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PDE4D-TARGETED SMALL MOLECULES FROM COMPUTATIONAL SCREENING FOR DEMENTIA

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Abstract

Phosphodiesterase 4D enzymes hydrolyze cyclic adenosine monophosphate (cAMP), a messenger molecule that signals neurons and the release of neurotransmitters, which are crucial to the development of memory and cognition. Inhibiting PDE4D offers promising drug treatments for neurological diseases, including dementia and other illnesses associated with memory, disorders that impair mental abilities, limit daily functioning, and complicate the lives of those affected. The goal of this study was to determine binding sites on the PDE4D protein using geometric and energetic methods and identify promising locations for binding. The PDE4D enzyme was virtually screened for interactions between the protein and the compound, revealing fitting compounds for these interactions. The docking of compounds onto the protein and their energetic interactions were quantified, providing further details on each interaction and its strength. To ensure the compounds would be absorbed and excreted properly, each molecule was analyzed according to ADME drug guidelines to become candidates for drug treatment. The toxicity levels of the compounds raise risks to the human body; by ensuring that the toxicity was harmless, 1 lead compound was successfully discovered for potential treatment, along with 2 backup compounds that met all other criteria in all experiments. These criteria included low drug and RMSD scores in the pharmacophore maps, negative ΔG values in docking of the molecules, 0 violations of Lipinski's rule and guidelines of ADME, and a high LD50. These compounds would inhibit the catalysis of cAMP, improving cognitive symptoms of dementia and associated disorders.

Keywords: *Drug discovery; dementia; small molecules; virtual screening; PDE4D.*

Introduction

Dementia is a neurological disorder characterized by a decline in mental abilities, some of which are categorized as memory loss and cognitive functioning (Arvanitakis, Z., Shah, R.C., Bennett, D.A., 2019). Over time, the progression of dementia and dementia-

related diseases, such as Alzheimer's, vascular dementia, Lewy body dementia, and frontotemporal dementia, can lead to impairment of daily functioning, especially amongst older populations (Chouliaras L., O'Brien J.T., 2023). In 2021, an estimated 57 million people suffered from dementia, with the pro-

jected value expected to increase to 139 million by 2050. The yearly cost of dementia is estimated to be well above \$1.3 trillion USD and is expected to rise to \$2.8 trillion USD by 2030 (Guerchet, M.G., Prince, M.P., Prina, M.P., 2020). The complications of dementia place psychological and emotional stress on its patients, as well as economic burdens on health care institutions to develop treatments and provide care (Liu W., Deng W., Gong X., Ou J., Yu S., Chen S., 2025).

The pathways of dementia consist of cholinesterase, amyloid, and tau. The cholinesterase enzyme specializes in the breakdown of the neurotransmitter acetylcholine (ACh). ACh is associated with both voluntary and involuntary movement, as well as strengthening cognitive processes related to memory and thinking (Moehle M. S., Conn P. J., 2019; Hasselmo M. E., 2006). The inhibition of cholinesterase limits the hydrolysis of ACh, increasing its functions and alleviating the associated decline in memory (Sharma K., 2019). Amyloid precursor proteins can be processed into amyloid- β , which is overproduced in dementia patients and can aggregate and clump in the brain (Clarkson, J.C., 2021). Tau proteins, located on neuron axons, can detach and form abnormal tau proteins that cannot maintain their own structure and clump as well (Clarkson, J.C., 2021). Both the aggregation of amyloid and tau contribute to the degradation of neurons, leading to cognitive decline (Gulisano W., Maugeri D., Baltrons M. A., Fà M., Amato A., Palmeri A., D'Adamio L., Grassi C., Devanand D. P., Honig L. S., Puzzo D., Arancio O., 2018).

Phosphodiesterase's (PDEs) are part of a family of enzymes that can be classified into 11 subgroups (PDE1 to PDE11). These enzymes are coded by 24 different genes, and they can be found in tissues located in the heart, brain, kidneys, endothelial cells, and immune cells. PDEs can stop cyclic nucleotide signaling through the hydrolysis of both cAMP and cGMP, both intracellular cellular messengers (Bizzi M. F., Bolger G. B., Korbonits M., Ribeiro-Oliveira A. Jr., 2019; Fan T., Wang W., Wang Y., Zeng M., Liu Y., Zhu S., Yang L., 2024). The specific subgroup, PDE4, selectively degrades cAMP and consists of 4 isoforms: PDE4A, PDE4B, PDE4C, and PDE4D, which are encoded by separate genes

(Fan T., Wang W., Wang Y., Zeng M., Liu Y., Zhu S., Yang L., 2024).

PDE4D enzymes hydrolyze cyclic adenosine monophosphate (cAMP), a secondary messenger crucial to cellular signaling in inflammatory immune responses, cardiovascular regulation, and brain cognition (Bhat A., Ray B., Mahalakshmi A. M., Tuladhar S., Nandakumar D. N., Srinivasan M., Essa M. M., Chidambaram S. B., Guillemin G. J., Sakharkar M. K., 2020). Through hydrolysis, the PDE4D molecule breaks cAMP into inactive 5'AMP. Through this degradation, the downstream signaling pathways are turned off and are unable to perform normal functions, such as triggering immune responses or activating genes (Olsen C. M., Liu Q. S., 2016). PDE4D is mainly located in the brain and is involved in cAMP signalling related to physiological processes involved with memory consolidation and neuroplasticity (Donders Z., Skorupska I. J., Willems E., Mussen F., Broeckhoven J. V., Carlier A., Schepers M., Vanmierlo T., 2024). These are associated with many neurodegenerative diseases in the central nervous system, including but not limited to dementia, Alzheimer's, multiple sclerosis, spinal cord injuries, and traumatic brain injuries (Donders Z., Skorupska I. J., Willems E., Mussen F., Broeckhoven J. V., Carlier A., Schepers M., Vanmierlo T., 2024).

Figure 1. A model of the PDE4D-cAMP pathway and the hydrolysis of cAMP (Gooderham M., 2013)

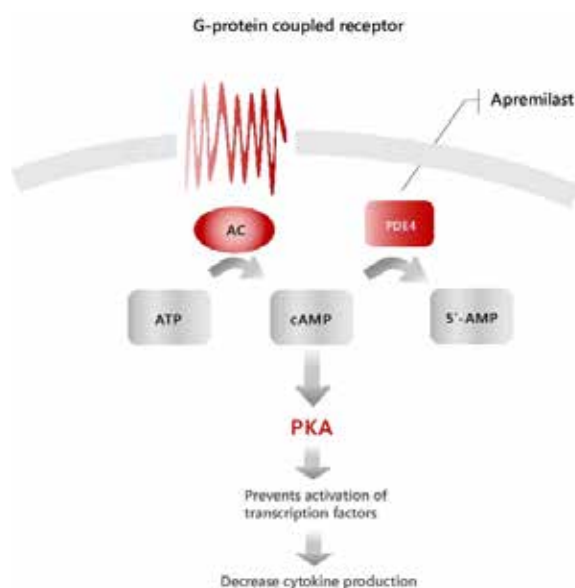


Figure 1 demonstrates the PDE4D hydrolysis of cAMP and the pathway that it follows. AC is represented as Adenylyl Cyclase, an essential enzyme that converts ATP, a primary energy-carrying molecule, to cAMP. cAMP molecules regularly bind to subunits of PKA, Protein Kinase A, and change their shape to be able to release catalytic subunits that attach to target proteins. The PDE4D protein catalyzes the hydrolysis of cAMP, breaking it down into 5'AMP. 5'AMP is considered inactive in cellular signaling; the degradation of cAMP prevents it from being able to send signals necessary for cognitive development.

