Original Research Article

Leveraging Computational Tools to Enhance Small-Molecule Drug Design for Cystic Fibrosis and Beyond

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ABSTRACT

Small-molecule drugs, organic compounds that interact with proteins and enzymes, represent a powerful approach for treating genetic diseases. These drugs are designed to alter specific molecular disease targets and do so with reduced off-target effects. Furthermore, these drugs offer ease in consumption through oral delivery. New technology, especially computational tools, have enabled the enhanced modeling of design of small-molecule drugs. These advancements are particularly effective in the treatment of genetic disease, where targeting mutated proteins can restore function and reduce disease progression. This project uses SwissDock molecular docking to assess how modifying chemical structures of the cystic fibrosis small-molecular drug Ivacaftor can enhance the drug's interaction with the mutated CFTR protein. Among the 4 modified Ivacaftor analogs tested alongside the unmodulated structure, hydroxyl-group-deleted Ivacaftor was shown to have the lowest average SwissParam score and thus the highest binding affinity to the mutated CFTR protein. The broader implications of this work highlight how structure-based drug design can lead to the development of more effective treatments for genetic disease and be applied for the treatment of other conditions, ultimately expanding the scope of precision medicine.

Keywords: Small-molecule drug design; computational tools; cystic fibrosis; Ivacaftor; SwissDock

INTRODUCTION

Small-molecule drugs account for over 90% of all approved therapeutics and remain the cornerstone of modern medicine due to their stability, ease of storage, straightforward consumption protocols, and versatility. As low-molecular-weight synthetic chemicals, these drugs are designed to affect organic processes inside the body (1). A common method in which small-molecule drugs affect biological processes is through forming complexes with target proteins, altering their interaction with other compounds in the body (2). Thus, molecular structure analysis is essential in the development of such drugs, as the structure of a molecule fundamentally determines its interaction with biological targets, significantly impacting its therapeutic potential (3). In processes such as structure-based virtual screening, 3D structural information of the target and candidate molecules are crucial to conduct molecular docking and scoring, laying the foundation for further experimental tests (4).

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In the field of small-molecule drugs, computational methods are currently marking a paradigm shift in the process of drug development. Traditional methods of drug discovery show a 90% failure rate in clinical trials, most likely due to the lack of predictability when solely utilizing in vitro models (5). The use of computational methods such as machine learning and AI can significantly reduce such problems, as models trained to predict structure-based molecular properties can inform critical decisions in selecting and testing molecular drug candidates (6, 7). Existing algorithms that predict ligand-receptor interactions have been shown to increase hit rates in virtual screening as well, proving their potential in aiding small-molecule drug design (8).

In this research, we examine the process of designing and optimizing small-molecule drugs through computational modeling of molecular structures, providing insight into the modern development of structure-based drug design. Specifically, we conduct original molecular docking of the small-molecule drug Ivacaftor, a treatment for cystic fibrosis, a genetic disorder characterized by a mutated, dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) protein to assess the binding affinity of its chemical modulations to the mutated CFTR protein (9). Through such computational modeling, we investigated the potential of further optimization of the drug. Such information conveys the importance of structural analysis in more efficient small-molecule drug design, enabling the treatment of otherwise intractable diseases.

LITERATURE REVIEW

Mechanism of Small-molecule Drugs

Small-molecule drugs interact with target proteins in several different ways. The three most common roles they take on are enzyme inhibitors, receptor agonists or antagonists, or ion channel modulators (1).

Enzyme Inhibitors

Enzymes are specialized proteins that act as catalysts of biochemical reactions through binding to a substrate molecule, in which differentiated chemical properties enable binding to target substrates (10). Due to their important role in metabolic pathways, mutated enzymes are often the cause of diseases, especially genetic disorders. Enzymes with genetic mutations can be dysfunctional, alter signaling pathways, and cause a range of other bodily symptoms (11). Enzyme inhibitors can block such abnormal enzyme function through binding to the enzyme, enzyme-substrate complex, or both structures. This binding inhibits enzyme-facilitated metabolism, and thus prevents symptoms from appearing (12).

