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Section 1. Preventive medicine

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DISCOVERY OF SMALL MOLECULE INHIBITORS OF MAO-B FOR ALZHEIMER'S DISEASE USING PHARMACOPHORE-BASED VIRTUAL SCREENING

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Abstract

Effective therapies are needed to mitigate Alzheimer's disease (AD), a neurodegenerative dementia that harms cognitive function in over 10% of people older than 65. Although monoamine oxidase B (MAO-B) is a critical therapeutic target for AD, only three MAO-B inhibitors (rasagiline, selegiline, and safinamide) are currently approved, and they are mainly used for treating Parkinson's Disease. To identify novel MAO-B inhibitors as treatments for AD, *in silico* drug discovery was employed as a cost-effective and efficient approach for screening a vast chemical space. Geometric, energetic, and machine learning methods were used to evaluate potential binding sites, which were subsequently assessed with molecular docking for 20 potential MAO-B inhibitors identified from pharmacophore mapping. These 20 molecules were then analyzed for their pharmacokinetic and toxicological properties via ADMET prediction, and Z56776036 and Z1980993192 were selected as the two most promising drug candidates. These lead compounds had high binding affinity (docking scores below –9 kcal/mol), strong ADME profiles, and low toxicity (LD50 values above 1000 mg/kg). This experiment proposes an innovative method of MAO-B inhibitor discovery. It represents a promising starting point for future work focused on further testing of the 2 lead compounds through *in vitro* screening and additional *in silico* discovery of lead compounds using the methodology of this project.

Keywords: *Alzheimer's disease, monoamine oxidase B, pharmacophore mapping, molecular docking, ADMET prediction, drug discovery*

Introduction

Alzheimer's disease (AD), a type of neurodegenerative dementia, impacts memory and cognitive abilities in over 57 million people worldwide alongside other dementias (*The Lancet*, 2022). It is estimated that 1 in 9 people over the age of 65 have Alzheimer's, and researchers predict that over 152 million people worldwide will have dementia by 2050 (*The Lancet*, 2022; Alzheimer's Associ-

ation, 2024). Additionally, healthcare costs for AD patients are projected to reach almost $1 trillion in 2050 (Alzheimer's Association, 2024). Therefore, treating AD remains a significant concern both for public health and economic stability.

Current FDA-approved drugs for AD include acetylcholinesterase inhibitors (AChEIs), NMDA receptor antagonists, and monoclonal antibodies targeting Aβ proteins (Zhang et al., 2024). AChEIs, such as donepezil, galantamine, and rivastigmine, are the main type of pharmacological treatment for AD (Zuliani et al., 2024). Although they mildly mitigate cognitive decline, AChEIs remain mostly symptomatic (Zuliani et al., 2024). Memantine, the only NMDA receptor antagonist currently approved for AD treatment, is prescribed for patients with moderate to severe AD (Balázs et al., 2021). Usually a second-line treatment after AChEIs, memantine has a small benefit in moderate to severe AD, but not in mild AD (Balázs et al., 2021). Finally, newer drugs for Aβ proteins include aducanumab, lecanemab, and donanemab (Ebell et al., 2024). Cognitive benefits are statistically significant but small, and risks such as edema or hemorrhage remain prevalent with these drugs (Ebell et al., 2024). As a result, many monoclonal antibodies for AD have been discontinued due to their side effects. Since current AD treatments have many drawbacks, researchers have been exploring more promising pathways for AD to develop new drugs.

Two main pathways for AD are the amyloid-β (Aβ) pathway and the neuroinflammation pathway, which have gained more attention in recent years over older pathways such as the cholinergic hypothesis. The amyloidogenic pathway occurs when β-secretase cleaves the amyloid precursor protein (APP) instead of α-secretase, forming soluble APP-β and a C-terminal fragment (CTFβ) instead of soluble APP-α (Hampel et al., 2021). As a result, Aβ peptides are produced, and they clump together in extracellular Aβ plaques that cause aberrant signaling between neurons (Hampel et al., 2021). Additionally, the amyloidogenic pathway can lead to tau hyperphosphorylation, which causes neurofibrillary tangles that are correlated with cognitive decline (Hampel et al., 2021). The neuroinflammation pathway involves the activation of microglia and astrocytes, which lead to inflammatory signaling with neurotoxic effects (Liew et al., 2023). Positive feedback loops as well as Aβ or tau buildup contribute to neuroinflammation, either through further glial activation or increased Aβ accumulation via RIPK1 kinase (Liew et al., 2023; Doig, 2018).

Monoamine oxidase B (MAO-B) can offer insights into new AD treatment through the Aβ and neuroinflammation pathways. MAO-B is an enzyme that breaks down monoamine neurotransmitters such as serotonin, dopamine, and norepinephrine, which regulate mood, cognition, and behavior (Behl et al., 2021). MAO-B works by removing the amino group of the neurotransmitter and oxidizing it, producing an inactive metabolite, an aldehyde (R-CHO), ammonia (NH_3), and hydrogen peroxide (H_2O_2) as a byproduct (Behl et al., 2021). Normally, MAO-B controls mood and motor activity, acting as a metabolic barricade against amines. However, the overexpression of MAO-B enzymes can lead to oxidative stress due to H_2O_2 buildup, causing Aβ plaques and neurofibrillary tangles (Behl et al., 2021). High levels of MAO-B, which breaks down non-hydroxylated amines, are found in astrocytes surrounding Aβ plaques (Behl et al., 2021). The oxidative stress caused by MAO-B overactivity may lead to the Aβ and tau pathways by triggering neuroinflammation, potentially causing Aβ plaques or neurofibrillary tangles.

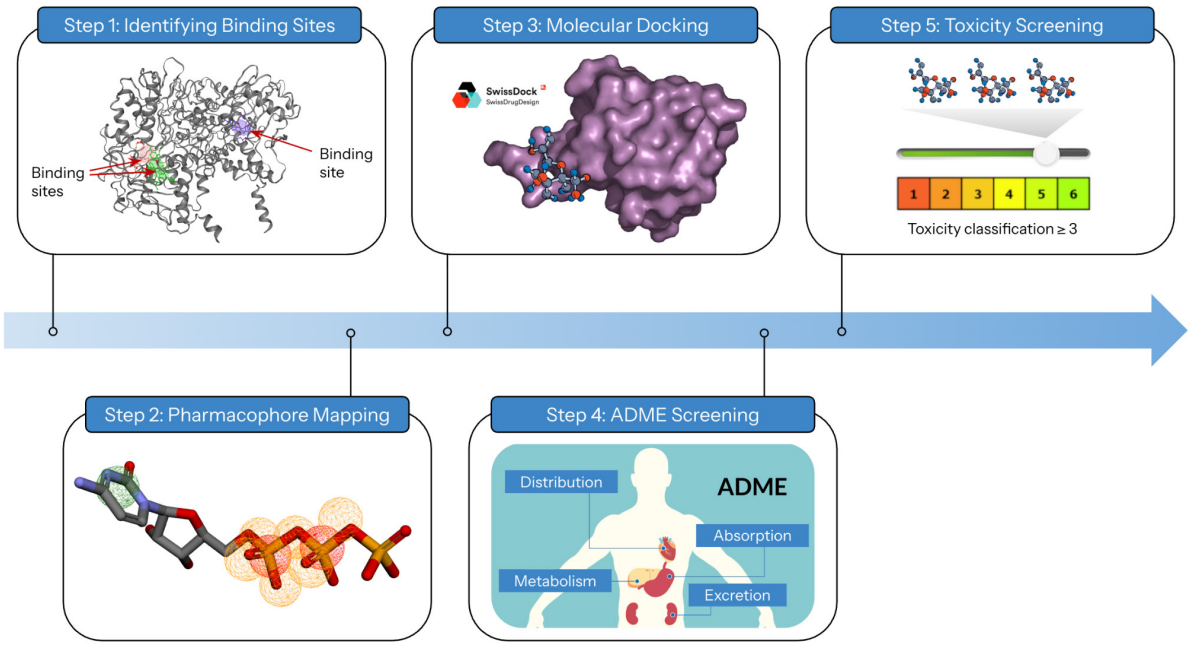
Because MAO-B is linked to two key pathways for Alzheimer's disease, monoamine oxidase inhibitors (MAOIs), an early class of antidepressants, are being explored as a promising new form of AD treatment. In Alzheimer's disease, the glutamate-GABA balance is disrupted, causing oxidative stress, and MAOIs can restore that balance by increasing GABA levels (Behl et al., 2021). MAOIs also sequester aldehydes and inhibit primary amine oxidase (PrAO), an enzyme that also produces aldehydes promoting Aβ plaque formation (Behl et al., 2021). These mechanisms indicate that MAOIs may be able to reduce oxidative stress in the brain, making them a powerful candidate for early AD treatment.

Researchers have explored various methods for developing MAOI-based AD treatment.

A study by Da Costa et al. used *in silico* preclinical screening to identify 4 lead compounds as potential MAO-B inhibitors, although these compounds have yet to undergo further testing (2024). Additionally, some propargylamines, such as selegiline and rasagiline, can be used as irreversible MAO-B inhibitors by binding covalently to the coenzyme flavin adenine dinucleotide (FAD), permanently disabling MAO-B (Chatzipieris et al., 2024). Newer compounds with internal alkynes are being explored as a way to avoid unwanted side effects of irreversible inhibition, such as upregulation of the GABA-synthesizing enzyme diamine oxidase (DAO) (Chatzipieris et al., 2024). Park et al. developed KDS2010 in 2019, a MAO-B inhibitor that is both reversible and highly selective (2019). By testing on APP/PS1 mice, the researchers discovered that KDS2010 reduces astrocytic GABA levels, bypassing the challenges of selegiline increasing DAO activity (Park et al., 2019). Beyond inhibitors for MAO-B alone, studies have also developed potential AD treatments involving drugs aimed at more than one target. Through *in silico* and *in vitro* analysis, Svobodova et al. tested 24 N-methylpropargylamino-quinazoline derivatives as multi-target directed ligands (MTDLs) for cholinesterases, monoamine oxidases, and N-methyl-D-aspartate receptors (NMDARs) in AD (2023). However, MTDLs face the challenge of selectivity, as they are designed to inhibit more than one enzyme. The potential of MAO-B inhibitors still remains largely unexplored, and no MAO-B inhibitors have passed clinical trials as of 2025. This research aims to improve upon existing work for MAO-B inhibitor discovery and focus on systematic pharmacophore-based *in silico* screening, which is cost-effective and offers rapid results.

