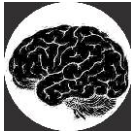
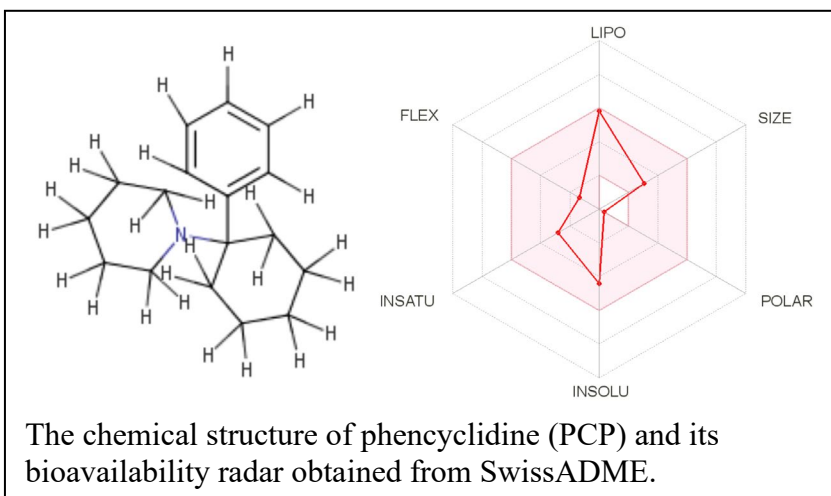

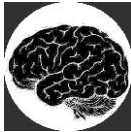
	<p align="center">SKILLS CENTER STANDARD OPERATING PROCEDURE</p>	<p align="center">A BIOFIZZ  PRODUCTON</p>
<p align="center"><i>In-silico</i> Drug Screening 2</p>	<p align="center">Effective Date: 03/05/2021</p> <p align="center">Module Hours 2</p>	<p align="center">Revision # 1.0</p> <p align="center">M. Guzie Checked by A. Siclair</p>

BACKGROUND

When it comes to designing an effective therapeutic drug, there are more factors to consider beyond the compound's affinity for the target. While a drug's ability to bind to the target molecule is of utmost importance in a drug screening assay and a good starting place for identifying potentially useful drugs, high target affinity is only one of the necessary characteristics of a good drug. An effective drug must be able to bind its target with high specificity, limiting any undesired reactions with other molecules in the body that could be harmful. Considering the vast amount of unique biological macromolecules in the body, this seems like a nearly impossible feat: designing a relatively small yet unique enough compound which will not have any adverse reactions in the body and only bind the target molecule. Fortunately, vast and quickly growing databases of known proteins are accessible to molecular biologists and pharmacologists attempting to design suitable medicines, and *in-silico* analysis allows for compounds to be assessed for unwanted binding and other important pharmacological properties as well.

Other important properties to consider of the compound involve its ability to reach the target area of the body in adequate concentrations, without being degraded and being in an active form, as well as being able to stay in that area of the body for long enough in which it will exert its biologically therapeutic effects. Yet still, drug designers must also assess the half life of the drug and how long it will remain in the body; if the body cannot degrade it in an adequate and timely manner, it may be toxic. These characteristics can be summarized by the ADME parameters: absorption, distribution, metabolism, and excretion. These four factors can indicate how a drug will be processed by the body and therefore have been a set standard for drug design. Computer models have allowed for the analysis of these qualities in compounds, greatly decreasing costs and offering insight into a drug's possible undesired effects before entering physical trials. The Swiss Institute of Bioinformatics created a web-based program which allows anyone to assess these qualities of a compound. In this module, the study of *in-silico* drug screening will be continued by assessing the pharmacokinetics of compounds



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using SwissADME (Daina, et al., 2017).

1. PURPOSE

The purpose of this procedure is to recognize the importance of *in-silico* analysis, and how it can be used to assess the kinetic and metabolic qualities of drug candidates in the body.

2. SCOPE



This procedure applies to qualified skills center users.

3. RESPONSIBILITY

- 3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
- 3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
- 3.3. It is the responsibility of the user to become trained in the use of this application.

