From Theory to Practice: Use of Computational Methods for Nitrosamine Assessments

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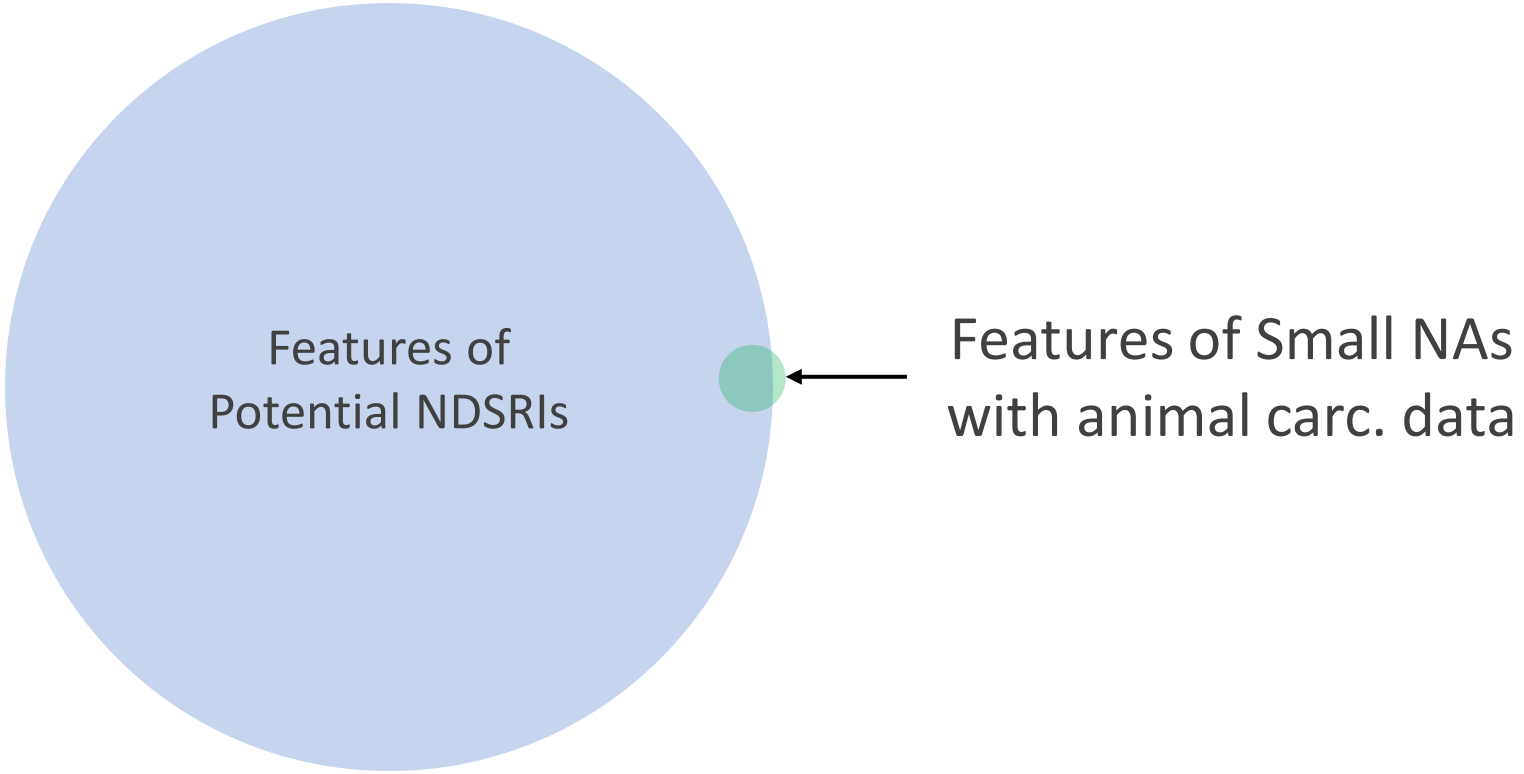
Possible Computational Approaches for Nitrosamines

- QSAR modeling between structure and carcinogenicity TD_{50} directly.
- SAR techniques to identify activating or mitigating features.
- Identification of analogs/surrogate chemicals that best represent the steric and electronic features of an untested example.
- Quantum mechanical calculations.
- Modeling metabolic activation of Nitrosamines.

Challenges

- Lack of experimental data for NDSRIs.
- The majority of experimental carcinogenicity data is from small nitrosamines and is not robust.
- A large number of NDSRIs may potentially form NAs.
- The structural diversity of NDSRIs is much larger than that of small nitrosamines with animal carcinogenicity data.

