07)	0.82 (0.77-0.870	2.11 (1.99-2.24)	0***
1j	5.12 (4.78-5.47)	6.34 (6.00-7.00)	2.40 (2.16-2.65)	3.77 (3.43-4.39)	4.39 (3.94-4.83)	6.37 (5.80-7.60)	0.08 (0.06-0.11)	0.62 (0.57-0.67)	3.95 ± 0.08***
1k	4.80 (4.51-5.09)[c]	6.08 (5.78-6.59)	3.23 (3.01-3.62)	5.08 (4.57-6.11)	1.40 (NaN-NaN)[d]	1.52 (NaN-NaN)	0.09 (0.06-0.12)	1.09 (1.01-1.18)	3.91 ± 0.03***
Galantamine	> 0.0139	> 0.0139	> 0.0139	> 0.0139	> 0.0139	> 0.0139	> 0.0139	> 0.0139	4.12 ± 0.05
Controls	0.33 (0.32-0.35)[e]	0.47 (0.45-0.50)[e]	0.10 (0.08-0.11)[e]	0.16 (0.15-0.20)[e]	0.015 (0.013-0.018)[e]	0.034 (0.029-0.041)[e]	0.013 (0.012-0.014)[e]	0.034 (0.032-0.04)[e]	4.32 ± 0.10[f]

[a]LC_{50}: concentration capable of killing 50% of the worm population or inhibiting egg hatching; [b]LC_{90}: concentration capable of killing 90% of the worm population or inhibiting egg hatching; [c]lower limit (lw) and upper limit (up) with 95% confidence interval; [d]not a number. [e]Nimitz®; [f]dimethyl sulfoxide (DMSO). *Dunnett post hoc ANOVA (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

As in the case of nematode worms, nematode eggs were susceptible to the selenoacetylenes. Compounds **1a** and **1j** were the most toxic to *C. elegans* and *M. incognita* egg hatching (2.16 and 0.08 mmol L^{-1}, respectively), whilst compounds **1b** and **1h** were the least toxic (5.96 and 2.05 mmol L^{-1}, respectively). Galantamine was not toxic to the tested nematode worms or eggs.

Effect of selenoacetylenes on Aβ-induced paralysis in *C. elegans* CL4176 worms

The mobility curves indicate the assay efficiency in assessing how Aβ affects *C. elegans* by comparing the CL4176 strain and its control CL802 (Figure S32, SI section). Our results show that treatment with the selenoacetylenes reduced the percentage of paralyzed worms compared to untreated worms in vehicle MeOH (negative control) and worms treated with the positive control (galantamine at 0.05 g L^{-1}).

The worm paralysis curves reveal that the evaluated compounds, including galantamine, reduced the percentage of paralyzed worms compared to the vehicle MeOH (negative control) (log-rank Mantel-Cox test, $p < 0.001$) (Figure S32). The negative and positive controls paralyzed 80 and 45% of the worm population after 28 h, indicating that galantamine reduced the percentage of paralyzed worms due to its protective effect against Aβ-induced paralysis in *C. elegans* (Dunnett's test, $p < 0.05$) (Figure 2). In the presence of compounds **1f** (0.21 mmol L^{-1}), **1e** (0.13 mmol L^{-1}), **1c** (0.19 mmol L^{-1}), **1j** (0.17 mmol L^{-1}), or **1i** (0.18 mmol L^{-1}) at 0.05 g L^{-1}, the percentage of paralyzed *C. elegans* worms was only 2.4, 8.9, 10.3, 15, and 15.6%, respectively (Figure 2) (Dunnett's test, $p < 0.05$).

Selenoacetylenes increase *C. elegans* BA17 longevity

The five selenoacetylenes (**1c**, **1e**, **1f**, **1i**, and **1j**) that reduced the percentage of *C. elegans* worm paralysis also

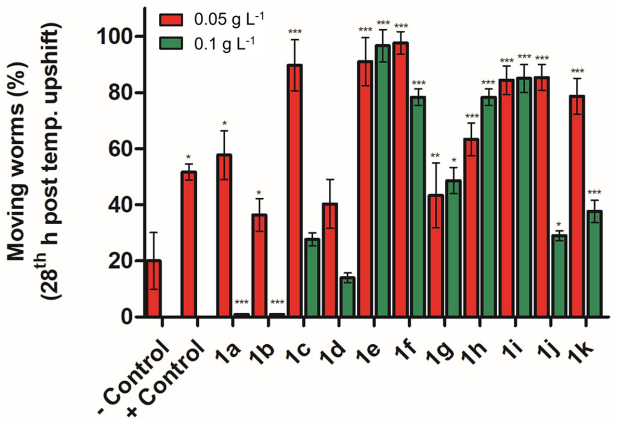


Figure 2. Paralysis in *C. elegans* CL4176 worms treated with selenoacetylenes. The data represent the mean ± SD (standard deviation) of moving worm. Vehicle MeOH (– control), galantamine at 0.05 g L^{-1} (+ control). Bars followed by * represent statistical difference in relation to the vehicle (– control) as revealed by Dunnett's test (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

increased the worm longevity (Table 5). Compounds **1c** and **1e** provided the highest increase (22.7%), while compounds **1i** and **1j** resulted in slightly lower increase (18.2%).