4. DEFINITIONS

- 4.1. Pharmacokinetics: The study of a substance's overall movement within the body.
- 4.2. ADME Parameters: A set of pharmacokinetic properties which are used to analyze how a substance will be absorbed, distributed, metabolized, and excreted from the body. *In-silico* screening now allows these to be examined earlier in the drug design process.
- 4.3. Absorption: The process of a drug moving from its site of delivery into the bloodstream.
- 4.4. Distribution: The reversible transfer of a drug from one location to another within the body.
- 4.5. Metabolism: The biotransformation of pharmaceutical substances in the body so that they can be eliminated more easily.
- 4.6. Excretion: The removal of drugs and their metabolites from the body.
- 4.7. Lipophilicity: The ability of a chemical compound to dissolve in fats, oils, lipids, and non-polar solvents.
- 4.8. Size: The size of a molecule, usually quantified by its molecular weight measured in Daltons.
- 4.9. Polarity: The overall charge distribution in a given molecule.

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- 4.10. Solubility: The ability of a substance to dissolve in a solvent.
- 4.11. Saturation: The point at which a solution of a substance can dissolve no more of that substance.
- 4.12. Flexibility: The ability of a molecule's bonds to extend, shorten, and rotate in three-dimensional space.

5. MATERIALS/EQUIPMENT

- 5.1. A computer to perform the procedure.
- 5.2. The SwissADME database.



6. PROCEDURE

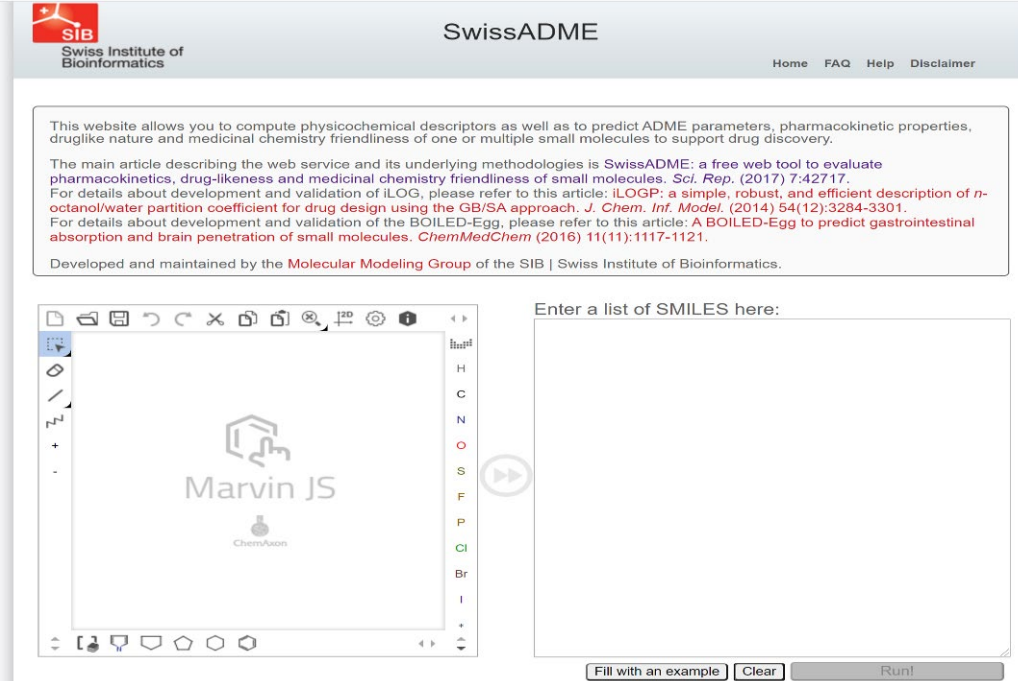
In this procedure, the web-based SwissADME program will be used to assess the pharmacokinetic and metabolic properties of a ligand that are important to the development of an effective, safe drug.

6.1. Running a Pharmacokinetic Analysis of a Compound

6.1.1. Go to the SwissADME program on the Swiss Institute of Bioinformatics website to run an ADME analysis on a compound: <http://www.swissadme.ch/index.php>.



6.1.2. The picture below shows the format of the program. On the left is the input box and on the right is the SMILES list of molecules to be ran for assessment.

