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Research Paper

Determination of small molecules as inhibitors of Interleukin-1β in cancer immunotherapy

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Abstract: *This study focused on interleukin-1β (IL-1β), a cytokine involved in inflammatory response. Chronic forms of inflammation due to IL-1β promote an environment conducive to metastasis and cancer growth, as well as other inflammatory and autoimmune diseases. There are currently monoclonal antibodies, such as canakinumab, that act as IL-1β blockers to be used in immunotherapy. However, small molecule inhibitors, which exhibit properties much more favorable to druggability, have been difficult to identify. Past studies have attempted to identify small molecules to inhibit Interleukin-1β through fragment-based screening and nuclear magnetic resonance studies. Moreover, these studies have mostly employed much smaller databases of molecules, allowing fewer possibilities for effective drug design. Through a unique combination of pharmacophore-based screening and molecular docking techniques, this study focused on discovering several promising IL-1β inhibitors for future testing and drug design. The study identified candidate molecules from an online database of over 18 million compounds using pharmacophore maps showing favorable interactions with binding sites on Interleukin-1β. The top candidates were then narrowed down through a combination of tests based on molecular docking and physical properties relating to druggability. The resulting small molecules identified by the screening provide a very promising starting point for future research in developing inhibitors of Interleukin-1β to be used in immunotherapy. Further development of these small molecules requires additional verification through biophysical screening techniques in a laboratory setting.*

Keywords: *IL-1β, small molecules, virtual screening, cancer, molecular docking, pharmacophore-based screening, drug properties*

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I. Introduction

Cancer

Cancer is a common disease leading to millions of deaths each year and is the second leading cause of death globally[1]. As a result, it has been the subject of extensive study. The National Cancer Institute provides general information about cancer[2]. It can begin in many different types of cells as genetic changes, which can originate from errors during cell replication, damage to DNA by harmful substances, or genes inherited from parents. Many types of unusual tissues, called tumors, can occur in the body, but most are benign and will not spread to other parts of the body through metastasis. However, some tumors that contain cells with certain genetic mutations become "cancerous," growing rapidly and ultimately negatively impacting health. Most mutations that lead to cancer can be classified under three general classes of genes responsible for regulating the cell cycle: proto-oncogenes, tumor suppressor genes, and DNA repair genes. Although the general mechanisms behind cancer are understood, cures for cancer are limited by the huge diversity in types of cancer and different cells within cancerous tumors.

Immunotherapy

There are a number of modern approaches to treating cancer, but it has mostly been dominated by three traditional approaches: surgery, chemotherapy, and radiotherapy. A review of tumor treatment strategies by researchers at the University of Hong Kong notes that patients treated with the three traditional approaches face "onerous physical and psychological challenges"[3], as they often have many adverse effects that are not limited to cancer cells. As a result, significant progress has been made in a number of alternative treatments[4]. Immunotherapy in particular has been the subject of recent research and attention due to the way that it takes advantage of the body's natural dynamic system to fight cancer—it has the potential to improve existing treatment regimens significantly. In their natural state, immune cells can effectively hunt down and fight foreign substances,

but face challenges in identifying cancer cells. Immunotherapy boosts the immune response and offers more precise targeting than traditional treatments. Several different types of immunotherapy are used, including monoclonal antibodies, which mimic normal antibodies and direct immune cells toward specific antigens on cancer cells, cytokines, proteins that are injected into the body to stimulate immune response, and immune system modulators, which improve the immune response against cancer[5]. While there are clear advantages to immunotherapy, it is still a field under development and has "obvious complexity and uncertainty," as a result of factors such as drug resistance, overstimulation of the immune system, and high costs[6].

Interleukin-1β

The specific target of this paper is interleukin-1β (IL-1β), a cytokine involved in inflammatory response. IL-1β is an alarm cytokine that initiates and promotes local inflammation in response to pathogens and other signs of danger[7]. In high concentrations, however, it can cause chronic inflammation and the production of carcinogenic nitric oxide and reactive oxygen species, supporting a tumor microenvironment. Attempts have been made to find immune system modulators that inhibit IL-1β, thus reducing tumor likelihood. A research paper published in Cancer Immunology Research by Diwanji et al.[8] targeted this protein using the monoclonal antibodies canakinumab and gevokizumab, which have specifically been designed to inhibit IL-1β. These treatments were tested on mouse samples both alone and in combination with existing cancer treatments. Tumors were then analyzed with flow cytometry, IHC, and RNA sequencing techniques after the treatment. Although reductions in tumor size were observed with canakinumab and gevokizumab individually and in combination with other treatments, none of these results were significant. The treatments were also found to impact populations of immune cells around the tumor, and the researchers suggest that IL-1β inhibition results in a "less immunosuppressive" cancer phenotype. Based on results from the experiment, the report concluded that canakinumab and gevokizumab are not useful as individual treatments, but have much potential in tandem with other cancer therapies due to the significant effects of IL-1β inhibition on the tumor microenvironment. Another paper published in Nature Communications by Hommel et al. noted that no effective low-weight inhibitors (discussed in the next section) of IL-1β existed; as such, they attempted to create one[9]. The researchers first used fragment-based screening and Nuclear Magnetic Resonance (NMR) to identify one compound out of a library with 3,452 compounds that could bind to IL-1β. Next, the researchers tested derivatives of the compound with 1H–1C–HMQC NMR, changing different substituents and eventually finding a compound they labeled (S)-2. (S)-2 was then tested with cells, and the researchers found that it could selectively bind and inhibit IL-1β and not IL-1α, making it useful as a potential drug candidate. X-ray crystallography was then used to investigate the three-dimensional binding structure, leading to the discovery of a new binding site resulting from an excited conformation of the interleukin, which offers potential for future targeting and inhibition. The results of these studies highlight the importance of IL-1β as a therapeutic target in reducing cancer and offer promise for the development of novel small-molecule inhibitors.

