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Creation of a Calibration Set for Natural Product Virtual Target Screening

Jacob Wilson
Biomedical Sciences and Psychology
University of South Florida
4202 E. Fowler Avenue
Tampa, FL 33620

Faculty Advisors: Dr. Wayne Guida and Dr. Wesley Brooks

Abstract

Virtual screening (VS) is a computational method of drug design, which involves docking a collection of potentially efficacious molecules against a protein of interest which is known to be relevant in a disease. A few molecules which bind particularly well to the protein are identified and considered molecules of interest (MOI) and potential drugs. The problem with drugs discovered through this method is that they might have a high binding affinity for other proteins and those interactions may cause adverse effects. Virtual Target Screening (VTS) is a method which is used to identify the potential of an MOI to bind to other proteins. It is accomplished by virtually docking the MOI against a collection of proteins which are known to be prevalent and physiologically important in vivo. In order to determine the significance of an MOI's interaction with each protein, the protein's average binding affinity for drug-like molecules must be determined by prescreening each protein against a collection of small synthetic molecules, which is often referred to as a calibration set. Since there is a new interest in the use of medicinal natural products, an effort was begun to adapt VTS to also work with natural products in addition to the synthetic molecules. Natural products, compared to synthetic molecules, can be larger, contain greater variety and complexity of aromatics, and provide a greater representation of the diversity of chemical space. This project developed a natural product calibration set which was then run against several distinct proteins with known inhibitors. The ability of VTS to identify those inhibitors relative to the calibration set was tested to validate the calibration set. When such functionality is added to VTS, it will allow the identification of potential adverse interactions of natural product drug candidates and to repurpose existing natural product drugs.

Keywords: Drug Design, Natural Product, Calibration Set

1. Introduction

The use of natural products and their derivatives as medicines has been common throughout history. Natural products are defined as compounds which are derived from a biological source. This biological source could be a plant, micro-organism, or animal.[1] Secondary metabolites are natural products which are biosynthesized by an organism. They are not usually essential for the functioning of the organism, but instead are typically the result of adaption to the surrounding environment.[2] Natural products were first used with the biological source which contained them; however, as technology advanced, so did the methods by which humans were able to extract the specific bioactive natural products from the biological source.[3]

1.1. Historical Overview

The first recorded medicinal use of natural products was the use of oils from cypress and myrrh by the ancient Mesopotamians to treat coughs and colds.[2] However, paleoanthropological studies in the mountains of Iraq have

suggested that natural products might have been used medicinally more than 60,000 years ago by Neanderthals.[4] The Greeks and Chinese also greatly utilized natural products, each producing pharmaceutical records which still survive to this day. For instance, the Greek physician Galen is thought to have produced over 30 books on the use of nature as a source of therapeutics and is also known for producing complex formulas and directions on how to compound drugs.[5]

While these cultures were quite advanced in their ability to extract bioactive products from nature around them, they had nowhere near the technology which has been developed in the past hundred years. It would be expected that with the advance in technology would come an advance in the level of investigation into the therapeutics which have already been determined to be effective. This, however, was not the case. With the advent of molecular science and great advances in organic chemistry in the 1950's came a decrease in interest in natural products and an increase in rational, synthetic drug design.[6] Natural products were not abandoned altogether, however. They have often served as a scaffold on which chemists can design a synthetic drug. This occurrence is best illustrated by the use of salicylic acid, a natural product which is extracted from the bark of the willow tree, as inspiration for the synthesis of acetylsalicylic acid, aspirin.[7]

Lately, there has been a surge of interest in the use of natural products as therapeutics.[8] This shift in interest is thought to be due to science's realization that modern approaches to drug discovery and development are not perfect and should be used in conjunction with other techniques which have previously worked.[6]