Through the inhibition of PDE4D, the process of hydrolysis of cAMP occurs much less frequently, increasing cAMP levels and enhancing its function (Adili A., Dilihu-maer A., Zhu H., Tang H., 2024). PDE4D inhibitors provide a potential therapeutic target for dementia by improving cAMP signaling focused on memory and cognition.

PDE4D inhibitors are possible therapeutic targets for neurological disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, and sleep disorders. The link between PDE4D and cAMP signaling can be used to examine specific PDE4D inhibitors while minimizing adverse side effects (Bhat A., Ray B., Mahalakshmi A. M., Tuladhar S., Nandakumar D. N., Srinivasan M., Essa M. M., Chidambaram S. B., Guillemin G. J., Sakharkar M. K., 2020). PDE4D inhibitors are also able to enhance cAMP levels and pathways focused on neurodegenerative diseases in the central nervous system. PDE4D inhibitors can be effective therapeutic targets for these neurological diseases. The development of PDE4D inhibitors can be used as drugs to prevent and treat these diseases (Adili A., Dilihu-maer A., Zhu H., Tang H., 2024).

Methodology

Analysis of Binding Sites

Geometric Method

To begin searching for viable binding sites on PDE4D for small molecules using geometric methods, ProteinPlus (<https://proteins.plus/>), a Structure-Based Modeling Support Server, was visited. After loading the site, the PDB code for PDE4D was entered and searched: **2PW3**. Once the protein had

been found, the site displayed both an image of the protein and a list of analysis tools. DoGSiteScorer was selected, and all settings remained unchanged. The "Analysis detail" was set to "Pocket(s)", the "Binding site prediction granularity" was set to "and drugability", no ligands were set or entered, and the "Chain" was set to "all". The data on the volume, surface area, and drug score of the top 5–10 binding sites were recorded and subsequently evaluated for consistency.

Machine Learning Methods

To further narrow down the available and compatible binding sites, another website, Prankweb (<https://prankweb.cz/>), was used to analyze the PDE4D protein binding sites. After the site was loaded, the same PDB code, 2PW3, was entered and searched for. The initial search settings stayed the same: "Experimental structure", "Use original structure", and "Use conservation" were selected. The rankings, score values, and # residues (or amino acids) were recorded, along with select amino acids from each binding site.

Pharmacophore-based Virtual Screening

To virtually screen the proteins for compounds compatible with binding sites, a pharmacophore map tool, Pharmit (<https://pharmit.csb.pitt.edu/>) analyzed the interactions. The PDB code, 2PW3, was input into the search, and the ligand was set to "CMP". To better view the interactions, the Ligand and Receptor, located under Visualization, were set to "None", and the opacity of the protein was lowered to its minimum. Around 3–5 interactions, located under Pharmacophore, were selectively chosen by turning them on or off, and compounds were searched from the databases MolPort, Enamine, ChEMBL36, and ChemSpace. Specific compounds and their RMSD (Root Mean Square Deviation) were recorded, along with images of the pharmacophore maps and compounds.

Molecular Docking using SwissDock

Once binding sites and compounds were selected, SwissDock (<https://swissdock.ch/>) was visited to continue experimenting in discovering viable interactions between the protein and the small molecules. The

site required a ligand, the target, and a defined search space. Using each of the IDs obtained from Pharmit, the SMILES (Simplified Molecular Input Line Entry System) was searched for each compound using their respective websites. These SMILES codes were input into Swissdock, and the ligand was prepared. The PDB code, **2PW3**, was input as the target; the chains were set to “Select all”; the heteratom(s) were set to “None”, and the target was prepared. To define the search space, the box was centered at (Clarkson, J.C., 2021; Chouliaras L., O’Brien J.T., 2023, 35) Å and its size was set to (25, 28, 25) Å. These values were used for all compounds across all calculations. Each compound and its SMILES were entered individually, along with the PDB code and defined search space, and the parameters were checked. Values for the SwissParamScore, or ΔG values (kcal/mol), and images for each compound and all interactions were recorded for further evaluation.

Drug Properties and ADME

Using the results obtained from the SwissDock experiment, 8 compounds with the lowest ΔG values and their SMILES code were input into SwissADME (<https://www.swissadme.ch/>). All SMILES were entered into the list, and the tool was run. Each molecule provided a chart, and the values for the molecular weight (g/mol), number of H-bond acceptors, number of H-bond donors, and Consensus LogP were recorded. To ensure that all values met the criteria of Lipinski’s Rule, the number of violations was also considered, along with other values (classification of solubility and GI absorption) to verify that the data results were aligned.

Toxicity Prediction

The 6 compounds obtained from the SwissADME testing underwent further testing to determine toxicity levels to the human body. To assess these toxicity levels, ProTox 3.0 (<https://tox.charite.de/protox3/index.php?site=home#>) was visited, and Tox prediction was selected. Each compound and its SMILES were entered into “Canonical SMILES”, and the “smiles” button was clicked so the compounds could be run through the system, and an image of the compound was

displayed. All models for toxicity were selected through the “All” button, and the tool was run by clicking “Start Tox-Prediction”. The Predicted LD50, Predicted Toxicity Class, images of the Inactive and Active Network Charts, and Average toxicity Radar Charts were recorded.

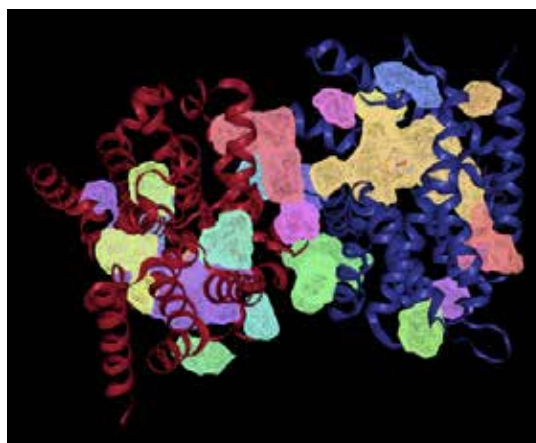
Results and Discussion

Binding Sites

Binding sites in proteins are 3D pockets that host interactions between attaching ligands and the designated protein. For the small molecules to bind properly to the protein, the shape, energetic interactions, and chemical interactions must be considered for the molecule to bind properly and for the protein to carry out its function. If the binding site is too small, then the compound will be unable to attach to the site; if the binding site is too large, then the compound will continue to move within the pocket, leading to weaker interactions. Most small molecules are usually under 1000 Da in size, and because of this, most binding sites are between 500–1000 Daltons (Da) to accommodate these compounds. It is estimated that 85% of proteins do not have binding sites, and it is important to first determine if the PDE4D protein has binding sites before determining location, sizes, and interactions.