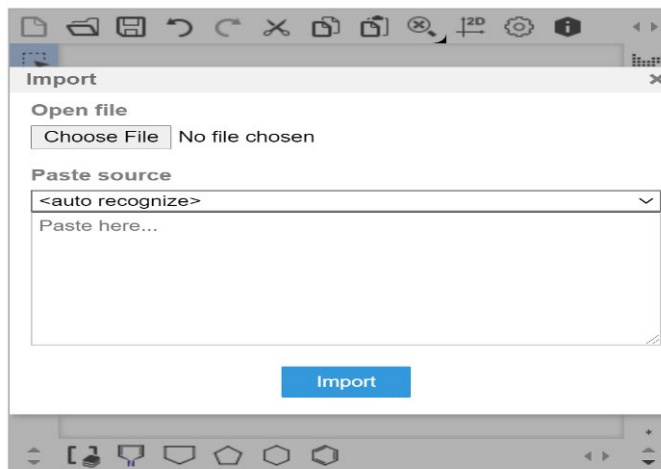
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The screenshot shows the SwissADME web application. At the top, there is a header with the SIB logo and the text 'Swiss Institute of Bioinformatics'. Below the header, there is a navigation menu with links for 'Home', 'FAQ', 'Help', and 'Disclaimer'. The main content area contains a paragraph of text describing the service and its purpose, followed by a list of references and a note about the development and maintenance of the tool. Below the text, there is a large text input field labeled 'Enter a list of SMILES here:'. To the left of this field is a 'Marvin JS' molecular sketcher window, which is currently empty and shows the 'ChemAxon' logo. At the bottom of the page, there are three buttons: 'Fill with an example', 'Clear', and 'Run!'.

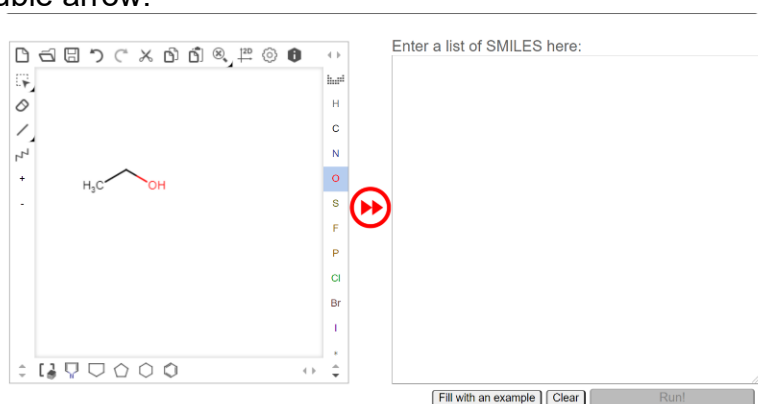
Molecules can be drawn from scratch in the Marvin molecular sketcher, or they can be uploaded/imported directly (and then edited in the molecular sketcher if desired.) By clicking on the “Import” button, a file can be uploaded or imported (shown below). The “Paste Source” box will list the file types which will be recognized. One of these file types is SMILES; SMILES files can be downloaded from the ZINC ligand database: <https://zinc.docking.org/>. Note that “Name” is also an option for the “Paste Source” box- a structure can be imported simply by searching it by name.

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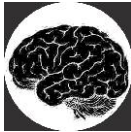


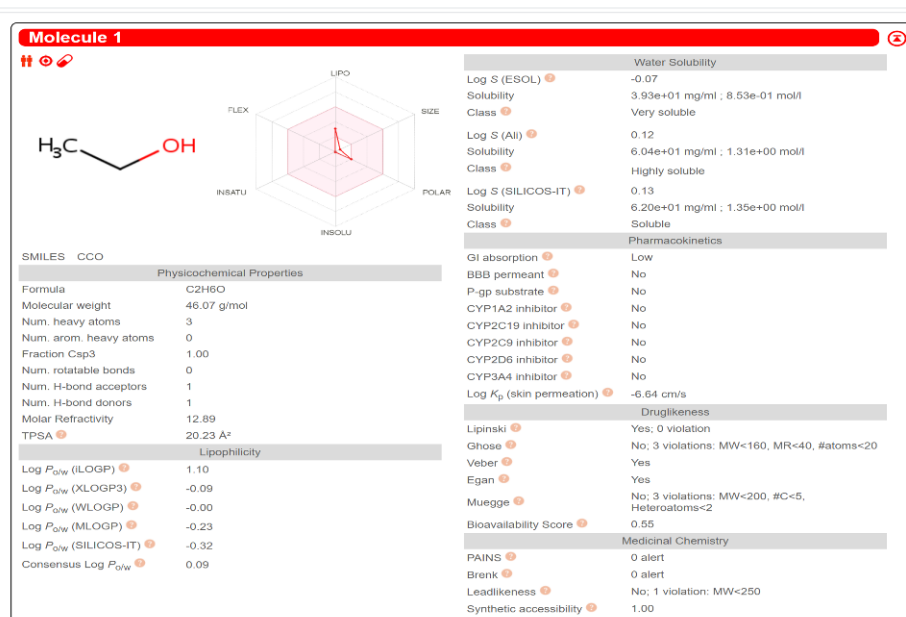
Any of these options are suitable methods for importing a compound. Choose a molecule and either draw it or import it.

