FORECASTER Suite 2018 Tutorial

Predicting Sites of Metabolism – IMPACTS

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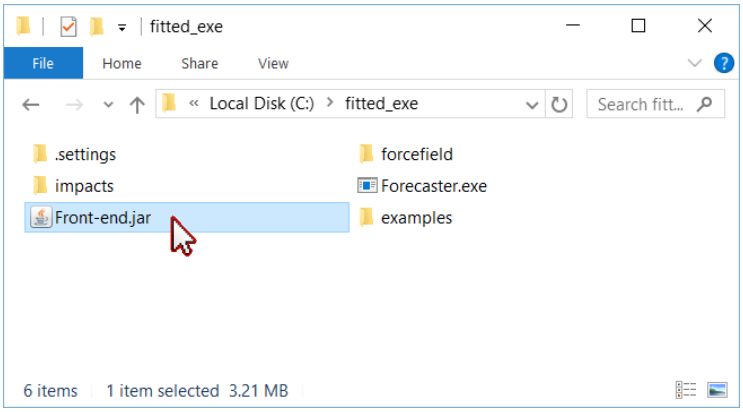
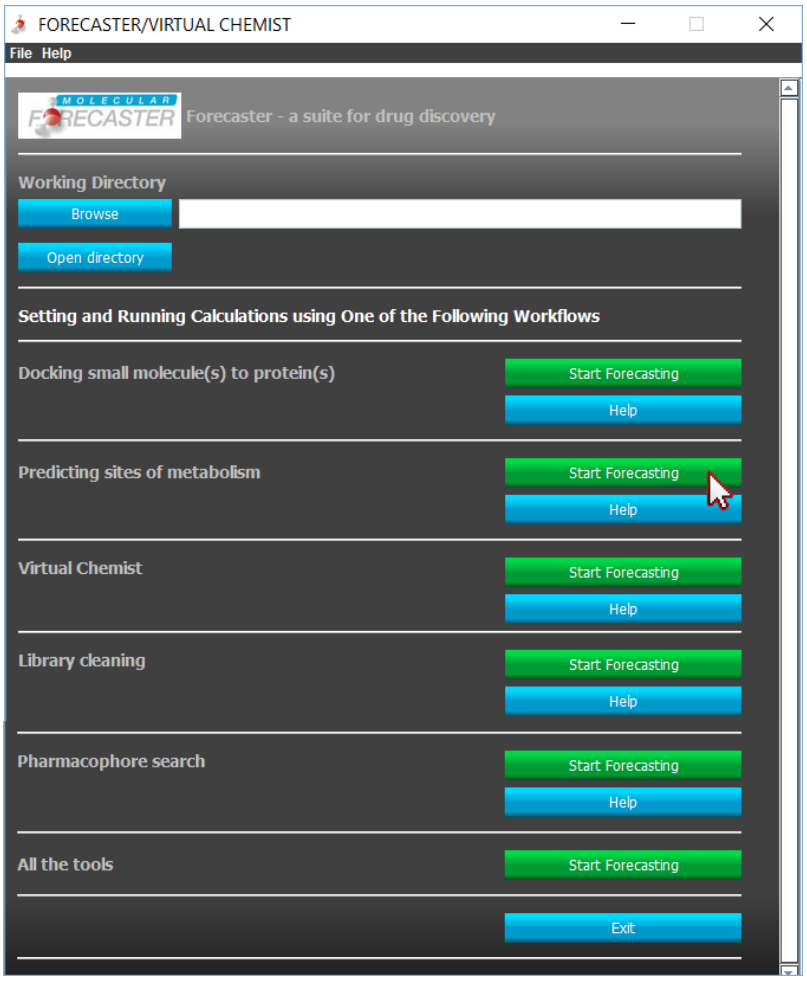
I. Running FORECASTER in Windows with the GUI

From a single molecule drawn in 2D, we will predict the two possible sites of metabolism (SOM) from the CYP450 using IMPACTS. The example will use ibuprofen and the 2C9 cytochrome P450 structure. Under windows, the graphical user interface (GUI) can be started by double clicking on the `Front-end.jar` file in the `fitted_exe` folder.