Aspirin, a drug commonly utilized for its anti-inflammatory, analgesic, and antipyretic properties, is one of the most widely used small-molecule drugs on-market (13). Aspirin is an enzyme inhibitor that specifically binds to cyclooxygenase-2 (COX-2), an enzyme that produces prostaglandins (PGs) that cause inflammation, pain, and fever (14). When introduced in the body, aspirin binds to the COX-2 enzyme covalently, forming an irreversible modification. This binding occurs at Ser530, a region near the enzyme's active site, therefore prevents the binding between COX-2 and its natural substrate, blocking the metabolic pathway that causes target symptoms (15).

Receptor Agonists and Antagonists

Receptors are specialized proteins that detect and pass on chemical messages in and among cells. Cellular responses are initiated as ligands with matching chemical structure bind to their binding sites. While agonists are ligands that initiate cellular response, antagonists do not cause any cellular response when bound to the receptor (16). These ligands are crucial in regulating numerous body functions, which include but are not limited to growth, metabolism, and reproduction (17). As a result, small-molecule drugs that mimic the structure of ligands can target a wide range of diseases, including diabetes, cardiovascular disorders, neurodegenerative diseases, and inflammatory conditions (18, 19).

An example of a receptor agonist small-molecule drug is Orforglipron, which is typically used to treat type-2 diabetes and obesity. Specifically belonging to a group of GLP-1 receptor agonists, the drug binds to GLP-1 receptors that increase glucose uptake by the muscles and decrease glucose production in the liver once the hormone GLP-1 binds to it (20, 21). By binding to the same receptor site as native GLP-1, the drug initiates the same process of glucose level regulation through initiating a signaling cascade involving increased cAMP production, protein kinase activation, and resulting in increased insulin release and inhibited glucagon secretion. Such a process ultimately aids in lowering blood glucose levels (22, 23).

Ion Channel Modulators

Ion channels are proteins embedded in the cell membrane that form pathways for ions to flow through

(24). By controlling the concentration of ions, these channels set the basis for processes such as neural signal transmission, muscle contraction, insulin secretion, and more (25). When abnormal ion channels are formed, such bodily functions are disrupted due to the gain or loss of ion currents, leading to diseases classified as channelopathies (26). Small-molecule drugs can treat channelopathies through the inhibition or modulation of ion currents once bound, altering them to a normal level (27, 28).

Diazepam is a widely used ion channel modulator drug that targets the GABA-A receptor chloride ion channels, which are crucial in the proper function of ganglia networks (29, 30). Facilitating rapid inhibitory signaling across the central nervous system, these ion channels are activated through the binding of the gamma-aminobutyric acid (GABA). Once activated, negatively charged chloride ions flow through, creating hyperpolarization and thus lowering the membrane potential (31). The absence of GABA and the consequential dysfunction of GABA-A receptor ion channels, therefore, can lead to lack of inhibition, resulting in hyperexcitability and the overactivity of seizures (32, 33). In such cases, Diazepam binds to an allosteric site between subunits of the ion channel, increasing the frequency of the chloride flows instead of GABA (29).

Computational Tools in Small-molecule Drug Development

Different mechanisms of small-molecule drugs still require accurate analysis of chemical structures of both the target protein and the drug candidate. Computational tools have become integral to modern drug design because they enable scientists to model and predict molecular interactions before conducting laboratory experiments. These tools accelerate drug development processes, reduce cost, and increase clinical trial success rates from approximately 40-65% to 80-90% (34, 35).

In traditional approaches to small-molecule drug development, an extensive compound library (possibly exceeding 600,000 compounds) with diverse chemical structures are tested experimentally to determine binding capacity to a given target protein (1, 36). High-throughput screening (HTS) is commonly utilized in this process, which rapidly identifies compounds that result in active interaction with the target region (37). However, hit rates with HTS are typically low, ranging around 1% or lower (1). Additionally, HTS fundamentally has the risk of presenting false positive results as in vitro experiments cannot fully reflect the biological activity of compounds in living systems (38).

Computational tools complement HTS by prioritizing promising active compounds for experimental validation and clinical development. By utilizing data including substructures, common chemical features, or historical HTS data through techniques such as machine learning, these tools can predict binding likelihood and eliminate false positives, thereby improving efficiency and accuracy in drug discovery (38, 39). Two examples of such computational tools that are both accessible and widely used in structure-based drug design are SwissDock and AlphaFold. These programs enable researchers to model drug-protein interactions, predict molecular binding sites, and optimize drug candidates prior to synthesis.