The curves in Figure 3 show that compounds **1c**, **1e**, **1f**, **1i**, and **1j** extended the *C. elegans* BA17 worm survival (Figure 3a) compared to the vehicle MeOH (negative control) (log-rank Mantel-Cox test, $p < 0.05$). Galantamine did not interfere with *C. elegans* BA17 worm survival (log-rank Mantel-Cox test, $p < 0.05$).

Protection against oxidative and thermal stress

Compounds **1c**, **1e**, **1f**, **1i**, and **1j** significantly increased *C. elegans* N2 survival in hours following acute oxidative stress induced by H_2O_2 (Table 6). Compound **1c** was the most efficient (227.3% higher compared to the vehicle MeOH (negative control)), followed by compounds **1f**, **1e**, **1j**, and **1i** (209.1, 190.9, 190.9, and 172.7%, respectively, compared to the vehicle MeOH (negative control)).

Table 5. Longevity of *C. elegans* BA17 worms treated with selenoacetylenes. Data are represented as mean ± SD (standard deviation) of the worms lifespan

Compound	Average lifetime / days	Median lifetime / days	Average lifetime increase / %
1c	27.00 ± 0.00*	25	22.7
1e	27.00 ± 0.00*	23	22.7
1f	26.33 ± 0.58*	23	19.7
1i	26.00 ± 0.00*	24	18.2
1j	26.00 ± 0.00*	25	18.2
Positive control	21.67 ± 0.58	18	–1.52
Negative control	22.00 ± 0.00	19	

Means followed by * indicate statistical difference by Dunnet's test ($p < 0.001$) compared to the vehicle MeOH (negative control). Galantamine at 0.05 g L^{-1} (positive control).

Table 6. *C. elegans* N2 survival after oxidative and thermal stress in the presence of selenoacetylenes. Data are represented as mean ± SD (standard deviation) of the worms lifetime

Treatment	Compound	Average lifetime / h	Median lifetime / h	Average lifetime increase / %
H_2O_2	**1c**	12.00 ± 1.15*	8	227.3
	1e	10.67 ± 1.15*	10	190.9
	1f	11.33 ± 0.00*	10	209.1
	1i	10.00 ± 0.00*	10	172.7
	1j	10.00 ± 0.00*	10	190.9
	positive control	4.50 ± 0.00	3.5	22.7
	negative control	3.67 ± 0.29	2.5	
35 °C	**1c**	66.00 ± 0.00*	54	83.3
	1e	60.00 ± 0.00*	36	66.7
	1f	62.00 ± 3.46*	48	72.2
	1i	60.00 ± 0.00*	42	66.7
	1j	62.00 ± 3.46*	54	72.2
	positive control	38.00 ± 3.46	24	5.6
	negative control	36.00 ± 0.00	21	

Means followed by * indicate statistical difference by Dunnet's test ($p < 0.001$) compared to the vehicle MeOH (negative control). Galantamine at 0.05 g L^{-1} (positive control).

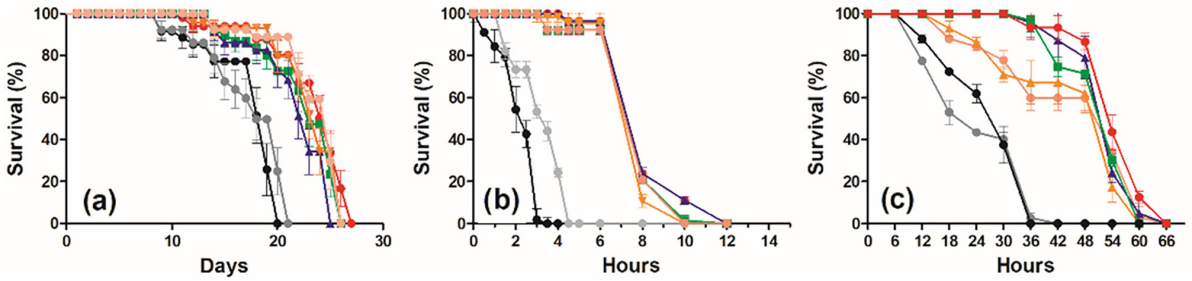


Figure 3. *C. elegans* worm survival. (a) *C. elegans* BA17 worm survival in days; (b) *C. elegans* N2 survival in hours after H_2O_2 oxidative stress; (c) *C. elegans* N2 survival in hours after heat shock. The curves show significant differences (log-rank Mantel-Cox test, $p < 0.05$) compared to treatment with the vehicle MeOH (negative control), as determined by the log-rank test (Mantel-Cox). (●) **1c**, (■) **1e**, (▲) **1f**, (▼) **1i**, (●) **1j**, (●) vehicle MeOH (negative control), (●) galantamine at 0.05 g L^{-1} (positive control).

The selected selenoacetylenes promoted thermotolerance in *C. elegans* N2 (Dunnett's test, $p < 0.05$) (Table 6). Worms treated with the selenoacetylenes resisted heat shock, which increased their survival as assessed in hours. Worms treated with compound **1c** had 83.3% longer survival on average compared to worm survival in the control group vehicle MeOH (negative control) (Table 6). The other selenoacetylenes increased worm survival after heat shock by between 66.7 and 72.2%. Galantamine did not protect *C. elegans* N2 against heat shock (Dunnett's test, $p < 0.05$).

