

In Silico Evaluation of Competitive Binding of Food Preservative Combinations at AChE, MAO-B and NMDA Receptors and Their Network Overlap with Neurodegeneration Pathways

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ABSTRACT

Background: Food preservatives such as sodium benzoate, potassium sorbate, sodium propionate, sodium nitrite, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are consumed together in packaged foods, yet regulatory limits and most neuropharmacological evaluations assess them as isolated chemicals. **Objective:** To evaluate, in silico, the individual and competitive pairwise binding affinities of these food preservatives at human acetylcholinesterase (AChE), monoamine oxidase B (MAO-B), and NMDA receptor (GluN2B), and identify their systems-level mechanistic overlap with Alzheimer's and Parkinson's disease pathways. **Methods:** Molecular docking (AutoDock Vina) was validated through native ligand redocking (RMSD < 2.0 Å) and compared against standard clinical references (donepezil, selegiline, memantine). Pairwise competitive binding was evaluated via sequential co-docking simulations. Biological target profiles (SwissTargetPrediction) were intersected with Alzheimer's and Parkinson's datasets (GeneCards, DisGeNET), mapped onto protein-protein interaction (STRING, Cytoscape cytoHubba), and characterized via KEGG and GO functional enrichment. **Results:** BHT and BHA displayed the strongest predicted solitary binding across all neuro-targets (AChE: -6.4 and -5.9 kcal/mol; MAO-B: -6.8 and -6.2 kcal/mol; NMDA: -6.3 and -5.8 kcal/mol), closely approaching standard reference drugs. Polar organic preservatives exhibited lower solitary binding (-2.6 to -5.5 kcal/mol). Sequential docking demonstrated prominent steric hindrance for bulky phenolic pairs, whereas small planar preservatives (benzoate) co-docked cooperatively with BHA ($\Delta\Delta G$ up to -0.8 kcal/mol). Network pharmacology highlighted 10 critical hub genes (AKT1, TP53, TNF, CASP3, EGFR, SRC, ESR1, PTGS2, MAPK3, MTOR) significantly converging on neuroactive ligand-receptor interactions, apoptosis, and neuroinflammation pathways ($p < 10^{-5}$). **Conclusion:** Dietary preservatives, especially synthetic phenolic antioxidants and multi-preservative combinations, demonstrate direct computational affinity for critical central neuroreceptors and converge on apoptotic and neuroinflammatory cascades, underscoring the urgent need for empirical multi-preservative neurotoxicological risk assessments.

Keywords: food preservatives, sodium benzoate, molecular docking, network pharmacology, acetylcholinesterase, MAO-B, NMDA receptor, neurodegeneration, synergistic binding

INTRODUCTION

Synthetic food preservatives are widely utilized across modern food manufacturing to prevent microbial spoilage, delay enzymatic decay, and impede oxidative degradation. Among the most prevalent chemical additives are organic acid salts (sodium benzoate, potassium sorbate, sodium propionate), reactive nitrogen precursors (sodium nitrite), and synthetic sterically hindered phenolic antioxidants

(butylated hydroxyanisole [BHA] and butylated hydroxytoluene [BHT]). Global regulatory bodies—including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA), and the US FDA—determine acceptable daily intakes (ADIs) based largely on single-substance toxicological evaluations. However, real-world human dietary habits entail concurrent exposure to multi-additive cocktails present in carbonated

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beverages, sauces, processed meats, and confectionery products.

Existing experimental literature reveals a complex and often contradictory picture regarding the neuropharmacological profiles of food additives. At supra-physiological dosages, rodent models demonstrate that sodium benzoate and sodium nitrite trigger systemic oxidative stress, hippocampal neuronal loss, and cognitive deficits. Conversely, clinical and pre-clinical trials have demonstrated that sodium benzoate acts as a D-amino acid oxidase (DAAO) inhibitor, enhancing synaptic D-serine availability and ameliorating cognitive and psychotic symptoms in clinical schizophrenia and Alzheimer's models. This striking duality highlights an urgent necessity to delineate how preservatives engage key central nervous system (CNS) enzymes and receptors at the atomic level.

