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Extension of the CADRE Platform: A Quantum-Mechanical Tool for Predicting Carcinogenic Potency of *N*-Nitroso Impurities in Pharmaceuticals

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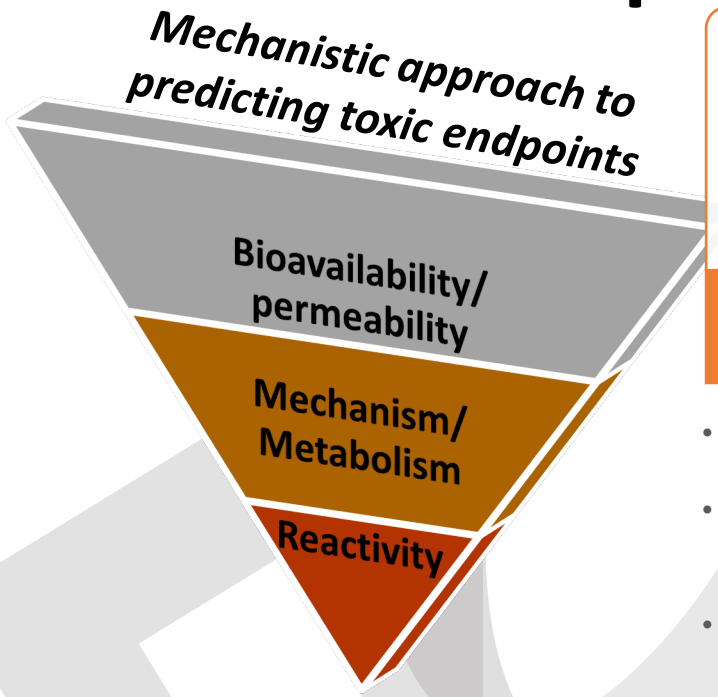
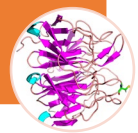
May 5th, 2023

CADRE: Computer-Aided Discovery and REdesign



Relies on Quantum Mechanics calculations

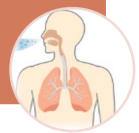
Skin sensitization



- *>90% external predictivity*
- *ECETOC category predictions*

- Kostal, J. et al, *Chem. Res. Toxicol.* **2016**, *29*, 58-64
- Roland, C. D. et al, *ACS Appl. Polym. Mater.* **2021**, 3, 2, 730–736
- Graham JC et al, *Chem. Res. Toxicol.* **2022**, 35, 6, 1011–1022

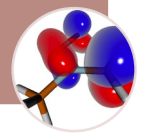
Respiratory sensitization



- *>80% external predictivity*
- *Binary predictions*

- Voutchkova et al, *Chem. Res. Toxicol,* **2022,** *35*, 2097–2106

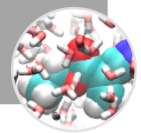
Nitrosamine carcinogenicity



- *>77% external predictivity*
- *COC potency cat predictions*

- Kostal, J. et al, *Chem. Res. Toxicol.* **2012**, *25,* 2780
- Kostal, J. et al, *Chem. Res. Toxicol.* **2023**, 36 (2), 291-304

Ecotoxicity

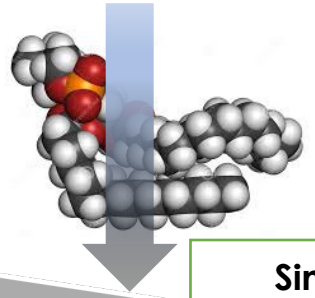


- *>80% external predictivity*
- *acute + chronic*
- *EPA's Safer Choice cat predictions*

- Kostal, J. et al. *PNAS*, **2015**, *112*, 6289-6294
- Melnikov, F. et al, *Green Chem.* **2016**, *18*, 4432-4445
- Thakore, R. R. et al, *Tetrahedron*, **2021**, *87,* 132090

- Service-based platform supported by high-performance computing
- **Integrating molecular simulations, expert systems and quantum mechanics in highly-predictive octox and ecotox models**
- **Over 10 years of broad use by major pharmaceutical and personal care industries** (4,000+ specialty/non-specialty chemicals tested to date)
- Externally validated on APIs/intermediates, pesticides, dyes, fragrances, extractables/leachables, peptide couplers, surfactants and polymers
- **Accepted for PMNs by US EPA and in registration under ECHA/REACH**
- Used in design/re-design strategies in new-product development