Methodology

Figure 1. *Overview of methodology with 5 main steps*



MAO-B Binding Sites

To detect potential binding sites on the MAO-B protein that are both geometrically and energetically viable, computational tools such as DoGSiteScorer, FTSite, and P2Rank were used. DoGSiteScorer detects binding pockets, identifies their geometric and physicochemical properties, and predicts protein druggability to assign a drug score using a support vector machine (Volkamer et al., 2012). MAO-B's PDB code, 6FWC, was applied for DoGSiteScorer on https://proteins.plus/ with default settings (Reis et al., 2018). The "DoGSiteScorer" tab was selected on the website, and all settings were left as default. FTSite uses molecular probes to determine energetically favorable binding sites, as locations where the probes bind are more likely

to be optimal binding sites for ligands (Ngan et al., 2011). Code 6FWC was used on FTSite (https://ftsite.bu.edu/) with default settings. P2Rank uses machine learning to classify Solvent Accessible Surface points, which are regularly spaced points that encode geometric and physicochemical properties (Krivák & Hoksza, 2018). Code 6FWC was inputted on P2Rank with default settings on PrankWeb (https://prankweb.cz/) (Jendele et al., 2019).

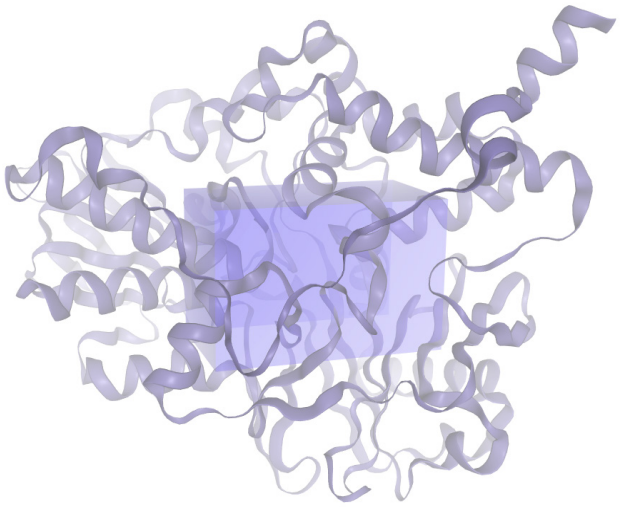
Pharmacophore Mapping

Pharmit (https://pharmit.csb.pitt.edu/) was used to generate a pharmacophore map and virtually screen small molecules to match the map (Sunseri & Koes, 2016). Pharmit filters through large drug databases and ranks results via energy minimization (Sunseri & Koes, 2016). To perform virtual screening with pharmacophore mapping in Pharmit, PDB code 6FWC was entered, "FAD" was selected from the adjacent dropdown menu, binding site waters were ignored, and the Enamine database was used. Pharmacophoric features of MAO-B were selected in areas likely to be optimal binding sites, creating 3 maps that captured different areas of the MAO-B enzyme (see Figure 8). Pharmit scanned the Enamine database and produced a list of molecules that matched the pharmacophore maps, from which 20 molecules were selected that had the least root mean square deviation.

Molecular Docking

After identifying the 20 top compounds from the Enamine database, molecular docking with SwissDock was used to test the ability of these compounds to dock on MAO-B (Grosdidier et al., 2011; Bugnon et al., 2024). On swissdock.ch, docking with attractive cavities was used with the SMILES code from Enamine (enaminestore.com) as the ligand and code 6FWC as the target. Chain B of the MAO-B enzyme was selected, and none of the heteroatoms were kept. Chain B was chosen because FTSite detected more potential binding sites on Chain B than Chain A. The search space was then defined with box center (20Å, 128Å, 18Å) and box size (15Å, 17Å, 27Å), as shown in Figure 2. The process was repeated for all 20 compounds.

Figure 2. *Setup of the SwissDock molecular docking. The purple ribbon represents chain B of the MAO-B protein, while the box represents the area where docking was simulated*



ADME Screening

Based on the results of the molecular docking, the top 10 drug candidates were chosen by most negative SwissParam score. SwissADME was used to determine various physicochemical features for the 10 compounds, as it identified their absorption, distribution, metabolism, and excretion (ADME) capabilities in the human body (Daina et al., 2017). To perform screening with SwissADME, the SMILES code obtained from the Enamine database was entered at http://www.swissadme.ch/. The molecular weight, number of hydrogen bond acceptors, number of hy-

drogen bond donors, and consensus LogP value was used for each drug candidate to determine whether the compound satisfied Lipinski's Rule of Five. Finally, the water solubility (Insoluble < Poorly < Moderately < Soluble < Very < Highly), gastrointestinal (GI) absorption classification, and blood-brain barrier (BBB) permeability were evaluated for each compound. For the screening, the consensus LogP value was used instead of the iLogP value, because the iLogP value was often an outlier from the other calculated LogP values. The ESOL Log S value was used for water solubility. Through this analysis, the number of drug candidates was narrowed down from 10 to 7.

Toxicity Screening

After the ADME screening, toxicity screening was performed on the 7 remaining compounds using ProTox 3.0 (https://tox.charite.de/protox3/). Tox Prediction was selected and the SMILES from the Enamine database was pasted in. All fields in the model prediction box were checked before starting the prediction. Only compounds that had an LD50 value greater than 400 mg/kg and a toxicity classification above 3 were kept, which narrowed the number of compounds down from 7 to 3. Then, examining the network and toxicity radar charts determined the final 2 safest potential candidates.

Results and Discussion

MAO-B Binding Sites

In order to determine potential drug candidates, binding sites on the protein that drugs could bond to were detected. A geometric method (DoGSiteScorer), an energetic method (FTSite), and a machine learning method (P2Rank) were used to predict potential binding sites on the MAO-B protein. DoGSiteScorer, the geometric method, was able to detect 38 potential binding sites. Table 1 shows all of the sites that had a drug score ≥ 0.5, of which there were 17. Two sites, P_0 and P_1, were noticeably larger than the others, although a smaller site (P_5) was ranked first by drug score.

Table 1. *The top 17 binding sites detected by DoGSiteScorer that had drug score ≥ 0.5, sorted by drug score*

Name	Volume (Å³)	Surface Area (Å²)	Drug Score
P_5	411.14	396.08	0.87
P_0	2077.85	1722.15	0.81
P_1	2042.91	1725.6	0.81
P_2	553.72	721.08	0.81
P_3	548.35	748.07	0.79
P_4	460.78	845.28	0.74
P_9	298.12	385.62	0.73
P_11	293.54	173.93	0.72
P_6	370.36	672.77	0.67
P_7	308.87	381.52	0.67
P_10	294.01	326.82	0.65
P_8	306.82	585.96	0.63
P_14	269.67	420.62	0.56
P_12	290.38	514.82	0.54
P_13	277.1	520.46	0.51
P_15	260.66	345.48	0.51
P_20	162.97	247.66	0.5

As seen in Figures 3 and 4, P_0 (yellow) and P_1 (purple) are much larger than the other binding sites. Figure 3 shows the top 3 binding sites, including P_0 and P_1 as well as P_5, which is much smaller but has the highest drug score assigned by DoGSiteScorer. Figure 4 shows all 17 potential binding sites.

Figure 3. *Top 3 binding sites identified by DoGSiteScorer. The red and blue ribbons represent the protein structure, while the green, yellow, and purple represent the binding sites (P_5, P_0, and P_1 respectively)*

