PHARMACIA

Pharmacia 73: e192975
DOI 10.3897/pharmacia.73.e192975

Research Article

Caffeine-derived compounds as multifunctional neuroprotective agents: antioxidant activity in subcellular models and MAO-A/B inhibition

Alime Garip[1], Magdalena Kondeva-Burdina[1], Virginia Tzankova[1], Javor Mitkov[2], Emilio Mateev[2], Alexander Zlatkov[2], Denitsa Stefanova[1]

1 *Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, 1431 Sofia, Bulgaria*
2 *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Sofia, 1431 Sofia, Bulgaria*

Corresponding author: Alime Garip (alime_1999@abv.bg)

Received 25 March 2026 ♦ **Accepted** 15 April 2026 ♦ **Published** 7 May 2026

Citation: Garip A, Kondeva-Burdina M, Tzankova V, Mitkov J, Mateev E, Zlatkov A, Stefanova D (2026) Caffeine-derived compounds as multifunctional neuroprotective agents: antioxidant activity in subcellular models and MAO-A/B inhibition. Pharmacia 73: e192975. https://doi.org/10.3897/pharmacia.73.e192975

Abstract

Oxidative stress, mitochondrial dysfunction, and dysregulated monoamine metabolism are key mechanisms underlying neuronal damage in neurodegenerative disorders, including Parkinson's disease. Caffeine and its derivatives have attracted interest due to their antioxidant and neuroprotective properties. This study evaluated the *in vitro* neuroprotective potential of five caffeine-derived compounds (AL0, AL3, AL4, AL9, and AL10) using subcellular models of oxidative injury. Monoamine oxidase (MAO) inhibitory activity was assessed using recombinant human MAO-A and MAO-B enzymes. Rat brain synaptosomes, mitochondria, and microsomes were exposed to 6-hydroxydopamine (6-OHDA), tert-butyl hydroperoxide (t-BuOOH), and iron/ascorbate (Fe^{2+}/AA), respectively. Neurotoxicity and oxidative stress were evaluated via MTT assay, malondialdehyde (MDA) levels, and reduced glutathione (GSH). Compounds AL0 and AL9 demonstrated consistent antioxidant effects, significant neuroprotection against multiple insults, and selective MAO-B inhibition, indicating their potential as lead structures for novel neuroprotective agents.

Keywords

antioxidant activity, caffeine derivatives, monoamine oxidase inhibition, neuroprotection, oxidative stress, Parkinson's disease, *in vitro* model

Introduction

Neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) represent an expanding global health burden. They are characterized by progressive neuronal dysfunction and loss of vulnerable neuronal populations. Among these, PD is the second most common neurodegenerative disorder worldwide, affecting more than 10 million people, with incidence increasing sharply with age (Poewe et al. 2017). Although the etiology of PD is multifactorial, converging evidence consistently implicates oxidative stress, mitochondrial dysfunction, impaired proteostasis, and dysregulated neurotransmitter metabolism as central drivers of disease progression (Jenner and Olanow 2006; Hwang 2013).

A key contributor to oxidative stress in the dopaminergic system is the enzyme monoamine oxidase-B (MAO-B). Localized primarily in glial cells and mitochondria, MAO-B

Copyright *Garip A et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

PENSOFT

catalyzes the oxidative deamination of dopamine, generating hydrogen peroxide (H_2O_2) and reactive aldehydes as by-products (Youdim et al. 2006). Elevated MAO-B expression in aging and PD brains further amplifies reactive oxygen species (ROS) generation and contributes to progressive dopaminergic neurodegeneration (Saura et al. 1994). Consequently, MAO-B inhibitors remain clinically validated therapeutic agents for early-stage PD, improving dopamine availability while simultaneously reducing oxidative load (Youdim and Bakhle 2006). MAO-A also contributes to monoamine metabolism, and dual inhibition of MAO-A/B has been increasingly explored as a strategy for achieving broader neuroprotective effects (Bortolato et al. 2008).

In parallel with enzymatic sources of ROS, damaged mitochondria represent a major generator of oxidative stress in neurons. Complex I defect, impaired oxidative phosphorylation, membrane depolarization, and increased superoxide production have been strongly associated with PD pathogenesis (Schapira 2008). Mitochondrial dysfunction reduces ATP generation, promotes lipid peroxidation, and decreases antioxidant defenses, particularly the levels of reduced glutathione (GSH), one of the most essential endogenous neuroprotective molecules (Dias et al. 2013). Synaptosomes and microsomal membranes are similarly susceptible to ROS-mediated injury due to their high metabolic activity and polyunsaturated lipid content (Ayala et al. 2014). Therefore, compounds that can stabilize mitochondrial function, reduce lipid peroxidation, and restore GSH balance hold strong therapeutic potential.

Caffeine, one of the most widely consumed psychoactive compounds, has attracted considerable scientific and therapeutic interest due to its neuroprotective, antioxidant, and adenosine receptor–modulating properties. Epidemiological studies consistently report that caffeine intake is associated with a reduced risk of developing Parkinson's disease, suggesting a potential disease-modifying effect (Ross et al. 2000; Ascherio et al. 2001). Mechanistically, caffeine exhibits mild MAO inhibition, enhances mitochondrial function, scavenges ROS, and attenuates neuroinflammation (Chen and Chern 2011). In recent years, structural modification of the caffeine molecule has emerged as a promising approach to enhance its biological activity, improve selectivity, and generate new multifunctional derivatives with stronger antioxidant and neuroprotective effects (Duarte et al. 2019).

The present study focuses on five structurally modified caffeine derivatives (compounds AL0, AL3, AL4, AL9, and AL10), previously identified for their low intrinsic toxicity and promising neuroprotective capacity in neuronal cell lines (Stefanova et al. 2026). Considering the multifactorial nature of oxidative neuronal injury, in the present study, a set of complementary subcellular *in vitro* models was applied, each representing a distinct component of oxidative stress–mediated neurodegeneration. The selected caffeine derivatives were first tested for their MAO-A and MAO-B inhibitory properties. Considering the central role of MAO-B in PD-associated oxidative stress, the identification of caffeine-based molecules with enhanced MAO-B inhibition and favorable selectivity profiles represents an important approach in neuroprotective drug development. In addition to their enzymatic effects, the compounds were evaluated in a set of complementary subcellular *in vitro* models representing different mechanisms of oxidative injury, including synaptosomes, mitochondria, and microsomes. Synaptosomal preparations were used to assess dopaminergic terminal vulnerability and reduced glutathione (GSH) depletion induced by 6-hydroxydopamine (6-OHDA), a widely used model of dopaminergic neurodegeneration (Xue and Bian 2015). Isolated mitochondria were employed to examine oxidative damage induced by tert-butyl hydroperoxide (t-BuOOH), a generator of reactive radical species leading to lipid peroxidation and mitochondrial oxidative stress (Brand et al. 2004). Microsomal fractions were used in the iron/ascorbate (Fe^{2+}/AA)-induced lipid peroxidation model to evaluate membrane susceptibility to hydroxyl radical–mediated oxidative injury via Fenton chemistry (Halliwell and Gutteridge 1992). Together, these subcellular systems enabled the assessment of the antioxidant and neuroprotective potential of the investigated caffeine derivatives.

Therefore, the aim of the present study was to investigate the neuroprotective, antioxidant, and MAO-inhibitory activities of the selected caffeine derivatives using a multi-model subcellular approach. By integrating enzymatic assays with synaptosomal, mitochondrial, and microsomal systems, it was sought to determine whether these compounds possess the multimodal properties required for effective neuroprotection.

Materials and methods

Chemistry

Synthesis of the evaluated molecules

The synthesis of the evaluated azomethine caffeine derivatives has been described in detail previously (Stefanova et al. 2026).

Molecular docking study

The xanthine-based hydrazide compound AL0 was constructed using ChemDraw and prepared for molecular docking using the LigPrep module in Schrödinger (Schrödinger Release 2026-1: LigPrep, Schrödinger, LLC, New York, NY, USA). The preparation included generation of the 3D structure, addition of hydrogen atoms, assignment of bond orders, generation of ionization states at physiological pH (7.0 ± 0.2), and energy minimization using the OPLS4 force field.