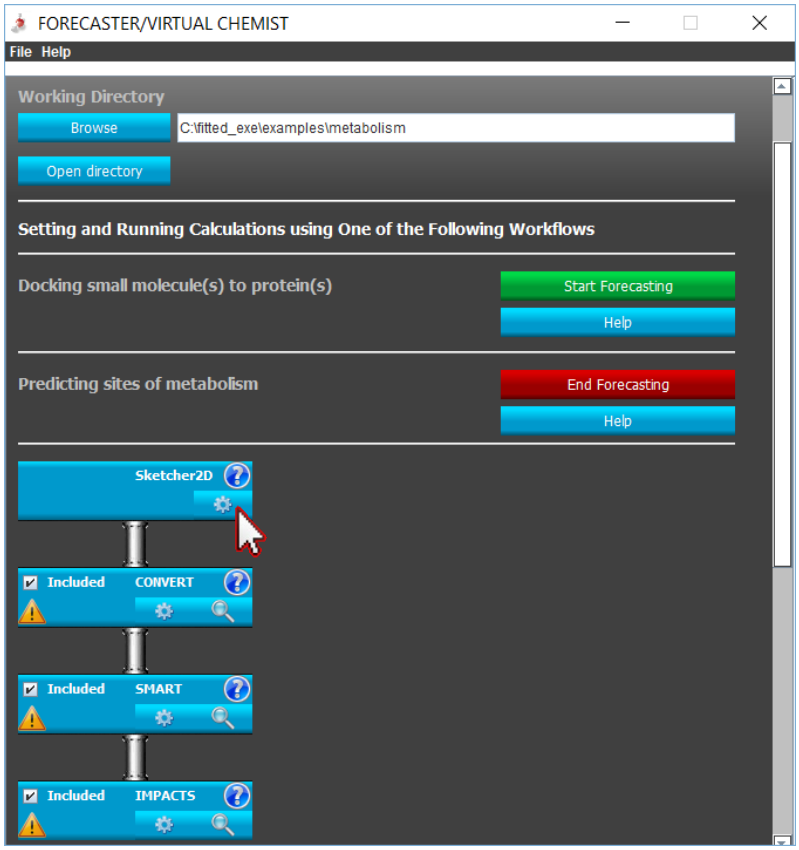


The GUI will open with Java and a list of workflows will be available. Clicking the **Start Forecasting** button will expand the workflow for the prediction of sites of metabolism.