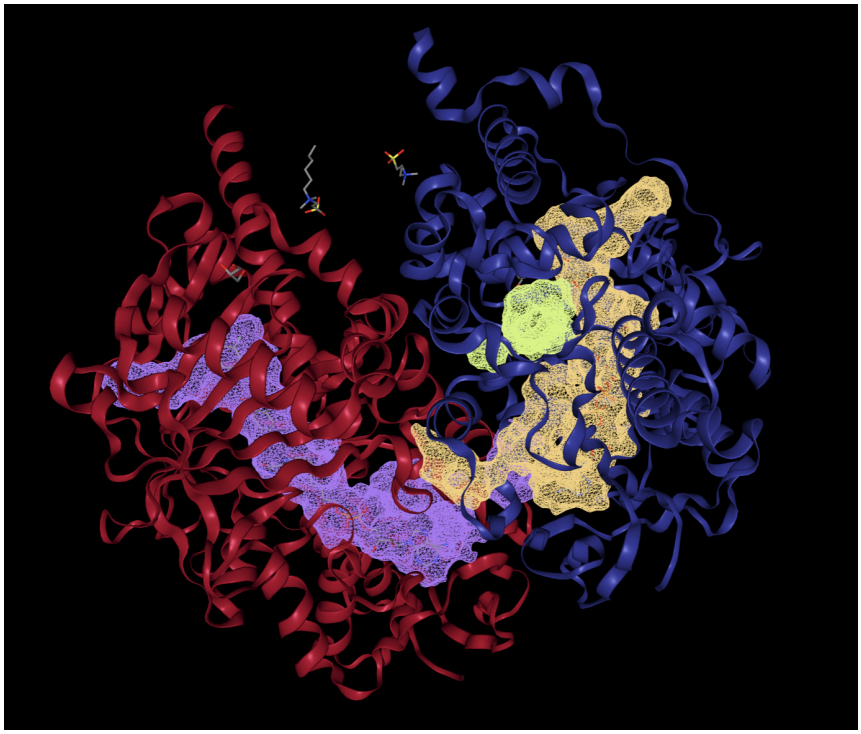
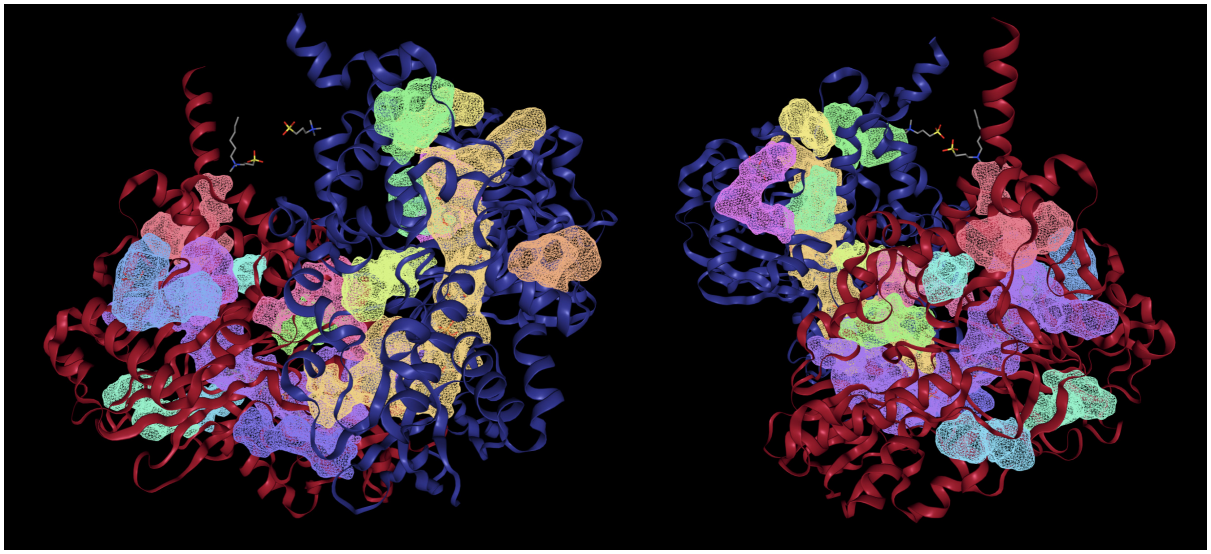
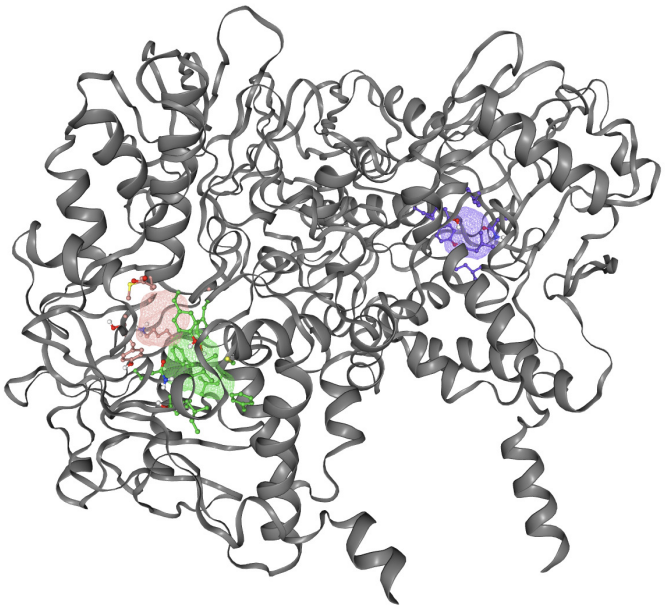


Figure 4. *All 17 potential binding sites identified by DoGSiteScorer, shown on the protein from two different angles*



The energetic method, FTSite, was able to detect 3 possible binding sites, as shown in Figure 5. Two sites were on the side colored blue on DoGSiteScorer, and one site was on the side colored red (see above). Since FTSite is based on energetic rather than geometric favorability, it identified less potential binding sites.

Figure 5. *Results of the FTSite energetic binding site prediction for the MAO-B protein. The gray ribbon represents the protein structure, while the green, red, and purple represent potential binding sites.*



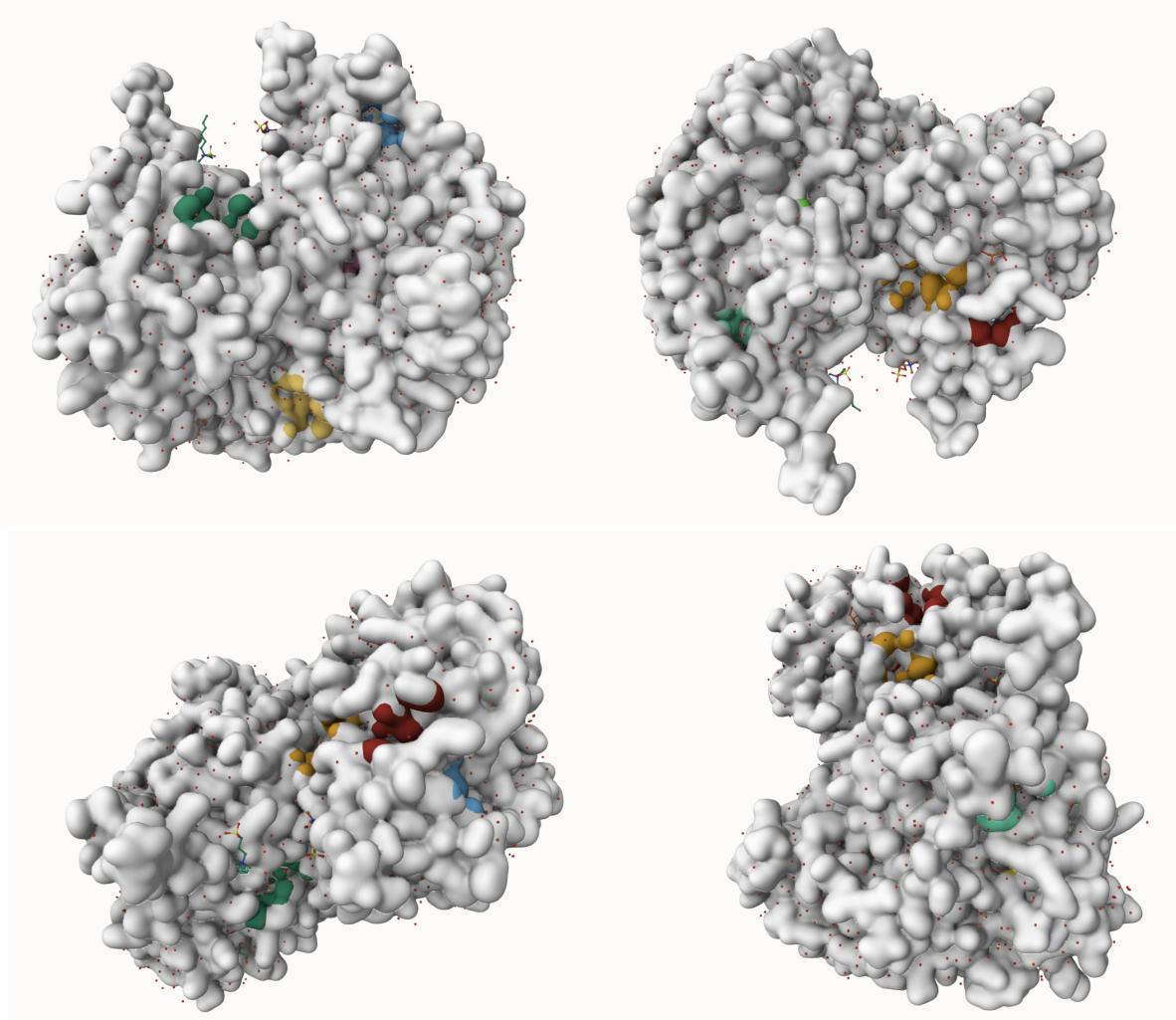
Finally, the machine learning method, P2Rank, was able to detect 12 binding sites, as shown in Table 2. Sites were ranked by score, and the data also included the number of residues for each site. The top two binding sites were consistent with DoGSiteScorer, as they were much larger than the rest. On PrankWeb, these two sites also had significantly higher scores and larger numbers of residues.

Table 2. *12 potential binding sites for the MAO-B protein detected by P2Rank, sorted by score.*

Rank sorted ascending	Score	# of residues
1	51.19	56
2	45.43	50
3	3.87	14
4	3.62	15
5	3.34	14
6	3.24	7
7	2.70	13
8	2.49	11
9	2.39	14
10	1.95	8
11	1.35	5
12	1.04	13

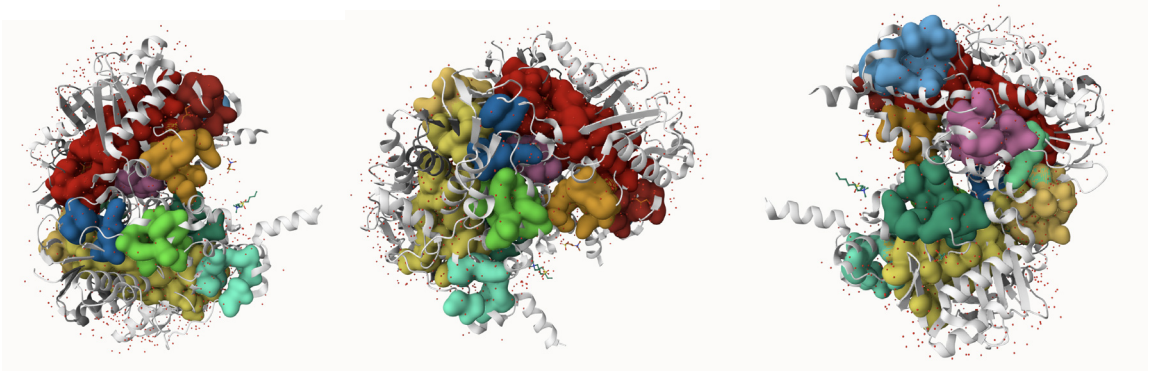
Figures 6 and 7 show various binding sites colored on the protein. Figure 6 shows the binding sites on the surface, and figure 7 shows them inside the MAO-B enzyme. As seen in figure 6, binding sites tended to be in concave pockets on the surface.

