

# **FORECASTER Suite 2019**

## **IMPACTS, SMART, AND CONVERT**

### **Tutorial**

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## ***I. What will be Covered in this Tutorial***

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This example demonstrates the site of metabolism prediction using our IMPACTS module. You will draw a small molecule using our Sketcher, convert it to 3D (using CONVERT), and model how it would be oxidized by cytochrome P450 2C9 using IMPACTS.

**Why use this workflow?** Cytochrome P450s (CYPs) are the major metabolizing enzyme found in the liver; they account for approximately 75-80% of all xenobiotic metabolism. Consequently, they are of extreme importance to drug discovery. If a drug candidate is metabolized too quickly, then perhaps it cannot exhibit its therapeutic potential in vivo and, conversely, if metabolized too slowly, then perhaps it acts erratically with off-targets due to a concentration build-up. Additionally, the oxidized metabolite may itself be toxic and is worthy of consideration. Knowing how a drug will be metabolized by CYPs is a first step in approaching these potential obstacles.

**How long will the tutorial take?** The tutorial itself is run at your own pace. The workflow takes a few minutes to complete, depending on your computing hardware. A standard laptop should handle the experiment in 3-5 minutes. The engine behind IMPACTS is largely similar to docking and thus the timeframe for a calculation is similar to FITTED.

### **FAQ.**

1. IMPACTS uses a trivalent approach to predicting sites of metabolism; it considers ligand reactivity, structural orientation within the CYP, and the transition state energy of the complex.
2. This tutorial demonstrates oxidation by CYP 2C9, but you will see that you can model the seven major CYPs.
3. The output from IMPACTS is provided in both 2D and 3D. The 3D transition state complex is especially useful for design purposes—a medicinal chemist can creatively make changes to the molecule:
  - a. Strengthening interactions with the CYP in binding site regions not close to the heme reactive center to perhaps increase oxidation by further stabilizing the transition state.
  - b. Disrupt interactions with the CYP in binding site regions not close to the heme reactive center to lessen the oxidation or favor a different site of metabolism.
  - c. Block the site of metabolism in an attempt to increase the half-life of the drug. This does not necessarily require 3D structures.

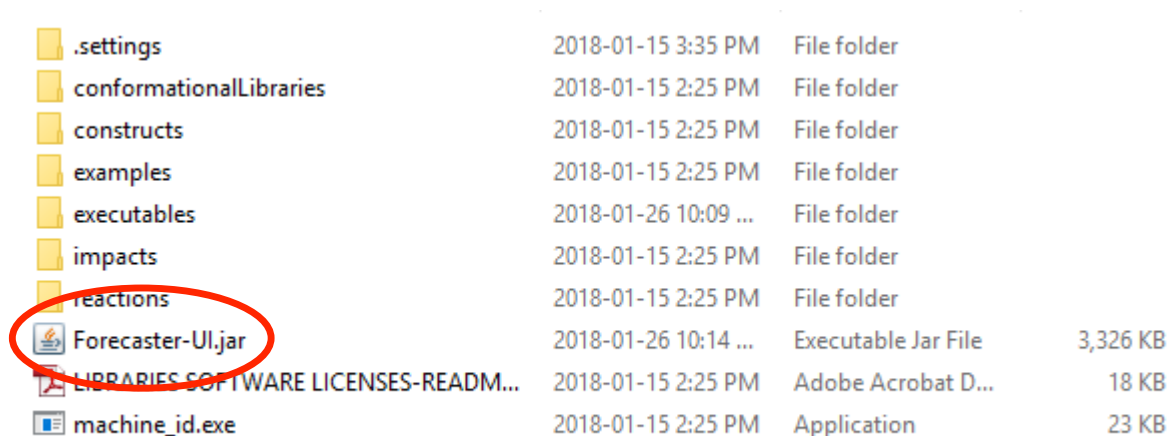
## II. Running FORECASTER with the User Interface

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The user interface (UI) can be started by double clicking on the `Forecaster-UI.jar` file in the `Forecaster` folder. This will open the main window.