The worm survival curves demonstrate the oxidative (Figure 3b) and thermal (Figure 3c) protection effects of compounds **1c**, **1e**, **1f**, **1i**, and **1j**, which increased the worm lifespan compared to the vehicle MeOH (negative control) (log-rank Mantel-Cox test, $p < 0.0001$). Galantamine (positive control) did not provide any oxidative or thermal protection to *C. elegans* N2.

In vivo antioxidant activity

Treatment with the selenoacetylenes increased GCS-1 expression in *C. elegans* LD1171 compared to the vehicle MeOH (negative control) (Dunnett's test, $p < 0.05$). Compound **1f** regulated GCS-1 expression the most effectively (208% increase), followed by compounds **1c** and **1e**, which increased GCS-1 expression by 164 and 140%, respectively (Figure 4). In contrast, galantamine (positive control) did not induce GCS-1 expression in *C. elegans* LD1171 compared to the negative control (Dunnett's test, $p < 0.05$) (Figure 4).

Discussion

The selenoacetylenes evaluated herein delayed Aβ-induced paralysis and enhanced resistance to oxidative and thermal stress in *C. elegans* worms. We found that

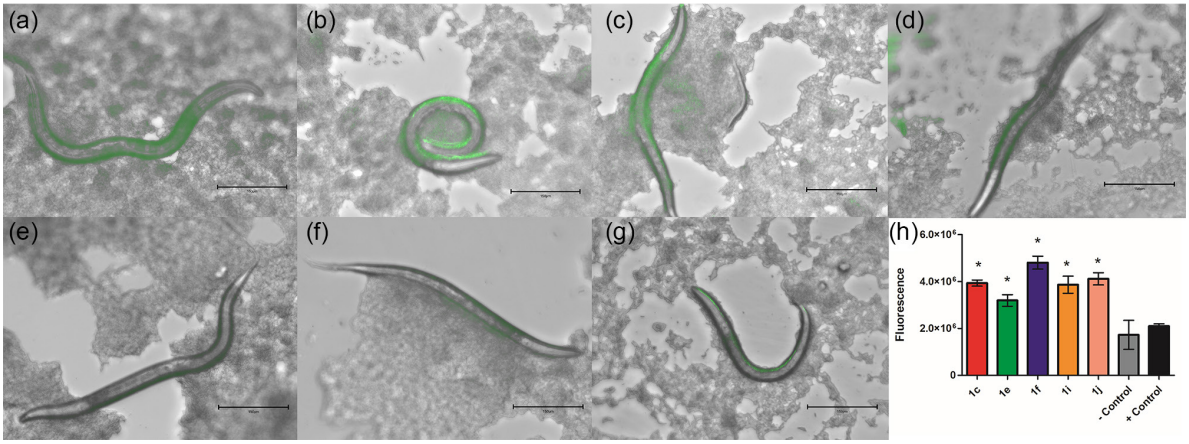


Figure 4. Quantification of fluorescence in *C. elegans* LD1171 intestines. Fluorescence images in worms: compound (a) **1c**; (b) **1e**; (c) **1f**; (d) **1i**; (e) **1j**; (f) vehicle MeOH (negative control); (g) galantamine at 0.05 g L^{-1} (positive control); (h) mean fluorescence values expressed as corrected total cell fluorescence (CTCF), * represents statistical difference compared to the negative control (Dunnett's test, $p < 0.05$). Scale bar = 0.15 mm.

treatment with the selenoacetylenes was associated with various health benefits in *C. elegans* worms, including increased longevity, and that these compounds displayed low toxicity in different model organisms. In addition, the selenoacetylenes exerted more pronounced effects compared to the positive control galantamine, a compound that can reduce cognitive and memory impairment and is recommended for treating AD.[40]

We verified that selenoacetylene toxicity varied depending on the employed biological model, and that the selenoacetylene structure influenced the biological activity profile. The presence of the phenyl group in the selenoacetylene molecule was important for antiprotozoal activity, as in the case of indazole derivatives.[83] Compounds **1g** and **1i** bear the but-3-in-2-ol radical, which was essential for increasing the antiprotozoal activity. Treatment with these compounds significantly reduced *T. pyriformis* cells, with 100% cell mortality being achieved for compound **1i**.