The crystal structure of human MAO-B in complex with safinamide (PDB ID: 2V5Z) was obtained from the Protein Data Bank and processed using the Protein Preparation Wizard in Maestro (Schrödinger Release 2026-1). The preparation included the addition of missing hydrogen atoms; assignment of protonation states at pH 7.0 ± 0.4 using PROPKA;

removal of water molecules located more than 5 Å from the FAD cofactor; optimization of the FAD geometry; completion of missing side chains using Prime; and restrained energy minimization to 0.3 Å RMSD using the OPLS4 force field.

The receptor grid was generated by centering on the co-crystallized ligand, safinamide. Compound AL0 was docked into the MAO-B active site using Glide in extra precision (XP) mode. Docking parameters included generation of 10,000 initial poses, retention of up to 100 poses for energy minimization, and output of 10 poses per ligand.

The top-ranked docking pose was further refined using the Induced Fit Docking (IFD) protocol, allowing flexibility of residues within 5 Å of the ligand (Tyr326, Ile199, Phe343, Tyr398, and Tyr435). Binding free energies were calculated using MM/GBSA rescoring with the VSGB 2.0 solvation model and OPLS4 force field, retaining explicit water molecules present in the binding site.

Measurement of MAO-A and MAO-B enzyme activity

Monoamine oxidase activity of recombinant human MAO-A (*h*MAO-A) and MAO-B (*h*MAO-B) was determined using a fluorometric assay based on Amplex® UltraRed reagent, as previously described (Bautista-Aguilera et al. 2014) with minor modifications (Kasabova-Angelova et al. 2020). In the presence of horseradish peroxidase (HRP), Amplex® Red reacts stoichiometrically (1:1) with hydrogen peroxide to produce the fluorescent compound resorufin.

The assay allows sensitive detection of hydrogen peroxide at concentrations as low as 10 pM in a total volume of 100 µL.

Control samples included enzyme solutions (*h*MAO-A or *h*MAO-B) in reaction buffer, enzyme solutions supplemented with hydrogen peroxide, and reaction buffer alone. The tested compounds were added at a final concentration of 1 µM.

The compounds and enzymes were added to a 96-well plate (eight replicates per compound) and incubated for 30 min at 37 °C in the dark. The reaction was initiated by adding 50 µL of a reaction mixture containing Amplex® Red reagent, HRP, and tyramine as a substrate in reaction buffer.

Fluorescence was measured at regular intervals (0, 30, 60, 90, 120, and 150 min) to monitor reaction kinetics at a constant temperature of 37 °C. Measurements were performed using a Synergy™ 2 Multi-Mode Microplate Reader (BioTek Instruments Inc., USA) at excitation/emission wavelengths of λex/λem = 570/690 nm.

IC_{50} values and selectivity index (SI)

Half-maximal inhibitory concentrations (IC_{50}) for the tested compounds, as well as for the reference inhibitors chlorgyline and selegiline, were determined after 2 h of incubation. The results are summarized in Table 2.

The selectivity index (SI) for MAO-B inhibition was calculated using the following equation:

$$\text{SI:hMAOB selectivity index} = IC_{50}(\text{hMAOA})/IC_{50}(\text{hMAOB})$$

Animals

Three experimental animals were used in this study. The animals were obtained from the National Breeding Centre of the Bulgarian Academy of Sciences (Sofia, Bulgaria) and maintained under standard laboratory conditions, including a 12 h light/dark cycle, an ambient temperature of 20–25 °C, and free access to food and water. Food was withdrawn 12 h prior to the experiments.

All experimental procedures were conducted in accordance with national and European regulations for the protection and welfare of laboratory animals. The study protocol was approved by the Bulgarian Food Safety Agency (Permission No. 190, approved on 6 February 2020, valid until 2026).

Brains were removed immediately after decapitation and kept on ice until further processing.

Subcellular in vitro studies

Two types of buffers were used for the isolation of rat brain synaptosomes and mitochondria. Buffer A was 5 mM HEPES and 0.32 M sucrose; Buffer B was 290 mM NaCl, 0.95 mM $MgCl_2 \times 2H_2O$, 10 mM KCl, 2.4 mM $CaCl_2 \times 2H_2O$, 2.1 mM NaH_2PO_4, 44 mM HEPES, and 13 mM D-glucose. For gradient centrifugation, Percoll reagent was used. A stock solution of 90% Percoll was used to prepare 16%, 10%, and 7.5% solutions. All solutions were used as described below. Immediately after harvesting, synaptosomes, microsomes, and mitochondria were incubated with the examined compounds for 1 h. The tested compounds (AL0, AL3, AL4, AL9, AL10) were characterized by lower intrinsic neurotoxicity and good neuroprotective effects on the SH-SY5Y cell line. For assessing the possible neurotoxicity of these compounds, the subcellular fractions were incubated with 100 µM for 1 h, and to evaluate their neuroprotective and antioxidant effects in different models of neurotoxicity, the subcellular fractions were pre-incubated with 50 µM for 30 min and subsequently incubated together for 1 h with the neurotoxic agents. The protein measurement of all subcellular fractions was performed by the method of Lowry et al. (1951).

Rat brain synaptosomes – isolation and incubation

The synaptosomes were prepared from rat brains by multiple subcellular fractionations using a Percoll gradient (Taupin et al. 1994). Model of 6-OHDA-induced neurotoxicity. This *in vitro* model resembles the neurodegenerative processes occurring in PD. Dopamine metabolism and oxidation lead to the formation of reactive oxygen species (ROS) and reactive quinones. These processes were catalyzed by the MAOB enzyme. They induce dopamine neurotoxicity and neurodegeneration (Stokes et al. 2002). The synaptosomes were incubated with 150 µM 6-OHDA for 1 h.

Synaptosomal viability

After the incubation, the synaptosomes were centrifuged three times at 15,000 × g for 1 min. The MTT test was performed to determine synaptosomal viability by the method described by Mungarro-Menchaca et al. (2002). Determination of reduced glutathione (GSH). The level of GSH was determined by measuring the non-protein SH-groups after precipitation of the proteins with trichloroacetic acid (TCA). The presence of thiols in the supernatant was determined using Ellman reagent. The resulting yellow color was measured spectrophotometrically (λ = 412 nm) (Robyt et al. 1971).

Rat brain microsomes – preparation

The brain was homogenized in 9 vol. of 0.1 M Tris buffer, containing 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 0.2 mM EDTA, 1.15% KCl, and 20% (v/v) glycerol at pH 7.4. The homogenate was centrifuged twice at 17,000 × g for 30 min. The supernatants from both centrifugations were combined and centrifuged twice at 100,000 × g for 1 h. The resulting pellet was frozen in Tris until needed (Ravindranath and Anandatheerthavarada 1990). $FeSO_4$/ascorbic acid-induced lipid peroxidation (LPO). The LPO was initiated with a solution of 20 μM iron sulfate and 0.5 mM ascorbic acid (Mansuy et al. 1986). Malondialdehyde (MDA) assay. The quantity of the lipid peroxidation product MDA was assessed using the method described by Deby and Goutier (1990).

$FeSO_4$/ascorbic acid-induced lipid peroxidation (LPO)

Non-enzymatic lipid peroxidation was induced in the isolated microsomal fractions using an iron/ascorbate pro-oxidant system. Microsomes were incubated with the tested substances under experimental conditions for 30 min, followed by initiation of lipid peroxidation by the addition of ferrous sulfate ($FeSO_4$, 20 μM) and ascorbic acid (0.5 mM). The incubation was continued for 60 min at 37 °C, as described by Mansuy et al. (1986).

Malondialdehyde (MDA assay)

The extent of lipid peroxidation was assessed by determining the formation of malondialdehyde (MDA) using the thiobarbituric acid-reactive substances (TBARS) method, as described by Deby and Goutier (1990). Following incubation, the reaction was terminated by the addition of trichloroacetic acid and thiobarbituric acid. The samples were heated in a boiling water bath for 30 min to allow the formation of the MDA–TBA adduct, then cooled and centrifuged. The absorbance of the resulting colored complex was measured spectrophotometrically, and MDA levels were used as an index of lipid peroxidation.

Rat brain mitochondria – isolation

Rat brain mitochondria were isolated by differential centrifugation using a Percoll density gradient, according to the method described by Sims and Anderson (2008). Briefly, brain tissue was homogenized under ice-cold conditions and subjected to successive centrifugation steps to remove nuclei and cellular debris, followed by gradient centrifugation to obtain a purified mitochondrial fraction. The isolated mitochondria were washed and resuspended in the appropriate incubation buffer for subsequent experimental procedures.