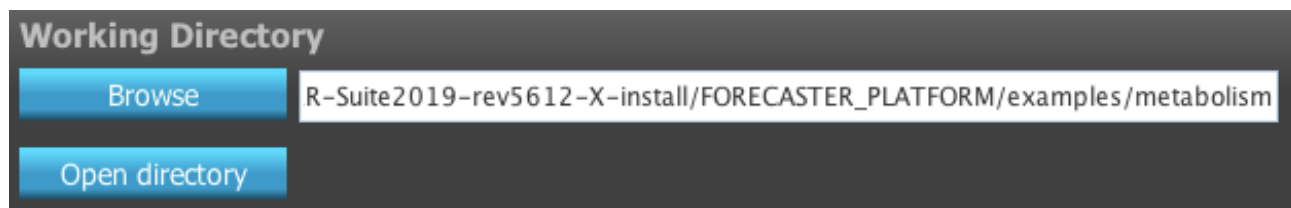
Under Linux and Mac OSX, it is recommended to launch it from a terminal window by typing the command below. Make sure that you are located in the folder where this jar file is.

```
Forecaster@Linux/Forecaster:~$ java -jar Forecaster-UI.jar
```

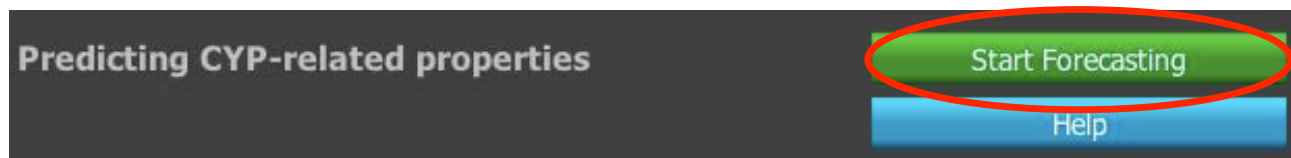


.settings	2018-01-15 3:35 PM	File folder	
conformationalLibraries	2018-01-15 2:25 PM	File folder	
constructs	2018-01-15 2:25 PM	File folder	
examples	2018-01-15 2:25 PM	File folder	
executables	2018-01-26 10:09 ...	File folder	
impacts	2018-01-15 2:25 PM	File folder	
reactions	2018-01-15 2:25 PM	File folder	
Forecaster-UI.jar	2018-01-26 10:14 ...	Executable Jar File	3,326 KB
LIBRARIES SOFTWARE LICENSES-READM...	2018-01-15 2:25 PM	Adobe Acrobat D...	18 KB
machine_id.exe	2018-01-15 2:25 PM	Application	23 KB

The first step is to set the working directory. Click **Browse**, under **Working Directory**. You will be prompted to navigate to the folder where you will save various files while working with FORECASTER. For this tutorial, go to “examples” and then to “metabolism”.



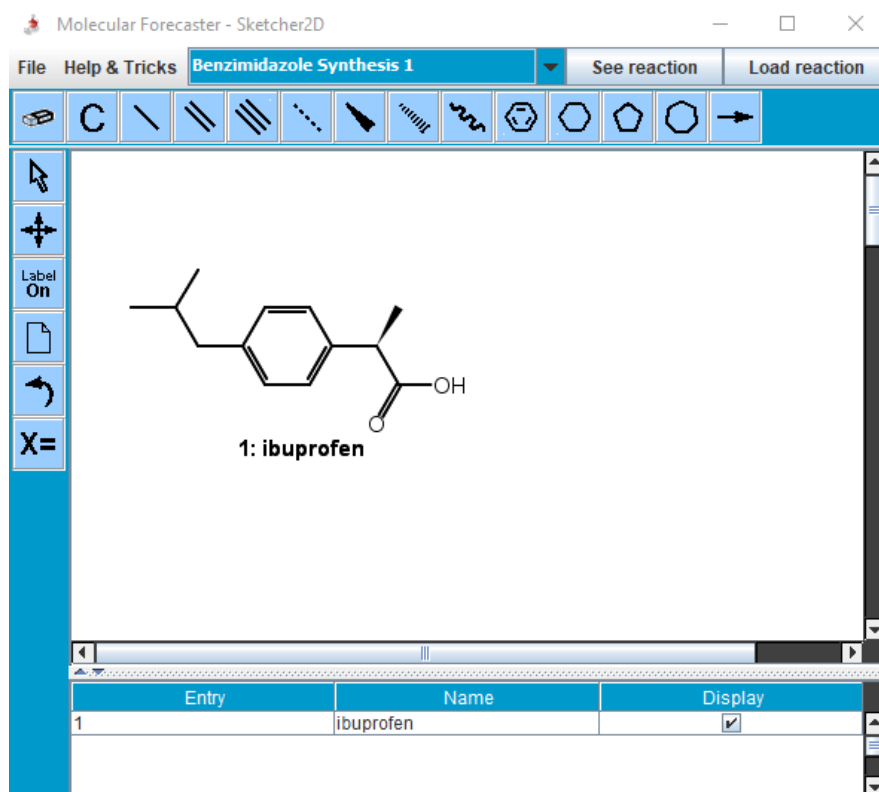
You may then click **Start Forecasting** to expand the “Predicting CYP-related properties” workflow.