SwissDock

SwissDock is a public computational tool that is part of the SwissDrugDesign Project, providing small-molecule docking results preceded by automatized ligands and target preparations. The current service provides two separate mechanisms for docking: Attracting Cavities (AC) that provides higher accuracy and AutoDock Vina that offers faster docking speed (40).

The AC algorithm identifies cavities within the protein's 3D structure and evaluates the optimal orientation of the ligand based on geometric fit and non-covalent forces such as hydrogen bonds, van der Waals interactions, and electrostatic attraction. This is accomplished by generating cloud points on the protein structure and rotating the ligand at various angles to consider the optimal geometrical positioning in the cavity when bonded. Here, cloud points are abstract points used in computation that represent the target protein's structure to simplify the optimization of the ligand position. Once the relative position of the ligand is optimized, the whole target molecule is reintroduced to finalize the positioning, ensuring maximum bond stability and equilibrium (40). Close analysis of ligand positioning is divided into two steps: the non-covalent intermolecular forces that act upon the ligand and target are first considered, followed by the prediction of a chemical reaction that leads to covalent bond formation (41).

AutoDock Vina, on the other hand, utilizes a specific Vina scoring function that approximates the binding affinity of the ligand and protein through intermolecular and intramolecular forces involved in the docking (42). More specifically, the 2024 version of the service is known to account for van der Waals interactions, hydrogen bonds, hydrophobic factors, and

conformational entropies. Such information is processed through trilinear interpolation, a computational technique that approximates data points in a three-dimensional space, then once again uses the whole target structure to finalize optimal docking poses (40, 43).

AlphaFold

Apart from molecular docking, protein structure prediction also greatly benefits from computational tools. Before the emergence of computational systems, tertiary protein structures were largely determined through experimental techniques including X-ray crystallography, nuclear magnetic resonance, and electron cryomicroscopy. Such methods made protein structure determination complex and often inaccurate. As a result, only approximately 0.1% of known protein sequences entered in UniProtKB have a resolved tertiary structure as of January 2023 (44).

AlphaFold is an artificial neural network-based model that greatly assists in the prediction of protein structures. Predictions are drawn by incorporating artificial intelligence trained with evolutionary, physical, and geometric factors that determine protein structure. Specifically, it firstly utilizes multiple sequence alignments (MSAs) to predict the most likely structure according to the given amino acid sequence, referring to existing data in the Protein Data Bank (PDB). After initial construction, the structure is refined to satisfy previously inputted factors that affect protein structure formation (45). In addition, AlphaFold also offers protein-ligand interaction predictions, allowing researchers to model drug binding without prior experimental structures. The tool solely requires input of the protein sequence and the SMILES ligand code, which is a line representation of the ligand's chemical structure (46). Such input information is then run through a trained model to determine polymer structure and the appropriate interactions (47). As a result, it is advantageous in real-world applications where pre-existing test results are rare.

Cystic Fibrosis, the CFTR Protein, and Ivacaftor

Cystic Fibrosis and the CFTR Protein

Cystic fibrosis is a chronic genetic disorder, typically characterized by chronic lung infections, pancreatic insufficiency, sinusitis, and infertility, among other symptoms. It is a recessive autosomal disorder caused by a mutation in the *Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)* gene, with over 2,000 disease-causing variations (48). The gene is responsible for encoding the CFTR protein, otherwise called the cAMP-dependent chloride channel (49). This ion channel protein is primarily activated by the phosphorylation of its R domain by protein kinase A (PKA), which initiates ATP binding that opens the pores (50). As chloride ions flow through the channel, causing absorption of ions into the cell and diffusion out of the cell, the resulting osmotic gradient allows passive water secretion in the form of sweat and moisture. A key step to this process is the reabsorption of chloride ions into the cell, which creates an appropriate gradient for secretion. Mutated CFTR proteins with a different structure, however, cannot initiate the normal flow of chloride ions, leading to an abnormally high concentration of chloride ions outside the cells. Such depolarization of the apical membrane potential ultimately leads to less osmotic action. As a result, organs such as lungs lack the fluid for proper hydration, and the patient experiences common symptoms such as recurrent lung infections, pancreatitis, congested sinuses, and reproductive challenges (48, 51).