Geometric Method Analysis of Binding Sites

The geometric method is used to determine the size of the binding sites that will fit a specific compound. By using this experiment, the binding sites can be located, and the interactions can be studied. Using the ProteinPlus site and DoGSiteScorer as a search engine for binding sites on the PDE4D protein, key quantitative values: volume, surface area, and the drug scores, were measured and recorded. As the geometric experiment analyzes the 3D structure of the binding site, these values are necessary in determining size. The protein was searched for using the PDB code, a unique 4-character alphanumeric identifier stored in the Protein Data Bank that references a specific protein and its 3D structure. A detailed image of all available binding sites based on size, depth, and drug score can be seen in Figure 2.

Figure 2. Geometric image of the PDE4D protein and available binding sites

The red and blue coils represent the PD-E4D protein, and all other color-coded 3D shapes represent the binding sites. Each binding site in a protein corresponds with values in size and success in binding with small molecules. This protein has 23 available binding sites for small molecules, ranging in size and shape. The binding sites are distributed and scattered across both chains; some are larger,

and some are smaller. Using the geometric method, the volume would characterize the size and depth of the binding sites, while the surface area would characterize its overall shape and available area to bind. The druggability score is also provided, a computational method that predicts the likelihood of a small molecule binding to the target protein. All values can be viewed below in Table 1.

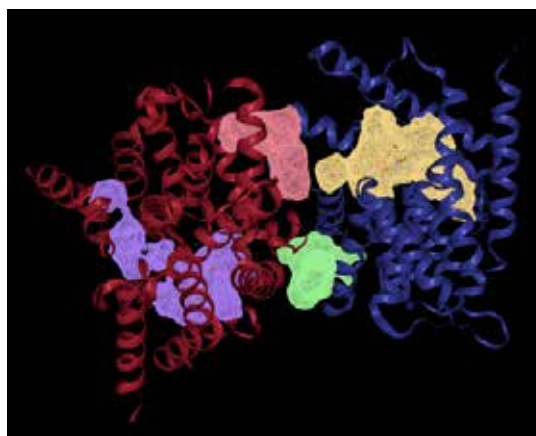
Table 1. Geometric data on 8 of 23 binding sites on the PDE4D Protein

Color	Name	Volume (Å ³)	Surface Area (Å ²)	Drug Score
	P_0	1183.88	1045.22	0.81
	P_1	1135.12	1036.62	0.81
	P_16	119.07	332.86	0.42
	P_17	118.54	246.43	0.43
	P_2	754.98	970.0	0.79
	P_3	659.81	813.43	0.76
	P_5	184.5	213.71	0.45
	P_9	168.14	383.07	0.4

It is known that the drug score ranges from 0 to 1. Scores above 0.5 are considered acceptable and indicate that the corresponding binding site on the protein is suitable and compatible for a small molecule. A higher drug score indicates there is a high structural probability that a small molecule can effectively bind to the target protein and perform its designated function. The PDE4D protein provides promising binding sites: P_0, P_1,

P_2, and P_3 all have drug scores from 0.76 to 0.81. The range of the drug scores is 0.59, with the lowest score being 0.22 and the highest score(s) being 0.81. The high values for volume and surface area, which correlate with high drug score values, will determine the success of the binding sites. Once selected, these specific, acceptable binding sites and their locations on the PDE4D protein can be viewed in Figure 3.

Figure 3. *Geometric image of the PDE4D protein and the most compatible binding sites*



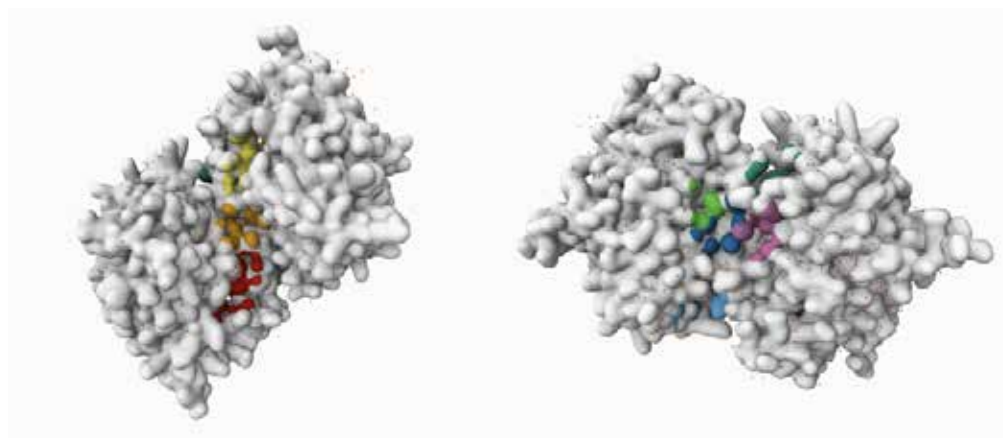
Experimenting through Machine Learning Methods

Cellular energetics play an important role in the binding of small molecules to these pockets. As the geometry, shape, and size of the binding sites have been analyzed, it is important to consider how the protein and small molecules interact energetically as well. Rather than analyzing both the geometry and energy individually and then comparing, it is

possible to combine them through machine learning methods, which account for both geometric and energetic properties.

The PrankWeb site used a structured method to analyze a 3D model of the protein and provided possible binding sites based on geometric structure and energy availability for small molecules. These binding sites can be viewed on the PDE4D protein in Figures 4 and 5.

Figure 4 and Figure 5. *PDE4D protein and its available binding sites based on both geometric and energetic properties*



All binding sites located in both Figures 4 and 5 indicate areas on the PDE4D protein where it is compatible with small molecules. Each color symbolizes a binding site that is compatible with a small molecule, indicating that the target protein can be bound to. The color-coded binding sites in Figure 4 represent the numbers ranked as 1, 2, and 3 in Table 2. The color-coded binding sites in Figure 5 represent the numbers ranked as 4, 5, 6, 7, and 8 in Table 2.

Discovering binding sites using the machine learning method would provide scores like the geometric method and the Protein-Plus tool, but on a much larger scale. Similarly, a higher score indicates a higher compatibility as a binding site. Each binding site will have a specific number of residues, or amino acids, that are available to interact with the small molecule. These values, along with information on the scores and number of residues are listed in Table 2.