### III. Sites of metabolism prediction

#### 1. SKETCHER2D and CONVERT: The conversion of the ligand from 2D to 3D

CONVERT will convert a 2D molecule into an energy-minimized 3D structure. The 2D molecule can either be imported as a file (supported input formats are mol and sdf) or drawn directly using the integrated sketcher. In this example, the file “ibuprofen.sdf” is included in the tutorial folder, or you may draw the molecule with the sketcher. Click the gear icon in the SKETCHER2D box to open the sketcher window.



Once the Ibuprofen molecule is drawn, the file is saved by clicking on **File** and **Save structure (sdf)**. After saving, the sketcher can be closed.

Clicking on the gear icon in the CONVERT box will open the parameters section. The newly created ligand file (ibuprofen.sdf) should automatically be selected as the **Input file**. The **Output file** can be set to any desired name and the **Mode** to “2D to 3D (mol2)”. You can also select a **Solvent** for different protonations. CONVERT adds hydrogens when they are missing, but will not change the ionization state if hydrogens are already included.

### Settings

Source of molecule structures	From working directory
Input file	ibuprofen.sdf
Output file name	molecule3D
Mode	2D to 3D (mol2)
Solvent	Water
Generate tautomers	No

Once all the parameters are set, the keyword file needs to be written by clicking the **Save** button. Clicking the **Exit** button will close the CONVERT parameters section and return to the main workflow. A green check should now appear in the CONVERT box.

### Running CONVERT

Edit Parameter File	
<b>Save</b>	parameters-convert.txt

## 2. SMART: The setup of the ligand for sites of metabolism prediction

SMART will setup the ligand (3D molecules with hydrogen atoms only) for the SOM prediction with IMPACTS. Once again, click on the gear icon to open the parameters. The **Source of ligand structures** should be set to “From CONVERT box above”. The file that will be created by CONVERT will automatically be selected as the **Input file**. The **Output file name** can be filled with any desired name. The **Mode** should be set to “Interaction with CYPs”.

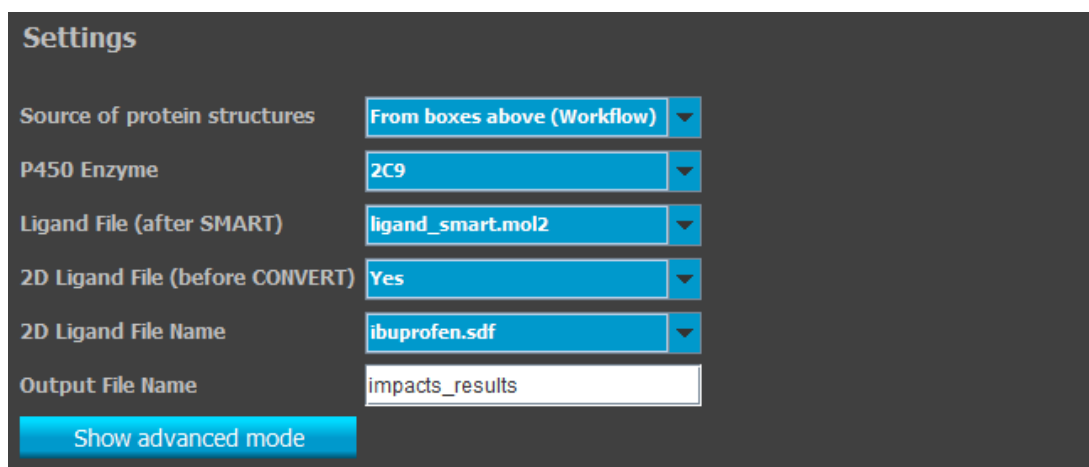
### Settings

Source of ligand structures	From CONVERT box (Workflow)
Output file name	ligand_smart
Mode	Interaction with CYPs

Once the settings are complete, the keyword file needs to be written by clicking the **Save** button. Clicking the **Exit** button will close the SMART parameters section and return to the main workflow. A green check should now appear in the SMART box.