Ivacaftor and Its Mechanisms

Ivacaftor is a small-molecule drug known to treat cystic fibrosis. As an ion channel modulator, the drug directly enhances ion channel gating and restores CFTR protein function, thereby reducing symptoms (52). In particular, as a channel potentiator, Ivacaftor binds directly to the ion channel on two identified sites: one is located in the cytosolic loop 4 of CFTR, while the other is located in the hinge-like structure in tm8, or the eighth-helical segment of the second membrane-spanning domain (53). Once directly bound to the protein, the drug increases the ion channel's open probability, allowing the flow of chloride ions to form a normal gradient (54).

Figure 1 presents the three-dimensional structure of Ivacaftor. Since the hydrogen bonds between the protein and the drug are the most crucial for proper drug function, it can be implied that the hydroxyl group attached to the benzene ring, the secondary amine group, and the amide group would be the central functional groups that interact with the CFTR protein (55).

Based on such information, the research conducts SwissDock molecular docking on Ivacaftor variants, characterized by modulations of functional groups. Through such computational modeling, the study aims to evaluate how the selected chemical modulations of Ivacaftor affect predicted binding affinity to the mutated CFTR protein.

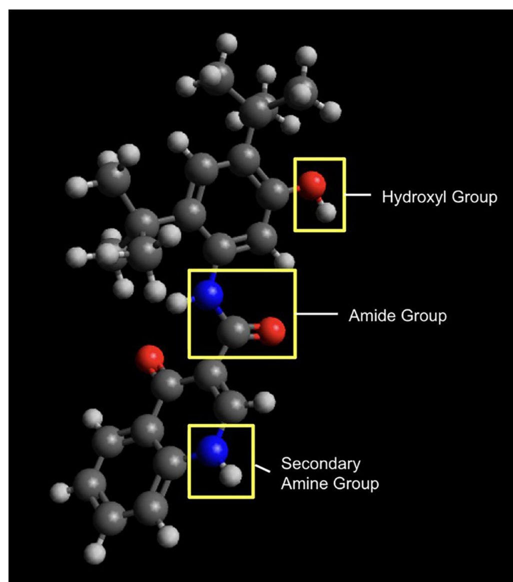

Figure 1. ***Three-dimensional Ivacaftor structure.*** *Dark gray spheres indicate carbon atoms, light gray spheres indicate hydrogen atoms, red spheres indicate oxygen atoms, and blue spheres indicate nitrogen atoms. The labels indicate the three functional groups present in the molecule.*

METHODS AND MATERIALS

In order to assess the predicted effectiveness of Ivacaftor with functional group variations, molecular docking was conducted for five total structures: the original Ivacaftor molecule and four versions with functional group modulations.

Firstly, the unmodulated structure of Ivacaftor was collected from PubChem (CID: 16220172), a database of chemical information (56). Using the open source Visualizer, a tool developed by the Swiss Federal Institute of Technology's cheminformatics department, the functional group modulations were designed and the corresponding SMILES codes were obtained for molecular docking (57). Excluding the unmodulated Ivacaftor molecule, four different modulations were tested: O-methylation, N-methylation on the secondary amide nitrogen, phenyl ring methylation on the ortho position relative to the hydroxyl group, and the removal of the hydroxyl group.

After modulation, SwissDock was used for molecular docking (40, 58). Between the AC mechanism and AutoDock Vina mechanism, the AC mechanism was utilized for maximum accuracy. The corresponding SMILES code for each Ivacaftor variation was submitted as the ligand, and the target protein was selected as the full CFTR protein with a sequence mutation from glutamic acid to glutamine in residue 1371 (PDB ID "6O2P.") (55) The attached Ivacaftor ligand was removed as a heteroatom in SwissDock. Regarding docking parameters, five were present in the AC mechanism: box center and box size were set with default parameters (153, 155, 152 Å and 20 20 20 Å respectively), the number of random initial conditions was set to 1, sampling exhaustivity was low, and cavity prioritization was buried. For each docking, the minimum SwissParam score, which corresponds to the minimum binding free energy, was recorded for results (59, 60). Furthermore, the optimal positions of each ligand were provided, in which the results of the trial that produced the lowest SwissParam score for each modulation were visualized through UCSF Chimera.

5 trials were conducted for each modulation, and all results were averaged to ensure consistency. Furthermore, independent t-tests were conducted between each pair of Ivacaftor molecules to determine statistical significance.

