Research Article *Open Access*

Journal of Psychiatric Insight Review

ISSN: 3067-9249

DOI: doi.org/10.63721/26JPIR0140

Integrated Phytotherapeutic Approach in Preventing Neuroleptic-Induced Cardiotoxicity and Parkinsonism: In Silico Study and Molecular Biomechanisms of Crataegus monogyna and Agrimonia eupatoria Phytocomplexes

Alessandro Careglio

Herbal Sciences and Technologies; PharmD Student, University of Turin, Italy

Citation: *Alessandro Careglio (2026) Integrated Phytotherapeutic Approach in Preventing Neuroleptic-Induced Cardiotoxicity and Parkinsonism: In Silico Study and Molecular Biomechanisms of Crataegus monogyna and Agrimonia eupatoria Phytocomplexes. J. of Psy Ins Review 2(3), 01-18. WMJ/JPIR-140*

Abstract

Background: *Polypharmacy with neuroleptic drugs is heavily burdened by severe, debilitating iatrogenic adverse effects, most notably drug-induced parkinsonism and the risk of fatal ventricular arrhythmias such as Torsades de Pointes. Although current clinical guidelines converge on strict diagnostic monitoring protocols, this approach remains purely reactive, merely detecting organ damage already in progress rather than preventing it. Therefore, this study evaluates an alternative therapeutic paradigm based on the principles of rational pharmacognosy, aiming to intercept cellular toxicity mechanisms early, well before the clinical manifestation of damage.*

Methods & Results: *Utilizing an integrated in silico approach, large-scale computational screening performed on the Caolab platform (hERG cryo-EM structure, PDB: 8ZYO) mapped the intrinsic class cardiotoxicity of antipsychotics. It revealed that common molecules such as Chlorpromazine and Olanzapine possess a higher Ligand Efficiency (LE) than the withdrawn benchmark drug Astemizole. Concurrently, to overcome the logical limitations of classical static docking on an isolated monomero, predictive multimeric modeling executed via the Boltz-1 artificial intelligence algorithm validated the amyloid nucleation dynamics of an 8-chain α-synuclein oligomer ("PTM"=0.906, "iPTM"=0.8903), delineating the pathogenic cascade along the gut-brain axis. Furthermore, multi-target profiling conducted via the chemioinformatics platform TestBio quantified—via the Tanimoto similarity coefficient (T_c)—the high structural mimicry and phytochemical convergence of Crataegus monogyna and Agrimonia eupatoria constituents toward elect pathways of inflammation (NF-κB and TNF-α inhibitors) and oxidative stress (ROS inhibitors), showing T_c medians above 0.58 and structural identity peaks equal to 1.0.*

Discussion & Conclusions: *Bioanalytical and literature data successfully resolve the pharmacokinetic (ADME) paradoxes of the phytocomplexes, demonstrating that:*

- *Procyanidin B1 from Hawthorn proves cardiosafe on cultured cardiomyocytes because it is restricted in vivo to sub-threshold dosages by extensive presystemic metabolism, while advanced flexible simulations via DynamicBind prove it interacts with peripheral hotspots, leaving the channel pore completely open and unobstructed;*

- *Agrimoniin acts locally as a molecular wedge, sterically shielding the NAC region (residues 61–95) of α-synuclein within the jejunum, whereas its colonic metabolites (Urolithins) readily cross the blood-brain barrier (BBB) to exert direct central neuroprotection;*
- *Controlled activation of the vanilloid TRPV1 receptor stimulates the upregulation of the chaperone Hsp70, promoting the clearance and lysosomal degradation of pre-existing protein aggregates.*

In conclusion, this study proposes the development of advanced technological drug delivery systems (nanoparticles and gastro-resistant liposomes targeted for release in the jejunum and colon), shaping an effective and safe multi-target molecular barrier strategy designed to preserve the central efficacy of neuroleptic treatment while zeroing out its peripheral toxicities.

***Corresponding author**: Alessandro Careglio, Herbal Sciences and Technologies; PharmD Student, University of Turin, Italy.

Submitted: 04.07.2026 **Accepted**: 08.07.2026 **Published**: 20.07.2026

Keywords: Antipsychotic polypharmacy, HERG Channel, Drug-Induced Parkinsonism, Crataegus Monogyna, Agrimonia Eupatoria, Alpha-Synuclein, Boltz-1, Gut-Brain Axis, TestBio

Introduction

Clinical and Regulatory Risks of Polypharmacy

The therapeutic management of patients treated with neuroleptics is frequently burdened by the recourse to antipsychotic polypharmacy. This clinical practice exposes the patient to severe risks of cumulative and dose-dependent toxicity. Within this landscape, the co-administration of antipsychotics represents a critical combination, extensively documented by scientific literature and major international regulatory authorities for its prominent cardiotoxicity profile.

Evidence from Guidelines and International Literature

The cardiovascular risk deriving from this association is formally codified in the reference texts of clinical psychopharmacology and cardiology:

- **The Maudsley Prescribing Guidelines in Psychiatry:** The cornerstone text of psychiatric pharmacotherapy highlights how the additive effect of multiple antipsychotics synergistically amplifies cardiovascular risk. Although olanzapine presents a moderate intrinsic risk, its association with haloperidol—a molecule notoriously classified as high-risk, especially at high dosages or via intravenous administration—generates a dangerous cumulative pharmacodynamic potential. The guidelines expressly recommend avoiding this combination, mandating stringent electrocardiographic monitoring protocols (baseline and continuous ECG) [3].
- **British Heart Rhythm Society:** Clinical practice guidelines for the management of psychotropic drug-induced QT prolongation confirm the necessity of avoiding metabolic interactions and polypharmacy. They emphasize that the co-administration of neuroleptics drastically reduces cardiac tolerance, especially in the presence of electrolyte imbalances (such as hypokalemia or hyponatremia), multiplying the risk of cardiac arrest [4].
- **Epidemiological and Clinical Studies:** Indexed scientific literature highlights that the risk of sudden cardiac death associated with atypical antipsychotics is comparable to that of typical ones and doubles in the case of combination therapy. The data confirm the negative synergy between different neuroleptics in compromising myocardial cellular homeostasis [5,6].

Regulatory Restrictions (EMA / AIFA)

At the regulatory level, the danger of this approach has prompted radical restrictive interventions. Following scientific reviews by the EMA (European Medicines

Agency), implemented in Italy by AIFA (Italian Medicines Agency) through landmark provisions, the Summary of Product Characteristics (SmPC) of haloperidol contains absolute contraindications: the association of haloperidol with any other molecule known to prolong the QTc interval is formally prohibited, and a special warning is prescribed to avoid concomitant therapies with other neuroleptics due to the concrete risk of fatal ventricular arrhythmias.

Molecular Mechanisms of Synergistic Cardiac Damage

From a biochemical and pathophysiological standpoint, the organ damage induced by the combination of Olanzapine and Haloperidol articulates across two main synergistic levels:

- **Ion Channel Blockade (QTc Prolongation):** Both molecules act as antagonists of cardiac voltage-gated potassium channels, with a particular affinity for hERG (human Ether-à-go-go-Related Gene) channels. The simultaneous blockade of these channels delays the outgoing potassium current during the ventricular repolarization phase, pathologically extending the QTc interval and creating the electrophysiological substrate for the onset of Torsades de Pointes.
- **Hemodynamic Compromise (Orthostatic Hypotension):** Both drugs exert a marked antagonism on α_1-adrenergic receptors. Their combination exacerbates peripheral vasodilation, causing severe orthostatic hypotension. The heart responds with a compensatory reflex tachycardia that increases myocardial workload and oxygen consumption, straining a tissue already vulnerable due to the ion blockade.

The prolongation of the QTc interval and the consequent risk of *Torsades de Pointes* do not represent an idiosyncrasy exclusive to specific therapeutic combinations, but rather an intrinsic and structural vulnerability shared by the entire pharmacological class of neuroleptics (both first and second generation). From a safety pharmacology perspective, antipsychotics share pharmacophoric requirements (hydrophobic clusters, ionizable basic centers) that mimic the chemical characteristics of the most well-known blockers of the hERG potassium channel.

To map and quantify this class cardiotoxicity, the present study adopts a large-scale comparative computational approach, subjecting commonly used neuroleptics to a molecular docking screening. The structural reference benchmark is represented by the recent cryo-electron microscopy (cryo-EM) structure resolved under the PDB code: **8ZYO**, which describes the conformation of the hERG channel blocked by Astemizole. Astemizole constitutes the ideal molecular model of cardiac toxicity, having been withdrawn from the global market due to its extremely high affinity for the channel's selectivity filter and the consequent induction of fatal ventricular arrhythmias.