Tert-butyl hydroperoxide (t-BuOOH)-induced oxidative stress in brain mitochondria

Oxidative stress in isolated brain mitochondria was induced by incubation with tert-butyl hydroperoxide (t-BuOOH). Mitochondrial suspensions were incubated with 75 μM t-BuOOH for 1 h at 37 °C, as previously described (Karlsson et al. 2000). After the incubation period, mitochondria were processed immediately for the assessment of oxidative stress markers.

Lipid peroxidation assay

Lipid peroxidation in isolated brain mitochondria was assessed by measuring the formation of malondialdehyde (MDA) using the thiobarbituric acid-reactive substances (TBARS) method, as described by Shirani et al. (2019). Following incubation, the reaction was terminated by the addition of 0.3 mL of 0.2% thiobarbituric acid (TBA) and 0.25 mL of 0.05 M sulfuric acid. The samples were heated at 100 °C for 30 min to allow the formation of the MDA–TBA adduct, then cooled and centrifuged at 3,500 × g for 10 min. The absorbance of the resulting supernatant was measured spectrophotometrically at 532 nm, and the amount of MDA formed was used as an index of lipid peroxidation.

Measurement of GSH content

The reduced glutathione (GSH) content in isolated brain mitochondria was determined using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), according to Shirani et al. (2019). After incubation, mitochondrial suspensions were mixed with 0.04% DTNB in 0.1 M phosphate buffer (pH 7.4). The formation of the yellow-colored product was monitored by measuring the absorbance at 412 nm, which is proportional to the GSH concentration.

Statistical analysis

The *h*MAOA and *h*MAOB activities were normalized as a percentage of the untreated control set at 100%, and the results were expressed as mean values and standard deviation (±SD) (GraphPad Prism). Statistical analysis

was performed by one-way analysis of variance (ANOVA) with a post hoc multiple comparisons procedure (Dunnett's test) to assess statistical differences in the case of normal distribution. Values of $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant.

Statistical analysis of the results obtained from brain microsomes, synaptosomes, and mitochondria was performed using the statistical program "MEDCALC." Results were expressed as mean ± SEM for six experiments. The significance of the data was assessed using the non-parametric Mann–Whitney test. Values of $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant.

Results

Chemistry

Based on the intriguing results obtained for a series of caffeine-based azomethines, synthesized in the laboratory and evaluated for their antioxidant properties (Stefanova et al. 2026), the best-performing molecules from the group were subjected to additional evaluation of their possible MAO-inhibiting effects, together with assessment of their neurotoxicity and neuroprotective effects at cellular and subcellular levels. The structures of the target compounds are presented in Fig. 1.

Molecular docking

Molecular docking studies were carried out to elucidate the potential binding affinities and to identify the key amino acid residues involved in the stabilization of the most active MAO-B inhibitor, compound AL0. The docking experiments were performed using the Glide XP mode, followed by induced-fit docking (IFD) and MM/GBSA rescoring to refine the predicted binding poses and evaluate the ligand–receptor interactions more accurately. The results, including the docking scores, binding free energies, and the amino acid residues participating in intermolecular stabilization, are summarized in Table 1.

Table 1. Summary of molecular docking results for MAO-B (PDB: 2V5Z).

Compound	Glide kcal/mol	IFD kcal/mol	MM/GBSA	Intermolecular stabilization
AL0	−8.75	−11.76	−59.14	Water-mediated H-bond with Cys172 and Tyr188
*Safinamide	−11.42	−13.59	−93.26	H-bonds (Gln206; H_2O)
**Selegiline	−9.76	−10.48	−76.17	p-p stacking Tyr326, FAD N5 covalent binding

*Co-crystallized (PBD:2V5Z); selective MAO-B inhibitor.
**Irreversible MAO-B inhibitor.

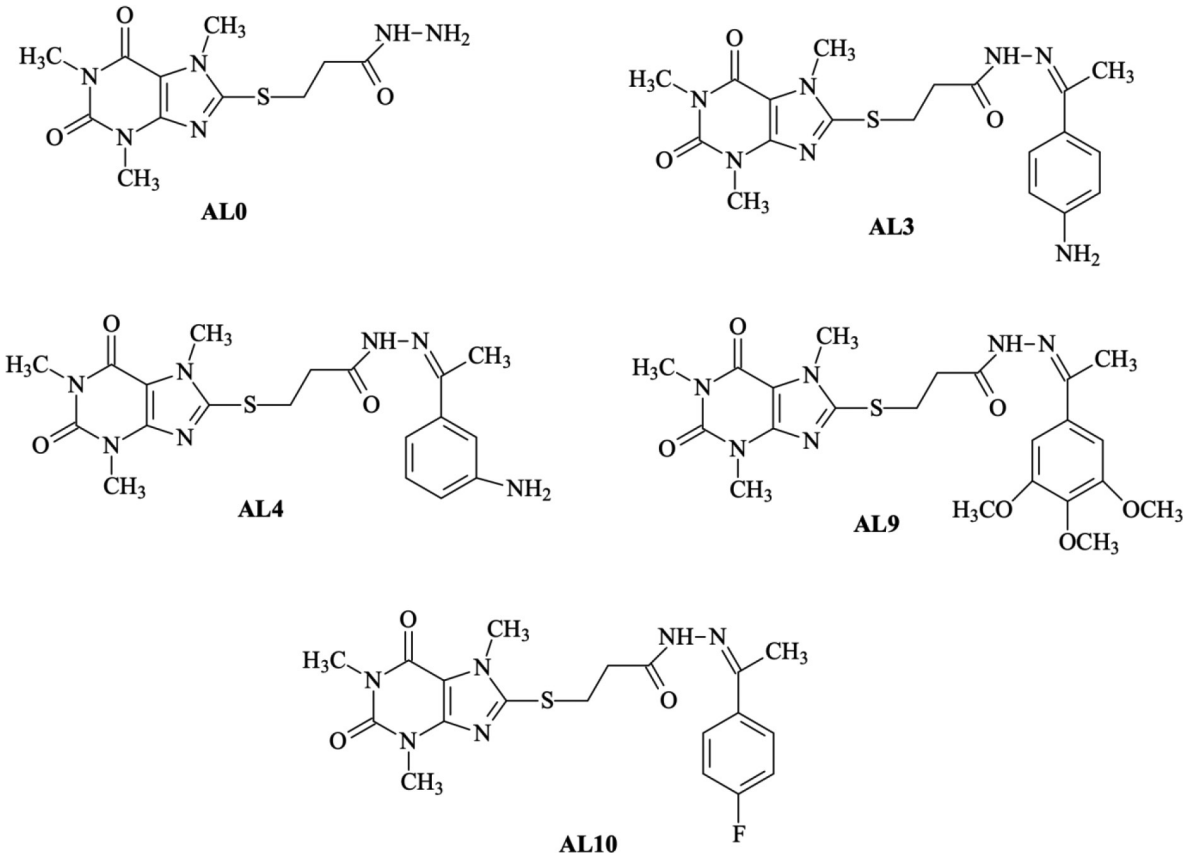

Figure 1. Structures of the evaluated AL0, AL3, AL4, AL9, and AL10.

To gain further insights into the active conformation of the xanthine-based hydrazide AL0, its docking pose was analyzed (Fig. 2).

*h*MAOA and *h*MAOB inhibition assay

The inhibitory activities of compounds AL0, AL3, AL4, AL9, AL10, and caffeine (1 μM) toward recombinant human *h*MAO-A and *h*MAO-B are summarized in Table 2.