Three macromolecular targets play pivotal, well-established roles in neurodegenerative cascades:

- (1) Acetylcholinesterase (AChE), which regulates cholinergic neurotransmission and whose peripheral anionic site accelerates amyloid-beta aggregation; (2) Monoamine Oxidase B (MAO-B), an outer mitochondrial membrane enzyme that governs dopamine metabolism and whose hyperactivation produces cytotoxic reactive oxygen species (ROS) in Parkinson's disease; and
- (3) N-Methyl-D-Aspartate (NMDA) Receptors (GluN1/GluN2B), where channel disruption or unmodulated allosteric stimulation precipitates excitotoxic intracellular calcium overload. Most computational and toxicological evaluations assess single additives against single targets, leaving the questions of competitive active-site occupancy and synergistic pathway engagement unanswered.

Therefore, this investigation establishes a comprehensive *in silico* paradigm combining crystallographic molecular docking, sequential competitive co-docking simulations, pharmacokinetic ADMET profiling, and systems-level network pharmacology to evaluate individual and combined preservative interactions across AChE, MAO-B, and NMDA targets.

MATERIALS AND METHODS

2.1 Ligand Preparation

Three-dimensional chemical structures of six food preservatives—Sodium Benzoate (benzoic acid form, PubChem CID: 243), Potassium Sorbate (sorbic acid form, CID: 5281515), Sodium Propionate (propionic acid form, CID: 1032), Sodium Nitrite (CID: 24526), BHA (CID: 24667), and BHT (CID: 31404)—along with standard clinical neuro-therapeutics: Donepezil (CID: 3152, AChE inhibitor), Selegiline (CID: 26757, MAO-B inhibitor), and Memantine (CID: 4054, NMDA channel blocker) were obtained from NCBI PubChem in SDF format. Structures were imported into Open Babel within PyRx 0.8, assigned physiological ionization states at pH 7.4, energy-minimized using the MMFF94 force field (200 conjugate gradient steps), and converted into PDBQT format with atomic Gasteiger partial charges and defined rotatable bonds.

2.2 Protein Preparation

High-resolution X-ray crystal structures of human target proteins were retrieved from the RCSB Protein Data Bank (PDB): Human Recombinant AChE in complex with Donepezil (PDB ID: 4EY7, resolution 2.35 Å); Human MAO-B in complex with Selegiline/deprenyl analog (PDB ID: 2Z5X, resolution 1.65 Å); and Human NMDA Receptor GluN1/GluN2B ligand-binding/channel domain (PDB ID: 4PE5, resolution 2.50 Å). Protein cleanup was carried out using BIOVIA Discovery Studio Visualizer 2021 and PyMOL 2.5: water molecules, co-factors, and non-receptor crystallographic ligands were removed; polar hydrogens and Kollman charges were added; and structures were saved as rigid receptor PDBQT files.

2.3 Docking and Protocol Validation

Docking calculations were executed using AutoDock Vina v.1.2.0 via PyRx. Grid boxes were centered on the co-crystallized native inhibitor coordinates: AChE (4EY7: $x = -13.98$, $y = -43.88$, $z = 27.91$; size 24x24x24 Å); MAO-B (2Z5X: $x = 52.82$, $y = 157.34$, $z = 25.11$; size 22x22x22 Å); and NMDA (4PE5: $x = -13.25$, $y = -18.72$, $z = -10.45$; size 22x22x22 Å). An exhaustiveness level of 32 was maintained. Protocol validation was verified by extracting and re-docking native co-crystallized ligands, ensuring an RMSD <

2.0 Å relative to experimental crystallographic conformations.

2.4 Combination (Competitive) Docking

To computationally model simultaneous binary exposure within the catalytic binding pocket, a sequential co-docking workflow was implemented. For each preservative pair (A + B), Ligand A was first docked into the apoprotein. The lowest energy conformation (Pose 1) was merged into the receptor coordinate file, generating a modified 'Target+Ligand A' complex. Ligand B was subsequently docked into this modified pocket using identical grid boundaries. Binding energy shifts were computed as $\Delta\Delta G = \Delta G (\text{Ligand B in [Receptor+A]}) - \Delta G (\text{Ligand B alone})$, where negative values denote cooperative pocket stabilization and positive values reflect competitive steric occlusion.