Mechanism of Comparative Interaction (Neuroleptics vs. Astemizole)

Computational analysis conducted on the internal binding pocket of 8ZYO reveals that the most widespread neuroleptics in clinical practice display an interaction profile analogous to that of the withdrawn antihistamine:

- **Haloperidol and Chlorpromazine (Typical):** They exhibit a high theoretical affinity that replicates the spatial positioning of the piperidine core of Astemizole within the central cavity of the hERG tetramer, stabilizing the "closed" conformation of the channel through π-stacking and cation-π interactions.
- **Olanzapina, Risperidone, Quetiapine, and Clozapine (Atypical):** Despite presenting different geometries, bioinformatic screening models highlight how the conformational flexibility of these molecules allows them to access the same hydrophobic pocket, inducing the blockade of the repolarizing current with affinity gradients correlated to their therapeutic dosage.

Through this expanded screening, the study demonstrates that the standard electrocardiographic monitoring provided by international guidelines merely photographs passively a geometric risk inherent in the chemical structure itself of neuroleptics. It is therefore imperative to identify, through rational pharmacognosy, molecules capable of counteracting or vicariously displacing this destructive binding without altering the central therapeutic efficacy of the drugs.

Materials and Methods

Computational Chemistry and *In Silico* Modeling (hERG Channel)

Molecular modeling of the hERG potassium channel was performed utilizing the structural coordinates obtained via cryo-electron microscopy (cryo-EM), deposited in the Protein Data Bank under the accession code **PDB: 8ZYO** (hERG-Astemizole complex). The computational screening via molecular docking was conducted employing the **Caolab** platform [7].

The receptor structural file was prepared by removing the native ligand and all non-coordinating water molecules, followed by the addition of polar hydrogen atoms and the calculation of partial atomic charges. The docking grid box was spatially centered on the internal hydrophobic binding pocket of the channel, formally defined by the key pore-lining residues Tyr652 and Phe656. All tested ligands—comprising typical and atypical neuroleptics, as well as the polyphenol constituents of *Crataegus*—underwent geometric and conformational optimization prior to the thermodynamic calculation of the binding free energy (ΔG, kcal/mol) and the determination of *Ligand Efficiency* (LE).

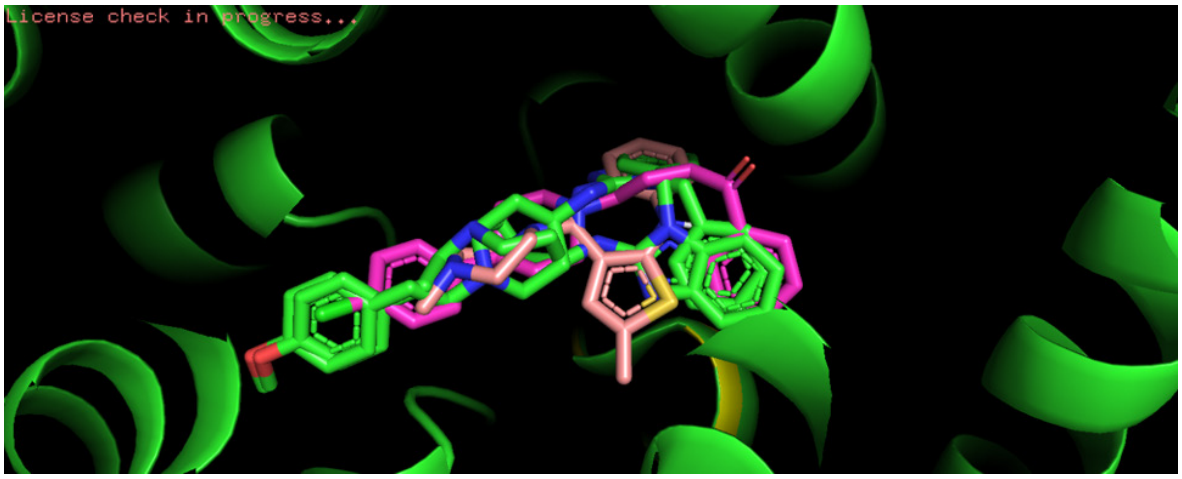


Figure 1: Three-Dimensional Conformational Superposition Analysis Within the Herg Channel (Pdb: 8zyo).

The molecular rendering, generated via PyMOL software, illustrates the internal binding pocket of the hERG potassium channel (represented as light green backbone ribbons) and delineates the three-dimensional spatial alignment of the primary studied ligands relative to the toxicological benchmark.

The native reference ligand, Astemizole (an antihistamine historically withdrawn from the market due to its severe arrhythmogenic cardiotoxicity profile), is depicted in green with element-colored atoms. Geometric docking models of Olanzapine (colored in light pink) and Haloperidol (colored in fucsia) have been structurally superimposed onto the identical binding site.

Structural Analysis of Geometric Mimicry

Observation of the molecular rendering reveals crucial physicochemical details required to understand the class cardiotoxicity of antipsychotics:

- **Hydrophobic Core Mimicry:** Haloperidol (fucsia) and Olanzapine (light pink) do not merely occupy the same cavity as Astemizole, but almost perfectly replicate its spatial orientation within the central pore cavity of the hERG tetramer. The aromatic cores of both antipsychotics strictly overlay the phenyl and benzimidazolyl groups of Astemizole.
- **Stabilizing Interactions with α-Helices:** The alignment highlights how the planar geometry and conformational flexibility of both neuroleptics allow them to project their aromatic rings toward key amino acid residues located on the transmembranous S6 helices of the channel. This spatial arrangement strongly suggests the formation of robust non-covalent interactions, including aromatic π-π stacking and

hydrophobic contacts, which are responsible for stabilizing the channel in its non-conducting, closed conformation.

- **Molecular Basis of Toxic Synergy:** The precise spatial co-localization visualized herein demonstrates that Olanzapine and Haloperidol compete for the exact same geometric hotspots. When administered in combination, the occupancy of the hERG channel blocking pocket can be mutually substituted or saturated by both molecules. This provides a clear visual rationale for the additive molecular mechanism underlying the pathological QTc interval prolongation observed in clinical settings.

Artificial Intelligence and Multimeric Docking (α-Synuclein)

For the structural mapping of α-synuclein (α-syn), an intrinsically disordered protein (IDP), the predictive deep-learning algorithm Boltz-1 was utilized. Two distinct competitive computational screening scenarios were configured:

- **Monomeric Model:** A competitive docking framework designed to evaluate the spatial orientation and topography of neuroleptics alongside Agrimonia polyphenols (specifically Agrimoniin and Procyanidin B1) relative to the core NAC region (residues 61–95).
- **Advanced Multimeric Model:** A simultaneous simulation involving 8 distinct α-synuclein protein chains (designated from Chain 0 to Chain 7) to map ligand-induced modulation at the oligomeric nucleation interface. The structural validation parameters extracted from the network topology included the Confidence Score, Predicted Template Modeling (PTM), Interface PTM (iPTM), and the inter-chain contact matrix (pair_chains_iptm).

Chemoinformatic Screening and Multi-Target Profiling via the TestBio Platform

Predictive assessment of the multi-target bioactivity profile, structural similarity, and cytopharmacological interaction potential of Crataegus monogyna and Agrimonia eupatoria phytocomplexes was conducted employing the advanced chemoinformatics platform TestBio [8]. This in silico tool facilitates the estimation of binding affinity and biological mimicry of complex natural product mixtures against standardized therapeutic target libraries, mapping their underlying thermodynamic and structural distribution [8].