MAO-B inhibition prevents dopamine breakdown, thereby increasing its synaptic availability, while excessive MAO-B activity can elevate ROS production and contribute to dopaminergic neuronal degeneration in Parkinson's disease. All tested compounds exhibited statistically significant dual inhibition of *h*MAO-A and *h*MAO-B, comparable to the classical inhibitors chlorgyline (*h*MAO-A, 55% inhibition) and selegiline (*h*MAO-B, 55% inhibition). Among the derivatives,

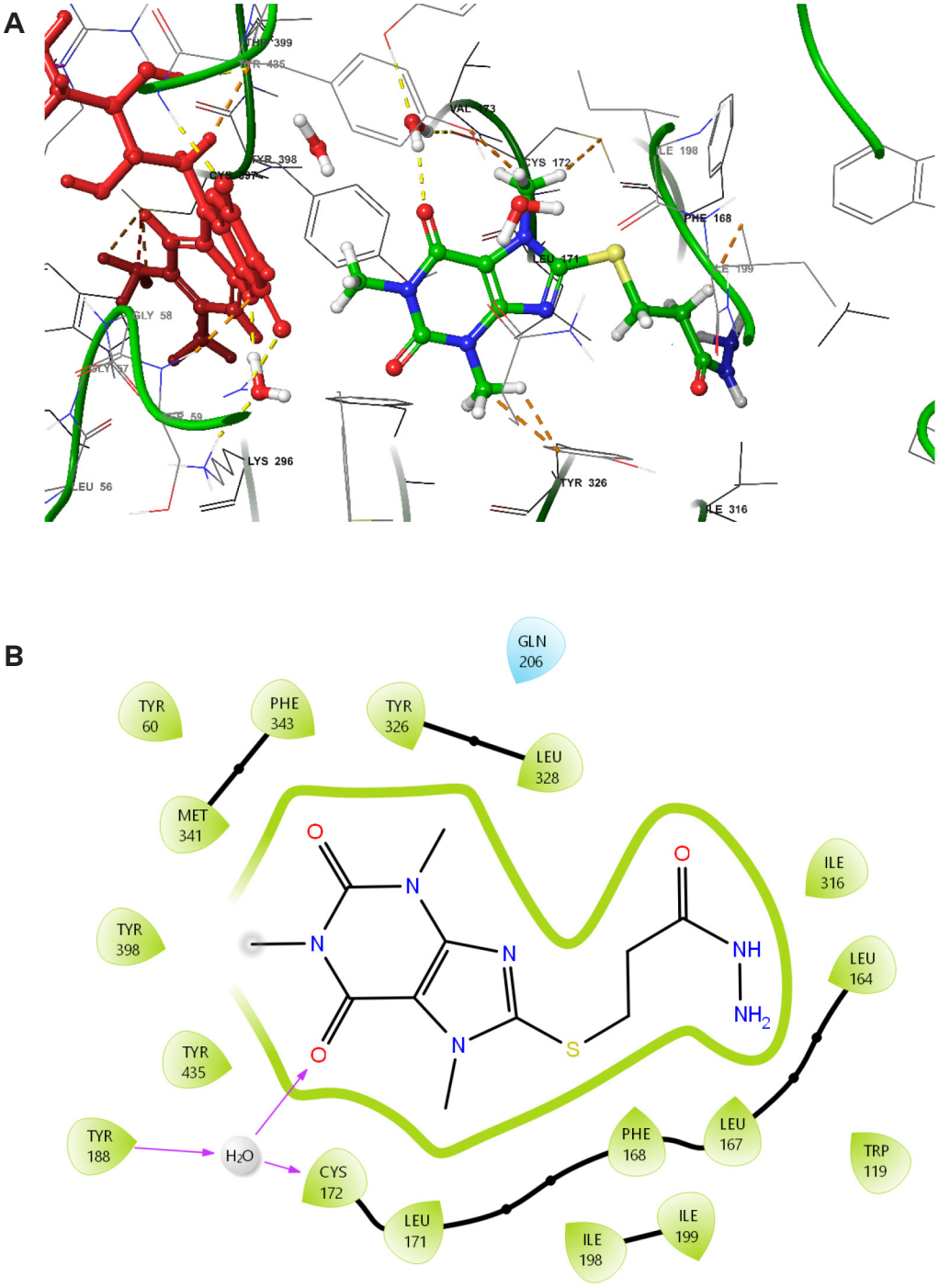

Figure 2. Major intermolecular interactions between the active site of MAO-B and compound AL0: **A.** 3D representation; **B.** 2D interaction diagram. Compound AL0 is shown in green, the FAD cofactor is displayed in red, and the key amino acid residues involved in stabilization are labeled.

Table 2. *In vitro* inhibitory activity of compounds AL0, AL3, AL4, AL9, AL10, and caffeine (1 μM) against recombinant human monoamine oxidase A and B (*h*MAO-A and *h*MAO-B). Values are expressed as mean ± SD from at least three independent experiments performed in quadruplicate. ** $P < 0.01$ and *** $P < 0.001$ vs. control (pure *h*MAO-A); ** $P < 0.01$ and *** $P < 0.001$ vs. control (pure *h*MAO-B).

Compound	*h*MAOA activity	% Inhibition	*h*MAOB activity	% Inhibition
Control	100% ± 7.8	-	100% ± 7.1	-
AL0	65% ± 7.1 **	↓ 35	50% ± 7.1 **	↓ 50
AL3	75% ± 6.9 **	↓ 25	60% ± 7.1 **	↓ 40
AL4	70% ± 7.2 **	↓ 30	60% ± 7.1 **	↓ 40
AL9	60% ± 6.8 **	↓ 40	50% ± 7.1 **	↓ 50
AL10	70% ± 7.3 **	↓ 30	65% ± 7.1 **	↓ 35
Caffeine	85% ± 7.1 **	↓ 15	75% ± 7.1 **	↓ 25
Chlorgyline	45% ± 6.6 ***	↓ 55	-	-
Selegiline	-	-	45% ± 6.6 ***	↓ 55

compound AL9 displayed the strongest *h*MAO-A inhibition (40%) and, together with compound AL0, the highest *h*MAO-B inhibition (50%). Compounds AL3 and AL4 inhibited *h*MAO-A by 25–30% and *h*MAO-B by 40%, and compound AL10 reduced *h*MAO-A and *h*MAO-B activity by 30% and 35%, respectively. Caffeine itself showed moderate inhibition (*h*MAO-A, 15%; *h*MAO-B, 25%). These results demonstrate that the caffeine derivatives possess dual MAO-A/B inhibitory properties with preferential activity toward MAO-B, supporting their potential to attenuate dopamine-associated oxidative stress in Parkinson's disease.

IC_{50} values and selectivity index (SI)

The half-maximal inhibitory concentrations (IC_{50}) of the active compounds, as well as those of the reference inhibitors chlorgyline and selegiline, were determined following 2 h exposure, as described. The results are summarized in Table 3.

AL0 and AL9 exhibited the highest SI values, indicating stronger selectivity toward *h*MAO-B.

Table 3. IC_{50} values (μM ± SEM) and selectivity indices (SI) of compounds AL0, AL3, AL4, AL9, and AL10; caffeine; and the reference inhibitors chlorgyline and selegiline against recombinant human monoamine oxidase A and B (*h*MAO-A and *h*MAO-B). Values are expressed as mean ± SEM from at least three independent experiments performed in quadruplicate.

Compounds	IC_{50}, (μM ± SD)[a] *h*MAOA	IC_{50}, (μM ± SD)[a] *h*MAOB	SI[b]
AL0	0.221 ± 0.10	0.121 ± 0.10	1.83
AL3	0.433 ± 0.10	0.244 ± 0.10	1.77
AL4	0.421 ± 0.10	0.266 ± 0.10	1.58
AL9	0.225 ± 0.10	0.121 ± 0.10	1.86
AL10	0.411 ± 0.10	0.399 ± 0.10	1.03
Caffeine	0.491 ± 0.10	0.411 ± 0.10	1.19
Selegiline	-	0.120 ± 0.085	-
Chlorgyline	0.131 ± 0.088	-	-

Effects of the compounds AL0, AL3, AL4, AL9, AL10, and caffeine on isolated rat brain synaptosomes

The effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine on isolated rat brain synaptosomes were evaluated at a concentration of 100 μM. All compounds induced slight but statistically significant reductions in synaptosomal viability and reduced glutathione (GSH) levels compared with untreated controls (Fig. 3). Specifically, compounds AL0 and AL9 decreased synaptosomal viability by approximately 15% and 20% and GSH levels by 25%, whereas compound AL3 reduced viability by 25% and GSH by 35%, and compound AL4 decreased viability by 30% and GSH by 40%. Compounds AL10 and caffeine both reduced synaptosomal viability and GSH levels by approximately 30%. These results indicate that, while minor neurotoxic effects are observed at this concentration, the compounds maintain an overall favorable profile for further neuroprotective evaluation.