CADRE: relying on explicit modeling of molecular interactions



CHEMICAL STRUCTURE

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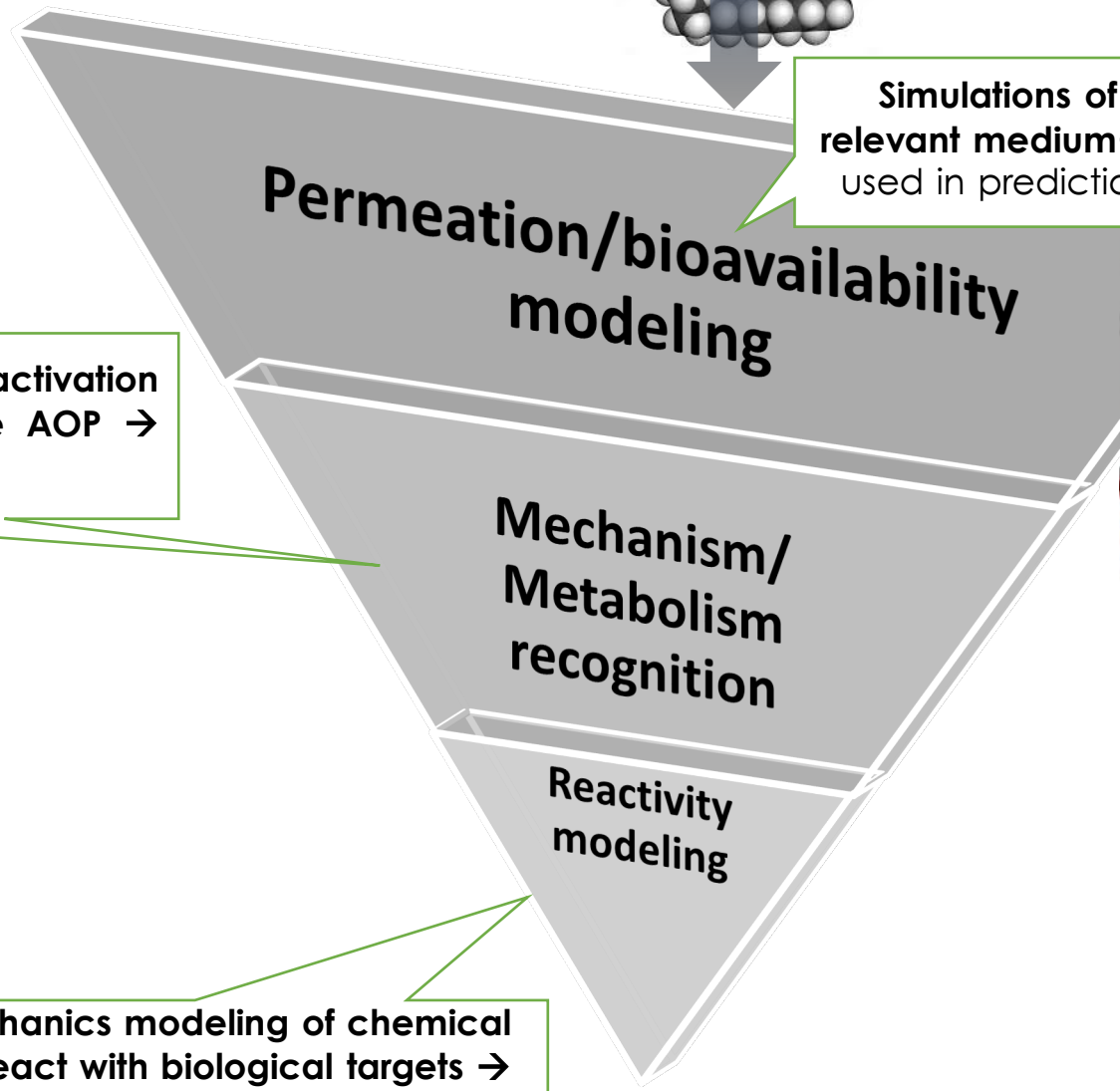
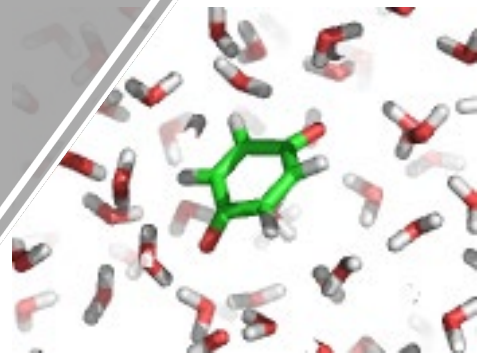
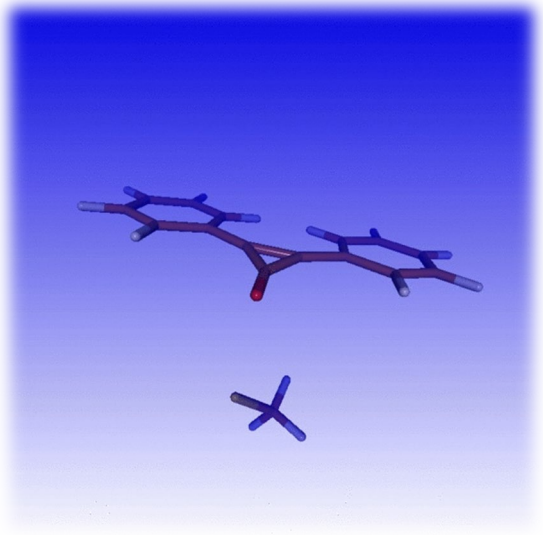
Simulations of chemical behavior in relevant medium→ interaction parameters used in prediction of barrier permeation





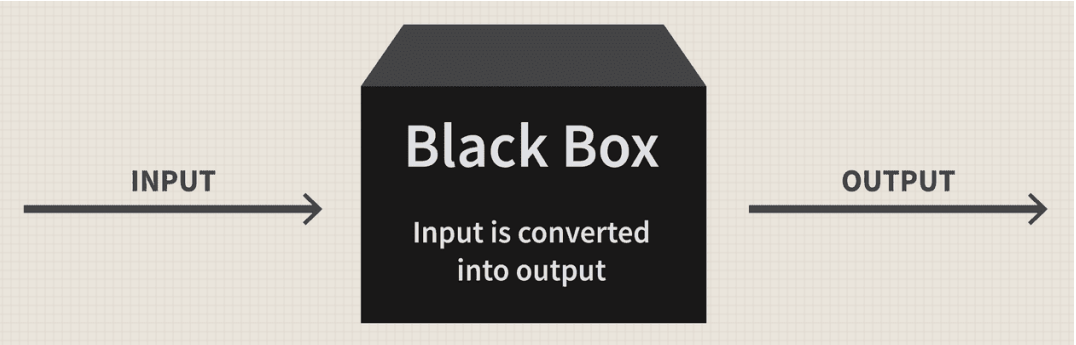
Mechanistic-pattern recognition for metabolic activation and/or target reactivity relevant to KEs in the AOP → determination of 'toxic possibility'



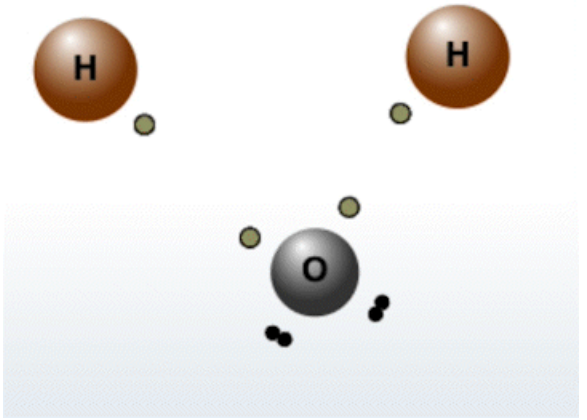
Quantum-mechanics modeling of chemical propensity to react with biological targets → potential and potency(!) classification of toxic response

Why trust quantum mechanics? Why not use readacross, ToxFix expert systems, traditional QSAR, AI/ML etc.