Table 2. Binding sites ranked 1–8 with corresponding scores, number of residues (amino acids), and specific amino acids

Binding Site	Rank	Score	# of Residues	Amino acids
	1	73.93	37	Serine 123, Asparagine 124, Histidine 75
	2	73.61	35	Isoleucine 128, Methionine 188, Phenylalanine 287
	3	7.34	12	Leucine 184, Serine 142, Asparagine 146
	4	3.98	12	Arginine 176, Aspartic acid 162
	5	2.96	10	Leucine 136, Arginine 176
	6	1.50	8	Methionine 137, Alanine 150
	7	1.47	8	Lysine 154, Alanine 150
	8	0.85	5	Leucine 134, Histidine 147

A higher score corresponded with a higher rank and number of amino acids. Specific amino acids were selected to demonstrate some components of the binding site that would interact energetically with the small molecule.

In general, scores above 5 are acceptable, with high values indicating that the binding sites of the proteins are compatible with the small molecules. Both binding sites 1 and 2, as ranked in Table 2, had scores of 73.93 and 73.61. The third binding site may also be considered, as it has a score of 7.34, but is unnecessary compared to the high values of 1 and 2. Most binding sites have between 10 and 20 amino acids; however, the first binding site has 37 amino acids, and the second binding site has 35 amino acids. Values well above the average range indicate that these binding sites are larger than the average. This information indicates that there are many subareas and sub pockets for the small molecules to bind to, increasing both their compatibility and the corresponding score.

Virtual Screening

The 2 methods of screening for compounds include physical screening and virtual screening. The purpose of screening for proteins is to narrow down the available compounds from millions to 10–20. Physical screening requires the target protein

and small molecules in large quantities and takes time to test the protein with different compounds to determine which compounds are most compatible with the binding sites. Virtual screening can screen for millions of compounds through online databases in a shorter time, providing accessible details on the compounds and their compatibilities. Because binding sites have already been located and their sizes and interactions have been recorded from the first experiments, it is possible to move on with virtual screening to determine specific compounds that will bind with the proteins.

Experimenting with Pharmacophore Maps

Pharmacophore maps are 3D maps of the interactions between the target protein and the binding compound. The components of a pharmacophore map consist of the number of interactions that occur, at which points, the relative distance between the interactions, and the types of interactions of the amino acids. Types of interactions include polar, non-polar, hydrophilic, and hydrophobic. By manipulating which interactions are occurring, it is possible to screen for compounds based on these selections.

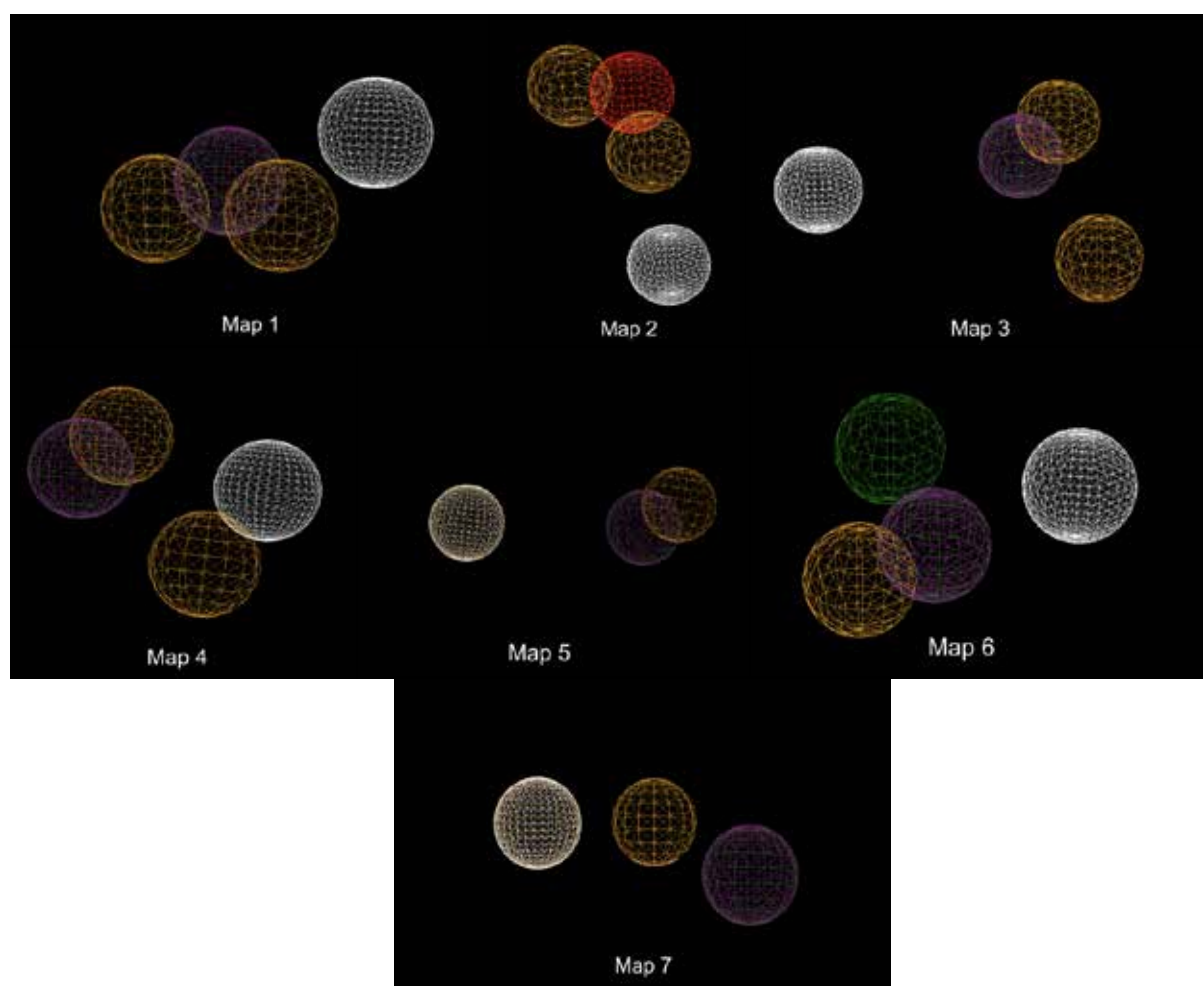
The RMSD (Root Mean Square Deviation) is a numerical value that measures the difference between a model's predicted values and

the actual values. In the pharmacophore experiments, an RMSD below 0.2 was the target, with values below 0.1 being ideal. A lower RMSD indicates that the overlay between the map and the compound is closer to perfect, with a score of 0 indicating no deviation at all.

The interactions selected were based on the conditions of number, relative distance, and type. In the pharmacophore map, each interaction was represented by a sphere, and its color represented the type of interaction. A lower number of interactions was ideal, as too many interactions would provide compounds with an RMSD above 0.2, as it would be difficult to find a compound

that could perform all interactions precisely. The distances between interactions were minimized as much as possible, with some interactions overlaying one another, and the diversity of interactions was also selected for. Different types of interactions are more accurate for natural compounds, as compounds do not interact uniformly. Interactions were selected based on these components, and compounds that matched these interactions were searched for from servers including MolPort, Enamine, etc. These results can be viewed in Table 3, which includes the name, RMSD, pharmacophore map, and compound.