2.5 Pharmacokinetic and ADMET Prediction

Physicochemical descriptors, Lipinski's Rule of Five compliance, gastrointestinal absorption, and blood-brain barrier (BBB) penetration were determined using SwissADME. Quantitative organ toxicities, hepatotoxicity, and acute oral rat lethal dose 50 (LD50 in mg/kg) classifications were modeled via ProTox-II and pkCSM toxicology platforms.

2.6 Network Pharmacology, PPI, and Enrichment

Putative human macromolecular targets of the six preservatives were predicted using SwissTargetPrediction (Homo sapiens, probability score ≥ 0.1). Genes associated with 'Alzheimer's Disease' and 'Parkinson's Disease' were harvested from GeneCards (score ≥ 15.0) and DisGeNET (score ≥ 0.2). Target-disease intersections were identified using InteractiVenn and mapped into STRING 11.5 (high confidence score ≥ 0.70). Protein-protein interaction (PPI) networks were visualized in Cytoscape v3.9.1, and top hub bottleneck genes were extracted using cytoHubba. Gene Ontology (GO) and KEGG enrichment analyses were conducted via DAVID Bioinformatics Resources with Benjamini-Hochberg false discovery rate corrections (FDR $p < 0.05$).

RESULTS

3.1 Docking Validation

Redocking native co-crystallized inhibitors reproduced crystallographic binding poses with high fidelity across all three target receptors (Table 1), demonstrating RMSD values significantly below the 2.0 Å threshold and confirming the validity of the docking parameters.

Target	PDB ID	Native Ligand	Resolution (Å)	RMSD (Å)	Validated (< 2.0 Å)
AChE	4EY7	Donepezil (E20)	2.35	0.84	Yes
MAO-B	2Z5X	Safinamide analog	1.65	0.96	Yes
NMDA	4PE5	Ifenprodil analog	2.50	1.12	Yes

3.2 Binding of Individual Preservatives

Predicted binding free energies (Table 2) showed that sterically bulky, lipophilic preservatives (BHT and

BHA) exhibited the strongest affinity across all three central targets, while low-molecular-weight polar organic acids displayed modest binding scores.

Compound	AChE (4EY7)	MAO-B (2Z5X)	NMDA (4PE5)	Strongest Target
Sodium Benzoate	-5.5	-5.2	-4.9	AChE
Potassium Sorbate	-4.8	-4.6	-4.2	AChE
Sodium Propionate	-3.8	-3.7	-3.4	AChE
Sodium Nitrite	-2.8	-2.6	-2.5	AChE
BHA	-5.9	-6.2	-5.8	MAO-B
BHT	-6.4	-6.8	-6.3	MAO-B
Donepezil (Ref)	-11.5	-8.1	-7.4	AChE
Selegiline (Ref)	-6.8	-7.8	-5.9	MAO-B
Memantine (Ref)	-5.7	-5.4	-7.2	NMDA

3.3 Key Molecular Interactions

Detailed active-site binding analysis (Table 3) showed that BHT and BHA engaged in extensive

hydrophobic and pi-pi stacking contacts with aromatic residues within AChE and MAO-B, while

sodium benzoate predominantly formed hydrogen bonds and electrostatic interactions.

Target	Ligand	dG (kcal/mol)	H-Bond Residues	Hydrophobic / Pi Residues
AChE	BHT	-6.4	Tyr124 (2.68 Å)	Trp86 (pi-alkyl), Tyr337 (pi-pi), Phe338
AChE	Benzoate	-5.5	Ser203 (2.12 Å), Gly121	Trp86 (pi-pi T-shaped), Tyr341, Phe295
MAO-B	BHT	-6.8	Cys172 (2.45 Å)	Phe343, Tyr398 (pi-pi), Leu171, Ile199
MAO-B	BHA	-6.2	Tyr435 (2.20 Å)	Phe168 (pi-alkyl), Trp119, Ile316, Tyr326
NMDA	BHT	-6.3	Glu236 (2.54 Å)	Phe176 (pi-alkyl), Trp223, Ile133, Val181
NMDA	Benzoate	-4.9	Arg115 (1.98 Å), Thr116	Phe114 (pi-pi), His113

3.4 Combination Docking

Sequential competitive docking of 15 preservative pairs (Table 4) revealed that combining two bulky phenolic preservatives (BHA + BHT) caused severe

steric clash (positive delta-delta G), whereas pairing small planar molecules (benzoate) with phenolic antioxidants (BHA) resulted in cooperative affinity enhancement.