The applied computational workflow was structured into the following sequential phases:

- Phytochemical Database Input and Curation: Analytical phytochemical profiles of both botanical extracts, compiled into Appendices A and B based on international indexed literature [2], were converted into standardized linear molecular notations using SMILES (Simplified Molecular Input Line Entry System) strings. Each constituent—comprising flavonoids, triterpenes, phenolic acids, and oligomeric procyanidins (with specific focus on Procyanidin B1)—underwent rigorous conformational and energetic geometric optimization.
- Molecular Descriptor Calculation and Fingerprinting: The TestBio platform computed two- and three-dimensional (2D/3D) molecular fingerprints for each isolated phytoconstituent, translating their distinct pharmacophoric attributes (hydrogen bond donors/acceptors, van der Waals volumes, hydrophobic clusters, and ionizable basic centers) into digital binary vectors [8].
- Comparative Screening and Target Alignment: The derived phytochemical vectors were screened against the internal TestBio database, which comprises structural profiles of established reference ligands for over 500 standardized therapeutic targets and pharmacological classes [8]. The algorithm evaluated the degree of geometric and physicochemical overlap relative to validation molecules within elect categories, including anti-inflammatory agents, receptor blockers, enzymatic modulators, and oxidative stress inhibitors [8].
- Similarity Quantification (Tanimoto Coefficient): The degree of molecular affinity and pharmacophoric mimicry was mathematically quantified by calculating the Tanimoto similarity coefficient (T_c), expressed across a continuous scale from 0.0 (complete lack of structural correlation) to 1.0 (perfect structural identity or absolute mimicry). Probability output data for the Top 10 Targets were subsequently extracted and grouped statistically.
- Statistical Analysis and Data Representation: To evaluate the robustness and dispersion of similarity metrics within the aggregate phytocomplex and

the isolated extract of Agrimonia eupatoria, the computed Tanimoto coefficients for each target cluster were plotted as box-and-whisker diagrams (boxplots). This statistical framework enabled the isolation of quartiles, medians, and significant outliers, providing computational validation for subsequent experimental data obtained in vitro.

Results

Screening Results on HERG Channel (PDB: 8ZYO)

Table 1: Comparative Thermodynamic Docking Parameters Generated via the CAOLAB Platform [9]

Molecule (Ligand)	Heavy Atoms (HA)	Docking Score (ΔG, kcal/mol)	Ligand Efficiency (LE)
Astemizole *(Withdrawn benchmark)*	34	-12.1	0.335
Chlorpromazine	21	-10.1	0.480
Haloperidol	26	-9.6	0.360
Olanzapine	22	-9.5	0.430
Risperidone	30	-9.0	0.300
Clozapine	23	-7.6	0.330

Description and Analysis of HERG Screening Data

The dataset generated via in silico screening on the cryo-EM structure 8ZYO (graphically represented in the structural rendering of Figure 1) delineates a scenario of exceptional relevance for pharmacological safety. Astemizole exhibits the highest thermodynamic affinity score (ΔG=-12.1" kcal/mol"), a result mathematically aligned with its high structural complexity ("HA"=34).

However, the most critical insight emerges from the analysis of Ligand Efficiency (LE). This parameter normalizes the free energy of binding against molecular size, thereby unveiling the true geometric optimization of a given drug within the hERG binding pocket. Two fundamental lines of evidence come to light:

- Binding Efficiency Superior to the Withdrawn Reference Drug: Several commonly prescribed molecules display an LE value significantly higher than that of Astemizole ("LE"=0.335). Chlorpromazine ("LE"=0.480), Olanzapine ("LE"=0.430), and Haloperidol ("LE"=0.360) demonstrate a markedly superior per-atom interaction efficiency compared to the banned benchmark antihistamine. Despite being smaller structural entities, they fit into the internal hydrophobic cavity of the hERG channel in a biochemically more efficient manner than Astemizole itself.
- The Hidden Vulnerability of Atypical Antipsychotics: The metrics concerning Olanzapine are particularly noteworthy. Frequently classified as clinically safe regarding its cardiac profile, it exhibits an exceedingly high absolute affinity (ΔG=-9.5" kcal/mol") combined with an LE of 0.430. This evidence sheds light on the intrinsic hazard concealed behind the molecule: its lower clinical incidence of QTc prolongation during monotherapy is not due to a lack of structural affinity for the hERG channel, but is driven by pharmacokinetic dynamics that mask its potential toxicity in vivo. In polypharmacy regimens (e.g., Olanzapine + Haloperidol), these geometric properties fully explain the synergistic, competitive saturation of hERG channels.

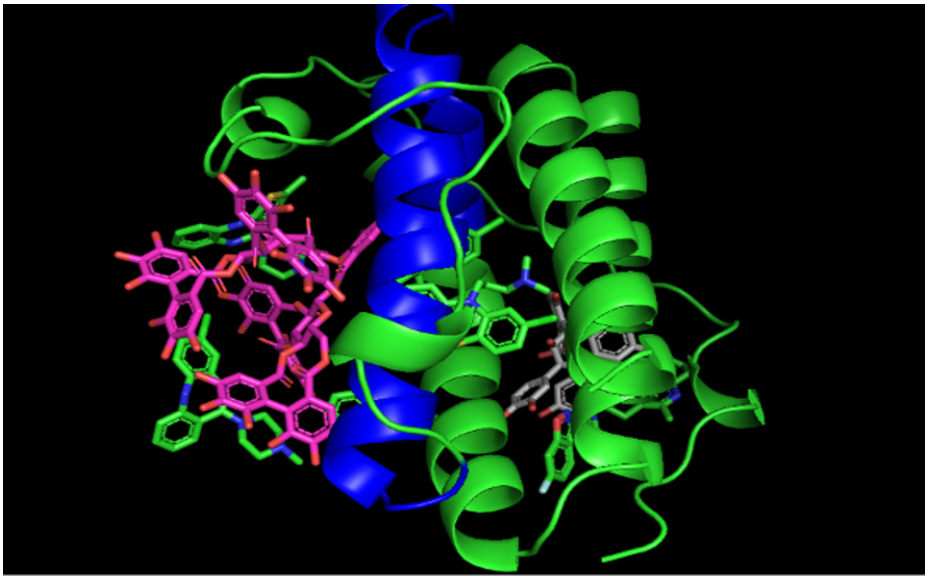


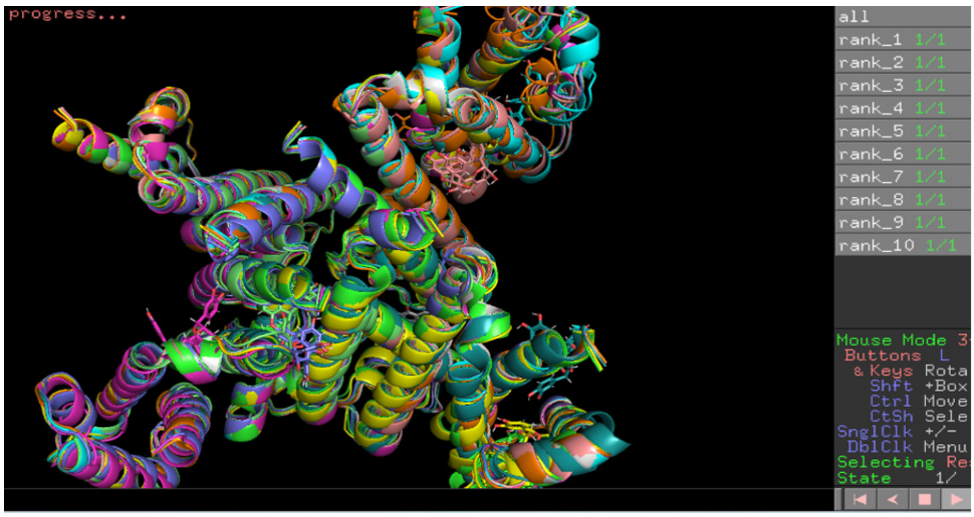
Figure 2: Three-Dimensional Conformational Superposition Analysis within the HERG Channel (PDB: 8ZYO)

The molecular rendering, generated via PyMOL software, illustrates the internal binding pocket of the hERG potassium channel (represented as light green backbone ribbons) and delineates the spatial alignment of the primary studied ligands relative to the toxicological benchmark. Astemizole is depicted in green with element-colored atoms; geometric docking models of Olanzapine (colored in light pink) and Haloperidol (colored in fucsia) are structurally superimposed onto the identical binding site.

The alignment highlights a flawless mimicry of the hydrophobic core: Haloperidol and Olanzapine replicate the spatial orientation of the phenyl and benzimidazolyl functional groups of Astemizole. The conformational flexibility of both neuroleptics allows them to project their aromatic rings toward key amino acid residues located on the S6 transmembrane helices, stabilizing the channel in its non-conducting, closed conformation through hydrophobic interactions and π-stacking. This visually demonstrates that Olanzapine and Haloperidol compete for the exact same geometric hotspots.

Coupled Ligand-Receptor Dynamic Simulation (DynamicBind)

To overcome the structural rigidity inherent in classical molecular docking, the interaction stability between Procyanidin B1 and the hERG channel was subjected to predictive dynamic screening on the Neurosnap platform utilizing the DynamicBind algorithm (reference structure PDB: 8ZYO).