Figure 6. *Results of the P2Rank binding site prediction for the MAO-B protein. The gray represents the protein structure, while the colors represent potential binding sites. The protein is visualized by "surface" and the pockets are visualized by "surface (atoms)," shown from four different angles*



As shown in figure 7, the light yellow and red binding sites are significantly larger than the rest, suggesting that they might correspond to the sites P_0 and P_1 identified by DoGSiteScorer. These two sites also had distinctively higher scores of 51.19 and 45.43, compared with the rest of the sites with scores between 1 to 4.

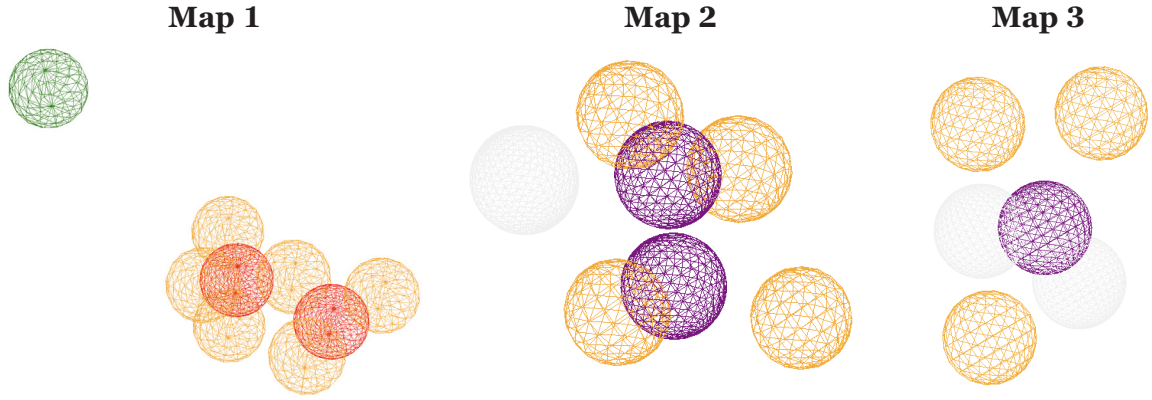
Figure 7. Results of the P2Rank binding site prediction with the protein visualized by "cartoon" and the pockets visualized by "surface (residues)," shown from three different angles



Pharmacophore Mapping

After potential binding sites were found, a pharmacophore map for the MAO-B protein that showed key features for binding was created. Instead of using the complete pharmacophore map for MAO-B, different combinations of pharmacophoric features were selected from Pharmit to create three maps with 6 to 9 features each, as shown in Figure 8. Each map focused on a distinctive aspect of MAO-B's pharmacophoric structure. The screening of the Enamine database for the first map only resulted in 3 potential drug candidates, so the second and third maps were added to find more compounds, producing 12 and 5 candidates respectively.

Figure 8. *Pharmit pharmacophore maps (1, 2, and 3 from left to right) generated from the MAO-B protein used to run virtual screening. Green spheres represent hydrophobic interactions, dark orange spheres represent negative ions, light orange spheres represent hydrogen acceptors, purple spheres represent aromatic interactions, and white spheres represent hydrogen donors*



Tables 3–5, which summarize the 20 compounds identified from the pharmacophore mapping experiment, show that the features of the compounds align closely with those of the pharmacophore maps. A lower RMSD is better, as it means the compound deviates less from the map. Compounds yielded from the third pharmacophore map had the lowest RMSD scores, whereas those from the first map had the highest RMSD scores. As shown in Figure 8, Map 1 is larger and more spread out than Map 2 and Map 3. Although the compounds generated from Map 1 had higher RMSDs, they were still included to test if a larger map might lead to drugs with better docking capabilities despite having worse matches.

Table 3. *Name, root mean square deviation (RMSD), and structure of drug candidates detected by Pharmit's virtual screening of the Enamine database with the first pharmacophore map*

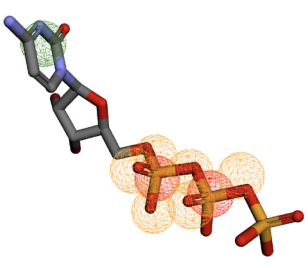
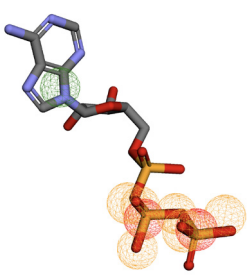
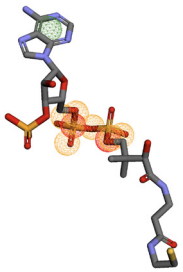
Name	Z4164535231	Z3810976496	Z3196311517
Structure			
RMSD	0.440	0.460	0.512

Table 4. *Name, root mean square deviation (RMSD), and structure of drug candidates detected by Pharmit's virtual screening of the Enamine database with the second pharmacophore map*

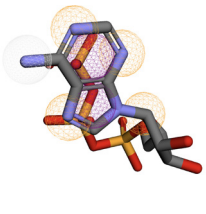
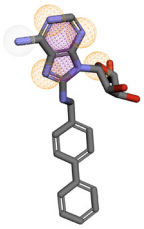
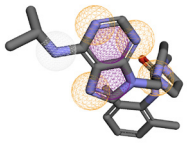
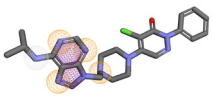
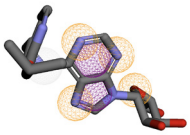
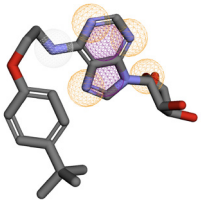
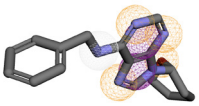
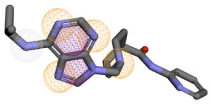
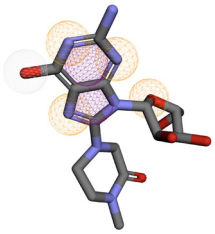
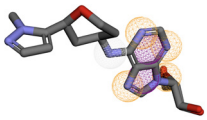
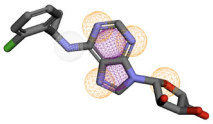
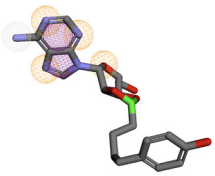
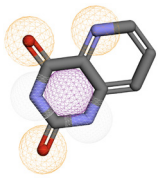
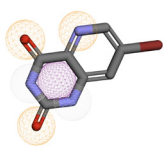
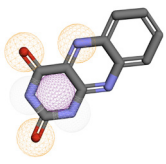
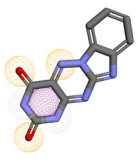
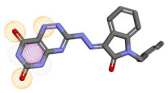
Name	Z3810976496	Z2065614619	Z1082764572	Z1082764448
Structure				
RMSD	0.045	0.050	0.051	0.052
Name	Z1980993192	Z1980914346	Z56780075	Z1082766136
Structure				
RMSD	0.056	0.061	0.062	0.063
Name	Z7911919636	Z4122876582	Z1980908378	Z4097793914
Structure				
RMSD	0.067	0.068	0.072	0.077

Table 5. *Name, root mean square deviation (RMSD), and structure of drug candidates detected by Pharmit's virtual screening of the Enamine database with the third pharmacophore map.*

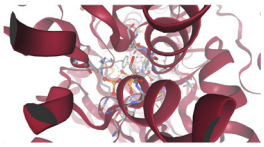
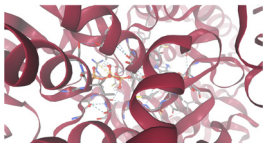
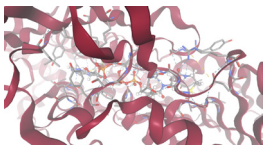
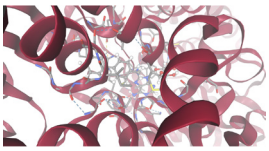
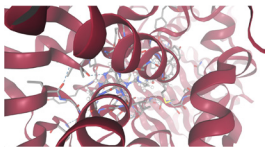
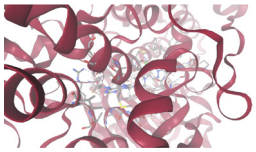
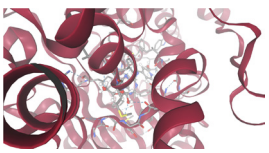
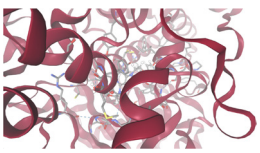
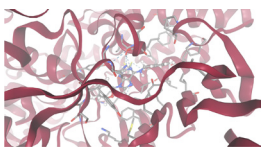
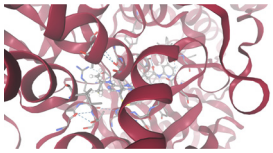
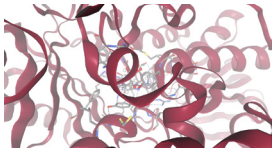
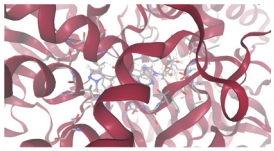
Name	Z1201626990	Z1269216848	Z56790788	Z56758453	Z56776036
Structure					
RMSD	0.027	0.027	0.027	0.028	0.029

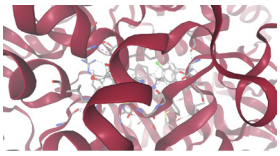
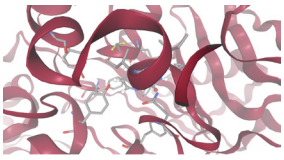
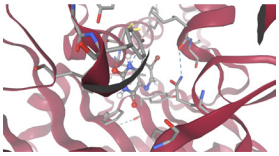
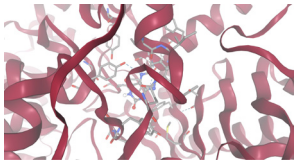
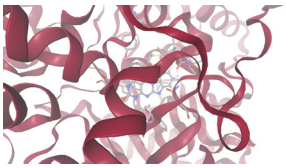
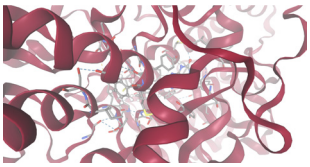
Molecular Docking