Small Molecules

Hommel et al. noted the importance of small molecule inhibitors, which are a major focus of immunotherapy[9]. Many current treatments in immunotherapy are not very effective, and this is often due to poor pharmacokinetic properties. Small-molecule treatments have unique chemical properties that allow for greater membrane permeability to reach targets, oral bioavailability, low costs, and access to a diverse range of targets[10]. Therefore, these treatments have shown increasing promise either working in tandem with other therapies or individually[11]. As a result, small-molecule treatments have been developed for a wide variety of targets, including immune response pathways, immune checkpoints, and metabolic pathways[12]. While Liu et al. noted that there are still challenges, such as identifying patient-specific dosages for small molecule drugs, their study also recognizes the great potential, aided by computational modeling techniques discussed in the next section[10].

Virtual Screening

The process of drug discovery features several key steps, beginning with the identification of a target and ending with clinical development of a drug. In between, large numbers of candidates must be meticulously evaluated and tested to ensure that the final drug is effective and safe, creating a multi-billion dollar process that often takes over 10 years[13]. Computational modeling has revolutionized modern drug discovery, streamlining the early stages of finding therapeutic candidates. The utility of computer-aided drug discovery is highlighted by three developments: the availability of 3D structures, the explosion of digital libraries, and new computing approaches and processing to take advantage of available information[14]. One advantage that computing-based techniques have over traditional ones lies in the screening process, in which numerous compounds are tested to find "hits" for further testing. Virtual screening techniques draw from libraries several orders of magnitude larger than traditional high-throughput screening, which increases both the number of hits and the possibility of hits with more advantageous physical properties.

Pharmacophore Screening

Pharmacophore-based virtual screening is a specific technique that has been successfully used in virtual screening. It relies on pharmacophoric features (**Figure 1.1**), which represent specific parts of a molecule with certain properties (e.g., hydrogen bond donors, hydrophobic, aromatic features). Because these features are what make interactions between two molecules possible, "similar biological events can be triggered by chemically divergent molecules."[15] This makes pharmacophore mapping extremely useful in searching for small molecules. Several recent studies have used pharmacophore mapping for protein inhibition for a variety of targets, such as Glycogen Synthase Kinase-3[16], Estrogen Receptor Beta[17], and Glutaminyl Cyclase[18]. However, none have targeted IL-1β.

Figure 1.1 shows seven main pharmacophoric feature types represented by geometric spheres[15].

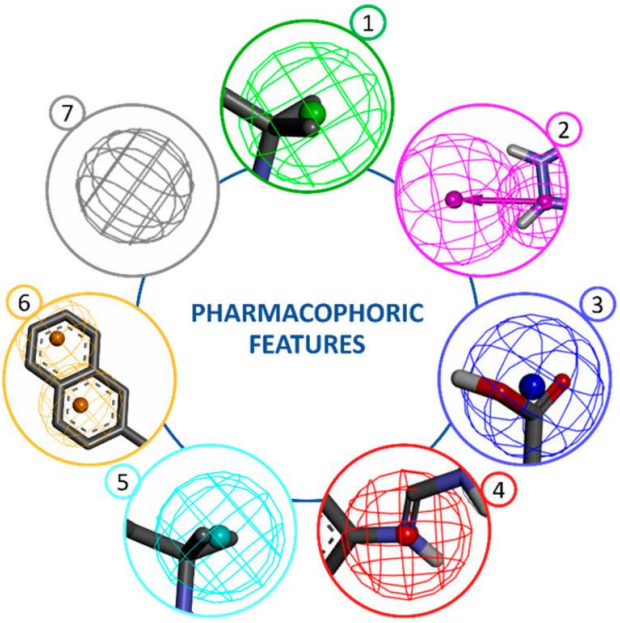


Figure 1.1 Pharmacophoric Features

Research Gap

The gap in current research that this study addresses has been pointed out in other studies, but has not been filled yet, and the methods to do so have also been explored, but not in relation to the target of this study. Although several studies have targeted the IL-1β protein, none has successfully found a small molecule inhibitor, which is in high demand and is growing in popularity as a therapeutic. Although other studies have applied pharmacophore screening to identify small molecule inhibitors, pharmacophore-based techniques have not been applied to IL-1β before. This methodology may be advantageous to past studies due to the versatility of pharmacophore features and the large chemical libraries that these techniques use. By addressing this gap, more effective cancer therapeutics could be created, and a greater understanding of the range of uses for pharmacophore mapping could be obtained. Thus, this research project aimed to answer the question: How might pharmacophore-based virtual screening methods be used to identify novel small-molecule inhibitors of the carcinogenic Interleukin-1β protein?

II. Methods

There are a number of different techniques used in virtual screening. Pharmacophore mapping, in particular, was selected for its past use in small-molecule protein inhibition. The expert advisor for this project contributed by introducing some of the specific applications used, which are all web-based and free. Each of these methods and the research papers behind them is cited in references.

Binding Site Verification

The first step in finding small molecule inhibitors to bind to IL-1β was checking for possible binding sites. Three methods were used to verify the existence of binding sites in the molecule: a geometric method, an energetic method, and a machine-learning method. The tools used for these methods were DoGSiteScorer[19,20,21,22], FTSite[23,24,25,26], and PRankWeb[27,28,29,30], respectively. A major part of this project was structures from the Protein Data Bank (PDB)[31,32,33]. The three-dimensional crystal structures of a protein or protein complex, containing all of the amino acids in the protein, are described by a PDB code that is widely recognized. For these experiments,

the four-character code 1IOB was used for the structure of IL-1β. This represents a crystal structure of the free IL-1β protein so that binding sites can be identified.