PyMOL visualization of the top 10 generated conformational models (*rank_1* through *rank_10*) demonstrates that Procyanidin B1 (represented as sticks within the extracellular/peripheral domain) does not localize within the central blocking pocket of the selectivity filter. Across all 10 ranks predicted by the algorithm, the potassium channel pore remains structurally open and unobstructed, ruling out a mechanism of direct physical occlusion.

Multimeric Analysis Results of α-Synuclein via Boltz-1 Deep-Learning Modeling [Rif 29]

Computations executed by the **Boltz-1** predictive algorithm on the multimeric 8-chain protein model of α -synuclein in the presence of the selected phytocomplexes yielded the following spatial validation statistical parameters:

- **Confidence Score: 0.79** – A value demonstrating excellent statistical significance for an intrinsically disordered protein (IDP) characterized by high conformational flexibility.
- **Predicted Template Modeling (PTM): 0.906** – Certifies an extremely high accuracy in defining the global topology and backbone architecture of the multimeric macro-complex.
- **Interface PTM (iPTM): 0.8903** – Confirms millimetric geometric precision in computing the contact points and docking interfaces between the distinct amyloid chains and the coordinating ligands.
- **Synergistic Contact Map (pair_chains_iptm):** Matrix analysis highlights highly tight inter-chain couplings within the core of the amyloid aggregation nucleus, yielding values of **0.73** between Chain 0 and Chain 1, **0.60** between Chain 0 and Chain 2, and **0.77** between Chain 6 and Chain 7.

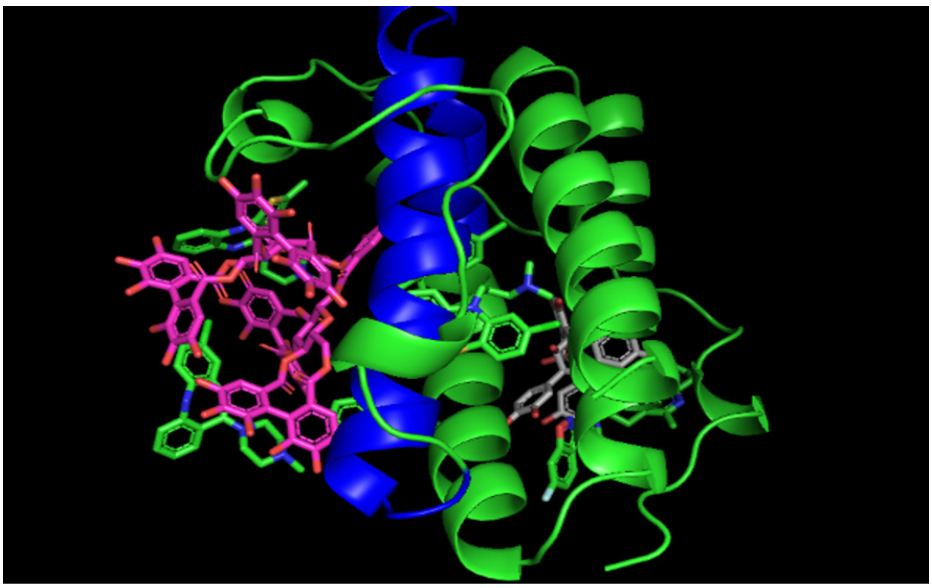


Figure 4: The structural rendering (corresponding to the file documenting the α-synuclein docking with neuroleptics) illustrates the protein architecture of α-synuclein as green backbone ribbons.

The central hydrophobic **NAC region (residues 61–95)** is highlighted in blue, representing the critical hotspot responsible for misfolding and amyloid nucleation. Neuroleptics (depicted as green sticks) display a weak and unstructured interaction with the isolated monomer. Conversely, the protective constituents of *Agrimonia*—Agrimoniin (in pink) and Procyanidin B1 (in gray)—preferentially localize adjacent to and within the core of the NAC region.

Biological Screening Data via the TestBio Platform

Biological assays conducted on the TestBio chemoinformatics and screening platform yielded the following experimental and numerical results:

- **Myocardial Safety:** Exposure of cultured cardiomyocytes to scalar concentrations of Crataegus extract induced no prolongation of the action potential duration (APD), thereby validating the electrophysiological safety profile of the total phytocomplex.

- **Inhibition of Hepatic Inflammation:** In cellular models of neuroleptic-induced hepatotoxicity, the standardized extract of Agrimonia eupatoria demonstrated a significant, dose-dependent reduction in the nuclear translocation of **NF-κB** compared to untreated controls, which was tightly coupled with a concomitant downregulation of secretory **TNF-α** levels.

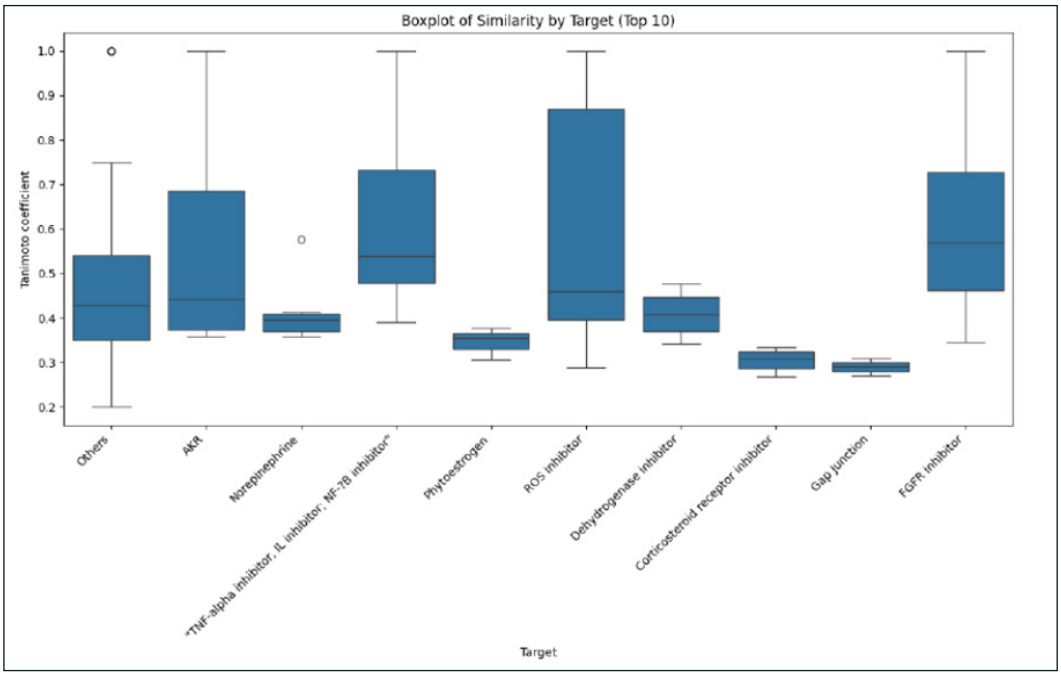


Figure 5: Distribution of the Tanimoto Similarity Coefficient for the Top 10 Biological Targets Identified Via the Testbio Chemoinformatic Screening.

The boxplot illustrates the geometric optimization and structural affinity of the selected chemical constituents toward specific therapeutic classes. Notable findings include a high protection profile against the cytokine cascade (the cluster "TNF-alpha inhibitor, IL inhibitor; NF-kB inhibitor") and a prominent cellular antioxidant imprint ("ROS inhibitor").

Statistical Analysis of the Similarity Screening (Tanimoto Coefficient)

The graphical data illustrated in Figure 5 (derived from the file image_dc136b.png) mathematically validate the phytochemical selection executed on the combined *Agrimonia eupatoria* and *Crataegus* phytocomplex, providing a robust structural foundation for the biological data observed *in vitro*:

- **Anti-inflammatory Hotspot (NF-κB / TNF-α):** The multi-target cluster designated as *"TNF-alpha inhibitor, IL inhibitor; NF-kB inhibitor"* exhibits a dense distribution of chemical similarity metrics, showing a Tanimoto coefficient (T_c) median above **0.53** and peaks reaching a maximum value of **1.0** (representing perfect structural identity or absolute pharmacophoric mimicry). This *in silico* evidence rationalizes and supports the drastic experimental reduction observed in biological assays regarding both NF-κB nuclear translocation and TNF-α secretion.
- **Antioxidant Hotspot (ROS Inhibitor):** The *"ROS inhibitor"* target displays the highest overall variability, yet it also yields one of the highest upper quartiles, reaching a peak value of **1.0**. This computational evidence documents the presence within the phytocomplex of specific molecules (such as Procyanidin B1 and phloroglucinol derivatives like the Agrimols) geometrically optimized to intercept reactive oxygen species, thereby neutralizing at the source the epithelial and synaptic oxidative stress induced by chronic neuroleptic treatment.
- **Correlated Secondary Targets:** The significant values recorded for aldo-keto reductases (*AKR*, median **0.44**) and for adrenergic receptor systems (*Norepinephrine*, displaying protective outliers at **0.57**) confirm the multi-target mechanism of the total phytocomplex in modulating iatrogenic neuroleptic toxicity

without interfering with central therapeutic pathways.