The neurotoxin 6-hydroxydopamine (6-OHDA) is widely used to model Parkinson's disease–related dopaminergic neurodegeneration. In the present study, an *in vitro* synaptosomal model was employed, in which 6-OHDA was used to induce oxidative damage and depletion of reduced glutathione (GSH) in isolated rat brain synaptosomes. Administered alone at 150 μM, 6-OHDA produced a strong neurotoxic effect, reducing both synaptosomal viability and GSH levels by approximately 50% compared with control synaptosomes (Fig. 4). In this model, all tested compounds (50 μM) demonstrated statistically significant neuroprotective activity. Compounds AL0 and AL9 provided the strongest protection, maintaining synaptosomal viability and GSH levels at approximately 85% of control values (toxic 6-OHDA group). Compound AL3 preserved synaptosomal viability to approximately 80% of the control level and GSH levels to approximately 70%, while compounds AL4, AL10, and caffeine showed moderate but statistically significant protective effects, maintaining viability at approximately 75% and GSH levels at approximately 70% compared with the 6-OHDA-treated group (Fig. 4).

Effects of the compounds AL0, AL3, AL4, AL9, AL10, and caffeine on isolated rat brain mitochondria

The effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine on isolated rat brain mitochondria were evaluated at a concentration of 100 μM. All compounds induced slight but statistically significant alterations, as evidenced by increased malondialdehyde (MDA) production and decreased reduced glutathione (GSH) levels compared with untreated controls (Fig. 5). Compounds AL0 and AL9 exhibited the mildest toxic effects, reducing GSH by approximately 30% and increasing MDA by 25%. In contrast, compounds AL3, AL4, AL10, and caffeine caused more pronounced changes, with GSH levels

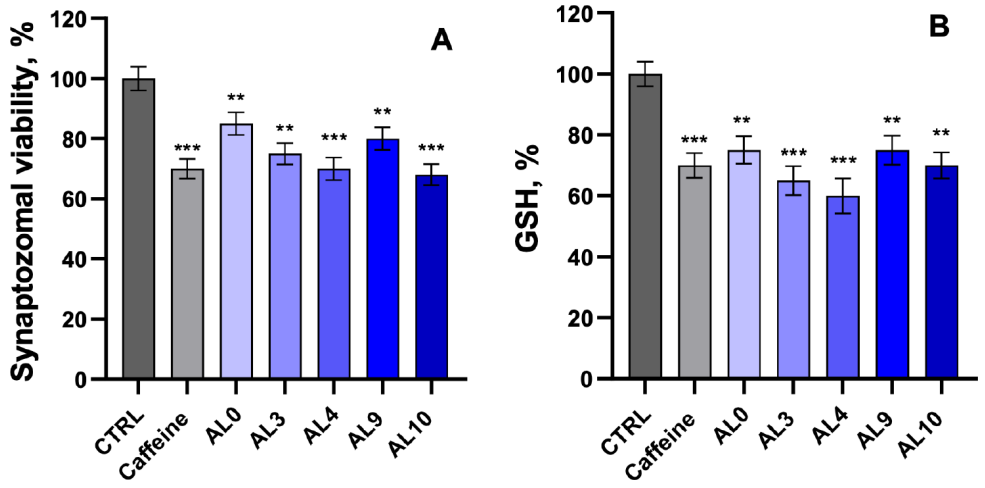

Figure 3. Effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine (100 μM) on synaptosomal viability (**A**) and reduced glutathione levels (GSH) (**B**) in isolated rat brain synaptosomes. The compounds were administered alone at 100 μM. ** $P < 0.01$; *** $P < 0.001$ vs. control (non-treated synaptosomes). Data are presented as mean ± SEM from at least three independent experiments ($n = 3$).

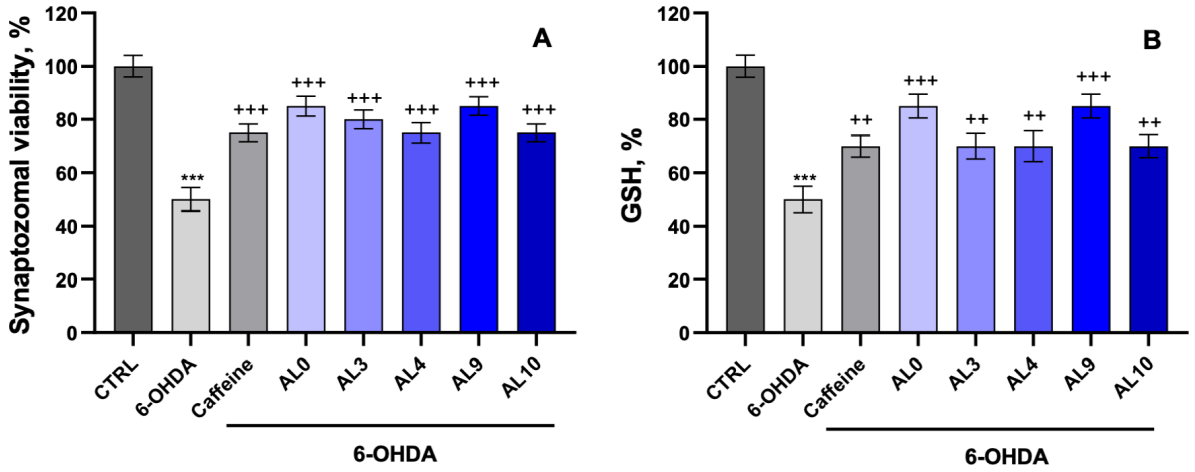

Figure 4. Effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine (50 μM), co-incubated with 6-OHDA (150 μM), on synaptosomal viability (**A**) and reduced glutathione (GSH) levels (**B**) in isolated rat brain synaptosomes. Values are expressed as mean ± SEM from at least three independent experiments. *** $P < 0.001$ vs. control (non-treated synaptosomes); ++ $P < 0.01$; +++ $P < 0.001$ vs. 6-OHDA-treated group.

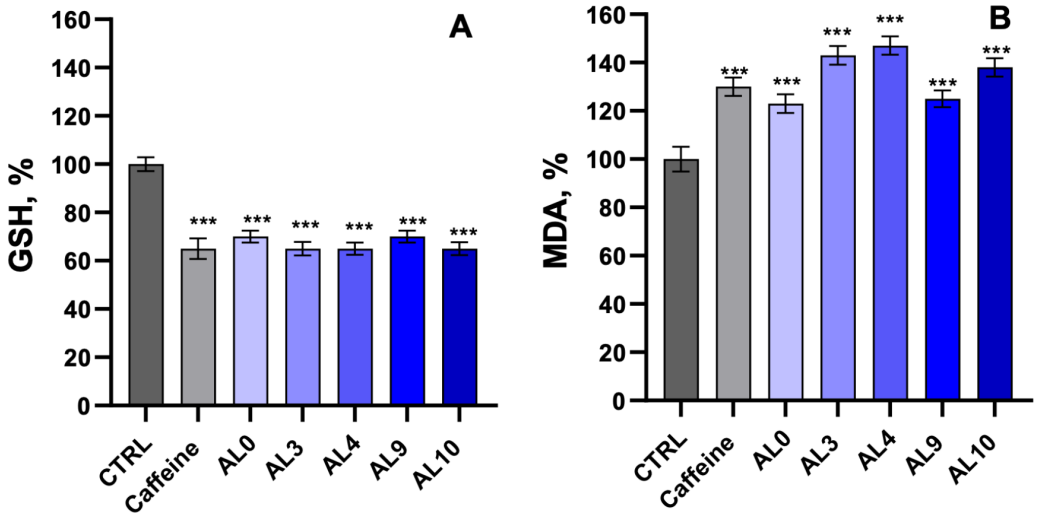

Figure 5. Effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine (100 μM), administered alone, on reduced glutathione (GSH) levels (**A**) and malondialdehyde (MDA) production (**B**) in isolated rat brain mitochondria. Values are expressed as mean ± SEM from at least three independent experiments. *** $P < 0.001$ vs. control (non-treated mitochondria).

decreasing by ~35% and MDA production increasing by 43%, 47%, and 38% relative to control. These findings indicate that, while minor mitochondrial stress is observed at this concentration, compounds AL0 and AL9 maintain the most favorable profile, supporting their potential for further neuroprotective evaluation.