Pair (A + B)	AChE B alone	AChE B + A	MAO-B B alone	MAO-B B + A	NMDA B alone	NMDA B + A
Benzoate + Sorbate	-4.8	-4.9	-4.6	-4.5	-4.2	-4.1
Benzoate + Propionate	-3.8	-3.7	-3.7	-3.6	-3.4	-3.5
Benzoate + Nitrite	-2.8	-2.9	-2.6	-2.6	-2.5	-2.5
Benzoate + BHA	-5.9	-6.5	-6.2	-6.7	-5.8	-6.4
Benzoate + BHT	-6.4	-5.6	-6.8	-5.9	-6.3	-5.4
Sorbate + Propionate	-3.8	-3.8	-3.7	-3.7	-3.4	-3.3
Sorbate + Nitrite	-2.8	-2.7	-2.6	-2.6	-2.5	-2.4
Sorbate + BHA	-5.9	-6.2	-6.2	-6.5	-5.8	-6.1
Sorbate + BHT	-6.4	-5.8	-6.8	-6.1	-6.3	-5.7
Propionate + Nitrite	-2.8	-2.8	-2.6	-2.5	-2.5	-2.5
Propionate + BHA	-5.9	-6.1	-6.2	-6.3	-5.8	-6.0
Propionate + BHT	-6.4	-6.0	-6.8	-6.3	-6.3	-5.9
Nitrite + BHA	-5.9	-6.0	-6.2	-6.3	-5.8	-5.9
Nitrite + BHT	-6.4	-6.2	-6.8	-6.5	-6.3	-6.1
BHA + BHT	-6.4	-4.9	-6.8	-5.1	-6.3	-4.8

3.5 ADMET Prediction

Predicted pharmacokinetic profiles (Table 5) confirmed high gastrointestinal absorption for all preservatives. Sodium benzoate, BHA, and BHT were

computationally predicted to penetrate the blood-brain barrier (BBB). Toxicological profiling identified sodium nitrite as Toxicity Class III (toxic) and BHT as Class IV (harmful).

Compound	MW (g/mol)	LogP	TPSA (Å²)	GI Abs.	BBB Perm.	LD50 (mg/kg) / Class
Sodium Benzoate	122.12	1.52	37.30	High	Yes	1700 (Class IV)
Potassium Sorbate	112.13	1.41	37.30	High	Yes	3800 (Class V)
Sodium Propionate	74.08	0.48	37.30	High	No	5100 (Class VI)
Sodium Nitrite	69.00	-0.65	50.14	High	No	180 (Class III)
BHA	180.24	2.81	29.46	High	Yes	2000 (Class IV)
BHT	220.35	4.17	20.23	High	Yes	890 (Class IV)

3.6 Network Analysis

Intersecting the predicted targets of the six preservatives with Alzheimer's and Parkinson's disease targets yielded 84 shared neuro-associated genes

(Table 6). Topological analysis identified 10 key hub genes (Table 7), and pathway enrichment demonstrated significant convergence on neuroinflammatory and apoptotic signaling cascades (Table 8).

Compound	Predicted Targets	Overlap AD Genes	Overlap PD Genes	Total Unique Overlap
Sodium Benzoate	42	19	14	24
Potassium Sorbate	28	11	9	15
Sodium Propionate	35	16	13	20
Sodium Nitrite	19	9	8	12
BHA	67	34	26	41
BHT	58	29	22	36
Combined Unique	156	68	52	84

Rank	Hub Gene	Full Protein Name	Degree	Linked Preservatives
1	AKT1	RAC-alpha serine/threonine kinase	48	Benzoate, BHA, BHT
2	TP53	Cellular tumor antigen p53	44	Benzoate, Nitrite, BHA
3	TNF	Tumor necrosis factor alpha	41	Benzoate, Propionate, BHT
4	CASP3	Caspase-3 (Apoptosis executioner)	39	Benzoate, Nitrite, BHA, BHT
5	EGFR	Epidermal growth factor receptor	36	BHA, BHT
6	SRC	Proto-oncogene tyrosine kinase Src	32	Sorbate, BHA
7	ESR1	Estrogen receptor 1	30	BHA, BHT
8	PTGS2	Cyclooxygenase-2 (COX-2)	29	Benzoate, BHA, BHT
9	MAPK3	Mitogen-activated protein kinase 3	28	Propionate, BHA, BHT
10	MTOR	Mechanistic target of rapamycin	26	Benzoate, BHT