Just like selenoacetylenes, other selenium derivatives such as β-selenoamines[84] and selenium-xylofuranosides[85] have low toxicity in *C. elegans*, and their biological activity depends on the substituent groups. The structure-activity relationship is crucial when selecting compounds with different biological activities as well as the concentrations to be evaluated in assays.[53] The toxicity (LC_{90}) of compounds in *C. elegans* is on average 10 times higher than their effective concentrations employed during neuroprotection assays, indicating that the selenoacetylenes evaluated herein are safe.[86] These results are promising because toxicity is one of the side effects of therapeutic agents used in AD therapy.[35]

Specific inhibition of the Aβ toxic species is the key for developing new therapeutic drugs to treat AD and has been validated in transgenic *C. elegans*.[86] *C. elegans* CL4176 expresses human Aβ in muscle cells. Deposition of Aβ aggregates in *C. elegans* CL4176 muscles depends on temperature, paralyzing the worms, and results in a clear and easily observable phenotype, that is, an Aβ-dependent paralysis phenotype.[87] Selenoacetylenes **1c**, **1e**, **1f**, **1i**, and **1j** specifically protected *C. elegans* CL4176 against Aβ-induced toxicity *in vivo*. Compounds **1c**, **1e**, **1f**, **1i**, and **1j** reduced the percentage of paralyzed worms more effectively than galantamine, known for decreasing Aβ production and Aβ-induced toxicity.[40]

This information adds evidence to the efficacy of compounds that modulate Aβ plaque formation by inhibiting their production, aggregation, and even disaggregation, to interrupt or to delay AD progression.[88-90] Other selenium derivatives such as *N*-γ-(L-glutamyl)-L-selenomethionine[91] and selenoesters[92] attenuate Aβ aggregation in *C. elegans*. These data contribute to identifying and characterizing new anti-AD agents.

The antioxidant property of diphenyl diselenide is related to its ability to reduce the percentage of Aβ-induced paralysis in *C. elegans* worms.[93] Notably, selenoacetylenes **1c**, **1e**, **1f**, **1i**, and **1j** exerted antioxidant activities and protected against thermal stress by increasing the survival time of worms subjected to H_2O_2 and heat shock. Although galantamine exerted a mild effect on the survival of worms subjected to H_2O_2, it did not affect the survival of worms subjected to heat shock. The antioxidant properties of selenium compounds affect aging and longevity positively.[85] Chaperones known as heat shock proteins (HSPs) assist conformational changes, protein folding, and protein aggregation. HSP70 plays an important neuroprotective role in AD by preventing plaque formation and Aβ aggregation.[94] How selenoacetylenes **1c**, **1e**, **1f**, **1i**, and **1j** affect chaperone expression needs to be investigated further.

Another possible antioxidant mechanism of selenoacetylenes is activation of protective signaling

pathways, similarly to selenonein (2-selenyl-Nα, Nα, Nα-trimethyl-L-histidine);[95] they could also act through GPx-like antioxidant activity, as observed in diselenides.[96] Here, we verified that the selenoacetylenes activated GCS-1 expression, promoting antioxidant protection. Oxidative stress is one of the main mechanisms of aging, and upregulating antioxidant enzymes is pivotal for protecting against oxidative stress.[97,98]

In *C. elegans*, aging is associated with physiological and neurological decline, resembling aging in mammals, including humans.[99] Aging can induce stress, reduce the overall health, and trigger age-related neurological diseases. Compounds with anti-aging properties can increase the ability of an organism to reduce stress.[77] Selenoacetylenes **1c**, **1e**, **1f**, **1i**, and **1j** extended the *C. elegans* lifespan, indicating that they are good candidates for anti-aging treatment. Other organoselenium compounds, such as diethyl-2-phenyl-2-tellurophenyl vinyl phosphonate and organoselenotriazoles, also increase *C. elegans* longevity through antioxidant mechanisms.[100,101]

Conclusions

In conclusion, this study has demonstrated that selenoacetylenes **1c**, **1e**, **1f**, **1i**, and **1j** have a neuroprotective effect in *C. elegans*, used as a model for studying AD. Compared to the commercial drug galantamine, the tested selenoacetylenes reduce the percentage of paralyzed worms, promote longevity, and significantly increase survival after oxidative and thermal stress. These data highlight the need to investigate the molecular mechanisms associated with the action of these compounds and to evaluate their activities in mammals.

Supplementary Information

Supplementary data with high-performance liquid chromatography (HPLC) (Figures S1-S11) and nuclear magnetic resonance spectroscopy (NMR) (Figures S12-S31) profiles of selenoacetylenes **1a**-**1k** are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

We would like to thank our colleagues in the research group at the Laboratory of Biotechnology and Microbial Ecology (LABEM, UFMT, Cuiabá, MT, Brazil). We acknowledge the financial support from the Foundation for Research Support of the State of Mato Grosso (FAPEMAT) for providing scholarships, under grant number 0356280/2021.

Author Contributions

Maria Isabela S. Figueiredo was responsible for conceptualization, data analysis, formal analysis, investigation, methodology validation, visualization and writing (original draft, review and editing); Ivani S. Mello and Sabrina K. Targanski for some trials with model organisms; Juliana S. Duarte, Leonardo G. Vasconcelos and André Luiz A. Stein for the methodology, synthesis and characterization of selenoacetylenes by NMR and HPLC; Marcos Antonio Soares for conceptualization, resources, supervision, formal analysis, investigation support and original writing support.