After molecular docking was assessed for each of the 20 compounds, most had a SwissParam score around –8, –9, or –10. During the docking simulation, the inhibitor was given the freedom to rotate around a defined box on the target and dock wherever possible. SwissDock then quantified the energy of

the interaction to determine how favorable binding would be. The top SwissParam score represented the Gibbs free energy (ΔG) of the best interaction in kilocalories per mole. The more negative the ΔG value, the more spontaneous the interaction, meaning that it consumed less energy and was more favorable. 10 compounds were selected by the most negative SwissParam score. The compound that performed the best was Z3196311517, followed by Z1082764448, the only two candidates that had SwissParam scores below –10. The SMILES of compound Z4097793914 was not accepted by SwissDock, likely due to the presence of boron, so it was removed from the screening. Compound Z3810976496 was found to be repeated, so its duplicate was taken out. The names and SwissParam scores of the top 10 compounds are bolded in Table 6 below. Although the three candidates identified from the first pharmacophore map had worse RMSD scores than the other seventeen, their SwissParam scores were all in the top 10 compounds for the molecular docking experiment. Compound Z3196311517 had the worst RMSD score, but it also had the most negative SwissParam score. The candidates identified from the third pharmacophore map generally had more positive SwissParam scores, likely due to their smaller size.

Table 6. *Results of molecular docking with SwissDock. In the residue interaction figures, the red ribbons represent the protein structure, the blue represents hydrogen bonds, and the yellow represents ionic interactions. The top 10 drug candidates are bolded*

Name	**Z4164535231**	**Z3810976496**	**Z3196311517**
SwissParam Score (kcal/mol)	**-9.8520**	**-9.9056**	**-10.7236**
Residue Interaction Figure			
Name	**Z2065614619**	**Z1082764572**	**Z1082764448**
SwissParam Score (kcal/mol)	**-9.4043**	**-9.7102**	**-10.6647**
Residue Interaction Figure			
Name	**Z1980993192**	**Z1980914346**	Z56780075
SwissParam Score (kcal/mol)	**-9.4726**	**-9.6217**	-8.5754
Residue Interaction Figure			
Name	**Z1082766136**	Z7911919636	Z4122876582
SwissParam Score (kcal/mol)	**-9.6193**	-8.2044	-8.9549
Residue Interaction Figure			

Name	Z1980908378	Z1201626990	Z1269216848
SwissParam Score (kcal/mol)	-8.7293	-6.4432	-6.7312
Residue Interaction Figure			
Name	**Z56790788**	**Z56758453**	**Z56776036**
SwissParam Score (kcal/mol)	-7.2137	-7.7093	**-9.7320**
Residue Interaction Figure			

ADME Screening

After performing ADME screening on the top 10 remaining drug candidates, the top 7 were selected based on whether they satisfied Lipinski's Rule of Five, a set of four rules that predict oral bioavailability (Lipinski et al., 1997). The molecular weight (MW), number of hydrogen bond (H-bond) acceptors, number of H-bond donors, and consensus LogP value for each drug candidate were used to evaluate their adherence to Lipinski's Rule of Five. Although they were all in the top 10, none of the three drug candidates selected from the first pharmacophore map satisfied Lipinski's Rule of Five, likely due to their large size. As a result, compounds Z1082764448, Z56776036, Z1082764572, Z1980914346, Z1082766136, Z1980993192, and Z2065614619 were selected as the top 7 potential MAO-B inhibitors. Out of these seven, compound Z1082764448 had the most negative SwissParam score. Table 7 summarizes the results of the ADME screening.

Table 7. *Physicochemical properties of 10 potential MAO-B inhibitors identified by SwissADME, sorted by SwissParam score. Green highlighting indicates which compounds satisfy Lipinski's Rule of Five ("RoF" column)*

Molecule	Mol. Weight	# H-bond acceptors	# H-bond donors	LogP Value	RoF	Water Solubility	GI absorption	BBB permeant
Z3196311517	767.53 g/mol	19	9	-3.20	No	Highly soluble	Low	No
Z1082764448	479.97 g/mol	6	1	2.44	Yes	Moderately soluble	High	No
Z3810976496	551.14 g/mol	16	5	-9.72	No	Highly soluble	Low	No
Z4164535231	563.15 g/mol	17	7	-9.79	No	Highly soluble	Low	No
Z56776036	414.38 g/mol	7	3	1.52	Yes	Soluble	Low	No
Z1082764572	436.55 g/mol	6	2	2.05	Yes	Moderately Soluble	High	No
Z1980914346	443.50 g/mol	8	4	1.54	Yes	Moderately Soluble	High	No

Molecule	Mol. Weight	# H-bond acceptors	# H-bond donors	LogP Value	RoF	Water Solubility	GI absorption	BBB permeant
Z1082766136	394.47 g/mol	6	2	1.59	Yes	Soluble	High	No
Z1980993192	416.43 g/mol	9	4	0.35	Yes	Soluble	Low	No
Z2065614619	448.47 g/mol	7	5	1.26	Yes	Moderately Soluble	Low	No

Toxicity Screening

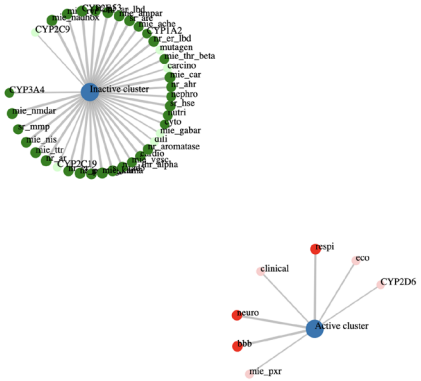
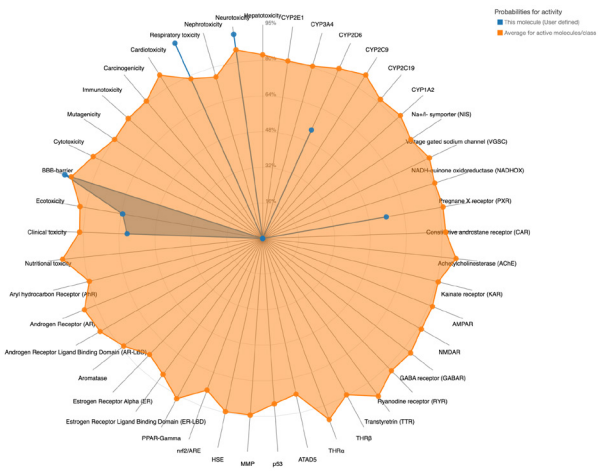
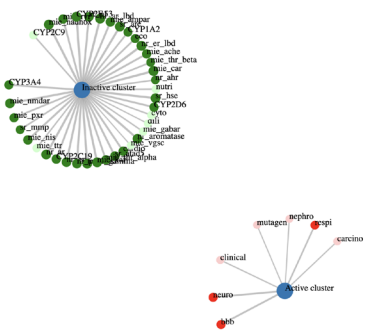
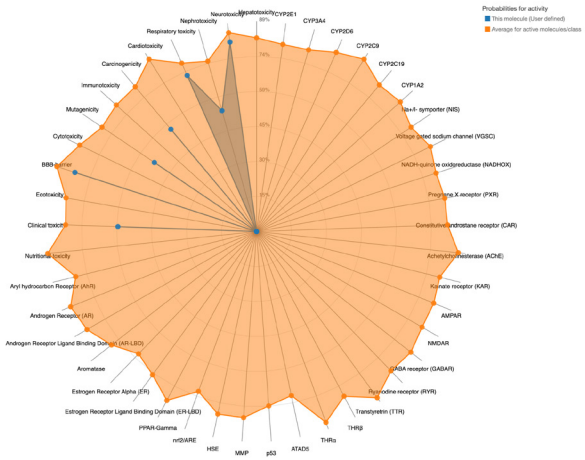
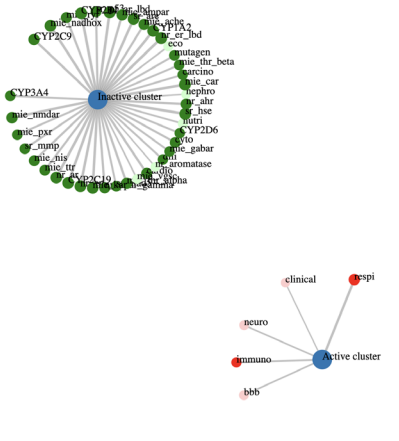
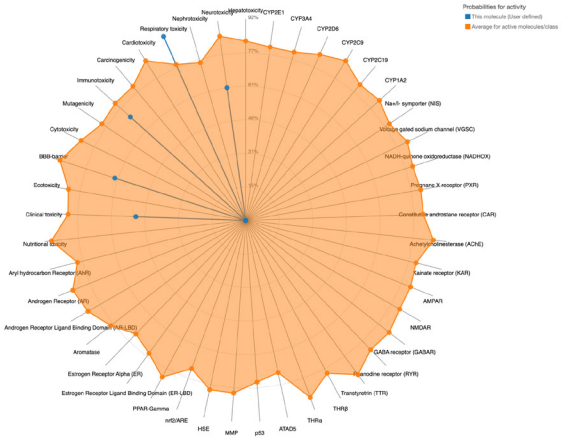
After the ADME screening, the remaining seven molecules were evaluated with ProTox-3.0 to determine their toxicity classification and LD50 value. Only compounds Z1082764448, Z56776036, and Z1980993192 were determined to be acceptably safe, as they had LD50 value above 400 mg/kg and toxicity classification above 3. The rest were deemed too toxic and eliminated from the screening. Results of the ProTox screening are shown in Table 8.