Chemical Space Around >N-N=O

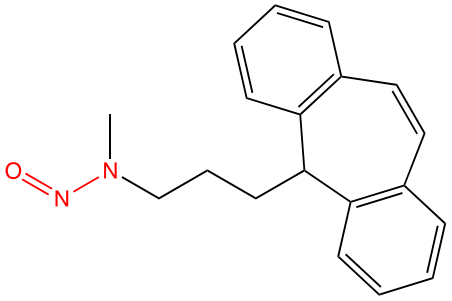


- 6277 NDSRIs.*
- 84 Small Nitrosamines.

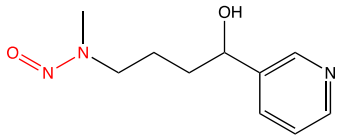
**Schlingemann et al, J. Pharm. Sci. 2022, 112 (5), 1287−1304.*

Importance of Local similarity

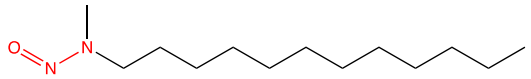
- Local similarity measures focus on structural features near the reactive functionality (e.g., the N-Nitrosamine moiety).
- Due to differences in complexity and size, global similarity measurements may not yield the best analogs.
- The most relevant analogs exhibit local similarity to the N-nitrosamine feature.



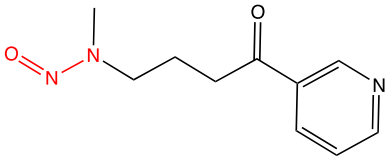
N-Nitroso protriptyline
QUERY CHEMICAL



Local Sim. = **0.775**
Global Sim. = 0.550
TD50 = 0.103 mg/kg/day



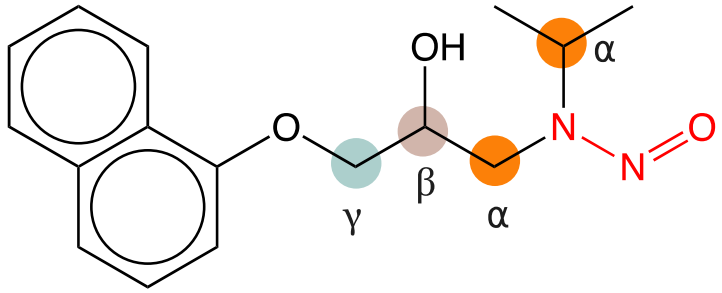
Local Sim. = **0.700**
Global Sim. = 0.339
TD50 = 0.537 mg/kg/day



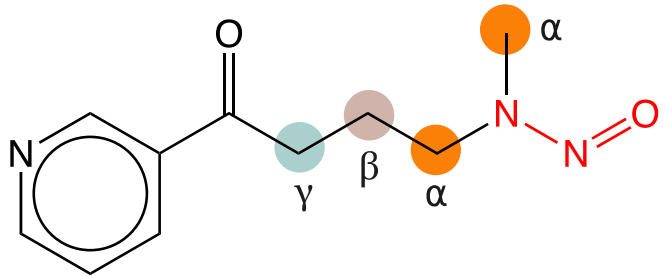
Local Sim. = **0.675**
Global Sim. = 0.326
TD50 = 0.142 mg/kg/day

*Chakravarti, S. K., Saiakhov, R. D. **Computing similarity between structural environments of mutagenicity alerts**, *Mutagenesis* 34, 55-65, 2018.