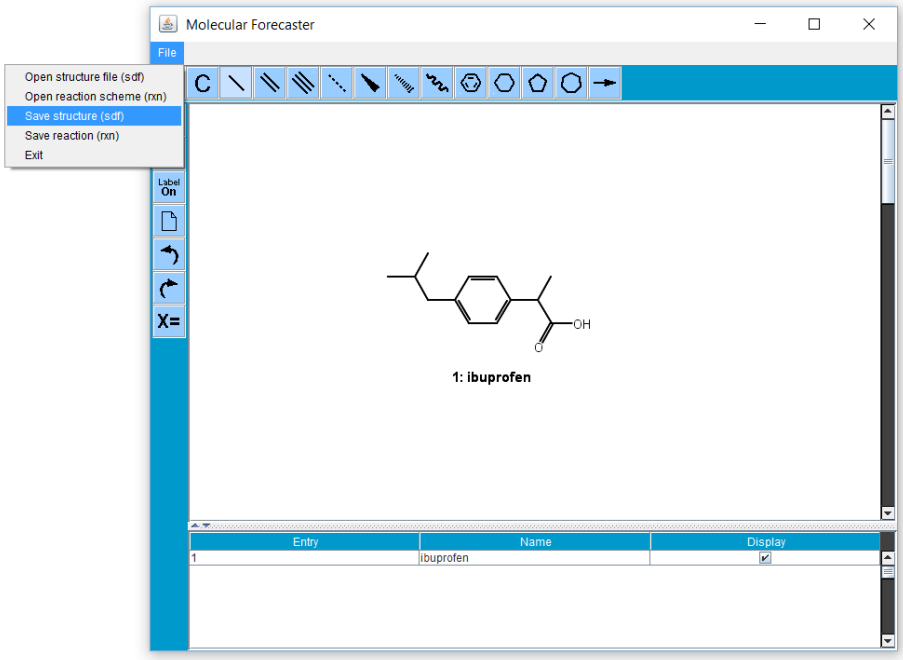


II. Sites of metabolism prediction

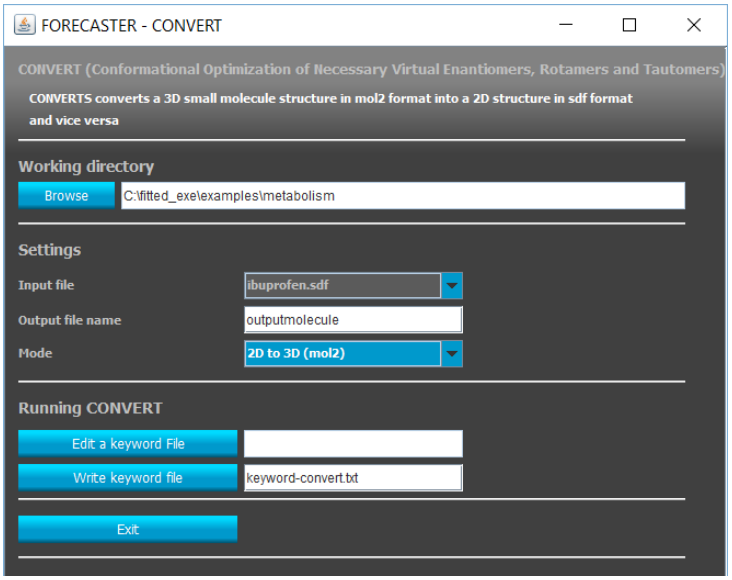
CONVERT: The conversion of the ligand from 2D to 3D



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 2D sketcher. In this example, we will draw the molecule with the sketcher. The first step is to set the working directory. This is done by clicking the ***Browse*** button at the top left of the GUI. You will be prompted to navigate to (or create) the desired folder. We will be using `C:\fitted_exe\examples\metabolism` for the current example.



Clicking the gear icon in the Sketcher2D box within the sites of metabolism workflow will open the sketcher window. Once the ibuprofen molecule is drawn, the file is saved by clicking on **File** and **Save structure (sdf)**. After providing a filename (`ibuprofen.sdf`), the sketcher can be closed. Clicking on the gear icon in the CONVERT box will open the parameters section. The newly created ligand

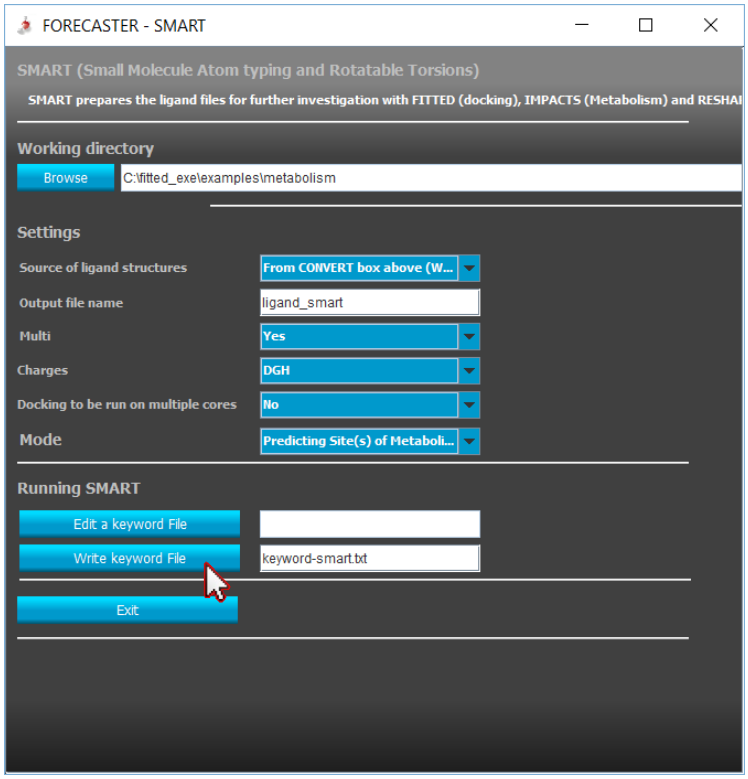


file (`ibuprofen.sdf`) should be available in the **Input file** menu. The **Ouput file** can be set to "outputmolecule" and the **Mode** to "2D to 3D (mol2)". Once all the parameters are set, the keyword file needs to be written by clicking the **Write keyword file** button. Clicking the **Exit** button will close the CONVERT parameters section and return to the main workflow. A green check should now appear in the CONVERT box. The program will not run until the complete workflow is ready.