Table 8. *Toxicological properties of 7 potential MAO-B inhibitors identified by ProTox. Green highlighting indicates which compounds have both LD50 value > 400 mg/kg and toxicity classification > 3*

Name	LD50 (mg/kg)	Toxicity Classification
Z1082764448	2520	5
Z56776036	3000	5
Z1082764572	175	3
Z1980914346	29	2
Z1082766136	175	3
Z1980993192	1000	4
Z2065614619	13	2

For the remaining 3 drug candidates, the network chart and toxicity radar chart were assessed for additional toxicological analysis. The network chart shows the active (toxic) and inactive clusters, while the toxicity radar chart compares the active elements of the compound against their acceptable limits. Compounds passed the screening if they had at most one element exceeding average toxicity. Compound Z1082764448 exceeded the average toxicity for three elements (BBB by 3%, respiratory toxicity by 17%, and neurotoxicity by 7%), and compound Z1980993192 exceeded the average toxicity for one element (respiratory toxicity by 14%). As a result, only compounds Z56776036 and Z1980993192 passed the toxicity screening, although Z1980993192 has the limitation of its respiratory toxicity. Z56776036 is considered to be the safest of the top two molecules. Table 9 shows the network and toxicity radar chart results.

Table 9. *Network charts and toxicity radar charts generated by ProTox. The blue dots represent the toxicity of the compound for a specific element, while the orange dots represent the average toxicity of FDA approved drugs for that element*

Name	Network Chart	Toxicity Radar Chart
Z108...		
Z567...		
Z198...		

Conclusion

MAO-B is a critical therapeutic target for the neurodegenerative dementia Alzheimer's disease, as it causes Aβ plaques and neurofibrillary tangles, two key pathways to AD. This experiment focused on identifying novel MAO-B inhibitors with *in silico* screening to optimize for high binding affinity, druglike pharmacological properties, and low toxicity. Using computational methodology, po-

tential binding sites were identified on the MAO-B protein with DoGSiteScorer, FTSite, and PrankWeb. DoGSiteScorer was able to identify 38 binding sites, FTSite identified 3, and PrankWeb identified 12. Pharmacophore mapping was then used to screen the Enamine database, yielding 20 potential drug candidates. The 20 candidates were subsequently evaluated via molecular docking, and 10 were determined to be bioactive. SwissADME then identified that 7 of the top 10 adhered to Lipinski's Rule of Five. Finally, for toxicity prediction, ProTox was used to identify the 2 lead compounds with toxicity classification above 3 and at most one active element exceeding average toxicity. This experiment successfully discovered two promising MAO-B inhibitors as treatment for Alzheimer's disease. The final candidates, Z56776036 and Z1980993192, had excellent docking scores of –9.7320 and –9.4726 respectively, strong ADME profiles, and high LD50 values of 3000 mg/kg and 1000 mg/kg respectively. This study represents a valuable starting point for future work involving additional pharmacophoric screening targeting different areas of the MAO-B protein, as well as molecular docking for Chain A of the MAO-B enzyme with SwissDock. Further testing of the 2 lead compounds through *in vitro* or *in vivo* screening is needed to confirm this paper's findings, and additional *in silico* discovery of lead compounds can be conducted using the effective, low-cost methodology described in this paper.

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STATISTICAL MODELING OF INTERMITTENT AMLODIPINE DOSING USING MONTE CARLO SIMULATION AND REAL-WORLD BLOOD PRESSURE DATA

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Abstract

Hypertension affects over 1.28 billion adults worldwide, yet treatment adherence remains low. Amlodipine, a calcium channel blocker with a long half-life, is commonly prescribed once daily, but many patients self-modify their dosing schedules. This study evaluated the effect of intermittent amlodipine dosing on blood pressure (BP) control using real-world data and Monte Carlo simulation. Blood pressure readings were collected over three months from a patient on varying dosing regimens (once daily [QD], once every other day [QOD], once every 2 days [Q2D], once weekly [Q7D], and once every 10 days [Q10D]). Statistical comparison showed no significant difference in systolic or diastolic blood pressure among dosing regimens. Monte Carlo simulations (N=1000) revealed normally distributed systolic BP values with overlapping distributions across dosing schedules. Predicted proportions of systolic BP exceeding 120 mmHg ranged from 12.2% to 27.2%, while SBP >140 mmHg occurred only twice across all regimens. Coefficient of variation for SBP was <10% in all scenarios. Findings suggest intermittent dosing has limited impact on BP control due to amlodipine's long half-life, supporting flexibility in individualized dosing schedules.

Keywords: *hypertension; amlodipine; intermittent dosing; blood pressure variability; real-world data; Monte Carlo simulation*

Introduction

Hypertension is a major global health burden, affecting an estimated 1.28 billion adults aged 30–79 years, with two-thirds residing in low- and middle-income countries (WHO, 2025). In the United States, nearly half of adults have hypertension, yet only 21% achieve adequate control. Medication adherence remains a major challenge; real-world studies report that fewer than 55% of patients adhere to prescribed antihypertensive regimens (Wogen et al., 2003).

Amlodipine, a dihydropyridine calcium channel blocker, is widely prescribed due to its long elimination half-life (30–50 hours) and sustained antihypertensive effect. The recommended dose ranges from 5 to 10 mg once daily (Wang et al., 2023). However, patients frequently self-adjust their dosing schedules, raising concerns about efficacy

and safety. While amlodipine's pharmacokinetics suggest tolerance to missed doses, systematic evaluations of intermittent dosing schedules remain scarce.

This case study investigates the impact of varying amlodipine dosing frequencies on blood pressure control using real-world data and Monte Carlo simulation. The study aims to determine whether alternative dosing schedules maintain therapeutic effectiveness and support a personalized medicine approach.

Methods

Data Collection

Blood pressure data were collected prospectively over three months from one patient receiving amlodipine 5 mg. The patient followed several dosing regimens: once daily (QD), once every other day (QOD), every 2 days (Q2D), weekly (Q7D), and every 10 days (Q10D).

Measurements were taken using a calibrated home BP monitor (Microlife®). For each assessment, at least three readings were obtained at 3-minute intervals in the morning after 15 minutes of seated rest. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded.

Data Analysis

Observed BP values were averaged per dosing schedule. Comparisons between regimens were conducted using ANOVA. Monte Carlo simulations (N=1000 iterations) were employed to predict distributions of SBP under each dosing schedule, incorporating variability. Primary endpoints included:

- Proportion of SBP readings <120 mmHg and <140 mmHg.
- Variability of SBP (coefficient of variation, CV%).

Results

Observed Data

Comparison of the actual BP data across various treatment schedules did not reveal a statistically significant difference (ANOVA p-value >0.05) in SBP and DBP despite small fluctuations were observed among various dosing schedules (Figure 1 and Figure 2).

Figure 1. *Measured SBP (Orange) and DBP (Yellow) Readings over Time and their Moving Averages*

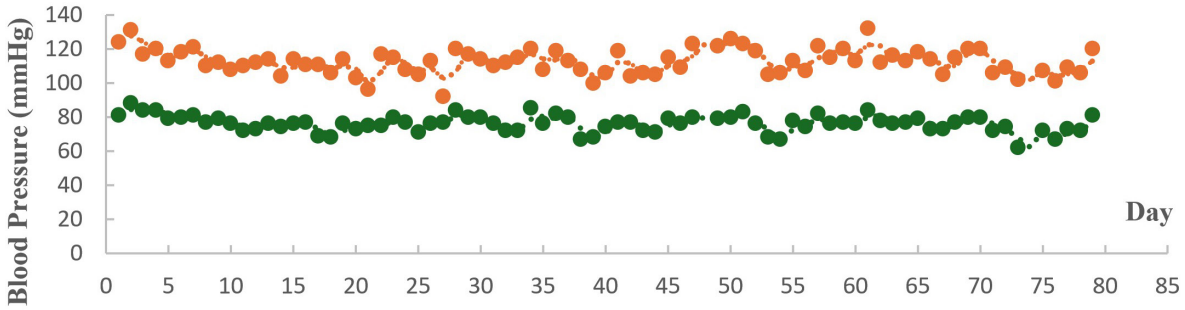
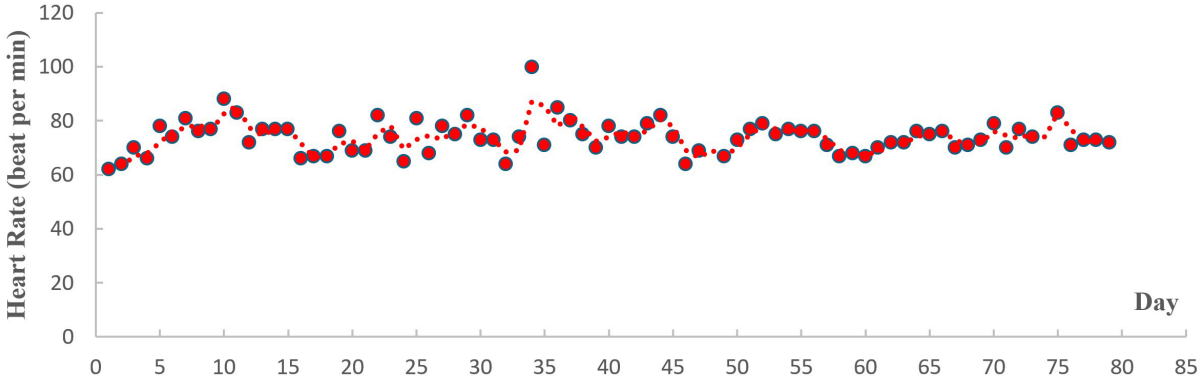


Figure 2. *Measured HR (Red) Readings over Time and their Moving Averages (Dotted Lines)*



Monte Carlo Simulation

Monte Carlo simulations (N = 1000 times) indicated that SBP data had a normal distribution with a central tendency (Figure 3) and overlapping of data distribution among various dosing schedules (Figure 4).

Monte Carlo simulations also showed that the proportion above SBP target (120 mmHg based on the American Heart Association guideline) was comparable, ranging from 12.2% to 27.2% given the inherent variability in BP (Table 1).