Matching Substitution Patterns of the Query and a Surrogate



Query NDSRI



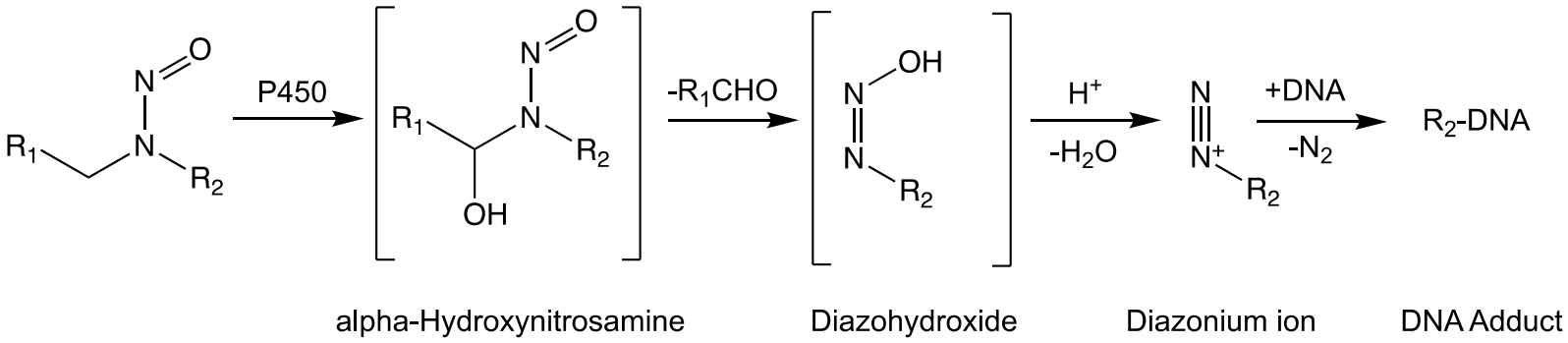
Surrogate (TD_{50} = 0.142 mg/kg/day)

Position	R_1	R_2
α ●	CH_2	$CH(CH_3)_2$
β ●	CH-OH	-
γ ●	CH_2	-
Cyclic?	No	

Position	R_1	R_2
α ●	CH_2	CH_3
β ●	CH_2	-
γ ●	CH_2	-
Cyclic?	No	

*Red colored entries are mismatched substituents

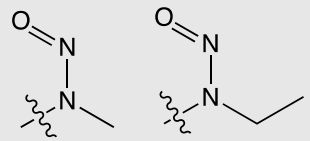
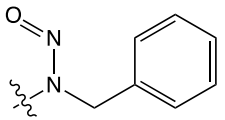
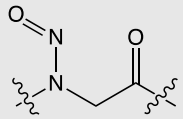
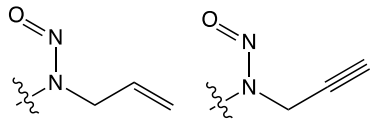
Effects of Substituents



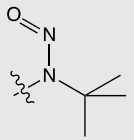
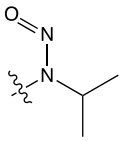
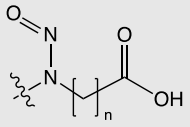
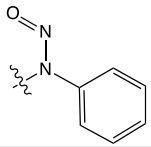
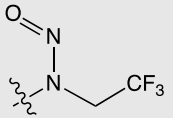
- On α-hydroxylation (R_1 substituents).
- On reactivity of the diazonium species (R_2 substituents).

Effect of Substituents on α-Hydroxylation

Potentially increase carcinogenicity*

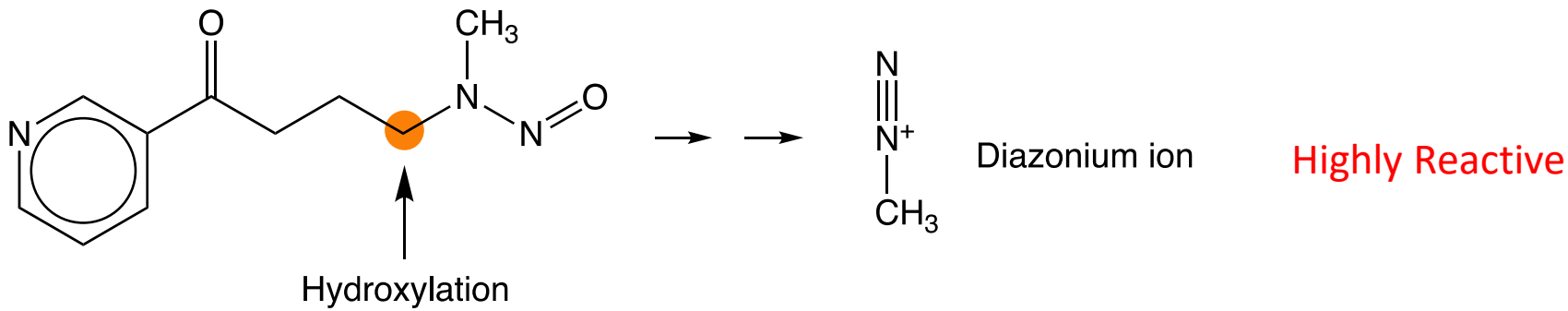
Substitution	Comment
	**Small alkyl chains** (methyl and ethyl)
	**Benzylic** (increased stability of the CYP generated radical)
	**β-Carbonyl** (increased acidity of the α-carbon H)
	**Allylic or propargylic** (increased acidity of the α-carbon H)