Once the workflow will be complete, this step will generate a new ligand (in mol2 format) named `ouputmolecule.mol2` and an output file named `outputmolecule.out`. At this stage, you can make sure there is no problem during the 3D conversion of the ligand. For this purpose, click on the magnifying glass on the CONVERT box of the workflow.

SMART: The setup of the ligand for sites of metabolism prediction

SMART will setup the ligand (3D molecules only) for the SOM prediction with IMPACTS. Since we are preparing a workflow, the files generated from the previous programs should be made available for the next step even if they are not yet physically present in the working directory. Thus, the **Source of ligand structures** should be set to "From CONVERT box above". The file that will be created by CONVERT (`outputmolecule.mol2`) will be automatically selected as the **Input file**. The **Output file name** can be filled with any desired name, "ligand_smart" is suggested. The atomic partial charges method can be selected under **Charges**. The methods available are DGH (recommended), none (no charge), and Input (will keep input file charges). The **Mode** should be set



to “Predicting Site(s) of Metabolism”. Once the settings are complete, the keyword file needs to be written by clicking the **Write keyword file** 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. The program will not run until the complete workflow is ready.

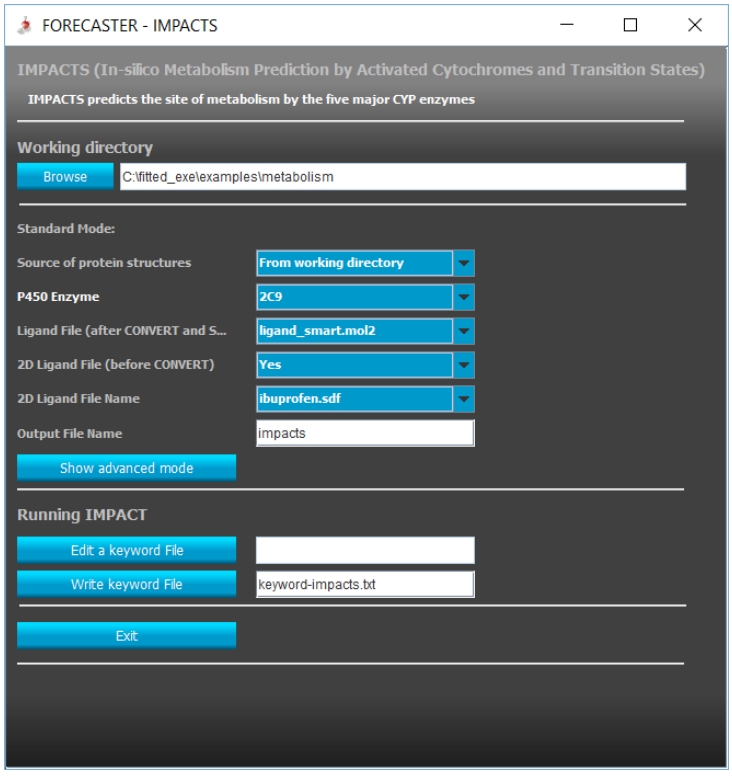
Later when the workflow will be run, it is recommended to open the output file and verify the potential errors after the workflow has completed. This action will generate a new ligand (in FITTED mol2 format) named `ligand_smart.mol2` and an output file named `ligand_smart.out`.

IMPACTS: Sites of metabolism predictions

The **Source of structures** should be set to “From working directory”. The required CYP450 protein structures will be copied automatically to the working directory upon launching of the workflow. For this tutorial, the **P450 Enzyme** should be set to “2C9”.