There were only two predicted episodes of SBP exceeding 140 mmHg across all dosing schedules.

Descriptive statistics of Monte Carlo simulation results indicated that the SBP data variability was generally less than 10% for all treatment schedules (coefficient of variation [CV]% = 5.1 to 7.0%) (Table 2).

Figure 3. *Distribution of Predicted SBP Values Using Monte Carlo Simulation for various Dosing Schedules*

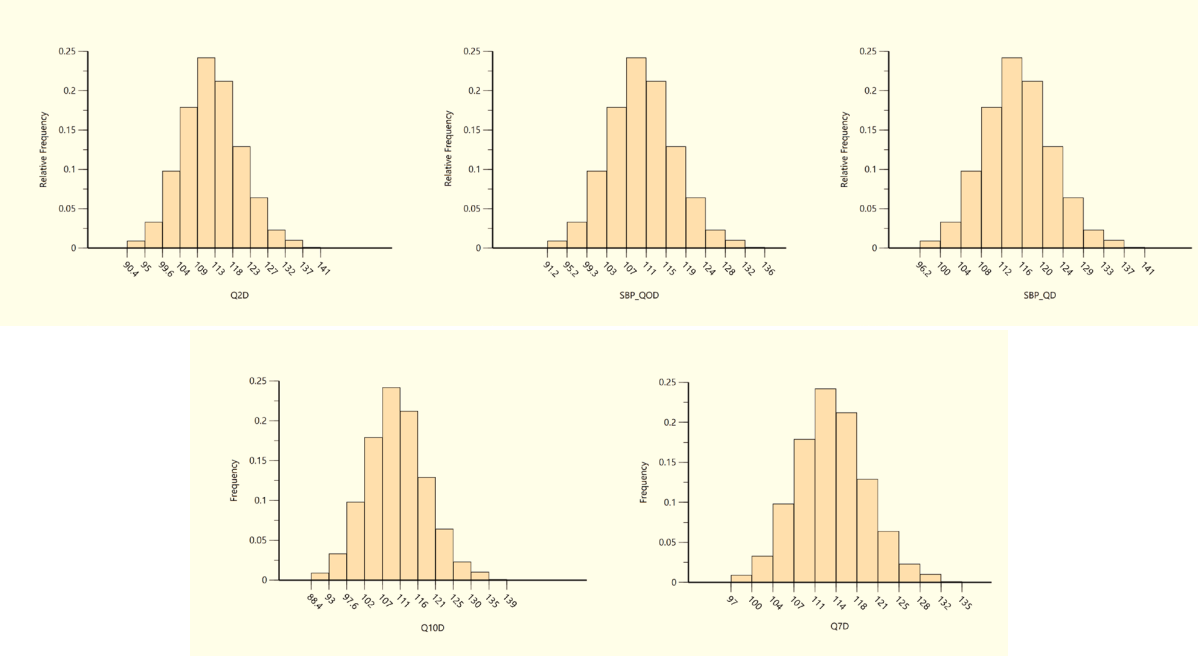


Figure 4. *Box Plots of Predicted SBP Values Using Monte Carlo Simulation for various Dosing Schedules*

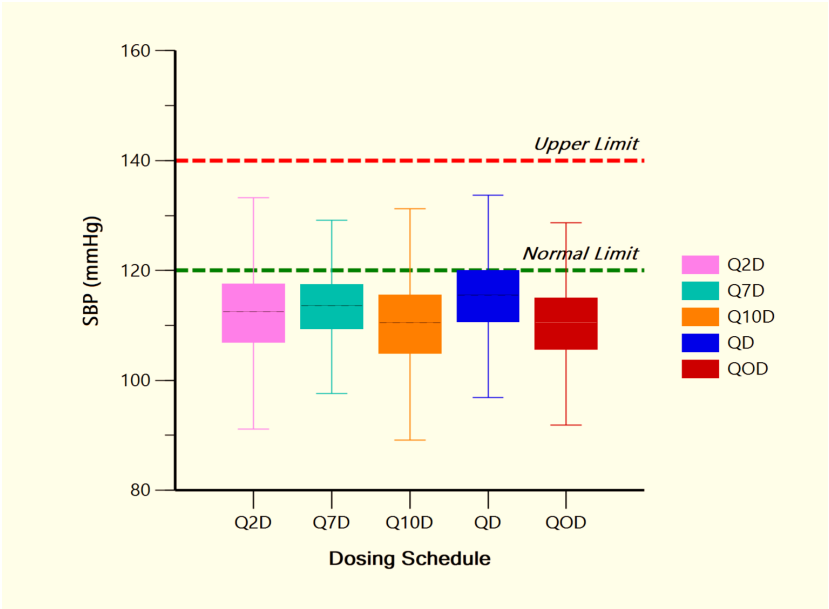


Table 1. *Percentage of Predicted SBP Values for various Dosing Schedules that are over Cutoffs*

% of simulations	QD	QOD	Q2D	Q7D	Q10D
≥ 120 mmHg	27.2	9.8	18.2	15.7	12.2
≥ 140 mmHg	0.01	0	0.01	0	0

Table 2. *Descriptive Statistics of Predicted SBP for various Dosing Schedules Based on Monte Carlo Simulation*

Variable	Dosing Schedule	N	Mean	SD	CV%	Min	Median	Max	Range
SBP	QD	1000	116	6.81	5.89	96	115	141	44
SBP	QOD	1000	111	6.81	6.16	91	110	136	44
SBP	Q2D	1000	113	7.78	6.92	90	112	141	51
SBP	Q7D	1000	114	5.83	5.14	97	114	135	38
SBP	Q10D	1000	111	7.78	7.04	88	110	139	51

Discussion

This case study suggests that intermittent dosing of amlodipine has limited impact on BP control. Due to its long half-life and sustained receptor binding, the drug maintains antihypertensive effects even with missed or delayed doses. These findings align with pharmacokinetic expectations and highlight the drug's forgiving profile compared to shorter-acting agents.

Importantly, results support flexibility in dosing schedules, which may improve adherence for patients who struggle with strict daily regimens. While this study is limited by its single-patient design, reliance on home monitoring, and simulation-based extrapolation, it provides preliminary evidence that alternative schedules may maintain efficacy.

Future studies with larger cohorts and prospective clinical trials are warranted to validate these findings and explore personalized dosing strategies.

Conclusion

Intermittent dosing of amlodipine, including schedules as infrequent as once weekly, did not significantly compromise blood pressure control in this case study. Monte Carlo simulations further support the robustness of antihypertensive effects across regimens. Personalized dosing approaches may enhance adherence and treatment outcomes compared to rigid once-daily prescribing.

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OPTIMIZING ATHLETIC PERFORMANCE THROUGH EVIDENCEBASED NUTRITIONAL SUPPLEMENTATION: UPDATED EVIDENCE AND PRACTICAL APPLICATIONS

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Abstract

Optimizing athletic performance and post-exercise recovery necessitates a comprehensive approach that integrates dietary strategies with training demands and physiological responses. Athletes commonly use dietary supplements, although their usefulness varies depending on the supplement type, sport features, and individual reactivity. This narrative review critically assesses evidence from randomized controlled trials, systematic reviews, and meta-analyses published between 2015 and 2024, with a focus on supplements commonly used in individual sports. The findings show that creatine monohydrate consistently improves high-intensity power output and neuromuscular adaptation, whereas protein and essential amino acids help with muscle remodeling and recuperation.

Caffeine and β-alanine enhance cognitive focus, absorption capacity, and prolonged high-intensity performance. The importance of dietary nitrates and omega-3 fatty acids in lowering inflammation, accelerating healing, and improving cardiovascular function is further supported by new research. Notwithstanding these benefits, problems with optimal dosage, scheduling strategies, inter-individual variability, supplement quality, and regulatory compliance still exist. This study emphasizes the need for individualized, evidence-based supplementation plans that consider training phase, safety requirements, and sport specificity.

Keywords: *ergogenic nutrition, dietary supplements, sport performance, recovery strategies, creatine, amino acids, caffeine, omega-3 fatty acids, nitrates, individualized nutrition*

Introduction

Optimal physiological adaptation and competitive performance are becoming increasingly vital in modern sports, where modest gains can determine success. As training demands rise across all athletic disciplines,

nutritional supplementation has emerged as an important strategy for increasing performance and recovery. Supplement use among athletes has skyrocketed over the last two decades, owing mostly to emerging scientific data confirming the efficacy of certain compounds *(Maughan et al., 2018, Peeling et al., 2018)*. This increase reflects the rising recognition that nutrition is critical for exercise adaptation, metabolic efficiency, and post-exercise recovery *(Beck, 2015)*. However, despite widespread adoption, translating research into practice remains inconsistent, which is compounded by differences in study designs, demographic variables, and sport-specific demands on athletes *(Amawi, 2024)*.

The evidence for certain ergogenic compounds has been strengthened by recent systematic reviews, study designs, and systematic meta-analyses. Across a variety of demographics, it has been demonstrated that creatine, whey protein, essential amino acids, β-alanine, caffeine, and dietary nitrates enhance athletic performance or recovery *(Fernández Lázaro, 2024; Scapec, 2024; Morton, 2018; Saunders, 2016)*. One of the most researched supplements, creatine monohydrate, has been shown time and time again to enhance resistance training adaptations and high-intensity exercise performance *(Kreider, 2017; Wax, 2021)*. It has also been demonstrated that protein and essential amino acid supplements enhance muscle hypertrophy, strength, and recuperation *(Morton, 2018)*.

Caffeine *(Grgic, 2019; Pickering & Grgic, 2019; Scapec, 2024)* and β-alanine *(Saunders, 2016; Suszter, 2020)* are two supplements with evidence-based ergogenic potential. Caffeine improves strength, muscular endurance, and repeated-sprint ability, while β-alanine improves high-intensity performance through increased muscle carnosine availability. Omega-3 fatty acids, branched-chain amino acids (BCAAs), leucine, and various antioxidants have also received attention for their potential roles in reducing inflammation, oxidative stress, and muscle damage, thereby aiding recovery *(Clemente Suárez, 2023; Fernández Lázaro, 2024; Fouré & Bendahan, 2017; Martinho, 2022; Plotkin, 2021; Thielecke & Blannin, 2020)*. However, the evidence for these recovery-oriented supplements is more diverse and frequently constrained by methodological discrepancies or small study numbers.