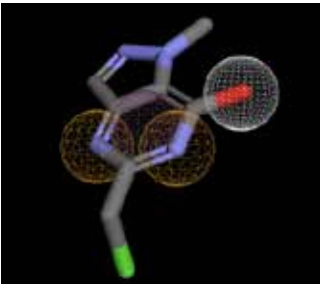
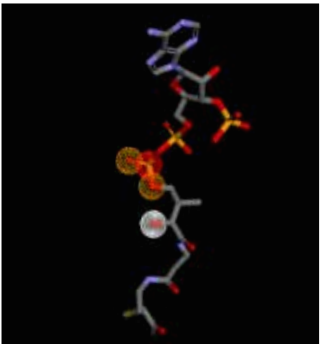
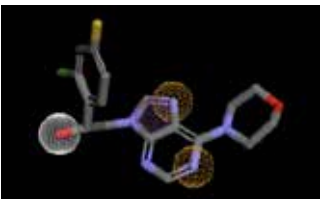
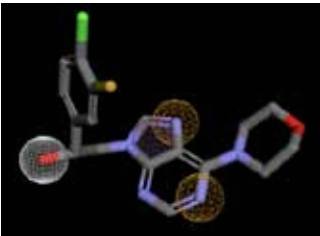
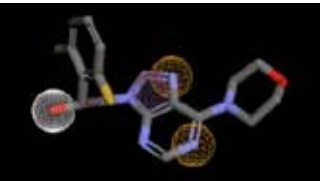
Figure 6. *Pharmacophore maps of interactions between compounds and the PDE4D protein*

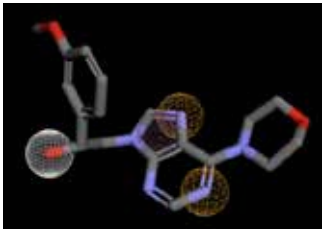
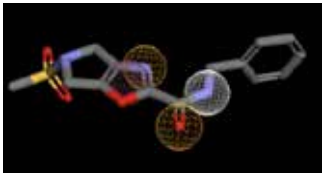
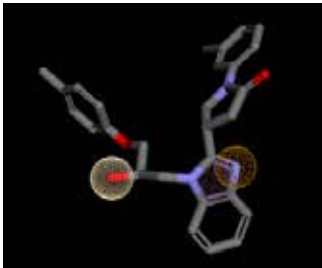
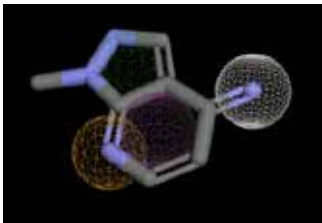
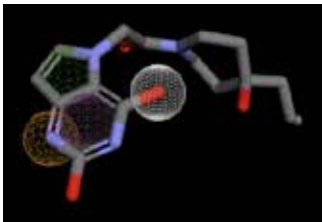
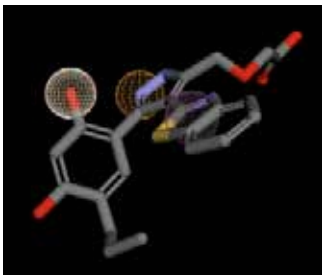


Each map below corresponds with specific compounds listed below in Table 3. The spheres indicate the interactions, and the colors indicate the type of interaction. Each map contains around 3–5 interactions. Yellow spheres represent hydrogen acceptor in-

teractions, red spheres represent anion interactions, purple spheres represent aromatic interactions, white spheres represent hydrogen donor interactions, and green spheres (overlaid with purple) represent hydrophobic interactions.

Table 3. *Compounds and values obtained from the pharmacophore map of interactions between molecules and proteins.*

Name		RMSD	Pharmacophore Map & Compound
Map 1	MolPort-046–855–508	0.024	
	MolPort-053–297–972	0.028	
Map 2	MolPort-053–223–721	0.041	
Map 3	Z1729094043	0.024	
	Z1675621302	0.025	
	Z1334538364	0.025	

Name		RMSD	Pharmacophore Map & Compound
	Z1345952503	0.025	
Map 4	CHEMBL3084286	0.042	
	CHEMBL3441070	0.042	
Map 5	MolPort-010–705–550	0.001	
Map 6	Z1901071406	0.024	
	Z6289672314	0.027	
Map 7	MolPort-000–479–672	0.012	

Multiple combinations were experimented with through the pharmacophore maps. Some compounds provided promising RMSD values of 0.001, while the highest value was 0.041. The compounds MolPort-010–705–550, CSSS00160257030, and CSC027798714 had the lowest RMSD values of 0.001 and 0.002, compared to all other compounds, which had RMSD values between 0.01 and 0.05. The pharmacophore maps with overlapping interactions of hydrogen donors and hydrogen acceptors (Maps 5 and 7) provided the lowest values, while the maps including anion interactions and multiple hydrogen acceptor interactions in proximity revealed higher RMSD values.

The experimentation with virtual screening revealed that there were many available compounds with acceptable RMSD values. All scores were below the maximum value of 0.2; values below 0.1 are considered exceptional, indicating that all compounds are candidates for further experimentation

Molecular Docking Experimenting

Molecular Docking is a computational tool used to determine how the binder interacts with the target protein. The protein continues to rotate until the compound can dock on any recognized binding site on the surface, and the energetic interaction between the two is recorded.

Using 3D modeling, molecular docking predicts all possible angles and areas where the ligand can bind to the protein, and is represented by ΔG , or Gibbs Free Energy, a numerical value used to quantify the energy of the interaction.

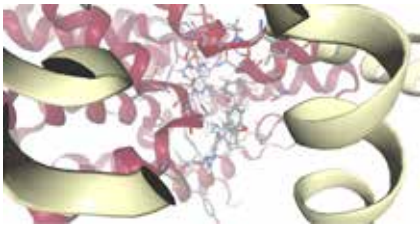
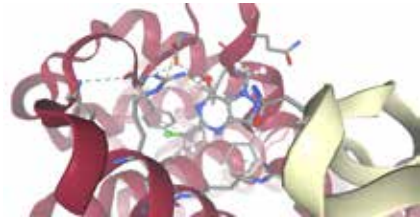
SwissDock