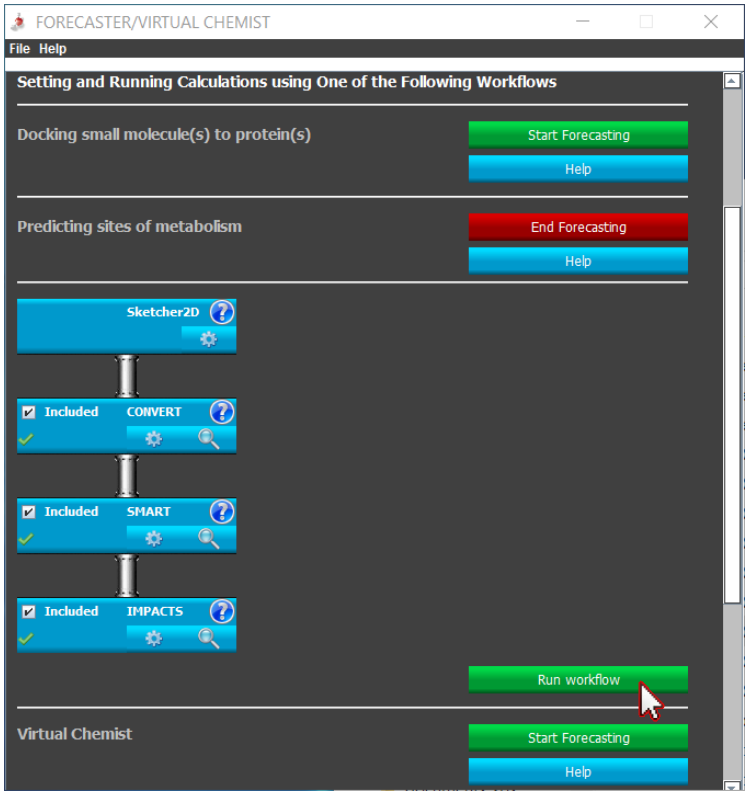
The **Ligand file** is the ligand that will be prepared by SMART (`ligand_smart.mol2`). The **2D Ligand File** should be set to “yes” and **2D Ligand File Name** to the input 2D molecule (`ibuprofen.sdf`). This 2D file (when available) will be used to create the metabolite representations in 2D (can be viewed in the sketcher). The **Output File Name** can be filled with any desired name but “impacts” is suggested.

The keyword may then be written by clicking the **Write keyword file** button. Clicking the **Exit** button will close the IMPACTS parameters section and return to the main workflow. A green check should now appear in the IMPACTS box. The program will not run until the execution of the workflow is launched.

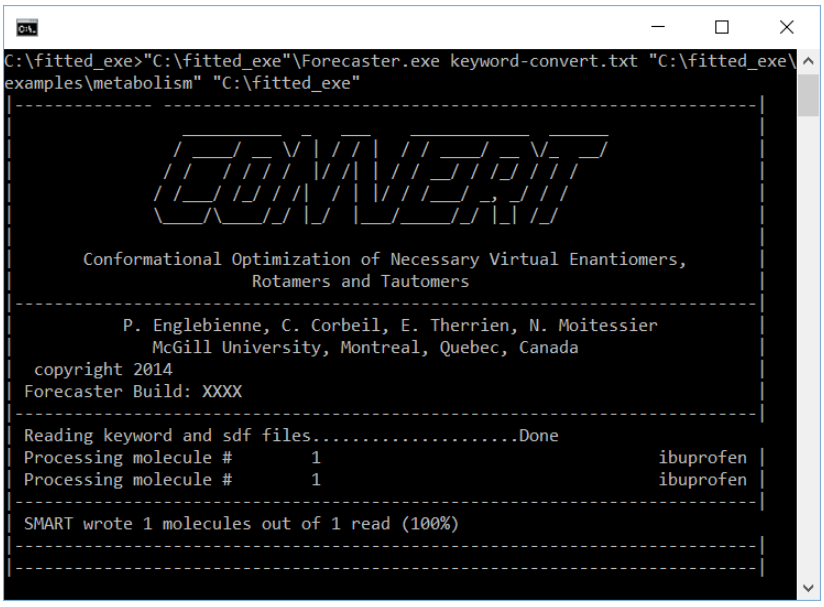


Workflow: Running the sites of metabolism workflow

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) and once the complete workflow is complete, the terminal window will close.