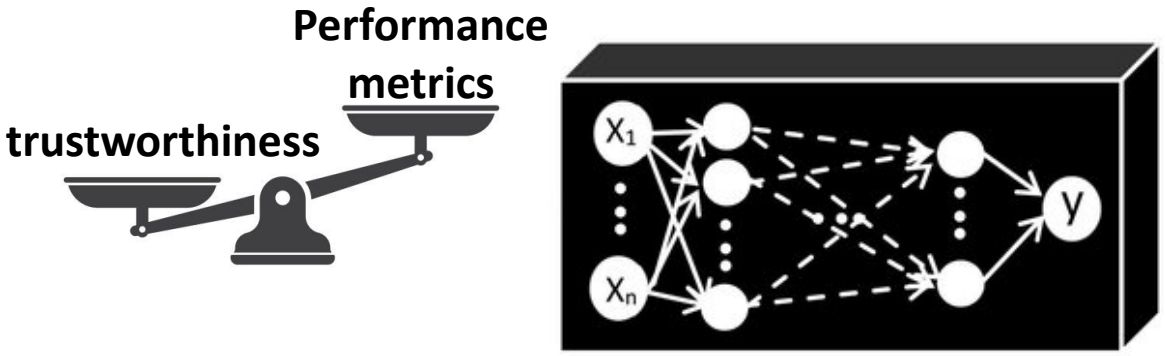
- Quantum mechanics is NOT a



- Quantum mechanics describes changes to electronic structure of molecules: making and breaking bonds → this is the underpinning of all metabolic processes!



- AI/Machine learning is not the solution in predictive toxicology! Relationships between inputs/outputs in neural networks are inscrutable



https://elearning.cpp.edu/learning-objects/making-molecules/

Nitrosamines: building on existing knowledge

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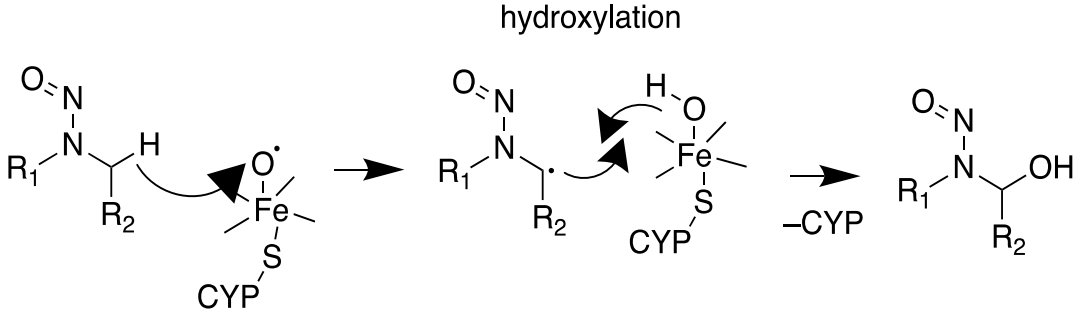

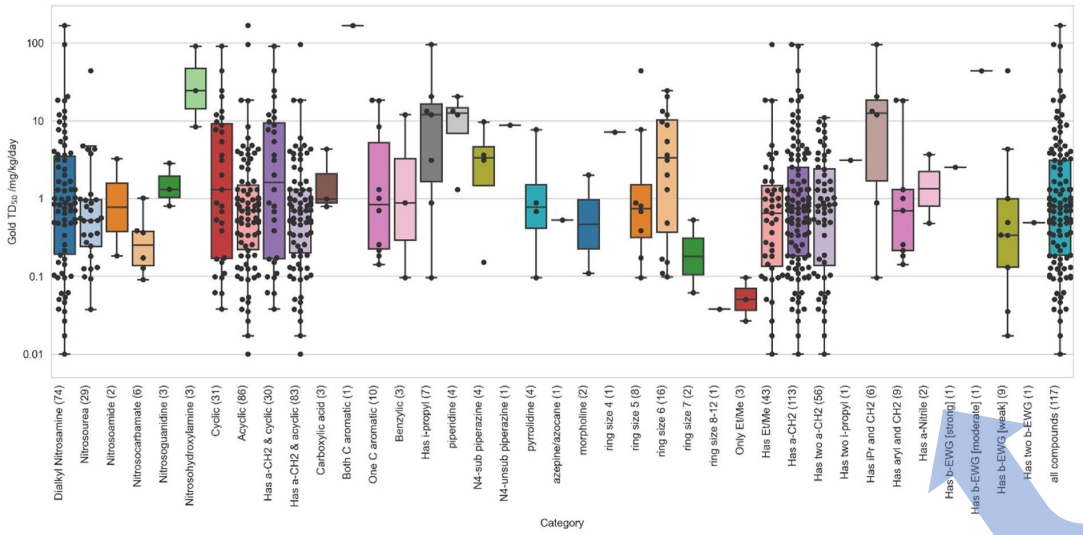

- (Ames) mutagenicity: high sensitivity in predicting rodent carcinogenicity → P450 metabolic activation controls DNA alkylation rates
- Having more (accessible) H's on alpha Cs ↑ toxicity
  - This promotes P450 hydroxylation (proposed to be the rate–determining step)
- Competing hydroxylations on beta/gamma positions can impede hydroxylation on alpha Cs (↓ toxicity)
- Competing metabolism: e.g., oxidation to aldehydes (↑ toxicity) or detoxification/ glucuronidation, etc. (↓ toxicity)