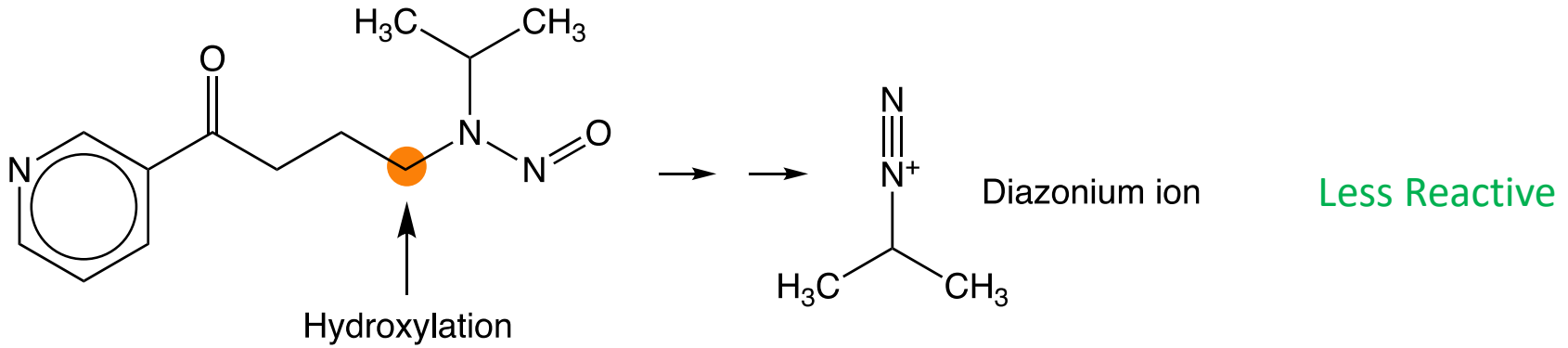
Potentially decrease carcinogenicity*

Substitution	Comment
	***tert*-Butyl** (absence of a α-carbon H)
	**Isopropyl** (steric hindrance to the α-carbon hydroxylation)
	**Carboxylic** (resistance towards oxidative metabolism)
	**Aromatic ring** (absence of a α-carbon H)
	**Strong electron withdrawing** (reduces α-carbon hydroxylation)

*Ponting, et al. "Strategies for Assessing Acceptable Intakes for Novel *N* -Nitrosamines Derived from Active Pharmaceutical Ingredients." *Journal of Medicinal Chemistry*, November 28, 2022. https://doi.org/10.1021/acs.jmedchem.2c01498.