1.2. Computational Chemistry

Computational Chemistry has greatly advanced and expedited the ability to develop new therapeutics. Computational Chemistry was first utilized in the late 1950's and early 1960's and used simple calculations which were run on large mainframe computers. As computing devices evolved, so did Computational Chemistry. Theoretical scientists were still, however, unable to visualize their results. This limited their ability to share their data with those outside of the field. Additionally, several commonly used compounds in medicinal chemistry were too large to be successfully modeled by computational measures. This, however, all changed with the advent of the microprocessor. After which, computational chemistry was able to visualize the results as well as model vastly larger compounds, making the results increasingly generalizable to scientists outside the field of Computational Chemistry.[9]

1.2.1. virtual screening

One of the pitfalls of medicinal chemistry is the size of the chemical space one must search through to find potential new therapeutic agents. To attempt to test each potential agent by assay would be prohibitively time consuming and costly. This problem is remedied through the use of Virtual Screening (VS).[10] VS is a computational method which computationally docks a collection of potentially efficacious molecules against a target protein or receptor which is relevant to the disease being researched.[11]

The VS process begins with a database of virtually depicted compounds which are thought to have potential as a therapeutic agent. Each compound in this database is then prepared by various programs to ensure they are each structurally correct as well as that all relevant tautomers and ionic states are included in the screening process. The target protein must then be prepared to ensure that it is ready for docking. Special care must be paid to the intended binding site on the target protein, such as an enzymatic active site, as it is an integral part of the docking process. The target must also be analyzed to ensure that all residues are correctly protonated. Lastly, each compound in the database is docked to the assigned binding site of the target. The docking mechanism varies depending on the program that is being used. Each program utilizes different algorithms and therefore proceeds differently. Each docking run is then scored based on how well it fit with the active site and how many interactions and bonds the ligand had with the active site.[11,12]

In VS, if a certain molecule has a high binding affinity for the target, then it is considered a Molecule of Interest (MOI) and may be modified to further improve its binding affinity and, by extension, its therapeutic potential.[13] This affords scientists a good indication of how to proceed. It, however, is not exact and is merely a good starting direction.[10]

1.2.2. molecular mechanics

Molecular Mechanics (MM) is the most common system behind VS systems. MM deals with the potential energy of a molecule as well as the attractive and repulsive forces between the atoms in the structure. These energy

calculations are then used to determine the conformation at which the structure would be most at ease. These energy determinative formulas and the energetic values of various elemental arrangements are written into the VS code.[9]

Lipinski's Rule of Five is a colloquial guideline to deal with the physicochemical properties of the compound and was originally intended to be used to determine the likelihood of a compound being absorbed by the body. The use of these guidelines, however, has been stretched by medicinal chemists and the guidelines are now used to determine the overall drug-likeness of a compound. The first rule is that the compound should have a molecular weight under 500. The second rule deals with the compound's lipophilicity and states that the compound should have a logP less than five. The third rule states that there should be fewer than 5 hydrogen donor groups on the molecule. The fourth rule states that there should be fewer than ten groups on the molecule that can accept hydrogen atoms and form a hydrogen bond.[14]

1.2.3. virtual target screening

The problem with drugs discovered through these means is that they might also have a high binding affinity for other proteins and receptors which are physiologically important *in vivo*. This could produce disastrous side effects. These side effects can be detected and evaluated through the use of Virtual Target Screening (VTS). VTS is a method which screens potentially efficacious MOIs against a variety of proteins to determine the selectivity of the MOI for its original intended target protein and therefore its likelihood of unintended binding *in vivo*. This method is used to determine the potential adverse effects the drug might have in the body, but also can be used to determine potential areas of interest in drug repurposing.[15]