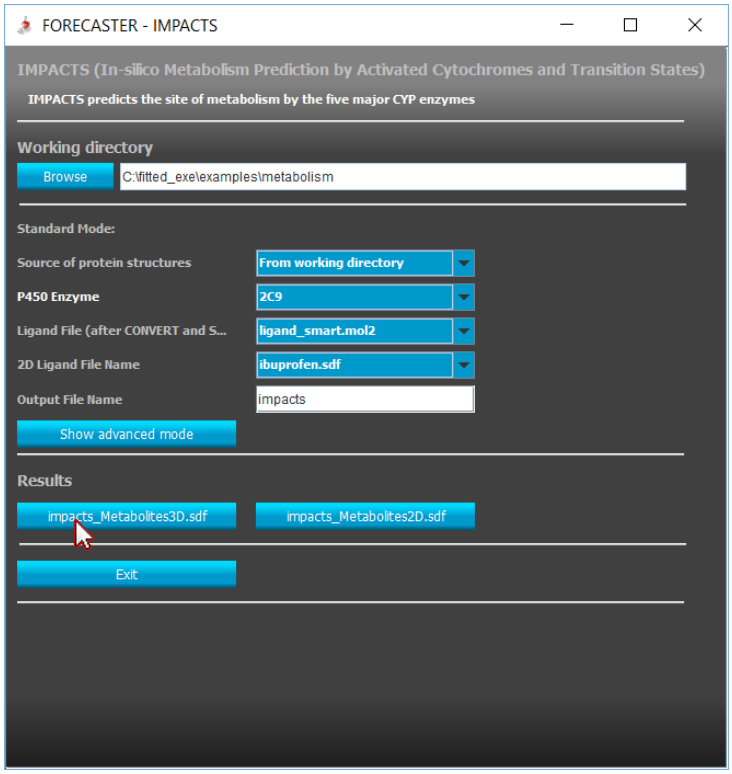


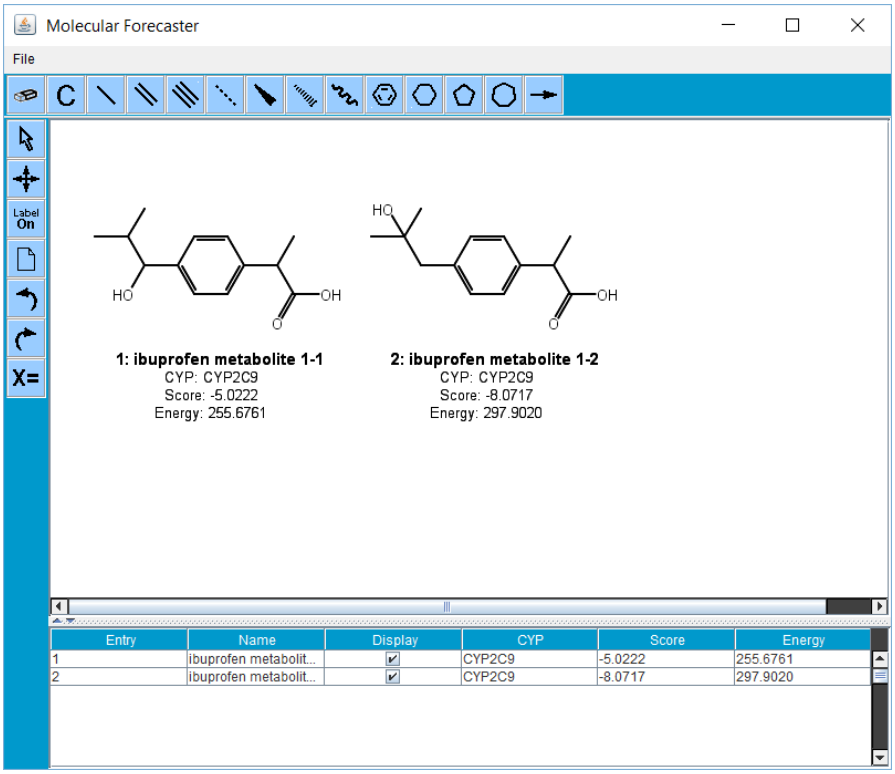
Once the prediction is complete, the results files will be available in the working directory. The IMPACTS program will generate two structural files. The file `impacts_Metabolites3D.sdf` contains the 3D conformation of the oxidized molecule in complex with the heme's iron. This files can be visualized in your favorite 3D graphical program along with the corresponding P450 protein structure. The file `impacts_Metabolites2D.sdf` contains 2D structures of the 2 possible metabolites. This file can be visualized in your favorite 2D sketcher program. 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).