RESULTS

In this experiment, four different functional group modulations were conducted on the Ivacaftor molecule: O-methylation, N-methylation on the secondary amide nitrogen, phenyl ring methylation on the ortho position relative to the hydroxyl group, and the removal of the hydroxyl group. These modulations each target the three functional groups of Ivacaftor in distinct ways, providing space for better comparison between the models. As functional groups heavily impact a chemical's properties, significant differences in results were expected. In order to compare the impact of modulated Ivacaftor on the bonding with the CFTR protein, molecular docking through SwissDock was utilized. The docking generated results on the modulated Ivacaftor molecules' optimal binding position and SwissParam score, which indicate the binding free energy for all tested docking clusters.

Figure 2-A presents the unmodulated Ivacaftor molecule bound to the mutated CFTR protein's specific cavity, which acts as the standard for comparison. Considering this specific protein orientation as the base position, Ivacaftor is bound to a cavity on its front side, slightly shifted to the right. In the cavity, as seen in Figure 3, the benzene ring's carbon and hydrogen bonds primarily bond with the CFTR protein, as represented through the two yellow lines connecting Ivacaftor with the protein.

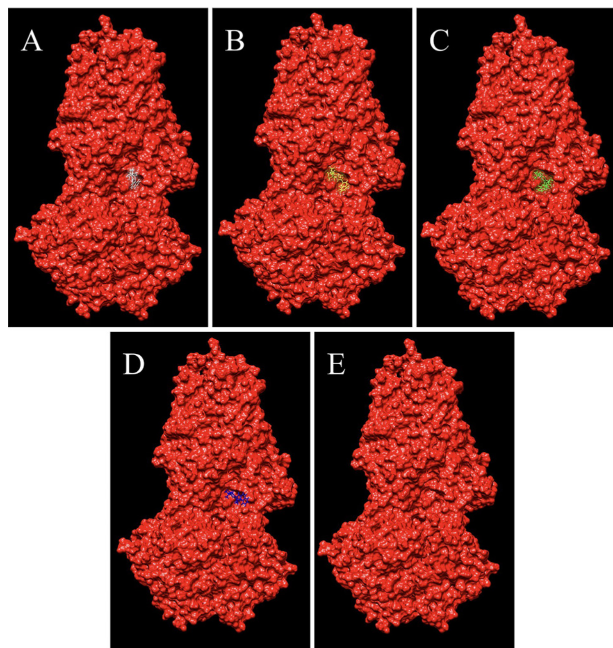

***Figure 2.* Ivacaftor molecules bound to mutated CFTR protein.** *The CFTR protein is in surface representation, base position, and red in all panels. Figure 2-A displays unmodulated Ivacaftor, in white, bound to the CFTR protein. Figure 2-B displays O-methylated Ivacaftor (Ivacaftor-mut1), in yellow, bound to the CFTR protein. Figure 2-C displays N-methylated Ivacaftor (Ivacaftor-mut2), in green, bound to the CFTR protein. Figure 2-D displays phenyl-methylated Ivacaftor (Ivacaftor-mu3), in blue, bound to the CFTR protein. Figure 2-E displays hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4), in pink, bound to the CFTR protein. Note that Ivacaftor-mut4 is invisible in base position.*

O-methylated Ivacaftor (Ivacaftor-mut1) generally displays a similar binding position to unmodulated Ivacaftor, but the orientation of the atoms differ (Figure 2-B). Here, tert-butyl groups and one hydrogen attached to the carbon ring with the secondary amine group formed bonds with the cavity, forming a total of 11 bonds (Figure 4). The N-methylated Ivacaftor (Ivacaftor-mut2) is also shown to bind to the same cavity as the previous two ligands, yet has a different ligand positioning (Figure 2-C). Different bonds are also shown, as while the tert-butyl groups still bond with the CFTR protein, the additional hydrogen that forms a bond is a part of the six-carbon ring connected to the hydroxyl group, which is different from that of the Ivacaftor-mut1 and CFTR protein complex. In total, 9 bonds are formed between Ivacaftor-mut 2 and the CFTR protein (Figure 5). Although the phenyl-methylated Ivacaftor (Ivacaftor-mut3) seems to be bound in the similar cavity, it is bound to the CFTR protein only with the tert-butyl group neighboring the hydroxyl group, forming a total of 7 bonds (Figure 2-D, 6). Lastly, the Ivacaftor with a hydroxyl group deletion (Ivacaftor-mut4) most effectively binds to a new cavity that isn't visible in the base position (Figure 2-E). Instead, it binds to a deeper cavity, almost invisible when displaying the CFTR protein with surface representation (Figure 7). Regarding bonds, its tert-butyl group neighboring the amide group and 2 carbons of the benzene ring bond with the CFTR protein, forming a total of 5 bonds (Figure 8).