Effect of Features on Reactivity of the Diazonium Species

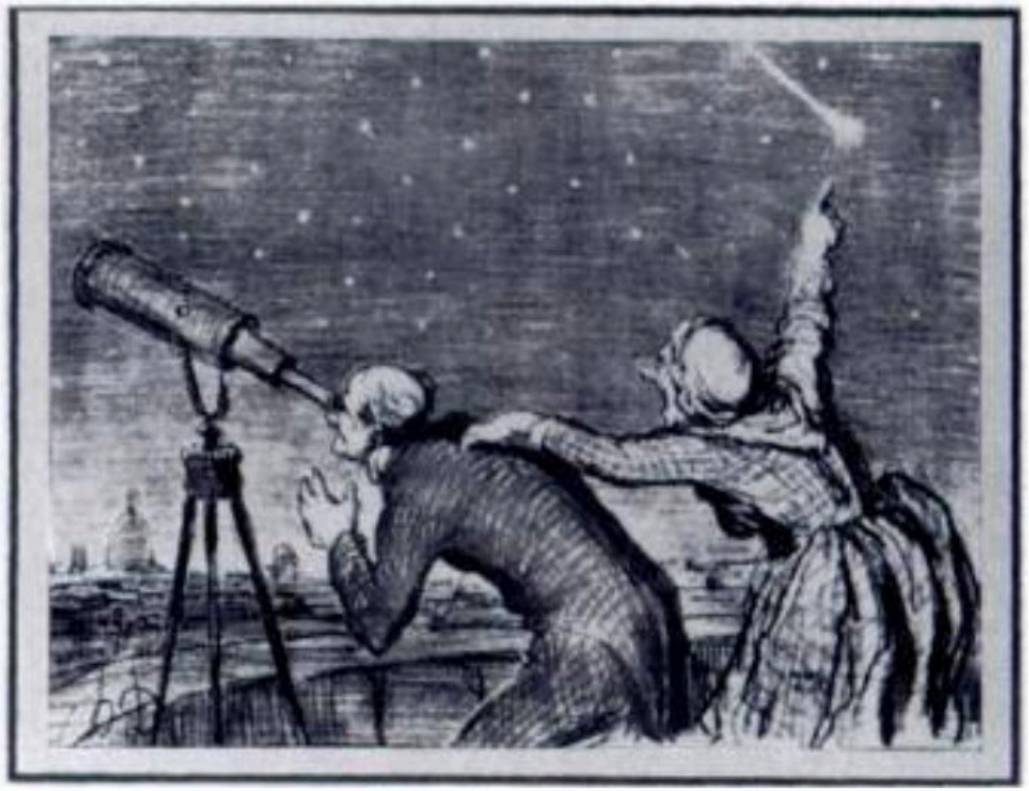




Can We Model α-Hydroxylation?

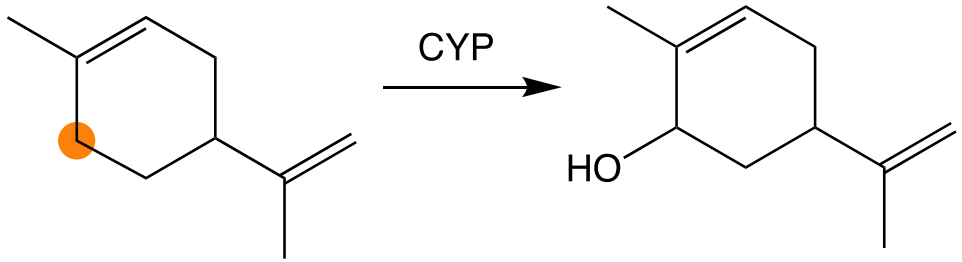
- To calculate the **likelihood** of hydroxylation based on structural features, for NAs in with liable α-carbon(s).
- To compute quantitative effects of structural features on α-hydroxylation.
- α-Hydroxy nitrosamine is unstable and transforms to reactive diazonium ion (in two steps).
- Large scale experimental observation of α-hydroxylated nitrosamines is not possible.
- Possible solution: Quantum Mechanics.

Can We Explore a More Obvious Location?



Picture credit: Meyers, M A. “Glen W. Hartman Lecture. Science, Creativity, and Serendipity.” *American Journal of Roentgenology* 165, no. 4 (October 1995): 755–64.

CYP-Mediated C-Hydroxylation Happens in Other Types of Compounds Too!