References

1. Hung, C.-W.; Chen, Y.-C.; Hsieh, W.-L.; Chiou, S.-H.; Kao, C.-L.; *Ageing Res. Rev.* **2010**, *9*, S36. [Crossref]
2. Zia, A.; Pourbagher-Shahri, A. M.; Farkhondeh, T.; Samarghandian, S.; *Behav. Brain Funct.* **2021**, *17*, 6. [Crossref]
3. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S. G.; Croteau, D. L.; Bohr, V. A.; *Nat. Rev. Neurol.* **2019**, *15*, 565. [Crossref]
4. Calabrese, V.; Scapagnini, G.; Colombrita, C.; Ravagna, A.; Pennisi, G.; Stella, A. M. G.; Galli, F.; Butterfield, D. A.; *Amino Acids* **2003**, *25*, 437. [Crossref]
5. Zheng, S.-Q.; Huang, X.-B.; Xing, T.-K.; Ding, A.-J.; Wu, G.-S.; Luo, H.-R.; *J. Gerontol., Ser. A* **2017**, *72*, 464. [Crossref]
6. Wang, Y.; Xu, E.; Musich, P. R.; Lin, F.; *CNS Neurosci. Ther.* **2019**, *25*, 816. [Crossref]
7. Kanfi, Y.; Naiman, S.; Amir, G.; Peshti, V.; Zinman, G.; Nahum, L.; Bar-Joseph, Z.; Cohen, H. Y.; *Nature* **2012**, *483*, 218. [Crossref]
8. Guerreiro, R.; Bras, J.; *Genome Med.* **2015**, *7*, 106. [Crossref]
9. Procaccini, C.; Santopaolo, M.; Faicchia, D.; Colamatteo, A.; Formisano, L.; De Candia, P.; Galgani, M.; De Rosa, V.; Matarese, G.; *Metabolism* **2016**, *65*, 1376. [Crossref]
10. Pohl, F.; Lin, P. K. T.; *Molecules* **2018**, *23*, 3283. [Crossref]
11. D'Anca, M.; Fenoglio, C.; Serpente, M.; Arosio, B.; Cesari, M.; Scarpini, E. A.; Galimberti, D.; *Front. Aging Neurosci.* **2019**, *11*, 232. [Crossref]
12. United Nations, *World Population Prospects 2022: Summary of Results*, https://www.un.org/development/desa/pd/content/World-Population-Prospects-2022, accessed in July 2024.
13. United Nations; *World Population Prospects The 2017 Revision, Key Findings and Advance Tables*; United Nations: New York, USA, 2017. [Link] accessed in July 2024
14. Chen, X.; Barclay, J. W.; Burgoyne, R. D.; Morgan, A.; *Chem. Cent. J.* **2015**, *9*, 65. [Crossref]
15. World Health Organization (WHO), *Dementia: Number of People Affected to Triple in Next 30 Years*, https://www.who.int/news/item/07-12-2017-dementia-number-of-people-affected-to-triple-in-next-30-years, accessed in July 2024

