Toxicity of Reactive Xenobiotics Evaluated with Glutathione Nucleophilicity

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Introduction

Reactive metabolites formed through Cytochrome P450-mediated Phase I metabolism can be detoxified through conjugation reactions with the antioxidant glutathione (GSH). The conjugation reactions can occur through several pathways including 1,4-Michael addition reactions and nucleophilic aromatic substitutions (SNAr). Glutathione conjugation of xenobiotics results in increased hydrophilicity and excretion of the conjugate. Predicting the reactivity of compounds to glutathione conjugation is highly important for a more complete understanding of how xenobiotics are metabolised. In this poster we illustrate that quantum-mechanical (QM) density functional theory (DFT) calculations and QSAR approaches can identify the experimentally-determined GSH adducts of reactive species.

Data sets

- 45 literature compounds identified as reactive towards GSH, corresponding to 664 potential sites of conjugation, 69 of which are observed experimentally
- 29 literature compounds that were postulated as unreactive

Methods

DFT approach

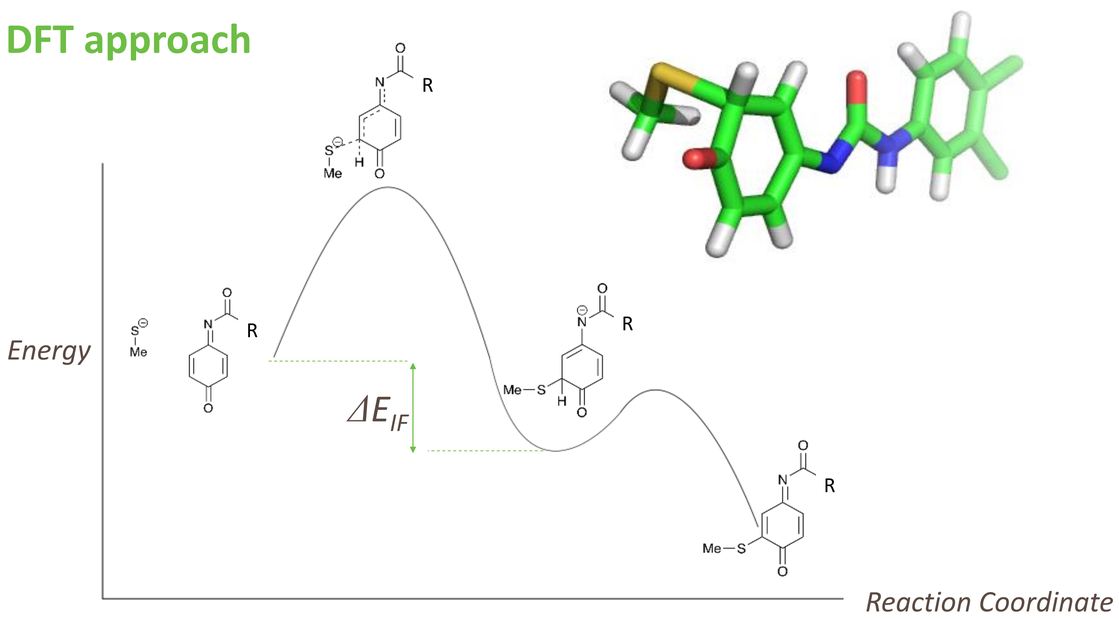

Figure 1: Optimised geometry of the MeS^- intermediate with quinone-imine triclocarban metabolite at the reactive site. This site forms a stable, associated intermediate.

- Intermediate formation energy ΔE_{IF} calculated at each carbon in the molecule (see Figure 1)
- DFT calculations performed with B3LYP functional and 6-31G* basis set
- Not all calculations lead to stable, associated intermediates (see Figure 2)
- If a site produced a stable, associated intermediate then the molecule was deemed to be “reactive”. If none of the sites produced a stable, associated intermediate the molecule was considered “unreactive”
- If multiple sites on the same compound formed stable, associated intermediates, the site with the larger ΔE_{IF} (the more stable intermediate) was deemed the site of conjugation

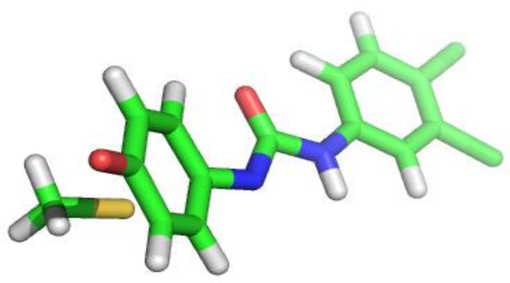

Figure 2: Optimised geometry of the MeS^- intermediate with quinone-imine triclocarban metabolite at an unreactive site ortho to the acylated imino nitrogen. A stable, associated intermediate is not formed.