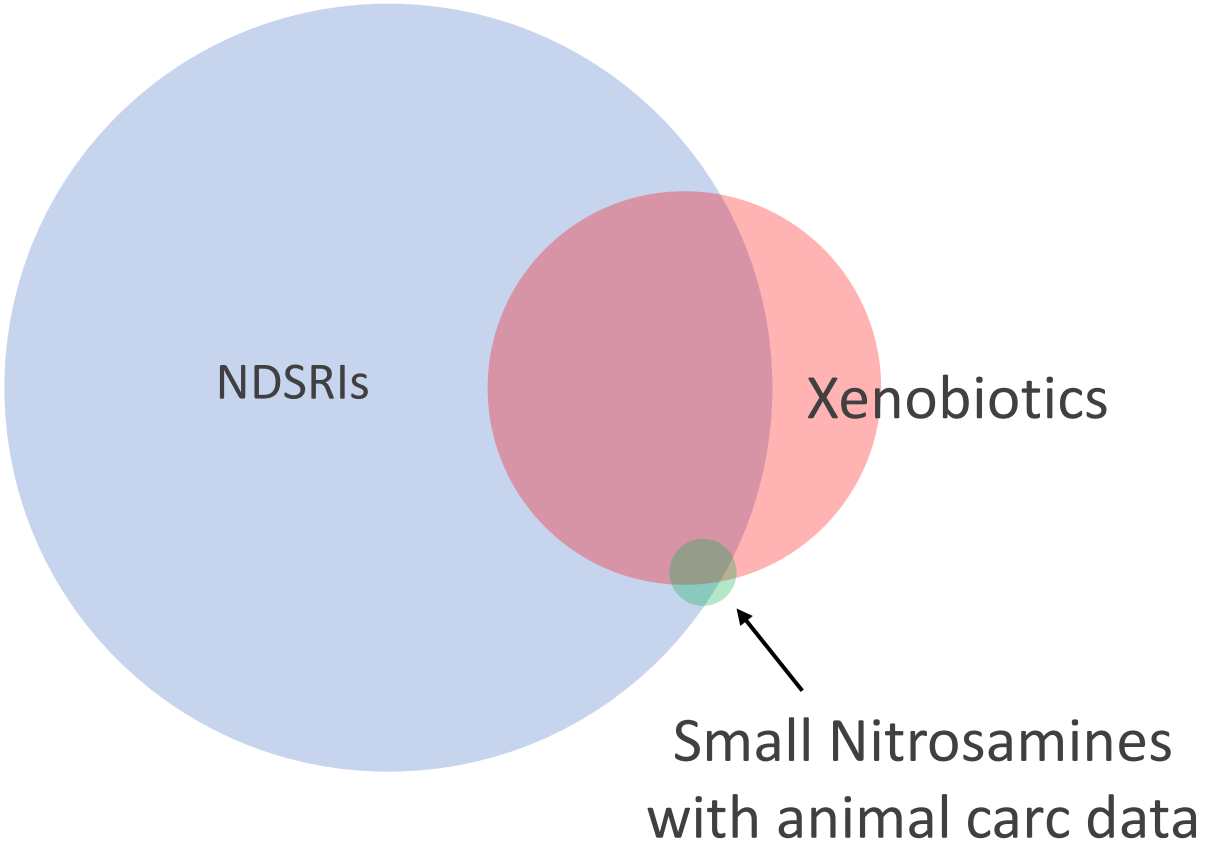
Substrate Metabolite

- Aliphatic C-hydroxylation is a common Phase-I metabolism process in xenobiotics mediated by CYP enzymes.
- Xenobiotic metabolism datasets are readily available.
- The likelihood of hydroxylation depends on the structural environment of the carbon atom.
- The resulting hydroxylated metabolites can be observed experimentally.