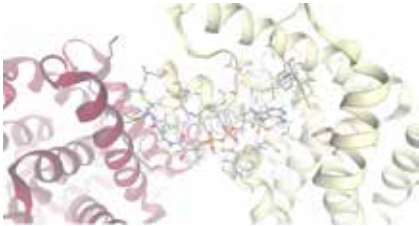
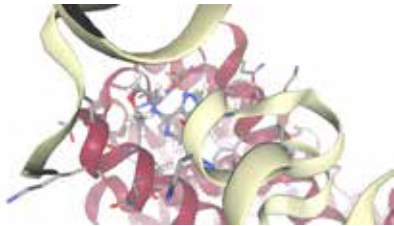
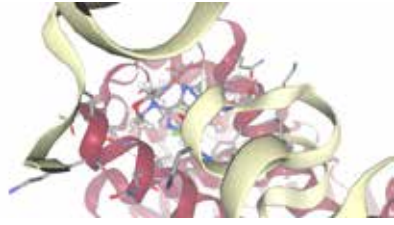
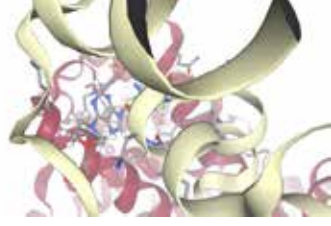
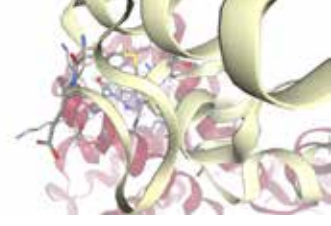
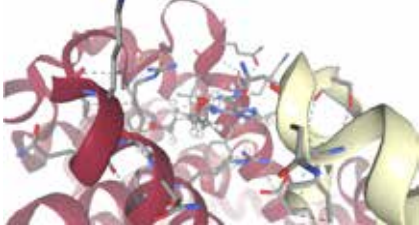
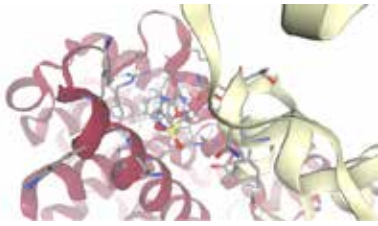
SwissDock, the tool used in this experiment, was used to analyze the 15 compounds obtained from the pharmacophore maps and virtual screening. Each compound corresponded with a ΔG value and an image of interactions with the protein.

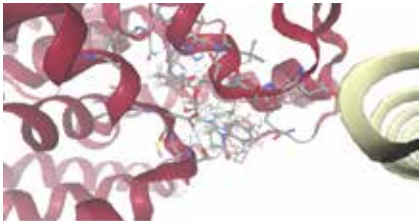
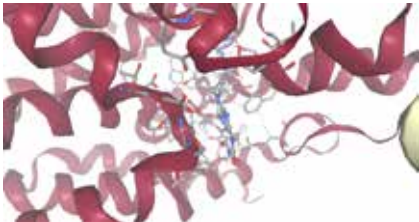
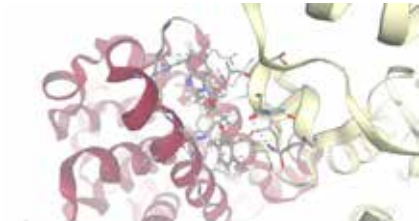
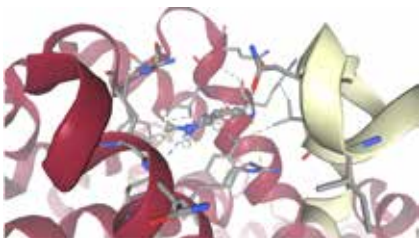
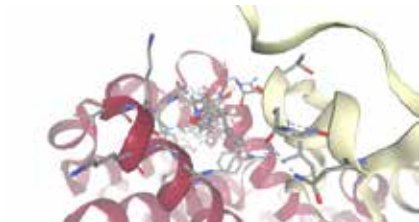
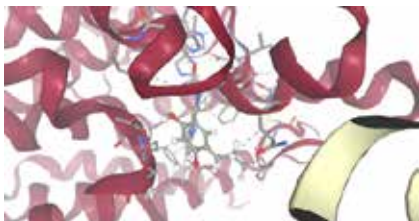
ΔG values are used to measure the thermodynamic favorability of chemical reactions and physical processes to determine if the reaction is spontaneous. A negative ΔG value, or $\Delta G < 0$, indicates that the reaction is spontaneous and will proceed, while a positive ΔG value, or $\Delta G > 0$, indicates the reaction is not spontaneous and will not proceed. All ΔG values for each compound were negative and were collected across all experiments. The lowest ΔG values from each compound can be viewed in Table 4.

Most of the binding sites were in the center of the protein, as discovered through experimentation using machine learning methods and Prankweb. Because of this, the search space was focused on the center of the protein, and all compounds tested would be included in these parameters.

Table 4. *SwissDock experimentation with select compounds and interactions with the PDE4D protein.*

Compound	ΔG value (kcal/mol)/ SwissParam Score	3D interaction pose of the compounds with PDE4D
MolPort-046–855–508	-10.4409	
MolPort-053–297–972	-6.4946	

Compound	ΔG value (kcal/mol)/ SwissParam Score	3D interaction pose of the compounds with PDE4D
MolPort-053–223–721	-10.3763	
Z1729094043	-7.9418	
Z1675621302	-7.7547	
Z1334538364	-7.7980	
Z1345952503	-7.6475	
CHEMBL3084286	-7.5887	
CHEMBL3441070	-7.5722	

Compound	ΔG value (kcal/mol)/ SwissParam Score	3D interaction pose of the compounds with PDE4D
MolPort-010–705–550	-7.9100	
CSSS00160257030	-8.3064	
CSC027798714	-8.2924	
Z1901071406	-6.2654	
Z6289672314	-7.5757	
MolPort-000–479–672	-8.1858	

Each compound and its respective ΔG value and images are listed. The lowest ΔG value was taken from each cluster, or binding site, and was recorded. Each image shows the compound location and interaction with the target protein, represented by the color-coded dashed lines. Blue represents hydrogen

bonding, yellow represents ionic interactions, orange represents cation- π interactions, gray represents hydrophobic contacts, and green represents π -stacking interactions.

The ΔG values can be classified into 3 categories: strong, moderate, and weak. A ΔG value is considered strong when it is negative

and of high magnitude. Similarly, a moderate ΔG value would have a moderate magnitude, and a weak ΔG value would have a low magnitude and value. Any ΔG value above 0 would not be considered, as it would imply that the interaction is unfavorable and not spontaneous.

Using this information on ΔG , the compounds with the strongest negative values were MolPort-046–855–508, MolPort-053–223–721, CSSS00160257030, Z1901071406, and MolPort-000–479–672. As noticed with the pharmacophore maps, most of the compounds with the lowest RMSD scores also corresponded with the strongest ΔG values. However, the strongest ΔG values were viewed from the first and third compounds, MolPort-046–855–508 and MolPort-053–223–721, which had ΔG values of -10.4409 and -10.3763 (Pharmacophore Map 1).

All ligands interacted with the protein target, as viewed through the dashed lines. Most interactions were hydrogen bonding or hydrophobic contact, with occasional ionic interactions, cation- π interactions, and π -stacking interactions. This corresponds with the results of the pharmacophore maps: the maps with the lowest RMSD scores had hydrogen donor and hydrogen acceptor interactions, as well as overlapping aromatic and hydrophobic interactions. The correlation between screening of the compounds in pharmacophore maps and their interactions with the target protein indicates that the interaction type dictates lower RMSD scores and ΔG values.