DoGSiteScorer

The geometric method, using DoGSiteScorer, is based primarily on binding site size and searches for possible binding pockets based on the three-dimensional structure of the proteins, calculates their physical properties, then assigns a druggability factor based on each pocket's volume, hydrophobicity, and enclosure. At the website proteins.plus, the geometric method with DoGSiteScorer was used by entering the PDB code and pressing "Go," then selecting the DoGSiteScorer tool and pressing "Calculate." DoGSiteScorer also has the option to add a ligand, but it is not necessary for binding site identification. Each of the binding sites identified by the tool was recorded.

FTSite

Next, the energetic method, using FTSite, searches each protein for ligand binding spots based on interaction energies. These binding sites are likely targets for small molecules. FTSite is based on FTMap, a server that utilizes 16 different probes with different chemical properties in billions of different positions to identify regions most likely to bind to other macromolecules. At the website ftsite.bu.edu, the FTSite tool was used by entering the PDB code and waiting for the tool to compute possible bonding sites. All of the results were recorded.

PrankWeb

Finally, the machine-learning method is based on a combination of previous factors, using the PrankWeb tool at prankweb.cz to find binding sites. At the website, the PDB code was entered, and the resulting information about possible binding sites and amino acid residues located at each site was recorded.

Pharmacophore Map Creation

After verifying the existence of promising binding sites in the protein target, the next step was identifying pharmacophore maps that fit the binding. The PDB code used for pharmacophore map identification was 4G6J, a structure based on the interactions of IL-1β with the drug Canakinumab. The tool PocketQuery[34] creates pharmacophore maps from the binding sites of Canakinumab with IL-1β. These pharmacophore maps can then be used with ZINCPharmer[35,36] to identify small molecules that match the pharmacophore maps and can likely bind to IL-1β, mimicking the interactions with Canakinumab.

At the PocketQuery website http://pocketquery.csb.pitt.edu/, the PDB code for the protein complex was entered. Next, the resulting pharmacophore maps were ranked by selecting "Score" on the top-right of the page so that the scores closest to 1 are at the top. Small molecules were then identified for the top pharmacophore maps by clicking on each pharmacophore map and selecting "Export" and "Send to ZINCPharmer." The ZINCPharmer tool requires at least three features on the pharmacophore map to search for small molecules that can bind effectively. If a pharmacophore map had too many features and no molecule was identified, some of them were deselected. The three top pharmacophore maps were then selected, and they were used to search for small molecules with the "Submit Query" button. For each of the three pharmacophore maps, ZINCPharmer searched for matching small molecules, providing a Root Mean Square Deviation (RMSD) value for each one. The names of the molecules with the lowest RMSD values were recorded. In this experiment, twenty-one small molecules, seven from each of the three pharmacophore maps, were selected.

Energetic Analysis

After identifying small molecules, energetic analyses of interactions with IL-1β through molecular docking were performed using the SwissDock tool at the website swissdock.ch. First, the SwissDock tool requires a SMILES ID for each small molecule. This was obtained through the ZINCPharmer website by clicking on a small molecule's name, and then copying the SMILES ID into SwissDock. In particular, this experiment used the Attracting Cavities docking engine, which allows the small molecule and the protein target to interact freely. After pressing the "Prepare ligand" button, a protein ID was entered. This experiment used the protein ID 1IOB, a crystal structure of free IL-1β that can bind with potential small molecules in any way. In this case, there was only one option for both "chains to keep" and "heteroatoms to keep", so these options were selected, and "Prepare target" was pressed. Next, it is necessary to specify a Search Space for the molecular docking. This experiment used the Center settings of 12, 15, 2 and Size settings of 33, 26, 29 to maximize the features of the protein in the Search Space.

Figure 2.1 shows the Search Space window of the protein IL-1β using the tool SwissDock[37,38,39] and the PDB code 1IOB.

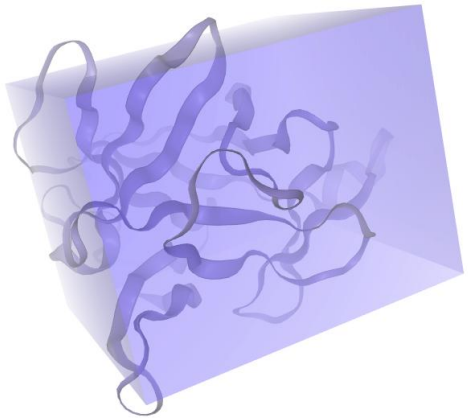


Figure 2.1 SwissDock Search Space

After specifying the Search Space, no extra parameters were needed. After clicking "Check parameters" and then "Start Docking," the docking process began. SwissDock tests the interaction of the target protein and each small molecule by probing binding sites across the protein with multiple different orientations. Once the docking finished, SwissDock showed a new page with a viewer and a table with the top orientations. The table was ordered by the highest (i.e., most negative) SwissParam Score (equivalent to Gibbs Free Energy), and the top interaction energy for each small molecule was recorded.

Druglike Properties

In order to verify that the top small molecules were druggable and followed Lipinski's rule, SwissADME[40], a tool for drug design, was used. SwissADME is at the website swissadme.ch, and also requires a SMILES ID for each small molecule. These were already obtained in the previous experiment. The five small molecules with the most negative Gibbs Free Energy were selected as the most promising candidates for drug design. Each SMILES ID was inputted so that SwissADME could calculate the four druggability-related properties of each drug to verify if they followed Lipinski's rule. The final results were recorded.

III. Results

Binding Site Verification

Potential binding sites in the protein were identified through 3 different methods: a geometric method, an energetic method, and a machine-learning method.

DoGSiteScorer

The DoGSiteScorer tool from proteins.plus was used, and seven binding sites were identified.

Table 3.1 shows the volume, surface area, and drug score of the 7 binding sites identified by DoGSiteScorer on IL-1β.