### 3. IMPACTS: Sites of metabolism predictions

The **Source of structures** should be set to “From SMART box above”. The required P450 enzyme (CYP) protein structures will be copied automatically to the working directory when the workflow is started. For this tutorial, the **P450 Enzyme** should be set to “2C9”. The **Ligand file** is the ligand prepared by SMART (`ligand_smart.mol2`, if the default name was used). The **2D Ligand File** should be set to “yes” and **2D Ligand File Name** to the input 2D molecule (`ibuprofen.sdf`). This 2D file (when available) will be used to create the metabolite representations in 2D (can be viewed in the sketcher). The **Output File Name** can be filled with any desired name; “`impacts_results`” is suggested.



Settings

Source of protein structures	From boxes above (Workflow)
P450 Enzyme	2C9
Ligand File (after SMART)	ligand_smart.mol2
2D Ligand File (before CONVERT)	Yes
2D Ligand File Name	ibuprofen.sdf
Output File Name	impacts_results

Show advanced mode

The keyword may then be written by clicking the **Save** button. Click the **Exit** button to return to the main workflow. A green check should now appear in the IMPACTS box.

### 4. Workflow: Running the sites of metabolism workflow

Once all the included steps are ready, the workflow can be executed by clicking the **Run workflow** button.



The programs run in a terminal (e.g., dos in Windows) and once the complete workflow is complete, the terminal window will close automatically. Do not close it manually.

## IV. Results Analysis

During the prediction process, several files will be generated. CONVERT will generate a new ligand. Smart will generate a new ligand (in FITTED mol2 format) and an output file (out format). To verify the integrity of any of these files, click the **magnifying glass** of the corresponding step.

IMPACTS will generate two structural files. The file `impacts_Metabolites3D.sdf` contains the 3D conformation of the oxidized molecule in complex with the heme's iron. This file can be visualized by clicking the **magnifier icon** in the IMPACTS box. Clicking the **Metabolites in 3D / Binding site** button will open the results in the 3D viewer. Within this 3D viewer, a table of data with the corresponding sites of metabolism will be available to easily visualize and analyze the predicted 3D poses. Alternatively, the results are compatible with most 3D graphical program.

Entry	Name	Display	none	IMPACTS_ID	IMPACTS_R...	IMPACTS_C...	IMPACTS_S...	IMPACTS_S...	IMPACTS_S...	IMPACTS_E...	IMPACTS_M...
1	CYP2C9 bi...	<input checked="" type="checkbox"/>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	ibuprofen m...	<input checked="" type="checkbox"/>	n/a	impacts_M...	1	CYP2C9	24	1.1000	-5.0222	255.6761	0.0000
3	ibuprofen m...	<input type="checkbox"/>	n/a	impacts_M...	2	CYP2C9	26	1.1097	-8.0717	297.9020	0.0000

The file `impacts_Metabolites2D.sdf` contains 2D structures of the two possible metabolites. Clicking the **Metabolites in 2D** button will open the results in a 2D viewer. Within this 2D viewer, a table of data with the score and energy values of the corresponding sites of metabolism will be available to easily visualize the results. Alternatively, the 2D results can also be visualized in your favorite 2D sketcher program.



Molecular Forecaster - Sketcher2D

File Help & Tricks Benzimidazole Synthesis 1 See reaction Load reaction

**1: ibuprofen metabolite 1-1**  
CYP: CYP2C9  
Score: -7.3528  
Energy: 248.5732

**2: ibuprofen metabolite 1-2**  
CYP: CYP2C9  
Score: -7.8528  
Energy: 292.6432

Entry	Name	Display	CYP	Score	Energy
1	ibuprofen metabolit...	<input checked="" type="checkbox"/>	CYP2C9	-7.3528	248.5732
2	ibuprofen metabolit...	<input checked="" type="checkbox"/>	CYP2C9	-7.8528	292.6432

The other output files generated are `impacts.log` (log file for potential errors), `impacts.out` (detailed output file), and `impacts-results.txt` (summary results text file).