Rules and structural alerts have limited predictivity due to confounding factors and complex dependencies

Cross, K. P.; Ponting, D. J., Developing Structure-Activity Relationships for N-Nitrosamine Activity. *Comput Toxicol* **2021,** *20*, 100186

Model development overview

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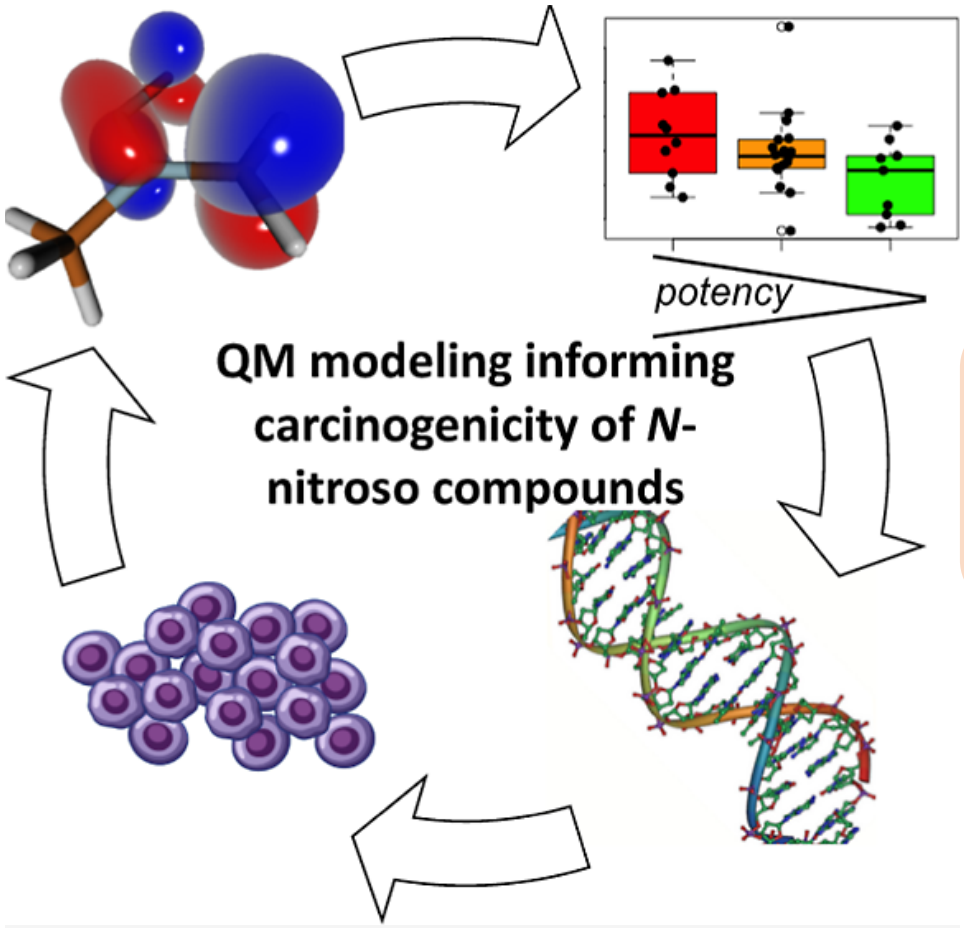

Lhasa carcinogenic database

Nitrosamines, nitroso-ureas, -amides, -carbamates, -imines and -hydroxylamines

([OX1]=[NX2][NX3]([#6])[#8,#6,#1])

	Potent COC	COC	Not a COC
Acceptable Intake Range (ng/day)	26.5	150	1500
TD_{50} (mg/kg/day)	0.0265	0.15	1.5
Range (mg/kg)	< 0.15	0.15-1.5	>1.5

Kostal et al. Quantum-Mechanical Approach to Predicting the Carcinogenic Potency of N-Nitroso Impurities in Pharmaceuticals. *Chem. Res. Toxicol.* **2023**, 36, 2, 291–304 https://doi.org/10.1021/acs.chemrestox.2c00380

Understanding key SARs from electronic structure

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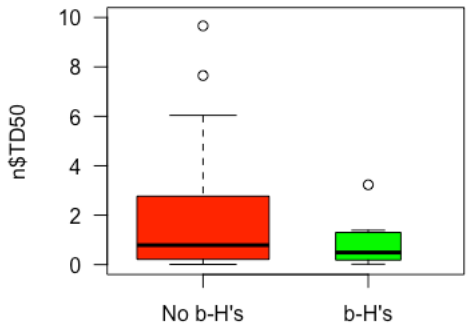
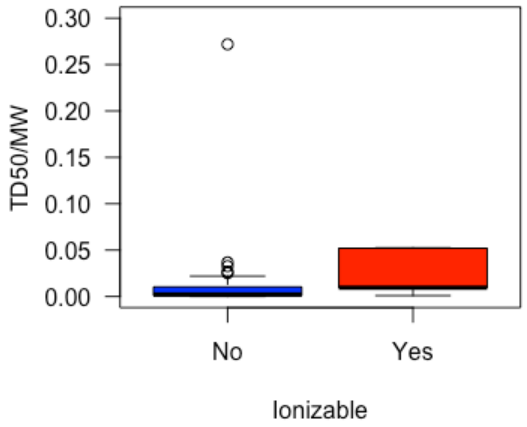
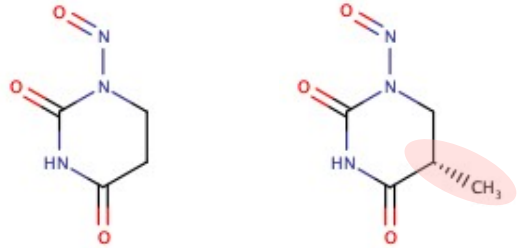
Beta Hs can increase potency!!
(15/18 are COCs)