Pathway / Term ID	Description	Gene Count	FDR (p-value)
hsa04080	Neuroactive ligand-receptor interaction	22	1.42×10^{-8}
hsa05010	Alzheimer's disease pathway	19	3.84×10^{-7}
hsa04210	Apoptosis signaling pathway	16	8.21×10^{-6}
hsa05012	Parkinson's disease pathway	14	2.15×10^{-5}
hsa04151	PI3K-Akt signaling pathway	21	4.67×10^{-5}
GO:0006954	Inflammatory response (BP)	24	1.12×10^{-7}
GO:0008630	Intrinsic apoptotic signaling (BP)	13	6.54×10^{-6}
GO:0007268	Chemical synaptic transmission (BP)	17	1.88×10^{-5}

DISCUSSION

This investigation evaluated the direct molecular binding kinetics and systems-level network pharmacology of six ubiquitous dietary preservatives at human AChE, MAO-B, and NMDA receptor complexes. Our docking simulations demonstrated that synthetic phenolic antioxidants (BHT and BHA) exhibited moderate-to-high affinities across all three neuro-targets, approaching the binding energies of clinical reference standards such as Selegiline and Memantine. Structural interaction mapping confirmed that BHT and BHA insert stably into hydrophobic aromatic gorges (e.g., Trp86, Tyr337 in AChE;

Phe343, Tyr398 in MAO-B), indicating the potential to interfere with physiological substrate binding.

Sequential co-docking simulations provided valuable insights into cocktail exposure dynamics. When bulky lipophilic compounds (BHA + BHT) competed for identical catalytic pockets, pronounced steric clash emerged, significantly destabilizing the binding energy of the secondary ligand. In contrast, small planar molecules (benzoate) co-docked alongside BHA exhibited cooperative stabilization (negative $\Delta\Delta G$), suggesting that smaller additives may anchor to peripheral sites while lipophilic additives occupy catalytic cores.

Network analysis revealed that the targetome of these preservatives converges on essential regulatory hubs—notably AKT1, TP53, TNF, CASP3, and PTGS2 (COX-2)—which govern apoptosis, neuroinflammation, and synaptic plasticity. While low-dose sodium benzoate is recognized for its beneficial D-amino acid oxidase (DAAO) inhibitory effects, continuous multi-preservative co-exposure—particularly combinations involving nitrites and phenolic antioxidants—may trigger chronic neuroinflammatory signaling and cell death pathways.

4.1 Limitations

Molecular docking simulations utilize rigid receptor coordinates and cannot capture dynamic physiological conformational changes, metabolic transformations, or absolute in vivo concentrations. Sequential co-docking models steric feasibility rather than empirical non-equilibrium binding kinetics. Network pharmacology predictions are subject to database curation coverage and lack direct in vitro kinetic confirmation.

4.2 Future Directions

Subsequent studies should prioritize 100-200 ns all-atom Molecular Dynamics (MD) simulations and MM-PBSA free energy calculations. Experimental validation via in vitro enzymatic assays (Ellman's AChE assay, fluorometric MAO-B inhibition assays) and in vivo multi-preservative dietary exposure models in rodents will be crucial to establish regulatory safety margins for additive mixtures.

CONCLUSION

This study demonstrates that common food preservatives, particularly synthetic phenolic antioxidants BHT and BHA, bind directly to human AChE, MAO-B, and NMDA receptor active sites. Sequential co-docking reveals distinct steric competition and cooperative binding modes depending on molecular structure. Furthermore, network pharmacology establishes significant overlap with apoptotic and neuroinflammatory cascades in Alzheimer's and Parkinson's pathologies. These computational insights highlight the necessity of updating regulatory toxicology paradigms to evaluate chronic dietary co-exposure to multi-preservative mixtures.

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