Table 3.1 DoGSiteScorer Results

Name	Volume (Å^3)	Surface Area (Å^2)	Drug Score
Site 1	343.94	348.52	0.76
Site 2	248.51	287.05	0.51
Site 3	273.15	473.85	0.5
Site 4	190.34	362.33	0.43
Site 5	198.59	453.2	0.4
Site 6	122.69	296.96	0.25
Site 7	112.38	280.63	0.16

Figure 3.1 shows the binding sites identified on IL-1β with PDB code 1IOB by DoGSiteScorer. (Key: Site 1: Orange, Site 2: Green, Site 3: Purple, Site 4: Blue, Site 5: Reddish-orange, Site 6: Lime, Site 7: Magenta)

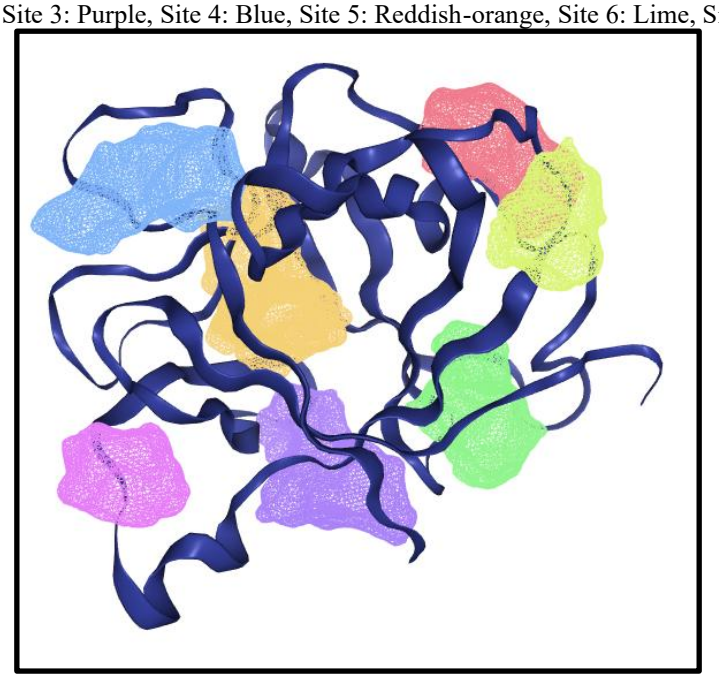


Figure 3.1 DoGSiteScorer Binding Sites

FTSite

The FTSite method identified three possible binding sites on the protein.

Figure 3.2 shows the FTSite energy-based binding sites identified on the IL-1β structure with PDB code 1IOB.

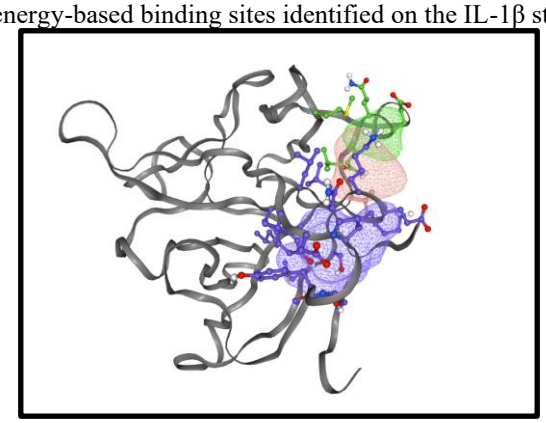


Figure 3.2 FTSite Binding Sites

PrankWeb

The final method was the machine-based PrankWeb. One binding site, with 4 residues (LEU10, LEU18, LEU69, and LEU122) was identified using the prankweb.cz tool.

Figure 3.3 shows one binding site with four leucine residues identified by Prankweb on IL-1β.