Ionized lowers potency by decreasing bioavailability

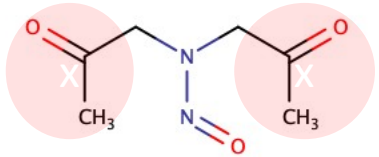




TD_{50}: 0.11
SASA (α-C): 4.1

TD_{50}: 31.2
SASA (α-C): : 1.9

TD_{50}: 68.7
Log S: -1.2



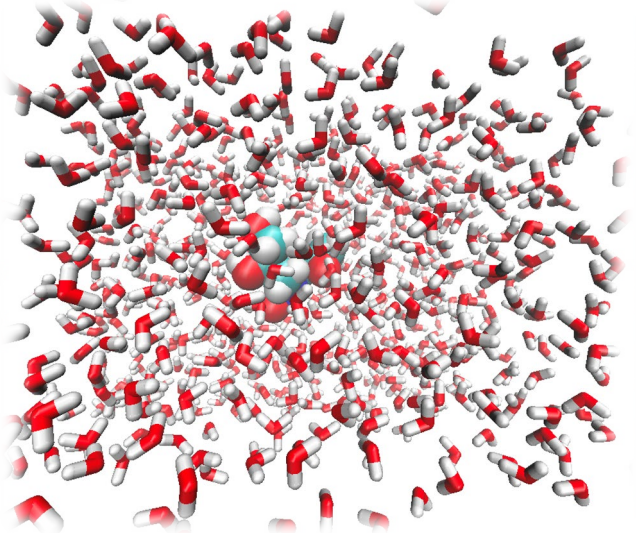
TD_{50}: 0.18
Log S: -0.065

Predictive model scoping:

- 92 QM variables (both global and local parameters derived from calculations and simulations in aqueous media)

Key effects considered:

- Ionization
- Hydrogenation at different positions



Understanding key SARs from electronic structure

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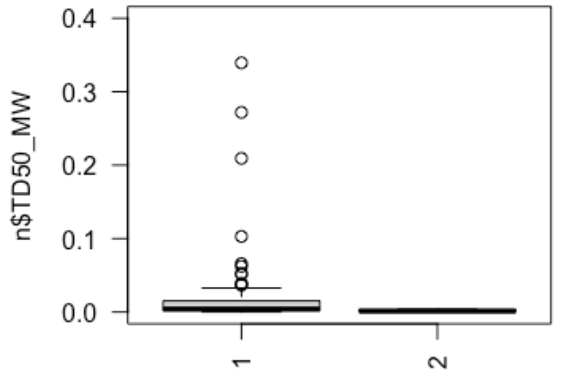
Multiple NNO groups → increased potency



TD_{50}: 0.134
Δμ (eV): -4.21

TD_{50}: 2.7
Δμ (eV): -3.67

- **Multiple NNO groups**

Understanding key SARs from electronic structure

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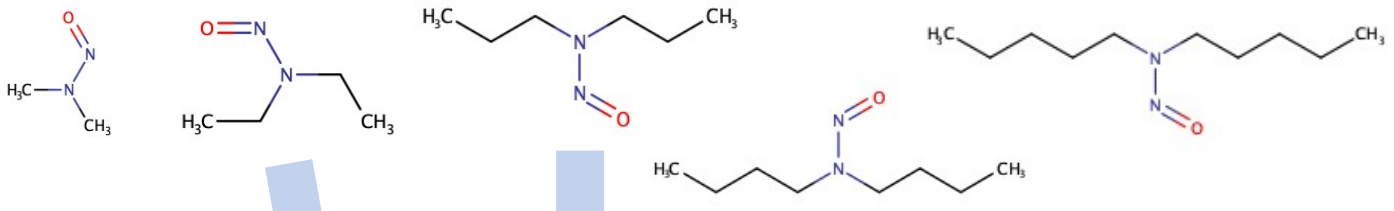
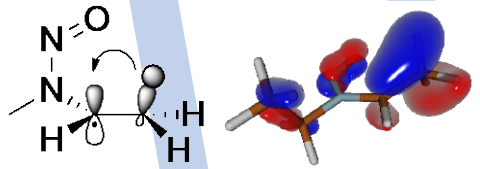


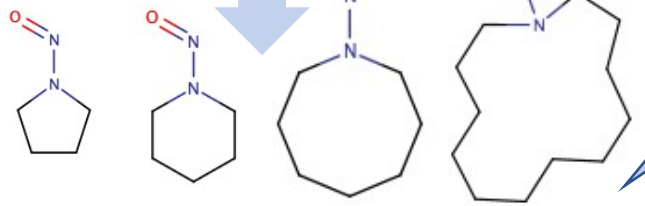
- Size and cyclization

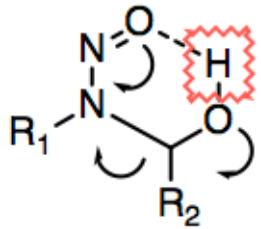
TD_{50}: 0.18 0.0177 0.186 0.691 4.09

Activity:



Steric hindrance cannot explain increase in activity



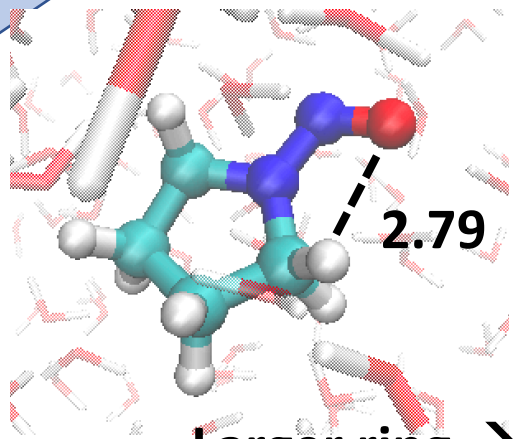


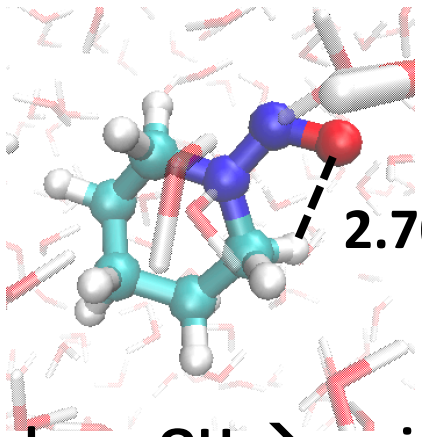
Proton transfer in heterolysis step

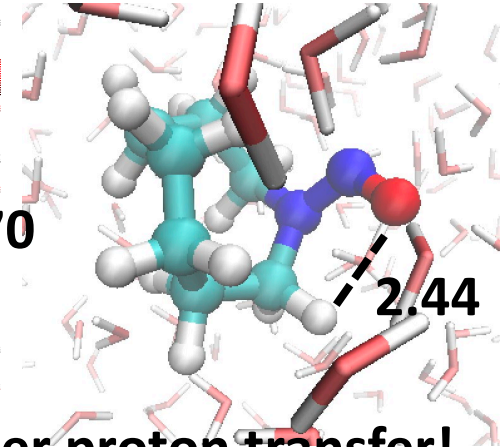
TD_{50}: 2.02 1.12 0.038 10.9

SASA (α-C): 22.7 22.4 20.1 18.2

Activity:







Larger ring → closer OH → easier proton transfer!

The model development process: capturing mechanistic complexity and uncertainty using QM descriptors

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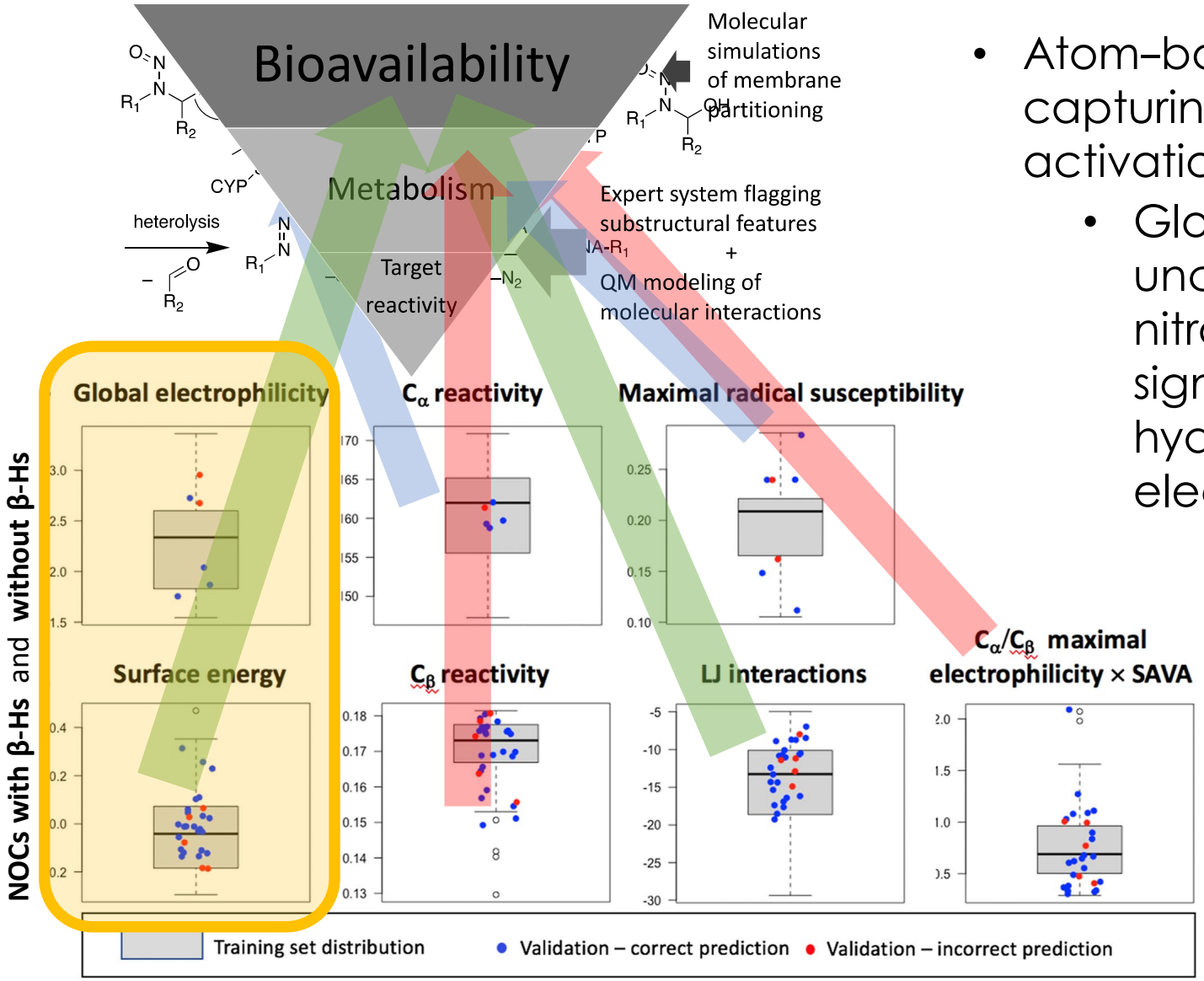

- Atom-based QM descriptors capturing key events in NOC activation
  - Global QM descriptors capturing uncertainty and competing *N*-nitroso mechanisms (e.g., 1,3-sigmatropic shift for nitrosamides, hydrolysis of nitrosoureas or direct electrophilicity of some NOCs)
    - Hybrid QM/MM Monte Carlo simulations in aqueous phase capturing bioavailability

Math is in the paper!!