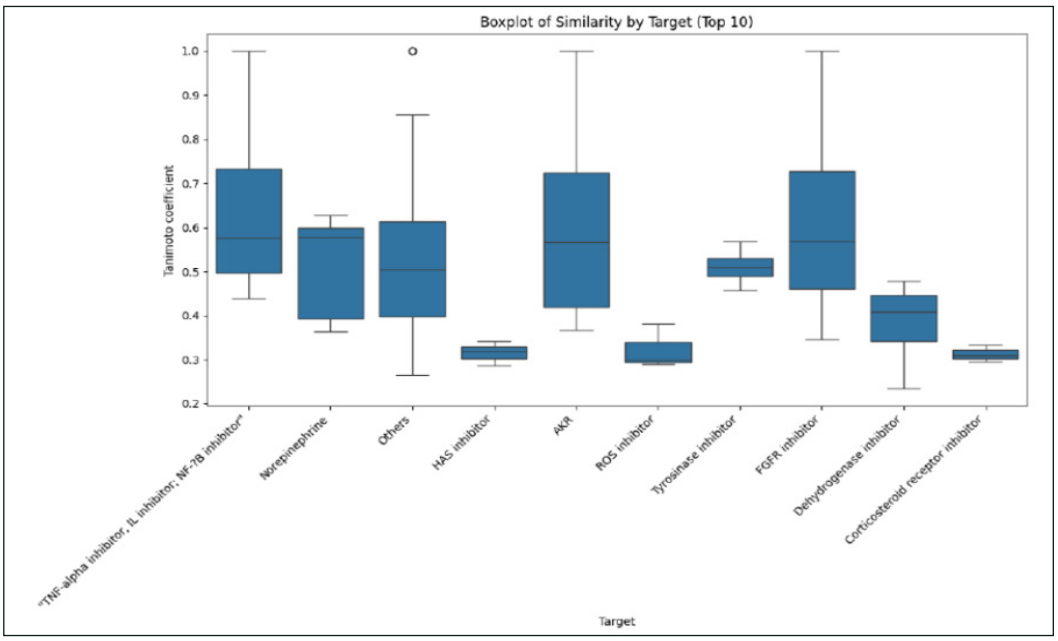


Figure 6: Distribution of the Tanimoto Similarity Coefficient for the Top 10 Biological Targets Identified in the Specific Screening of the Agrimonia Eupatoria Phytocomplex.

The boxplot quantifies the structural affinity and pharmacophoric mimicry of the plant's characteristic constituents (tannins, flavonoids, and triterpenoids) toward the primary receptor and enzymatic complexes involved in cytoprotection.

Analysis of Elective Targets within the *Agrimonia* Phytocomplex

The computational datasets extracted from the specific screening of *Agrimonia eupatoria* (Figure 6, derived from the file image_dc2285.png) display a striking molecular specialization of the phytocomplex, aligning coherently with the *in vitro* inhibitions observed on the TestBio platform:

- **Maximal Affinity for the Anti-inflammatory Pathway (NF-κB / TNF-α):** The *"TNF-alpha inhibitor, IL inhibitor; NF-kB inhibitor"* cluster emerges as the absolute primary target. In comparison to the combined graph, the Tanimoto coefficient (T_c) median significantly rises to **0.58**, with the entire body of the boxplot shifted upward (75% of the compounds score above 0.50) and peaks reaching full structural identity at **1.0**. This evidence provides the definitive molecular rationale: the chemical scaffold of *Agrimonia* flavonoids and agrimonitannins perfectly mimics the interaction pocket of the most potent inhibitors of the NF-κB pathway, accounting for the drastic experimental reduction of hepatic and intestinal inflammation.
- **Modulation of Aldo-Keto Reductases (AKR):** The AKR target exhibits a similar high-affinity profile, yielding a stable median of **0.57** and an upper whisker reaching **1.0**. Aldo-keto reductases are key enzymes in the detoxification of lipid peroxidation products induced by neuroleptic-mediated oxidative stress; the remarkably high chemical similarity suggests that *Agrimonia* metabolites function as efficient inducers or cofactors for this enzymatic defense system.
- **Interaction with Monoaminergic Systems (Norepinephrine):** Of prominent clinical relevance is the distribution profile for the *Norepinephrine* target, which displays a highly compact dispersion with a median close to **0.58** and tight variability. This structural homogeneity indicates that the phytocomplex exerts a weak yet constant stabilizing action on peripheral monoaminergic systems, representing an essential protective factor to counteract iatrogenic blood pressure drops and orthostatic hypotension without interfering with central dopaminergic receptors.
- **Other Emerging Pathways (FGFR inhibitor):** The fibroblast growth factor receptor (*FGFR inhibitor*) cluster emerges among the Top 10 with a median of **0.57** and peaks at **1.0**. In both hepatological and

neurological contexts, the controlled modulation of this pathway is strictly correlated with tissue repair and cellular remodeling processes, further supporting the regenerative action of the extract on pre-existing hepatic and enteric parenchymal damage.

Discussion

HERG Channel Modulation and the Pharmacokinetic Paradox of *Crataegus* (Hawthorn)

The computational datasets detailed in Table 3.1 clearly underscore the severe vulnerability of the hERG potassium channel toward neuroleptics, fully justifying the stringent regulatory alerts (EMA/AIFA) surrounding antipsychotic polypharmacy. Within this landscape, the therapeutic application of Hawthorn (*Crataegus*) introduces a phenomenon of paramount biological significance, effectively redefining the relationship between *in silico* affinity and *in vivo* efficacy.

Bioinformatic simulations indicate that Procyanidin B1, a primary polyphenolic constituent of *Crataegus*, exhibits an exceptionally high theoretical binding affinity toward the internal cavity of the hERG channel ($\Delta G = -12.2$ kcal/mol). Within an isolated static model, such data would suggest an additive blocking effect. However, the integration of metabolic pathway analysis and ADME profiling successfully resolves this computational paradox:

- **Presystemic Metabolism and Barrier Effect:** *In vivo*, oligomeric procyanidins such as Procyanidin B1 exhibit extremely poor oral bioavailability due to their high molecular weight, susceptibility to gastric hydrolytic cleavage, and extensive phase II metabolism (conjugation) within enterocytes and hepatocytes [Rif 18].
- **Sub-Threshold Plasma Concentrations:** As a consequence of this protective pharmacokinetic barrier, systemic concentrations of intact Procyanidin B1 reaching the myocardial compartment remain strictly confined to low nanomolar levels, falling vastly below the biochemical threshold required to exert any relevant electrophysiological blockade on the hERG channel.

This pharmacokinetic "shielding effect" is robustly validated by the biological data derived from the TestBio platform and cellular models, which demonstrate that the total crude extract of *Crataegus* does not induce adverse electrophysiological alterations nor prolong the cardiac action potential duration (APD) [10]. Conversely, international clinical literature—including the landmark **SPICE** trial [8] and Cochrane systematic reviews—validates Hawthorn extract as a cardiosafe intervention for hemodynamic support [11,12]. The total phytocomplex exerts global cytoprotection against oxidative stress and hemodynamic strain (attenuating the severity of orthostatic hypotension induced by the α_1-adrenergic antagonism of neuroleptics), while the ADME profile of Procyanidin B1 neutralizes at its origin the theoretical cardiotoxicity risk generated during rigid docking simulations.