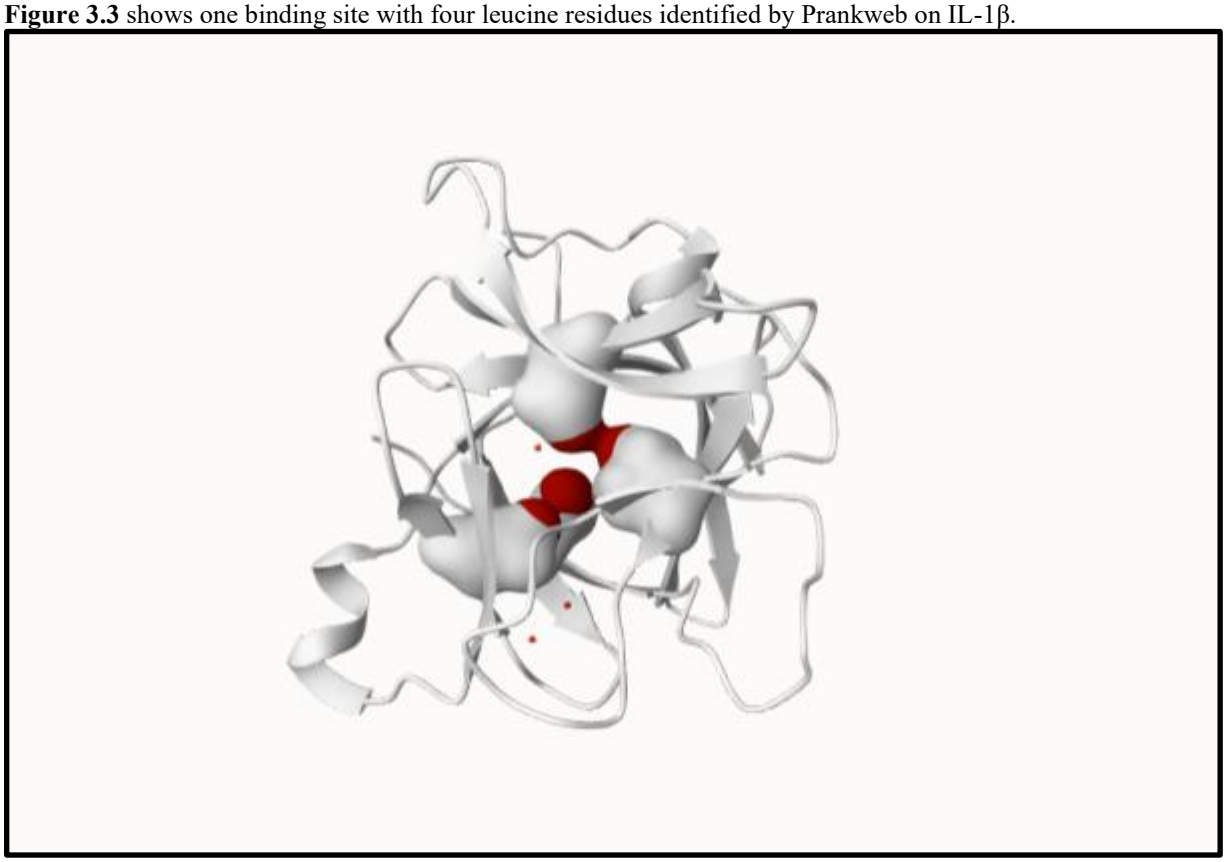


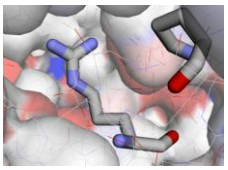
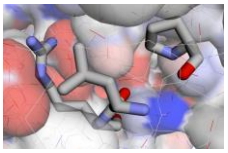
Figure 3.3 PrankWeb Binding Sites

Pharmacophore Map Creation

The three highest-scoring maps that met the criteria were selected from PocketQuery, two from the heavy chain of Canakinumab, and one from the light chain. Seven small molecules were selected from each map.

Table 3.2 shows the three maps collected from the interaction of IL-1β with Canakinumab, the number of residues each map was based on, and a score.

Table 3.2 Pharmacophore Maps

Map Number	Chain	Number of Residues	Score	Image
Map #1 (kept 2nd hydrogen)	Heavy	2	0.983362	
Map #2 (kept 1st hydrogen)	Heavy	3	0.976374	

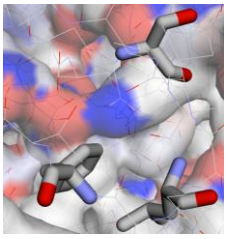
Map #3	Light	3	0.974554	

Figure 3.4 shows the pharmacophore maps and residues of the three maps identified from PocketQuery on ZINCPharmer in order from Map #1 to Map #3 (left to right). These maps are based on the interactions of IL-1β with the monoclonal antibody Canakinumab, with the PDB code 4G6J.

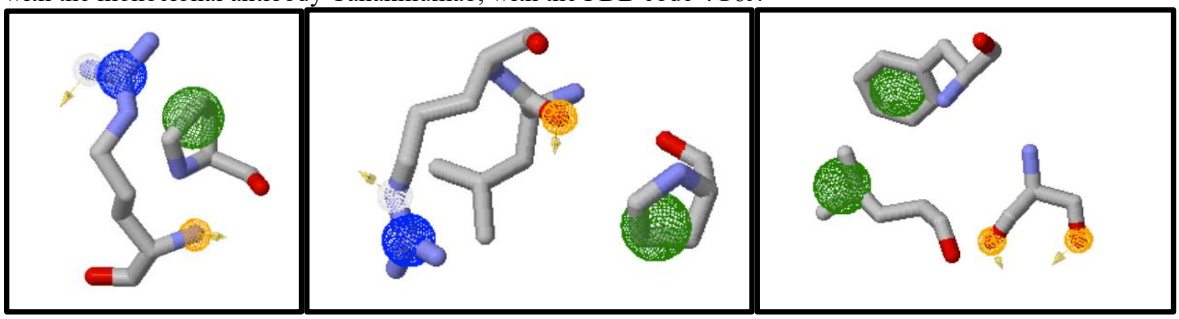
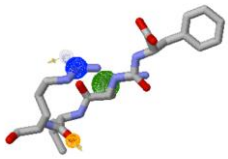
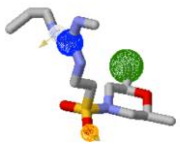
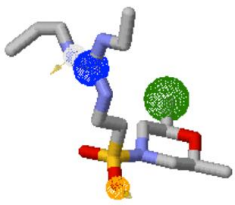


Figure 3.4 Pharmacophore Map Structures

Table 3.3 shows the RMSD of the top seven small molecules resulting from Map #1, the names of the molecules, their molecular masses, and chemical structures.

Table 3.3 Map #1 Small Molecules

Name	RMSD	Molecular Mass (daltons)	Image
ZINC04533954	0.105	606	
ZINC73507050	0.170	321	
ZINC73486367	0.170	335	

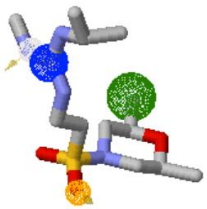
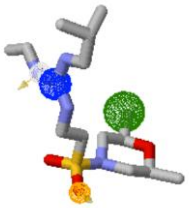
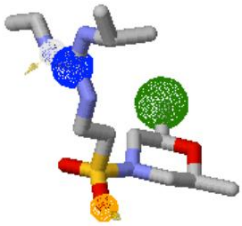
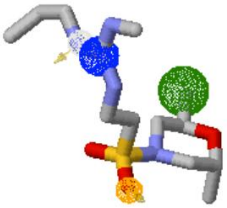
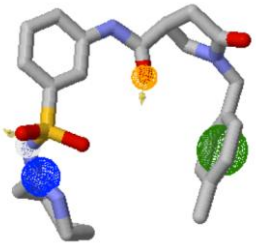
ZINC72625042	0.170	321	
ZINC73316448	0.170	350	
ZINC72613293	0.170	335	
ZINC73507052	0.172	321	

Table 3.4 shows the RMSD of the top seven small molecules resulting from Map #2, the names of the molecules, their molecular masses, and chemical structures.