Important uncertainties still exist despite advancements in the scientific literature. These include long-term safety concerns (*Garthe & Ramsbottom, 2020*), timing in relation to training, interindividual responsiveness (*Pickering & Grgic, 2019*), and optimal dosing protocols *(Ribeiro, 2021)*. Athletes must conform to international anti-doping regulations, therefore regulatory issues continue to be crucial to applied practice *(World Anti-Doping Agency, WADA, 2024*). These difficulties highlight the necessity of thorough, current, and sport-specific evidence syntheses.

Therefore, **the goal of this review** is to gather and analyze high-quality research that has been published between 2015 and 2024, with a focus on controlled trials, systematic reviews, and meta-analyses that look into nutritional supplements for athletic performance and recuperation. Dose-response correlations, sport-specific applications, interindividual variation, molecular pathways, and regulatory compliance are the main topics of discussion. In order to help practitioners, coaches, and athletes take nutritional supplements safely and effectively in sports, the review attempts to offer a thorough, evidence-based framework.

Research Methodology

This study was conducted as a narrative review using systematic search procedures to collect high-quality data on dietary supplements and athletic performance. The PubMed/MEDLINE, Scopus, Web of Science, and SportDiscus databases were searched extensively for publications published between January 2015 and December 2024.

Only systematic reviews, meta-analyses, and randomized controlled trials with competitive, well-trained, or healthy athletes were included. Eligible studies had to demonstrate objective performance or recovery outcomes, as well as well-specified supplementation protocols. Studies that used observational methodologies, clinical populations, multi-ingredient supplements without distinguishing individual effects, or had no performance-related outcomes were ex-

cluded. To locate more relevant articles, reference lists for relevant reviews and position statements were manually reviewed.

Supplement-Specific Evidence

Creatine monohydrate continues to be the most common ergogenic aid. Numerous meta-analyses and research studies reveal significant improvements in maximal strength, peak power, repeated sprint ability, and lean mass gains when combined with resistance training. *(Kreider et al., 2017; Mielgo-Ayuso et al., 2019; Wax et al., 2021)*. Lower dose regimens may have longer-term benefits, although effective protocols typically include a loading phase (≈20 g/day for 5–7 days) followed by maintenance (3–5 g/day) *(Ribeiro et al., 2021)*. According to position statements *(Kreider et al., 2017),* no consistent adverse effects have been detected at recommended doses, indicating long-term safety in healthy populations. Supplementing with protein, particularly whey or premium complete proteins, promotes muscle protein synthesis, recuperation, and adaptability in strength. *(Jäger et al., 2017; Beck et al., 2015)*.

Consuming 20–40 g of protein right after exercise improves the anabolic response. Protein supplementation is especially helpful during energy shortage or high-volume training stages. According to *Plotkin et, al* (2021), total daily protein consumption continues to be the dominant determinant for maximal hypertrophy or strength improvements, but isolated BCAA supplementation gives a slight benefit. Supplementing with beta alanine boosts intramuscular carnosine, improving buffering capacity and postponing tiredness during one to four minutes of high-intensity exercise *(Saunders et al., 2016; Suszter et al., 2020)*. Usually, protocols give 4–6 g daily for 4–8 weeks. Anaerobic and repeated sprint workouts yield the greatest advantages, whereas endurance activities yield mixed results. The most frequently reported side effect is mild paresthesia; in healthy people, there are no major adverse events.

Caffeine's ergogenic and neuromodulator characteristics have been extensively explored. Acute doses of 3–6 mg/kg administered 30–60 minutes before exercise consistently enhance anaerobic power, reaction time, perceived effort, and endurance performance *(Grgic et al., 2019)*. Recent RCTs *(Scapec et al., 2024)* suggest additional benefits for power output and muscle endurance. Variations in reaction between individuals may be influenced by genetic variants (such as CYP1A2), habitual intake, and stimulant sensitivity *(Pickering & Grgic, 2019)*. Long-term omega-3 supplementation (1–2 g/day EPA + DHA) supports anti-inflammatory responses, improves joint health, reduces muscular soreness, and may improve recovery *(Fernández Lázaro et al., 2024; Thielecke & Blannin, 2020)*.

Similarly, exercise-induced oxidative stress may be reduced by antioxidants and polyphenols, but excessive consumption may impair adaptive signaling *(Clemente Suárez, et al., 2023)*. There is little proof that omega-3 directly improves performance, but it is consistent for long-term musculoskeletal health and recuperation.

Particularly in recreational or lightly trained athletes, recent research indicates modest gains in time-trial performance and fatigue resistance. Supplementing with nitrate may be particularly helpful during multi-day tournaments or high-volume endurance training.

Results and Discussion

This review clearly demonstrates that, despite the wide availability of nutritional supplements marketed to enhance athletic performance, only a limited number are supported by strong and consistent scientific evidence. The results of the literature analysis indicate that **creatine monohydrate, caffeine, protein/essential amino acids, and β-alanine** currently represent the supplements with the most robust ergogenic support, whereas other supplements show moderate or context-dependent effects. Creatine monohydrate emerges as the most extensively researched and reliable ergogenic aid in sports nutrition. Experimental studies and expert consensus statements consistently report significant improvements in maximal strength, power output, and repeated high-intensity exercise performance. These benefits are primarily attributed to increased intramuscular phosphocreatine stores, enhanced ATP resynthesis, and

improved training quality over time *(Kreider et al., 2017; Wax et al., 2021)*. Collectively, these findings support the use of creatine as a foundational supplement, particularly in strength- and power-based sports. Protein intake and essential amino acids play a central role in supporting exercise-induced muscle hypertrophy and post-exercise recovery. Evidence indicates that **total daily protein intake** is a more critical determinant of adaptation than precise timing or isolated amino acid supplementation. Meta-analytical findings confirm that protein supplementation, when combined with resistance training, significantly enhances muscle mass and strength gains, especially in physically active and trained individuals (*Morton et al., 2018; Jäger et al., 2017)*. Caffeine demonstrates consistent ergogenic effects across a wide range of performance outcomes, including endurance capacity, muscular strength, power output, and reductions in perceived exertion. These effects are largely mediated through central nervous system stimulation, increased motor unit recruitment, and altered pain perception. However, substantial inter-individual variability exists, influenced by genetic factors, habitual caffeine intake, and dosing strategies, underscoring the importance of individualized supplementation protocols *(Grgic et al., 2019; Pickering & Grgic, 2019)*. β-Alanine supplementation has been shown to improve performance in high-intensity efforts lasting approximately 1–10 minutes by increasing intramuscular carnosine concentrations and enhancing buffering capacity against exercise-induced acidosis. The magnitude of these benefits depends on supplementation duration, total dose, and baseline carnosine levels, indicating that structured loading protocols are essential to maximize its ergogenic potential *(Saunders et al., 2016)*. Dietary nitrates, commonly consumed as beetroot juice, demonstrate moderate improvements in exercise economy and endurance performance, particularly in recreational and sub-elite athletes. These effects are attributed to increased nitric oxide bioavailability and improved mitochondrial efficiency. However, evidence in elite populations remains inconsistent, likely due to ceiling effects associated with already optimized physiological systems *(Peeling et al., 2018)*. Omega-3 fatty acids and antioxidant supplements primarily influence recovery-related outcomes, including inflammation, muscle soreness, and cellular stress, rather than directly enhancing performance. While these supplements may be beneficial during periods of intensified training or rehabilitation, emerging evidence suggests that chronic high-dose antioxidant supplementation may blunt training-induced physiological adaptations by attenuating exercise-related oxidative signaling pathways *(Clemente Suárez et al., 2023)*. Branched-chain amino acids (BCAAs) and isolated leucine are frequently promoted for muscle hypertrophy and recovery; however, current evidence indicates that their effects are inferior to those of complete protein sources containing all essential amino acids. Whole-protein supplementation more effectively stimulates muscle protein synthesis and supports long-term hypertrophic adaptations, particularly in resistance-trained individuals *(Fouré & Bendahan, 2017; Plotkin et al., 2021)*. Overall, **the findings of this review** confirm that nutritional supplementation can meaningfully enhance athletic performance only when grounded in strong scientific evidence, appropriate dosing, and sport-specific application. A major challenge remains the translation of laboratory-based findings into applied sport settings, where inter-individual variability, training status, and competitive demands substantially influence supplementation outcomes *(Amawi et al., 2024)*. Persistent misconceptions regarding supplement timing and synergistic combinations continue to exist, despite evidence suggesting that **total intake and chronic adaptation** are often more influential than acute timing strategies *(Ribeiro et al., 2021)*. Regulatory compliance also represents a critical consideration, as contamination risks and evolving anti-doping regulations pose ethical and career-threatening risks for athletes. Consequently, supplementation strategies should prioritize third-party tested products and evidence-based selection *(Maughan et al., 2018; WADA, 2024)*. **Although** this review synthesizes high-quality evidence, methodological heterogeneity across studies limits direct comparison and generalizability. Future research should place greater

emphasis on underrepresented populations, including female athletes, youth and master athletes, long-term safety outcomes, and precision nutrition approaches that account for individual genetic responsiveness.

Conclusions

This review confirms that only a limited number of nutritional supplements provide consistent and meaningful ergogenic benefits. **Creatine monohydrate, caffeine, protein/essential amino acids, and β-alanine** demonstrate the strongest evidence for enhancing strength, power, endurance, and training adaptations when appropriately dosed and applied. Other supplements show moderate or context-dependent effects, with limited direct influence on performance, and chronic high-dose antioxidant use may impair long-term adaptations. Overall, supplementation should support – not replace – sound nutrition and training practices and must be individualized according to sport demands and athlete characteristics. Given the potential risks related to contamination and anti-doping regulations, evidence-based selection and third-party testing remain essential for safe and effective supplement use.

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