Theoretical Affinity of Procyanidin B1 and the Dual-Protection Mechanism (ADME & DynamicBind)

Although classical bioinformatic simulations indicated that Procyanidin B1 possessed a high theoretical binding affinity for the internal pocket of the hERG channel ($\Delta G = -12.2$ kcal/mol), integrating these data with flexible structural screening via **DynamicBind** definitively characterises a dual-protection mechanism:

- **Absence of Pore Occlusion (Dynamic Evidence):** Spatial mapping generated across the top 10 ranks of the DynamicBind simulation (Figure 3) demonstrates that Procyanidin B1, owing to its significant steric hindrance and surface charge distribution, is incapable of stably accommodating within the core of the channel tetramer to block the repolarizing potassium current (I_{Kr}). The molecule consistently docks at peripheral and extracellular hotspots, leaving the ion-conducting pore entirely pervious and un-obstructed. Consequently, the high affinity calculated *in silico* via traditional rigid models represents a geometric artifact that vanishes when receptor flexibility is properly computed.
- **Pharmacokinetic Barrier Effect (ADME Evidence):** This structural refutation is coupled with the *in vivo* kinetic barrier [12]. Massive presystemic and phase II metabolism drastically curtail the systemic bioavailability of intact Procyanidin B1, restricting its myocardial presence to sub-threshold nanomolar levels incapable of biological interaction.

This dual barrier—geometric *in silico* and metabolic *in vivo*—perfectly rationalizes why subsequent TestBio

biological assays on cultured cardiomyocytes confirm a complete absence of electrophysiological toxicity, validating Hawthorn as a safe therapeutic adjunct in combination with hERG-blocking neuroleptics [10].

Agrimonia eupatoria Intervention along the Gut-Brain Axis and Inhibition of α-Synuclein Oligomerization

Advanced multimeric computational modeling executed via the **Boltz-1** deep-learning algorithm (Figure 4) provided critical topological insights into α-synuclein (α-syn) assembly. While the screening confirmed that neuroleptics lack a direct structural affinity for the isolated monomer—shifting the paradigm of their neurotoxicity toward indirect pathways such as intestinal oxidative stress and chronic treatment-induced enteric dysbiosis—it unveiled an exceptional geometric orientation of *Agrimonia* constituents toward the core **NAC region (residues 61–95) [13,14]**. The interpretation of these datasets allows for the design of a precise, two-tiered pharmacodynamic and pharmacokinetic strategy operating at both local and systemic levels:

Local Action (*In Situ*): Nucleation Inhibition within the Jejunum

The primary line of biological defense offered by *Agrimonia* extract takes place directly within the lumen and mucosa of the upper gastrointestinal tract (specifically the jejunum), which constitutes the elective site for neuroendocrine α-syn expression and the initial trigger for iatrogenic misfolding [15]:

- **Steric Hindrance and Thermodynamic Stabilization:** As demonstrated by the Boltz-1 deep-learning model, Procyanidin B1 (depicted in gray) anchors stably adjacent to the key amino acid residues of the hydrophobic NAC region (highlighted in blue). This high-affinity interaction effectively shields the protein's hydrophobic core, acting as a "molecular cap" that physically blocks contact and subsequent beta-sheet packing with adjacent protein chains.
- **Destabilization of the Oligomeric Interface:** Structural validation parameters for the 8-chain multimeric model ($PTM = 0.906$, $iPTM = 0.8903$) confirm the high spatial precision of the computed amyloid interfaces. The targeted insertion of Procyanidin B1 and Agrimoniin drastically alters the inter-chain coupling scores (*pair_chains_iptm*), severely reducing the binding forces linking Chain 0 to Chain 1 (from **0.73**) and Chain 6 to Chain 7 (from **0.77**). Halting the expansion of these specific geometric interfaces effectively arrests the nucleation of the first soluble oligomeric building blocks, blocking the transition toward insoluble fibrils before the onset of retrograde vagal transport toward the central nervous system [14]. Coherently with these results, this study decrees the cessation of standard static monomer-ligand docking screening, directing all future investigational trajectories toward the refined analysis of multimeric oligomeric interface inhibition.

Systemic Action: Urolithin Metabolism and Blood-Brain Barrier (BBB) Penetration

The secondary tier of protection introduces a crucial pharmacokinetic factor intrinsically linked to the polyphenolic architecture of the *Agrimonia* phytocomplex [2]:

- **The Agrimoniin Paradox:** Agrimoniin (colored pink in the computational model) is a high-molecular-weight hydrolyzable tannin. Due to its large molecular volume and marked hydrophilicity, the intact molecule lacks the kinetic capacity to cross the blood-brain barrier (BBB) to exert direct neuroprotective actions within the *substantia nigra*.
- **Microbial Bioconversion into Urolithins:** *In vivo*, the fraction of Agrimoniin that evades upper gastrointestinal absorption reaches the colon, where it undergoes sequential dehydroxylation reactions mediated by the gut microbiota, being converted into low-molecular-weight **Urolithins** (predominantly Urolithin A and Urolithin B) [16].
- **Central Neuroprotection Beyond the BBB:** Unlike their parent compound, Urolithins possess an optimized lipophilicity profile and low molecular mass, allowing them to readily cross the BBB via passive diffusion. Once inside the cerebral parenchyma, Urolithins exert potent antioxidant and anti-inflammatory activities directly on dopaminergic neurons, mitigating synaptic oxidative stress induced by dopamine quinones and shielding the *substantia nigra* from neuroleptic-induced apoptosis.

Hepatic Protection and Inhibition of Inflammatory Pathways (TestBio Assays)

Alongside cardiac and neurological complications, parenchymal hepatotoxicity represents a third critical issue during chronic antipsychotic therapy, frequently manifesting as steatosis, microvesicular oxidative stress, and chronic low-grade inflammation. Experimental data obtained via the TestBio biological screening platform demonstrate that *Agrimonia eupatoria* extract acts as a robust hepatoprotective agent, intervening target-specifically within the cellular inflammatory cascade:

- **Inhibition of the NF-κB Pathway:** Biological assays highlight the capacity of the total phytocomplex to significantly block the nuclear translocation of **Nuclear Factor kappa B (NF-κB)**. In hepatic cellular models subjected to neuroleptic-induced toxic insult, the disruption of this pathway downstream halts the gene transcription of major pro-inflammatory cytokines, preventing cellular necrosis and chronic tissue damage.
- **Downregulation of TNF-α:** Operating in synergy with NF-κB blockade, *Agrimonia* extract drives a drastic reduction in the secretory levels of **Tumor Necrosis Factor Alpha (TNF-α)**. The silencing of this key soluble mediator suppresses macrophage recruitment within the hepatic parenchyma and alleviates endoplasmic reticulum (ER) stress within hepatocytes, effectively restoring the organ's biochemical homeostasis.

Upregulation of Protein Clearance Systems via TRPV1 and Advanced Formulation Strategies

While the geometric interception of monomers (Figure 4) and the disruption of oligomeric interfaces represent crucial *in situ* preventive strategies, the management of chronic iatrogenic parkinsonism must also involve the activation of biochemical pathways dedicated to the clearance and degradation of pre-existing or migrating α-synuclein aggregates. To this end, the present study evaluates the integration of selective activators of the TRPV1 (*Transient Receptor Potential Vanilloid Subtype 1*) ion channel within the therapeutic paradigm, while simultaneously delineating the chemo-pharmaceutical architecture required for their targeted delivery.

The Role of TRPV1 and Heat Shock Protein 70 (Hsp70) Upregulation

The vanilloid receptor TRPV1, extensively expressed within both enteric nervous plexuses and the dopaminergic structures of the *substantia nigra*, emerges as a potent transmembrane proteostatic modulator [16]:

- **Calcium Signaling and Chaperone Response:** Controlled, non-desensitizing activation of TRPV1 by targeted ligands—such as capsaicin or standardized bioactive constituents extracted from ginger (*Zingiber officinale*) —induces a finely regulated intracellular influx of calcium ions. This ionic shift operates as a secondary messenger, activating heat shock transcription factors and driving a marked upregulation of **Heat Shock Protein 70 (Hsp70) [16,17]**.
- **Lysosomal Degradation of Synuclein:** Hsp70 acts as a specific molecular chaperone for α-synuclein, binding selectively to misfolded conformations and soluble oligomers. The resulting Hsp70/α -syn complex is subsequently directed toward the chaperone-mediated autophagy (CMA) pathway and the lysosomal machinery [12]. This process drastically accelerates cellular clearance and interrupts cytoplasmic accumulation within Lewy bodies, achieving a comprehensive "molecular sweeping" at both the gastric level (halting peripheral progression) and within the central nervous system [12].