Figure 9 displays a visual overview of the relative positionings of the unmodulated Ivacaftor (in white)

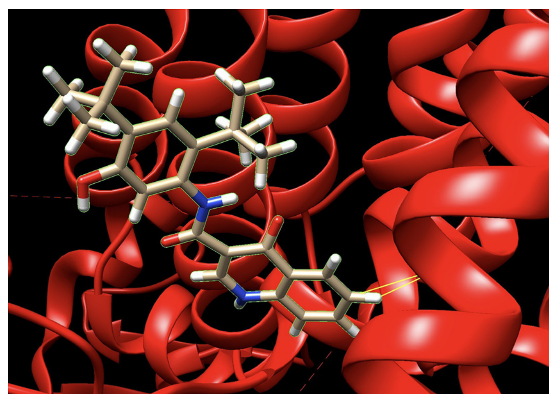

***Figure 3.* Bonding of unmodulated Ivacaftor molecule bound to mutated CFTR protein, close up.** *The CFTR protein is in red, shown to form bonds with the benzene ring structure of Ivacaftor, represented through the yellow line.*

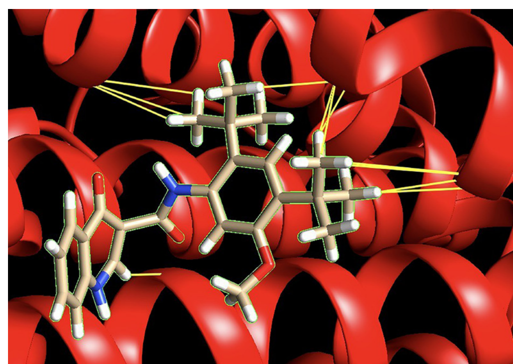

***Figure 4.* Bonding of O-methylated Ivacaftor (Ivacaftor-mut1) molecule bound to mutated CFTR protein, close up.** *The CFTR protein is in red, shown to form bonds with the tert-butyl groups and a hydrogen of the six-carbon ring containing a secondary amine group of Ivacaftor-mut1, represented through the yellow line.*

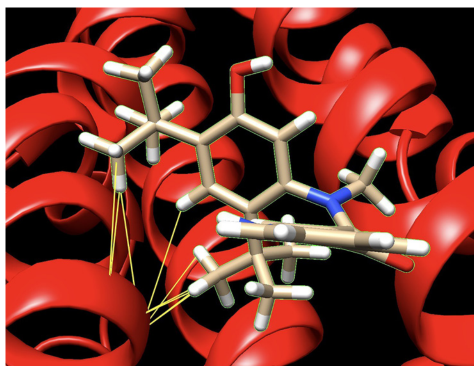

***Figure 5.* Bonding of N-methylated Ivacaftor (Ivacaftor-mut2) molecule bound to mutated CFTR protein, close up.** *The CFTR protein is in red, shown to form bonds with the tert-butyl groups and a hydrogen of the six-carbon ring containing a hydroxyl group of Ivacaftor-mut2, represented through the yellow line.*

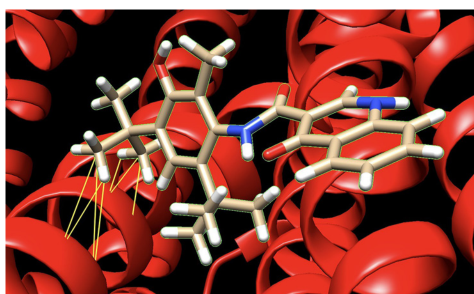

***Figure 6.* Bonding of phenyl-methylated Ivacaftor (Ivacaftor-mut3) molecule bound to mutated CFTR protein, close up.** *The CFTR protein is in red, shown to form bonds with the tert-butyl group neighboring the hydroxyl group of Ivacaftor-mut3, represented through the yellow line.*