Neuroprotective effects in the t-BuOOH-induced oxidative stress model

Exposure of isolated rat brain mitochondria to tert-butyl hydroperoxide (t-BuOOH, 75 μM) alone induced pronounced oxidative stress, decreasing mitochondrial GSH levels by approximately 50% and increasing MDA production by ~150% relative to untreated controls (Fig. 6). In this model, all tested compounds (50 μM) exhibited significant neuroprotective and antioxidant activity. Compounds AL0 and AL9 showed the strongest protective effects, restoring GSH levels to approximately 85% of the control level and reducing MDA production by approximately 38% compared with the t-BuOOH-treated group. In contrast, compounds AL3, AL4, AL10, and caffeine exhibited moderate protection, restoring GSH levels to approximately 70% of the control level and decreasing MDA production by approximately 70% relative to the t-BuOOH-treated group (Fig. 6). These results highlight the superior antioxidant and mitochondrial-protective potential of compounds AL0 and AL9.

Effects of the compounds AL0, AL3, AL4, AL9, AL10, and caffeine on isolated rat brain microsomes

The effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine were evaluated on isolated rat brain microsomes at a concentration of 100 μM. All compounds induced slight but statistically significant pro-oxidant effects, as reflected by increased malondialdehyde (MDA) levels compared with untreated controls. Compounds AL0 and AL9 caused a moderate increase in MDA production by 15% and 23%, whereas compounds AL3, AL4, AL10, and caffeine elicited stronger pro-oxidant effects, elevating MDA levels by 42%, 30%, 28%, and 38% compared with control (Fig. 7).

To further assess antioxidant potential, the iron/ascorbate (Fe^{2+}/AA) system, a classical non-enzymatic model of lipid peroxidation mediated by Fenton chemistry, was employed. Fe^{2+}/AA alone increased MDA production by ~139% compared with control microsomes. In this model, all tested compounds (50 μM) exhibited significant antioxidant activity. Compounds AL0 and AL9 showed the strongest protective effects, reducing MDA production by ~55–60%, while compounds AL3, AL4, AL10, and caffeine decreased MDA levels by ~45–50% relative to Fe^{2+}/AA-treated microsomes (Fig. 7). These findings indicate that compounds AL0 and AL9 possess the most prominent antioxidant capacity in microsomal membranes.

Discussion

Neurodegenerative diseases such as Parkinson's disease (PD) are characterized by progressive neuronal dysfunction and loss, largely driven by oxidative stress, mitochondrial impairment, and disturbances in neurotransmitter metabolism (Alqahtani et al. 2023). In this context, multifunctional small molecules combining antioxidant, neuroprotective, and enzyme-modulating properties represent a promising strategy for counteracting neurodegenerative processes. The present study provides comprehensive *in vitro* evidence that caffeine-derived compounds possess neuroprotective properties. Their beneficial effects were observed across several mechanistically relevant models of oxidative stress, mitochondrial dysfunction, and membrane lipid peroxidation.

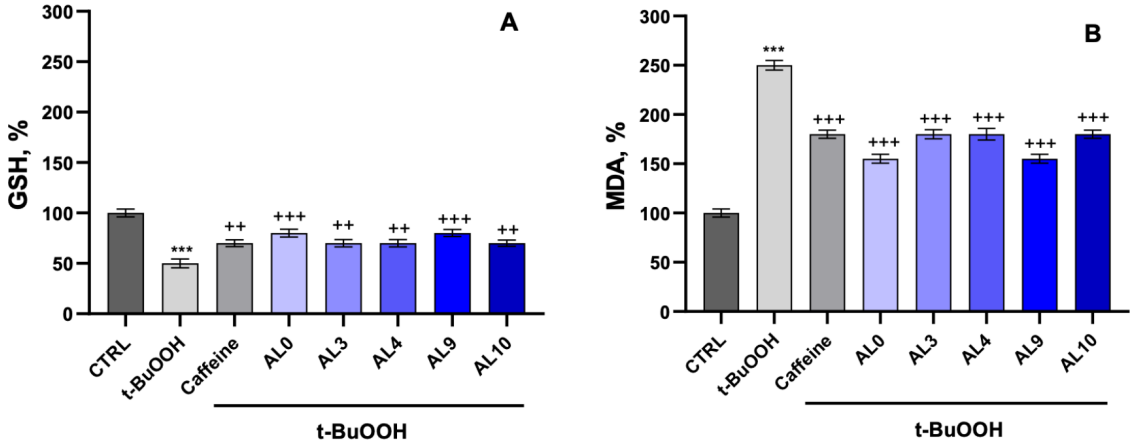

Figure 6. Effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine (50 μM), co-incubated with tert-butyl hydroperoxide (t-BuOOH, 75 μM), on reduced glutathione (GSH) levels (**A**) and malondialdehyde (MDA) production (**B**) in isolated rat brain mitochondria. Values are expressed as mean ± SEM from at least three independent experiments. *** $P < 0.001$ vs. control (non-treated mitochondria); ++ $P < 0.01$; +++ $P < 0.001$ vs. t-BuOOH-treated group.

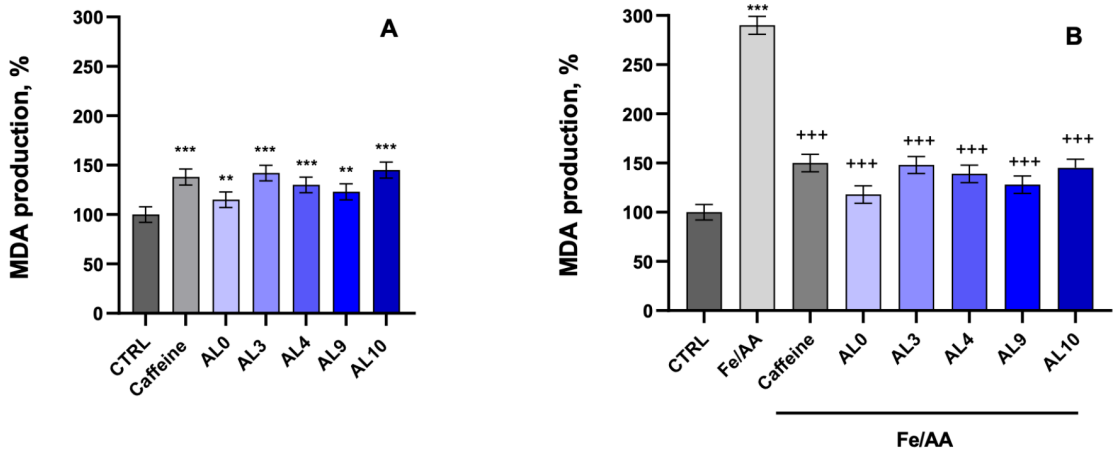

Figure 7. Effects of compounds AL0, AL3, AL4, AL9, AL10, and caffeine, administered alone (100 μM) (**A**) and co-incubated at 50 μM with the Fe^{2+}/ascorbic acid (Fe^{2+}/AA) system (**B**), on malondialdehyde (MDA) production in isolated rat brain microsomes. Values are expressed as mean ± SEM from at least three independent experiments. ** $P < 0.01$; *** $P < 0.001$ vs. control (non-treated microsomes); +++ $P < 0.001$ vs. Fe^{2+}/AA-treated group.

Molecular docking

Compound AL0 demonstrates competitive binding affinity within the MAO-B active site, with Glide XP and IFD scores (–8.75 and –11.76 kcal/mol, respectively) approaching those of established inhibitors selegiline (–9.76/–10.48 kcal/mol) and safinamide (–11.42/–13.59 kcal/mol). The initial Glide XP score indicates favorable recognition of the xanthine scaffold, while IFD refinement reveals enhanced binding through induced-fit accommodation near the FAD cofactor. Importantly, the water-mediated hydrogen bonding network in AL0 stabilization, involving Cys172 and Tyr188 amino acids, positions the ligand effectively within the substrate cavity, supporting its classification as a promising reversible MAO-B inhibitor candidate.

However, MM/GBSA recovery reveals substantially lower binding free energy for AL0 (–59.14 kcal/mol) compared to safinamide (–93.26 kcal/mol) and selegiline (–76.17 kcal/mol). This discrepancy suggests that, while AL0 achieves good initial docking poses, its predicted solvation and entropic penalties are higher, potentially due to suboptimal hydrophobic enclosure or conformational strain in the water-bridged complex. Safinamide benefits from optimal Gln206 hydrogen bonding and extensive surface burial, while selegiline's covalent FAD engagement provides irreversible stabilization absent in AL0's reversible profile. Despite the modest MM/GBSA score, AL0's docking simulations establish it as a viable xanthine-based lead for MAO-B inhibition, given the non-covalent binding mode.