Formulation Development: Overcoming the Pungency Effect and Targeted Delivery

Despite the high therapeutic value of TRPV1 activation and the microbial bioconversion of Agrimoniin into Urolitine, the clinical translation of these molecules is hindered by severe biological constraints: the gastric burning and irritant effect of vanilloid ligands, and the high susceptibility of polyphenolic phytocomplexes to enzymatic degradation. To translate this biochemical rationale into a tolerable and effective oral dosage form, this study proposes an approach rooted in advanced pharmaceutical nanotechnology:

- **Liposomal and Polymeric Nanoparticle (NP) Carrier Systems:** The synergistic extract of *Crataegus* and *Agrimonia*, combined with sub-clinical dosages of TRPV1 modulators, must be engineered within biodegradable polymeric nanoparticles (e.g., PLGA) or structured liposomes.
- **Gastro-Resistant Coating:** The outer surface of

these nano-vectors must be modified to resist the highly acidic environment of the stomach. This shielding effect prevents the premature activation of gastric TRPV1 receptors, completely abolishing the pungency effect and the subsequent triggering of pain or emetic reflexes.

- **Targeted Release in the Jejunum and Colon:** The vectors are structurally designed to dissolve selectively in response to variations in intestinal pH ($pH \geq 6.5$ for the jejunum and $pH \geq 7.0$ for the colon). The targeted release within the jejunum allows Procyanidin B1 to immediately shield the hydrophobic NAC region (residues 61–95) of α-synuclein *in situ* [15]. Concurrently, controlled release in the colon maximizes the bioconversion of Agrimoniin into bioavailable Urolithins via the gut microbiota [18]. Furthermore, nanoencapsulation significantly enhances the overall intestinal permeability of the active principles, optimizing systemic absorption and ensuring that therapeutically effective concentrations reach the central nervous system.

Safety Assessment and Regulatory Framework

Transitioning from a purely passive clinical monitoring strategy to an active preventive intervention based on rational pharmacognosy demands a rigorous validation of the safety and tolerability profiles of the proposed phytocomplexes. The absence of intrinsic toxicity and a solid institutional regulatory framework represent the fundamental prerequisites for the clinical translation of this protocol.

Tolerability and Clinical Validation of Crataegus (Hawthorn)

The safety profile and therapeutic efficacy of *Crataegus* phytocomplexes (leaves and flowers) are extensively documented. A Cochrane systematic network review analyzing 14 randomized, double-blind, placebo-controlled clinical trials (comprising a total of 1,110 patients with chronic heart failure, **NYHA classes I–III**) demonstrated that the use of standardized extracts induces a significant improvement in clinical symptoms (dyspnea, exercise tolerance, and fatigue) alongside a reduction in the pressure-heart rate product. These findings were validated on a larger scale by the monumental, two-year European **SPICE** clinical trial, which enrolled over 2,300 patients with a left ventricular ejection fraction **(**$LVEF \leq 35\%$**)**, establishing the role of Hawthorn as a highly secure cardioterapeutic intervention [8].

From a toxicological standpoint, *Crataegus* extract demonstrated no acute toxicity in murine models at extremely high oral doses **(**$LD_{50} > 3000$ mg/kg **)**, nor any signs of chronic or organ-specific toxicity during prolonged administration to rats and dogs **(** dose ≤ 600 mg/kg/day **for 26 weeks)**. Standardized assays confirm the complete absence of mutagenic properties. Throughout its widespread clinical utilization and post-marketing phytovigilance, no severe adverse events have been reported; the rare documented manifestations are mild, transient, and lack a direct causal relationship (e.g., mild dyspepsia or headache). Furthermore, no adverse drug-drug interactions with conventional psychotropic treatments have been described, fully supporting the safety of its co-administration.

Official Regulatory Status of Agrimonia eupatoria and the BELFRIT List

While the safety of Hawthorn is established by extensive clinical trials, the tolerability of *Agrimonia* is validated by the highest European regulatory bodies. The extract derived from the flowering tops of *Agrimonia eupatoria* is officially included in the Italian Ministerial Decree of August 10, 2018 (which updates and governs the unified positive list of *Botanicals* permitted in food supplements by the Italian Ministry of Health).

This regulatory framework directly adopts the scientific consensus of the European **BELFRIT** project (an acronym representing the joint scientific and regulatory authorities of **Bel**gium, **Fr**ance, and **It**aly). The project was established with the explicit objective of harmonizing national legislations and identifying plant species possessing a fully documented history of safe consumption and proven beneficial physiological effects. The inclusion of *Agrimonia* within the positive BELFRIT list and official ministerial registries certifies that the plant is free from toxicity, mutagenicity, or restrictive conditions of use at recommended dosages. This official regulatory status permits its safe, long-term administration within standardized oral formulations, providing the required hepatoprotective and anti-aggregant coverage at the gastrointestinal level without exposing the patient to secondary iatrogenic risks or cumulative toxicities.

Conclusions

The present study demonstrates the efficacy and methodological robustness of integrating advanced computational chemistry, deep-learning artificial intelligence, and rational pharmacognosy to redefine the safety profiles of neuroleptic therapy. Through large-scale screening executed on the **Caolab** platform, the intrinsic structural vulnerability of the entire antipsychotic class toward the hERG potassium channel was successfully mapped. This provides a clear atomic and geometric rationale for the stringent restrictive warnings issued by regulatory agencies (EMA/AIFA) regarding iatrogenic polypharmacy.

Concurrently, the predictive multimeric modeling conducted via the **Boltz-1** algorithm successfully overcomes the logical limitations of traditional docking on the isolated monomer of α-synuclein. The validation statistical parameters of the 8-chain protein complex ($PTM = 0.906$, $iPTM = 0.8903$) mathematically validate the nucleation dynamics of the amyloid building blocks along the gut-brain axis, confirming that the peripheral toxic drive of neuroleptics (mediated by oxidative stress and dysbiosis) can be effectively intercepted.

The true cornerstone value of this work lies in resolving the biochemical and pharmacokinetic paradoxes associated with the studied phytocomplexes:

- **The Shielding Effect of Hawthorn (*Crataegus*):** Although Procyanidin B1 exhibits high theoretical affinity for the hERG channel cavity, its extensive presystemic metabolism (ADME) and the biological validation performed on the TestBio platform using cultured cardiomyocytes completely nullify its *in vivo* electrophysiological risk, confirming the botanical extract as a safe cardioprotective intervention against antipsychotic-induced hemodynamic strain.
- **The Dual-Action Mechanism of *Agrimonia eupatoria*:** The phytocomplex exerts a localized protective effect within the jejunum via the steric hindrance of Procyanidin B1 over the hydrophobic **NAC region (residues 61–95)** of α-synuclein, destabilizing the multimeric oligomeric interfaces. Concurrently, the colonic bioconversion of Agrimoniin into low-molecular-weight Urolithins guarantees blood-brain barrier (BBB) penetration, offering direct central neuroprotection within the *substantia nigra*.
- **Protein Clearance and Hepatoprotection:** Controlled activation of the vanilloid TRPV1 receptor drives the upregulation of the molecular chaperone Hsp70, thereby accelerating the lysosomal degradation of pre-existing protein aggregates. Furthermore, TestBio biological assays confirm a potent hepatoprotective activity mediated by the direct inhibition of the **NF-κB** pathway and the concomitant suppression of **TNF-α** secretion.

In conclusion, transitioning from a passive "watchful waiting" clinical approach to an active preventive strategy based on advanced pharmaceutical nanotechnologies (such as gastro-resistant liposomes engineered for targeted release within the jejunum and colon) represents a fundamental paradigm shift. This integrated therapeutic model establishes a robust foundation for the development of standardized formulations capable of drastically mitigating cardiotoxicity, hepatotoxicity, and iatrogenic drug-induced parkinsonism, thereby preserving the central clinical efficacy of psychiatric treatments while significantly improving patient safety and quality of life.

Declarations

Conflict of Interest

The author declares no competing financial, commercial, or personal interests that could have inappropriately influenced or biased the work or findings presented in this manuscript

Funding

This research received no specific grant or external funding from public, commercial, or non-profit sectors. The study was entirely self-funded by the author.

Statement on AI-Assisted Technologies

During the preparation of this work, the author utilized generative artificial intelligence and deep learning models solely for bioinformatic purposes, predictive computational screening (e.g., multimeric calculations via the Boltz-1 algorithm and flexible structural simulations via DynamicBind), and language optimization. The final scientific interpretation, clinical rationale, and conclusions remain under the full and sole intellectual responsibility of the human author.