Kostal et al. Quantum-Mechanical Approach to Predicting the Carcinogenic Potency of N-Nitroso Impurities in Pharmaceuticals. *Chem. Res. Toxicol.* **2023**, 36, 2, 291–304 https://doi.org/10.1021/acs.chemrestox.2c00380

Model performance

	CAT 1 Potent COC	CAT 2 COC	CAT 3 Not a COC
TD_{50} (mg/kg)	< 0.15	0.15-1.5	>1.5

Compound	Accuracy	LOO Accuracy	External Validation based on less reliable Gold CPDB
No beta hydrogens	92%	83%	71% for 3 potency categories 86% for cat 1&2 vs. cat 3
Beta hydrogens	94%	89%	79%
Overall	**93%**	**87%**	**77%**

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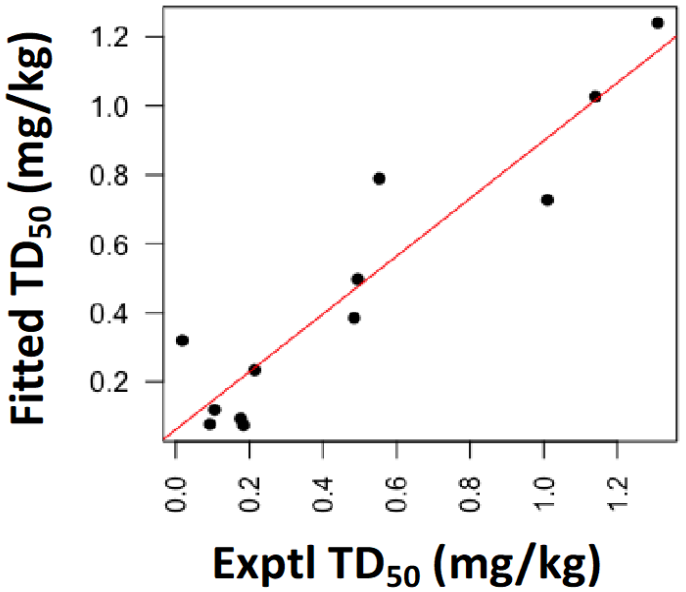

Confirmatory evidence for potent nitrosamines without β-Hs (TD_{50} < 1.5 mg/kg)
→ R^2 = 85% and R^2_{adj} = 82%

- Rigorous external testing: training/test set ratios for both LDA models were considerably more stringent (1.7) than the accepted industry standard (4)!!

Kostal et al. Quantum-Mechanical Approach to Predicting the Carcinogenic Potency of N-Nitroso Impurities in Pharmaceuticals. *Chem. Res. Toxicol.* **2023**, 36, 2, 291–304
https://doi.org/10.1021/acs.chemrestox.2c00380

How robust are these models when applied to complex pharmaceuticals?

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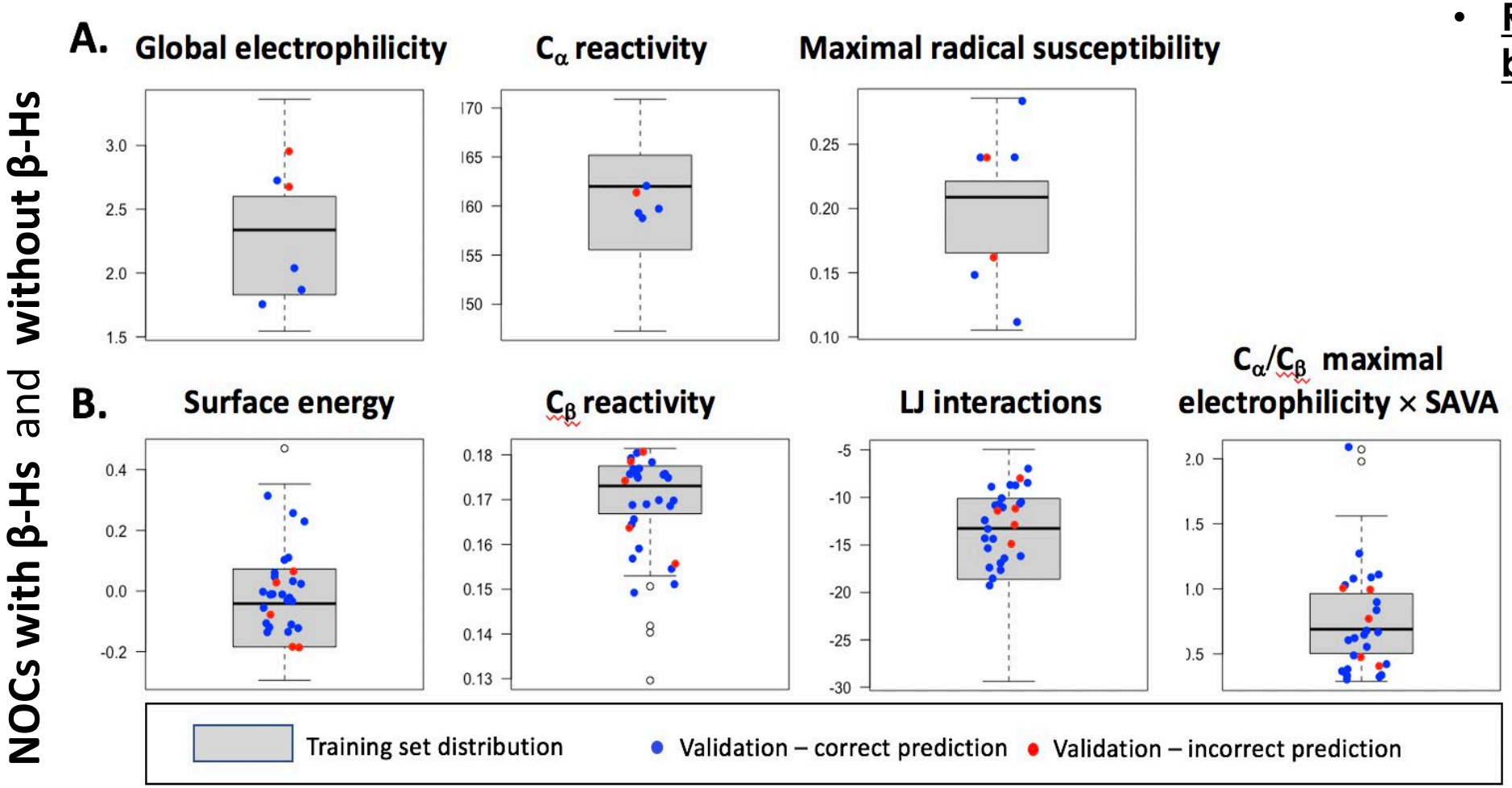