QSAR approach

- Data matrix comprised of 664 potential sites from the 45 reactive molecules set
- The rows were split 80:10:10 into independent training:validation:test sets
- 3 splits were performed to estimate the sensitivity of model prediction to set split
- 8-fold oversampling was used within the training set to correct for the bias toward unreactive sites vs reactive sites
- Descriptors were generated from AM1 semi-empirical QM calculations
- The Auto-Modeller™ module of StarDrop™ [1] was used to generate classification models using Random Forests, Gaussian Processes, and Decision Tree methods

Results

DFT approach

- A stable intermediate was found at all experimentally observed site of GSH conjugation (no false negative predictions)
- Only 2 false positive site predictions were made in the reactive set (i.e. sites where conjugation had not been observed experimentally)
- For the unreactive set, 10 compounds were predicted as false positives

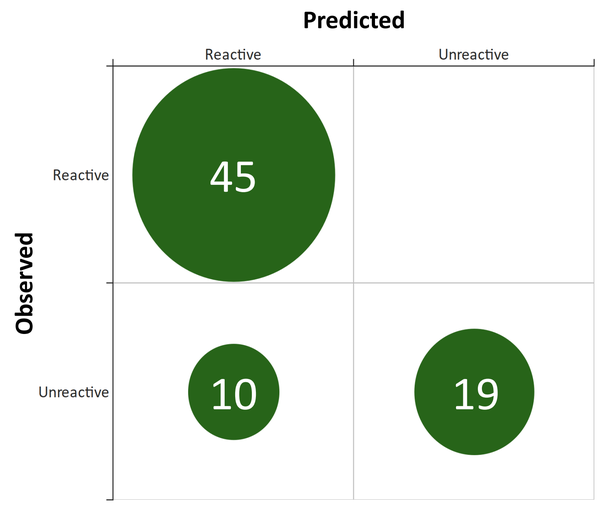

Figure 3: Confusion matrix covering the DFT results for the reactive and unreactive molecules (Kappa = 0.7)

These results rely on considering *both* the ΔE_{IF} energies *and* the state of association of the intermediate formed, as it was found that with some compounds (such as dehydroretronecine shown in Figure 4) the dissociated intermediates had lower energies than the associated ones.

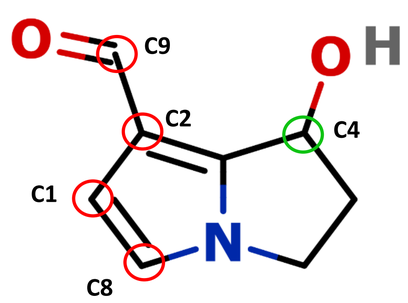

Figure 4: Structure of the dehydroretronecine aldehyde metabolite. Red circles denote sites not experimentally observed as GSH reactive, green circles denote experimentally observed reactive sites

Position	ΔE_{IF} (kjmol^{-1})	Associated intermediate?	Experimentally Observed
C1	-78.97	No	No
C2	-125.86	No	No
C4	-41.65	Yes	Yes
C8	-78.67	No	No
C9	-126.56	No	No

Table 1: Energies shown for different intermediates of dehydroretronecine with MeS- at 6-31G basis set and whether the associated intermediate is stable during the optimisation*

QSAR approach

- Test set Kappa values of 0.77 +/- 0.14 were achieved for different set splits (Kappa of ~0.6 without oversampling the training set)
- Important descriptors demonstrate that a mixture of electronic and accessibility factors determine the reactive vs unreactive classification

Descriptor	Importance
Fnn(+)	1
Angle to adjacent non-H atoms	0.96
Atom centred charge	0.95
Euclidean Distance to nearest heavy atom	0.77
No. of Electrons	0.65
Orbital Coefficient LUMO	0.54

Table 2: Important descriptors from Random Forest models to classify sites as reactive or unreactive. Fnn(+) is the nucleophilic Fukui number

- Unlike DFT, the QSAR models produce both false negative and false positive predictions
- The higher speed of the QSAR approach makes it more appropriate for compound prioritisation in the design process
- Some sites were found to be commonly mis-predicted, possibly indicating that the coverage of site property space is more sparse for these

Conclusions

We have shown two complementary methodologies for the successful prediction of xenobiotic susceptibility to conjugation by GSH. The DFT approach uses high-level QM theory to model the reactivity of each carbon site within a molecule and is able to correctly identify the reactive sites with no false negatives. The QSAR approach provides a fast screening approach with a good categorisation ability which should be maintained as the data sets used to create the models are expanded to cover a greater domain of applicability.

References

[1] StarDrop: www.optibrium.com/stardrop