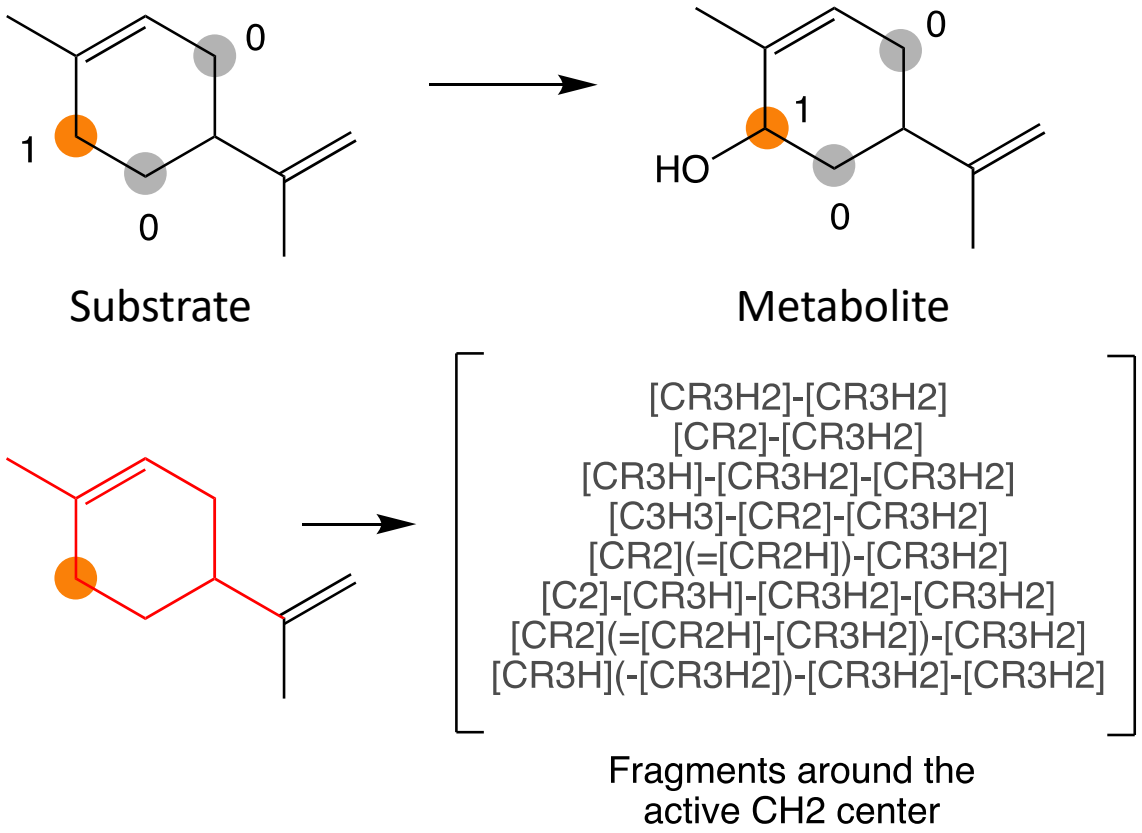
Reasonable Assumptions

- More labile carbon atoms will produce hydroxylated metabolites more frequently.
- The nitrosamine group is just another modulator of hydroxylation.
- The >N-N=O group's contribution towards hydroxylation is same in all NAs.
- The relative difference in NAs is due to other features around the >N-N=O group.

Better Coverage of the NDSRI Features



A Slightly Different Type of QSAR

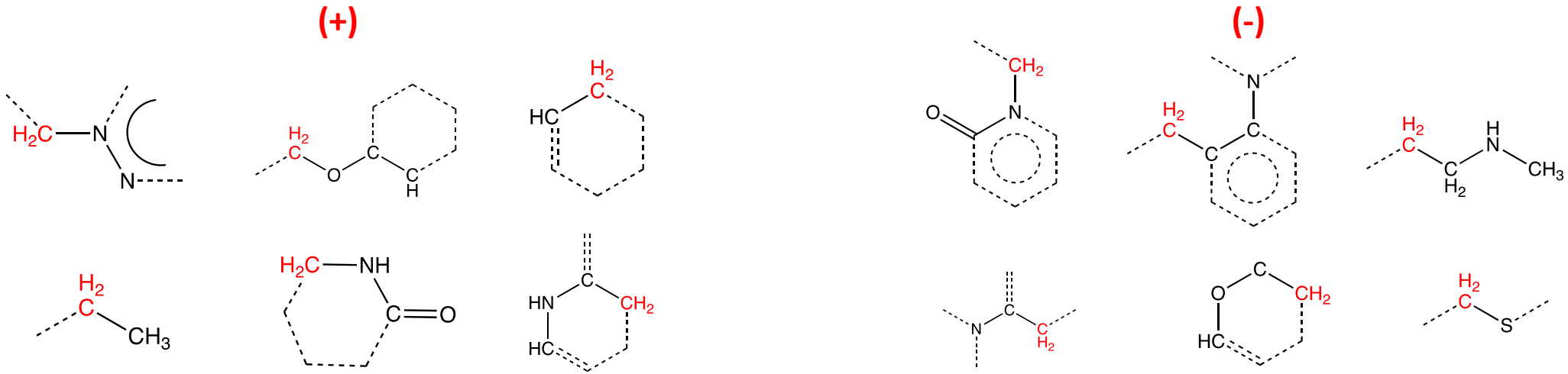


- Training set – 839 Active and 2691 Inactive CH2 centers from 620 substrate-metabolite pairs.
- 10-fold Validation performance: ~84% ROC-AUC

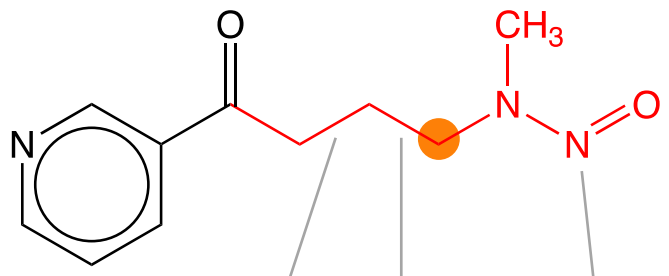
Chakravarti S. **Computational Prediction of Metabolic alpha-Carbon Hydroxylation Potential of N-Nitrosamines: Overcoming Data Limitations for Carcinogenicity Assessment**. *ChemRxiv.* March 14, 2023;

Features Affecting -CH_2- Hydroxylation in Xenobiotics

- Molecular fragments with positive and negative impact on CH2 hydroxylation:
  - 127 fragments with positive contribution (more than 0.100).
  - 209 fragments with negative contribution (less than -0.100).