16. Cai, H.; Cong, W.-N.; Ji, S.; Rothman, S.; Maudsley, S.; Martin, B.; *Curr. Alzheimer Res.* **2012**, *9*, 5. [Crossref]
17. Prince, M.; Wimo, A.; Guerchet, M.; Ali, G. C.; Wu, Y.-T.; Prina, M.; Chan, K. Y.; Xia, Z.; *World Alzheimer Report 2015*; Alzheimer Disease International: London, UK, 2015. [Link] accessed in July 2024
18. Cummings, J.; *Am. J. Geriatr. Psychiatry* **2021**, *29*, 375. [Crossref]
19. Nitrini, R.; Bottino, C. M. C.; Albala, C.; Capuñay, N. S. C.; Ketzoian, C.; Rodriguez, J. J. L.; Maestre, G. E.; Ramos-Cerqueira, A. T. A.; Caramelli, P.; *Int. Psychogeriatrics* **2009**, *21*, 622. [Crossref]
20. Herrera, E.; Caramelli, P.; Silveira, A. S. B.; Nitrini, R.; *Alzheimer Dis. Assoc. Disord.* **2002**, *16*, 103. [Crossref]
21. de Melo, S. C.; Champs, A. P. S.; Goulart, R. F.; Malta, D. C.; Passos, V. M. A.; *Arq. Neuro-Psiquiatr.* **2020**, *78*, 762. [Crossref]
22. Ferreira-Vieira, T. H.; Guimaraes, I. M.; Silva, F. R.; Ribeiro, F. M.; *Curr. Neuropharmacol.* **2016**, *14*, 101. [Crossref]
23. Selkoe, D. J.; Hardy, J.; *EMBO Mol. Med.* **2016**, *8*, 595. [Crossref]
24. Lewis, J.; Dickson, D. W.; *Acta Neuropathol.* **2016**, *131*, 27. [Crossref]
25. Briggs, C. A.; Chakroborty, S.; Stutzmann, G. E.; *Biochem. Biophys. Res. Commun.* **2017**, *483*, 988. [Crossref]
26. Kumar, A.; Singh, A.; *Front. Pharmacol.* **2015**, *6*, 206. [Crossref]
27. Calsolaro, V.; Edison, P.; *Alzheimer's Dementia* **2016**, *12*, 719. [Crossref]
28. Selkoe, D. J.; *Neuron* **1991**, *6*, 487. [Crossref]
29. Mattson, M. P.; *Nature* **2004**, *430*, 631. [Crossref]
30. Rayman, M. P.; *Lancet* **2012**, *379*, 1256. [Crossref]
31. Cong, W.; Bai, R.; Li, Y.-F.; Wang, L.; Chen, C.; *ACS Appl. Mater. Interfaces* **2019**, *11*, 34725. [Crossref]
32. Malar, D. S.; Prasanth, M. I.; Jeyakumar, M.; Balamurugan, K.; Devi, K. P.; *J. Biochem. Mol. Toxicol.* **2021**, *35*, e22632. [Crossref]
33. Crismon, M. L.; *Ann. Pharmacother.* **1994**, *28*, 744. [Crossref]
34. Rogers, S. L.; Friedhoff, L. T.; *Dementia* **1996**, *7*, 293. [Link] accessed in July 2024
35. McKeith, I.; Del Ser, T.; Spano, P.; Emre, M.; Wesnes, K.; Anand, R.; Cicin-Sain, A.; Ferrara, R.; Spiegel, R.; *Lancet* **2000**, *356*, 2031. [Crossref]
36. Jann, M. W.; Shirley, K. L.; Small, G. W.; *Clin. Pharmacokinet.* **2002**, *41*, 719. [Crossref]
37. Simoni, E.; Daniele, S.; Bottegoni, G.; Pizzirani, D.; Trincavelli, M. L.; Goldoni, L.; Tarozzo, G.; Reggiani, A.; Martini, C.; Piomelli, D.; Melchiorre, C.; Rosini, M.; Cavalli, A.; *J. Med. Chem.* **2012**, *55*, 9708. [Crossref]
38. Parsons, C. G.; Danysz, W.; Dekundy, A.; Pulte, I.; *Neurotoxic. Res.* **2013**, *24*, 358. [Crossref]
39. Matesanz, A. I.; Caballero, A. B.; Lorenzo, C.; Espargaró, A.; Sabaté, R.; Quiroga, A. G.; Gamez, P.; *Inorg. Chem.* **2020**, *59*, 6978. [Crossref]
40. Nordberg, A.; *Alzheimer Dis. Assoc. Disord.* **2006**, *20*, S12. [Crossref]
41. Papp, L. V.; Lu, J.; Holmgren, A.; Khanna, K. K.; *Antioxid. Redox Signaling* **2007**, *9*, 775. [Crossref]
42. Gitler, A. D.; Dhillon, P.; Shorter, J.; *Dis. Models Mech.* **2017**, *10*, 499. [Crossref]
43. Sosa, M. A. G.; De Gasperi, R.; Elder, G. A.; *Hum. Genet.* **2012**, *131*, 535. [Crossref]
44. Wu, T.; Xu, H.; Liang, X.; Tang, M.; *Chemosphere* **2019**, *221*, 708. [Crossref]
45. Harrington, A. J.; Hamamichi, S.; Caldwell, G. A.; Caldwell, K. A.; *Dev. Dyn.* **2010**, *239*, 1282. [Crossref]
46. Kaletta, T.; Hengartner, M. O.; *Nat. Rev. Drug Discovery* **2006**, *5*, 387. [Crossref]
47. Domínguez-Álvarez, E.; Gajdács, M.; Spengler, G.; Palop, J. A.; Marć, M. A.; Kieć-Kononowicz, K.; Amaral, L.; Molnár, J.; Jacob, C.; Handzlik, J.; Sanmartín, C.; *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2821. [Crossref]
48. Calahorro, F.; Ruiz-Rubio, M.; *Invertebr. Neurosci.* **2011**, *11*, 73. [Crossref]
49. Li, J.; Le, W.; *Exp. Neurol.* **2013**, *250*, 94. [Crossref]
50. Mangiavacchi, F.; Dias, I. F. C.; Di Lorenzo, I.; Grzes, P.; Palomba, M.; Rosati, O.; Bagnoli, L.; Marini, F.; Santi, C.; Lenardao, E. J.; Sancineto, L.; *Pharmaceuticals* **2020**, *13*, 211. [Crossref]
51. Murdolo, G.; Bartolini, D.; Tortoioli, C.; Piroddi, M.; Torquato, P.; Galli, F.; *Selenium and Cancer Stem Cells*; Academic Press Inc., 2017.
52. Rayman, M. P.; *Hormones* **2020**, *19*, 9. [Crossref]
53. Domínguez-Álvarez, E.; Plano, D.; Font, M.; Calvo, A.; Prior, C.; Jacob, C.; Palop, J. A.; Sanmartín, C.; *Eur. J. Med. Chem.* **2014**, *73*, 153. [Crossref]
54. Kim, C.; Lee, J.; Park, M.-S.; *Arch. Pharm. Res.* **2015**, *38*, 659. [Crossref]
55. Pacuła, A. J.; Kaczor, K. B.; Antosiewicz, J.; Długosz, A.; Janecka, A.; Janecki, T.; Wojtczak, A.; Ścianowski, J.; *Molecules* **2017**, *22*, 492. [Crossref]
56. Jung, C.-H.; Washburn, M. P.; Wells, W. W.; *Biochem. Biophys. Res. Commun.* **2002**, *291*, 550. [Crossref]
57. Sarma, B. K.; Mugesh, G.; *Chem. - Eur. J.* **2008**, *14*, 10603. [Crossref]
58. Lopes, A. M.; Dahms, H.-U.; Converti, A.; Mariottini, G. L.; *Environ. Monit. Assess.* **2021**, *193*, 285. [Crossref]
59. Tinkov, O. V.; Grigorev, V. Y.; Grigoreva, L. D.; *SAR QSAR Environ. Res.* **2021**, *32*, 541. [Crossref]
60. Piatek, M.; Sheehan, G.; Kavanagh, K.; *Antibiotics* **2021**, *10*, 1545. [Crossref]
61. Ferraz, C. A.; Pastorinho, M. R.; Palmeira-de-Oliveira, A.; Sousa, A. C. A.; *Environ. Pollut.* **2022**, *292*, 118319. [Crossref]