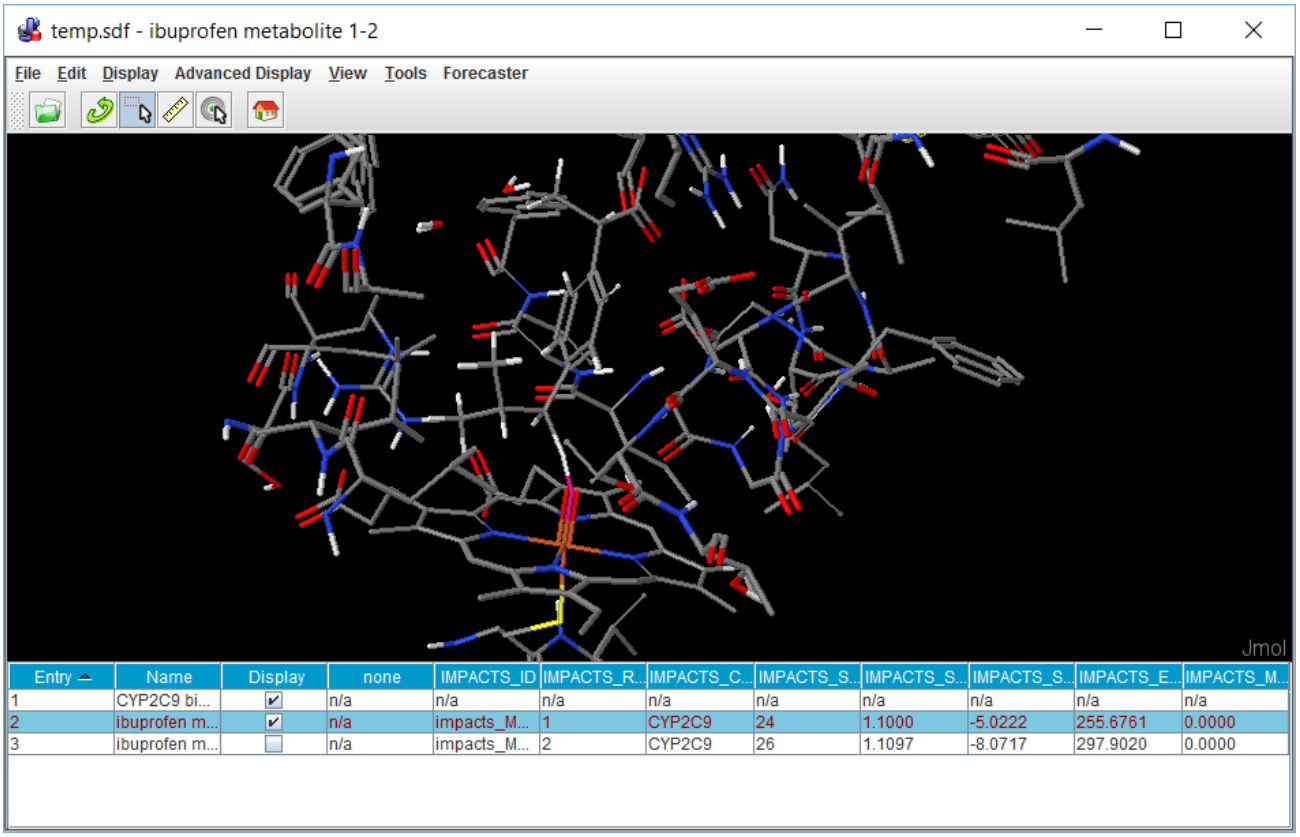


Alternatively, the results can be visualized by clicking the magnifier icon in the IMPACTS box. Clicking the **impacts_Metaboolites3D.sdf** button will open the results in the 3D viewer. Within this 3D viewer, a table of data with the corresponding sites of metabolism will be available to easily visualize and analyze the predicted 3D poses. In addition, clicking the **impacts_Metabolites2D.sdf** 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.







Entry	Name	Display	none	IMPACTS_ID	IMPACTS_R...	IMPACTS_C...	IMPACTS_S...	IMPACTS_S...	IMPACTS_S...	IMPACTS_E...	IMPACTS_M...
1	CYP2C9 bi...	☑	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	ibuprofen m...	☑	n/a	impacts_M...	1	CYP2C9	24	1.1000	-5.0222	255.6761	0.0000
3	ibuprofen m...	☐	n/a	impacts_M...	2	CYP2C9	26	1.1097	-8.0717	297.9020	0.0000