α-Hydroxylation Prediction for Nitrosamines



frag #1

frag #2

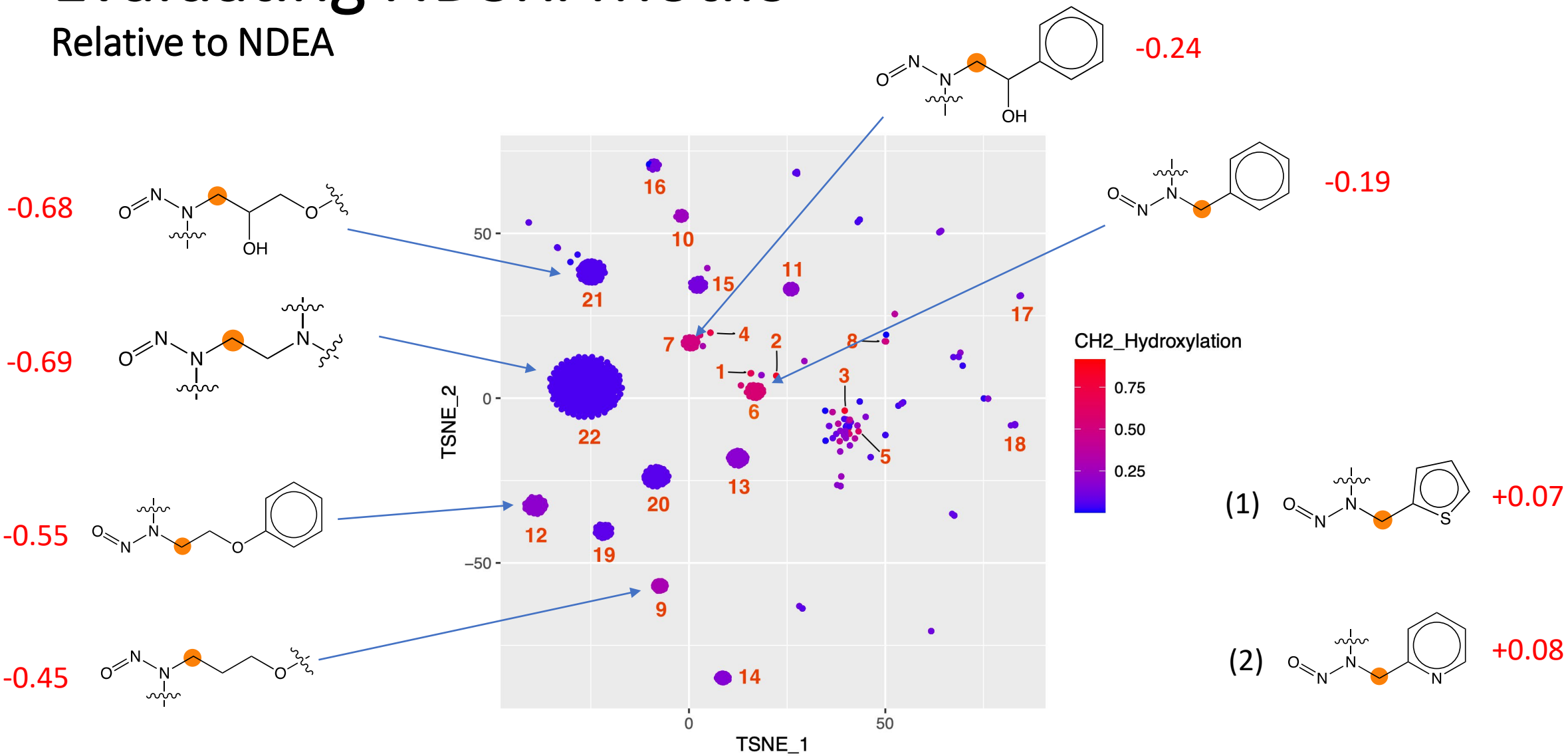
Nitrosamine Constant

$$p^{\alpha\text{-}Hydroxylation} = f(2.111 - 0.998 + \ldots + 2.00 - 1.126)$$

Regression Constant

Evaluating NDSRI Motifs

Relative to NDEA



A Small Step Forward

- Now we have the ability to quantitatively estimate the effects of a large number of specific chemical features on α-hydroxylation.
- The xenobiotic metabolism dataset is expandable, unlike the small nitrosamine dataset.
- This has the potential to improve the surrogate selection process.
- Applications extend beyond pharmaceuticals.

Limitations

- We found little overall correlation between predicted α-CH2 hydroxylation and carcinogenicity TD50 of nitrosamines.
- ‘Uncovered features’ in some query nitrosamines can lead to unreliable predictions.
- This may not fully capture the metabolic chemistry unique to nitrosamines.
- The analysis is entirely reliant on the xenobiotic metabolism data, i.e., the substrate-metabolite pairs.

Thank You!

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