62. Agarrayua, D. A.; Funguetto-Ribeiro, A. C.; Trevisan, P.; Haas, S. E.; Ávila, D. S.; *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2023**, *263*, 109477. [Crossref]
63. Anderson, B.; Nicely, P.; Gilbert, K.; Kosaka, R.; Hunt, J.; Phillips, B.; *Overview of Freshwater and Marine Toxicity Tests: A Technical Tool for Ecological Risk Assessment*; California Environmental Protection Agency: Davis, 2004. [Link] accessed in July 2024
64. Jia, W.; Su, Q.; Cheng, Q.; Peng, Q.; Qiao, A.; Luo, X.; Zhang, J.; Wang, Y.; *Oxid. Med. Cell. Longevity* **2021**, *2021*, 9966223. [Crossref]
65. Bieber, L. W.; da Silva, M. F.; Menezes, P. H.; *Tetrahedron Lett.* **2004**, *45*, 2735. [Crossref]
66. Brenner, S.; *Genetics* **1974**, *77*, 71. [Crossref]
67. Sutphin, G. L.; Kaeberlein, M.; *J. Visualized Exp.* **2009**, *27*, e1152. [Crossref]
68. Ramarao, N.; Nielsen-Leroux, C.; Lereclus, D.; *J. Visualized Exp.* **2012**, *70*, e4392. [Crossref]
69. Megaw, J.; Thompson, T. P.; Lafferty, R. A.; Gilmore, B. F.; *Chemosphere* **2015**, *139*, 197. [Crossref]
70. Maurya, R.; Dubey, K.; Singh, D.; Jain, A. K.; Pandey, A. K.; *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109375. [Crossref]
71. Porta-de-la-Riva, M.; Fontrodona, L.; Villanueva, A.; Cerón, J.; *J. Visualized Exp.* **2012**, *64*, e4019. [Crossref]
72. Boyd, W. A.; Smith, M. V.; Freedman, J. H.; *Methods in Molecular Biology*, vol. 889; Harris, C.; Hansen, J., eds.; Humana Press: Totowa, NJ, USA, 2012, p. 15. [Crossref]
73. Nitao, J. K.; Meyer, S. L.; Chitwood, D. J.; *J. Nematol.* **1999**, *31*, 172. [Link] accessed in July 2024
74. R Core Team; *R: A Language and Environment for Statistical Computing*, version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria, 2023.
75. Pacheco, A. G.; Rebelo, M. F.; *Mar. Environ. Res.* **2013**, *91*, 41. [Crossref]
76. Dostal, V.; Link, C. D.; *J. Visualized Exp.* **2010**, *44*, e2252. [Crossref]
77. Phulara, S. C.; Pandey, S.; Jha, A.; Chauhan, P. S.; Gupta, P.; Shukla, V.; *GeroScience* **2021**, *43*, 791. [Crossref]
78. Bonomo, L. F.; Silva, D. N.; Boasquivis, P. F.; Paiva, F. A.; Guerra, J. F. C.; Martins, T. A. F.; Torres, Á. G. J.; de Paula, I. T. B. R.; Caneschi, W. L.; Jacolot, P.; Grossin, N.; Tessier, F. J.; Boulanger, E.; Silva, M. E.; Pedrosa, M. L.; Oliveira, R. P.; *PLoS One* **2014**, *9*, e89933. [Crossref]
79. Lin, C.; Zhang, X.; Xiao, J.; Zhong, Q.; Kuang, Y.; Cao, Y.; Chen, Y.; *Food Funct.* **2019**, *10*, 1398. [Crossref]
80. Rasband, W. S.; *ImageJ*, version 1.54d; U. S. National Institutes of Health, Bethesda, Maryland, USA, 2023.
81. Guerrero-Rubio, M. A.; Hernández-García, S.; García-Carmona, F.; Gandía-Herrero, F.; *Antioxidants* **2021**, *10*, 438. [Crossref]
82. GraphPad; *Prism*, 5.0 GraphPad Software, CA, USA, 2007.
83. Rodríguez-Villar, K.; Yépez-Mulia, L.; Cortés-Gines, M.; Aguilera-Perdomo, J. D.; Quintana-Salazar, E. A.; Del Angel, K. S. O.; Cortés-Benítez, F.; Palacios-Espinosa, J. F.; Soria-Arteche, O.; Pérez-Villanueva, J.; *Molecules* **2021**, *26*, 2145. [Crossref]
84. Stefanello, S. T.; Gubert, P.; Puntel, B.; Mizdal, C. R.; de Campos, M. M. A.; Salman, S. M.; Dornelles, L.; Avila, D. S.; Aschner, M.; Soares, F. A. A.; *Toxicol. Rep.* **2015**, *2*, 961. [Crossref]