Table 3.4 Map #2 Small Molecules

Name	**RMSD**	**Molecular Mass (daltons)**	**Image**
ZINC09714530	0.100	484	

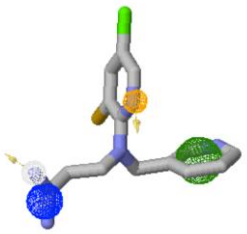
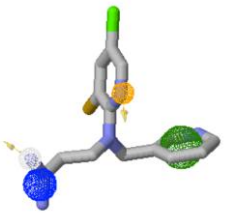
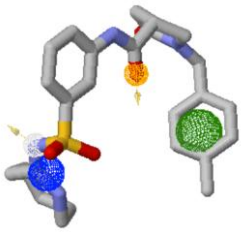
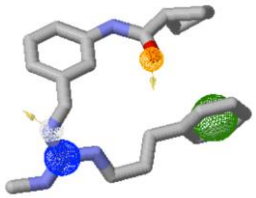
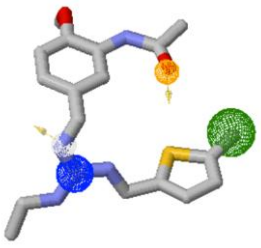
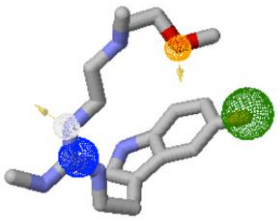
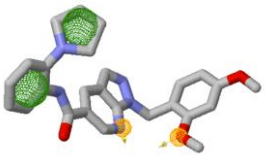
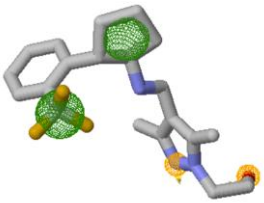
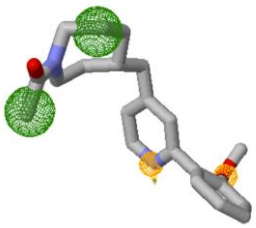
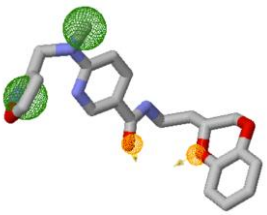
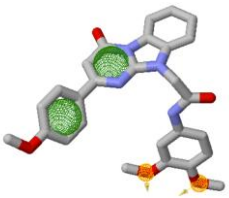
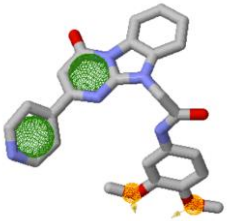
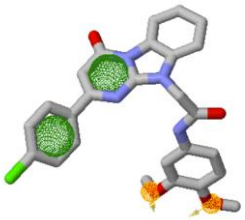
ZINC94052025	0.193	309	
ZINC94561077	0.193	309	
ZINC09714528	0.203	484	
ZINC73423551	0.218	380	
ZINC80720409	0.221	376	
ZINC84634631	0.225	351	

Table 3.5 shows the RMSD of the top seven small molecules resulting from Map #3, the names of the molecules, their molecular masses, and chemical structures.

Table 3.5 Map #3 Small Molecules

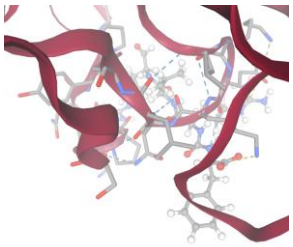
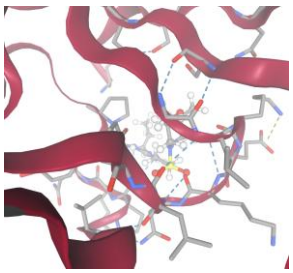
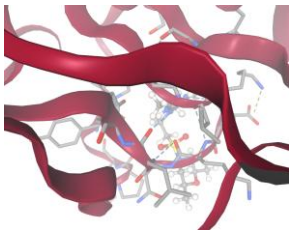
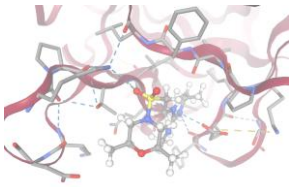
Name	RMSD	Molecular Mass (daltons)	Image
ZINC29101302	0.137	458	
ZINC92855724	0.155	381	
ZINC72365797	0.155	338	
ZINC12302607	0.171	395	
ZINC21663767	0.177	485	
ZINC40484421	0.177	455	

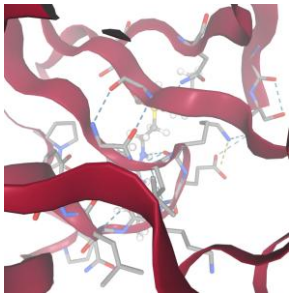
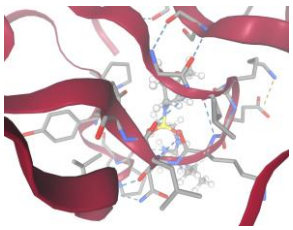
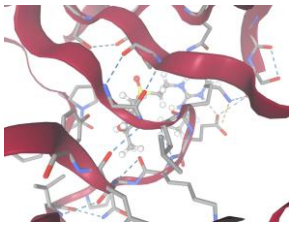
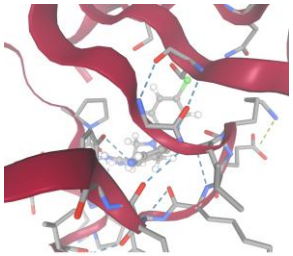
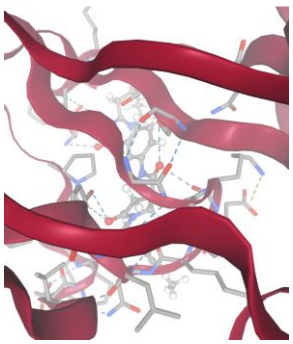
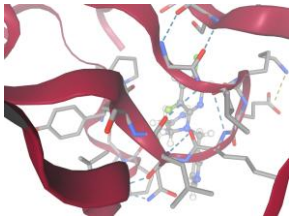
ZINC21663769	0.177	489	