In order for the VTS score to have meaning, there needs to be a benchmark for a significant interaction with the target. Typically this benchmark is determined by comparing the docking score of the MOI to the docking score of a known inhibitor of the target. However, this can be particularly difficult if there is no known inhibitor for the compound or if the inhibitor is a weak or covalent inhibitor. Due to these conditions, a second method was developed in which a set of compounds known to have general drug-like characteristics is used to determine the average docking score of the proteins so that significance can be assigned to the docking score of the MOI. This set of compounds is referred to as a calibration set. For each protein in the VTS collection, if the MOI is within the top 5% of the compounds in the calibration set, then it is thought the MOI has a significant docking score and might have an interaction with the targeted protein in the VTS collection. Each protein in the VTS collection is tested in a similar manner with the MOI. This approach has previously been validated repeatedly.[11,16,17]

The renewed interest in the use of natural products as therapeutics, has been accompanied by an increased interest in the adaptation of new computational techniques to be utilized with natural products.[18,19,20] This is the philosophy with which artemisinin was discovered. Artemisinin is an antimalarial which was ubiquitous in traditional Chinese medicine. Youyou Tu, the discoverer of artemisinin, was awarded the 2015 Nobel Prize in Medicine for the discovery, validating this philosophy.

In order for this philosophy to continue, more techniques need to be adapted for use with the natural products. There is a current effort to adapt VTS for use with natural products. A necessary part of this effort is the creation of a novel natural product calibration set. This calibration set would need to be created as the existing synthetic, small molecule calibration sets would not contain the same range of structures and characteristics. This is due to natural products displaying far greater chemical diversity and occupying a far larger region of chemical space than synthetic compounds.[21]

2. Methods

The compounds in the calibration set were partially obtained through literature searches for books, articles, and reviews on Phytochemistry, Bioactive Natural Products, and Eastern Medicine. Additionally, natural product diversity sets were also utilized. The structure for each compound, which was not contained in a diversity set, was obtained from PubChem. The structures for the compounds contained in the diversity set were present in the set file and therefore did not need to be obtained.

The ligands were prepared by way of the LigPrep utility of Schrodinger's Maestro interface prior to the inception of screening.[22] LigPrep generates 3D tautomer and stereoisomers of each compound by varying the ionic state, ring conformation, and stereochemistry of each compound. Thereby allowing the screening to accommodate the different forms of each compound which might be present if the compound were *in vivo*.

The Glide utility of Schrodinger's Maestro interface was utilized to test the validity of the calibration set by screening two proteins with known affinity for compounds in the set.[23]

The M4 muscarinic adrenergic receptor (M4) and adenosine deaminase (ADA) were chosen to serve in the experimental validation. Atropine and Cordycepin were chosen to serve as the positive control for the proteins, respectively. Each compound was also chosen to serve as the other's negative control. The protein structures were obtained by way of their PDB identification numbers (5DSG and 3IAR, respectively) and were optimized with the Protein Preparation Wizard in Schrodinger's Maestro interface (Sastry et al., 2013). The Protein Preparation Wizard prepares the protein structure by ensuring all missing atoms, residues and side chains are completed with the Prime utility of Schrodinger's Maestro interface; that all non-standard residues are identified and the proper bond orders and protonation states are determined and applied; and that the structure is properly optimized and minimized.[24]

Descriptive statistics—which included measures of central tendency, dispersion, and shape—were used to summarize and report the results of the validation experiments. The IBM SPSS Suite (23) was used to obtain these descriptive statistics.

3. Results

The final calibration set which was utilized in the concept validation experiment contained 1080 compounds a majority of which belong to the terpene, alkaloid, flavonoid, peptide, and fatty acid chemical classes.

The first validation experiment was performed on M4 and produced the results in Table 1. The results are abridged as the full data table would be prohibitively long. 1841 compounds out of the over eight thousand compounds which were run were able to be docked and scored. The results of this experiment had a normal distribution, with skewness of .870 (*SE*= .057). The mean glide score in this experiment was -7.756 with a standard deviation of 1.546. The positive control for this experiment was atropine which scored in the top 3% of compounds with a score of -10.0698. The negative control was cordycepin which scored in the top 34% of compounds with a score of -8.557.