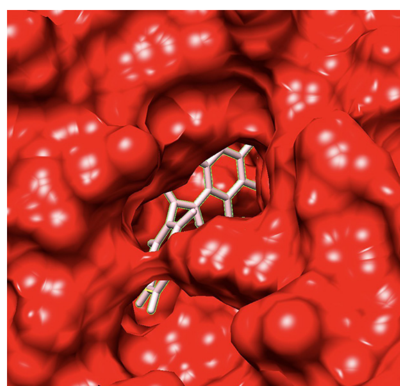

***Figure 7.* Bonding position of hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) molecule bound to mutated CFTR protein, close up.** *The CFTR protein is in surface representation and red. Ivacaftor-mut4 is represented in stick representation and pink, barely visible as it is bound inside the surface of the CFTR protein.*

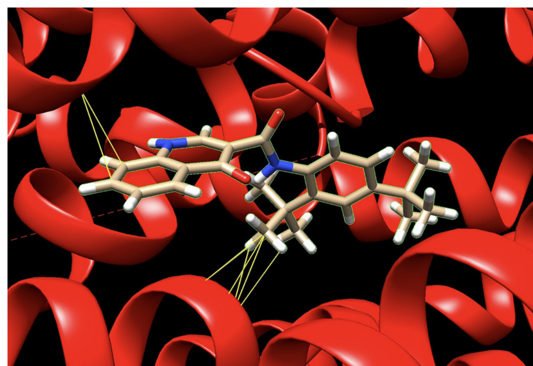

***Figure 8.* Bonding of hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) molecule bound to mutated CFTR protein, close up.** *The CFTR protein is in red, shown to form bonds with the tert-butyl group neighboring the amide group and 2 carbons of the benzene ring of Ivacaftor-mut4, represented through the yellow line.*

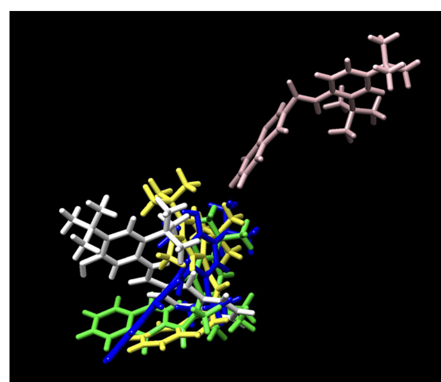

***Figure 9.* Relative positioning of unmodulated Ivacaftor and all 4 modulated Ivacaftors.** *Ivacaftor and its variants were loaded onto Chimera to compare relative positioning once bound to the CFTR protein. The unmodulated Ivacaftor is in white, Ivacaftor-mut1 is in yellow, Ivacaftor-mut2 is in green, Ivacaftor-mut3 is in blue, and Ivacaftor-mut4 is in pink.*

along with all the modulated Ivacaftor structures once they are bound to the CFTR protein. Although Ivacaftor-mut1, Ivacaftor-mut2, and Ivacaftor-mut3 are placed in a somewhat similar positioning to the unmodulated Ivacaftor, they nevertheless show distinct orientations among each other. On the other hand, Ivacaftor-mut4 displays a distant position from the other Ivacaftor structures.

The relative binding free energies of each Ivacaftor modulation and CFTR protein binding process was assessed through Swissparam scores produced by SwissDock, which are summarized in Table 1. Low

Table 1. *Summary of SwissDock calculations of SwissParam scores for 5 different versions of Ivacaftor molecules. Minimum SwissParam score indicates the lowest SwissParam score selected in each simulation trial, in which their average values and standard deviations were calculated as shown below.*