All compounds are exceptional candidates, as all ΔG values are negative values with large magnitudes. The best 8 compounds out of these 15 were selected for further testing and experimentation: MolPort-046–855–508, MolPort-053–223–721, Z1729094043, Z1334538364, MolPort-010–705–550, CSSS00160257030, Z1901071406, and MolPort-000–479–672, were selected.

Drug Treatments using ADME

SwissADME is a computational tool used in pharmacokinetics to analyze the chemical properties of a designated drug and determine whether it can function adequately

ly in the human body. Pharmacokinetics is the study of how the body interacts with the administered drug. SwissADME evaluates compounds and drug candidates and how the interaction between the molecule and the human body is affected. The acronym ADME stands for Absorption, Distribution, Metabolism, and Excretion. These are the 4 key stages a drug must go through to be processed by the body, determining its effectiveness and how long it remains in the body. Absorption, the first stage, occurs when the drug is absorbed into the bloodstream through the stomach. The drug is circulated by the bloodstream until it reaches the target protein. Both absorption and distribution occur before the molecule binds to its target. If either step does not occur, or does not occur efficiently, the drug is unable to reach its target and perform its function. The next stages, metabolism and excretion, occur after the compound has bound to the protein and ensure that the drug remains in the body for the necessary amount of time. If the drug remains in the body for an extended period, its effects may become toxic. If the drug does not remain in the body long enough, it cannot treat the protein to its fullest extent. Metabolizing enzymes detect the drug and decompose it into small units, which are then excreted from the body.

Experimenting through SwissADME using Lipinski's Rule

The ADME process is critical in determining how the drug moves through the body, how it is processed, and how it is excreted. Through SwissADME, values are obtained and analyzed according to Lipinski's Rule, a set of criteria in pharmacology that evaluates whether a drug has acceptable absorption and permeation. These criteria include fewer than 5 hydrogen bond (H-bond) donors and 10 hydrogen bond acceptors, a molecular mass less than 500 Daltons (Da), and a calculated LogP value below 5. A molecule larger than 500 Da would be ineffective, as it would take too long to bind to a protein and be processed, leading to the drug remaining longer in the body, while a molecule too small will be unable to bind to the protein or be processed more quickly. The ideal time for a drug to remain in the body is around 8–10 hours, with an accepted range of 2–10 hours.

The calculated LogP, or log of Partitioning, measures how well a drug dissolves in fat compared to water. A high LogP indicates that it is more likely to dissolve in fat, while a lower LogP indicates that it is more likely to dissolve in water.

All criteria of Lipinski's rule must be met for the compound to qualify as a drug treat-

ment. Any violation would indicate that the drug has an unfavorable ADME profile and would not be compatible with the human body. Approximately 80–90% of compounds fail to meet the guidelines of ADME and Lipinski's Rule and are therefore eliminated as candidates.

Table 5. Analysis of compounds and values through SwissADME testing for violations of Lipinski's Rule

Compound	Molecular Weight (g/mol)	Number of H-bond acceptors	Number of H-bond donors	(Consensus) LogP	Violations of Lipinski's Rule	Solubility Classification	Bioavailability Score
MolPort-046-855-508	826.95	10	3	5.09	Disagree; 2 violations	Poorly soluble	0.17
MolPort-053-223-721	823.60	20	9	-4.24	Disagree; 3 violations	Very soluble	0.11
Z1729094043	377.80	6	1	1.40	Agree; 0 violation	Soluble	0.55
Z1334538364	361.35	7	1	1.16	Agree; 0 violation	Soluble	0.55
MolPort-010-705-550	455.55	4	1	3.70	Agree; 0 violation	Moderately soluble	0.55
CSSS00160257030	636.13	5	2	2.48	Disagree; 1 violation	Poorly soluble	0.55
CSC027798714	416.51	4	1	4.33	Agree; 0 violation	Moderately soluble	0.55
Z1901071406	148.17	2	2	0.36	Agree; 0 violation	Soluble	0.55
MolPort-000-479-672	439.48	7	4	3.71	Agree; 0 violation	Moderately soluble	0.11

Information such as the classification of solubility and bioavailability score were also included to provide further data on whether the drug would be processed efficiently through the body. A drug's solubility determines how likely it is to dissolve in liquid, and the bioavailability score measures what percent of the substance successfully reaches the bloodstream and can bind to the target protein. Not all drugs can reach the target, as they can bind to the plasma or are unable to reach the protein. High levels in both solubil-

ity and bioavailability are optimal. Although they have no impact on the outcome of Lipinski's rule, the data adds confirmation or rejection of the compounds.

The compounds MolPort-046–855–508, MolPort-053–223–721, and CSSS00160257030 violated at least one of Lipinski's rules. All three of these compounds were eliminated.

Trends observed throughout the data were that higher classifications of solubility (soluble, very soluble, etc.) corresponded

with lower LogP values. In addition, the lower the number of H-bond donors and acceptors, the lower the molecular weight. It was also observed that almost all selected compounds that met Lipinski's Rule requirements had a bioavailability score of 0.55, with the only exception being MolPort-000-479-672. Throughout the data, it has been observed that compounds obtained from Enamine have been the most reliable through all experiments.

The remaining 6 compounds (Z1729094043, Z1334538364, MolPort-010-705-550, CSC027798714, Z1901071406, and MolPort-000-479-672) met all the criteria. Z1334538364 had the lowest LogP value and molecular weight, and both MolPort-010-705-550 and CSC027798714 had the lowest number of H-bond donors and acceptors. All compounds ranged from moderately soluble to soluble and had high bioavailability scores except MolPort-000-479-672, which had a lower score and the highest number of H-bond donors and acceptors. Z1901071406 had the lowest molecular weight out of all compounds tested. These 5 compounds meet the guidelines of ADME and Lipinski's Rule, providing promising candidates for drug treatment.

Predicting Toxicity of Compounds in the Human Body

Pharmacodynamics is the study of how drugs impact the human body through the examination of chemical and molecular mechanisms of drug action, binding with a target protein. All foreign substances entering the human body contain some level of toxicity, in which a certain amount can cease human functioning and bodily processes. The 3 main types of toxicity include: acute toxicity, organ toxicity, and impacted proteins, pathways, and endpoints. Acute toxicity refers to short-term toxicity effects, typically lasting from a few minutes to a few days after the drug has been taken. Organ toxicity refers to the accumulation of the drug in the organs over time, which can range from months to years after the drug is taken, or if the drug is taken repeatedly over a period. Once a lethal amount has accumulated in the body, possible effects include organ damage and death. Both the liver and the kidney are the most susceptible to harmful drug effects, as they manage the

ADME metabolic stages and filter toxins from the body for excretion. The proteins that the drug can bind to, the pathways that it travels to reach the target or to be excreted, and the endpoints from where they are excreted can all be affected by the movement of the drug through the body. All these categories must be considered when screening for a drug and its toxic side effects on the human body.