6.1.3. Once a molecule is in the input box, the transfer button (red double arrow) will become active. Select this button to move the molecules from the input box into the SMILES box. The picture below shows a sketch of ethanol which will be transferred to the SMILES list by using the red double arrow.



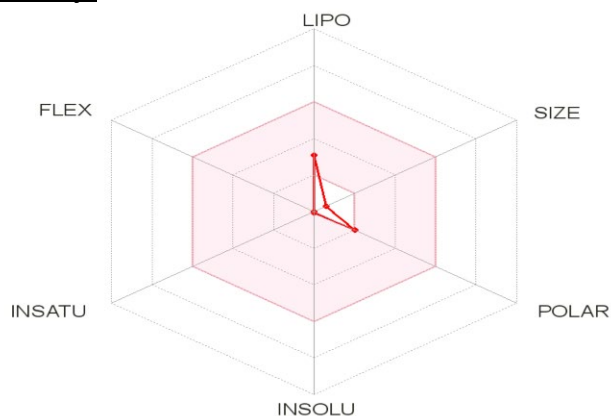
6.1.4. Once the molecule(s) have made their way to the SMILES box, hit the red “Run!” button to run an analysis. Note that multiple molecules can be inputted into the SMILES box and be ran at once. Results will be shown in the same web page, below the input panel as such:

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SwissADME results for ethanol. Hovering the cursor over the small orange question marks will give more information about the meaning of the value and how it was obtained.

6.1.5. The diagram next to the structure of the molecule is called the Bioavailability Radar. This radar takes 6 factors into account to determine a molecules overall ability to act as an effective drug in the body.



The values are (starting at the top and going clockwise) lipophilicity, size, polarity, solubility, saturation, and flexibility (see DEFINITIONS section for more explanation of these properties). The shaded pink area represents the regions in which a compound has to fall within to be considered “Drug-like”, that is, active and available in the body for enough time to access

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cells and make a distinctive chemical change. The ideal range for each value represented by the shaded pink region is:

lipophilicity: between -0.7 and +5.0

size: molecular weight between 150 and 500 g/mol

polarity: topological polar surface area between 20 and 130 Å²

solubility: log *S* not higher than 6

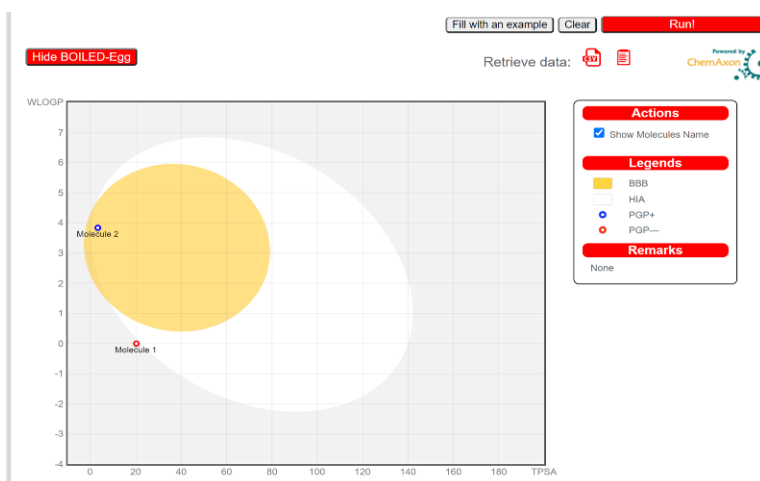
saturation: fraction of carbons in the sp³ hybridization not less than 0.25

flexibility: no more than 9 rotatable bonds.