The second validation experiment was performed on ADA and produced the results in Table 2. The results are abridged as the full data table would be prohibitively long. 556 compounds out of the over eight thousand compounds which were run were able to be scored. The results of this experiment had a normal distribution. with skewness of .549 (*SE*=.104). The mean glide score in this experiment was -5.44 with a standard deviation of 1.929. The positive control for this experiment was cordycepin which scored in the top 1% of compounds with a score of -9.133. The negative control was atropine which failed to produce results due to an inability to successfully minimize a viable docked structure. Which means that the compound, under standard conditions, would not bind to the protein in any of its tested forms.

Table 1: Results of screening the calibration set against the M4 muscarinic receptor.

ID	compound	Docking score
1	zotarolimus	-13.069656
2	cinchonidine	-11.904168
3	epicatechin gallate	-11.484274
4	smenospongine	-11.463371
5	cinchonidine	-11.313664
6	cinchonidine	-11.282499
8	cinchonidine	-10.865375
9	cinchonidine	-10.849771
10	cinchonidine	-10.829259
50	xanthohumol	-10.112581
51	cyanidin	-10.099655
52	tagitinin A	-10.097405

53	tagitinin A	-10.075704
54	retamine	-10.074217
55	**atropine**	**-10.0698**
56	coronaridine	-10.064226
57	codonopsine	-10.062986
58	(+)-B-hydrastine	-10.036566
59	cytisine	-10.02159
60	andrographolide	-10.009365
600	globulol	-8.595357
601	castanospermine	-8.593696
602	globulol	-8.591996
603	cularimine	-8.590668
604	aromadendrene	-8.590198
605	libertelenone A	-8.588863
606	bilobalide	-8.588075
607	tagitinin A	-8.585593
608	marrubin	-8.584779
609	phscion	-8.584485
610	curcumol	-8.583148
611	bilobalide	-8.583094
612	coronaridine	-8.582211
613	oxirapentyn	-8.579403
614	pannarine	-8.578128
615	erysonine	-8.577951
616	vincamine	-8.5775
617	guggulsterone	-8.576687
618	sorbicatechol B	-8.573372
619	guignardone I	-8.571961
620	asperuloside	-8.570495
621	libertelenone A	-8.569874
622	pyrethrosin	-8.56955
623	curcumol	-8.567171
624	sclareolide	-8.566422
625	hyodeoxycholic acid	-8.566392
626	diaporthein B	-8.565806
627	pyrethrosin	-8.564583
628	curcumol	-8.564456
629	harmine	-8.558875
630	marrubin	-8.558026
631	**cordycepin**	**-8.557376**

632	curcumol	-8.556963
633	pterostilbene	-8.55469
634	xohimbine hydrochloride	-8.553337
635	curcumol	-8.553222
636	chlorogenic acid	-8.552832
637	bilobalide	-8.552409
638	hispidulin	-8.549823
639	hispidulin	-8.549823
640	aesculin	-8.549679
641	bengenin	-8.549282
642	cedronin	-8.548042
643	artesunate	-8.54668
644	bengenin	-8.546206
645	libertelenone A	-8.543378
646	pyrethrosin	-8.541361
647	variolin B	-8.541068
648	aesculin	-8.538916
649	bactobolin	-8.537859
650	libertelenone A	-8.534627

The calibration set was screened against the muscarinic receptor to determine how well the positive nd negative control would bind to this receptor. The positive control scored in the top 3% of all the compounds in the set. The negative control scored in the top 34% of all the compounds in the set.