The simulations demonstrated that AL0 is positioned near the FAD cofactor. This binding pose positions the planar xanthine scaffold optimally within the enzyme's bipartite substrate cavity, which is lined by aromatic "gate" residues and hydrophobic side chains (Milczek et al. 2011). The 2D interaction diagram illustrates that AL0 is engaged in several non-covalent interactions, including hydrogen bonds, π–π stacking, and extensive van der Waals contacts, collectively contributing to the stabilization of the ligand–enzyme complex and supporting its high predicted binding affinity.

The xanthine ring system of AL0 is enveloped by a cluster of aromatic residues, including Tyr398, Tyr435, Tyr188, Phe343, Tyr326, and Trp119, which form hydrophobic interactions with the purine-like heterocycle. Tyr326, a critical gating residue, facilitates access to the FAD-proximal region, while Tyr398 and Tyr435 contribute hydrogen bonding. Additional hydrophobic stabilization arises from van der Waals contacts with an extensive array of aliphatic and sulfur-containing residues, such as Met341, Tyr60, Ile198, Ile199, Ile316, Ile164, Leu165, Leu167, Leu171, Leu172, and Cys172. These interactions effectively bury the ligand, minimizing desolvation penalties and promoting tight packing within the cavity of MAO-B.

A notable feature of the binding mode is the involvement of a bridging water molecule, which mediates a hydrogen bond between the ligand's carbonyl moiety and Tyr188/Cys172. This water-bridged interaction underscores the role of structured solvent networks and highlights the importance of explicitly including water molecules in docking simulations.

A key finding is the dual inhibitory activity toward *h*MAO-A and *h*MAO-B, with most compounds exhibiting significant inhibition at low micromolar concentrations. MAO-B inhibition remains a well-established therapeutic approach in PD, as excessive MAO-B activity contributes to dopamine depletion, hydrogen peroxide production, and secondary oxidative injury (Guglielmi et al. 2022). Compounds AL0 and AL9 emerged as the most potent inhibitors, showing ~40% inhibition of MAO-A and ~50% inhibition of MAO-B, with selectivity indices indicating a preferential effect toward MAO-B. These results suggest that the structural modifications in these derivatives enhance both neuroprotection and modulation of monoaminergic metabolism. The neuroprotective properties of the compounds were confirmed in the 6-OHDA synaptosomal model, which mimics dopaminergic neurodegeneration. 6-OHDA selectively enters dopaminergic neurons via the dopamine transporter and, once in the cytosol, undergoes auto-oxidation to generate reactive oxygen

species (ROS) and induce mitochondrial dysfunction. Under the catalytic action of MAO-B, 6-OHDA is further converted to *p*-quinone, amplifying ROS formation and markedly reducing intracellular GSH levels, thereby contributing to dopaminergic neurodegeneration (Xue and Bian 2015). As expected, 6-OHDA alone reduced synaptosomal viability and GSH levels by ~50%. All caffeine derivatives provided statistically significant protection, with compounds AL0 and AL9 showing the strongest effects, maintaining ~85% of viability and GSH. This dual preservation supports the notion that these molecules act both upstream and downstream of oxidative insult, stabilizing redox balance and mitigating excitotoxic mechanisms. In isolated mitochondria, all compounds induced mild toxicity when applied alone at 100 μM, consistent with the sensitivity of this system. However, under oxidative challenge with t-BuOOH, all derivatives demonstrated significant antioxidant and mitochondrial-protective effects, reducing MDA formation and preserving GSH levels. Compounds AL0 and AL9 again displayed superior activity, reducing MDA by ~38% and restoring GSH to ~70% of control levels. These findings indicate that the derivatives may function as radical scavengers and membrane stabilizers, mitigating oxidative damage propagated through peroxyl, alkoxyl, and methyl radicals.

Microsomal membranes, rich in polyunsaturated fatty acids, were also protected by the caffeine derivatives. While slight pro-oxidant effects were observed when compounds were applied alone at 100 μM, all derivatives showed strong antioxidant activity under Fe^{2+}/AA-induced oxidative stress, reducing MDA levels by 45–50%, with compounds AL0 and AL9 again demonstrating the greatest protective effect. This context-dependent activity highlights their ability to respond to acute oxidative challenges.

Across all models, a consistent pattern emerged: compounds AL0 and AL9 exhibited superior neuroprotective, antioxidant, and MAO-inhibitory properties, whereas compounds AL3, AL4, AL10, and caffeine showed moderate but significant activity. This structure–activity relationship indicates that specific chemical modifications of the caffeine core enhance interactions with cellular membranes, free radical scavenging, mitochondrial redox modulation, and dual MAO inhibition. The parallel improvements in synaptosomal and mitochondrial parameters further support the concept of multimodal neuroprotection, targeting metabolic, enzymatic, and oxidative pathways simultaneously. Mechanistically, the data suggest that compounds capable of restoring GSH levels, attenuating ROS-mediated lipid peroxidation, and reducing MAO-B-driven oxidative stress hold therapeutic potential for PD. The caffeine derivatives evaluated in the study exhibit complementary neuroprotective and antioxidant activities across distinct biological systems, supporting their development as multifunctional therapeutic agents. Further *in vivo* studies, pharmacokinetics, and toxicity profiling are required to provide solid information for future drug development based on caffeine scaffolds.

Conclusion

The present study demonstrates that caffeine-derived compounds AL0 and AL9 exhibit the most promising neuroprotective profile among the tested molecules (AL0, AL3, AL4, AL9, AL10, and caffeine). Overall, the docking-derived interaction profile of AL0 reveals efficient accommodation within the MAO-B binding pocket, characterized mostly by a dense network of hydrophobic interactions. This binding mode aligns closely with established MAO-B inhibitors, such as safinamide. These findings position AL0 as a promising lead compound for the development of xanthine-based MAO-B selective inhibitors, particularly for neuroprotective applications in neurodegenerative diseases, with opportunities for further optimization through side-chain modifications to enhance potency or isoform selectivity. At 1 μM, both compounds showed potent dual inhibition of MAO-A and MAO-B, with marked selectivity toward MAO-B, a key contributor to oxidative stress and dopamine depletion. At higher concentrations (100 μM), all compounds induced only mild but statistically significant decreases in synaptosomal viability and GSH levels, as well as moderate increases in MDA production in mitochondria and microsomes, indicating a relatively favorable safety profile. In different models of oxidative neuronal injury—6-OHDA in synaptosomes, t-BuOOH in mitochondria, and Fe^{2+}/AA in microsomes—compounds AL0 and AL9 consistently provided the strongest protection, preserving glutathione levels, reducing lipid peroxidation, and mitigating oxidative damage. Caffeine-derived compounds AL0 and AL9 demonstrated the most promising neuroprotective profile, exhibiting dual MAO-A/B inhibition with marked MAO-B selectivity, mild intrinsic toxicity, and significant protection against oxidative stress in synaptosomes, mitochondria, and microsomes. These findings support their potential as multifunctional candidates for the development of neuroprotective agents targeting neurodegenerative diseases.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: Permission No. 190, Bulgarian Food Safety Agency (approved on February 6, 2020; valid until 2026).

Use of commercially available immortalised human and animal cell lines: SH-SY5Y human neuroblastoma cell line (Sigma-Aldrich, ECACC, catalogue No. 94030304).

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

Funding

This study was financed by the European Union—NextGenerationEU through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01. Alime Garip is gratefully acknowledged for funding the in vitro cytotoxicity tests, sponsored by the Medical Science Council of the Medical University of Sofia under the project "Young Researcher 2025," Д-218/4 June 2025.

Author contributions

Alime Garip: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. Magdalena Kondeva-Burdina: Conceptualization, Supervision, Writing – review & editing. Virginia Tzankova: Conceptualization, Supervision, Writing – review & editing. Yavor Mitkov: Chemical synthesis, Methodology, Writing – review & editing. Emilio Mateev: Chemical synthesis, Data curation. Alexander Zlatkov: Conceptualization, Supervision, Writing – review & editing. Denitsa Stefanova: Conceptualization, Methodology, Supervision, Writing – review & editing, Project administration.