The rest of the data obtained under “Physiological Properties”, “Lipophilicity”, “Water Solubility”, “Pharmacokinetics”, “Drug-Likeness”, and “Medicinal Chemistry” give numerical values applying to some of these properties assessed in the bioavailability radar. (Daina, et al., 2017).


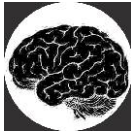
6.2. Analysis of The Boiled-Egg

6.2.1. Beneath the Marvin sketch input box, there will be a red button which says “Show BOILED-Egg.” Select this option.



The BOILED-Egg model takes lipophilicity and polarity into account when assessing the passage of a molecule into the gastrointestinal tract (HIA, Human Intestinal Absorption, represented by white oval) and the passage of a molecule into the brain (BBB, Blood Brain Barrier, represented by the yellow circle YOLK of the egg).

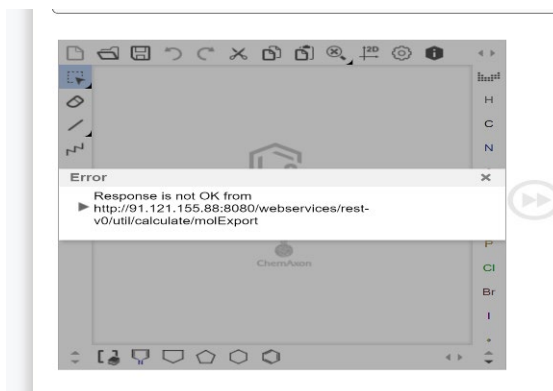
6.2.2. In this picture, the points are labeled (“Show Molecules Name” selected) but the structure of the molecule can also be observed by hovering the mouse over the points. Here, molecule 1 (red) is ethanol and the diagram indicates that it is passively absorbed by the

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intestines but not the blood brain barrier (most likely due to polarity.) Molecule 2 (blue) is phencyclidine, an aromatic compound consisted of all hydrocarbons except one nitrogen atom, constituting its non-polarity which allows for its passage into the brain through the blood brain barrier. As ethanol is smaller, and its oxygen is not hidden by many non-polar residues, its overall topological polar surface area has a greater effect on the molecule's polarity.

7. TROUBLE SHOOTING

7.2. "Error: Response is not OK."





Obtaining this result when trying to import a ligand file means that the file being imported is not supported/recognized by SwissADME. To browse which files are supported, go through the "Paste Source" box when in the import screen. The easiest fix to this solution would be: trying to import a molecule by name, simply drawing the molecule, or searching the molecule on the ZINC database (<https://zinc.docking.org/>) and downloading the ligand as a SMILES file then importing it.

8. REFERENCES

Daina, A., Michielin, O., & Zoete, V. (2017, March 03). *Swissadme: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules*. Retrieved from <https://www.nature.com/articles/srep42717>.

9. MODULE MASTERY TASK

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Select several unique compounds (4) for analysis using SwissADME following the parameters below.

9.1. If you have already completed the *In-Silico* Drug Screening 1 SOP, select the drug candidate you analyzed in that module and run its analysis through SwissADME. If not, select any drug compound of interest. Based on the results from the bioavailability radar, physiological properties, and BOILED-Egg diagram, do you think this drug would be effective getting to the targeted area of the body? Write a one paragraph response on whether you think your drug would be an effective candidate based on the results from BOTH *in-silico* drug screening 1 (target-ligand binding) and *in-silico* drug screening 2 (pharmacokinetic properties) and why.

9.2. Select three compounds with the anticipation of getting a BOILED-Egg diagram with one molecule in the yolk, one molecule in the white, and one molecule outside of the entire egg. Use what you know about physiological properties, specifically lipophilicity and polarity, to select three compounds which you think will yield these results. Take a screenshot of the BOILED-Egg diagram for submission, along with a conclusive sentence on each molecule describing why you picked that molecule, and what properties allowed you to make the judgement of where it would land in the BOILED-Egg diagram.