Ivacaftor Molecule Type	Minimum SwissParam Score (mean)	SD (Standard Deviation)
Unmodulated	-6.3036	0.31681
O-methylated (Ivacaftor-mut1)	-6.5646	0.33465
N-methylated (Ivacaftor-mut2)	-6.4156	0.43912
Phenyl-methylated (Ivacaftor-mut3)	-6.3107	0.23162
Hydroxyl-group-deleted (Ivacaftor-mut4)	-6.7588	0.24564

scores indicate low binding free energy, which typically leads to more binding affinity and stability. On the other hand, high scores indicate high binding free energy, which is associated with less stability during bonding. The ligand that showed the lowest average score, therefore the lowest binding free energy and highest stability, was the hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4), with an average SwissParam score of -6.7588. This result was significantly higher than that of unmodulated Ivacaftor, which had the lowest average SwissParam score of -6.3036 (p=0.0348). Phenyl-methylated Ivacaftor (Ivacaftor-mut3) also displayed a significantly lower average Swissparam score of -6.3107 than hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) (p=0.0179). However, O-methylated Ivacaftor (Ivacaftor-mut1) and N-methylated Ivacaftor (Ivacaftor-mut2) generated SwissParam scores that were statistically insignificant compared to both the hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) and the unmodulated Ivacaftor, with p-values less than 0.05.

DISCUSSION

This experiment displays how small-molecule drug evaluation can be conducted through computational tools, specifically through molecular docking algorithms. The variance in ligand binding positions and orientation despite singular function group modulations present prove that such docking and evaluation is valuable for identifying possible protein-ligand binding sites and interactions. Furthermore, the SwissParam scores provide specific information on the effectiveness of each protein ligand interaction, suggesting a potential method into how computational calculations can aid the selection and testing of more effective small-molecule drugs.

Among the functional group modulations in this empirical study, the hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) generated the lowest average minimum SwissParam score, potentially implying that it likely forms the most stable interactions with the target CFTR protein. On the other hand, the finding that the unmodulated Ivacaftor molecule had the highest average minimum SwissParam score, which was unexpected as it has been selected as the small-molecule drug to be sold on market. However, the development process of Ivacaftor did not solely include computational study, though high-throughput screening was involved. Instead, in-vitro studies with recombinant cell lines and human bronchial epithelial cell cultures along with clinical trials were also conducted to ensure realistic effectiveness and applicability of the drug candidates (61). Furthermore, as seen through the t-test, some pairs among the 5 tested Ivacaftor variants showed statistically insignificant difference in SwissParam scores, suggesting that such computational scoring systems alone cannot dictate drug development processes.

Moreover, although hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) could be a potential drug candidate to test for effectiveness against cystic fibrosis, it cannot be concluded that it will be an effective drug. As seen in the visualizations of protein-ligand interactions, hydroxyl-group-deleted Ivacaftor (Ivacaftor-mut4) was bound to a different cavity as to unmodulated Ivacaftor. As the original drug binds to a specific site on the protein to initiate the ion channel's function, it is not guaranteed that the interaction between the modulated Ivacaftor and the protein would have identical or improved effects

Additionally, all tested modulations may have competent binding and function, but could also display higher off-target binding or other toxicities not captured by this study's modeling. In fact, the reason that unmodulated Ivacaftor was selected as the most appropriate on-market drug could be the lack of such side effects along with competent potency in treating cystic

fibrosis. With that said, further evaluation and in-vitro experimentation of each functional group modulation would provide more tangible evidence on their effectiveness, and solely relying on molecular docking to judge drug efficacy would be an unreliable assessment.

CONCLUSION

The use of computational tools can greatly aid in small-molecule drug optimization through more efficient evaluations of chemical interactions and docking visualizations, especially benefiting the treatment of genetic disorders such as cystic fibrosis. Examining the detailed mechanisms of small-molecule drugs proved the importance of protein-ligand bonding and involved interactions, while reviewing the current use of computational tools in small-molecule drug development offered insight into possible improvements in related drug development procedures. Lastly, the molecular docking of Ivacaftor with functional group modulations with the mutated CFTR protein proved that computational tools indeed could assist the assessments of drug candidates, primarily based on the protein-ligand interaction predictions. From this process, the hydroxyl-group-deleted Ivacaftor structure displayed the highest average binding affinity to the mutated CFTR protein, suggesting further experimentation on the specific modulation to investigate whether it could be applied as an improved version of the existing Ivacaftor drug.

Similar to the optimization of Ivacaftor, common utilization of computational tools can effectively assist small-molecule drug development and optimization in the pharmaceutical industry. Due to its speed and efficiency, such tools can significantly benefit patients suffering from intractable diseases, specifically genetic disorders that produce mutated proteins. With further development, advanced computational tools could even open doors for precision medicine for a wider audience, significantly improving healthcare in the future.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

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