85. Wollenhaupt, S. G. N.; Soares, A. T.; Salgueiro, W. G.; Noremberg, S.; Reis, G.; Viana, C.; Gubert, P.; Soares, F. A.; Affeldt, R. F.; Lüdtke, D. S.; Santos, F. W.; Denardin, C. C.; Aschner, M.; Avila, D. S.; *Food Chem. Toxicol.* **2014**, *64*, 192. [Crossref]
86. Crowder, C. M.; *Trends Neurosci.* **2004**, *27*, 579. [Crossref]
87. Link, C. D.; Taft, A.; Kapulkin, V.; Duke, K.; Kim, S.; Fei, Q.; Wood, D. E.; Sahagan, B. G.; *Neurobiol. Aging* **2003**, *24*, 397. [Crossref]
88. Scott, J. D.; Li, S. W.; Brunskill, A. P. J.; Chen, X.; Cox, K.; Cumming, J. N.; Forman, M.; Gilbert, E. J.; Hodgson, R. A.; Hyde, L. A.; Jiang, Q.; Iserloh, U.; Kazakevich, I.; Kuvelkar, R.; Mei, H.; Meredith, J.; Misiaszek, J.; Orth, P.; Rossiter, L. M.; Slater, M.; Stone, J.; Strickland, C. O.; Voigt, J. H.; Wang, G.; Wang, H.; Wu, Y.; Greenlee, W. J.; Parker, E. M.; Kennedy, M. E.; Stamford, A. W.; *J. Med. Chem.* **2016**, *59*, 10435. [Crossref]
89. Eketjäll, S.; Janson, J.; Kaspersson, K.; Bogstedt, A.; Jeppsson, F.; Fälting, J.; Haeberlein, S. B.; Kugler, A. R.; Alexander, R. C.; Cebers, G.; *J. Alzheimer's Dis.* **2016**, *50*, 1109. [Crossref]
90. Ivanoiu, A.; Pariente, J.; Booth, K.; Lobello, K.; Luscan, G.; Hua, L.; Lucas, P.; Styren, S.; Yang, L.; Li, D.; Black, R. S.; Brashear, H. R.; McRae, T.; *Alzheimer's Res. Ther.* **2016**, *8*, 24. [Crossref]
91. Chang, C.-H.; Wei, C.-C.; Ho, C.-T.; Liao, V. H.-C.; *Phytomedicine* **2021**, *92*, 153733. [Crossref]
92. Pereira, F. S. O.; Barbosa, F. A. R.; Canto, R. F. S.; Lucchese, C.; Pinton, S.; Braga, A. L.; de Azeredo, J. B.; Quines, C. B.; Ávila, D. S.; *NeuroToxicology* **2022**, *88*, 14. [Crossref]
93. Zamberlan, D. C.; Arantes, L. P.; Machado, M. L.; Golombieski, R.; Soares, F. A. A.; *Neuroscience* **2014**, *278*, 40. [Crossref]
94. Gammazza, A. M.; Bavisotto, C. C.; Barone, R.; de Macario, E. C.; Macario, A. J. L.; *Curr. Pharm. Des.* **2016**, *22*, 4040. [Crossref]
95. Rohn, I.; Kroepfl, N.; Aschner, M.; Bornhorst, J.; Kuehnelt, D.; Schwerdtle, T.; *J. Trace Elem. Med. Biol.* **2019**, *55*, 78. [Crossref]
96. Nascimento, V.; Ferreira, N. L.; Canto, R. F. S.; Schott, K. L.; Waczuk, E. P.; Sancineto, L.; Santi, C.; Rocha, J. B. T.; Braga, A. L.; *Eur. J. Med. Chem.* **2014**, *87*, 131. [Crossref]
97. Tan, L.; Wang, S.; Wang, Y.; He, M.; Liu, D.; *Toxicol. Lett.* **2015**, *235*, 75. [Crossref]

98. Huang, C.; Xiong, C.; Kornfeld, K.; *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 8084. [Crossref]
99. Ma, X.; Cui, X.; Li, J.; Li, C.; Wang, Z.; *J. Funct. Foods* **2017**, *39*, 287. [Crossref]
100. Avila, D. S.; Benedetto, A.; Au, C.; Manarin, F.; Erikson, K.; Soares, F. A.; Rocha, J. B. T.; Aschner, M.; *Free Radicals Biol. Med.* **2012**, *52*, 1903. [Crossref]
101. Soares, A. T. G.; Rodrigues, L. B. L.; Salgueiro, W. G.; Dal Forno, A. H. C.; Rodrigues, C. F.; Sacramento, M.; Franco, J.; Alves, D.; Oliveira, R. P.; Pinton, S.; Ávila, D. S.; *J. Trace Elem. Med. Biol.* **2019**, *53*, 34. [Crossref]

Submitted: April 6, 2024
Published online: July 22, 2024