ID	Compound	Docking Score
1	sodium Danshensu	-9.421654
2	inosine.cdx	-9.362321
3	cyanidin	-9.312395
4	sodium Danshensu	-9.279765
5	**cordycepin**	**-9.132812**
6	sinigrin	-9.121926
7	delphinidin	-9.055094
8	guanosine	-9.049348
9	inosine	-9.046362
10	sinigrin	-8.998283
11	caffeic acid	-8.994107
12	inosine	-8.978397
13	makaluvic acid C	-8.970154
14	ferulic acid	-8.951257
15	ascochitine	-8.944649

16	piceatannol	-8.912427
17	guanosine	-8.89786
18	sinigrin	-8.897427
19	guanosine	-8.858956
20	inosine	-8.829351
21	arbutin	-8.785006
22	inosine	-8.765665
23	fumalic acid	-8.712759
24	arbutin.cdx	-8.691102
25	salicylic acid	-8.688791
26	inosine	-8.581025
27	guanosine	-8.56912
28	inosine	-8.548509
29	pelargonidin	-8.533512
30	guanosine	-8.529205
31	pyridoxamin	-8.502568
32	guanosine	-8.484077
33	guanosine	-8.475023
34	arbutin	-8.406821
35	3-Indolebutyric acid	-8.402934
36	balcalein	-8.282186
37	baicalein	-8.280543
38	inosine	-8.272413
39	arbutin	-8.24549
40	sinapic acid	-8.236322
41	gentisic acid	-8.179438
42	resveratrol	-8.16517
43	arbutin	-8.147971
44	guanosine	-8.141108
45	guanosine	-8.135229
46	alvatic acid	-8.096304
47	sinigrin	-8.089617
48	ribavarin	-8.074883
49	inosine	-7.998646
50	(-)-sydonic acid	-7.959159

Figure 2: Results of screening the calibration set against Adenosine Deaminase. The calibration set was screened against the enzyme to determine how well the positive and negative control would bind to this receptor. The positive control scored in the top 1% of all the compounds in the set. The negative control failed to successfully dock to the enzyme.

4. Discussion

The concept validation experiments supported the effectiveness of the calibration set as the positive control for each experiment were high scoring. However, the experiment did elucidate some areas of improvement. Many of the compounds which were run were not able to be scored. Upon further examination, it was determined that some of these failures were due to size. Due to these failures, it is suggested that future iterations of this project be either screened for a molecular weight under 500 Daltons or run against Lipinski's rules. This could be accomplished by either running the set through QikProp to fully run against Lipinski's rules or manually establishing molecular weight parameters that are in line with Lipinski's rule on molecular weight when preparing the Glide run. Additionally, another method needs to be determined to test larger molecules. This is a very important experimental direction which needs to be pursued.

Additionally, the size and diversity of the calibration set needs to be increased so as to increase the predictive power of calibration set. The set would need to be expanded to include a more representative diversity of alkaloids in addition to expanding the set to be more representative of the diversity which is present in the chemical space as a whole. The next set will need to be around 5,000 compounds. This would allow the calibration set to be used with more confidence and for the results to have more importance.

Lastly, a more robust validation system needs to be designed to further test the construct. The current system was sufficient for what it was designed, but would not be functional for much more. The new system would need to possess not only a greater diversity of compounds, but would also need to possess negative controls which are structurally similar to the positive control, but are not relevant with regards to the target protein. This would allow the experiment to test the calibration set not only to see if the positive control passes, but would also test to make sure that it passed for the right reason. This would further empower the calibration set.

Once the calibration set is functional and sufficiently powerful, it will be a very useful tool for determining the potential effectiveness of MOIs, and in conjunction with the rest of the natural product VTS system, would aid in the discovery and analysis of novel therapeutics which are derived from natural products.

5. Conclusion

The work involved in this project is a first step in a direction which seems to be a promising venture. The natural product calibration set which was designed for the Virtual Target Screening system was sufficiently effective and passed the controls, albeit not with the desired power in the case of the negative control for the first test. The further directions which were outlined in the discussion section would empower the calibration set and allow it to be useful for the interpretation of glide scores from VTS.

If this line of experimentation proves fruitful and the procedure's predictive value receives repeated biochemical validation, it would prove to be extremely beneficial to the field of drug design as it would allow for the minimization of the time required to produce a novel therapeutic. This minimized time investment would allow clinicians to utilize drugs on an accelerated timeline and should lead to a decreased cost of drug design and development.

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