- **Robust predictivity beyond AD:**
  - exceeding the distribution of descriptor values in the training set **has no negative impact external predictivity**
  - no correlation between different NOC classes and outlier rates → **equal applicability to all NOCs**

Kostal et al. Quantum-Mechanical Approach to Predicting the Carcinogenic Potency of N-Nitroso Impurities in Pharmaceuticals. *Chem. Res. Toxicol.* **2023**, 36, 2, 291–304
https://doi.org/10.1021/acs.chemrestox.2c00380

CADRE applied to nitrosamines on the EMA list

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N-nitroso compound (CAS number)	AI (ng/day)	TD_{50} (mg/kg/day)	Source	Measured potency	Predicted potency
N-Nitrosodimethylamine (62-75-9)	96.0	0.177	LCDB	2	2
N-nitrosodiethylamine (55-18-5)	26.5	0.0177	LCDB	1	1
N-nitrosoethylisopropylamine (16339-04-1)	26.5	N/A	-	-	1
N-nitrosodiisopropylamine (601-77-4)	26.5	N/A	-	-	2
N-nitroso-N-methyl-4-aminobutyric acid (61445-55-4)	96.0	0.982	CPDB	2	2
1-Methyl-4-nitrosopiperazine (16339-07-4)	26.5	N/A	-	-	2
N-Nitroso-di-n-butylamine (924-16-3)	26.5	0.691	CPDB	2	2
N-nitroso-N-methylaniline (614-00-6)	34.3	0.106	LCDB	1	1
N-nitroso-morpholine (59-89-2)	127	0.135	LCDB	1	1
N-nitroso-varenicline (2755871-02-2)	37.0	N/A	-	-	3
N-nitrosodipropylamine (621-64-7)	26.5	0.186	CPDB	2	2
N-nitrosomethylphenidate (55557-03-4)	1300	N/A	-	-	3
N-nitrosopiperidine (100-75-4)	1300	1.12	LCDB	2	2
N-nitrosorasagilene (2470278-90-9)	18	N/A	-	-	2
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3- a]pyrazine	37	N/A	-	-	3
N-nitroso-1,2,3,6-tetrahydropyridine (55556-92-8)	37	0.0599	LCDB	1	2
N-nitrosonortriptyline (55855-42-0)	8		-	-	3
N-methyl-N-nitrosophenethylamine, (13256-11-6)	8	0.00797	LCDB	1	2

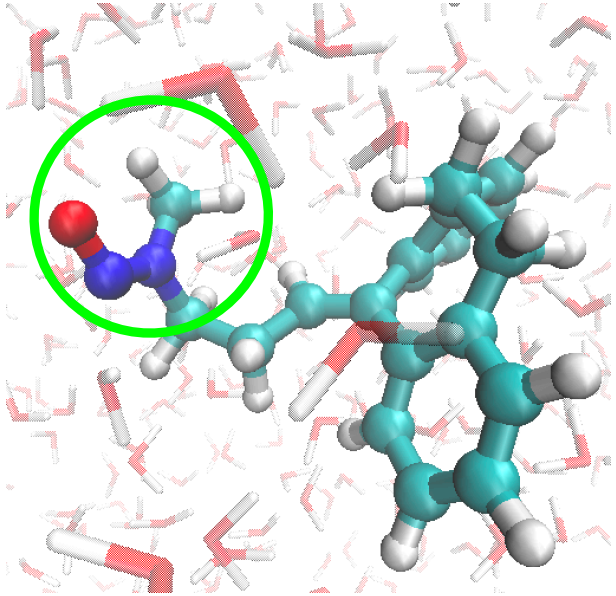
European Medicines Agency. Nitrosamine Impurities Report **2020**, https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities (accessed July 31, 2022).

Read-across vs. CADRE example

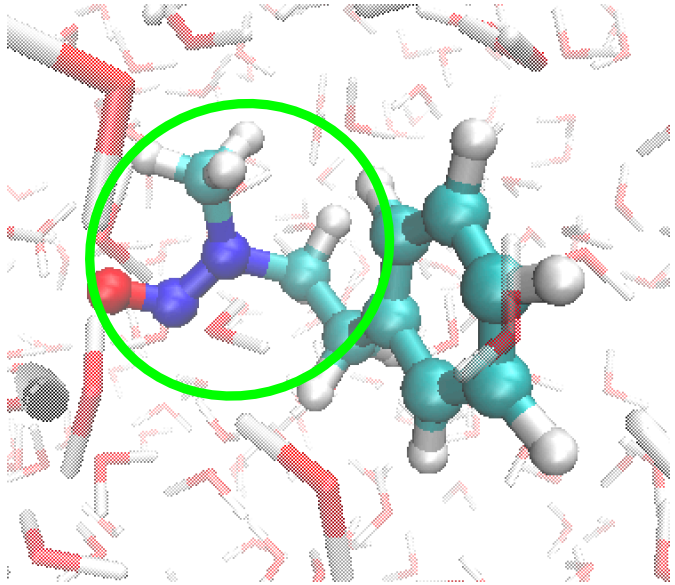
It's 'similar' so why is it less potent?

Steric effects in hydroxylation step from QM/MM MC simulations:

Syn (alpha C-H less accessible)



Anti (alpha C-H more accessible)



THANK YOU – QUESTIONS?

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