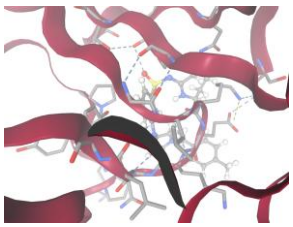
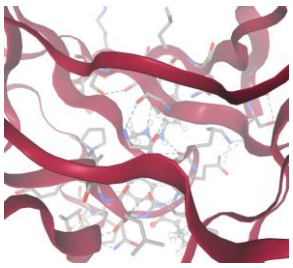
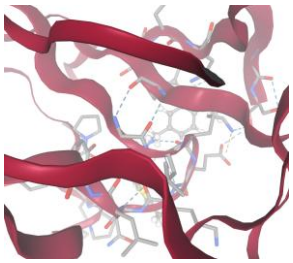
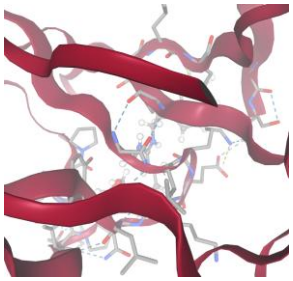
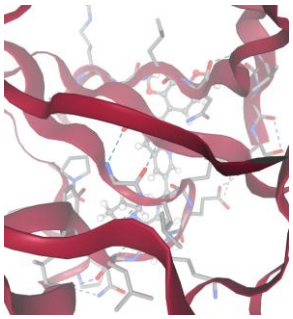
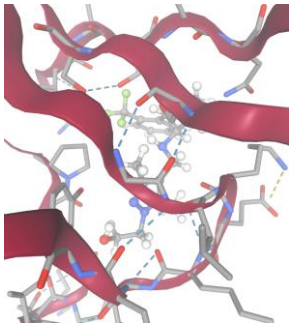
Energetic Analysis
The energies of the top 21 small molecules were analyzed using molecular docking and the SwissDock tool.

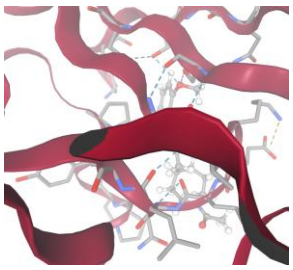
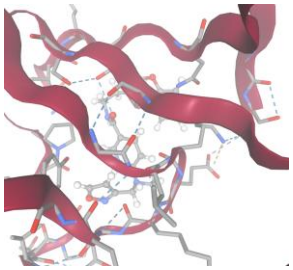
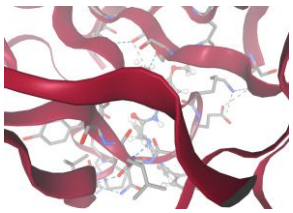
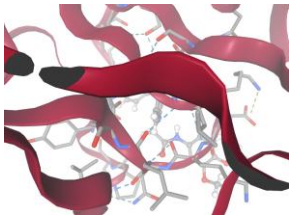
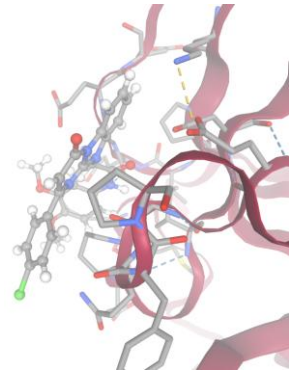
Table 3.6 shows the SwissDock results for each molecule, including the name of the small molecule, the pharmacophore map it was identified from, the Gibbs Free Energy, and an image of the small molecule binding to IL-1β.

Table 3.6 Molecular Docking Results

Name	**Pharmacophore Map**	**Gibbs Free Energy (kcal/mol)**	**Image**
ZINC04533954	Map 1	-7.4258	
ZINC73507050	Map 1	-6.9300	
ZINC73486367	Map 1	-6.9701	
ZINC72625042	Map 1	-7.1456	

ZINC73316448	Map 1	-6.8828	
ZINC72613293	Map 1	-7.1022	
ZINC73507052	Map 1	-6.9513	
ZINC09714530	Map 2	-6.4430	
ZINC94052025	Map 2	-7.5875	
ZINC94561077	Map 2	-6.6235	

ZINC09714528	Map 2	-7.5970	
ZINC73423551	Map 2	-6.9708	
ZINC80720409	Map 2	-7.5970	
ZINC84634631	Map 2	-6.9119	
ZINC29101302	Map 3	-7.5857	
ZINC92855724	Map 3	-7.0775	

ZINC72365797	Map 3	-6.9693	
ZINC12302607	Map 3	-7.0976	
ZINC21663767	Map 3	-7.4437	
ZINC40484421	Map 3	-7.3192	
ZINC21663769	Map 3	-7.7568	

Druglike Properties

A final verification of the top five small molecules from the 21 identified by the SwissDock tool is Lipinski's rule, which is often used to ensure that a drug will have good absorption and permeation in the body. Lipinski's rule examines four properties of the small molecule: the number of hydrogen bond donors, the number of hydrogen bond acceptors, molecular mass, and CLogP. The number of hydrogen bond donors must be less than five, and the number of hydrogen bond acceptors must be less than ten. This check prevents the molecule from having an excess of hydrogen bond interactions in the body, allowing it to travel more easily. Next, the molecular mass must be less than 500 daltons. This checks to make sure that the molecule is not too big in order to pass through different systems in the body. Finally, CLogP, which must be less than five, examines the lipophilicity of the small molecule. A value larger than zero means that the drug is lipophilic, but if CLogP is too large, the small

molecule will be unable to permeate different systems of the body effectively, getting stuck in hydrophobic regions.