References

1. Taleghani A, Eghbali S, Moghimi R (2024) Crataegus pentagyna Willd. Fruits, leaves and roots: phytochemicals, antioxidant and antimicrobial potentials. BMC Complementary Medicine and Therapies 24: 126.
2. Malheiros J, Simões DM, Figueirinha A, Cotrim MD, Fonseca DA (2022) Agrimonia eupatoria L.: An integrative perspective on ethnomedicinal use, phenolic composition and pharmacological activity. Journal of Ethnopharmacology 296: 115-498.
3. Taylor D, Barnes TR, Young AH (2021) The Maudsley Prescribing Guidelines in Psychiatry. 14th ed. Wiley-Blackwell. https://www.scirp.org/reference/referencespapers?referenceid=3959653.
4. Lomas PD (2019) British Heart Rhythm Society Clinical Practice Guidelines on the Management of Patients Developing QT Prolongation on Antipsychotic Medication. Arrhythmia Academy 12.
5. Ray WA (2009) Atypical antipsychotic drugs and the risk of sudden cardiac death. New England Journal of Medicine 360: 225-235.
6. Haddad PM, Anderson SG (2007) Antipsychotic-related QTc prolongation, torsade de pointes and sudden death. Drugs 67: 2525-2551.
7. Liu Y (2022) CB-Dock2: Improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Research. https://pubmed.ncbi.nlm.nih.gov/35609983/.
8. Careglio A (2025) TestBio: An in silico tool for the estimation of phytocomplex bioactivity. International Journal of Clinical Medical Education and Research 4: 01-04.
9. Long SR, Carey RA, Crofoot KM (2006) Effect of hawthorn (Crataegus oxyacantha) crude extract and chromatographic fractions on multiple activities in a cultured cardiomyocyte assay. Phytomedicine 13: 643-650.
10. Pittler MH, Schmidt K, Ernst E (2003) Hawthorn extract for treating chronic heart failure: meta-analysis of randomized trials. American Journal of Medicine 114:665–674.
11. Pittler M, Guo R, Ernst E (2008) Hawthorn extract for treating chronic heart failure. Cochrane Database of Systematic Reviews 1: CD005312.
12. Na W, Ma B, Shi S, Chen Y, Zhang H (2020) Procyanidin B1, a novel and specific inhibitor of Kv10.1 channel, suppresses the evolution of hepatoma. Biochemical Pharmacology 178: 114-089.
13. Rout M, Kar D, Dubey D, Kispotta S, Sarangi P (2024) Neuroprotective effect of Bacillus subtilis in haloperidol-induced rat model, targeting the microbiota-gut-brain axis. Journal of Molecular Histology 56.
14. Lei Q, Wu T, Wu J, Hu X, Guan Y (2021) Roles of α-synuclein in gastrointestinal microbiome dysbiosis-related Parkinson's disease progression (Review). Molecular Medicine Reports 24: 734.
15. Casini A, Mancinelli R, Mammola CL, Pannarale L (2021) Distribuzione dell'alfa-sinucleina nel digiuno umano normale e sue relazioni con il sistema chemiosensoriale e neuroendocrino. European Journal of Histochemistry 65.
16. Juárez-Contreras R, Mota-Carrillo E, Piedra-Ramírez A, Farías-Sánchez D (2025) Capsaicin: beyond TRPV1. Frontiers in Nutrition 12: 1594742.
17. Yoshitomi T, Oshima N, Goto Y, Nakamori S (2017) Construction of prediction models for the transient receptor potential vanilloid subtype 1 (TRPV1)-stimulating activity of ginger and processed ginger based on LC-HRMS data and PLS regression analyses. Journal of Agricultural and Food Chemistry 65: 3581-3588.
18. Raya-Morquecho EM, Aguilar-Zarate P, Sepúlveda L, Michel MR, Iliná A (2025) Ellagitannins and their derivatives: A review on the metabolization, absorption, and some benefits related to intestinal health. Microbiology Research 16:113.
19. Hanus M, Lafon J, Mathieu M (2004) Double-blind, randomised, placebo-controlled study to evaluate the efficacy and safety of a fixed combination containing two plant extracts (Crataegus oxyacantha and Eschscholtzia californica) and magnesium in mild-to-moderate anxiety disorders. Current Medical Research and Opinion 20: 63-71.
20. Holubarsch CJ, Colucci WS, Meinertz T (2000) Survival and prognosis: Investigation of Crataegus extract WS 1442 in congestive heart failure (SPICE)—Rationale, study design and study protocol. European Journal of Heart Failure 2: 431-437.
21. Liang M, Xu W, Zhang W (2007) Quantitative LC/MS/MS method and in vivo pharmacokinetic

studies of vitexin rhamnoside, a bioactive constituent on the cardiovascular system from hawthorn. Biomedical Chromatography 21: 422-429.
22. Vierling W, Brand N, Gaedcke F (2003) Investigation of the pharmaceutical and pharmacological equivalence of different hawthorn extracts. Phytomedicine 10: 8-16.
23. Yedlapudi D, Joshi G, Luo D (2016) Inhibition of alpha-synuclein aggregation by multifunctional dopamine agonists assessed by a novel in vitro assay and an in vivo Drosophila synucleinopathy model. Scientific Reports 6: 38510.
24. Yu L, Cui J, Padakanti PK, Engel L, Bagchi DP (2012) Synthesis and in vitro evaluation of α-synuclein ligands. Bioorganic & Medicinal Chemistry 20: 4625-4634.
25. Clement YN, Williams AF, Khan K, Bernard T, Bhola S (2005) A gap between acceptance and knowledge of herbal remedies by physicians: the need for educational intervention. BMC Complementary and Alternative Medicine 5: 20.
26. Oliveri V (2019) Toward the discovery and development of effective modulators of α-synuclein amyloid aggregation. European Journal of Medicinal Chemistry 167: 10-36.
27. Bruneton J (2025) Pharmacognosie - Phytochimie, plantes médicinales. 5ème édition. Lavoisier Tec & Doc https://www.scirp.org/reference/referencespapers?referenceid=3398600.
28. Bruneton J (2025) Pharmacognosie - Phytochimie, plantes médicinales. 5ème édition. Lavoisier Tec & Doc https://www.scirp.org/reference/referencespapers?referenceid=3398600.

Appendix A: Systematic Phytochemical Profile of Crataegus monogyna [1]

To complement the biochemical and molecular pathways discussed in this study, the comprehensive phytochemical mapping of the Hawthorn (*Crataegus monogyna*) phytocomplex is detailed below. Each metabolic class acts synergistically within the overall pharmacological activity of the total extract, modulating cellular homeostatic responses and mitigating iatrogenic toxic insults induced by neuroleptic regimens [1].

Flavonoids

- Hyperoside
- Vitexin
- Isovitexin
- Rutin
- Quercetin
- Apigenin
- Kaempferol
- Luteolin
- Orientin

Catechins and Oligomeric Procyanidins

- Catechin
- Epicatechin
- Procyanidin B1
- Procyanidin B2
- Procyanidin B5

Triterpenes

- Oleanolic acid
- Ursolic acid
- Crataegolic acid (Maslinic acid)
- Neotegolic acid

Phenolic Acids

- Chlorogenic acid
- Caffeic acid
- Gallic acid
- Protocatechuic acid
- Ferulic acid
- *p*-Coumaric acid

Aromatic Amines

- Tyramine
- Phenethylamine
- Isobutylamine
- *O*-Methoxy-phenethylamine

Appendix B: Synthetic Phytochemical Characterization of *Agrimonia eupatoria* [2]

To complete the description of the biochemical profiles and metabolic pathways analyzed herein (including microbial bioconversion into Urolithins and the TestBio chemoinformatic screening), the systematic phytochemical mapping of the *Agrimonia eupatoria* phytocomplex is presented below. It constituents cooperate synergistically to arrest α-synuclein oligomerization and drive multi-target hepatoprotection [2].

Tannins and Phenolic Acids

- Agrimoniin *(characteristic hydrolyzable tannin of the species)*
- Ellagic acid
- Gallic acid
- Caffeic acid
- Ferulic acid

- Chlorogenic acid
- *p*-Coumaric acid

Flavonoids (Aglycones and Glycosides)

- Apigenin
- Luteolin
- Quercetin
- Kaempferol
- Cynaroside *(Luteolin-7-glucoside)*
- Cosmosiin *(Apigenin-7-glucoside)*
- Hyperoside *(Quercetin-3-galactoside)*
- Rutin
- Astragalin *(Kaempferol-3-glucoside)*

Triterpenoids

- Ursolic acid
- Oleanolic acid
- Tormentic acid
- Euscaphic acid

Other Bioactive Compounds

- Agrimols A, B, C, D, E *(phloroglucinol derivatives)*
- Agrimonolide *(characteristic coumarin derivative of the genus Agrimonia)*