Author ORCIDs

Alime Garip https://orcid.org/0009-0008-9216-1544
Emilio Mateev https://orcid.org/0000-0002-5885-7213
Alexander Zlatkov https://orcid.org/0000-0002-1695-8454

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Alqahtani T, Deore SL, Kide AA, Shende BA, Sharma R, Chakole RD, Ghosh A (2023) Mitochondrial dysfunction and oxidative stress in Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis: An updated review. Mitochondrion 71: 83–92. https://doi.org/10.1016/j.mito.2023.05.007

Ascherio A, Zhang SM, Hernán MA, Kawachi I, Colditz GA, Speizer FE, Willett WC (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Annals of Neurology 50: 56–63. https://doi.org/10.1002/ana.1052

Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity 2014: 1–31. https://doi.org/10.1155/2014/360438

Bautista-Aguilera OM, Esteban G, Bolea I, Nikolic K, Agbaba D, Moraleda I, Iriepa I, Samadi A, Soriano E, Unzeta M, Marco-Contelles J (2014) Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil–indolyl hybrids as multipotent cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer's disease. European Journal of Medicinal Chemistry 75: 82–95. https://doi.org/10.1016/j.ejmech.2013.12.028

Bortolato M, Chen K, Shih JC (2008) Monoamine oxidase inactivation: From pathophysiology to therapeutics. Advanced Drug Delivery Reviews 60: 1527–1533. https://doi.org/10.1016/j.addr.2008.06.002

Brand MD, Affourtit C, Esteves TC, Green K, Lambert AJ, Miwa S, Pakay JL, Parker N (2004) Mitochondrial superoxide: Production, biological effects, and activation of uncoupling proteins. Free Radical Biology and Medicine 37: 755–767. https://doi.org/10.1016/j.freeradbiomed.2004.05.034

Chen JF, Chern Y (2011) Impacts of methylxanthines and adenosine receptors on neurodegeneration: Human and experimental studies. Handbook of Experimental Pharmacology 200: 267–310. https://doi.org/10.1007/978-3-642-13443-2_10

Deby C, Goutier R (1990) New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. Biochemical Pharmacology 39: 399–405. https://doi.org/10.1016/0006-2952(90)90043-K

Dias V, Junn E, Mouradian MM (2013) The role of oxidative stress in Parkinson's disease. Journal of Parkinson's Disease 3: 461–491. https://doi.org/10.3233/JPD-130230

Duarte AI, Moreira PI, Oliveira CR (2019) Insulin in central nervous system: More than just a peripheral hormone. Journal of Aging Research 2019: 1–14. https://doi.org/10.1155/2019/3840173

Guglielmi P, Carradori S, D'Agostino I, Campestre C, Petzer JP (2022) An updated patent review on monoamine oxidase (MAO) inhibitors. Expert Opinion on Therapeutic Patents 32: 849–883. https://doi.org/10.1080/13543776.2022.2083501

Halliwell B, Gutteridge JMC (1992) Biologically relevant metal ion-dependent hydroxyl radical generation: An update. FEBS Letters 307: 108–112. https://doi.org/10.1016/0014-5793(92)80911-Y

Hwang O (2013) Role of oxidative stress in Parkinson's disease. Experimental Neurobiology 22: 11–17. https://doi.org/10.5607/en.2013.22.1.11

Jenner P, Olanow CW (2006) The pathogenesis of cell death in Parkinson's disease. Neurology 66(Suppl. 4): S24–S36. https://doi.org/10.1212/WNL.66.10_suppl_4.S24

Karlsson J, Emgard M, Brundin P, Burkitt MJ (2000) trans-Resveratrol protects embryonic mesencephalic cells from tert-butyl hydroperoxide. Journal of Neurochemistry 75: 141–150. https://doi.org/10.1046/j.1471-4159.2000.0750141.x

Kasabova-Angelova A, Kondeva-Burdina M, Mitkov J, Georgieva M, Tzankova V, Zlatkov A (2020) Neuroprotective and MAO-B inhibitory effects of a series of caffeine-8-thioglycolic acid amides. Brazilian Journal of Pharmaceutical Sciences 56: e18255. https://doi.org/10.1590/s2175-97902019000318255

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Mansuy D, Sassi A, Dansette PM, Plat M (1986) A new potent inhibitor of lipid peroxidation in vitro and in vivo, the hepatoprotective drug anisyldithiolthione. Biochemical and Biophysical Research Communications 135: 1015–1021. https://doi.org/10.1016/0006-291X(86)91029-6

Milczek EM, Binda C, Rovida S, Mattevi A, Edmondson DE (2011) The 'gating' residues Ile199 and Tyr326 in human monoamine oxidase B

function in substrate and inhibitor recognition. FEBS Journal 278: 4860–4869. https://doi.org/10.1111/j.1742-4658.2011.08386.x

Mungarro-Menchaca X, Ferrera P, Morán J, Arias C (2002) β-Amyloid peptide induces ultrastructural changes in synaptosomes and potentiates mitochondrial dysfunction in the presence of ryanodine. Journal of Neuroscience Research 68: 89–96. https://doi.org/10.1002/jnr.10193

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag A-E, Lang AE (2017) Parkinson disease. Nature Reviews Disease Primers 3: 17013. https://doi.org/10.1038/nrdp.2017.13

Ravindranath V, Anandatheerthavarada HK (1990) Preparation of brain microsomes with cytochrome P450 activity using calcium aggregation method. Analytical Biochemistry 187: 310–313. https://doi.org/10.1016/0003-2697(90)90461-H

Robyt JF, Ackerman RJ, Chittenden CG (1971) Reaction of protein disulfide groups with Ellman's reagent. Archives of Biochemistry and Biophysics 147: 262–269. https://doi.org/10.1016/0003-9861(71)90334-1

Ross GW (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA 283: 2674. https://doi.org/10.1001/jama.283.20.2674

Saura J, Luque JM, Cesura AM, Prada MD, Chan-Palay V, Huber G, Löffler J, Richards JG (1994) Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography. Neuroscience 62: 15–30. https://doi.org/10.1016/0306-4522(94)90311-5

Schapira AH (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. The Lancet Neurology 7: 97–109. https://doi.org/10.1016/S1474-4422(07)70327-7

Shirani M, Alizadeh S, Mahdavinia M, Dehghani MA (2019) The ameliorative effect of quercetin on bisphenol A-induced toxicity in mitochondria isolated from rats. Environmental Science and Pollution Research 26: 7688–7696. https://doi.org/10.1007/s11356-018-04119-5

Sims NR, Anderson MF (2008) Isolation of mitochondria from rat brain using Percoll density gradient centrifugation. Nature Protocols 3: 1228–1239. https://doi.org/10.1038/nprot.2008.105

Stefanova D, Garip A, Tzankova V, Kostov S, Mateev E, Zlatkov A, Mitkov Y (2026) Design and synthesis of caffeine-based derivatives with antioxidant and neuroprotective activity: In vitro evaluation and SwissADME profiling. Antioxidants 15: 217. https://doi.org/10.3390/antiox15020217

Stokes AH, Freeman WM, Mitchell SG, Burnette TA, Hellmann GM, Vrana KE (2002) Induction of GADD45 and GADD153 in neuroblastoma cells by dopamine-induced toxicity. NeuroToxicology 23: 675–684. https://doi.org/10.1016/S0161-813X(02)00093-1

Taupin P, Zini S, Cesselin F, Ben-Ari Y, Roisin M (1994) Subcellular fractionation on Percoll gradient of mossy fiber synaptosomes. Journal of Neurochemistry 62: 1586–1595. https://doi.org/10.1046/j.1471-4159.1994.62041586.x

Xue X, Bian J-S (2015) Neuroprotective effects of hydrogen sulfide in Parkinson's disease animal models. In: Methods in Enzymology: 169–186. https://doi.org/10.1016/bs.mie.2014.11.015

Youdim MBH, Bakhle YS (2006) Monoamine oxidase: Isoforms and inhibitors in Parkinson's disease and depressive illness. British Journal of Pharmacology 147(S1): S287–S296. https://doi.org/10.1038/sj.bjp.0706464

Youdim MBH, Edmondson D, Tipton KF (2006) The therapeutic potential of monoamine oxidase inhibitors. Nature Reviews Neuroscience 7: 295–309. https://doi.org/10.1038/nrn1883