Table 3.7 shows the Gibbs Free Energy and four criteria for each small molecule important for druggability according to Lipinski's rule: number of hydrogen bond donors, number of hydrogen bond acceptors, molecular mass, and CLogP.

Table 3.7 Final Small Molecule Druglike Properties

Name	Gibbs Free Energy (kcal/mol)	H-bond Donors	H-bond Acceptors	Mass (daltons)	CLogP	Druglike?
ZINC21663769	-7.7568	1	5	488.92	3.64	Yes
ZINC09714528	-7.5970	2	5	482.60	2.78	Yes
ZINC80720409	-7.5970	4	2	375.51	2.51	Yes
ZINC94052025	-7.5875	2	3	308.76	1.77	Yes
ZINC29101302	-7.5857	1	5	457.52	3.49	Yes

IV. Discussion

Data Analysis and Discussion

Table 4.1 shows the average RMSD, average molecular mass, and molecular mass standard deviation values for each map.

Table 4.1 Map Molecules Data

Map	Average RMSD	Average Molecular Mass (daltons)	Molecular Mass Standard Deviation (daltons)
Map #1	0.161	369.85	104.68
Map #2	0.193	384.71	73.50
Map #3	0.164	428.71	57.73

Table 4.2 shows the average Gibbs Free Energy, Gibbs Free Energy Standard Deviation, and amino acid residues for each map.

Table 4.2 Map Interactions

Map	Average Gibbs Free Energy (kcal/mol)	Gibbs Free Energy Standard Deviation (kcal/mol)	Amino Acid Residues
Map #1	-7.058	0.186	Arginine, Proline
Map #2	-7.104	0.490	Arginine, Leucine, Proline
Map #3	-7.3214	0.291	Leucine, Proline, Serine

The top five molecules in terms of Gibbs Free Energy interaction with Interleukin-1β all came from Maps #2 and #3, with three molecules from Map #2 and two from Map #3. Map #3 overall had the highest average Gibbs Free Energy but a lower standard deviation in Gibbs Free Energy than Map #2, so larger values for the few top molecules from Map #2 likely accounted for this discrepancy. All three of these maps, which were the top pharmacophore maps, included a proline residue. Maps #1 and #2 shared an arginine residue, and Map #2 and Map #3 shared a leucine residue, which is consistent with the leucine residues identified in the possible binding sites in the free protein. The average molecular mass of the inhibitors increases monotonically from Map #1 to Map #3, while the standard deviation decreases monotonically. This may be notable because several of the top molecules were close to 500 daltons in mass, although they did not surpass this limit for Lipinski's rules for druggability. However, one of these molecules from Map #2 had a significantly lower molecular weight, around 300 daltons. This molecule also had a CLogP value of 1.77, which was less than all of the other molecules. This means that although this molecule is lipophilic, its hydrophobic interactions are not as strong as the other molecules. Values of CLogP that are too high could lead to decreased druggability as a result of reduced permeability in hydrophilic regions of the body. All five of the molecules with the highest Gibbs Free Energy of

interaction passed Lipinski's rules, meaning that they could potentially be effectively adapted as drugs to inhibit Interleukin-1β.

Implications, Limitations, and Future Considerations

Interleukin-1β plays a central role in promoting tumor development through inflammation, and the identification of five small-molecule inhibitors in this project (ZINC21663769, ZINC09714528, ZINC80720409, ZINC94052025, and ZINC29101302) demonstrates the potential for expanding immunotherapy treatments outside monoclonal antibodies. In particular, the top small molecule with the ZINC ID: ZINC21663769 and a Gibbs Free Energy of interaction with Interleukin-1β of -7.7568 kcal/mol is particularly promising. These small molecules, all passing Lipinski's test and exhibiting favorable Gibbs Free Energies (-7.7568 to -7.5857 kcal/mol), offer an encouraging basis for oral or low-cost alternatives to antibodies that may target tumors more effectively. The results of this project also demonstrate the potential of an *in silico* pipeline for small molecule drug discovery—combining DoGSiteScorer, FTSite, PrankWeb pocket identification, PocketQuery pharmacophore mapping, ZINCPharmer screening, SwissDock docking, and SwissADME drug-likeness evaluation—that could be used to target other proteins in the future.

For future work, also attempting to target Interleukin-1β, there are a few important lessons from this project to consider. More *in silico* methods, such as molecular dynamics, could be used to further test the effectiveness of each small molecule. Most importantly, however, a major limitation of computational modeling is that it cannot be the sole method behind drug creation. Before any of these five molecules can be used to create inhibitory drugs, all of them must still be verified in a laboratory. Physical interactions, such as the binding affinity and kinetics, can be evaluated through techniques such as surface plasmon resonance (SPR) and microscale thermophoresis (MST). Eventually, the ability of these small molecules to modulate the immune system can be evaluated in animal testing, using established mouse models of immuno-oncology.

Despite the positive results of this study, there are several limitations to computational modeling that must be acknowledged. First, the binding energies from molecular docking may not reliably correlate with *in vitro* or *in vivo* affinities, requiring further testing discussed earlier. Second, although pharmacophore mapping uses a large database, there are many potential small molecules not considered. Additionally, the binding of these small molecules through pharmacophore mapping might overlook potential cryptic binding sites, which are unpredictable and only exist due to conformational changes while binding to the protein. Moreover, the absence of a control re-docking experiment (benchmarking against a known IL-1β inhibitor) limits understanding of the accuracy of this technique. Finally, docking and ADME filters do not account for complex biological factors such as cellular uptake, metabolic stability, or immunogenicity, which must also be tested.

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