ProTox 3.0 Experimenting and Toxicity Levels

To determine whether the compound or drug is toxic or not, its toxicity levels can be predicted through its LD50, or Lethal Dose 50%. LD50 is measured through acute toxicity and refers to the amount of substance required to kill 50% of the tested population and is measured in mg/kg. A lower LD50 would indicate that the drug is more toxic, as it would take a smaller amount of the drug to kill an organism. On the other hand, a higher LD50 would be optimal and indicate the drug is less toxic, leading to a more flexible range for doses. The ProTox 3.0 tool predicts the LD50 for the entered compound, as well as the Toxicity class (measured on a scale from 1–6), which serves to validate the LD50. The 6 compounds obtained from the SwissADME experiments, and their values and images are displayed below in Table 6.

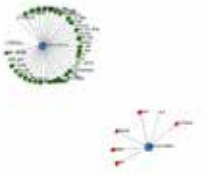
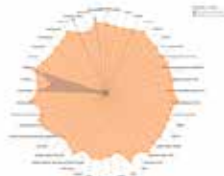
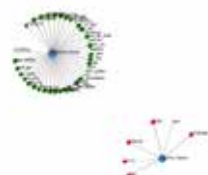
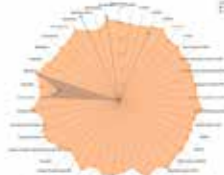
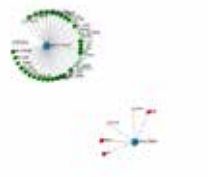
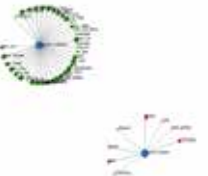
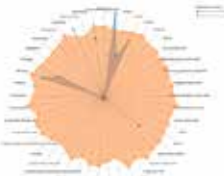
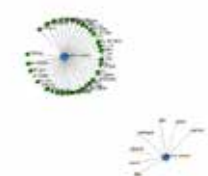
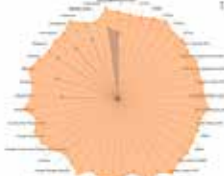
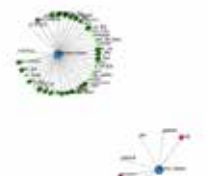
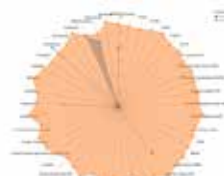
All compounds selected were based on the SwissParam scores, or ΔG values from the SwissDock experiments. Because these values determine how likely the interaction between the compound and protein is to happen, the compounds with the better SwissParam scores, and meeting the criteria for LD50 and having low toxicity, would be selected as the main compounds. The second and third best, nontoxic compounds with better SwissParam scores would be selected as backups for future testing, in the case that the lead compound fails experiments in the future.

Any other values below an LD50 of 300 mg/kg would be considered toxic and therefore eliminated as a possible drug treatment. Therefore, compounds Z1729094043 and Z1334538364 were eliminated, as they had an LD50 of 130 mg/kg, well below the acceptable value. All other compounds had an LD50 ranging from 1000 to 1500, indicating that they were less toxic to the human body. The toxicity class value also corresponds with

the LD50 and would assist in validating the results. A value from 1–2 would be the most toxic, and a value from 3–6 would be less toxic and safer for the human body. Both com-

pounds mentioned above had a value of 3. This would not be considered very toxic but is on the lower end of the acceptable values on the scale.

Table 6. LD50, toxicity class, cluster, and radar charts obtained from analysis of compounds through ProTox 3.0

Compound	LD50 (mg/kg)	Toxicity Class (1–6)	Inactive and Active Network Chart	Average toxicity Radar Chart
Z1729094043	130 mg/kg	3		
Z1334538364	130 mg/kg	3		
MolPort-010-705-550	1000 mg/kg	4		
CSC027798714	1000 mg/kg	4		
Z1901071406	1000 mg/kg	4		
MolPort-000-479-672	1500 mg/kg	4		

The active and inactive clusters charts demonstrate which elements of toxicity are most impacted by the drug. If the model is active, it indicates that the compound is toxic to that specific part of the body, and if the model is inactive, the drug is not toxic. Some vital organs, pathways, proteins, and endpoints are more crucial to human functioning than others. If these areas are impacted too much by the toxicity of the drug, then the compound would be too lethal for human consumption. Each compound had around 5–8 active elements in each chart, with the most active elements being “clinical”, appearing in all active clusters, as well as “bbb” (Blood Brain Barrier) and “neuro” appearing frequently in most active charts.

The active elements can be further analyzed through the Toxicity Radar Chart. The orange dots are the standard FDA-approved drugs and their toxicity levels: if 2 or more blue dots exceed the orange line, then the compound would be considered too toxic and would be excluded from the final data. If only one blue dot exceeds the orange area, it is still acceptable, as it may be considered a limitation of the tool. All toxicity radar charts for compounds Z1729094043, Z1334538364, MolPort-010–705–550, and CSC027798714 had 2 or more points exceeding the orange line and were therefore eliminated from the data.

Only compounds Z1901071406 and MolPort-000–479–672 met all criteria: having an LD50 above 300 mg/kg, a toxicity class in the acceptable range, and a radar chart with 1 or fewer blue dots exceeding the orange dots. MolPort-000–479–672 was selected as the lead compound, as it had the highest LD50, with compounds Z190107140 and MolPort-010–705–550 being selected as backups.

Conclusion

PDE4D inhibition elevates cAMP levels, thereby improving learning, memory, and cognition. The discovery of compounds and molecules that can inhibit the PDE4D enzyme holds potential as future drug candidates for treatment, especially targeted

towards dementia and other neurological diseases. By inhibiting the hydrolysis of cAMP, PDE4D inhibitors enhance neural signaling and memory improvement. It is important to consider the pharmacokinetics and pharmacodynamics between the compound and the human body, ensuring the drug can perform its designated function without harming the body over time. All these requirements were considered throughout the study and were used to determine a lead compound and 2 compounds that could progress towards future physical screening.

Using geometric and energetic methods, the computational tools DoGSiteScorer, Protein Plus, and Pharmit were used to determine available binding sites on the protein based on their volumes, surface area, drug scores, pharmacophore interactions, and RMSD scores. By selecting certain interactions, compounds were compiled through online databases including MolPort, Enamine, and ChemSpace. 15 compounds were selected for further experimentation to quantify the energy of each interaction through SwissDock. These compounds were analyzed through SwissADME to determine if they met the guidelines of ADME and Lipinski's Rule, with only 6 compounds selected. By determining the LD50 of each compound, active and inactive clusters, 3 final compounds were selected. These compounds, having met all criteria of all experiments, are most promising as drug treatments for inhibiting PDE4D enzymes.

Limitations of this study occurred in the pharmacophore maps and ProTox 3.0 analysis of toxicity levels. In the pharmacophore maps, available interactions between the proteins and compounds were limited and overlapping, leading to slightly higher RMSD scores and less reliable compounds. In ProTox 3.0, the average similarity and prediction accuracy percentages compared the compound to the server's database, leading to similar LD50 values and toxicity classes across compounds. To ease these limitations, a higher number of compounds could be tested in the future to discover ones with high accuracy.

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